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Cover Art: Male nurse frog of *Andinobates opisthomelas* transporting a single tadpole at the newly localized type locality in the rural district (*vereda*) of Santa Inés, Valdivia, Antioquia, Colombia. Photo: Juan Pablo Durango.

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On the Type Locality of Andinobates opisthomelas (Boulenger, 1899) and Colostethus pratti (Boulenger, 1899), with a Redescription of A. opisthomelas Based on Newly Collected Topotypes

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South American Journal of Herpetology, 38, 2026, 45–60

On the type locality of *Andinobates opisthomelas* (Boulenger, 1899) and *Colostethus pratti* (Boulenger, 1899), with a redescription of *A. opisthomelas* based on newly collected topotypes

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Abstract. *Andinobates opisthomelas* (Boulenger, 1899) and *Colostethus pratti* (Boulenger, 1899) were both described on the basis of specimens from "Santa Ines, N. of Medellín, Republic of Colombia; altitude 3800 feet [1,160 m]." Unfortunately, as Colombia's political divisions were still taking form, this information is inadequate to ensure a clear understanding of the type locality. Over time, the taxonomy of these species advanced without identifying the precise location of the type locality. In addition, several independent evolutionary lineages closely related to *A. opisthomelas* have been identified and described as new species. Thus, we aim to clarify the exact geographical location of the type locality of *A. opisthomelas* and redescribe the species on the basis of newly collected topotypic material. Based on historical records, contemporaneous taxonomic literature, and administrative cartography, we identify the rural district (*vereda*) of Santa Inés, in the municipality of Valdivia, department of Antioquia, as the type locality of *A. opisthomelas* and *C. pratti. Andinobates opisthomelas* sensu stricto is readily diagnosed from all congeners on the basis of its dorsal skin texture, dorsal coloration, toe fringes, and advertisement call, and morphometric comparisons with populations across the northwestern Andes reveal significant differentiation. The correct voucher number of the lectotype of *A. opisthomelas* is BMNH 1947.2.15.22, not BMNH 1947.2.15.29 as reported previously.

Keywords. Acoustics; Integrative taxonomy; Morphology; Tadpoles; Geographical history.

INTRODUCTION

Since its proposal in 2011, the Andean poison frog genus *Andinobates* Twomey et al. in Brown et al., 2011 has undergone significant taxonomic and systematic progress (Amézquita et al., 2013; Batista et al., 2014; Márquez et al., 2017; Chaves-Portilla et al., 2021). However, while new species continue to be described, a cornerstone problem remains unresolved: the location of the type locality of *Andinobates opisthomelas* (Boulenger, 1899)—the oldest available name and one of the most widely distributed species of the genus—is unclear, which inhibits taxonomists' ability to properly assign the name and determine which, if any, lineages must be recognized as new species. At the time *A. opisthomelas* was named, species descriptions were typically brief, comprising at most two or three paragraphs. As knowledge of biodiversity has grown and cryptic diversity has emerged as a common scenario in dendrobatid frogs (e.g. Posso-Terranova and Andrés, 2018), the number of characters and level of detail required to differentiate species also grew (Braby et al., 2024; Ruedas et al., 2025). For example, different sets of characters and new methodological approaches were used to describe *A. cassidyhornae* Amézquita et al., 2013 and *A. victimatus* Márquez et al., 2017, both of which were previously considered to be populations of *A. opisthomelas.* Neither study reviewed the type material of *A. opisthomelas* or included specimens from the type locality, despite presenting morphological and molecular evidence suggesting that *Andinobates* represents a complex of cryptic similar species. In order to determine if additional, unnamed species of *Andinobates* exist, it is imperative to first clarify the identity of *A. opistethomelas* sensu stricto, for which it is necessary to precisely identify the type locality. The problem is compounded by the fact that the same type locality is shared with *Colostethus pratti* (Boulenger, 1899), which also comprises a complex of similar species (Grant et al., 2006, 2017; Ibáñez D. et al., 2017).

The type specimens of *Andinobates opisthomelas* and *Colostethus pratti* were collected by Antwerp Edgar Pratt in "Santa Ines, N. of Medellín, Republic of Colombia, altitude 3800 feet [1,160 m]" (Boulenger, 1899:275). Identifying the precise location of Pratt's "Santa Ines" has proven challenging, as Colombia's political and administrative landscape was still taking shape at the time of Pratt's collections, making it difficult to unambiguously match this historical toponym to any present-day administrative unit.

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To enable taxonomic progress, we used historical records to locate the type locality of *Andinobates opisthomelas* and *Colostethus pratti* as precisely as possible. We then provide a detailed description of the adult and larval morphology of *A. opisthomelas* on the basis of the type series and newly collected topotypical material. Additionally, to estimate morphological variation and distribution of *A. opisthomelas,* we compared morphometric data from topotypes with specimens from other localities across the northwestern Andes.

MATERIALS AND METHODS

Type locality

To identify the geographic location of Santa Ines, we reconstructed Pratt’s itinerary through Colombia using contemporaneous taxonomic literature and traced the historical origin and administrative evolution of place names north of Medellín. We reviewed the localities of specimens of other taxa (i.e., plants, mammals, butterflies, and snakes) collected by Pratt and housed in natural history collections. We also consulted the Royal Geographical Society (RGS), of which Pratt was a member, and the Natural History Museum in London, where the specimens were deposited. Finally, we conducted an extensive search focused in the name “Santa Inés” in historical and contemporary maps (most from international and Colombian geoportals, but also printed maps), online museum databases, historical catalogues (Gabriel, 1932), gazetteers (Paynter, 1997; Gardner, 2008; Falling Rain Software, 2017), and official documents on the origin of geopolitical and administrative units to the north of Medellín.

Fieldwork

Field surveys were conducted at two localities currently named Santa Inés, both located north of Medellín. The first locality corresponds to a small region known as “Santa Inés” (but not a formal administrative unit) in the municipality of Guadalupe, Antioquia (coordinates: 6.88305, -75.18555), 800–1,000 m above sea level (masl), surveyed once on 15–18 November 2021. The second locality corresponds to the rural district (*vereda*) of Santa Inés in the municipality of Valdivia, department of Antioquia (7.10812, -75.47118), 300–1,700 masl, surveyed on three occasions: 17–20 February 2023, 12–16 June 12–16 2023, and 14–16 February 2024.

We conducted surveys along linear transects of variable length exclusively during daytime hours, with each transect surveyed over 6–8 h. Along each transect, two complementary methodologies were employed: visual encounter surveys with manual capture, and audio strip transects (Heyer et al., 1994). A variety of microhabitats were systematically inspected, including areas adjacent to water bodies (streams, ponds, lagoons, floodplains, and artificial ponds), vegetation substrates (roots, trunks, branches, and leaves), and beneath stones.

Each captured specimen was placed separately in a transparent, non-reusable polyethylene bag (40 × 30 cm). For every individual encountered during the surveys, the date, time, habitat, and taxonomic identification were recorded, and all specimens were photographed; some specimens were also recorded calling (see below). Following euthanasia via cardiac injection of 3% xylocaine, tissue samples were collected and stored in 96% ethanol. Voucher specimens were fixed in 7% formalin and preserved in 70% ethanol following Heyer et al. (1994). Tadpoles were euthanized by immersion in a eugenol solution and subsequently fixed in 7% formalin.

Advertisement calls were recorded in WAV format at a sampling rate of 44.1 kHz and 24-bit resolution using a Zoom H5 digital recorder coupled with a Marantz SG-9P shotgun microphone, positioned approximately 1 m from each calling individual. Temperature and humidity data were not collected during recordings. Two recordings were deposited in the Banco de Sonidos OcainaCua (BSOC) of the Museo de Ciencias Naturales de La Salle (BSOC011_001, BSOC011_002).

Redescription

To characterize the morphology of *Andinobates opisthomelas* and close relatives, we built a morphological matrix of 36 continuous and discrete variables scored for 304 specimens from different localities and seven natural history museums. To compare and quantify coloration patterns, we photographed all examined specimens in dorsal, ventral, and lateral views; additionally, curators provided photographs of key specimens we were unable to examine personally. Specimens examined (adults and tadpoles) are listed in Appendix 1. For hand morphology, we followed Fabrezi and Alberch (1996), in considering finger I of other tetrapods to be absent in anurans and number the remaining four fingers starting with finger II. We followed the enumeration format used by Edwards (1971, 1974) and Brown et al. (2006) for the presentation of diagnostic character states. Character states follow Grant et al. (2006, 2017). Measurements of adult preserved specimens were made with a Mitutoyo CD-6’’ ASX digital caliper to 0.01 mm in a dissecting microscope. Larval measurements were made with the software ImageJ 1.54 g (Schneider et al., 2012) with photographs taken on a stacking set using a Canon t7i and a Stackshot Macro Rail Package. Unless otherwise noted, measurements and proportions are given only for adults, as determined by an approximate cut-off of SVL < 13 mm for adult females based on follicular development of investigated individuals and the presence of vocal slits in adult males (Duellman and Trueb, 1994). Morphological data given in the redescription are based only on specimens from the region of the inferred type locality. Throughout the text, descriptive statistics are summarized as mean ± SD.

We followed, Myers and Duellman (1982), Coloma (1995), and Brown et al. (2006) for measurements definitions and terminology: snout–vent length (SVL), head length (HL), head width (HW), snout length (SL), eye-to-nostril distance (EN), inter-narial distance (IND), nostril-mouth length (NML), nostril-snout length (NS), tympanum-mouth length (TML), tympanum-nostril length (TNL), eye length (EL), interorbital distance (IOD), upper eyelid width (UE), horizontal diameter of tympanum (HTYD), vertical diameter of tympanum (VTYD), tympanum-eye distance (TED), forearm length (FOL), humerus length (HUL), hand length (HaL), width of fourth finger disc (W4FD), width of fourth finger (W4F), tibia length (TL), thigh length (THL), hand length (HaL), foot length (FOT), width of third toe disc (W3TD), width of third toe (W3T), width of fourth toe disc (W4FD), width of fourth toe (W4T).

To assess morphological variation in *Andinobates opisthomelas* sensu lato, we defined four operational taxonomic units (OTUs) as follows: one for the area of the newly delimited type locality (*A. opisthomelas* sensu stricto) and three based on ventral color patterns and geographical distribution. We also included the previously described species *A. bombetes* and *A. cassidyhornae* in the analysis as these two are diagnosable species from *A. opisthomelas* a can shed light on expected variation in these measurements among congeners. We tested the normality of each variable through the Shapiro-Wilk test. We conducted exploratory descriptive analysis to summarize character variation and evaluate outliers through visual inspection of boxplots. We log-transformed our data to reduce the variation of the scale and the heteroscedasticity of the data and eliminate the relationship between the means of the variables and their variances (Sokal and Rohlf, 2012). To explore the variation in all morphometric variables, we performed a principal components analysis (PCA) based on the correlation matrix and employed the PCA results to select variables to perform single analyses between the OTUs. We performed ANOVA tests to identify differences between the OTUs, then used *t*-tests with Bonferroni *P*-corrections to identify differences between the OTUs (Appendix S1). Analyses were conducted using the *stats* package of R software version 4.2.2 (R Core Team, 2023). Boxplot and PCA graphics were made using the R package *ggplot2* (Wickham, 2016) for visualization of statistical results.

Examined material (Appendix 1) is deposited in the following institutions (acronyms in parentheses): Museo de Ciencias Naturales de la Salle, Medellín, Colombia (MCNS); Colección de herpetología, Colecciones Biológicas de la Universidad CES, Medellín, Colombia (CBUCES-D); Colección Biológica Universidad EAFIT, Medellín, Colombia (EAFIT-Am). Other museum acronyms follow Sabaj (2020) and Frost (2024). All specimens were inspected directly except those from the type series of *Andinobates opisthomelas,* which were inspected via photographs kindly provided by the museum curator.

Tadpole morphology

We examined tadpoles of *Andinobates opisthomelas* taken from nurse frogs collected at the inferred type locality. For tadpole staging we followed Gosner (1960). We used terminology and measurements of larval specimens from Altig and McDiarmid (1999) and Altig (2007), as follows: body length (BL), total length (TL), tail length (TAL), body width (BW), body high (BH), tail muscle height (TMH), maximum tail height (MTH), tail muscle width (TMW), interorbital distance (IOD), internarial distance (IND), eye nostril distance (END), and oral disc width (ODW).

Acoustics

We used Raven Pro software 1.6.4 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023) to analyze calls, applying a Hann window with 50% overlap and a discrete Fourier transform size of 512 samples following Köhler et al. (2017). The temporal features of the call (call duration, note duration, and inter-note interval) were manually measured on oscillograms, and power spectra were used to obtain the spectral features of the call (dominant frequency, low frequency, and high frequency), which were measured at 20 dB below the peak intensity of the dominant frequency. Oscillograms and spectrograms were plotted using R software version 4.2.2 (R Core Team, 2023) with the package *Seewave* version 2.1.5 (Sueur et al., 2021), using a Hanning window type with 90% overlap and a fast Fourier transform window length of 512 samples. Audio files in WAV format were imported with the *tuneR* package version 1.3.3 (Ligges et al., 2023). We used a note-centered approach (defining uninterrupted units of sound as notes and their entirety as call) and the terms and definitions for the acoustic parameters as defined by Köhler et al. (2017).

RESULTS

Type locality

According to the Colombian National Administrative Department of Statistics (DANE, 2020), 27 administrative units bearing the name “Santa Inés” are currently recognized. However, restricting the search to localities north of Medellín, only one candidate emerges: the rural district (*vereda*) of Santa Inés, in the municipality of Valdivia, Antioquia, located in the lower Cauca Valley (Fig. 1). As outlined below, historical records strongly support this location as the type locality.

Edgar Antwerp Pratt was an English explorer and naturalist who collected plants, insects, and vertebrates. He was elected Fellow of the Royal Geographical Society (RGS) in December 1891 (Beolens et al., 2011, 2013). Although the RGS holds no records of Pratt’s travels in Colombia in the 1890s (RGS, pers. comm.), it is possible to piece together his movements across the country using references in the taxonomic literature.

