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Skull morphology of bottlenose dolphins from different ocean populations with emphasis on South America

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Abstract

The bottlenose dolphin, genus *Tursiops*, is cosmopolitan occurring in tropical and temperate regions, with morphological variation between and within different oceans. Since the genus' taxonomy has been under discussion for a long time, this work aimed at analyzing the cranial variability of *T. truncatus* from different regions of the world. Geometric Morphometrics analyses were performed in 201 skulls of adult specimens, on dorsal, ventral, and lateral views, from the Eastern North Pacific, Eastern North Atlantic, Eastern South Atlantic, and Western South Atlantic oceans. The results indicate differences between individuals that inhabit the Atlantic and Pacific oceans. Within the Atlantic Ocean, there is an evident longitudinal differentiation of specimens from the eastern and western regions. A latitudinal separation was also observed, considering specimens from the North and South Atlantic Ocean. In the Western South Atlantic statistical differences were found between two morphological groups, identified as *T. gephyreus* (sensu Lahille, 1908) and *T. truncatus*, and the cross-validation presented 98% as minimum confidence for correct classification of these two groups. The present study provides strong morphological support to consider these two lineages as separate species.

KEYWORDS
biogeography, morphological comparison, Odontoceti

1 | INTRODUCTION

The bottlenose dolphin, *Tursiops* spp., is one of the most well-known dolphin species and has been the basis of many studies of cetacean behavior. Two species are currently recognized: *T. aduncus* in coastal waters of the Indo-Pacific region and *T. truncatus* with a cosmopolitan distribution in both coastal and oceanic waters (Wang, 2018; Wells & Scott, 2018). Nevertheless, there is still much discussion regarding the genus taxonomy. During most of the 20th century, there was consense that it is a polytypic species, despite researchers stressing the need for a full genus review (Hershkovitz, 1966; Jefferson, Leatherwood, & Webber, 1993; Ross, 1977; Walker, 1981; Wells & Scott, 2018). Similarly, the International Whaling Commission's Scientific Committee has recommended a global revision of the genus (IWC, 1995, 2009) and from 2015 to 2018 this revision has been performed, confirming the status of the two already recognized species and concluding that "future taxonomic questions should be examined within an appropriately wide and inclusive geographic context" (IWC, 2019, p. 50).

The genus *Tursiops* has a worldwide distribution, occurring in tropical and temperate regions, in almost all oceans (Leatherwood & Reeves, 1990), some internal seas (e.g., Black Sea), and estuarine areas (Fruet, Secchi, Di Tullio, & Kinas, 2011; Odell & Asper, 1990; Simões-Lopes & Fabian, 1999). They occur along South America's Atlantic coast (Lodi et al., 2016), from the Caribbean Sea (Pardo et al., 2009) in the north, along all the Brazilian coast (Siciliano, Moreno, Silva, & Alves, 2006), reaching south up to the Chubut Province, in Argentina, with few records in Tierra del Fuego (Goodall et al., 2008; Goodall et al., 2011). Bottlenose dolphins are common both in coastal waters in the Brazilian, Uruguayan and Argentinian coasts (Pinedo, Rosas, & Marmontel, 1992) as well as in offshore areas (Di Tullio, Gandra, Zerbini, & Secchi, 2016; Moreno et al., 2009; Moreno et al., 2017).

Differences between offshore and coastal morphotypes of bottlenose dolphins have been identified in areas of the Atlantic and Pacific oceans (Mead & Potter, 1995; Waerebeek, Reyes, Read, & Mckinnon, 1990; Walker, 1981; Wells & Scott, 2018). For instance, coastal forms in the Atlantic Ocean and elsewhere tend to be smaller, lighter in color and with larger fins. However, there are exceptions such as the offshore forms of the eastern North Pacific Ocean, which are smaller than the coastal forms in this region (Wells & Scott, 2018). In the Indo-Pacific region, individuals of these two adjacent environments are actually different species, with *Tursiops truncatus* (Montagu, 1821) using more offshore areas, and *T. aduncus* (Ehrenberg, 1833) inhabiting more coastal habitats (Hale, Barreto, & Ross, 2000; Möller & Beheregaray, 2001; Ross, 1977; Wang, Chou, & White, 1999; Wang, Chou, & White, 2000a; Wang, Chou, & White, 2000b).

In Argentinian waters, *Tursiops gephyreus* was comprehensively described in 1908, based on two specimens collected on the La Plata River estuary (Lahille, 1908). Afterward, this name was used for all bottlenose dolphins in the southwestern Atlantic up to the 1970s, when *Tursiops* was considered a polymorphic species and researchers reverted to using *T. truncatus* without explicit justification (Hershkovitz, 1966). More recently it has been suggested that bottlenose dolphins from southern South America should be recognized as a subspecies (Barreto, 2000; Costa, Rosel, Daura-Jorge, & Simões-Lopes, 2016) or a separate species (Barreto, 2004; Wickert, 2013; Wickert, von Eye, Oliveira, & Moreno, 2016). Nevertheless, comparisons of specimens from this area to bottlenose dolphins from other areas of its distribution must be performed in order to understand the relationship among the different morphotypes.

Geometric morphometrics has been used to access the morphological variations in skulls of dolphins, being successfully applied to verify interspecific (Amaral, Coelho, Marugán-Lobón, & Rohlf, 2009), ontogenetic (del Castillo, Flores, & Cappozzo, 2014; del Castillo, Segura, Flores, & Cappozzo, 2016; del Castillo, Viglino, Flores, & Cappozzo, 2017; Parés-Casanova & Fabre, 2013; Sydney, 2010; Sydney, Machado, & Hingst-Zaher, 2012), sexually dimorphic (del Castillo et al., 2014; Monteiro-Filho, Monteiro, & Reis, 2002) and geographic variation (Jefferson & Waerebeek, 2004; Monteiro-Filho et al., 2002; Perrin, Thieleking, Walker, Archer, & Robertson, 2011). However, until now, this has not been done for bottlenose dolphins in South America. Thus, we studied skull variations in bottlenose dolphins from different oceanic regions, using 2D-geometric morphometrics, in order to differentiate *Tursiops truncatus* and *Tursiops gephyreus*. Our aim was to provide sound morphological information to help clarifying the species status.

2 | MATERIALS AND METHODS

Two-dimensional geometric morphometrics analysis (GM) was used to assess the skull variations among specimens of the genus *Tursiops*. Both, landmarks and semilandmarks were used to describe the skull morphology of the specimens (Bookstein, 1991; Bookstein, 1997; Gunz, Mitteroeker, & Bookstein, 2005; MacLeod, 2013; Rohlf & Marcus, 1993). Here, we use the terms *truncatus* and *gephyreus* (sensu Wickert et al., 2016) to refer, respectively, to the morphotypes designated to the taxonõmic unit *T. truncatus* and *T. gephyreus* (sensu Lahille, 1908 and Wickert et al., 2016).

A sample of 201 well-preserved adult skulls of bottlenose dolphins from different oceanic regions was analyzed. Skulls were considered adults when the specimens' age, assessed through growth layer groups in teeth (Hohn, Scott, Wells, Sweeney, & Irvine, 1989), was higher than 5 years (Barreto, 2016) or had fused bones, closed sutures and closed alveoli (Tavares et al., 2010). A full list of specimens, sex, locality, and museums is available in supplemental online material, Appendix I.

The GM-analyses encompassed five geographic groups for comparisons (Figure 1): Eastern North Pacific Ocean (ENPO) (California, *N* = 30), Western South Atlantic Ocean—*truncatus* (WSAO—t) (Atlantic coast of South America, from Ceará, Brazil to Patagonia, Argentina, *N* = 57), Western South Atlantic Ocean—*gephyreus* (WSAO—g) (Atlantic coast of South America, from Paraná, Brazil to Buenos Aires Province, Argentina *N* = 71), Eastern North Atlantic Ocean (ENAO) (United Kingdom,

FIGURE 1 Distribution of samples used in this study. ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO, Western South Atlantic Ocean, including *gephyreus* and *truncatus* forms. Circles indicate the approximate location where animals were collected and may indicate more than one animal
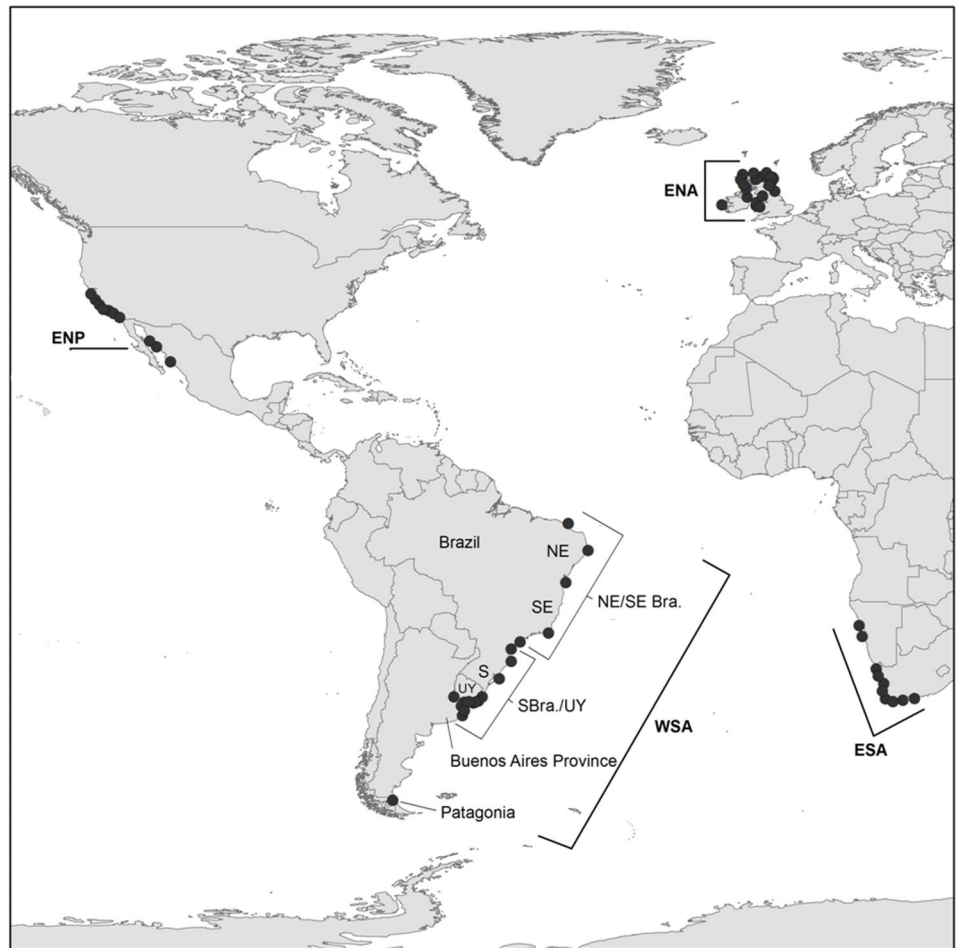
including the holotype of the species *T. truncatus*, referred as *Delphinus truncatus* Montagu 1821, N = 23), and Eastern South Atlantic Ocean (ESAO) (Namibia and South Africa, N = 20).

