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MORPHOLOGICAL DESCRIPTION AND ANCESTRAL STATE RECONSTRUCTION OF THE HEMIPENES OF FIVE *ANOLIS* SPECIES (SQUAMATA: DACTYLOIDAE) DISTRIBUTED IN THE TRANS-ANDEAN REGION IN COLOMBIA

DESCRIPCIÓN MORFOLÓGICA Y RECONSTRUCCIÓN ANCESTRAL DE CARACTERES DE LOS HEMIPENES DE CINCO ESPECIES DE *ANOLIS* (SQUAMATA: DACTYLOIDAE) DISTRIBUÍDAS EN LA REGIÓN TRANS-ANDINA DE COLOMBIA

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Resumen.– La morfología de los genitales masculinos, los hemipenes, ha sido de gran importancia y bastante útil para la identificación, diferenciación y taxonomía de Squamata, especialmente para especies con morfología externa similar, asimismo ha sido ampliamente utilizado para el establecimiento de relaciones filogenéticas. En los Andes Colombianos se encuentran 29 especies de *Anolis* pertenecientes al grupo fuscoauratus, lagartos con morfología muy similar, lo que los hace normalmente muy difíciles de identificar. Aquí estudiamos y comparamos la morfología hemipenial de cinco especies de *Anolis* distribuidos en los Andes Colombianos: *Anolis antonii*, *A. fuscoauratus*, *A. mariarum*, *A. tolimensis* y *Anolis* candidata 1. Para establecer si los caracteres hemipeniales pueden ser útiles para diferenciar entre estas especies realizamos una reconstrucción filogenética y ancestral de caracteres, incluyendo las especies anteriormente mencionadas junto con otras cercanas filogenéticamente. Nuestras observaciones evidenciaron que la presencia de la cresta de piel en la cara asulcada, su tamaño y extensión, así como el tamaño de los lóbulos respecto a la longitud total del hemipene, y la presencia de proyecciones carnosas pueden ser ampliamente útiles para la identificación de especies de *Anolis*. También presentamos la primera descripción de este órgano para *A. antonii* y *A. tolimensis*, y encontramos tres morfologías diferentes para *A. mariarum*. Finalmente, soportamos la propuesta de la nueva especie *Anolis* candidata 1, debido a su morfología hemipenial distinta a las demás especies del género, especialmente debido a su tamaño y prominencia de la cresta de piel.

Palabras clave.– Estructuras hemipeniales, genitales masculinos, región neotropical, taxonomía..

Abstract.– Morphology of the male genitalia, the hemipenis, have shown to be very important and useful for identification, differentiation and taxonomy of squamates, especially for species that have very similar external morphology, it has also been used in the establishment of phylogenetic relationships. In the Colombian Andes, there are 29 species of *Anolis* belonging to the fuscoauratus group, lizards with very similar external morphology, which usually makes them very difficult to identify. Here we studied and compared the hemipenial morphology of five species of *Anolis* distributed on Colombian Andes: *Anolis antonii*, *A. fuscoauratus*, *A. mariarum*, *A. tolimensis* and a recently proposed species from the eastern cordillera, *Anolis* candidate 1. In order to establish if hemipenial characters would be useful to differentiate these species, we also made a phylogenetic and ancestral state reconstruction, including the mentioned species and others phylogenetically close. Our observations showed that presence of skin ridge on the asulcate face, its size and extension, as well as lobes size with respect to the total length of the hemipenis, and presence of fleshy projections can be very useful for identification of Anolis species. We also presented the first description of this organ for *A. antonii* and *A. tolimensis*. Also, we found three different morphologies for *A. mariarum*. Finally, we support the *Anolis* candidate one due to its different hemipenial morphology with respect to other species of the genus, which mainly differs for its size and prominence of its skin ridge.

Keywords.– Hemipenial structures, male genitalia, Neotropical region, taxonomy..

INTRODUCTION

Males of Squamata order have copulatory organs called hemipenes, which are paired and retracted towards base of tail (Böhme & Ziegler, 2009; Pough et al., 2016). During copulation, these are everted inside the female cloaca to transfer sperm through the sulcus spermaticus (Arnold, 1986; Böhme & Ziegler, 2009). These organs show great morphological variation in some groups such as *Siphlophis*, *Anolis*, and *Iphisa* (Zaher & Prudente, 1999; Köhler, 2009; Albano de Mello et al., 2023), and evolve rapidly compared to external characters, allowing differentiation between taxa (Arnqvist, 1997; Hosken & Stockley, 2004; Köhler et al., 2003; Köhler & Sunyer, 2008; Albano de Mello et al., 2023). However, in some groups such as Chamaeleonidae and Elapidae families this organ is very conservative (Klaver & Böhme, 1986; Keogh, 1999), hemipenes are often considered a source of useful characters in taxonomy and phylogeny of a variety of species (Köhler & Sunyer, 2008; Böhme & Ziegler, 2009; Köhler et al., 2012; da Silva et al., 2013; Gredler et al., 2014; D'Angiolella et al., 2016; Klaczko et al., 2017; Albano de Mello et al., 2023), especially in those with similar external morphology where identification is often challenging (Quipildor et al., 2018a; De-Lima et al., 2019). As mentioned above, hemipenial morphology can be highly variable. These organs might be unilobed or bilobed and exhibit ornamentations such as spines, ridges, papillae, or horns. Additionally, these organs exhibit a wide variation in size, thickness, and length of both trunk and lobes, as well as in the degree of development of sulcal lips, the presence of folds, flounces, wrinkles, and other characters (Dowling & Savage, 1960; Kluge, 1982; Böhme & Ziegler, 2009; Vitt & Caldwell, 2014; Gredler et al., 2015; Pough et al., 2016; Gilman et al., 2018; De-Lima et al., 2019).

Hemipenial morphology in anole lizards provided relevant evidence to identify and resolve the taxonomy of cryptic anole species (Köhler & Sunyer, 2008; Köhler & Vesely, 2010; Köhler et al., 2010, 2012; D'Angiolella et al., 2016). Köhler et al. (2012) studied the populations of *A. tropidogaster* distributed in Central America, which show very similar external morphology but exhibit a very distinct in their hemipenial morphology. Using the variation in the hemipenial morphology, Köhler et al. (2012) differentiated those populations into two species: *A. tropidogaster*, which has large, bulbous, and bilobed hemipenes, and *A. gaigei*, with small, thin, and unilobed hemipenes. D'Angiolella et al. (2016) described the hemipenial morphology of 12 species of *Anolis*, revealing a general pattern in which the hemipenes of most species are bilobed, although some are unilobed. Their lobes are calyculate, either globular or tubular, and, in most of the species, present a naked area or nude disk, depending on whether sulcal lips are present or not. The *sulcus spermaticus* is bifurcated, deep, and extended to the lobes (D'Angiolella et al., 2016).

According to Moreno-Arias et al. (2021) and Uetz et al. (2023), among the *Anolis* species distributed in Colombia, are *A. antonii* (Western and Central Cordilleras), *A. fuscoauratus* (Amazonian region with its occurrence confirmed in the Magdalena Valley by Grisales et al., 2017), *A. mariarum* (in the Western and Central Cordilleras), and *A. tolimensis* (Central and Eastern Cordilleras). All these species were previously nested in the fuscoauratus group (Williams, 1976; Ayala & Williams, 1988; Savage, 2002; Nicholson, 2002), which now is recognized as polyphyletic (Nicholson, 2002; Poe, 2004; Nicholson et al., 2012).

Acevedo et al. (data not published) recently studied disjunct populations of *A. tolimensis* distributed in Central and Eastern Cordillera using an integrative taxonomic approach that includes molecular, morphological, and ecological information. They found that these populations correspond to a species complex with at least three new species, one of which is referred to as Anolis candidate 1. This species is distributed in the Eastern Cordillera of Colombia specifically in the localities of San Antonio, Tena, and Silvania, at Cundinamarca department.

Based on the aforementioned information, we describe and compare the morphology of the hemipenes of *A. antonii*, *A. fuscoauratus*, *A. mariarum A. tolimensis*, and *Anolis* candidate 1 to determine whether this morphology provides valuable taxonomic evidence for differentiating these species. We also evaluated the hypothesis proposed by Acevedo et al. (data not published), which points out that the populations of *A. tolimensis* distributed in the Central Cordillera differs from *Anolis* candidate 1, which is distributed in the Eastern Cordillera. Additionally, we described and optimized morphological characters through ancestral reconstruction to determine if they provide helpful and systematic information for the group.

MATERIALS AND METHODS

Our study aimed to examine hemipenes of 18 specimens from five species of the genus Anolis (analyzed specimens by species in parenthesis): *A. antonii* (5), *A. fuscoauratus* (5), *A. mariarum* (5), *A. tolimensis* (1), and *Anolis* candidate 1 (2) proposed by Acevedo et al. (data not published). These species are distributed in the cordilleras of Colombia, except for *A. fuscoauratus*, which is found across the Amazon basin (Fig. 1). We used the specimens deposited at the Colección Zoológica "José Ricardo Cure Hakim" of the Universidad Militar Nueva Granada (CZCH-UMNG) (2 specimens) and the Colección de Herpetología del Instituto de Ciencias Naturales, Bogotá, Colombia (ICN-MHN-Her) (18 specimens) (Table 1).

Preparation and description of hemipenes

Due to the size and fragility of these organs in the species studied, we used only specimens with fully or partially everted hemipenes.

We followed techniques from Pesantes (1994) and Zaher & Prudente (2003). First, we made an incision at the base of the tail to extract the structure. Then, the organs were immersed in 2 % potassium hydroxide for 30-second intervals until they became translucent and soft. We filled the organs with colored petroleum jelly and everted them with the help of insulin needles with rounded tips. We took photographs of the sulcate and asulcate views using a camera attached to a Stereomicroscope. Descriptions were made following the terminology proposed

