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Phenotypic changes across a geographic gradient: the case of three sympatric dolphin species
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Corresponding Author:	Giulia Guidarelli Universita degli Studi del Molise Pesche, Isernia ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universita degli Studi del Molise
Corresponding Author's Secondary Institution:	
First Author:	Giulia Guidarelli
First Author Secondary Information:	
Order of Authors:	Giulia Guidarelli
	Paolo Colangelo
	de Francesco Maria Carla
	Nicolosi Paola
	Anna Loy
	Meloro Carlo
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Phenotypic changes in the mammalian mandible can occur at different spatial and temporal scale. We investigated mandibular size and shape variation in three extant closely related dolphins (Cetacea, Odontoceti): Tursiops truncatus, Stenella coeruleoalba and Delphinus delphis in order to test the hypothesis that similar phenotypic changes occur across the same geographical gradient. Our data included 219 specimens (51 S. coeruleoalba, 104 T. truncatus and 64 D. delphis) representatives of the following geographic locations: the Mediterranean Sea, the eastern north Atlantic and the North Sea. Each mandibular specimen was photographed laterally and spatial positioning of eight homologous 2D landmarks was recorded. After applying generalised Procrustes analysis (GPA), intraspecific variation was first investigated between sexes and among populations to allow further pooling of samples. Size and shape differences among populations and species were investigated through multivariate ordination techniques (PCA), Procrustes ANOVA and allometric analyses. In all the three species, Mediterranean populations clearly differed from the extra-Mediterranean ones in terms of direction of phenotypic changes, amount and patterns of mandible shape changes. Among the three, the direction of geographic phenotypic changes was significantly similar in the striped and common dolphin, while the bottlenose dolphin was the most diverging species, differing also in size and allometric trajectory. Shape variation of the two former species highlighted a morphological convergence in the Atlantic, and a phenotypic divergence in the Mediterranean. Shape differentiation among the three dolphins was interpreted in the light of feeding adaptation and interspecific competition.

Authors:

Guidarelli Giulia[1], Colangelo Paolo[2], de Francesco Maria Carla[1], Nicolosi Paola[3], Meloro Carlo[4], Loy Anna[1]

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Affiliations:

[1]*Environmetrics Lab, Dipartimento di Bioscienze e Territorio, Università del Molise, Contrada Fonte Lappone s.n., 86090, Pesche (IS), Italy.*

[2]*CNR - Institute for Ecosystem Study, Largo Tonolli 50, 28922 Verbania Pallanza (VB), Italy.*

[3]*Museo di Zoologia, Università degli Studi di Padova, Via Jappelli 1/a, 35121 Padova, Italy.*

[4]*Research Centre in Evolutionary Anthropology and Palaeoecology, School of Natural Sciences and Psychology, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK.*

Corresponding author:

Giulia Guidarelli, g.guidarelli@studenti.unimol.it, + 39 338 59 31 985

Orcid ID: 0000-0002-1248-4917

Abstract

Phenotypic changes in the mammalian mandible can occur at different spatial and temporal scale. We investigated mandibular size and shape variation in three extant closely related dolphins (Cetacea, Odontoceti): *Tursiops truncatus, Stenella coeruleoalba* and *Delphinus delphis* in order to test the hypothesis that similar phenotypic changes occur across the same geographical gradient. Our data included 219 specimens (51 *S. coeruleoalba*, 104 *T. truncatus* and 64 *D. delphis*) representatives of the following geographic locations: the Mediterranean Sea, the eastern north Atlantic and the North Sea. Each mandibular specimen was photographed laterally and spatial positioning of eight homologous 2D landmarks was recorded. After applying generalised Procrustes analysis (GPA), intraspecific variation was first investigated between sexes and among populations to allow further pooling of samples. Size and shape differences among populations and species were investigated through multivariate ordination techniques (PCA), Procrustes ANOVA and allometric analyses. In all the three species, Mediterranean populations clearly differed from the extra-Mediterranean ones in terms of direction of phenotypic changes, amount and patterns of mandible shape changes. Among the three, the direction of geographic phenotypic changes was significantly similar in the striped and common dolphin, while the bottlenose dolphin was the most diverging species, differing also in size and allometric trajectory. Shape variation of the two former species highlighted a morphological convergence in the Atlantic, and a phenotypic divergence in the Mediterranean. Shape differentiation among the three dolphins was interpreted in the light of feeding adaptation and interspecific competition.

Keywords: Geometric morphometrics, mandible, *Stenella coeruleoalba*, *Tursiops truncatus*, *Delphinus delphis*, phenotypic change vectors.

Introduction

The common dolphin *Delphinus delphis*, the striped dolphin *Stenella coeruleoalba* and the bottlenose dolphin *Tursiops truncatus* are small cetaceans belonging to the subfamily Delphininae of the family Delphinidae that comprises 37 species (Committee for Taxonomy 2016). The Delphininae likely arose from an extremely rapid radiation during mid to late Miocene, leading to an unsolved phylogenetic tree and therefore to a still debated taxonomy (McGowen, Spaulding, and Gatesy 2009; Steeman et al. 2009). To date, incomplete lineage sorting (Amaral et al. 2012) and hybridization (Kingston, Adams, and Rosel 2009) limited progress on molecular analyses. Also, morphological investigations have not succeeded in solving the evolutionary relationships among the species, so that more efforts are still needed to clarify the systematic of this group (McGowen, Spaulding, and Gatesy 2009; McGowen 2011; Amaral et al. 2012; Perrin, Rosel, and Cipriano 2013).

The three small dolphins are widely distributed in tropical and temperate waters of the Pacific, Atlantic and Indian Oceans, and in most seas of the world including the Mediterranean Sea. The Mediterranean is the deepest and the largest basin on Earth and it is a marine biodiversity hotspot with high percentages of endemic species (Bianchi and Morri 2000; Danovaro and Pusceddu 2007). This semi-enclosed basin is generally characterized by high sea temperature, high salinity, great seabed topographic variety and homothermy, with water temperatures remaining constant from 300-500 m to the bottom (Coll et al. 2010). The presence of diverse oceanographic dynamics and water circulation patterns result in high variability in productivity in both space and time (Otero and Conigliaro 2012). The Atlantic Ocean has denser and cooler

waters, characterized by low salinity and lower sea temperatures, decreasing with depth. The Strait of Gibraltar is the narrow and shallow passage between the Mediterranean and the Atlantic Ocean. Excluding the Suez Canal, it constitutes the only source of oceanic water and, throughout the history, it has been the main source of biota to the Mediterranean Sea (Coll et al. 2010). Many studies tested the hypothesis of the Strait of Gibraltar acting as a phylogeographical barrier for both invertebrates and vertebrates and three main phylogeographic explanations were presented: lack of population structure, Atlantic and Mediterranean separation and genetic boundaries not associated with Gibraltar (Quesada, Beynon, and Skibinski 1995; Pérez-Losada, Guerra, and Carvalho 2002; Duran et al. 2004; Cimmaruta, Bondanelli, and Nascetti 2005; Patarnello, Volckaert, and Castilho 2007). When the Atlantic-Mediterranean transition is analysed at a finer geographical scale, some species show a drastic genetic change in correspondence of an oceanographic front located from Almeria (Spain) to Oran (Morocco), called the Almeria-Oran Front. Specifically, this phenomenon was observed in *Sepia officinalis* (Pérez-Losada, Guerra, and Carvalho 2002), *Mytilus galloprovincialis* (Quesada, Beynon, and Skibinski 1995), *Paracentrotus lividus* (Duran et al. 2004), *Dicentrarchus labrax* (Naciri et al. 1999) and *Merluccius merluccius* (Cimmaruta, Bondanelli, and Nascetti 2005) (Patarnello, Volckaert, and Castilho 2007).

