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Evolutionary analysis of genes associated with the sense of balance in semi-aquatic mammals

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Abstract

Background Semi-aquatic mammals represent a transitional phase in the evolutionary spectrum between terrestrial and aquatic mammals. The sense of balance is crucial for mammalian locomotion, and in semi-aquatic mammals, the structural foundation of this sense (the vestibular system) shows distinct morphological adaptations to both aquatic and terrestrial environments compared to their terrestrial counterparts. Despite this, the precise molecular mechanisms driving these adaptations remain elusive. Our study endeavors to unravel the genetic components associated with the sense of balance in semi-aquatic mammals and to examine the evolutionary trajectories of these genes, shed light on the molecular mechanisms underlying the adaptive evolution of balance perception in semi-aquatic mammals.

Results We selected 42 mammal species across 20 orders, 38 families, and 42 genera for analysis. We analyzed a comprehensive set of 116 genes related to the vestibular system's development or function. Our findings indicate that 27 of these genes likely experienced adaptive evolution in semi-aquatic mammals. Particularly, genes such as *SLC26A2*, *SOX10*, *MYCN*, and *OTX1* are implicated in collectively orchestrating morphological adaptations in the semicircular canals to suit semi-aquatic environments. Additionally, genes associated with otolith development, including *SLC26A2*, *OC90*, and *OTOP1*, likely regulate otolith sensitivity across various locomotor modes. Moreover, genes linked to vestibular disorders, such as *GJB2*, *GJB6*, and *USH1C*, may provide a molecular foundation for averting vertigo amidst intricate locomotor scenarios in semi-aquatic mammals.

Conclusions Our research offers insights into the molecular mechanisms underlying the evolution of the sense of balance in semi-aquatic mammals, while also providing a new research direction for the adaptive evolution of mammals undergoing a secondary transition to an aquatic lifestyle.

Keywords Semi-aquatic mammals, Vestibular system, Sense of balance, Adaptive evolution, Molecular basis

Background

The sense of balance is vital to the survival of animals, allowing for spatial orientation and stable posture during movement [1]. It is essential for perceiving self-motion, maintaining postural control, and stabilizing during locomotion, often considered the animal's sixth sense [2, 3]. The vestibular system serves as the anatomical cornerstone for generating, perceiving, and exercising balance. A part of this system comprises three semicircular

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canals (the anterior, posterior, and horizontal canals) that detect rotational movements in all directions Another component of the vestibular system includes two otolith organs—the utricle and the saccule, which are positioned horizontally and vertically, respectively, and are responsible for detecting linear acceleration and head tilt in the horizontal and vertical planes [1, 2, 4].

The vestibular system is crucial for maintaining balance and has undergone significant evolutionary selection related to locomotion [5]. Specifically, the size and morphological variability of the semicircular canals within the bony labyrinth, such as roundness, orthogonality, and aspect ratio, are closely associated with locomotor mode [1, 6]. A comparative study of these canals in mammals with diverse locomotor behaviors, including burrowing, arboreal, slithering, and subterranean living, reveals a correlation between canal morphology and locomotor modes. Moreover, there is a significant difference in the declination angle of the semicircular canals between subterranean-living taxa and slithering taxa [7].

With the secondary adaptation of certain vertebrates to aquatic environments, their vestibular system undergoes alterations, leading to distinctions from their terrestrial relatives. A morphological comparison of semicircular canals in musteloids across different habitats showed notable differences between semi-aquatic and terrestrial species. Semi-aquatic otters and minks exhibit elliptical anterior semicircular canals, increased curvature of the horizontal semicircular canals, and reduced angles between posterior and horizontal canals. All of these morphological features correlate with the animal's sensitivity to detecting head movements [8]. Cetaceans, have uniquely small semicircular canal arcs relative to body weight, thus reducing the sensitivity of their vestibular system and likely matching their rapid body-rotation behavior [9]. Similarly, aquatic manatees and semi-aquatic seals exhibit reduced semicircular canals [10–12]. Since the Mesozoic era, numerous reptiles transitioned from terrestrial to aquatic habitats, including the extinct thalattosuchians. Marine-dwelling thalattosuchians species displayed smaller labyrinth, wider semicircular canal diameters, and enlarged vestibules akin to those seen in other marine reptiles and cetaceans, aiding in swimming [11]. The canal shape variations in amniotes also suggest a pattern linked to the transition from terrestrial to aquatic locomotion. Physical differences exist between air and water, so the vestibular system differs in terrestrial and aquatic species [13].

The vestibular system is essential for balance and spatial orientation, and extensive research using mice and zebrafish has investigated its genetic basis. These studies seek to understand how genes influence vestibular development and behavior, as well as the molecular causes of vestibular disorders in humans [4, 14, 15].Proper development of the vestibular system relies on the precise temporal expression and regulation of various genes [16]. Gene deletions or mutations can disrupt semicircular canal formation, as seen in mice with BMP2 gene knockouts lacking all three canals [17], or the absence of specific canals due to mutations in genes like *OTX1* [18] and *FGF10* [19]. Furthermore, specific genes play a role in the formation of otoliths, hair cells, and other vestibular structures. For example, mutations in *SLC26A4* [20] and *ATP2B2* (*PMCA2*) [21] affect otolith structure. Similarly, mutations in *CELSR1* [22] and *MITF* [23] impact vestibular hair cell growth, with the latter specifically influencing saccule hair cells. The comprehensive role of genes in vestibular development is highlighted by the abnormalities in otoliths, semicircular canals, and hair cells in zebrafish embryos lacking the *HDAC1* gene [24].

Although the structural features of the vestibular system in semi-aquatic mammals differ significantly from those of terrestrial animals, the genes that are dependent on the normal development of the vestibular system have been studied extensively. Nonetheless, the molecular underpinnings behind these structural disparities remain enigmatic. Therefore, we curated a gene set linked to the sense of balance and conducted evolutionary analyses to elucidate the molecular basis of the modified vestibular system features in semi-aquatic mammals.

Results

Sequences retrieval of genes associated with the sense of balance

We selected 12 semi-aquatic mammals and 30 terrestrial mammals across the entire mammalian range as our research subjects. These 42 species belong to 42 genera, 38 families, and 20 orders (Table 1). Through literature search, we compiled a set of 116 genes related to the sense of balance (refer to Additional file 1: Table S1 for the gene list and corresponding references). Based on the constructed gene sets, an extensive collection of 4731 sequences was obtained from 42 mammalian species. Among these, 3746 sequences were directly retrieved from the NCBI database (refer to Additional file 1: Table S2 for the respective sequence search version numbers). Furthermore, a total of 985 sequences were retrieved through a comprehensive BLAST search, encompassing unannotated genomes as well as target sequences that were not previously explored within annotated genomes (see Additional file 2 for detailed raw data of all sequences). Finally, through cluster analysis and bidirectional BLAST analysis based on combinations of datasets from different species, we confirmed that all obtained sequences are orthologous genes of the 116 genes (Additional file 3).

