Taxonomy, Species Limits, and Phylogenetic Relationships of *Anoura* Gray 1838 (Chiroptera: Phyllostomidae)

By

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Para mis padres, todo su amor, apoyo y cariño me han llevado hasta donde estoy.

Para Cruz, Epifania, Justo y Mariano, sin saberlo, pusieron en mí la semilla del campo…

Para Hernán, seguirá cabalgando, con las manos firmes en las riendas y los pies firmes en los estribos…

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Abstract

This dissertation addresses several aspects of the diversity and species limits in the nectarivorous bat genus *Anoura* Gray 1838 (Chiroptera: Glossophaginae). *Anoura* has a history of taxonomic and nomenclatural changes. The first two species to be described, *Anoura caudifer* and *A. geoffroyi*, are also the species with the highest morphological variation (varying in terms of skull size and forearm length and overlapping with closely related species). These two species showed dental characteristics that separate them; these dental characteristics along with differences in body size would later be used to establish two species complexes within *Anoura*. Early in the 20th century and after the description of *A. caudifer* and *A. geoffroyi*, *A. aequatoris* was described based solely on two specimens. Later came the descriptions of *A. cultrata* and *A. latidens* in 1960 and 1984 respectively; these two species were at the moment the only two species that had discrete dental characters separating them from *A. caudifer* and *A. geoffroyi*, yet they shared a unique premolar dental morphology and larger body size, associating them to *A. geoffroyi.* By the end of the 20th century *A. luismanueli* was described from Venezuela; its smaller size resembles the small size of the specimen of *A. aequatoris*, but no comparison was made with this species or to *A. caudifer* from the central Andes. After *A. luismanueli* came the description of *A. fistulata*. This species has a unique soft tissue morphology enabling it to feed from plants with extremely long corollas, making it the third species with a discrete character aiding in its identification. The last two species of small-bodied *Anoura* to be described were *A. cadenai* and *A. javieri,* which have particular combination of skull and body size aiding in their diagnosis. The last described

species of large-bodied *Anoura* was *A. carishina*, described using only five specimens possessing dental characters identical to the diagnostic characters of *A. latidens.* The smaller species of the genus in terms of skull and general body size (*A. caudifer, A. aequatoris, A. cadenai, A. fistulata* and *A. luismanueli*) were included in the *A. caudifer* species complex given their premolar morphology, which led to grouping the large-bodied *A. geoffroyi, A. carishina* and *A. latidens* in the *A. geoffroyi* species complex. Studies based on classical and geometric morphometrics have determined that *Anoura* currently has 10 recognized species; these taxonomic revisions have not included all closely related species. The study of their phylogenetic relationships has focused on the position of *Anoura* within the Glossophaginae and on the broader understanding of the evolution of Noctilionoidea. However, if we want to understand the species limits within the genus, it is necessary to include both morphometric and genetic approaches. In Chapter 1, I investigate the identity of *Anoura carishina* and its position in the morphospace of the large-bodied *Anoura* using craniodental and external variables. I analyze traits thought to be diagnostic for these species, including 1) an elliptical Fourier transformation analysis of the shape of the third upper premolar (P4); 2) a comparison of the area of the second (P3) and third (P4) upper premolars; and 3) a comparison of maxillary toothrow angles. I find that *A. carishina* is morphologically indistinguishable from *A. latidens*, and that there is broad overlap in morphology between *A. latidens* and the *A. geoffroyi* species complex. Overall, results suggest that a stable taxonomy for the group should consider *A. carishina* as a junior synonym of *A. latidens,* and that, although *A. latidens* is distinguishable from *A. geoffroyi*, further genetic and taxonomic research is needed to clarify species limits within the *A. geoffroyi* species complex. In Chapter 2, I

study the species limits of *Anoura* from a statistical perspective, based on characters that were generally used to describe species in the genus. I examine the morphological species limits of *Anoura* using Gaussian Mixture Models in order to find groups among 581 individual specimens in the phenotypic space defined by 12 cranial and 11 external morphological characters. The morphometric analyses using Gaussian Mixture Models do not support a clear separation within either large-bodied or small-bodied *Anoura* species. I find that the morphospace generated by the shape of the P4 separates *A. geoffroyi* from *A. latidens*, with the type specimen of *A. carishina* nested well within the morphospace of *A. latidens*. However, both species shared part of the morphospace, which (in agreement with Chapter 1) provides further evidence that *A. carishina* should be treated as a junior synonym of *A. latidens*.This study also provides new localities for *A. latidens* in South America, expanding its range to Northern Bolivia. In Chapter 3, I address the phylogenetic relationships within *Anoura* by sequencing ultraconserved elements (UCEs) of the genome and inferring species trees under quartet-based methods and multispecies coalescent models. Phylogenetic analyses obtained four main well-supported clades supporting the monophyly of the small-bodied *Anoura* species, the monotypic status of *A. caudifer,* and the invalidation of *A. aequatoris* and *A. peruana* as independent species. Results also showed polyphyletic patterns indicating putative hybridization/introgression events. This dissertation presents a thorough taxonomic revision, providing a dichotomous key and the most complete phylogenetic hypothesis to date for *Anoura*. Morphometric analyses from Chapter 1 and 2 conclude that currently the diversity in *Anoura* is overestimated, with high morphological overlap within the large

and small-bodied *Anoura*, while molecular analyses corroborate the monophyly of the proposed large- and small-bodied species complexes within *Anoura*.

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CHAPTER 1.

Large *Anoura* (Chiroptera: Glossophaginae) taxonomy, taxonomic status of *Anoura carishina*, and implications for the distribution of *Anoura latidens* in Colombia.

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Abstract

The *Anoura geoffroyi* species complex is composed of three large-bodied species: *A. geoffroyi*, *A. peruana*, and *A. carishina.* Several inconsistencies arise from the description of *A. carishina,* and given the lack of comparison to the dentition and external characters of *A. latidens*, here we compare the taxonomic characters of these species. To understand the position of *A. carishina* in the morphospace occupied by large-bodied *Anoura*, we conducted a principal component analysis on 12 craniodental and 11 external morphological characters. One dataset (n = 202) includes only the 12 craniodental measurements; the second dataset (n = 125) includes all 23 craniodental and postcranial measurements. We complemented our results with further analysis of traits thought to be

diagnostic for these species, including 1) an elliptical Fourier transformation analysis of the shape of the third upper premolar (P4), 2) a comparison of the area of the second (P3) and third (P4) upper premolars, and 3) a comparison of maxillary toothrow angles. We found that *A. carishina* is morphologically indistinguishable from *A. latidens*, and that there is broad overlap in morphology between *A. latidens* and *A. geoffroyi*. However, several characters found in *A. latidens* are lacking in *A. geoffroyi*, including a triangular shape to the P4 caused by a medial-internal cusp enclosed by the base of the tooth, a lack of development of the anterobasal cusp in the P3, a smaller braincase, and a shorter rostrum. We reassessed the distribution of *Anoura latidens* in Colombia, adding new records and correcting previously published records that were misidentified. Overall, our results suggest that a stable taxonomy for the group should consider *A. carishina* as a junior synonym of *A. latidens,* and that, although *A. latidens* is distinguishable from *A. geoffroyi*, further molecular and taxonomic work is needed to clarify species limits within the *A. geoffroyi* species complex.

Key words: Chiroptera, Colombia, distribution, elliptical Fourier transformation, morphometry, nectarivorous bat, shape analysis

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Anoura is one of the most speciose genera in the phyllostomid subfamily Glossophaginae. It is comprised of 10 currently recognized species, although not all are widely accepted species (Tamsitt and Valdivieso 1966; Handley 1984; Mantilla-Meluk and Baker 2006; Griffiths and Gardner 2008; Jarrín-V and Kunz 2008; Mantilla-Meluk and Baker 2010; Pacheco et al. 2018). The genus is subdivided into two groups based on dental morphology and size (Allen 1898; Griffiths and Gardner 2008), with five small-bodied species (*A. caudifer*, *A. aequatoris*, *A. cadenai*, *A. fistulata and A. luismanueli*) and five large-bodied species (*A. carishina*, *A. cultrata*, *A. geoffroyi*, *A. peruana* and *A. latidens*). Mantilla-Meluk and Baker (2010) designated three of these large-bodied species (along with their subspecies) as the *A. geoffroyi* species complex, including *A. carishina*, *A. geoffroyi geoffroyi*, *A. geoffroyi lasiopyga* and *A. peruana,* elevating *A. peruana* to a separate species rather than a subspecies of *A. geoffroyi.* To date, the inferred phylogenetic relationships within the genus only include four species, *Anoura caudifer, A. cultrata, A. geoffroyi* and *A. latidens*; however, the relationships between *Anoura geoffroyi* and *A. latidens* are unclear, with *A. geoffroyi* and *A. latidens* being sister taxa (Dávalos et al. 2014; Rojas et al. 2016) or *A. latidens* being the sister clade to *A caudifer, A. cultrata* and *A. geoffroyi* (Carstens et al. 2002).

The most recently described *Anoura* species is *Anoura carishina* Mantilla-Meluk and Baker 2010, only known to date from the five specimens of the type series deposited at the Mammal Collection Alberto Cadena García at Instituto de Ciencias Naturales (Universidad Nacional, Bogotá, Colombia). Its known distribution is limited to 3 localities in the western slopes of the southern Colombian Andes and the Sierra Nevada de Santa Marta, a mountain system isolated from the Andes in the north of Colombia.

The type ICN-14530 and paratype ICN-14531 are from Taminango, Nariño department (1.67º, -77.32º). The two other localities are San Pedro de La Sierra, Sierra Nevada de Santa Marta, department of Magdalena (10.90º, -74.04º) for paratypes ICN-5224, 5225 and Cali, Pance, department of Valle del Cauca (3.32º, -76.63º) for paratype ICN-5938. *Anoura carishina* was described as a large *Anoura* with the following diagnostic characters: greatest length of skull less than 24.5 mm, small canines, P4 teeth with a wide triangular base, and complete zygomatic arches (although they are broken in several of the type series collections; (Mantilla-Meluk and Baker 2010)). However, in the description it was only explicitly compared to the subspecies of *Anoura geoffroyi* (*A. g. geoffroyi*, *A. g. lasiopyga*) and *A. peruana* - it was not compared to *A. latidens*, a species with which it bears resemblance in dental morphology, size, and coloration.

Anoura latidens Handley 1984 is described as a large species of *Anoura,* distinguishable from *A. geoffroyi* by a relatively short rostrum, an inflated braincase, nearly parallel maxillary toothrows, and smaller and more robust premolars, which have a quadrangular appearance when viewed from above. More specifically, Handley (1984) states that the third upper premolar (P4) has a medial-internal cusp enclosed in the triangular base of the tooth (rather than an abruptly protruding cusp as in *A. geoffroyi*) and that the second upper premolar (P3) possesses a reduced anterobasal cusp. The holotype is from Pico Ávila, Caracas, Venezuela, and the species has been reported from at least 14 localities in Venezuela (Handley 1976, 1984; Linares 1986, 1998), where it occupies a variety of ecosystems with an altitudinal range from 50 to 2600 meters above sea level. Outside of Venezuela *A. latidens* has only been registered in a handful of

localities in Colombia, Guyana, and Peru (Handley 1984; Linares 1998; Solari et al. 1999; Lim and Engstrom 2001), suggesting a wide yet discontinuous distribution.

In Colombia, *Anoura latidens* is distributed in the Andean region (eastern, central, and western mountain ranges) and the inter-Andean valleys (Alberico et al. 2000; Solari et al. 2013). The first record for Colombia was mentioned in the species description (Handley 1984) as collected by Nicéforo María in 1923 in San Juan de Rioseco, department of Cundinamarca, on the western slope of the Cordillera Oriental (eastern mountain range) above the inter-Andean valley of the Magdalena river at a height of 1000 meters above sea level. Later Muñoz (2001) attributed the first record to Wilson & Reeder (1993) and added a new locality in the Cordillera Oriental (eastern mountain range) in the municipality of Gramalote, Norte de Santander department, however they did not give a catalog number for this collection supposedly located in the Museo de Ciencias Naturales de La Salle. Two other localities are reported by Rivas-Pava et al. (2007) based on three specimens deposited at Museo de Historia Natural de la Universidad del Cauca (MHNUC) from the municipalities of Acevedo (Huila department) and Argelia (Cauca department). The most recent recorded locality was Reserva Forestal Bosque de Yotoco (Valle del Cauca department) in the southwestern Andes, with one specimen deposited in the Instituto de Ciencias Naturales (ICN) mammal collection (Mora-Beltrán and López-Arévalo 2018). With only 5 localities, little is known about the taxonomic and conservation status of the populations of *A. latidens* occurring in Colombia .

In this study we use morphometric approaches to reevaluate the taxonomy of the *A. geoffroyi* species complex. We focus particularly on the extent to which *A. carishina*

and *A. latidens* are distinguishable from each other and from other species in the complex. We also examine all known Colombian records of *A. latidens* to evaluate its distribution within the country.

MATERIALS AND METHODS

We measured 260 individuals from the *A. geoffroyi* species complex, including 5 *A. carishina,* 48 *A. peruana,* 59 *A. latidens*, and 148 *A. geoffroyi* (106 *A. g. geoffroyi* and 42 *A. g. lasiopyga*) (See Supplementary Data SD1 for specimens reviewed and measured). We measured 12 cranial and 11 postcranial characters to the nearest 0.01 mm. Craniodental characters included: greatest length of skull (GLS, distance from the most posterior point of the skull to the most anterior point of the premaxilla not including incisors), condylobasal length (CBL, distance from the most posterior point of the condyles to the most anterior point of the premaxilla not including incisors), postorbital breadth (PB, minimum interorbital distance measured across the frontals), braincase breadth (BCB, greatest breadth of the braincase, not including the mastoid and paraoccipital processes), height of braincase (HBC, distance from the ventral border of the foramen magnum to the parietal), mastoid breadth (MB, greatest width at the mastoid processes), maxillary tooth-row length (MTRL, distance from the most posterior point of the third upper molar to the most anterior point of the upper canine), palatal length (PL), breadth across third upper molars (M3-M3), breadth across upper canines (C-C), mandibular length (MANL, distance from the condyles to the anterior face of the mandible) and mandibular tooth-row length (MANTRL, distance from canine to the third mandibular molar). Postcranial measurements included: forearm (FA, measured from the olecranon to the articulation of the wrist), length of 3rd (D3MC), 4th (D4MC) and 5th

(D5MC) metacarpals, length of the 1st and 2nd phalanxes of 3rd (D3P1, D3P2), 4th (D4P1, D4P2) and 5th (D5P1, D5P2) digit, and length of the tibia (Tibia). Measurements were selected based on their frequent use in bat taxonomy (Handley 1960; Nagorsen and Tamsitt 1981; Handley 1984; Velazco 2005; Mantilla-Meluk and Baker 2006; Velazco and Patterson 2008; Mantilla-Meluk and Baker 2010; Velazco and Simmons 2011). Note that our measurement of the greatest length of the skull differs from that in the description of *Anoura carishina* (Mantilla-Meluk and Baker 2010). We measured the greatest length of the skull from the posterior-most point of the occipital to the anterior-most point in the premaxilla (excluding incisors), the same measurement used in all other *Anoura* descriptions (Handley 1960, 1984; Molinari 1994; Muchhala et al. 2005). In contrast, the description *A. carishina* and the comparison of this taxon to subspecies of *Anoura geoffroyi* (*A. g. geoffroyi*, *A. g. lasiopyga*) and *A. peruana* are based on measurements of the greatest length of the skull taken from the posterior-most point of the occipital to the anterior-most point of the nasal bones. To explore the morphometric variation of morphometric characters, we perform a principal component analysis (PCA) for 2 data sets. One dataset (n = 202) includes only the 12 craniodental measurements; the second dataset (n = 125) includes all 23 craniodental and postcranial measurements.

To test the reliability of dental characters distinguishing *A. latidens* and *A. carishina* from *A. geoffroyi,* we traced the contour of the premolars from digital photographs of the ventral view of the skull of 70 *A. latidens, 36 A. geoffroyi*, 7 *A. peruana* and *5 A. carishina.* We took each photograph next to a band of millimeter paper in order to standardize measurements. We selected the contour of the P3 and P4 using ImageJ (Schneider et al. 2012), and obtained the area of this contour using the "Measure"

function. To quantify the shape of the P4 (irrespective of size) we transformed every contour image of the P4 to a binary image in Image J (Schneider et al. 2012) and then employed an elliptical Fourier transformation on these images. Using SHAPE v1.3 (Iwata and Ukai 2002) this contour was transformed into chain code, assigning a string of code that represents the perimeter of every image of the third upper premolar, which was then used to create a harmonic or elliptical Fourier descriptor (EFDs) series. This approach allowed us to quantify the shape using 20 harmonics, which were used as input for a PCA.

Aside from tooth morphology, Handley (1984) argued that the arrangement of maxillary toothrows was important to distinguish *A. latidens* from *A. geoffroyi*. In particular, *A. latidens* would have nearly parallel maxillary toothrows while *A. geoffroyi* would have less paralleled toothrows. To quantify this character, we used ImageJ to overlay lines over images of the occlusal view of the maxillae for 5 *A. latidens,* 34 *A. geoffroyi*, 4 *A. peruana* and 66 *A. carishina.* Specifically, these lines connected the metastyle of the third upper molar (M3) to the most anterior point of the canines for each toothrow (See Supplementary Data SD 3, Fig. 3). We then measured the angle between these lines.

We tested for significant differences between *A. geoffroyi*, *A. latidens*, *A. peruana* and *A. carishina* in 1) craniodental measurements (including those related to rostrum length and an inflated braincase) 2) P4 and P3 size (i. g. total surface area), 3) the shape of P4 (EFD principal components) and 4) the toothrow angle using a multivariate analysis of variance (MANOVA) followed by Bonferroni-corrected posthoc tests to test for significant differences in the central tendency of morphometric variables between

species taxa following the methods previously used to describe and support the validity of species in *Anoura*.

To assess the geographical distribution of *A. latidens* we reviewed the published records and examined the skulls of specimens labeled as *A. geoffroyi* and *A. caudifer* in the following collections: Colección de Mamíferos Alberto Cadena García at Instituto de Ciencias Naturales de la Universidad Nacional de Colombia (ICN), Instituto de Investigación en Recursos Biológicos Alexander von Humboldt (IAvH), Museo Universidad Distrital Francisco José de Caldas (MHNUD), Museo de Historia Natural de la Universidad del Cauca (MHNUC), Colección Teriológica Universidad de Antioquia (CTUA), National Museum of Natural History (USNM), Muséum d'Histoire Naturelle de la Ville de Genève (MHNG), American Museum of Natural History (AMNH), and Field Museum of Natural History (FMNH).

RESULTS

Morphological revision. — We found that the type series of *Anoura carishina* is a mixed series composed of four specimens diagnosable as *A. latidens* and one specimen diagnosable as *A. geoffroyi.* The type specimen of *A. carishina* (ICN 14530) shows the dental characters provided in the description of *A. latidens* (Handley 1984). The type specimen ICN 14530 has molars and premolars with the anterobasal cusp of the second upper premolar (P3) reduced and the medial-internal cusp of the third upper premolar (P4) enclosed in a triangular base. When comparing the type of *A. latidens* to the type series of *A. carishina* we found that specimens ICN 14530,14531, 5224 and 5225 possess both characteristics, while specimen ICN 5839 possessed neither and is instead

diagnosable as *A. geoffroyi* (Fig. 1). In our review of the type material, we also discovered that the specimen labeled as the holotype of *A. carishina* in Figure 4 of Mantilla-Meluk and Baker (2010) is in fact ICN-5225, while the specimen labeled as ICN-5225 is actually the type (ICN-5225 is a female paratype that possessed both auditory bullae, while ICN 14530 is a male specimen lacks the right auditory bulla; see Supplementary Data SD 3, Supplementary Fig. 1).

In our review of previously-published records of *Anoura latidens* in Colombia, we found that only 2 are valid, including specimen AMNH-69187 used in the species description (Handley 1984) and ICN 22807 from Reserva Forestal Bosque de Yotoco, municipality of Yotoco, department of Valle del Cauca (Mora-Beltrán and López-Arévalo 2018). The *A. latidens* specimens reported by Rivas-Pava et al. (2007) from the municipalities of Acevedo (department of Huila; MHNUC-M0722, 0723) and Argelia (department of Cauca; MHNUC-M1552) actually correspond to individuals of *A. geoffroyi*, while there is no record of the *A. latidens* specimen reported by Muñoz (2001) in the mammal collection of Colegio San Jose de la Salle. The only two records of Glossophagine bats from the locality of Gramalote (Norte de Santander, Colombia) in the mammal collection of Colegio San Jose de la Salle (specimens CSJ-m 168 and 169) that could be putative records of *A. latidens* were are diagnosable as *Glossophaga soricina.*

On the other hand, among all of the collections we reviewed, we found a total of three *Anoura latidens* specimens that were misidentified as other *Anoura* species. Specimens ICN 4398, ICN 11195, and MHNUD 587 coincide with the dental characters of *A. latidens* proposed by Handley (1984). ICN 4398 is an adult male, preserved as a skin and extracted skull. This record is located in the inter-Andean valley of the Cauca

River, between the Cordillera Central and Cordillera Occidental (central and western mountain ranges). ICN 11195 is an adult male, preserved as a skin and extracted skull. It was collected in Parque Regional Natural Ucumarí, Vereda la Suiza, city of Pereira, department of Risaralda. This locality is situated in the protected area Santuario de Fauna y Flora Otún Quimbaya and resides in the western slope of the Cordillera Central (central mountain range) at an elevation of 1900 meters. MNHUD 587 is an adult male, preserved as a skin and extracted skull. It was collected in Vereda La Huerta, municipality of La Vega, department of Cundinamarca on the western slope of the Cordillera Oriental (eastern Andes) at an elevation of 980 meters (see Supplementary Data SD1).

Morphometric analyses. — The type series of *A. carishina* overlaps in the morphospace of both *A. latidens* and the *A. geoffroyi* species complex (*A. g. geoffroyi*, *A. g. lasiopyga* and *A. peruana*) in most of its measurements (Fig. 2, Supplementary Data SD2). For the dataset with all measurements (Fig. 2. A), our principal component analysis shows that less than 50% of the variation is explained by the first two principal components of the PCA (PC1 33.24%, PC2 10.68%). We recovered similar results when only craniodental measurements (Fig. 2. B) were taken into account (PC1 40.01 %, PC2 17.19%) (see Supplementary Data 3, Supplementary Fig. 1 for the distribution of *A. g. geoffroyi*, *A. g. lasiopyga* and *A. peruana* in the morphospace).

A separate multivariate analysis of variance on the centroids of PC1 showed no significant differences (Bonferroni corrected *P* value = 1.0) between *Anoura latidens* (PC1 $\bar{X}$=-0.0732) and *A. carishina* (PC1 $\bar{X}$=-0.0886) with the *A. geoffroyi* species complex being significantly different from *A. latidens* and putative *A. carishina*

(*P*=0.001, $\bar{X}$=-0.0732). While PC2 showed no significant differences (*P*=0.120) between *A. latidens* (PC2 $\bar{X}$=0.007) *A. carishina* (PC2 $\bar{X}$=0.0591), only *A. carishina* was significantly different from *A. geoffroyi* (*P*=0.028, PC2 $\bar{X}$=-0.044).

The upper last premolar (P4) shape variation was explained by the first two principal components of 20 EFDs (PC1 71.83% and PC2 13.07 (Fig. 3). We see that the type specimen of *Anoura carishina* (ICN 14530) is in the center of the morphospace occupied by *A. latidens*, with the position of the *A carishina* paratype diagnosable as *A. g. geoffroyi* (ICN 5938) closer to the morphospace of *A. g. geoffroyi*. Despite evidencing different morphological clusters corresponding to *A. g. geoffroyi* (with *A. peruana* immersed in its morphospace) and *A. latidens*, the morphospace of the shape of P4 does not show a clear separation between them, with some specimens of *A. g. geoffroyi, A. peruana* and *A. latidens* occupying the space between clusters (Fig. 3).

The multivariate analysis of variance (MANOVA) on morphometric measurements showed overall significant differences for each measurement (Pillai's Trace and Wilks' Lamda *P*<0.001); however, differences in postorbital breadth (PB; $F_{3,121}$=1.023, *P*=0.385) and forearm length (FA; $F_{3,121}$=0.223, *P*=0.881) were not significant across all species taxa comparisons (Table 2). Bonferroni corrected *P* values show significant differences between *A. latidens* and *A. carishina* only in height of braincase (HBC; *P*=0.030), while *A. g. geoffroyi* and *A. latidens* have significant differences in the means of all variables, with the exception of postorbital breadth (PB; *P*=1.0), height of braincase (HBC; *P*=0.166), and forearm length (FA; *P*=1.0). Of particular relevance are significant differences in measurements related to the overall shorter rostrum and less inflated braincase of *A. latidens*, as these features were

highlighted by Handley (1984) in the description of this taxon. Specifically, *A. latidens* has a shorter greatest length of skull (GLS), palate length (PL), maxillary toothrow length (MTRL), braincase breadth (BCB) and mastoid breadth (MB) in comparison to *A. geoffroyi* and *A. peruana* (see Table 2, SD2). Between these latter two taxa, *Anoura peruana* only showed significant differences with *A. geoffroyi* in height of braincase (HBC; P=0.043). Our results point to a lack of statistical evidence and significance when contrasting *A. latidens* and *A. carishina*.

Our MANOVA on premolar shape and toothrow angle (Table 3) showed significant differences between species taxa in the area of P4 ($F_{3,105,}$= 14.878, P<0.001), PC1 of P4 shape (EFDs; $F_{3,105}$=103.508, P<0.001) and toothrow angles (TRA, $F_{3,105}$=3.157, P=0.028). Bonferroni-corrected posthoc tests show that *A. latidens* has a larger P4 area ($\bar{X}$= 0.69 mm^2) than *A. carishina* ($\bar{X}$= 0.61 mm^2, P=0.049), *A. g. geoffroyi* ($\bar{X}$= 0.61 mm^{2,} P<0.001), and *A. peruana* ($\bar{X}$= 0.56 mm^2, P=0.002). The first principal component of the P4 shape showed significant differences between *A. g. geoffroyi* and both *A. carishina* and *A. latidens,* and between *A. peruana* and *A. latidens (P*<0.001), while *A. peruana* was not different from *A. g. geoffroyi* (P=0.112) or *A. carishina* (P=0.079). Notably, *A. carishina* is not significantly different from *A. latidens* for any of these traits except P4 area, and the four specimens of *A. carishina* diagnosable as *A. latidens* fall completely within the range of *A. latidens* variation in P4 area (Fig. 3). Even though toothrow angle was significantly different overall (Table 3, TRA, $F_{3,105}$=3.157, P=0.028) only a Fisher's least significant difference posthoc test showed differences between *A. g. geoffroyi* and *A. latidens* (P=0.011).

DISCUSSION

Upon revision of the type material of *Anoura carishina* and *A. latidens* we found that the type series of *A. carishina* is a mixed group of four specimens corresponding to *A. latidens* and one to *A. g. geoffroyi.* Our analyses of craniodental measurements and premolar shape of individuals of all species and subspecies in the *Anoura geoffroyi* complex (*A. geoffroyi*, *A. latidens*, *A. carishina*, and *A. peruana*) find no support for *Anoura carishina* as an entity morphologically distinct from *A. latidens*. Our results also clarify the characters that distinguish *A. latidens* from *A. geoffroyi* (shorter rostrum, less inflated braincase, less parallel toothrows, the triangular base of the last upper premolar and reduced anterobasal cusp of the second upper premolar) expand the known distribution of *A. latidens* in Colombia, and raise issues regarding the conservation status of this species in the country.

