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The evolution of genital shape variation in female cetaceans*

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Male genital diversification is likely the result of sexual selection. Female genital diversification may also result from sexual selection, although it is less well studied and understood. Female genitalia are complex among whales, dolphins, and porpoises, especially compared to other vertebrates. The evolutionary factors affecting the diversity of vaginal complexity could include ontogeny, allometry, phylogeny, sexual selection, and natural selection. We quantified shape variation in female genitalia using 2D geometric morphometric analysis, and validated the application of this method to study soft tissues. We explored patterns of variation in the shape of the cervix and vagina of 24 cetacean species ($n = 61$ specimens), and found that genital shape varies primarily in the relative vaginal length and overall aspect ratio of the reproductive tract. Extensive genital shape variation was partly explained by ontogenetic changes and evolutionary allometry among sexually mature cetaceans, whereas phylogenetic signal, relative testis size, and neonate size were not significantly associated with genital shape. Female genital shape is diverse and evolves rapidly even among closely related species, consistent with predictions of sexual selection models and with findings in invertebrate and vertebrate taxa. Future research exploring genital shape variation in 3D will offer new insights into evolutionary mechanisms because internal vaginal structures are variable and can form complex spirals.

KEY WORDS: Allometry, cetacean, evolution, geometric morphometrics, natural selection, ontogeny, phylogeny, sexual selection, vagina.

Research on genital morphology has largely focused on exploring the often extreme morphological variation found in male intromittent organs, with female genitalia considered less variable than males and subsequently not as well studied (Ah-King et al. 2014; Brennan 2016). This oversight is surprising as morphological variation in male and female genitalia should covary because of close mechanical interactions during copulation (Brennan and Prum 2015). Recent studies have quantified previously unreported variation in female genital morphology in several taxa, validating the value of exploring female reproductive tract morphology (flies, Puniamoorthy et al. 2010; Yassin and Orgogozo 2013; waterfowl, Brennan et al. 2007; watersnakes, Showalter et al. 2013; cetaceans, Orbach et al. 2017a). Because female genital adaptations can be subtle, careful quantification of morphological attributes is critical to identify patterns and the underlying evolutionary drivers of variation (Brennan and Prum 2015).

Morphological variation in female genitalia, sometimes assessed concurrently with male genital variation, may reflect sexual selection pressures operating during mating via female mate choice, intrasexual male competition, or sexual conflict (Hosken and Stockley 2004; Simmons 2014; Brennan and Prum 2015). In addition, morphological variation in female genitalia may also be influenced by natural selection that prevents interspecific mating (lock and key hypothesis; Masly 2012), ensures successful oviposition, or facilitates an easy parturition of neonates or egg-laying (Brennan 2016). Female vaginal shapes and structures can also

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exhibit ontogenetic variation, as reproductive organs are often not functional at birth in vertebrates and change into their adult form during sexual maturation (e.g., rats, Berdnikovs et al. 2007; snakes, Showalter et al. 2013).

Cetaceans (whales, dolphins, and porpoises; ~90 extant species) are a speciose clade of mammals that evolved from a common terrestrial ancestor. Whales, dolphins, and porpoises exhibit a diverse array of adaptations to aquatic living (reviewed in Berta et al. 2015), including social organizations ranging from solitary in some large baleen whales to highly gregarious in some small toothed whales. Their diverse life histories and adaptations, coupled with the unusual genital complexity of females, make cetaceans a particularly interesting group to investigate patterns of morphological evolution. Recent work on cetaceans has documented widespread variation in female genital structures called vaginal folds, which appears to be unparalleled in other mammalian taxa (Orbach et al. 2017a). With the exception of artiodactyls, the closest relatives to cetaceans, other mammalian groups do not have vaginal folds (Pabst et al., 1998). These folds are muscular protrusions of the vaginal wall into the lumen, and their function remains unclear (reviewed in Clarke et al. 1994; Orbach et al. 2017a). The vaginal folds of cetaceans vary in number, thickness, positioning, shape, and size across species and appear to be under strong selection pressures because fold lengths are positively allometric with body size (Orbach et al. 2017a). However, quantifying vaginal fold attributes is difficult, as morphological characteristics may vary within a species (Orbach et al. 2016, 2017a). In addition to these folds, cetacean genitalia vary across species in the relative length, width, and proportions of the cervix and vaginal regions (Fig. 1), herein referred to broadly as genital shape. However, this variation has yet to be systematically described and examined.

Studies of shape variation have revolutionized our understanding of morphological evolution, particularly concerning complex structures. Several studies have used either Fourier analysis or geometric morphometric approaches to examine shape variation in insect genitalia (Arnqvist 1998; Garnier et al. 2005; Pizzo et al. 2006; Simmons and Garcia-Gonzalez 2011), and more recently, vertebrate genitalia (Evans et al. 2011; Heinen-Kay and Langerhans 2013; Showalter et al. 2013; Simmons and Firman 2014). However, quantitative studies of female genital shape variation have not been previously reported in mammals.

We use a geometric morphometric approach to analyze 2D genital shape variation in female cetaceans. We examine several hypotheses that may explain genital shape variation including ontogeny, geography, evolutionary (ontogenetic-controlled) allometry, phylogeny, testes mass, and neonate size. We predicted ontogeny would influence the shape of cetacean genitalia and reveal which dimensional aspects are most important for copulation or parturition as animals become sexually mature. Geography was predicted to influence cetacean genital shape as interpopulation variation in testes mass and mating systems are known to occur in spinner dolphins (*Stenella longirostris*; Perrin and Mesnick 2003). Despite findings that vaginal length scales isometrically to body length in cetaceans, we predicted that evolutionary allometry would affect genital shape variation because of the positively allometric relationship between vaginal folds and body length (Orbach et al. 2017a). We also predicted that shared evolutionary history would affect genital shape because a moderate phylogenetic signal was reported between vaginal length and body length across the cetacean clade (Orbach et al. 2017a) and morphological traits are expected to be more similar among closely related species (e.g., Kamilar and Cooper 2013). Sexual and natural selection can influence genital shape (e.g., Hosken and Stockley 2004; Langerhans et al. 2005; Reinhardt 2010; House et al. 2013). Using testes size as a proxy for postcopulatory competition, we predicted that species with larger relative testes (higher sperm competition) may have longer and narrower vaginas to make insemination more difficult. Cetaceans have larger testes-to-body size ratios than similar-sized terrestrial mammals, suggesting they engage in sperm competition (Kenagy and Trombulak 1986; Aguilar and Monzon 1992), although there is extensive interspecific variation. In addition, we predicted that neonate size may influence vaginal shape, so that relatively larger neonates may be associated with a shorter and wider vagina to facilitate parturition. Finally, because vaginal shape may be under selection from both of these traits simultaneously (Brennan and Prum 2015), we examined the interaction between relative testes mass, relative neonate size, and genital shape.

