RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 2: 194-203 (February 2026)

Evolution and Adaptation of *Aquaporin* Genes in Rodents along a Habitat Moisture Gradient: A Case Study of *Eothenomys miletus*

Lijuan Cao[1], Wenrong Gao[2], Wanlong Zhu[1], Tongtong Gu[1]

10.18805/IJAR.BF-2080

ABSTRACT

Background: To elucidate the adaptive evolution of aquaporin (AQP) genes in arid habitats, this study performed phylogenetic and selection pressure analyses on AQP1 to AQP11 across 15 rodent species, with subsequent experimental validation of candidate genes under positive selection.

Methods: In the present study, phylogenetic trees were constructed using MEGA-X and the iTOL online platform, while selection pressure analysis was performed *via* PAML and the Datamonkey website. Finally, using *Eothenomys miletus* inhabiting the Hengduan Mountains as the research subject, water stress experiments were conducted by measuring AQP expression levels through ELISA.

Result: Phylogenetic reconstruction using MEGA-X and iTOL indicated that species from humid environments clustered into a distinct clade, implying that moisture availability may have influenced the divergence of *AQP* genes. Selection analyses implemented in PAML identified one positively selected site each in AQP1, AQP5 and AQP7. Furthermore, signals of positive selection were detected in AQP10 and AQP11 among species from arid habitats. Given the substantial existing literature on AQP1, AQP5 and AQP7 and the limited phenotypic consequences of AQP10 deficiency in mice, this study focused on validating AQP11. After subjecting the endemic species of the Hengduan Mountains, *E. miletus*, to water deprivation treatment, the protein expression levels of AQP11 were measured using enzyme-linked immunosorbent assay (ELISA). Findings demonstrated significantly elevated AQP11 levels in seven tissues-kidney, brain, liver, heart, small intestine, white adipose tissue (WAT) and brown adipose tissue (BAT)-in the water-deprived group compared to controls. Based on existing evidence, we propose that AQP11 is involved in cellular water stress responses across multiple organs by mediating H_2O_2 transport across the endoplasmic reticulum membrane. Thus, it is hypothesized that the AQP11 gene may play a critical role in the adaptation of rodents to arid environments. This research offers a theoretical basis for further elucidating the mechanisms by which organisms adapt to environmental water stress.

Key words: *Aquaporin*, *Eothenomys miletus,* Phylogenetic tree, Rodents, Selection pressure, Water stress.

Abbreviations: AQP: Aquaporin; BAT: Brown adipose tissue; *E. miletus*: *Eothenomys miletus*; ER: Endoplasmic reticulum; *O. torridus*: *Onychomys torridus*; WAT: White adipose tissue.

INTRODUCTION

Aquaporins (AQPs) constitute a class of membrane proteins ubiquitously distributed in both prokaryotic and eukaryotic cells (Liu *et al*., 2024; Wang *et al*., 2024). They function as specialized channels that facilitate the rapid transmembrane transport of water molecules, thereby playing a pivotal role in water secretion and absorption processes (Ishibashi *et al*., 2017). Beyond their fundamental water permeability, AQPs participate in a wide range of physiological functions, including cell proliferation, apoptosis and neural signal transduction (Verkman, 2012). To date, 13 AQP subtypes (designated AQP0 to AQP12) have been identified in mammals. These sub-types are categorized into three major functional groups: orthodox AQPs (selective for water), aquaglyceroporins (permeable to water and small solutes such as glycerol) and superaquaporins (also known as unorthodox AQPs) (Rojek *et al*., 2008; Zhang *et al*., 2012). The *AQP* gene family has undergone complex evolutionary processes across different species. Its structural and functional diversity is closely associated with adaptations to environments characterized by varying water availability, such as those with distinct precipitation regimes (Gui *et al*., 2021).

Global warming alters atmospheric circulation and ocean current patterns, leading to a redistribution of global

[1]School of Life Sciences, Yunnan Normal University, Kunming, 650500, China.

[2]School of Biological Resources and Food Engineering, Qujing Normal University, Qujing, 655011, China.

Corresponding Author: Wanlong Zhu, Tongtong Gu; School of Life Sciences, Yunnan Normal University, Kunming, 650500, China. Email: zwl_8307@163.com; 240041@ynnu.edu.cn

ORCIDs: 0009-0006-5629-9250, https://orcid.org/0000-0001-9521-7613; 0000-0001-8261-4089, https://orcid.org/0009-0007-6017-0393

How to cite this article: Cao, L., Gao, W., Zhu, W. and Gu, T. (2026). Evolution and Adaptation of *Aquaporin* Genes in Rodents along a Habitat Moisture Gradient: A Case Study of *Eothenomys miletus*. *Indian Journal of Animal Research*. **60(2):** 194-203. doi: 10.18805/IJAR.BF-2080.

Submitted: 14-10-2025 **Accepted:** 15-12-2025 **Online:** 25-12-2025

precipitation and an exacerbation of regional water crises (Benz *et al*., 2024). Against this backdrop, understanding the genetic mechanisms underlying organismal adaptation to hydrological stress has become a critical research focus. Accumulating evidence indicates that environmental adaptive selection is a major driver of functional diversification in *AQPs*. This diversity in *AQP* genes may originate from various evolutionary mechanisms, including whole-genome duplication, local gene replication and horizontal gene transfer (Ishibashi *et al*., 2017). For instance, cucumber genome-wide scans have revealed that the promoter regions of AQP genes contain an abundance of stress-responsive elements, with some of these genes having undergone purifying selection, reflecting adaptation to environmental challenges (Lai *et al*., 2022). In reptiles, positive selection signals identified in the *AQP* genes of terrestrial species suggest a role in adapting to arid environments (Zang *et al*., 2019; Zhang *et al*., 2024). Additionally, research indicates that *AQP11* and its orthologs may have played a pivotal role in the aquatic-to-terrestrial transition of vertebrates, as observed in amphibians and fish. Furthermore, *AQP11* has been demonstrated to perform essential functions in terrestrial reptiles (Lorente-Martínez *et al*., 2023). Consequently, investigating the evolutionary patterns of *AQP* genes under water stress will enhance our understanding of the molecular mechanisms that enable organisms to cope with and adapt to environmental pressures, thereby providing insights into ecological resilience.

