Evolution of ion channels in the water-to-land transition of vertebrates

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Abstract

The transition of vertebrates from aquatic to terrestrial environments represents one of the most profound evolutionary events in their history, involving extensive physiological and morphological innovations. Key adaptations included the transformation of fins into limbs with digits to enable efficient terrestrial locomotion, the ability to perceive novel environmental stimuli, and the emergence of reproductive strategies suited to life on land, processes in which ion channels played fundamental roles. Accordingly, understanding the genetic basis of vertebrate terrestrialization requires investigating the evolution of this group of membrane proteins. Our analyses reveal that the proportion of ion channels is highly conserved, representing approximately 1.4% to 1.6% of total protein-coding genes in most lineages, with a notable increase to ~1.9% in teleost fishes. Our natural selection analyses revealed an overrepresentation of specific ion channel gene families, including TRP, RyR, HTR3, and HCN. We identified 29 ion channel genes showing signatures of positive selection, many of which are associated with key physiological functions such as nociception and thermosensation. We also detected an elevated rate of gene turnover in the common ancestor of terrestrial vertebrates, indicative of substantial genomic remodeling through gene gain and loss. Together, these findings suggest that, despite overall conservation in the proportions of ion channels, specific gene families underwent changes that were likely critical to meeting the physiological demands of terrestrial life. These results provide a foundation for future comparative and functional studies aimed at elucidating the molecular mechanisms underlying major environmental transitions.

Keywords: TRP, Gene Copy Number Variation, Terrestrialization, Gene Family, Tetrapods

Introduction

One of the most fascinating challenges in evolutionary biology is understanding the genetic underpinnings behind phenotypic transformations. Vertebrates have successfully colonized an exceptional range of environments, a diversification that required profound morphological and physiological changes (Ashley-Ross et al., 2013). The growing availability of high-quality genomes now offers an outstanding opportunity to uncover the molecular changes underlying those phenotypic transformations. Well-studied examples include the transition from land to water in cetaceans (whales, dolphins and porpoises) (Nery et al., 2013; Tsagkogeorga et al., 2015; McGowen et al., 2012; Sun et al., 2013), the conquest of the deserts by rodents (Rocha et al., 2021; Yuan et al., 2025), adaptation to subterranean habitats in fossorial mammals (Bondareva et al., 2023; Du et al., 2015; Jiang et al., 2020), and the terrestrialization of invertebrates groups (Martínez-Redondo et al., 2023; Aristide & Fernández, 2023). Among vertebrates, the colonization of land, dated to ~408-352 million years ago (Kumar et al., 2022), represented one of the most significant events during their evolutionary history (Ashley-Ross et al., 2013; MacIver & Finlay, 2022). This transition was enabled by a suite of morphological and physiological innovations that allowed early vertebrates to meet the challenges of terrestrial life. For example, fins transformed into digit-bearing limbs capable of supporting body weight and locomotion on land, lungs evolved to replace or complement gills for aerial respiration, sensory systems were rewired to detect airborne sounds, odors, and visual cues, and reproductive strategies became less-water dependent, most notably with the origin of the amniote egg (Cupello et al., 2022; Clack, 2009; Clack, 2006).

Ion channels are multimeric proteins embedded in the plasma membrane (Catacuzzeno et al., 2025) that form selective pores, regulating the passage of specific ions (e.g., sodium, potassium, and calcium) across the cell membrane and generating electrical signals that are crucial for nerve signaling and muscle contraction (Barker et al., 2017). These proteins play a key role in the major adaptive challenges associated with colonizing new environments. For example, in locomotion, ion channels such as voltage-gated sodium channels are crucial for generating and propagating action potentials along neurons that initiate movement (Marban et al., 1998). The expansion of voltage-gated sodium channels in early tetrapods is linked to the evolution of new sensory receptors and more complex motor control required for terrestrial locomotion (Zakon et al., 2011; Zakon, 2012). In the sensory system, ion channels also play key roles, TRP channels, for instance, participate in cold, heat, and chemical detection (M. Zhang et al., 2023; Willis, 2009; Saito & Tominaga, 2017; Lu et al., 2022), mechanically-gated ion channels like PIEZO responds to physical force, such as pressure, touch, and sound waves (Delmas & Coste, 2013; Hamed et al., 2024), and cyclic nucleotide-gated (CNG) channels participate in visual and olfactory systems, converting sensory stimuli into electrical signals (Pifferi et al., 2006). Finally, in reproduction, ion channels such as CATSPER (1-4), KCNU1, and members of the TRP channel family participate in fundamental processes of fertility, including sperm capacitation, motility, and the acrosome reaction (Shukla et al., 2012; Dubey et al., 2024; H. Wang et al., 2021). Thus, given their central roles in the emergence of key physiological and morphological traits, reconstructing their evolutionary history is essential for understanding vertebrate evolution.

To study the evolution of ion channels during the colonization of land by vertebrates, we designed a bioinformatic pipeline to annotate the entire repertoire of ion channels across the genomes of species representing all major groups of jawed vertebrates. After identifying the ion channels in each species, we inferred homologous relationships to investigate the role of positive selection and gene copy number variation. Our main results show that, on average, vertebrates possess a relatively consistent proportion of ion channels, ranging from 1.4% to 1.9% of their total protein-coding genes. Furthermore, we identified 29 ion channels showing signatures of positive selection that are associated with physiological functions such as nociception, thermosensation, and others. Importantly, we also detected a high rate of gene turnover in the ancestor of terrestrial vertebrates, potentially reflecting adaptive genomic changes associated with life on land. These results highlight that, despite the overall conservation of ion channel proportions, specific gene families experienced adaptive changes that were likely crucial for meeting the physiological demands of terrestrial life.

Material and Methods

Taxonomic sampling, DNA sequences, and ion channel annotation

We assembled a dataset of 86 species spanning all major groups of jawed vertebrates. Our sampling included cartilaginous fishes, bony fishes, coelacanths, lungfish, amphibians, reptiles, birds, and mammals (Fig. 1). Species were chosen from genome databases using strict quality filters. We included only assemblies deposited in NCBI RefSeq v.228 (Sayers et al., 2025), assembled at the chromosome level, and sequenced at ≥30x coverage. With a few exceptions, all 86 selected species met these criteria (Supplementary Table 1).

