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Phylogeography of the African Helmeted Terrapin, *Pelomedusa subrufa*: Genetic Structure, Dispersal, and Human Introduction

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The African Helmeted Terrapin, *Pelomedusa subrufa,* is currently recognized as a single species, and is found throughout sub-Saharan Africa, as well as on the Arabian Peninsula and in Madagascar. A preliminary genetic study of *Pelomedusa* based on mitochondrial and nuclear DNA is presented to determine the genetic diversity within *Pelomedusa*, and whether the disjunct populations are natural or anthropogenic. The monophyly of *Pelomedusa* is in question, as *Pelusios* may be nested within *Pelomedusa*. Within *Pelomedusa* there are three subgroups: Western, Eastern, and Southern. The Western clade is composed of two monophyletic groups divided by ecoregion, one through the Sahel region of Africa and the other in the savanna region of West Africa. The Eastern clade contains a disjunct population on the Arabian Peninsula, and divergence dating estimates indicate that this population was probably established through a natural dispersal event. The Southern clade includes a disjunct population in Madagascar, and this Malagasy population likely is the result of a recent human-mediated introduction, since sequence divergence compared to other individuals in the Southern clade is relatively low.

The African Helmeted Terrapin, *Pelomedusa subrufa,* is widespread over sub-Saharan Africa in semi-arid regions and savanna habitats (Loveridge 1941). *Pelomedusa* is a monotypic genus, and three subspecies are traditionally recognized, although there are some who have dismissed the validity of all three subspecies (Boycott and Bourquin 2008). In addition to continental Africa, there are two disjunct populations, one in Madagascar and another on the Arabian Peninsula, which are separated from the mainland by the Mozambique Channel and the Red Sea, respectively. The details surrounding the establishment of these disjunct populations are unknown; Malagasy and Arabian populations of *Pelomedusa* may be the result of a natural event or human introduction.

Pelomedusa subrufa is expected to be genetically diverse since other vertebrates with pan-African distributions show high levels of genetic structure (lizards of the genus *Agama* [Leaché et al. 2009] and bats of the genus *Otomops* [Lamb et al. 2008]). Within *Agama*, three regional clades were recovered (East, West, and South) with evidence of a Sahel radiation within the Western clade and a "biogeographic corridor" between East and West Africa (Leaché et al. 2009). Lamb et al. (2008) recovered three well-supported bat clades: a northeastern Africa including Yemen, an African clade excluding the northeast, and a Malagasy clade.

This and a concurrent study (Vargas-Ramirez et al. 2010) are the first to investigate the genetic structure within *Pelomedusa* across its range. By studying *Pelomedusa* genetically, we can gather preliminary data on genetic diversity, infer patterns of distribution, and test the validity of populations in Madagascar and the Arabian Peninsula. In this paper, a phylogeographic study of

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Pelomedusa subrufa is presented based on mitochondrial (*12S* rRNA, *16S* rRNA, *CytB* and *CO1*) and nuclear (1097 bp from RNA fingerprint protein 35 intron 1) DNA.

1. Materials and Methods

1.1 Taxon Sampling and Laboratory Protocols

A total of 25 specimens were sequenced for this study, of which 19 are *Pelomedusa subrufa* and six are outgroup samples (one *Erymnochelys madagascariensis*, two *Platemys platycephala*, and three *Pelusios castaneus*). Previous to our work, only two known-locality *Pelomedusa* were sequenced and available on GenBank, both which we include in our analyses. Details on these specimens, including GenBank numbers, can be found in Table 1.

Total cellular DNA was extracted using a standard salt extraction protocol (Sambrook and Russell 2001). Four mitochondrial loci (12S rRNA [*12S*], 16S rRNA [*16S*], cytochrome b [*CytB*], and cytochrome oxidase 1 [*CO1*]) and one nuclear locus (RNA fingerprint protein 35 [*R35*]) were sequenced using the primer pairs indicated in Appendix I. For some samples where the DNA was degraded, internal primers were developed (Appendix II) to allow amplification of smaller, overlapping segments of the chosen genes. The samples were PCR amplified using standard thermocycler conditions. PCR amplified products were purified with ExoSAP-IT (USB Corp.) or gel extracted and purified using the Gel-ase enzyme (EPICENTRE Biosystems) when multiple products were amplified. The purified PCR products were cycle-sequenced using Big Dye Terminator v.3.1, and then sequenced using an ABI 3730 automated sequencer.

