

RESEARCH ARTICLE

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

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<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, Ståhls 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 poganosa* 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 stereomicroscope 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 stereomicroscope 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. poganosa* 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 barbiellini* (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
Rearing	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)

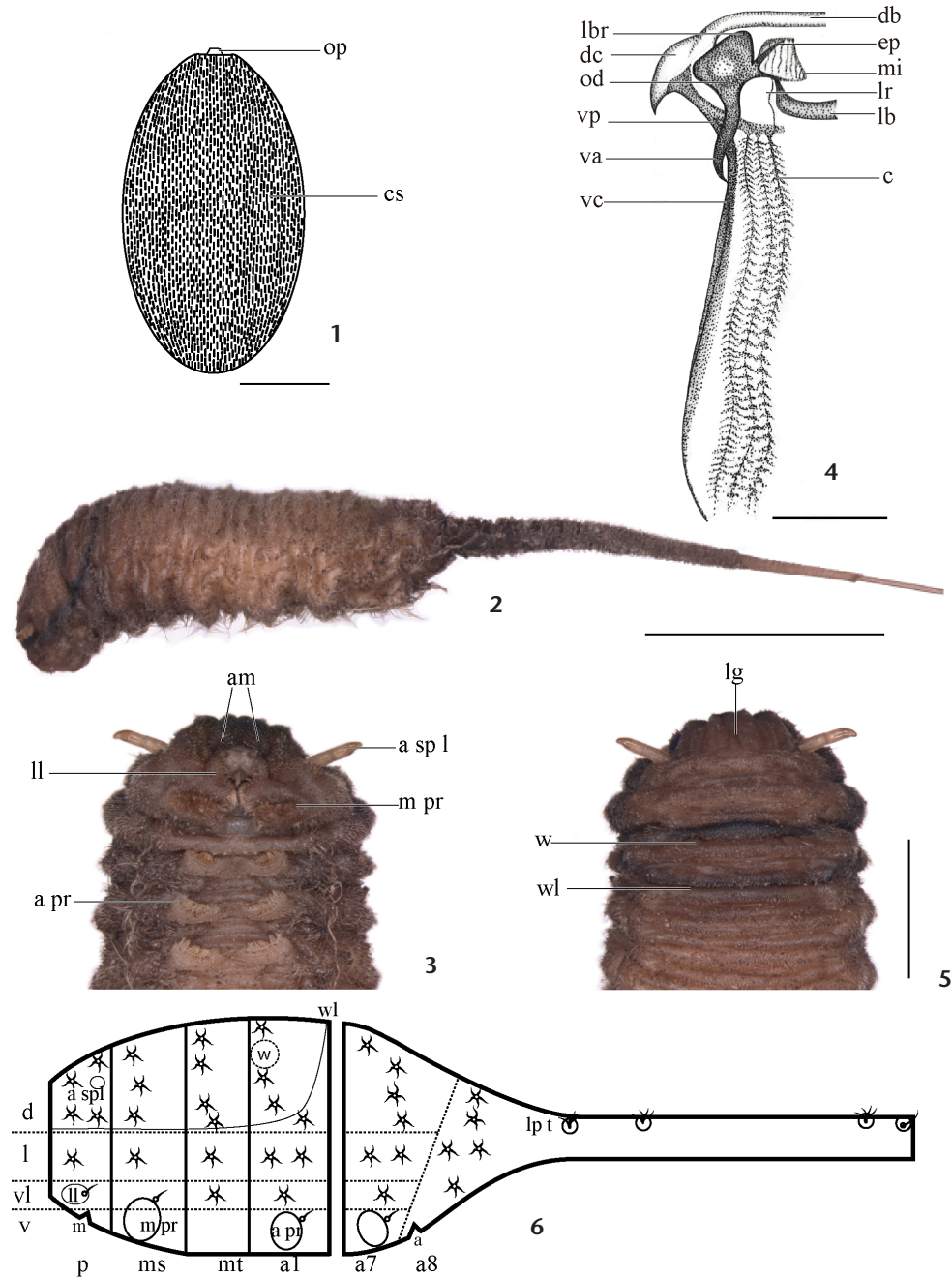
Length: 11.50 ± 0.58 mm; width 4.0 mm (n = 3). Overall appearance: long-tailed, sub-cylindrical in cross-section with flattened ventral surface; truncate anteriorly, tapering posteriorly to anus. Cuticle transparent when alive; beige to dark-brown after fixation; covered with tiny spicules directed backwards 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 pogonos*: (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: $7,18 \pm 1,32$ mm; width: $4,40 \pm 0,62$ 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.

