JOURNAL OF MORPHOLOGY 00:00–00 (2016)

Skull Ontogeny and Modularity in Two Species of *Lagenorhynchus*: Morphological and Ecological Implications

Daniela L. del Castillo,[1,2]* Mariana Viglino,[2,3] David A. Flores,[2,4,5] and Humberto L. Cappozzo[1,2,6]

[1]*Laboratorio de Ecología, Comportamiento y Mamíferos Marinos, División Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina*
[2]*CONICET. Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina*
[3]*Instituto Patagónico de Geología y Paleontología, CCT CONICET-CENPAT, Boulevard Brown 2915 (U9120ACD), Puerto Madryn, Chubut, Argentina*
[4]*Unidad Ejecutora Lillo, CONICET-Fundación Miguel Lillo, Miguel Lillo 251, Tucumán 4000, Argentina*
[5]*Instituto de Vertebrados, Fundación Miguel Lillo, Miguel Lillo 251 (CP4000), Tucumán, Argentina*
[6]*Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico, Departamento de Ciencias Naturales y Antropología, Fundación Azara, Universidad Maimonides, Hidalgo 775 7mo (C1405BDB), Buenos Aires, Argentina*

ABSTRACT Comparisons of skull shape between closely related species can provide information on the role that phylogeny and function play in cranial evolution. We used 3D-anatomical landmarks in order to study the skull ontogeny of two closely related species, *Lagenorhynchus obscurus* and *Lagenorhynchus australis*, with a total sample of 52 skulls. We found shared trends between species, such as the relative compression of the neurocranium and the enlargement of the rostrum during ontogeny. However, these are common mammalian features, associated with prenatal brain development and sensory capsules. Moreover, we found a posterior displacement of the external nares and infraorbital foramina, and a strong development of the rostrum in an anteroposterior direction. Such trends are associated with the process of telescoping and have been observed in postnatal ontogeny of other odontocetes, suggesting a constraint in the pattern. Interspecific differences related to the deepness of facial region, robustness of the feeding apparatus and rostrum orientation may be related with the specific lifestyles of *L. obscurus* and *L. australis*. We also tested the presence of three different modules in the skull (basicranium, neurocranium, rostrum), all of which presented strong integration. Only the rostrum showed a different ontogenetic trajectory between species. Even though we detected directional asymmetry, changes in this feature along ontogeny were not detectable. Because asymmetry may be related to echolocation, our results suggest a functional importance of directional asymmetry from the beginning of postnatal life. J. Morphol. 000:000–000, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: directional asymmetry; modularity; geometric morphometrics; Delphinidae; ontogeny; *Lagenorhynchus*

INTRODUCTION

Toothed whales (Cetacea: Odontoceti) show a large diversity in skull shape, size, asymmetry and sometimes basic construction of the feeding apparatus and teeth. They also show variation in the facial region (including bony origin for nasofacial muscles implicated in echolocation), nasal passages, acoustic system and air sinuses (e.g., Miller, 1923; Mead, 1975; Oelschläger, 2000). Cranford et al. (1996) documented considerable diversity in shape, size and position of the melon and associated fatty-tissues between odontocetes species, even though they share some basic similarity in component parts. The authors proposed that these differences influence the sound quality and propagation pathway, as well as beam formation strategies when they echolocate. Another morphological comparison of closely related taxa was presented by Fraser and Purves (1960), who compare the dissociation of the tympanoperiotic bones from the skull and development of the air sinuses system in Cetacea. In this work, the authors accounted for the evolution of these characters within the order by comparing closely related genera and

---

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: Cetacean Society International and Society for Marine Mammalogy; Contract grant sponsor: Agencia Nacional de Promoción Científica y Tecnológica, Argentina PICT 2012-1583.

*Correspondence to: Daniela L. del Castillo, Laboratorio de Ecología, Comportamiento y Mamíferos Marinos, División Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina.
E-mail:dld.castillo@gmail.com

Received 20 April 2016; Revised 28 September 2016;
Accepted 21 October 2016.

DOI 10.1002/jmor.20629

families. Both studies showed that the analysis of the morphological diversity of the skull of closely related species can help us understand how it is influenced by phylogenetic history, habitat adaptation and the possible functional implications on the skull (Mead and Potter, 1995).

The genus *Lagenorhychus* Gray 1828 includes six species (*Lagenorhynchus acutus, Lagenorhynchus albirostris, Lagenorhynchus australis, Lagenorhynchus cruciger, Lagenorhynchus obliquidens* and *Lagenorhynchus obscurus*) that have an antitropical distribution (Leatherwood et al., 1991) and a fossil record dating from the Pleistocene of Europe (Uhen et al., 2007). Several recent molecular studies suggest that the genus, as currently recognized, is an artificial grouping (e.g., Cipriano, 1997; McGowen et al., 2009; Banguera-Hinestroza et al., 2014). Phylogenetic studies suggest that the North Atlantic *L. acutus* and *L. albirostris* comprise the earliest diverging lineages within Delphinidae, and outside of the clade Lissodelphinae, whereas the remaining four species are members of the subfamily Lissodelphininae, which includes the genera *Lissodelphis* and *Cephalorhynchus*. Because taxonomy is beyond the scope of the present work, we will continue using the genus name *Lagenorhynchus* as described.

The Peale's dolphin, *L. australis* Peale 1848, is restricted to the coastal and shallow shelf waters of southern South America, and has the smallest range of any species in the genus. It is fairly common from the San Jorge Gulf in the southwestern Atlantic Ocean, to the south of the archipelago of Tierra del Fuego, Cape Horn and the Burdwood Bank and up the Chilean coast as far as Valdivia (Goodall et al., 1997; Goodall, 2008). Peale's dolphin seldom strands, therefore some aspects of their life history are unknown (e.g., Goodall et al., 1997; Lescrauwaet, 1997; Schiavini et al., 1997; Boy et al., 2011). The species is strongly associated with kelp beds (*Macrocystis pyrifera*) and captures small octopuses assisted by their wide flat lips (Goodall et al., 1997; Goodall, 2008; Berta, 2015). Its diet also includes demersal and bottom fish, shrimps and cephalopods (Schiavini et al., 1997). Adults can reach lengths of 190–218 cm, and group size is usually small, from 2 to 5 animals, but aggregations of up to 100 have been recorded (Goodall, 2008).

The dusky dolphin, *L. obscurus* Gray 1828 inhabits cool temperate waters of the Southern Hemisphere, with well documented occurrences along the coasts of Southwest Africa, Argentina, Chile, Peru, New Zealand and Australia (Van Waerebeek, 1993; Van Waerebeek et al., 1995). Dusky dolphins are found principally in neritic waters above continental shelves and slopes but can also be observed over deep water if close to continents or islands. This species preys on a wide variety of fish and squid (Würsig and Würsig, 2009). The average adult size is 185 cm and school size varies from 3 to 5 individuals to a dozen or so, and occasionally up to about 2000 (Van Waerebeek and Würsig, 2008).

This article focuses on the ontogenetic variation of the cranial morphology and modularity of two closely related species of this genus that inhabit cool waters of the southern hemisphere (*L. obscurus* and *L. australis*). Knowledge about their growth patterns will allow comparison with other odontocete species (e.g., Sydney et al., 2012; del Castillo et al., 2014), and will increase the morphological data of this genus for future ecological, taxonomic and phylogenetic analyses. Our aim was to investigate shape changes in the skull of *L. obscurus* and *L. australis* along their ontogenetic trajectories and compare them. We hypothesized that, because these species are sympatric in South America (Garaffo et al., 2011), both species have different lifestyles and fine-scale habitat use (Goodall et al., 1997; Würsig and Würsig, 2009), which may result in different cranial morphology and growth patterns linked to functional demands. We applied a three-dimensional geometric morphometric approach, which has been demonstrated to be a powerful tool for the study of geometrical shape in biological structures and may provide insights into their evolutionary history and intraspecific variation (Monteiro-Filho et al., 2002; Amaral et al., 2009). Another goal was to test which skull region differ the most in the growth pattern between species, because specific parts of the skull in cetaceans are subject to different functional and evolutionary constraints (e.g., Guidarelli et al., 2014; Frandsen and Galatius, 2013). Based on previous studies in cetacean cranial development (e.g., Perrin, 1975; del Castillo et al., 2014, in press), we hypothesize that the rostrum will be the region that differ the most, both in size and shape during growth. To test this hypothesis, we worked with the concept of modules (Olson and Miller, 1958) and the possibility of testing them individually. Modules are composed by characters related ontogenetically or functionally which have high mutual influence, compared with those without functional commitments or unshared ontogenetic patterns. Modularity studies are uncommon in cetaceans, and have been mainly applied in evolutionary and ecological approaches (Sydney et al., 2012; Guidarelli et al., 2014). In this report, we also tested the presence of modules and their ontogenetic variation between both species.

