Molecular phylogeny of *Lytorhynchus diadema* (Reptilia, Colubridae) populations in Saudi Arabia

Ahmed Alshammari¹, Ahmed Badry², Salem Busais³, Adel A. Ibrahim⁴, Eman El-Abd⁵

¹ Department of Biology, Faculty of Science, Ha’il University, 81451, Hail, Saudi Arabia
² Department of Zoology, Faculty of Science, Al-Azhar University, Nasr City, P.O. Box: 11751, Cairo, Egypt
³ Department of Biology, Faculty of Education, Aden University, Khormaksar, 6235 Aden, Yemen
⁴ Department of Zoology, Faculty of Science, Suez University, 43527 Suez, Egypt
⁵ Department of Radiation Sciences, Medical Research Institute, Alexandria University, Alexandria, Egypt

http://zoobank.org/4CD5D696-CF13-4768-89BE-C47C3DCDD294

Corresponding author: Ahmed Badry (ahmedbadry@azhar.edu.eg)

Academic editor: Peter Mikulíček ♦ Received 6 September 2021 ♦ Accepted 14 November 2021 ♦ Published 21 December 2021

Abstract

This study presents the molecular phylogenetic relationships among *Lytorhynchus diadema* (Duméril, Bibron & Duméril, 1854) populations in Saudi Arabia relative to populations from Africa and Asia. This phylogenetic analysis was based on mitochondrial 16S and 12S rRNA partial gene fragments using Neighbor-joining, Maximum Parsimony, and Bayesian methods. The results strongly support the monophyly of *Lytorhynchus* based on two concatenated genes and the 12S rRNA gene separately. Also, a significant separation is observed between the Arabian samples from Saudi Arabia, Yemen, and Oman, and the African populations from Egypt, Tunisia, and Morocco.

Key Words

Colubridae, *Lytorhynchus*, mtDNA, phylogeny, Saudi Arabia

Introduction

The genus *Lytorhynchus* Peters, 1863 contains six described species encompassing a vast geographical distribution and range of habitats (Leviton and Anderson 1970; Leviton 1977; Torki 2017; Uetz et al. 2021). The Diademed sand Snake, *Lytorhynchus diadema* (Duméril, Bibron & Duméril, 1854), is a non-venomous colubrid snake (Anderson 1898). It is a nocturnal species that inhabits vegetated sand dunes, gravel plains, and salt flats called “sabkha” (Gasperetti 1988; Amr and Disi 2011; Ibrahim 2013). The range of *L. diadema* extends from Morocco in the west across North Africa towards Arabia and Iran (Gasperetti 1988; Al-Sadoon 1989; Schätti and Gasperetti 1994; Baha El Din 2006; Sindaco et al. 2013; Al-Sadoon et al. 2017; Alshammari et al. 2017). Although it was previously suggested that *L. gaddi* Nikolosky, 1907 was a subspecies of *L. diadema* (Leviton et al. 1992), Schätti and Gasperetti (1994) defined it as separate species, therefore increasing the number of species in the genus to seven.

To assess the geographic variation and genetic diversity within the range of *L. diadema*, samples from Saudi Arabia were collected, sequenced, and compared with samples from across Arabia and North Africa. The partial mitochondrial 16S and 12S rRNA sequences were also compared to two congeners of *L. maynardi* (Alcock and Finn 1896) and *L. gaddi*.

Materials and methods

Snakes were collected from the Ha’il and Ta’if provinces of Saudi Arabia (Fig. 1, Table 1) according to the ethical
rules stated in the New York Academy of Sciences (1988) and DNA was extracted from blood samples as described by Alshammari et al. (2015). Partial sequences of 12S and 16S rRNA (lengths of 518–629 and 423 bp for 16S and 12S rRNA, respectively) were amplified by PCR using sequence-specific primers, visualized, and sequenced according to Pook et al. (2009) and Alshammari et al. (2015). The obtained sequences were analyzed and submitted to GenBank (Table 1). Additional sequences of *L. diadema* from Egypt, Tunisia, Morocco, Yemen, and Oman, as well as available data sequences for other species of genus *Lytorhynchus* from Iran and Pakistan (Table 1), were downloaded from GenBank. Additional sequences of other genera were retrieved from GenBank to investigate the monophyly and phylogenetic position of *Lytorhynchus* within Colubrinae. *Coelognathus flavolineatus* (Schlegel, 1837) was used as an outgroup (MG673301 and AY039162).

