Comparative genomics provides insights into the aquatic adaptations of mammals

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The ancestors of marine mammals once roamed the land and independently committed to an aquatic lifestyle. These macroevolutionary transitions have intrigued scientists for centuries. Here, we generated high-quality genome assemblies of 17 marine mammals (11 cetaceans and six pinnipeds), including eight assemblies at the chromosome level. Incorporating previously published data, we reconstructed the marine mammal phylogeny and population histories and identified numerous idiosyncratic and convergent genomic variations that possibly contributed to the transition from land to water in marine mammal lineages. Genes associated with the formation of blubber (*NFIA*), vascular development (*SEMA3E*), and heat production by brown adipose tissue (*UCP1*) had unique changes that may contribute to marine mammal thermoregulation. We also observed many lineage-specific changes in the marine mammals, including genes associated with deep diving and navigation. Our study advances understanding of the timing, pattern, and molecular changes associated with the evolution of mammalian lineages adapting to aquatic life.

marine mammals | comparative genomics | aquatic adaptation

Species invasions into novel habitats mark major transitions in the evolution of life on Earth. Some of these are relatively ancient, such as the vertebrate transition from the oceans to life on land (~375 Mya) or the evolution of arboreal vertebrate species (~160 Mya). When divergent lineages transition to the same novel habitat, it provides an opportunity to investigate the mechanisms that permit these adaptations and the relationship between similar phenotypes among lineages and the underlying genetic basis. Convergent processes may utilize homologous genomic regions in different lineages to achieve similar phenotypes (1). Alternatively, distinct, genomic processes may be possible (2), or genetic drift may lead to different options for divergent lineages. Relatively recent transitions may be the most informative, on the assumption that extended periods of evolution may obscure the relationship between genomic differences and the original adaptations. A system well suited to this investigation is the adaptation of divergent, terrestrial mammalian lineages to life in aquatic environments.

Marine mammals, broadly defined as mammals whose terrestrial predecessors entered the sea and who obtain all or most of their food from a marine environment, comprise at least 129 extant species divided into three orders (3). Cetartiodactyla includes cetaceans (whales, dolphins, and porpoises); Carnivora includes pinnipeds (walruses, sea lions, and seals), sea otters, and polar bears; and Sirenia includes sea cows (now extinct), manatees, and dugongs (3). Of these, cetaceans, pinnipeds, and sirenians are considered the oldest groups of marine mammals (3). In contrast, sea otters and the polar bear emerged relatively recently so much so that the polar bear can still hybridize with terrestrial sister taxa (3–5). The most species-rich group of marine mammals

Significance

Divergent lineages can respond to common environmental factors through convergent processes involving shared genomic components or pathways, but the molecular mechanisms are poorly understood. Here, we provide genomic resources and insights into the evolution of mammalian lineages adapting to aquatic life. Our data suggest convergent evolution, for example, in association with thermoregulation through genes associated with a surface heat barrier (*NFIA*) and internal heat exchange (*SEMA3E*). Combined with the support of previous reports showing that the UCP1 locus has been lost in many marine mammals independently, our results suggest that the thermostatic strategy of marine mammals shifted from enhancing heat production to limiting heat loss.

Author contributions: S. Li conceived and led the study; S. Li, K.W., and G.F. designed this project and research aspects; Y.Y., Y.Z., P.Z., C.L., J.W., Haiyu Gao, and G.F. performed data analysis including genome assembly, annotation, gene family, chromosome evolution, and UCE and manuscript writing; P.Z., A.R.H., M. Lin, L.D., Z.Y., W.L., X.W., H.K., J.Z., R.R.d.F., and M.H.R. performed sample collection; Y.Z., Haiyu Gao, L.D., M. Liu, W.L., and H.K. collected samples and made contributions to figure designing; M. Lv, Haoyang Gao, Y.S., X.G., J.G., and L.M. performed data filtering, genome assembly and annotation; Y.Q., S.P., and Q.X. performed sequencing libraries construction; and A.R.H., I.S., S.P., S. Li, K.W., S.M.-Y.L., X.X., and H.Y. performed manuscript amending. All other authors revised and read the manuscript.

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is Cetacea, which comprises ~90 species (3). Cetaceans, pinnipeds, and sirenians represent an exceptional case of convergent evolution—the emergence of similar phenotypic traits in species separated by millions of years of evolution (6). In these separate lineages of marine mammals, phenotypic convergence is observed in all major physiological systems (7, 8). The degree to which convergence is reflected at the molecular level can now be partially answered using genomics. However, the interpretation of such results has hitherto been restricted by the limited number of high-quality genomes from marine mammals (6, 9). Remaining uncertainties include the phylogenetic relationships between and within marine mammal groups and their demographic history. To address these questions, we assembled and annotated 17 marine mammal genomes (11 cetaceans and six pinnipeds). Based on more comprehensive genomic data, we identified many putative genetic innovations for the aquatic adaptation of mammals, including those associated with thermoregulation and skeletal systems.

Results

Genome Sequencing, Assembly, and Annotation. We performed the sequencing and de novo assembly of 17 marine mammal genomes (11 cetaceans and six pinnipeds) (*SI Appendix*, Table S1). Among these, 14 were assembled by Supernova (10) with 10× Genomics data (average scaffold N50 = 28.66 Mb and contig N50 = 142.33 kb) (Table 1 and *SI Appendix*, Tables S1–S3). The remaining three genomes were assembled using Illumina paired-end reads (*SI Appendix*, Tables S1–S3). Eight of the assemblies were further improved by Hi-C chromosome anchoring (*SI Appendix*, Fig. S1). The assembled genomes of the 17 marine mammal species range in size from 2.37 to 2.62 Gb, which is similar to *k*-mer–based estimations using GCE (11) (*SI Appendix*, Table S4) and those of published marine mammal genomes (*SI Appendix*, Table S5). More than 95% of each species' short reads could be mapped to their respective assembly (*SI Appendix*, Fig. S2). BUSCO (Benchmarking Universal Single-Copy Orthologs) (version 3.0.2) (12) was used to assess the quality of the assemblies, revealing an average genome completeness of 90.98% (*SI Appendix*, Table S6). Analysis of syntenic relationships, comparing genome assemblies of closely related species, also showed high continuity of these genomes (*SI Appendix*, Fig. S3).

