

Convergent evolution of olfactory and thermoregulatory capacities in small amphibious mammals

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Olfaction and thermoregulation are key functions for mammals. The former is critical to feeding, mating, and predator avoidance behaviors, while the latter is essential for homeothermy. Aquatic and amphibious mammals face olfactory and thermoregulatory challenges not generally encountered by terrestrial species. In mammals, the nasal cavity houses a bony system supporting soft tissues and sensory organs implicated in either olfactory or thermoregulatory functions. It is hypothesized that to cope with aquatic environments, amphibious mammals have expanded their thermoregulatory capacity at the expense of their olfactory system. We investigated the evolutionary history of this potential trade-off using a comparative dataset of three-dimensional (3D) CT scans of 189 skulls, capturing 17 independent transitions from a strictly terrestrial to an amphibious lifestyle across small mammals (Afrosoricida, Eulipotyphla, and Rodentia). We identified rapid and repeated loss of olfactory capacities synchronously associated with gains in thermoregulatory capacity in amphibious taxa sampled from across mammalian phylogenetic diversity. Evolutionary models further reveal that these convergences result from faster rates of turbinal bone evolution and release of selective constraints on the thermoregulatory-olfaction trade-off in amphibious species. Lastly, we demonstrated that traits related to vital functions evolved faster to the optimum compared to traits that are not related to vital functions.

olfaction | thermoregulation | heat loss | aquatic habitat | turbinal bones

The adaptive radiation of mammals is characterized by the colonization of a variety of habitats in association with morphological innovations (1, 2). Among the most spectacular patterns of mammalian evolution is the multiple invasions of aquatic habitats (3). Several mammalian lineages, including the ancestors of whales and manatees, became fully aquatic (3), whereas several groups of rodents, afrotherians, carnivorans, and others evolved an amphibious lifestyle. These amphibious mammals are adapted to live both in water and on land, a circumstance that is predicted to lead to evolutionary trade-offs (3). For instance, aquatic habitats are a challenge to mammalian thermoregulation because warm organisms lose heat quicker in water than in air due to the high thermal inertia of water (4, 5). Similarly, olfaction is particularly inefficient underwater because it requires inhalation (3, 6).

In mammals, the rostrum contains bony structures named turbinates that contribute to heat conservation and olfaction (7). Anteriorly, the respiratory turbinates are lined with a vascular epithelium that helps conserve heat during respiration (7). Posteriorly, the olfactory turbinates are covered by olfactory receptors and connected to the olfactory bulb, representing a critical component of mammalian olfaction (7–9). This anteroposterior functional

partitioning has been documented in histological, airflow dynamic, and performance test studies (9–13). It was previously hypothesized that the number and the shape of turbinal bones are conserved across species while their relative size and complexity are more labile, with variation related to species ecology (14–23). For example, dietary specializations are correlated with relative turbinal surface area in some Carnivora and Rodentia (19, 22).

Important functions such as thermoregulation should be under strong selective pressure in amphibious organisms. Indeed, Van Valkenburgh et al. (16) demonstrated that some aquatic Carnivora have huge respiratory turbinal bones that limit heat loss. In contrast, because mammals usually do not smell underwater (6), olfaction should be under relaxed selective pressures. It was previously shown that some amphibious mammals have a reduced

Significance

In the evolutionary history of mammals, invasion of aquatic habitats is associated with profound morphological changes. Because mammalian systems of olfaction and thermoregulation are challenged by aquatic environments, it was previously hypothesized that amphibious mammals have reduced olfactory capacity but enhanced thermoregulatory capacity. Using newly acquired three-dimensional (3D) computed tomography (CT) scans of nasal cavities from terrestrial and amphibious mammals, we found strong statistical support for this hypothesis. Our results show a strong trade-off between olfactory and thermoregulatory capacities in amphibious mammals, with morphological changes that occurred 5.4 times faster than the background rate. The rapid rate of morphological change and convergent patterns we identified demonstrate the adaptation experienced by mammals during the many transitions to amphibious habits.

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The authors declare no competing interest.

