

Supplementary Material 2. Morphological changes associated with species positions in the morpho-space and anatomical interpretation of differences.

Morphological changes associated with species positions in the morpho-space

Information on shape changes along the first two axis of variation (Principal Components, PC; Figure 3) has been integrated to understand morphological differences among species. It should be noted that changes detailed below correspond to movement of certain landmarks with respect to other. Thus, changes are always relative. When describing shape changes, unless stated otherwise, comparisons are made with respect to the consensus shape, located in the origin of coordinates. Shape changes associated with minimum and maximum values of the first two components are described in Supplementary Tables S2 and shown in Supplementary Figure S3. Accordingly, shape changes for each species have been compiled in Table 4. This table contains the description of particular morphologies of vertebral structures. Within each region, species are organized in such a way they show an increase in flexibility (Table 4; Figure 4). Mean morphology of each region for the four species is depicted in Figure 5 (A-I).

In this study, the cervical region corresponded to the complex atlas-axis, thus inferences did not include the remaining cervical vertebrae. In all these species Cv3 through Cv7, are highly compressed and in contact with each other (MCM, personal observation). Slight morphological differences were evident between species in our analyses of the cervical region. Commerson's dolphin (*Cephalorhynchus commersonii*; $PC1 < 0$, $PC2 \geq 0$; Figure 3) had longer centra with relatively smaller faces and a shorter neural spine. Transverse processes were long in proportion to centrum face. The extremes of the transverse processes had a ventral position. The dusky dolphin (*Lagenorhynchus obscurus*) showed PC values associated with the consensus shape ($PC1 \cong 0$, $PC2 \cong 0$).

Peale's dolphin (*Lagenorhynchus australis*; $PC1 > 0$, $PC2 \cong 0$; Figure 3) morphology resulted in slightly anteroposterior compressed centrum with big faces. Relatively higher neural processes, and shorter but robust transverse processes when compared to Commerson's and dusky dolphin. Vertebral morphology of the hourglass dolphin (*Lagenorhynchus cruciger*) was highly variable. Mean shape for this species resulted to have large centrum faces, with robust neural process and long robust transverse processes (Table 4, Figure 5A).

At the beginning of the thorax (Th), two groups were differentiated along PC1: one group consisting of only Commerson's dolphin, and the other group consisting of Peale's dolphin and the hourglass dolphin; the dusky dolphin's equal frequency ellipse partially overlapped both groups (Figure 3). PC2 allowed further separation between Peale's dolphin and the hourglass dolphin. Commerson's dolphin ($PC1 < 0$, $PC2 \cong 0$; Figure 3, Table 4, Figure 5B) vertebra had relatively small faces, with the anterior f being concave. In addition, neural process was slightly higher (due to a higher neural arch), and transverse processes were short with extremes placed closer to the anteroposterior axis, both horizontally and vertically. Pre-zygapophyses were conspicuous. Peale's dolphin PC scores ($PC1 > 0$, $PC2 > 0$; Figure 3; Table 4; Figure 5B) were associated with slightly disk shaped centra with larger faces, and shorter but wider neural process. Transverse processes were long and more anteriorly inclined. Their extremes were placed more ventrally, closer to the anteroposterior axis. Vertebral morphology of the dusky dolphin was the most similar to the consensus and differed from the vertebral morphology of both the hourglass dolphin and Peale's dolphin in the degree of anteroposterior compression. The hourglass dolphin ($PC1 > 0$, $PC2 \leq 0$; Figure 3; Table 4; Figure 5B) had strongly compressed centra with relatively large faces. In general, vertebrae were more robust,

with long slender processes. Pre-zygapophyses were high but they did not protrude notably.