Taxonomic status of A. carishina— Different lines of evidence lead us to formally treat *Anoura carishina* as a junior synonym of *A. latidens.* First, the triangular base of the third upper premolar P4 of the type specimen of *A. carishina* (ICN 14530) and 3 paratypes is indistinguishable from *A. latidens*, as demonstrated by our analyses of tooth shape (Fig. 3). Second, we found that all four of these specimens lack a developed anterobasal cusp in the second upper premolar (P3). And finally, none of the 18 morphological measurements differ between *A. latidens* and the *A. carishina* specimens (Table 2 and 3) with the exception of height of the brain case (HBC; P=0.030) and P4 area (P=0.049), and in both of these cases there is still extensive overlap in the range of measurements (HBC: 7.14-8.07 mm for *A. latidens* vs. 7.72-8.30 mm for *A. carishina*; P4 area: 0.56-

0.86 mm^2 for *A. latidens* vs. 0.50-0.70 mm^2 for *A. carishina*). In light of the lack of statistical evidence supporting the morphological diagnosis of *A. carishina*, the holotype and three of the paratypes are diagnosable as individuals of *A. latidens*. The fourth paratype (ICN 5938) presents a developed anterobasal cusp in the second upper premolar (P3) and lacks a medial internal cusp enclosed in the base of the third upper premolar (P4), supporting its diagnosis as *A. geoffroyi*.

Diagnosis of A. latidens and A. geoffroyi— Our morphometric analysis of craniodental measurements shows that the morphospace of *A. latidens* partially overlaps with that of *A. g. geoffroyi* and *A. peruana*. Of the traits mentioned by Handley (1984) to diagnose *A. latidens* from *A. geoffroyi,* we found several to be diagnostic characters and useful in separating *A. latidens* from the *A. geoffroyi* species complex. These characters include a more robust and more triangular third upper premolar (P4; see Fig. 3), a reduced anterobasal cusp of second upper premolar (P3), and a shorter rostrum (in terms of GLS, PL, MANL; Table 2, Supplementary Data SD2). We add to this list mastoid breadth (MB) and mandibular tooth row length (MANTRL), which are also smaller for *A. latidens* (Table 2, Supplementary Data SD2). Toothrow angle, which Handley (1984) suggested is more parallel in *A. latidens* showed significant differences after a Fisher's least significant difference posthoc test (P=0.011). Contrary to Handley (1984) we found that *A. g. geoffroyi* has more parallel toothrows (TRA $\bar{X}$=13.39º) than *A. latidens* (TRA $\bar{X}$=14.01º). Finally, although Handley (1984) suggested that *A. latidens* has a more inflated braincase, we found that its braincase (BCB, Table 2, Supplementary Data SD2) is in fact significantly less inflated than *A. geoffroyi* and *A. peruana*.

Distribution and implications for the conservation of Anoura latidens in Colombia — By combining the 2 valid previously-published records of *Anoura latidens* in Colombia (Handley 1984; Mora-Beltrán and López-Arévalo 2018) with the 7 records we found here, we report *A. latidens* in 7 localities across the country (Fig. 4, Supplementary Data SD1). With the exception of the Sierra Nevada de Santa Marta, all localities fall within highly altered ecosystems (IAvH 2004). Vereda El Hormiguero (ICN 4398) is located in a sugar cane agricultural system, even at the time of the capture of the specimen (Arata et al. 1967). San Juan de Rioseco (AMNH 69187) and Vereda La Huerta (MHNUD 587) are mountainous areas with a landscape composed of ranching pastures, small agricultural fields, and fragments of natural forests. Vereda La Suiza (ICN 11195) presents a heterogeneous forest cover composed of fragments of natural forests, secondary forests, and reforested areas; it is part of the Santuario de Fauna y Flora Otún Quimbaya, registered in the Colombian National System of Protected Areas (SINAP) (Estrada-Villegas et al. 2010). Reserva Forestal Bosque de Yotoco (ICN 22807) is a protected reserve in the Valle del Cauca department on the eastern slopes of the Western Cordillera. All records are located in the Andean region and the Sierra Nevada de Santa Marta between 590 and 1690 m a.s.l. (Fig. 4, Supplementary Data SD1). In Venezuela, *A. latidens* has a similar elevational distribution, with records from 50 to 2240 meters above sea level and the majority (81%) located between 1000-1500 m a.s.l. (Handley 1984; Linares 1986; Soriano et al. 2002).

Assessing the conservation status of *Anoura latidens* in Colombia under the conventional parameters (variation in population size, size of distribution range and habitat loss) becomes a challenge given its discontinuous distribution. The distribution of

A. latidens is immersed in highly transformed environments and not associated with natural vegetation cover. Local abundances are also unknown, but its limited presence in Colombian mammal collections suggests a pattern of low abundance in the Colombian Andes. Adding to this issue, *A. latidens* is sympatric to *A. geoffroyi*, and only craniodental features are useful for its diagnosis, it is likely that they are misidentified during fieldwork, as suggested by the fact that all new records for Colombia were previously identified as *A. geoffroyi*. In summary, *Anoura latidens* is a species with a relative broad distribution from Venezuela to the Central Andes of Peru, and unknown population numbers inhabiting highly disturbed ecosystems. It is crucial to coordinate strategies with the different bat conservation programs in South America to encourage research and conservation on this species leading to effective strategies.

This study provides evidence that *A. carishina* should be treated as a junior synonym of *A. latidens*, given extensive overlap in morphology, including key traits such as 1) shape of the upper third premolar (P4), 2) craniodental measurements and 3) the presence of the anterobasal cusp in the second upper premolar (P3). We found support for several characters suggested by Handley (1984) to distinguish *A. latidens* from *A. geoffroyi*, including a shorter rostrum, more robust premolars, and triangular shape to P4 (with medial-internal cusp being enclosed by the base of the tooth), while we detected no differences in toothrow angle. Finally, contrary to Handley (1984), we find that the braincase of *A. latidens* is in fact significantly less inflated than that of *A. geoffroyi*. Given the high morphological overlap between *A. geoffroyi* subspecies and *A. peruana*, we recommend further taxonomic work combining both morphological and molecular approaches to better understand the species limits of this species complex.

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SUPPLEMENTARY DATA

Supplementary Data SD 1—Database of specimens examined and their geographical information including localities and geographical coordinates. Specimens revised and

identified but not measured are indicated with an asterisk (*)

Supplementary Data SD 2—Summary measurements of *A. carishina, A. g. geoffroyi*, *A. g. lasiopyga*, *A. peruana* and *A. latidens.*

Supplementary Data SD 3— Supplementary Figure 1 Type Series of *A. carishina*, A) Type specimen ICN 14530, B) ICN 14531, C) ICN 5224, D) ICN 5225 E) ICN 5398. Supplementary Figure 2. PCA analyses between the different species/subspecies of the *A. geoffroyi* species complex, Top) using 12 craniodental and 11 postcranial measurements Bottom) using only the 12 craniodental measurements. Supplementary Figure 3. Depiction of toothrow angle measurement.

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FIGURES

Fig. 1. Skull morphology of A) *A. latidens* type AMNH 370119, B) *A. carishina* type ICN 14530 and C) *A. carishina* paratype ICN 5938. Note the robust molars and premolars in the first two, in contrast to the slender premolars of the *A. carishina* paratype ICN 5938.

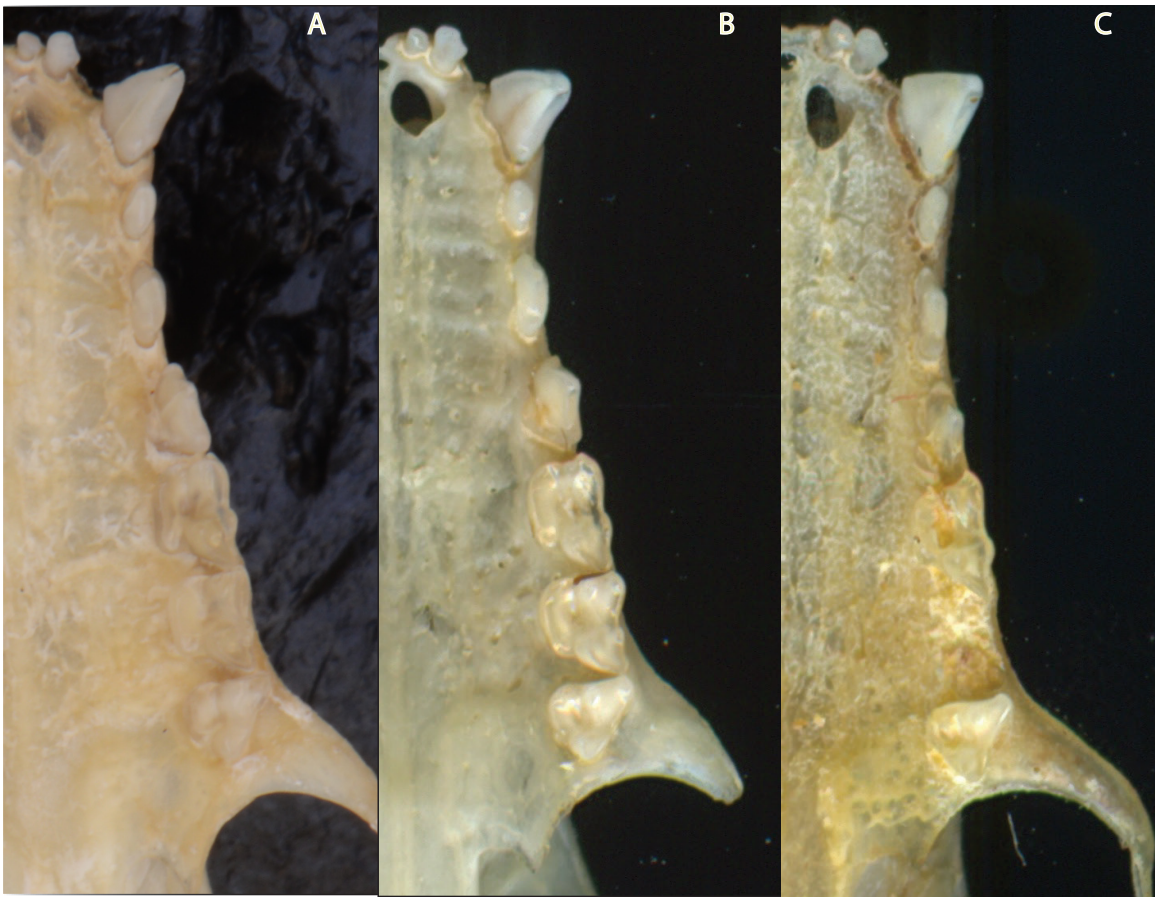


Fig. 2. A) PCA analyses using 12 craniodental and 11 postcranial measurements of *Anoura* specimens. B) PCA analyses using only the 12 craniodental measurements of *Anoura carishina*, *A. latidens* and *A. geoffroyi* species complex specimens.

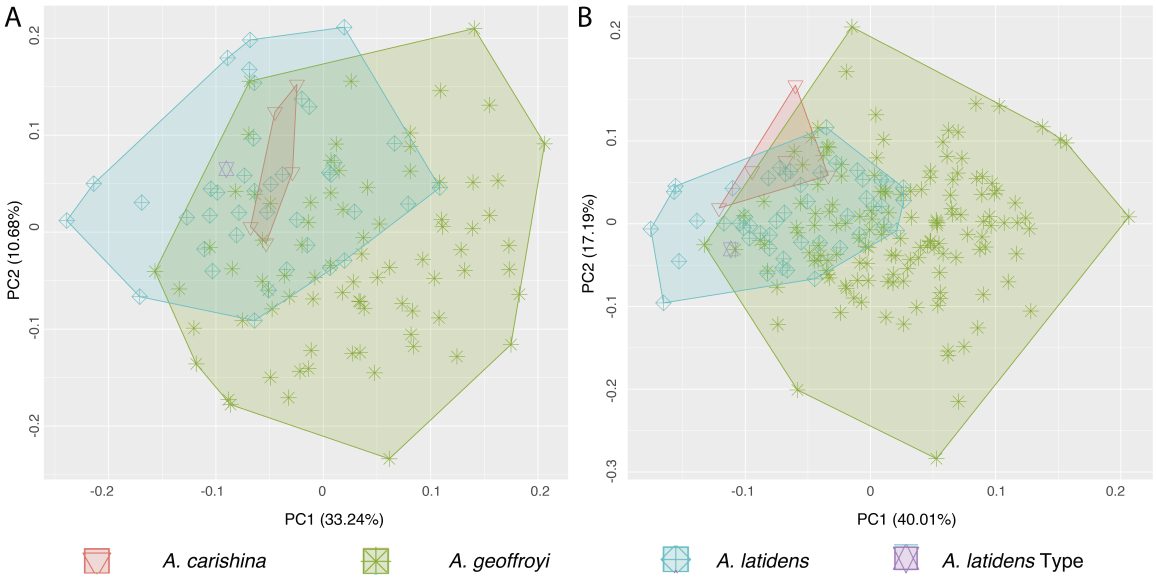


Fig. 3. A) Mean (long-dashed lines), -2SD (short-dashed lines), and + 2SD (solid lines) contour shapes of the third premolar (P4) in our sample (with all three super-imposed to the left), showing the variation explained by each of the elliptical Fourier descriptor (EFD) principal components. B) Scatterplot of EFD PC1 vs. P4 area. Note that the *Anoura carishina* type specimen (ICN 14530) is nested well within the morphospace of *A. latidens.*

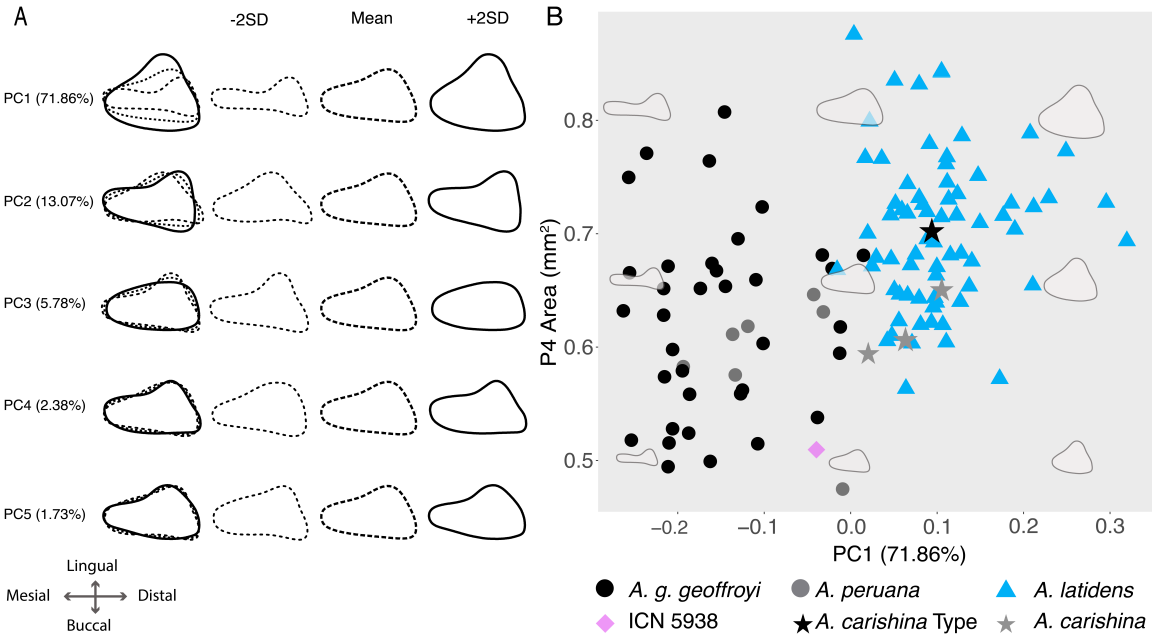
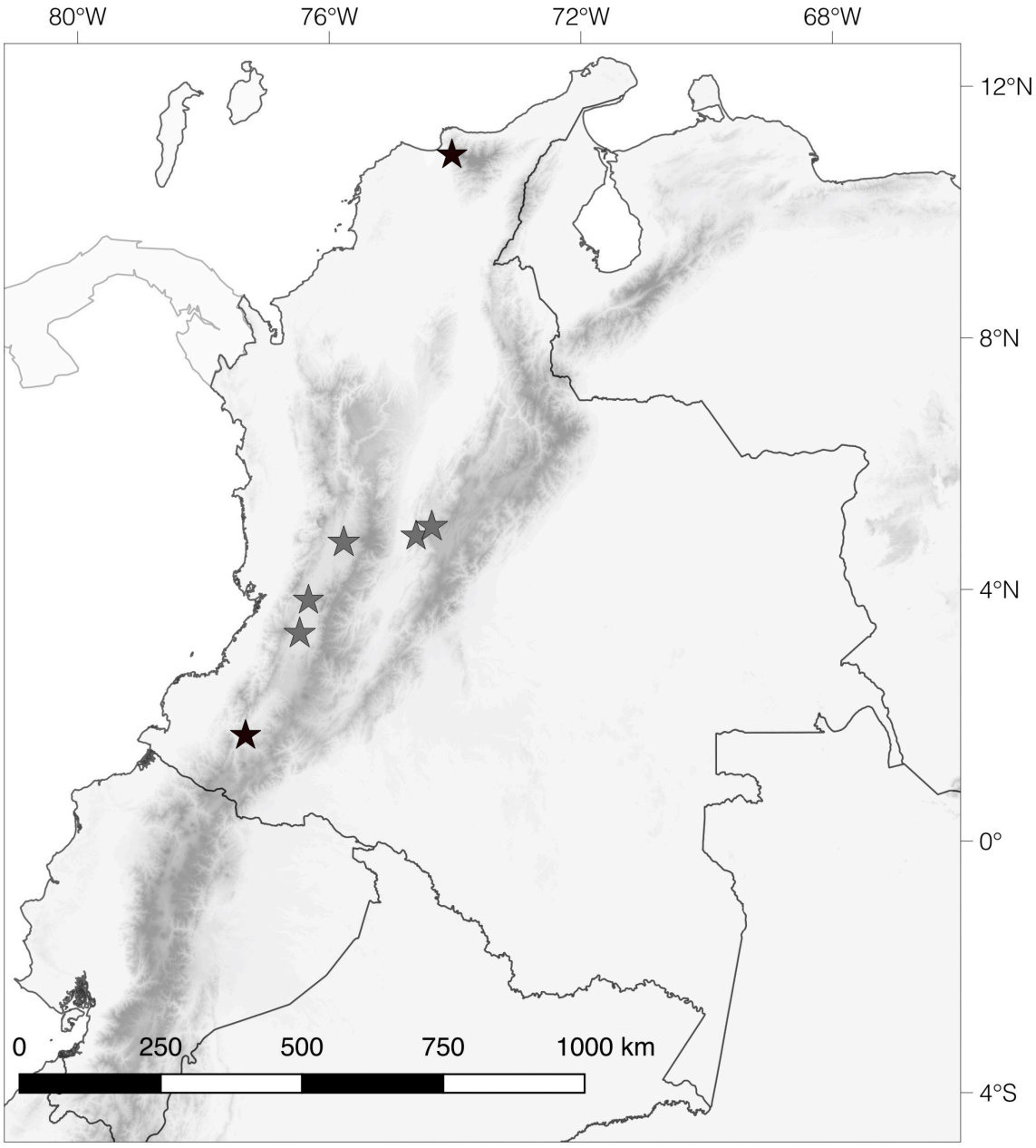


Fig. 4. Distribution of *Anoura latidens* in Colombia. Black stars show localities of specimens previously attributed to *A. carishina*, while grey stars show all other records.



Tables

Table 1. Measurements (mm) of the type specimen of *Anoura latidens*, and the type series of *A. carishina*, see methods for measurement abbreviations.

	A. latidens Type USNM 370119	*A. carishina* Type ICN 14530	*A. carishina* ICN 5224	*A. carishina* ICN 5225	*A. carishina* ICN 14531	*A. carishina* ICN 5938
GLS	24.05	24.08	24.44	24.05	23.90	24.12
CBL	23.27	23.35	23.65	23.53	23.45	23.52
ZW	10.66	10.95	9.93	9.97	10.59	10.70
PB	4.81	5.24	4.91	4.86	5.19	5.15
BCB	9.50	10.03	9.81	9.35	9.82	9.88
MB	9.99	10.11	9.75	10.02	10.17	10.22
MTRL	9.06	9.09	9.32	9.18	9.01	9.28
PL	13.44	12.27	12.52	12.71	12.87	13.11
PPL	8.79	9.57	9.01	9.40	9.17	8.71
M3-M3	5.94	6.31	6.22	5.91	6.09	6.06
C-C	4.09	4.46	4.39	4.06	4.16	4.52
CW	6.08	6.23	5.89	5.90	5.73	6.26
HBC	7.54	8.30	8.04	7.91	7.83	7.72
MANL	16.89	17.15	17.46	17.00	17.27	17.36
MANTRL	9.35	9.71	9.48	9.48	9.39	9.63
MH	4.44	4.67	5.06	4.57	4.45	4.69
FA	42.69	43.09	44.15	43.79	41.14	41.07
D3MC	39.53	39.32	39.24	39.86	38.22	39.11
D3P1	13.21	13.69	13.48	13.00	13.47	12.81
D3P2	21.18	20.42	20.50	21.18	21.01	20.47
D4MC	37.88	37.09	38.97	38.37	36.43	37.73
D4P1	9.73	9.64	10.20	10.07	10.26	9.97
D4P2	13.32	14.24	13.65	15.03	14.11	14.08
D5MC	33.57	32.64	33.56	33.07	30.89	32.62
D5P1	7.81	8.20	8.20	8.00	8.68	8.06
D5P2	11.92	11.62	12.65	13.22	12.34	12.61
Tibia	14.97	13.64	15.05	15.40	14.73	14.34

Table 2. MANOVA *F* values and *P*-values for Bonferroni-corrected posthoc tests of morphometric variables between *Anoura peruana* (*n*=5), *A. carishina* (*n*=5), *A. geoffroyi* (*n*=75) and *A. latidens* (*n*=40), with significant *P*-values in bold. See methods for measurement abbreviations.

Variable	MANOVA *F*	MANOVA *P*	*A. latidens -. A. carishina*	*A. geoffroyi -. A. carishina*	*A.peruana -. A. carishina*	*A. geoffroyi - A. latidens*	*A. peruana - A. latidens*	*A. geoffroyi - A.peruana*
GLS	**33.013**	**0.000**	1.000	**0.001**	**0.001**	**0.000**	**0.000**	1.000
CBL	**25.771**	**0.000**	1.000	**0.001**	**0.006**	**0.000**	**0.001**	1.000
PB	**1.023**	0.385	1.000	1.000	0.867	1.000	1.000	0.607
BCB	**5.587**	**0.001**	1.000	1.000	1.000	**0.001**	1.000	0.354
HBC	**5.625**	**0.001**	**0.030**	0.295	**0.005**	0.166	0.500	**0.043**
MB	**9.297**	**0.000**	1.000	**0.047**	1.000	**0.000**	1.000	0.255
PL	**21.262**	**0.000**	1.000	**0.001**	**0.001**	**0.000**	**0.000**	0.787
MTRL	**9.982**	**0.000**	1.000	0.087	0.120	**0.000**	**0.003**	0.415
M3.M3	**3.094**	**0.030**	1.000	1.000	1.000	**0.021**	0.902	1.000
C.C	**17.085**	**0.000**	1.000	0.058	1.000	**0.001**	1.000	0.387
MANL	**5.034**	**0.003**	1.000	0.515	0.211	**0.009**	0.850	1.000
MANTRL	**14.744**	**0.000**	1.000	**0.012**	**0.002**	**0.000**	**0.000**	0.417
FA	**0.223**	0.881	1.000	1.000	1.000	1.000	1.000	1.000

Table 3. MANOVA *F* and *P*-values for Bonferroni-corrected posthoc tests of P3 and P4 area, toothrow angles (TRA) and Principal components 1 and 2 of P4 shape between *Anoura peruana* (*n*=4), *A. carishina* (*n*=5)*, A. g. geoffroyi* (*n*=34) and *A. latidens* (*n*=66), with significant *P*-values in bold. See methods for measurement abbreviations.

