

RESEARCH ARTICLE

Every hooked beak is maintained by a prey: Ecological signal in cephalopod beak shape

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Abstract

1. Beaks are among the few hard parts of coleoid cephalopods and are informative for species identification. Although mandible shape has been shown to be adaptive in many vertebrate taxa, it has been suggested that the shape of coleoid beaks does not bear any ecological signal. Yet, previous studies only explored beak shape in 2D and none have provided an in-depth investigation of the potential relationship with ecological variables such as habitat use or diet.
2. The goal of the present study was to understand whether variation in cephalopod beak shape reflects ecology and/or is more driven by phylogenetic relatedness as suggested previously.
3. We imaged 101 lower and 108 upper beaks in 3D using underwater photogrammetry and micro-CT scanning. Our 3D morphometric analysis conducted on 75 species of cephalopod shows that there is a significant but moderate phylogenetic signal. However, comparative phylogenetically informed analyses demonstrate that beak shape is also driven by ecology.
4. We detected significant differences in beak shape between species inhabiting different habitats (pelagic, benthic or demersal) and of different trophic levels. Our results further suggest that beak shape variation can be summarized along a continuum between two main functions: fast closing versus hard biting.
5. These results provide novel insights into the drivers of beak shape diversity in coleoid cephalopods and suggest that beak shape has evolved adaptively in relation to diet and habitat use.

KEYWORDS

3D geometric morphometrics, beak, cephalopod, ecology, phylogenetic signal

1 | INTRODUCTION

Previous studies have demonstrated strong ecomorphological relationships in musculoskeletal systems that provide direct fitness advantages to the feeding (Brassard et al., 2020; Dollion et al., 2017;

Michaud et al., 2020) or locomotor systems (Botton-Divet et al., 2016; Fabre et al., 2015; Verde Arregoitia et al., 2017). The jaws of vertebrates have been the subject of many ecomorphological studies due to their direct effect on growth and survival (Anderson et al., 2008; Wainwright, 1988). The beak of Darwin's finches, for

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example, is a textbook example of how natural selection through the availability of different trophic resources (Grant & Grant, 1995; Grant & Grant, 2002; Schluter, 2000) drives variation in function (Herrel et al., 2005b) which, in turn, drives variation in shape (Herrel et al., 2005a). The shape of a biological structure reflects the importance of phylogenetic, functional and developmental constraints, and can be used to understand the drivers of phenotypic variation (Dryden & Mardia, 2016). Surprisingly, the ecomorphological relationships of beaks in cephalopods remain poorly studied despite showing a great diversity in form.

Cephalopods are a class of cosmopolitan marine molluscs with a long evolutionary history dating back to the early Cambrian (Hildenbrand et al., 2021; Kröger, 2013; Kröger et al., 2011). Divided into two sub classes, Coleioidea and Nautiloidea, their diversity is represented by about 800 extant species (Jereb et al., 2014; Jereb & Roper, 2005, 2010). Cephalopods occupy key roles in marine ecosystems (Clarke, 1996; Piatkowski et al., 2001), and play pivotal roles in trophic webs by being positioned between lower trophic levels and top predators (Boyle & Rodhouse, 2005). Numerous studies have attempted to identify cephalopod diets, yet stomachs are often empty or prey are unidentifiable (Ibáñez et al., 2008, 2021). The high digestion rate and strong prey reduction by means of the beaks (Clarke, 1962) considerably reduce the ability of identifying prey (Boyle & Rodhouse, 2005) from stomach contents. Stable isotopes measured in muscles (Guerreiro et al., 2015; Hanlon & Messenger, 2018) or beaks (Cherel & Hobson, 2005; Xavier et al., 2007), by contrast, have revealed the trophic position of a wide range of species. These studies have demonstrated that cephalopods are typically mesopredators with low to high trophic levels depending on the ecosystem they live in.

The beaks, being among the few hard parts of coleoids (Miserez et al., 2008), are not easily digested and are frequently found in the stomachs of their predators (Boyle & Rodhouse, 2005; Furness et al., 1984). These beaks have provided critical information on many species, including poorly known ones (Cherel et al., 2009). Despite their critical role in interacting with prey and allowing the inclusion of different types of prey into the diet, the variability in beak shape and its possible relationships with trophic ecology and habitat use has never been investigated. Rather, previous studies suggested that the diet of coleoids is not diversified enough for the beaks to reflect dietary adaptations. Consequently, beak shape is considered to reflect phylogenetic relatedness (Clarke & Maddock, 1988). However, palaeontological studies have hypothesized the relationships between beak and radula shape and diet (Gasiorowski, 1973; Kruta et al., 2011; Saunders et al., 1978; Tanabe & Fukada, 1999). These studies have suggested that ammonites possessing blunt-edged lower jaws, a slit rather than a thickening along the mid-line, and relatively small lower jaws were likely incapable of processing large prey. Ammonites with a short rostrum and upper jaws that are relatively blunt and weakly tanned and having a multicuspid radula have been suggested to be zooplankton feeders. In contrast, jaws with a calcareous covering and a robust radula, as found in nautilids, have been suggested to be suited for a scavenging life style. Moreover, some authors have suggested that the beaks of para-larvae of

recent octopods, argonauts and some squid show features that facilitate prey ingestion (Franco-Santos et al., 2013; Franco-Santos & Vidal, 2014, 2020). Moreover, diet can vary greatly during ontogeny in ommastrephid squid, ranging from detritivory in paralarvae to carnivory in adults (Fernández-Álvarez et al., 2018).

There are, however, few in-depth quantitative studies of beak shape beyond analyses of linear dimensions (Mangold & Fioroni, 1966). Moreover, previous studies using 2D geometric morphometrics (Neige & Dommergues, 2002; Tanabe et al., 2015) have suggested a strong relationship between beak shape and phylogeny. Yet, this relationship has not been quantified to date despite the advent of well-supported molecular phylogenies (Anderson & Lindgren, 2021; Fernández-Álvarez et al., 2021; Lindgren et al., 2012; Sanchez et al., 2018; Strugnell & Nishiguchi, 2007; Uribe & Zardoya, 2017). Despite the fact that beaks are complex 3D objects, the shape of the beak has never been quantified in 3D, likely because of the complexity of acquiring the form of these objects. Composed of chitin, water and a protein complex (Miserez et al., 2008), they are difficult to image and are dry sensitive and deform easily when dehydrated (Roscian et al., 2021).

The goals of the present study were (a) to characterize the variation in the shape of the upper and lower beak across a diverse sample of coleoid species using 3D geometric morphometric approaches; (b) to quantitatively test for the presence of phylogenetic signal in beak shape; and (c) to test whether beak shapes differ between species occupying different habitats, living at different depths and occupying different trophic levels using comparative phylogenetically informed analyses. If the beak of cephalopods has evolved adaptively in response to the constraints exerted by different prey resources present in different habitats or at different depths, then this should be reflected in its shape.

