

## Research Article



# Tracing molecular adaptation of mudskippers from water to land transition: Insight from the molecular dynamics simulation of collagen type-I

Lagzian M.<sup>1</sup>; Ghanbarifardi M.<sup>1\*</sup>

Received: June 2022

Accepted: February 2023

### Abstract

Molecular adaptation of mudskippers to land living is undoubtedly a complex multifactorial phenomenon, and the skin is one of the most influential factors. This study explores the behavior of collagen type-I (CT1) from the structural and functional point of view using an in-silico approach and discusses its possible role in the adaptation to aerial respiration. The approach was started by finding the CT1 triple helix gene and protein of *Boleophthalmus pectinirostris*, *Periophthalmus magnuspinnatus*, and *Danio rerio* (as the reference sample), followed by homology modeling and 100 ns molecular dynamics simulation. Subsequently, the trajectories were analyzed for RMSD, RMSF, Rg, H-bond, and SASA. The results all together showed that the flexibility of the CT1 of *Danio rerio* is considerably greater than *Boleophthalmus*, and the latter is more flexible than *Periophthalmus*. This difference in flexibility is also evident in the morphology of their skin since Zebrafish's skin is more elastic than the two mudskippers. The skin of *Boleophthalmus* is as well slightly softer than *Periophthalmus*. Our findings demonstrate that the more terrestrial species have the less flexible CT1 in the skin that saves them from desiccation. However, the skin structure of mudskippers makes a balance between the capability of respiration and saving them from desiccation. The results reveal one of the phenotypic adaptations of mudskippers for living on land, which is congruent with the morphological and physiological distinctive features.

**Keywords:** Mudskipper, Collagen, Molecular Dynamics, Water to Land Transition

<sup>1</sup>-Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran

\*Corresponding Author's Email: mehdi.ghanbarifardi@science.usb.ac.ir

## Introduction

Mudskippers, classified as Oxudercinae, are tropical and subtropical fishes distributed in shallow sublittoral, littoral, and supralittoral zones of the Indo-Pacific and western Africa. Ten genera, including *Apocryptes*, *Apocryptodon*, *Boleophthalmus*, *Oxuderces*, *Parapocryptes*, *Periophthalmodon*, *Periophthalmus*, *Pseudapocryptes*, *Scartelaos*, and *Zappa* are representing mudskippers (Murdy, 1989; Jaafar and Larson, 2008). These fish are commonly associated with soft-bottom habitats, especially mangroves, and mudflats (Ghanbarifardi *et al.*, 2016). Within the Indo-Pacific, mudskippers are spread from the Middle East and East Africa to Japan/South Korea, and Australia (Murdy, 1989; Jaafar and Larson, 2008).

Regarding geological periods, mudskippers are the most recently evolved fishes that are active on land. The most terrestrial genera, *Periophthalmodon* and *Periophthalmus*, are usually exposed to air. Some species of these genera remain on land even during high tide and present stressful reactions, such as a lowered heart rate and a substantial drop in blood oxygen levels when forcibly kept in water (Graham, 1997; Polgar *et al.*, 2010). Moreover, *Boleophthalmus* and *Scartelaos* are also adapted to terrestrial life, to some extent (Polgar *et al.*, 2017). Mudskippers have as well experienced changes in genomes, gene expressions, and protein structures for living in harsh intertidal environments. Whole-genome sequencing of four representative mudskippers (*Boleophthalmus*

*pectinirostris*, *Periophthalmodon schlosseri*, *Periophthalmus magnuspinnatus*, *Scartelaos histophorus*) elucidates the molecular mechanisms that underlying morphological and physiological terrestrial adaptations of these fishes. After diverging from other teleosts, mudskippers have acquired 684 genes that are not present in other analyzed teleosts (You *et al.*, 2014). These include expanded immune system gene clusters, elaborated pathway containing nine key genes for greater ability to detoxify ammonia than many other aquatic species, two new variants of long wavelength-sensitive opsin protein to enhance aerial vision (Tytler and Vaughan, 1983; You *et al.*, 2014), and two novel categories of vomeronasal receptors for improved olfaction (Boschat *et al.*, 2002; Leinders-Zufall *et al.*, 2004; You *et al.*, 2014). Furthermore, they retain the typical piscine respiratory and circulatory anatomy despite their ability to endure periods out of water; however, these fishes resemble amphibians in their reliance on the skin as a gas-exchange organ and are expected to cope with regulating cutaneous blood perfusion according to external conditions and internal necessities (Jaafar and Murdy, 2017).

Collagen, the main structural protein of vertebrates, is present in several types of tissues, including bone, cartilage, and especially skin (Brodsky and Persikov, 2005). 29 types of collagen molecules have been recognized so far. However, collagen Type-I is the most abundant form in the skin (Yang *et al.*, 2014). The quaternary structure of collagen Type-I is

composed of three polypeptides (alpha chains), each twisted in a left-handed helix and arranged together in a right-handed super-helix structure; this is stabilized by the existence of inter-chain crosslinks. Molecules of collagen Type-I of fishes are typically constituted from two alpha chains,  $\alpha 1$  and  $\alpha 2$  that have a similar molecular weight of about 120kDa (Kimura and Ohno, 1987; Bailey *et al.*, 1998; Gómez-Guillén *et al.*, 2002). More importantly, this molecule is one of the main governing factors in the morphological strength of the skin.

The present study aims to examine and compare collagen type-I of two mudskippers and zebrafish using molecular dynamics simulations and investigate if probable structural differences are related to terrestrial and aquatic adaptations.

