The Utility of Captive Animals in Actualistic Research: A Geometric Morphometric
Exploration of the tooth row of *Alligator mississippiensis* suggesting ecophenotypic
influences and functional constraints

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Short Title: **CAPTIVE VS. WILD *ALLIGATOR* SNOUT SHAPE**

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Abstract

Captive broad snouted crocodylians are generally thought to have wider, shorter rostra than their wild counterparts. Interpreted to reflect morphological change in response to the conditions of captivity, this qualitative pattern could affect the utility of these animals in a variety of fields of research. However, due to relative ease of access and availability of life history data, captive animals are often utilized in actualistic research. Thus, this issue should be addressed in more detail. Here we explore snout shape variation between captive and wild members of *Alligator mississippiensis* using 2D morphometric techniques. Several landmark schemes are used to assess the utility of different aspects of morphology in distinguishing the groups. While statistical analyses consistently differentiated between the groups, the area of morphospace occupied by wild members of *A. mississippiensis* generally overlapped with the larger area encompassing the captive specimens. This indicates that the captive condition is not as uniform as previously thought and instead encompasses a large spectrum of morphologies, ranging from the stereotypical broad, shortened snouts to outlines that are indistinguishable from the wild morphotype. These results align well with the interpretation that this change reflects an extreme example of ecophenotypy, since ranched, farmed, or zoo organisms are held in an array of enclosures, ranging from indoor, climate controlled pens to outdoor, more natural areas. This variation in environments should be reflected in different reactions to the animals' surroundings, resulting in a broad spectrum of morphotypes. While wild specimens are still preferred, especially for fine scale analyses, these results indicate that not all captive members of *Alligator mississippiensis* exhibit the extreme morphological alterations often

cited in the literature. Weighing the conditions in which the animals are held and exploring the possibility of morphological differences against the benefits of using captive specimens should be part of any actualistic study.

Keywords: Crocodylia, intraspecific variation, ecophenotypy, rostral morphology, captivity

Introduction

Captive animals often differ morphologically from their wild relatives (e.g. Guay and Iwaniuk, 2008; Hard et al., 2000; McPhee, 2004; O'Regan and Kitchener, 2005; Zuccarelli, 2004). Differences in behavior (e.g. Flemming et al., 1996; Geiser and Ferguson, 2001; McPhee, 2003), functional performance (e.g. Erickson et al., 2004), and rate and type of pathologies (Fitch and Fagan, 1982; Munson et al., 2005) all have been noted between captive and wild individuals. Even so, animals kept in farms and zoos are regularly used to study a wide variety of topics, from physiology to functional morphology to taphonomy (e.g. Schachner et al., 2013). Within the bite mark literature, captive animals have been used extensively (e.g. D'Amore and Blumenschine, 2009; Drumheller, 2012; Drumheller and Brochu, 2014; Haynes, 1983; Marean and Spencer, 1991; Njau and Blumenschine, 2005; Pickering and Wallis, 1997; Willey and Snyder, 1989) even though the potential for biases has been recognized in some taxa (Haynes, 1982).

65 However, captive animals have a variety of characteristics that make them
66 attractive as research specimens. Living organisms held in zoological parks or farms are
67 often more accessible for research. When dealing with threatened or endangered species,
68 these animals may be the only viable source of data. Also, many of these facilities keep
69 long-term veterinary and physiological records of their animals. Some of these variables,
70 such as mass or sex, can be collected in the field, but others, such as exact age and
71 measurements through ontogeny, may be difficult to impossible to collect from wild
72 animals. Furthermore, captive specimens have habitats and routines that include regular
73 human interaction, a potentially complicating factor if this fosters novel behaviors, but one
74 which can make data collection safer for animals and researchers alike. Therefore, if
75 captivity can be shown to have a negligible effect on ensuing data, these animals can prove
76 to be productive sources of information.

77 Thanks to highly successful conservation efforts and extensive research collections,
78 *Alligator mississippiensis* (Daudin, 1802) is one of the most rigorously studied species of
79 extant crocodylians (Rowe et al., 1999). Therefore, this group is a strong candidate for
80 studying the validity of using captive crocodylians as proxies for their wild – living or
81 extinct – relatives. Among crocodylians, a variety of morphological differences between
82 captive and wild individuals have been cited anecdotally in the literature. Captive *A.*
83 *mississippiensis* are said to have wider, blunter snouts, teeth that splay outwards (Figure 1),
84 and larger fat deposits (Erickson et al., 2004). This pattern is generally thought to apply to
85 other generalized and blunt-snouted taxa. Captive tube-snouted crocodylians, such as
86 *Gavialis gangeticus* and *Tomistoma schlegelii*, often exhibit unusual bending along the

rostrum, both laterally and dorsally. Previous morphometric analyses of *A. mississippiensis* have also revealed that captive animals exhibit a raised lip along the margins of the skull table, comparatively short basioccipitals and wide lacrimals, closely set eyes, and an upturned rim around the orbits (Sadleir, 2009). Prey acquisition behavior among captives does not substantially deviate from that seen in the wild (SKD personal observation, Cleuren and De Vree, 1992; 1999), but bite force performance does differ between captive and wild individuals, becoming more pronounced among older, larger animals, suggesting that this difference may stem from morphological changes driven by the conditions of their disparate environments, i.e. a possible ecophenotypic pattern (Erickson et al., 2004). Such observations raise concerns over the use of farm or zoo animals in research, and some institutions have responded by limiting or avoiding accession and curation of captive specimens (SKD personal observation).

This study aims to explore the effects of captivity on the morphology of *A. mississippiensis* and assess the potential implications for actualistic research. We utilize two-dimensional geometric morphometric methods to explore differences in tooth placement and snout shape between captive and wild members of *A. mississippiensis*. Several different analytical techniques and landmarking schemes are used to quantify these differences allowing us to assess the potential influence of ecophenotypy and functional constraints.

Materials and Methods

The specimens used in this study are held in three collections: the American Museum of Natural History (AMNH), Florida Museum of Natural History (FLMNH), and a research collection from the Rockefeller Wildlife Station (RWS) held at the Field Museum of Natural History (FMNH). Associated metadata were often limited (i.e. details of animal sex, age, etc. were rarely recorded), so only specimens with known provenance and which lacked extensive pathologies, postmortem damage, and remaining soft tissue obscuring areas of interest were included in the analysis (Supporting information, Table S1). We took images of the ventral view of each cranium, representing 19 captive and 66 wild individuals, with an Olympus® Stylus™ brand, model 760 still-image camera. Both camera and specimen were oriented on a copy stand using a two-way level centered on the suture between the specimens' maxillae in order to ensure a uniform camera angle during image and data collection.