In the mid thorax (Thm) the analyses showed three groups along PC1. One group consisted in Commerson's dolphin ($PC1 > 0, PC2 \cong 0$), the second group was formed by Peale's dolphin and the dusky dolphin ($PC1 \cong 0$), and the third group included only the hourglass dolphin ($PC1 > 0, PC2 > 0$; Figure 3). PC2 allowed further separation between Peale's dolphin and the dusky dolphin ($PC2 < 0$ and $PC2 \cong 0$, respectively). Commerson's dolphin values ($PC1 > 0, PC2 \cong 0$; Figure 3) indicated long centra with small faces, and long robust neural processes (Table 4; Figure 5C). Transverse processes were short and robust, with anterior and ventral inclination, and pre-zygapophyses notably anterior with respect to the centrum. Once again, vertebral morphology of the dusky dolphin was the most similar to the consensus shape. When compared to Commerson's and the dusky dolphin, Peale's dolphin ($PC1 \cong 0, PC2 < 0$; Figure 3; Table 4; Figure 5C) had slightly shorter centra. Its neural process was slightly shorter but more robust. Transverse processes were longer with dorsally placed extremes, and well developed pre-zygapophyses placed further from each other than in any of the other three species. Finally, the hourglass dolphin ($PC1 > 0, PC2 > 0$; Figure 3; Table 4; Figure 5C) had compressed centra with large faces. Neural process was short and slender, and transverse processes were long and oriented more perpendicular to the neural process both vertically and horizontally. Prezygapophyses were well developed but due to transverse process orientation, their extremes were placed closer to the centrum.

At the thorax - torso boundary (ThTa) three groups were observed. Two groups were represented by Commerson's and Peale's dolphin, with negative PC1 values ($PC1 < 0$) but opposite values in PC2 ($PC2 > 0$ and $PC2 < 0$, respectively). The third group was formed by dusky and hourglass dolphins ($PC1 > 0, PC2 \cong 0$; Figure 3). Commerson's

dolphin ($PC1 < 0$, $PC2 > 0$; Figure 3; Table 4; Figure 5D) centra were long with small faces. Due to changes in the centrum axis orientation, the neural spine resulted to be more caudally inclined. Transverse processes were slightly shorter but robust and posteriorly inclined (Table 4; Figure 5D). Metapophyses were well developed and placed higher on the neural process. Peale's dolphin values ($PC1 < 0$, $PC2 < 0$; Figure 3; Table 4; Figure 5D) had long centra with large faces, specially the posterior one. Neural process was high and robust, with a more perpendicular orientation with respect to the anteroposterior axis. Transverse processes were long and strongly oriented caudally. Metapophyses were average and placed lower on the neural process. Values for the dusky dolphin and the hourglass dolphins ($PC1 > 0$, $PC2 \cong 0$; Figure 3; Table 4) resulted in highly compressed centra and medium sized faces. Neural process presented intermediate height and slender, with slightly more posterior orientation than in Peale's dolphin (Figure 5D). Transverse processes were long and slender with inclination slightly less posterior and less ventral. Metapophyses were less developed and in a similar position than in Peale's dolphin (Table 4; Figure 5D).

In the anterior-mid-torso boundary (TaTm) we identified two distinct groups along PC1. The first group consisted only of Commerson's dolphin with negative PC1 values ($PC1 < 0$), and the second group included partially overlapping equal frequency ellipses for Peale's dolphin, the dusky dolphin, and the hourglass dolphin (Figure 3). There were slight differences in PC1 values among these three species, increasing from Peale's to dusky, and to hourglass dolphin. Peale's dolphin differed also its PC2 values. Values obtained for Commerson's dolphin ($PC1 < 0$; $PC2 \cong 0$; Figure 3) corresponded with long centra; and a long robust neural process curved anteriorly (Table 4; Figure 5E). Transverse processes were of average length and oriented posteriorly. Metapophyses were not particularly well developed and placed relatively far from the centrum. Peale's

dolphin ($PC1 \cong 0$, $PC2 < 0$) had slightly longer centra and robust neural processes with well-developed metapophyses. Transverse processes length and orientation fell in between Commerson's dolphin and the other two species of *Lagenorhynchus*. Due to their orientation, the extremes were placed more ventrally (Table 4; Figure 5E). The dusky dolphin ($PC1 \cong 0$, $PC2 \cong 0$) showed values associated with anteroposterior compressed centra; slender and long neural spine in relation to the centrum. The poorly developed metapophyses were placed high. Transverse processes were long, slender and curved anteriorly. Their extremes were located more anteriorly and dorsally. All these features, except for metapophyses development, were more evident in the hourglass dolphin ($PC1 > 0$, $PC2 \cong 0$; Table 4; Figure 5E)

