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Offshore and coastal common bottlenose dolphins of the western South Atlantic face-to-face: What the skull and the spine can tell us

Ana P. B. Costa,[1] Department of Biology, University of Louisiana at Lafayette, 410 E. St. Mary Boulevard, Lafayette, Louisiana 70503, U.S.A.; **Patricia E. Rosel,** National Marine Fisheries Service, 646 Cajundome Boulevard, Lafayette, Louisiana 70506, U.S.A.; **Fábio G. Daura-Jorge and Paulo C. Simões-Lopes,** Laboratório de Mamíferos Aquáticos (LAMAQ), Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, PO Box 5102, Florianópolis, SC, 88040-970, Brazil.

Abstract

The taxonomy of *Tursiops truncatus* in the western South Atlantic is not resolved. Two different hypotheses have been proposed: (1) offshore and coastal ecotypes with a parapatric distribution, and (2) two species, *T. truncatus* and *T. gephyreus*, living in sympatry. To test these hypotheses, we examined a total of 100 physically mature skulls and 35 vertebral columns from the suggested overlap zone in southern Brazil. In all skulls, 24 measurements, four alveoli counts and two categorical variables were analyzed. Vertebral formula was determined and five measurements were taken from selected vertebrae. Multivariate analyses were conducted for skull and vertebral data. Results revealed the presence of two well-separated groups. Specimens of Group1 had smaller skulls and shorter body lengths, but more vertebrae, than Group2. The morphological characteristics of each group corresponded well with two ecotypes of common bottlenose dolphins reported in other ocean basins. Therefore, we assigned the specimens of Group1 to the offshore ecotype, and Group2 to the coastal ecotype. Differences in the geographic locations and ratio of strandings supported the parapatric hypothesis. The significant morphological differentiation observed suggests the presence of different subspecies, but an additional independent line of evidence is needed to hypothesize whether they represent different species.

Key words: ecotype, skeletal morphology, South Atlantic, subspecies, *Tursiops truncatus*.

The genus *Tursiops* Gervais 1855 is a morphologically diverse group. At least 20 nominal species of *Tursiops* have been named (Hershkovitz 1966), with many of these species reflecting geographic variability within the genus (Mead and Potter 1990, Hale *et al*. 2000, Perrin *et al*. 2011). The high variability, global distribution, and the possibility of hybridization with several other odontocetes (Wells and Scott 1999) have resulted in a confused taxonomy for the genus and the specific status of many of the different forms remains unresolved. For several decades only *Tursiops truncatus* (Montagu 1821), the common bottlenose dolphin, was considered a valid

[1]Corresponding author (e-mail: abc2978@louisiana.edu).

species (*e.g.*, Wells and Scott 1999). More recently, the Indo-Pacific bottlenose dolphin, *T. aduncus* (Ehrenberg 1832), was recognized based on genetic differentiation, external morphology, and cranial morphology (Ross 1977, 1984; Rice 1998; Wang *et al.* 1999, 2000). Most recently, the Burrunan dolphin, *T. australis*, was proposed by Charlton-Robb *et al*. (2011) based on genetic analyses and external and cranial morphologies. However, it is not currently recognized by the Society for Marine Mammalogy's Committee on Taxonomy (Committee on Taxonomy 2016). The taxonomy of the species in the western South Atlantic Ocean (wSA) has also been controversial. Lahille (1908) described *T. gephyreus* based on two specimens found in La Plata River (Argentina).

Studies of cranial morphology, tooth diameter, hemoglobin profiles, genetic analyses, and ecological patterns have demonstrated the presence of two common bottlenose dolphins ecotypes or morphotypes—coastal and offshore—in many parts of the world (Duffield *et al*. 1983, Hersh and Duffield 1990, Kenney 1990, Van Waerebeek *et al*. 1990, Mead and Potter 1995, Hoelzel *et al*. 1998, Natoli *et al*. 2004, Sanino *et al*. 2005, Rosel *et al*. 2009, Perrin *et al*. 2011). In the western South Atlantic Ocean both coastal and offshore types have been previously suggested based on differences in color pattern (Simões-Lopes and Daura-Jorge 2008, Cremer *et al*. 2009, Lodi 2009), skull morphology (Toledo 2013) and genetic analyses (Costa *et al*. 2015). However, the presence and distribution of these two ecotypes in the wSA are not well defined.

More recently, two different hypotheses, based on skull morphology, have been proposed for the taxonomy and distribution of *Tursiops* in the wSA. Taking into consideration the oceanographic features of the continental shelf of the wSA, visual sightings, and the number of strandings of different morphological types of common bottlenose dolphins along the coast, Toledo (2013) suggested the presence of offshore and coastal ecotypes with a parapatric distribution along southern Brazilian and northern Argentinian coasts. In contrast, other studies using stranding records and skull morphology comparisons to skulls described by Lahille (1908) suggested that both ecotypes are in fact different subspecies (Barreto 2000) or even species (Wickert 2010) living in sympatry. Both studies suggested a latitudinal difference between the two forms—*T. truncatus* in the north and *T. gephyreus* in the south, with a sympatric zone (27°S–35°S) in waters of southern Brazil.

Our study aims to improve the understanding of the presence of different morphotypes in southern Brazil, as well as to contribute to the taxonomic resolution of the genus for the western South Atlantic Ocean. We use morphological analyses of skull and vertebral column characters to examine the degree of differentiation among *Tursiops* stranded in southern Brazil and to identify morphological characteristics that may help determine whether the types have a sympatric or parapatric distribution.

Methods

Samples

We examined 100 physically mature skulls and 35 vertebral columns of *Tursiops truncatus* deposited in four museum collections: Laboratório de Mamíferos Marinhos e Tartarugas Marinhas at Universidade Federal de Rio Grande (FURG); Laboratório de Zoologia at Universidade do Estado de Santa Catarina (UDESC); Laboratório de Mamíferos Aquáticos at Universidade Federal de Santa Catarina (UFSC); Acervo

Biológico Iperoba at Universidade da Região de Joinville (UNIVILLE) (Table S1). The specimens were collected in Brazil during stranding events between 1971 and 2013 from the northern coast of the state of Santa Catarina (26°S) to the southern coast of the state of Rio Grande do Sul (33°S).

We measured only skulls defined as physically mature, approximated by fusion (*i.e.*, no movement) of the maxillae to the cranium (Ross and Cockcroft 1990). In addition, 85 of the 100 skulls also exhibited fusion of distal premaxilla/maxilla further supporting they were physically mature (see Perrin and Heyning 1993); the maxillary tips of 11 were broken so fusion could not be assessed and four did not exhibit fusion of the premaxilla and maxilla. Of the 35 specimens for which both skull and complete or nearly complete postcranial skeletons were available, 17 also exhibited a physically mature vertebral column (Pattern 3: complete epiphyseal fusion through the entire vertebral column, according to Costa and Simões–Lopes 2012). All 35 postcranial skeletons were used to define the vertebral formula, but only the 17 physically mature vertebral columns were measured.

Cranial Measurements

Cranial measurements were taken with dial and digital calipers to the nearest millimeter following Perrin (1975), with the addition of two measurements (DPT: distance between pterygoids and WAC: width of alveoli cavity). In all skulls, 24 cranial measurements (Fig. S1), four alveoli counts and two categorical variables (CRCA: presence of scars of *Crassicauda* sp. and FEXO: presence of *fenestra* on exoccipital) were recorded (Table S2). Highest counts of teeth or alveoli were made for both left and right upper and lower rows.

Vertebral Column Measurements

Assembly of the spine and vertebral counts were performed to define the vertebral formula. The regions of the vertebral column were defined whenever possible according to the classical system based on Rommel (1990): cervical (C), thoracic (T), lumbar (L), and caudal (Ca). When the thoracic and lumbar regions could not be defined with precision due to lack of some vertebral ribs, both regions were assembled together as the thoracic–lumbar (T/L) region. The first caudal vertebra was identified as the first vertebra that bears hemal arch facets on its posterior ventral border (*e.g.*, Rommel 1990).

