





Variation in cranial morphology of bottlenose dolphins (genus *Tursiops*) off South Africa

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ABSTRACT

Taxonomy plays an important role in conservation biology. Despite the variety of methods used to differentiate units, some groups, such as Delphinidae within the Cetacea have proven difficult to untangle. This study aimed to shed light on morphological variation of the genus *Tursiops* in South African waters using geometric morphometrics and to distinguish morphological groups and variation in these groups. A total of 241 crania of *Tursiops* spp. were analyzed using a suite of 2-dimensional landmarks defined on photographs of the specimens. Results revealed two distinct morphological groups, with the smaller cluster comprised mainly of specimens from the cold temperate region off the west coast and the larger cluster comprised of specimens mainly from the warm temperate and subtropical regions off the south and east coast, respectively. We suggest that these groups correspond to different species of *Tursiops*, but this result requires further support. These groups were treated as separate entities and sexual dimorphism and geographic variation were assessed within each group. While sexual dimorphism and geographic variation were not significant within Cluster D1 and V1, they were significant within Clusters D2 and V2. The few Cluster 1 specimens found in the warm temperate and subtropical regions, relative to the number of Cluster 2 specimens, could be an indication of an offshore distribution for this group in these regions. Alternatively, the smaller cluster may also be indicative of a potentially small population size.

Key words: *Tursiops* spp., bottlenose dolphin, taxonomy, cranial morphology, geometric morphometrics.

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Taxonomy is an important aspect of conservation biology, as defining units, such as species, (Vane-Wright 2013) forms the basis of designing and implementing conservation strategies. Different methods of defining taxonomic units have been used, with morphological and geographic data being the original methods utilized by taxonomists. More recently, molecular analyses have been frequently used in taxonomy, especially to clarify problematic groups. However, morphological methods remain relevant since they provide additional insights into mechanisms driving species differentiation (Adams *et al.* 2004).

The family Delphinidae is a complex group regarding clarification of its taxonomic units, even with the variety of different techniques applied to date (Natoli *et al.* 2006, Pinela *et al.* 2011). *Tursiops* (bottlenose dolphins) is one of the problematic genera in this family (Pinela *et al.* 2011). Throughout the 20th century, taxonomic work led to the description of a large number of species within a genus for small cetaceans, particularly in the genus *Tursiops* (reviewed in Pinela *et al.* 2011). Due to the controversial taxonomic status of the genus, the phylogeny of *Tursiops* has been extensively studied for more than four decades. This genus has a cosmopolitan distribution with morphological differences recorded both between and within regions (Natoli *et al.* 2006, Pinela *et al.* 2008, Perrin *et al.* 2013).

Subsequently, the genus *Tursiops* was considered to be monospecific until the late 1970s when the existence of a second species, *T. aduncus*, was described (Ross 1977). Rigorous investigations were employed to examine the genus using external morphology (Ross 1977, Wang *et al.* 2000a), skeletal morphology (Ross 1977, Wang *et al.* 2000b), and later molecular mitochondrial DNA (mtDNA) analysis (Wang 1999, Natoli *et al.* 2004). Based on these assessments, *T. aduncus* is now an accepted species (Ross 1977; Wang *et al.* 2000a, 2000b; Committee on Taxonomy 2016). Several molecular phylogenetic analyses have not supported a sister species relationship between the two *Tursiops* species; however, recent publications, such as McGowen (2011) and Amaral *et al.* (2012) do so, though with weak support. The *Tursiops* genus has furthermore been extensively studied with regards to the population structure of both species. There are still uncertainties concerning the distribution of the genus in South African waters, although *T. truncatus* is assumed to be widely dispersed, particularly along the Atlantic coast, and *T. aduncus* is distributed inshore along the Indian Ocean coast (Findlay *et al.* 1992, Best 2007). *Tursiops aduncus* has been described as having strong population substructuring along parts of the South African coastline, but with low genetic diversity within groups (Natoli *et al.* 2004). In addition, it was suggested that representatives of this species occurring in South African waters were significantly differentiated from *T. aduncus* occurring elsewhere, which was possibly deserving status as a separate species (Natoli *et al.* 2004).

The regional variation in both *T. truncatus* and *T. aduncus* in terms of size, coloration, shape, and foraging strategies, is thought to be due to adaptations to the different environments in which the two species

occur. Parapatric offshore and inshore forms as well as various ecotypes have been described in some regions using either morphological or genetic criteria (Connor *et al.* 2000, Tezanos-Pinto *et al.* 2008), but hitherto these lines of evidence have not been combined. For example, coastal and offshore forms of *T. truncatus* have been identified off both the Pacific and the Atlantic coasts of the United States (Perrin *et al.* 2011, Vollmer and Rosel 2013). These forms were identified using morphological and ecological factors. Despite this separation, elsewhere in the world confusion between *T. aduncus* (which is generally a more coastal species) and the inshore form of *T. truncatus* is evident (Reeves *et al.* 2004). For example, in South African specimens, sequences from the mitochondrial control region from a population reported to constitute the inshore ecotype of *T. truncatus* were shown to match a sequence of the *T. aduncus* holotype, which was collected along the Ethiopian coast of the Red Sea (Perrin *et al.* 2007).

Morphology and Tursiops Taxonomy

Cranial morphology has been a useful tool to investigate sexual dimorphism (de Oliveira *et al.* 2005, Bigoni *et al.* 2010, Frandsen and Galatius 2013, Conry *et al.* 2016), geographical variations (Sanvicente-Anorve *et al.* 2004, Murphy and Rogan 2006), allometry (del Castillo *et al.* 2014, Torre *et al.* 2014) and inshore *vs.* offshore variations (Perrin *et al.* 2011) within many species of cetaceans. An example of its value within the Cetacea is *D. delphis* in southern Australia comprising a single morphologically variable species (Bell *et al.* 2002). Similarly, when considering *Tursiops*, Mead and Potter (1995) used cranial and external morphology to differentiate populations of *T. truncatus* off the Atlantic coast of North America. No overlap in cranial morphology with regards to shape and size was reported between inshore and offshore populations. In terms of body size, the offshore population was reported to be approximately 15% larger than the inshore population. Cranial morphology was also investigated in *T. truncatus* from the Gulf of Mexico (Turner and Worthy 2003). Cranial variation based on sex, geographic location, and ontogeny was revealed (Turner and Worthy 2003). In terms of geographic location, significant variation was observed between adult females from Texas and from Florida. In addition, sexual dimorphism was reported in the Texas populations but not in the Florida population (Turner and Worthy 2003).

