A morphologically atypical great fruit-eating bat *Artibeus lituratus* from Paraguay

Un murciélago frutero grande *Artibeus lituratus* morfológicamente atípico de Paraguay

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Abstract

Introduction: Morphological abnormalities and their underlying causes are well documented and understood in humans and some domesticated animals but are less often reported within wild populations. This is likely because many abnormalities lead to the early death of individuals and typically are only encountered within large samples of specimens. Here, we report an adult female great fruit-eating bat *Artibeus lituratus* (Chiroptera, Phyllostomidae) collected from Paraguay with notable skull and post-cranial abnormalities.

Methods: Specimens, including the atypical *A. lituratus*, were collected from eastern Paraguay and prepared as skin and skull museum voucher specimens and deposited in the Natural Science Research Laboratory of the Museum of Texas Tech University. Sequences of Cytochrome *b* (Cyt *b*) were generated and uploaded to BLAST to confirm species identification. We then quantitatively compared the atypical individual with 15 typical females collected from the same locality using 13 wing measurements and 13 skull measurements.

Results: The Cyt *b* sequence of the atypical specimen was a 100 % match to *A. lituratus*. The atypical specimen was much smaller from the perspective of overall body size and wing morphology. The skull was also qualitatively different, much smaller and less robust than other female *A. lituratus* from this site. Mastoids and the sagittal crest were greatly reduced, and the frontal shield was absent in the atypical individual.

Discussion and conclusions: We encourage reports of morphological abnormalities to be made as determining rates of abnormalities within populations may indicate their overall health.

Key words: Morphological deformity; morphometrics; New World bats; skeletal deformity; skull morphology; wing morphology.

Resumen

Introducción: Las anormalidades morfológicas y sus causas subyacentes están ampliamente conocidas y documentadas en humanos y animales domésticos; sin embargo, no se reportan frecuentemente en poblaciones silvestres. Este fenómeno se debe a que muchas anormalidades conducen a la muerte temprana del individuo y generalmente solo se las observan cuando se colecta un gran número de especímenes. Aquí reportamos una hembra adulta del murciélago frutero *Artibeus lituratus* (Chiroptera, Phyllostomidae) de Paraguay con anormalidades craneales y post-craneales notorias.

Metodología: Los especímenes, incluyendo al individuo atípico de *A. lituratus*, fueron preparados como ejemplares científicos de piel y cráneo, y depositados en el Natural Science Research Laboratory de la Universidad de Texas Tech. Las secuencias de Citocromo *b* (Cyt *b*) fueron cargadas en BLAST para la identificación de la especie. Comparamos cuantitativamente al individuo atípico con otras 15 hembras normales colectadas en la misma localidad usando 13 medidas alares y 13 craneales.

Resultados: La secuencia del Cyt *b* del espécimen atípico obtuvo 100 % de coincidencia con *A. lituratus*. El ejemplar atípico fue de tamaño menor en las medidas convencionales del cuerpo y morfología de alas. El cráneo fue más pequeño y menos robusto comparado con otras hembras de *A. lituratus* del mismo sitio. Los huesos mastoideos y las crestas sagitales fueron notablemente reducidos y el escudo frontal estuvo ausente en el individuo atípico.

Discusión y conclusión: Recomendamos que las anormalidades morfológicas se reporten con mayor frecuencia ya que la obtención de la tasa de anormalidad puede indicar el estado de salud poblacional.

Palabras claves: Deformidad esquelética; deformidad morfológica; morfología alar; morfología craneal; morfometría; murciélagos del Nuevo Mundo.

Introduction

Morphological abnormalities and their underlying genetic and/or environmental causes are well documented and understood in humans and some domesticated animals (Mortier *et al.* 2019). Because many abnormalities can lead to an individual being aborted early in development, dying shortly after birth, or the complete dysfunction of systems essential to survival (Kunz and Chase 1983; Eissa *et al.* 2021), reports of morphological abnormalities in wild populations are rare and are typically only encountered within large samples of specimens. For example, only 2 individuals with deformed tails were detected from a sample of 150,000 Mexican free-tailed bats (*Tadarida brasiliensis*; Mitchell and Smith 1966). Atypical individuals have been reported in wild populations of fish (Eissa *et al.* 2021), amphibians (Soto-Rojas *et al.* 2017), reptiles (Clark *et al.* 2011), birds (Pourlis 2011), and mammals (Kunz and Chase 1983; Stephens *et al.* 2018; Esquivel *et al.* 2021). This indicates that morphological abnormalities do not always result in immediate death (Pourlis 2011; Stephens *et al.* 2018; Eissa *et al.* 2021).

The Neotropical bat family Phyllostomidae is one of the most diverse families of bats (Order Chiroptera) with over 227 species in 61 genera (Simmons and Cirranello 2022). The genus *Artibeus* is one of the most species rich genera within Phyllostomidae with 13 species, all of which are frugivorous. The great fruit-eating bat (*Artibeus lituratus*) is the largest New World fruit bat and ranges from México through Argentina (Larsen *et al.* 2013). Herein, we report a case of an individual *A. lituratus* collected during a field study in Paraguay (Stevens *et al.* 2004) with notable skull and post-cranial abnormalities (Figure 1) for which we characterize, report, and make quantitative comparisons with typical *A. lituratus* individuals from the site where it was collected.

