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Comprehensive Genome-Wide Analysis and Habitat-Specific Expression of Aquaporin 3 Genes in *Tenualosa ilisha* from Bangladesh

Afroza Pervin, Md. Nazmul Hasan, Atiqur Rahman Sunny, Md Toasin Hossain Aunkor, Asif Iqbal, Md Jahangir Alam and Md. Faruque Miah*

Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
***Corresponding Author: faruque-btc@sust.edu**

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ABSTRACT

Identification, characterization, and functional investigation of the *TiAQP3* gene family in Hilsa (*Tenualosa ilisha*) were the main objectives of the research. Four *TiAQP3* genes were identified and confirmed based on the existence of conserved aquaporin domains. Diverse amino acid lengths, weights, and isoelectric points were observed in the identified genes. TiAQP3 protein were found in the plasma membrane, highlighting their conserved role in transportation of water. Ten preserved motifs, including critical NPA boxes, were discovered, demonstrating functional conservation. The *TiAQP3* genes are closely related to aquaporin genes from other migratory fish, indicating similar methods for maintaining osmotic balance. Structural modelling revealed retained secondary and tertiary characteristics, including six transmembrane helices critical for selective water transport. The expression of the *TiAQP3* gene in *T. ilisha* differs among tissues and habitats, indicating their role in osmoregulation. Higher expression was observed in gill tissue of fish from Chandpur, Bhola, and Haor. Kidney tissue from Cox's Bazar sample showed highest expression, indicating the need for enhanced renal function in response to osmotic stress. Lower level expression in muscle and liver tissues indicates their limited involvement in osmoregulation. This study lays the groundwork for further research into how aquaporins help aquatic organisms adapt to their environment and their evolutionary history.

INTRODUCTION

Teleostean species have an osmoregulatory mechanism that enables them to regulate salt and water balance despite varying environmental conditions. Epithelial tissues, especially in the gills and intestines, are very important for detecting changes in salinity, reducing osmotic stress, and sometimes helping to keep infections at bay **(Fiol & Kültz, 2007; Sunny *et al.*, 2025a)**. In reaction to environmental changes, macromolecules such as proteins trigger intricate processes, including variations in cell volume, adjustments in cytoskeletal organisation, and the remodelling of entire tissues **(Henry *et al.*, 2003; Fiol & Kültz, 2007)**. Aquaporins (AQPs), tiny membrane proteins

(26–34 kDa), belong to the major intrinsic protein (MIP) class and are recognized as integral membrane pore proteins that facilitate water transport across all biological membranes. They are essential for enabling water transfer between cells **(Agre, 2006)**. Aquaporins (AQPs) are hydrophobic proteins that facilitate the rapid passive transport of water across membranes **(Huang *et al.*, 2006)**. They also enable the transport of ions and other solutes. Various AQP isoforms have been examined in gills, kidneys, and intestinal epithelium, demonstrating differential expression in reaction to alterations in ambient salinity **(Giffard-Mena *et al.*, 2007)**. These findings indicate that aquaporins are essential for osmoregulation. The AQP protein was first found and isolated from the membranes of human erythrocytes in 1988. It was then given the name AQP1 in 1992 **(Benga, 2012)**. Currently, the aquaporin family comprises 17 members (Aqp0–16), categorized into four groups: classical aquaporins (AQP-0, -1, -2, -4, -5, -6, -14, -15), primarily facilitating water transport; aquaglyceroporins (AQP-3, -7, -9, -10, -13), which transport water, urea, and glycerol; ammoniaporins (AQP-8, -16), responsible for the transport of water, urea, ammonia, and H_2O_2; and unorthodox aquaporins (AQP-11, -12) **(Zhan *et al.*, 2023)**. Members of the aquaporin superfamily are thought to have six transmembrane helices connected by five loops, which are labelled A–E. The amino and carboxy termini are located in the cytoplasm. The "hourglass" hypothesis **(Agre *et al.*, 2001)** posits that loops B (cytoplasmic) and E (extracellular) protrude into the membrane from opposing sides and converge at the protein's centre. These loops constitute the lining of the aqueous pore, establishing a narrow, perpetually open conduit for water transport. The conserved Asn-Pro-Ala (NPA) motif and neighbouring residues on loops B and E are vital for water transport activity, enabling crucial interactions within the pore **(Wspalz *et al.*, 2009)**. AQP3, an aquaglyceroporin, serves as a channel that permits the passage of water, glycerol, urea, and ammonia/ammonium. Teleosts like the European sea bass (Dicentrarchus labrax) **(Giffard-Mena *et al.*, 2007)** and Atlantic salmon (Salmo salar) **(Madsen *et al.*, 2015)** have it in their gills and kidneys. AQP3 was initially found in the European eel (*Anguilla anguilla*) **(Cutler & Cramb, 2000)** and subsequently characterized in the Osorezan dace (*Tribolodon hakonensis*) **(Hirata *et al.*, 2003)** and Mozambique tilapia (*Oreochromis mossambicus*) **(Hirata *et al.*, 2003)**. AQP3 is important for keeping the water balance, osmoregulation, and other bodily functions that fish need to survive in water **(Hara-Chikuma & Verkman, 2005)**. Fish inhabit settings characterized by diverse salt levels, including freshwater, seawater, and brackish waters. AQP3 regulates water transport in response to environmental changes, enabling the fish to either absorb or excrete water as needed **(Madsen *et al.*, 2015)**. AQP3 facilitates water absorption in freshwater fish, mitigating dehydration by extracting water through the gills and skin. In marine fish, it facilitates the excretion of surplus salt and the regulation of water loss via the gills and kidneys. AQP3 is abundantly produced in the gills and kidneys of fish, facilitating the regulation of osmotic pressure. It facilitates the transport of water and tiny solutes (e.g., glycerol) across cell membranes, crucial for sustaining