In this study, we examined variation in cranial morphology to differentiate morphological groups of bottlenose dolphins, *Tursiops* spp., inhabiting South African waters. Our aim was to use museum specimens of *Tursiops* spp. collected along the South African coastline to determine whether there is evidence of different groups occurring in South African waters. A further aim was to investigate the degree of variation within each group identified in relation to local and global habitat distribution.

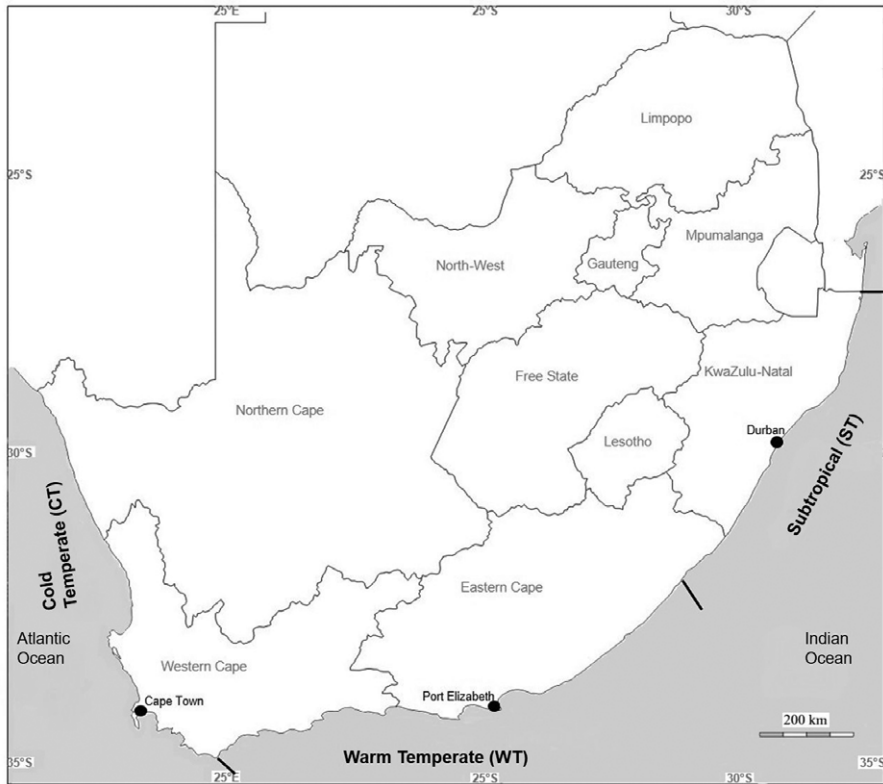


Figure 1. Map of South Africa showing the three geographic regions.

MATERIALS AND METHODS

Cranial Morphology

Crania of *Tursiops* spp. were obtained from the Graham Ross Marine Mammal collection at the Port Elizabeth Museum and the Iziko South African Museum in Cape Town. These specimens were collected from various sources, including animals retrieved as bycatch in the shark nets off the coast of KwaZulu-Natal (some 80% of specimens) and dolphins stranded between St Helena Bay on the West coast of South Africa and the Mozambique border on the east coast (Fig. 1). These locations lie in the three coastal regions of South Africa, namely: cold temperate (Namibian coast towards Cape Agulhas), warm temperate (Cape Agulhas–Mzamba), and the subtropical region (Mzamba to the Mozambique border; Fig. 1).

A total of 241 crania (males = 125, females = 116, cold temperate [CT] = 19, warm temperate [WT] = 53, subtropical [ST] = 169) were examined (Fig. 1). Only adult specimens were used for cranial morphology analysis. Maturity was determined using the degree of fusion between the maxillae and premaxillae (Perrin and Heyning 1993). We

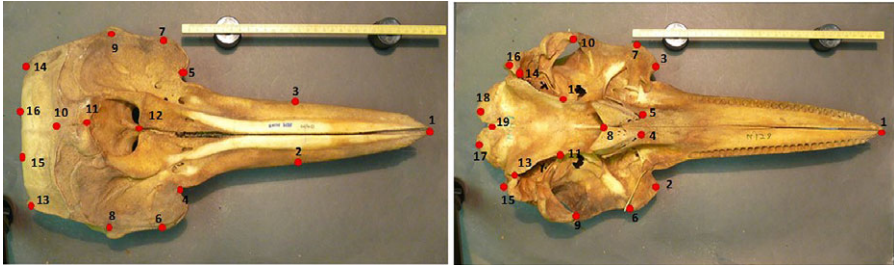


Figure 2. Two sets of landmarks digitized on each *Tursiops* spp. cranium on dorsal and ventral aspects.

assumed that crania in which at least 50% of the length of the dorsal aspect of the rostrum was fused came from mature individuals. For crania, both dorsal and ventral aspects of each specimen were photographed. For this, each specimen was placed in a standard orientation, designed to minimize the vertical distance between all landmarks. This was achieved by placing a spirit level on specific areas of the skull to ensure standard positioning for all skulls. Specimens were photographed against a dark background to emphasize their outlines, using a Panasonic Lumix DMC-FZ50 digital camera with 35–420 mm zoom lens set at 35 mm. A tripod and an extension were used to position the camera lens at an arbitrary standard height of 76 cm above the premaxillary foramina and the greater palatine foramina for dorsal and ventral surfaces of the crania, respectively. A spirit level was also used to ensure that the camera was always at a 90° angle to the skull. A 30 cm ruler was placed at an elevated level alongside each skull to provide a measure of scale at a plane close to that of the landmarks.

The resulting JPEG images were cropped and converted into TPS files using tpsUtil and imported into TpsDig 2.05 (Rohlf 2006). A set of landmarks on each aspect (dorsal [D] and ventral [V]) were digitized onto the photograph of each skull (Rohlf 2006). These landmarks were chosen to catch the shape variation throughout the cranium and were homologous among the crania (Fig. 2). A description of the landmarks is given in Table 1.

