Convergent evolution of olfactory and thermoregulatory capacities in small amphibious mammals

Quentin Martinez[a,1], Julien Clavel[b,c], Jacob A. Esselstyn[d,e], Anang S. Achmadi[f], Camille Grohé[g,h], Nelly Pirot[i,j], and Pierre-Henri Fabre[a,k]

[a]Institut des Sciences de l'Évolution de Montpellier (ISEM), CNRS, Institut de recherche pour le développement (IRD), Université de Montpellier (UM), UMR 5554, 34095 Montpellier, France; [b]Department of Life Sciences, The Natural History Museum, SW7 5DB London, United Kingdom; [c]Univ. Lyon Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, UMR CNRS 5023, Université Claude Bernard Lyon 1, École Nationale des Travaux Publics de l'État (ENTPE), F-69622 Villeurbanne, Cedex, France; [d]Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803; [e]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803; [f]Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), 16911 Cibinong, Indonesia; [g]Division of Paleontology, American Museum of Natural History, New York, NY 10024; [h]Laboratoire Paléontologie Évolution Paléoécosystèmes Paléoprimatologie (PALEVOPRIM, UMR 7262, CNRS-Institut écologie et environnement [INEE]), Université de Poitiers, 86073 Poitiers, Cedex 9, France; [i]Institut de Recherche en Cancérologie de Montpellier (IRCM), INSERM, U1194 UM, Institut du Cancer de Montpellier (ICM), F-34298 Montpellier, Cedex 5, France; [j]Réseau d'Histologie Expérimentale de Montpellier, UMS3426 CNRS-US009 INSERM-UM, 34298 Montpellier, France; and [k]Mammal Section, Department of Life Sciences, The Natural History Museum, SW7 5DB London, United Kingdom

Edited by David B. Wake, University of California, Berkeley, CA, and approved February 28, 2020 (received for review October 11, 2019)

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 turbinals that contribute to heat conservation and olfaction (7). Anteriorly, the respiratory turbinals are lined with a vascular epithelium that helps conserve heat during respiration (7). Posteriorly, the olfactory turbinals 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.

Author contributions: Q.M. and P.-H.F. designed research; Q.M., J.C., J.A.E., N.P., and P.-H.F. performed research; J.C. and N.P. contributed new reagents/analytic tools; Q.M., J.C., and P.-H.F. analyzed data; Q.M., J.C., J.A.E., A.S.A., C.G., and P.-H.F. wrote the paper; A.S.A. provided museum specimens; and C.G. provided 3D CT scans.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

Data deposition: The 3D CT scan surfaces are available via MorphoSource (https://www.morphosource.org/Detail/ProjectDetail/Show/project_id/974).

[1]To whom correspondence may be addressed. Email: quentinmartinezphoto@gmail.com.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1917836117/-/DCSupplemental.

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. 2*A*). 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. 2*B*), and the emergence of a new respiratory turbinal (Fig. 2*A*).