appropriate hydration levels **(Edwards & Marshall, 2012)**. AQP3 is present in the skin of certain fish species, where it enhances water permeability, particularly in amphibious fish that can thrive in both aquatic and terrestrial environments. The Hilsa fish, also known as "Hilsa" or "Ilish," is an important species for the environment and the economy, especially in the Bay of Bengal and the rivers of Bangladesh, India, and nearby areas **(Shamsuzzaman *et al.*, 2020; Sunny *et al.*, 2025)**. Hilsa is a euryhaline species that can move between freshwater and saltwater **(Sunny *et al.*, 2021)**. This makes it an interesting subject for studying osmoregulation and the function of aquaporin (AQP) proteins like AQP3. The complete genome of Hilsa has recently been published, enabling the identification of gene families and the assessment of their functions under various conditions. To investigate the physiological and molecular roles of AQP3 in Hilsa, with an emphasis on how it affects osmotic control during anadromous migration. This study aimed to contribute to a deeper understanding of Hilsa biology and support conservation efforts by revealing mechanisms that underpin their adaptability to varying environmental conditions, considering the species' ecological and economic value.

MATERIALS AND METHODS

Identification of AQP3 Genes in *Tenualosa ilisha*

The available amino acid sequence of AQP3 from the anadromous fish *Gasterosteus aculeatus* (three-spined stickleback), retrieved from the ENSEMBL database (https://asia.ensembl.org/index.html), was used as a query in the TBLASTn search tool against the whole-genome shotgun contig database of NCBI, with the search restricted to *T. ilisha*. Potential gene sequences are selected using the FGENESH, the fastest and most accurate gene finder tool (http://www.softberry.com/) from the identified contigs. Finally, the AQP3 genes were selected based on the presence of functionally conserved domains using the NCBI Conserved Domain Database and Interpro. Further, the predicted genes were confirmed using BLASTp against the NCBI non-redundant (NR) protein sequence database.

Characterization of *TiAQP3* genes

The molecular weight (MW, kDa) and isoelectric point (pI) of each TiAQP3 protein were calculated using the ExPASy Prot-Param tool (https://web. expasy.org/protparam/) **(Gasteiger *et al.*, 2005)**. The subcellular localization of the encoded proteins was predicted by using CELLO v.2.5 (http://cello.life.nctu.edu.tw/) **(Yu *et al.*, 2006)**. Moreover, the length of protein and CDS, as well as the number of exons and introns of all genes, were estimated by the FGENESH. Potential transmembrane helices in the encoded amino acid sequences were predicted using TMHMM Server v2.0 (http://www.cbs.dtu.dk/services/ TMHMM/). To display the conserved residue, multiple protein sequence alignments of all predicted TiAQP3 proteins were constructed using

Multlin tool (http://multalin.toulouse.inra.fr/multalin/multalin.html) with the default parameters.

Gene structure, motif analysis, and phylogenetic analysis

Gene structure was constructed using the GSDS 2.0 (Gene Structure Display Server) tool (http://gsds.gao-lab.org/) **(Hu *et al.*, 2015)** by contrasting the genomic DNAs to their commensurate cDNAs of the predicted genes. The conserved motifs for each TiAQP3 protein were investigated using the MEME Suite (Multiple Expectation Multiple Em for Motif Elicitation) version 5.1.0 (http://meme-suite.org/tools/meme) with all default options except motif number **(Bailey & Elkan, 1995)**. The sequences of AQP3 genes from several representative fishes, including zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), along Hilsa, were used for phylogenetic analysis. The amino acid sequences of AQP3 genes of selected species were retrieved from the NCBI database. Phylogenetic analyses were conducted using MEGA 11 based on the maximum likelihood method and JTT (Jones-Taylor-Thornton) model **(Tamura *et al.*, 2021)**. Bootstrapping with 500 replications was conducted to evaluate the phylogenetic trees. Then, the online tool Interactive Tree of Life (http://itol.embl.de/) was used to display, annotate, and decorate phylogenetic trees.

Secondary and tertiary structures prediction of *TiAQP3* genes

The secondary structures of the selected proteins were built using the online tool NPS@SOPMA (https://npsa-prabi.ibcp.fr/) with default parameters. The structure investigation comprised the quantity and percentage of alpha helixes, extended strands, and random coils presented in various colors. Using Swiss-Model (https://www.swissmodel.expasy.org/), 3D models of tertiary structures **(Gasteiger *et al.*, 2003)** were predicted. The Biovia Discovery Studio Visualizer client 2016 software was applied to visualize the protein structure's PDB data of the *TiAQP3* genes.

Experimental sample collection

Hilsa fish samples, including gills, liver, kidney, and muscle tissue were collected from regions in Bangladesh with different salinity levels: Cox's Bazar (marine), Chandpur (freshwater), Bhola (brackish), and Haor (freshwater). The samples were immediately preserved in liquid nitrogen.

