Patterns of morphological variation of the vertebral column in dolphins

M. Viglino, D. A. Flores, M. D. Ercoli & A. Álvarez

División Mastozoología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' – CONICET, Ciudad Autónoma de Buenos Aires, Argentina

Abstract

Cetaceans swim by the alternate action of their epiaxial and hypaxial muscles and their propulsive movements are confined to the vertical plane. Changes in the shape and mechanical properties of vertebrae strongly affect their function during oscillatory swimming. The first objective of this study was to provide a quantitative characterization of vertebral morphology in representatives of the Delphinidae and Pontoporiidae families. A novel morphometric approach was applied, using nine vertebral measurements and three indices. The second objective was to assess the relationship between morphology and both habitat and size through regression analyses. The phylogenetic structure of the distribution of characters was also explored by estimating phylogenetic signal. No relationship was found between morphology and habitat or size, but vertebral measurements and indices showed a significant phylogenetic signal. Morphological profiles indicated that coastal and oceanic delphinid species had a conservative regionalization of the vertebral column. All delphinid species showed discoidal centra morphology, while *Pontoporia blainvillei* presented a spool-shaped morphology. Differences in vertebral morphology and inferred muscular architecture between *P. blainvillei* and delphinids could indicate distinct dynamics of vertebral movement during swimming. However, other complex and specific functional relationships and life-history traits may also be influencing vertebral morphology. The detailed study of the complex evolutionary history of lineages could bring to light other clarifying dimensions for understanding morphological evolution in odontocetes.

Introduction

The cetacean body plan originated in the late-middle Eocene to the late Oligocene within basal Pelagiceti (Basilosauridae) that presented anatomical features that indicate they were fully aquatic (Uhen, 2010). In living forms, this body plan includes a fusiform body, a pair of flippers, a dorsal fin (although it is sometimes absent) and flukes (Slijper, 1961). Skeletal modifications for life in water include telescopic modification and loss of symmetry of the skull (McLeod et al., 2007); flexible and large rib cage with reduced sternum; transformation of thoracic limbs into flippers (Cooper et al., 2007); hind-limb reduction (except for pelvic remains; Thewissen et al., 2006); and absence of the sacrum.

Cetaceans swim by the alternate action of their robust epiaxial and hypaxial muscles (Pabst, 1993) and by propulsive oscillatory movements that are confined to the vertical plane with the greatest amplitude at the flukes and caudal peduncle (e.g. Slijper, 1961; Fish, 1993). The shape and the mechanical properties of vertebrae strongly affect their function during

oscillatory swimming (Slijper, 1946; Wainwright, 2000; Buchholtz & Schur, 2004). For example, several odontocete species show an increase in the number of articulating vertebrae and high neurapophyses that touch one another when the back is overstretched, as well as the shortening and/or fusion of cervical vertebrae, which results in a rigid anterior portion of the column and a posterior region in which mobility is concentrated (Slijper, 1946, 1961). At the same time, shape and size of the vertebral bodies are influenced by bending resistance as well as by the development of zygapophyses, neural spine and neural arches (Slijper, 1946).

Little has been studied about vertebral morphology in cetaceans since Slijper (1946) made a detailed description of vertebrae and muscles of mammals. Long et al. (1997) studied vertebral mechanical properties of *Delphinus delphis* and proposed vertebral designs for stiff and flexible regions of the vertebral column. Stiff regions include vertebrae with short centra and intervertebral discs and long neural spines and transverse processes, while flexible regions are characterized by vertebrae with long centra and intervertebral discs and

short neural spines and transverse processes. Buchholtz (2001, 2007) studied centra length in extant cetacean species and used this to infer swimming style and its evolution in extinct species. Buchholtz & Schur (2004) described centra shape variation along the vertebral column of delphinids and discussed its evolutionary and phylogenetic implications. According to them, coastal species could represent the plesiomorphic condition, with a lower vertebral count and spool-shaped centra, while offshore species could represent the derived condition that includes a higher vertebral count and discoidal centra. More recent studies have focused on the regionalization of the vertebral column, especially in *Lagenorhynchus* species (Buchholtz, Wolkovich & Cleary, 2005; Marchesi, 2012). To summarize, previous studies suggest that there is some relationship between vertebral morphology and habitat, as well as between vertebral morphology and phylogenetic relationships. However, no study has yet quantified vertebral morphology profiles nor applied statistical methods to assess the relationship between morphology and the variables proposed.

Based on previous studies (Slijper, 1946; Buchholtz & Schur, 2004) and on the lack of analytical approaches in the bibliography, the first goal of this study was to provide a quantitative characterization of vertebral morphology along the column on representatives of the Delphinidae and Pontoporiidae families. For this, a novel morphometric approach was taken. As a second goal, the relationship between morphology and habitat was quantified through regression analyses. Since there is marked variation in size among these groups, size was included as another potential explanatory variable. In addition, the phylogenetic structure of the distribution of characters was also explored through the estimation of a phylogenetic signal. Based on the available theoretical background, a significant association between vertebral morphology and both habitat selection and phylogenetic relationships were expected.

Materials and methods

Sample

A total of nine postcranial skeletons of seven species were examined, all of which are deposited at the Mammal Collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN; Buenos Aires, Argentina) and the Osteological Collection of Marine Mammals of the Centro Nacional Patagónico (CNPMAMM; Chubut, Argentina). Since this study required disarticulated skeletons in order to take the vertebral measurements chosen (see below) and the invasiveness on museum material that this implied, the total number of postcranial skeletons measured was inevitably reduced. Individuals measured were complete or had less than 10% of their vertebrae missing. The following Delphinidae species were included: *D. delphis*, *Lagenodelphis hosei*, *Lagenorhynchus obscurus*, *Cephalorhynchus commersonii*, *Lagenor. cruciger* and *Tursiops truncatus*. *Pontoporia blainvillei*, from the Pontoporiidae family, was also included. Pontoporiidae has a close phylogenetic relationship with Delphinidae and occupies a more basal position within the
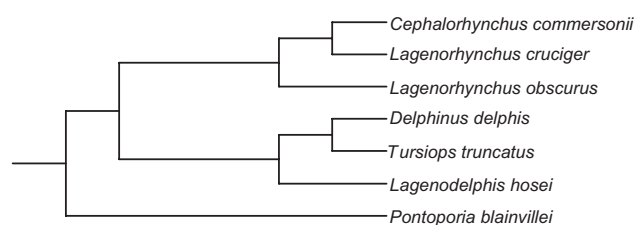
Figure 1 Phylogenetic relationships as proposed by McGowen *et al.* (2009) of the species included in this study.