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

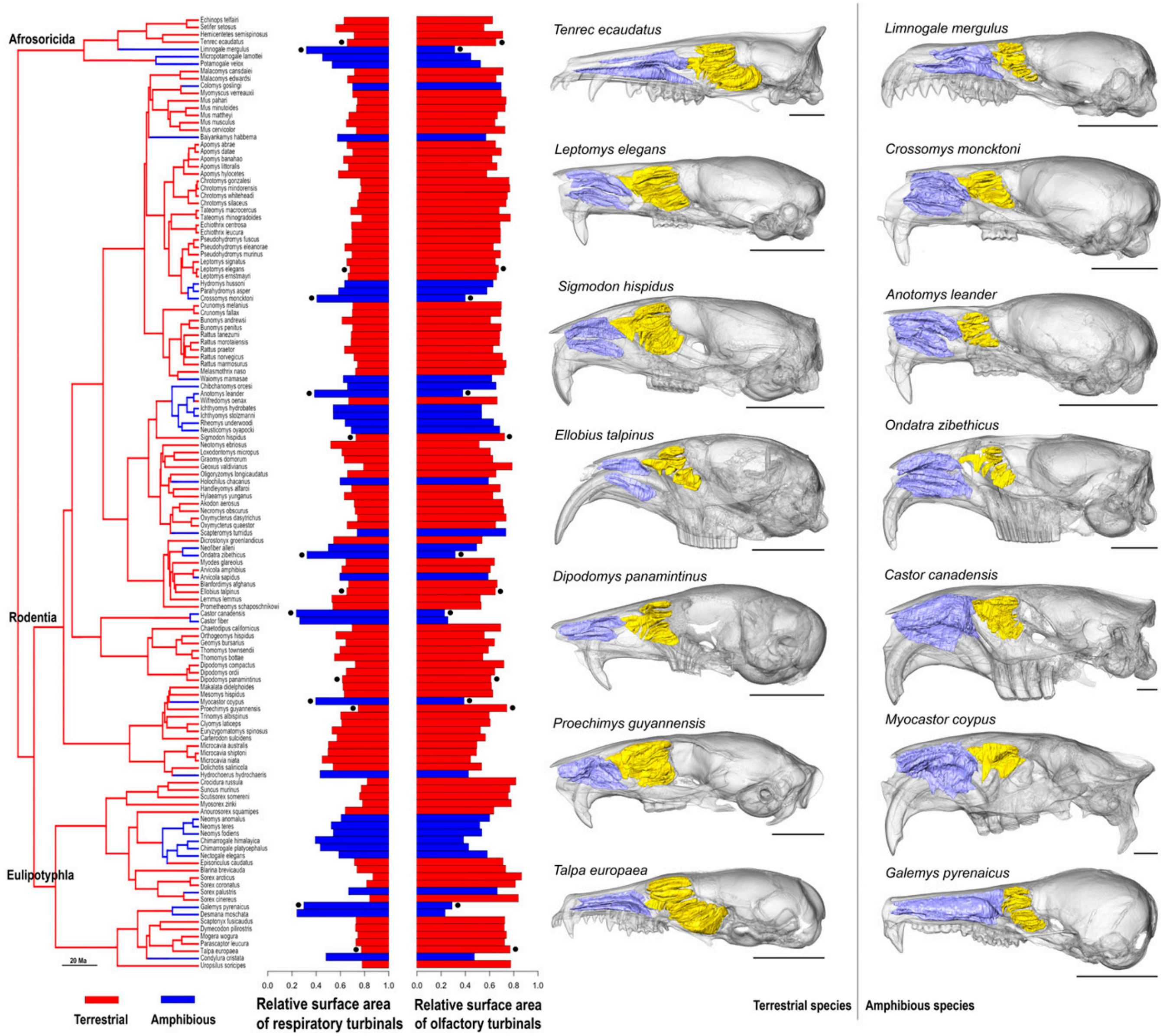

Fig. 1. Loss of olfactory and gain of thermoregulatory capacities in amphibious mammals. Phylogeny of the sampled species with barplots of the relative surface area of olfactory and respiratory turbinals based on ratios; blue = amphibious; red = terrestrial. Black circles highlight illustrated species.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