The vertebral count was characterized through visual inspection, with the terminal small, triangular caudal element being counted as one fused vertebra (Kemper 2004). Specimens with complete or nearly complete vertebral columns (*i.e.*, missing the last six or fewer caudal vertebrae) were analyzed and the number of missing vertebrae for a nearly complete vertebral column was estimated by comparison to complete specimens. In addition, three specimens missing more than six of the last caudal vertebrae were also included.

Vertebral measurements were also taken with digital calipers to the nearest millimeter. Seven vertebrae were chosen for each physically mature specimen: Atlas–axis (C_{1-2}), first thoracic (T_1), tenth thoracic (T_{10}), first lumbar (L_1), eighth lumbar (L_8), first caudal (Ca_1), and eighth caudal (Ca_8). Five measurements were taken for each of the seven vertebrae chosen (Table S2). Total external body lengths (TL) from field measurements were available for 61 out of 100 dolphins, including 13 of the 17 specimens with physically mature vertebral columns.

Statistical Analyses

A principal component analysis (PCA) was performed to determine if the cranial measurements supported the presence of different groups of *Tursiops truncatus* in the western South Atlantic Ocean. PCA is a multivariate analysis without *a priori* identification of groups or clusters and it was used to identify positions of the specimens along multivariate axes. The clusters in the PCA were highlighted with 95% confidence ellipses. We also carried out a discriminant function analysis (DFA) to confirm the separated clusters, estimate the probability that each specimen belonged to each cluster, and verify the variables that best explain the separation of the groups. All measures were log transformed prior to conducting the PCA and DFA as a way to reduce the variance between the measurements and assume a more conservative approach (Quinn and Keough 2002). A stepwise discriminant function analysis was used to determine which measurements were the most important for discriminating the groups. Since multivariate analyses are sensitive to missing data, we omitted from the PCA and DFA three of the 24 cranial measurements (measurements of mandible: LLLT, LLRM, and HLRM) because the mandible was not available for 24 specimens, and also omitted 22 specimens with missing data for some skull characters. The statistical significance of DFA clustering was assessed through Wilks' λ, Pillai's Trace, and Hotelling-Lawley tests (Quinn and Keough 2002). A second PCA was conducted using only the most important skull characters defined by DFA in an attempt to assign to each cluster the 22 specimens with incomplete data for cranial measurements. The DFA was repeated to confirm the second PCA clustering. PCA was also employed to verify whether vertebral measurements supported the cranial clustering. One vertebral measurement (GWV_T1) was omitted from the PCA due to the presence of missing values. The DFA was not applied to vertebral data since the sample size was much smaller than the number of independent variables.

The unpaired two-sample *t*-test (or Welch's test) was used to assess whether mean measurements of the mandible (LLLT, LLRM, and HLRM) were different between Groups. Assumptions of normality and homogeneity were assessed for the three measurements (Quinn and Keough 2002). The nonparametric Wilcoxon rank sum test (or Wilcoxon-Mann-Whitney test) was used to test for differences between Groups in the presence of scars of *Crassicauda* sp. in the skulls and for alveoli counts between Groups, since these variables failed to meet at least one of the assumptions for conducting *t*-tests, even after log transformation. In addition, due to small sample sizes, the permutation *t*-test was employed to determine whether mean total external body length (TL) of dolphins with physically mature vertebral columns (*i.e.*, physically mature adults) was different between Groups. In order to correct for small sample sizes (Ludbrook and Dudley 1998), the observed difference in the means between the groups for TL ($\Delta\mu$) was compared to the distribution of difference of the means estimated by randomly permuting each sample's assignment in 1,000 iterations (Manly 1991). All the statistical analyses described above were conducted using R version 3.1.1 (R Core Team 2014).

The presence of sexual dimorphism in cranial measurements was tested solely for the specimens of known sex (19 females, 23 males) in one of the groups defined by PCA (Group2, see Results) using one-way multivariate analysis of variance (one-way MANOVA). Assumptions of the MANOVA, *e.g.*, no multivariate outliers (tested using the Mahalanobis distance), multivariate normality (see below), homogeneity of variance-covariance of matrices (tested using Box's *M* test), no multicollinearity (tested using Pearson's correlation), were assessed for the 24 cranial measurements

and four tooth counts (Quinn and Keough 2002). The tooth counts were removed from the data set due to violations of multivariate normality, even after log transformation, as well as seven cranial measurements (LR, LRN, LRIN, LLRM, POW, PROW, and WZP) due to high correlation ($r > 0.81$) with other measurements. Therefore, the MANOVA was performed using 17 cranial measurements with no violations of its assumptions. Missing values in the Group2 data set were replaced by mean values for each measurement by sex as in Kemper (2004). It was not possible to test sexual dimorphism in Group1 defined by the PCA (see Results) due to the small sample size for this group (6 females, 3 males). The MANOVA analyses were conducted in IBM SPSS Statistics version 23.0 (IBM Corp. 2015), with the exception of the test for multivariate normality, which was conducted in R version 3.1.1 (R Core Team 2014) using R package MVN (Korkmaz *et al*. 2014).

Results

Cranial Analyses

We used 78 skulls and 21 cranial measurements for the first PCA and DFA analyses. The first PCA of the cranial measurements clustered the specimens from the wSA into two groups (Group1 = 23 specimens; Group2 = 55 specimens), with the first two components explaining 74.2% of the variance (Fig. 1). The most informative variables for PC1 (loadings > 0.20) were associated with width and length of the skull, with the skull length to width ratio for Group1 being smaller than for Group2. For PC2 the most informative variables corresponded with aspects of the shape of the skull: DPT, LAOLL, LNSO, and WIN.

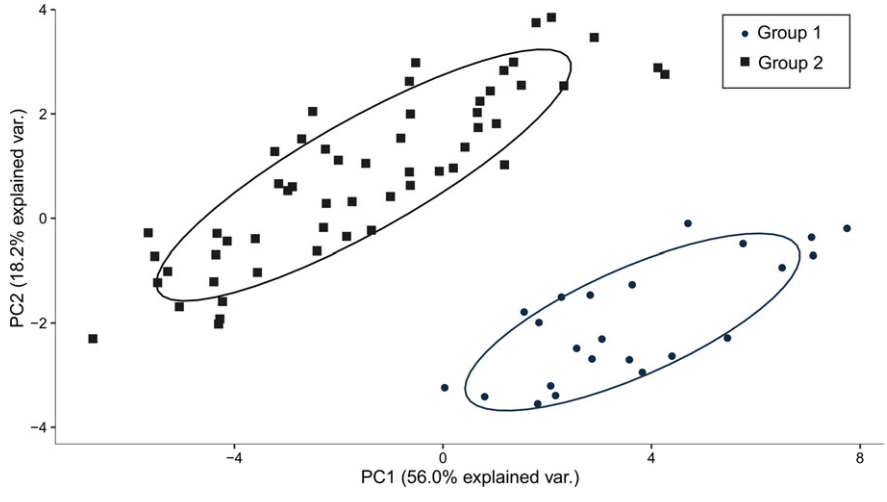

Figure 1. Scatter plot of the principal component 1 (PC1) and 2 (PC2) scores from the first principal component analysis of 21 skull measurements and 78 common bottlenose dolphin skulls from the western South Atlantic Ocean. Group1: offshore, Group2: coastal. Ellipses correspond to the 95% confidence interval.

The DFA complemented the PCA results, clustering the skulls into two well-separated groups. The percentage of factor scores classified to the correct group was 100% and all maximum posterior probabilities were equal to 1, providing confidence in sample classifications. The linear discriminant analysis (LDA) ability to separate the groups was equal to 1. The most important skull variables that best explained the separation of the groups were: DPT, LRN, LNSO, LAOLL, WPM, WEN, and LRIN. The statistical significance of the DFA clustering was confirmed by all statistical tests used (Wilks' $\lambda = 0.025$, $P < 10^{-16}$; Pillai's Trace = 0.975, $P < 10^{-16}$; Hotelling–Lawley = 38.569, $P < 10^{-16}$).