Table 1 List of 42 mammals selected for this study

Order	Family	Genus	Species	Abbreviation
Monotremata	Tachyglossidae	*Tachyglossus*	*Tachyglossus aculeatus*	Tacu
Didelphimorphia	Didelphidae	*Monodelphis*	*Monodelphis domestica*	Mdom
Dasyuromorphia	Dasyuridae	*Sarcophilus*	*Sarcophilus harrisii*	Shar
Diprotodontia	Phascolarctidae	*Phascolarctos*	*Phascolarctos cinereus*	Pcin
	Phalangeridae	*Trichosurus*	*Trichosurus vulpecula*	Tvul
Cingulata	Dasypodidae	*Dasypus*	*Dasypus novemcinctus*	Dnov
Pilosa	Megalonychidae	*Choloepus*	*Choloepus didactylus*	Cdid
Hyracoidea	Procaviidae	*Procavia*	*Procavia capensis*	Pcap
Proboscidea	Elephantidae	*Elephas*	*Elephas maximus*	Emax
Macroscelidea	Macroscelididae	*Elephantulus*	*Elephantulus edwardii*	Eedw
Tubulidentata	Orycteropodidae	*Orycteropus*	*Orycteropus afer*	Oafe
Afrosoricida	Tenrecidae	*Echinops*	*Echinops telfairi*	Etel
Eulipotyphla	Talpidae	*Condylura*	*Condylura cristata*	Ccri
	Erinaceidae	*Erinaceus*	*Erinaceus europaeus*	Eeur
	Soricidae	*Sorex*	*Sorex araneus*	Sara
Scandentia	Tupaiidae	*Tupaia*	*Tupaia belangeri chinensis*	Tbel
Primates	Hominidae	*Homo*	*Homo sapiens*	Hsap
	Hylobatidae	*Nomascus*	*Nomascus leucogenys*	Nleu
	Cercopithecidae	*Macaca*	*Macaca mulatta*	Mmul
Carnivora	Mustelidae	*Enhydra*	*Enhydra lutris*	Elut
	Mustelidae	*Lutra*	*Lutra lutra*	Llut
	Mustelidae	*Aonyx*	*Aonyx cinereus*	Acin
	Mustelidae	*Neovison*	*Neovison vison*	Nvis
	Mustelidae	*Martes*	*Martes flavigula*	Mfla
	Otariidae	*Zalophus*	*Zalophus californianus*	Zcal
	Odobenidae	*Odobenus*	*Odobenus rosmarus divergens*	Oros
	Phocidae	*Phoca*	*Phoca vitulina*	Pvit
	Ursidae	*Ursus*	*Ursus maritimus*	Umar
	Felidae	*Acinonyx*	*Acinonyx jubatus*	Ajub
	Canidae	*Vulpes*	*Vulpes ferrilata*	Vfer
Perissodactyla	Equidae	*Equus*	*Equus caballus*	Ecab
Pholidota	Manidae	*Manis*	*Manis javanica*	Mjav
Artiodactyla	Hippopotamidae	*Hippopotamus*	*Hippopotamus amphibius*	Hamp
	Suidae	*Sus*	*Sus scrofa*	Sscr
	Bovidae	*Bos*	*Bos taurus*	Btau
Lagomorpha	Leporidae	*Oryctolagus*	*Oryctolagus cuniculus*	Ocun
	Ochotonidae	*Ochotona*	*Ochotona princeps*	Opri
Rodentia	Muridae	*Mus*	*Mus musculus*	Mmus
	Bathyergidae	*Heterocephalus*	*Heterocephalus glaber*	Hgla
	Cricetidae	*Mesocricetus*	*Mesocricetus auratus*	Maur
	Castoridae	*Castor*	*Castor canadensis*	Ccan
	Hydrochaeridae	*Hydrochoerus*	*Hydrochoerus hydrochaeris*	Hhyd

Note: Species scientific names and abbreviations labeled in red indicate that the species is a semi-aquatic mammal; all other species are terrestrial mammals

We used the obtained orthologous genes to construct a phylogenetic tree based on the Maximum Likelihood (ML) method and the Bayesian Inference (BI) method. The results indicate that the topological structures of the phylogenetic trees produced by both methods are consistent with the topological structure obtained from the TimeTree database (http://www.timetree.org/). We have refined the ML tree and presented it (Fig. 1). The subsequent analyses are all based on the topology of this tree.