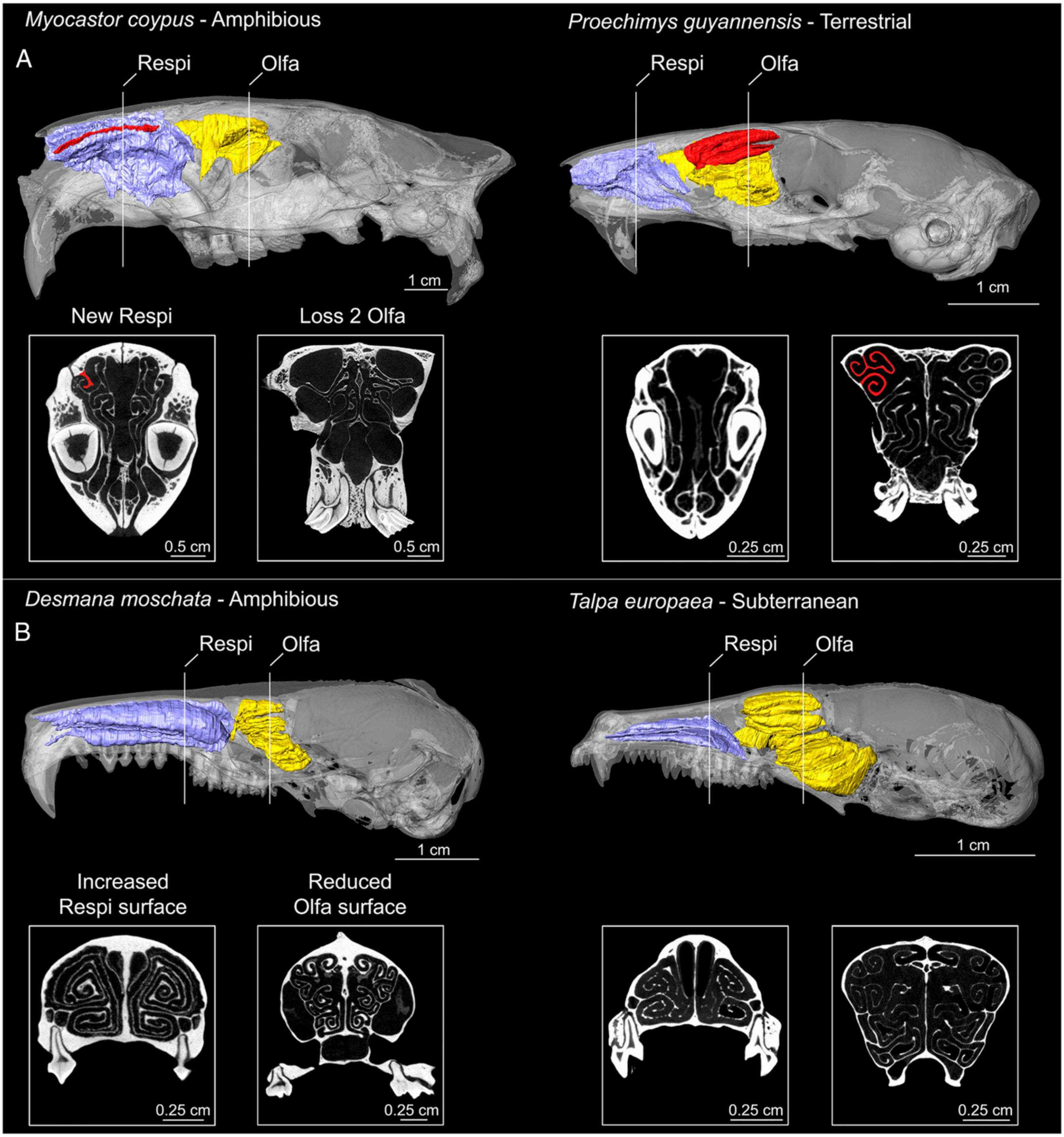

Fig. 2. Loss and gain of anatomical structures. 3D representations of turbinal bones and coronal cross section showing two mechanisms of adaptation to the amphibious environment: (*A*) emergence of new respiratory turbinals and loss of some olfactory turbinals as seen in the amphibious *Myocastor coypus* 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

	Relative olfactory turbinal surface area		Relative respiratory turbinal surface area		Bivariate model: relative olfactory and respiratory surface area	
Model	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 2*B*). 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. 2*A*). 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