We employed de novo– and homology-based prediction methods to annotate the genes and repeat sequences of the assembled genomes (*SI Appendix*, Tables S7 and S8). Annotated protein-coding genes ranged from 20,083 to 20,947 per species (Table 1). The average gene lengths were similar to those of closely related species (*SI Appendix*, Fig. S4), and we recovered an average 96.44% of the BUSCO Mammalia gene set (4,104 genes) (Table 1). Overall, we generated high-quality genome sequences for 17 marine mammals, providing a good foundation for developing a better understanding of aquatic adaptation in marine mammals across three divergent ancestral lineages.

Phylogeny and Demographic History of Marine Mammals. Combining published genome data with our 17 genomes, we were able to provide a detailed phylogenomic reconstruction of marine mammal species. Two nucleotide datasets were used (*SI Appendix*, Table S9): ortholog sequences from whole-genome alignment and reciprocal best hit ortholog genes from gene annotations. The maximum-likelihood trees generated from the alignments of the individual loci of the two datasets were used as input for the coalescent-based phylogenetic method ASTRAL-III (13), and these two datasets generated a consensus topology (*SI Appendix*, Fig. S5 and Fig. 1*A*). The overall phylogenetic relationship of three lineages of marine mammals is consistent with previous studies (8, 14–16). For cetaceans, they support the monophyly of Physeteroidea + Kogiidae, Delphinidae, Monodontidae + Phocoenidae, and Ziphiidae among odontocete taxa, with Physeteroidea as the most basal clade of odontocetes, consistent with a recent large-scale phylogenomic analysis of cetaceans (17). For pinnipeds, there is support for a sister group relationship between Musteloidea and Pinnipedia and the monophyly of Odobenidae + Otariidae, consistent with studies based on mitochondrial DNA (18).

We further assessed divergence times for each marine mammal phylogenetic tree node (*SI Appendix*, Fig. S7). The divergence time between Cetacea and Hippopotamidae was estimated to be ~55.5 Mya, which coincides with the Paleocene–Eocene

Table 1. Assembly statistics for the 17 novel marine mammal genomes generated for this study

Species	Common name	Genome size (Gb)	Contig N50 (Kb)	Scaffold N50 (Mb)	Repeat (%)	Gene number	Gene BUSCO completeness (%)
Balaenoptera edeni	Bryde's whale	2.37	66.6	103.91	47.22	20,809	92.40
Balaenoptera musculus	Blue whale	2.43	79.13	8.28	35.84	20,083	97.90
Kogia sima	Dwarf sperm whale	2.59	55.05	26.52	39.10	20,300	96.80
Kogia breviceps	Pygmy sperm whale	2.56	54.19	21.62	32.98	20,947	94.40
Tursiops aduncus	Indo-Pacific bottlenose dolphin	2.41	105.24	104.71	39.03	20,188	99.00
Lagenorhynchus obliquidens	Pacific white-sided dolphin	2.46	281.8	30.04	40.18	20,502	96.90
Lagenorhynchus australis	Peale's dolphin	2.39	54.71	0.38	39.31	20,417	93.80
Grampus griseus	Risso's dolphin	2.66	259.31	103.28	42.54	20,534	98.20
Pseudorca crassidens	False killer whale	2.43	152.55	32.5	37.83	20,380	99.40
Neophocaena phocaenoides	Indo-Pacific finless porpoise	2.62	8.27	0.43	40.99	20,215	88.60
Mesoplodon densirostris	Blainville's beaked whale	2.47	29.93	0.8	45.98	20,686	96.10
Zalophus californianus	California sea lion	2.48	95.48	139.61	36.17	20,375	94.80
Otaria byronia	South American sea lion	2.45	137.42	144.69	40.90	20,286	95.30
Arctocephalus australis	South American fur seal	2.49	110.5	140.1	36.13	20,398	99.00
Phoca vitulina	Harbor seal	2.39	133.1	38.77	36.24	20,537	99.10
Phoca largha	Spotted seal	2.39	172.73	59.25	39.73	20,139	98.80
Pusa sibirica	Baikal seal	2.43	147.18	157.52	36.34	20,413	98.90