Odontoceti suborder (McGowen, Spaulding & Gatesy, 2009; Fig. 1). Table 1 lists species, specimen number, sample size, habitat and total length for each species. Species were considered of coastal habitat if found mainly on waters above the continental shelf, while oceanic species were considered as those mainly found on waters beyond this shelf (Forcada, 2008). However, this is not a strict discretization because of the high variation in habitat use by some species. Individuals were aged as adults by the degree of epiphyseal fusion (Perrin, 1975). Regions of the skeleton were determined following Rommel (1990) and the first caudal vertebra was defined as the first that possessed a pair of ventral apophyses that articulate with a chevron bone (DeSmet, 1977).

Vertebral measurements and indices

A set of vertebral measurements were used to describe vertebral morphology following Buchholtz *et al.* (2005). The cervical region was excluded because the elements there are usually fused or articulated (Rommel, 1990; Berta, Sumich & Kovacs, 2006). The measurements considered in this study were maximal vertebral width (VW), maximal vertebral height (VH), centrum width (CW), centrum height (CH), centrum length (CL), neurapophysis height (NH; neural arch + neural spine; see Rommel, 1990), neurapophysis inclination (NI), metapophysis height (MH) and transverse process inclination (TPI; Fig. 2). All measurements, except for NH, NI, MH and TPI, were obtained using a digital caliper with an accuracy of 0.01 mm. CL was measured ventrally and CW and CH were measured on the anterior centrum face. NI and TPI were measured from left lateral and dorsal images of vertebrae using TPSDIG version 2.16 (Rohlf, 2010). Angles corresponding to NI and TPI were measured with an accuracy of 0.01° and were considered of anterior inclination if <90° and of posterior inclination if >90°. In those cases in which two consecutive vertebrae or less were damaged, the measurements for these vertebrae were calculated as the average of the measurements of the two adjacent nondamaged vertebrae (McShea, 1993).

The moment of resistance (MR) was calculated in order to study which part of the column suffers the stress of water resistance the most, as indicated by Slijper (1946). It was defined as

$$MR = CW \times CH^2$$

Table 1 Species included in the present study, with collection number, sample size (*n*), habitat, total length and geometric mean (see Materials and methods)

Family	Species	Collection number	*n*	Habitat	Total length (cm)[h]	Geometric mean
Delphinidae	*Delphinus delphis*	MACN 20530	1	Oceanic[a]	270	34.59
	Lagenodelphis hosei	CNPMAMM 642	1	Oceanic[b]	270	44.64
	Lagenorhynchus obscurus	CNPMAMM 597, 780	2	Oceanic[c]	210	35.01
	Cephalorhynchus commersonii	CNPMAMM 616	1	Coastal[d]	152	31.13
	Lagenorhynchus cruciger	CNPMAMM 640, 641	2	Oceanic[e]	187	41.16
	Tursiops truncatus	CNPMAMM 642	1	Coastal[f]	400	64.13
Pontoporiidae	*Pontoporia blainvillei*	MACN 24823	1	Coastal[g]	175	24.50

Sources for habitat: [a]Perrin (2008); [b]Dolar (2008); [c]Cassens *et al.* (2005); [d]Goodall (1994); [e]Goodall (2008); [f]Wells & Scott (2008); [g]Crespo (2008).
Source for total length: [h]Bastida & Rodríguez (2003).
Underlined collection number indicates which specimen was selected for posterior analysis for species with *n* = 2.
CNPMAMM, Osteological Collection of Marine Mammals of the Centro Nacional Patagónico; MACN, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'.
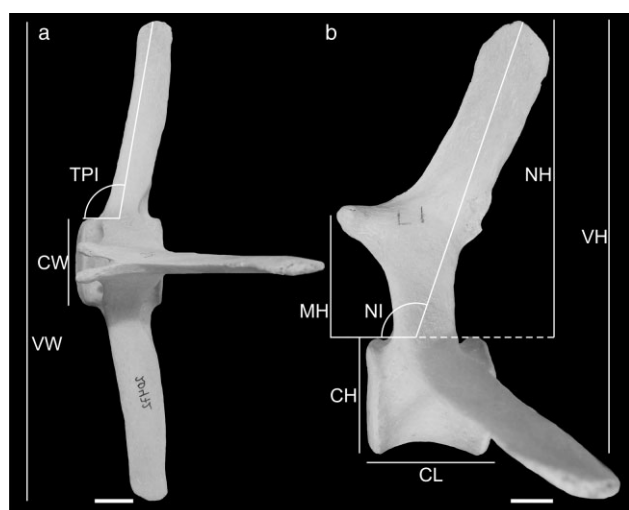
Figure 2 Vertebral measurements used in the present study. (a) Dorsal view; (b) left lateral view. VW, maximal vertebral width; VH, maximal vertebral height; CW, centrum width; CH, centrum height; CL, centrum length; NH, neurapophysis height; NI, neurapophysis inclination; MH, metapophysis height; TPI, transverse process inclination. Scale: 20 mm.

Relative centrum length (RCL) provides a summary of centrum dimensional change along the column. Higher values of RCL indicate more flexibility in the sagittal plane, while lower values indicate reduced mobility through a limited angle of possible deflection at intervertebral joints (Buchholtz, 2001). RCL was defined as:

$$RCL = 2CL/(CW + CH)$$

An additional vertebral index was calculated in order to assess proportional variations in shape that were not contemplated in previous indices and was defined as centrum length over centrum height (CL/CH).

Vertebral measurements (which were previously standardized; see Phylogenetic comparative methods) and indices of each species were plotted against percentage total length (%TL) in order to obtain morphological profiles. Total length was measured from the first thoracic vertebra to the last caudal vertebra. Given that there are intraspecific variations in the vertebral formula, one specimen per species was included in the statistical analysis. A visual inspection of the morphological profiles of the specimens for a same species showed that they were similar. The selection criteria used was to include adult specimens with the largest number of vertebrae (Table 1).

Phylogenetic comparative methods

Phylogenetic comparative methods were applied in order to detect the influence of phylogeny on vertebral morphology variation. A phylogenetic effect can be defined as the tendency for closely related species to resemble each other more than distantly related species (Felsenstein, 1985). More specifically, the 'phylogenetic signal' is maximized when the degree of interspecific divergence for a continuous trait increases as a linear function of phylogenetic distance (Martins & Hansen, 1997).

Before performing these analyses, linear measurements were standardized to control for the effect of size. The size variable chosen was the geometric mean (Mosimann, 1970) of all linear measurements of all vertebrae, because it is an isometric factor, and it was calculated for each species (Table 1). Then, to accomplish the first goal, all morphological profiles of each species were fitted into polynomial curves (considering $P < 0.05$) in order to summarize the main changes of each variable with a defined set of parameters. With this procedure, it is possible to compare species with different number of vertebrae and summarize morphological differences with the same number of parameters. The order of the curves was estimated as the number of inflexion points plus two (since the second derivative indicates the number of inflexion points of a given curve). Polynomial fitting was carried out using the basic package for R (R Development Core Team, 2009).