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olfactory bulb and cribriform plate, two components of olfaction, compared to their terrestrial relatives (24–26). Aquatic vertebrates also have a smaller repertoire of functional olfactory receptor (OR) genes than terrestrial vertebrates (27–32). Nevertheless, how pervasive, consistent, and strong these putative convergences and trade-offs are remains unknown.

We analyzed turbinal morphology in amphibious mammals to test for consistent anatomical adaptations enhancing heat conservation and for simultaneous release from selective pressures on olfactory structures. In total, we compared 17 independently derived amphibious lineages to their close terrestrial relatives in order to illuminate the evolution of thermoregulatory-olfactory trade-offs during major mammalian land-to-water transitions.

Results

Adaptation and Convergence. The relative surface area of olfactory and respiratory turbinals is significantly associated with ecological

lifestyle ($P < 0.0001$ in both cases; *SI Appendix, Table S1*). Most amphibious species have reduced olfactory turbinals and expanded respiratory turbinals as compared to their close terrestrial relatives (Fig. 1). The relative reduction of olfactory surface area is affected by the relative reduction of some olfactory turbinals (Figs. 1 and 2) and by the loss of other olfactory turbinals, as seen in *Myocastor coypus*, which lost two frontoturbinals (Fig. 24). The relative increase of respiratory surface area is driven by a relative expansion of the size of respiratory turbinals (Figs. 1 and 2), an increase in complexity (Fig. 2B), and the emergence of a new respiratory turbinal (Fig. 24).

The best-fitted model of morphological evolution for the relative surface area of respiratory turbinals is based on an Ornstein-Uhlenbeck process (OUM) that describes the evolution toward distinct optimal values for species with terrestrial and amphibious lifestyles (Table 1). This is also the case for size-corrected estimates of the relative surface area of the respiratory turbinals