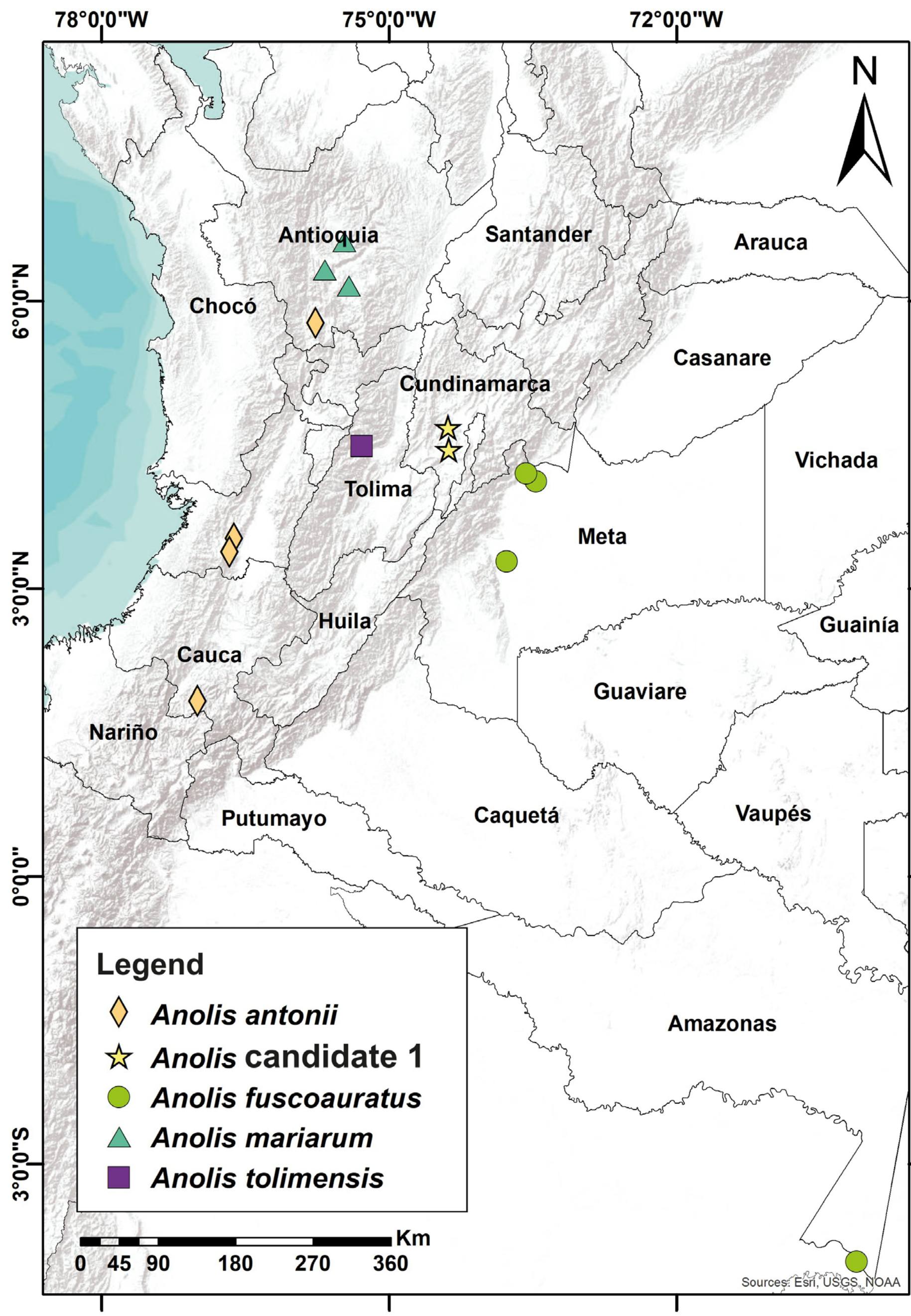

Figura 1. Localidades de colecta de los especímenes del grupo fuscoauratus usados en este estudio.

Figure 1. Collection localities of the specimens of fuscoauratus group used in this study

Tabla 1. Número de colecta, localidad, y estado de hemipenes de los especímenes de *Anolis* preparados en el trabajo. Las abreviaciones son: EP, Evertidos parcialmente; ET, Evertidos totalmente; ICN, Colección de Herpetología del Instituto de Ciencias Naturales, Bogotá, Colombia; CZCH-UMNG-R, Colección de Herpetología José Ricardo Cure Hakim, Universidad Militar Nueva Granada, Cajicá, Colombia.

Table 1. Collection number, range, locality, and hemipenes status of *Anolis* specimens prepared in this work. The abbreviations are as follows: EP, Partially Everted; ET, Totally Everted; ICN, Colección de Herpetología del Instituto de Ciencias Naturales, Bogotá, Colombia; CZCH-UMNG-R, Colección de Herpetología José Ricardo Cure Hakim, Universidad Militar Nueva Granada, Cajicá, Colombia.

Collection number	Species	Mountain range	Location	Hemipenes status
ICN3775	*Anolis antonii*	Western	Valle del Cauca, Km 18 Cali vía el mar	EP
ICN3778	*Anolis antonii*	Central	Antioquia, Jericho	ET
ICN3786	*Anolis antonii*	Western	Cauca, Bolívar	EP
ICN3788	*Anolis antonii*	Western	Valle del Cauca, Peñas Blancas	ET
ICN4436	*Anolis antonii*	Western	Valle del Cauca, Peñas Blancas	EP
ICN4437	*Anolis fuscoauratus*	Not in cordillera	Meta, San Juan de Arama	ET
ICN8614	*Anolis fuscoauratus*	Not in cordillera	Meta, San Juan de Arama	ET
ICN11069	*Anolis fuscoauratus*	Not in cordillera	Amazonas, Leticia	EP
ICN11069	*Anolis fuscoauratus*	Not in cordillera	Meta, Villavicencio	EP
ICN11073	*Anolis fuscoauratus*	Not in cordillera	Meta, Villavicencio	ET
ICN3807	*Anolis mariarum*	Central	Antioquia, Santa Rosa de Osos	EP
ICN3808	*Anolis mariarum*	Central	Antioquia, Km 9 SE Palmitas	ET
ICN3791	*Anolis mariarum*	Central	Antioquia, Rionegro	ET
ICN3793	*Anolis mariarum*	Central	Antioquia, Rionegro	ET
ICN3799	*Anolis mariarum*	Central	Antioquia, Rionegro	ET
ICN3785	*Anolis tolimensis*	Central	Tolima, Llanitos	ET
ICN5966	*Anolis* candidate 1	Eastern	Cundinamarca, Tena	ET
CZCH-UMNG-R:185	*Anolis* candidate 1	Eastern	Cundinamarca, Silvania	EP

by Klaver and Böhme (1986), Branch (1986), Zaher (1999), and D'Angiolella et al. (2016). Based on the morphological descriptions, five characters and their states were defined to reconstruct their evolutionary history.

Phylogenetic reconstruction and ancestral states

We performed the phylogenetic reconstruction using ND2 gene sequences generated by Grisales-Martínez et al. (2017). We also included the sequence of *Anolis* candidate 1, a new species proposed by Acevedo et al. (data not published), along with other species closely related to *Anolis fuscoauratus*, based on the phylogenetic hypothesis by Poe et al. (2017) (Table 2). The selection criteria for the chosen species were based on the availability of hemipenes descriptions in the literature (view Appendix 1 to see characteristics of all species used). We also used *A. cristatellus*, *A. stratulus*, and *A. auratus* as outgroups, selected for their close phylogenetic relationship to the chosen species according to Poe et al. (2017) and Nicholson et al. (2018). We performed the sequence alignment with MAFFT v7 using the default parameters and conducted maximum likelihood and ultrafast bootstrap analyses (BS) with 1,000 replicates using IQ-TREE (http://iqtree.cibiv.univie.ac.at).

We performed ancestral state reconstruction (AR) (Table 3) using the R v4.3.1 (https://www.r-project.org) with the phytools package (Revell, 2012). Equal-rates (ER) model was used instead of all-rates-different (ARD) model, based on AIC value selection (Revell & Harmon, 2022). We defined the character states based on characteristics observed and recorded in the literature. As we identified three hemipenial morphologies for Anolis mariarum,

Tabla 2. Secuencias con el número de acceso de GenBank usadas la reconstrucción filogenética en este estudio. El grupo externo está indicado por un asterísco (*).

Table 2 . Sequences with GenBank accession numbers used in the phylogenetic reconstruction for this study. Outgroup are indicated by an asterisk (*).

Access code	Species	Voucher	Reference
MF449477	*Anolis antonii*	CZPD5006	Grisales-Martínez et al., 2017
MF449498	*Anolis fuscoauratus*	MHUAR12433	Grisales-Martínez et al., 2017
Not published	*Anolis tolimensis*	MCAM79	Acevedo, 2023
MF449486	*Anolis mariarum*	MHUAR11077	Grisales-Martínez et al., 2017
Not published	*Anolis candidate 1*	MCAM015	Acevedo, 2023
*AY909740	*Anolis auratus*	CIEZAH1163	Grisales-Martínez et al., 2017
MF449501	*Anolis urraoi*	MHUAR12517	Grisales-Martínez et al., 2017
AY909778	*Anolis sericeus*	LACM7069	Grisales-Martínez et al., 2017
AY909783	*Anolis tropidonotus*	LDW11998	Grisales-Martínez et al., 2017
AF294285	*Anolis trachyderma*	Not reported	Grisales-Martínez et al., 2017
AY909782	*Anolis tropidogaster*	JMS203	Grisales-Martínez et al., 2017
AY909772	*Anolis polylepis*	JMS46	Grisales-Martínez et al., 2017
AY909770	*Anolis kemptoni*	JMS35	Grisales-Martínez et al., 2017
AY909735	*Anolis altae*	MVCFC14383	Grisales-Martínez et al., 2017
KJ954107	*Anolis humilis*	Not reported	Grisales-Martínez et al., 2017
KJ953945	*Anolis quaggulus*	Not reported	Grisales-Martínez et al., 2017
*KX173775	*Anolis cristatellus*	Not reported	Winchell et al., 2016
*AF055929	*Anolis stratulus*	USNM321908	Jackman et al., 1999
AY909762	*Anolis isthmicus*	MF0191	Grisales-Martínez et al., 2017

Tabla 3. Probabilidad de reconstrucción de estados de carácter de los hemipenes basados en este estudio y revisión de literatura, junto con su valor de AIC para los modelos ER y ARD. Los números de nodo corresponden con aquellas mostrados de la Figura 5 a Figura 9. Números en negrita corresponden a Clado 1(Nodo 16), Clado 2 (Nodo 13), Clado 3 (Nodo 10), Clado 4 (Nodo 7), Clado 5 (Nodo 5), y grupo interno (Nodo 4). La sumatoria de las probabilidades debe igualar uno. Si la sumatoria no iguala uno, los valores faltantes corresponden a un valor de incertidumbre en la reconstrucción de los estados de carácter. Las abreviaciones son: A=Dos tercios de la longitud total, B = Mitad de la longitud total, y C = Un tercio de la longitud total.

Table 3. Probability of state character reconstruction of hemipenes based on this study and literature review, along with their AIC values for ER and ARD models. Node numbers correspond to those shown in Figure 5 to Figure 9. Bold numbers correspond to Clade 1 (node 16), Clade 2 (Node 13), Clade 3 (Node 10), Clade 4 (Node 7), Clade 5 (Node 5), and ingroup (Node 4). Sum of the probabilities should total one. If the sum does not equal one, the missing value corresponds to uncertainty value in the reconstruction of the character states. Abbreviations are as follows: A= Two thirds of the total length, B= Half of the total length, and C= One third of the total length.

Node	Skin ridge		Lobes form		Lobes size			Type of hemipenes		Flounces	
	Present	Absent	Globular	Tubular	A	B	C	Bilobed	Unilobed	Present	Absent
1	0.37	0.31	0.50	0.50	0.25	0.25	0.25	0.99	0.01	0.30	0.50
2	0.36	0.20	0.50	0.50	0.25	0.25	0.26	1.00	0.00	0.16	0.70
3	0.38	0.41	0.50	0.50	0.25	0.25	0.25	1.00	0.00	0.44	0.40
4	0.51	0.40	0.50	0.50	0.26	0.25	0.25	1.00	0.00	0.48	0.44
5	0.53	0.41	0.50	0.50	0.43	0.19	0.19	1.00	0.00	0.06	0.91
6	0.58	0.39	0.50	0.50	0.26	0.26	0.24	1.00	0.00	0.68	0.30

Tabla 3 (cont.). Probabilidad de reconstrucción de estados de carácter de los hemipenes basados en este estudio y revisión de literatura, junto con su valor de AIC para los modelos ER y ARD. Los números de nodo corresponden con aquellas mostrados de la Figura 5 a Figura 9. Números en negrita corresponden a Clado 1(Nodo 16), Clado 2 (Nodo 13), Clado 3 (Nodo 10), Clado 4 (Nodo 7), Clado 5 (Nodo 5), y grupo interno (Nodo 4). La sumatoria de las probabilidades debe igualar uno. Si la sumatoria no iguala uno, los valores faltantes corresponden a un valor de incertidumbre en la reconstrucción de los estados de carácter. Las abreviaciones son: A=Dos tercios de la longitud total, B = Mitad de la longitud total, y C = Un tercio de la longitud total.