The information contained in the parameters of the curves was compared using a principal component analysis (PCA)

that provided independent and standardized variables that summarized principal changes between the curves that represent each species (i.e. PCs). The PCs that accounted for more than 95% of the variance of the original parameters were retained for a comparative analysis (see below). PCA was carried out using the MASS package for R (Venables & Ripley, 2002).

The relationship between vertebral morphology (all vertebral measurements and indices) and the habitat variables (i.e. coastal and oceanic) and size (i.e. total body length) were assessed through ordinary least squares (OLS) regression analyses. Habitat was fitted as a dummy variable with two categories: coastal and oceanic (see Legendre & Legendre, 2003). In order to take phylogenetic structure into account, a phylogenetic generalized least squares (PGLS) regression model was used. This method is suitable for dealing with phylogenetic nonindependent comparative data (Martins & Hansen, 1997). Regressions were carried out using the APE package for R (Paradis, Claude & Strimmer, 2004). The phylogenetic covariance matrix was obtained from a recently published phylogeny (McGowen *et al.*, 2009), considering a length of '1' for all branches.

Finally, the influence of phylogeny on patterns of morphological variation was evaluated by calculating the phylogenetic signal of the PCs corresponding to vertebral measurements and indices. A multivariate phylogenetic signal (*tree length*) was calculated using the function 'Mtree-ace.R' written by Dr. Adrien Perrard (2012) and kindly provided to us by the author and by following the method described by Klingenberg & Gidaszewski (2010). This function required the APE package for R. This test relies on mapping the phylogeny within the multivariate space of the morphological measurements and indices by computing internal node values, minimizing the squared parsimony (or matching a Brownian model of evolution) and calculating the tree length. The significance is tested by permuting the values at the tips of the phylogeny and comparing the squared tree length of the phylogeny within the morphospace to a null distribution of squared tree lengths computed with these permutations. A significant tree length implies that the phylogenetic tree is one of the shortest trees linking the values in the morphospace, so the position of the species in the morphospace is congruent with the phylogeny, that is, there is a significant phylogenetic signal in the data.

Results

Vertebral morphological patterns

The fit of the curves was over 75% for the majority of the morphological profiles, although some measurements showed a poorer fit (RCL for *Lagenor. cruciger* and NI for *P. blainvillei*). The most complex curves were CW, CL, MR, RCL and CL/CH, which adjusted to a fifth degree polynomial curve. Alternatively, the curves for WV, CH, NH, MH, TPI and NI adjusted to a fourth degree polynomial curve (Supporting Information Appendix S1).

Thoraco-lumbar transition was found at 20–25 %TL for all delphinids. However, this transition was found at 35 %TL for

P. blainvillei. The caudal region of all species began at approximately 50 %TL. The highest (HV) and widest (WV) vertebrae (Fig. 3a,b) in delphinids were found at approximately 30–35 %TL and 20–25 %TL, respectively. In contrast, the highest and widest vertebrae for *P. blainvillei* were found at 40–45 %TL. All delphinid species presented low CL values (in accordance with discoidal shaped centra; Fig. 3c) except at the last portion of the column. Comparatively, *P. blainvillei* presented the highest CL values, in accordance with spool-shaped centra, at the thoracic, lumbar and anterior caudal regions. The posterior CL values were similar to delphinids. CH and CW showed similar patterns of variation for all delphinids. The same happened for *P. blainvillei*, although it presented slightly higher values for these indices at the lumbar and anterior caudal regions (40–75 %TL; Figs 3d and 4a). Accordingly, *P. blainvillei* also showed the highest values for CL/CH which represent the most elongated vertebrae (Fig. 4b). No clear pattern was observed for metapophysis height (MH) but all species showed a similar trend in which this measurement increased up to the lumbar region and then decreased up to the disappearance of the process (Fig. 4c).

All delphinid species showed the same pattern for neurapophysis inclination (NI), that is, a posterior inclination for the first portion of the column, an anterior inclination up to the middle portion and again a posterior inclination for the rest of the column (Fig. 4d). The synclinal vertebra (i.e. NI = 90°; Slijper, 1946) was found at approximately 50 %TL. In contrast, *P. blainvillei* did not present a synclinal point (Fig. 4d) and all its neurapophyses showed a posterior inclination. Neurapophysis height (NH; Fig. 5a) varied in a similar way in all delphinid species. *P. blainvillei* showed the lowest values, in accordance with the higher degree of inclination of these processes in this species. TPI showed a similar pattern in most delphinids, where the first vertebrae showed an anterior inclination that rapidly changed to a posterior inclination. Toward 30–50 %TL, this changed again to an anterior inclination up until this process disappeared (Fig. 5b). *P. blainvillei* showed the second change of inclination at 60 %TL.

MR showed a gradual increment up to 75 %TL (or slightly earlier in *P. blainvillei*), followed by a steep decrease (Fig. 5c). *T. truncatus* showed the highest values, while other delphinids and *P. blainvillei* showed the lowest. The RCL in all delphinids increased at the beginning of the column, followed by a slight decrease and then a new increase that peaked at the last portion of the column. *T. truncatus* presented the highest values for delphinids, followed by *C. commersonii* and *Lagenod. hosei*. The lowest values were presented by *Lagenor. cruciger*, *D. delphis* and *Lagenor. obscurus*. In contrast, *P. blainvillei* showed the highest values of all, with an increase that peaked at approximately 40 %TL (Fig. 5d).

