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New distribution records, morphology and natural history notes of the endemic Colombian lizard *Anolis limon*

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ABSTRACT – *Anolis limon* is a species endemic to the inter-Andean valley of the Magdalena River, Colombia. We report three new localities for *A. limon* in the department of Tolima, Colombia, and provide data on its morphology, natural history and behaviour. Specimens were collected during field surveys of the eastern slope of the Central Cordillera in the Colombian Andes and their external features (scales) and internal characteristics (hemipenes) were compared with literature descriptions. We detected some variation in body size and meristic data between populations of *A. limon*. Furthermore, the distribution of *A. limon* was limited to the eastern slope of the Central Cordillera, located in the middle of the Magdalena region. This zone is highly fragmented and degraded, which may affect *A. limon* populations, as other animal species from these localities have disappeared in the last decade. Consequently, an evaluation of the conservation status of *A. limon* is needed.

INTRODUCTION

The genus *Anolis* (Daudin, 1892) has 440 species (Uetz et al., 2024) making it one of the most diverse groups of reptiles (Moreno-Arias & Calderón-Espinosa, 2016). In Colombia, the genus has 79 species (Uetz et al., 2024), nine of which have been registered in the Tolima department (Llano-Mejía et al., 2010), and six for the municipality of Falan, Tolima (Gallego et al., 2008).

Anolis limon is an endemic species from the inter-Andean valley of the Magdalena River of Colombia (Choco-Magdalena biogeographic province), reported in the departments of Antioquia and Caldas, between 520 and 1093 m a.s.l. (Velasco & Hurtado-Gómez, 2014; Rojas-Morales et al., 2019; Ramírez-Chaves et al., 2021). The external and internal morphology of this species was detailed in the original description by Velasco & Hurtado-Gómez (2014). However, to date there is no description of hemipenial morphology, which in squamate reptiles is an important systematic and taxonomic trait (Köhler et al., 2007; Klaczko et al., 2015).

Recently, the potential distribution of *A. limon*, with data on its habitat and threats, has been summarised by Moreno-Arias et al. (2021). Nevertheless, there is still a lack of knowledge about its natural history, morphological variation and ecology. Herein, we report three new localities for *A. limon* in the department of Tolima, Colombia, as well as expanding our understanding of morphological variation in the species including, for the first time, data on hemipenial morphology. We also provide some comments on its natural history, behaviour and current distribution.

MATERIALS AND METHODS

Field surveys were conducted in the municipalities of Falan and Armero (Department of Tolima), on the eastern slope of the Central Cordillera in the Colombian Andes. Falan area is characterised by its heterogeneous landscape between agricultural fields, mainly of *Annona muricata*, *Theobroma cacao*, *Persea americana*, and remnants of tropical humid forests (Agenda Ambiental del Municipio de Falan, 2011). The Armero zone appears to be a transition area between a tropical dry forest and humid forest.

The *A. limon* specimens were collected during two field trips, from 10 October to 16 November 2020 (Falan) and 21 June 2023 (Armero). The lizards were found during visual encounter surveys, between 18:00 h and 23:00 h, following common transit trails near water sources used by the locals covering different types of habitats and arboreal strata, where captures were made manually (Leal & Losos, 2000). The individuals collected were sacrificed with 2% roxicaine, fixed with a 10% formalin and stored in 70% alcohol (Angulo et al., 2006). The specimens were deposited in the zoological collection of the University of Tolima (CZUT) and the Natural History Museum of the University of Cauca (MHNUC). Collection permit was authorised by the Ministerio de Ambiente, Vivienda y Desarrollo Territorial (ANLA Resolutions 02191 of 2018).