The PCA and DFA analyses were repeated using 96 skulls (including 18 out of the 22 with missing data) and six of the seven most important skull variables that best explained the separation of the groups. The variable DPT was not included due to a high number of specimens with missing data for this measurement. Four skulls with missing data for some of these six characters were also omitted. The second PCA placed the new specimens into the two groups, clustering three of the 18 skulls in Group1, and the remaining 15 in Group2 (Group1 = 26; Group2 = 70) (Supporting Information Table S3). The first two components explained 82.6% of the variance observed (Fig. 2). It is important to note that the second PCA exhibited less separation between the two groups (Fig. 2) when compared to the first PCA (Fig. 1) because there was a reduction in the number of variables ($n = 6$), with two of these variables being associated with the shape of the skull (LNSO and LAOLL) and not the size. The DFA confirmed the PCA clustering with 100% of factor scores classified

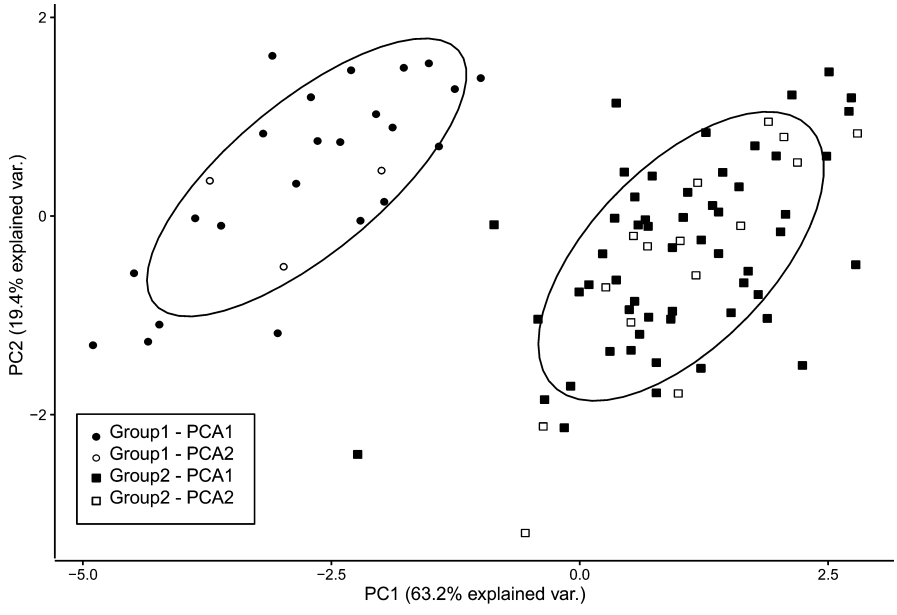

Figure 2. Scatter plot of the principal component 1 (PC1) and 2 (PC2) scores from the second principal component analysis of six skull measurements and 96 common bottlenose dolphin skulls from the western South Atlantic Ocean. Group1: offshore, Group2: coastal. Ellipses correspond to the 95% confidence interval.

to the correct group and all maximum posterior probabilities larger than 0.99. The statistical significance of the clustering was confirmed by all statistical tests used (Wilks' $\lambda = 0.081$, $P < 10^{-16}$; Pillai's Trace = 0.919, $P < 10^{-16}$; Hotelling–Lawley = 11.317, $P < 10^{-16}$).

Comparative Anatomy of the Skull

Group1—The interparietal bone was clearly visible in the vertex of the skull. The vertex of the skull (nasals, frontals, interparietal, and nuchal crest) was longer than in the specimens of Group2 (*i.e.*, larger distance between end of nasals to the hindmost point of supraoccipital, LNSO) (Fig. 3A, B). The nasals were almost symmetrical. Together, these features resulted in a less pronounced telescoping of the skull. The antorbital notch was deeper in this group. A convex pharyngeal crest and a narrow Eustachian notch (*sensu* Toledo 2013) were evident in lateral view. The specimens of Group1 showed a flattened ascending process of the maxilla, while in the specimens of Group2 the ascending process was concave.

The posterior border of the pterygoid hamulus was oriented almost 90° (obtuse apex in Ross 1977) to the sagittal plane of the skull (Fig. 3C). The medial expansion of the pterygoids put them into contact, *i.e.*, there was almost no distance between the pterygoid hamuli (DPT). There was broad separation of the occipital condyles at the level of the basioccipital (Fig. 4). Group1 skulls presented slightly narrower alveolar cavities (WAC), broader internal nares (WIN), a greater lacrimal process (LAOLL) (see Supporting Information Table S3), and shorter mandible (LLLT, HLRM, and LLRM: $P < 10^{-5}$). Scars from *Crassicauda* sp. were observed in 14 out 26 skulls (53.8%) clustered in Group1.

Group2—Elements of the vertex of the skull were compressed in an anteroposterior direction. The vertex was shorter, and appeared protuberant in the lateral view. The interparietal bone was not visible in the vertex of physically mature skulls. There was a marked asymmetry between the nasal bones (symmetric in Group1). The left nasal was smaller and more compressed in the anteroposterior direction. All these characters made Group2 skulls more telescoped than was seen in specimens of Group1 (Fig. 4).

The overall skull (CBL), as well as the rostrum (LR, LRN, and LRIN), was longer than the specimens of Group1 (see Table S3). The premaxillary sac fossa (*sensu* Toledo 2013) or prenarial triangle (*sensu* Perrin 1975) was visually deeper and concave in Group2. This concavity was also marked in both right and left maxillae. The antorbital notch was shallower than in specimens of Group1. Laterally, the pharyngeal crest was straight (nonconvex), and the Eustachian notch was broad. The posterior border of the pterygoid hamulus was oriented in an acute angle (subacute apex in Ross 1977) in relation to the sagittal plane of the skull. The pterygoids never came into contact with each other (Fig. 3D). The external nares (WEN) and premaxillae (WPM) were broader, and the upper left tooth row (LULT) was longer than in Group1.

All skulls of Group2 exhibited the presence of one *fenestra* in the left and/or right side of the skull at the level of the exoccipital, near to the temporal crest. The same structure was not present in the specimens of Group1. Scars from *Crassicauda* sp. were observed in 30 out of 70 skulls (42.9%) classified in this group; there was no significant difference between the Groups (Wilcoxon-test = 1010; $P = 0.3419$), though this may be a reflection of the small sample size in Group1.