Data Analysis

Prior to all analyses, the raw coordinates were transformed into Procrustes coordinates by superimposition using the program MorphoJ (Klingenberg 2011) to remove information on position, size, and rotation. The program PAST (Hammer *et al.* 2001) was used to construct phenetic clusters to attribute specimens to groups and assess size variation, while MorphoJ (Klingenberg 2011) was used for shape variation analyses. Clusters defined were henceforth treated as separated entities. Hierarchical cluster analysis (Sheets *et al.* 2006) was used to indicate the existence of different possible groups. A linear discriminant analysis was also used to further analyze the shape differences between major clusters detected to assess differences and how well these clusters could be

Table 1. Description of landmarks used for the analysis of phenology, size, and shape, for both dorsal and ventral aspects

Landmark number	Description
Dorsal cranial view	
1	Rostral tip
2 and 3	Midpoint between the anteriormost point of antorbital notch and rostral tip/ midpoint of the rostrum; right and left
4 and 5	The antorbital notch; right and left
6 and 7	Intersection between the frontal bone and the zygomatic process; right and left
8 and 9	Intersection between the parietal bone and the frontal interparietal suture; right and left
10	Anteriormost point of the nuchal crest
11	Midpoint of the nasal bone suture
12	Posteriormost point in the premaxilla bone
13 and 14	Posteriormost point on the curve of the parietal bone; right and left
15 and 16	Posteriormost point on the occipital condyle; right and left
Ventral cranial view	
1	Rostral tip
2 and 3	Anteriormost point on the antorbital notch in the maxilla; left and right
4 and 5	Anteriormost point of the palatine surface of the pterygoid; left and right
6 and 7	Intersection between the frontal bone and the zygomatic process; left and right
8	Anteriormost point between the two pterygoid hamuli
9 and 10	Intersection between the parietal bone and the frontal interparietal suture; left and right
11 and 12	External most point of the suture between the basioccipital crest and the pharyngeal crest (choanae); left and right
13 and 14	Posteriormost point on the paraoccipital process curve of the parietal bone; left and right
15 and 16	Posteriormost point on the curve of the occipital bone; left and right
17 and 18	Posteriormost point on the edge of the supraoccipital bone; left and right
19	Midpoint of the intercondyloid notch

separated based on skull shape. To determine differences in size, centroid size (the square root of the sum of the squared distances from each landmark to the centroid of the configuration; Zelditch *et al.* 2004) was calculated. Data were tested for normality using the Shapiro-Wilk test. A 2-way analysis of variance (Pares-Casanova and Fabre 2013) was used to compare size between sexes and between regions. For shape, the data were corrected for allometry by performing shape analysis on the residuals of a multivariate linear regression of shape (Procrustes coordinates)

Table 2. Discriminant Function Analysis (DFA) pairwise Mahalanobis, Procrustes distances, pairwise group allocation tables and the leave-one-out cross-validation classification between sexes for dorsal and ventral aspects of *Tursiops* spp. crania from South Africa. Associated *P* values generated from permutation tests (1,000 permutations)

Groups	Dorsal			Ventral		
	Malanobis distance	<i>P</i>	Procrustes distance	Mahalanobis distance	<i>P</i>	Procrustes distance
Females-males	1.12	< 0.001	0.01	0.735	0.064	0.01
			< 0.001	Females-males		0.001
	Group allocated to					
	Group allocated to			Group allocated to		
	True group	Female	Male	Total	% Correct	
Females-males						
	Female	80	34	114	70	
	Male	35	82	117	70	
Discriminant function						Discriminant function
	Female	77	37	114	67.5	
	Male	37	80	117	68	
Cross-validation						Cross-validation
	Female					Female
	Male					Male
						Total
						Female
						Male
						32
						73
						105
						69.5
						64
						59
						55

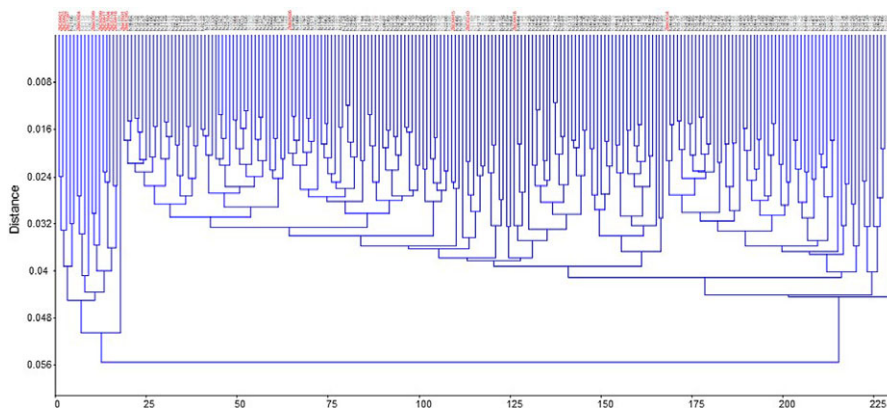


Figure 3. UPGMA phenogram for the dorsal aspect of both male and female *Tursiops* spp. crania from South Africa. Regions are rendered: red = cold temperate, grey = warm temperate, black = subtropical.

on centroid size. A simple linear regression was chosen over a regression on $\log(\text{CS})$ because the amounts of explained variance of regressions when using centroid size and $\log(\text{centroid size})$ as dependent variables were almost identical. Discriminant function analysis using jackknife/leave-one-out cross-validation was used to investigate (1) differences between groups, (2) sexual dimorphism in the whole sample, (3) variation of shape between regions (cold temperate CT, warm temperate WT, and subtropical ST; Fig. 1), and (4) to investigate sexual dimorphism within regions. Lastly, overlap in the distribution of the clusters between the dorsal and the ventral aspects was assessed using the G -test for goodness-of-fit (Sokal and Rohlf 1969).

RESULTS

There were no clear sex related differences in size or shape of the skulls. Sexual size dimorphism was not significant for either dorsal ($F = 0.541$, $df = 1$, $P = 0.463$) or ventral ($F = 0.977$, $df = 1$, $P = 0.324$) aspects and although shape dimorphism was significant in both aspects (Table 2), there was substantial overlap between females and males. As a result, the sexes were pooled for all further analyses.

Cluster Analysis and Cranial Size Variation

Phenetic clusters of both the dorsal and the ventral aspects of crania showed that the specimens separated into two groups (Fig. 3, 4). The first group was represented by Cluster D1 (Dorsal aspect 1) and Cluster V1 (Ventral aspect 1). This first group comprised of crania mainly from the cold temperate region (52%) and a few individuals from the warm temperate (35%) and subtropical (13%) regions. The second group was represented by Cluster D2 and Cluster V2. Most specimens, mainly from the subtropical and warm temperate regions, separated into these groups

