

ITERATIVE HABITAT TRANSITIONS ARE ASSOCIATED WITH MORPHOLOGICAL CONVERGENCE OF THE BACKBONE IN DELPHINOIDS

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ABSTRACT

Living cetaceans are ecologically diverse and have colonized habitats ranging from rivers and estuaries to the open ocean. This ecological diversity is strongly associated with variation of vertebral morphology. Interestingly, intraspecific ecological specialization between coastal and offshore environments has also been described for several species of extant delphinoids (Monodontidae, Phocoenidae, and Delphinidae). These apparent similar ecological specialisations between and within species provide a framework to compare ecomorphological patterns below and above the species level. Here, we investigated the tempo of habitat transitions during the delphinoid evolutionary history and we quantified the effect of habitat on the vertebral morphology in all delphinoids and between bottlenose dolphin (*Tursiops truncatus*) ecotypes of the western North Atlantic Ocean. Our comparative analyses highlight iterative habitat transitions and associated morphological convergences of the axial skeleton in delphinoids, both occurring at high evolutionary rates. Moreover, morphological modifications between coastal and offshore bottlenose dolphin ecotypes are similar in direction and magnitude to those observed in the entire Delphinidae family. Ecomorphological patterns currently observed at the intraspecific level might reflect macroevolutionary patterns that contributed to diversification.

Introduction

Understanding and identifying factors promoting organismic diversity and disparity is a central question in evolutionary biology. Across the tree of life, some ecomorphological transitions appear to occur repeatedly, often resulting in convergence among species occupying similar ecological niches. Repeated adaptive radiations over space or time have been described in terrestrial organisms such as *Anolis* lizards and canids (Van Valkenburgh [1991](#); Losos et al. [1998](#); Slater [2015](#)). In the aquatic environment, iterative transitions between nearshore and offshore environments have been documented in a variety of invertebrate and vertebrate clades

and seem to be a recurring pattern (Bayer and McGhee [1984](#); Lindgren et al. [2012](#); Velez-Juarbe et al. [2012](#); Frédérich et al. [2013](#); Bribiesca-Contreras et al. [2017](#)). For instance, multiple transitions between shallow and deep-sea habitats shaped ophiuroids evolutionary history (Bribiesca-Contreras et al. [2017](#)). In marine mammals, similar habitat partitioning between shallower and deeper habitats, reflected by differences in body size, rostral deflection, and tusk morphology, has been suggested in three different fossil assemblages of sea cows (Dugongidae) (Velez-Juarbe et al. [2012](#)).

Similarly to other aquatic organisms, extant cetaceans diversified along the onshore-offshore environmental gradient. The ecological diversity of cetaceans is associated with marked variation in their backbone morphology (Buchholtz and Schur [2004](#); Buchholtz et al. [2005](#); Marchesi et al. [2018](#), [2020](#); Gillet et al. [2019](#)). Coastal species are characterised by a small body size and low count of elongated vertebrae while species living further offshore either retained a low vertebral count with a large body size (i.e., baleen whales, sperm whales, beaked whales) or retained a small body but with an extremely high number of discoidal vertebrae (i.e., delphinoids) (Gillet et al. [2019](#)). The widespread colonization of nearshore and offshore environments is particularly striking in delphinoids (i.e., narwhal, beluga, porpoises, and dolphins), a monophyletic clade grouping more than half of living cetacean species. Moreover, habitat partitioning can also be found at the intraspecific level since coastal and offshore ecotypes have been described for several delphinoids, such as common bottlenose dolphins (*Tursiops truncatus*) (Mead and Potter [1995](#); Gaspari et al. [2015](#)), common dolphins (*Delphinus delphis*) (Natoli et al. [2006](#); Amaral et al. [2012](#); Segura-García et al. [2016](#)), spotted dolphins (*Stenella attenuata*) (Leslie and Morin [2018](#)), spinner dolphins (*Stenella longirostris*) (Perrin et al. [1999](#); Andrews et al. [2013](#)), and narrow-ridge finless porpoises (*Neophocaena asiaeorientalis*) (Zhou et al. [2018](#)). In these species, coastal ecotypes are genetically divergent from the more widely distributed offshore ecotypes and exhibit distinct morphological traits (coloration patterns, body size, and skull shape) (Andrews et al. [2013](#); Segura-García et al. [2016](#); Leslie and Morin [2018](#); Costa et al. [2019](#)). However, the frequency of such ecomorphological transitions and the resulting level of vertebral convergence are still unknown.

Habitat specialisation has been particularly studied in common bottlenose dolphins for which coastal and offshore ecotypes have been described for most of its geographical range. These ecotypes differ in morphology, feeding preferences, parasitic load, and haemoglobin profile, and are

clearly identified based on genetic data (Walker [1981](#); Hersh and Duffield [1990](#); Mead and Potter [1995](#); Hoelzel et al. [1998](#); Torres et al. [2003](#); Natoli et al. [2004](#); Perrin et al. [2011](#); Caballero et al. [2012](#); Moura et al. [2013](#); Louis et al. [2014](#); Gaspari et al. [2015](#); Costa et al. [2016](#); Segura-García et al. [2018](#)). Genetic studies suggest that coastal populations emerged from offshore animals that specialised in the exploitation of resources in shallower habitats (Natoli et al. [2004](#); Moura et al. [2013](#)). In addition, a study focusing on the vertebral morphology of common bottlenose dolphins in the western South Atlantic concluded that coastal individuals have fewer but more elongated vertebrae (Costa et al. [2016](#)). Interestingly, this ecomorphological pattern mirrors the one described within the entire delphinoid clade (Gillet et al. [2019](#)), suggesting that similar drivers may shape the cetacean backbone above and below the species level. However, to date, only a few empirical studies have highlighted that macroevolutionary patterns could be partly explained and predicted by microevolutionary processes (Hulsey et al. [2006](#); Pointer and Mundy [2008](#); Rolland et al. [2018](#)).

Modifications in vertebral count and shape are known to be associated with developmental changes, leading to direct functional adaptations (Long et al. [1997](#); Woltering [2012](#); Buchholtz and Gee [2017](#)). Therefore, vertebral morphology seems to be a valuable trait to explore the repetition and similarity of ecomorphological patterns within delphinoids at the inter- and intraspecific levels. In the present work, we aim to test whether similar processes of backbone disparification act at the micro- and macroevolutionary scales in delphinoids by using a suite of morphometric and phylogenetic comparative methods. We hypothesize that multiple independent transitions along the coastal-offshore gradient involved similar modifications of vertebral shape both within and among species, ultimately leading to convergence. The wealth of species for which ecotypes have been described suggest that habitat transitions occur readily in delphinoids. We hence also hypothesize that habitat transitions and associated vertebral modifications should occur at high evolutionary rates at the macroevolutionary scale. To this purpose, we investigated the tempo of habitat transitions during the delphinoid evolutionary history and we quantified the effect of habitat on vertebral morphology at three different taxonomic levels: 1) within the whole delphinoid clade, 2) within Delphinidae and Phocoenidae, and 3) among bottlenose dolphin ecotypes of the western North Atlantic Ocean. We demonstrate that habitat transitions occurred frequently and rapidly, and were accompanied by convergent vertebral modifications. Moreover, habitat-related evolution of the backbone at the family level mirrors patterns at the intraspecific level suggesting continuity between micro- and macroevolutionary processes.