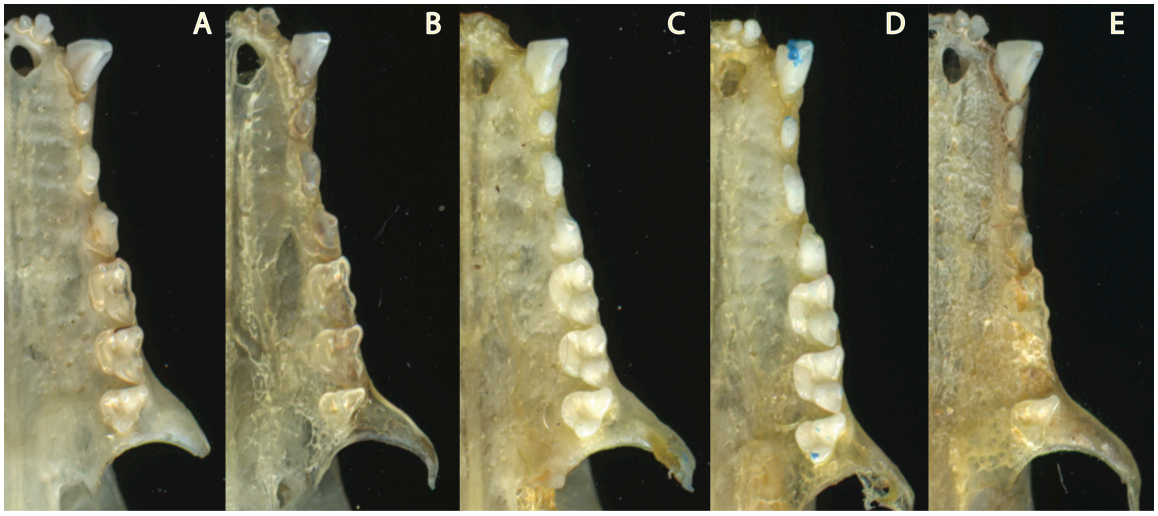
Variable	**MANOVA *F***	**MANOVA *P***	***A. latidens - A .carishina***	***A. g. geoffroyi - A. carishina***	***A.peruana - A. carishina***	***A. g. geoffroyi - A. latidens***	***A. peruana - A. latidens***	***A. g. geoffroyi - A. peruana***
P3 area	0.952	0.418	1.000	1.000	1.000	0.641	1.000	1.000
P4 area	14.878	**0.000**	**0.049**	1.000	1.000	**0.000**	**0.002**	**1.000**
P4 Shape PC1	103.508	**0.000**	0.678	**0.000**	0.079	**0.000**	**0.000**	0.122
P4 Shape PC2	0.340	0.797	1.000	1.000	1.000	1.000	1.000	1.000
TRA	3.157	**0.028**	1.000	1.000	1.000	0.066	0.407	1.000

SUPPLEMENTARY DATA

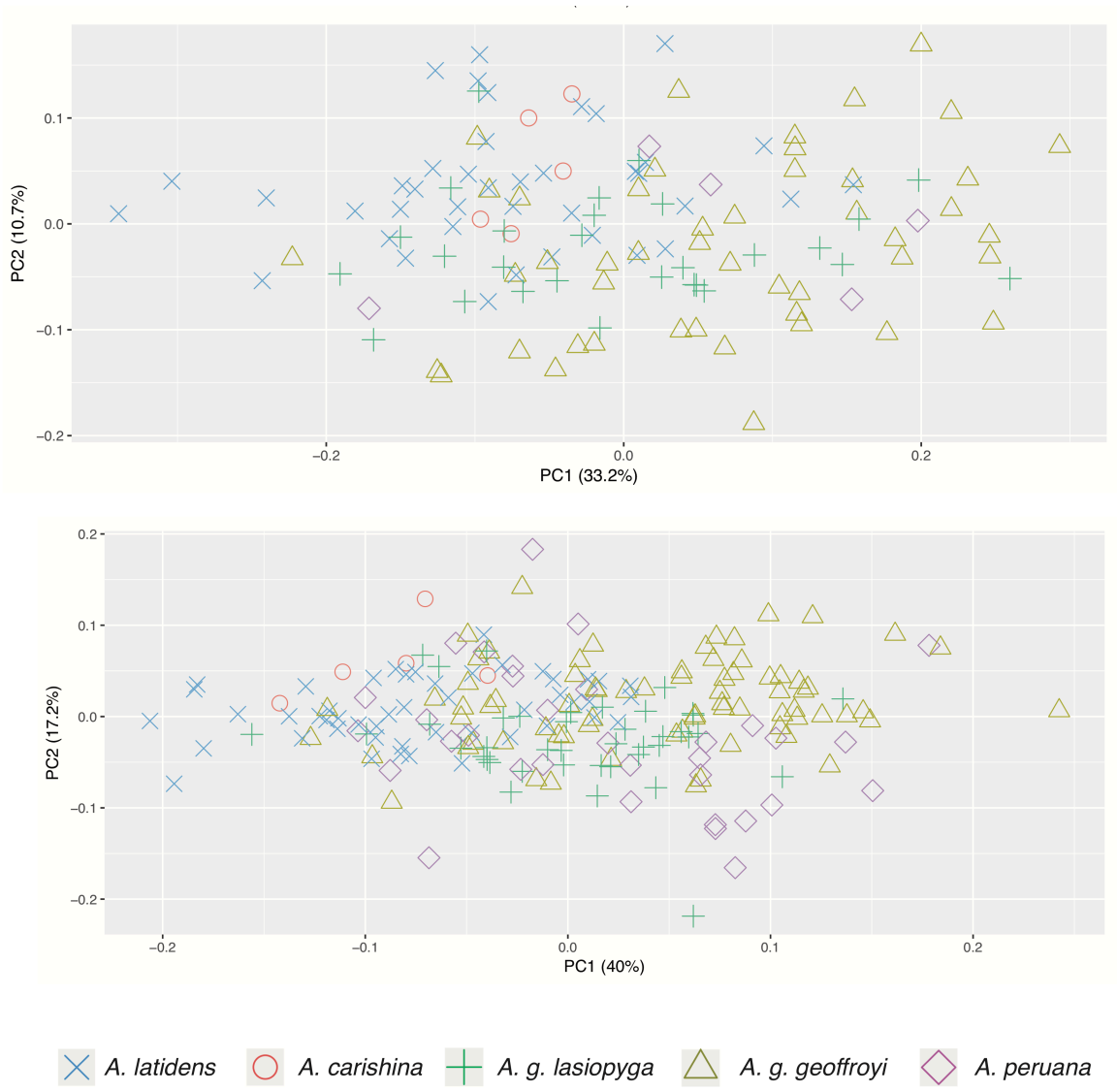
Supplementary Data SD 1—Database of specimens examined and their geographical information including localities and geographical coordinates. Specimens revised and identified but not measured are indicated with an asterisk (*)

Supplementary Data SD 2—Summary measurements of *Anoura carishina, A. g. geoffroyi*, *A. g. lasiopyga*, *A. peruana* and *A. latidens.*

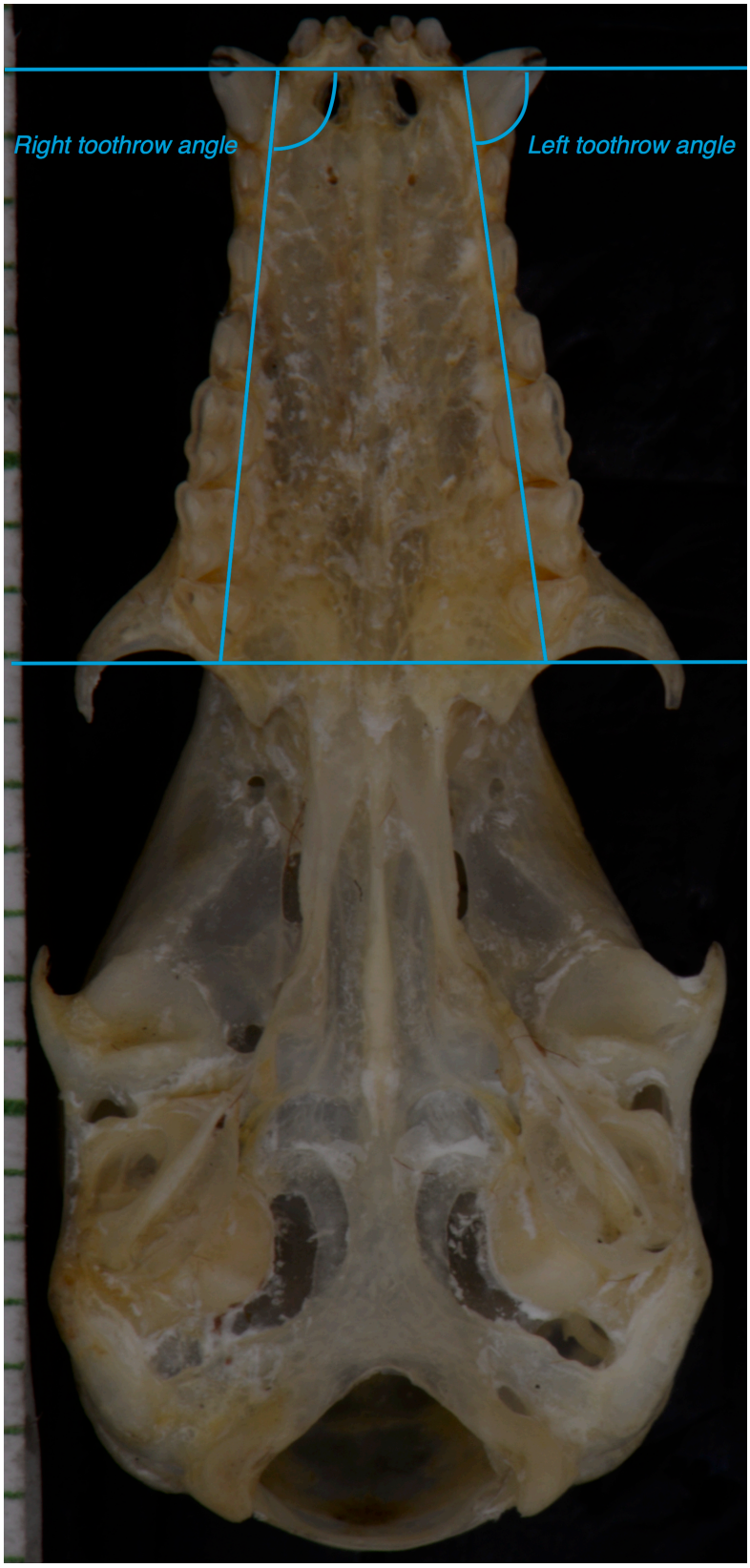
Supplementary Data SD 3— Supplementary Figure 1 Type Series of *Anoura carishina*, A) Type specimen ICN 14530, B) ICN 14531, C) ICN 5224, D) ICN 5225 E) ICN 5398.



Supplementary Figure 2. PCA analyses discriminating between the different species/subspecies of the *Anoura geoffroyi* species complex, Top) using 12 craniodental and 11 postcranial measurements Bottom) using only the 12 craniodental measurements.



Supplementary Figure 3. Depiction of toothrow angle measurement.



CHAPTER 2.

Testing species limits of *Anoura* Gray 1838 (Phyllostomidae: Glossophaginae) using morphology and Gaussian Mixture Models

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Abstract

Anoura is one of the most diverse genera in the nectarivorous bat subfamily Glossophaginae; however, species limits and phylogenetic relationships within the genus remain uncertain. Recently, small- and large-bodied species of *Anoura* were separated in the *A. caudifer* species complex (small-bodied) and the *A. geoffroyi* species complex (large-bodied) based on morphological characters. We present a morphological study of all currently accepted species taxa, including the type series of *A. cadenai, A. carishina* and *A. latidens,* as well as specimens previously used to elevate *A. g. peruana and A. c. aequatoris* to species level. Our data includes 12 cranial and 11 external morphological postcranial characters for 581 *Anoura* specimens. We perform multivariate statistical analyses identical to those used by earlier authors to describe *A. cadenai* and *A. carishina* and elevate *A. g. peruana* and *A. c. aequatoris* to species level. We then use Gaussian Mixture Models (GMMs) to infer groups based on the multivariate normal distribution of the morphometric

variables. GMMs provide a statistical framework supported by Bayesian information criterion (BIC) scores in which a probabilistic model is built using multivariate normal distributions of morphological traits to infer the number of groups in the dataset without using *a priori* species or group assignments. Analyses based on GMMs show that both large- and small-bodied *Anoura* species groups segregate into 2 to 3 clusters lacking clear geographical or phylogenetic divergence. These findings suggest that the current, morphology-based taxonomic arrangement for *Anoura* likely overestimates the number of species, and that there is little support for the use of *A. peruana* and *A. aequatoris* as species taxa. After evaluating our results in light of biogeography of the group, we propose to keep the current taxonomic arrangement following Griffiths and Gardner (2008), and specifically to not recognize *A. aequatoris* or *A. peruana* as independent species. Our results suggest that phylogenetic work is needed in order to clarify the diversity and interrelationships of this genus.

Key words: Chiroptera, elliptical Fourier descriptors, Gaussian, Glossophaginae, Mclust, morphometry, nectarivorous bat

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Leaf-nosed nectarivorous bats in the phyllostomid subfamilies Glossophaginae and Lonchophyllinae have an important role as pollinators in the Neotropics (Fleming et al. 2005). They show a higher degree of specialization than old world pollinating bats (Fleming and Muchhala 2008), possess unique adaptations to nectarivory (Muchhala 2006), and influence evolution of the plants they pollinate (Muchhala 2008; Muchhala and Thomson 2009, 2010). *Anoura* Gray 1838 is the most diverse genus within the subfamily Glossophaginae, with 10 nominal species distributed from central Mexico to northern Argentina, Paraguay and southern Brazil (Griffiths and Gardner 2008; Pacheco et al. 2018). The exact number of *Anoura* species is debated based on disagreements over morphological species limits and their relation to the geographical distributions of the different species (Jarrín-V and Kunz 2008; Jarrin-V 2012; Jarrín-V and Coello 2012; Calderon-Acevedo et al. 2018). However, *Anoura* can be readily subdivided into two groups based on patterns of variation in dental morphology and overall body size. Specifically, an enlarged paracone in the first upper premolar (P1) and an undeveloped anterobasal cusp in the first lower premolar (p1) (Allen 1898; Griffiths and Gardner 2008) separates the small-bodied *Anoura* taxa, including *A. cadenai, A. caudifer*, *A. fistulata*, *A. javieri*, *A. luismanueli*, and *A. aequatoris*, from the large-bodied taxa of the genus, including *A. geoffroyi*, *A. latidens*, *A. peruana* and *A. cultrata.* Cranial measurements overlap broadly between many *Anoura* species taxa (Jarrín-V and Kunz 2008; Jarrín-V and Coello 2012; Calderón-Acevedo and Muchhala 2018), and only 3 of them are supposed to have diagnostic craniodental or soft morphology characters: *A. cultrata* has an enlarged and blade-like first lower premolar (Handley 1960); *A. latidens* has broad premolars and molars, with the internal cusp of the third upper premolar (P4) enclosed in the broad triangular base of the tooth and an undeveloped anterobasal cusp of

the second upper premolar (P3) (Handley 1984); and *A. fistulata*, which has unique adaptations to nectarivory such as a glossal tube, a tongue 150% the size of its body and an enlarged lower lip (Muchhala et al. 2005).

To summarize the current taxonomic and nomenclatural understanding of *Anoura*, there are two species limits hypotheses for the genus: 1) a conservative taxonomy considering *A. aequatoris* as a junior synonym of *A. caudifer*, *A. peruana* as subspecies of *A. geoffroyi*, and *A. carishina* as a junior synonym of *A. latidens* (Simmons 2005; Griffiths and Gardner 2008) and 2) an alternative taxonomy ascribing species rank to *A. aequatoris* and *A. peruana,* and considering *A. carishina* an independent species from *A. latidens* (Mantilla-Meluk and Baker 2006; Mantilla-Meluk et al. 2010; Pacheco et al. 2018). In this study we use a large dataset of measurements to rigorously statistically test these two proposed species limits hypotheses. In the following paragraphs we provide more historical context, detailing the studies that led to the current species hypotheses for the small and large-bodied *Anoura* species.

Inferring species limits among small-bodied *Anoura* has been challenging due to a lack of obvious diagnostic characters and extensive phenotypic variation. For example, craniodental measurements, fur color, uropatagium size and the presence of a fringe of hair in the uropatagium vary extensively within and between small-bodied *Anoura* species taxa (Tamsitt and Valdivieso 1966; Nagorsen and Tamsitt 1981; Jarrín-V and Kunz 2008; Calderón-Acevedo and Muchhala 2018). *Anoura caudifer* (Geoffroy Saint-Hilaire 1818) was described from "the vicinity of Rio de Janeiro" in Brazil*,* but it is distributed from Panama to Argentina and Brazil and shows wide morphological variation through its geographic distribution, with most craniodental measurements overlapping with all other small *Anoura* (Tamsitt and Valdivieso 1966; Calderón-

Acevedo and Muchhala 2018). Subsequent to the description of *A. caudifer*, Lönnberg (1921) described *A. aequatoris* as *Lonchoglossa wiedi aequatoris*, a small morph of *A. caudifer* with a characteristic fringe of fur in the uropatagium; however, it was described using only two specimens from the region of Ilambo (Illambo), Gualea, in the Pichincha province of Ecuador. After its description, *Anoura aequatoris* was treated as a valid taxon by several authors (Sanborn 1933; Cabrera 1958) who suggested that the larger morphs of *A. caudifer* did not have an Andean distribution, and therefore that the small-bodied *A. caudifer* in the Andes represented a distinct species (Sanborn 1933). However, Tamsitt and Valdivieso (1966) found no support for the suggestion that these small-bodied *Anoura* constitute either a separate species or a subspecies of *A. caudifer.* Tamsitt and Valdivieso (1966) showed that this species is highly variable in morphology, with both smaller and larger specimens distributed in the Atlantic forest, Amazonian and Andean localities. Based on this evidence, Griffiths and Gardner (2008) disregard the putative *A. aequatoris* as a standalone species or subspecies of *A. caudifer* and only recognized *A. caudifer* (Geoffroy Saint-Hilaire 1818), *A. luismanueli* (Molinari 1994), *A. fistulata* (Muchhala et al. 2005), and *A. cadenai* (Mantilla-Meluk and Baker 2006) as valid monotypic species.

Subsequent to Tamsitt and Valdivieso (1966) synonymizing *A. aequatoris* with *A. caudifer*, Mantilla-Meluk and Baker (2006) elevated *A. aequatoris* back to species level based on a discriminant analyses that used craniodental measurements and their relation to geographical distribution. Specifically, based on a sample of 33 *Anoura* specimens from the Colombian Andes they proposed the existence of a small-bodied taxon corresponding to *A. aequatoris*. They also described *A. cadenai* and reassessed the distribution of *A. luismanueli* for Colombia. Concerns arose from the description of *A.*

cadenai and the elevation to species level of *A. aequatoris,* given the circularity of the analyses, since the grouping variable (geography) was not independent from the variables used in the linear discriminant analysis (Jarrín-V and Kunz 2008). Thus, the *a priori* assignment of individuals to morphological species groups based on which of the three cordilleras of the Colombian Andes they occur in, and using this variable to generate the morphospace of small-bodied *Anoura* adds redundancy in their findings, biasing the analysis towards classifying specimens in morphological species groups that completely match the *a priori* group assignments (Jarrín-V and Kunz 2008).

The most recent taxonomical revision of small-bodied *Anoura* produced the description of a new species, *A. javieri* from southwestern Peru (Pacheco et al. 2018) a species taxa thought to be characterized by large forearm and skull length and a poorly developed uropatagium in comparison to *A. caudifer*. In addition, it revised the morphological species limits of small-bodied *Anoura* and supported the species level status of *A. aequatoris*. In their taxonomic revision Pacheco et al. (2018) found significant differences in the central tendency of the craniodental measurements of *A. caudifer* and specimens assigned to *A. aequatoris* from Peru, however this is a local perspective of the morphological variation of small-bodied *Anoura* and lacks comparisons to northern Andean *A. caudifer* and putative *A. aequatoris*.

Large *Anoura* taxonomy has also undergone recent changes. Griffiths and Gardner (2008) recognized only 3 large species, *A. geoffroyi* (Gray 1838), *A. cultrata* (Handley 1960), and *A. latidens* (Handley 1984), as well as 3 subspecies of *A. geoffroyi*: *A. g. geoffroyi* from Brazil to the Amazon and part of the Andes of Peru, Ecuador and Colombia*, A. g. lasiopyga* from Central America and Mexico and *A. g. peruana* from the Andes of Peru, Ecuador, Colombia and Venezuela. The last taxonomic revision by

Mantilla-Meluk and Baker (2010) described the new species *A. carishina*, recognized *A. peruana* as a formal species rather than a subspecies of *A. geoffroyi*, and referred to these large species (along with their subspecies) as the *A. geoffroyi* species complex (i. e. *A. carishina*, *A. peruana*, *A. geoffroyi geoffroyi*, and *A. geoffroyi lasiopyga*). Calderón-Acevedo et al., (in review) found that *A. carishina* is a junior synonym of *A. latidens* based on the characteristic shape of the third upper premolar (P4), and diagnosed the type and 3 paratypes of *A. carishina* as *A. latidens* and 1 paratype as *A. geoffroyi*. This work also concludes that *A. latidens* should be considered part of the *A. geoffroyi* species complex.

In this study, we look at the different proposed taxonomies (conservative and alternative) for the genus *Anoura* by separately analyzing each species complex under a framework based on the application of Gaussian mixture models (McLachlan and Peel 2000) to species delimitation (Cadena et al. 2018) . This approach estimates the best statistically supported clustering scheme based on the normal distributions of phenotypic characters, with no a priori assignment of specimens to species. Given the large differences in dental characters and body size that separate the small-bodied (*A. caudifer*) and large-bodied (*A. geoffroyi*) species complexes, we treat the two groups separately, and analyze the species limits within each. We evaluate the conservative taxonomy of Griffiths and Gardner (2008) and the alternative taxonomy of Mantilla-Meluk and Baker (2006, 2010), while addressing the following questions: 1) What is the number of morphological groups in each species complex? 2) What morphometric characters discriminate between the morphological groups, and what is the location of each morphological group in the morphospace of each species complex?

MATERIALS AND METHODS

Morphological measurements and taxonomic sampling —We examined 560 *Anoura* specimens, including 195 *A. caudifer*, 47 *A. aequatoris,* 18 *A. cadenai*, 22 *A. luismanueli,* 9 *A. fistulata, 9 A. javieri,* 107 *A. g. geoffroyi,* 42 *A. g. lasiopyga,* 48 *A. peruana*, and 63 *A. latidens*, amounting to 300 specimens in the *A. caudifer* species complex and 260 in the *A. geoffroyi* species complex. We did not include *A. cultrata* in our sample of specimens since we believe that its limits are well defined by unique first lower premolar morphology, a character that enables the correct identification of this species both in the field and museum collections (Nagorsen and Tamsitt 1981; Griffiths and Gardner 2008).

In each specimen we measured 12 craniodental characters and, where possible, 11 postcranial characters to the nearest 0.01 mm. We selected this set of characters because they have previously been used to understand phenotypic variation and species boundaries in *Anoura* (Handley 1960; Tamsitt and Valdivieso 1966; Nagorsen and Tamsitt 1981; Handley 1984; Molinari 1994; Simmons and Voss 1998; Mantilla-Meluk and Baker 2006, 2010; Pacheco et al. 2018). Craniodental characters included: greatest length of skull (GLS, distance from the most posterior point of the skull to the most anterior point of the premaxilla not including incisors), condylobasal length (CBL, distance from the most posterior point of the condyles to the most anterior point of the premaxilla not including incisors), zygomatic width (ZW, measured at the zygomatic processes), postorbital breadth (PB, minimum interorbital distance measured across the frontals), braincase breadth (BCB, greatest breadth of the braincase, not including the mastoid and paraoccipital processes), height of braincase (HBC, distance from the ventral border of the foramen magnum to the parietal), maxillary tooth-row length (MTRL,

distance from the most posterior point of the third upper molar to the most anterior point of the upper canine), palatal length (PL), breadth across third upper molars (M3-M3), breadth across upper canines (C-C), mandibular length (MANL, distance from the condyles to the anterior face of the mandible) and mandibular tooth-row length (MANTRL, distance from canine to the third mandibular molar). Postcranial measurements included: forearm (FA, measured from the olecranon to the articulation of the wrist), length of 3rd (D3MC), 4th (D4MC) and 5th (D5MC) metacarpals, length of the 1st and 2nd phalanxes of 3rd (D3P1, D3P2), 4th (D4P1, D4P2) and 5th (D5P1, D5P2) digit, and length of the tibia (Tibia).

We obtained measurements during visits to the collections of 7 museums, including the American Museum of Natural History (AMNH, New York, USA), Colección Teriológia Universidad de Antioquia (CTUA, Medellín, Colombia), Museo de Ciencias Naturales de la Salle (CSJ-m, Instituto Tecnológico Metropolitano, Medellín, Colombia), Colección de Mamíferos Alberto Cadena García (ICN, Instituto de Ciencias Naturales, Universidad Nacional, Bogotá Colombia), Colección Mastozoológica Universidad Distrital Francisco José de Caldas (MHNUD, Bogotá, Colombia), Field Museum of Natural History (FMNH, Chicago, USA) and the National Museum of Natural History (USNM, Washington D.C. USA). We supplemented the measurements of *A. javieri* and *A. luismanueli* with data from their respective descriptions (Molinari 1994; Pacheco et al. 2018). See Supplementary Data SD1 for the full list of specimens measured and geographical information on collecting localities.

Statistical differences in central tendency of morphological traits—We tested for significant differences among previously proposed species taxa in the central tendency of

morphometric variables with a multivariate analysis of variance (MANOVA) followed by Bonferroni-corrected post-hoc tests. This allows us to test previous taxonomies under the same framework recently employed to describe *Anoura* species taxa (i.e. species differing significantly in central tendency of the previously mentioned morphological measurements).

Clustering analyses —Previous studies of morphometrics for the genus *Anoura* have used classical analyses such as principal component analyses (PCA) and linear discriminant analysis (LDA) to delimit morphological groups (Mantilla-Meluk and Baker 2006, 2010; Mantilla-Meluk et al. 2012; Mantilla-Meluk et al. 2014). These analyses may help understand the phenotypic variation within a group; however, PCAs do not provide statistical support for the existence of groups in morphospace; moreover, sample sizes of previous studies were small in relation to the number of variables used (Jarrín-V and Kunz 2008; Jarrín-V and Coello 2012) and thus may not represent the morphological variation of each of the species complexes through their geographical ranges. In addition, LDAs were used to support the supposed morphological groups present in the morphospace of by PCAs using a variable (for example, geographical distribution or species name) as a prior. This is useful when investigating groups with established species limits that also rely on discrete characters for their diagnosis. However, this is not always the case in *Anoura* (Jarrín-V and Kunz 2008; Jarrin-V 2012; Jarrín-V and Coello 2012), as many species overlap in measurements (Calderón-Acevedo and Muchhala 2018; Calderon-Acevedo et al. 2018).

For our study, we began by conducting a PCA analysis containing all species in the genus. This confirmed a clear separation between the small- and large-bodied *Anoura*

species complexes, thus we conducted a second PCA and further analyses separately for each complex. For each, we analyzed two datasets, one containing only the 12 craniodental measurements and a smaller one in terms of sample size, containing all 23 variables. After identifying the dispersion of our data in the morphospace, we proceeded to fit Gausian mixture models (GMMs, McLachlan and Peel 2000) and thus estimate the number of groups among the specimens in each particular dataset. We used the principal component scores obtained from the variance-covariance matrix of log-transformed morphometric data as input for GMMs.

GMMs provide systematists with a useful statistical approach to go beyond graphical analyses. They can be used to determine the number of normal distributions underlying a particular dataset of continuous variables, and examine which combination of such distributions is best suited to explain phenotypic variation. The parameters of GMMs include means and variance-covariance matrices, which describe the phenotypes of groups detected among a sample of specimens. Since GMMs do not need prior information to assign specimens to groups, they provide an objective approach to test hypotheses about species limits, by evaluating support using Bayesian Information Criterion (here on referred to as BIC). These characteristics make GMMs a powerful tool to elucidate species limits (Guillot et al. 2012; Edwards and Knowles 2014).

All statistical analyses were conducted in R (R Core Team 2018) using a series of packages devised for multivariate clustering. After we calculated the principal components on a variance-covariance matrix of log transformed morphological measurements for each data set and species complex, we reduced the dimensionality of the datasets by performing variable selection for clustering models (Raftery and Dean 2006; Maugis et al. 2009a; Maugis et al. 2009b) and selected a subset of the principal

components using the R package clustvarsel (Scrucca and Raftery 2014). We then used the R package Mclust v5.4.2 (Scrucca et al. 2016) to find the best fitting clustering model (based on BIC scores) for our data. We then compared results of our unsupervised analyses (i.e, with no *a priori* species assignment) to a model-based discriminant analysis classification (using the function MclustDA) which assigns groups (species) to each individual based on a taxonomic hypothesis, and tested the following hypotheses for taxonomical arrangements: 1) A conservative species limits hypothesis following Griffiths and Gardner (2008), where *A. aequatoris* and *A. peruana* are not valid species, and 2) an alternative hypothesis proposed by Mantilla-Meluk and Baker (2006, 2010), where *A. aequatoris* and *A. peruana* are valid species. Note that previous results show that *A. carishina* is a synonym of *A. latidens* (Calderón-Acevedo et al., in review) and therefore in both hypotheses we treat the specimens from the *A. carishina* type series as *A. latidens.*

After inferring the morphological clusters present in our dataset using GMMs, we evaluated the gaps separating clusters of specimens and also species-taxa in the multivariate morphological space defined by selected principal components, and calculated the proportion of non-overlapping phenotypes between each pair of morphological clusters as described in Zapata and Jiménez (2012) and Aguilar et al. (2016). This approach begins by estimating the ridgeline manifold, a curve defined in morphological space that contains all of the critical points of the probability density of a pair of morphological clusters, namely minima (i.e., gaps), maxima and saddle points. This implies a remarkable reduction of dimensionality with no loss of information about the existence of and location of morphological gaps. In particular,

inspecting the probability density function along a single morphological dimension (the ridgeline manifold) reveals gaps between two clusters defined on any arbitrarily high number of morphological dimensions (Ray and Lindsay 2005). Finally, we estimated the proportion of non-overlapping phenotypes between each pair of morphological groups or species taxa in each species complex, using the mixing proportions of the distribution describing two morphological groups, the probability density function describing the morphological variation with the mean and variance of each morphological group.. If the probability density function along the ridgeline manifold is bimodal one can estimate s plot of β (which is a proportion of the multivariate distribution with values varying from 0 to 1) along the ridgeline manifold. Then the tolerance ellipsoids for each group of samples are estimated with different values of β, every tolerance region ellipsoid shares one single point along the ridgeline manifold corresponding to the different values of α with another ellipsoid defining a tolerance region of a second distribution (Zapata and Jiménez, 2012). The overlap of these ellipsoid tolerance regions for different proportions of β and values of α is inspected in a plot that shows the estimated phenotypic overlap between two morphological groups or between two species-taxa.