The skulls were photographed in three different views: dorsal, ventral, and lateral. The camera was fixed in a standard distance (1 m), with a small aperture to increase the depth of field. The rostrum was kept parallel to the ground during the shots of dorsal and ventral views. A total of 201 images of skulls in dorsal view were analyzed, 176 in lateral view, and 153 in ventral view (Table 1). Sample size differences among the views are related to the exclusion of specimens with broken or badly preserved skull structures, or positional problems during the photo acquisition and only detected during image processing.

Landmark selection prioritized the use of type I and II landmarks to describe the skull traits (Bookstein, 1991) and was partially based on Monteiro-Filho et al. (2002) and Sydney et al. (2012). Anatomical nomenclature of the bottlenose dolphin's skull bones follows Mead and Fordyce (2009). Curved structures, as well as hard to mark points (e.g., rostrum outline) were described using semilandmarks. We followed MacLeod (2013) analyzing landmarks and semilandmarks together without any a priori mathematical adjustment. Fifteen landmarks and six semilandmarks were used in the dorsal analysis (Figure 2); sixteen landmarks and six semilandmarks were used in the lateral analysis (Figure 2); and thirty-one landmarks were used to describe the ventral view of the skull (Figure 2). MakeFan6 (Sheets, 2002) was used to create the frames to plot the

TABLE 1 Sample size analyzed of the *Truncatus* groups for the different skull views

Skull view	Groups					
	ENPO	WSAO−t	WSAO−g	ENAO	ESAO	Total
Dorsal	30	57	71	23	20	201
Lateral	26	52	63	19	16	176
Ventral	22	42	58	17	14	153

Abbreviations: ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO−g, Western South Atlantic Ocean—*gephyreus*; WSAO−t, Western South Atlantic Ocean—*truncatus*.
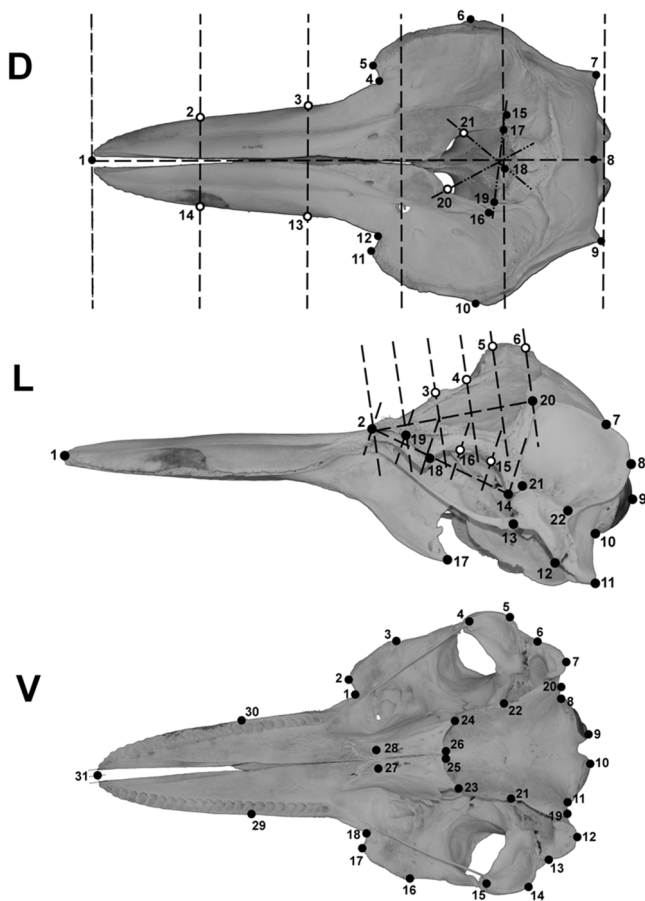
FIGURE 2 Landmarks (full circles) and semilandmarks (open circles) for dorsal (D), lateral (L), and ventral (V) views of _T. truncatus'_ skull. Combs and fans are represented

semilandmarks. Landmarks and semilandmarks were acquired through TPSDig2, version 2.0 (Rohlf, 2004). A full description of landmarks and semilandmarks is presented in supplemental online material, Appendix II.

Skulls of vertebrates have an internal plane of symmetry and this is an example of what Klingenberg, Barluenga, and Meyer (2002) call "object symmetry." However, biological structures are rarely perfectly symmetrical and, therefore, the degree of asymmetry on these features should be addressed through the study of the symmetric and asymmetric components (Klingenberg et al., 2002). Skulls of the Delphinidae family (including _Tursiops_) are left-skewed (Leatherwood & Reeves, 1990; Jefferson & LeDuc, 2018), thus, as in other studies where the analyzed structures are symmetrical objects (e.g., Barros, 2013; Barros et al., 2017; Trevisan, Marochi, Costa, Santos, & Masunari, 2016), we used the symmetrical components to access the general differences among the morphotypes, but the asymmetric components were also considered to better understand the overall variations among the skulls.

The Procrustes correction, that is, orthogonal least square superposition (Rohlf & Slice, 1990), of the raw coordinates was performed in order to adjust the position and size of the specimens. A Principal

Component Analysis (PCA) was used to ordinate the specimens in the morphospace after the Procrustes superimposition and allowed the detection of outliers (Viscosi & Cardini, 2011).

The variation in the size of the different groups was tested using a univariate ANOVA of the log-transformed Centroid Size (logCS) values (Klingenberg, 2011). This analysis was performed after testing for normality (Shapiro–Wilk's test) and homoscedasticity (Levene's test), as well as skewness and kurtosis. The statistical significance (α-level = .05) of the shape differences were tested through Canonical Variate Analysis (CVA) over the "procrusted coordinates" of the symmetrical components. The _P_-values for all the pairwise tests were based on Procrustes distances (Klingenberg, 2011). Furthermore, we regressed shape (i.e., the Procrustes coordinates) on size (logCS) to test the influence of skull size on shape.

Considering the two morphotypes recognized for the South American Coast (_truncatus_ and _gephyreus_), a Discriminant Function Analysis (DFA) with leave-one-out cross-validation among the groups WSAO—t and WSAO—g was performed. This allowed us to obtain the correct-classification percentages of the Procrustes distances and evaluate phenotypic divergence among the groups.

All Geometric Morphometrics methods were performed in MorphoJ (Klingenberg, 2011). Shape changes across the morphotypes observed in the CVA roots were represented using wireframes (Viscosi & Cardini, 2011).

3 | RESULTS

The one-way ANOVA based in the configurations' centroids found significant size differences among _Tursiops_ groups in both dorsal, lateral, and ventral views of the skull (Dorsal—$F_{4,\ 199}$ = 220.8, $p < .0001$; Lateral—$F_{4,\ 174}$ = 16.71, $p < .0001$; Ventral—$F_{4,\ 152}$ = 127.2, $p < .0001$). Tukey's _post hoc_ test revealed that almost all groups vary significantly for size, and the differences are more expressive when observing the dimensions of the dorsal view of the skull (Table 2). In dorsal and ventral view, the _gephyreus_ morphotype (WSAO—g) has the largest skull, while the population representing the Eastern North Atlantic Ocean smaller (Figure 3).

The regression of the Procrustes coordinates on logCS indicated a significant influence of skull size on the shape of the skull in all views (Dorsal—$p < 0.0001$; Lateral—$p < .0001$; and Ventral—$p < .0001$). However, a high percentage of prediction was obtained only for the dorsal view (Dorsal—Total Sums of Squares [TSS] = 0.5306, Predicted = 32.72%; Lateral—TSS = 0.9185, Predicted = 4.24%; Ventral—TSS = 0.3628, Predicted = 12.92%). We thus described the allometric variation in shape only for the dorsal view of the skull.

