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Sistemática de *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 y *A. lemniscatus* Boulenger 1898 (Squamata, Sauria, Anolidae) del Ecuador

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DECLARACIÓN DE APORTES DE TERCEROS

En la elaboración de esta tesis titulada "*Sistemática de Anolis binotatus Peters 1863, A. bitectus Cope 1864 y A. lemniscatus Boulenger 1898 (Squamata, Sauria, Anolidae) del Ecuador*", he contado con la colaboración y apoyo de diversas personas e instituciones, cuyas contribuciones han sido fundamentales para el desarrollo y culminación de este trabajo de investigación. A continuación, detallo sus aportes específicos:

1. **Dr. Omar Torres-Carvajal, Director de tesis**. Supervisión y orientación académica durante todo el proceso de investigación. Revisión crítica, edición y sugerencias para la estructura y contenido del manuscrito. Enseñanza de herramientas bioinformáticas para la elaboración de análisis filogenéticos.
2. **Dr. Santiago Ron.** Museo de Zoología QCAZ. Lector de tesis. Comentarios y sugerencias del plan de tesis.
3. **Dr. Santiago Burneo.** Museo de Zoología QCAZ. Lector de tesis. Comentarios y sugerencias del plan de tesis.
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5. **Dr. Steven Poe**. Universidad de New Mexico, Estados Unidos. Aporte con la matriz de secuencias de los genes mitocondriales y nucleares para todas las especies del clado Draconura. Revisión, edición y sugerencias del manuscrito.
6. **Máster Valentina Posse**. Instituto Nacional de Biodiversidad (INABIO). Preparación de hemipenes y elaboración de resultados sobre la variación morfológica de los hemipenes. Revisión y sugerencias del manuscrito.
7. **Axel Fläschendräger.** Universidad Martín Lutero de Halle-Wittenberg, Alemania. Revisión de caracteres y fotografía de especímenes tipo. Revisión y sugerencias del manuscrito.

Agradezco profundamente a todas las personas e instituciones mencionadas por su valiosa colaboración y apoyo, sin los cuales esta investigación no habría sido posible.

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A todos ustedes, este logro es también suyo.

RESUMEN

Hace más de un siglo se describieron tres especies de lagartijas con una morfología similar en el Ecuador: *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 y *A. lemniscatus* Boulenger 1898. Nosotros estudiamos la genética, el material tipo y la variación morfológica en toda el área de distribución de estas formas para evaluar el estatus taxonómico de estos nombres. Nuestros análisis filogenéticos, basados en los genes COI, ND2 y RAG1, revelaron la existencia de tres clados geográficamente estructurados (norte, central y sur) similares en morfología y coloración, que no correspondían a las tres especies en cuestión. Del mismo modo, no encontramos ninguna justificación para reconocer estas tres especies basándonos en el examen del material tipo y en las comparaciones de morfología a lo largo del rango geográfico de los especímenes asignados a las tres especies. Aunque había diferencias significativas en el número de escamas entre la interparietal y los semicírculos supraorbitales entre los clados moleculares recuperados, no consideramos que estas diferencias sean diagnósticas. Por lo tanto, proponemos que *A. binotatus* es un sinónimo senior de *A. bitectus* y de *A. lemniscatus*. Proporcionamos una nueva descripción de *A. binotatus*.

Palabras clave: Draconura, filogenia, Norops, sinonimia, tipos.

ABSTRACT

More than a century ago, three species of lizard with similar morphology were described from Ecuador: *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 and *A. lemniscatus* Boulenger 1898. We studied the genetics, type material and morphological variation throughout the range of these forms to evaluate the taxonomic status of these names. Our phylogenetic analyses based on COI, ND2 and RAG1 genes revealed three geographically structured clades (northern, central and southern) similar in morphology and coloration that did not correspond to the three species in question. Similarly, we found no justification for recognizing all three species based on examination of type material and comparisons of morphology across the geographic range of specimens assigned to the three species. Although there were significant differences in the number of scales between the interparietal and supraorbital semicircles among recovered molecular clades, we do not consider these differences as diagnostic. Therefore, we propose that *A. binotatus* is a senior synonym of both *A. bitectus* and *A. lemniscatus*. We provide a new description of *A. binotatus*.

Keywords: Draconura, Norops, phylogeny, synonymy, types.

OBJETIVO GENERAL

Determinar en base a evidencia morfológica y filogenética la validez taxonómica de *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 y *A. lemniscatus* Boulenger 1898.

OBJETIVOS ESPECÍFICOS

- Determinar la posición filogenética de *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 y *A. lemniscatus* Boulenger 1898.

- Describir la morfología de las especies validadas en el estudio.

- En caso de que se concluya que dos o más especies son la misma especie, proponer una sinonimia.

DESCRIPCIÓN DEL MANUSCRITO

El presente manuscrito, titulado "*Systematics of Ecuadorian anoles: Anolis bitectus Cope, 1864 and A. lemniscatus Boulenger, 1898, two junior synonyms of A. binotatus Peters, 1863 (Squamata, Iguanidae, Anolinae)*", ha sido presentado para consideración de publicación de la Revista **Vertebrate Zoology**. El manuscrito ha sido escrito siguiendo los lineamientos de la revista que se encuentran disponibles en el siguiente **enlace**.

La revista Vertebrate Zoology se encuentra indexada en la base de datos de Scopus, de acuerdo a la información del siguiente **enlace**.

MANUSCRITO

A continuación, a manera de anexo, se incluye en manuscrito enviado a la revista científica Vertebrate Zoology.

Systematics of Ecuadorian anoles: *Anolis bitectus* Cope, 1864 and *A. lemniscatus* Boulenger, 1898, two junior synonyms of *A. binotatus* Peters, 1863 (Squamata, Iguanidae, Anolinae)

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Journal: Vertebrate Zoology

ABSTRACT

More than a century ago, three species of lizard with similar morphology were described from Ecuador: *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 and *A. lemniscatus* Boulenger 1898. We studied the genetics, type material and morphological variation throughout the range of these forms to evaluate the taxonomic status of these names. Our phylogenetic analyses based on COI, ND2 and RAG1 genes revealed three geographically structured clades (northern, central and southern) similar in morphology and coloration that did not correspond to the three species in question. Similarly, we found no justification for recognizing all three species based on examination of type material and comparisons of morphology across the geographic range of specimens assigned to the three species. Although there were significant differences in the number of scales between the interparietal and supraorbital semicircles among recovered molecular clades, we do not consider these differences as diagnostic. Therefore, we propose that *A. binotatus* is a senior synonym of both *A. bitectus* and *A. lemniscatus*. We provide a new description of *A. binotatus*.

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Hace más de un siglo se describieron tres especies de lagartijas con una morfología similar en el Ecuador: *Anolis binotatus* Peters 1863, *A. bitectus* Cope 1864 y *A. lemniscatus* Boulenger 1898. Nosotros estudiamos la genética, el material tipo y la variación morfológica en toda el área de distribución de estas formas para evaluar el estatus taxonómico de estos nombres. Nuestros análisis filogenéticos, basados en los genes COI, ND2 y RAG1, revelaron la existencia de tres clados geográficamente estructurados (norte, central y sur) similares en morfología y coloración, que no correspondían a las tres especies en cuestión. Del mismo modo, no encontramos ninguna justificación para reconocer estas tres especies basándonos en el examen del material tipo y en las comparaciones de morfología a lo largo del rango geográfico de los especímenes asignados a las tres especies. Aunque había diferencias significativas en el número de escamas entre la interparietal y los semicírculos supraorbitales entre los clados moleculares recuperados, no consideramos que estas diferencias sean diagnósticas. Por lo tanto, proponemos que *A. binotatus* es un sinónimo senior de *A. bitectus* y de *A. lemniscatus*. Proporcionamos una nueva descripción de *A. binotatus*.

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Introduction

Anoles (*Anolis*) are a fascinating group of Neotropical lizards that includes 449 species occurring from the southern United States, through Central America, Caribbean islands, and South America south to Paraguay (Uetz et al. 2023). Due to their ecological diversity and abundance, anoles have long been subject to studies on evolutionary ecology (Losos 2009), and many taxonomic, phylogenetic, and biogeographic studies on anoles have been published (Poe 2004, Nicholson et al. 2012, Castañeda and Queiroz 2013, Poe et al. 2017, Prates et al. 2020).

Nearly ten percent of anole species occur in Ecuador (Torres-Carvajal et al. 2024), where the study of *Anolis* systematics has been intensive. Over the past 15 years, studies on Ecuadorian anoles have focused largely on the clade *Dactyloa,* resulting in the discovery and description of many species including *Anolis otongae* Ayala-Varela & Velasco, 2010; *A. podocarpus* Ayala-Varela & Torres-Carvajal, 2010; *A. poei* Ayala-Varela et al., 2014; *A. dracula* Yánez-Muñoz et al., 2018; and *A. nemonteae* Ayala-Varela et al., 2021. However, another important and diverse Ecuadorian clade of *Anolis* is *Norops* Wagler, 1830 (Losos 2009) , which includes a diverse sample of species distributed throughout Ecuador.

Several taxonomic subgroups have been recognized within Norops (e.g. Nicholson et al 2012). However, phylogenetic validation of these groups has been difficult. *Norops* contains three subclades—*Draconura* Wagler, 1830, found in mainland Central and South America; *Placopsis* Gosse, 1850, from Jamaica, and *Trachypilus* Fitzinger, 1843, sourced in Cuba (Poe et al. 2017). *Draconura* is one of the least studied clades of anoles (Andrews 1971, Fitch et al. 1976, Nicholson 2002, Vitt et al. 2002, 2003). Of the 45 *Anolis* species recognized in Ecuador (Torres-Carvajal et al. 2024), 16 belong to the *Draconura* clade, of which 11 occur west of the Andes along the Pacific lowlands and adjacent Andean slopes.

The systematics of Ecuadorian species within *Draconura* is poorly known (Poe et al. 2017). The taxonomic identity of three species, *Anolis binotatus*, *A. bitectus*, and *A. lemniscatus*, has been particularly problematic as they have been reported to share many morphological characters including males with a large orange dewlap and females without a dewlap, a brown lateral stripe extending longitudinally from the eye to the groin, ventral scales larger than dorsal scales, and several enlarged middorsal scale rows. Moreover, all three species purportedly co-occur along the Pacific coast of Ecuador (VertNet 2020), and their type localities are geographically close: *Anolis binotatus* Peters, 1863, described from Guayaquil; *A. bitectus* Cope, 1864 from "West Equador"; and *A. lemniscatus* Boulenger, 1898 from “Chimbo”. Moreover, phylogenetic studies have included only one specimen each of *A. binotatus* and *A. bitectus* (Poe 2004, Nicholson et al. 2005, Poe et al. 2017).

This study aims to clarify the taxonomic status of *Anolis binotatus*, *A. bitectus*, and *A. lemniscatus* through molecular phylogenetic analyses and a comprehensive review of specimens of the three species including type material. Our molecular phylogenetic analyses resulted in three geographically correlated clades. We tested whether these clades were diagnosable according to morphology in order to investigate potential species status.

Methods

Review of specimens

We examined 153 specimens identified as *Anolis binotatus*, *A. bitectus*, and *A. lemniscatus* and nine type specimens (Appendix 1) deposited in the collections of the British Museum of Natural History, United Kingdom (**BMNH**), Museum of Comparative of Zoology, Harvard University, United States (**MCZ**), Natural History Museum of Berlin, Germany (**ZMB**), Instituto Nacional de Biodiversidad del Ecuador (**DHMECN**), Escuela Politécnica Nacional del Ecuador (**EPN**), Fundación Gustavo Orcés, Ecuador (**FHGO**), and Museo de Zoología de la Pontificia Universidad Católica del Ecuador (**QCAZ**). We recorded external morphological characters following the terminology proposed by Williams et al. (1995), Poe (2004), and Köhler (2014). Measurements were taken with digital calipers to the nearest 0.1 mm. Complete tails were measured with a ruler, regenerated or broken tails were not measured. Scale counts were made on the left side if applicable. Sex was determined by the presence of both everted hemipenes and/or dewlaps in males (assumed to be absent in females). We followed Savage (1997) and D'Angiolella et al. (2016) for hemipenial characters. The right hemipenis of specimens QCAZ 3438, 13120, and 14365 were removed with a subcaudal incision and prepared using the method described by Pesantes (1994).