After selecting the species, we downloaded their protein-coding sequences (.faa file extension) from the Ensembl v.113 (Harrison et al., 2024) and NCBI RefSeq v.228 (Sayers et al., 2025) databases. For each gene, only the longest transcript isoform was retained. We then predicted transmembrane segments with TMHMM v2.0 (Krogh et al., 2001) and kept proteins with ≥2 transmembrane domains. Conserved domains were annotated using the NCBI Batch Web CD-search Tool (Marchler-Bauer et al., 2011) against the CDD database v.3.21 (J. Wang et al., 2023) with an E-value threshold of 0.01. To identify ion channels, we compiled a list of ion channel-associated CDD entries and intersected it with the CD-search results using an in-house Perl script, yielding a preliminary set of ion channels per species. Finally, we manually curated these sets, consulting NCBI and GeneCards (Stelzer et al., 2016) to remove putative false positives and produce the final per-species ion channel lists (Ion_channel_per_species_list.zip), which were used in downstream analysis (Fig. 2).

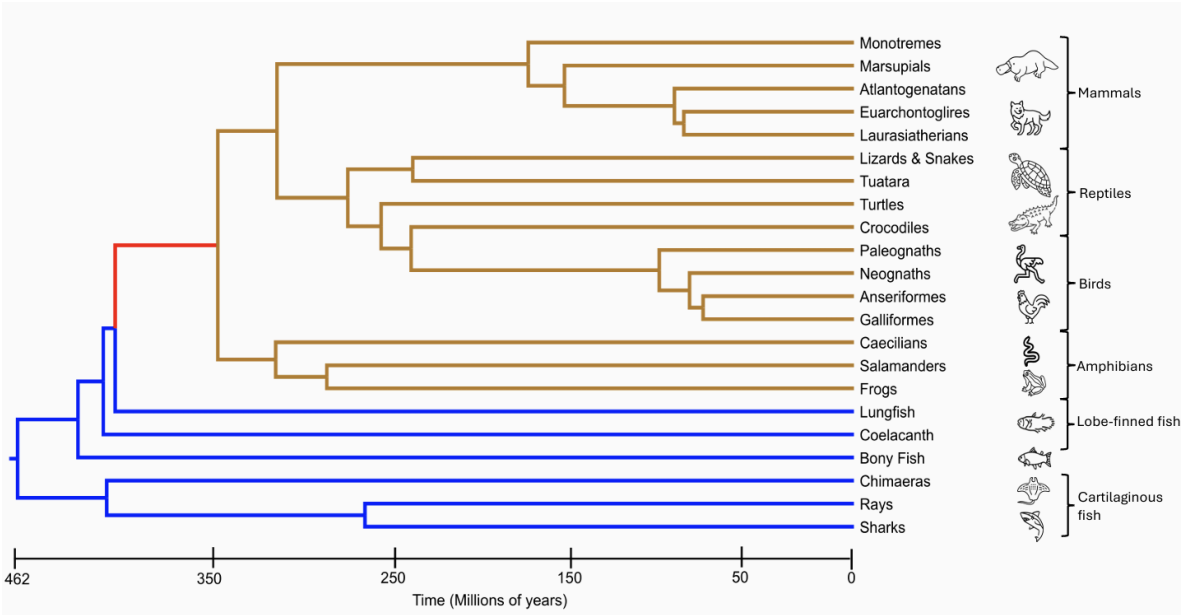

Figure 1. Time-calibrated phylogenetic tree illustrating the jawed vertebrate lineages included in our study. Blue branches represent aquatic lineages, brown branches indicate terrestrial lineages, and the red branch highlights the ancestral lineage that underwent the transition from aquatic to terrestrial environments. Animal silhouettes were obtained from Flaticon (www.flaticon.com), and evolutionary relationships and divergence times were derived from the timetree database (https://timetree.org/).

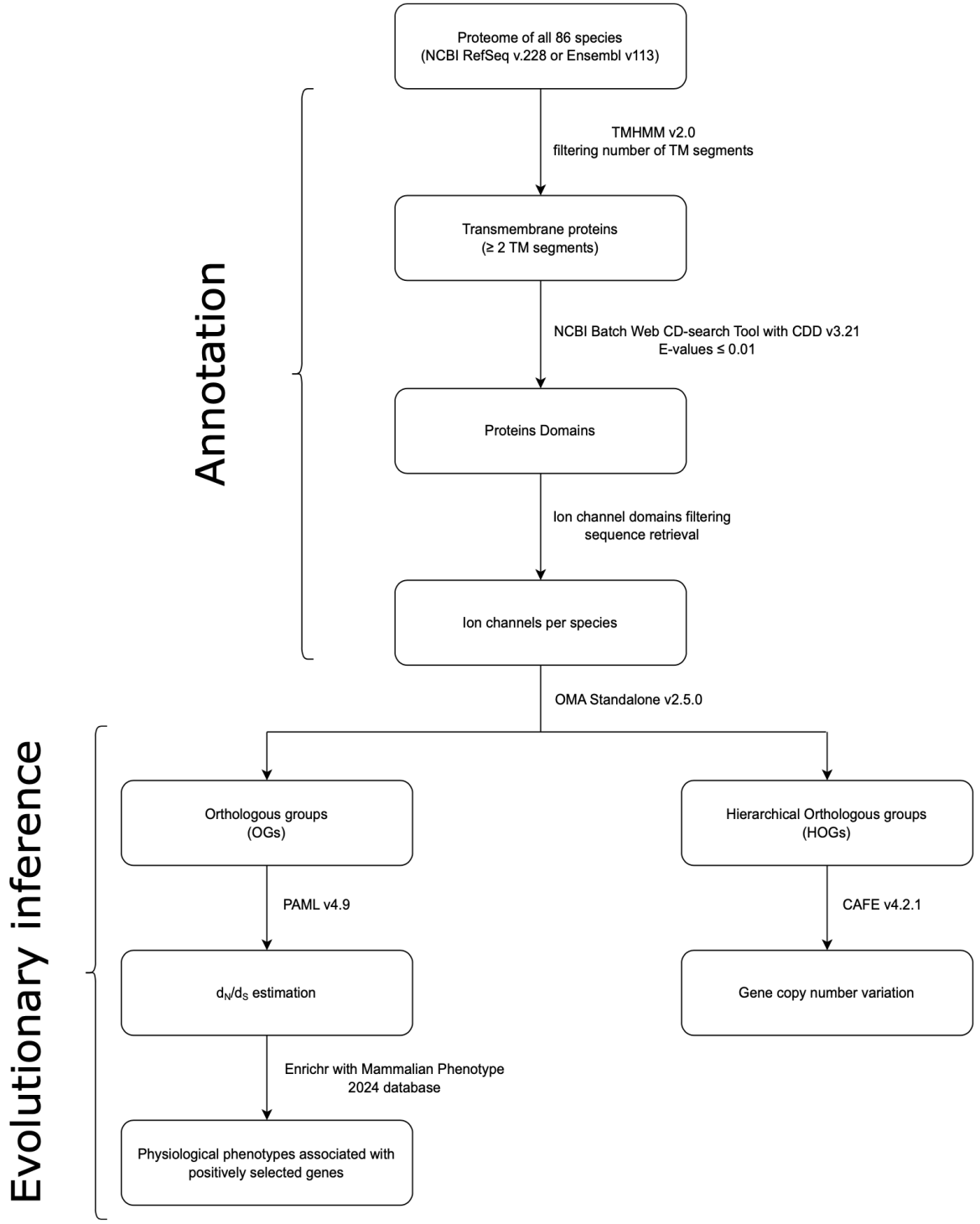

Figure 2. Workflow summarizing the annotation and evolutionary inference pipeline used in this study. TM: Transmembrane; d_N/d_S: ratio of non-synonymous to synonymous substitution rates.