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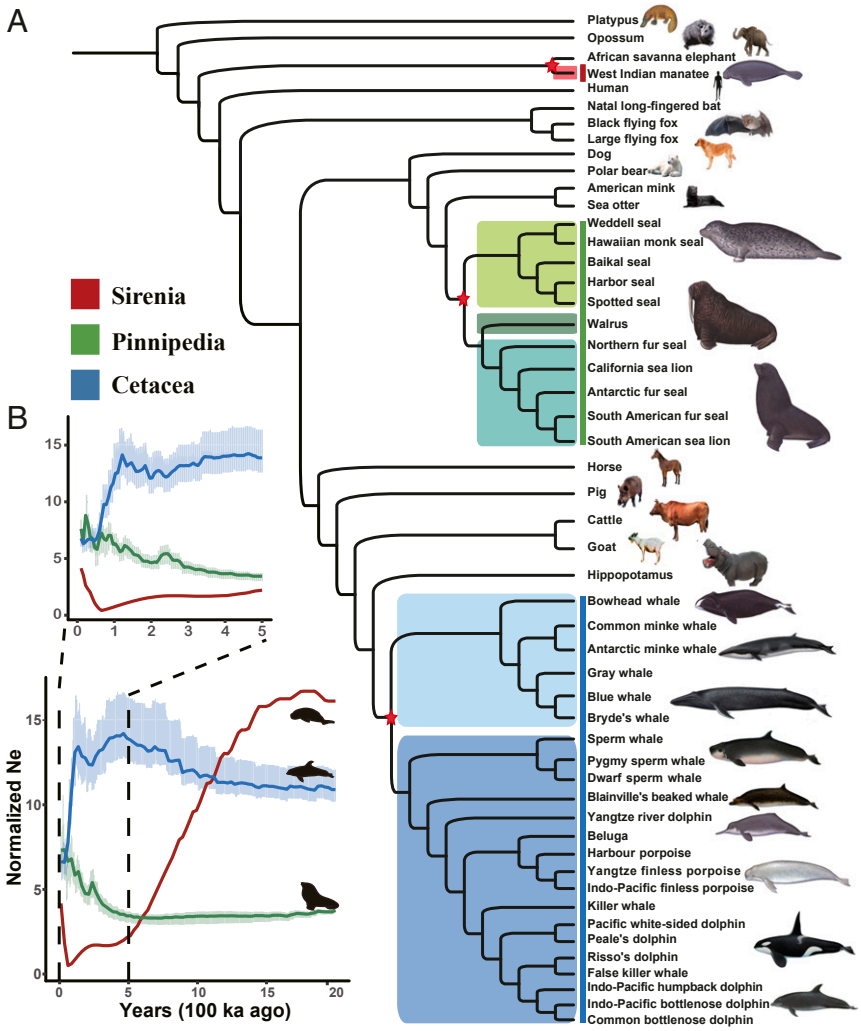

Fig. 1. Phylogeny and population changes of marine mammals. (*A*) A maximum likelihood phylogenetic tree of 35 marine mammal species and 16 outgroup mammal species. Three lineages of marine mammals are distinguished by columns of different colors: Cetacea (blue), Pinnipedia (green), and Sirenia (red). Red stars represent the species differentiation node mentioned in the main text. (*B*) Population size history of three lineages of marine mammals. The normalized effective population size (N_e) of each species was estimated using pairwise sequentially Markovian coalescent. The N_e for each group of marine mammals is shown.

transition and a global temperature rise, which possibly prompted terrestrial mammals to enter the sea (19). The initial split of Mysticeti (baleen whales) and Odontoceti (toothed whales) was about ~37.7 Mya. The emergence of Pinnipedia was estimated to be 27.4 Mya, while the divergence time between Odobenidae and Otariidae was about 18.6 Mya. The divergence time of sirenians and the African savanna elephant, their closest land relative, was estimated to be ~63.9 Mya.

We also reconstructed the demographic histories of cetaceans, pinnipeds, and sirenians (*SI Appendix*, Table S10). The three marine mammal lineages were found to experience different historical changes in population size (see normalized average effective population size, N_e, in Fig. 1*B* and individual species profiles in *SI Appendix*, Fig. S8). Specifically, the N_e of cetaceans experienced a rapid decline during the last 500,000 y. Consistently, the heterozygosity rate of most cetaceans is even lower than the endangered giant panda [~1.32‰ (20, 21)] (*SI Appendix*, Table S11), highlighting the ongoing conservation needs of cetacean species.

Genome Evolution of Marine Mammals. We compared the genome sizes of the three marine mammal lineages with their terrestrial relatives: Cetacea versus Ruminantia, Pinnipedia versus Canidae, and Sirenia versus Proboscidea. The average genome size of Pinnipedia (2.4 Gb) and Sirenia (3.1 Gb) was similar to their terrestrial sister taxa (Fig. 2*B*). In contrast, the genome size of cetaceans ranged from 2.4 to 2.6 Gb and displayed a decreasing trend compared to Ruminantia (~2.8 Gb in reindeer, cattle, and goat), their most closely related lineage (Fig. 2*B*). Consistent with the genome size comparisons, pinnipeds and sirenians present similar repeat contents to their terrestrial sister taxa, while cetacean genomes have ~10% fewer repeats than ruminants. Five subtypes of repeats are more abundant in ruminant species (*SI Appendix*, Table S12), including LINE/RTE-BovB, LTR/ERV1, LTR/ERVK, SINE/Core-RTE, and SINE/tRNA-Core-RTE. In addition to several reported large fragments in ruminant genomes (22), we found 11 large (>1.5 Mb) deletions and three large insertions (*SI Appendix*, Tables S13–S15) in cetaceans, compared to their terrestrial counterpart cattle.

Based on the eight chromosome-level genome assemblies that we generated (*SI Appendix*, Fig. S1) and two publicly available chromosome-level genomes [(sperm whale (23) and Indo-Pacific humpback dolphin (24)], we reconstructed the ancestral chromosomes of Cetacea (using the Indo-Pacific bottlenose dolphin