MATERIALS AND METHODS

Data Collection

We analyzed a sample of 52 skulls representing ontogenetic series of *L. obscurus* ($n = 32$) and *L. australis* ($n = 20$), deposited at the Museo Acatushún de Aves y Mamíferos Marinos (Ushuaia Argentina), and at the National Museum of Natural History, Smithsonian Institution (Washington; see Supporting Information, Table S1). Because sexual dimorphism in the skull shape has not been reported for the species (Van Waerebeek, 1993; Boy et al., 2011), both sexes were pooled.

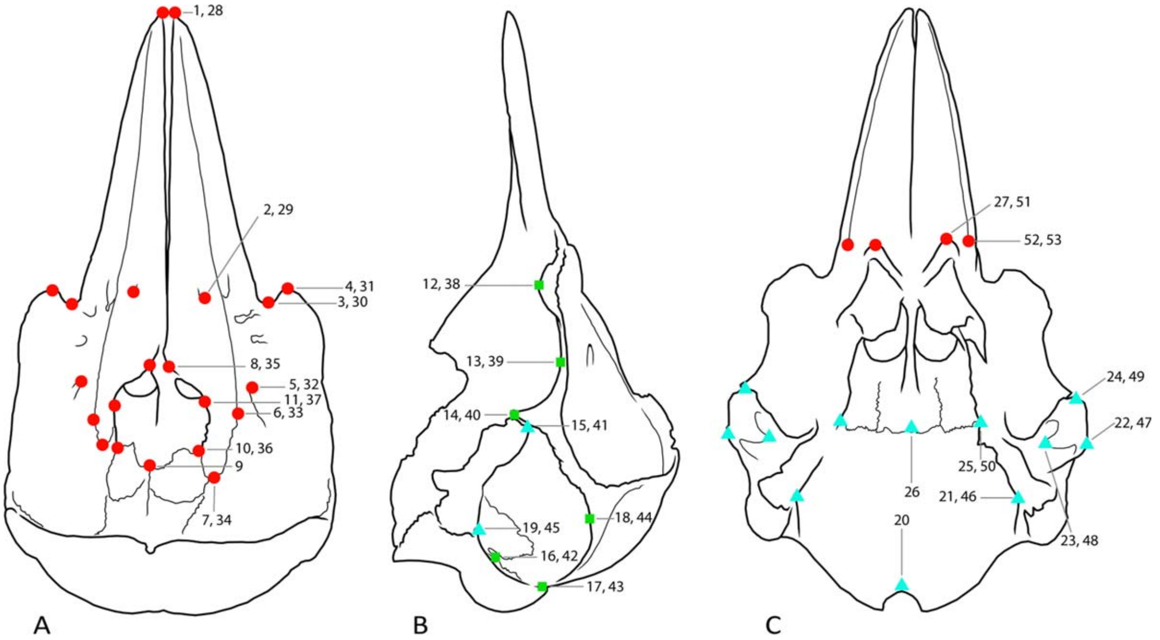

Fig. 1. Landmarks in dorsal (**A**), lateral (**B**) and ventral (**C**) views of the skull. Circles: landmarks assigned to rostrum; Squares: landmarks assigned to neurocranium; Triangles: landmarks assigned to basicranium.

A 3D digitizer Microscribe MX6DOF System (GoMeasured3D, Amherst, VA) was used to record 55 landmarks on each skull (Fig. 1, Supporting Information, Table S2), which were chosen following Sydney et al. (2012) with some modifications (del Castillo et al., 2014, in press). We chose landmarks that represented the complete skull and allowed us to see important features of the development in each cranial module (see below). Each skull was digitized at least twice, and the configurations were defined as the average of both digitalizations per specimen.

Ontogenetic and Shape Analyses

Superimposition of landmark configurations were performed by a Generalized Procrustes Analysis (Goodall, 1991; Rohlf, 1999) and size was measured as the Centroid Size (CS), defined as the square root of the sum of squared distances of each landmark from the centroid (see Supporting Information, Table S1). Both analyses were done using MorphoJ 1.05e (Klingenberg, 2011).

In order to study the shape ontogeny of both species, we considered CS as a proxy of age, so this relationship was statistically tested. For *L. australis*, age data by GLGs (Growth Layer Groups, counted in teeth) was available for almost all specimens (75% of the total sample; Boy et al., 2011). The regression of CS against age revealed a significant relationship ($n = 15$, $P < 0.05$; Supporting Information, Fig. S1). In the case of *L. obscurus*, age data was not available, and maturity was determined using skull sutures following Van Waerebeek (1993). Skull sutures as maturity indicators are often used in the literature (e.g., Galatius, 2005; Chen et al., 2011; del Castillo et al., 2015). An ANOVA was performed to test that mature specimens had larger sizes than immature ones ($n = 32$, $\mathrm{df} = 31$, $F = 12.77$, $P < 0.01$; Supporting Information, Fig. S2). According to these results, we found CS to be a valid proxy of age for both species. For these analyses, we used the Infostat software version 2016 (Di Rienzo et al., 2008).

We used log-transformed CS as a measure of size because preliminary analyses showed that it resulted in better linear relationships than untransformed CS (e.g., del Castillo et al., 2014, in press; see below). To study how shape changed in relation to size that is common to both species, we performed a pooled within-group regression of the Procrustes coordinates against logCS. This analysis allows the estimation of a common allometric component, which is an approximation of the average direction of shape development among the species. The common allometric component is estimated by a pooled regression of the shape variables, corrected for their species mean, against size (Mitteroecker et al., 2004; Galatius, 2010). To visualize the allometric relationships, we plotted the regression scores (the projection of shape data onto the direction of the regression vector in the shape tangent space; Drake and Klingenberg, 2008) against logCS. For this analysis, we used MorphoJ 1.05e (Klingenberg, 2011).

In order to detect species-specific shape changes during ontogeny, we performed regressions of shape against logCS for the two species separately. To visualize the allometric relationships, we plotted the regression scores of each species (the projection of shape data onto the direction of the regression vector in the shape tangent space; Drake and Klingenberg, 2008) againts logCS. The angles between regression vectors were compared using VecCompare, which compares the between-group angle with the 95% confidence interval (CI) of within-group ranges assessed by a bootstrap procedure (4,900 resamples). We considered that if the observed between-group angle was larger than the CI ranges of the two within-group angles, the directions of the two compared vectors were significantly different at a $P = 0.05$. Such regressions were performed for the whole set of landmarks as well as for the subsets defined by the three cranial modules (see below) in order to assess differences between trajectories. For these analyses, we used the IMP Packages version 6a (Sheets, 2001).

To study differences in shape between species that were not associated with skull size, we performed a size-corrected PCA, which is a PCA on the residuals of the regression of shape against logCS (Klingenberg, 2011) (i.e., residuals of the pooled within-group regression, see above). This procedure was performed with MorphoJ 1.05e (Klingenberg, 2011).

