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Vertebral morphology in extant porpoises: radiation and functional implications

Running title: Vertebral morphology in porpoises

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The datasets presented in this study can be found in online repositories, https://doi.org/10.6084/m9.figshare.17054546

ABSTRACT

Vertebral morphology has profound biomechanical implications and plays an important role in adaptation to different habitats and foraging strategies for cetaceans. Extant porpoise species (Phocoenidae) display analogous evolutionary patterns in both hemispheres associated with convergent evolution to coastal versus oceanic environments. We employed 3D geometric morphometrics to study vertebral morphology in five porpoise species with contrasting habitats: the coastal Indo-Pacific finless porpoise (*Neophocaena phocaenoides*); the mostly coastal harbor porpoise (*Phocoena phocoena*) and Burmeister's porpoise (*Phocoena spinipinnis*); and the oceanic spectacled porpoise (*Phocoena dioptrica*) and Dall's porpoise (*Phocoenoides dalli*). We evaluated the radiation of vertebral morphology, both in size and shape, using multivariate statistics. We supplemented data with samples of an early-radiating delphinoid species, the narwhal (*Monodon monoceros*); and an early-radiating delphinid species, the white-beaked dolphin (*Lagenorhynchus albirostris*). Principal component analyses were used to map shape variation onto phylogenies, and phylogenetic constraints were investigated through permutation tests. We established links between vertebral morphology and movement patterns through biomechanical inferences from morphological presentations. We evidenced divergence in size between species with contrasting habitats, with coastal species tending to decrease in size from their estimated ancestral state, and oceanic species tending to increase in size. Regarding vertebral shape, coastal species had longer centra and shorter neural processes, but longer transverse processes, whilst oceanic species tended to have disk-shaped vertebrae with longer neural processes. Within Phocoenidae, the absence of phylogenetic constraints in vertebral morphology suggests a high level of evolutionary lability. Overall, our results are in accordance with the hypothesis of speciation within the family from a coastal ancestor, through adaptation to particular habitats. Variation in vertebral morphology in this group of small odontocetes highlights the importance of environmental complexity and particular selective pressures for the speciation process through the development of adaptations that minimize energetic costs during locomotion and prey capture.

Keywords: ~~porpoise~~, ~~vertebral~~ column, eco-morphology, geometric morphometrics, biomechanics, ~~radiation~~.

Research Highlights

We studied vertebral morphology across phylogeny in porpoises. We evidenced morphologies with biomechanical relevance according to each species' habitat. Our results agree with the hypothesis of speciation through adaptation to habitats.

Graphical Abstract

By applying 3D geometric morphometrics, we studied five functionally relevant vertebrae along the vertebral column of five porpoise species, we made an ecomorphological characterization of the vertebral column of extant porpoises. Differences were translated in different degrees of vertebral column flexibility. We found a lack of phylogenetic signal in vertebral morphology and morphological resemblance between species with similar habitat requirements.

1. INTRODUCTION

Cetacean feeding strategies are associated with particular requirements regarding locomotion. Body adaptations of cetaceans that evolved in different habitats may reflect particular selective pressures (Ballance, 2018). Considering that the cetacean body is not rigid and shows variable flexibility (Long, Pabst, Shepherd & McLellan, 1997; Pabst, 1993, 2000), greater column flexibility has been associated with greater maneuverability (Fish & Rohr, 1999; Fish, 2002). However, a less flexible (more rigid) morphology would minimize energy requirements and increase efficiency during prolonged swimming (Fish & Rohr, 1999). Factors affecting locomotion involve a complex interaction between the axial skeleton and its associated muscles, tendons, ligaments, and the subdermal connective tissue sheath (Long et al., 1997; Pabst, 1990). Nevertheless, a morphological characterization of the vertebral column is useful to analyze the locomotor performance of cetacean species inhabiting different habitats and using various foraging strategies (Buchholtz, 2001; Marchesi, Mora, Dans, Coscarella & González-José, 2020).

There are variations in vertebral structure that may reinforce or limit movements between adjacent vertebrae. They may be grouped into considerations of the shape of the centrum, its spacing, the structure and orientation of the vertebral processes, the number of vertebrae, and the development of accessory structures (Buchholtz & Schur, 2004; Marchesi, Mora, Pimper & Goodall, 2017; Marchesi et al., 2020a). Buchholtz and Schur (2004) summarized vertebral centrum features that would affect the mechanical properties of the vertebral column. These authors discussed four possible mechanical scenarios that could affect vertebral column regions: areas of potential elastic stability, with short centrum length and low convexity of the faces; areas of potential rigidity, with long centra and low convexity of the faces; areas with high rotation potential but low vertical displacement, with short centrum length and highly convex faces; and areas with high rotation potential and vertical displacement potential, with long centrum length and highly convex faces (see Buchholtz & Schur, 2004; Marchesi, Boy, Dans, Mora & González-José, 2020). Particular vertebral morphologies with adaptive biomechanical implications have been reported for coastal and pelagic cetaceans (see, Buchholtz & Schur, 2004; Gillet, Frédérich & Parmentier, 2019; Marchesi et al., 2020a, 2020b; Woodward, 2006).

Porpoises are among the smallest cetaceans and represent an interesting evolutionary lineage within Delphinoidea (Figure 1; Chehida et al., 2020; McGowen, Tsagkogeorga, Álvarez-Carretero, dos Reis & Struebig, 2020; Steeman et al., 2009). They split from Monodontidae during the Miocene (~15 Mya), and diversified until the early Pleistocene (6 – 2 Mya, Chehida et al., 2020). Extant porpoises are divided into three genera, of which *Phocoena* is paraphyletic (Figure 1B). Although the crown group of porpoises constitutes a relatively homogenous family of odontocetes, there are variations concerning habitat preference, size, life history and morphology (Galatius, Berta, Frandsen & Goodall, 2011). Porpoises inhabit both hemispheres in riverine, coastal, and pelagic habitats (Read, 2018). Most species are cold-water tolerant, with the finless porpoises as exceptions (Neophocaena spp.; Committee on Taxonomy, 2021). These latter species inhabit shallow warm tropical and subtropical waters and river systems (Chehida et al. 2020; Kasuya, 1999). The critically endangered vaquita (*Phocoena sinus*) differs from other porpoise species by having an extremely localized geographical distribution in the upper Gulf of California (Morell, 2017). The remaining species of the family constitute one of the best know examples of antitropical distribution (Chehida et al., 2020). In the Northern Hemisphere, the harbor porpoise (*Phocoena phocoena*) and Dall's porpoise (*Phocoenoides dalli*) occupy distinct habitats.

P. phocoena prefers habitats that are generally less than 100m deep (Jefferson, Webber & Pitman, 2015). They usually forage near the sea bottom, but may also do some pelagic feeding at night (Bjørge & Tolley, 2018). Recently, studies have reported that, at least in one population, individuals perform seasonal travels to offshore temperate waters where they feed on mesopelagic fish at depths around 400m (Nielsen et al. 2018; Nielsen, Teilmann & Heide-Jørgensen, 2019). In contrast, *P. dalli* prefers deep offshore waters of the North Pacific, but is also found in deep nearshore and inshore waters along the west coast of North America. It preys on schooling mesopelagic fishes and squid (Houck and Jefferson, 1999; Read, 2018). This pattern of habitat preferences is mirrored in the Southern Hemisphere by Burmeister's porpoise (*Phocoena spinipinnis*) and the spectacled porpoise (*Phocoena dioptrica*). *Phocoena spinipinnis* inhabits coastal waters of South America both in the Atlantic and Pacific oceans (Jefferson et al., 2015), mainly in shallow coastal waters (5-130 m deep), up to 50 km from shore (Reyes, 2018). It is a generalist in terms of foraging, feeding on demersal and pelagic fish but also on benthonic prey (Jefferson et al., 2015; Muñoz Moreda, 2019). On the other hand, *P. dioptrica* is circumpolar in sub-Antarctic and Antarctic waters. It is one of the world's most poorly-known cetaceans, being considered an oceanic species that occasionally comes near shore (Goodall & Brownell, 2018). For this species, an isotopic continuum with the offshore hourglass dolphin (*Lagenorhynchus cruciger*) has been reported, suggesting that both species feed in cold oceanic waters near the Antarctic Convergence (Riccialdelli, Newsome, Fogel & Goodall, 2010). More recently, stomach content and isotopic analyses on skin and bone have suggested that *P. dioptrica* prefers feeding in oceanic cold waters resources, but can also feed over the shelf on neritic prey, partially overlapping with *P. spinipinnis*, at least in certain parts of its distribution (Muñoz Moreda, 2019).