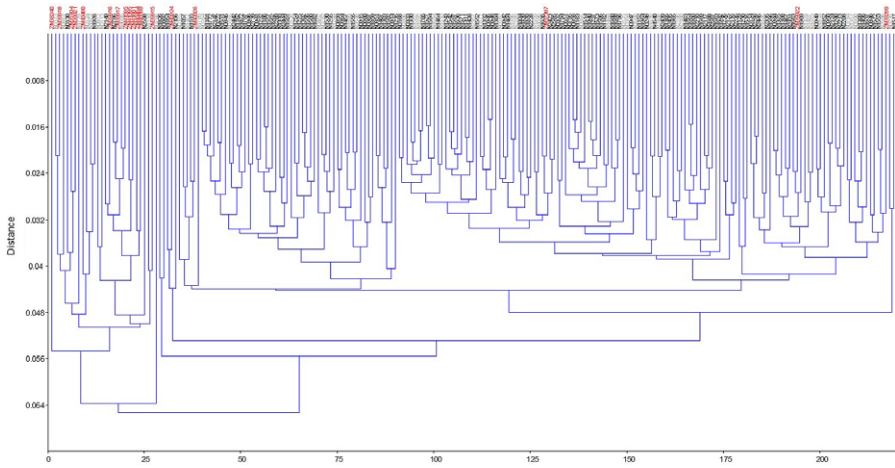


Figure 4. UPGMA phenogram for the ventral aspect of both males and females *Tursiops* spp. crania from South Africa. Regions are rendered: red = cold temperate, grey = warm temperate, black = subtropical.

(Fig. 3, 4). Cluster D1 and Cluster D2 were significantly different ($F = 461.6$, $df = 1$, $P < 0.001$) in terms of centroid size, with Cluster D1 being much larger (Fig. 5).

In the ventral aspect, a similar trend to the dorsal aspect of *Tursiops* crania was observed (Table 3, Fig. 5). Significant differences were found between clusters V1 and V2 ($F = 601.4$, $df = 1$, $P < 0.001$). The box and whisker plots also showed that Cluster V1 crania were larger compared to Cluster V2 crania (Fig. 5). Some ($n = 9$) of the specimens that were found in Cluster D1 were also found in Cluster V1.

The distribution of crania between the clusters was not significantly different between the dorsal and the ventral aspects ($GG = 1.781$, $df = 1$, $P = 0.182$). This indicates that crania found in one cluster as assessed by one aspect were more likely to be found in the same cluster for the other aspect.

Cranial Shape

Discriminant function analysis of both the dorsal and ventral aspects showed significant differences in shape between clusters (Table 4). The reliability of the discrimination between these groups was then tested using the leave-one-out cross-validation. More than 90% of specimens were correctly classified to their original cluster in both dorsal and ventral aspects (Table 4). The small number of “incorrectly” classified specimens showed that there was very little shape overlap between the two clusters and thus supports the presence of two different groups of *Tursiops* spp. (Table 4). Figure 6 shows the shape differences between the two clusters in both aspects, with Cluster 1 specimens characterized by a broader braincase, elongated hamular crests, and shorter widened rostra. In contrast, Cluster 2 specimens were characterized by a reduced

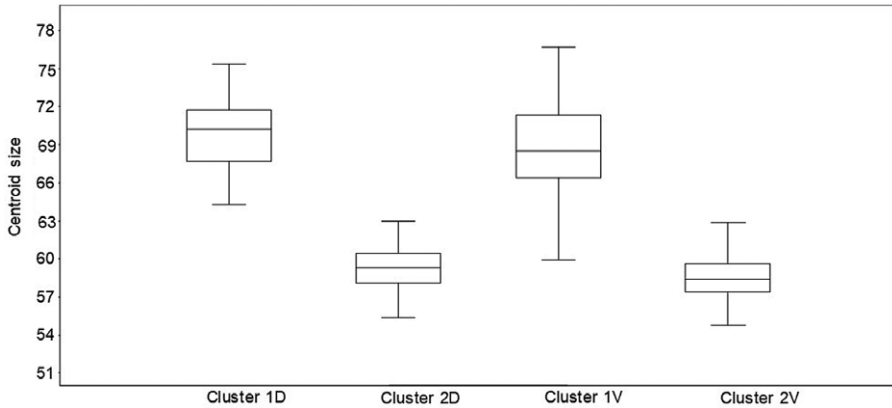


Figure 5. Box whisker plot showing cranial size variations between the two clusters in the dorsal and ventral aspects of *Tursiops* spp. crania from South Africa.

braincase, but with extended supraoccipital and exoccipital bones, reduced hamular crests, and slender but elongated rostra (Fig. 7).

Cranial Size and Shape Variation Within Clusters

Clusters D1 and V1—In terms of cranial size in Clusters D1 and V1 ($n_D = 18$, $n_V = 28$), sexual dimorphism was not significant in the dorsal ($F = 2.804$, $P = 0.314$) and ventral ($F = 1.008$, $P = 0.250$) aspect. Only one specimen was from the Subtropical region, resulting in this region being excluded from analysis. No significant differences were found between the warm and cold temperate regions for the dorsal aspect ($F = 1.159$, $P = 0.082$). Similarly, no significant differences between sexes and between regions were evident in the ventral aspect ($F = 1.304$, $P = 0.289$). In addition, cranial shape differences between the sexes and between regions were examined within each cluster. In cluster 1, sexual dimorphism was not significant in either the dorsal or the ventral aspects of *Tursiops* spp. crania.

Cluster D2 and V2—The two-way ANOVA revealed significant differences between regions in the dorsal aspect of Cluster D2 specimens (Table 3). Crania from the cold temperate region were significantly larger than those from the warm temperate and the subtropical regions (Table 3, Fig. 6). Sexual dimorphism was not significant in this cluster (Table 3). As with the dorsal aspect, significant differences between regions in Cluster V2 were observed (Table 3). Crania from the cold temperate region were larger compared to the crania from the subtropical and the warm temperate regions (Fig. 6). Sexual dimorphism was again not significant in this aspect (Table 3).

In Cluster 2, sexual dimorphism with regard to shape was significant in both the dorsal and ventral aspects of *Tursiops* spp. (Table 5). In this cluster, significant differences between the sexes were detected in both aspects, with females possessing longer rostra compared to males (Table 5). Furthermore, significant differences in regions were found

Table 3. Two-way ANOVA between regions and between sexes for cranium centroid size in Cluster 2 specimens of *Tursiops* spp. crania from South Africa.

	Sum of squares	df	<i>F</i>	<i>P</i>
Dorsal				
Region	63.75	2	13.01	<0.000
Sex	5.905	1	2.41	0.122
Region and sex	16.43	2	3.353	0.0370
Within	490	200		
Total	575.1	205		
Ventral				
Region	99.64	2	18.46	<0.000
Sex	2.638	1	0.977	0.324
Region and sex	10.75	2	1.991	0.14
Within	499.3	185		
Total	612.7	190		

between the subtropical and the warm temperate regions in the dorsal aspect, and the cold temperate and subtropical regions (Table 5). The leave-one-out cross-validation showed that <70% of specimens were correctly assigned to their sex (Table 5). Between regions, >70% specimens were correctly assigned except for the cold temperate region, in which <50% of specimens were correctly assigned to their region.