1.2 Phylogenetic Analysis

Sequencher v. 4.7 (Gene Codes) was used to align and edit complementary sequences. Multiple sequence alignments were generated using Muscle v3.7 (Edgar 2004).

Phylogenetic analyses were run on three different datasets: *R35* only (nuclear locus), combined mitochondrial loci (*12S*, *16S*, *CO1*, *CytB*), and combined mitochondrial and nuclear loci. Datasets were partitioned by gene and codon position to account for different models of evolution (Brandley et al. 2005). Each dataset was analyzed under maximum likelihood (ML) using RAxML (Stamatakis et al. 2008) and Bayesian inference (BI) using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). ML analyses were run under the GTRMIX model of substitution and 1000 bootstrap replicates. For BI analyses, models of nucleotide substitution for each partition were estimated using MrModeltest v2.3 (Nylander 2004). Tests of stationarity were run with the online program AWTY (Wilgenbusch et al. 2004), and the appropriate burn-in was removed before combining runs.

1.3 Divergence Dating Analyses

The problems associated with divergence dating have been highlighted (Graur and Martin 2004; Parham and Irmis 2008). We heed their warnings and use conservative divergence dating methods to estimate separation times of the Arabian and Malagasy populations from the mainland. We calculated divergence dates using two methods, first by incorporating uncertainty from the geologic column into our fossil calibration date in BEAST v1.5.2 (Drummond and Rambaut 2007), and next by using a rate of evolution (Weisrock et al. 2001).

BEAST was run on the combined mitochondrial and nuclear dataset, partitioned by gene and codon, and run for 20 million generations. An uncorrelated log-normal clock model was used along with a speciation yule process tree prior and a normal prior frequency distribution. A fossil *Pelusios* (Williams 1954) was used as a calibration point for the divergence between *Pelusios-Pelomedusa* (Near et al. 2005), whose minimum divergence time is estimated to be 23.1-15.9 mya (Parham and Irmis 2008).

TABLE 1. Locality data, voucher numbers, and GenBank accession numbers for specimens used in this study. Detailed locality data can be obtained from the institutions. MVZ=Museum of Vertebrate Zoology, ULM=University of Louisiana at Monroe, R=Museum of Comparative Zoology, FGZC= Frank Glaw Zoological Collection, LSU=Louisiana Sate University, ZFMK= Zoologiches Forschungs Museum Alexander Koenig.

