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Phylogeography of the East African Serrated Hinged Terrapin *Pelusios sinuatus* (Smith, 1838) and resurrection of *Sternothaerus bottegi* Boulenger, 1895 as a subspecies of *P. sinuatus*

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***Abstract.*—*Pelusios sinuatus* is distributed in East Africa from southern Ethiopia and Somalia to northeastern South Africa. Inland it reaches westernmost Zimbabwe, Rwanda, and Burundi. Despite this wide range, which spans in north-south direction across 3,500 km and in east-west direction more than 1,500 km, no geographic variation has been described. However, using phylogenetic and haplotype network analyses of mitochondrial and nuclear DNA (2,180 bp and 2,132 bp, respectively), phylogeographic variation is herein described, with two distinct genealogical lineages. One occurs in the northern and central parts of the distribution range, and the other is in the south. Terrapins representing the southern lineage attain a smaller maximum body size than terrapins from the northern and central parts of the range. The distribution ranges of the two lineages abut in the border region of Botswana, South Africa, and Zimbabwe. We conclude that each lineage represents a distinct subspecies, with the nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838) occurring in the south and the newly recognized subspecies *Pelusios sinuatus bottegi* (Boulenger, 1895) in the central and northern distribution range. We found phylogeographic structuring within each subspecies and propose that the differentiated population clusters should be recognized as Management Units.**

Keywords. management units, Pelomedusidae, systematics, taxonomy, Testudines, turtle

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Introduction

The freshwater turtle genus *Pelusios* comprises 17 species distributed across sub-Saharan Africa, with most likely introduced populations on Madagascar, the Seychelles, and Guadeloupe in the Lesser Antilles (Fritz et al. 2011, 2013; Stuckas et al. 2013; TTWG 2017). All species of *Pelusios* are side-necked turtles characterized by a plastral hinge that allows partial or complete closure of the anterior shell opening (Bramble and Hutchison 1981; Branch 2008). Together, *Pelusios* and its African-Arabian sister genus *Pelomedusa*, which has 10 formally recognized species (Petzold et al. 2014; TTWG 2017), constitute the family Pelomedusidae that is sister to the New World-Madagascan family Podocnemididae. Pelomedusids and podocnemidids are both of Gondwanan origin (de Broin 1988; Noonan 2000) and, together with the Australasian and South American family Chelidae, represent the chelonian suborder Pleurodira (side-necked turtles; TTWG 2017).

In addition to a number of species with localized narrow distribution, *Pelusios* also includes species with wide distributions (Branch 2008; TTWG 2017). The Serrated Hinged Terrapin, *P. sinuatus* (Smith, 1838), has one of the largest distribution ranges of all *Pelusios* species. It occurs in East Africa from southern Ethiopia and Somalia southwards to Eswatini (formerly Swaziland) and northeastern South Africa (Branch 2008; TTWG 2017; Fig. 1). The Serrated Hinged Terrapin has the largest body size in its genus and may reach up to

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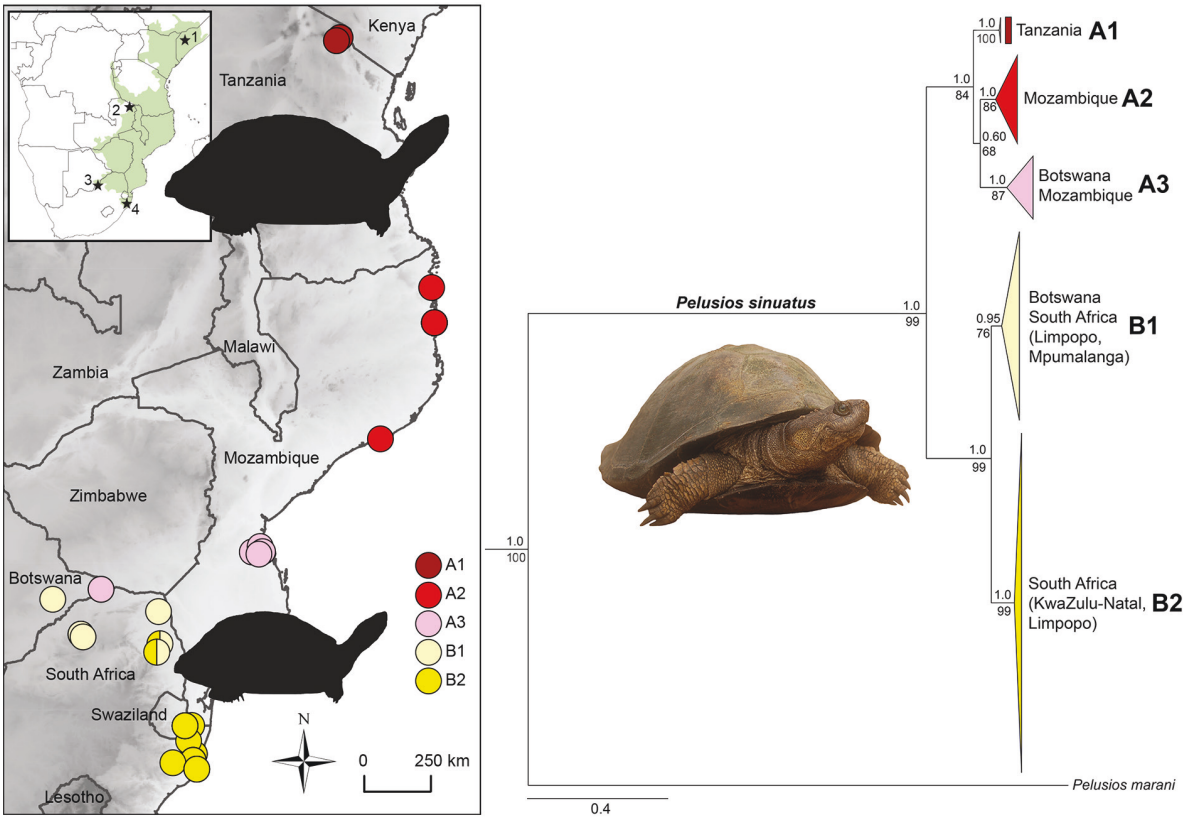

Fig. 1. Left: Sampling sites and mitochondrial identity of *Pelusios sinuatus* used in the present study. Divided symbols indicate syntopic occurrences of the respective subclades. Terrapin shapes symbolize differences in maximum sizes of the northern and southern *P. sinuatus* (see Discussion section). **Inset**: Distribution range of *P. sinuatus* according to the TTWG (2017) with the type localities of taxa referred to this species. (1) *Sternothaerus bottegi* Boulenger, 1895: Bardere (Bardera), Somalia; (2) *Pelusios sinuatus leptus* Hewitt, 1933: Isoka, Zambia; (3) *Sternotherus sinuatus* Smith, 1838 – restricted type locality (Broadley, 1981): confluence of Crocodile and Marico Rivers, Limpopo (Transvaal); (4) *Pelusios sinuatus zuluensis* Hewitt, 1927: Mzinene River (Umsinene River, Zululand), KwaZulu-Natal. **Right:** Bayesian tree for 61 *Pelusios sinuatus* using 2,180 bp of mitochondrial DNA. Clades are collapsed to cartoons showing the deepest genetic divergence within each clade. Outgroup *Pelomedusa variabilis* was removed for clarity. Numbers above the nodes are posterior probabilities; below the nodes, thorough bootstrap values under ML. Full trees are available from https://figshare.com/s/52a7af23cff3aa08ea75. **Inset:** Adult *Pelusios sinuatus*, Bonamanzi, KwaZulu-Natal, South Africa.