Homology inference

Once the ion channels were identified for each species, we inferred homologous relationships using OMA Standalone v2.5 (Altenhoff et al., 2019), which identified Orthologous Groups (OGs) and Hierarchical Orthologous Groups (HOGs). To maximize the number of OGs available for analysis, we employed a non-exact search strategy (Nery et al., 2013). In this procedure, we specified the species/clades that must be represented in each orthologous group, as well as the minimum number of species per orthologous group. Specifically, we required that OGs include at least one amniote, the lungfish, one amphibian, and one bony fish, with a minimum of 11 species per orthologous group. This approach ensured an appropriate phylogenetic coverage while maintaining sufficient data density for robust comparative analyses. Using the OGs files as input, we estimated the ratio of non-synonymous (d_N) to synonymous (d_S) substitution rates to assess the role of natural selection. The HOGs files, in turn, were used to analyze gene copy-number variation. For the d_N/d_S analysis, amino acid sequences were aligned with MAFFT v7.490 (Katoh & Standley, 2013) with the FFT-NS-2 strategy, and codon-based nucleotide alignments were obtained by employing the amino acid alignments as templates, using the software PAL2NAL (Suyama et al., 2006).

Molecular evolution analyses

Codon-aligned nucleotide sequences generated with PAL2NAL were used as input for PAML v4.9 software (Z. Yang, 2007) in Phylip format. We estimated the ratio of non-synonymous (d_N) to synonymous (d_S) substitution rates ($\omega = d_N/d_S$) using the branch-site model approach. This approach compares two nested models. In the first (alternative) model, the branches of interest, called foreground branches, are allowed to have a class of sites evolving under positive selection ($\omega_2 > 1$), while the remaining branches, called background branches, are modeled with two site classes: conserved sites with $0 < \omega_1 < 1$ and neutral sites with $\omega_1 = 1$ (Álvarez-Carretero et al., 2023). In our analysis, the ancestral branch of tetrapods was labeled as the foreground branch to test for adaptive changes potentially associated with the vertebrate transition from water to land. For the second (null) model, the same model structure is retained, but the ω_2 parameter for the foreground branch is fixed to 1, thus not allowing positive selection. The alternative and null models were compared using a likelihood ratio test (LRT) based on their log-likelihood values. Statistical significance was assessed using the associated p-values, which were further corrected for multiple testing using the false discovery rate (FDR).

Physiological phenotypes associated with the positively selected genes

After identifying ion channels showing the signature of positive selection, we sought to infer the physiological processes potentially associated with them. To this end, we used the Enrichr platform (https://maayanlab.cloud/Enrichr/) (Evangelista et al., 2023), which identifies overrepresented biological processes and pathways from a given list of gene symbols by querying approximately 240 functional annotation libraries. In this study, we compared the ion channels with signatures of positive selection using the MGI Mammalian

Phenotype 2024 database (Baldarelli et al., 2024), which includes 11,625 genes grouped into 5,003 phenotypic categories and integrated into Enrichr. The platform applies False Discovery Rate (FDR) correction to adjust p-values, and only categories with adjusted p-values ≤ 0.01 were considered statistically significant.

Gene family turnover rate analyses

We used the program CAFE v4.2.1 (Han et al., 2013) to identify gene family expansions and contractions in ion channels across vertebrate lineages. The program CAFE implements stochastic birth-death models of gene family evolution along a phylogeny. As input, we provided the Hierarchical Orthologous Groups (HOG) inferred with OMA, along with our time-calibrated phylogenetic tree in Newick format. Two models were evaluated: in the first, two λ (turnover rate) parameters were estimated separately for terrestrial and aquatic vertebrates. In the second, three λ turnover rate parameters were estimated, one for terrestrial vertebrates, one for aquatic vertebrates, and one for the ancestor of tetrapods. Initially, the analysis across all 86 species failed, likely due to the large number of input taxa. To resolve this issue, we reduced the dataset to 66 species while maintaining key species relevant to the water-to-land transition (Supplementary File S2). This reduced dataset allowed CAFE to successfully estimate both likelihood ratios and λ values for the gene family turnover rates across the phylogeny.

Results and Discussion

Annotation and comparative distribution of ion channels in jawed vertebrates

After completing the annotation stage, we found that the 23 sampled mammals possess, on average, 20,868 protein-coding genes, of which approximately 300 (1.4%) encode ion channels. This value is higher than the one we previously estimated for the group (Uribe et al., 2024), a difference likely attributable to changes in the E-value threshold and updates to CDD libraries related to ion channels in version 3.21 (J. Wang et al., 2023). Among sauropsids (birds and reptiles, n=32), the mean number of protein-coding genes was 18,084, with about 287 (1.6%) identified as ion channels (Fig. 3). The eight amphibian genomes contained an average of 21,718 protein-coding genes, with 319 (1.5%) corresponding to ion channels (Fig. 3). In lungfish, we detected 22,039 protein-coding genes, with 329 (1.5%) corresponding to ion channels (Fig. 3), while the coelacanth genome contained 19,737 protein-coding genes, including 332 (1.7%) ion channels (Fig. 3). For the 10 teleost species included in our sampling, the mean number of protein-coding genes was 22,828 genes, with 442 (1.9%) classified as ion channels (Fig. 3). This proportion of ion channels is markedly greater than that observed in other jawed vertebrates and is likely attributable to the additional round of whole genome duplication that occurred in the ancestor of teleosts (Amores et al., 1998; Kuraku & Meyer, 2009; Jaillon et al., 2004). Finally, in cartilaginous fish (n=11), we identified an average of 18,812 protein-coding genes, of which 300 (1.6%) correspond to ion channels (Fig. 3) (Supplementary Table 1).

