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Can habitat characteristics shape vertebral morphology in dolphins? An example of two phylogenetically related species from southern South America

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Abstract

Fast swimming pelagic cetacean species have osteological characteristics that promote a more stable spine in comparison to that of coastal species. The Peale's dolphin (*Lagenorhynchus australis*) and the hourglass dolphin (*Lagenorhynchus cruciger*) have a close phylogenetic relationship and are found in coastal and pelagic waters in the Southern Hemisphere, respectively. The aim of this work was to study the relationship between the vertebral column's morphology and its flexibility, across these species of contrasting habitats. Vertebral counts and multiple measurements of each vertebra were used to infer intervertebral flexibility. Bivariate plots and discriminant multivariate analyses were employed to compare each functional region along the vertebral column. Both species displayed a regionalization of the column into three stable regions and two flexible areas, which statistically differ in the proportion of the skeleton occupied in each species. While the Peale's dolphin has rounder vertebrae, associated with higher flexibility, the hourglass dolphin has disk-shaped vertebrae and strongly inclined processes related to high stability. Although the species are closely related phylogenetically, vertebral morphology is influenced by a diverse set of ecological and behavioral factors, reflecting a high degree of vertebral plasticity within the genus.

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Key words: *Lagenorhynchus*, Peale's dolphin, hourglass dolphin, contrasting habitats, biomechanics, vertebral morphology, flexibility, maneuverability, stability.

The genus *Lagenorhynchus* currently includes six species: the white-beaked dolphin (*L. albirostris*), the Atlantic white-sided dolphin (*L. acutus*), the Pacific white-sided dolphin (*L. obliquidens*), the dusky dolphin (*L. obscurus*), the Peale's dolphin (*L. australis*), and the hourglass dolphin (*L. cruciger*) (Committee on Taxonomy 2016). This genus, though, is considered to be polyphyletic (LeDuc *et al.* 1999, Pichler *et al.* 2001, Harling-Cognato and Honeycutt 2006, McGowen 2011, Banguera-Hinestroza *et al.* 2014), and its phylogenetic status among the other cetacean families has been broadly discussed (Caballero *et al.* 2008). Only the latter four species listed above are closely related to each other, based on mitochondrial and nuclear DNA data studies (LeDuc *et al.* 1999, Harling-Cognato and Honeycutt 2006, McGowen 2011, Banguera-Hinestroza *et al.* 2014). These studies suggest that these species should be placed together in a separate genus, *Sagmatias* and strongly support the monophyly of the clade conformed by *L. australis* and *L. cruciger*. Comparative analyses of cranial morphology and vertebral formulas also support these relationships (Miyazaki and Shikano 1997). However, it should be noted that these latter two species show important differences in anatomy and habitat ecology (Goodall *et al.* 1997*a*, *b*, *c*).

L. australis is the most coastal of the three *Lagenorhynchus* species inhabiting the Southern Hemisphere (Goodall *et al.* 1997*a*, *b*; Brownell *et al*. 1998). It inhabits two types of coasts on each side of the South American continent. In the south, it frequents fiords and channels in association with kelp beds (*Macrocystis pyrifera*), where it feeds on demersal and benthic prey (Goodall *et al.* 1997*a*, *b*; Viddi and Lescrauwaet 2005). Along the eastern coast, it inhabits open coasts of the continental shelf, feeding on demersal and pelagic prey (Schiavini *et al.* 1997). Riccialdelli *et al.* (2010) utilized stable isotopes analyses to suggest both benthic (*e.g.*, *Eleginops maclovinus*) and pelagic (*e.g.*, *Odonthestes* spp.) prey as its main food resource. Even though it has been sighted in offshore waters of the Argentinean continental shelf, its abundance is negatively correlated with depth; supporting a preference for shallow waters (Dellabianca *et al.* 2016).

L. cruciger is an oceanic and circumpolar species (Goodall *et al.* 1997*c*, Riccialdelli *et al.* 2010, Dellabianca *et al.* 2012, Santora 2012). Its basic biology and trophic ecology are poorly known due to its oceanic behavior and the small number of specimens found dead on beaches (Brownell and Donahue 1998, Fernández *et al.* 2003, Jefferson *et al.* 2008). Based on the few specimens analyzed, it has been suggested that this species feeds mainly on fish and squid that undertake diurnal migrations (Goodall *et al.* 1997*c*, Fernández *et al.* 2003). Sightings of this species have been mainly reported for areas where water depths are greater than 1,500 m (Goodall 1997, Santora 2012).

There is an association between the flexibility and maneuverability of the cetacean body and swimming speed, food habits, and habitat utilization patterns; as was stated by Fish (2002) for odontocetes and by Woodward (2006) for large whales. Fast swimming oceanic dolphins are characterized to have a relatively stable morphological configuration (*e.g.*, Long *et al.* 1997, Pabst 2000, Fish 2002, Buchholtz and Schur 2004). Systems that stabilize the body in cetaceans can be either active or passive; the latter do not require energy and are basically determined by morphology, including vertebral shape (Long *et al.* 1997, Fish *et al.* 2003).

In this regard, the vertebral column of dolphins is characterized as flexible and highly variable (Long *et al.* 1997, Pabst 2000, Buchholtz and Schur 2004). Vertebral column flexibility is affected by several factors such as the muscles and ligaments; the composition, size, and shape of the intervertebral disks; and the morphology and interference of the vertebrae (Gal 1993, Long *et al*. 1997, Koob and Long 2000). Thus, the force transmission pattern along the length of the vertebral column, and its subsequent movements, are strongly influenced by regional variations in morphology (Gal 1993). The morphological variability along the spine, reinforcing or limiting movements, can be described by investigating the number of vertebrae, centrum shape and size, process structure and orientation, and accessory structures such as metapophyses (Buchholtz and Schur 2004). These latter authors identified certain morphological and ecological attributes, such as low vertebral count, spool-shaped centra, and coastal habits, as primitive; they predicted that a high vertebral count would be associated with disk-shaped centra and pelagic habits. Thus, the shape of the centra, curvature of articular faces, number of intervertebral joints, and vertebral process orientation and length, can be used to determine regions of stability and flexibility within the cetacean vertebral column (Slijper 1936, Buchholtz 2001; Table 1). According to Buchholtz and colleagues (Buchholtz 2001, Buchholtz and Schur 2004, Buchholtz *et al.* 2005), the relative centrum length (RCL) is an accurate descriptor of vertebral morphology that relates to the three variables (*i.e.*, length, height, and width) of a centrum (Table 2). A value near 1 indicates a vertebral centrum with smaller faces and less contact area between adjacent vertebrae, allowing greater angular movements and higher flexibility of the area (Long *et al.* 1997, Buchholtz 2001, Buchholtz and Schur 2004). On the other hand, lower values ($RCL < 0.75$) are associated with large, flat centrum faces; greater contact between adjacent vertebrae; long processes; and stable regions (Buchholtz, 2001, Buchholtz and Schur 2004, Woodward 2006; Table 1). This information offers important clues to the proportion of the column involved in the oscillation and displacement of the flukes, and how the swimming style can vary among species (Buchholtz 2001, Buchholtz and Schur 2004).