55 cm in shell length (Spawls et al. 2002). In contrast to other *Pelusios* species, *P. sinuatus* is a deep-water terrapin that occurs in perennial rivers, lakes, and larger man-made water bodies in savannah regions. During the rainy season, Serrated Hinged Terrapins move overland, and they colonize smaller water bodies, like pans and waterholes (Broadley and Boycott 2009). In eight other widespread *Pelusios* species, Fritz et al. (2013) and Kindler et al. (2016) found that phylogeographic patterns were not correlated with habitat type, with some species displaying pronounced phylogeographic structuring and others not. Among the studied savannah species, *P. rhodesianus* showed a deep phylogeographic structure and could actually represent a species complex (Kindler et al. 2016), whereas phylogeographic structuring in *P. nanus* and *P. subniger* was negligible. However, the westernmost studied population originally identified as *P. subniger* (in Democratic Republic of the Congo) was found to represent a genetically distinct undescribed species. Unfortunately, samples from the northern distribution area of *P. subniger* were not available for study, so nothing is known about the genetic identity of the northern populations (Fritz et al. 2013; Kindler et al. 2016). Another savannah species (*P. castanoides*), with a distribution range similar to that of *P. sinuatus,* was characterized by moderate phylogeographic variation (Fritz et al. 2013).

Until now, neither the morphological nor the phylogeographic variation of *P. sinuatus* has been studied systematically. The species has traditionally been regarded as monotypic (Ernst and Barbour 1989; TTWG 2017; Wermuth and Mertens 1961, 1977), even though Hewitt (1927, 1933) had described two subspecies from South Africa and Zambia that were soon synonymized (Loveridge 1936). However, using mitochondrial and nuclear DNA sequences of only two samples from KwaZulu-Natal (South Africa) and another one from Botswana, Fritz et al. (2011) found two genetic lineages, suggesting that an in-depth investigation of genetic variation may reveal further differentiation. The present paper presents the first assessment of the genetic variation of *P. sinuatus* across its range. The results are discussed with respect to taxonomy and two subspecies are recognized within *P. sinuatus*. To this end, *Sternothaerus bottegi* Boulenger, 1895 is resurrected from the synonymy of *P. sinuatus* (Smith, 1838), in which it was placed soon after its description (Calabresi 1916; Siebenrock 1916).

Materials and Methods

Sampling, chosen loci, and general data evaluation strategy: Sixty-one samples of *Pelusios sinuatus* from Botswana, Mozambique, South Africa, and Tanzania were studied, including previously published data for three terrapins (Appendix 1). The same mitochondrial and nuclear DNA fragments were targeted as in earlier studies on *Pelusios* (Fritz et al. 2011; Kindler et al. 2016). Three mitochondrial DNA fragments were sequenced (12S, cyt *b*, and ND4 with adjacent DNA coding for tRNAs). In addition, two protein-coding nuclear genes (Cmos and Rag2) and intron 1 of the nuclear R35 gene were sequenced. Details of DNA isolation, PCR, and sequencing are described in Kindler et al. (2016). The 12S sequences obtained were up to 398 bp long (with gaps); the cyt *b* sequences were up to 913 bp; and the mtDNA sequences comprising the partial ND4 gene plus adjacent DNA coding for tRNAs were up to 869 bp long. All nuclear DNA blocks could be sequenced directly. Cmos sequences had lengths of up to 358 bp; R35 sequences, up to 1,101 bp; and Rag2 sequences, up to 673 bp. Sequences were aligned and inspected using BioEdit 7.0.5.2 (Hall 1999). All sequences aligned perfectly and gaps occurred only in sequence blocks not coding for proteins.

Mitochondrial DNA is maternally inherited, whereas nuclear loci are inherited biparentally. Moreover, mtDNA is prone to introgression, including across species borders, which often leads to conflicting results for the two marker systems (Currat et al. 2009; Funk and Omland 2003; Kehlmaier et al. 2019; Sloan et al. 2017; Toews and Brelsford 2012). To avoid the risk of such distortion, mitochondrial and nuclear sequence data were examined separately.

Phylogenetic analyses: Individual mtDNA fragments were concatenated for phylogenetic analyses, and this data set was combined with previously published sequences, resulting in an alignment of 2,180 bp length. The dataset included 61 sequences of *Pelusios sinuatus* and, as outgroups, one sequence each of *Pelomedusa variabilis* and *Pelusios marani*. European Nucleotide Archive (ENA) accession numbers and collection sites are given in Appendix 1. The best partitioning scheme was determined using PartitionFinder (Lanfear et al. 2012) and the Bayesian Information Criterion (BIC). Three partitioning schemes were tested: (1) unpartitioned, (2) partitioned by mtDNA fragment, and (3) partitioned by gene and codon position with DNA not coding for proteins (i.e., 12S and DNA coding for tRNAs) corresponding to one additional partition each. According to the results of PartitionFinder, scheme (3) was selected.

Phylogenetic relationships were inferred using Bayesian and Maximum Likelihood (ML) approaches. Bayesian trees were obtained with MrBayes 3.2.6 (Ronquist et al. 2012) using the partitioning scheme and evolutionary models shown in Table 1 and default parameters. Two parallel runs, each with four chains, were conducted. The chains ran for 10 million generations, with every 500th generation sampled. The calculation parameters were analyzed using a burn-in of 2.5 million generations to assure that both runs converged. Subsequently, only the plateau of the remaining trees was sampled using the same burn-in, and a 50% majority rule consensus tree was generated. Tracer 1.7 (Rambaut et al. 2018) served to check for convergence of the runs using the Effective Sample Sizes (ESS) of parameters, and resulted in ESSs over 200 after discarding the burn-in. In addition, phylogenetic relationships were inferred under ML using RAxML 7.2.8 (Stamatakis 2006) and the GTR+G substitution model across all partitions. Five independent ML searches were performed using different starting conditions and the fast bootstrap algorithm to explore the robustness of the results by comparing the best trees. Then, 1,000 non-parametric thorough bootstrap replicates were calculated and the values were plotted against the best tree.