Total RNA extraction, cDNA synthesis, and RT-PCR

The collected samples were used for total RNA extraction with the TIANGEN RNA Easy Fast Tissue/Cell Kit, following the manufacturer’s instructions. RNA quality was assessed using a NanoDrop spectrophotometer. The total RNA was then used to synthesize cDNA according to the manufacturer’s protocol for the PrimeScript™ First Strand cDNA Synthesis Kit. The synthesized cDNA served as the template for the real-

time PCR (RT-PCR) assay using a gene-specific primer (forward-5'-TCATTCTGGTGATGTTTGG-3' and reverse-5'-CTGGCCAGACACCAAGA-3'). The gene-specific primer was designed using the Primer 3 tool based on the conserved region of the *TiAQp3* genes (excluding *TiAQP3-1*). For each cDNA sample, three biological and technical replicates were performed during the real-time PCR. The housekeeping gene EF1α was used as an internal control to normalize cDNA levels. The RT-PCR amplifications were conducted in a 10µL reaction volume, consisting of 5µL of master mix, 0.3µL of forward primer, 0.3µL of reverse primer, 100ng of cDNA template, and nuclease-free water to make up the remaining volume. PCR was performed using a CFX 96 (BioRad) machine with the following thermal profile: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 45 seconds. The relative expression levels of the target gene were calculated using the $2^{-\Delta\Delta Ct}$ method **(Rao *et al.*, 2013)**.

Statistical analysis

Technical replication was used to find the mean value of expression at different treatments. MS Office 365 and GraphPad Prism 8.4.3 were used to analyze the data. A one-way ANOVA and a Bonferroni post hoc test assessed the significant differences ($P \leq 0.05$). To represent the significant differences, different means were labeled with asterisk marks.

RESULTS

Identification, verification, and characteristics of Hilsa AQP3 genes

AQP3 of anadromous fish, three-spined stickleback (*G. aculeatus*), was used as a query sequence to search the Hilsa AQP3 genes in the public database NCBI using the BLASTn tool. We identified four *TiAQP3* genes in Hilsa, which confirmed the presence of the conserved AQP3 domain. Identified *AQP3* genes were assigned a unique name (*TiAQP3-1-TiAQP3-4*). The identified *AQP3* genes were named (*TiAQP3-1* to *TiAQP3-4*) using the prefix 'Ti' for *T. ilisha*, followed by '*AQP3'* for aquaporin 3. All the identified *TiAQP3* genes were further analyzed in terms of their nucleotide sequence, cDNA, CDS, and amino acid (AA) length, exon number, molecular weight (MW), isoelectric point (pI), and subcellular localization (Table 1). All the *TiAQP3* genes encoded proteins with variable lengths from 280 to 304 amino acids (aa). For example, *TiAQP3-1* encoded a protein with 295 aa, while *TiAQP3-3* encoded a protein that is 304 aa long, and each protein contains AQP3 domains. The maximum MW of kDa was found for *TiAQP3-3*, while the minimum was kDa for *TiAQP3-2*. The maximum pI was found for *TiAQP3-2* (6.90), while the minimum was 5.30 for *TiAQP3-1*. Previous reports suggested that different TiAQP3 proteins have distinct subcellular localizations. Subcellular localization showed that all of the proteins were localized in the plasma membrane (Table 1).

Table 1. Features of identified *AQP3* genes in *Tenualosa ilisha*

SL No.	Gene name	Contig ID	Position		Gene Length (bp)	Protein length	CDS length (bp)	pI	MW (kd)	Localization
			Start	End						
1	*TiAQP3-1*	SCED01007086.1	14020	19993	5973 bp	295 aa	888 bp	5.30	31.10991	Plasma Membrane
2	*TiAQP3-2*	SCED01007058.1	146892	152953	6061 bp	280 aa	843 bp	6.90	30.34739	Plasma Membrane
3	*TiAQP3-3*	QYSC01119003.1	204908	211015	6107 bp	304 aa	915 bp	6.42	32.83936	Plasma Membrane
4	*TiAQP3-4*	PYXC01002552.1	4302617	4308699	6082 bp	304 aa	915 bp	6.42	32.82533	Plasma Membrane

Exon/intron and conserved motif analysis

The exon/intron position of a gene family may play a major role in the process of evolution. The exon/intron position and diversity of exon/intron patterns of all *TiAQP3* genes were determined by comparing the CDS sequences with the respective genomic DNA sequences using GSDS (Gene Structure Display Server). The results showed that the exon/intron number in the *TiAQP3* genes was the same (all the genes contain 6 exons and 5 introns), but their orientations were different (Fig. 1). According to previous reports, AQP3 proteins contain many highly conserved motifs. Therefore, in a comparative analysis of conserved motifs of TiAQP3 proteins using the MEME, we found 10 conserved motifs in all the TiAQP3 proteins. The arrangement of the motifs was different, but most of the identified motifs were similar, indicating the conservatism of the Hilsa AQP3 protein sequences (Fig. 2).

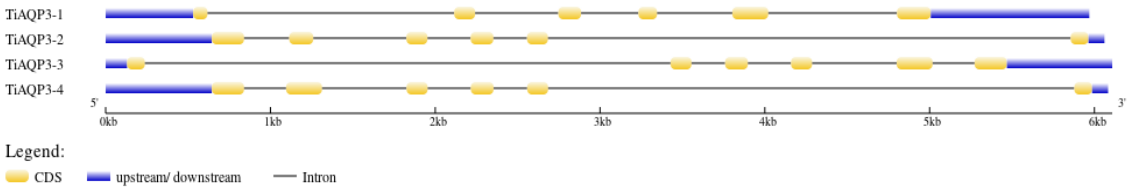

Fig. 1. Schematic representation of the gene structure of the *TiAQP3* family. The exons (coding regions, CDS) are marked in yellow, while upstream/downstream regions are highlighted in blue. Introns are represented as black lines. The scale bar at the bottom represents the genomic length in kilobases (kb)