To capture the morphology of the snout and tooth row, we collected three sets of landmarks: snout outline, alveolar positions, and sutural intersections of the bones of the snout. Images for the analysis were compiled using tpsUTIL, version 1.60 (Rohlf, 2010). We performed landmark and semilandmark digitization in tpsDIG2, version 2.18 (Rohlf, 2010). Snout shape was assessed using a semilandmark approach to outline analysis (Bookstein, 1996; 1997). As employed in this study, semilandmarks are non-discrete anatomical loci representing a biologically homologous curve within a single species, in this case *A. mississippiensis*. We chose not to slide our semilandmarks for this study (i.e. allow the semilandmarks to move in order to minimize Procrustes distance or bending energy between individual specimen configurations and the Procrustes reference

configuration). The justification for sliding of semilandmarks has been questioned for a number of reasons (see MacLeod, 2013). Currently employed sliding protocols only allow the semilandmark to travel along a line tangent to the outline curve, rather than along the curve itself. Thus, any sliding moves the semilandmark off of the original outline; forcing it away from the actual biological curve we are interested in quantifying (MacLeod, 2013). Additionally, when curves have been well sampled, there is little space between semilandmarks within which they can slide, resulting in only minor differences from the original semilandmark placement (Tennant and MacLeod, 2014). Snout profile outlines were traced from the posterior-most point of the right maxilla, around the right to left premaxillae, to the posterior-most point of the left maxilla of 84 *A. mississippiensis* crania. We then resampled this curve for 60 equidistant points (Figure 2). We deemed this resolution sufficient to faithfully represent the outline of these structures based on visual inspection of the digitized specimens in tpsDIG2.

Landmarks selected to assess tooth position included all five premaxillary and the first seven maxillary teeth of the same 84 skulls (Figure 2). More distal maxillary teeth were not sampled, following the landmark selection scheme outlined by Sadleir (2009), for multiple reasons related to ontogeny. Tooth placement and, to a lesser extent, tooth number vary somewhat through ontogeny. More importantly, in young individuals, alveoli are often not differentiated distal to the seventh maxillary tooth, making accurate landmark placement problematical. The center of the socket was selected for each landmark instead of a feature of the tooth itself for two reasons: crocodylians continuously shed teeth, making eruption and wear patterns irregular and the vast majority of the specimens

available for this study had lost most, if not all, of their teeth during natural decay or the collection and cleaning processes.

The third set of landmarks assessed differences in sutural positions of the bones of the snout (Figure 2). This data set includes far fewer specimens relative to the outline and alveolar data sets (n=40; includes one specimen excluded from the outline and alveolar data sets due to pathological closure of some alveoli). This decrease in sample size is due to either insufficient closure of the secondary palate (particularly in the region of the intersection of the premaxillae and maxillae), or damage, that hinders accurate landmark placement. We concentrated on ventral cranial landmarks, as differences between dorsal cranial features in captive and wild individuals have one, already been explored using these techniques and two, yield results that are driven by features other than snout shape (Sadleir, 2009).

Variation due to scale, orientation, and position of the digitized specimens was removed using generalized least squares Procrustes superimposition in the software package MorphoJ v. 1.06d (Klingenberg, 2011). Procrustes superimposition translates landmark configurations by positioning their centroid to the origin of a Cartesian coordinate space and scaling the landmark configurations to unit centroid size. The average of all landmark configurations is used as a reference, and then all configurations are rotated to minimize summed squared distances between homologous landmarks (Gower, 1975; Rohlf and Slice, 1990; Zelditch et al., 2004), converting the two-dimensional coordinates to Procrustes shape coordinates. *Alligator* skulls are bilaterally symmetrical, so the total shape variation of the sample will include both symmetrical and asymmetrical

components. The symmetrical component of shape variation was isolated in MorphoJ and all further analyses were performed on the symmetrical component of shape. Specimens utilized in this study span a broad size range (6.6 cm – 55.8 cm as measured from quadrate condyle to tip of snout). To remove the overall effects of size on shape (i.e. allometry), Procrustes shape coordinates were regressed on log-transformed centroid size. The amount of shape variation accounted for by the regression is quantified as a percentage of the total Procrustes sums of squares (Klingenberg and McIntyre, 1998). We employed a permutation method to test the significance of the correlation between size and shape against a null hypothesis of complete independence using MorphoJ (Drake and Klingenberg, 2008). To explore the distribution of the captive and wild group shapes, we performed a principal component analysis (PCA) on the residuals from this regression in MorphoJ. Symmetrization, regression, and PCA were applied to the snout outline and alveoli positions, snout outline only, and alveoli positions only, to test for differences in signal between data sets. We applied the same analytical procedures to the reduced sutural intersection data set.

To assess differences between captive and wild groups, we performed a linear discriminant analysis (LDA) for each of the four data sets. Discriminant analysis requires an inversion of the pooled within-group variance-covariance matrix (Sheets et al. 2006). Because of this requirement, the number of variables cannot exceed the number of specimens. The number of variables in the snout outline, alveoli positions, and combined snout outline and alveoli position data sets approached, or exceeded the sample size. Thus, LDA of the shape scores directly was not possible. PCA was used to reduce the statistical

dimensionality of the data, by utilizing a limited number of PC scores. We employed the method of Sheets et al. (2006) for objectively selecting an appropriate number of PCs to include by maximizing cross-validation success rate. This involves performing the discriminant analysis and cross-validation iteratively, varying the number of PCs up to the maximum number of PCs generated in each analysis (which, in all cases was less than the number of specimens) and selecting the number of axes that produce the highest success rate. In instances where multiple numbers of PCs achieved the same maximum success rate, we selected the lowest number. We performed discriminant analyses of the selected PCs in PAST v.3.04 (Hammer et al. 2001). Given the small number of variables relative to the degrees of freedom in the sutural intersection data set, the LDA was performed directly on the regression residuals (Procrustes coordinates vs. log-transformed centroid size) in MorphoJ, rather than on PC axes.