RESULTS

The exploratory PCA showed a separation between the small- and large-bodied *Anoura* species complexes, with the first two principal components explaining 77.8% of the total variation in the complete dataset and 84.1% in the craniodental dataset (Fig 1. A and B). A separate PCA on each species complex showed that the first two principal components explain 50.16% of the total variation in the complete dataset and 72.97% in

the craniodental dataset of the *A. caudifer* species complex, while in another PCA for the *A. geoffroyi* species complex the first two principal components explain 43.29% of the total variation in the complete dataset and 84.8% in the craniodental dataset. Variable selection using clustvarsel (Scrucca and Raftery 2014) on the PCA of each species complex reduced the number of variables used as input for our models: the complete dataset of the *A. caudifer* complex was reduced from 23 to 12 variables, and the craniodental dataset was reduced from 12 to 9, while the complete dataset of the *A. geoffroyi* species complex was reduced from 23 to 14 and the craniodental dataset was reduced from 12 to 10 principal components. The best unsupervised GMMs subdivide the *Anoura caudifer* species complex into 3 morphological clusters and the *A. geoffroyi* species complex into two morphological clusters (Fig. 2, Table 1). Other unsupervised Mclust models with lower support than the best model (empty circles in Fig. 2) assume the same number of morphological groups as there are species in each taxonomic hypotheses; however, the unsupervised models are still better supported than the modeling of taxonomic classifications (conservative and alternative) in both species complexes. This means that although a model might assume five morphological clusters, these morphological clusters differ from the taxonomic hypothesis that recognizes only five species. Below we detail the results for all analyses in each species complex.

Anoura caudifer *species complex* — Our GMM analyses on both datasets (craniodental and all measurements) support 3 morphological clusters (Fig 2 A and B). Craniodental measurements clustered specimens in groups of 3, 98 and 135 individuals. Group 1 (G1) with 3 specimens is composed of *A. aequatoris* and *A. cadenai*; group 2 (G2, 98 individuals) contains representatives of all species while group 3 (G3, 135 individuals) contains all species but *A. fistulata*. In the case of the complete dataset,

individuals were clustered in 2 groups of 4 specimens each (groups 1 and 3) and one group with 176 individuals (G2). Group 1 is composed of *A. aequatoris* and *A. cadenai* and group 3 contains only *A. caudifer* specimens while group 2 contains representatives of all species. In both datasets, one group is much larger than the other two and mostly composed of *Anoura caudifer,* although all species are present in the largest cluster of both datasets (Fig. 3A).

Our model-based classifications found that the conservative taxonomy, where *A. caudifer* is monotypic (i.e., not recognizing *A. aequatoris* as a valid species or subspecies), has better support in both datasets than the alternative taxonomy, where *A. aequatoris* is a separate entity (Fig 2 A and B). The classification assigns most specimens to *A. caudifer*; the only species taxa that had most of its specimens correctly assigned was *A. javieri* with only 1 out of 9 specimens assigned to *A. caudifer* (See SD 3 for the classification tables of the modeled based discriminant analysis).

The proportion of non-overlapping phenotypes between the three morphological clusters from our unsupervised GMM analysis in both datasets is low. Comparisons of the complete dataset of group 1 (G1) and group 2 (G2) is 0.015%, G1 and G3 is 4.5%, with the highest proportion of non-overlapping phenotypes between G2 and G3 being 40.3%. In regards to the craniodental dataset we find similar results, G1 and G2 0.73%, G1 and G3 1.4%, G2 and G3 0.002%. These results point out the high phenotypic overlap between the morphological groups and show the morphological homogeneity across the different morphological groups of the *A. caudifer* species complex. The overlapping phenotypes between the species taxa showed similar results, when doing comparisons between all pairs of species taxa most comparisons had 0% non-overlapping phenotypes, with only comparisons of *A. fistulata-A. aequatoris* (0.03%), *A. fistulata-A. cadenai*

(0.07%), *A. fistulata-A. caudifer* (0.04%) and *A. fistulata-A. luismanueli* (0.01%) having small proportions of non-overlapping phenotypes. Comparisons of *A. javieri* and other species were not computed since the sample size of *A. javieri* is less than the number of variables analyzed with its variance-covariance matrix having a negative determinant.

The MANOVA showed overall significant differences between species taxa for each measurement (Pillai's Trace and Wilks' Lambda $P<0.001$), with the exception of the postorbital breadth ($P=0.573$ $F=0.768$) (Table 2). However, posthoc tests between specific pairs of species showed some differed more than others (Table 2). *Anoura aequatoris* showed no differences compared to *A. luismanueli* in most of its measurements, except for width of the canines and length of the upper and lower toothrows, with *A. aequatoris* having a more robust rostrum (C.C $\bar{X}$=4.0), and longer upper (MTRL $\bar{X}$=8.07) and lower (MANTRL $\bar{X}$=8.43) toothrows (Supplementary Material SD2). *Anoura caudifer, A. fistulata* and *A. javieri* only differentiate from *A. aequatoris* in variables related to the length of the rostrum and toothrows (Table 2), with *A. aequatoris* having a shorter rostrum and toothrows than the former species (See SD 2). The larger species of the *A. caudifer* species complex, *A. cadenai*, *A. fistulata* and *A. javieri* show little difference across their measurements, with the exception of *A. javieri* possessing a narrower palate across the molars (M3.M3 $\bar{X}$= 5.38) than *A. cadenai* (M3.M3 $\bar{X}$= 5.89) or *A. fistulata* (M3.M3 $\bar{X}$= 5.67).

Anoura geoffroyi species complex —Gaussian mixture model analysis on both datasets supported 2 morphological clusters in the species complex, grouped in 111 and 16 specimens for the complete dataset, and 195 and 9 specimens for the cranial dataset. Both morphological clusters include specimens attributed to *A. g. geoffroyi, A. g. lasiopyga, A.*

peruana and *A. latidens*. When fitting our data to the modeled discriminant functions of the conservative and alternative taxonomical arrangements, we find higher support for the conservative species hypothesis of two morphological clusters within the *A. geoffroyi* species complex, with most specimens assigned correctly to either *A. geoffroyi* or *A. latidens.* The alternative species limits proposed by Mantilla-Meluk and Baker (2010), including *A. geoffroyi*, *A. latidens*, and *A. peruana*, had lower statistical support (Δ BIC>300). Finally, we tested a third hypothesis recognizing *A. peruana, A. latidens,* and the subspecies of *A. geoffroyi* (*A. g. geoffroyi* and *A. g. lasiopyga*) as valid entities and found its support to be the lowest. Similar to our results with the *A. caudifer* species complex, for the *A. geoffroyi* species complex we also find frequency of non-overlapping phenotypes between the two morphological clusters in both datasets. The proportion of non-overlapping phenotypes in the complete dataset is 1.15e-07%, with 0.0013% in the craniodental dataset. Just as in the *A. caudifer* species complex, there is high phenotypic overlap in the morphological groups of the *A. geoffroyi* species complex, showing that the traits commonly used to describe and diagnose species in the genus *Anoura* do not correspond to morphological groups that match either the conservative or the alternative taxonomy. .The proportion of non-overlapping phenotypes between all species taxa and subspecies of the *A. geoffroyi* species complex was similar to the result of the *A. caudifer* species complex. Only the comparison between *A. latidens* and *A. geoffroyi lasiopyga* found a minimal percentage of 0.03 of non-overlapping phenotypes.

The MANOVA showed overall significant differences for each measurement (Pillai's Trace and Wilks' Lambda $P<0.001$) within the species complex. There were no significant differences between *A. g. geoffroyi, A. g. lasiopyga* and *A. peruana*,

supporting the conclusion that these should be treated as a single taxon. However, all of these species/subspecies have significant differences with *A. latidens.* Specifically, the most salient difference is that *Anoura latidens* presents a robust skull while *A. geoffroyi* and associated species present a slender, longer skull (Supplementary material SD2, SD3 Table 2).

DISCUSSION

Previous work suggests that there are 8 to 10 species of *Anoura*, however no recent study has tested the morphological differentiation of recently described species. Jarrín-V and Kunz (2008) discussed concerns regarding the taxonomical history of *Anoura* and the lack of a statistical framework for the appropriate description of species limits within the genus (Jarrín-V and Kunz 2008; Jarrín-V and Coello 2012). Our results show that the linear measurements used previously to describe and delimit species in *Anoura* account for fewer morphological groups than species taxa, with no particular pattern of a species taxa belonging in a unique morphological group, (i.e. all morphological groups are composed of more than one species taxa). Specifically, GMM analyses suggest that there are 3 morphological clusters in the *A. caudifer* species complex and 2 in the *A. geoffroyi* species complex. Although the best supported GMMs supported fewer morphological groups than actual described species taxa and do not correspond to the conservative or alternative taxonomies, when we constrained the models to test the different arrangements we find that the alternative taxonomy is inflated in both species complexes, having less statistical support than a more conservative taxonomy. Each morphological group assumed by our best supported GMM is composed of several nominal species, and the proportion of non-overlapping phenotypes between the morphological groups is low for both of the species complexes and all species taxa.

This means that the phenotypes present in each group present a wide overlap between them and do not correspond to the species taxa of *Anoura*, supporting our previous findings using PCAs or GMMs, that there is a high morphometric overlap within each species complex. Therefore, despite the fact that the conservative taxonomy has lower support than the unsupervised GMMs. Our results point out that measurements used to describe morphological groups within the *A. caudifer* and *A. geoffroyi* species complexes fail to separate species taxa in equivalent morphological groups. Further studies should focus on the problem of the species limits of *Anoura* species that lack discrete morphological characters that can separate from similar species

We support previous results of Tamsitt and Valdivieso (1966) and Calderón-Acevedo and Muchhala (2018) in finding that *A. caudifer* is a species that covers all of the morphospace of small-bodied *Anoura.* In contrast to the suggestion that the putative *A. aequatoris* and *A. luismanueli* are distinct (Mantilla-Meluk and Baker 2006), we find that the morphospace of both are immersed in the morphospace of *A. caudifer* (SD3 Table 1.). A further revision and comparison of Venezuelan specimens of *A. luismanueli* to *A. caudifer* specimens from the Andes of Colombia, Ecuador and Peru is necessary to better understand the extent of morphological variation within *A. luismanueli*. In terms of the taxonomy of the *Anoura geoffroyi* species complex, our results show how morphologically overlapping this group is, with only the discrete characters that separate *A. latidens* from other species in the *A. geoffroyi* species complex being useful to discriminate between these species taxa. The arguments of Mantilla-Meluk and Baker (2010) in favor of splitting *A. geoffroyi* focus on the position of *A.g. peruana* in the morphospace generated from a PCA of craniodental and external measurements, yet their sample sizes are low. Other characters purported to separate *A. geoffroyi* from *A.*

peruana, such as a completely formed zygomatic arch and paler coloration are known to vary within species (Sanborn 1933), and are subjective as they are not readily quantifiable. Our results show that, with the addition of more specimens to morphometric analyses, there is a high phonotypical overlap between the two groups assumed by the GMMs within the *A. geoffroyi* species complex. In fact, we find that *A. g. geoffroyi*, *A.g. lasiopyga* and *A.g. peruana* share the same morphospace and even overlap in part with that of *A. latidens* (Fig. 3. SD3 Table 2). Additionally, our MANOVAS show that although the subspecies of *A. geoffroyi* are distinct from *A. latidens*, they do not differ between themselves. The lack of differences between the *A. geoffroyi* subspecies lead us to formally treat *A. geoffroyi* as a single species based on morphology using the same analyses previously used to elevate *A. peruana* to species level; however, further molecular analyses are needed to understand the relationships between the Andean, Amazonian and Central American populations of *A. geoffroyi*, and thus we advocate for the use of the conservative taxonomy of Griffiths and Gardner (2008).

The morphological measurements used in delimiting species within *Anoura*, although useful for separating the small- and large-bodied species complexes, fail to discriminate between some species (or subspecies) taxa within each species complex. Several morphological characteristics of each species taxa are useful for identifying them, however, a continued practice of using principal component analyses and linear discriminant analyses relying on geographical distribution and *a priori* species assignment based on geography alone can lead researchers to draw species limits using circular reasoning, particularly when grouping variables are not independent from variables used to define the morphospace. Gaussian mixture models do not support the

species limits with both species complexes, species limits that were previously proposed using the common morphometric characters as input in PCAs and LDAs

Our GMMs results show that a conservative taxonomy following Griffiths and Gardner (2008) has higher statistical support provided by BICs. However, both taxonomies are minimally supported in comparison to the best supported model of our GMMs. Specifically, we find that the *A. caudifer* species complex consists of 3 morphological groups while the *A. geoffroyi* species complex is composed only of 2 groups, and these morphological groups do not correspond to any of the taxonomic hypotheses tested. Using morphometric measurements to delimit *Anoura* species shows the high overlap within both species complexes, suggesting that except for those species with readily-diagnosable discrete characters (i.e. *Anoura fistulata*, *A. cultrata* and *A. latidens*) the limits of small-bodied *Anoura* and the subspecies of *A. geoffroyi* remain unclear. We are aware of the limitations of our study given the low sample size of some taxa of the *A. caudifer* species complex, increasing samples size may help clarify the taxonomic status of some groups, such as *A. luismanueli.* . A more fruitful approach will integrate molecular phylogenetic approaches with morphology to understand the cryptic diversity of *Anoura*.

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SUPPLEMENTARY DATA

Supplementary Data SD1—Database of specimens examined and their geographical information including localities and geographical coordinates.

Supplementary Data SD2—Summary measurements of all *Anoura* species

Supplementary Data SD3—Classification tables from modeled discriminant analysis.

Supplementary Data SD4—Key to the species of *Anoura* based on this study and the taxonomic revision of Griffiths and Gardner (2008), Mantilla-Meluk and Baker (2006, 2010) and Pacheco et al. (2018).

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FIGURE LEGENDS

Fig 1. A) Principal component analyses of all measurements and B) Principal component analyses of craniodental measurements. The blue polygon on the left side of the morphospace denotes the *Anoura geoffroyi* species complex, while the red polygon on the right side of the morphospace denotes the *A. caudifer* species complex.

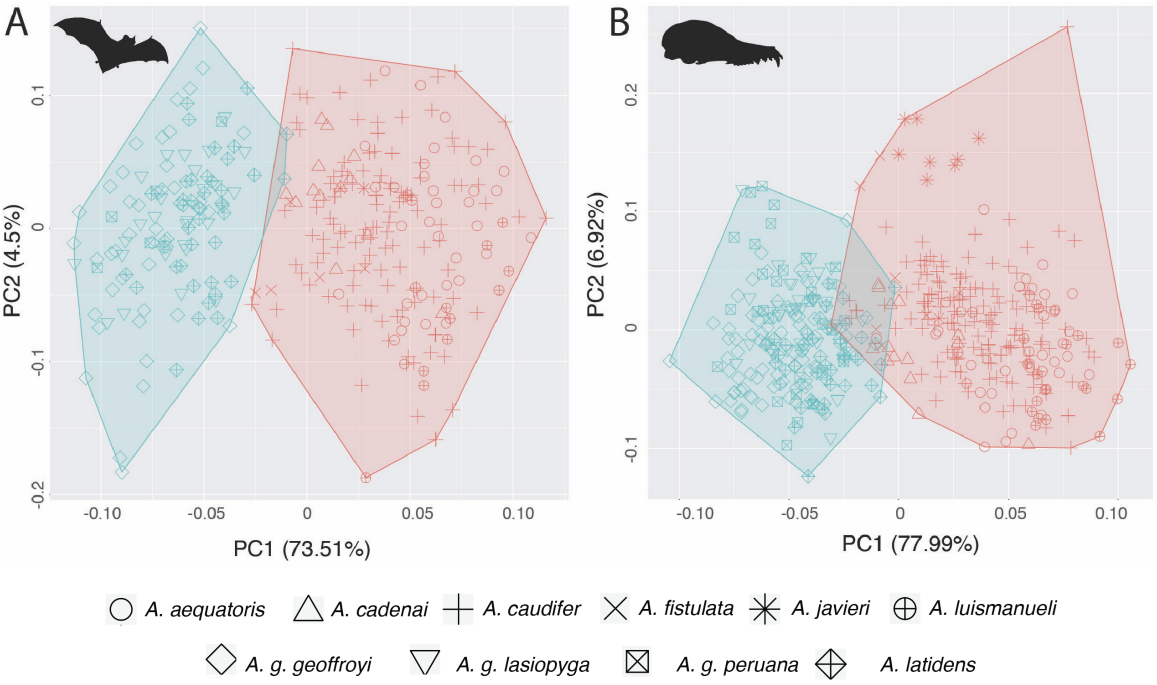


Fig. 2. Clustering analyses show that species-limit hypotheses with fewer taxa are better supported by morphometric data under unsupervised modeling (e.g, without *a priori* species assignments for specimens). Upper panels correspond to the *Anoura caudifer* species complex for the skull dataset (A) and for all measurements (B), while lower panels correspond to the *Anoura geoffroyi* species complex for the skull dataset (A) and for all measurements (D). Empty circles represent unsupervised (no *a priori* species assignment) morphological models, explaining from 1 to 10 morphological clusters; while filled triangles represent the conservative taxonomy of Griffiths and Gardner (2008), filled diamonds represent the alternative taxonomy of Mantilla-Meluk and Baker (2006, 2010), empty diamonds correspond to a model-based discriminant analysis classification taking in account all subspecies within the *A. geoffroyi* species complex taken as valid taxa. Note that unsupervised models for the same number of species as the conservative or alternative hypotheses have higher support than the supervised modeled taxonomical arrangements.

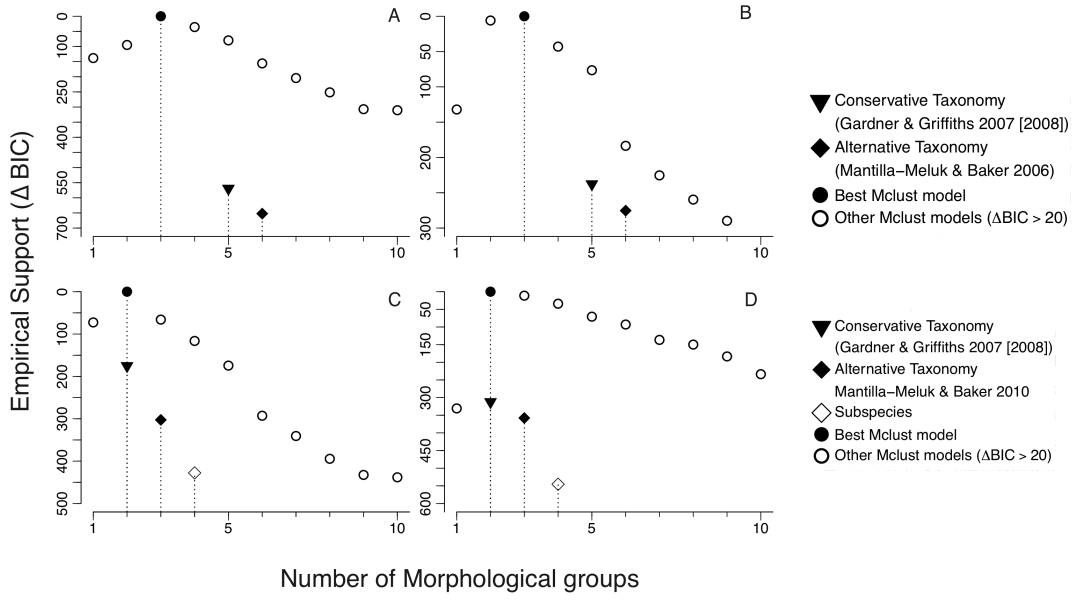
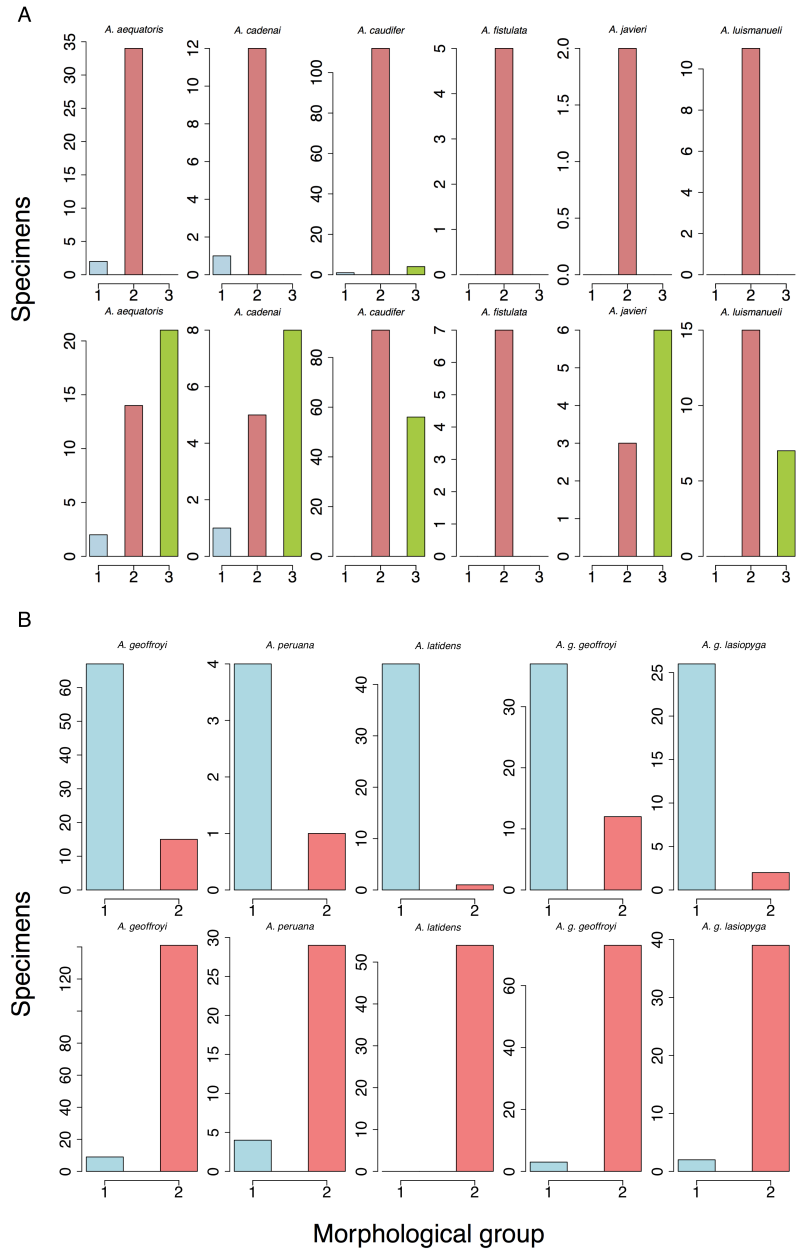


Figure 3. A) Distribution of the specimens attributed to the *A. caudifer* species complex in unsupervised morphological clusters. Top: all measurements dataset, Bottom: skull measurements dataset. B) Distribution of the specimens attributed to the *A. geoffroyi* species complex in unsupervised morphological clusters. Top: all measurements dataset, Bottom: skull measurements dataset.



Tables

Table 1. BIC scores of Gaussian mixture Models per species complex and number of morphological clusters and taxonomical hypothesis. EMP= best supported model, GG= conservative taxonomy of Griffiths and Gardner (2008); MMB= alternative taxonomy of Mantilla-Meluk and Baker (2006,2010). G= other empirical models explaining 2 to 6 morphological groups.

A. caudifer species complex

Complete dataset			Skull measurements		
Model	BIC	ΔBIC	Model	BIC	ΔBIC
EMP	10244.46	0	EMP	10354.69	0
G2	10149.58	94.88	G2	10348.66	6.03
G4	10208.73	35.73	G4	10311.69	43
G5	10164.68	79.78	G5	10278.22	76.47
G6	10088.6	155.86	G6	10171.16	183.53
GG	9675.226	569.234	GG	10117.01	237.68
MMB	9592.229	652.231	MMB	10079.41	275.28

A. geoffroyi species complex

Complete dataset			Skull measurements		
Model	BIC	ΔBIC	Model	BIC	ΔBIC
EMP	7866.088	0	EMP	9567.283	0
G3	7800.186	65.902	G3	9555.996	11.287
G4	7749.767	116.321	G4	9533.217	34.066
G5	7691.633	174.455	G5	9496.523	70.76
GG	7690.908	175.18	GG	9255.006	312.277
MMB	7563.472	302.616	MMB	9209.49	357.793

Table 2. MANOVA *F*- and, *P*-values, with Bonferroni-corrected posthoc tests *P*-values of species pair comparisons, based on morphometric variables between *A. aequatoris* (*n*=37)*, A. cadenai* (*n*=14), *Anoura caudifer* (*n*=147), *A. fistulata* (*n*=6) *A. javieri* (*n*=9) and *A. luismanueli* (*n*=22). Significant *P*-values in bold, grey cells indicate significant *P*-values with no measurement overlap between species. See methods for measurement abbreviations.

	MANOVA *F*	MANOVA *P*	*A.aeq-A.cad*	*A.aeq-A.cau*	*A.aeq-A.fis*	*A.aeq-A.jav*	*A.aeq-A.lui*
GLS	29.545	**0.001**	**0.000**	**0.001**	**0.000**	**0.000**	**0.026**
CBL	29.934	**0.001**	**0.000**	**0.000**	**0.000**	**0.001**	0.146
ZW	9.111	**0.001**	**0.000**	1.000	1.000	1.000	0.585
PB	1.367	0.238	1.000	1.000	0.666	1.000	1.000
BCB	9.084	**0.001**	**0.007**	0.707	**0.000**	1.000	1.000
MTRL	30.023	**0.001**	**0.000**	**0.001**	**0.000**	**0.000**	**0.005**
PL	19.288	**0.001**	**0.010**	**0.001**	**0.000**	**0.000**	**0.025**
M3M3	8.039	**0.001**	**0.000**	0.770	1.000	1.000	1.000
CC	14.770	**0.001**	**0.000**	0.516	0.245	0.269	**0.016**
HBC	5.560	**0.001**	0.071	1.000	1.000	**0.013**	1.000
MANL	31.349	**0.001**	**0.000**	**0.009**	**0.000**	**0.000**	0.431
MANTRL	32.203	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**

	MANOVA *F*	MANOVA *P*	*A.cad-A.cau*	*A.cad-A.fis*	*A.cad-A.jav*	*A.cad-A.lui*	*A.cau-A.fis*
GLS	29.545	**0.001**	**0.035**	1.000	**0.028**	**0.000**	**0.001**
CBL	29.934	**0.001**	**0.006**	1.000	0.102	**0.000**	**0.000**
ZW	9.111	**0.001**	**0.000**	1.000	**0.000**	**0.000**	**1.000**
PB	1.367	0.238	1.000	1.000	1.000	1.000	1.000
BCB	9.084	**0.001**	0.124	0.196	1.000	**0.001**	**0.000**
MTRL	30.023	**0.001**	**0.001**	1.000	0.776	**0.000**	**0.004**
PL	19.288	**0.001**	1.000	0.393	0.855	**0.000**	**0.010**
M3M3	8.039	**0.001**	**0.000**	0.124	**0.000**	**0.000**	1.000
CC	14.770	**0.001**	**0.001**	1.000	**0.000**	**0.000**	1.000
HBC	5.560	**0.001**	**0.029**	1.000	**0.000**	0.622	1.000
MANL	31.349	**0.001**	**0.000**	1.000	0.165	**0.000**	**0.000**
MANTRL	32.203	**0.001**	**0.000**	1.000	1.000	**0.000**	**0.002**

	MANOVA *F*	MANOVA *P*	*A.cau-A.jav*	*A.cau-A.lui*	*A.fis-A.jav*	*A.fis-A.lui*	*A.jav-A.lui*
GLS	29.545	**0.001**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
CBL	29.934	**0.001**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
ZW	9.111	**0.001**	0.734	**0.003**	0.535	0.077	1.000
PB	1.367	0.238	1.000	1.000	0.982	0.285	1.000
BCB	9.084	**0.001**	1.000	0.063	**0.004**	1.000	1.000
MTRL	30.023	**0.001**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
PL	19.288	**0.001**	**0.013**	**0.000**	1.000	**0.000**	**0.000**
M3M3	8.039	**0.001**	1.000	1.000	1.000	1.000	1.000
CC	14.770	**0.001**	**0.004**	**0.000**	**0.004**	**0.000**	1.000
HBC	5.560	**0.001**	**0.004**	1.000	**0.008**	1.000	**0.005**
MANL	31.349	**0.001**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
MANTRL	32.203	**0.001**	**0.000**	**0.000**	1.000	**0.000**	**0.000**

Table 3. MANOVA *F* values and *P*-values for Bonferroni-corrected posthoc tests of skull morphometric variables between *Anoura geoffroyi geoffroyi* (*n*=68), *A. g. lasiopyga* (*n*=40) *A. peruana* (n=31) and *A. latidens* (*n*=49), with significant *P*-values in bold. See methods for measurement abbreviations.