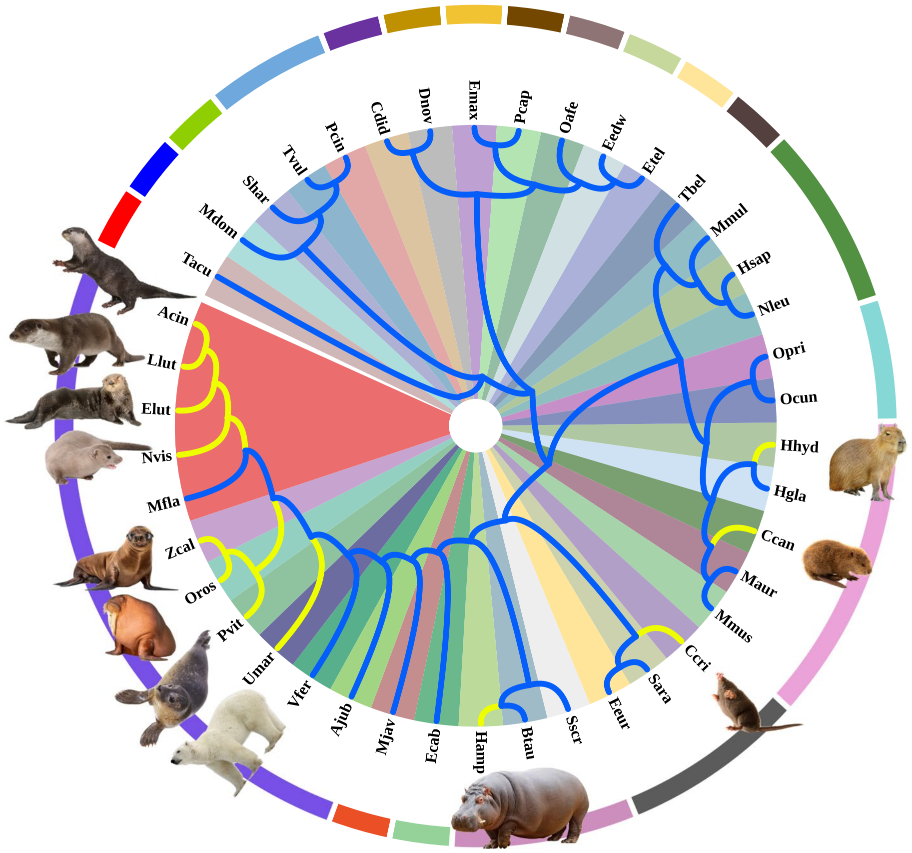

Fig. 1 Phylogenetic tree of 42 species constructed using the maximum likelihood (ML) method. The ends of the evolutionary branches denote the abbreviated Latin names of the 42 species. The branches highlighted in yellow represent the lineages of semi-aquatic species, with the corresponding animal illustrations indicating the semi-aquatic species investigated in this study, while the other branches represent terrestrial species. The varying background colors of the evolutionary branches indicate the different families to which the species belong. The outermost arcs of different colors represent the different orders to which the species belong. The full names corresponding to the abbreviated Latin names of the species, as well as the details of the families and orders to which they belong, can be found in Table 1

Selection pressure analysis

Using the branch-site model, we successfully identified two genes, *EYA1* and *SLC26A2*, under positive selection in semi-aquatic mammals (Table 2). In heterozygous mice, reducing the expression of the *EYA1* gene to only 21% of the normal level resulted in the absence of vestibular sensory formation. Notably, the *EYA1* gene showed exclusive expression during the differentiation of hair cells, highlighting its pivotal role in the development of vestibular sensory and hair cells [25]. Similarly, when the *SLC26A2* gene was knocked down in zebrafish, notable abnormalities in the morphology of otolith and semicircular canals occurred due to apoptosis, leading to imbalanced swimming behavior [26].

Recognizing the potential for positive selection in genes related to balance in terrestrial mammals, we

Table 2 List of positively selected genes in semi-aquatic group mammals (branch-site model)

Gene Name	Model	lnL	2ΔlnL	*p*_Value	Level Parameters	Positively selected sites (BEB)
EYA1	ma	−12,352.896			w0 = 0.029, w1 = 1.000, w2 = 20.619	175 S 0.981*
	ma0	−12,359.559	13.327	< 0.001	w0 = 0.029, w1 = 1.000, w2 = 1.000	
SLC26A2	ma	−21,685.825			w0 = 0.066, w1 = 1.000, w2 = 3.630	22 I 0.901; 492 E 0.885; 582 A 0.932; 593 T 0.865
	ma0	−21,687.810	3.970	0.046	w0 = 0.066, w1 = 1.000, w2 = 1.000	

Note: lnL represents log-likelihood values, and the * symbol represents posterior probability (BEB) greater than 95%

performed an extensive site model analysis on all terrestrial species using two distinct methods (comparing between the M8 and M7 models, and the M2 and M1 models). The results of the two methods (Additional file 1: Table S3 and S4) showed that a total of 16 genes exhibited significant positive selection signals in terrestrial group species but did not contain positively selected genes in the semi-aquatic group, indicating that the two genes detected in semi-aquatic mammals were specifically favored for their adaptation to the semi-aquatic mode of locomotion.

Divergent evolution genes are characterized by their experience of significantly distinct selection pressures across various evolutionary clades. To compare the evolutionary patterns of genes related to sense of balance in semi-aquatic and terrestrial groups, we utilized the Clade model C (CmC) for our analysis. The results demonstrated that 17 genes in the semi-aquatic group were classified as divergent evolution genes (Additional file 1: Table S5). In addition to the *SLC26A2* gene identified through positive selection, several other genes, including *CASP3* [27], *COG4* [28], *OTX1* [18], *SOX10* [29], and *TSKU* [30], are involved in the formation of the semicircular canals. Genes like *NOXO1* [31], *OC90* [32], *OTOP1* [33], and *SLC26A4* [20] contribute to the formation of otoliths. The absence of the *NEUROD1* gene leads to abnormalities in vestibular function [34]. The divergent evolution gene *WNT3A*, along with related genes like *WNT1*, plays a crucial role in promoting the development of the vestibule in the inner ear. Interestingly, double mutant mouse embryos lacking both *WNT3A* and *WNT1* genes exhibit a complete absence of vestibular structures [35]. Abnormalities in divergent evolution gene *HES1* and related genes like *HES5* result in an overproduction of hair cells in the utricle and saccule [36]. Furthermore, the *CDH23* [37], *GJB6* [38], *KCNA10* [39], and *USH1C* [40] genes have been linked to various types of human vestibular dysfunction disorders.

Rapidly evolved genes are protein-coding genes that undergo a higher rate of amino acid substitutions between species than average. These genes, along with positively selected genes, often play a key role in adaptive evolution in response to changing environments [41, 42]. In this study, we identified 11 genes that are undergoing rapid evolution according to the Branch model (Additional file 1: Table S6). Of these, eight were also detected by the branch-site model and Clade model C. Furthermore, the rapidly evolved genes *ADGRG6* (*GPR126*) [43], *HDAC1* [24], and *ILDR1* [44] are implicated in significant roles during the development of the semicircular canals.

Identification of genes with correlations between evolutionary rates and habitats

In phylogenetics, where each gene may demonstrate variable evolutionary rates across species, establishing correlations between gene evolutionary rates and diverse movement patterns necessitates thorough consideration. To mitigate the influence of phylogenetic factors on gene evolutionary rates, we employed the Phylogenetic Independent Contrast (PIC) approach. Our investigations revealed significant correlations between evolutionary rates and locomotor modes for 10 genes (Fig. 2). Among these, *SOX10*, *GATA2* [45], and *MYCN* [46] genes are associated with semicircular canal formation, while *OC90*, *OTOP1*, and *SIX1* [47] genes are linked to otolith formation. Furthermore, the *SLC26A2* gene is implicated in both semicircular canal and otolith formation, whereas *GJB2* [38], *GJB6*, and *USH1C* genes are associated with vestibular disorder.