To study the asymmetric component of shape, we performed a Procrustes Anova in MorphoJ 1.05e (Klingenberg, 2011), which allows detecting significance of different sources of variation, such as interindividual variation, fluctuating asymmetry (FA) and directional asymmetry (DA). Fluctuating asymmetry refers to nonpathological asymmetries of bilaterally symmetric characters, that is, deviations in either direction from the perfect bilateral symmetry of low magnitude and a zero mean value (Palmer, 1994). Directional asymmetry occurs when one side of a bilateral trait always develops more than the other side (Van Valen, 1962), and affects odontocete cetaceans' skull morphology on a wide range of magnitudes (Ness, 1967; MacLeod et al., 2007). We performed a regression of asymmetric component of shape against logCS to study its ontogenetic development for both species separately, and compared results.

Modularity

In order to test modularity (Olson and Miller, 1958) and its ontogenetic variation in both species, landmarks were assigned to three defined cranial modules: basicranium (landmarks 15, 19, 41, 45, 20–26, 46–50), rostrum (landmarks 1–11, 27–37, 51–53) and neurocranium (landmarks 12–14, 16–18, 38–40, 42–44; see Fig. 1). We proposed these modules based on empirical evidence about modularity and covariation of characters in the mammal skull (e.g., Cheverud, 1982; Hallgrímsson et al., 2007). The mammal skull comprises three partially independent and embryologically distinct units: the basicranium (derived from the chondrocranium), which provides support for the braincase; the neurocranium (the dermatocranial bones of the cranial vault), which provides support and protection for the brain, and the rostrum (derived initially from the splanchnocranium with subsequent development of dermatocranial elements), which is related to feeding apparatus.

Hypotheses regarding the boundaries of modules were tested by comparing the strength of covariation among all possible partitions of landmarks and this procedure was performed using MorphoJ 1.05e (Klingenberg, 2011). RV-coefficient (Escoufier, 1973) is a scalar measure of the strength of the association between two subsets of landmarks in a configuration. This coefficient takes values between 0 (no correlation between the two blocks of variables) and 1 (maximum correlation between the two blocks of variables; Klingenberg, 2009). To determine whether there was support for modularity, we followed the procedures described by Klingenberg (2009). We compared the RV-coefficient for our three hypothesized modules to RV-coefficients estimated from 10,000 random partitions. Evidence of modularity exists when the RV-coefficient for a given modularity hypothesis is less than the RV-coefficients of 95% of the random contiguous partitions of the structure. Since the modules proposed did behave as such (see Results), species-specific ontogenetic regressions (see above) were performed on each module separately as well.

RESULTS

Ontogenetic and Shape Analyses

The pooled within-group regression of shape against logCS predicted 10% of the total variance (Fig. 2). Common ontogenetic trajectory described a lengthening of the rostrum, a backward displacement of dorsal infraorbital foramina and external nares, and a reduction in the relative size of the latter. A widening and anteroposterior enlargement of the antorbital notches was also observed. The trajectory also showed a dorsal displacement and an anteroposterior shortening of the temporal fossa as well as an enlargement of the mandibular fossa of the squamosal. A relative anteroposterior shortening of the posterior region of the braincase and a relative reduction of the size of the orbits were also observed.

When performing regression of shape against logCS for both species separately, differences in the directionalities of ontogenetic vectors were detected ($P < 0.05$). In *L. obscurus* the regression of the symmetric component of shape against logCS was significant ($P < 0.05$), and size explained 13.9% of the shape changes (Fig. 3). In *L. australis*, the regression was also significant ($P < 0.05$), and size explained 8.2% of the shape changes (Fig. 3). *L. obscurus* displayed during its ontogeny a stronger development of the rostrum in an anteroposterior direction than in *L. australis*, as well as a more concave posterior portion of the facial region. Also, in *L. australis* the rostrum widened while in *L. obscurus* it became narrower. The external nares showed a marked displacement to the posterior region of the skull in *L. obscurus*, while in *L. australis* this displacement was much shorter (Fig. 4). The temporal fossa deepened and shortened anteroposteriorly in *L. obscurus*, whilst the orbit shortened more anteroposteriorly than in *L. australis*. The basioccipital bone widened more and underwent greater anteroposterior shortening in *L. australis* than in *L. obscurus*.

Size-corrected PCA (Fig. 5) documents that *L. obscurus* has a more elongated rostrum and a more concave posterior portion of the facial region than *L. australis*, thus being more aligned with the rest of the skull. The lateral margin of the external nares was formed by the premaxilla and maxilla in both species, but in *L. obscurus* the contribution of the maxilla was greater. This difference could be related either to a more anterior position of the nares in the cranium or to a more posterior displacement of the premaxilla. The temporal fossa was proportionally larger and presented a more ventral position in *L. australis* than in *L. obscurus*, and the cranium was wider in the former as well. Orbits were less concave in *L. obscurus* and the postorbital process of the frontal was more ventrally and anteriorly placed in *L. australis*. The posterior part of the skull was shorter anteroposteriorly in *L. obscurus*.

Procrustes Anova analyses (Table 1) showed that both species had DA, and this factor accounted for 25% of total variation in shape in *L. obscurus* and 43% in *L. australis*. FA represented 9.5% and 8.5% of total variation in shape for both species, respectively. Regressions of the Asymmetric component of shape against logCS were not significant ($P = 0.63$ for *L. obscurus*; $P = 0.81$ for *L. australis*), indicating that the asymmetric component of shape did not change nor increase during postnatal ontogeny.

Modularity

The RV coefficient between the three predefined modules (rostrum, basicranium and neurocranium

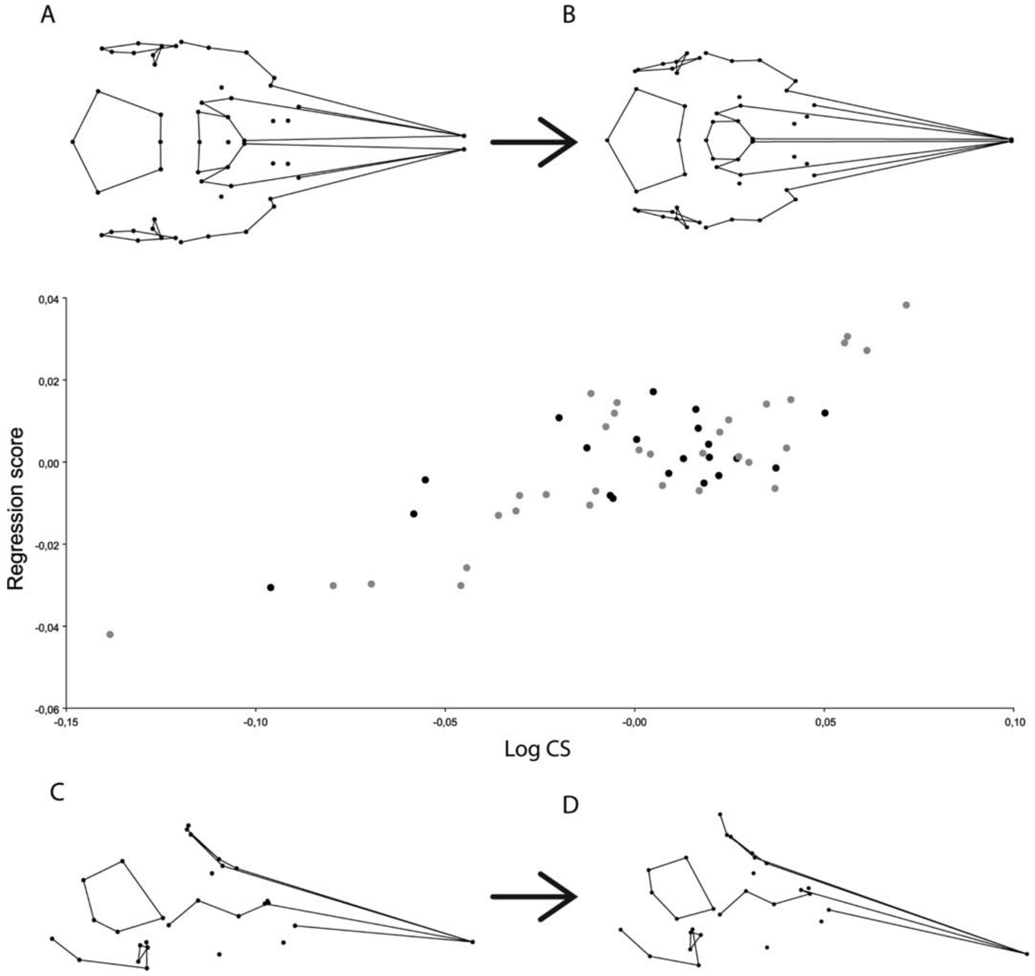

Fig. 2. Scatter plot of the pooled-within group regression of the Regression scores against logCS for both species, to study shape change in relation to size. Black points: *L. australis*; Grey points: *L. obscurus*. (**A, B**) Dorsal view and (**C, D**) lateral view. Shape changes are represented by increasing (right) and decreasing (left) logCS values two times.

subsets) was 0.837. This value suggests a strong integration of the cranium. However, the RV coefficient between the three subsets was lower than those for most other subdivisions of the landmarks ($P < 0.05$; Fig. 6). Therefore, the basicranium, neurocranium and rostrum do have a degree of modular separation in spite of the strong integration.

