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Discovery of a rare and striking new pierid butterfly from Panama (Lepidoptera: Pieridae)

SHINICHI NAKAHARA^{1,6}, JOHN R. MACDONALD², FRANCISCO DELGADO³ & PABLO SEBASTIÁN PADRÓN^{1,4,5,7}

¹McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, 32611, USA

²Mississippi Entomological Museum, Department of Biochemistry, Entomology and Plant Pathology, Mississippi State University, Mississippi, 39762, USA

³Centro Regional Universitario de Azuero, Universidad de Panamá, Chitre, Panama

⁴Laboratorio de Entomología, Escuela de Biología, Ecología y Gestión, Universidad del Azuay, Cuenca, Ecuador

⁵Instituto Nacional de Biodiversidad, Sección invertebrados, Quito, Ecuador.

⁶Departamento de Entomología, Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Apartado 14-0434 Lima-14, Peru

⁷Corresponding author. E-mail: sebastianpadronm@yahoo.com

Abstract

We here describe and name a distinctive new pierid species in the subfamily Pierinae, *Catantixia sibyllae* Nakahara, Padrón & MacDonald, **n. sp.** from western Panama. *Catantixia sibyllae* **n. sp.** is known from only two male specimens collected at two sites which are approximately 130 km apart in western Panama. This new species is the only species in the genus without markings in the median area of both surfaces of forewing and hindwing, and our molecular data suggest that the Peruvian species *C. lisa* Baumann & Reissinger, 1969 is its sister species.

Resumen

Se describe y nombra una nueva especie distintiva de pierido en la subfamilia Pierinae, *Catantixia sibyllae* Nakahara, Padrón & MacDonald, **n. sp.** del oeste de Panamá. *Catantixia sibyllae* **n. sp.** es conocida actualmente solo de dos especímenes machos colectados en dos sitios separados por 130 km en el oeste de Panamá. Esta nueva especie es la única dentro del género sin ninguna marca en el área media de las dos superficies de las alas anterior y posterior, nuestros datos moleculares sugieren que la especie peruana *C. lisa* Baumann & Reissinger, 1969 es la especie hermana de este nuevo taxón.

Key words: *Catantixia*, DNA barcodes, Pierinae, taxonomy

Introduction

The genus *Catantixia* Butler, 1870 is not only one of the largest pierid genera, but also one of the largest Andean butterfly radiations, with 97 species and over 250 subspecies recognized (Lamas, 2004; Lamas & Bollino, 2004; Bollino & Costa, 2007; Bollino, 2008; Bollino & Boyer, 2008; Radford & Willmott, 2013; Bollino & Padrón, 2016; Bollino & Costa, 2017). Members of *Catantixia* can be found throughout Central and South America, almost exclusively in mountainous areas. Most species are confined to restricted elevational bands (pers. obs.), and some species can be found above 3000 m. The greatest diversity is found at middle elevations between 1500–2000 m in the eastern Andes of Colombia, Ecuador, Peru and Bolivia, where over 30 species can be found in some areas (PSP, unpublished data). Although the majority of species are restricted to the tropical Andes, some *Catantixia* species occur outside of the Andean region, including the 8 species occurring exclusively in Central America, one of which

is the type species for the genus, *Euterpe nimbice* Boisduval, 1836, described from Veracruz, Mexico. Fabricius (1793) introduced the oldest available name for a species in the genus, *Papilio sisamnus* Fabricius, 1793, and since then the group has received attention from many taxonomists, resulting in many names that can be difficult to apply correctly. Genitalic characters are uninformative for species-level classification of the group (e.g., Lamas & Bollino, 2004), and some species are very rare in collections, making the taxonomic decisions for some taxa somewhat arbitrary. Consequently, the taxonomy of *Catantixia* has remained unstable, despite historical efforts to understand the group (e.g., Klots, 1933), with the cited species diversity of the genus varying from 76 to 94, depending on the author's taxonomic viewpoint (Eitschberger & Racheli, 1998; Lamas 2004). However, as a result of ongoing DNA study of *Catantixia* by PSP and collaborators, combined with recent studies on the group (Radford & Willmott, 2013; Bollino & Padrón, 2016; Bollino & Costa, 2017), we believe that the taxonomy of the group will soon reach a reasonable degree of stability, and generic relationships will be elucidated, enabling us to better understand this diverse Neotropical butterfly radiation.

During the course of studying *Catantixia* specimens in European and American museums, we encountered a single specimen of a distinctive undescribed pierid butterfly from Santa Fé, Panama in the Lepidoptera collection at the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM). Subsequently, JM collected the second known specimen of the species during a trip to western Panama in May, 2018. The DNA “barcode” sequence obtained from this second specimen influenced our decision to place it in the genus *Catantixia*. The purpose of this article, therefore, is to describe and name this highly distinctive new species of *Catantixia* from Panama, to contribute towards the aforementioned ongoing molecular work, in addition to improving our understanding of the true species richness of the group.

