

UNIVERSIDADE FEDERAL DO PARANÁ

ANGELA MARIA ECHEVERRY TOBÓN

CONTRIBUIÇÃO AO CONHECIMENTO DAS ESPÉCIES DE *QUICHUANA*
KNAB (SYRPHIDAE: DIPTERA) COLETADAS EM BROMELIACEAE DA MATA
ATLÂNTICA DO ESTADO DO PARANÁ E UTILIZAÇÃO DE DNA-BARCODING
PARA ASSOCIAÇÃO DOS ESTÁGIOS DE DESENVOLVIMENTO DAS
ESPÉCIES

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2022

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ESPÉCIES

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A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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LUCIANE MARINONI

Presidente da Banca Examinadora

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MARIO ANTONIO NAVARRO DA SILVA

Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica

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MARCOANDRE SAVARIS

Avaliador Externo (ESCOLA SUPERIOR DE AGRICULTURA - LUIZ DE QUEIROZ - USP)

Assinatura Eletrônica

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TATIANA ALEJANDRA SEPULVEDA VILLA

Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

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*«Somos nuestra memoria, somos ese quimérico museo de formas
inconstantes, ese montón de espejos rotos».*

(BORGES, J.L. 1969, p. 7)

RESUMO

Quichuana Knab, 1913 (Diptera: Syrphidae) é um gênero Neotropical, endêmico, com 50 espécies descritas, sete das quais são relatadas para o Brasil. Os adultos são visitantes florais onde se alimentam de néctar e pólen, enquanto as larvas são aquáticas e estão associadas à matéria orgânica em decomposição depositada em fitotelmata de diversas plantas. Neste estudo, descrevemos e ilustramos pela primeira vez *Quichuana* **sp. nov.**, (terceiro instar larva, pupário e macho) e os estágios imaturos de *Quichuana pogonosa* (ovo, terceiro instar larva e pupário) a partir de material coletado na fitotelmata das bromélias *Alcantarea imperialis* Carrière e *Aechmea distichantha* Lem., do Paraná, Brasil; novos dados de interações tróficas, ciclo de vida e habitats larvais são dados, melhorando substancialmente o conhecimento sobre os estágios imaturos e a história natural do gênero, constituindo também uma linha de base para futuros estudos morfológicos comparativos. Sequências do gene mitocondrial citocromo c oxidase I (COI) foram geradas para ambas as espécies e uma análise das composições de nucleotídeos e divergências dessas sequências foi feita junto com as de outras cinco do conjunto de dados do Barcode of Life Data Systems (BOLD) para estudar a divergência intraespecífica e interespecífica. Os resultados obtidos a partir de dados moleculares e morfológicos apoiam a delimitação de *Quichuana* **sp. nov.**, e foi possível associar as larvas de terceiro estágio de ambas as espécies com seus correspondentes adultos. Também podemos detectar que *Q. pogonosa* possui uma variedade que aqui é chamada de *Q. pogonosa* **var.**

Palavras-chave: Mosca-das-flores, estágios imaturos, fitotelmata, Taxonomia integrativa.

ABSTRACT

Quichuana Knab, 1913 (Diptera: Syrphidae) is a Neotropical endemic genus, with 50 described species, seven of which are reported from Brazil. The adults are visitors of flowers where they feed on nectar and pollen, while the larvae are aquatic and are associated with decaying organic matter, deposited in phytotelmata of various plants. In this study, are describe and illustrate for the first time *Quichuana* **sp. nov.** (third instar larva, puparium, and male adult); as well the immature stages (egg, third instar larva, and puparium) of *Quichuana pogonosa*, from material collected in the phytotelmata of the bromeliads *Alcantarea imperialis* Carrière and *Aechmea distichantha* Lem., from Paraná, Brazil; new data on trophic interactions, life cycle, and larval habitats are given, substantially improving knowledge about immature stages and the natural history of the genus, also constituting a baseline for future comparative morphological studies. Mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences were generated for both species and an analysis of nucleotide compositions and divergences of these sequences was done along with those of five others from the Barcode of Life Data Systems (BOLD) dataset to study intraspecific and interspecific divergence. The results obtained from molecular and morphological data support the delimitations of a *Quichuana* **sp. nov.** and it was possible to associate the third instar larvae of both species with their corresponding adults. We also could detect that *Q. pogonosa* has a variety we herein call *Q. pogonosa* **var.**

Keywords: Flower flies, immature stages, phytotelmata, integrative taxonomy.

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INTRODUÇÃO GERAL

Syrphidae é uma família de Diptera, com mais de 6.400 espécies descritas em todo o mundo (Pape e Evenhuis 2021). Os sirfídeos habitam a maioria dos ecossistemas terrestres sendo encontrados em todos os continentes, exceto na Antártica e em algumas ilhas oceânicas remotas (Thompson et al. 2010). De acordo com Thompson et al. (2010), 82 gêneros e 1.800 espécies ocorrem na Região Neotropical, compreendendo mais de 30% da diversidade mundial da família, porém, existem muitas espécies para serem descobertas e descritas.

As moscas da família Syrphidae são conhecidas como "hoverflies" ou "flower flies", por serem excelentes voadoras com a habilidade de pairar no ar, e ao fato de que a maioria dos adultos são visitantes frequentes de flores, atuando como polinizadores importantes em ambientes naturais e agrícolas (Dunn et al. 2020; Doyle et al. 2020). Os membros desta família apresentam uma grande variedade de formas, cores e tamanhos, com comprimento do corpo que varia entre 4 e 35 mm (Sommaggio 1999); muitas espécies apresentam notáveis semelhanças com a morfologia, padrão de coloração e comportamento de adultos de Hymenoptera aculeata, tais como abelhas e vespas, fornecendo um exemplo de mimetismo batesiano (Sommaggio 1999; Leavey et al. 2020).

Syrphidae podem ser rapidamente diferenciados de outros Diptera pelos seguintes caracteres morfológicos dos adultos: (i) asa com célula basal alongada (R, BM e CuP), (ii) célula R_{4+5} peciolada, (iii) células apicais fechadas, e (iv) presença de uma longa veia longitudinal, denominada veia espúria, que ocorre entre os setores radial e medial, esta veia pode apresentar vários graus de esclerotinização, sendo inconspícua em algumas poucas espécies (Thompson et al. 2010).

Atualmente os representantes da família estão classificados em quatro subfamílias: Eristalinae, Microdontinae, Pipizinae e Syrphinae (Mengual e Rojo 2015; Young et al. 2016; Pauli et al. 2018). Por muito tempo, as relações filogenéticas dentro da família foram baseadas principalmente no estudo dos caracteres morfológicos dos adultos (Ståhl et al. 2003); no entanto análises separadas e combinadas de dados moleculares, caracteres morfológicos dos

estágios imaturos e adultos, e alguns aspectos de sua biologia, forneceram a classificação atual, gerando um contexto sólido para estudos evolutivos (Rotheray e Gilbert 1999; Ståhls et al. 2003; Mengual et al. 2008; Young et al. 2016; Moran et al. 2021).

Assim como todos os dípteros ciclorrafos, as larvas dos sirfídeos passam por três instares ou estágios antes de se desenvolverem em pupários (Rotheray 1993). O terceiro ínstar e pupários de Syrphidae podem ser distinguidos dos outros dípteros pela seguinte combinação de caracteres: (i) dorso do protórax com dobras longitudinais (imperceptível em Microdontinae e alguns Syrphinae), (ii) tubos dos espiráculos posteriores fundidos em um único tubo de respiração esclerotinado (separado apenas em algumas espécies não aquáticas), (iii) comprimento do tubo variável entre subfamílias, sendo reduzidos nas subfamílias Syrphinae, Microdontinae e Pipizinae, e alongado e retrátil em Eristalinae (Rotheray et al. 1993; Pérez-Bañon et al. 2003).

Ao contrário de outros dípteros, a diversificação da morfologia dos sirfídeos imaturos é tão impressionante quanto a sua capacidade de adaptação a diferentes ambientes, o que tem desencadeado em um conjunto variado de estruturas especializadas para alimentação, respiração e movimentação (Rotheray et al. 1993). De acordo com Souba et al. (2020), a maior diversidade ecológica de Syrphidae é encontrada nos estágios larvais, já que têm necessidades bióticas e abióticas muito variáveis, diferentes daquelas dos adultos.

Três grupos funcionais podem ser reconhecidos em Syrphidae de acordo com a biologia das larvas: fitófagos, que se desenvolvem em tecidos de plantas vivas agindo como minadores de folhas, raízes e caules; saprófagos, que filtram microorganismos em vários materiais orgânicos em decomposição e zoófagos, alimentando-se de outros artrópodes, com alguns casos conhecidos de parasitoidismo (Rotheray e Gilbert 2011; Ricarte et al. 2007; Thompson et al. 2010; Mengual e Rojo 2015).

As larvas de Microdontinae são predadoras, parasitas e parasitóides, alimentando-se exclusivamente de ovos, larvas e pupas de formigas (Reemer e Ståhls 2013; Pérez-Lachaud et al. 2013; Scarparo et al. 2017). As larvas de

Pipizinae são predadoras, com preferência por Sternorrhyncha (Hemiptera) (Mengual e Rojo 2008). As larvas de Eristalinae apresentam uma grande variedade de hábitos alimentares, com espécies fitófagas e predadoras, sendo a maioria delas saprófaga que se desenvolvem em ambientes úmidos ou aquáticos, com grandes quantidades de matéria orgânica em decomposição (Martins et al. 2010; Arcaya e Mengual 2016). Finalmente, Syrphinae que de acordo com Vujić et al. (2013) é um grupo generalista.

As larvas de Eristalinae são facilmente reconhecidas por apresentarem o processo respiratório posterior alongado que pode atingir o dobro do comprimento do corpo em espécies da tribo Eristalini, pelo que são popularmente conhecidas como “larvas cauda-de-rato”. Esse processo telescópico permite a respiração de oxigênio atmosférico, em ambientes aquáticos ou semiaquáticos (Rotheray e Gilbert 1999), como os contidos em plantas terrestres com modificações para o armazenamento de água, assim como as bromélias que retêm água nas axilas internas das folhas para formar fitotelmas (poças de plantas) (Frank e Lounibos 2009).

As plantas da família Bromeliaceae são comuns em florestas úmidas, como a Mata Atlântica, onde se apresentam como uma das famílias de epífitas com maior riqueza de espécies (Freitas et al. 2016). No caso de Syrphidae, apesar da considerável falta de conhecimento sobre as suas associações com estas plantas, de acordo com Marcos-García et al (2012), espécies de *Ocyptamus* Macquart, 1834; *Quichuana* Knab, 1913 e *Copestylum* Macquart, 1846 parecem dever sua alta radiação à sua associação com bromélias (Bromeliaceae).

Apesar da grande importância das formas imaturas em termos de ocupação de ambientes diferenciados e diversidade morfológica, a maior parte dos trabalhos taxonômicos realizada para a família se fundamenta no estudo dos adultos, principalmente de sua morfologia. Da mesma forma, as sequências de DNA que são uma ferramenta valiosa para reconhecer e descrever espécies, são pouco utilizadas. Dessa forma, pelo exposto, buscamos preencher essas lacunas de conhecimento, com informação taxonômica dos estágios imaturos, dados sobre sua biologia e dados moleculares.

Os resultados desse estudo foram divididos em dois capítulos, seguindo o formato de artigos científicos. Em ambos os capítulos apresentamos dados biológicos e morfológicos de duas espécies do gênero *Quichuana* obtidos a partir da criação de larvas coletadas nas bromélias *Aechmea distichantha* Lem. e *Alcantarea imperialis* Carrière.

Embora duas espécies da tribo *Eristalini* pertencentes ao gênero *Eristalis* Latreille, 1804, e *Lejops* Rondani, 1857, também tenham sido coletadas e criadas, nossos estudos se concentraram apenas nas espécies de *Quichuana*, uma vez que informações sobre hábitos, morfologiae e biologia de estágios imaturos são praticamente inexistentes.

O gênero de mosca-das-flores, *Quichuana* Knab (1913) é endêmico da Região Neotropical. Atualmente são conhecidas 50 espécies distribuídas do México à Argentina, sete espécies foram registradas para o Brasil. Seus representantes podem ser facilmente distinguidos pela presença de pilosidade no olho; sub-basais, dorsais, arista nu; pós-pronoto com pilosidade; metafêmur posterior com placa basoventral de sétulas pretas; veia da asa R₄₊₅ fortemente sinuosa e célula R₁ variando de aberta a fechada na margem da asa, mas nunca peciolada (Thompson et al. 2010).

Enquanto as moscas adultas costumam visitar flores em busca de néctar e pólen, registros publicados indicam que as larvas que apresentam o processo respiratório posterior alongado (Pérez-Bañón et al. 2003) são aquáticas ou semi-aquáticas e estão associadas a matéria orgânica em decomposição depositada em fitotelmas de plantas das famílias Bromeliaceae, Heliconiaceae, Poaceae e Marantaceae. (Walker 1857, Picado 1913, Shannon 1925, 1927, Lane e Carrera 1944, Seifert e Seifert 1976, 1979, Seifert e Florence 1976, Thompson 1981, Thompson et al. 2010, Ricarte et al. 2012, Reemer 2016).

A maioria das mosca-das-flores aquáticas parecem depender das fitotelmas contidos em plantas, principalmente bromélias, para completar seu ciclo de vida. Portanto, sua conservação não pode ser alcançada sem conservar sua planta hospedeira.

As bromélias são muito sensíveis à variação ambiental e o ecossistema onde vivem e estão sendo fortemente afetadas pelas mudanças climáticas, e outras pressões antrópicas (Sabagh et al 2017). Portanto, é prioritário gerar informações que permitam estabelecer estratégias para sua conservação.

No primeiro capítulo, descrevemos a morfologia dos estágios imaturos de *Quichuana pogonosa* Fluke, 1937 incluindo ovo, larva de terceiro ínstar e pupário de amostras coletadas em fitotelmata das bromélias *Aechmea distichantha* e *Alcantarea imperialis* nos Municípios de Balsa Nova e Curitiba, Paraná, Brasil; fornecemos as sequências do mitochondrial cytochrome c oxidase subunit I (COI) gene e dados sobre a história de vida da espécie.

No segundo capítulo são acrescentadas informações sobre os imaturos de *Q. pogonosa* e sua variabilidade intraespecífica. Também, é descrita uma nova espécie criada a partir de larvas obtidas da fitotelmata da bromélia *Aechmea distichantha* no município de Balsa Nova, Paraná, Brasil. São apresentadas ilustrações e dados preliminares sobre seu ciclo de vida. Análises das sequências do gene mitocondrial citocromo c oxidase I (COI) foram realizadas para essas espécies, juntamente com as de outras cinco sequências de espécies de *Quichuana* do conjunto de dados Barcode of Life Data systems (BOLD), para estudar a divergência em nível intraespecífico e interespecífico.

Análises moleculares juntamente com a morfologia confirmaram a *Quichuana* **sp. nov.**, como novidade para a ciência e conseguimos associar a larva de terceiro ínstar ao adulto; além de uma indicação de que as espécies *Quichuana angustiventris* (Macquart, 1855), *Quichuana calathea* Shannon, 1925 e *Quichuana picadoi* Knab, 1913 são espécies intimamente relacionadas.

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CHAPTER I

**Description of the immature stages, biology and DNA- *barcoding* of
QUICHUANA POGONOSA (Diptera: Syrphidae) collected in Bromeliaceae in
Paraná, Brazil**

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RESEARCH ARTICLE

Description of the immature stages, biology and DNA-barcoding of *Quichuana pogonosa* (Diptera: Syrphidae) collected in Bromeliaceae in Paraná, Brazil

Angela Echeverry¹, Diego de S. Souza^{1,2}, Luciane Marinoni¹

¹Laboratório de Estudos em Diversidade de Insetos da Região Neotropical, Departamento de Zoologia, Universidade Federal do Paraná. Caixa Postal 19020, 81531-980 Curitiba, PR, Brazil.

²Departamento de Entomologia, Museu Nacional, Universidade Federal do Rio de Janeiro. Quinta da Boa Vista, São Cristóvão, 20940-040 Rio de Janeiro, RJ, Brazil.

Corresponding author: Angela Maria Echeverry (angela.mariae@hotmail.com)

<http://zoobank.org/488A6108-C58C-4C52-9555-6672E65DA862>

ABSTRACT. *Quichuana* Knab, 1913 is a Neotropical genus of flower flies (Diptera: Syrphidae) with 50 valid species. Adults of this genus are flower visitors and their larvae are usually associated with the phytotelmata of bromeliads and heliconias, actively participating in the recycling of nutrients in forest environments. Despite their importance in ecosystem dynamics, *Quichuana* larvae are poorly known. Herein we describe the immature stages of *Quichuana pogonosa* Fluke, 1937 from samples collected from the phytotelmata of two terrestrial bromeliad species in the state of Paraná, Brazil. We provide illustrations of the egg, third instar larva and puparium, as well as information on the life cycle of the species. Additionally, we describe and illustrate the male genitalia and present the DNA-barcoding based on larva and adult specimens.

KEY WORDS. Atlantic Forest, hoverflies, morphology, phytotelmata, taxonomy.