2 | MATERIALS AND METHODS

2.1 | Material acquisition

We investigated 75 species belonging to 61 genera covering all the major families of coleoids including Octopoda (17 species), Sepiida (12), Spirulida (1), Myopsida, (4), Oegopsida (40) and one Vampyromorpha (Table S1). Species were selected to encompass all habitat types and depth levels, known diets, and the major shapes described previously by Clarke (1986). When possible, species included in the molecular phylogenies from Lindgren et al. (2012) and Sanchez et al. (2018) were selected. All samples were from collections, no live specimens have been used for the study. In all, 67 buccal masses were dissected and scanned using the X-ray micro-CT facilities at the National Museum of Natural History in Paris, France and at the Natural History Museum in London, U.K. (Table S2). The resulting scans were segmented using Materialize Mimics (v.21.0). In all, 116 beaks housed at the Centre d'Etudes Biologiques de Chizé, France were reconstructed with the underwater photogrammetry protocol described in Roscian et al. (2021). A total of 108 upper, and

101 lower beaks of adult specimens were reconstructed in three dimensions with both techniques. As upper and lower beaks were not always available for each species, the sample differs slightly depending on the species considered. Some beaks could not be modelled in 3D because of a lack of contrast in the CT data or they because they were not available.

Information on the ecology of the species was retrieved from the literature and included the type of habitat (pelagic, benthic and demersal), the type of substrate (sandy/muddy, rocky mixed versus pelagic habitat), mean depth, trophic level, stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and diet (Table S3). Although some ecological variables such as habitat or mean depth were available for all species, others such as stable isotopes, trophic levels or diets were more scattered and present for only a third of the species included in our study (Xavier et al., 2015).

2.2 | Morphometric data and analyses

To quantify beak shape, we selected 7 landmarks, 8 curves and 150 sliding semi-landmarks on the lower beak surface; 5 landmarks, 7 curves and 293 sliding semi-landmarks on the surface of the upper beak including landmarks described in the 2D landmark protocols of Neige and Dommergues (2002) and Tanabe et al. (2015) (Table 1). Some of the landmarks defined by Neige and Dommergues could not be used because of their uncertain position on the beak surface in 3D.

Semi-landmarks on curves and surfaces were added to represent the whole shape (Figure 1).

A quantification of the repeatability of the placement of the anatomical landmarks was performed with three specimens of the same species for both upper and lower beaks to ensure that the measurement variability was lower than the inter-individual variability. Each landmark was digitized 10 times for each specimen on different days. The landmarks were then aligned using a generalized Procrustes analysis (GPA) and visualized with a principal component analysis. With the validated landmarks, semi-landmarks were used and slid by minimizing the bending energy in R (v 4.0.0) using the 'slider3D' function in the MORPHO package (Schlager, 2017). The contribution of size to shape diversity for both upper and lower beaks was tested using a Procrustes ANOVA with permutation (Klingenberg, 2016). When

species were represented by two individuals, a mean conformation was calculated after the GPA. A principal component analysis (PCA) and phylogenetic principal component analysis (phyloPCA) were computed for lower and upper beaks separately using the 'dudi.pca' function of the ADE4 package and 'gm.prcomp' function in the GEOMORPH package. To investigate the entire shape variability and differences between morphological groups, a hierarchical clustering was performed using the Ward distances on the PC scores representing 90% of the explained variances. To quantify the phylogenetic signal in the beak shape, a K-mult test ('physignal' function) was used (Adams, 2014). The influence of the different habitats (pelagic, benthic or demersal) was investigated using phylogenetic MANOVAs ('procD.pgls' function). PGLS regressions were calculated for numerical values such as mean depth, stable isotope values and trophic level (Clavel & Morlon, 2020). All these analyses were performed on the Procrustes coordinates. The morphological disparity ('morphol.disparity' function from the GEOMORPH package) was also calculated when the phyloMANOVA was significant to identify which groups showed greater disparity (Zelditch et al., 2012). These analyses were performed on the complete sample and on a subsample containing only species of Oegopsida, the clade best represented in our study and showing high morphological disparity. This allowed us to test whether the results also held for this group only.

2.3 | Phylogeny

2.3.1 | Sequence acquisition

Our dataset is very similar to the dataset used in Sanchez et al. (2018), where the authors sampled markers for 124 genus-level representatives of cephalopods, sometimes leading to the combination of genes coming from different species to maximize genus-level marker sampling.

We expanded the taxon sampling by adding representatives of *Filippovia*, *Neorossia*, *Semirossia*, *Stigmatoteuthis* and *Taningia*. These taxa were added either to be more representative within a given family or because ecological data were available for these genera. The *actin* sequences were removed from the sample as there is evidence for numerous paralog copies (Carlini et al., 2000). The 28S rRNA marker,

TABLE 1 Description of landmarks used based on the Neige and Dommergues (2002) nomenclature

Upper beak		Lower beak	
Point	Description	Point	Description
1	Anterior tip of rostrum	1	Anterior tip of rostrum
2	Maximal curvature of the hood	2	Maximal curvature of the hood
3	Posterior end of lateral wall	3	Posterior end of lateral wall
4	Left projection of contact wing/lateral wall	4	Left maximal curvature of lateral wall
5	Right Projection of contact wing/lateral wall	5	Right maximal curvature of lateral wall
		6	Left projection of contact wing/lateral wall
		7	Right Projection of contact wing/lateral wall