It has been suggested that anti-tropical phocoenid species display analogous evolutionary adaptations in the northern and southern hemispheres associated with convergent evolution to coastal versus oceanic environments (Chehida et al., 2020). For instance, for *P. dioptrica* and *P. dalli*, possible parallel offshore evolution has been accompanied by a convergent highly contrasted countershading coloration pattern with a white ventral side and black dorsal side in both species (Perrin, 2018). Studies investigating convergence in morphology among porpoises have focused on the skull and have reported specific characteristics related to coastal and pelagic habitats (Galatius et al., 2011). Regarding the vertebral column, some descriptive works have been made (see Barnes, 1985; Buchholtz, 2001; Hamilton, 1941; Howell, 1927; Perrin, Goodall & Cozzuol, 2000; Yoshida, Shirakihara, Takemura & Shirakihara, 1994). Postcranial characters have been included to resolve the systematics within the family, but the resultant phylogenies were not congruent with previous or more recent ones (Barnes, 1985; Chehida et al., 2020; Fajardo-Mellor, Berta, Brownell, Boy & Goodall 2006). Galatius et al. (2011) reported similar degrees of paedomorphosis in terms of epiphyseal fusion of the vertebral column for coastal porpoise species. They proposed that this would be a convergent adaptation toward that particular habitat, although the driver behind this was suggested to be life-history adaptation with the resultant morphology as a by-product. The vertebral morphology of *P. dalli* has been considered one of the most extreme among cetaceans, with particularly high vertebral counts, strongly compressed centra, and exceptionally long neural and transverse processes (Barnes, 1985). To date, no studies have focused on the shape of the vertebrae of this family of small odontocetes.

Understanding how particular selective pressures may influence morphology, and the mechanisms, which may affect morphological features, are central topics in ecomorphological research (see Meloro, Raia, Carotenuto & Cobb, 2011; Monteiro & Nogueira, 2011; and references therein). In this study, we employed three-dimensional geometric morphometrics to analyze vertebral morphology of five closely related odontocete species belonging to the family Phocoenidae. We evaluated the radiation of both size and shape of the vertebrae and analyzed differences among species from a biomechanical point of view. We expected to find morphological and functional convergence between species with similar habitats and thus morphological divergence between sister species pairs in both hemispheres. In addition, we assessed the existence of phylogenetic constraints in vertebral morphology. In absence of these constraints, other factors, such as the biomechanical demands of each habitat, could have been involved in determining vertebral morphology in extant porpoise species. To study the family within a broader phylogenetic context, we supplemented data on porpoises with samples of a basal delphinoid species from a coastal habitat, the narwhal (*Monodon monoceros*), and a basal delphinid species from a pelagic/shelf habitat with a high vertebral count, the white-beaked dolphin (*Lagenorhynchus albirostris*; see Figure 1A).

2. Materials and Methods

2.1. Samples and vertebrae selection

The specimens studied are housed in various mammalogy collections and listed in supplementary online material 1. Classification into ontogenetic groups was done based on the degree of fusion of the vertebral epiphyses (Goodall et al., 1988; Lockyer, Goodall & Galeazzi, 1988; Perrin, 1975). Due to a low number of adult specimens, we included both sub-adults and adults in the analyses. Given a low number of complete specimens available for this study we included specimens disregarding their geographic origin and/or sex. Despite this, specimens of a particular species had been collected in the same geographical area, and both sexes were represented (supplementary online material 1).

The regionalization pattern of the vertebral column of cetaceans differs notably from that of terrestrial mammals (Buchholtz, 2007; Buchholtz & Gee, 2017; Buchholtz & Schur, 2004; Crovetto, 1991; Slijper, 1936). Given this, we employed a functional approach to vertebral column regional subdivision that provides a detailed analysis of the variation within each region, focusing on functional aspects that could be masked under the traditional criterion (e.g., Buchholtz, 1998; Buchholtz & Schur, 2004; Marchesi, Mora, Crespo, Boy & González-José, 2018). Based on this functional criterion, traditional lumbar and caudal regions are divided into three functional regions: torso (trunk), tailstock, and fluke (Figure 2).

We analyzed skeletons qualitatively to define these functional regions for each species (supplementary online material 2). Based on this, we selected a maximum of five vertebrae for each specimen, representing particular functional regions and their boundaries for a total of 190 vertebrae (Table 1). The number of vertebrae included for each region does not correspond with the number of specimens studied because some specimens were incomplete (lacking caudal vertebrae) or damaged (lacking epiphyses). Due to variation in vertebral count both within regions (especially in the torso) and between species (supplementary online material 1), we chose the vertebrae based on their position with respect to the functional regions described previously (Table 1, Figure 2). The first thoracic vertebra was selected to represent the anterior thorax (Th), the last thoracic vertebra represented the thorax-anterior torso boundary (ThTo). We chose the central vertebra in the torso to represent the mid-torso (Tm). Caudally, the vertebra with a neural process perpendicular to the body axis after which neural process have caudal orientation was chosen to represent the synclinal point (SP; *sensu* Slijper, 1946). This vertebra was equivalent to the boundary between mid- and posterior torso employed in previous studies on dolphins (Marchesi et al. 2020a, 2020b; Marchesi, Dans, Mora & González-José, 2021). Finally, the central vertebra of the tailstock (TS) was chosen (Figure 2). In regions with an even number of vertebrae, where the central vertebra could not be selected, we used the vertebra immediately anterior to the middle of the region; for example, in a region with four vertebrae, the second vertebra was chosen.

2.2. 3D-Geometric morphometrics

Using a Microscribe G2X, we digitized one of three original 3D-landmark configurations containing 28, 35, or 41 landmarks, depending on the region (supplementary online material 3). For each region, landmark configurations were superimposed by Generalized Procrustes Analysis (GPA; Goodall, 1991; Rohlf & Slice, 1990). The centroid size (CS), which is the square root of the summed squared distances of each landmark from the centroid of the landmark configuration, was used as size variable. Shape variables are the new coordinates that describe the location of each specimen in a curved space related to Kendall’s shape space and represent the difference between the consensus and each sample (Slice, 2001). For shape analyses, we worked with the symmetric component of shape (Klingenberg, 2011). Unless otherwise stated, analyses were performed in MorphoJ 1.07a (Klingenberg, 2011).

To investigate radiation in size within Phocoenidae we plotted CS on a recently suggested phylogeny (see Chehida et al., 2020; Figure 1B). At the same time, a permutation test was performed to test the null hypothesis of no phylogenetic signal in relation to size (Klingenberg & Gidaszewski, 2010).