INTRODUCTION

Syrphidae, commonly known as flower flies or hoverflies, is one of the most diverse families of Diptera, with more than 6,200 species worldwide (Young et al. 2016), of which 1,800 species from 60 genera have been recorded from the Neotropical region (Thompson et al. 2010). Adults of this family play an important role as pollinators in both natural and agricultural ecosystems, since they are mostly flower visitors, feeding on pollen, nectar or honeydew (Rotheray and Gilbert 2011, Klecka et al. 2018, Doyle et al. 2020). Syrphid larvae have very diverse feeding habits, including phytophagous, saprophagous, and zoophagous (Rotheray and Gilbert 1999, Wagner et al. 2008).

Syrphids are currently classified into four subfamilies, according to Mengual et al. (2015): Eristalinae, with saprophagous and phytophagous larvae (Campoy et al. 2017, Zorić et al. 2019, Pérez-Bañón et al. 2020); Microdontinae, whose larvae are closely associated with ants (Reemer 2013); Pipizinae, comprising predatory larvae (Mengual et al. 2015); and Syrphinae, with predatory larvae of a wide range of soft-bodied arthropods, and some plant miners (Nishida et al. 2002).

Quichuana Knab, 1913 is a Neotropical genus of Eristalinae (Eristalini: Helophilina) with 50 valid species distributed from Mexico to Argentina (Thompson et al. 2010). The taxonomy of *Quichuana* is well-resolved based on the adult morphology (Thompson et al. 2010, Ricarte et al. 2012). The genus was revised by Hull (1946), Thompson (1972) and Ricarte et al. (2012). Posteriorly, Montoya et al. (2017) described two additional species. Most of the taxonomic work was based on the adult stage, and little is known about the immature stages (Rotheray and Gilbert 2008).

Rearing observations indicate that the larvae of *Quichuana* species are aquatic, saprophagous and develop in tanks of bromeliads, inflorescences of heliconias species, and foci of wet rot in woody and non-woody plants (Walker 1857, Picado 1913, Shannon 1925, 1927, Lane and Carrera 1944, Seifert and Seifert 1976, 1979, Seifert and Florence 1976, Thompson 1981, Thompson et al. 2010, Ricarte et al. 2012, Reemer 2016).

Morphological, behavioral and ecological characters of the immature stages are important for understanding evolutionary relationships among species of Syrphidae (Rotheray and Gilbert 1999, Ståhl et al. 2003, Mengual et al. 2015). With the goal to increase the understanding of the taxonomy of *Quichuana*, we

describe the morphology of the immature stages of *Quichuana pogonosa* Fluke, 1937 – a species restricted to Southern Brazil (Fluke and Plaumann 1937, Morales and Köhler 2008) – including egg, third instar larva and puparium. Also, we provide the DNA-barcoding and data on the life history of the species.

MATERIAL AND METHODS

Collecting of samples

The specimens were obtained from the phytotelmata of bromeliads at two localities in the state of Paraná, Brazil: 12 eggs and one adult female were collected from *Alcantarea imperialis* (Carrière) Harms J.R. Grant, in the neighborhood of Jardim das Américas, urban area of Curitiba, 25°27'37.3"S, 49°13'36.8"W, 14.xii.2020, A. Echeverry and D. Vanegas leg.; and third instar larvae were collected from the phytotelmata of *Aechmea distichantha* Ruiz and Pav., at the farm 'Chácara Bonita', rural area of Balsa Nova, 25°31'05.6"S, 49°35'11.4"W, 06.ix.2020, A. Echeverry and J. Paludo leg. Below, these locations will be referred to as site 1 and site 2, respectively. The identification of bromeliads species was confirmed using the catalog 'Flora do Brasil' (Faria et al. 2020, Versieux 2020).

The samples from *Alcantarea imperialis* were collected in situ without disassembling the plant. Eggs and larvae were sorted directly in the water tanks using Pasteur pipettes and brushes, and the adults foraging on the leaves were collected with an entomological net. The bromeliad, *Aechmea distichantha* had to be disassembled and the leaves removed one by one, from the outermost part to the inflorescence, exposing the larvae. All syrphid specimens examined in this study were deposited in the Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil (DZUP).

Rearing the immatures

The samples were reared at the Laboratory of Studies on Diversity of Insects of the Neotropical region (Taxonlab), Universidade Federal do Paraná. Eggs and larvae were placed individually in plastic containers (12 cm diameter, 11 cm height). They were closed on top with a veil to allow air exchange. Leaves and water from the bromeliad from which samples had been collected were put in the respective plastic containers. The containers were kept at room temperature and the samples sprayed daily with distilled water to maintain local humidity. Each container was examined daily until pupation. Then the puparia and the substrate were transferred to another container without water to prevent the proliferation of fungi. The adults were euthanized four hours after emergence. The four hour wait allowed their cuticle to harden and their wings to become fully extended. During the wait period, the adults were fed with honey and water.

Morphological study of immatures

Third instar larvae selected for the morphological study were recognized by the presence of a pair of discs with differ-

entiated cuticles on the dorsal surface of the first abdominal segment (Hartley 1961). Third instar larvae were immersed in nearly boiling water for three minutes to extend the body tissues and were then put in 70% ethanol.

Two eggs and three first instar larvae were directly preserved in 70% ethanol. The puparia were immersed in warm 10% KOH for 30 minutes to extract the cephalopharyngeal skeleton, which was then immersed in glacial acetic acid to remove the excess KOH, and were stored in microvials containing glycerine.

The length of the larvae and puparia were measured from the anterior margin of the prothorax to the anterior margin of abdominal segment 8. The length of the abdominal segment 8, including the posterior respiratory process, is presented separately. The terminology used in the descriptions follow Rotheray (1993) for the general morphology of the third instar larva, Marcos-García and Pérez-Bañón (2001) for the chaetotaxy and puparium, Campoy et al. (2017) for the eggs and Campoy et al. (2020) for the cephalopharyngeal skeleton.

Specimens were studied using a Nikon SMZ800 stereo-microscope and illustrations were made using a drawing tube fixed to the stereomicroscope. Photographs were taken using a camera Leica DFC-500 digital camera on a Leica MZ16 stereoscope and mounted using the software Leica LAS 3D Viewer and LAS Montage v.4.7.

Morphological study of adults

Adult specimens were euthanized by freezing and were pinned dry. Male genitalia were cleared in 10% KOH, washed in 5% acetic acid and then stored in microvials containing glycerine. The microvials were pinned with the specimen.

The identification of adults was determined using the key to *Quichuana* species (Ricarte et al. 2012) and the original description of *Q. pogonosa* by Fluke and Plaumann (1937). The identification was confirmed by Dr Antonio Ricarte (Universidad de Alicante, Spain). The terminology used to describe the male genitalia follows Cumming and Wood (2017).

DNA extraction, amplification and sequencing of DNA-barcoding

Two third instar larvae and two adults were preserved in absolute ethanol for the molecular experiments. The right hind leg of the adults and a portion of the abdominal segment 6 of the larvae were used for DNA extraction. Total genomic DNA of these samples was extracted using a non-destructive method with the aid of Invitrogen – PureLink Genomic DNA Mini-Kit, following the manufacturer's protocol. Specimens used for DNA extraction were deposited at DZUP.

Amplification of the cytochrome c oxidase subunit 1 (cox1), using the primers LCO1490 and HCO2198 (Folmer et al. 1994) and the kit MyTaq™ DNA Polymerase (Bioline), was performed in a total volume of 25 µL, consisting of 1 µL of genomic DNA, 5 µL of 5 MyTaq Reaction Buffer (containing 15 mM MgCl₂ and 5 mM dNTPs), 0.5 µL of each 0.01 mM primer, 0.1 µL of MyTaq DNA Polymerase and 17.9 µL of ultrapure wa-

ter. PCR cycle conditions were as follows: initial denaturation at 94 °C for 3 min, 5 cycles of annealing temperature at 94 °C for 30 s, 47 °C for 40 s, 72 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 52 °C for 40 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using 5 M ammonium acetate precipitation with isopropanol and sequenced in both directions using the BigDye Terminator v. 3.1 technology (Applied Biosystems, Foster City CA, U.S.A.).

RESULTS

Collecting and rearing of immatures

In total, 23 samples of *Q. pogonosa* were collected, including 12 eggs and one adult female specimen from Site 1 and two third instar larvae from Site 2. Details on the rearing of these samples are presented in Table 1. In addition, one larva of *Lejops barbiellinii* (Ceresa, 1934), two larvae *Eristalis* sp. and one larvae of *Quichuana* sp. were collected in co-occurrence with *Q. pogonosa* in Site 1.

Table 1. Total number of specimens of *Quichuana pogonosa* collected, reared and fixed in this study.

	Egg	Larva		Puparium	Adult
		First instar	Third instar		
Collected	12*	–	7	3	1 female
Rearred	8	8	5	9	12 (8 females, 4 males)
Fixed	2	3	3	12	13

* Two eggs lost.

TAXONOMY

Quichuana pogonosa Fluke, 1937

Type locality: Brazil, Santa Catarina, Nova Teutônia. Distribution: Venezuela, Brazil. Holotype male, American Museum of Natural History, New York. Type locality: Brazil, Santa Catarina, Nova Teutônia. Distribution: Venezuela, Brazil.

Fluke and Plaumann 1937: 5 (fig. 14, head of female); 11 (original descriptions); Hull 1946: 2 (key to species); 15 (fig. 8, face of female paratype); Thompson 1972: 135 (fig. 50, male genitalia); Morales and Köhler 2008: 44 (list), 46 (list); Ricarte et al. 2012: 102 (fig. 55, right antennae, lateral inner view), 108 (diagnostic features), 125 (key to species).

Egg (Fig. 1)

Length: 0.94 ± 0.04 mm; width: 0.51 ± 0.07 mm ($n = 2$). White colour, oval in shape, twice as long as wide. Opercular region flatten. Chorionic surface with microsculpture made up of longitudinal rectangular units evenly distributed throughout the egg. Operculum as a truncated cone.

Examined material. Site 1: 10 exemplars (two unhatched eggs and 8 shells from hatched eggs; all deposited together under DZUP 690901).

First instar larva

Length: 2.63 ± 0.09 mm; width: 0.83 ± 0.05 mm ($n = 3$).

Examined material. Site 1: 3 exemplars (all deposited together under DZUP 690902).

Third instar larva (Figs 2–6)

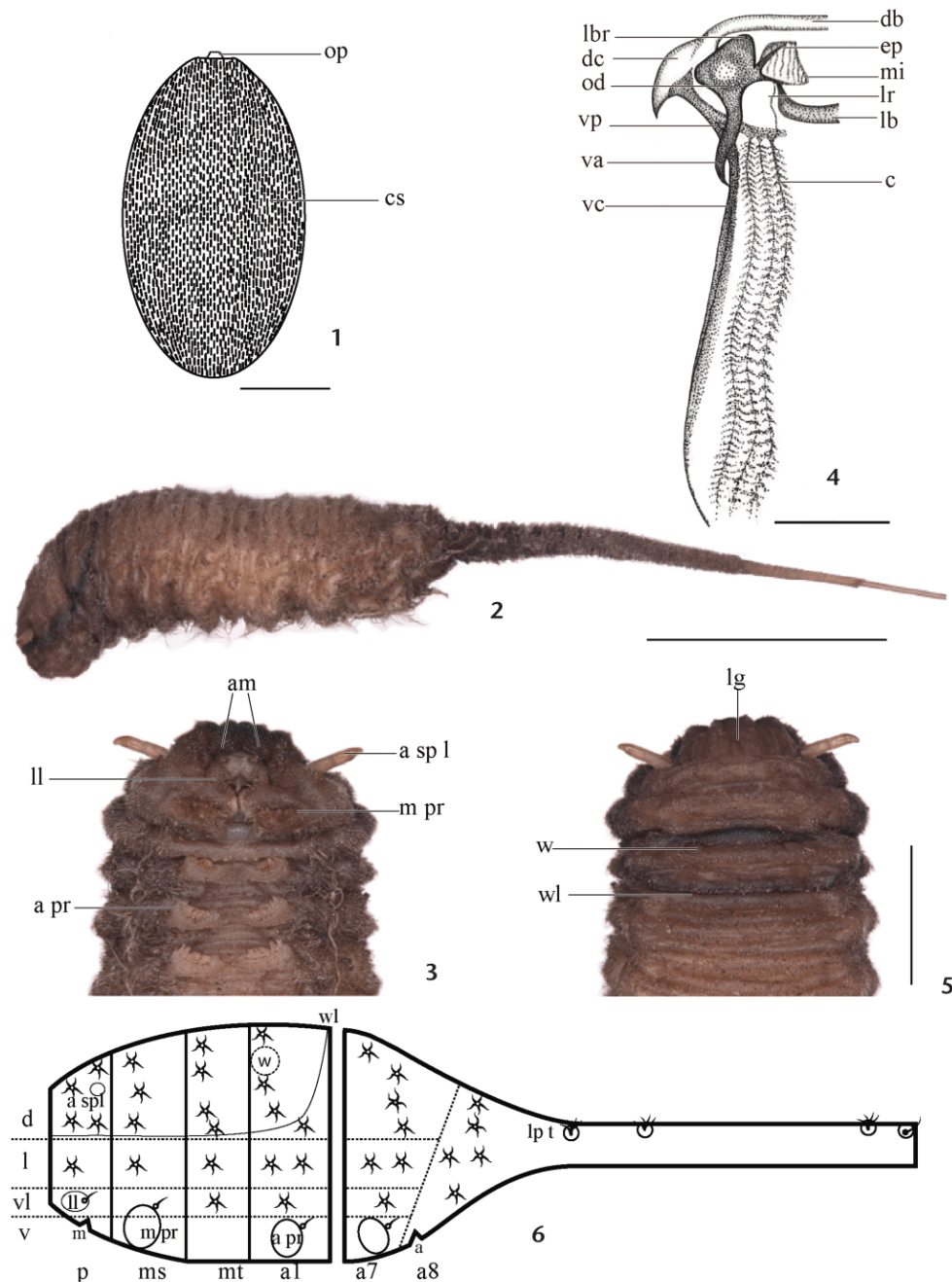
Length: 11.50 ± 0.58 mm; width 4.0 mm ($n = 3$). Overall appearance: long-tailed, sub-cylindrical in cross-section with flattened ventral surface; truncate anteriorly, tapering posteriorly to anus. Cuticle transparent when alive; beige to dark-brown after fixation; covered with tiny spicules directed backwards and beige-colored at base and dark-brown at apex.

Head. Mouthparts internal: base of papilla with pair of fleshy projections; apex of each projection with well-developed antennomaxillary organ divided at base. Cephalopharyngeal head skeleton: mandibles and mandibular lobes internal. Cibarial camera located in the ventral cornu, with six transverse branched ridges. Ventral cornu elongate. Dorsal cornu triangular, shorter than ventral cornu, attached to dorsal bridge. Dorsal bridge joining the two dorsal cornua. Vertical plate sclerotized. Mandibular lobes ovoid at base, coupled with mandibles. Labrum slightly sclerotized.

Thorax. Dorsal surface of prothorax with four longitudinal grooves, dorso-apical margin curving down towards front of head, with sclerotized hooked spicules directed backwards. Opercular suture as a line on dorsolateral margin, from prothorax to abdominal segment 1. Dorso-lateral surface with a pair of brown and shiny spiracles; length: 0.81 ± 0.11 mm; width: 0.40 mm; distance between spiracles: 1.36 ± 0.18 mm; slightly acuminate, curved tips and crenate at margin; retractile into inverted integumental pockets; with 12 to 14 facets at apex surrounding about half the circumference. Lateral lips well-developed, apex triangular with conical hooks, inner margin with six long setae with forked apex; base enlarged with conical hooks; inner margin covered with very fine pubescence, as a first filter anterior to oral cavity. Dorsal lip with a tuft of long unpigmented setae, situated below antennomaxillary organs; ventral lip not clearly visible. Ventral surface with well-developed prolegs bearing 16–18 crochets arranged in two semicircular rows, crochets on first row long, crochets on second row about half the length of crochets on first row. Several rows of spicules ventral to second row.

Abdomen. Segment 1 with primordia of pupal spiracles as two circular patches with weak pubescence (see w in Figs 5, 6). Segments 1–6 with well-developed conic prolegs; with two semicircular lines bearing 16–18 crochets each, directed posteriorly; prolegs of segment 6 with large crochets directed anteriorly. Segment 8 length: $13.83 \text{ mm} \pm 7.61$ mm; width at base: 3 mm; width at apex: 1 mm. Posterior respiratory process with four pairs of weakly developed lappets with one sensilla on each one; light brown, ringed at base; apical spiracular disc with two apertures surrounded by eight long plumose interspiracular setae.

