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# Morphological variation and modularity in the mandible of three Mediterranean dolphin species

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**Abstract**

Geometric morphometric studies of dolphins have traditionally concentrated on the skull, while the mandible and its modularity have been little explored. We investigated the mandible variability and modularity in three strictly related Mediterranean dolphins: *Stenella coeruleoalba*, *Delphinus delphis* and *Tursiops truncatus*. The aims were to describe the interspecific differences in the size and shape of the mandible as a whole, and of its structural modules, and to detect the influence of adaptive pressures on trait variation. Data were collected on 96 specimens from the Mediterranean Sea. Eight and 10 two-dimensional landmarks were recorded respectively in the lateral and medial sides of the mandible. After General Procrustes Analysis (GPA) transformation, measurement error, sexual dimorphism and ontogenetic allometry were first investigated to allow further pooling of samples. Univariate analysis of variance (ANOVA) of centroid size was used to evaluate size differences among the species; multivariate ordination, classification and clustering methods were used to investigate interspecific variation of shape variables. Different subsets of landmarks representing distinct mandibular subunits were tested for modular integration through the RV coefficient; two-block partial least squares statistics was used to explore the patterns of covariation between modules. Size and shape differences in the whole mandible of the three species evidenced a clear morphological divergence of *Tursiops truncatus* and a close similarity of *Stenella coeruleoalba* and *Delphinus delphis*. The analysis of modularity identified the corpus and the ramus, with its internal foramen, as distinct modules. The corpus and ramus patterns of variation could discriminate between *T. truncatus* vs. the other two species. The mandibular foramen was the only trait able to discriminate each species, and the corresponding shape differences were related to selective pressures toward the differentiation of communication patterns.

**Keywords:** Stenella coeruleoalba, Tursiops truncatus, Delphinus delphis, *geometric morphometrics, covariation*

## Introduction

The Delphinidae is one of the 10 families of odontocete cetaceans. It comprises 17 genera and 36 species of living dolphins (Bianucci 2013; Committee on Taxonomy 2013), distributed in all oceans, estuarine waters and most seas of the world. In this family, interspecific and intraspecific morphological variations have been studied with both traditional and geometric morphometric (GM) approaches (Murphy & Rogan 2006; Westgate 2007; Yao et al. 2008; Amaral et al. 2009; Kurihara & Oda 2009; Loy et al. 2011; Barroso et al. 2012; Parés-Casanova & Fabre 2013). Together with molecular data, these methods proved to be highly powerful and useful also in discovering new species (Heyning & Perrin 1994; Wang et al. 2000; Monteiro-Filho et al. 2002).

Despite this large number of studies, the phylogenetic relationships within the subfamily Delphininae have not been clarified yet. In particular, the phylogeny of the clade formed by the genera *Delphinus*, *Stenella* and *Tursiops* has not been satisfactorily resolved by either molecular or morphological analyses. The difficulties in solving the delphinine relationships derive from the recent and rapid radiation of this clade that occurred between about 5 and 10 Ma (Zhou et al. 2011; Bianucci 2013). Since the first phylogenetic analysis among members of the Delphinidae (LeDuc et al. 1999), the genera *Tursiops* and *Stenella* have proven paraphyletic even

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if no clear relationships were identified among the species up to now (MacGowen et al. 2009; McGowen 2011; Amaral et al. 2012). In addition, no cladistic analyses based on morphological data have been developed so far, and morphological synapomorphies for the family Delphinidae or the subfamily Delphininae have not been identified (Perrin et al. 2013). In order to clarify the evolutionary history within the *Delphinus*-*Stenella*-*Tursiops* group, it is therefore crucial to study the taxonomic distribution of new molecular or morphological characters.

Many traditional and geometric morphometric studies have concentrated on cetacean skull elements (Perrin 1975; Amaral et al. 2009; Kurihara & Oda 2009; Loy et al. 2011) and on terrestrial mammal mandibles (Klingenberg et al. 2003; Raia 2004; Monteiro & Nogueira 2010; Meloro & O'Higgins 2011; Meloro et al. 2011; Prevosti et al. 2012), while just one geometric morphometric study analyzed the shape of the mandible across all major lineages of odontocetes (Barroso et al. 2012) and none investigated the possibility that this possesses a modular structure (Klingenberg 2008), likely corresponding to a subdivision of functions between its anterior and posterior parts, chiefly involved in feeding and hearing, respectively.

As a matter of fact, the odontocete mandible is involved in a variety of functions related to feeding. Toothed whales have different feeding strategies that depend on the kind of prey, prey availability, abundance distribution and depth (Werth 2007; Bearzi et al. 2009). Unlike in other mammals, the odontocete mandible also constitutes a key component of the hearing apparatus (Nummela et al. 2007).

Toothed whales possess highly adapted ears and they use a combination of sound emission and sensing in foraging and socializing, and in detecting and localizing objects in the environment (Nummela 2009). In particular, in the vast vocal repertoire, whistles are very important because they can be used to convey information on species identity, on individual and population identity and on the behavioural state of the caller (Gannier et al. 2010). So far, two different hypotheses have been proposed for sound reception. The first considers that the sound passes through a fatty pad (called the "acoustic window") into the thin external lamina ("pan bone") of the mandibular ramus, and enters the internal mandibular fat body (MFB), which in turn allows transmission caudally to the bony ear complex (Norris 1968). The expanded mandibular foramen, which houses the MFB, poses a dimensional limit to the lipid tissues and may partially determine sound reception characteristics in toothed whales (Barroso et al. 2012). Under the second hypothesis, called "the gular pathway hypothesis" (Cranford et al. 2008), the sound enters the head from below and between the lower jaws, passes through the opening of the mandibular foramen and continues towards the bony ear complex, conveyed by the specialized mandibular lipid tissues. Despite these differences, both hypotheses recognize the role of the mandible as an essential component of the sound reception apparatus.

We analyzed the interspecific morphological variability and modularity of the mandible in three Mediterranean dolphins, the striped dolphin *Stenella coeruleoalba*, the common dolphin *Delphinus delphis* and the common bottlenose dolphin *Tursiops truncatus*. Since the evolutionary history of these closely related species is still debated, our major aims were to find new morphological characters useful to distinguish the species, to identify functional modules, and to interpret their variation in the light of phylogeny and functional adaptations (McGowen 2011; Amaral et al. 2012). The analyses were based on a two-dimensional geometric morphometric approach (Bookstein 1991; Rohlf & Marcus 1993; Zelditch et al. 2004). This technique allows to analyze separately the two component of variation of forms, i.e. size and shape, and to visualize the results as shape changes of specific regions of the biological structures under exam (Rohlf & Marcus 1993). Size and shape variation of the whole mandible and of each module, together with the pattern of covariation between modules, were investigated across the three species.

