Molecular Identification of Reptiles from Tabuk Region of Saudi Arabia Through DNA Barcoding: A Case Study

Bishal Dhar, Mohua Chakraborty, Madhurima Chakraborty, Sorokhaibam Malvika, N. Neelima Devi, Abdulhadi A. Aloufi, Subrata Trivedi, and Sankar K. Ghosh

Abstract The deserts of Saudi Arabia provide an excellent habitat for reptiles. Although reptiles show significant vertebrate diversity, only few barcoding studies have been conducted on reptiles. In this case study, we collected different reptile species from the Tabuk region of Saudi Arabia and performed DNA barcoding in order to validate those species. We performed DNA sequencing for the COI region of 21 species belong to the order squamata. The BOLD Identification System (IDS) was used to establish species identity of the developed sequences. We searched both the private and published data in BOLD for available sequences through the "All Barcode Records" search engine. The Neighbour Joining tree of all the species under this study was constructed and the phylogenetic reconstruction was done using K2P distance model as per the standard protocol of DNA barcode. It was observed that *Chamaeleo chamaeleon* clusters with three *Diplometopon zarudnyi* sequences, of them two sequences have been generated in the lab and one sequence have been extracted from the database. *Eurylepis taeniolatus* also formd distinct branch in vicinity of three sequences of *Myrophis platyrhyncus*. This case study demonstrated the effectiveness of COI barcodes for reptile species from Saudi Araba in discriminating species recognized through prior taxonomic work contributing to the growing library of DNA barcodes of animal species of the world. Some species groups with overlapping barcodes identified in this study were good candidates for further studies of phylogeography and speciation processes. Further phylogenetic work on

B. Dhar · M. Chakraborty · M. Chakraborty · S. Malvika · N. N. Devi
Department of Biotechnology, Assam University, Silchar, Assam, India

A. A. Aloufi
Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Faculty of Science, Department of Biology, Taibah University, Medinah, Saudi Arabia

S. Trivedi
Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

S. K. Ghosh (✉)
University of Kalyani, Kalyani, Nadia, West Bengal, India

S. Trivedi et al. (eds.), *DNA Barcoding and Molecular Phylogeny*,
https://doi.org/10.1007/978-3-030-50075-7_16

these species will reveal which of these highly divergent and geographically separated populations should be treated as belonging to the same species or sister species.

Keywords DNA barcoding · Reptile · Tabuk · Squamata · COI · BOLD

1 Reptiles: A Fundamental Component of Biodiversity

Reptiles are a group of vertebrate animals that comprises snakes, lizards, crocodiles, turtles, etc. These groups of animals have originated in and around 310–320 million years ago, in the late Carboniferous period (Laurin and Reisz 1995) (http://www.ucmp.berkeley.edu/carboniferous/carboniferous.php). Reptiles either have four limbs or like snakes, which had descended from four-limbed ancestors. Reptiles, contrasting to amphibians, do not have an aquatic larval stage (Sander 2012). Reptiles play an important role in the food webs of the ecosystems, filling up the critical role of both predator and prey. Reptiles have been hunted or traded, particularly as food, traditional medicines, leathers as well as decorative materials (http://www.endangeredspeciesinternational.org/reptiles3.html). Modern-day reptiles (Squamata) are the most diverse order of reptiles with more than 9600 species (Sander 2012).

Saudi Arabia Saudi Arabia occupies most of the Arabian Peninsula, with the Red Sea and the Gulf of Aqaba to the west and the Persian Gulf to the east (Figure 1). Saudi Arabia contains the world's largest continuous desert, which is known as the Rub Al-Khali or Empty Quarter. It has a land area of 2,149,690 sq. km (http://www.factmonster.com/country/saudi-arabia.html). The desert features a subtropical, hot and arid climate throughout the year, very similar to the Sahara Desert, which is actually an extension of the Sahara Desert over the Arabian Peninsula. The temperatures swing between very high heat and seasonal night time freezes. The desert of Saudi Arabia provides an excellent refuge for reptiles from the savage extremes of climate, because even a few inches of sand offer excellent insulation against heat and cold.

