

**Supporting Information**

**Disentangling the *Pelomedusa* complex using type specimens and historical DNA  
(Testudines: Pelomedusidae)**

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**ZOOTAXA**

**TABLE S1.** Used samples, GenBank sequences and their accession numbers. Mitochondrial lineages sensu Vargas-Ramírez *et al.* (2010) indicated. Sequences generated by Vargas-Ramírez *et al.* (2010) start with FN; sequences from the data set of Wong *et al.* (2010), with DQ or GU; and sequences generated by Fritz *et al.* (2011), with FR. Sequences starting with HG were produced for the present study. Abbreviations for vouchers: BMNH – The Natural History Museum, London; LSUMZ – Louisiana State University, Museum of Natural Sciences; MCZ – Museum of Comparative Zoology, Cambridge, Mass.; MNHN – Muséum National d’Histoire naturelle, Paris; MTD – Museum für Tierkunde, Senckenberg Dresden; MVZ – Museum of Vertebrate Zoology, Berkeley; PEM – Port Elizabeth Museum; Rax – Collection C. Raxworthy; SMF – Senckenberg Museum, Frankfurt am Main; ZFMK – Zoologisches Forschungsmuseum Alexander Koenig, Bonn; ZSM – Zoologische Staatssammlung München.

Sample/Voucher	Provenance	mtDNA lineage	GenBank accession numbers			Remarks
			12S	cyt <i>b</i>	ND4 + tRNAs	
<i>Pelomedusa subrufa</i> :						
MTD T 5601	Benin: Atakora Department: Pendjari National Park, near Batia; N10°54.756 E1°29.112	III	HG934005	FN645232	FN645291	—
MTD T 5562	Botswana: Central District: Mashatu Game Reserve; S22°15.78 E28°41.04	VIII	—	FN645261	FN645320	—
MTD T 5139	Burkina Faso: Hauts-Bassins: Bobo-Dioulasso; N11°10.68 W4°17.4	III	HG934006	FN645224	FN645283	—
MTD T 5183	Cameroon: Extreme North Province: Maroua; N10°35.76 E14°19.44	I	FR716884	FN645215	FN645274	—
ZFMK 15171	Cameroon: Extreme North Province: Mokolo, Margui-Wandala; N10°44.48423 E13°48.0009	I	—	GU213858	—	—
MTD T 4243	Central African Republic: Ouham Prefecture: Zémio; N5°2.04282 E25°8.00442	IV	FR716887	FN645234	FN645293	—
MTD T 5220	Democratic Republic of Congo: Haut Katanga Province: Kalakundi Mine; S11°12.48 E27°22.92	VIII	HG934007	FN645264	FN645323	—
SMF 7947	Eritrea: eastern slope of coastal mountains; approx. N16°11 E38°51	X (new)	HG934008	—	—	Lectotype <i>Pentonyx gehafie</i>
SMF 7948	Eritrea: eastern slope of coastal mountains; approx. N16°11 E38°51	X (new)	HG934009	—	HG934054	Paralectotype <i>Pentonyx gehafie</i>
SMF 33054	Eritrea: eastern slope of coastal mountains; approx. N16°11 E38°51	X (new)	HG934010	HG934049	HG934055	Paralectotype <i>Pentonyx gehafie</i>
Rax 2055	Ghana: Central Region: Muni Lagoon, Winneba; N5°21.23333 W0°42.2	II	DQ283264	—	—	—
LSUMZ 87102	Ghana: Greater Accra Region: approx. 34 km N of Accra; N5°50.346 W0°06.51	II	GU213789	—	—	—
MVZ 245226	Ghana: Greater Accra Region: approx. 10 mi N of Accra; N5°43.683 W0°10.396	II	GU213790	FN645222	FN645281	—