The wireframes demonstrated that smaller specimens (logCS close to 4.1) with more negative regression scores tend to have a large and robust neurocranium and a robust but short snout, the temporal crest is markedly pronounced, and the distance between the nasal opening and the posterior limit of the neurocranium is long due to the forward position of this trait. Larger specimens (logCS close to 4.6) with more positive regression scores tend to have a narrower skull

TABLE 2 Results of Tukey's *post hoc* test for the size differences between the pairs of the *Truncatus* groups analyzed (dorsal, lateral, and ventral views of the skull), showing the Q values (lower diagonal) and the p values (upper diagonal)

Dorsal view (N = 201)	ENAO	ENPO	ESAO	WSAO—g	WSAO—t
ENAO (N = 23)	–	**<.0001**	**<.0001**	**<.0001**	**<.0001**
ENPO (N = 30)	7.25	–	**<.01**	**<.0001**	**<.0001**
ESAO (N = 20)	12.7	5.451	–	**<.0001**	**<.0001**
WSAO—g (N = 71)	33.19	25.94	20.49	–	**<.0001**
WSAO—t (N = 57)	23.06	15.81	10.36	10.13	–
Lateral view (N = 176)					
ENAO (N = 19)	–	**<.0001**	0.377	**<.05**	0.9993
ENPO (N = 26)	8.03	–	**<.0001**	0.06723	**<.0001**
ESAO (N = 16)	2.54	10.56	–	**<.0001**	0.2528
WSAO—g (N = 63)	4.32	3.7	6.86	–	**<.05**
WSAO—t (N = 52)	0.33	7.7	2.87	3.9	–
Ventral view (N = 153)					
ENAO (N = 17)	–	1	**<.0001**	**<.0001**	**<.0001**
ENPO (N = 22)	7.25	–	**<.0001**	**<.0001**	**<.0001**
ESAO (N = 14)	12.7	5.451	–	**<.0001**	**<.0001**
WSAO—g (N = 58)	33.19	25.94	20.49	–	**<.01**
WSAO—t (N = 42)	23.06	15.81	10.36	10.13	–

Note: Significant values are highlighted in bold.
Abbreviations: ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO—g, Western South Atlantic Ocean—*gephyreus*; WSAO—t, Western South Atlantic Ocean—*truncatus*.

profile, with an elongated and slim rostrum. The posterior portion of the neurocranium is rounded and anteriorly oriented while the nasal opening is posteriorly oriented (Figure 4).

The CVA showed significant statistical shape differences between all analyzed groups in dorsal, lateral, and ventral skull views (*p*-values ranging from <.001 to <.0001). Statistical results of CVA are summarized in Table 3.

The CVA of the GM analysis of the skulls' dorsal view produced four canonical roots. However, the percentages of variation related to CV3 and CV4 were low (less than 5%). Thus, the morphological discrimination between groups was based on both CV1 and CV2. The CV1 (52.61% of the explained variation) indicates differentiation between the skull morphology of the group composed by the *gephyreus* morphotype (WSAO—g) from the one formed by the *truncatus* morphotype. In contrast, the CV2 (38.9%) showed a longitudinal factor that distinguished the skull morphology of geographical groups composed by the *truncatus* morphotype specimens located at the Western South Atlantic Ocean (WSAO-t) from those at the Eastern North and South Atlantic Oceans (ENAO and ESAO). The specimens of the Eastern North Pacific Ocean (ENPO) remained close to what is the mean shape for the observed variation axes (Figure 5).

Considering the geographic groups established, along the CV1 axis, the WSAO—g specimens presented negative scores and the skull pattern associated with these values tends to present a narrower skull profile, marked by a slim rostrum and neurocranium. The back portion of the neurocranium is also forwardly oriented while the nasal opening is posteriorly oriented. The proximal tips of the premaxillae are close to the nasal opening and they do not extend past the posterior border of the nasal opening, which is rounded (Figure 5—see CV1−).

However, almost all specimens from the other geographical groups composed by *truncatus* morphotypes presented positive scores on the CV1. Their skulls seem to be more robust with a shorter and broader rostrum and neurocranium. The temporal crest is markedly pronounced and the distance between the nasal opening and the posterior limit of the neurocranium is long due to the forward position of this trait. The proximal tips of the premaxillae are more laterally deviated from the border of the nasal opening and extend past the posterior limit of the nasal opening, which is not curved (Figure 5—see CV1+).

According to the variation explained by the CV2 axis, all specimens of ENAO and almost all of ESAO exhibited negative scores, which could be translated in a longer and more robust rostrum, and a longitudinally compressed but laterally robust neurocranium, when compared to the other groups. The nasal opening is discrete and with a straight posterior margin. The proximal tips of the premaxillae are close to the nasal opening, and the antorbital maxillary notch is laterally pronounced (Figure 5—see CV2−). The WSAO—t specimens exhibited positive scores in the CV2. In comparison with specimens from the ESAO, their skull morphology seems to be characterized by a relatively narrow rostrum with a longitudinally elongated but laterally narrowed neurocranium. The nasal opening is larger, with the posterior border gently curved. The proximal tips of the premaxillae are anteriorly farther from the nasal opening and the antorbital notch is close to the rostrum and anteriorly oriented (Figure 5—see CV2+).

For the GM analysis for the skulls' lateral view, only the first three CV-roots (CV1, CV2, and CV3) were used. These 3 CV-roots presented higher percentages of variation and were used as the basis to differentiate the groups. As observed in the dorsal view analysis, the
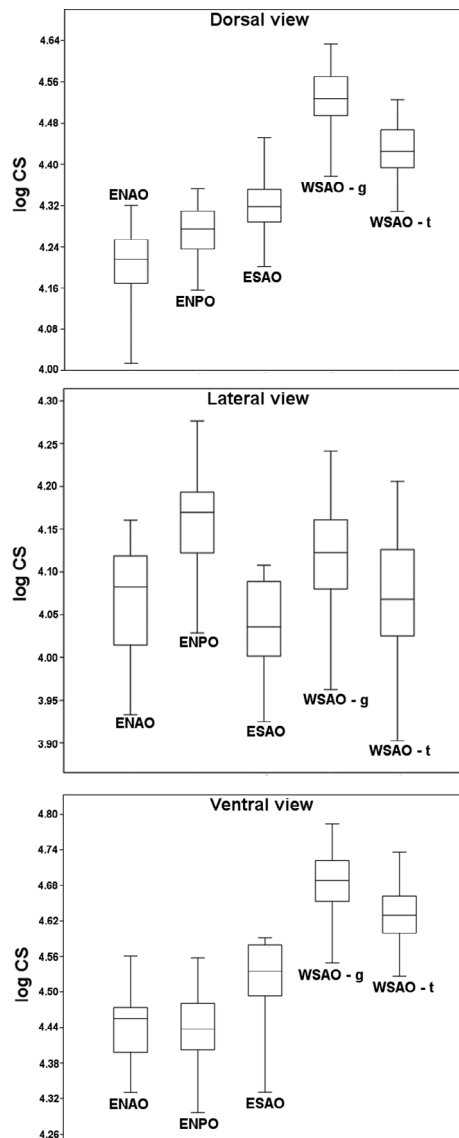
FIGURE 3 Box plot of the log-transformed centroid sizes for the dorsal, lateral, and ventral skull views in five groups of *Tursiops*. Horizontal line = median; box = first interquartile; vertical bar = second interquartile

morphological differences between the individuals denoted by CV1 (60.16% of the variation) are strongly related to the distinction between the *gephyreus* and *truncatus* morphotypes. The CV2 (22.17%) distinguished the *truncatus* morphotype geographical groups, in a longitudinal component within the Atlantic Ocean (i.e., Western from Eastern). The CV3 (11.07%) showed a latitudinal factor segregating specimens from the Eastern North Atlantic and those from the Eastern South Atlantic. The specimens of the Eastern North Pacific Ocean remained close to what is the consensus figure for the observed variation axes (Figure 6).

Specimens located at the negative CV1 scores (WSAO—g) have a larger area occupied by the temporal fossae in the neurocranium, in contrast to the other groups. The pterygoid hamulus is more developed and discretely displaced forward, and the occipital condyle is

elevated and less posteriorly projected. The ventral portion of the lacrimal bone appears to be broad and dorsally oriented (Figure 6—see CV1−). Conversely, the geographical groups formed by *truncatus* morphotypes (i.e., WSAO—t, ENAO, ESAO, and ENPO) have a smaller area occupied in the neurocranium by the temporal fossae. The pterygoid hamulus is developed and discretely backward projected and the occipital condyle is lowered and backward projected. The ventral portion of the lacrimal bone is also broad but ventrally oriented (Figure 6—see CV1+).

The skull variation in the CV2 indicated that the WSAO—t specimens, located at negative scores, have a broader and ventrally oriented lacrimal bone when compared to the other groups. Their temporal fossae seem to be smaller, backward and ventrally oriented and the occipital condyle is elevated and less posteriorly projected. The pterygoid hamulus is short and less projected ventrally, narrowing the distance between the tip of this bone and the basicranium (Figure 6—see CV2−). On the other hand, specimens from Eastern Atlantic Ocean (ENAO and ESAO) have a skull pattern with the rostrum slightly turned downwards, the transition between the rostrum and the neurocranium seems to be flattened except for a frontal "stop" in the neurocranium that causes an abrupt projection of the vertex. The occipital condyle is well pronounced posteriorly, and the pterygoid bone is long and ventrally projected, expanding the distance between the tip of this bone and the basicranium (Figure 6—see CV2+).