The aim of this work was to study the relationship between the vertebral column's flexibility, as inferred from its morphology, and the habitat characteristics of *L. australis* and *L. cruciger*, which have distinctive ecological attributes. We compare between species (1) the proportion of length of a particular vertebral region and the number of vertebrae in each region, (2) osteological features to identify potential differences in the flexibility of the vertebral regions, and (3) results obtained in this study with information regarding their habitat. Our working hypothesis is that *L. australis* displays vertebral features that have been identified to enhance flexibility, whereas *L. cruciger* should show vertebral features associated with enhanced stability.

Table 1. Combination of vertebral features in flexible and stable areas of the vertebral column for cetaceans based on Buchholtz and Schur (2004) and Woodward (2006). RCL: Relative Centrum Length, NP: Neural processes; TP: Transverse processes.

	Flexible area	Stable area
Centrum facets	Small and round (convex)	Large and flat (concave)
Centrum morphology	Spool-shaped	Discoidal
RCL	High	Low
NP	Short and inclined	Long and erect
TP	Short	Long
Metapophyses	Placed low on the NP	May be absent; placed high on the NP

Table 2. Description of the variables included in the study.

Variables	Code	Description
Direct measurements		
Centrum length	CL	Measured ventrally
Centrum width	CW	Measured on the anterior face
Centrum height	CH	Measured on the anterior face
Neural process height	NPH	Vertical distance from tip of neural process to a horizontal line on the dorsal surface of the vertebra
Neural arch length[a]	NAL	Length of the midline from the metapophyses to a horizontal line on the dorsal surface of the vertebra
Neural arch inclination[a]	NAI	Angle between the NAL midline and a horizontal line
Neural spine length[a]	NSL	Length of the midline from tip of the neural process to a horizontal line at the metapophyses
Neural spine inclination[a]	NSI	Angle between the NPL midline and a horizontal line
Neural process lenght[a]	NPL	Sum NAL + NSL
Neural process width at metapophyses[a]	MPW	Length of the horizontal line that crosses the spine at the metapophyses
Neural process width[a]	NPW	At a midpoint between the metapophyses and the center
Metapophyses height[a]	MH	Vertical line from the dorsal surface of the center to the point of insertion of the metapophyses.
Transverse process length[a]	TPL	Length of the midline from the center to the tip of the process
Transverse process inclination[a]	TPI	Angle between the TPL midline and a horizontal line
Transverse process width[a]	TPW	Maximum distance between the tips of a transverse process
Calculated measurements		
Relative centrum length	RCL	Following Buchholtz *et al.* (2001): $CL_i/[(1/2). (CW_i + CH_i)]$.
Relative neural process height	RNPH	NPH_i/CH_i
Transverse process relative width	RTPW	TPW_i/CW_i
Relative Metapophyses height	RMH	MH_i/NPH_i
Metapophyses development	MD	MPW_i/NPW_i

[a] Measurements taken by employing photographs.

MATERIALS AND METHODS

A total of 23 specimens of both sexes, 14 of *L. australis* and 9 of *L. cruciger*, were measured in this study. Specimens were provided by the Goodall collection (RNP) from the Museo Acatushún de Aves y Mamíferos Marinos Australes, (AMMA, Ushuaia, Argentina); Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN, Buenos Aires, Argentina); Centro Nacional Patagónico (CENPAT, Puerto Madryn, Argentina); and the Instituto de la Patagonia de la Universidad de

Magallanes (IPPA, Punta Arenas, Chile) (Table S1). The species included in this work are considered rare, with postcranial skeletons not commonly available for study.

The specimens were classified as physically immature and mature based on the degree of fusion of the vertebral epiphyses according to the criterion proposed by Perrin (1975) and modified by Goodall *et al.* (1988) and Lockyer *et al.* (1988). Specimens included in this study were subadults and adults, belonging to classes 2, 2b, 3, and 3b (Table S1).

To analyze the postcranial skeleton, the column was divided into series based on two different criteria (Fig. 1). The traditional criterion follows Rommel (1990) and divides the skeleton into cervical, thoracic, lumbar, and caudal vertebrae. The functional criterion for cetaceans, proposed by Buchholtz and Schur (2004), considers the vertebral morphology and the change in the direction of the neural spines. It is not widely employed probably due to the amount of work required to identify these regions in museum specimens. Based on this later criterion, traditional lumbar and caudal regions are divided into three functional regions: torso, tail stock, and fluke. The torso comprises all vertebrae between the thoracic region (last rib bearing vertebra) and the first vertebra where CH > CW, the anterior limit of the tail stock (Fig. 1, Table 2). The torso was also divided into three subregions: anterior, mid-, and posterior torso; vertebrae where the neural spine changes its inclination are employed to define the anterior and posterior limits of the mid-torso. Tail stock vertebrae are those where CH > CW and fluke vertebrae are characterized by CW < HC. In order to analyze and quantify the existence of heterogeneity within the thoracic region, in the present work this area was also separated into three sectors: a, b, and c (Fig. 1); the separation was based only on vertebral number allocating four vertebrae in the first two sectors and five or six vertebrae in the last sector, depending on the thoracic count. Thorax subdivisions were employed only for the discriminant analyses (see below). This criterion of functional subdivision provides a thorough analysis of the morphological variations within each area, focusing on those with functional implications for swimming that could be masked under the traditional criterion (Buchholtz and Schur 2004).

On each specimen, measurements were made on every postcervical vertebra (from the first thoracic vertebra) for a total of 15 osteological variables in lateral (left) and dorsal view, following Buchholtz (2001), Buchholtz and Schur (2004), Buchholtz *et al.* (2005), and Woodward (2006) (Fig. 2, Table 2). For the cervical vertebrae, only centrum length was measured. Linear measurements were taken directly using a digital caliper to the nearest 0.1 mm, and indirectly from lateral and dorsal photographs containing a scale bar and using software Image J (Ferreira and Rasband 2012, Schneider *et al.* 2012). Indirect measurements were also employed to obtain angular values. For the vertebral processes, angular values >90° were considered as an anterior inclination, whereas values <90° were considered as a posterior inclination (Fig. 2). Based on these measurements, five relative variables were calculated (Table 2).