Materials and methods

Morphological work. The male abdomen of the holotype was soaked overnight in a 10% KOH solution, dissected and examined, and then stored in glycerine vial after study. The genitalia were stained with chlorazol black prior to examination to better visualize membranous structures. Drawings of the genitalia were done using a camera lucida attached to a Leica MZ 16 stereomicroscope at 50X and 25X magnification. The wing venation was drawn using the same microscope at 7.1X magnification. Adult images were taken with a Canon EOS REBEL T3i and Canon 100mm IS macro lens against a standard grey background for the holotype, while the paratype specimen was photographed with a Leica DFC digital camera mounted on a Leica Z16 Microscope where motorized z-stepping was used, and stacks were merged using Leica Application Suite V 4.1.0 with Montage Module. Images of head morphology and abdomen were taken by Helicon Focus 6.7.1 using Canon EOS 6D, subsequently stacked using Helicon Remote (ver. 3.8.7 W). We follow a modified version of Miller (1970: 46) for the terminology of wing venation (Fig. 2), Neild (1996) for wing area, and Nakahara *et al.* (2018) for genitalia.

Examined collections. *Catantixia* and other relevant taxa were studied in the following public and private collections in order to record distribution data and study morphology: **AMNH:** American Museum of Natural History, New York, USA; **INABIO:** Instituto Nacional de Biodiversidad, Quito, Ecuador (formerly MECN); **MABO:** Maurizio Bollino collection, Lecce, Italy; **MASI:** Mark Simon collection, Gainesville, USA; **MEM:** Mississippi Entomological Museum, Mississippi State University, Mississippi, USA; **MGCL:** McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA; **MNHU:** Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt Universität, Berlin, Germany; **MUSM:** Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; **MZUJ:** Zoological Museum, Jagellonian University, Krakow, Poland. **NHMUK:** Natural History Museum, London, UK (formerly BMNH); **PIBO:** Pierre Boyer collection, Le Puy-Sainte-Réparate, France; **PUCE:** Museo Pontificie Universidad Católica del Ecuador, Quito, Ecuador; **SMNS:** Staatliches Museum für Naturkunde, Stuttgart, Germany; **USNM:** National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Molecular work. We extracted genomic DNA from a single leg removed from the paratype male (LEP-68704) collected in May 2018. Qiagen's DNeasy Blood & Tissue Kit was used following the manufacturer's protocol with a final elution volume of 50 µl l. We amplified the DNA “barcode” (part of the mitochondrial gene *cytochrome oxidase I*) (Hebert *et al.*, 2003), using the primers LCO (forward, GGTCAACAAATCATAAAGATATTGG) and HCO (reverse, TAAACTTCAGGGTGACCAAAAATCA) (Folmer *et al.*, 1994). All PCR reactions were

conducted in a 20 µl volume comprising 2 µl DNA, 0.5 µl BSA (20 mg/mL), 6.7 ddH₂O, 0.4 µl of each primer (10 µl M), and 10 µl OneTaq® Hot Start Quick-Load® 2X Master Mix with Standard Buffer with the following reaction condition: 1 min at 94°C followed by 5 cycles of 30 s at 94°C, 40 s at 45°C, 1 min at 68°C, followed by 35 cycles of 30 s at 94°C, 40 s at 51°C, 1 min at 68°C, followed by 5 min at 68°C. PCR products were sequenced by Eurofins Genomics LLC (Louisville, USA) using the same primers as in the PCR.

COI sequences were manually edited using BioEdit (Hall, 1999) and were aligned using the ClustalW program in SeaView Version 4.7 (Gouy *et al.*, 2010). We obtained a 626 bp sequence for the paratype male (LEP-68704). This sequence was included in a dataset comprising 272 COI sequences representing approximately 80 *Catantacta* species, in addition to 25 COI sequences of related genera including *Archonias* Hübner, [1831], *Charonias* Röber, 1908, *Leodonta* Butler, 1870, *Eucheira* Westwood, 1834, *Melete* Swainson, [1831], *Neophasia* Behr, 1869, and *Pereute* Herrich-Schäffer, 1867. *Melete lycimnia* (Cramer, 1777) was used as outgroup to perform a Bayesian Inference (BI) analysis in order to infer the relationships of the new species. BI was performed in MrBayes 3.2 (Ronquist *et al.*, 2012), using the GTR+G+I model and Markov chain Monte Carlo simulations for 5,000,000 generations, with tree sampling every 100 generations (50,000 generations), until the standard deviation of split frequencies was below 0.01. First 12,500 (25%) generations were excluded as burn-in. FigTree (Drummond and Rambaut, 2007) was used to draw the tree topology. As this dataset forms an integral part of forthcoming molecular phylogenetic study on *Catantacta* (Padrón *et al.* in prep.), we present the relevant portion of the trimmed down tree and refer to the rest as “unpublished data”. GenBank accession number for *C. sibyllae* n. sp. paratype is MK036766 (DNA voucher: LEP-68704); for its sister species, *C. lisa* is MK036765 (DNA voucher: LEP-15611, this specimen is from Nueva Cajamarca, San Martín, Peru, in MZUJ). The genetic distance between these two species was calculated based on the Tamura-Nei model using Geneious (version 11.15).