In the description of *Leptognathus pratti* Boulenger, 1897 (now *Dipsas pratti*), Boulenger (1897:523) reports material collected by Pratt in “Medellín, Republic of

Colombia." That same year, *Nectomys russulus* Oldfield, 1897 (now a junior synonym of *Sigmodontomys alfari* Allen, 1897; Wilson and Reeder, 2005) was described on the basis of a specimen collected by "Mr. Pratt the 7th of July of 1897 in Valdivia, Colombia, alt. 1200 meters" (Oldfield, 1897:548). Shortly thereafter, *Dendrobates* (currently *Andinobates*) *opisthomelas* Boulenger, 1899 and *Phyllobates* (currently *Colostethus*) *pratti* Boulenger, 1899 were described from "Santa Inés, North of Medellín, altitude 3800 feet [~1,200 masl]" (Boulenger, 1899:275) Together, these records establish that Pratt obtained material from at least three localities in western Colombia—Medellín, Valdivia, and Santa Inés—within a narrow temporal window around 1897.

Further support for Pratt's itinerary derives from additional vertebrate collections associated with the Cauca Valley. *Sciurus versicolor* Oldfield, 1900 (now a synonym of *S. granatensis* Humboldt, 1805) was described from a specimen collected by Pratt in "Colombia–Valdivia, Lower Cauca" (Oldfield, 1900:386) and later Oldfield (1902) referenced a *Potos* Geoffroy Saint-Hilaire and Cuvier, 1795 specimen also collected by Pratt in Valdivia. Numerous publications on invertebrates corroborate his extensive collecting activity in Valdivia (Grose-Smith, 1898, 1902; Joicey and Rosenberg, 1915; Gabriel, 1932) and the broader Cauca Valley region (Arrow, 1911; Joicey and Talbot, 1921), with several of these records sharing the same elevation and date data reported in the descriptions of *Andinobates opisthomelas* and *Colostethus pratti.*

Given the localities reported in the literature, we propose that Pratt's route to Medellín followed *La Trocha,* the principal overland road of the period, which ran from Montería through Cáceres, Valdivia, and Yarumal before reaching Medellín (Parsons, 1997). Although other historical routes existed—such as the *Camino de Islitas* from the east and the *Camino de Popayán* from the south—these are inconsistent with Pratt's known collecting localities in Valdivia and Santa Inés, both situated north of Medellín. Heading south from Montería, *La Trocha* passed directly through these localities, making it the most geographically coherent route for his itinerary. This route would have taken him directly through the lower Cauca Valley, where the bulk of his Colombian collections originated.

Prior to the formal establishment of Valdivia as a municipality in 1912 by the 8th Ordinance of the Antioquia Departmental Assembly, the area had been occupied since 1574, when Andrés de Valdivia founded the original settlement of Valdivia (Parsons, 1997; Restrepo, 2015). Before the parish of Valdivia was established in 1895, religious services were held in a nearby hamlet called Santa Inés, a settlement belonging to Pedro Vásquez, a prominent local merchant who housed settlers on his lands (Restrepo, 2015). At the time of Pratt's visit, all these settlements fell within the jurisdiction of the municipality of Yarumal.

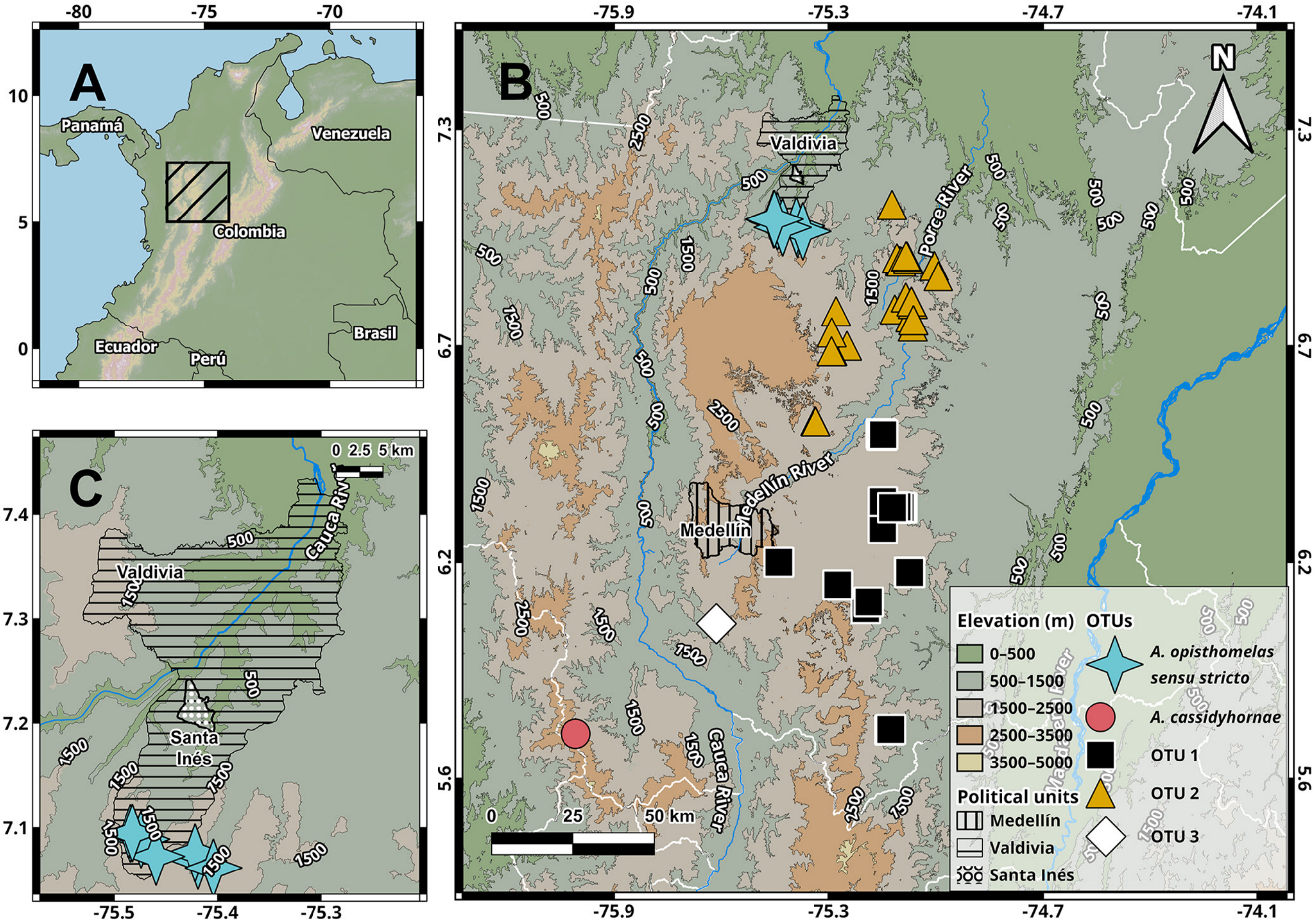

Figure 1. (A) Location of the study area within Colombia. **(B)** Distribution records of *Andinobates opisthomelas* sensu lato and related species reviewed in this study across the northern Colombian Andes. **(C)** Detail of Santa Inés in the Municipality of Valdivia, Antioquia.

When Valdivia was formally constituted as a municipality in 1912, Santa Inés was incorporated as one of its rural districts, or *veredas.*

On the basis of the above considerations, we conclude that the type locality of *Andinobates opisthomelas* and *Colostethus pratti* corresponds to the rural district (*vereda*) of Santa Inés in the current-day municipality of Valdivia (7.10812, -75.47118). The exact boundaries of Santa Inés prior to this administrative transition, however, remain unclear. At present, Santa Inés encompasses approximately 11.3 km²—the most refined figure obtainable.

Species account

Andinobates opisthomelas (Boulenger, 1899)

Figs. 2–4

Partial chresonymy

Dendrobates opisthomelas Boulenger, 1899
Minyobates opisthomelas–Myers, 1987
Ranitomeya opisthomelas–Grant et al., 2006
Andinobates opisthomelas–Twomey et al., 2011

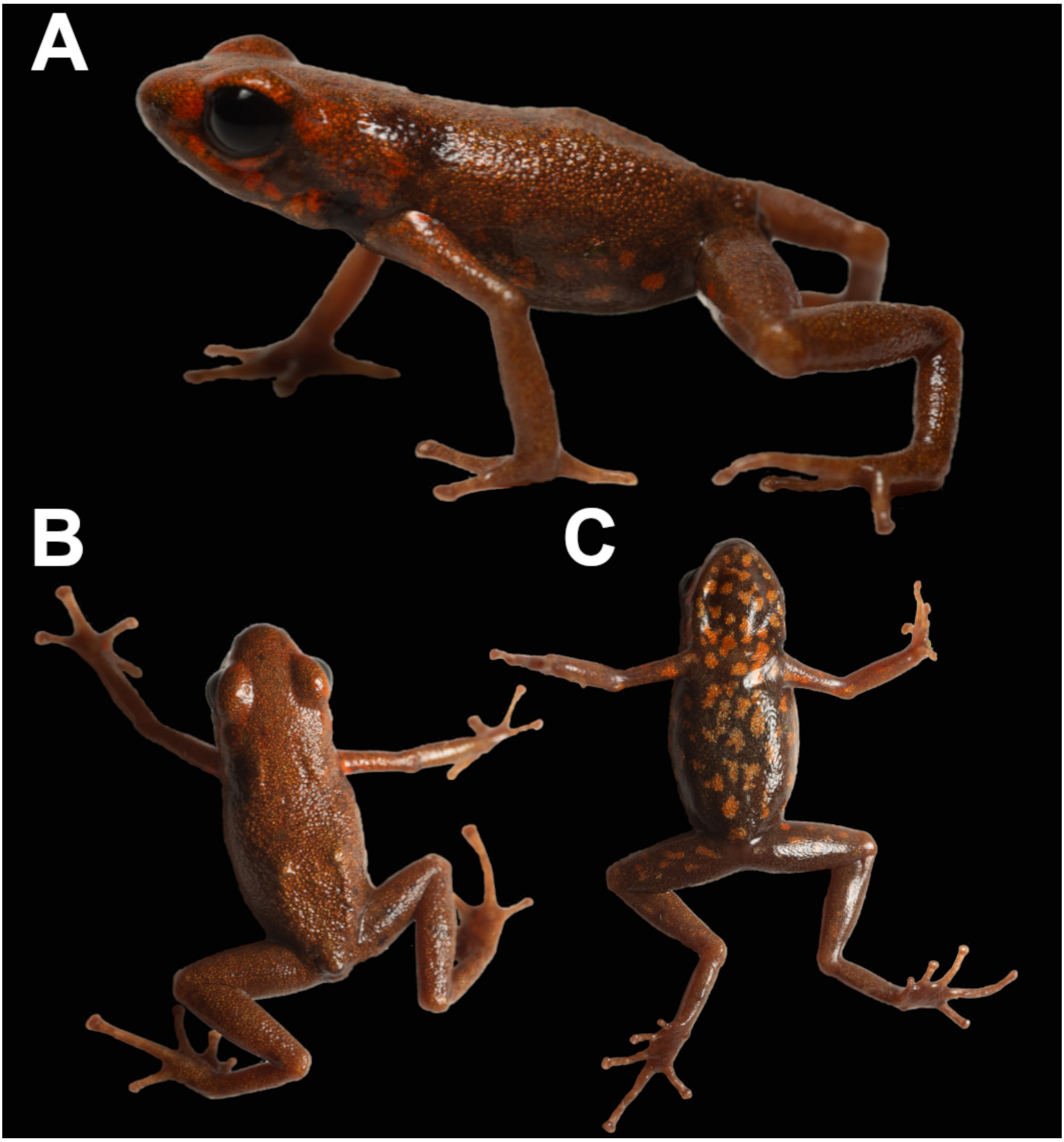

Figure 2. Adult female of *Andinobates opisthomelas* in life from type locality (CBUCES-D 877, SVL = 17.14 mm) in **(A)** lateral view, **(B)** dorsal view, and **(C)** ventral view.

Lectotype

BMNH 1947.2.15.22 (Fig. 4A–B; voucher number misreported as BMNH 1947.2.15.29 by Silverstone, 1975; see Discussion).

Type locality

Rural district (*vereda*) of Santa Inés, municipality of Valdivia, department of Antioquia, ca. 7.10812, -75.47118 (Fig. 1).

Diagnosis

Based on specimens from type series and topotypic specimens: (1) small, adult males SVL 16.32 ± 1.12 mm; (2) dorsal skin strongly granular, smoother ventrally; (3) five well-developed, externally visible toes; (4) expanded discs on toes III–V; (5) tarsal keel present but variable variable in size and shape; (6) lateral fringes on pre- and postaxial edges of toes III and IV; (7) long, buzz-like advertisement call; (8) dorsal coloration conspicuously colorful; (9) dorsal color red with irregular dark brown stippling, without stripes; (10) abdomen and throat dark brown with discrete red spotting/reticulation/marbling; (11) groin brown with gold stippling; (12) testes pale cream with brown reticulation.

