Volume 9, Issue 1, January 2017

Open Access

Sudan Journal of Science (SJS)

[Molecular Studies of Some Herpetofauna of Sudan]

This study aimed to survey and identifies some species of reptiles from various regions in the Sudan using molecular information. Genomic DNA was extracted from the tail tips and amplified by polymerase chain reaction (PCR) using primers for cytochrome-b mitochondrial gene. PCR products were sequenced and the resulting cytochrome-b gene sequences were aligned with homologous sequences obtained from the GenBank datsaabase. Phylogenetic trees were constructed to set up relationships of the gecko and lizard species. The phylogenetic relationships were compared to relevant species recorded from different world locations

Open Access

Molecular Studies of Some Herpetofauna of Sudan

Mukhtar M. Hassan[1], Sumaia M. Abukashawa[1]

[1]Department of Zoology, Faculty of Science, University of Khartoum, P.O. Box 321, Postal Code 11115, Sudan.

Abstract

This study aimed to survey and identifies some species of reptiles from various regions in the Sudan using molecular information. Genomic DNA was extracted from the tail tips and amplified by polymerase chain reaction (PCR) using primers for cytochrome-b mitochondrial gene. PCR products were sequenced and the resulting cytochrome-b gene sequences were aligned with homologous sequences obtained from the GenBank database. Phylogenetic trees were constructed to set up relationships of the gecko and lizard species. The phylogenetic relationships were compared to relevant species recorded from different world locations.

Keywords: Sudan, Geckos, Lizards, DNA sequence, phylogeny

1. Introduction

Sudan is an important place for genetic studies of all organisms including reptiles due to the big gap of research in Sudan. Utong (2012) and Utong and Abukashawa (2013) studied the characterization of the Agamid lizards (*Uromastyx spp*.) from Kassala State based on morphology, cytogenetic and mitochondrial gene sequences.

Conservation biologists have begun to recognize the significance of maintaining biodiversity (Erwin, 1991). Understanding herpetofauna is an important part of documenting biodiversity of regions. Preservation of necessary habitat in natural areas is an important step in species conservation and management. The preservation of species genetic structure at the population level is not widely recognized, but potentially critical for ensuring long-term survival (Frankel and Soulé, 1981). Over the past decades, researchers have placed increasing emphasis on the role that genetics plays in wildlife preservation and using genetic information to preserve variation within species (Haig, 1998). Simply stated, increased genetic variation within local populations may enhance species' ability to adapt to environmental conditions changing (Mayr, 1963).

Genetic work on some species of reptiles has been done in some parts of Africa. Phylogenetic relationships among species in the Sphaerodactylid lizard of the genus *Pristurus* were investigated by Papenfuss *et al.* (2009) in Mauritania, the Horn of Africa, the Arabian Peninsula, the Socotra Archipelago, Jordan and costal Iran. This study show the similarity of the genetic structure among this genus which depends on the effects of natural barriers on genetic structure.

The Red Sea is a potential barrier separating Arabia and Africa and its formation starting in the early Miocene may have driven allopatric divergence in several complexes whose distributions occur on both sides. (Matthew and Theodore (2010) examined the phylogenetic of *Stenodactylus*, a gecko complex distributed across Afro-Arabia, and found that *Stenodactylus* is a complex with deep divergences spanning tens of millions of years, and whose biogeography may have been influenced by major geological events, such as the formation of the Red Sea. Looking at recent timescales, *Stenodactylus* shows interesting patterns, including paraphyly with respect to *Tropiocolotes* and signals of divergence between sand dwelling lineages separated by the Oman Mountains.

The rates of base substitution on mitochondrial genome is higher than that on nuclear DNA that leads to causes of faster evolutionary rates in species (Manadvilli*et al*., 2002). Many studies of vertebrates' cytochrome b gene have focused on inheritance and evolution in lizard species (Elmerot*et al*., 2002). Therefore, complete sequencing and accurate polymerase chain reaction (PCR) studies on such genes may provide good knowledge on evolutionary and phylogenetic classification of reptiles.

Reptiles are among the most remarkable components of global biodiversity. Herpetofauna is also utilized as an indicator to environmental changes. Sudan is an important place for genetic studies of all organisms including reptiles due to the big gap of research in Sudan. Studies of reptiles in Sudan are very limited compared to research on the herpetofauna of Africa and Arabia. There is no systematic map of their distribution in the country and no thorough genetic studies of their relationships are published in literature.

Objectives of the study are to identify the cytochrome b mitochondrial gene structure of some reptile species and estimate levels of genetic relationships and diversity between the collected taxa. The results of the present study of some reptiles species is expected to contribuate to mapping of the herpetofauna of Sudan.

2. Material and methods

2.1 Study areas

Ten locations in eight states, each representing a different habitat in the Sudan were selected for reptile sampling (**Figure 1**).They were Red Sea State represented by Port-Sudan and Dongonab Bay Islands, River Nile State represented by El Muswwarat area, Khartoum State represented by Khartoum locality, Shambat (Bahri locality) and Tuti Island, Gezira State represented by Wad Madani and Rufaa

localities, Gedarif State represented by Doka and Kassala State represented by New Halfa, and Elsawagi of Kassala city.

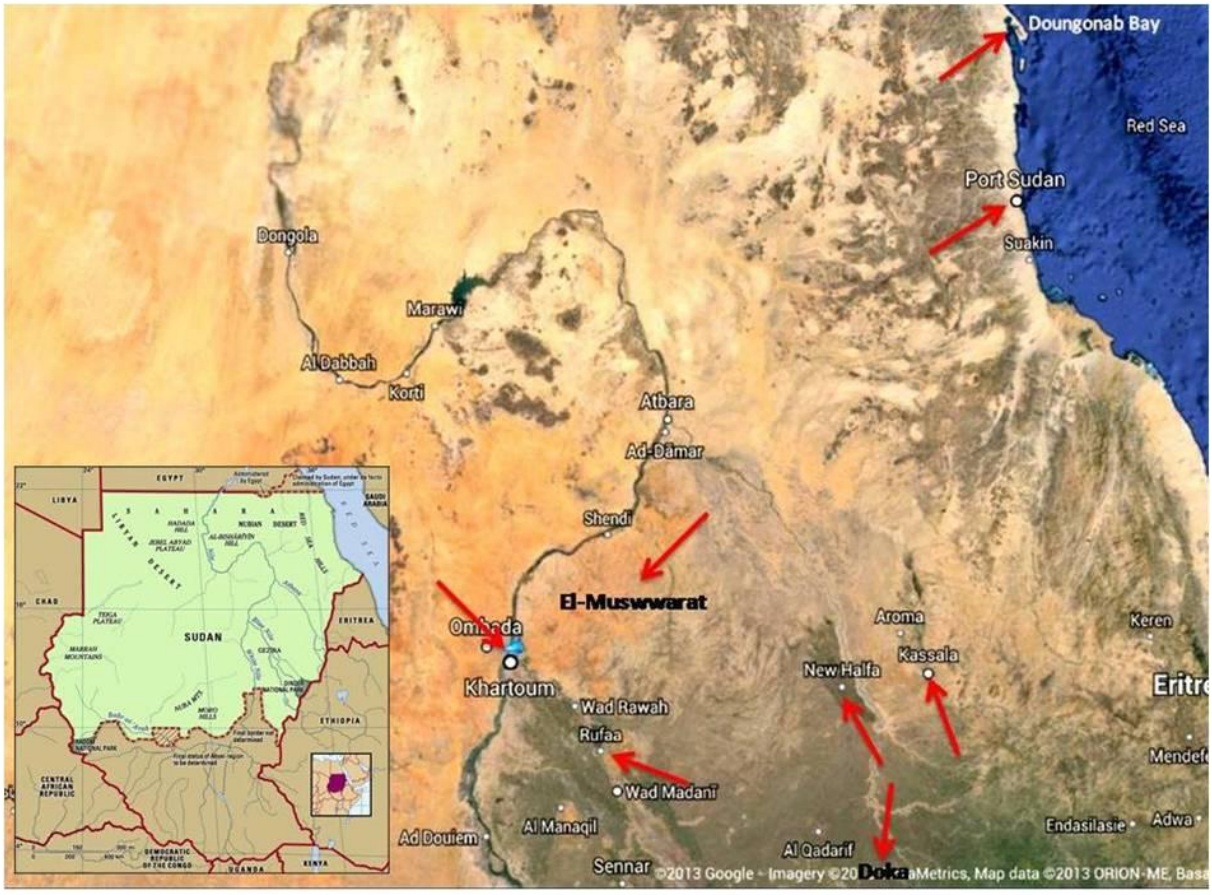

Figure (1): Map of Sudan showing the various locations of the study areas indicated by the arrows.