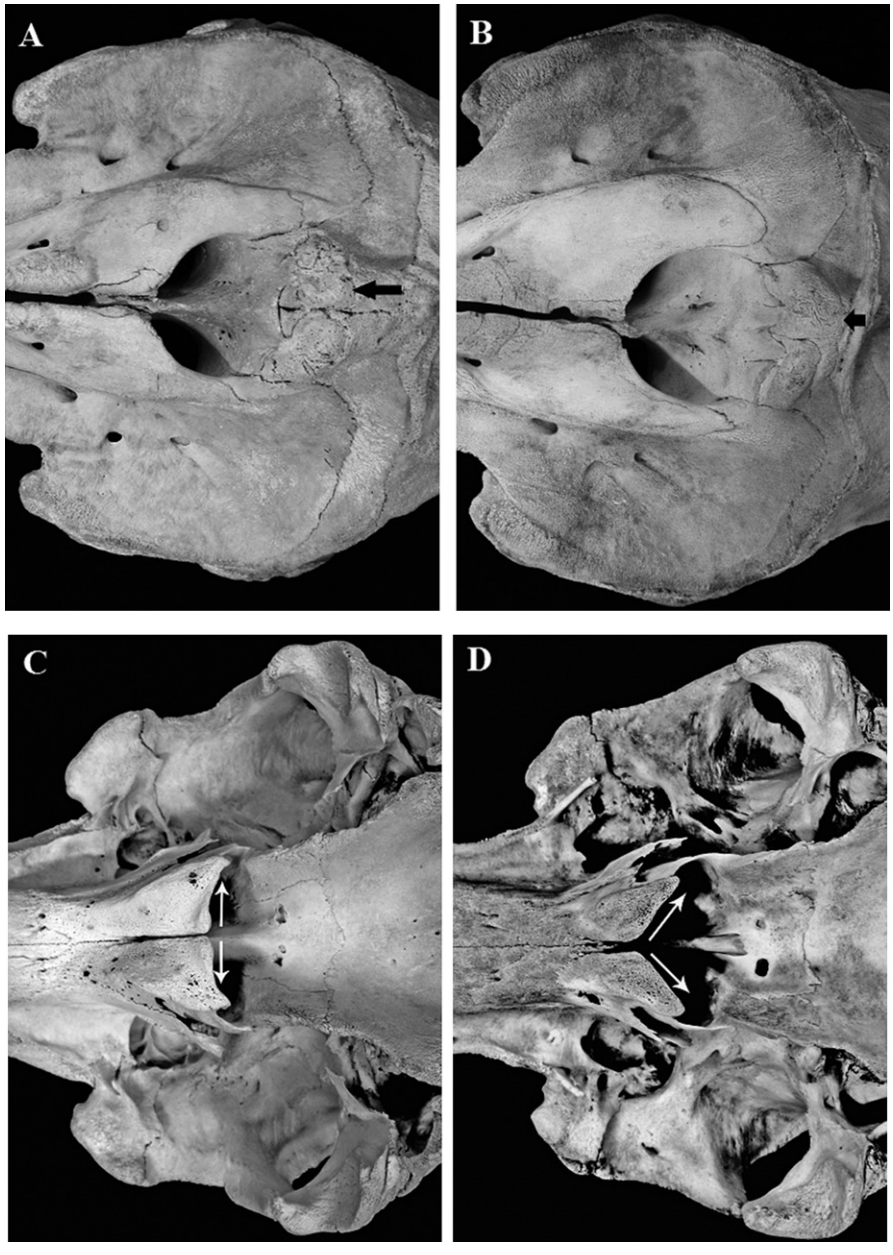

Figure 3. Dorsal view of the vertex of the skulls of common bottlenose dolphins of (A) Group1 (offshore, UFSC1287) and (B) Group2 (coastal, UFSC1089), and ventral view of the pterygoid hamulus of skulls of common bottlenose dolphins of (C) Group1 (offshore, UFSC1322) and (D) Group2 (coastal, UFSC1249) from the western South Atlantic Ocean. Black arrows indicate the distance from the junction between the sutures of the nasals to hindmost point of margin of supraoccipital crest. White arrows indicate the oriented angle of pterygoid hamuli.

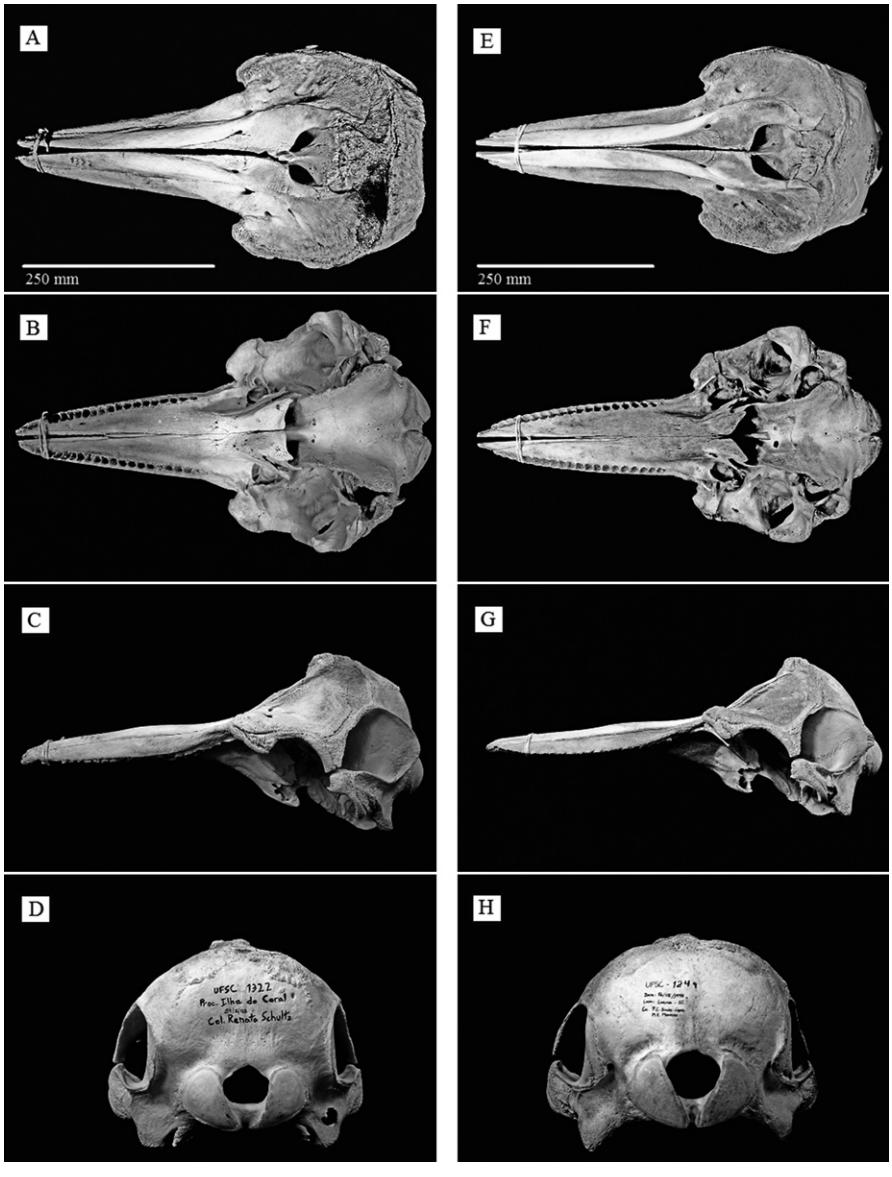

Figure 4. Dorsal, ventral, left lateral and occipital views of physically mature skulls of common bottlenose dolphins of Group1 (offshore, UFSC1322, A–D) and Group2 (coastal, UFSC1249, E–H) from the western South Atlantic Ocean. Scale bar of 250 mm applies only to the dorsal view.

Possible signs of pedomorphism, *i.e.*, slow suture fusion and smaller morphological structures (see Barnes 1985, Galatius and Gol'din 2011), were observed in both groups. The dorsal view of Group1 skulls exhibited a shorter rostrum, a less telescoping skull and a longer vertex of the skull with visible interparietal and frontal bones,

which resembles the juvenile skulls of both Groups (not used in this study). On the other hand, in the occipital view of Group2, the skull had a more rounded shape and the presence of *fenestra* can indicate an incomplete fusion of the occipital complex (*i.e.*, exoccipital, parietal, and supraoccipital bones). However, in this study we did not have an age series of skulls with which to investigate the skeletal ontogeny of the Groups. Further analyses are needed to examine whether heterochrony may play a role in the morphological variation observed.

Alveoli Counts

Group1 dolphins exhibited slightly more alveoli than Group2 with, on average, 22 alveoli in the tooth rows of the mandible (left side: 20–24; right side: 19–26) and 23 on the maxilla (left side: 20–26; right side: 21–25). Group2 dolphins exhibited, on average, 21 alveoli in the mandible (left/right sides: 18–23) and 22 in the maxilla (left/right sides: 20–24). However, caution is needed when considering alveoli/tooth counts since the first teeth in the tip of the rostrum may not have well-defined alveoli. Significant differences between Groups were found only in the maxilla (TUL and TUR, $P < 0.05$). Specimens of Group2 exhibited slightly larger alveoli cavities (mean 12.41 mm) than Group1 (mean 10.57 mm; see Table S3).