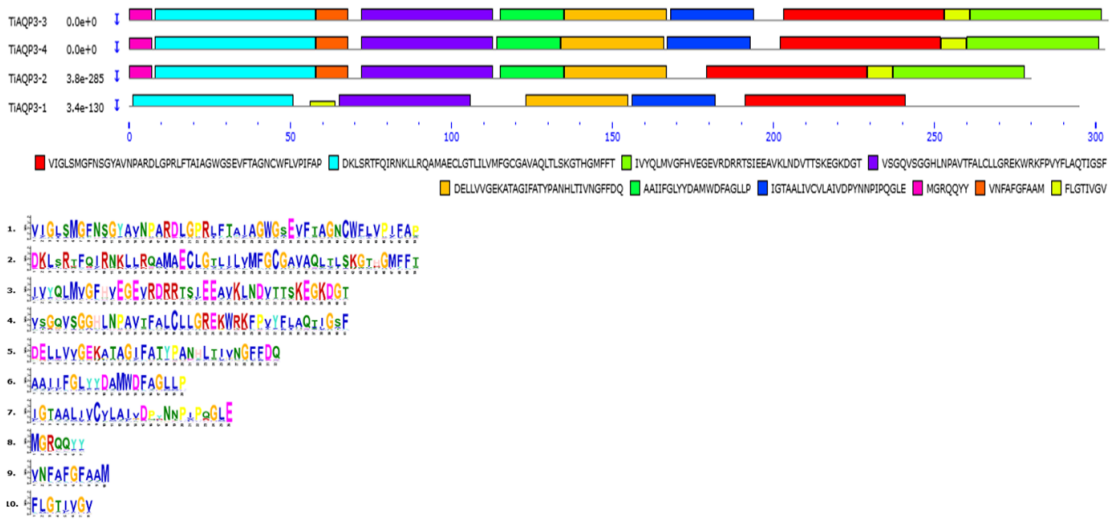

Fig. 2. Conserved motifs of TiAQP3 protein sequences. The colored boxes indicate specific conserved regions or motifs with their corresponding amino acid sequences detailed below. The scale at the top shows the relative positions of residues in the sequences

Multiple sequence alignment (MSA) and phylogenetic tree analysis

The multiple sequence alignment of 4 Hilsa *TiAQP3(1-4)* genes reveals conserved regions essential for function, especially in transmembrane domains. Highly conserved motifs, e.g., asparagine-proline-alanine (NPA boxes), are found, which are vital for water transportation (Fig. 3). There are also conserved hydrophobic and polar residues that are essential for structural integrity and selective permeability. This analysis suggests the evolutionary conservation of AQP3 proteins, with variability in extracellular and intracellular loops indicating diverse environmental adaptations in this fish species. The phylogenetic tree of *TiAQP3(1-4)* genes presents the evolutionary relationships among Hilsa AQP3 genes with other migratory 25 fish species. Result analysis revealed that *TiAQP3-1* clusters closely with *Nibea albiflora, Sparus aurata, Fundulus heteroclitus and Eptatretus stoutii* and *TiAQP3-2*, *TiAQP3-3* and *TiAQP3-4* clusters closely with *Clupea harengus, Poeciliopsis prolifica, Alosa sapidissima, Danio rerio, Gobiocypris rarus, Pseudaspius hakonensis, Cyprinus carpio, Misgurnus mizolepis,* and *Myxocyprinus asiaticus;* all are teleost fish (Fig. 4). The results indicated a common ancestry and functional conservation of AQP3 genes. Migratory species such as *Clupea harengus, Cyprinus carpio,* and *Misgurnus mizolepis*, show proximity to Hilsa AQP3 genes in the tree, suggesting similar adaptations for osmoregulation during migrations between freshwater and marine environments.