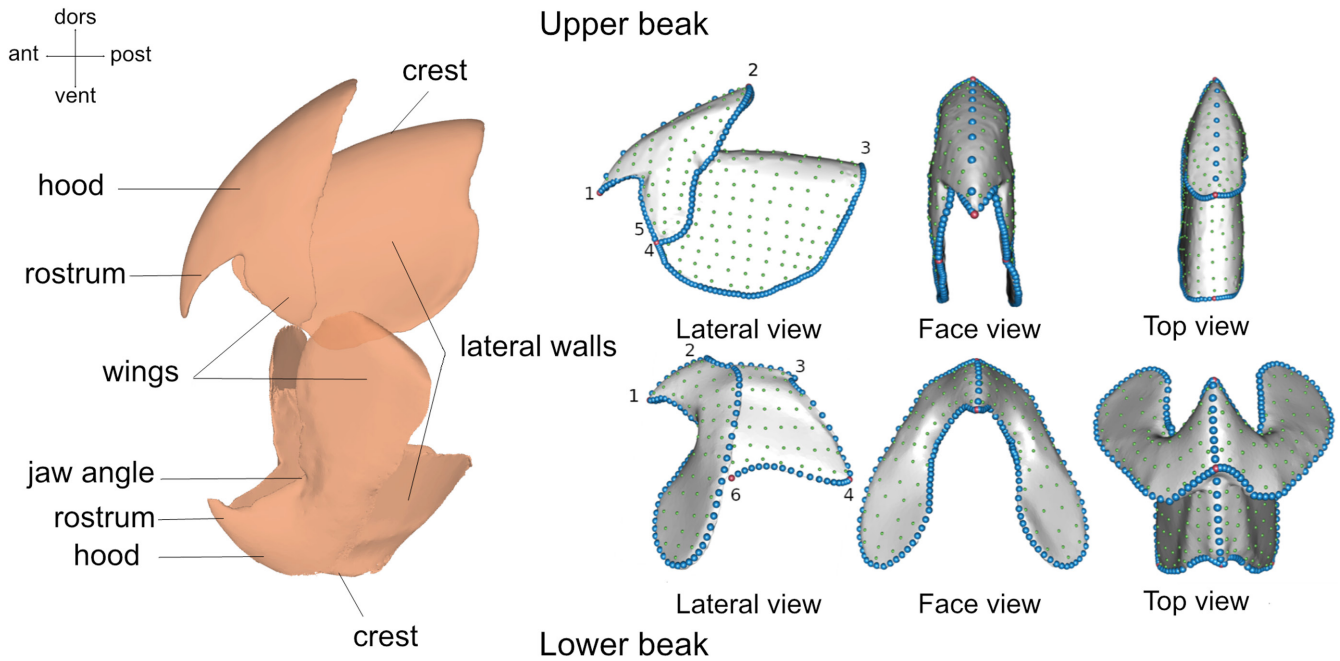


FIGURE 1 Anatomy of upper and lower beaks (left) oriented with the anterior part towards the left and the dorsal part towards the top in lateral view. Landmarks (large red spheres), curves (large blue spheres) and sliding semi-landmarks on the template (small green spheres) for upper and lower beaks (right). The numbers indicate the landmarks used in the geometric morphometric analyses. These numbers are described in [Table 1](#)

surprisingly not used in Sanchez et al. (2018), was added to the study, as it is well represented in GenBank and as it has traditionally been used in phylogenetic studies of molluscs (Strugnell & Nishiguchi, 2007; Zaharias et al., 2020). Four additional full mitochondrial genomes were added.

Overall, we sampled 129 genus-level representatives, 6 nuclear markers (28S rRNA, 18S rRNA, *Histone h3*, *octopine dehydrogenase*, *pax-6* and *rhodopsin*), 3 mitochondrial markers (cytochrome c oxidase subunit I [*cox1*], 12S rRNA and 16S rRNA) and 22 full mitochondrial genomes (see Supporting Information 4 for a complete list of all GenBank IDs).

2.3.2 | Sequence alignment

Sequences were aligned using MAFFT v7.471 (Kato & Standley, 2013), and manually checked and trimmed if necessary to ensure reasonable missing data proportions. Mitochondrial genomes were split into markers, including most tRNA portions. We used the MAFFT's E-INS-i iterative refinement method to align the 28S rRNA, 18S rRNA, 12S rRNA and 16S rRNA, specifically designed for RNA polymerase alignments. Protein-coding genes (*Histone h3*, *octopine dehydrogenase pax-6*, *rhodopsin* and the 13 protein-coding mitochondrial genes) were aligned using the MAFFT's G-INS-i algorithm. Subsequently, we translated the alignments in amino acids and ensured that the alignments were codon-based, that is, with inserts and deletions being multiple of 3. All 22 tRNA mitochondrial genes were aligned using MAFFT G-INS-i.

We used the AMAS python tool (Borowiec, 2016) to generate a concatenated alignment and a partition file of all 43 markers.

2.3.3 | Tree estimation

We used IQ-TREE version 2.1.2 (Minh et al., 2020) to estimate a phylogeny from the concatenated alignments using partition models (Chernomor et al., 2016). We allowed ModelFinder (Kalyaanamoorthy et al., 2017) to select individual models for each marker following a BIC criterion. We also ran 1,000 ultrafast bootstraps (UFBoot) (Hoang et al., 2018) to get support values.

To assess the relationship between phylogeny and beak shapes, we subsampled the tree to include the 75 species for which we had information on shape using the PHYTOOLS package in R. We replaced names of genera by species analysed when a single species was present. When two species within a genus were present, we manually added them as sister taxa using TreeGraph 2 (Stöver & Müller, 2010). For the four species of *Histioteuthis*, species relationships were randomly resolved.

In both cases, we assigned a branch length of 0.01 to minimize biases.

3 | RESULTS

3.1 | Phylogenetic analysis

Our final concatenated matrix contains a total of 129 genus-level representatives and is composed of 23,826 sites, with a total of 73.8% missing data. In comparison, the concatenation matrix from Sanchez et al. (2018) was composed of 15,713 sites and 71.7% missing data. About 61.9% of the edges were well supported

(UFBoot > 95%), 16.7% of the nodes have medium support (80% < UFBoot < 95%) and 21.4% of the nodes were poorly supported (UFBoot < 80%).

We compared our newly generated phylogeny with the ML phylogeny from Sanchez et al. (2018) using the phylogeny from Anderson and Lindgren (2021) as a reference. We counted the number of false-negative edges, that is, the number of edges in Sanchez et al. (2018) or in our phylogeny that are not found in tree C75-A1 from Anderson and Lindgren (2021). Our results show that, of the 26 informative edges that are comparable, our phylogeny fails to retrieve eight edges, while Sanchez et al. (2018) fail to retrieve 19 edges from the C75-A1 tree. Our analysis recovered the monophyly of the Octopodiformes and Decapodiformes with high support (UFBoot 97%, Supporting Information 5). Within the Decapodiformes, the unequivocal monophyly of Sepiida, Myopsida, Oegopsida and Bathyteuthida is well supported (Figure 2). Relationships within these Decapodiformes, including Idiosepiida and Spirula, are known to be unstable depending on sampling and the method used to resolve relationships (Anderson & Lindgren, 2021). In contrast to Anderson and Lindgren (2021), our result is consistent with the topology of Strugnell et al. (2017), Tanner et al. (2017) and Uribe and Zardoya (2017) in placing Sepiida as a sister taxon to all other Decapodiformes. Relationships between the Myopsida and the Spirula + Bathyteuthoidea + Oegopsida clades are less well supported (UFBoot < 80%) but are consistent with Lindgren et al. (2012), Strugnell et al. (2017) and Tanner et al. (2017). Our analysis failed to recover Sepiolida as monophyletic by branching *Idiosepius* as sister to the Sepidiariidae. Sepiolidae is, however, monophyletic although the topology at the subfamily level differs slightly from the recent hypothesis proposed by Sanchez et al. (2021). The relationships between the Octopoda subclades are poorly supported as in previous studies and are similar to those found by Lindgren et al. (2012).

3.2 | Morphospace variation

Allometry was low but significant for upper beaks but absent for lower beaks (Table 2). The PCA performed on both lower and upper beaks presents a substantial shape variation on the first axis (45.3% and 53.2%, respectively). The PCA on lower beaks (Figure 3, left side) shows a clear separation between orders of coleiods on the first two axes. Indeed, Octopodiformes, represented by the suborders Cirrata and Incirrata, are grouped together on the negative part of the first axis.