For the regressions of shape against logCS of the three modules separately, differences between species in the directionalities of ontogenetic vectors of the rostrum were detected ($P < 0.05$). However, no diference in the directionalities of the ontogenetic vectors were found for the basicranium or for the neurocranium ($P > 0.05$).

DISCUSSION

Ontogenetic and Shape Analyses of Both Species

There are highly conserved trends in the ontogenetic development of the mammalian skull, and our results were in agreement with these trends. Features observed in our sample, such as the relative compression of the neurocranium and the enlargement of the rostrum (which is proportionately larger in advanced stages of postnatal development) are common features in mammals (e.g., Ito and Miyazaki, 1990; Galatius et al., 2011; Flores et al., 2015) that are associated with the accelerated prenatal brain and sensory capsule (Hanken and Hall, 1993) development. Other ontogenetic trends observed in both species were expected since they are related to specific adaptations of odontocetes. For instance, the posterior displacement of the external nares and infraorbital foramina, and the strong development of the rostrum in an anteroposterior direction are associated with the process of telescoping (Miller, 1923), and have been observed in postnatal ontogeny of several species, such as *Cephalorhynchus commersonii, L. albirostris, Phocoena phocoena* and *Pontoporia blainvillei* (Galatius, 2010; del Castillo et al., 2014, in press). Even though

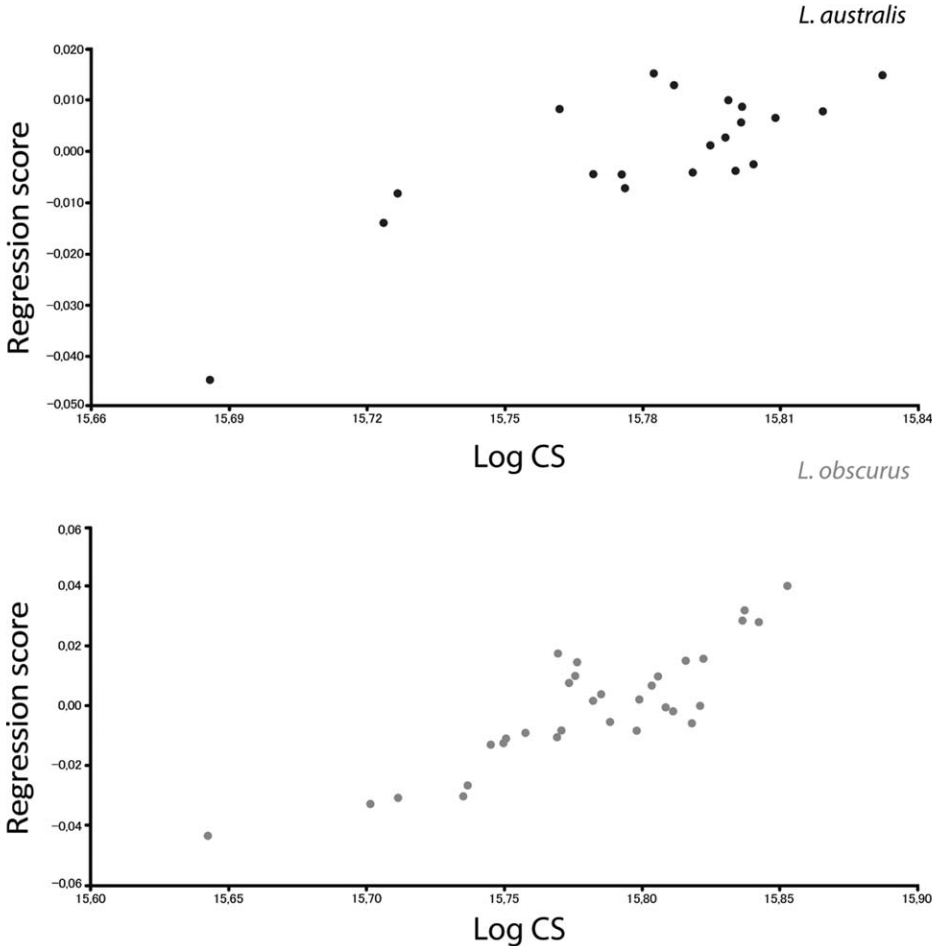

Fig. 3. Scatter plot of the regressions of Regression scores against logCS of *L. australis* (black points), and *L. obscurus* (grey points), to study species-specific shape changes during ontogeny.

telescoping is known to develop during prenatal growth, Rauschmann et al. (2006) noted that this process is not fully accomplished in perinatal dolphins, so it is expected to find telescoping-related ontogenetic trends during postnatal growth.

The development of the external nares and the premaxillary sac fossa in early stages of ontogeny of our sample is also in agreement with trends found in other species. Rauschmann et al. (2006) noted that the nasal area of perinatal *Stenella attenuata* is similar to that of adults in terms of structure, and del Castillo et al. (2014, in press) observed a development of premaxillae and external nares in *P. blainvillei* and *C. commersonii*. In addition, Frainer et al. (2015) found that the sound generating structures and auditory sense of *P. blainvillei* might already be functional at birth. Thus, the observed trends suggest that the development of the external nares and the posterior portion of the premaxilla are common features among odontocetes, probably associated with the early development of breathing and sound production apparatuses, albeit they have a continuous postnatal maturation (Rauschmann et al., 2006; Cozzi et al., 2015).

Differences between Species in Shape Analysis and Modularity

Although both species have similar skull morphology and general ontogenetic trends, several differences were noted. *L. obscurus* had a proportionally longer and narrower rostrum, as well as a more pronounced telescoping, evidenced by the position of the external nares and the dorsal infraorbital foramina. This species had a more subtle slope on the rostrum, which makes it more aligned with the rest of the skull than in *L. australis*. The premaxillary sac fossa displayed a stronger concavity in *L. obscurus* and also the temporal fossa had a more dorsal position. Some of these differences, such as those regarding rostrum length and inclination, and concavity of premaxillary sac fossa were also noted by Galatius and Goodall (2016).