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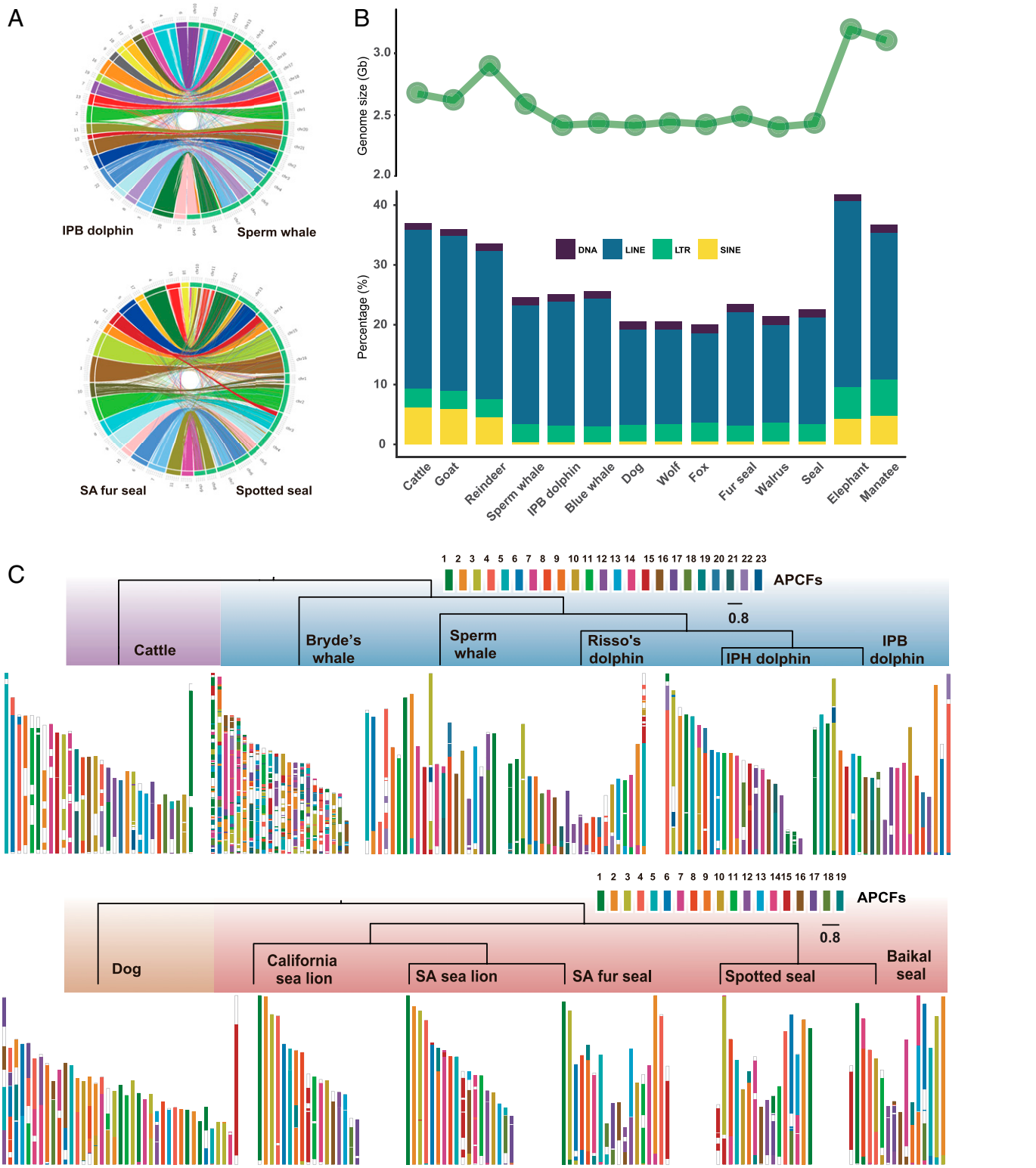

Fig. 2. Structural characteristics and chromosome evolution of marine mammal genomes. (*A*) Circos plot of representative genomes of marine mammals: sperm whale, Indo-Pacific bottlenose dolphin (IPB dolphin), South American fur seal (SA fur seal), and spotted seal. (*B*) Genome sizes and transposable element content analysis of representative genomes of marine mammals. We selected three Ruminantia species, three cetacean species, three Canidae species, three pinniped species, an elephant, and a manatee. (*C*) Chromosome evolution of Cetacea and Pinnipedia. We reconstructed 23 and 19 ancestral chromosomes of Cetacea and Pinnipedia, respectively. The chromosome assignment to ancestral chromosomes is shown by colored bars, Indo-Pacific humpback dolphin (IPH dolphin).

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as the reference genome) and Pinnipedia (using the South American sea lion as the reference genome) with DESCHRAMBLER (25) at 300-kb resolution (Fig. 2*C*). In Cetacea, we identified 1,308 conserved segments and reconstructed 23 ancestral predicted chromosome fragments (APCFs), with a total length of 2.09 Gb. In Pinnipedia, we identified 194 conserved segments and reconstructed 19 APCFs, with a total length of 1.84 Gb. We traced back the source of these APCFs for both lineages and found there are fewer chromosome rearrangement events in Pinnipedia than in Cetacea (Fig. 2*C*).

Evolution of Genes and Gene Families. We next assessed the expansion and contraction of gene families, positively selected genes (PSGs), and rapidly evolving genes (REGs) in the three marine mammal lineages. In total, 44, 29, and 212 gene families were identified as expanded, and 87, 15, and 12 gene families were contracted in the ancestor node of Cetacea, Pinnipedia, and Sirenia, respectively (*SI Appendix*, Fig. S9). Functional enrichment analysis of these gene families revealed that "olfactory transduction" is the only shared contracted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (*SI Appendix*, Table S16). Several expanded gene family-associated KEGG pathways are shared among two types of marine mammals: "thermogenesis" and "oxidative phosphorylation" in Cetacea and Pinnipedia and neural plasticity (as suggested by the "alcoholism" pathway) and "estrogen signaling" in Pinnipedia and Sirenia (*SI Appendix*, Table S17).

To assess the selective pressures acting on marine mammal genomes, we estimated the d_N/d_S ratio (ω) using 7,252 orthologous, protein-coding genes. When compared with terrestrial outgroups, marine mammal branches always had a higher d_N/d_S ratio (*SI Appendix*, Fig. S10). We identified 5, 11, and 16 PSGs and 21, 17, and 295 REGs in the ancestral branches of Cetacea, Pinnipedia, and Sirenia, respectively (*SI Appendix*, Tables S18 and S19 and Fig. S9) (χ^2 test, $P < 0.05$). We found that cystic fibrosis transmembrane conductance regulator (*CFTR*) underwent rapid evolution in both Pinnipedia and Sirenia. *CFTR* plays a vital role in the transport of various ions across the cell membrane, water transport, and fluid homeostasis (26, 27).