For identification of the species, we followed the taxonomic keys and descriptions available in Batista et al. (2015), Velasco & Hurtado-Gómez (2014) and Williams (1988). The morphological character terminology used in this work is based on Köhler (2014). All specimens were

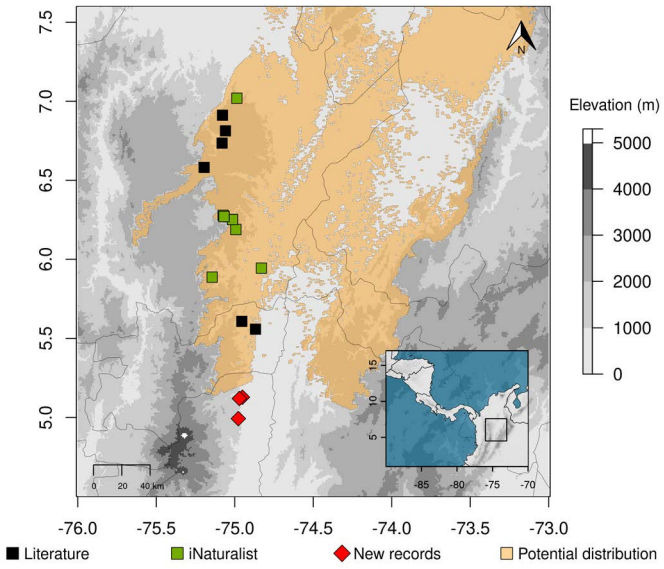

Figure 1. Distribution map of *Anolis limon* from literature, iNaturalist and new records. The potential distribution was obtained from BioModels of the Humboldt Institute (Moreno-Arias et al., 2019).

measured from the tip of the snout to the vent (SVL) with a digital calliper to the nearest 0.1 mm. For the distribution map, we compiled reports from published literature (Velasco & Hurtado-Gómez, 2014; Rojas-Morales et al., 2019; Ramírez-Chaves et al., 2021) and iNaturalist (https://www.inaturalist.org/. Accessed on 8 January 2024.). Only iNaturalist records in which the species was recognisable and had "Research Grade" were used. In Figure 1, we show the potential distribution of *A. limon*, this is based on a climatic-level distribution model sourced from the BioModelos dataset at the Humboldt Institute (Moreno-Arias et al., 2019) which corresponds precisely to the one proposed as a potential distribution for *A. limon* by Moreno-Arias et al. (2021).

Two hemipenes were also prepared. An inverted hemipene was everted following the procedures outlined by Zaher (1999), although to soften the tissue we used warm water instead of KOH. The second hemipene was prepared on a freshly sacrificed specimen, following the procedure proposed by Zaher & Prudente (2003). The hemipenes were treated with an alcoholic alizarin solution to contrast surface ornamentation and to reveal deep calcified structures (Harvey & Embert, 2008). Terminology for hemipenial morphology follows Köhler et al. (2007), Myers et al. (1993), Angiolella et al. (2016) and Savage (1997).

RESULTS

Three adult females, an adult male and a juvenile female were collected in the locality of Cúcuta (5.128479°, -74.951279°; 915 m a.s.l.), in a small gallery forest next to the river Murillo, Falan. Another adult male and three females were collected in the locality of Piedecuesta, around the Normal Superior school of Falan (5.120412°, -74.970604°; 1069 m a.s.l.), within a gallery forest. Additionally, another male