As for small delphinids, genetic studies have identified population boundaries between Mediterranean and North Atlantic populations (Natoli et al. 2008; Garcia-Martinez et al. 1999; Garcia-Martinez et al. 1995; Gaspari et al. 2007; Bourret, Macé, and Crouau-Roy 2007; Natoli et al. 2005). In *Delphinus delphis*, mtDNA revealed significant genetic differentiation between the Alboran Mediterranean population and the Atlantic Ocean (Galicia and Portugal) (Natoli et al. 2008). Both mtDNA (Garcia-Martinez et al. 1995; Garcia-Martinez et al. 1999) and nuclear markers (Valsecchi et al. 2004; Gaspari et al. 2007; Bourret, Macé, and Crouau-Roy 2007) revealed a distinct separation between Mediterranean and North Atlantic stocks of the striped dolphin *Stenella coeruleoalba*. Natoli et al. (2005) found a clear genetic differentiation from the Black Sea to Scotland (North-East Atlantic Ocean) for the bottlenose dolphin *Tursiops truncatus*. Main differences were found between Scotland and southern North Atlantic, and between the Mediterranean and the Black Sea. Genetic differentiation was also observed in mtDNA of the western Mediterranean *Tursiops* populations compared to the adjacent Atlantic ones (Galicia and Portugal), supporting evidences of a genetic boundary at the Almeria-Orán front. Differences in oceanographic parameters (ocean floor topography, surface salinity, temperature and productivity) were claimed as potential drivers of genetic structure (Natoli et al. 2005).

A high degree of morphometric differentiation was found among small delphinid species across different geographical areas (Bell, Kemper, and Conran 2002; Wang, Chou, and White 2000; Perrin 1984; Perrin 1975; Heyning and Perrin 1994). Regarding the Mediterranean and the North East Atlantic, researches focused on different areas, making comparison among studies complex. Murphy et al (2006) studied the morphological variation of *Delphinus delphis* in eastern North Atlantic and found a latitudinal cline in size, with northern mature males being slightly larger in mandible length, skull width and total body length compared to dolphins from the northwest coast of Spain. The Portuguese population showed segregation in morphometric characteristics (skull width in males and orbital measurements in females) suggesting the mixing of common dolphins off the Portuguese coast with common dolphins in the Mediterranean and farther south. Westgate (2007) analysed the cranial morphology of short-beaked common dolphins from the eastern and western North Atlantic and detected subtle but significant differences both in males and females, with rostral width as an important discriminating variable. The occipital region of the skull also supports the separation of the Mediterranean common dolphins from the Irish, British and Danish populations (Nicolosi 2011). Distinct cranial features were detected in the coastal French and Mediterranean striped dolphins compared to the Scottish stocks (Loy et al. 2011). In this case, shape differences involved mainly the rostral and occipital regions of the skull. By counterpart, morphological variation of the bottlenose dolphin has never been investigated in this geographical area.

The mandible is a skull component that has been studied in both terrestrial (Klingenberg, Mebus, and Auffray 2003; Raia 2004; Meloro et al. 2008; van Heteren 2009; Meloro 2011; Prevosti et al. 2012; van Heteren et al. 2016) and aquatic mammals (Barroso, Cranford, & Berta, 2012; Guidarelli, Nicolosi, Fusco, de Francesco, & Loy, 2014; Heyning & Perrin, 1994; Nummela, Kosove, Lancaster, & Thewissen, 2004; Wang et al., 2000). In cetaceans, mandibular variation has been little explored. Only Barroso, Cranford, and Berta (2012) analysed mandibular shape across all major odontocetes lineages, while Guidarelli et al. (2014) focused on the interspecific variation and modularity in three Mediterranean dolphins. In spite of its structural simplicity, the mandible has proved to be an informative mammalian skull component, useful in discriminating species (Guidarelli et al. 2014), identifying ecological adaptations (Meloro 2011; Meloro and O'Higgins 2011; Prevosti et al. 2012), intraspecific geographic variation (Murphy et al. 2006; Westgate 2007; Loy et al. 2008) and phylogenetic signal at macroevolutionary scale (Meloro et al. 2008; Figueirido, Palmqvist, and Pérez-Claros 2009; Meloro et al. 2011; Barroso, Cranford, and Berta 2012; van Heteren et al. 2016).

Taking into account the evidences of both genetic and morphological segregation between the Mediterranean and the eastern North Atlantic populations observed in many cetacean taxa, and considering the specific conditions of the Mediterranean and the Atlantic environments, we explored the mandibular morphology of *D. delphis*, *T. truncatus* and *S. coeruleoalba* to test the following hypotheses: 1. the three dolphin species exhibit significant differences in mandibular size and shape between the Mediterranean and the extra-Mediterranean stocks 2. morphological variation in the three species follows similar trajectories of size and shape changes across discrete geographic areas.

Materials and methods

Data were collected on a total of 220 specimens belonging to *Delphinus delphis* (n= 64), *Stenella coeruleoalba* (n = 51) and *Tursiops truncatus* (n = 104). Each species is represented by populations coming from three broad geographic areas: the Mediterranean Sea (MS), the North East Atlantic (AO) and the North Sea (NS) (Fig. 1, Table 1) (Online Resource 1).

Only adult individuals were selected. When age information was not available, the mandibular length and the shape of the coronoid crest were used as proxies of age (Perrin 1975; Mead and Fordyce 2009) . Mandibles were photographed in lateral view with a Nikon 3100 camera set at a fixed distance from the object (1.5 m). Eight landmarks (Fig. 2) were recorded to provide adequate coverage of the mandibular morphology.

Landmarks were digitized on the right hemi-mandible using the software tpsDig2 version 2.26 (Rohlf 2003). We selected the right hemi-mandible because there were more undamaged specimens available. To measure the two nested sources of error associated with imaging and digitizing, ten specimens were photographed twice and, on each image, the eight-landmarks were digitized twice on two different days. A Procrustes analysis of variance (ANOVA) was first run in MorphoJ version 1.06b to test for image and digitizing error (Klingenberg 2011, http://www.flywings.org.uk/MorphoJ_page.htm).