Rodentia, the most species-rich and widely distributed order of mammals, possess complex mechanisms for regulating water metabolism (Liu *et al*., 2014). Their diversification across ecological gradients has resulted in the evolution of diverse strategies for water acquisition and conservation. Given the central role of *AQPs* in maintaining water balance and facilitating environmental adaptation (Beall *et al*., 2004; Bo *et al*., 2016; Li and Guo, 2011; Wang *et al*., 2009), this study investigates adaptive changes in *AQP* genes across rodent species distributed along a habitat humidity gradient. Due to the limited research on *AQP0* and *AQP12* in rodents, we focus on the evolutionary patterns of *AQP1* to *AQP11* in arid-adapted species, followed by functional validation of positively selected *AQP* genes.

Since the Tertiary Period, the orogenic uplift of the Tibetan Plateau and the formation of the Hengduan Mountains have transformed the regional paleoclimate from warm and humid to arid and cold (Geng and Zhu, 2024; Li *et al*., 2014). This region is now characterized by mild annual temperature variation, high diurnal temperature range and pronounced vertical climatic zonation (Ren *et al*., 2023). These heterogeneous topographic and climatic conditions have likely acted as key drivers of genetic and phenotypic differentiation in local species (Luo *et al*., 2024). In rodents, different functional categories of *AQPs* may face distinct selection pressures and their evolutionary patterns may correlate with habitat humidity gradients. To address this research gap, we therefore conducted water-deprivation experiments on *Eothenomys miletus*, a species endemic to the Hengduan Mountains, to elucidate how AQP expression underpins its adaptability to varying rainfall regimes. The complex paleoclimatic changes in this region provide the context for studying adaptive evolution, while research on this specific species can directly uncover mechanisms of local adaptation, addressing a gap left by previous studies that often focused on widely distributed or model species. The findings of this study furnish valuable understanding of the potential mechanisms by which rodents may cope with growing water scarcity under global warming conditions.

MATERIALS AND METHODS

Data acquisition for *AQP* genes

The coding sequence (CDS) regions of *AQP1* to *AQP11* genes from 15 rodent species were obtained from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov). Ecological metadata, including species habitat characteristics, were obtained from the Animal Diversity Web (https://animaldiversity.org) (Table 1).

Phylogenetic analysis of *AQP* genes

The amino acid sequence information of the *AQP* genes were aligned using MUSCLE software embedded in MEGA X (Kulikov *et al*., 2024). The most appropriate amino acid substitution model was selected using ModelGenerator (Keane *et al*., 2004). Maximum likelihood (ML) phylogenetic analysis was performed in MEGA X, with 1000 bootstrap replicates employed to estimate node support (Kumar *et al*., 2016). Ultimately, the resulting tree was graphically presented and annotated through the Interactive Tree of Life (iTOL) online resource (https://itol.embl.de).

Selection pressure analysis of *AQP* genes

Position-specific selection pressures acting on *AQP* genes were assessed using the site models (M7 and M8) implemented in PAML 4.7. To enhance the reliability of positively selected site identification across the 15 rodent species, two additional models-the Fixed Effects Likelihood (FEL) and Single-Likelihood Ancestor Counting (SLAC) approaches-accessible via the Datamonkey web server (http://www.datamonkey.org), were also employed. The strength of natural selection was quantified by calculating the ratio of nonsynonymous (dN) to synonymous (dS) substitution rates ($\omega = dN/dS$). Selective pressures were interpreted as follows: $\omega<1$ indicates purifying selection; $\omega>1$ indicates positive selection and $\omega = 1$ suggests neutral evolution. Branch-specific models in PAML 4.7 were applied to test for positive selection acting along specific lineages of rodent *AQP* genes (Zhang *et al*., 2025). Finally, branch-site models were used to examine whether *AQP* genes in rodent species inhabiting arid environments have undergone positive selection.

Table 1: NCBI numbers for *AQP1-11* genes in 15 rodent species.