## Materials and methods

### *Sample*

A total of 96 specimens from the three species of dolphins occurring in the Mediterranean Sea (13 *D. delphis*, 42 *S. coeruleoalba*, 41 *T. truncatus*) were used for studying the lateral side of the mandible, whereas 88 specimens (eight *D. delphis*, 45 *S. coeruleoalba*, 35 *T. truncatus*) were used for the study of the medial side. All the specimens (110 in total, as 73 of them provided data on both mandible sides) are from the Mediterranean Sea (Figure 1, Table I). Only adult specimens were selected. When independent age information was not available, the length of the mandible was used as a proxy of age (Perrin 1975), and small mandibles were excluded from the analyses.

Mandibles were photographed in lateral and medial view with a Nikon 3100 digital camera at a fixed distance from the subject (1.5 m).

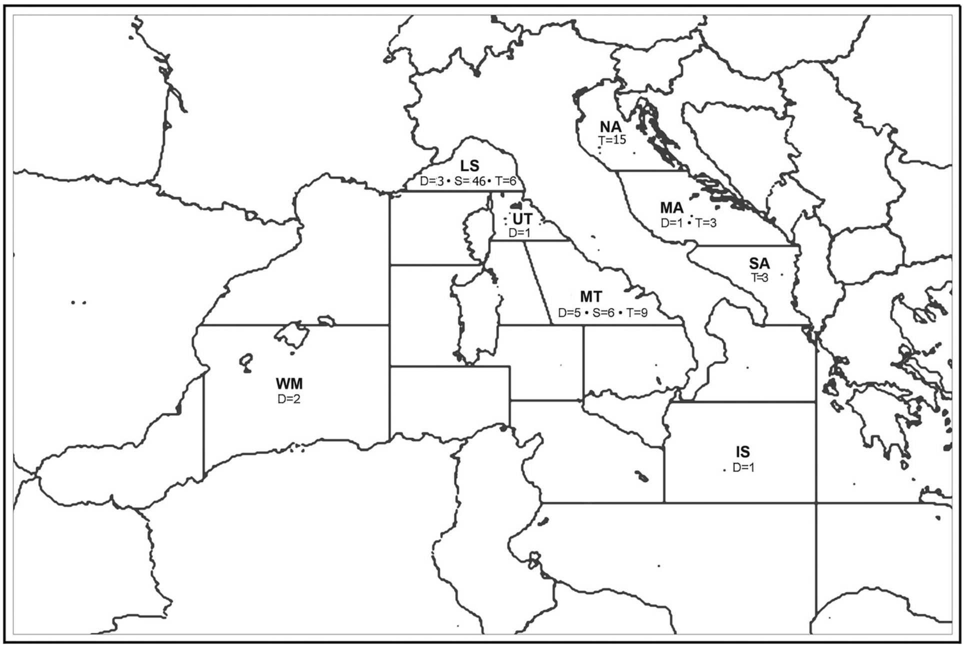

Figure 1. Location of samples. WM = Western Mediterranean Sea; LS = Ligurian Sea; UT = Upper Tyrrhenian; MT = Middle Tyrrhenian; IS = Ionian Sea; NA = Northern Adriatic; MA = Middle Adriatic; SA = Southern Adriatic. D = *Delphinus delphis*; S = *Stenella coeruleoalba*; T = *Tursiops truncatus*. Sample sizes refer only to samples of known locality.

Table I. Details on sample numbers and collections. *D* = *Delphinus delphis*; *S* = *Stenella coeruleoalba*; *T* = *Tursiops truncatus*; *m* = males; *f* = females; nd = sex not defined in the museum label. The number for each sample refers to the lateral (first number) and medial (second number) view.

| Institution | Df | Dm | Dnd | Sf | Sm | Snd | Tf | Tm | Tnd | n |
|---|---|---|---|---|---|---|---|---|---|---|
| Museo Civico di Storia Naturale di Milano | 1-0 | 1-1 | 2-1 | 12-12 | 21-24 | 3-3 | 1-1 | 3-2 | 2-0 | 46-44 |
| Museo Civico di Storia Naturale di Genova | | | 3-0 | | | | | | | 3-0 |
| Museo Civico di Zoologia di Roma | | 3-4 | 1-1 | 1-1 | 2-2 | 3-3 | 2-2 | 4-4 | 2-2 | 18-19 |
| Museo di Storia Naturale, Università di Pisa (Calci) | | | | | | | | 1-0 | 0-1 | 1-1 |
| Museo Zoologico-Università di Firenze | | | | | | | 1-1 | 2-1 | 4-3 | 7-5 |
| Fondazione Cetacea (Riccione) | | | | | | | 4-4 | 4-4 | | 8-8 |
| Dipartimento di Veterinaria -Università di Padova | | | | | | | 2-2 | 6-5 | 3-3 | 11-10 |
| Muséum National d'Histoire Naturelle (Paris) | | | 2-1 | | | | | | | 2-1 |
| Total for sex | 1-0 | 4-5 | 8-3 | 13-13 | 23-26 | 6-6 | 10-10 | 20-16 | 11-9 | |
| Total for species | | **13-8** | | | **42-45** | | | **41-35** | | **96-88** |

We identified eight and 10 landmarks on the lateral and on the medial side of the mandible, respectively (Figure 2, Table II). Landmarks were digitized using TpsDig2 (Rohlf 2013, available at http://life.bio.sunysb.edu/morph/).

At variance with the rest of the skull, odontocete mandibles are symmetrical (Barroso et al. 2012). Therefore we chose to analyze only the right mandible as more undamaged samples were available compared with the left mandibles.

To evaluate the reliability of landmark positioning, a repeatability test was conducted on 10 images, repeating data acquisition three times on three consecutive days and performing a Procrustes analysis of variance (ANOVA) in MorphoJ (version 1.05f, available at http://www.flywings.org.uk/MorphoJ_page.htm).