To remove the effect of size-dependent shape variation (allometry) in the multivariate analysis of shape, we performed a multivariate pooled within-group regression (pooled by species) of the individual Procrustes coordinates on the logarithm of CS. We used the pooled regression to accommodate differences between species and to avoid imbalance caused by different sample sizes between the species. This type of regression considers the structure of the group to separate components predicted by size and residual components of shape variation (Klingenberg, 2016). The shape variation not affected by allometric scaling remains preserved in the residuals of such a regression, which can be used as allometry-free shape variables (Klingenberg, 2016). After performing the regression, we computed the average residuals for each species (average size-corrected shape), and used them for assessing the major components of variation by Principal Components Analysis (PCA). Two independent PCAs were done, one including only the porpoises and one containing all seven species. Then, we mapped shape variables (PC-scores) onto the corresponding phylogeny using squared-change parsimony (Maddison, 1991; Figures 1A, 1B). This way, ancestral and node shapes were estimated. For both analyses, a permutation test was performed for the null hypothesis of no phylogenetic signal in the data, in the same way as with CS.

3. RESULTS

3.1. Size

Plotting CS onto the porpoise phylogeny showed a clear pattern of divergence between *N. phocaenoides* and the other four species, with this tropical species showing a clear decrease from estimated ancestral size (Figure 3; node 1). In most regions, except for the Tm, there was a clear divergence pattern between sister species (*P. phocoena* - *P. dalli* and *P. spinipinnis* - *P. dioptrica*). In general, species considered to be coastal (*P. phocoena* and *P. spinnipinis*) tended to be smaller compared to the estimated ancestor of each sister clade (Figure 3, node 3 and 4), while their oceanic sister species (*P. dalli* and *P. dioptrica*) tended be larger. In the Tm, the divergence pattern was only evident for *P. phocoena* and *P. dalli* and the Southern Hemisphere species retained size in comparison to their estimated immediate ancestor (Figure 3, node 3). For all regions, permutation tests failed to reject the null hypothesis of absence of phylogenetic signal in CS (Table 2).

3.2. Shape

Species-specific shapes of each region for the five Phocoenidae species in comparison with the species grand mean shape is depicted in Figures 4A–E. Based on that, complete descriptions of particular shapes of vertebral structures are summarized in Table 3. Within each region, species are organized in such a way that they show an increase in flexibility from right to left. We included inferences regarding the biomechanical implications of the particular morphologies in supplementary onlne material 4.

In all regions, *N. phocaenoides* had the longest centra and the smallest centrum faces (Figure 4A-E, Table 3). For this species, neural spines were the shortest, except for the TS, and transverse processes were the longest and most robust, especially in the ThTo and the SP. In the Th and SP, transverse processes showed a greater anterior inclination. There were well-developed metapophyses placed high on the neural process in all regions except for the TS. At the other end of the spectrum, *P. dalli* had highly compressed centra with large faces. Neural processes were particularly long for this species, having a strong anterior inclination in most regions. Transverse processes were relatively short except in the Th and Tm. In this latter region, transverse processes were relatively long in comparison to the centra, and less anteriorly inclined compared to the grand mean shape. In this species, metapophyses were absent or poorly developed and placed low on the neural process.

Phocoena phocoena, *P. spinipinnis*, and *P. dioptrica* fell in between these extremes (Figure 4, Table 3). *Phocoena phocoena* had longer centra and smaller faces than *P. spinipinnis*; and *P. dioptrica* had relatively shorter centra with larger faces than the other two species. Morphology of the neural process in these three species varied depending on the region. *Phocoena phocoena* neural processes were long in relation to the centra, with particularly strong anterior inclination in the Tm. *Phocoena spinnipinis* had relatively shorter neural processes, except for the Th. In the Tm, this species had a posteriorly inclined neural process contrasting with the other two species of this paraphyletic genus. *Phocoena dioptrica* neural processes were the closest to the grand mean shape in the ThTo and SP, being longer in the Tm and shorter in the extremes of the column (Th and TS). The relative length of transverse processes decreased from *P. phocoena* (longer than mean shape), to *P. spinipinnis* (longer or equal to mean shape), and *P. dioptrica* having the shortest transverse processes among these species. Transverse process inclination varied considerably within each species between regions. In the anterior torso (ThTo), *P. phocoena* had the most posteriorly inclined transverse processes, *P. spinipinnis* had transverse processes almost perpendicular to the antero-posterior axis, while transverse process inclination for *P. dioptrica* was close to that of the grand mean shape. In the Tm and at the SP, these three species showed variable degrees of strong anterior inclinations. Finally, metapophyses had greater development both in *P. phocoena* and *P. dioptrica*, being smaller than the grand mean in *P. spinipinnis*.

3.3. Phylogeny

For all regions, the PCA involving only Phocoenidae showed that the first two principal components explained over 70 % of the total variance (Figure 5A). Contributions of each PC varied depending on the region. In most regions (Th, ThTo and SP), PC1 explained over 80% of the variance. Both at the Tm and the TS, contributions of PC2 to species distributions in the morpho-space were greater than in the other regions. Shape changes associated with negative and positive values of the first two components are shown in Figure 6 and described in supplementary online material 5. As a generalization, changes along the first two PCs translated into changes in centrum length (along the antero-posterior axis), relative size of centrum faces, length and orientation of processes and the development of zyga- and metapophyses.

The mapping of PC-scores on the phylogeny for porpoises (Figure 5A) showed differences depending on the region. In all cases, *P. dalli* was clearly separated from the rest of the family, and especially from *N. phocaenoides,* which was located at the opposite extreme of PC1. *P. phocoena*, P. *spinipinnis* and *P. dioptrica* varied in position along the first two PCs. Overall, there was a clear morphological divergence between sister species (*P. spinnipinis*-*P. dioptrica* and *P. phocoena*-*P. dalli*). *P. spinipinnis* was located closer to *P. phocoena* than to its sister species in three of the studied regions (Th, SP and TS). In the two remaining regions (ThTo and Tm), shape divergence between *P. spinipinnis* and *P. dioptrica* was evident but the two species were in neighboring areas of the morphospace. Permutation tests failed to reject the null hypothesis of no phylogenetic signal in all regions (Table 2).

PCA involving species of the three families (Phocoenidae, Monodontidae, and Delphinidae) also showed clear differences between species, with the first two PCs explaining over 80% of the total variance (Figure 5B). There was great differentiation between *M. monoceros* and the remaining species. On the contrary, for most regions, *L. albirostris* was located in similar areas of the morpho-space as *P. dalli*. Permutation tests failed to reject the null hypotheses of no phylogenetic signal in all regions except for the Tm (Table 2).

