

A new species of bannerwing damselfly, *Polythore albistriata* sp. nov. (Odonata: Polythoridae)

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All relevant data are within the paper.

Abstract. The traditional method of classifying the twenty-one species within the South American genus *Polythore* has been relying on wing color patterns and male genital ligula shape. However, recent molecular research has shown that wing color patterns can vary significantly within some species, making it an insufficient means of species diagnosis by itself in some cases. In this study, we employ a combined approach of morphological and molecular data to describe a new *Polythore* species found in the southeastern Colombian Andes. Our analysis includes detailed illustrations and pictures of diagnostic features, haplotype networks for two barcode genes, *COX1* and *NADH I*, a distribution map, and a brief discussion of the conservation status of the species. We found wing color pattern, male genital ligula morphology, and *NADH I* barcode sequences to be sufficiently diagnostic to identify the species as new. Our results highlight the importance of integrating multiple data sources for accurate species identifications and descriptions in this genus.

Key words. Colombia, Neotropics, wing color patterns, barcodes

<https://zoobank.org/References/5cd9eb36-bd44-495b-858f-b6e0f9834621>

Resumen. El método tradicional para clasificar las veintinueve especies dentro del género suramericano *Polythore* ha dependido del uso de patrones de coloración de las alas y la forma de la lígula genital del macho. Sin embargo, investigaciones moleculares recientes han demostrado que los patrones de coloración de las alas pueden variar significativamente dentro de algunas especies, lo que los convierte en un medio insuficiente para el diagnóstico a nivel de especie en algunos casos. En este estudio, empleamos un enfoque combinado de datos morfológicos y moleculares para describir una nueva especie de *Polythore* encontrada en el sureste de los Andes colombianos. Nuestro análisis incluye ilustraciones detalladas y fotografías de características diagnósticas, redes de haplotipos para dos genes de código de barras, *COX1* y *NADH I*, un mapa de distribución y una breve discusión sobre el estado de conservación de la especie. Encontramos que el patrón de coloración de las alas, la morfología de la lígula genital, y las secuencias del fragmento *NADH I* son de valor diagnóstico para identificar la nueva especie. Nuestros resultados destacan la importancia de integrar múltiples fuentes de datos para la identificación y descripción precisa de especies en este género.

Palabras claves. Código de barras, Colombia, Neotrópico, patrones de coloración alar

Introduction

The Neotropical bannerwing damselflies of the genus *Polythore* Calvert, 1917 (Odonata: Polythoridae) comprise 21 described species distributed across South America (Börzsöny, 2013; Garrison et al., 2011; Paulson et al., 2023; Rojas-Riaño, 2011). Rojas-Riaño (2011) suggested that the presence of supplementary sectors between RP2 and IR2 proximal to pterostigma was a wing venation character diagnostic of males of this genus. Classical molecular phylogenetic reconstructions for the family Polythoridae always recovered *Polythore* as a monophyletic genus with two major clades—Amazonian and Andean (Sánchez et al., 2018, 2020). Traditionally, its taxonomy and identification has been relying on morphological characters such as wing coloration and the secondary male structures (i.e., ligula) (Bick & Bick 1985, 1990, 1986). Despite the traditional use of wing coloration patterns to distinguish species in the genus, it has been demonstrated that this character is uninformative for some of them, either because it is highly variable within species, or invariant between multiple species. This finding indicates that wing color pattern is a phenotypic convergence, raising the possibility that this genus includes both polytypic and cryptic species (Sanchez et al., 2010, 2015). Bick & Bick (1985, 1986) proposed six groups within the genus *Polythore*, based mostly on the wing coloration, but the latest phylogenetic hypotheses show that these groups are not natural (Sánchez et al., 2018, 2020).

Here we describe a new species with a wing color pattern that is similar to that of *P. williamsoni* from Peru, but with unique ligula characters and a geographical distribution contained largely within the southeastern corner of the Eastern Cordillera of Colombia. This new species (called *Polythore* sp. nov. 1 in the phylogenetic hypotheses by Sánchez et al., 2018, 2020) clusters with northeastern Andean species, including *P. concinna*, *P. procera*, and *P. derivata*, within the Andean clade (Sánchez et al., 2018, 2020).

Material and methods

All specimens referred to here are deposited in the ANDES-E, Universidad de Los Andes (Bogotá), the Colección Entomológica de la Universidad de Antioquia CEUA (Medellín), Museo de Colecciones Biológicas de la Universidad del Atlántico, Barranquilla, (UARC), and the Colección de Artrópodos de la Universidad del Rosario (CAUR) Bogotá, in Colombia.

For diagnosis, the papers by Bick & Bick (1985, 1986, 1990) and Rojas-Riaño (2011) were used and specimens were compared with those preserved at CEUA. For descriptions, scaled pictures of the specimens were used for measuring them with the software ImageJ 1.53t. Total length and abdominal length do not include appendages.

Laboratory photographs were taken with an Olympus SZX9 stereoscope and its mounted Canon EOS 90D

camera and the DinoScopeLite Edge Digital Microscope AM4115ZT. Lateral scans of the specimens and their excised wings were made with an EPSON Scanner Perfection V550, using the scanning frame and protocol proposed by the Target Odonata Wing Digitization project (<https://github.com/willkuhn/towd>). In addition, we dissected specimens to expose and visualize the male ligula with a Hitachi S-4700 field emission scanning electron microscope (FE-SEM) at the Imaging Facility of the American Museum of Natural History, New York, USA. All imagery was then processed with the software Adobe Photoshop v. 24.2.0.

In addition to specimens identified as *Polythore* sp. nov in Sánchez et al. (2018, 2020) for which two mitochondrial barcoding genes had been sequenced (~ 799 bp Cytochrome Oxidase I and ~ 548 bp *NADH* subunit I dehydrogenase), two additional specimens (CAUR-VOD2 and VOD3) were sampled for genetic material, using about a quarter of the thorax and following the DNeasy Qiagen Blood and Tissue Kit manufacturer's protocol. We amplified and sequenced *NADH* subunit I dehydrogenase as per the protocols published in Sanchez et al. (2020). The sequences were compared with previously published sequences of *P. procera* (COI: MF109967, FJ514997, FJ514995; ND1: MF109939, MF109920, OM515610), *P. concinna* (COI: MF109951; ND1: MF109927), and *P. derivata* (COI: MF109959, MF109970; ND1: MF109941) for both fragments. We created Minimum Spanning Networks for each locus in PopArt (Leigh & Bryant, 2015).