A generalized Procrustes analysis (GPA) was performed to translate, scale and rotate all landmark configurations in order to minimize the average distances of configurations from the reference. The reference in GPA is an iteratively computed mean configuration named consensus (Rohlf and Slice 1990). These operations remove all sources of variation that are not shape differences and separate shape from size. The size variable is the centroid size (CS), that is the square root of the summed squared distances of each landmark from the centroid of the landmark configuration. Shape variables

are 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). These differences are measured as Procrustes distances. Since most of methods of multivariate statistics assume a Euclidean space, after superimposition shape coordinates are projected from Kendall's space onto a Euclidean space tangent to the consensus. Multivariate analyses can be run in this tangent space in which linear distances among configurations approximate original Procrustes distances in Kendall's space (Zelditch et al. 2004).

To assess shape and size differentiation between sexes (intraspecific variation) we performed a Procrustes ANOVA as implemented in the R package *geomorph* (Adams & Otárola-Castillo, 2013) in R Studio version 0.98.1103 (R Studio) with 9,999 number of iterations, using Procrustes distances and centroid size values respectively (*D. delphis*: f = 11, m = 21; *S. coeruleoalba*: f = 18, m = 27; *T. truncatus*: f = 36, m = 40).

A One-way non-parametric multivariate analysis of variance (MANOVA) on Procrustes coordinates was run to test for significant differences among different populations within each species (software PAST, version 2.17c, HAMMER et al. 2001). The Procrustes ANOVA was employed to test the null-hypothesis (H_0) that there is no significant interspecific size and shape difference among species and populations.

The function procD.allometry (R package *geomorph*) was used first to investigate intraspecific allometry in each species, and then to test for significant interspecific differences among allometric trends with natural log-transformed centroid size (lnCS) as the covariate predictor, performing an ANOVA for homogeneity of slopes.

To statistically compare patterns of phenotypic changes among species' populations from the three selected geographic areas, we employed the function trajectory.analysis (R package *geomorph*). Phenotypic change along a geographic gradient is represented by a trajectory connecting sample from three different seas along a latitudinal gradient (Collyer and Adams 2013; Meloro et al. 2014). The phenotypic trajectory analysis computes pairwise comparisons among the geometric attributes of the trajectories, describing the shape (*S*), the magnitude (*D*) and the orientation (*Θ*) of phenotypic changes. For each species, the differences in shape are computed as the deviations between corresponding geographical levels across two scaled and aligned phenotypic trajectories expressed as Euclidean distance. The magnitude is the path-length distance, derived from the sum of the Euclidean distances of sequential geographical levels within each trajectory and defines the amount of shape change; the orientation is the direction of its first principal component (PC1). When phenotypic change is quantified across two levels, the change is represented by a vector connecting the phenotypic means of the two levels. Compared to trajectories, vectors can be mathematically described by two attributes: magnitude and direction of phenotypic change. Trajectory and vector's attributes are calculated for each species and then statistically compared to infer how patterns of phenotypic change diverge. The variation in attributes is assessed via permutation and the extent to which they are concordant allows to detect differences or similarities among patterns of phenotypic variation (Adams and Collyer 2009). Trajectories are visualized in the space of principal components (PC1 and PC2) and display the phenotypic change that occurs from one geographic region to another. For each pairwise comparison, attribute differences are significant if the *P values* from a generally high number of permutations (9,999) are less than a type I error rate of α < 0.05. An analysis of variance (ANOVA) was performed with type I sums of squares (SS) and a randomized residual permutation procedure (RRPP) (Collyer, Sekora, and Adams 2015).

Results

Measurement error

The Procrustes ANOVA run among distinct sampling replicates proved that the shape variables and centroid size values obtained through each session of data acquisition were not significantly different. For both size and shape variation, differences among individuals significantly accounted for the 97% of total variance. Differences among replicates due to imaging were non-significant and explained about 0.7% while the digitalization error accounted for less than 1.7%.

Intraspecific shape and size variation

In each species, sexual dimorphism was not significant neither in size nor shape (Table 2). The analyses provided no evidence of sexual dimorphism for the mandibular structure. Therefore, all subsequent analyses were conducted on pooled samples of both sexes, including the specimens of unknown sex.

In all three species, non-parametric MANOVA performed on Procrustes coordinates showed significant differences between populations (*D. delphis*: TSS = 0.033, WSS = 0.028, F = 6.138, $P < 0.001$; *S. coeruleoalba*: TSS = 0.022, SSW = 0.019, F = 3.064, $P < 0.01$; *T. truncatus*: SST = 0.083, SSW = 0.075, F = 5.554, $P < 0.001$). Post hoc tests revealed non-significant pairs for populations of North Sea vs Atlantic Ocean in *D. delphis* and *S. coeruleoalba* (see Table 3). In the striped dolphin, pairwise comparison between the Atlantic Ocean and the Mediterranean Sea population was significant only without Bonferroni correction ($P < 0.05$) (Table 3). In the bottlenose dolphin, significant shape differences in all populations pairs were detected (Table 3). The CVA plot (Fig. 3) displays the discrimination among the three populations and highlights the differentiation between the Mediterranean and the North Sea specimens. Wireframe graphs show that morphological changes are concentrated in the ramus, which is wider, with more developed condylar and coronoid processes compared to the Mediterranean configuration.

Interspecific shape and size variation

Shape. The first two PCs extracted from shape variables accounted for the 75% of total variance (Fig. 4). Species were best discriminated along the first axis (63% of variance), with the bottlenose dolphin clearly separated in correspondence of the negative scores. Shape changes along the first PC concern the alveolar groove length and the ramus' expansion that are inversely correlated: on the positive scores, the common dolphin and the striped dolphin show a narrow jaw morphology characterized by a longer tooth row and a reduced ramus width, whereas the bottlenose dolphin has a massive mandible with a shorter dental groove and a wider ramus, distinctly developed along the dorso-ventral axis. Procrustes ANOVA highlighted interspecific differences among species and populations together with the interaction between the two factors (Table 4).

Size. ANOVA test with permutation was run to test for significant interspecific differences among means and then a multiple comparison test among species was conducted. Size (Centroid Size) was significantly different among taxa (F = 57.987, df = 2, *P*-value < 0.01) (Fig. 5) and populations (F = 14.140, df = 2, *P*-value < 0.01) but after pairwise comparisons the bottlenose dolphin was the only significantly different species with respect to the other small dolphins (*P*-value < 0.001). A more complex model including both species and population as factors detected a significant interaction between the two independent variables (F = 4.664, df = 4, *P*-value < 0.01).

Allometry

A significant association was detected between size (lnCS) and shape in the whole sample with size explaining 19% of the total shape variance (df = 1; SS = 0.053; MS = 0.053; R^2 = 0.193; F = 87.497; Z = 21.929; P < 0.01). The null hypothesis of parallel slopes among species was rejected based on a significance criterion of alpha = 0.05 (group allometries: df = 210; SSE = 0.124; SS = 0.004; R^2 = 0.017; F = 3.748; Z = 2.992; P < 0.05). Figure 6 shows allometric trajectories as the first principal component of predicted shape values on lnCS (Adams and Nistri 2010; Adams, Rohlf, and Slice 2013).