Chaetotaxy. Prothorax with four pairs of dorsal sensilla, one pair of lateral and one pair ventrolateral on lateral lips. Mesothorax with three pairs of dorsal sensilla, one pair lateral and one pair



Figures 1–6. *Quichuana pogonosa*: (1) egg; (2) habitus of third instar larva, lateral view (DZUP 690903); (3) third instar larva, ventral view (DZUP 690903); (4) cephalopharyngeal skeleton of third instar larva, lateral view; (5) third instar larva, dorsal view (DZUP 690903); (6) map of the chaetotaxy of the third instar larva in lateral view showing the position of the sensillae. (a1, a7, a8) abdominal segments a2–a6 are suppressed from the figure for clarity; (a) anus; (am) antennomaxillary organs; a pr, abdominal prolegs; (a sp l) anterior spiracles larval; (c) cibarium; (cs) chorionic surface; (d) dorsal; (dc) dorsal cornu; (db) dorsal bridge; (ep) epipharyngeal plate; (l) lateral; (lb) labial bridge, (lbr) labrum; (lg) longitudinal grooves; (lr) labial rods; (ll) lateral lip; (lp t) lappets; (m) mouthparts; (mi) mandibular lobes; (m pr) mesothoracic proleg; (ms) mesothorax; (mt) metathorax; (od) optical depression; (op) operculum; (p) prothorax; (v) ventral; (va) ventral arm; (vc) ventral cornu; (vl) ventrolateral; (vp) vertical plate; (w) primordia of pupal spiracles; (wl) line of weakness after puparia formation bounding the operculum. Scale bars: 1, 4 = 0.5 mm, 2 = 5 mm, 3, 5 = 2 mm.

ventrolateral very close to proleg. Metathorax with four pairs of dorsal sensilla, one pair lateral, one pair ventrolateral and one pair ventral. Abdominal segments 1–6 with four pairs of dorsal sensilla, two pairs lateral, one pair ventrolateral and one pair posterior to each proleg. Abdominal segment 8 with two pairs of dorsal sensilla, two pairs lateral and one pair ventrolateral. Ventrally, with long setae on ventrolateral margins and posterior to anus.

Examined material. Site 1: 3 exemplars (DZUP 690903, DZUP 690904, DZUP 690905).

Puparium (Figs 7–10)

Length: 7.18 ± 1.32 mm; width: 4.40 ± 0.62 mm ($n = 12$). Subcylindrical in cross-section; truncated anteriorly; tapered posteriorly, flattened ventrally. Integument brown, weakly rough, with transverse folds and wrinkles exhibiting larval mouth, prolegs and anal papillae.

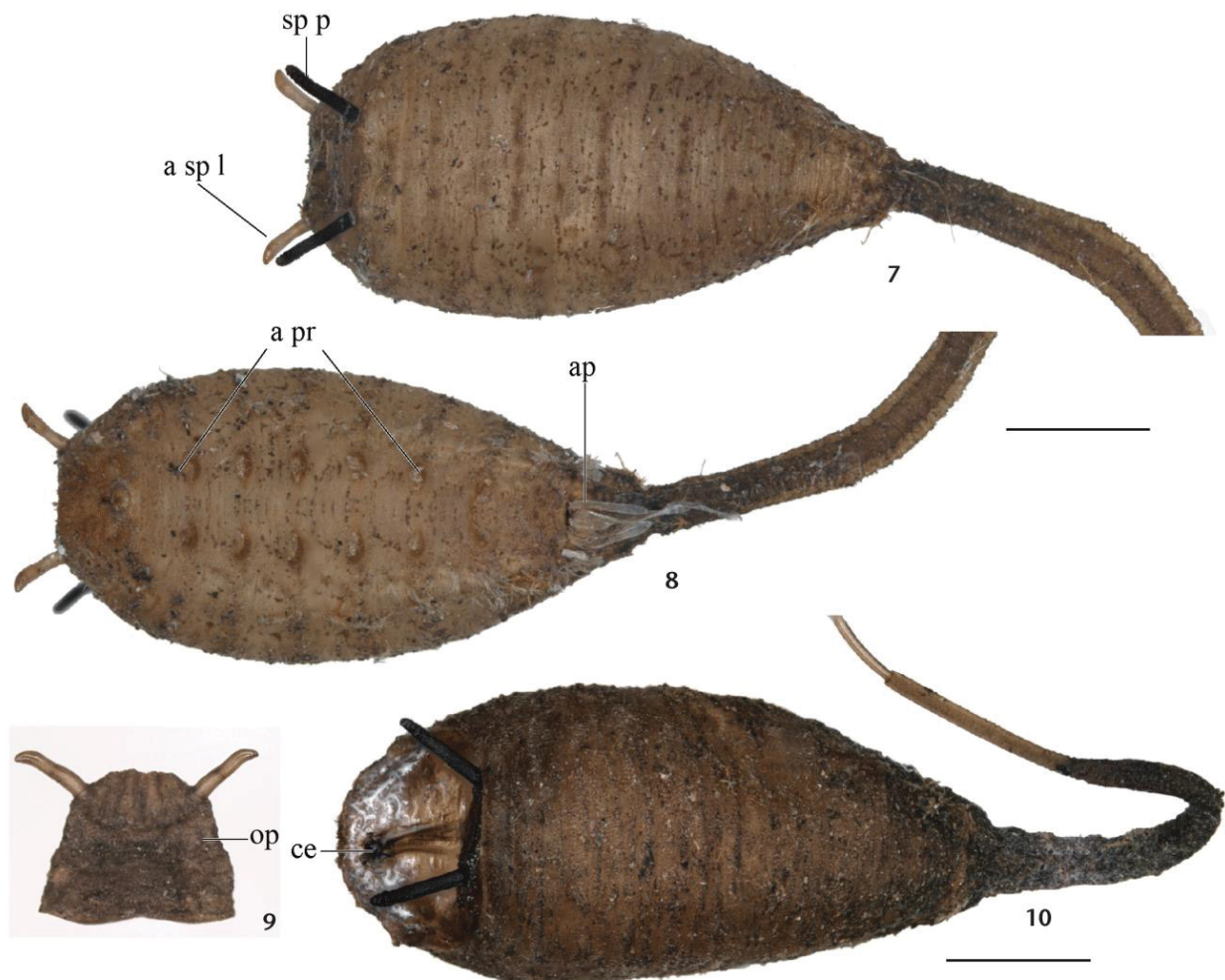
Pupal spiracles. Length: 1.41 ± 0.19 mm; width: 0.24 ± 0.02 mm; distance between each respiratory process: 1.29 ± 0.36 mm. Subcylindrical, projecting towards center of operculum, black, slightly curved at apex, apical surface bearing irregularly spaced and circular-shaped tubercles.

Examined material. See examined material under 'Adults'.

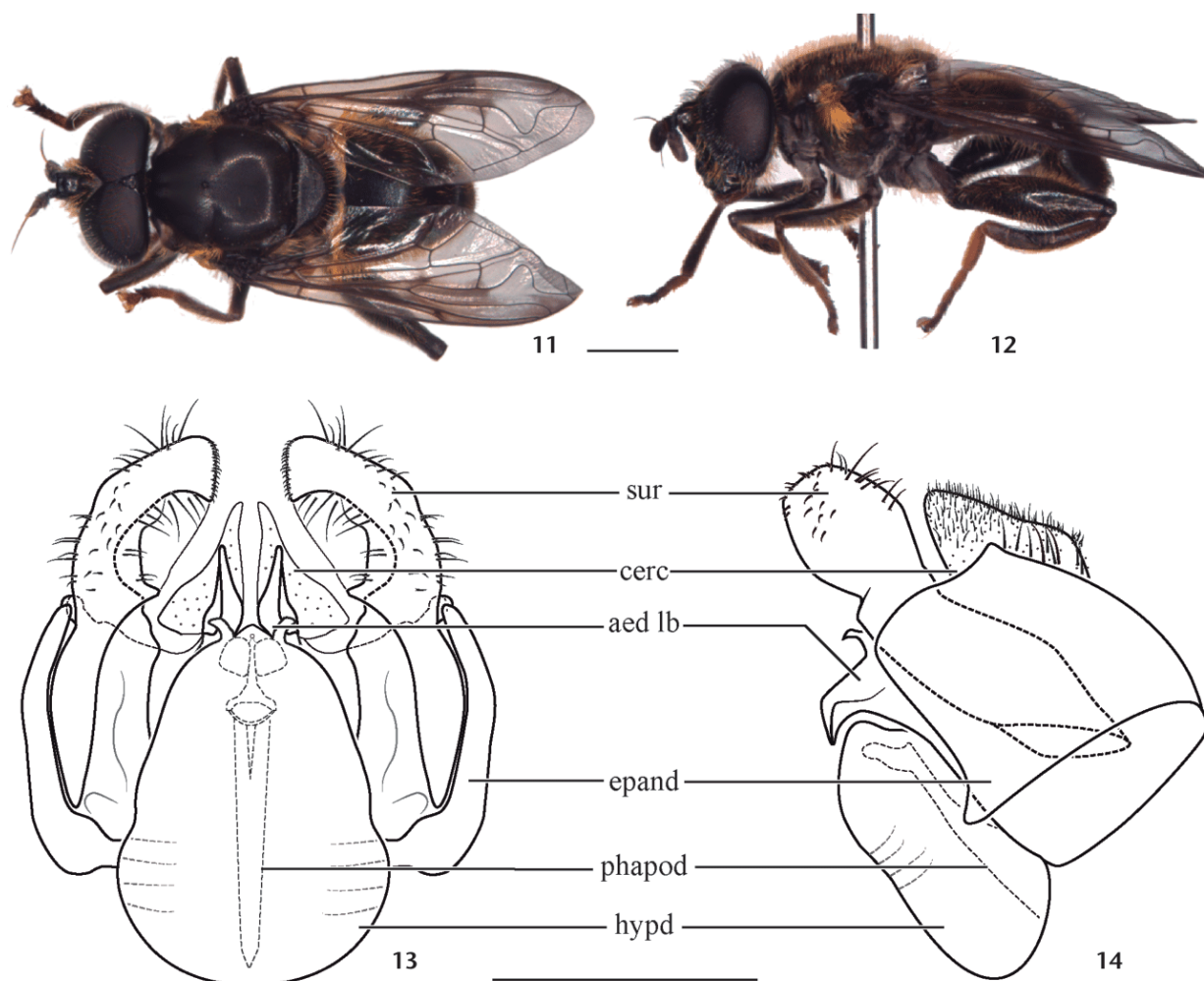
Adults (Figs 11–14)

Male genitalia. Surstylus elongate, curved, with truncate apex; ventral and dorsal surfaces with long dark brown bristles near apex; basal surface with short and spaced dark brown bristles. Cercus triangular bearing setulae. Hypandrium piriform; apex with a pair of hook-like projections. Epandrium elongated, about four times as long as wide. Aedeagal lobe triangular.

Examined material. Site 1: 1 female (collected in oviposition) (DZUP 690907); 1 female, emergence 15.xii.2020



Figures 7–10. *Quichuana pogonosa*: (7) pupa, dorsal view (DZUP 690906); (8) pupa, ventral view (DZUP 690906); (9) operculum (DZUP 690914); (10) puparium, dorsal view (DZUP 690914). (a sp l) anterior spiracles larval; (a pr) abdominal prolegs; (ap) anal papillae; (ce) cephalopharyngeal skeleton; (op) operculum; (sp p) pupal spiracles. Scale bars: 2 mm.



Figures 11–14. *Quichuana pogonosa*, male (DZUP 690906): (11) habitus dorsal; (12) habitus lateral; (13) genitalia, ventral view; (14) genitalia lateral view. (aed lb) aedeagal lobe, (cerc) cercus, (epand) epandrium, (hypd) hypandrium, (phapod) phallosome, (sur) surstylus. Scale bars: 11, 12 = 2 mm, 13, 14 = 1 mm.

(DZUP 690908), 1 male, emergence 15.xii.2020 (DZUP 690909); 1 male, pupation 4.i.2021, emergence 15.i.2021 (DZUP 690910); 1 female, pupation 22.i.2021, emergence 2.ii.2021 (DZUP 690911); 1 female, pupation 4–5.ii.2021, emergence 15.ii.2021 (DZUP 690912); 1 female, pupation 8.ii.2021, emergence 18.ii.2021 (DZUP 690913); 1 female, pupation 19.ii.2021, emergence 3.iii.2021 (DZUP 690914); 1 male, pupation 19.ii.2021, emergence 3.iii.2021 (DZUP 690915); 2 females (DZUP 690916), (DZUP 690917); 1 male (DZUP 690918); Site 2: 1 male, pupation 8.ix.2021, emergence 22.ix.2021 (DZUP 690906).

Life history (Figs 15–20)

A female was observed in situ ovipositing on the water surface of the phytotelmata of *Alcantarea imperialis*. In the same

plant there were other long-tailed larvae at different stages of *Quichuana*, *Eristalis* Latreille, 1804, and *Lejops* Rondani, 1857. Pupae and puparia of different species were also observed together attached to dry leaves.

At the laboratory, the collected eggs hatched 56 hours after they were laid. The newly hatched larvae feed on their eggshell. First and second instar larvae were observed feeding on small invertebrates, zooplankton and organic matter in the water column. The third instar larvae were observed feeding on organic matter down the bottom of the container. When at rest, the larvae remained hidden at the bottom of the tank, between the sediment and the leaves, using their prolegs to adhere to the leaves. The posterior respiratory process remained extended with the spiracular disc in contact with the water surface, allowing them to breathe and feed at the same time.



Figures 15–20. *Quichuana pogonosa*: (15) egg; (16) first instar larvae next to shells; (17–18) habitus of third instar larvae; (19) pupae aggregation; (20) puparia of *Quichuana pogonosa* (left) and *Eristalis* sp. (right).

It took the larvae 61–62 days to complete their development. When the third instar larvae were ready to pupate, they migrated to the dry leaves. The pupae adhered to the leaves using their anal papillae. The pupal spiracles appeared 24 hours after pupation. The pupal stage lasted 11–13 days.

DNA-barcoding

In order to facilitate the identification of immature stages, sequences of the barcoding region (cox1; 658 bp) were generated for two larvae and two adult specimens. The sequences were deposited on GenBank under the following accession numbers: MZ389870 and MZ389871 (larvae), MZ389868 and MZ389869 (adults). Intra-specific variation among these sequences ranged from 0 to 0.046% for both p-distances and K2P estimations (Table 2).

Table 2. Pairwise distance between cox1 sequences of *Quichuana pogonosa*. K2P distances, diagonal superior; p-distances, diagonal inferior.

DZUP	GenBank	Locality	Sample	Adult	Adult	Larva	Larva
690906	MZ389868	Site 2	Adult male		0	0.0046	0.0015
690913	MZ389869	Site 1	Adult female	0		0.0046	0.0015
690903	MZ389870	Site 1	Larva	0.0046	0.0046		0.0031
690904	MZ389871	Site 1	Larva	0.0015	0.0015	0.003	

DISCUSSION

This work provides the first description of the immature stages of a *Quichuana* species. To date, only one identification key, for the Neotropical long-tailed Syrphidae, includes third instar larvae of *Quichuana* (Pérez-Bañón et al. 2003).

Larvae of *Q. pogonosa* in their third instar share similarities with other saprophagous species of Syrphidae from the Neotropics, such as: (i) cephalopharyngeal skeleton with developed pharyngeal ridges. This allows the filtration of microorganisms that are suspended in the water; (ii) prolegs with two lines of crochets; (iii) retractable anterior spiracles; and (iv) posterior respiratory process elongate, with weak lappets (Morales and Marinoni 2008, Pérez-Bañón et al. 2013). Similarly, the pattern of rectangular units on the chorion of the egg is a potential diagnostic character for the genus. These sculptures have been reported in other flower flies and present distinctive patterns in different genera (Pérez-Bañón and Marcos-García 1998, Sasaki and Mikami 2007, Ureña and Hanson 2010, Campoy et al. 2017).

Adult females belonging to different species of Syrphidae, including species of the same genus, may oviposit in the same bromeliad. This complicates the identification of immature stages and their association with the respective adults.

There was little variation among the DNA-barcoding sequences. These data, along with the morphology described here for adults and immature stages (mainly chaetotaxy), provide robust evidence to associate adults and immature stages of *Q. pogonosa*. We provide the first sequences for *Q. pogonosa*

deposited in the GenBank. Sequences of the following five fully identified species had been previously deposited in the BOLD system: *Q. angustiventris* (Macquart, 1855), *Q. calathea* Shannon, 1925, *Q. cincta* (Bigot, 1883), *Q. fasciata* (Sack, 1941), *Q. picadoi* Knab, 1913 and *Q. subcostalis* (Walker, 1860).

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CHAPTER II

DESCRIPTION OF A NEW SPECIES OF THE GENUS *QUICHUANA* KNAB, 1913 AND INTRASPECIFIC VARIABILITY OF *QUICHUANA POGONOSA* Fluke, 1937

Description of a new species of the genus *Quichuana* Knab, 1913 and
intraspecific variability of *Quichuana pogonosa* Fluke, 1937

Angela M. Echeverry¹, Luciane Marinoni¹

¹ *Laboratório de Estudos em Diversidade de Insetos da Região Neotropical, Departamento de Zoologia, Universidade Federal do Paraná. Caixa Postal 19020, 81531-980 Curitiba, PR, Brazil.*

Corresponding author: Angela Maria Echeverry (angela.mariae@hotmail.com)

ABSTRACT

The genus *Quichuana* Knab, 1913 (Diptera: Syrphidae) is distributed in the Neotropics. Adults have been extensively studied, while little is known about the immature stages in terms of morphology and life history. The larvae are saprophagous and develop inside bromeliads phytotelmata mainly.

