DOI: 10.1002/ajp.23616

EMERGING METHODS AND TECHNOLOGIES



Check for updates

Measuring mantled howler monkey (Alouatta palliata) testes via parallel laser photogrammetry: Expanding the use of noninvasive methods

Austen J. Ehrie^{1,2} | Alec A. Iruri-Tucker² | Yasmin B. Lord³ | Heidi G. Williamson⁴ | Kevin D. Hunt^{2,3} | P. David Polly^{2,5} | Courtney L. Fitzpatrick¹ | Michael D. Wasserman^{2,3}

Correspondence

Austen J. Ehrie, 424 Nagle St, Biological Sciences Building West, Lab 119, College Station, TX 77840, USA.
Email: ajehrie@tamu.edu

Funding information

Indiana University Hutton Honors College; Indiana University Center for the Integrative Study of Animal Behavior; NSF IRES, Grant/Award Number: 1854114

Abstract

Parallel laser photogrammetry (PLP), which consists of attaching two or three parallel laser beams at a known inter-beam distance to a camera, can be used to collect morphological measurements of organisms noninvasively. The lasers project onto the photo being taken, and because the inter-beam distance is known, they act as a scale for image analysis programs like ImageJ. Traditionally, this method has been used to measure larger morphological traits (e.g., limb length, crown-rump length) to serve as proxies for overall body size, whereas applications to smaller anatomical features remain limited. To that end, we used PLP to measure the testes of 18 freeliving mantled howler monkeys (Alouatta palliata) at La Selva Biological Station, Costa Rica. We tested whether this method could reliably measure this relatively small and globular morphology, and whether it could detect differences among individuals. We tested reliability in three ways: within-photo (coefficient of variation [CV] = 4.7%), between-photo (CV = 5.5%), and interobserver (intraclass correlation = 0.92). We found an average volume of 36.2 cm³ and a range of 16.4-54.4 cm³, indicating variation in testes size between individuals. Furthermore, these sizes are consistent with a previous study that collected measurements by hand, suggesting that PLP is a useful method for making noninvasive measurements of testes.

KEYWORDS

howler monkeys, La Selva Biological Station, male sex characteristics, remote measurement, reproductive ecology

Abbreviations: AE, Austen Ehrie; ASP, American Society of Primatologists; BIACUC, Bloomington Institutional Animal Care and Use Committee; CV, coefficient of variation; HW, Heidi Williamson; ICC, intraclass correlation; IRES, International Research Experience for Students; MINAE, Ministry of Environment and Energy; PLP, parallel laser photogrammetry; SINAC, National System of Conservation Areas.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. American Journal of Primatology published by Wiley Periodicals LLC.

¹Department of Biology, Texas A&M University, College Station, Texas, USA

²Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, Indiana, USA

³Department of Anthropology, Indiana University, Bloomington, Indiana, USA

⁴Department of Ecology and Conservation Biology, Texas A&M University, College Station, Texas, USA

⁵Department of Geology, Indiana University, Bloomington, Indiana, USA

^{1.} Author Austen Ehrie's current affiliation 2. Author Austen Ehrie's past affiliation.

1 | INTRODUCTION

Among primate species, the testes exhibit variation in size that reflects characteristics of the mating system. For instance, species that live in multimale multifemale mating systems tend to have larger testes relative to body size compared to those in one-male, multifemale systems, whereas species living in pair-bonded systems have the smallest ratio (Dixson & Anderson, 2004; Harcourt et al., 1995; Kenagy & Trombulak, 1986). This relationship between mating system and testes size is a consequence of sperm competition—that is, when females mate with multiple males per ovarian cycle, males experience selection to increase their sperm production to maximize the probability of paternity (Firman & Simmons, 2008; Moller, 1989; Takihara et al., 1987). Therefore, accurate measurements of testes size are critical for assessing the fundamentals of sperm competition among primate species.

Measurements of testes in wild primates can also provide insight into the effects of anthropogenic disturbances on reproductive anatomy and physiology. For instance, some pesticides act as androgen antagonists (Lemaire et al., 2004; Mnif et al., 2011; Peiris & Dhanushka, 2017), and because the development of male primary sex characteristics depends on the production and successful binding of androgens (Vom Saal, 1983), testes size can reflect reproductive abnormalities. These abnormalities can include testes shrinkage and testicular dysgenesis, which have been linked to impaired spermatogenesis, poor sperm quality, and altered hormone levels (Marshall et al., 1978; Mehrpour et al., 2014).

Despite the important information that testes measurements provide, these data are difficult to obtain from free-living animals. Many studies of sperm competition in nonhuman primates have relied on measurements using captive animals and museum specimens (Dunn et al., 2015; Harcourt et al., 1995; Kappeler, 1997; Lüpold et al., 2019). Although these methods can result in accurate measurements, they are limited in scope and the important variation found within and between wild populations has remained unavailable. An accurate and precise noninvasive method can capture this complexity because the data come from a wild population of living, actively reproducing individuals in real time.

It is furthermore imperative that measurements of testes be collected noninvasively because invasive methods can pose health risks to animals. A traditional approach to collecting morphometric measurements is to dart individuals and put them under anesthesia to make manual measurements (Fedigan, 2010; Sapolsky & Share, 1998). Although this method has produced important data, it can cause physical distress and injury to primates in several ways, including injuries caused directly by the darts. A survey of primatologists found that across primate studies using darting techniques, 43 out of 2092 (2.1%) primates were injured, with 33 (1.6%) of them considered serious or fatal (Cunningham et al., 2015). These risks are heightened for arboreal species because they can collide with branches or the ground when falling from the canopy. In addition, darting and capture triggers a hormonal stress response (i.e., increased cortisol) and can provoke agitation and other stress-induced behaviors (Bethell et al., 2012; Wasserman, Chapman, et al., 2013). Last, given the close relatedness of humans and nonhuman primates, there have been several instances of

disease transfer (Adrus et al., 2019; Dolz et al., 2019; Illia et al., 2022; Köndgen et al., 2008; Obanda et al., 2019; Wallis & Rick Lee, 1999). This risk of disease transfer can be mitigated with noninvasive methods that maintain greater distances between researchers and subjects.