TABLE S1 CONTINUED

Sample/Voucher	Provenance	mtDNA lineage	Accession numbers			Remarks
			12S	cyt <i>b</i>	ND4 + tRNAs	
MVZ 245428	Ghana: Greater Accra Region: approx. 10 mi N of Accra; N5°43.683 W0°10.396	II	—	FN645221	FN645280	—
MVZ 245228	Ghana: Greater Accra Region: Shai Hills Production Preserve, dam 1 km E of camp; N5°52.476 W0°3.1548	II	HG934011	FN645223	FN645282	—
MVZ 245229	Ghana: Greater Accra Region: Shai Hills Production Preserve, pond 0.5 km W of camp; N5°52.818 W0°2.268	II	FR716885	FN645217	FN645276	—
MVZ 245230	Ghana: Greater Accra Region: Shai Hills Production Preserve, pond 0.5 km W of camp; N5°52.818 W0°2.268	II	HG934012	FN645218	FN645277	—
MVZ 245429	Ghana: Greater Accra Region: Shai Hills Production Preserve, pond 0.5 km W of camp; N5°52.818 W0°2.268	II	HG934013	FN645219	FN645278	—
LSUMZ 86932	Ghana: Northern Region: 2.5 km SW of Buipe; N8°46.126 W1°28.639	II	GU213788	—	—	—
MTD T 5134	Ivory Coast: Vallée du Bandama: Bouaké; N7°41.22 W5°1.92	II	HG934014	FN645216	FN645275	—
MTD T 5526	Kenya: Kakamega: Kakamega; N0°17.04 E34°44.52	V	HG934015	FN645240	FN645299	—
MTD T 5527	Kenya: Kakamega: Kakamega; N0°17.04 E34°44.52	V	FR716888	FN645241	FN645300	—
MTD T 5521	Kenya: Marsabit: South Horr; N2°6.12 E36°47.31	V	HG934016	FN645235	FN645294	—
MTD T 5522	Kenya: Marsabit: South Horr; N2°6.12 E36°47.31	V	HG934017	FN645236	FN645295	—
MTD T 5523	Kenya: Marsabit: South Horr; N2°6.12 E36°47.31	V	HG934018	FN645237	FN645296	—
MTD T 5524	Kenya: Marsabit: South Horr; N2°6.12 E36°47.31	V	HG934019	FN645238	FN645297	—
MTD T 5525	Kenya: Marsabit: South Horr; N2°6.12 E36°47.31	V	HG934020	FN645239	FN645298	—
MTD T 5224	Madagascar: Analaiva (at main road east of Morondava); S20°19.998 E44°29.994	VIII	—	FN645251	FN645310	—
MTD T 5062	Madagascar: between Ambondro and Tsihombe; S25°15.588 E45°38.112	VIII	HG934021	FN645262	FN645321	—
MTD T 5061	Madagascar: between Ambondro and Tsihombe; S25°22.998 E45°42.57	VIII	HG934022	FN645253	FN645312	—
ZSM 177/2004	Madagascar: between Ambondro and Tsihombe; S25°22.998 E45°40.242	VIII	HG934023	FN645252	FN645311	—
MTD T 6018	Madagascar: Ifaty near Toliara; S23°9 E43°37.002	VIII	—	FN645257	FN645316	—
MTD T 6019	Madagascar: Ifaty near Toliara; S23°9 E43°37.002	VIII	—	FN645258	FN645317	—
MTD T 6020	Madagascar: Ifaty near Toliara; S23°9 E43°37.002	VIII	—	FN645259	FN645318	—
MTD T 5227	Madagascar: just north of Andranomena; S20°10.23 E44°26.22	VIII	FR716891	FN645254	FN645313	—

TABLE S1 CONTINUED

Sample/Voucher	Provenance	mtDNA lineage	Accession numbers			Remarks
			12S	cyt <i>b</i>	ND4 + tRNAs	
MTD T 5228	Madagascar: just south of Andranomena; S20°11.49 E44°25.44	VIII	—	FN645255	FN645314	—
MTD T 5225	Madagascar: Kirindy creek near Alan Amborompotsy; S20°2.85 E44°38.91	VIII	HG934024	FN645256	FN645315	—
MTD T 5226	Madagascar: Kirindy creek near Alan Amborompotsy; S20°2.85 E44°38.91	VIII	HG934025	FN645260	FN645319	—
MTD T 5221	Malawi: Mt Mulanje; S15°55.38 E35°42.9	VIII	—	FN645263	FN645322	—
MCZ R-184287	Namibia: Kunene: Kamanjab; S19°37.46667 E14°50.3	VIII	GU213786	GU213845	—	—
PEM R14953	Namibia: Otjozondjupa: Quickborn; S21°11.29218 E17°8.31503	VIII	HG934026	—	—	Lectotype <i>Pelomedusa galeata damarensis</i>
PEM R14954	Namibia: Otjozondjupa: Quickborn; S21°11.29218 E17°8.31503	VIII	HG934027	HG934050	—	Paralectotype <i>Pelomedusa galeata damarensis</i>
MVZ 238883	Niger: Agadez Region: Tchirozérine Department: Tafadek, 40 km N Agadez; N17°23.208 E7°57.378	III	FR716886	FN645231	FN645290	—
MVZ 238885	Niger: Agadez Region: Tchirozérine Department: Tafadek, 40 km N Agadez; N17°23.208 E7°57.378	III	HG934028	FN645230	FN645289	—
MVZ 238878	Niger: Dosso Region: Dogondoutchi Department: 57 km SSW (by Niamey Rd.) Dogondoutchi; N13°14.232 E3°48.96	III	GU213792	FN645233	FN645292	—
MVZ 238887	Niger: Tillabéri Region: Kollo Department: Guéssélbodi, 30 km SE Niamey on road to Dosso; N13°24.792 E2°21.21	III	GU213795	FN645225	FN645284	—
MVZ 238879	Niger: Tillabéri Region: Say Department: Moli Haoussa, 15 km NW (by Tamou Rd.) Tapoa; N12°31.92 E2°20.028	III	HG934029	FN645228	FN645287	—
MVZ 238880	Niger: Tillabéri Region: Say Department: Moli Haoussa, 15 km NW (by Tamou Rd.) Tapoa; N12°31.92 E2°20.028	III	HG934030	FN645226	FN645285	—
MVZ 238881	Niger: Tillabéri Region: Say Department: Moli Haoussa, 15 km NW (by Tamou Rd.) Tapoa; N12°31.92 E2°20.028	III	HG934031	FN645227	FN645286	—
MVZ 238882	Niger: Tillabéri Region: Say Department: Moli Haoussa, 15 km NW (by Tamou Rd.) Tapoa; N12°31.92 E2°20.028	III	HG934032	FN645229	FN645288	—
ZFMK 19836	Nigeria: between Bahuda and Sokoto; N13°4.44768 E5°21.2952	III	HG934033	GU213860	—	—
MTD T 592	Saudi Arabia: close to Yemeni border; N16°55.776 E42°54.276	VII	HG934034	FN645246	FN645305	—