Table 1. A list of *Lytorhynchus* samples collected from Saudi Arabia used in this study and GenBank accession numbers of 16S rRNA and 12S rRNA previously used in phylogenetic studies with the relative sources.

| Species     | Site          | Country   | Latitude, Longitude | 16S     | 12S     | Reference       |
|-------------|---------------|-----------|---------------------|---------|---------|-----------------|
| *L. diadema*| Ta’if         | Saudi Arabia | 21.388, 40.531    | HQ267793 | HQ658442 | This study       |
|             | Ha’il         |           | 27.528, 41.739     | HQ267794 | -       |                 |
|             | Har’il        |           | 27.528, 41.739     | HQ267795 | HQ658430 |                 |
|             | Har’il/Al-Fatkh  |           | 27.456, 41.293     | HQ267796 | HQ658425 |                 |
|             | Ha’il         |           | 27.528, 41.739     | -       | HQ658422 |                 |
|              | North Sinai   | Egypt     | 31.045, 33.416     | KX909295 | KX909261 | Tamar et al. 2016 |
|              | -             | Egypt     | -                   | -       | -       | Carranza et al. 2004 |
|              | Djebel        | Tunisia   | 35.762, 9.647      | AY188064 | -       | Nagy et al. 2003 |
|              | -             | Morocco   | -                   | KX909294 | -       | Tamar et al. 2016 |
|              | Wal Wafi      | Oman      | 22.308, 59.221     | KX909293 | KX909259 |                 |
|              | Jabal Mafluq  | Yemen     | 16.629, 43.984     | -       | -       | Schätti and Monsch 2004 |
| *L. gaddi*   | Bampur        | Iran      | 49.236, 31.273     | KX909296 | KX909262 | Tamar et al. 2016 |
|              | Hatay         | Pakistan  | 29.389, 65.684     | -       | KX909286 |                 |
| *L. maynardi*| Hatay         | Pakistan  | 29.389, 65.684     | -       | KX909317 | KX909285 |
Phylogenetic analyses

FinchTV 1.4.0, was used to screen and analyze sequences. Sequences were aligned using ClustalW in Mega 6 using the default settings (Tamura et al. 2013). The aligned 12S and 16S sequences were concatenated and combined into a single alignment using the Mesquite v3.2 software (Maddison and Maddison 2018), and the nucleotide composition was calculated. To estimate the sequence divergence for the whole data set, genetic distances were calculated using Mega 6. Phylogenetic analyses were performed on the combined data set (n=15), as well as separate analyses on the individual gene performed to determine the signal in the individual gene. The Maximum-parsimony (MP) and neighbor-joining (NJ) analyses were performed with Paup v4 (Swofford 2001) with heuristic searches using stepwise addition followed by tree bisection reconnection (TBR) branch swapping (Swofford et al. 1996). In all alignments, gaps were treated as missing characters. Confidence within the nodes was evaluated using 1000 bootstrap replicates (Felsenstein 2002) with random addition of taxa. MrModeltest 2.3 (Nylander 2004) was used to select the best-fit models of nucleotide evolution supported by Akaike information criterion (AIC) (Akaike 1973). The geographic structure was inferred using Bayesian inference (BI) implemented with MrBayes 3.1.2 (Ronquist et al. 2012). Analyses were run for one million generations and the output parameters were visualized to determine stationarity and convergence using Tracer 1.4 (Rambaut and Drummond 2007).