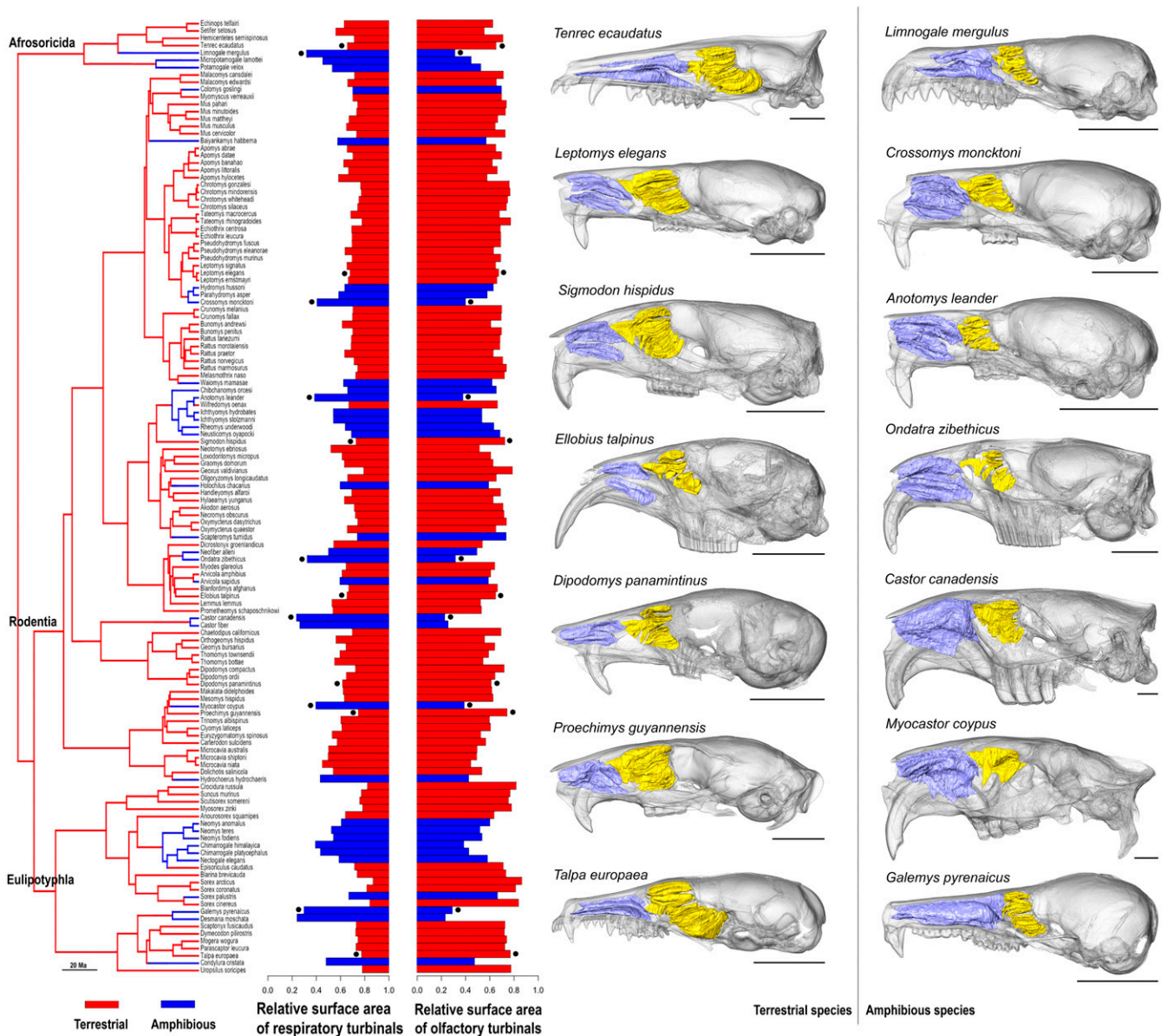


Fig. 1. Loss of olfactory and gain of thermoregulatory capacities in amphibious mammals. Phylogeny of the sampled species with barplots of the relative surface area of olfactory and respiratory turbinals based on ratios; blue = amphibious; red = terrestrial. Black circles highlight illustrated species. Respiratory turbinals are blue and olfactory turbinals are yellow. (Scale bars, 1 cm.)