We produced species distribution maps in ArcGIS 10.8 software (ESRI 2019) and we estimated a minimum convex polygon to determine the area of occurrence of the lineages inferred by phylogenetic analyses as explained below.

DNA sequencing and phylogenetic analyses

We generated 46 new DNA sequences from 16 individuals originally identified in collections as *Anolis binotatus*, *A. bitectus*, or *A. lemniscatus* (Table 1) including records from or near the type localities of both *A. binotatus* and *A. lemniscatus* (note that the type locality of *A. bitectus* is vague, i.e. "West Equador"). Sequences included the nuclear recombination-activating gene 1 (RAG1, 987 nucleotides) and mitochondrial genes cytochrome c oxidase I (COI, 655 nucleotides) and NADH dehydrogenase subunit 2 (ND2, 1036 nucleotides). Primers and amplification protocols were taken from Kumazawa and Nishida (1993), Folmer et al. (1994), Macey et al. (1997), Schulte II and Cartwright (2009). We added the new sequences to the *Draconura* dataset presented by Poe et al. (2017). In addition, we obtained from GenBank sequences of *Anolis aequatorialis*, *A. bitectus*, *A. fraseri* and *A. parilis*, as well as *Polychrus marmoratus*, *Pristidactylus scapulatus*, and *Urostrophus gallardoi*, which were used to root the trees. Our final data matrix contained 158 taxa and 2,678 aligned nucleotides (

http://purl.org/phylo/treebase/phylows/study/TB2:S31693?x-access-code=347a054ebb30212b19c0defc42459f35&format=html).

Data were assembled and aligned in Geneious Prime 2020.2.2 (https://www.geneious.com) under default settings for MUSCLE 3.8.425 (Edgar 2004). Sequences were translated into amino acids for confirmation of alignment. After partitioning the concatenated dataset by codon position for each gene (i.e., 9 partitions total), we used IQ-TREE (Trifinopoulos et al. 2016) for phylogenetic inference under the maximum likelihood criterion. We used MODELFINDER (Kalyaanamoorthy et al. 2017) to infer models of evolution. Branch support was addressed under both ultrafast bootstrapping (UFBoot, Minh et al. 2013) and Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT, Guindon et al. 2010). We used FigTree version 1.4.4 (Rambaut 2018) to examine the topology of the tree. Finally, uncorrected genetic distances among species of the clade *Draconura* were calculated in PhyML 3.0 (Guindon et al. 2010) for COI and ND2.

Morphological analyses

The following measurements were taken for statistical analyses: snout–vent length (SVL), head length (HL), head width (HW), head height (HH), humerus length (HUL), ulna length (UL), hand length (HDL), femur length (FL), tibia length (TIL), foot length (FOL), toe length (ToL), toepad width (TPW), snout length (SL), interparietal length (IL), and ear-opening measured vertically (ED). Additionally, we recorded the following meristic characters: dorsals in 5% SVL (dorsals5), ventrals in 5% SVL (ventrals5), middorsal scales (middr), lamellae under phalanges II and III of fourth toe (lam), scales between second canthals (snsc), scales between supraorbital semicircles (sosc), scales between interparietal and supraorbital semicircles (ip.sosc), postrostrals (pr), loreal rows (lorr), supralabials to center of eye (suplbls2), and postmentals (pm).

Measurements and meristic characters were *ln*-transformed. To remove the effect of body size, we calculated the ratios of the *ln*-transformed measurements against the *ln*-transformed SVL. We performed a Linear Discriminant Analysis (LDA) to determine differences in measurements and scale counts among the major clades recovered in the phylogenetic analysis. For the analyses of morphology, no a-priori assignment to recognized taxa (i.e., *A. binotatus*, *A. bitectus* or *A. lemniscatus*) was done; instead, the inferred phylogenetic lineages were used as groups. We performed a MANOVA test to evaluate quantitative differences in characters with a normal distribution; a Kruskal-Wallis test was used for differences in variables without a normal distribution. For post hoc analyses we used the standard Bonferroni correction test for variables with homogeneity of variances and the Games-Howell test for variables without homogeneity of variance. The statistical analyses were performed in RStudio version 2023.6.2.561.5 (RStudio Team 2020) and IBM SPSS Statistics version 21 (IBM Corp. 2012).

Species delimitation

The taxonomic conclusions of this study are based on external morphological features and color pattern and inferred phylogenetic divergences and genetic distances. We consider this information as operational species delimitation criteria following the evolutionary species concept (Simpson 1951, 1961; de Queiroz 1998, 2007). We tested for phylogenetic structure across the range of the three purported species using mitochondrial and nuclear DNA data. Recovered lineages were tested for diagnosability using morphological data and evaluated with respect to the type localities of the three species.

Results

Phylogenetic relationships

Data partitions and models of evolution are presented in Table 2. Maximum Likehood analyses positioned *Anolis binotatus* sensu lato (i.e., including *A. bitectus* and *A. lemniscatus*) within a clade highly congruent with the *Draconura* clade of Poe et al. (2017) with maximum support (Fig. 1). The sister species of *A. binotatus* sensu lato is *A. gracilipes* (SH-aLRT = 100, UFBoot = 100), and together these form a sister clade to *A. notopholis*. *Anolis binotatus* sensu lato is composed of three well-supported subclades herein named **northern clade** (SH-aLRT = 91.6, UFBoot = 100), **central clade** (SH-aLRT = 100, UFBoot = 100), and **southern clade** (SH-aLRT = 100, UFBoot = 100); the latter two are sister taxa (SH-aLRT = 99.4, UFBoot = 100). The type-localities of *A. binotatus* and *A. lemniscatus* are within the "central" and "southern" clades, respectively.

Interspecific genetic distances among sampled species of the *Draconura* clade range from 0.02 (*A. mariarum*/*A. antonii*) to 0.53 (*A. gracilipes*/*A. humilis*) for ND2 and from 0.03 (*A. roatanensis*/*A. bicaorum*) to 0.34 (*A. auratus*/*A. aquaticus, A. auratus*/*A. gracilipes*, *A. auratus*/*A. taylori*) for COI. Genetic distances between *Anolis binotatus* and *A. gracilipes* are 0.23 (ND2) and 0.18 (COI). Genetic distances among northern, central and southern subclades within *A. binotatus* are 0.14–0.17 for ND2 and 0.10–0.14 for COI.

Morphology

The linear discriminant model with morphometric variables demonstrated an accuracy of 70% in correctly assigning individuals to the three *A. binotatus* molecular clades (northern, central, and southern). The model with meristic variables exhibited a higher level of precision, with an accuracy of 77% in correctly predicting clade assignment (Fig. 2).

The first model demonstrated trace proportions of 0.76 for the first linear discriminant function (LD1) and 0.24 for the second linear discriminant function (LD2), while the latter model exhibited trace proportions of 0.59 for LD1 and 0.41 for LD2.

The MANOVA and Kruskal-Wallis tests did not indicate statistically significant differences between the three *A. binotatus* clades, except in the number of scales between the interparietal and supraorbital semicircles (Chi^2=10.519, $p<0.01$).

We have examined the holotype of *A. binotatus* (ZMB 4685), syntypes of *A. bitectus* (BMNH 1946.8.5.83 and 1946.8.20.11), and syntypes of *A. lemniscatus* (BMNH 1946.8.8.35–39, MCZ 16783, Appendix 1), and we find these to be nearly identical in scoreable traits (Table 3, Fig. 3). Only the number of middorsal rows are slightly higher in *A. binotatus* (14) vs. *A. bitectus-A. lemniscatus* (10–12), which we interpret as intraspecific variation. Similarly, dorsal, lateral and ventral coloration patterns are similar across female type specimens.

Taxonomic Conclusions

Both the phylogenetic and morphological analyses (Figs 1, 2), which included examination of type material, failed to distinguish *A. binotatus*, *A. bitectus* and *A. lemniscatus* as three separate species. Therefore, here we recognize *Anolis bitectus* and *A. lemniscatus* as junior synonyms of *A. binotatus.* All examined specimens agree well with the description of *A. binotatus* provided by Peters (1863).

***Anolis binotatus* Peters, 1863**

Anolis bitectus — Cope, 1864; Boulenger, 1885; Peters, 1967; Peters & Donoso-Barros, 1970; Williams et al., 1995; Torres-Carvajal, 2001; Poe, 2004; Miyata, 2013. Type specimens: BMNH 1946.8.5.83 and 1946.8.20.11.

Anolis binotatus — Bocourt, 1873; Peters, 1967; Peters & Donoso-Barros, 1970; Williams et al., 1995; Torres-Carvajal, 2001; Poe et al., 2017.

Anolis lemniscatus — Boulenger, 1898; Peters & Donoso-Barros, 1970; Williams et al., 1995; Gemel et al., 2019. Type specimens: BMNH 1946.8.8.35–39, MCZ 16783, NMW 12812:1–3.

Anolis tropidogaster — Peters, 1967.

Norops binotatus — Nicholson, 2002; Nicholson et al., 2012.

Norops bitectus — Nicholson, 2002.

Norops lemniscatus — Nicholson, 2002.

Holotype.—ZMB 4685, Guayaquil, Guayas province, Ecuador.

English common names:

Two-marked Anoles, Roof Anoles, West Ecuadorian Anoles, White-Ribbon Anoles.

Spanish common names:

Anolis de dos marcas, anolis de dorso cubierto, anolis de cinta blanca.

Diagnosis

Anolis binotatus belongs to the *Digilimbus* clade, *Draconura* clade, and *Norops* clade of Poe et al., 2017 based on work presented here and the phylogenetic tree of Poe et al. (2017). Within *Draconura*, *A. binotatus* forms a clade with *A. datzorum* Köhler et al., 2007, *A. gracilipes* Boulenger, 1898, *A. notopholis* Boulenger, 1896 and *A. salvini* Boulenger, 1885. *Anolis binotatus* is readily distinguished from these forms by its enlarged middorsal scale rows (middorsals not distinctly enlarged in *A. datzorum* and *A. salvini*); its whitish-brown dorsum, with a straight brown stripe behind eye extending above tympanum, and by having 7−14 enlarged middorsal scale rows (greenish brown dorsum with an irregular brown stripe behind eye extending above tympanum, and 9−17 enlarged middorsal scale rows in *A. gracilipes*); and its lack of a black line from eye to nostril below canthus rostralis (present in *A. notopholis*).