The distribution map was composed from the Shuttle Radar Topography Mission's digital elevation models with 30-m spatial resolution, downloaded from <http://srtm.csi.cgiar.org> (Jarvis et al., 2008), and a shape file of Colombian departmental political divisions, using QGIS v.3.12.3 (QGIS Development Team, 2020). Elevation data and geo-coordinates were taken in the field with a Garmin Etrex GPS. Also, if the species was unambiguously identifiable and the location was precisely recorded, data published on social media platforms are included. These citizen scientist records are highlighted in the text and the map.

Abbreviations for structures are as follows: FW: forewing, HW: hindwing, Pt: pterostigma, S1–10: abdominal segments one to ten. Terminology follows Garrison et al. (2010).

Results

Polythore albistriata new species (Figs 1–10)

Examined material

13 males, five females.

Holotype

CEUA 43176, male, from COLOMBIA, Huila Department, Estrecho del Magdalena, 1,400 m a.s.l., Lat. 1.9280280, Long. -76.2975000, collected with an aerial net in the forest, July 21, 2008, C. Bota leg.

Paratypes

Huila Department, San Agustin Municipality. One male, Parque Arqueológico Mesitas, 1,763 m a.s.l., Lat. 1.885500, Long. -76.294222, collected dead in a spider web over a rocky creek, July 20, 2008, C. Bota leg. (CEUA 81599). One male, at the outlet of the Naranjos River, 1,580 m a.s.l., Lat. 1.872000, Long. -76.299500, July 23, 2008, C. Bota leg. (CEUA 43180). Two males, from the type locality, September 2011, E. Realpe leg. One male, from the type locality, Sept. 2014, E. Realpe and M. Sánchez leg. (ANDES-E—DNA Voucher PM 1–2). One female, from the type locality, September 6, 2016, Y. Cano leg. (ANDES-E 22915). One female and two males, small creek on the route towards el Quebradón, 1,737 m a.s.l., Lat. 1.880111, Long. -76.309889, September 5–6, 2016. E. Realpe and Y. Cano leg. (ANDES-E). One male, El Pital Municipality, Vereda El Recreo, 2,020 m a.s.l., Lat. 2.283333, Long. -75.900000, edge of a gallery forest, July 7, 2013, C. Bota leg. (CEUA 74238). One male, Palestina Municipality, Natural National Park Cueva de los Guacharos, 2,300 m a.s.l., Lat. 1.6154240, Long. -76.1024390, on a trail, February 1, 2018, M. Ramírez and M. Fonseca leg. (ANDES-E 23380).

Caqueta Department. Three males, Florencia Municipality, near Puente Caraño, 652 m a.s.l., Lat. 1.736250, Long. -75.638250, October 11–14, 2014, C. Salazar leg. (CAUR-DNA Voucher VOD1-3).

Putumayo Department. Three females, Sibundoy Municipality, high sector of the road detour towards Mocoa-Sibundoy, 1,453 m a.s.l., Lat. 1.080122, Long. -76.731226, January 16, 2013, L. Pérez and J. Montes leg. (UARC-SAIA_0558-0560).

Localities recorded by citizen scientists in the Facebook group “Libélulas de los Andes Colombianos”. Huila Department: Municipality Baraya, La Troja township, 1,560 m a.s.l., Lat. 3.189512, Long. -74.938244, recorded by Michael Garcia. Municipality Palestina, Los Robles Reserve, 2,100 m a.s.l., Lat. 1.674236, Long. -76.147542, October 28, 2021, recorded by Joe Thompson. Neiva Municipality, 1,800 m a.s.l., Lat. 2.884386 Long. -75.066875, recorded by Arias H Dilmer.

Etymology

This name is a combination of two Latin roots that describe the wing color pattern of this species. The plural *albis* signifies “white”, and the singular feminine *striata* means a mark or a line.

Description of holotype

Head. Black except for the following pale yellow; two small, almost symmetric spots in the central part of the labrum, base of mandibles, genae, two small symmetric spots close to the lateral edge on the anteclypeus, and two transverse bands on the antefrons, starting under the scape and reaching the eye margin, two symmetric, ovoid spots on postfrons on each side of the median

ocellus and two symmetric, quadrangular spots at the base of the postocular lobes; labium cream on base and black on apex. Anterior part of head except the antefrons smooth and shiny; dorsal part of head including antefrons with some pruinescence and a sculptured (punctuated), iridescent surface. Rear of head black with two symmetric, elongated, cream spots in the middle.

Thorax. Prothorax black, except for two symmetric, pale yellow bands on each side of the pronotal medium lobe, propleural external sides with pale yellow, thin bands. Pronotal medium lobe divided by a medial sulcus, pronotal posterior lobe rounded and convex.

Pterothorax. Black except for the following pale yellow areas: a transversal, thin stripe in the dorsal portion of the mesepisternum with perpendicularly bent distal ends, ending close to the antehumeral suture; a transversal stripe starting on the mesinfraepisternum, crossing the mesepisternum just above the antehumeral stripe and then descending to the metepisternum in the distal ¼ of its length; a transverse stripe starting on the mesocoxa, crossing the base of the mesinfraepisternum, continuing over the interpleural suture to half of its length where it continues on the dorsal part of the metepisternum, bending down close to the antealar suture; a transverse stripe on the metapleural suture, bifurcated towards its proximal end with the upper branch starting on the metepisternum and the lower branch starting on the metacoxa; a transverse stripe in the ventral area of the metepimeron, bifurcating towards its distal end with the upper branch on the metepimeron and the lower branch on the venter of the metathorax (Fig. 1). Venter of pterothorax ocher, with a black spot shaped like a “W” (Fig. 2).

Legs. Black except for proximal spots in the external ½ of the mesofemur and ¾ of the metafemur; armature increasing towards the joint between the femora and the tibiae; the protibia apical third bears tibial combs on its external sides.

Wings. Veins brown, a transverse white band in the middle of the wings, extending to the Pt along the subcostal space (Figs 3A, 4A, 5A), see measurements of the wings and the white stripe in Table 1.

Abdomen. Black except for a pale yellow transverse lateral line that starts on S1 and ends in the middle of S3, with a small gap in the apical portion of S3, and a lateroapical spot on S4. Genital ligula with an inner fold proximal to flexure (Fig. 6F); in lateral view, two lateroapical subquadrate processes, with a short-curved flagellum on the apical corner, apex of the flagellum thickened (Figs 6A, C, E, F); in ventral (ectal) view, apex concave with two bluntly pointed processes (Figs 6A, C, E, F). Cercus black, twice as long as S10, with a ventral, curved, bluntly pointed process at the cercus’ midlength. Paraprocts undeveloped.

Measurements. Total length 5.4 cm, abdomen 4.4 cm.