Decapodiformes, including Sepiida, Myopsida and Oegopsida, are grouped together along this axis. The Vampyromorpha, characterized by its single species *Vampyroteuthis infernalis*, is positioned between Octopoda and Decapoda on the first axis. For the upper beaks (Figure 3, right), the separation between orders on the first two axes of the PCA is less clear. Oegopsida and Incirrata occupy two largely distinct parts of the morphospace with little overlap

between them, Sepiida, Cirrata, Myopsida and Vampyromorpha are more overlapping.

The phylomorphospace of the lower beaks contains less variation on the first principal components (Figure 4) and the orders are no longer clearly separated on this axis. Along the first one (22.12% of the explained variance) variation is situated mostly in the proportion of the hood and the width, height and opening of the wings compared to the length of the lateral walls. Species on the negative part have a sharp rostrum, a relatively short hood and tall lateral walls. The reduction of the height of the lateral walls is accompanied by an increase in their length. *Vampyroteuthis infernalis* is distant from other species and its hood is the largest of the species in our sample. Cirrata and Oegopsida are positioned towards the positive part of the first axis and have a broad, rounded hood, lower yet longer lateral walls, and a round and flat crest. The shape of the wings, their curvature, length, width and the opening of the walls are the main features describing variation along the second axis. The hierarchical clustering shows that the main differences remain between Octopodiformes and Decapodiformes in the phyloPCA (Figure S1).

The first two axes of the phylogenetic PCA on upper beaks show that the differences between coleoid orders are still important (Figure 5). Indeed, Oegopsida (centre, right bottom group) and Incirrata (upper left group) occupy two different parts of the morphospace. However, the other groups are not clearly separated. On the first axis, representing 28.4% of the overall variability, the hood and rostrum become larger and more pointed towards the positive side while they are extremely reduced and rounded towards the negative part of the axis. The lateral walls also become straighter when moving towards the positive part of the axis. In addition, the opening width of the beak, which describes the distance between the inner parts of the wings and the lateral walls, decreases from the negative to the positive part of the first axis. On the second axis (16.6% of the variance), the major differences between beaks are similar to the first axis. Hoods are more extended and pointed towards the negative part of the axis and reduced and rounded towards the positive side. Furthermore, the length and height of the lateral walls in comparison to the hood increases from bottom to top as beaks become wider. The hierarchical clustering shows a clear difference between Oegopsida and the other groups and a lower but still significant difference between Myopsida and Octopodiformes (Figure S2).

A Kmult test for the upper and lower beaks shows that beak shape has a moderate but significant phylogenetic signal ($K = 0.25$ and 0.21 for lower and upper beaks, respectively, $p = 0.001$). This result indicates that beak shape within the taxa studied is not structured solely by the phylogeny (Table 2).

3.3 | Ecological signal

The phylogenetic MANOVA revealed that lower beaks are significantly different depending on the living environment of species (Table 2) with pelagic species differing in shape from benthic and

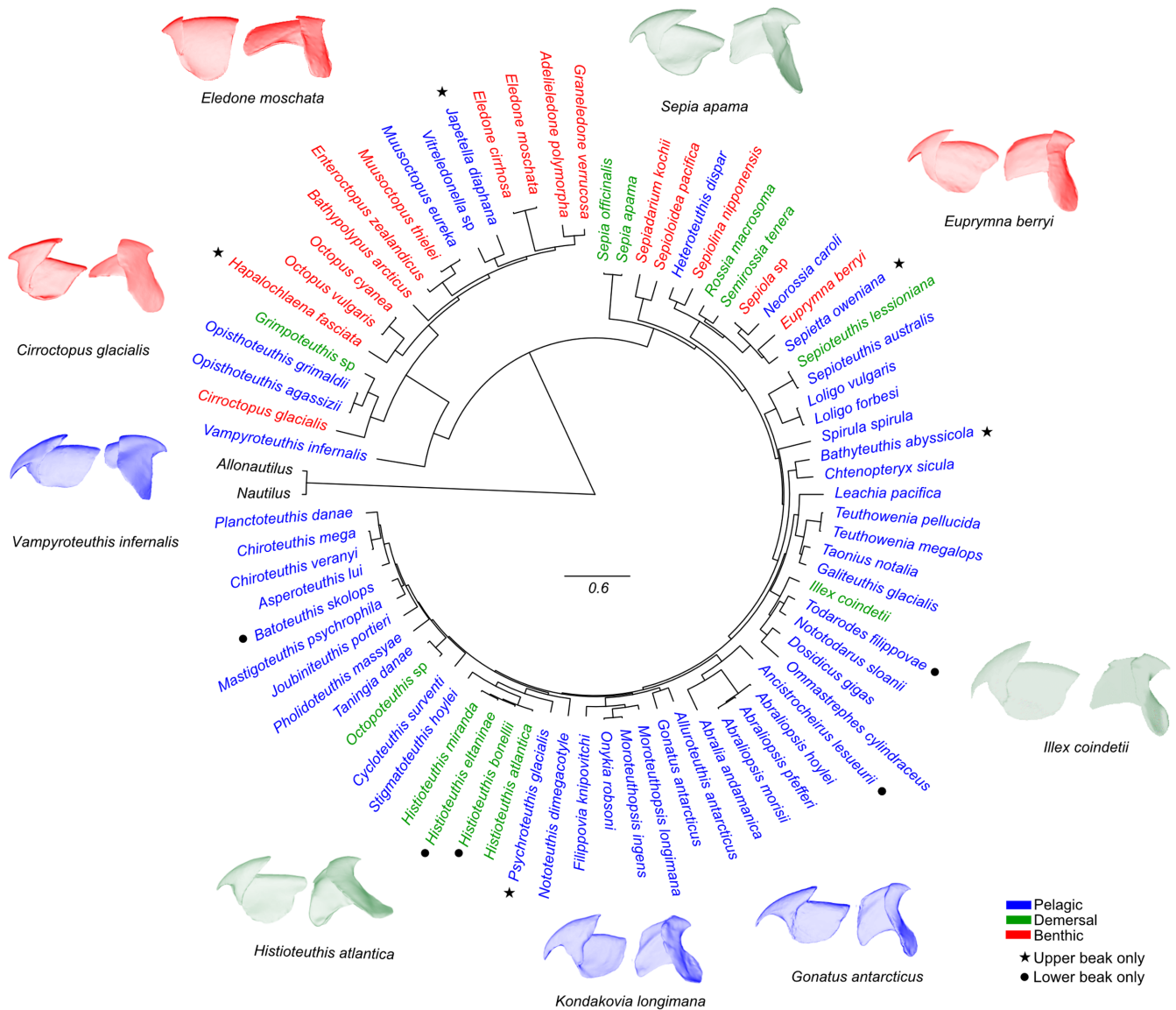


FIGURE 2 Subsampled phylogeny including the 75 species studied based on the newly generated molecular phylogeny (Supporting Informations 4 and 5) with habitat mapped for each species: benthic (red), demersal (green) and pelagic (blue). Black dots indicate species for which only the lower beak is included in the analyses as the black star for upper beaks. Lower and upper beaks shape representative of the different taxonomic groups and of the three habitats assessed in the PhyloPCA are illustrated

demersal species (Figure 6). Pelagic species have short indented lateral walls with a large jaw angle, on average, whereas benthic species have longer and straighter walls with a rounder rostrum and a narrow jaw angle. These characteristics are also representative of oceanic squids on one hand and the benthic octopuses on the other hand. In addition, species using different types of substrates (i.e. soft, mixed, or pelagic) also show significant differences in beak shape. Lower beak shape also predicted trophic level and the $\delta^{13}\text{C}$ values are also significantly related to beak shape. In contrast, the upper beaks shape show no significant relationship with any ecological parameters except with the types of substrates but the signal is weak. The morphological disparity is not different between the three tested habitat groups for either the upper, nor the lower beak.