Material and Methods

DATA COLLECTION

Reproductive tracts were obtained opportunistically from fresh (<24 h postmortem) or moderately decomposed deceased female cetaceans. Sexually immature and mature specimens were collected from marine mammal response networks and research institutions in the United States, New Zealand, and South Africa under government authorization (see Ethics statement). Most specimens were collected from toothed whales because of their smaller sizes compared to baleen whales. The marine mammal stranding networks provided information on the total body length (Norris 1961) and sexual maturity state of specimens (based on regional asymptotic body lengths or presence of *corpora albicantia* or *corpora lutea* on the ovaries; Perrin and Donavan 1984).

Intact reproductive tracts (from the ovaries to the external urogenital slit) were excised from the postmortem animals, frozen immediately, and transferred to necropsy facilities. Specimens were defrosted and oriented in a dorsal recumbency. A single longitudinal incision was made consistently down the ventral

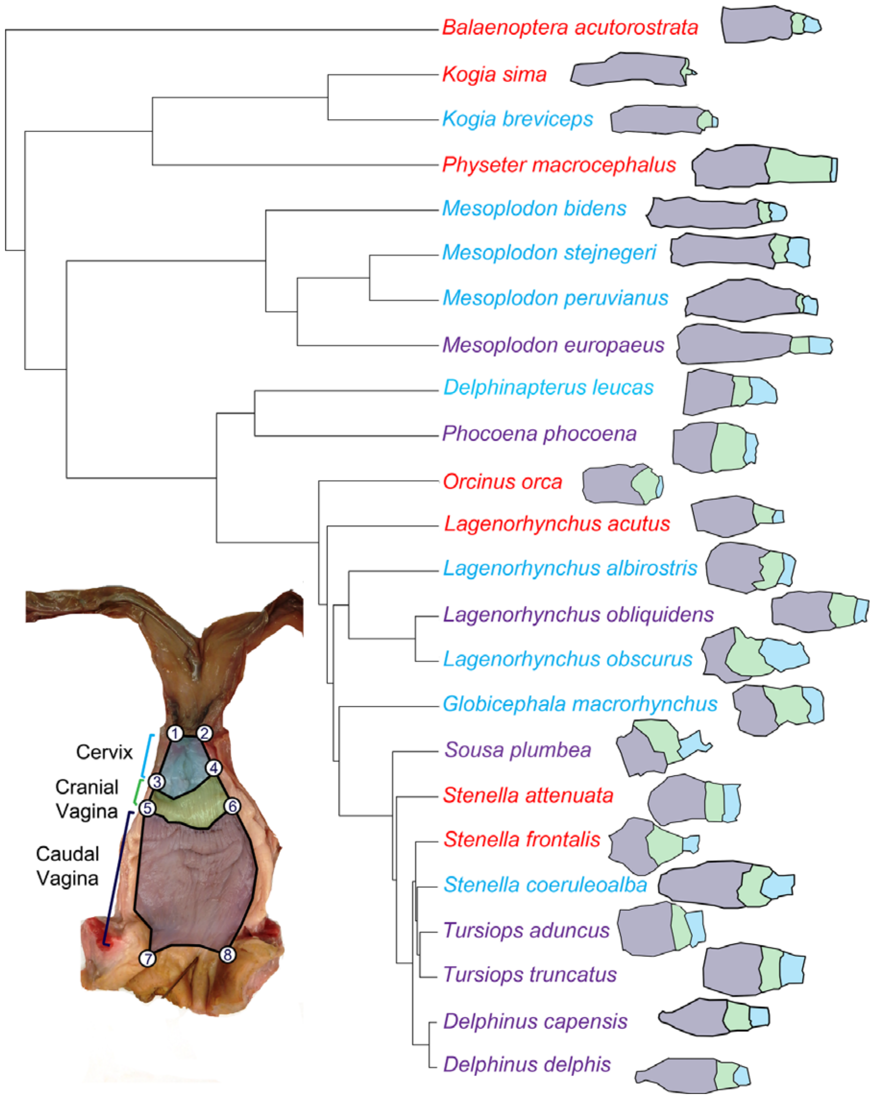

Figure 1. Cetacean genital shapes mapped onto a phylogenetic tree. Phylogeny showing the cetacean taxa examined in our analysis with the corresponding female reproductive tract shape. The cervix is oriented on the right side and the vaginal opening is on the left side. All genital shapes were subjected to general Procrustes analysis. In species with more than one specimen, a consensus (mean) shape is presented. Species in which there were only sexually immature specimens are red, only sexually mature specimens are blue, and a combination of sexually immature and mature specimens are purple. A labeled reproductive tract is depicted including the order that the landmarks were applied across specimens.

midline, from the clitoris through to the internal bifurcation of the uterine horns (Orbach et al. 2016). To further ensure the incision was exactly midline through the ventral wall of the reproductive tract, the incision was consistently made through the midpoint of the bladder (connected to the ventral vaginal wall). The vaginal walls were splayed open to reveal the contours of the cervix, vaginal folds, and clitoris. As there can be extensive asymmetry within the cetacean reproductive tract, specific landmarks found on the dorsal midline of reproductive tracts were aligned in a straight line to ensure consistent orientation for measurements and digitization. Specifically, the internal bifurcation of the uterine horn was aligned in the same straight line as the cranial limit of the vagina (Orbach et al. 2016). To quantitatively capture variation in genital shape across a range of species and body sizes, each reproductive tract was photographed in a standardized bird's-eye view using digital cameras with a minimum resolution of 10.1 megapixels. The focal plane of the camera was positioned parallel to the midline of the reproductive tract, with the long axis of the vaginal canal oriented such that the vaginal opening was at the bottom of the image. Scales were positioned in both transverse and coronal (frontal) planes. Given that the uterine horns' positioning was not a primary concern of this study, they were not digitized and their positioning was not oriented during photography. Although soft tissue is harder to align in a repeatable manner compared to hard tissue, the vaginal structure was robust and maintained its shape during imaging (see error analysis next). Because the tissues were collected from recently postmortem specimens and stored frozen, they were not subjected to the shrinkage typically in association with formalin or ethanol preservation (Fox et al. 1985).