Species name	*AQP1*	*AQP2*	*AQP3*	*AQP4*	*AQP5*	*AQP6*	*AQP7*	*AQP8*	*AQP9*	*AQP10*	*AQP11*
Apodemus sylvaticus	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	52173	52160	52176	52155	52160	52160	52176	52174	52187	521809	52158
	780.1	182.1	262.1	467.1	183.1	181.1	263.1	794.1	865.1	50.1	315.1
Arvicanthis niloticus	XM_0	XM_0	XM_0	>XM_	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	34519	34517	34503	03451	34517	34517	34503	34512	34491	345007	34513
	367.1	053.1	924.1	7845.1	052.1	244.1	508.1	699.1	820.1	95.1	529.1
Arvicola amphibius	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	38320	38343	38347	38331	38343	38343	38348	38331	38321	383116	38314
	281.1	467.1	752.1	137.1	370.1	492.1	194.1	729.1	699.1	32.2	038.1
Cricetulus_griseus	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	03503	03507	03511	03502	03507	03507	07652	03514	35458	035016	35461
	923.5	835.4	964.5	224.3	837.3	842.4	475.4	321.4	466.1	58.1	166.1
Dipodomys_ordii	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	13035	13018	13026	13014	13018	13018	13026	13011	13009	130150	13014
	125.1	798.1	706.1	845.1	761.1	750.1	562.1	496.1	945.1	30.1	019.1
Ictidomys tridecemlineatus	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	05319	05324	05326	21728	05325	05325	05326	40277	05316	217305	05323
	169.4	931.3	192.4	932.1	110.4	111.2	191.4	041.1	618.4	66.2	237.3
Myodes glareolus	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	48440	4844	48420	48447	48444	48444	48420	48460	48424	484567	48435
	399.1	4591.1	547.1	917.1	539.1	589.1	473.1	395.1	145.1	08.1	111.1
Mus musculus	NM_0	NM_0	NM_0	NM_0	NM_0	NM_1	NM_0	NM_0	NM_0	-	NM_1
	07472.	09699.	16689.	01308	09701.	75087.	01378	01109	22026.		75105.
	2	3	2	641.1	4	5	638.1	045.1	3		3
Octodon degus	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	04626	23722	04631	23713	04637	04637	23716	23715	23712	046298	04632
	526.2	718.1	918.2	994.1	797.2	798.2	112.1	812.1	649.1	10.1	529.2
Onychomys torridus	XM_0	XM_0	XM_0	>XM_	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0
	3618	36209	36179	03620	36209	36209	361785	36175	36194	361913	36190
	1519.1	029.1	876	5144.1	028.1	043.1	37.1	028.1	383.1	38.1	868.1
Perognathus longimembris	XM_0	XM_0	XM_0	>XM_	XM_0	XM_0	XM_0	XM_0	XM_0	XM_04	XM_0
pacificus	48339	48364	48363	04836	48364	48364	48363	48332	48332	83568	48361
	524.1	782.1	390.1	3523.1	797.1	771.1	271.1	686.1	593.1	77.1	098.1
Peromyscus leucopus	XM_0	XM_0	XM_0	>XM_	XM_0	XM_02	XM_0	XM_0	XM_0	XM_0	XM_0
	28864	28874	28884	02889	28874	28874	28884	28867	28865	288908	28877
	100.2	394.2	043.2	2014.2	393.2	392.2	044.2	892.2	626.2	37.1	458.2

Table 1: Continue...

Table 1: Continue...

Phodopus roborovskii	XM_0	XM_0	XM_0	>XM_	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	
	51202	51189	51187	05120	51189	51189	51187	51192	51197	51199	51192	
	874.1	084.1	553.1	2136.1	880.1	881.1	549.1	899.1	912.1	250.1	278.1	
Rattus norvegicus	NM_0	NM_0	NM_0	>NM_0	NM_0	NM_0	NM_0	XM_0	NM_0	XM_0	NM_1	
	12778.	12909.	31703.	0114	12779.	22181.	01429	06230	22960.	391037	73105.	
	2	3	2	2366.2	2	2	387.1	187.5	2	79.2	1	
Rattus rattus	XM_0	XM_0	XM_0	>XM_	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	XM_0	
	32906	32890	32905	03288	32890	32890	32890	32892	32911	328969	32892	
	446.1	976.1	027.1	5627.1	977.1	978.1	240.1	702.1	040.1	38.1	972.1	

Water deprivation experiment design

Wild-caught *E. miletus* individuals were collected from farmland and shrubland habitats in Xiaguan Town, Dali City, Yunnan Province, China (GPS coordinates: 25°34′54″ N, 100°14′6″E; altitude: 2061 m). Following capture, all animals were disinfected and cleared of ectoparasites by local health authorities prior to transportation. Thereafter, the animals were transferred to the laboratory animal facility of Yunnan Normal University. They were individually housed in standard cages (260 mm × 160 mm × 150 mm) under a 12-hour light:12-hour dark photoperiod and a consistent surrounding temperature held at 25±1°C. All animals were provided with a standard laboratory diet (produced by Kunming Medical University) and were acclimatized for four weeks with food and water available *ad libitum*. Only healthy, non-reproductive adult individuals were selected for the experiment. Post-acclimation, the animals were randomly assigned to one of two experimental groups: a water-deprived treatment group (n = 6), which received standard feed but no water access and a control group (n = 6), which maintained *ad libitum* access to both food and water. Pre-experiment body weights did not differ significantly between the two groups ($p>0.05$). At the conclusion of the experimental period, all animals were humanely euthanized *via* CO, asphyxiation. Target tissues were promptly dissected, forthwith flash-frozen in liquid nitrogen and preserved at -80°C for subsequent molecular assays. As *AQP1*, *AQP5* and *AQP7* have been extensively characterized in previous literature and because *AQP10* deficiency has been reported to produce minimal phenotypic consequences in murine models (Morinaga *et al*., 2002; Rebez and Jacob, 2024), the present study focused on the functional validation of *AQP11*.

ELISA for AQP11 protein in *E. miletus*

AQP11 expression levels were quantified in seven tissue types-kidney, brain, liver, heart, small intestine, white adipose tissue (WAT) and brown adipose tissue (BAT)-using ELISA. Frozen tissue samples of *E. miletus*, stored at -80°C, were analyzed. The measurements were performed using a commercially available rat AQP11 ELISA Kit (Baika Biological Technology Co., Kunming, China), strictly following the manufacturer's protocol.

Data processing and analysis

SPSS Statistics version 27.0 was employed for all statistical analyses. First, the distribution of the data was assessed using normality tests. With the exception of the AQP11 expression data in BAT, which deviated from a normal distribution, all other datasets satisfied the assumption of normality. Based on the distribution characteristics of the data, appropriate statistical methods were selected for between-group comparisons: For data meeting the normality assumption, Variations in the expression levels of AQP11 between the control and treatment groups within the same tissue type were assessed via an independent-

samples t-test. In the case of BAT data with a non-normal distribution, the non-parametric Mann Whitney U test was employed. To compare AQP11 expression levels among different tissues within the same experimental group, one-way analysis of variance (one-way ANOVA) was used. Prior to conducting the ANOVA, the assumption of homogeneity of variance was verified. To identify specific group differences after a significant ANOVA, we applied Tukey's HSD post hoc test for pairwise comparisons.

All boxplot figures were generated using OriginPro 2024 software.