(http://www.saudiaramcoworld.com/issue/196805/the.toadhead.from.najad.and.other.reptiles.htm).

Fig. 1 Study site (Saudi Arabia) (http://www.operationworld.org/saud)

DNA Barcoding and Species Identification The ability to accurately identify and describe species is indispensable for any biological research, but the traditional morphological-based taxonomic approaches have only managed to explain 1–1.5 million species over the past 250 years (Chapple and Ritchie 2013; Mora et al. 2011), which is around 10% of the Earth's predicted eukaryotic diversity, a very meagre amount (Mora et al. 2011). It is estimated that dogging overwhelming and cumbersome approaches would not accomplish a comprehensive inventory of the world's biodiversity (Chapple and Ritchie 2013; Packer et al. 2009) and maybe for much longer given the sharp decline in the number of specialist taxonomists (Rodman and Cody 2003; Wheeler et al. 2004). The DNA barcoding approach was initiated in 2003 by Paul Hebert and his team (Hebert et al. 2003) in the University of Guelph, Ontario, as a way to overcome the existing taxonomic 'impediments' (Hebert et al. 2003). DNA barcoding has been a promising tool for the rapid and accurate identification of various species and inventorying species diversity (Hebert et al. 2003; Dawnay et al. 2007). It has been instrumental in the identification of existing species and the discovery of new species. DNA barcoding can be helpful in species diagnosis because sequence divergences are generally much lower among individuals of the same species than between species (Hebert et al. 2003). The distinction between intra- and inter-specific divergences, termed the 'barcoding gap' (Meyer and Paulay 2005), enables unknown sequences to be assigned to an existing species or flagged as a suspected new species. DNA barcoding use sequence variations in short regions (648-bp) of cytochrome c oxidase I (*COI*) to aid species identification and discovery in large assemblages of life (Hebert et al. 2003; Savolainen et al. 2005). A significant advantage of the DNA barcoding approach is that it works in situations where morphological approaches become confounding (Armstrong and Ball 2005; Chapple and Ritchie 2013), species with multiple life stages (Hebert et al. 2004) and sexual dimorphism, variable or plastic morphology (Smith et al. 2006, 2007; Burns et al. 2008). DNA barcoding is not only a powerful tool for species identification but also can play a vital role in wildlife forensics and conservation genetics (Wolinsky 2012). The occurrence of cryptic species is relatively common in nature. Cryptic species are those species that are morphologically similar but genetically distinct. DNA barcoding can be a very effective tool in the assessment of these cryptic species (Hebert et al. 2004). DNA barcoding can also be very effective for molecular phylogenic studies (Ajmal Ali et al. 2014).

2 Identification of Reptiles from Tabuk Region of Saudi Arabia through DNA Barcoding: A Case Study

2.1 BLAST Result Analysis

A total of 21 reptile sequences from the order Squamata have been collected from Tabuk Region of Saudi Arabia and sequenced. The BLAST search results of these sequences have been detailed in Table 1. A Neighbour Joining (NJ) tree has been

Table 1 Similarity match with GenBank sequences using nucleotide BLAST. The result showed the closest match with the available database sequence. The similarity of the sequences is expressed in terms of percentage of identity with *E* value

