

A new dwarf green anole (Squamata: Dactyloidae) of the *Anolis carolinensis* species group, from western Cuba

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Abstract

Green anoles of the *Anolis carolinensis* group are divided into the *carolinensis* and *isolepis* subgroups according to a consensus of molecular phylogenies. Species in the *Anolis isolepis* subgroup (*A. altitudinalis*, *A. isolepis*, *A. oporinus*, and *A. toldo*) are endemic to Cuba and the highest diversity is concentrated in forested areas of eastern Cuba. Here, we describe a new species of this subgroup from western Cuba based on genetic and morphological differences from other species. Our phylogenetic analysis, based on DNA sequences, includes all of the known species and suggests that the new species is more closely related to *A. altitudinalis*, *A. oporinus*, and *A. toldo* than to the widespread *A. isolepis*. In addition, we provide a new hypothesis on the taxonomic status of *A. incredulus* and recommend that it no longer be considered as a species in the *A. carolinensis* group. Due to the lack of a diagnosis, and poor condition, of the only available specimen, we consider *A. incredulus* as a *species inquirenda*. New morphological and genetic data of recently collected specimens of *A. oporinus* and *A. toldo* will provide complementary information about these species known from one or a few specimens.

Keywords: Caribbean, West Indies, *Anolis angusticeps*, *Anolis incredulus*, ecomorph.

Resumen

Los anolis verdes del grupo *Anolis carolinensis* se dividen en dos subgrupos: *carolinensis* e *isolepis*, según un consenso de filogenias moleculares. Las especies del subgrupo *Anolis isolepis* (*A. altitudinalis*, *A. isolepis*, *A. oporinus* y *A. toldo*) son endémicas de Cuba y la diversidad se concentra en las áreas boscosas del este de Cuba. El objetivo principal de este trabajo es describir una nueva especie dentro de este subgrupo del occidente de Cuba, con suficiente distinción genética y morfológica para merecer reconocimiento como un taxón separado. Nuestro análisis filogenético, basado en secuencias de ADN, incluye todos los taxones inclusivos conocidos y sugiere que la nueva especie está más estrechamente relacionada con *A. altitudinalis*, *A. oporinus* y *A. toldo* que con la extendida *A. isolepis*. Además, proporcionamos una nueva hipótesis sobre el estado taxonómico de *A. incredulus* y recomendamos dejar de considerarlo dentro de la radiación de *A. carolinensis*. Debido a la falta de diagnóstico y al mal estado del único ejemplar disponible, se propone considerar a *A. incredulus* como *species inquirenda*. Nuevos datos morfológicos y genéticos de ejemplares de *A. oporinus* y *A. toldo* colectados recientemente, brindarán información complementaria sobre estas especies que han sido conocidas, hasta la fecha, de uno o muy pocos ejemplares.

Key words: Caribe, Antillas, *Anolis angusticeps*, *Anolis incredulus*, ecomorfo.

Introduction

The *Anolis carolinensis* species series (or species group), the green anoles, has been divided into two subgroups based on molecular data: *carolinensis* and *isolepis* (Burnell and Hedges 1990; Glor *et al.* 2004, 2005; Nicholson *et al.* 2005, 2012; Poe *et al.* 2017; Cádiz *et al.* 2018). Nicholson *et al.* (2012, 2018) listed 14 species and a total of 17 species and subspecies in this clade. These authors have established the Cuban archipelago as the center of

radiation of the *carolinensis* species group. The four species in the *Anolis isolepis* subgroup (*Anolis altitudinalis* Garrido, *A. isolepis* [Cope], *A. oporinus* Garrido and Hedges, and *A. toldo* Fong and Garrido) are endemic to Cuba. Poe *et al.* (2017) also considered “the name *Pseudoequestris* Varona (1985) is appropriate for the largest crown clade containing *A. isolepis* but not *A. carolinensis*”; but this name, originally proposed as a subgenus, has not been ever used. Species in the *A. isolepis* species subgroup have been considered in the trunk-crown ecomorph by Losos (2011) and Nicholson *et al.* (2012).

Anolis isolepis is among the rarest anoles on the island (Barbour and Ramsden, 1919; Ruibal, 1964; Garrido and Hedges, 2001). It was described by Edward D. Cope (Cope 1861) from specimens collected by Charles Wright in the surroundings of Monte Verde, Yateras, Guantánamo (see also Underwood, 1905). *Anolis altitudinalis* was first described as a subspecies of *A. isolepis* by Garrido (1985) and later raised to species status by Garrido and Hedges (2001). *Anolis toldo*, described by Fong and Garrido (2000), and *A. oporinus* by Garrido and Hedges (2001), were the last discoveries and both have local distributions in the mountains of the eastern region. Most species in the subgroup inhabit forested areas and only *A. isolepis* is distributed in the central part of the island, from 0–1306 m above sea level (Schwartz and Henderson, 1991; Rodríguez Schettino *et al.*, 2010). The first mention of *Anolis isolepis* in the west was recently made by Kanamori *et al.* (2022) in a genomic study. A photograph of one individual from Sierra del Rosario (Artemisa Province) is in Uetz *et al.* (2022). *Anolis isolepis* is sympatric with *A. altitudinalis*, *A. toldo*, and *A. oporinus* (Fong and Garrido 2000; Garrido and Hedges 2001). Finally, Nicholson *et al.* (2012, 2018) listed the small-sized *Anolis incredulus* Garrido and Moreno within the radiation of green anoles of the *A. carolinensis* species group following the suggestion of Garrido and Moreno (1998), but its morphology is quite different and the affinities of the species deserve reconsideration.

The main objective of this study is to describe a new species of the *isolepis* subgroup collected in Western Cuba. We also show its relationships with other species in the subgroup using new DNA sequences and a more comprehensive sampling. In addition, we provide a new perspective on the taxonomic status of *A. incredulus* and include complementary morphological and genetic information from recently collected specimens of *A. oporinus* and *A. toldo*.

Materials and Methods

Laboratory methods. The total DNA was extracted from muscle tissues of the tail or leg using standard phenol-chloroform methods. We amplified and sequenced one mitochondrial gene, NADH dehydrogenase subunit 2 (ND2, 1036 bp), from all individuals and two nuclear genes, zinc finger protein 521 (ZNF521, 634 bp) and fibro-sin-like 1 (FBRSL1, 488 bp). Primer sequences and amplification conditions have been described previously (Cádiz *et al.* 2013).

Data analysis. In the phylogenetic analysis, we considered each gene and codon position as a different partition. The substitution models for each partition were selected using Kakusan4 (Tanabe, 2011) with PAUP* (Swofford, 2002) and Treefinder (Jobb *et al.* 2004) based on Akaike’s information criterion (AIC) and Bayesian information criterion 2 (BIC2) for the maximum-likelihood (ML) method and Bayesian inference (BI) method, respectively. The resulting GTR + Gamma+Invariant model (Tamura and Nei 1993) was applied to each of the regions. Reconstruction of the ML tree was calculated using Treefinder and the confidence level of each node was estimated with 1000 bootstrap replicates. Reconstruction of the BI tree was calculated using MrBayes 3.2.7a (Ronquist and Huelsenbeck 2003) by performing 10 million generations of MCMC sampling with a frequency of 1000. The MCMC convergence was verified using Tracer 1.5 (Rambaut and Drummond 2007) and the first 2000 trees were discarded as burn-in. Both LM and BI trees were highly congruent.