North and South groups of the Eastern Atlantic Ocean segregate in the CV3. Specimens from the South (ESAO) exhibited negative scores for this axis. This means, in reference to the other groups, that their skulls have a rostrum slightly turned downwards, the transition between the rostrum and the neurocranium seems to be flattened except for a frontal concavity in the neurocranium that causes a projection of the vertex. The occipital condyle is well pronounced posteriorly and the pterygoid bone is short and posteriorly projected, narrowing the distance between the tip of this bone and the basicranium (Figure 6—see CV3−). Specimens from the ENAO show a skull morphology marked by an accentuated concavity between the rostrum and the neurocranium and, opposed to the specimens from Eastern South Atlantic, they presented a frontal bone projection instead of a depression. Also, in these specimens, the occipital condyle is not markedly projected posteriorly, which is not evident in the other groups. The pterygoid bone is downwardly projected, increasing distance between the tip of this bone and the basicranium (Figure 6—see CV3+).

The CVA of the GM analysis of the skulls' ventral view produced four canonical roots. The percentage of variation related to CV4 was low (nearly 6%), so the morphological comparisons between groups were based on CV1, CV2, and CV3 axes. As observed in the analyses of the dorsal and lateral skull views, the CV1 (62.86% of the explained variation) indicated differentiation between the skull morphology of the geographical groups composed by the *gephyreus* morphotype (WSAO—g) from those of the *truncatus* morphotype. The CV2 (21.08%) showed a longitudinal factor distinguishing the *truncatus* specimens located at the Western South Atlantic Ocean (WSAO-t)

FIGURE 4 Regression scatterplot and wireframes of the deformation related to the values of the log-transformed centroid size. The light grey in the wireframes represents the mean shape for the data set
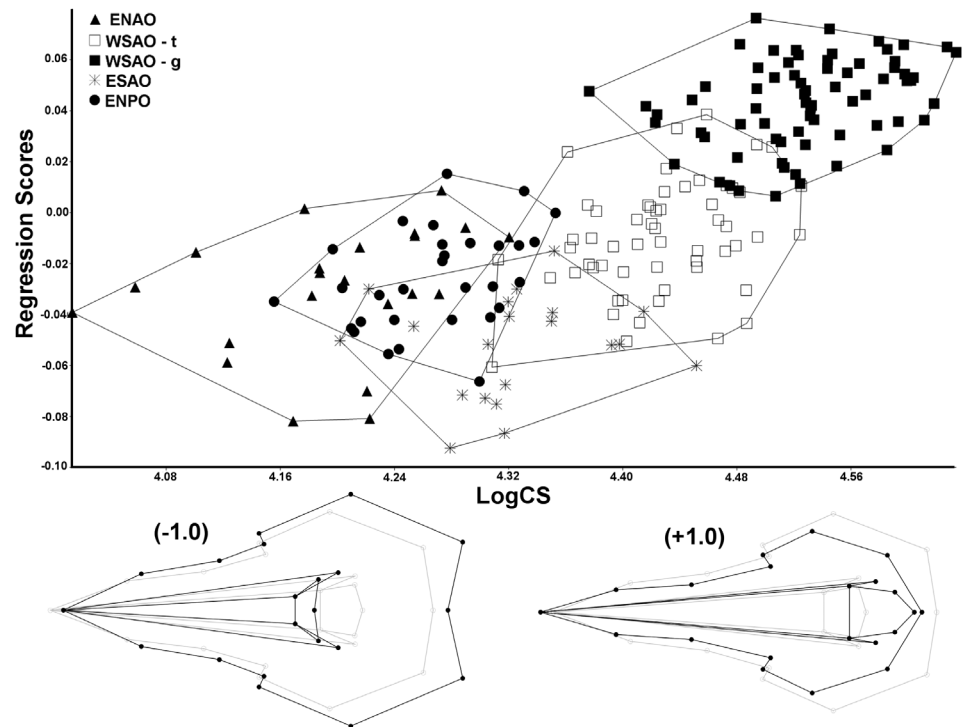

TABLE 3 Results of the CVA for the morphometric variation found between the pairs of the *Truncatus* groups analyzed (dorsal, lateral, and ventral views of the skull), showing the Procrustes distances (lower diagonal) and *p*-values (upper diagonal)

Dorsal view (N = 201)	ENAO	ENPO	ESAO	WSAO−g	WSAO−t
ENAO (N = 23)	–	<.0001	<.001	<.0001	<.0001
ENPO (N = 30)	0.0352	–	<.00001	<.0001	<.0001
ESAO (N = 20)	0.0298	0.0409	–	<.0001	<.0001
WSAO−g (N = 71)	0.0781	0.0716	0.0977	–	<.0001
WSAO−t (N = 57)	0.0467	0.0233	0.0542	0.0605	–
Lateral view (N = 176)					
ENAO (N = 19)	–	<.0001	<.0001	<.0001	<.0001
ENPO (N = 26)	0.0848	–	<.0001	<.0001	<.0001
ESAO (N = 16)	0.0489	0.0677	–	<.0001	<.0001
WSAO−g (N = 63)	0.0591	0.0684	0.0621	–	<.0001
WSAO−t (N = 52)	0.0722	0.0575	0.0817	0.0596	–
Ventral view (N = 153)					
ENAO (N = 17)	–	<.0001	<.0001	<.0001	<.0001
ENPO (N = 22)	0.0549	–	<.0001	<.0001	<.0001
ESAO (N = 14)	0.0734	0.0450	–	<.0001	<.0001
WSAO−g (N = 58)	0.0776	0.0374	0.0639	–	<.0001
WSAO−t (N = 42)	0.0552	0.0359	0.0648	0.0446	–

Note: Significant values are highlighted in bold.
Abbreviations: ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO−g, Western South Atlantic Ocean—*gephyreus*; WSAO−t, Western South Atlantic Ocean—*truncatus*.

from those at the Eastern portion of the Atlantic Ocean (ENAO and ESAO). The CV3 (10.13%) demonstrated a latitudinal factor that distinguishes the North (ENAO) from the South specimens (ESAO) in the Eastern Atlantic Ocean. The specimens of the Eastern North Pacific Ocean (ENPO) remained close to what is the mean shape for the observed variation axes (Figure 7).

Specimens of the *gephyreus* morphotype from the Western South Atlantic Ocean (WSAO−g) exhibited negative CV1 scores denoting a narrower rostrum in comparison to the others. The paraoccipital processes are laterally pronounced, and the tips of the occipital condyles are close to each other and posteriorly projected. The choana opening is elongated and the palatine surface of the pterygoid is short, with
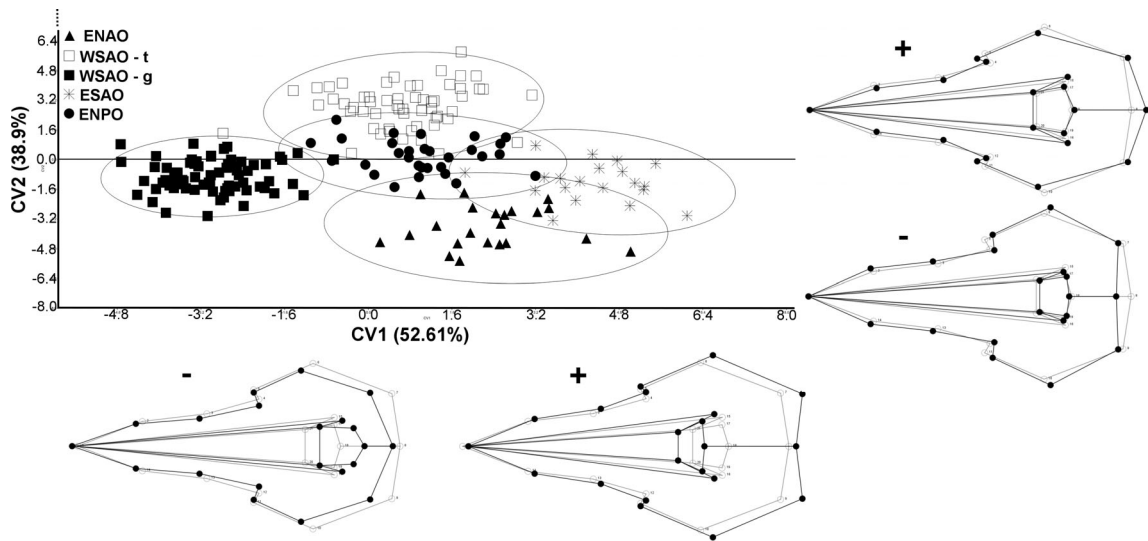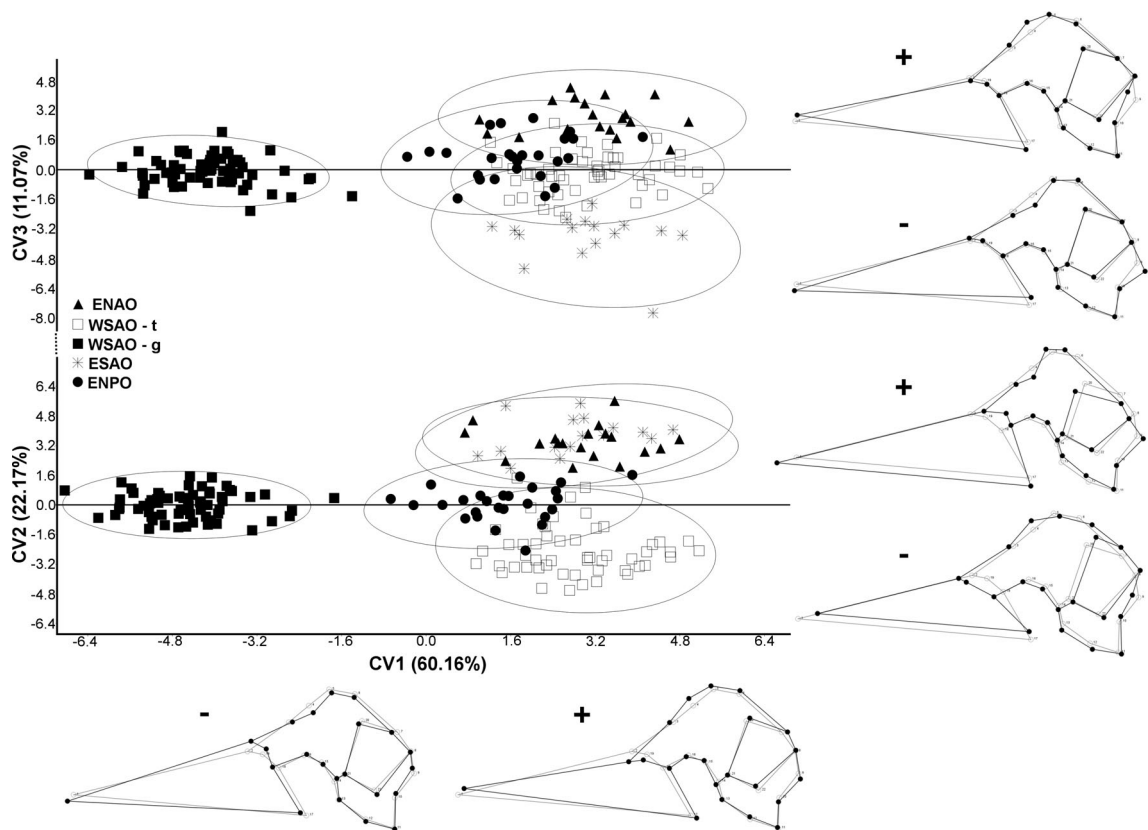
FIGURE 5 CVA scatterplots of the first pair of canonical variates roots (CV1 vs. CV2) for the dorsal skull view, showing the variation in the shape of the *Tursiops* groups through the wireframes, relative to the negative and positive extremes of the CV axes. ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO—g, Western South Atlantic Ocean—*gephyreus*; WSAO—t, Western South Atlantic Ocean—*truncatus*