## Materials and methods

### *Software and high-performance computing (HPC) system*

All classical sequence analyses including but not limited to sequence extraction, alignment, general nucleotide analyses, gene finding, exonic region prediction, and protein sequence analyses were conducted on Geneious Prime 2020.1.1 (Biomatters Ltd, New Zealand). All lower-level structural analyses, including homology modeling, were performed on Molsoft ICM-Pro 3.9-2c (Molsoft LLC, USA). Higher-level constructional evaluations, including molecular dynamics simulation (MD), were carried out by Molecular Operating Environment (MOE) 2020.09 (CCG, Canada), NAMD 2.14 molecular dynamics engine (Phillips

*et al.*, 2020), and VMD 1.9.4 (Humphrey *et al.*, 1996). All high-level computations were run on the high-performance cloud computing service provided by the University of Sistan and Baluchestan.

### *Mudskippers species and collagen type-I genes identification*

Searching the NCBI genome database (<https://www.ncbi.nlm.nih.gov/nuccore>) was shown there are four whole-genome sequenced mudskippers, including *Scartelaos histophorus*, *Boleophthalmus pectinirostris*, *Periophthalmodon schlosseri*, and *Periophthalmus magnuspinnatus* (You *et al.*, 2014). However, their assembly status was limited to the Scaffold level, which means the scaffolds are all unplaced, and also there is a high probability of many unresolved gaps in the sequence contigs. Since these genomes are roughly annotated, the tBLASTn search was as well used in addition to a normal search to identify the accurate location of collagen Type-I genes. As described earlier, the collagen Type-I triple helix is the most abundant type of this molecule comprised of two  $\alpha 1$ -fibers twisted around an  $\alpha 2$ -fiber. Therefore, there are two distinct genes for producing the complete helix. *Danio rerio* collagen Type-I reference mRNAs (NM\_199214.1 for  $\alpha 1a$  & NM\_182968.2 for  $\alpha 2$ ) were retrieved from GenBank and translated to their corresponding proteins. These proteins were used as the reference molecule for comparison purposes and also used as an input to run a tBLASTn search against nucleotide collections of

the mentioned mudskippers. Afterward, the potential gene regions were analyzed for exon-intron coding segments. All the exonic intervals were joined together and subsequently translated to the protein. The proteins were annotated by InterProScan analyses (Jones *et al.*, 2014). Finally, the first collagen triple helix repeat (58 amino acids in length) was extracted from each protein and used for deducing the homology models.

#### *Homology modeling*

As described above, all homology modeling steps were conducted by Molsoft ICM-Pro 3.9-2c. Initially, the identified repeated segments for the collagen triple helix were blasted to find an optimal template for the structural alignment, which is crucial for inferring a correct homology model. A low-resolution structure that belonged to collagen Type-I (PDB ID 3HR2) was predicted as the best template for modeling the collagen molecule investigated here. Since the template structure was only comprised of carbon  $\alpha$  trace, it could not be used as a correct template. Therefore, the structure was initially repaired accordingly before proceeding further to homology modeling. It should be noted that this is the only actual collagen Type-I structure file, and other suggested templates were artificial collagen-like peptides. That justifies why this template was used despite some issues with its structure.

Following the preparation of the template, it was aligned and justified to the above-indicated collagen segments. Subsequently, a multiple chains structure

builder with a full refinement algorithm of ICM-Pro 3.9-2c was used to make the homology model. To create the final functional collagen molecule, iHyd-PseCp server (Qiu *et al.*, 2016) was used to predict potential hydroxylation sites for proline amino acids. To build the final models, those spotted prolines were converted to hydroxyproline on each homology model. The best homology model was chosen based on the DOPE score. The quality and suitability of the models for subsequent computational analyses were assessed by evaluating their Ramachandran plots and SAVES server v6.0 analyses (Colovos and Yeates, 1993).

#### *Molecular dynamics simulation*

Tracing molecular adaptation of the collagen Type-I with two utterly different living environments was accomplished by MD simulation according to the method described elsewhere (Heidarpour Saremi *et al.*, 2021). Initially, all 3D macromolecular structures of the modeled collagens were corrected for probable unresolved atomic data. Subsequently, the structures were dissolved in TIP3P water molecules. A layer mode with an 8 Å margin was used to reduce the final size of the systems. All systems charges were neutralized by adding 0.1M counter ions (NaCl). Then an NPT ensemble (isobaric-isothermal conditions) with Amber10EHT all-atoms forcefield was used to perform the MD simulation: the systems were relaxed by energy minimization for 1 ns to remove any steric clashes or unusual geometry, which would artificially raise the energy

of the system. That was followed by a 5 ns heating step (0 -300K) to prevent the systems from getting trapped in the local minima. After that, all systems were equilibrated at the target temperature for 10 ns. Finally, the production step was conducted for 100 ns with a 2-fs time step. All light bond lengths (excluding water molecules) were constrained using the SHAKE algorithm. The systems were sampled each 2-ps and analyzed for RMSD (root-mean-square deviation), RMSF (root-mean-square fluctuation), Rg (radius of gyration), H-bond, and SASA (solvent accessible surface area) changes. All pre-MD steps were carried out on MOE 2020.09, and the simulations were done by NAMD 2.14 GPU accelerated version. All trajectories were analyzed by VMD 1.9.4a53.