Phylogenetic comparative methods

Table 2 shows the results of both OLS and PGLS regression analyses. Both analyses showed that there was no significant relationship between vertebral morphology and habitat or between vertebral morphology and total length, except for a
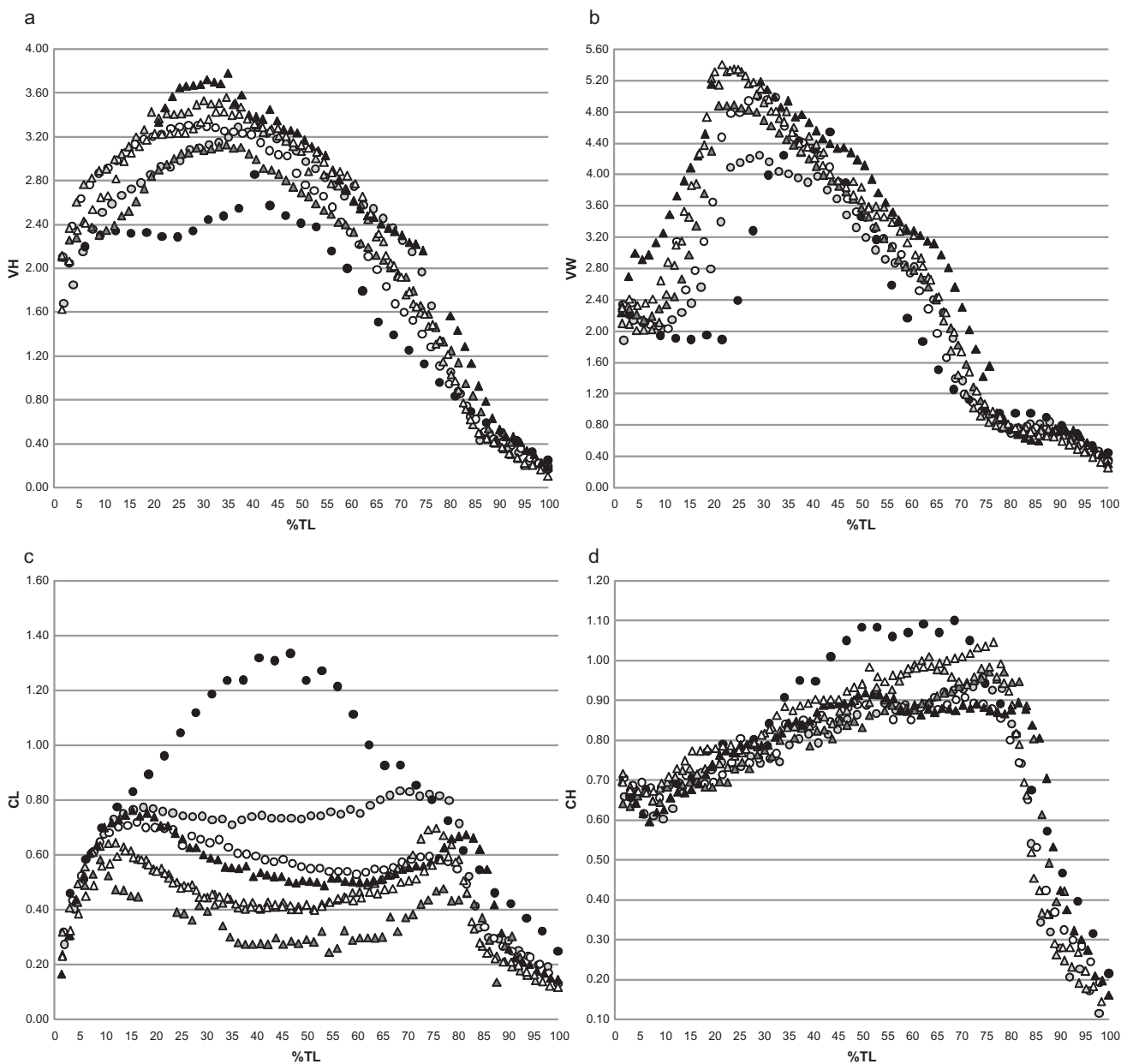
Figure 3 Morphological profiles of: (a) VH, (b) VW, (c) CL and (d) CH (for abbreviations, see Fig. 2). Species habitats: coastal (circles) and oceanic (triangles). Species symbols: (●) *Pontoporia blainvillei*; (◐) *Cephalorhynchus commersonii*; (○) *Tursiops truncatus*; (▲) *Lagenorhynchus obscurus*; (△) *Lagenorhynchus cruciger*; (△) *Delphinus delphis*; (▲) *Lagenodelphis hosei*.

significant relationship found for MR and total length in the PGLS analysis.

Regarding the multivariate phylogenetic signal, all vertebral measurements and indices showed a statistically significant signal, except for MH and MR (Table 3).

Discussion

The present study proposes a novel mode of quantification that makes it possible to incorporate the variations of verte-