Species	Voucher #	Locality	12S	16S	CO1	CytB	R35
1) *Platemys platycephala*	MVZ 247579	Suriname	GU213780	GU213801	GU213826	GU213842	GU213861
2) *Platemys platycephala*	MVZ 247580	Suriname	GU213781	GU213802	n/a	GU213843	n/a
3) Erymnochelys madagascariensis	MVZ 238759	Madagascar	GU213782	GU213803	n/a	GU213844	GU213862
4) *Pelusios castaneus*	MVZ 250330	Sierra Leone	GU213783	GU213804	n/a	n/a	n/a
5) *Pelusios castaneus*	MVZ 250331	Sierra Leone	GU213784	GU213805	n/a	n/a	GU213863
6) *Pelusios castaneus*	ULM 178	Guinea	GU213785	GU213806	n/a	n/a	GU213864
7) *Pelomedusa subrufa*	R-184287	Namibia	GU213786	GU213807	n/a	GU213845	GU213865
8) *Pelomedusa subrufa*	FGZC 324	Madagascar	GU213787	GU213808	n/a	GU213846	GU213866
9) *Pelomedusa subrufa*	LSU 20145	Ghana	GU213788	GU213809	GU213827	n/a	GU213867
10) *Pelomedusa subrufa*	LSU 20315	Ghana	GU213789	GU213810	GU213828	n/a	GU213868
11) *Pelomedusa subrufa*	MVZ 245226	Ghana	GU213790	GU213811	GU213829	GU213847	GU213869
12) *Pelomedusa subrufa*	MVZ 245229	Ghana	GU213791	GU213812	GU213830	GU213848	GU213870
13) *Pelomedusa subrufa*	MVZ 238878	Niger	GU213792	GU213813	GU213831	GU213849	GU213871
14) *Pelomedusa subrufa*	MVZ 238879	Niger	GU213793	GU213814	GU213832	GU213850	GU213872
15) *Pelomedusa subrufa*	MVZ 238883	Niger	GU213794	GU213815	GU213833	GU213851	GU213873
16) *Pelomedusa subrufa*	MVZ238887	Niger	GU213795	GU213816	GU213834	GU213852	GU213874
17) *Pelomedusa subrufa*	MVZ 236628	Yemen	GU213796	GU213817	GU213835	GU213853	GU213875
18) *Pelomedusa subrufa*	MVZ 241329	Somalia	GU213797	GU213818	GU213836	GU213854	GU213876
19) *Pelomedusa subrufa*	MVZ 241330	Somalia	GU213798	GU213819	GU213837	GU213855	GU213877
20) *Pelomedusa subrufa*	MVZ 241331	Somalia	GU213799	GU213820	GU213838	GU213856	GU213878
21) *Pelomedusa subrufa*	MVZ 241332	Somalia	GU213800	GU213821	GU213839	GU213857	GU213879
22) *Pelomedusa subrufa*	ZFMK 15171	Cameroon	n/a	GU213822	GU213840	GU213858	n/a
23) *Pelomedusa subrufa*	ZFMK 17076	Senegal	n/a	GU213823	GU213841	GU213859	n/a
24) *Pelomedusa subrufa*	ZFMK 19836	Nigeria	n/a	GU213824	n/a	GU213860	n/a
25) *Pelomedusa subrufa*	ZFMK 54523	Malawi	n/a	GU213825	n/a	n/a	n/a
26) *Pelomedusa subrufa*	GENBANK	Ghana	DQ283264	DQ283264	n/a	n/a	n/a
27) *Pelomedusa subrufa*	GENBANK	Togo	n/a	AF113639	AF113663	n/a	n/a

Weisrock et al. (2001) reviewed the rate of evolution of mitochondrial DNA in a fragment including *ND1*, *ND2* and *CO1* and found it was consistent in a range of vertebrates at a rate of 0.57-0.69% per lineage per million years. Although there were no empirical data on turtles, we calculate divergence dates by applying the fastest rate (0.69% per lineage; 1.38% pair-wise rate) to our *CO1* dataset to obtain a minimum date of divergence.

2. RESULTS

Of the 2180 bp of mitochondrial DNA (*12S*, *16S*, *CytB*, *CO1*) sequenced, 591 sites were phylogenetically informative, while 70 of the 1097 bp of nuclear DNA (*R35*) were phylogenetically

informative. Since some of the tissue samples were old or degraded, we were unable to compile a complete dataset. However, we were able to maintain our geographic coverage by sequencing a minimum of two mitochondrial markers for each sample. Although missing data may be a problem, our results seem relatively robust, as all phylogenetic analyses produced similar phylogenies. Herein, we only present the results of the ML analysis performed on the concatenated mitochondrial and nuclear dataset (Fig. 1). The monophyly of the family Pelomedusidae (*Pelomedusa* and *Pelusios*) is strongly supported (ML bootstrap = 99%; posterior probability = 1.0). Within *Pelomedusa* three geographic clades appear: Western, Eastern, and Southern (Figs. 1 and 2). Total variation between these three major clades of *Pelomedusa* is at most an uncorrected pair-wise difference of ~15% (mtDNA). However, the monophyly of the genus *Pelomedusa* is equivocal due to the position of *Pelusios*. *Pelusios* is either the sister group to the Southern *Pelomedusa* clade in the combined data analyses (weakly supported, Fig. 1), or forms an unresolved polytomy with the Southern and Eastern + Western *Pelomedusa* clades in our *R35* analyses (not shown).