GEOMETRIC MORPHOMETRIC ANALYSES

2D geometric morphometric techniques were used to capture the genital shape of the cervix, cranial vagina, and caudal vagina. We selected a geometric morphometric approach over measuring the more traditional Euclidean distances because we were interested in describing how the overall outline shapes varied rather than how individual landmarks varied with respect to one another. Photographs of reproductive tracts were imported into tpsDIG2 (Rohlf 2006; Fig. S1), and landmarks were used to outline the cranial and caudal bounds of the cervix, largest vaginal fold, and vaginal opening (Figs. 1 and S2). We traced the shape of the borders of these three regions using 10 semilandmark curves with 128 semilandmarks (Fig. S2). The cervix was delineated from the cranial vaginal region by the caudal end of the ectocervix (*portio vaginalis*; Fig. S2). The cranial vaginal region was delimited from the caudal vaginal region by the largest vaginal fold (the fold of greatest protrusion into the vaginal lumen from the vaginal wall; Fig. S2). The caudal demarcation of the caudal vaginal region was the cranial limit of the vulva; a natural change in tissue color was used to extend the transverse line from the cranial limit of the vulva laterally to the bisected clitoris (Orbach et al. 2016; Fig. S2). We included the cervix in our analysis as it may play a role in copulation because the penis tip penetrates the cervix in some artiodactyls (Bravo et al. 1996).

Landmark configurations were imported into R (R Core Development Team 2017) and subjected to general Procrustes analysis (GPA) using the R package *geomorph* (Adams and Otarolá-Castillo 2013). GPA translates, rescales, and rotates all landmark configurations into a common orientation (Zelditch et al. 2012). Semilandmark curves were slid such that bending energy between semilandmarks was minimized (Perez et al. 2006). Although we found substantial asymmetry in the genitalia, the asymmetry is biological and unrelated to mounting, landmarking error, or preservation (see error analysis next). Therefore, we chose to use the raw asymmetric data rather than correcting for asymmetry using the symmetrical component of variation because the latter would eliminate potentially important information relating to true biological shape.

ERROR ANALYSIS

To reduce landmark error and eliminate interobserver error, all specimen photography and landmarking were performed by one researcher (DNO). Intraobserver landmark error was calculated for each individual landmark using the method described by Singleton (2002) and for each individual specimen using the method described by Lockwood et al. (2002). We landmarked a single sexually mature common bottlenose dolphin (*Tursiops truncatus*) specimen not used in any other analysis (specimen ID: MARS2017-137) 11 times to create an error dataset (Tables S1 and S3). This specimen was remounted prior to taking each photograph to simulate the error inherent in the mounting process and in the photography process as a whole, in addition to error in the landmarking process.

We performed a GPA on the error dataset, calculated the consensus shape using the gpagen and mshape functions in the R package *geomorph* (Adams and Otárola-Castillo, 2013), and exported the landmark data into Microsoft Excel version 12.3.3 (Microsoft, 2008). The details of the GPA analysis can be found in Table S1. After calculating error for individual landmarks, we included the error sample subset in the original dataset to qualitatively evaluate how the error subset plotted in morphospace. This allowed us to evaluate whether there was substantial scatter in our error dataset relative to other specimens. Given that many of our assertions were based on Principal component analysis (PCA), this was a valuable check on our data capture process. Finally, following Lockwood et al. (2002) and Hedrick and Dodson (2013), we calculated the Euclidean distances between PC1, 2, 3, and 4 (PC variance > 5%) for both nonerror specimens and error specimens and the mean of the error sample for each PC. This allowed us to quantitatively evaluate the amount of scatter of the error subset within the total specimen morphospace.

OVERALL GENITAL SHAPE

Overall geometric morphometric patterns of the cervix and vagina were visualized by overlaying the reproductive tract shapes subjected to GPA onto a cetacean phylogenetic tree (McGowen et al. 2009). A consensus (average) genital shape was used when there was more than one specimen per species. PCAs were run on all cetacean specimens to visualize general genital shape trends in the data.

ONTOGENETIC ALLOMETRY

Intraspecific ontogenetic and geographic patterns were explored using common bottlenose dolphins (*T. truncatus*) and harbor porpoises (*Phocoena phocoena*). We had large representative samples of sexually immature and mature specimens and different regions of stranding for these two species. The ontogenetic and geographic allometric analyses were run separately for common bottlenose dolphins and harbor porpoises using log-transformed centroid size and the common allometric component (CAC) method developed by Mitteroecker et al. (2004). Centroid size is the squared root of the sum of the square distances of a set of landmarks from their centroid (Bookstein 1991). The CAC is a vector of regression scores generated by a pooled regression of shape variables on size, corrected for species means. Our application of this method to two cetacean species is similar to that used by Drake and Klingenberg (2008) for single taxa. Based on the results of the ontogenetic allometric analyses, all subsequent tests were restricted to inclusion of sexually mature cetaceans only.

EVOLUTIONARY ALLOMETRY

We examined evolutionary allometric relationships to determine if body size and vaginal size in sexually mature cetaceans influence genital shape. Evolutionary allometry was assessed by plotting the CAC against the log-transformed centroid size of the landmark configurations. This approach was repeated by plotting the CAC against the log-transformed total body length of the specimens.

PHYLOMORPHOSPACE

The genital shape morphospace of sexually mature specimens was explored using a PCAs. We then examined the effect of phylogeny on our data using a recent time-calibrated cetacean tree (McGowen et al. 2009) by calculating the *K*-test statistic developed by Blomberg et al. (2003) and modified for high-dimensional morphometric data by Adams (2014). The mean genital shape was calculated for species with more than one sexually mature specimen following Sherratt et al. (2014). The *K*-statistic was analyzed using the physignal function in *geomorph* (Adams and Otarolá-Castillo 2013). A phylogeny was overlaid on the PC morphospace to visualize the relationship between phylogeny and genital shape using the plotGMPhyloMorphoSpace function in *geomorph* (Adams and Otarolá-Castillo 2013).