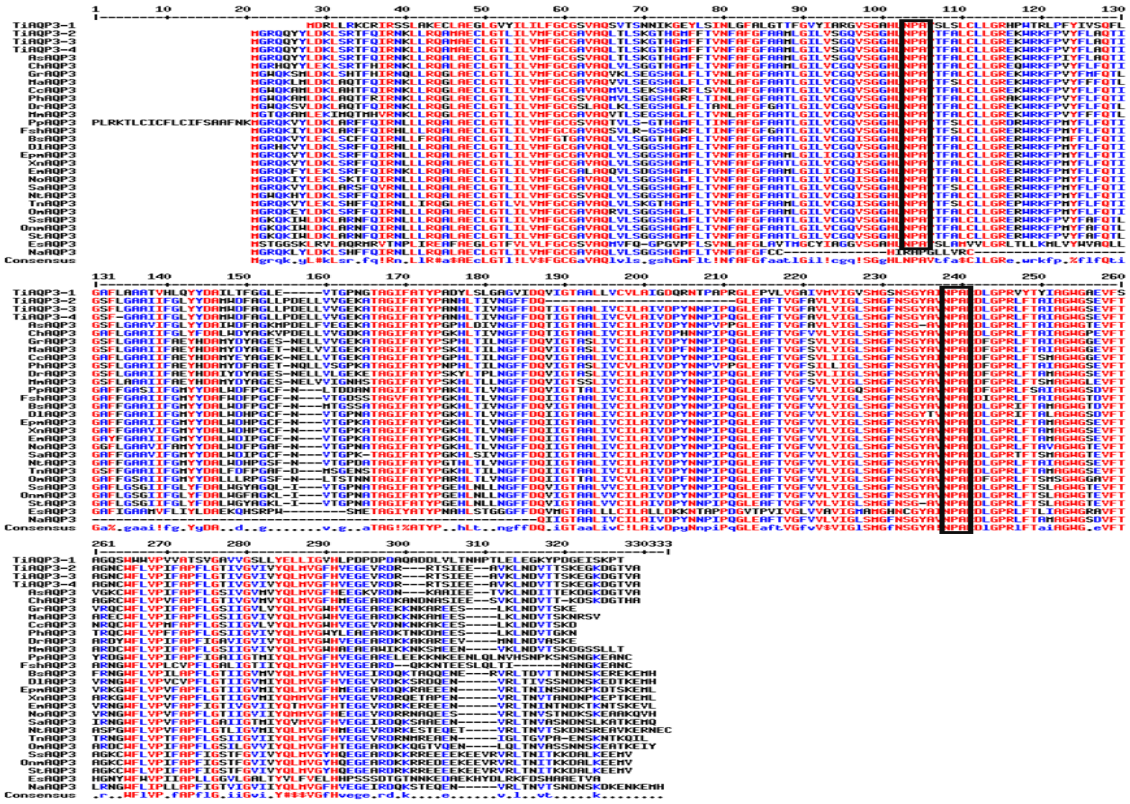

Fig. 3. Sequence alignment of aquaporin-3 (AQP3) proteins from various species. The conserved residues are highlighted, and the consensus sequence is presented at the bottom. Specific conserved motifs critical for function water transport (NPA regions) are marked with boxes

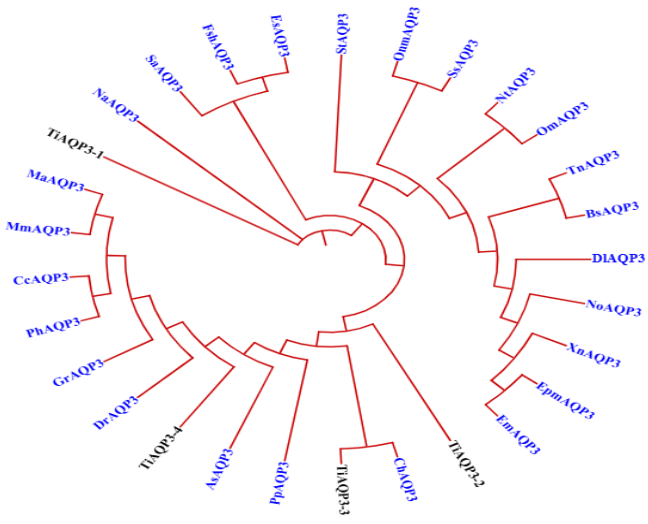

Fig. 4. Phylogenetic tree showing the evolutionary relationship among aquaporin-3 (AQP3) proteins across different species. Red lines represent evolutionary branches, and blue labels AQP3 genes from different fish species

Protein secondary structure and tertiary structure

The secondary structure features of *TiAQP3-1, TiAQP3-2, TiAQP3-3,* and *TiAQP3-4* highlight conserved characteristics typical of aquaporins (Supp. Fig. 1). Alpha-helices dominate the structures, ranging from 34.58% in *TiAQP3-1* to 38.49% in *TiAQP3-3*, forming the core of the transmembrane domains. Beta-sheets are consistent, contributing approximately 19% across all variants, while coils vary from 41.79% to 45.76%, reflecting their role in structural flexibility and loop regions. Notably, no 3-10 helices are predicted. Each gene encodes six membrane-spanning motifs (MSMs), characteristic of aquaporin channels, essential for water transport across membranes (Supp. Table 1). These shared features indicate structural conservation, crucial for their function, while slight differences in coil and alpha-helix content may suggest minor variations in stability or membrane interactions.

The tertiary structures of *TiAQP3-1*, *TiAQP3-2*, *TiAQP3-3,* and *TiAQP3-4* demonstrate high-quality 3D models, as reflected in their PROCHECK, ERRAT, and Z-score values. Most residues are in the most favored regions (92.0–93.9%), with minimal residues in the additional, generously allowed, or disallowed regions, ensuring accurate backbone geometry and stereochemical quality. ERRAT values range from 95.769 to 98.208, indicating high structural reliability, while Z-scores (-4.09 to -4.85) suggest consistency with known membrane protein structures (Supp. Table 2 and Fig. 2). The 3D structures display six transmembrane alpha-helices, characteristic of aquaporins, forming a hydrophilic pore for water transport (Fig. 5). The dipole arrangement of the helices, along with conserved motifs like NPA, contributes to selectivity and stability. Slight variations among the *TiAQP3* isoforms may reflect functional specialization.