Variation in male paratypes

In some of the paratypes the thin thoracic middorsal stripe is connected to the thin antehumeral stripe; the “W”-shaped spot on the venter of the pterothorax varies, with some forming a “U” and others being parenthesis-shaped “()” (Figs 2 A, B). Sometimes the abdomi-

nal lateroapical stripe extends to S4, sometimes there is a proximal spot on S5. The wing’s white stripe exhibits variation in width and in the starting and ending points within it (Table 1, Fig. 5A).

Measurements. Total length 5.2–5.5 cm, abdomen 4.2–4.5 cm (Fig. 3A), right wings see Table 1.

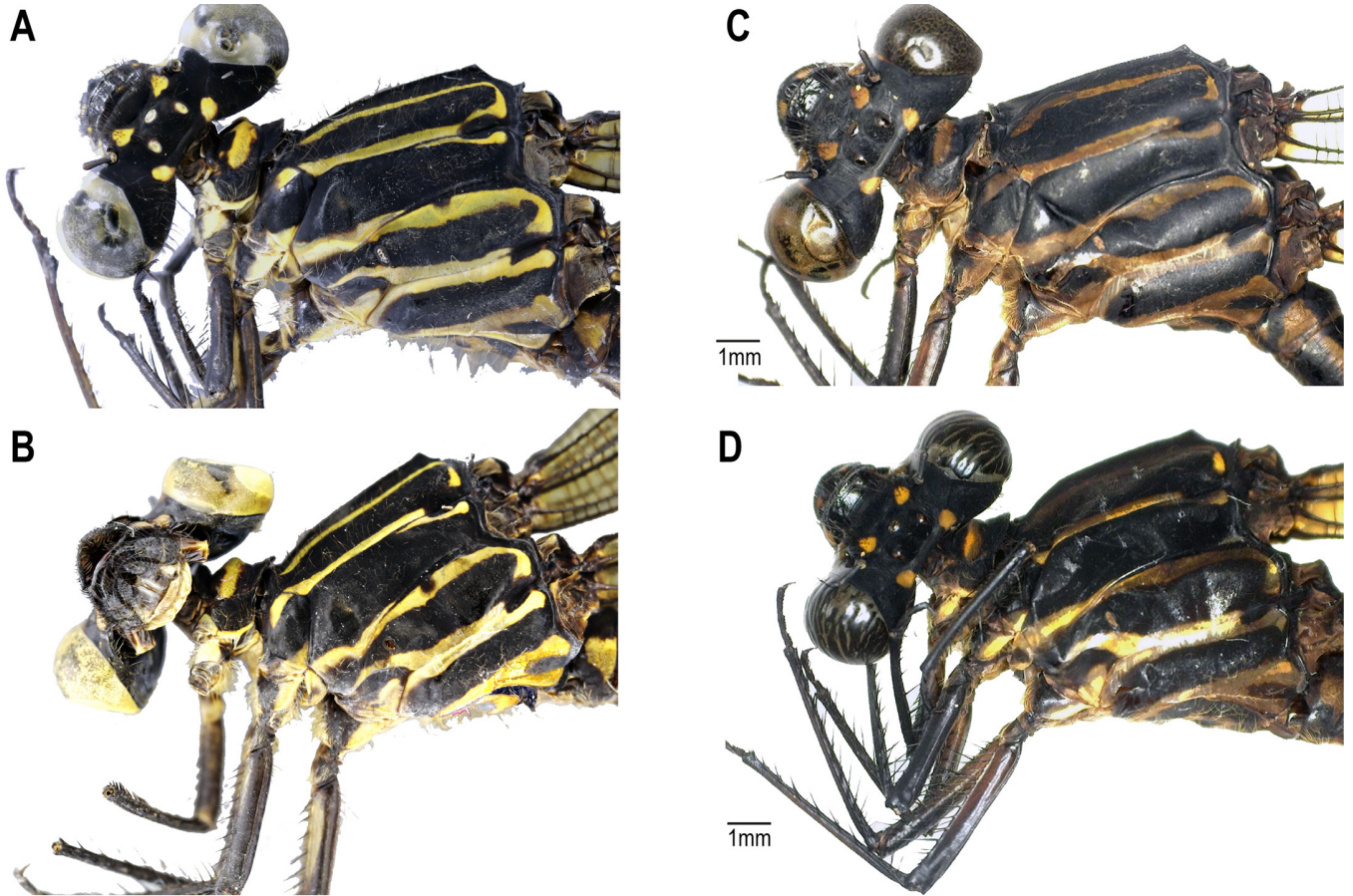


Figure 1. A, B – *Polythore albistriata* thorax in lateral view, male, holotype: A – head in dorsal view; B – head in ventral view. C, D – Variation of thorax in lateral, and head in dorsal view: C – allotype; D – male, paratype.

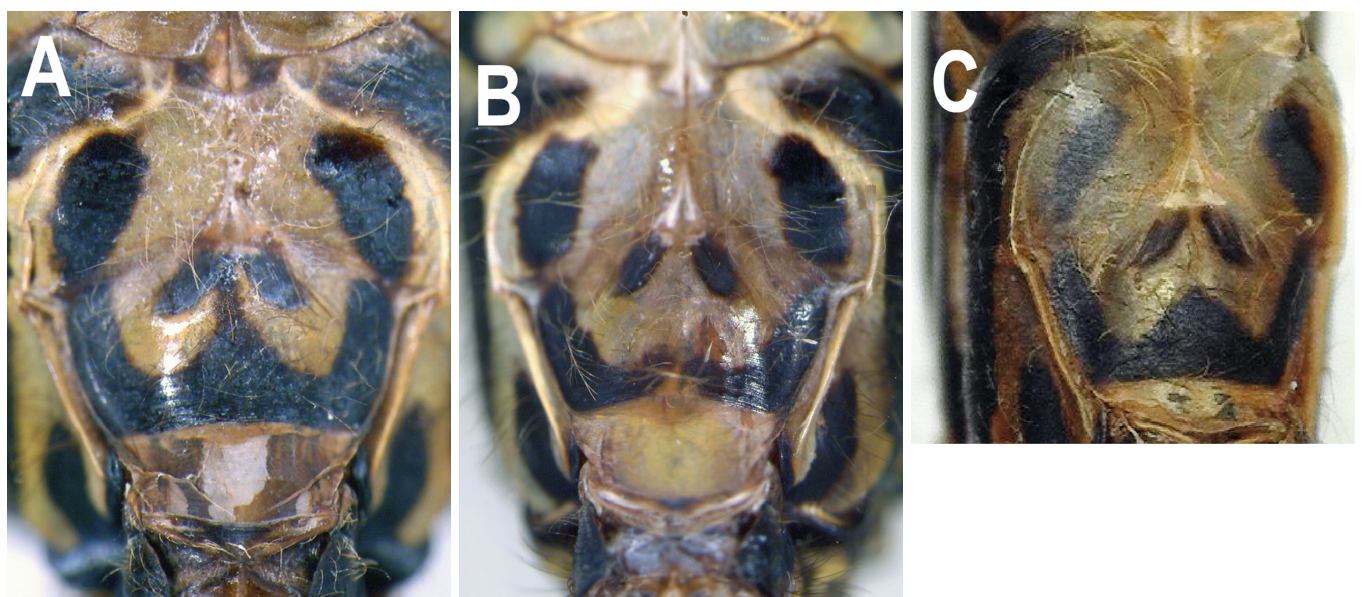


Figure 2. *Polythore albistriata*, ventral view of the thorax: A – male, holotype; B – male, paratype; C – female, paratype.