TESTES MASS AND NEONATE SIZE

We assessed the effects of maximum testes mass and average neonate size on genital shape variation. The residuals of maximum combined left and right testes mass and maximum sexually mature male mass (g), and the residuals of average neonate body length at birth and average mother body length at birth (cm) were calculated based on data obtained from an extensive literature review (Table S2). Two species (*Mesoplodon peruvianus* and *Mesoplodon stejnegeri*) were excluded from the analysis because we were unable to find published data on testes mass for these species. We ran a phylogenetic general least squares (pgls) model in the R package *caper* (Orme 2013) and evaluated the effect of both testes and neonate residuals and their interaction on vaginal shape.

Results

ERROR ANALYSIS

We found that error ranged between 2.22% (landmark 8) and 7.77% (landmark 4), with a mean percent error of 5.11% (Table S1). This error was relatively small, but larger than that typically found in hard tissue analyses (e.g., Foth et al. 2016). We then examined whether our error sample substantially diverged qualitatively and quantitatively in morphospace using Euclidean distances between specimens and the mean of the error sample. We found that all error specimens overlapped in the same region of morphospace and that no other specimens overlapped with the error sample (Fig. 2). The Euclidean distances demonstrated that all error specimens were more similar to one another than to any nonerror specimens (Table S3; Fig. 2).

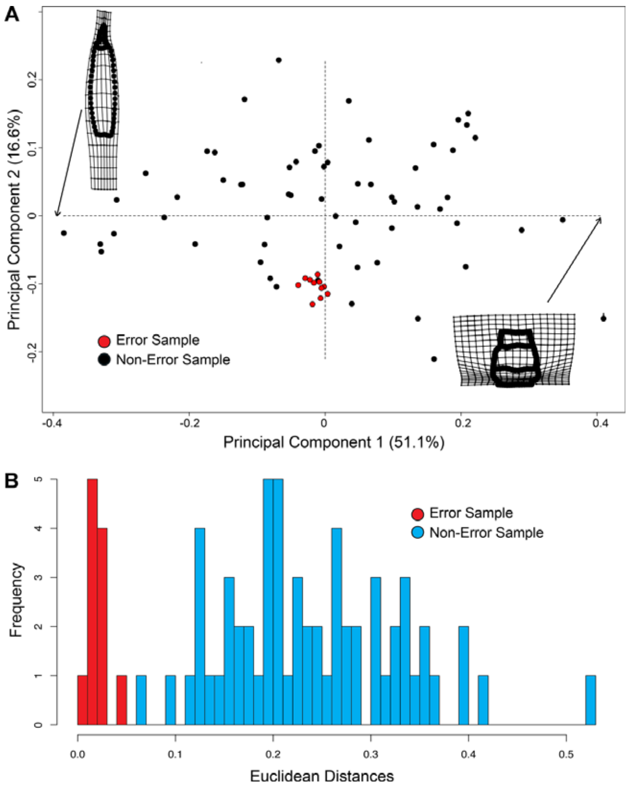

Figure 2. **Error analysis demonstrating limited intraspecimen measurement error. (A) Principal component analysis of complete cetacean sample ($n = 61$) and error subset sample ($n = 11$). This demonstrates that in PC morphospace, members of the error subset cluster more closely to other members of the error subset than to other specimens. (B) Distribution of Euclidean distances between each specimen and the mean of the error subset dataset. Error samples were more similar to one another than any was to nonerror samples.**

OVERALL GENITAL SHAPE

Sixty-one reproductive tracts were obtained, representing 24 species and seven families of marine mammals (Table S4; Fig. S1). Accordingly, our data have representation from 26% of extant cetacean species (McGowen et al. 2009), with the diversity of reproductive tract shapes depicted in Figures 1 and S1. However, only one baleen whale specimen was obtained. In the PCA of all specimens, principal component 1 (PC1) and PC2 accounted for

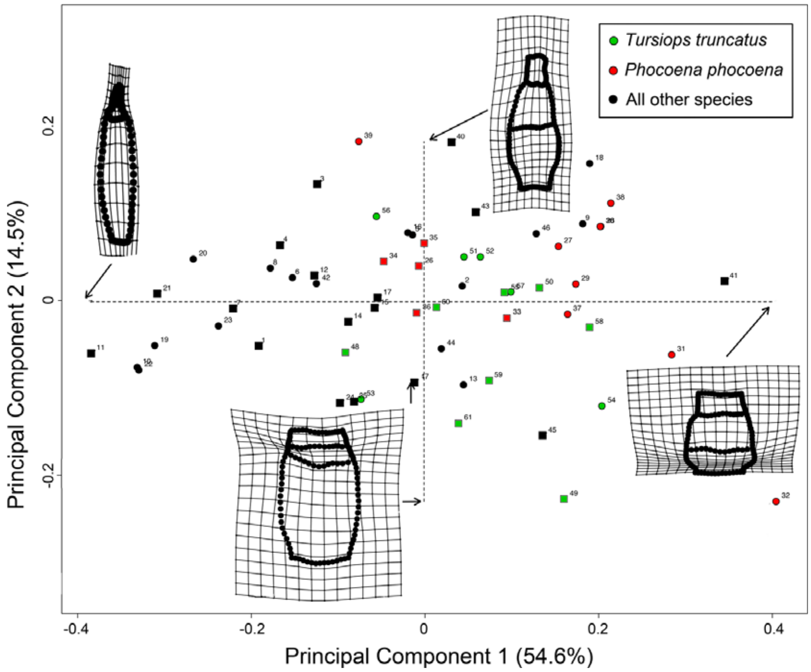

Figure 3. **Principal components analysis of cetacean genital shapes for all specimens ($n = 61$). Common bottlenose dolphin (*Tursiops truncatus*) and harbor porpoise (*Phocoena phocoena*) specimens are highlighted to demonstrate the extent of intraspecific variation driven by ontogenetic allometry. Sexually immature specimens are represented by squares, whereas sexually mature specimens are represented by circles. Specimen numbers in the plot refer to specimens in Table S4.**

69.1% of the total variance (Table S4). As the remaining PCs after PC2, each accounted for less than 10% of the variance (Table S4), only the patterns found in PC1 and PC2 are described. PC1 explained 54.6% of the total genital shape variation and was driven by the relative length of the cranial and caudal vaginal regions (Fig. 3). PC2 explained 14.5% of the total genital shape variation and was driven by a change in the overall aspect ratio (the ratio of width to height) of the cervix and vagina (Fig. 3).