Anolis binotatus is a brown, small anole (maximum recorded SVL 54.1 mm in males, 55.2 mm in females). Compared to other anoles belonging to the Draconura clade from western Ecuador, it can be distinguished from *A. granuliceps* Boulenger, 1898 (character states in parentheses) by having a larger size, keeled ventral scales, and a large dewlap past arms well on to chest in males (maximum SVL 49 mm, smooth or very faintly keeled ventral scales, and a small dewlap to arms in males of *A. granuliceps*). *Anolis binotatus* differs from *A. lynchi* Miyata, 1985 in having larger head scales, 6−15 scales across the snout between the second canthals (smaller head scales, 16−29 between second canthals in *A. lynchi*). *Anolis binotatus* differs from *A. lyra* Poe et al., 2009 in having an orange dewlap in males and no dewlap in females (male dewlap red with dark central blotch; female dewlap present, usually blue or white with dark central blotch in *A. lyra*), and in possessing fewer toe lamellae, 10−15 (16−21 in *A. lyra*). *Anolis binotatus* differs from *A. maculiventris* Boulenger, 1898 in having keeled ventral scales (smooth in *A. maculiventris*), and from *A. parvauritus* Williams, 1966 in its brown dorsum (green in *A. parvauritus*). *Anolis binotatus* is most similar morphologically to *A. gracilipes* but differs from it in having 7−14 enlarged middorsal scale rows (9−17 in *A. gracilipes*); larger ventral scales, 3−7 in 5% SVL (5−11 in 5% SVL in *A. gracilipes*); a straight brown stripe behind eye extending above tympanum (irregular brown stripe behind eye extending above tympanum in *A. gracilipes*), a "V" shaped band on the snout, pointing backward (band absent in *A. gracilipes*), and dorsum of body whitish brown (greenish brown in *A. gracilipes*;see also genetic distances in the Phylogenetic Relationships section above). Finally, *A. sagrei* Duméril & Bibron, 1837 (*Trachypilus* clade), a species recently introduced in Ecuador (Amador et al. 2017) is similar to *A. binotatus* in having a brown body dorsum, and ventral scales keeled and larger than dorsals. However, *A. sagrei* differs from *A. binotatus* in lacking enlarged middorsal scale rows, having an orange, yellow, or red dewlap with a yellow border, and possessing a laterally more strongly compressed tail.

Description and variation

SVL in males 45.0–54.1 mm, and 45.3–55.2 mm in females. Variation in ratios: head length/SVL 0.22–0.30; head width/SVL 0.12–0.16; HH/SVL 0.10–0.14;

interorbital length/SVL 0.02–0.05; interparietal length/SVL 0.02–0.05; ear height/SVL 0.01–0.03; humerus length/SVL 0.13–0.22; ulna length/SVL 0.11–0.26; hand length/SVL 0.13–0.19; femur length/SVL 0.27–0.36; tibia length/SVL 0.23–0.31; foot length/SVL 0.29–0.41; 4th toe length/SVL 0.17–0.33; 4th toe width/SVL 0.01–0.02. Head scales in supraocular disc and frontal region keeled (single or multiple), rugose, or smooth; 6−15 scales between second canthals; 4−8 scales bordering rostral posteriorly; divided anterior nasal in contact or separated by 1–2 scales from rostral, or anterior nasal in contact or separated by one scale from rostral, or circumnasal separated by one or two scales from rostral; supraorbital semicircles separated by 1–3 scales; supraocular disc with 1–8 gradually enlarged scales; supraocular edge continuous or broken; 1–4 elongate superciliaries followed by gradually smaller scales; 5−8 loreal rows; >15 loreal scales; mid-snout without or with parallel scale rows; rostral with smooth or cleft dorsal edge; frontal region of head with a depression; rostral overlapping the mental or even; interparietal larger than ear opening, separated by 1−4 scales from semicircles; ear round with normal edge or nub; transparent scales in lower eyelids absent; preoccipital scale usually absent; suboculars in contact with supralabials or separated by one scale; 7−10 supralabials counted up to a point below center of eye; 4−7 postmentals; no enlarged sublabials or 1−2 in contact with infralabials; mental divided partially or completely; mental extending further back posteriorly than rostral along edge of mouth, or rostral extending further than mental or even; posterior edge of mental dented, straight or concave; no nuchal crest; dorsal scales keeled; 7−14 middorsal scale rows larger than flank scales; 4−8 middorsal scales in a longitudinal segment representing 5% of SVL; flank scales smooth, homogeneous in size, barely separated by skin; ventral scales larger than dorsals, keeled or slightly keeled, imbricate, and arranged in diagonal or transversal rows; 3−7 midventral scales in a longitudinal segment representing 5% of SVL; inconspicuous axillary pocket present or absent; toepads overlapping distal phalanx in all toes; 10−16 lamellae under phalanges II and III of fourth toe; supradigitals with multiple keels; tail crest absent or serrate; tail laterally compressed or round, with a single or double row of middorsal scales; insolitus tail (Poe 2004) absent; enlarged postcloacal scales absent (in males and females); when adpressed against body the hindlimb reaching posterior to ear, or to ear, or to eye, or between ear-eye, or between eye-snout, or to snout, or past snout.
Nuchal and dorsal folds absent in preserved specimens but present in males in life; dewlap large, extending posteriorly behind forelimbs (in males), with 6−7 longitudinal rows of one elongate scale each, same size as ventrals, and separated by naked skin (Table 4). Intraspecific variation in morphological characters in *Anolis binotatus* is presented in Table 5.

Color in Life

Adult male QCAZ 14151, Bosque Protector Cerro Blanco, Guayaquil, Guayas province (Fig. 4A, B, undisturbed color pattern): dorsum of head, body, and tail whitish brown; dorsum of limbs light brown; dorsum of head with a dark brown interorbital stripe, a dark brown stripe in the form of an inverted “V” in the frontal area, and a small dark brown spot in the center of the snout; dorsum of body with a whitish-brown chevron pattern; dorsum of forelimbs (at the level of the ulna) with two faint dark brown transverse bands delimited with a thin white stripe; dorsum of hindlimbs with dark brown transverse bands delimited with a thin white stripe: two bands on thigh and two on shank; tail with reddish brown transverse bands delimited with a thin white stripe; flanks of head with dark brown radial stripes extending from ocular region, lips cream with two well-defined dark brown stripes and a wide dark brown band from postocular region, across neck over tympanum to groin; ventral aspect of head cream; ventral aspect of body, limbs, and tail yellowish cream; pupil with yellowish cream halo and dark brown iris; dewlap skin solid orange-yellow with yellowish white scales (Fig. 4A).

Adult male QCAZ 3437, San Pablo, Chimborazo province (Fig. 4C, D, G, undisturbed color pattern): general color pattern similar to male QCAZ 14151, but dorsum of snout without a small dark brown spot; orange-brown iris; a cream lip region with three well-defined dark brown stripes; ventral aspect of body, limbs, and tail cream; and dewlap skin solid yellow with white scales (Fig. 4C).

Adult male QCAZ 17041, La Victoria, Chimborazo province (Fig. 4E, F, H, undisturbed color pattern): general color pattern similar to males QCAZ 3437 and 14151, but dorsum of the forelimbs without faint dark brown bands; nuchal fold whitish brown; and tongue pinkish cream.

Adult female QCAZ 16283, Cerro El Mate, Guayas province, (Fig. 5A, B, C, undisturbed color pattern): dorsum of head, body, and tail whitish brown; dorsum of limbs light brown; dorsum of the head with a dark brown interorbital band, and a dark brown band in the form of an inverted “V” in the frontal area; dorsum of the body with a whitish-brown vertebral band with a dark brown border; dorsum of the forelimbs (at the level of the ulna) with a well-defined dark brown transverse band; dorsum of hindlimbs with dark brown transverse bands: two bands on thigh and two on shank; tail with brown transverse bands; flanks of head with dark brown radial stripes extending from the ocular region, a cream lip region with three well-defined dark brown stripes and a wide dark brown band from the postocular region across the neck over the tympanum to the groin level; ventral aspect of head whitish cream; ventral aspect head, body, limbs whitish cream; ventral aspect of tail whitish cream with copper brown transverse bands; pupil with light brown halo and dark brown iris; gular skin orange with cream scales.

Adult female QCAZ 15868, Las Playas, Bolívar province (Fig. 5D, E, F, undisturbed color pattern): general color pattern similar to female QCAZ 16283, but dorsum of forelimbs without dark brown transverse bands; ventral aspect of head whitish brown; and iris orange-brown.

Adult female QCAZ 17044, La Victoria, Chimborazo province (Fig. 5G, H, I, disturbed color pattern): general color pattern similar to female QCAZ 15868, but dorsum of body with a dark brown vertebral region; pupil with halo yellowish cream and dark brown iris; tongue pinkish cream.

Adult female, iNaturalist, Cerro Mutiles, Esmeraldas province (Fig. 5J, K, undisturbed color pattern): flanks of head with a stripe dark brown in lip region.

Juvenile male QCAZ 14153, Centro Científico Río Palenque, Los Ríos province (Fig. 5L, undisturbed color pattern): general color pattern similar to adult male QCAZ 14151, but dorsum of nuchal grey with a dark brown transverse band from ocular region; dorsum of the body with a grey chevron pattern; ventral aspect of head dirty cream; ventral aspect of limbs cream with faint gray reticulations; iris orange-brown; dewlap skin solid yellowish orange with cream scales.

Hemipenis (Fig. 6)

QCAZ 13120, El Triunfo, Guayas province, central clade: Fully everted hemipenis wider (4.46 mm) than long (3.43 mm, 1.62 mm truncus length), bilobed, with globular lobes that represent more than half the hemipenis length; sulcus spermaticus unforked and deep, with well-developed sulcal lips and opening into a wide and elongate naked area extending laterally from the base of the lobes to the apex; lobes densely calyculate outside naked area; enlarged and irregular calyces cover asulcate surface of lobes; skin ridge present in asulcate surface on lobular crotch (crotch flap); truncus with transverse folds (Fig. 6A).

Except for size, we found no variation in the other two examined hemipenes, which belong to specimens from the southern clade (Figs 6B, C). QCAZ 3438, San Pablo, Chimborazo province: fully everted hemipenis wider (5.06 mm) than long (3.06 mm, 1.74 mm truncus length). QCAZ 14365, E of Shurimal, Azuay province: fully everted hemipenis wider (4.25 mm) than long (4.03 mm, 0.88 mm truncus length).

Distribution and natural history

Anolis binotatus occurs along the Pacific slopes of the Andes and nearby lowlands in Ecuador between 3−1,354 m in elevation (Fig. 7). It has been recorded in Esmeraldas, Manabí, Pichincha, Santo Domingo de los Tsáchilas, Cotopaxi, Guayas, Bolívar, Chimborazo, Los Ríos, Cañar, Azuay, and El Oro provinces. This species occurs in Dry Shrub, Deciduos Forest, Choco Tropical Rainforest, Western Foothill and Western Montane (Ron 2020). Specimens of *Anolis binotatus* were collected mainly in secondary forest, along the edge of roads, forest and rivers, cacao and rubber plantations, and pastures. During the day, individuals were found active between 9:00 and 17:00 on leaflitter, rocks, twigs, and grass, 0.2–2.0 m above ground. At night, individuals were found between 19:00 and 23:00 h sleeping horizontally or vertically with heads up or down on leaves, ferns, twigs, shrubs, or cane leaves, grass, fallen trees, and leaflitter, 0.3–2.7 m above ground. *Anolis binotatus* generally occurs in sympatry with *A. fasciatus*, *A. festae*, *A. chloris A. princeps*, *A. peraccae* and *A. gracilipes* in the lowlands (Reyes-Puig and Ríos-Alvear 2016); *A. poei*, *A. aequatorialis* and *A. fasciatus* (Ayala-Varela et al. 2014) in highlands, such as Telimbela, Bolívar province; *A. fasciatus* and *A. nemonteae* at the Buenaventura Reserve in southern Ecuador (Yánez-Muñoz et al. 2013, Garzón-Santomaro et al. 2019, Ayala-Varela et al. 2021); and *A. festae* and *A. sagrei* on

Santay Island, Guayas province (Cruz-García et al. 2023). The smallest individual QCAZ 1474 (SVL = 22.9 mm) was collected on 3 August 1992.

An adult female (SVL 52.6 mm, FHGO 9064) collected on 24 March 2013 presented two oviductal eggs (13.95 mm x 4.92 mm; 6.50 mm x 4.10 mm).

Conservation

Anolis binotatus is endemic to Ecuador. Its range is estimated to cover an area of 49,434 km² in the lowlands and adjacent foothills of the Andes in western Ecuador. Most individuals have been collected in agricultural or anthropogenic areas. However, there are records in protected areas, such as Manglares Churute Ecological Reserve, Pedro Franco Dávila Ecological Reserve, Buenaventura Biological Reserve, Tito Santos Reserve, Jama Coaque Reserve, Lalo Loor Reserve, El Cajas Reserve, Bosque Protector Cerro Blanco, Bosque Protector Balao, Centro Científico Río Palenque, and Área Nacional de Recreación Isla Santay, which suggest that many populations of *A. binotatus* are well protected.

