Official journal website:
amphibian-reptile-conservation.org

Amphibian & Reptile Conservation
13(1) [General Section]: 104–110 (e171).

Genetic diversity of Egyptian populations of the African Common Toad (*Sclerophrys regularis*, Reuss 1833)

Lamiaa Elsayed Mokhtar Deef

Department of Zoology, Faculty of Science, Damietta University, New Damietta, Damietta, EGYPT

Abstract.*—Genomic data have become invaluable for answering questions in biological conservation and for gaining high resolution in population genetic studies. A molecular dataset has been integrated to provide genetic variation and baseline genetic information, using mitochondrial cytochrome oxidase subunit I (COI) gene analysis of the African Common Toad (*Sclerophrys regularis*), order Anura, family Bufonidae. In the present study, mitochondrial DNA sequence data were analyzed for *Sclerophrys regularis* from many localities in Egypt. Based on COI sequences, the phylogenetic tree was constructed using the Maximum Likelihood (ML) method. Results show that Egyptian *Sclerophrys regularis* populations have very high genetic diversity and gene flow among them. The haplotype diversity was 1.000 for all studied regions, except for Gharbiya and Beni Suef populations which were0.900 and 0.833, respectively. The low haplotype diversity values in these two regions could indicate a possible genetic barrier between the South and North River Nile that is restricting gene flow, such as water sources, climatic conditions or distances between habitats. At present, there is insufficient data to determine the evolutionary significant units (ESU) for the conservation of *Sclerophrys regularis*. More exhaustive studies should examine the more variable genetic markers and the ecology of this species to establish a conservation strategy.

Keywords. Amphibian, Anura, Bufonidae, COI gene, DNA sequence, haplotype

Citation: Deef LEM. 2019. Genetic diversity of Egyptian populations of the African Common Toad (*Sclerophrys regularis*, Reuss 1833). *Amphibian & Reptile Conservation* 13(1) [General Section]: 104–110 (e171).

Received: 13 June 2018; **Accepted:** 27 June 2018; **Published:** 10 March 2019

Introduction

Amphibians play a pivotal role in properly functioning ecosystems, sharing in nutrient cycling, bioturbation, energy flow, food webs, and other ecosystem dynamics (Hocking and Babbitt 2014; Cortés-Gomez et al. 2015). Indeed, these animals provide additional ecosystem services valuable to humans, such as regulating pests, serving as a food source, functioning as models for medical research, and giving enjoyment and intangible contributions that vary across cultures (Warkentin et al. 2009). Because of their critical importance in functional ecosystems, anthropogenic efforts toward the maintenance of amphibian diversity are essential. As a prerequisite, amphibian diversity first needs to be measured accurately so that improvement scan be documented and restoration measures implemented in disturbed systems.

Stuart et al. (2004) and the International Union for Conservation of Nature (IUCN) (2015) reported that amphibians are the most threatened group of vertebrates assessed to date. In spite of this fact, amphibian species are among the most poorly known vertebrate groups in many geographic areas (Pino-Del-Carpio et al. 2014).

Recently, declines of native amphibian populations have received a great deal of attention. Reports indicate that diseases, pollution, habitat destruction, predation, and competition with exotic species may be related to amphibian declines (Houlahan et al. 2000; Kiesecker et al. 2001; Stuart et al. 2004; Whiles et al. 2013). One of the most important families of the class Amphibia is the Bufonidae, which is distributed in all parts of the world except for Antarctica. Due to their wide distribution, bufonids are frequently used as model organisms in experimental biology studies. The genus *Sclerophrys* (previously part of the genus *Bufo*) is a widespread and well-known bufonid genus consisting of 17 species (Saad et al. 2009), including the African Common Toad, *Sclerophrys regularis* Reuss, 1833 (Borkin 1999: 338; Ibrahim 2001; Baha El Din 2006). Recently, the name *Amietophrynus regularis* (Reuss, 1833) was applied (Borkin and Litvinchuk 2013; Ibrahim 2013a, b), however, the generic name should be replaced by the senior valid name *Sclerophrys* Tschudi, 1838 (Ohler and Dubois 2016) providing the combination *Sclerophrys regularis* (Reuss, 1833). The African Common Toad is a large, strong toad with warty skin. The dorsal surface is dark olive-brown in color with

Correspondence. *lamiaadeef@yahoo.com*; *lamiaadeef@du.edu.eg*

dark, often symmetrically arranged patches on the back. Smaller dark blotches occur on the upper lip and the eyelids and dark markings separate the warts on the flanks. The undersides of both sexes are white to beige and the throats of males are black (Rödel 2000).