Results

Genetic divergence

Across all combined sequences, there were 766 aligned nucleotides. Of these, 620 bases (80.9%) were constant; 138 (18.0%) were variable, and 94 (12.2%) were parsimony informative. Within the 766 bp, 44 polymorphic segregating sites were detected. Divergence among Lytorhynchus samples ranged from 0 to 0.04 (Table 2). For the 16S rRNA sequences, there have been 413 aligned nucleotides. Of these, 313 bases (75.7%) were constant; 98 (23.7%) were variable, and 72 (17.4%) were parsimony informative. Within the 413 bp, 62 polymorphic segregating sites were detected. Divergence among Lytorhynchus samples ranged from 0 to 0.04 (Suppl. material 1: Table S1). For the 12S rRNA sequences, there have been 352 aligned nucleotides of which 255 (72.4%) were constant, 95 (26.9%) were variable, and 65 (18.4%) were parsimony informative. Within the 413 bp, 30 polymorphic segregating sites were detected. Divergences among Lytorhynchus samples ranged from 0 to 0.08 (Suppl. material 1: Table S2).

Discussion

The current study has documented for the first time higher diversification of the 12S rRNA gene in inferring the phylogenetic relationship of L. diadema irrespective of the 50% lower polymorphism of the 12S rRNA than 16S rRNA. Thus, more samples and multigene concatenation

Table 2. Uncorrected pairwise distances among Lytorhynchus samples based on concatenated mitochondrial 12S rRNA and 16S rRNA sequences. Standard error estimates are shown above the diagonal. SA = Saudi Arabia.
The taxonomic status of *L. gaddi* has been discussed previously (Leviton et al. 1992; Schätti and Gasperetti 1994; Amr and Disi 2011). The divergence between *L. gaddi* and species of genus *Lytorhynchus* ranged from 4–5%, 2–4%, 5–6% for the two concatenated genes, 16S and 12S, respectively (Table 2, Suppl. material 1: Tables S1, S2). *Lytorhynchus gaddi* is distributed in the coastal zones bordering Iran and Oman (Shafiei et al. 2015). It can be distinguished from *L. diadema* by numerous morphologic features as suggested by Leviton and Anderson (1970). Our results revealed that *L. diadema* from Arabia and North Africa nested as a sister group to the specimens of *L. gaddi* from Iran in all phylogenetic analyses, and it was predicted to have diverged from *L. gaddi* (Fig. 2). Also, our result supports the species status of *L. diadema*, *L. maynardi*, and *L. gaddi* and this divergence might be due to vicariant events during the Miocene that might have supported the diversification among the Arabian and Eurasian taxa (Rögl 1999; Harzhauser and Piller 2007; Tamar et al. 2016).

Our phylogenetic results indicate a distinct geographic division between the Arabian populations from Saudi Arabia, Yemen, and Oman and those from North African populations from Egypt, Tunisia, and Morocco, with a genetic divergence of 4–8%. A similar geographical separation based on morphological and molecular data was previously detected by Lawson et al. (2005) among *Lytorhynchus* and other colubrids. Previous studies reported an association between the Red Sea formation and the speciation between Arabian and African lineages in various faunal groups (Sanmartín 2003; Amer and Kumaza 2010; Derriocourt 2005; Tamar et al. 2016; Saleh et al. 2018; Alqahtani and Badry 2020a, b). The diversification of the Arabian species is likely primarily due to the progressive aridification events during the Late Pleistocene and the early Holocene, as suggested by other researchers (Bray and Stokes 2004; Lowe et al. 2014). Moreover, palaeoclimatic effects also would have had a marked contribution to the distribution and the speciation of numerous species as reported previously (Lourenço 2020).

In conclusion, this study demonstrated a clear geographic division within the species *L. diadema*, with strong support for a monophyletic relationship, sister to *L. gaddi*. Additional detailed morphological and molecular revisions are required to clarify the relationships between Saudi *L. diadema* and other species of this genus.