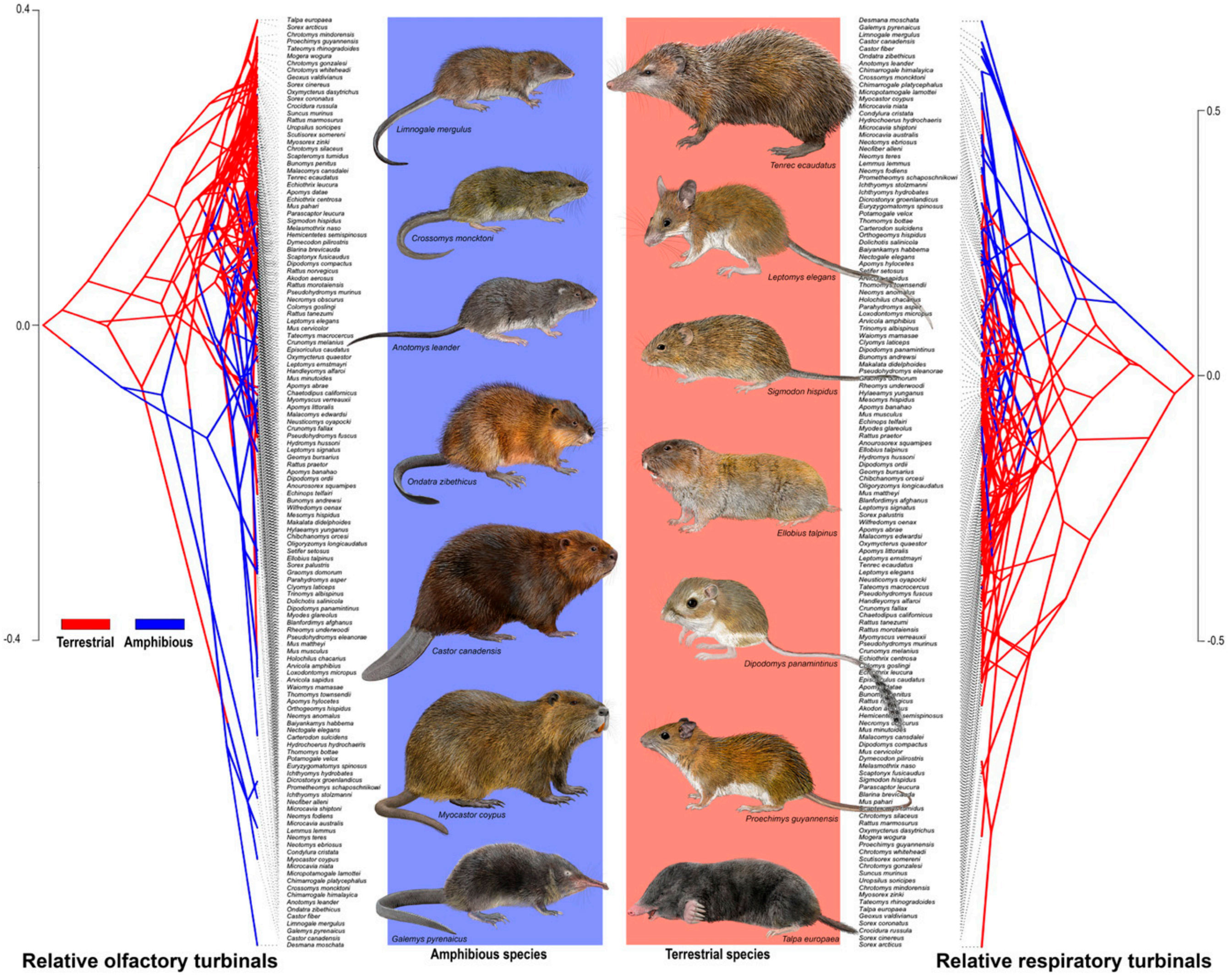

Fig. 3. Convergent loss of olfactory and gain of thermoregulatory capacities in amphibious mammals. Phenograms based on the residual of phylogenetic generalized least squares regressions for the relative surface area of olfactory and respiratory turbinals. Phenograms with branches crossing and concentrating in a given area indicate convergent lineages. Blue = amphibious; red = terrestrial. Illustrations by Toni Llobet and Lynx Editions. Reprinted with permission from refs. 73–75.

compared to their terrestrial relatives (Figs. 1–3), an adaptation that minimizes heat loss in the aquatic environment. Due to the great thermal conductivity of water (41), heat loss is about two to four times higher in water than in air for the same temperature (5). This factor is even more important in small amphibious mammals than in fully aquatic mammals because the former generally paddle at the air-water interface, an energetically demanding form of locomotion (42–44). To respond to the energetic and thermal constraints of the aquatic environment, some small amphibious mammals developed many anatomical, physiological, and behavioral features compared to their terrestrial relatives, such as (1) a larger body size (6, 45), (2) a higher metabolic rate (45–47), (3) denser fur and fat (45, 48), (4) Hardarian glands to waterproof the fur (45, 49), and (5) an energy-rich carnivorous diet (45).

Our evidence for the enlargement of respiratory turbinals in amphibious mammals is consistent with histological studies showing a thickening of the epithelium of respiratory turbinals in amphibious shrews (38) and of the bony structures of the respiratory turbinals in extinct aquatic mammals (50, 51). Our results also show a gradient of increasing relative surface area of respiratory turbinals with greater aquatic specialization. For instance, within Talpidae, this gradient increases from the nonamphibious (*Dymecodon*, *Mogera*, *Parascaptor*, *Scaptonyx*, *Talpa*, and *Uropsilus*) to the occasionally amphibious (*Condylura*) and finally to the fully amphibious (*Desmana* and *Galemys*; Fig. 1 and *SI Appendix*, Fig. S2).

Our results also suggest that temperature may contribute to the size of respiratory turbinals. For example, the Russian desman (*Desmana moschata*) lives in colder water and has a larger relative respiratory turbinal surface area (Fig. 1) than the Pyrenean desman (*Galemys pyrenaicus*). The importance of temperature was previously suggested via a respiratory turbinal comparison between the leopard seal (*Hydrurga leptonyx*) and the extinct tropical

Table 2. Fast morphological evolution of small amphibious mammals

Variables	Observed rate ratio	TER rate	AMP rate	LRT
Relative olfactory turbinal surface area	5.425	0.001	0.004	<0.001
Relative respiratory turbinal surface area	1.426	0.003	0.004	<0.001

AMP = amphibious; LRT = likelihood-ratio test; TER = terrestrial.