Herein we describe and illustrate a new species reared from larvae collected in the phytotelmata of the bromeliad *Aechmea distichantha* from the Municipality Balsa Nova, Paraná, Brazil; we apply DNA barcodes to associate larvae and adult, and described larva stage and puparium.

We also present preliminary data about the life cycle. In addition, a study of the intraspecific variability of *Quichuana pogonosa* reared from immature collected in the phytotelmata of bromeliads *Aechmea distichantha* and *Alcantarea imperialis*, in the municipalities of Balsa Nova and Curitiba, Paraná, Brazil, respectively is presented. The (COI) gene sequences obtained in this study were analyzed along with those of five other *Quichuana* species sequences, obtained from the Barcode of Life Data Systems (BOLD).

Comparatively, low divergences of (COI) sequences were also found between the species *Q. angustiventris* (Macquart, 1855), *Q. calathea* Shannon 1925, and *Q. picadoi* Knab, 1913 indicating are closely related species.

KEY WORDS. Hoverflies, DNA-*barcoding*, immature stages, morphology, phytotelmata, taxonomy.

INTRODUCTION

Syrphids of the genus *Quichuana* Knab, 1913 (Eristalinae: Eristalini: Helophilina) comprise 50 described species (Ricarte et al. 2012; Montoya et al. 2017) occurring in the Neotropical Region, from Mexico to Argentina (absent only in the Chilean subregion) (Thompson et al. 2010). Seven species occur in Brazil, including five endemic: *Quichuana angustiventris* (Macquart, 1855); *Quichuana bezzii* Ceresa, 1934; *Quichuana borgmeieri* Lane & Carrera, 1944 *Quichuana invenusta* Ricarte, 2012; *Quichuana parissii* Ceresa, 1934; *Quichuana pogonosa* Fluke, 1937 and, *Quichuana undulatipila* Ricarte & Hancock, 2012.

Most of the knowledge about the taxonomy of the Syrphidae species is based on the morphology of the adult, while most of the immature stages are still unknown or have not been described, despite the fact that they are diverse in morphology and ecology (Rotheray & Gilbert, 1999); It is necessary to balance the knowledge of the different stages of development since provides useful data to conserve species, management of species that are economically useful (Rotheray 2003) and, additional characters for phylogenetic studies (Rotheray & Gilbert 1999; Ståhls et al 2003; Young et al 2016).

Morphological characters of larvae and puparium have been used together with other data sets, such as breeding sites, habitats, and geographic distribution, in search of patterns that may explain the diversification within the family Syrphidae and resolve species limits (Ståhls et al. 2003, Mengual et al. 2008, Láska et al.2006).

The adults are distinguished from other members of the subfamily Eristalinae by the presence of pile eyes; sub-basal, dorsal, bare arista; pilose postpronotum; posterior metafemur with basoventral patch of black setulae; wing vein R₄₊₅ strongly sinuous and cell R₁ varying from open to close at the wing margin, but never petiolate (Thompson et al. 2010).

Ricarte et al. (2012) recognized two groups of species within *Quichuana* mainly on the basis of abdomen width of the adults: 1. species with a narrow or slender abdomen, entirely black to dark brown; basoflagellomere elongated, bf > 1.5; the length of the eye contiguity ranging from less to slightly more than pollinose triangle; face pollinose, but has at least a central shiny stripe of variable

length, and 2. species with robust or broad abdomen, *Eristalis*-like, usually with conspicuous red abdomen markings; the length of the eye contiguity conspicuously longer than that of the ocellar triangle; basoflagellomere round to oval, $bf < 1.5$, and face is extensively shiny.

The only reports about the immature stages of *Quichuana* species are about the larvae in phytotelmata of Bromeliaceae, Heliconiaceae, Marantaceae, and Poaceae families (Walker 1857, Picadoi 1913, Shannon 1925, 1927, Lane & Carrera 1944, Seifert & Florence 1976, Seifert & Seifert 1976, 1979, Thompson 1981, Thompson et al. 2010, Ricarte et al. 2012, Reemer 2016). Detailed information is only available life history for *Quichuana aurata* (Walker 1987) and morphological and biological data for *Quichuana pogonosa* (Echeverry et al. 2021).

According to Rotheray & Gilbert (2011), the third instar of *Quichuana* species can be distinguished by the presence of hook-shaped sclerotized spicules directed backward on the anterior margin of the prothorax and prolegs of segment 6 with large crochets directed anteriorly.

Ricarte et al. (2012) indicated that the larvae of the slender abdomen species have the front of the thorax coated with sclerotized spicules and larvae of the broad abdomen species have a complex arrangement of spicules and large hooks on the front of the thorax.

Over the last two decades, traditional morphological taxonomy studies have been integrated with molecular analyzes such as DNA-*barcoding*, which have accelerated the diagnosis and identification of Syrphidae and provided a high taxonomic resolution (Pérez-Bañón et al. 2003; Mengual et al. 2006; Milankov et al. 2008; Marcos-García et al. 2011).

So far, there is only one published research using DNA-*barcoding* to associate the adults with immature stages, and only six the COI gene of *Quichuana* are available in the Barcode of Life Data Systems (BOLD), of which five have identification. According to Shearer & Coffroth (2008), DNA barcodes can be used at any stage of life (larvae, pupae, or adult), allowing both intraspecific identification and interspecific association at different stages of development.

Considering the above, the objectives of this study are: 1) to describe a new species reared from larvae obtained from the phytotelmata of the bromeliad *Aechmea distichantha* along with its immature stages, 2) to describe the intraspecific variability found in *Quichuana pogonosa*, 3) to generate the COI gene sequences for both species based on adult and immature specimens, in order to be able to associate the unknown immature sample collected in bromeliads with the adult fly, by comparing their COI gene sequences and, 4) to study the intra and interspecific divergence of the *Quichuana* species analyzed in terms of nucleotide composition and intraspecific.

MATERIAL AND METHODS

Collecting and identification of samples

Two specimens of *Quichuana pogonosa* one male and one female were reared from two larvae obtained from phytotelmata of the bromeliad *Alcantarea imperialis* Carrière in the urban area of Curitiba, 25°27' 37.3"S, 49°13'36.8"W. One male of *Quichuana* **sp. nov.**, was reared from two larvae obtained from phytotelmata of the bromeliad *Aechmea distichantha* Lem. at rural area of Balsa Nova, 25°31'05.6"S, 49°35'11.4"W. In both cases, the bromeliads were disassembled *in situ* and the leaves were removed one by one, from the outermost part to the inflorescence, exposing the larvae. The larvae were collected using a brush, while a single adult female of *Quichuana pogonosa* was collected with an entomological net, near the inflorescence of the bromeliad.

For the identification of the bromeliad species, the catalog 'Flora do Brasil' was used (Faria et al. 2020, Versieux 2020). The identification was confirmed by Dr. Fabrício Schmitz Meyer of the Herbário do Departamento de Botânica da Universidade Federal do Paraná (UPCB).

To identify the Syrphidae species the keys of Ricarte et al. (2012) and Montoya et al. (2017) were used as well as the original descriptions. Morphology of male genitalia was also used to confirm and support our observations.

All syrphid specimens examined in this study were deposited in the Coleção Entomológica Padre Jesus Santiago Moure (DZUP) Universidade

Federal do Paraná, Curitiba, Brasil and the information was databased. The data are available at Taxonline – Network of Biological Collections of Paraná (<http://taxonline.bio.br>).

Rearing the immatures

Rearing took place at the Laboratory of Studies on Diversity of Insects of the Neotropical Region (Taxonlab), Department of Zoology, Universidade Federal do Paraná. Larvae were kept in cylindrical plastic containers (12 cm in diameter, 11 cm in height), along with small amounts of tank water, decaying organic matter of the collected material, and small pieces of dried bromeliad leaves to facilitate pupation. They were kept at room temperature in natural light and water was sprayed periodically in small amounts to keep the moisture. The containers were checked daily. As soon as the larvae turned into pupae they were removed and placed individually in a container without humidity to avoid the proliferation of fungi. The containers were inspected daily until the emergence of the adults.

Morphological study of the immature stages

Descriptions are based on preserved specimens and follow the morphological terminology used for third instar larva and the positions of sensillae larval segment proposed by Rotheray (1991) and Pérez-Bañón (2000); for puparium follows Rotheray & Gilbert (1999) and Rotheray (2019); for cephalopharyngeal skeletons follows Campoy et al. (2020). The third instar larva of *Quichuana* **sp. nov.**, was recognized by the presence of one pair of discs on the dorsal surface of the first abdominal segment (Hartley 1961) (Fig. 5f).

Larvae for the morphological study were killed and preserved directly in 70% ethanol.

The integuments of puparium were cleaned with brushes to remove debris. Cephalopharyngeal skeletons were removed with entomological pins from the antero-ventral margin of puparia. After the dissection, the cephalopharyngeal skeleton were cleaned KOH for 10–15 min. Finally, the cephalopharyngeal skeletons were washed in 5% acetic acid and preserved in microtubes with glycerine. The length of early stages was measured from the anterior margin of

the prothorax to the anus, ventrally; the length of the abdominal segment 8, including the posterior respiratory process, is presented separately. Height and width were measured at their maxima.

Morphological study of adults

Body length was measured following Ricarte et al. (2012) as follows. Adult body length was determined by measuring the distance between the apices of the frontal prominence on the head and the abdomen with species characterized as small (≤ 10 mm), medium (10–12 mm) or large (> 12 mm). Abdomen width was determined by estimating the dorsal length of the abdomen from the base of tergum II to the apex of abdomen ('l' in Fig. 1a), divided by the maximum width ('W' in Fig. 1b), with abdomens characterized as narrow (> 1.6), moderately broad (1.4–1.6), or broad (< 1.4). The shape of the abdomen is characterized as tapering, parallel-sided, waisted, or oval. Species with narrow and tapering, parallel-sided, or waisted abdomens are referred as slender. The size of the basoflagellomere (bf) was expressed by measuring the distance between the tip of the basoflagellomere and the most prominent point of the pedicel ('l' in Fig. 1b) divided by the distance between the base of the arista across the basoflagellomere ('W' in Fig. 1b). The wing length was measured from wing insertion to the wing apex. Colour characteristics are described from dry-mounted specimens.

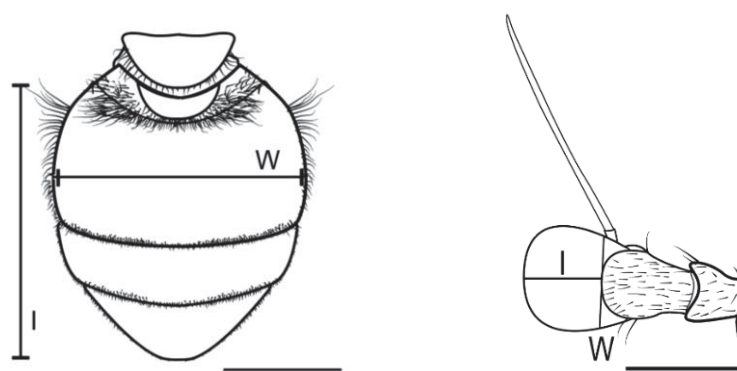


Figure 1. (a) *Quichuana* sp. female, abdomen, dorsal view; (b) *Quichuana pogonosa* var. female, (DZUP 690925), right antenna, lateral inner view. Scale bar: scale bar a = 2 mm, b = 1 mm.

The descriptions of morphological characters are based on previous descriptions of the genus (Ricarte et al. 2012).

At the beginning of the species descriptions, we add the information of the labels. The identification and locality were transcribed, where: "\n" represents the end of a line of the same label, "" represents the beginning and end of the data of the label. The information contained between parentheses "()" represents the color of the label.

Male genitalia were dissected with ophthalmic scissors, cleared in 10% KOH for 12–24 hours, washed in 5% acetic acid to neutralize the KOH, rinsed in water, and examined within several drops of glycerin, stored in microvials with glycerine, and pinned with the corresponding specimen. The terminology used for genitalia follows Cumming & Wood (2017).

For the precise identification, diagnostic characters are provided for *Quichuana pogonosa* and *Quichuana* **sp. nov.**, and compared with related species.

Drawings and photos

Morphology of adults and immature stages were observed using a Nikon SMZ800 stereomicroscope. Illustrations were drawn using a drawing tube fixed to the stereomicroscope and Illustrator CC. Photographs were taken using a Leica DFC-500 digital camera on a Leica MZ16 stereoscope and mounted using the software Leica LAS 3D Viewer and LAS Montage v.4.7. Anterior spiracles, pupal spiracles, spiracular disk of posterior respiratory processes, and egg chorion, were mounted on standard stubs and coated with gold using a Denton Vacuum Desk IV coater. Scanning electron micrographs were produced using a JEOL JSM-6360LV microscope in the Centre of Electron Microscopy of the Universidade Federal do Paraná (CME/UFPR).

DNA extraction, amplification, and sequencing of DNA-barcoding

Total genomic DNA of these samples was extracted using a non-destructive method, the right hind leg of the adults, and a portion of the abdominal segment 6 of the larvae were used for DNA extraction using Invitrogen – PureLink Genomic DNA Mini-Kit, following the manufacturer's protocol. Specimens used

for DNA extraction were deposited at DZUP. The DNA barcode fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using primer pair LCO1490 and HCO2198 (Folmer et al. 1994); and the kit MyTaqTM DNA Polymerase (Bioline), was performed in a total volume of 25 μ L, consisting of 1 μ L of genomic DNA, 5 μ L of 5 MyTaq Reaction Buffer (containing 15 mM MgCl₂ and 5 mM dNTPs), 0.5 μ L of each 0.01 mM primer, 0.1 μ L of MyTaq DNA Polymerase and 17.9 μ L of ultrapure water. PCR cycle conditions were as follows: initial denaturation at 94 °C for 3 min, 5 cycles of annealing temperature at 94 °C for 30 s, 47 °C for 40 s, 72 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 52 °C for 40 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using 5 M ammonium acetate precipitation with isopropanol and sequenced in both directions using the BigDye Terminator v. 3.1 technology (Applied Biosystems, Foster City CA, U.S.A.).

Molecular analysis

The sequences were edited using Geneious® 9.1.3 (Biomatters Ltd.). Alignments for the molecular analysis were produced with the sequences produced in this study and, additionally, five sequences were obtained in the Barcode of Life Data Systems (BOLD). A complete list of taxa used in this study, including their source, (BOLD) accession numbers, and voucher institutions is presented in Table 1.

The COI gene sequences were aligned using (Molecular Evolutionary Genetics Analysis), version MEGA 7 (Kumar et al. 2016). The nucleotide compositions and sequence divergences within and between species were calculated using the distance model Kimura 2-Parameter (K2P) (Kimura, 1980), The COI gene sequences were clustered using Neighbor-joining analyses. Sequences are deposited on the Barcode of Life Dataystems (BOLD) at <http://www.boldsystems.org>.

Table 1. List of specimens used for molecular work processed in the study.

Species	BOLD No. COI -5'	Sample	Neotropical Distribution (Ricarte et al. 2012)
<i>Quichuana pogonosa</i>	MZ389868	Adult male	Brazil
<i>Quichuana pogonosa</i>	MZ389869	Adult female	Brazil
<i>Quichuana pogonosa</i>	MZ389870	Larva	Brazil
<i>Quichuana pogonosa</i>	MZ389871	Larva	Brazil
<i>Quichuana</i> sp.1	NS	Adult male	Brazil
<i>Quichuana</i> sp.1	NS	Adult female	Brazil
<i>Quichuana</i> sp. nov.	NS	Adult male	Brazil
<i>Quichuana</i> sp. nov.	NS	Larva	Brazil
<i>Copestylum</i> sp.	NS	Adult male	Brazil
<i>Copestylum</i> sp.	NS	Larva	Brazil
<i>Eristalinus</i> sp.	NS	Larva	Brazil
<i>Quichuana angustiventris</i>	ASIND1931-12, ASIND 1934-12, ASIND1935-12, ASIND 2639-12, ASIND 2640-12	Adult female	Brazil, Colombia, Costa Rica, Peru Trinidad, Venezuela, Surinam
<i>Quichuana angustiventris</i>	ASIND1933-12	Adult male	Brazil, Colombia, Costa Rica, Peru Trinidad, Venezuela, Surinam
<i>Quichuana angustiventris</i> *	ASIND2638-12	Adult male	Costa Rica
<i>Quichuana calathea</i>	ASIND1936-12	Adult male	Colombia, Costa Rica, Cuba, Panama

<i>Quichuana picadoi</i>	ASIND1947-12, ASIND1949-12, ASIND1950-12, ASIND1951-12	Adult female	Colombia, Costa Rica, Mexico, Trinidad, Surinam
<i>Quichuana picadoi</i>	ASIND1948-12	Adult male	Colombia, Costa Rica, Mexico, Trinidad, Surinam
<i>Quichuana fasciata</i>	ASIND1942-12	Adult male	Peru
<i>Quichuana fasciata</i>	ASIND1944-12, ASIND 1945-12	Adult female	Peru
<i>Quichuana fasciata</i>	ASIND2647-12, ASIND2648-12, ASIND2650-12, ASIND2651-12, ASIND2652-12		Peru
<i>Quichuana</i> sp. ASIND	ASIND1906-12, ASIND1908-12	Adult male	Costa Rica
<i>Quichuana</i> sp. ASIND	ASIND1911-12, ASIND1913-12, ASIND1921-12, ASIND1925-12, ASIND1902-12	Adult female	Costa Rica
<i>Quichuana</i> sp. ASIND	ASIND2645-12, ASIND2643-12, ASIND2646-12	-	Costa Rica
<i>Quichuana</i> sp.	FFECU375-16	-	Ecuador

NS: not submitted. * "*Quichuana angustiventris*" possible identification error.

