DOI: https://doi.org/10.3897/arphapreprints.e101467

Genetic diversity among sea snakes of the genus *Hydrophis* (Elapidae, Reptilia) in the Persian Gulf and Gulf of Oman

Seyyed Saeed Hosseinian Yousefkhani, Amaal Yasser, Murtada Naser, Mohsen Rezaie-Atagholipour, Majid Askari Hesni, Fariba Yousefabadi, Eskandar Rastegar-Pouyani

Genetic diversity among sea snakes of the genus *Hydrophis* (Elapidae, Reptilia) in the Persian Gulf and Gulf of Oman

Seyyed Saeed Hosseinian Yousefkhani[1], Amaal Yasser[2, 3], Murtada Naser[2, 4], Mohsen Rezaie-Atagholipour[5], Majid Askari Hesni[6], Fariba Yousefabadi[7], Eskandar Rastegar Pouyani[8, *]

1. Department of Animal Science, School of Biology, Damghan University, Damghan, Iran

2. Marine Science Centre, University of Basrah, Basra, Iraq

3. Australian Rivers Institute, Griffith University, 170 Kessels Road, Nathan, Queensland 4111 Australia

4. School of Environment and Science, Griffith University, 170 Kessels Road, Nathan, Queensland, 4111, Australia

5. Environmental Management Office, Qeshm Free Area organization, Qeshm Island, P. O. Box 7951614465, Hormozgan Province, Iran

6. Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman Province, Iran

7. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

8. Department of Biology, Hakim Sabzevari University, Sabzevar, Iran

* Corresponding author's Email: Eskandar Rastegar Pouyani: rastegarpouyani45@gmail.com

Abstract

The Persian Gulf and Gulf of Oman are two important marine ecosystems in southern Iran with rich biodiversity. Sea snakes of the genus *Hydrophis* are important components of the animal diversity in this area. Ten species of the genus *Hydrophis* have been distinguished in the region and their genetic structure was compared with other populations in south and southeast Asia. We found that five species (including *H. platurus, H. cyanocinctus, H. spiralis, H. schistosus* and *H. gracilis, H. lapemiodes*) show high genetic similarity with conspecific populations in the Indian Ocean and Australia. *Hydrophis curtus* from southern Iran shows a high level of differentiation from other populations in Sri Lanka and Australia. *Hydrophis curtus* in our study shows variation and the Iranian samples of the species of 0.6% and 6% genetic distance from other populations in Sri Lanka for 16S and COI gene fragments, respectively. This means the variability between Iranian and southeast Asia populations may reveal new genetic lineages and the need of further morphological evaluations to re-evaluate their taxonomic position.

Keywords

True sea snake, Indian Ocean, microhabitat adaptation, dispersal, variation.

Introduction

Sea snakes are known as part of the marine ecosystems and play a significant role in the food chain as both predators for small marine fauna and prey for larger predators (Voris 1972, Udyawer et al. 2018). Their range of distribution is global due to their habitat, which includes seas, oceans and gulfs. Various studies have been conducted on sea snakes in Southeast Asia, India, Indonesia, and northern Australia, each with a specific purpose (Ukuwela et al. 2012, Ukuwela et al. 2016). True sea snakes of the group *Hydrophinii* have shared common ancestor since approximately six million years ago, but the major speciation events and radiation within the group and the genus *Hydrophis* took place over the last 3.5 million years ago (Sanders et al. 2013).

The Persian Gulf and Gulf of Oman are known as areas with high biological diversity and are located in the south of Iran (Owfi et al. 2016) and share a herpetofauna with the Indian Ocean (Rezaie-Atagholipour et al. 2016). So far, 10 species of the genus *Hydrophis* have been recorded in these regions as: *H. platurus*, *H. schistosus*, *H. curtus*, *H. viperinus*, *H. spirlais*, *H. cyanocinctus*, *H. ornatus*, *H. lapemoides*, *H. gracilis* and *H. cantoris* (Rajabizadeh 2019). Among these species, *H. platurus* has a wider distribution range toward the eastern Africa (Heatwole 1999), but the westernmost ranges of the other species reaches to the Persian Gulf (Rezaie-Atagholipour et al. 2016). The geomorphological structure of the Persian Gulf and water salinity in the region are important factors that suggest the need to examine marine snakes within other areas such as the Indian Ocean of Pacific region (Sheppard et al. 2010). One study examined genetic diversity and gene flow within *Hydrophis curtus* and found high genetic diversity (Ukuwela et al. 2014), illustrating the importance of the Indonesia-Australia region for biodiversity (Voris 1972, Dunson 1975). Full evaluation of true sea snakes in Indo-Pacific region

revealed that speciation in last three million years was influenced by the sea level changes which made barriers among marine basins (Ukuwela et al. 2016).