Field work. From 2009 to present, a total of 22 trips to various parts of Panama were made by JM and colleagues. An estimated 378 days were spent in the field sampling a wide range of localities, including (among others): *Chiriquí*: Volcán, Santa Clara, Mt. Totumas, Bambito, Río San Catalina, Río San Felix, Concepción, Boquete, Fortuna; *Bocas del Toro*: Fortuna cabins; *Veraguas*: Santiago, Santa Catalina; *Herrera*: Ocu, Las Minas, Chepo; *Cocle*: El Valle de Antón, El Copé; *Panamá Oeste*: Cerro Campana; *Panamá*: Veracruz, Cerro Ancón, Cerro Azul, Cerro Jefe, Canopy Tower near Gamboa, Cerro Pelado, El Llano/Cartí Rd., Río Mamoni, Bayano; *Colón*: Gatun area, old Ft. Sherman area, Santa Rita Arriba, Gamboa Pipeline Rd., Achiote Rd., old Ft. Gulick area; *Darién*: Cerro Chucanti, Metetí, El Real, Parque Nacional de Darién/Rancho Frío, Parque Nacional de Darién/Rancho Plástico vic. of Cerro Pirre. Sampling took place during all months of the year except October and November, which tend to be very rainy.

Results

DNA barcodes placed *Catantacta sibyllae* n. sp. as sister to *C. lisa* Baumann & Reissinger, 1969 with a high support (posterior probability = 100; Fig. 1). The *Catantacta* clade, including the type species (*Euterpia nimbice*) and *C. lisa*, was recovered as a well-supported clade based on the much larger data set analyzed (unpublished data). The genetic distance between *C. sibyllae* n. sp. and *C. lisa* was calculated as 8.8% based on the COI barcode.

Catantacta sibyllae Nakahara, Padrón & MacDonald, new species

(Figs 2–6)

Diagnosis. *Catantacta sibyllae* n. sp. is easily distinguished from all *Catantacta* species by the median area of both surfaces of forewing and hindwing being entirely black with no markings. *Catantacta sibyllae* n. sp. is readily distinguished from its sister species, *C. lisa*, by the lack of broad white median band on both sides of dorsal forewing and hindwing. *Catantacta sibyllae* n. sp. is further distinguished from *C. lisa* by its rather prominent submarginal and marginal spots on both sides of dorsal forewing and hindwing. Also, these spots on the ventral surface appear more whitish in *C. sibyllae* n. sp., whereas more yellowish in *C. lisa*. The genitalia appear not to be informative in this group, thus diagnostic genitalic characters are not provided here.

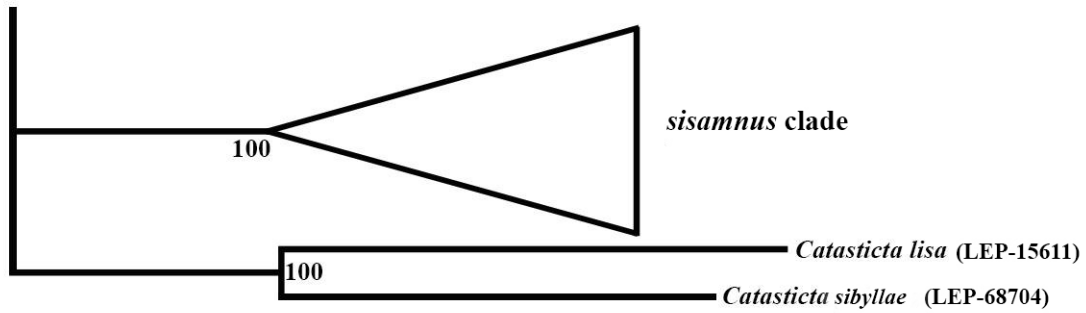


FIGURE 1. BI phylogenetic tree showing relationship of *Catasticta sibyllae* n. sp. (node support is posterior probability).

Description. MALE: Forewing length, holotype: 30 mm; paratype: 33 mm.

Head (Figs 5a, b): Eyes naked, with white scales at base; frons covered with brown scales, adorned with black long hair-like scales and white long hair-like scales, area around antennae base similarly with black long hair-like scales and white long hair-like scales; labial palpi roughly twice as long as eye depth, ventrally with white long hair-like scales and brown long hair-like scales, laterally with black scales with ventral and dorsal margin with white scales; antennae approximately half of forewing length, brown, ventrally scattered with white scales, with ca. 35 antennomeres ($n=1$, HT), distal 9–10 antennomeres composing rather prominent club.

Thorax: Dorsally brown with light coloured long hair-like scales; ventrally brown with light coloured long hair-like scales and brown scales.