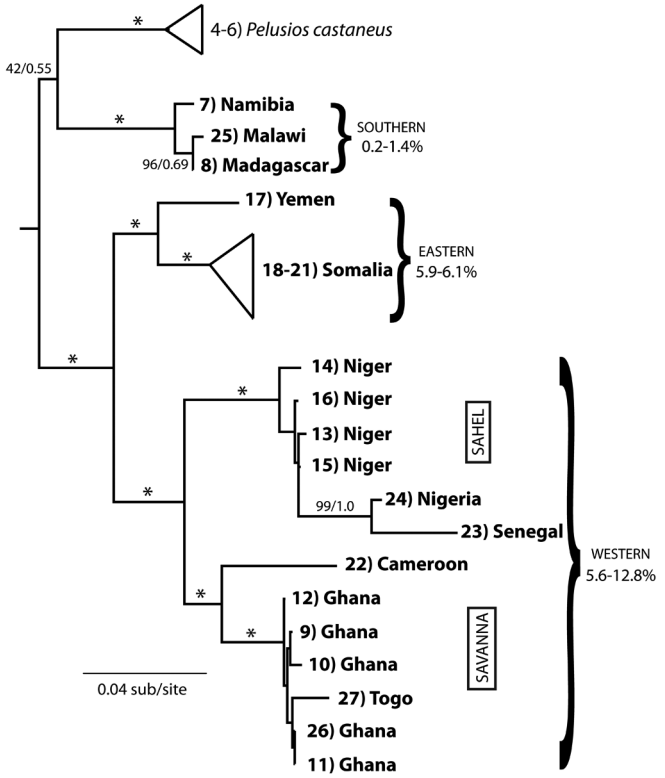

FIGURE 1. Phylogenetic inference from the maximum likelihood (ML) analysis on the combined mitochondrial and nuclear DNA analysis. ML and Bayesian analyses on mitochondrial only and nuclear only datasets produced similar trees. Support values on nodes are ML bootstraps/Bayesian posterior probabilities, while stars (*) represent full support (100/1.0). *Pelomedusa* clades are labeled according to the text and Figure 2. Ranges of percentages next to clades represent the uncorrected pair-wise sequence divergence between important specimens in the group—Southern: Madagascar and southern Africa, Eastern: Yemen and eastern Africa, Western: Sahel and Savanna. Numbers next to taxa correspond to the numbers in Table 1 and Figure 2.

2.1 The Western Clade

The Western clade consists of samples from Ghana, Togo, Cameroon, Niger, Nigeria, and Senegal (Fig. 1). Within this clade, there is further substructure, with one group consisting of Cameroon, Ghana, and Togo, and a second group consisting of Niger, Nigeria, and Senegal. These two groups differ by an uncorrected pair-wise difference of ~5.6–12.8%.

2.2 The Eastern Clade

The Eastern clade consists of individuals on the Horn of Africa (Somalia) and on the Arabian Peninsula (Yemen). There is strong support for this group from all phylogenetic analyses (ML bootstrap = 100%, Bayesian posterior probability = 1.0, Fig. 1). There is approximately 300km, including the Red Sea, between Somali and Yemeni specimens. The minimum sequence divergence between the Yemen and Somali specimens is 0.8% (*R35*) and 5.9% (mtDNA).

2.3 The Southern Clade

The Southern clade consists of samples from Malawi, Namibia, and Madagascar. In all cases, the Madagascar specimen is nested within the Malawi and Namibia specimens (ML bootstrap = 100%; Bayesian posterior probability = 1.0, Fig. 1). This is despite the long geographic distance (800-1600km) and the crossing of the Mozambique Channel. The percentage sequence divergence between the Madagascar individual and the other members of the Southern clade is ~0.4% (*R35*) and 0.5–1.4% (mtDNA).

2.4 Divergence Dating

Plots of likelihood scores indicated that BEAST analyses had reached stationarity. The *Pelomedusa* populations of particular interest are the disjunct populations in Yemen and Madagascar. 95% confidence intervals of the divergence dates were large, 14.1–0.8 mya for Yemen + Eastern Africa and 10.1–0.0006 mya for Madagascar + Southern Africa.