Resubstitution and cross-validation are two standard methods for estimating classification success rate in LDA. The first uses the discriminant function to assign individual specimens to groups through resubstitution (RS). Previous workers have demonstrated that classification rates based on RS consistently “overfit” the discriminant axis to the data (anomalously high classification success rates) because this estimate of success rate is based on the very same data used to generate the discriminant function (Kovarovic et al., 2011; Sheets et al., 2006; Solow, 1990). In light of this issue, we also performed a leave-one-out cross-validation to assess the reliability of the group membership classifications. As sample size differed greatly between the captive and wild groups, a method to assess classification success rate relative to this difference in sample

size was needed. We utilized Morrison's (1969) proportional chance criterion (C_{pro}), to assess whether classification based on the discriminant function performs better than randomly assigning samples to groups in proportion to their size (McGarigal et al., 2000). Cross-validation and RS success rates were compared against C_{pro} scores to test whether these rates exceeded those expected under random chance.

To visualize the changes in morphology associated with the discriminant axis (characteristics separating groups), we imported the discriminant scores into MorphoJ. The size-corrected shape scores were then regressed onto the discriminant scores to generate wireframes of the shape for each group mean and the shape of the most maximally divergent specimen in each group.

To test the significance of the shape differences between captive and wild groups, we performed a permutational MANOVA (Anderson, 2001) on the regression residuals for each data set based on the null hypothesis that the measured groups were indistinguishable. A permutational MANOVA was required as the data were not normally distributed. We performed the MANOVA using the Euclidian distance measure in PAST with 10,000 replicates of group membership permutation.

Patterns of ontogenetic shape change between captive and wild *Alligator* were also tested. We compared ontogenetic trajectories by dividing the data into captive and wild groups. Because both captive and wild data sets poorly sample very small individuals, the smallest individuals, regardless of group membership, were pooled and included in both data sets (*i.e.* the smallest captive was added to the wild group and the smallest two wild

individuals were added to the captive group). For the reduced sutural intersection landmark data set, only the smallest captive was added to the wild data set (the two smallest wild specimens were unusable for this analysis as the secondary palate had yet to fully close). As shape differences between captive and wild *Alligator* are expected to result primarily from environmental effects, the differences are assumed to be minimal in the youngest/smallest individuals. Differences in shape between captive and wild individuals of the smallest size class should represent natural variation, rather than environmentally induced shape change. This methodology provides the same ontogenetic shape starting point for each group, and yields comparable group size ranges. (While no previous studies of the effects of captivity on crocodylians have noted differences in neonates, this assumption has not yet been fully tested. Therefore, we performed a second set of analyses, keeping the smallest captive and wild individuals separate. This yielded similar results, and the majority of the calculated difference stemmed from the variation in smallest sampled size between the groups, not their ensuing ontogenetic trajectories.) The Procrustes coordinates of each group were regressed on log-transformed centroid size **separately** in MorphoJ to linearize the exponential relationships often observed in growth-series data. We utilized a permutation test to assess significance of the regression based on the null hypothesis of complete independence between the variables, also implemented in MorphoJ. From the regression, shape scores were calculated as a means of visualizing the relationship between size and shape (Drake and Klingenberg, 2008). These shape scores are variables associated with the shape change predicted by the regression (including the residual variation along that direction in shape space). To test the hypothesis of equivalent

rates of ontogenetic change, we then regressed the shape scores for the captive and wild samples against log-transformed centroid size in an analysis of covariance (ANCOVA) in PAST for each set of landmarks.

Results

Comparison of captive vs. wild shape:

In all analyses the first two principal component (PC) axes accounted for a great majority of the variation (74.6% - 93.5%; see Supporting Information, Table S2 for detailed results). These two PC axes define a lower-dimensional shape subspace that will be used to describe the major aspects of variation among captive and wild *Alligator* specimens. The first two PC axes of the combined analysis of snout outline and alveoli landmark data encompass 82.96% of the variation in the sample (PC1: 63.96%; PC2: 19.0%). A bivariate plot of these two axes (Figure 3A) shows broad overlap of the captive and wild specimens, with captives generally exhibiting more negative values along PC1. The 95% equal frequency ellipse for the captive sample shows a larger variance along both axes than the wild sample. The first PC axis is associated with the length and breadth of the snout, with more positive values representing a longer and narrower snout, while negative values indicate a shorter and broader snout (Figure 3B). Associated with the elongation of the snout is an elongation of the diastema separating the fifth premaxillary alveolus from the first maxillary alveolus. The second PC axis is associated with, in part, the length of the tooth row, relative to the length of the snout. More negative values along

PC2 show a more posteriorly elongated tooth row, placing the maximum curvature of the snout lateral to the 4th maxillary tooth in a more posterior position.

The first two PC axes of the outline-only analysis account for 93.5% of the sample variance (PC1: 85.4%; PC2: 8.1%). A bivariate plot of PC1 vs. PC2 shows extensive overlap between captive and wild samples (Figure 3C). However, captive samples tend towards shorter, broader snouts (negative values on PC1), while wild samples possess longer, narrower snouts (positive values along PC1). The two groups show no separation along PC2. The 95% equal frequency ellipses show that the captive sample exhibits higher variance along PC1 despite smaller sample size, while captive and wild samples show similar variance along PC2. Variation along PC1 primarily reflects differences in snout length and breadth, with a lesser contribution of the extent of festooning along the maxillary margin (Figure 3D). Principal component axis two describes differences in the maximum width of the snout around the level of the 5th maxillary tooth and differences in width at the posterior end of the maxilla.

For the analysis of alveolar positions, the first two PC axes account for 76.98% of the variation (PC1: 61.38%; PC2: 15.6%). Both captive and wild samples again show broad overlap along PC1 and PC2 (Figure 3E). On average, captives show more negative values along PC1 and slightly more negative values along PC2. The captive sample also shows greater variance along PC1, but lower variance along PC2. The first PC axis primarily describes the length and breadth of the tooth row with a minor contribution from variation in the distance between the left and right first premaxillary teeth (Figure 3F). The second PC axis involves variance in length of the diastema between the fifth premaxillary

alveolus and first maxillary alveolus and the breadth of the posterior region of the tooth row.

The first two PC axes of the analysis of sutural intersection data account for 74.64% of the variation (PC1: 59.94%; PC2: 14.7%). On a bivariate plot of PC1 vs. PC2, the captive and wild samples overlap extensively, with captives generally having more negative values along PC1 and more positive values along PC2 (Figure 3G). The captive sample also exhibits much greater variance along PC1 and slightly greater variance along PC2 than does the wild sample. The first PC axis is primarily associated with variation in snout breadth, with minor contributions from variation in the length of the premaxilla and anterioposterior position of the anterior margin of the suborbital fenestrae (Figure 3H). Axis two encompasses variation in the anteroposteior position of the midline maxilla/palatine suture and the anterior border of the suborbital fenestrae. It also includes a minor component of snout length.