2.2 DNA Extraction

Sixty one reptiles were collected by hand. Minute portions of the tail tips were cut and preserved in absolute ethanol (100%) for subsequent extraction of DNA. Reptiles were then released at their site of capture. The total genomic DNA was extracted from tissues using the potassium acetate protocol (Dellaporta*et al*., 1993). The tail tips samples were placed separately in 200µl lyses buffer (1% SDS, 50mM Tris/HCl pH 8.0, 25mM NaCl, 25mM ethylene diamine tetra acetic acid (EDTA)/ pH8.5). An amount of 1.66 µl proteinase K was added and the tail tips were crushed slightly to facilitate the introduction of proteinase K into tissues. The samples were then incubated overnight at 37 °C. After incubation, the samples were put in a warm bath at 68°C for 30 minutes, and 100µl of potassium acetate were then added. The samples were kept on ice for about 60minutes, and tubes were inverted

occasionally. Samples were then centrifuged at 13,000 rpm for 15 minutes and the supernatant was transferred to new tubes. This step was repeated three times. Finally, 600 µl of cold absolute ethanol was added and the tubes were kept overnight at -20°C to precipitate the extracted DNA.

For the collection of DNA, the samples were spun in a centrifuge for about 10minutes at 13,000rpm. In this step, the supernatant was discarded and the pellets containing the precipitated DNA were left on the bottom of the tubes. These pellets were then washed in 100 µl 70% ethanol and centrifuged three times at 13,000 rpm for 10, 7 and 5 minutes respectively. After each washing the ethanol was discarded. The last washing was performed by adding 100 µl of cold absolute ethanol and centrifuged at 14000 rpm for 10 minutes. The DNA pellets were left for 30 minutes to allow excess ethanol to evaporate before being dissolved in 40 µl of double distilled water. Dissolved DNA was stored at -20°C till use.

2.3 DNA Amplification and Sequencing

Cytochrome b primers were designed for PCR amplification of 399 bp fragments of cytochrome b (cyt-*b*) mitochondrial genes (Kocher *et al*. 1989). Two (forward b-F and reverse b-R) primers were used for DNA amplification:

Cyt b-F 5'- AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'

Cyt b-R 5 '-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'

PCR reactions were carried out using G-STORM 482 thermocycler.PCR reactions were set-up in Maxime PCR PreMix tubes (i-Star Taq) in a total volume 25 µl using Maxime PCR PreMix Kit(i-Star Taq) consisting of 2.5U i-Star Taq™ DNA polymerase. Reactions were carried out in a G-STORM system 482 Thermal Cycler in a volume of 25 µl containing 2 µL of template DNA, 1 µl of each forward and reverse primers (10 pmol/ µl), 1 µl of each dNTP (2.5mM), 1 µl $MgCl_2$ (2.5 mM), 1 µl of 1x PCR buffer, µl of Taq DNA Polymerase (1 U) and 15 µl of distilled water. Amplification conditions were as follows: denaturation at 94°C for 3 min followed by cycling of 1min at 94°C, annealing for 1min at 53°C and extension for 1min at 72°C. Thirty-five amplification cycles were performed followed by a final extension of 10 min at 72°C.The PCR products were then stored at 4 °C for later use.

2.4 Phylogenetic Trees Construction

Sequences were analyzed using BioEdit, MEGA 5.05 and CLC workbench version 7. The phylogenetic relationships were inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.46211403 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary

distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1^{st} +2^{nd} +3^{rd} +Noncoding. All positions containing gaps and missing data were eliminated.There were a total of 305 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Thompson *et al*., 1994; Tamura *et al*., 2007).

Nucleotide sequences from the studied mitochondrial cytochrome-*b* were aligned together with sequences obtained from GenBank database. All the phylogenetic tree process was analyzed using CLC workbench 7 and MEGA5.05 computer software, through Neighbour-joining (NJ) method to construct the comparison and infer the evolutionary phylogenetic tree relationships between taxa genes sequences. The analysis were accomplished by Kimura 2-parameter model and supported by degree of internal branches in each tree by 1000 bootstrap replications.

2.5 Molecular Identification of Species

All the aligned sequences were compared with the sequences registered in GenBank computer package, to infer the relationships between examined species on the basis of molecular base components. The partial sequences from different species of reptile's individuals were aligned using BioEdit and CLC Main Work Bench version 7for identification and investigation of similarity between the aligned sequences, a total of 940 sites for the gene sequences were used to infer similarities and differences.

3. Results

3.1 Amplification of mtDNA cytochrome-*b* gene

The PCR products of the mitochondrial cyt-*b* gene resulted in fragments of ~399 bp observed in all specimens **(Plates 1 and 2**).

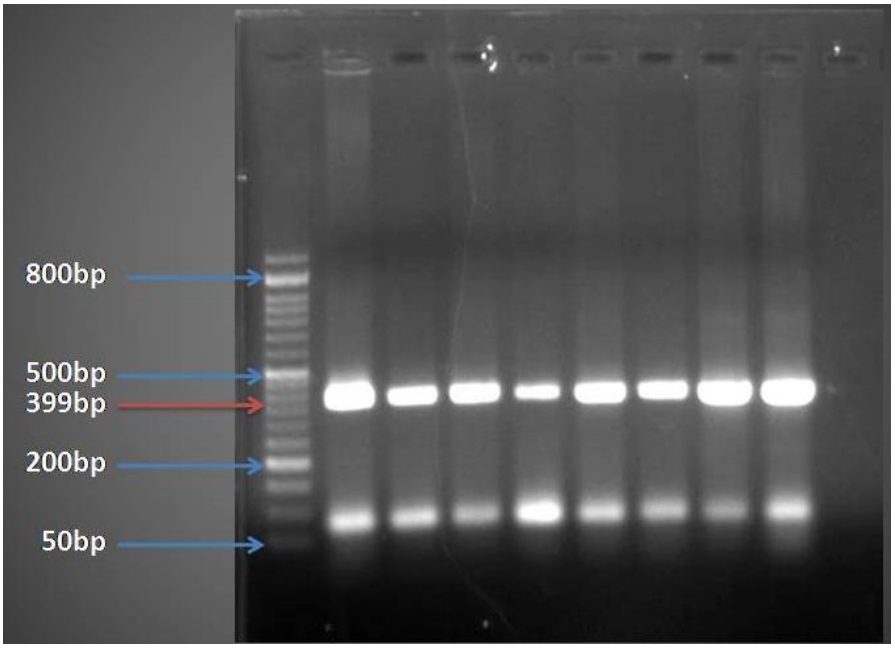

Plate 1: Result of gel electrophoresis of genomic DNA amplification of mtDNA cytochrome-b gene.

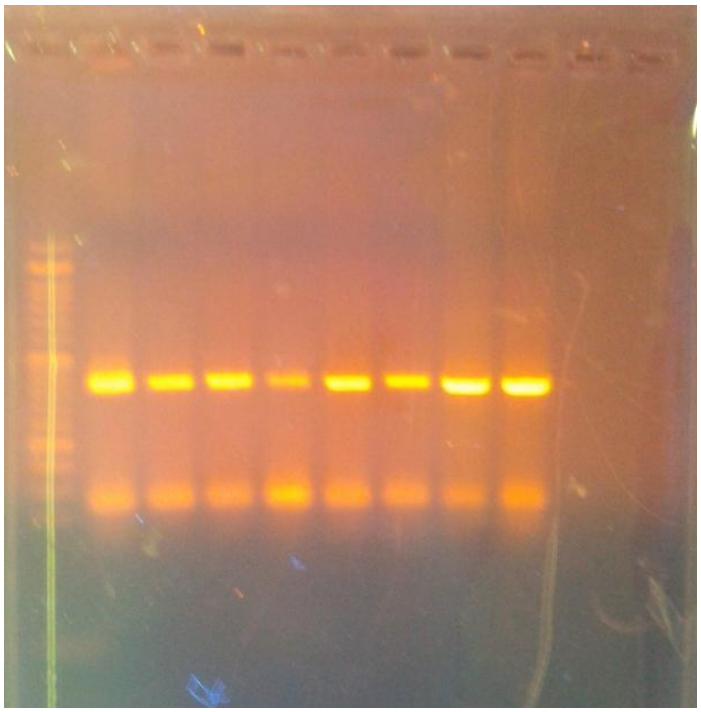

Plate 2: Result of gel electrophoresis of genomic DNA amplification without UV-filter.