For each specimen, total centrum length (TCL) was calculated as the sum of CL for the whole vertebral column (Table S1). TCL is not the real length of the vertebral column, since intervertebral disc space was not accounted for. For each region, the length, the number of vertebrae, and the proportion of TCL it represented were determined. For incomplete specimens, TCL was estimated following Buchholtz *et al.* (2005), with the regression equation calculated for each species as follows: *L. australis*: $y = 45.057x + 25.693$, $r^2 = 0.9304$, $n = 7$; *L. cruciger*: $y = 38.195x + 37.414$, $r^2 =$

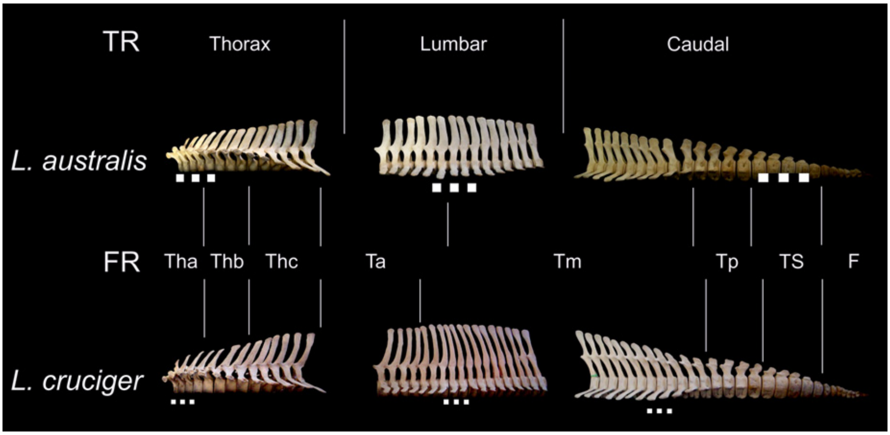

Figure 1. Vertebral series for the analysis of postcranial skeleton for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*), according to the traditional series (TR) and the functional series (FR). Cervical region is not shown. Th (a, b, c): thorax (a, b, c); Ta: anterior torso; Tm: mid-torso; Tp: posterior torso; TS: tail stock; F: fluke. Scale = 10 cm.

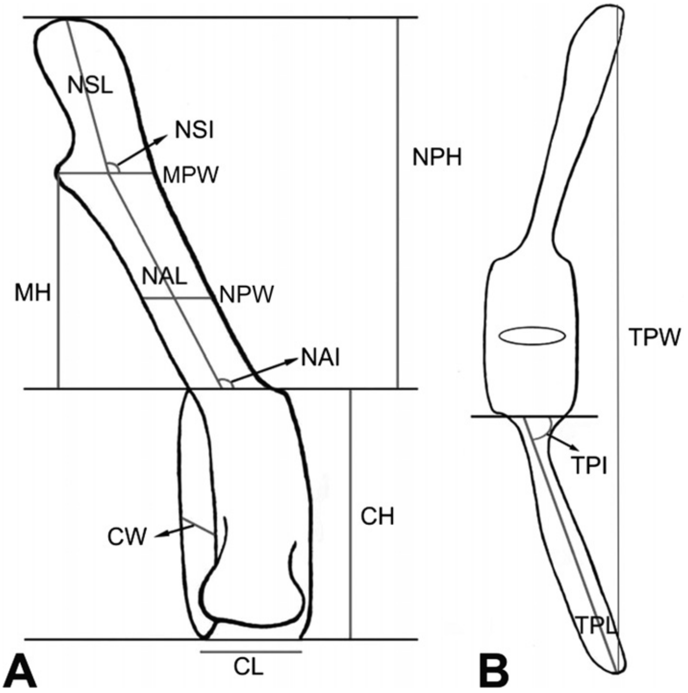

Figure 2. Vertebral variables included in the study. A, left lateral view. B, dorsal view. Codes are explained in Table 2.

0.9015, $n = 6$, where y = TCL (in cm), x = average CL (in cm) for vertebrae numbers 8–21, and n = number of complete specimens measured.

Vertebral count varies among individuals and species (Goodall *et al.* 1997*a*, *c*; Miyazaki and Shikano 1997) (Table S1). For that reason, the values employed for the statistical analyses were the mean values of each variable estimated for both the traditional and functional regions, and also for the subregions.

A Kolmogorov-Smirnov test was used to analyze the normality for all variables, while a Shapiro-Wilks test was employed in order to test homoscedasticity (Statistica v7.0, Statsoft Inc., Tulsa, OK). Mean centrum length and interspecific variation in the proportion of TCL for each particular region in both species were determined, and tested for differences between species using a Mann-Whitney *U* test. Interspecific differences in vertebral counts for each region were evaluated by comparing the mode of the number of vertebrae for each region.

Interspecific variation in the components of the vertebral column was evaluated with bivariate plots, analyzing the different variables in relation to the total centrum length (TCL). Graphic representations were generated using Microsoft Excel 2007.

Morphological differentiation between species was also evaluated by discriminant multivariate analysis (DA) (Bookstein *et al.* 1985, Strauss 1985, dos Reis *et al.* 1990). The following log transformed variables (Table 2) were used in the canonical discriminant analysis (CDA): RCL, RNPH, RTPW, MD, MH, NAL, NSL, NPL, and TPL. Angular variables (NAI, NSI, and TPI) were also analyzed with no data transformation (CDA_{AV}). Wilk's lambda values (λ) were used as classification criteria to obtain the best discriminant models. The percentage of cases properly classified into groups (classification matrix) and the significance values for each model were also evaluated. Square Mahalanobis distances between groups were calculated based on the best discriminant model for each vertebral region (dos Reis *et al.* 1990). Variables or specimens with missing data were excluded because multivariate analyses are particularly sensitive to this (Tabachnick and Fidel 1996). All statistical analyses were performed with the software Statistica v7.0 (Statsoft Inc., Tulsa, OK).

Results

For the analyses involving processes, several specimens were not measured. Three *L. australis* specimens had damaged neural spines and transverse processes, and two other specimens had their transverse processes broken. Therefore, the number of *L. australis* specimens used in the analyses of the processes was eleven and nine for the neural and transverse processes, respectively. For *L. cruciger* analyses, all the variables were measured.

Variation of the Vertebral Regions

Using the traditionally defined regions, the cervical and thoracic traditional regions presented the same modal number of vertebrae for the two species, whereas the lumbar and caudal regions differed in this number (Table 3A). As it was expected based on size differences between species (Table S1), all the traditional regions in *L. australis* were longer than those in *L. cruciger*, but only the cervical region displayed a significant difference in the relative percentage of the skeleton that it occupies (Table 3A). For the functional regions (Table 3B), interspecific differences in the modal number of vertebrae were observed only in the anterior and mid-subregions of

Table 3. Regional distribution of the column for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*). (A) Traditional regions; (B) Functional regions.

	Cervical			Thoracic			Lumbar			Caudal		
Species	V	CLr **	% *	V	CLr **	%	V	CLr **	%	V	CLr **	%
(A) Traditional regions												
L. australis	7	5.01	3.78	13	31	24.2	16	38.1	28.9	31	58.8	44.1
L. cruciger	7	4.16	3.56	13	27.1	23.2	18	34.4	29.4	33	51.7	44.2

							Torso																	
	Neck			Thorax			Anterior			Middle			Posterior			Total torso			Tail stock			Fluke		
Species	V	CLr	% *	V	CLr	%	V	CLr **	% **	V	CLr **	% **	V	CLr *	% **	V	CLr	% *	V	CLr	% **	V	CLr	% **
(B) Functional regions																								
L. australis	7	5.01	3.78	13	31	24.2	6	17.8	13.4	19	43.9	33	4	10.9	8.21	29	72.6	54.61	5	13.6	10.2	12	10.7	8.08
L. cruciger	7	4.16	3.56	13	27.1	23.2	4	8.23	7.03	26	45.7	39.1	4	8.84	7.55	34	62.8	53.68	5	14.2	12.1	13	9.17	7.84

V: mode of the number of vertebrae in each region; CLr: mean value of centrum length for each region; %: mean value of the percentage of total centrum length (TCL) that each region represents. The levels of significance obtained by the Mann-Whitney test for the difference of the mean values of each region are shown: $*P < 0.05$; $**P < 0.01$.