ONTOGENETIC ALLOMETRY

Ontogeny had a significant effect on variation in female reproductive tract shape in common bottlenose dolphins (*T. truncatus*; $n = 14$, $F_{12} = 2.52$, $P = 0.027$) and in harbor porpoises (*P. phocoena*; $n = 14$, $F_{12} = 8.05$, $P < 0.001$), the two species for which we had a large enough sample size to explore ontogenetic trends. The relative width of the cervix and vagina change with size (Fig. 4A and B), as does the relative length of the cranial vagina among harbor porpoises (Fig. 4B). However, ontogeny is a stronger predictor of genital shape in *P. phocoena* ($R^2 = 0.402$) compared to *T. truncatus* ($R^2 = 0.174$). PC1 divided reproductive tract shapes distinctly by reproductive state in *P. phocoena* (Fig. 4D; Table S5), where sexually mature specimens had wider genitals overall, and the cranial vaginal region was the most prominent. In *T. truncatus*, sexually mature females vary along PC1, whereas sexually immature females vary along PC2 (Fig. 4C; Table S6). Sexually mature females have wide cervices, but vary in the relative size of the cervix and cranial vagina. Sexually immature females vary in the relative width of the cervix and relative length of the caudal vagina. The extensive degree of intraspecific variation in reproductive tract shape, largely attributed to ontogenetic factors, is highlighted in Figure 3, which also emphasizes that specimens for both *T. truncatus* and *P. phocoena* are found throughout the morphospace for cetaceans as a whole. Based on the clear effects of ontogenetic allometry on cetacean genital shape, only sexually mature specimens were used in subsequent analyses. The effects of population could not be disentangled from the effects of body size among our samples because sexually immature specimens were coincidentally obtained primarily from different populations than sexually mature specimens (Fig. 4E and F).

EVOLUTIONARY ALLOMETRY

Thirty-one of the 61 specimens were sexually mature and included in the analysis of evolutionary allometry and phylogeny. Evolutionary allometry was a significant predictor of reproductive tract shape using the log-transformed total body length of specimens ($F_{29} = 8.07$, $P < 0.001$, $R^2 = 0.21$), but not the log-transformed centroid size of the vagina ($F_{29} = 2.15$, $P = 0.095$, $R^2 = 0.07$; Table S7). Reproductive tracts were overall narrower and with longer caudal vaginal regions as specimens increased in body length (Fig. 5).

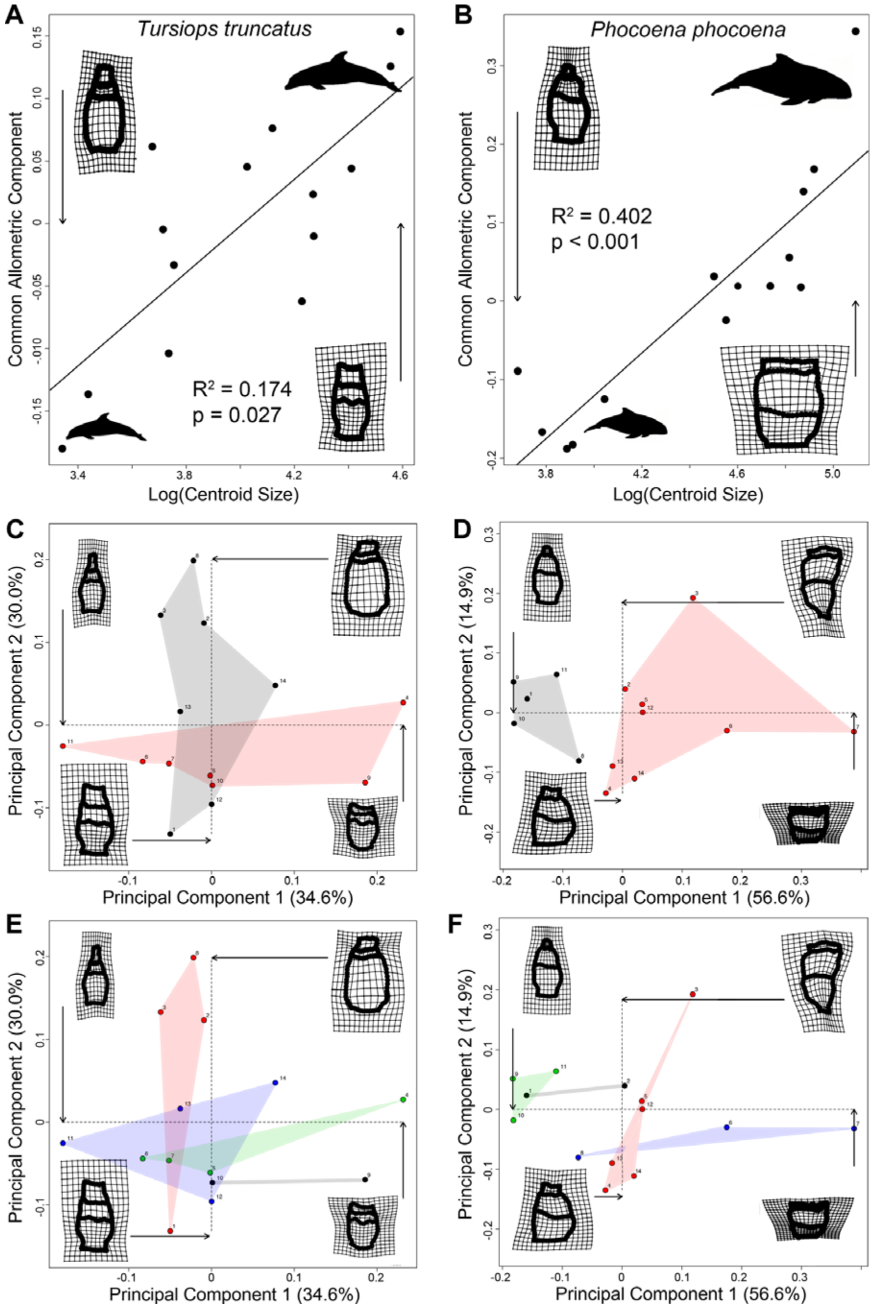

Figure 4. Within species ontogenetic allometry of cetacean genital shapes. Ontogenetic trajectory plotting genital shape against centroid size with regression line for (A) common bottlenose dolphins (*Tursiops truncatus*) and (B) harbor porpoises (*Phocoena phocoena*). Shape trends divided into sexually immature (black) and sexually mature (red) specimens within (C) *T. truncatus* and (D) *P. phocoena*. (E) Shape trends splitting *T. truncatus* specimens into populations (North Carolina—red; Texas—green; Florida—black; Virginia—blue). (F) Shape trends splitting *P. phocoena* specimens into populations (California—red, Massachusetts—green, Alaska—black, Oregon—blue).