3.2 Cytochrome-b Gene Sequencing

Approximately 336 bp from cyt-*b* gene were sequenced from different species. The base composition analysis showed that there were more A-T base pairs than G-C base pairs and more A and C content than T and G base content (Table I)

The evaluation of DNA sequences generated by Kemura's 80-parameter model of the evolutionary distance and differences between two sequences resulted in distance and differences between intra-species and inter-species alignments for cyt-*b* (Tables II, III and IV).

Table (I): The Molecular base composition for cyt-*b* gene sequences.

DNA Region	**Length (bp)**	**Base Percentage (%)**						**Base composition**			
		A	**T**	**C**	**G**	**A+T**	**G+C**	**A**	**T**	**C**	**G**
Cytochrome-b	336	27.08	26.19	27.8	19.64	53.27	46.73	91	88	91	66

Table (II): Pairwise mean distances and differences comparison of the representative ***Tarentolaannularis***species based on mitochondrial cytochrome-b gene sequences. **1**= ***Tarentola annularis*** (AF364322.1), and **2-11**= ***Tarentola annularis*** (cyt-b).

		1	2	3	4	5	6	7	8	9	10	11
Tarentola annularis (AF364322.1)	1		43	30	113	36	40	129	39	38	40	58
Tarentola annularis 14 (cyt b)	2	0.09		18	91	16	4	105	20	23	4	30
Tarentola annularis 17 (cyt b)	3	0.08	0.02		91	10	15	109	22	15	15	43
Tarentola annularis 38 (cyt b)	4	0.41	0.30	0.31		90	90	107	101	96	90	106
Tarentola annularis 40 (cyt b)	5	0.09	0.03	0.02	0.31		13	107	18	15	13	41
Tarentola annularis 41 (cyt b)	6	0.09	0.00	0.02	0.29	0.02		102	16	19	0	28
Tarentola annularis 43 (cyt b)	7	0.45	0.37	0.35	0.35	0.36	0.36		110	114	102	106
Tarentola annularis 44 (cyt b)	8	0.06	0.02	0.01	0.31	0.01	0.02	0.36		9	16	38
Tarentola annularis 48 (cyt b)	9	0.07	0.01	0.00	0.30	0.00	0.01	0.35	0.01		19	47
Tarentola annularis 56 (cyt b)	10	0.09	0.00	0.02	0.29	0.02	0.00	0.36	0.02	0.01		28
Tarentola annularis 57 (cyt b)	11	0.11	0.05	0.06	0.33	0.07	0.05	0.37	0.05	0.06	0.05	

Table (III): Pairwise mean distances and differences comparison from the representative ***Hemidactylus robustus*** species based on mitochondrial cytochrome-b gene sequences
1= ***Hemidactylus robustus*** (KC818849.1), and **2-19**= ***Hemidactylus robustus*** (cyt-b)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Hemidactylus robustus (KC818848.1)	1		13	14	13	17	13	14	13	13	13	15	13	13	13	13	13	13	13	15
Hemidactylus robustus 21	2	0.05		2	0	5	0	2	0	0	0	14	0	0	0	0	0	0	0	14
Hemidactylus robustus 22	3	0.05	0.01		2	3	2	0	2	2	2	16	2	2	2	2	2	2	2	16
Hemidactylus robustus 23	4	0.05	0.00	0.01		5	0	2	0	0	0	14	0	0	0	0	0	0	0	14
Hemidactylus robustus 24	5	0.06	0.01	0.01	0.01		5	3	5	5	5	19	5	5	5	5	5	5	5	19
Hemidactylus robustus 25	6	0.05	0.00	0.01	0.00	0.01		2	0	0	0	14	0	0	0	0	0	0	0	14
Hemidactylus robustus 26	7	0.05	0.01	0.00	0.01	0.01	0.01		2	2	2	16	2	2	2	2	2	2	2	16
Hemidactylus robustus 27	8	0.05	0.00	0.01	0.00	0.01	0.00	0.01		0	0	14	0	0	0	0	0	0	0	14
Hemidactylus robustus 28	9	0.05	0.00	0.01	0.00	0.01	0.00	0.01	0.00		0	14	0	0	0	0	0	0	0	14
Hemidactylus robustus 29	10	0.05	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00		14	0	0	0	0	0	0	0	14
Hemidactylus robustus 30	11	0.05	0.05	0.06	0.05	0.06	0.05	0.06	0.05	0.05	0.05		14	14	14	14	14	14	14	0
Hemidactylus robustus 31	12	0.05	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.05		0	0	0	0	0	0	14
Hemidactylus robustus 32	13	0.05	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.05	0.00		0	0	0	0	0	14

Table (IV): Pairwise mean distances and differences comparison from the representative ***Trachylepis quinquetaeniata*** species based on mitochondrial cytochrome-*b* gene sequences
1= ***Trachylepis quinquetaeniata*** (AF153593.1), and **2-12**= ***Trachylepis quinquetaeniata*** cyt-b)

		1	2	3	4	5	6	7	8	9	10	11	12
T. quinquetaeniata (AF153593.1)	1		17	22	15	14	15	16	16	19	17	18	65
Trachylepis quinquetaeniata	2	0.06		10	3	10	10	1	1	13	2	12	51
Trachylepis quinquetaeniata	3	0.07	0.02		7	15	17	9	9	19	10	18	52
Trachylepis quinquetaeniata	4	0.05	0.01	0.02		8	10	2	2	12	3	11	50
Trachylepis quinquetaeniata	5	0.05	0.03	0.04	0.02		2	9	9	13	10	13	57
Trachylepis quinquetaeniata	6	0.05	0.03	0.04	0.03	0.00		9	9	11	10	11	57
Trachylepis quinquetaeniata	7	0.06	0.00	0.02	0.00	0.03	0.03		0	12	1	11	50
Trachylepis quinquetaeniata	8	0.06	0.00	0.02	0.00	0.03	0.03	0.00		12	1	11	50
Trachylepis quinquetaeniata	9	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00		13	1	58
Trachylepis quinquetaeniata	10	0.06	0.01	0.02	0.01	0.03	0.03	0.00	0.00	0.00		12	51
Trachylepis quinquetaeniata	11	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00		59
Trachylepis quinquetaeniata	12	0.11	0.06	0.07	0.06	0.08	0.08	0.06	0.06	0.06	0.06	0.06	

Table (V): The similarity and the species with highest homology compared with DNA sequences registered in GenBank database

Species' mtDNA gene sequence	**Species with highest homology (Accession number)**	**Query coverage**	**Max. identity**	**Max. score**
Cytochrome *b*	*Tarentola annularis* (AF364322.1)	100%	100%	1264
	Hemidactylus robustus (KC818849.1)	100%	100%	2071
	Trachylepis quinquetaeniata (AF153593.1)	100%	100%	1201

Inferred similarities and differences between the aligned sequences are shown in figures (2, 3, and 4).