We have adopted the threat category proposed by Ayala et al. (2020), who considered *Anolis binotatus* to be a species of Least Concern. Although, like most other anole species, *A. binotatus* is subject to multiple threats of habitat loss and degradation and its population status is unclear, *A. binotatus* is widely distributed and appears tolerant to current levels of disturbance within its range. Therefore, we believe its status is best considered as Least Concern.

Discussion

Anolis binotatus was described by Peters in 1863 from "Guayaquil, Ecuador"; shortly after, Cope described *A. bitectus* from "West Equador" in 1864, and Boulenger described *A. lemniscatus* from "Chimbo, Ecuador" in 1898. Despite morphological similarity, neither Cope (1864) nor Boulenger (1898) compared the newly described species with *A. binotatus*. The type material of *A. bitectus* (BMNH 1946.8.5.83 and 1946.8.20.11) was collected by Louis Fraser, a naturalist and curator of the Zoological Society Museum of London, who made expeditions to several localities in Ecuador, mainly Gualaquiza, Morona Santiago province in eastern Ecuador, and Pallatanga, Chimborazo province in western Ecuador (Cope 1864, Ortega et al. 2014). As Cope (1864) mentioned "West Equador" as the type locality of *A. bitectus*, it is likely that the type specimens of this species were collected in or near Pallatanga (Fig. 7). It is also worth noting that the type locality of *A. lemniscatus*, Chimbo, Ecuador, does not longer exist under that name. Boulenger (1898:108) described Chimbo as "Puente del Chimbo", the railway terminus about 70 miles from Guayaquil, at an elevation of about 1000 feet". The locality is a small town that no longer exists. Nowadays this area corresponds to the town of Bucay called Antonio General Elizalde, Guayas province, Ecuador, just west of the town of Cumandá, Chimborazo province, at 2.202ºS, 79.141ºW, 290 m (see Paynter 1993). Therefore, the type localities of *A. bitectus*, *A. binotatus*, and *A. lemniscatus* are geographically close (Fig. 7) without any major biogeographical barriers among them, which further supports our conclusion that all three species names are synonyms.

On the other hand, our knowledge of these three clades of *Anolis binotatus* (northern, central and southern clades) will become clearer as future studies increase their genomic scope.

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Appendix I

Specimens examined (datum WGS84)

Type material (9 specimens)

***Anolis binotatus* (1).** ECUADOR — **Guayas** • Guayaquil; 2.1887ºS, -79.8900ºW; ZMB4685.
***Anolis bitectus* (2).** ECUADOR — **Chimborazo** • "West Equador" [Pallatanga]; 2.0202ºS, 78.9737ºW; BMNH 1946.8.5.83, 1946.8.20.11.
***Anolis lemniscatus* (6).** ECUADOR — **Guayas** • "Chimbo" [Bucay]; 2.202ºS, 79.141ºW; alt. 290 m; BMNH 1946.8.8.35–39, MCZ16783.

Other specimens

***Anolis binotatus* (144 specimens).** ECUADOR — **Azuay (23)** • Recinto La López; 3.0873ºS, 79.7149ºW; alt. 425 m; QCAZ5003–5005 • Recinto Santa Marta, finca La Envidia; 3.0565ºS, 79.6806ºW; alt. 403 m; EPN10780 • Recinto Santa Marta, orilla del Río Tenguel; 3.0561ºS, 79.6941ºW; FHGO8721 • Río Patul; 2.5678ºS, 79.3665ºW; alt. 400 m; QCAZ2196 • Road E from Shurimal (17,4 km int main road); 3.0216ºS, 79.6494ºW; alt. 784 m; QCAZ14362 • Road E from Shurimal (19,0 km int main road); 3.0216ºS, 79.6494ºW; alt. 1000 m; QCAZ14363 • Road E from Shurimal (22,0 km int main road); 3.0308ºS, 79.646ºW; alt. 1155 m; QCAZ14364–14365 • Sector Gaby; 3.0583ºS, 79.7218ºW; alt. 314 m; DHMECN10355, EPN10724, 10758, 10768–10769, FHGO8456 • Sector Papagrande; 3.0618ºS, 79.5204ºW; alt. 757 m; FHGO8454, 8457, EPN10746–10747 • Sector Puntudo; 3.0618ºS, 79.5204ºW; alt. 599 m; FHGO8455 • Tamarindo; 2.7833ºS, 79.55ºW; alt. 400 m; FHGO861, 865. — **Bolívar (7)** • Balsapamba; 1.7672ºS, 79.1788ºW; alt. 465 m; QCAZ3894 • Las Playas; 1.1663ºS, 79.1307ºW; alt. 783 m; QCAZ15868 • Puente en vía San José del Tambo-Chillanes, ca. 8 km E San José del Tambo; 1.9418ºS, 79.1717ºW; alt. 538 m; QCAZ16617 • Río Telimbela; 1.6597ºS, 79.1638ºW; alt. 1208 m; QCAZ6784–6785 • Santa Rosa de Agua Clara; 2.1948ºS, 79.1113ºW; alt. 424 m; QCAZ7859, 7861. — **Cañar (7)** • Cañar-Azuay, 2 km S Manta Real, Río Patul; 2.5580ºS, 79.3618ºW; alt. 350 m; QCAZ1473 • Cañar-Azuay, 2 km S Manta Real, Río Patul; 2.5573ºS, 79.3617ºW; alt. 350 m; QCAZ1474 • Manta Real; 2.5536ºS, 79.3642ºW; alt. 259 m; QCAZ4131, 4139 • Manta Real; 2.5515ºS, 79.3496ºW; alt. 250–600 m; DHMECN1628 • Manta Real, Río Patul; 2.5586ºS, 79.3618ºW; alt. 350 m; QCAZ907 • El Cajas reserve, límite provincial entre Cañar y Azuay, Río Patul; 2.5589ºS, 79.3504ºW; alt. 350 m; QCAZ143. — **Chimborazo (18)** • Mayaguan; 2.2051ºS, 79.1080ºW; alt. 343 m; QCAZ3431, 3433–3435 • Recinto La Victoria; 2 km E Cumandá/Bucay, alrededores del Río Blanco; 2.2041ºS, 79.1111ºW; alt. 361 m; QCAZ17041 • Recinto La Victoria, 2 km E Cumandá/Bucay, alrededores del Río Blanco; 2.2033ºS, 79.1113ºW; alt. 355 m; QCAZ17040 • Recinto La Victoria, 2.5 km E Cumandá/Bucay, puente La Victoria; 2.1984ºS, 79.1085ºW; alt. 351 m; QCAZ17042 • Recinto La Victoria, 2.5 km E Cumandá/Bucay, puente La Victoria; 2.1964ºS, 79.1085ºW; alt. 351 m; QCAZ17043 • Recinto La Victoria, 2.5 km E Cumandá/Bucay, puente La Victoria; 2.1963ºS, 79.1084ºW; alt. 419 m; QCAZ17024, 17044–17045 • Recinto La Victoria, alrededores del Río Chimbo y balneario El Hueco de la

Mano de Dios; 2.1953ºS, 79.1064ºW; alt. 364 m; QCAZ17048–17050 • Recinto La Victoria, alrededores del Río Chimbo y balneario El Hueco de la Mano de Dios; 2.1950ºS, 79.1066ºW; alt. 380 m; QCAZ17046–17047 • San Pablo (vía Pallatanga - Bucay); 2.1453ºS, 79.0413ºW; alt. 650 m; QCAZ3437–3438. — **Cotopaxi (12)** • La Maná; 0.9405ºS, 79.2208ºW; alt. 220 m; FHGO1058 • La Maná; 0.9383ºS, 79.2253ºW; alt. 210 m; QCAZ4245 • La Maná, Río Calope, comuna San Pedro; 0.9879ºS, 79.2129ºW; alt. 300 m; QCAZ3047–3051, 3053 • Las Minas; 1.2042ºS, 79.2588ºW; alt. 216 m; QCAZ15869 • Puente de La Maná; 0.9374ºS, 79.2232ºW; alt. 218 m; QCAZ4242–4244. — **El Oro (6)** • 24 km SW of Piñas; 3.648ºS, 79.8233ºW; alt. 232 m; QCAZ14361 • Alrededores La Avanzada; 3.5655ºS, 79.9194ºW; alt. 231 m; DHMECN11354, 11356 • Bella María, Cerca del Valle Hermoso; 3.5116ºS, 79.8202ºW; alt. 282 m; QCAZ8990 • Cerro de Arcos, sendero Ñalacapac; 3.6974ºS, 79.8018ºW; alt. 927 m; DHMECN10868 • Buenaventura biological reserve; 3.6454ºS, 79.7634ºW; alt. 800 m; DHMECN2579. —**Esmeraldas (8)** • Bosque Protector Balao; 0.9334ºN, 79.7196ºW; FHGO9064 • Cerro Mutiles; 0.9002ºN, 79.6050ºW; iNaturalist record • Esmeraldas, Codesa; 0.9344ºN, 79.6930ºW; alt. 83 m; QCAZ8399 • Estación Terminal Marítimo OCP; 0.9365ºN, 79.7213ºW; alt. 146 m; FHGO6573 • Estación Terminal Marítimo OCP; 0.9386ºN, 79.7214ºW; alt. 146 m; FHGO6571 • Estación Terminal Marítimo OCP; 0.9394ºN, -79.723ºW; alt. 191 m; FHGO6574 • Estación Terminal Marítimo OCP; 0.9403ºN, 79.7208ºW; alt. 135 m; FHGO6572 • Estación Terminal Marítimo OCP; FHGO6575 • Quinindé; 0.2377ºN, 79.4304ºW; iNaturalist record • Quinindé; 0.2967ºN, 79.2920ºW; iNaturalist record • Recinto El Paisaje; 0.1034ºN, 79.4228ºW; alt. 172 m; DHMECN9317. — **Guayas (25)** • Bosque Protector Cerro Blanco, caseta Jaguar; 2.1355ºS, 80.0860ºW; alt. 378 m; QCAZ17985 • Bosque Protector Cerro Blanco, quebrada Canoa; 2.1773ºS, 80.022ºW; alt. 66 m; QCAZ14152 • Centro Shuar Tsuer Entsa, Balao Chico; 2.7904ºS, 79.6419ºW; alt. 459 m; QCAZ16355 • Cerro de Hayas, Río Ochoa, 1.8 km ESE de Cooperativa 23 de Noviembre; 2.7244ºS, 79.6196ºW; alt. 143 m; QCAZ17116 • El Piedrero, 15 km del Triunfo vía a Pallatanga; QCAZ3847–3848 • El Triunfo, entrada del Cristo Peregrino, empacadora de las calles, finca de Julio Álvarez; 2.3277ºS, 79.3990ºW; alt. 46 m; QCAZ13120 • ESPOL, campus Gustavo Galindo, Km 35 vía Perimetral; 2.1514ºS, 79.9821ºW; alt. 445 m; FHGO12669, 12671–12672 • ESPOL, campus Gustavo Galindo, Km 35 vía Perimetral; 2.1510ºS, 79.9657ºW; alt. 153 m; FHGO12705 • Isla Santay; 2.2097ºS, 79.8723ºW; alt. 3 m; QCAZ17986 • Isla Santay, sendero Huaquillas; 2.2254ºS, 79.8661ºW; alt. 4 m; QCAZ17866 • Naranjal en la rivera del Río Chacayacu, Paují; 2.6800ºS, 79.5816ºW; alt. 114 m; QCAZ6903 • Manglares-Churute ecological reserve, cerro El Mate; 2.4505ºS, 79.6192ºW; alt. 333 m; QCAZ16283 • Río Congo; 0.9992ºS, 79.5957ºW; alt. 65 m; DHMECN18049–18050 • Eco Hostería Monoloco; 2.4427ºS, 79.5614ºW; alt. 240 m; DHMECN17767–17768 • El Piedrero, rancho Alemán; 2.3401ºS, 79.2119ºW; alt. 377 m; DHMECN17773 • El Piedrero, rancho Alemán; 2.3364ºS, 79.2116ºW; alt. 284 m; DHMECN17771–17772 • El Piedrero, rancho Alemán; 2.3278ºS, 79.2077ºW; alt. 200 m; DHMECN17770 • Hacienda Ithaca; 1.9894ºS, 79.4613ºW; alt. 24 m; DHMECN17766 • Las Pavas; 2.3613ºS, 79.2735ºW; alt. 129 m; DHMECN17769. — **Los Ríos (26)** • 14–22 km S Quevedo; 1.1606ºS, 79.4328ºW; MCZ150360 • 2 km ONO Miraflores, vía San José del Tambo-Montalvo; 1.9022ºS, 79.2787ºW; alt. 29 m; QCAZ16618 • 50.7 km S Santo