bral morphological profiles into statistical analyses, including phylogenetic comparative methods. Previous studies have analyzed morphological profiles and evaluated differences among species and among phylogeny mainly by visual inspection (e.g. Buchholtz & Schur, 2004; Buchholtz *et al.*, 2005). Such evaluation was improved in the present study by calculating the phylogenetic signal through the mapping of the phylogenetic tree within a morphospace. Phylogenetic regressions helped to elucidate the relationship between vertebral morphology, size, habitat and phylogeny. These variables had
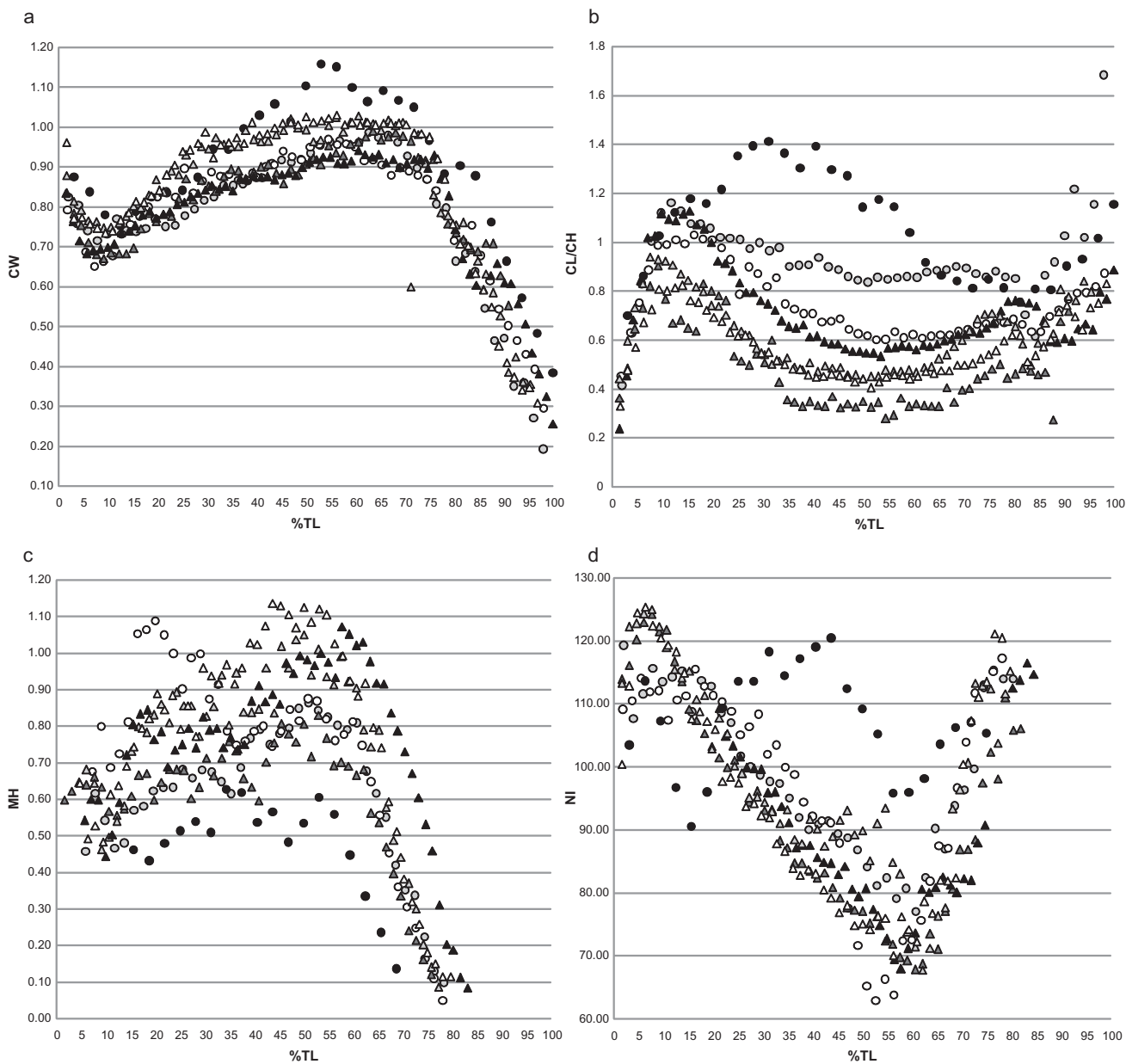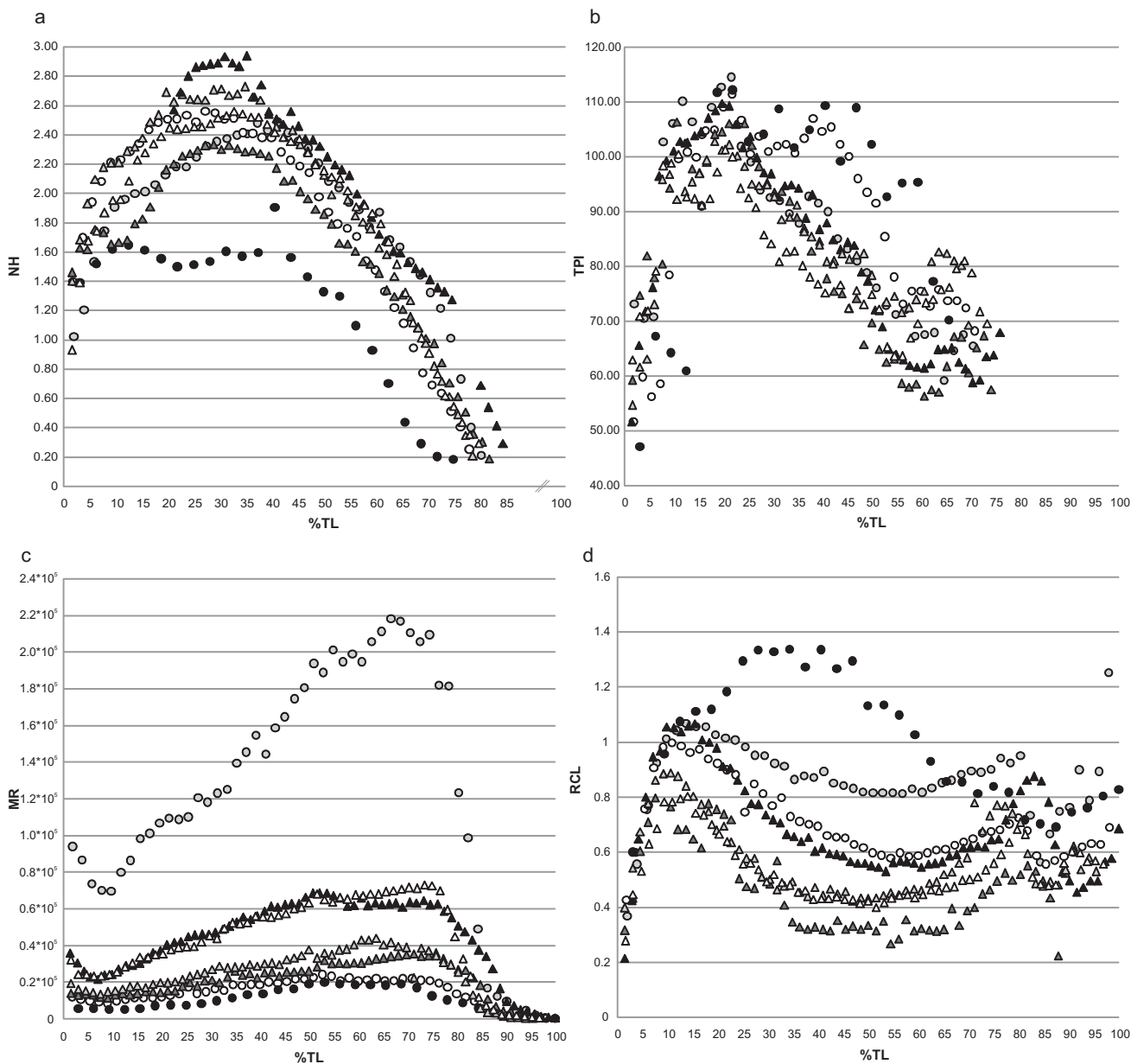
Figure 4 Morphological profiles of (a) CW, (b) CL/CH, (c) MH and (d) NI (for abbreviations, see Fig. 2). Species habitat: coastal (circles) and oceanic (triangles). Species symbols: (●) *Pontoporia blainvillei*; (◉) *Cephalorhynchus commersonii*; (○) *Tursiops truncatus*; (▲) *Lagenorhynchus obscurus*; (▲) *Lagenorhynchus cruciger*; (△) *Delphinus delphis*; (▲) *Lagenodelphis hosei*.

been previously proposed as related (e.g. Buchholtz & Schur, 2004) but this had never been tested statistically.