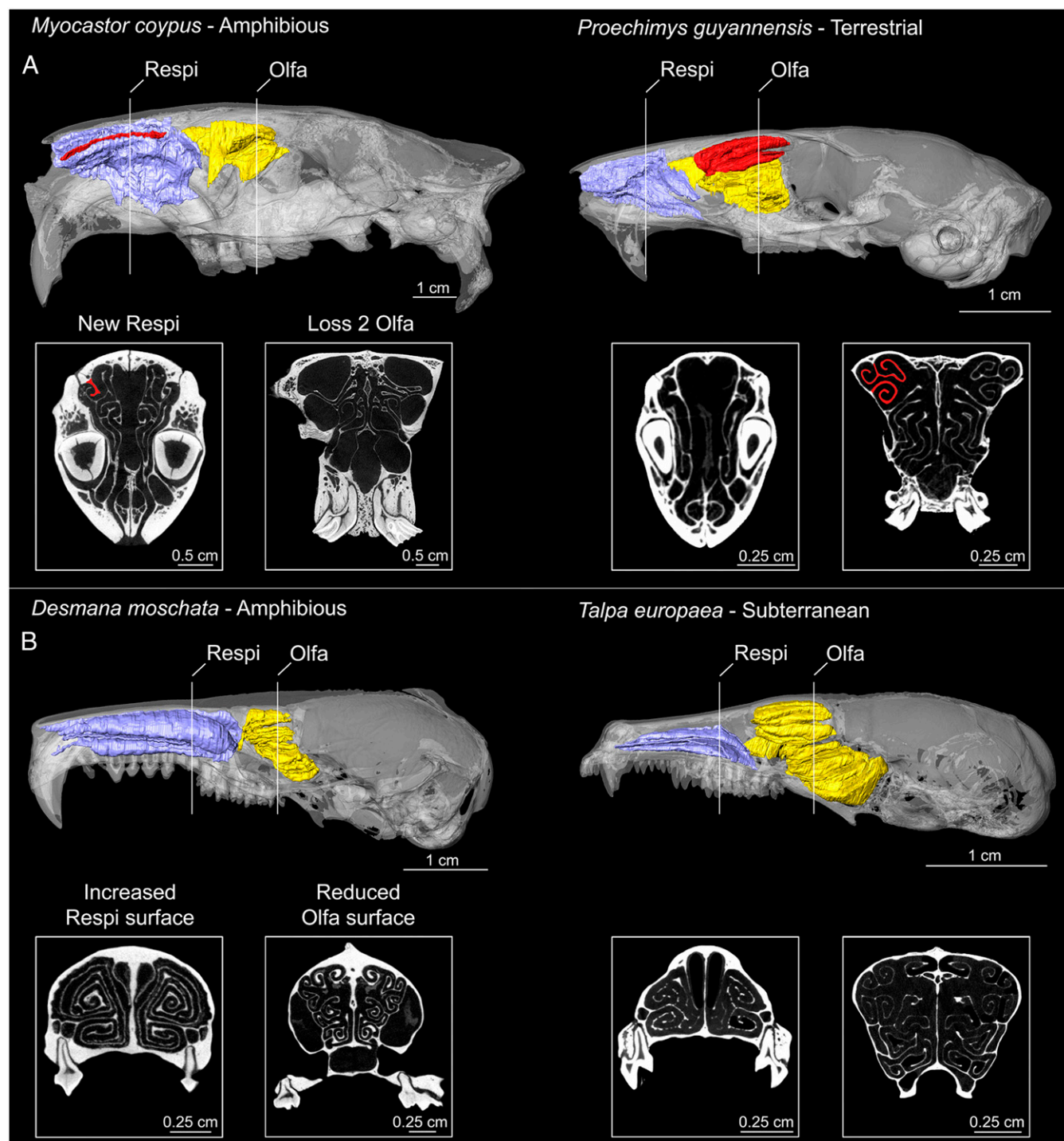


Fig. 2. Loss and gain of anatomical structures. 3D representations of turbinal bones and coronal cross section showing two mechanisms of adaptation to the amphibious environment: (A) emergence of new respiratory turbinals and loss of some olfactory turbinals as seen in the amphibious *Myocastor coypus* and its close terrestrial relative *Proechimys guyannensis*. (B) Increase in surface area and complexity of respiratory turbinals and reduction in olfactory turbinals, as seen in the amphibious *Desmana moschata* and its close terrestrial relative *Talpa europaea*. Respi = respiratory turbinals, Olfa = olfactory turbinals. Red = turbinals not shared between amphibious and terrestrial relatives; blue = respiratory turbinals; yellow = olfactory turbinals.

(SI Appendix, Table S2), indicating that this pattern is not driven by allometric effects. In contrast, the best model for the evolution of the relative surface area of the olfactory turbinals is a Brownian motion model with multirate and multiselective regimes (BMMm), which illustrates mean phenotype and evolutionary rate differences between amphibious and terrestrial lineages (Table 1). The estimated

rates of the BMMm model further show that the amphibious lineages were evolving faster than their terrestrial relatives (see below). The second-best-fitted model (but also the best model for the size-corrected relative surface area of olfactory turbinals; SI Appendix, Table S2) is an ecological release (ER) model consistent with a release of selective pressures on olfactory turbinals associated

Table 1. Mean results from models of turbinal bone evolution fitted to 100 stochastic character maps of amphibious and terrestrial lifestyles

Model	Relative olfactory turbinal surface area		Relative respiratory turbinal surface area		Bivariate model: relative olfactory and respiratory surface area	
	AIC	AICw	AIC	AICw	AIC	AICw
BM1	−60.353	0.000	30.554	0.000	−273.975	0
BMM	−104.670	0.000	23.697	0.000	−367.927	0
BM1m	−96.136	0.000	−4.240	0.286	−309.068	0
BMMm	−124.436	0.980	−3.504	0.192	−395.461	1
OU1	−66.957	0.000	20.143	0.000	−289.787	0
OUM	−99.446	0.005	−5.390	0.501	−319.622	0
ER	−103.035	0.015	7.958	0.020	−351.664	0

Model fits were compared using differences in the AIC. See [SI Appendix, Table S2](#) for size-free results.

with colonization of the aquatic environment (Table 1). This scenario is also supported by bivariate models of correlated evolution between the relative surface area of respiratory and olfactory turbinals that favor the BMMm and the ER model (Table 1). These models show a strong negative association between the respiratory and olfactory turbinal surface area, typical of evolutionary trade-offs (average correlation of -0.92) and favoring a scenario with release from selection on this trade-off in amphibious mammals.