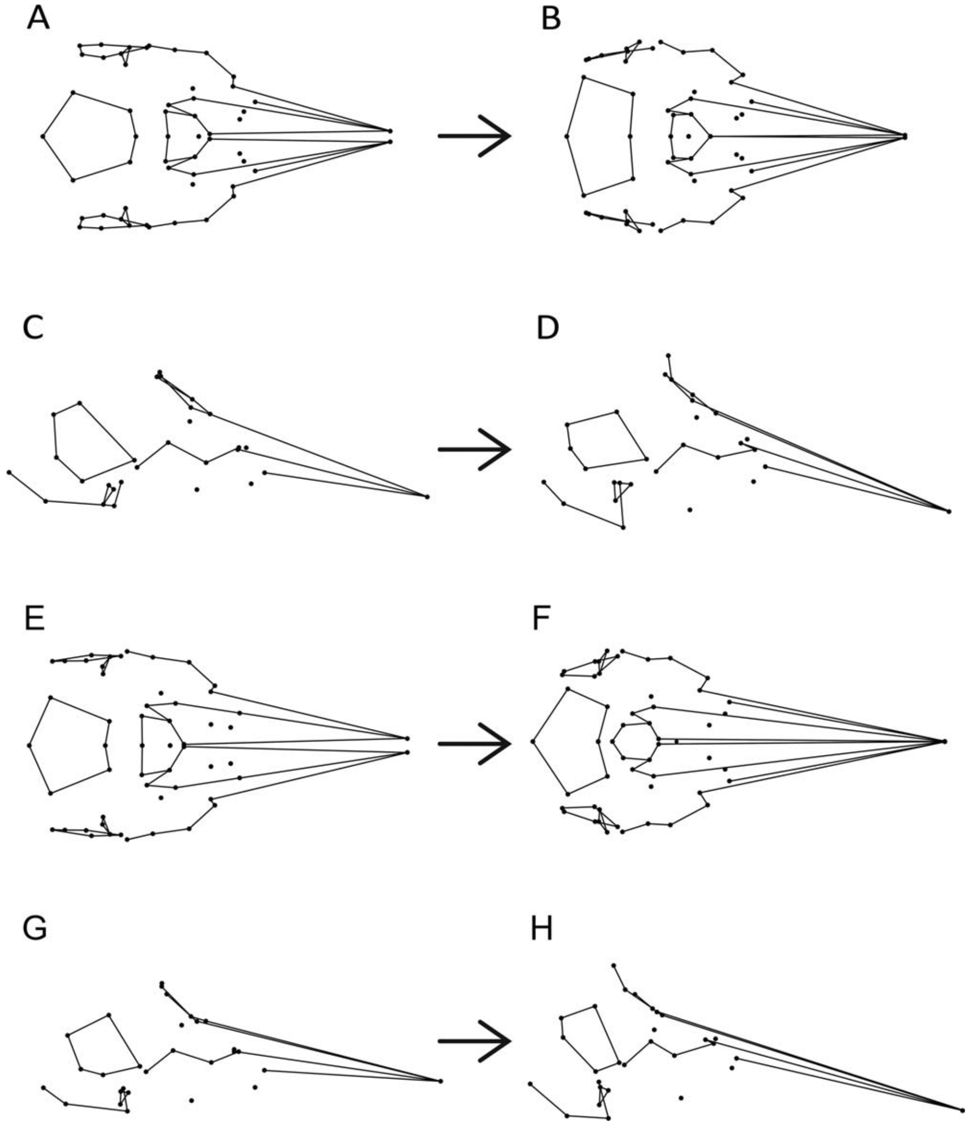

Fig. 4. Shape changes along the allometric trajectories in dorsal (**A, B, E, F**) and lateral views (**C, D, G, H**), for *L. australis* (A, B, C, D) and for *L. obscurus* (E, F, G, H). Shape changes are represented by increasing (right) and decreasing (left) logCS values two times.

Regarding the feeding apparatus, Perrin (1975) proposed that robust feeding apparatuses are advantageous in coastal waters, where available prey may be larger, more irregularly and heavily constructed than offshore. Smaller feeding apparatuses that allow greater maneuverability are advantageous in pelagic habitats where the major diet items are small squid and small, streamlined, fast-moving fishes. Previous studies have shown that *L. obscurus* and *L. australis* use different food resources. For example, on the Patagonian coast, *L. australis* is associated with coastal kelp beds (*Macrocystis pyrifera*; Lescrauwaet, 1997) and its main prey are a variety of small fish (e.g., *Genypterus blacodes*, *Pleoticus muelleri, Loligo gahi, Illex argentinus, Merluccius hubbsi)*, and small octopuses (Schiavini et al., 1997), that is, mainly demersal and bottom taxa. *L. obscurus*, instead, is a mesopelagic feeder and its main prey is a pelagic fish, *Engraulis anchoita* (Alonso et al., 1998). Our study shows that *L. australis* possessed a more robust feeding apparatus (wider rostrum and deeper temporal fossa), than *L. obscurus*. Moreover, rostrum morphology in *L. australis* (short and wide) could suggest a greater capability of suction feeding than *L. obscurus* (Werth, 2006), which would be in accordance with the diet for this species. Differences in feeding apparatus morphologies and diets may indicate how partitioning of ecological niches may have reduced the occurrence of competition for food resources when these species are in direct sympatry (Bearzi, 2005).

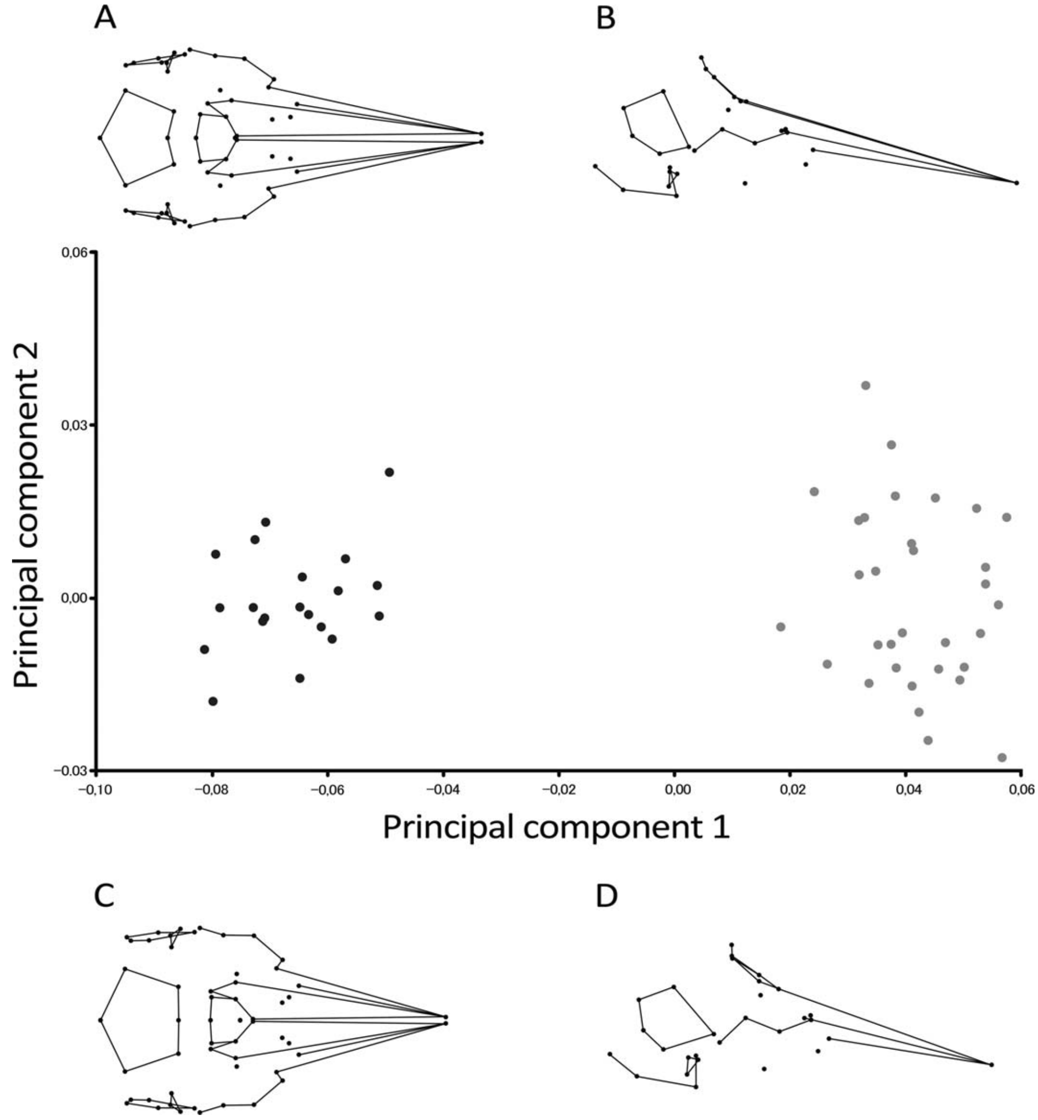

Fig. 5. PC1 and PC2 of the size-corrected PCA, to study differences in shape that are not associated with skull size. Black points and (**C, D**): *L. australis*. Grey points and (**A, B**): *L. obscurus*. (A, C) dorsal view and (B, D) lateral view. Shape changes are represented by increasing (right) and decreasing (left) logCS values two times. PC1 and PC2 explain 65,69% and 3,98% of variance, respectively.