Sample code	Reptiles vouchered	Species match in BLAST	*E* value	Identity	Accession
001(F)	*Chamaeleo chamaeleon*	Diplometopon zarudnyi voucher MVZ 234273	0	99%	AY605474.1
2R(F)	*Chalcides ocellatus*	*Sceloporus virgatus* voucher AMNH herpetology 137,700	2.00E-133	82%	KU985944.1
		Sceloporus virgatus voucher AMNH herpetology 137,699	2.00E-133	82%	KU985908.1
		Hydrobates pelagicus voucher NHMO-BC33	1.00E-130	82%	GU571435.1
		Hydrobates pelagicus voucher NHMO-BC32	1.00E-130	82%	GU571434.1
3R(F)	*Scincus mitranus*	Oligosoma maccanni isolate OMA7	1.00E-125	82%	KC349736.1
		Oligosoma maccanni isolate OMA2	1.00E-125	82%	KC349722.1
		Oligosoma maccanni isolate OMA15	1.00E-125	82%	KC349720.1
5R(F)	*Eurylepis taeniolatus*	*Myrophis platyrhynchus* voucher MFL356	2.00E-133	82%	GU224964.1
		Myrophis platyrhynchus voucher MFL354	2.00E-133	82%	GU224963.1
		Myrophis platyrhynchus voucher MFL353	2.00E-133	82%	GU224956.1
7(f)	*Stellagama stellio*	Stellagama stellio voucher ZMMU R-11324	0	92%	KF691700.1
8(F)	*Stellagama stellio*	Stellagama stellio voucher ZMMU R-11324	0	91%	KF691700.1
009(f)	*Pseudotrapelus aqabensis*	Pseudotrapelus aqabensis isolate C-5-33	0	100%	KP994947.1
		Pseudotrapelus dhofarensis isolate C-4-242,743	0	91%	KP994946.1
		Pseudotrapelus jensvindumi isolate C-7-236,932	0	90%	KP994949.1
		Pseudotrapelus jensvindumi voucher CAS:225340	0	90%	KP979760.1
		Pseudotrapelus dhofarensis voucher ZISP:26351	0	90%	KP979759.1
10(F)	*Pseudotrapelus aqabensis*	Pseudotrapelus aqabensis isolate C-5-33	0	99%	KP994947.1
		Pseudotrapelus dhofarensis isolate C-4-242,743	0	91%	KP994946.1
		Pseudotrapelus jensvindumi isolate C-7-236,932	0	90%	KP994949.1
		Pseudotrapelus jensvindumi voucher CAS:225340	0	90%	KP979760.1

(continued)

Table 1 (continued)

Sample code	Reptiles vouchered	Species match in BLAST	*E* value	Identity	Accession
12(F)	*Diplometopon zarudnyi*	Diplometopon zarudnyi voucher MVZ 234273	0	99%	AY605474.1
13(F)	*Rhagerhis moilensis*	Mimophis mahfalensis voucher REPT_M12473	2.00E-167	86%	JQ909478.1
16(F)	*Cerastes gasperettii*	*Cerastes cerastes*	0	89%	EU852311.1
19(F)	*Cyrtopodion scabrum*	*Auriparus flaviceps* voucher FMNH 394359	1.00E-111	80%	DQ432755.1
		Hemidactylus pumilio voucher IBES5021	1.00E-110	80%	KU567474.1
21(F)	*Stenodactylus doriae*	*Cephalopholis cyanostigma* voucher UG0456	1.00E-111	80%	KP194176.1
22(F)	*Stenodactylus doriae*	*Cyanopica cyanus*, isolate: YIO318–10	2.00E-118	80%	AB843453.1
25(F)	*Mesalina brevirostris*	*Monasa morphoeus* voucher LGEMA-3306	1.00E-125	82%	JN801821.1
		Monasa morphoeus voucher LGEMA-9860	1.00E-120	81%	JN801823.1
26R(F)	*Acanthodactylus opheodurus*	*Conger conger* voucher CSFOM-031	1.00E-125	82%	KJ709504.1
		Conger conger voucher re 2 hg 190,506 E	1.00E-125	82%	JN231238.1
		Conger conger voucher FCFOPB064–17	1.00E-125	82%	JQ775006.1
27(F)	*Phoenicolacerta kulzeri khazaliensis*	Phoenicolacerta kulzeri	0	89%	FJ460596.1
29(f)	*Acanthodactylus opheodurus*	Monasa morphoeus voucher LGEMA-3428	2.00E-129	82%	JN801822.1
30(f)	*Hemidactylus flaviviridis*	*Hemidactylus homoeolepis* voucher CN1034	1.00E-160	85%	KU567377.1
060(f)	*Diplometopon zarudnyi*	Diplometopon zarudnyi voucher MVZ 234273	0	99%	AY605474.1
063(F)	*Stellagama stellio*	Cerastes cerastes	0	89%	EU852311.1

