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The impact of genetic restoration on cranial morphology of Florida panthers (*Puma concolor coryi*)

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The breeding population of the endangered Florida panther (*Puma concolor coryi*) is confined to the southern portion of the Florida peninsula. During the 20th century, a combination of isolation and small population size resulted in increased inbreeding. This ultimately led to a genetic restoration program in 1995 to alleviate correlates of inbreeding depression and avert extinction of the Florida panther. Genetic restoration involved the release of 8 female Texas pumas into the wild Florida population, a management initiative that has significantly improved the outlook for recovery. It is unknown whether genetic restoration changed the distinctive cranial morphology of the Florida panther; and whether some metrics that were historically used to differentiate Florida panthers from other subspecies of *P. concolor* are still valid. We used a high-resolution digital imaging system to compare cranial morphology from several groupings of Florida panthers that were defined by genetic restoration to elucidate any morphological changes this event may have had on cranial profile. Our results indicated that cranial measurements of Florida panthers were not significantly altered by genetic restoration.

Key words: cranial morphology, digital analysis, digital imagery, Florida panther, genetic restoration, *Puma concolor coryi*

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The federally endangered Florida panther (*Puma concolor coryi*) once ranged throughout the southeastern United States before becoming isolated in the southern tip of peninsular Florida in the 20th century due to varied anthropogenic factors (Land et al. 2004; Kautz et al. 2006; Onorato et al. 2010; Fig. 1). As with many subspecies cataloged within the early 20th century, Florida panthers were uniquely identified among 30 different subspecies of *P. concolor* via multiple morphometric and pelage characteristics (Nelson and Goldman 1929; Young and Goldman 1946). The initial morphometric description by Young and Goldman (1946) of the Florida panther as a subspecies was supported by Wilkins et al. (1997), who quantified the defining characters of the panther. Florida panther crania exhibited a broad, flat frontal region and a highly arched nasal profile termed the "Roman nose" (Young and Goldman 1946). Other characteristics frequently associated with the Florida panther population during the early years (1981–1995) of field research included a cowlick (middorsal whorl of hair) as well as a deformity of the distal caudal vertebrae resulting in a 90° bend referred to as a kinked tail (Onorato et al. 2010). O'Brien et al. (1990) described the tail kink and cowlick as traits of the Florida panther, whereas Wilkins et al. (1997) concluded that both traits are found in other subspecies of puma, but were more common in Florida panthers. During the 1980s and early 1990s, the panther population was thought to comprise approximately 20–30 individuals (McBride et al. 2008). The isolation and small size of the population resulted in extensive inbreeding, which decreased the genetic heterozygosity of the population and exacerbated certain genetic defects including low sperm quality and quantity, cryptorchidism, atrial septal defects, and reduced immunity to infectious diseases (Roelke et al. 1993; Barone et al. 1994, Johnson et al. 2010).

In an effort to mitigate the decline in genetic diversity, multiple government agencies, including The United States Fish and Wildlife Service, the Florida Fish and Wildlife Conservation Commission, and the National Park Service, as well as academics from varied disciplines of wildlife conservation, agreed to release 8 female pumas (*P. c. stanleyana*) from Brewster County, Texas (Fig. 1, bottom), into southern Florida in 1995 (Johnson et al. 2010; Onorato et al. 2010). Texas pumas were chosen for this genetic

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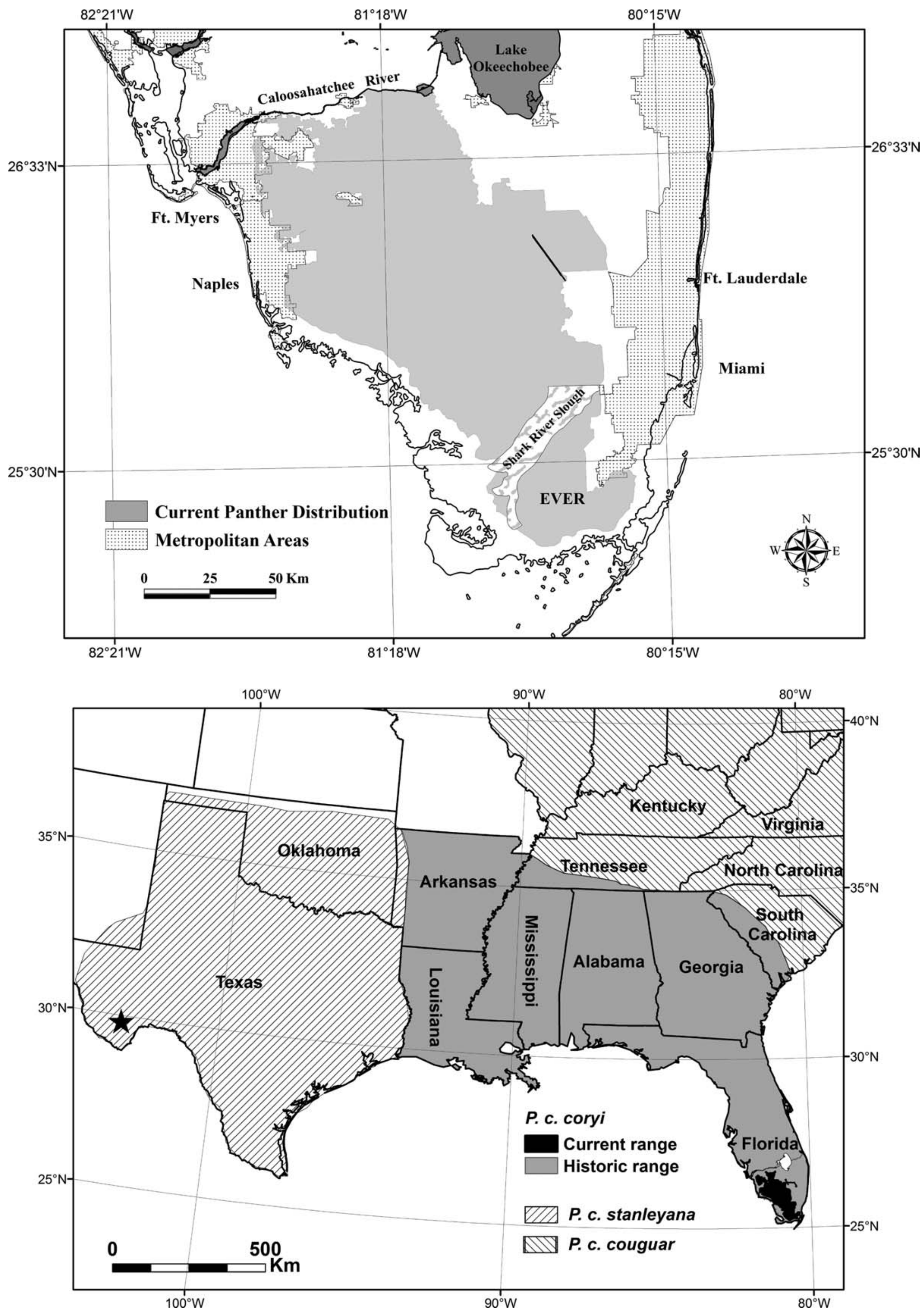