Separate estimates of evolutionary divergence (i.e. genetic distances) among clades, unrelated to tree construction, were conducted in MEGA X (Kumar *et al.* 2018; Stecher *et al.* 2020). The number of base substitutions per site was calculated by averaging over all sequence pairs between groups. Analyses were conducted using the

Kimura 2-parameter model (Kimura 1980). The rate variation among sites was modeled with a gamma distribution (shape parameter = 4). All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 2160 positions in the final dataset after combining the sequences from all three genes.

Morphological analysis. Measurements were taken with a digital caliper Vinca DCLA 0605. Most measurements and scale counts follow Köhler (2014), as detailed in the next paragraph, with few modifications (indicated by an asterisk and/or commented) and a free use of abbreviations. The color names and color reference number are according to Köhler (2012).

The following measurements were taken: (1) snout-vent length (**SVL**), from the tip of snout to the anterior margin of the cloacal opening; (2) axilla to groin distance, measured on straight positioned specimen with limbs positioned at 90° angle with body; (3) tail length, from the posterior margin of the cloacal opening to the tail tip; (4) head length (**HL**)*, from the tip of snout to the angle of jaw, where the caliper in vertical position anchors the end of the mandible; (5) head width (**HW**), at the level of posterior margins of eyes; (6) snout length (**SL**), from the tip of snout to anterior border of the ocular orbit; (7) thigh length or femoral length (**FL**), measured from a medial point at the anterior margin of cloacal opening to the middle of knee (thigh positioned 90° angle with body); (8) shank length or tibia length (**TL**), taken from the middle of knee to heel when thigh and shank are positioned forming a 90° angle; (9) subdigital pad width, at the widest point of the fourth toe.

The following scale counts were taken: (10) total number of fourth toe lamellae, counted from the basal lamella at the level of joint of toes third and fourth to the distal smaller lamella at the end of the digital pad; (11) number of scales between first canthals, canthals not included in count, considering first canthal scales those situated anterior to the largest supraciliary scale on the upper anterior corner of the ocular orbit; (12) number of supralabial scales, counting as first supralabial the one behind the rostral scale and the last one would be that below the middle of the eye; (13) number of infralabial scales, taken similarly, but first infralabial is the one behind mental scale; (14) number of postrostral scales, those bordering rostral posteriorly, excluding first supralabial scales; (15) postmental scales, includes first sublabial scales in contact with the mental scale besides the much smaller interstitial scales among them; (16) number of internasal scales, as those between the circumnasal scales but excluding them; (17) number of scales between supraorbital semicircles, those situated in the narrowest point between the semicircles; (18) number of scales between supraorbital semicircles and the interparietal scale, the minimum number of scales located between the interparietal and the semicircles; (19) total number of loreal scales, counted within the imaginary triangle defined by canthals above, supralabial bellow and the anterior border of eye; (20) number of ventral scales in the axilla to groin distance (**VAG**)*, we excluded from count the two scales respectively situated immediately at both sides of the imaginary sagittal plane, then the count was done following the transversal arrangements of scales and thin entomological pins were used as landmarks for count; (21) number of dorsal scales in the axilla to groin distance (**DAG**)*, similar to 19.

Collection abbreviations. ANSP, Academy of Natural Sciences of Philadelphia, United States; BSC.H, herpetological collection at Centro Oriental de Ecosistemas y Biodiversidad (BIOECO), Santiago de Cuba CZACC, collection of the Instituto de Ecología y Sistemática (IES), La Habana, Cuba; MHNH, Museo de Historia Natural Carlos de La Torre, Holguín; MNHNCu, Museo Nacional de Historia Natural de Cuba, La Habana; USNM, United States National Museum of Natural History, Washington D.C.

Results

Molecular phylogeny. The topology of the obtained tree recovers the two subgroups of Cuban green anoles, *carolinensis* and *isolepis*, recognized by other authors. Most nodes are highly supported (Bayesian posterior probabilities > 90%). We included all of the previously known species of the *A. isolepis* subgroup (*A. altitudinalis*, *A. isolepis*, *A. oporinus* and *A. toledo*). *Anolis isolepis* was sequenced from distant localities representing most of its geographic range in the east but we lack genetic samples from central Cuba where the species also occurs. The first specimens of this subgroup collected in Cordillera de Guaniguanico, western Cuba, represent an undescribed

taxon (Fig. 1) which is, unexpectedly, the sister species to *Anolis altitudinalis* + *A. oporinus* + *A. toldo*, three anoles with restricted distributions in the east. The phylogeny also shows that *Anolis toldo* and *A. oporinus* are sister species.