**TABLE S1 CONTINUED**

Sample/Voucher	Provenance	mtDNA lineage	Accession numbers			Remarks
			12S	cyt <i>b</i>	ND4 + tRNAs	
MTD T 593	Saudi Arabia: close to Yemeni border; N16°55.776 E42°54.276	VII	HG934035	FN645247	FN645306	—
MTD T 594	Saudi Arabia: close to Yemeni border; N16°55.776 E42°54.276	VII	HG934036	FN645248	FN645307	—
MTD T 595	Saudi Arabia: Wadi Turaba near Tai'if; N20°42.24 E41°22.86 (questionable locality)	VII	HG934037	FN645249	FN645308	—
ZFMK 17076	Senegal: Dakar; N14°42.03048 W17°26.84257	III	HG934038	GU213859	—	Neotype <i>Pelomedusa gasconi</i>
MVZ 241329	Somalia: Awdal Region: 4 km N (by road) Borama; N9°58.23 E43°8.76	VI	GU213797	FN645242	FN645301	—
MVZ 241330	Somalia: Awdal Region: 4 km N (by road) Borama; N9°58.23 E43°8.76	VI	GU213798	FN645243	FN645302	—
MVZ 241331	Somalia: Awdal Region: 4 km N (by road) Borama; N9°58.23 E43°8.76	VI	GU213799	FN645244	FN645303	—
MVZ 241332	Somalia: Awdal Region: Rugi, 30 km NE (by road) Borama; N9°58.188 E43°25.95	VI	FR716889	FN645245	FN645304	—
MNHN 9506	South Africa: “Le Cap”	IX	HG934039	HG934051	HG934056	Lectotype <i>Pentonyx capensis</i>
MTD T 5218	South Africa: Eastern Cape: Port Elizabeth; S33°51.06 E25°34.86	IX	—	FN645265	FN645324	—
MTD T 5219	South Africa: Eastern Cape: Port Elizabeth; S33°51.06 E25°34.86	IX	FR716892	FN645266	FN645325	—
MTD T 5505	South Africa: Eastern Cape: Sterkstroom; S31°34.398 E26°30.036	IX	—	FN645268	FN645327	—
MTD T 5563	South Africa: Gauteng Province; S25°54.84 E28°7.86	IX	HG934040	FN645272	FN645331	—
BMNH 1849.1.30.27	South Africa: KwaZulu-Natal: “Natal”	IX	HG934041	—	—	Lectotype <i>Pelomedusa nigra</i>
BMNH 1862.12.4.4	South Africa: KwaZulu-Natal: “Natal”	IX	HG934042	—	—	Paralectotype <i>Pelomedusa nigra</i>
BMNH 1862.12.4.5	South Africa: KwaZulu-Natal: “Natal”	IX	HG934043	—	—	Paralectotype <i>Pelomedusa nigra</i>
MTD T 5508	South Africa: KwaZulu-Natal: Ndumo; S26°56.0772 E32°16.90512	IX	—	FN645269	FN645328	—
MTD T 5509	South Africa: KwaZulu-Natal: Ndumo; S26°56.0772 E32°16.90512	IX	HG934044	FN645270	FN645329	—
PEM R14962	South Africa: Northern Cape: Besondermeid, Steinkopf (Namaqualand); S29°14.5962 E17°44.1495	IX	HG934045	HG934052	HG934057	Holotype <i>Pelomedusa galeata devilliersi</i>
MTD T 5510	South Africa: Northern Cape: Bothithong; S27°1.002 E23°45.084	IX	HG934046	FN645271	FN645330	—