Parsimony networks: For each mitochondrial and nuclear DNA fragment, a parsimony network was constructed using Popart (http://popart.otago.ac.nz). Since the underlying TCS algorithm is sensitive to missing data, a few individuals represented by short sequences were excluded. In addition, for achieving complete coverage, the lengths of mtDNA sequences were trimmed, resulting in an alignment of 348 bp length for 12S, 784 bp for cyt *b*, and 737 bp for ND4 + DNA coding for tRNA. For network construction of nuclear data, heterozygous sequences of R35 were phased using the Phase algorithm in DnaSP 5.10 (Librado and Rozas 2009), and two identical copies for homozygous sequences of all loci were included. Nuclear DNA sequences for the networks had the same lengths as given above.

Uncorrected *p* distances and isolation by distance for mtDNA: Uncorrected *p* distances were calculated for the mitochondrial cyt *b* gene alone as well as for the mtDNA alignment of concatenated sequences using MEGA 7.0.21 (Kumar et al. 2016) and the pairwise deletion option. The distances of the concatenated sequence data were used to examine for a positive correlation between geographic and genetic distances (isolation by distance). For this purpose, Mantel tests as implemented in IBD 1.52 (Bohonak 2002) were run for three data sets using genetic and spatial distances. The latter were obtained via the Geographic Distance Matrix Generator 1.2.3 (http://biodiversityinformatics.amnh.org/open_source/gdmg/index.php). The significance of the slope of the reduced major axis (RMA) regression was assessed by 30,000 randomizations. One data set included the sequences for all 61 terrapins. The other two data sets included the sequences for each clade of *P. sinuatus* (clades A and B) identified in the present study.

Table 1. Partitioning and evolutionary models used for MrBayes.

Subset	nst	rates	Model
1-398 401-1311\3 1314-1998\3 1999-2180	6	gamma	SYM + gamma
399-1311\3 1312-1998\3	6		SYM
400-1311\3 1313-1998\3	6	gamma	SYM + gamma

Body size: Using Vernier calipers (accuracy 0.1 mm), straight carapace length of adult terrapins was recorded as a measure of body size during fieldwork in South Africa (n = 11). Also measured were specimens in the Museum for Comparative Zoology, Cambridge, Massachusetts, USA (MCZ 39383, South Africa), the Museum für Naturkunde, Berlin, Germany (ZMB 158, Mozambique; ZMB 5517, 5518, 15689, 16158, 16242, Tanzania; ZMB 22416, Burundi), the Museum für Tierkunde (Museum of Zoology), Senckenberg, Dresden, Germany (MTD D 49650, South Africa), and the Paläontologisches Institut und Museum, Zürich, Switzerland (PIMUZ A/III527, Tanzania). Measurements were divided into northern and southern groups according to the two genetic clades identified in the present study. Since males and females could not be distinguished in all samples, the two sexes were combined for analysis. After testing for whether the data were parametric, the body sizes of the groups were compared using a *t*-test as implemented in SigmaPlot 13.0 (Systat Software, Inc., San Jose, California, USA).

Results

Phylogenetic and haplotype network analyses of mtDNA: Both tree-building approaches delivered the same topology, corresponding to two geographically widespread clades, A and B, both of which showed substructuring and received high support values (Fig. 1). Clade A corresponded to the samples from the north and center of the distribution range of *Pelusios sinuatus* (Tanzania, Mozambique, and Botswana), and clade B to samples from the south (Botswana and South Africa). Clade A consisted of three subclades and clade B, of two. All subclades of clade A and one subclade of clade B were well supported under both Bayesian and ML analyses; the second subclade of clade B was moderately supported. In Botswana and northeastern South Africa, records of terrapins representing clades A and B are only separated by a distance of approximately 200 km, and in northeastern South Africa, representatives of subclades B1 and B2 were found in two sites syntopically.

In haplotype networks of the three mtDNA fragments, no shared haplotypes occurred for the two clades (Fig. 2). Four 12S haplotypes were found for clade A, with two private haplotypes for subclade A2 that differed by one mutation step each from a shared haplotype that included sequences of subclades A1 and A3. Another private haplotype of subclade A3 also differed by one mutation step from the previously mentioned shared haplotype. This shared haplotype was separated by three mutation steps from a common haplotype containing sequences of subclades B1 and B2, and a second rare haplotype for subclade B1 differed by one mutation step from this common haplotype. Haplotype networks of the two other mtDNA fragments showed more differentiation, with no shared haplotypes between any clades or subclades. For the cyt *b* fragment, haplotypes of clades A and B were separated by a minimum of 21 steps. Within haplotypes of clade A, up to 10 mutations occurred, with each subclade corresponding to one distinct haplotype. Within haplotypes of clade B, a loop occurred that connected three of the four haplotypes of subclade B1; and the four haplotypes of this subclade were separated by a minimum of four steps from the three haplotypes of subclade B2, each of which differed by one mutation step. With respect to the mtDNA fragment containing the partial ND4 gene and adjacent DNA coding for tRNAs, haplotypes of clades A and B differed by a minimum of 13 mutations. Subclade A1 was represented by one haplotype. Subclade A2 consisted of three haplotypes that each differed by a maximum of three mutations; and subclade A3 had four haplotypes that differed by up to four steps. Within the individual haplotypes of clade A, a maximum of nine steps occurred, and the three subclades were separated by a minimum of 4–7 steps. The three haplotypes of subclade B1 differed by a maximum of two mutations and the two haplotypes of subclade B2, by one mutation, and the two subclades were distinct by a minimum of six steps.

Haplotype network analyses of nuclear loci: The three nuclear loci showed distinctly less variation compared to the mtDNA fragments. Often, shared haplotypes between distinct clades and subclades were found (Fig. 3). For the Cmos gene, the 15 haplotypes found differed by a maximum of nine mutation steps. Clades A and B shared two haplotypes, even though the vast majority of phased sequences of clades A and B corresponded to unique haplotypes for each clade. A generally similar picture was revealed for the Rag2 locus, with one shared haplotype of clade A and clade B, six unique additional haplotypes of clade A, and three further unique haplotypes of clade B. The maximum number of mutations between the Rag2 haplotypes was seven. For intron 1 of the R35 gene, no shared haplotypes were found for clades A and B. A total of 15 haplotypes occurred that were partially connected over a loop. In a direct line (not across the loop), the haplotypes differed by up to seven mutations. Of the 15 haplotypes, six corresponded to clade A and nine to clade B.