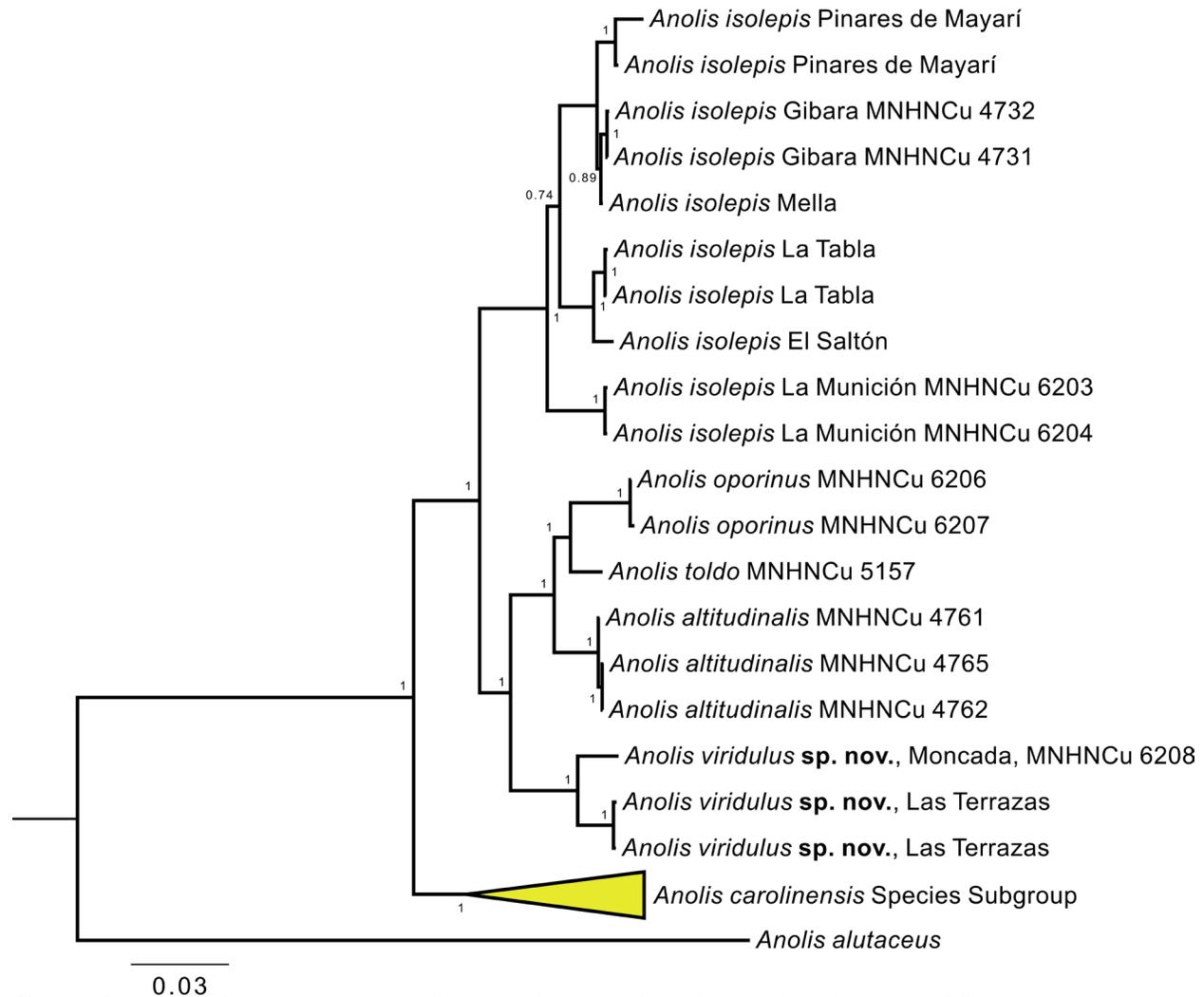


Figure 1. Bayesian tree (50% majority consensus) based on three genes (one mitochondrial and two nuclear). The node supports are the Bayesian posterior probabilities.

The new species has a high genetic sequence divergence of 7–9% with *A. isolepis* from six localities (Fig. 1). The range of intraspecific divergences in *A. isolepis* was between 0.4 and 5.5%, representing a genetic structure that should be studied in the future. The new species diverges 6.3% from *A. altitudinalis*, 6.3% from *A. toldo* and 7.2% from *A. oporinus*. A similarly high genetic distance (7.3–7.5%) is observed among *A. altitudinalis* and *A. isolepis* from populations along the Sierra Maestra mountain range, and from sympatric populations of *A. toldo* and *A. isolepis* of Yateras (7.0%). Genetic sequence divergences from any species in the *A. isolepis* species group and the *A. carolinensis* subgroup is > 9%. Genetic divergence between the closely related, but allopatric, *A. oporinus* and *A. toldo* was intermediate (3.6%), as well as *A. altitudinalis* vs. *A. oporinus* (4.8%), and *A. altitudinalis* vs. *A. toldo* (3.5%), which curiously are in the range of intraspecific distances of *A. isolepis*. Here, we describe the new species from western Cuba.

Anolis viridulus sp. nov.

Western Dwarf Green Anole

(Fig. 2, 4A, 5A, 6A)

Holotype. Adult male MNHNCu 6021, collected on the roadside near the entrance to the Estación Ecológica (22.849732, -82.932877), Las Terrazas, Reserva de la Biosfera (Biosphere Reserve) Sierra del Rosario, Cordillera de Guaniguanico, Candelaria Municipality, Artemisa Province, by Luis M. Díaz and Otelmi (Otis) Campa on 21 January 2022.

Paratypes (n=9). Adult male MNHNCu 6020, same data as holotype; adult females MNHNCu 6022–23, same data as holotype; juvenile male MNHNCu 6024, same data as holotype; juvenile female MNHNCu 6025; male MNHNCu 6209 same locality as holotype but collected on 10 November 2014 by Antonio Cádiz; adult male MNHNCu 6208, surroundings of Cueva de Santo Tomás (22.554167, -83.837837), El Moncada, 18 km W of the town of Viñales, Sierra de los Órganos, Pinar del Río Province, collected by Luis M. Díaz and Jeff Lemm on 13 June 2019; adult male MNHNCu 6026, *Idem.*, collected by Luis M. Díaz and Eliosbel (Elio) García on 8 February 2022.

Diagnosis. A small *Anolis* (males maximum SVL 44.6 mm; females 43.7 mm) of the *carolinensis* group, *isolepis* subgroup, according to the inferred phylogenetic position in Fig. 1. Like the other green anoles of the subgroup (Fig. 3), *Anolis viridulus* **sp. nov.** has a compact build, high and laterally depressed body; head surface relatively flat, with large multicarinate scales (Fig. 4); head canthal and frontal crests absent to barely developed; short limbs; short tail which is 1–1.2 times longer than body or even shorter than it, tapering abruptly from the base; darker hands and feet, with brown-barred digits; body scales round shaped, mostly isomorphic, smooth to keeled (see Fig. 3); and squeaky vocalizations when lizards are handled. *Anolis viridulus* **sp. nov.** is smaller than *Anolis altitudinalis* (42.1–44.6 mm SVL in adults *A. viridulus* **sp. nov.** vs. 46.0–52.0 mm in adult *A. altitudinalis*) and differs from it by lacking both a broad white supralabial stripe extended above the forearm insertion and a supraxillary spot which is best defined in dark color phase or in preserved specimens (Fig. 3 and 6); besides this, the new species has lower total count of fourth toe subdigital scales (27–30 in *A. viridulus* **sp. nov.** vs. 31–35 in *A. altitudinalis*); gorgetal scales of *A. altitudinalis* are bigger and more spaced than in *A. viridulus* **sp. nov.**, and the dewlap reaches more intense shades of yellow (Fig. 6). *Anolis viridulus* **sp. nov.** external morphology is similar to that of *Anolis isolepis* (Fig. 3B–D), particularly to individuals from eastern populations (e.g. Sierra Maestra and Cabo Cruz) that lack the white supralabial line usually present elsewhere. Both species overlap in size but *A. viridulus* **sp. nov.** averages larger than *A. isolepis* (mean = 43.4 mm SVL in *A. viridulus* **sp. nov.** vs. 39.2 mm [35.9–43.0] in *A. isolepis*) and have modally 4 postmental scales (4–5) vs 6 (4–6) in the later (n=34) (Fig. 5); scales between second canthals modally 3 (2–5) in *A. viridulus* **sp. nov.** vs. 5 (3–7) in *A. isolepis*. *Anolis oporinus* and *A. toledo* are two larger sister species (maximum SVL 52.8 mm in *A. oporinus* and 61.2 mm in *A. toledo*), with a straight wide postlabial white line shortly extended beyond the forearms insertion; conspicuous body bands or just middorsal transversal bars on a background with longitudinally arranged marbling and different shades of green in life (but the coloration is much paler in *A. oporinus* than *A. toledo*); belly may have lateral intrusions from the body transversal patterns; preserved specimens do not turn blue; higher number of ventral and dorsal scales (VAG 50–59 and DAG 63–76 in *A. viridulus* **sp. nov.** vs VAG 63–68 and DAG 79–82 in *A. oporinus* and *A. toledo*); higher number of loreal scales (10–14 in *A. viridulus* **sp. nov.** vs. 15–16 in *A. oporinus* and *A. toledo*); postmental scales 6; and comparatively more conspicuously keeled and multicarinate scales than the new species (Fig. 3 E, F).