Materials and Methods

DATA SAMPLING

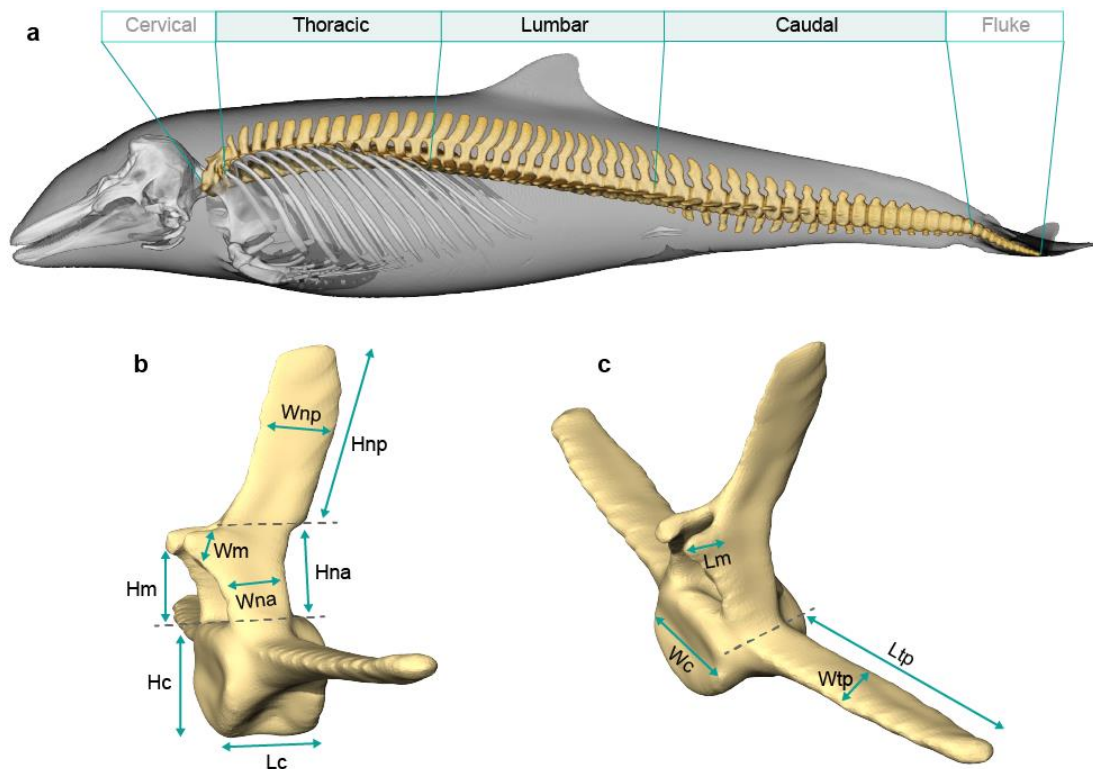
Vertebral shape data were collected on 122 specimens from 37 delphinoid species (out of 46 extant species (Committee on Taxonomy [2021](#))) housed in eight natural history museums. For most species, data were collected on three specimens, and only one species (*Orcaella brevirostris*) was represented by a single specimen (Online Resource 1, Table S1). Only morphologically mature

specimens with vertebral epiphyses fused to the centrum were included in our dataset. To further investigate ecomorphological patterns among ecotypes, vertebral shape was collected on common bottlenose dolphins (*T. truncatus*) specimens from the western North Atlantic (WNA) where divergence from offshore to coastal ecotypes is thought to have occurred first in this species. The monophyletic coastal ecotype is ecologically and morphologically differentiated from the offshore ecotype, suggesting that it could be an example of incipient speciation (Moura et al. 2013). Coastal WNA specimens are generally smaller, have larger flippers, and possess proportionally longer snout and smaller internal nares than offshore WNA specimens (Hersh and Duffield 1990; Mead and Potter 1995). Nine WNA specimens were sampled, among which six were classified as belonging to the coastal or offshore ecotype: three coastal specimens (1 male, 2 females) and three offshore specimens (2 males, 1 female). Ecotype information was retrieved from the museum database (Smithsonian National Museum of Natural History, USNM), which determined ecotype information for each specimen based either on sampling location for bycaught animals (one specimen in our dataset) or on osteological morphology for stranded animals (see Online Resource 1, Table S1 for specimen accession numbers and ecotype classifications). For analyses at the species level, vertebral shape data of the nine specimens was used to calculate *T. truncatus* species mean, while only the six specimens with defined ecotypes were used for intraspecific analyses.

The vertebral shape of each specimen was quantified by taking twelve linear measurements with digital calipers on every vertebra of the thoracic, lumbar, and caudal regions (Fig. 1). Vertebral regions were defined following the description of Rommel (1990), and the first fluke vertebra was identified as the first vertebra with a centrum height at least 5% lower than the centrum width. Fluke vertebrae were not included in our analyses, allowing the inclusion of

specimens missing the few last vertebrae. The mean value of each measurement was calculated for each vertebral region (see methods in Gillet et al. 2019). Specimen mean regional measurements were \log_{10} -transformed and phylogenetically size-corrected with the R-function *phyl.resid.intra*, using the \log_{10} -transformed total centrum length (TCL, i.e., the sum of the vertebral centrum length of all vertebrae of each specimen) as a proxy for body length (R version 4.0.5) (López-Fernández et al. 2014; R Core Team 2017). Vertebral shape residuals resulting from the size-correction regression on mean regional data were used in all subsequent shape analyses except analyses of variance and analyses of tempo of morphological evolution for which principal component (PC) scores were used. All phylogenetic analyses were conducted based on the cetacean consensus time-calibrated tree from McGowen et al. (2020).

Fig. 1 Vertebral morphometrics. a. 3D model of harbour porpoise (*Phocoena phocoena*) skeleton and backbone regions (from Rommel 1990). Measurements were taken on every thoracic, lumbar, and caudal vertebra; b–c. Harbour porpoise first lumbar vertebra in left lateral and dorsolateral views. Measurement abbreviations: the first capital letter is the type of measurement (L: length, H: height, W: width), and the subsequent lowercase letter(s) correspond(s) to the vertebral part (c: centrum, np: neural process, na: neural arch, m: metapophysis, tp: transverse process).



Specimens were classified according to two factors: phylogenetic group and ecology. Phylogenetic groups correspond to the three families of delphinoids: Monodontidae (belugas and narwhals), Phocoenidae (porpoises), and Delphinidae (dolphins). To investigate the ecomorphological patterns at the intraspecific level, WNA *T. truncatus* specimens were not included in the Delphinidae group and were instead considered as a fourth distinct phylogenetic group. Ecological data for each species were collected from synthetic bibliographic works (Perrin et al. 2009; Berta 2015; IUCN 2017). Each species was classified in one of the following categories: (i) rivers, bays, and estuaries (i.e., Rivers & bays), (ii) continental shelf (i.e., Coasts), (iii) continental slope and offshore waters (i.e., Offshore), and (iv) mixed lifestyle between continental shelf and offshore waters (i.e., Mixed) (Online Resource 1, Table S2).

HABITAT TRANSITIONS

Presence of ecotypes in several delphinoids suggests that ecological transitions are not constrained and it might be expected that numerous habitat transitions should also have occurred during the diversification of delphinoids. We tested this prediction by studying the frequency of habitat transitions across delphinoid phylogeny. Models of discrete trait evolution were used to establish the importance and frequency of ecological transitions during their evolutionary history. In order to use the most comprehensive analysis, ecological data were also gathered for some additional delphinoid species not included in the morphological dataset (*Lagenorhynchus cruciger*, *Lissodelphis peronii*, *Sotalia fluviatilis*, *Phocoena sinus*). Exact information about habitat preference and phylogenetic position of fossil taxa is rarely known. Consequently, models were run on extant

species only. However, to provide a reasonable root value to the models, an analysis of habitat transition was first run on the entire cetacean clade and the estimated ecological state probability of the most recent common ancestor (MRCA) of delphinoids was used as root value for the habitat transition analysis of delphinoids (see Online Resource 1, Supplementary Analysis I). Four different macroevolutionary models were tested using the function *fitMk* from the *phytools* R- package (Revell [2012](#)): (i) an equal rates model (ER) where all transitions between habitats have the same rate; (ii) an all rates different (ARD) model for which all habitat transition have different rates; (iii) an ordered model (ORD1) for which only the following reciprocal transitions are possible and have different rates: rivers-coasts, coasts-mixed, mixed- offshore; (iv) a second ordered model (ORD2) similar to ORD1 but in which reciprocal coasts-offshore transitions are also possible. The model with the highest weighted Akaike information criterion (AIC) was conserved for simulations of the stochastic mapping which was calculated using the *make.simmap* function (R-package *phytools*). The mapping was repeated 1,000 times and averaged results of all simulations are presented.