Materials and methods

Specimens, including an atypical adult female *A. lituratus*, were collected from the Reserva Natural del Bosque Mbaracayú, in the Departamento de Canindeyu in eastern Paraguay. The habitat was interior Atlantic Forest and further details as to the site and other bat species encountered can be found in Stevens *et al.* (2004). The atypical individual was captured on 20 November 1997, prepared as a skin and skull museum voucher specimen, and deposited into the Natural Science Research Laboratory (NSRL) at Texas Tech University (TTU 94274). We took 13 wing measurements including length of the forearm, thumb, and the elements of each digit (2-1, 3-1, 3-2, 3-3, 3-4, 4-1, 4-2, 4-3, 5-1, 5-2, 5-3) and 13 skull measurements including greatest length of skull (GLS), condylo-basal length (CBL), breadth of upper canines (BUC), breadth of the post-orbital constriction (POC), breadth of braincase (BBC), breadth across mastoids (BAM), breadth of upper molars (BUM), length of maxillary tooth row (LMT), height of cranium (HOC), total length of mandible (TLM), breadth of lower canines (BLC), breadth of lower molars (BLM) and breadth of the coronoid process (COP). Measurements were taken from the atypical *A. lituratus* individual as well as 15 typical female individuals collected from the same locality. Each measurement was taken 3 times using EZ Cal® 6” fractional digital calipers and then the mean was calculated for each individual. The mean and standard deviation of each measurement was taken from 15 typical *A. lituratus* individuals and compared to the measurements of the atypical individual (Table 1). A principal components analysis was used to illustrate morphological relationships of typical and the atypical specimens.

The atypical individual of *A. lituratus* was so different that at first its species-specific designation was unclear. Moreover, 3 *Artibeus* spp. are syntopic in Eastern Paraguay (Owen *et al.* 2022) and typical *A. fimbriatus* and *A. planirostris* are more similar to typical *A. lituratus* than to the atypical individual. Thus, species identification could not be confirmed morphologically due to the nature of the abnormalities. We sequenced the mitochondrial gene Cytochrome *b* (Cyt *b*) to confirm species identification as *A. lituratus*. Genomic DNA was extracted from a wing punch taken from the atypical *A. lituratus* specimen (TTU 94274) using a DNEasy Blood and Tissue Extraction Kit (Qiagen Inc., Valencia, California). We amplified Cyt *b* using HotStarTaq (Qiagen Inc., Valencia, California) and primers 400F (Edwards *et al.* 2001) and 700H (Peppers *et al.* 2002) and the thermal profile: hot start of 80 °C, initial denaturation at 95 °C for 2 min, followed by 34 cycles of denaturation at 95 °C for 30 s, annealing 42 °C for 45 s, extension at 73 °C for 1 min, and a final extension at 73 °C for 15 min. Amplification products were purified using ExoSAP-IT (Applied Biosystems, Foster City, California). Purified products were sequenced using ABI Prism Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Thermofisher, Waltham, MA, USA). Sequence products were purified using Sephadex columns (Princeton Separation, Adelphia, New Jersey) and centrifugation, followed by dehydration and resuspension in formamide. Purified sequencing products were analyzed using an ABI Prism 310 automated sequencer (Biotechnology Resource Center, Institute of Biotechnology, Cornell University, Ithaca, New York). Sequences were aligned and annotated in Sequencher 4.10.1 software (Gene Codes Corporation, Ann Arbor, Michigan) and final sequences were uploaded to BLAST® (Madden 2002) for species confirmation.

Results

The atypical specimen had lower body and wing measurements compared to other *A. lituratus* females captured at the same site (Table 1). For example, the average forearm length for the 15 typical individuals was 15 % larger (71.38 mm) than for the atypical female (60.58 mm). The skull was also qualitatively different, much smaller and less robust than other female *A. lituratus* from this site. Mastoids and the sagittal crest were greatly reduced, and the frontal shield was absent in the atypical individual. The first two principal components for wing, cranial and mandibular characteristics accounted for between 67 and 91 percent of the variability among individual *A. lituratus* (Figure 2). The atypical individual was very different with respect to typical individuals in all three ordination spaces. The Cyt *b* sequence for the atypical specimen was a 100 % match to *A. lituratus* based on a BLAST search.

Discussion

This atypical individual was one of more than 4,300 *A. lituratus* (< 0.001 %) captured at the Reserva Natural del Bosque Mbaracayú during our field effort (Stevens *et al.* 2004). Despite the severity of the phenotypic abnormalities, the atypical female *A. lituratus* was captured while foraging and was lactating, which suggests successful reproduction. The survival of this individual suggests that outliers may survive in natural populations more frequently than previously thought; however, being able to record such anomalies represents substantial collecting efforts, as has been suggested in previous case reports (Mitchell and Smith 1966; Kunz and Chase 1983).