### Geometric morphometrics

A Generalized Procrustes Analysis (GPA) was performed to remove differences in scaling, rotation and orientation from the raw landmark coordinates (Rohlf & Slice 1990). GPA provides two new sets of variables: the shape variables and the centroid size values (CS). Centroid size is the mean squared distance from each landmark to the centroid of the landmark configuration, and it expresses the overall size of the landmark configuration (Zelditch et al.

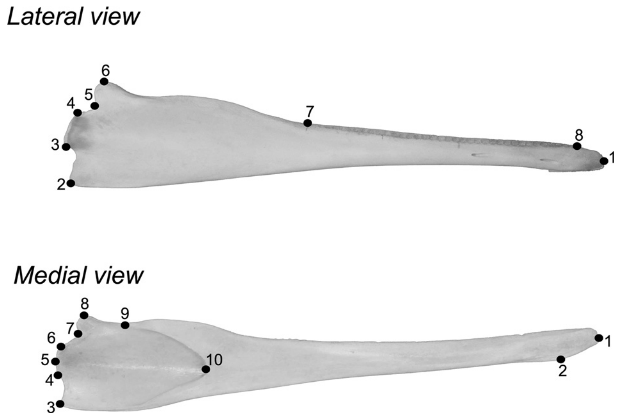

Figure 2. Location of landmarks on the lateral (top) and medial (bottom) sides of a mandible of *Stenella coeruleoalba*.

Table II. Description of the mandibular landmarks.

| Number | Description |
|---|---|
| *Lateral view* | |
| **1** | 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 |
| *Medial view* | |
| **1** | Tip of the mandible |
| **2** | Gnathion, the lowest point of the midline of the mandibular symphysis |
| **3** | Posterior ventral tip of the angular process |
| **4** | Ventral extreme point of the condylar process |
| **5** | Caudal extreme of the condyle |
| **6** | Dorsal extreme point of the condylar process |
| **7** | Most concave point of the mandibular notch |
| **8** | Tip of the coronoid process |
| **9** | Flexion point where medial wall intersects lateral wall on dorsal side |
| **10** | Most anterior point of the internal mandibular foramen |

2004). Shape variables are new coordinates in the Kendall's shape space representing the difference between the "consensus specimen" and each sample. The "consensus specimen" is a collection of mean coordinates for each landmark, and the deviation of each configuration from the "consensus specimen" corresponds to the Procrustes distance. GPA is then followed by projection of the shape coordinates onto a Euclidean space that is tangent to the Kendall's shape space. Multivariate analyses can be run in this tangent space in which linear distances between specimens approximate the Procrustes distances in the Kendall's shape space (Adams et al. 2004). Size was compared between sexes (intraspecific sex variation) and across species (interspecific variation) by an ANOVA with the software SPSS (version 2.1). Intraspecific allometry was investigated through a multivariate regression of shape on centroid size. This operation allows to test and to partition out the shape component of variation that is predicted by size. In case of significant allometry, only the residual component of variation was used for subsequent interspecific analyses of shape variation (Zelditch et al. 2004). We performed a multivariate analysis of covariance (MANCOVA) using SPSS, with log-transformed centroid size (lnCS) as the covariate to test for differences in slope among species. We used lnCS as a measure of size because our size range was relatively large, and it resulted in a better linear relationship than untransformed CS (Klingenberg et al. 2012).

To assess shape differentiation between sexes (intraspecific variation) we performed Hotelling's $T^2$ tests on shape variables followed by two-group multivariate permutations using the software PAST (version 2.7, Hammer et al. 2001). Interspecific shape variation was investigated by a principal component analysis (PCA) run with the software MorphoJ on shape variables, followed by a MANOVA on the principal component (PC) scores accounting for the 95% of the total variance. Wireframe graphs were produced to illustrate variation along principal component axes. Procrustes distances were used to explore the morphometric relationships among the species through unweighted pair group method with arithmetic mean (UPGMA) with the software PAST. *Delphinus delphis* sample could not be included in sexual dimorphism analyses because female specimens were not available.

Sexual dimorphism was analysed only for lateral sides because of the larger sample size, whereas the interspecific variability was investigated on both lateral and medial sides.

### *Modularity*

Landmarks 1, 7 and 8 describe the mandibular corpus, and landmarks 3–7 describe the mandibular ramus (Figure 2). These subsets of landmarks represent two distinct mandibular subunits that were tested for modularity. A similar partition was studied in Klingenberg et al. (2003), describing the two regions in the mouse mandible, and in Meloro et al. (2011) in the mandible of 97 different carnivores.

Hypotheses concerning the boundaries of modules were tested by comparing the strength of covariation among all possible partitions of landmarks (contiguous and non-contiguous partitions). RV coefficient (Escoufier 1973) is a scalar measure of the strength of the association between two subsets of landmarks in a configuration. The RV coefficient takes values between 0 and 1 inclusive. It is 0 if there is no correlation between the two blocks of variables, and it is 1 when they are maximally correlated to one another (Klingenberg 2009). Interspecific variation of the best module configurations were then analyzed separately for both lateral and medial sides. GPA was repeated independently for each module data set. Then PCA and canonical variate analysis (CVA) were run to explore shape variation among species.

Lastly, patterns of covariation between modules were examined with a two-block partial least squares analysis (2B-PLS, Rohlf & Corti 2000). Unlike regression analysis, one of the main properties of 2B-PLS is that it does not assume variables' (shape variables of each subset) dependence; moreover, this procedure can be used even if the blocks have different numbers of landmarks. However, PLS only examines covariation between modules without exploring any other possible partition. 2B-PLS provides an overall measure of association and it finds pairs of orthogonal axes which account for the maximum amount of covariation between the two sets of variables under examination (Klingenberg et al. 2003). For each pair of axes, PLS then computes a singular value (SV) and a *P*-value of the associated permutation test, the proportion of covariation for which the pair of axes accounts, the correlation between the PLS scores for each pair of axes and, just in case, an associated permutation *P*-value. In the present study, we treated the subsets as entirely separated configurations by performing two independent Procrustes fits before running the 2B-PLS. Using this approach, the anatomical connection of the two subsets is ignored and covariation between the subsets is recorded only if there are joint changes of shape within each subset (Klingenberg 2009). To investigate the pattern of covariation of the posterior and the anterior part of the mandible, 2B-PLS was performed between the ramus and the corpus (landmarks of the lateral side), and between the corpus (landmarks of the lateral side) and the mandibular foramen (landmarks of the medial side). Modularity analyses were all performed in MorphoJ.