MORPHOSPACE OF VERTEBRAE

In order to explore the morphological variation across habitats and phylogeny, vertebral shapes were projected into a phylomorphospace. To do so, vertebral shape residuals of each specimen were firstly implemented in a regular principal components analysis (PCA) (*prcomp* function in R). As a few variables had a variance substantially larger than other variables (Online Resource 1, Table S3), the PCA was run on the correlation matrix. Mean PC scores were then computed for every species, except WNA *T. truncatus* specimens, and subsequently projected in the final phylomorphospace.

The effect of habitat on vertebral shape was quantitatively tested by running MANOVAs and phylo-MANOVAs with residual randomization permutation procedure (RRPP; 10,000 permutations) (*lm.rpp* and *manova.update* functions from the *RRPP* R-package v.0.6.0; Collyer and Adams [2018, 2019](#)). Subsequent pairwise comparisons among habitats were tested using the *pairwise* function (*RRPP* R-package). Following the Jolliffe cut-off, only PCs 1 to 7, accounting for 88% of the total variance, were conserved for variance analyses as they possess eigenvalues higher than 0.7. In addition, phylogenetically-corrected analyses of variance were also conducted on an incremental number of PCs (results presented in Online Resource 1). Regular MANOVAs were run on specimen PCs 1–7 values for the whole Delphinoidea clade and for each phylogenetic group (i.e., Delphinidae, Phocoenidae, and WNA *T. truncatus*) except Monodontidae, as both species have been classified in the same habitat category (i.e., mixed). Phylo-MANOVAs only support values for one specimen per species, so species average scores for PCs1-7 were calculated and used as input. Given that Phocoenidae comprises a restricted number of species (five species in our dataset, out of seven currently recognized; Committee on Taxonomy [2021](#)) and that the phylogenetic relationships at the specimen level are unknown in our WNA *T. truncatus* dataset, phylo-MANOVAs were run only for the whole Delphinoidea clade and for the Delphinidae family based on a Brownian motion model. To further investigate morphological differences between ecological groups within each phylogenetic group, non-phylogenetic and phylogenetically-corrected ANOVAs and associated pairwise tests were run on the 7 first PCs individually. Analyses were run on specimen values (ANOVA: functions

anova and *pairwise.t.est* from R-package *stats*, phyloANOVA: function *phylANOVA.intra* from López-Fernández et al. (2014) with 10,000 simulations, no pairwise test available) and on species-averaged values (ANOVA: functions *anova* and *pairwise.t.est* from R-package *stats*, phyloANOVA: function *phylANOVA* from R-package *phytools*). Finally, the effect of small sample size for WNA *T. truncatus* ecotypes was assessed using simulations (see Online Resource 1, Supplementary Analysis II).

VERTEBRAL SHAPE CONVERGENCE

To assess the iterative nature of morphological evolution in delphinoids, the amount of phenotypic convergence between species having similar ecology was tested using a Euclidean distance-based approach (Stayton 2015). Convergence was calculated on species averaged shape residuals. As the mixed habitat category encompasses species with variable ecology, phenotypic convergence was only tested for rivers (4 species), coasts (9 species), and offshore (17 species) habitat categories. Convergence was estimated and statistically tested independently for each habitat category using the function *convratsig* from the R-package *convevol* with 500 iterations.

The convergence analysis returns four values of convergence (C1 to C4) corresponding to different manners of quantifying convergence. C1 represents the proportion between the morphological distance between two species (D_{tip}) and the maximum morphological distance that occurred during their evolution since their divergence from their MRCA (D_{max}), with larger values indicating greater levels of convergence. C1 is expressed as a ratio to allow comparison between different datasets; however, it implies that no distinction is made between species with absolute small phenotypic differences and species with absolute large phenotypic differences converging to the same percentage. Hence, C2 corresponds to the difference between D_{max} and D_{tip} and represents the absolute amount of convergence achieved. C3 corresponds to the proportion between the absolute level of convergence (C2) and the total amount of morphological evolution (i.e., the total morphological branch length in a morphospace) of the species of interest since they diverged from their MRCA. Finally, C4 represents the proportion between C2 and the total morphological branch length of the entire smallest clade containing the species of interest (Stayton 2015).

Within Delphinidae, several sister species have a similar habitat ecology and this could affect the estimation of convergence (Stayton 2015). To account for this, an additional set of analyses of convergence were run using a randomized subsampling of species. Three clusters of closely related species classified in the same habitat category were defined prior to analyses. These included a coastal cluster (*C. hectori*, *C. eutropia*, *C. commersonii*, *C. heavisidii*, *L. australis*), offshore cluster 1 (*S. bredanensis*, *G. griseus*, *P. crassidens*, *F. attenuata*, *P. electra*, *G. macrorhynchus*, *G. melas*), and offshore cluster 2 (*S. coeruleoalba*, *D. delphis*, *L. hosei*, *S. clymene*). One species of each cluster was randomly selected and conserved for subsequent convergence analysis. The randomized subsampling was repeated 30 times, and for each subsampling, convergence was evaluated and statistically tested with 500 iterations. Convergence values and *P*-values reported in results correspond to the median values of the 30 subsamplings.

PHENOTYPIC TRAJECTORIES

An analysis of phenotypic trajectory (PTA) (Collyer and Adams [2013](#)) was used to test the hypothesis of shared morphological trajectories between Delphinidae, Phocoenidae and WNA *T. truncatus* along the river-coast-offshore habitat gradient. This analysis allows testing whether direction and magnitude of habitat-related morphological modification patterns are similar at inter- and intraspecific levels. As PTA performs its own PCA, the analyses were run on specimen vertebral shape residuals with the *trajectory.analysis* function from the *RRPP* R-package. Given the substantial differences in variance levels among residuals (Online Resource 1, Table S3), the analysis was run on the correlation matrix by scaling residuals with the R-function *scale* prior to analysis. Similarly to the convergence analysis, PTA was only calculated on the three clearly defined habitat categories (rivers, coasts, and offshore) for each phylogenetic group (i.e., Delphinidae, Phocoenidae and WNA *T. truncatus*). PTA requires that every group possesses the same ecological categories. Given that there is no riverine ecotype for WNA *T. truncatus*, two distinct PTAs were performed. The first PTA investigated phenotypic trajectories along the rivers-coasts-offshore ecological gradient for Delphinidae and Phocoenidae, while the second PTA investigated trajectories along the coasts-offshore gradient for Delphinidae, Phocoenidae and WNA *T. truncatus*. The significance of the results of each PTA was calculated from 10,000 simulations and with a randomized residual permutation procedure (RRPP).

TEMPO OF EVOLUTION

The existence of morphologically distinct ecotypes at the intraspecific level suggests that ecomorphological transitions can occur quickly. To test this hypothesis, evolutionary rates of ecological states (i.e., habitat) and vertebral morphology were modelled using the method developed by Shi et al. ([2021](#)) which relies on model-averaging rather than selecting a single best model to estimate evolutionary rates and allows to estimate evolutionary rates of multivariate traits without a priori on rate shift positions. For both ecological and morphological traits, a series of models in which rates are allowed to vary among pre-defined partitions on the phylogeny were fitted. An initial single rate (i.e., one-partition) in which rates are uniform across the phylogeny model was fitted, then all possible multiple rate models with two, three, four, and five partitions, respectively defined by one, two, three, and four rate shifts, were fitted. Here, we limited the number of partitions in order to avoid an oversplitting of our phylogenetic tree into too small subclades, which could lead to misleading conclusions (Shi, personal communication). Branch-specific rates were then computed by averaging results from all models scaled by their Akaike weight and visualized on the phylogeny using the *BAMMtools* R-package (Rabosky et al. [2014](#)).

For habitat traits, all transitions were allowed and transition rates between states were forced as equal for all transitions within the same partition. Models were fitted using the *diversitree* R-package (Fitzjohn [2012](#)). For vertebral shape data, rates of each fitted model were estimated based on a Brownian Motion model using the *mvMORPH* R-package (Clavel et al. [2015](#)) and only models that converged were retained for computing average weighted evolutionary rates. As this approach requires a dataset with fewer variables than observations, analyses were run on the species-average

of the three first PCs, representing 68.59% of the total variance. This limitation on dimensionality also implies that among all possible multirate models, only models in which all partitions have at least four tips were retained for analysis. In order to interpret evolutionary rates estimated for delphinoids in a broader context, ecological and morphological evolutionary rates were also estimated for the entire cetacean clade using a similar approach (see Online Resource 1, Supplementary Analysis III).