FIG. 1.—Geographical representation of the current breeding range of the Florida panther in southern Florida (top) and the distribution of 3 subspecies of *Puma concolor* described in the text (bottom). The historic ranges for *P. c. stanleyana*, *P. c. couguar*, and *P. c. coryi* are from Young and Goldman (1946). *P. c. couguar*, the eastern cougar, is extinct. The map also depicts the location where female Texas pumas were collected in western Texas (★) for release in southern Florida as part of the genetic restoration project. EVER = Everglades National Park.

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augmentation because the ranges of the 2 subspecies historically shared a common border—where they presumably interbred, logically making them the most closely related, extant population (Young and Goldman 1946; Johnson et al. 2010). Five of the 8 Texas females mated with wild Florida panthers; the last Texas puma was removed from the wild in 2003 (Land et al. 2004; Johnson et al. 2010).

By all indications, genetic restoration was successful in terms of increasing genetic variation and decreasing the correlates of inbreeding (e.g., cryptorchidism, atrial septal defects, and low fecundity) that typified the Florida panther population prior to 1995 (Johnson et al. 2010). In addition, genetic restoration has played a significant role in the increased population size of the Florida panther (McBride et al. 2008; Johnson et al. 2010), although the breeding population remains restricted to areas south of the Caloosahatchee River in southern Florida (Fig. 1, top). Because of the genetic restoration project, the occurrence of defining morphological characteristics such as kinked tails and cowlicks as well as genetic correlates of inbreeding decreased, in some cases dramatically, in admixed (i.e., mixed-heritage offspring) panthers born after 1995 (Johnson et al. 2010; Onorato et al. 2010). Although genetic restoration with Texas puma females reduced the occurrences of these traits in Florida panthers, we do not know whether it changed the characteristic skull morphology of Florida panthers described by Young and Goldman (1946) and quantified by Wilkins et al. (1997).

To determine whether genetic restoration has altered the prototypical cranial morphology of Florida panthers, we examined crania from time periods both before and after the introduction of the Texas female pumas in 1995. We wanted to assess whether genetic restoration had changed the distinguishing skull morphology described by Wilkins et al. (1997), who used traditional skull measuring techniques (e.g., calipers) and whose sample of skulls did not include any panthers born after genetic restoration. Our study included samples of both pre– and post–genetic restoration panthers and utilized a novel method for measuring cranial morphology via a high-resolution digital imaging system.

We measured and compared the nasal profiles (i.e., Roman nose) and other cranial characteristics based on sex, historic versus recently born panthers (era analysis; born prior to genetic restoration in 1995 versus after), nonadmixed versus admixed Florida panthers (ancestry analysis; as assigned via genetic data), and Florida panthers versus Texas pumas. The historic category included all panthers born prior to genetic restoration in 1995, whereas recent panthers were those born after the restoration in 1995. We used genetic data provided by Johnson et al. (2010) to classify each panther in the historic and recent categories as either nonadmixed or admixed; all historic panthers were nonadmixed. In the Florida panther literature panthers that are of pure Florida origin have been labeled "canonical" panthers, but we referred to them here as nonadmixed Florida panthers (Johnson et al. 2010; Onorato et al. 2010). Admixed panthers, by contrast, are those that have a 10% threshold of Texas ancestry as determined by Johnson et al. (2010).

The morphometric study of Wilkins et al. (1997) predicted that male crania should be larger than female crania for both Florida panthers and Texas pumas; Texas puma crania should be larger than Florida panther crania; and there should be significant differences between the nasal profiles of Florida panthers and Texas pumas. It is more difficult, however, to predict the impact of genetic restoration on the cranial measurements and nasal profiles of nonadmixed and historic panthers in comparison to recent and admixed panthers. Historically, gene flow between the Texas subspecies and the Florida panther did not impact the cranial morphology sufficiently to negate subspecific designations via morphology (Young and Goldman 1946). In addition, the short period of time that the pure Texas females spent in the wilds of southern Florida assisted in controlling the level of introgression (Johnson et al. 2010; Onorato et al. 2010), in effect limiting the likelihood of significant changes in skull morphology resulting from this management initiative. The findings of previous studies leads us to predict that there will be no significant differences between the skull morphology of nonadmixed, admixed, historic, or recent Florida panther groupings.