PHYLOMORPHOSPACE

PC1 explained 72.7% of the morphospace variance in the reproductive tract shape of sexually mature species ($n = 17$) and shows a narrowing and lengthening of the caudal vaginal region (Fig. 6; Table S8). Phylogeny was not a significant predictor of reproductive tract shape among sexually mature cetaceans ($K_{\mathrm{mult}} = 0.125$, $P = 0.821$).

TESTES MASS AND NEONATE SIZE

The residuals of maximum combined left and right testes mass to maximum male body mass did not significantly affect female genital shape ($F_{13} = 1.47$, $P = 0.62$, $R^2 = 0.10$). Similarly, the residuals of average neonate to mother length at birth did not have a significant effect on reproductive tract shape variation ($F_{15} = 2.54$, $P = 0.642$, $R^2 = 0.14$). The interaction between relative

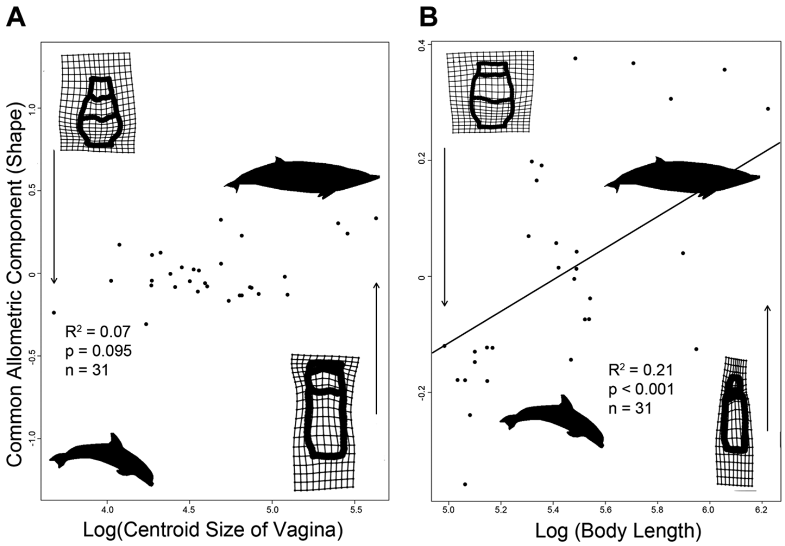

Figure 5. Evolutionary allometry of cetacean genital shapes. (A) Evolutionary allometric trajectory between shape (CAC) and the log-transformed centroid size of the vagina and (B) between shape (CAC) and log-transformed body length with regression line.

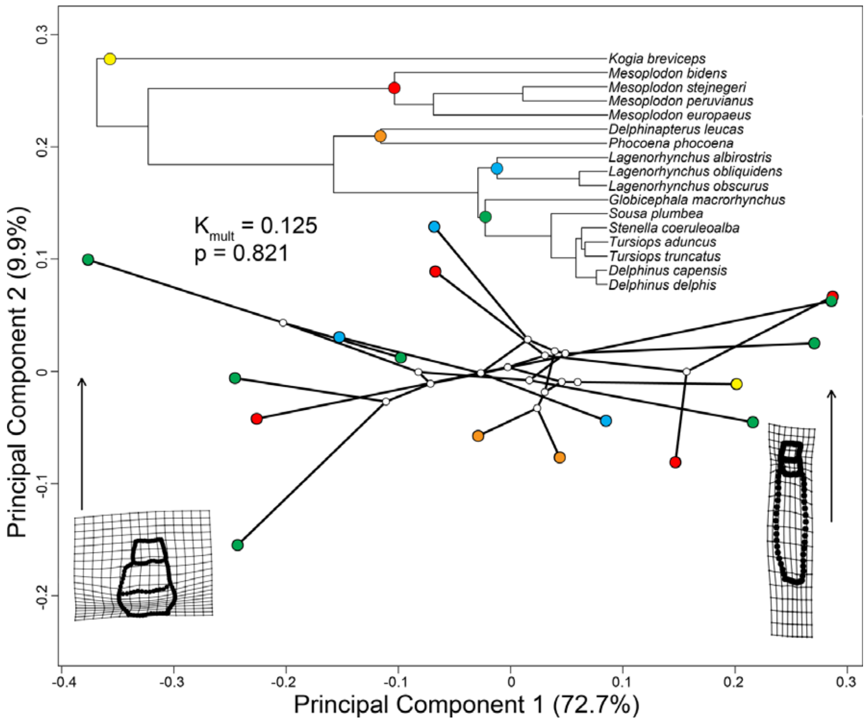

Figure 6. Phylomorphospace of cetacean genital shapes. Visualization of the morphospace of sexually mature specimens with phylogeny overlain. Colors indicate monophyletic clades on inset cladogram.

testes mass and relative neonate size also did not significantly affect female reproductive tract shape variation ($F_3 = 3.34$, $P = 0.243$, $R^2 = 0.36$; Table S2).

Discussion

Our research examined the evolution of female genital shape in a clade where extensive genital diversity has been documented but comparative measurements have been limited to linear dimensions (Orbach et al. 2017a). As shape can be a large component of morphological evolution and provide insights into functionality (Evans and Sanson 2003), the application of a 2D geometric morphometric approach enabled us to quantitatively analyze fine-scale morphological shape variation and explore potential evolutionary drivers. Our results are consistent with previous studies in several taxa, where extensive variation in female genitalia has been reported, underscoring the need for more careful quantification of female morphological variation (reviewed in Brennan and Prum 2015). For example, in insects, the shape of female genitalia has been shown to evolve rapidly (e.g., flies; Puniamoorthy et al. 2010), and females can be more variable in shape than males (Scarab beetles, *Pyllophaga hirticula*; Polihronakis 2006). Even when male genital shape changes faster than female shape, female shape can be extremely variable (stink bugs; Genevcius et al. 2017). In cases where morphological variation in female genitalia are apparently subtle, careful quantification of shape variation has shown significantly different vaginal shapes despite some overlap in morphospace (watersnakes, *Nerodia sipedon* and *N. fasciata*; Showalter et al. 2013), and significant morphological differences (Yassin and Orgogozo 2013). Evidence that female genital shape evolves rapidly and covaries with male genital variation is well established in several taxa (reviewed in Brennan and Prum 2015), and our study provides a first step in investigating this pattern more broadly in mammals, where no comparative studies of female genital shape across a clade have been conducted.