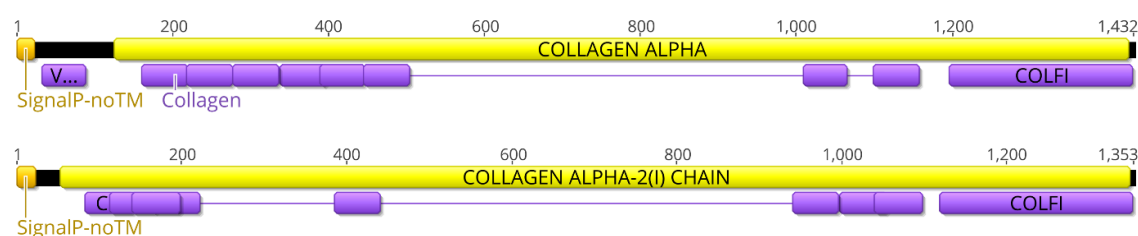
## Results

### *Collagen type-I gene and protein candidates*

As described in section 2.2, there are four different species of mudskippers with their whole genome sequenced. However, the tBLASTn output of *D. rerio* collagen Type-I against them returned positive

results only for the two species, including *B. pectinirostris* and *P. magnuspinnatus*. That was not surprising because, as mentioned earlier, these whole genomes just assembled at the scaffold level, which means some unplaced contigs plus unresolved gap segments. As a result, it is very likely that in the two other species not mentioned above (*S. histophorus* and *P. schlosseri*), the collagen locus is located in these regions and could not be identified by the BLAST result.

The identified collagen genes were encoded for the proteins, and their preliminary information was given in Table 1. As it can be seen, these proteins are primarily similar in their properties, including size and weight. That is very important in interpreting MD results because these specifications can influence them. For better understanding, the annotated primary structures of  $\alpha 1$  and  $\alpha 2$  subunits of collagen Type-I protein from *B. pectinirostris* are also shown in Figure 1. Multiple sequence alignments between all subunits listed in Table 1 have shown no more than 53% identity and 63% similarity (according to Blosom62) among them.



**Figure 1:** A schematic representation of pro-collagen Type-I subunits. The  $\alpha 1$  was displayed at the top and the  $\alpha 2$  at the bottom. The mature subunits are formed by removing signal peptides and black segments from both ends. The yellow segments indicated the mature collagen Type-I subunits interval. The collagen triple helix repeats are shown in violet color.

**Table 1: Summary of identified collagen Type-I protein subunits.**

Organism	Subunit	Mature Protein Size*	Mol. Weight (kDa)	Repeat Unit Size*	Repeat Unit Weight (kDa)
<i>D. rerio</i>	$\alpha 1$	1311	122.6	58	4.9
	$\alpha 2$	1295	121.3	58	5.3
<i>B. pectinirostris</i>	$\alpha 1$	1302	121.5	59	5.3
	$\alpha 2$	1295	121.5	57	5.2
<i>P. magnuspinnatus</i>	$\alpha 1$	1316	123	59	5.3
	$\alpha 2$	1296	120.8	59	5.1

\*Size in number of amino acids

*Homology models*

The PDB id 3HR2 was used as the highest similar template for homology models. As mentioned earlier, it belongs to a low-resolution structure of naturally-occurring collagen Type-I fibrils from *Rattus norvegicus*. Its subunits have around 78% and 64% identity to the identified  $\alpha 1$  and  $\alpha 2$  subunits of collagen Type-I from the three investigated species. Although this is not a high level of sequence identity, it is still in the standard range for homology modeling. The predicted models for the collagen Type-I molecule are shown in Fig. 3. Generally, all models are comparable, and the SAVES6.0 server analysis verified the quality of the models as suitable resolution structures. In this regard, the RMSD value between *B. pectinirostris*, *P. magnuspinnatus*, and *D. rerio* and the template are 2.16, 3.94, and 2.96 Å, respectively. Although, a highly successful homology model was defined as one having less than 2Å difference from the template. However, considering the low resolution of the template (5.16 Å) and medium sequence identity, the obtained results are very promising. A combined Ramachandran plot for all three models is also shown in Figure 2, indicating that less than 2.5% of the

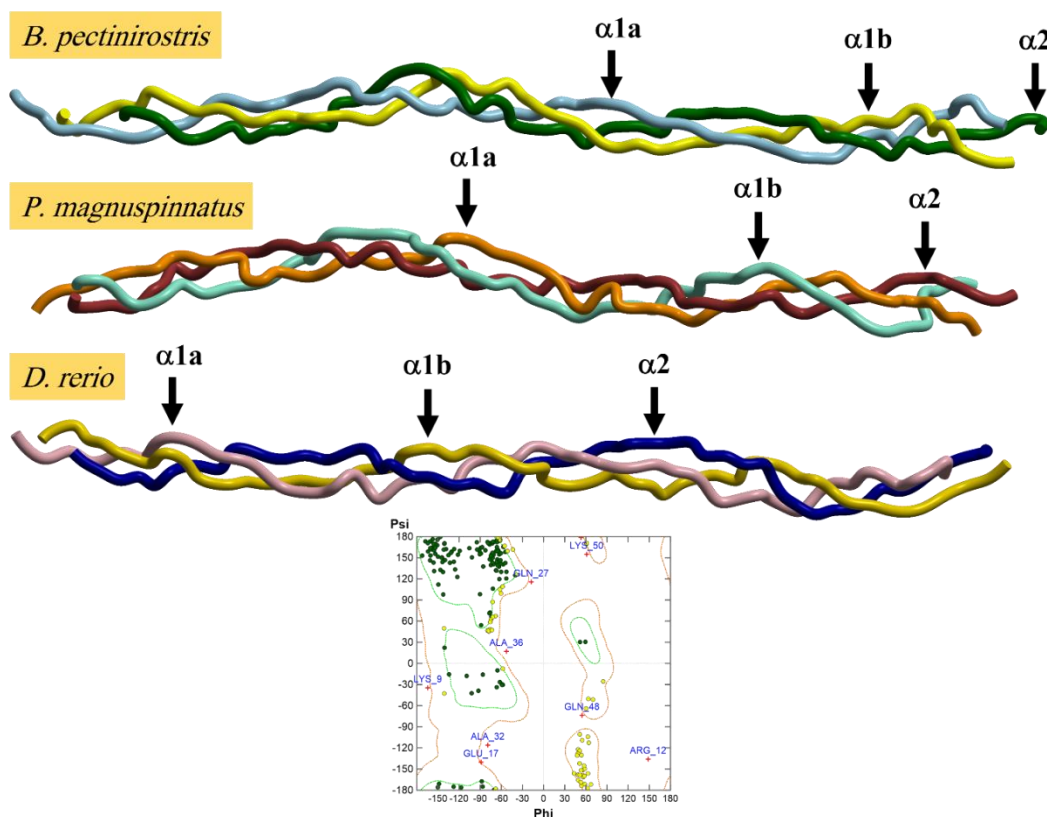
residues have resided outside the allowable areas. Considering all the above results, this strongly supports the geometric fitness of the modeled collagens.