Conserved Noncoding Elements and ATAC-Seq. We identified 4,518,724 and 4,341,059 conserved noncoding elements (CNEs) in Cetacea and Pinnipedia, respectively. We further performed assay for transposase-accessible chromatin sequencing (ATAC-seq) (28) of two cetaceans (Indo-Pacific bottlenose dolphin and Risso's dolphin) and two pinnipeds (Baikal seal and South American sea lion) to identify CNEs associated with open chromatin (i.e., accessible to the transcriptional machinery). A total of 1,158 and 1,684 genes in Cetacea and Pinnipedia, respectively, have CNEs with ATAC-seq signal peaks within 3 kb upstream or downstream (*SI Appendix*, Tables S21 and 22). Of these genes, 371 have CNE peaks in both marine orders (*SI Appendix*, Table S23 and Fig. S11). Although further experimental work could be a worthwhile attempt to assess the contribution of these CNEs, our results provide a valuable resource for further studies on gene regulation in marine mammal species.

Signals of Convergent Evolution among Marine Mammals. The evolution of marine mammals, the adaptation of terrestrial mammalian lineages to life histories dependent on the marine environment, is considered a seminal example of convergent evolution. The degree to which convergence is reflected at the molecular level can be addressed using genomics. Understanding this phenomenon addresses key questions about redundancy, pleiotropy, and the relationship between genotype and phenotype. We applied the "Convergence at Conservative Sites" method (29) to investigate convergent genes in the three lineages of marine mammals. Orthologous genes were assigned by synteny alignment (*SI Appendix*, *SI Materials and Methods*). We identified 195 convergent amino acid substitutions in 172 genes among marine mammals (*SI Appendix*, Tables S24). Only three genes (*FAM20B*, *NFIA*, and *KYAT1*) share convergent amino acid substitution in all three marine mammal lineages. Six genes (*HERC1*, *MITF*, *EPG5*, *FAT1*, *SYNE1*, and *ATM*) show convergent mutations at different amino acid positions in cetacean manatee and pinniped manatee. For example, *MITF* has an L10F substitution in cetaceans and sirenians (the manatee) and a T570A substitution in pinnipeds and the manatee. Among the 94 genes with convergent amino acid substitutions in the fully aquatic cetaceans and Sirenia, but not between the amphibious pinnipeds in either cetaceans or Sirenia, five genes are within the KEGG pathway "dopaminergic synapse" (though the adjusted *P* value is not significant at the 0.05 level: $P = 0.51$; *SI Appendix*, Table S25). Previous studies indicate that *UCP1* has been independently lost in many marine mammals, especially in cetaceans and sirenians (30, 31). We confirm and extend this inference, showing that a functional *UCP1* is present in most pinnipeds, except for the Antarctic fur seal, which is the most polar of the species included in this assessment (*SI Appendix*, Table S26 and Fig. S12).

Genetic Changes Related to Cetacean Traits. Cetaceans have many unique biological characteristics, including echolocation, deep diving, and large variation in body size. The molecular basis of echolocation has been well studied previously (32–34). However, based on more comprehensive data, we systematically reanalyzed the 504 hearing-related gene sequences in 40 species, including two groups of echolocating bats (group M: big brown bat, Natal long-fingered bat, Brandt's bat, and little brown bat and group G: greater horseshoe bat) and 16 toothed whales (group T) (*SI Appendix*, Fig. S13). A total of 64 genes were identified as convergent genes, most reported in previous studies (*SI Appendix*, Table S27).

We next compared the four whale species with the best diving abilities to 20 comparatively shallow-diving species to study the genetic basis of deep diving in cetaceans. The deep-diving species are sperm whale (reported to dive to 1,860 m for >1 h) (35), Blainville's beaked whale (1,251 m for 57 min) (36, 37), and dwarf and pygmy sperm whales [species in the family Kogiidae with highly similar ecology and habitat (up to 1,425 m for 43 min) (38–40)]. We retrieved 1,803 genes from HypoxiaDB, a hypoxia-regulated protein database (41), and observed 39 genes with at least one specific amino acid change unique to the deep-diving group (*SI Appendix*, Table S28). MB encodes myoglobin, a protein critical for oxygen storage and transport (42). Deep-diving species have amino acid residue changes associated with elevated myoglobin net surface charge and maximal dive time (43). Compared with background branches, 45 genes showed significantly higher d_N/d_S ratios in deep-diving species (*SI Appendix*, Table S29) (χ^2 test, $P < 0.05$). We detected 45 REGs in deep-diving cetaceans. Of these, three genes (*SETX*, *GIF*, and *TMPRSS11D*) had d_N/d_S values above 1, indicating positive selection. Seven REGs (*CEP170*, *DHCR7*, *DSP*, *GBE1*, *PLD1*, *SETX*, and *TMPRSS11D*) have shared amino acid mutations in the four deep-diving species.

Cetacean bodyweight spans orders of magnitude from 50 kg (the vaquita, *Phocoena sinus*) up to 180,000 kg (the blue whale, *Balaenoptera musculus*) (44). We selected a set of 1,528 genes involved in body size development and estimated their d_N/d_S ratios in cetaceans with large body size: the blue whale (3) and the sperm whale (3). Compared to the background, we found 102 REGs (with significantly higher d_N/d_S) in giant cetaceans (*SI Appendix*, Table S30 and Fig. S14) (χ^2 test, $P < 0.05$). These REGs were enriched in the Hedgehog and Wnt signaling pathways essential for bone development (45) (*SI Appendix*, Table S31). Additional bone development–related genes with a higher d_N/d_S in giant cetaceans include *BMP1* in the TGF-β signaling pathway and the Notch signaling pathway genes *SNW1* and *CTBP2*.

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Discussion

Convergent Evolution in Marine Mammals for Aquatic Adaptation.