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 (*Anotomys 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 ($\sigma 2$) 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 "halflife" in *mvMORPH* (64).

ACKNOWLEDGMENTS. We thank the editor and two anonymous reviewers for their helpful and constructive reviews. We thank R. Allio, S. Ferreira-Cardoso, J. Claude, F. Condamine, F. Delsuc, E. Eveque, L. Hautier, R. Lebrun, C. Molinier, K.C. Rowe, I. Ruf, and N. Upham for different types of support, including interesting discussions. Three-dimensional data acquisitions were performed using the micro-computed tomography (μ-CT) facilities of the MRI platform member of the national infrastructure France-BioImaging supported by the French National Research Agency (Grant ANR-10-INBS-04, "Investments for the future"), and those of the Laboratoire d'Excellence Centre Méditerranéen de l'Environnement et de la Biodiversité (LabEx CeMEB, ANR-10-LABX-0004), LabEx Centre d'étude de la biodiversité amazonienne (CEBA, ANR-10-LABX-25-01), and Digital and Hardware Solutions and Modeling for the Environment and Life Sciences (NUMEV, ANR-10-LABX-0020). We acknowledge the Réseau d'Histologie Expérimentale de Montpellier (RHEM) facility supported by a Sites de Recherche Intégrée sur le Cancer (SIRIC) Montpellier Cancer Grant (Institut National Du Cancer [INCa] Inserm Direction générale de l'offre de soins [DGOS] 12553), the European Regional Development Foundation, and the Occitanian Region (Grant FEDER-FSE 2014-2020 Languedoc Roussillon) for processing our animal tissues, histology technics, and expertise. Thanks to M. Broyon, Y. Glasson, and F. Abella from the RHEM MRI platform for slide digitalization, and to F. Ahmed, B. Clark, V. Fernandez, and R. P. Miguez for access to the computed tomography facilities at the Natural History Museum London. A. Evans and M. McCurry helped with CT scanning at Monash University. We are grateful to the following people for granting access to specimens: S. Agret, F. Bonhomme, F. Catzeflis, J. Claude, F. Darinot, J. J. Duquesne, R. Gibert, S. Ginot, G. Le Minter, F. Poitevin, P. Tortosa, N. Simmons, N. Vazzoler-Antoine, and F. Veyrunes. We thank T. Llobet and Lynx Edicions for use of their illustrations. Funding was provided by the Synthesis of Systematic Resources (SYNTHESYS) Project, which is financed by European Community Research Infrastructure Action (FP7: Grants GB-TAF-5737 and GB-TAF-6945 to the National History Museum London), by Agence Nationale de la Recherche (Défi des autres savoirs, Grants DS10, ANR-17-CE02-0005 RHINOGRAD 2017), "Projets Exploratoires Premier Soutien (PEPS), adaptation, adaptabilite (shrewnose)," by a Frick postdoctoral fellowship, the National Science Foundation (Grants DEB-1754393 and DEB-1257572), and SHREWNOSE (Grant ANR-10-LABX-0025-01). This is a contribution of ISEM 2020-035 SUD, Université de Montpellier, CNRS, École Pratique des Hautes Études (EPHE), IRD, Montpellier, France.