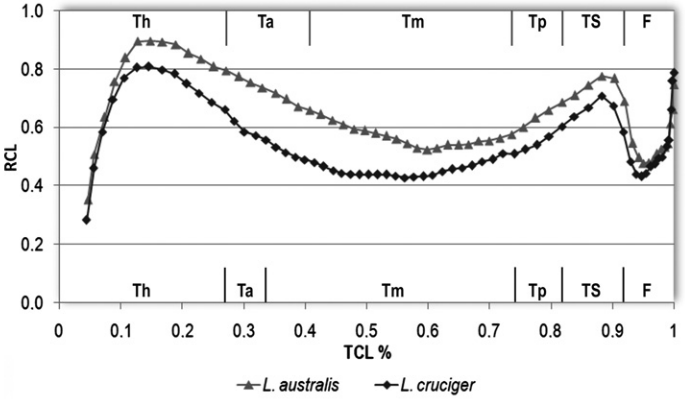

Figure 3. Variation in the mean values of relative centrum length (RCL) with the proportion of total centrum length (TCL %) for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*). The different regions of the vertebral column are separated by vertical lines; above for *L. australis*, and below for *L. cruciger*. Th: thorax; Ta: anterior torso; Tm: mid-torso; Tp: posterior torso; TS: tail stock; F: fluke.

the torso. The anterior and posterior torso were significantly longer in *L. australis*, and the mid-torso was significantly longer in *L. cruciger*. The mean value of the proportion of the skeleton occupied by each region displayed significant differences between species for all functional regions except the thorax. The anterior and posterior torso occupied a greater proportion of the skeleton in *L. australis*, while the mid-torso represented a larger proportion in *L. cruciger*. *L. australis* displayed significantly higher percentage values for the total torso and flukes, while *L. cruciger* displayed significantly higher percentage values for the tailstock.

Variation in Vertebral Morphology

The pattern of variation of the mean value for each character, showing differences between species and their relative position in the functional regions may be seen in Figures 3–6. The mean values of relative centrum length (RCL) tended to be greater for *L. australis* than for *L. cruciger* along the length of the skeleton (Fig. 3). The regions that displayed maximum RCL mean values were in the same relative position in both species. In *L. australis*, only a small portion of the mid-torso had RCL mean values below 0.6. In *L. cruciger*, all three subregions of the torso had RCL mean values below 0.6. These trends in the torso are translated into differences in vertebral shape between species, having *L. cruciger* vertebrae markedly disk-shaped all along the torso.

For both species metapophyses appeared at a relatively low position, and remained low all along the thorax and the anterior torso (Fig. 4). In the mid-torso, the relative position of the metapophyses increased. Variation of RMH in the posterior torso may be exaggerated due to the length and inclination of the neural processes in those areas. Main differences between species were in the posterior half of the mid-torso, where metapophyses were placed lower in *L. australis* than in *L. cruciger*, and in the posterior torso, where an opposite pattern was observed. For both species, metapophyses

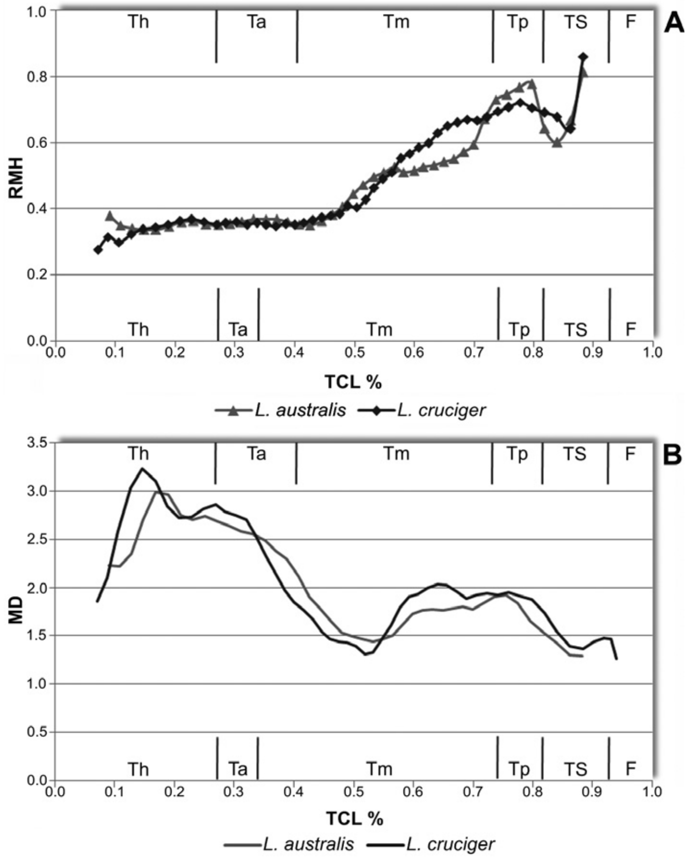

Figure 4. Variation in the: (A) mean values of relative metapophyses height (RMH); and (B) trend line for metapophyses development (MD), with the proportion of total centrum length (TCL %) for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*). The different regions of the vertebral column are separated by vertical lines, see Figure 3.

development (MD) values tended to be higher in both the thoracic region and the anterior torso than in other areas of the column. There was an area of minimum values in the mid-torso and then an increase in the posterior half of that region. In *L. australis*, metapophyses were better developed in the posterior torso than in the mid-torso. In contrast, *L. cruciger* showed similar values of MD from the posterior half of the mid-torso up to the begging of the tail stock (Fig. 4).

Development of the processes relative to the centrum size was estimated as the relative width of the transverse process (RTPW) and the relative height of the neural process (RNPH) (Fig. 5). RTPW values tended to be lower in *L. australis* than in *L. cruciger* (maximum mean value RTPW = 5.25 and 6.03, respectively), particularly in the thorax and the anterior torso (Fig. 5A). Along the mid-torso, the decrease of the RTPW mean values in *L. australis* tended to be less uniform than in *L. cruciger*, especially in the second half of the mid-torso (between 56% and 74% of the total centrum length). Even though the degree of development of the neural process was similar in