Uncorrected *p* distances and isolation by distance for mtDNA: Sequence divergences of the mitochondrial cyt *b* gene are often used to distinguish between chelonian taxa (e.g., Iverson et al. 2013; Kindler et al. 2012, 2016; Petzold et al. 2014). The uncorrected *p* distance between clade A and clade B of *P. sinuatus* amounted to 2.80% on average, while the within-group values were 1.05% and 0.28%, respectively. Between the individual subclades of clade A, divergences ranged between 1.31% and 1.54%, with no variation within those two clades for which sequences of more than one individual were available. Subclades B1 and B2 differed by only 0.57%, with within-group divergences of 0.06% and 0.02%, respectively (Table 2).

The IBD test for all data revealed a statistically significant correlation of genetic and geographic distances (Z = 22487528, r = 0.65, $p \leq 0.0001$; n = 61). When the two clades were analyzed separately, a statistically significant correlation was also found for clade A (Z = 1387859, r = 0.58, $p \leq 0.0003$; n = 17), but not for clade B (Z = 9009469796357, r = 0.06, $p \leq 0.0777$; n = 44).

Body size: The mean straight carapace length (± SD) for the samples of the northern and southern clades,

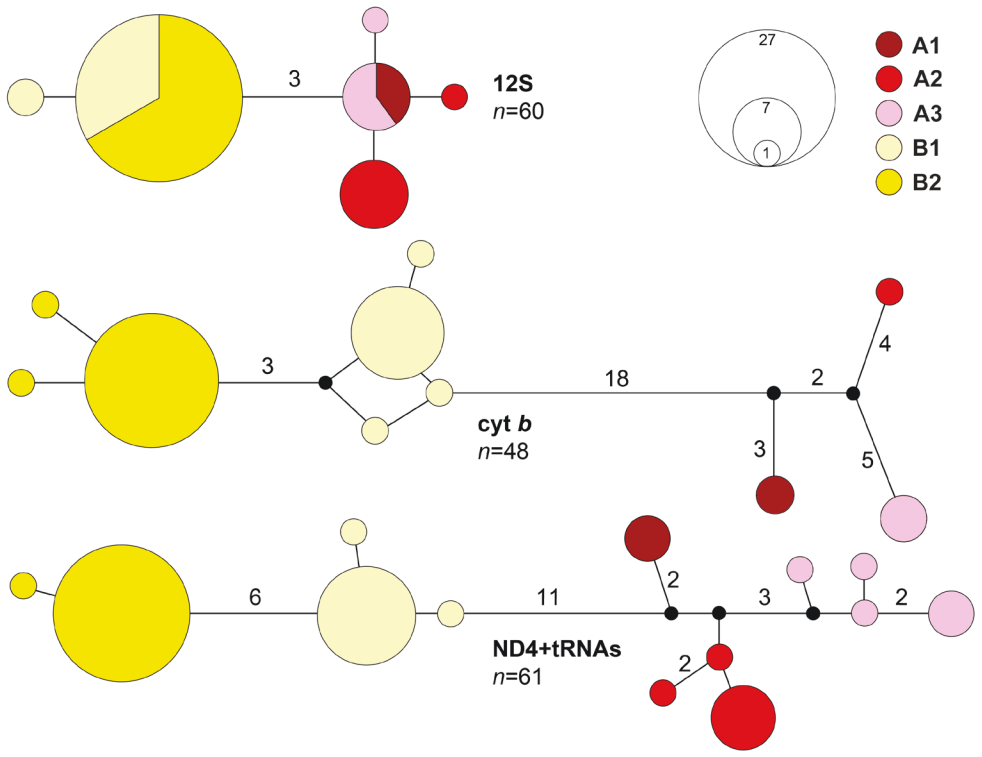

Fig. 2. Parsimony networks for individual mtDNA fragments of *Pelusios sinuatus*. Symbol size is proportional to haplotype frequency. Each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by numbers of substitutions along the lines. Colors correspond to Fig. 1. Small black circles represent missing node haplotypes.

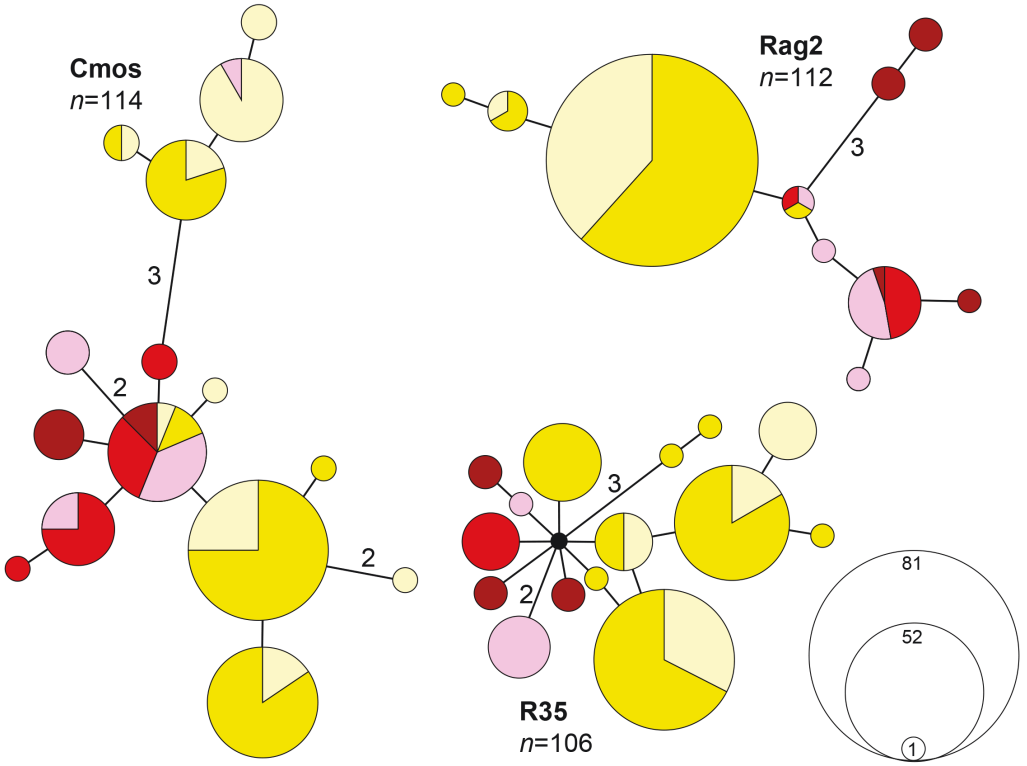

Fig. 3. Parsimony networks for nuclear loci of *Pelusios sinuatus*. Symbol size is proportional to haplotype frequency. Each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by numbers of substitutions along the lines. Colors correspond to Fig. 1. The small black circle represents a missing node haplotype. Sample sizes refer to phased nuclear sequences, i.e., each individual is represented twice.