constructed using the developed sequences along with the downloaded BLAST hits of individual sequences. Only those BLAST hits have been considered which have the highest scores, and E_value is close to 0. Among them, only eight sequences have conspecific sequences available in the database. Remaining sequences showed a match with the closest available relative in the database like congeneric or confamilial species. In some rare cases, in the absence of true phylogenetic relative in the database, the closest hit showed random matches with species belonging to completely different taxa, like Aves and Anguilliformes. However, these cases were associated with high E-value which makes the hit false positive. As in the case of

Acanthodactylus opheodurus, in the absence of conspecific sequence, BLAST generated hit with 98% query coverage and 82% similarity with conger sequences which belongs to the phylum Aves. The E-Value of the match was however high with 1.00E-125 that showed a random match. The taxonomic details of Blast hits are given in Table 2.

3 Species Identification Using BOLD

The BOLD Identification System (IDS) was used to establish species identity of the developed sequences. This identification system for COI accepts sequences from the 5′ region of the mitochondrial Cytochrome c oxidase subunit I gene and returns a species-level identification when one is possible. We searched both the private and published data in BOLD for available sequences through the "All Barcode Records" search engine. The search returns every COI barcode record on BOLD with a minimum sequence length of 500 bp including unvalidated library and records without species-level identification. This also includes many species represented by only one or two specimens as well as all species with interim taxonomy. Further, the "Species Level Barcode Records" was used to extract a list of the nearest matches and that provided a probability of placement to a taxon.

Among the twenty-one COI barcode sequences developed in the lab, species status for only five sequences could be confirmed using the BOLD identification system. For most of the remaining sequences, conspecific sequences were not available in the BOLD database. Table 3 shows a detailed description of similarity match of the sequences using the BOLD identification system. Top five matches of the sequences using the "All Barcode Records" search were displayed for each of the sequences. In the case of 001(F), *Chamaeleo chamaeleon* fifteen COI sequences were available in the BOLD database. However, the top five similarity match did not show close identity with any of these sequences. Instead, the sequence showed 99.81% similarity with *Diplometopon zarudnyi* and IDS identified the sequence as *Diplometopon zarudnyi*. Such incongruency in the similarity may be because of the presence of hybrid sequences or mislabelled sequence. Conspecific sequences for 2R (F) *Chalcides ocellatus* were not available in the BOLD database. 3R(F) *Scincus mitranus* showed 95.4% similarity with congeneric sequence *Scinus scinus* available in a private database. Three sequences of *Eurylepis taeniolatus* were found in early release section; however, they showed an average of 87% match with the 5R (F) *Eurylepis taeniolatus.* Four sequences of *Stellagama stellio* were present in the database. They showed 88%–96% similarity with 7(F) *Stellagama stellio* and IDS did not identify species status of the sequence. However, 8(F) *Stellagama stellio* was identified up to species level as it showed 98.5% similarity with database conspecific sequence. Developed sequences of *Pseudotrapelus aqabensis* 9(F) and 10(F)) showed 99% similarity with database sequences and were identified correctly by IDS. 12(F) *Diplometopon zarudnyi* showed 99% similarity with database sequence and was identified correctly up to species level. *Rhagerhis moilensis and Mesalina*

