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A New Fossil *Anolis* Lizard in Hispaniolan Amber: Ecomorphology and Systematics

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Abstract

Pre-Pleistocene fossils of *Anolis* lizards are rare, although 20 Miocene fossils preserved in amber from the island of Hispaniola have been reported on previously. Using light-microscopy and computed-tomography imaging, we studied a new amber fossil *Anolis* lizard from Hispaniola. The fossil is likely a juvenile and preserves a largely intact left forelimb, including both scales and skeletal elements, as well as some additional skin, skin impressions, and fragmentary skeletal elements from other parts of the body. Using measurements and lamella counts from the forelimb of the fossil and other juvenile anoles, discriminant function analysis and three Euclidean-distance criteria derived from a principal components analysis consistently support classification of the fossil as a member of the trunk ecomorph category, and those results, in combination with two scale characters preserved in the fossil, suggest that it is a member of the *Anolis distichus* series within the *Ctenonotus* clade. These results represent only the third case of a well-supported assignment of an *Anolis* fossil to the trunk ecomorph category and the first to the *A. distichus* series. They also highlight that such assignments can sometimes be inferred from highly incomplete fossils.

Anolis lizards provide a well-known example of the ecomorph phenomenon, in which organisms exhibit species-specific quantifiable morphological adaptations related to ecological specialization, in this case to use of structural habitats (e.g., Losos, 2009; Losos et al., 1998; Williams, 1972, 1983). Each anole ecomorph is named for the structural habitat, or habitats, that are most commonly occupied by the lizards assigned to that ecomorph, and six or seven are commonly recognized—crown giant, trunk-crown, trunk, twig, trunk-ground, grass-bush, and, recently recognized, ground (Huie et al., 2021). In the Greater Antilles of the Caribbean Sea, where the ecomorph phenomenon in *Anolis* lizards is best documented, members of the various ecomorph categories have evolved largely independently through convergent evolution between lineages on different islands (Losos, 2010; Losos et al., 1998; Mahler et al., 2013).

The fossil record of *Anolis* lizards prior to the Pleistocene is generally poor (Losos, 2009); however, one exception concerns amber deposits on the island of Hispaniola, from which some 20 fossil specimens of *Anolis* lizards have been studied (Sherratt et al., 2015). Amber fossils often preserve not only skeletal elements but also skin, often, the preserved parts are intact, and sometimes, strongly supported inferences can be made about the systematic relationships and ecological morphology of the fossils. The first three amber fossil anoles reported on in the scientific literature (de Queiroz et al., 1998; Polcyn et al., 2002; Rieppel, 1980) were all inferred to be Hispaniolan green anoles (members of the *Anolis chlorocyanus* species group of Williams, 1976 = *Anolis chlorocyanus* series of Burnell & Hedges, 1990) and members of the trunk-crown ecomorph category; most specimens analyzed to date that can be assigned to an ecomorph with a high degree of confidence have been assigned to that category (Sherratt et al., 2015). Only a few specimens have been assigned to three other of the ecomorph categories—namely, trunk (2 specimens), trunk-ground (2), and twig (1) (Sherratt et al., 2015). Here, we report on a fragmentary new specimen that nonetheless appears to be assignable to one of the rarer ecomorphs as well as to a small subclade of anoles with reasonably high degrees of confidence.

Materials and Methods

The specimen is cataloged in the paleontology collection of the Staatliches Naturhistorisches Museum Braunschweig under the number 8198. It was studied with a combination of optical microscopy, including photography, and x-ray computed tomography. The specimen was examined and photographed using a Keyence VHX-6000 digital microscope with an overhead ring light. Stacks of images were recorded at different focal lengths, then each stack was assembled into a single composite image using the microscope's built-in software. All photographs presented here are composite images.