Comparisons. *Andinobates opisthomelas* sensu stricto is most similar to *A. cassidyhornae,* but differs by having the abdomen and throat dark brown with discrete red spotting/reticulation/marbling (vs. solid black with few irregular spots reaching midway up the flanks in *A. cassidyhornae*). From *A. victimatus, A. opisthomelas* differs by having larger size (16.32 ± 1.12 mm vs. 14.23 ± 0.67 mm), the abdomen and throat dark brown with discrete red spotting/reticulation/marbling (vs. red), finger and toe tips dark brown (vs. light gray/tan pigmentation), and a longer advertisement call (2.1 ± 0.17 s vs. 0.49 ± 0.06 s). From *A. geminisae, A. opisthomelas* differs by having the abdomen and throat dark brown with discrete red spotting/reticulation/marbling (vs. orange). From *A. bombetes, A. opisthomelas* differs by having strongly granular dorsal skin (vs. smooth), a red dorsum with irregular dark brown stippling or reticulation and no stripes (vs. red or orange dorsolateral stripes), the abdomen and throat dark brown with discrete red spotting/reticulation/marbling (vs. mottled black on pale green, blue-green, or yellow ventral coloration), and lateral fringes present on the pre- and postaxial edges of toes III and IV

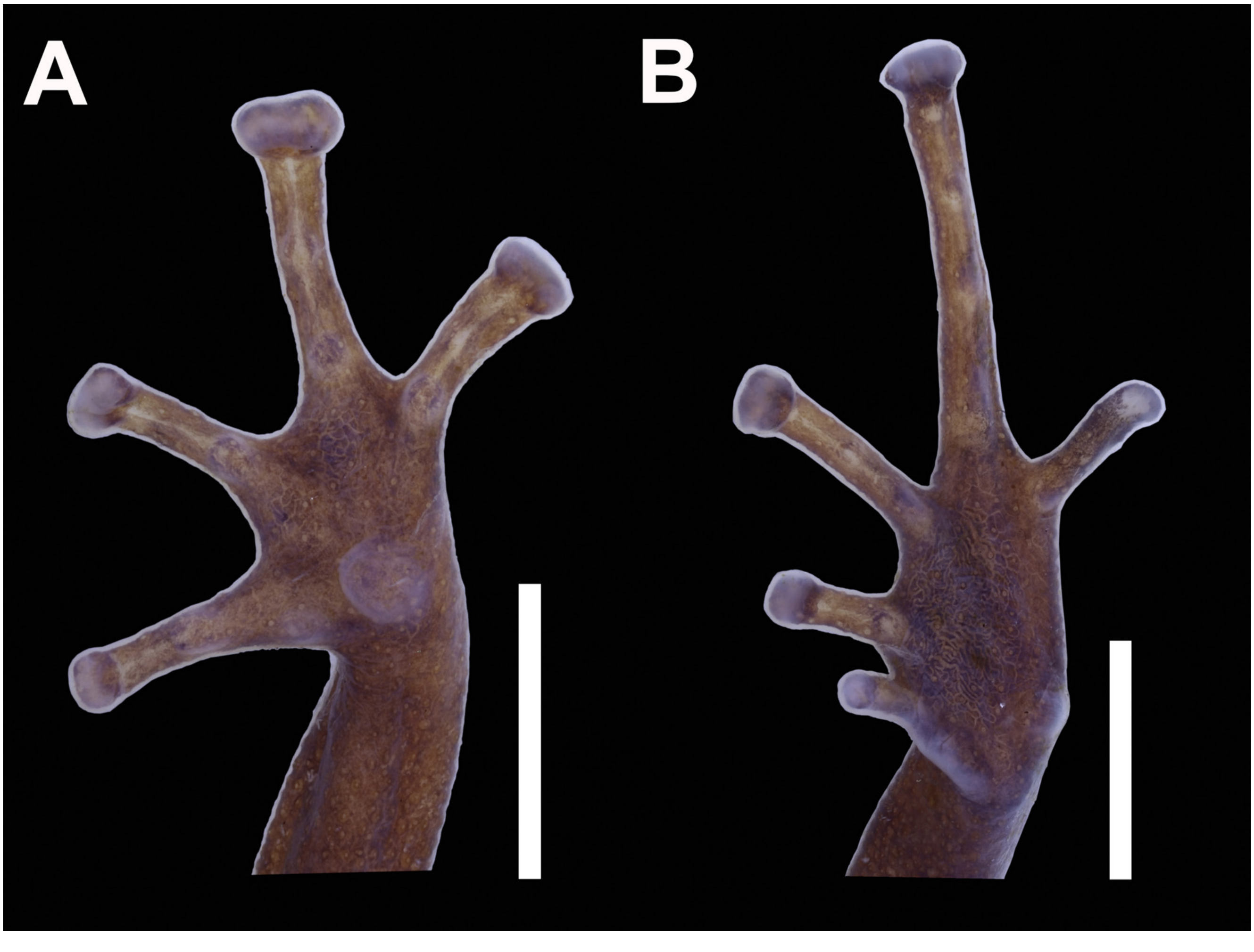

Figure 3. Ventral views of **(A)** hand and **(B)** foot of *Andinobates opisthomelas* from the type locality (CBUCES-D 875). Scale bars = 2 mm.

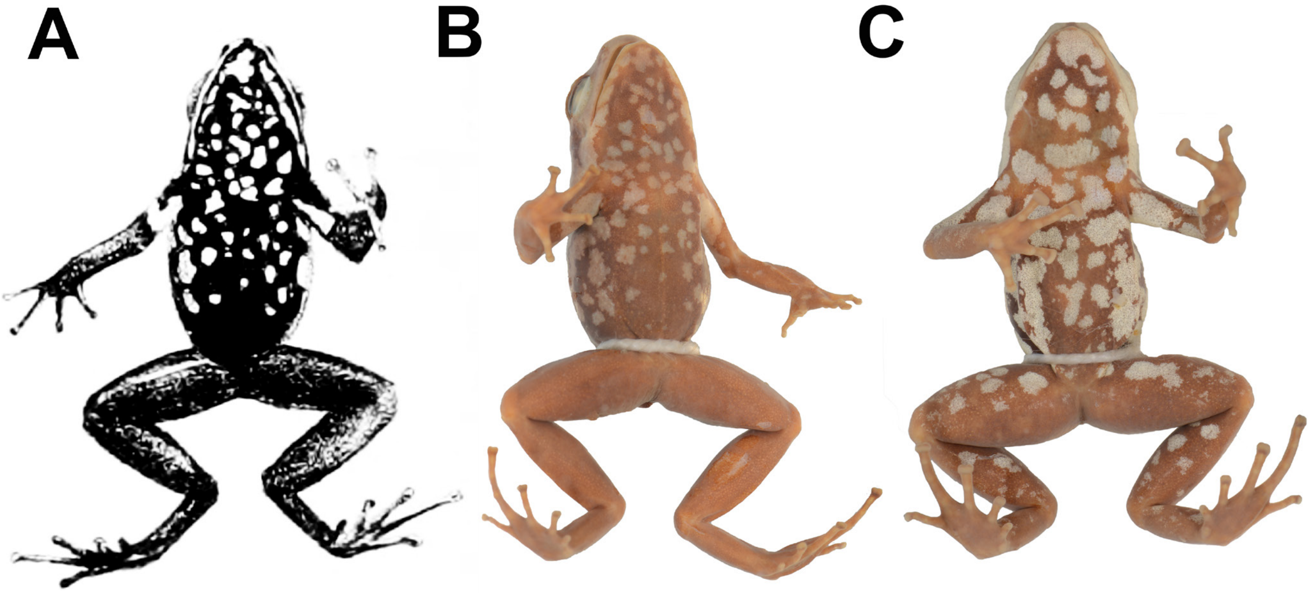

Figure 4. (A) Reproduction of the illustration from Boulenger (1899: plate IX, fig. 4). **(B)** BMNH 1947.2.15.22 (18.3 mm SVL), with the same unique ventral pattern and limb position (albeit reflected) as the specimen illustrated by Boulenger. **(C)** BMNH 1947.2.15.29 (18.0 mm SVL), with the same ventral pattern as the specimen illustrated by Silverstone (1975: 19, fig. 12N).

(vs. absent). From *A. daleswansoni* and *A. dorisswansoni, A. opisthomelas* differs by having five well-developed, externally visible toes (vs. first and second toes fused). From *A. dorisswansoni, A. opisthomelas* further differs by having a red dorsum with irregular dark brown stippling or reticulation (vs. irregular red or yellow-orange blotches on a dark brown or black background), the abdomen and throat dark brown with discrete red spotting/reticulation/marbling (vs. irregular red or yellow-orange blotches on a dark brown or black background), and testes pale cream with brown reticulation (vs. dark brown). From *A. daleswansoni, A. opisthomelas* further differs by having the dorsum and hindlimbs red with irregular dark brown stippling or reticulation towards the lower dorsal region and hindlimbs (vs. red cephalic hood and ochre lower dorsal region and hindlimbs). From *A. fulguritus, A. viridis,* and *A. altobueyensis, A. opisthomelas* differs by having a red dorsum with irregular dark brown stippling or reticulation (vs. yellow to green). From *A. minutus, A. opisthomelas* differs by having a red dorsum with irregular dark brown stippling or reticulation (vs. yellow/golden with black stripes) and an inner metatarsal tubercle present (vs. absent). From *A. tolimensis* and *A. supata, A. opisthomelas* differs by having a red dorsum with dark brown irregular stippling (vs. solid yellow), unexpanded toe discs (vs. expanded on toes III–V), and testes pale cream with brown reticulation (vs. brown). From *A. abditus, A. opisthomelas* differs by having a conspicuously red dorsum with dark brown stippling (vs. dark brown inconspicuous coloration), the groin and axilla brown with some gold-like stippling (vs. bright orange spots), and lateral fringes present on the pre- and postaxial edges of toes III and IV (vs. absent).

Description (6 females, 11 males, 15 unsexed). Small, adult SVL 16.32 ± 1.12 mm; males smaller than females. Adult males 15.76 ± 1.03 mm SVL (Table 1); vocal slits present; testes pale cream with brown reticulation (Fig. 5B); dorsal tadpole transport. Adult females 17.13 ± 1.55 mm SVL; mature ova brown, oviducts swollen, convoluted. Large intestine pigmented anteriorly, dark reticulated dots

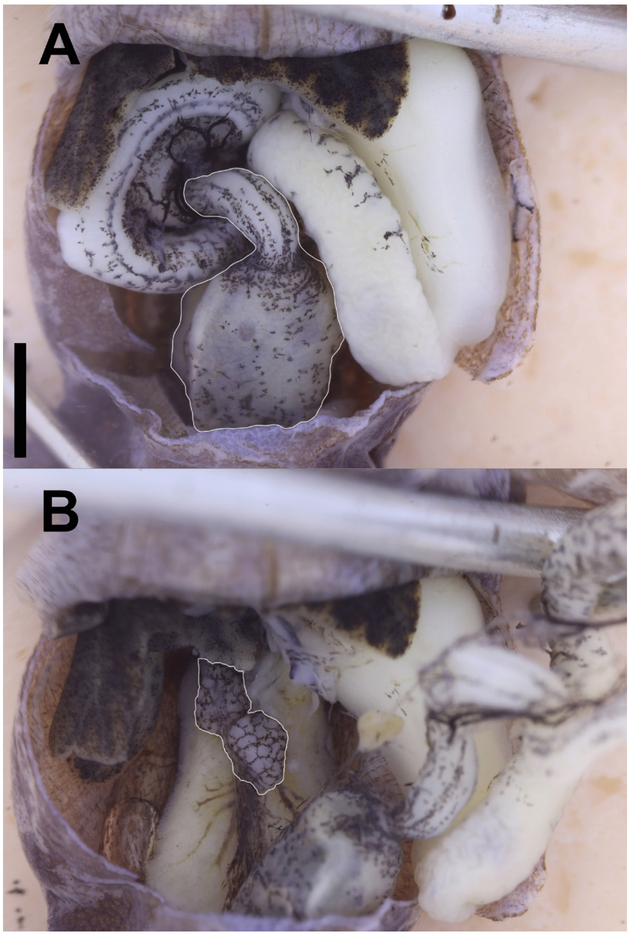

Figure 5. (A) Large intestine and **(B)** testis coloration of *Andinobates opisthomelas* from the type locality (CBUCES-D 1299). Scale bars = 2 mm.

Table 1. Range of measurements (mm) and descriptive statistics (mean ± SD) for topotypic material of *Andinobates opisthomelas* examined in this study. See text for abbreviation definitions.