Domingo; 0.5565ºS, 79.3705ºW; alt. 173 m; QCAZ9804 • Centro Científico Río Palenque; 0.5920ºS, 79.3622ºW; alt. 175 m; QCAZ9822–9823 • Centro Científico Río Palenque, alrededores de la estación; 0.5881ºS, 79.3629ºW; alt. 210 m; QCAZ14153–14154 • Hacienda Cerro Chico, 43 km (by road) N Quevedo, vía a Santo Domingo; 0.6432ºS, 79.4223ºW; alt. 120 m; QCAZ17025 • Hacienda Cerro Chico, 43 km (by road) N Quevedo, vía a Santo Domingo; 0.6432ºS, 79.4229ºW; alt. 155 m; QCAZ17026 • Hacienda Cerro Chico, 43 km (by road) N Quevedo, vía a Santo Domingo; 0.6415ºS, 79.4216ºW; alt. 134 m; QCAZ17051–17055 • Hacienda Cerro Chico, 43 km (by road) N Quevedo, vía a Santo Domingo; 0.6412ºS, 79.4215ºW; alt. 162 m; QCAZ17056 • Juan Montalvo, orilla del Río Cristal; 1.7940ºS, 79.2843ºW; alt. 65 m; QCAZ6900–6901 • Pichilingue; 1.1029ºS, 79.4647ºW; MCZ77377 • Recinto Jauneche, Pedro Franco Dávila ecological reserve; 1.2464ºS, 79.6591ºW; alt. 33 m; QCAZ4488–4491 • Río Palenque; 0.5893ºS, 79.3681ºW; alt. 200 m; QCAZ10564 • Sector Río Baba; EPN13204 • "Vintanas" [Ventanas]; 1.4408ºS, 79.4627ºW; MCZ57449 • La Libertad, Río Lulo Grande, Proyecto STADLER, Bloque Coronado; 0.5055ºS, 79.3580ºW; alt. 256 m; DHMECN16956. — **Manabí (9)** • Carbonera; 0.0789ºN, 79.9890ºW; alt. 49 m; DHMECN18679 • Carbonera; 0.0796ºN, 79.9890ºW; alt. 35 m; DHMECN18681 • Chone; 0.4808ºS, 80.0844ºW; iNaturalist record • Estación 19; 0.8740ºS, 80.1352ºW; FHGO11129 • Jama Coaque, 3.12 km O Camarones; 0.0837ºS, 80.1497ºW; alt. 74 m; QCAZ11436 • Lalo Loor reserve; 0.0784ºS, 80.1481ºW; alt. 50 m; QCAZ10757, 10762 • Tito Santos reserve; 0.1496ºS, 80.1996ºW; alt. 199 m; EPN8265–8266. — **Pichincha (3)** • **"**Río Pachijal" [Pachijal river near to the Recinto Pachijal]; 0.1594ºN, 78.9376ºW; alt. 505 m; FHGO9162–9164. — **Santo Domingo de los Tsáchilas (1)** • La Concordia, finca de Germán Cortez; 0.0022ºN, 79.4104ºW, alt. 144 m; QCAZ11242***.***

Legend of tables

Table 1. Vouchers, locality data, and GenBank accession numbers of taxa and gene regions sequenced in this study.

Table 2. Data partitions used in phylogenetic analyses. Numbers in parentheses indicate codon position. Charset of sites, number of sequences (seq), sites, selected model, number of unique site patterns (USP), invariable sites (IVS) and parsimony-informative sites (PIS) are indicated.

Table 3. Comparison of type specimens for *Anolis binotatus*, *A. bitectus* and *A. lemniscatus*. Summary of meristic characters, measurements (mm) and color patterns in preservative. For each quantitative character, range and sample size (N) are given. (*) Variation reported by Williams et al. (1995).

Table 4. Summary of meristic characters, ratios, measurements (mm) and color patterns of *Anolis binotatus*. For each quantitative character, range and sample size (N) followed by mean ± standard deviation are given.

Table 5. Sexual variation in meristic characters, ratios, and measurements (mm) in *Anolis binotatus*. Range and size (in parentheses) followed by mean ± standard deviation are given.

Legend of figures

Figure 1. Phylogeny of the clade *Draconura* (upper left) with a close-up of *Anolis binotatus* and closely related species. Maximum Likelihood tree based on an analysis of 158 taxa and 2,678 characters including two mitochondrial genes (COI, ND2) and one nuclear gene (RAG1). Numbers above branches correspond to SH-aLRT/UFboot support values. GenBank accession numbers along with locality data are presented in Table 1 for newly sequenced specimens. Voucher number and province name are presented for specimens of *A. binotatus*. Colors represent northern (green), central (red), and southern (blue) clades discussed in the text.

Figure 2. Scatterplots of LD1 and LD2 generated by discriminant function analyses on the morphometric (**A**) and meristic (**B**) variables recorded in this study for *Anolis binotatus*, *A. bitectus* and *A. lemniscatus*. Samples were assigned to three clades within *A. binotatus* based on the phylogenetic tree presented in Figure 1.

Figure 3. Type specimens of taxa included in this study. *Anolis binotatus*, ZMB 4685, female **holotype** (**A**, **D**); *A. bitectus*, BMNH 1946.8.20.11, female **syntype** (**B**, **E**); *A. lemniscatus*, BMNH 1946.8.8.38, female **syntype** (**C**, **F**) and BMNH 1946.8.8.39, male **syntype** (**G**). Scale bars = 5 mm. Photographs by A. Fläschendräger (**A**, **D**), and J. Streicher (**B**, **C**, **E**, **F**, **G**).

Figure 4. Variation of color in life of adult males of *Anolis binotatus*. Bosque Protector Cerro Blanco, Guayaquil, Guayas province, QCAZ 14151 (**A**, **B**); San Pablo, Chimborazo province, QCAZ 3437 *(***C**, **D**, **G**), 15 km W Pallatanga; La Victoria, QCAZ 17041 (**E**, **F**, **H**), to 2.6 km E of type locality of *A. lemniscatus*. Photographs by F. Ayala (**A**, **B**, **C**, **D**, **G**), and F. Gordon (E, F, H).

Figure 5. Variation of color in life of adult females and juvenile of *Anolis binotatus*. Guayas, Cerro El Mate, QCAZ 16283 (**A**, **B**, **C**) to 42 km SW of type locality of *A. binotatus*; Las Playas, Bolívar province, QCAZ 15868 (**D**, **E**, **F**) to 95 km N Pallatanga; La Victoria, Chimborazo province, QCAZ 17044 (**G**, **H**, **I**) to 2.6 km E of type locality of *A. lemniscatus*; Cerro Mutiles, Esmeraldas province, iNaturalist (**J**, **K**); Centro Científico Río Palenque, Los Ríos province, juvenile male, QCAZ 14153 (**L**). Photographs by F. Ayala (**A**, **B**, **C**, **L**), G. Pazmiño (**D**, **E**, **F**), F. Gordon (**G**, **H**, I), and M. Yánez-Muñoz (**J**, **K**).

Figure 6. Hemipenes of *Anolis binotatus i*n asulcate (left), and sulcate (right) views. **A**) QCAZ 13120 (SVL = 47.33 mm) from Guayas province (central clade), **B**) QCAZ 3438 (SVL = 53.93 mm) from Chimborazo province (southern clade), **C**) QCAZ 14365 (SVL = 47.25 mm) from Azuay province (southern clade). Ca = calyces, CF = crotch flap, SS = sulcus spermaticus. Photographs by F. Ayala. Scale bar = 1 mm.

Figure 7. Distribution of *Anolis binotatus* in Ecuador. Records belonging to the northern clade are shown in green, central clade in red, and southern clade in blue. Minimum convex polygons are shown for each clade. Type localities of *A. binotatus* (yellow star), and *A. lemniscatus* (aqua star) are indicated. Pallatanga, herein proposed as type locality of *A. bitectus* is also shown (white star).

Table 1. Vouchers, locality data, and GenBank accession numbers of taxa and gene regions sequenced in this study.

No.	Species	Voucher	Province	Locality	COI	ND2	RAG1
1	*Anolis aequatorialis*	QCAZ6855	Pichincha	Mindo	JN112720.1	JN112662.1	JN112593.1
2	*Anolis aequatorialis*	QCAZ6883	Pichincha	Río Chisinche	JN112721.1	JN112663.1	JN112594.1
3	*Anolis binotatus*	QCAZ5004	Azuay	Recinto La López	X	X	-
4	*Anolis binotatus*	QCAZ14365	Azuay	E Shurimal	X	X	X
5	*Anolis binotatus*	QCAZ6784	Bolívar	Río Telimbela	X	X	X
6	*Anolis binotatus*	QCAZ4139	Cañar	Manta Real	X	X	X
7	*Anolis binotatus*	QCAZ17024	Chimborazo	Puente La Victoria (antiguo puente del Río Chimbo)	X	X	X
8	*Anolis binotatus*	QCAZ3438 POE	Chimborazo	San Pablo (vía Pallatanga - Bucay)	X	X	-
9	*Anolis binotatus*	QCAZ8399	Esmeraldas	Esmeraldas, Codesa	X	X	X
10	*Anolis binotatus*	QCAZ14152	Guayas	Bosque Protector Cerro Blanco	X	X	X
11	*Anolis binotatus*	QCAZ17025	Los Ríos	Hacienda Cerro Chico, 43 km N Quevedo	X	X	X

No.	Species	Voucher	Province	Locality	COI	ND2	RAG1
12	*Anolis binotatus*	QCAZ17026	Los Ríos	vía a Sto. Domingo Hacienda Cerro Chico, 43 km N Quevedo vía a Sto. Domingo	X	X	X
13	*Anolis binotatus*	QCAZ6900	Los Ríos	Juan Montalvo en la orilla del Río Cristal	X	X	X
14	*Anolis binotatus*	QCAZ4488	Los Ríos	Recinto Jauneche, Pedro Franco Dávila ecological reserve	X	X	X
15	*Anolis binotatus*	QCAZ11436	Manabí	Jama Coaque, 3.12 km W Camarones	X	X	X
16	*Anolis binotatus*	QCAZ11242	Santo Domingo de los Tsáchilas	La Concordia, finca de Germán Cortez	X	X	X
17	*Anolis binotauts*	DHMECN10868	El Oro	Sendero Ñalacapac	X	X	X
18	*Anolis binotauts*	DHMECN11354	El Oro	La Avanzada	X	X	X
19	*Anolis bitectus*	KU218372	Manabí	Río Coaque	-	AY909743.1	-
20	*Anolis bombiceps*	QCAZ4851	Pastaza	Comunidad Santa Rosa	X	X	-