Convergence in the relative surface area of olfactory and respiratory turbinals in amphibious taxa is supported by three of four of Stayton's (33) convergence indices (C1: $P = 0.003$, C2: 0.001 , C3: $P = 0.005$, and C4: $P = 0.188$; [SI Appendix, Table S3](#)). Phenograms for the relative surface area of olfactory and respiratory turbinals show convergences in most clades, with amphibious species evolving toward a lower relative surface area of olfactory turbinals (Fig. 3, *Left*) and a greater relative surface area of respiratory turbinals (Fig. 3, *Right*).

Phylogenetic Half-Life and Evolutionary Rates. The phylogenetic half-life of the total turbinal surface area and that of the relative surface area of olfactory and respiratory turbinals were estimated under the best-fitted OU models (respectively, 0.88 , 0.15 , and 0.18 ; [SI Appendix, Table S4](#)). They are all lower than that of skull length (1.41 ; [SI Appendix, Table S4](#)), indicating that the turbinal bone surface area evolved faster than skull length, a body-size-related trait that is itself associated with features of species ecology.

The evolutionary rate of the relative surface area of olfactory turbinals is 5.4 times faster in amphibious species than in terrestrial ones (likelihood ratio test [LRT]: $P < 0.001$; Table 2 and [SI Appendix, Tables S5 and S6](#)). The evolutionary rate of the relative surface area of respiratory turbinals is 1.4 times faster in amphibious species as compared to terrestrial ones (LRT: $P < 0.001$; Table 2 and [SI Appendix, Tables S5 and S6](#)).

Discussion

Convergent Olfactory Losses in Small Amphibious Mammals. Olfaction is a key function for mammals which was hypothesized to be under strong selective pressure (16, 17). Mammals usually do not smell underwater (6), suggesting that olfaction may be less important to amphibious species than to their terrestrial relatives. Our results show that amphibious mammals adapted to the aquatic environment through at least two types of morphological changes in their olfactory system: 1) the reduction of the relative surface area of the olfactory turbinals and 2) the loss of some olfactory turbinals (Fig. 2).

Using turbinal bones and phylogenetic comparative methods, we report that 17 lineages of small amphibious mammals

convergently experienced a reduction of their olfactory turbinal bones (Figs. 1 and 3), suggesting relaxed selective pressures on olfactory anatomical structures in amphibious placentals. We found reduced olfactory turbinals in all three studied orders (Afrosoricida, Eulipotyphla, and Rodentia; Fig. 1). In Afrosoricida, the amphibious *Microgale mergulus* has less than half the olfactory turbinal surface area of its terrestrial counterpart, *Tenrec ecaudatus* (Fig. 1). The largest quantitative differences between an amphibious lineage and its close terrestrial relatives are found within the Talpidae (Eulipotyphla). Both amphibious desman species (*Desmana* and *Galemys*) have, respectively, less than a third and less than half of the relative olfactory turbinal surface area compared to the subterranean mole *Talpa europaea* (Figs. 1 and 2B). This is somewhat surprising given that some Eulipotyphla, such as the star-nosed mole (*Condylura cristata*), water shrews (*Sorex palustris*), and the Russian desman (*Desmana moschata*), are known to sniff and smell underwater (34–36). However, the large difference may be partially related to the earthworm dietary specializations of subterranean moles. In rodents, earthworm specialists have significantly larger and more complex olfactory turbinals than do carnivores and omnivores (22). A similar pattern of olfactory turbinal reduction was also found in Rodentia. For example, the amphibious North American beaver (*Castor canadensis*) has less than half the olfactory turbinal surface area of its close terrestrial relative, the panamint kangaroo rat (*Dipodomys panamintinus*, Fig. 1).