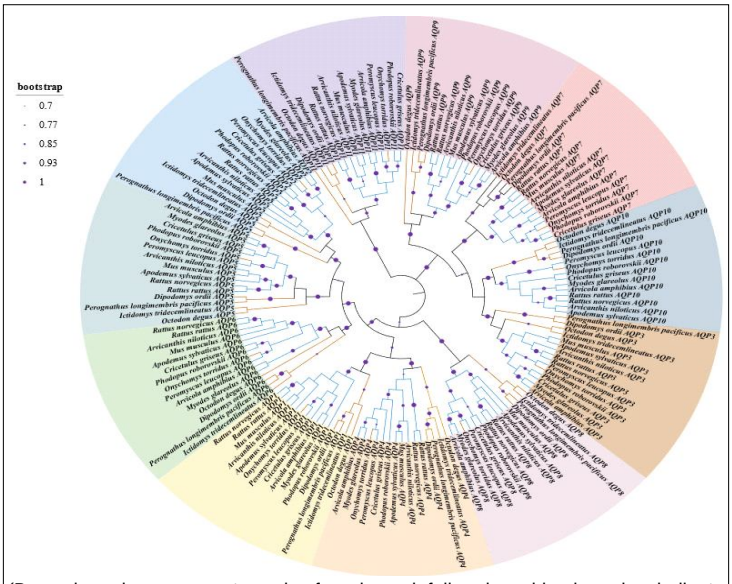
RESULTS AND DISCUSSION

Phylogenetic analysis of *AQP* genes

AQPs are critical membrane proteins that regulate water homeostasis in organisms and are classified into three major categories: classical water-selective AQPs (AQP0, 1, 2, 4, 5 and 6). Among these, AQP2, 5 and 6 are functionally important in the skin, bladder, salt glands and kidneys across amphibians, sauropsids and mammals. (Morinaga *et al*., 2002; Nishimura and Yang, 2013). Deficiencies in these AQPs can result in severe physiological defects (Ikeda *et al*., 2002; Noda and Sasaki, 2006; Oshio *et al*., 2006). Classical aquaglyceroporins (AQP3, 7, 9 and 10) are involved in osmoregulation and energy metabolism through glycerol transport (Hara-Chikuma, 2005; Sohara *et al*., 2006) and play crucial roles in metalloid homeostasis (Bienert *et al*., 2008). Unorthodox AQPs (AQP11 and AQP12) are considered "superaquaporins" within the AQP subfamily (Ishibashi, 2006). Additionally, AQP8 is capable of transporting water, urea, ammonia and free radicals (Bienert *et al*., 2007).

A total of 164 *AQP* genes were phylogenetically clustered into three major groups: *AQP1*, *2*, *4*, *5*, *6* and *8* formed one cluster; *AQP3*, *7*, *9* and *10* grouped together; while AQP11 formed a distinct separate branch. This phylogenetic structure corresponds exactly to the three recognized *AQP* subfamilies (Fig 1). Furthermore, *AQP* genes within each subfamily exhibited clearly resolved, species-specific phylogenetic relationships. Notably, rodent species inhabiting humid environments consistently clustered together within the phylogenetic tree.

Within this research, phylogenetic trees were reconstructed based on AQP genes from 15 rodent species. The resulting clustering patterns revealed a correlation with species ecotypes. Specifically, lineages from humid habitats frequently formed independent clades, suggesting that adaptation to arid environments may have driven unique evolutionary changes in rodent *AQP* genes to fulfill physiological requirements under water stress. Interestingly, *O. torridus*-which inhabits arid regions including deserts, sand dunes and savannas in the western and southwestern United States and northern Mexico, where rainfall is relatively low-was found to group within a clade comprising species from more humid environments across the *AQP1-11* gene tree (Harold and Egoscue, 1960). This anomalous positioning suggests that *O. torridus* may have evolved alternative physiological mechanisms for coping with water scarcity, potentially involving regulatory changes in AQP expression or function rather than rapid gene evolution. This contrasts with the rapid evolutionary pattern observed in *AQP* genes among other drought-adapted species, such as the genomic data from the comparatively arid and humid groups of Liangzhou donkeys, where the *CYP4A11* gene was identified as a



(Brown branches represent species from low-rainfall regions; blue branches indicate species from high-rainfall regions).

Fig 1: Phylogenetic tree of *AQP1-11* genes.

key factor in their adaptation to arid environments (Wang *et al*., 2022). This suggests that rodents may employ diverse molecular evolutionary pathways in responding to drought stress. However, the specific reasons still require subsequent experimental verification.

Positively selected sites in rodent *AQP* genes

To explore the molecular basis of adaptation in arid-adapted rodents, we identified sites under positive selection. Analysis of positively selected sites across the *AQP* gene family in the 15 rodent species identified a single positively selected site in each of three genes: site 131 in *AQP1*, site 111 in *AQP5* and site 8 in *AQP7*. No significant signals of positive selection were detected in the remaining AQP genes analyzed (Table 2).

Signatures of positive selection were detected in *AQP1*, *AQP5* and *AQP7*. The selection on *AQP1*, *AQP5* and *AQP7* aligns with their established roles in mediating adaptive responses to environmental stress in other mammals, such as enhanced renal water reabsorption in camels (Wang *et al*., 2014) and thermoregulation in goats (Kaushik *et al*., 2024). Notably, *AQP7*-the sole glycerol channel in adipose tissue-is closely linked to adipocyte morphology and physiology, obesity development and the maintenance of energy balance and glucose homeostasis (Miranda *et al*., 2010). Therefore, the positively selected sites identified in *AQP1*, *AQP5* and *AQP7* in this study may therefore represent adaptive modifications that enhance responses to environmental water variability by regulating water metabolism, thermoregulatory capacity and energy balance.