MATERIALS AND METHODS

Selection of specimens.—The majority of specimens used in this study reside in the Florida Museum of Natural History (FLMNH) Mammal Collection in Gainesville, Florida. The remaining specimens were loaned from Sul Ross State University (SRSU) in Alpine, Texas. The main criteria for selecting specimens were condition of cranium, collection locality, age class, estimated date of birth, and ancestry as delineated via genetic data. The limiting factor for this study was the condition of the cranium. A large portion of the panther skulls in the FLMNH collection were damaged at death and could not be fully measured because of extensive fracturing related to vehicle collisions or intraspecific encounters. All Florida panthers ($n = 20$ each for males and females from the FLMNH) were collected west of Shark River Slough, Florida (Fig. 1, top), the core habitat where the highest concentration of nonadmixed panthers historically persisted. The Texas puma specimens ($n = 8$, 1 male and 2 females from the FLMNH, 3 males and 2 females from SRSU) were collected west of the Pecos River in western Texas (Fig. 1, bottom) to match the geographic origins of the female pumas used for genetic restoration. Two of the Texas female skulls we measured were from individuals released into southern Florida (TX103 and TX107). All sampled specimens were classified as adults if the basialisphenoid fissure was fused and exhibited completely developed crania. We used 1995, the year genetic restoration was initiated, as the dividing point when selecting panther specimens. All curatorial data pertaining to the crania used in this study are provided in Appendix I.

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Within the Florida panther group, we further categorized each specimen as either historic or recent (era group) and nonadmixed or admixed (ancestry group) in order to quantify changes to the unique cranial morphology of Florida panthers resulting from genetic admixture with Texas pumas. The main purpose of the era grouping was to use a temporal scale to assign specimens to a category without relying on genetic information, whereas the ancestry grouping was based on known pedigree and genetic data from Johnson et al. (2010). Historic panthers were estimated to have been born prior to 1995 ($n = 10$ each for males and females), whereas recent panthers were born after 1995 ($n = 10$ each for males and females). Our historic sample was composed of individuals born from 1971 to 1991, whereas our recent sample included skulls from panthers born from 1996 to 2005. Nonadmixed grouping of Florida panthers were defined as having no direct non-Florida relatives or $< 10\%$ non-Florida ancestry as delineated via genetic data (Johnson et al. 2010). The admixed grouping of panthers included descendants of the introduced female Texas pumas. All Florida panthers included in the aforementioned historic era group ($n = 10$ each for males and females) and 4 panthers from the recent era group ($n = 2$ each for males and female) were classified as nonadmixed via genetic data. The remaining recent category specimens ($n = 8$ each for males and females) were designated as admixed panthers according to the same genetic data (Johnson et al. 2010; Appendix I).

Photography and measurement collection.—We used a high-resolution digital imaging system (Visionary Digital, Palmyra, Virginia) that utilized a Canon 5D Mark II 20 megapixel camera body (Canon USA Inc., Melville, New York) mounted on a software-controlled copy stand to photograph the specimens following the suggestions of Pearcy and Wijttin (2010) in regard to consistency in photographing crania for morphological analysis. The camera was set to manual mode with an aperture range of F10–12 and a shutter speed of 1/200. We used a pair of adjustable halogen bulb lamps to provide the necessary lighting and a black background. We selected the Canon 50-mm 2.8f lens (Canon USA Inc.) at a 1:8 focal length to photograph specimens. The lens was calibrated with micrometer scales in Photoshop CS3 (Adobe Systems Inc., San Jose, California) at each incremental focal length by taking an image of an ultrafine ruler. The resulting accuracy of measurement was approximately 6 μm.

We photographed each cranium dorsally, ventrally, and laterally detached from the dentary. In dorsal photographs, the cranium rested on the occipital condyle and the canines. If the canines were absent, the cranium rested on the incisors or dental alveolus and the pterygoid processes. We used the middle of the temporal line as a focus to center the cranium in the camera's field of view. For ventral photographs, the cranium rested on the frontal bone with the posterior of the palatine centered in the camera's field of view. We used a foam block to stabilize and level the cranium for lateral pictures. The cranium was aligned so that the zygomatic arch was centered, the lingual edge of the maxillary toothrow was parallel to the edge of camera's field of view, and the 2 maxillary toothrows were on the same vertical plane as the camera. For each photograph we created a 100-mm scale bar using Photoshop CS3 and selected the ruler tool to collect 15 cranial measurements in millimeters for each specimen. Descriptions and abbreviations for each measurement are provided in Appendix II.

The Roman nose nasal profile of each Florida panther specimen was traced over the lateral image in a new canvas layer using the brush tool in Photoshop CS3 starting at the tip of the nasals and proceeding to the temporal line (Fig. 2). The outline of the nasal profile was then moved to a new image file and rotated in relation to a horizontal plane so the terminals were in contact with the plane. The images were quantified using ImageJ software (Rasband 2009) by recording the y-value at 2-mm increments along the curve using the software's multipoint tool. The values were then normalized to remove variation due to size and establish the high ($y = 1$) and low ($y = 0$) points of the slope to permit a graphical comparison (Fig. 3).

Tests of precision for cranial measurements.—It was impractical to photograph a large group of specimens in a single day. If the focus on the lens was not calibrated consistently or if a different focal point on the specimen was selected, the image would be slightly altered and therefore the measurements would differ. To account for variation resulting from taking skull images on different days, we photographed a single male specimen (FP135) on 5 consecutive days and recorded each of the 15 cranial measurements for each photograph to determine the error associated with photographing specimens over time (multiple picture test). An intraclass correlation coefficient (ICC) test (Winer 1971; Baik et al. 2006) was used to statistically assess precision as it related to measurements recorded from images taken on different days. To verify the precision of measurements collected using the imaging software, we randomly selected 5 male specimens (FP07, FP25, FP99, UCFP45, and UCFP89) and recorded each of the 15 cranial measurements 5 times for each cranium (multiple measurement test). We statistically assessed the precision of the multiple measurements using an ICC test, as described for the multiple picture test.