Iterative LDA followed by cross-validation identified the optimal number of PCs to include for each data set (Table 1; full results of the iterative analyses available in Supporting Information, Table S3). The discriminant function was generally successful in distinguishing captive from wild specimens (RS classification success rates: 95.24% to 80%; Table 1). The high classification success rate of RS demonstrates some amount of overfitting as cross-validation success rates are uniformly lower (90.48% to 70%; Table 2), though the difference is small. In all analyses the correct classification rates exceed those expected by chance. Results of the permutational MANOVA indicate significant differences between group means of captive and wild specimens in all cases (Table 1).

The morphological features associated with the discriminant functions are shown in Figure 4. In all cases, the features distinguishing the groups are subtle. For the combined snout outline and alveoli position data set, these features include increased undulations along the maxillary margins, slight shortening and broadening of the snout, and slight posterior expansion of the alveoli positions in captives relative to wild specimens (Figure 4A). In the outline analysis, captives show broader snouts with a greater undulation of the maxillary margin and a slight decrease in snout length (Figure 4B). Analysis of the alveoli positions demonstrates a slight narrowing of the premaxillary portion of the tooth row, a decrease in length of the diastema between the last premaxillary and first maxillary alveolus, and a broadening of the posterior portion of the tooth row among captive specimens (Figure 4C). The morphological features differentiating groups in the sutural intersection data include an overall broadening of the snout, a slightly more posteriorly positioned midline premaxilla/maxilla suture, and anterior margins of the suborbital fenestrae somewhat more anterolaterally placed in captives relative to wild specimens (Figure 4D).

Patterns of Ontogenetic Shape Change:

Regression of Procrustes shape coordinates on log-transformed centroid size indicated a variable, yet significant, amount of shape variance attributable to size in all data sets (range: 6.37-32.89%; Table 2). Analyses of covariance (ANCOVA) indicate that patterns of shape change differ significantly between captive and wild samples for all data

sets except the sutural intersections (**Table 1**). For the significant data sets, the captives tend to change shape at a faster rate than wild specimens (Figure 5). The variation within given size classes appears to be greater among captive specimens as well, though it has been suggested that at least part of the difference seen in captive morphologies is related to an accelerated growth rate, and therefore size should not be considered a trustworthy proxy for age in these animals (Sadleir, 2009). The large sample of farmed captives from Rockefeller Wildlife Station, all culled at approximately the same size, exhibit a very wide spread along the shape score axis; much larger than the similar number of wild specimens of comparable size.

Discussion

Most, though not all, of the analyses comparing snout shapes across captive and wild groups yielded statistically significant results. However, there is a great deal of overlap between the groups in all cases. At face value, these results are unexpected. One of the principle differences often cited between captive and wild crocodylians is snout shape. General wisdom holds that among generalist crocodylians, like *A. mississippiensis*, captive individuals will exhibit shorter, wider snouts (Erickson et al., 2004). Previous analyses of *A. mississippiensis* crania demonstrated strongly divergent morphologies between the two groups, though much of this signal was preserved elsewhere in the skull (Sadleir, 2009). If the differences between these animals are so evident that they can be observed

366 qualitatively, why would a quantitative method have more difficulty differentiating
367 between the groups?

368 The problem seems to stem from the fact that captive vs. wild morphologies have
369 been treated as a binary condition, when a spectrum would better describe the observed
370 patterns. Across the board, these analyses demonstrate that captive animals occupy a larger
371 area of morphospace than their wild counterparts (Figure 3). However, there is also a great
372 deal of overlap between the groups, and the area of morphospace occupied by the wild
373 animals falls largely within the range of the captive sample. These results may reflect a
374 combination of compounding factors in the development of the crocodylian snout:
375 ontogeny, ecophenotypy, and functional constraints.

376 Differences between juvenile and adult crocodylians long have been recognized
377 (McIlhenny, 1935). These qualitative observations of morphology and behavior since have
378 been subjected to more rigorous analysis, both within and between captive and wild
379 animals. Erickson and colleagues (2004) recognized that bite forces in captive and wild *A.*
380 *mississippiensis* were highly similar among juveniles, but that differences arose in older,
381 larger animals. Potential explanations given for differences in bite forces, cranial
382 morphology (Sadleir, 2009), biomechanical performance of humeri (Meers, 2002) and
383 geometric dimensions of femora (Farlow et al., 2005) in captives of different crocodylian
384 species were all related to the long-term effects of unnatural habitats, behavior, and diet
385 (Meers, 2002; Sadleir, 2009), pressures which act over time and ontogeny. During growth,
386 the ecological pressures of captivity introduce increasingly aberrant morphologies the
387 longer the conditions persist. Thus, the differences in captive morphologies should be

viewed as a rather extreme kind of ecophenotypy. This interpretation is further supported by the results of most of the ANCOVA analyses (Figure 5). The changes in shape when size is taken into account were statistically significant in all analyses except the sutural intersections (Table 1). Among the significant analyses, shape change among captives occurred at a higher rate than wild specimens throughout ontogeny.

However, even this explanation still oversimplifies the conditions that could lead to the observed patterns in crocodylian snout shape. Qualitative observations and in-depth morphometric analyses of populations within single species have revealed the breadth of variation possible under natural conditions (Sadleir, 2009), and yet all captives are often lumped together as a single state. Living conditions for crocodylians at zoological parks and farms can range from bare concrete enclosures to fenced off areas of largely unchanged natural habitats. Animals that are held in indoor facilities often live under highly climate controlled conditions, with unchanging temperature and light exposure (Sadleir, 2009), while those kept in open air enclosures experience essentially the same light and temperature changes as their wild counterparts. Differences in captive diet and behavior level have been noted by researchers (Farlow et al., 2005; Meers, 2002), but have not yet been surveyed or studied in much detail. With so much possible variation in the conditions of captivity, a broad spectrum of captive animals' morphological reactions to these pressures would be expected, and in fact, has been observed (Figure 6; Figure 7).

Further interesting patterns emerge when we compare the relative strength of the signal generated across the different analyses of snout shape: alveoli position, snout outline, alveoli plus outline, and sutural intersections (Table 1). The MANOVA results for

all four analyses were statistically significant. The LDA successfully differentiated the sample into the a priori defined captive and wild groups. This was further supported when cross-validation analyses correctly classified specimens at rates exceeding 70% in all cases. Yet the cross-validation rate differed between analyses with the lowest success rates coming from the analyses of alveoli position (75%) and of sutural intersections (70%). When placed in an ontogenetic context, all analyses yielded statistically significant results except the exploration of suture contacts.