## Results

### Measurement error

The ANOVA run among distinct sampling replicates proved that the shape variables obtained through each session of data acquisition were not significantly different ($F = 0.5$, $P > 0.10$). Therefore all analyses were conducted using the initial landmark configuration.

### Intraspecific variation

*Sexual dimorphism.* Hotelling's $T^2$ tests results revealed a non-significant sexual dimorphism for the shape of the mandible both in *T. truncatus* (Hotelling's $T^2 = 12.27$, $F = 0.35$, $P = 0.97$) and in *S. coeruleoalba* (Hotelling's $T^2 = 11.93$, $F = 0.41$, $P = 0.95$). After the two-group permutations (9,999 randomizations), results did not change when using Euclidean or Mahalonibis distances, both in *T. truncatus* (Mahalanobis distance = 2.24, $P = 0.93$; Euclidean distance = 0.007, $P = 0.83$) and in *S. coeruleoalba* (Mahalanobis distance = 5.59, $P = 0.14$; Euclidean distance = 0.005, $P = 0.71$). Similarly, ANOVA on centroid size did not detect any significant difference in size between male and female samples, either in *T. truncatus* ($F = 1.27$, $P > 0.05$, $N = 29$) or in *S. coeruleoalba* ($F = 0.76$, $P > 0.05$, $N = 36$). Moreover, regression analysis did not show any significant difference in the allometric trajectories between males and females in either species. To sum up, the analyses provided no evidence of sexual dimorphism for the investigated characters. Therefore, all subsequent analyses were conducted on pooled samples of both sexes, including the specimens of unknown sex.

*Allometry.* Analysis of allometry, which for the nature of our samples reflects a mix of both static and ontogenetic allometry, revealed a significant allometric component in *T. truncatus*, with CS accounting for 11.6% of the total shape variance (10,000 permutation runs, $P < 0.05$), whereas in *S. coeruleoalba* and *D. delphis* the effect of size on shape was not statistically significant (10,000 permutation runs, $P > 0.05$). Therefore, only for *T. truncatus*, further interspecific comparisons were based on the residuals from regression in order to remove the influence of intraspecific size variation on shape data. MANCOVA confirmed the significant differences in slope between the species allometric trajectories (Wilks' lambda = 0.570; $F = 2.139$; df = 24, 158; $P = 0.003$).

### Interspecific variation

ANOVA showed significant differences among the three species in terms of size ($F = 38.37$, df = 2, 1153, $P < 0.0001$). The Levene test indicates that variances in CS are unequal between groups (Levene = 3.390, df = 2, 93; $P = 0.038$) and, for this reason, a Dunnett's T3 post hoc test was used for pairwise comparisons among species. In particular, just the comparison between the largest species

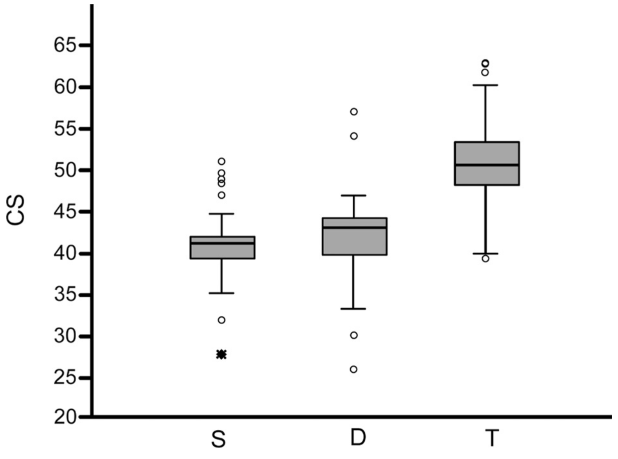

Figure 3. Box plot of centroid size (CS) for the three species. *D* = *Delphinus delphis*; *S* = *Stenella coeruleoalba*; *T* = *Tursiops truncatus*.

*T. truncatus* and the other two species was statistically significant (Figure 3).

The first two principal components of shape variation of lateral and medial sides of the mandible accounted for 76.54% and 60.58% of the shape variation, respectively, and showed a clear separation among species, especially between *T. truncatus* and the other two species (Figure 4).

Most of the shape variation concerned the length of the alveolar row, the height of the ramus (distance between the angular and the coronoid process), and the width of the mandibular foramen on the medial side (Figure 4b). *T. truncatus* has an extremely robust mandible, characterized by a relatively short tooth row, an enlarged foramen and a greater distance between coronoid and angular processes with respect to both *D. delphis* and *S. coeruleoalba*. The lateral side was more effective in distinguishing also between *S. coeruleoalba* and *D. delphis*, the latter showing the most divergent shape along PC1 (Figure 4a). The mandibles of these two species are distinguished by the different position of the angular process and the first alveolus. In *D. delphis*, the mandible is even more elongated than in *S. coeruleoalba*, having a longer alveolar groove and a less developed ramus. The morphological differences along PC1 on the medial side (accounting for 43.46% of variation) are mainly involved in the symphysis and in the mandibular foramen whose role was further investigated in the modularity analysis (see below). As expected, *D. delphis* and *S. coeruleoalba* share the more slender morphology, while *T. truncatus* has a dorso-ventrally developed foramen, a larger symphysis and, on the whole, a mandible which looks more massive.

MANOVA on lateral sides run on the first seven PCs (95% of total variance) detected a clear difference among the three species (Wilks' lambda = 0.1223, df = 14, 174, $F = 23.11$, $P < 0.0001$). Post hoc pairwise comparisons were significant after Hotelling's $T^2$ with or without applying Bonferroni correction ($P < 0.001$) in all cases. MANOVA on the medial sides run on the first eight PCs (95% of total variance) again detected significant discrimination among species (Wilks' lambda = 0.0388, df = 16, 56, $F = 39.75$, $P < 0.0001$) with Hotelling's $T^2$ confirming differences between all pairs. The UPGMAs produced from Procrustes distances (Figure 5) supported a closer morphological similarity between *S. coeruleoalba* and *D. delphis*.