Sexual Dimorphism

The MANOVA found no significant sexual dimorphism in Group2 when considering the 17 cranial measurements together (Wilks' $\lambda = 0.445$; Pillai's Trace = 0.555; Hotelling–Lawley = 1.250; $P = 0.099$). However, significant differences were found in two measurements when comparing the cranial measurements individually (independent ANOVAS, with Bonferroni correction, $P < 0.003$), where males were larger than females in the LLPTF and WAC measurements (Table S4).

Visual Identification of the Four Specimens with Missing Data

The strong separation of the two clusters provided by multivariate analyses and the morphological description in the previous sections permitted us to assign to a group the four remaining skulls with missing data. The specimens UFSC1099, UFSC1261, UNIVILLE230, and UNIVILLE241 had a longer distance between nasals and supraoccipital (LNSO) (range: 41.5-51.1 mm), the nasals were almost symmetrical, a convex pharyngeal crest and a narrow Eustachian notch were evident in lateral view, the posterior border of the pterygoid hamulus was oriented almost 90° to the sagittal plane of the skull, and there was no space between the pterygoid hamuli (DPT). These characters were consistent with Group1. The vertebral column was available for the four specimens (but only UNIVILLE 241 was physically mature), confirming their clustering with Group1 (see below).

Vertebral Column Analyses and Morphology

The PCA of the vertebral column measurements revealed two well–separated groups that corresponded 100% with the two groups defined for the skulls. The first two components explained 80.9% of the total variation (Fig. 5). The most informative variables for PC1 (loadings > 0.18) were associated with width and height of the vertebrae, with the vertebra height to width ratio for Group1 smaller than for

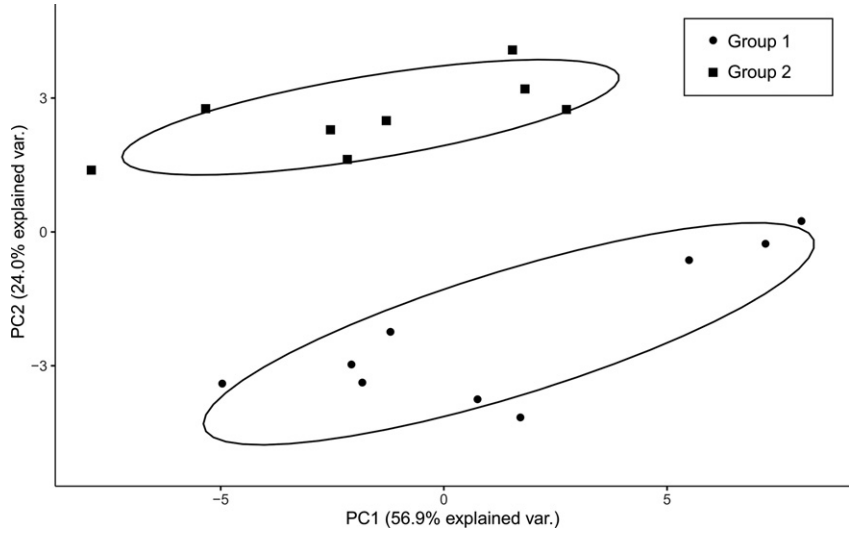

Figure 5. Scatter plot of principal component 1 (PC1) and 2 (PC2) scores from the principal component analysis of 17 common bottlenose dolphin vertebral columns from the western South Atlantic Ocean. Group1: offshore, Group2: coastal. Ellipses correspond to the 95% confidence interval.

Group2. For PC2, the variables that best differentiated the groups were: LVB_T_1, LVB_L_8, LVB_Ca_1, LVB_Ca_8 (positive loadings >0.18) and GWV_$C_{1\text{-}2}$, GWV_T_{10}, GWV_L_1, GWC_L_8, WNC_T_1, WNC_T_{10}, WNC_L_1, WNC_L_8, WNC_Ca_1, WNC_Ca_8 (negative loadings >0.18) (Table S5). The physically mature vertebral column UNIVILLE241 was clustered by PCA in Group1, confirming the assignment provided by visual inspection of the skull.

The vertebrae of specimens of Group1 were broader (GWV) and had a broader neural channel (WNC). The height (HVB) to width (WVB) ratio of the vertebral body was larger in Group2, and they had a longer centrum (LVB).

There were differences in the number of vertebrae between Groups. Half of the specimens of Group1 (9 out of 18) had the vertebral formula $C_7 + T_{13} + L_{16} + Ca_{29} = 65$. However, differences primarily in the total number of the lumbar and caudal vertebrae were found in the other nine animals: $C_7 + T_{13–14} + L_{14–17} + Ca_{28–30} = 62–68$. The vertebral columns from UFSC1099, UFSC1261 and UNIVILLE230 presented high counts in the thoracic ($T_{13–14}$), lumbar ($L_{15–17}$) and caudal (Ca_{28}) regions, also confirming the assignment to Group1 provided by visual inspection of the skull. While Group1 had a variable total number of vertebrae, Group2 showed a fairly consistent pattern in the vertebral formula with lower counts in each region (except the cervical) and the vertebral formula $C_7 + T_{12} + L_{13} + Ca_{26} = 58$ ($n = 15$). Two exceptions were found due to differences in the number of lumbar vertebrae ($L_{12–14}$) and consequently in the total count (TC = 57–59). Though Group2 dolphins generally had fewer vertebrae, the statistical analyses of the morphometric data revealed that they were significantly larger in external total body length than the specimens of Group1 ($t = -3.561$; $P = 0.0026$).

Discussion

Morphological Differentiation and Ecological Implications

The examination of 100 skulls and 35 vertebral columns of common bottlenose dolphins that stranded along the southern Brazilian coast revealed two distinct forms. Multivariate analyses identified two well separated clusters, where the morphological characteristics of each group correspond well with two ecotypes of common bottlenose dolphins reported in different parts of the world (see Van Waerebeek *et al*. 1990, Mead and Potter 1995, Perrin *et al*. 2011). Therefore, we assigned the specimens of Group1 as the offshore ecotype, and Group2 as the coastal ecotype (see below).

The differences in skull morphology found between the two groups may be related to feeding and ecological habits, and are in agreement with differences in diet, echolocation, and dive depth between coastal and offshore common bottlenose dolphins (*T. truncatus*) identified in other studies (Walker 1981, Barros and Odell 1990, Hersh and Duffield 1990). We observed that specimens of Group2 exhibited slightly larger tooth alveoli than specimens of Group1, as well as a longer rostrum and mandible. These characteristics are similar to those cited for coastal common bottlenose dolphins in many other areas of the world, and they are hypothesized to be adaptations for feeding on larger prey (*e.g.*, nearshore sciaenid and mugilid fishes) than the offshore common bottlenose dolphins, which usually feed on squids (Walker 1981, Van Waerebeek *et al*. 1990, Perrin *et al*. 2011). Perrin *et al*. (2011) also noted that coastal common bottlenose dolphins in the eastern North Pacific have fewer teeth in the jaws, probably because large teeth occupy more space, agreeing with our observations for Group2.

Other skull features may also be related to ecological differences between the two groups and further support our hypothesis. Larger internal nares (WIN) and narrower external nares (WEN) as seen in Group1 have both been suggested as adaptations to improve air exchange in the deeper diving offshore common bottlenose dolphins in other ocean basins (Mead and Potter 1995, Perrin *et al*. 2011). Perrin *et al*. (2011) also suggested that different echolocation and hearing abilities may explain the variation in shape observed in the pterygoid hamuli, reflecting different feeding habitats. However, further studies are needed to verify differences in echolocation between coastal and offshore common bottlenose dolphins. Wahlberg *et al*. (2011) investigated differences in echolocation between the coastal species *T. aduncus* from the Indian Ocean and *T. truncatus* from the Atlantic Ocean, revealing that the coastal *T. aduncus* had clicks with higher frequency and directionality than *T. truncatus*, which may reflect differences in morphological structures involved in sound production (Wahlberg *et al*. 2011).