Crocodylians' snouts are their main tool for interacting with their environments, so much so that major differences in overall snout shape are often used as shorthand for the ecological role of different taxa (Brochu, 2001; Busby, 1995; McHenry et al., 2006; Pierce et al., 2008; Sadleir and Makovicky, 2008). Ultimately, an individual crocodylian must maintain a snout and mouth morphology that is functional enough to perform the daily requirements of, for example, feeding. The patterns observed between analyses yielding significant results and those that did not suggest that a functional constraint such as this might be in play.

When the covariation between size and shape are taken into account, the comparison of sutural contacts between captive and wild *Alligator* yielded a p-value of 0.9406. This analysis was performed on a reduced data set, due to problems with full sutural closure in some of the specimens (Table S1). Nevertheless, the results provide a clear, robust signal: the points of contact between the major bones of the snout vary little between these groups. This leaves snout outline and tooth position to explain the majority of the differences between the two groups. Each of these variables in isolation and when

combined can differentiate captive and wild specimens. In general, captive animals exhibit slightly shorter, blunter snouts with an increased undulation to the lateral margins of the maxilla. The tooth row flares posteriorly, and the spacing between the last premaxillary tooth and the first maxillary tooth also changes between the groups.

Visually, it seems that tooth position is shifting in order to accommodate the changes to the surrounding bone, but the pattern is more complicated than the alveoli simply following the outline of the snout. For example, the margin of the captive animals' snouts has a more distinct lateral undulation than their wild counterparts (Figure 4). The teeth do not directly follow the curvature of the undulation in the captives though, and instead the space between the teeth increases as the tooth row extends further posteriorly in order to bridge the distance made by the increased curvature. The individual position of each tooth changes in the captives, but they are collectively doing so in a manner that cleaves closer to the line of their wild counterparts' tooth rows than the lateral margins of the jaws might suggest.

This dovetails interestingly with another oft cited feature of captive crocodylians, and one that this study was not able to directly address: tooth splay. Qualitatively, it has been noted that the teeth of captive crocodylians, at least those in the anterior half of the snout, splay outward instead of occupying their usual, more dorso-ventral orientation (Erickson et al., 2004). The current data set makes measuring tooth splay difficult, because the vast majority of teeth have fallen out of their sockets during preparation for accession into skeletal collections. Left with only the alveoli to observe, quantitatively assessing tooth splay in this sample becomes challenging. Preliminary measurements of the diameter

of the first five maxillary teeth indicate that the teeth are rotating in place rather than migrating towards the outer margin of the snout, resulting in a widening of the alveoli in the labio-lingual axis (Drumheller, 2012). These findings align with the results presented here, and may suggest an explanation for tooth splay. The teeth do not initially follow the lateral expansion and increased curvature of the margins of the snout, possibly being functionally constrained by the need to maintain some level of occlusion with the teeth of the lower jaw. To do otherwise could inhibit the primary feeding and grasping utility of this anatomical structure (Brochu, 2001; Busby, 1995; McHenry et al., 2006; Pierce et al., 2008; Sadleir and Makovicky, 2008). However, the laterally expanding margins of the skull increasingly overhang the tooth row, a process which could start to force the teeth to accommodate the expansion by rotating in the labial direction – i.e. splaying. This pattern requires further exploration though, preferable utilizing living animals or specimens preserved with teeth in situ.

This study also does not address the rate and type of pathology in captive and wild crocodylians. While none of the wild specimens utilized in this study exhibited obvious significant pathologies in the tooth row, two of the 18 sampled captive specimens did. However, prior research indicates that wild *A. mississippiensis* are certainly not immune from injuries and infections that affect the tooth row (Erickson, 1996). Broken teeth and related alveolar damage and infections commonly result in partial to complete edentulism in wild members of many crocodylian species, including *A. mississippiensis*. In fact, one survey of crocodylian dental conditions directly compared captive and wild specimens, and found that the wild animals actually exhibited significantly more pathologies than their

captive counterparts (Erickson, 1996). While differences in rate and type of pathologies between captive and wild animals have been observed in other taxa (Fitch and Fagan, 1982; Munson et al., 2005), without a more in depth study among crocodylians, all that can be confidently stated is that one, both captive and wild crocodylians experience these types of pathologies and two, any animals exhibiting major pathologies in the snout should probably be excluded from analyses related to jaw use and function.

With regards to the utility of captive animals in bite force, mechanics, and mark studies, the results of this analysis were mixed. However, taken in context, they can provide suggestions on how to approach working with these animals. Even among the analyses that produced statistically significant results, wild and captive *Alligator* ranges exhibited wide overlap, with the captive animals occupying an area of morphospace that encompassed, but also extended beyond, the wild range (Figure 3), a pattern that is not entirely explained by ontogeny (Figure 5). When viewed as an example of ecophenotypy, a likely explanation for this pattern takes form. When the conditions of captivity more closely mimic wild conditions (e.g. outdoor, more natural enclosures), the ensuing morphology should cleave more closely to the wild morphotype. Similarly, less natural conditions (e.g. divergent climate conditions, artificial indoor enclosures) would be expected to result in more divergent morphologies (Figure 7).

This indicates that captive crocodylians should not be wholesale avoided by researchers, especially for gross scale studies of function, if the individuals have been kept under healthier, more naturalistic conditions in their captivity and if existing data sets sampling both captive and wild animals yield congruent results, such as within the

crocodylian bite mark literature (e.g. Drumheller and Brochu, 2014; Njau and Blumenschine, 2006) or when a method exists for standardizing data collected between the groups, such as within the bite force literature (Erickson et al., 2004). Wild animals are still preferable for studies in which the potential biasing effects of captivity have not yet been explored, such as systematic analyses, or in which biases previously have been identified, such as morphometric (Sadleir, 2009) and biomechanical analyses (Erickson et al., 2004). This paper should serve as a case study and a suggestion for how potentially biasing effects of captivity can be addressed and assessed in the future when determining whether captive animals will serve as a reasonable proxy for their wild counterparts.

Author Contributions

SKD collected the data and performed preliminary analyses. EWW performed the final analyses. SKD and EWW generated the figures and wrote the manuscript. RWS contributed to the analyses used, improved the manuscript, and provided access to specimens.