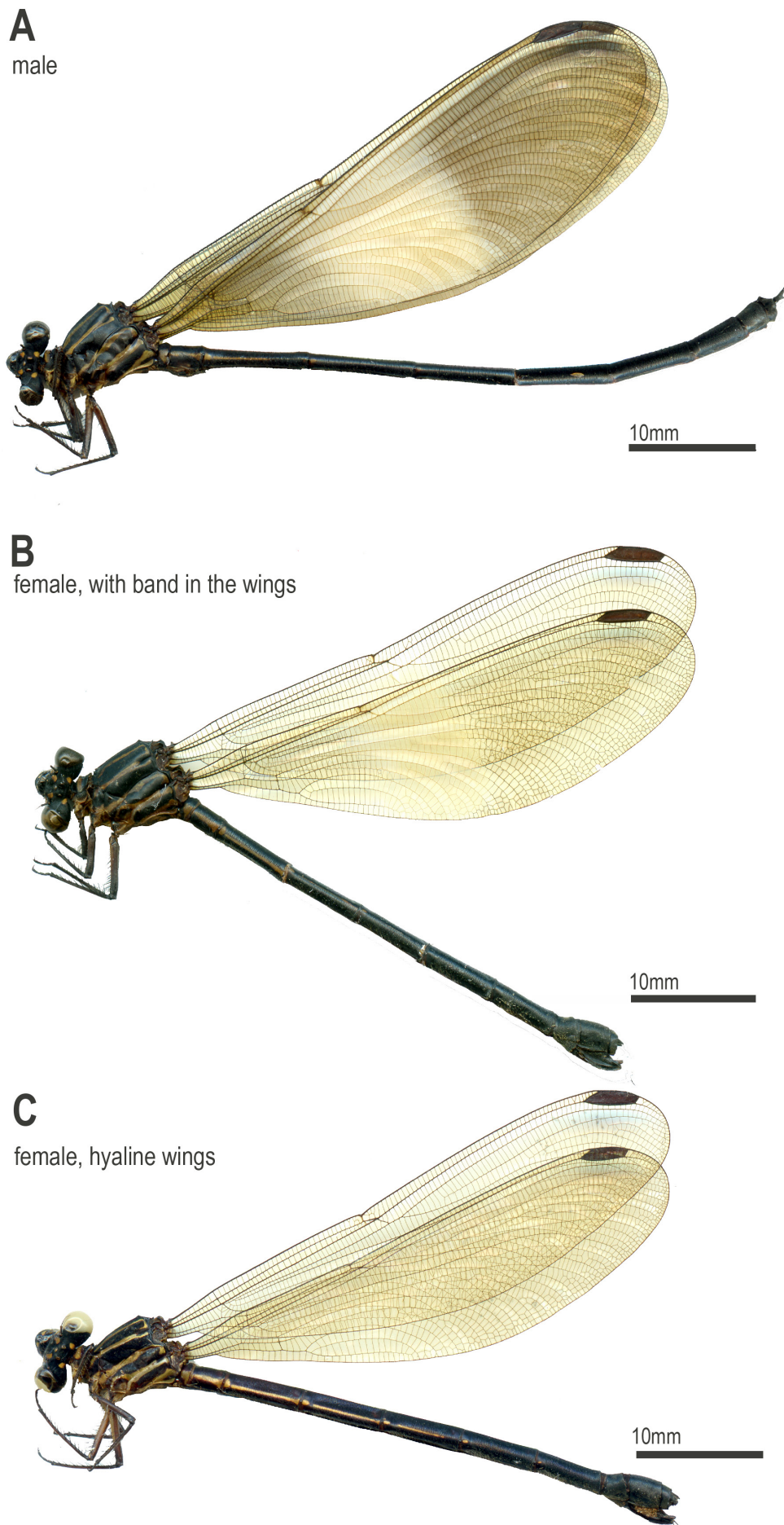


Figure 3. *Polythore albistriata*, lateral fully body scans: A – ANDES-E 22917, male, paratype; B – ANDES-E 22915, allotype; C – UARC-SAIA 0558, female, paratype with hyaline wings.