No.	Species	Voucher	Province	Locality	COI	ND2	RAG1
21	*Anolis fraseri*	QCAZ14393	Cotopaxi	South of Pucayacu	MW514058.1	MW512712.1	MW512704.1
22	*Anolis fraseri*	QCAZ3431	Chimborazo	Mayaguan	-	-	X
23	*Anolis fraseri*	QCAZ3441	Chimborazo	La Victoria	MW514056.1	MW512710.1	MW512702.1
24	*Anolis fraseri*	QCAZ6862	Pichincha	Mindo	JN112743.1	JN112683.1	JN112616.1
25	*Anolis fraseri*	QCAZ6867	Manabí	Bilsa ecological reserve	JN112744.1	JN112684.1	JN112617.1
26	*Anolis fraseri*	QCAZ8025	Cotopaxi	San Francisco de Las Pampas	MW514057.1	MW512711.1	MW512703.1
27	*Anolis fuscoauratus*	QCAZ5025	Morona Santiago	Comunidad Shaime	X	X	-
28	*Anolis gracilipes*	QCAZ8071	Esmeraldas	Alto Tambo, 5 km via al Placer	X	X	-
29	*Anolis granuliceps*	QCAZ4537	Esmeraldas	Via Durango-Playón de San Francisco	X	X	-
30	*Anolis maculiventris*	QCAZ4733	Pichincha	Límite de Otongachi	X	X	-
31	*Anolis maculiventris*	QCAZ14895	Carchi	Chical	X	X	-
32	*Anolis parilis*	QCAZ10178	Esmeraldas	4 km W at Alto Tambo	KU316043.1	KU316054.1	-
33	*Anolis parilis*	QCAZ15058	Esmeraldas	Tesoro Escondido reserve	MW514059.1	MW512713.1	MW512705.1

No.	Species	Voucher	Province	Locality	COI	ND2	RAG1
34	*Anolis parilis*	QCAZ15386	Esmeraldas	Durango	MW514060.1	MW512714.1	MW512706.1
35	*Anolis parvauritus*	QCAZ4532	Esmeraldas	Durango	X	X	-
36	*Anolis parvauritus*	QCAZ5047	Esmeraldas	Tundaloma Lodge	X	X	-
37	*Anolis scypheus*	QCAZ5104	Pastaza	Villano	X	X	-
38	*Polychrus marmoratus*	QCAZ10223	Sucumbíos	1.6 km S Jivino Verde	-	KY982434.1	KY982465.1
39	*Pristidactylus scapulatus*	PT4810	Argentina	Río Negro	JN112790.1	AF528732.1	JN112660.1
40	*Urostrophus gallardoi*	FBC36	Argentina	Córdoba	JN112791.1	AF528735.1	JN112661.1

Table 2. Data partitions used in phylogenetic analyses. Numbers in parentheses indicate codon position. Charset of sites, number of sequences (seq), sites, selected model, number of unique site patterns (USP), invariable sites (IVS) and parsimony-informative sites (PIS) are indicated.

Partition	charset	seq	sites	model	USP	IVS	PIS
COI (1)	1–655	111	219	TIM3e+I+G4	118	147	59
COI (2)	2–655	111	218	F81+F+I+G4	85	189	5
COI (3)	3–655	111	218	TN+F+I+G4	218	1	217
ND2 (1)	656–1691	146	346	GTR+F+I+G4	323	86	233
ND2 (2)	657–1691	146	345	TPM3u+F+I+G4	278	159	154
ND2 (3)	658–1691	146	345	TIM2+F+I+G4	345	3	341
RAG1 (1)	1692–2678	27	329	HKY+F+G4	71	269	33
RAG1 (2)	1693–2678	27	329	HKY+F+G4	64	280	29
RAG1 (3)	1694–2678	27	329	K2P+G4	111	209	73
All	–	158	2678	–	1613	1343	1144

Table 3. Comparison of type specimens for *Anolis binotatus*, *A. bitectus* and *A. lemniscatus*. Summary of meristic characters, measurements (mm) and color patterns in preservative. For each quantitative character, range and sample size (N) are given. (*) Variation reported by Williams et al. (1995).

Character	Holotype of *A. binotatus* (ZMB 4685 female)	Syntypes of *A. bitectus*	Syntypes of *A. lemniscatus*	This study (*A. binotatus*)
Scales between second canthals	8 (1)	8–13 (20)*	8–11 (5)	7–15 (81)
Postrostrals	6(1)	4–9 (20)*	4–6 (4)	4–8 (81)
Scales between supraorbital semicircles	1 (1)	2–3 (2)	1–2 (6)	1–3 (81)
Loreal rows	6 (1)	7 (2)	5–7 (6)	5–8 (81)
Scales between interparietal and semicircles	2 (1)	3–4 (2)	2–3 (6)	1–4 (81)
Supralabials to below centre of eye	7 (1)	8 (2)	7–10 (5)	7–10 (81)
Postmentals	6 (1)	5–6 (2)	4–6 (6)	4–7 (81)
Middorsal rows	14 (1) keeled	11 (2) keeled	10–12 (3) keeled	7–14 (81) keeled
Ventra body scales	Keeled, imbricate	Keeled, imbricate	Keeled, subimbricate	Keeled, subimbricate
Lamellae under phalanges II–III of fourth toe	16	13–14 (2)	12–16 (3)	10–15 (81)
Ventral larger dorsals scales	Present	Present	Present	Present

A stripe behind the eye, passing above the tympanum	Present	Present	Present	Present
Snout-vent length	44.0 (1)	52.0–52.0 (2)	45.0–52.0*	45.0–55.2 (81)

Table 4. Summary of meristic characters, ratios, measurements (mm) and color patterns of *Anolis binotatus*. For each quantitative character, range and sample size (N) followed by mean ± standard deviation are given.

Character	*A. binotatus*
Scales between second canthals	7–15 (81) 9.65 ± 1.36
Postrostrals	4–8 (81) 6.17 ± 0.82
Scales between supraorbital semicircles	1–3 (81) 1.35 ± 0.57
Loreal rows	5–8 (81) 6.81 ± 0.79
Scales between interparietal and semicircles	1–4 (81) 1.91 ± 0.57
Supralabials to below center of eye	7–10 (81) 8.41 ± 0.81
Postmentals	4–7 (81) 5.23 ± 0.89
Lamellae under phalanges II–III of fourth toe	10–15 (81) 13.05 ± 1.24
Middorsals rows	7–14 (81) 10.22 ± 1.36
Middorsals in 5% SVL	4–7 (81) 5.12 ± 0.55
Midventrals in 5% SVL	3–7 (81) 4.43 ± 0.76
Head length/SVL	0.22–0.30 (81) 0.26 ± 0.016
Head width/SVL	0.12–0.16 (81) 0.14 ± 0.007
Head height/SVL	0.10–0.14 (81) 0.12 ± 0.007
Snout length/SVL	0.09–0.13 (81) 0.11 ± 0.007
Interorbital length/SVL	0.02–0.05 (81) 0.03 ± 0.004
Interparietal length/SVL	0.02–0.05 (81) 0.03 ± 0.005
Ear height/SVL	0.01–0.03 (81) 0.02 ± 0.005
Humerus length/SVL	0.13–0.22 (81) 0.18 ± 0.018
Ulna length/SVL	0.11–0.26 (81) 0.15 ± 0.026
Hand length/SVL	0.13–0.19 (81) 0.16 ± 0.012
Femur length/SVL	0.27–0.36 (81) 0.31 ± 0.021

Tibia length/SVL	0.23–0.31 (81) 0.27 ± 0.017
Foot length/SVL	0.29–0.41 (81) 0.35 ± 0.022
4th toe length/SVL	0.17–0.33 (81) 0.21 ± 0.024
4th toe width/SVL	0.01–0.02 (81) 0.01 ± 0.003
Snout-vent length	45.00–55.22 (81) 49.02 ± 2.54
Dewlap color in males	Solid orange skin; scales white
Maximum SVL	55

Table 5. Sexual variation in meristic characters, ratios, and measurements (mm) in *Anolis binotatus*. Range and size (in parentheses) followed by mean ± standard deviation are given.

Character	Males	Females
Scales between second canthals	7–13 (47) 9.41 ± 1.28	8–15 (34) 10.00 ± 1.40
Postrostrals	5–7 (47) 5.94 ± 0.71	4–8 (34) 6.50 ± 0.86
Scales between supraorbital semicircles	1–3 (47) 1.24 ± 0.52	1–3 (34) 1.51 ± 0.60
Loreal rows	5–8 (47) 6.55 ± 0.74	6–8 (34) 7.17 ± 0.73
Scales between interparietal and semicircles	1–3 (47) 1.89 ± 0.38	1–4 (34) 1.95 ± 0.75
Supralabials to below center of eye	7–10 (47) 8.49 ± 0.83	7–10 (34) 8.29 ± 0.77
Postmentals	4–7 (47) 5.18 ± 0.87	4–7 (34) 5.30 ± 0.92
Lamellae under phalanges II–III of fourth toe	10–15 (47) 12.97 ± 1.37	11–15 (34) 13.16 ± 1.07
Middorsals rows	7–14 (47) 10.10 ± 1.43	8–12 (34) 10.31 ± 1.26
Middorsals in 5% SVL	5–7 (47) 5.34 ± 0.53	4–6 (34) 4.83 ± 0.44
Midventrals in 5% SVL	3–7 (47) 4.72 ± 0.81	3–5 (34) 4.06 ± 0.45
Head length/SVL	0.22–0.30 (47) 0.26 ± 0.018	0.23–0.29 (34) 0.26 ± 0.013
Head width/SVL	0.12–0.16 (47) 0.14 ± 0.007	0.13–0.15 (34) 0.14 ± 0.006
Head height/SVL	0.11–0.14 (47) 0.12 ± 0.007	0.10–0.12 (34) 0.11 ± 0.005
Snout length/SVL	0.10–0.13 (47) 0.11 ± 0.007	0.09–0.12 (34) 0.11 ± 0.006
Interorbital length/SVL	0.03–0.05 (47) 0.03 ± 0.004	0.02–0.04 (34) 0.03 ± 0.005
Interparietal length/SVL	0.02–0.05 (47) 0.03 ± 0.005	0.02–0.03 (4) 0.03 ± 0.003
Ear height/SVL	0.01–0.03 (47) 0.02 ± 0.005	0.03–0.04 (34) 0.03 ± 0.005
Humerus length/SVL	0.13–0.22 (47) 0.18 ± 0.019	0.15–0.22 (34) 0.18 ± 0.015
Ulna length/SVL	0.13–0.26 (47) 0.16 ± 0.026	0.11–0.17 (34) 0.14 ± 0.014
Hand length/SVL	0.13–0.19 (47) 0.16 ± 0.013	0.14–0.18 (34) 0.15 ± 0.011
Femur length/SVL	0.27–0.36 (47) 0.32 ± 0.020	0.27–0.35 (34) 0.30 ± 0.017
Tibia length/SVL	0.24–0.31 (47) 0.28 ± 0.016	0.23–0.29 (34) 0.26 ± 0.015
Foot length/SVL	0.29–0.41 (47) 0.35 ± 0.024	0.31–0.38 (34) 0.34 ± 0.015
4th toe length/SVL	0.17–0.28 (47) 0.21 ± 0.023	0.17–0.33 (34) 0.21 ± 0.025

4th toe width/SVL	0.01–0.02 (47) 0.01 ± 0.003	0.01–0.02 (34) 0.01 ± 0.002
Snout-vent length	45.0–54.1 (47) 48.57 ± 2.40	45.3–55.2 (34) 49.65 ± 2.62
Dewlap color	Solid orange skin; scales white	Absent dewlap
Maximum SVL	54	55

Figure 1. Phylogeny of the clade *Draconura* (upper left) with a close-up of *Anolis binotatus* and closely related species. Maximum Likelihood tree based on an analysis of 158 taxa and 2,678 characters including two mitochondrial genes (COI, ND2) and one nuclear gene (RAG1). Numbers above branches correspond to SH-aLRT/UFboot support values. GenBank accession numbers along with locality data are presented in Table 1 for newly sequenced specimens. Voucher number and province name are presented for specimens of *A*. *binotatus*. Colors represent northern (green), central (red), and southern (blue) clades discussed in the text.