1.1 | Description

Photogrammetry is broadly defined as the use of photographic images to obtain measurements and information from physical objects in an environment (Bell et al., 1997). Though specific techniques vary, many require an object of a known size to be in the frame of the photo for proper scaling (Domb & Pagel, 2001; Kurita et al., 2012). Others may require specifications of the camera (e.g., focal length of the camera lens) or the distance between the photographer and the study subject (Fitzpatrick et al., 2014; Galbany et al., 2016). While these techniques can result in proper morphological estimates, they can be labor intensive and are not always feasible when applied to arboreal animals.

Parallel laser photogrammetry (PLP) is a form of photogrammetry in which parallel lasers of a known inter-beam distance are projected onto the visual field of a digital photograph so that they can act as a scale to make measurements of other objects within the image (Bergeron, 2007; Rothman et al., 2008). This technique has gained popularity in the last two decades because it allows researchers to collect precise and accurate morphometric measurements without disturbing the animal. Unlike other forms of photogrammetry, PLP does not require an object of known size to be in the frame or other information for scaling. Furthermore, it has been used for a wide variety of taxa, including ungulates (Bergeron, 2007; Kumar et al., 2012; Weisgerber et al., 2015), fishes and marine mammals (Durban & Parsons, 2006; Leurs et al., 2015; O'Connell & Leurs, 2016; Rizzo et al., 2017; Rogers et al., 2017; Rohner et al., 2011; Webster et al., 2010), and both terrestrial and arboreal primates (Anzà et al., 2022; Barrickman et al., 2015; Brown et al., 2022; Chappell et al., 2015; Galbany et al., 2016, 2017; Lu et al., 2016; Richardson et al., 2022: Rothman et al., 2008: Sandel et al., 2022: Schuppli et al., 2016; Wright et al., 2019, 2020).

Most studies using this method have focused on larger body parts such as limb, trunk, fin, or total length as proxies for overall body size to make predictions about body growth. However, some researchers have attempted to measure smaller body parts such as the body length of darters (Rizzo et al., 2017) and the flanges of male Bornean orangutans (Brown et al., 2022). In the present study we demonstrate that PLP can measure testes, and to our knowledge, we are the first to do so for any primate.

1.2 | Example

1.2.1 | Ethical statement

We conducted all research in accordance with safety protocols derived from Indiana University's Bloomington Institutional Animal Care and Use Committee (BIACUC), Laser Safety Program, and

were distinguished from juveniles and sub-adults by body size, a defined beard and mantle, and descended testes (Glander, 1980).

Biosafety Committee. We conducted all research in accordance with the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Nonhuman Primates and Code for Best Practices in Field Primatology. Costa Rica's Ministry of Environment and Energy (MINAE) and National System of Conservation Areas (SINAC) granted us permission to conduct this research.

1.2.2 Study system and site

We studied mantled howler monkeys (Alouatta palliata), which are found in regions ranging from Mexico to Ecuador (Dias, 2005; Papworth & Mejia, 2015). In contrast to their golden brown and black pelage, the scrotums of males are large and palish white (Glander, 1980; Jones, 1999), allowing for ease of photo capture. monkeys Furthermore, mantled howler mate vear-round (Glander, 1980), making seasonal fluctuations of testes size unlikely (Kelaita et al., 2011). Our study occurred at La Selva Biological Station (N10°25'50.243, W84°00'25.095) in Costa Rica. La Selva is a lowland tropical rainforest, with large-scale cultivation of banana and pineapple surrounding the forest (Shaver et al., 2015; Sigel et al., 2006). Data were collected from May 28 to July 20, 2022.

A 2022 census detected 25 groups of wild mantled howler monkeys in this region (Schreier et al., in review), and we further found that each group typically consists of 1-4 adult males. We ultimately tracked 13 of these groups at random, and by using group demographic information (e.g., age-sex classes) in tandem with our photos, we were able to identify individual adult males. Adult males

1.2.3 Laser apparatus

The laser apparatus used in this study was the same device Barrickman et al. (2015) used to measure trunk and limb length of mantled howler monkeys (Figure 1a-c). An aluminum box was constructed to house two green lasers (Apinex, Montreal, model GM-CW02) within two parallel aluminum barrels at a fixed horizontal distance of 4 cm. Green lasers were chosen because they are the most visible in the canopy. The lasers were held in the barrels by four small screws, allowing for slight modifications when needed, and PVC struts were attached to the lateral sides of the aluminum box for additional structural support. The lasers were powered using a standard battery pack located in the back interior of the aluminum box. A tripod bolt ran through the middle of the apparatus so that it could attach to the underside of our camera (Canon-EOS Rebel T7 DSLR with EF-S 18-55 mm and EF 75-300 mm lenses). The lasers were oriented horizontally, as this orientation allows both lasers to be of the same distance from apparatus to study target.

Barrickman et al. (2015) also tested whether the angle of the surface onto which the lasers are projected or the angle between the photographer and arboreal subject would result in measurement discrepancy. In both cases, it was not necessary for the angles to be perfectly perpendicular relative to the photographer as small deviations were tolerated and resulted in low error. However, it is

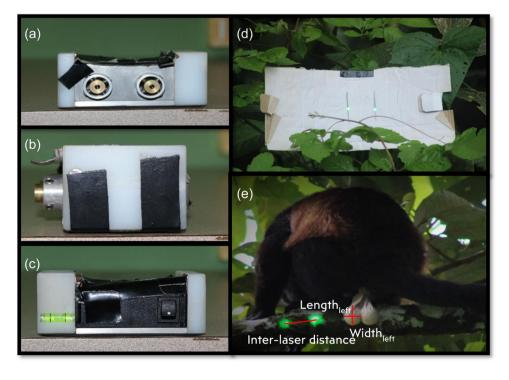


FIGURE 1 PLP apparatus and photo capture technique. (a-c) Apparatus used in this study. (d) Demonstration of the calibration method. (e) Demonstration of an ideal photo capture and measuring technique. [Color figure can be viewed at wileyonlinelibrary.com]

still recommended that deviations from perpendicularity be minimal, and we made the effort to follow this recommendation in our own photo capture protocol. For further detail on the design of this apparatus, as well as tests of accuracy, see Barrickman et al. (2015).