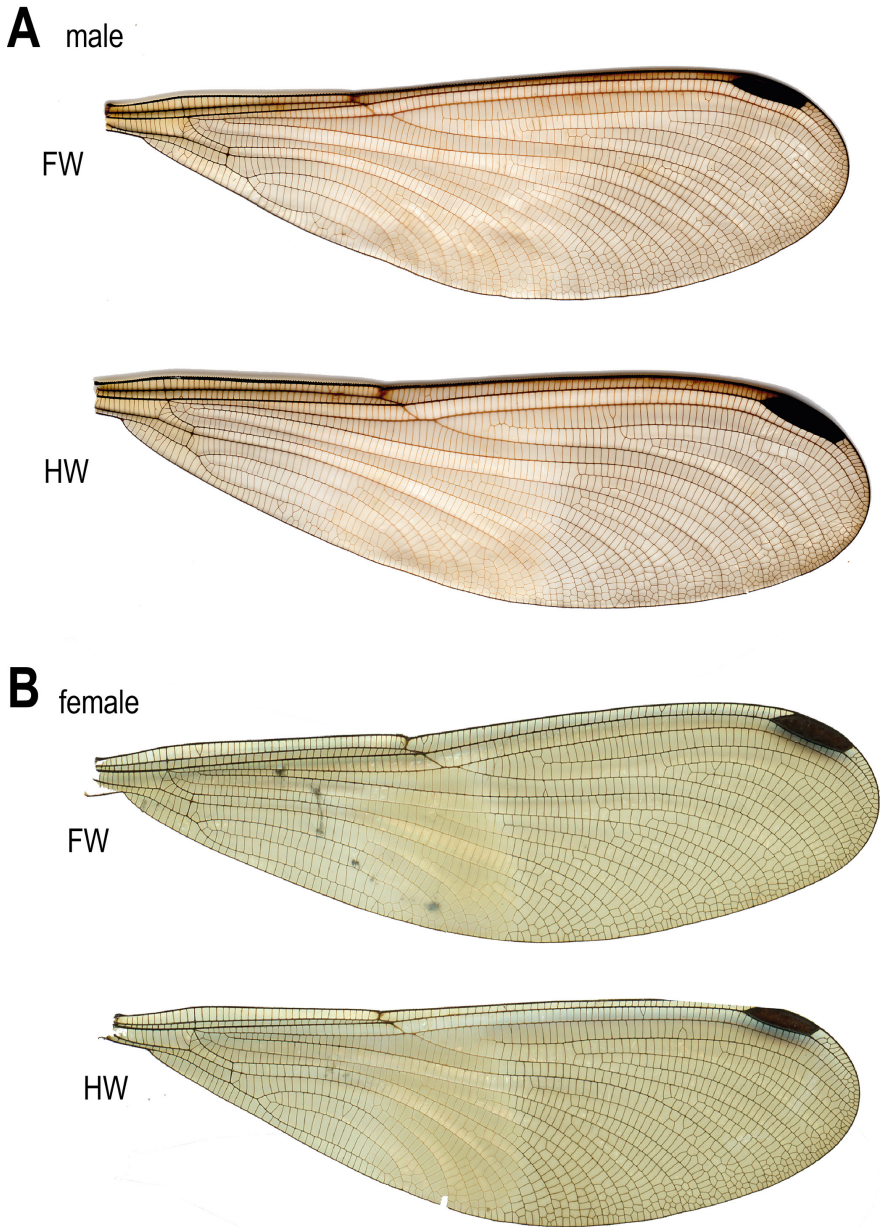


Figure 4. *Polythore albistriata*, high-resolution scans of excised right wings: A – CAUR-PM2, male, paratype; B – ANDES-E 22915, allotype.

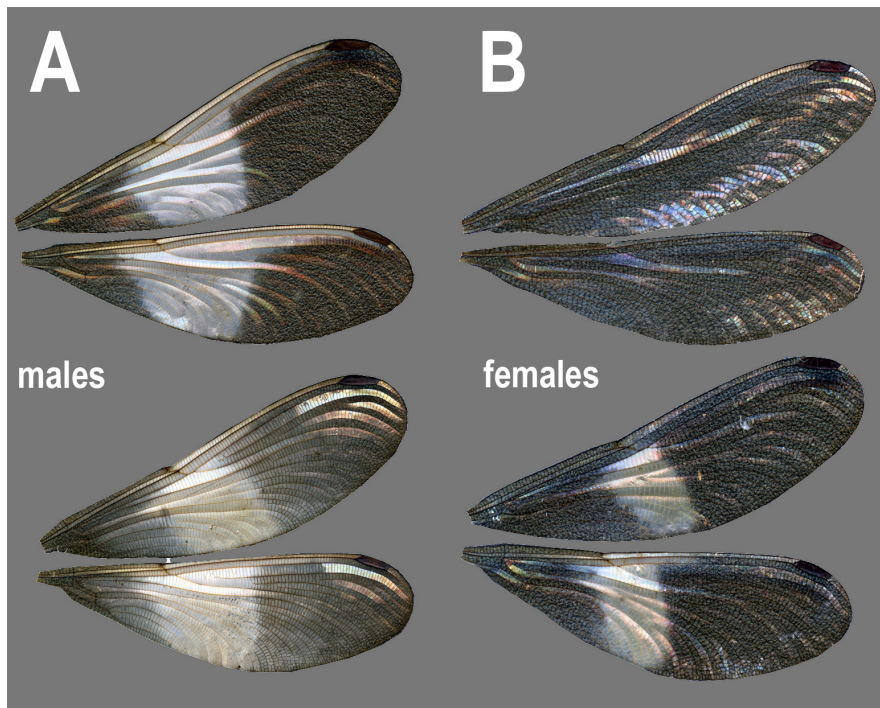


Figure 5. *Polythore albistriata*, variation of the white wing band against a contrasting background: A – males; B – females.