Acknowledgements

We are also grateful to Professor Dr. Ann V. Paterson, Department of Natural Sciences, Williams Baptist College, Walnut Ridge, Arkansas, USA, and Dr. Sarah Du Plessis, School of Biosciences, Cardiff University, Wales, UK, for their help with professional English editing and proofreading.
References

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki F (Eds) Proceedings of the Second International Symposium on Information Theory. Academia Kiado, Budapest, 267–281.

Alqahtani AR, Badry A (2020a) Interspecific phylogenetic relationship among different species of the genus Bothus (Scorpiones: Bothidae) inferred from 16S rDNA in Egypt and Saudi Arabia. Zoology in the Middle East 66(2): 178–185. https://doi.org/10.1080/09397140.2020.1742991

Alqahtani AR, Badry A (2020b) Genetic diversity among different species of the genus Leptomorphus (Scorpiones: Bothidae) in Saudi Arabia and the Middle East. Saudi Journal of Biological Sciences 27(12): 3348–3353. https://doi.org/10.1016/j.sjbs.2020.08.048

Al-Sadoon MK (1989) Survey of the reptilian fauna of the kingdom of Saudi Arabia. 1- The snake fauna of the central region. Journal of King Saud University 1(Science 1, 2): 53–69.

Al-Sadoon MK, Paray BA, Al-Otaibi H (2017) Survey of the reptilian fauna of the Kingdom of Saudi Arabia. VI. The snake fauna of Turayf region. Saudi Journal of Biological Sciences 24(4): 925–928. https://doi.org/10.1016/j.sjbs.2017.02.007

Alshammari AM, Busais SM, Ibrahim AA (2017) Snakes in the Province of Ha’il, Kingdom of Saudi Arabia, including two new records. Herpetozoa 30(1/2): 59–63.

Alshammari AM, El-Abd E, Ciccozzi M, Presti AL, Giovanetti M, Celli E (2015) Single-gene versus double-gene tree analyses in molecular classification of Saudi venomous snakes. Arabian Journal for Science and Engineering 40(1): 37–49. https://doi.org/10.1016/s1336-0141-9-y

Amer SA, Kumazawa Y (2005) Mitochondrial DNA sequences of the Afro-Arabian spiny-tailed lizards (genus Uromastyx; family Agamidae): phylogenetic analyses and evolution of gene arrangements. Biological Journal of the Linnean Society 85: 247–260. https://doi.org/10.1111/j.1095-8312.2005.00485.x

Amr ZS, Disi AM (2011) Systematics, distribution and ecology of the snakes of Jordan. Vertebrate Zoology 61(2): 179–266.

Anderson J (1898) Zoology of Egypt. Vol. 1. Reptilia and Batrachia. Quaritch, London, 370 pp.

Baha El Din SM (2006) A Guide to Reptiles and Amphibians of Egypt. American University in Cairo Press, Egypt.

Bray HE, Stokes S (2004) Temporal patterns of arid-humid transitions in the south-eastern Arabian Peninsula based on optical dating. Geomorphology 59: 271–280. https://doi.org/10.1016/j.geomorph.2003.07.022

Carranza S, Arnold E N, Wade E, Fahd S (2004) Phylogeography of the false smooth snakes, Macroprotodon (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from Northwest Africa. Molecular Phylogenetics and Evolution 33(3): 523–532. https://doi.org/10.1016/j.ympev.2004.07.009

Derricourt R (2005) Getting “Out of Africa”: Sea crossings, land crossings in the Hominin migrations. Journal of World Prehistory 19: 119–132. https://doi.org/10.10109/j.prehoty.2005.07.016

Duméril AMC, Bibron G, Duméril AHA (1854) “Épétologie générale ou Histoire Naturelle complète des Reptiles. 71, Paris, [six vols] 780 pp. https://doi.org/10.5962/bhl.title.118797

Felsenstein J (2002) Bootstraps and testing trees. Transition 5(10): 20.