The African Common Toad is often found near rivers, where it also breeds. Furthermore, it is distributed across a wide geographic range that makes this species an ideal candidate for a geographic analysis of its genetic variability. This toad is abundant, found in both moist and dry savanna, forest margins, montane grassland, and agricultural habitats. It is distributed widely in Sub-Saharan Africa, with its range extending to the oases in Algeria and Libya and into northern Nilotic Egypt (Frost 2007).

In Egypt, this toad is adaptable, but the molecular characterization of this species remains unclear (Sakr et al. 2014). Presently, pollution, habitat destruction, predation, and competition with exotic species may be contributing to the decline of this species, drawing attention to the study of its genetic variation. Therefore, the purpose of this study was to elucidate the genetic variability of *Sclerophrys regularis* populations in Egypt and to provide baseline genetic information, using mitochondrial cytochrome oxidase subunit I (COI) gene analysis.

Material and Methods

Study area and sample collection

Egypt covers an area of about one million km^2 (Fig. 1) in the central part of the great Palearctic desert belt which extends from the Atlas Mountains in the west to the Gobi Desert in the east. Most of Egypt is occupied by some of the driest deserts of the world, only interrupted by the Nile Valley and Delta, and a few small oases. Saleh (1997) divided Egypt into four habitats for amphibians: The Western Desert, the Eastern Desert, the Sinai Peninsula, and the Nile Valley and Delta.

In the present study, fourteen locations were selected as the study areas, representing each of the different habitats of Egypt that are suitable for this toad:1. North Coast of Egypt, Matrouh, Alexandria, Damietta, and Ismailia (Marine and Coastal Habitat, toads were captured from parks and around buildings); 2. Arish and Sharm El-Shaikh (Marine and Coastal Habitat of the Sinai Peninsula, toads were captured from agricultural lands); 3. Gharbiya, Cairo, Bani Sweif, Menia, Sohag, Qena, and Aswan (the Nile Valley, Delta and the Eastern Desert, toads were captured from slow-flowing pools along streams and swamps, and around building and roads); 4. Siwa Oasis (the Western Desert, toads were captured from farms).

Sixty-nine African Common Toads were obtained from the fourteen locations in Egypt, with between four and seven toads obtained per locality (Fig. 1). These locations were numbered from 1 to 14, with the number of toads from each shown in parentheses: 1. Sharm El-Shaikh (4); 2. Arish (4); 3. Ismailia (5); 4. Damietta (6); 5. Alexandria (5); 6. Matrouh (4); 7. Gharbiya (5); 8. Cairo (4); 9. Siwa Oasis (7); 10. Beni Suef (4); 11. Menia (5); 12. Sohag (5); 13. Qena (6), and 14. Aswan (5).Toads were sampled from April 2015 to March 2016. The specimens were caught in special nets or picked up by volunteers during nocturnal and diurnal surveys.

Fig. 1. Map of the sampled localities.

DNA extraction, PCR amplification, and mitochondrial DNA sequencing

Samples of muscle tissues from the toads were taken immediately and frozen at -80 ºC. DNA was extracted using a GeneJET™ kit Genomic DNA Kit#K0721. COI gene was amplified using primers FE1 (5′- GGT CAA CAA ATC ATA AAG ATA TTG G -3′) and RE1 [(5′- TAA ACT TCA GGG TGA CCA AAG AAT CA -3′)]. The polymerase chain reactions (PCR) consisting of ~50 ng of template DNA were carried out in volumes of 15 µl with 1× PCR Buffer: 2 mM MgCl, 0.5 µM each of FE1 and RE1, 0.2 mM dNTP, and 0.6 U Taq DNA Polymerase. The thermocycling conditions to amplify DNA using the polymerase chain reaction (PCR) were: 1 cycle 96 °C/3 min; 35 cycles 95 °C/30 s, 55 °C/45 s, 72 °C/1.5 min; and 1 cycle 72 °C/7 min.