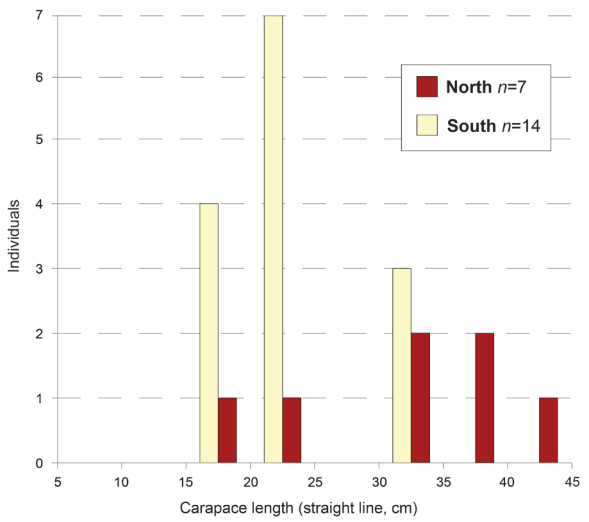

Fig. 4. Carapace lengths of adult *Pelusios sinuatus* from the northern and the southern distribution range (museum specimens and wild-caught terrapins), corresponding to clades A (north) and B (south).

respectively, were 31.8 ± 7.9 cm (n = 7) and 24.0 ± 5.5 cm (n = 14), with terrapins from the northern clade being significantly larger than those from the southern clade (t_{19} = 2.66, p = 0.0156).

Discussion

The present study is the first assessment of the phylogeography for the Serrated Hinged Terrapin (*Pelusios sinuatus*), which is widely distributed in East Africa (TTWG 2017; inset in Fig. 1). In north-south direction, the distribution area extends over approximately 3,500 km and in east-west direction, over more than 1,500 km. Within this large area, two mitochondrial clades (A and B) with parapatric distribution and substantial geographic substructure were discovered (Figs. 1 and 2). In contrast to mitochondrial DNA, the slower evolving nuclear DNA has not reached complete lineage sorting for the Cmos and Rag2 loci (Fig. 3), even though haplotype sharing between clades A and B was restricted. For intron 1 of the R35 gene, no shared haplotypes occurred. Thus, mitochondrial and nuclear markers show largely concordant differentiation patterns.

Clade A was found in the northern and central parts of the distribution range (Tanzania, Mozambique, and Botswana), and clade B, in the south (Botswana and South Africa). Close to the border region of Botswana, Zimbabwe, and South Africa the two clades abut, which explains why Fritz et al. (2011) had already discovered the two clades using only three samples from that region. Nearby, to the southeast, sites were found with syntopic occurrences of the two otherwise parapatric mitochondrial subclades within clade B. This implies that the correlation of genetic and geographic distances for the whole data set cannot result from isolation by distance alone, because then neither distinct clades nor contact zones would be expected (Figs. 1 and 2). This is also supported by the absence of evidence for isolation by distance in the southern clade B. Therefore, we conclude

Table 2. Uncorrected *p* distances (means, expressed as percentages) between and within mitochondrial subclades of *Pelusios sinuatus* using the cyt *b* gene (913 bp). Below the diagonal are between-group values; on the diagonal, within-group divergences are in bold.

	n	A1	A2	A3	B1	B2
A1	2	**0**				
A2	1	1.31	—			
A3	3	1.53	1.31	**0**		
B1	15	2.83	2.85	2.63	**0.06**	
B2	27	2.75	2.97	2.75	0.57	**0.02**

that the observed genetic divergence is, at least in part, caused by vicariance and subsequent dispersal, and that the correlation of geographic and genetic distances results mainly from our patchy sampling. A future challenge is to close the large sampling gaps in order to locate additional contact zones, especially between the northern subclades.

The north-south differentiation of *P. sinuatus* is similar to that in another terrapin species. *Pelusios castanoides* has a continental distribution range similar to *P. sinuatus* but occurs also on Madagascar and the Seychelles (TTWG 2017), although it is unclear whether the latter populations are native. In *P. castanoides*, a sample from the north of the distribution range (Kenya) was distinct from samples from South Africa and southern Mozambique (Fritz et al. 2013), suggesting a shared biogeographic history with *P. sinuatus*.

To the best of our knowledge, *P. sinuatus* from different parts of the distribution range have never been compared morphologically, but northern terrapins (clade A) seem to grow to a larger maximum size than southern ones (clade B). According to de Witte (1952), *P. sinuatus* reaches 46.5 cm in Lake Tanganyika. Branch (2008) reports a maximum shell length of 48.5 cm for *P. sinuatus*, and Spawls et al. (2002) mention up to 55 cm for upland Kenya. Serrated Hinged Terrapins of such size are never seen in South Africa. This is confirmed by the measurements of wild animals and collection material reported here (Fig. 4) that show a statistically significant difference between the mean carapace lengths of terrapins representing clades A and B. Seven adult museum specimens from Tanzania and Burundi (clade A) had straight carapace lengths from 19.6 cm to 40.0 cm (i.e., the largest specimens are still distinctly below the published maximum size). In contrast, 14 adult museum specimens and wild terrapins from the distribution range of clade B (Mozambique and South Africa) ranged between 17.5 cm and 34.7 cm. Thus, it appears that northern and southern terrapins differ morphologically, at least with respect to their maximum size, but more measurements are required and further studies warranted for comparing variations in additional morphological characters. The size variation of *P. sinuatus* is reminiscent of other turtles in which size increases with latitude (Ashton and Feldman 2003), either within the same species (e.g., *Chelonoidis chilensis*: Fritz et al. 2012a; *Testudo graeca*: Werner et al. 2016) or in distinct species of the same genus (*Pelodiscus* spp.: Farkas et al. 2019). However, there are exceptions. For instance, in Leopard Tortoises (*Stigmochelys*

Table 3. Average uncorrected *p* distances (percentages) of 795 bp of the cyt *b* gene of *Pelusios* species from Kindler et al. (2016). *Pelusios subniger* includes a putative undescribed species from the Democratic Republic of the Congo (n = 2). It differs from other *P. subniger* by an average distance of 3.13% (Petzold et al. 2014). The relationship of *P. carinatus* and *P. rhodesianus* is unclear; some populations assigned to the latter species (*P. rhodesianus* A) could be conspecific with *P. carinatus* (Kindler et al. 2016). Values for sympatrically occurring species pairs in boldface and red.