4. DISCUSSION

4.1. Eco-morphology

It has been suggested that cetacean body morphology is subject to a trade-off between drag during routine movements and the work required to maneuver (Weihs, 2002). In this sense, flexibility and slow precise maneuvering is observed in cetaceans in complex habitats and for demersal and bottom feeding, whereas torso rigidity or elastic stability and high-speed maneuvers are expected in cetaceans from the pelagic environment (Fish, 2002). Features signaling differences in the flexibility of the vertebral column with biomechanical implications have already been reported for mysticetes (Woodward, 2006) and several odontocete species (Buchholtz, 2001; Buchholtz & Schur, 2004; Costa, Rosel, Daura-Jorge & Simões-Lopes, 2016; Gillet et al. 2019, Marchesi et al., 2017, 2020a, 2020b). Buchholtz (2001) classified cetaceans into anatomical/functional groups based on vertebral morphology patterns along the torso and tailstock. This author included all phocoenids, together with *Orcinus*, in a group characterized by reduced centrum length throughout the torso and a modest increase of relative centrum length in the tailstock vertebrae; suggesting that high flexibility would be mostly restricted to the final portion of the vertebral column. In this regard, despite following the general pattern described by Buchholtz (2001), our detailed analyses showed considerable morphological variation within porpoises with biomechanical and functional implications that can be associated with adaptation towards particular habitats (e.g., riverine, coastal, and oceanic; supplementary online material 4). In accordance to its early divergence, the coastal tropical species, *N. phocaenoides,* showed osteological features that would indicate relatively high flexibility all along the vertebral column including spool-shaped vertebrae, a low number of intervertebral joints (low total count) and neural processes that do not interfere with those of adjacent vertebrae (Figure 7). These features would be highly beneficial in complex habitats where high flexibility and maneuverability are necessary (Fish, 2002; supplementary online material 4). For *P. phocoena, P. spinipinnis,* and *P. dioptrica,* vertebral morphologies varied between the two more coastal species, and between the coastal species and the oceanic species. In the coastal species, *P. phocoena and P. spinipinnis,* differences in vertebral morphology could be signaling different preferences for feeding in offshore waters (Figure 7; supplementary online material~~i~~ 4). Both species had features implying greater maneuverability than in the oceanic species, *P. dioptrica*. With different morphology to what we expected based on its proposed habitat, *P. dioptrica* poses a conundrum. This species has traditionally been considered to feed in cold-water oceanic habitats, but its vertebral morphology lacks typical features signaling a strong adaptation to oceanic fast swimming such as those observed in this study for *P. dalli,* or those previously reported for oceanic dolphins (Gillet et al., 2019; Marchesi et al., 2020a). Based on vertebral morphology, we propose that this species could be favoured to exploit the neritic habitat over the continental shelf more frequently than previously reported. This would agree with the partial overlap in prey preference between the two Southern Hemisphere species evidenced by stomach content and stable isotope analyses (Muñoz Moreda, 2019). Finally, the oceanic, fast-swimming *P. dalli* has been considered to represent the most extreme features within the family (Barnes, 1985), having the greatest total vertebral count among cetaceans and exceeding the other porpoises by over 50% (Barnes, 1985; Slijper, 1936; supplementary online material 1). As it has been proposed for dolphins (Buchholtz & Schur, 2004; Gillet et al., 2019, Marchesi et al., 2020a), highly disk-shaped vertebrae with large faces and extremely long neural processes indicate a derived vertebral column, in which flexibility is restricted to the final portion of the body. In this species, the elastically stable torso could be functioning as a spring storing potential energy; thus, saving energy during prolonged swimming (supplementary online material 4; Long et al., 1997; Marchesi et al., 2020a; Pabst, 1996). As in other pelagic cetacean species, fluke displacements would be produced almost exclusively by flexions of the tailstock that oscillates from a highly stable region (Buchholtz, 2001; Long et al., 1997; Marchesi et al., 2017; Marchesi et al., 2020). The relatively low potential for vertical displacement in the tailstock of this species suggests a relatively low amplitude, but high fluke oscillation frequency (Figure 7). This would be in accordance with previous kinematic studies that reported differences in these parameters between *P. phocoena* and *Lagenorhynchus acutus*, with the fast-swimming dolphin showing high fluke oscillation frequency and lower amplitude than the coastal porpoise (Curren, Bose & Lien, 1994). Moreover, in *P. dalli,* the relatively stable tailstock could be acting in series with the rigid torso to improve propulsion (see Long et al., 1997; Pabst, 1996). At the same time, a stable tailstock could result in less energy dissipation due to reduced movement between adjacent vertebrae; thus, translating most of the energy to the fluke, and allowing efficient swimming movements over longer distances when moving and feeding in the oceanic environment. In addition, the highly laterally compressed tailstock vertebrae would help minimize resistance during vertical displacement (Fish & Hui, 1991; Slijper, 1961). While further research is needed, we hypothesize that flexibility of the vertebral column of Dall's porpoise would be even more restricted than in other small, pelagic odontocetes (supplementary online material 5).

4.2. Phylogeny

Crown Phocoenidae species seem to have undergone a rapid radiation during the late Miocene until the early Pleistocene (between 6 and 2 myr) due to dispersal events influenced by geological, oceanic and climatic reorganization followed by allopatric speciation (Chehida et al., 2020; McGowen, Spaulding & Gatesy, 2009; Steeman et al., 2009;). The finless porpoises, *Neophocaena spp.*, was the first clade to diverge among crown Phocoenidae and the remaining porpoise species diverged ~ 4.0 Mya. For the two sister clades with antitropical distribution (*P. phocoena* - *P. dalli* and *P. spinipinnis* - *P. dioptrica*), it has been proposed that symmetric evolution took place in both hemispheres resulting in analogous ecological adaptations (Chehida et al., 2020; Galatius, 2010). Moreover, phocoenids display skull shape features, as well as other morphological adaptations, related to their coastal and oceanic habitats (Galatius et al., 2011). Our findings agree with this hypothesis, although various morphologies may serve similar biomechanical and functional purposes. By employing tridimensional geometric morphometrics we were able to detect varying degrees of morphological and functional convergences between species with analogous habitats.

We found the coastal *N. phocaenoides* to be smaller than the estimated common ancestor of crown Phocoenidae. For the two sister clades of anti-tropical species, there was divergence in size between species with different habitat preferences, in most regions of the vertebral column of porpoises. Coastal species tended to decrease in size from their estimated last common ancestor (Figure 3). Small size in porpoises, as well as several paedomorphic characters, have been suggested to be related to adaptation of life history to habitats with abundant and predictable food resources, such as many coastal habitats (Galatius et al., 2011). Coastal habitats serve as nurseries for fish and invertebrates that move further offshore as they grow (Beck et al., 2001), additionally favoring smaller individuals with greater maneuverability (Domenici, 2001). Oceanic species showed larger sizes than their coastal sister species. In these species, large size is thought to increase travel speed, minimizing transit time between patchy resources while also providing greater energy storage to deal with less predictable resources (Galatius et al., 2011). With the observed vertebral morphology of these species, the increased size could be translated into longer neural processes related to a greater relative development of swimming muscles. These conclusions regarding size should be treated carefully since our sample might be biased regarding sex for some species and sexual dimorphism in size has been reported for porpoise species (Amano, 2018; Goodall & Brownell 2018; Jefferson, 2018; Read, 1999; Reyes, 2018). Despite this, they are supported by data on body length and mass for the respective species (see Würsig, Thewissen & Kovacs, 2018).

Vertebral morphologies of the narwhal (*Monodon monoceros*) and the white-beaked dolphin (*Lagenorhynchus albirostris*) were notably different from consensus and from each other (supplementary online material 6). The vertebral morphology of the narwhal (*Monodon monoceros*) was clearly different from Phocoenidae; showing great morphological differences with respect to their estimated last common ancestor (Figure 5B). In agreement with our hypothesis, the oceanic dolphin, *L. albirostris*, was located in the proximity of *P. dalli* for most regions; indicating morphological convergence and adaptation towards open water fast-swimming. In most regions, vertebral morphology in Delphinoidea families (Monodontidae, Phocoenidae, and Delphinidae; see Waddell, Milinkovitch, Bérubé & Stanhope, 2000) did not show phylogenetic constraints within our sample, thus suggesting evolutionary lability. At the supra-family level, the only region showing the presence of a phylogenetic signal in vertebral morphology was the Tm; suggesting an early evolutionary constraint in the morphology of this region. The relevance of this region for the biomechanics of the vertebral column has previously been addressed (see Buchholtz, 2001; Marchesi et al., 2020). This result could be suggesting an early establishment of a set of morphological features particular to each family that was involved in early diversification of the delphinoids (~ 20 Mya). This hypothesis requires further testing by including a larger number of delphinoid species and other odontocete species. Within Phocoenidae, variation of vertebral morphology did not contain a phylogenetic signal in any of the studied regions (Table 2), but rather reflected ecomorphological adaptations (see above). Again, this implies a measure of evolutionary lability, which also supports the hypothesis of allopatric speciation by a founding event across the Equator as well as adaptation to different habitats. Moreover, in some regions of the vertebral column, there was morphological, and thus functional, similarity between species with similar habitat requirements (Figures 4, 5; supplementary online material 4).