The specimen was scanned using micro-computed tomography (µCT) on the Imaging Beamline P05 (Lytaev et al., 2014) that is operated by Helmholtz-Zentrum Hereon at the PETRA III storage ring (Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany). Scans were performed using a photon energy of 18 keV with a sample-to-detector distance of 100 mm. We recorded the projections with a custom 20

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MP CMOS imaging system and an effective pixel size of 1.28 µm (Lytaev et al., 2014). For each tomographic scan, 3601 projections were recorded at equal intervals between 0 and π. We conducted a reconstruction by applying a transport-of-intensity phase-retrieval approach and using the filtered back-projection algorithm carried out with a custom reconstruction pipeline (Moosmann et al., 2014) using MATLAB (Mathworks, 2022) with application of the ASTRA Toolbox (van Aarle et al., 2015, 2016). We binned raw projections twice for subsequent processing, which resulted in an effective pixel size of the reconstructed volume (voxel) of 2.56 µm. To decrease the burden on computer memory, we converted all the stacks into 8-bit tiffs, which we downscaled by 50%; we also cropped the empty space around the specimen using the 'scale' and 'crop' functions in Fiji (Schindelin et al., 2012). We used Drishti version 2.6.6 to reconstruct the projections as volumes (Limaye, 2012). We took measurements from the scan using the 3D Slicer software, version 5.2.2 (Kikinis et al., 2013) equipped with the SlicerMorph toolkit (Rolfe et al., 2021). A TIFF stack of the scan is deposited at www.morphdbase.de.

Because the amber specimen is likely a juvenile, and most existing *Anolis* ecomorphological data sets are for adults, (e.g., Armstead & Poe, 2015; Huie et al., 2021; Losos et al., 1998; Poe & Anderson, 2019) or were measured by a different researcher (Sherratt et al., 2015), JMH collected new data for 43 juvenile anoles representing 14 Hispaniolan ecomorph species (Supplementary Data: Appendix 1). In addition, because the best-preserved part of the fossil is the left forelimb, our study focused on that limb. Thus, JMH measured the length of the upper arm, lower arm, and the hand (from wrist to tip of the fourth finger), as well as the width of the pad on the fourth finger. He also counted the number of subdigital lamellae (laterally expanded subdigital scales) on the fourth finger from the base of the digit to the distal terminus of the adhesive pad (i.e., the lamellae underlying the proximal three phalanges of digit IV, which has five phalanges in total; the subdigital scales under the penultimate phalanx are not lamellar, while the terminal = ungual phalanx is small and associated primarily with the claw). We measured the soft tissues rather than the bones of both the fossil and the modern specimens.

To assess the amber anole as a member of an ecomorph class, we first size corrected the continuous variables using forelimb length (sum of the upper arm, lower arm, and hand measurements) as a proxy for size. The discrete variable, lamella count, was not size corrected but was log transformed. We then assessed the ecomorph assignment of the fossil using the same approaches as Huie et al. (2021). First, we performed a discriminant function analysis (DFA) using the size-corrected, and log-transformed, variables and trained with the ecomorph species using the MASS package (Venables & Ripley, 2002) in R Statistical Software version 4.3.2 (R Core Team, 2021). We then performed a principal components analysis (PCA), also using R version 4.3.2, and, because of the small number of variables, used only the first two PC axes to calculate Euclidean distances between the specimens and ecomorph centroids. Second, we calculated Euclidean distances between each member of an ecomorph and the ecomorph centroid, between all pairs of specimens representing the same ecomorph, and between the fossil and both each ecomorph centroid and the members of each ecomorph. We then applied the following three distance criteria to determine whether the fossil could be assigned to one or more of the ecomorphs: (1) Centroid distance (CD); the fossil was considered assignable to an ecomorph if the Euclidean distance from the fossil to the centroid of that ecomorph was ⩽ the distance of the furthest member of that ecomorph to the centroid. (2) Mean pairwise distance (MPD); the fossil was considered assignable to an ecomorph if the average Euclidean distance of the fossil to all members of that ecomorph was ⩽ the largest average pairwise distance among the members of that ecomorph. (3) Nearest-neighbor distance (NND); the fossil was considered assignable to an ecomorph if the Euclidean distance of the fossil to its nearest member of that ecomorph was ⩽ the largest nearest-neighbor distance among the members of that ecomorph.

Comparisons of scale characters were also made with specimens representing several species of Hispaniolan anoles representing every ecomorph for which the fossil had a nonzero DFA assignment probability or satisfied at least one of the Euclidean-distance criteria (Supplementary Data: Appendix 2).

Results

The amber piece containing the fossil (Fig. 1A) is irregularly shaped and measures 24 mm in length by 21 mm in width and 10 mm in thickness. It was collected from an unknown mine in the Dominican Republic and purchased by PM from Ambra Greco (Viale Ugo Foscolo, 9, 20900 Monza MB, Italy). Although the geological formation is unknown, all currently known Dominican amber is from two formations, La Toca and Yanigua (Penney, 2010). The age of Dominican amber has been debated (reviewed by Penney, 2010), but relatively recent estimates give the age as Early to Middle Miocene or 15–20 Ma (Iturralde-Vinent, 2001; Iturralde-Vinent & Macphee, 1996).