Table 3 (cont.). Probability of state character reconstruction of hemipenes based on this study and literature review, along with their AIC values for ER and ARD models. Node numbers correspond to those shown in Figure 5 to Figure 9. Bold numbers correspond to Clade 1 (node 16), Clade 2 (Node 13), Clade 3 (Node 10), Clade 4 (Node 7), Clade 5 (Node 5), and ingroup (Node 4). Sum of the probabilities should total one. If the sum does not equal one, the missing value corresponds to uncertainty value in the reconstruction of the character states. Abbreviations are as follows: A= Two thirds of the total length, B= Half of the total length, and C= One third of the total length.

Node		Skin ridge		Lobes form		Lobes size			Type of hemipenes		Flounces	
		Present	Absent	Globular	Tubular	A	B	C	Bilobed	Unilobed	Present	Absent
7		0.54	0.42	0.50	0.50	0.26	0.26	0.24	0.99	0.01	0.68	0.29
8		0.51	0.44	0.50	0.50	0.30	0.30	0.20	1.00	0.00	0.93	0.05
9		0.61	0.37	0.50	0.50	0.26	0.27	0.24	1.00	0.00	0.69	0.29
10		0.52	0.44	0.50	0.50	0.27	0.27	0.23	1.00	0.00	0.58	0.38
11		0.11	0.87	0.50	0.50	0.34	0.28	0.19	1.00	0.00	0.13	0.85
12		0.99	0.01	0.50	0.50	0.30	0.30	0.21	1.00	0.00	0.94	0.05
13		0.99	0.00	0.50	0.50	0.30	0.30	0.21	1.00	0.00	0.94	0.05
14		0.99	0.00	0.50	0.50	0.23	0.32	0.23	1.00	0.00	0.85	0.10
15		0.97	0.01	0.50	0.50	0.22	0.29	0.25	1.00	0.00	0.84	0.09
16		1.00	0.00	0.50	0.50	0.35	0.32	0.18	1.00	0.00	0.98	0.01
17		1.00	0.00	0.50	0.50	0.59	0.14	0.18	1.00	0.00	0.98	0.02
18		1.00	0.00	0.50	0.50	0.78	0.07	0.09	1.00	0.00	0.97	0.03
AIC values	**ARD**	**41.96**		**26.31**			**65.51**		**15.38**		**42.23**	
	ER	**35.64**		**26.95**			**51.51**		**15.67**		**39.49**	

we assumed that the specimen we used had morphology type 1, as it is the accepted morphology and reported in literature.

RESULTS

Morphological description of hemipenes

All characters observed and used in hemipenes descriptions are shown in Figure 2. The hemipenes of *A. antonii A. fuscoauratus*, *A. mariarum*, *A. tolimensis*, and *Anolis* candidate 1 (Fig. 3) are bilobed (either globular or tubular), with calyculated lobes and have a deep and bifurcated sulcus spermaticus extending from the base of the hemipenis to the apex of the lobes. We found differences among species in total length of organ, size of lobes, presence and extent of skin ridges on asulcate face, and presence of fleshy projections at the base of lobes (Fig. 3). In addition to these differences, we identified three different morphological types in *A. mariarum* (see below). In all five species, except for *A. mariarum* type 2, a skin ridge is present on the asulcate face, from the base of lobes. This ridge is prominent in *A. mariarum* type 1 and 3, *A. fuscoauratus* and *Anolis* candidate 1. In *Anolis* candidate 1, *A. fuscoauratus*, *A. mariarum*, and *A. tolimensis* the trunk constitutes the major proportion of the total length of the hemipenes, unlike *A. antonii*, where the lobes constitute the major proportion. Another difference between *A. antonii* and the other species is that *A. antonii* has tubular lobes with lateral fleshy projections and does not have flounces. In contrast, the other species have globular lobes and lack lateral fleshy projections and horizontal flounces (Fig. 3A) (see Appendices 2-6 for photographs of all the hemipenes studied). We will now present detailed descriptions of morphological variation in the hemipenes of each studied species:

Anolis antonii (Fig. 3A)

Hemipenes with tubular lobes. The lobes constitute approximately two-thirds of the total length of the hemipenis trunk and have lateral fleshy projections extending from the middle of the trunk to middle of the lobes. On the sulcate face, sulcus spermaticus has well-developed sulcal lips that open into a nude disk at the apex of the lobe. Outside this naked disk, lobes are densely calyculated. On asulcate face, a prominent skin ridge extends from the base of the trunk to the crotch of lobes, and adjacent calyces are elongated. The trunk has no flounces.

Anolis fuscoauratus (Fig. 3B)

Hemipenes with globular lobes. The lobes are approximately one-third the size of the trunk and do not have fleshy projections. On the sulcate face, *sulcus spermaticus* features poorly developed sulcal lips that open into a nude disk at the apex of lobes. Lobes are calyculated outside this naked disk. On the asulcate face, a medial skin ridge extends from the distal part of the trunk to the crotch of lobes, and the adjacent calyces are elongated. The trunk exhibits three to five flounces, which are more prominent on the lateral faces.

Anolis tolimensi (Fig. 3C)

Hemipenes with globular lobes. Lobes constitute about half the hemipenis length and do not have fleshy projections. On the sulcate face, *sulcus spermaticus* has well-developed sulcal lips that open into a nude disk at the apex of lobes. Outside this naked disk, lobes are densely calyculated. On asulcate face, a skin ridge

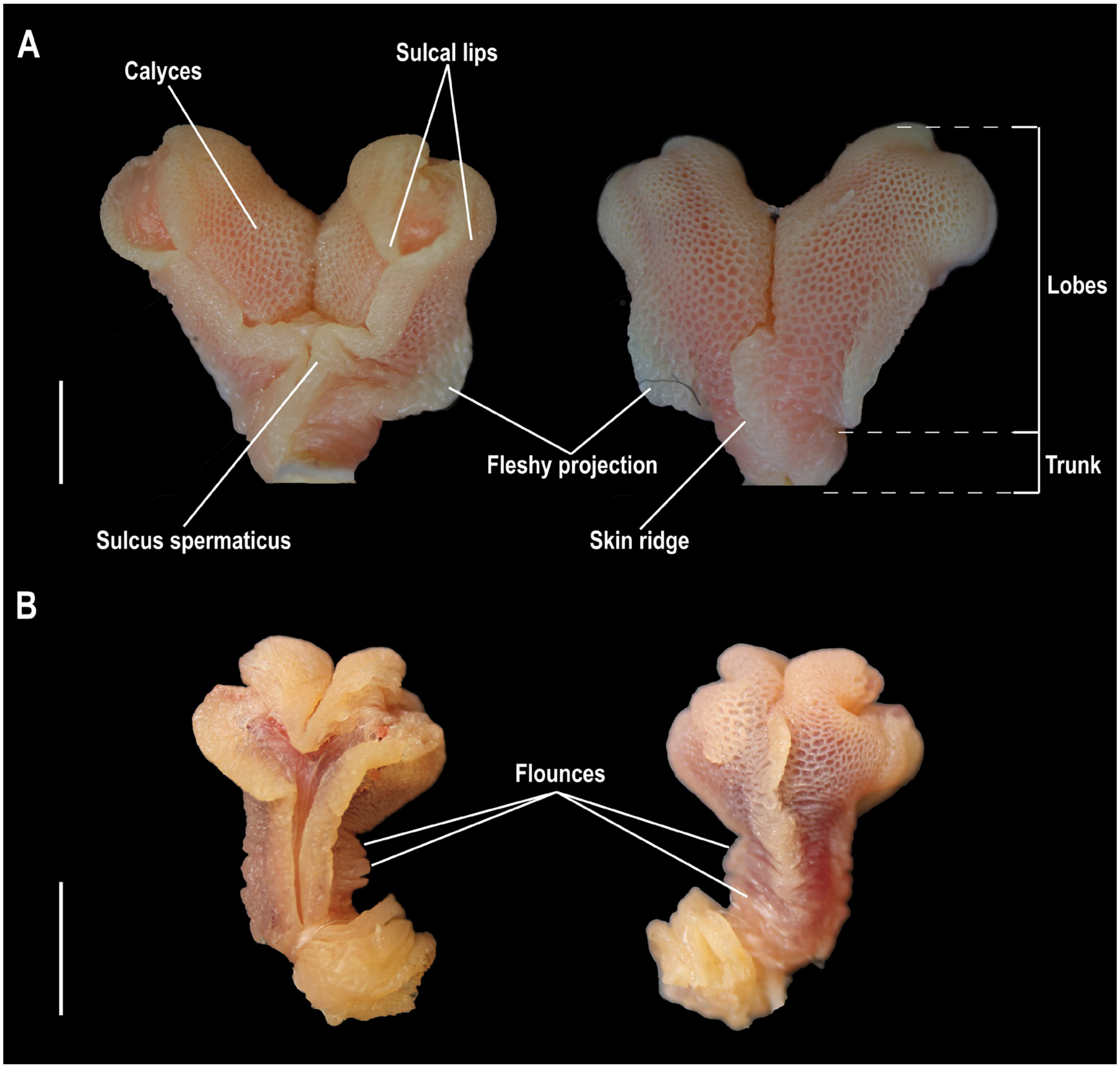

Figura 2. Características morfológicas de la estructura hemipenial en las caras sulcada (izquierda) y asulcada (derecha) de las especies de *Anolis*, (A) *Anolis antonii*, voucher ICN3786, (B) *Anolis tolimensis*, voucher ICN3785 (Escala = 2mm). Fotos: Stiven Melo-Torres.

Figure 2. Morphological characteristics of hemipenial structure on both sulcate (left) and asulcate (right) faces of *Anolis* species, (A) *Anolis antonii*, voucher ICN3786, (B) *Anolis tolimensis*, voucher ICN3785 (Scale = 2mm). Photos: Stiven Melo-Torres.