Legs: Foreleg black, foretarsus longer than tibia, foretarsus and tibia scattered with whitish scales ventrally, in addition to multiple spines, dorsally stripe of white scales present, tibial spurs absent, femur slightly longer than tibia, ventrally brown, dorsally stripe of white scales present; midleg and hindleg not examined.

Wing venation (Fig. 4): Base of forewing subcostal vein not swollen; four forewing radius veins present; origin of M_1 not associated with discal cell; base of cubitus not swollen; forewing recurrent vein absent; middle portion of m_2 - m_3 portion reduced; hindwing humeral vein developed.

Wing shape (Figs 2, 4): Forewing subtriangular, appearing somewhat elongated with costal margin convex in angular manner and concave outer margin, inner margin rather straight; hindwing rounded, appearing slightly elongated, costal margin curved, angles inwards at base, outer margin curved, inner margin slightly concave near tornus, anal lobe almost straight, slightly round.

Wing pattern (Fig. 2): *Dorsal Forewing:* ground colour black; white submarginal spots slightly suffused with black scales, present in all cells from R_2+R_3 to 2A, spot in cell $R_2+R_3-R_4$ elongated, appearing more as costal streak, spot in cell R_4-R_5 small, similar in appearance, spots in cells from R_4 to Cu_2 subsquared, except for streak-like spot in cell M_2-M_3 , spot in cell Cu_2-2A appearing somewhat doubled; marginal spots slightly suffused with black scales present in all cells from R_4 to 2A, those in cells from R_4 to Cu_1 teardrop-like with black suffusion visible on basal side, spot in cell Cu_1-Cu_2 squared, spot in cell Cu_2-2A smallest, distance between submarginal spots in corresponding cells closer towards posterior end as outer margin traverses towards body; fringe blackish; *Dorsal hindwing:* ground colour similar to forewing; white submarginal spots slightly suffused with black scales, present in all cells from $Sc+R$ to 2A, spots in cells Cu_1-Cu_2 and Cu_2-2A smaller, in particular, spot in cell Cu_2-2A appearing as white hint; marginal spots present in all cells from $Sc-R_1$ to 2A, spot in $Sc+R_1-R_5$ elongated and streak-like, spots in cells $Rs-M_1$, M_1-M_2 and M_2-M_3 rather trapezoidal, spots in cells M_3-Cu_1 , Cu_1-Cu_2 and Cu_2-2A rather rectangular; fringe black; *Ventral forewing:* ground colour and general wing pattern similar to dorsal forewing except as follows: ground colour slightly paler; marginal whitish spots appearing larger; submarginal whitish spots appearing larger and more pointier towards base, submarginal spot in cell Cu_2-2A appearing as two spots; *Ventral hindwing:* ground colour and general wing pattern similar to dorsal hindwing except as follows: ground colour slightly paler; yellow costal streak at base of costal cell, just distal of humeral vein; reddish patch just basal of humeral vein above subcostal vein; yellow patch at base of discal cell; reddish patch at base of anal cell; submarginal spots smaller and slightly yellow; marginal spots somewhat creamy, additional spot present in cell 2A-3A.