FIGURE 6 CVA scatterplots of the first two pairs of canonical variates roots (CV1 vs. CV2 and CV1 vs. CV3) for the lateral skull view, showing the variation in the shape of the *Tursiops* groups through the wireframes, relative to the negative and positive extremes of the CV axes. ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO—g, Western South Atlantic Ocean—*gephyreus*; WSAO—t, Western South Atlantic Ocean—*truncatus*

FIGURE 7 CVA scatterplots of the first two pairs of canonical variates roots (CV1 vs. CV2 and CV1 vs. CV3) for the ventral skull view, showing the variation in the shape of the *Tursiops* groups through the wireframes, relative to the negative and positive extremes of the CV axes. ENAO, Eastern North Atlantic Ocean; ENPO, Eastern North Pacific Ocean; ESAO, Eastern South Atlantic Ocean; WSAO—g, Western South Atlantic Ocean—*gephyreus*; WSAO—t, Western South Atlantic Ocean—*truncatus*
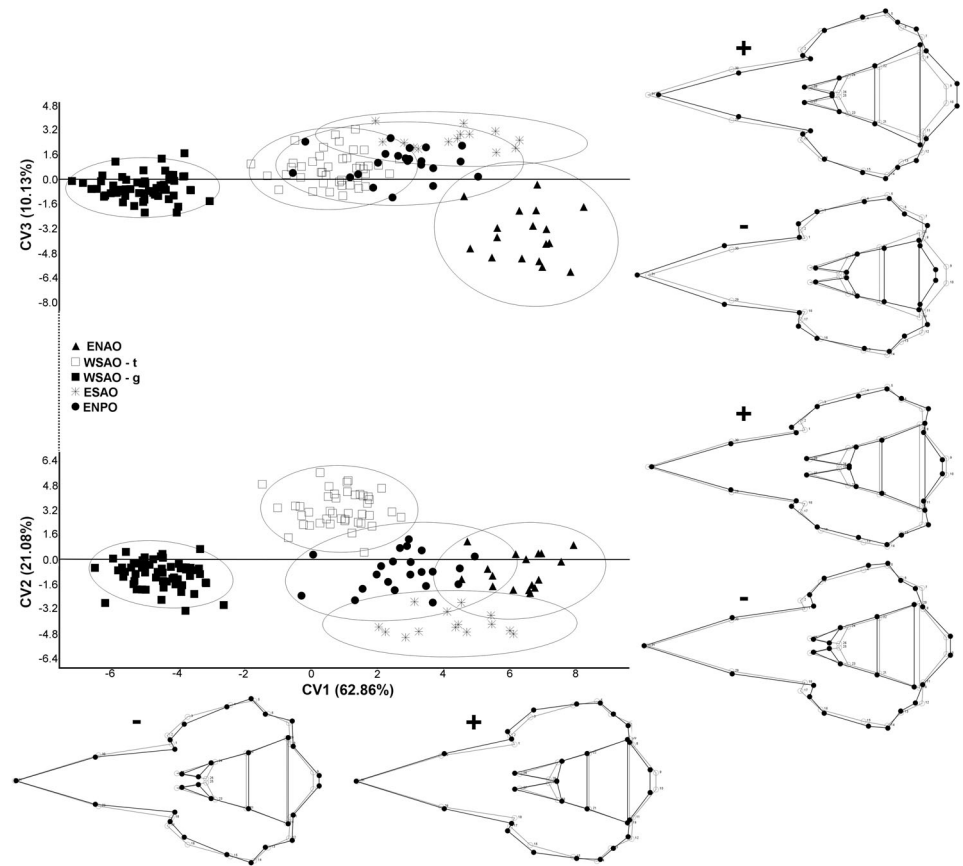
the pterygoid tips transversely separated from each other (Figure 7—see CV1−). The geographical groups composed by the *truncatus* morphotype tended to have positive CV1 scores. This denotes that they have a broader rostrum and neurocranium across the ventrolateral crest, occipital condyles more apart from each other and anteriorly positioned, an elongated palate roof and reduced interpterygoid space as main distinguishing features from the *truncatus* morphotype (Figure 7—see CV1+).

Individuals from the Eastern Atlantic Ocean (ENAO and ESAO) have negative CV2 scores and this denotes a broader rostrum and neurocranium across the ventrolateral crest, occipital condyles more apart from each other and projected posteriorly, a reduced palate roof and interpterygoid space (Figure 7—see CV2−). In opposition, *truncatus* specimens from the Western Atlantic Ocean (WSAO—t) exhibit positive CV2 scores denoting a narrower rostrum. The landmarks of the ventrolateral crests tend to align, suggesting a more flattened shape of the anterior portion of the ventral neurocranium. The paraoccipital processes are more laterally pronounced, and the tips of the occipital condyle are discreetly close to each other and anteriorly positioned. The choana opening is shortened, the palatine surface of the pterygoid is elongated, and the pterygoid tips are closer to each other (Figure 7—see CV2+).

Specimens of the Eastern North Atlantic Ocean (ENAO) have negative CV3 scores presenting a more elongated and broader rostrum, a developed palatine surface of the pterygoid and its hamulus, marked posteriorly projection of the paraoccipital processes

and reduction of the occipital condyles. The basicranium bones are longitudinally and laterally shortened (Figure 7—see CV3−). However, southern specimens (ESAO) have positive CV3 scores denoting the longitudinal and lateral hypertrophy of the basicranium bones, reduction of the palatine surface of the pterygoid, and robust and posteriorly projected occipital condyles. The rostrum seems to be relatively short and narrow. The paraoccipital processes are laterally and anteriorly positioned (Figure 7—see CV3+).

The cross-validation percentage from the Discriminant Function Analysis performed between WSAO—t and WSAO—g showed that all animals, independent of the area where they were collected, were easily separated in *truncatus* or *gephyreus* morphotypes, with more than 98% of certainty (Table 4).

TABLE 4 Discriminant Function Analysis (DFA) results based on Procrustes Distances followed by cross-validation percentage of species morphotypes analyzed (gephyreus and truncatus groups) in the West South Atlantic Ocean (South American Coast) for different cranial orientations

Skull view	DFA *p*-values	Cross-validation (%)
Dorsal	**<.0001**	98–100
Lateral	**<.0001**	100–100
Ventral	**<.0001**	98–100

Note: Significant values are highlighted in bold.

4 | DISCUSSION

4.1 | Geographic variation

Bottlenose dolphins are distributed globally and are known to exhibit morphological variations between the different regions where they occur. Morphotypes (or sub-species and species) identified in the Eastern Pacific, Western Atlantic, Black Sea, and Indian oceans, as well as in southern Australia, differ in coloration, body dimensions, skull structures and genetic markers (Barreto, 2000; Charlton-Robb et al., 2011; Fruet et al., 2017; Ross & Cockcroft, 1990; Simões-Lopes & Daura-Jorge, 2008; Viaud-Martinez, Brownell Jr, Komnenou, & Bohonak, 2008; Walker, 1981; Wickert, 2013; Wickert et al., 2016). Furthermore, within specific regions (e.g., Atlantic and Pacific coasts of the USA, coast of Peru) identification of different morphotypes, usually related to coastal and pelagic environments have also been identified (Costa et al., 2016; Hoelzel, Potter, & Best, 1998; Mead & Potter, 1995; Ngqulana, Pistorius, Galatius, Plön, & Hofmeyr, 2019; Perrin et al., 2011; Waerebeek et al., 1990). However, until now all studies focused on comparisons within specific areas, without comparisons between oceanic basins. The only trans-oceanic study that has been published dealt with genetic differences, and observed that coastal populations have less genetic variability and, in most cases, were significantly different from pelagic groups (Natoli, Peddemors, & Hoelzel, 2004).