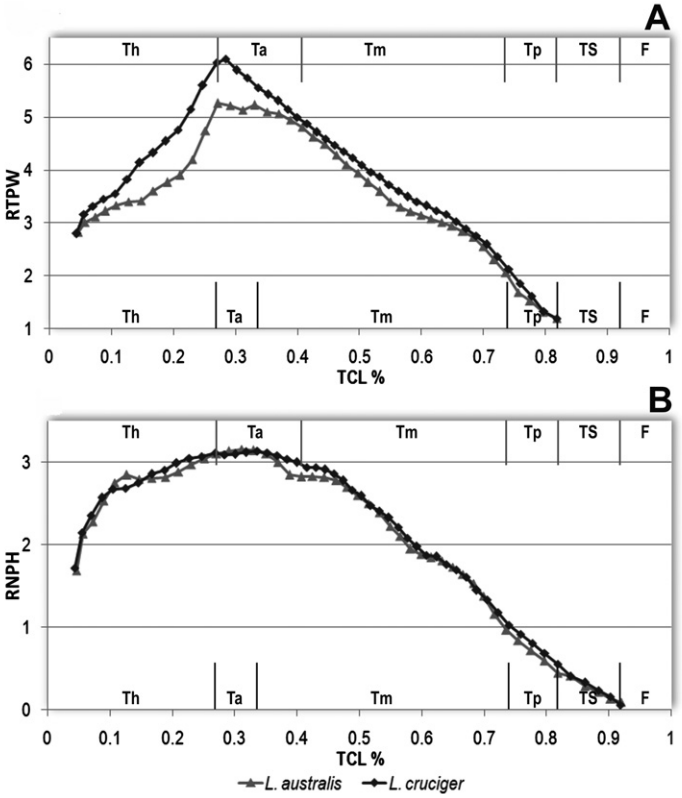

Figure 5. Variation in the mean values of: (A) relative transverse process width (RTPW); and (B) relative neural process height (RNPH) versus the proportion of total centrum length (TCL %) for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*). The different studied regions of the vertebral column are separated by vertical lines, see Figure 3.

both species, the variation pattern of the RNPH values tended to be slightly different for each species, being less uniform in *L. australis* (Fig. 5B).

In both species, mean values of the neural spine inclination (NSI) in the first region of the thorax were <90° (posterior inclination) (Fig. 6A, 7). NSI values decreased, creating an area of a maximum posterior inclination of the neural spines (*L. australis*: minimum NSI = 57.3°; *L. cruciger*: minimum NSI = 54.2°) in the mid-thorax. The first vertebra at which the NSI is 90°, signaling the anterior/mid-torso transition, was located more caudally in *L. australis* than in *L. cruciger* (Fig. 7, position A). NSI values increased and remained similar all along the mid-torso, with lower values in *L. australis* (97.3° < NSI < 101.5°) than in *L. cruciger* (103.7° < NSI < 107.1°). The second change in the mean values of the inclination of the neural spines signals the transition between the mid- and the posterior torso, which occurred at the same relative position in both species (Fig. 6A, position B).

Mean values for the neural arch inclination (NAI) also displayed interspecific variation along the vertebral column, decreasing along the length of the thorax with a region of minimum values in the anterior torso (88° < NAI < 89°), and tending to

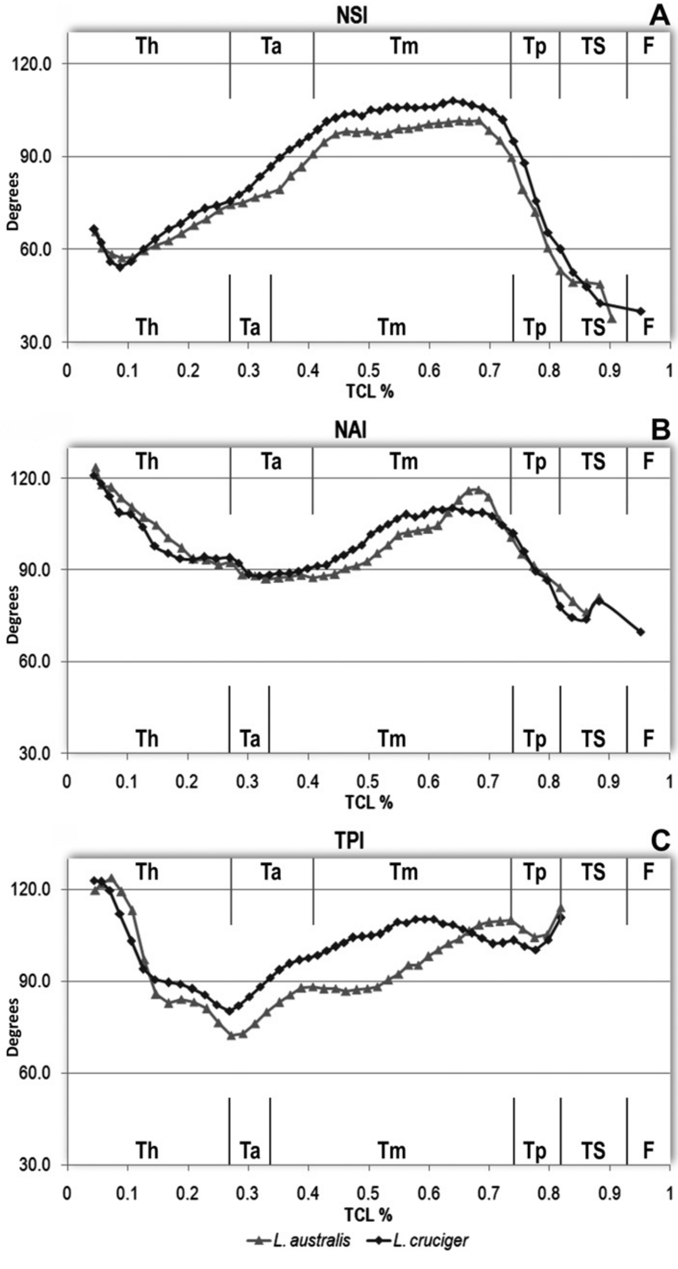

Figure 6. Variation in the inclination mean values with the proportion of total centrum length (TCL %) for the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*). (A) neural spine inclination (NSI), (B) neural arch inclination (NAI), and (C) transverse process inclination (TPI). The different regions of the vertebral column are separated by vertical lines, see Figure 3. Angular values <90° indicate a posterior inclination, values >90° indicate an anterior inclination.