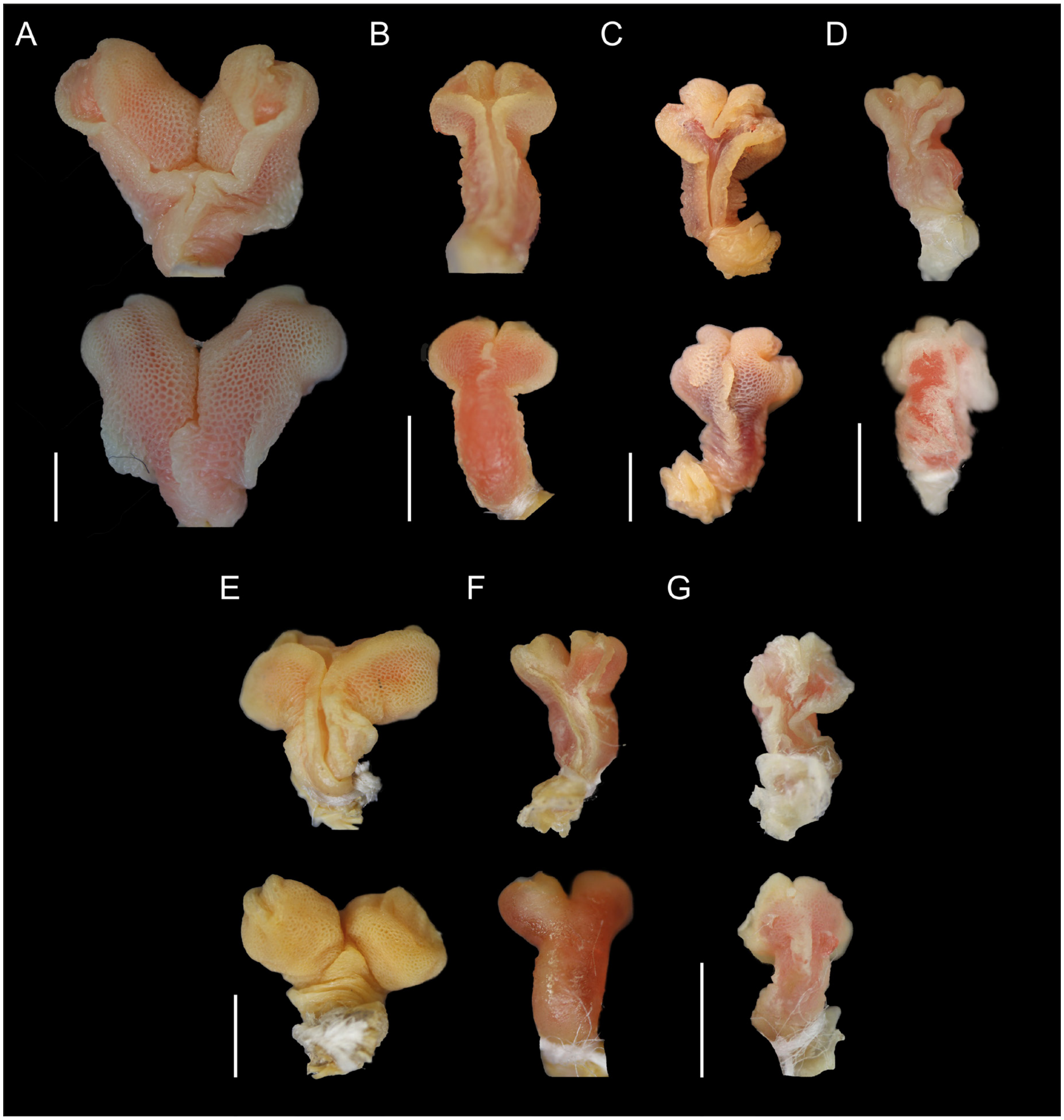

Figura 3. Hemipenes de (A) *A. antonii*, (B) *A. fuscoauratus*, (C) *A. mariarum* tipo 1, (D) *A. mariarum* tipo 2 (E). *A. mariarum* tipo 3 (F) *A. tolimensis*, (G) *Anolis* candidata 1, cara asulcada (arriba) y cara asulcada (abajo) (Escala = 2mm). Fotos: Stiven Melo-Torres.

Figure 3. Hemipenes of (A) *A. antonii*, (B) *A. fuscoauratus*, (C) *A. mariarum* type 1, (D) *A. mariarum* type 2 (E). *A. mariarum* type 3 (F) *A. tolimensis*, (G) *Anolis* candidate 1, sulcate face (top) and asulcate face (bottom) (Scale = 2mm). Photos: Stiven Melo-Torres.

extends from the middle of the trunk to the crotch of lobes. It is very prominent near the crotch of the lobes and becomes shallower as it extends along the trunk; the calyces adjacent to the skin ridge are elongated. Trunk has three to four horizontal flounces.

Anolis canditate (Fig. 3D)

Hemipenes with globular and calyculate lobes. Lobes comprise approximately one-half to one-third of total length of the hemipenis and lack fleshy projections. On the sulcate face, *sulcus spermaticus* features poorly developed sulcal lips that open into a naked disk at the apex of lobes. Trunk presents two flounces, which are more noticeable in the lateral regions. There is a skin ridge that is shallow and present only at the base of the lobes.

Anolis mariarum (Fig. 3E-G)

All individuals of this species have hemipenes with globular lobes and without fleshy projections. On the sulcate face, *sulcus spermaticus* has well-developed and deep sulcal lips, which are shallower on the type 2, and opens into a naked disk at the apex of the lobes. Outside this nude disk, lobes are calyculated, these are shallower in type 2. The specimens exhibit three different types of hemipenial morphology: Type 1 (Fig. 3E): Lobes constitute approximately half of the total length of the organ. On the asulcate face, a skin ridge extends from the distal part of the trunk to the crotch of the lobes, and adjacent calyces are elongated. Trunk has two to three flounces on each side. Type 2 (Fig. 3F): Lobes constitute approximately one-third of the total length of the hemipenis. There is no skin ridge on the asulcate face, and the trunk does not present horizontal flounces. Type 3 (Fig. 3G): Lobes constitute approximately one-third to one-quarter of total length of the hemipenis. A skin ridge is present on the asulcate face, extending from the middle of the trunk to the crotch of the lobes, and with the adjacent calyces enlarged. Trunk does not present flounces.

Phylogenetic analyses

The maximum likelihood analysis shows five clades excluding the external group (Figure 4). Clade 1, formed by *A. tolimensis*, *Anolis* candidate 1, *A. antonii*, and *A. mariarum* (BS 94 %) conforms to a South American monophyletic group. Clade 2 nest *A. fuscoauratus*, *A. urraoi*, *A. kemptoni*, and *A. altae* (BS 40 %), belonging to South America and Central America. Clade 3 is formed by *A. polylepis*, *A. humilis*, and *A. quaggulus* (BS 90 %), all these species are from Central America. Clade 4 contains *A. trachyderma*, *A. tropidogaster*, and *A. tropidonotus* (BS 65 %), as well as Clade 2, these species are distributed in South America and Central America. Finally, Clade 5 relates *A. sericeus* and *A. isthmicus* (BS 100 %), species from Central America (Fig. 4).

Definition of morphological characters

Based on the hemipenes of the studied species and images and descriptions published in the literature, we defined five characters for ancestral reconstruction: Character 1: Type of hemipenes (0 = bilobed; 1 = unilobed); Character 2: Type of lobes (0 = globular; 1 = tubular); Character 3: Proportion of the length of lobes relative to the total length of the hemipenes (0 = half of the total length [1/2]; 1 = one-third of the total length [1/3]; 2 = two-thirds of the total length [2/3]); Character 4: Skin ridge (0 = presence; 1 = absence); and Character 5: Horizontal flounces (0 = presence; 1 = absence).

Ancestral states reconstruction of characters

Below, we present the results of the ancestral state reconstruction analysis, using the morphological characters defined herein.

Character 1. Type of hemipenes (Fig. 5, Table 3)

The ancestral state of all clades of the fuscoroids group is hemipenes bilobed with a probability value of 100 % at each node. The hemipenes unilobed appear twice as independent events.

Character 2. Type of lobes (Fig. 6, Table 3)

The globular and tubular states reconstruction (AR 50 %) is maintained throughout all nodes in the reconstruction.

Character 3. The Proportion of the length of lobes relative to the total length of the hemipenes (Fig. 7, Table3)

The ancestral state estimated for the South American Clade is lobes with two-thirds (2/3) of the total length of the hemipenes (Clade 1 - node 16, AR 35 %). Also, the ancestral state of the clade (A. mariarum + A. antonii) is lobes with two-thirds (1/2) of the total length of the hemipenes (node 18, AR 78 %). The ancestral state for the South American + Central American Clades (Clade 2 – node 13 and Clade 4 – node 7) is ambiguous for Clade 2, (AR 30 % for both states. The ancestral state of the Central American Clades is, for Clade 3, lobes with half (½) of the total length of the hemipenes (node 10, AR 27 %; and for the Clade 5, lobes with two-thirds (2/3) of the total length of the hemipenes (node 5, AR 43 %).

Character 4. Skin ridge (Fig. 8, Table 3)

The ancestral node reconstruction of skin ridge shows the presence of ornamentation in the ingroup (node 4, AR 51 %), Clade 1 (node 16, AR 99 %), Clade 2 (node 13, AR 99 %), Clade 3 (node 10, AR 51 %), Clade 4 (node 7, AR 54 %), and Clade 5 (node 5, AR 53 %).

Character 5. Flounces (Fig. 9, Table 3)

The ancestral node reconstruction of the horizontal flounces shows the presence of these flounces in the ingroup group (Node 4, AR 48 %), Clade 4 (node 7, AR 68 %), Clade 3 (node 10, AR 58 %), Clade 2 (node 13, AR 94 %), Clade 1 (node 16, AR 98 %). The ancestral states of the Central American Clade (Clade 5 - node 5, AR 91 %) are different, showing the absence of the reconstruction of the horizontal flounces.

DISCUSSION

Hemipenial morphology in *Anolis*

According to Köhler et al. (2012) *Anolis* hemipenes vary in their morphology, being either bilobed or unilobed, and the length of the lobes, while other characters, such as ornamentations, appear static within the same species. Also, according to D'Angiolella et al. (2016), anoles hemipenes may be bilobed or unilobed, and lobes, either globular or tubular with presence of ornamentations such as calyces or skin projections. In our results, we found that main variations in hemipenial morphology of *A. mariarum*, *A.*