Results

HABITAT TRANSITIONS

Based on the habitat stochastic mapping on the entire cetacean tree, the probabilities of the estimated ancestral state of MRCA of delphinoids were 0.785 for the mixed habitat, 0.210 for the coastal habitat, and 0.005 for the offshore environment (Online Resource 1, Fig. S1). Accordingly, these values were used as priors during the modeling of habitat transitions in delphinoids. The best macroevolutionary model to investigate habitat transitions in delphinoids was the ER model (weighted AIC: ER = 0.967, ORD1 = 0.013, ORD2 = 0.020, ARD < 0.001). The posterior most probable ecological state for the MRCA of delphinoids was the mixed habitat (mixed = 0.92, coasts = 0.08) (Fig. 2a). On average, the stochastic mapping detected 29.01 habitat shifts during delphinoid evolutionary history (Fig. 2b and Online Resource 1, Table S4). The most frequent habitat transitions were from offshore to mixed (14.53% of all transitions), followed by coasts to offshore (13.07%), offshore to coasts (12.52%), and offshore to rivers and bays (11.70%).

A mean of 4.4 habitat transitions occurred in the Phocoenidae family and half of them (55.83%) were from coasts to another habitat. The three most common transitions were from coasts to mixed (21.23%), coasts to offshore (21.23%), and coasts to rivers and bays (13.36%) (Online Resource 1, Table S4). A coastal ecology was the most probable ancestral state for Phocoenidae (rivers = 0.21, coasts = 0.55, mixed = 0.12, offshore = 0.11) (Fig. 2a). In Delphinidae, an average of 19.28 changes occurred and half of them (49.80%) were from offshore to another habitat. The most common habitat transitions were from offshore to mixed (18.83%), offshore to coasts (15.58%), offshore to rivers and bays (15.39%), and coasts to offshore (13.37%). The most probable ancestral state for the delphinid family was offshore habitat (rivers = 0.13, coasts = 0.10, mixed = 0.24, offshore = 0.53).

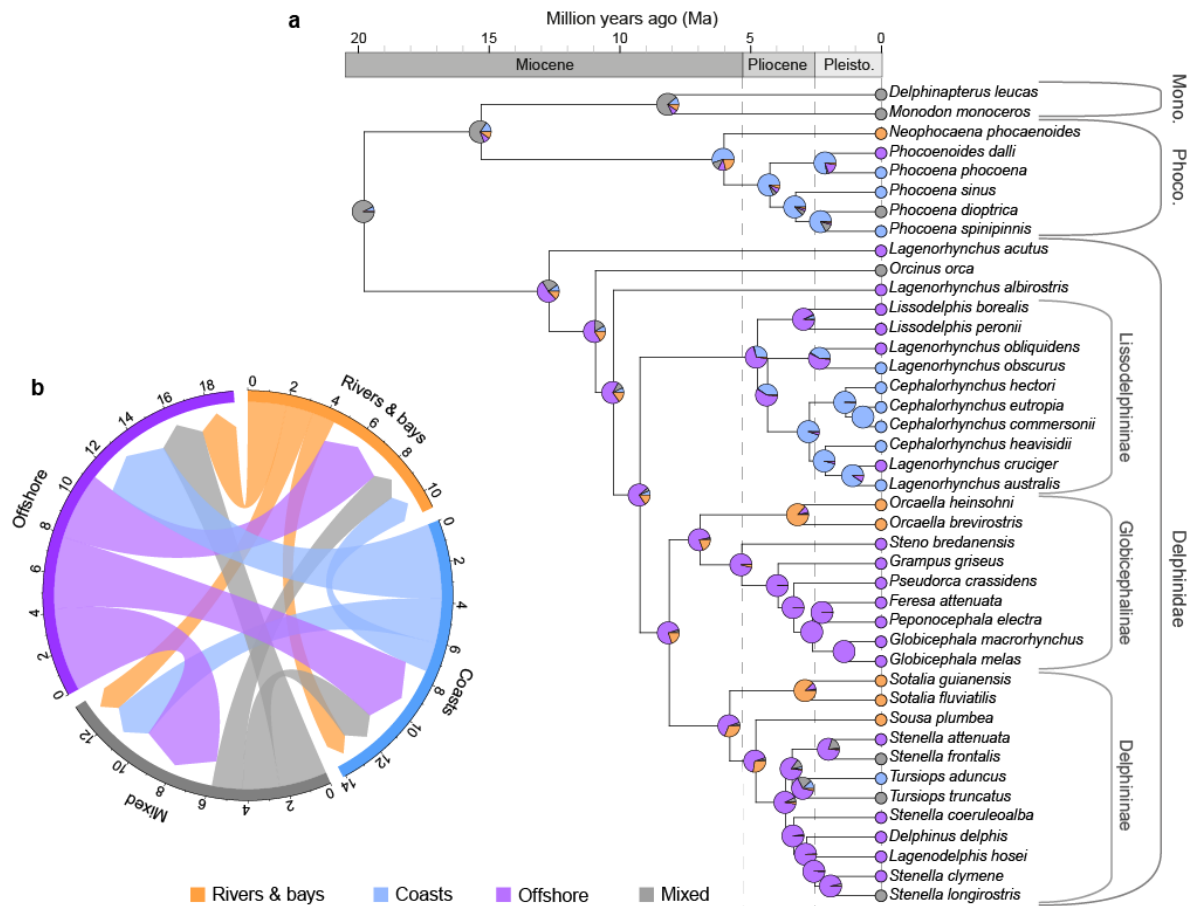
MORPHOSPACE OF VERTEBRAE

The two first PCs of the PCA account together for 57% of the total shape variation (Fig. 3). High values on PC1 correspond to more discoidal vertebral centra, narrower vertebral processes (neural spines, neural arches, and transverse processes) and smaller metapophyses. Positive PC2 values are associated with longer neural spines, and higher neural arches and metapophyses (Online Resource 1, Fig. S2 and Table S3). Species distribution on the morphospace follows a rivers-coasts-offshore ecological gradient along PC1 with riverine species having lower PC1 scores than offshore species.

This ecomorphological gradient along PC1 is found at different taxonomic levels. Delphinidae and Phocoenidae species inhabiting rivers and bays have comparable vertebral morphologies whereas offshore species of both families all possess rostrocaudally shortened vertebrae. Moreover, this tendency is also observed at the intraspecific level. Ecotypes of WNA *T. truncatus* are distributed in the same area of the morphospace occupied by Delphinidae in agreement with the ecomorphological partitioning observed at the interspecific level. The coastal ecotype has a similar morphology to other coastal delphinoids (low PC1 scores) while offshore ecotypes show shortened vertebrae as observed in offshore delphinoids (high PC1 scores). The visual exploration of the morphospace also reveals that the three delphinoids families occupy distinct regions of the morphospace.

Regular MANOVAs detected a significant effect of habitat on vertebral morphology in the entire clade of Delphinoidea ($Z = 8.47$, $P = 0.0001$), as well as within each family (Delphinidae: $Z = 7.49$, $P = 0.0001$, Phocoenidae: $Z = 5.56$, $P = 0.0001$). Pairwise comparisons between rivers, coasts and offshore environments were always significant and only mixed habitat was not always significantly different from other habitats (Online Resource 1, Table S5). Phylo-MANOVAs strengthened, at least partially, the habitat effect on vertebral morphology. Tests were both significant for Delphinoidea ($Z = 2.63$, $P = 0.005$) and Delphinidae ($Z = 2.53$, $P = 0.007$). Pairwise comparisons highlighted that offshore species are significantly different from riverine and coastal species (Online Resource 1, Table S5). While the regular MANOVA did not detect a significant difference between coastal and offshore WNA *T. truncatus* ecotypes ($Z = 1.72$, $P = 0.084$), ANOVAs found a significant difference on PC1 ($F = 11.79$, $P = 0.026$) and PC3 ($F = 37.25$, $P = 0.004$). The sensitivity analysis showed that the probability of finding significant differences among six WNA *T. truncatus* randomly sampled among a population where there is no significant effect of habitat was relatively poor (Online Resource 1, Supplementary Analysis II and Fig. S5). Non-phylogenetic and phylogenetic ANOVAs on individual PCs always highlighted a significant effect of habitat on vertebral shape for PC1 except for Phocoenidae when accounting for phylogenetic relationships (see Online Resource 1, Tables S6 and S7). Phylo-ANOVAs and phylo-MANOVAs on an incremental number of PCs highlighted that most of the ecomorphological signal is concentrated on PC1 (Online Resource 1, Table S8).