Positive selection in *AQP* genes of rodents from arid habitats

Various ω ratio models were employed to assess whether positive selection has acted on *AQP* genes in rodents. Significant heterogeneity in ω ratios among branches was identified for *AQP2* ($p = 0.0002$*), *AQP3* ($p = 0.0074$*), *AQP5* ($p = 0.0203$*), *AQP6* ($p = 0.0034$*), *AQP9* ($p < 0.0001$), *AQP10* ($p < 0.0001$) and *AQP11* ($p = 0.0001$*). In contrast, the remaining *AQP* genes exhibited no significant branch-specific variation in ω ratios (Table 3).

Branch-site model A revealed positive selection acting on rodent AQP genes. Specifically, sites under significant positive selection were identified in *AQP10* (site 294, $p = 0.0020$*) and *AQP11* (site 184, $p = 0.0037$*) in species inhabiting arid environments. No significant signals of positive selection were detected in the other *AQP* genes (Table 4). Consequently, in environments with lower water content, AQP10 and AQP11 assume a more significant role in rodents. However, the specific reasons for this phenomenon require further investigation.

AQP11 expression in tissues of *E. miletus*

AQP11 is an unconventional AQP that localises to the endoplasmic reticulum (ER) and is implicated in regulating subcellular water distribution among organelles, particularly in maintaining osmotic balance in critical

Table 2: Site model testing of positive selection sites in reptile *AQP* genes.

Gene	No. of species	LnL M7	LnL M8	P-value	M8	FEL	SLAC	Total no. of sites
AQP1	15	-3093.61	-3077.39	9.03206E-08	120,131	131	0	1
AQP2	15	-3506.81	-3506.82	0.999850011	0	0	0	0
AQP3	15	-3252.67	-3252.52	0.86376892	0	0	0	0
AQP4	15	-3614.02	-3608.67	0.004738664	0	300	0	0
AQP5	15	-3353.01	-3345.42	0.000509286	111	111	265	1
AQP6	15	-3724.58	-3724.58	0.997253778	0	9	0	0
AQP7	15	-3932.24	-3925.87	0.001706177	8,137	8,152,194,208,210,210	224	1
AQP8	15	-4149.07	-4149.05	0.978386982	0	15,27,38	260	0
AQP9	15	-4743.12	-4741.24	0.152544336	0	6,54,93,195,202,277	295	0
AQP10	15	-5067.43	-5056.50	1.79279E-05	7,277	19,35,96,98,276,279,286	0	0
AQP11	15	-4124.33	-4120.90	0.032314152	0	66,237,265	0	0

Table 3: Phylogenetic analysis of positive selection in the *AQP* gene branch.

Gene	Model	np	LnL	2ΔlnL	*P*-value
AQP1	Model 0	55	-3176.76	31.4996	0.173005337
	Model 2	30	-3192.51		
AQP2	Model 0	55	-3510.70	57.7217	0.000213802*
	Model 2	30	-3539.56		
AQP3	Model 0	55	-3277.33	45.4702	0.007388162*
	Model 2	30	-3300.07		
AQP4	Model 0	55	-3637.07	35.5600	0.078546423
	Model 2	30	-3654.85		
AQP5	Model 0	55	-3377.84	41.5090	0.020281613*
	Model 2	30	-3398.59		
AQP6	Model 0	55	-3734.98	48.3352	0.003399588*
	Model 2	30	-3759.15		
AQP7	Model 0	55	-4034.62	28.5123	0.284814847
	Model 2	30	-4048.87		
AQP8	Model 0	55	-4213.26	33.2906	0.123961526
	Model 2	30	-4229.90		
AQP9	Model 0	55	-4803.90	63.2883	3.62875E-05*
	Model 2	30	-4835.54		
AQP10	Model 0	51	-5108.32	87.4770	1.8774E-09*
	Model 2	28	-5152.06		
AQP11	Model 0	55	-4126.92	59.7635	0.000112694*
	Model 2	30	-4156.81		

Note: * indicates $p<0.05$.

Table 4: Selection pressure analysis of *AQP* gene orthologous sites in rodents.

Gene	Model	np	LnL	2ΔlnL	*P*-value
AQP1	Model A	32	-3089.62	0	1
	Model A null	31	-3089.62		
AQP2	Model A	32	-3513.16	7.1545	0.007477614*
	Model A null	31	-3516.73		
AQP3	Model A	32	-3254.33	10.4262	0.001242401*
	Model A null	31	-3259.54		
AQP4	Model A	32	-3609.81	0	1
	Model A null	31	-3609.81		
AQP5	Model A	32	-3359.47	0	1
	Model A null	31	-3359.47		
AQP6	Model A	32	-3736.11	0.0922	0.761398723
	Model A null	31	-3736.15		
AQP7	Model A	32	-3926.60	8.5983	0.003364769*
	Model A null	31	-3930.91		
AQP8	Model A	32	-4159.04	0	1
	Model A null	31	-4159.04		
AQP9	Model A	32	-4745.70	0	1
	Model A null	31	-4745.70		
AQP10	Model A	30	-5056.82	9.5741	0.001973416*
	Model A null	29	-5061.60		
AQP11	Model A	32	-4115.71	8.4435	0.003663505*
	Model A null	31	-4119.94		

Note: N indicates $p<0.05$.