In general, the genital shape of cetacean reproductive tracts varies primarily on the relative length of the caudal and cranial vagina, followed by some variation in the aspect ratio of the vagina and cervix. Ontogenetic factors largely contribute to the overall intraspecific genital shape variation (Fig. 3). The separation between the cranial and caudal vagina was delimited by the largest vaginal fold. This geometrically homologous separation was a consistent and obvious landmark across specimens. Further, the largest fold is under strong mechanical and likely sexual selection pressure; the largest fold stops progression of the penis shaft during simulated intromission, while the penis tip likely continues into the cranial vagina (Orbach et al. 2017b). Therefore overall vaginal shape variation is likely to be associated with penis morphology in cetaceans.

ONTOGENY

Ontogeny significantly influences the genital morphology of common bottlenose dolphins (*T. truncatus*) and harbor porpoises (*P. phocoena*; Fig. 4). As our results differ from findings that used linear measurements to explore ontogenetic changes in the genitalia of *T. truncatus* (Orbach et al. 2016), we advocate the value of using multiple analytical tools to explore patterns of variation in morphology. Ontogenetic genital shape changes have the potential to reveal which aspects of genitalia are most important to perform a copulatory or reproductive function. For example, sexually mature watersnakes have a bifurcation in their vaginas that is not well-developed in sexually immature females, and males have bifurcated hemipenes, suggesting that the bifurcation of females is necessary for copulatory function (Showalter et al. 2013). Similarly, in this study, we observed an extension and broadening of the cranial vagina in sexually mature females (Fig. 4C and D) that may be associated with the role of the penis tip in sperm deposition proximate to the cervix. In both *P. phocoena* and *T. truncatus*, the penis shaft stops at the largest vaginal fold that divides the vagina into the cranial and caudal regions, while the penis' filiform tip can continue cranially in the vagina (Orbach et al 2017b). Therefore the shape change in the cranial vagina may accommodate movement of the penis tip. As ontogeny was a stronger predictor of genital shape in *P. phocoena* compared to *T. truncatus*, we suggest ontogenetic effects may vary with the extent of vaginal complexity. In *T. truncatus*, there is typically only one fold, but in *P. phocoena* there are several folds concentrated mainly in the cranial region of the vagina (Orbach et al. 2017a). The allometric relationship suggests that reproductive tract shape changes occur gradually, rather than being punctuated by events leading to vaginal and cervical distention, such as first sexual intercourse or first parturition (Fig. 4A and B).

The cervices of *T. truncatus* and *P. phocoena* also widen as females become sexually mature, consistent with reports of ontogenetic changes in tissue composition related to the functional role of the cervix in reproduction in various mammalian taxa (El-Banna and Haffez 1972; Kress and Mardi 1992). To our knowledge, this is the first study to show an ontogenetic change in the shape of the cervix. In alpaca (*Vicugna pacos*), the tip of their fibroelastic penis penetrates through the cervix (Bravo et al. 1996). However, the penis tip in cetaceans is unlikely to reach all the way to the cervix even at full distention (Orbach et al. 2017b).

GEOGRAPHY

We predicted geography would influence cetacean genital shape. However, the roles of geographic and ontogenetic allometry on reproductive tract shape were confounded in our data, as sexually immature specimens were coincidentally collected from different populations than sexually mature specimens and ontogeny accounted for a large percentage of overall shape variation (Fig. 4C and D). The opportunistic nature of specimen acquisition hindered our ability to collect specimens of both maturity states from the same populations. Although geographic divergence and associated natural selection pressures can influence male genital morphology (e.g., landsnail, *Helix aspersa*; Madec and Guiller 1994; guppies, *Poecilia reticulata*; Evans et al. 2011; deer mice, *Peromyscus maniculatus* and *Peromyscus oreas*; Sullivan et al. 1990), geographic effects on female genitalia have not been well-documented and further research is warranted.

EVOLUTIONARY ALLOMETRY AND PHYLOGENETIC PATTERNS

Evolutionary allometry was a significant predictor of female genital shape in cetaceans (Fig. 5), just as it was a predictor of vaginal length (Orbach et al 2017a). However, vaginal size was not associated with vaginal shape. Accumulating evidence suggests that genital shape, not just vaginal length, should be considered in analyses of genital scaling patterns. Size and shape components are important considerations for the functional morphology of genitalia, and both can vary allometrically with body size. For example, the shape of both male and female genitalia varies more rapidly than genital size (e.g., *Ontophagus* beetles, Macagno et al. 2011; *O. taurus*, Simmons et al 2009; *Mus musculus*, Simmons and Firman 2014), suggesting that changes in size are more constrained than changes in shape; this may facilitate mechanical fit during copulation (reviewed in Brennan and Prum 2015).

The lack of a phylogenetic signal in our data (Figs. 1 and 6; Table S8) suggests that vaginal shape evolves rapidly even among closely related species, some of which had very different genital shapes (Figs. 1 and 5). A high degree of morphological divergence among closely related species is predicted by the "lock and key" mechanism of genital evolution (Masly 2012), but is not inconsistent with other models (Brennan and Prum 2015). However, in the absence of studies of penile morphology, it is impossible to distinguish the evolutionary mechanism responsible for this rapid divergence (Brennan and Prum 2015). In at least *P. phocaena* and *T. truncatus*, sexual conflict may partially drive genital evolution because the largest vaginal fold acts as a barrier to further intromission of the penis shaft into the vagina (Orbach et al 2017b). Vaginal barriers to penile penetration have been shown to result from sexual conflict in waterfowl (Brennan et al 2007).