Description of holotype (see Figs. 2, 4, and 5). SVL 44.6 mm; axilla-groin length 18 mm; head length 16.1 mm; head width 6.8 mm; snout length 7.1 mm; snout width 5.8 mm; ear opening width 0.5 mm; ear opening height 0.7 mm; thigh length 10.6 mm; shank length 7.2 mm; dorsal head scales large (the largest is about seven times the size of dorsal scales on body), multicarinate; dorsal surface of head relatively flat, with very shallow prefrontal and parietal depressions; rostral canthus distinct, but no canthal or frontal ridges are well defined; postrostrals 5; internasals 6; parietal scale width 0.6 mm; parietal scale length 1.0 mm; interparietal separated from supraorbital semicircles by 2/3 (left/right) scales; scales surrounding the interparietal 9, smooth to very shallowly multicarinate, the anterior scales are larger than posterior ones; 3/4 (left/right) moderately enlarged supraocular scales; scale between supraorbital semicircles 1; supralabials to center of eye 9; infralabials to center of eye 9; one superciliary scale enlarged followed by granular scales; no scales rows between suboculars and supralabial; scales between first

and second canthals 5; three loreal rows near the base of snout gradually reduced to one row; total loreal scales 14; temporal scales large, smooth to very shallowly multicarinate, 9 between the row of scales bordering the orbit and the anterior edge of the ear opening; rostral scale slightly overlapping the mental; mental divided, slightly broader than rostral; postmental scales 4; dorsal scales rounded to slightly oval, smooth (mostly) to slightly keeled (dorsal scales on anterior part of body), juxtaposed, with pale interstitial skin, and in transversal to diagonal rows; although not very distinctive, at least 5 middorsal rows of scales are slightly narrower, smaller and tightly arranged than lateral scales, but the transition is very gradual; 74 dorsal scales in the axilla-groin distance, 33 in a distance equivalent to the snout length on mid belly; 59 ventral scales in the axilla-groin distance, 27 in a distance equivalent to the snout length on mid belly; ventral scales rounded, smooth, juxtaposed, arranged in diagonal to transversal rows; chin scales long, smooth to slightly keeled, gradually giving place to smooth gular scales; sublabial scales shallowly multicarinate, separated from infralabial scales by a row of intercalate scales after third infralabial; forelegs and hind legs with smooth scales; supradigital scales shallowly multicarinate; two enlarged postcloacal scales more than twice the length of surrounding scales, separated each other by one smaller scale; dewlap small, narrow in preserved specimen, round when the animal fully distended it in life (Fig. 2); scales on dewlap narrowly separated and barely defining rows (about 15 scales from base to edge), middle gorgetals oval to semicircular in shape; fourth toe width 1.1 mm; fourth toe subdigital lamellae 28 in total, 17 along the widest distal portion of digit or adhesive pad (phalanges II-III); the digital adhesive pad is very distinct, and its profile is projected forward under the distal phalange; tail regenerated, 42.8 mm in length, with swollen tip, scales on ventral surface much enlarged than dorsals, keeled; external length of the hemipenial bulges 7.8 mm, giving a gross appearance to the base of tail.

Variation. Table 1 shows the variation of metric and meristic characters. Because many individuals have regenerated tail, its length is not included in the table. Tail length/SVL: 0.9 (male MNHNCu 6208), 1.1 (male MNHNCu 6026) and 1.2 (female MNHNCu 6023). The only individual with 5 postmental scales is the juvenile male MNHNCu 6024. All males have enlarged postcloacal scales narrowly separated by a row of two consecutive smaller scales.



Figure 2. *Anolis viridulus* sp. nov. (A) Holotype male (MNHNCu 6021) from the type locality at Las Terrazas, Sierra del Rosario while displaying to another male in captivity; (B) adult paratype male MNHNCu 6026 from Moncada, Viñales. Photos: L. M. Díaz.

Table 1. Measurements, counts, and morphological ratios of *Anolis viridulus* **sp. nov.** and the other species of the *isolepis* species subgroup of the *carolinensis* group.

	<i>A. viridulus</i> sp. nov.	<i>A. altitudinalis</i>	<i>A. isolepis</i>	<i>A. oporinus</i>	<i>A. toledo</i>
SVL	M: 41.32 (23.15–43.39) F: 39.81 (33.07–43.36)	M: 47.79 (47.35–48.40) F: 46.95 (46.54–47.37)	M: 40.96 (36.60–43.00) F: 39.29 (35.98–43.14)	M: (none) F: 48.59 (45.65–52.8)	M: (none) F: 55.51
Head length	14.16 (11.30–16.04)	16.24 (14.06–17.44)	13.53 (12.41–15.19)	16.67 (15.47–17.94)	18.90
Head width	7.28 (6.40–8.35)	8.38 (7.83–9.02)	6.96 (5.07–7.72)	8.73 (8.31–9.42)	9.7
Snout length	6.31 (4.84–7.45)	7.37 (6.70–7.91)	6.14(5.33–7.02)	7.45 (6.82–8.27)	8.34
Axilla–groin distance	16.00 (12.84–18.14)	18.51 (16.74–19.64)	15.68(12.89–17.88)	21.89 (20.54–24.40)	22.33
Thigh length	8.96 (6.93–10.72)	10.17 (9.19–10.63)	8.76 (7.48–10.22)	9.90 (9.49–10.65)	11.85
Shank length	6.96 (6.02–7.79)	7.77 (7.50–8.19)	6.74 (5.97–7.70)	7.53 (7.36–7.73)	9.16
Fourth toe width	0.99 (0.82–1.13)	1.00 (0.73–1.27)	0.94 (0.79–1.11)	1.06 (0.93–1.27)	1.31
Fourth toe total subdigital lamellae	27 (27–30)	33 (19–35)	28 (26–31)	31 (31–31)	34
Postrostral scales	6 (5–6)	5 (5–9)	5 (5–7)	6 (5–6)	5
Postmental scales	4 (4–5)	4 (4–6)	6 (4–6)	2 (2–2)	4
Internasal scales	6 (4–6)	4 (4–6)	6 (4–6)	6 (5–6)	4
Scales between supraocular semicircles	1 (0–1)	1 (1–1)	1 (0–2)	1 (1–1)	1
Scales between first canthaks	5 (2–5)	3 (3–5)	5 (3–6)	5 (3–5)	5
Scales between second canthaks	3 (2–5)	4 (3–4)	5 (3–7)	4 (3–5)	3
Supralabials	8 (7–9)	8 (8–9)	7 (6–9)	8 (7–8)	7
Infralabials	7 (6–9)	8 (7–9)	7 (5–8)	7 (6–8)	7
Loreal scales	12 (10–14)	11 (9–13)	12 (7–17)	15 (14–16)	15
Scales between interparietal and supraorbital semicircles	2 (1–2)	1 (0–1)	1 (1–4)	1 (1–2)	1
Ventral scales	54 (50–59)	48 (48–63)	55 (49–67)	68 (63–69)	74
Dorsal scales	63 (63–76)	70 (65–78)	64 (56–90)	80 (79–82)	87
HL/SVL	0.35 (0.32–0.37)	0.34 (0.30–0.36)	0.34 (0.30–0.36)	0.34 (0.33–0.35)	0.34
HW/HL	0.51 (0.42–0.58)	0.52 (0.49–0.56)	0.51 (0.39–0.56)	0.52 (0.51–0.54)	0.51
SL/HL	0.44 (0.42–0.47)	0.45 (0.44–0.48)	0.45 (0.42–0.49)	0.45 (0.44–0.46)	0.44
FL/SVL	0.22 (0.18–0.25)	0.21 (0.19–0.22)	0.22 (0.19–0.24)	0.20 (0.20–0.21)	0.21
TL/SVL	0.18 (0.16–0.19)	0.16 (0.15–0.17)	0.17 (0.14–0.19)	0.15 (0.14–0.16)	0.16