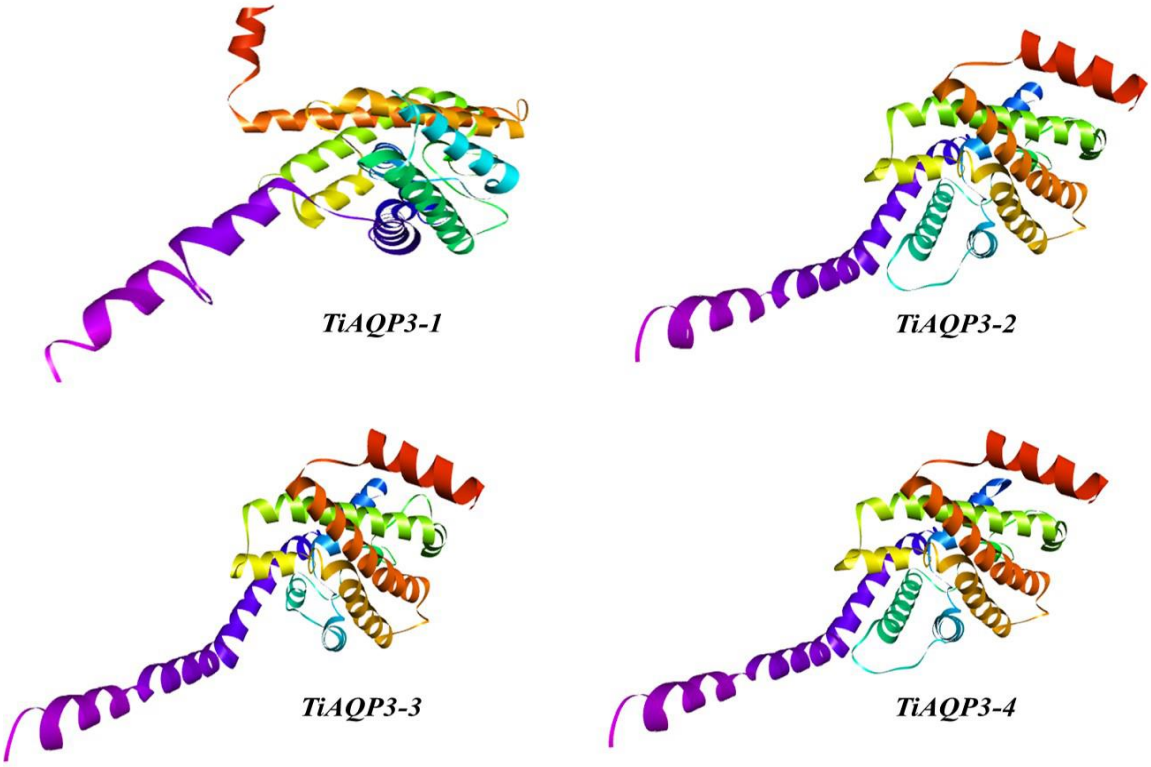

Fig. 5. Predicted 3D structural models of the four aquaporin-3 (AQP3) proteins from Hilsa species, namely TiAQP3-1, TiAQP3-2, TiAQP3-3, and TiAQP3-4. Each model is color-coded to represent secondary structural elements such as alpha-helices and beta-sheets

Expressions of *TiAQP3* gene in different habitats

The expression of the *TiAQP3* gene in *T. ilisha* exhibits tissue- and habitat-specific variations, highlighting its critical role in osmoregulation and environmental adaptation. In gill tissue, *TiAQP3* expression is significantly higher in fish from Chandpur, Bhola, and Haor compared to Cox's Bazar, as indicated by the statistical significance. This suggests that fish in these habitats experience greater osmoregulatory demands, possibly due to variations in salinity or water quality, necessitating increased aquaporin activity for water transport and ion balance. In kidney tissue, the highest *TiAQP3* expression is observed in fish from Cox's Bazar, significantly exceeding levels in the other habitats. This elevated expression may reflect the need for enhanced renal water reabsorption and ion homeostasis in response to environmental stressors such as higher salinity or osmotic gradients unique to this region.

In contrast, *TiAQP3* expression in muscle and liver tissues is relatively low across all habitats, with no significant differences observed. This indicates that *TiAQP3* has a limited functional role in these tissues, which are less directly involved in osmoregulation compared to gill and kidney tissues. The consistent low expression in muscle and liver suggests that these tissues are not primary sites for water transport or ion regulation.

Overall, the findings underscore the importance of gill and kidney tissues in mediating the habitat-specific osmoregulatory adaptations of *T. ilisha*. The differential expression of *TiAQP3* in these tissues reflects the species' ability to adjust to varying environmental conditions, ensuring survival and physiological homeostasis in diverse aquatic habitats.