	Females (*n* = 6)	**Males (*n* = 11)**	**Unsexed (*n* = 15)**	**Combined (*n* = 32)**
SVL	14.52–18.96 (17.13 ± 1.55)	14.14–17.24 (15.76 ± 1.03)	14.33–18.03 (16.40 ± 0.90)	14.14–18.96 (16.32 ± 1.12)
IND	2.17–2.56 (2.35 ± 0.16)	1.91–2.31 (2.17 ± 0.13)	1.97–2.58 (2.28 ± 0.16)	1.91–2.58 (2.26 ± 0.16)
UE	1.28–1.76 (1.51 ± 0.18)	1.15–1.47 (1.34 ± 0.10)	1.19–1.61 (1.46 ± 0.11)	1.15–1.76 (1.43 ± 0.13)
IOD	1.83–2.27 (2.1 ± 0.18)	1.79–2.13 (1.93 ± 0.13)	1.62–2.25 (1.95 ± 0.18)	1.6–2.27 (1.97 ± 0.18)
HW	4.86–6.04 (5.39 ± 0.44)	4.46–5.21 (4.92 ± 0.27)	4.76–5.73 (5.18 ± 0.30)	4.46–6.04 (5.14 ± 0.34)
HL	4.41–5.78 (5.05 ± 0.53)	4.36–4.85 (4.53 ± 0.17)	4.42–5.17 (4.87 ± 0.22)	4.36–5.78 (4.81 ± 0.33)
SL	2.27–2.78 (2.435 ± 0.188)	2.03–2.63 (2.317 ± 0.187)	2.14–2.85 (2.461 ± 0.163)	2.03–2.85 (2.41 ± 0.169)
NS	1.09–1.26 (1.17 ± 0.06)	0.91–1.22 (1.10 ± 0.10)	1.05–1.32 (1.19 ± 0.08)	0.91–1.32 (1.16 ± 0.09)
EN	1.3–1.57 (1.448 ± 0.093)	1.29–1.43 (1.359 ± 0.056)	1.34–1.61 (1.443 ± 0.082)	1.29–1.61 (1.423 ± 0.08)
TNL	3.53–4.12 (3.84 ± 0.21)	3.26–3.8 (3.59 ± 0.17)	3.47–4.01 (3.74 ± 0.17)	3.26–4.12 (3.71 ± 0.19)
TML	0.32–0.51 (0.40 ± 0.08)	0.3–0.7 (0.52 ± 0.16)	0.4–0.68 (0.56 ± 0.08)	0.3–0.7 (0.51 ± 0.12)
NML	0.53–0.67 (0.60 ± 0.05)	0.37–0.65 (0.55 ± 0.101)	0.46–2.42 (0.70 ± 0.48)	0.37–2.42 (0.64 ± 0.33)
EL	2.13–2.4 (2.26 ± 0.11)	1.93–2.26 (2.07 ± 0.12)	1.94–2.38 (2.15 ± 0.12)	1.93–2.4 (2.14 ± 0.13)
TED	0.35–0.49 (0.44 ± 0.05)	0.34–0.55 (0.43 ± 0.07)	0.32–0.52 (0.43 ± 0.06)	0.32–0.56 (0.43 ± 0.06)
HTYD	0.93–1.26 (1.11 ± 0.11)	0.86–1.12 (0.98 ± 0.10)	0.9–1.27 (1.10 ± 0.10)	0.86–1.27 (1.07 ± 0.11)
VTYD	1.06–1.44 (1.20 ± 0.13)	0.88–1.22 (0.10 ± 0.12)	0.9–1.34 (1.08 ± 0.12)	0.88–1.44 (1.07 ± 0.13)
TL	7.18–8.48 (7.79 ± 0.51)	6.7–7.91 (7.23 ± 0.39)	6.78–8.39 (7.33 ± 0.51)	6.7–8.48 (7.36 ± 0.50)
THL	7.21–8.23 (7.83 ± 0.39)	6.5–7.98 (7.13 ± 0.48)	6.82–8.18 (7.40 ± 0.43)	6.5–8.23 (7.38 ± 0.47)
HaL	4.26–4.78 (4.43 ± 0.18)	3.69–4.69 (4.28 ± 0.36)	3.52–4.94 (4.22 ± 0.43)	3.52–4.94 (4.27 ± 0.35)
FOL	4.41–5.58 (4.86 ± 0.39)	3.89–4.5 (4.22 ± 0.25)	4.05–5.16 (4.48 ± 0.30)	3.89–5.58 (4.50 ± 0.35)
HUL	4.05–4.9 (4.50 ± 0.38)	3.55–4.56 (4.00 ± 0.41)	3.59–4.94 (4.07 ± 0.36)	3.53–4.94 (4.12 ± 0.40)
W4FD	0.6–0.86 (0.68 ± 0.10)	0.54–0.75 (0.65 ± 0.07)	0.49–0.97 (0.69 ± 0.13)	0.49–0.97 (0.69 ± 0.11)
W4F	0.3–0.45 (0.37 ± 0.06)	0.26–0.48 (0.37 ± 0.08)	0.26–0.48 (0.36 ± 0.06)	0.26–0.48 (0.36 ± 0.06)
FOT	6.6–7.22 (6.96 ± 0.28)	5.92–7.36 (6.67 ± 0.57)	5.73–8 (6.71 ± 0.60)	5.73–8 (6.71 ± 0.51)
W3TD	0.53–0.71 (0.62 ± 0.07)	0.5–0.57 (0.54 ± 0.03)	0.5–0.72 (0.61 ± 0.07)	0.5–0.72 (0.60 ± 0.06)
W3T	0.29–0.46 (0.39 ± 0.06)	0.28–0.42 (0.36 ± 0.05)	0.24–0.52 (0.38 ± 0.08)	0.24–0.52 (0.38 ± 0.07)
W4TD	0.53–0.71 (0.64 ± 0.06)	0.51–0.64 (0.58 ± 0.06)	0.5–0.79 (0.61 ± 0.07)	0.5–0.79 (0.61 ± 0.07)
W4T	0.32–0.49 (0.39 ± 0.06)	0.36–0.46 (0.40 ± 0.04)	0.3–0.45 (0.37 ± 0.06)	0.3–0.49 (0.38 ± 0.06)

posteriorly (Fig. 5A). Dorsum strongly granular, especially on anterior body, sacral region, and dorsal surface of hindlimbs. Belly and flanks slightly granular. Head wider than long, snout short and rounded in lateral and dorsal views (Fig. 2). Eye diameter ca. 41% of head length. Canthus rostralis rounded, loreal region concave. Nares small, rounded, near tip of snout, directed laterad, separated from orbits by less than eye diameter. Pupil oval. Tympanum rounded, slightly angled anteroposteriad, separated from eye by half tympanum diameter. Tympanic ring prominent anteroventrally, concealed posterodorsally. Vomerine, maxillary, and premaxillary teeth absent. Choanae small, rounded. Tongue attached anteriorly, free along most of its extension; medial lingual process absent. Dorsal surface of finger and toe discs with paired dermal scutes. Fingers lacking webbing, slightly compressed dorsoventrally, with or without fringes (Fig. 3); relative lengths of adpressed fingers IV > V > III > II; finger II barely passing subarticular tubercle of finger III; finger III reaching proximal edge of distal subarticular tubercle of finger IV; finger V reaching distal half of distal subarticular tubercle of finger IV. Discs of fingers III–V moderately expanded, finger II disc unexpanded. Finger and toe discs truncate (but toe V disc rounded), oval, slightly more expanded on fingers than toes (Fig. 3). Subarticular tubercles 1–1–2–2 (sometimes 1–1–1–2, with distal subarticular tubercle on finger IV absent) on fingers; distal and basal subarticular tubercles of fingers subequal, rounded, slightly protuberant. Supernumerary tubercles absent. Palmar tubercle large, rounded; thenar tubercle oval, almost three times smaller than palmar tubercle. Five well-developed toes, without webbing; relative lengths of adpressed toes IV > III > V > II > I. Toe I disc unexpanded, toes II to IV discs moderately expanded, toe V disc weakly expanded. Subarticular tubercles of toes 1–1–2–3–2, prominent. Basal tubercles larger than distal tubercles; supernumerary tubercles absent. Inner metatarsal tubercle elongated, almost twice length of outer metatarsal tubercle; outer metatarsal tubercle rounded, conical. Inner tarsal tubercle small followed by a keel.

Morphometric analysis (Table 1). The first two components in the PCA explained 57.9% of the total variation in the data (Fig. 7). Populations of *Andinobates opisthomelas* show low variation along principal component axes. Remarkably, *A. bombetes* and *A. cassidyhornae* show a clear separation along PC1, but not on PC2. However, both species fall in the variation exhibit in *A. opisthomelas* sensu stricto (Fig. 7). The three OTUs overlap widely, although there seems to be a pattern of segregation, as the type locality specimens extend to the positive half of the PC1 axis, OTU3 tends to the negative half of the PC1 axis, and OTU1 lies between them on the PC1 axis (Fig. 7). The variables with the highest PC1 loadings were SVL, TL, TNL, FOT, HaL, and THL. For these variables, our ANOVA indicated that the morphological variation among the six OTUs was significantly different (Fig. 8), and the *P*-values for the pair-wise difference were highly significant for all comparisons ($P < 0.0001$). The *t*-test shows that OTU2 and *A. bombetes* differ in SVL, TL, TNL, FOT, HaL, and THL (Fig. 8). Apparently OTU2 and *A. opisthomelas* sensu stricto differ in all these variables (Figs. 7–8). Notably, OTU3 falls inside the variation of those populations and has no significative difference with any of them (Figs. 7–8).

Coloration in life. The dorsum and flanks are red with a dark brown irregular stippling that becomes reticulated in denser stippled regions. This stippling seems to be the result of aggregations of melanophores that form reticulations when they connect. The red disappears on the limbs

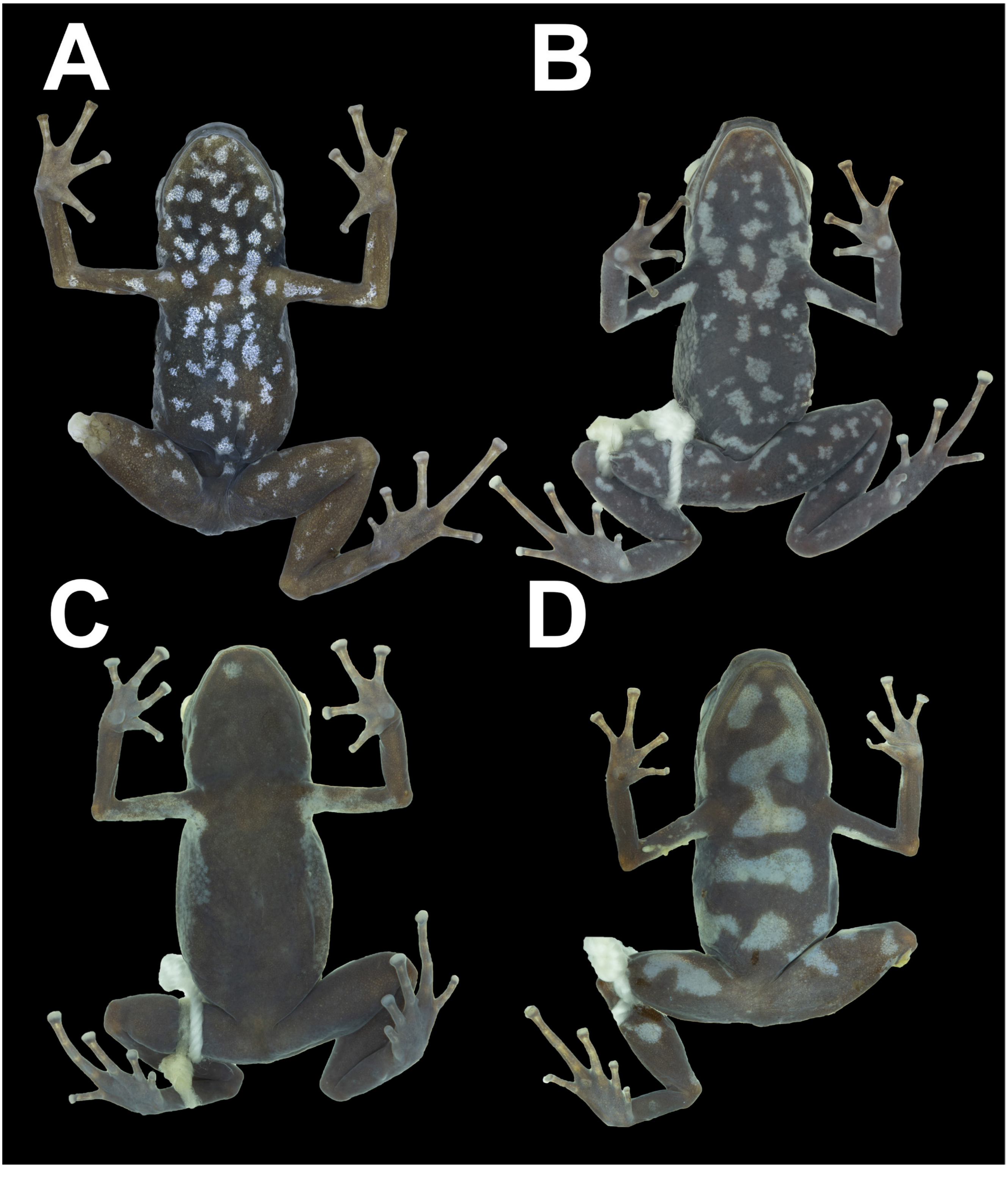

Figure 6. Ventral coloration pattern of *Andinobates opisthomelas* from **(A)** Santa Inés, Valdivia (CBUCES-D 877; 17.1 mm SVL; topotype), **(B)** Fredonia (MHUA-A12376; 17.1 mm SVL; OTU3), **(C)** El Carmen de Viboral (MHUA-A12359; 18.3 mm SVL; OTU1) and **(D)** Amalfi (MHUA-A09440; 15.5 mm SVL; OTU2).

(including fingers and toes) and sacral region and some gold stippling appears over the brown. The ventral surface of the limbs is the same color as the dorsal surface. The abdomen and throat are dark with discrete red spotting/reticulation/marbling (Fig. 2).

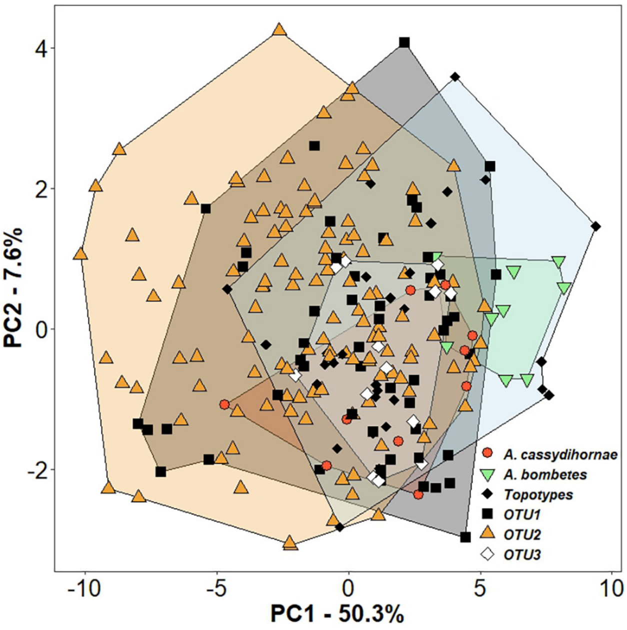

Figure 7. Bivariate plot of the first two components (PC1 and PC2) from a principal components analysis using a correlation matrix of 28 morphological variables.

Coloration in preservative. In recently preserved specimens, all red fades to yellow and over time becomes silvery white (Fig. 6A). All the brown turns black or ochre. After a long time in preservative, the entire body becomes red ochre, with light red spots in life becoming grullo.

Vocalization (five males, one not collected, n = 26). Recordings made in February and June of 2023 in the rural district of San Fermín, municipality of Valdivia, department of Antioquia, at ca. 10:00. Specimen coordinates, museum numbers, and associated audio recordings are provided in Appendix 2.