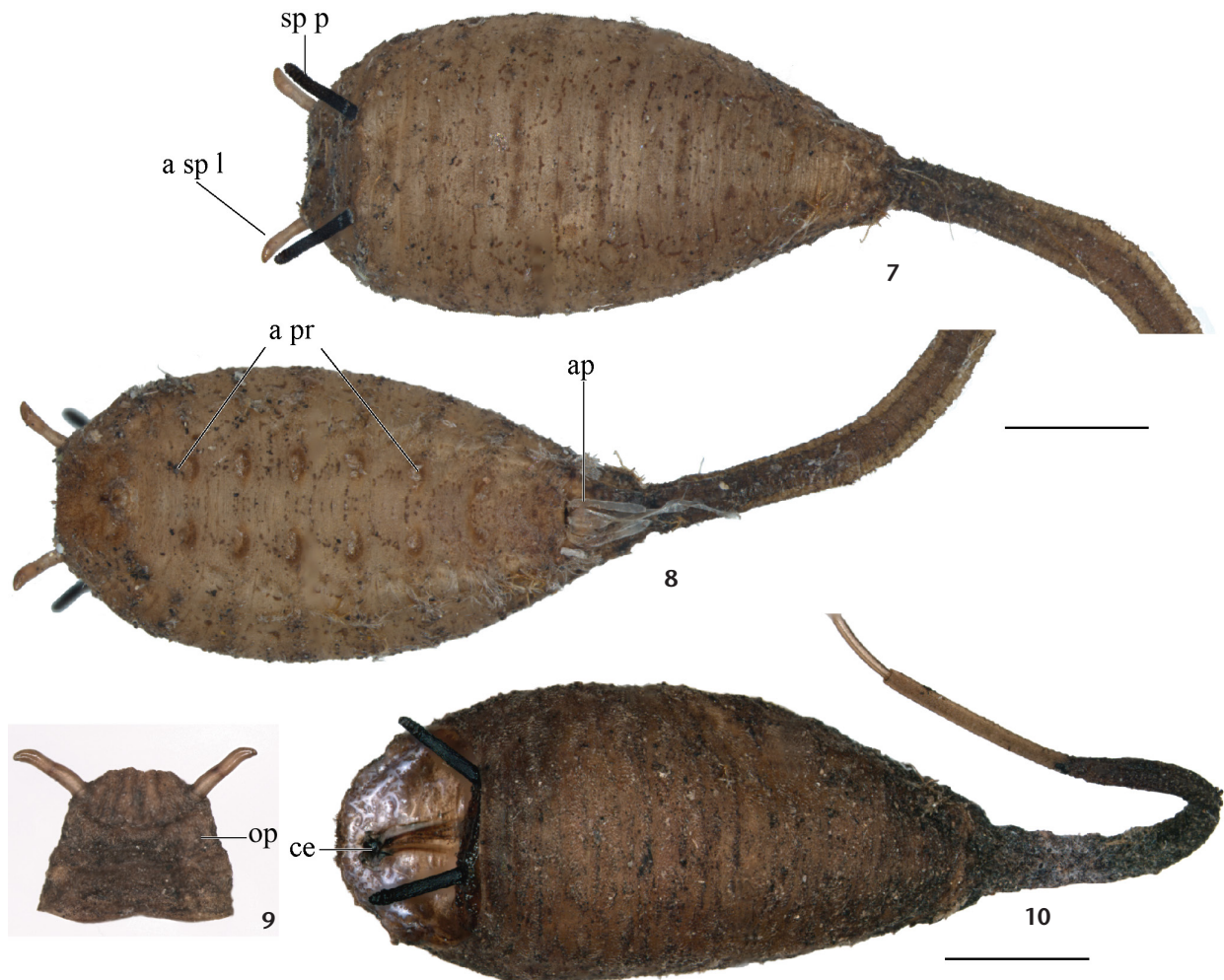
Pupal spiracles. Length: $1,41 \pm 0,19$ mm; width: $0,24 \pm 0,02$ mm; distance between each respiratory process: $1,29 \pm 0,36$ mm. Subcylindrical, projecting towards center of operculum, black, slightly curved at apex, apical surface bearing irregularly spaced and circular-shaped tubercles.