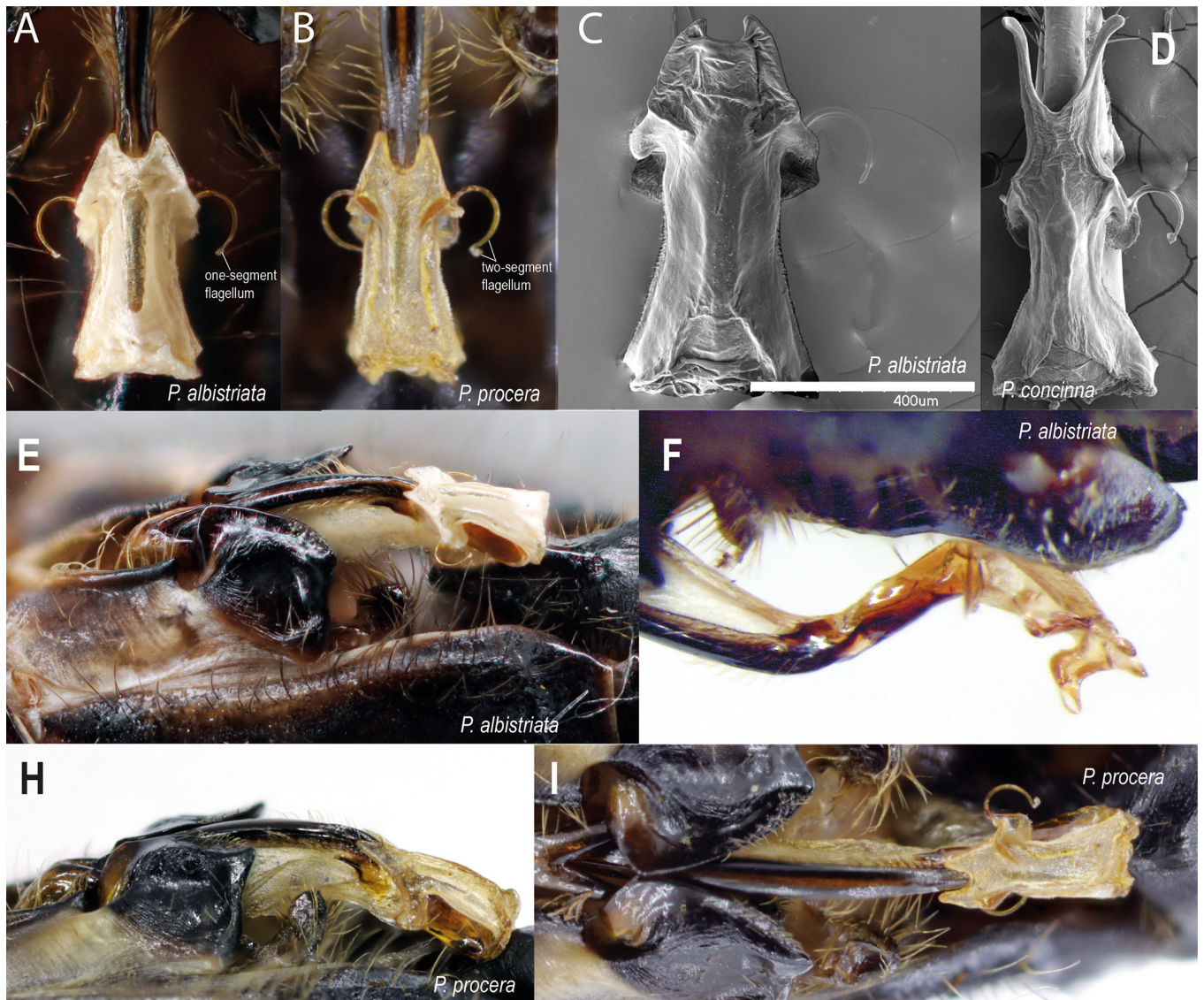


Figure 6. A–D: male genital ligula of closely related *Polythore* species in ventral (ectal) views: A – one-segment flagellum, *P. albistriata*, paratype; B – two-segment flagellum, *P. procera*; C – SEM, *P. albistriata*, paratype; D – SEM, *P. concinna*. E, F – *Polythore albistriata*, paratype: E – in lateroventral view; F – lateral view in open position. H, I – *P. procera*: H – in lateral view; I – in ventrolateral view. A and E by Camilo Flórez.

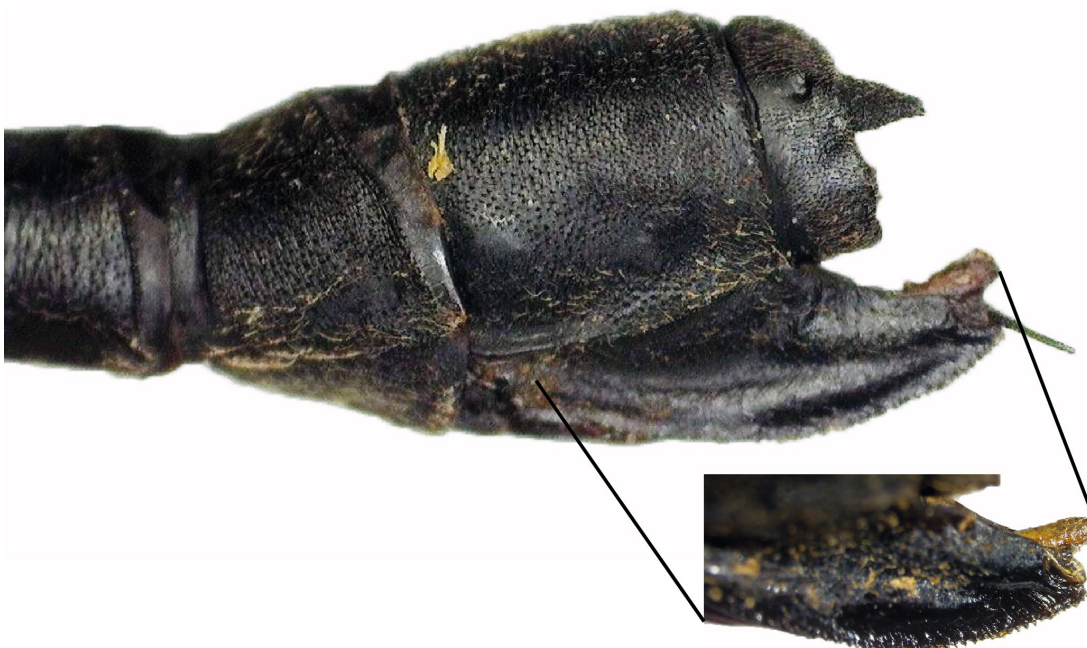


Figure 7. *Polythore albistriata*, ovipositor.

Table 1. Measurements of the right wing and width of the white stripe (mm). The measurements were taken at the widest point in the middle of each white stripe.

Specimen	HW	FW	Start point	End point	White stripe width	Sex
CEUA74238	40.62	42.87	11.64	26.59	14.96	M
CEUA43180	35.88	37.64	9.305	21.25	11.6	M
Holotype CEUA43176	37.32	40.516	11.21	25.86	14.65	M
ANDES23430	42.46	45.23	11.07	28.46	17.39	M
ANDES23380	36.52	37.76	7.29	21.74	14.45	M
ANDES22917	42.05	43.73	12.13	26.38	14.25	M
ANDES22914	41.28	43.31	10.18	28.01	17.83	M
	42.457	45.227	12.129	28.457	17.832	MAX
	35.88	37.638	7.287	21.245	11.6	Mean
	39.448	41.578	10.402	25.469	15.018	MIN
UARC-SAIA0560	35.43	37.76	9.39	16.17	6.77	F
ANDES22918	37.52	39.19	13.22	19.94	6.72	F
ANDES22915	35.99	38.08	12.84	20.01	7.16	F
UARC-SAIA0558	35.09	37.31	NA	NA	NA	F
UARC-SAIA0559	34.82	36.94	NA	NA	NA	F
	39.447	41.578	13.222	25.47	15.018	MAX
	34.816	36.935	7.287	16.171	6.718	Mean
	36.311	38.357	10.628	20.567	9.457	MIN

Allotype description and variation in female paratypes

Head. Anteclypeus similar to that of holotype, almost black.

Legs as in the holotype.

Thorax and abdomen. Body coloration as in the holotype (Figs 1B, C), but the lateral stripe on the abdomen starts in S1 and terminates in the distal quarter of S5. The ventral coloration of the thorax varies from as in the holotype to a “U”-shaped spot (Fig. 2C). Ovipositor almost black, with a series of small denticles ventrally. Ovipositor stylus tip is the most distal part of the body (Fig. 7).

Wings. The pale band is variable in size and some females have hyaline wings (Figs 3B, C, 4B, 5B).

Measurements. Total length 4.9–5.3 cm, abdomen 3.9–4 cm, right wings see Table 1.