The PCR products were separated on 1.0% agarose gels, bands were visualized by ethidium bromide staining and viewed with an ultraviolet light source. The amplified products were purified using a GeneJET™ kit (Thermo K0701) according to the manufacturer's instructions. Sequencing was performed using an ABI 3730xl DNA sequencer.

Data Analysis

For sequence alignment, MEGA version 7.0 (Kumar et al. 2016) and Geneious version 5.3 (Drummond et al. 2010) were used, followed by visual editing. Numbers of haplotypes, haplotype diversity (h) and nucleotide di-

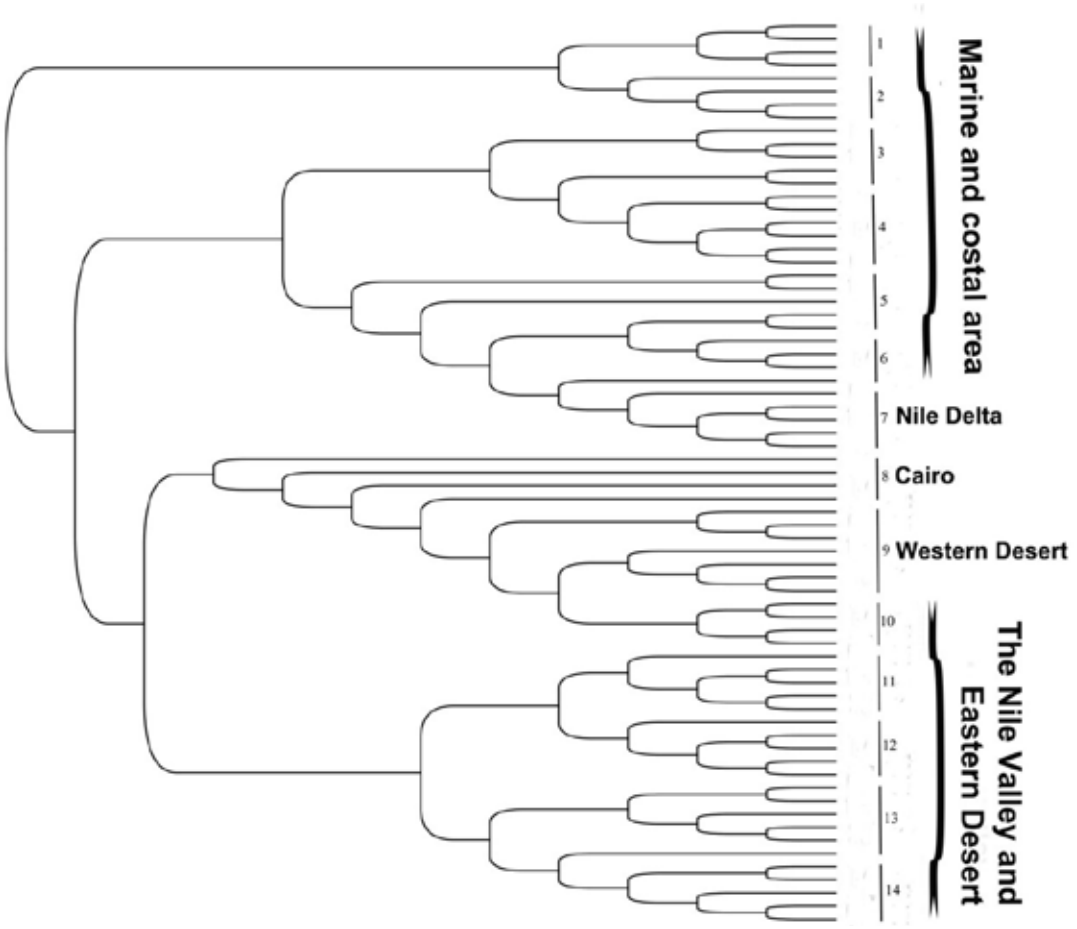

Fig. 2. Phylogenetic tree of African Common Toad, using COI haplotypes based on the Maximum Likelihood method. Numbers refer to localities mentioned in the text: 1. Sharm El-Shaikh; 2. Arish; 3. Ismailia; 4. Damietta; 5. Alexandria; 6. Matrouh; 7. Gharbiya; 8. Cairo; 9. SiwaOasis; 10. Bani Sweif; 11. Menia; 12. Sohag; 13. Qena; 14. Aswan.