FIGURE 2. Map of specimen localities used in this study. Shapes correspond to the genetic clades found in Figure 1. Circles represent the Western clade, with filled-in circles representing samples in the Sahel and empty circles representing samples in the Savanna. Red squares represent the Southern clade and black stars represent the Eastern clade. Numbers next to taxa correspond to the numbers in Table 1 and Figure 1

For analyses using a 1.38%/million years rate of sequence evolution, we were able to calculate the divergence time of the Yemen sample, but not the Madagascar sample, as *CO1* sequences were unavailable for Madagascar and southern Africa samples. The percent sequence divergence between Yemen and Somalia was a minimum of 4.3%. By dividing by 1.38%/million years, we estimate the divergence of Yemeni and Somali *Pelomedusa* to be ~2.4 mya. This date is nested within the divergence date estimate from BEAST.

3. DISCUSSION

Our sampling for this study is by no means complete, as *Pelomedusa* is found throughout sub-Saharan Africa. Relative to other animal groups, turtles are relatively under-collected since their secretive lifestyle makes them difficult to capture and their large size makes them difficult to transport. However, we have compiled all known, available samples for this study and use this opportunity to provide preliminary phylogeographic data on this understudied taxon. Our phylogenetic analyses put the sister relationship of *Pelusios* and *Pelomedusa* into question. *Pelusios* was either nested within *Pelomedusa* or placed in a polytomy with two *Pelomedusa* clades. These results could have several explanations: 1) an artifact of the markers we have used (i.e., incomplete lineage sorting), 2) a rapid radiation early in the history of the family Pelomedusidae, resulting in a

hard polytomy, or 3) *Pelusios* diverging from within *Pelomedusa*. This result does not seem to be an artifact of the markers used, as Vargas-Ramirez et al. (2010) found similar results with different markers. Therefore, the relationship between *Pelusios* and *Pelomedusa* appears to be more complicated than previous believed and should be further studied.

Divergence date estimates from both the BEAST and the molecular rate of evolution analyses are concordant, as the point estimate is nested within the BEAST range. Despite the uncertainty in the wide range from BEAST, these analyses provide valuable insight, and are incorporated into the discussion below.

3.1 The Western Clade: The Sahel and the Savanna

Pelomedusa samples in the Western clade do not always show close affinity to geographic neighbors; although the Nigerian sample is only ~200km from the Cameroon sample, Nigeria is more closely related to samples ~1000km away in Senegal. Instead of geography, the substructure of the Western clade seems to be based on ecoregion. The first group consists of individuals from Niger, Nigeria, and Senegal, and is found in the Sahel region of Africa. The Sahel region is just south of the Sahara Desert, and is a dry region that stretches across Africa from the Atlantic Ocean to the Red Sea (Fig. 2). The second group consists of individuals from Cameroon, Ghana, and Togo. These countries occur in the wetter savanna region to the south of the Sahel. This pattern is also seen in the lizard genus *Agama* (Leaché et al. 2009). The persistence of this biogeographic pattern between taxa suggests that there is relatively low interaction between the Sahel and savanna species.

3.2 The Eastern Clade: The Population in Yemen

Populations of *Pelomedusa* on the Arabian Peninsula are restricted to Yemen and Saudi Arabia (Boycott and Bourquin 2008). Due to the current political instability of the area, additional sampling from the area is difficult. With our current sampling we are able to gain insight into the relationship of the two populations, but this should be considered a working hypothesis until additional data are collected. As expected based on geography, the Yemeni specimen is most closely related to the population from neighboring Somalia.

Timing and geologic history must be considered when evaluating the mechanisms of isolated populations. These populations can naturally occur through vicariance or dispersal, or be a result of artificial, human transport. Although we can never definitively determine the cause of a disjunct population, analyzing geologic and molecular data can help us evaluate the likelihood of a particular event occurring.