Examined material. See examined material under 'Adults'.

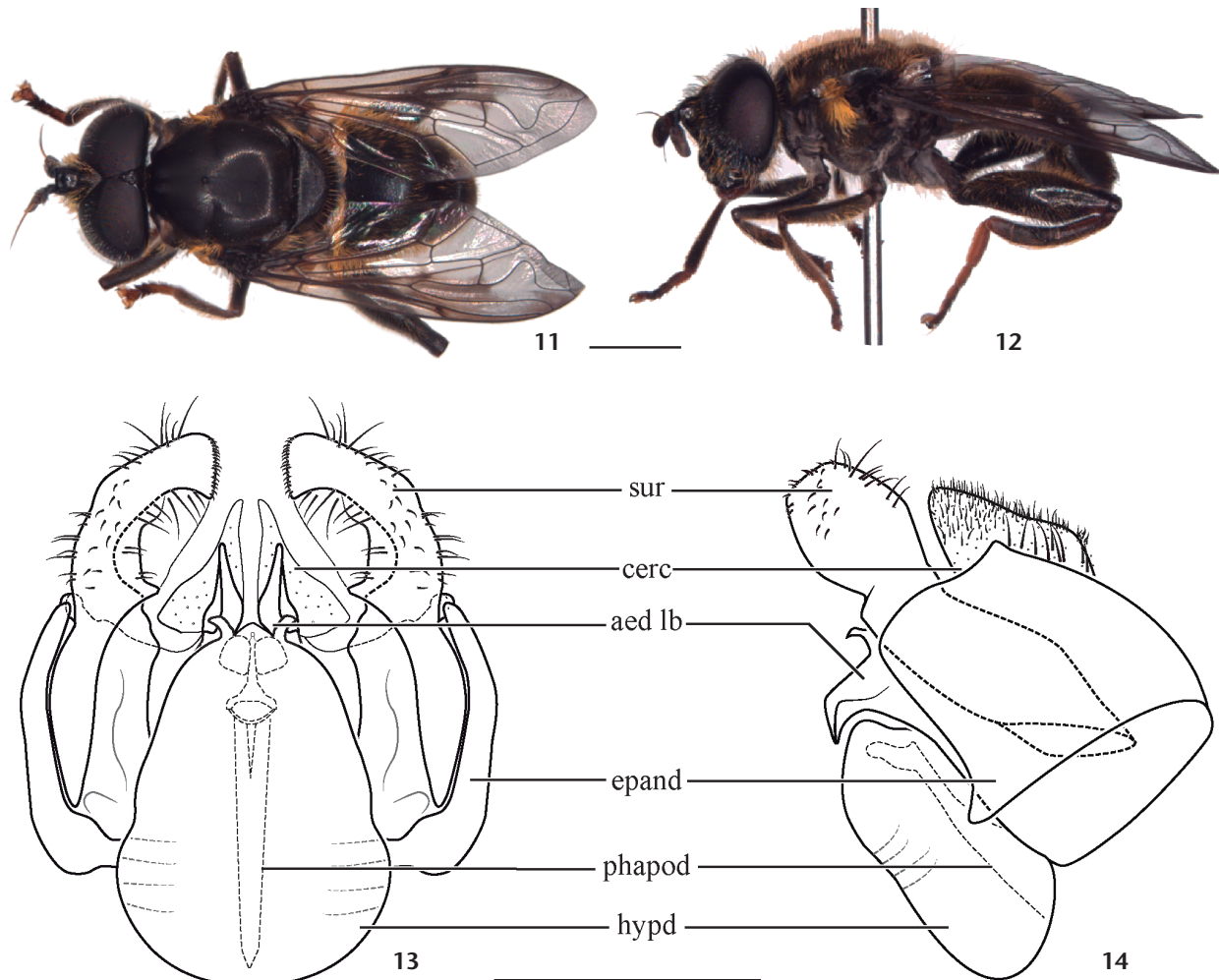
Adults (Figs 11–14)

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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