In the bottlenose dolphin, CS accounted for 9% of the total shape variance (1000 permutation runs, P < 0.001) while in the common and the striped dolphins the effect of size on shape was not significant (1000 permutation runs, P > 0.05).

Trajectories and vectors analyses

First, we analysed phenotypic trajectories change in the three species along the geographic gradient from the Mediterranean to the North Sea (Fig. 7, Table 5), including three areas: the Mediterranean Sea, the North-East Atlantic and the North Sea. The analysis evidenced that the three species display similar magnitude and direction of phenotypic change (Table 5).

Each taxon exhibits the same amount of phenotypic change along the trajectory from the Mediterranean to the North East Atlantic. In the striped dolphins shape changes are mainly expressed along the second PC, while for the common and the bottlenose dolphin shape variation is distributed along the first PC (Fig. 7). The magnitude of shape changes is nearly similar among species except for the common dolphin which shows a shorter path (Table 5).

Since most pairwise comparisons did not show any significant difference between the North East Atlantic and the North Sea populations, a phenotypic change vector (PCV) method (Adams and Collyer 2009) was used combining these two in a single population (extra-Mediterranean). ANOVA performed on the Procrustes distances detected significant differences among Species, Seas and their interaction (Table 6).

The analysis of phenotypic vectors between the Mediterranean Sea and the extra Mediterranean showed the same low and not significant amount of shape change among the three taxa ($D_{D, S}$ = 0.001, P = 0.745; $D_{D, T}$ = 0.002, P = 0.490; $D_{S, T}$ = 0.001, P = 0.770), while the direction of phenotypic change was significantly different between the bottlenose dolphin and the other two species ($\Theta_{D, T}$ = 75.730°, P = 0.015; $\Theta_{S, T}$ = 99.681°, P = 0.005). The angle between the striped and the common dolphin was not significantly different from random expectation even if it was particularly large ($\Theta_{D, S}$ = 71.177°, P = 0.070).

Figure 8 compares species' shape configurations corresponding to the two geographic areas as deformation grids associated to the population mean on PC1 and PC2: the Mediterranean Sea and the North Atlantic Ocean. The Mediterranean common dolphins have a longer corpus with a thinner mandibular tip and a smaller ramus compared to the Atlantic stock. Morphological variation is concentrated on landmarks corresponding to the end of the alveolar line and to the angular and coronoid processes. Striped dolphin's shape changes involve the position of the angular and the condylar processes. In the Atlantic group, specimens display a more robust mandible with the angular process that expands posteriorly, and a shorter corpus expanded along the dorso-ventral axis. In the Mediterranean population, the angular process moves anteriorly and the ramus profile is more rounded. The Mediterranean stocks of the bottlenose dolphins show a different morphological pattern. Compared to the Mediterranean specimens the Atlantic population has a shorter

corpus and a wider ramus which is characterized by an expansion of the angular and condylar processes along the antero-posterior axis.

Discussion

The results of our analyses confirmed significant differences in the shape of the mandible between the Mediterranean populations and the extra-Mediterranean ones, whereas slighter discrimination was found between the North east Atlantic and the North Sea stocks of dolphins. The only exception is represented by the striped dolphin, whose Mediterranean population is significantly different from the North Sea but not from the North East Atlantic stock. The non-significant difference observed between these two striped dolphin's populations could be related to the small sample size that prevented from finding a statistically supported separation.

The three small dolphins undergo similar amount of morphological variation across the geographic gradient but the bottlenose dolphin's shape changes follow a different direction. Indeed, many aspects of shape and size variability indicate a more pronounced differentiation of *T. truncatus* compared to the other small delphinids: it differs in both size, allometric trajectory and direction of shape change vectors while, on the other hand, the striped and common dolphins share similar mandibular size values, same direction of shape change and allometric trajectories. These findings are partially consistent with the original question, as we found a significant morphological separation between the Mediterranean and the extra-Mediterranean stocks in the three species. In contrast, patterns of phenotypic change are shared just by the two small delphinids. In and out the Mediterranean, the striped and the common dolphins share similar patterns of phenotypic changes even if these are more evident in the former species. Striped and common dolphins from the extra-Mediterranean area have a similar morphology defined by an expanded angular process along the antero-posterior axis and a slender shape compared to the bottlenose dolphin. When the Mediterranean populations are concerned, the mandible has a restricted angular process and a well-pronounced mandibular notch. However, when the two geographic areas are compared, the two small dolphins seem to diverge in the Mediterranean Sea and to converge out from the basin.

Interspecific shape change variation was in accordance with previous results (Guidarelli et al. 2014) that described a clear discrimination of the bottlenose dolphin from the other two species. The higher similarity between the two small dolphins compared to the bottlenose dolphin is in accordance with phylogenies provided by molecular data (Amaral, Sequeira, and Coelho 2007; Amaral et al. 2012) who hypothesized a closer phylogenetic relationship between the striped and common dolphin. *T. truncatus'* mandible is large and massive with a shorter alveolar groove while *S. coeruleoalba* and *D. delphis* are characterized by a slender and longer tooth row (Fig. 4). At the intraspecific level, the bottlenose dolphin in the extra-Mediterranean population displays an extended ramus with pronounced condylar and coronoid processes and a wide coronoid crest. The Mediterranean population shows a relatively slender morphology with a restricted ramus and a thinner mandibular symphysis (Figs. 3, 8).

Differences in feeding apparatus morphologies and diets revealed in both terrestrial (Adams and Rohlf 2000) and marine vertebrates (del Castillo et al. 2017) may indicate how partitioning of ecological niches reduce the occurrence of competition for food resources when the species are in direct sympatry (M. Bearzi 2005). Therefore, mandibular shape changes could be explained in terms of adaptive processes (e.g., convergence and divergence) related to interspecific interactions and feeding ecology. The common dolphin is mainly considered a neritic predator feeding on epipelagic and mesopelagic shoaling fish (e.g., anchovy Engraulidae, sardine *Sardina pilchardus*) in both the Mediterranean Sea and the Atlantic Ocean (G. Bearzi et al. 2003; Spitz, Rousseau, and Ridoux 2006; Silva 1999). However, even when living in the