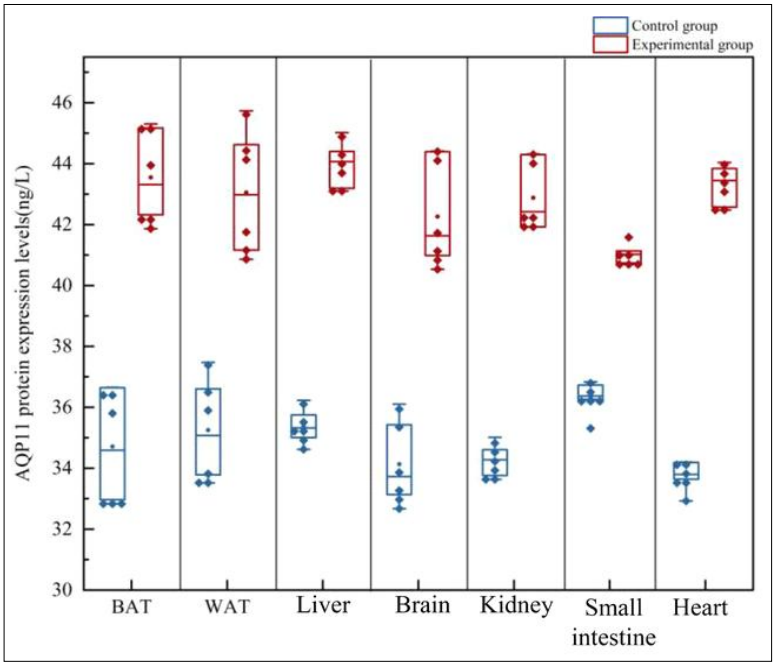


Fig 2: Box plot showing AQP11 expression levels in seven tissues of *E. miletus.*

organs such as the kidneys (Morishita *et al*., 2004). It functions as a peroxiporin, facilitating the transport of H_2O_2 across the ER membrane. Expressed in multiple tissues-including kidney, liver, intestine and brain-AQP11 contributes to organellar redox homeostasis and participates broadly in cellular stress responses (Bestetti *et al*., 2020; Markou *et al*., 2022; Yakata *et al*., 2007).

Compared with the control group, water-deprived *E. miletus* exhibited significantly higher AQP11 expression (p<0.05) in the kidney, brain, liver, heart, small intestine, WAT and BAT (Fig 2). Post-hoc analysis further revealed that expression in the small intestine was significantly lower than in the other six tissues (p<0.05), indicating the most pronounced differential expression among all tissues examined.

We quantified AQP11 expression in *E. miletus* under water-restricted conditions and observed significant upregulation across all seven tissues examined, with the most pronounced increase in the kidney. In mammals, AQP11 deficiency is associated with ER-derived vacuolation in proximal tubules, polycystic kidney disease and early renal failure, highlighting its essential role in renal tubule development and ER homeostasis (Michałek and Grabowska, 2019; Morishita *et al*., 2005; Schwartz and Johnson, 1971). The marked renal upregulation suggests a protective mechanism against osmotic stress, potentially through enhanced H_2O_2 efflux from the ER, thereby mitigating redox imbalance and preventing ER stress-induced pathology.

Notable upregulation was also observed in the brain and heart, indicating a previously underappreciated role for AQP11 in supporting neural and cardiovascular function during dehydration. In the brain, AQP11 is localised to the choroid plexus epithelium and brain capillary endothelium, suggesting a possible role in water regulation at the blood-brain barrier (Benga and Huber, 2012). Its activity may involve interplay with other AQPs, such as AQP4, in maintaining cerebral water balance (Zhang *et al*., 2022). Although the cardiac mechanism remains unclear, our data imply AQP11 involvement in systemic adaptation to osmotic stress.

In metabolic tissues, strong upregulation in BAT points to a role in alleviating ER stress *via* H_2O_2 transport, which may be critical for sustaining thermogenesis and energy balance under metabolic challenge (Calamita and Delporte, 2021). Similarly, hepatic upregulation likely supports the clearance of H_2O_2 generated during oxidative protein folding, thereby promoting hepatocyte function under high ER load (Ishibashi *et al*., 2021). By contrast, AQP11 expression in the small intestine showed only a mild response to water stress, suggesting that other mechanisms may predominately regulate hydric balance in this organ under drought conditions.

CONCLUSION

AQPs are phylogenetically widespread across most species and investigations of *AQP* genes in rodents are likely to provide valuable insights for related human studies. Focusing on rodents with adaptations to arid habitats, this research identified a positively selected site within *AQP11*, suggesting that the gene may be critical for adaptive responses to xeric conditions. In *E. miletus*, AQP11 expression was significantly up-regulated across all seven tissues examined, supporting the hypothesis that AQP11 acts as a key physiological regulator of endoplasmic

reticulum redox homeostasis-mediating H_2O_2 efflux and facilitating adaptive responses to water deprivation and other stressors in multiple organ systems. This study has certain limitations. Specifically, as the research focused on particular rodent species, the generalizability of the conclusions to a broader range of species requires further verification. Additionally, the relatively limited sample size may affect the statistical power of some analyses. Future studies could employ gain- and loss-of-function experiments while expanding both sample sizes and the range of species examined to more directly validate the specific role of AQP11 in adaptation to arid environments.

ACKNOWLEDGEMENT

This work was supported by the National Natural Scientific Foundation of China (No. 32560262; 32500436), the Special Basic Cooperative Research Programs of Yunnan Provincial Undergraduate Universities' Association (NO. 202301BA070001-076).

Ethical approval

All animal procedures were within the rules of Animals Care and Use Committee of School of Life Sciences, Yunnan Normal University. This study was approved by the committee (13-0901-011).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Beall, M., Wang, S.B., Yang, B.X., Chaudhri, N., Amidi, F. and Ross, M. (2004). Aquaporin gene expression in murine fetal membranes and placenta. *American Journal of Obstetrics and Gynecology*. **191:** S139.

Benga, O. and Huber, V.J. (2012). Brain water channel proteins in health and disease. *Molecular Aspects of Medicine*. **33:** 562-578.

Benz, S.A., Irvine, D.J., Rau, G.C., Bayer, P., Menberg, K., Blum, P., Jamieson, R.C., Griebler, C. and Kurylyk, B.L. (2024). Global groundwater warming due to climate change. *Nature Geoscience*. **17:** 545-551.

Bestetti, S., Galli, M., Sorrentino, I., Pinton, P., Rimessi, A., Sitia, R. and Medraño-Fernandez, I. (2020). Human aquaporin-11 guarantees efficient transport of H_2O_2 across the endoplasmic reticulum membrane. *Redox Biology*. **28:** 101326.