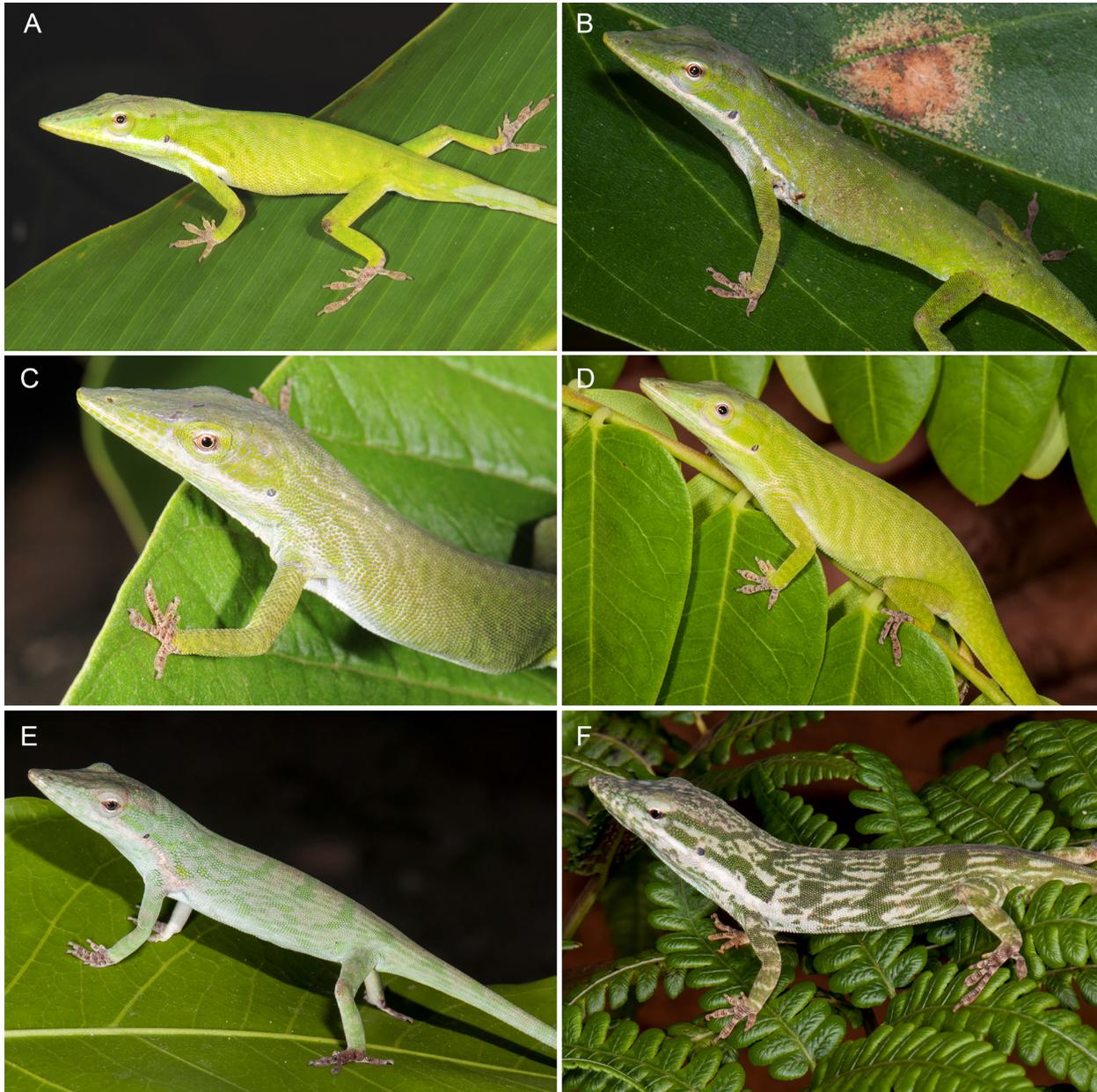


Figure 3. Other species of the *Anolis isolepis* subgroup. **(A)** *Anolis altitudinalis* (MNHNCu 4762), adult male from La Platica, Sierra Maestra, Granma Province; **(B)** *Anolis isolepis* (no voucher), adult male from La Melba, Holguín Province; **(C)** *A. isolepis* (MNHNCu 6203), adult male from La Municipión, Yateras, Guantánamo Province; **(D)** *A. isolepis* (MNHNCu 4731), adult female, from Los Hoyos, Gibara, Holguín; **(E)** *Anolis oporinus* (MNHNCu 6206), adult female from La Pimienta, Carsos de Baire, Granma Province; **(F)** *Anolis toledo* (MNHNCu 5157), adult female from Cupeyal del Norte, Yateras, Guantánamo Province. Photos: L. M. Díaz.