The presence of scars of *Crassicauda* sp. in the skulls of common bottlenose dolphins is a useful character to identify offshore ecotypes in the western North Atlantic. According to Mead and Potter (1995), 74% of the skulls of the offshore common bottlenose dolphins examined (n = 38) in the western North Atlantic presented bone lesions caused by this parasite, whereas only 1.6% of 183 skulls of the coastal ecotype had *Crassicauda* lesions. For the wSA, no significant difference in the presence of *Crassicauda* lesions was observed between the ecotypes, suggesting that it is not a useful character to differentiate them in this region.

Sexual dimorphism was not documented for the coastal samples (Group2) using the MANOVA analysis. The presence of sexual dimorphism for bottlenose dolphins

has been detected in other ocean basins (Turner and Worthy 2003, Perrin *et al*. 2011), but it is not a rule (Hersh *et al*. 1990, Van Waerebeek *et al*. 1990, Wang *et al*. 2000, Turner and Worthy 2003, Kemper 2004), reflecting the great geographic variation of the genus *Tursiops*.

Osteological studies utilizing vertebral column data are not commonly conducted in cetacean taxonomy, although their inclusion may improve the ability to differentiate distinct forms of the same species (Ross and Cockcroft 1990). Generally, *T. aduncus* (Chinese waters: TC = 59–61, n = 19, see Wang *et al*. 2000; Australian waters: TC = 57–62, n = 30, see Kemper 2004) has fewer vertebrae than *T. truncatus* (Chinese waters: TC = 63–67, n = 20, see Wang *et al*. 2000; Australian waters: TC = 61–66, n = 8, see Kemper 2004). The species *T. aduncus* is thought to be restricted to coastal waters, and interestingly the wSA coastal dolphins also exhibited fewer vertebrae than the parapatric offshore dolphins. However, Rommel (1990) found more than 60 vertebrae for both coastal and offshore common bottlenose dolphins (n = 10; both ecotypes together) in the western North Atlantic. In the present study we observed that, although smaller in total external body length, the specimens of Group1 had more vertebrae than Group2. A similar trend was observed by Jefferson and Rosenbaum (2014) when *Sousa* species where compared, though these findings were based on small sample sizes. Buchholtz and Schur (2004) cited that larger cetacean species (*e.g.*, *Orcinus orca*) have a lower total count than smaller species (*e.g.*, *Lagenorhynchus acutus*) and suggested total count and vertebral length are inversely related: species with more vertebrae exhibit a reduction in the length of vertebral body, as we saw in Group1. Morphometric comparisons of condylobasal length (CBL) and total external body length among common bottlenose dolphins from different ocean basins suggest that coastal dolphins from the wSA are the largest common bottlenose dolphins examined to date from the Pacific and Atlantic coasts of the Americas (Table 1). This

Table 1. Comparison of condylobasal lengths (CBL) and reported maximum total external body lengths (TL) of coastal and offshore *Tursiops truncatus* from different ocean basins. In our study, Group1 is considered the offshore ecotype and Group2 the coastal ecotype from the western South Atlantic Ocean.

	Coastal				Offshore				
Ocean basin	n	CBL (mm)	n	TL (cm)	n	CBL (mm)	n	TL (cm)	Reference
Western South Atlantic	70	533–609	49	366	30	495–567	14	310	This study
Western North Atlantic	72	350–510	72	290	33	350–530	33	310	Mead and Potter 1995[a]
Eastern South Pacific	4	507–542	12	308	15	494–542	33	305	Van Waerebeek *et al*. 1990
Eastern North Pacific	29	471–548	17	333	12	479–570	14	310	Perrin *et al*. 2011, Perrin and Reilly 1984

[a]Mead and Potter (1995) provided modes rather than ranges. It is the only work listed in the table that did not specify whether all the skulls measured were physically mature.

pattern differs significantly from what is seen in the western North Atlantic Ocean, where the coastal ecotype is smaller than the offshore ecotype (Mead and Potter 1995). Barros (1991) suggested that the larger common bottlenose dolphins from the Brazilian coast are found in an area influenced by the cold waters of Malvinas/Falkland current. Along the U.S. Pacific coast, the larger ecotype is also found in more nearshore waters, a region also influenced by cold current and strong upwelling zones (Huyer 1983). This pattern would be in line with Bergmann's rule (Meiri and Dayan 2003). Furthermore, differences in size between offshore (Group1) and coastal (Group2) common bottlenose dolphins of the wSA may be attributed to areas of higher productivity that provide a stable environment for reaching greater size in the coastal series (see below) as has been suggested for other dolphin species, with the larger forms being found in cold and high productivity environments (see Di-Méglio *et al*. 1996, Danil and Chivers 2007).

Overall, the morphological differentiation between the two wSA clusters identified by the multivariate analysis was significant, with no overlap between them and corresponded well with ecological habits expected for coastal and offshore morphotypes of common bottlenose dolphins. Some characters (LR, LNSO, DPT, symmetry of the nasals, shape of the pharyngeal crest, and Eustachian notch) were very helpful in the identification of skulls with limited data. These variables, as well as the vertebral column, will be the most useful for visual identification of specimens. In addition, preliminary evidence for color pattern differentiation between both forms was also observed in the present study and seemed also to be in agreement with what has been suggested in the literature for coastal and offshore common bottlenose dolphins (see Supporting Information for additional discussion).

Geographic Distribution

The stranding records of our samples revealed a geographic trend for both ecotypes (Fig. 6). Specimens of Group2 usually stranded inside or near the entry of sheltered waters, and were more concentrated south of the latitude 27°S, while Group1 individuals usually stranded to the north of the latitude 28°S and outside sheltered waters. Furthermore, there were significantly more skulls from Group2 than Group1 in the data set (70:30), suggesting the ecotypes do not strand with the same frequency, and supporting the parapatric hypothesis. Offshore carcasses are much less likely to reach the beach (see Perrin *et al*. 2011). If the groups were sympatric, a more even ratio of both types would be expected.

Comparison of our results with the morphological study of Toledo (2013) suggested that the specimens assigned (according to cranial morphology) by Toledo (2013) as offshore have similar morphological characteristics with our Group1, while the specimens assigned as coastal are similar to our Group2. In addition, there were 24 skulls common to both studies and there were no differences in ecotype assignment for these skulls between the two studies. Toledo (2013) measured 44 skulls collected between latitudes 2°50′S and 23°05′S and all corresponded to the offshore form. The first stranding records of the coastal form in the Toledo study appeared around latitude 23°51′S, with most of the records for this form being found along the southern Brazilian coast and the northern coast of Argentina (29°S–42°S) (see Toledo 2013). However, sightings from field studies suggested that the coastal form can be found as far south as 43°S (Coscarella *et al*. 2012). The southern limit of the offshore form in the wSA is less well defined, being also found in higher latitudes (south of 23°S), but in lower numbers than the coastal form as seen in our study.