All analyses were also performed on a subsample containing only Oegopsida (Table 2). When examining only Oegopsida, allometry is significant for both beaks, different from the overall analysis. Similar levels of phylogenetic signal and relationships between shape and trophic ecology were recovered, however. These results are congruent with the signal obtained for coleoids as a whole and confirm the role of ecology in driving beaks shape, including within a clade of closely related, mostly pelagic, species.

4 | DISCUSSION

Our principal component analysis performed on 3D morphometric data reveals patterns similar to those reported in Neige and

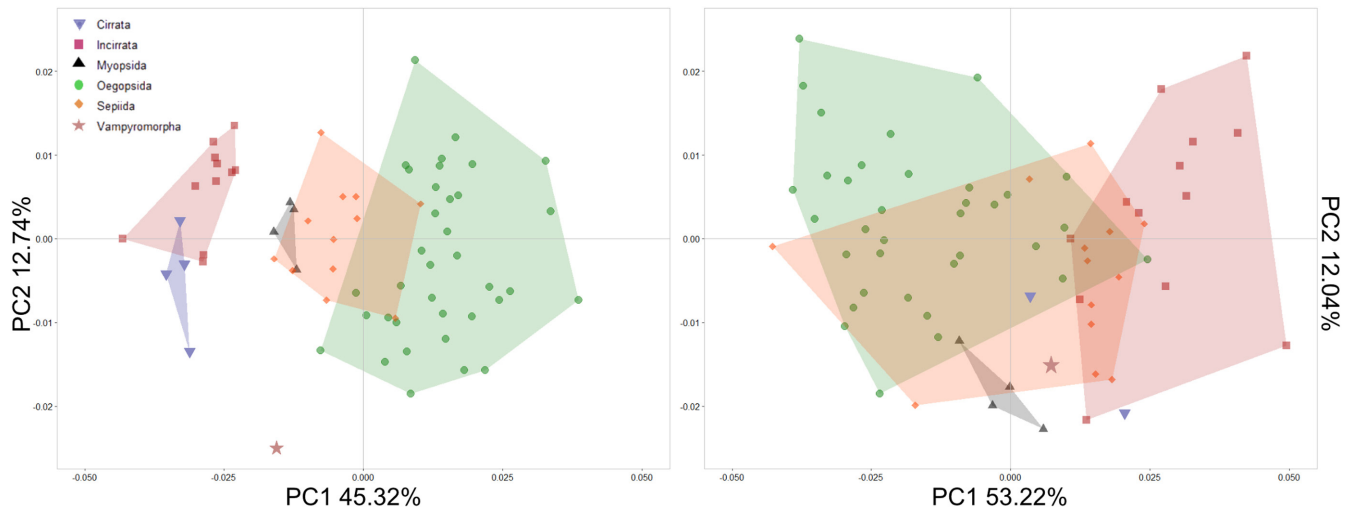


FIGURE 3 Morphospace of the lower ($N = 70$, left) and upper beaks shape ($N = 71$, right) constructed from the first two PCs. H: Cirrata, Octopodiformes; Incirrata, Octopodiformes; N: Myopsida, Decapodiformes; Sepiida, Decapodiformes; •: Oegopsida and Bathyteuthida (*Bathyteuthis abyssicola* for upper beak only) and *Cthenopteryx sicula*, Decapodiformes; F: *Vampyroteuthis infernalis*, Vampyromorpha. The coloured polygons outline the morphological space occupied by the different orders. See Supporting Information 6 for details on other principal component variances