Thermoregulation strategies. Most mammals are endothermic, and it would have been a challenge for them to shift from a terrestrial environment to an aquatic environment with a faster heat transfer rate and (usually) lower temperatures. Previous phenotypic observations of marine mammals have shown that all marine mammals have blubber, a subcutaneous, insulating layer of fat and connective tissue, which is essential for limiting heat loss (46). It should also be noted that the thickness of the blubber varies in species according to their habitat (47). For example, in the case of sirenians, the extinct Steller's sea cows distributed at the Arctic Circle had 8- to 10-cm thick blubber, while West Indian manatees living in Florida have 1- to 2-cm thick blubber (48). A convergent amino acid substitution was observed in cetaceans, pinnipeds, and sirenians in the domain region of NFIA, in a site highly conserved across vertebrates. It should also be noted that *NFIA* is among only three genes that have convergent amino acid substitution in all the three marine mammal lineages. The *NFIA* gene is essential for determining the fate of multipotent precursors into skeletal muscle and adipocyte (white and brown) precursors (49). The up-regulation of *NFIA* can differentiate multipotent precursors into brown adipocytes and vice versa toward muscle cells and white adipocytes (50). We propose that the convergent NFIA amino acid substitution in three marine mammal lineages is associated with blubber development.

In addition to thermal insulation, it is also important for marine mammals to rapidly transfer heat from heat-producing tissues to other parts of the body. The heat transfer of marine mammals is carried out by a countercurrent heat exchange system achieved by extraordinarily well-developed retia mirabilia—vascular structures composed of interwoven arteries and veins. The retia mirabilia of marine mammals are mainly found in poorly insulated regions and effectively limits heat loss to the aquatic environment through the mutual exchange of heat between venous and arterial blood. We observed that five genes with vascular development roles [*MFN2*, *FILIP1L*, *PTPRB*, *THBS1*, and *SEMA3E* (51–55)] exhibited convergent genomic variations in multiple, not all, marine mammal lineages (Fig. 3*A*). Within these genes, *SEMA3E* (encoding semaphorin 3E) is essential for vascular patterning and angiogenesis (55–58) and has a convergent site in cetaceans and pinnipeds (Fig. 3*D*). In mice, *SEMA3E* mediates endothelial cell positioning and patterning of the developing vasculature. Its presence results in highly branched plexus forms, which coincides with the retia mirabilia phenotype (55–58) (Fig. 3*D*). We hypothesize that these genetic changes contribute to the development of the retia mirabilia of marine mammals, ensuring better heat transfer in the body and allowing for a balanced overall temperature—to reduce heat loss and avoid excessively high or low body temperatures.

Heat production is another essential aspect of thermoregulation. Most mammals have a small but highly specialized tissue, brown adipose tissue, which provides heat by consuming white adipose cells and is the most important organ for thermogenesis