Identification of convergent evolution genes

Different clades of semi-aquatic mammals may have undergone similar molecular adaptations to facilitate their transition to aquatic locomotion. We considered the likelihood of multiple amino acids with comparable biochemical properties conferring similar fitness at a given locus, we employed the PCOC (Profile Change with One Change) method to identify signals of convergent evolution by detecting shifts in amino acid properties. Our analysis revealed convergent evolution in six genes, each exhibiting changes in amino acid properties at least one locus in semi-aquatic species (Fig. 3). These genes include, in addition to those previously mentioned (*OTOP1* and *SLC26A2*), *CHD7*, which plays a crucial role in the development of semicircular canals. The complete deletion of *CHD7* results in hypoplasia of the semicircular canals and vestibular sensory organs [48]. Furthermore, the *GSDME* (*DFNA5*) gene, which also influences semicircular canal development [49], showed convergent evolution. Specific convergent sites of amino acid changes include amino acid 2152 of *CHD7*, amino acids 236 and 650 of *GSDME*, amino acid 263 of *OTOP1*, and amino acids 404 and 744 of *SLC26A2*.

Overview of different gene sets

To investigate the evolutionary patterns of genes associated with the sense of balance in mammals transitioning to a semi-aquatic locomotor mode, we delved into 27 genes implicated in maintaining balance during semi-aquatic adaptation. This gene set consisted of two positively selected genes, 17 divergent evolution genes, 11 rapidly evolved genes, 10 genes correlating

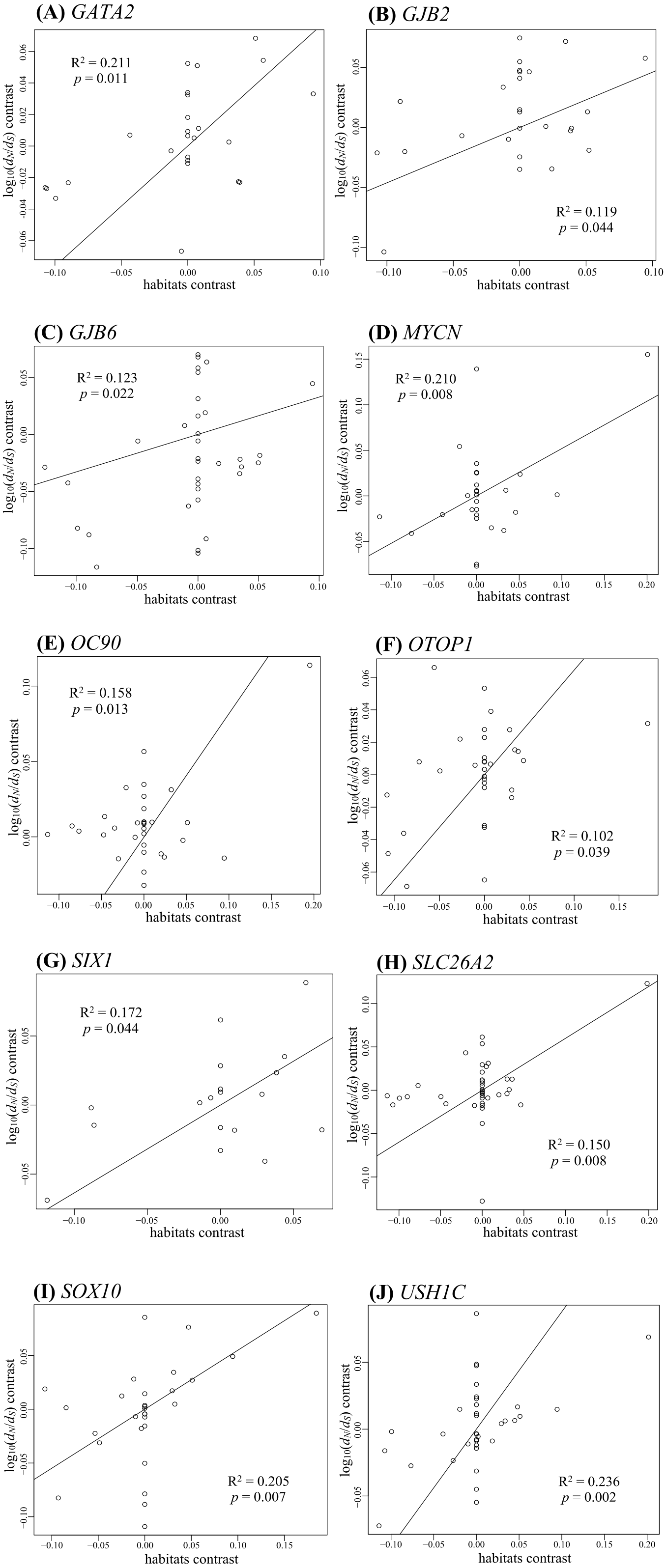

Fig. 2 Genes with significant correlation between evolutionary rate and habitat type. (**A**)—(**J**) represent the genes *GATA2*, *GJB2*, *GJB6*, *MYCN*, *OC90*, *OTOP1*, *SIX1*, *SLC26A2*, *SOX10*, and *USH1C*, respectively. The horizontal axis represents habitat types corresponding to different modes of locomotion, that is, the contrast between semi-aquatic and terrestrial habitats. The vertical axis represents the contrast of evolutionary rates of genes in species with different modes of locomotion, with values transformed by log10. All *p* values for the correlations are less than 0.05