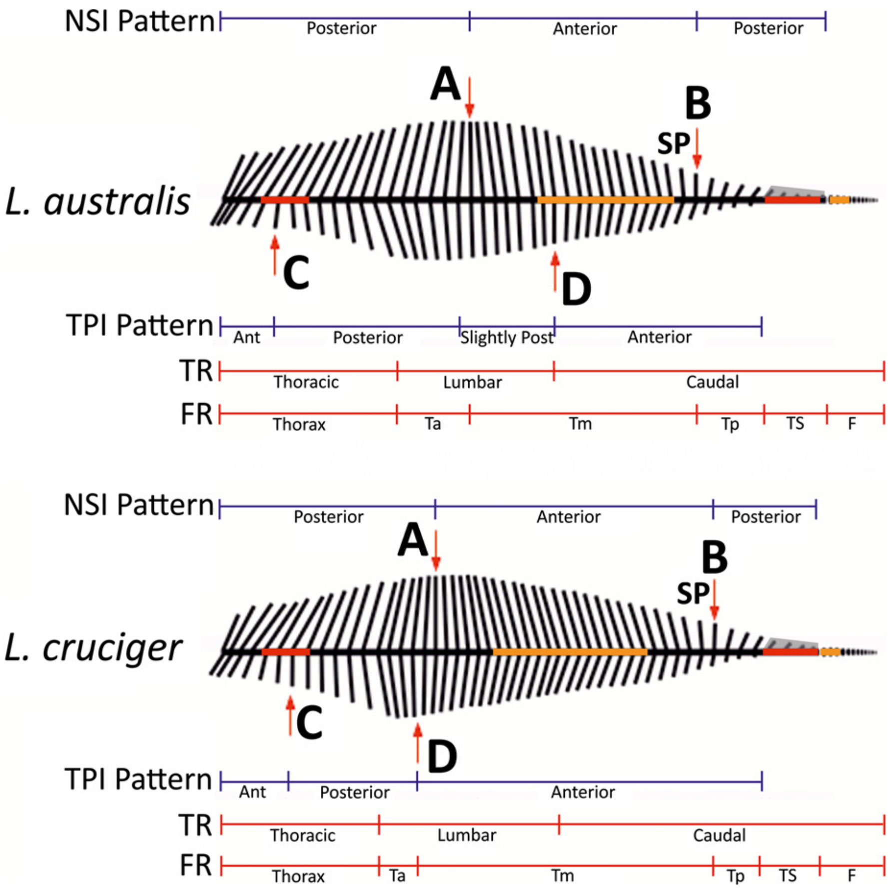

Figure 7. Schematic of the neural spines (superior lines) and the transverse processes (inferior lines) in the Peale's dolphin (*L. australis*) and the hourglass dolphin (*L. cruciger*), from T-1 (8th vertebrae) through all caudal vertebrae. The scheme is drawn in proportion to total centrum length, angles are accurate. Red bars: flexible zones; orange bars: stables zones; gray shade: tail stock; NSI: neural spine inclination; TPI: transverse process inclination; SP: synclinal point; TR: traditional regions; FR: functional regions; T(a, m, p): anterior, middle, and posterior torso; TS: tail stock; F: fluke.

involve a greater proportion of the skeleton in *L. australis* than in *L. cruciger* (15% and 10% of the TCL, respectively; Fig. 6B). Maximum values of NAI (NAI = 116.4°) for *L. australis* were found in the last third of the mid-torso; and tended to be greater than those observed in the similar region for *L. cruciger*. In this latter species, the highest NAI values were observed throughout the caudal half of the mid-torso ($93.8° < \text{NAI} < 109.9°$).

Mean values for transverse process inclination (TPI), as observed in NSI, had two points along the column where the values of the angles inverted (Fig. 6C; Fig. 7, positions C and D). In the anterior region of the thorax, the transverse processes had inclination values that were higher than 90° (anterior inclination). Inclination values of this region tended to be slightly higher in *L. australis* ($97.1° < \text{TPI} < 123.9°$) than in *L. cruciger* ($93.9^\circ < \mathrm{TPI} < 119.6^\circ$). These values decreased to a minimum (*L. australis*: TPI = 72.2°; *L. cruciger*: TPI = 80.3°) at the boundary thorax/anterior torso. In the cephalic half of the torso, the transverse processes of *L. australis* were inclined posteriorly towards the tail, increasing in the anterior torso but close to 90° in the anterior half of the mid-torso. Maximum anterior inclination values ($108.4^\circ < \mathrm{TPI} < 109.8^\circ$) were observed only in the last region of the mid-torso. In *L. cruciger* the change in the processes orientation was in the middle section of the anterior torso, with a larger proportion of the skeleton with transverse processes anteriorly inclined ($95.7^\circ < \mathrm{TPI} < 110.3^\circ$). In this species, TPI values reached a maximum in the posterior half of the mid-torso.

Discriminant models of the vertebral column by regions showed high interspecific differentiationin morphological characters involved in the posterior thoracic region and the torso; especially in the mid-torso (maximum values of Mahalanobis distances [DH] between groups; considering the species as grouping variable; Table 4). The anterior area of the thorax did not display multivariate differentiation between

Table 4. Models of the variables for interspecific differentiation. (A) linear variables; (B) angular variables.

R	*N*	λ	*F*(v/df)	PS	%C	VS	DH
(A) Linear variables							
Tha	18	0.29	2.15 (9/8)	*P* = 0.15	94		0.15
Thb	18	0.08	13.42 (8/9)	**	100	RCL* RNPH* NPL*	42.41
Thc	18	0.073	18.03 (7/10)	**	100	RCL** RNPH*	44.99
Ta	18	0.041	25.88 (8/9)	**	100	RCL** RNPH* TPL*	81.81
Tm	18	0.022	63.05 (7/10)	**	100	RCL** RNPH** RTPW* NAL*	156.94
Tp	17	0.15	4.4 (9/7)	*	100		20.05
TS	18	0.26	4.12 (7/10)	*	94,44	RCL*	10.38
(B) Angular variables							
Tha	19	0.78	1.4 (3/15)	*P* = 0.29	63.15		0.99
Thb	19	0.69	2.2 (3/15)	*P* = 0.13	73.68		0.13
Thc	19	0.42	6.8 (3/15)	**	90	TPI*	4.88
Ta	19	0.57	3.65 (3/15)	*	84	TPI*	2.62
Tm	19	0.3	11.73 (3/15)	**	94.8	TPI**	8.42
Tp	19	0.89	0.58 (3/15)	*P* = 0.63	63.16		0.42
TS	19	0.98	0.14 (2/16)	*P* = 0.86	57.89		0.068

R: corporal region; Th (a, b, c): thorax a, b and c; T(a, m, p): anterior, mid- and posterior Torso; TS: tail stock; *N*: number of individuals; λ: Wilks' Lambda; *F*: Fisher's *F*, (v/df): number of variables/degrees of freedom; PS: model significance, including all the variables; %C: percentage of correct classification of the cases; VS: significance of each variable; DH: Mahalanobis' square distance between groups. Asterisk indicates differences statistically significant (*$P < 0.05$) and highly significant (**$P < 0.001$).

species. Although, the posterior torso displayed significant discrimination between species, none of the variables explained the observed interspecific differentiation, with DH between groups as one of the smallest for all the proposed models (Table 4A). The tail stock region also showed discrimination between species by differences in the relative centrum length.

Regarding the angular variables (CDA_{AV}), TPI explains the multivariate discrimination in the thorax c, and in the anterior and mid-torso of the vertebral column. None of the other variables were significant in the other regions studied (Table 4B). The different tendencies in processes inclination observed graphically were not supported by the discriminant models.

DISCUSSION

Interspecific Variation Relative to the Locomotor Performance

Factors affecting locomotion and swimming involve a complex interaction between the axial skeleton and its associated muscles, ligaments, and subdermal connective sheath (Long *et al.* 1997, Pabst 2000, Buchholtz and Schur 2004). Thus, morphological characterization of the vertebral column is a useful step to gain insights into the locomotor performance of cetacean species occurring in contrasting environments. Here, we present a complete characterization for each functional region along vertebral column of two little-studied dolphin species of the genus *Lagenorhynchus* occurring in contrasting habitats, *L. australis* and *L. cruciger*, with emphasis on the morphological differences of biomechanical importance.