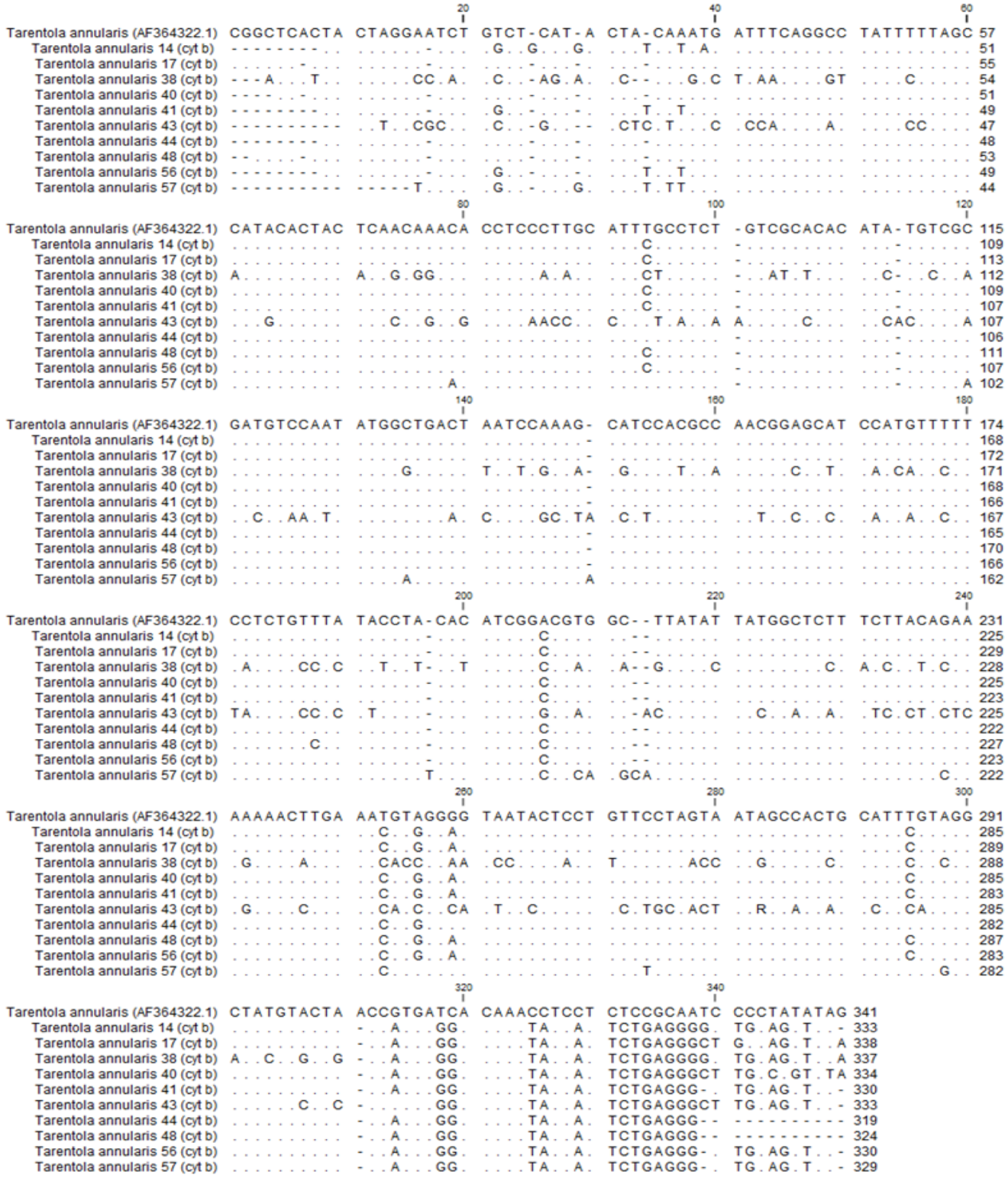

Figure (2): partial alignment of mtDNAcyt-b gene of ***Tarentola annularis*** collected from different part of Sudan. All sequences aligned with reference from GenBank with accession number: ***Tarentola annularis*** (AF364322.1). All sequences shown for forward strand and dots indicate identity with reference and dashes denoted a deletion or un-sequenced bases.

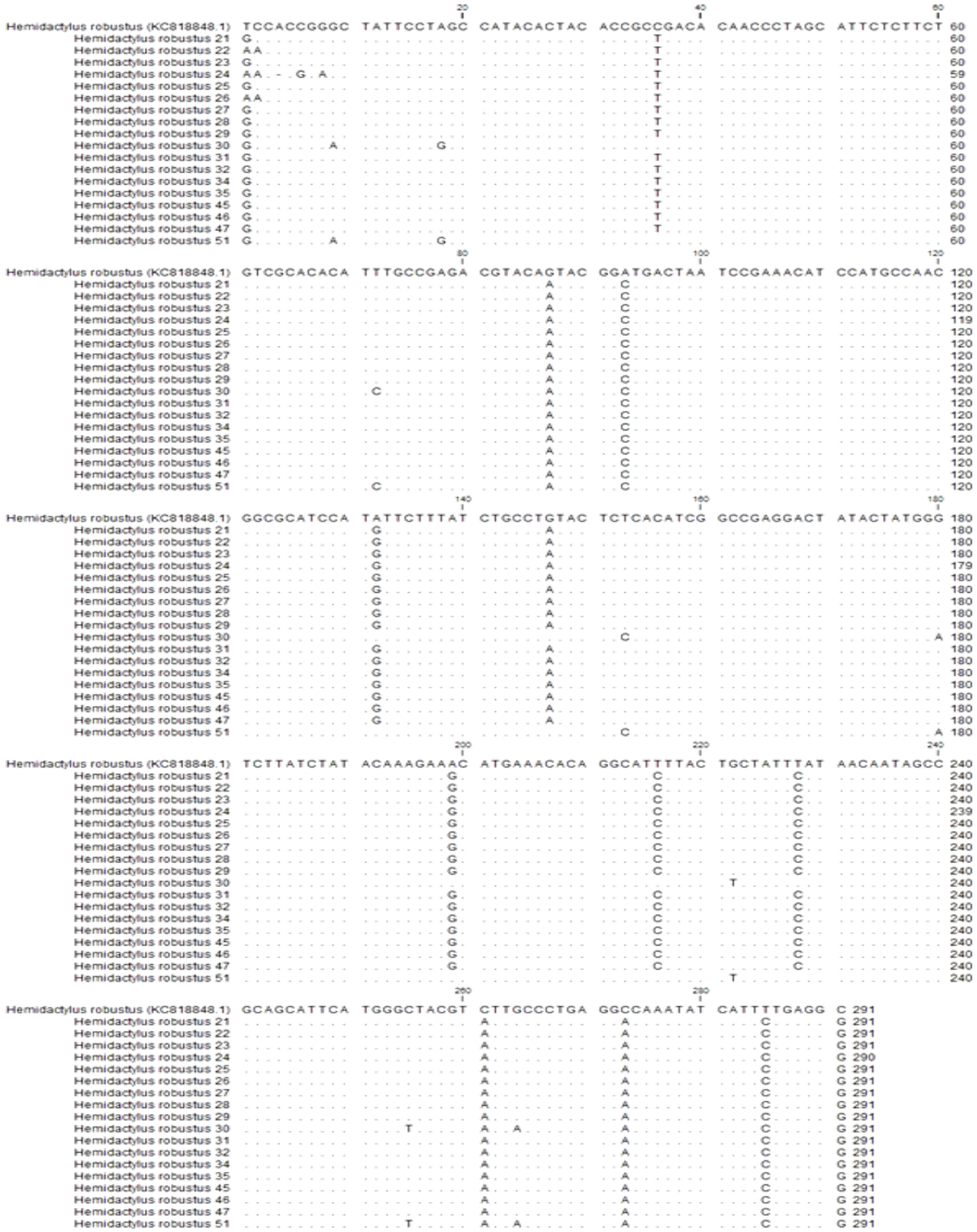

Figure (3): partial alignment of mtDNAcyt-b gene of ***Hemidactylus robustus*** collected from different Islands in Sudanese Red Sea coast. All sequences aligned with reference from GenBank with accession number: ***Hemidactylus robustus*** (KC818848.1).
All sequences shown for forward strand and dots indicate identity with reference and dashes denoted a deletion or un-sequenced bases.