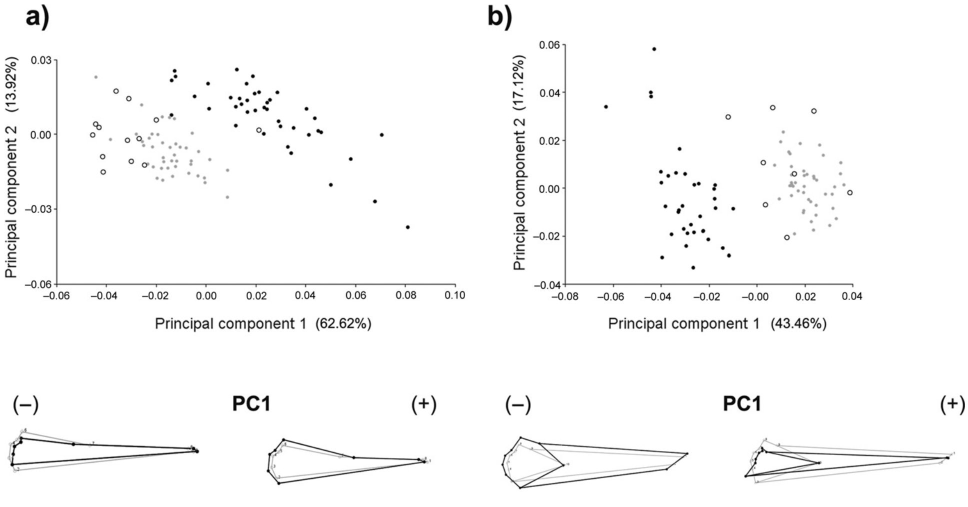

Figure 4. Scatter plot of the two first principal component (PC) scores (percentage given in parentheses) obtained from shape variables: (**a**) lateral side, (**b**) medial side. Wireframe graphs for the extremes of each axis are shown; grey line refers to the consensus configuration, black line represents the configuration corresponding to the extreme of each axis. White circles: *Delphinus delphis*, grey circles: *Stenella coeruleoalba*, black circles: *Tursiops truncatus*. Scale factor = 0.1.

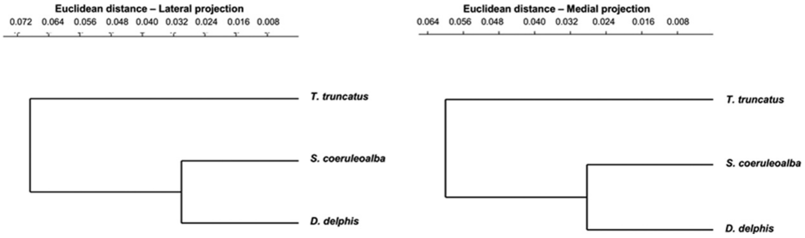

Figure 5. Unweighted pair group method with arithmetic mean (UPGMA) phenogram computed from Procrustes distances among lateral and medial sides of the mandible shape of the three species.

*Analysis of modularity*

The partition of landmarks of the lateral side of the mandible into two sets, corresponding to the ramus and the corpus, yielded the lowest RV coefficient among the 56 partitions considered (Figure 6). This result indicates that the a priori division reflected the modularity of the mandibular structure better than any other partition.

The distribution of PC1 and PC2 scores (summing up to 70% of the variation) computed on the ramus showed a clear distinction of *T. truncatus* from *S. coeruleoalba*, whereas *D. delphis* samples overlapped with the other two species (Figure 7). Shape changes associated to the average scores for the two axes for each species were produced through TpsRelw (version 1.53, Rohlf 2013), and indicate that the diagnostic characters are the angular and the condylar processes. *T. truncatus* showed a well-developed condylar process, while in *S. coeruleoalba* the angular process is more expanded.

PCA run on the corpus did not show a similar clear separation among the three species, that resulted in a poor diagnostic power.

In contrast, the shape of the mandibular foramen could clearly discriminate among the three species, i.e. this trait was diagnostic also for *D. delphis*

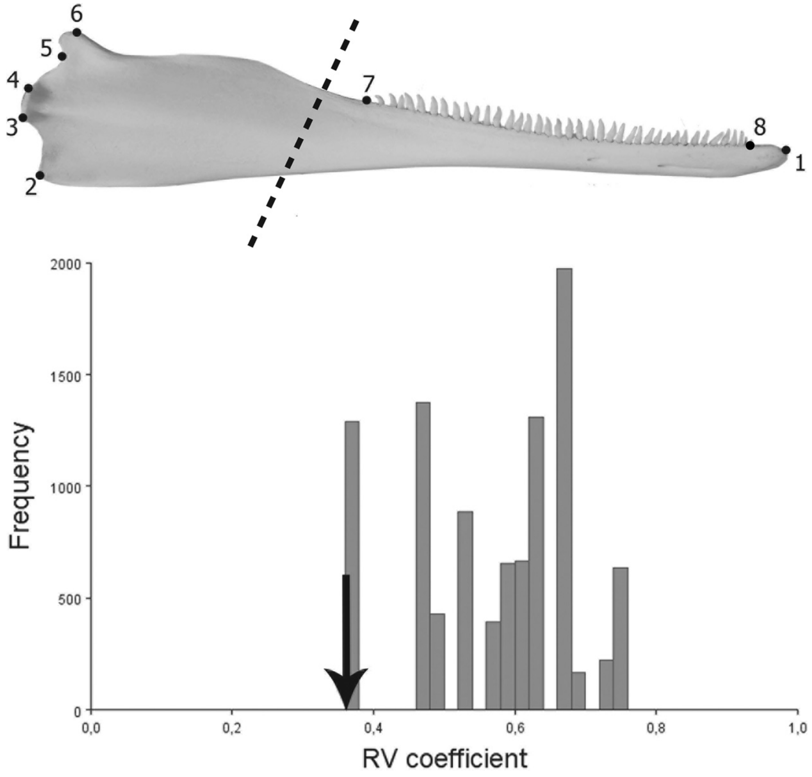

Figure 6. Top: mandibular modules in the lateral view: corpus (landmarks 1, 7, 8) and ramus (landmarks 2–6). Bottom: distribution of the RV coefficients for the 56 contiguous and non-contiguous partitions that were evaluated. The black arrow indicates the RV coefficient (RV = 0.46) for the tested hypothesis.