Comparative tests for cranial morphology.—We used a multivariate analysis of variance (MANOVA) to assess whether there were significant differences between the 15 cranial measurements for sex (males and females), era (historic and recent panthers), ancestry (nonadmixed and admixed panthers), and location (Texas and Florida). First, we tested to determine if there were confounding interactions between era and sex (era*sex; e.g., were differences between sex constant across era) and between ancestry and sex (ancestry*sex). Then, we tested for significant differences between the 15 cranial measurements within the era group (historical versus recent) and within the ancestry group (nonadmixed versus admixed). Finally we compared the 15 cranial measurements of Florida panthers and Texas pumas.

The normalized y-intercepts of the Florida panther nasal profiles were compared using a 1-way analysis of variance

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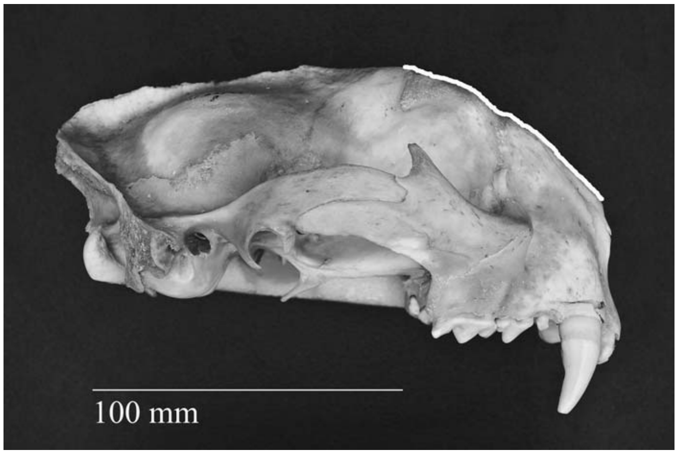

Fig. 2.—Measurement process for the nasal profile. Overlay of hand-drawn line using Photoshop CS3 starting from the nasal bones and ending at the temporal line. The line was then rotated in relation to a horizontal plane so the terminals were in contact with the plane. Specimen shown is FP04, a male, nonadmixed Florida panther.

(ANOVA) framework to determine if differences existed between sex, era, and ancestry groups using simple contrasts. Comparisons with the Texas puma nasal profiles also were completed to determine if the profile slopes of admixed panthers fall between nonadmixed panthers and Texas pumas. All statistical analyses were computed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina).

Results

Tests of precision for cranial measurements.—The ICC test results of the multiple picture test indicated that our measurements should provide a reliable estimate >99% of the time for each cranium sampled (ICC value = 0.99951), regardless of whether pictures were taken for skulls on the same day or a number of days. The estimate of ICC for our multiple measurement test indicated that our measuring protocol produced precise measurements >95% of the time for all 15 cranial measurements, thereby providing reliable measures for subsequent analyses (Table 1).

Comparative tests for cranial morphology.—The MANOVA framework to test interactions for the 15 cranial measurements between era (historic and recent)*sex (e.g., were differences between sex constant across era) and ancestry (nonadmixed and admixed)*sex were not significant for Florida panthers ($F_{15,13} = 0.85$, $P = 0.6185$ and $F_{15,13} = 0.90$, $P = 0.5845$, respectively). Therefore, we combined all male panthers and all females to determine whether cranial measurements differed significantly by sex. Results for this main-effects test showed that there was a significant difference between the cranial measurements of male and female panthers ($F_{15,13} = 26.87$, $P < 0.0001$). Results from univariate tests that