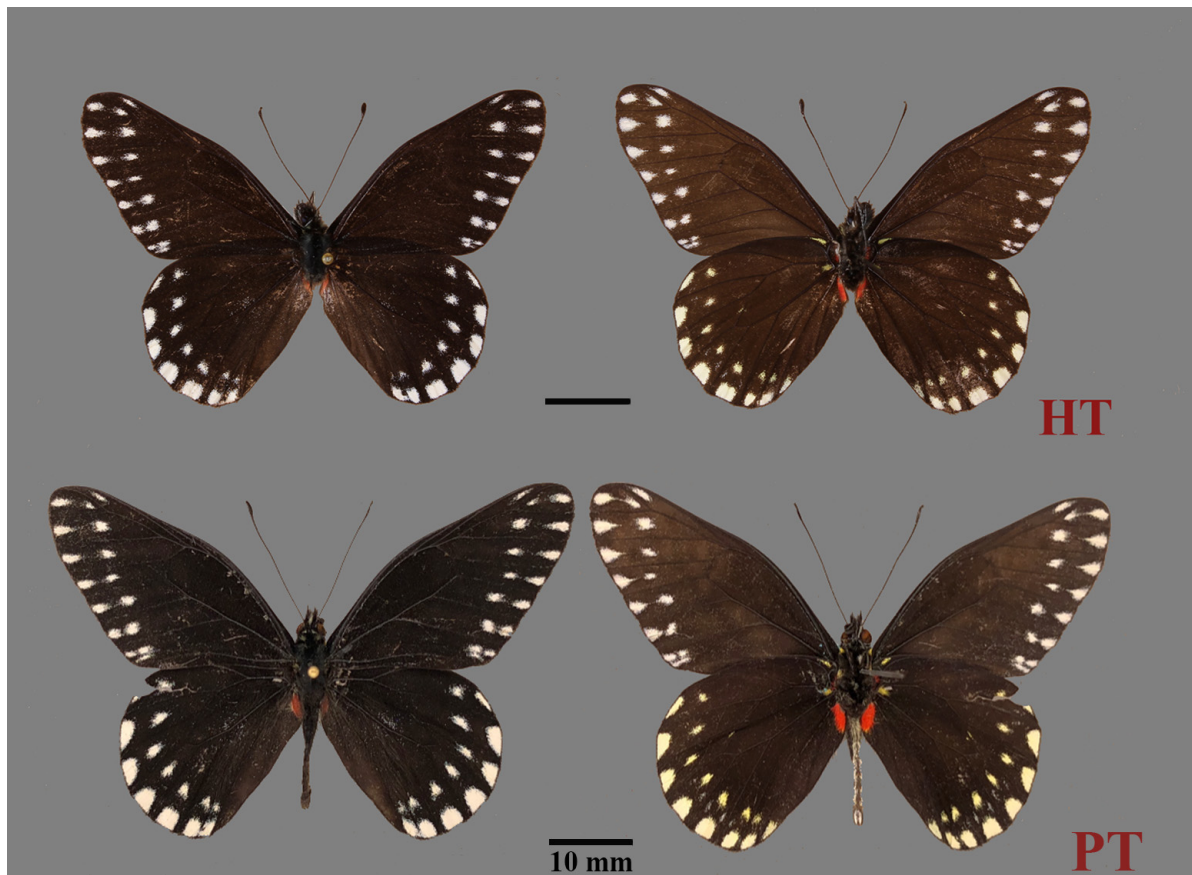


FIGURE 2. *Catastieta sibyllae* n. sp. holotype male (dorsal on left, ventral on right); paratype male (dorsal on left, ventral on right).

Abdomen (Fig. 5c): Dorsally and laterally black, ventrally with brown scales, white scales and white long hair-like scales, extending along ventral margin of valva; eighth sternite appearing as small plate.

Genitalia (Fig. 3): Tegumen somewhat trapezoidal in lateral view; uncus shorter than tegumen in lateral view with convexed ventral margin, ending in a blunt point, with tuft of hair-like setae visible at base; ventral surface of anal tube weakly sclerotized (this structure is referred to as “gnathos” in Radford & Willmott (2013)); combination of ventral arms of tegumen and dorsal arms of saccus rather straight, middle portion broadens in lateral view; appendices angulares apparently absent; saccus short, broad and straight, similar to uncus in length; juxta appearing as tall plate in posterior view, narrow long stripe in lateral view; valva round, overall appearing somewhat as bean-shaped in lateral view, anterior margin rounded and positioned above uncus, ventral margin curved with hair-like setae present along margin, dorsal margin basal of costa concave, costa pointy, terminating in blunt end; phallus curved, phallobase about one-fourth of phallus in length with trapezoidal winglet ventrally, ductus ejaculatorius visible, manica covering more than half of aedeagus, vesica not examined as phallus not separated.

FEMALE: Unknown or unrecognized.

Types. Holotype: Male with following labels written verbatim: PANAMÁ: Veraguas Prov. Santa Fe 800m 13-IX-1981 Gordon B. Small// Genitalic vial SN-17-177 S. Nakahara// USNM ENT 00804432//FOTO// (USNM).

Paratype: Male with following labels written verbatim: PANAMA: Bocas del Toro; vic. Fortuna Cabins; ca. 910m N 08° 46' 40.86" W 082° 11' 2502" May 17, 2018 John R. MacDonald// DNA voucher LEP-68704// (MEM).

Etymology. The specific epithet is in recognition of Maria Sibylla Merian, a European naturalist and entomologist who embarked on a voyage to South America in the late seventeenth century and conducted empirical research focused on butterflies. Her research, in the form of numerous paintings of insects, served as a foundation for the scientific study of insects, now known as “entomology”. This specific epithet is regarded as a latinized feminine noun in the genitive case.