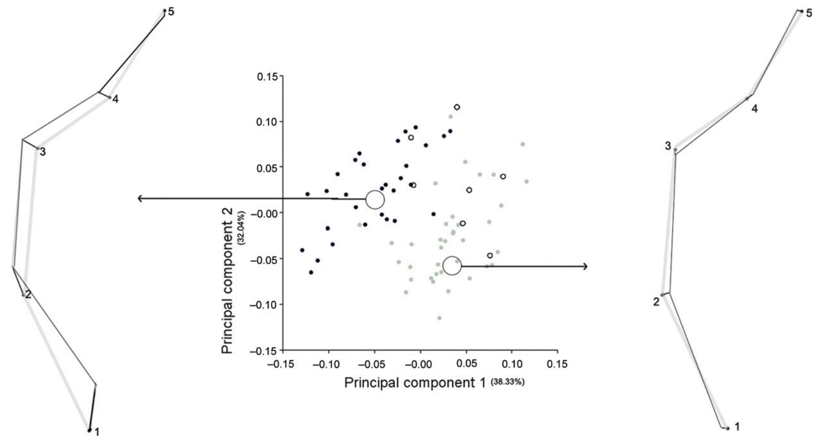

Figure 7. Scatter plot of the first two principal component (PC) scores obtained from the ramus' shape variables. Wireframe graphs correspond to the consensus configuration (grey line) and to the average scores on both PC axes (black line) for *Stenella coeruleoalba* and *Tursiops truncatus*. White circles: *Delphinus delphis*, grey circles: *S. coeruleoalba*, black circles: *T. truncatus*.

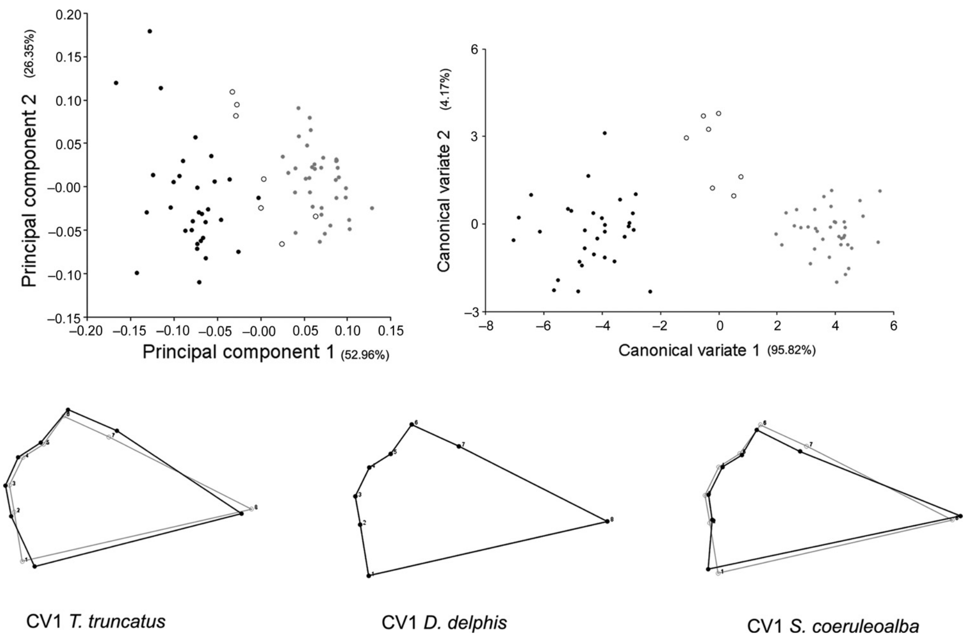

Figure 8. Scatter plot of the first two principal component scores (left) and of the first two canonical variates (CV; right) obtained from the mandibular foramen's shape variables. White circles: *Delphinus delphis* specimens, grey circles: *Stenella coeruleoalba*, black circles: *Tursiops truncatus*. In wireframe graphs, the grey line represents the consensus configuration, while the black line refers to the configurations corresponding to the extreme negative value (*T. truncatus* specimens), the extreme positive value (*S. coeruleoalba* specimens) and the medial value (*D. delphis* specimens) of the first canonical variate.

(Figure 8). Shape changes related to variation along the first canonical variate (CV1, 96% of cumulative variation) evidenced that the foramen in *T. truncatus* is round shaped and differs in the posterior ventral tip of the angular process that moves anteriorly, while in *S. coeruleoalba* the foramen is narrower with a posteriorly retracted angular process; in *D. delphis* it shows an intermediate shape between the two.

### *Module covariation*

The low RV coefficient for the distinction of ramus and corpus indicated a pronounced independence of the two modules (RV = 0.14, 10,000 permutation runs $P = 0.0003$). 2B-PLS between the shape variables of these two modules showed a significant covariation (correlation = 0.46, 10,000 random permutations, $P < 0.001$). Concordant shape

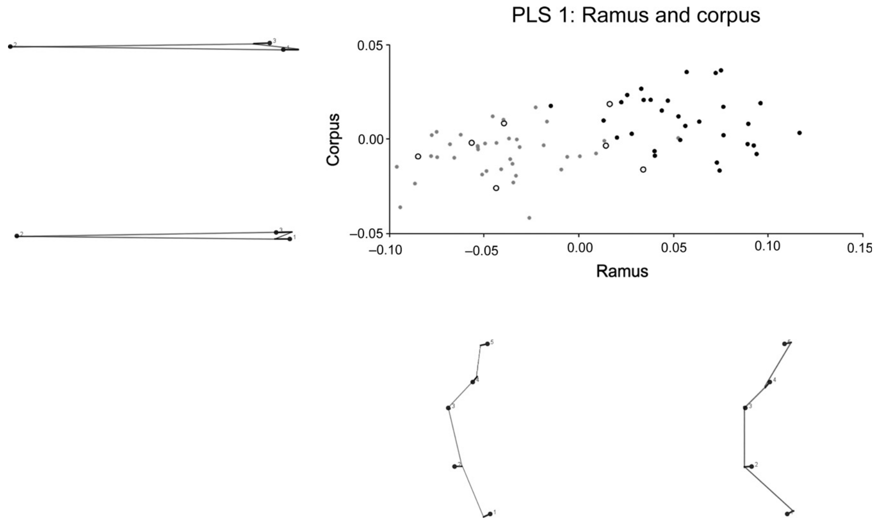

Figure 9. Plot of the first partial least square (PLS1) scores illustrating the pattern of maximum covariation between ramus (landmarks 2–6) and corpus (landmarks 1, 7, 8) in the three species. Wireframe graphs display the shape variation in correspondence to the extreme values of each axis. White circles: *Delphinus delphis* specimens, grey circles: *Stenella coeruleoalba*, black circles: *Tursiops truncatus*. Scale factor = 0.1.

changes along the first partial least square (PLS1) for the ramus and the corpus, accounting for 95% of total covariance, showed a clear distinction between the covariation of the two modules in *T. truncatus* with respect to *S. coeruleoalba* and *D. delphis* (Figure 9). This result suggests that the two groups (*T. truncatus vs. S. coeruleoalba-D. delphis*) do not share the same pattern of integration of corpus and ramus.