1. K. V. Kardong, "Introduction" in *Vertebrates: Comparative Anatomy, Function, Evolution*, D. W. C. Brown, Ed. (McGraw-Hill, 1998), chap. 1, pp. 1–47.
2. G. R. McGhee, *Convergent Evolution: Limited Forms Most Beautiful* (MIT Press, 2011).
3. J. G. M. Thewissen, S. Nummela, *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates* (University of California Press, 2008).
4. G. W. Molnar, Survival of hypothermia by men immersed in the ocean. *J. Am. Med. Assoc.* **131**, 1046–1050 (1946).
5. R. M. Smith, J. M. Hanna, Skinfolds and resting heat loss in cold air and water: Temperature equivalence. *J. Appl. Physiol.* **39**, 93–102 (1975).
6. H. Pihlström, "Comparative anatomy and physiology of chemical senses in aquatic mammals" in *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates* (University of California Press, Berkeley, 2008), pp. 95–109.
7. V. Negus, *The Comparative Anatomy and Physiology of the Nose and Paranasal Sinuses* (Livingstone, 1958).
8. H. Breer, J. Fleischer, J. Strotmann, The sense of smell: Multiple olfactory subsystems. *Cell. Mol. Life Sci.* **63**, 1465–1475 (2006).
9. A. W. Barrios, G. Núñez, P. Sánchez Quinteiro, I. Salazar, P. Chamero, Anatomy, histochemistry, and immunohistochemistry of the olfactory subsystems in mice. *Front. Neuroanat.* **8**, 63 (2014).
10. K. J. Ressler, S. L. Sullivan, L. B. Buck, A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* **73**, 597–609 (1993).
11. H. Pihlström, M. Fortelius, S. Hemilä, R. Forsman, T. Reuter, Scaling of mammalian ethmoid bones can predict olfactory organ size and performance. *Proc. Biol. Sci.* **272**, 957–962 (2005).
12. J. R. Harkema, S. A. Carey, J. G. Wagner, The nose revisited: A brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. *Toxicol. Pathol.* **34**, 252–269 (2006).
13. B. A. Craven, E. G. Paterson, G. S. Settles, The fluid dynamics of canine olfaction: Unique nasal airflow patterns as an explanation of macrosmia. *J. R. Soc. Interface* **7**, 933–943 (2010).
14. I. Ruf, "Vergleichend-ontogenetische Untersuchungen an der Ethmoidalregion der Muroidea (Rodentia, Mammalia). Ein Beitrag zur Morphologie und Systematik der Nagetiere," PhD dissertation, Universität Tübingen, Tübingen, Germany (2004).
15. I. Ruf, Comparative anatomy and systematic implications of the turbinal skeleton in Lagomorpha (Mammalia). *Anat. Rec. (Hoboken)* **297**, 2031–2046 (2014).
16. B. Van Valkenburgh *et al.*, Aquatic adaptations in the nose of carnivorans: Evidence from the turbinates. *J. Anat.* **218**, 298–310 (2011).
17. P. A. Green *et al.*, Respiratory and olfactory turbinal size in canid and arctoid carnivorans. *J. Anat.* **221**, 609–621 (2012).
18. T. E. Macrini, Comparative morphology of the internal nasal skeleton of adult marsupials based on X-ray computed tomography. *Bull. Am. Mus. Nat. Hist.* **365**, 1–91 (2012).
19. B. Van Valkenburgh *et al.*, Respiratory and olfactory turbinals in feliform and caniform carnivorans: The influence of snout length. *Anat. Rec. (Hoboken)* **297**, 2065–2079 (2014).
20. K. K. Yee, B. A. Craven, C. J. Wysocki, B. Van Valkenburgh, Comparative morphology and histology of the nasal fossa in four mammals: Gray squirrel, bobcat, coyote, and white-tailed deer. *Anat. Rec. (Hoboken)* **299**, 840–852 (2016).
21. A. A. Curtis, N. B. Simmons, Unique turbinal morphology in horseshoe bats (Chiroptera: Rhinolophidae). *Anat. Rec. (Hoboken)* **300**, 309–325 (2017).
22. Q. Martinez *et al.*, Convergent evolution of an extreme dietary specialisation, the olfactory system of worm-eating rodents. *Sci. Rep.* **8**, 17806 (2018).