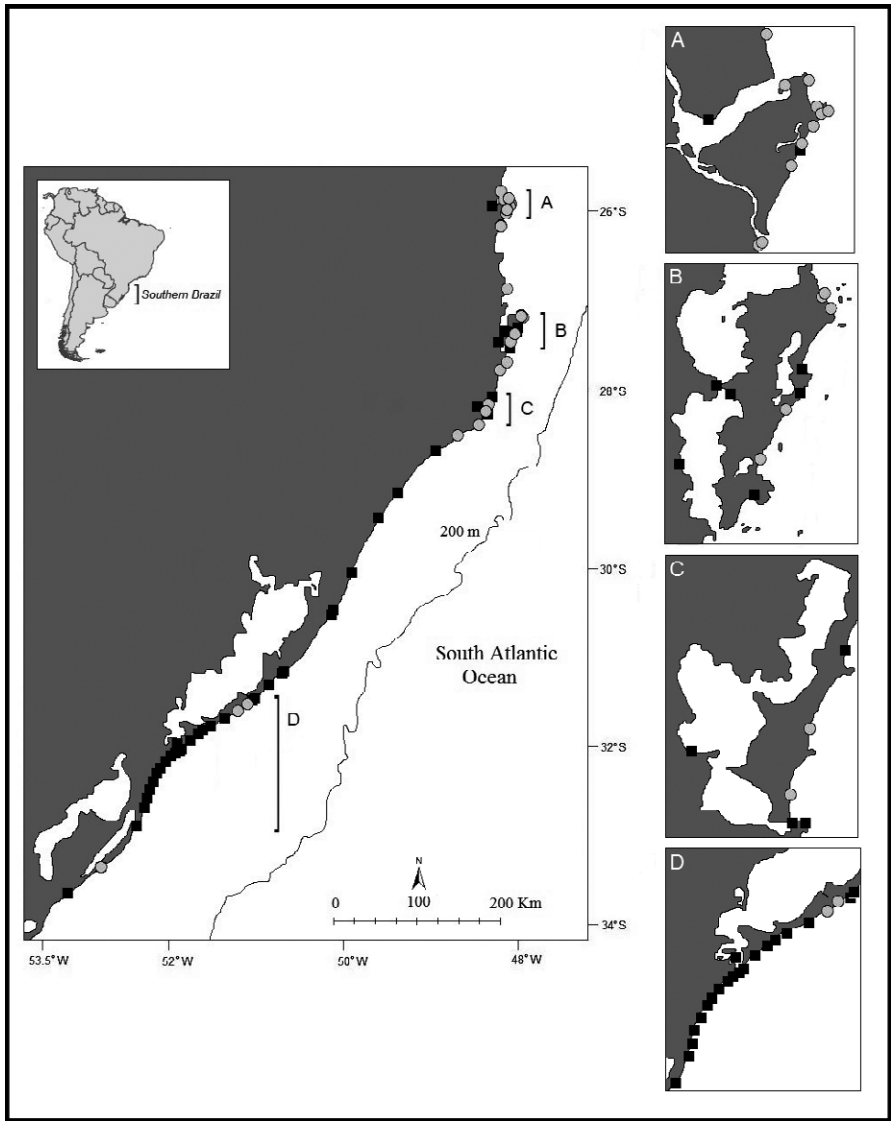

Figure 6. Distribution of strandings of common bottlenose dolphins along the southern Brazilian coast used in this study (gray circles = Group1–offshore; black squares = Group2–coastal). A: Babitonga Bay; B: Island of Santa Catarina (Florianópolis); C: Complex Estuarine of Santo Antônio dos Anjos (Laguna); D: Patos Lagoon.

There were also records of eight stranded common bottlenose dolphins in Tierra del Fuego (53°S–55°S), considered morphologically similar to those from the northern coast of Brazil (Goodall *et al*. 2011), *i.e.*, the offshore form, suggesting the offshore form may range along the entire coast. The total vertebral count was verified for seven of these eight specimens and ranged from 64 to 67 vertebrae (APBC, personal observation) in line with vertebral counts of the offshore form from further north.

Oceanographic features of the western South Atlantic Ocean may explain the restricted distribution of the Group2 coastal ecotype. The continental shelf is very narrow (30–160 km) north of latitude 22°S. South of this latitude it widens and becomes the western South Atlantic Shelf with its widest point, the Patagonian Shelf, between 41°S and 55°S (Bisbal 1995, Palma *et al*. 2008). North of latitude 22°S, the limitation of the distribution of the coastal ecotype is most likely due to the absence of appropriate coastal habitat. Estuarine/coastal resident communities of common bottlenose dolphins in the wSA are found solely south of latitude 27°S (Würsig 1978, Simões-Lopes 1991, Wedekin *et al*. 2008, Laporta 2009, Vermeulen and Cammareri 2009, Fruet *et al*. 2011, Daura-Jorge *et al*. 2013). Seasonal presence of common bottlenose dolphins along the coast between 23°S and 27°S may be related to periods of high productivity and resource availability (see Monteiro-Filho *et al*. 1999, Simões-Lopes and Fábian 1999, Simões-Lopes and Daura-Jorge 2008, Santos *et al*. 2010).

The infrequent records of the coastal form in the widest portion of the continental shelf, south of 43°S, may be influenced by other environmental variables (*i.e.*, marine currents, surface temperatures, productivity, turbidity), as has been demonstrated for franciscana dolphins (Mendez *et al*. 2010). The Brazil and Malvinas/Falkland currents are the two major currents in the western South Atlantic Ocean. The warm Brazil current flows from north to south along the coast of South America until it meets the cold, nutrient rich subantarctic waters of the Malvinas/Falkland current to form the subtropical convergence zone (35°S–40°S), one of the most biologically productive ocean areas in the world (Bisbal 1995, Palma *et al*. 2008). South of latitude 43°S (southern limit of the coastal form distribution), the cold Maldivas/Falkland current (water temperature: 4°C–15°C, see Seelinger *et al*. 1997) is the sole current influencing this area (see Palma *et al*. 2008), and could restrict the distribution of coastal common bottlenose dolphins, which appear to favor warmer water temperatures, in the range of 10°C–32°C (Bastida and Rodríguez 2005).

According to Moura *et al*. (2013), offshore common bottlenose dolphins may have colonized coastal habitats released by climatic changes during the Eemian and Holocene in many parts of the world. In the western South Atlantic, the Last Glacial Maximum took place around 24,000 yr ago exposing a large portion of the continental shelf (see Ponce *et al*. 2011); the area was later inundated by the sea, reaching a Mid-Holocene sea-level highstand and decreasing towards present (see Nagai *et al*. 2014), which led to the formation of several sheltered areas along the coast (see Ponce *et al*. 2011). Entry into these areas may have been an opportunity for the divergence of coastal and offshore bottlenose dolphin ecotypes (Moura *et al*. 2013).

Taxonomy of the Genus in the Western South Atlantic Ocean

In the western South Atlantic Ocean, Lahille (1908) described a new species of *Tursiops*, *T. gephyreus*, based on two specimens from the estuary of La Plata River, Argentina (35°11′ S). Comparing Lahille's description and drawings with our findings, *T. gephyreus* would be considered as belonging to the coastal ecotype due to the following characteristics: longer skull and rostrum (CBL, LR, LRN, LRIN; see Table S6), the vertex of the skull was shorter, the Eustachian notch was broad, *fenestra* were visible in the occipital view, the posterior border of the pterygoid hamulus was oriented in an acute angle in relation to the sagittal plane of the skull and the pterygoids never came into contact with each other. Furthermore, the total vertebral count was 58 vertebrae.

However, at this time *T. truncatus* is the only species recognized for the South Atlantic Ocean, though some authors have proposed that *T. gephyreus* be formally recognized as either a subspecies (Barreto 2000) or species (Wickert 2010). These authors suggested both *T. truncatus* and *T. gephyreus* (or the subspecies *T. truncatus truncatus* and *T. truncatus gephyreus*) are present in the wSA, with a sympatric overlapping zone along the southern Brazilian coast (27°S–35°S). The primary differences between our study and Barreto (2000) and Wickert (2010) are (1) we made no *a priori* groupings of the skulls before performing the multivariate analyses, thereby removing any preconceived expectation of the geographic contribution to resultant groupings and (2) we are proposing that the two morphological forms have a parapatric distribution that is longitudinally based (*i.e.*, coastal *vs*. offshore) rather than latitudinally based (*i.e.*, north *vs*. south).