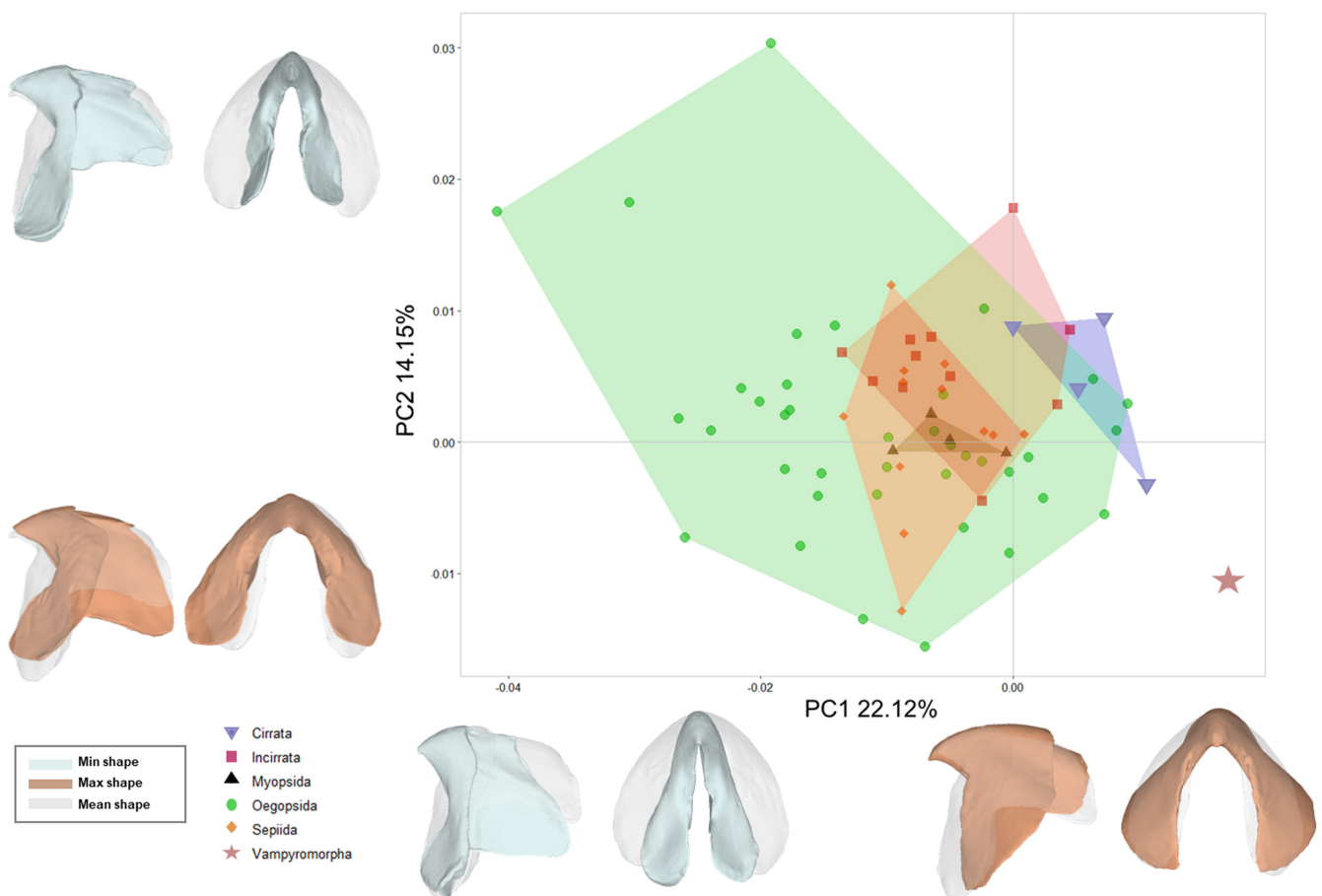


FIGURE 4 Phylogenetic PCA using GLS-centring on mean specimens of lower beaks. $N = 70$. The minimum, maximum and mean shapes are real specimens positioned at the extremities of the axes and compared to a specimen positioned at 0,0 (grey outline). The specimen representing the minimum of PC1 is *Taonius notalia*, for PC2 this is *Dosidicus gigas*. The specimen representing the maximum of PC1 is *Vampyroteuthis infernalis* and for PC2 this is *Teuthowenia pellucida*. See Supporting Information 6 for details on the other principal components and the proportion of variance explained

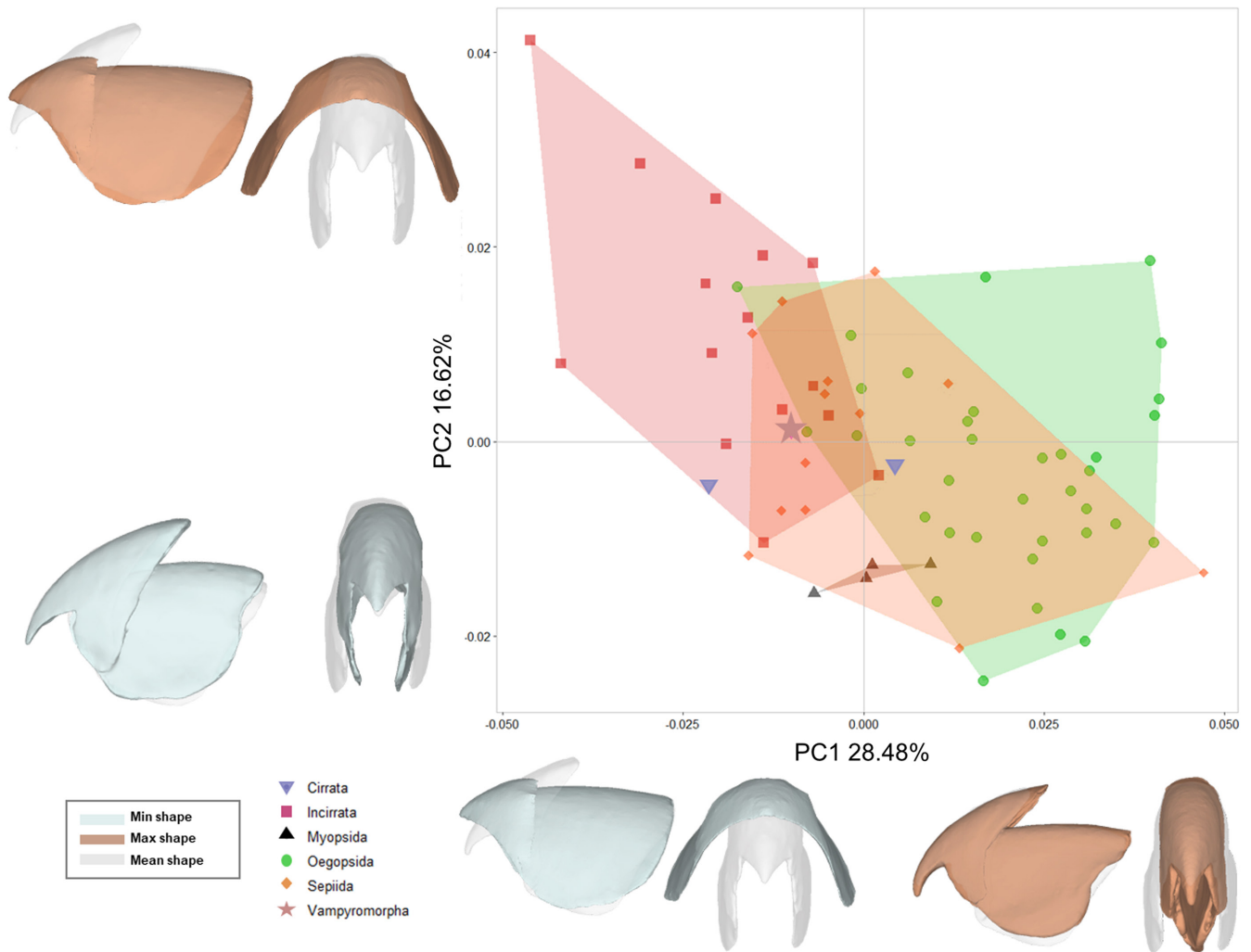


FIGURE 5 Phylogenetic PCA using GLS-centring on mean specimens of upper beaks. $N = 71$. The minimum, maximum and mean shapes are real specimens positioned towards the extremities of the axes and compared to a specimen in the middle of the plot (0,0; grey outline). The specimen representative of the minimum PC1 is *Japetella diaphana*, for PC2 this is *Ommastrephes cylindraceus*. The specimen representing the maximum of PC1 is *Sepia apama* and *Japetella diaphana* for PC2. See Supporting Information 6 for details on the other principal components the proportion of variance explained

Dommergues (2002) and Tanabe et al. (2015) who documented 2D shape differences between higher level clades of cephalopods without, however, explicitly investigating the impact of phylogeny and ecology on beak shape. For the lower beaks, the orders of cephalopods are easily distinguishable on the first axis and their main differences are the roundness of the rostrum, the opening of the jaw angle and the proportion of the hood relative to the lateral walls. The 3D morphometric analyses are not congruent with the findings of Neige and Dommergues (2002) with respect to the upper beak. Indeed, the distinction between orders is more blurred because of a large overlap between groups (Figure 3). The difference between Octopods and Oegopsids is still clear and these clades are positioned on opposite parts of the morphospace. Although our results are generally in agreement with previous 2D studies that have shown clusters of beak shapes according to the major clades, the phylogenetic signal is only moderate for both beaks. This result is at odds with, Clarke and Maddock (1988) who claimed that the shape of the lower beak only

reflects phylogenetic history and did not show any ecological signal. Rather, we suggest that part of the variation of the beaks shape can be explained by other factors such as function or ecology.