versity (π) were calculated from aligned DNA sequences by DnaSP version 6.0 (Rozas et al. 2017). Within and among population genetic diversities were estimated by calculating Nei's nucleotide diversity (Pi) indices using DnaSP, version 6.0. Within-population gene diversity (H), gene diversity in total populations [$H_T = H_S + D_{ST}$, D_{ST}, gene diversity between populations and Hs, the average intrapopulation diversity (Nei 1973)], and a measure of population differentiation (G_{ST}), were calculated according to the methods described by Pons (1996). MEGA version 7.0 was used to construct a Maximum Likelihood tree.

Results

Partial COI fragments of 654 bp were obtained from the 69 individual Egyptian toads from the 14 populations and some of the sequences were deposited in GenBank (Accession numbers KF665552, KF665569, KF665599, KF665651, KF665716, KF665756, KF665824, KY079472, KY079473, KY079476, KY079477, KY079478, KY079479, KY079480, KY079483, KY079484, KY079487, KY079488).

Haplotype information is shown in Table 1. For COI data of *Sclerophrys regularis*, 67 haplotypes were recovered. Of the 14 populations, the Siwa Oasis population had seven haplotypes; Damietta and Qena had six; Matrouh, Arish, Sharm El-Shaikh, Cairo, and Gharbiya each had four; and Beni Suef three.

The average values of haplotype diversity (h) and nucleotide diversity (π) of the toads are shown in Table 2. The haplotype diversity was 1.000 for each of the studied regions except for the Gharbiya and Beni Suef populations, which were 0.900 and 0.833, respectively, indicating lower haplotype diversity values in these two localities. However, nucleotide diversity was the highest in Cairo at 0.71177. Damietta and Beni Suef had the lowest nucleotide diversities at 0.27339 and 0.23012, respectively.

Total genetic diversity ($H_T = 0.999$) was higher than the average intrapopulation diversity ($H_S = 0.00001$) resulting in high levels of genetic differentiation ($G_{ST} = 0.99899$) as shown in Table 3. These results indicate that this toad has high levels of genetic variation among its Egyptian populations and distinct population structures at all but two of the locations, Beni Suef and Gharbiya.

Based on COI sequences, a phylogenetic tree was constructed using the Maximum Likelihood method (Fig. 2). The tree shows two distinct clades: the first clade comprises the Sharm El-Sheikh and Arish populations and the

Table 1. Haplotype numbers of the study localities of African Common Toad (*Sclerophrys regularis*).

Population	Haplotype numbers
Sharm El-Shaikh	4
Arish	4
Ismailia	5
Damietta	6
Alexandria	5
Matrouh	4
Gharbiya	5
Cairo	4
Siwa Oasis	7
Bani Sweif	4
Menia	5
Sohag	5
Qena	6
Aswan	5

Table 2. The average values of haplotype diversity (h) and nucleotide diversity (π) of the African Common Toad (*Sclerophrys regularis*).

Population	Haplotype diversity (h)	Nucleotide diversity (π)
Sharm El-Shaikh	1.000	0.53517
Arish	1.000	0.56702
Ismailia	1.000	0.47080
Damietta	1.000	0.27339
Alexandria	1.000	0.50122
Matrouh	1.000	0.42355
Gharbiya	0.900	0.38746
Cairo	1.000	0.71177
Siwa Oasis	1.000	0.47306
Bani Sweif	0.833	0.23012
Menia	1.000	0.41116
Sohag	1.000	0.55214
Qena	1.000	0.56381
Aswan	1.000	0.47523

second clade comprises individuals from all other studied localities. In the constructed phylogenetic trees of this species based on COI sequences, haplotypes of the second clade consist of two main sub-groups. The first sub-group represents the haplotypes of Ismailia, Damietta, Alexandria, Matrouh, and Gharbiya populations, which includes one cluster of the Ismailia and Damietta populations and a second cluster of the Alexandria, Matrouh and Gharbiya populations. The second sub-group consists of haplotypes of Cairo, Siwa Oasis, Bani Sweef, Menia, Sohag, Qena, and Aswan populations, which includes one cluster of Cairo, Siwa Oasis and Beni Suef populations, a second cluster of the Menia and Sohag populations, and a third cluster of the Qena and Aswan populations.