Many factors may result in morphological abnormalities including genetic mutations (Pourlis 2011; Eissa *et al.* 2021), environmental factors such as pollution (Pourlis 2011; Eissa *et al.* 2021), illness or parasites, nutritional deficiencies, stress (Eissa *et al.* 2021), and trauma or injury (Stephens *et al*. 2018; Eissa *et al.* 2021), among others. Examples of abnormalities specific to the skeletal system include conjoined twins (Nogueira *et al.* 2017), dental anomalies such as polydontia or oligodontia (Esquivel *et al.* 2021), the gain or loss of limbs or digits, and deformities of the skull or skeleton (Kunz and Chase 1983; Gonçalves *et al*. 2011).

Although intensive effort is needed to capture atypical individuals, reporting such abnormalities within wild populations is worthwhile and could have multiple benefits. For example, in some instances, skeletal anomalies such as gain or loss of teeth or changes in teeth or skull morphology can represent ecological adaptations that enhance fitness, are selected and ultimately lead to adaptive evolutionary changes (Esquivel *et al.* 2021). Finally, while unlikely at this large (66,000 ha) and fairly undisturbed forest fragment in Paraguay, deformities may serve as an indicator of poor health of populations and indicate underlying stressors such as environmental degradation and mismanagement (Pourlis 2011; Soto-Rojas *et al.* 2017; Eissa *et al.* 2021) or low genetic diversity and inbreeding (Clark *et al.* 2011).

Therefore, morphological abnormalities detected within wild populations should be reported in order to determine the frequency in populations. This will provide information on the quality of habitat and health of populations while enabling better monitoring and management efforts (Pourlis 2011; Soto-Rojas *et al.* 2017; Eissa *et al.* 2021). Understanding factors resulting in abnormalities, such as those of the individual reported here, could help implement conservation efforts that help decrease the prevalence of such abnormalities in wild bat populations.

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Table 1. Summary of measurements taken from the atypical female *Artibeus lituratus* individual to 15 typical female *A. lituratus* individuals collected from the Reserva Natural del Bosque Mbaracayú, in the Departamento de Canindeyu in eastern Paraguay. Number abbreviations refer to digit components with the first number indicating the digit number and the second number indicating the specific element of that digit (*e. g.*, 3-1 refers to the metacarpal, 3-2 to the first phalanx, 3-3 to the second phalanx, and 3-4 to the third phalanx of the third digit).

	Population		Atypical *A. lituratus*
Character	Mean	S.D.	Mean
Forearm	71.38	2.97	60.58
Thumb	13.56	1.05	13.13
2-1	56.1	4.01	50.89
3-1	67.32	2.16	60.25
3-2	22.52	0.98	17.62
3-3	34.79	3.92	28.03
3-4	21.73	1.55	20.88
4-1	65.6	2.04	60.78
4-2	18.74	0.74	14.82
4-3	25.09	1.35	19.75
5-1	67.56	1.96	61.28
5-2	14.28	0.74	11.31
5-3	20.6	1.05	16.06
Length of skull	31.36	0.38	29.37
Condylo-basal length	29.76	0.47	28.40
Breadth across upper canines	7.66	0.37	7.53
Post-orbital constriction	6.64	0.31	6.22

Breadth of braincase	13.41	0.39	12.89
Breadth across mastoids	16.7	0.57	14.16
Breadth across upper molars	13.5	0.53	12.16
Length of maxillary tooth row	12.47	0.39	12.11

Figures

Figure 1. Picture of the skull of an atypical female *Artibeus lituratus* (right) compared to a typical female *A. lituratus* (left) collected from the Reserva Natural del Bosque Mbaracayú, in the Departamento de Canindeyu in eastern Paraguay.

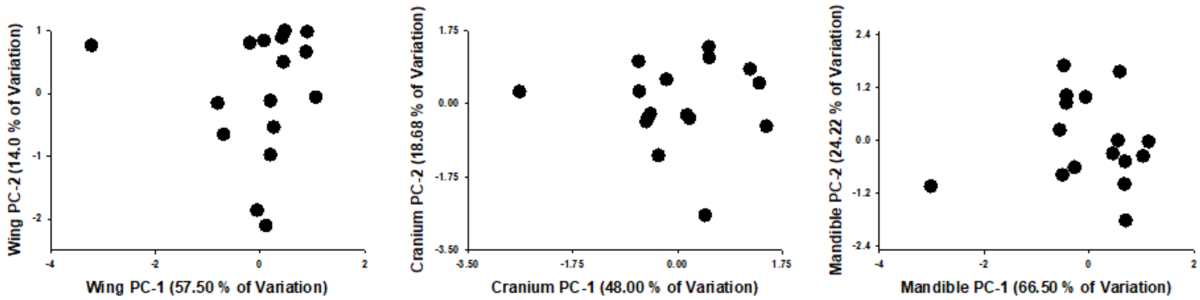

Figure 2. Results of principal components analysis comparing the atypical female *Artibeus lituratus* individual to 15 typical female *A. lituratus* individuals collected from the Reserva Natural del Bosque Mbaracayú, in the Departamento de Canindeyu in eastern Paraguay.