The phylomorphospace of lower beaks no longer grouped beaks according to taxonomy except for the distinction between Octopodiformes and Decapodiformes. The shape variation highlights features that cannot be captured in two dimensions. The opening width of the beak, the tilting of the wings and the shape of lateral walls are major components of shape variation in lower beaks.

These variations are also associated with differences in the musculature of the buccal mass Kear (1994). The reduction of the lateral wall length is associated with an increase in its width which may help maintain a large enough surface for muscle insertion, for example. Moreover, the tilt of the wings is associated with the insertion of the main mandibular muscle and consequently this affects the overall muscle volume that can be contained within the beak. These variations in the shape of the lower beaks and their

TABLE 2 Statistical tests applied to lower (LB) and upper beaks (UB) for the whole sample (top) and for a subsample with Oegopsida (bottom). *N* is the number of samples, *df* the degrees of freedom, *P* is the *p*-value. **P* ranging between 0.05 and 0.01; ***P* < 0.01

Parameter	Test	<i>N</i> LB;UB	<i>df</i>	Statistic	LB statistic	LB <i>P</i>	UB statistic	UB <i>P</i>
<i>All specimens</i>								
Allometry	Multiple regression	101;108	1	R^2	0.02	0.065	0.13	0.001**
Phylogenetic signal	Kmult	70;71	–	<i>K</i>	0.25	0.001**	0.21	0.001**
Environment	phyloMANOVA	70;71	2	<i>F</i>	2.28	0.008**	0.80	0.644
Type of substrate	phyloMANOVA	70;71	2	<i>F</i>	4.13	0.001**	2.78	0.003**
Mean depth	PGLS	70;71	1	R^2	0.02	0.349	0.02	0.129
Trophic level	PGLS	24;21	1	R^2	0.23	0.002**	0.03	0.579
$\delta^{13}\text{C}$	PGLS	25	1	R^2	0.17	0.013*	0.03	0.7
$\delta^{15}\text{N}$	PGLS	25	1	R^2	0.08	0.135	0.04	0.47
$\delta^{13}\text{C}:\delta^{15}\text{N}$	PGLS	25	1	R^2	0.1	0.091	0.03	0.622
<i>Oegopsida</i>								
Allometry	Multiple regression	56;58	1	R^2	0.04	0.014*	0.14	0.001**
Phylogenetic signal	Kmult	38;36	–	<i>K</i>	0.31	0.001**	0.28	0.02*
Environment	phyloMANOVA	38;36	2	<i>F</i>	5.78	0.001**	0.87	0.463
Mean depth	PGLS	38;36	1	R^2	0.03	0.317	0.02	0.607
Trophic level	PGLS	16	1	R^2	0.28	0.003**	0.04	0.756
$\delta^{13}\text{C}$	PGLS	17	1	R^2	0.07	0.264	0.04	0.753
$\delta^{15}\text{N}$	PGLS	17	1	R^2	0.03	0.713	0.06	0.425
$\delta^{13}\text{C}:\delta^{15}\text{N}$	PGLS	17	1	R^2	0.04	0.575	0.05	0.562

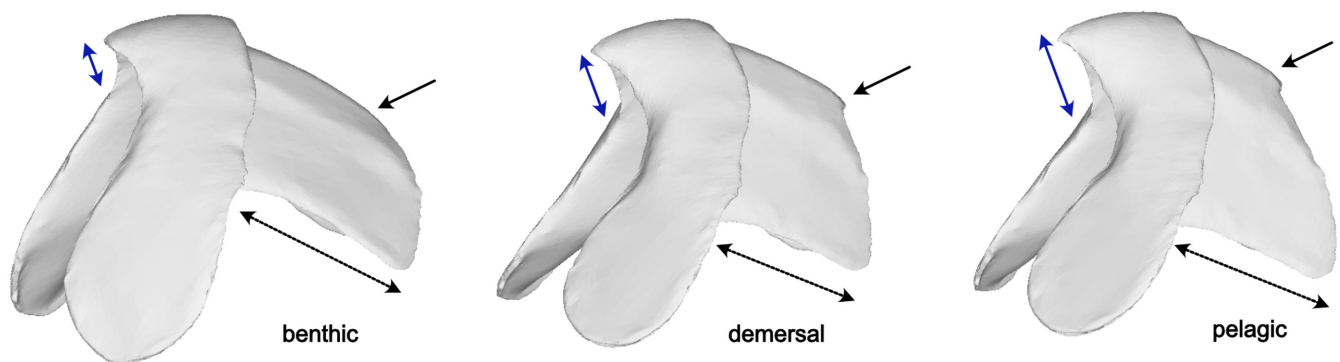


FIGURE 6 Morphological disparity of lower beaks for benthic, demersal and pelagic species. The biggest difference between the demersal and pelagic species resides in the length of the crest and shape of the lateral walls. Demersal species have a longer crest (black arrow) and longer and straighter lateral walls (double black arrow) than pelagic species. Pelagic species have short, indented lateral walls with a large jaw angle (double blue arrow), on average, whereas benthic species have a longer and straighter crest and lateral walls with a rounder rostrum and a narrow jaw angle

relationship with the muscle insertions are congruent with the mechanical hypotheses developed by Kear (1989). As the lower beaks of benthic octopods are characterized by a small and rounded rostrum with a reduced hood, they might be associated with a strong bite. Indeed, the bite point is closer to the pivot point described by Uyeno and Kier (2005). Based on the lever arms, the closing speed is likely relatively low, yet the force high. This is congruent with our knowledge of their diet consisting primarily of crustaceans and bivalves (see Table S3 for details and reference on diet). Whereas

the radula has been suggested to be the main actor in drilling holes and in scraping the flesh of bivalves (Nixon, 1979), the beaks could be used to maintain hard prey such as crabs or lobsters and may be effectively used to crush these prey (Voight, 2000). The shape corresponding to the opposite side of the PCA represents a radically divergent type of bite, suggested to be faster but weaker as observed in some Oegopsids. These squid-like shapes have a longer rostrum and hood and consequently the distance between the pivot point and the bite point is greater. Most Sepiids and