	MANOVA *F*	**MANOVA *P***	***A.g.geo-A.g.las***	***A.g.geo-A.per***	***A.g.las-A.per***
GLS	33.673	**0.000**	0.348	1.000	1.000
CBL	31.58	**0.000**	0.892	1.000	0.394
ZW	13.337	**0.000**	**0.000**	**0.000**	0.249
PB	4.541	**0.004**	1.000	**0.002**	0.081
BCB	5.931	**0.001**	0.491	0.304	1.000
MTRL	5.715	**0.001**	1.000	1.000	1.000
PL	17.626	**0.000**	1.000	1.000	1.000
M3M3	3.602	**0.015**	0.596	0.502	1.000
CC	25.027	**0.000**	0.647	0.086	1.000
HBC	10.174	**0.000**	**0.000**	0.500	**0.019**
MANL	18.265	**0.000**	**0.000**	1.000	**0.001**
MANTRL	20.073	**0.000**	1.000	0.760	0.137

	MANOVA *F*	**MANOVA *P***	***A.g.geo-A. lat***	***A.g.las-A.lat***	***A.per-A.lat***
GLS	33.673	**0.000**	**0.000**	**0.000**	**0.000**
CBL	31.58	**0.000**	**0.000**	**0.000**	**0.000**
ZW	13.337	**0.000**	**0.004**	1.000	1.000
PB	4.541	**0.004**	1.000	1.000	**0.039**
BCB	5.931	**0.001**	**0.000**	0.251	0.717
MTRL	5.715	**0.001**	**0.008**	**0.003**	**0.012**
PL	17.626	**0.000**	**0.000**	**0.000**	**0.000**
M3M3	3.602	**0.015**	1.000	**0.046**	**0.044**
CC	25.027	**0.000**	**0.000**	**0.000**	**0.000**
HBC	10.174	**0.000**	0.053	**0.033**	1.000
MANL	18.265	**0.000**	**0.000**	1.000	**0.000**
MANTRL	20.073	**0.000**	**0.000**	**0.000**	**0.000**

Table 4. MANOVA *F* values and *P*-values for Bonferroni-corrected posthoc tests of skull morphometric variables between *Anoura geoffroyi geoffroyi* (*n*=68), *A. g. lasiopyga* (*n*=40) *A. peruana* (n=31) with significant *P*-values in bold. See methods for measurement abbreviations.

	MANOVA *F*	**MANOVA *P***	***A.g.geo-A.g.las***	***A.g.geo-A.per***	***A.g.las-A.per***
GLS	1.724	0.182	0.218	1.000	0.581
CBL	1.680	0.190	0.515	1.000	0.245
ZW	17.389	**0.000**	**0.000**	**0.000**	1.000
PB	6.811	**0.002**	0.978	**0.001**	**0.040**
BCB	2.490	0.087	0.266	0.168	1.000
MTRL	0.221	0.802	1.000	1.000	1.000
PL	0.358	0.700	1.000	1.000	1.000
M3M3	1.883	0.156	0.371	0.319	1.000
CC	2.970	0.055	0.398	0.067	1.000
HBC	12.696	**0.000**	**0.000**	0.337	**0.020**
MANL	10.234	**0.000**	**0.000**	1.000	**0.001**
MANTRL	2.359	0.098	0.917	0.449	0.096

Table 5. MANOVA *F* of skull morphometric variables between *Anoura geoffroyi* (*n*=139) and *A. latidens* (n=49) with significant *P*-values in bold.

	MANOVA *F*	**MANOVA *P***
GLS	96.236	**0.000**
CBL	90.147	**0.000**
ZW	0.401	0.527
PB	0.182	0.670
BCB	12.391	**0.001**
MTRL	16.711	**0.000**
PL	52.497	**0.000**
M3M3	6.422	**0.012**
CC	66.587	**0.000**
HBC	0.299	0.585
MANL	27.682	**0.000**
MANTRL	53.974	**0.000**

SUPPLEMENTARY DATA

Supplementary Data SD2—Summary measurements of all *Anoura* species

		A. aequatoris	*A. cadenai*	*A. caudifer*	*A. fistulata*	*A. javieri*	*A. luismanueli*
GLS	$\bar{X}$	21.95	23.08	22.40	23.77	24.00	21.24
	SD	0.57	0.64	0.71	0.93	0.54	0.46
	n	46	18	193	9	9	22
CBL	$\bar{X}$	21.27	22.58	21.76	23.24	23.39	20.62
	SD	0.73	0.63	0.73	0.80	0.55	0.59
	n	46	18	187	9	9	22
ZW	$\bar{X}$	9.26	9.89	9.39	9.68	9.09	9.00
	SD	0.37	0.36	0.47	0.28	0.43	0.20
	n	44	16	176	9	9	22
PB	$\bar{X}$	4.55	4.59	4.58	4.69	4.55	4.53
	SD	0.15	0.11	0.13	0.21	0.11	0.20
	n	47	18	193	9	9	22
BCB	$\bar{X}$	8.84	9.06	8.91	9.34	8.93	8.79
	SD	0.26	0.11	0.20	0.16	0.16	0.16
	n	46	17	192	9	9	22
MTRL	$\bar{X}$	8.07	8.62	8.25	8.77	8.85	7.74
	SD	0.25	0.28	0.29	0.29	0.37	0.31
	n	45	18	187	9	9	22
PL	$\bar{X}$	11.46	12.06	11.85	12.88	12.69	10.69
	SD	0.75	0.77	0.73	0.84	0.46	0.47
	n	45	16	181	8	9	22
M3.M3	$\bar{X}$	5.43	5.89	5.49	5.67	5.38	5.41
	SD	0.27	0.18	0.28	0.24	0.05	0.22
	n	43	17	180	9	9	22
C.C	$\bar{X}$	4.00	4.24	4.05	4.15	3.84	3.84
	SD	0.17	0.16	0.17	0.24	0.14	0.10
	n	46	18	190	8	9	22
HBC	$\bar{X}$	7.02	7.21	6.98	7.18	6.65	7.07
	SD	0.33	0.25	0.28	0.18	0.22	0.37
	n	46	18	191	7	9	22
MANL	$\bar{X}$	15.73	16.89	16.04	17.42	17.65	15.22
	SD	0.80	0.50	0.59	0.71	0.53	0.59
	n	46	18	189	9	9	22
MANTRL	$\bar{X}$	8.43	9.07	8.63	9.23	9.18	8.06
	SD	0.27	0.31	0.31	0.34	0.33	0.33
	n	46	18	187	9	9	22

		A. aequatoris	*A. cadenai*	*A. caudifer*	*A. fistulata*	*A. javieri*	*A. luismanueli*
FA	$\bar{X}$	35.42	36.79	35.92	37.34	37.15	35.03
	SD	1.04	0.76	1.28	0.66	0.58	0.64
	n	45	16	163	7	8	12
D3MC	$\bar{X}$	35.14	36.08	35.54	36.56	36.03	33.91
	SD	1.15	1.00	1.55	0.84	0.07	0.96
	n	45	16	162	6	2	12
D3P1	$\bar{X}$	11.65	12.84	12.12	13.31	12.05	11.53
	SD	0.66	0.51	0.64	0.54	0.04243	0.80
	n	45	16	162	6	2	12
D3P2	$\bar{X}$	18.50	19.17	18.79	20.27	19.96	18.68
	SD	0.75	0.96	1.40	1.01	0.03	1.13
	n	45	16	161	6	2	11
D4MC	$\bar{X}$	33.14	34.12	33.74	34.78	34.91	32.16
	SD	1.06	1.46	1.62	1.23	0.51	1.06
	n	45	16	162	6	2	12
D4P1	$\bar{X}$	8.71	8.90	8.96	9.45	8.78	8.78
	SD	0.61	0.43	0.58	0.86	0.50	0.47
	n	45	16	161	6	2	12
D4P2	$\bar{X}$	11.39	12.07	11.74	12.94	11.96	11.76
	SD	0.75	0.67	0.96	1.06	0.09	0.76
	n	45	16	160	6	2	11
D5MC	$\bar{X}$	29.11	29.67	29.37	30.85	30.42	27.55
	SD	1.20	1.12	1.72	1.78	0.2687	0.94
	n	45	16	162	6	2	12
D5P1	$\bar{X}$	7.45	7.83	7.68	7.64	7.44	7.38
	SD	0.44	0.43	0.53	0.41	0.12	0.35
	n	45	16	161	6	2	12
D5P2	$\bar{X}$	10.57	10.96	10.62	11.78	10.84	10.34
	SD	0.80	0.79	0.78	0.81	0.23	0.77
	n	45	16	160	6	2	11
Tibia	$\bar{X}$	11.86	11.87	12.15	13.17	13.01	11.40
	SD	0.85	0.79	0.88	1.38	0.10	0.54
	n	44	16	148	6	2	12

		A. g.geoffroyi	*A. g.lasiopyga*	*A. peruana*	*A. latidens*	*A. cultrata*
GLS	$\bar{X}$	25.21	24.96	25.05	24.21	24.47
	SD	0.61	0.37	0.76	0.48	0.63
	n	104	42	48	62	20
CBL	$\bar{X}$	24.53	24.35	24.39	23.66	23.80
	SD	0.59	0.37	0.77	0.46	0.66
	n	100	41	48	62	20
ZW	$\bar{X}$	10.78	10.36	10.47	10.54	10.11
	SD	0.40	0.24	0.39	0.29	0.26
	n	88	40	44	56	20
PB	$\bar{X}$	5.07	5.03	4.93	5.04	5.03
	SD	0.23	0.15	0.16	0.15	0.22
	n	104	42	48	63	20
BCB	$\bar{X}$	9.84	9.78	9.73	9.70	9.79
	SD	0.24	0.16	0.21	0.21	0.23
	n	99	42	47	62	20
MB	$\bar{X}$	10.44	10.26	10.29	10.17	10.22
	SD	0.31	0.18	0.36	0.24	0.24
	n	100	41	48	60	20
MTRL	$\bar{X}$	9.53	9.56	9.48	9.26	8.57
	SD	0.29	0.71	0.29	0.19	0.40
	n	103	42	48	63	20
PL	$\bar{X}$	13.57	13.48	13.51	12.89	12.15
	SD	0.59	0.37	0.64	0.49	0.75
	n	96	41	35	57	19
M3.M3	$\bar{X}$	6.16	6.06	6.03	6.21	5.84
	SD	0.27	0.20	0.16	0.17	0.20
	n	99	42	48	61	19
C.C	$\bar{X}$	4.63	4.54	4.60	4.31	4.90
	SD	0.24	0.21	0.29	0.18	0.21
	n	102	42	46	63	20
HBC	$\bar{X}$	7.69	7.35	7.53	7.56	8.05
	SD	0.35	0.23	0.50	0.23	0.47
	n	97	41	48	61	20
MANL	$\bar{X}$	18.01	17.50	17.95	17.34	17.47
	SD	0.53	0.77	0.67	0.40	0.46
	n	97	42	37	62	20
MANTRL	$\bar{X}$	10.00	9.91	10.04	9.63	9.07
	SD	0.31	0.23	0.29	0.20	0.39
	n	97	42	37	62	19

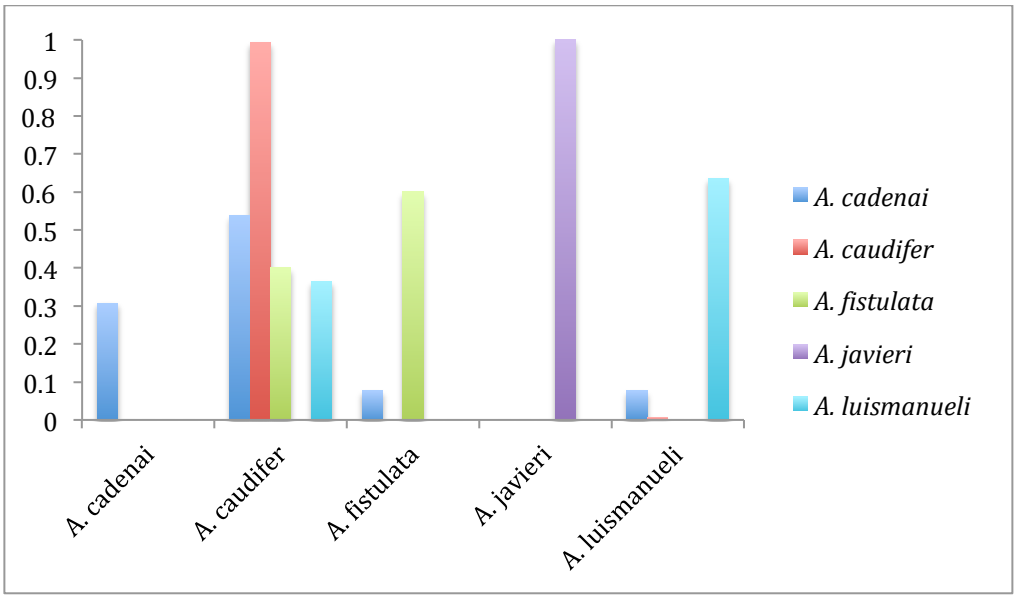
		A. g.geoffroyi	*A. g.lasiopyga*	*A. peruana*	*A. latidens*	*A. cultrata*
FA	$\bar{X}$	42.69	42.50	43.36	43.13	41.83
	SD	1.34	1.27	1.48	1.39	1.00
	n	96	42	32	53	15
D3MC	$\bar{X}$	40.69	40.56	41.82	40.20	40.55
	SD	1.43	1.27	0.82	1.23	0.98
	n	94	30	12	53	15
D3P1	$\bar{X}$	13.66	13.65	14.34	13.34	13.96
	SD	0.92	0.74	0.83	0.69	0.83
	n	94	30	12	53	15
D3P2	$\bar{X}$	21.99	21.89	22.26	20.96	22.58
	SD	1.61	0.98	1.00	0.89	0.75
	n	94	30	12	53	15
D4MC	$\bar{X}$	38.95	38.94	40.44	38.65	39.10
	SD	1.55	1.36	1.00	1.19	1.35
	n	93	29	12	53	15
D4P1	$\bar{X}$	10.29	10.16	10.51	10.08	10.52
	SD	0.68	0.56	0.79	0.69	0.59
	n	93	29	12	53	15
D4P2	$\bar{X}$	13.88	13.52	13.58	13.37	14.08
	SD	0.87	0.87	0.62	0.74	0.73
	n	93	29	12	53	15
D5MC	$\bar{X}$	33.72	33.40	34.20	33.52	34.37
	SD	1.39	1.18	1.19	1.13	0.82
	n	93	30	12	53	15
D5P1	$\bar{X}$	8.68	8.77	9.21	8.50	8.42
	SD	0.61	0.47	1.12	0.52	0.56
	n	93	30	11	53	15
D5P2	$\bar{X}$	12.21	12.09	11.99	11.99	13.01
	SD	0.80	0.80	0.87	0.67	0.82
	n	93	30	11	53	15
Tibia	$\bar{X}$	14.34	13.78	13.39	14.28	14.57
	SD	1.05	0.70	0.73	0.88	0.73
	n	76	30	18	54	15

Supplementary Data SD3. Classification tables from modeled discriminant analysis.

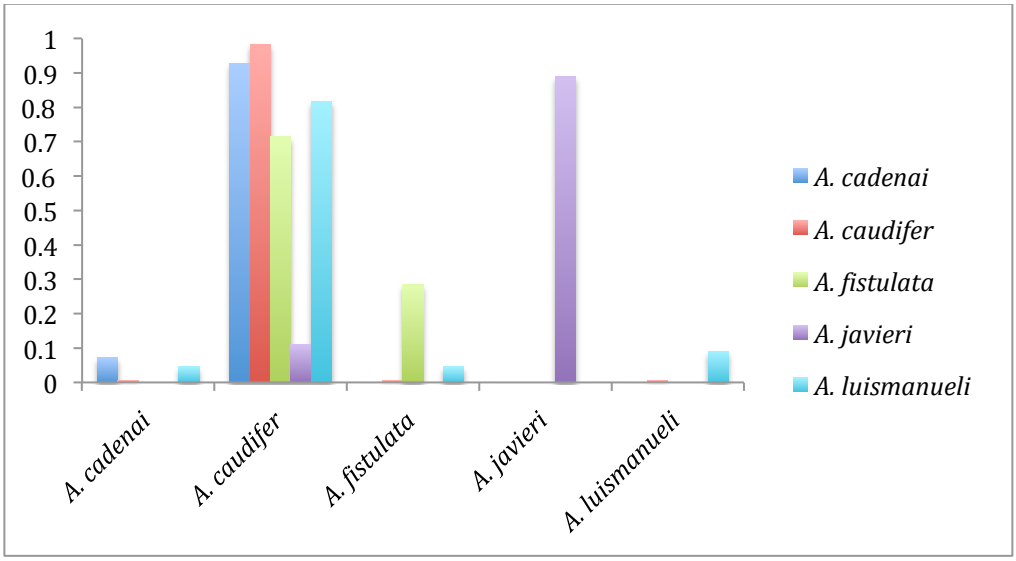
***Anoura caudifer* species complex**

Conservative classification (Griffiths and Gardner 2007 [2008])

A) All morphometric measurements dataset

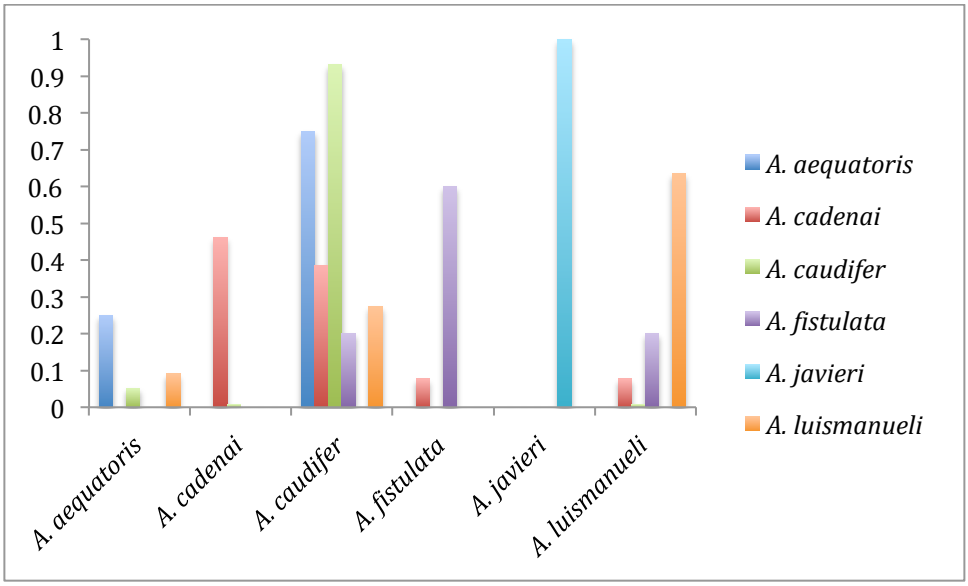


B) Craniodental measurements dataset

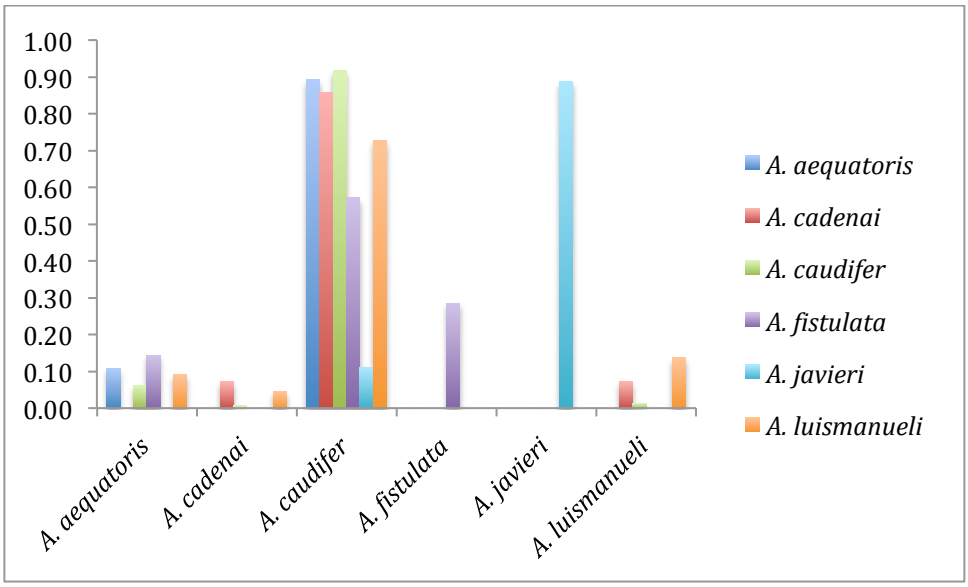


Alternative classification (Mantilla-Meluk and Baker 2006)

C) All morphometric measurements dataset



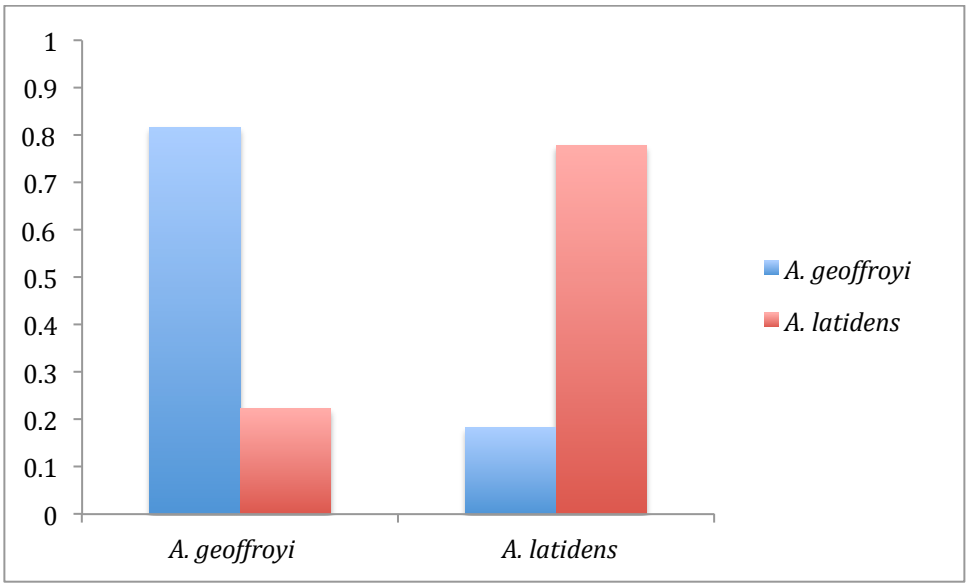
D) Craniodental measurements dataset



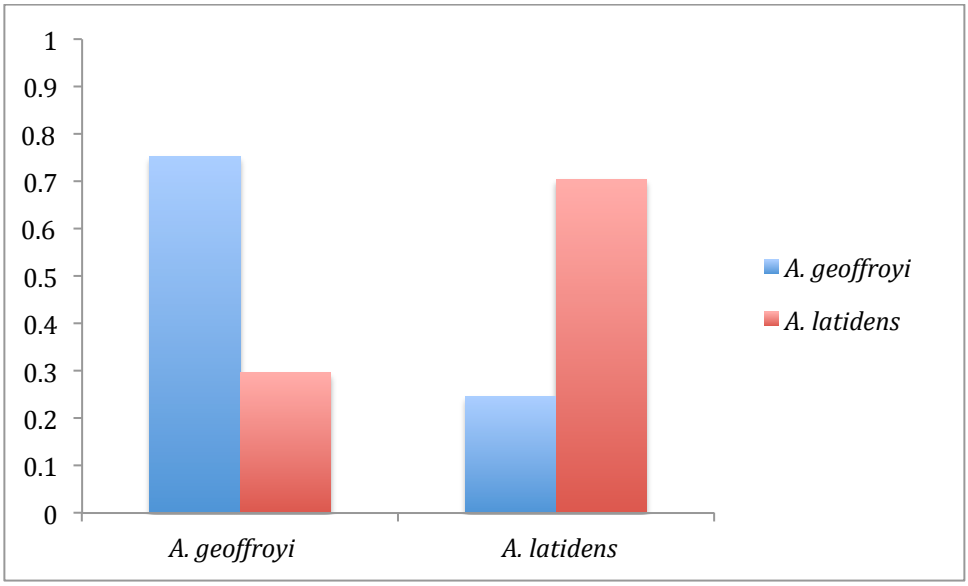
***Anoura geoffroyi* species complex**

Conservative classification (Griffiths and Gardner 2007 [2008])

A) All morphometric measurements dataset

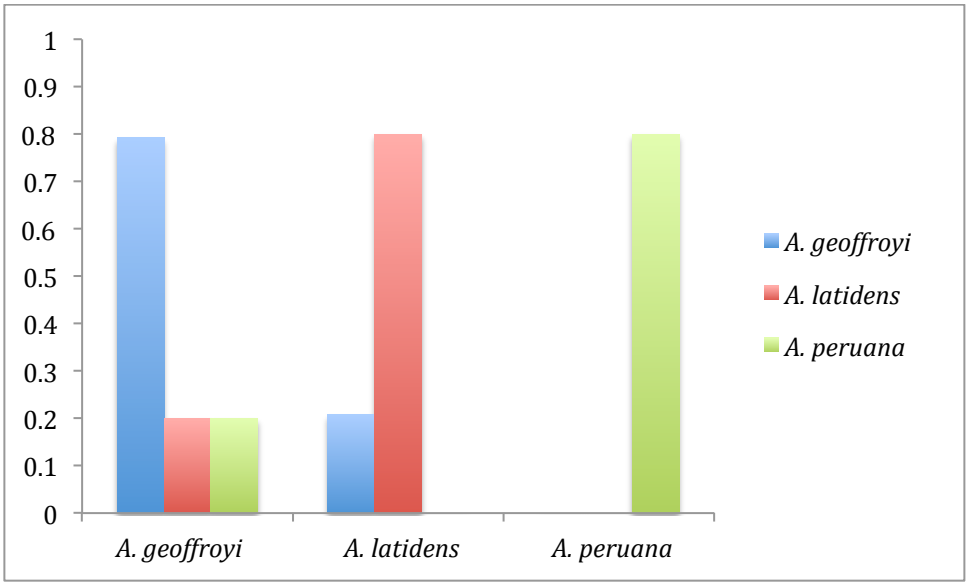


B) Craniodental measurements dataset

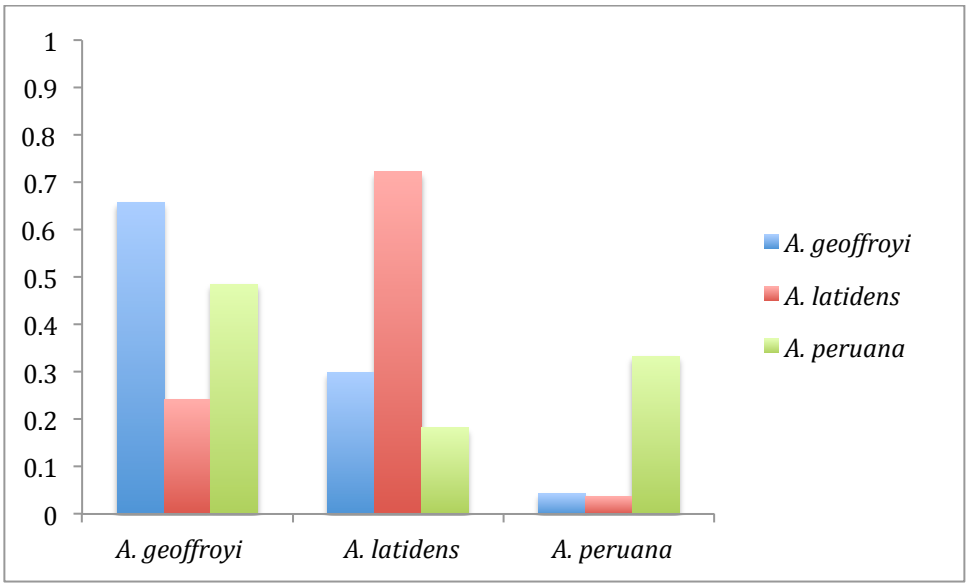


Alternative classification (Mantilla-Meluk and Baker 2010)

C) All morphometric measurements dataset



D) Craniodental measurements dataset



Supplementary Data SD4—Key to the species of *Anoura* based on this study and the taxonomic revision of Griffiths and Gardner (2008), Mantilla-Meluk and Baker (2006, 2010), Pacheco et al. (2018)

Key to the species of *Anoura*

1.First lower premolar (p1) enlarged, bladelike and different in shape from the second and third lower premolars, canines with an anteriomedial sulcus. *Anoura cultrata*

1'. First lower premolar (p1) the same size and similar in shape to the second and third lower premolars; canines without an anteriomedial sulcus. 2

2. Last upper premolar (P4) without medial-internal cusp; first lower molar (m1) lacks antero-external cristid; tail usually present; forearm usually less than 40mm. 3

2'. Upper last premolar (P4) with medial-internal cusp developed; first lower molar (m1) with antero external cuspid and cristid; tail absent; forearm more than 40.0 mm. 4

3. Forearm more than 33.5 mm; uropatagium and legs sparsely haired; upper toothrow length more than 8.30; palate length more than 12 mm; slope between the frontal and nasal bones low. 5

3'. Forearm less than 34 mm, uropatagium and legs covered with dense hair; palate length less than 11.50; upper tooth row length less than 8.22 mm; slope between the frontal and nasal bones steep. *Anoura luismanueli*

4. Broad molars and premolars; medial-internal cusp of the last upper premolar (P4) enclosed by the base of the tooth, giving it a triangular shape. *Anoura latidens*.