	n	*ada*	*bec*	*bro*	*car*	*c'us*	*c'es*	*cha*	*cup*	*gab*	*mar*	*nan*	*nig*	*rho* A	*rho* B	*sin*	*sub*	*upe*	*wil*
adansonii	1																		
bechuanicus	2	11.59																	
broadleyi	7	3.05	13.15																
carinatus	15	9.92	12.91	11.17															
castaneus	19	6.52	13.70	7.55	11.48														
castanoides	29	9.57	11.50	9.88	10.99	11.17													
chapini	8	6.03	15.91	10.17	**13.95**	4.34	12.35												
cupulatta	4	10.98	10.79	11.24	11.26	**12.14**	10.76	15.03											
gabonensis	24	12.73	13.62	13.68	**12.84**	**13.52**	12.54	**12.42**	13.09										
marani	5	12.80	11.21	12.68	13.01	13.57	12.50	**15.35**	11.82	**13.75**									
nanus	26	11.59	**14.99**	15.07	15.31	15.36	12.21	12.94	14.09	**12.42**	15.25								
niger	2	11.76	13.38	13.90	12.25	**15.17**	11.85	15.77	**9.10**	**12.73**	14.14	13.07							
rhodesianus A	6	10.81	**13.45**	11.39	**2.49**	**11.83**	**11.12**	**10.92**	12.28	**11.43**	14.19	**13.24**	12.57						
rhodesianus B	14	7.93	**14.23**	11.93	**6.22**	**12.39**	**12.12**	**10.63**	13.44	**12.09**	14.58	**12.41**	12.77	4.04					
sinuatus	2	14.02	11.42	13.05	12.52	14.01	**13.60**	16.32	11.26	13.95	11.02	15.73	14.34	13.92	13.96				
subniger	41	14.57	5.33	12.25	12.70	13.50	**11.89**	15.28	10.92	13.15	11.21	**13.72**	11.64	13.50	13.55	**10.08**			
upembae	3	10.98	1.38	13.15	13.04	14.41	11.50	16.54	10.91	14.01	11.82	**14.99**	13.56	**14.56**	**13.54**	11.80	**5.43**		
williamsi	2	9.15	10.91	10.87	10.23	11.03	3.89	**15.00**	11.01	14.11	11.33	15.73	13.69	**13.97**	**10.77**	**13.31**	12.28	11.31	

pardalis) the northernmost and southernmost populations comprise large-sized individuals, while tortoises from geographically intermediate populations are medium-sized (Fritz et al. 2010; Spitzweg et al. 2019). Another pattern is found in continental *Trachemys* species once considered conspecific, with taxa having the largest body sizes in Central America and distinctly smaller-sized North and South American congeners (Ernst and Barbour 1989; Legler and Vogt 2013; Vargas-Ramírez et al. 2017). Clearly, further research is needed for a better understanding of the described size variation in *P. sinuatus* and other turtle species, but we concur with Joos et al. (2017) and Spitzweg et al. (2019) that many factors beyond latitude act in concert on such variation.

An open question remains how the genetic and morphological differentiation patterns of *P. sinuatus* relate to taxonomy. The concordant variation of different genetic and morphological characters justifies recognizing each clade within *P. sinuatus* as a distinct taxon. However, without entering the debate about species concepts and species conceptualization (e.g., de Queiroz 2007; Zachos 2016), we are reluctant to assign species status to either clade. In our understanding, restricted gene flow and largely isolated gene pools represent unambiguous traits of distinct species. In contrast to other cases (e.g., Kindler et al. 2017; Spinks et al. 2014; Vamberger et al. 2015), patchy sampling prevents sound conclusions here, particularly the lack of comprehensive sampling from the putative contact zone of clades A and B. Yet, in times when legislative restrictions make biodiversity research virtually impossible for many widely distributed species (Neumann et al. 2018; Prathapan et al. 2018), researchers are often forced to use the evidence available as a starting point.

The mitochondrial divergence of the two clades (Figs. 1 and 2), together with concordant variation in the nuclear loci (Fig. 3), provide two important insights: (1) the two mitochondrial clades represent distinct genealogical lineages; and (2) mitochondrial introgression plays no obvious role here, allowing the application of mtDNA to infer taxonomic differentiation. Uncorrected *p* distances of the mitochondrial cyt *b* gene have frequently been used as a 'taxonomic yardstick' to decide which taxonomic rank should be applied to turtle taxa (e.g., Iverson et al. 2013; Kindler et al. 2012, 2016; Petzold et al. 2014; Spinks et al. 2004), analogous to the widely applied barcoding approach (e.g., Hebert et al. 2003). However, as pointed out by Fritz et al. (2012b) and Kindler et al. (2012), the wide range of genetic divergences between different turtle species (differing by one order of magnitude) prevents the application of a rigid threshold across all turtle groups. Instead, thresholds for different groups need to be adjusted individually using unambiguous, ideally sympatric, species that are closely related to the taxa in question. Thus, previously published cyt *b* data for other *Pelusios* species (Kindler et al. 2016; Petzold et al. 2014) can serve here for comparison. Also, for these species a wide range has been reported (pairwise average divergences between species vary from 1.38% to 16.54%), even though the low value of 1.38% between the allopatric *P. bechuanicus* and *P. upembae* has been suggested to indicate their conspecificity (Kindler et al. 2016). When only divergences of sympatric species are considered, the values range between 2.49% and 15.35% (Table 3). Yet, the lowest value refers to *P. carinatus* and populations of *P. rhodesianus* that could actually be conspecific with *P. carinatus* (Kindler et al. 2016).

If this value is disregarded, the lowest value between unambiguous sympatric species amounts to 5.43% (*P. subniger* vs. *P. upembae*), and this value is much higher than the divergence between clades A and B of *P. sinuatus* (2.80%) found here.

In view of this relatively low value, we suggest subspecies status for the Serrated Hinged Terrapins from the southernmost and more northerly parts of the distribution range. The name *Sternotherus sinuatus* Smith, 1838 is clearly referable to the southern subspecies, while the oldest name for the northern subspecies is *Sternothaerus bottegi* Boulenger, 1895 (Fig. 1). Accordingly, the smaller-sized southern populations represent the nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838), and the large-sized northern subspecies is to be named *Pelusios sinuatus bottegi* (Boulenger, 1895) nov. comb. Another name, *Pelusios sinuatus zuluensis* Hewitt, 1927 (type locality: Mzinene River, KwaZulu-Natal, South Africa) clearly is a junior synonym of the nominotypical subspecies. A fourth name, *Pelusios sinuatus leptus* Hewitt, 1933 (type locality: Isoka, Zambia) can be identified with the northern subspecies, and is thus a junior synonym of *P. s. bottegi*.