Most small mammals in our sampling have relatively conserved turbinal morphology, consisting of a set of six to eight olfactory turbinals and two to three respiratory turbinals (Fig. 1 and [SI Appendix, Fig. S1](#)). The amphibious coypu (*Myocastor coypus*) is an exception. This species lost two olfactory turbinals, and we showed the presence of a new respiratory turbinal bone not seen in its close terrestrial relatives such as *Proechimys guyannensis* (Fig. 2A). The relative surface area of the olfactory turbinals in the coypu also decreased, and this species has about half of the relative surface area of olfactory turbinals measured in the terrestrial *Proechimys guyannensis* (Fig. 1). Further studies should assess the role and significance of both the reduction of the surface area of the olfactory turbinals and the loss of some olfactory turbinals (Fig. 2).

Our results are consistent with those of studies on the olfactory bulb brain, another major component of olfaction. Indeed, the olfactory bulb of some amphibious mammals is smaller compared to their terrestrial relatives (24). This organ is involved in detection and discrimination of odor molecules (37). Our results are also consistent with histological studies in eulipotyphlan water shrews. *Neomys fodiens* and *Sorex palustris* water shrews have a lower relative number of olfactory receptors than their closely related terrestrial species (38), and we quantified that these two species also have reduced olfactory turbinals (Fig. 1). Our observation of repeated reduction of olfactory surface area is also consistent with convergent enrichment in pseudogenes as well as reduction of the number of functional OR genes in amphibious and aquatic vertebrate genomes (26–31, 39). We demonstrated that small amphibious mammals convergently lost a part of their olfactory capacities. Altogether, our observations suggest that olfactory turbinal bones can be used as reliable proxies for olfactory capacities in mammals and used to infer that the ecology of fossil mammals provides critical information on the timing and onset of aquatic transitions.

Efficient Heat Conservation Capacities in Small Amphibious Mammals. Olfactory turbinal bone reduction might result from a trade-off between the sizes of the respiratory and olfactory turbinals (16, 22). We found strong support for this hypothesis, indicated by the negative association between the respiratory and olfactory turbinals (see *Results*) for the ecological release model.

Respiratory turbinal bones are essential to moisten and warm the air before it enters the lungs (7, 40). We showed that small amphibious species convergently evolved larger respiratory turbinals

monk seal (*Monachus tropicalis*), two species existing different thermal environments (16). However, the importance of temperature in the relative size of turbinals has never been tested using convergent species. In Rodentia, the Ecuadorian fish-eating rat (*Aotomys leander*) lives in cold-water torrents at high elevation (up to 4,000 m) and has relatively larger respiratory turbinals than the Oyapock's fish-eating rat (*Neusticomys oyapocki*; Fig. 1 and *SI Appendix*, Fig. S3), which lives in lowland streams (below 500 m, refs. 52 and 53). We found a similar example in Australo-Papuan murinae with the earless water rat (*Crossomys moncktoni*) that lives and dives in cold-water torrents up to 3,500 m (54) and has relatively larger respiratory turbinals than the western water rat (*Hydromys hussoni*; Fig. 1 and *SI Appendix*, Fig. S3) that lives below 1,800 m (55). Hence, the convergent evolution of relative surface area of respiratory turbinals reveals fine ecological variation.