In the present study, we describe skull variations in *Tursiops* along its geographic distribution. The skulls, when analyzed in dorsal, ventral and lateral views, indicated a clear differentiation between the groups, in a pattern similar to what was observed by Natoli et al. (2004) on the molecular level. Our results indicate a clear distinction between individuals that inhabit the Atlantic and Pacific oceans. Additionally, within the Atlantic Ocean, there is an evident differentiation of specimens from the eastern (United Kingdom + South Africa/Namibia) and western (South America) regions. A latitudinal separation was also observed, considering specimens from the North (United Kingdom) and South (South Africa/Namibia) Atlantic Ocean.

We analyzed specimens that for the most part were stranded animals, accumulated over time in museum collections. Therefore, it was not possible to ascertain if they were from coastal or oceanic populations if these existed in that area. However, it can be assumed that each collection should have specimens from oceanic and coastal animals. Thus, the variation observed in this work probably reflects the aggregated variability of bottlenose dolphins in each sampled region.

It is possible that the separation detected between east/west and north/south animals may be related to the existence of differences between coastal and oceanic morphotypes. In South Africa, there are two species of bottlenose dolphins, the Common bottlenose dolphin, *T. truncatus*, and the Indo-Pacific bottlenose dolphin, *T. aduncus*, with the former occupying areas further offshore than the latter, which typically inhabits coastal environments (Hale et al., 2000; Ross, 1977; Wang, 2018). In Scottish waters, where most of the specimens from the United Kingdom used in this study are from, there are also bottlenose dolphins in coastal and oceanic environments (Cheney et al., 2013). In the Atlantic coast of South America, there are also bottlenose dolphins in both environments, and it has been proposed that the *truncatus* morphotype is more pelagic when compared with the *gephyreus* morphotype that apparently has a restricted coastal distribution (Barreto, 2000; Costa et al., 2016; Wickert et al., 2016). Therefore, except for South African and Southwestern Atlantic specimens, it is unknown if the specimens analyzed were from coastal or pelagic populations, and this might have contributed to the observed differences. However, as stated before, the approach used here allowed to evaluate the cumulative variability of bottlenose dolphins in each region, indicating overall differentiation between large areas.

4.2 | Status of *Tursiops gephyreus* Lahille, 1908

In South America's Atlantic coast, the taxonomic status of bottlenose dolphins has been under discussion since the 1970s. However, only in the early 2000's more in-depth studies on skull morphology indicated that two morphologically distinct forms existed and that they deserved taxonomic differentiation (Barreto, 2000, 2004). Recently, other studies used a larger set of samples, confirming the previously observed differences and the need to taxonomically separate the two distinct lineages (Costa et al., 2016; Wickert et al., 2016).

One of the characters pointed out in previous works that differentiated the two morphological groups, and corroborated in the present analysis, is skull size, with animals from the *gephyreus* group being larger. The results reported here also support previous descriptive analyses, that identified the ascending process of the right premaxilla as a diagnostic character for *T. gephyreus* (Wickert, 2013; Wickert et al., 2016). These works identified that in this species the right premaxilla has a falciform shape, reaching the posterior margin of the external bony nares, while in *T. truncatus* the premaxilla is straighter, projecting posteriorly from the margin of the narial opening. The same characteristic was observed in the present study for animals in the *truncatus* and *gephyreus* groups (Figure 8).

This study and previous studies were performed with skulls that were collected in its majority from stranded animals. The distribution of specimens from both morphotypes suggests a distribution of the *gephyreus* group south of Paraná State in Brazil, approximately at 25.6°S, and for the *truncatus* group strandings are common north of central Rio Grande do Sul State, at 31.0°S. Therefore, in the area between 25.6°S and 31.0°S, both morphotypes probably occur in sympatry, or at least parapatry if there is any longitudinal separation in their distribution (Costa et al., 2016). However, analysis of photo-identified dolphins along the coast of southern and southeastern Brazil observed a strong differentiation in dorsal fin shapes and coloration of both morphotypes in the field, reporting that both forms were not seen together (Simões-Lopes et al., 2019). The latter authors prefer to use the term "ecotype" to separate the two groups, and identified recognizably distinct distribution patterns between them, but with an area of overlap in their distribution, along the shallow and nearshore waters of the southern Brazilian coast. Thus, if interbreeding was
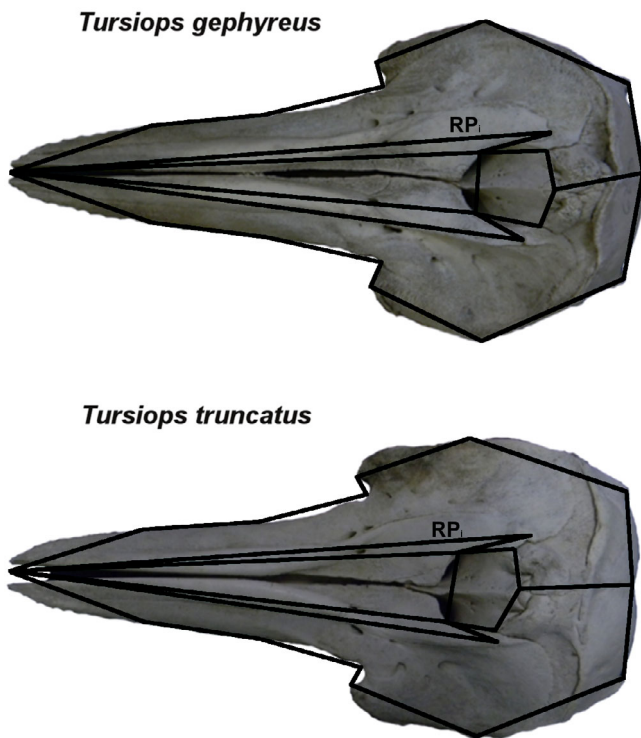
FIGURE 8 Skull morphology of *Tursiops gephyreus* and *Tursiops truncatus* correlated with the wireframe (black lines) representing the general shape deformation. Note that in *T. gephyreus* the right premaxilla (RP) has a falciform shape, reaching the posterior margin of the external bony nares, while in *T. truncatus* the premaxilla is straighter, projecting posteriorly from the margin of the narial opening

occurring between the two morphotypes, at least in this area specimens should exhibit intermediate characters. However, all animals, independent of the area where they were collected, were easily separated in the two morphological groups by the Discriminant Function Analysis, with more than 98% of certainty.

The results reported here from the analysis of the two morphotypes along South America's Atlantic Coast as well with other oceanic regions support the separation of animals from Southern Brazil, Uruguay, and Argentina (WSAO—g, *T. gephyreus*) from specimens of NE/SE/S Brazil (WSAO—t, *T. truncatus*). The present study is the most comprehensive morphometrical approach to bottlenose dolphins' skulls hitherto, providing morphological support to consider these two lineages as separate species (Wickert et al., 2016).

Moreover, the study performed by Fruet et al. (2017) using both nuclear (microsatellite) and mitochondrial molecular markers from specimens from the SWA found minimal connectivity between lineages (both current and historical), suggesting they are following discrete evolutionary trajectories. More recently, de Oliveira et al. (2019) also separated bottlenose dolphins from WSAO in two "biological units," northern and southern. The northern unit seems to occur in a wide range of depths, including offshore waters and is consistent with the *T. truncatus* morphology. The southern unit was described as

having a coastal distribution, occurring in very shallow waters and estuaries, and being consistent with the previous description of *T. gephyreus*. The results from the present work, not only corroborates the differences within the WSAO observed in previous works with different methodologies, but also compares with specimens from more distant areas, making it clear that *Tursiops gephyreus*, Lahille, 1908 should be recognized with full species status as proposed by Lahille in 1908.

The recognition of *Tursiops gephyreus* as a full species also has implications for conservation, because this lineage is undergoing a strong population decline in Argentina (Coscarella, Dans, Degrati, Garaffo, & Crespo, 2012) and facing several threats from anthropogenic activities in Southern Brazil (see Fruet et al., 2016). This is particularly serious because *T. gephyreus*, has a more restricted pattern of occurrence, limited distribution (only occurs in coastal waters of Southern Brazil, Uruguay, and Central Argentina; Wickert et al., 2016), small population size (Coscarella et al., 2012; Vermeulen & Bräger, 2015) and much lower genetic diversity (Fruet et al., 2017). The list of the endangered fauna of Rio Grande do Sul State in Brazil, has been updated in 2014 according to IUCN criteria and the coastal-estuarine population, referred herein to *T. gephyreus* was classified as "vulnerable" due to high anthropogenic pressure and declining habitat quality (Rio Grande do Sul, 2014). Moreover, the IUCN *Red List of Threatened Species* 2019 added the Lahille's Bottlenose dolphin as Vulnerable (VU—D1) in the global assessment (Vermeulen, Fruet, Costa, Coscarella, & Laporta, 2019). In conclusion, independent of its taxonomic status, it is essential that conservation strategies should be implemented quickly to avoid that this discrete evolutionary lineage of *Tursiops* from the SWA becomes extinct in the near future since there are no more than 400 mature individuals of Lahille's bottlenose dolphin in SWA.