Principal component analysis of the mid-torso (Tm) showed three distinct groups along the first component. One group contained Commerson's dolphin, the second group consisted of Peale's dolphin, and the third group was formed by the dusky dolphin and the hourglass dolphin (Figure 3). Commerson's dolphin values ($PC1 < 0$, $PC2 \cong 0$) were associated with long centra. Neural process was robust and strongly inclined forward. Transverse processes were short and curved, the anterior border being convex and the posterior one concave. Metapophyses were well-developed (Table 4; Figure 5F). Peale's dolphin showed values ($PC1 \cong 0$, $PC2 < 0$; Figure 3; Table 4; Figure 5F) that resulted slightly shorter centra than in Commerson's dolphin. Neural process was long, wide and less inclined forward than in Commerson's dolphin. Metapophyses were slightly developed and placed low on the neural process. Transverse processes were slightly longer than in Commerson's dolphin with a perpendicular orientation with respect to the anteroposterior axis. Both dusky and hourglass dolphins showed values ($PC1 > 0$, $PC2 \cong 0$; Figure 3; Table 4; Figure 5F) that indicate notably compressed centra and long slender neural processes. Metapophyses were reduced or absent. Both species had long slender

transverse processes strongly inclined forward with more dorsally placed extremes (Table 4; Figure 5F).

When analyzing the mid-posterior torso boundary (TmTp) two groups were distinguishable along PC1: one group comprising Peale's dolphin, the dusky dolphin and the hourglass dolphin ($PC1 < 0$), and the other group consisting of Commerson's dolphin ($PC1 > 0$; Figure 3). Species within the first group could be further separated according to PC2 values: the ellipse for the hourglass dolphin ($PC2 \cong 0$) only partially overlapped the ellipses of Peale's ($PC2 < 0$) and the dusky dolphin ($PC2 > 0$). Commerson's dolphin values ($PC1 > 0$, $PC2 \cong 0$; Figure 3; Table 4; Figure 5G) signaled compression of the centra and relatively small faces. Neural process in this species had average length and strong forward inclination. Metapophyses were well developed and placed high on the process. Transverse processes were relatively long and strongly inclined forward (Table 4; Figure 5G). On the other extreme, Peale's dolphin showed values ($PC1 < 0$, $PC2 < 0$) associated with slightly elongated centra and large faces. Neural process was relatively long; with forward inclination less marked than in Commerson's dolphin. Transverse processes were relatively long and strongly inclined forward. Its extremes had a more ventral position. Metapophyses showed to be the smallest in comparison with the other three species (Table 4; Figure 5G). For the dusky dolphin ($PC1 < 0$, $PC2 > 0$) values indicated slightly compressed centra. Neural process was slender and less inclined forward than in Commerson's dolphin. Metapophyses were well developed and placed relatively distant to the longitudinal axis. Transverse processes were relatively short and less inclined forward than in the other *Lagenorhynchus* species (Table 4; Figure 5G). The hourglass dolphin had values ($PC1 < 0$, $PC2 \cong 0$) associated with relatively longer centra and larger faces than in Dusky dolphin. Neural process was almost perpendicular to the longitudinal axis and the metapophyses were well developed and place far from the

centrum axis. Transverse processes were short but robust with similar inclination than in dusky dolphin (Table 4; Figure 5G).

For the final region of the torso (Tp) a pattern similar to the mid-posterior torso boundary was observed (Figure 3). Commerson's dolphin showed values ($PC1 > 0$, $PC2 \cong 0$) associated with anteroposterior compression and small convex faces (Table 4; Figure 5H). Articular processes for chevrons resulted to be well developed. Neural process was short in proportion to centrum height. There were well developed metapophyses placed at great distance of the longitudinal axis. Due to changes in relative position of the centrum and neural process, metapophyses result much anterior than the vertebrae they belong. Transverse processes were well developed. Peale's dolphin values ($PC1 < 0$, $PC2 < 0$; Figure 3) resulted in slightly long centra with a markedly convex posterior face (Table 4; Figure 5H). Neural process was well developed in relation to the centrum height. Metapophyses were placed low on the neural spine. Transverse processes showed the consensus shape. Values for the dusky dolphin ($PC1 < 0$, $PC2 > 0$) signalled compressed centra with a convex posterior face (Table 4; Figure 5H). Tall neural process and well developed metapophyses of medium height. Transverse processes were the smallest of all species. The hourglass dolphin had values ($PC1 < 0$, $PC2 \cong 0$) that indicate relatively big slightly convex faces. The degree of anteroposterior compression falls in between dusky and Peale's dolphin. Neural process was better developed than in Commerson's dolphin when compared with centrum height but was relatively shorter and slender and with smaller metapophyses than in the dusky dolphin. Transverse processes were better developed than in the other *Lagenorhynchus* species (Table 4; Figure 5H).