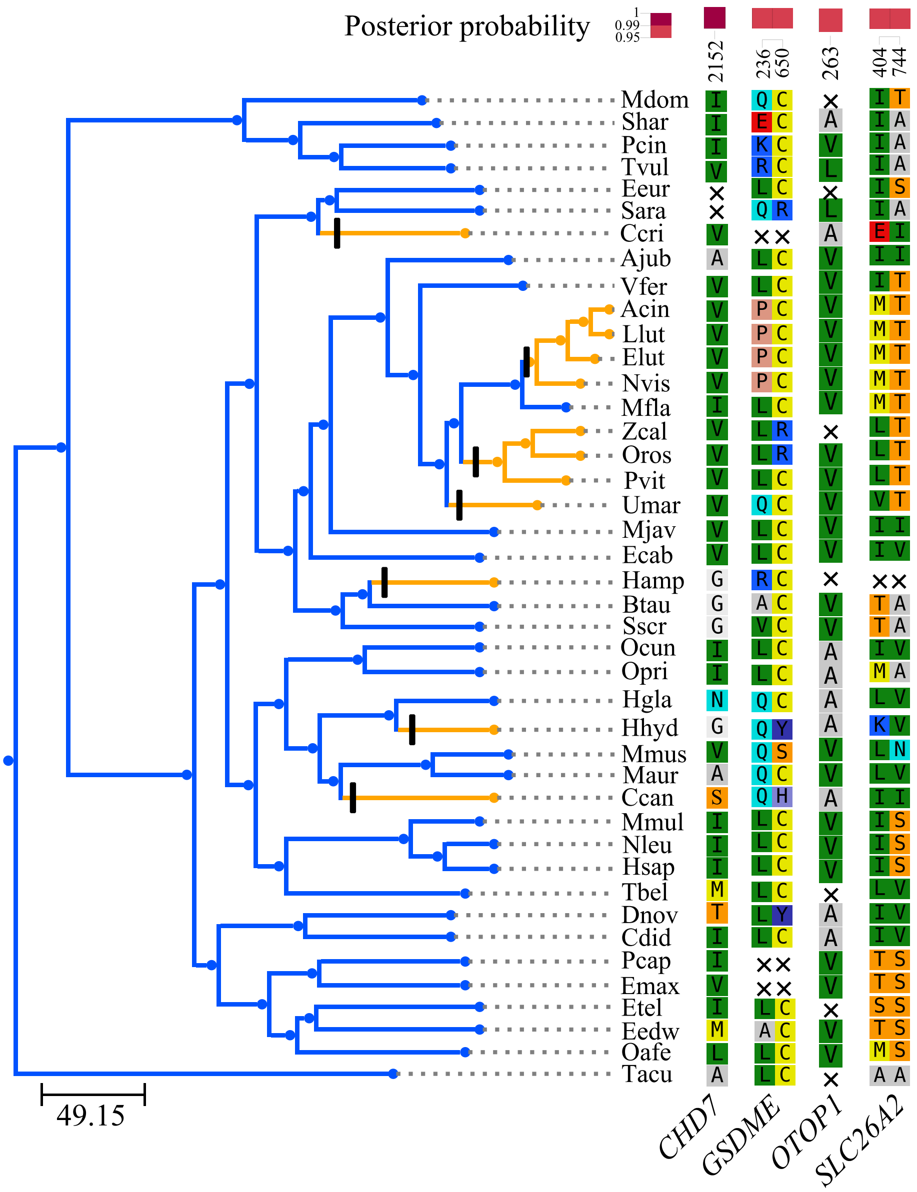

Fig. 3 Genes with convergent substitutions of amino acids exhibiting similar biochemical characteristics in semi-aquatic mammals. The left side of the figure shows a phylogenetic tree of 42 species, with the ends of the branches representing their abbreviated Latin names, corresponding to the full names listed in Table 1. Yellow highlights denote branches of semi-aquatic mammals, while black vertical lines demarcate the semi-aquatic species into different evolutionary clades, indicating independently occurring secondary aquatic adaptations. The right side lists the amino acid positions with convergent substitutions: the 2152nd of *CHD7*, the 236th and 650th of *GSDME*, the 263rd of *OTOP1*, and the 404th and 744th of *SLC26A2*. An "×" with no background color indicates that the gene was not identified in this species, and this evolutionary branch was excluded from the analysis of that gene. The posterior probabilities for each convergent site are all greater than 0.95

with semi-aquatic habitat, and six genes exhibiting convergent evolution (Fig. 4). Notably, the *SLC26A2* gene, crucial for the normal development of otoliths and semicircular canals, was consistently identified across all five datasets. Additionally, *OTOP1* was present in four datasets — divergent evolution genes, rapidly evolved genes, genes correlating with semi-aquatic habitat, and genes undergoing convergent evolution. Furthermore, the *GJB6* and *USH1C* genes were identified by all three methods, whereas the *CASP3*, *CDH23*, *KCNA10*, *OTX1*, *OC90*, and *SOX10* genes were concurrently detected by two methods. These ten genes are proposed as promising

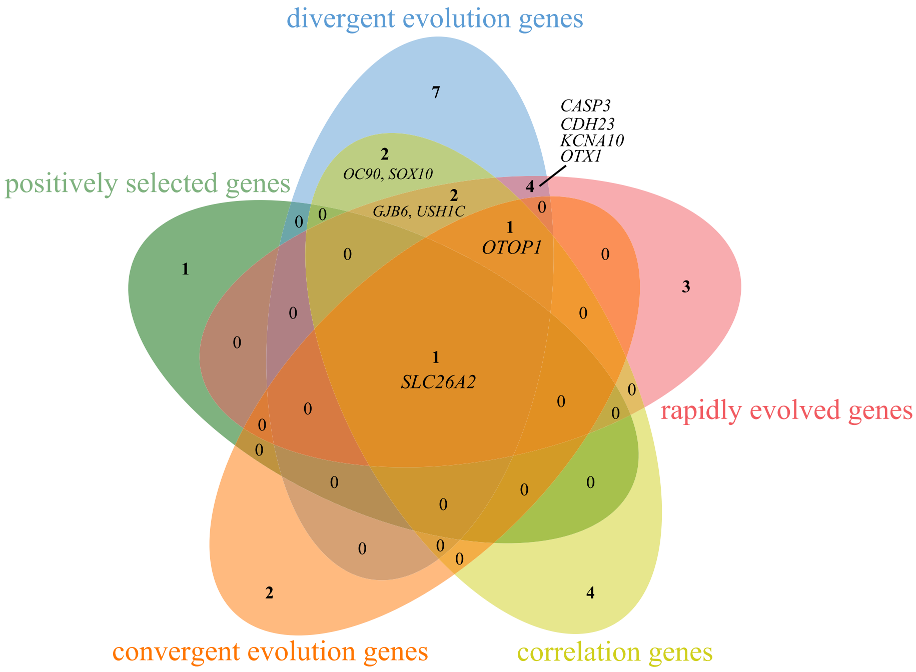

Fig. 4 Venn diagram showing the overlap between positively selected genes, divergent evolution genes, rapidly evolved genes, genes with semi-aquatic correlation, and convergent evolution genes. The *SLC26A2* gene is included in all five datasets. The *OTOP1* gene is present in the genes with correlation, convergent evolution genes, divergent evolution genes, and rapidly evolved genes. The *GJB6*, *USH1C*, *OC90*, *SOX10*, *CASP3*, *CDH23*, *KCNA10* and *OTX1* genes have also been detected by at least two methods

candidates that underwent adaptive evolution in semi-aquatic mammals to maintain locomotor balance during terrestrial ambulation and aquatic swimming.