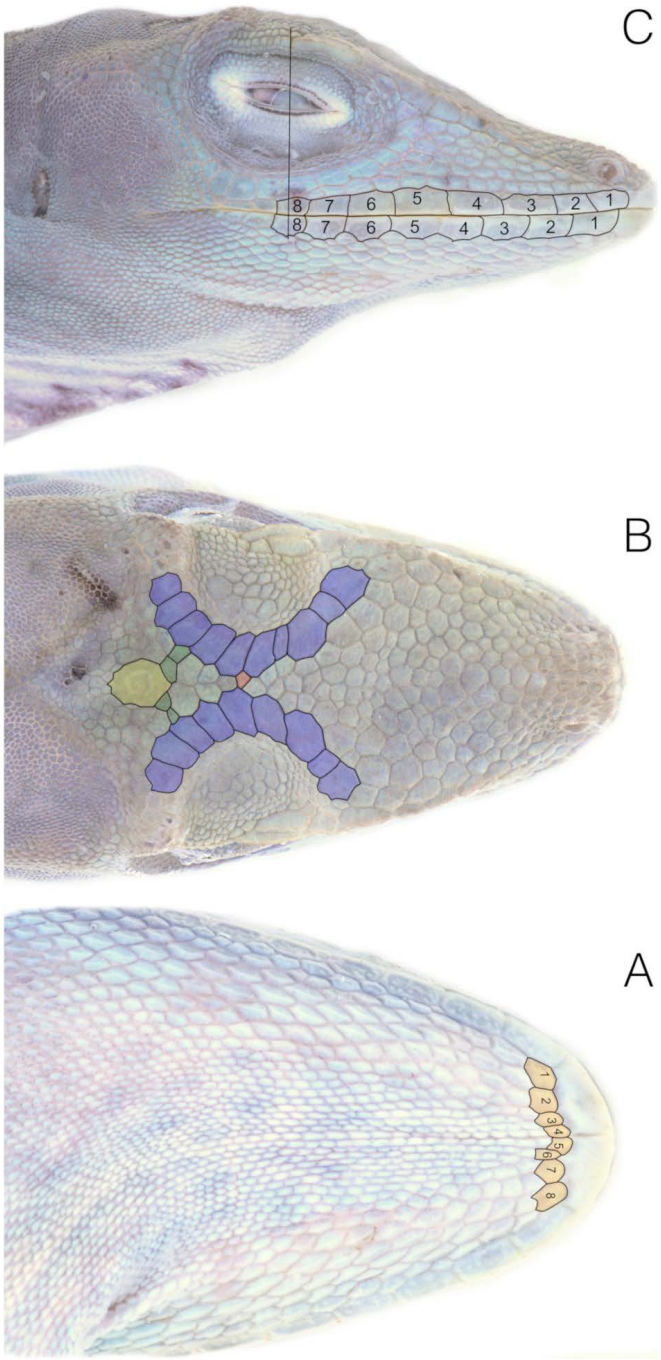

Figure 2. Scales of head of *Anolis limon* - **A.** Ventral view, postmental scales, **B.** Dorsal view, red = scales between supraorbital semicircles, blue = supraorbital semicircles, yellow = interparietal scale, and green = scales between interparietal scale and supraorbital semicircles, **C.** Lateral view, supralabial and infralabial scales to centre of eye

was collected in the locality of La Parroquia, municipality of Armero (4.9929691°, -74.9772454°; 1076 m a.s.l.) in a gallery forest. All specimen collection sites are indicated in Figure 1.

Identification

These individuals were identified as *A. limon* by having mostly green dorsal colouration, smooth ventral scales, dewlap in males light tan or yellow with green light transverse scales, interparietal scale separated from supraorbital semicircles by

Figure 3. Colouration in life of *Anolis limon* from Falan, Tolima - **A.** Adult male (CZUT-R 797), **B.** Juvenile female (CZUT-R 805) and **C.** Adult female (CZUT-R 799)

2–4 scales and 2–3 scales between supraorbital semicircles (Velasco & Hurtado-Gomez, 2014). However, we observed some variation in these meristic characters and body size, in comparison to other reported data for this species (Table 1, Figs. 2 & 3).

Colouration in life (Fig. 3)

The dorsal colouration pattern observed in adult females was similar to that described by Velasco & Hurtado-Gomez (2014). Under non-stressful conditions, the males displayed faint and slightly bluish bands on a green background (Fig. 3A). After the induction of stress, the aforementioned light bands underwent a transformation, taking on a pale green hue, while the inter-band spaces transitioned to a black colouration. This stress-induced colouration pattern aligns with the described pattern in life for males (Velasco & Hurtado-Gomez, 2014). The juvenile female (Fig. 3B) has a similar dorsal pattern to that of adult females (Fig. 3C), although with fewer black spots. This pattern consists of dark spots and a few lateral white spots on a light green background colour. Nevertheless, the dewlap in juveniles was greenish-yellow, whereas in adult females, it exhibited a fading green colour.