PCA analyses for the most caudal area that we analyzed, the tail stock (TS), showed a similar pattern of interspecific differences as was seen in the two previous regions (TmTp and Tp). There were two distinguishable groups along PC1, and PC2 values

allowed separation between the dusky dolphin and the other *Lagenorhynchus* species (Figure 3). Commerson's dolphin values ($PC1 > 0$, $PC2 \cong 0$) indicated a highly compressed centrum with small and highly convex faces. Neural process was well developed in proportion to centrum height. Metapophyses had an averaged development (Table 4; Figure 5I). Peale's dolphin and the hourglass dolphin resulted the most similar to each other ($PC1 < 0$, $PC2 < 0$; Figure 3). Centra were moderately compressed antero-posteriorly and laterally. Peale's dolphin differed in the high degree of face convexity, being the highest in this species. Neural process was short in proportion to centrum height, the hourglass dolphin had the shortest neural processes. Metapophyses were almost absent (Table 4; Figure 5I). The dusky dolphin ($PC1 < 0$, $PC2 > 0$) showed values associated with longer and laterally highly compressed centra. Face convexity fell in between the convexity observed for the other two *Lagenorhynchus* species. Metapophyses had the greatest development (Table 4; Figure 5I).

Anatomical interpretation of morphological differences

The shape of the centrum, the curvature of the faces, the number of intervertebral joints, and the orientation and length of the processes both neural and transverse may be used to determine stable and flexible regions along the vertebral column (Slijper 1936; Crovetto, 1991; Buchholtz, 2001; Marchesi et al., 2017). Moreover, changes in processes development and orientation affects insertion sites influencing muscle length and mechanical advantage (see Buchholtz and Schur, 2004). For each studied region, we have made biomechanical interpretations of differences in relation with possible effects on axial muscles.

Despite PCA of the cervical region (atlas-axis complex, Cv) showed a relatively well-preserved morphology (Figure 3), there were statistical differences among species

(Table 5). Long centra with small faces in Commerson's dolphin might be indicating a cervical region relatively more flexible than in the other three species (Table 4). A greater length of the transverse processes affects the lever arm for the *m. scalenus dorsalis* fibers that relate the cervical region to the ribs, possibly affecting lateral movement of the head. Morphology of the complex atlas-axis in the dusky dolphin suggests an intermediate flexibility and development of muscle attachment sites (Figure 3; Figure 4; Table 4). For Peale's dolphin, slightly compressed centra would indicate greater stability than in Commerson's dolphin. Moreover, a robust neural process might be associated with larger attachment sites for muscles participating in extension of the vertebral column. The morphology of the neural process affects insertion sites for the *m. splenius* that relates the cervical region with the skull. More importantly, it affects origin sites for two muscles highly involved in extending the column: the *m. longissimus cervicis*, that relates the cervical region with the dorsal side of the transverse processes from the anterior half of the thorax; and the *m. multifidus*, involved in providing rigidity to the deep tendon (see Pabst, 1990). Mean shape for the hourglass dolphin would indicate high stability (compressed centra), and large insertion sites and mechanical advantage for the muscles that insert in this region (Figure 4; Table 4, Figure 5A).