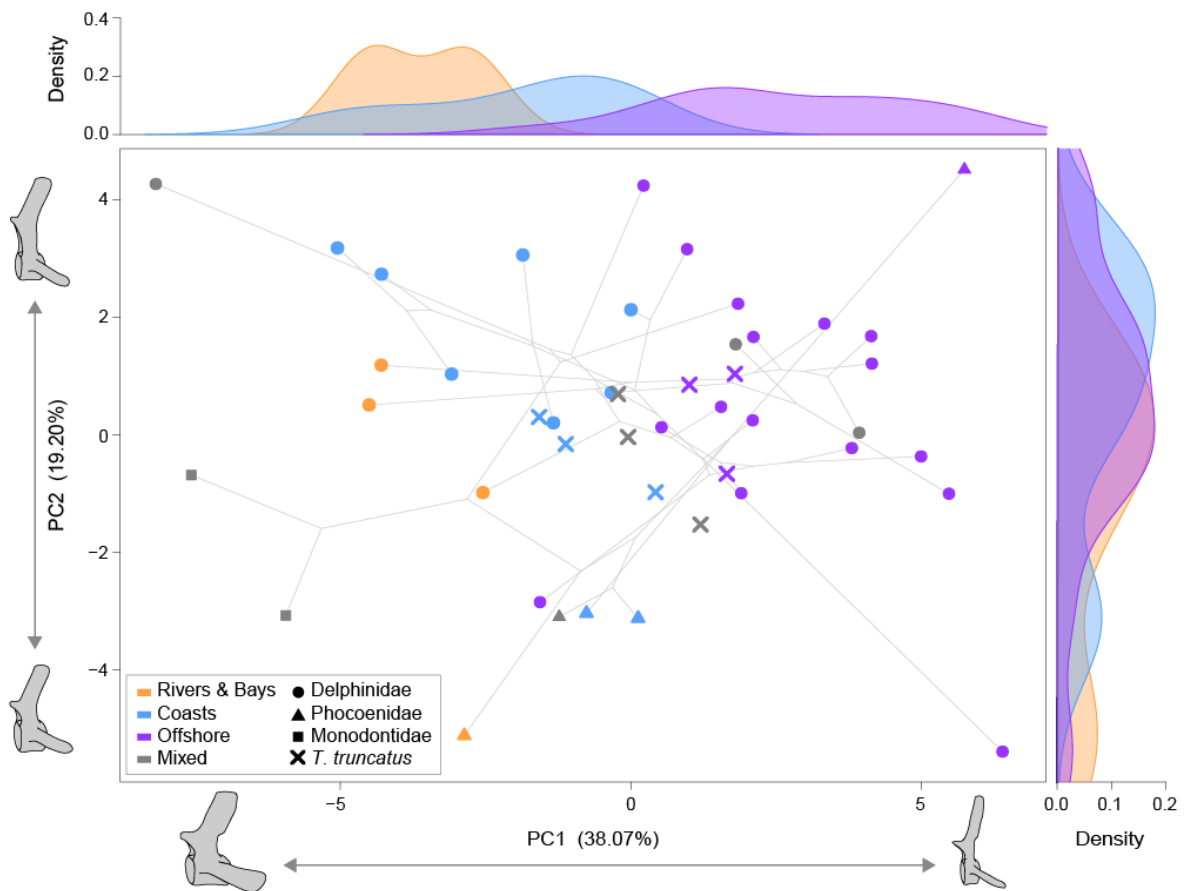
Fig. 2 *Habitat transitions during delphinoid evolutionary history* a. Ancestral ecological state reconstruction performed using an ER stochastic mapping plotted on delphinoid time-calibrated tree from McGowen et al. (2020). Posterior probabilities (based on 1,000 simulations) of each node state are indicated by pie charts. Mono: Monodontidae, Phoco: Phocoenidae; b. Estimated number of transitions from one habitat (base of the arrows) to another one (tip of the arrows).



VERTEBRAL SHAPE CONVERGENCE

Distance-based analyses of convergence found a significant convergence for each habitat category (Online Resource 1, Table S9). The highest level of convergence is achieved in riverine and coastal species with an average of 46% and 38% of convergence, respectively (rivers: $C1 = 0.456$, $P < 0.001$; coasts: $C1 = 0.375$, $P < 0.001$). This corresponds to 27% of the total evolution of riverine species ($C3 = 0.274$, $P < 0.001$) and 19% of the total evolution of coastal species ($C3 = 0.189$, $P < 0.001$). The level of convergence of each of the two groups corresponds to 1.6% and 1.3% of the total evolution of the entire delphinoid clade (river: $C4 = 0.016$, $P = 0.012$; coasts: $C4 = 0.013$, $P = 0.018$). Offshore species presented a lower level of convergence with an average of 21% ($C1 = 0.209$, $P < 0.001$) which corresponds to 14% of the total evolution of offshore species ($C3 = 0.135$, $P < 0.001$) and to 1.1% of the total evolution of delphinoids ($C4 = 0.011$, $P < 0.001$). Analyses of convergence using species subsampling found comparable results with slightly lower convergence levels for coastal species (Online Resource 1, Table S9).

Fig. 3 Phylomorphospace of delphinoid vertebral shape. Typical vertebral shapes corresponding to PC1 and PC2 minimal and maximal values are shown along each axis. Symbol shapes correspond to phylogenetic groups and symbol colours correspond to habitat categories. Density plots on top and right sides of the phylomorphospace show differences in morphology between riverine, coastal, and offshore species along PC1 and PC2, respectively