4'. Narrow molars and premolars; the medial-internal cusp of the last upper premolar (P4) protrudes from the narrow base of the tooth; P4 lacking a triangular shape. *Anoura geoffroyi*

5. Glossal tube present; xiphoid process wide, greatest length of skull more than 22 mm; width of braincase more than 9.1 mm; lower lip protrudes 3 mm beyond upper lip. *Anoura fistulata*

5'. Glossal tube absent; xiphoid process not developed; width of brain- case less than 9.15 mm; lower lip protrudes less than 3 mm beyond upper lip. 6

6. Keel along midline of mesopterygoid fossa flattened posteriorly; uropatagium sparsely haired; posterior projection of pterygoids short, extending to the anterior projection of each bulla; upper canines are robust and resemble those of *A. cultrata*. *Anoura cadenai*

6’. Keel along midline of mesopterygoid fossa not flattened posteriorly and extending onto septum between the basisphenoid pits; uropatagium well haired; posterior projection of pterygoids long, extending behind the anterior projections of each bulla; upper canines are slender and delicate. 7

7. Uropatagium greater than 4 mm at the knee joint, semicircular in shape, molar and premolars wide, palatal process present; rostrum relatively short. *Anoura caudifer*

7’. Uropatagium shorter than 4 mm at the knee joint, “V” shaped; molars and premolars narrow; palatal process absent; rostrum long and delicate. *Anoura javieri*

CHAPTER 3.

Genome-wide ultraconserved elements resolve phylogenetic relationships among leaf-nosed bats in the genus Anoura Gray 1838

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Abstract

Anoura Gray 1838 is the most speciose genus within the Neotropical nectarivorous bat subfamily Glossophaginae (Chiroptera: Phyllostomidae). Currently, 8 to 10 species of *Anoura* are recognized based on patterns of morphological variation; however, previous taxonomic revisions and phylogenetic studies used limited taxon sampling, of either three or four species, and thus focused primarily on the position of *Anoura* within

Glossophaginae. In this study we (1) resolve phylogenetic relationships of 8 species of *Anoura* from species trees inferred based on genome-wide sequencing of 2039 ultraconserved element loci for 42 individuals, (2) estimate the diversification times within *Anoura* using a Penalized Likelihood approach and (3) infer historical biogeographic patterns within the genus. Our results identified four well-supported clades supporting the monophyly of small-bodied *Anoura* species (previously recognized as the genus *Lonchoglossa*), the monotypic status of *A. caudifer,* the nested positions of specimens attributed to "*A. aequatoris*" within *A. caudifer* and "*A. peruana*" within *A. geoffroyi*, species complexes and polyphyletic patterns indicating possible hybridization-mediated introgression events or other evolutionary processes requiring further study. Our dated phylogeny suggests that the diversification of *Anoura* began in the Miocene, 9 million years ago, with its extant species appearing in the past 4 million years. We identified the central and northern Andes as the ancestral range of the genus, with more recent dispersal and/or founder event speciation in the Amazon and Brazilian Atlantic forest in the past 2.5 Ma.

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1. Introduction

With 214 species, the Neotropical leaf-nosed bat family Phyllostomidae is among the most speciose groups of bats. As such, it exhibits the richest diversity in feeding guilds and adaptations of any bat family (Baker et al., 2012; Burgin et al., 2018). Within the nectarivorous subfamily Glossophaginae, the most species rich genus is *Anoura* Gray 1838*,* which is composed of 8 to 10 species (Griffiths and Gardner, 2008; Pacheco et al., 2018). *Anoura* has the widest geographical distribution of any glossophagine genus (Griffiths and Gardner, 2008), likely due in part to high metabolic rates enabling them to maintain constant body temperatures and inhabit large elevational gradients (Soriano et al., 2002). This group is also well known for containing the mammal species with the longest tongue relative to body size (*A. fistulata*; Muchhala, 2006; Calderón-Acevedo and Muchhala, 2018). To date, studies of phylogenetic relationships and species limits within *Anoura* have employed limited taxon sampling, generally including only 3 or 4 species (*A. caudifer, A. cultrata, A. geoffroyi* and *A. latidens* (Carstens et al., 2002; Datzmann et al., 2010; Rojas et al., 2012; Rojas et al., 2016). The position of *Anoura* within Glossophaginae has been inferred in several broader phylogenetic studies (Griffiths, 1982; Baker et al., 1989; Wetterer et al., 2000; Baker et al., 2003); however, there are currently only three existing infrageneric phylogenetic hypotheses (summarized in Fig. 1): (1) Carstens et al. (2002): (*A. latidens,* (*A. caudifer, A. geoffroyi*)); (2) Dávalos et al. (2014): (*A. caudifer*, (*A. geoffroyi, A. latidens*)); and (3) Rojas et al. (2016): (*A. cultrata*, (*A. caudifer*, (*A. geoffroyi, A. latidens*))). The former two phylogenetic hypotheses (Carstens et al., 2002; Dávalos et al., 2014) were based on morphological and molecular data whereas the latter was based solely on molecular data (Rojas et al., 2016).

One conspicuous morphological pattern of variation within *Anoura* is the clear divergence in body size between large- and small-bodied *Anoura*, which is associated with dental morphology in that only the small-bodied *Anoura* possess an enlarged paracone and reduced paracrista in the first upper premolar (Allen, 1898; Mantilla-Meluk and Baker, 2006, 2010; Pacheco et al., 2018). Several previously proposed nomenclatural schemes reflect this divergence; for example, the genus *Lonchoglossa* Peters 1868 was previously used to refer to individuals belonging to the small-bodied species *A. caudifer* before this generic name was synonymized with *Anoura* (Sanborn, 1933, 1943). In earlier studies, specimens from the genus *Anoura* were treated as comprising two distinct genera, *Lonchoglossa* and *Anoura*, on the basis of differences in overall body size and dental characters (Sanborn, 1933, 1943; Husson, 1962). However, other authors advocated for the treatment of *Anoura* and *Lonchoglossa* as one cohesive genus (Simpson, 1945; Cabrera, 1958). More recently, Mantilla-Meluk and Baker (2006, 2010) referred to the small-bodied *Anoura* species as the '*A. caudifer* species complex' (including *A. aequatoris, A. cadenai, A. caudifer, A. fistulata, A. javieri*, and *A. luismanueli*) and the large-bodied species as the '*A. geoffroyi* complex' (including *A. geoffroyi, A. carishina*, and *A. peruana*; note that *A. cultrata* and *A. latidens* were not placed in either complex). Calderon-Acevedo et al. (in review) showed that the large-bodied taxon *A. carishina* represents a junior synonym of *A. latidens,* and that *A. latidens* belongs in the *A. geoffroyi* species complex. Previous phylogenetic studies of *Anoura* included only one species of small-bodied *Anoura*; therefore, no studies to date have tested for reciprocal monophyly of the small-bodied and large-bodied *Anoura*. The most recent taxonomic revision of small-bodied *Anoura* by Pacheco et al. (2018) discussed the possibility of using the available name *Lonchoglossa* Peters 1868 as a genus rank to refer

to the *A. caudifer* species complex but noted an infrageneric phylogeny was needed to confirm whether this represents a monophyletic group. Without a formal phylogenetic hypothesis for the entire genus, dental characters alone cannot be assumed to reflect synapomorphies of small-bodied *Anoura.*

In this study, we use ultraconserved elements (UCEs) to infer the phylogenetic relationships of *Anoura* species. Ultraconserved elements are sequences longer than 200 bp that are widely conserved across different vertebrate groups, yet have highly variable flanking regions (Bejerano et al., 2004), making them useful for resolving evolutionary relationships at different depths of divergence. Data from UCEs have proven useful for resolving higher-order mammalian relationships (McCormack et al., 2012; Esselstyn et al., 2017) as well as shallower species- and population-level patterns of evolutionary divergence (Giarla and Esselstyn, 2015; Jackson et al., 2017; Morales et al., 2017; Van Dam et al., 2017; Lima et al., 2018; Andermann et al., 2019). Ours is the first genome-scale study of *Anoura* and the first study to infer phylogenetic relationships for the genus based on near complete species-level sampling (9 of 10 nominal species in the genus; Griffiths and Gardner, 2008; Pacheco et al. 2018; Calderon-Acevedo et al. in review). We infer species trees using robust summary-statistic and quartet-based methods that have been shown to be statistically consistent under the multispecies coalescent model (Chifman and Kubatko, 2014; Mirarab et al., 2014; Zhang et al., 2017), and we assess the impact of taxon sampling on our phylogenetic inferences through a sensitivity analysis comparing results from datasets with varying levels of taxonomic completeness. We use our results to test previous hypotheses of *Anoura* relationships (Fig. 1); to evaluate taxonomic arrangements for the genus; to test the monophyly of *Anoura*, *Lonchoglossa*, and the *A. caudifer* and *A. geoffroyi* species complexes; and to infer the diversification

times and biogeographic patterns of *Anoura*. By comparing gene trees from concatenation and MSC models against our congruent species tree results, we also find evidence of polyphyly consistent with confounding factors such as incomplete lineage sorting or hybridization-mediated introgression, indicating several fruitful areas for future research.

2. Materials and methods

2.1. Taxon sampling

We obtained tissue samples from 42 individuals representing 9 nominal species of *Anoura* (*A. aequatoris, A. cadenai, A. caudifer, A. luismanueli, A. fistulata, A. carishina, A. latidens, A. geoffroyi* and *A. peruana*) from the northern Andes, the Amazon rainforest, and the Caribbean region (Fig. 2; Suppl. Table S1). Our sampling includes part of the type series of *A. cadenai* (*n* = 3) and *A. carishina* (*n* = 4) as well as specimens treated as *A. aequatoris* (*n* = 3) and *A. peruana* (*n* = 4) by previous authors. We also sampled two *Glossophaga* from the Colombian Andes to use as outgroups, including *G. soricina* (*n* = 1) and *G. longirostris* (*n* = 1). Two *Anoura* specimens were morphologically similar but could not be confidently assigned by the authors or other taxonomic experts to any nominal *Anoura* species based on external anatomical characters or tooth characters; therefore, here we treat these specimens as a putative candidate species, '*Anoura* sp. A'. We collected 7 samples in the wild, including 5 samples (Acul222, Acul226, AculNM83, AfisNM49, and AfisNM95) from specimens that were captured and released (thus are not supported by voucher specimens), and two from specimens deposited in the Muchhala Lab at the University of Missouri-St. Louis (Ageo251 and Ageo252). Samples from museum voucher specimens included in our

study came from the following collections: Colección Teriológica Universidad de Antioquia (CTUA, Medellín, Colombia), Colección de Mamíferos Alberto Cadena García (ICN, Instituto de Ciencias Naturales, Universidad Nacional, Bogotá Colombia), Colección de Mamíferos Museo de Ciencias Naturales de la Salle (CSJ-m Instituto Tecnológico Metropolitano, Medellín, Colombia), Field Museum of Natural History (FMNH, Chicago, USA) and the Abilene Christian University Natural History Collection (ACUNHC, Abilene, TX, USA).

Species assignment to nominal taxa followed the recommendations of Griffiths and Gardner (2008), Calderon-Acevedo et al. (in review) and Chapter 2 of this dissertation in not recognizing *A. aequatoris, A. carishina* and, *A. peruana* as distinct species. Specifically, we treated *A. carishina* as a synonym of *A. latidens* and *A. peruana* as a synonym of *A. geoffroyi.* We also treat *A. aequatoris* as a synonym of *A. caudifer*, rather than a separate species or subspecies rank, given that *A. caudifer* shows high morphological variation across its geographical distribution (Tamsitt and Valdivieso, 1966; Calderón-Acevedo and Muchhala, 2018), and the characters used to separate *A. caudifer* and *A. aequatoris* have previously been shown to be unreliable (Jarrín-V and Kunz, 2008). Supplementary data SD1 Table 1 lists the voucher catalog numbers, geographical sampling localities, as well as NCBI BioProject and BioSamples accession numbers for all samples included in this study.

2.2. DNA extraction, UCE sequencing, and data processing

We extracted whole genomic DNA from ethanol-preserved tissues and museum skins using the Puregene DNA isolation kit (Gentra System, Minneapolis, MN). Tissue samples from museum specimens were prepared for extraction using a series of daily

ethanol washes. Samples were immersed and vortexed in 99% ethanol with a subsequent 70% ethanol wash for 4 days to remove contaminants (Velazco and Patterson, 2013; Giarla and Esselstyn, 2015). Whole genomic DNA samples were sent to Rapid Genomics LLC (Gainesville, FL) for library preparation and target enrichment of over 2386 UCEs in the tetrapod 2.5K probe set (Faircloth et al., 2012), followed by multiplexed paired-end (2×100bp) sequencing of the UCEs on Illumina HiSeq 3000 PE100 machines. We demultiplexed and assembled the UCE reads using the software program phyluce v1.6 (Faircloth, 2016, 2017). These demultiplexed sequence reads were subjected to quality control to remove low quality bases and adapter sequences in Trimmomatic (Lohse et al., 2012; Del Fabbro et al., 2013), as implemented in the program Illumiprocessor (Faircloth, 2013). Subsequent to this, we performed a *de novo* read assembly to obtain larger contigs using ABySS v1.5.2 (Simpson et al., 2009) with the default k-mer value of 35. After probes and UCEs were matched, we aligned UCE contigs with MAFFT v7 (Katoh and Standley, 2013) using the default settings. We phased the final aligned contigs using the program 'phyluce_snp_bwa_multiple_align' in phyluce, and then we extracted biallelic single nucleotide polymorphism (SNP) data from the BAM files using the phyluce program 'phyluce_snp_phase_uces'. Finally, phased SNPs were realigned across samples for each UCE locus using MAFFT v7 (Katoh and Standley, 2013).

The final, full dataset contained 2039 phased UCE loci for all 42 individuals (thus n = 84 phased sequences per locus). To facilitate a sensitivity analysis evaluating the effects of taxon sampling on our phylogenomic species tree results, we split the concatenated alignment and filtered individual loci based on four different levels of taxonomic completeness, as follows (name, followed by taxonomic threshold percentage and number of loci in parentheses): 70p (70%, 1839 loci), 80p (80%, 1432 loci), 90p

(90%, 432 loci), and 95p (95%, 100 loci). All loci in each filtered dataset met the sampling value threshold (%), while loci not meeting this threshold were excluded. To make it possible to objectively test the validity of our assignment of individuals to species/lineages (see above) using population-level methods, we generated a reduced SNP dataset for population genomics analyses. We converted the full dataset alignment into variant call format (VCF) v4.1, evaluated the number of SNPs and then subsampled the data to 1 SNP per UCE locus (n = 2039 SNPs) using the 'FASTA2VCF' function in PIrANHA v0.3a2 (Bagley, 2019). As SNPs within a given locus are under strong linkage disequilibrium, subsampling was conducted to remove the effects of linkage disequilibrium on the downstream genetic analyses. To test for global patterns of genetic structure and genetic differentiation among species based on our assignment of individuals to species, we performed principal components analysis (PCA) on the reduced SNP dataset using the smartpca program in the EIGENSOFT package (Patterson et al., 2006; Price et al., 2006). The Tracy–Widom statistic was used to test for the presence of significant population structure, by species, and analysis of variance (ANOVA) statistics were used to test for significant pairwise divergences of species along significant eigenvectors (Patterson et al., 2006). Tests were considered significant at the $\alpha = 0.05$ level. A PCA on craniodental morphometric measurements was conducted on the same specimens used in our analyses to explore the morphospace of the *Anoura* specimens used in our analysis. Raw reads generated and used during this research are available under BioProject PRJNA529738, available at http://www.ncbi.nlm.nih.gov/bioproject/529738. Supplementary material, aligned sequences, trees and input files are available from the Mendeley Data accession for this project, which can be found at: http://dx.doi.org/10.17632/xhxbf5hyyt.1

2.3. Phylogenomic analyses

We estimated the 'best' maximum-likelihood (ML) gene tree for every UCE locus in RAxML v8.2.12 (Stamatakis, 2014) while specifying the GTR+Γ model and gauging nodal support based on 100 bootstrap pseudoreplicates, using the MAGNET v1.1.0 pipeline available in PIrANHA (Bagley, 2019). Subsequently, gene trees and bootstrap trees were used to estimate the species tree of every dataset (e.g., 70p, 80p, 90, 95p, and the full dataset) using the best tree and multilocus bootstrapping approaches available in ASTRAL-III v5.6.3 (Mirarab et al., 2014; Zhang et al., 2017). ASTRAL-III computes a species tree from gene trees using the species assignment as a prior by estimating quartet relationships from all of the supplied gene trees and then finding the species tree that agrees with the largest amount of the estimated quartets. ASTRAL-III also provides branch support in the form of local posterior probabilities and internal branch lengths in coalescent units of gene tree discordance (Sayyari and Mirarab, 2016).

We inferred the optimum partitioning scheme for the full dataset, including the optimum number of data subsets and their DNA substitution models in PartitionFinder v2.1.1, using the 'rcluster' algorithm and the Bayesian information criterion (BIC) (Lanfear et al., 2014; Lanfear et al., 2017). We then used SVDquartets v1.0 (Chifman and Kubatko, 2014, 2015) to obtain estimates of the species tree as well as the multispecies coalescent gene tree, or 'lineage tree', based on quartet assembly methods. We conducted independent runs of SVDquartets on the full dataset while partitioning the data into (1) all 2039 UCE loci and (2) the optimum data subsets identified by PartitionFinder. In each SVDquartets analysis, we exhaustively sampled quartets and estimated node support using 500 non-parametric bootstrapping pseudoreplicates. SVDquartets uses singular value decomposition (Eriksson, 2005) to infer the relationships within sets of four

random taxa from the complete dataset. After inferring the relationships between sets of quartets, SVDquartets estimates the three valid splits present in each quartet by computing an SVD score, then all estimated quartets and their SVD scores are used to infer the species tree.

2.4. Divergence time estimation

To obtain estimates of the divergence times of *Anoura* lineages, we use the penalized likelihood (PL) framework (Sanderson, 2002) implemented in the software program treePL (Smith and O'Meara, 2012). Given a tree topology with branch lengths in substitutions/site, treePL uses a semi-parametric PL approach to estimate rates of gene evolution on different branches of the tree, greatly reducing the computation time for large, genome-wide multilocus datasets. We obtained divergence times in treePL by calibrating the best ML tree from a concatenated 'supermatrix' analysis of the full dataset in RAxML (-f a x options, with 100 rapid bootstrapping iterations) using two secondary calibration points based on divergence dates inferred by Rojas et al. (2016) in a broader, multilocus analysis of evolution in Noctilionoidea. Calibration points included the upper and lower 95% credible intervals of the most recent common ancestor (MRCA) date estimates for the divergence of *Anoura* and *Glossophaga* (22.21–17.22 million years ago, Ma) and of all *Anoura* species (9.75–5.03 Ma). We ran treePL multiple times with random seeds, using the 'thorough' run mode and the leave one out cross validation procedure.

2.5. Historical Biogeography

To reconstruct the geographic distributions and dispersal events of *Anoura* species over our time-calibrated tree we used the R package 'BioGeoBEARS' (Matzke, 2013a,

2016). We coded five geographical areas representing the geographical distribution of our samples: B=Brazil's Atlantic forest, A=Amazonia, C=Central Andes of Peru and Ecuador, N= Northern Andes in Colombia and L= Lesser Antilles. We conducted two analyses, one including a pruned species tree (including only one tip per species and coding the areas using the distribution of the species) and our complete time-calibrated tree (with tips for each specimen and coding the areas based on the geographic locality of the sample). We tested six models: Dispersal Extinction Cladogenesis (DEC, Ree and Smith, 2008), a likelihood version of the parsimony Dispersion-Vicariance model (DiVA, Ronquist, 1997) called DIVALIKE and a likelihood version of the BayArea model (Landis et al., 2013) called BAYAREALIKE, as well as the versions of these models allowing for founding event speciation (+J models, Matzke, 2013b; Matzke, 2014). The maximum likelihood framework implemented in BioGeoBEARS on the DIVALIKE and BAYAREALIKE models allows a direct comparison of model fit using statistical tools. We used Akaike Information Criterion (AIC, Akaike, 1973, 1998) and a second order AIC correcting for small sample sizes (AICc, Anderson and Burnham, 2004; Burnham and Anderson, 2004) to statistically compare the fit of the models to our data.

The DEC model (Ree and Smith, 2008) assumes that daughter lineages inherit the ancestral area state if the MRCA is limited to a single area or if it has a widespread distribution, with one daughter lineage inhabiting only one area from the subset of possible areas of the MRCA's ancestral distribution. On the other hand, DiVA (Ronquist, 1997) reconstructs ancestral distributions without assumptions about the relationships between areas and assumes that speciation depends on vicariance events. Finally, the BayArea model assumes that there is no range evolution at cladogenesis and thus the ancestral range is inherited by both daughter species. This model also allows for the

inclusion of a large number of areas (Landis et al., 2013; Matzke, 2013b). The +J version of the mentioned models adds a jump speciation/dispersal parameter (J), accounting for founder event speciation (Matzke, 2014). Founder event speciation implies that a daughter species "jumps" to a new area outside of the MRCA's ancestral area, allowing for dispersal events over large distances (Paulay and Meyer, 2002; Templeton, 2008).

3. Results

3.1. Ultraconserved elements data processing and assignment validation

We obtained a total of 179,809,722 raw forward and reverse reads, with an average of 4,281,184 reads per individual (range: 765,774 – 7,704,220), 499,242 contigs per individual (range: 88,262 – 857,926), and 1891 UCE loci per individual (range: 1423 – 1965). The matrix of sequences for all 2039 UCE loci in the full dataset contained 986,712 aligned nucleotides, with slightly elevated A and T frequencies (A: 29.6%; C: 20.5%; G: 20.5%; T: 29.4%). Overall, this dataset was highly informative, with 51,103 variant sites or SNPs, of which 38,047 were parsimony-informative sites (range 0 – 129 parsimony-informative sites per UCE locus).

We used PCA to independently test for genetic structure and differentiation of species in our assignment scheme used during species tree inference in ASTRAL-III and SVDquartets. While PC1 (which explained 43.31% of observed genetic variation) only significantly differentiated *A. cadenai* from *A. caudifer* based on ANOVA statistics ($p = 4.7 \times 10^{-4}$), the two species complexes species were differentiated along PC2 (which explained 5.9% of observed genetic variation) (Fig 3, supplementary figure, Fig. S1). Within the *Anoura caudifer* species complex the smaller species, *A. caudifer* and *A. luismanueli* are differentiated from the larger *A. cadenai* and *A. fistulata*; while within the

large-bodied *Anoura A. cultrata* is differentiated from the *A. geoffroyi* species complex. PC2 also differentiated the four main clades in our ASTRAL-III species tree from the analysis of the full dataset (Fig. 3, supplementary figure, Fig. S1), showing that clusters also diverged in the genetic space into two broader groups reflecting the previously hypothesized division between the *A. caudifer* and *A. geoffroyi* species complexes (Mantilla-Meluk and Baker, 2006, 2010) (Fig. 3A). The PCA on craniodental measurement of the specimens shows that species differentiate in the morphological space (Supplementary figure Fig. S3B). Principal component 1 of the morphological PCA explains 75.09 %, while PC2 explains 8.36 of the morphological variation. Species of *Anoura* differentiate along PC1, with the two species complexes occupying separate sides of the morphospace.

3.2. Patterns of relationships estimated from UCEs

The ASTRAL-III species tree estimated from the full UCE dataset contained four main clades. *Anoura* initially splits into the *A. caudifer* species complex (clade 1 and 2) and all other species (clade 3 and 4). The *A caudifer* complex includes the medium-bodied species *A. cadenai, A. fistulata* and *A.* sp A in clade 1, and the small-bodied *A. caudifer* and *A. luismanueli* in clade 2. The remaining species are all large-bodied, including the *A. geoffroyi* complex with *A. latidens* and *A. geoffroyi* in clade 3, which is sister to clade 4 containing only *A. cultrata* (Fig.3B). Local posterior probability support values were 1.0 for all splits with the exception of the node between *A. cultrata* and the clade containing *A. geoffroyi* and *A. latidens,* which had a local posterior probability of 0.98. The ASTRAL-III species trees computed from the reduced datasets, with taxon completeness at 70%, 80%, and 90%, had similar topologies to that of the full dataset,

suggesting that missing data had a minimal impact on our species tree inferences (See supplementary material S2). However, the ASTRAL-III species tree inferred for the 95p dataset reconstructed *A. cultrata* as sister to the *A. caudifer* species complex rather than clade 3, with low posterior support of 0.41 (supplementary figure, Fig. S2).

The species tree inferred using SVDquartets was identical to our ASTRAL-III species tree in terms of topology. It had strongly supported relationships with 100% bootstrap support, with two exceptions. First, the node corresponding to the clade of larger-bodied *Anoura* (including *A. cultrata* in clade 3 and *A. latidens* and *A. geoffroyi* in clade 4) had bootstrap support ranging from 71% to 72% (Fig. 4A). Second, whereas *Anoura* was strongly supported as monophyletic in the ASTRAL-III results, it never received bootstrap support greater than 50% in the SVDquartets species tree analysis.