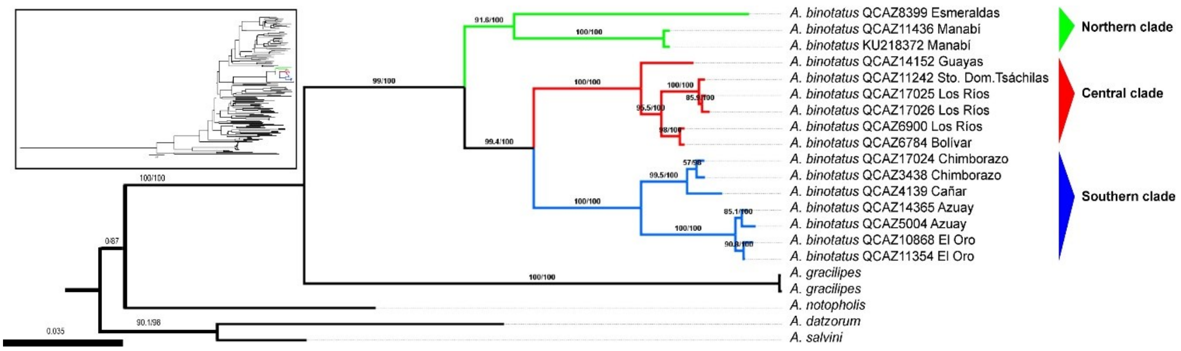

Figure 2. Scatterplots of LD1 and LD2 generated by discriminant function analyses on the morphometric (**A**) and meristic (**B**) variables recorded in this study for specimens identified as *Anolis binotatus*, *A. bitectus* and *A. lemniscatus*. Samples were assigned to three clades within *A. binotatus* based on the phylogenetic tree presented in Figure 1.

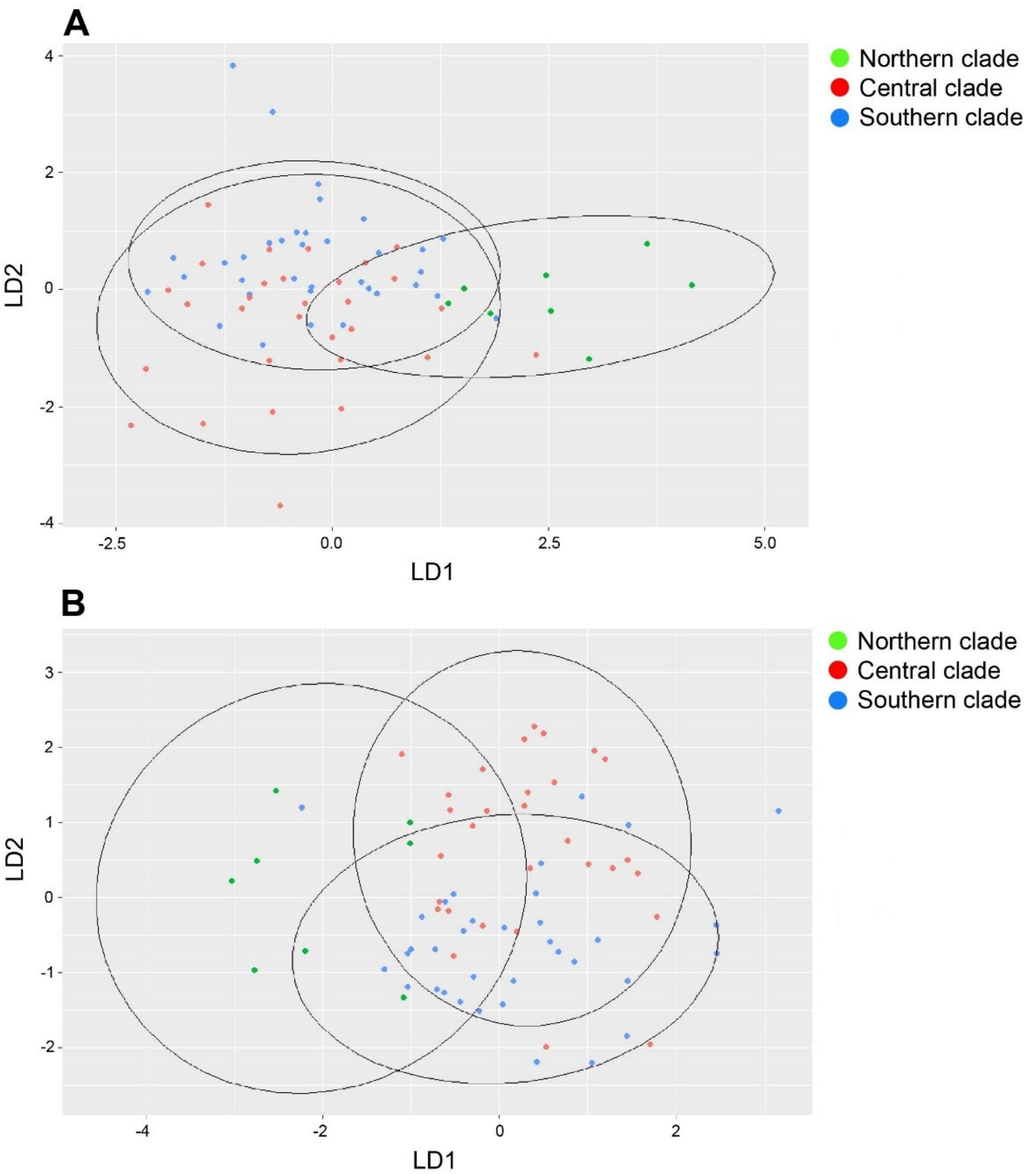

Figure 3. Type specimens of taxa included in this study. *Anolis binotatus*, ZMB 4685, female **holotype** (**A**, **D**); *A. bitectus*, BMNH 1946.8.20.11, female **syntype** (**B**, **E**); *A. lemniscatus*, BMNH 1946.8.8.38, female **syntype** (**C**, **F**) and BMNH 1946.8.8.39, male **syntype** (**G**). Scale bars = 5 mm. Photographs by A. Fläschendräger (**A**, **D**), and J. Streicher (**B**, **C**, **E**, **F**, **G**).

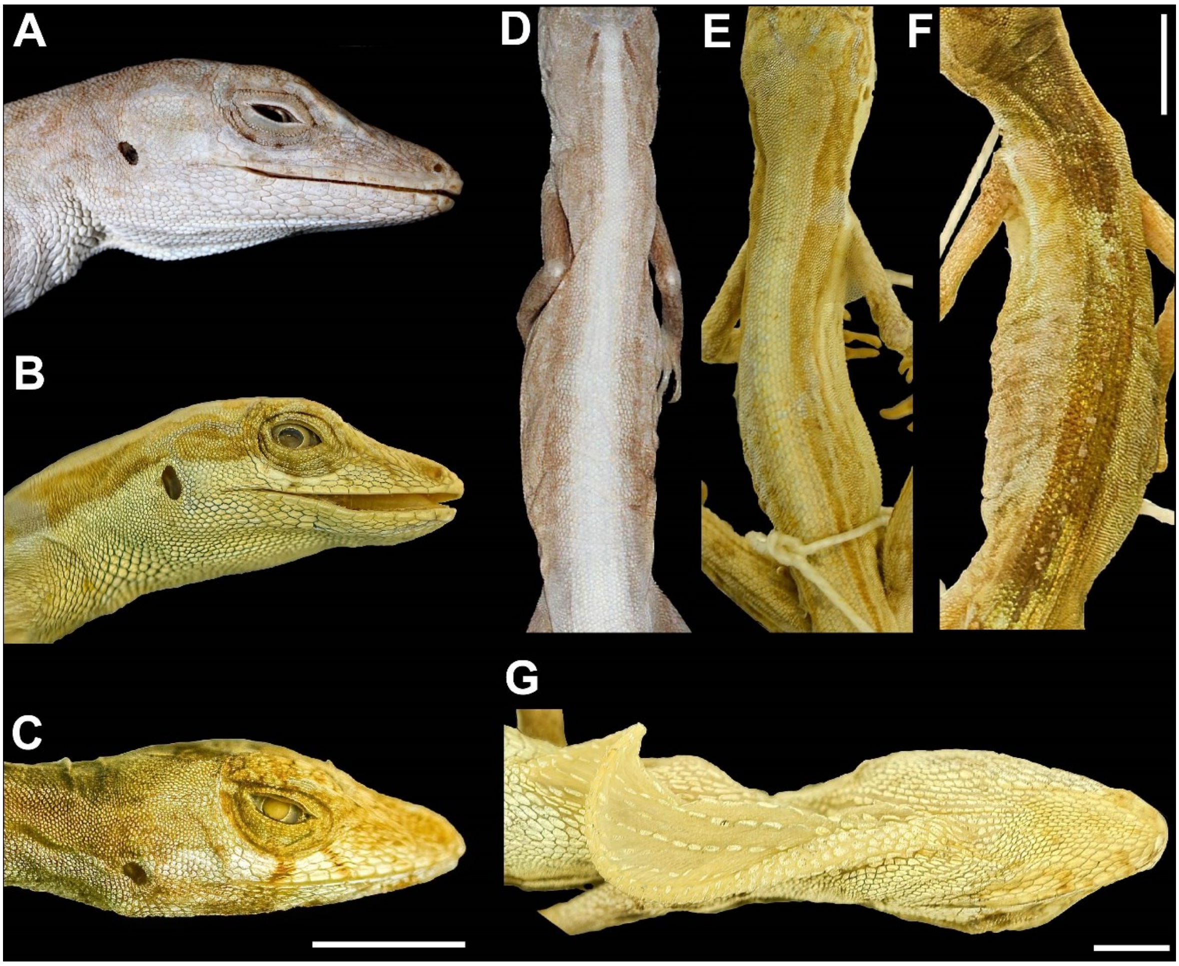

Figure 4. Variation of color in life of adult males of *Anolis binotatus*. Bosque Protector Cerro Blanco, Guayaquil, Guayas province, QCAZ 14151 (**A**, **B**); San Pablo, Chimborazo province, QCAZ 3437 *(***C**, **D**, **G**) to 15 km W Pallatanga; La Victoria, QCAZ 17041 (**E**, **F**, **H**) to 2.6 km E of type locality of *A. lemniscatus*. Photographs by F. Ayala (**A**, **B**, **C**, **D**, **G**), and F. Gordon (E, F, H).