*Molecular dynamics analysis*

Since collagen Type-I is one of the main components for the structure and function of the skin, potential molecular events that may lead to differences between mudskippers (*Boleophthalmus pectinirostris* and *Periophthalmus magnuspinnatus*), and a fully aquatic fish (*Danio rerio*) were investigated by analyzing the behavior of this molecule during a course of 100 ns using molecular dynamics simulation. The obtained results are described below.

*RMSD*

The root-mean square deviation of backbone atoms of three different collagen molecules are shown in Figure 4a. The trajectories reach a steady state after 5 ns (RMSD fluctuation < 1Å), indicating all systems were converged. The collagen Type-I triple helix from *D. rerio* demonstrates significantly higher flexibility than both examined mudskippers, as observed in the results.



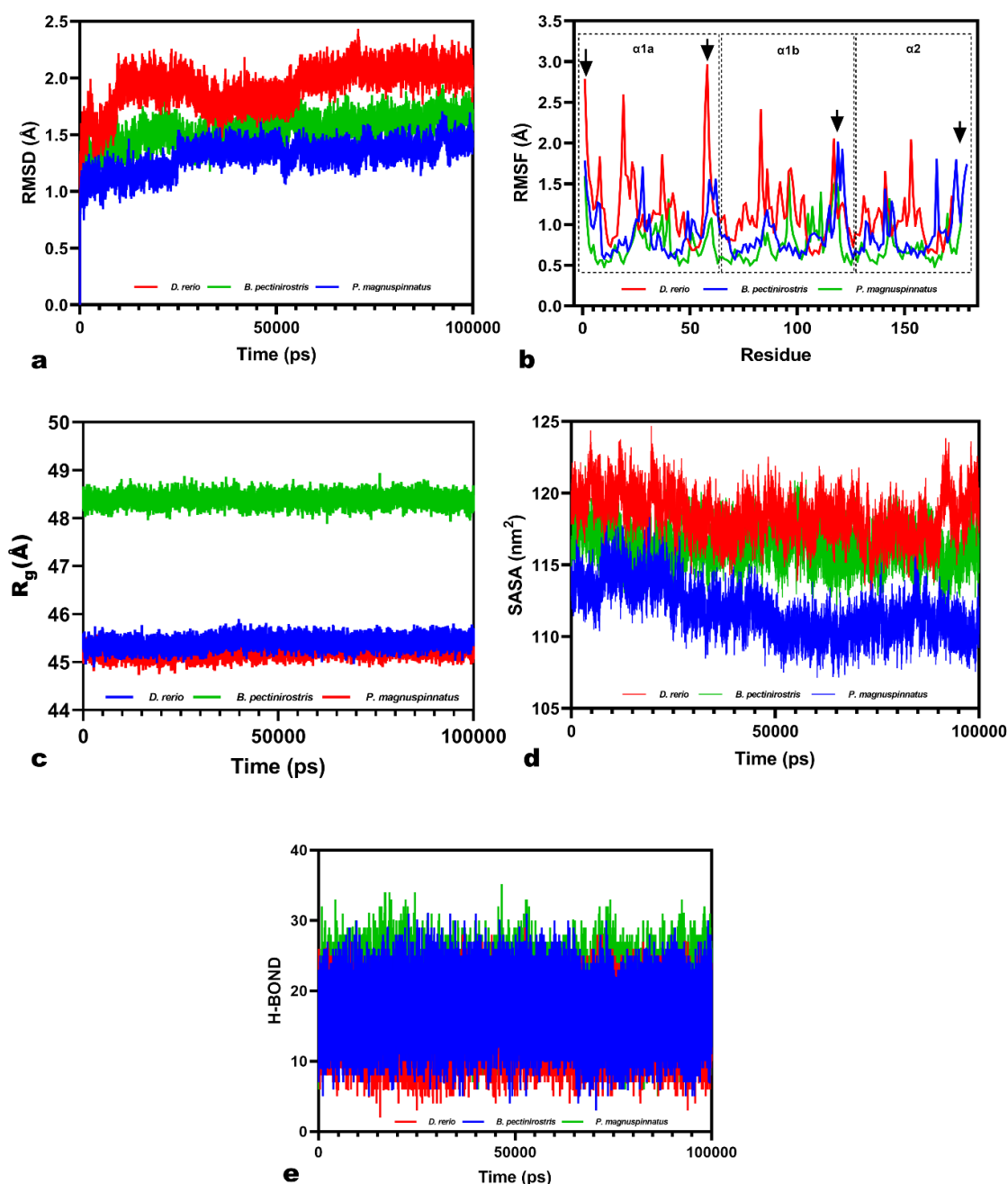
**Figure 2:** The homology models of the collagen Type-I triple helix repeat from the three different investigated species. The combined Ramachandran plot was given at the bottom of the image.