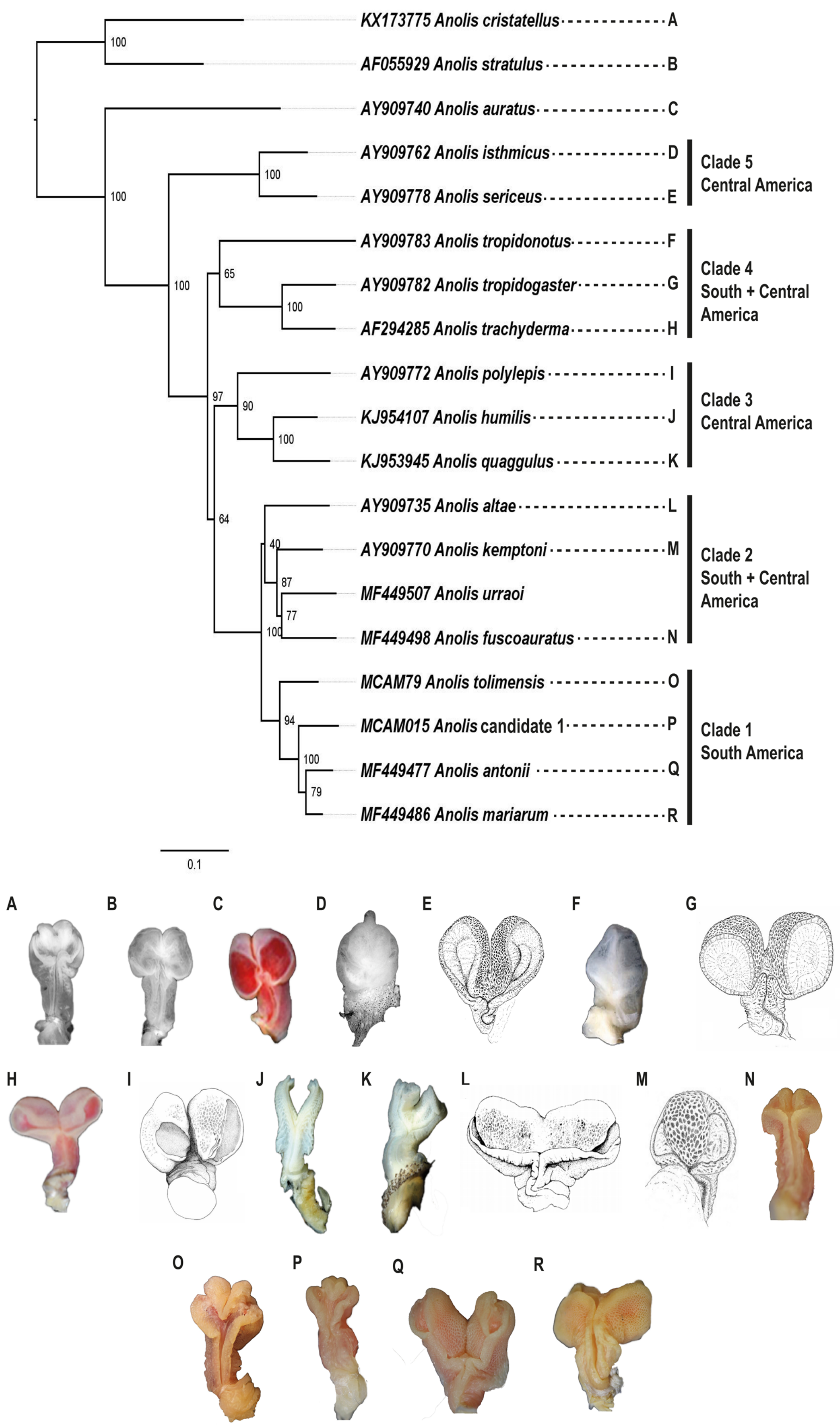

Figura 4. Árbol de máxima verosimilitud con el soporte mostrando las relaciones filogenéticas de las especies Andinas y otras relacionadas del grupo fuscoauratus. Aquí presentamos los clados asociados con las áreas de Centro y Sur América. Abajo se encuentran los hemipenes para cada especie en la topología indicada por letras (A-R).

Figure 4. Maximum likelihood tree with bootstrap node support showing phylogenetic relationships of Andean and other related species of the fuscoauratus group. We presented clades associated with Central and South American areas. Below are the hemipenes for each species in the topology indicated by letters (A-R).

tolimensis, *A. antonii*, *A. fuscoauratus* and *Anolis* candidate 1 are the shape of lobes, globular or tubular, along with its size respect to the total length of the hemipenis and the entire size of this organ, which agree with those previously mentioned for the genus (Köhler et al., 2012; D'Angiolella et al., 2016).

Diagnostic characters of species

We present the first description of the hemipenes of *A. antonii,* which shows a low intraspecific morphological variation considering its wide distribution in the western cordillera. The main variation is the skin ridge size on the asulcate face. For its recognition, we highlight tubular lobes and the presence of fleshy projections at the base of lobes (Figure 3A), a feature not observed in any other of the species we studied. Features such as fleshy projections and skin ridges are helpful for species identification, such as the case of *A. auratus*, *A. chrysolepis*, *A. ortonii*, A. scypheus, and *A. tandai*, which all possess fleshy projections on lobes of hemipenis (D'Angiolella et al., 2016).

In the case of *A. fuscoauratus*, it has a similar morphology as described by D'Angiolella et al. (2016) (Fig. 3B), being the most informative characters of the size of lobes, which is one-third

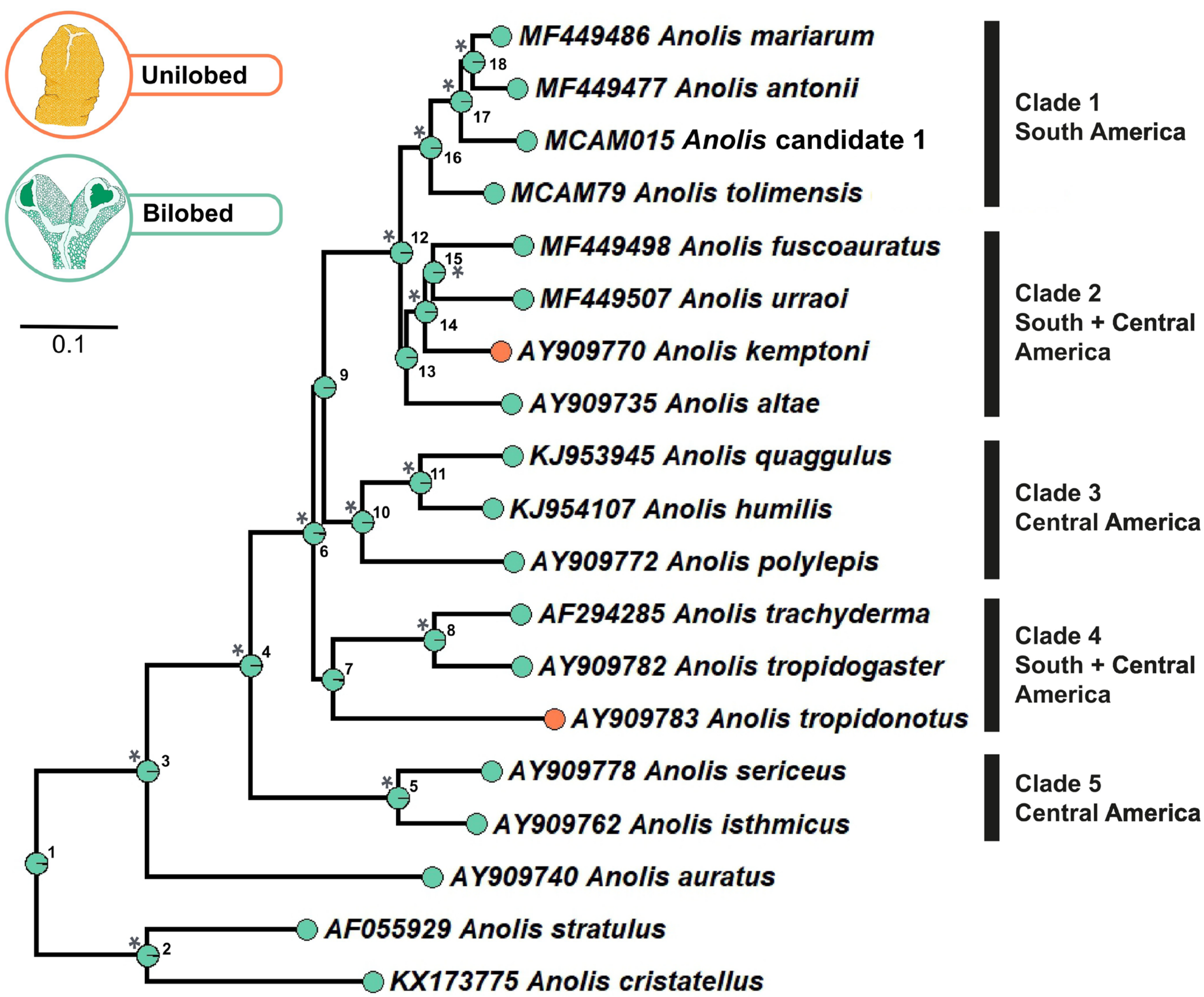

Figura 5. Reconstrucción ancestral de caracteres del tipo de hemipenes (carácter 1). Los diagramas sectoriales muestran la probabilidad de cada estado de carácter. El asterisco (*) indican los clados bien soportados. Presentamos los números de nodo junto a cada nodo de la filogenia.

Figure 5. Character state reconstruction of type of hemipenes (character 1). Pie charts show probability of each state of character. Asterisk (*) indicates well-supported clades. We presented the node numbers next to each node of the phylogeny.

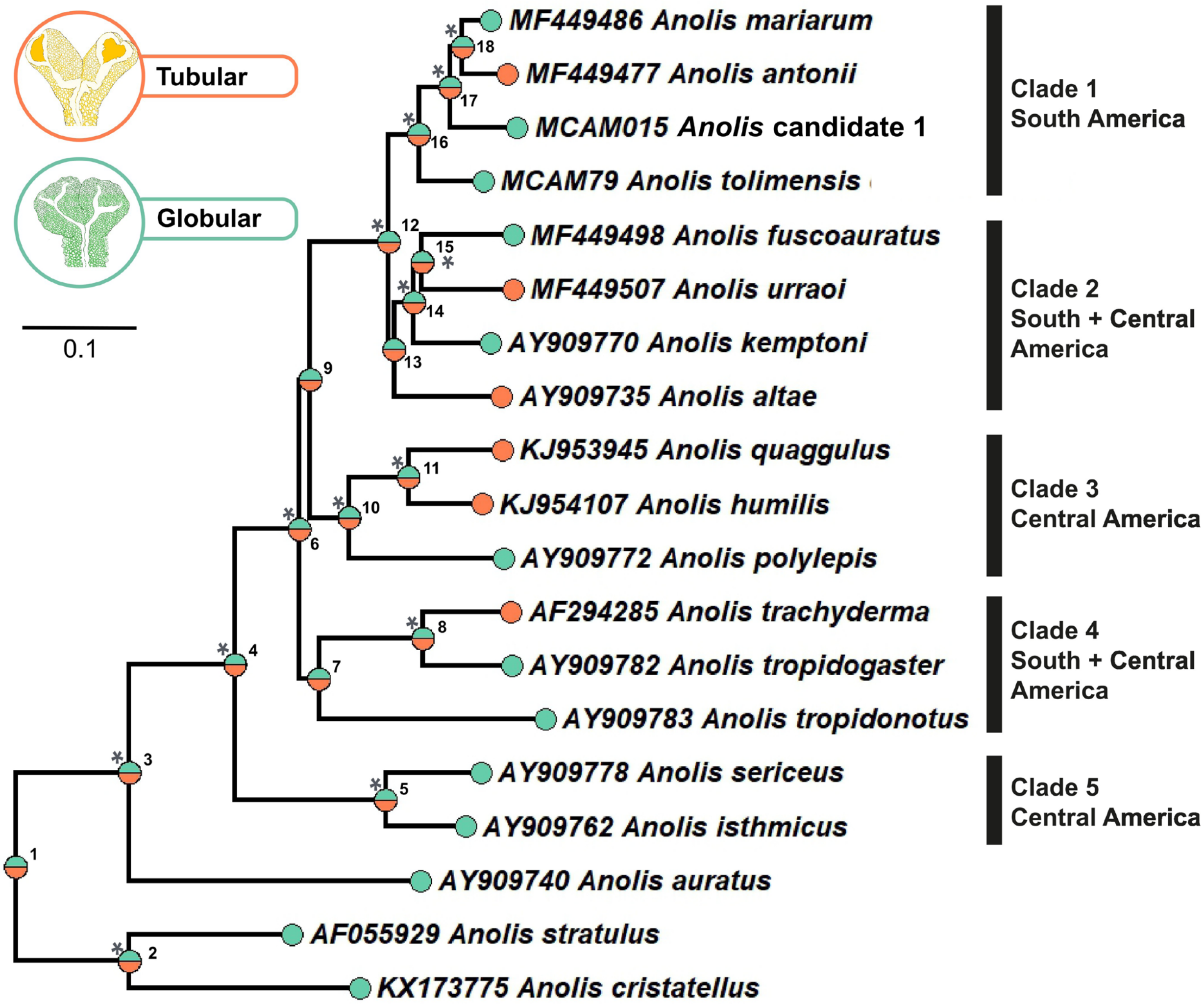

Figura 6. Reconstrucción ancestral de caracteres del tipo de lóbulos (caracter 2). Los diagramas sectoriales muestran la probabilidad de cada estado de caracter. El asterisco (*) indican los clados bien soportados. Presentamos los números de nodo junto a cada nodo de la filogenia.