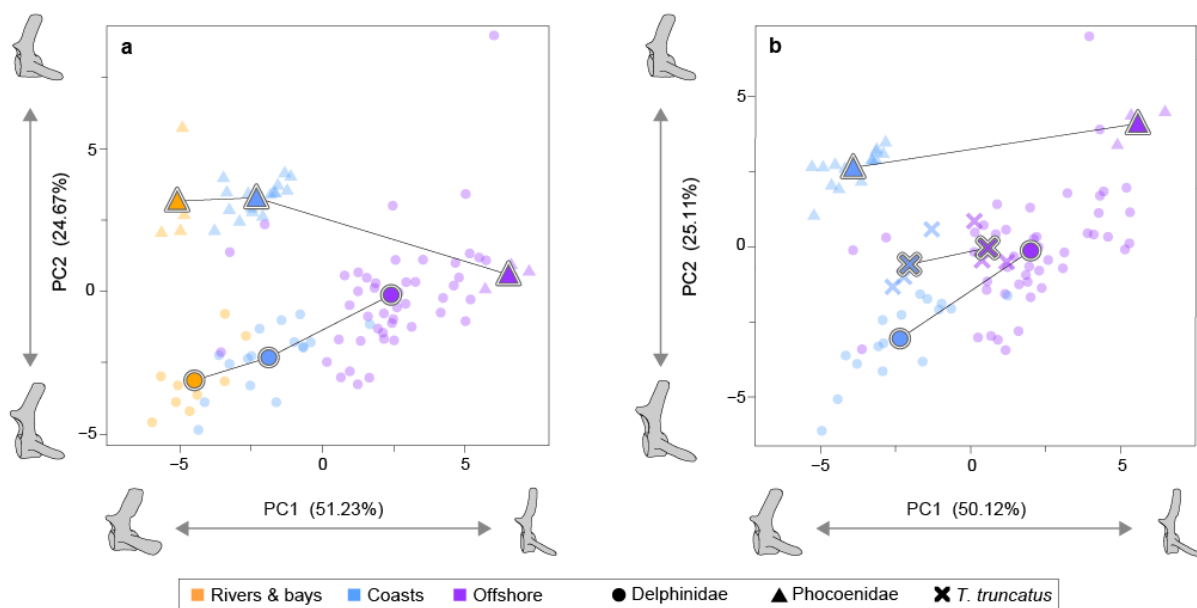
PHENOTYPIC TRAJECTORIES

On the PCA projection of the PTA for Delphinidae and Phocoenidae along the rivers-coasts-offshore gradient (Fig. 4a), riverine specimens have lower PC1 values than their offshore counterparts. It corresponds to more elongated vertebral centra and wider vertebral apophyses (neural processes, neural arches and transverse processes) in riverine species (Online Resource 1, Fig. S3). Phocoenidae have higher PC2 scores than Delphinidae, corresponding to lower neural arches and metapophyses and, to a lower extent, smaller metapophyses in the caudal region. The statistical comparison of trajectories highlighted a significant difference between the two families for their trajectory lengths (length difference (Δd) = 5.760, effect size (Z) = 2.531, P = 0.002), directions (angle difference ($\Delta\theta$) = 59.88°, Z = 2.847, P = 0.004), and their overall trajectory shapes (shape difference (ΔD) = 0.304, Z = 1.721, P = 0.040) (Online Resource 1, Table S10).

On the PCA projection of the coasts-offshore PTA of Delphinidae, Phocoenidae and WNA *T. truncatus* (Fig. 4b), offshore specimens have higher scores on PC1 corresponding to shorter vertebral centra and narrower vertebral apophyses (neural processes, neural arches, and transverse processes), mainly in the thoracic and lumbar regions (Online Resource 1, Fig. S3). Delphinidae and WNA *T. truncatus* ecotypes occupy similar regions of the morphospace and have lower PC2 scores

than Phocoenidae, which correspond to higher metapophyses, longer transverse processes in the thoracic region, and larger metapophyses in the caudal region. Within Phocoenidae and Delphinidae, coastal species also tended to have lower PC2 scores than offshore species of the same family. The pairwise comparisons of phenotypic trajectories demonstrated a significant difference between Delphinidae and Phocoenidae both in trajectory length ($\Delta d = 6.405$, $Z = 3.118$, $P < 0.001$) and direction ($\Delta\theta = 68.45^\circ$, $Z = 2.890$, $P = 0.001$). The PTA also highlighted a significant difference between Phocoenidae and WNA *T. truncatus* in trajectory length ($\Delta d = 8.874$, $Z = 3.212$, $P < 0.001$) but not in direction ($\Delta\theta = 53.27^\circ$, $Z = 0.952$, $P = 0.174$). Interestingly, there were no significant differences in trajectory length ($\Delta d = 2.469$, $Z = 0.893$, $P = 0.191$) and direction ($\Delta\theta = 47.63^\circ$, $Z = 1.089$, $P = 0.144$) between Delphinidae and WNA *T. truncatus* (Online Resource 1, Table S10).

Fig. 4 Ecomorphological trajectories. *a.* Morphospace and phenotypic trajectories of Phocoenidae and Delphinidae along the river-coast-offshore gradient; *b.* morphospace and phenotypic trajectories of Phocoenidae, Delphinidae and WNA *T. truncatus* along the coast-offshore gradient. Small symbols correspond to specimens. Large symbols connected by lines correspond to mean PC scores of each phylogenetic group and habitat category. Lines connecting large symbols represent the trajectory of each phylogenetic group along the ecological gradient



TEMPO OF EVOLUTION

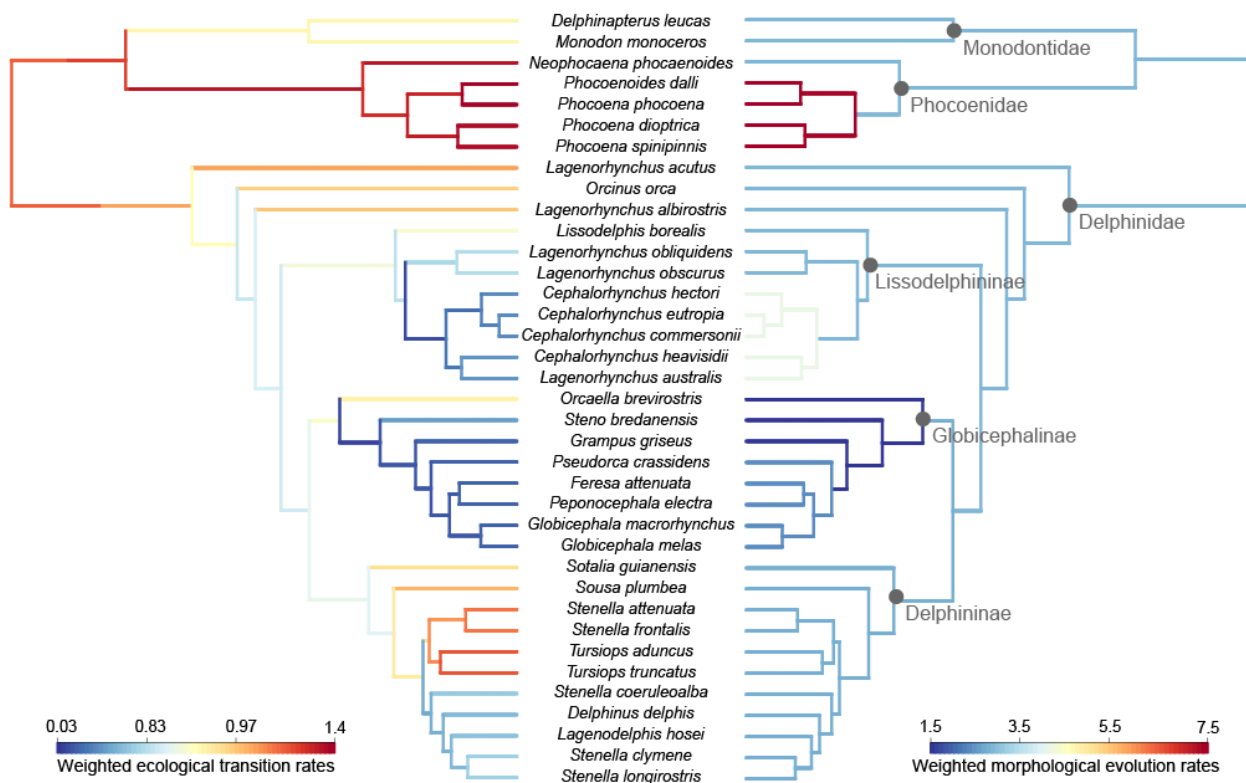
For ecological transition rates, 59,536 single and multirate models were fitted in total, each of them individually accounting for less than 0.5% of the total Akaike weight. The five best models comprise one three-partition and four five-partition models. Average weighted evolutionary rates highlight high transition rates in Phocoenidae, basal Delphinidae, and Delphininae and lower rates in Lissodelphinidae and Globicephalinae (Fig. 5).

Among the 9,109 single and multirate models fitted to estimate vertebral shape evolutionary rates based on the three first PCs, 7,419 models converged and were used to calculate the average weighted rates. The best model accounts for 92.66% of the total Akaike weight. This model highlighted higher evolutionary rates for Phocoenidae and all *Cephalorhynchus* species (Fig. 5). Despite the lower morphological evolutionary rates of Delphinidae compared to Phocoenidae, analyses of evolutionary rates for all cetaceans show that delphinoids have higher morphological evolutionary rates than other cetaceans (Online Resource 1, Fig. S4).

Discussion

Numerous transitions between riverine, coastal and offshore habitats occurred repeatedly and rapidly during delphinoid evolutionary history. Our quantification of vertebral shape demonstrates that vertebral morphology is strongly associated with habitat, regardless of the taxonomic level. Moreover, the occurrence of multiple independent habitat transitions resulted in moderate but significant vertebral shape convergence among species or ecomorphs living in similar habitats.