DISCUSSION

In his seminal paper, Ross (1977) suggested that the genus *Tursiops* be divided into two species based on evidence from the cranial morphology of South African specimens. More research has since been carried out on the species worldwide (Ross 1977; Wang *et al.* 2000a, 2000b; Natoli *et al.* 2004). However, Ross' work was based on relatively few samples, mostly from warm temperate and subtropical regions, and his assessment was based on traditional morphometrics. We have further assessed the taxonomic status of this genus in the region using a greatly expanded sample size, which gives full geographic coverage along the entire South African coastline, using the latest methods of morphometric analysis. We have further combined evidence for cranial variation between sexes and between regions within each species.

Interspecific Differences

The results of this study show evidence of the presence of two groups of *Tursiops* spp. off the coast of South Africa. However, without further support, such as would be provided by genetic data, our results remain speculative. Significant differences in both cranial size and shape between the groups were observed. Cluster 1 crania were significantly larger, having broader braincases, wider and proportionately shorter rostra than Cluster 2 crania. The latter crania had smaller braincases with narrow, but longer rostra.

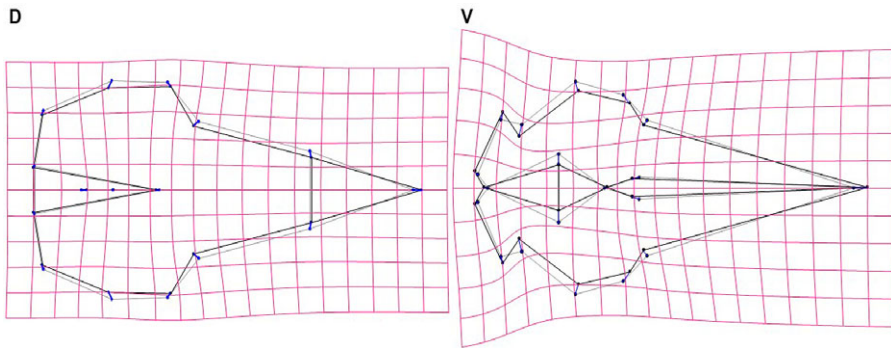


Figure 6. Box whisker plot showing cranial size variation between the three regions within cluster 2 specimens in the dorsal and ventral aspects of *Tursiops* spp. crania from South Africa.

Characteristics of the cranial morphology of these two groups are in accordance with studies elsewhere, both off South Africa and in most ocean basins where these dolphins are distributed. Ross (1977) indicated that the length of the rostrum was proportionately shorter in *T. truncatus* and that the proximal portion of the rostrum was distinctly broader and deeper than in *T. aduncus*. He also described the braincase in *T. aduncus* as narrower than that of *T. truncatus*. These differences have been reflected in comparisons of sympatric *Tursiops* spp. elsewhere (Wang 1999, Wang *et al.* 2000b, Kurihara and Oda 2007). Sympatric *Tursiops* species examined from Chinese, Japanese and South Australian waters showed similar patterns, where *T. aduncus* specimens had longer rostra and narrower braincases than those of *T. truncatus* specimens (Wang *et al.* 2000b, Kemper 2004, Kurihara and Oda 2007), as reported in Ross (1977). Kurihara and Oda (2007) examined a total of 72 crania of *Tursiops* spp. from the Indian and western Pacific Oceans to clarify the systematics of this genus and found that *T. aduncus* crania were smaller than those of *T. truncatus*. Thus, differences reported in this study agree with a consistent, global pattern.

All the specimens used in this study have been sourced as stranded or bycatch animals. Therefore, the predominant group found in a region is likely to have an inshore distribution due to the proximity of their habitat to the coast. The more distantly distribution of offshore animals may account for their lower numbers. Specimens in the cold temperate region were predominantly in Clusters D1 and V1. The few specimens found in the warm temperate and subtropical regions, relative to the number of *T. aduncus*, could be an indication of an offshore distribution for this species in these regions. Alternatively, this species has a potentially much smaller population in these regions. *T. aduncus* seemed to dominate the sample in warm temperate and the subtropical regions, as has previously been reported (Ross 1977,1984; Findlay *et al.* 1992).

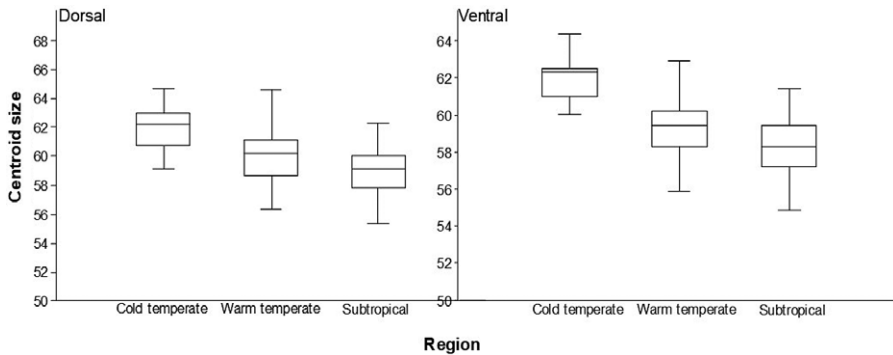


Figure 7. Visualization of shape differences associated with discriminant vectors between specimens in the dorsal (D) and ventral (V) aspects of the crania of *Tursiops* spp. from South Africa. lollipop showing the direction of change, outline of Cluster 1, outline of Cluster 2.

Sexual Dimorphism

We found no significant sexual dimorphism in cranial size and shape in Clusters D1 and V1. This could be an artifact of the relatively small sample size of this group. In contrast, sexual dimorphism in cranial shape was found to be significant in Clusters D2 and V2. Several studies have reported that sexual dimorphism was not significant in either *T. truncatus* or *T. aduncus* (Ross 1977, Wang 1999, Wang *et al.* 2000b, Turner and Worthy 2003, Kemper 2004). However, in other studies sexual dimorphism has been reported in *T. truncatus* (Perrin *et al.* 2011, Duras *et al.* 2014). These contrasting results clearly reflect the variability in cranial morphology and levels of sexual dimorphism in the study species.

Geographical Variation

There were no significant differences in cranial size and shape between the three regions for Clusters D1 and V1, once again possibly reflecting the small sample sizes for each region. There were, however, significant differences between the regions in cranial shape and size in Clusters D2 and V2, with the cold temperate region having bigger crania compared to crania from the other two regions. These differences in cranial size and shape have been ascribed to varying environmental conditions, such as water temperature, prey availability, feeding strategies, fluctuating environment, mortality, and an offshore/inshore distribution (Bell *et al.* 2002, Duras *et al.* 2014).