Fast Evolution of the Turbinals in Small Amphibious Mammals. We demonstrated that small amphibious mammals convergently reduced their olfactory turbinal bones and increased their respiratory turbinal bones, producing differential olfactory and thermoregulatory capacities. We hypothesized that differential evolutionary rates between amphibious and terrestrial species reflect the relaxed selective pressures for the relative size of olfactory turbinals and the strong selection for respiratory turbinals. Indeed, we demonstrated that the evolutionary rates of olfactory and respiratory turbinals were 5.4 and 1.4 times faster in amphibious species than in terrestrial ones. Rapid evolution in this case was likely fostered by a trade-off in which relaxed selection on a previously important trait (olfactory turbinals) provided physical space within the nasal cavity for expansion of a newly important trait (respiratory turbinals). Furthermore, because the shift between foraging in water and in terrestrial environments is abrupt, we suggest that the morphological changes occurred quickly to adapt to new sensorial and physiological environments. Indeed, vertebrates can evolve faster when they are confronted with rapid environmental modifications (56–58). The consistent (17 times) and highly convergent loss of olfactory capacities and the gain of thermoregulatory capacities at the order level is surprising. We showed that morphological traits related to vital functions such as olfaction and thermoregulation evolved faster to the selective optimum—the average phenotype expected to be optimal for both amphibious and terrestrial lineages—compared to morphological traits unrelated to vital functions such as skull length. Together, these results demonstrate that the shift to the aquatic environment played an important role in the morpho-anatomical shaping of small amphibious mammals.

Materials and Methods

Data Acquisition. Undamaged skulls belonging to 130 species of Afrosoricida, Eulipotyphla, and Rodentia were selected from the following: American Museum of Natural History (AMNH), Centre de Biologie et de Gestion des Populations (CBGP), Field Museum of Natural History (FMNH), Museums Victoria (NMV), Museum Zoologicum Bogoriense (MZB), Natural History Museum London (NHMUK), Natural History Museum of Paris (MNHN), Naturalis Biodiversity Center of Leiden (RMNH), Royal Museum for Central Africa (RMCA), Smithsonian Institution National Museum of Natural History (NMNH), and University of Montpellier (UM). In total, our sample included 17 evolutionarily independent colonizations of the aquatic environment framed by closely related terrestrial species. Skulls were scanned using X-ray microtomography (*SI Appendix*, Table S7). We segmented left turbinals from each individual with Avizo Lite 9.0.1 (VSG Inc.). Segmentation followed turbinal descriptions presented for Rodentia (14, 22), Lagomorpha (15), and Marsupialia (18). Following Martinez et al. (22), we segmented the branching of the lamina semicircularis that is covered by the olfactory epithelium (9). Based on morphological, histological, airflow dynamic, and performance tests, we partitioned the turbinal bones into two functional parts: thermoregulatory and olfactory (Fig. 1 and *SI Appendix*, Fig. S2). To refine this functional partitioning, we performed turbinal bone histology on representative specimens of the following species: *Tenrec ecaudatus* (Afrosoricida), *Suncus murinus* (Eulipotyphla), *Talpa europaea* (Eulipotyphla), and *Mus musculus domesticus* (Rodentia, *SI Appendix*, Figs. S4 and S5).

Adaptation and Convergence. We computed phylogenetic ANCOVA (analysis of covariance) to determine if differences between amphibious and terrestrial lifestyles explain the variation in olfactory and respiratory turbinal surface using the total surface area of the turbinals as a covariate. We used the residuals of the phylogenetic generalized least squares (PGLS) regression of the olfactory and thermoregulatory turbinal surface area on the total surface area as relative surface area measures in downstream comparative analyses (respectively, the relative surface area of olfactory and respiratory turbinals). To consider another proxy of size, we also used residuals from a PGLS regression of the olfactory and thermoregulatory turbinal surface areas on skull length (see results in *SI Appendix*, Tables S1, S3, and S5 and Figs. S6 and S7). In order to obtain size-free estimates of relative surface area for both respiratory and olfactory turbinals, we also computed the residuals of a linear model (generalized least squares [GLS]) with the olfactory surface or respiratory surface area as the response variable and skull length and total surface area as covariates (see results in *SI Appendix*, Table S6 and Fig. S8). Prior to comparative analyses, data averages were taken when multiple individuals were available. The PGLS regressions were performed using the “gls” function in the R package *nlme* and the “corBrownian” structure in the R package *ape* (59). We used a maximum clade credibility (MCC) phylogeny obtained from 1,000 trees sampled in the posterior distribution of Upham et al. (60) and pruned to match the species in our dataset. The MCC tree was constructed in TreeAnnotator v.1.8.2 (61).