oceanic domain, the common dolphin seems to select a particular prey type, which is small shoaling migrating mesopelagic fish, rather than a particular prey species (Pusineri et al. 2007). In the Mediterranean Sea, *D. delphis* is often observed in association with *S. coeruleoalba* (G. Bearzi et al. 2003) with a decreasing eastward gradient of relative abundance of common dolphin likely relative to its capacity to form single species group (Frantzis and Herzing 2002). No evidence of food competition exists between the two species because contrarily to the common dolphin, the Mediterranean diet of the striped dolphin is mainly based on demersal (Lahaye et al. 2006) and pelagic cephalopods (e.g. Ommastrephidae, Histioteuthidae, Onychoteuthidae) (Wurtz and Marrale 1993; Meotti and Podestà 1997). The taller shape of the striped dolphin's ramus observed in the Mediterranean stock is concordant with its teuthophagous diet since from an evolutionary point of view, more robust mandibles are correlated with a suction feeding strategy rather than a raptorial behaviour (Johnston and Berta 2011; Werth 2000). Outside the basin, the striped dolphin is mainly observed in oceanic waters feeding primarily on small mesopelagic fish (Spitz et al. 2006; Pusineri et al. 2007) whereas the common dolphin lives in neritic areas occurring mostly over the continental shelf (Silva 1999), likely limiting the interspecific competition for food resources. The bottlenose dolphin shows a clear different pattern of shape change across the geographic gradient compared with the other two dolphins (Fig. 8, Table 5). Since its significant allometric component, *T. truncatus'* shape change is clearly related to size variation. The Atlantic population's jaw morphology is more robust compared to the Mediterranean one and is characterized by well-developed bony processes and a prominent mandibular notch, possibly reflecting muscle insertion for a stronger musculature (Mead and Fordyce 2009). The species is generally considered an opportunistic feeder and a top predator of coastal and shelf habitat, however, in the Mediterranean its diet is primarily based on demersal prey such as European hake *Merluccius merluccius*, European Conger *Conger conger*, common cuttlefish *Sepia officinalis*, common octopus *Octopus vulgaris* (Blanco, Raga, and Salomón 2001; G. Bearzi, Fortuna, and Reeves 2009) while the importance of hake in the Mediterranean diet contrasts with the greater importance of gadids (e.g., whiting *Merlangius merlangius*) in the East Atlantic (Santos et al. 1994; Blanco, Raga, and Salomón 2001) and with a diet inclusive of big prey such as haddock *Melanogrammus aeglefinus* and large salmonids *Salmo salar* and *Salmo trutta* in northern latitudes including the Black Sea (Wilson, Thompson, and Hammond 1997; Santos Vázquez 1998; Santos et al. 2001). Despite high level of sympatry with the common dolphin in the Mediterranean neritic habitat, associations between the two species have been rarely observed (G. Bearzi et al. 2005). Whether this could be related to different prey preferences is not known, however remarkably different feeding strategies have been observed: the bottlenose dolphin performs long dives (up to eight min) preying demersal species while the common dolphin performs shorter dives (less than two min) and prefers small epipelagic fish. Our morphological data supports this dietary segregation since *D. delphis'* slender mandibular shape is typically related to the raptorial feeding strategy while *T. truncatus'* blunted jaw (i.e., shorter length, higher ramus and reduced dentition) corresponds to a suction feeder structure.

Finally, despite the observed differences between the bottlenose dolphin mandibular morphology and the other two small species, it is worth noting that they display some common patterns of geographic phenotypic change: first, the Mediterranean populations are always clearly different from the Atlantic ones; second, the amount of phenotypic change is significantly similar among species. From an evolutionary point of view, after the isolation of the Mediterranean Basin during the Messinian salinity crisis, which occurred in the late Miocene (5.59-5.33 Ma) (Krijgsman et al. 1999), the Atlantic waters rapidly refilled the basin (Garcia-Castellanos et al. 2009). In fact, while the Italian Miocene fossil record is almost negligible, the Italian Pliocene fossils testify the Mediterranean colonization by the Atlantic oceanic fauna (Cagnolaro et al. 2015). Sharing the same semi-enclosed basin could have led to a more pronounced interspecific niche segregation within the basin compared to the Atlantic Ocean, with species displaying habitat partitioning to avoid direct competition: here the mandibular structure divergence observed in the Mediterranean stocks of the striped and the

common dolphins seems to support this hypothesis while, on the other hand, the Mediterranean morphological divergence of the bottlenose dolphin could be related to the marked difference of prey type (e.g., lack of big and large prey living in northern latitudes). Future researches should concentrate on these aspects of evolutionary ecology to link the observed anatomical differences to different functional demand related to distinct diet preferences. Last but not least, we underline the role of geometric morphometrics to investigate the morphological variability of species both at the intraspecific and interspecific level and to identify functional important anatomical changes.

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Figure 1 Location of samples. NS = North Sea; AO = eastern North Atlantic; MS= Mediterranean Sea; D = *Delphinus delphis*; S = *Stenella coeruleoalba*; T = *Tursiops truncatus.*
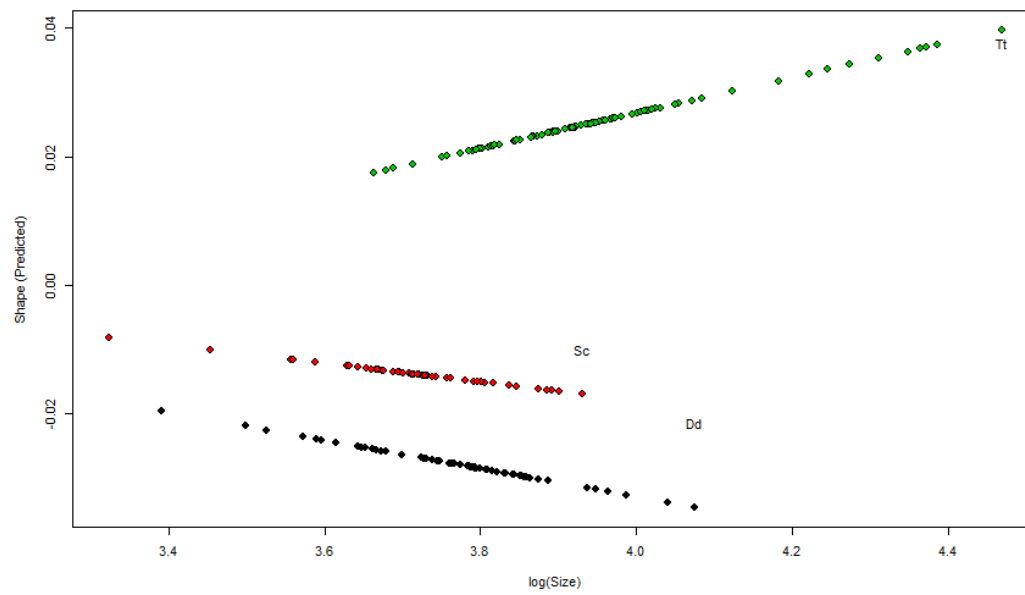
Figure 1 Allometric trajectories are shown as the first principal component of predicted shape values on log-transformed centroid size (lnCS). Green, red and black dots represent respectively *Tursiops truncatus*, *Stenella coeruleoalba* and *Delphinus delphis*.