1.2.4 | Photo capture and image analysis

With the parallel laser apparatus and camera, we took photos of adult males while their testes were in view. Given the aluminum material of the apparatus and the high humidity of the study site, slight deviations in the inter-beam distance were expected. We therefore calibrated the lasers throughout observation days to maintain an inter-beam distance of 4 cm by taking photos of a piece of carboard that had a 4 cm length marking (Figure 1d). We took photos from various distances up to 20 m and if the projected lasers did not align with the markings, the lasers were adjusted accordingly. Mantled howler monkeys have multiple predictable resting periods throughout the day, providing ample opportunity for calibration. In cases where calibration revealed a need for adjustment, we excluded the photos captured since the previous calibration from subsequent analyses. Photos taken where the lasers were correctly calibrated were evaluated based on predetermined criteria: (1) Lasers are projected onto a plane perpendicular to the photographer, (2) Lasers are projected onto a solid surface (e.g., tree branch or the howler monkey itself) with minimal distortion. (3) Minimal obstruction so that the circumference of the testes can be detected, (4) Testes are perpendicular to the photographer, and (5) Photo is of overall good quality (e.g., minimal blurriness and proper focus). Each photo received a score from one to five, determined by the number of criteria satisfied (see Figure 2 for photo examples). Only photos with a minimal score of three were measured and used in analysis, resulting in measurements from 18 individuals. For each individual, we measured one to four photos.

We made measurements using the publicly accessible image analysis software ImageJ (v. 2.3.21). Lines were drawn between the centers of the laser points (inter-laser distance) and a distance was retrieved in pixels. Because the actual distance is known to be 4 cm (inter-beam distance), a pixel to centimeter conversion scale was set for each photograph so that estimates for the lengths and widths of the testes was returned in centimeters (Figure 1e). When making the measurements we did not include scrotal folds and extra scrotal skin. The following formula for a prolate spheroid was used to calculate the volume of a single testis (Dunn et al., 2015; Kelaita et al., 2011): testis volume (cm³) = $[(\pi)(L)(W^2)]/6$. Both testis volumes were then added together for a total testes volume. Austen Ehrie (AE) measured each photo five times and then calculated the average. If an individual had multiple photos, the final testes measurement was the average of the photo averages. All usable photos were measured a second time by Heidi Williamson (HW) for an interobserver analysis. Because this analysis resulted in high reliability (see below), subsequent analyses and data presented used the measurements made by AE.

Photo Score	Score Description	Photo Example
1	Criteria 2, 3, 4, and 5 not satisfied.	
2	Criteria 2, 3, and 4 not satisfied	
3	Criteria 4 and 5 not satisfied. Testes not perfectly perpendicular to photographer and testes are out of focus.	
4	Criterion 3 not satisfied. Some overlap between testes.	
5	All 5 criteria satisfied.	

FIGURE 2 Photo scoring method. All photos were given a score from 1 to 5 based on how many of the following criteria were satisfied. (1) Lasers are projected onto a plane perpendicular to the photographer. (2) Lasers are projected onto a solid surface with minimal distortion. (3) Minimal obstruction so that the circumference of the testes can be detected. (4) Testes are perpendicular to the photographer. (5) Photo is of overall good quality. [Color figure can be viewed at wileyonlinelibrary.com]

1.2.5 | Statistical analysis

A concern going into this study was that the PLP technique would not be able to detect biologically meaningful differences in testes size between males given the relatively small size of the testes, even though a priori evidence indicates that differences should exist in this species (Kelaita et al., 2011). Among individual testes measurements the assumption of equal variance was violated, so to examine whether there were differences between individuals we performed a nonparametric Kruskal–Wallis test.

We tested for measurement repeatability between the five measurements for a photo (hereafter within-photo repeatability), as well as between photos for an individual (hereafter between-photo repeatability), by calculating coefficient of variation (CV) percentage scores. For a sample set, these scores reflect how

variable the measurements are around the mean ($[\sigma/\mu] \times 100\%$); a lower percentage reflects lower error (i.e., high repeatability). Within-photo repeatability error was higher compared to other studies using this metric (see below). We hypothesized that this error could have been because of poor photo quality (i.e., if photos of a lower quality resulted in a higher repeatability error). To test this, we ran a simple linear model with CV scores as the response variable and photo quality score as the independent variable.

We tested interobserver reliability of the final measurements in two ways. First, we used an intraclass correlation (ICC) score under the two-way random effects, absolute agreement, single rater conditions for a comprehensive assessment (Koo & Li, 2016) using the R package *IRR*. Here the statistical output is a value ranging from 0 to 1 where a score closer to 1 indicates a higher degree of reliability. We then calculated an absolute error percentage for each individual by subtracting observer 1's measurements and dividing the difference by observer 1's measurements. All statistical tests were performed in R (v. 4.2.2).

Although the ideal validation of our method would be to compare PLP measurements of testes size with manual measurements taken directly from the same individuals, this validation was not possible for this study. Instead, we compared our PLP measurements with the manual measurements reported in Kelaita et al. (2011). Even though these measurements come from a different population, the goal was to determine whether our testes volume overlap with the distribution of known sizes collected by hand. In other words, we wanted to confirm that it is plausible to see our measurements in nature.

1.2.6 | Results

Across the 18 individuals, the average testes volume was 36.2 cm^3 , with a range of $16.4-54.4 \text{ cm}^3$ (Figure 3). The Kruskall–Wallis test confirmed that testes volume did differ among some individuals ($\chi^2 = 180.07$, p < 0.0001, df = 17).

The average between-photo CV was 5.5%, with a range from 1.7% to 15.5%. The average within-photo CV was 4.7%, with a range from 2.0% to 8.4%. This higher than expected within-photo error was not predicted by photo quality (R^2 = 0.01, $F_{(1,36)}$ = 0.31, p = 0.58; see Figure S1). The comprehensive interobserver analysis showed an ICC score of 0.92 with a 95% confidence interval of 0.80–0.97, indicating good to excellent reliability (Koo & Li, 2016). Individual interobserver error rates showed a mean error of 6.70%, with a range of 0.24%–37.2%. Two of the individuals were outliers (18.2% and 37.2%), and with their exclusion the mean error is 4.08% and the range is 0.24%–8.5%.