One of the most noteworthy results was the strong phylogenetic signal on vertebral morphology, especially for VW, VH, NH and TPI. These variables are related to the muscular architecture of the main axial muscles that are involved in caudal (propulsive) movements (i.e. mm. multifidi and m. longissimus). A strong phylogenetic signal could be explained by the fact that *P. blainvillei* has a different muscular arrangement compared with delphinids (Strickler, 1980). Tendon fibers of insertion of the mm. multifidi in delphinids form a deep tendon that serves as a temporary skeletal element from which m. longissimus originates (Pabst, 1993), whereas, in *P. blainvillei*, this deep tendon does not exist and the m. longissimus presents its usual bony origins (Strickler, 1980). Thus, in this study we hypothesize that the derived muscular configuration of delphinids, absent in *P. blanvillei* (see Strickler, 1980), could influence the arrangement of

Figure 5 Morphological profiles of (a) NH, (b) TPI, (c) MR and (d) RCL (for abbreviations, see Fig. 2). Species habitats: coastal (circles) and oceanic (triangles). Species symbols: (●) *Pontoporia blainvillei*; (◉) *Cephalorhynchus commersonii*; (○) *Tursiops truncatus*; (▲) *Lagenorhynchus obscurus*; (△) *Lagenorhynchus cruciger*; (△) *Delphinus delphis*; (▲) *Lagenodelphis hosei*.

neurapophyses. These processes in the thoracic and anterior lumbar regions increased in height and had a posterior inclination, favoring the mechanical advantage of the bundles of mm. multifidi (Slijper, 1946), which, in turn, tense the deep insertion tendons (Pabst, 1993). Conversely, neurapophyses in the middle-posterior lumbar and anterior caudal regions of delphinids tilted anteriorly and metaphophyses remained high, which could suggest the loss of the configuration of the deep tendons and an improvement of the mechanical advantage for m. longissimus (Slijper, 1961). Although CL is known

to influence the inclination of neurapophyses in most mammals (Slijper, 1946), this variable remained roughly constant along portions of the column where this process changed its inclination in delphinids (CL; Fig. 3c). The nearly vertical and posterior inclination of neurapophyses in *P. blainvillei* could be increasing the mechanical advantage of mm. multifidi (Slijper, 1946, 1961) along the entire vertebral column (supported by the fact that it has the lowest MH values) and may be related to the absence of the particular myological arrangement displayed by delphinids. It is possible

Table 2 Ordinary (OLS) and phylogenetic (PGLS) regression analyses of vertebral measurements and indices against variables of habitat and size (see Materials and Methods for explanation)

Measurements and indices	OLS				PGLS			
	Versus habitat		Versus size		Versus habitat		Versus size	
	$F_{(2,4)}$	P	$F_{(4, 8)}$	P	$F_{(2, 4)}$	P	$F_{(4, 8)}$	P
VH	0.354	0.7221	0.120	0.9715	0.537	0.6215	0.398	0.8054
VW	2.485	0.1989	1.126	0.4090	0.054	0.9477	0.016	0.9994
CL	0.296	0.7590	0.105	0.9777	0.875	0.4839	0.860	0.5268
CH	1.505	0.3257	0.911	0.5018	2.019	0.2476	0.147	0.9591
CW	0.879	0.4824	0.365	0.8270	1.035	0.4343	0.976	0.4716
NH	0.332	0.7354	0.168	0.9487	0.232	0.8030	0.563	0.6966
MH	1.173	0.3972	0.996	0.4629	1.207	0.3890	0.162	0.9521
TPI	2.026[*]	0.2884	0.965[+]	0.5166	5.222[*]	0.1040	1.833[+]	0.2398
NI	1.541	0.3189	0.806	0.5548	0.282	0.7680	0.120	0.9713
MR	0.920	0.4691	0.436	0.7795	14.054	**0.0155**	3.715	0.0540
RCL	0.202	0.8250	0.018	0.9992	2.135	0.2340	1.002	0.4602
CL/CH	0.224	0.8080	0.025	0.9985	2.108	0.2371	0.769	0.5747

Bold numbers indicate the measurement/index has a statistically significant relation to the corresponding variable.
[*]d.f. = (3,3); [+]d.f. = (6,6).

Table 3 Phylogenetic signal of the PCs corresponding to vertebral measurements and indices

Measurements and indices	Tree length	P
VH	11.095	**0.0006**
VW	13.708	**0.0005**
CL	20.435	**0.0259**
CW	13.583	**0.0017**
CH	27.471	**0.0257**
MH	25.232	0.1045
NH	19.460	**0.0140**
NI	20.186	**0.0000**
TPI	14.777	**0.0023**
MR	29.571	0.1713
RCL	18.615	**0.0205**
CL/CH	16.613	**0.0015**

Tree length represents the phylogenetic multivariate signal calculated. Bold numbers indicate statistically significant tree length.

that this muscular system could be favored since it has a large amount of short bundles, which may be necessary to allow the mobility of a vertebral column composed of long elements and few mobility points.

Considering this, a strong phylogenetic signal could be interpreted as an effect of the inclusion of *P. blainvillei*, as this taxon represents one of the basal families within Odontoceti (McGowen *et al.*, 2009). It also differs from delphinids in some ecological aspects such as being one of the smallest cetacean species and having a slow swimming speed, similar to porpoises and other riverine dolphins (Bordino, Thompson & Iñíguez, 1999). To test this effect, the data was reanalyzed excluding *P. blainvillei*. Although OLS and PGLS regression analyses showed the same results, phylogenetic signal was lost in some functionally important measurements such as CL, CW, NH and RCL (results not shown). These results thus

support the conclusion that phylogenetic structure is very important to understand the differences between both vertebral column 'models' and their morphological evolution and that this phylogenetic structure is mainly contained in the basal node of divergence of Pontoporiidae and Delphinidae. Therefore, future studies should increase the number of delphinid species and include other outgroup species (e.g. *Inia geoffrensis*) in order to test whether the phylogenetic signal still persists or whether it becomes nonsignificant (e.g. in case they have patterns similar to delphinids).

Odontocetes have a locomotion characterized by oscillatory movements carried out mainly by the flukes and caudal region of the vertebral column. Therefore, the analysis of centrum and neurapophyses and transverse process morphology is an important task required to infer regions of stability or flexibility within the vertebral column (Slijper, 1946; Long *et al.*, 1997; Buchholtz, 2001; Buchholtz & Schur, 2004). Morphological profiles indicated that delphinid species of both habitats (i.e. coastal and oceanic) had a conservative regionalization of the vertebral column. This regionalization included an anterior rigid portion composed by the thoracic and anterior lumbar regions, and a more flexible posterior portion composed by the posterior lumbar and caudal regions. Both portions were characterized by posteriorly and anteriorly tilted neurapophyses, respectively (Fig. 4d; Slijper, 1946; Buchholtz & Schur, 2004). In contrast, *P. blainvillei* presented a more flexible column, with all neurapophyses inclined anteriorly (Fig. 4d). RCL profiles of delphinids (Fig. 5d) concurred with pattern 3 for these species as described by Buchholtz (2001; i.e. a rigid thoracic, lumbar and anterior caudal regions and a flexible posterior caudal region), while *P. blainvillei* concurred with pattern 2 for beaked whales and river dolphins (i.e. flexible thoracic, lumbar and anterior caudal regions and a more flexible posterior caudal region). Terminal caudal vertebrae include the peduncle and tail fluke, highly specialized structures compared with terrestrial

mammals, which play a crucial role in caudal oscillation during swimming (e.g. Long *et al.*, 1997; Pabst, 2000). This specialization was reflected in the conservative pattern observed across the sample, through a clear decrease of the CL of the caudal elements as well as vertebrae that were laterally compressed (indicating the peduncular region; Rommel, 1990) and dorsoventrally compressed (indicating the presence of a tail fluke; Rommel, 1990) in the posterior end of the caudal region (Figs 3c and 4a,b). This differs from the long rod-like caudal vertebrae present in most terrestrial mammals (e.g. Thewissen *et al.*, 2001; Uhen, 2004, 2010; Buchholtz, 2007).