Lateral view
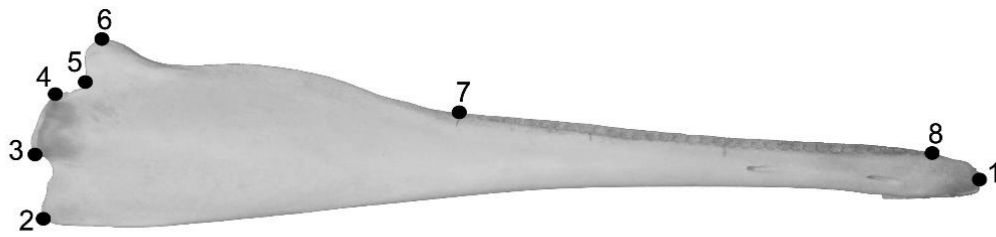
Figure 1 Location of landmarks on the right hemi-mandible. Landmark descriptions as follow: 1. Most anterior tip of the mandible; 2. Posterior ventral tip of the angular process; 3. Ventral extreme point of the condylar process; 4. Dorsal extreme point of the condylar process; 5. Most concave point of the mandibular notch; 6. Tip of the coronoid process; 7. Most posterior end of alveolar groove; 8. Most anterior end of alveolar groove.
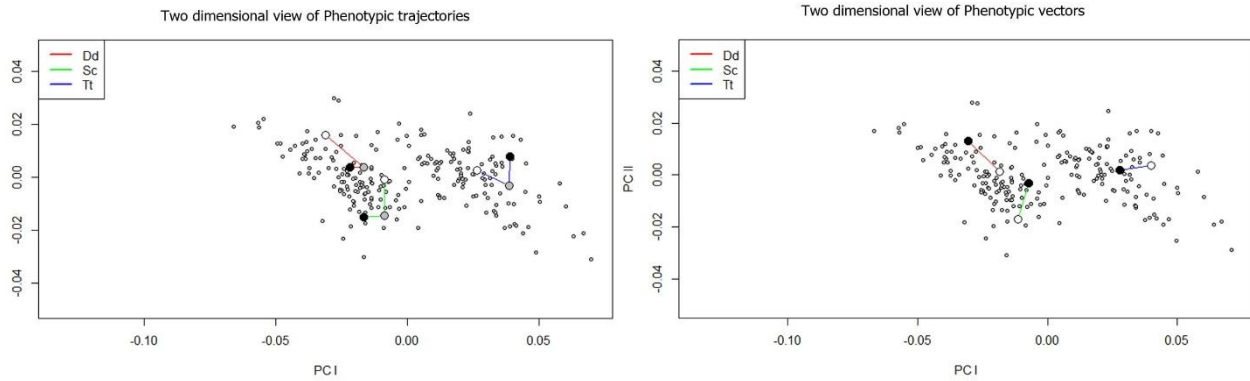
Figure 1 Left: geographic trajectories for the three species *D. delphis* (Dd, red) *S. coeruleoalba* (Sc, green) and *T. truncatus* (Tt, blue) across three geographical areas: white, grey and black points represent respectively the mean shape for the Mediterranean Sea, the North East Atlantic Ocean and the North Sea. Right: geographic vectors for the three species *D. delphis* (Dd, red) *S. coeruleoalba* (Sc, green) and *T. truncatus* (Tt, blue) across two geographical areas: white, and black points represent respectively the mean shape for the Mediterranean Sea and the North Atlantic Ocean. Trajectories and vectors are displayed along the first two principal component axes summarizing 75 % of cumulative variance.