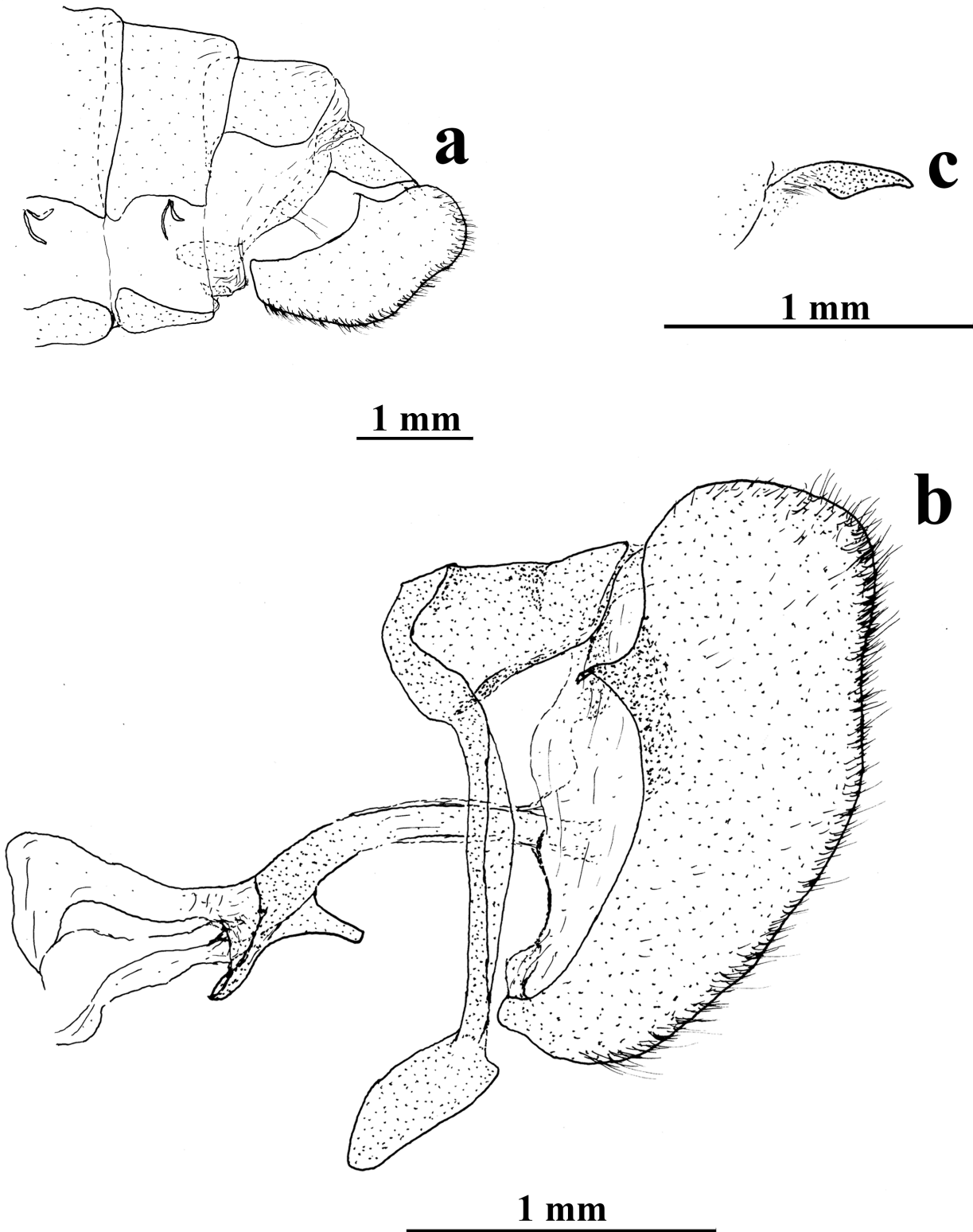


FIGURE 3. *Catasticta sibyllae* n. sp. male genitalia, dissection based on the holotype (genitalic vial: SN-17-177): a) lateral view of genitalia *in situ* at posterior end of abdomen; b) genitalia in lateral view; c) lateral view of uncus, with valva removed.