Table 2 Taxonomic details of the BLAST hit results in NCBI

BLAST hits	Taxonomy
AY605474.1| Diplometopon_zarudnyi	Chordata; Reptilia; Squamata; Trogonophidae; Diplometopon;
DQ432755.1| Auriparus_flaviceps	Chordata; Aves; Passeriformes; Remizidae; Auriparus
EU852311.1|Cerastes_cerastes	Squamata; Viperidae;
FJ460596.1| Phoenicolacerta_kulzeri	Squamata; Lacertidae; Phoenicolacerta;
GU571434.1| Hydrobates_pelagicus	Chordata; Aves; Procellariiformes; Hydrobatidae; Hydrobates;
GU571435.1| Hydrobates_pelagicus	Chordata; Aves; Procellariiformes; Hydrobatidae; Hydrobates;
GU224956.1| Myrophis_platyrhynchus	Chordata; Actinopterygii; Anguilliformes; Ophichthidae; Myrophinae; Myrophis;
GU224963.1| Myrophis_platyrhynchus	Chordata; Actinopterygii; Anguilliformes; Ophichthidae; Myrophinae; Myrophis;
GU224964.1| Myrophis_platyrhynchus	Chordata; Actinopterygii; Anguilliformes; Ophichthidae; Myrophinae; Myrophis;
JN231238.1|Conger_conger	Chordata; Actinopterygii; Anguilliformes; Congridae; Congrinae; Conger;
JN801821.1| Monasa_morphoeus	Chordata; Aves; Galbuliformes; Bucconidae; Monasa
JN801822.1| Monasa_morphoeus	Chordata; Aves; Galbuliformes; Bucconidae; Monasa
JN801823.1| Monasa_morphoeus	Chordata; Aves; Galbuliformes; Bucconidae; Monasa
JQ909478.1| Mimophis_mahfalensis	Chordata; Reptilia; Squamata; Lamprophiidae; Psammophiinae; Mimophis
JQ775006.1|Conger_conger	Chordata; Reptilia; Squamata; Lamprophiidae; Psammophiinae; Mimophis
KC349720.1| Oligosoma_maccanni	Scincidae
KC349722.1| Oligosoma_maccanni	Scincidae
KC349736.1| Oligosoma_maccanni	Scincidae
KF691700.1|Stellagama_stellio	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Stellagama
AB843453.1| Cyanopica_cyanus	Chordata; Aves; Passeriformes; Corvidae; Cyanopica;
KJ709504.1|Conger_conger	Chordata; Aves; Passeriformes; Corvidae; Cyanopica;
KP979759.1| Pseudotrapelus_dhofarensis	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Pseudotrapelus;
KP979760.1| Pseudotrapelus_jensvindumi	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Pseudotrapelus;
KP994946.1| Pseudotrapelus_dhofarensis	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Pseudotrapelus;

(continued)

Table 2 (continued)

BLAST hits	Taxonomy
KP994947.1| Pseudotrapelus_aqabensis	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Pseudotrapelus;
KP994949.1| Pseudotrapelus_jensvindumi	Chordata; Reptilia; Squamata; Agamidae; Agaminae; Pseudotrapelus;
KP194176.1| Cephalopholis_cyanostigma	Chordata; Actinopterygii; Perciformes; Serranidae; Epinephelinae; Cephalopholis;
KU567377.1| Hemidactylus_homoeolepis	Chordata; Reptilia; Squamata; Gekkonidae; Hemidactylus
KU567474.1| Hemidactylus_pumilio	Chordata; Reptilia; Squamata; Gekkonidae; Hemidactylus
KU985908.1| Sceloporus_virgatus	Chordata; Reptilia; Squamata; Phrynosomatidae; Sceloporinae; Sceloporus

brevirostris did not have any conspecific sequences available in the database. However, *Mesalina brevirostris* showed 98% similarity with *Acanthodactylus boskianus* and hence was identified as the same species. *Cyrtopodion scabrum* has a conspecific sequence available in the database but IDS did not show significant similarity with these sequence. 21(F) *Stenodactylus doriae* showed 81–89% similarity with the available database sequences while 22(F) *Stenodactylus doriae* showed 91% similarity with the sequences. 63(F) *Stellagama stellio* did not show match with any of the available database sequences.