Monteiro-Filho et al. (2002) proposed for the Amazon species *Sotalia fluviatilis* that a rostrum with a downward inflection was related to the need to scan riverbed bottoms in search for food and avoid obstacles (e.g., tree trunks and branches). Like *Sotalia*, we found that *L. australis*, which is strongly associated with kelp beds, also showed a downward inflection in comparison with *L. obscurus*.

TABLE 1. Procrustes Anova of shape asymmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (i.e., dimensionless)

	Effect	SS	MS	df	*F*	*P* (param.)
	Individual	0.12	5.0-05	2,449	6.16	<0.01
L. obscurus	Side (DA)	0.05	6.6 E −04	76	81.44	<0.01
	Ind * Side (FA)	0.02	8.2 E −06	2,356	3.7	<0.01
	Residual	0.01	2.2-06	4,495		
	Individual	0.05	3.6-05	1,501	5.05	<0.01
L. australis	Side (DA)	0.05	7.0 E −04	76	98.34	<0.01
	Ind * Side (FA)	0.01	7.13-06	1,444	5.33	<0.01
	Residual	0.01	1.34 E −06	3,100		

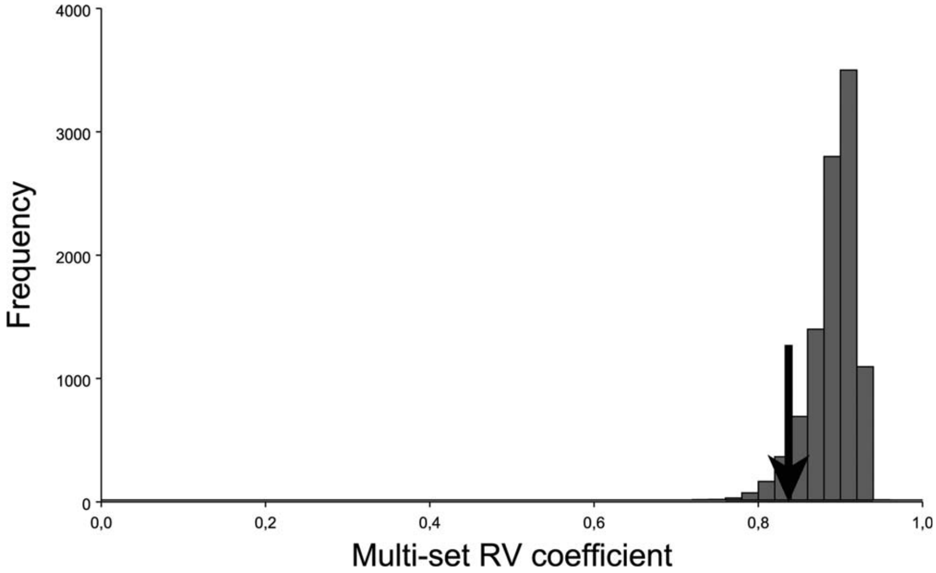

Fig. 6. Analysis of modularity of the skull for *L. australis* and *L. obscurus*. The arrow indicates the value of the RV coefficient for the subdivision of landmarks into modules proposed (for more details, see materials and methods section) and the histogram represents the distribution of RV coefficients for 10,000 alternative partitions of landmarks into anatomically contiguous subsets.

Galatius et al. (2011) noted that deeper facial region in more pelagic porpoises may provide more space for a larger melon, capable of transmitting more directional and powerful sound beams, which can be advantageous in deeper waters (Cranford et al., 2008; McKenna et al., 2012). We observed that *L. obscurus*, which inhabits coastal and continental shelf waters possessed a deeper facial region than *L. australis*, which inhabits mainly shallow coastal waters (Würsig and Würsig, 2009). However, since connective tissues and muscles acting on the melon can actively manipulate melon shape and sound characteristics (Harper et al., 2008; McKenna et al., 2012), more data is needed to fully understand the functional morphology and evolution of odontocete biosonar and its correlation with skull morphology.

Autonomy and integration are terms that refer to the degree of covariation among the components of a module (set of related traits): autonomy refers to the relative independence of modules, while morphological integration studies how these traits co-vary with each other (e.g., Klingenberg, 2009; Marroig et al., 2009; Goswami and Polly, 2010). Our results showed that the three subsets of landmarks proposed did behave as different modules, as expected (although see Sydney et al., 2012 for other module hypotheses). However, the high RV-coefficient suggested a strong integration between modules. This result is in general accordance to what other authors have found as a general trend for mammalian skulls (e.g., Goswami, 2006; Marroig et al., 2009; Goswami and Polly, 2010), although these authors used other modules than those proposed here. Even though the exploration in a more taxonomically diverse sample is necessary, the high integration of the skull in our study reflects the conservative morphology of the delphinid skull, in relation to their strong functional compromise. In this sense, high levels of integration imply a lower capacity for evolutionary response, which can be interpreted as a lower evolutionary flexibility in phenotypic terms (Marroig et al., 2009). Goswami and Polly (2010) evaluated two models to test the influence of modularity in morphological evolution: constraint (high correlation among traits limits morphological disparity) and facilitation (high correlation among traits promotes morphological disparity). They found that the constraint model is mainly present across large time scales in the evolution of the placental mammalian skull. However, the skull modularity and high correlation between modules (high RV-coefficient value) found in our study would be in accordance with the facilitation model proposed by the authors. Comparisons of ontogenetic trajectories for the three proposed modules agreed with our hypothesis, as only the rostrum showed angular differences between both species. Perrin (1975) suggested that the greater selective pressure acting on the skull at intraspecific level is associated with feeding strategies. Based on this hypothesis, we expected that the same would occur between closely related species and could be another strategy for sympatric species to reduce competition. It would be interesting to see which model (constraint or facilitation) would best explain morphological variation across cetacean skull evolution both at a small scale pattern (i.e., interspecific and intergeneric) and at a large scale pattern (i.e., between families and suborders), within a phylogenetic hypothesis framework.

Asymmetry

The percentage of variance explained by DA in our study was greater when comparing with other non-cetacean mammal species and it was greater in comparison with the percentage of variance explained by FA. This result agreed with our expectations, considering the well documented asymmetrical condition in odontocete skulls (e.g., Ness, 1967; Cranford et al., 1996; del Castillo et al., in press). In our study, DA accounted for 25% of total variation of shape in *L. obscurus* and 43% in *L. australis*, whilst FA was 9.5% and 8.5%, respectively. Recent studies on humans, mice and macaques have shown that DA is lower than FA (e.g., Willmore et al., 2005; Burgio et al., 2009; Lotto and Gonzalez, 2014). Even though these works did not use our same set of landmarks, they used a set of cranial landmarks that represented the whole skull, for which the levels of DA and FA are suitable for comparisons with our results. Recently, del Castillo et al. (in press), found that in *Cephalorhynchus commersonii* (same set of landmarks) DA accounted for 34% of the total variance, and FA represented only 10%. Furthermore, Yurick and Gaskin (1988) observed that the degree of asymmetry was not correlated with skull length in *Phocoena phocoena*. In accordance with this, we found that the regression analysis of the asymmetric component of shape against size was not significant for any of the analyzed species. A similar result was also found for *C. commersonii* (del Castillo et al., in press), where, even though this regression was significant, the percentage explained by size was extremely low (1.2%), indicating that changes in postnatal ontogeny are almost negligible. Taking into account that DA does not increase during postnatal ontogeny, and that it is widely assumed that DA present in odontocete skulls is related to echolocation, it can be inferred the importance of this functional feature in cetaceans from the beginning of postnatal life (Haddad et al., 2012; Berta et al., 2014).