RESULTS AND DISCUSSION

Molecular analyses

We obtained 658-bp nucleotide characters for *Quichuana* **sp. nov.**, and *Quichuana pogonosa* **var.** The intraspecific variability *Quichuana* was low, with a maximum K2P distance of 2.2% (Table 2), and interspecific pairwise divergences for species of *Quichuana* divergences ranged from 5% to 15% (Table 3).

Our analysis suggests five separated clades within the inner group supported by values higher than 99.8%; the resulting neighbor-joining tree is shown in (Fig. 2). The specimens of *Quichuana* **sp. nov.**, is resolved in a separate branch, clearly divergent from all other *Quichuana* species. With the addition of this new species, the total number of known species for the genus is increased to 51 total, and nine for the Brazilian fauna. Consequently, Brazil is the third richest country in terms of *Quichuana* species.

When analyzing the other clades our results suggest that *Q. angustiventris*, *Q. calathea*, *Q. picadoi*, and *Quichuana* sp. FFCU375-16 are closely related species as they are separated by an average pairwise interspecific divergence of 2.2% (Table 3). This similarity can thus indicate a possible more recent diversification of these taxa.

This result reveals a congruence with the morphological analysis carried out by Ricarte et al. (2012). According to them, this species group, which includes *Quichuana quixotea*, has a limited number of structural characters enough variables to be used for distinguishing species, some of them are chromatic; male genitalia additional are remarkably homogeneous. Molecular markers for other genes that include a greater number of sequences would certainly help to characterize different species.

In a previous analysis of the morphological characteristics and using the identification keys proposed by Ricarte et al. (2012) we could not, *a priori*, recognize the identity of the taxa, so we called *Quichuana* sp. 1 (as appear in Table 1). The specimens have various morphological characteristics that are different from the species described so far. Such that we even considered describing them as belonging to a new species. However, after performing the molecular-distance-based analysis we observed that both specimens (male and female) of *Quichuana* sp. 1 appeared in the same clade of *Quichuana pogonosa*. The distance among the specimens is 0.5% (Table 3), considered to indicate that both specimens of *Quichuana* sp. 1 are actually *Quichuana pogonosa*. With this evidence, we are compelled to believe that those specimens are a variety of *Quichuana pogonosa* and from now on we are going to treat *Quichuana* sp1. as *Quichuana pogonosa* **var.**

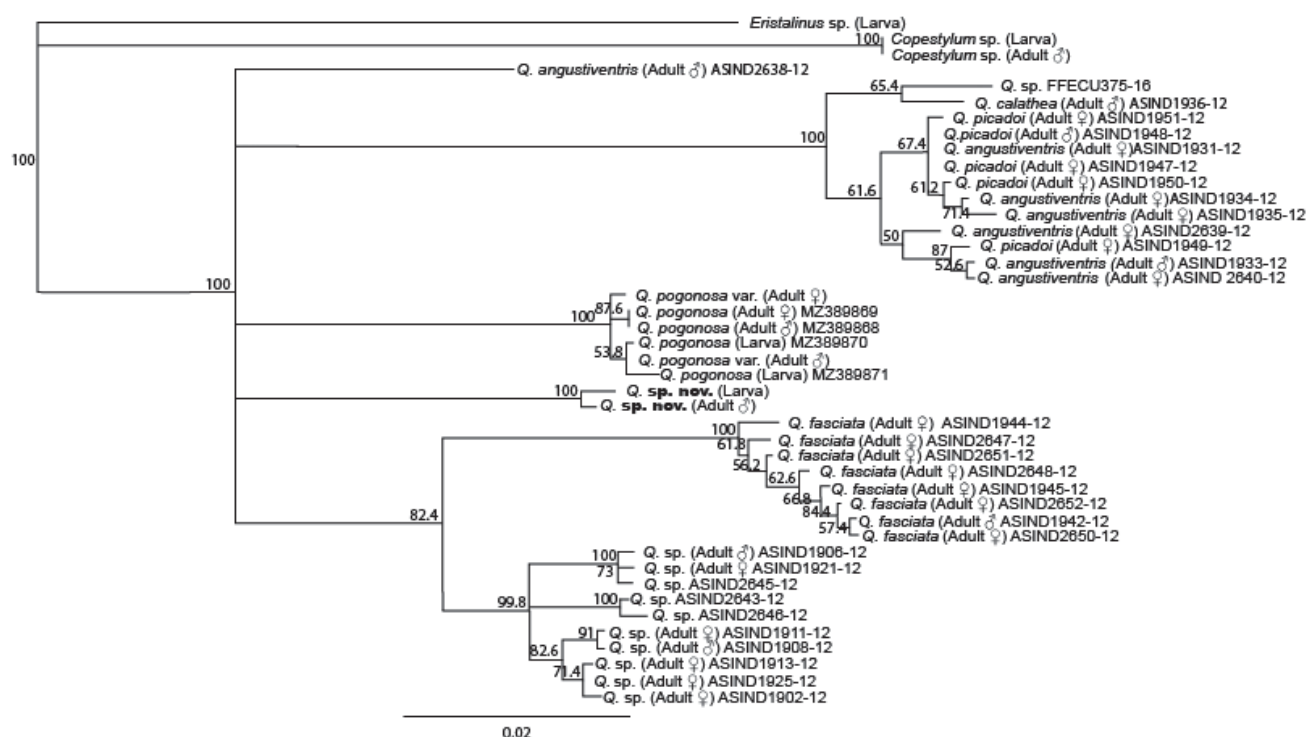


Figura 2. Neighbor-joining distance tree based on the COI dataset of eight *Quichuana* species, reconstructed based on Kimura 2-parameter model.

Herein we provide two new COI gene sequences of two specimens of *Quichuana pogonosa* var.; two sequences for *Quichuana* sp. nov., and one of a species of the genus *Copestylum* (*Copestylum* sp.). In this way, the number of species in Barcode of Life Data Systems (BOLD) of the genus *Quichuana* is increased from six to seven, and of the genus *Copestylum* from 111 to 112. Data obtained in the present study support the utility of COI barcode analysis for the association of unidentified developmental stages with adults.

Table 2. Range of intraspecific genetic distance of *cox1* sequences of *Quichuana* species, and outgroups *Copestylum* sp.

Species	n	K2P-distances
<i>Quichuana pogonosa</i>	4	0 – 0.005
<i>Quichuana pogonosa</i> var.	2	0 – 0.002
<i>Quichuana</i> sp. nov.	2	0 – 0.005
<i>Copestylum</i> sp.	2	0
<i>Eristalinus</i> sp.	1	0
<i>Quichuana angustiventris</i>	6	0 – 0.022

<i>Quichuana angustiventris</i> *	1	0
<i>Quichuana calathea</i>	1	0
<i>Quichuana picadoi</i>	5	0 – 0.017
<i>Quichuana fasciata</i>	8	0 – 0.014
<i>Quichuana</i> sp. ASND	10	0 – 0.019
<i>Quichuana</i> sp.	1	0

Table 3. Maximum pairwise genetic distance of between of *Quichuana* species based on Kimura-2-parameter model (K2P).

Species	n	<i>Q. pog</i>	<i>Q. sp. nov.</i>	<i>Cop sp</i>	<i>Q. pog var.</i>	<i>Q. ang</i>	<i>Q. cal</i>	<i>Q. fas</i>	<i>Q. sp.</i>	<i>Q. pic</i>	<i>Q. ASND</i>	<i>Eristalis</i>
<i>Q. pog</i>	4											
<i>Q. sp. nov.</i>	2	0.060										
<i>Cop sp.</i>	2	0.122	0.129									
<i>Q. pog var.</i>	2	<u>0.005</u>	0.057	0.124								
<i>Q. ang</i>	6	0.099	0.087	0.151	0.097							
<i>Q. cal</i>	1	0.094	0.082	<u>0.155</u>	0.092	0.023						
<i>Q. fas</i>	8	0.082	0.075	0.151	0.080	0.097	0.099					
<i>Q. sp.</i>	1	0.095	0.085	0.153	0.094	0.029	0.017	0.097				
<i>Q. pic</i>	5	0.094	0.082	0.145	0.092	0.020	0.025	0.099	0.028			
<i>Q. ASND</i>	10	0.073	0.052	0.124	0.072	0.090	0.085	0.058	0.084	0.086		
<i>Eristalis</i>	1	0.111	0.109	0.133	0.108	0.144	0.147	0.129	0.144	0.140	0.111	
<i>Q. ang*</i>	1	0.058	0.050	0.111	0.057	0.082	0.077	0.078	0.077	0.077	0.052	0.099

Numbers underlined indicate the highest interspecific distance and the lowest interspecific distance. n = No. sequences. *Q. pog* = *Quichuana pogonosa*; *Q. sp. nov.* = *Quichuana sp. nov.*; *Cop sp.* = *Copestylum sp.*; *Q. pog var.* = *Quichuana pogonosa var.*; *Q. ang* = *Quichuana angustiventris*; *Q. cal* = *Quichuana calathea*; *Q. fas*: *Quichuana fasciata*; *Q. sp* = *Quichuana* sp. corresponds to the sequence of the (BOLD) FFcu375-16; *Q. pic* = *Quichuana picadoi*; *Q. ASND* corresponds to the following sequences of the Barcode of Life Data Systems (BOLD): ASIND

1902-12, ASIND 1906-12, ASIND 1908-12, ASIND 1911-12, ASIND 1913-12, ASIND 1921-12, ASIND 1925-12, ASIND 2643-12, ASIND 2645-12, ASIND 2646-12; *Eristali*: *Eristalinus* sp.; *Q. ang**: corresponds to the sequence of the (BOLD) ASIND2638-12 "*Quichuana angustiventris*".

Morphological analysis

Immatures

The larvae studied have been found living underwater in phytotelmata of bromeliads with the spiracular disk of the posterior respiratory process tube in the air, and the anterior spiracles contracted into pockets of the inverted integument, which probably helps protect the spiracles from being blocked.

The larvae of *Quichuana* **sp. nov.**, and larvae of *Quichuana pogonosa* described by Echeverry et al. (2021) show striking similarities. Both have large prolegs and same number and arrangement crochets on the abdominal prolegs, which enable the larvae to grip the bottom sediment without floating to the surface (Rotheray, 1993). However, the base of the prolegs differs in color and shape, being black, spherical in *Quichuana* **sp. nov.**, white-cream, and conical in *Q. pogonosa*. Additionally, the spicules of the mesothoracic prolegs in *Quichuana* **sp. nov.**, cover a larger área, such suggests an enhanced ability to grip.

The mouthparts of these two species are similar to that of other saprophagous syrphids (Rotheray 1993), with well-developed pharyngeal ridges, pre-oral filtering setae, and lateral lips, allowing them to filter micro-organisms from fluids. The notable feature of the cephalopharyngeal skeleton of *Quichuana* **sp. nov.**, is presence of two optic depressions, and the ventral arm with a rounded apex, whereas *Quichuana pogonosa* var., has a one optic depression and, the apex of the ventral arm is acute. Also can be distinguished by the antenno-maxillary organs that are separated by a conspicuous groove in *Quichuana* **sp. nov.**, while in *Q. pogonosa* they are short and bulbous.

The Size and distribution of sensilla, spicules and setae show a slight variation between these two species: *Quichuana*. **sp. nov.** has a smaller number of sensillae than *Q. pogonosa*. Large spicules are present on the dorsum of the prothorax margin of both species, It probably enables access to micro-organisms

present in decaying plant material that has accumulated tank of the bromeliad (Rotheray et al. 2007).

Regarding the puparium, the shape, arrangement of openings, and surface sculpture of the pupal spiracle are the main diagnostic characteristics of both species. There was no differences in the posterior spiracle, of the taxa studied this feature, however, can be useful for distinguishing the genus

With the immature stages of only two species described, many more will need to be studied before we can characterize the immature stages of the *Quichuana* genus.

Adults

The material recorded in this work confirms the faunal importance of the Atlantic Forest for the *Quichuana* species, since it seems to provide a unique habitat, rich in density and abundance of bromeliads.

For these two species an integrative approach, of morphological characters and DNA sequences, was applied to achieve and confirm species delimitation

In Table 4 the morphological intraspecific variation between *Quichuana pogonosa* Fluke, 1937 and *Quichuana pogonosa* var. male and female is presented.

As explained before, morphological differences initially made the identification of *Quichuana pogonosa* var., difficult, since the studied specimens were not fit to the original description of the species. In summary, the results presented herein identified that the length of the body, the width of the abdomen, the proportion of the basoflagellomere, as well as the coloration can vary among individuals of *Quichuana pogonosa* adults.

After dissecting and clarifying the male genitalia, we were able to associate these individuals with the name *Q. pogonosa*, since the upper lobe of the hypandrium of the male genitalia is very distinctive, demonstrating once again that it has considerable taxonomic value within the genus.

It is necessary to analyze the proportion of basoflagellomere and the frontal prominence as a character to separate *Quichuana pogonosa* from *Quichuana borgmeieri* Lane & Carrera, 1944, a species with which it can be easily confused, since the range of difference between the proportions of basoflagellomere is very narrow, and the prominence frontal is little high in *Quichuana pogonosa* var.

Quichuana sylvicola Knab, 1913 is also very similar to *Q. pogonosa*, being able to differentiate them by only three characters: the basoflagellomere is elongated (bf = 2.3), with a ridge between the forehead and the frontal prominence, and the alula is entirely microtrichose, however, the crest was only observed in females of *Quichuana pogonosa* var.

Future morphological, behavioral, and molecular studies in populations of *Quichuana pogonosa* could show whether the intraspecific variation detected is related to adaptive processes, sexual selection, or varies in response to environmental conditions.

Table 4. Morphological intraspecific variation between *Quichuana pogonosa* Fluke, 1937 and *Quichuana pogonosa* var.; males and females.

Character	<i>Q. pogonosa</i> ♂	<i>Q. pogonosa</i> ♀	<i>Q. pogonosa</i> var. ♂	<i>Q. pogonosa</i> var. ♀
Body length	7.9– 9 mm (N=2)	9.6– 11.5 mm (N=3)	6.9 mm	7.4– 7.6 mm
Basoflagello- mere	bf = 1.6, (N=2)	bf = 1.4–1.7, (N = 3)	bf = 1.1	bf = 1.3, (N= 2)
Frons	Black, with dome	Black, without dome	Brown, with dome	Light brown, without dome
Face (Frontal prominence)	Conspicuous	Conspicuous	Little high	Conspicuous, ridge between the forehead and the frontal prominence

Scutum	Two medial, greyish pollinose stripes	Two medial, greyish pollinose stripes	Two medial, greyish pollinose stripes	Three medial, black stripes
Wings	Infusate, with a pigmented anterior margin, tapering towards the Apex	Hyaline, with a pigmented anterior margin, tapering towards the Apex	Hyaline	White
Legs	Black	Black	Light brown	Light yellow
Abdomen	Slender to moderated	Slender to moderated	Broad (1.1)	Broad (1.3)

TAXONOMY

Family Syrphidae Latreille, 1802

Subfamily Eristalinae Newman 1834

Genus *Quichuana* Knab, 1913

Quichuana Knab, 1913: 14. Type species: *Quichuana sylvicola* Knab, 1913 (original designation). Type locality: Peru.

Diagnosis of *Quichuana* species.

Pilose eyes (holoptic males); sub-basal, dorsal, bare arista; pilose postpronotum; vein R₄₊₅ strongly sinuate; cell R₁ open; metafemur with basoventral patch of black setulae (Thompson et al. 2010).

Quichuana sp. nov.

(Figures 1–6)

Description of *Quichuana* sp. nov.

Type material. Holotype. 1♂, “Brazil: Paraná, Balsa Nova / 25°31'05.6"S, 49°35'11.4"W / 06.ix.2020/ A. Echeverry, J. Paludo, leg”, “Reared from larva

collected in phytotelmata of bromeliad: / *Aechmea distichantha*", "pupation 8.ix.2020 / emergence 23.ix.2020" / "(DZUP 690922)". Paratype. third instar larva, "Brazil: Paraná, Balsa Nova / 25°31'05.6"S, 49°35'11.4"W / 06.ix.2020 / A. Echeverry, J. Paludo, leg", "Collected in phytotelmata of bromeliad / *Aechmea distichantha*", "(DZUP 690923)". Male with genitalia in a plastic micro-vial attached to the pin.