In *T. truncatus*, the decrease in the length of the alveolar groove and the expansion of the tip of the mandible in the ramus are associated with an expansion of the condylar process and the retraction of the coronoid and the angular processes in the corpus. In *S. coeruleoalba* and *D. delphis*, a longer tooth row and a reduction of the tip of the mandible is associated with a reduction of the condyle and an expansion of the mandibular notch, the coronoid and the angular processes of the corpus.

Also, covariation of foramen vs. corpus gave a low RV coefficient (RV = 0.18, 10,000 permutation runs, $P < 0.0001$), confirming the morphological independence of the two modules (Figure 10). The first SV accounted for the maximum covariance (98% of total covariance) and the correlation between PLS1 shape changes was also significant (correlation = 0.5, $P = 0.0001$), but contrary to the pattern of covariation of ramus *vs.* corpus, in this case, the three species show a similar integration pattern, with no clear distinction among species.

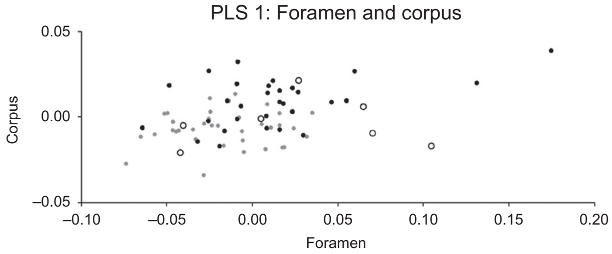

Figure 10. Plot of the first partial least square (PLS1) scores showing the pattern of maximum covariation between the mandibular foramen (medial view, landmarks 3–10) and corpus (lateral view, landmarks 1, 7, 8) in the three species. White circles: *Delphinus delphis* specimens, grey circles: *Stenella coeruleoalba*, black circles: *Tursiops truncatus.* Scale factor = 0.1.

## Discussion

This study investigated the pattern of mandibular shape differentiation among *Stenella coeruleoalba*, *Delphinus delphis* and *Tursiops truncatus*. Our main purposes were to detect morphological differences in the mandibular structure of these closely related species, and to evaluate whether the double function of the mandible, feeding and acoustic, could be reflected in its modular organization and differentiation. The functional subdivision and module integration of the mandible was tested here for the first time in aquatic mammals. We recognized two semi-independent units, the ramus and the corpus, corresponding to a similar subdivision observed in the mandible of the mouse and in those of other carnivores (Klingenberg et al. 2003; Meloro et al. 2011). In addition to the putatively primitive functions of these modules, i.e. the anterior component (the corpus) directly interacting with the food (e.g. by grabbing and processing it) and the posterior ramus supporting the masticatory muscles (Meloro et al. 2011), the ramus of odontocetes is also involved in acoustic sensing (Perrin 1975). Therefore, the pattern of interspecific variations is discussed in the light of these two functions.

### *Intraspecific variation*

In agreement with other GM studies on the same species, we found no sexual dimorphism in either shape or size of the mandible (Amaral et al. 2009; Loy et al. 2011). This allowed us to pool the samples and to include sex-undetermined specimens in successive analyses. Allometry analysis revealed a significant allometric component in *T. truncatus*, whereas this factor was not statistically significant for *S. coeruleoalba*. A positive allometry between the rostral length and the width of the temporal fossa in the adult specimens of *T. truncatus* was found by Kurihara and Oda (2009), and it was related to an extension of the range for food catching and an increase in mouth-closing speed. Even if our sample is very limited and our study was not targeted to the study of allometric growth, the differences observed in the strength of allometry across the three taxa is also in agreement with the hypothesis that "mosaic heterochrony" could be an important factor in the morphological evolution of the Delphinidae (Sydney et al. 2012).

### *Interspecific variation*

Both size and shape of the mandible differ among the three species, with the greatest differences found between *T. truncatus* and the other two smaller species, *S. coeruleoalba* and *D. delphis*. The UPGMA phenograms computed from both the lateral and the medial sides of the mandible agree on a closer morphological similarity between *S. coeruleoalba* and *D. delphis*, while *T. truncatus* was found to be the most divergent taxon. Divergence of *T. truncatus* is also evident in the significant effect of the allometric component and in the pattern of covariation between the ramus and the corpus.

This pattern is concordant with the phylogeny provided by Amaral et al. (2007, 2012), Alfonsi et al. (2013) and Bianucci (2013). These authors hypothesized a recent separation of the three species, with *D. delphis* and *S. coeruleoalba* as sister species, and *T. truncatus* in a relatively basal position (Amaral et al. 2007, 2012). However, other phylogenetic hypotheses have recently emerged from an extensive analysis of both mitochondrial and nuclear gene sequences, by McGowen et al. (2009). These authors hypothesized a closer phylogenetic relationship between *S. coeruleoalba* and *T. truncatus*, while *D. delphis* would belong to a different clade among the Delphininae. According to this phylogenetic hypothesis, the morphological similarities found between *S. coeruleoalba* and *D. delphis* should be interpreted either as a primitive condition within the Delphininae, or as a case of possibly adaptive convergence. Since our study focused on three taxa only, and in the absence of comparative data from other Delphinidae taxa, this question cannot be solved at present.

The pattern of interspecific morphological variation that emerged from the analyses of the whole mandible and of its functional modules, i.e. the corpus and the ramus with its internal foramen, allowed to depict species-specific morphologies that can be discussed with reference to specific differences in feeding habits and acoustic performances.