The fossil lizard (Fig. 1B) is oriented diagonally within the amber piece relative to both its width and its length. The fossil consists of a mostly intact (skin and skeleton) left forelimb, parts of the right forelimb, the posterior part of the head, skin from the left side of the body posterior to the insertion of the forelimb, and skin (scale) impressions from the ventral surface of the body, the posterior lateral surface of the body on left side, the lateral surface of the body on the right side, and the right temporal region of the head. The main skeletal elements preserved (Fig. 1C) consist of most of the bones of the left forelimb (humerus, radius, ulna, and some of the metacarpals and phalanges), the ventral part of the left quadrate bone and posterior part of the lower jaw (articular and most of the surangular and prearticular bones), and fragments of at least four ribs (both bony dorsal and cartilaginous ventral components) associated with the skin on the left side of the body. The radius and ulna are displaced distally relative to the skin, and some of the metacarpals are also displaced from their presumed natural positions. The proximal and distal ends of most of the limb bones (diaphyzes) appear flat or concave and adjacent phalanges are separated by gaps, suggesting that the epiphyses were mostly or entirely cartilaginous

TABLE 1. Component loadings (above) and standard deviation and proportion of variance explained by the components (below) from the PCA. Pad width and lamella count are for manual digit IV.

	PC1	PC2	PC3	PC4	PC5
Upper arm	0.013	0.023	0.190	0.804	0.563
Lower arm	0.013	0.132	0.650	−0.529	0.530
Hand	−0.019	−0.133	−0.711	−0.272	0.634
Pad width	−0.251	0.952	−0.176	0.017	0.002
Lamella count	−0.968	−0.242	0.071	0.004	0.002
Standard deviation	0.22427	0.13542	0.06140	0.03405	0.00178
Proportion of variance	0.68367	0.24928	0.05124	0.01576	0.00004
Cumulative proportion	0.68367	0.93295	0.98420	0.99996	1.00000

TABLE 2. Ecomorph assignment of the fossil anole. The table gives discriminant-function-analysis probabilities and Euclidean distances for the amber fossil *Anolis* specimen regarding its classification in the six traditional ecomorph categories (Losos, 2009; Williams, 1972). Abbreviations: CG = crown giant, GB = grass-bush, TC = trunk-crown, TG = trunk-ground, Tr = trunk, Tw = twig, DFA = discriminant-function-analysis-classification probability, CD = centroid distance, MPD = mean pairwise distance, NND = nearest-neighbor distance, ns = (criterion) not satisfied. Euclidean-distance values are given only when the fossil satisfied the criterion based on the corresponding distance measure (see Methods). Best supported assignments according to each criterion (highest value for DFA, lowest for Euclidean distances) are in bold.

	Ecomorph					
Criterion	CG	GB	TC	TG	Tr	Tw
DFA	0	0.034	0.002	0.213	**0.751**	0
CD	ns	0.201	ns	0.169	**0.042**	ns
MPD	ns	0.213	0.285	0.173	**0.095**	ns
NND	ns	0.089	0.117	0.073	**0.007**	ns

(see Maisano, 2001). Some poorly preserved bones of the right forelimb are also present. The retroarticular process of the lower jaw is highly fragmented but appears to have been well-developed; the presence and size of the angular process could not be determined.

The dorsal and lateral scales are small and granular. The ventral scales (impressions) are much larger (at least 4x larger than the dorsals by area); they are smooth and subimbricate (the posterior borders slightly overlap the scale or scales behind them) and are arranged in transverse rows (Fig. 1B). The supra-digital scales are smooth or perhaps faintly keeled. The scales on the dorsal surface of the manus (metacarpal region) are large and overlapping. The subdigital scales associated with the antepenultimate phalanx and the phalanges proximal to it, when present, are wide and lamellar, forming the ventral surface of pads on digits II–V. There are 19 subdigital lamellae on the underside of manual digit IV (counted using the method of Köhler, 2014). Indications of dark transverse markings are present on the upper and lower forelimb.