Gasperetti J (1988) Snakes of Arabia. Fauna of Saudi Arabia 9: 169–450.

Harzhauser M, Piller WE (2007) Benchmark data of a changing sea—palaeogeography, palaeobiogeography and events in the Central Paratethys during the Miocene. Palaeogeography, Palaeoclimatology, Palaeoecology 253(1-2): 8–31. https://doi.org/10.1016/j.palaeo.2007.03.031

Heise PJ, Maxson LR, Dowling HG, Hedges SB (1995) Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. Molecular Biology and Evolution, 12(2): 259–265.

Ibrahim A (2013) Herpetology of the Suez Canal Zone. Vertebrate Zoology 63: 87–110.

Kelly CM, Barker NP, Villet MH (2003) Phylogenetics of advanced snakes (Caenophidia) based on four mitochondrial genes. Systematic Biology 52(4): 439–459. https://doi.org/10.1080/10635150390218132

Kraus F, Brown WM (1998) Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences. Zoological Journal of the Linnean Society 122(3): 455–487. https://doi.org/10.1111/j.1099-3642.1998.tb02159.x

Lawson R, Sadowski JB, Crother BJ, Burbirink FT (2005) Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 37(2): 581–601. https://doi.org/10.1016/j.ympev.2005.07.016

Leviton AE (1977) A new Lytorhynchus snake. Journal of the Saudi Arabian Natural History Society 19: 16–25.

Leviton AE, Anderson SC (1970). Review of the snakes of the genus Lytorhynchus. California Academy of Sciences. Proceedings of the California Academy of Sciences ser. 4, 37: 249–274.

Lourenço WR (2020) First record and description of a new species of Leirus Ehrenberg from Kuwait (Scorpiones: Bothiidae). Serket 17(2): 143–149.

Lowe G, Yağmur EA, Kovářík F (2014) A review of the genus Leirus Ehrenberg, 1828 (Scorpiones: Bothiidae) with description of four new species from the Arabian Peninsula. Euscorpius 191: 1–129. https://doi.org/10.18590/euscorpius.2014.vol191.1

Maddison WP, Maddison DR (2018) Mesquite: A Modular System for Evolutionary Analysis. McDowell SB (1987) Systematics, p. 3–50. Snakes: ecology and evolutionary biology. McGraw Hill, New York.

Nagy ZT, Joger U, Wüster W (2009) When continents collide: phylogeny, historical biogeography and systematics of the medically important viper genus Echis (Squamata: Viperidae). Molecular Phylogenetics and Evolution 35(3): 792–807. https://doi.org/10.1016/j.ympev.2009.08.002

Rajabizadeh M, Pyron RA, Nazarov R, Poyarkov NA, Adriaens D, Herrel A (2020) Additions to the phylogeny of colubrine snakes in Southwestern Asia, with description of a new genus and species (Serpentes: Colubridae: Colubrinae). PeerJ 8: e9016. https://doi.org/10.7717/peerj.9016
Ahmed Alshammari et al.: Molecular phylogeny of Lytorhynchus diadema populations from Saudi Arabia

Rögl F (1999) Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geologica Carpathica 50(4): 339–349.

Rambaut A, Drummond AJ (2007) Tracer v1. 4: MCMC trace analyses tool. http://tree.bio.ed.ac.uk/software/tracer

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Lartet B, Liu L, Suchard MA, 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

Rossman DA, Eberle WG (1977) Partition of the genus Natrix, with preliminary observations on evolutionary trends in natricine snakes. Herpetologica: 34–43.

Saleh M, Younes M, Basuony A, Abdel-Hamid F, Nagy A, Badry A (2018) Distribution and phylogeography of Blanford’s Fox, Vulpes cana (Carnivora: Canidae), in Africa and the Middle East. Zoology in the Middle East 64(1): 9–26. https://doi.org/10.1080/09397140.2017.1419454

Sanmartín I (2003) Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). Journal of Biogeography 30(12): 1883–1897. https://doi.org/10.1046/j.0305-0270.2003.00982.x

Schätti B, Gasperetti J (1994) A Contribution to the Herpetology of Southwest Arabia. Fauna of Saudi Arabia 14: 348–423.