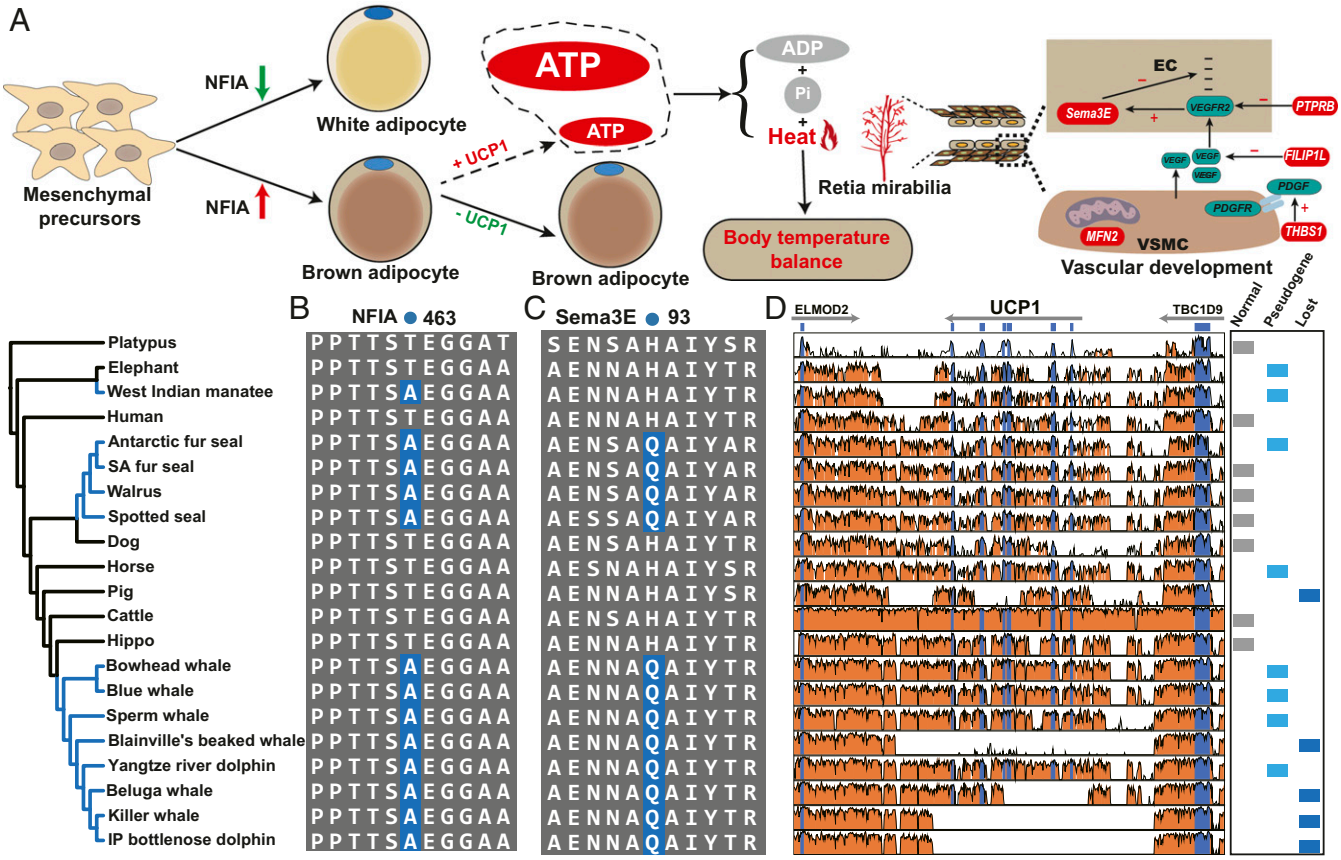

Fig. 3. Convergent evolution of thermoregulation in marine mammals. (*A*) Schematic diagram of thermoregulation in marine mammals. Up- or down-regulation of nuclear factor I A (NFIA) affects the cell fate of mesenchymal precursors, the integrity of UCP1 gene affects the fate of brown adipocyte, and the well-developed retia mirabilia in marine mammal aids in the heat transfer to maintain body temperature balance. VSMC, vascular smooth muscle cell; EC, endothelial cell. (*B*) A unique amino acid change in the *NFIA* gene of marine mammals. Shared amino acid change are highlighted in blue, IP, Indo-Pacific. (*C*) A unique amino acid change in the Sema3E gene of cetaceans and pinnipeds. Blue highlighting indicates the shared amino acid change. (*D*) VISTA sequence conservation plot of the *UCP1* gene, using goat (ARS1) as a reference.

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in mammals. However, a previous study based on 133 mammals showed that mammals adapted to extreme cold conditions, including cetaceans and sireneans, tend to show the inactivation of *UCP1* (30). Consistent with the previous study (30), our newly sequenced cetacean genomes suggest that *UCP1* is reduced to a pseudogene in baleen whales and lost in toothed whales (*SI Appendix*, Table S26). In addition, the newly sequenced pinnipeds suggested that all but the Antarctic fur seal have intact *UCP1* coding sequences. Our observations on *NFIA*, *SEMA3E*, and *UCP1* lead us to suggest that limiting heat loss to the environment is more critical than increasing heat production in fully aquatic marine mammals.

Altered skeletal systems. During the transition from terrestrial to aquatic habitats, the skeleton of marine mammals also underwent remarkable modifications in bone morphology and soft tissue distribution (7, 59). On the one hand, the change in body shape allowed for better swimming ability. On the other hand, a larger size and a fusiform shape are beneficial for maintaining body temperature. In vertebrates, most bones develop through a process of endochondral ossification, during which chondrocytes secrete a cartilage matrix rich in proteoglycans (PGs) (60), and long bones (such as the forelimb) develop from intermediate cartilage that is progressively replaced by bone (61). The genes *XYLT1* and *FMA20B* are the two most critical genes for PG synthesis, in which *XYLT1* initiates glycosaminoglycan side chain outgrowth onto PG protein cores by transferring xylose to serine residues (62, 63), while *FAM20B* phosphorylates xylose on glycosaminoglycan side chains (62). Mutations in these two genes usually have severe skeletal effects, especially in forelimb development (62–66). Interestingly, the gene *FAM20B* is among the only three genes with convergent amino acid substitutions in the three marine mammal lineages, while the gene *XYLT1* has convergent substitutions in cetaceans and sirenians (Fig. 4*A*). Therefore, these genetic innovations may play pivotal roles in the musculoskeletal system of marine mammals.