In addition, the phylogenetic tree generated in the current study using the Maximum Likelihood method revealed that individuals of Sharm El-Shaikh and Arish populations have the greatest genetic distances from the other studied populations. The other individuals examined are included in a single branch. On the other hand, the tree showed that haplotypes of Ismailia and Damietta populations are grouped in one branch while haplotypes of Alexandria, Matrouh and Gharbiya populations are in a different branch next to each other in one clade and are more closely related to each other. In contrast, haplotypes of Cairo, Siwa Oasis and Beni Suef populations are situated in another branch close to the haplotypes of the Menia, Sohag, Qena, and Aswan populations (Fig. 2).

Table 3. Genetic differentiation (G_{ST}) and diversity parameters (H_T, H_S) for the combined mtDNA sequences in all studied populations of the African Common Toad (*Sclerophrys regularis*).

Zoon	H_S	H_T	G_{ST}
Sharm El-Shaikh	0.031	1.000	0.969
Arish	0.031	1.000	0.969
Ismailia	0.016	1.000	0.984
Damietta	0.009	1.000	0.991
Alexandria	0.016	1.000	0.984
Matrouh	0.031	1.000	0.969
Gharbiya	0.026	0.900	0.874
Cairo	0.031	1.000	0.969
Siwa Oasis	0.005	1.000	0.995
Bani Sweif	0.050	0.833	0.783
Menia	0.016	1.000	0.984
Sohag	0.016	1.000	0.984
Qena	0.009	1.000	0.991
Aswan	0.016	1.000	0.984
Total	0.00001	0.999	0.99899

Discussion

It is well-established that the long-term evolution of a species produces its genetic variation and this genetic variation represents its evolutionary potential for survival and development (Soltis et al. 1992; Gitzendanner and Soltis 2000). During a long evolutionary history, high levels of genetic variation are expected to accumulate. At present, there is insufficient data to determine the evolutionary significant units (ESU) for the conservation of *Sclerophrys regularis*. However, it seems prudent to protect and preserve all of the habitats of *Sclerophrys regularis* due to its high genetic variation and wide distribution. More exhaustive studies should focus on more highly variable genetic markers and on the ecology of *Sclerophrys regularis* to establish an effective conservation strategy. As expected, these toads were found to have high genetic diversity and genetic differentiation at the species level.

The results provide insight into the genetic variability of *Sclerophrys regularis* using mitochondrial cytochrome oxidase subunit I (COI) gene analysis. Avise et al. (1987) and Moritz (1994) reported that mitochondrial DNA genes have been used extensively in evolutionary biology to measure genetic variation within populations, especially in those that diverged recently, and to assess the conservation value of specific populations or areas. In particular, mtDNA is useful for phylogenetic studies because it has maternal inheritance, a mutation rate 10 times faster than nuclear DNA, and a low recombination rate (Brown et al. 1979; Masuda and Yoshida 1994). As a barcoding gene, COI is also widely used for many taxa (including amphibians) for species identification and taxonomic discovery (Marshall 2005; Salvolainen et al. 2005; Little and Stevenson 2007) because it often yields deeper phylogeny than other genes, such as cytochrome *b* (Lynch and Jarrell 1993; Simmons and Weller 2001).

The results indicate that the Siwa Oasis population and the Damietta and Qena populations had the largest numbers of haplotypes. On the other hand, the Matrouh, Arish, Sharm El-Sheikh, Cairo, and Beni Suef populations had the lowest numbers of haplotypes, suggesting a possible genetic barrier between South and North River Nile which restricts gene flow, such as water sources, climatic conditions or distances between habitats. In addition, a high haplotype diversity was found in all studied locations, except for the Gharbiya and Beni Suef populations which had low haplotype diversity values. This may be related to dry climate and limited water sources in the two locations.