23. I. K. Lundeen, E. C. Kirk, Internal nasal morphology of the Eocene primate *Rooneyia viejaensis* and extant Euarchonta: Using μCT scan data to understand and infer patterns of nasal fossa evolution in primates. *J. Hum. Evol.* **132**, 137–173 (2019).
24. M. R. Sánchez-Villagra, R. J. Asher, Cranio-sensory adaptations in small faunivorous semiaquatic mammals, with special reference to olfaction and the trigeminal system. *Mammalia* **66**, 93–109 (2002).
25. D. J. Bird, A. Amirkhanian, B. Pang, B. Van Valkenburgh, Quantifying the cribriform plate: Influences of allometry, function, and phylogeny in Carnivora. *Anat. Rec. (Hoboken)* **297**, 2080–2092 (2014).
26. D. J. Bird *et al.*, Olfaction written in bone: cribriform plate size parallels olfactory receptor gene repertoires in Mammalia. *Proc. Biol. Sci.* **285**, 20180100 (2018).
27. T. Kishida, S. Kubota, Y. Shirayama, H. Fukami, The olfactory receptor gene repertoires in secondary-adapted marine vertebrates: Evidence for reduction of the functional proportions in cetaceans. *Biol. Lett.* **3**, 428–430 (2007).
28. Y. Niimura, On the origin and evolution of vertebrate olfactory receptor genes: Comparative genome analysis among 23 chordate species. *Genome Biol. Evol.* **1**, 34–44 (2009).
29. T. Kishida, T. Hikida, Degeneration patterns of the olfactory receptor genes in sea snakes. *J. Evol. Biol.* **23**, 302–310 (2010).
30. T. Kishida *et al.*, Loss of olfaction in sea snakes provides new perspectives on the aquatic adaptation of amniotes. *Proc. Biol. Sci.* **286**, 20191828 (2019).
31. A. C. Beichman *et al.*, Aquatic adaptation and depleted diversity: A deep dive into the genomes of the sea otter and giant otter. *Mol. Biol. Evol.* **36**, 2631–2655 (2019).
32. A. Liu *et al.*, Convergent degeneration of olfactory receptor gene repertoires in marine mammals. *BMC Genomics* **20**, 977 (2019).
33. C. T. Stayton, The definition, recognition, and interpretation of convergent evolution, and two new measures for quantifying and assessing the significance of convergence. *Evolution* **69**, 2140–2153 (2015).
34. K. C. Catania, Olfaction: Underwater "sniffing" by semi-aquatic mammals. *Nature* **444**, 1024–1025 (2006).
35. K. C. Catania, J. F. Hare, K. L. Campbell, Water shrews detect movement, shape, and smell to find prey underwater. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 571–576 (2008).
36. Y. F. Ivlev, M. V. Rutovskaya, O. S. Luchkina, The use of olfaction by the Russian desman (*Desmana moschata* L.) during underwater swimming. *Dokl. Biol. Sci.* **452**, 280–283 (2013).
37. S. Firestein, How the olfactory system makes sense of scents. *Nature* **413**, 211–218 (2001).
38. R. Larochelle, G. Baron, Comparative morphology and morphometry of the nasal fossae of four species of North American shrews (Soricinae). *Am. J. Anat.* **186**, 306–314 (1989).
39. S. Hayden *et al.*, Ecological adaptation determines functional mammalian olfactory subgenomes. *Genome Res.* **20**, 1–9 (2010).
40. M. L. Noback, K. Harvati, F. Spoor, Climate-related variation of the human nasal cavity. *Am. J. Phys. Anthropol.* **145**, 599–614 (2011).
41. T. J. Dawson, F. D. Fanning, Thermal and energetic problems of semiaquatic mammals: A study of the Australian water rat, including comparisons with the platypus. *Physiol. Zool.* **54**, 285–296 (1981).
42. F. E. Fish, R. V. Baudinette, Energetics of locomotion by the Australian water rat (*Hydromys chrysogaster*): A comparison of swimming and running in a semi-aquatic mammal. *J. Exp. Biol.* **202**, 353–363 (1999).
43. T. M. Williams, The evolution of cost efficient swimming in marine mammals: Limits to energetic optimization. *Philos. Trans. R. Soc. B Biol. Sci.* **354**, 193–201 (1999).