Surprisingly, the RMSD trajectory presented in Figure 3a highlights that the Collagen Type-I molecule of *B. pectinirostris* exhibits more flexibility than that of *P. magnuspinnatus*.

#### RMSF

Root-mean-square fluctuation of all residues of Collagen Type-I molecules from the investigated species during 100 ns MD simulations demonstrated in Figure 3b. Even by excluding a few residues at both ends of each helix of the Collagen Type-I molecule (black arrows in Fig. 3b), which have shown high RMSF values, *D. rerio* represented considerably higher fluctuation ( $1.141 \pm 0.39 \text{ \AA}$ ) comparing the two other

species of mudskippers. All three molecules showed more considerable fluctuation at both ends' residues due to the terminal flexibility of proteins (Medvedev *et al.*, 2014). However, some patches in the middle of the *D. rerio* collagen Type-I molecule showed RMSF values similar to the ends' residues. These patches are predominantly made of Ala amino acids with a few charged residues, which mimics a loop region that mostly corresponds to the largest RMSF values. Similar to the RMSD plot, *B. pectinirostris* have shown a bit higher RMSF values ( $0.897 \pm 0.29 \text{ \AA}$ ) compared to *P. magnuspinnatus* ( $0.745 \pm 0.22 \text{ \AA}$ ), which exactly corresponds to the greater amount of its flexibility.



**Figure 3:** Time evolution plot of RMSD (a), RMSF (b),  $R_g$  (c), SASA (d) and intramolecular H-bonds (e) of three investigated collagen Type-I molecules during 100 ns MD simulation at 310K. In the RMSF plot, the boundary of each subunit of triple helix is indicated above it. For more detail, please refer to the text.

### The Radius of Gyration ( $R_g$ )

This parameter reflects the radius of a macromolecule relative to its center of mass (an index of the molecule compactness) and its variation directly connected to its stability. When a molecule (protein) becomes unstable, its

$R_g$  value increases significantly and vice versa (Rhodes, 2006). As shown in Fig. 3c, the  $R_g$  trajectories for all three molecules have a completely steady-state and almost identical standard deviation during the whole 100 ns MD simulation ( $45.41 \pm 0.116$  Å for *D. rerio*,  $45.22 \pm 0.111$  Å



for *P. magnuspinnatus*, and  $48.39 \pm 0.112 \text{ \AA}$  for *B. pectinirostris*). Although Rg values do not show any direct information about the flexibility of a molecule, it shows all molecules have similar stability; therefore, any difference in their flexibility is not due to structural instability. The greater value of Rg for *B. pectinirostris* collagen Type-I is due to its longer length ( $170 \text{ \AA}$ ) comparing two other species ( $\approx 160 \text{ \AA}$ ).

#### Solvent Accessible Surface Area (SASA)

It is a decisive factor in the structural stability and folding of a protein, and it is in close relation to its flexibility (Marsh, 2013). High flexible proteins have greater SASA values than more rigid structures, which is completely according to the data demonstrated in Figure 3d. The figure shows that collagen Type-I from *D. rerio* demonstrate considerably higher SASA value ( $118 \pm 1.49 \text{ nm}$ ) than *P. magnuspinnatus* ( $111 \pm 1.72 \text{ nm}$ ).

#### Intramolecular H-bonds

As shown in Figure 4e, the average of intramolecular h-bonds for *P. magnuspinnatus* collagen Type-I is higher than the two other molecules ( $18.53 \pm 3.68$ ) during the 100 ns MD simulation. On the other hand, *D. rerio* shows the lowest number of H-bonds ( $14.67 \pm 3.28$ ) during the same time. Likewise, the average number of H-bonds for *B. pectinirostris* is placed between these two species ( $16.79 \pm 3.28$ ).

### Discussion

One of the influential factors in fish adaptations to environmental conditions

is their skin (including dermis and epidermis) (Rakers *et al.*, 2010; Tunnah *et al.*, 2022). Based on RMSD and RMSF analyses, higher flexibility may cause lower strength of connective tissue in the skin layers containing this collagen molecule. That can potentially increase the average water loss by diffusion through the skin in Zebrafish. However, given that this species spends their entire life in the water, this will not cause harm to them. On the other hand, both mudskippers have shown a more rigid collagen Type-I structure compared to *D. rerio*. That is entirely consistent with real-life conditions that these fishes are encountered. They spend much time out of the water; Therefore, they are more at risk of dehydration. That is so important because these creatures depend on oxygen to be absorbed through their skin by diffusion to survive in non-aqueous environments. This process requires a wet contact surface. As a result, rapid dehydration can be fatal due to impairing oxygen uptakes. For this reason, these animals use two methods to avoid body dehydration: Firstly, they primarily live in mudflats when they are not in water; Secondly, they have evolved morphological changes like improved skin structure to reduce passive water loss as much as possible (Jaafar and Murdy, 2017). Since the collagen Type-I structure is one of the governing factors for skin behavior, a more rigid triple helix structure can better fit the modified skin mentioned above. Interestingly, the RMSD trajectory in Fig. 4a has indicated higher flexibility for the collagen Type-I molecule of *B. pectinirostris* than *P.*

*magnuspinnatus*. That is completely in accordance with the different lifestyles between these species. In this regard, *B. pectinirostris* is known to spend more time underwater than *P. magnuspinnatus* (Jaafar and Murdy, 2017). As a result, it does not need to be very strict in maintaining the body's hydration level. Reduction in collagen synthesis is an important part of skin altering during air exposure since fine collagen during terrestrial excursions may be beneficial for locomotion, and thin skin could facilitate aerial respiration (Dong *et al.*, 2021; Dorostghoal *et al.*, 2022).