The anterior thorax (Th) showed a somewhat conservative morphology (Figure 3). For the four species, vertebrae were notably compressed in this region, thus reinforcing their stability at this section. At the same time, the forward inclination of the transverse processes would suggest that the orientation of the ribs would cause a great interference in thorax rotation. Despite this pattern, morphology was significantly different for each species (Table 5). Small centrum faces in Commerson's dolphin indicate a relatively greater flexibility, although interference caused by well-developed pre-zygapophyses would limit the potential for dorsoventral flexion (Rommel and Reynolds, 2018). Robust,

highly compressed vertebrae with large faces in the hourglass dolphin are associated with increased stability. Moreover, transverse processes development in this species could be interpreted as larger attachment sites for the *m. longissimus cervicis*. Both Peale's dolphin and the dusky dolphin showed morphologies that indicate an intermediate degree of flexibility in this region, being the former species more similar to hourglass dolphin and the latter species, more similar to Commerson's dolphin (Figure 4; Table 4; Figure 5B).

In the species studied here, the mid thorax (Thm) is a potentially rigid area, having relatively long centra and small flat faces (Buchholtz and Schur, 2004; Marchesi et al., 2019). Despite this, there were particular morphologies for each species (Figure 3; Table 5). Commerson's dolphin had the longest centra and the smallest centrum faces. Neural spine development in this species would indicate a smaller insertion area for the superficial tendon and the *m. multifidus*. This situation, in addition to the upper position of the transverse processes (higher neural arch) could be translated in a smaller area for epaxial muscles (Table 4; Figure 5C). Strongly compressed centra and face dimensions in the hourglass dolphin indicates a more stable area (Table 4; Figure 5C). In addition, neural spines development in this species would be indicating greater development of insertion sites for the superficial tendon and the *m. multifidus*. The great development of the transverse processes would indicate a greater relative development of the *m. longissimus*, involved in changing the relative position of the head (in conjunction with the *m. semispinalis*) and in the extension of the vertebral column (in conjunction with the *m. semispinalis*; see Pabst, 1990). The dusky dolphin and Peale's dolphin fell in between these extremes, with Peale's dolphin being more similar to the hourglass dolphin than to the dusky dolphin.

At the boundary between the thorax and the torso (ThTa), groups observed by PCA were statistically different groups (Figure 3; Table 5). Commerson's dolphin, long centra

with small faces and short transverse processes indicate a potentially flexible region in comparison to the other species (Table 4; Figure 5D). At the same time, the markedly posterior inclination of the neural process may affect the length of the *m. multifidus* fibers and high well-developed metapophyses signal large insertion sites for the deep tendon. Neural arch height and metapophyses position also could mean a greater area available for the *m. longissimus*. In the case of Peale's dolphin, the large dimension of the centrum faces might offset the potential flexibility of long centra (Table 4; Figure 5D). In this species, neural process development could be indicating a great development of the *m. multifidus*. For the group consisting of the dusky dolphin and the hourglass dolphin, antero-posteriorly compressed centra would be associated with increased elastic stability (Buchholtz and Schur, 2004; Marchesi et al., 2019). The degree of development of insertion sites for the *m. multifidus* would be smaller than in the other two species due to the shorter neural process length. However, their inclination might result in a greater distance from the origin to the insertion site of the muscle fibers, increasing mechanical advantage for this muscle. The length of the transverse processes might be associated with a greater development of the *m. longissimus* and the *m. hypaxialis lumborum*. In addition, the perpendicular orientation of the transverse processes to the antero-posterior axis might translate into a greater distance from the origin to the insertion site when comparing to Commerson's and Peale's dolphin (Table 4; Figure 5D).

At the anterior-mid-torso boundary (TaTm), the observed differences by PCA were confirmed by the significance of the MD (Figure 3; Table 5). Each species showed morphologies associated with distinct degrees of stability (Table 4; Figure 5E). Long centra with relatively robust neural processes in Commerson's dolphin could signal a potential flexible region and a notable development of the epaxial muscles, respectively. In this species, the slight reduction of the metapophyses would signal a lesser

development of the deep tendon. This region in Peale's dolphin would be slightly less flexible than in Commerson's dolphin and metapophyses development might be related with larger insertion sites for the deep tendon. At the other extreme, highly compressed centra of the dusky dolphin and the hourglass dolphin suggest a highly stable area (Table 4; Figure 5E). In these species, the morphology of the processes and could translate into a relatively larger m. *multifidus* (low metapophyses) and a more anterior origin of the m. *longissimus* and the m. *hypaxialis lumborum* fibers. The greater development of metapophyses in the hourglass dolphin could result in enlarge insertions sites for the deep tendon and an increase mechanical advantage for the muscles acting on the fluke.