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Table Captions

Table 1. Results of linear discriminant analyses, permutational MANOVA, and ANCOVA. C_{pro} = Proportional chance criterion (classification success threshold expected from randomly assigning

samples to groups in proportion to their size). P-values in bold are non-significant. For graphical representation of morphology associated with LDA see Fig. 4; For ANCOVA plots, see Fig. 5.

Data set	% predicted	p-value
Outline + Alveoli: total sample	7.36	0.0020
Outline + Alveoli: captive	19.75	0.0167
Outline + Alveoli: wild	5.35	0.0158
Outline only: total sample	7.91	0.0047

Linear Discriminant Analysis

Data set	Classification success rate (%)		
	C_{pro}	Resubstitution	Cross-validation
Outline + Alveoli (11 PCs)	66.32	92.86	90.48
Outline only (15 PCs)	66.32	95.24	89.29
Alveoli only (9 PCs)	66.32	80.95	75.00
Sutural landmarks	56.12	80.0	70.00

Data set	Per. MANOVA		ANCOVA	
	F-value	p-value	F-value	p-value
Outline + Alveoli	19.22	<0.0001	6.73	0.0112
Outline only	24.11	<0.0001	5.27	0.0242
Alveoli only	4.57	0.0092	7.75	0.0066
Sutural landmarks	9.43	0.0004	0.01	0.9406

Table 2. Results of regression of shape scores on log-transformed centroid size. The % predicted is the percentage of the Procrustes sums of squares predicted by the regression. P-values are derived from permutation tests assessing significance of the regression based on the null hypothesis of complete independence between the variables.

Outline only: captive	20.54	0.028863
Outline only: wild	6.37	0.0193
Alveoli only: total sample	11.17	<0.0001
Alveoli only: captive	27.46	0.005564
Alveoli only: wild	6.37	0.0072
Sutural landmarks: total sample	29.71	<0.0001
Sutural landmarks: captive	30.90	0.008665
Sutural landmarks: wild	32.89	<0.0001

Figure Captions

Figure 1: *Alligator mississippiensis*. (A) FLMNH 61483, captive adult. (B) FLMNH 34886, wild adult. (C) Generalized ‘captive’ morphotype with arrows indicating the regions commonly cited as differing from the wild form. Scale bars = 10 cm.

673

674 Figure 2: *Alligator mississippiensis*. Landmark schemes used in this analysis: Dashed gray
 675 line indicates region of snout used for semilandmark outline analysis; white circles indicate
 676 24 alveolar landmarks; gray circles indicate landmarks used for sutural intersection

677 analysis.

A) Outline + Alveoli

B)

C) Outline only

D)

E) Alveoli only

F)

G) Sutural landmarks

H)

678

679

680 Figure 3: Results of principal component analyses: (A) Morphospace related to the first
681 two PC axes for the combined snout outline and alveoli position data; (B) Shape change
682 associated with positive and negative extremes of the first two PC scores; (C)
683 Morphospace related to the first two PC axes for the snout outline data; (D) Shape change
684 associated with positive and negative extremes of the first two PC axes; (E) Morphospace
685 related to the first two PC axes for the alveoli position data; (F) Shape change associated
686 with positive and negative extremes of the first two PC axes; (G) Morphospace related to
687 the first two PC axes for the sutural intersection data; (H) Shape change associated with
688 positive and negative extremes of the first two PC axes. Percentages on PC axes indicate
689 percentage of total variance explained by that PC. Ellipses represent 95% equal frequency
690 ellipse for each group. For PC plots, black circles represent wild specimens, gray squares
691 represent captive specimens. For the shape change wireframes, black represents positive
692 change along the PC axis, while gray indicates negative change. **Arrows on (B) and (F)**
693 **indicate the location of the diastema between the fifth premaxillary and first maxillary**
694 **teeth.**

Figure 4: Wireframes demonstrating shape change between group means (left column) and between maximally divergent specimens (i.e. range; right column) along the discriminant axis. (A) Combined outline and alveoli analysis; (B) Snout outline analysis; (C) Alveoli position analysis; (D) Sutural intersection landmark analysis. Black wireframes indicate wild samples, while gray indicates captive. See Table 1 for numerical results of the discriminant analysis.

Figure 5: Results of ANCOVA comparing patterns of ontogenetic shape change. (A) Combined outline and alveoli analysis; (B) Snout outline analysis; (C) Alveoli position analysis; (D) Sutural intersection landmark analysis. Black circles indicate wild specimens; gray squares indicate captive specimens. See Table 1 for numerical results of the ANCOVA.

Figure 6: *Alligator sinensis*, range of variation. From left to right: wild FMNH 31302, 'typical' captive FMNH 197946, 'extreme' captive FMNH 229974. Scale bars = 5 cm.

713

714 Figure 7: *Alligator mississippiensis*, diversity of captive cranial morphologies. Left to
715 right, top to bottom: FLMNH 34886 (wild exemplar), FLMNH 35129, FLMNH 61483,
716 AMNH 9112, FLMNH 95386, AMNH 7141, AMNH 119220, FMNH RWSF 7, FMNH
717 RWSF 4, AMNH 138124. Scale bars = 2 cm.

718 Table S1. Specimens utilized in this study. * indicates specimens included in sutural landmark data
719 set; † indicates specimen excluded

720 from outline and alveolar data sets.

721

Specimen #	Provenance	Designation	Notes
AMNH 7141*	Reported as captive, no specific provenance	Captive	
AMNH 9112*	Reported as captive, no specific provenance	Captive	Pathologies in tooth row
AMNH 31563*	Reported as captive, no specific provenance	Captive	Pathologies in tooth row
AMNH 119220*	Central Park Zoo, NY	Captive	
AMNH 138124*	Reported as captive, no specific provenance	Captive	
FMNH 284886	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS1
FMNH 284887*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS2
FMNH 284888	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS3
FMNH 284889	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS4
FMNH 284892	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS7
FMNH 284893	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS8
FMNH 284894	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS9
FMNH 284895*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS10
FMNH 284896	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS11
FMNH 284897	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS12
FMNH 284898*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS13
FMNH 284899*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS14
FMNH 284900	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS15
FMNH 284901	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS16
FMNH 284902*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS17
FMNH 284903	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS18
FMNH 284904*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS19
FMNH 284905*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS20