Mediterranean populations   Extra-Mediterranean populations

D. delphis

S. coeruleoalba

T. truncatus
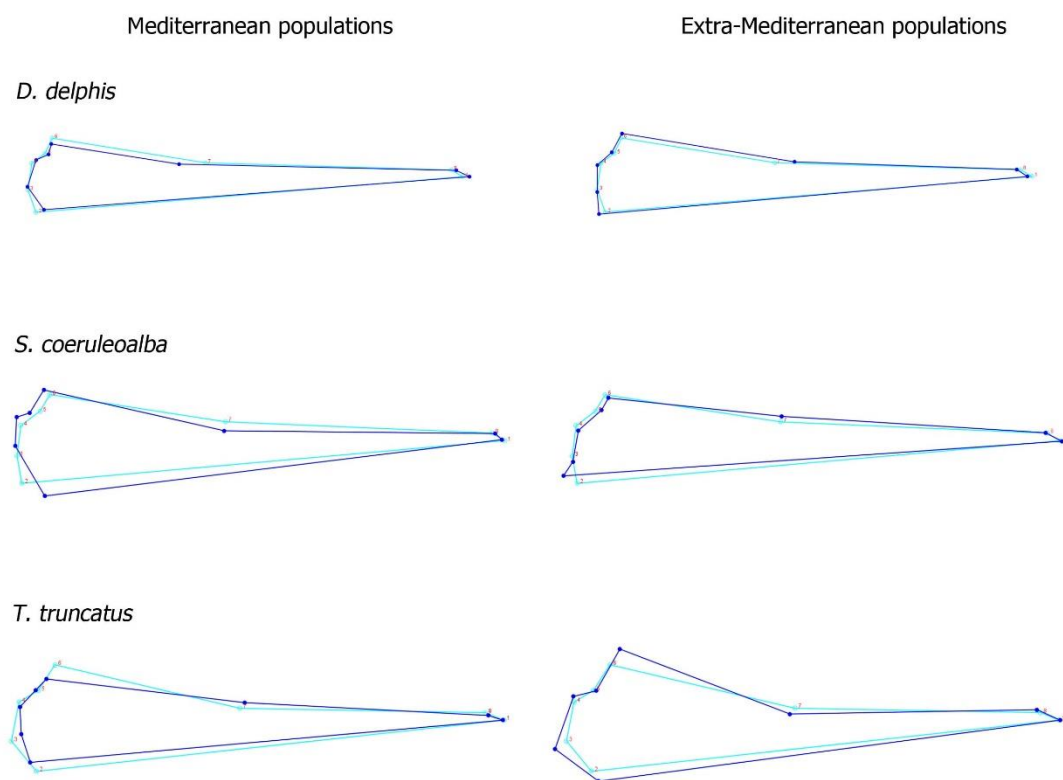
Figure 8 Deformation grids, produced with TpsRelw, show features of mean shape configurations corresponding to the Mediterranean and extra-Mediterranean populations of the three species. Wireframe grids show shape changes relative to the PC1. Scale factor=5.
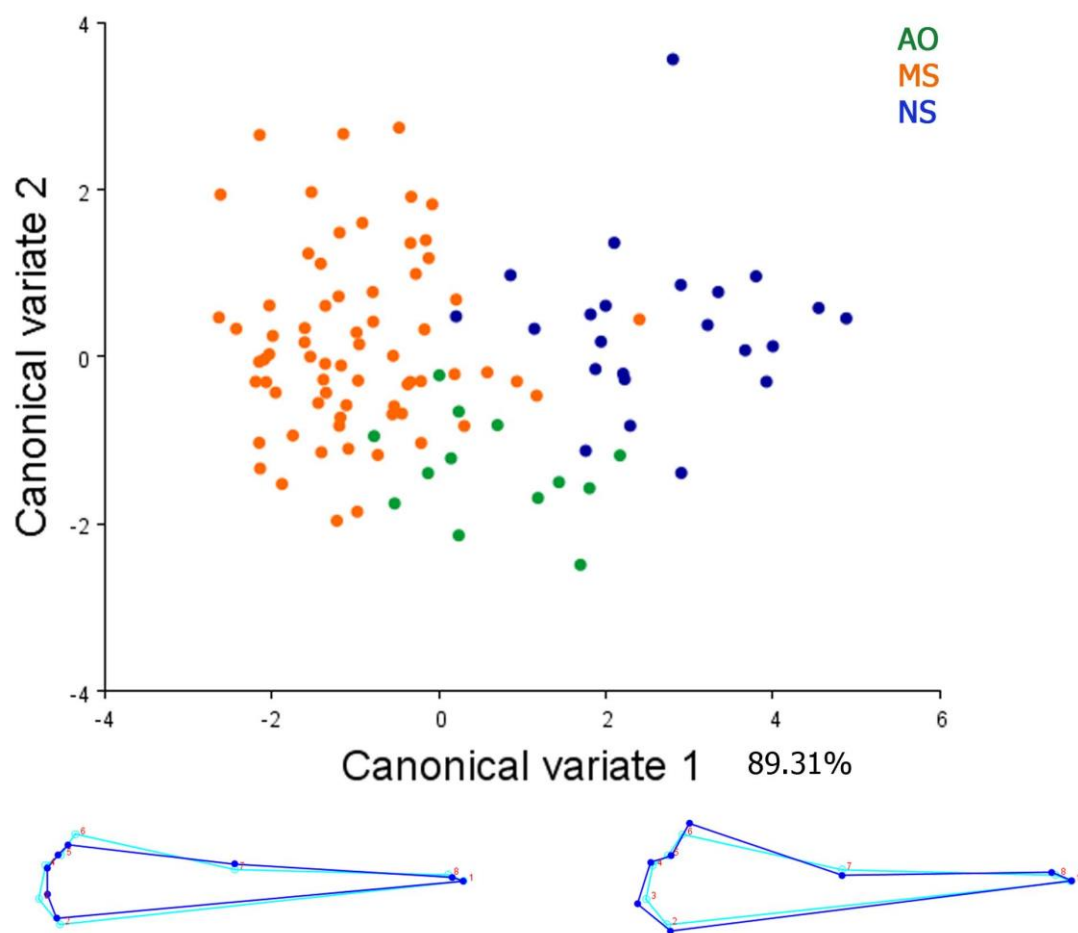
Figure 1 Scatter plot of CV scores for the three populations of *Tursiops truncatus*. Shape differences are displayed as contour plots of deviations (dark blue) from the mean shape (light blue) at the extremes of the axis showing the higher separation among the seas. Scale factor=8.
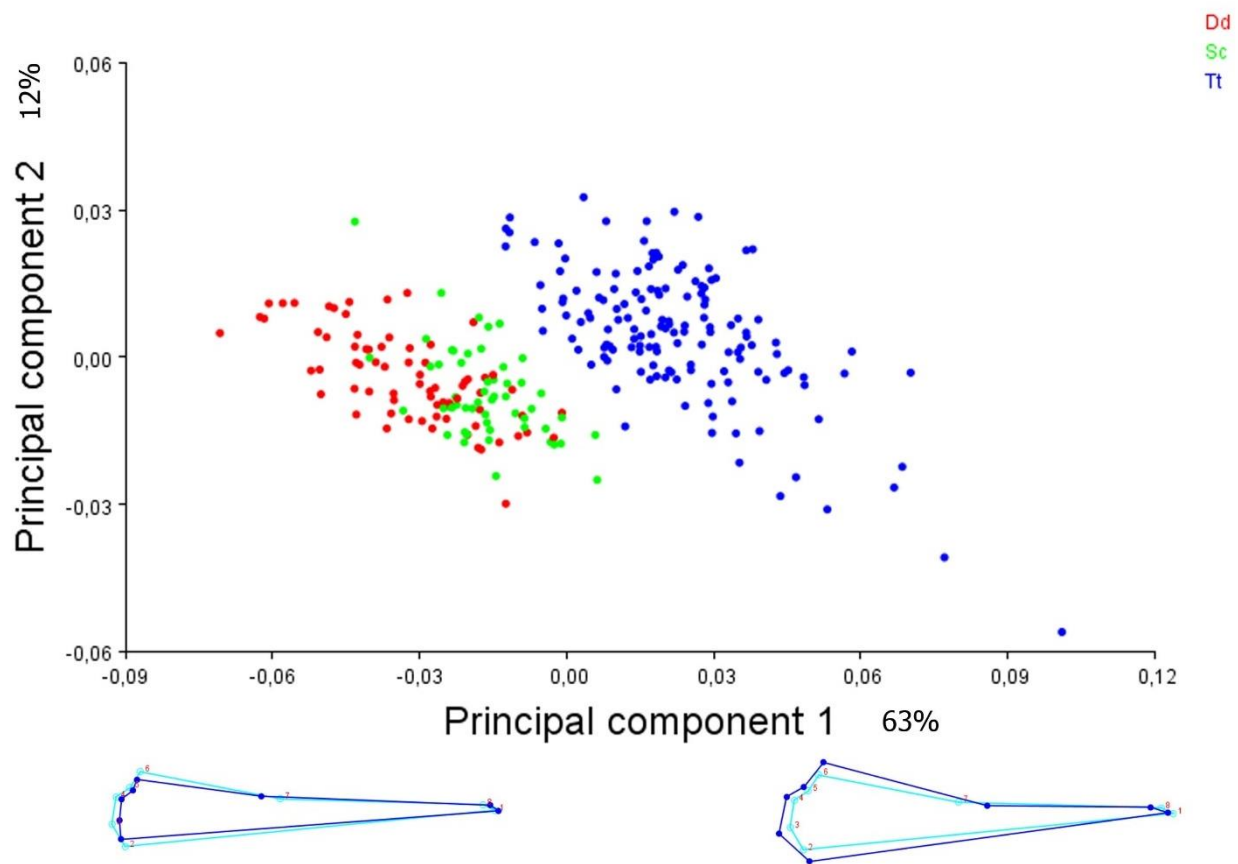
Figure 1 Shape variation of *Delphinus delphis* (red) *Stenella coeruleoalba* (green) and *Tursiops truncatus* (blue) along the first two principal components axes, summarizing 75% of cumulative variance. Wireframe graphs for the extremes of each axis are shown, light blue line refers to the consensus configuration, blue line represents the configuration corresponding to the extreme of the axis. Scale factor = 0.1.
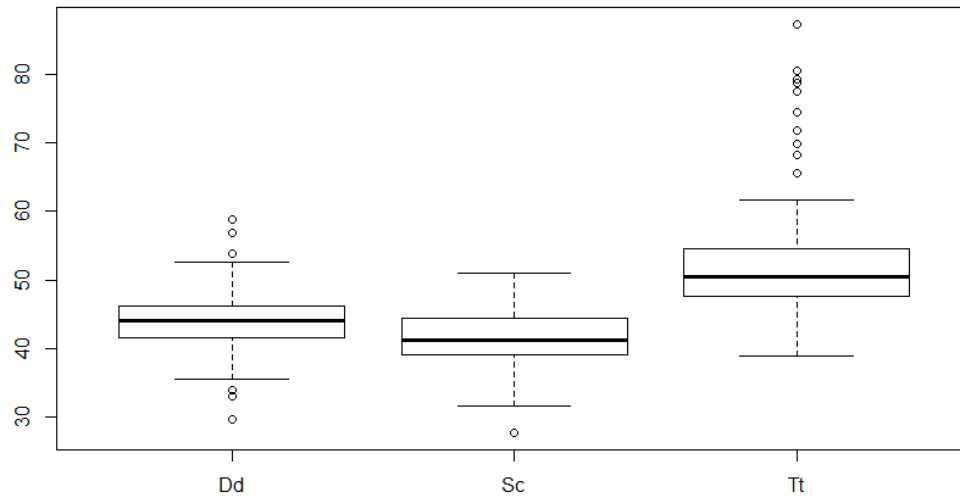
Figure 1 Box plot for centroid size (CS) for the three species. Dd = *Delphinus delphis*; Sc = *Stenella coeruleoalba*; Tt = *Tursiops truncatus*.