AUTHOR CONTRIBUTIONS

Fernando Sicuro: Conceptualization; data curation; formal analysis; investigation; methodology; supervision; validation; visualization. **Janaína Wickert:** Data curation; investigation; validation; visualization. **Ignacio Moreno:** Conceptualization; data curation; investigation; validation; visualization; writing-original draft. **Oscar Rocha-Barbosa:** Conceptualization; supervision; validation; visualization. **André Barreto:** Conceptualization; data curation; investigation; validation; visualization; writing-original draft; writing-review and editing.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

Amaral, A. R., Coelho, M. M., Marugán-Lobón, J., & Rohlf, F. J. (2009). Cranial shape differentiation in three closely related delphinid cetacean species: Insights into evolutionary history. *Zoology*, *112*, 38–47.

Barreto, A. S. (2000). *Variação craniana e genética de Tursiops truncatus (Delphinidae, Cetacea) na costa Atlântica da América do Sul*. PhD thesis. Brasil: Fundação Universidade Federal do Rio Grande at Rio Grande do Sul.

Barreto, A. S. (2004). *Tursiops* in Atlantic South America: Is *Tursiops gephyreus* a valid species? *Proceedings of the Symposium on Cetacean Systematics*, La Jolla, 28–29 April 2004.

Barreto, A. S. (2016). Growth of the skull of the bottlenose dolphin, *Tursiops truncatus*, in the Southwestern Atlantic. *Latin American Journal of Aquatic Mammals*, *11*, 199–211.

Barros, H. M. D. R. (2013). *Variação geográfica de Trichechidae (Mammalia: Sirenia): Análise morfométrica e citogenética*. DSc thesis. Pernambuco, Brazil: Universidade Federal de Pernambuco.

Barros, H. M. D. R., Meirelles, A. C., Luna, F. O., Marmontel, M., Cordeiro-Estrela, P., Santos, N., & Astúa, D. (2017). Cranial and chromosomal geographic variation in manatees (Mammalia: Sirenia: Trichechidae) with the description of the Antillean manatee karyotype in Brazil. *Journal of Zoological Systematics and Evolution Research*, *55*, 73–87.

Bookstein, F. L. (1991). *Morphometric tools for landmark data: Geometry and biology*. Cambridge, UK: Cambridge University Press.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: Morphometrics of group differences in outline shape. *Medical Image Analysis*, *1*, 225–243.

Charlton-Robb, K., Gershwin, L. A., Thompson, R., Austin, J., Owen, K., & McKechnie, S. (2011). A new dolphin species, the burrunan dolphin *Tursiops australis* sp. nov., endemic to southern Australian coastal waters. *PLoS One*, *6*, e24047.

Cheney, B., Thompson, P. M., Ingram, S. N., Hammond, P. S., Stevick, P. T., Durban, J. W., ... Quick, N. J. (2013). Integrating multiple data sources to assess the distribution and abundance of bottlenose dolphins *Tursiops truncatus* in Scottish waters. *Mammal Review*, *43*, 71–88.

Coscarella, M. A., Dans, S. L., Degrati, M., Garaffo, G. V., & Crespo, E. A. (2012). Bottlenose dolphins at the southern extreme of the southwestern Atlantic: Local population decline? *Journal of the Marine Biological Association of the United Kingdom*, *92*, 1843–1849.

Costa, A. P., Rosel, P. E., Daura-Jorge, F. G., & Simões-Lopes, P. C. (2016). Offshore and coastal common bottlenose dolphins of the western South Atlantic face-to-face: What the skull and the spine can tell us. *Marine Mammal Science*, *32*, 1433–1457.

de Oliveira, L. R., Fraga, L. D., Ott, P. H., Siciliano, S., Lopes, F., Almeida, R., ... Bonatto, S. L. (2019). Population structure, phylogeography, and genetic diversity of the common bottlenose dolphin in the tropical and subtropical southwestern Atlantic Ocean. *Journal of Mammalogy*, *100*, 564–577.

del Castillo, D. L., Flores, D. A., & Cappozzo, H. L. (2014). Ontogenetic development and sexual dimorphism of franciscana dolphin skull: A 3D geometric morphometric approach. *Journal of Morphology*, *275*, 1366–1375.

del Castillo, D. L., Segura, V., Flores, D. A., & Cappozzo, H. L. (2016). Cranial development and directional asymmetry in Commerson's dolphin, *Cephalorhynchus commersonii* commersonii: 3D geometric morphometric approach. *Journal of Mammalogy*, *97*, 1345–1354.

del Castillo, D. L., Viglino, M., Flores, D. A., & Cappozzo, H. L. (2017). Skull ontogeny and modularity in two species of Lagenorhynchus: Morphological and ecological implications. *Journal of Morphology*, *278*, 203–214.

Di Tullio, J. C., Gandra, T. B. R., Zerbini, A. N., & Secchi, E. R. (2016). Diversity and distribution patterns of cetaceans in the subtropical southwestern Atlantic outer continental shelf and slope. *PLoS One*, *11*, e0155841.

Fruet, P. F., Secchi, E. R., Di Tullio, C. J., & Kinas, P. G. (2011). Abundance of bottlenose dolphins, *Tursiops truncatus* (Cetacea: Delphinidae), inhabiting the Patos Lagoon estuary, southern Brazil: Implications for conservation. *Zoologia (Curitiba)*, *28*, 23–30.

Fruet, P. F., Secchi, E. R., Di Tullio, J. C., Simões-Lopes, P. C., Daura-Jorge, F., Costa, A. P. B., ... Möller, L. M. (2017). Genetic divergence between two phenotypically distinct bottlenose dolphin ecotypes suggests separate evolutionary trajectories. *Ecology and Evolution*, *7*, 9131–9143.

Fruet, P. F., Zappes, C. A., Bisi, T. L., Simões-Lopes, P. C., Laporta, P., Loureiro, J. D., & Flores, P. A. C. (2016). Report of the working group on interactions between humans and *Tursiops truncatus* in the Southwest Atlantic Ocean. *Latin American Journal of Aquatic Mammals*, *11*, 79–98.

Goodall, R., Dellabianca, N., Boy, C., Benegas, L., Pimper, L., & Riccialdelli, L. (2008). Review of small cetaceans stranded or incidentally captured on the coasts of Tierra del Fuego, Argentina, over 33 years. *IWC Paper Scientific Committee Document* SC/60/SM21.

Goodall, R. N. P., Marchesi, M. C., Pimper, L. E., Dellabianca, N., Benegas, L. G., Torres, M. A., & Riccialdelli, L. (2011). Southernmost records of bottlenose dolphins, *Tursiops truncatus*. *Polar Biology*, *34*, 1085–1090.

Gunz, P., Mitteroeker, P., & Bookstein, F. L. (2005). *Semilandmarks in three dimensions* (p. 73–98). In D. E. Slice (Ed.), *Modern Morphometrics in physical anthropology*. Chicago, IL: University of Chicago Press.

Hale, P. T., Barreto, A. S., & Ross, G. J. B. (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.

Hershkovitz, P. (1966). *Catalog of living whales*. Washington, DC: Smithsonian Institution, US National Museum Bulletin, Government Printing Office.

Hoelzel, A. R., Potter, C. W., & Best, P. B. (1998). Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society of London B: Biological Sciences*, *265*, 1177–1183.

Hohn, A. A., Scott, M. D., Wells, R. S., Sweeney, J. C., & Irvine, A. B. (1989). Growth layers in teeth from known-age, free-ranging bottlenose dolphins. *Marine Mammal Science*, *5*, 315–342.

IWC—International Whaling Commission. (1995). Report of the Scientific Committee. Annex G. Report of the sub-committee on small cetaceans. *RIWC*, *45*, 165–186.

IWC—International Whaling Commission. (2009). Report of the Scientific Committee. *Journal of Cetacean Research and Management*, *11*(Suppl. 2), 1–78.

IWC—International Whaling Commission. (2019). Report of the Scientific Committee. *Journal of Cetacean Research and Management*, *20*(Suppl.), 1–78.

Jefferson, T. A., Leatherwood, S., & Webber, M. A. (1993). *Marine mammals of the world*. Rome: Food & Agriculture Org.

Jefferson, T. A., & LeDuc, R. (2018). Delphinids, overview. *Encyclopedia of Marine Mammals*, 242–246. https://doi.org/10.1016/b978-0-12-804327-1.00101-1

Jefferson, T. A., & Waerebeek, K. (2004). Geographic variation in skull morphology of humpback dolphins (*Sousa* spp.). *Aquatic Mammals*, *30*, 3–17.

Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources*, *11*, 353–357.

Klingenberg, C. P., Barluenga, M., & Meyer, A. (2002). Shape analysis of symmetric structures: Quantifying variation among individuals and asymmetry. *Evolution*, *56*, 1909–1920.

Lahille, F. (1908). Nota sobre un delfín. *Anales del Museo Nacional de Buenos Aires*, *16*, 347–367.

Leatherwood, S., & Reeves, R. R. (1990). *The bottlenose dolphin*. San Diego, CA: Academic Press.

Lodi, L., Domit, C., Laporta, P., Tullio, J. C. D., Martins, C. C. A., & Vermeulen, E. (2016). Report of the working group on the distribution of *Tursiops truncatus* in the Southwest Atlantic Ocean. *Latin American Journal of Aquatic Mammals*, *11*, 29–46.

MacLeod, N. (2013). Landmarks and semilandmarks: Differences without meaning and meaning without difference. *Palaeontology Association News*, *82*, 32–43.

Mead, J. G., & Fordyce, R. E. (2009). The therian skull: A lexicon with emphasis on the odontocetes. *Smithsonian Contributions to Zoology*, *627*, 1–248.