Fig. 5 Phylorates of delphinoids. Weighted evolutionary rates of habitat transitions (left) and vertebral shape (right) of delphinoids averaged from single and multirate models. Warm colours indicate high 1–3 from the PCA evolutionary rates while cool colours correspond to low evolutionary rates. Vertebral shape evolutionary rates were calculated using PCs



ECOMORPHOLOGY OF THE AXIAL SKELETON IN DELPHINOIDS

Within delphinoids, the principal direction of vertebral morphological variance (represented by PC1) correspond to a progressive rostrocaudal shortening of vertebrae following a gradual transition from nearshore to offshore habitats (Fig. 3). This general ecomorphological trend agrees with previous studies on cetacean vertebral morphology (see for instance, Buchholtz and Schur 2004; Marchesi et al. 2017, 2021; Gillet et al. 2019). The spool-shaped vertebral morphology of riverine species corresponds to increased backbone flexibility which is coherent with high manoeuvrability capacities needed in shallow and complex habitats. Conversely, offshore species have short vertebral centra and long and narrow vertebral processes, leading to increased backbone rigidity and efficient fast swimming (Long et al. 1997; Buchholtz 2001). Besides shortening of the vertebral centrum and processes along the rostrocaudal axis, the PTA showed at a finer level that, within Delphinidae and Phocoenidae, pelagic species tend to have metapophyses located lower above the vertebral centrum along the entire backbone, smaller metapophyses in the caudal region and shorter transverse processes in the thoracic region. Previous studies on two dolphins (*Lagenorhynchus cruciger* and *L. australis*) and on five porpoises found relatively similar results (Marchesi et al. 2017 and Marchesi et al. 2021, respectively). However, these studies highlighted that delphinid metapophyses were lower in the offshore species only in the second half of the caudal region but were higher in the anterior caudal region. Metapophyses were also smaller in the offshore species but only in the lumbar region, while they were larger in the caudal region. Surprisingly, transverse processes were longer in the pelagic *L. cruciger*, in opposition to our results. In porpoises, most results followed the same trend, except transverse processes that were shorter for offshore species only in the posterior thoracic but longer in the anterior thoracic region. These subtle differences could reflect finer morphological variation among some closely related species, while our study highlights more general trends observed at a broader taxonomic level.

The vertebral morphology of WNA *T. truncatus* clearly differs between coastal and offshore ecotypes as highlighted by the ANOVAs and the PCA (Fig. 3 and Online Resource 1, Table S5). Moreover, the position of coastal and offshore ecotypes falls within the same subspaces of morphospace occupied by coastal and offshore delphinids. The similarity of the phenotypic trajectory directions between WNA *T. truncatus* and Delphinidae along the coastal-offshore transition further highlights the similarity of their ecomorphological trajectories (Fig. 4). Consequently, offshore WNA *T. truncatus* have more discoidal centra and narrower apophyses than coastal individuals. While specimen availability and preservation state prevented us from collecting data on a larger sample size for *T. truncatus*, the sensitivity analysis shows that chances of finding such results on a few specimens sampled from a larger simulated population where there is no actual difference between coastal and offshore ecotypes are fairly low (Online Resource 1, Supplementary Analysis II). Moreover, our results are in accordance with vertebral shape modifications identified on a large number of offshore and coastal ecotypes from the Southwestern Atlantic (Costa et al. 2016). These substantial vertebral modifications are also coherent with previous studies that showed divergences between WNA ecotypes based on genetics, ecology, as well as external and skull morphology (Mead and Potter 1995; Hoelzel et al. 1998; Torres et al. 2003; Natoli et al. 2004; Moura et al. 2013). Furthermore, lengths of the phenotypic trajectory in the morphospace of WNA *T. truncatus* and delphinids are equivalent,

suggesting that morphological modifications at the intraspecific level are as important as at the family level. These results support the previously suggested hypothesis of incipient speciation between the two WNA *T. truncatus* ecotypes (Hoelzel et al. [1998](#); Moura et al. [2013](#)). While coastal and offshore ecotypes have also been described in other delphinids such as common dolphins (*D. delphis*), spotted dolphins (*S. attenuata*), and spinner dolphins (*S. longirostris*), these ecological differences were mainly identified based on molecular data, body colour pattern, and/or skull shape (Perrin et al. [1999](#); Natoli et al. [2006](#); Amaral et al. [2012](#); Andrews et al. [2013](#); Segura-García et al. [2016](#); Leslie and Morin [2018](#)). Investigating the axial skeleton of these species at the intraspecific level would allow to test whether the morphological adaptation of their backbone follows the same ecomorphological pattern observed in WNA *T. truncatus* and the whole delphinoid clade.

MORPHOLOGICAL DIFFERENCES AMONG DELPHINOID FAMILIES

While delphinoids generally exhibit a gradual transition from spool-shaped to disk-shaped vertebrae along the rivers-coasts-offshore ecological gradient, the three families occupy distinct areas of the morphospace (Figs. [3](#) and [4](#)). Monodontidae differs from Delphinidae (except killer whales, *Orcinus orca*) and Phocoenidae by having more spool-shaped vertebrae which could provide greater vertebral flexibility (Long et al. [1997](#); Buchholtz [2001](#)). This morphology could be associated with their specialized ecology requiring manoeuvrability to swim in shallow waters, feed on benthic preys, and/or swimming close to sea ice (Heide-Jørgensen [2018](#); O’Corry-Crowe [2018](#)). Conversely to Monodontidae, Phocoenidae and Delphinidae diversified in various habitats (rivers, coasts, offshore). In both families, evolutionary transitions between habitats correspond to vertebral shape changes and species living in the same habitat show morphological convergence (Figs. [3](#) and [4](#) and Online Resource 1, Tables S5 and S9).

Despite Phocoenidae and Delphinidae having diversified into similar habitats following the same general morphological trend (i.e., vertebral shortening in offshore waters), a thorough analysis of the backbone shows differences in morphospace occupation and ecomorphological trajectories (Figs. [3](#) and [4](#)). Coastal and riverine phocoenids differ from delphinids by having shorter neural arches and spines as well as lower metapophyses. Conversely, offshore phocoenids (Dall's porpoises, *Phocoenoides dalli*) tend to have longer neural arches and spines and higher metapophyses than most delphinids. Furthermore, the rostrocaudal shortening of vertebral centra and narrowing of vertebral apophyses along the coastal-offshore transition are largely greater in porpoises than in delphinids, as illustrated by the PTA (Fig. [4](#)). Dall's porpoises have a remarkably specialised vertebral column, as they possess the highest vertebral count of any cetaceans, with 97 vertebrae for an average body length of 2 m (Amano and Miyazaki [1996](#); Buchholtz et al. [2005](#); Gillet et al. [2019](#)). This morphology could be related to the highly active and fast-swimming behaviour of this small-sized species and could reflect a peculiar exploitation of resources (Ridgway and Johnston [1966](#); Law and Blake [1994](#)). The two principal dorsal muscles involved in dorsal bending (i.e., the musculus multifidus and the m. longissimus) insert on metapophyses and the distal extremity of neural spines (Slijper [1936](#); Pabst [1990](#)). Hence, the lower metapophyses and shorter neural spines of coastal and riverine phocoenids imply that the lever arm of their dorsal muscles is smaller than

delphinids. In contrast, the vertebral morphology of offshore porpoises indicates a stiffer backbone with larger lever arm than most delphinids. These morphological differences probably impact backbone biomechanics and could denote differences in swimming abilities between Phocoenidae and Delphinidae.