Figure 6. Character state reconstruction of type of lobes (character 2). Pie charts show probability of each state of character. Asterisk (*) indicates well-supported clades. We presented the node numbers next to each node of the phylogeny.

of total length of the organ and presence on asulcate face of a shallow and short skin ridge extending from the base of lobes to the crotch. In addition, D'Angiolella et al. (2016) report pigmentation for this species on the hemipenes, although this feature varies among specimens, and we did not observe such pigmentation.

We present the first description of the hemipenes for *A. tolimensis* (Fig. 3C). The skin ridge helps identify this species for other species like *A. trachyderma*, *A. brasiliensis*, and *A. planiceps* (D'Angiolella et al., 2016). We observed that it extends prominently from the base of the lobes to the crotch, reducing in size as it reaches the trunk. It is also essential to highlight the deep sulcal lips becoming thicker at the apex of the lobes, which are useful in the identification of other *Anolis* species such as *A. pseudokemptoni* (Köhler et al., 2007), *A. gruuo* (Ponce & Köhler, 2008), *A. auratus*, *A. brasiliensis*, *A. chrysolepis*, among others (D'Angiolella et al., 2016).

For *Anolis* candidate 1 (Fig. 3D), in conjunction with the molecular and morphological analysis data presented by Acevedo et al. (data not published), and the hemipenial morphology found in this work, we support the hypothesis that it is a distinct species from *A. tolimensis*. Among the main differences, we highlight the skin ridge on asulcate face of *Anolis* candidate 1 being shallower than in *A. tolimensis*, which is very prominent near the crotch of the lobes (Fig. 2C). In contrast, *Anolis* candidate 1 maintains the same size throughout its length. We also remark on the size of the lobes of *A. tolimensis*, which are approximately half of the total length of the hemipenis and larger respect to those of *Anolis* candidate 1, where lobes are one-third of the total length of the hemipenis. Likewise, it is essential to mention that *A. tolimensis* hemipenes are more prominent than that for *Anolis* candidate 1 (± 5 mm and ± 3 mm, respectively). This is similar to what is reported by Köhler et al. (2006), who mention that one of the main differences between the hemipenes of the species is their size.

For *A. mariarum*, we described three types of hemipenial morphology, which present variation in hemipenis size, depth of sulcal lips, flounces, and presence of skin ridge (Fig. 3E-G). Type 1, from Km 9 SE Palmitas, Antioquia, hemipenis size is similar to that reported by Rubio & Agudelo (2020), being ~5 mm and the one in literature being ~6 mm. Its lobes are also half of total length of hemipenis, with a skin ridge on the asulcate face and flounces on the trunk, characteristics that are useful for identifying this species (Rubio & Agudelo, 2020). On the other hand, in type 2, from Santa Rosa de Osos, Antioquia, the lobes are smaller, representing one-third of the total length of the

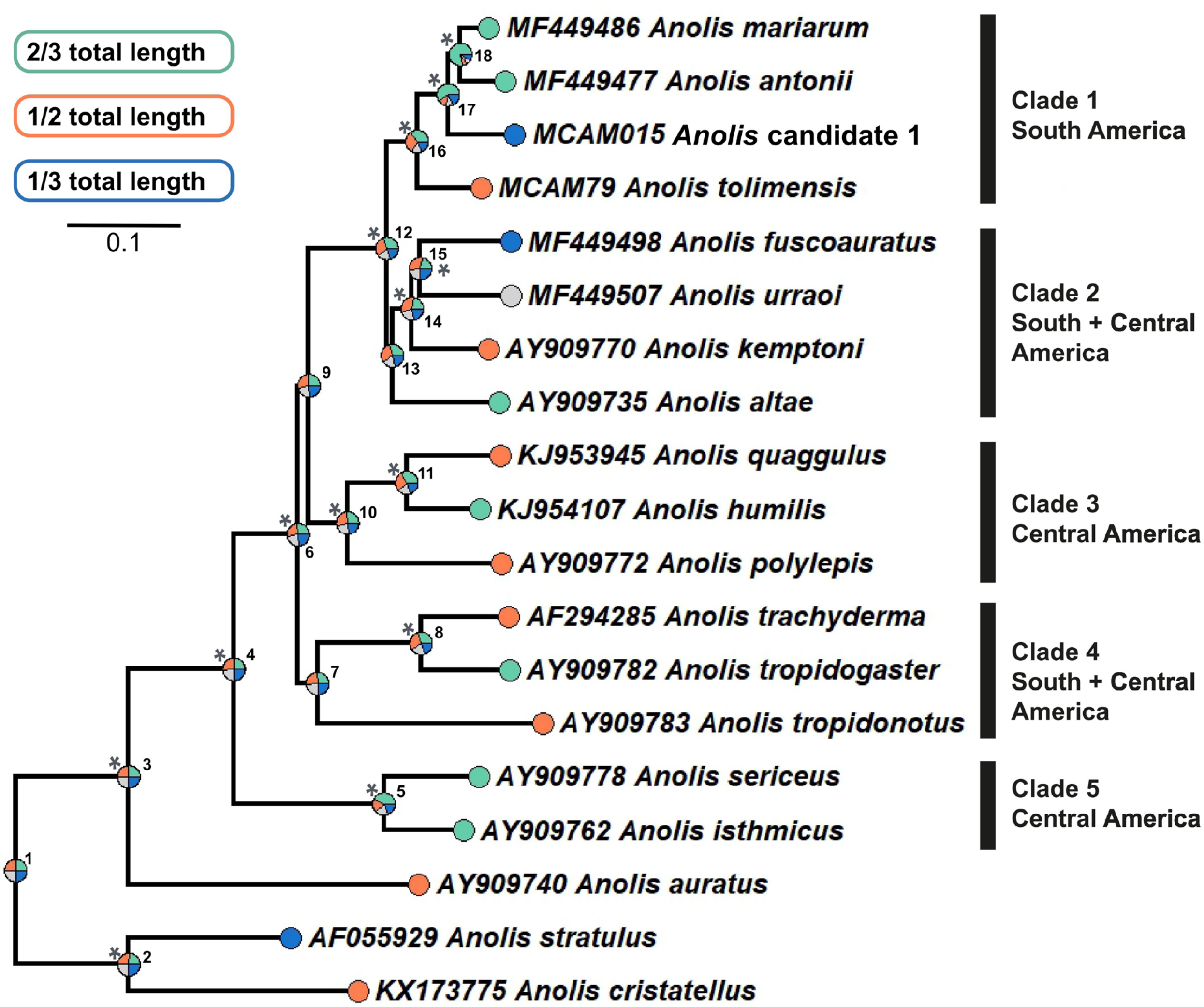

Figura 7. Reconstrucción ancestral de caracteres de la proporción de la longitud de los lóbulos con relación a la longitud total del hemipene (carácter 3). Los diagramas sectoriales muestran la probabilidad de cada estado de carácter. Color gris indica que no hay información disponible para el carácter. El asterisco (*) indican los clados bien soportados. Presentamos los números de nodo junto a cada nodo de la filogenia.

Figure 7. Character state reconstruction of proportion of the length of lobes relative to total length of hemipenes (character 3). Pie charts show the probability of each state of character. Gray color indicates no information available for the character. Asterisk (*) indicates well-supported clades. We presented the node numbers next to each node of the phylogeny.

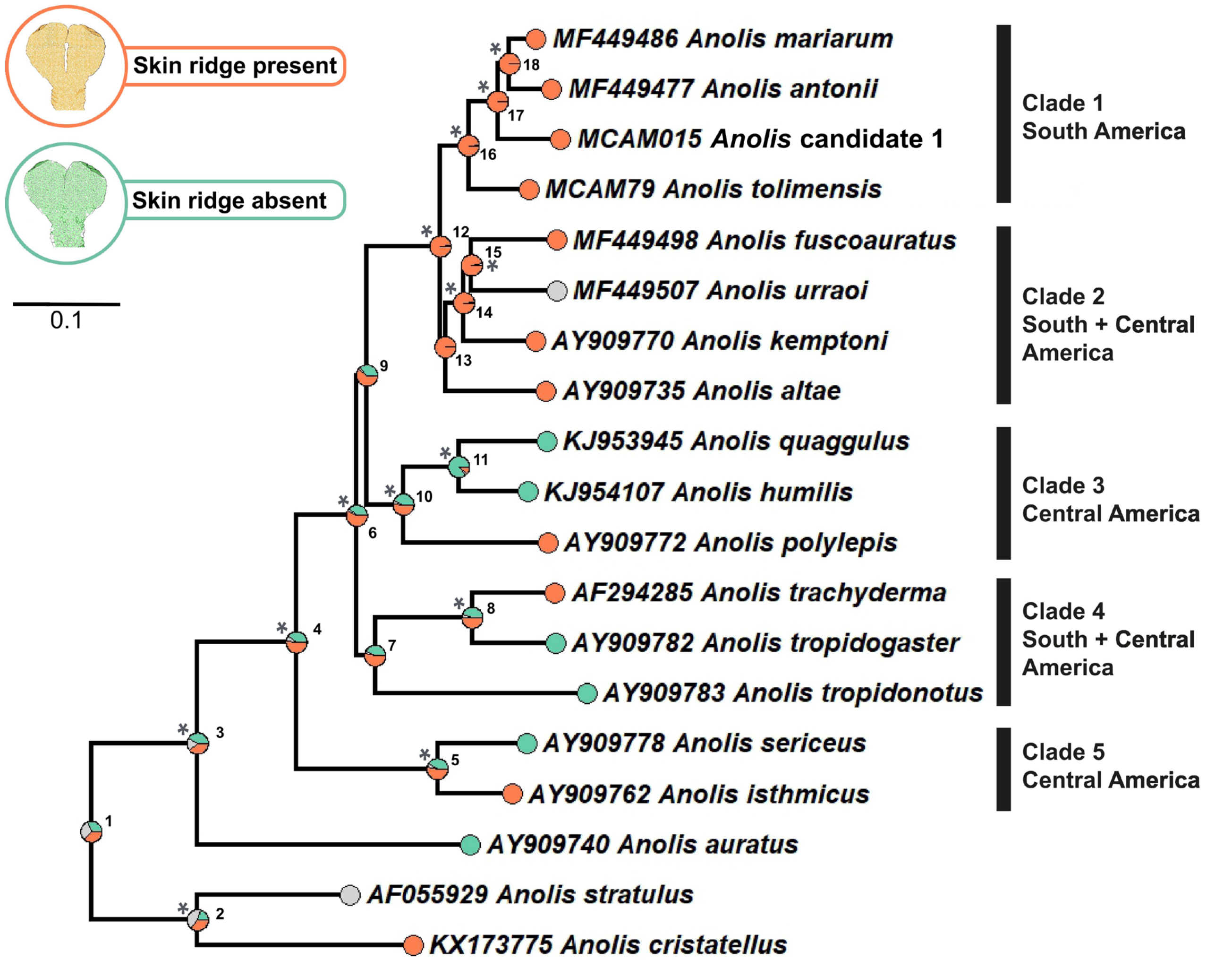

Figura 8. Reconstrucción ancestral de caracteres de crestas de piel en la cara asulcada de los hemipenes (carácter 4). Los diagramas sectoriales muestran la probabilidad de cada estado de carácter. Color gris indica que no hay información disponible para el carácter, mientras que el gris en los terminales representa incertidumbre. El asterisco (*) indican los clados bien soportados. Presentamos los números de nodo junto a cada nodo de la filogenia.