In this study, available genetic markers of different species of sea snakes of the genus *Hydrophis* from the Persian Gulf and the Gulf of Oman were compared to different populations in other areas of the Indian Ocean, Southeast Asia, and Indonesia. Examining the genetic structure of the species in the Persian Gulf and comparing them with other populations can illustrate the degree of genetic connection between these populations.

Materials and Methods

Tissue sampling and DNA extraction

Tissue samples were obtained from the specimens collected in the southern coastal regions of Iran (Persian Gulf and Gulf of Oman) during 2013 field work (Rezaie-Atagholipour et al. 2016) and the samples were preserved in 96% ethanol. A total of 31 samples used in this study belong to seven species from four localities in southern Iran (Table S1; Fig. 1) and 27 sequences were downloaded from GenBank. Also, sequences of *Ephalophis greyae* were downloaded from GenBank and used as the outgroup. Total genomic DNA was extracted from muscle tissue using the standard proteinase K – salt method and the quality and concentration of extracted DNA were measured using Nanodrop 1000.

Mitochondrial and nuclear fragment sequences

Two mitochondrial sequences, 16SrRNA (*16S*) (Kocher et al. 1989) and Cytochrome Oxidase-subunit 1(*COI*), and one anonymous nuclear marker (*G1888*) (Bertozzi et al. 2012) were used to reconstruct the molecular phylogenetic relationship between Iranian and Southeast Asia species of sea snake. Two of these markers were obtained from previous successful studies (Lukoschek

and Keogh 2006, Sanders et al. 2013), but COI is a new marker in the present study. The following primers were used for each gene fragments; 16S: 16SL 5'-CGCCTGTTT ATCAAAAACAT-3'/ 16SH 5'-CCGGTCTGAACTCAGATCACG-3'; COI: RepCOIF 5'-TNTTMTCAACNAACCACAAAGA-3'/ RepCOIR 5'-ACTTCTGGRTGKCCAAARAATCA-3'; G1888: G1888F 5'- CAGGGCCTTGCCTTGTGCCA-3'/ G1888R 5'-ACCTCTGCGCACTATGACTCTTGA-3' (Bertozzi et al. 2012). All DNA sequences were amplified using a standard PCR protocol as denaturation at 94℃ for 5 minutes, annealing temperatures of 49 ℃ for mitochondrial fragments and 52 ℃ for anonymous nuclear marker for 45 seconds, and elongation at 72℃ for 70 seconds by 36 cycles and the final elongation for 8 minutes. Sequencing of the PCR products was performed by the Kodon genetic group in Tehran, Iran. Sequences of the same species and the same genetic fragments from Southeast Asia and the outgroup were downloaded from GenBank and added to the dataset (Table S1). Obtained sequences were aligned under the ClustalW algorithm that implemented within Bioedit 7.0.9.0 (Hall 1999) and the protein coding genes were translated to amino acid sequences using MEGA 6.0 (Tamura et al. 2013) to check for internal stop codons and determine the correct reading frame. The sequences generated in this study will be deposited in GenBank database and will be added into the Table S1. Uncorrected genetic distance (*P* distance) was calculated using MEGA 6.0 for *16S*. The sequences of the COI gene fragment were not sufficient and we did not have more than one sequence for representatives from each lineage, so it could not be used to calculate genetic distance.

Molecular phylogenetic analyses and haplotype network

Two mitochondrial and one nuclear fragment were concatenated (total length: 1723 bp; *16S*: 523 bp; *COI*: 708 bp; *G1888*: 492 bp) and phylogenetic trees were reconstructed using Bayesian

Inference (BI) and Maximum Likelihood (ML) methods. The best substitution model for the concatenated alignment was TVM+I+G. RaxML 7.4.2 (Stamatakis 2006) as implemented in RaxmlGUI 1.3 (Silvestro and Michalak 2012) was employed for ML analyses. The substitution model was set as GTR+G+I in RaxMl software for the molecular phylogenetic reconstruction. Bootstrapping was set as 1000 replicates to find the node support and the analysis run as heuristic search method (Felsenstein 1985). MrBayes 3.2.1 (Ronquist et al. 2012) was used to run the BI analysis and the number of generations were set as 10^7 with a sample frequency of every 1000 generations.