In the PCA of ecologically relevant morphological characters, the first two PCs explained 93.3 % of the variation (Table 1). The loadings of both of those PCs primarily involved pad characters, with lamella number loading most heavily on PC1 and pad width loading most heavily on PC2 (Table 1). When the specimens of ecomorph species and the fossil are plotted in the morphological space defined by the first two PCs (Fig. 2), the ecomorphs are reasonably well separated. That separation reveals, for example, that crown giant anoles have many lamellae while twig and grass-bush anoles have few lamellae, and that trunk-crown and twig anoles have wide pads while crown giant, trunk-ground, and grass-bush anoles have narrow ones (for their body sizes; see also Fig. S1). The fossil is positioned along the upper border of the space occupied by trunk anoles, very close to one of the trunk anole specimens (USNM 329155, a specimen of *A. distichus*), which is an area of the space not overlapped by any of the other ecomorphs.

The DFA of the same ecologically relevant characters (Table 2) classified the fossil as a trunk anole with the highest probability (P = 0.751) and a trunk-ground anole with the second highest probability (P = 0.213), with those two assignments accounting for > 0.96 of the probability. The remaining fraction was accounted for by the grass-bush (P = 0.034) and trunk-crown (P = 0.002) ecomorphs; assignment to the crown-giant and twig ecomorphs had zero probability.

Comparing the fossil to the modern ecomorph species under the three Euclidean-distance criteria (Table 2), the fossil satisfied the three Euclidean-distance criteria for

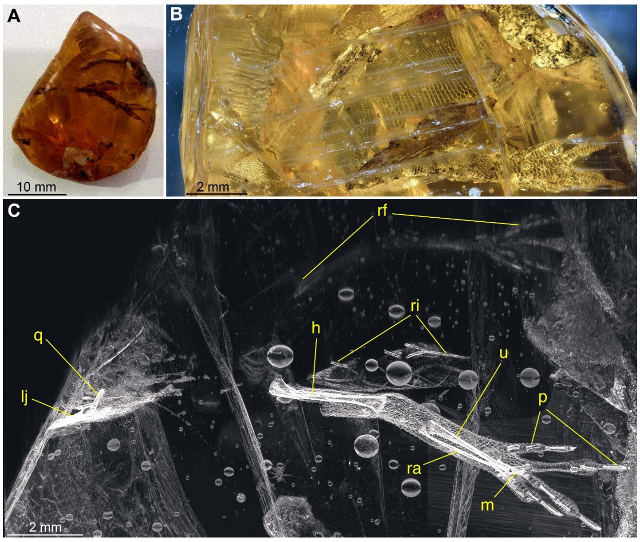

FIG. 1. SNHMB.G 8198, a fossil *Anolis* lizard preserved in Miocene amber from the Dominican Republic. (A) Photograph of the entire amber piece containing the fossil lizard. (B) Photograph of the fossil lizard in dorsal view showing the impressions of the ventral scales (center), the supinated left forelimb (lower right), and the lateral body skin (between the ventral scale impressions and the limb). (C) Radiographic image (composite z-projection) of the fossil lizard generated from the μCT data. Osseous elements include parts of the left quadrate (q) and lower jaw (lj), the left humerus (h), ulna (u) radius (ra), metacarpals (m), and phalanges (p), bones of the right forelimb (rf; faint), and parts of several left ribs (ri).

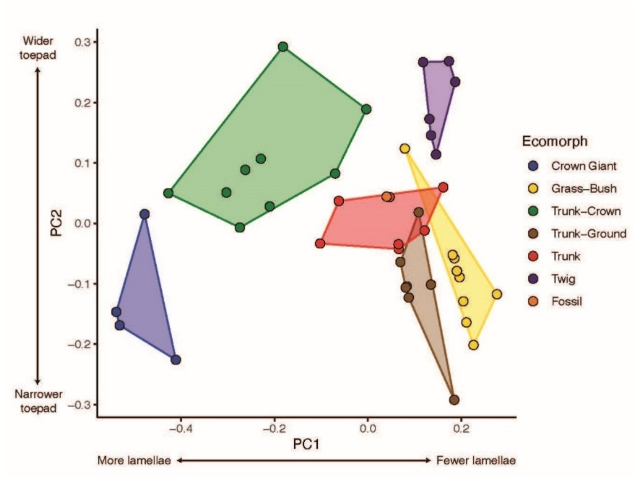

FIG. 2. Location of the amber fossil *Anolis* (SNHMB.G 8198) relative to representatives of modern ecomorph species in the morphological space defined by the first two principal components (PC) for the forelimb data. Modern specimens representing the different ecomorphs and the fossil are represented by color-filled circles as indicated in the key on the right, with the species assigned to the same ecomorph united within a minimum convex polygon shaded with a similar (but lighter) color. The orange-filled circle representing the fossil anole is positioned near the middle of the upper edge of the polygon for the trunk ecomorph, partially overlapping the circle representing one of the trunk-ecomorph members.