Mead, J. G., & Potter, C. W. (1995). Recognizing two populations of the Bottlenose Dolphin (*Tursiops truncatus*) of the Atlantic Coast of North America—Morphologic and ecologic considerations. *International Marine Biology Research Institute*, *5*, 31–44.

Möller, L. M., & Beheregaray, L. B. (2001). Coastal bottlenose dolphins from southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. *Marine Mammal Science*, *17*, 249–263.

Monteiro-Filho, E. L. D. A., Monteiro, L. R., & Reis, S. F. (2002). Skull shape and size divergence in dolphins of the genus *Sotalia*: A tridimensional morphometric analysis. *Journal of Mammalogy*, *83*, 125–134.

Moreno, I. B., Ott, P. H., Tavares, M., Oliveira, L. R. D., Danilewicz, D., Siciliano, S., & Bonnato, S. L. (2009). *Os cetáceos com ênfase no golfinho-nariz-de-garrafa, Tursiops truncatus (Montagu, 1821)*. In D. D. L. Viana (Ed.), *O Arquipélago de São Pedro e São Paulo: 10 anos de Estação Científica* (pp. 286–292). SECIRM: Brasília, DF.

Moreno, I. B., do Amaral, K. B.; de Camargo, Y. R., Dorneles, D. R., Frainer, G., Heissler, V. L., … Wickert, J. C. (2017). *Cetáceos da Ilha da Trindade e Arquipélago de Martin Vaz*. In S. C. Abrantes (Ed.), *Protrindade: Programa de pesquisas científicas na Ilha da Trindade. 10 anos de pesquisas*. Brasília, DF: SECIRM.

Natoli, A., Peddemors, V. M., & Hoelzel, A. R. (2004). Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology*, *17*, 363–375.

Ngqulana, S. G., Pistorius, P., Galatius, A., Plön, S., & Hofmeyr, G. G. (2019). Variation in cranial morphology of bottlenose dolphins (genus *Tursiops*) off South Africa. *Marine Mammal Science*, *35*, 617–636.

Odell, D. K., & Asper, E. D. (1990). Distribution and movements of freeze-branded bottlenose dolphins in the Indian and Banana Rivers, Florida. In S. Leatherwood & R. R. Reeves (Eds.), *The bottlenose dolphin* (pp. 515–540). San Diego, CA: Academic Press.

Pardo, M. A., Mejía-Fajardo, A., Beltrán-Pedreros, S., Trujillo, F., Kerr, I., & Palacios, D. M. (2009). Odontocete sightings collected during offshore cruises in the western and southwestern Caribbean Sea. *Latin American Journal of Aquatic Mammals*, *7*, 57–62.

Parés-Casanova, P. M., & Fabre, L. (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., Thieleking, J. L., Walker, W. A., Archer, F. I., & Robertson, K. M. (2011). Common bottlenose dolphins (*Tursiops truncatus*) in California waters: Cranial differentiation of coastal and offshore ecotypes. *Marine Mammal Science*, *27*, 769–792.

Pinedo, M. C., Rosas, F. C. W., & Marmontel, M. (1992). *Cetáceos e pinípedes do Brasil: Uma revisão dos registros e guia para identificação das espécies*. Manaus, AM: UNEP/FUA.

Rio Grande do Sul (2014). Decreto 51.797, "Declara as Espécies da Fauna Silvestre Ameaçadas de Extinção no Estado do Rio Grande do Sul". State decree, published September 8th, 2014. Available at http://www.al.rs.gov.br/filerepository/repLegis/arquivos/DEC%2051.797.pdf

Rohlf, F. J. (2004). *tpsDig, digitize landmarks and outlines. Ver. 2.0*. Stony Brook: Department of Ecology and Evolution, State University of New York.

Rohlf, F. J., & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, *39*, 40–59.

Rohlf, J. F., & Marcus, L. F. (1993). A revolution in morphometrics. *Trends in Ecology & Evolution*, *8*, 129–132.

Ross, G. J. B. (1977). The taxonomy of bottlenosed dolphins *Tursiops* species in South Africa waters, with notes on their biology. *Annals of the Cape provincial Museums. Natural History*, *11*, 135–194.

Ross, G. J. B., & Cockcroft, V. G. (1990). *Comments on Australian bottlenose dolphins and the taxonomic status of Tursiops aduncus (Ehrenberg, 1832)*. In S. Leatherwood & R. R. Reeves (Eds.), *The bottlenose dolphin* (pp. 101–128). San Diego, CA: Academic Press.

Sheets, H. D. (2002). *IMP software series: Computer software and manual*. Buffalo, NY: Canisius College.

Siciliano, S., Moreno, I. B., Silva, E. D., & Alves, V. C. (2006). *Baleias, botos e golfinhos da Bacia de Campos*. Rio de Janeiro, RJ: ENSP/FIOCRUZ.

Simões-Lopes, P., Daura-Jorge, F., Lodi, L., Bezamat, C., Costa, A., & Wedekin, L. (2019). Bottlenose dolphin ecotypes of the western South Atlantic: The puzzle of habitats, coloration patterns and dorsal fin shapes. *Aquatic Biology*, *28*, 101–111.

Simões-Lopes, P. C., & Daura-Jorge, F. G. (2008). *Os parceiros da sobrevivência: A interação entre botos e pescadores no sul do Brasil*. Florianópolis, SC: Editora Insular.

Simões-Lopes, P. C., & Fabian, M. E. (1999). Residence patterns and site fidelity in bottlenose dolphins, *Tursiops truncatus* (Montagu) (Cetacea, Delphinidae) off Southern Brazil. *Revista Brasileira de Zoologia*, *16*, 1017–1024.

Sydney, N. V. (2010). *Ontogenia e assimetria craniana do boto-cinza, Sotalia guianensis (Cetacea: Delphinidae)*. MSc thesis. São Paulo, Brasil: Universidade de São Paulo at.

Sydney, N. V., Machado, F. A., & Hingst-Zaher, E. (2012). Timing of ontogenetic changes of two cranial regions in *Sotalia guianensis* (Delphinidae). *Mammalian Biology—Zeitschrift für Säugetierkunde*, *77*, 397–403.

Tavares, M., Moreno, I. B., Siciliano, S., Rodriguez, D., Santos, M. C. O., Lailson-Brito, J., & Jr Fabián, M. E. (2010). Biogeography of common dolphins (genus *Delphinus*) in the Southwestern Atlantic Ocean. *Mammal Review*, *40*, 40–64.

Trevisan, A., Marochi, M. Z., Costa, M., Santos, S., & Masunari, S. (2016). Effects of the evolution of the Serra do Mar mountains on the shape of the geographically isolated populations of *Aegla schmitti* Hobbs III, 1979 (Decapoda: Anomura). *Acta Zoológica*, *97*, 34–41.

Vermeulen, E., & Bräger, S. (2015). Demographics of the disappearing bottlenose dolphin in Argentina: A common species on its way out? *PLoS One*, *10*, e0119182.

Vermeulen, E., Fruet, P., Costa, A., Coscarella, M., & Laporta, P. (2019). *Tursiops truncatus ssp. gephyreus. The IUCN Red List of Threatened Species* 2019: e.T134822416A135190824. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T134822416A135190824.en.

Viaud-Martinez, K. A., Brownell, R. L., Jr., Komnenou, A., & Bohonak, A. J. (2008). Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. *Biological Conservation*, *141*(6), 1600–1611.

Viscosi, V., & Cardini, A. (2011). Leaf morphology, taxonomy and geometric morphometrics: A simplified protocol for beginners. *PLoS One, 6,* e25630.

Waerebeek, K. V., Reyes, J. C., Read, A. J., & Mckinnon, J. S. (1990). *Preliminary observations of bottlenose dolphins from the Pacific coast of South America* (p. 143–154). In S. Leatherwood & R. R. Reeves (Eds.), *The bottlenose dolphin*. San Diego, CA: Academic Press.

Walker, W. A. (1981). Geographical variation in morphology and biology of bottlenose dolphins (*Tursiops*) in the eastern North Pacific. National Marine Fisheries Service Southwest Fisheries Center Administrative Report No.LJ-81-03C (unpublished).

Wang, J. Y. (2018). Bottlenose Dolphin, *Tursiops aduncus*, Indo-Pacific Bottlenose Dolphin. *Encyclopedia of Marine Mammals,* 125–130.

Wang, J. Y., Chou, L. S., & White, B. N. (1999). Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters. *Molecular Ecology, 8,* 1603–1612.

Wang, J. Y., Chou, L. S., & White, B. N. (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., Chou, L. S., & White, B. N. (2000b). Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Zoology, 252,* 147–162.

Wells, R. S., & Scott, M. D. (2018). Bottlenose dolphin, *Tursiops truncatus*, common bottlenose dolphin. *Encyclopedia of Marine Mammals,* 118–125. https://doi.org/10.1016/b978-0-12-804327-1.00072-8

Wickert, J. C. (2013). Tursiops no Oceano Atlântico Sul Ocidental: Redescrição e revalidação de *Tursiops gephyreus* Lahille, 1908 (Cetartiodactyla: Delphinidae). In *MSc thesis*. Brazil: Universidade Federal do Rio Grande do Sul at Rio Grande do Sul. Available at http://hdl.handle.net/10183/183391

Wickert, J. C., von Eye, S. M., Oliveira, L. R., & Moreno, I. B. (2016). Revalidation of *Tursiops gephyreus* Lahille, 1908 (Cetartiodactyla: Delphinidae) from the southwestern Atlantic Ocean. *Journal of Mammalogy, 97,* 1728–1737.

SUPPORTING INFORMATION

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