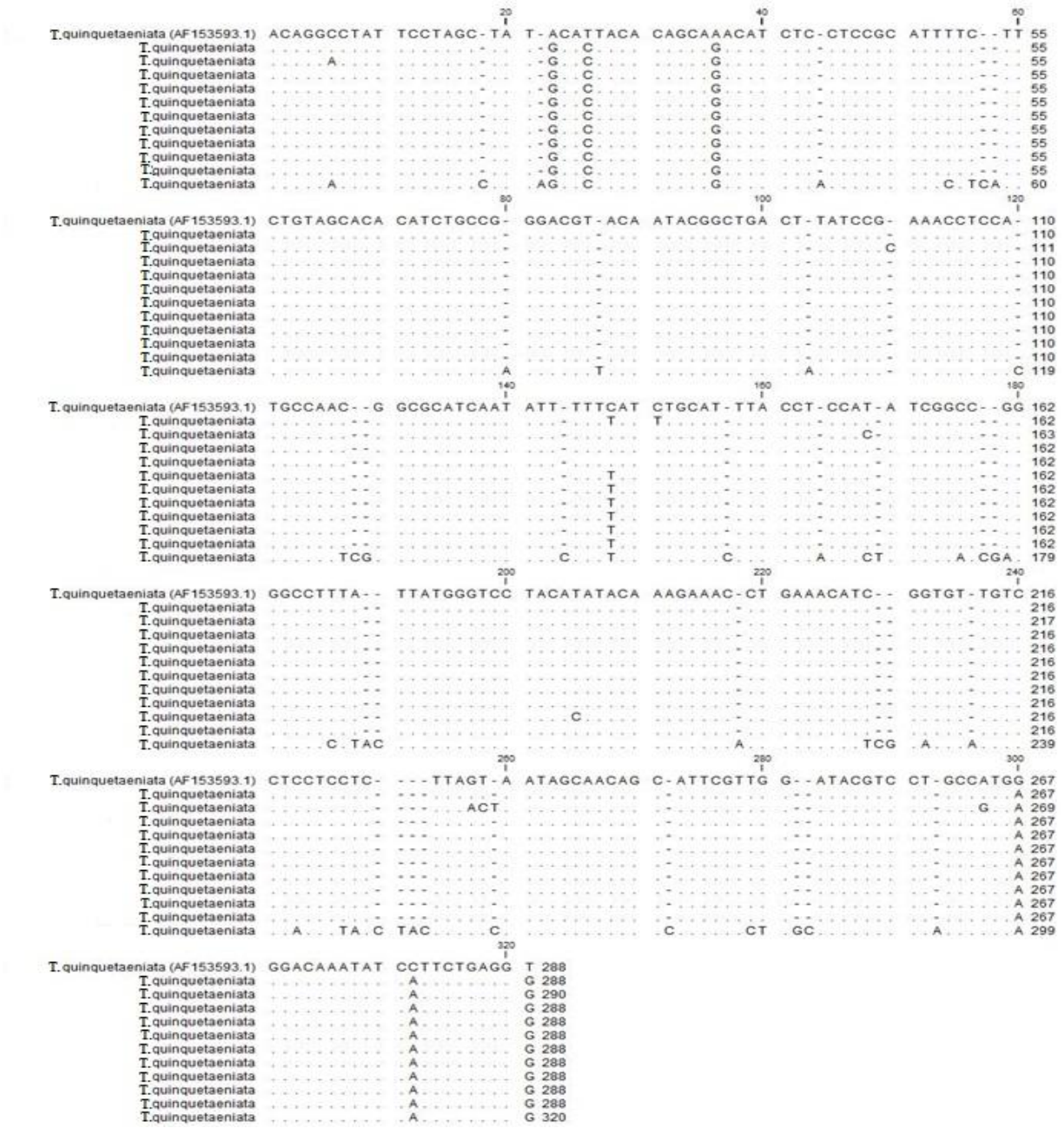

Figure (4): partial alignment of mtDNA cyt-b gene of ***Trachylepis quinquetaeniata*** collected from Khartoum state, Tuti Island, Sudan.
All sequences aligned with reference from GenBank with accession number: ***Trachylepis quinquetaeniata*** (AF153593.1).
All sequences shown for forward strand and dots indicate identity with reference and dashes denoted a deletion or un-sequenced bases.

3.3 Phylogenetic Relationships

Nucleotide sequences from the studied mitochondrial cytochrome-*b* aligned with sequences obtained from the GenBank database in different species are shown below.

3.3.1 *Tarentola annularis*:

A total of 10 individuals of *Tarentola annularis*were aligned together with the reference sequenced obtained from GenBank (accession number: AF364322.1) and the tree was constructed to infer the relationships of these species (**Figure 6**). The population of the *Tarentola annularis*was found to be separated into two main groups: one group consisted of two individuals one from Khartoum and one from Port-Sudan. The other group was divided into two groups; the first one is represented by one individual from Al-Gezira State (Rufaa). The second groups were divided into three major clades. The one which is collected from Doka is related to that one obtained from GenBank (AF364322.1).

Cluster-I illustrate that *T.annularis* from Khartoum is sister to *T.annularis* Port-Sudan. Cluster-II comprised of two sub-clusters, Sub-cluster 1 showed the *T.annularis* from Al-Gezira. Sub-cluster 2 consist of three groups, group-1 showed *T. annularis* from El-Musswarat, Khartoum and Dongonab Bay grouped together, group-2 showed the *T. annularis* from Doka with *T. annularis* (AF364322.1l) from GenBank, group-3 contains *T. annularis* from Khartoum North, El-Musswarat and Al-Gezira (Rufaa).

3.3.2 *Hemidactylus robustus*

Hemidactylus sp. were recorded in the Islands of Dongonab bay in the Sudan Red Sea coast. They were found under the shrubs of halophytes. Sequences of the 18 individuals compared to the sequence of the GenBank (accession number: KC818848.1) confirmed that *Hemidactylus sp.* in Sudan is *Hemidactylus robustus* (**Figure 7**). The populations were separated into two groups: the first group was divided into two; one group is represented by *Hemidactylus robustus*reference from GenBank clustered with two *Hemidactylus robustus* sequences from individuals from Saadallh Island at Dongonab Bay. The second group is divided into clades. These clades are distantly related to the first group that includes the *Hemidactylus robustus* reference obtained from the GenBank.

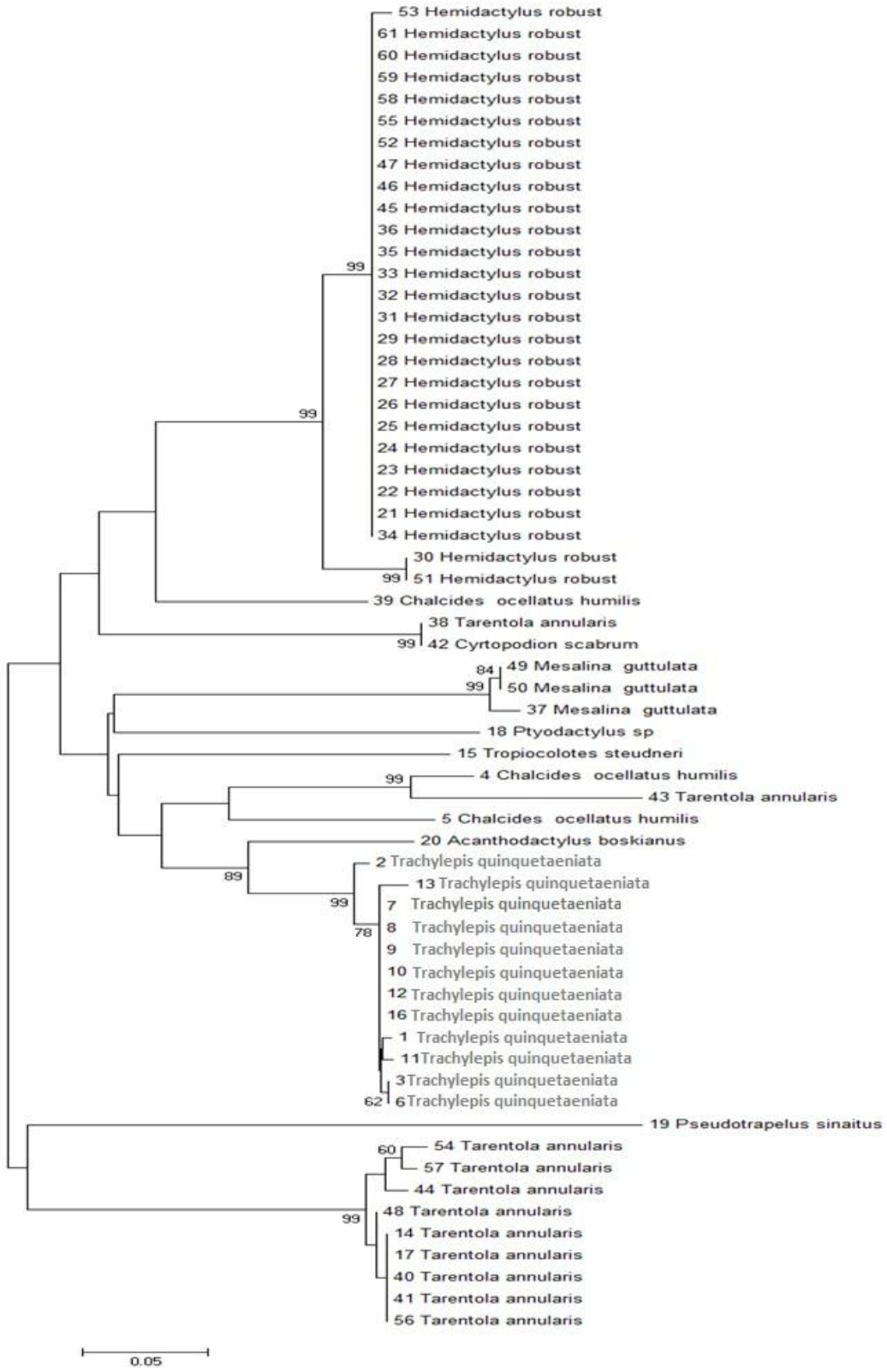

Figure (5): Phylogenetic relationships of the different collected reptiles.