Bienert, G.P., Moller, A.L.B., Kristiansen, K.A., Schulz, A., Moller, I.M., Schjoerring, J.K. and Jahn, T.P. (2007). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *Journal of Biological Chemistry*. **282:** 1183-1192.

Bienert, G.P., Schüssler, M.D. and Jahn, T.P. (2008). Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends in Biochemical Sciences*. **33:** 20-26.

Bo, S., Tian, H., Yan, L., Zhao, K. and Ma, T. (2016). Expression of AQP-1 in the developing renal tubules of mice. *Journal of Xi'an Jiaotong University (Medical Sciences)*. **37:** 59-62.

Calamita, G. and Delporte, C. (2021). Involvement of aquaglyceroporins in energy metabolism in health and disease. *Biochimie*. **188:** 20-34.

Geng, Y. and Zhu, W.L. (2024). Comparative study of thermoregulatory and thermogenic characteristics of three sympatric rodent species: The impact of high-temperature acclimation. *Indian Journal of Animal Research*. **58(12):** 2057-2063. doi: 10.18805/IJAR.BF-1840.

Gui, Y.J., Shi, S., Luo, Y.J., Muni Re, M., Hao, J.H., Luo, T., Li, B., Zhang, X.B., Wang, C. and Wang, X.J. (2021). Composition and geographical distribution of the rodent fauna in Xinjiang. *Chinese Journal of Hygienic Insecticides and Equipments*. **27:** 557-564.

Hara-Chikuma, M. (2005). Aquaporin-3 functions as a glycerol transporter in mammalian skin. *Biology of the Cell*. **97:** 479-486.

Harold, J. and Egoscue. (1960). Laboratory and field studies of the northern grasshopper mouse. *Journal of Mammalogy*. **41:** 99-110.

Ikeda, M., Beitz, E., Kozono, D., Guggino, W.B., Agre, P. and Yasui, M. (2002). Characterization of aquaporin-6 as a nitrate channel in mammalian cells: Requirement of pore-lining residue threonine 63. *Journal of Biological Chemistry*. **277:** 39873-39879.

Ishibashi, K. (2006). Aquaporin subfamily with unusual NPA boxes. *Biochimica et Biophysica Acta*. **1758:** 989-993.

Ishibashi, K., Morishita, Y. and Tanaka, Y. (2017). The evolutionary aspects of aquaporin family. *Advances in Experimental Medicine and Biology*. **969:** 35-50.

Ishibashi, K., Tanaka, Y. and Morishita, Y. (2021). The role of mammalian superaquaporins inside the cell: An update. *Biochimica et Biophysica Acta*. **1863:** 183617.

Kaushik, R., Goel, A. and Rout, P.K. (2024). Gene expression analysis of Aquaporin genes in ruminants during growth phase in response to heat stress. *International Journal of Biological Macromolecules*. **281:** 136262.

Keane, T.M., Naughton, T.J. and Mcinerney, J.O. (2004). Model generator: Amino acid and nucleotide substitution model selection. National University of Ireland.

Kulikov, N., Derakhshandeh, F. and Mayer, C. (2024). Machine learning can be as good as maximum likelihood when reconstructing phylogenetic trees and determining the best evolutionary model on four taxon alignments. *Molecular Phylogenetics and Evolution*. **200:** 108181.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. **33:** 1870-1874.

Lai, M.X., Du, C.X. and Fan, H.F. (2022). Identification and bioinformatics analysis of AQP gene family in *Cucumis sativus*. *Journal of Zhejiang A and F University*. **39:** 318-328.

Li, X.H., Zhu, X., Niu, Y. and Sun, H. (2014). Phylogenetic clustering and overdispersion for alpine plants along elevational gradient in the Hengduan Mountains Region, southwest China. *Journal of Systematics and Evolution*. **52:** 280-288.

Li, X.M. and Guo, M. (2011). Morphology of principal cells and expression of aquaporin-2, -3 in collecting duct during mouse kidney development. *Acta Anatomica Sinica*. **42:** 389-393.

Liu, J., Lu, Z.C., Wang, X.Y., Jia, G.X. and Zhang, P.J. (2024). From land to sea: Unique evolution in reproductive strategies of marine mammals. *The Innovation Life*. **2:** 100090.

Liu Z., Xu, Y.C., Rong, K., Jin, Z.M. and Ma, J.Z. (2014). The current progress in rodents molecular phylogeography. *Frontiers in Ecology and the Environment*. **34:** 307-315.

Lorente-Martínez, H., Agorreta, A., Irisarri, I., Zardoya, R., Edwards, S.V. and Mauro, D.S. (2023). Multiple instances of adaptive evolution in aquaporins of amphibious fishes. *Biology (Basel)*. **12:** 846.

Luo, M., Yang, W.Y., Bai, L., Zhang, L., Huang, J.W., Cao, Y.H., Xie, Y.H. and Tong, L.P. *et al*. (2024). Artificial intelligence for life sciences: A comprehensive guide and future trends. *The Innovation Life*. **2:** 100105.

Markou, A., Unger, L., Abir-Awan, M., Saadallah, A., Halsey, A., Balklava, Z. and Conner, M. *et al*. (2022). Molecular mechanisms governing aquaporin relocalisation. *Biochim. Biophys. Biochimica et Biophysica Acta-Biomembranes*. **1864:** 183853.

Michałek, K. and Grabowska, M. (2019). Investigating cellular location of aquaporins in the bovine kidney. A new view on renal physiology in cattle. *Research in Veterinary Science*. **125:** 162-169.