2 | COMPARISON AND CRITIQUE

2.1 | Measurement repeatability and reliability

The between-photo repeatability error (\bar{x} = 5.5%) in this study is comparable to that of other studies: average error ranged from 5.4% (glabella to rump) to 18.0% (distal hindlimb length) in mantled howler monkeys (Barrickman et al., 2015), 1.6%–5.1% (forearm length) and 3.5% (flange width) in Bornean orangutans (Brown et al., 2022), and 3.7% (forearm length) in Assamese macaques (Anzà et al., 2022). This relatively low error suggests that even if the same individuals were

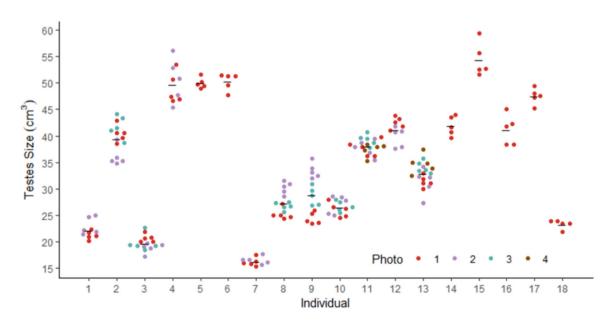


FIGURE 3 Testes measurements for all 18 individuals. Within-photo repeatability is indicated by measurements of same color and between-photo repeatability is indicated by measurement of differing color. Individual averages are denoted by a black line. [Color figure can be viewed at wileyonlinelibrary.com]

Within-photo repeatability error ($\bar{x} = 4.7\%$) was higher than the few previous studies that reported such data: 0.28%-0.98% for gelada shoulder-rump length (Lu et al., 2016) and 0.28%-0.74% and 0.62% for Bornean orangutan forearm length and flange width (Brown et al., 2022), respectively. We hypothesized that this could have been because of poor photo quality, but our analysis of the relationship between photo quality score and within-photo error did not support this hypothesis. Instead, our within-photo error was likely inflated as a consequence of measuring a three-dimensional phenotype as opposed to a twodimensional one. Here is a simplified example: when comparing measurements of the same photo with the dimensions of 3×2 cm and 4 × 3 cm, respectively, the difference in volume is 12.5 cm (18.8 cm³ vs. 6.3 cm³; difference of 66.5%), whereas the difference when considering only the length or width is 1 cm (difference of 33.3%). In our data set, when just considering the length or width of either testis, the average repeatability error decreased to 2.7%. Though still relatively high, this error could be due to the small size and irregular shape of the testes. When making measurements we tried to not include scrotal folds and extra scrotal skin, but these elements still made pinpointing the precise edges of the testes difficult and likely led to discrepancies between measurements.

Though our comprehensive interobserver assessment indicates high reliability, we did find substantial error rates for two individuals. For each of them we only had one usable photo, and both were of a lower quality. Were we to use this data set for analyses in the context of sperm competition or endocrine disruption, we would exclude these two individuals. For future studies, we suggest that the researchers determine a maximum error value for interobserver reliability before using the measurements for subsequent analyses; this value will be context-dependent and take into consideration the study species and the number of observers. Nevertheless, the interobserver error rates among the rest of the sample population $(\bar{x} = 4.08\%)$ remained relatively low and consistent with previous studies: 3.62% across anatomy in mantled howler monkeys Barrickman et al. (2015) and 4.74% across anatomy in Bornean orangutans (Brown et al., 2022). Both of these studies also show that error rate is higher for smaller anatomy compared to larger anatomy.

For complete validation of PLP accuracy, manual measurements of the same individuals are ideal. Because this validation was not possible for this study, we instead compared our PLP derived measurements with the manually derived measurements reported in Kelaita et al. (2011). Though the averages differ (36.2 cm³ vs. 22.7 cm³), this discrepancy could be because of population differences. More importantly, however, was that the range of our measurements (16.4–54.4 cm³) was encompassed by the range of manual measurements (11.4–61.2 cm³), indicating that PLP can capture biologically relevant testes size for this species (i.e., it is unlikely that our measurements were overestimates or underestimates). Furthermore, other studies show high accuracy when comparing PLP and manual measurements of the same individuals for other anatomy and taxa (Barrickman et al., 2015; Bergeron, 2007;

Galbany et al., 2016; Rizzo et al., 2017; Rogers et al., 2017; Rothman et al., 2008; Wright et al., 2019).

The tests of measurement repeatability and reliability presented in this study suggest that PLP can precisely measure testes noninvasively, despite the challenges associated with measuring such anatomy in an arboreal environment. However, we want to stress the importance of capturing multiple high-quality photos per individual.

2.2 | Considerations for photo capture

Capturing photos that had the testes and lasers in clear view and in the appropriate position was difficult in this arboreal species because the researcher is always below and relatively far from the study subject. Moreover, there is obstruction from foliage and a bright background from the light sky relative to the low light conditions in the forest. Furthermore, mantled howler monkeys can spend up to 67.5% of their time resting (Schreier et al., 2021), during which males lay on top of their testes, blocking them from view. Anecdotally, we found the best time for photo capture to be when males were feeding or moving between resting trees, as they would move around the terminal branches with their testes hanging below them. It may also be necessary to use additional camera accessories that can limit the impact of distance or background. However, there was no evidence of fundamental flaws with the PLP apparatus itself.

2.3 | Individual variation

We documented significant variation in testes size across 18 individuals within one population at La Selva Biological Station, Costa Rica. The difference between the males with the smallest and largest testes was over a threefold difference in size. While there has yet to be an explicit empirical study to characterize the causal factors of within-population variation in this species, several could be at play. First, for many nonhuman primate species absolute testes size scales to absolute body size (i.e., larger males have larger testes [Harcourt et al., 1981]). Adult male mantled howler monkeys vary in overall body size (Kelaita et al., 2011), and if testes size increases isometrically with body size, the individuals in our study population with larger testes may be larger males overall. Future studies should aim to collect both testes and body measurements via PLP to test this hypothesis. Second, as previously discussed, sperm competition is a potential contributor. Mantled howler monkeys live in groups ranging on average between 8 and 23 individuals with an average female-to-male sex ratio of 4:1 (Kelaita et al., 2011). In the La Selva population, the number of males within a group ranged from 1 to 4, so males that live in groups with other males may have larger testes than males that do not. Third, exposure to endocrine-active chemicals, whether dietary or anthropogenic, could affect morphological development and final size through direct effects on hormone receptors or indirectly through altering endogenous hormone

levels (Steiniche et al., 2023; Wasserman et al., 2012; Wasserman, Milton, et al., 2013). In a previous report by Wang et al., (2019, 2020), pesticides with androgen antagonistic properties were detected in both air samples as well as the fecal samples of mantled howler monkeys in La Selva, Costa Rica. However, a direct correlational analysis comparing endogenous pesticide presence and testes size has yet to be undertaken. For our current study, we also collected demographic, geographic, and environmental data, as well as fecal samples from the specific males examined here for pesticide and hormone concentration analyses. Future studies will examine the relative contributions of these factors to the variation of testes size documented here, and the use of PLP will facilitate research on within-population variation in other species.