For the Tm, the three groups evidenced by PCA showed morphologies that would suggest decrease in flexibility from Commerson's dolphin to the group formed by the dusky dolphin and the hourglass dolphin, with Peale's dolphin showing an intermediate flexibility (Figure 3; Figure 4; Table 4; Figure 5F). The most divergent morphology was observed in Commerson's dolphin (Table 5). Morphology of the neural process in Commerson's dolphin would be related with an increased attachment sites and mechanical advantages for swimming muscles, especially the m. *longissimus*. In this species, metapophyses development could be related with the deep tendon performing notable work in this region. For Peale's dolphin, close to perpendicular orientation of the processes could affect rotational potential for this area. In the two most stable species (dusky and hourglass dolphins), long processes (neural and transverse) with forward inclination could translate into well-developed dorsal (m. *multifidus* and *longissimus*) and ventral muscles (m. *hypaxialis lumborum*) with high mechanical advantage due to fiber length. At the same time, the reduced metapophyses might be signaling a reduced effect of the deep tendon (Table 4; Figure 5F).

Shape changes for the TmTp allowed to differentiate three groups with variable degrees of vertebral column stability (Figure 3; Figure 4; Figure 5G, Table 4; Table 5). The most stable group morphology was observed for the Commerson's dolphin with compressed vertebrae and small centrum faces that might offset at least partially the stability provided by the centrum compression. At the same time, the length and notable anterior inclination of its processes would signal a greater interference among adjacent vertebrae, increasing stability in this region. Well-developed transverse processes would affect the insertion area for dorsal (m. *intertransversarius caudae medialis* and *lateralis*), lateral (m. *intertransversarius caudae dorsalis* and *ventralis*) and ventral muscles (m. *hypaxialis lumborum* and *intertransversarius caudae ventralis*; see Pabst, 1990). The second more stable group was composed by the dusky dolphin, with slightly compressed centra and midsized faces. In this species, process development may suggest a relatively greater development of the m. *longissimus* and of the deep tendon insertion sites than in the other species. The third group was formed by Peale's dolphin and the hourglass dolphin with vertebral morphology that would indicate relatively less stability in comparison the other two species (Figure 4; Table 4; Figure 5G). Neural processes for these species showed similar morphology; except for metapophyses development that was smaller in Peale's dolphin. Transverse processes morphology also varied between these two species. Peale's dolphin showed development and inclination similar to that of Commerson's dolphin, whereas hourglass dolphin's transverse processes resembled those of the dusky dolphin.

Shape analyses for the final region of the torso (Tp) showed that Commerson's dolphin is significantly different from the rest of the species, Peale's and the hourglass dolphin were not statistically different, and this latter species was not different from the dusky dolphin (Table 5). A highly stable region in Commerson's dolphin is inferred due

to the presence of slightly compressed centra, well developed neural process and high metapophyses of notably anterior position (Table 4; Figure 4; Figure 5H). Small faces dimension might partially counteract stability allowing a greater movement potential. The development of the processes, both neural and transverse, would agree with an important development of the muscles involved in dorsoventral flexion of the column. In the dusky dolphin, convex faces compensate at least partially the high compression degree of the centra and the development of the processes could be translated in an intermediate development of the swimming muscles. On the other extreme, Peale's dolphin showed features associated with greater flexibility such as slightly long centra with convex faces, short neural processes but with large insertion sites for the fibers of the *m. extensor caudae medialis* and transverse processes of medium size. The hourglass dolphin, falls in between the other two *Lagenorhynchus* species and has relatively large faces that might restrict flexibility given by long centra. Neural process development was similar to Peale's dolphin but transverse processes were larger.

Finally, the most caudal region studied, the tail stock (TS), also showed morphological differences from a relatively stable region in Commerson's dolphin up to flexible areas in the *Lagenorhynchus* species (Figure 3; Table 5; Figure 4; Figure 5I). The dusky dolphin showed features in this region that would indicate the greatest degree of flexibility when compared to the other three species analyzed such as long centra, convex faces. Moreover, a marked dorsoventral elongation of the centra could be related with a greater displacement of the fluke. The great development of metapophyses in this species could be translated in an increased advantage for muscles and tendons acting on this region.