Consistent with previous reports (Hughes & Friedman, 2008; Lovell et al., 2014; G. Zhang et al., 2014), we observed that birds possess fewer protein-coding genes than other vertebrate groups, with values ranging from 14,765 in the common swift (*Apus apus*) to 18,018 in the chicken (*Gallus gallus*). This reduction also extends to transmembrane proteins, which range from 2,032 in the white-throated tinamou (*Tinamus guttatus*) to

3,358 in the North Island brown kiwi (*Apteryx mantelli mantelli*). These findings are consistent with the reduced genome size of birds, which is hypothesized to increase the cell surface relative to volume, thereby maximizing gas exchange and facilitating the metabolic demands of powered flight, a pattern also observed in bats (L. Wu et al., 2021; Gregory, 2002; Gregory, 2001). Despite this overall reduction, the relative abundance of ion channels remains stable at approximately 1.7%, comparable to other jawed vertebrates (1.4% to 1.9%), highlighting the essential role of these proteins in maintaining life.

Thus, overall, our comparative analyses show that the relative abundance of ion channels varies little among jawed vertebrate lineages, highlighting their fundamental and evolutionarily conserved role in vertebrate physiology.

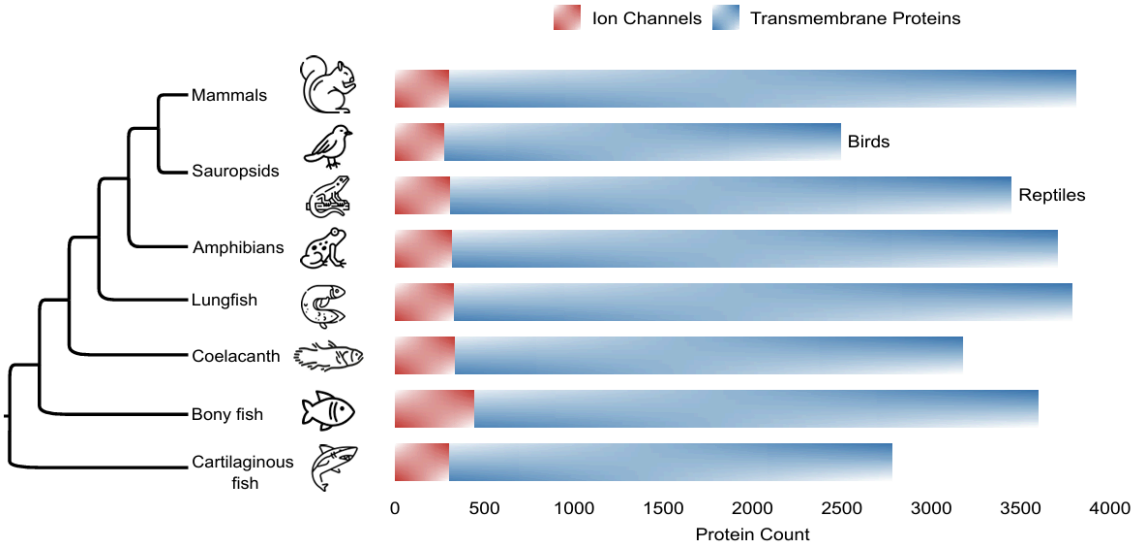

Figure 3. Ion channel and transmembrane protein abundance across jawed vertebrates.

Overrepresentation of RyR, HCN, HTR3, and TRP gene families under positive selection

We analyzed a final set of 168 1:1 orthologous groups, each comprising between 11 to 86 species per group (Fig. 4). Notably, over 95% of these groups included more than 30 species (Fig. 4). Within this dataset, we identified 29 ion channels exhibiting signatures of positive selection (Supplementary Table 3), suggesting potential adaptive changes associated with the water-to-land transition.

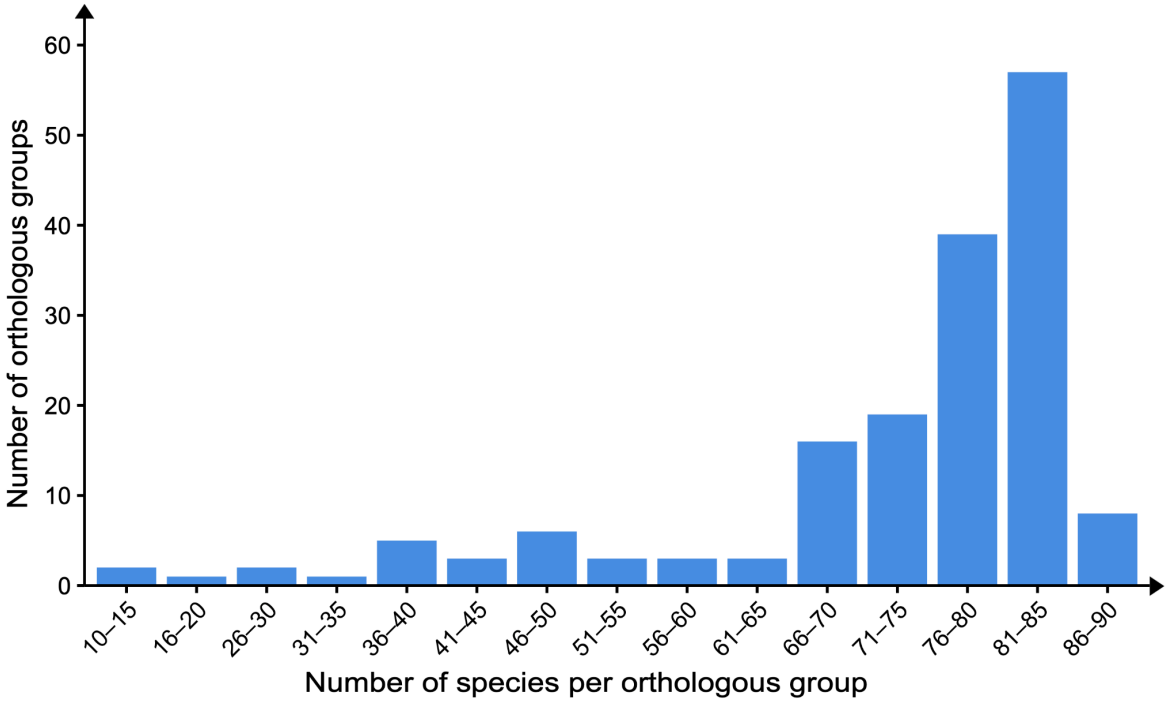

Figure 4. Distribution of orthologous groups according to the number of species they contain.