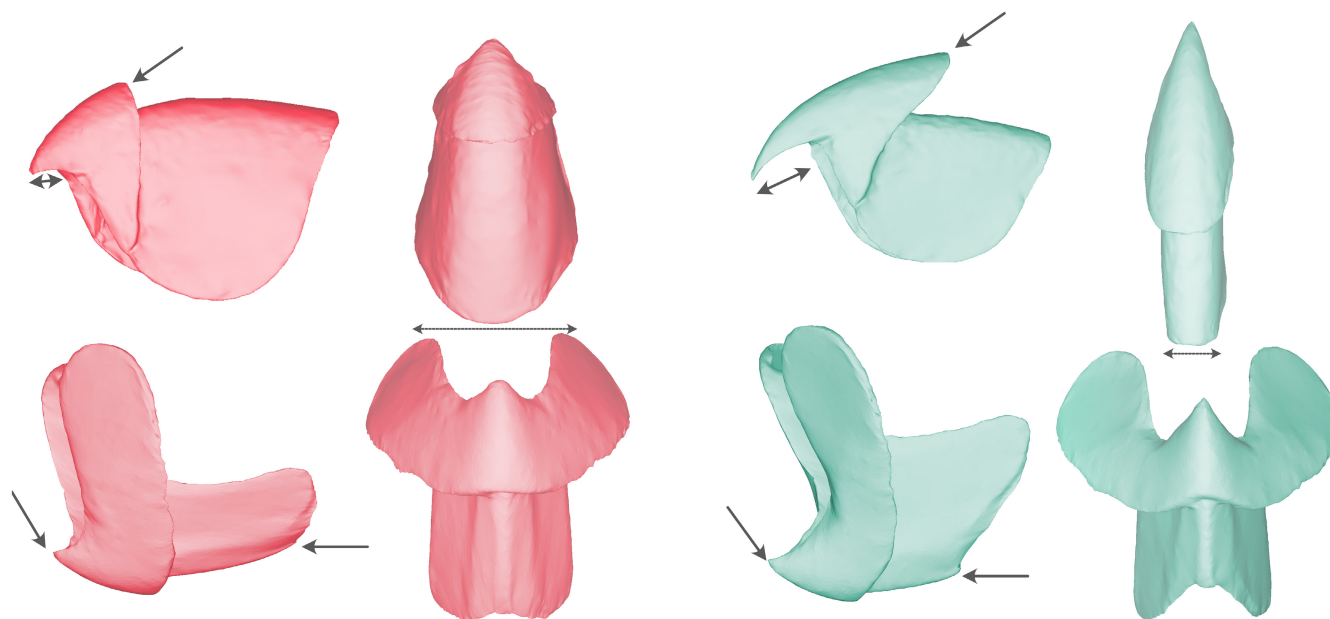


FIGURE 7 Mean shapes of Octopodiformes (left; shapes in pink) and Decapodiformes (right; shapes in blue) beaks based on the hierarchical clustering using all phyloPCA axes

Myopsids fall between these extremes and have beak shapes that suggest that they are stronger than fast with a small and round rostrum. In Oegopsids, a wide range of variation in the shape of the lower beak is present and is likely associated with an equally large variation in function (Figure 7).

For the upper beaks, in addition to the length of the hood and rostrum, the 3D shape analysis shows that the width of beak is also a major feature of its shape (Figure 5). The posterior elongation of the hood and the curvature of the crest are associated with the insertion of the superior mandibular muscle (Kear, 1994). The phylomorphospace of the upper beaks still shows phylogenetic signal, despite a greater overlap between clades. These observations are in line with the suggestions of Neige and Dommergues (2002), that the upper beak is more informative than previously thought for systematic purposes despite the fact that it has been suggested to be the principal actor in reducing prey (Uyeno & Kier, 2005, 2007). The major differences observed also reflect the type of bites described earlier for lower beaks. In addition, the length and the tip of the rostrum of upper beaks can be associated with two main functions: a short and rounded rostrum with a reduced hood is associated with crushing, while a long, pointed rostrum with a wide hood is likely used as a piercing tool. The former is observed in most of the benthic Octopods and the latter is represented in some Oegopsids.

Irrespective of the phylogenetic signal in the data, species that used different habitats differed significantly in the shape of the lower beak, especially pelagic and benthic species (Figure 6). The lower beak shape was also significantly associated with trophic level. These results remain when analysing data for the subsample of Oegopsida, suggesting that ecology is a major driver of

beak shape in coleoid cephalopods. These data suggest that diet is an important driver of beak shape (Table 2). Unfortunately, data on diet are scarce and partial for most species (Xavier et al., 2015) due to their high digestion rate, pre-digestive secretions, and the use of their beaks and radula to turn prey into small pieces. Stables isotopes and trophic levels can only be considered as a proxy for diet and cannot provide insights into how beaks are used to deal with different types of prey. Improving our knowledge of diet is essential to better understand the relationships between beak shape and diet. Recent developments in meta-barcoding of gut contents of several species (Fernández-Álvarez et al., 2018) are promising in this respect. Moreover, species with unusual beak shapes such as Stauroteuthidae, which has a very small but extremely curved rostrum or *Tremoctopus*, where the rostrum is mostly reduced, need to be added to the dataset. Moreover, functional data on prey such as hardness or size are needed to better understand variation in beak shape.

This study highlights the importance of habitat as a driver of variation in beak shape variability. The mean shape of the lower beaks of the benthic group faithfully represents the main characteristics of benthic octopods. The short, rounded, thick rostrum of these animals is likely more resistant to the forces exerted when breaking shells, corresponding to the 'crushing' function and strong beak described above. This use of the beak is also related to the hunting behaviour of benthic octopods. Their long and extremely mobile arms allow them to capture, maintain and manipulate prey without needing to kill it rapidly. Moreover, they also have venom that is used to kill and/or predigest prey. In contrast, the mean shape of lower beaks in pelagic species, similar to the beak of Oegopsida such as *Dosidicus gigas*, is likely more efficient at

piercing and tearing fish and cephalopods. These species hunt by projecting the tentacles and rapidly bringing back the prey towards the beak to kill it.

Our first observations on the variation in beak shape, the association thereof with information on muscle insertions, lever arms, habitats and diet allow us to validate some broad functional categories. Indeed, while some species have strong and crushing beaks, others appear more optimized for speed. Future quantitative analyses of the buccal mass musculature are essential to be able to evaluate variation in performance and possible relationship to beak shape. Biomechanical models or in vivo measurements could be used to quantify bite force and may shed further light on the association between form and function of the cephalopod beak. Although the movements of the beaks and muscle activity patterns have been investigated to some degree (Boyle et al., 1979; Uyeno & Kier, 2007), the precise function of the beaks in cephalopods remains poorly understood. Thus, future studies focusing on a better understanding of the anatomy and function of the buccal complex will be critical to improve inferences on the role that beak shape plays in transferring forces to prey and allowing cephalopods to deal with different types of prey. As both beaks work together to reduce prey, the covariation between the two structures could shed further light on beak function.

AUTHORS' CONTRIBUTIONS

M.R. performed the data acquisition, the analyses and wrote the initial draft of the paper; A.H. and I.R. supervised the project, discussed the results and contributed to writing of the manuscript; Y.C. provided specimens and expertise on cephalopod beaks; P.Z. constructed the phylogeny and wrote the phylogenetic results of the manuscript; R.C. provide expertise for morphometric analyses and statistics; I.K. provides expertise on cephalopod ecology; V.F. scanned the NHM specimens. All authors contributed to the drafting and revision of the final manuscript.

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CONFLICT OF INTEREST

We have no conflict of interest to declare. Antony Herrel is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

GeneBank IDs used to construct the phylogeny, Newick files, and data with references are available on InDoRES: <https://doi.org/10.48579/PRO/BVWXJL>. Scans from computed tomography are available in the archive of the AST-RX platform in Paris and in National History Museum imaging archive in London, UK.

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