The relationships among lineages and subclades of the genus *Hydrophis* were assessed with *16S rRNA* mitochondrial locus, because the number of sequences of *16S* covered all species. Sequence alignment was entered to DNAsp v. 5.0. (Librado and Rozas, 2009) and created *.rdf extension file for Network 1.2.1. The haplotype network was calculated using Median Joining method.

Results

Our dataset includes 59 samples containing two mitochondrial gene fragments 16S (495 bp; 77 *V*; 40 *Pi*) and COI (661 bp; 158 *V*; 112 *Pi*) and one nuclear anonymous fragment G1888 (375 bp; 265 *V*; 250 *Pi*) totaling 1531 bp. Both the ML and BI trees show similar topology and therefore we present only the BI tree (Fig. 2). The reconstructed molecular phylogenies show variation within the genus *Hydrophis* (Fig. 2). Our results show that all species of genus *Hydrophis* in the Persian Gulf and Gulf of Oman cluster with the species in Southeast Asia except *H. curtus* and *H. lapemiodes*. *Hydrophis curtus* in the western part of its distribution range (Persian Gulf and

Gulf of Oman) shows clear divergence from Indonesian samples (Ukuwela et al. 2014). *Hydrophis lapemiodes,* also, shows minor variation between Iranian and Southeast Asia populations, but not as distinct genetic lineage.

Tree topology was used to group the sequences and calculate the uncorrected genetic distance (*P* distance) among the lineages (Table 1). Mean genetic distance in *16S rRNA* is relatively low (average 3.25% among all lineages) in *Hydrophis,* but due to the few sequences of COI, we only calculate genetic distance among some clades that revealed relatively high values. Iranian lineages of *H. curtus* are differentiated from those in Southeast Asia by 0.6% and 6% in *16S* and COI gene fragments, respectively (Table 1 and 2). Two other distinct species, *H. platurus* and *H. viperinus* show the genetic differentiation in 16S as 0.6% and COI as 5.1% (see Table 1 and 2). The haplotype network calculated for the *16SrRNA* fragment confirmed the relationship among lineages in the concatenated phylogenetic tree (Fig. 3). We calculated the haplotype network based on the 16S gene fragment, because the number of sequences completely covered the studied taxa. The Iranian clade of *H. curtus* had a separate haplotype (Haplotype number 2), but the other samples of *H. curtus* considered had a different distinct haplotype (Haplotype number 16). Limited number of COI sequences prohibited us from obtaining genetic distance estimates among all taxa, but the few numbers of sequences did reveal some differentiation between the Persian Gulf and Southeast Asia.

Discussion

Sea snakes are one of the most interesting reptiles in biogeographic and phylogeographic studies (Ukuwela et al. 2022). The biogeographical study of the genus *Hydrophis* indicated that the true sea snake's diversification is the result of sea level changes during last 2.5 million years (Ukuwela et al. 2015) and most species of this genus were distributed globally. The Persian Gulf, Gulf of

Oman, and Indian Ocean are the most important regions in South and southwest Asia for species of the genus *Hydrophis*. Our molecular results indicate that the sea snakes in the Persian Gulf and Gulf of Oman have a shared genetic structure with Indian Ocean and Southeast Asia taxa (Fig. 2). Among them, *H. curtus* has relatively differentiated more than others, so that it may represent genetically distinct entities at the species level. Genetic variations among Persian Gulf populations of *H. curtus* and other populations in southeast Asia (0.6% for 16S and 6% for COI) indicate that it may representative a new genetic lineage in SW Asia (Table 1 and 2). This variation among these taxa indicates that the threshold of genetic variation may indicate there are distinct populations at the species level, although this variation has not yet been established in morphology.

Recently, a study was done on the genetic structure of *H. curtus* in Southeast Asia that revealed the high variation among its populations. The phylogeny indicated that this species was affected by climatic fluctuations in late Pliocene and early Pleistocene (Ukuwela et al. 2014, Ukuwela et al. 2022). Adding the Iranian dataset confirms that *H. curtus* populations in the western part its range (Persian Gulf) have a relative genetic variability greater than those populations in Sri Lanka and southeast Asia. Other species of the genus *Hydrophis* have not yet been studied, but our study indicates that their differentiation requires conducting a comprehensive study. However, the variation among populations of these species imply a pattern of variation in the Indian Ocean and West Pacific regions that may occur either in Sri Lanka or in the Persian Gulf as well (Voris 2000, Lambeck et al. 2002).