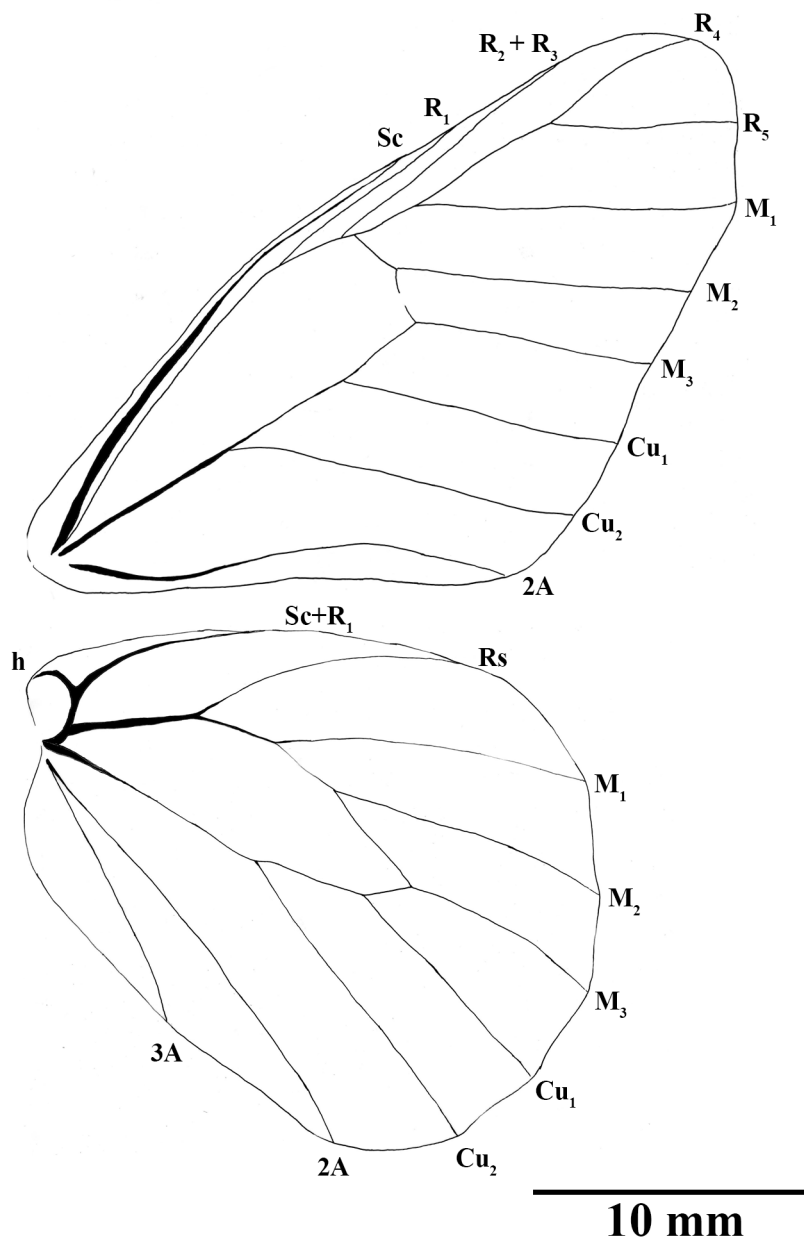


FIGURE 4. *Catasticta sibyllae* n. sp. wing venation based on the holotype.

Distribution and habitat (Fig. 6). This species is known to date from two sites situated in western Panama, namely Bosque Reserva Palo Seco, Bocas del Toro (08° 46' 40.86" N 082° 11' 25.02" W) and Santa Fé, Veraguas (precise coordinates unavailable, but the town of Santa Fe lies at 08° 29' 59.99" N 81° 03' 60.00" W). Both sites are somewhat on the border of two vegetation types, sub-montane rainforest and montane rainforest, near the continental divide (Blandin, 2017).

Biology. JM observed the paratype male nectaring on flowers of *Sommeria donnell-smithii* (Rubiaceae) at about 5–6 m off the ground. Lekking behavior, often seen in this genus, was not observed with this individual. The approximate time of collection was 11:00 am. Other butterflies nectaring or perching on this tree were *Glutophrissa drusilla* (Cramer, [1777]) (Pieridae), *Enantia albania* (Bates, 1864) (Pieridae), *Panhiades ochus* (Godman & Salvin, 1887) (Lycaenidae), *Cupathecla cupentus* (Stoll, 1781) (Lycaenidae), *Necyria duellona beltiana* Hewitson, 1870 (Riodinidae), *Adelpha leucophthalma leucophthalma* (Latreille, [1809]) (Nymphalidae), *Marpesia merops* (Doyère, [1840]) (Nymphalidae). Other species observed in the vicinity included: *Catasticta*

sisamnus smalli Eitschberger & Racheli, 1998 (Pieridae), *Archonias brassolis approximata* (Butler, 1873) (Pieridae), *Thestius epopea* (Hewitson, 1870) (Lycaenidae), *Morpho helenor veragua* LeMoult & Réal, 1962 (Nymphalidae), and *Oressinoma typhla* Doubleday, [1849] (Nymphalidae).



FIGURE 5. *Catasticta sibyllae* n. sp. morphology, all images based on the holotype: a) head in lateral view; b) antennal club in dorsal view; c) abdomen in ventral view.

Discussion

The discovery of a new pierid species as distinctive as *C. sibyllae* n. sp. is remarkable, especially considering that this species is known from an area of low endemism for the group. Given the diversity of the genus in the tropical Andes, in addition to high endemism in that region, new Andean *Catasticta* taxa have been fairly regularly discovered even during the past decade (Bollino, 2008; Radford & Willmott, 2013; Bollino & Padrón, 2016; Bollino & Costa, 2017). However, the last species-level taxon of *Catasticta* discovered outside of the Andean region dates back more than 30 years, when *C. oaxaca* Beutelspacher, 1984 was named and described from Oaxaca, Mexico. Despite our examination of some of the largest repositories for *Catasticta* in Europe and the Americas (see Methods), we found no additional specimens of *C. sibyllae* n. sp..