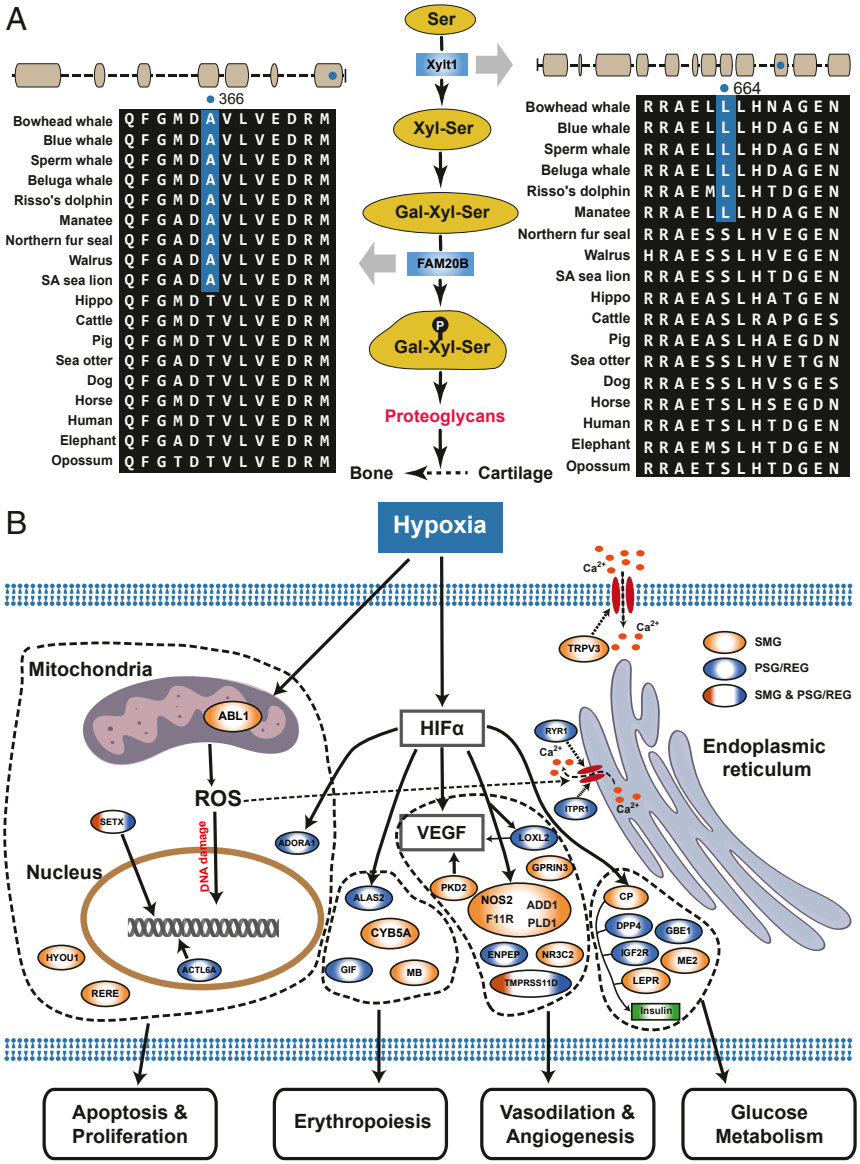

Fig. 4. Various genomic changes potentially related to aquatic adaptations. (*A*) PG biosynthesis pathway. Genes with roles in the PG biosynthesis pathway have unique amino acid changes (highlighted in blue) in marine mammals. Ser, serine; Xyl, xylose; Gal, galactose; P, phosphorylate and SA, South American. (*B*) Diagram of genes that specifically change in the deep-diving group is divided into four parts according to the gene functions, highlighting specific mutation genes (SMGs) (orange) and PSGs/REGs (blue).

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Red blood cell oxygen transport. The efficient uptake, transport, and storage of oxygen is also a challenge for marine mammals (67). Previous studies have shown that the red blood cells of marine mammals have higher hemoglobin content and tolerate rapid hydrostatic pressure changes during deep diving (68–71). The red cell membrane cytoskeletal network consists of spectrin, actin, and protein 4.1R (72). Protein 4.1R in mature red blood cell is a key component of the erythroid membrane skeleton, regulating red cell morphology and mechanical stability (73–76). The gene *EPB41* (encoding the protein 4.1R) shares a convergent site in cetaceans and pinnipeds and is under rapid evolution in the sirenian lineage (*SI Appendix*, Fig. S15). The convergent substitution may mediate the efficient oxygen storage of marine mammals.

Specific genetic innovations in three marine mammal lineages. Although the three marine mammal lineages share the convergent, evolved genes associated with their aquatic adaptation, we also observed many lineage-specific changes. For the echolocation ability specific to toothed whales, we conducted a systematic convergent evolutionary analysis. Most of the convergent genes we observed have been identified in previous works, but we did find several convergent genes (*OTOS*, *ATP2B2*, and *PGAP1*) associated with echolocation that have not been revealed before. Within them, *OTOS*, which encodes otospiralin in the fibrocytes of the cochlea and vestibule, is essential for the survival of the neurosensory epithelium (77, 78). Cetaceans exhibit a wide range of diving behaviors, ranging from short, shallow dives to long, deep dives (79). The challenge of diving is to overcome the damage caused by hypoxia, and glutathione is an antioxidant that protects important cell components from reactive oxygen species induced by hypoxia (80–82). A previous study indicated that several genes in the glutathione metabolism pathway have cetacean-specific amino acid changes (83). We found that the gene *GSR* (glutathione-disulfide reductase) is among 21 REGs in cetaceans using our dataset (*SI Appendix*, Table S19). The data suggest that the common ancestor of cetaceans may have provided the genetic basis for deep dives. By examining genes specifically changed in deep-diving species, we observed that multiple genes that have interactions with HIF-α, which functions as the primary regulator of cellular and systemic homeostatic response to hypoxic stress (84, 85), were under positive selection or had unique amino acid substitutions in deep-diving species. For example, the gene *ALAS2* is under positive selection in deep-diving species. This gene is regulated by HIF-α in erythroid cells (86) and encodes a protein that catalyzes the first and rate-limiting reaction in the heme biosynthetic pathway. We assigned the PSGs, REGs, and specific mutation genes in the deep-diving species to their respective, related biological processes to provide a resource for subsequent studies (Fig. 4*B*).

For the 11 PSGs in pinnipeds, three PSGs are dim-light, vision-related genes (*RP1*, *CRYGN*, and *MYO7A*) (87–89), which may reflect enhanced, dim-light vision as an adaptation to underwater predation (90). In comparison, genes involved in cone-mediated vision (i.e., in bright light) are pseudogenized in mysticetes and deep-diving odontocetes (91). This is consistent with pinnipeds mainly employing vision for navigation, while cetaceans rely on hearing (including echolocation in odontocetes). The REGs of sirenians are enriched in a function associated with gastric acid secretion (*SI Appendix*, Table S20), possibly reflecting their strict herbivorous diets.

Conclusions

The shift from a terrestrial to an aquatic habitat in several independent lineages is considered a key example of macroevolutionary transitions in the history of mammals. We have generated a comprehensive genomic dataset of marine mammals, providing 17 high-quality cetacean and pinniped genomes. Our exploration of three dozen genomes provides a well-resolved phylogeny and insights into the demography and genome evolution of marine mammals. We document numerous idiosyncratic and convergent genomic variations possibly contributing to the transition to aquatic life in cetaceans, pinnipeds, and sirenians—providing a rich resource for further experimental and computational research.

Methods

Details of samples and materials are shown in *SI Appendix, SI Materials and Methods*. Sequencing libraries including whole-genome sequencing, 10× Genomics, Hi-C, and ATAC were described in *SI Appendix, SI Materials and Methods*. Tools and parameters for genome assembly and annotation are shown in *SI Appendix, SI Materials and Methods*. Genome evolution analyses including genome sizes, gene flow, phylogenetic trees, demographic history, gene families, and PSGs are also detailed in *SI Appendix, SI Materials and Methods*.

Data Availability. Genome assembly and raw sequencing data have been deposited in China National GeneBank Nucleotide Sequence Archive (CNP0000758). All other study data are included in the article and/or supporting information.

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