All delphinid species presented CL values lower than CW and CH (Figs 3c,d and 4a) values, which is consistent with a discoidal morphology of the centrum, but *P. blainvillei* presented a predominant spool-shaped vertebral morphology, with CL values higher than CH and CW values (Figs 3c,d and 4a). Spool-shaped morphology and a low total number of vertebrae have been proposed as a plesiomorphic condition associated with a coastal habitat, while discoidal morphology and a higher total number of vertebrae could represent a derived condition associated with an oceanic habitat (Buchholtz & Schur, 2004). However, this association is not sustained by the results obtained through our quantitative approach (according to the classification scheme considered) and by the fact that no significant relationship between vertebral morphology and habitat was found. Moreover, *P. blainvillei* was the only coastal species with spool-shaped morphology, which suggests a phylogenetic value for this character. As already observed for transverse processes and neurapophyses, CL, CH and CW converge to a similar pattern in the caudal region for all species, except for *P. blainvillii*, which displayed longer centra (Fig. 3c). The shape of vertebral centra is, in some degree, related to the mobility (stiffness vs. flexibility) of the column in different regions and/or species. As Buchholtz & Schur (2004) and Long *et al.* (1997) proposed, spool-shaped centra (i.e. *P. blainvillei*, especially at 50 %TL) could be associated with a more flexible region, while discoidal centra (typical of delphinids) could be related to a stiffer region. Thus, the differences found in muscular and vertebral morphology for *P. blainvillei* could indicate a distinct dynamic of vertebral movement during swimming.

Although morphological profiles were mostly similar across delphinid species, some interesting combinations of characters occurred within this family. For instance, *T. truncatus* showed lower VW and VH and higher CL (in concordance with RCL and CL/CH; Figs 5d and 4b) in the anterior and/or middle region of the column, which suggests more flexibility of the thoracic and lumbar areas than the rest of the delphinids. Shorter neurapophyses and transverse process (and longer centra) would allow for freer movement between contiguous vertebrae (Buchholtz & Schur, 2004). Similar morphological patterns were observed in other species not included in this analysis, such as *Feresa attenuata* and *Pseudorca crassidens* (M.V. pers. obs.). Strikingly, these species do not share habitat or size and do not form a natural clade (McGowen *et al.*, 2009) so their similarities should be subject of future studies. Other variations with possible functional consequences were detected. Although maximal VW exhibited

a peak between 20 and 30 %TL in delphinids, *Lagenor. obscurus*, *Lagenor. cruciger* and *Lagenod. hosei* presented a proportionally more accentuated peak than *D. delphis*, *C. commersoni* and specially *T. truncatus*, which reveals variations also in the development of transverse processes in the thoracic region (Fig. 3b). Similarly, delphinids also varied their CL in the lumbar and anterior caudal regions (between 25 and 75 %TL), where *T. truncatus* showed the most elongated centra compared with the remainder species, especially with respect to *D. delphis* (Fig. 3c).

According to our results, neither habitat nor size was found as explanatory variables of vertebral morphology (except for MR, which is directly influenced by size because of its own definition). Thus, more complex and specific functional relationships and life-history traits may also be influencing vertebral morphology. However, data such as hunting techniques or maximum swimming speed are rare or anecdotal (LeDuc, 2008). Furthermore, the detailed study of the complex evolutionary history of lineages (e.g. Steeman *et al.*, 2009; Moura *et al.*, 2013) could bring to light other clarifying dimensions for understanding morphological evolution in odontocetes. For example, future studies could make use of the quantitative approach presented in this study and include fossil specimens with available postcranial evidence (e.g. *Albireo whistleri* Barnes, 1984; *Notocetus vanbenedeni* de Muizon, 1987) as well as other outgroups of Delphinidae, such as *Inia geoffrensis*. Currently, there is a need to broaden our knowledge on locomotion, evolutionary history, habitat use and other ecological aspects of odontocetes. New variables could be included and tested in future studies using this quantitative approach, together with a broader sample of species and extinct and living representatives of the diverse families of odontocetes in order to shed some light on these unknowns.

Acknowledgments

References

Barnes, L.G. (1984). Fossil odontocetes (Mammalia: Cetacea) from the Almejas Formation, Isla Cedros, Mexico. *PaleoBios* **42**, 1–46.

Bastida, R. & Rodríguez, D. (2003). *Mamíferos marinos de Patagonia y Antártida*. 1st edn. Buenos Aires: Vazquez Mazzini.

Berta, A., Sumich, J.L. & Kovacs, K.M. (2006). *Marine mammals: evolutionary biology*. 2nd edn. New York: Academic Press.

Bordino, P., Thompson, G. & Iñíguez, M. (1999). Ecology and behaviour of the franciscana (*Pontoporia blainvillei*) in Bahía Anegada, Argentina. *J. Cetacean Res. Manage* **1**, 213–222.

Buchholtz, E.A. (2001). Vertebral osteology and swimming style in living and fossil whales (Order: Cetacea). *J. Zool.(Lond.)* **253**, 157–190.

Buchholtz, E.A. (2007). Modular evolution of the cetacean vertebral column. *Evol. Dev.* **9**, 278–289.

Buchholtz, E.A. & Schur, S.A. (2004). Vertebral osteology in Delphinidae (Cetacea). *Zool. J. Linn. Soc.* **140**, 383–401.

Buchholtz, E.A., Wolkovich, E.M. & Cleary, R.J. (2005). Vertebral osteology and complexity in *Lagenorhynchus acutus* (Delphinidae) with comparison to other delphinoid genera. *Mar. Mamm. Sci.* **21**, 411–428.

Cassens, I., Van Waerebeek, K., Best, P.B., Tzika, A., Van Helden, A.L., Crespo, E.A. & Milinkovitch, M.C. (2005). Evidence for male dispersal along the coasts but no migration in pelagic waters in dusky dolphins (*Lagenorhynchus obscurus*). *Mol. Ecol.* **14**, 107–121.

Cooper, L.N., Berta, A., Dawson, S.D. & Reidenberg, J.S. (2007). Evolution of hyperphalangy and digit reduction in the cetacean manus. *Anat. Rec.* **290**, 654–672.

Crespo, E.A. (2008). Franciscana dolphin (*Pontoporia blainvillei*). In *Encyclopedia of marine mammals*: 466–469. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

DeSmet, W.M.A. (1977). The regions of the cetacean vertebral column. In *Functional anatomy of marine mammals*: 59–80. Harrison, J. (Ed.). New York: Academic Press.