Diagnosis

Bick & Bick (1985, 1986) proposed six groups within the genus based mostly on their wing colorations, but the

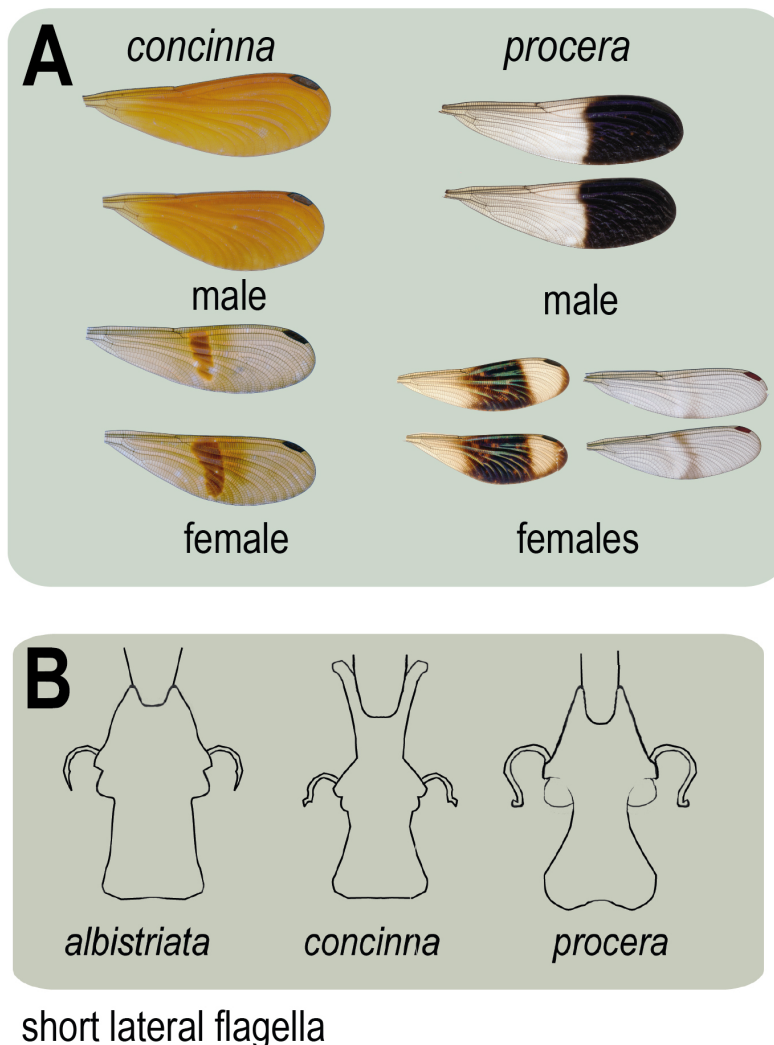


Figure 8. Diagnostic features of the two most closely related species, *Polythore procera* and *P. concinna*: A – Male and female wing color patterns; B – male genital ligula.

latest phylogenetic hypotheses show that these groups are not natural (Sánchez et al., 2018, 2020). They (Fig. 3) recovered *P. albistriata* sp. nov. (as *Polythore* sp. nov.) as closely related to *P. concinna* and *P. procera*. Males of these three species can be identified by their strongly contrasting wing coloration: *Polythore concinna* has smoky amber wings (Fig. 8A), *P. procera* has a white band in its middle third followed by a black band in the distal area (Fig. 8A), while *P. albistriata* has a white band in the middle third only (Figs 4, 5). Also, the shape of the genital ligula differs between these species: *Polythore concinna* has two very distinctive, enlarged processes on the apex, while *P. procera* and *P. albistriata* have similar ligula that can be differentiated by the apical lobes being more elongated in *P. procera* than in *P. albistriata*, and the lateral flagella in *P. procera* are two-segmented while in *P. albistriata* the apices of the flagella are thickened but not segmented into two (Figs 8B, 6). The females can be distinguished by the color of their wings: *Polythore concinna* has amber-brown bands, *P. procera* sometimes shows a white band, but it is always followed by a black band that varies in size (Fig. 8A), sometimes being very narrow and sometimes covering the entire apex of the wing, while *P. albistriata* may sport a white band or have totally hyaline wings (Figs 3, 8A). The species *P. williamsoni* and *P. mutata* have similar wing colorations as *P. albistriata*, with transverse white bands, but they differ in their genital ligula. The ligula of *P. mutata* has angulate anterolateral lobes, extremely short flagella, and wider lateroapical processes than *P. albistriata*, while *P. williamsoni* has ear-shaped two-segmented, long lateral flagella and wider lateroapical processes as well (Sánchez et al., 2018). In addition, the geographical distributions of these two species are completely disjunctive from *P. albistriata*; *P. mutata* is mainly distributed in the Amazon foothills, and *P. williamsoni* in the southeastern Andes of Peru.

DNA barcode identification

Two mitochondrial gene sequences that belong to *Polythore albistriata* sp. nov. were previously published on NCBI GenBank (see Table 2 for Accession Numbers). In addition, the Minimum Spanning Networks reveal that the haplotypes of *P. albistriata* are divergent from the most closely related species (Fig. 9). However, it is important to highlight that the *COI* is highly diverse across all sampled haplotypes for all the species we compared. There may be more mutations between two specimens of *P. albistriata* coming from different geographic populations (18 mutational steps) than between some of the specimens from other species that are geographically closer to them (e.g., 6, 28 or 38 mutational steps between *P. procera* vs. *P. albistriata* haplotypes; see Fig. 8), suggesting that this gene fragment might be affected by introgression across close geographic populations, possibly indicating recent gene flow or incomplete lineage sorting across these species. The *ND1*

Table 2. NCBI GenBank Accession numbers of the Cytochrome Oxidase I (*COI*) and NADH Dehydrogenase 1 (*ND1*) from Sánchez et al., (2018, 2020) and this study.

Specimen DNA voucher	Cytochrome Oxidase I (<i>COI</i>)	NADH Dehydrogenase 1 (<i>ND1</i>)
ANDES-E PM	MF109968	MF069489
CAUR-VOD1	MF109947	MF109921
CAUR-VOD2	NA	OM515597
CAUR-VOD3	NA	OM515598

locus seems more stable at species level, clearly merging all the haplotypes of *P. albistriata* and showing divergence from the haplotypes of *P. concinna*, *P. procera* and *P. derivata* (Fig. 9). Based on our results we suggest that for DNA identification in this genus *NADH* subunit I Dehydrogenase is a better barcode at species level, while Cytochrome Oxidase I can be a useful barcode for population-level signatures within a narrow geographical range.