Our next step was to assess whether the 29 channels showing the signature of natural selection were non-randomly distributed among gene families. To this end, we first classified each of the 168 analyzed ion channels into their respective gene families (Supplementary Table 3) and then compared the observed distribution of the 29 positively selected ion channels against their expected family level counts (Fig. 5). Our analysis revealed that ryanodine receptors (RyR), hyperpolarization-activated cation channels (HCN), 5-hydroxytryptamine receptor 3 (HTR3) and transient receptor potential channels (TRP) families accumulated more positively selected genes than expected, with observed/expected ratios ranging from 2.3 to 3.9 (Fig. 5). In contrast, families such as TMEM16, CHRN, CLC, and KCN were under-represented with ratios below 1 (Fig. 5).

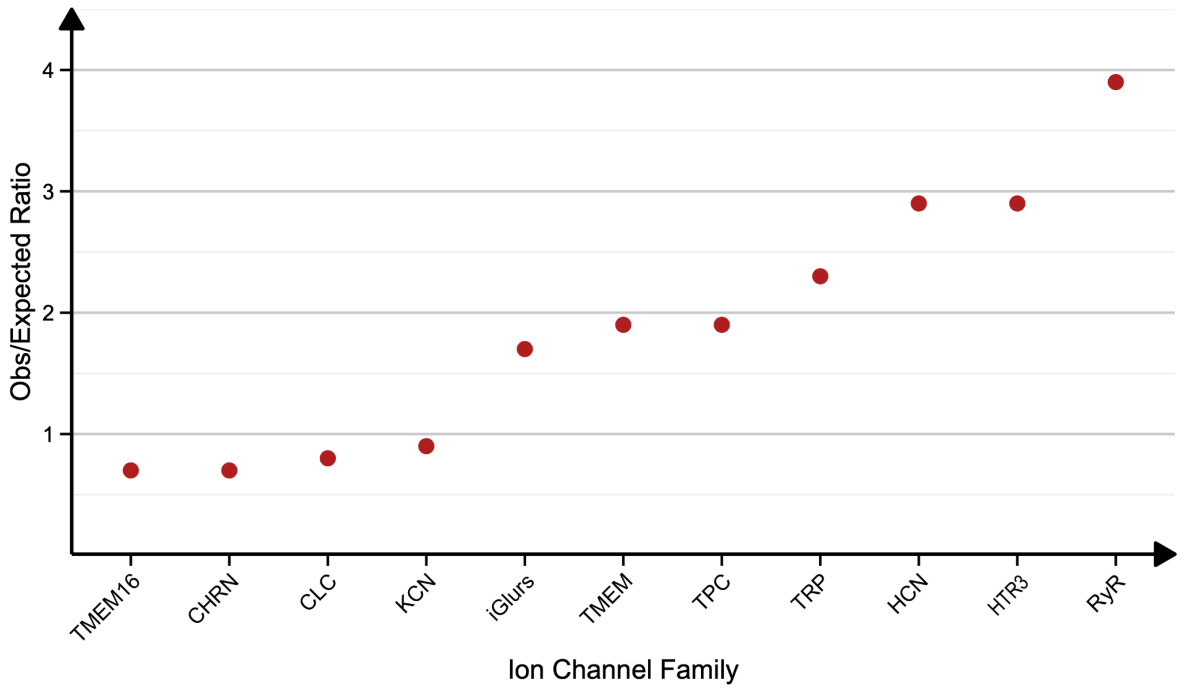

Figure 5. Enrichment analysis of ion channel gene families under positive selection. The plot shows the observed-to-expected ratio for each ion channel family among the 29 genes exhibiting signatures of positive selection.

The most represented ion channel families perform key physiological functions in vertebrates. For instance, RyRs regulate intracellular calcium levels and are essential for excitation-contraction coupling in both cardiac and skeletal muscle cells, as well as for diverse processes including development, signaling, learning, and memory (Lanner et al., 2010). By increasing cytoplasmic calcium concentration, RyRs trigger muscle contractions that sustain coordinated movement and normal cardiac rhythm (H.-T. Yang et al., 2002; Chagovetz et al., 2019). HCN channels are primarily expressed in the heart and the central nervous system, where they play fundamental roles in regulating cardiac and neuronal rhythmicity by generating the pacemaker current (Wahl-Schott & Biel, 2009). This inward cation current is activated during membrane hyperpolarization and carries both sodium and potassium ions, contributing to the depolarization process that underlies the rhythmic beating of the heart (Scicchitano et al., 2012). In the central nervous system, HCN channels are involved in critical functions including learning, memory, and motor control (Z. Zhang et al., 2024). HTR3 channels are expressed in both the central and peripheral nervous systems, where they mediate fast synaptic signaling by opening in response to the neurotransmitter serotonin (Barnes et al., 2009). By allowing the influx of cations such as Na^+ and Ca^{2+}, HTR3 channels depolarize postsynaptic neurons and participate in diverse physiological processes, including pain sensation, motor regulation, anxiety control, and emesis (Thompson & Lummis, 2006). The last highly represented group, TRP ion channels, constitutes a large and diverse family of non-selective cation channels expressed in many tissues, particularly in sensory neurons (Nelson et al., 2011; Kauer & Gibson, 2009). They serve as key molecular sensors, detecting a broad spectrum of environmental and physiological stimuli, including temperature, pain, mechanical stress,

phototransduction, and chemical irritants (M. Zhang et al., 2023). Owing to their central roles in these essential processes, TRP channels have been widely studied across fields ranging from evolutionary biology to medicine (Saito et al., 2011; Ribelayga, 2010; Yue et al., 2015; Lee et al., 2021; L. Yue & Xu, 2021; Cabezas-Bratesco et al., 2022; Flores-Aldama et al., 2020; Cancino et al., 2025). Together, these findings suggest that adaptive molecular evolution during the water-to-land transition was mainly concentrated in ion channel gene families central to excitability, sensory perception, and the coordination of vital physiological processes.

Functional enrichment of positively selected ion channels during the vertebrate water-to-land transition

We next used the 29 ion channels showing signatures of positive selection as input to an enrichment analysis using the Enrichr tool to gain a more detailed understanding of the biological functions potentially affected during the vertebrate water-to-land transition (Supplementary Table 4). The resulting enriched phenotypic associations clustered into four major physiological categories: 1) neurological system, 2) sensory perception, 3) immune system, and 4) lethality (Fig. 6). Each physiological category is composed of a set of physiological features described by functional or characteristic terms. For example, premature death is a physiological feature of the lethality category.