The advertisement call of *Andinobates opisthomelas* at the type locality consists of a single pulsed call composed of 194 ± 27 pulses/call (Fig. 9B). The pulses were single or paired (Fig. 9C). Call duration is 2.1 ± 0.17 s (Fig. 9B) with an interval between adjacent calls of 15.4 ± 3.95 s and total call rate of ca. 4 calls/minute. The call exhibits frequency modulation during the first second, with the dominant frequency rising progressively before stabilizing at 5554.13 ± 0.17 Hz (Fig. 9B). The call also shows amplitude modulation throughout its duration, with amplitude increasing gradually during the first

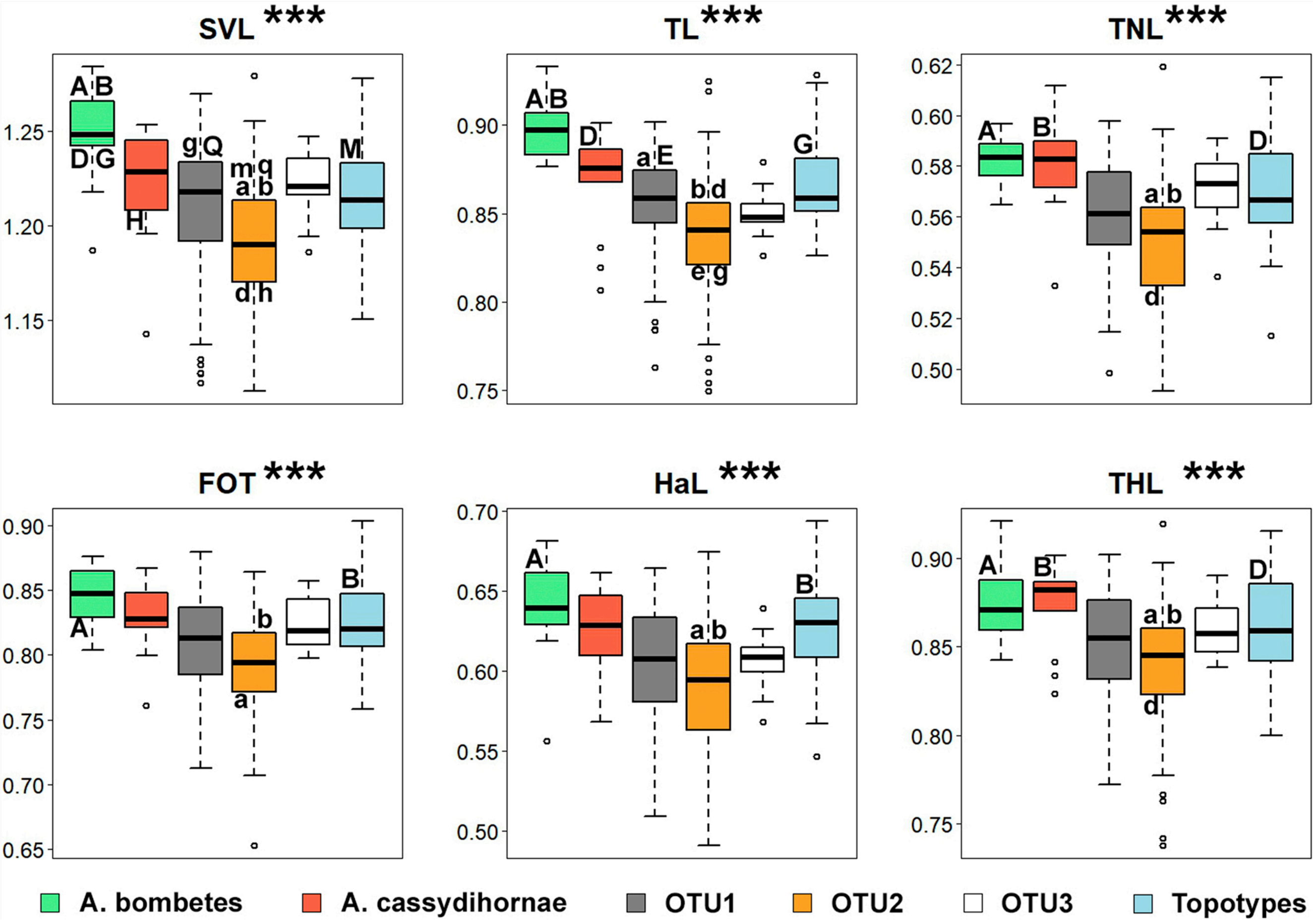

Figure 8. Boxplot of the six most influential variables on the PCA. *** indicates significative differences in the ANOVA analysis ($P < 0.0001$). Statistically significant differences in the t-test for morphometric variables between OTUs are denoted as upper case and lower-case pairs. Definitions of abbreviations can be found in methodology section.

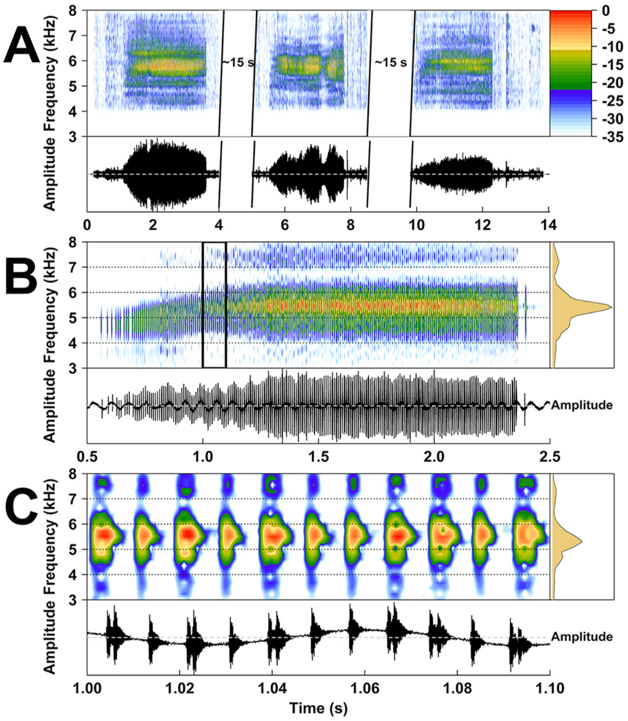

Figure 9. Advertisement call of *Andinobates opisthomelas* from the type locality (CBUCES-D 1299). **(A)** Spectrogram and oscillogram of three consecutive calls showing the inter-call interval (ca. 15 s). **(B)** Spectrogram and oscillogram of a single call with the mean frequency spectrum; the rectangle indicates the section shown in panel C. **(C)** Zoomed spectrogram and oscillogram showing the internal pulse structure of the call.

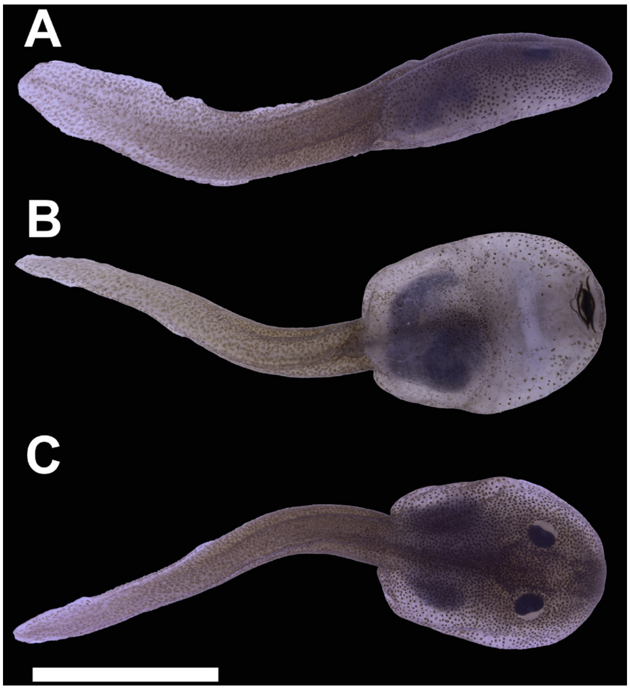

Figure 10. Preserved tadpole of *Andinobates opisthomelas* from the type locality in **(A)** lateral, **(B)** ventral, and **(C)** dorsal views (CBUCES-D 1303; Gosner stage 25). Scale bar = 2 mm.

second before reaching a plateau. The band frequency ranges from 4906.12 ± 237.02 Hz at the lower limit to 6350.43 ± 594.08 Hz at the upper limit (Fig. 9B). The call begins with 1–3 pulses separated by a pause from subsequent pulses, after which the pulse rate remains relatively constant at approximately 90 pulses/second, until a final pause followed by a terminal click concludes the call.

Tadpole morphology **(*n* = 5, Figs. 10–11).** Information from back-riding tadpoles. CBUCES-D 1304: stage 23, and CBUCES-D 870, 1303, 1314–15: stage 25. In dorsal view, body oval, snout rounded (Fig. 10C); in lateral view, body elliptical, depressed (BH/BW = 0.47), snout rounded (Fig. 10A). Eyes dorsal, large, directed anterolaterally. Nares elliptical, located dorsally, directed anterolaterally,

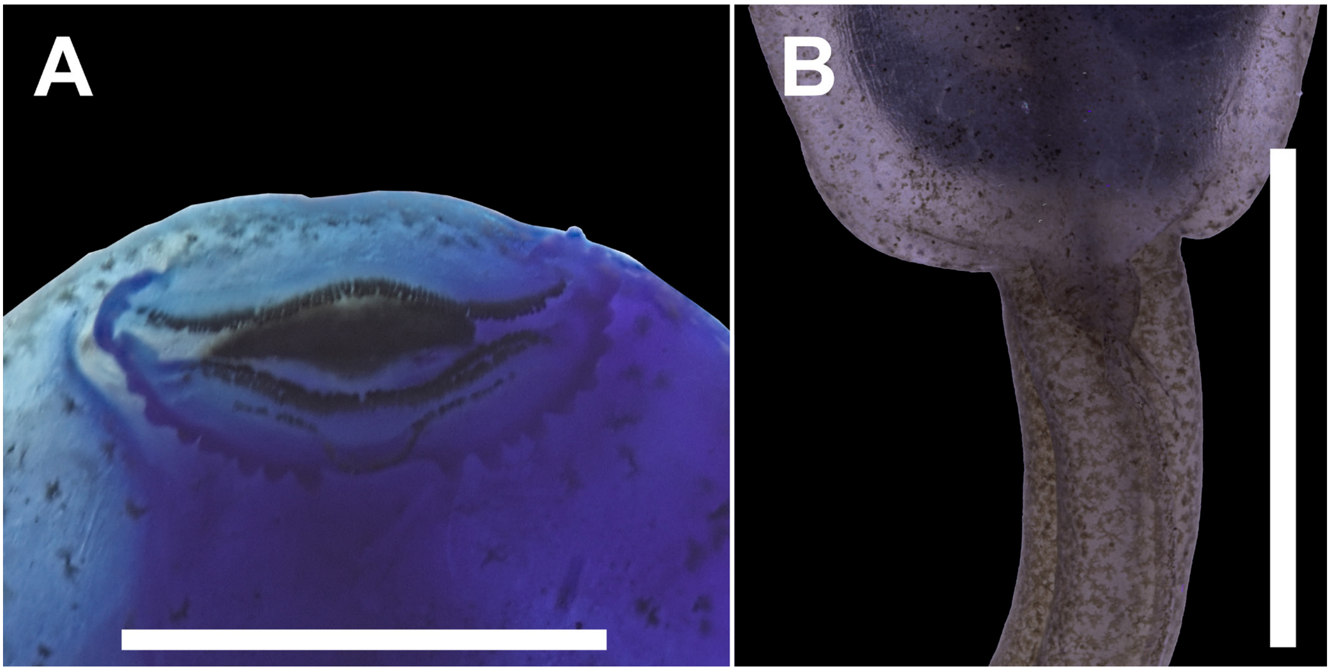

Figure 11. (A) Ventral view of the body anterior region showing oral disc and **(B)** ventral view of the tail proximal region of a preserved tadpole of *Andinobates opisthomelas* from the type locality (CBUCES-D 1303; Gosner stage 25). Arrow indicates the vent tube. Scale bars = 1 mm.

located between snout and eyes. Spiracle sinistral, lateral, located ventral to midline of body; inner wall present, fused to body, opening round, smaller than spiracle width. Vent tube medial with lateral displacement, tubular, positioned at level of ventral fin, right margin equal to left, opening elliptical (Fig. 11B). Tail long (TAL/TL = 0.56); caudal musculature not reaching round tip; dorsal fin almost straight, low, originating on body/tail junction, ventral fin arched, low (Fig. 10A). Oral disc anteroventral, laterally emarginated, bordered by single row of conical, alternating marginal papillae; upper lip with large diastema (Fig. 11A). Labial tooth row formula 2(2)/3(1); A1 and A2 lengths subequal; A2 gap small; P1, P2, and P3 lengths subequal (Fig. 11A). Jaw sheaths massive, serrate, dark, well keratinized; upper and lower jaw sheath V-shaped. Lateral line stitches indistinguishable.

In preserved specimens, body light brown and evenly pigmented with dark brown stippling in dorsal view, light brown with dark brown stippling in posterolateral view, slightly translucent, with dense dark brown stippling increasing posteriorly covering intestines in ventral view. Tail light brown anteriorly, pearl grey posteriorly with dark brown stippling. Fins translucent (Fig. 10).

Tadpole measurements (mm). CBUCES-D 1303: TL = 6.494; BL = 2.385; TAL = 3.663; BW = 1.852; BH = 0.884; TMH = 0.549; MTH = 0.819; TMW = 0.577; IOD = 0.524; IND = 0.458; END = 0.404; ODW = 1.028.

Distribution. *Andinobates opisthomelas* sensu lato inhabits the humid premontane and montane forest of the northern range of the Cordillera Central, 5.7–7.1°N, 1,300–2,400. *Andinobates opisthomelas* sensu stricto is only known from the southern part of the municipality of Valdivia and northern region of the municipality of Yarumal, Antioquia (Fig. 1).

Natural history. *Andinobates opisthomelas* is diurnal. It inhabits leaflitter and is commonly found beneath moss or fallen vegetation near streamlets and rivers in preserved forests, although it can also be found in disturbed areas far from water bodies. Males were observed calling beside streams, perched under moss walls. Calling activity occurs throughout the day, peaking in early morning and decreasing by midday. Males transport one or occasionally two tadpoles; we recorded a single male calling while carrying a tadpole.

Variation. *Andinobates opisthomelas* sensu lato exhibits a high level of variation in ventral pattern of coloration. The three non-topotypic OTUs we defined were: populations from the highlands of east of the Medellín river (OTU1; Fig. 1) that possess a wide dark brown ventral band with some incursions of red and irregular ventral blotches (Fig. 6C); populations from the highlands of central and northeastern regions of the Medellín river (OTU2; Fig. 1) with large, round, pale blue or white ventral blotches (Fig. 6D); and populations from the highlands southeast of the Medellín river (OTU3; Fig. 1), with dark background and discrete pale spotting/reticulation/marbling and occasionally yellow dorsal coloration (Fig. 6B).