4 Neighbour-Joining (NJ) Clustering

The Neighbour Joining tree of all the species under this study is constructed as shown in Fig. 2. The phylogenetic reconstruction was done using K2P distance model as per the standard protocol of DNA barcode. As observed in this case, 001F_*Chamaeleo chamaeleon* clusters with three *Diplometopon zarudnyi* sequences; of them, two sequences (12F) and 60(F)) have been generated in the lab and one sequence, AY605474, has been extracted from the database. Such clustering could be possible because of either the presence of mislabelled or misidentified sequence or there could be the possibility of species introgression. 2R(F) *Chalcides ocellatus* clusters separately as no conspecific sequence is available in the database. However, they align close to (KU985908, KU985944) *Sceloporus virgatus* belonging to the same order Squamata but different family Phrynosomatidae. 3RF_*Scincus mitranus* clusters separately but close to three confamilial database sequences of *Oligosoma maccanni* (KC349720, KC349736, KC349722). *Eurylepis taeniolatus* also forms distinct branch in the vicinity of three sequences of *Myrophis platyrhyncus*(GU224956, GU224963-64), which are Anguilliformes. 7R and 8R *Stellagama stellio* clusters together along with another database sequence (KF691700) of the same species. However, 63R *Stellagama*

Table 3 Species identification using BOLD-IDS (Barcode of Life Datasystem-Identification system) search engine. The developed sequences of the specimen are checked for similarity match in the Public Record Barcode Database of BOLD-IDS for comprehensive species identification

Voucher ID	Vouchered specimen	Top hit (similarity)	Status	Species Identification
001(F)	Chamaeleo chamaeleon	Diplometopon zarudnyi (99.81)	Private	No
2R(F)	Chalcides ocellatus	No match		No
3R(F)	Scincus mitranus	No match		No
5R(F)	Eurylepis taeniolatus	No match		No
7(f)	Stellagama stellio	No match		No
8(F)	Stellagama stellio	Stellagama stellio (98.51)	Early-release	Species identified
009(f)	Pseudotrapelus aqabensis	Pseudotrapelus aqabensis (98.9)	Published	Species identified
10(F)	Pseudotrapelus aqabensis	Pseudotrapelus aqabensis (99.45)	Published	Species identified
12(F)	Diplometopon zarudnyi	Diplometopon zarudnyi (99.82)	Private	Species identified
13(F)	Rhagerhis moilensis	No match		No
16(F)	Cerastes gasperettii	No match		No
19(F)	Cyrtopodion scabrum	No match		No
21(F)	Stenodactylus doriae	No match		No
22(F)	Stenodactylus doriae	No match		No
25(F)	Mesalina brevirostris	Acanthodactylus boskianus (98.38)	Early-release	No
26R(F)	Acanthodactylus opheodurus	No match		No
27(F)	Phoenicolacerta kulzeri khazaliensis	No match		No
29(f)	Acanthodactylus opheodurus	Acanthodactylus boskianus (99.46)	Early-release	Genus identified
30(f)	Hemidactylus flaviviridis	No match		No
060(f)	Diplometopon zarudnyi	Diplometopon zarudnyi (99.48)	Private	Species identified
063(F)	Stellagama stellio	No match		No

stellio clusters separately and close to 16(F) *Cerastes gasperettii*. 009F and10R *Pseudotrapelus aqabensis* clusters together with conspecific sequence KP994947 from database. Moreover, four database sequences (KP979760, KP994949, KP979759, KP994946) from three congeneric species of Pseudotrapelus clusters distinctly under the same node. As conspecific sequences are not present in the database, 13(F) *Rhagerhis moilensis* shows closest hit with *Mimophis mahfalensis,* which belong to the same family. In the NJ tree as well the two sequences form close cluster distinct from other families. 19 (F*) Cyrtopodion scabrum* forms subcluster with three sequences of Hemidactylus genus where sequences (KU567377,