Examining cranial variation between regions within each group gives insight into the environmental processes in these regions (Perrin 1984, Galatius and Gol'din 2011). Potential for such environmentally determined differences in populations of *Tursiops* spp. is present in South Africa. Two very different marine habitats are associated with the two current systems: the Agulhas Current along the eastern and

Table 4. Discriminant function analysis (DFA) pairwise Mahalanobis, Procrustes distances between clusters and the leave-one-out cross validation classification for dorsal and ventral aspects of *Tursiops* spp. crania from South Africa. Associated *P* values generated from permutation tests (1,000 permutations)

		Dorsal			Ventral							
Groups	Malanobis distance	<i>P</i>	Procrustes distance	<i>P</i>	Mahalanobis distance	<i>P</i>	Procrustes distance	<i>P</i>				
1-2	5.082	<0.001	0.043	<0.001	1-2	6.12	<0.001	0.049	<0.001			
Group allocated to												
		Cluster 1	Cluster 2	Total	% Correct	1-2	Discriminant function	True group	Cluster 1	Cluster 2	Total	% Correct
1-2	Cluster 1	17	1	18	94	1-2	Discriminant function	Cluster 1	27	1	28	96
	Cluster 2	2	211	213	99			Cluster 2	0	191	191	100
Cross-validation	Cluster 1	16	2	18	89	Cross-validation		Cluster 1	26	2	105	59
	Cluster 2	2	211	213	99			Cluster 2	0	191	191	100

Table 5. The discriminant function analysis pairwise group allocation tables and the leave-one-out cross-validation classification in Cluster 2 for both dorsal and ventral aspects of *Tursiops* spp. crania from South Africa. Abbreviations as follows: CT = cold temperate, ST = subtropical, WT = warm temperate

Groups	Dorsal			Ventral		
	Malanobis distance	P	Procrustes distance	Mahalanobis distance	P	Procrustes distance
Females-males	1.180	< 0.001	0.009	0.921	0.006	0.009
CT-ST	1.829	0.228	0.012	2.939	0.007	0.021
CT-WT	2.326	0.175	0.012	3.234	0.085	0.018
ST-WT	1.152	< 0.001	0.007	1.091	0.010	0.007
	Group allocated to					
F-M	True group	Females	Males	Total	% Correct	
Discriminant function	Females	82	25	107	76.6	
	Males	32	74	106	70	
Cross-validation	Females	74	33	107	69	
	Males	33	73	106	69	
	Group allocated to					
CT-ST	True group	CT	ST	Total	% Correct	
Discriminant function	CT	5	1	6	83	
	ST	24	140	164	85	
Cross-validation	CT	3	3	6	50	
	ST	26	138	164	84	
	True group					
Discriminant function	CT	5	0	5	100	
	ST	10	134	144	93	
Cross-validation	CT	1	4	5	20	
	ST	16	128	144	89	
	True group					
CT-WT	True group	CT	WT	Total	% Correct	
Discriminant function	CT	6	0	6	100	
	WT	7	36	43	84	
Cross-validation	CT	3	3	6	50	
	WT	8	35	43	81	
	True group					
Discriminant function	CT	5	0	5	100	
	WT	1	41	42	97.6	
Cross-validation	CT	2	3	5	40	
	WT	8	34	42	81	
	True group					
ST-WT	True group	ST	WT	Total	% Correct	
Discriminant function	ST	124	40	164	75.6	
	WT	12	31	43	72	
Cross-validation	ST	115	49	164	70	
	WT	17	26	43	60.5	
	True group					
Discriminant function	ST	103	41	144	71.5	
	WT	12	30	42	71	
Cross-validation	ST	99	45	144	68.8	
	WT	16	26	42	62	

southern coast and the Benguela Current on the west coast. The Agulhas Current on the eastern seaboard of South Africa derives its water from the tropics and subtropics and is characterized by warm water, low nutrients and is thus low in biological productivity (Ansorge and Lutjeharms 2007). The Benguela Current on the other hand is characterized by wind driven coastal upwelling of cool, subthermocline water rich in nutrients and thus high biological productivity (Ansorge and Lutjeharms 2007). These habitats differ in temperatures, primary productivity, and prey availability (Ansorge and Lutjeharms 2007). These environmental differences may explain variances in species distribution (Best 2007, Perrin *et al.* 2007) and the regional variation in the cranial size and shape found in this study within *T. aduncus*.

Geographical differences have been found in *T. truncatus* off Florida, in the Black Sea, and in the Adriatic Sea, with specimens having larger crania in the cold regions compared to the warmer regions (Đuras *et al.* 2014). Differences in cranial size of Dall's porpoise (*Phocoenoides dalli*) populations corresponded with temperature and the distribution of primary productivity, indicating that food quantity might affect their overall cranial size (Amano and Miyazaki 1992). Hale *et al.* (2000) found that *T. aduncus* crania from southeast Africa, east and south China Sea and eastern Australia varied in cranial size, being significantly smaller in the tropical regions compared to subtropical and temperate regions, reflecting differences in seawater temperature.

In odontocetes, cranial variations have been found mostly in characters that are associated with feeding, such as the number and size of teeth, length and breadth of the rostrum, and the size of the temporal fossa, indicating that variation is related to prey availability and foraging strategies (Perrin 2009). Spinner dolphin (*Stenella longirostris*) populations were found to differ in the eastern tropical Pacific in features such as dorsal fin shape, color patterns, fluke shape, and cranial morphology (Perrin 2009). Crania of Hector's dolphin (*Cephalorhynchus hectori*) from the North Island of New Zealand were significantly larger than those from the South Island and that their rostra differed significantly (Baker *et al.* 2002). Harbor porpoises (*Phocoena phocoena*) in different regions differ in adaptations for pelagic and demersal prey (Galatius and Gol'din 2011, Galatius *et al.* 2012). Significant differences reported in the rostral dimensions between the inshore and the offshore *S. longirostris* suggested that a wider and longer rostrum would be advantageous when pursuing larger, demersal prey in shallow waters, while a smaller rostrum would be preferred in catching fast, pelagic prey (Perrin 1975). Furthermore, significant differences in the crania involving the posterior-most region and most muscle and ligament attachments may also be due to varying foraging strategies, behavior, and vocalization (Conry *et al.* 2016).