The inferred lineage tree based on the multispecies coalescent (Fig. 4B) yielded the same clades obtained in the ASTRAL-III (Fig. 3B) and SVDquartets species trees (Fig. 4A) and had high bootstrap support for all internal branches between the four clades, with lower support for the branch leading to clades 3 and 4. Relationships within clade 1 (*A. fistulata* as the sister taxa to *A.* sp A and *A. cadenai*) as well for clade 2 (*A. caudifer* and *A. luismanueli*) remained the same as the SVDquartets species tree. However, one sample of *A. caudifer,* Aaeq210 (previously attributed to *A. aequatoris*), was inferred as sister to our only sample of *A. luismanueli*, Alui212, and other specimens previously designated as *A. aequatoris* were nested within *A. caudifer* in the SVDquartets tree topology (Fig. 4B). We also found in this topology that *A. caudifer* formed a cohesive species despite its large geographic distribution, with sample Acau271 from Brazil sister to all other Andean individuals of *A. caudifer.*

The MSC lineage tree from SVDquartets suggested several noteworthy points that beget further research in *Anoura* phylogenetic relationships. We find that *Anoura cultrata* samples form the sister clade to the *A. caudifer* species complex, with 100% bootstrap support. However, one individual that is morphologically diagnosable as *A. latidens* (sample Alat90; ICN-4398) was nested among the *A. cultrata* samples. This individual, from the Cordillera Occidental of Colombia, has all of the diagnostic traits of *A. latidens* and lacks the characteristic blade-like first lower premolar (p1) of *A. cultrata*. It falls immediately sister to a lineage containing sample Acul208 (ICN-21196) from the western slope of the Cordillera Central, which comes from a specimen lacking the dental characteristics of *A. latidens* (i.e. it does not possess a triangular last upper premolar (P4) with the base of the tooth enclosing the medial-internal cusp), and which possesses a unique, blade-like first lower premolar. Also in the SVDquartets lineage tree, *A. geoffroyi* was inferred as reciprocally monophyletic to *A. latidens*, whereas samples from Peru previously attributed to the nominal taxa *A. peruana* interdigitated with the Caribbean and Andean samples of *A. geoffroyi*.

3.3. Divergence times of *Anoura* species

Ultrametricizing the branches of our concatenated RAxML tree using PL analysis in treePL allowed us to estimate divergence times, yielding a chronogram revealing that *Anoura* is a relatively recent Neogene genus. We date the most recent common ancestor (MRCA) of *Anoura to* around ~9.3 Ma in the Miocene corresponding to the initial divergence between the large- and small-bodied *Anoura* clades (clades 1 and 2 vs. clades 3 and 4), with subsequent diversification events within lineages occurring over the Pliocene to Pleistocene (Quaternary). Within the large-bodied clade, the divergence of its

three species dated to approximately 8.72 Ma in the late Miocene, and diversification within the *A. geoffroyi* species complex is marked by an initial divergence at the MRCA ~5.14 Ma in the Pliocene, while diversification within the *A. cultrata* clade started around 2.07 Ma in the Pleistocene. We also infer that diversification within *A. geoffroyi* and *A. latidens* took place since around 3.41 Ma and 1.72 Ma, respectively. Within the small-bodied *Anoura caudifer* species complex, we infer that its two major lineages diverged around 6.15 Ma in the latest Miocene. Within this complex, while the MRCA of *A. caudifer* indicates genetic variation has arisen since ~3.05 Ma in the Pliocene, all other species speciated or experienced intraspecific genetic divergence soundly within the Pleistocene epoch (MRCAs: *A. fistulata*, 2.56 Ma; *A.* sp A, 1.50 Ma; *A. cadenai*, 1.06 Ma; *A. luismanueli*, 0.71 Ma).

3.4 Biogeographic reconstruction

The model that best fits both our complete and pruned ultrametric trees was the DEC+J model with the highest Likelihood (complete tree: lnL=-40.09; pruned tree: lnL=-20.69) and lowest AIC (all specimens=86.17; pruned tree=47.37) and AICc (all specimens=86.84; pruned tree=53.37) scores (Table 1). We find contrasting results in the AIC and AICc scores when analyzing our pruned tree, in which the DEC model has a better AIC and AICc support than the DEC+J model despite the DEC+J model having a better likelihood (Table 1). The addition of the J parameter increased the Likelihood of the DEC, DIVALIKE and BAYAREALIKE models, showing the importance of accounting for founder event speciation (Table 1).

In both analyses (pruned species tree and complete tree) the DEC+J model infers the range of the MRCA of *Anoura* as occupying all possible areas. This pattern is present

also in the MRCA of *A. cultrata* and the *A. geoffroyi* species complex as well as the MRCA of the *A. caudifer* species complex (Fig. 6). Clade 1 (*A. cadenai*, *A. fistulata*, and *A.* sp A) shows a fragmentation event from the ancestral range, with *A. fistulata* remaining in the central and northern Andes while the MRCA of *A. cadenai* and *A.* sp A remains in the northern Andes and the Amazons. Clade 2 (*A. caudifer* and *A. luismanueli*) shows a dispersal event to the Brazilian Atlantic forest in *A. caudifer* and a fragmentation of the original range, while *A. caudifer* remains present in all the areas *A. luismanueli* is restricted to the northern Andes. For Clade 3, the *A. geoffroyi* species complex is reconstructed as having a wide distribution with subsequent fragmentation of the MRCA of *A. latidens* and *A. geoffroyi*; when looking in detail at the reconstructed ranges using the complete tree, we see that *A. geoffroyi* had an ancestral area in the northern Andes with multiple dispersal events to the central Andes and the Lesser Antilles. Clade 4, *A. cultrata,* is restricted to the central and northern Andes in our study although it is (like *A. geoffroyi*) distributed in Central America as well.

4. Discussion

Our phylogenomic analysis provides support for four well-established clades within the glossophagine bats of the genus *Anoura*. The phylogenetic relationships among these major lineages are consistent with previous taxonomic assignments of their constituent species into species complexes, and our results strongly support the monophyly of the *A. caudifer* and *A. geoffroyi* species complexes proposed by Mantilla-Meluk and Baker (2006, 2010). Our findings also provide robust genomic evidence corroborating morphological evidence supporting the treatment of *A. carishina* as a junior synonym of *A. latidens* (Calderon-Acevedo et al., in review), given the position of the

type specimen of *A. carishina* as falling within the *A. latidens* clade, with the paratype of *A. carishina* previously identified as *A. geoffroyi* nested within the *A. geoffroyi* clade (Figs. 4A and 5). Another taxonomically relevant finding is that specimens previously attributed to *A. aequatoris* interdigitate with *A. caudifer*, which provides additional support for the treatment of *A. caudifer* as a monotypic species. The biogeographic patterns inferred from our analysis are preliminary but represent a first step in understanding the evolution of a genus where several species have sympatric distribution, with up to five species present in some localities of the Central and Northern Andes Griffiths and Gardner, 2008; Pacheco et al., 2018). Our analyses show that the most recent common ancestor of *Anoura* most likely had a wide distribution covering all areas where the genus currently occurs, with subsequent fragmentation of ancestral populations that eventually reached reproductive isolation and evolved into independent species. Interestingly, *Anoura caudifer* is sympatric with all species in the *A. caudifer* species complex (e.g*., A. cadenai, A. fistulata, A. caudifer, A. javieri* and *A. luismanueli*), thus there is no clear scenario of reproductive isolation due to vicariance, even when taking into account the restricted distributions of *A. cadenai* (central and western Andes of southern Colombia), *A.* sp A (Amazon) and *A. fistulata* (central and northern Andes). This is evident in the *A. geoffroyi* species complex as well, where you can find the complete distribution range of *A. latidens* being immersed in the distribution of *A. geoffroyi* (Handley, 1984; Griffiths and Gardner, 2008). Thus, reproductive isolation leading to speciation cannot be readily explained by geographic isolation alone, and therefore speciation with gene flow and other factors such as mate-choice and ecological speciation may have played important roles.

4.1. Phylogenetic relationships in *Anoura*

Our results support the reciprocal monophyly of small- and large-bodied *Anoura*. We find that the two larger species of the *A. caudifer* species complex (*A. fistulata* and *A. cadenai*) are closely related and include *A.* sp A. *Anoura fistulata* is a cohesive species across the eastern and western Andes of Ecuador, and is the sister clade to *A. cadenai* and *A.* sp A. *Anoura caudifer* remains cohesive, with a sample from Brazil being sister to all Andean individuals. *Anoura caudifer* is the most variable species within the genus, exhibiting variation in size and skull shape through its range (Jarrín-V and Kunz, 2008; Jarrín-V and Coello, 2012; Calderón-Acevedo and Muchhala, 2018), this variation in size could reflect a geographic trend of size variation as seen in *A. cultrata* (Nagorsen and Tamsitt, 1981; Tamsitt and Nagorsen, 1982).

Our results regarding the *Anoura geoffroyi* species complex and *A. cultrata* (large-bodied *Anoura*) provide a better understanding of these taxa. Large-bodied *Anoura* remain together in two clades (clades 3 and 4, Fig. 3). The use of the term *A. geoffroyi* species complex is appropriate when referring to *A. latidens* and *A. geoffroyi*, and it should not include *A. cultrata* until its position regarding both species complexes is clarified. Moreover, our results regarding the position of Alat90 within the *A. cultrata* clade shows a polyphyletic pattern, possibly indicating introgression or hybridization events during the past 2 Ma (Figs. 4A and 5). Sample Alat90 has all the characteristics of *Anoura latidens* and lacks the blade-like lower premolar unique to *Anoura cultrata* (Handley, 1960, 1984; Jarrín-V and Kunz, 2008), suggesting this is not simply a case of misidentification. We are not sure as to the nature of this event; however further incongruence analyses comparing mitochondrial and nuclear genes could help resolve the particular position of this *Anoura latidens* individual within *A. cultrata.*

Both approaches used to infer the species tree yielded congruent results. However, our lineage tree calculated from SVDquartets contrasts with our species trees and the concatenated ML tree in the inferred placement of *A. cultrata.* In both species trees and the ML tree, *A. cultrata* is sister to the *A. geoffroyi* species complex (Figs 3B, 4A and 5), while in our lineage tree *A. cultrata* is sister to the *A. caudifer* species complex.

4.2. Divergence times and historical biogeography of *Anoura*

The divergence time results provide a new perspective into the biogeographic patterns and evolution of *Anoura* in the past 9 Ma. We generally agree with Rojas et al. (2016) in dating the MRCA of extant species of *Anoura* but provide the first divergence time estimation of *Anoura* including more than 4 species. Although the Andes began to form from 30 to 20 Ma, the uplift accelerated during the last 10 Ma (Gregory-Wodzicki, 2000; Garzione et al., 2008; Hoorn et al., 2010; Mora et al., 2010; Garzione et al., 2014). It is during this accelerated Andean uplift that *Anoura* appears, and in the past 4 Ma it diversified into its extant species (Fig. 5). *Anoura* diversified at the same time as other species of nectarivorous bats (Rojas et al., 2016) but apparently at a faster rate, being the most speciose genus of nectarivorous bats; however it has diversified at a slower rate when compared to other subfamilies of Phyllostomidae like Stenodermatinae, the most species rich subfamily (Velazco and Patterson, 2008; Velazco and Simmons, 2011; Velazco and Patterson, 2013; Rojas et al., 2016).

Our ancestral area reconstruction provides insights into how species of the *A. caudifer* and *A. geoffroyi* species complexes attained their current distributions. Despite the fact that most species within the genus are sympatric, we see several speciation events

within this sympatric distribution. However, given the constraints of our biogeographic analyses, we are aware that the ultrametric trees used as input in our ancestral area reconstruction lack specimens and dates for clades that were not included (i.e. *A. cultrata* and *A. geoffroyi* from Central America, and *A. latidens* from the central Andes). Our results suggest that the ancestor of *Anoura* had a wide geographical range that fragmented over time into the present distribution. However, there are not clear patterns of geographical barriers having an effect on speciation. For instance, *Anoura* occupies a wide range of habitats from Central America to central South America, although some species have relatively restricted ranges they still have sympatric distributions. *Anoura luismanueli* is known only from the northern Andes while *A. cadenai* is registered in the central and western Cordilleras of Colombia (Mantilla-Meluk and Baker, 2006; Mantilla-Meluk et al., 2009) and *A. fistulata* has been reported from the southern Colombia with several localities in the western and eastern Andes of Ecuador and Peru (Gárate-Bernardo and Carrasco-Rueda, 2011; Calderón-Acevedo and Muchhala, 2018). In the case of the *A. geoffroyi* species complex, *A. latidens* has a narrower distribution range, present in the Venezuelan lowlands, northern and central Andes (Calderón-Acevedo and Muchhala in review) while *A. geoffroyi*, is distributed from the Brazilian Atlantic forest, the Amazons, Central and Northern Andes, Lesser Antilles and Central America (Griffiths and Gardner, 2008). Despite the narrow distributions of some species, there are localities in which 5 or more species of *Anoura* can be identified (Handley, 1960, 1976, 1984; Alberico et al., 2000; Pacheco et al., 2018) with access to the same resources, and *A. caudifer* and *A. geoffroyi* are always sympatric to the other members of their species complexes.

An example of the inter Andean valleys or the Amazons not being a geographic barrier that has diminished gene flow or promoted speciation is found in *Anoura cadenai*

and *A. caudifer*. *Anoura cadenai* is distributed across the inter Andean valleys of Colombia (Fig. 2) and *A. caudifer* is a cohesive lineage across the Andes with Brazilian Atlantic forest specimens coupled with north Andean specimens. Thus the diverse geographical landscape of the northern Andes (where most of the species are present and most of our samples come from) does not seem to present strong barriers to dispersal or vicariance processes that could explain allopatric speciation in the genus. Another example is the possible introgression/hybridization between *A. cultrata* from the western Colombian Andes and *A. latidens* from the central Colombian Andes.

We propose that the next step in understanding speciation in *Anoura* should focus on different alternatives, rather than vicariant events, which could explain how these species have evolved in sympatry. Echolocation provides bats with a unique tool aiding in spatial location and foraging (Fenton and Ratcliffe, 2004), and can be used to determine species identity in cryptic species complexes (Kingston and Rossiter, 2004; Siemers and Schnitzler, 2004; Murray et al., 2012). Echolocation also plays a role in female mate-choice (Puechmaille et al., 2014), which can lead to reproductive isolation and stop gene flow between sympatric species. Echolocation in *Anoura* has been studied in a behavioral framework of *A. geoffroyi* (Chase, 1983; Ortega and Alarcón-D, 2008), but there is no comparison between the characteristics of echolocation calls between species of *Anoura.* Another aspect that has received little attention in glossophagine bats is the use of social communication in different formats (i.e. social calls, olfaction, visual cues). Bats rely on social calls to communicate an individual's identity, group membership, sex, body condition (Chaverri et al., 2018), and signal individual location within roosts (Chaverri et al., 2010; Furmankiewicz et al., 2011). These factors can

influence ecological speciation and should be included in future studies of *Anoura* speciation.

4.3. Taxonomic implications

This study presents several taxonomic implications for *Anoura* and corroborates previous findings using morphology regarding the monophyly of *Lonchoglossa* (Pacheco et al., 2018) and the identity of *A. peruana* (Calderón-Acevedo et al. in review) and *A. aequatoris* (Calderón-Acevedo and Muchhala, 2018). Our phylogenomic results provide evidence for the monophyly of the *Anoura caudifer* and *A. geoffroyi* species complexes, making suitable the use of the name *Lonchoglossa* to refer to this clade. However, we suggest that this could be applied as a subgenus rank rather than elevating the *A. caudifer* species complex to the genus level. The use of subgenera in mammalian taxonomy provides a classification tool, governed by the International Code of Zoological Nomenclature, allowing mammalogists to correctly refer to monophyletic clades without creating nomenclatural and taxonomical instability (Teta, 2018). The most recent taxonomic revisions of the genus point to the need to split the Glossophaginae tribe Anourina (Baker et al., 2003; Baker et al., 2016; Cirranello et al., 2016) into *Anoura* and *Lonchoglossa* (Pacheco et al., 2018). Using *Lonchoglossa* as a generic name for the *A. caudifer* species complex would imply that *A. cultrata* should receive a new generic name, with *A. geoffroyi* and *A. latidens* remaining under the genus *Anoura*. However, given the uncertainty as to where *A. cultrata* is placed in the phylogeny based on out different analyses, we suggest that the genus rank name of *Anoura* should remain for all current species and that the name *Lonchoglossa* could be applied as a subgenus in reference to the *Anoura caudifer* species complex. This classification would reflect the

phylogenetic relationships between the small-bodied *Anoura* (*A. caudifer* species complex).

We find support for synonymizing *A. carishina* to *A. latidens, A. peruana* to *A. geoffroyi* and *A. aequatoris* to *A. caudifer* from the phylogenetic position of the type specimen of *A. carishina* and the interdigitated pattern of *A. peruana* with *A. geoffroyi* and *A. aequatoris* with *A. caudifer* (Fig. 4 B). First, we find that the type specimen of *Anoura carishina* nests within the clade of *A. latidens*, further supporting morphological work suggesting that this name should be treated as a junior synonym of *A. latidens* (Calderon-Acevedo et al., in review). Second, we find that Peruvian samples of *A. geoffroyi*, which were previously ascribed to *A. peruana*, in fact interdigitate with Andean and Caribbean samples diagnosed as *A. g. geoffroyi* (Fig. 4; Supplementary data S1). This result supports previous morphological work showing that the traits used to separate *A. peruana* from *A. geoffroyi* (Mantilla-Meluk and Baker, 2010) are not reliable to separate these species (Calderon et al., in review), and thus that *A. peruana* should be regarded as a synonym of *A. geoffroyi* until a more complete geographic sampling that includes central American samples of *A. g. lasiopyga* can clarify the diversification observed in *A. geoffroyi*. We suggest that population level studies are necessary to understand the relationships and interdigitated pattern of northern and central Andean populations of *A. geoffroyi* and eventually elucidate the evolution of this clade. Finally, we find that the interdigitated pattern seen between *A. caudifer* and *A. aequatoris* supports the monotypic status of *A. caudifer* and that *A. aequatoris sensu* Mantilla-Meluk and Baker (2006) is a synonym of *A. caudifer*.

5. Conclusions

We present the most complete phylogenomic perspective of *Anoura* and elucidate the relationships between the *A. caudifer* and *A. geoffroyi* species complexes. Our results support the monophyly of the *A. caudifer* species complex, allowing for the use of the name *Lonchoglossa* as a subgenus rank when referring to the small-bodied *Anoura*. We support the previous morphological findings and formally synonymize *A. carishina* to *A. latidens, A. peruana* to *A. geoffroyi*, and *A. aequatoris* to *A. caudifer.* Our biogeographic inferences provide a previously unknown time frame for all species of *Anoura* with the exception of *A. javieri*, and show that the MRCA of *Anoura* most likely had a widespread distribution. We find that geographic barriers have not had a major effect in speciation within *Anoura* and other mechanisms could be behind reproductive isolation and promote speciation. The addition of *Anoura javieri* and a better geographical sampling of *A. luismanueli* specimens as well as including samples covering the complete distribution of all species would improve our understanding of *Anoura* evolution. Our future directions include the exploration of the ancestral distributions ranges using ecological modeling (i.e. MaxEnt) that takes into account the effect of glaciation periods in *Anoura* distribution; population level studies within *A. caudifer* and *A. geoffroyi*; and studies focusing on differences in ecology, echolocation and mate choice between *Anoura* species, which may improve our understanding behind species limits of morphologically similar but genetically differentiable species.

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Appendix A. Supplementary material

Supplementary figures and data associated with this article, aligned sequences, trees and input files are available from the Mendeley Data accession for this project, which can be found at: http://dx.doi.org/10.17632/xhxbf5hyyt.1

Supplementary Data S1— Supplementary Table 1.Voucher numbers, Species assignment, geographic records and BioProject and Biosample accession numbers of specimens used in this study

Supplementary Data S2— Supplementary Figure 1. Smartpca complementary results; Supplementary Figure 2. ASTRAL-III species trees of the full and reduced UCE datasets;

Supplementary Figure 3. Comparison between A) smartPCA on sequence data, and B) PCA on morphometric measurements.

Supplementary Data S3— PartitionFinder2 results for the complete UCE dataset.

Supplementary Data S4— Aligned sequences

Supplementary Data S5— Input files

Supplementary Data S6— Trees

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Figures

Fig. 1. Previous phylogenetic hypotheses of *Anoura*

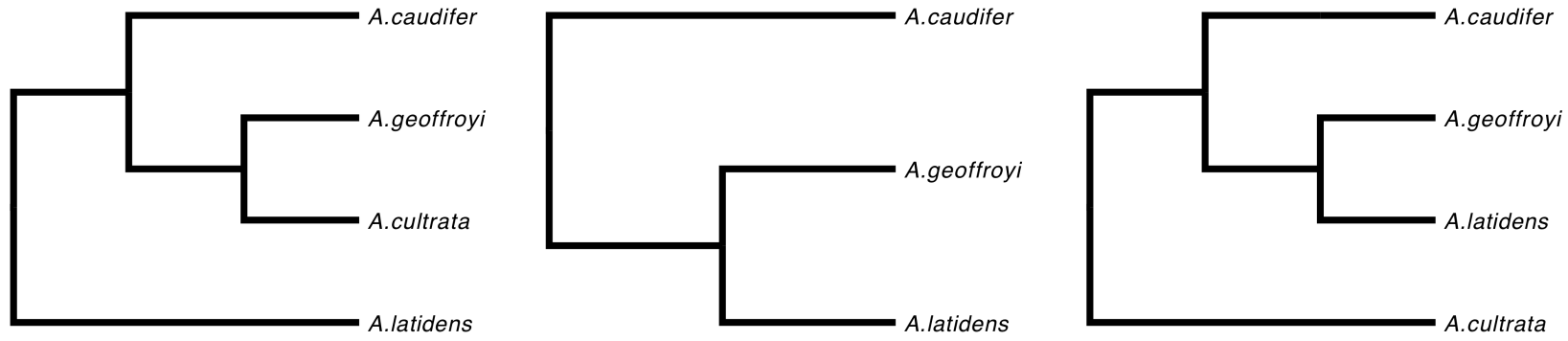


Fig. 2. A) Distribution of the *A. caudifer* species complex geographic extent of our samples. B) Distribution of the *A. geoffroyi* species complex and geographic extent of our samples. Distribution data of *Anoura* obtained from Rojas et al., (2018).

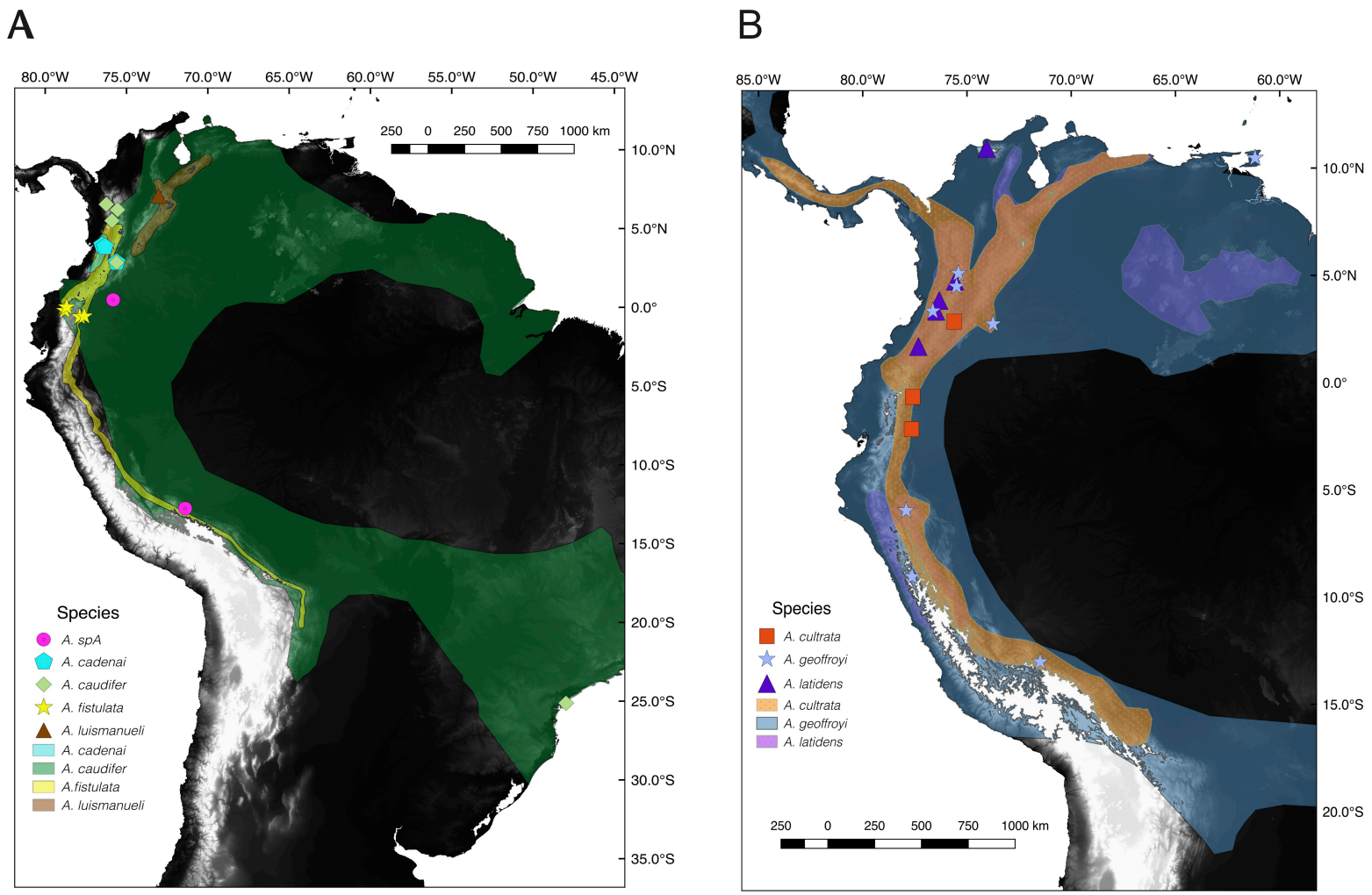


Fig. 3. A) Smartpca analysis showing the different clades present in *Anoura.* B) ASTRAL III species tree

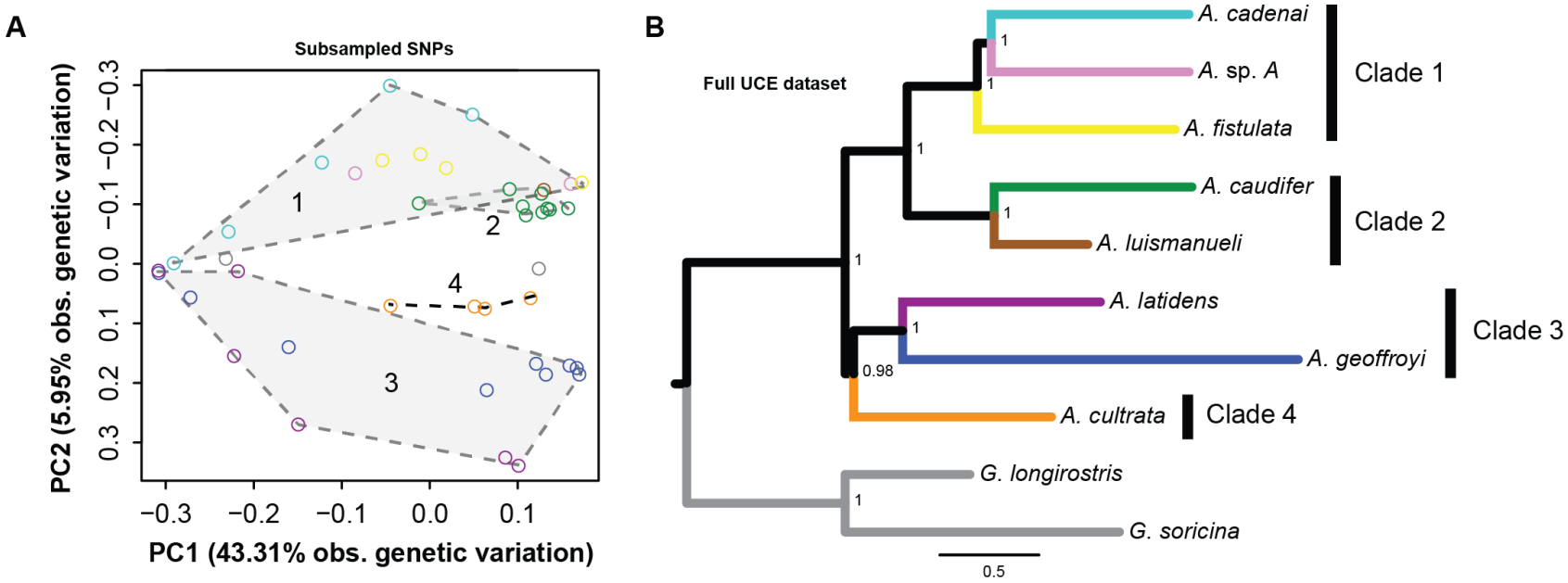


Fig. 4. SVDquartets full data analysis A) SVDquartets species tree. B) Lineage tree of *Anoura* based on a full data analysis of 2039 UCEs, nodal support values are expressed in the following format: Bootstrap support using subsets derived from PartitionFinder 2 / Bootstrap support treating each loci as a separate partition. G1, G2 and G3 correspond to the morphological Groups from Chapter 2