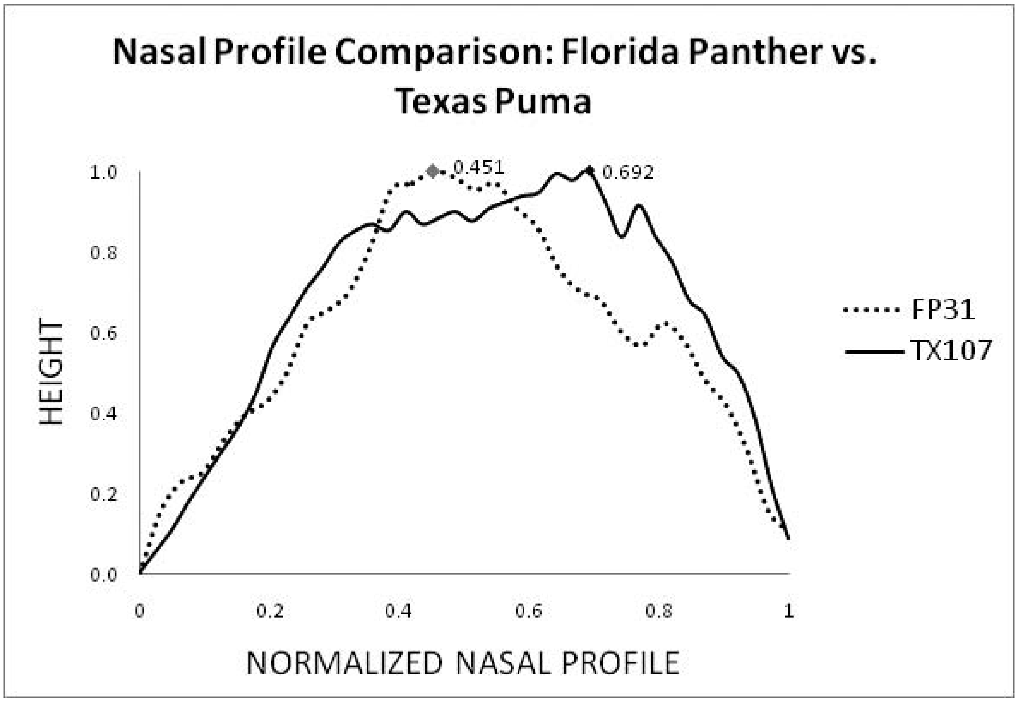

Fig. 3.—Images of normalized nasal profiles of a Florida panther and a Texas puma. The nasal profiles of Florida panther FP31 (dashed line) and Texas puma TX107 (solid line) show the location of the highest point of the each cranium (y = 1) and the highly arched Roman nose in the Florida panther. The nasal profiles were created using the brush tool of Photoshop CS3 and quantified using ImageJ software measured at 2-mm increments along the x-axis. The profiles were then normalized on the x- and y-axes. The anterior end of the cranium (nasal bones) is located at x = 0, the inflection point of the nasal arc is found at y = 1, and the posterior end of the cranium (frontal region) is located at x = 1. The closer the inflection point is to x = 0, the more pronounced the nasal region is in the cranium.

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Table 1.—Intraclass correlation coefficient (ICC) test results assessing cranial measurement precision for Florida panther crania. Five male specimens (FP07, FP25, FP99, UCFP45, and UCFP89) were randomly selected to verify the precision of measurements collected using Photoshop CS3. The 15 cranial measurements were recorded 5 times each for each cranium. All ICC values were >0.95. Abbreviations of cranial measurements are provided in Appendix II.

Cranial measurement	ICC
CBL	0.98954
CRH	0.97926
CW	0.97989
MASB	0.99664
MXTR	0.99049
PAL	0.99271
PAW	0.99630
POC	0.98415
POPB	0.95858
PW	0.99098
TL	0.99773
UCL	0.98856
UCW	0.99214
WC	0.98875
ZB	0.98756

assess this difference for each measure revealed that 9 of 15 measurements were significantly larger for males than for females (Table 2).

The comparison of era (historic versus recent panthers; by sex) assessed differences between cranial measurements from panthers born in the historic preintrogression era (prior to 1995) to those born in the recent postrestoration (after 1995) era and accounted for the constant difference between sexes. This comparison did not incorporate genetic information associated with the specimens. MANOVA results indicated that cranial measurements from historic and recent Florida panthers were not significantly different ($F_{15,13} = 1.09$, $P = 0.4428$). The comparison of ancestry (nonadmixed versus admixed panthers; by sex), which relied on genetic designations of specimens, also took into account the constant difference between sexes. MANOVA results indicated that there was no significant difference in cranial measurements for nonadmixed versus admixed Florida panthers ($F_{15,13} = 0.65$, $P = 0.7909$).

We subsequently compared the 15 cranial measurements of Florida panthers to those of Texas pumas. Because our previous tests with Florida panthers determined there were no significant differences between era and ancestry, we combined all panthers for our comparison with the Texas pumas. A MANOVA framework was used to test for an interaction between location (Texas versus Florida) and sex (e.g., were differences between sex constant across locations). This interaction was significant ($F_{15,19} = 3.75$, $P = 0.0039$), indicating that differences between the sexes were not constant across locations. The main effects test comparing males and females for the entire sample combined (male Texas and Florida skulls versus female Texas and Florida skulls) revealed a significant difference ($F_{15,19} = 4.71$, $P = 0.0010$), further reiterating the conclusion that male and female cranial morphologies were significantly different. A test for the main effect of location comparing Texas and Florida crania, taking into account differences resulting from the sex of a specimen, revealed significant differences in cranial measurements ($F_{15,19} = 6.80$, $P < 0.0001$).

Table 2.—Univariate tests comparing all male ($n = 20$) versus all female ($n = 20$) Florida panthers (*Puma concolor coryi*) for each of the 15 skull measurements. Nine of 15 cranial measurements were significantly (*) larger for males than females. Abbreviations for cranial measurements are provided in Appendix II.

Measure	F_{19}	P
CBL	22.42	0.0001*
CRH	27.43	0.0001*
CW	0.81	0.3756
MASB	66.65	0.0001*
MXTR	5.41	0.0267*
PAL	150.32	0.0001*
PAW	2.35	0.1354
POC	0.27	0.6069
POPB	11.84	0.0017*
PW	0.55	0.4642
TL	10.47	0.0029*
UCL	8.17	0.0075*
UCW	0.11	0.7372
WC	0.72	0.4033
ZYB	10.99	0.0023*

The mean height of the nasal profile contour measurements for the different groupings of Florida panthers ranged from 0.450 to 0.531, whereas the Texas puma average profile was 0.544. All but 1 of the Florida panther normalized height values fell within the range of values for the Texas pumas, although the means were consistently lower for all Florida panther groupings (Fig. 4). The contrasts from a 1-way ANOVA revealed that males and females do not differ significantly ($t = 0.33$, $P = 0.7462$) in nasal profile contour measurements. We combined the sexes for subsequent Roman nose analyses and failed to find significant differences between the nasal profiles within era ($t = 1.29$, $P = 0.2061$), ancestry ($t = 1.64$, $P = 0.1086$), nonadmixed and Texas ($t = 1.62$, $P = 0.1132$), or admixed and Texas comparisons ($t = 0.27$, $P = 0.7907$).