To assess the evolution of the turbinal surface area in relation to ecological lifestyles, and to investigate the potential changes in evolutionary dynamics of the olfactory and thermoregulatory turbinal surface area, we used univariate and bivariate phylogenetic models of trait evolution. We focused on two models, Brownian motion (BM) and Ornstein–Uhlenbeck (OU, refs. 62–64), both implemented in the R package *mvMORPH* (ref. 64, functions “mvBM” and “mvOU”). BM processes describe the accumulation of infinitesimal phenotypic change along the branches of a phylogenetic tree (with the amount of change controlled by the rate parameter σ); OU processes describe selection toward an optimal trait value (parameter θ , or two optima associated with terrestrial or amphibious lifestyles—hereafter called selective regimes) and add to the BM process an extra parameter (α) that describes the strength of selection toward the optimal trait value (62, 63). The macroevolutionary optimum estimated by these models can be seen as the average phenotype toward which the lineages have evolved in both the amphibious and terrestrial species. More specifically, we applied a single-rate BM model (BM1), a model with regime-specific rates (BMM), an OU model with a single selective regime (OU1), and an OU model with regime-specific optima (OUM) to our trait data. We also considered BM models (BM1m and BMMm) that allow different ancestral states for the different lifestyles (using the option “smean=FALSE” in the function “mvBM”). BM1m has distinct trait means per regime but a single rate, while BMMm has distinct means and rates (65–67). In addition, we considered an ER model (using “mvSHIFT” in *mvMORPH*) which combines BM and OU processes. In the ER process function, the terrestrial species evolve under selective pressures to maintain the evolutionary trade-off (modeled by an OU process) while amphibious species are released from these pressures (modeled by a BM process). This scenario matched our expectation that olfactory turbinals are not valuable underwater and that thermoregulation becomes more important. The reconstructed history of the terrestrial and amphibious selective regimes on which BMM, OUM, and the ER model were fitted was obtained from 100 stochastic character maps using the function “make.simmap” in the R package *phytools* (68). Model fits were compared using the Akaike information criterion.

We quantified the level of convergences in turbinal surface area for the amphibious species using the C indexes proposed by Stayton (33). We ran these analyses with the R package *convevol* (69, 70), performing 1,000 simulations. Finally, we mapped the evolution of relative olfactory and respiratory surface areas on the branches of the phylogeny using the “phenogram” function in *phytools* (68). This phenogram projects the phylogeny related to a phenotype trait. Phenograms with branches crossing and concentrating in a given area indicate convergent evolution.

Evolutionary Rates and Phylogenetic Half-Life. We compared the rates of morphological evolution (σ^2) estimated from the BMM and BMMm fit for the relative surface area of turbinals between amphibious and terrestrial species. The significance of the difference in rates between the amphibious and terrestrial species was assessed by comparing the fit of BMM to a model with a common rate for the two lifestyles (BM1 and BM1m) using both the Akaike information criterion and likelihood ratio tests.

We found that an OU model best fit the relative surface area of the respiratory turbinals (*SI Appendix*, Table S5); thus, interpreting the difference in rates of phenotypic evolution estimated by the BM model can be misleading

because young clades may appear to evolve more quickly than older ones under a homogeneous OU process (67, 71). To assess if the differences in evolutionary rates were not artifactual and could be interpreted biologically, we ran simulations under the best-fit OU model maximum likelihood parameter estimates to compute a null distribution of expected rate differences. This null distribution of rate differences between terrestrial and amphibious species obtained from 100 simulated traits (in the OU model) was then compared to the rate differences estimated on the empirical data (*SI Appendix, Table S5*).

To test if turbinal bones evolved faster to the optimal trait value associated with each lifestyle as compared to skull length—a size-related trait that often correlates with multiple features of species ecology and life history—we estimated the phylogenetic half-life from the Ornstein-Uhlenbeck process that describes the time necessary for our morphological trait to evolve halfway from the ancestral state to the primary optimum (62). Compared to the evolutionary rates we obtained from the BM models described previously, this measure could be interpreted as a rate of “adaptation” to the different lifestyles (62, 72). We estimated the phylogenetic half-life, with the function “half-life” in *mvMORPH* (64).

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