Conclusion

While *Tursiops* is locally of little concern, further information of the distribution of two morphological distinct groups in in South African waters is relevant in determining management strategies. Characteristics

that distinguish *T. truncatus* from *T. aduncus* appear to be broadly similar across their distribution. However, variation within each of the species seem to vary between different ocean basins. For example, in some regions, sexual dimorphism in cranial size has been reported for both species, while in other regions this does not apply. Geographical variation has been reported in some populations of each species in different ocean basins and even though it has been mentioned that most of the differences would be associated with feeding or foraging strategies, some differences appear to be associated with differences in temperature. In colder regions, species tend to attain larger sizes as compared to animals of the same species in warmer areas (Amano and Miyazaki 1992). Geographic variation also plays a role in sexual dimorphism, with it reportedly not being significant in areas of low productivity compared to areas of high productivity (Amano and Miyazaki 1992). Cranial morphology informs on more than the taxonomic status of a species, it also reflects differences in localities, and how they are affected by different environmental factors, such as temperature. Future studies such as genetics to confirm the species designations of the genus *Tursiops* in South Africa are being carried out.

ACKNOWLEDGMENTS

We would like to thank Port Elizabeth Museum at Bayworld, Iziko South African Museum and their past and present staff for access to crania used in this study. We are also grateful to the KwaZulu-Natal Sharks Board for providing access to the dolphins and for making their facilities available. Thanks to the National Research Foundation (South African) for the Innovation and Scarce Skills: Doctoral scholarship for the first author (Reference: SFH1208157902). This presents the Africa Earth Observatory Network's (AEON) publication number 212.

LITERATURE CITED

- Adams, D. C., F. J. Rohlf and D. E. Slice. 2004. Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology* 71:5–16.
- Amano, M., and N. Miyazaki. 1992. Geographic variation and sexual dimorphism in the skull of Dall's porpoise, *Phocoenoides dalli*. *Marine Mammal Science* 8:240–261.
- Amaral, A. R., J. A. Jackson, L. M. Möller, L. B. Beheregaray and M. M. Coelho. 2012. Species tree of a recent radiation: The subfamily Delphininae (Cetacea, Mammalia). *Molecular Phylogenetics and Evolution* 64:243–253.
- Anson, I. J., and J. R. E. Lutjeharms. 2007. The cetacean environment off Southern Africa, Pages 5–14 in P. B. Best, ed. *Whales and dolphins of the Southern African subregion*. Cambridge University Press, Cambridge, U.K.
- Baker, A. N., A. N. H. Smith and F. B. Pichler. 2002. Geographical variation in Hector's dolphin: Recognition of new subspecies of *Cephalorhynchus hectori*. *Journal of the Royal Society of New Zealand* 32:713–727.
- Bell, C. H., C. M. Kemper and J. G. Conran. 2002. Common dolphins *Delphinus delphis* in Southern Australia: A morphometric study. *Australian Mammalogy* 24:1–10.
- Best, P. B. 2007. *Whales and dolphins of the South African subregion*. 1st edition. Cambridge University Press, Cambridge, U.K.

- Bigoni, L., J. Velemínska and J. Bruzek. 2010. Three-dimensional geometric morphometric analysis of cranio-facial sexual dimorphism in a Central European sample of known sex. *HOMO: Journal of Comparative Human Biology* 61:16–32.
- Committee on Taxonomy. 2016. List of marine mammal species and subspecies. Society for Marine Mammalogy. Available at <http://www.marinemammalscience.org> (accessed on 29 May 2017).
- Connor, R. C., J. Mann, P. L. Tyack and H. Whitehead. 2000. The social lives of whales and dolphins. Pages 1–6 *in* J. Mann, R. C. Connor, P. L. Tyack and H. Whitehead, eds. *Cetacean societies: Field studies of dolphins and whales*. University of Chicago Press, Chicago, IL.
- Conry, D. S., P. A. Pistorius, S. Plön and G. J. G. Hofmeyr. 2016. Sexual dimorphism in striped dolphin (*Stenella coeruleoalba*) crania from South Africa. *Marine Mammal Science* 32:1254–1271.
- de Oliveira, L. R., E. Hingst-Zaher and J. S. Morgante. 2005. Size and shape sexual dimorphism in the skull of the South American fur seal, *Arctocephalus australis* (Zimmermann, 1783) (Carnivora: Otariidae). *Latin American Journal of Aquatic Mammals* 4:27–40.
- del Castillo, D. L., D. A. Flores and H. L. Cappozzo. 2014. Ontogenetic development and sexual dimorphism of franciscana dolphin skull: A 3D geometric morphometric approach. *Journal of Morphology* 275:1366–1375.
- Duras, M., D. D., Brnic, T. Gomercic and A. Galov. 2014. Craniometry of bottlenose dolphins (*Tursiops truncatus*) from the Adriatic Sea. *Veterinarski Arhiv* 84:649–666.
- Findlay, K. P., P. B., Best, G. J. B. Ross and V. G. Cockcroft. 1992. The distribution of small odontocete cetaceans off the coasts of South Africa and Namibia. *South African Journal of Marine Science* 12:237–270.
- Frandsen, M. S., and A. Galatius. 2013. Sexual dimorphism of Dall's porpoise and harbor porpoise skulls. *Mammalian Biology - Zeitschrift für Säugetierkunde* 78:153–156.
- Galatius, A., and P. E. Gol'din. 2011. Geographic variation of skeletal ontogeny and skull shape in the harbour porpoise (*Phocoena phocoena*). *Canadian Journal of Zoology* 89:869–879.
- Galatius, A., C. C. Kinze and J. Teilmann. 2012. Population structure of harbour porpoises in the greater Baltic region: Evidence of separation based on geometric morphometric comparisons. *Journal of the Marine Biological Association of the United Kingdom* 29:388–355.
- Hale, P. T., A. S. Barreto and G. J. B. Ross. 2000. Comparative morphology and distribution of the *aduncus* and *truncatus* forms of bottlenose dolphin *Tursiops* in the Indian and Western Pacific Oceans. *Aquatic Mammals* 26:101–110.
- Hammer, O., D. A. T. Harper and P. D. Ryan. 2001. PAST: Palaeontological statistics software package for education and data analysis. *Paleontological Electronica* 4:1–9.
- Kemper, C. M. 2004. Osteological variation and taxonomic affinities of bottlenose dolphins, *Tursiops* spp., from South Australia. *Australian Journal of Zoology* 52:29–48.
- Klingenberg, C. P. 2011. MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11:353–357.
- Kurihara, N., and S. Oda. 2007. Cranial variation in bottlenose dolphins *Tursiops* spp. from the Indian and western Pacific Oceans: Additional evidence of two species. *Acta Therologica* 52:403–418.
- McGowen, M. R. 2011. Toward the resolution of an explosive radiation—A multilocus phylogeny of oceanic dolphins (Delphinidae). *Molecular Phylogenetics and Evolution* 60:345–357.