Dolar, M.L.L. (2008). Fraser's dolphin (*Lagenodelphis hosei*). In *Encyclopedia of marine mammals*: 469–471. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1–5.

Fish, F.E. (1993). Power output and propulsive efficiency of swimming bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* **185**, 179–193.

Forcada, J. (2008). Distribution. In *Encyclopedia of marine mammals*: 316–321. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Goodall, R.N.P. (1994). Commerson's dolphin *Cephalorhynchus commersonii* (Lacépéde 1804). In *Handbook of marine mammals, the first book of dolphins*: 241–267. Ridgway, S.H. & Harrison, R. (Eds). New York: Academic Press.

Goodall, R.N.P. (2008). Hourglass dolphin (*Lagenorhynchus cruciger*). In *Encyclopedia of marine mammals*: 573–575. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Klingenberg, C.P. & Gidaszewski, N.A. (2010). Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* **59**, 245–261.

LeDuc, R. (2008). Delphinids, overview. In *Encyclopedia of marine mammals*: 298–302. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Legendre, P. & Legendre, L. (2003). *Numerical ecology*. 2nd edn. Amsterdam: Elsevier Science B.V.

Long, J.H. Jr., Pabst, A., Shepherd, W.R. & McLellan, W.A. (1997). Locomotor design of dolphin vertebral columns: bending mechanics and morphology of *Delphinus delphis*. *J. Exp. Biol.* **200**, 65–81.

Marchesi, M.C. (2012). *Análisis de la variación morfométrica en la columna vertebral de dos especies de delfines del género* Lagenorhynchus *en asociación a hábitats contrastantes*. Undergraduate thesis, Universidad Nacional de Mar del Plata at Mar del Plata, Buenos Aires, Argentina.

Martins, E.P. & Hansen, T.F. (1997). Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**, 646–667.

McGowen, M.R., Spaulding, M. & Gatesy, J. (2009). Divergence date estimation and a comprehensive molecular tree of extant cetaceans. *Mol. Phylogenet. Evol.* **53**, 891–906.

McLeod, C.D., Reidenberg, J., Weller, M., Santos, M.B., Herman, J., Goold, J. & Pierce, G.J. (2007). Breaking symmetry: the marine environment, prey size, and the evolution of asymmetry in cetacean skulls. *Anat. Rec.* **290**, 539–545.

McShea, D.W. (1993). Evolutionary change in the morphological complexity of the mammalian vertebral column. *Evolution* **47**, 730–740.

Mosimann, J.E. (1970). Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distributions. *J. Am. Stat. Assoc.* **65**, 930–945.

Moura, A.E., Nielsen, S.C., Vilstrup, J.T., Moreno-Mayar, J.V., Gilbert, M.T.P., Gray, H.W., Natoli, A., Möller, L. & Hoelzel, A.R. (2013). Recent diversification of a marine genus (*Tursiops* spp.) tracks habitat preference and environmental change. *Syst. Biol.* **62**, 865–877.

de Muizon, C. (1987). The affinities of *Notocetus vanbenedeni*, an early Miocene platanistoid (Cetacea, Mammalia) from Patagonia, southern Argentina. *Am. Mus. Novit.* **2904**, 1–27.

Pabst, D.A. (1993). Intramuscular morphology and tendon geometry of the epaxial swimming muscles of dolphins. *J. Zool.(Lond.)* **230**, 159–176.

Pabst, D.A. (2000). To bend a dolphin: convergence of force transmission designs in cetaceans and scombrid fishes. *Am. Zool.* **40**, 146–155.

Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 169–178.

Perrard, A. (2012). *Systematics & geometric morphometrics: evolution of the wing venation within the genus* Vespa *(Hymenoptera: Vespidae)*. PhD thesis, Museum National d'Histoire Naturelle at Paris, France.

Perrin, W.F. (1975). Variation of spotted and spinner porpoise (genus *Stenella*) in the eastern Pacific and Hawaii. *Bull. Scripps Inst. Oceanogr. Univ. California* **21**, 1–206.

Perrin, W.F. (2008). Common dolphin (*Delphinus delphis* and *D. capensis*). In *Encyclopedia of marine mammals*: 255–260. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

R Development Core Team (2009). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.r-project.org/ (accessed on May 2013).

Rohlf, F.J. (2010). TpsDig, Ver. 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rommel, S. (1990). Osteology of the bottlenose dolphin. In *The bottlenose dolphin*: 29–49. Leatherwood, S. & Reeves, R.R. (Eds). San Diego: Academic Press.

Slijper, E.J. (1946). Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of mammals. *Verh. K. Ned. Akad. Wet. Afd. Natuurk. Sect. 2* **42**, 1–128.

Slijper, E.J. (1961). Locomotion and locomotory organs in whales and dolphins (Cetacea). *Symp. Zool. Soc. London* **5**, 77–94.

Steeman, M.E., Hebsgaard, M.B., Fordyce, R.E., Ho, S.Y., Rabosky, D.L., Nielsen, R., Rahbek, C., Glenner, H., Sørensen, M.V. & Willerslev, E. (2009). Radiation of extant cetaceans driven by restructuring of the oceans. *Syst. Biol.* **58**, 573–585.

Strickler, T.L. (1980). The axial musculature of *Pontoporia blainvillei*, with comments on the organization of this system and its effect on fluke-stroke dynamics in the Cetacea. *Am. J. Anat.* **157**, 49–59.

Thewissen, J.G.M., Williams, E.M., Roe, L.J. & Hussain, S.T. (2001). Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature* **413**, 277–281.

Thewissen, J.G.M., Cohn, M.J., Stevens, L.S., Bajpal, S., Heyning, J. & Horton, W.E. Jr. (2006). Developmental basis for hind-limb loss in dolphins and origin of the cetacean bodyplan. *Proc. Natl Acad. Sci. USA* **103**, 8414–8418.

Uhen, M.D. (2004). Form, function, and anatomy of *Dorudon atrox* (Mammalia, Cetacea): an archaeocete from the Middle to Late Eocene of Egypt. *Pap. Paleontol.* **34**, 1–238.

Uhen, M.D. (2010). The origin(s) of whales. *Annu. Rev. Earth Planet. Sci.* **38**, 189–219.

Venables, W.N. & Ripley, B.D. (2002). *Modern applied statistics with S*. 4th edn. New York: Springer.

Wainwright, S.A. (2000). The animal axis. *Am. Zool.* **40**, 19–27.

Wells, R.S. & Scott, M.D. (2008). Common bottlenose dolphin (*Tursiops truncatus*). In *Encyclopedia of marine mammals*: 249–255. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Supporting Information

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

Appendix S1. Curve fitting of morphological profiles of the species included in the present study.