Diagnosis. Small-sized species with abroad abdomen, $bf = 1.5$, basoflagellomere oval-shaped (Fig. 3c). The specie is characterized by alula microtrichose except for a semicircular area in the middle; lateral margin of the terga I–II with conspicuously long pile (Fig. 3b); black pile on the apex of the metafemur; metatibia markedly curved and dark brown, except for a slightly brown median triangular mark (Fig. 3d); superior lobe of hypandrium curved forwards in profile view, nearly rounded at the apex (Figs. 3e,f).

Description of adult

Male (holotype). Body length 7.0 mm; abdomen width: 1.2; wing length XX mm *Head* (Figs 3a,c). Eye black with straight, sparse, yellowish pile. Ocellar triangle with both long, forward-directed black and white pile, yellowish pollinosity on the anterior corner. Occiput black with yellowish pile intermixed with a row of postocular black setae, white pollinose on the lower half, and black pollinose on the upper half. Frontal triangle shiny black, without dome, with anteriorly directed, yellowish-white pile hairs. Antenna black, arista reddish; scape and pedicel black, with black setula; basoflagellomere black, yellow pollinose. Face shiny black, with long pile and, yellowish pollinosity on the eye margin and gena; face with a central, shiny, black stripe comprising the facial tubercle and reaching the insertion the antenna; lateral stripes bare, outlined with yellowish pollinosity (Fig. 3c).

Thorax (Figs 3a–b,d). Shiny black scutum, except for the brown callus humeral and posterior callus. Scutum with two medial brownish gray, pollinose stripes extending beyond the transverse suture; scutum coated with yellowish backward directed pile. Supra-alar area with many black bristles. Scutellum dark brown, with yellowish pile. Pleura dark brown, white pollinose. Posterior anepisternum, anepimeron, and katepisternum, with long yellow pile. Anterior

and posterior spiracle black. *Legs*: brown pale pilose. Coxas black, white pollinose. Trochanter dark brown, white pollinose. Pro- and mesofemur dark brown except, apical third light brown. Metafemur light brown, except for the anterior apical third shiny black, swollen; apical third of the metafemur ventrally bearing spiny black pile. Pro- and mesotibia dark brown; metatibia, conspicuously curved, dorsoventral flattened, dark brown except for a slightly brown triangular mark on the middle of the length of the tibia. Tarsomeres black, white pollinose with few scattered strong black setae. *Wing membrane* slightly pigmented light-brown on the anterior basal half; extensively, with small bare at the basal area of cell BM. Alula microtrichose, except for a bare semicircular area on the anterior margin. Veins reddish. Calypter white margins with long pile light-yellow. Haltere light-brown. Tegula shiny black, with strong black setae.

Abdomen (Figs. 3a–b). Shiny dark brown, covered with short yellowish pile inclined backward. Terga I–II black pollinose; lateral margins of terga I–II with long pale-yellow pile; Sternites II–IV with long pale yellow scattered pile. *Male genitalia* (Figs. 3e–f). Rounded Hypandrium at the ventral-basal margin, with two pairs of processes hooked on the front; superior lobes of hypandrium round at the apex. Surstylus elongated, sinuous in shape with long pile posteriorly. Epandrium with projection dark sclerotized at the point of articulation with the hypandrium. Cercus sub-triangular-shape.

Female. Unknown.

Distribution. Brazil (Paraná, Balsa Nova).

Remarks. *Quichuana* **sp. nov.**, is very similar to *Quichuana undulatipila* Ricarte & Hancock, 2012, can be separated by following characters: *Quichuana* **sp. nov.**, has black pile on the apex of the metafemur, and on *Q. undulatipila* these are absent; pile on lateral margins of terga I–II smooth, on *Q. undulatipila* wavy. Additional material examined puparium and cephalofaringeal skeleton stored in a plasticmicro-vial, attached to each specimen.

Description of the third instar larva

(Figure 5–6)

Length 16.0 mm; width 3.10 mm. *Overall appearance* (Figs. 5a,c): Long-tailed, sub-cylindrical shape in cross-section, flattened ventral surface, truncate anteriorly, tapering posteriorly to anus. Cuticle white-cream when alive, brown after fixation. Dorsal body surface coated with light brown setae, directed backward. Ventrolateral margin of the abdominal segment 1–7 with two lines of long setae. *Head* (Pseudocephalon) (Fig. 5d). Internal mouth-hooks. Papilla base with antenno-maxillary organs separated by a conspicuous groove; located between the mouth and the dorsal surface of the prothorax. *Cephalopharyngeal skeleton* (Fig. 5b). Dorsal cornu short rounded apically, slightly sloping back, and tapered posteriorly. Dorsal bridge joining the two dorsal cornua. Ventral arm long, rounded at the apex. Ventral cornu elongated, with the cibarium located at the base, cybar filter with six branching transverse divided into two rows of fine filaments. Mandibular lobes enlarged, with internal ridges; epipharyngeal plate pointed, heavily sclerotized; labrum sclerotized with two optic depressions.

Thorax (Figs. 5d–f). Lateral lips well-developed, triangular-shaped, lower margin coated in long densely aggregated setae, inner margin with conical brown hooks, gradually tapering into the preoral cavity. Dorsal lip situated below the antenna-maxillary organs, with a tuft of long setae. Ventral lip not clearly seen. Dorsoapical margin of prothorax curved toward the front of the head, with four longitudinal grooves and sclerotized hooked spicules directed backward, opercular suture weakly marked. Dorsolateral surface of the prothorax with a pair of light-brown, shining spiracles, retractile into inverted integumental pockets. Ventral surface of mesothorax with well-developed proleg with 16–18 crochets arranged in two rows, the first row with long and thin crochet and the second row with short and thick sclerotized crochets.

Abdomen (Figs 5a,c–f). Dorsal surface of the first abdominal segment with primordia of pupal spiracles, differentiated as an area of the cuticle as two circular patches with little pubescence, through which the pupal spiracle will be pushed. Segments 1–6 with well-developed prolegs with about 16–18 crochets directed facing backward, with the distal third sclerotized, anterior crochets long and slender, posterior smaller and thicker, followed by two rows of spicules, prolegs of segment 6 with crochets directed forward. Segment 8 with three pairs of lappets, each lappet with one sensillum, first at the base, second almost in the

middle, and third pairs of lappets at the end of the segment. *Posterior respiratory process* (Figs. 5a, 6f). Length: 27.7 mm; width: at base: 3.0 mm, at the tip 1.0 mm, shiny, sclerotized, and pale yellow in color, ringed at the base; spiracular disc with 4 pairs of half circle shape spiracular openings, surrounded by four short inter-spiracular setae. *Chaetotaxy* (Fig. 5f). Prothorax with two pairs of dorsal sensilla, one pair of lateral. Mesothorax with two pairs of dorsal sensilla, one pair lateral, two pair ventrolateral, and one pair ventral close to proleg. Metathorax with two pairs of dorsal sensilla, two pairs lateral, one pair ventrolateral. Abdominal segments 1–6 with three pairs of dorsal sensilla, two pairs lateral, two pairs ventrolateral, and one pair posterior to each proleg. Abdominal segment 8 with five pairs of dorsolateral sensillae.

Description of puparium

(Figures 4–5)

Length 7.0 mm; width 3.0 mm; height 25 mm. Sub-cylindrical in cross-section; truncated anteriorly; tapering gradually from middle posteriorly to a posterior respiratory process; flattened ventrally. Surface of the integument light brown in color, rough, exhibiting larval segmentation and disposition of the sensillae. Puparium surface keeps characteristics of the larva; exposed anal papillae. *Pupal spiracles* (Figs. 4a, 6c–d,f). Length 1.3 mm; width 0.1 mm; length tip only 0.5 mm, separated by a distance 1 mm. Cylindrical in shape with a rounded tip, toward center of the upper part of operculum, distal half curved toward the lateral margins of the puparium, black in color; reticulated basal surface. Tubercles rounded, irregularly arranged on not more than a quarter of spiracle apex, each tuber with 4–6 spiracular openings. *Anterior larval spiracles* (Figs. 4b, 6a–b). Length 0.7 mm; width 0.1 mm separated by a distance 1.2 mm. Pale yellow in color, cone-shape, with pointed slightly recurved tips, spiracular openings (16) arranged in a semicircle of the ventral surface margin. *Posterior respiratory process*. See the description of the larva.

Biology. Larvae were collected in the *A. distichantha* bromeliad, plants commonly found in areas of the Brazilian Cerrado and Atlantic Forest (NCFlora 2012). Larvae were collected in tanks containing a high concentration of decomposing plant matter, coexisting with other *Quichuana pogonosa* larvae.

Comparison of the intraspecific variability of *Quichuana pogonosa* Fluke, 1937.

Material examined: 1♂, "Brazil: Paraná, Curitiba / 25°27'37.3"S, 49°13'36.8"W / 14.xii.2020 / A. Echeverry, D. Vanegas, leg.", "Reared from larva collected in phytotelmata of bromeliad: / *Alcantarea imperialis*", "pupation 04.i.2021 / emergence 15.i.2021" / "(DZUP 690924)": 1♀, "Brazil: Paraná, Curitiba / 25°27'37.3"S, 49°13'36.8"W / 14.xii.2020 / A. Echeverry, D. Vanegas, leg.", "Collected in flight over / bromeliad *Alcantarea imperialis*", "(DZUP 690925)": 1♀. "Brazil: Paraná, Curitiba / 25°27'37.3"S, 49°13'36.8"W / 14.xii.2020 / A. Echeverry, D. Vanegas, leg.", "Reared from larva collected in phytotelmata of bromeliad: / *Alcantarea imperialis*", "pupation 07.i.2021 / emergence 19.i.2021" / "(DZUP 690926)". Male with genitalia in a plastic micro-vial attached to the pin.

Diagnosis. Light brown species (Figs 7–8), small-sized with a broad abdomen. bf up to 1.3; basoflagellomere nearly round (Fig. 1); frontal prominence little high (7–8a); wing without pigmented; scutum of the female with three brownish-gray pollinose stripes (Fig. 7b).

(Figures 7–10)

Male. Small-sized species. Body length 6.9 mm; abdomen width 1.1 mm. *Head* (Fig. 7a,c). Eye black with pile light-yellow, anterior and posterior margins bare. Ocellar triangle with long, forward-directed black pile, and light-yellow pollinosity on the anterior corner. Occiput black with long light-yellow pile intermixed with a line of rows of black setae, white pollinose on the lower half. Frontal triangle shiny black, with a dome in the upper half covered with long black pile directed forward. Dorsal surface of the frontal triangle with long, light-yellow pile, shorter in the margins of the eyes; frontal prominence shiny black, with black and light-yellow pile intermixed. Antenna with arista reddish. Scape and pedicel black, with black setula. Basoflagellomere black pollinose, bf = 1.3. Gena and postgena shiny black, bare. Face shiny black, yellowish pollinosity, with light-yellow pile; face with a central shiny black stripe, bare and smooth comprising the facial tubercle, not reaching to the insertion the antenna; lateral stripes, outlined with yellowish pollinosity and covered with scattered light-yellow pile.

Thorax (Fig.7a–b). Shiny black scutum, except for the lightly brown callus humeral and posterior callus; scutum coated with light-yellow backward directed pile, with one or two black bristles above the supra-alar area; scutum with two medial, white pollinose stripes, extending over the anterior three-quarters. Scutellum slightly brown in color, with longer light-yellow pile. Pleura lightly brown, white pollinose. Anterodorsal anepimeron with short white pile; posterodorsal anepimeron and notopleura with a tuft of yellow pile. Katepisternum dorsally and anepisternum posteriorly covered with long yellowish pile. Anterior and posterior spiracle blackish *Legs* (Fig.7a,d). Pro- and mesocoxae brown, white pollinose, with light-yellow pile, densely aligned on the anteroapical margin; metacoxa black, white pollinose, with light-yellow pile on the margin anterior. Pro- and mesotrochanter brown, white pollinose, with one hair dorsocentral; metatrochanter shiny black, with light-yellow pile. Pro-and mesofemur white pollinose lightly brown dorsally, black ventrally, with light-yellow pile longer on the posterior surface; metafemur mainly lightly brown, except base and ventral surface of the apical third, swollen, excavated on the apical third, with light-yellow pile; apicodorsal area of the metafemur bearing short, black, adpressed hairs, apical third of the metafemur ventrally bearing spiny black pile. Pro- and mesotibia lightly brown dorsally, covered by shorts yellow pile, black ventrally with scattered black hairs; metatibia conspicuously curved, brown, except for a slightly brown triangular mark on the middle of the length of the tibia; tarsomeres brown with surface densely covered by short reddish hairs, interspersed with few scattered strong black setae; obscured dorsally. *Wing* (Fig.7a). Hyaline, black microtrichose, with areas bare in basal-anterior cell R between vein spuria and M, three third basal half of cell BM, basal anterior area of CuP and anal lobe. Veins brown. Haltere light yellow. Tegula shiny black, with black pile with and two or three light-yellow pile. Alula microtrichose except for a central area. Calypter hyaline, covered with short, black pile, margins black, with long pile light yellow.

Abdomen (Fig 7b). Brown, covered with light yellow pile inclined backward, longer on the lateral margins. Tergum I with a mustache of yellow pile, with a central bare gap of about one-third of the tergum width; terga II–IV with black pile, restricted at the posterior margin *Male genitalia* (Figs 7e–f). Superior lobe of

hypandrium truncated apically with two invaginations, outer surface with one expansion. Hypandrium with two pairs of hooked processes near the ventral margin, the upper short and thin and the lower long and thick. Surstylus with rounded apex in lateral view and long pile posteriorly. Cercus subtriangular in shape (tapered), approximately as long as its width at base in lateral view and with slight excavation.

Female (Length 7.6 mm; abdomen width 1.3, bf = 1.1 mm. same as the male, except for the lighter coloring (Figs 8a–d). Frons without a dome, with a median white pollinose band, only with pile light yellow. Face with tubercle more prominent than in the male (Figs 8c). Basoflagellomere nearly square (Figs 8c). Scutum with three brownish-grey pollinose stripes one central and two medials (Fig 8b). Wing white with hyaline veins (Figs 8d).

Description of puparium

(Figure. 9)

Male. Length 7.0 mm; width 3.0 mm; height 3.0 mm. Sub-cylindrical in cross-section; truncated anteriorly; tapering gradually from middle posteriorly to a posterior respiratory process; flattened ventrally. Surface of the integument light brown in color, rough, exhibiting larval segmentation. Ventral-apical surface of puparium with three longitudinal grooves, covered with sclerotized hooked spicules directed backward, progressively longer and thicker. Lateral lips well developed, cone-shaped, covered with short spicules densely aggregated, directed toward the margin of the mouth opening; upper margin of the dorsal lip without spicules, visible mouth opening. Remains of mesothoracic prolegs with crochets arranged in two semicircular rows. Abdominal segment 1–6 with well-developed prolegs; with crochets in two parallel rows, with the distal third sclerotized; crochets in anterior row bigger than those in a posterior row, followed by a patch of black, small spicules; crochets of last pair of prolegs directed forward. Vento-lateral margin of the abdominal segment 1–7 with two lines of long setae densely aggregated. Margins of anal opening covered with long setae; anal opening exposing 10 anal papillae. Segment 8 extended as in long-tailed length 9.75 mm. *Pupal spiracle* (Figs 9c, 10c–e). Length 1.0 mm; width 0.25 mm (length tip 0.5 mm), separated by a distance similar to the length of one spiracle.

Subcylindrical with a rounded tip, projecting toward center of the upper part of operculum, curved backward (sinuoso), distal half curved toward the lateral margins of the puparium; black in color; apical third surface bearing irregularly-spaced rounded tubercles, each tubercle bearing 5–6 oval spiracular openings. *Anterior larval spiracle* (Figs 9b, 10). Length 1.5 mm; width 0.15 mm, projecting from the lateral margins of the lower part of the operculum, tips, pale yellow in color, cone-shape, with pointed slightly recurved tips, spiracular openings (17) arranged in a semicircle of the ventral surface. *Posterior respiratory process* (Fig 10f). Length 30 mm; width 0.15 mm; shiny, sclerotized, light brown in color. Spiracular disc with four pairs of spiracular openings and four pairs of well-developed branched inter-spiracular setae.

Cephalopharyngeal skeleton. no variation with that already described for *Quichuana pogonosa*.

Female. Same as male, except for the lighter coloring, the larger and the pupal spiracles not curved. Length 8.7 mm; width 4.5 mm; high 3.75 mm; anterior larval spiracles: 1.1 mm; width 0.25 mm; pupal spiracles length 1.5 mm; width 0.35 mm, separated by 1.25 mm.

Biology. Larvae were found in *A. imperialis* bromeliad tanks, water-filled containing abundant organic matter. *A. imperialis* is an endemic species of the Atlantic Forest of Brazil (Palma et al. 2007). Larvae of *Q. pogonosa*, *Eristalis* Latreille, 1804, and *Lejops* Rondani, 1857., were found coexisting in the same tank.

Distribution. Brazil (Paraná, Curitiba).

Remarks. Females of *Quichuana pogonosa* var. Similar to the male, except normal sexual dimorphism and wing white in color on female, hyaline in male; scutum of the female with three brown-grey pollinose stripes, two in males. Other additional material examined: male genitalia stored in a plastic microtube, puparium, and cephalofaringeal skeleton stored in a plastic tube, attached to each specimen.