This assessment is in line with the recent proposal to use the subspecies category for naming lineages that qualify for the genetic criteria of Evolutionarily Significant Units (ESUs; Moritz 1994). Accordingly, subspecies should correspond to distinct mtDNA lineages (except for cases of mitochondrial capture), and they should be diagnosable by nuclear genomic evidence. However, in contrast to species, subspecies are genetically less divergent and capable of large-scale gene flow with other subspecies. Applying subspecies names for such lineages facilitates communication within and beyond science, particularly in legislation and conservation (Kindler and Fritz 2018). In this vein, the recognition of two subspecies of *P. sinuatus* not only reflects their genetic divergence but also will contribute in the medium term to their conservation. Currently, *P. sinuatus* is not considered to be imperiled (IUCN category "Least Concern," Rhodin et al. 2018). However, in many African countries freshwater habitats are increasingly threatened by progressing land use and, consequently, the numbers of Serrated Hinged Terrapins are dwindling. Furthermore, we propose to treat the subclades A1–A3 within *P. s. bottegi* and subclades B1 and B2 within *P. s. sinuatus* as distinct Management Units in the sense of Moritz (1994), i.e., as populations with significant mitochondrial divergence.

Conclusions

Serrated Hinged Terrapins (*Pelusios sinuatus*) show concordant variation in mitochondrial and nuclear marker genes, corresponding to two distinct genealogical lineages in the southernmost and more northerly parts of the distribution range. Each lineage displays phylogeographic structuring. Terrapins representing the two lineages differ also in body size, with individuals from the northern and central parts of the distribution reaching larger sizes than terrapins from the southern parts. Considering the degree of genetic differentiation compared to other *Pelusios* species, we conclude that the two lineages should be regarded as distinct subspecies. The nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838) corresponds to populations in the south (South Africa and parts of Botswana), and the resurrected taxon *Pelusios sinuatus bottegi* (Boulenger, 1895) nov. comb. to populations from the northern and central distribution range. A contact zone of the two subspecies is identified in the border region of Botswana, South Africa, and Zimbabwe. The genetically differentiated population clusters within each subspecies should be treated as distinct Management Units. Further research is needed to find out whether additional diagnostic morphological characters for the two subspecies exist. In addition, denser sampling would allow a fine-scale phylogeography for the species including an assessment of gene flow between the two subspecies and the Management Units within each subspecies. Such research could contribute significantly to the development of long-term management plans for this species. However, the current legislative situation makes progress unlikely because multiple countries are involved and obtaining sampling permits for biodiversity research often becomes a major, if not insurmountable, administrative obstacle.

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Appendix 1. Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt *b*	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
14050	LR594053	LR594111	LR594157	LR594218	LR594272	LR594325	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14051	LR594054	LR594112	LR594158	LR594219	LR594273	LR594326	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14052	LR594055	LR594113	LR594159	LR594220	LR594274	LR594327	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14053	LR594056	LR594114	LR594160	LR594221	LR594275	LR594328	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14054	LR594057	LR594115	LR594161	LR594222	LR594276	LR594329	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14055	LR594058	LR594116	LR594162	LR594223	LR594277	LR594330	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14056	LR594059	LR594117	LR594163	LR594224	LR594278	LR594331	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
5564	FR716875	n/a	FR716984	FR717028	FR717076	FR717121	Botswana: Mashatu Game Reserve	-22.212308	29.136038	A1
5565	LR594060	n/a	LR594164	LR594225	LR594279	LR594332	Botswana: Mashatu Game Reserve	-22.212308	29.136038	A1
7044	LR594061	n/a	LR594165	LR594226	LR594280	LR594333	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7045	LR594062	n/a	LR594166	LR594227	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7046	LR594063	n/a	LR594167	LR594228	n/a	LR594334	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7047	LR594064	n/a	LR594168	LR594229	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7048	LR594065	n/a	LR594169	n/a	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7049	LR594066	n/a	LR594170	LR594230	n/a	LR594335	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
17104	LR594067	LR594118	LR594171	LR594231	LR594281	LR594336	Mozambique: Cabo Delgado: Rio Diquide	-11.884827	40.460208	A2
9891	LR594068	n/a	LR594172	LR594232	LR594282 LR594283	LR594337	Mozambique: Sofala province, along road 428	-20.923567	34.6662	A1

Appendix 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt *b*	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
11100	LR594069	n/a	LR594173	LR594233	LR594284	LR594338	Mozambique: Sofala province, along road 428	-21.007317	34.539433	A1
6956	LR594070	LR594119	LR594174	LR594234	LR594285 LR594286	LR594339	Mozambique: Sofala: NE of Rio Save Game Reserve	-20.933333	34.316667	A1
6959	LR594071	LR594120	LR594175	LR594235	LR594287	LR594340	Mozambique: Sofala: NE of Rio Save Game Reserve	-20.7425	34.586567	A1
5215	LR594072	n/a	LR594176	LR594236	LR594288	LR594341	Mozambique: Zambesia: Moebase	-17.059513	38.699233	A2
17003	LR594073	LR594121	LR594177	LR594237	LR594289	LR594342	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17004	LR594074	LR594122	LR594178	LR594238	LR594290	LR594343	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17005	LR594075	LR594123	LR594179	LR594239	LR594291	LR594344	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17006	LR594076	LR594124	LR594180	LR594240	LR594292	LR594345	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17010	LR594077	LR594125	LR594181	n/a	n/a	n/a	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.145899	31.591881	B2
9143	LR594078	LR594126	LR594182	LR594241	LR594293	LR594346	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve, Waterlily Dam	-28.05809	32.29412	B2
14040	LR594079	LR594127	LR594183	LR594242	LR594294	LR594347	South Africa: KwaZulu-Natal: Jozini, Kwa Nyamazane Conservancy	-27.391656	32.139994	B2
16199	LR594080	LR594128	LR594184	LR594243	LR594295	LR594348	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16200	LR594081	LR594129	LR594185	LR594244	LR594296	LR594349	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16202	LR594082	LR594130	LR594186	LR594245	LR594297	LR594350	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16203	LR594083	LR594131	LR594187	LR594246	LR594298	LR594351	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
10614	LR594084	n/a	LR594188	LR594247	LR594299	LR594352	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.885692	32.223672	B2
10615	LR594085	LR594132	LR594189	LR594248	LR594300	LR594353	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.874944	32.231997	B2
14041	LR594086	LR594133	LR594190	LR594249	LR594301	LR594354	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.891275	32.299	B2