Figure 8. Character state reconstruction of skin ridges on asulcate face of the hemipenes (character 4). Pie charts show probability of each state of character. Gray color at the terminals indicates that there is no information available for the character, while gray at the nodes represents degree of uncertainty. Asterisk (*) indicates well-supported clades. We presented the node numbers next to each node of the phylogeny.

hemipenis. Still, despite the total length of the hemipenis, close to the one reported in the literature (~5 mm), it does not have a skin ridge on the asulcate face nor flounces in the trunk. Finally, type 3, collected in Rionegro, Antioquia, is similar to hemipenes of *Anolis* candidate 1, as it has a skin ridge on asulcate face, extending from the middle of trunk to the crotch of lobes, also its lobes are one-third of total length of the hemipenis and its size is ~3 mm, but skin ridge is shallower in A. mariarum type 3. Unlike morphotype 1, type 3 hemipenis is smaller (~3 mm) and skin ridge is shallower.

This variation could correspond to specimens that are not *A. mariarum*, but other species of the genus not enough studied, as has been shown for other cryptic species within the genus like *A. humilis*, *A. quaggulus*, and *A. marsupialis* (Köhler et al., 2003; Köhler et al., 2015), *A. monteverde* and *A. altae* (Köhler, 2009), and *A. tropidonotus*, *A. spilorhipis*, *A. mccraniei* and *A. wilsoni* (Köhler et al., 2016). However, this possibility needs further investigation using hemipenial and body morphology, along with molecular data, to identify correctly and deeply understand variations observed in this species

Genital characters in lizards show high variation and evolve approximately six times faster compared to other morphological characters (Klaczko et al., 2015; Quipildor et al., 2020). This may be a consequence of the coevolution of male and female genitalia so that reproduction is species-specific, coevolution of genitalia pleiotropic effects, or by sexual selection by females or males, depending on their ability to stimulate the female or to transport sperm for reproduction (Arnqvist, 1997). It has also been evidenced in other lizards of the genera *Anolis*, *Tropidurus*, and in other animals (Eberhard, 2010; Klaczko & Stuart, 2015; De-Lima et al., 2019; House et al., 2019), being an important cause for speciation processes (Klaczko & Stuart, 2015). This could explain the distinct morphologies that we observed in our study and the differences concerning other species (Figure 4). On the other hand, Johnson et al. (2014) mention that evolution of hemipenes in the genus *Anolis*, as well as its size, may be related to the frequency of reproductive activities, due to the activity of the muscles that control the movement of the hemipenis at the time of copulation. Gilman et al. (2018), add that testosterone levels may also cause variations in male genitalia, leading to changes in size and ornamentations as observed among the four species in our study especially in *A. fuscoauratus* and *A. mariarum*. Additionally, in the genus *Anolis* a correlation between forelimb length and hemipenis shape has been evidenced, this being a

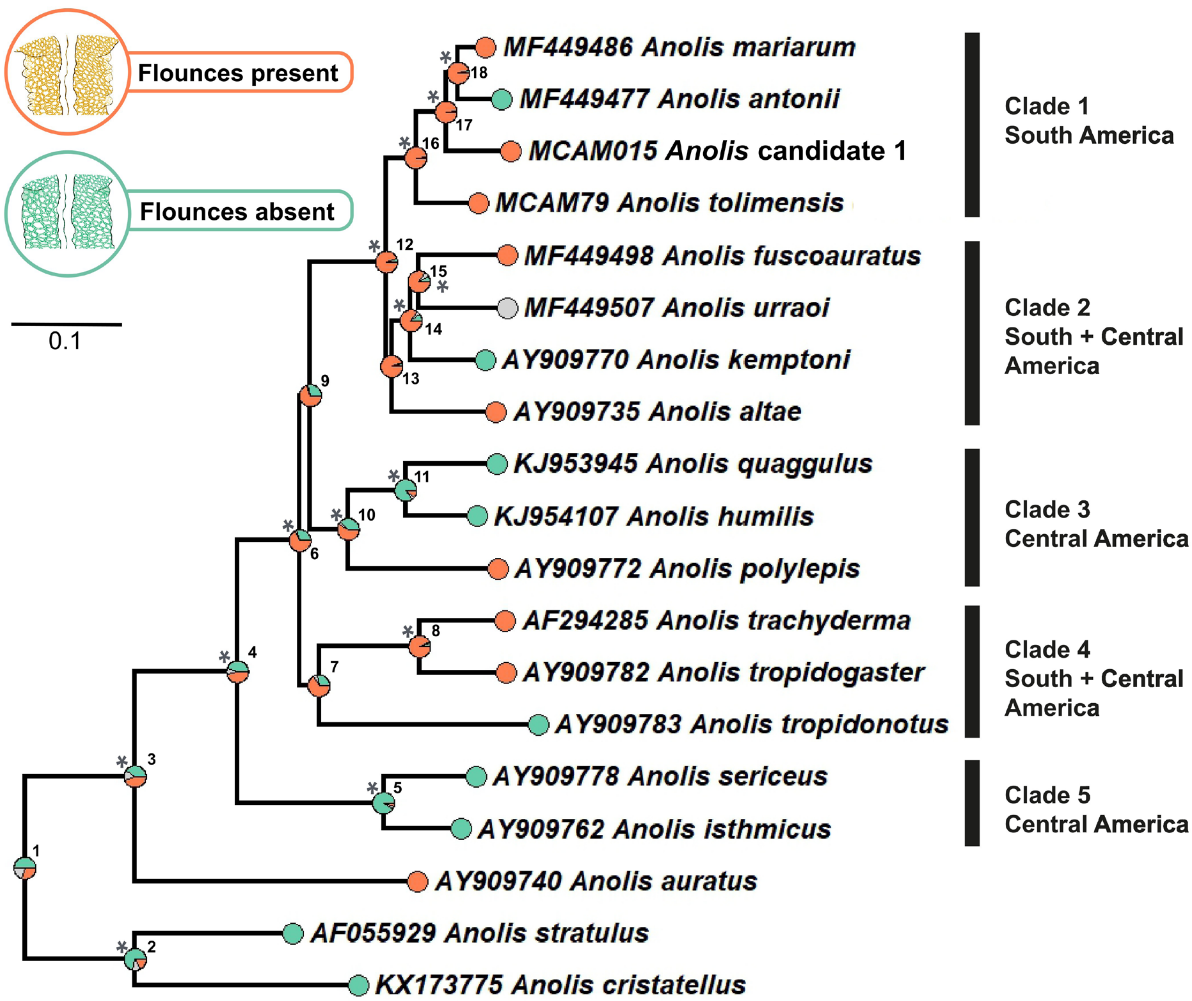

Figura 9. Reconstrucción ancestral de caracteres de crestas de piel en la cara asulcada de los hemipenes (carácter 4). Los diagramas sectoriales muestran la probabilidad de cada estado de carácter. Color gris indica que no hay información disponible para el carácter, mientras que el gris en los terminales representa incertidumbre. El asterisco (*) indican los clados bien soportados. Presentamos los números de nodo junto a cada nodo de la filogenia.

Figure 9. Character states reconstruction of flounces. Pie charts show probability of each state of character. Gray color indicates no information available for the character, while gray at the nodes represents degree of uncertainty. Asterisk (*) indicates well-supported clades. We presented the node numbers next to each node of the phylogeny.

pleiotropic effect, so these characters would evolve together due to their relatedness to the same set of genes (Klackzko et al., 2017).

In addition, it has been reported that the uplift of the Andes has generated speciation processes in several families of reptiles and amphibians, due to changes in climatic, environmental, and feeding conditions (Santos et al., 2009; Nunes et al., 2012; Castroviejo-Fisher et al., 2014; Hutter et al., 2017; Esquerré et al., 2018; Torres-Carvajal et al., 2020; Boschman & Condamine, 2022; Ocampo et al., 2022), as well as for the genus *Anolis* (Calderón-Espinosa & Barragán-Contreras, 2014; Vargas-Ramírez & Moreno-Arias, 2014; Poe et al., 2017). All these changes and speciation processes could be implicated in the variations in the morphology of the hemipenes in *Anolis*, although this requires further investigation.

Taxonomic and phylogenetic implications

Ancestral reconstruction of characters has been used to deduce, understand, and determine the character states of a common ancestor from the states of the descendants. This methodology provides a better understanding of the evolutionary history of the organism under study (Giannasi et al., 2000). For example, Williams (1972) used species of *Anolis* from the Caribbean Islands to demonstrate the displacement of external characteristics such as body coloration and habit, among other adaptive features, generating a possible connection between ecological adaptation hypotheses and these characters, recognizing the importance of linking osteological and molecular evidence to confirm common ancestors. Subsequently, this reconstruction process was used to infer the evolution of body size in species of the *A. roquet* group and to relate it to the hypothesis of joint evolution caused by interspecific competition (Giannasi et al., 2000) and to study the patterns of chromosomal changes in some species of the genus (Castiglia et al., 2013).

As for our results, the absence or presence of skin ridge in the South American clade (Clade 1) does not represent relevance to differentiate within the South American species because it had a probability of 99 % of presenting the state of character. The same occurs for all clades with the presence of flounces, where we got probability ranges between 96-99 %. In comparison, for the Central American + South American clades (Clades 2 and 4) and the Central American (3 and 5), we found a fifty-fifty chance for skin ridge to be present, so we see it as a valuable character for the differentiation between *Anolis* species as mentioned by some authors (Köhler & Vesely, 2010; D'Angiolella et al., 2016).

As for the lobe size concerning total length of the hemipenes, it seems to represent a key character to differentiate the species belonging to clade 2 (Central American + South American), since the three different character states are present in these species. As for the character state of 1/3 length, we observed that it has appeared independently in some clades, such as 1 and 2. These character shifts may be due to different events, as described in the study Austin et al. (2010), where they found that the variation in body size of *Tribolonotus* may be due to ecological factors coupled with competition between species located on this island, the same may be occurring in species separated by mountain ranges; however, further studies would be required to verify this.

For the type of hemipenes, we see a probability of presenting both states, bilobed and unilobed, so it is a character that does not follow a trend in its evolution. However, it is useful to separate between species, as in our case, the separation between *A. antonii* from the others of the study, due to its tubular hemipenes and large size concerning the others. Likewise, these characters have also been helpful for the separation of other species of the *Anolis tropidonotus* complex (Köhler et al., 2015; Köhler et al., 2016).

CONCLUSIONS

In this study, we provided a comparative description of the hemipenes of five *Anolis* species. Among them, we present the first description of hemipenes of *A. antonii* and *A. tolimensis*. These descriptions allowed us to differentiate between the five species studied. Key characteristics for their differentiation include the presence of skin ridge, its size, prominence, and extent, as well as the type of lobes, and the presence of fleshy projections at the base of the lobes, such as those observed in *A. antonii*. Additionally, we remark on the description of three different morphologies in *A. mariarum*. Type 1 corresponds to the morphology reported in the literature, while the other types could refer to new species. However, this requires further investigation including specimens of the entire distribution range. We also provide a description of the hemipenes morphology of *Anolis* candidate 1, which supports the hypothesis that this is a distinct species from *A. tolimensis*. Furthermore, we emphasize the importance of using these characters in phylogenetic and taxonomic studies to identify and classify species, especially those with similar external morphology.