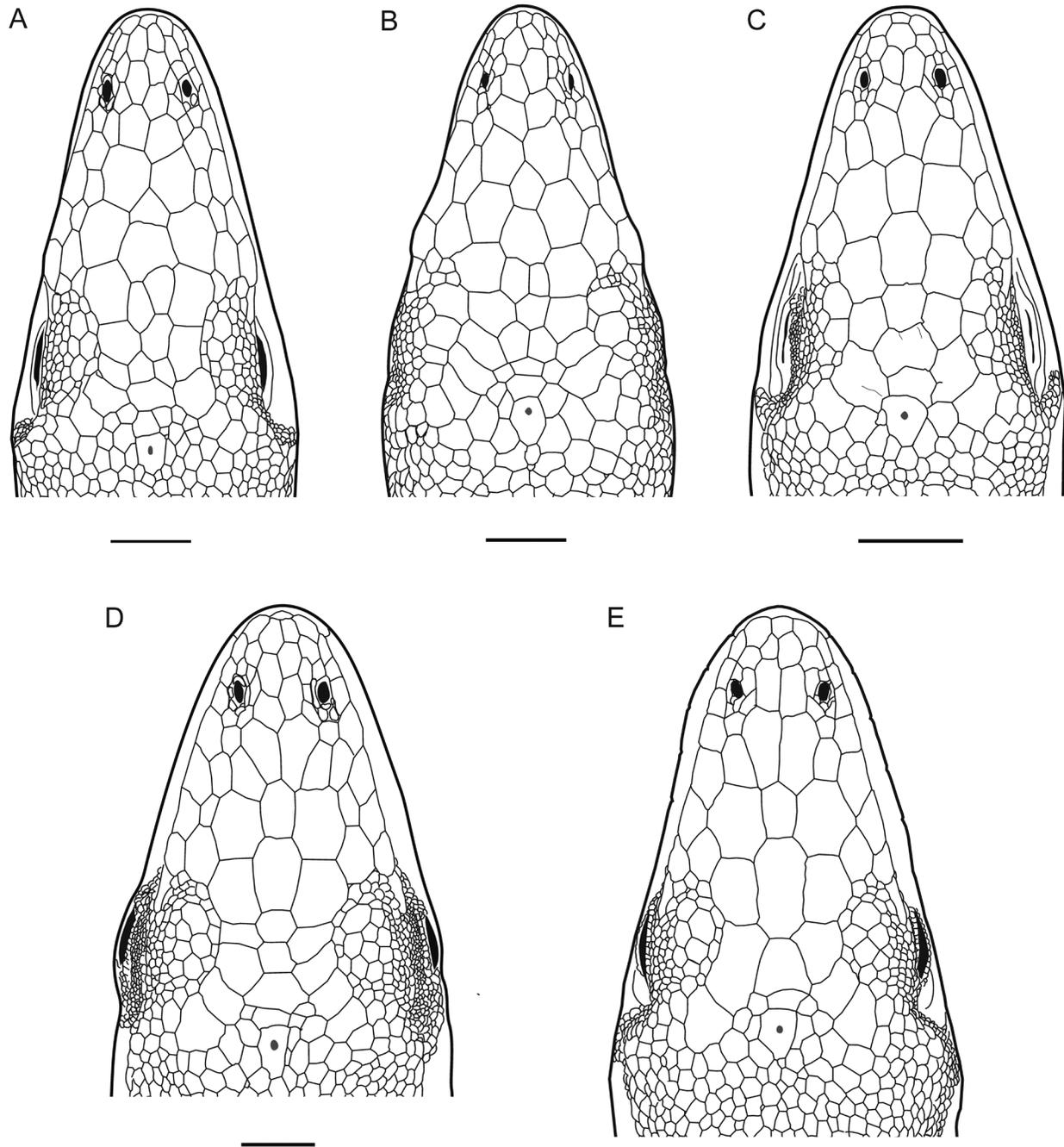


Figure 4. Head scales of lizards in the *Anolis isolepis* subgroup. Only disposition but not texture is illustrated. **(A)** *A. viridulus* **sp. nov.**, holotype male MNHNCu 6021, from Las Terrazas, Sierra del Rosario; **(B)** *A. isolepis*, male MNHNCu 6203, from La Muni3n, Yateras, Guant3namo; **(C)** *A. altitudinalis*, male MNHNCu 4765, from La Platica, Sierra Maestra, Santiago de Cuba; **(D)** *A. oporinus*, female MNHNCu 6207, from La Pimienta, Santiago de Cuba; **(E)** *A. toledo*, female MNHNCu 5157, from Cupeyal del Norte, Guant3namo. Scale bar = 2 mm. Illustrations: L. M. D3az.

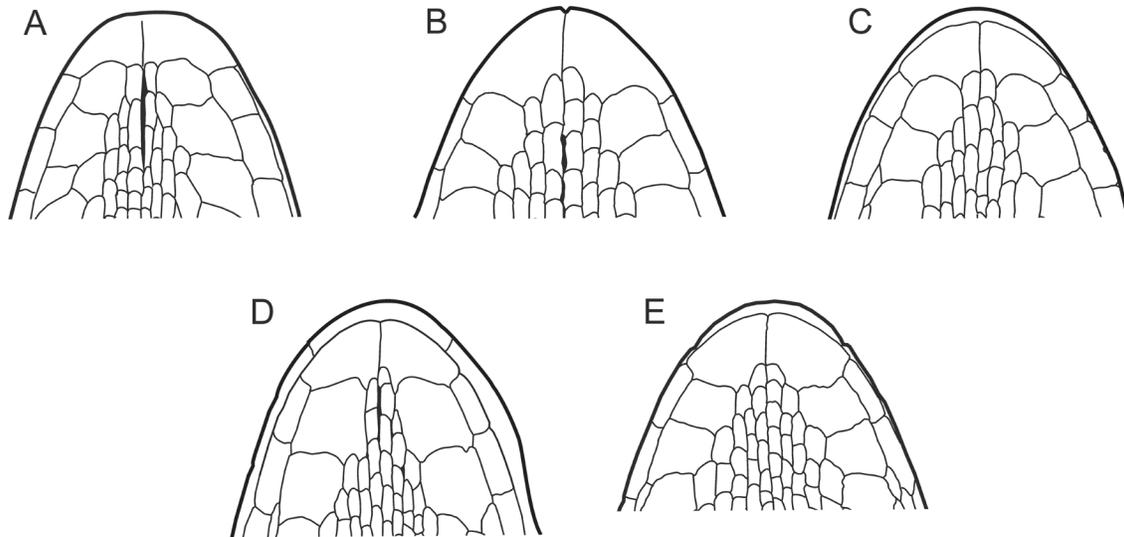


Figure 5. Postmental scales in five species of *Anolis* in the *A. isolepis* subgroup. (A) *Anolis viridulus* (holotype); (B) *A. isolepis* (MNHNCu 6197); (C) *A. altitudinalis* (MNHNCu 4763); (D) *A. oporinus* (MNHNCu 6206); (E) *A. todo* (MNHNCu 5157).

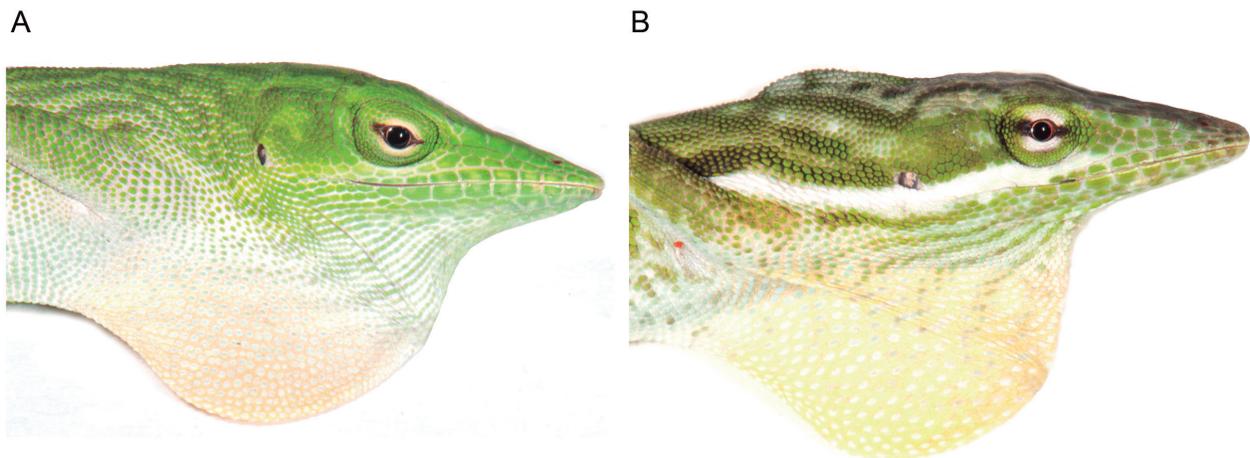


Figure 6. Lateral view of head pattern and dewlap coloration of *Anolis viridulus* **sp. nov.** (paratype MNHNCu 6209) and *A. altitudinalis* (no voucher), from La Platica, Sierra Maestra. Photos: Luis M. Díaz.

Color in life. Color references in Köhler (2012; see Fig. 2 and 6). Both sexes are uniformly yellow green (color 103), or opaline green (color 106) to brown depending on metachromatic phase. These lizards may show diagonal stripes on body variably defined by having a more contrasting green or changing to brown when lizards are highly stressed. Belly white with well-defined edges, which are sharper in females than males. Male dewlap white, with a pale pinkish-buff (color 3, unsaturated) or light peach in the interstitial skin of distal half. Hands and feet with differentiated coloration with respect body and limbs, consisting in a mosaic of brown (color 19), buff (colors 1–3) and green on digital tips, with dark spots and transversal bars. Paratype MNHNCu 6026 with aligned suprascapular white dots and a contrasting supraxillary green spot (Fig. 2B).