Appenidx 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

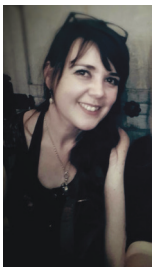
Sample	12S	cyt *b*	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
13590	LR594087	LR594134	LR594191	LR594250	LR594302	LR594355	South Africa: KwaZulu-Natal: Ndumo Game Reserve: Mabayani	-26.865118	32.240964	B2
5216	FR716876	FR716937	FR716985	FR717029	FR717077	FR717122	South Africa: KwaZulu-Natal: Phinda Game Reserve	-27.843744	32.335521	B2
5217	FR716877	FR716938	FR716986	FR717030	FR717078	FR717123	South Africa: KwaZulu-Natal: Phinda Game Reserve	-27.843744	32.335521	B2
17014	LR594088	LR594135	LR594192	LR594251	LR594303	LR594356	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17015	LR594089	LR594136	LR594193	LR594252	LR594304	LR594357	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17016	LR594090	LR594137	LR594194	LR594253	LR594305	LR594358	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17028	LR594091	LR594138	LR594195	LR594254	LR594306	LR594359	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17029	LR594092	LR594139	LR594196	LR594255	LR594307	LR594360	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17030	LR594093	LR594140	LR594197	LR594256	LR594308	LR594361	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17031	LR594094	LR594141	LR594198	LR594257	LR594309	LR594362	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17038	LR594095	LR594142	LR594199	LR594258	LR594310	LR594363	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B1
17039	LR594096	LR594143	LR594200	LR594259	LR594311	LR594364	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17040	LR594097	LR594144	LR594201	LR594260	LR594312	LR594365	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17041	LR594098	LR594145	LR594202	LR594261	LR594313	LR594366	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17042	LR594099	LR594146	LR594203	LR594262	LR594314	LR594367	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B1
17061	LR594100	LR594147	LR594204	LR594263	LR594315	LR594368	South Africa: Limpopo: Lapalala	-23.89392	28.29516	B1
17068	LR594101	LR594148	LR594205	LR594264	LR594316	LR594369	South Africa: Limpopo: Lapalala	-23.89392	28.29516	B1
16206	LR594102	LR594149	LR594206	LR594265	LR594317	LR594370	South Africa: Limpopo: Palabora Mining Company, near Loolo Dam, S of Phalaborwa	-24.018889	31.140833	B1
16197	LR594103	LR594150	LR594207	LR594266	LR594318	LR594371	South Africa: Limpopo: Palabora Mining Company, SE of Phalaborwa	-24	31.210556	B1
16196	LR594104	LR594151	LR594208	n/a	LR594319	LR594372	South Africa: Limpopo: Palabora Mining Company: Cleveland Nature Reserve, in Olifants River near picnic site	-24.03056	31.19306	B2
17073	LR594105	LR594152	LR594209	LR594267	LR594320	LR594373	South Africa: Limpopo: Vaalwater	-23.98716	28.37323	B1

Appenidx 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt *b*	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
13585	LR594106	LR594153	LR594210	LR594268	LR594321	LR594374	South Africa: Mpumalanga: Kruger National Park: Shingwedzi River near Shingwedzi Camp	-23.107365	31.439066	B1
13586	LR594107	n/a	LR594211	n/a	n/a	n/a	South Africa: Mpumalanga: Kruger National Park: Shingwedzi River near Shingwedzi Camp	-23.107365	31.439066	B1
16214	LR594108	LR594154	LR594212	LR594269	LR594322	LR594375	Tanzania: Kilimanjaro Region: Moshi	-3.34261	37.319831	A3
16269	LR594109	LR594155	LR594213	LR594270	LR594323	LR594376	Tanzania: Manyara Region: Kikuletwa Hotsprings	-3.443532	37.193393	A3
16270	LR594110	LR594156	LR594214	LR594271	LR594324	LR594377	Tanzania: Manyara Region: Kikuletwa Hotsprings	-3.443532	37.193393	A3
	Outgroups									
	Pelusios marani									
5214	FR716869	FR716928	FR716979	n/a	n/a	n/a				
	Pelomedusa variabilis									
—	AF039066	AF039066	AF039066	n/a	n/a	n/a				

Melita Vamberger is a Slovenian herpetologist and evolutionary biologist working at the Senckenberg Natural History Collections, Dresden, Germany. Melita studied Biology at the University of Ljubljana, Slovenia, focusing on the natural history of the European Pond Turtle (*Emys orbicularis*). After her diploma, Melita moved to Germany for her Ph.D. at the University of Leipzig, studying the phylogeography and hybridization of two closely related freshwater turtle species (*Mauremys caspica* and *M. rivulata*). Melita's main interests are speciation, gene flow, adaptation, and evolution of different turtle taxa using an integrative approach that combines genetic and ecological methods, especially in the Western Palearctic and sub-Saharan Africa.

Margaretha D. Hofmeyr is Professor Emeritus at the Biodiversity and Conservation Biology Department, University of the Western Cape, South Africa. Margaretha is an ecophysiologist by training, and first studied large ungulates before switching to chelonians. Her ecophysiological studies revealed that South African tortoises have many unique characteristics, which stimulated further interest in their genetic diversity and systematics. Margaretha has published extensively on the ecology and phylogeography of sub-Saharan tortoises and turtles, and she is closely involved in conservation projects for threatened tortoises. This work resulted in her being awarded the 2015 Sabin Turtle Conservation Prize. Margaretha is a member and Regional Vice-Chair for Africa of the IUCN/SSC TFTSG and coordinated the 2014 and 2018 Red List Assessments for South African tortoises and freshwater turtles.



Courtney A. Cook is a Senior Lecturer in the Water Research Group, Unit for Environmental Sciences and Management, North-West University, South Africa. Courtney is a parasitologist focusing on the biodiversity, taxonomy, and phylogeny of protozoan blood parasites of ectothermic vertebrates (amphibians, reptiles, and fish), with several authored and co-authored scientific articles in this area. Her M.Sc. and Ph.D. both focused exclusively on these parasites infecting tortoises of South Africa, which inspired a keen interest in these animals, particularly with respect to their taxonomy and phylogeny, both important aspects in understanding the associated host-parasite relationships. Courtney was recently awarded a Y-rating by the South African National Research Foundation, identifying her as a promising young researcher in her field from a global perspective.

Edward C. Netherlands is a dual Ph.D. candidate between the North-West University, South Africa, and Katholieke Universiteit Leuven, Belgium. His Ph.D. forms part of the VLIR-UOS program for the development of tools for the sustainable utilization and management of aquatic resources in South Africa. Edward's research interests focus on the molecular biology, ecology, and taxonomy of herpetofauna and their associated parasites. He is also passionate about teaching the importance of conservation to young minds and non-scientists. Ed has authored or co-authored several scientific articles and a bilingual frog field guide (in English and Zulu). Edward also received the Research Excellence for Next Generation Researchers Award as a final year Ph.D. Candidate from the National Research Foundation in South Africa.

Uwe Fritz is the head of the Museum of Zoology, Senckenberg Natural History Collections in Dresden, Germany, and Extraordinary Professor for zoology at the University of Leipzig, Germany. Uwe has worked for many years on the taxonomy, systematics, and phylogeography of turtles and tortoises, and has also studied snakes and lizards to a lesser extent. He is particularly interested in hybridization patterns and gene flow in the contact zones of distinct taxa. Uwe has authored or co-authored numerous scientific articles, mainly in herpetology, and has edited proceedings and books, among them the two turtle volumes of the *Handbook of Amphibians and Reptiles of Europe*.