2.4 | Conclusion

We have demonstrated that the PLP method precisely captures biologically meaningful variation in testes size in a wild population of nonhuman primates and holds great promise for future applications. To validate the results presented in this study, we encourage a follow-up study in a captive environment. This approach will allow for a direct comparison between PLP derived and manually derived testes measurements from the same individuals and prevent harm towards a wild population. The ongoing development of this reliable and noninvasive method for measuring the testes of wild animals will open up key avenues for research, with potential applications for questions that are focused on the basic biology of nonhuman primates as well as those that are focused on the effects of anthropogenic disturbance on primate populations.

AUTHOR CONTRIBUTIONS

Austen J. Ehrie: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (equal); Resources (equal); Software (equal); Supervision (supporting); Validation (lead); Visualization (lead); Writing—original draft (lead); Writing—review & editing (lead). Alec A. Iruri-Tucker: Conceptualization (supporting); Data curation (lead); Formal analysis (supporting); Investigation (supporting); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Writing-original draft (supporting). Yasmin B. Lord: Conceptualization (supporting); Data curation (lead); Formal analysis (supporting); Investigation (supporting); Methodology (equal); Project administration (equal); Supervision (supporting); Writing-original draft (supporting). Heidi G. Williamson: Data curation (equal); Formal analysis (equal); Methodology (equal); Software (equal); Validation (equal); Writing—review & editing (supporting). Kevin D. Hunt: Conceptualization (equal); Project administration (equal); Supervision (supporting); Writing-review & editing (equal). P. David Polly: Conceptualization (supporting); Data curation (supporting); Methodology (equal); Project administration (equal);

Software (equal); Validation (supporting); Writing—review & editing (equal). Courtney L. Fitzpatrick: Formal analysis (supporting); Supervision (equal); Validation (supporting); Visualization (supporting); Writing—original draft (equal); Writing—review & editing (equal). Michael D. Wasserman: Conceptualization (equal); Data curation (equal); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Resources (lead); Supervision (lead); Validation (supporting); Visualization (supporting); Writing—original draft (supporting); Writing—review & editing (equal).

ACKNOWLEDGMENTS

This study was conducted as part of the NSF IRES (International Research Experience for Students) grant #1854114. We want to thank the Organization for Tropical Studies and La Selva Biological Station staff for accommodating the living experience of the field researchers and allowing them to enter the forest for data collection. The utilization of parallel laser photogrammetry could not have been realized without the apparatus borrowed from Dr. Amy Schreier of Regis University. We thank Dr. Adam Fudickar for help in overall study design as well as Dr. Elizabeth George for helping with data visualization. This research experience would not have been as fulfilling without the camaraderie and insight from fellow IRES members Eric Johnson, Isabel Pacheco, Nyla Pete, Catharine Reed, and Wyatt Reed. Additional and much appreciated funding came from Indiana University from the Center for the Integrative Study of Animal Behavior undergraduate summer research grant and the Hutton Honors College undergraduate research grant.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All photos, raw measurements, data files, and R scripts are publicly available and can be found at the following link. https://zenodo.org/doi/10.5281/zenodo.10413240.

ORCID

Austen J. Ehrie http://orcid.org/0009-0000-6429-1650

REFERENCES

Adrus, M., Zainudin, R., Ahamad, M., Jayasilan, M. A., & Abdullah, M. T. (2019). Gastrointestinal parasites of zoonotic importance observed in the wild, urban, and captive populations of non-human primates in Malaysia. *Journal of Medical Primatology*, 48(1), 22–31. https://doi.org/10.1111/jmp.12389

Anzà, S., Berghänel, A., Ostner, J., & Schülke, O. (2022). Growth trajectories of wild Assamese macaques (*Macaca assamensis*) determined from parallel laser photogrammetry. *Mammalian Biology*, 102, 1–15. https://doi.org/10.1007/s42991-022-00262-2

Barrickman, N. L., Schreier, A. L., & Glander, K. E. (2015). Testing parallel laser image scaling for remotely measuring body dimensions on mantled howling monkeys (*Alouatta palliata*). *American Journal of Primatology*, 77(8), 823–832. https://doi.org/10.1002/ajp.22416

0982345, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ajp.23616 by Indiana University, Wiley Online Library on [18/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons Licensea