Other species of the genus *Hydrophis*, (*H. platurus, H. cyanocinctus, H. spiralis, H. ornatus, H. schistosus* and *H. gracilis*) included the analyses, clearly placed within their conspecific Southeast Asian clades that may indicate a high level of gene flow and similarity in genetic structure (Fig.

2). We assume that different responses to local adaptation between *H. curtus* and other six other taxa make them more variable.

The total diversity of the true sea snakes of the genus *Hydrophis* in the Persian Gulf and Gulf of Oman is 100 species (Rezaie-Atagholipour et al. 2016). According to the literature, *H. schistosus, H. viperinus, H. lapemiodes, H. cantoris, H. gracilis, H. spiralis* and *H. platurus* were abundant in Gulf of Oman as opposed to the Persian Gulf, which means the species prefer to not travel far from the Hormoz Strait, maybe due to the high salinity of Persian Gulf. But, *H. curtus* and *H. ornatus* are mostly distributed in the Persian Gulf and also in the Gulf of Oman. Our results revealed the variation between Persian Gulf and Southeast Asia population of *H. curtus* (6% genetic distance in COI gene fragment) and reveals a new distinct genetic lineage. Distinctive ecological structure of Persian Gulf may reinforce new adaptations to *H. curtus* as the westernmost population and differentiate it from the Southeast Asia clade. In general, biodiversity conservation needs information from local populations. Only then can we encourage conservationists to reassess the conservation status of the species in the Persian Gulf and the Gulf of Oman, because the latest status of most species is least concern and one is data deficient.

Acknowledgements

We thank from L. Lee Grismer, Ann Paterson and Kamelia Algiers who edited the early draft of the manuscript. This study was partially supported by Iran national Science Foundation (INSF) with project number of 99025967.

References

196 Bertozzi T, Sanders KL, Sistrom MJ, Gardner MG (2012) Anonymous nuclear loci in non-model
197 organisms: making the most of high-throughput genome surveys. Bioinformatics 28:
198 1807–1810. https://doi.org/10.1093/bioinformatics/bts284

199 Dunson WA (1975) *The Biology of Sea snakes*. University Park Press, Baltimore, USA

200 Felsenstein J (1985) Confidence limits on phylogenies: an approach using the
201 bootstrap. Evolution 39: 783–791. https://doi.org/10.2307/2408678

202 Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis
203 program for Windows 95/98/NT. In Nucleic acids symposium series 41: 95–98.

204 Heatwole H (1999) *Sea Snakes*. University of New South Wales Press. Sydney, Australia

205 Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and
206 speciation. Biological journal of the Linnean Society 58: 247–276.
207 https://doi.org/10.1006/bijl.1996.0035

208 Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989)
209 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with
210 conserved primers. Proceedings of the National Academy of Sciences 86: 6196–6200.
211 https://doi.org/10.1073/pnas.86.16.6196

212 Lambeck K (1996) Shoreline reconstructions for the Persian Gulf since the last glacial
213 maximum. Earth and Planetary Science Letters 142: 43–57. https://doi.org/10.1016/0012-
214 821X(96)00069-6

215 Lambeck K, Esat TM, Potter EK (2002) Links between climate and sea levels for the past three
216 million years. Nature 419: 199–206. https://doi.org/10.1038/nature01089

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Lukoschek V, Keogh JS (2006) Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biological journal of the Linnean Society 89: 523–539. https://doi.org/10.1111/j.1095-8312.2006.00691.x

Owfi F, Braulik GT, Rabbaniha M (2016) Species diversity and distribution pattern of marine mammals of the Persian Gulf and Gulf of Oman-Iranian waters. Iranian Journal of Fisheries Sciences 15: 927–944.

Rajabizadeh M (2019) *The snakes of Iran.* pp. 456, Iranshenasi press, Tehran, Iran.