Directional asymmetry in odontocete cetacean skull is believed to be functionally linked to echolocation, and several studies (e.g., Mead, 1975; Yurick and Gaskin, 1988; Cranford et al., 1996) show that the anatomy of the epicranial complex varies considerably between different species of odontocetes, as does the variety of sounds emitted by them. It is still unknown if these differences are responsible for this variety of sounds or if there is a combination of different factors involved. Therefore, we consider it insufficient to make inferences about the functional implications of the DA observed in echolocation and sound production only from osteological information, because the soft anatomy of this region for these two species has not been studied in detail. Another hypothesis has been proposed, where skull asymmetry is related to prey size (MacLeod et al., 2007). Differences between species in levels of asymmetry presumably relate to niche differences in prey size preferences that lead to differing levels of selective pressure for asymmetry. Because *L. australis* and *L. obscurus* are sympatric but possess different diets, the significant differences of DA detected between both species could be explained with this hypothesis. However, more information on soft structures and diet across all the distribution of both species is necessary to support this interpretation. In recent phylogenetic studies (May-Collado and Agnarsson, 2006; McGowen et al., 2009) *L. australis* is closely related to *C. commersonii* (with a DA of 34%) (del Castillo et al., in press). As a first interpretation, we suggest that DA could be influenced by phylogenetic relationships, because the more closely related *L. australis* and *C. commersonii* present higher DA values. However, a phylogenetic analysis mapping this character should be made in order to test this proposition, shedding light on the evolution of DA in cetaceans.

ACKNOWLEDGMENTS

We especially thank Natalie Goodall for access to the Acatushún collection and her hospitality, as well the many volunteers that work on the museum. We thank the editor, Morgan Churchill and an anonymous reviewer for their suggestions, they greatly improved the manuscript. We also thank Agustín Dib for drawing the figures, and to Agustina Gomez Laich for grammar corrections. Finally, warm thanks to Charley Potter for permission and access to material under his care, and especially for his hospitality and help during sampling in Smithsonian Institution. Author Contributions: Study design was done by del Castillo, Viglino, Flores, Cappozzo. Acquisition of data was done by del Castillo. Analysis of data was done by del Castillo, Viglino. Manuscript preparation was done by del Castillo, Viglino, Flores, Cappozzo.

LITERATURE CITED

Alonso MK, Crespo EA, Garcia NA, Pedraza SN, Coscarella MA. 1998. Diet of dusky dolphins, *Lagenorhynchus obscurus*, in waters off Patagonia, Argentina. Fish Bull 96:366–374.

Amaral AR, Coelho MM, Marugán-Lobón J, Rohlf FJ. 2009. Cranial shape differentiation in three closely related delphinid cetacean species: Insights into evolutionary history. Zoology 112:38–47.

Banguera-Hinestroza E, Hayano A, Crespo E, Hoelzel AR. 2014. Delphinid systematics and biogeography with a focus on the current genus *Lagenorhynchus*: Multiple pathways for antitropical and trans-oceanic radiation. Mol Phylogenet Evol 80:217–230.

Bearzi M. 2005. Dolphin sympatric ecology. Mar Biol Res 1: 165–175.

Berta, A. 2015. Whales, Dolphins, and Porpoises: A Natural History and Species Guide. Chicago: University of Chicago Press. 288 p.

Berta A, Ekdale EG, Cranford TW. 2014. Review of the cetacean nose: Form, function, and evolution. Anat Rec 297:2205–2215.

Boy CC, Dellabianca N, Goodall RNP, Schiavini AC. 2011. Age and growth in Peale's dolphin (*Lagenorhynchus australis*) in subantarctic waters off southern South America. Mamm Biol 76:634–639.

Burgio G, Baylac M, Heyer E, Montagutelli X. 2009. Genetic analysis of skull shape variation and morphological integration in the mouse using interspecific recombinant congenic strains between C57BL/6 and mice of the *Mus spretus* species. Evolution 63:2668–2686.

Chen YJ, Chou LS, Watson A. 2011. The maturation of skulls in postnatal Risso's Dolphins (*Grampus griseus*) from Taiwanese waters. Taiwania 56:177–185.

Cheverud JM. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

Cipriano F. 1997. Antitropical distribution and speciation in dolphins of the genus *Lagenorhynchus*: A preliminary analysis. In: Dizon AE, Chivers SJ, Perrin WF, editors. Molecular Genetics of Marine Mammals. Lawrence, KA: Society for Marine Mammalogy. pp 305–316.

Cozzi B, Podestà M, Vaccaro C, Poggi R, Mazzariol S, Huggenberger S, Zotti A. 2015. Precocious ossification of the tympanoperiotic bone in fetal and newborn dolphins: An evolutionary adaptation to the aquatic environment? Anat Rec 298:1294–1300.

Cranford TW, Amundin M, Norris KS. 1996. Functional morphology and homology in the odontocete nasal complex: Implications for sound generation. J Morphol 228:223–285.

Cranford TW, Mckenna MF, Soldevilla MS, Wiggins SM, Goldbogen JA, Shadwick RE, Krysi P, St Leger J, Hildebrand JA. 2008. Anatomic geometry of sound transmission and reception in Cuvier's beaked whale (*Ziphius cavirost*ris). Anat Rec 291:353–378.

del Castillo DL, Flores DA, Cappozzo HL. 2014. Ontogenetic development and sexual dimorphism of franciscana dolphin skull: A 3D geometric morphometric approach. J Morphol 275:1366–1375.

del Castillo DL, Flores DA, Cappozzo HL. 2015. Cranial suture closure in the franciscana dolphin, *Pontoporia blainvillei* (Gervais and D'Orbigny, 1844). Mast Neotr 22:144–148.

del Castillo DL, Segura V, Flores DA, Cappozzo HL. 2016. Cranial development and directional asymmetry in Commerson's dolphin, *Cephalorhynchus commersonii commersonii*: 3D geometric morphometric approach. J Mammal 97:1345–1354.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2008. InfoStat, Vertion 2008, Grupo InfoStat, FCA. Argentina: Universidad Nacional de Córdoba.

Drake AG, Klingenberg CP. 2008. The pace of morphological change: Historical transformation of skull shape in St Bernanrd dogs. Proc R Soc Lond Biol 275:71–76.

Escoufier Y. 1973. Le traitement des variables vectorielles. Biometrics 29:751–760.

Flores DA, Abdala F, Martin GM, Giannini NP, Martinez JM. 2015. Post-weaning cranial growth in shrew opossums (Caenolestidae): A comparison with bandicoots (Peramelidae) and carnivorous marsupials. J Mammal Evol 22:285–303.

Frainer G, Huggenberger S, Moreno IB. 2015. Postnatal development of franciscana's (*Pontoporia blainvillei*) biosonar relevant structures with potential implications for function, life history, and bycatch. Mar Mam Sci 31:1193–1212.

Frandsen MS, Galatius A. 2013. Sexual dimorphism of Dall's porpoise and harbor porpoise skulls. Mamm Biol 78:153–156.

Fraser FC, Purves PE. 1960. Hearing in cetaceans: Evolution of the accessory air sacs and structure of the outer and middle ear in recent cetaceans. Bull Br Mus Nat Hist Zool 7:1–140.

Galatius A. 2005. Sexually dimorphic proportions of the harbor porpoise (*Phocoena phocoena*) skeleton. J Anat 206:141–154.

Galatius A. 2010. Paedomorphosis in two small species of toothed whales (Odontoceti): How and why? Biol J Linn Soc 99:278–295.

Galatius A, Goodall RNP. 2016. Skull shapes of the Lissodelphininae: Radiation, adaptation and asymmetry. J Morphol 277: 776–785.

Galatius A, Berta A, Frandsen MS, Goodall RNP. 2011. Interspecific variation of ontogeny and skull shape among porpoises (Phocoenidae). J Morphol 272:136–148.

Garaffo GV, Dans SL, Pedraza SN, Degrati M, Schiavini A, González R, Crespo EA. 2011. Modeling habitat use for dusky dolphin and Commerson's dolphin in Patagonia. Mar Ecol Prog Ser 421:217–227.