In addition, the trend of the *D. rerio* SASA trajectory is toward higher values, indicating the flexibility has increased, while for the *P. magnuspinnatus*, the opposite is true. Likewise, the SASA range for *B. pectinirostris* collagen Type-I has resided between two other species with a greater tendency to the *D. rerio*, which is alike to the results of RMSD analysis. Interestingly, all investigated collagen Type-I molecules had almost similar molecular weights. The obtained result is contrary to an investigation that indicated the SASA parameter is a direct function of molecular weight (Yang *et al.*, 2014). So, there is another reason for the observed difference between SASA values in these molecules. In this regard, deep inspections of amino acid composition indicated a greater hydrophobic content for *P. magnuspinnatus* than two other molecules. That is a driving force in determining the extent to which a protein interacts with the solvent (Durham *et al.*,

2009). Therefore, it may be the reason for differences between the SASA values.

Hydrogen bonds are one of the most important governing factors in protein stability and rigidity. More rigid structures benefit from a higher number of intramolecular H-bonds besides other stabilizing forces (Hubbard and Kamran-Haider, 2010). The hydrogen bonds, including N-H (Gly)  $\cdots$  O=C (X) hydrogen bond, O-H (Hyp in Y)  $\cdots$  O=C (peptide backbone) hydrogen bond and water mediated hydrogen bond, among  $\alpha$  chains strengthen collagen triple helix. Every individual polypeptide  $\alpha$ -chain of collagen is fixed through hydrogen bonding, which is formed between the hydroxyl groups of Gly and Hyp residues within two neighboring triplets (Zhang *et al.*, 2020). Our result shows *P. magnuspinnatus* collagen Type-I bears the most intramolecular h-bonds regarding the other two species; moreover, the least intramolecular h-bonds is observed *D. rerio* collagen Type-I, the fully aquatic fish. The observed result of this evaluation is consistent with the findings of RMSD, RMSF, and SASA analyses.

Given the results obtained from MD simulations, they all agree that the flexibility of the collagen Type-I molecules is a direct function of the amount of water available in the environment of the investigated fishes. Since maintaining the skin hydrated is critical for the respiration and gas exchange of those fishes that thrive on land under desiccation, it may be an adaptation mechanism for controlling the body's hydration level as much as

possible. The MD results are also in accordance with a previous study that indicated Mudskipper skin is modified to minimize desiccation (Theresa, 2011), and it contains a special type of cells (swollen mid cells) that act as both a water-loss barrier and water warehouse (Zhang *et al.*, 2003). Two other papers about Aquaporins (AQPs), a type of water channel, in mudskippers (Finn *et al.*, 2014; Lorente-Martínez *et al.*, 2018) indicated that aquaporins appear to have been crucial in the water-to-land transition of tetrapods. This channel is responsible for the transport of water and small uncharged solutes (e.g., glycerol and ammonia) through the membranes of the cells, especially those that resided at the surface layers of the skin. Selective changes that have occurred in that molecule and collagen Type I (the present study) of mudskippers (magnified in more terrestrialized genera, *Periophthalmus* and *Periophthalmodon*) could be related to their adaptation to an amphibious lifestyle (Lorente-Martínez *et al.*, 2018). In accordance with the present study, a recent skin histological examination of two mudskippers, *Periophthalmus waltoni* and *Boleophthalmus dussumieri* proved that epidermis in *B. dussumieri* is thicker significantly, mucous cells are also observed only in the epidermis of *B. dussumieri*; moreover, capillaries in the dorsal and ventral parts of the body are located nearer to the epidermal surface in *P. waltoni* (Dorostghoal *et al.*, 2022). These three key characteristics are important histological adaptations for *P. waltoni* to conduct aerial respiration

successfully.

This study investigated the molecular basis of mudskippers' adaptation for water to land transition and avoiding desiccation while having aerial respiration using computational structural biology methods, including homology modeling and molecular dynamics simulation on the collagen Type-I (CT1) as one of the most important components of the skin. The approach indicated a clear connection between the behavior of CT1 and the ability of the species to benefit from atmospheric oxygen. More specifically, the connection is inversely related to the flexibility of the CT1 of the investigated species. For example, CT1 from *D. rerio*, as a fully aquatic species entirely independent of atmospheric oxygen, showed the highest flexibility. Conversely, *P. magnuspinnatus* mostly lived on the land and is highly dependent on aerial oxygen absorption and retains body water by showing the lowest flexibility of CT1 in its exclusive skin.

Interestingly, the flexibility of CT1 from *B. pectinirostris*, which is more dependent on the aquatic environment for life, has a greater tendency towards *D. rerio* than *P. magnuspinnatus*. In other words, this is more likely that adjusting the flexibility of CT1 is a mechanism for controlling the skin's permeability to water. In conclusion, mudskippers could be used as live representatives to examine evolutionary steps of water-to-land adaptation, such as exchanging aerial gases and prohibiting evaporation. However, many known genomic changes in mudskippers have not been examined in the transcriptome, translation, and

protein structure levels. The protein structures of these fishes, like CT1 in this study, should be compared to the other teleosts' proteins in future studies.

### Acknowledgments

The authors are thankful to the University of Sistan and Baluchestan Research Council for their valuable help throughout this work.