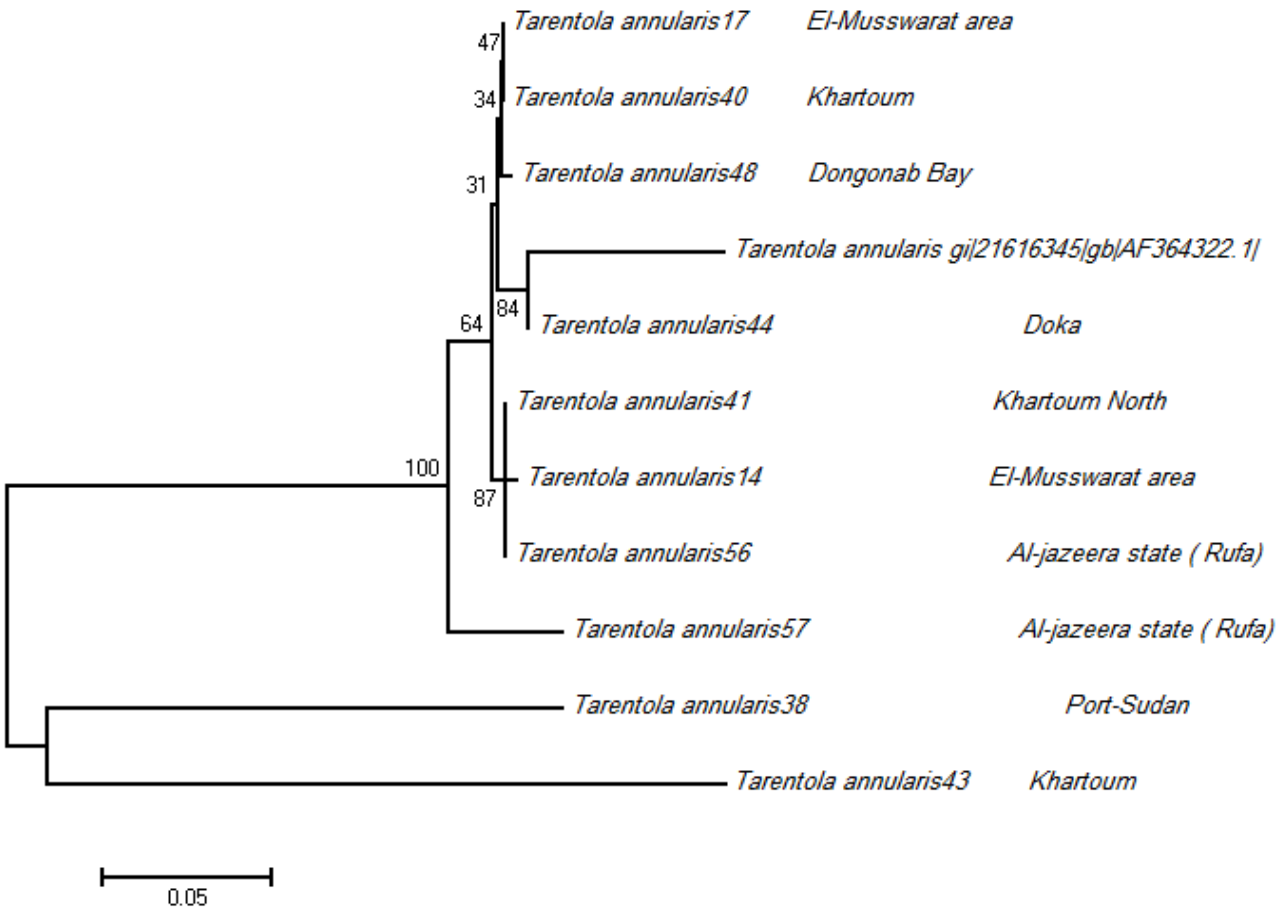

Figure (6): Neighbor joining phlyogenetic tree of mitochondrial cytochrome *b* gene of ***Tarentola annularis*** individuals. The sequences of species with accession number AF364322.1 were obtained from GenBank. Values at the nodes indicate bootstrap support of 1000 replication.

The tree shows the individuals and the sites of collection.

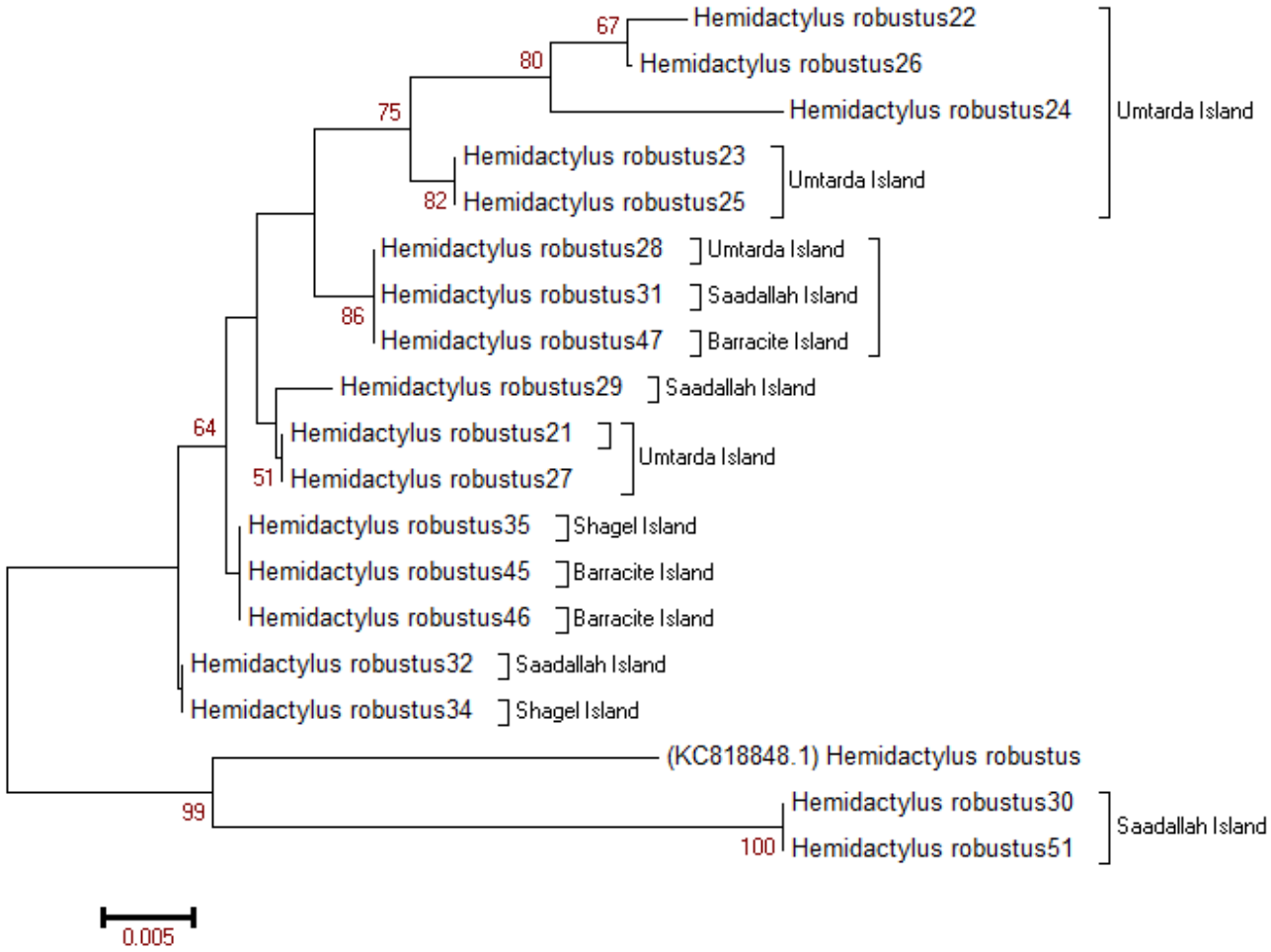

Figure (7): Neighbor joining phlyogenetic tree of mitochondrial cytochrome-*b* gene of ***Hemidactylus robustus*** individuals. The sequences of species with accession number KC818848.1were obtained from GenBank. Values at the nodes indicate bootsrap support of 1000 replication.

The tree shows the individuals and the sites of collection.

3.3.3 *Trachylepis quinquetaeniata*:

A total of 11sequences from different individuals of *Trachylepis quinquetaeniata* collected from Tuti Island Khartoum were aligned together with the reference obtained from the GenBank (accession number: AF153593.1) and the phylogenetic tree was constructed to infer the relationships of these individuals (**Figure 8**). The collected individuals were divided into two main groups: the first group represents only one individual of this lizard species. The second group was divided into two groups, group one represented by only one individual and two divided into two clades.