Goodall C. 1991. Procrustes methods in the statistical analysis of shape. J R Stat Soc 53:285–339.

Goodall RNP. 2008. Peale's dolphin. *Lagenorhynchus australis*. In: Perrin WF, Würsig B, Thewissen JGM, editors. Encyclopedia of Marine Mammals. San Diego: Academic Press. pp 844–847.

Goodall RNP, de Haro JC, Fraga F, Iñíguez MA, Norris KS. 1997. Sightings and behavior of Peale's dolphins, *Lagenorhynchus australis*, with notes on dusky dolphins, *L. obscurus*, off southernmost South America. Rep Int Whal Commn 47:757–775.

Goswami A. 2006. Cranial modularity shifts during mammalian evolution. Am Nat 168:270–280.

Goswami A, Polly PD. 2010. The influence of modularity on cranial morphological disparity in Carnivora and Primates (Mammalia). PLoS One 5:e9517.

Guidarelli G, Nicolosi P, Fusco G, de Francesco MC, Loy A. 2014. Morphological variation and modularity in the mandible of three Mediterranean dolphin species. Ital J Zool 81: 354–367.

Haddad D, Huggenberger S, Haas-Rioth M, Kossatz LS, Oelschläger HH, Haase A. 2012. Magnetic resonance microscopy of prenatal dolphins (Mammalia, Odontoceti, Delphinidae)–Ontogenetic and phylogenetic implications. Zool Anz 251:115–130.

Hallgrímsson B, Lieberman DE, Liu W, Ford-Hutchinson AF, Jirik FR. 2007. Epigenetic interactions and the structure of phenotypic variation in the cranium. Evol Dev 9:76–91.

Hanken J, Hall BK. 1993. The Skull: Functional and Evolutionary Mechanisms, Vol. 3. Chicago: The University of Chicago Press. 468 p.

Harper CJ, McLellan WA, Rommel SA, Gay DM, Dillaman RM, Pabst DA. 2008. Morphology of the melon and its tendinous connections to the facial muscles in bottlenose dolphins (*Tursiops truncatus*). J Morphol 269:820–839.

Ito H, Miyazaki N. 1990. Skeletal development of the striped dolphin (*Stenella coeruleoalba*) in Japanese waters. J Mammal Soc Jpn 14:79–96.

Klingenberg CP. 2009. Morphometric integration and modularity in configurations of landmarks: Tools for evaluating a priori hypotheses. Evo Dev 11:405–421.

Klingenberg CP. 2011. MorphoJ: An integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357.

Leatherwood S, Grove JS, Zuckerman AE. 1991. Dolphins of the genus *Lagenorhynchus* in the tropical South Pacific. Mar Mamm Sci 72:194–197.

Lescrauwaet AK. 1997. Notes on the behavior and ecology of the Peale's dolphin, *Lagenorhynchus australis*, in the Strait of Magellan. Chile. Rep Int Whal Commn 47:747–755.

Lotto FP, Gonzalez PN. 2014. Inestabilidad del desarrollo en estructuras cráneo-faciales de poblaciones humanas sudamericanas. Rev Arg Antropol Biol 16:17–29.

MacLeod CD, Reidenberg JS, Weller M, Santos MB, Herman J, Goold J, Pierce GJ. 2007. Breaking symmetry: The marine environment, prey size, and the evolution of asymmetry in cetacean skulls. Anat Rec 290:539–545.

Marroig G, Shirai LT, Porto A, Oliveira FB, Conto V. 2009. The evolution of modularity in the mammalian skull II: Evolutionary consequences. Evol Biol 36:136–148.

May-Collado L, Agnarsson I. 2006. Cytochrome b and Bayesian inference of whale phylogeny. Mol Phylogenet Evol 38:344–354.

McGowen MR, Spaulding M, Gatesy J. 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Mol Phylogenet Evol 53:891–906.

McKenna MF, Cranford TW, Berta A, Pyenson ND. 2012. Morphology of the odontocete melon and its implications for acoustic function. Mar Mam Sci 28:690–713.

Mead JG. 1975. Anatomy of the external nasal passages and facial complex in the Delphinidae (Mammalia: Cetacea). Smithson Contrib Zool 207:1–72.

Mead JG, Potter CW. 1995. Recognizing two populations of the bottlenose dolphin (*Tursiops truncatus*) off the Atlantic coast of North America: Morphologic and ecologic considerations. IBI Rep 5:31–44.

Miller GS. 1923. The telescoping of the cetacean skull. Smith Misc Coll 76:1–71.

Mitteroecker P, Gunz P, Bernhard M, Schaefer K, Bookstein FL. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. J Hum Evol 46:679–698.

Monteiro-Filho ELA, Moteiro LR, Furtado dos Reis S. 2002. Skull shape and size divergence in dolphins of the genus *Sotalia*: A tridimensional morphometric analysis. J Mammal 83:25–134.

Ness AR. 1967. A measure of asymmetry of the skulls of odontocete whales. J Zool 153:209–221.

Oelschläger HHA. 2000. Morphological and functional adaptations of the toothed whale head to aquatic life. Hist Biol Int J Paleobiol 14:33–39.

Olson EC, Miller RL. 1958. Morphological Integration. Chicago: University of Chicago Press.

Palmer AR. 1994. Fluctuating asymmetry analyses: A primer. In: Markow TA, editor. Developmental Instability: Its Origins and Evolutionary Implications. Netherlands: Springer. pp 335–364.

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

Rauschmann MA, Huggenberger S, Kossatz LS, Oelschläger HH. 2006. Head morphology in perinatal dolphins: A window into phylogeny and ontogeny. J Morphol 267:1295–1315.

Rohlf FJ. 1999. Shape statistics: Procrustes method for the optimal superimposition of landmarks. Syst Zool 39:40–59.

Schiavini ACM, Goodall RNP, Lescrauwaet AK, Koen Alonso M. 1997. Food habits of the Peale's dolphin, *Lagenorhynchus australis*, review and new information. Rep Int Whal Commn 47:827–834.

Sheets DH. 2001. IMP, integrated morphometric package. http://www.canisius.edu/~sheets/morphsoft.html.

Sydney NV, Machado FA, Hingst-Zaher E. 2012. Timing of ontogenetic changes of two cranial regions in *Sotalia guianensis* (Delphinidae). Mamm Biol 77:397–403.

Uhen MD, Fordyce RE, Barnes LG. 2007. Odontoceti. In: Janis CM, Gunnell GF, Uhen MD, editors. Evolution of Tertiary Mammals of North America. New York: Cambridge University Press. pp 566–606.

Van Valen L. 1962. A study of fluctuating asymmetry. Evolution 16:125–142.

Van Waerebeek K. 1993. Geographic variation and sexual dimorphism in the skull of the dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828). Fish Bull 91:754–774.

Van Waerebeek K, Würsig B. 2008. Dusky Dolphin *Lagenorhynchus obscurus*. In: Perrin WF, Wursig B, Thewissen JGM, editors. Encyclopedia of Marine Mammals. San Diego: Academic Press. pp 335–338.

Van Waerebeek K, van Bree PJH, Best PB. 1995. On the identity of *Prodelphinus* Petersii Lutken, 1889 and records of dusky dolphins *Lagenorhynchus obscurus* (Gray, 1828) from the Indian and southern mid-Atlantic Oceans. S Afr J Mar Sci 16:25–35.

Werth AJ. 2006. Mandibular and dental variation and the evolution of suction feeding in Odontoceti. J Mammal 87:579–588.

Willmore KE, Klingenberg CP, Hallgrímsson B. 2005. The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. Evolution 59:898–909.

Würsig B, Würsig M. 2009. The Dusky Dolphin: Master Acrobat Off Different Shores, 1st ed. San Diego: Academic Press. 416 p.

Yurick DB, Gaskin DE. 1988. Asymmetry in the skull of the harbour porpoise *Phocoena phocoena* (L.) and its relationship to sound production and echolocation. Can J Zool 66:399–402.