5. CONCLUSION

This is the first detailed tridimensional morphological study on vertebrae of phocoenids. Our results suggest that the vertebral morphology of extant porpoises displays features of biomechanical relevance in relation to each species' preferred habitat. Moreover, we demonstrated clear divergence, both in size and shape, between sister species of porpoises. This likely reflects the energetic and biomechanical requirements of each species' habitat (e.g. coastal and oceanic). Overall, our results are in accordance with the hypothesis of speciation from a coastal ancestor, through adaptation to particular habitats and founder event(s) across the Equator. Moreover, the absence of phylogenetic signal in vertebral morphology may reflect evolutionary lability that could have been involved in the rapid radiation of porpoises. The observed variation in vertebral morphology of these small odontocetes highlights the importance of environmental complexity and selective pressures in the development of adaptations that minimize energetic costs and enhance foraging in specific habitats. Future studies targeting a larger sample size and other odontocetes would allow us to assess the existence of phylogenetic signal in vertebral morphology both at inter- and intrafamily levels. This could help elucidate the extent to which habitat and biomechanical requirements influence morphology in these mammals of axial locomotion.

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Table 1. Sample size for each region of the studied species: finless porpoise (*Neophocaena phocaenoides*), Burmeister's porpoise (*Phocoena spinipinnis*), spectacled porpoise (*Phocoena dioptrica*), harbor porpoise (*Phocoena phocoena*), Dall's porpoise (*Phocoenoides dalli*), narwhal (*Monodon monoceros*), and white-beaked dolphin (*Lagenorhynchus albirostris*). The number of the vertebra (counted from the first cervical vertebra) employed to characterize each particular region for each species is shown between parenthesis. See Figure 2 for region names.

	Th	ThTo	Tm	SP	TS
N. phocaenoides	2 (8)	1 (20)	2 (32)	2 (40)	2 (47)
P. phocoena	8 (8)	5 (20)	8 (33)	6 (41)	8 (49)
P. spinipinnis	7 (8)	7 (20)	5 (34)	6 (45)	8 (51)
P. dioptrica	11 (8)	9 (20)	15 (35)	13 (40)	15 (50)
P. dalli	5 (8)	5 (24)	6 (47)	6 (66)	5 (75)
M. monoceros	1 (8)	1 (18)	1 (27)	2 (32)	2 (39)
L. albirostris	3 (8)	3 (22)	4 (46)	3 (65)	3 (73)
Total	37	31	41	38	43

Table 2. Results (*p*-values) for the permutation test performed when plotting the phylogeny for Phocoenidae onto centroid size (CS) and PC-scores (P), as well as for plotting PC scores of all seven species (P+M+D). See Figure 2 for references on region name

	Th	ThTo	Tm	SP	TS
CS	0.462	0.465	0.367	0.419	0.337
P	0.134	0.337	0.272	0.614	0.669
P+M+D	0.054	0.623	0.016	0.551	0.445

Table 3. Species-specific shapes of the five Phocoenidae species. Unless stated otherwise, comparisons are made to the grand mean shape. Within each region, species are arranged in such a way that long centra is depicted on the left side and compressed centra on the right side. Thus, species position is related to variable degrees of flexibility/stability of a region. F: face; NP: neural process; TP: transverse processes; ZP: zygapophyses; MZ: metapophyses; CF: Chevron articulation faces; NA: neural arch; NS: neural spine; +: more; -: less; V: ventral; D: dorsal; Ext.: extremes; Post.: posterior; Ant.: anterior; Incl.: inclination; ⊥: perpendicular to; A-P: antero-posterior axis; D-V: dorso-ventral axis. Lat: lateral. Th: anterior thorax; ThTo: limit thorax-torso; Tm: mid-torso; SP: synclinal point: TS: tailstock.

	Flexiblility ◄				► **Stability**
	Long ◄				► **Compressed**
Th	*N. phocaenoides*	*P. phocoena*	*P. spinipinnis*	*P. dioptrica*	*P. dalli*
F	Smaller	Smaller	Close to consensus	Larger	Largest
NP	Shorter due to short NS + Post. Incl.	Longer (long NS, short NA) + Post. Incl.	Close to consensus ⊥ A-P	Shorter. (short NS, large NA) + Ant. Incl.	Longest (longest NS) + Ant. Incl.
TP	Shorter, + Robust. ⊥ D-V	Long in relation to centrum + Robust	Ext. of + D position + Ant. Incl.	Short in relation to centrum - Ant. Incl.	Longest. + V Incl.
ZP	Largest. Pre-ZP further from A-P	Larger	Slightly closer to A-P	Larger Post-ZP	
ThTo	*N. phocaenoides*	*P. phocoena*	*P.spinipinnis*	*P. dioptrica*	*P. dalli*
F	Smaller	Overall smaller centra	Smaller	Close to consensus	Largest
NP	Shortest (Short NS)	Long in relation to centrum Average inclination (Post.)	Slightly shorter + Post. Incl.	Close to consensus	Longest (both NA and NS) Ant. Incl.
TP	Longest. Most Robust.	Longer, + Narrow. + Post. Incl.	Average length. - Post. Incl.	Close to consensus	Shortest. + Post. Incl.
MP	Well developed. High on NP	Larger. Higher. Close to A-P	Smaller. Lower	Larger. Higher. Far from A-P	Smaller. Far from A-P
Tm	*N. phocaenoides*	*P. phocoena*	*P. spinipinnis*	*P. dioptrica*	*P. dalli*
F	Smaller	Smaller	Close to consensus	Larger	Largest in relation to centrum length
NP	Shortest (Short NS). ⊥ A-P	Slightly shorter. + Ant. Incl.	Shorter. + Post. Incl.	Slightly longer. ⊥ A-P	Longest. Strong Ant. Incl.
TP	Longer. - Ant. Incl. Curved ventrally	Longer. + Robust. + Ant. Incl.	Average length. + Ant. Incl.	Shorter. + Ant. Incl.	Longer. Slender. – Ant. Incl.
MP	Largest. High on NP	Higher in relation to NP	Smaller	Average development. High	Small/absent
SP	*N. phocaenoides*	*P. phocoena*	*P. spinnipinis*	*P. dioptrica*	*P. dalli*
F	Smallest	Smaller	Smaller	Close to consensus	Largest
NP	Shortest. Slightly Post. Incl.	Shorter. Slightly Ant. Incl.	Shorter. + Post. Incl.	Consensus length. + Robust ⊥ A-P	Longest (long NS) Slightly Ant. Incl.
TP	Longest. + Robust. Strong Ant. Incl.	Longer. +Robust. Ant. Incl.	Longer. + Robust. - Ant. Incl.	Shorter. + Ant. Incl.	Shortest. + Narrow
MP	Largest. Higher	Consensus	Smaller	Larger. High	Almost absent. Low
TS	*N. phocaenoides*	*P. phocoena*	*P. spinnipinis*	*P. dioptrica*	*P. dalli*
F	Smaller. Short on D-V Slightly convex	Smaller. Laterally compressed Highly convex	Slightly smaller Highly convex	Slightly larger Highly convex	Largest in relation to centrum. Longer on D-V. Laterally compressed. Least convex.
NP	Longer. + Robust	Longer. + Robust	Longer. + Post. Incl.	Shorter	Longest. – Post. Incl.
MP	Absent	Slightly longer	Smaller	Consensus	Absent
CF	Ant.: smaller Post.: Larger	Slightly smaller	Slightly larger	Consensus	Smaller in relation to consensus Large in relation to centrum

Figure 1. Phylogenetic three showing relationships among species based on McGowen et al (2020) and Chehida et al. (2020). A) Phylogenetic relationships between delphinoid families: Monodontidae, Phocoenidae, and Delphinidae. B) Phylogenetic relationships among the Phocoenidae species included in this study.