Color in alcohol. Specimens quickly turns blue, with pure white belly. Some of them show darker blue diagonal stripes that form a pattern similar to when they were green.

Etymology. The species name is a diminutive version of the Latin word for “green,” alluding to the vividly green, small size, and compact body of this anole. The common name follows proposed guidelines (Hedges *et al.* 2019).

Distribution (Fig. 7). The species is known from Las Terrazas, Sierra del Rosario, Artemisa Province, and

from Moncada, Viñales, Pinar del Río Province, 90 km apart (air distance); both localities are in the Cordillera de Guaniguanico. Estimated extent of occurrence (EOO): 860 km².

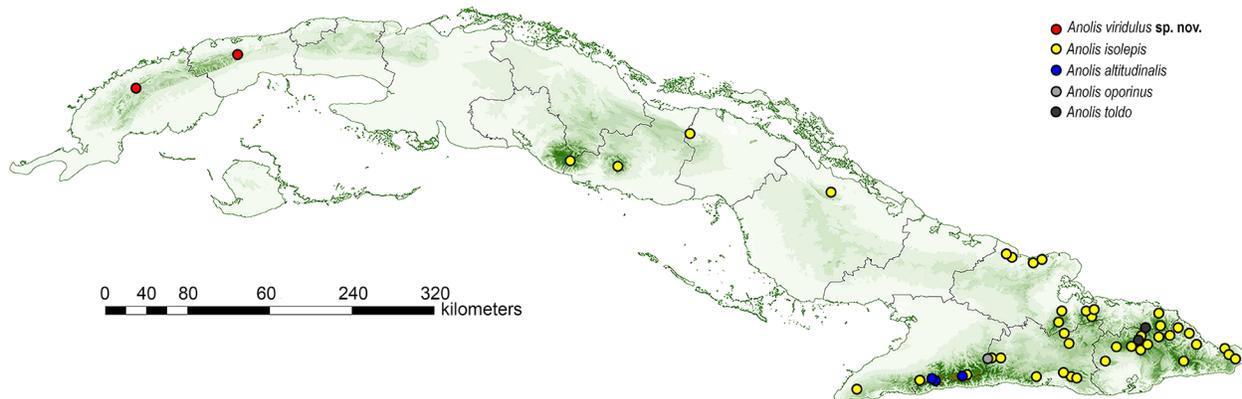


Figure 7. Distribution of species in the *Anolis isolepis* subgroup of the *Anolis carolinensis* group. Records are from the compilation by Rodríguez Schettino *et al.* (2013), author's visual records, and specimens examined in collections

Notes on ecology and behavior. At Las Terrazas, Sierra del Rosario, lizards were collected on *Calophyllum antillanum* (Calophyllaceae) and *Cojoba arborea* (Fabaceae), perched 1.6 m to 4 m high, while roosting at night on leaves. Most trees at the area were planted by reforestation programs during the last six decades. One juvenile was observed, but not collected, on a leaf of *Psidium guajava* (Myrtaceae) 1.8 m high. Two males (MNHN Cu 6020 and 6021), kept in captivity for two days, were territorial and demonstrated threatening behavior towards each other, raising the body and flattening it laterally while the small dewlap was extended. Both individuals were found roosting in the wild in the same tree not far from each other. Very likely, they were engaged in territorial assertions during the day. Tail amputations and regenerations in most males are suggestive of strong physical territorial interactions. These lizards are distinctive by walking upright on their four legs when they are on the surface of horizontal leaves, with their backs somewhat curved and body laterally compressed. The two males from Moncada (Viñales) were respectively collected at night while roosting on leaves of the same tree *Zanthoxylum martinicense* (Rutaceae) in different years, about 3–4 m high. The tree was surrounded by small coffee and banana plantations on the border of a karstic hill ("mogote") with natural vegetation complex. At Las Terrazas, annual average temperature is 25°C (18.0–31.3°C), annual total precipitation 994 mm, relative humidity 66–81%, average daily sun hours per month 10.1–10.3 in May to July, 7.1–7.6 from November to January (<https://es.climate-data.org/americas-del-norte/cuba/artemis/las-terrazas-506058/>).

Conservation. The species occurs in the protected areas Reserva Ecológica de Recursos Manejados Sierra del Rosario and Parque Nacional Viñales.

Discussion

From a biogeographic perspective, the closest relationships of *A. viridulus sp. nov.* to eastern highland species with restricted distributions, instead of the widespread *A. isolepis*, is a puzzle. A more precise hypothesis about the biogeographical origin of *A. viridulus sp. nov.* in the west is needed, especially considering the gap that the central region represents in our analysis. In fact, any study with lizards in the *A. isolepis* subgroup is limited by the scarcity of specimens in collections and the rarity in the wild compared to other anoles. At La Pimienta, *Anolis isolepis* is the "common" species while *A. oporinus* is extremely rare and sporadically found after a long search; in fact, the latter was only known from one specimen for twenty-two years. *Anolis toledo* is considerably harder to find than sympatric *A. isolepis* and is known from only three individuals. Furthermore, the number of specimens of *A. altitudinalis* in collections probably does not exceed fifteen. It could be possible that central Cuba harbors two dwarf green anole species, one in the subclade *A. isolepis* and another closer to *A. altitudinalis*, both coexisting in some forested areas. However, everything is very speculative until broader phylogeographic studies are carried out in the central region of the island.



Figure 8. Habitat of *Anolis viridulus* sp. nov. at the type locality. Each photo was taken on both sides of the same road. (A) The tree line is dominated by *Calophyllum antillanum*; (B) trees are mainly *Cojoba arborea*. Las Terrazas, Sierra del Rosario, Cordillera de Guaniguanico. Photos: L. M. Díaz.

We did not include *Anolis incredulus* in the molecular phylogeny of Cuban green anoles because DNA sequences of this species were unavailable. The only known specimen from Pico Cuba, Sierra Maestra, eastern Cuba, is the female holotype (Fig. 9 A–B), which is in a very poor state of preservation, almost destroyed. Collection efforts subsequent to species description have been unsuccessful. The small size of *A. incredulus* and its supposed green coloration suggest that it could be superficially confused with species of the *A. carolinensis* species group, as proposed by Garrido and Moreno (1998) and later listed by Nicholson *et al.* (2018), particularly the members of the *A. isolepis* subgroup. However, the external morphological characters of this specimen are sufficiently evident to allow us to make the decision not to further consider the relationship of this species with those of the *Anolis carolinensis* group. Instead of this, all the external characters of this specimen show that it is a twig anole related to *Anolis angusticeps*, a widely distributed lizard that also occurs at low to mid altitudes of the Sierra Maestra mountain range. The diagnostic white spot on the neck mentioned by Garrido and Moreno (1998) is not actual skin pigment in such a bleached specimen, but rather it is the calcified endolymphatic sacs visible as a result of chemical reactions with formalin (Fig. 9B). The only remnant of pigment is a dark spot on the scapular area, which coincides with a similar pattern in species of the *A. angusticeps* group. In the holotype *A. incredulus*, according to Garrido and Moreno (1998), the thigh length was 7.5 mm, then the ratio FL/SVL = 0.2. Now it is impossible to repeat this measurement because the holotype is dismembered and decapitated. Estrada and Hedges (1995) reported the same ratio (0.2) for *Anolis alayoni*. The only character that remains diagnostic is the low number of ventral scales in the snout length, lower than counts by Garrido (1975). Like in other species of the *A. angusticeps* group there are 2 to 3 enlarged supraocular scales surrounded by granular scales, also illustrated by Garrido and Moreno (1998), although the scalation definition was not very accurate probably due to specimen condition. The holotype of *A. oporinus* also has 17 granular temporal scales between the posterior margin of ocular orbit and the ear opening; in the *A. isolepis* subgroup, for example, they are much bigger and, in consequence, fewer (9–11). All scales are granular and smooth in *A. oporinus*, similar