Rezaie-Atagholipour M, Ghezellou P, Hesni MA, Dakhteh SMH, Ahmadian H, Vidal N (2016) Sea snakes (Elapidae, Hydrophiinae) in their westernmost extent: an updated and illustrated checklist and key to the species in the Persian Gulf and Gulf of Oman. ZooKeys 622: 129–164. https://doi.org/10.3897/zookeys.622.9939

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sanders KL, Lee MS, Bertozzi T, Rasmussen AR (2013) Multilocus phylogeny and recent rapid radiation of the viviparous sea snakes (Elapidae: Hydrophiinae). Molecular Phylogenetics and Evolution 66: 575–591. https://doi.org/10.1016/j.ympev.2012.09.021

Sheppard CRC, Al-Husiani M, Al-Jamali F, Al-Yamani F, Baldwin R, Bishop J, Benzoni F, Dutrieux E, Dulvy NK, Durvasula SRV, Jones DA, Loughland R, Medio D,

Nithyanandan M, Pilling GM, Polikarpov I, Price ARG, Purkis S, Riegl B, Saburova M, Namin KS, Taylor O, Wilson S, Zainal K (2010) The Gulf: A young sea in decline. Marine Pollution Bulletin 60: 13–38. https://doi.org10.1016/j.marpolbul.2009.10.017

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Udyawer V, Barnes P, Bonnet X, Brischoux F, Crowe-Riddell JM, D'anastasi B, Fry BG, Gillett A, Goiran C, Guinea ML, Heatwole H, Heupel MR, Hourston M, Kangas M, Kendrick A, Koefoed I, Lillywhite HB, Lobo AS, Lukoschek W, McAuley R, Nitschke C, Rasmussen AR, Sanders KL, Sheehy C, Shine R, Somaweera R, Sweet SS, Voris HK (2018) Future directions in the research and management of marine snakes. Frontiers in Marine science 5: 1–16. https://doi.org/10.3389/fmars.2018.00399

Ukuwela KDB, de Silva A, Sivaruban A, Sanders KL (2022) Diversity, distribution, and natural history of the marine snakes of Sri Lanka. Marine Biodiversity 52: 1–15. https://doi.org/10.1007/s12526-022-01259-3

Ukuwela KD, de Silva A, Fry BG, Sanders KL (2014) Multilocus phylogeography of the sea snake *Hydrophis curtus* reveals historical vicariance and cryptic lineage diversity. Zoologica Scripta 43: 472–484. https://doi.org/10.1111/zsc.12070

Ukuwela KD, Lee MS, Rasmussen AR, De Silva A, Fry BG, Ghezellou P, Sanders KL (2016) Evaluating the drivers of Indo-Pacific biodiversity: speciation and dispersal of sea snakes (Elapidae: Hydrophiinae). Journal of Biogeography 43: 243–255. https://doi.org/10.1111/jbi.12636

Ukuwela KD, Sanders KL, Fry BG (2012) *Hydrophis donaldi* (Elapidae, Hydrophiinae), a highly distinctive new species of sea snake from northern Australia. Zootaxa 3201: 45–57. https://doi.org/10.11646/zootaxa.3201.1.3

Voris HK (1972) The role of sea snakes (Hydrophiidae) in the trophic structure of coastal ocean communities. Journal of Marine Biological Association of India 14: 429–442.

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of biogeography 27: 1153–1167. https://doi.org/10.1046/j.1365-2699.2000.00489.x

Table 1: *P* distance among different lineages of Sea snake in 16S gene fragment.

1: *H. spiralis*; 2: *H. curtus*_Iran; 3: *H.cyanocinctus*; 4: *H. gracilis*; 5: *H. ornatus*_Iran; 6: *H. platurus*; 7: *H. schistosus*; 8: *H. curtus*; 9: *H. ornatus*; 10: *H. viperinus*; 11: *H. obscurus*; 12: *H. lapemiodes*; 13: *H. brooki*.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	1.8												
3	1.8	1.9											
4	3.6	2.5	3.7										
5	1.7	1.6	1.6	3.4									
6	1.4	1.3	1.3	3.1	0.9								
7	2.3	1.6	2.2	3.4	1.9	1.6							
8	1.2	0.6	1.3	2.5	0.9	0.6	1.6						
9	1.9	1.8	1.8	3.6	0.6	1.2	1.9	1.2					
10	1.4	1.3	1.3	3.1	0.9	0.6	1.6	0.6	1.2				
11	2.6	1.9	2.5	3.1	2.2	1.9	2.2	1.9	2.4	1.9			
12	1.7	1.6	1.6	3.4	0.6	0.9	1.9	0.9	0.8	0.9	2.2		
13	3.6	2.8	3.5	4.6	3.1	2.8	1.9	2.8	3.4	2.8	3.5	3.1	

Table 2: *P* distance among different lineages of Sea snakes in COI gene fragment.