Most features of the anterior thoracic region were similar in both species. This was to be expected as the thorax has a major function in assisting breathing during locomotion in dolphins (Cotten *et al.* 2008) and differences in thorax flexibility have not been found in species with different habits (Piscitelli *et al.* 2010). In the thorax, two highly stable areas were separated by a flexible zone with a change in direction of the transverse processes (see Fig. 7). For certain species, such as the bottlenose dolphin (*Tursiops truncatus*) and the common dolphin (*Delphinus delphis*), thoracic stability is provided by the development and morphology of neural and transverse processes and by the great development of the metapophyses (Long *et al.* 1997, Pabst 2000). General morphology of *L. australis*, in conjunction with the inclination of both neural and transverse processes, would produce the required interference between adjacent vertebrae, which could counteract the relative high flexibility of this species. Moreover, there are seven double-headed ribs on each side for *L. australis* (MCM, personal observation on 11 specimens) and no more than six double-headed ribs in *L. cruciger* (Marchesi *et al.* 2016). These extra double-headed ribs could further counteract the higher flexibility of the thorax of *L. australis*. Cotten *et al.* (2008) proposed that intercostal muscles may provide stability to the thorax during locomotion but no muscular analysis were done for the studied species. In both species studied here, the development and morphology of processes and the presence of rib facets are the only osteological evidence of the thorax-torso discontinuity (Fig. 1, Fig. S1). The transverse processes reach their maximum relative length at the boundary of the thorax-torso (Fig. 5), increasing the surface area of attachment for the axial muscles, longissimus and multifidus. It is in the anterior torso that the longissimus muscle develops large forces that are transmitted to the posterior region of the column (Pabst 1993).

According to Long *et al.* (1997), in cetaceans, the design of a stiff vertebral segment is characterized by the presence of short vertebra and relatively large intervertebral disc diameter (*i.e.,* wide centra). For the torso, both species exhibited disk-shaped vertebrae and little variation in the morphology of the vertebral centra (Fig. 3). This disk-shaped morphology is found in a more anterior position in *L. cruciger*, likely increasing stability in this region.

As described by Buchholtz *et al.* 2005, the term "lumbarization" refers to vertebrae that have centrum dimensions, neural process heights, and neural spine inclinations typical of the lumbar region. Hence, the classical lumbar series in the species studied here are expanded because their high number of vertebrae and the "lumbarization" of adjacent vertebrae, especially in *L. cruciger*. This expanded mid-region provides skeletal support for muscles involved in the production of forces acting on the tailstock, the longissimus and its caudal extension, the extensor caudae lateralis (Pabst 1990). Consequently, lumbar stability may help to reduce recoil and drag movements, while long neural processes provide an increased mechanical advantage to generate greater locomotor muscle forces (Woodward 2006). Thus, the high differentiation in the anterior and mid-torso between species denotes the most important differences from a biomechanical perspective (Table 4A).

On the other hand, the metapophyses in cetaceans provide insertion sites for the tendinous systems of the longissimus and multifidus muscles; the main effectors of the column movement (Pabst 2000). An increase in their height (HM) enlarges the lever arm of the muscles, increasing the mechanical advantage for propulsion but limiting angular rotation (Buchholtz and Schur 2004). In the posterior half of the mid-torso, the difference in the relative height of the metapophyses may be indicating greater angular rotation potential in *L. australi*s that could be translated in a greater movement potential of the posterior body. The opposite was observed for *L. cruciger* where high metapophyses might be limiting angular rotation, thus enhancing stability of the region in this species. The trend of similar metapophyseal development (DM) along most of the vertebral column could be indicating that the attachment sites for the locomotory muscles are similar in both species (Fig. 4).

Furthermore, the morphology and inclination of the processes in the vertebrae of the mid-torso also reflect important functional differences between species (Fig. 5, 6). Variation within the subareas of the mid-torso in *L. australis* appears to be associated with a functional regionalization, which would allow more flexibility in comparison to *L. cruciger*. Only the posterior half of the mid-torso of the former species presents characteristics associated with stability. This would represent an advantage, increasing the dorso-ventral and rotational movement of the tail, and thus, maneuverability. On the other hand, *L. cruciger* has osteological characteristics associated with stability all along the mid-torso. Its long and strongly inclined processes are translated into a highly stable torso that functions as a unit in the generation of the propulsive forces.

The transition between the mid- and posterior regions of the torso is signaled by the reversion of the processes orientation, creating the synclinal point; where the angular divergence, and the distance between adjacent spines is the greatest (Buchholtz and Schur 2004). This area represents the transition between the stable mid-torso and the flexible tail stock, and it could be the main area where the forces for changing the direction of the flukes are generated. The slight increase in the length of the transverse processes in *L. australis* just before the synclinal point seem to reflect the greater development of the muscles involved in the rotational and lateral movements of the tail, giving to this species certain advantage for the rotation of the flukes.

The tail stock is a region of high flexibility and rotational potential, with vertebral centra that are long, tall, and with convex faces. Due to the absence or reduction of processes, movement interference in this region is highly reduced (Fig. 5). It should be noted that many cetacean species swim by oscillating the caudal third of their body (Fish 1993), where the column is less stiff when compared to the lumbo-caudal joint (Long *et al.* 1997). In this region, the elongation of the centra maximizes the possible vertical displacement allowing the dorso-ventral movements of the tail stock (Long *et al.* 1997, Buchholtz and Schur 2004). Vertebral morphology of the tail stock region differs between the species studied, although little statistical differentiation of this region between both species was observed (Table 4). Higher values of RCL in *L. australis* indicate that this region could be more flexible than in *L. cruciger* (Fig. 3). In the former species, the trend of the development of the neural processes and metapophyses could be associated with a greater potential movement of the fluke.

Even though the fluke has a first portion with vertebral morphology that denotes a high stability (Fig. 7), *L. australis* seems to have a higher degree of flexibility than *L. cruciger*. Long (1992) found that the precaudal intervertebral disks of the blue marlin (*Makaira nigricans*) are less stiff than the caudal joints and he proposed that this high rigidity might accelerate the undulatory wave during swimming. Similarly, this small stable area in *L. australis* and *L. cruciger* would accelerate the oscillation of the tail. On the other hand, the greater stability of this area might be counteracted by the high degree of flexibility of the intervertebral disks in both the species studied. At least in the common dolphin, the intervertebral joint of the base of the fluke is less stiff than any other in the body (Long *et al.* 1997). In addition, in the spinner dolphin (*Stenella attenuatta*) and the common dolphin, the thickness of the intervertebral disks is greater at the ends of the column than in the middle area (Crovetto 1991, Long *et al.* 1997). The species included in this study have a similar pattern in the thickness variation of the intervertebral disks (MCM, personal observation) and this could be counteracting stability provided by vertebral morphology in these species of dolphins. Fluke vertebrae morphology seems to be highly conservative in both species studied, being vertebrae similar for both species. The vertebrae centra of the fluke are short, low, and wide, with rectangular section; and are immersed in the cartilage that supports the flukes. A similar pattern has been observed in the caudal area of the bottlenose dolphin (MCM, personal observation), a species with coastal and off-shore forms that have been found to have differences in vertebral morphology (Costa *et al.* 2016), suggesting that fluke morphology might be highly preserved among delphinid taxa.

Functional Morphological Analysis

In this work, we propose that morphological differences in the column design in these cetacean species may have been molded by the selection pressures associated with their preferred habitat characteristics (complex coastal habitats *vs*. pelagic environments), which directly impact their behavioral performance. As was suggested by Fish (2002), features affecting the flexibility and maneuverability could be primarily associated with both feeding behaviors and type of habitat. There is a tradeoff between the energetic cost of recoil movements during routine movements and the labor required to maneuver (Weihs 2002). In this sense, flexibility with slow and precise maneuvering is observed in cetacean species occurring in more complex habitats, whereas high-speed maneuvers are performed by cetaceans in the pelagic environment (Fish 2002).