Taxonomic remarks

Silverstone (1975:32) explicitly designated “the specimen illustrated in the original description” as the lectotype of *Andinobates opisthomelas;* however, the voucher number he reported for that specimen, BMNH 1947.2.15.29, is incorrect. As evidenced by comparing the unique patterns of ventral spotting and position of the limbs, the specimen illustrated by Boulenger (1899: plate IX, fig. 4) corresponds to BMNH 1947.2.15.22 (Fig. 4B), whereas BMNH 1947.2.15.29 (Fig. 4C) matches the specimen illustrated by Silverstone (1975:19, fig. 12N).

We examined MHUA-A 3382 (SVL 15.7 mm) and 3864 (SVL 14.9 mm) from Tierralta Cordoba, suspected by Márquez et al. (2017) to be *Andinobates victimatus,* and their small size and ventral red coloration lead us to corroborate that they are indeed *A. victimatus.* We also examined MHUA-A 00510–18 from the municipality of Andes and conclude that their ventral color pattern falls within the variation of *A. cassidyhornae.* Thus, we restrict the distribution of *A. opisthomelas* to the northern portion of the Cordillera Central in Antioquia.

DISCUSION

The uncertainty surrounding the type locality of *Andinobates opisthomelas* stems from multiple converging factors. First, Colombia’s political and administrative divisions were actively changing at the time of Pratt’s collections in the late 19th century—new municipalities were being established, traditional place names were being reassigned, and settlement boundaries were in flux—making it particularly difficult to map historical toponyms onto present-day administrative units. Second, although Boulenger’s description was adequate by the standards of his time, the geographic context available to him was insufficient to unambiguously pin the type locality to a specific place recognizable by future researchers. Third, the collector himself, A.E. Pratt, left no known travel records documenting his route through Colombia, leaving his itinerary and the precise locations of his collections without direct documentary support. These factors left the type locality of the oldest described species of *Andinobates* effectively unresolved for more than a century.

Our results provide a substantially more precise and historically grounded delimitation of the type locality of *Andinobates opisthomelas* than previously available. Amézquita et al. (2013:165) precisely placed the type locality to the northwest of Medellín and stated that “Santa Inés corresponds to the now extinct population from the type locality of *A. opisthomelas.*” However, they did not provide any supporting information, such as administrative divisions, coordinates, or elevational data, to justify their placement of the locality not their claim that the population was extinct. In contrast, we present evidence from contemporaneous taxonomic literature, historical cartography, and administrative records to identify the rural district (*vereda*) of Santa Inés, municipality of Valdivia, Antioquia, as the type locality—a designation fully consistent with Boulenger's geographic and elevational data (ca. 1,200 masl), corroborated by Pratt's documented collecting activity in the same area around 1897, and further supported by the collection of new individuals from this locality whose morphology and coloration are concordant with the type series. Given that Boulenger (1899) reported the same type locality for *Colostethus pratti,* our findings clarify the type locality of that species as well. This represents the most precise and documented delimitation of the type locality to date, refined to an approximate area of 11.3 km².

To the best of our knowledge, no published studies addressing *Andinobates* systematics (e.g., Grant et al., 2017, Márquez et al., 2017) have used sequences, morphology, or bioacoustics from topotypic material of *A. opisthomelas.* This is relevant, as current understanding of the diversity and relationships of *Andinobates* is supported by molecular and acoustic evidence, so the relationships of *A. opisthomelas* sensu stricto with its congeners is pending evaluation. Here we partially address the question by analyzing morphological and bioacoustic variation from type and topotypical material.

The individual pulses of the advertisement call of *Andinobates opisthomelas* are composed of single and paired linked pulses (Fig. 9C), resembling its sister genus *Ranitomeya,* whose pulses are composed of several linked pulses (Brown et al., 2011). This trait has not been reported in other *Andinobates* species; however, Brown et al. (2011) suggest that linked pulses could be an artifact of recording quality.

The advertisement call of *Andinobates opisthomelas* is structurally similar to that of *A. geminisae,* as both species present a pause at the beginning and end of the call (Batista et al., 2014). This similarity is noteworthy, as no other known advertisement call of an Andean *Andinobates* species shares this characteristic. *Andinobates geminisae* is part of a lowland clade inhabiting the rainforests of Panama and Colombia, whereas *A. opisthomelas* is from higher elevations in the western Andes. It should be noted, however, that advertisement calls remain undescribed for several species, and this pause may be an overlooked feature in previously described calls. We found no evidence that this trait is influenced by temperature or humidity. Although we were careful describing the acoustic repertoire of topotypical material, we refrain from drawing strong conclusions from our comparisons given the lack of control over these variables. Nevertheless, our acoustic data retains comparative value, as not all call parameters are equally sensitive to environmental factors.

In the original description of *Andinobates opisthomelas,* Boulenger described the coloration as follows: "Head and body lemon-yellow above, obscured with dark brown or blackish on the coccygeal region, black round the vent; arms yellow, forearms, hands, and hind limbs blackish; throat and belly black, with white spots" (Boulenger, 1899). Silverstone (1975:32) suggested the yellow coloration was an artifact of preservation, noting that "In preserved specimens of *Dendrobates,* red fades to yellow before becoming white or gray; thus Boulenger, in his original description, stated that the dorsum of the types was yellow. The types (from Santa Inés) have light ventral spots…". Our observations corroborate Silverstone's hypothesis, as we have observed that in recently preserved specimens of *A. opisthomelas* sensu stricto, the red dorsal coloration fades progressively to yellow before eventually becoming silvery white, with yellow persisting longer in the dorsal region. Given that the type series was collected by Pratt in Valdivia, Antioquia, and subsequently transported to London before being examined by Boulenger, a considerable amount of time in preservative had elapsed before the description was prepared, during which the original red coloration would have already transitioned to yellow.

To our knowledge, the only known populations of *Andinobates opisthomelas* exhibiting white to light blue ventral spots in life are those from northwest of Medellín, in the municipalities of Anorí and Amalfi, Antioquia (OTU2), and one population from southern Antioquia in the municipality of Fredonia (OTU3). Our statistical analysis shows that there is a high degree of segregation between *A. opisthomelas* sensu stricto and the OTU2 (Figs. 9–10). This population (OTU2) has shown genetic divergence in comparison with OTU1 (Amézquita et al., 2013). However, the lack of differences between *A. opisthomelas, A. bombetes,* and *A. cassidyhornae* suggest that morphometrics is not a tool that can aid in the solving of the taxonomy of the genus.

To evaluate the potentially confounding effect of sexual dimorphism on the morphometric analysis, we conducted a separate PCA using only sexed specimens (Appendix S2). Due to insufficient numbers of sexed individuals, this assessment was only possible for OTU2 (*n* = 91); OTU1, OTU3, and the topotypes lacked adequate sexed samples for this analysis. Within OTU2, males and females showed extensive overlap across both PC1 and PC2, with no evidence of consistent sex-based segregation in morphospace. Additionally, a PCA including all sexed individuals across OTUs revealed a similar pattern of broad overlap between sexes. These results suggest that sexual dimorphism does not represent a major source of morphometric variation in this dataset, supporting the inclusion of all specimens regardless of sex in the primary analysis. Nevertheless, we acknowledge that the absence of sufficient sexed material for OTU1, OTU3, and the topotypes prevents a definitive assessment of sexual dimorphism in these groups, and this remains a limitation of the present study.

As with other clades inside Dendrobatoidea, species of *Andinobates* are highly polytypic, with some species having individuals of the same population exhibiting a wide range of variations in the same character. These levels of variation make it difficult to understand the taxonomic diversity inside the genus, thus any taxonomic decision must be taken cautiously.

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ONLINE SUPPLEMENTARY INFORMATION

The following Supporting Information is available for this article online:

Appendix S1. Morphometric statistical comparisons among OTUs.

Appendix S2. Assessment of sexual dimorphism effects on morphometric variation.

APPENDIX 1: MATERIAL EXAMINED

Countries (uppercase), departments (bold), municipalities (underlined), and catalog numbers (plain text separated by commas) are listed in alphabetical order. Museum acronyms follow Frost (2023) and Sabaj (2020) as specified in the Materials and Methods section.

Andinobates bombetes (*n* = 14): COLOMBIA: **Valle del Cauca:** Yotoco: CSJ-h 2034–2041, CSJ-h 2043–2045, CSJ-h 2048–2050.

Andinobates cassydihornae (*n* = 18): COLOMBIA: **Antioquia:** Andes: LACM 71962–71970, MHUA-A 00510–00518, MHUA-A 06167–68.

Andinobates opisthomelas (*n* = 294): COLOMBIA: **Antioquia:** Alejandría: MHUA-A 13347 Amalfi: CSJ-h 2178, MHUA-A 00114–00115, MHUA-A 01509, MHUA-A 01953–01954, MHUA-A 01956–01957, MHUA-A 01959–01962, MHUA-A 01965–01966, MHUA-A 02940–02982, MHUA-A 02988, MHUA-A 03077–03079, MHUA-A 03085, MHUA-A 03095, MHUA-A 03138, MHUA-A 04374, MHUA-A 09427, MHUA-A 09433, MHUA-A 09440, MHUA-A 09447–09455, MHUA-A 09457–09465, MHUA-A 09467–09470, MHUA-A 09472, MHUA-A 09474, MHUA-A 09476–09479, MHUA-A 09482, MHUA-A 09486, MHUA-A 09488, MHUA-A 09492–09493; Angostura: MHUA-A 07766; Anorí: MHUA-A 01955, MHUA-A 01963–01964, MHUA-A 02763–02765, MHUA-A 02927–02939, MHUA-A 02983–02987, MHUA-A 03216, MHUA-A 03307, MHUA-A 03309–03310, MHUA-A 03312–03313, MHUA-A 03315–03320, MHUA-A 03950–03954, MHUA-A 04101–04102, MHUA-A 04138, MHUA-A 04317, MHUA-A 05344–05349, MHUA-A 11398, MHUA-A 11728–11729, MHUA-A 11740, MHUA-A 13020; Argelia de Maria: MHUA-A 11237–11238; Carolina del Príncipe: CSJ-h 2165, CSJ-h 2167, CSJ-h 2169, MHUA-A 02660–02661, MHUA-A 12149–12150, MHUA-A 13219, MHUA-A 13292; Cocorná: MHUA-A 10264; Concepción: MHUA-A 13299, MHUA-A 13313–13314; Don Matías: MHUA-A 12180–81, MHUA-A 13225, MHUA-A 13231; El Carmen de Viboral: MHUA-A 10256, MHUA-A 10263, MHUA-A 12357–12362; Fredonia: MHUA-A 12374–12386; Granada: MHUA-A 10697–10705, MHUA-A 10817, MHUA-A 12739–12740; Guatapé: MHUA-A 00096, MHUA-A 00103, MHUA-A 00154, MHUA-A 00159, MHUA-A 00605–00608, MHUA-A 00756–00757, MHUA-A 00760, MHUA-A 01000, MHUA-A 02567–02570, MHUA-A 04115–04116, MHUA-A 04675, MHUA-A 08039, MHUA-A 08041–08043, MHUA-A 08045, MHUA-A 08049–08050, MHUA-A 08054, MHUA-A 08085, MHUA-A 08090, MHUA-A 08096, MHUA-A 08101, MHUA-A 08322, MHUA-A 08821, MHUA-A 11386; Rionegro: MHUA-A 10994–10995; Llano Grande: LACM 43898–43899; San Rafael: LACM 43880-43897, LACM 61067; Santo Domingo: EAFIT-Am 0670–0685; Valdivia: CBUCES-D 1299–1312, CBUCES-D 335, CBUCES-D 869, CBUCES-D 871–878, MHUA-A 07012–07014, BMNH 1947.2.15.21–1947.2.15.29, BMNH 1947.2.15.31–1947.2.15.34; Yarumal: CSJ-h 2179–2182, MHUA-A 00148, MHUA-A 04386, MHUA-A 06802–06805, MHUA-A 12363–12365, MHUA-A 12373.

APPENDIX 2

Coordinates, in decimal degrees, from topotypical material of *Andinobates opisthomelas* sensu stricto examined in this study. Recorded individuals are indicated by an astrisk (*).

COLOMBIA: **Antioquia:** Yarumal: 7.025824, -75.404928 (CSJ-h 2179–2182); 7.068611, -75.418889 (MHUA-A 00148, 04386); 7.075923, -75.421706 (MHUA-A 06802–06805); 7.0614667, -75.40415 (MHUA-A 12363–12365); 7.072391 -75.459573 (MHUA-A 12373); Valdivia: 7.108124, -75.4711858 (CBUCES-D 335, 869, 871 (BSOC011_002)*–873*–877*, 878, 1299 (BSOC011_001)*–1312); 7.088877, -75.473911 (MHUA-A 07012–07014).

Appendix S1. Morphometric statistical analyses among OTUs

This appendix summarizes the statistical analyses conducted to evaluate morphometric differences among Operational Taxonomic Units (OTUs) and reference taxa. We performed one-way analyses of variance (ANOVA) for each morphometric variable, followed by pairwise comparisons using t-tests with Bonferroni correction.

Table S1. One-way ANOVA for morphometric variables across OTUs (df between = between-group degrees of freedom; df within = within-group degrees of freedom; F = F statistic; p = p-value).

Variable	df between	df within	F value	p-value
SVL	5	288	13.61	<0.001
IND	5	285	9.48	<0.001
UE	5	281	4.99	<0.001
IOD	5	284	8.25	<0.001
HW	5	285	9.61	<0.001
HL	5	286	3.01	0.011
SL	5	285	16.38	<0.001
NS	5	285	13.39	<0.001
EN	5	285	14.48	<0.001
TNL	5	285	13.85	<0.001
TML	5	286	12.50	<0.001
NML	5	283	4.84	<0.001
EL	5	286	15.48	<0.001
TED	5	286	8.05	<0.001
HTYD	5	286	7.82	<0.001
VTYD	5	286	4.03	0.0015
TL	5	286	17.90	<0.001

THL	5	286	8.32	<0.001
HaL	5	280	10.55	<0.001
FOL	5	285	10.59	<0.001
HUL	5	275	5.68	<0.001
W4FD	5	281	9.02	<0.001
W4F	5	280	9.03	<0.001
FOT	5	264	10.69	<0.001
W3TD	5	265	5.81	<0.001
W3T	5	264	3.37	0.0057
W4TD	5	257	8.54	<0.001
W4T	5	257	7.60	<0.001

Table S2. Pairwise t-tests (Bonferroni-adjusted p-values) among OTUs in long format (Group 1 vs Group 2; p = adjusted p-value; Significance: ns, *, **, ***).