FMNH 284906*	Rockefeller Wildlife Refuge, LA	Wild	Field number RWS21
FMNH 284875	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF1
FMNH 284876*	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF2
FMNH 284877	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF3
FMNH 284878*	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF4
FMNH 284879	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF5
FMNH 284880*	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF6
FMNH 284881*	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF7
FMNH 284882	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF8
FMNH 284883	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF9
FMNH 284884*	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF10
FMNH 284885	Rockefeller Wildlife Refuge, LA	Captive	Field number RWSF11
FLMNH 10941*	Everglades, FL	Wild	
FLMNH 11764	Alachua County, FL	Wild	Disarticulated
FLMNH 18126*	Wauberg River, Alachua County, FL	Wild	
FLMNH 33549	Oklawaha River near Eureka, FL	Wild	Disarticulated
FLMNH 34788*	Alachua County, FL	Wild	Disarticulated
FLMNH 34825	Rockefeller Wildlife Refuge, LA	Wild	Disarticulated
FLMNH 34886*	Columbia County, FL	Wild	
FLMNH 35129*	"Albert," FLMNH mascot	Captive	
FLMNH 35144	Rockefeller or Sabine Wildlife Refuge, LA	Wild	Disarticulated
FLMNH 35150	Rockefeller or Sabine Wildlife Refuge, LA	Wild	Disarticulated
FLMNH 35152	Rockefeller or Sabine Wildlife Refuge, LA	Wild	Disarticulated
FLMNH 35153	Rockefeller Wildlife Refuge, LA	Wild	Disarticulated

FLMNH 35154	Rockefeller Wildlife Refuge, LA	Wild	
FLMNH 35155*	Loxahatchee Wildlife Refuge, FL	Wild	
FLMNH 37230*	Loxahatchee Wildlife Refuge, FL	Wild	
FLMNH 37231*	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 37467	Jefferson County, FL	Wild	Disarticulated
FLMNH 37468*	Alachua County, FL	Wild	Disarticulated
FLMNH 38031	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 38972	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 38973	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 38974*	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39098	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39099	Rockefeller Wildlife Refuge, LA	Wild	Disarticulated
FLMNH 39106*	Savannah River, Aiken County, SC	Wild	
FLMNH 39619*	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39620	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39621	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39622	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39623*	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39624	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 39644	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 40535	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 40769	Bivins Arm River, Alachua County, FL	Wild	Disarticulated
FLMNH 40816	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 40817	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated

FLMNH 42474	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 42475	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 42523	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 42538	Loxahatchee Wildlife Refuge, FL	Wild	Disarticulated
FLMNH 42872*	Sopchoppy River, Wakulla County, FL	Wild	
FLMNH 43151*	Ochlocknee River, FL	Wild	Disarticulated
FLMNH 56386*	FL Fish and Game	Wild	Disarticulated
FLMNH 61483*†	St. Augustine Alligator Farm	Captive	
FLMNH 87885	Broward County Water Conservation Area, FL	Wild	Disarticulated
FLMNH 87886*	Broward County Water Conservation Area, FL	Wild	
FLMNH 95386*	Katharine Ordway Preserve, CT	Captive	
FLMNH 109040*	Bivins Arm Lake, Gainesville, FL	Wild	
FLMNH 121384*	Pasco County, FL	Wild	
FLMNH 153430*	Sumpter County, FL	Wild	

722 Table S2. Eigenvalues and percent variance accounted for by each PC from Principal Component
723 Analyses of *Alligator mississippiensis* skulls.

724

725

Outline only

PC	Eigenvalues	% Variance	Cumulative %
1	0.00049054	85.401	85.401

2	0.00004645	8.086	93.487
3	0.00001435	2.498	95.985
4	0.00001113	1.938	97.923
5	0.00000463	0.806	98.729
6	0.00000227	0.396	99.125
7	0.00000139	0.242	99.367
8	0.00000071	0.124	99.491
9	0.00000062	0.108	99.598
10	0.00000043	0.076	99.674
11	0.00000034	0.059	99.733
12	0.00000023	0.04	99.773
13	0.00000018	0.032	99.805
14	0.00000015	0.026	99.831
15	0.00000013	0.022	99.852
16	0.00000011	0.019	99.871
17	0.0000001	0.017	99.888
18	0.00000008	0.015	99.902
19	0.00000006	0.011	99.913
20	0.00000006	0.011	99.924
21	0.00000006	0.01	99.934
22	0.00000005	0.009	99.943
23	0.00000005	0.008	99.951
24	0.00000004	0.007	99.958
25	0.00000003	0.006	99.964
26	0.00000003	0.005	99.969

27	0.00000003	0.005	99.974
28	0.00000002	0.004	99.978
29	0.00000002	0.003	99.981
30	0.00000002	0.003	99.984
31	0.00000001	0.002	99.987
32	0.00000001	0.002	99.988
33	0.00000001	0.001	99.989
34	0.00000001	0.001	99.99
35	0.00000001	0.001	99.992
36	0.00000001	0.001	99.993
37	<0.00000001	0.001	99.993
38	<0.00000001	0.001	99.994
39	<0.00000001	0.001	99.995
40	<0.00000001	0.001	99.995
41	<0.00000001	0.001	99.996
42	<0.00000001	<0.001	99.996
43	<0.00000001	<0.001	99.997
44	<0.00000001	<0.001	99.997
45	<0.00000001	<0.001	99.998
46	<0.00000001	<0.001	99.998
47	<0.00000001	<0.001	99.998
48	<0.00000001	<0.001	99.999
49	<0.00000001	<0.001	99.999
50	<0.00000001	<0.001	99.999
51	<0.00000001	<0.001	99.999

52	<0.00000001	<0.001	99.999
53	<0.00000001	<0.001	100
54	<0.00000001	<0.001	100
55	<0.00000001	<0.001	100
56	<0.00000001	<0.001	100
57	<0.00000001	<0.001	100
58	<0.00000001	<0.001	100

Alveoli Only

PC	Eigenvalues	% Variance	Cumulative %
1	0.00056205	61.375	61.375
2	0.00014288	15.602	76.978
3	0.0000819	8.943	85.921
4	0.00003381	3.692	89.613
5	0.00002016	2.202	91.815
6	0.00001753	1.915	93.73
7	0.00001244	1.358	95.088
8	0.0000105	1.147	96.235
9	0.00000743	0.812	97.047
10	0.00000507	0.553	97.6
11	0.00000398	0.435	98.035
12	0.00000346	0.377	98.413
13	0.0000028	0.305	98.718
14	0.00000236	0.258	98.976