### References

- Bailey, A.J., Paul, R.G. and Knott, L., 1998.** Mechanisms of maturation and ageing of collagen. *Mechanisms of Ageing and Development*, 106(1-2), 1-56. DOI: 10.1016/S0047-6374(98)00119-5
- Boschat, C., Pélofi, C., Randin, O., Roppolo, D., Lüscher, C., Broillet, M.C. and Rodriguez, I., 2002.** Pheromone detection mediated by a V1r vomeronasal receptor. *Nature Neuroscience*, 5(12), 1261-1262. DOI: 10.1016/S0047-6374(98)00119-5
- Brodsky, B. and Persikov, A.V., 2005.** Molecular Structure of the Collagen Triple Helix. *Advances in Protein Chemistry*, 70, 301-339. DOI: 10.1016/S0065-3233(05)70009-7
- Colovos, C. and Yeates, T.O., 1993.** Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Science*, 2(9), 1511-1519. DOI: 10.1002/pro.5560020916
- Dong, Y.W., Blanchard, T.S., Noll, A., Vasquez, P., Schmitz, J., Kelly, S.P., Wright, P.A. and Whitehead, A., 2021.** Genomic and physiological mechanisms underlying skin plasticity during water to air transition in an amphibious fish. *Journal of Experimental Biology*, 224(2), 1-10. DOI: 10.1242/jeb.235515
- Dorostghoal, M., Jazayeri, A. and Ashiri, S. 2022.** Histological study on the skin structure in two mudskippers, *Periophthalmus waltoni* and *Boleophthalmus dussumieri* in relation to their terrestrial life. *Applied Microscopy*, 52(1), 1-6. DOI: 10.1186/s42649-022-00081-2
- Durham, E., Dorr, B., Woetzel, N., Staritzbichler, R. and Meiler, J., 2009.** Solvent accessible surface area approximations for rapid and accurate protein structure prediction. *Journal of Molecular Modeling*, 15(9), 1093-1108. DOI: 10.1007/s00894-009-0454-9
- Finn, R.N., Chauvigne, F., Hlidberg, J.B., Cutler, C.P. and Cerda, J., 2014.** The lineage-specific evolution of aquaporin gene clusters facilitated tetrapod terrestrial adaptation. *PLoS One*, 9(11), e113686. DOI: 10.1371/journal.pone.0113686
- Ghanbarifardi, M., Esmaeili, H.R., Gholami, Z., Aliabadian, M. and Reichenbacher, B., 2016.** Molecular phylogeny of three mudskippers (Gobiidae) from the Persian Gulf and Gulf of Oman (Makran). *Journal of Applied Ichthyology*, 32(3), 416-420. DOI: 10.1111/jai.12999
- Gómez-Guillén, M.C., Turnay, J., Fernández-Díaz, M.D., Ulmo, N., Lizarbe, M.A. and Montero, P., 2002.** Structural and physical properties of gelatin extracted from different marine species: a

- comparative study. *Food Hydrocolloids*, 16(1), 25-34. DOI: 10.1016/S0268-005X(01)00035-2
- Graham, J.B., 1997.** Air-Breathing Fishes. Academic Press. San Diego, USA. p 299.
- Heidarpour Saremi, L., Ebrahimi, A. and Lagzian, M., 2021.** Identification of new potential cyclooxygenase-2 inhibitors: insight from high throughput virtual screening of 18 million compounds combined with molecular dynamic simulation and quantum mechanics. *Journal of Biomolecular Structure and Dynamics*, 39(5), 1717-1734. DOI: 10.1080/07391102.2020.1737574
- Hubbard, R.E. and Kamran-Haider, M., 2010.** Hydrogen Bonds in Proteins: Role and Strength. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0003011.pu b2
- Humphrey, W., Dalke, A. and Schulten, K. 1996.** VMD: Visual molecular dynamics. *Journal of Molecular Graphics*, 14(1), 33-38. DOI: 10.1016/0263-7855(96)00018-5
- Jaafar, Z. and Larson, H.K., 2008.** A new species of mudskipper, *Periophthalmus takita* (Teleostei: Gobiidae: Oxudercinae), from Australia, with a key to the genus. *Zoological Science*, 25(9), 946-952. DOI: 10.2108/zsj.25.946
- Jaafar, Z. and Murdy, E.O., 2017.** Fishes Out of Water: Biology and ecology of mudskippers. CRC Press, Taylor & Francis Group, England. p 410
- Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A. F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.-Y., Lopez, R. and Hunter, S., 2014.** InterProScan 5: genome-scale protein function classification. *Bioinformatics*, 30(9), 1236-1240. DOI: 10.1093/bioinformatics/btu031
- Kimura, S. and Ohno, Y., 1987.** Fish type I collagen: tissue-specific existence of two molecular forms, ( $\alpha 1$ ) $2\alpha 2$  and  $\alpha 1\alpha 2\alpha 3$ , in Alaska pollack. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 88(2), 409-413. DOI: 10.1016/0305-0491(87)90320-8
- Leinders-Zufall, T., Brennan, P., Widmayer, P., Chandramani-S., P., Maul-Pavicic, A., Jager, M., Li, X. H., Breer, H., Zufall, F. and Boehm, T., 2004.** MHC class I peptides as chemosensory signals in the vomeronasal organ. *Science*, 306(5698), 1033-1037. DOI: 10.1126/science.1102818
- Lorente-Martínez, H., Agorreta, A., Torres-Sánchez, M. and San Mauro, D., 2018.** Evidence of positive selection suggests possible role of aquaporins in the water-to-land transition of mudskippers. *Organisms Diversity and Evolution*, 18, 499-514. DOI: 10.1007/s13127-018-0382-6
- Marsh, J.A., 2013.** Buried and accessible surface area control intrinsic protein flexibility. *Journal of*