Table 1 Details on sample numbers and collections. D = *Delphinus delphis*; S = *Stenella coeruleoalba*; T = *Tursiops truncatus*; MS = Mediterranean Sea, AO = North East Atlantic; NS = North Sea.

Museum	*D. delphis*			*S. coeruleoalba*			*T. truncatus*		
	MS	AO	NS	MS	AO	NS	MS	AO	NS
Museo Civico di Storia Naturale di Milano	3			36			1		
Museo Civico di Storia Naturale di Genova	3								
Museo Civico di Zoologia di Roma	4			6			6		
Museo di Storia Naturale, Università di Pisa (Calci)							1		
Museo Zoologico, Università di Firenze							5		
Accademia dei Fisiocritici, Siena							24		
Naturalis Biodiversity Center, Leiden		2	2						
Royal Belgian Institute of Natural Sciences, Bruxelles									3
Zoological Museum, University of Copenhagen		4	6						1
National History Museum of Scotland, Edinburgh		1	17		4	4		1	19
University of Haifa, Israel	6						24		
Natural History Museum, Tel Aviv University	1								
Aquário Vasco da Gama, Lisboa								6	
Museu Nacional de História Natural e da Ciência, Lisboa		5						6	
Muséum National d'Histoire Naturelle, Paris	3	3							
Naturhistoriska riksmuseet, Stockholm		2	2			1			
Zoological Museum, Barcelona							7		
Total for population	20	17	27	42	4	5	68	13	23
Total for species	**64**			**51**			**104**		

Table 1 ANOVA based on a randomized residual permutation procedure (RRPP) with 1000 random permutations. Sex as independent variable and Procrustes distances and Centroid Size values as dependent variable for shape and size respectively.

		Df	SS	MS	Rsq	F	Z	Pr(>F)
D. delphis	Size	1	8.320	8.321	0.018	0.536	0.330	0.450
	Shape	1	0.000	0.000	0.027	0.828	0.696	0.491
S. coeruleoalba	Size	1	5.920	5.918	0.006	0.280	0.172	0.625
	Shape	1	0.000	0.000	0.018	0.804	0.685	0.534
T. truncatus	Size	1	327.000	326.970	0.032	2.441	1.424	0.115
	Shape	1	0.001	0.001	0.022	1.685	1.394	0.133

Table

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Table 1 Pairwise comparisons among *D. delphis, S. coeruleoalba* and *T. truncatus*' populations. Above and below the diagonal P-values of Bonferroni corrected and uncorrected significance respectively. AO = Atlantic Ocean, MS = Mediterranena Sea, NS = North Sea.

D. delphis	AO	MS	NS
AO	0	**< 0.001**	0.2
MS	**< 0.001**	0	**< 0.001**
NS	0.066	**< 0.001**	0
S. coeruleoalba			
AO	0	0.14	1
MS	**0.046**	0	**0.009**
NS	0.352	**0.003**	0
T. truncatus			
AO	0	**0.026**	**0.049**
MS	**0.008**	0	**< 0.001**
NS	**0.016**	**< 0.001**	0

Table 1 ANOVA based on a randomized residual permutation procedure (RRPP) with 1000 random permutations. Procrustes distances as dependent variable and Species, Sea and the interaction between the two as independent, categorical variables. Here, "Sea" identifies populations coming from the three geographic areas.

	Df	SS	MS	Rsq	F	Z	Pr(>F)
Species	2	0.138179	0.069090	0.48020	110.5364	26.1997	0.001 **
Sea	2	0.007516	0.003758	0.02612	6.0126	4.9682	0.001 **
Species x Sea	4	0.008298	0.002075	0.02884	3.3192	3.0464	0.001 **
Residuals	214	0.133758	0.000625				
Total	222	0.287752					

Table 1 Pairwise comparisons of geometric attributes of phenotypic trajectories for each species across the three geographic areas. S = trajectory shape difference; D = amount of mandible shape changes; Θ = direction of phenotypic changes.

	S	D	Θ
Delphinus - Stenella	$S_{D, S} = 0.351, P = 0.525$	$D_{D, S} = 0.0005, P = 0.980$	$\Theta_{D, S} = 80.022°, P = 0.255$
Delphinus – Tursiops	$S_{D, T} = 0.431, P = 0.150$	$D_{D, T} = 0.003, P = 0.775$	$\Theta_{D, T} = 94.704°, P = 0.095$
Stenella – Tursiops	$S_{S, T} = 0.190, P = 0.815$	$D_{S, T} = 0.004, P = 0.745$	$\Theta_{S, T} = 109.147°, P = 0.130$

Table 1 ANOVA based on a randomized residual permutation procedure (RRPP) with 1000 random permutations. Procrustes distance is the dependent variable, and species, sea and the interaction between the two are the independent, categorical variables. Here, "Sea" represents populations coming from two geographic areas.

	Df	SS	MS	Rsq	F	Z	Pr(>F)
Species	2	0.136589	0.068295	0.49393	114.752	25.5011	0.001 **
Sea	1	0.005989	0.005989	0.02166	10.064	7.8506	0.001 **
Species x Sea	2	0.006596	0.003298	0.02385	5.541	4.6910	0.001 **
Residuals	214	0.127362	0.000595				
Total	219	0.276536					

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