In all phylogenetic analyses, the individual from Yemen is the sister to the Somalia clade, and is different by an uncorrected pair-wise difference of ~6%. Our divergence dating analyses estimate a date of 14.1-1 mya (BEAST) or 2.4 mya (molecular rate of evolution) for the minimum divergence time. Although there is a wide range in dates, these data indicate that the event was relatively old, most likely before the Pleistocene. These data indicate that *Pelomedusa* populations on the Arabian Peninsula are most likely naturally occurring, and not a result of human introduction.

If not anthropogenic, then we can consider natural mechanisms of vicariance or dispersal to explain this biogeographic pattern. Based on the *Pelusios* fossil from our divergence dating analyses, we estimate the age of *Pelomedusa* to be 23.6-13.9 mya. Based on this estimate, the formation of the Red Sea 26-25 mya (Gass 1977) predates the divergence of the family *Pelomedusa*, making a vicariance event highly unlikely.

The remaining possible mechanisms of *Pelomedusa* on the Arabian Peninsula involve a dispersal event, either over land or water. The African-Arabian distribution is not unique to *Pelomedusa*. This geographic pattern is also seen in hamadryas baboons (*Papio*; Winney et al. 2004), snakes (*Naja* [Trape et al. 2009], *Echis* [Pook et al. 2009], *Coluber* [Leviton 1986]), spiny-tailed

lizards (*Uromastyx*; Amer and Kumazawa 2005), and skinks (*Chalcides*; Greenbaum et al. 2006). In each of these taxa, dispersal over land bridges has been hypothesized either via a northerly route through Egypt and the Sinai peninsula (*Uromastyx*, *Echis*), or via a southerly route (*Papio*, *Chalcides*) across a land bridge over the Strait of Bab el Mandeb. The hypothesis that a population could have dispersed around the Sinai Peninsula is less plausible in *Pelomedusa* because its current range does not extend up to the Red Sea's northern coast (Boycott and Bourquin 2008), and there is no fossil evidence to suggest that the historic range of *Pelomedusa* ever spanned that region (Wood 1973). In the southern end of the Red Sea, Africa and Arabia were intermittently connected and disconnected during subsequent movement of the Arabian and African plates. Although the geology of the Red Sea and the timing of land bridges are still controversial (Leviton 1986; Rohling et al. 1998; Siddall et al. 2003; Fernandes et al. 2006), our divergence dates infer that the establishment of *Pelomedusa* on the Arabian Peninsula was before the Pleistocene, making it unnecessary to consider Pleistocene fluctuations in sea level (Rohling et al. 1998; Siddall et al. 2003). However, Fernandes et al. (2006) estimate the presence of a land bridge between 12-6 mya, which cannot be discounted for *Pelomedusa*.

A natural over-water dispersal was not considered for other African-Arabian distributed taxa since the Red Sea is a likely barrier to dispersal for non-aquatic species. For the aquatic *Pelomedusa*, additional possibilities of rafting or swimming across the Red Sea are possible, as some freshwater turtles have been shown to be surprisingly salt tolerant (Dunson and Moll 1980; Dunson and Seidel 1986). Lower sea levels in the past, shortening the over-water distance between Somalia and Yemen, would make these scenarios even more plausible.

Our data suggest that the Arabian population of *Pelomedusa* is not the result of either an anthropogenic or vicariance event, but rather a pre-Pleistocene, naturally occurring dispersal event over a land bridge or water in the southern end of the Red Sea. Further sampling around the Red Sea coupled with refined geologic data may help us resolve the biogeographic history of *Pelomedusa* on the Arabian Peninsula.

3.3 The Southern Clade: The Population in Madagascar

The population in Madagascar is most closely related to the specimens from Malawi and Namibia, its nearest geographic neighbors included in this study. Similar to the analysis for the Eastern clade, both timing and geology of the region need to be considered to evaluate the cause of the disjunct population in Madagascar.

There are two traditional hypotheses to account for naturally occurring populations in Madagascar: continental drift vicariance and over water dispersal. The continental drift vicariance hypothesis requires that the population in Madagascar was already established at least 165 million years ago (Rabinowitz et al. 1983), before the split from the main Gondwana land mass. This seems an impossible scenario because the breakup of Madagascar from mainland Africa predates the origin of the genus *Pelomedusa* by more than 130 million years.