Figure 2. Skeleton of the spectacled porpoise (*Phocoena dioptrica*) showing the "traditional" and functional regions (white text) and the representative vertebrae employed in the study (grey text). Cv: cervical region; Th: anterior thorax; ThTo: boundary between thorax and torso; Tm: mid-torso; TS: tailstock; SP: synclinal point (sensu Slijper, 1936).

Figure 3. Plots, one for each region studied, resulting from mapping centroid size (CS) on the phylogeny proposed by Chehida et al. (2020) for the five Phocoenidae species studied. Numbers signal estimated nodes. Cv: cervical region; Th: anterior thorax; ThTo: boundary between thorax and torso; Tm: mid-torso; TS: tailstock; SP: synclinal point Np: *Neophocaena phocaenoides*; Pdi: *Phocoena dioptrica*; Pda: *Phocoenoides dalli*; Pp: *Phocoena phocoena*; Ps: *Phocoena spinipinnis*.

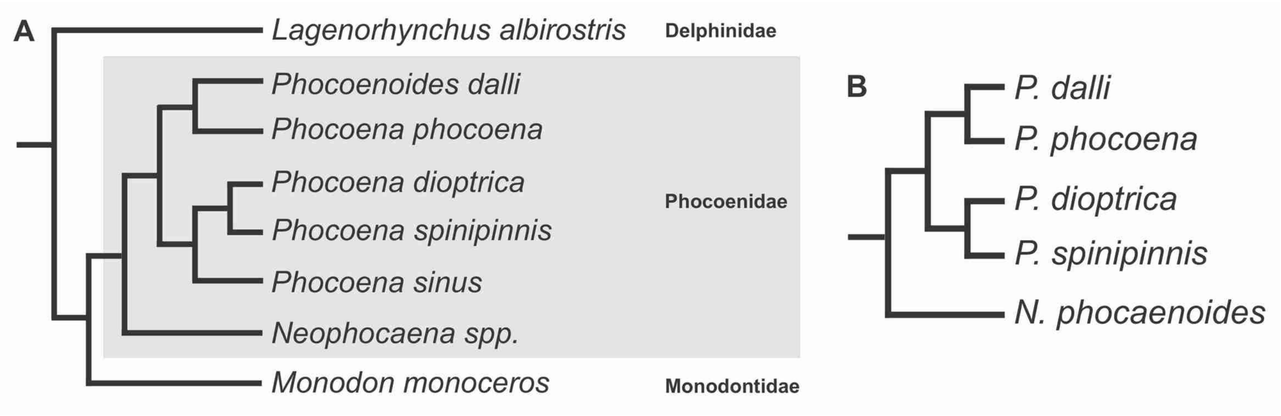
Figure 4. Mean species-specific shape (black outline and markers) for each region studied (A–E) of the five Phocoenidae species compared to the grand mean of all species (grey outline and markers). Within each region, anterior, dorsal, and lateral views are organized from top to bottom. Correction for allometric effects has been performed. Np: *Neophocaena phocaenoides*; Pdi: *Phocoena dioptrica*; Pda: *Phocoenoides dalli*; Pp: *Phocoena phocoena*; Ps: *Phocoena spinipinnis*.

Figure 5. Plots of the first two principal components (PC1 vs PC2), one for each region studied, resulting from mapping phylogeny on shape (PC-scores), and the reconstructed scores for nodes and root (open circle) for (A) Phocoenidae species, and (B) the three odontocete families: Phocoenidae

(P), Monodontidae (M), and Delphinidae (D). The percentage of variance explained by each component is included. In B, a grey polygon encompassing Phocoenidae was drawn for visual purposes. SP: synclinal point (sensu Slijper, 1936); Th: anterior thorax; ThTo: boundary between thorax and torso; Tm: mid-torso; TS: tailstock.

Figure 6. Shape changes associated with PCs 1 and 2 of the principal component analyses of mean shapes for the five Phocoenidae species. Mean shape of all species is indicated with gray outline and markers, changes associated with negative (-; scale factor: -0.1) and positive (+; scale factor: 0.1) are indicated with black outline and markers. Within each region anterior (A), dorsal (D), and left lateral (L) views are organized from top to bottom. Correction for allometric effects has been performed. SP: synclinal point (sensu Slijper, 1936); Th: anterior thorax; ThTo: boundary between thorax and torso; Tm: mid-torso; TS: tailstock.

Figure 7. Schematics representing the inferred degrees of flexibility/stability along the vertebral column of the Phocoenidae species. Black vertical lines signal the boundaries for functional regions. Colored figures correspond to the relative position of the studied vertebrae with respect to functional regions (see Figure 2). Numbers above colored figures indicate the vertebral count for that functional region in each species (see supplementary online material 1). Numbers within colored figures indicate the number of the vertebra along the vertebral column employed for each species, lack of numbers indicates the absence of differences when compared to *N. phocaenoides*. In the cervical region (Cv), fused + unfused vertebrae are denoted. Np: *Neophocaena phocaenoides*; Pdi: *Phocoena dioptrica*; Pda: *Phocoenoides dalli*; Pp: *Phocoena phocoena*; Ps: *Phocoena spinipinnis*.



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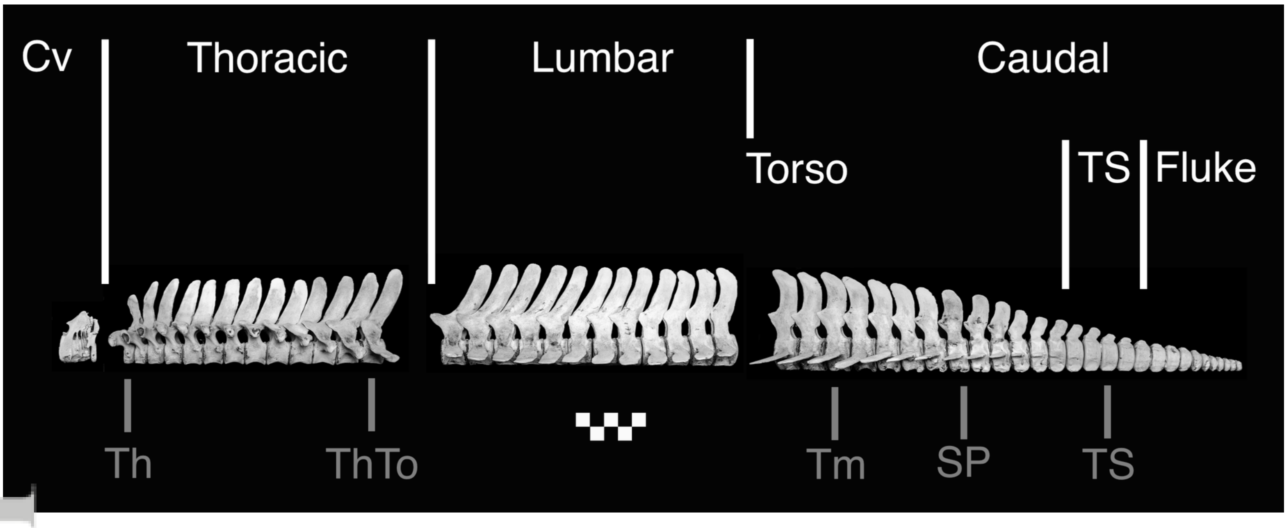