1: *H. curtus*_Iran; 2: *H.cyanocinctus*_Iran; 3: *H. gracilis*_Iran; 4: *H. lapemiodes*_Iran; 5: *H. ornatus*_Iran; 6: *H. platurus*_Iran; 7: *H. schistosus*_Iran; 8: *H. viperinus*_Iran; 9: *H. brooki*; 10: *H. lapemiodes*; 11: *H. schistosus*; 12: *H. cyanocinctus*; 13: *H. obscurus*; 14: *H. curtus*; 15: *H. ornatus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2	6.7														
3	11.2	9.1													
4	7.2	4.7	9.4												
5	5	4.9	10.1	4.7											
6	5.3	6.3	9.8	6	4.3										
7	5.5	5.4	10.4	5.6	4.9	5.8									
8	6.2	5.4	10.4	5.4	3.7	5.5	5.1								
9	8.4	8.3	11.3	7.6	6.9	6.7	8.3	6.9							
10	7	2	9.4	4.9	5.1	6.5	5.6	5.6	8.1						
11	6.1	5.8	11	6.5	5.8	6.1	1.6	5.8	9.2	6					
12	6.2	1.4	9.5	5.1	4.3	5.7	5.8	5.1	8.3	1.6	6.1				
13	5.5	5.4	9.1	5.6	5.1	5.5	5.4	5.4	7.1	5.6	6.3	5.1			
14	6	5.9	10.9	6.4	4.9	5.3	5.4	6.1	7.4	6.1	6.3	5.8	5.6		
15	4.8	4.7	9.9	4.4	0.7	4	4.7	3.4	6.6	4.9	5.5	4.1	4.9	4.7	

289 **Figure 1:** A) map of the world and the selected region as study area; B) localities in south and
290 southeastern Asia where samples used in this study; C) Localities in southern Iran where samples
291 collected: 1: Bushehr; 2: Larak Island; 3: Jask; 4: Beris and Pasabandar.

292

293 **Figure 2:** Molecular phylogenetic tree of the genus *Hydrophis*. Red and green clades indicate
294 distinct populations of *H. curtus* and *H. ornatus* of Persian Gulf from other populations in Indian
295 Ocean. Samples with an asterisk symbol (*) were downloaded from GenBank.