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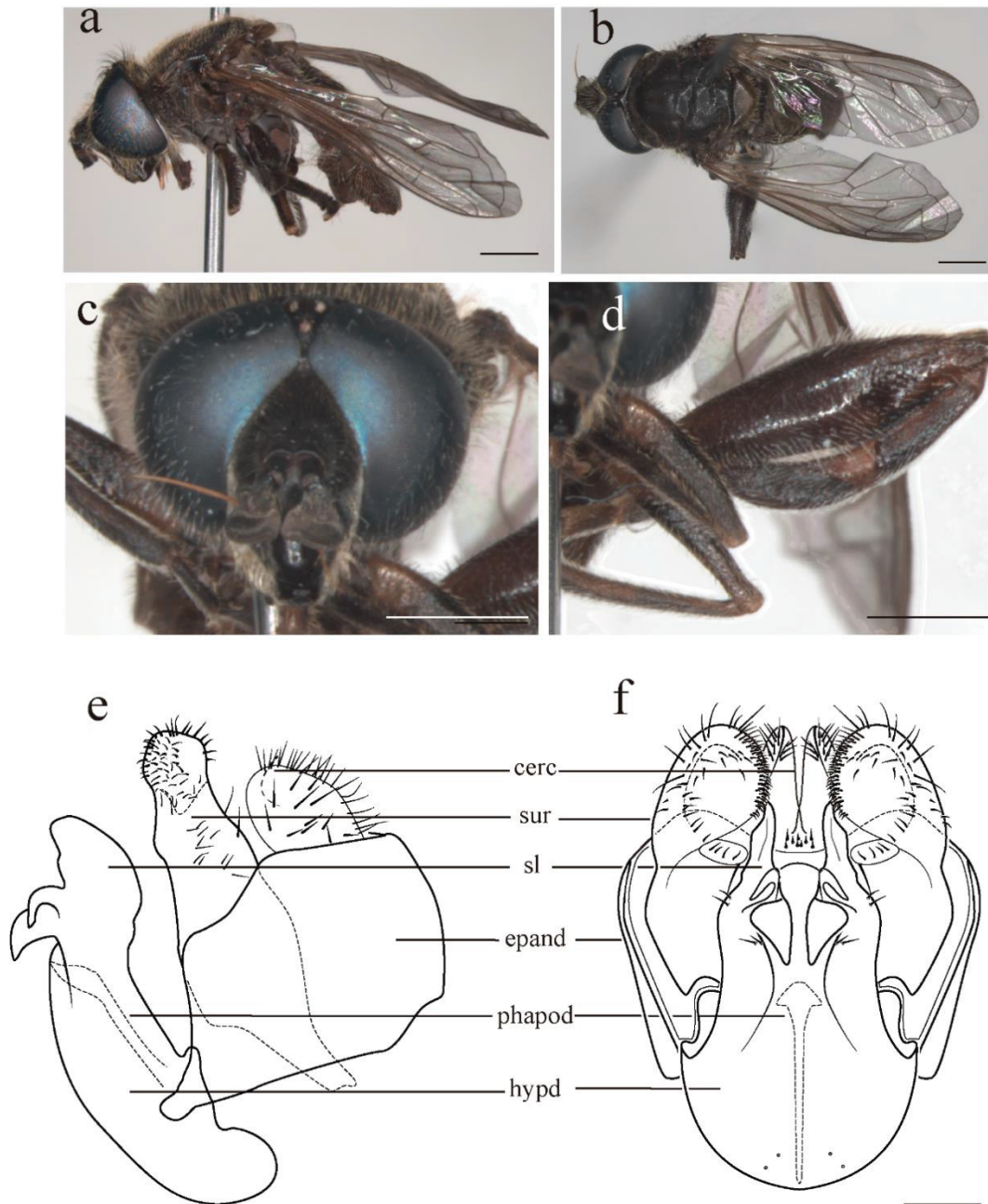


Figure 3. *Quichuana* sp. nov., male, holotype (DZUP 690922): (a) habitus lateral; (b) habitus dorsal; (c) head, frontal view; (d) posterior leg; (e) genitalia, lateral view; (f) genitalia ventral view. (cerc) cercus; (epand) epandrium; (hypd) hypandrium; (phapod) phallodeme; (sur) surstylus; (sl) superior lobe. Scale bars: a, b, c, d = 1 mm, e, f = 500 µm.

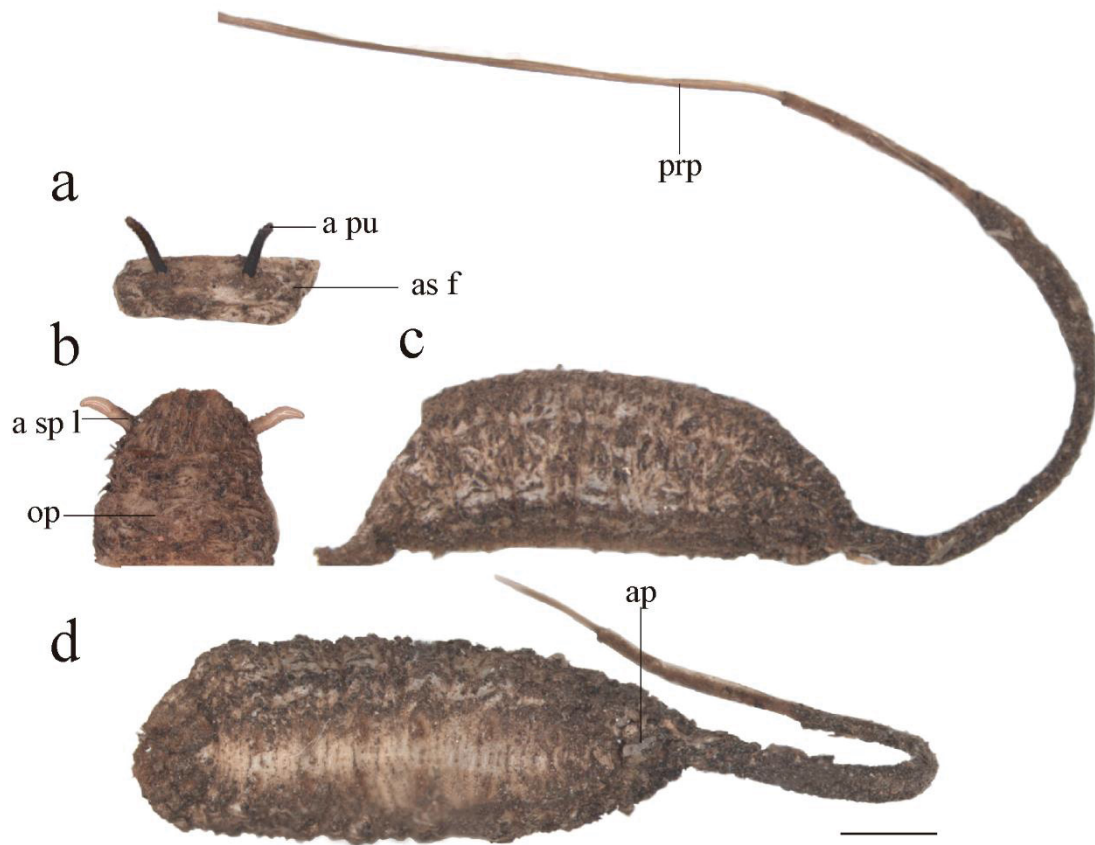


Figure 4. *Quichuana* **sp. nov.** male (DZUP 690922): (a) first abdominal segment of puparium anterior; (b) operculum; (c) puparium lateral view; (d) puparium ventral view. (a pu) anterior pupal spiracles; (as f) first abdominal segment of puparium; (a sp l) anterior spiracles larval; (ap) anal papillae; (op) operculum; (prp) posterior respiratory process. Scale bars: a, b = 0.5 mm, c, d = 1 mm.

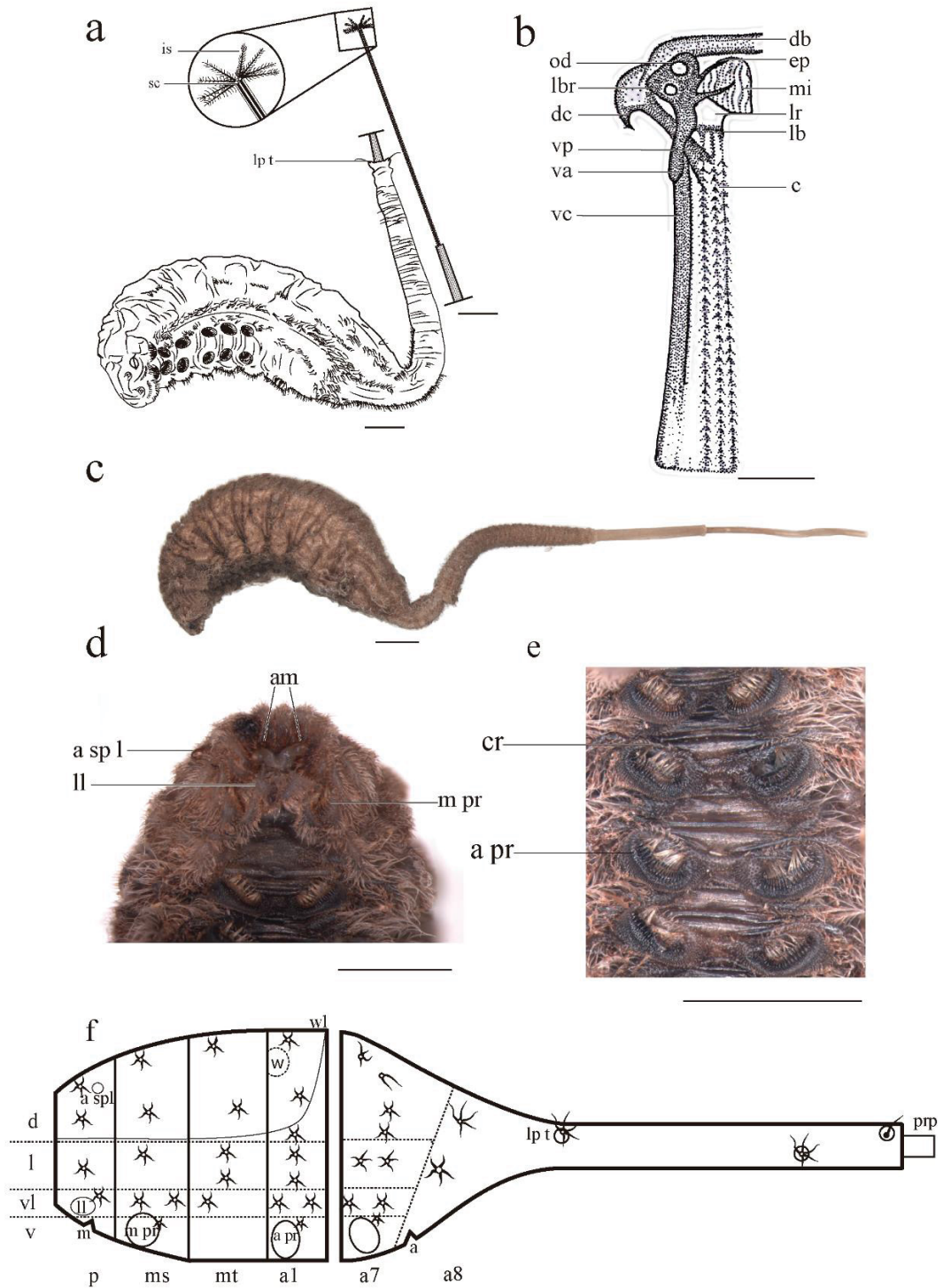


Figure 5. *Quichuana* sp. nov. third instar larva (DZUP 690923); (a) third instar larva, lateral view showing posterior respiratory process; (b) cephalopharyngeal skeleton, lateral view; (c) third instar larva, lateral view; (d) third instar larva, ventral view; (e) prolegs; (f) map of the chaetotaxy of the third instar larva in lateral in lateral view, showing the position of the sensillae. (am) antenno-maxillary organs; (a spl) anterior spiracle larval; (a pr) abdominal prolegs; (c) cibarium; (cr) crochets; (cs) central scars; (dc) dorsal cornu; (db) dorsal bridge; (ep)

apopharyngeal plate; (is) interspiracular setae; (ll) lateral lips; (lp t) lappets; (lr) labial rods; (mi) mandibular lobes; (m pr) mesothoracic prolegs; (od) optical depression; (va) ventral arm; (vc) ventral cornu; (vp) vertical plate; (a1, a7, a8) abdominal segments, a2-a6 are suppressed from figure for clarity; (a) anus; (a pr) abdominal prolegs; (d) dorsal; (l) lateral; (ll) lateral lips; (lp t) lappets; (m) mouthparts; (mt) metathorax; (m pr) mosothoracic prolegs; (p) prothorax; (v) ventral; (vl) ventrolateral; (w) primordia of pupal spiracles; (wl) line of weakness after puparium formation bounding the operculum. Scale bars: a, c, d, e = 1 mm, b = 0.4 μ m.

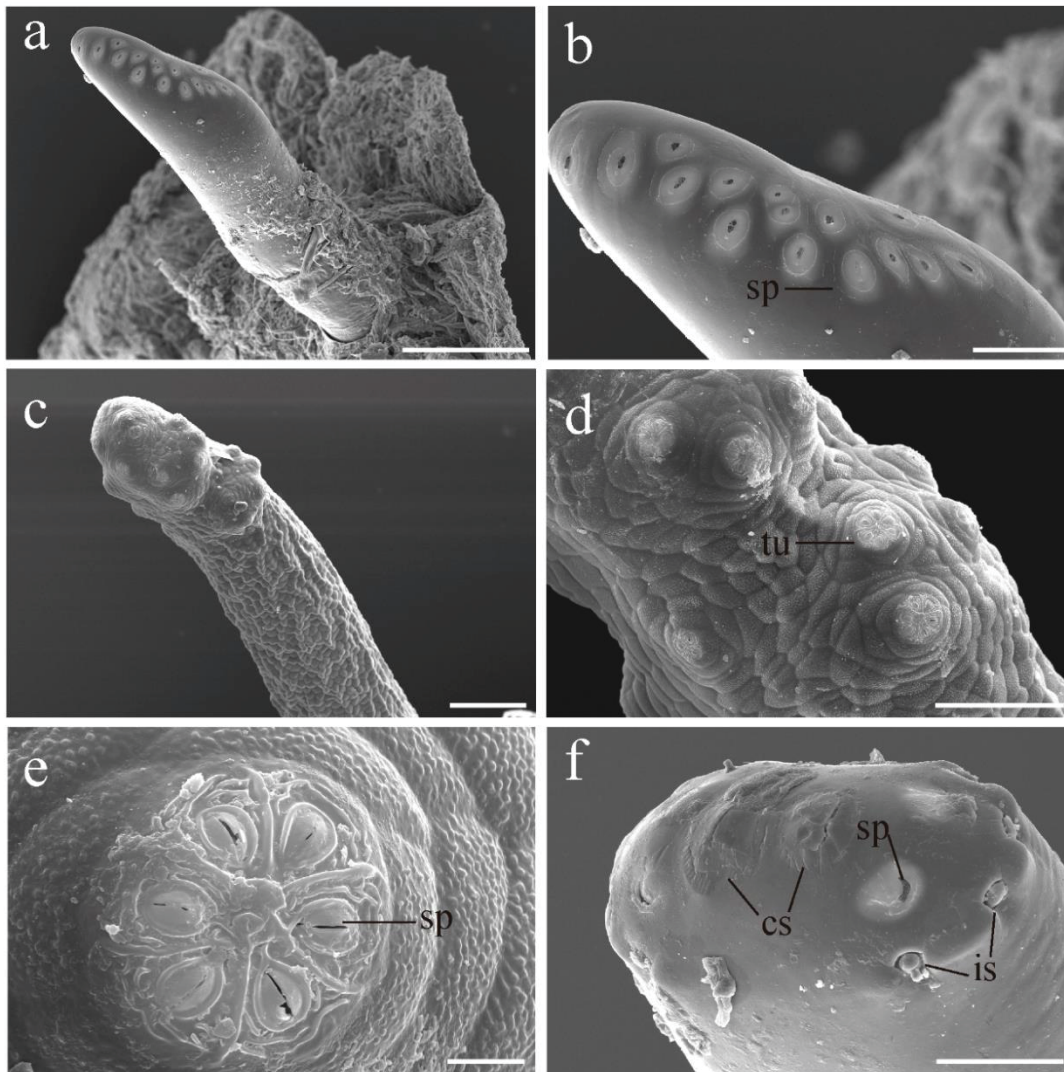


Figure 6. *Quichuana* sp. nov. (a) anterior spiracle, lateral view; (b) anterior spiracle, apical view; (c) pupal spiracle, lateral view; (d) pupal spiracle, apical view; (e) tubercle of pupal spiracles; (f) espiracular disk of posterior respiratory processes. (cs) central scars; (is) interspiracular branched setae; (sp) spiracular openings; (tu) tubercle spiracular. Scale bars: a = 200 μ m, b = 100 μ m, c, d = 50 μ m, e, f = 20 μ m.