Hemipenial morphology (n = 2, Fig. 4). Moderately bilobed with the sulcus spermaticus bifurcating at the base of lobes. The branches of the sulcus spermaticus continue to the tips of lobes where it opens into a broad naked area. The sulcal lips in the truncus are poorly developed and the sulcus spermaticus wide. Surface of lobes strongly calyculate. The asulcate process is sub-triangular and well-developed, beginning within the crotch and extending to less than half of the truncus, covered by fused calyces that transform into transverse folds, with a more elevated medial portion.

Natural history and behaviour notes

All individuals were caught at night, sleeping on leaves of *Carludovica palmata* or *Cyathea* sp, mostly at heights between 1.60 m to 2 m above ground, although one individual was observed at 6 m. During the day, a female was perching on a leaf of *C. palmata*. One juvenile was collected in October 2020, and two more juveniles were observed in June 2023. A female captured in October 2020 (CZUT-R 801) deposited an egg inside the capture container and when the preserved specimen was examined another egg was detected. In addition, two collected females also contained two eggs, one in each oviduct, while another female only contained one egg. Some individuals emitted a low squeak when caught, while others attempted to bite. Additionally, all individuals changed their colour to a brown tone when captured or stressed.

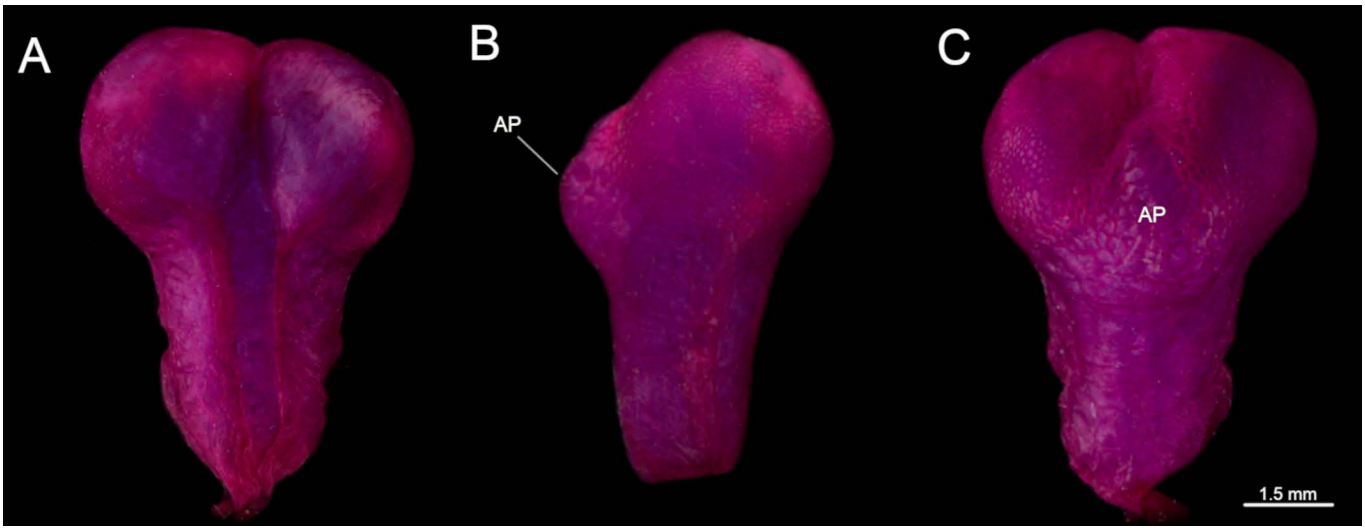

Figure 4. Hemipene of *Anolis limon* (CZUT-R 797), AP = Asulcate process

Table 1. Variation in meristic characters and body size in *Anolis limon*

Characters	Velasco & Hurtado-Gómez (2014) (n = 6)	Current study (n = 10)
Postrostrals	5–7	7–9
Postmentals (Fig. 2A)	6–7	6–8
Scales between interparietal scale and supraorbital semicircles (Fig. 2B)	2–4	1–3
Scales between supraorbital semicircles (Fig. 2B)	2–3	1–2
Supralabials to centre of eye (Fig. 2C)	8–9	8–10
Infralabials to centre of eye (Fig. 2C)	7–9	7–10
Lamellae under third and four phalanges of fourth toe	20–22	20–23
SVL (mm)	Males = 74.5–78.6 Females = 71.2–81.8	Males = 76.2–87.1 Females = 76.1–85.3 Juvenile = 46.2

DISCUSSION

Anolis limon had been only known in the Departments of Antioquia and Caldas (Rojas-Morales et al., 2019; Velasco & Hurtado-Gómez, 2014), from 520 to 1800 m a.s.l. (Fig. 1). Therefore, the new records reported here extend the geographical distribution of this species to the department of Tolima, 64 km south-west in a straight line from its closest previous locality (Norcasia, Caldas). Additionally, these new localities are outside of the potential distribution calculated for this species (Fig. 1) (Moreno-Arias et al., 2021). The most similar