Discussion

We compared the cranial morphology from several groupings of Florida panthers that were defined by genetic restoration, to elucidate any physical effects this event had on the historically unique Florida panther cranial profile. We also compared panther groups to Texas puma crania to determine whether admixed panthers resulting from genetic restoration were more similar to Florida panthers or to Texas pumas. These comparisons were made with data collected using a novel technique via high-resolution digital imaging that collected very precise measurements. Given that morphology played a pivotal role in the historic delineation of the subspecies *P. c. coryi*, assessing how genetic restoration may

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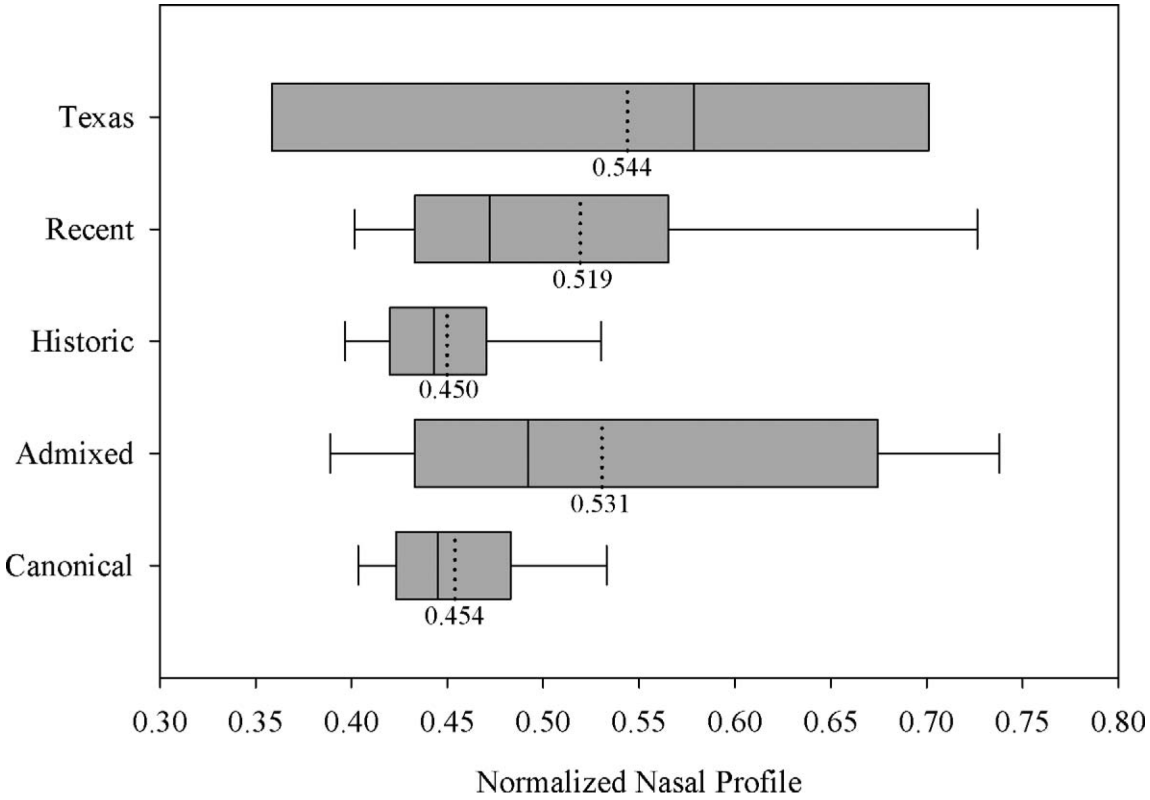

FIG. 4.—Box plot of normalized nasal profile median (solid lines) and mean (dotted lines) for values of x when $y = 1.0$ (the highest point of the cranium) for historic ($n = 20$), recent ($n = 20$), nonadmixed ($n = 24$), and admixed ($n = 16$) Florida panthers, and Texas pumas ($n = 8$). Whiskers depict the 95% confidence intervals. The sexes were combined for this comparison because we found no significant differences between sexes within each grouping. The 95% confidence intervals for Texas pumas could not be calculated because of small sample size.

have altered distinctive cranial traits is of interest to varied government agencies and conservation groups that are involved with Florida panther recovery.

Our analyses of Florida panther and Texas puma crania found significant differences between males and females for the 15 different cranial measurements. Results revealed that male crania were larger than female crania, supporting the previous findings of Gay and Best (1995) and Wilkins et al. (1997). Male pumas have significantly more robust crania and have a body mass 40–60% greater than females (Anderson 1983; Maehr and Moore 1992; Gay and Best 1995, 1996; Wilkins et al. 1997). Gay and Best (1995, 1996) determined that the variation in size was linked to sexual selection caused by inter- and intraspecific competition and to a lesser extent age, because puma crania continue to grow up to 9 years in males and 6 years in females.

We wanted to assess the impact of the genetic restoration project on the morphology of panther crania via 2 methods: comparison of specimens categorized into groups without using genetic data (era analysis) and comparison of specimens categorized into groups utilizing genetic data (ancestry analysis). Wilkins et al. (1997) was unable to sample admixed panthers or compare the ancestry of the specimens using genetic data because the data were not available at the time. The most recently collected specimen in their study was from 1990, 5 years prior to genetic restoration (Wilkins et al. 1997), whereas our most recent specimen was collected in 2005. Our study added 2 additional levels of scrutiny to the study conducted by Wilkins et al. (1997) by incorporating Texas–Florida admixed panthers and utilizing genetic ancestry information provided by Johnson et al. (2010). Our MANOVA took into account differences among the sexes and noted no significant differences for the 15 cranial measurements when compared between era (recent versus historic) or ancestry (admixed versus nonadmixed) Florida panther groupings. Therefore, whether we view differences from a genetic (ancestry) or temporal (era) perspective, these skull morphology measures have not changed significantly as a result of genetic restoration.