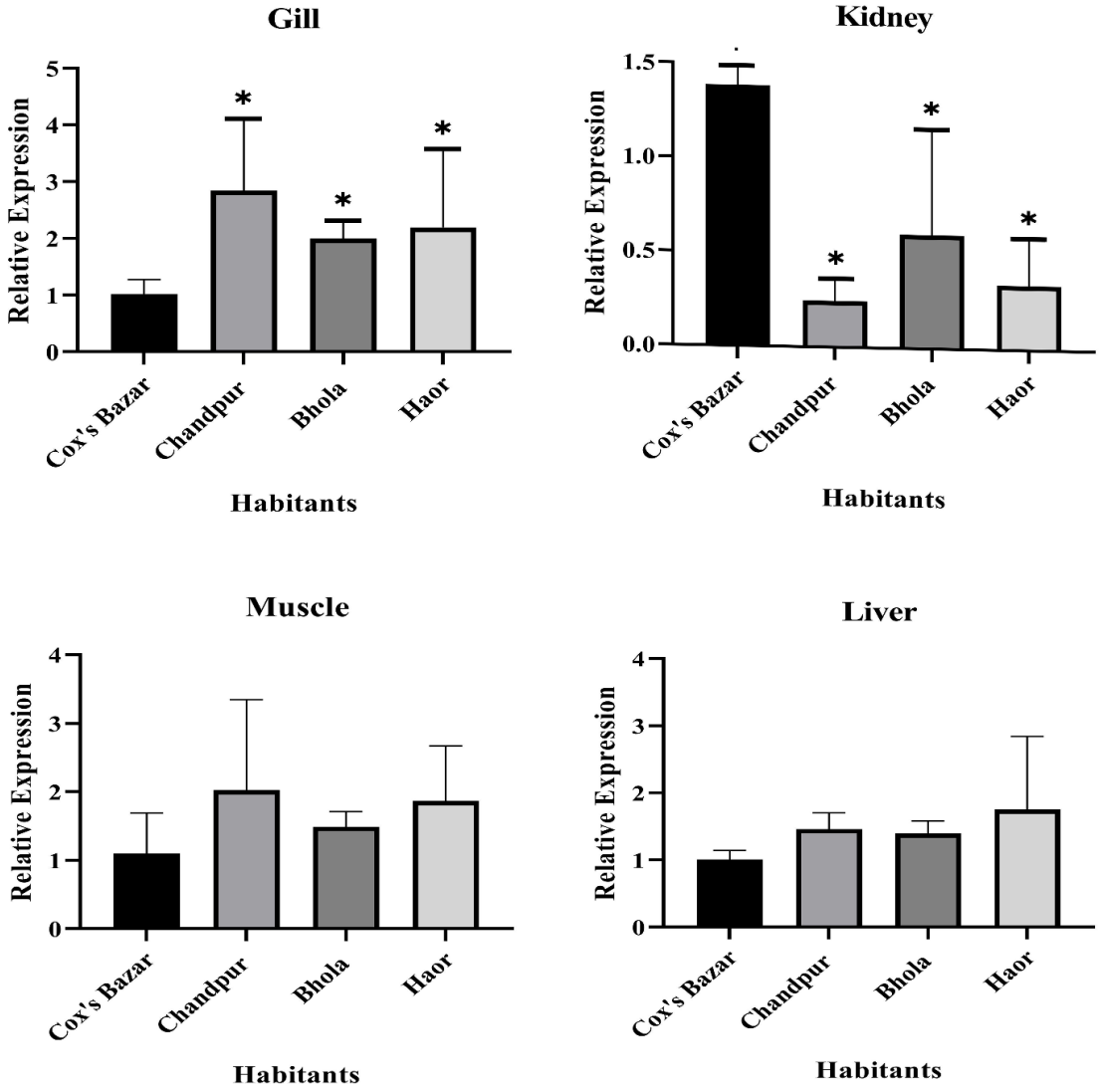

Fig. 6. Tissue-specific relative expression levels of *TiAQP3* genes in fish from different habitats. Expression levels in muscle, kidney, gill, and liver tissues are compared across Cox's Bazar, Chandpur, Bhola, and Haor regions. Asterisks (*) denote significant differences ($P < 0.05$)

DISCUSSION

The identification and characterization of the *TiAQP3* gene family in Hilsa is important to explore their functional and evolutionary roles. In this study, four TiAQP3

genes (*TiAQP3-1* to *TiAQP3-4*) were identified according to the presence of conserved aquaporin domains using the AQP3 sequence of the three-spined stickleback as a query. These proteins showed slide variations in amino acid lengths, molecular weights, and isoelectric points. But all the predicted genes were localized in the plasma membrane, indicating their conserved role in water transport. The exon-intron structures revealed all the genes share identical exon numbers (six exons and five introns) among the *TiAQP3* genes, though their orientations differed **(Dong *et al.*, 2016; Zhan *et al.*, 2023; Zhou *et al.*, 2023)**. This structural similarity indicates evolutionary conservation, while differences in orientation suggest potential functional deviations. The conserved motifs identified in these genes further support the functional consistency among *TiAQP3* proteins. Notably, the presence of highly conserved motifs, such as the NPA boxes, essential for water channeling, aligns with findings from other species **(Ishibashi, 2006)**. Phylogenetic analysis revealed that *TiAQP3* genes share close evolutionary relationships with *AQP3* genes from migratory fish species, such as *Clupea harengus* and *Cyprinus carpio*, suggesting shared mechanisms of osmoregulation critical for migration between freshwater and marine environments **(Dong *et al.*, 2016; Zhang *et al.*, 2021)**.

Structural analyses of TiAQP3 proteins highlight their functional reliability. Secondary structure predictions show alpha-helixes dominance in forming transmembrane domains, with conserved hydrophobic regions supporting selective water transport. The tertiary structures, validated by high-quality PROCHECK and ERRAT scores, confirm the structural integrity of the six transmembrane motifs and conserved hydrophilic pores. Subtle differences in alpha-helical and coil content among TiAQP3 isoforms may reflect adaptations to specific physiological needs **(Parry *et al.*, 1992)**.

Fig. (5) illustrates the relative expression levels of the *TiAQP3* gene in *T. ilisha* across four tissues: gill, kidney, muscle, and liver from different habitats Cox's Bazar, Chandpur, Bhola, and Haor. The *TiAQP3* gene shows significantly higher expression in the gills of fish from Chandpur, Bhola, and Haor (freshwater habitats) compared to Cox's Bazar (seawater). This elevated expression suggests increased *TiAQP3* activity in the gills, likely to meet the heightened osmoregulatory demands in freshwater environments. In the kidney, *TiAQP3* expression is highest in fish from Cox's Bazar, significantly surpassing levels observed in the other habitats. Fish from Chandpur, Bhola, and Haor show reduced but statistically significant kidney expression, indicating that kidney *TiAQP3* activity may be essential in Cox's Bazar due to salinity differences or freshwater stress adaptation.

In muscle tissue, *TiAQP3* expression is consistently low across all habitats, with no significant differences, implying that this gene plays a minimal role in muscle tissue compared to the osmoregulatory tissues of the gill and kidney. Similarly, the liver exhibits low *TiAQP3* expression across habitats, mirroring the trend observed in muscle and showing no significant variation. These findings suggest that *TiAQP3* has a negligible role in liver tissue under the tested conditions. Overall, the data underscore the

critical role of *TiAQP3* in gill and kidney tissues in facilitating osmoregulation and environmental adaptation in T. ilisha.