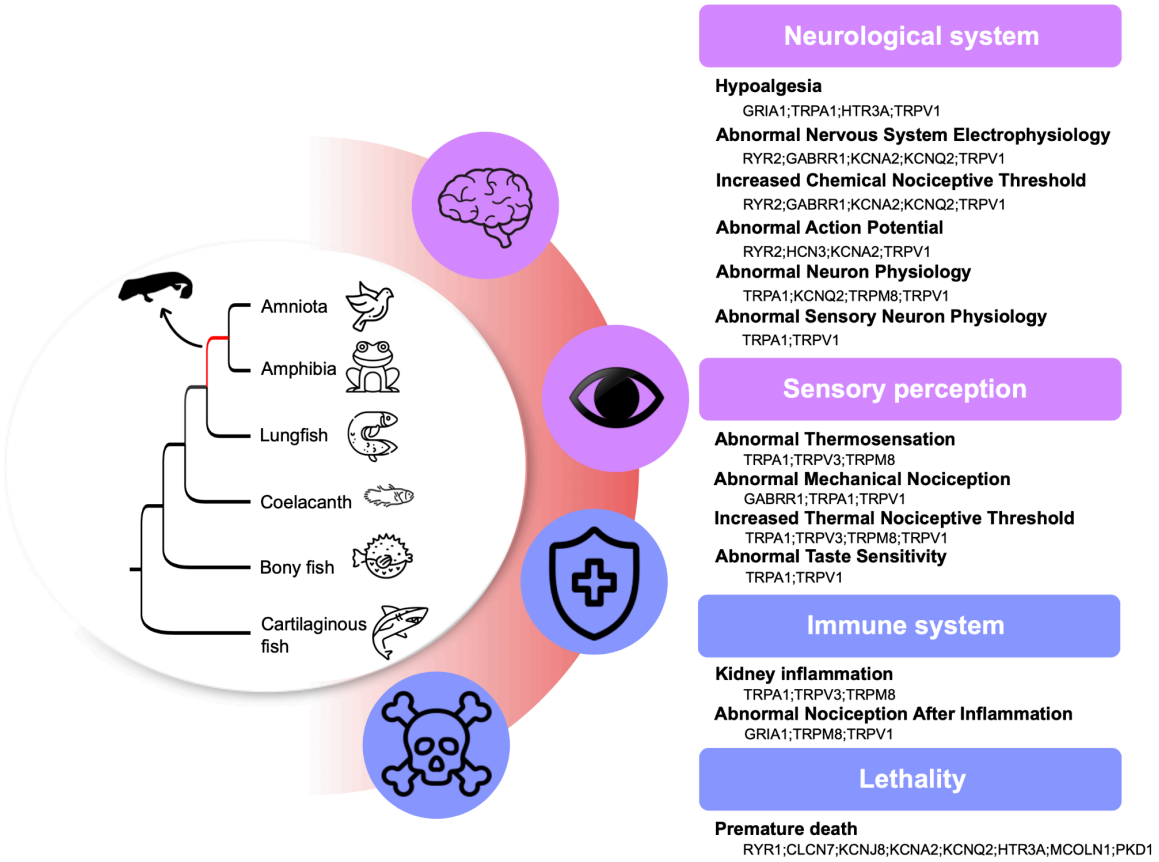

Figure 6. Enrichment of functional categories among ion channel genes under positive selection in the tetrapod ancestor.

Several ion channels were recurrently associated with multiple physiological features across these categories (Fig. 6), suggesting that a subset of ion channels may have played central roles in key adaptive processes during vertebrate evolution.

The physiological features associated with the neurological system category point to evolutionary changes in neuronal signaling and excitability, suggesting that adaptations in the nervous system were a key component of vertebrate terrestrialization. Ion channels most frequently linked to this category included RYR2, GABRR1, KCNA2, and KCNQ2. RYR2 encodes a calcium release channel located in the sarcoplasmic reticulum, essential for excitation-contraction coupling in cardiac muscle (Kobayashi et al., 2022). A comparative study shows that RYR2 exhibits distinct thermal sensitivities between endothermic and ectothermic vertebrates (Vornanen, 2006). In cold water fish, RYR2 maintains robust gating and calcium sensitivity even at temperatures as low as 2°C, whereas in mammals, gating slows and closure is impaired under similar conditions, indicating reduced function under cold stress. These differences highlight lineage-specific adaptations of RYR2 to thermal environments. GABRR1 encodes the rho-1 subunit of the ionotropic GABA receptor, a chloride channel expressed predominantly in the central nervous system and retina, where it contributes to inhibitory neurotransmission (Zhu et al., 2019). In the retina, these channels modulate glutamate release at bipolar cell terminals, thereby shaping the duration of visual signals (Popova, 2014). Such fine-tuning of visual inhibition may have aided adaptation to variable light environments during vertebrate evolution. KCNA2, a member of the voltage-gated potassium channel family KCNA, plays a pivotal role in neuronal excitability and neurotransmitter release (Hoegg & Meyer, 2007). In the cerebellum, Kv1.2 subunits encoded by KCNA2 regulate calcium spiking in Purkinje-cell dendrites and control GABA release from basket-cell terminals. Mutations in KCNA2 in mice cause chronic motor incoordination due to disrupted excitation-inhibition balance (Xie et al., 2010). These functions are essential for motor coordination, a capability critical for efficient terrestrial locomotion. Similarly, KCNQ2 encodes a potassium channel primarily expressed in the nervous system, where it regulates neuronal excitability and helps maintain membrane potential stability via M-current (Devaux et al., 2004; Ma et al., 2023). A mutation in KCNQ2 produces developmental and epileptic encephalopathy, characterized by severe early-onset seizures (Ye et al., 2023; Brun et al., 2022; M. Mao et al., 2025), underscoring its critical role in maintaining neuronal homeostasis.