- Bell, C. M., Hindell, M. A., & Burton, H. R. (1997). Estimation of body mass in the southern elephant seal, Mirounga leonina, by photogrammetry and morphometrics. Marine Mammal Science, 13(4), 669-682. https://doi.org/10.1111/j.1748-7692.1997.tb00090.x
- Bergeron, P. (2007). Parallel lasers for remote measurements of morphological traits. The Journal of Wildlife Management, 71(1), 289-292. https://doi.org/10.2193/2006-290
- Bethell, E. J., Holmes, A., MacLarnon, A., & Semple, S. (2012). Evidence that emotion mediates social attention in rhesus macaques. PLoS One, 7(8), e44387. https://doi.org/10.1371/journal.pone.0044387
- Brown, E. R., Laman, T. G., Kane, E. E., Harwell, F. S., Susanto, T. W., & Knott, C. D. (2022). Application of a parallel laser apparatus to measure forearms and flanges of wild Bornean orangutans (Pongo pygmaeus wurmbii). American Journal of Primatology, 84(12), e23445. https://doi.org/10.1002/ajp.23445
- Chappell, J., Phillips, A. C., van Noordwijk, M. A., Mitra Setia, T., & Thorpe, S. K. S. (2015). The ontogeny of gap crossing behaviour in Bornean orangutans (Pongo pygmaeus wurmbii). PLoS One, 10(7), e0130291. https://doi.org/10.1371/journal.pone.0130291
- Cunningham, E. P., Unwin, S., & Setchell, J. M. (2015). Darting primates in the field: A review of reporting trends and a survey of practices and their effect on the primates involved. International Journal of Primatology, 36(5), 911-932. https://doi.org/10.1007/s10764-015-9862-0
- Dias, P. D. (2005). Observation of parturition in the Mexican mantled howler monkeys (Alouatta palliata) on the island of Agaltepec, Veracruz State, Mexico. American Journal of Primatology, 65(1), 93-98. https://doi.org/10.1002/ajp.20100
- Dixson, A. F., & Anderson, M. J. (2004). Sexual behavior, reproductive physiology and sperm competition in male mammals. Physiology & Behavior, 83(2), 361-371. https://doi.org/10.1016/j.physbeh.2004. 08 022
- Dolz, G., Chaves, A., Gutiérrez-Espeleta, G. A., Ortiz-Malavasi, E., Bernal-Valle, S., & Herrero, M. V. (2019). Detection of antibodies against flavivirus over time in wild non-human primates from the lowlands of Costa Rica. PLoS One, 14(7), e0219271. https://doi.org/10.1371/ journal.pone.0219271
- Domb, L. G., & Pagel, M. (2001). Sexual swellings advertise female quality in wild baboons. Nature, 410(6825), 204-206. https://doi.org/10. 1038/35065597
- Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., Dengg, S., Fitch, W. T., & Knapp, L. A. (2015). Evolutionary trade-off between vocal tract and testes dimensions in howler monkeys. Current Biology, 25(21), 2839-2844. https://doi.org/10. 1016/j.cub.2015.09.029
- Durban, J. W., & Parsons, K. M. (2006). Laser-metrics of free-ranging killer whales. Marine Mammal Science, 22(3), 735-743. https://doi.org/10. 1111/j.1748-7692.2006.00068.x
- Fedigan, L. M. (2010). Ethical issues faced by field primatologists: Asking the relevant questions. American Journal of Primatology, 72(9), 754-771. https://doi.org/10.1002/ajp.20814
- Firman, R. C., & Simmons, L. W. (2008). The frequency of multiple paternity predicts variation in testes size among island populations of house mice. Journal of Evolutionary Biology, 21(6), 1524-1533. https://doi.org/10.1111/j.1420-9101.2008.01612.x
- Fitzpatrick, C. L., Altmann, J., & Alberts, S. C. (2014). Sources of variance in a female fertility signal: Exaggerated estrous swellings in a natural population of baboons. Behavioral Ecology and Sociobiology, 68, 1109-1122. https://doi.org/10.1007/s00265-014-1722-y
- Galbany, J., Abavandimwe, D., Vakiener, M., Eckardt, W., Mudakikwa, A., Ndagijimana, F., Stoinski, T. S., & McFarlin, S. C. (2017). Body growth and life history in wild mountain gorillas (Gorilla beringei beringei) from Volcanoes National Park, Rwanda. American Journal of Physical Anthropology, 163(3), 570-590. https://doi.org/10.1002/ajpa.23232
- Galbany, J., Stoinski, T. S., Abavandimwe, D., Breuer, T., Rutkowski, W., Batista, N. V., Ndagijimana, F., & McFarlin, S. C. (2016). Validation of

- two independent photogrammetric techniques for determining body measurements of gorillas. American Journal of Primatology, 78(4), 418-431. https://doi.org/10.1002/ajp.22511
- Glander, K. E. (1980). Reproduction and population growth in free-ranging mantled howling monkeys. American Journal of Physical Anthropology, 53(1), 25-36. https://doi.org/10.1002/ajpa.1330530106
- Harcourt, A. H., Harvey, P. H., Larson, S. G., & Short, R. V. (1981). Testis weight, body weight and breeding system in primates. Nature, 293(5827), 55-57. https://doi.org/10.1038/293055a0
- Harcourt, A. H., Purvis, A., & Liles, L. (1995). Sperm competition: Mating system, not breeding season, affects testes size of primates. Functional Ecology, 9, 468-476. https://doi.org/10.2307/2390011
- Illia, G., Jouliá, R. B., Citon, L., Oklander, L., & Kowalewski, M. (2022). Parasites and other infectious agents in non-human primates of Argentina. Current Tropical Medicine Reports, 9, 267-277. https:// doi.org/10.1007/s40475-022-00277-2
- Jones, C. B. (1999). Testis symmetry in the mantled howling monkey. Simetría de los testículos en el mono Congo. Neotropical Primates, 7(4), 117-119.
- Kappeler, P. M. (1997). Intrasexual selection and testis size in strepsirhine primates. Behavioral Ecology, 8(1), 10-19. https://doi.org/10.1093/ beheco/8.1.10
- Kelaita, M., Dias, P. A. D., Aguilar-Cucurachi, M. S., Canales-Espinosa, D., & Cortés-Ortiz, L. (2011). Impact of intrasexual selection on sexual dimorphism and testes size in the Mexican howler monkeys Alouatta palliata and A. pigra. American Journal of Physical Anthropology, 146(2), 179-187. https://doi.org/10.1002/ajpa.21559
- Kenagy, G. J., & Trombulak, S. C. (1986). Size and function of mammalian testes in relation to body size. Journal of Mammalogy, 67(1), 1-22. https://doi.org/10.2307/1380997
- Köndgen, S., Kühl, H., N'Goran, P. K., Walsh, P. D., Schenk, S., Ernst, N., Biek, R., Formenty, P., Mätz-Rensing, K., Schweiger, B., Junglen, S., Ellerbrok, H., Nitsche, A., Briese, T., Lipkin, W. I., Pauli, G., Boesch, C., & Leendertz, F. H. (2008). Pandemic human viruses cause decline of endangered great apes. Current Biology, 18(4), 260-264. https://doi. org/10.1016/j.cub.2008.01.012
- Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. Journal of Chiropractic Medicine, 15(2), 155-163. https://doi.org/10.1016/j. jcm.2016.02.012
- Kumar, S., Srivastava, D. K., & Ahamad, S. (2012). A simple technique to estimate linear body measurements of elephants. Current Science, 102, 26.
- Kurita, H., Suzumura, T., Kanchi, F., & Hamada, Y. (2012). A photogrammetric method to evaluate nutritional status without capture in habituated free-ranging Japanese macaques (Macaca fuscata): A pilot study. Primates, 53, 7-11. https://doi.org/10.1007/s10329-011-0280-4
- Lemaire, G., Terouanne, B., Mauvais, P., Michel, S., & Rahmani, R. (2004). Effect of organochlorine pesticides on human androgen receptor activation in vitro. Toxicology and Applied Pharmacology, 196(2), 235-246. https://doi.org/10.1016/j.taap.2003.12.011
- Leurs, G., O'Connell, C. P., Andreotti, S., Rutzen, M., & Vonk Noordegraaf, H. (2015). Risks and advantages of using surface laser photogrammetry on free-ranging marine organisms: a case study on white sharks Carcharodon carcharias. Journal of Fish Biology, 86(6), 1713-1728. https://doi.org/10.1111/jfb.12678
- Lu, A., Bergman, T. J., McCann, C., Stinespring-Harris, A., & Beehner, J. C. (2016). Growth trajectories in wild geladas (Theropithecus gelada). American Journal of Primatology, 78(7), 707-719. https://doi.org/10. 1002/aip.22535
- Lüpold, S., Simmons, L. W., & Grueter, C. C. (2019). Sexual ornaments but not weapons trade off against testes size in primates. Proceedings of the Royal Society B: Biological Sciences, 286(1900), 20182542. https://doi.org/10.1098/rspb.2018.2542