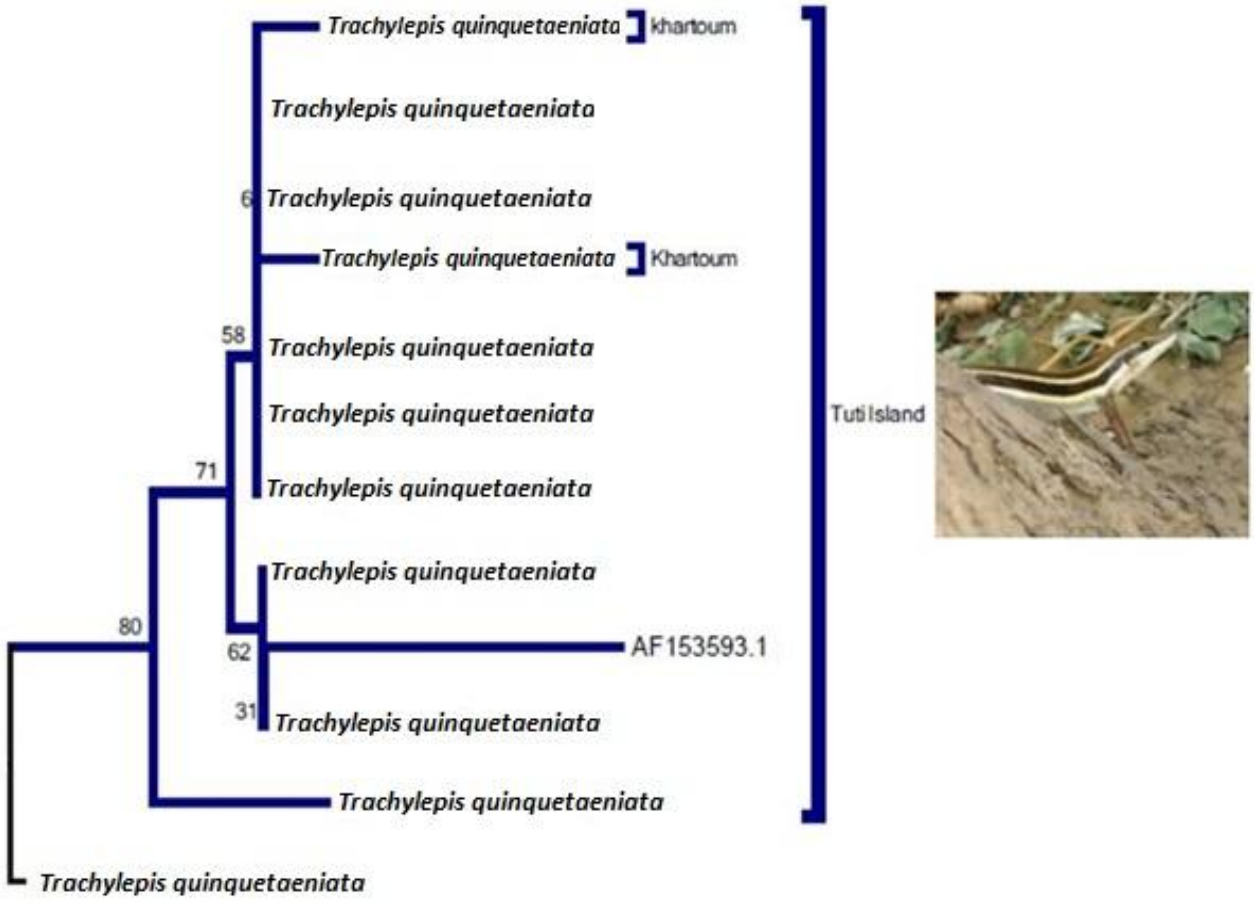

Figure (8): Neighbor joining phylogenetic tree of mitochondrial cytochrome-*b* gene of ***Trachylepis quinquetaeniata*** individuals. The sequences of species with accession number AF153593.1were obtained from GeneBank. Values at the nodes indicate bootstrap support of 1000 replication.

The tree shows the individuals and the sites of collection.

3.3.4 *Mesalina guttulata*

The three adult male specimens found on the 21st of May 2013 morning before sunrise camouflaged under the shrubs of halophytes were identified as *Mesalina guttulata* as the first record of the species in the Red Sea State. From the morphological features, the collected specimens can be described as a small, slim lizard with a long, narrow snout and a light brown-grey body. As its common name Small-spotted Lizard suggests, the upper parts covered with conspicuous light and dark spots, which sometimes form a lined pattern. The under parts of the small-spotted lizard are whitish. The body length (TL) was 78.3 mm; the tail length (TaL) was 31.8 mm.

The phylogenetic tree based on the cyt-b gene sequences (**Figure 9**) confirms that the collected specimens belong to *Mesalina guttulata*. The species of *Mesalina guttulata* from both Donganab Bay islands clustered with *M. guttulata* recorded by Kapli *et al*. (2008) from Libya.

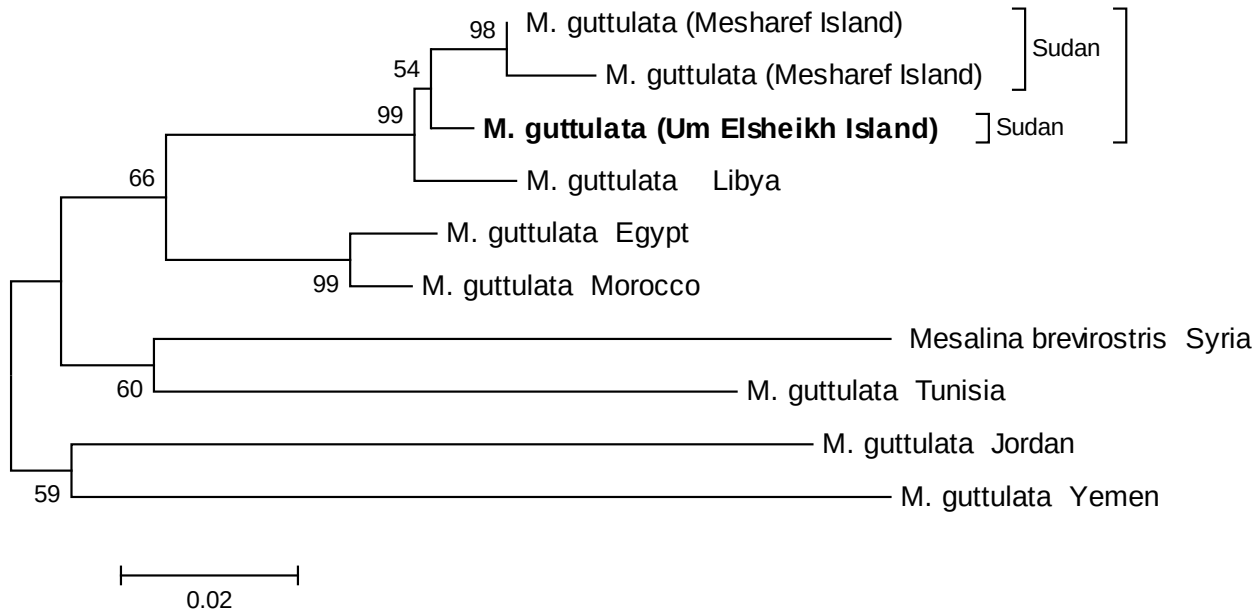

Figure (9): Neighborhood-joining dendogram showing relationships among *M. guttulata* based on cytochrome b sequences. Numbers on branches are bootstrap values over 1000 replicates.

4. Discussion

Reptiles, with 9,546 species (and ~2,800 subspecies), are the second richest class of tetrapod. The phylogeny of global reptiles is fast advancing with recent multiple studies enabling a deeper understanding of both the relationships among major clades and within species-rich lineages. The phylogenetic relationships among these major groups have been reported in a number of studies **(**Pough *et al*, 2004).

The phylogenetic analysis of *T. annularis* showed that the species from phylogenetic tree with 2 main clusters: Cluster-I illustrate that *T. annularis* from Khartoum is sister to that of Port Sudan species. Cluster- II comprised of two sub-clusters, Sub-cluster 1 represent *T. annularis* from Al-Gezira. Sub-cluster 2 consists of three groups, Group-1 showed that the cyt-*b* sequences of *T. annularis* from El-Musswarat, Khartoum and Dongonab Bay are grouped together. Group-2 showed the *T. annularis* collected from Doka clustered with *T. annularis* (AF364322.1l) from

GenBank. Group-3 contains *T. annularis* from Khartoum North, El-Musswarat and Al-Gezira State (Rufa). No surprise as squamates have been consolidated as the most successful lineage among living reptiles in terms of species richness, morphological and ecological diversity, and as one of the most successful orders among terrestrial vertebrates in general (Uetz, 2000; Uetz, 2003).