Besides biomechanical implications, morphological divergences between Phocoenidae and Delphinidae could also be explained by different evolutionary history. Based on our analyses, the MRCA of crown phocoenids probably lived in coastal waters (Fig. 2). Morphological investigations of the inner ear anatomy of extinct and extant phocoenids suggest that stem porpoises colonized both coastal and offshore environments, implying that ecological transitions already occurred early in the evolution of porpoises (Racicot et al. 2016). However, the uncertainty of stem porpoise phylogeny prevents us from drawing any solid conclusion on the ancestral state of crown porpoises. Some studies have identified *Semirostrum ceruttii*, a supposedly coastal species, as the sister-clade to crown porpoises (Racicot et al. 2014; Tanaka and Ichishima 2016), while others have identified *Piscolithax longirostris* and *P. tedfordi* as the closest relatives of crown porpoises (Colpaert et al. 2015; Lloyd and Slater 2021), the latter one being considered as a pelagic species based on its inner ear morphology despite having been found in coastal deposits (Racicot et al. 2016). Both scenarios nonetheless imply parallel, independent transitions between coastal and offshore environments in the two main clades of extant phocoenids. The coastal origin of crown Phocoenidae suggests that *Phocoenoides dalli* and *Phocoena dioptrica* have independently colonized more offshore waters in the Northern and Southern Hemispheres, respectively (Chehida et al. 2020). The parallel colonization of offshore environments by these two species was accompanied by convergence in external body coloration (i.e., strong countershading) and skull morphology (Galatius et al. 2011; Chehida et al. 2020). However, *P. dioptrica* retains a vertebral morphology more similar to coastal Phocoenidae (*Phocoena spinipinnis* and *Phocoena phocoena*) than to *P. dalli*, maybe in relation with its ability to swim in shallow waters as sightings have also been reported in coastal and estuarine environments (Goodall and Schiavini 1995; Goodall and Brownell 2018).

Conversely to Phocoenidae, our results suggest that the MRCA of crown delphinids and MRCA of each delphinid subfamily (Lissodelphininae, Globicephalinae, and Delphininae) likely lived in offshore environments (Fig. 2). Unfortunately, the extremely scarce fossil record of stem Delphinidae, with only two species currently described (Murakami et al. 2014; Kimura and Hasegawa 2020), and the absence of ecological investigation on these species preclude any comparison of our results with the fossil record. Assuming that offshore ecology is associated with long distance travels, these results would be consistent with the hypothesis of large-scale dispersal of early delphinids during the Miocene (Banguera-Hinestroza et al. 2014). More recent delphinids would have subsequently adapted to more coastal waters. Phocoenidae and Delphinidae thus radiated in similar environments but from different ecomorphological ancestral states. Environmental constraints drove the overall direction of morphological adaptation but ancestral state constrained the fine-scale orientation of these modifications, resulting in slightly different strategies. In addition, differences in morphospace occupation between the two families might also reflect fine-scale habitat partitioning between sympatric species. For instance, morphological variation could be linked to differences in prey size range or subtle differences in spatial distribution associated with

water depth or salinity (Bearzi [2005](#); Spitz et al. [2006](#); Smith et al. [2008](#)). The relationship between these ecological factors and vertebral morphology is not established yet but clearly possible, similarly to the demonstrated link between cranial shape variation and exploitation of different dietary resources between porpoises and dolphins (McCurry et al. [2017](#)).

PATTERNS OF ECOLOGICAL DIVERSIFICATION IN DELPHINOIDS

Ecological transitions between the open sea and shallower habitat seem to be iterative in delphinoids. At the intraspecific level, population partitioning based on habitat preference has been reported for multiple Delphinidae species, including *T. truncatus*, *Delphinus* spp., *Stenella longirostris*, and *Stenella attenuata* (Perrin et al. [1999](#); Natoli et al. [2006](#); Amaral et al. [2012](#); Andrews et al. [2013](#); Segura-García et al. [2016](#); Leslie and Morin [2018](#)). In bottlenose (*Tursiops* spp.) and common (*Delphinus* spp.) dolphins, coastal populations are thought to have emerged from globally distributed offshore populations through multiple independent founder events (Natoli et al. [2004](#), [2006](#); Moura et al. [2013](#)). At the interspecific level, speciation through ecological specialisation between coastal and offshore waters has been proposed for the sister species *Tursiops aduncus* and *T. truncatus* as well as for two pairs of *Lagenorhynchus* species in the Delphinidae family (Moura et al. [2013](#); Banguera-Hinestroza et al. [2014](#); Galatius and Goodall [2016](#)). In addition, parallel ecological speciation has been recently proposed for two pairs of Phocoenidae: *P. phocoena* and *P. dalli* in the Northern Hemisphere and *P. spinipinnis* and *P. dioptrica* in the Southern Hemisphere (Chehida et al. [2020](#)). The numerous ecological shifts retrieved by our stochastic mapping are consistent with these hypotheses and suggest that habitat specialisation played a role in the diversification history of the whole delphinoid clade (Fig. [2](#)). Repeated independent transitions between nearshore/benthic and offshore/pelagic ecologies appear to be a recurrent process in aquatic environments and have been reported for various marine taxa such as ammonites, cephalopods, ophiuroids, fishes, and sirenians (Bayer and McGhee [1984](#); Lindgren et al. [2012](#); Velez-Juarbe et al. [2012](#); Frédéricich et al. [2013](#); Bribiesca- Contreras et al. [2017](#)).

The similarity of ecological specialisation and vertebral shape modification patterns at the intra- and interspecific levels (Figs. [3](#) and [4](#)) suggests continuity between evolutionary processes occurring below and above the species level. It also implies that ecomorphological changes should occur rapidly at the macroevolutionary level. In general, delphinoids have higher ecological and morphological evolutionary rates than other cetaceans indicating that ecomorphological transitions occur faster in this group. Additionally, we demonstrated a discrepancy between the numerous habitat transitions occurring at high evolutionary rates in Phocoenidae and Delphinidae and their tempo of morphological evolution. The associated morphological modifications of the backbone only occur at high rates in Phocoenidae while rates are lower in most Delphinidae subfamilies. Delphinoids, and more specifically extant delphinoids, diversified relatively recently with a substantial increase in diversity within porpoises and all delphinid subfamilies during the Pliocene (Bianucci [2013](#)). This clade could then provide an empirical example in which macroevolutionary patterns still reflect microevolutionary patterns in contrast to older clades in

which large-scale and/or sudden events might have occurred leading to discrepancies between micro- and macroevolutionary processes (Erwin [2000](#); Uyeda et al. [2011](#)).

CONCLUSION

Ecological shifts between onshore and offshore habitats played a key role for delphinoid diversification. The iterative pattern of habitat transitions in delphinoids is associated with repeated invasion of similar areas of the morphospace, ultimately leading to vertebral shape convergence. The general mode of morphological evolution along the nearshore-offshore ecological gradient is similar in Phocoenidae and Delphinidae, supporting the idea that environmental constraints act in the same way for both clades. However, both families exhibit fine scale differences in evolutionary trajectory and the tempo of ecomorphological evolution differs between the two families, suggesting a decoupling of habitat transition and morphological evolution rates. The recurrent ecomorphological patterns observed in delphinoids could be due to developmental, genetic, biomechanical, or physiological constraints that can limit evolvability or, at least, drive morphological evolution in a specific direction associated with a combination of traits that are more readily evolvable (Sidlauskas [2008](#); Felice et al. [2018](#)). Additional studies are needed to investigate the factors underlying vertebral convergence in delphinoids.

In addition to habitat transitions observed at the macroevolutionary level, coastal-offshore ecotypes segregation observed in WNA *T. truncatus* reflects the general pattern of increased delphinoid disparity. By comparing the ecomorphological variations observed at the intra- and interspecific levels, we provide additional support to the hypothesis that coastal and offshore ecotypes described in several delphinoid species reflect ecological speciation processes. Beyond an illustration of similar ecomorphological patterns at the micro- and macroevolutionary levels, our comparative approach shows how morphology can iteratively evolve at different evolutionary scales, suggesting that ecomorphological patterns currently observed at the intraspecific level might reflect macroevolutionary processes that contributed to diversification.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10914-022-09615-7>.

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Authors' Contributions Amandine Gillet, Bruno Frédéricich, and Eric Parmentier; conceived the project. Amandine Gillet collected and analyzed data with input from Bruno Frédéricich and Stephanie E Pierce. The first draft of the manuscript was written by Amandine Gillet and all authors reviewed and edited it.

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Data Availability The datasets generated during and/or analysed during the current study are available in the Dryad repository, <https://doi.org/10.5061/dryad.7dj6850>.

Declarations

Competing Interests The authors declare they have no competing interest.

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