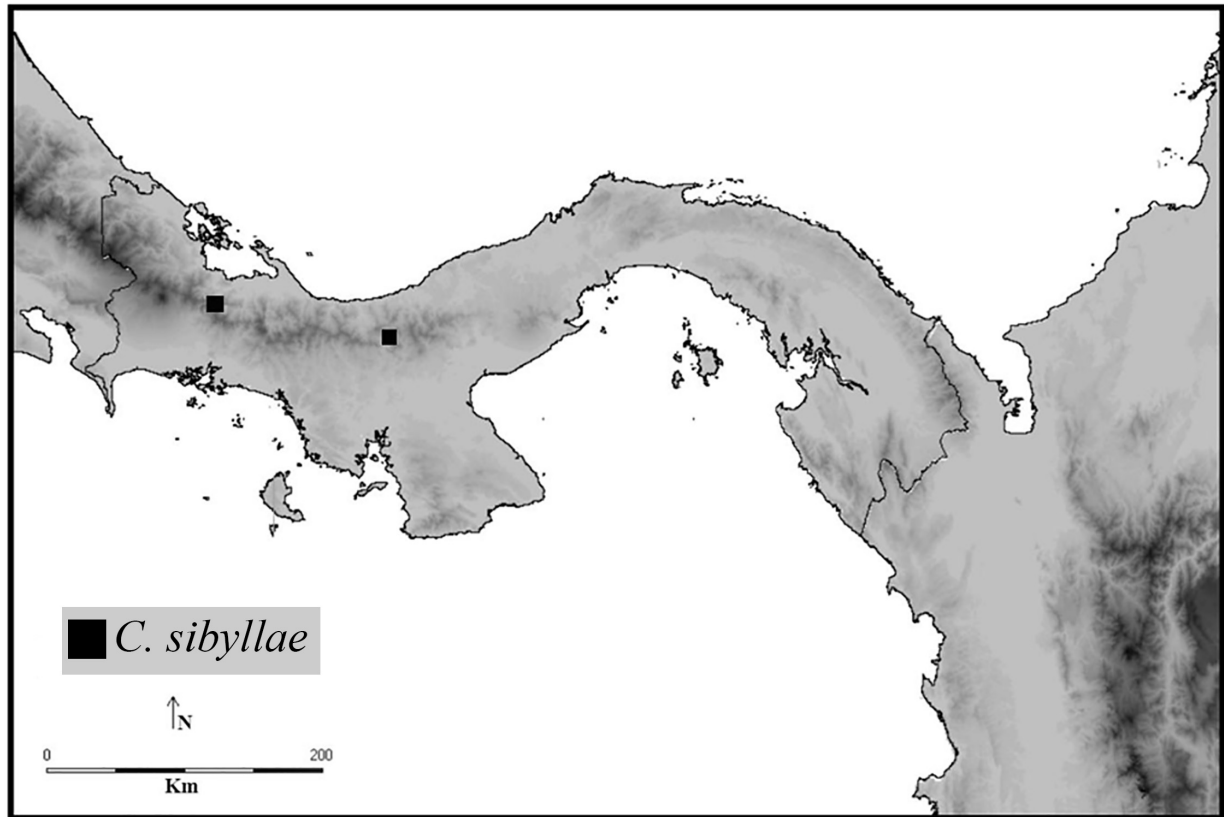


FIGURE 6. Distribution records for *Catasticta sibyllae* n. sp., Atlantic slope habitat image of Fortuna forest, where the paratype male was found (photo credit: Thomas J. Riley).

The discovery of the paratype male of *Catantixia sibyllae* **n. sp.** was made during an ongoing field project by JM and colleagues aimed at improving the knowledge of Panamanian butterflies. Although many of the areas surveyed in Panama are at elevations too low for the genus *Catantixia*, sufficient field work has been done at higher elevations to record all previously described species of *Catantixia* known from Panama, except *C. cerberus* Godman & Salvin, 1889. Typically, when found, *Catantixia* species tend to occur in numbers, although *C. sibyllae* **n. sp.** was encountered only as a solitary individual. A total of five trips corresponding to 20 days in the field have been spent at the Fortuna cabins location, where the paratype male was collected. Surveys at this location were spread over 5 months (January, February, May, July, and August, with 4 days of survey per each month) and 2 years. This fact, combined with the lack of specimens in existing collections, suggests that *C. sibyllae* **n. sp.** is a very rare species.

Both known locations of *C. sibyllae* **n. sp.** are near the continental divide, an area that tends to receive abundant moisture from rain and fog (Bagley & Johnson, 2014). Perhaps the foodplant (currently unknown) is restricted to this habitat, thus helping to explain the apparent narrow geographical range of this species. Interestingly, *Morpho helenor veragua*, another restricted-range taxon, is also found at both locations where *C. sibyllae* **n. sp.** occurs (Blandin, 2017). Whether this association is coincidental, or the two butterflies are linked through past biogeographical events occurring in this region remains unknown. The inference of *C. lisa* as the sister species is also worth noting, since this finding suggests speciation occurring horizontally without change in altitudinal, a diversification pattern indicated in several other studies on Andean radiations (e.g., Patton & Smith, 1992). *Catantixia lisa* is known from around 1000 m elevation in central to northern Peru, and this disjunct distribution perhaps warrants further investigation from a biogeographical perspective, in relation to other species possessing similarly disjunct distributions (e.g., *Euselasia andreae* Hall *et al.*, 1998 (Riodinidae); *Splendeptychia salvini* (Butler, 1867) (Nymphalidae)).

Clearly, any additional distributional and/or observational records for *C. sibyllae* **n. sp.** would be extremely valuable to improve our understanding of its distribution, biology, and biogeography, as well as to assess the conservation status of this rare and unique species.

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