- Mead, J. G., and C. W. Potter. 1995. Recognizing two populations off the bottlenose dolphin (*Tursiops truncatus*) of the Atlantic Coast of North America: Morphologic and ecologic considerations. *International Marine Biology Research Institute Reports* 5:31–44.
- Murphy, S., and E. Rogan. 2006. External morphology of the short-beaked common dolphin, *Delphinus delphis*: Growth, allometric relationships and sexual dimorphism. *Acta Zoologica* 87:315–329.
- Natoli, A., V. M. Peddemors and A. R. Hoelzel. 2004. Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology* 17:363–375.
- Natoli, A., A. Canadas, V. M. Peddemors, A. Aguilar, C. Vaquero, P. Fernandez-Piqueras and A. R. Hoelzel. 2006. Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.). *Journal of Evolutionary Biology* 19: 943–954.
- Pares-Casanova, P. M., and L. Fabre. 2013. Size and shape variability in the skull of the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821). *Anatomia, Histologia, Embryologia* 42:379–383.
- Perrin, W. F. 1975. Distribution and differentiation of populations of dolphins of the genus *Stenella* in the Eastern Tropic Pacific. *Journal of the Fisheries Research Board of Canada* 32:1059–1067.
- Perrin, W. F. 1984. Patterns of geographical variation in small cetaceans. *Acta Zoologica* 172:137–140.
- Perrin, W. F., 2009. Geographic variation. Pages 492–498 in W. F. Perrin, B. Würsig and J. G. M. Thewissen, eds. *Encyclopaedia of marine mammals*. 2nd edition. Academic Press, Burlington, MA.
- Perrin, W. F., and J. E. Heyning. 1993. Rostral fusion as a criterion of cranial maturity in the common dolphin, *Delphinus delphis*. *Marine Mammal Science* 9:195–197.
- Perrin, W. F., K. M. Robertson, P. J. H. van Bree and J. G. Mead. 2007. Cranial description and genetic identity of the holotype specimen of *Tursiops aduncus* (Ehrenberg, 1832). *Marine Mammal Science* 23:343–357.
- Perrin, W. F., J. L. Thieleking, W. A. Walker, F. I. Archer and K. M. Robertson. 2011. Common bottlenose dolphins (*Tursiops truncatus*) in California waters: Cranial differentiation of coastal and offshore ecotypes. *Marine Mammal Science* 27:769–792.
- Perrin, W. F., P. E. Rosel and F. Cipriano. 2013. How to contend with paraphyly in the taxonomy of the delphinine cetaceans? *Marine Mammal Science* 29:567–588.
- Pinela, A. M., A. Aguilar and A. Borrell. 2008. Occurrence of long-beaked and short-beaked forms of *Delphinus* spp. off NW Africa appears to reflect differential use of habitat rather taxonomy. IWC Scientific Committee Document SC/60/SM20.
- Pinela, A. M., A. Borrell and A. Aguilar. 2011. Common dolphin morphotypes: Niche segregation or taxonomy? *Journal of Zoology* 284:239–247.
- Reeves, R. R., W. F. Perrin, B. L. Taylor, C. S. Baker and S. L. Mesnick. 2004. Report of the Workshop on Shortcomings of Cetacean Taxonomy in Relation to Needs of Conservation and Management, April 30–May 2, 2004, La Jolla, California. U.S. Department of Commerce, NOAA Technical Memorandum NMFS NOAA-TM-NMFS-SWFSC 363. 74 pp.
- Rohlf, F. J. 2006. TpsDig: Digitize landmarks and outlines, version 2.05. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
- Ross, G. J. B. 1977. The taxonomy of bottlenose dolphins *Tursiops* species in South African waters, with notes on their biology. *Annals of the Cape Provincial Museums (Natural History)* 11:135–193.

- Ross, G. J. B. 1984. The smaller cetaceans of the east coast of southern Africa. *Annals of the Cape Provincial Museums (Natural History)* 15:173–410.
- Sanvicente-Anorve, L., J. L. Lopez-Sanchez, A. Aguayo-Lobo and L. Medrano-Gonzalez. 2004. Morphometry and sexual dimorphism of the coastal spotted dolphins, *Stenella attenuata graffmani*, from Bahia de Banderas, Mexico. *Acta Zoologica* 85:223–232.
- Sheets, H. D., K. M. Covino, J. M. Panasiewicz and S. R. Morris. 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Frontiers in Zoology* 3:15.
- Sokal, R. R., and F. J. Rohlf. 1969. *Biometry*. W. H. Freeman and Company, San Francisco, CA.
- Tezanos-Pinto, G., C. S. Baker, K. Russell, *et al.* 2008. A worldwide perspective on the population structure and genetic diversity of bottlenose dolphins (*Tursiops truncatus*) in New Zealand. *Journal of Heredity* 100:11–24.
- Torre, J., O. Vidal and R. L. Brownell, Jr. 2014. Sexual dimorphism and developmental patterns in the external morphology of the vaquita, *Phocoena sinus*. *Marine Mammal Science* 30:1285–1296.
- Turner, J. P., and G. A. J. Worthy. 2003. Skull morphometry of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico. *Journal of Mammalogy* 84:665–672.
- Vane-Wright, R. I. 2013. Taxonomy, Methods of. Pages 97–111 in S. Levin, ed. *Encyclopedia of biodiversity*. 2nd edition. Academic Press, Cambridge, U.K.
- Vollmer, N. L., and P. E. Rosel. 2013. A review of common bottlenose dolphins (*Tursiops truncatus truncatus*) in the northern Gulf of Mexico: Population biology, potential threats and management. *Southeastern Naturalist* 12:1–43.
- Wang, J. Y. 1999. The classification of sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. Ph.D. thesis, McMaster University, Hamilton, Canada. 116 pp.
- Wang, J. Y., L.-S. Chou, and B. N. White. 2000a. Differences in the external morphology of two sympatric species of bottlenose dolphins (genus *Tursiops*) in the waters of China. *Journal of Mammalogy* 81:1157–1165.
- Wang, J. Y., L.-S. Chou and B. N. White. 2000b. Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Zoology* 252:147–162.
- Zelditch, M. L., D. L. Swiderski, H. D. Sheets and W. L. Fink. 2004. *Geometric morphometrics for biologists: A primer*. Academic Press, New York, NY.

Received: 27 November 2017

Accepted: 24 August 2018