The nucleotide diversity was highest in the Cairo population, while the Damietta and Beni Suef populations had the lowest nucleotide diversity. High haplotype diversity and low nucleotide diversity in a species can often be explained by many singular haplotypes with few base substitutions.

Samples from the Sharm El-Shaikh and Arish localities had the greatest genetic distances to the other studied populations, which may be related to differences in habitat and climate specifically, the Sharm El-Shaikh and Arish regions are particularly arid (Borkin et al. 2016).

In Egypt, this toad mainly inhabits savannas. Borkin et al. (2016) reported that western Africa, the northern part of eastern Africa, and the Nile River valley in Egypt were the most suitable environments for *Sclerophrys regularis*. Distribution of this toad is restricted by the Sahara Desert in the north and, perhaps, by concurrent closely related species in the south, such as *Sclerophrys gutturalis* (Power 1927). The population at Sharm El-Sheikh is highly isolated from the native range of the species by the desert of Sinai. However, the coastal strip along the Red Sea in the southernmost part of the Sinai Peninsula has quite suitable environmental conditions for the survival of this species (Borkin et al. 2016).

Large agricultural reclamation projects using Nile water are being established in northwest Sinai. Some have suggested that *Sclerophrys regularis* will spread into this region (Baha El Din 2006). Recently, at the eastern side of the Suez Canal, *Sclerophrys regularis* was found in six localities in green fields east of the Bitter Lakes up to 9–10 km into Sinai, including the vicinities of villages Al-Taqaddom, Al-Abtal and Meet Abul Kum Al-Jadeeda (Ibrahim 2013a, b). As in the western bank of the Suez Canal, *Sclerophrys regularis* introduced from the River Nile is becoming quite common around freshwater irrigation canals.

Conclusions

The results from COI analysis show that *Sclerophrys regularis* has very high genetic diversity and gene flow among different Egyptian populations. Therefore, it seems reasonable to perform many additional studies on *Sclerophrys regularis* due to its high genetic variation and wide distribution. These data may indicate that the ecological niche of this species is somewhat broader than was revealed for populations within its native distributional range.

Literature Cited

Avise JC, Arnold J, Ball RM. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematic. *Annual Review of Ecology and Systematic*s 18: 489–522.

Baha El Din S. 2006. *A Guide to the Reptiles and Amphibians of Egypt*. The American University in Cairo Press, Cairo – New York. Available: https://www.amazon.com/Guide-Reptiles-Amphibians-Egypt/dp/9774249798 [Accessed: 1 June 2006].

Borkin LJ. 1999. Distribution of amphibians in North Africa, Europe, western Asia, and the former Soviet Union. Pp. 329–420 In: *Patterns of Distribution of Amphibians: A Global Perspective*. Editor, Duellman WE. The Johns Hopkins University Press, Baltimore,