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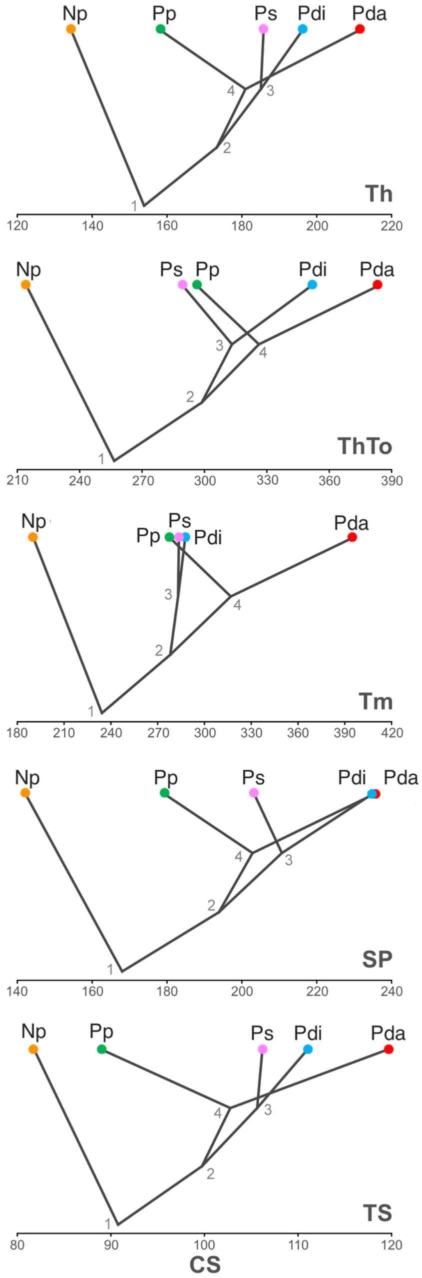

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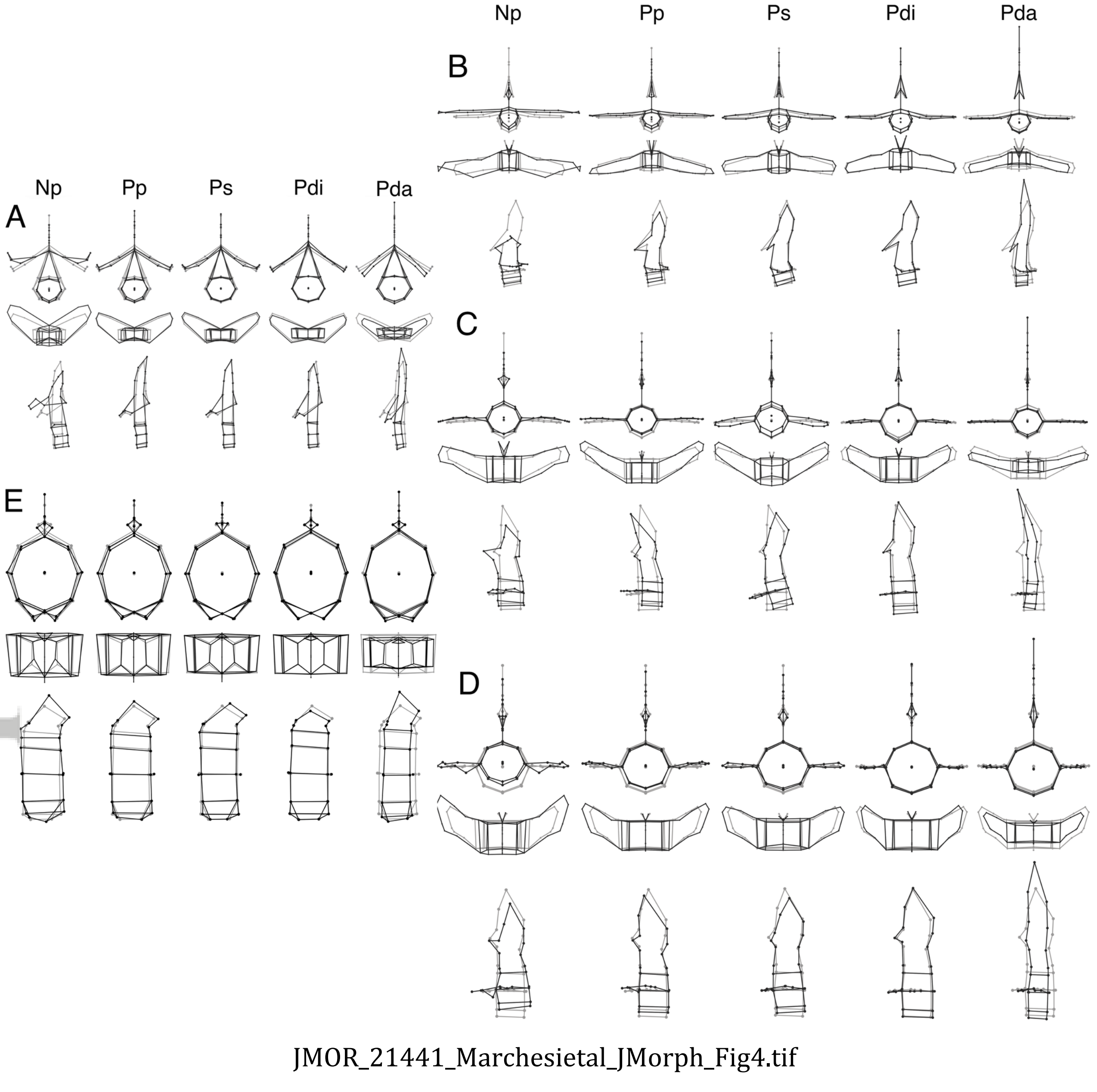