44. F. E. Fish, Mechanics, power output and efficiency of the swimming muskrat (*Ondatra zibethicus*). *J. Exp. Biol.* **110**, 183–201 (1984).
45. N. Dunstone, "Adaptations to the semi-aquatic habit and habitat" in *Behaviour and Ecology of Riparian Mammals*, N. Dunstone, M. L. Gorman, Eds. (Cambridge University Press, Cambridge, UK, 1998), pp. 1–16.
46. P. J. Stephenson, Resting metabolic rate and body temperature in the aquatic tenrec *Limnogale mergulus* (Insectívora: Tenrecidae). *Acta Theriol. (Warsz.)* **39**, 89–92 (1994).
47. R. W. Gusztak, "Diving physiology and aquatic thermo-regulation of the American water shrew (*Sorex palustris*)," M.S. thesis, University of Manitoba, Winnipeg, MB, Canada (2008).
48. W. J. Hamilton, Habits of the star-nosed mole, *Condylura cristata*. *J. Mammal.* **12**, 345 (1931).
49. H. J. Harlow, The influence of Hardarian gland removal and fur lipid removal on heat loss and water flux to and from the skin of muskrats, *Ondatra zibethicus*. *Physiol. Zool.* **57**, 349–356 (1984).
50. E. Amson, G. Billet, C. de Muizon, Evolutionary adaptation to aquatic lifestyle in extinct sloths can lead to systemic alteration of bone structure. *Proc. Biol. Sci.* **285**, 20180270 (2018).
51. E. Peri, P. D. Gingerich, G. Aringhieri, G. Bianucci, Reduction of olfactory and respiratory turbinates in the transition of whales from land to sea: The semiaquatic middle Eocene *Aegyptocetus tarfa*. *J. Anat.* **236**, 98–104 (2020).
52. R. S. Voss, *Systematics and Ecology of Ichthyomyine Rodents (Muroidea) : Patterns of Morphological Evolution in a Small Adaptive Radiation* (Bulletin of the American Museum of Natural History Series, vol. 188, American Museum of National History, 1988).
53. R. S. Voss, *Tribe Ichthyomyini Vorontsov, 1959. Mammals of South America. Vol. 2 Rodents 2* (The University of Chicago Press, 2015), pp. 279–291.
54. K. M. Helgen, "A taxonomic and geographic overview of the mammals of Papua" in *The Ecology of Papua,* A. J. Marshall, B. M. Beehler, Eds. (Periplus Editions, 2007), pp. 689–749.
55. G. G. Musser, E. Piik, A new species of *Hydromys* (Muridae) from western New Guinea (Irian Jaya). *Zool. Meded.* **56**, 153–166 (1982).
56. R. M. Bonett, A. L. Blair, Evidence for complex life cycle constraints on salamander body form diversification. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 9936–9941 (2017).
57. E. K. Baken, D. C. Adams, Macroevolution of arboreality in salamanders. *Ecol. Evol.* **9**, 7005–7016 (2019).
58. V. Millien, Morphological evolution is accelerated among island mammals. *PLoS Biol.* **4**, e321 (2006).
59. E. Paradis, J. Claude, K. Strimmer, APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
60. N. S. Upham, J. A. Esselstyn, W. Jetz, Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biol.* **17**, e3000494 (2019).
61. A. J. Drummond, M. A. Suchard, D. Xie, A. Rambaut, Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
62. T. F. Hansen, Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**, 1341–1351 (1997).
63. M. A. Butler, A. A. King, Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *Am. Nat.* **164**, 683–695 (2004).
64. J. Clavel, G. Escarguel, G. Merceron, mvMORPH : an R package for fitting multivariate evolutionary models to morphometric data. *Methods Ecol. Evol.* **6**, 1311–1319 (2015).
65. B. C. O'Meara, C. Ané, M. J. Sanderson, P. C. Wainwright, Testing for different rates of continuous trait evolution using likelihood. *Evolution* **60**, 922–933 (2006).
66. G. H. Thomas, R. P. Freckleton, T. Székely, Comparative analyses of the influence of developmental mode on phenotypic diversification rates in shorebirds. *Proc. Biol. Sci.* **273**, 1619–1624 (2006).
67. S. Joly *et al*., Greater pollination generalization is not associated with reduced constraints on corolla shape in Antillean plants. *Evolution* **72**, 244–260 (2018).
68. L. J. Revell, phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).
69. A. C. T. Stayton, M. C. T. Stayton, convevol: Analysis of convergent evolution. R package version 1.3. https://cran.r-project.org/web/packages/convevol/convevol.pdf. Accessed 23 March 2020.
70. M. L. Zelditch, J. Ye, J. S. Mitchell, D. L. Swiderski, Rare ecomorphological convergence on a complex adaptive landscape: Body size and diet mediate evolution of jaw shape in squirrels (Sciuridae). *Evolution* **71**, 633–649 (2017).
71. S. A. Price, J. J. Tavera, T. J. Near, P. C. Wainwright, Elevated rates of morphological and functional diversification in reef-dwelling Haemulid fishes. *Evolution* **67**, 417–428 (2013).
72. T. F. Hansen, *Adaptive Landscape and Macroevolutionary Dynamics. The Adaptive Landscape in Evolutionary Biology* (Oxford University Press, 2012).
73. D. E. Wilson, T. E. Lacher Jr., R. A. Mittermeier, Eds., "Lagomorphs and rodents I" in *Handbook of the Mammals of the World* (Lynx Editions, Barcelona, 2016), vol. 6.
74. D. E. Wilson, T. E. Lacher Jr., R. A. Mittermeier, Eds., "Rodents II" in *Handbook of the Mammals of the World* (Lynx Editions, Barcelona, 2017), vol. 7.
75. D. E. Wilson, T. E. Lacher Jr., R. A. Mittermeier, Eds., "Insectivores, sloths and colugos" in *Handbook of the Mammals of the World* (Lynx Editions, Barcelona, 2018), vol. 8.