The over water dispersal hypothesis relaxes the time frame by allowing a dispersal to happen any time after 165 mya. Although Raxworthy (2003) puts *Pelomedusa* on a short list of species that "may represent natural yet (in evolutionary time scales) recent immigrant species", this seems unlikely considering the *Pelomedusa's* life history; *Pelomedusa* is a bottom walker rather than a swimmer, and prefers very little current in the water (Raxworthy 2003). The strong currents (e.g. Siddall et al. 2003) and width of the Mozambique Channel make the scenario of a natural, over-water dispersal event less likely.

Divergence dating analyses estimates do not provide much insight on the dating of this event (10.1– 0.0006 mya). However, phylogenetic analyses show that the individual from Madagascar is genetically very similar to the rest of the southern clade (uncorrected pair-wise difference

0.3–1.5%), despite the long geographic distance (800–1600km). The relatively low sequence divergence across such a large distance points towards a recent split between the lineages in southern Africa and Madagascar. Therefore, we believe the Malagasy population is most likely the result of a recent, human-mediated introduction. Additional sampling in southern Africa and Madagascar, focusing on genetic diversity and relationships between geographic areas, may help to resolve this issue.

5. Conclusion

Our preliminary phylogeographic study reveals considerable diversity and genetic structure within *Pelomedusa subrufa*, currently recognized as a single species. Phylogeographic patterns of mainland African *Pelomedusa* mirror that found in another pan-African reptile (Leaché et al. 2009). Based on geologic and molecular data, the disjunct populations of *Pelomedusa* on the Arabian Peninsula and Madagascar seem to have different histories, with the former being an old dispersal event, while the latter seems to be a recent human introduction. The major conclusions of our study are corroborated by Vargas-Ramirez et al. (2010), a concurrent study that was undertaken in an independent laboratory that we were made aware of during the publishing of our study.

Since we were unable to obtain dense sampling across *Pelomedusa's* range, our work should be seen as preliminary. Data presented in this study provides a framework to direct future genetic surveys. There is a need to further explore the *Pelomedusa subrufa* populations across the natural water ways and populations in the hypothesized contact zones between the three subclades. Denser sampling through these key areas could further illuminate the genetic complexity within this pan-African turtle, and ultimately offer key insight into the biogeographical phenomenon across Africa.

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Appendix I

Flanking primers used to amplify and sequence molecular markers in this study.

Gene	Primer Pair	Reference
12S	N/12SA, P/12SB	Palumbi et al. 1991
16S	M89(L), M90(H)	Georges et al. 1999
CytB	Bcytb, L	Palumbi et al. 1991; Shaffer et al. 1997
CO1	M72(L), M73(H)	Georges et al. 1998
R35	R35ex1, R35ex2	Fujita et al. 2004

Appendix II

Internal primers specific to Pelomedusa used to amplify and sequence molecular markers in this study.

Gene	Primer	Sequence (5' -> 3')	Primer Pair
16S	16S.L	TAAGACGAGAAGACCCTGTG	M90(H)
	16S.H	GCTGTTATCCCTGGGGTA	M89(L)
CytB	Cytb.R2	CGGGTTARGGTWGSRTTGTC	Bcytb
	Cytb.F1	CCTWCCATGAGGMCAAATATC	Cytb.R1
	Cytb.R1	GKRTGAARTTYTCWGGGTCTG	Cytb.F1
	Cytb.F2	GGATCHAACAAYCCMACAGGACT	L
CO1	CO1.H	GGCTCATARTATWGGRGCTTC	M72(L)
R35	R35.R3	AGTTAACCTAATGCCTGCC	R35ex1
	R35.F1	TCCAGTTTTACATCAGTGTAACTC	R35.R2
	R35.R2	AGAATGACACTGAACAATTCC	R35.F1
	R35.F2	TGTGTAATGTATGGAAAGGATAA	R35.R1
	R35.R1	GTGACTTTGACAGATGCTAGAA	R35.F2
	R35.F3	GAAACCCAGTCTTGCCTT	R35ex2