To elucidate interactions among the 27 genes from the five datasets, we cross-referenced them with the STRING database. The analysis results indicate that there are significant interactions among the proteins of 21 genes ($p < 1.0e\text{-}16$) (Fig. 5), and they are significantly enriched in GO terms such as semicircular canal development (GO:0060872, FDR = 0.0002) and semicircular canal morphogenesis (GO:0048752, FDR = 0.0092).

Discussion

To comprehensively investigate the evolutionary patterns of genes associated with the sense of balance in semi-aquatic mammals and uncover the molecular evolutionary mechanisms driving structural adaptations of the vestibular system in these animals, we conducted an extensive study. Our research focused on 42 mammalian species, spanning 42 genera, 20 orders, and 38 families, and encompassed 12 semi-aquatic species alongside 30 terrestrial counterparts. Furthermore, we curated a list of 116 genes known to influence the development or functionality of the vestibular system through an exhaustive review of pertinent literature. This gene set can also serve as a reference for other similar studies, such as investigating the genetic basis of sense of balance evolution in mammals with other modes of locomotion (e.g., flight). It may even provide insights for medical fields, such as the treatment of vertigo.

Evolution of genes involved in semicircular canals development

The functional interplay between the overall size and morphological diversity of the semicircular canal and locomotor behaviors stands as a well-established phenomenon [1, 6]. Previous investigations have delineated significant morphological disparities in semicircular canals between semi-aquatic and terrestrial mammals [8, 10–12]. Through our analysis, we have identified 27 genes potentially pivotal for the adaptive evolution of the sense of balance in semi-aquatic mammals. Among these genes, 48.1% (13/27) — *ADGRG6*, *CASP3*, *CHD7*, *COG4*, *GATA2*, *GSDME*, *HDAC1*, *ILDR1*, *MYCN*, *OTX1*, *SLC26A2*, *SOX10*, and *TSKU* — are implicated in the development of semicircular canals. Of particular significance is the *SLC26A2* gene, notable for its involvement in positive selection, divergent evolution, rapidly evolved, semi-aquatic correlation, and convergent evolution. Similarly, the *SOX10* gene, categorized as both a divergent evolution gene and a gene with semi-aquatic correlation. These two genes emerge as potential candidates

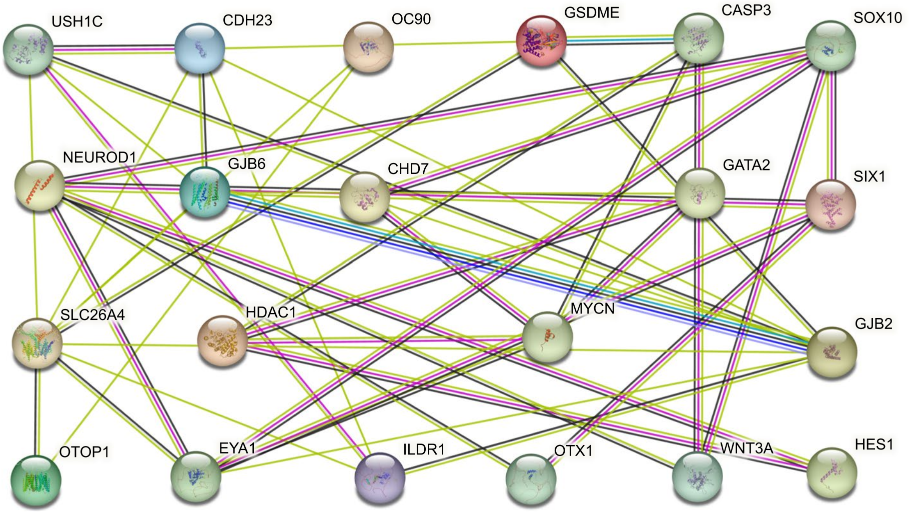

Fig. 5 The interaction network between proteins of 21 genes. Among the 27 candidate genes, there are significant interactions between the proteins of 21 genes ($p < 1.0e\text{-}16$). Each node in the network represents the protein of a gene. The filling in the nodes represents the 3D structure of the protein. The differently colored connecting lines between nodes represent different types of interaction evidence: "▬" for from database, "▬" for experimental validation, "▬" for text mining, "▬" for gene neighborhood, "▬" for gene fusions, "▬" for gene co-occurrence, "▬" for co-expression, and "▬" for protein homology

for regulating the diverse morphology of semicircular canals in semi-aquatic mammals during their adaptation to aquatic locomotion. Of the three semicircular canals, special attention is focused on the horizontal semicircular canal, which is closely associated with motor agility [8]. The *MYCN* and *OTX1* genes investigated in this study play a crucial role in the normal development of the horizontal semicircular canals. Given their correlation with semi-aquatic traits and rapid evolutionary rates, these genes may govern the development of the horizontal semicircular canal, thus enhancing the precise perception of semi-aquatic locomotor behaviors in semi-aquatic mammals.

The genes identified in association with semicircular canal development offer molecular insights into the morphological diversity of these canals in response to alterations in locomotor behaviors. Nevertheless, the complex morphogenesis of inner ear semicircular canals necessitates meticulous regulation, involving processes like cell proliferation and epithelial-mesenchymal transition [48]. The quest for identifying the precise regulatory mechanisms requires further investigation within the fields of genetics and physiology.

Evolution of genes involved in otolith development

In the utricle and the saccule, the two otolith organs, the presence of denser otoliths heightens sensitivity to gravitational forces and linear acceleration. This increased sensitivity is due to the inertia of otoliths, which resist movement and exert a shearing force on stationary cilia. This force triggers nerve impulses within the vestibulocochlear nerve [50]. Despite extensive comparative morphological analyses of the semicircular canals, there has been limited attention given to comparative anatomical investigations of mammalian otolithic organs. Prior research is constrained, with only one study highlighting the significantly thicker otolithic membranes in cetaceans compared to terrestrial and semi-aquatic mammals. However, this research lacks quantitative data and overlooks proportional effects [51].

In the present study, examination of 27 genes revealed that 25.9% (7/27) of them (*HDAC1*, *NOXO1*, *OC90*, *OTOP1*, *SIX1*, *SLC26A2*, and *SLC26A4*) are involved in otolith formation. Remarkably, among these genes, *SLC26A2* was consistently present in all five datasets, while the *OTOP1* gene appeared in four datasets simultaneously, and the *OC90* gene was also found concurrently in two datasets. The identification of adaptive evolutionary genes associated with otoliths suggests that otoliths may also have undergone selective pressures during the evolutionary process of mammalian locomotion. This implies that otoliths might have experienced modifications in their sensitivity to better suit a semi-aquatic lifestyle. Future research endeavors could explore the specific influence of these genes on otolith perception across different locomotor modalities.