The mandible of *T. truncatus* is large and massive, and has a shorter alveolar groove. These characteristics are likely linked to the unique feeding ecology of the two species belonging to the monophyletic genus *Tursiops* (McGowen et al. 2009; McGowen 2011). *T. truncatus* occurs in most coastal waters of the Mediterranean basin, where it primarily feeds on benthic and demersal preys, such as *Merluccius merluccius*, *Conger conger*, *Sepia officinalis*, *Octopus vulgaris* and a variety of other bony fishes and molluscs (Blanco et al. 2001; Cañadas et al. 2002; Bearzi et al. 2005; Azzellino et al. 2008; Bearzi et al. 2009). In most models of abundance distribution, *T. truncatus* populations show a preference for waters in a layer between 200 and 600 m in depth (Cañadas et al. 2002; Cañadas & Hammond 2006). Moreover, it is amply documented that *Tursiops* species have specific foraging behaviours, in particular the ability of prey handling and consumption, observed in a wild Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), able to catch and prepare the prey using an ordered sequence of behaviours in which the snout was used to hit and push the prey along the sand (Finn et al. 2009). The shape of the *T. truncatus* mandible observed in our analyses could correspond with a suction feeder structure, able to handle and beat the prey. Indeed, its shorter alveolar groove is an anatomical trait correlated with suction feeding (Werth 2006).

Considerable differences between *T. truncatus* and the other two species of the present study also emerged from the analysis of modularity. *T. truncatus* exhibits a distinctive pattern of variation in the ramus (Figure 9). This was reflected also in the results of 2B-PLS between the ramus and the corpus. In *T. truncatus*, the shortening of the tooth row is associated with an expansion of the ramus, while the two other species show a relatively long alveolar groove covarying with a slight reduction of the ramus width. These features may be related to different feeding strategies as well, with *T. truncatus* being mainly a suction feeder, while *S. coeruleoalba* and *D. delphis* are considered raptorial feeders.

*S. coeruleoalba* and *D. delphis* share mandibular features, such as a slender mandible and longer tooth row, that correlate with their shared grasping, raptorial feeding behaviour. Both species feed primarily on small epipelagic or mesopelagic schooling fish near the surface, and typical target preys are European anchovies *Engraulis encrasicolus* and sardines (European pilchard, *Sardina pilchardus*, and round sardinella, *Sardinella aurita*) (Cañadas et al. 2002; Pusineri et al. 2007; Cañadas & Hammond 2008; Moura et al. 2012). These two dolphins usually live in different habitats. *D. delphis* is a more oceanic species that prefers open and productive waters (Cañadas et al. 2002), feeding especially on lanternfish and squids (Otero & Conigliaro 2012). *S. coeruleoalba* is mainly neritic, but it can be found both offshore and in coastal waters, sharing the first habitat with *D. delphis* and the second with *T. truncatus*. It especially targets species of the Clupeidae family and some of the Gadidae family as well as some cephalopods (Bearzi et al. 2003).

The shape of the medial side of the mandible is the only feature which allows to clearly distinguish all three species. More specifically, the analysis of mandibular modules showed that the key diagnostic character is the shape of the mandibular foramen.

Our results partially contradict Barroso et al. (2012), who found that most of the shape variation in the mandible of odontocetes concerned the anterior (food-interacting) region, while the posterior mandibular foramen accounted only for a small portion of the total variation. Our results indicate that the shape of the mandibular foramen does vary at the species level, and we suggest to extend the analysis of modularity to other delphinid species to better evaluate possible selective pressures acting on this less-considered mandibular component.

The mandibular foramen constitutes a fundamental unit of the hearing apparatus, even if its involvement in sound reception pathways is still debated (Norris 1968; Cranford et al. 2008). *T. truncatus* displays a wider, rounded foramen, *S. coeruleoalba*, at the opposite, shows an elongated, flattened foramen, and *D. delphis* presents a foramen with an intermediate shape between the two. Gannier et al. (2010) studied whistles in five delphinid species in the western Mediterranean Sea, including the three species studied here, and found that some sound features, such as the maximal and the minimal frequencies and the frequency range, can usually allow to discriminate among the species. In particular, they noted that even *S. coeruleoalba* and *D. delphis* could be easily identified. This differentiation in whistles has been suggested to result from selective pressures against hybridization in sympatric species such as the Mediterranean dolphins (Bearzi et al. 2005). The observed clear shape differentiation in mandibular foramina could be associated to species-specific sound reception capabilities and communication patterns as well.

Despite the evident interspecific shape differences in the mandibular foramen, the pattern of covariation between this component and the anterior corpus was similar in the three species. The different pattern of morphological covariation exhibited by the lateral and by the medial sides of the mandible could indeed reflect the different functions in which the two sides are involved: the feeding function, that seems to mainly affect the external morphology of the ramus, and the hearing function, that likely influences the morphology of the internal foramen.

## Conclusion

This is the first study on modularity in the odontocete mandible to be run through a GM approach. Despite its structural simplicity, the dolphin's mandible and its functional modules, i.e. the corpus, the ramus and the internal foramen, were revealed to be highly informative cranial components, potentially indicative of the recent adaptive evolution of the species. However, at present, the lack of an agreed phylogeny for the three species (Amaral et al. 2007, 2009, 2012; McGowen et al. 2009; McGowen 2011), associated to a substantial deficiency in comparative data across the Delphinidae, does not allow us to clearly evaluate the phylogenetic component of shape variation. The morphology of the mandible, the role of the allometric component and the patterns of integration between the ramus and the corpus agreed in indicating *Tursiops truncatus* as the most divergent taxon, while *Stenella coeruleoalba* and *Delphinus delphis* showed more similar morphology and module integration patterns. The analysis of modularity allowed to clearly identify distinct modules reflecting specific adaptations in the three species. The ramus and the corpus were found to differ both in their morphology and in their covaration pathway in *T. truncatus* with respect to *S. coeruleoalba* and *D. delphis*, suggesting a similar feeding ecology in the two long-jawed species with respect to *T. truncatus*. The foramen was found to be the only reliable diagnostic character able to differentiate also between *S. coeruleoalba* and *D. delphis*. As the foramen is deeply involved in sound reception, the differences observed in its shape could be the result of selective pressure toward a differentiation of communication patterns in the three species. Extending the present analyses to other representative species of the Delphininae will likely contribute to illuminate the several still open questions on the evolution and adaptation of this very recently radiated group.

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