Intraspecific variation in shape was evident in our samples, although our samples sizes were too small to quantify this potentially confounding factor. Genital shape can be highly variable within species (e.g., scarab beetles, Polihronakis 2006; watersnakes, Showalter et al. 2013). We eliminated all sexually immature specimens from the evolutionary allometry and phylogeny analyses to minimize the effects of ontogenetic variation, but other aspects of reproductive state, such as pregnancy and parturition, may affect vaginal shape (e.g., *Homo sapiens*; Pendergrass et al. 2000).

TESTES MASS AND NEONATE SIZE

We found no significant correlations between genital shape variation and relative testes weight, relative neonate size, or their interaction. Although residual testes size is a good predictor of the intensity of sexual selection (Kenagy and Trombulak 1986), the vagina interacts directly with the penis during copulation and therefore penile morphology may be a better predictor of vaginal shape than testes size (e.g., waterfowl, Brennan et al. 2007). Future studies that assess the coevolution of penis length and vaginal shape in cetaceans at the interspecific level may find that sexual selection influences female genital shape evolution through penis shape directly. Cetacean species with large relative testes masses have comparatively long penises (Brownell and Ralls 1986; Dines et al. 2014), as do rodents and carnivores (Ramm 2007). Future studies that assess the relationship between testes mass and penis shape may yield interesting results. As vaginal shape morphology did not change relative to neonate size, shape may not be influenced by natural selection pressures associated with parturition.

ERROR ANALYSIS

Intraobserver landmarking error was small (5.11%). However, because error is calculated based on a ratio involving the distance from each landmark to the consensus centroid, landmarks closer to the centroid have higher error (von Cramon-Taubadel et al. 2007). This could explain why landmarks 3, 4, and 5, which were closest to the centroid, had the highest error. Regardless, even the error surrounding these landmarks is small relative to interspecimen differences (Fig. 2). Singleton's (2002) method is appropriate as it quantifies error for each landmark individually and thus we considered its advantages to outweigh its disadvantages in the present analysis. Soft tissue analyses using geometric morphometric approaches are rare and may be more prone to error than hard tissue analyses, so we examined the position of the error dataset in morphospace and quantified the Euclidean distances within and between the error sample and nonerror specimens. Given that there was no overlap in total shape between the error sample and other specimens either qualitatively in morphospace (Fig. 2), no overlap in Euclidean distances (Table S3, Fig. 2; Lockwood et al. 2002), and that individual landmark error was small (Table S1; Singleton 2002), we assert that error did not play a large role in our analysis and that the results demonstrating large within species shape divergence are real trends. This also suggests that the use of geometric morphometric techniques to quantify soft tissue shape can provide repeatable data, validating its potential application to other soft tissue systems.

Although our data include 26% of all extant cetacean species and could be expanded upon further, we believe our data are robust and representative of taxon-wide patterns. A larger sample size per species could further elucidate changes that may be associated with reproductive state. For example, vaginas are longer in pregnant compared to lactating and resting *T. truncatus*; however, sample sizes were too small to draw biologically relevant conclusions (Orbach et al. 2016). Our data included only one baleen whale species. Due to the larger sizes of baleen whales compared to toothed whales, obtaining baleen whale reproductive tracts is an obstacle that should be overcome in future research to explore the potential roles of additional life-history factors in genital morphology evolution. We also acknowledge that reproductive tract shape variation could reflect genetic drift rather than an adaptive function(s). However, because reproductive structures are immediately and critically important to an organisms' fitness, nonadaptive deviations could have devastating consequences for an individual. Alternatively, vaginal complexity may be under selection, and genital shape variation is a by-product that accommodates more vaginal folds. If so, we would expect to find most variation in the cranial vaginal region, where most folds are concentrated (Orbach et al. 2017a), but our results here do not support that hypothesis. The folds form a complex 3D structure in the female vaginal lumen (Orbach et al. 2017b), and therefore 3D morphometric analyses that incorporate spiraling patterns may better capture the complexity and extensive variation of female genitalia among cetaceans. For example, 3D casts of the vaginal lumen in humans have shown variation in shape, length, and width that may correlate with ethnicity and parturition history (Pendergrass et al. 2000). We recommend that reproductive tracts be collected and preserved intact to explore 3D genital shape patterns so that larger sample sizes can be obtained.

In conclusion, ontogeny and evolutionary allometry explain patterns of genital shape variation in cetaceans, while phylogeny, testes size, and neonate size do not. Rapid evolution of female genital shape, as suggested by the lack of phylogenetic signature reported here, seems to be a common feature of female genitalia across taxa and suggests an important role for sexual selection in their evolution. Ontogenetic patterns of genital variation may be a widespread feature of vertebrate genitalia. Future efforts that describe 3D shape variation and analyze male morphology are likely to be fruitful in further explaining the complexity of female genitalia among cetaceans.

AUTHOR CONTRIBUTIONS

PLRB conceived the project idea. BW and PLRB funded the specimen collection. DNO and SLM collected and dissected the specimens. DNO traced the photographs in TPS. BH wrote the statistical code, ran the analyses, and created the figures. DNO wrote the initial manuscript with feedback from coauthors. All authors contributed toward manuscript revisions.

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Ethics: Specimens in the United States of America were collected under National Marine Fisheries Service (NMFS) salvage permit letters to DNO. Specimens from New Zealand were imported to the United States of America under an institutional Convention on International Trade in Endangered Species of Wild Fauna and Flora permit (CITES; 14US690343/9). Specimens from South Africa were authorized by the South African Departments of Environmental Affairs and Agriculture, Forestry, and Fisheries to the Port Elizabeth Museum/Bayworld (RES2016-62). Specimens in South Africa were photographed on-site and not exported. This study was exempt from an Institutional Animal Care and Use Committee (IACUC) authorization as the specimens were deceased upon acquisition and the salvage materials were authorized from appropriate government agencies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Biological data on the body lengths or masses of specimens, males, testes, neonates, and mothers.
Table S2. Error analysis for individual landmarks using the method developed by Singleton (2002).
Table S3. Error analysis for whole specimens using the method developed by Lockwood et al. (2002).
Table S4. Principal components 1–4 for all specimens included in the study.
Table S5. Principal components for only harbor porpoise (*Phocoena phocoena*) data.
Table S6. Principal components for only common bottlenose dolphins (*Tursiops truncatus*) data.
Table S7. Principal components for sexually mature specimens only.
Table S8. Principal components analysis of mean phylogenetic signal.
Figure S1. Images of 61 specimens used for GM analyses.
Figure S2. Scheme of semilandmark and curve positioning and orientation for trace in TPS.