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
SVL	OTU1	A. bombetes A. cassydihornae OTU1	0.00091	***
SVL	OTU1	OTU2	1.00000	ns
SVL	OTU2	A. bombetes A. cassydihornae OTU1	7.9e-09	***
SVL	OTU2	OTU2	0.00468	**
SVL	OTU2	OTU3	0.00110 -	ns
SVL	OTU3	A. bombetes A. cassydihornae OTU1	0.53474	ns

Variable	Group 1	Group 2	p-value	Significance
SVL	OTU3	OTU2	1.00000	ns
SVL	OTU3	OTU3	1.00000 0.01149 -	ns
SVL	Topotypes	A. bombetes A. cassydihornae OTU1	0.01362	*
SVL	Topotypes	OTU2	1.00000	ns
SVL	Topotypes	OTU3	1.00000 0.00125 1.00000	ns
IND	OTU1	A. bombetes A. cassydihornae OTU1	2.8e-06	***
IND	OTU1	OTU2	1.00000	ns
IND	OTU2	A. bombetes A. cassydihornae OTU1	3.1e-09	***
IND	OTU2	OTU2	1.00000	ns
IND	OTU2	OTU3	1.00000 -	ns
IND	OTU3	A. bombetes A. cassydihornae OTU1	0.00064	***
IND	OTU3	OTU2	1.00000	ns
IND	OTU3	OTU3	1.00000 1.00000 -	ns
IND	Topotypes	A. bombetes A. cassydihornae OTU1	0.00052	***
IND	Topotypes	OTU2	1.00000	ns

Variable	Group 1	Group 2	p-value	Significance
IND	Topotypes	OTU3	1.00000 0.08365 1.00000	ns
UE	OTU1	A. bombetes A. cassydihornae OTU1	1.000	ns
UE	OTU1	OTU2	0.051	ns
UE	OTU2	A. bombetes A. cassydihornae OTU1	0.069	ns
UE	OTU2	OTU2	1.000	ns
UE	OTU2	OTU3	0.052 -	ns
UE	OTU3	A. bombetes A. cassydihornae OTU1	1.000	ns
UE	OTU3	OTU2	0.532	ns
UE	OTU3	OTU3	1.000 1.000 -	ns
UE	Topotypes	A. bombetes A. cassydihornae OTU1	1.000	ns
UE	Topotypes	OTU2	0.032	*
UE	Topotypes	OTU3	1.000 0.054 1.000	ns
IOD	OTU1	A. bombetes A. cassydihornae OTU1	0.16796	ns
IOD	OTU1	OTU2	0.63418	ns

Variable	Group 1	Group 2	p-value	Significance
IOD	OTU2	A. bombetes A. cassydihornae OTU1	0.00031	***
IOD	OTU2	OTU2	0.00273	**
IOD	OTU2	OTU3	0.03319 -	ns
IOD	OTU3	A. bombetes A. cassydihornae OTU1	1.00000	ns
IOD	OTU3	OTU2	1.00000	ns
IOD	OTU3	OTU3	1.00000 0.07892 -	ns
IOD	Topotypes	A. bombetes A. cassydihornae OTU1	0.00152	**
IOD	Topotypes	OTU2	0.00917	**
IOD	Topotypes	OTU3	0.28444 1.00000 0.14143	ns
HW	OTU1	A. bombetes A. cassydihornae OTU1	0.0040	**
HW	OTU1	OTU2	1.0000	ns
HW	OTU2	A. bombetes A. cassydihornae OTU1	7.1e-08	***
HW	OTU2	OTU2	1.0000	ns
HW	OTU2	OTU3	0.0010 -	ns

Variable	Group 1	Group 2	p-value	Significance
HW	OTU3	A. bombetes A. cassydihornae OTU1	0.0638	ns
HW	OTU3	OTU2	1.0000	ns
HW	OTU3	OTU3	1.0000 0.6929 -	ns
HW	Topotypes	A. bombetes A. cassydihornae OTU1	0.0015	**
HW	Topotypes	OTU2	1.0000	ns
HW	Topotypes	OTU3	1.0000 0.2953 1.0000	ns
HL	OTU1	A. bombetes A. cassydihornae OTU1	0.081	ns
HL	OTU1	OTU2	1.000	ns
HL	OTU2	A. bombetes A. cassydihornae OTU1	0.017	*
HL	OTU2	OTU2	1.000	ns
HL	OTU2	OTU3	1.000 -	ns
HL	OTU3	A. bombetes A. cassydihornae OTU1	1.000	ns
HL	OTU3	OTU2	1.000	ns
HL	OTU3	OTU3	1.000 1.000 -	ns
HL	Topotypes	A. bombetes A. cassydihornae OTU1	0.605	ns

Variable	Group 1	Group 2	p-value	Significance
HL	Topotypes	OTU2	1.000	ns
HL	Topotypes	OTU3	1.000 1.000 1.000	ns
SL	OTU1	A. bombetes A. cassydihornae OTU1	5.5e-09	***
SL	OTU1	OTU2	1.00000	ns
SL	OTU2	A. bombetes A. cassydihornae OTU1	7.8e-15	***
SL	OTU2	OTU2	0.76572	ns
SL	OTU2	OTU3	0.01592 -	ns
SL	OTU3	A. bombetes A. cassydihornae OTU1	0.00028	***
SL	OTU3	OTU2	1.00000	ns
SL	OTU3	OTU3	1.00000 0.18282 -	ns
SL	Topotypes	A. bombetes A. cassydihornae OTU1	5.4e-08	***
SL	Topotypes	OTU2	1.00000	ns
SL	Topotypes	OTU3	1.00000 0.14030 1.00000	ns
NS	OTU1	A. bombetes A. cassydihornae OTU1	6.6e-07	***
NS	OTU1	OTU2	1.000	ns

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
NS	OTU2	A. bombetes A. cassydihornae OTU1	2.5e-12	***
NS	OTU2	OTU2	1.000	ns
NS	OTU2	OTU3	0.011 -	ns
NS	OTU3	A. bombetes A. cassydihornae OTU1	4.7e-05	***
NS	OTU3	OTU2	1.000	ns
NS	OTU3	OTU3	1.000 1.000 -	ns
NS	Topotypes	A. bombetes A. cassydihornae OTU1	3.5e-06	***
NS	Topotypes	OTU2	1.000	ns
NS	Topotypes	OTU3	1.000 0.118 1.000	ns
EN	OTU1	A. bombetes A. cassydihornae OTU1	6.4e-11	***
EN	OTU1	OTU2	0.05995	ns
EN	OTU2	A. bombetes A. cassydihornae OTU1	6.6e-12	***
EN	OTU2	OTU2	0.04068	*
EN	OTU2	OTU3	1.00000 -	ns
EN	OTU3	A. bombetes A. cassydihornae OTU1	0.00032	***
EN	OTU3	OTU2	1.00000	ns

Variable	Group 1	Group 2	p-value	Significance
EN	OTU3	OTU3	1.00000 1.00000 -	ns
EN	Topotypes	A. bombetes A. cassydihornae OTU1	2.8e-05	***
EN	Topotypes	OTU2	1.00000	ns
EN	Topotypes	OTU3	0.06371 0.02504 1.00000	ns
TNL	OTU1	A. bombetes A. cassydihornae OTU1	0.017	*
TNL	OTU1	OTU2	0.024	*
TNL	OTU2	A. bombetes A. cassydihornae OTU1	5.1e-06	***
TNL	OTU2	OTU2	8.2e-06	***
TNL	OTU2	OTU3	0.016 -	ns
TNL	OTU3	A. bombetes A. cassydihornae OTU1	1.000	ns
TNL	OTU3	OTU2	1.000	ns
TNL	OTU3	OTU3	1.000 0.016	ns
TNL	Topotypes	A. bombetes A. cassydihornae OTU1	1.000	ns
TNL	Topotypes	OTU2	1.000	ns
TNL	Topotypes	OTU3	0.533 1.4e-05 1.000	ns

Variable	Group 1	Group 2	p-value	Significance
TML	OTU1	A. bombetes A. cassydihornae OTU1	1.0000	ns
TML	OTU1	OTU2	1.0000	ns
TML	OTU2	A. bombetes A. cassydihornae OTU1	0.0149	*
TML	OTU2	OTU2	0.0064	**
TML	OTU2	OTU3	3.6e-07 -	ns
TML	OTU3	A. bombetes A. cassydihornae OTU1	0.4733	ns
TML	OTU3	OTU2	0.3036	ns
TML	OTU3	OTU3	0.2026	ns
TML	Topotypes	A. bombetes A. cassydihornae OTU1	1.0000	ns
TML	Topotypes	OTU2	1.0000	ns
TML	Topotypes	OTU3	1.0000	ns
NML	OTU1	A. bombetes A. cassydihornae OTU1	0.01198	*
NML	OTU1	OTU2	1.00000	ns
NML	OTU2	A. bombetes A. cassydihornae OTU1	0.00013	***
NML	OTU2	OTU2	1.00000	ns
NML	OTU2	OTU3	1.00000 -	ns

Variable	Group 1	Group 2	p-value	Significance
NML	OTU3	A. bombetes A. cassydihornae OTU1	0.05042	ns
NML	OTU3	OTU2	1.00000	ns
NML	OTU3	OTU3	1.00000 1.00000 -	ns
NML	Topotypes	A. bombetes A. cassydihornae OTU1	0.07045	ns
NML	Topotypes	OTU2	1.00000	ns
NML	Topotypes	OTU3	1.00000 0.70914 1.00000	ns
EL	OTU1	A. bombetes A. cassydihornae OTU1	0.3048	ns
EL	OTU1	OTU2	1.0000	ns
EL	OTU2	A. bombetes A. cassydihornae OTU1	3.0e-07	***
EL	OTU2	OTU2	0.0865	ns
EL	OTU2	OTU3	2.3e-08 -	ns
EL	OTU3	A. bombetes A. cassydihornae OTU1	1.0000	ns
EL	OTU3	OTU2	1.0000	ns
EL	OTU3	OTU3	1.0000	ns

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
EL	Topotypes	A. bombetes A. cassydihornae OTU1	0.2773	ns
EL	Topotypes	OTU2	1.0000	ns
EL	Topotypes	OTU3	1.0000	ns
TED	OTU1	A. bombetes A. cassydihornae OTU1	8.8e-05	***
TED	OTU1	OTU2	1.0000	ns
TED	OTU2	A. bombetes A. cassydihornae OTU1	4.5e-06	***
TED	OTU2	OTU2	1.0000	ns
TED	OTU2	OTU3	1.0000 -	ns
TED	OTU3	A. bombetes A. cassydihornae OTU1	0.1810	ns
TED	OTU3	OTU2	1.0000	ns
TED	OTU3	OTU3	1.0000 1.0000 -	ns
TED	Topotypes	A. bombetes A. cassydihornae OTU1	0.2704	ns
TED	Topotypes	OTU2	1.0000	ns
TED	Topotypes	OTU3	0.0528 0.0021 1.0000	ns
HTYD	OTU1	A. bombetes A. cassydihornae OTU1	5.6e-08	***

Variable	Group 1	Group 2	p-value	Significance
HTYD	OTU1	OTU2	1.00000	ns
HTYD	OTU2	A. bombetes A. cassydihornae OTU1	2.1e-06	***
HTYD	OTU2	OTU2	1.00000	ns
HTYD	OTU2	OTU3	0.86282 -	ns
HTYD	OTU3	A. bombetes A. cassydihornae OTU1	3.3e-05	***
HTYD	OTU3	OTU2	1.00000	ns
HTYD	OTU3	OTU3	1.00000 1.00000 -	ns
HTYD	Topotypes	A. bombetes A. cassydihornae OTU1	0.00011	***
HTYD	Topotypes	OTU2	1.00000	ns
HTYD	Topotypes	OTU3	1.00000 1.00000 1.00000	ns
VTYD	OTU1	A. bombetes A. cassydihornae OTU1	0.0013	**
VTYD	OTU1	OTU2	0.5415	ns
VTYD	OTU2	A. bombetes A. cassydihornae OTU1	0.1106	ns
VTYD	OTU2	OTU2	1.0000	ns
VTYD	OTU2	OTU3	0.0725 -	ns

Variable	Group 1	Group 2	p-value	Significance
VTYD	OTU3	A. bombetes A. cassydihornae OTU1	0.3350	ns
VTYD	OTU3	OTU2	1.0000	ns
VTYD	OTU3	OTU3	1.0000 1.0000 -	ns
VTYD	Topotypes	A. bombetes A. cassydihornae OTU1	0.0316	*
VTYD	Topotypes	OTU2	1.0000	ns
VTYD	Topotypes	OTU3	1.0000 1.0000 1.0000	ns
TL	OTU1	A. bombetes A. cassydihornae OTU1	5.8e-06	***
TL	OTU1	OTU2	1.00000	ns
TL	OTU2	A. bombetes A. cassydihornae OTU1	4.5e-12	***
TL	OTU2	OTU2	0.00181	**
TL	OTU2	OTU3	0.00083 -	ns
TL	OTU3	A. bombetes A. cassydihornae OTU1	0.00027	***
TL	OTU3	OTU2	1.00000	ns
TL	OTU3	OTU3	1.00000 1.00000 -	ns

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
TL	Topotypes	A. bombetes A. cassydihornae OTU1	0.00698	**
TL	Topotypes	OTU2	1.00000	ns
TL	Topotypes	OTU3	0.68982 1.1e-06 1.00000	ns
THL	OTU1	A. bombetes A. cassydihornae OTU1	0.08003	ns
THL	OTU1	OTU2	0.11207	ns
THL	OTU2	A. bombetes A. cassydihornae OTU1	0.00077	***
THL	OTU2	OTU2	0.00124	**
THL	OTU2	OTU3	0.46400 -	ns
THL	OTU3	A. bombetes A. cassydihornae OTU1	1.00000	ns
THL	OTU3	OTU2	1.00000	ns
THL	OTU3	OTU3	1.00000 0.16204 -	ns
THL	Topotypes	A. bombetes A. cassydihornae OTU1	1.00000	ns
THL	Topotypes	OTU2	1.00000	ns
THL	Topotypes	OTU3	0.92962 0.00234 1.00000	ns