three to four of the six traditionally recognized ecomorphs, depending on the criterion. In all cases, the smallest Euclidean distances (indicating strongest support) were for the trunk ecomorph and the next smallest for the trunk-ground ecomorph followed by grass-bush and trunk-crown, except that the fossil did not satisfy the centroid-distance criterion for the trunk-crown ecomorph. The fossil did not satisfy any of the Euclidean-distance criteria for the crown-giant and twig ecomorphs.

Discussion

The amber fossil can be assigned unambiguously to the *Anolis* clade (sensu Poe et al., 2017) based on the morphology of the toepads. Although toepads have evolved multiple times in squamatan reptiles (Hagey et al., 2017; Miller & Stroud, 2021), the pads of anoles are distinctive in having the combination of a single row of lamellae and the claw separated from the pad by a nonlamellar segment associated with the penultimate phalanx (see figures in Griffing et al., 2022). In addition to the small size of the specimen and the fact that nearly all previously known fossil anoles preserved in amber appear to be juveniles (see measured and estimated SVLs in Sherratt et al., 2015: Table S2), the lack (or very small size) of ossified epiphyses indicates that the specimen is a juvenile (see Maisano, 2001).

Regarding its ecological morphology, the amber fossil is most likely a member of the trunk ecomorph category, as its DFA assignment probability for that category is more than three times greater than for any other ecomorph, and its Euclidean distances to the next closest ecomorph are 1.8 (MPD), 4.0 (CD), and 10.4 (NND) times greater than to the trunk ecomorph. Although there is some ambiguity in ecomorph assignment resulting from the small number of relevant traits that can be measured in the fossil, the only other ecomorph assignment that deserves further consideration is trunk-ground, as that is the only other assignment with more than a negligible DFA assignment probability (i.e., $P > 0.05$) and is the assignment that ranks second according to all three of the Euclidean-distance criteria.

Extant Hispaniolan trunk anoles are all members of the *Anolis distichus* series (Losos, 2009), which is part of the *Ctenonotus* subclade (sensu Poe et al., 2017), while the extant Hispaniolan trunk-ground anoles are all members of the *Anolis cybotes* series, which is equivalent to the *Audantia* subclade (sensu Poe et al., 2017). Members of these two clades differ in that those of the *distichus* series have smooth supra-digital scales while those of the *cybotes* series have keeled supradigitals (Cochran, 1941); moreover, based on our own observations, members of the *distichus* series have the ventral scales arranged in transverse rows (as do members of the *chlorocyanus* series of Hispaniolan trunk-crown anoles; see illustrations in Köhler & Hedges, 2016) while those of the *cybotes* series (= *Audantia*) have the ventrals arranged in oblique rows (see illustrations in Köhler et al., 2019). The fossil exhibits the conditions found in members of the *distichus* series for both characters.

Thus, assuming that the fossil is a member of one of the extant clades of Hispaniolan anoles, as most previously studied well-preserved Hispaniolan amber fossil anoles are inferred to be (Sherratt et al., 2015), its ecomorphological and taxonomic characters in combination support the inference that the fossil is a trunk anole of the *A. distichus* series. The age of the fossil (15–20 Ma) is within that of the *distichus* series, whose crown age is estimated to be 20.78 Ma and was not estimated using fossil calibrations within that clade (Poe et al., 2017). Our findings represent only the third case of a well-supported trunk ecomorph assignment for a fossil *Anolis* lizard (the other two were by Sherratt et al., 2015) and the first case of assignment to the *A. distichus* series (see Sherratt et al., 2015). They also highlight that such assignments can be inferred from only a small part of the body, in this case, primarily the forelimb, of a highly incomplete fossil.

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Supplementary Data

Supplementary data associated with this article can be found online alongside the manuscript.

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SUPPLEMENTARY MATERIALS

Figure S1

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Appendix 1

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Appendix 2

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