The distribution and the ecological status of *Hemidactylus sp.* are poorly known in Sudan. McCoy (1970) mentioned that *Hemidactylus turcicus* is native to coastal regions of the Mediterranean in Europe and Africa, the Red Sea in Egypt, Somalia, Arabian Peninsula, and Persian Gulf, to western India along the Indian Ocean. In this study according to the molecular data the species of *Hemidactylus sp* in Sudan is *Hemidactylus robustus*.

It is clear from both morphological and molecular data that the collected samples of *Mesalina* are *Mesalina guttulata* species. This novel record of the species highlights not only the need for more research on this taxonomic group but also the need for research on the herpetofauna of Dongonab Bay as it may add more to the biodiversity of this area which is declared as a Marine Protected Area (MPA) (Abukashawa and Mahmoud, 2015).

The phylogenic relationships among the species of the family Gekkonidae was based on morphological (Anderson, 1898 and Marx, 1968), karyological (Kawai *et al*., 2009) and molecular studies (Joger, and Bshaenia 2011).

In this study the fragments obtained from the amplified regions of mtDNA cyt-*b* gene sequenced gave approximately between 320-285 bp in different species of reptiles. These results correlate with other studies on DNA sequence analysis from *Uromastyx* species. For example Utong (2012) amplified and sequenced part of *Uromastyx* species mitochondrial DNA cyt-*b*, revealing approximately 285 bp. Gonzalez *et al*. (1996) reported similar results from the lizard genus *Gallotia* (Sauria: Lacertidae), in which the amplification of mtDNAcyt-b gene gave an amplicon of 307 bp. The analysis of bases composition percentages of cyt-*b* gene revealed more base composition of A-T base pairs (A=27.08%, T= 26.19%) than G+C (G= 19.64%, C= 27.8%) and more A (27.08%) and C (27.8%) than T (26.19%) and G (19.64%) base content. These findings are similar to the findings of Utong (2010) and Utong and Abukashawa (2013) on *Uromastyx* species where they reveal, the overall base compositions as, A= 34.64%, T= 23.51%, C= 29.12%, G= 15.09%. In this study the base compositions were found twisted similar to those of other vertebrate mtDNAs, with more A-T base pairs than G-C base pairs and more A and C contents than T and G contents.

The results of the genetic distances of DNA sequences between the species of reptiles showed show that the distance between intra-and inter-species alignments for cyt-b segments for *Tarentola annularis* sequences was 0.00-0.45, *Hemidactylus sp.* was 0.00-0.06 and for *Mabuya quinquetaeniata*is was 0.00-0.11. Based on these results the genetic distance between the species

is small. Utong (2010) and Utong and Abukashawa (2013) reported similar results on sequences from *Uromastyx* sp. in which the genetic distance between intra-species was ≤ 0.03.

5. Conclusions

The collection of reptiles from field trips to various locations in Sudan revealed different and new reptile species records for the country, emphasizing the poor knowledge of the biodiversity in these areas.

The cytochrome-b mitochondrial gene amplification proved high sensitivity, reliability and usefulness for species identification. The possibility of searching databases containing DNA sequences for *cyt b* constituted a significant advantage of this technique of species identification since it makes it possible to analyze biological material even of unknown origin.

Due to the poor knowledge of the biodiversity in the study area and in the Sudan as whole the following recommendations are made:

More studies on the molecular and evolutionary phylogenetic, relationships are recommended because data presented in this study provides a framework to direct future genetic surveys. There is an eminent need to increase studies on the biodiversity and the conservation of many regions in Sudan especially the Red Sea region.

REFERENCES

Abukashawa, S. M. A. and Mahmoud M. M. (2015). New Record Of The Small-Spotted Lizard, *Mesalina Guttulata* (Lichtenstein, 1823) From Dongonab Bay, Red Sea**.** *Russian Journal of Herpetology 22*, (3): 219-223

Anderson, J. (1898). Zoology of Egypt. Reptilia and Batrachia, Vol. 1. Quaritch, London B, 371 pp.

Dellaporta, S. L.; Wood, J. and Hicks, J. B. (1983). A plant mini preparation: version II. *Plant Mol. Biol. Rep*., **1**:19-20.

Elmerot, C.; Arnason, U.; Gojobori, T. and Janke, A., (2002). The mitochondrial genome of the puffer fish, *Fugu rubripes*, and ordinal teleostean relationships. *Gene*, **295**:163-172.

Erwin, T. L. (1991). An evolutionary basis for conservation strategies. *Science* **253**:750-752.

Frankel, O. H.; SouleÂ, M. E. (1981). Conservation and Evolution. Cambridge University Press, UK.

Gonzalez, P.; Pinto, F.; Nogales, M.; Jimenez-Asensio, J.; Hernandez M. and Cabrera, V. M. (1996). Phylogenetic relationships of the Canary Islands endemic lizards genus *Gallotia* (Sauria: Lacertidae), inferred from mithochondrial DNA sequences. *Molecular phylogenetics and evolution*. **6**(1):63-71.

Haig, S. (1998). Molecular contributions to conservation. *Ecology* **79**:413-425.

Joger U. and Bshaenia I. (2010). A new *Tarentola* subspecies (Reptilia: Gekkonidae) endemic to Tunisia *Bonn. Zool. Bull*., **57**: 267–274.

Kapli, P.; Lymberakis, P.; Poulakakis, N.; Mantziou, G.; Parmakelis, A.; Mylonas, M. (2008). Molecular phylogeny of three Mesalina (Reptilia: Lacertidae) species (*M. guttulata*, *M. brevirostris* and *M. bahaeldini*) from North Africa and the Middle East: Another case of paraphyly? *Mol. Phylogenet. Evol.* **49**: 102-110.

Kawai A J. Ishijima, C. Nishida, A. Kosaka, H. Ota, S. Kohno, Y. Matsuda. (2009). The ZW sex chromosomes of Gekkohokouensis (Gekkonidae, Squamata) represent highly conserved homology with those of avian species Chromosome, 118, 43–51.

Kocher, T. D.; Thomas,W. K.; Meyer,A.; Edwards, S. V.; Paabo, S.; Villablanca, F. X. and A. C. Wilson. (1989). Dynamics of Mitochondrial DNA Evolution in Animals: Amplification and Sequencing with Conserved Primers. *Proc. Nati. Acad. Sci. USA*. **86**: 6196-6200.

Mandavilli, B. S.; Santos, J. H. and Houten, B. V. (2002). Mitochondrial DNA repair and aging. *Mutation Res*., **509**:127-151.

Marx H., (1968). Checklist of the Reptiles and Amphibians of Egypt. U.S. Naval Medical Research Unit Number Three, Cairo 91pp (special publication).

Matthew, K. F. and Theodore J. P. (2010). Molecular systematics of Stenodactylus (Gekkonidae), an Afro-Arabian gecko species complex. *Museum of Comparative Zoology*,Harvard University, 26 Oxford Street Room 500B Cambridge, MA 02138, USA Elsevier Inc. doi:10.1016/j.ympev.2010.10.014

Mayr, E., (1963). Animal Species and Evolution. *The Belknap Press of Harvard University Press*, Cambridge, MA.

McCoy, C. J. (1970). *Hemidactylus turcicus*. *Cat. American Amphib*. Rept. **87**:1-2

Pough, F. H.; Andrews R. M.; Cadle J. E.; Crump M. L. and Savitzky A. H.. (2004) *Herpetology*. New Jersey: Pearson, Prentice Hall.

Tamura K.; Dudley J.; Nei M. and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*., **24**: 1596-1599.

Theodore, J. P.; Todd, J.; Aaron, B.; Bryan, L. S.; Michael, D. R. and James F. P. (2009). Phylogenetic Relationship among Species in the Sphaerodactylid Lizard Genus *Pristurus*. *Proceeding of the California Academy of Sciences*. Series 4, 60 (19): 675-68.

Thompson, J. D.; Higgins, D. G. and Gibson T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., **22** (22); 4673-80.

Uetz, P. (2000). How many reptile species? *Herpetological Review* **31**: 13–15.

Uetz, P. (2003). The EMBL Reptile Database. http://www.reptile-database.org/. Heidelberg, Germany.

Utong, J. A. (2012). Characterization of the Agamid Lizard (*Uromastyx* Spp.) Genome From Kassala State Based On Morphology, Cytogenetics And Mithochondrial Gene Sequences. **PhD thesis.** Faculty of Science, University of Khartoum.

Utong, J. A. M. and Abukashawa, S.M.A. (2013). Characterization of the Agamid Lizard Genus Uromastyx from Eastern Sudan Based on Morphology, Karyotypes and Mitochondrial DNA**.** *Asian Herpetological Research* 4(4): 268–281.