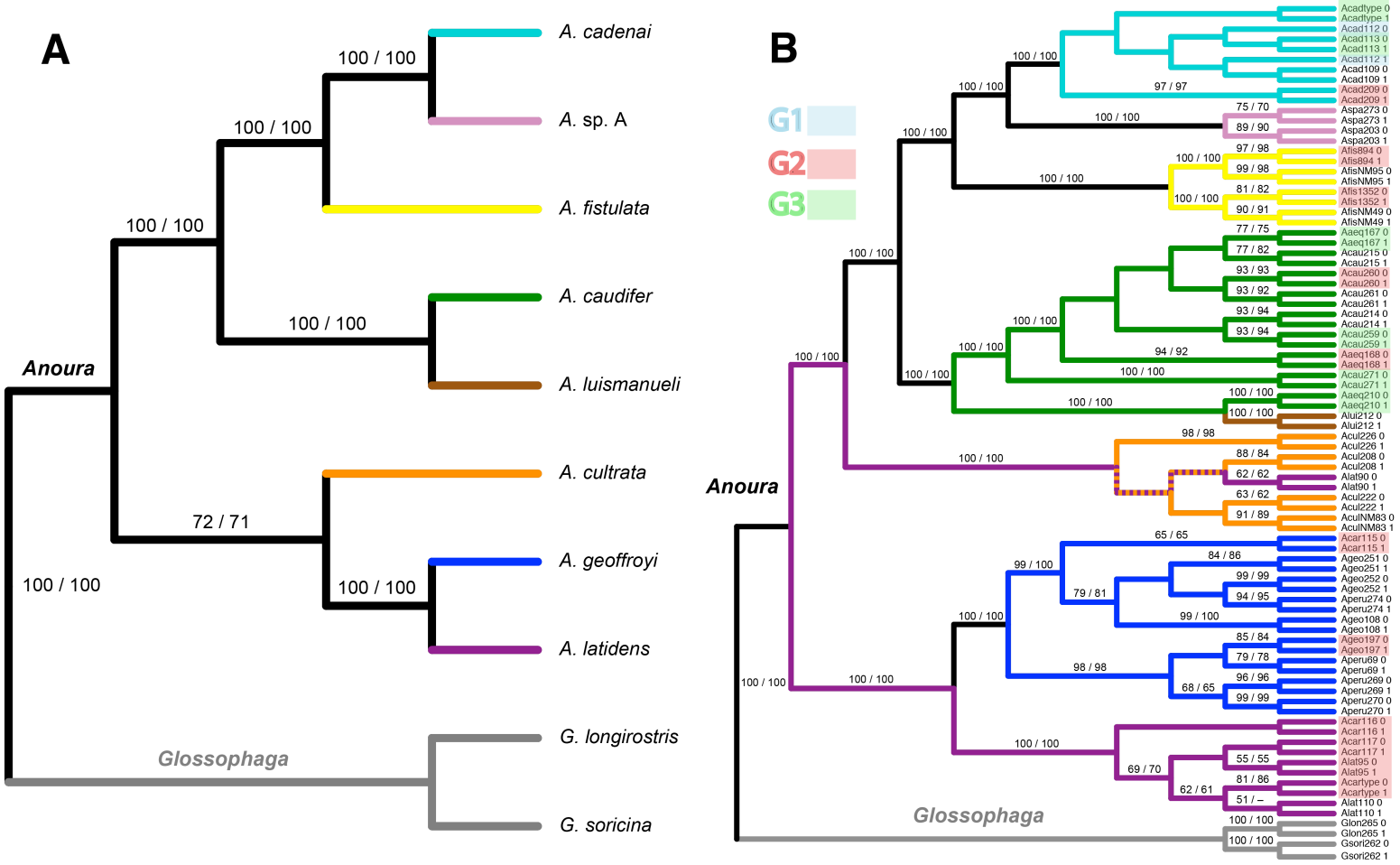


Fig. 5. Diversification time inference of *Anoura* using Penalized Likelihood.

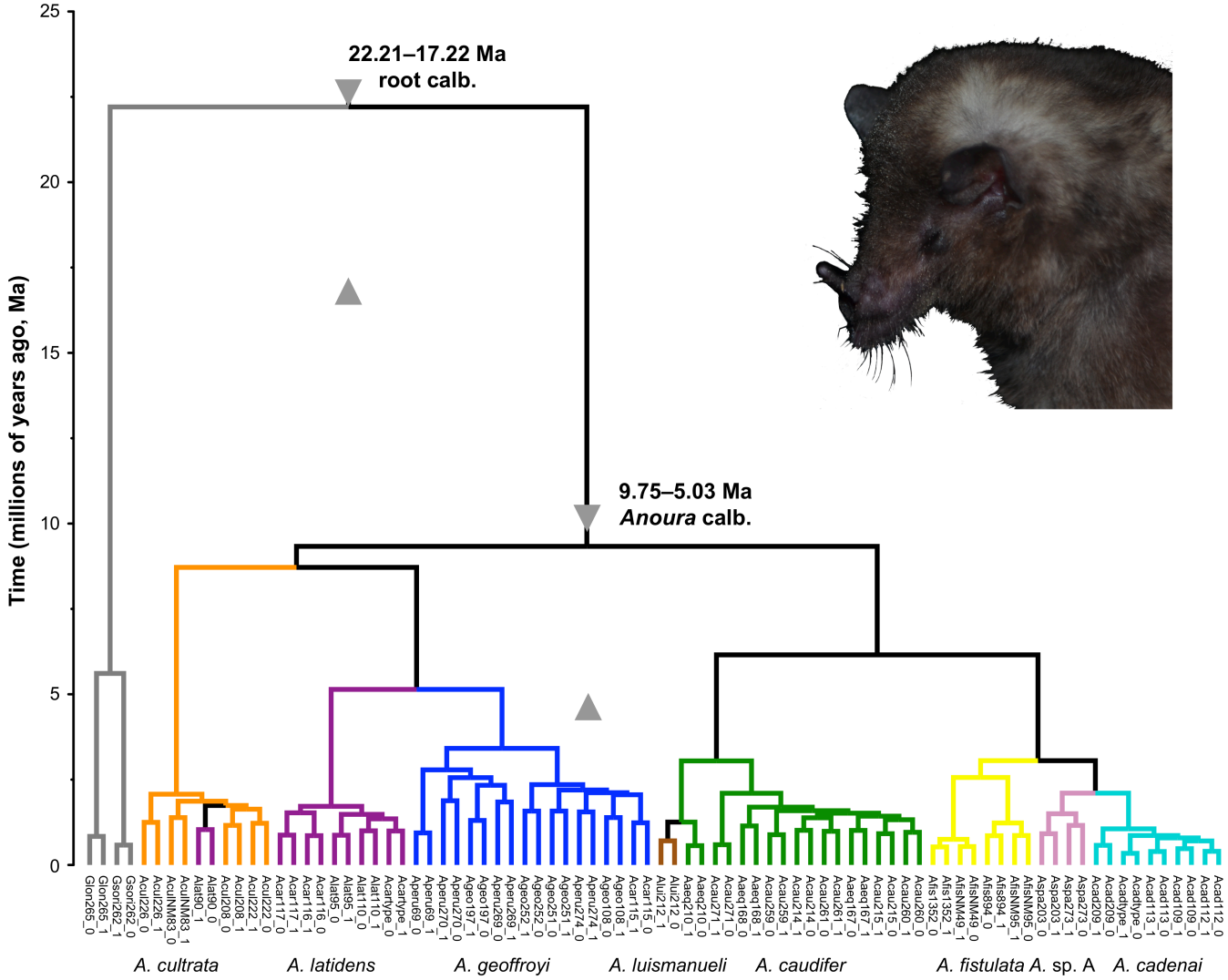
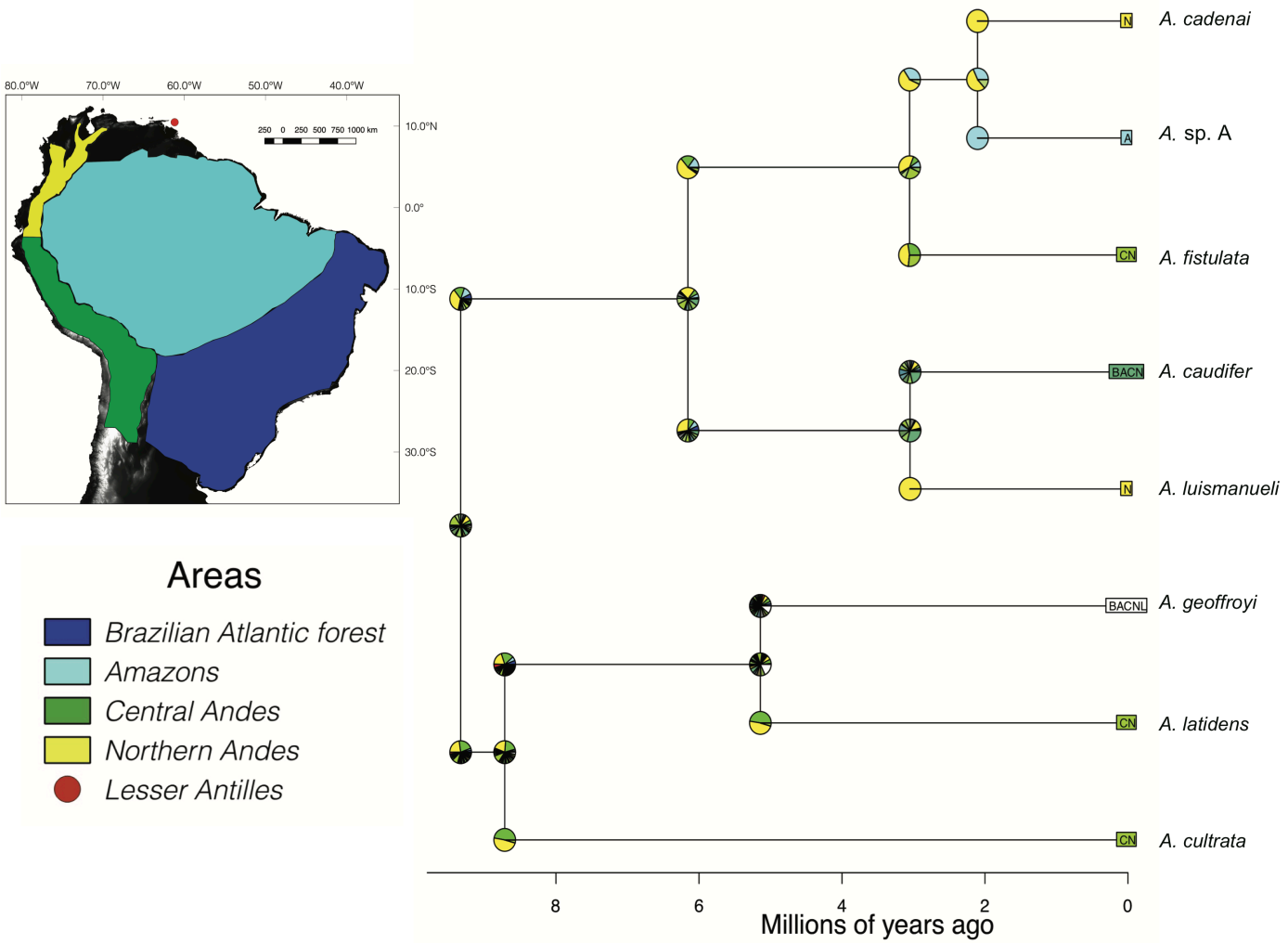


Fig. 6. Ancestral area reconstruction of *Anoura* under the DEC+J model.



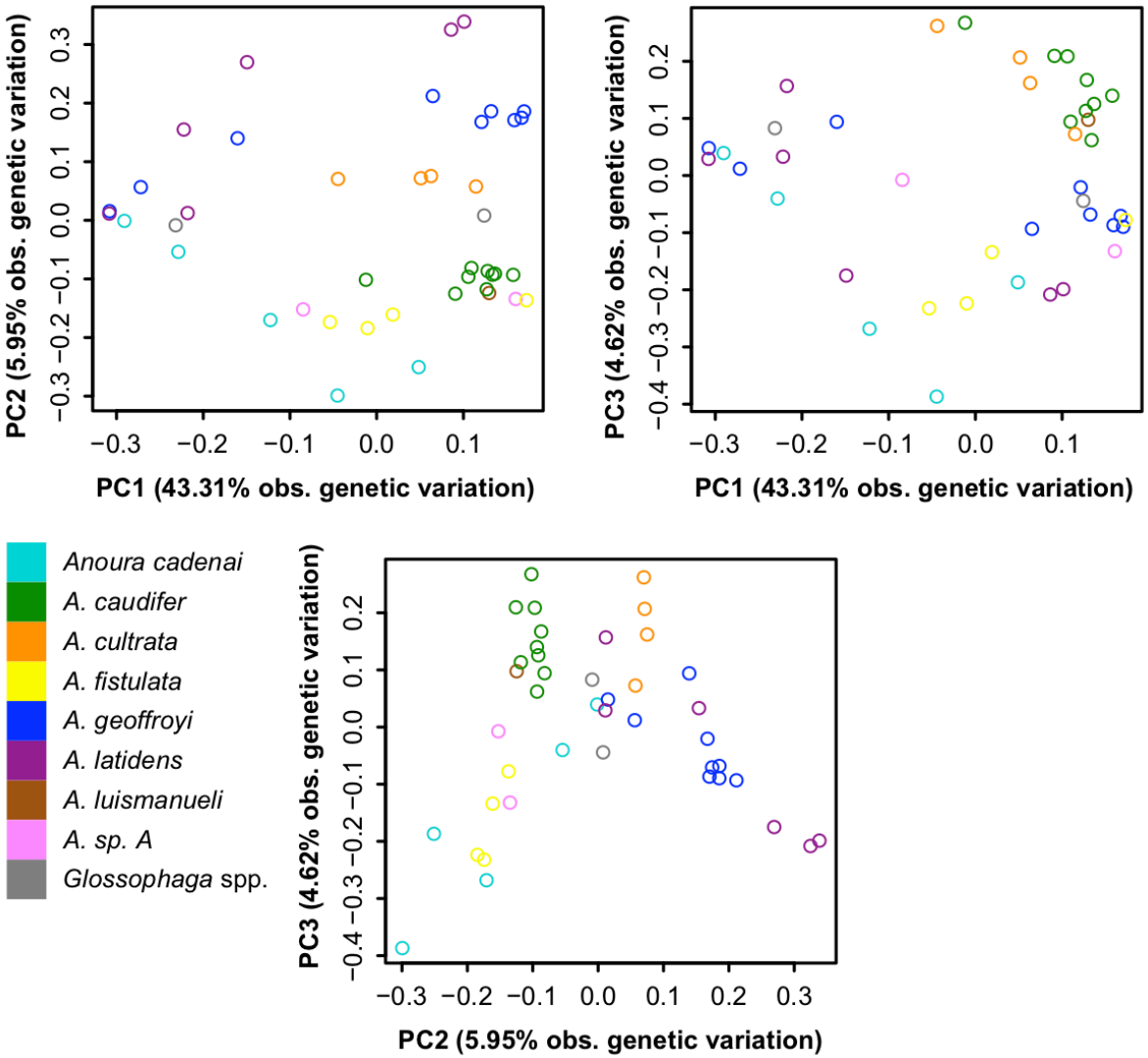
Supplementary material

Supplementary Data S1— Supplementary Table 1.Voucher numbers, Species assignment, geographic records and BioProject and Biosample accession numbers of specimens used in this study

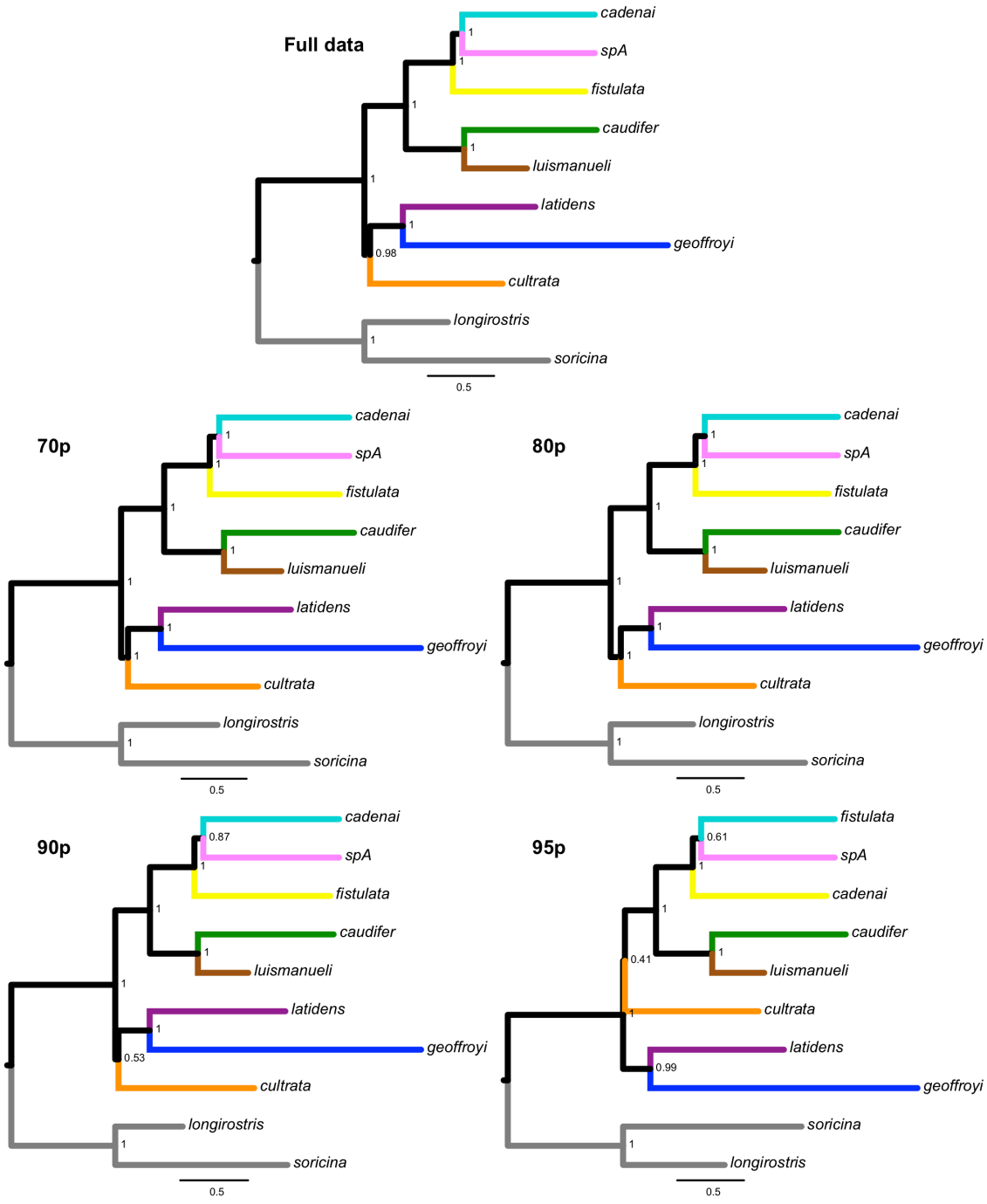
Nominal Species	This Study	Sequence code	GenBank Accession	Biosample	BioProject	Country	Province	Municipaily	Lat	Long
Anoura aequatoris	*Anoura caudifer*	Aaeq167	KCZT00000000	SAMN11318422	PRJNA529738	Colombia	Antioquia	Jardín	5.50	-75.89
Anoura aequatoris	*Anoura caudifer*	Aaeq168	KCZS00000000	SAMN11318423	PRJNA529738	Colombia	Antioquia	Jardín	5.50	-75.89
Anoura aequatoris	*Anoura caudifer*	Aaeq210	KCZR00000000	SAMN11318424	PRJNA529738	Colombia	Huila	Teruel	2.84	-75.61
Anoura cadenai	*Anoura cadenai*	Acadtype	KCZM00000000	SAMN11318426	PRJNA529738	Colombia	Valle del Cauca	Calima	3.93	-76.49
Anoura cadenai	*Anoura cadenai*	Acad112	KCZP00000000	SAMN11318427	PRJNA529738	Colombia	Valle del Cauca	Calima	3.93	-76.49
Anoura cadenai	*Anoura cadenai*	Acad113	KCZO00000000	SAMN11318428	PRJNA529738	Colombia	Valle del Cauca	Calima	3.93	-76.49
Anoura cadenai	*Anoura cadenai*	Acad109	KCZQ00000000	SAMN11318440	PRJNA529738	Colombia	Valle del Cauca	Yotoco	3.83	-76.33
Anoura cadenai	*Anoura cadenai*	Acad209	KCZN00000000	SAMN11318441	PRJNA529738	Colombia	Huila	Teruel	2.84	-75.61
Anoura caudifer	*Anoura caudifer*	Acau214	KCZH00000000	SAMN11318430	PRJNA529738	Colombia	Antioquia	Urrao	6.52	-76.25
Anoura caudifer	*Anoura caudifer*	Acau215	KCZG00000000	SAMN11318431	PRJNA529738	Colombia	Antioquia	Urrao	6.52	-76.25
Anoura caudifer	*Anoura caudifer*	Acau259	KCZF00000000	SAMN11318432	PRJNA529738	Colombia	Antioquia	Medellin	6.19	-75.55
Anoura caudifer	*Anoura caudifer*	Acau260	KCZE00000000	SAMN11318433	PRJNA529738	Colombia	Antioquia	Urrao	6.54	-76.24
Anoura caudifer	*Anoura caudifer*	Acau261	KCZD00000000	SAMN11318434	PRJNA529738	Colombia	Antioquia	Urrao	6.54	-76.24
Anoura caudifer	*Anoura caudifer*	Acau271	KCZC00000000	SAMN11318435	PRJNA529738	Brazil	Sao Paulo		-25.13	-47.97
Anoura cultrata	*Anoura cultrata*	Acul208	KCZB00000000	SAMN11318436	PRJNA529738	Colombia	Huila	Teruel	2.84	-75.61
Anoura cultrata	*Anoura cultrata*	Acul222	KCZA00000000	SAMN11318437	PRJNA529738	Ecuador	Napo	Pacto Sumaco	-0.67	-77.60
Anoura cultrata	*Anoura cultrata*	Acul226	KCYZ00000000	SAMN11318438	PRJNA529738	Ecuador	Napo	Pacto Sumaco	-0.67	-77.60
Anoura cultrata	*Anoura cultrata*	AculNM83	KCYY00000000	SAMN11318439	PRJNA529738	Ecuador	Morona Santiago		-2.17	-77.66
Anoura fistulata	*Anoura fistulata*	AfisNM49	KCYV00000000	SAMN11318442	PRJNA529738	Ecuador	Santo Domingo de los Tsachilas	Guajalito	-0.22	-78.80
Anoura fistulata	*Anoura fistulata*	AfisNM95	KCYU00000000	SAMN11318443	PRJNA529738	Ecuador	Napo	Cosanga	-0.60	-77.88
Anoura fistulata	*Anoura fistulata*	Afis1352	KCYW00000000	SAMN11318444	PRJNA529738	Ecuador	Napo	Sumaco	-0.57	-77.60
Anoura fistulata	*Anoura fistulata*	Afis894	KCYX00000000	SAMN11318445	PRJNA529738	Ecuador	Pichincha	Quito	0.01	-78.68
Anoura geoffroyi	*Anoura geoffroyi*	Acar115	KCZL00000000	SAMN11318446	PRJNA529738	Colombia	Valle del Cauca	Cali	3.33	-76.64

Anoura geoffroyi	*Anoura geoffroyi*	Ageo197	KCYS00000000	SAMN11318447	PRJNA529738	Colombia	Tolima	Cajamarca	4.48	-75.50
Anoura geoffroyi	*Anoura geoffroyi*	Ageo251	KCYR00000000	SAMN11318448	PRJNA529738	Trinidad	Sangre Grande		10.47	-61.18
Anoura geoffroyi	*Anoura geoffroyi*	Ageo252	KCYQ00000000	SAMN11318449	PRJNA529738	Trinidad	Sangre Grande		10.47	-61.18
Anoura geoffroyi	*Anoura geoffroyi*	Ageo108	KCYT00000000	SAMN11318456	PRJNA529738	Colombia	Meta	Vista Hermosa	2.73	-73.75
Anoura latidens	*Anoura latidens*	Alat90	KCYP00000000	SAMN11318450	PRJNA529738	Colombia	Valle del Cauca	Cali	3.32	-76.48
Anoura latidens	*Anoura latidens*	Alat95	KCYO00000000	SAMN11318451	PRJNA529738	Colombia	Risaralda	Pereira	4.73	-75.57
Anoura latidens	*Anoura latidens*	Alat110	KCYN00000000	SAMN11318452	PRJNA529738	Colombia	Valle del Cauca	Yotoco	3.83	-76.33
Anoura latidens	*Anoura latidens*	Acartype	KCZI00000000	SAMN11318453	PRJNA529738	Colombia	Nariño	Taminango	1.68	-77.33
Anoura latidens	*Anoura latidens*	Acar116	KCZK00000000	SAMN11318454	PRJNA529738	Colombia	Magdalena	San Pedro de la Sierra	10.91	-74.05
Anoura latidens	*Anoura latidens*	Acar117	KCZJ00000000	SAMN11318455	PRJNA529738	Colombia	Nariño	Taminango	1.68	-77.33
Anoura luismanueli	*Anoura luismanueli*	Alui212	KCYM00000000	SAMN11318457	PRJNA529738	Colombia	Santander	Piedecuesta	7.08	-73.03
Anoura peruana	*Anoura geoffroyi*	Aperu69	KCYL00000000	SAMN11318458	PRJNA529738	Colombia	Caldas	Manizales	5.09	-75.41
Anoura peruana	*Anoura geoffroyi*	Aperu269	KCYK00000000	SAMN11318459	PRJNA529738	Peru	Amazonas	Bongara	-5.97	-77.92
Anoura peruana	*Anoura geoffroyi*	Aperu270	KCYJ00000000	SAMN11318460	PRJNA529738	Peru	Ancash	Yungay	-9.05	-77.63
Anoura peruana	*Anoura geoffroyi*	Aperu274	KCYI00000000	SAMN11318461	PRJNA529738	Peru	Cusco	Paucartambo	-13.02	-71.49
Anoura sp A	*Anoura* sp A	Aspa273	KCYG00000000	SAMN11318425	PRJNA529738	Peru	Madre de Dios	Manu	-12.77	-71.39
Anoura sp A	*Anoura* sp A	Aspa203	KCYH00000000	SAMN11318429	PRJNA529738	Colombia	Putumayo	La Peinilla	0.47	-75.82
Glossophaga longirostris	*Glossophaga longirostris*	Glon265	KCYF00000000	SAMN11318462	PRJNA529738	Colombia	La Guajira	Barrancas	10.93	-72.75
Glossophaga soricina	*Glossophaga soricina*	Gsori262	KCYE00000000	SAMN11318463	PRJNA529738	Colombia	Huila	Paicol	2.44	-75.77

Supplementary Data S2— Supplementary Figure 1. Smartpca complementary results



Supplementary Figure 2. ASTRAL-III species trees of the full and reduced UCE datasets.



Supplementary Figure 3. Comparison between A) smartpca analysis showing the different clades present in *Anoura*, and B) PCA on morphometric measurements.