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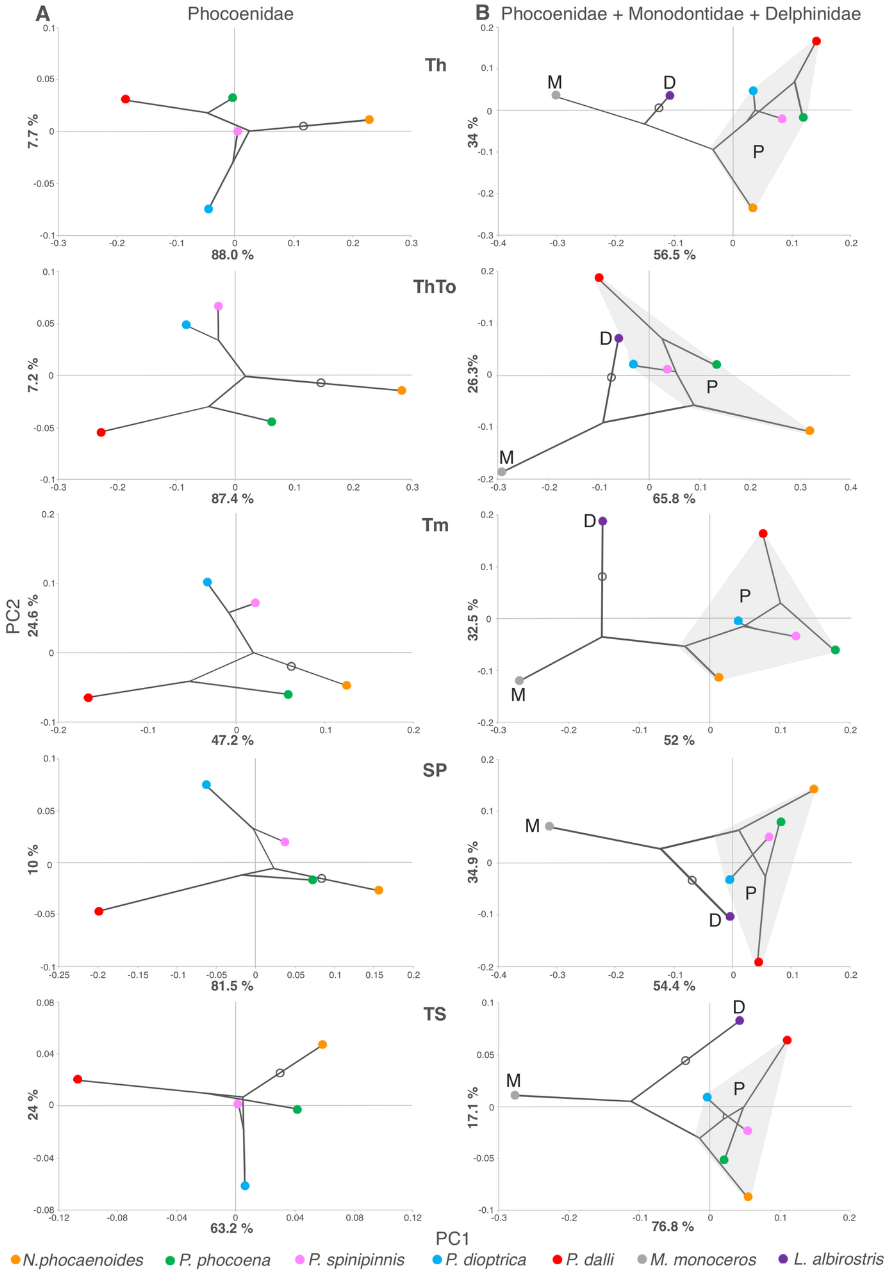

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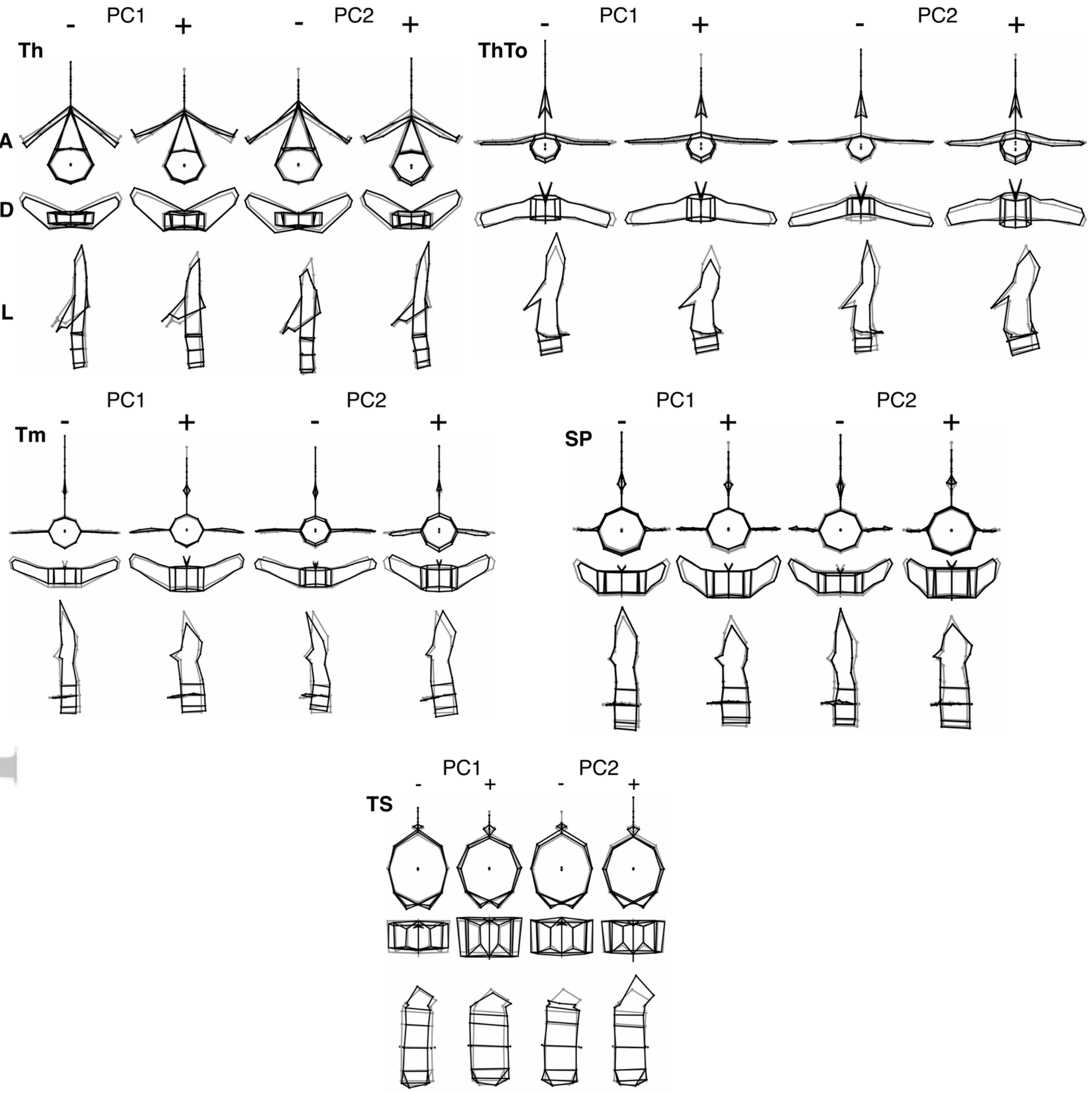

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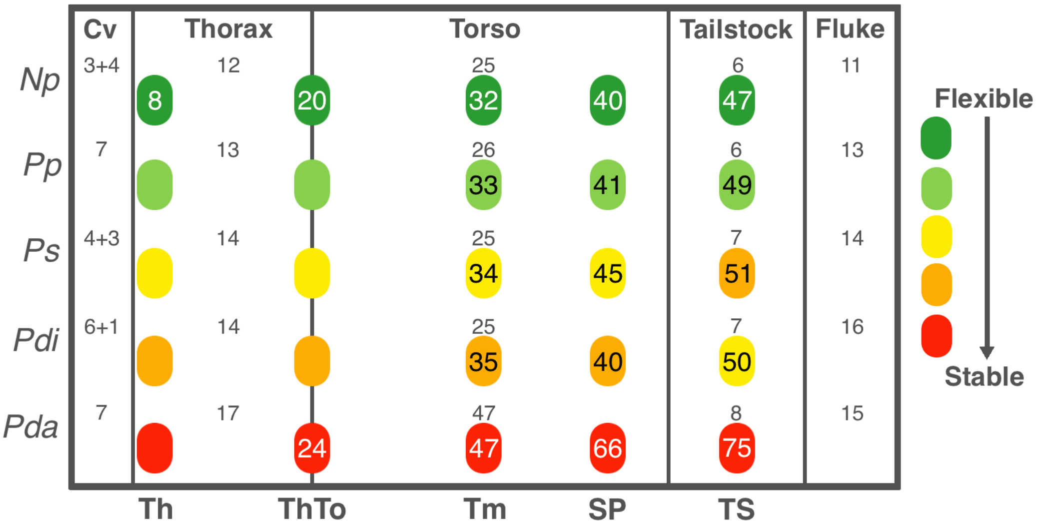

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