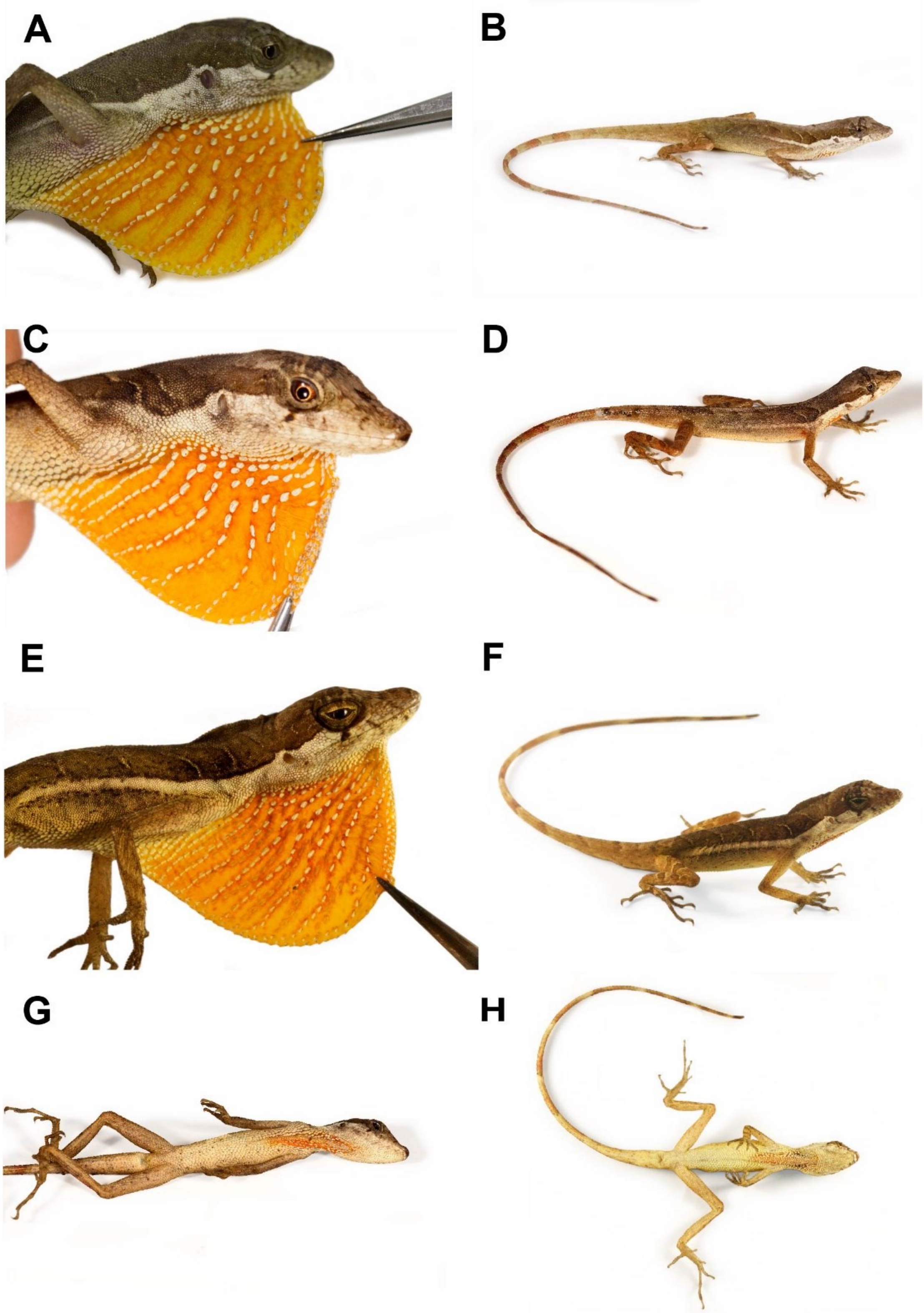

Figure 5. Variation of color in life of adult females and juvenile of *Anolis binotatus*. Guayas, Cerro El Mate, QCAZ 16283 (**A**, **B**, **C**) to 42 km SW of type locality of *A. binotatus*; Las Playas, Bolívar province, QCAZ 15868 (**D**, **E**, **F**) to 95 km N of Pallatanga; La Victoria, Chimborazo province, QCAZ 17044 (**G**, **H**, **I**) to 20.6 km E of type locality of *A. lemniscatus*; Cerro Mutiles, Esmeraldas province, iNaturalist (**J**, **K**); Centro Científico Río Palenque, Los Ríos province, juvenile male, QCAZ 14153 (**L**). Photographs by F. Ayala (**A**, **B**, **C**, **L**), G. Pazmiño (**D**, **E**, **F**), F. Gordon (**G**, **H**, I), and M. Yánez-Muñoz (**J**, **K**).

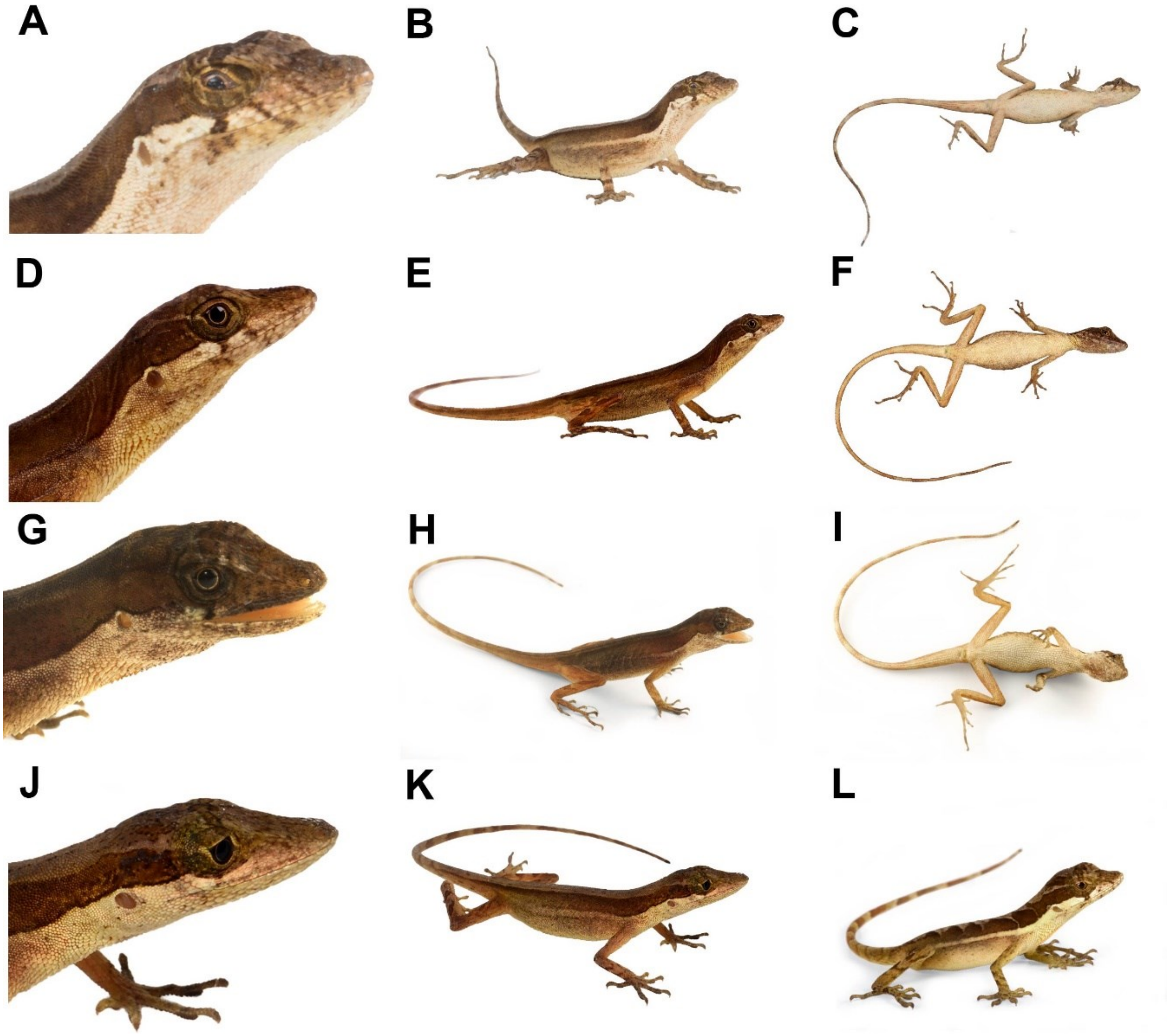

Figure 6. Hemipenes of *Anolis binotatus i*n asulcate (left), and sulcate (right) views. **A**) QCAZ 13120 (SVL = 47.33 mm) from Guayas province (central clade), **B**) QCAZ 3438 (SVL = 53.93 mm) from Chimborazo province (southern clade), **C**) QCAZ 14365 (SVL = 47.25 mm) from Azuay province (southern clade). Ca = calyces, CF = crotch flap, SS = sulcus spermaticus. Photographs by F. Ayala. Scale bar = 1 mm.

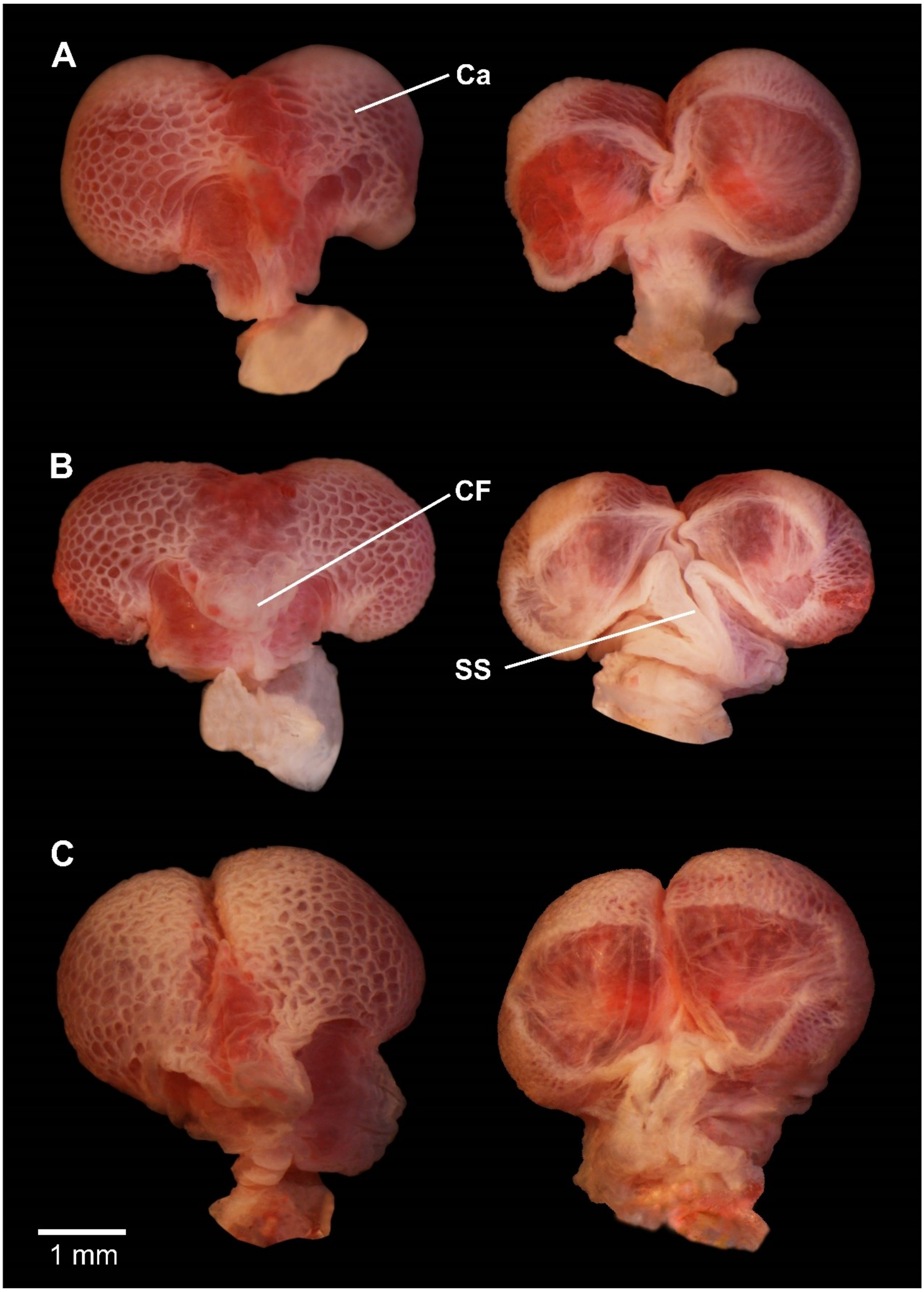

Figure 7. Distribution of *Anolis binotatus* in Ecuador. Records belonging to the northern clade are shown in green, central clade in red, and southern clade in blue. Minimum convex polygons are shown for each clade. Type localities of *A. binotatus* (yellow star), and *A. lemniscatus* (aqua star) are indicated. Pallatanga, herein proposed as type locality of *A. bitectus* is also shown (white star).