The sensory perception category was strongly represented by members of the TRP family, particularly the thermoTRP channels TRPV1, TRPA1, TRPM8, and TRPV3, which were associated with multiple physiological features (Fig. 6). These TRP ion channels are multimodal receptors, capable of being activated by temperature changes and a variety of sensory stimuli (Saito & Tominaga, 2017). TRPV1 is activated by high temperatures (>43°C), low pH, and capsaicin (Luo et al., 2019). In contrast, TRPM8 responds to low temperatures (<25°C) and compounds such as menthol and eucalyptol (Palchevskyi et al., 2023). TRPV3 is activated by warm temperatures (33-39°C) and natural compounds such as genariol and carvacrol (Kalinovskii et al., 2023). Lastly, TRPA1 is activated by noxious cold and chemical irritants, including cinnamaldehyde, menthol, and other electrophilic compounds (Kim et al., 2024). Those thermoTRP channels have been extensively studied for their evolutionary significance in vertebrate sensory systems (Lu et al., 2022; Saito & Tominaga, 2017; Laursen et al., 2016; Patapoutian et al., 2003; Vay et al., 2012; Kashio &

Tominaga, 2022; Castillo et al., 2018). For example, comparative studies on TRPV1 reveal that thermosensitivity varies among species according to their environmental niches (Saito & Saito, 2025). In squirrels and camels, which tolerate high temperatures, TRPV1 exhibits a higher activation threshold, thereby reducing nociceptive responses under heat stress (Laursen et al., 2016). Such adjustments likely prevent unnecessary pain signaling in persistently hot habitats while preserving the ability to detect damaging thermal extremes. Similarly, variation in TRPV1 and TRPA1 thermosensitivity among *Xenopus* frogs has been linked to differences in habitat temperature, suggesting a role for these channels in thermal niche specialization (Saito et al., 2019; Saito et al., 2016). In addition, the formation of the MHR1-3 domain in TRPM8 confers cold sensitivity, enabling vertebrates, particularly terrestrial tetrapods, to detect potentially harmful cold and trigger behavioral and physiological responses (Lu et al., 2022). Finally, TRPV3 is abundantly expressed in epidermal keratinocytes (Lei & Tominaga, 2025) and exhibits opposite temperature sensitivities across vertebrate lineages. In mammals, it is activated by warm temperatures, whereas in the western clawed frog, it responds to cold stimuli, illustrating that TRPV3 function diverged during vertebrate evolution (Saito et al., 2011). Collectively, these findings indicate that functional diversification within TRP channels paralleled the ecological shifts of vertebrates, facilitating their adaptation to a wide range of thermal niches during the water-to-land transition.

Additional enriched physiological categories included immune system and lethality, encompassing terms such as kidney inflammation, abnormal nociception after inflammation, and premature death (Fig. 6). Notably, TRP channels were prominent among the immune-related phenotypic terms (Fig. 6). These channels play key roles in inflammatory processes, acting in both pro- and anti-inflammatory pathways (Silverman et al., 2020; Gouin et al., 2017; Straub, 2014; C.-K. Wu et al., 2021). For example, TRPV1 and TRPA1, expressed in nociceptive neurons and immune cells, are activated by endogenous activators, such as acidified pH, prostaglandins, and oxidative aldehydes, all of which are produced during inflammation (Csekő et al., 2019). TRPV1 promotes neurogenic inflammation by triggering the release of proinflammatory neuropeptides (substance P and CGRP), whereas TRPA1 detects oxidative stress, contributing to chronic inflammatory pain (Straub, 2014). In contrast, TRPM8 exerts anti-inflammatory effects by counteracting TRPV1/TRPA1-mediated hyperexcitability, reducing neuropeptide release, and attenuating edema (Straub, 2014; Silverman et al., 2020; Premkumar, 2014). TRPV3, mainly expressed in keratinocytes, is activated by warm temperatures and endogenous lipids, and contributes to skin inflammation by regulating the release of interleukin-1α, prostaglandin E_2, and nitric oxide (S. M. Huang et al., 2008; Mandadi et al., 2009). GRIA1, which encodes a subunit of AMPA receptors, mediates fast excitatory transmission in the vertebrate central nervous system and contributes to synaptic plasticity and inflammatory pain via calcium-permeable AMPA receptor upregulation in the dorsal horn (Y.-C. Chen et al., 2001; Hartmann et al., 2004). Together, these interactions between ion channels modulate the balance between nociceptive sensitization and inflammation resolution, supporting vertebrate adaptation to new terrestrial challenges, including exposure to new pathogens, toxins, and physical stressors.

Finally, the lethality category, defined as death after weaning but before the normal life span (Baldarelli et al., 2024), included multiple ion channels whose mutations disrupt vital

physiological processes. For example, RYR1 mutations cause congenital myopathies leading to respiratory failure and premature death (Amburgey et al., 2013; O'Connor et al., 2023). CLCN7 mutations cause osteopetrosis, a severe, life-threatening disorder characterized by bone fragility and impaired hematopoiesis (X. Wang et al., 2023). KCNJ8 mutations are associated with cardiac channelopathies such as J-wave syndrome (JWS), which manifests as abnormal ECG patterns and lethal ventricular arrhythmias that can lead to sudden cardiac death (Medeiros-Domingo et al., 2010). Other mutations have been linked to sudden infant death syndrome (SIDS), particularly under conditions of infection or inflammation, reflecting its critical role in regulating vascular tone and the cardiac adaptive response to stress (Tester et al., 2011). Additionally, mutations in this channel contribute to Cantu syndrome, a multi-organ disease characterized by vascular abnormalities that may increase the risk of infant mortality (Nichols, 2016). Although mutations in KCNA2 are not linked to a defined human disease, they are associated with severe seizure disorders, and knockout experiments in mice have demonstrated premature death (Syrbe et al., 2015). Similarly, mutations in KCNQ2 cause a spectrum of developmental and epileptic encephalopathies (DEEs), including early infantile epileptic encephalopathies (EIEEs), which are marked by high mortality and morbidity during infancy (D. Y. Chen et al., 2018). HTR3A is a ligand-gated ion channel that mediates excitatory responses to serotonin in the nervous system. Mutations in this channel have been shown to cause premature death in mice from complications related to bladder overactivity, chronic urinary retention, and urinary tract outlet obstruction (Bhattacharya et al., 2004). For MCOLN1, a cation channel present in the membranes of lysosomes and endosomes that plays a fundamental role in the ability of cells to digest and recycle materials, mutations can cause mucolipidosis type IV (MLIV). MLIV is a lysosomal storage disease that exhibits progressive intellectual disability, motor impairment, and premature death (Boudewyn et al., 2017). Finally, PKD1 is an integral membrane protein that participates in diverse processes, including regulation of cell growth, division, and motility, as well as interactions with other cells, particularly in the kidney and liver. Mutations in PKD1 in Autosomal Dominant Polycystic Kidney Disease (ADPKD) can cause vascular abnormalities, particularly ruptured intracranial aneurysms, leading to serious health problems such as stroke, brain damage, coma, and even death (Rossetti et al., 2003). Taken together, these examples illustrate that mutations in ion channels, despite their functional and structural diversity, consistently disrupt core physiological processes essential for survival. Their recurrent association with premature death underscores the fundamental role of ion channels in maintaining organismal viability and highlights why they have been key targets of selective pressures during the vertebrate water-to-land transition.