Miranda, M., Escoteì, X., Ceperuelo-Mallafreì, V., Alcaide, M.J., Simoìn, I., Vilarrasa, N., Wabitsch, M. and Vendrell, J. (2010). Paired subcutaneous and visceral adipose tissue aquaporin-7 expression in human obesity and type 2 diabetes: differences and similarities between depots. *The Journal of Clinical Endocrinology and Metabolism*. **95:** 3470-3479.

Morinaga, T., Nakakoshi, M., Hirao, A., Imai, M. and Ishibashi, K. (2002). Mouse *aquaporin 10* gene (*AQP10*) is a pseudogene. *Biochemical and Biophysical Research Communications*. **294:** 630-634.

Morishita, Y., Matsuzaki, T., Hara-Chikuma, M. andoo, A., Shimono, M., Matsuki, A. and Kobayashi, K. *et al*. (2005). Disruption of aquaporin-11 produces polycystic kidneys following vacuolization of the proximal tubule. *Molecular and Cellular Biology*. **25:** 7770-7779.

Morishita, Y., Sakube, Y., Sasaki, S. and Ishibashi, K. (2004). Molecular mechanisms and drug development in aquaporin water channel diseases: Aquaporin superfamily (superaquaporins): Expansion of aquaporins restricted to multicellular organisms. *Journal of Pharmacological Sciences*. **96:** 276-279.

Nishimura, H. and Yang, Y. (2013). Aquaporins in avian kidneys: Function and perspectives. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. **305:** R1201-R1214.

Noda, Y. and Sasaki, S. (2006). Regulation of aquaporin-2 trafficking and its binding protein complex. *Biochimica et Biophysica Acta*. **1758:** 1117-1125.

Oshio, K., Watanabe, H., Yan, D., Verkman, A.S. and Manley, G.T. (2006). Impaired pain sensation in mice lacking Aquaporin-1 water channels. *Biochemical and Biophysical Research Communications*. **341:** 1022-1028.

Rebez, B.E. and Jacob, N. (2024). The role of aquaporin in stress physiology: A review. *Agricultural Science Digest*. **44(6):** 991-999. **doi:** 10.18805/ag.D-6121.

Ren, Y., Jia, T., Zhang, H. and Wang, Z.K. (2023). Comparative analyses of Pst and Fst of *Eothenomys miletus* from different areas in Yunnan province. *Sichuan Journal of Zoology*. **42:** 29-36.

Rojek, A., Praetorius, J., Frøkiaer, J.R., Nielsen, S.R. and Fenton, R.A. (2008). A current view of the mammalian aquaglyceroporins. *Annual Review of Physiology*. **70:** 301-327.

Schwartz, S.L. and Johnson, C.B. (1971). Pinocytosis as the cause of sucrose nephrosis. *Nephron*. **8:** 246-254.

Sohara, E., Rai, T., Sasaki, S. and Uchida, S. (2006). Physiological roles of AQP7 in the kidney: Lessons from *AQP7* knockout mice. *Biochimica et Biophysica Acta*. **1758:** 1106-1110.

Verkman, A.S. (2012). Aquaporins in clinical medicine. *Annual Review of Medicine*. **63:** 303-316.

Wang, G., Wang, F., Pei, H., Li, M., Bai, F., Lei, C. and Dang, R. (2022). Genome-wide analysis reveals selection signatures for body size and drought adaptation in Liangzhou donkey. *Genomics*. **114(6):** 110476.

Wang, J.B., Li, H.Y., Huang, Z.X., Shao, B.P. and Wang, J.L. (2014). Renal expression and functions of AQP1 and AQP2 in Bactrian camel (*Camelus bactrianus*). *Journal of Camel Practice and Research*. **21:** 153-160.

Wang, J.H., Zhang, L., Zhang, P.J. and Sun, B.J. (2024). Physiological processes through which heatwaves threaten fauna biodiversity. *The Innovation Life*. **2:** 100069.

Wang, K., Yan, L., Tian, H. and Guo, M. (2009). Expression of aquaporins 2, 3, 4 in mouse collecting duct during development. *Chinese Journal of Anatomy*. **32:** 629-631.

Yakata, K., Hiroaki, Y., Ishibashi, K., Sohara, E., Sasaki, S., Mitsuoka, K. and Fujiyoshi, Y. (2007). Aquaporin-11 containing a divergent NPA motif has normal water channel activity. *Biochimica et Biophysica Acta*. **1768:** 688-693.

Zang, Y., Chen, J., Zhong, H., Ren, J., Zhao, W., Man, Q., Shang, S. and Tang, X. (2019). Genome-wide analysis of the aquaporin gene family in reptiles. *International Journal of Biological Macromolecules*. **126:** 1093-1098.

Zhang, D., Tan, Y.J., Qu, F., Sheng, J.Z. and Huang, H.F. (2012). Functions of water channels in male and female reproductive systems. *Molecular Aspects of Medicine*. **33:** 676-690.

Zhang, J., Mu, Y., Zhu, W.L. and Yang, X.M. (2025). A rare mitochondrial genome of albino *Eothenomys eleusis* Thomas 1911 (Cricetidae: Arvicolinae) from southeastern Yunnan, China and its phylogenetic analysis. *Indian Journal of Animal Research*. **59(4):** 560-567. doi: 10.18805/IJAR.BF-1882.

Zhang, S.Z., Xie, D.X., Ma, C.J., Chen, Y., Li, Y.Y., Liu, Z.W., Zhou, T., Miao, Z.M., Zhang, Y.M., Zhang, L.Y. and Liu, Y.Q. (2022). Aquaporins: important players in the cardiovascular pathophysiology. *Pharmacological Research*. **183:** 106363.

Zhang Y.H., Wang, X., Yu, H.Y., Zhong, J., Qu, M., Zhang, Y., Shan, B.B., Qin, G., Zhang, H.X., Huang, L.M., Ma, Z.H., Gao, T.X. and Lin, Q. (2024). Mouthbrooding behavior and sexual immune dimorphism in Indian perch jaydia lineata. *The Innovation Life*. **2:** 100066.