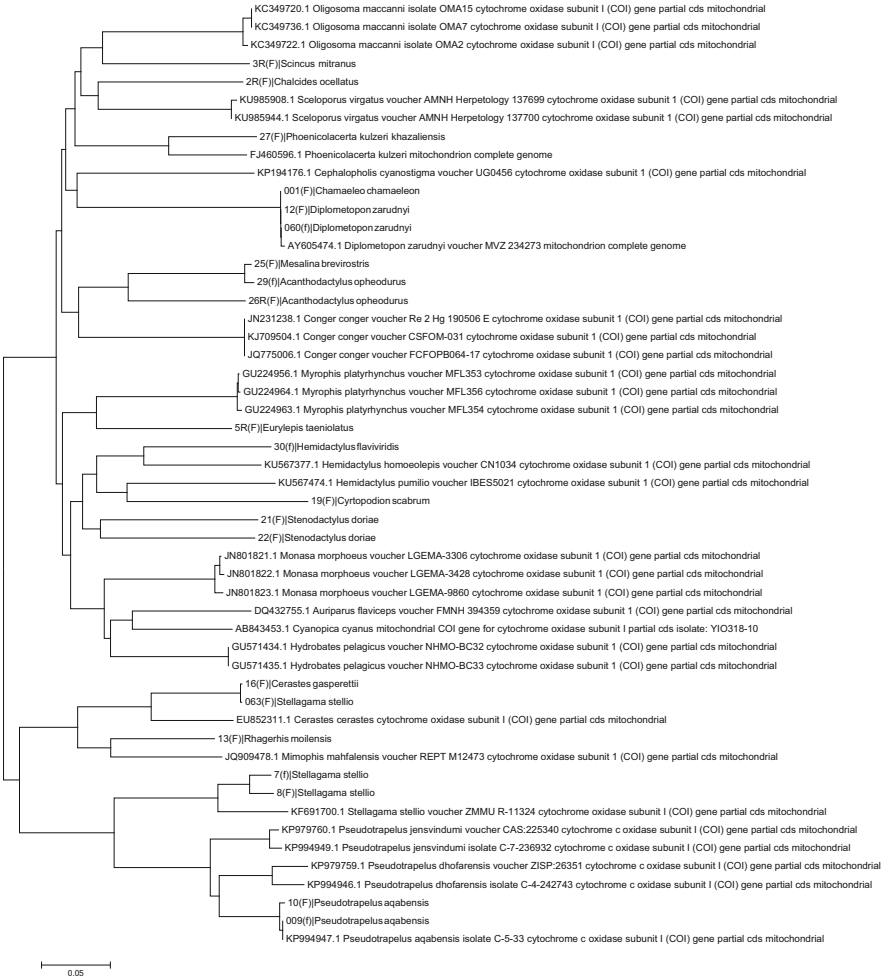

Fig. 2 Neighbour Joining tree of COI sequences of all the reptile species from Tabuk Region of Saudi Arabia along with the other database sequences as study replicates

KU567474) of two species were extracted from the database and one sequence, 30 (F) *Hemidactylys flavivirdis,* was developed in lab. Both of these genera belong to the same family Gekkonidae. 21(F) and 22(F) *Stenodactylus doriae* clusters together along with other sequences of Gekkonidae family. Species of Lacertidae family, 25 (F) *Mesalina brevirostris*, 26R (F) and 29 (F) *Acanthodactylus opheodurus* forms distinct cluster. However, 27(F) *Phoenicolacerta kulzeri khazaliensis ssp* forms separate cluster along with a conspecific database sequence FJ460596.