Finally, characters such as the shape of the lobes, presence of fleshy projections, size and type of hemipenes, and prominence and extent of the skin ridge on the asulcate face are the main characteristics that provide information to differentiate the species in our study or between groups of species. We do not consider the presence of flounces on the trunk or the presence or absence of the skin ridge as a valuable characteristic for the differentiation of the species. We found no significant patterns in all character states of the clades formed from Central and South America (2 and 4), Central America (3 and 5), and South America (1).

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APPENDICES

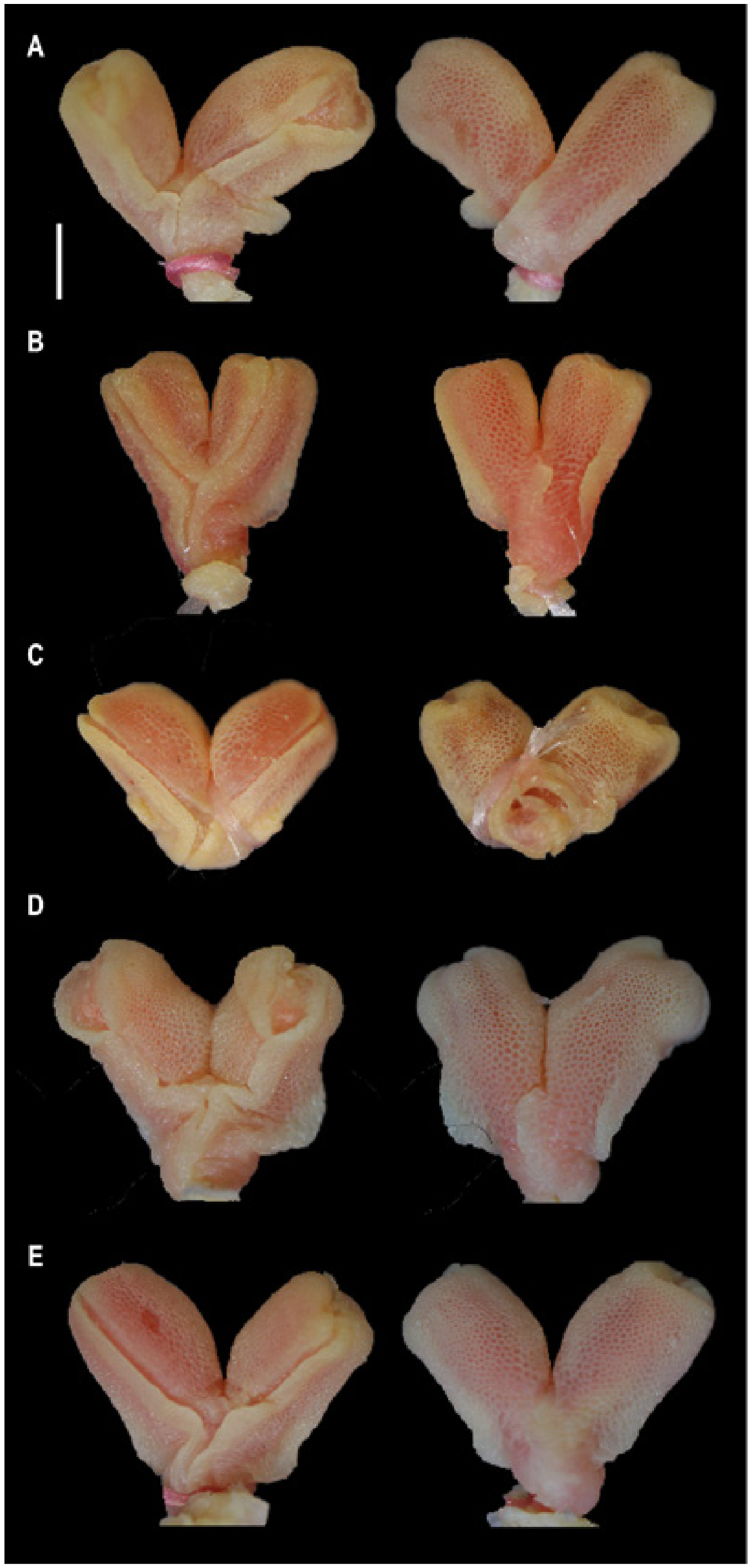
Appendix 1. Characters and their states for each species used in ancestral state reconstruction

Apéndice 1. Caracteres y sus estados para cada especie utilizada en la reconstrución de estados ancestrales.

Species	Hemipenis	Lobes	Size	Skin ridge	Flounces	Reference
Anolis mariarum	Bilobed	Globular	Two-thirds of total length	Yes	Yes	Our study
Anolis antonii	Bilobed	Tubular	Two-thirds of total length	Yes	No	Our study
Anolis candidate 1	Bilobed	Globular	One-third of total length	Yes	Yes	Our study
Anolis tolimensis	Bilobed	Globular	Half of total length	Yes	Yes	Our study
Anolis altae	Bilobed	Tubular	Two-thirds of total length	Yes	Yes	Köhler et al., 2009
Anolis fuscoauratus	Bilobed	Globular	One-third of total length	Yes	Yes	Our study
Anolis urraoi	Bilobed	Tubular	No inf.	No inf.	No inf.	Grisales-Martínez et al., 2017
Anolis kemptoni	Unilobed	Globular	Half of total length	Yes	No	Köhler et al., 2007
Anolis quaggulus	Bilobed	Tubular	Half of total length	No	No	Köhler et al., 2015
Anolis humilis	Bilobed	Tubular	Two-thirds of total length	No	No	Köhler et al., 2015
Anolis polylepis	Bilobed	Globular	Half of total length	Yes	Yes	Köhler et al., 2010
Anolis trachyderma	Bilobed	Tubular	Half of total length	Yes	Yes	Köhler et al., 2012
Anolis tropidogaster	Bilobed	Globular	Two-thirds of total length	No	Yes	D'Angiolella et al., 2016
Anolis tropidonotus	Unilobed	Globular	Half of total length	No	No	Köhler et al., 2016
Anolis sericeus	Bilobed	Globular	Two-thirds of total length	No	No	Köhler & Vesely, 2010
Anolis auratus	Bilobed	Globular	Half of total length	No	No	D'Angiolella et al., 2016
Anolis isthmicus	Bilobed	Globular	Two-thirds of total length	Yes	No	Köhler et al., 2014
Anolis stratulus	Bilobed	Globular	One-third of total length	No inf.	No	Klaczko et al., 2017
Anolis cristatellus	Bilobed	Globular	Half of total length	Yes	No	Klaczko et al., 2017

Appendix 2. Studied hemipenes of *Anolis antonii*, (A) ICN3774, (B) ICN3775, (C) ICN3778, (D) ICN3786, (E) ICN3788 (Scale = 2mm).

Apéndice 2. Hemipenes estudiados de *Anolis antonii*, (A) ICN3774, (B) ICN3775, (C) ICN3778, (D) ICN3786, (E) ICN3788 (Escala = 2mm).

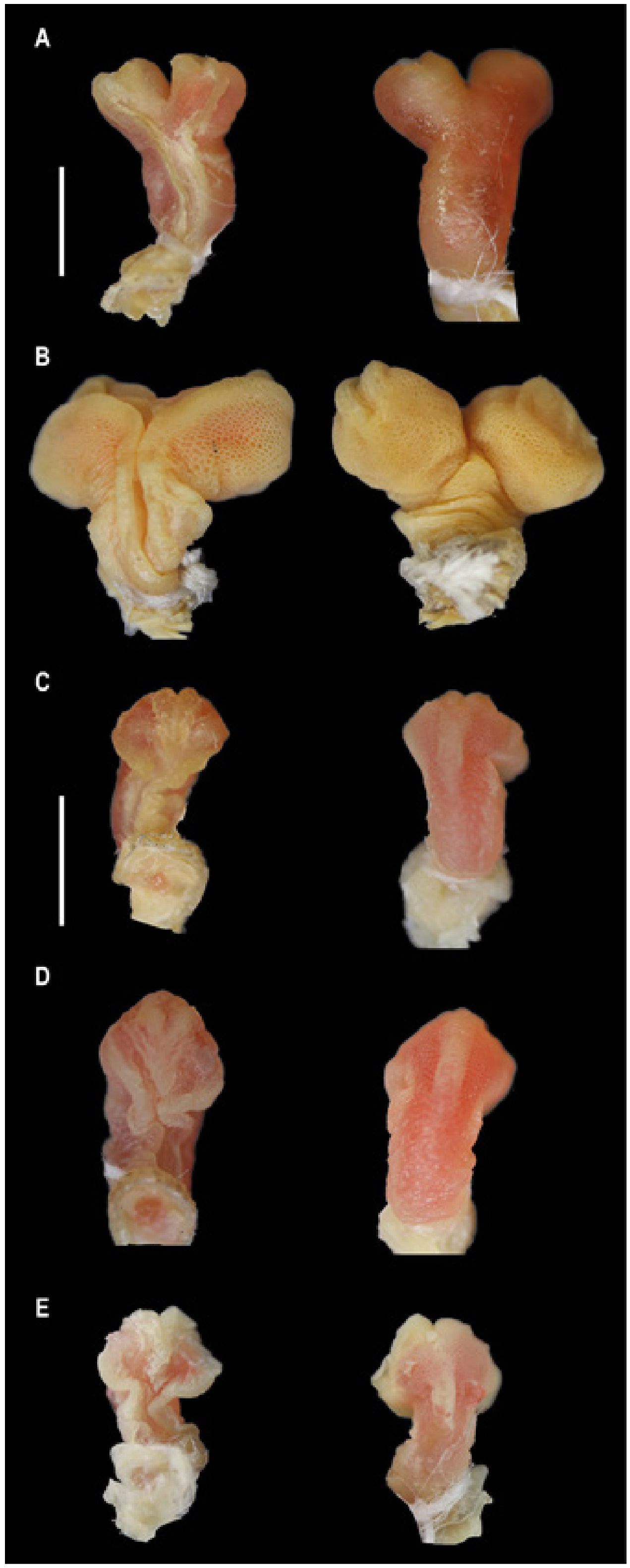


Appendix 3. Studied hemipenes of *Anolis fuscoauratus*, (A) ICN4436, (B) ICN4437, , (C) ICN8616, , (D) ICN11069, , (E) ICN11073 (Scale = 2mm).

Apéndice 3. Hemipenes estudiados de *Anolis fuscoauratus*, (A) ICN4436, (B) ICN4437, , (C) ICN8616, , (D) ICN11069, , (E) ICN11073 (Escala = 2mm).

Appendix 4. Studied hemipenes of *Anolis mariarum*, (A) ICN3807, (B) ICN3808, (C) ICN3791, (D) ICN3793, (E) ICN3799 (Scale = 2mm).

Apéndice 4. Hemipenes estudiados de *Anolis mariarum*, (A) ICN3807, (B) ICN3808, (C) ICN3791, (D) ICN3793, (E) ICN3799 (Escala = 2mm).



Appendix 5. Studied hemipenis of *Anolis tolimensis*, (A) ICN3785 (Scale = 2mm). /**Apéndice.** Hemipenes estudiados de *Anolis tolimensis*, (A) ICN3785 (Escala = 2mm).

Appendix 6. Studied hemipenis of *Anolis* candidate 1, (A-B) ICN5966, (C) CZCH-UMNG-R:185 (Scale = 2mm).

Appendix 6. Hemipenes estudiados de *Anolis* candidato 1, (A-B) ICN5966, (C) CZCH-UMNG-R:185 (Escala = 2mm).