- Marshall, S., Whorton, D., Krauss, R. M., & Palmer, W. S. (1978). Effect of pesticides on testicular function. *Urology*, 11(3), 257–259. https:// doi.org/10.1016/0090-4295(78)90128-0
- Mehrpour, O., Karrari, P., Zamani, N., Tsatsakis, A. M., & Abdollahi, M. (2014). Occupational exposure to pesticides and consequences on male semen and fertility: A review. *Toxicology Letters*, 230(2), 146–156. https://doi.org/10.1016/j.toxlet.2014.01.029
- Mnif, W., Hassine, A. I. H., Bouaziz, A., Bartegi, A., Thomas, O., & Roig, B. (2011). Effect of endocrine disruptor pesticides: A review. International journal of environmental research and public health, 8(6), 2265–2303. https://doi.org/10.3390/ijerph8062265
- Moller, A. P. (1989). Ejaculate quality, testes size and sperm production in mammals. Functional Ecology, 3, 91–96. https://doi.org/10.2307/ 2389679
- O'Connell, C. P., & Leurs, G. (2016). A minimally invasive technique to assess several life-history characteristics of the endangered great hammerhead shark *Sphyrna mokarran*. *Journal of Fish Biology*, 88(3), 1257–1264. https://doi.org/10.1111/jfb.12900
- Obanda, V., Maingi, N., Muchemi, G., Ng'ang'a, C. J., Angelone, S., & Archie, E. A. (2019). Infection dynamics of gastrointestinal helminths in sympatric non-human primates, livestock and wild ruminants in Kenya. *PLoS One*, *14*(6), e0217929. https://doi.org/10.1371/journal.pone.0217929
- Papworth, S., & Mejia, M. (2015). Population density of Ecuadorian mantled howler monkeys (Alouatta palliata aequatorialis) in a tropical dry forest, with information on habitat selection, calling behavior and cluster sizes. Studies on Neotropical Fauna and Environment, 50(2), 65-72. https://doi.org/10.1080/01650521.2015.1033939
- Peiris, D. C., & Dhanushka, T. (2017). Low doses of chlorpyrifos interfere with spermatogenesis of rats through reduction of sex hormones. Environmental Science and Pollution Research, 24(26), 20859–20867. https://doi.org/10.1007/s11356-017-9617-x
- Richardson, J. L., Levy, E. J., Ranjithkumar, R., Yang, H., Monson, E., Cronin, A., & McFarlin, S. C. (2022). Automated, high-throughput image calibration for parallel-laser photogrammetry. *Mammalian Biology*, 102, 1–13. https://doi.org/10.1007/s42991-021-00174-7
- Rizzo, A. A., Welsh, S. A., & Thompson, P. A. (2017). A paired-laser photogrammetric method for in situ length measurement of benthic fishes. North American Journal of Fisheries Management, 37(1), 16–22. https://doi.org/10.1080/02755947.2016.1235632
- Rogers, T. D., Cambiè, G., & Kaiser, M. J. (2017). Determination of size, sex and maturity stage of free swimming catsharks using laser photogrammetry. *Marine Biology*, 164, 213. https://doi.org/10. 1007/s00227-017-3241-7
- Rohner, C. A., Richardson, A. J., Marshall, A. D., Weeks, S. J., & Pierce, S. J. (2011). How large is the world's largest fish? Measuring whale sharks Rhincodon typus with laser photogrammetry. Journal of Fish Biology, 78(1), 378–385. https://doi.org/10.1111/j.1095-8649.2010. 02861.x
- Rothman, J. M., Chapman, C. A., Twinomugisha, D., Wasserman, M. D., Lambert, J. E., & Goldberg, T. L. (2008). Measuring physical traits of primates remotely: The use of parallel lasers. American Journal of Primatology, 70(12), 1191–1195. https://doi.org/10.1002/ajp. 20611
- Sandel, A. A., Derby, R. N., Chesterman, N. S., McNamara, A., Dudas, M. M., & Rawat, I. (2022). Parallel lasers and digital photography to estimate limb size of chimpanzees (*Pan troglodytes*) at Ngogo, Kibale National Park, Uganda. *Primates*, 63(3), 217–224. https://doi.org/10.1007/s10329-022-00982-6
- Sapolsky, R. M., & Share, L. J. (1998). Darting terrestrial primates in the wild: A primer. American Journal of Primatology, 44(2), 155–167. https://doi.org/10.1002/(SICI)1098-2345(1998)44:2<155::AID-AJP6>3.0.CO:2-V
- Schreier, A. L., Bolt, L. M., Russell, D. G., Readyhough, T. S., Jacobson, Z. S., Merrigan-Johnson, C., & Coggeshall, E. M. C. (2020). Mantled howler