Evolution of genes associated with vestibular disorders

A comprehensive investigation into the intricate molecular and cellular mechanisms governing vestibular morphogenesis is indispensable not only for elucidating the development of this complex organ but also for comprehending the etiology of vestibular abnormalities [52]. Within our study, we have discerned that 18.5% (5/27) of the genes (*CDH23*, *GJB2*, *GJB6*, *KCNA10*, and *USH1C*) constitute the genetic underpinning for vestibular disorders in humans. Notably, two of these genes (*GJB6* and *USH1C*) were concurrently identified in three datasets. The *CDH23* and *USH1C* genes are recognized as the causal agents for the USH1C and USH1D subtypes of Usher syndrome type I (USH1), respectively. It is important to note that individuals with USH1 often exhibit vestibular dysfunction, with delays in motor development serving as a clinical hallmark of congenital vestibular impairments [38]. In contradistinction to terrestrial animals, which navigate both terrestrial and aquatic terrains, semi-aquatic mammals confront the unique challenge of traversing both land and underwater domains in three dimensions. This necessitates the maintenance of stability during activities such as foraging or evading predators. Consequently, the vestibular system of semi-aquatic mammals is subjected to distinct demands to uphold physical balance [53]. We posit that the distinctive evolutionary trajectories of the *USH1C* and *CDH23* genes lay the groundwork for the adaptation of intricate locomotor patterns in semi-aquatic mammals. Understanding the mechanisms of these genes in semi-aquatic species could inform potential treatments for patients with USH1.

In instances of DFNB1 deafness stemming from mutations in *GJB2* and/or *GJB6*, the majority of patients manifest vestibular dysfunction. For many, alleviating vertigo requires reclining, while others endure vertigo severe enough to disrupt their daily activities [38]. Additionally, irregularities in vestibular otolith fragments can trigger the pathological sensation of vertigo [20]. Vertigo, characterized by the perception of motion in oneself or the environment, is a prevalent disorder of the human vestibular system. Approximately 7.4% of adults experience lifelong bouts of vertigo, significantly hampering their work performance and daily functionality, thus posing a considerable burden on healthcare systems [54]. Furthermore, beyond terrestrial habitats, semi-aquatic mammals, such as otter species like sea otters, partake in rapid rotational movements underwater [55]. Understanding the mechanisms employed by these creatures to manage vertigo holds promising implications for the advancement of treatment strategies for human vertigo.

Conclusion

Multiple mammalian clades have independently evolved to thrive in semi-aquatic environments. We investigated the evolutionary mechanisms of sense of balance related genes in these species, crucial for maintaining locomotive balance between land and water. Our dataset, comprising 116 genes linked to the vestibular system, was analyzed across 12 semi-aquatic and 30 terrestrial mammalian species. Our findings propose that *SLC26A2* and *SOX10* are key candidates for morphological changes in the semicircular canals, crucial for the evolution of the vestibular system in response to shifts in motor patterns. These genes, in conjunction with *MYCN* and *OTX1*, likely orchestrate semicircular canal formation to adapt to semi-aquatic habitats. Additionally, genes involved in otolith development, such as *SLC26A2*, *OC90*, and *OTOP1*, might modulate otolith sensory acuity and other functions across diverse movement modalities. Genes like *GJB2*, *GJB6*, and *USH1C*, linked to vestibular disorders, could constitute the molecular underpinnings enabling semi-aquatic mammals to mitigate vertigo amidst intricate movement patterns.

Methods

Species coverage and construction of gene set

Within the Mammalia class, there are approximately 140 species that exhibit a semi-aquatic lifestyle [50, 53]. In our study, we selected 12 semi-aquatic mammal species and 30 terrestrial mammal species for our research based on the publication status of semi-aquatic mammalian genomes, the availability of species genomes in our laboratory, the quality of genome assembly, and the phylogenetic distribution of the species. We have reviewed extensive literature and compiled nearly all genes known prior to the commencement of this study that are associated with the development of the vestibular system (semicircular canals, otoliths, and vestibular structures). These genes will be used as a gene set for subsequent analyses.

Sequence acquisition and construction of the phylogenetic tree

Among the 42 selected species (refer to Additional file 1: Table S7 for genomic details), genome sequencing was conducted in our laboratory for the *Aonyx cinereus*, *Lutra lutra*, *Neovison vison*, *Martes flavigula*, and *Vulpes ferrilata*. However, the genome sequences of the *Aonyx cinereus*, *Lutra lutra*, *Neovison vison* are not yet publicly available. Genome data for the remaining species were acquired from the NCBI database (https://www.ncbi.nlm.nih.gov/).

For annotated genomes, protein-coding sequences of the target genes were directly retrieved from the NCBI database. In cases where multiple protein-coding sequences were available for a gene, we selected the longest sequence after alignment. For unannotated genomes and target sequences not found in annotated genomes, we utilized the target gene sequences of *Homo sapiens*, *Mus musculus*, *Equus caballus*, *Manis javanica*, and *Enhydra lutris* as query sequences. Utilizing TBtools software [56] and GeneWise (https://www.ebi.ac.uk/Tools/psa/genewise/), we obtained the protein coding sequences of these target genes. Afterwards, we preserved only the longest transcript for each gene. In the BLAST process, we set a stringent E-value threshold of 1e-10. In cases where multiple homologous sequences were still present, we included them in the dataset for clustering analysis to ensure that the correct sequences were identified. Additionally, we conducted a bidirectional BLAST analysis using the RBH (Reciprocal Best Hit) method [57] to verify whether the genes we obtained are orthologous. Based on the identified occurrences of the target genes across the 42 species, we carried out multiple bidirectional BLAST analyses on various combinations of species datasets.

To facilitate subsequent analyses of molecular evolution, correlation, and convergent evolution, we concatenated the orthologous genes identified in all 42 species in the same order. We then performed sequence alignment using the PRANK program [58]. To improve the quality of the alignment, we subsequently trimmed the sequence alignment results using the Gblocks program [59], eliminating regions with poor alignment quality. Finally, we used IQ-TREE (-m GTR + F + I + G4 -bb 1000 -alrt 1000) and MrBayes (ngen = 1,000,000, samplefreq = 1000, nchains = 4) to construct the maximum likelihood (ML) and Bayesian inference (BI) trees, respectively.