Vertebral morphology along the column of both species studied here suggests a serial conformation of a stiff mid- and posterior torso, a flexible tail stock and the stable base just anterior to the insertion of the flukes (Fig. 7). According to Pabst (2000), this seems to be a functionally significant design that controls the flexural pattern of the body in vertebrates of steady swimming. Both species show a regionalization of the column into three stable regions (cervical region, mid-torso, and the base of the fluke) and two flexible areas (mid-thorax and tail stock). Buchholtz and Schur (2004), in their analyses of delphinids osteology, observed similar patterns, suggesting that more derived columns display a greater "regionalization" of the column than more primitive species. These authors qualified the vertebral column of the genus *Lagenorhynchus* as highly derived, based on total count and vertebral morphology. Curren *et al.* (1994) described the Atlantic white-sided dolphin (*L. acutus*) as a fast swimming species that produces wider turns when compared with a coastal species. The Pacific white-sided dolphin and the Commerson's dolphin (*Cephalorhynchus commersonii*), two species closely related to those included in this work, have restricted flexibility and produce faster and wider turns in comparison to cetaceans with high maneuverability such as the river dolphin (*Inia* sp.; Fish 2002). For the former two species, Buchholtz and Schur (2004) reported similar patterns of vertebral variation to those observed in this work, having the first one vertebral columns with characteristics that are considered derived, typical of fast-swimming species.

Costa *et al.* (2016) studied both coastal and offshore forms of bottlenose dolphins in the southwestern Atlantic and found significant differences in vertebral morphology that coincide with some of our findings. The offshore form showed vertebrae shorter (*i.e.*, disk-shaped) and higher total counts than the coastal form. As in that case, even though both species in our study are closely related and considered highly derived, there are substantial differences that allow us to characterize each species skeleton and associated them with differences in ecological habits.

In contrast to our results, Viglino *et al.* (2014) found no significant association between vertebral morphology and habitat in seven species of dolphins, including *L. cruciger*. This result could be explained by the low number of specimens studied, with the concomitant disregard of intraspecific variation, and by the large phylogenetic distances of the species analyzed. Our detailed description of vertebral morphology in these species, evidencing not only differences in the size of the structures but in their relative development, and our functional interpretations of the results reaffirm that association between vertebral morphology and habitat should be analyzed from a different approach than the traditional perspective.

Our results suggest that *L. australis* skeleton has vertebral features associated with greater flexibility (*e.g.*, longer, less discoidal vertebrae). The fact that the highly stable cervical region represents a greater proportion of the skeleton in *L. australis* than in *L. cruciger* might be indicating a trade-off between stabilizing the anterior part of the body and the area where the swimming muscles are inserted. The greater regionalization of *L. australis* column seems to reflect a greater diversity of movements in the mid-torso, suggesting a greater maneuverability. Dolphins are tunniform swimmers and they do not use a mobile wave to generate thrust (Long *et al.* 1997). Despite this, the greater differentiation of the torso into subregions, its lower stability, the great flexibility of the tail stock and the high stability at the base of the fluke indicate a type of swimming in which a larger area of the column is involved in movement. Given the morphology and inclination of the processes, the areas where the rotational potential is greater are larger in *L. australis* than in *L. cruciger*. These areas could allow this species to change the angle of attack of mandibles and tail with relative independence, increasing the diversity of head movements, and thus increasing the efficiency to capture prey in complex coastal habitats. The osteological features observed in *L. australis* specimens would be indicating that this species might sacrifice speed for maneuverability, a necessary requirement for its coastal habitat. A larger relative area of the fluke would benefit this coastal species since high maneuverability requirements need flukes with greater areas in relation to the body volume (Woodward 2006). Altogether, the greater flexibility leads to an increased maneuverability that could be beneficial for foraging in highly heterogeneous environments or for social behavior such as those of group feeding (Woodward 2006). The kelp beds of the algae *Macrocystis pyrifera*, where this species is frequently seen, and the coastal topography place numerous barriers to straight movements (Viddi and Lescrauwaet 2005). Moreover, capturing benthic prey requires turns in limited spaces that would be mostly restricted if the column was more stable, as it was observed for baleen whales by Woodward (2006).

L. cruciger has markedly disk-shaped vertebrae all along the column. The stability in the mid-torso is reinforced both for the "lumbarization" of the anterior and posterior vertebrae and for the higher vertebral count in the region. Besides this, the morphology of the processes and the metapophyses suggest a greater mechanical advantage for the swimming muscles inserted mainly in the mid- and posterior torso. At the same time, this morphology results in greater interference between adjacent vertebrae, furthermore increasing the stability and limiting notably rotational potential in this region. As the mid-torso involves a larger proportion of the skeleton than in *L. australis*, the stability of *L. cruciger* column would be enhanced, acting as an "oscillatory beam" and hypothetically storing potential energy. This elastic energy could replace part of the muscle work required to accelerate or decelerate the fluke, acting as a spring (Pabst 1996, Long *et al.* 1997). As in other pelagic cetacean species, fluke displacements are produced only by flexions of the peduncle that oscillates from a highly stable region. Even though in *L. cruciger* most of the skeleton is relatively stable, the flexible tail stock represents a larger proportion of the skeleton than in *L. australis*. This would increase the degree of vertical displacement of the fluke, increasing propulsion capacity.

The main results of this work are in agreement with those proposed by Fish and Rohr (1999) and by Fish (2002), who suggested that the more stable the design the more favored these animals can be when foraging or traveling in pelagic habitats. A morphological design mostly adapted for stability during swimming would contribute in minimizing energy expenditure and increasing thrust efficiency for steady swimming during feeding behaviors and migration (Fish and Rohr 1999, Fish 2002). The greater stability of *L. cruciger* could also be involved in reducing the effect of external forces while this species swims in the turbulent waters that it frequents.

Vertebral morphology of each species is distinctive and seems to be related with its trophic niche and the habitat it occupies. As both species are closely related phylogenetically, they would be reflecting a high degree of plasticity in vertebral morphology. Specializations of the vertebral columns seem to reflect particular adaptations to different habitats (coastal and oceanic), thus maximizing the available resources.

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Supporting Information

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Figure S1. Dorsal view of the thoracic, lumbar, and caudal regions of the Peale's dolphin (*L. australis*) (specimen RNP 269) and the hourglass dolphin (*L. cruciger*) (specimen RNP 2366). Scale = 10 cm.

Table S1. List of specimens included in the study. TC: total count; PM: physical maturity based on Goodall *et al.* 1988. TCL: total centrum length. TCL for incomplete animals is shown between parentheses and was estimated following Buchholtz *et al.* 2005. Vertebral formulas for *L. cruciger* from Marchesi *et al.* 2016. CNPMAMM: Centro Nacional Patagónico; IPMI: Instituto de la Patagonia; MACN: Museo Argentino de Ciencias Naturales: RNP: Museo Acatushún (see Materials and Methods).