296

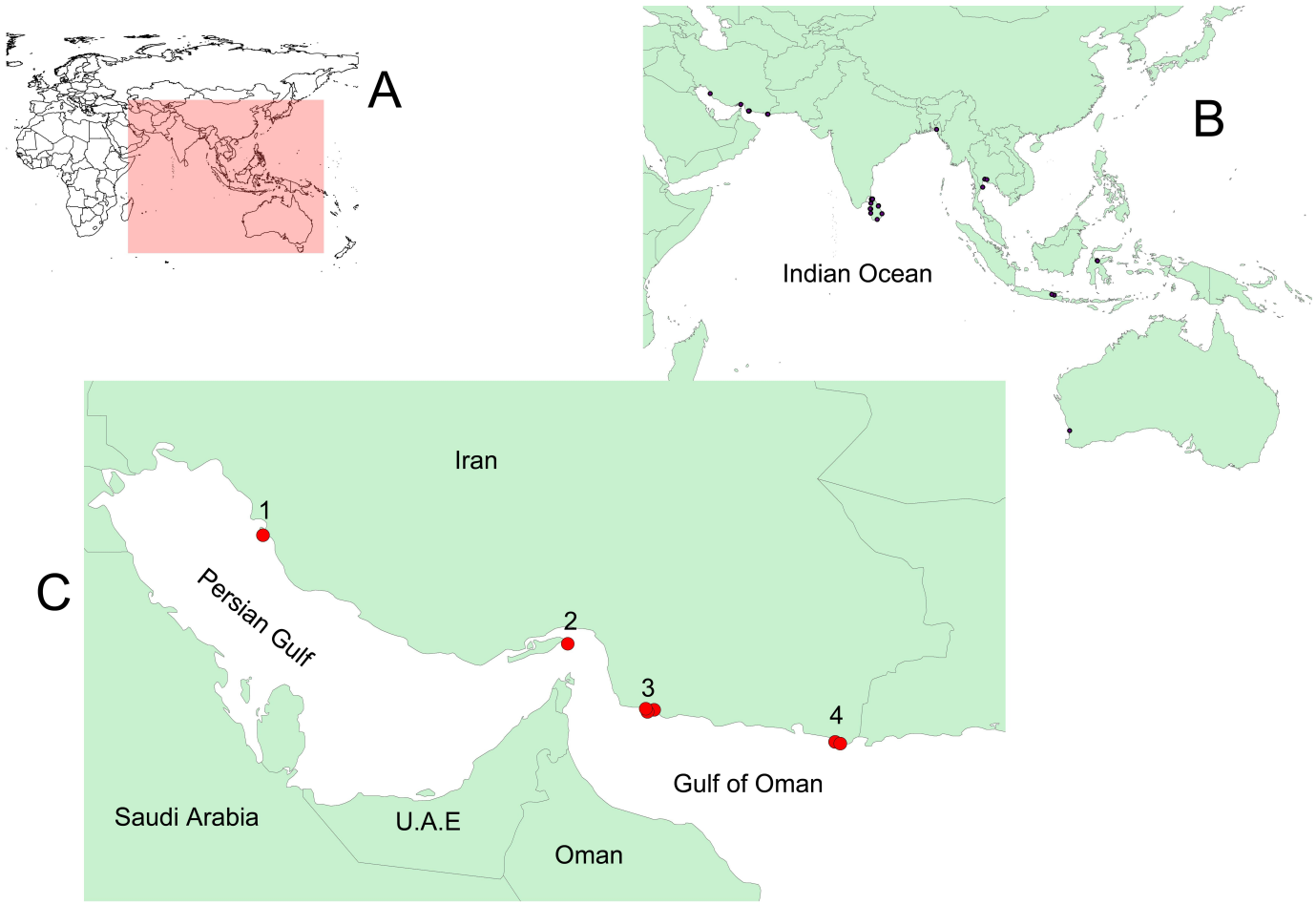
297

298 **Figure 3:** Haplotype network of the genus *Hydrophis* for 16S gene fragment. The haplotype
299 colors correspond with the color in molecular phylogenetic tree in Figure 2.

300

301

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 07/02/2023* DOI: https://doi.org/10.3897/arphapreprints.e101467




2_H_curtus
57_H_curtus
5_H_curtus
18_H_curtus
15_H_curtus
60_H_curtus
48_H_curtus
61_H_curtus
12_H_platurus
23_H_platurus
165965_H_platurus *
7_H_ornatus
3_H_ornatus
16_H_ornatus
Hlp002_H_lapemoides *
Hlp001_H_lapemoides *
KLS0017_H_lapemoides *
Ho16_H_ornatus *
MZB4253_H_ornatus *
KLS0173_H_ornatus *
MZB_4195_H_curtus *
MZB_4192_H_curtus *
MZB_4191_H_curtus *
4_H_cyanocinctus
6_H_cyanocinctus
56_H_cyanocinctus
63_H_cyanocinctus
KLS0170_H_cyanocinctus *
KLS0171_H_cyanocinctus *
KLS0093_H_spiralis *
KLS0071_H_spiralis *
55_H_spiralis
KLS0139_H_viperinus *
KLS0120_H_viperinus *
52_H_schistosus
53_H_schistosus
45_H_schistosus
50_H_schistosus
41_H_schistosus
51_H_schistosus
14_H_schistosus
H_schistosus_IR002 *
KLS0088_H_schistosus *
KLS0028_H_schistosus *
H_schistosus_IR001 *
ZMUCR661292_H_brooki *
ZMUCR661293_H_brooki *
Hb001_H_brookii *
R661175_H_obscurus *
33_H_gracilis
34_H_gracilis
31_H_gracilis
37_H_gracilis
36_H_gracilis
54_H_gracilis
39_H_gracilis
KLS0100_H_gracilis *
KLS0099_H_gracilis *
E_greyae_WAM157940 *
91/0.67
87/0.63
78/0.61
92/0.85
82/0.68
95/0.91
89/0.9
91/0.92
92/0.90
68/0.56
100/1.00
H. curtus Iran
H. ornatus Iran
0.3

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 07/02/2023* DOI: https://doi.org/10.3897/arphapreprints.e101467