Schätti B, Monsch P (2004) Systematics and phylogenetic relationships of whip snakes (Hierophis Fitzinger) and Zamenis andreana Werner, 1917 (Reptilia: Squamata: Colubrinae). Revue Suisse de Zoologie 111(2): 239–256. https://doi.org/10.5962/bhl.part.80237

Schlegel H (1837) Essai sur la physionomie des serpens. Partie Descriptive. La Haye (J. Kips, J. HZ. et W. P. van Stockum) 606 S. + xvi.

Schaffer S, Fahnini H, Sehhatiasabet ME, Moradi N (2015) Rediscovery of Maynard’s Longnose Sand Snake, Lytorhynchus maynardi, with the geographic distribution of the genus Lytorhynchus Peters, 1863 in Iran. Zoology in the Middle East 61(1): 32–37. https://doi.org/10.1080/09397140.2014.994309

Sindaco R., Venchi A, Grieco C (2013) The Reptiles of the Western Palearctic, Volume 2: Annotated Checklist and Distributional Atlas of the Snakes of Europe, North Africa, Middle East and Central Asia, with an Update to Volume 1. 1st Edizioni Belvedere, Latina, Italy.

Śmид J, Martínez G, Gebhart J, Aznar J, Gállego J, Góccmen B, De Pous P, Tamar K, Carranza S (2015) Phylogeny of the genus Rhynchocalamus (Reptilia; Colubridae) with a first record from the Sultanate of Oman. Zootaxa 4033: 380–392. https://doi.org/10.11646/zootaxa.4033.3.4

Swolford DL (2001) PAUP*. Phylogenetic Analyses Using Parsimony (and Other Methods), Version 4.06b. Sunderland (Massachusetts: Sinauer Associates. Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference. In: Hillis DM, Moritz C, Mable BK (Eds) Molecular systematics. Sunderland (Massachusetts): Sinauer Associates, 407–510.

Tamar K, Śmид J, Góccmen B, Meiri S, Carranza S (2016) An integrative systematic revision and biogeography of Rhynchocalamus snakes (Reptilia, Colubridae) with a description of a new species from Isra-el PeerJ 4: e2769. https://doi.org/10.7717/peerj.2769

Tamura K, Stecher G, Peterson D, Filipski A, Kuma K (2013) MEGA6: Molecular Evolutionary Genetics Analyses Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/msv115

Torki F (2017) Description of a new species of Lytorhynchus (Squa-mata: Colubridae) from Iran. Zoology in the Middle East 63(2): 109–116. https://doi.org/10.1080/09397140.2017.1299319

Uetz P, Freed P, Hosok J (2016) The reptile database. 2016. reptile-database.org

Vidal N, Kindl S, Wong A, Hedges B (2000) Phylogenetic relationships of Xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. Molecular Phylogenetics and Evolution 14: 389–402. https://doi.org/10.1006/mpev.1999.0717

**Supplementary material 1**

**Figures S1, S2; Tables S1, S2**

Authors: Ahmed Alshammari, Ahmed Badry, Salem Busais, Adel A. Ibrahim, Eman El-Abd

Data type: docx. file

Explanation note: Figure S1. Neighbor-Joining phylogenies of Lytorhynchus spp. DNA sequences fragment of the 16S region from Saudi Arabia. Figure S2. Neighbor-Joining phylogenies of Lytorhynchus spp. DNA sequences fragment of the 12S region from Saudi Arabia. Table S1. The uncorrected p distance of the sequence divergence of 12S mtDNA sequences between Lytorhynchus samples included in this study. Table S2. The uncorrected p distance of the sequence divergence of 12S mtDNA sequences between Lytorhynchus samples included in this study.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/herpetozoa.34.e74009.suppl1