Maryland, USA. 633 p.
Borkin LJ, Litvinchuk SN. 2013. Amphibians of the Palearctic: taxonomic composition. *Proceedings of the Zoological Institute RAS* 317(4): 494–541.
Borkin LJ, Goncharov AI, Litvinchuk SN. 2016. The Egyptian toad, *Sclerophrys regularis* (Reuss, 1833) at Sharm El-Sheikh, with comments on amphibians of the Sinai. *Russian Journal of Herpetology* 23(4): 283–292.
Brown WM, George MJ, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* 76: 1,967–1,971.
Cortés-Gomez AM, Ruiz-Agudelo CA, Valencia-Aguilar A, Ladle RJ. 2015. Ecological functions of neotropical amphibians and reptiles: a review. *Universitas Scientiarum* 20(2): 229–245.
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, et al. 2010. Geneious Version 5.1. Available: http://www.geneious.com [Accessed: 16 August 2010].
Frost DR. 2007. *Amphibian Species of the World: An Online Reference*. Version 5.0. Available: http:// research.amnh.org/herpetology/amphibia/index.html [Accessed: 13 May 2007].
Gitzendanner MA, Soltis PS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87: 783–792.
Hocking DJ, Babbitt KJ. 2014. Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology* 9(1): 1–17.
Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. 2000. Quantitative evidence for global amphibian population declines. *Nature* 404: 752–755.
Ibrahim AA. 2001. Geographic distribution: *Bufo regularis*. *Herpetological Review* 32(2): 112.
Ibrahim AA. 2013a. The herpetology of the Suez Canal Zone, Egypt. *Vertebrate Zoology* 63(1): 87–110.
Ibrahim AA. 2013b. Chapter 29. Amphibians of Egypt: a troubled resource. Pp. 107–117 In: *Amphibian Biology. Basic and Applied Herpetology.* Volume 27. Conservation and Decline of Amphibians: Eastern Hemisphere. Part 2. Mauritania, Morocco, Algeria, Tunisia, Libya and Egypt. 117 p.
IUCN (International Union for the Conservation of Nature). 2015. *IUCN Red List of Threatened Species. Version 4*. Gland, Switzerland, IUCN.
Kiesecker JM, Blaustein AR, Belden LK. 2001. Complex causes of amphibian population declines. *Nature* 410: 681–684.
Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1,870–1,874.
Little DP, Stevenson DW. 2007. A comparison of algorithms for the identification of specimens using DNA barcodes: examples from gymnosperms. *Cladistics* 23: 1–21.
Lynch M, Jarrell PE. 1993. A method for calibrating molecular clocks and its application to animal mitochondrial DNA. *Genetics* 135: 1,197–1,208.
Marshall E. 2005. Taxonomy. Will DNA barcodes breathe life into classification? *Science* 307: 1,037.
Masuda R, Yoshida MC. 1994. Nucleotide sequence variation of cytochrome *b* genes in three species of weasels *Mustela itatsi*, *Mustela sibirica*, and *Mustela nivalis*, detected by improved PCR product direct sequencing technique. *Journal of the Mammalogical Society of Japan* 19: 33–43.
Moritz C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* 3: 401–411.
Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* 70: 3,321–3,323.
Ohler A, Dubois A. 2016. The identity of the South African toad *Sclerophrys scapensis* Tschudi, 1838 (Amphibia, Anura). *PeerJ* 4: e1,553.
Pino-Del-Carpio A, Ariño AH, Villarroya A, Puig J, Miranda R. 2014. The biodiversity data knowledge gap: assessing information loss in the management of Biosphere Reserves. *Biological Conservation* 173: 74–79.
Pons OPR. 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics* 144: 1,237–1,245.
Rödel MO. 2000. *Herpetofauna of West Africa. Amphibians of the West African Savanna, Volume 1*. Edition Chimaira, Frankfurt, Germany. 335 p.
Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. *Molecular and Biology Evolution* 34: 3,299–3,302.
Saad YM, Hanaf MS, Essa MA, Guerges AA, Ali SF. 2009. Genetic signatures of some Egyptian *Clarias gariepinus* populations. *Global Veterinaria* 3: 503–508.
Sakr SA, Badawy GM, El-Borm HT. 2014. Ultrastructural and molecular changes in the developing small intestine of the toad *Bufo regularis*. *Scientific World Journal* 4(5): 1–13.
Saleh MA. 1997. *Amphibians and Reptiles of Egypt.* Publication of the National Biodiversity Unit, no. 6. Egyptian Environmental Affairs Agency, Cairo, Egypt. 234 p.
Salvolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R. 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 360: 1,805–1,811.
Simmons RB, Weller SJ. 2001. Utility and evolution of cytochrome *b* in insect. *Molecular Phylogenetics and Evolution* 20: 196–210.

Soltis PS, Soltis DE, Tucker TL, Lang FA. 1992. Allozyme variability is absent in the narrow endemic *Bensoniella oregona* (Saxifragaceae). *Conservation Biology* 6: 131–134.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, et al. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1,783–1,786.

Warkentin IG, Bickford D, Sodhi NS, Bradshaw CJA. 2009. Eating frogs to extinction. *Conservation Biology* 23(4): 1,056–1,059.

Whiles MR, Hall RO, Dodds WK, Verburg P, Huryn AD, et al. 2013. Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16: 146–157.

Lamiaa Elsayed Mokhtar Deef received her Master's degree in 2008 from the Faculty of Science, Mansuora University and then a Ph.D. degree in 2014 from the Faculty of Science, Damietta University. She is currently a lecturer at the same faculty at Damietta University. Her research interests include genetics, taxonomy, and phylogenetic studies.