This case study demonstrated the effectiveness of COI barcodes for reptile species from Saudi Arabia in discriminating species recognized through prior taxonomic work contributing to the growing library of DNA barcodes of animal species

of the world. The study showed that the partial COI gene enables accurate animal species identification where adequate reference sequence data exist. Some species groups with overlapping barcodes identified in this study were good candidates for further studies of phylogeography and speciation processes. Further phylogenetic work on these species will reveal which of these highly divergent and geographically separated populations should be treated as belonging to the same species or sister species.

References

Ajmal Ali M, Gyulai G, Hidvegi N, Kerti B, Al Hemaid FM, Pandey AK, Lee J (2014) The changing epitome of species identification - DNA barcoding. Saudi J Biol Sci 21(3):204–231. https://doi.org/10.1016/j.sjbs.2014.03.003

Armstrong KF, Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. Philos Trans R Soc Lond Ser B Biol Sci 360(1462):1813–1823. https://doi.org/10.1098/rstb.2005.1713

Burns JM, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PD (2008) DNA barcodes and cryptic species of skipper butterflies in the genus Perichares in area de Conservacion Guanacaste, Costa Rica. Proc Natl Acad Sci U S A 105(17):6350–6355. https://doi.org/10.1073/pnas.0712181105

Chapple DG, Ritchie PA (2013) A retrospective approach to testing the DNA barcoding method. PLoS One 8(11):e77882. https://doi.org/10.1371/journal.pone.0077882

Dawnay N, Ogden R, McEwing R, Carvalho GR, Thorpe RS (2007) Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Sci Int 173(1):1–6. https://doi.org/10.1016/j.forsciint.2006.09.013

Hebert PD, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc Biol Sci/The Royal Soc 270(1512):313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci U S A 101(41):14812–14817. https://doi.org/10.1073/pnas.0406166101

http://www.endangeredspeciesinternational.org/reptiles3.html

http://www.saudiaramcoworld.com/issue/196805/the.toadhead.from.najad.and.other.reptiles.htm

http://www.ucmp.berkeley.edu/carboniferous/carboniferous.php The Carboniferous Period

Laurin M, Reisz RR (1995) A reevaluation of early amniote phylogeny. Zool J Linnean Soc 113 (2):165–223

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biol 3(12):e422. https://doi.org/10.1371/journal.pbio.0030422

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B (2011) How many species are there on earth and in the ocean? PLoS Biol 9(8):e1001127. https://doi.org/10.1371/journal.pbio.1001127

Packer L, Gibbs J, Sheffield C, Hanner R (2009) DNA barcoding and the mediocrity of morphology. Mol Ecol Resour 9(Suppl s1):42–50. https://doi.org/10.1111/j.1755-0998.2009.02631.x

Rodman JE, Cody JH (2003) The taxonomic impediment overcome: NSF's partnerships for enhancing expertise in taxonomy (PEET) as a model. Syst Biol 52(3):428–435

Sander PM (2012) Reproduction in early amniotes. Science 337(6096):806–808

Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopedia of life: an introduction to DNA barcoding. Philos Trans R Soc Lond Ser B Biol Sci 360 (1462):1805–1811. https://doi.org/10.1098/rstb.2005.1730

Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PD (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies

(Diptera: Tachinidae). Proc Natl Acad Sci U S A 103(10):3657–3662. https://doi.org/10.1073/pnas.0511318103

Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PD (2007) DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. Proc Natl Acad Sci U S A 104(12):4967–4972. https://doi.org/10.1073/pnas.0700050104

Wheeler QD, Raven PH, Wilson EO (2004) Taxonomy: impediment or expedient? Science 303 (5656):285. https://doi.org/10.1126/science.303.5656.285

Wolinsky H (2012) Wildlife forensics. Genomics has become a powerful tool to inform conservation measures. EMBO Rep 13(4):308–312. https://doi.org/10.1038/embor.2012.35