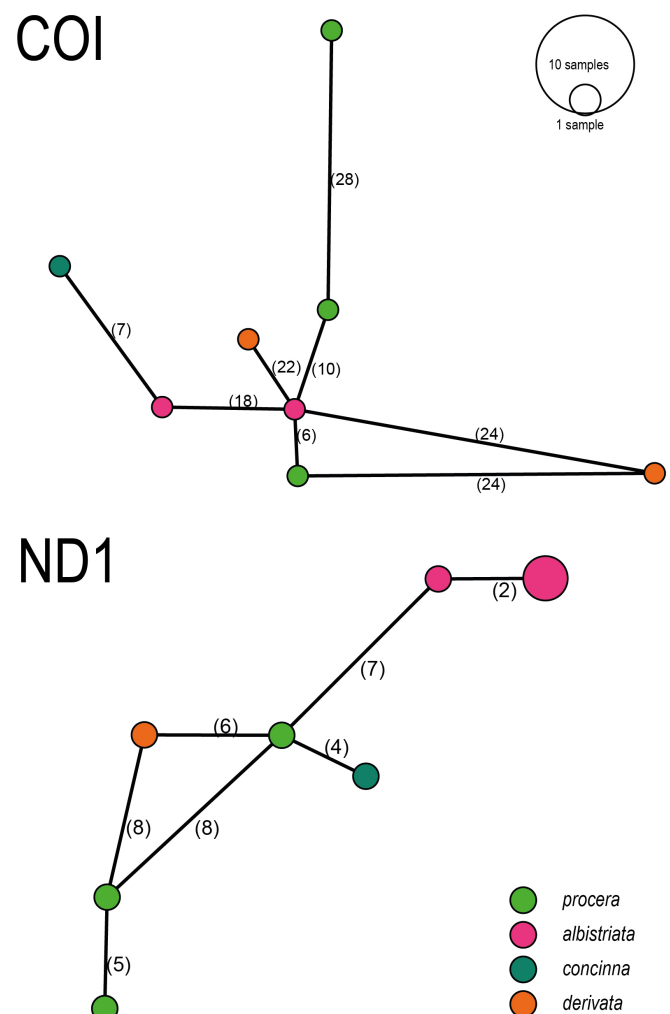


Figure 9. Minimum spanning haplotype networks for both barcode mitochondrial gene fragments—Cytochrome oxidase I (*COI*) and NADH subunit 1 dehydrogenase (*ND1*). Each circle represents a haplotype, and the numbers within the parentheses indicate the extent of mutations between them.

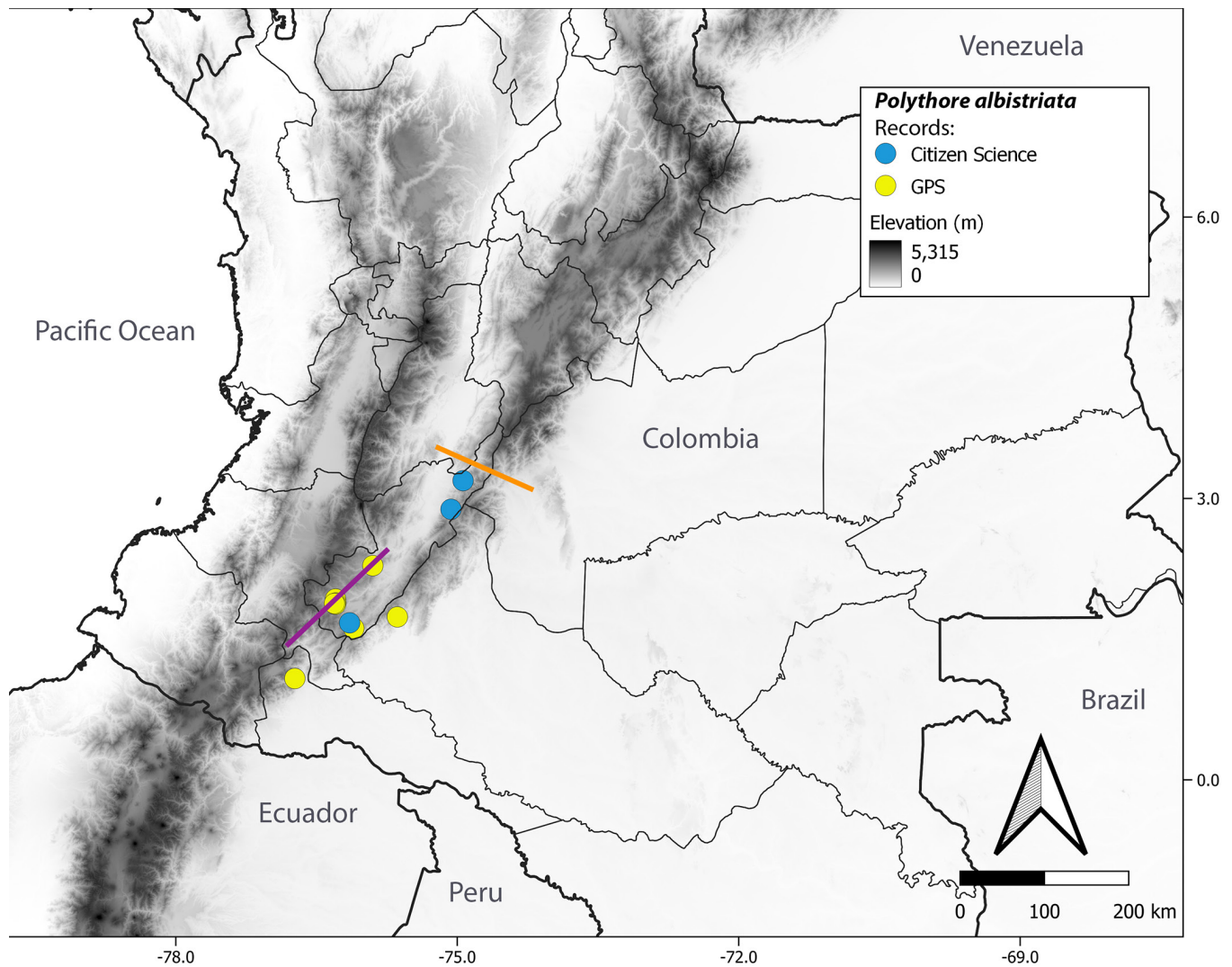


Figure 10. *Polythore albistriata* sp. nov. distribution map in southeastern Colombia near the Colombian Massif (purple) and the Eastern Cordillera Depression (orange). Collection-based (yellow) and citizen science data (blue).

Distribution

This new species occurs in the southeastern Colombian Andes between 1,500 and 2,300 m a.s.l.. It has been recorded mainly in the upper Magdalena Valley in both the Central and Eastern Cordilleras (Huila Department) and in some localities on the Amazon slope of the Eastern Cordillera (Caquetá and Putumayo Departments; Fig. 10). This distribution pattern concurs with the biogeographic limits determined by the Colombian Massif and the Las Cruces Pass found in a study on endemic Andean birds (Hazi et al., 2018).

Biology

This species inhabits rocky streams and small rivers in cloud forests. Individuals within the known populations are not very abundant.

Conservation status

The species is known from nine different locations within a relatively small area with significant pressures from

agriculture and urban expansion. Nevertheless, one of the locations lies inside the Colombian national park “Cueva de Los Guacharos” where the habitat for the species is protected.

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