Selection pressure analysis

To assess the impact of natural selection on genes associated with the sense of balance, we employed the codeml program within the PAML software package [60]. Initially, we utilized the branch-site model (Ma: model = 2, NSsites = 2, fix_omega = 0; null model Ma0: model = 2, NSsites = 2, fix_omega = 1, omega = 1) to identify potential positive selection among genes shared within semi-aquatic mammal subgroups. In this analysis, semi-aquatic species were designated as foreground branches, contrasting with terrestrial species as background branches. Each gene underwent thorough examination, with loci possessing a posterior probability (BEB) exceeding 0.8 considered indicative of positive selection [61]. This approach led to the identification of genes harboring at least one such locus as positively selected genes shared by the semi-aquatic group.

Concurrently, site model (M2a: model = 0, NSsites = 2; null model M1a: model = 0, NSsites = 1. M8: model = 0, NSsites = 8; null model M7: model = 0, NSsites = 7) analysis was independently conducted for each gene across all terrestrial mammals. This method aimed to isolate positively selected loci, thereby minimizing the influence of genes exhibiting positive selection across all species and facilitating the identification of genes specifically favored in semi-aquatic species.

Subsequently, to identify genes under divergent evolution and those that have rapidly evolved in semi-aquatic species following their adaptation to aquatic habitats, all species were categorized into semi-aquatic and terrestrial groups. We applied Clade model C (CmC) [62] (CmC: model = 3, NSsites = 2; null model M2a_rel: model = 0, NSsites = 22) to calculate the evolutionary rate for each gene within these groups. Genes displaying significantly different evolutionary rates between the two control groups were designated as divergent evolution genes. Additionally, we utilized the Branch model (M2: model = 2, NSsites = 0; null model M0: model = 0, NSsites = 0) to analyze all genes within the two groups. Genes exhibiting a significantly higher evolutionary rate compared to the terrestrial group were classified as rapidly evolved genes shared among the semi-aquatic group. Finally, the free ratio model (M1: model = 1, NSsites = 0; null model M0: model = 0, NSsites = 0) in the branch model was employed to ascertain the respective evolutionary rates of genes across all species for subsequent correlation analysis.

Investigating the correlation between gene evolutionary rates and habitats

To explore whether the evolutionary rates of different genes are linked to specific locomotor patterns in various habitats, we conducted correlation analyses using Phylogenetic Independent Contrast (PIC) methods [63]. PIC analyses are instrumental in mitigating phylogenetic biases when assessing diverse traits across species. Initially, we logarithmically adjusted the evolutionary rate of each gene along the terminal branches, as determined by the free ratio model. Subsequently, we categorized terrestrial and semi-aquatic species as 0 and 1, respectively, for correlation analyses between these two subgroups. The phylogenetic tree with branch lengths utilized in this study was obtained from the TimeTree database. All analyses were executed in R using the *ape* package [64], with specific commands detailed in accordance with the procedures outlined on website https://www.r-phylo.org/wiki/HowTo/Phylogenetic_Independent_Contrasts.

Convergent evolutionary analysis

Semi-aquatic mammals confront the dual challenge of adapting to two distinct locomotor modes: terrestrial movement and aquatic swimming, stemming from their secondary aquatic adaptation. Our investigation aims to discern whether species with independently evolving semi-aquatic adaptations share similar molecular traits in maintaining their sense of balance. We conducted an analysis of convergent evolution at the protein level employing the PCOC (Profile Change with One Change) method, which has exhibited superior efficacy in identifying convergence compared to other approaches. In this approach, convergence at a site is delineated as a shift from the biochemical properties of an ancestral branch amino acid to an amino acid with distinct biochemical properties shared by multiple convergent clades. Essentially, our objective was to discern convergence in amino acid properties as opposed to mere identical amino acid substitutions [65, 66]. PCOC operates as a phylogeny-based examination utilizing branch lengths derived from the TimeTree database. Semi-aquatic species are delineated as evolutionarily discrete entities based on their phylogenetic ties and are allocated to distinct clades, each representing independent secondary aquatic adaptations. These branches encompass: clade 1 (*Condylura cristata*), clade 2 (*Enhydra lutris*, *Lutra lutra*, *Aonyx cinereus*, and *Neovison vison*), clade 3 (*Zalophus californianus*, *Odobenus rosmarus divergens*, and *Phoca vitulina*), clade 4 (*Ursus maritimus*), clade 5 (*Hippopotamus amphibius*), clade 6 (*Castor canadensis*), and clade 7 (*Hydrochoerus hydrochaeris*). We set a posteriori probability threshold of 0.95 for the identification of convergent loci, categorizing genes manifesting at least one convergent locus as convergent evolutionary genes.

Protein interaction analysis

To investigate the protein interactions among positively selected genes, rapidly evolved genes, genes with semi-aquatic correlation, and convergent evolution genes obtained from the previous analysis, we utilized the STRING v12.0 database (https://string-db.org/). This database is specifically designed to gather, assess, and consolidate all publicly available information on protein interactions, supplementing it with computational predictions [67]. By using the STRING database, we can investigate whether there are significant interactions among the candidate genes obtained from the analysis results, to substantiate the validity of our findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-024-02345-9.

Additional file 1: Table S1. List of related genes for sense of balance and references. Table S2. The version number of the sequence downloaded from the NCBI database. Table S3. List of positively selected genes in terrestrial group mammals (site model: comparison between M8 model and M7 model). Table S4. List of positively selected genes in terrestrial group mammals (site model: comparison between M2 model and M1 model). Table S5. List of divergent evolution genes in semi-aquatic group mammals (Clade model C). Table S6. List of rapidly evolving genes in semi-aquatic group mammals (Branch model). Table S7. Information of 42 mammal genomes used in this study.

Additional file 2. The raw data of all sequences obtained in this study.

Additional file 3. The results of bidirectional BLAST with the RBH method on all identified orthologous gene sequences.

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Authors' contributions

Y.D.: Conceptualization, Data curation, Investigation, Writing—original draft. Q.W.: Conceptualization, Funding acquisition, Supervision. G.S. and X.G.: Investigation, Writing—review & editing. T.L. and L.W.: Validation, Resources. S.Z. and X.W.: Software, Methodology. Y.S. and L.S.: Investigation, Software, Data curation. H.Z.: Conceptualization, Funding acquisition, Project administration. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analyzed during the current study are available in Additional file 2 in Supplementary Information.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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