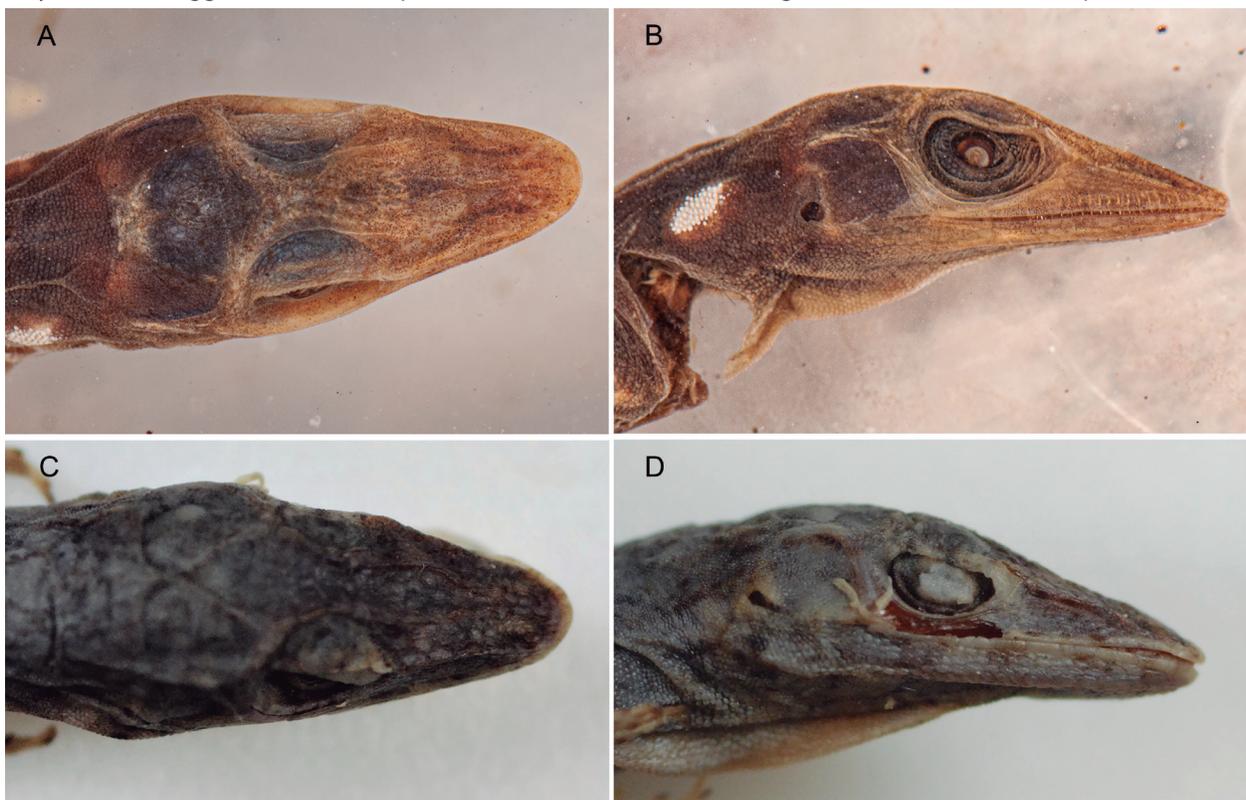


Figure 9. Head of the female holotype of *Anolis incredulus* Garrido and Moreno (CZACC 4.7394) in dorsal (A) and lateral (B) views, compared with the head of the holotype of *Anolis angusticeps* Hallowell (ANSP 7789), in dorsal (C) and lateral (D) views. Photos: Luis M. Díaz (A–B) and Ned S. Gilmore (C–D).

to species in the *A. angusticeps* group. The postmental scales are eight, number of scales between second canthals were six, postrostral scales seven, and there was one row of scales separating supraorbital semicircles. The reference to a green coloration was anecdotally given by the collector (Gerardo T. Albañir) in 1963 as a general appearance, without precise details, and is open to some subjectivity.

With the above mentioned morphological differences and the new hypothesis on the taxonomic relationships of *Anolis incredulus* we consider unnecessary to do further comparisons with members of the *Anolis carolinensis* clade. It is premature to make a decision on the validity of *A. incredulus* as a species because it could be a valid taxon. However, due to the poor species definition and the poor condition of the only known specimen, making it difficult to verify the status of many morphological characters, it would be better to consider it a *species inquirenda*. Future collecting effort and research may provide the evidence needed to re-evaluate its taxonomic status.

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Appendix 1

Specimens examined for comparisons

Anolis altitudinalis (n=5).—MNHNCu 4761–4765, La Platica, Sierra Maestra.

Anolis angusticeps.—ANSP 7789 (holotype male), Cienfuegos Province, Cuba.

Anolis isolepis (n=34).—MNHNCu 110, Arroyo Los Gatos, Jaguaní River, Holguín Province; MNHNCu 3789, Cabezas del Jaguaní, Holguín Province; MNHNCu 4731–4733, Los Hoyos, Gibara, Holguín Province; MNHNCu 5034–35, Cabo Cruz, Granma Province; MNHNCu 6190–94, La Pimienta, Santiago de Cuba Province; MNHNCu 2194, Gran Piedra, Santiago de Cuba Province; MNHNCu 6195–96, Idem.; MNHNCu 6203–04, La Municipión, Yateras, Guantánamo Province; MNHNCu 5037, Altiplanicie de El Toldo, Holguín Province; MNHNCu 6197–6202, 6205, Arroyo Bueno, La Melba, Moa, Holguín Province; MHNH 14–279, El Peñón, Estero Ciego, Rafael Freyre, Holguín Province; MHNH 14–260, Pinares de Mayarí, Holguín Province; MHNH 14–262, Playa Pesquero, Rafael Freyre, Holguín Province; MHNH 14–280, Arroyo Bueno, La Melba, Moa, Holguín Province; MHNH 14–278, Los Hoyos, Gibara, Holguín Province; MHNH 14–144, El Beril, Maisí, Guantánamo Province; MHNH 14–319, Cabonico, Mayarí, Holguín Province; MHNH 14–263, La Zoilita, Sierra del Cristal, Mayarí, Holguín Province; MHNH 14–317, Río piloto, Mayarí, Holguín Province; MHNH 14–316, Gran Piedra, Santiago de Cuba Province; MHNH 14–145, Patana Arriba, Maisí, Guantánamo Province; MHNH 14–261, Campamento Ruso, Yateras, Guantánamo Province.

Anolis oporinus (n=3).—MNHNCu 4522 (Field Number USNMFS 191409), holotype, and MNHNCu 6206–07, La Pimienta, Santiago de Cuba Province.

Anolis tordo (n=3).—BSC.H 1080 (holotype), Altiplanicie de El Toldo, Holguín Province; MNHNCu 5157, Cupeyal del Norte, Guantánamo.