Accelerated gene turnover rate in the tetrapod ancestor

In our copy number variation (CNV) analysis, we observed variation in gene turnover rates (Fig. 7). In the first model, we estimated two λ parameters, one for terrestrial and another for aquatic vertebrates. This analysis revealed that aquatic vertebrates exhibit a higher gene turnover rate ($\lambda = 0.00099$), which is 1.45 times faster than that of terrestrial vertebrates ($\lambda = 0.00068$). In the second model, we estimated three λ parameters, one for terrestrial vertebrates, one for aquatic vertebrates, and a third for the tetrapod ancestor (Fig. 7). Consistent with our two-λ model, aquatic vertebrates showed a higher evolutionary rate ($\lambda = 0.00099$), 1.47 times faster, than terrestrial vertebrates ($\lambda = 0.00067$).

However, the highest λ parameter was observed in the last common ancestor of tetrapods (λ = 0.00123), which is 1.83 times higher than that of terrestrial vertebrates (λ = 0.00067) and 1.24 times higher than that of aquatic vertebrates (λ = 0.00099). The faster gene turnover rate in aquatic vertebrates could be influenced by the whole genome duplication (WGD) that occurred in the common ancestor of all extant teleosts around 300 million years ago (Amores et al., 1998; Kuraku & Meyer, 2009; Jaillon et al., 2004).

CNV analysis examines differences in gene copy number across the genomes of sampled species along the phylogeny. This type of genetic variation represents a significant source of genetic diversity (F. Zhang et al., 2009). CNVs can shape phenotypic traits, influence adaptation to novel environments, and affect susceptibility to diseases, among other biological processes (Bhanuprakash et al., 2018). For example, the expansion of the amylase gene (AMY), which enables starch digestion, has been reported in several mammalian species that consume starch-rich diets, such as humans, mice, rats, pigs, and dogs (Pajic et al., 2019). Moreover, an intraspecific correlation has been observed in humans, with populations that historically consume more starch tending to have higher copy numbers of amylase genes (Perry et al., 2007). Another well-studied example is found in Antarctic icefishes (*Channichthyidae*), in which CNVs have shaped extreme physiological adaptations (Corliss et al., 2019). These species have lost hemoglobin and myoglobin genes, resulting in their “white blood”, while a novel antifreeze glycoprotein gene (AFGP) arose through the duplication and neofunctionalization of a digestive enzyme gene (L. Chen et al., 1997). Together, gene loss and duplication allowed these species to survive in subzero waters, underscoring the role of CNVs in vertebrate adaptation (Shin et al., 2024).

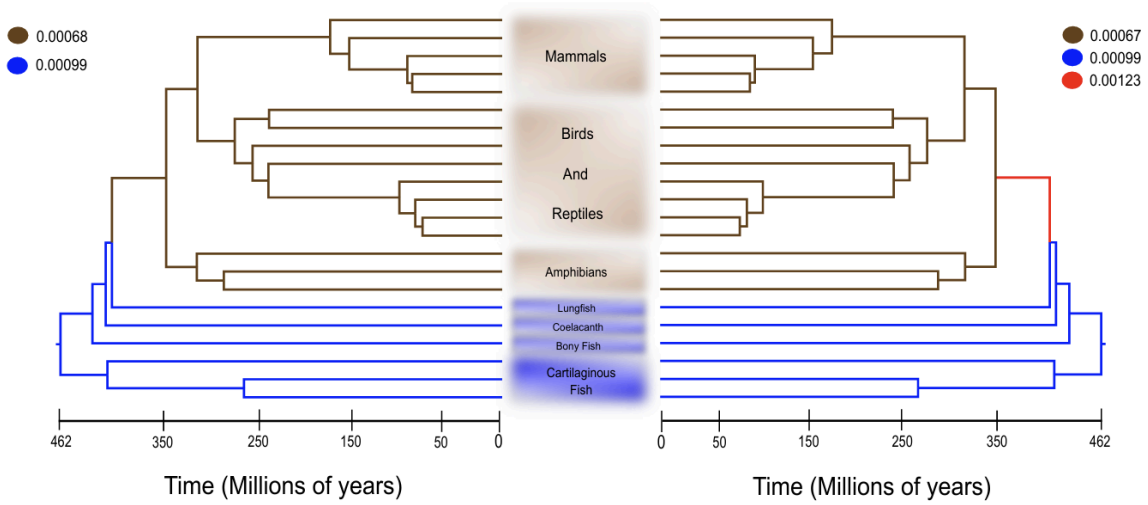

Figure 7. Estimated gene turnover rates of ion channels across jawed vertebrates. The first model (left) estimates separate gene turnover rates (λ) for ion channels in terrestrial vertebrates (brown branches) and aquatic vertebrates (blue branches). The second model (right) estimates separate λ values for terrestrial vertebrates (brown branches), aquatic vertebrates (blue branches), and the last common ancestor of tetrapods (red branch).

Together, these results indicate that the highest gene turnover occurred in the last common ancestor of tetrapods, suggesting an episode of intensified gene gain and loss during this evolutionary transition. This elevated λ contrast with the lower rates observed in both extant terrestrial and aquatic lineages, highlighting the tetrapod ancestor as a key period of genomic innovation. Such a pattern is consistent with the major ecological and physiological shifts associated with the water-to-land transition, where extensive remodeling of gene repertoires, including ion channels, may have been required.

Conclusions

In this study, we investigated the evolutionary dynamics of ion channels during the vertebrate transition from water to land, which occurred approximately 408–352 million years ago. It is well known that this major ecological shift required extensive physiological reorganization, involving processes in which ion channels play central roles, including air breathing, osmoregulation, terrestrial locomotion, neuromuscular control, sensory transduction, and host–pathogen interactions. Interestingly, despite surveying a broad diversity of jawed vertebrate lineages, we found that the relative proportion of ion channels is remarkably conserved (~1.4–1.9%), highlighting their fundamental role in vertebrate physiology. Our natural selection analyses revealed an overrepresentation of specific ion channel gene families, including TRP, RyR, HTR3, and HCN. Genes under positive selection were predominantly associated with the nervous and immune systems, sensory perception, and essential viability traits, and were implicated in key physiological processes, including thermosensation, pathogen response, and nociception. Our findings suggest that excitability and sensory pathways underwent substantial remodeling during vertebrate terrestrialization. Finally, our results indicate that gene copy number variation also played a pivotal role in this transition, as evidenced by the highest estimated λ value occurring in the common ancestor of terrestrial vertebrates.

Conflict of interest

No conflict of interest has been declared by the authors.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT (OpenAI) in order to improve grammar and language clarity. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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