Reeves *et al*. (2004) recommended that at least two independent lines of evidence be required in order to identify cetacean species, but for subspecies a single line would suffice. According to the authors, subspecies can be defined as groups that appear to be on independent evolutionary trajectories, with low gene exchange demonstrated by morphological differentiation or by genetic evidence. Other lines of evidence (*e.g.*, geographical or behavioral) can be used as complements whenever possible. Although there has long been debate over the usefulness of designating subspecies (Zink 2004, Phillimore and Owen 2006, Patten 2010, Remsen 2010) the subspecies category is recognized by the International Code of Zoological Nomenclature (ICZN 1999), and its concept includes geographical varieties within species with sufficient diagnostic distinctness (see Winker 2010). Several cetacean subspecies have been described using morphological differentiation. For example, a subspecies of spinner dolphin (*Stenella longirostris roseiventris*) was recognized in Southeast Asian waters based on morphological characters (Perrin *et al*. 1999), and differential color pattern demonstrated the presence of a new subspecies of Indo-Pacific humpback dolphin (*Sousa chinensis taiwanensis*) in Taiwan (Wang *et al*. 2015). Skull morphology differentiation and low mitochondrial gene flow between *Tursiops truncatus* from the Black Sea and the Mediterranean Sea supported the presence of the distinct subspecies *T. t. ponticus* in the Black Sea (Viaud-Martinez *et al*. 2008).

Based on the criteria cited above to describe different subspecies and given the great degree of morphological separation of the two ecotypes in the present study, consideration could be given to calling Group2 (coastal ecotype) *Tursiops truncatus gephyreus* following Lahille (1908), while Group1 (offshore ecotype) would remain as the nominate subspecies *T. t. truncatus*. According to Hershkovitz (1966), several species names have been previously utilized for bottlenose dolphins collected in the La Plata and Uruguay Rivers, but in some cases it is unclear how the type locality was determined: *Delphinus cymodoce* Gray 1846 (later *Tursio cymodoce*; see Gray 1868, 1871), *Tursio cymodice* (Figueira 1894), and *Delphinus (Tursio) cymodoce* (Burmeister 1867). However, it is important to note that *Tursio cymodice* and *D.* (*Tursio*) *cymodoce* were described through reference to Gray's findings, and therefore these two names should be considered as *Delphinus cymodoce* Gray 1846 (or *Tursio cymodoce*). Furthermore, in his original description of *D. cymodoce*, Gray did not provide a type locality, although in later publications he indicated it was from the Uruguay River. He likely did this simply following Burmeister's reference of two local specimens to species from an unknown locality. Flower (1883) and True (1889), both using the name *Tursio cymodice*, indicated Gray's specimen was a young animal for which the "distinguishing characters are those of immaturity" (Flower 1883), and stated that this name should "be expunged". In True (1889), the specimen number (355a) matches

the number assigned to the skull of *D. cymodoce* Gray 1846 deposited in the British Museum of Natural History, which suggests that Flower (1883) and True (1889) were both referring to the holotype specimen of *D. cymodoce* Gray 1846 (though the species name they used was *Tursio cymodice*), the specimen Flower suggested was too young to be used to identify a species and for which the original locality was unknown. Therefore, with the incongruence in the use of the names and localities and the only measurements available in the literature from the young skull of "*Tursio cymodice*" provided by True (1889), we believe that Lahille's (1908) description and name, *Tursiops gephyreus,* is the only name clearly borne by a holotype skull from the region.

The great morphological disparity between the ecotypes in the wSA suggests there is limited gene flow between them. Ongoing studies of genetic variation will help assess the degree of genetic divergence between the ecotypes before officially moving forward with taxonomic naming of coastal subspecies. In addition, given the broad geographic range of *Tursiops*, it is necessary to place any taxonomic study in the larger geographic context of the genus, including *T. aduncus*, to help clarify the degree of evolutionary separation between the two ecotypes in the wSA and improve our understanding of their taxonomic status.

Conclusions

The present study identified significant morphological differentiation between two ecotypes of bottlenose dolphins in the western South Atlantic Ocean and concluded they have a parapatric distribution, with one ecotype associated with offshore waters and the second associated with nearshore, coastal waters. The great degree of morphological differentiation between the ecotypes, revealed through skull and vertebral column analyses, suggested that the ecotypes might represent two distinct subspecies, *Tursiops truncatus truncatus* (offshore ecotype) and *T. t. gephyreus* (coastal ecotype). This name for the coastal subspecies follows from Lahille's (1908) description. Ongoing genetic studies, along with broader geographic sampling, will further clarify the genetic structure and levels of gene flow between the ecotypes, and will help confirm whether the ecotypes represent good subspecies or possibly should be elevated to the species level. Lastly, coastal populations are more susceptible to anthropogenic impacts, and the presence of coastal common bottlenose dolphins of restricted and possibly endemic distribution in the western South Atlantic Ocean, as suggested by the morphological analyses, reveals the importance of protecting this group as well as its habitat.

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Supporting Information

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Table S1. List of specimens analyzed in this study with sex, total body length (TL), date of collection, approximate latitude and longitude of the stranding location, vertebral column (if available for analyses), and the morphological form to which each specimen was classified based on cranial measurements. F: Female; M: Male; U: Unknown. FURG: Laboratório de Mamíferos Marinhos e Tartarugas Marinhas at Universidade Federal de Rio Grande; MORG: Museu Oceonográfico de Rio Grande (specimen located at FURG); UDESC: Laboratório de Zoologia at Universidade do Estado de Santa Catarina; UFSC: Laboratório de Mamíferos Aquáticos at Universidade Federal de Santa Catarina; UNIVILLE: Acervo Biológico Iperoba at Universidade da Região de Joinville.

Table S2. List of cranial and vertebral measurements, tooth count and categorical variables analyzed in this study with the respective abbreviations.

Table S3. Mean and range (in millimeters) of 21 cranial measurements considered in the first principal component analysis taken from common bottlenose dolphins of Groups1 and 2 from the western South Atlantic Ocean. Most informative variables for PC1 and PC2 are highlighted in bold (see Table S2 for measurements abbreviations).

Table S4. Individual significance for each of the 17 cranial measurements used in the sexual dimorphism statistical test performed with MANOVA. The results below were obtained from independent ANOVAS, with Bonferroni correction ($P < 0.003$) to avoid Type I error. MANOVA was only performed in the common bottlenose dolphins of known sex of Group2 from the western South Atlantic Ocean. Measurements with significant *P*-values ($P < 0.003$) are marked with *. Mean and range values are in millimeters (see Table S2 for measurements abbreviation).

Table S5. Mean and range (in millimeters) of five vertebral measurements taken from seven vertebrae of the vertebral column of common bottlenose dolphins of Groups1 and 2 from the western South Atlantic Ocean. Most informative variables for PC1 and PC2 are highlighted in bold. $C_{1–2}$: Atlas-axis; T_1: first thoracic; T_{10}: tenth thoracic; L_1: first lumbar; L_8: eighth lumbar; Ca_1: first caudal; Ca_8: eighth caudal.

Table S6. Comparison of cranial measurements of *Tursiops gephyreus* (from Lahille 1908) and coastal and offshore *Tursiops truncatus* (from the present study). In our

study, Group1 is considered the offshore ecotype and Group2 the coastal ecotype from the western South Atlantic Ocean. Sample sizes (*n*) for each group are provided.

Figure S1. Diagram of the 21 measurements used in the first Principal Component Analysis (PCA). List of cranial measurements with the respective abbreviations in Table S2.

Figure S2. Dorsal view of (A) coastal ecotype (Group2, UFSC1249) and (B) offshore ecotype (Group1, UFSC1415).

Figure S3. Color pattern of the offshore (Group1) specimen UFSC1415 (A–C), coastal (Group2) specimen UDESC0019 (D), and resident estuarine bottlenose dolphin of the same coastal population as UDESC0019 (E–F). A: falcate dorsal fin; B: W mark in the throat region; C: V mark in the genital region; D: triangular dorsal fin; E: throat region; F: V mark in the genital region. The arrows indicate cookiecutter shark bites (*Isistius* sp.).