15	0.0000019	0.208	99.184
16	0.00000171	0.186	99.37
17	0.00000154	0.168	99.539
18	0.00000111	0.121	99.66
19	0.00000096	0.105	99.764
20	0.00000091	0.099	99.864
21	0.0000007	0.077	99.941
22	0.00000054	0.059	100

Combined Outline + Alveoli data set

PC	Eigenvalues	% Variance	Cumulative %
1	0.00048782	63.955	63.955
2	0.00014494	19.002	82.957
3	0.00005081	6.661	89.618
4	0.00002552	3.346	92.964
5	0.00001228	1.61	94.574
6	0.0000096	1.258	95.832
7	0.00000635	0.833	96.665
8	0.00000511	0.67	97.336
9	0.0000038	0.499	97.834
10	0.0000026	0.341	98.175
11	0.00000219	0.287	98.462
12	0.00000192	0.252	98.714
13	0.00000134	0.175	98.889

14	0.00000124	0.163	99.052
15	0.00000085	0.112	99.163
16	0.0000008	0.105	99.268
17	0.00000072	0.094	99.362
18	0.0000005	0.066	99.428
19	0.00000047	0.062	99.489
20	0.00000045	0.059	99.548
21	0.00000037	0.048	99.596
22	0.00000034	0.044	99.641
23	0.00000031	0.04	99.681
24	0.00000027	0.036	99.716
25	0.00000023	0.03	99.746
26	0.00000021	0.028	99.774
27	0.00000018	0.023	99.797
28	0.00000017	0.022	99.819
29	0.00000016	0.021	99.84
30	0.00000013	0.017	99.857
31	0.00000012	0.016	99.873
32	0.00000011	0.014	99.887
33	0.00000009	0.012	99.899
34	0.00000009	0.012	99.911
35	0.00000008	0.011	99.921
36	0.00000008	0.01	99.932
37	0.00000007	0.009	99.941
38	0.00000006	0.008	99.949

39	0.00000005	0.006	99.956
40	0.00000004	0.005	99.961
41	0.00000004	0.005	99.966
42	0.00000003	0.004	99.97
43	0.00000003	0.004	99.974
44	0.00000003	0.004	99.977
45	0.00000003	0.003	99.981
46	0.00000002	0.003	99.983
47	0.00000002	0.002	99.986
48	0.00000002	0.002	99.988
49	0.00000001	0.002	99.99
50	0.00000001	0.002	99.991
51	0.00000001	0.001	99.992
52	0.00000001	0.001	99.994
53	0.00000001	0.001	99.995
54	0.00000001	0.001	99.996
55	<0.00000001	0.001	99.996
56	<0.00000001	0.001	99.997
57	<0.00000001	0.001	99.997
58	<0.00000001	<0.001	99.998
59	<0.00000001	<0.001	99.998
60	<0.00000001	<0.001	99.998
61	<0.00000001	<0.001	99.999
62	<0.00000001	<0.001	99.999
63	<0.00000001	<0.001	99.999

64	<0.00000001	<0.001	99.999
65	<0.00000001	<0.001	99.999
66	<0.00000001	<0.001	99.999
67	<0.00000001	<0.001	99.999
68	<0.00000001	<0.001	100
69	<0.00000001	<0.001	100
70	<0.00000001	<0.001	100
71	<0.00000001	<0.001	100
72	<0.00000001	<0.001	100
73	<0.00000001	<0.001	100
74	<0.00000001	<0.001	100
75	<0.00000001	<0.001	100
76	<0.00000001	<0.001	100
77	<0.00000001	<0.001	100
78	<0.00000001	<0.001	100
79	<0.00000001	<0.001	100
80	<0.00000001	<0.001	100
81	<0.00000001	<0.001	100
82	<0.00000001	<0.001	100

Sutural intersection data set

PC	Eigenvalues	% Variance	Cumulative %
1	0.00074968	59.942	59.942
2	0.0001839	14.704	74.646

3	0.00013095	10.47	85.116
4	0.00008863	7.087	92.203
5	0.00004238	3.388	95.591
6	0.00003483	2.785	98.376
7	0.00002031	1.624	100

726

727 Table S3. Results of iterative cross-validation success rate calculations from linear discriminant
728 analyses. Numbers in bold represent the highest success rate for each data set. When multiple
729 numbers of PCs yielded the same maximum success rate, we chose the smallest number of PCs,
730 though including the largest number showed no noticeable differences in morphology as expressed
731 through the wireframes.

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Cross-validation classification success rate

PCs	Outline + Alveoli	Outline only	Alveoli only
2	82.14	79.67	63.1
3	79.67	80.95	70.24
4	83.33	78.57	70.24
5	83.33	73.81	67.86
6	83.33	72.62	67.86
7	78.57	75	66.67
8	76.19	78.57	73.81
9	79.76	84.52	75
10	77.38	84.52	75
11	90.48	86.9	72.62
12	88.1	88.1	75

13	86.9	88.1	73.81
14	86.9	86.9	70.24
15	86.9	89.29	70.24
16	86.9	89.29	67.86
17	86.9	88.1	75
18	88.1	88.1	73.81
19	85.71	88.1	73.81
20	84.52	88.1	72.62
21	84.52	86.9	72.62
22	83.33	86.9	73.81
23	85.71	86.9	
24	85.71	86.9	
25	84.52	86.9	
26	83.33	85.71	
27	82.14	84.52	
28	84.52	83.33	
29	84.52	83.33	
30	85.71	78.57	
31	86.9	78.57	
32	84.52	79.76	
33	86.9	80.95	
34	85.71	79.76	
35	85.71	80.95	
36	85.71	83.33	
37	86.9	82.14	

38	86.9	80.95
39	84.52	78.57
40	85.71	78.57
41	84.52	78.57
42	84.52	79.76
43	83.33	82.14
44	83.33	85.71
45	82.14	82.14
46	82.14	82.14
47	80.95	80.95
48	80.95	80.95
49	79.76	79.76
50	79.76	77.38
51	77.38	75
52	77.38	75
53	76.19	75
54	78.57	75
55	79.76	75
56	84.52	77.38
57	84.52	73.81
58	82.14	72.62
59	79.67	
60	78.57	
61	77.38	
62	76.19	

63	72.62
64	72.62
65	77.38
66	76.19
67	75
68	75
69	78.57
70	76.19
71	73.81
72	73.81
73	72.62
74	70.24
75	63.1
76	61.9
77	59.52
78	54.76
79	53.57
80	48.03
81	46.43
82	44.05

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