- monkeys (Alouatta palliata) in a Costa Rican forest fragment do not modify activity budgets or spatial cohesion in response to anthropogenic edges. Folia Primatologica, 92(1), 49–57. https://doi.org/10.1159/000511974
- Schuppli, C., Forss, S. I. F., Meulman, E. J. M., Zweifel, N., Lee, K. C., Rukmana, E., Vogel, E. R., van Noordwijk, M. A., & van Schaik, C. P. (2016). Development of foraging skills in two orangutan populations: Needing to learn or needing to grow? *Frontiers in zoology*, 13, 43. https://doi.org/10.1186/s12983-016-0178-5
- Shaver, I., Chain-Guadarrama, A., Cleary, K. A., Sanfiorenzo, A., Santiago-García, R. J., Finegan, B., Hormel, L., Sibelet, N., Vierling, L. A., Bosque-Pérez, N. A., DeClerck, F., Fagan, M. E., & Waits, L. P. (2015). Coupled social and ecological outcomes of agricultural intensification in Costa Rica and the future of biodiversity conservation in tropical agricultural regions. Global Environmental Change, 32, 74–86. https://doi.org/10.1016/j.gloenvcha.2015.02.006
- Sigel, B. J., Sherry, T. W., & Young, B. E. (2006). Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. *Conservation Biology*, 20(1), 111–121. https://doi.org/10.1111/j.1523-1739.2005.00293.x
- Steiniche, T., Wang, S., Chester, E., Mutegeki, R., Rothman, J. M., Wrangham, R. W., Chapman, C. A., Venier, M., & Wasserman, M. D. (2023). Associations between faecal chemical pollutants and hormones in primates inhabiting Kibale National Park, Uganda. *Biology Letters*, 19(5), 20230005. https://doi.org/10.1098/rsbl. 2023.0005
- Takihara, H., Cosentino, M. J., Sakatoku, J., & Cockett, A. T. K. (1987). Significance of testicular size measurement in andrology: II. Correlation of testicular size with testicular function. *Journal of Urology*, 137(3), 416–419. https://doi.org/10.1016/S0022-5347(17) 44053-5
- Vom Saal, F. S. (1983). The interaction of circulating oestrogens and androgens in regulating mammalian sexual differentiation, In Hormones and Behaviour in Higher Vertebrates (pp. 159–177). Springer. https://doi.org/10.1007/978-3-642-69216-1_11
- Wallis, J., & Rick Lee, D. (1999). Primate conservation: The prevention of disease transmission. *International Journal of Primatology*, 20, 803–826. https://doi.org/10.1023/A:1020879700286
- Wang, S., Steiniche, T., Romanak, K. A., Johnson, E., Quirós, R., Mutegeki, R., Wasserman, M. D., & Venier, M. (2019). Atmospheric occurrence of legacy pesticides, current use pesticides, and flame retardants in and around protected areas in Costa Rica and Uganda. *Environmental Science & Technology*, 53(11), 6171–6181. https://doi. org/10.1021/acs.est.9b00649
- Wang, S., Steiniche, T., Rothman, J. M., Wrangham, R. W., Chapman, C. A., Mutegeki, R., Quirós, R., Wasserman, M. D., & Venier, M. (2020). Feces are effective biological samples for measuring pesticides and flame retardants in primates. *Environmental Science & Technology*, 54(19), 12013–12023. https://doi.org/10.1021/acs.est.0c02500
- Wasserman, M. D., Chapman, C. A., Milton, K., Gogarten, J. F., Wittwer, D. J., & Ziegler, T. E. (2012). Estrogenic plant consumption predicts red colobus monkey (*Procolobus rufomitratus*) hormonal state and behavior. *Hormones and Behavior*, 62(5), 553–562. https:// doi.org/10.1016/j.yhbeh.2012.09.005
- Wasserman, M. D., Chapman, C. A., Milton, K., Goldberg, T. L., & Ziegler, T. E. (2013). Physiological and behavioral effects of capture darting on red colobus monkeys (*Procolobus rufomitratus*) with a comparison to chimpanzee (*Pan troglodytes*) predation. *International Journal of Primatology*, 34(5), 1020–1031. https://doi.org/10.1007/s10764-013-9711-y
- Wasserman, M. D., Milton, K., & Chapman, C. A. (2013). The roles of phytoestrogens in primate ecology and evolution. *International Journal of Primatology*, 34, 861–878. https://doi.org/10.1007/ s10764-013-9699-3

- Webster, T., Dawson, S., & Slooten, E. (2010). A simple laser photogrammetry technique for measuring Hector's dolphins (*Cephalorhynchus hectori*) in the field. *Marine Mammal Science*, 26(2), 296–308. https://doi.org/10.1111/j.1748-7692.2009.00326.x
- Weisgerber, J. N., Medill, S. A., & McLoughlin, P. D. (2015). Parallel-laser photogrammetry to estimate body size in free-ranging mammals. Wildlife Society Bulletin, 39(2), 422–428. https://doi.org/10.1002/ wsb.541
- Wright, E., Galbany, J., McFarlin, S. C., Ndayishimiye, E., Stoinski, T. S., & Robbins, M. M. (2019). Male body size, dominance rank and strategic use of aggression in a group-living mammal. *Animal Behaviour*, 151, 87–102. https://doi.org/10.1016/j.anbehav.2019.03.011
- Wright, E., Galbany, J., McFarlin, S. C., Ndayishimiye, E., Stoinski, T. S., & Robbins, M. M. (2020). Dominance rank but not body size influences female reproductive success in mountain gorillas. *PLoS One*, 15(6), e0233235. https://doi.org/10.1371/journal.pone.0233235

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ehrie, A. J., Iruri-Tucker, A. A., Lord, Y. B., Williamson, H. G., Hunt, K. D., Polly, P. D., Fitzpatrick, C. L., & Wasserman, M. D. (2024). Measuring mantled howler monkey (*Alouatta palliata*) testes via parallel laser photogrammetry: Expanding the use of noninvasive methods. *American Journal of Primatology*, e23616. https://doi.org/10.1002/ajp.23616