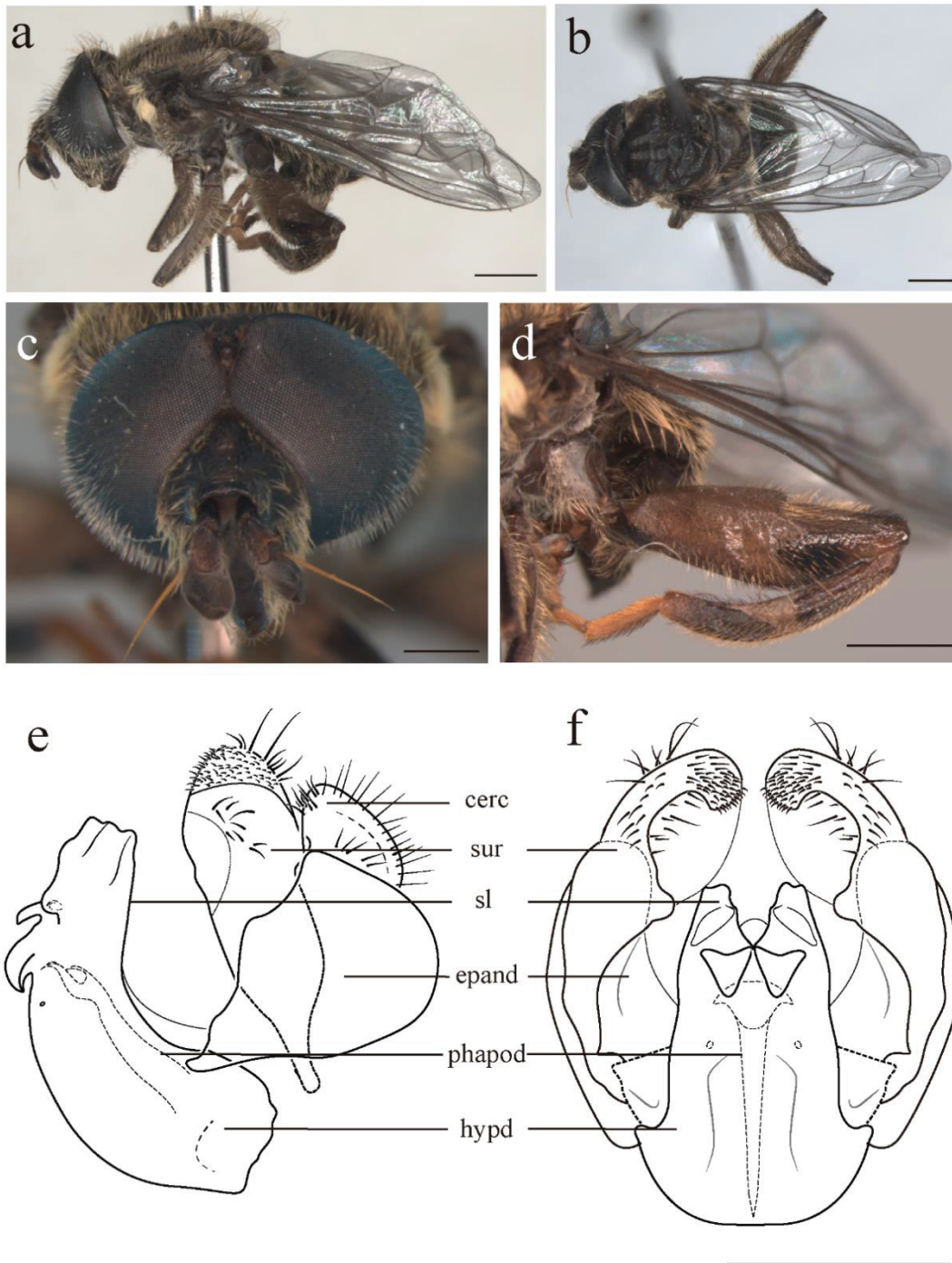


Figure 7. *Quichuana pogonosa* var. male, (DZUP 690924): (a) habitus lateral; (b) habitus dorsal; (c) head, frontal view; (d) posterior leg, lateral view; (e) genitalia lateral view; (f) genitalia ventral view. Scale bars: a, b, d = 1 mm, c = 0.5 mm, e, f = 1 mm.

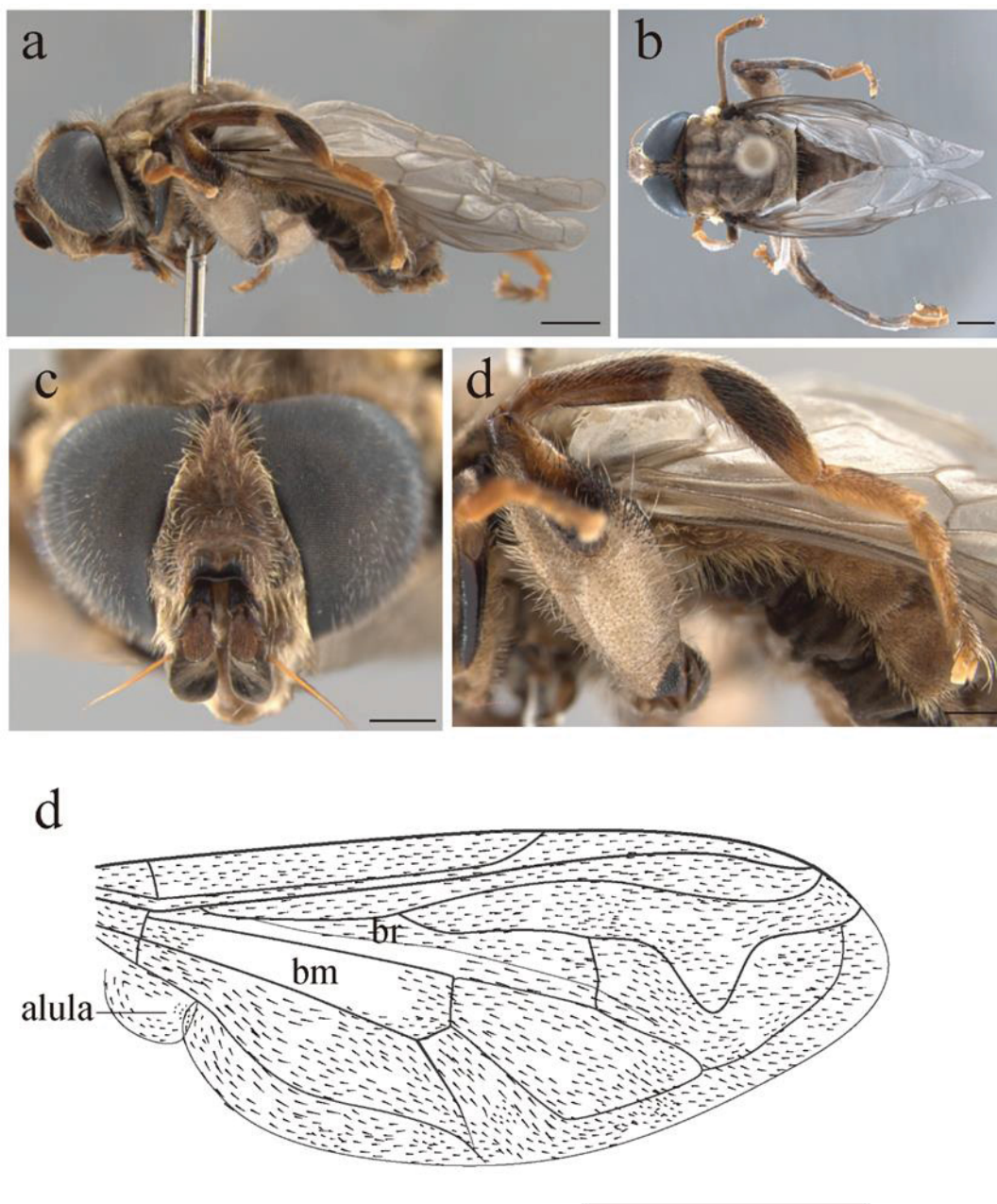


Figure 8. *Quichuana pogonosa* var. female, (DZUP 690925): (a) habitus lateral; (b) habitus dorsal; (c) head, frontal view; (d) posterior leg, lateral view; (e) right wing dorsal view; (bm) cell basal medial; (br) cell basal radial. Scale bars: a, b = 1 mm, c, d = 0.5 mm, e = 0.25 mm.

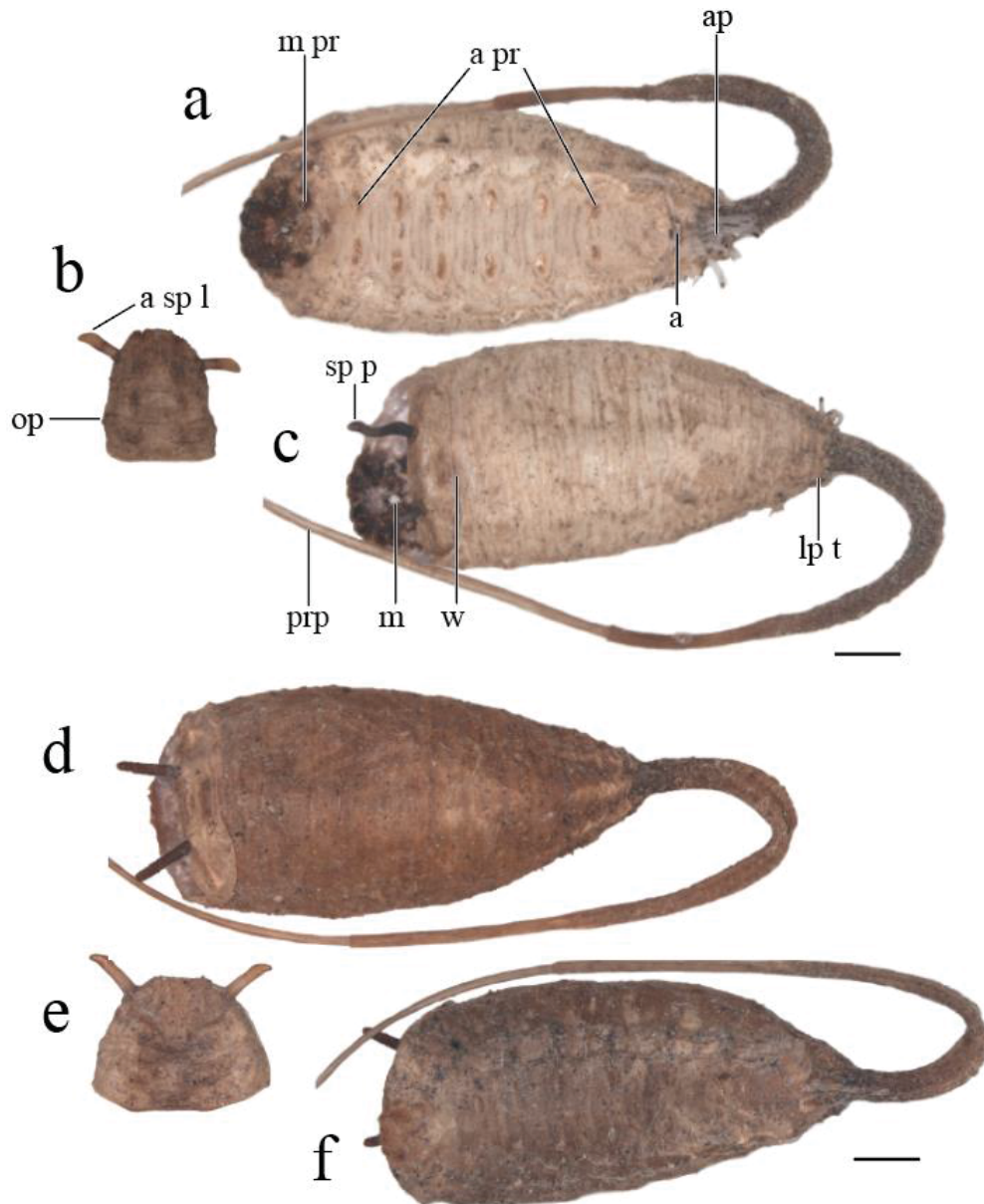


Figure 9. *Quichuana pogonosa* var. male (DZUP 690924). (a) puparium, ventral view; (b) operculum; (c) puparium dorsal view, *Quichuana pogonosa* var. female (DZUP 690925); (d) puparium dorsal view; (e) operculum; (f) puparium, ventral view. (a) anus; (ap) anal papillae; (a pr) abdominal prolegs; (a sp l) anterior spiracles larvae; (lp t) lappets; (m) mouthparts; (m pr) mesothoracic prolegs; (op) operculum; (prp) posterior respiratory process; (sp p) pupal spiracles; (w) primordia of pupal spiracles. Scale bars: 2 mm.

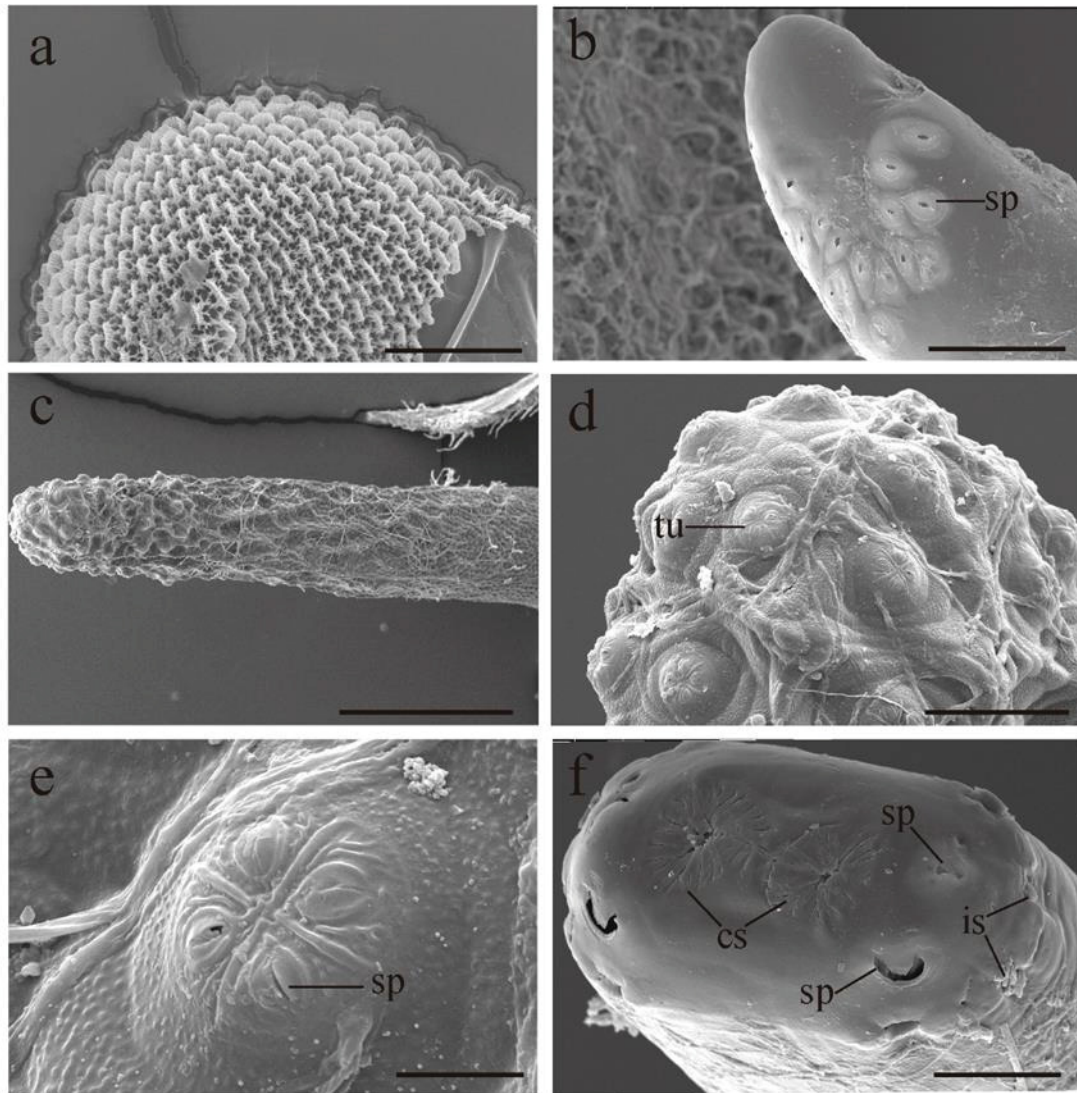


Figure 10. *Quichuana pogonosa* var. (a) details of egg chorion microsculpture; (b) anterior spiracle, ventral view; (c) pupal spiracle, lateral view; (d) pupal spiracle, apical view; (e) tubercle of pupal spiracles; (f) spiracular plate of posterior respiratory processes. (cs) central scars; (is) interspiracular branched setae; (sp) spiracular openings; (tu) tubercle spiracular. Scale bars: a, b = 100 μm , c = 500 μm , d, f = 50 μm , e = 10 μm .