- Molecular Biology*, 425(17), 3250-3263. DOI: 10.1016/j.jmb.2013.06.019
- Medvedev, K.E., Alemasov, N.A., Vorobjev, Y.N., Boldyreva, E.V., Kolchanov, N.A. and Afonnikov, D.A., 2014.** Molecular dynamics simulations of the Nip7 proteins from the marine deep- and shallow-water *Pyrococcus* species. *BMC Structural Biology*, 14, 23. DOI: 10.1186/s12900-014-0023-z
- Murdy, E.O., 1989.** A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *The Australian Museum*, 11, 1-93. DOI: 10.3853/j.0812-7387.11.1989.93
- Phillips, J.C., Hardy, D.J., Maia, J.D.C., Stone, J.E., Ribeiro, J.V., Bernardi, R.C., Buch, R., Fiorin, G., Henin, J., Jiang, W., McGreevy, R., Melo, M.C.R., Radak, B.K., Skeel, R.D., Singharoy, A., Wang, Y., Roux, B., Aksimentiev, A., Luthey-Schulten, Z., Kale, L.V., Schulten, K., Chipot, C. and Tajkhorshid, E., 2020.** Scalable molecular dynamics on CPU and GPU architectures with NAMD. *The Journal of Chemical Physics*, 153(4), 044130. DOI: 10.1063/5.0014475
- Polgar, G., Sacchetti, A. and Galli, P., 2010.** Differentiation and adaptive radiation of amphibious gobies (Gobiidae: Oxudercinae) in semi-terrestrial habitats. *Journal of Fish Biology*, 77(7), 1645-1664. DOI: 10.1111/j.1095-8649.2010.02807.x
- Polgar, G., Ghanbarifardi, M., Milli, S., Agorreta, A., Aliabadian, M., Esmaeili, H.R. and Khang, T.F., 2017.** Ecomorphological adaptation in three mudskippers (Teleostei: Gobioidae: Gobiidae) from the Persian Gulf and the Gulf of Oman. *Hydrobiologia*, 795(1), 91-111. DOI: 10.1007/s10750-017-3120-8
- Qiu, W.-R., Sun, B.-Q., Xiao, X., Xu, Z. C. and Chou, K.C., 2016.** iHyd-PseCp: Identify hydroxyproline and hydroxylysine in proteins by incorporating sequence-coupled effects into general PseAAC. *Oncotarget*, 7(28), 44310-44321. DOI: 10.18632/oncotarget.10027
- Rakers, S., Gebert, M., Uppalapati, S., Meyer, W., Maderson, P., Sell, A. F., Kruse, C. and Paus, R., 2010.** Fish matters: the relevance of fish skin biology to investigative dermatology. *Experimental Dermatology*, 19(4), 313-324. DOI: 10.1111/j.1600-0625.2009.01059.x
- Rhodes, G., 2006.** Crystallography Made Crystal Clear (Third Edition)., Academic Press, Elsevier, Burlington, USA, p 306.
- Theresa, F.D., 2011.** Direct evidence of cutaneous resistance to evaporative water loss in amphibious mudskipper (family Gobiidae) and rockskipper (family Blenniidae) fishes from Pulau Hoga, southeast Sulawesi, Indonesia. *Journal of experimental marine biology and ecology*, 406(1-2), 125-129. DOI: 10.1016/j.jembe.2011.05.032
- Tunnah, L., Turko, A. J. and Wright, P.A., 2022.** Skin ionocyte density of amphibious killifishes is shaped by phenotypic plasticity and constitutive

- interspecific differences. *Journal of Comparative Physiology B*, 192(6), 701-711. DOI: 10.1007/s00360-022-01457-2
- Tytler, P. and Vaughan, T., 1983.** Thermal ecology of the mudskippers, *Periophthalmus koelreuteri* (Pallas) and *Boleophthalmus boddarti* (Pallas) of Kuwait Bay. *Journal of Fish Biology*, 23(3), 327-337. DOI: 10.1111/j.1095-8649.1983.tb02912.x
- Yang, Y.J., Jung, D., Yang, B., Hwang, B.H. and Cha, H.J., 2014.** Aquatic proteins with repetitive motifs provide insights to bioengineering of novel biomaterials. *Biotechnology Journal*, 9(12), 1493-1502. DOI: 10.1002/biot.201400070
- You, X., Bian, C., Zan, Q., Xu, X., Liu, X., Chen, J., Wang, J., Qiu, Y., Li, W., Zhang, X., Sun, Y., Chen, S., Hong, W., Li, Y., Cheng, S., Fan, G., Shi, C., Liang, J., Tom Tang, Y., Yang, C., Ruan, Z., Bai, J., Peng, C., Mu, Q., Lu, J., Fan, M., Yang, S., Huang, Z., Jiang, X., Fang, X., Zhang, G., Zhang, Y., Polgar, G., Yu, H., Li, J., Liu, Z., Zhang, G., Ravi, V., Coon, S. L., Wang, J., Yang, H., Venkatesh, B., Wang, J. and Shi, Q., 2014.** Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nature Communications*, 5(1), 5594. DOI: 10.1038/ncomms6594
- Zhang, J., Taniguchi, T., Takita, T. and Ali, A., 2003.** A study on the epidermal structure of *Periophthalmodon* and *Periophthalmus* mudskippers with reference to their terrestrial adaptation. *Ichthyological Research*, 50, 310-317. DOI: 10.1007/s10228-003-0173-7
- Zhang, X., Xu, S., Shen, L. and Li, G., 2020.** Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. *Journal of Leather Science and Engineering*, 2(1), 1-29. DOI: 10.1186/s42825-020-00033-0