species to *A. limon* are *Anolis ibanezi* and *Anolis purpurecens* (*purpurecens* = *chocorum*), but *A. limon* may be distinguished from them by having an orange dewlap with white transverse scales in males (Velasco & Hurtado-Gomez, 2014). Particularly, in the department of Tolima, the most similar species recorded are *Anolis fraseri* and *Anolis frenatus*. However, *A. limon* has a medium size (SVL < 90 mm; SVL > 100 mm in *A. frenatus*), smooth head scales (rugose or keeled head scales in *A. frenatus*), and granular posterior superciliary scales (smooth and squarish posterior supraciliary scales in *A. fraseri*) (Williams, 1988).

The differences in body size and meristic data between the populations of *A. limon* (Table 1) may be attributed to microenvironmental factors, such as temperature, precipitation and latitude, since these characters are usually adaptive (Calsbeek et al., 2006; Malhotra & Thorpe, 1991). Variations in other characters, such as the dewlap, have been also related to abiotic and biotic variables (Baeckens et al., 2018; Nicholson et al., 2007; Vanhooydonck et al., 2009). Nevertheless, we did not observe any differences in this trait between the original description and the new specimens reported here. Regarding the hemipenial morphology, we provide the first description of the hemipenis of *A. limon*. As yet there is no detailed description of hemipenis for closely related species to *A. limon* (*A. ibanezi* and *purpurescens*); however, *A. limon* shares a bilobed condition with *A. ibanezi*, as indicated by Batista et al. (2015).

The municipalities of Falan and Armero, in the Tolima department, do not belong to the choco-magdalena biogeographic province (Hernández-Camacho et al., 1992). However, in these localities there are several species typical of this province, such as birds (*Capito hypoleucus*, *Habia gutturalis*, *Discosura conversii*), snakes (*Tantilla alticola*) and the lizard studied here (*A. limon*). Some authors suggest a potential extension of this biogeographic province to the south of Tolima (Chaparral) (Acosta-Galvis et al., 2006). Therefore, the new records of *A. limon* in the Tolima department provide evidence supporting the possibility that the limits of this biogeographic province in the Magdalena Valley are more extensive. Given the decline of several species from this province in the Tolima region over the last decade (such as *Trogon cupreicauda*, *Crypturellus erythrops solitarius* and *Crax alberti*) it is likely that threats such as deforestation, agricultural practices, hunting, and the absence of protected areas, may pose a risk to the conservation of *A. limon*.

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