Given that admixed Florida panthers comprise a majority of the panther population today (Johnson et al. 2010), it is important to note that the cranial morphology for these 15 different measures was not significantly different between admixed and the nonadmixed Florida panthers that dominated the population prior to 1995. Although nonadmixed panthers are still found in the population, admixed panthers comprised 75.2% of the 424 panthers handled between 1995 and 2008 (including 272 neonatal kittens—Johnson et al. 2010). The amount of Texas ancestry in admixed Florida panthers is variable (Johnson et al. 2010). Seven of the 16 sampled admixed Florida panthers in our study had >50% Texas ancestry, 5 had <50% Texas ancestry, and 4 had an unknown

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level of Texas ancestry (Johnson et al. 2010). One of the introduced female Texas pumas sampled from the FLMNH collection (TX107) had the 2nd highest genetic contribution to the Florida panther population during the genetic restoration project, after TX101 (Johnson et al. 2010). This female had the largest cranium of all the Texas pumas sampled in our study with the maximum measurement values for all measurements except for POC. Four of the admixed panthers in our study (FP86, FP91, FP120, and FP127) can trace their lineage back to TX107 either as direct offspring or as 2nd-generation offspring (i.e., a grandchild—Johnson et al. 2010). Eight of the admixed panthers in our study were sired by FP79, an F_1 admixed Florida panther whose dam is TX101 and whose sire was an unknown panther with a unique allele (Johnson et al. 2010). Even with this elevated contribution of Texas ancestry in our sample of admixed panthers, it still did not alter the cranial measurements sufficiently to reveal a significant difference between nonadmixed and admixed panthers. Perhaps sufficient levels of backcrosses between admixed and nonadmixed panthers prevented this distinction from developing or change will occur over a longer period of time. This is a topic for additional research.

Our analysis comparing Texas pumas to Florida panthers revealed that there were significant differences in the 15 skull measurements when accounting for the differences between males and females. Differences between the cranial measurements of Texas and Florida pumas could be attributed to minor disparities in diet because ungulate prey comprises the majority of prey for both groups, but Florida panthers have a greater total variety of prey species (Iriarte et al. 1990). Our results were similar to those of previous studies (Young and Goldman 1946; Gay and Best 1995; Wilkins et al. 1997) and provided further credence to the prediction that genetic restoration did not result in admixed panthers with cranial measurements similar to those of Texas pumas.

In light of our cranial measurement results, it was not surprising to find a lack of significant differences between the nasal profiles of nonadmixed and admixed panthers. Additionally, the contrasts from our 1-way ANOVA comparing nasal profiles between our recent and historic groupings of Florida panthers were consistent with the findings of Wilkins et al. (1997) comparing their recent and historic panther samples. However, the lack of significant differences in the nasal profiles of nonadmixed panthers and Texas pumas demands further scrutiny because Wilkins et al. (1997) detected significant differences. Our recorded mean nasal height for Texas pumas was only slightly higher than the mean reported by Wilkins et al. (1997). It is worth noting that a single nonadmixed panther (FP07) and 6 of the 16 admixed panthers (FP70, FP86, FP91, FP129, UCFP94, and UCFP103) had nasal profile values higher than the mean value for Texas pumas. The normalized height values for all but 1 of the Florida panthers fall within the broad range of values for the Texas pumas (Fig. 4).

Culver et al. (2000) concluded that North American pumas were genetically homogenous when compared to Central and South American pumas and warranted classification under a single subspecies. Our findings, at least in regard to nasal profiles, may support Culver's conclusions. That being said, a major difference between the study by Wilkins et al. (1997) and our study was that they compared the nasal profiles of 28 subspecies of *P. concolor*, totaling 286 specimens (including 28 Texas specimens and 36 Florida panthers). Conversely, although we sampled more Florida panthers ($n = 40$; composed of nonadmixed and admixed specimens), we only collected morphometric data from 8 Texas specimens. The lack of significant differences might be an artifact of this small sample size. We note that Wilkins et al. (1997) found no significant differences between the nasal profiles of *P. c. coryi* and 12 other subspecies of North and South American puma, including the extinct *P. c. couguar* (eastern cougar), whose range historically abutted most of the northern border of the Florida panther's range (see Fig. 1, bottom; Young and Goldman 1946). These comparisons reveal that nasal profiles alone are perhaps insufficient to delineate a subspecies.

Differences in the data collection methods between our study and that of Wilkins et al. (1997) also may have played a role in our disparate results in regard to nasal profiles of *P. c. stanleyana* and *P. c. coryi*. Wilkins et al. (1997) measured skulls with a carpenter's contour gauge and the program Sigma Scan (SYSTAT Software, Inc., Chicago, Illinois). Conversely, we used a high-resolution digital imaging system to photograph the specimens and the accuracy and precision of our measurements were likely greater than when using a contour gauge. Our ICC tests quantified the level of precision of our measurement techniques. A subsequent study could involve a direct comparison between the methods by measuring a set of the same skulls used by Wilkins et al. (1997) to determine if this was the root cause of the differing results.

The most substantive result of our study was that we did not observe significant differences in the cranial measurements or nasal profiles of nonadmixed versus admixed Florida panthers. From that morphometric perspective, we can conclude that admixed panthers that were the product of genetic restoration remain similar morphologically to nonadmixed panthers. Our results for comparisons between Florida panthers and the Texas pumas were more ambiguous, given that our cranial measurement comparisons agreed with historic analyses, but our nasal profile measurements revealed results that were contrary to previous studies. Whether or not one accepts the idea of subspecies, this topic will continue to be debated. Reanalyzing these data with new Florida panther specimens in the coming years will shape our understanding of the effects of genetic restoration on panther morphology and our decision-making process for the continued recovery of the Florida panther.

During the deliberation leading up to the genetic restoration project, the United States Fish and Wildlife Service decided that the resulting offspring from introduced Texas females and Florida panthers or their offspring were to be granted full protections under the Endangered Species Act (United States Fish and Wildlife Service 2008). This supersedes our results and establishes continued protection for the panther regardless of any contrary conclusions. Our findings, however, reveal that

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the genetic restoration did not significantly alter the skull morphology or change the uniqueness of the Florida panther.