The tissue distribution of AQP3 varies significantly across species, indicating that this aquaporin may perform tissue-specific physiological roles adapted to the unique demands of each organism. Studies using a polyclonal antibody derived from sea bream AQP3 revealed its presence in the gill, kidney, spleen, heart, brain, and liver of sea bream. In Mozambique tilapia, however, transcript analysis detected AQP3 expression in the brain, kidney, spleen, and gill but not in the liver **(Watanabe *et al.*, 2005)**. Similarly, in the European eel, AQP3 transcripts were abundant in the gills, while expression in the kidney, brain, heart, and liver was negligible **(Cutler & Cramb, 2002)**. These findings underscore the species-specific roles of AQP3 in osmoregulation and other physiological processes **(Deane & Woo, 2006)**. In freshwater salmon, AQP3 expression was noted in all organs except the liver, with the highest levels in the esophagus, gills, and muscles **(Tipsmark *et al.*, 2010)**. Elevated *AQP3* expression in the gills of freshwater (FW)-acclimated fish highlights its crucial role in osmoregulation in this tissue. For instance, FW-adapted eels exhibit several-fold higher AQP3 expression in gills compared to seawater (SW)-adapted eels **(Cutler & Cramb, 2002; Lignot *et al.*, 2002)**. Similarly, FW-acclimated sea bass showed an eight-fold increase in AQP3 expression, accompanied by a 3- to 11-fold rise in osmotic water permeability and branchial urea clearance compared to SW-acclimated fish. This indicates that AQP3 facilitates water and urea transport in gill tissues **(Lignot *et al.*, 2002)**.

Under freshwater conditions, AQP3 likely prevents cell swelling caused by water influx across the apical surface of branchial epithelial cells **(Cutler & Cramb, 2000)**. It achieves this by facilitating water efflux from the branchial epithelium to the serosal fluid and subsequently into the circulatory system **(Cutler & Cramb, 2000)**. The excess water is then excreted as abundant isotonic urine **(Nebel *et al.*, 2005)**. In contrast, in seawater-acclimated fish, water uptake and reabsorption occur primarily in the gut and kidney to offset osmotic water loss in a hypertonic environment. The effect of salinity on gill AQP3 expression has been widely studied, with findings generally consistent with those of Cutler and Cramb. AQP3 expression is commonly downregulated in the gills of seawater-acclimated fish, as observed in European seabass (*Dicentrarchus labrax*), silver seabream (*Sparus sarba*), Mozambique tilapia (*Oreochromis mossambicus*), Japanese eel (*Anguilla japonica*), Atlantic salmon (*S. salar*), Japanese medaka (*Oryzias latipes*), marine medaka (*Oryzias dancena*), and killifish (*Fundulus heteroclitus*) **(Deane & Woo, 2004; Watanabe *et al.*, 2005, Giffard-Menan*et al.*, 2007; Tipsmark *et al.*, 2010; Jung *et al.*, 2012; Kim *et al.*, 2014; Gu *et al.*, 2015)**.

In tilapia, *AQP3* mRNA was detected in the kidney, with higher expression in seawater-acclimated fish than in freshwater-acclimated fish. This contrasts with other aquaporins, such as eel AQP1, AQP1dup, and AQPe, which showed different expression patterns under similar conditions. In Atlantic salmon, *AQP3* expression in the kidney was

notably lower than AQP1. As an aquaglyceroporin, *AQP3* facilitates the transport of small molecules like glycerol, urea, and ammonia (NH3) in addition to water. Its increased expression during seawater acclimation suggests a role in maintaining renal fluid balance and enhancing reabsorption, consistent with similar findings in seawater-acclimated European seabass **(Giffard-Mena *et al.*, 2007)** and tilapia **(Tipsmark *et al.*, 2010)**. For *AQP3* to improve the reabsorptive capacity of the seawater nephron, it must be localized on both the apical and basal membranes of the same tubular cells **(Tipsmark *et al.*, 2010)**.

The upregulation of *AQP3* in seawater-acclimated fish implies a specialized role in osmoregulation, particularly in water and fluid reabsorption within the distal nephron. In marine teleosts, maintaining water balance under hyperosmotic conditions is essential due to constant osmotic water loss to the surrounding seawater. Increased renal *AQP3* expression likely facilitates efficient water reabsorption from the filtrate into the circulatory system, aiding in fluid conservation and homeostasis. This function aligns with the physiological adaptations observed in marine teleosts, where the kidney and associated structures are optimized to minimize water loss while preserving ionic balance in saline environments. Further research on *AQP3* localization and functional dynamics in specific nephron segments could provide deeper insights into its precise role in osmoregulation under varying salinity conditions.

CONCLUSION

This study provides a genome-wide analysis of the *TiAQP3* gene family in Hilsa. Four *TiAQP3* genes have been identified and analyzed using bioinformatic tools. Data analysis revealed that *TiAQP3* genes showed similar features like evolutionary conservation, structural integrity, functional significance, etc. within these genes as well as in the other species. The identification of conserved motifs, exon-intron structures, and phylogenetic relationships indicates their critical role in osmoregulation and adaptation to diverse aquatic habitats. The structural analyses confirm their reliability as functional water channels. Expression patterns in organs like the kidney and gill suggest their regulatory role in environmental adaptability, particularly in terms of migratory contexts. These findings advance our understanding of aquaporins in migratory fish and provide a basis for future research on environmental resilience and evolutionary adaptations in aquatic species.

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Availability of data and material

The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The Authors declare that there is no conflict of interest.

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