Variable	Group 1	Group 2	p-value	Significance
HaL	OTU1	A. bombetes A. cassydihornae OTU1	0.04806	*
HaL	OTU1	OTU2	0.91229	ns
HaL	OTU2	A. bombetes A. cassydihornae OTU1	0.00027	***
HaL	OTU2	OTU2	0.01409	*
HaL	OTU2	OTU3	0.12508 -	ns
HaL	OTU3	A. bombetes A. cassydihornae OTU1	0.53216	ns
HaL	OTU3	OTU2	1.00000	ns
HaL	OTU3	OTU3	1.00000 1.00000 -	ns
HaL	Topotypes	A. bombetes A. cassydihornae OTU1	1.00000	ns
HaL	Topotypes	OTU2	1.00000	ns
HaL	Topotypes	OTU3	0.02461 5e-07	ns
FOL	OTU1	A. bombetes A. cassydihornae OTU1	1.00000	ns
FOL	OTU1	OTU2	0.12502	ns
FOL	OTU2	A. bombetes A. cassydihornae OTU1	0.01862	*
FOL	OTU2	OTU2	0.00047	***
FOL	OTU2	OTU3	0.08208 -	ns

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
FOL	OTU3	A. bombetes A. cassydihornae OTU1	1.00000	ns
FOL	OTU3	OTU2	1.00000	ns
FOL	OTU3	OTU3	0.38853 0.00275 -	ns
FOL	Topotypes	A. bombetes A. cassydihornae OTU1	1.00000	ns
FOL	Topotypes	OTU2	1.00000	ns
FOL	Topotypes	OTU3	0.17108 9.7e-06 1.00000	ns
HUL	OTU1	A. bombetes A. cassydihornae OTU1	0.0022	**
HUL	OTU1	OTU2	0.7019	ns
HUL	OTU2	A. bombetes A. cassydihornae OTU1	7.9e-05	***
HUL	OTU2	OTU2	0.1335	ns
HUL	OTU2	OTU3	1.0000 -	ns
HUL	OTU3	A. bombetes A. cassydihornae OTU1	0.0699	ns
HUL	OTU3	OTU2	1.0000	ns
HUL	OTU3	OTU3	1.0000 1.0000 -	ns

Variable	Group 1	Group 2	p-value	Significance
HUL	Topotypes	A. bombetes A. cassydihornae OTU1	0.0703	ns
HUL	Topotypes	OTU2	1.0000	ns
HUL	Topotypes	OTU3	1.0000 0.5290 1.0000	ns
W4FD	OTU1	A. bombetes A. cassydihornae OTU1	0.0941	ns
W4FD	OTU1	OTU2	1.0000	ns
W4FD	OTU2	A. bombetes A. cassydihornae OTU1	4.3e-05	***
W4FD	OTU2	OTU2	0.0038	**
W4FD	OTU2	OTU3	0.0081 -	ns
W4FD	OTU3	A. bombetes A. cassydihornae OTU1	1.0000	ns
W4FD	OTU3	OTU2	1.0000	ns
W4FD	OTU3	OTU3	1.0000 0.0255 -	ns
W4FD	Topotypes	A. bombetes A. cassydihornae OTU1	0.1299	ns
W4FD	Topotypes	OTU2	1.0000	ns
W4FD	Topotypes	OTU3	1.0000 0.1070 1.0000	ns

Variable	Group 1	Group 2	p-value	Significance
W4F	OTU1	A. bombetes A. cassydihornae OTU1	0.0089	**
W4F	OTU1	OTU2	0.0115	*
W4F	OTU2	A. bombetes A. cassydihornae OTU1	3.5e-05	***
W4F	OTU2	OTU2	5.0e-05	***
W4F	OTU2	OTU3	0.4736 -	ns
W4F	OTU3	A. bombetes A. cassydihornae OTU1	1.0000	ns
W4F	OTU3	OTU2	1.0000	ns
W4F	OTU3	OTU3	1.0000 0.2487 -	ns
W4F	Topotypes	A. bombetes A. cassydihornae OTU1	0.0134	*
W4F	Topotypes	OTU2	0.0168	*
W4F	Topotypes	OTU3	1.0000 1.0000 1.0000	ns
FOT	OTU1	A. bombetes A. cassydihornae OTU1	0.0170	*
FOT	OTU1	OTU2	0.9126	ns
FOT	OTU2	A. bombetes A. cassydihornae OTU1	2.1e-05	***
FOT	OTU2	OTU2	0.0077	**

Variable	Group 1	Group 2	p-value	Significance
FOT	OTU2	OTU3	0.0700 -	ns
FOT	OTU3	A. bombetes A. cassydihornae OTU1	1.0000	ns
FOT	OTU3	OTU2	1.0000	ns
FOT	OTU3	OTU3	1.0000 0.0407	ns
FOT	Topotypes	A. bombetes A. cassydihornae OTU1	1.0000	ns
FOT	Topotypes	OTU2	1.0000	ns
FOT	Topotypes	OTU3	0.4072 4.3e-05 1.0000	ns
W3TD	OTU1	A. bombetes A. cassydihornae OTU1	0.62118	ns
W3TD	OTU1	OTU2	0.02218	*
W3TD	OTU2	A. bombetes A. cassydihornae OTU1	0.05888	ns
W3TD	OTU2	OTU2	0.00062	***
W3TD	OTU2	OTU3	1.00000 -	ns
W3TD	OTU3	A. bombetes A. cassydihornae OTU1	1.00000	ns
W3TD	OTU3	OTU2	1.00000	ns
W3TD	OTU3	OTU3	1.00000 0.30787 -	ns

Variable	Group 1	Group 2	p-value	Significance
W3TD	Topotypes	A. bombetes A. cassydihornae OTU1	1.00000	ns
W3TD	Topotypes	OTU2	0.44296	ns
W3TD	Topotypes	OTU3	1.00000 0.23233 1.00000	ns
W3T	OTU1	A. bombetes A. cassydihornae OTU1 OTU2 OTU3	1.00	ns
W3T	OTU2	A. bombetes A. cassydihornae OTU1 OTU2 OTU3	0.12	ns
W3T	OTU3	A. bombetes A. cassydihornae OTU1 OTU2 OTU3	1.00	ns
W3T	Topotypes	A. bombetes A. cassydihornae OTU1 OTU2 OTU3	1.00	ns
W4TD	OTU1	A. bombetes A. cassydihornae OTU1	0.0016	**
W4TD	OTU1	OTU2	0.0421	*
W4TD	OTU2	A. bombetes A. cassydihornae OTU1	3e-05	***
W4TD	OTU2	OTU2	0.0016	**

Variable	Group 1	Group 2	p-value	Significance
W4TD	OTU2	OTU3	1.0000 -	ns
W4TD	OTU3	A. bombetes A. cassydihornae OTU1	1.0000	ns
W4TD	OTU3	OTU2	1.0000	ns
W4TD	OTU3	OTU3	0.2361 0.0173 -	ns
W4TD	Topotypes	A. bombetes A. cassydihornae OTU1	0.0067	**
W4TD	Topotypes	OTU2	0.1138	ns
W4TD	Topotypes	OTU3	1.0000 1.0000 0.4707	ns
W4T	OTU1	A. bombetes A. cassydihornae OTU1	0.01926	*
W4T	OTU1	OTU2	0.17340	ns
W4T	OTU2	A. bombetes A. cassydihornae OTU1	0.00016	***
W4T	OTU2	OTU2	0.00284	**
W4T	OTU2	OTU3	0.71706 -	ns
W4T	OTU3	A. bombetes A. cassydihornae OTU1	1.00000	ns
W4T	OTU3	OTU2	1.00000	ns
W4T	OTU3	OTU3	0.80958 0.03197 -	ns

Variable	**Group 1**	**Group 2**	**p-value**	**Significance**
W4T	Topotypes	A. bombetes A. cassydihornae OTU1	0.00877	**
W4T	Topotypes	OTU2	0.07720	ns
W4T	Topotypes	OTU3	1.00000 1.00000 0.37497	ns

Appendix S2. Assessment of sexual dimorphism effects on morphometric variation.

To address the potential confounding effect of sexual dimorphism on morphometric comparisons among OTUs, we assessed morphological variation between males and females using PCA. Only 46.1% of the 297 specimens in the dataset could be sexed (137 of 297). The proportion of sexed specimens varied considerably among OTUs: A. bombetes had no sexed specimens (0%, n = 14), Topotypes had only 6 sexed individuals (16.2%, n = 37), A. cassydihornae had 6 sexed individuals (42.9%, n = 14), OTU1 had 25 sexed individuals but with a highly unbalanced sex ratio (17 males, 8 females; 38.5%, n = 65), and OTU3 had only 9 sexed individuals (69.2%, n = 13). Only OTU2 had sufficient and adequately balanced numbers of sexed specimens (33 males, 58 females; 59.1%, n = 154) to meet the assumptions required for PCA, confirmed through Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests. Consequently, two complementary analyses were performed: a PCA including all sexed specimens across OTUs (Figure S2.1), and a PCA restricted to OTU2 sexed specimens (Figure S2.2). In both analyses, males and females showed extensive overlap in morphological space, indicating that sexual dimorphism does not confound the among-OTU comparisons presented in the main text.

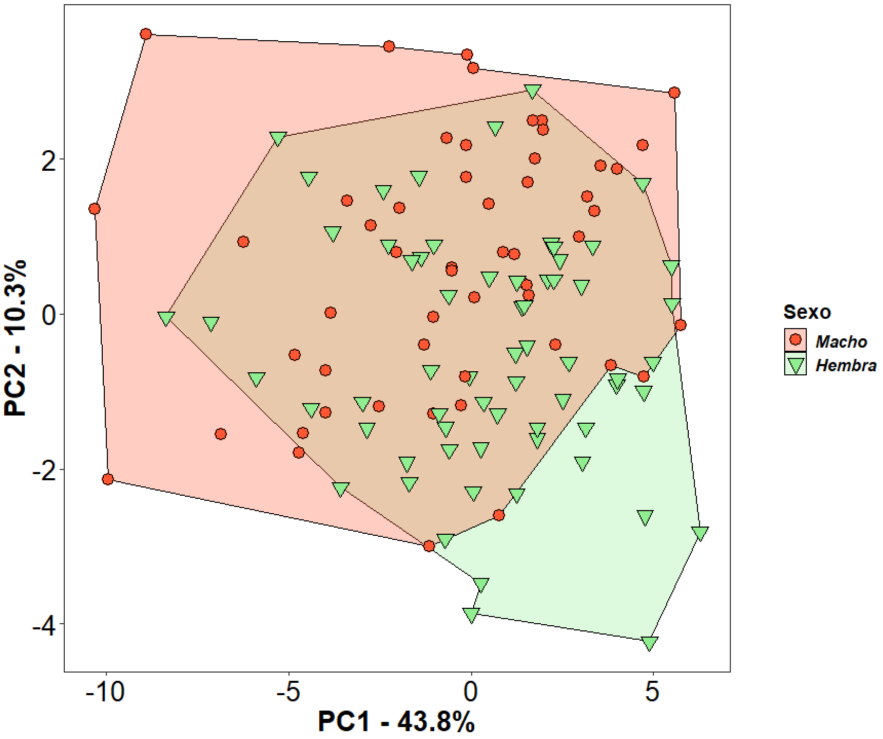

Figure S1. Bivariate plot of the first two components (PC1 and PC2) from a principal components analysis using a correlation matrix of 28 morphological variables for all sexed specimens across OTUs.

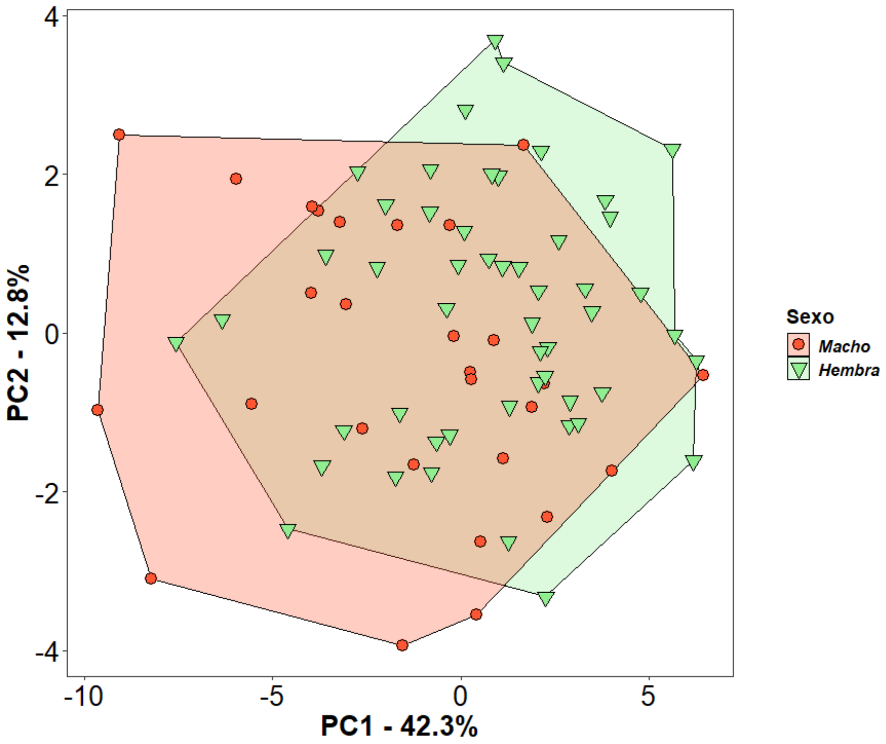

Figure S2. Bivariate plot of the first two components (PC1 and PC2) from a principal components analysis using a correlation matrix of 28 morphological variables restricted to sexed specimens of OTU2.