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APPENDIX I

Inventory of specimens of *Puma concolor* ssp. measured for this study. Catalog no. is the catalog number from the institution owning the specimens (UF = Florida Museum of Natural History, University of Florida, Gainesville, Florida; SRSU = Sul Ross State University, Alpine, Texas). Collectors no. is the collector's number assigned to the specimen on capture or collection by Florida Fish and Wildlife Conservation Commission or National Park Service field staff. Birth year can be either known (i.e., individual marked at the natal den as a kitten) or estimated by pelage or tooth wear, or both, characteristics of the individual when captured or collected. Death year is the year the carcass was collected. Grouping categories are described extensively in the text. M = male, F = female, UNK = unknown.

Catalog no.	Sex	Collectors no.	Birth year	Death year	Grouping
UF16374	M	FP 06	1975	1982	Nonadmixed, historic
UF19096	M	FP 01	1971	1983	Nonadmixed, historic
UF22409	M	FP 07	1975	1985	Nonadmixed, historic
UF22529	M	FP 04	1974	1985	Nonadmixed, historic
UF24314	M	FP 20	1983	1988	Nonadmixed, historic
UF24515	M	FP 25	1984	1988	Everglades[a]
UF26844	M	FP 34	1989	1993	Nonadmixed, historic
UF24646	M	FP 17	1980	1990	Nonadmixed, historic
UF26159	M	FP 29	1988	1992	Nonadmixed, historic
UF26841	M	FP 44	1990	1993	Nonadmixed, historic
UF26843	M	FP 50	1991	1993	Nonadmixed, historic
UF30937	M	UCFP 45	1999	2002	Nonadmixed[a]
UF31024	M	UCFP 50	1996	2003	Nonadmixed, recent
UF31178	M	UCFP 75	2003	2005	Admixed, recent
UF31252	M	UCFP 94	2004	2007	Admixed, recent
UF31296	M	FP 134	2002	2007	Admixed, recent
UF31297	M	UCFP 89	2002	2006	Admixed, recent
UF31395	M	FP 99	2000	2002	Nonadmixed, recent
UF31392	M	FP 135	2003	2006	Admixed, recent
UF31549	M	UCFP 103	2005	2008	Admixed, recent
UF31555	M	FP 127	2002	2007	Admixed, recent
UF31759	M	FP 131	1999	2008	Admixed, recent
UF10424	F	UCFP 06	1979	1981	Nonadmixed, historic
UF18798	F	FP 03	1973	1983	Nonadmixed, historic
UF19090	F	FP 05	1974	1983	Nonadmixed, historic
UF24267	F	FP 08	1974	1987	Nonadmixed, historic
UF26520	F	FP 205	1990	1991	Nonadmixed, historic
UF27148	F	FP 31	1981	1994	Nonadmixed, historic
UF27370	F	FP 38	1985	1994	Nonadmixed, historic
UF27618	F	FP 52	1991	1995	Nonadmixed, historic
UF28980	F	FP 40	1988	1998	Nonadmixed, historic
UF29261	F	UCFP 28	1969	1972	Nonadmixed, historic
UF31101	F	FP 91	1999	2003	Admixed, recent
UF31173	F	FP 136	2001	2005	Admixed, recent
UF31174	F	FP 120	2000	2005	Admixed, recent
UF31182	F	FP 69	1997	2005	Nonadmixed, rRecent
UF31192	F	FP 70	1997	2006	Admixed, recent
UF31220	F	FP 129	2001	2006	Admixed, recent
UF31393	F	FP 86	1999	2003	Admixed, recent
UF31025	F	UCFP 53	2000	2003	Admixed, recent
UF31651	F	UCFP 114	2004	2008	Nonadmixed, recent
UF31851	F	FP 140	2002	2009	Admixed, recent
UF29565	F	TX103	1991	1999	Texas
UF30142	F	TX107	1991	2001	Texas
SRSU533	M		UNK	1964	Texas
SRSU1076	M		UNK	1971	Texas
SRSU1604	M		UNK	1975	Texas
UF22534	M		UNK	1986	Texas
SRSU508	F		UNK	1964	Texas
SRSU2212	F		UNK	1983	Texas

[a] Specimen only used to determine measurement collection precision, not included in other comparative tests

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APPENDIX II

Definition of cranial measurement abbreviations. Abbreviations are taken from Wilkins et al. (1997).

Condylobasal length (CBL)—Anterior tips of premaxillae to posterior plane of occipital condyles.

Cranium height (CRH)—Vertical distance from lower border of maxillae to height of frontals, at vertical plane of postorbital processes.

Condyle width (CW)—Greatest width of occipital condyles.

Mastoid breadth (MASB)—Greatest posterior width of skull including outside margins of the mastoids.

Maxillary toothrow (MXTR)—Anterior alveolus of canine to posterior alveolus of carnassials.

Palatal length (PAL)—Anterior tips of premaxillae to posterior edge of palate.

Width of palate (PAW)—Greatest width of palate between outside margins of carnassials alveoli.

Postorbital constriction (POC)—Least distance posterior to the postorbital processes.

Postorbital process breadth (POPB)—Greatest width between the outer points of postorbital processes.

Pterygoid width (PW)—Least distance at outer edges of pterygoids.

Total length (TL)—Anterior tips of premaxillae to posterior point in median line over the foramen magnum.

Upper carnassial crown length (UCL)—Anteroposterior length of crown at cingulum.

Upper carnassial crown width (UCW)—Greatest width of carnassial.

Width at canines (WC)—Width at outside margins of canine aveoli.

Zygomatic breadth (ZYB)—Greatest distance between outside borders of zygomata.

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