**TABLE S1 CONTINUED**

Sample/Voucher	Provenance	mtDNA lineage	Accession numbers			Remarks
			12S	cyt <i>b</i>	ND4 + tRNAs	
MTD T 5897	South Africa: Western Cape: Chelance; S33°38.1852 E19°20.7072	IX	HG934047	HG934053	HG934058	—
MTD T 5484	South Africa: Western Cape: Swellendam District; S33°59.994 E20°22.08	IX	—	FN645267	FN645326	—
MVZ 236628	Yemen: Gwol Madram, Wadi Tuban; N13°19.908 E44°41.652	VII	FR716890	FN645250	FN645309	—
MVZ 230517	Unknown	II	HG934048	FN645220	FN645279	—
<b>Outgroup (<i>Pelusios sinuatus</i>):</b>						
MTD T 5216	South Africa: KwaZulu-Natal: Phinda; S27°51.21882 E32°18.43458	—	FR716876	—	—	—
MTD T 5506	South Africa: Limpopo: Phalaborwa; S24°2.16 E31°11.586	—	—	FN645273	FN645332	—

**TABLE S2.** Primer sequences for mtDNA fragments for fresh samples. Nucleotides in brackets represent variable positions.

Fragment	Primer	Primer sequence (5'–3')	Source
12S rRNA	L1091	AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT	Kocher <i>et al.</i> (1989)
	H1478	TGA CTG CAG AGG GTG ACG GGC GGT GTG T	
cyt <i>b</i>	mt-c-For2	TGA GG(AGC) CA(AG) ATA TCA TT(CT) TGA G	Fritz <i>et al.</i> (2006)
	mt-f-na	AGG GTG GAG TCT TCA GTT TTT GGT TTA CAA GAC CAA TG	
ND4+tRNAs	L-ND4	GTA GAA GCC CCA ATC GCA G	Stuart & Parham (2004)
	H-Leu	ATT ACT TTT ACT TGG ATT TGC ACC A	

**TABLE S3.** Primer sequences and lengths of amplified PCR products of mtDNA fragments of the 12S, *cyt b* and ND4 genes for historical samples. Nucleotides in brackets represent variable positions. Note that primer PeloND4\_268\_rev contains the nucleoside inosine which is capable of base-pairing with any natural nucleotide. Primer position numbers refer to consensus sequences used for primer design obtained from examples of the nine genetic lineages of Vargas-Ramírez *et al.* (2010). First generation primers with asterisks; second generation primers without asterisks.

Fragment	Primer	Primer sequence (5'–3')	Length of obtained DNA fragment [bp] <sup>1</sup>
12S fragment 1	Pelo12S_136_for*	AGG AGC CTG TTC (AT)AT AAG CG	109–112
	Pelo12S_283_rev*	GCT ACA CCT (CG)GA CCT GAC	
12S fragment 2	Pelo12S_266_for*	GTC AGG TC(CG) AGG TGT AGC	123–124
	Pelo12S_427_rev*	GTG TGT GCT TGT C(AG)C AG(CT) GC	
12S fragment 3	Pelo12S_160_for	CAG C(CT)T ATA TAC CGC CGT C	92
	Pelo12S_295_rev	TCT AT(AG) TTA GAA AAT GTA GCC C	
<i>cyt b</i> fragment 1	PeloCytB_126_for*	TTA (CT)T(CT) CT(CT) CA(CT) GAA A(CA)A GG	100
	PeloCytB_265_rev*	A(CT)G GTT A(CT)G GTT AAT AGT CC	
<i>cyt b</i> fragment 2	PeloCytB_207_for*	TCA TAC AAA GA(CT) CT(CT) (CT)TA GG	115
	PeloCytB_361_rev*	AAG AGG AA(CT) TAT CA(AG) TC(CTA) GG	
<i>cyt b</i> fragment 3	PeloCytB_203_for	CTT CT(CT) (AGT)TA CAA AGA CCT (AC)TT AGG	89
	PeloCytB_338_rev	GAT GTG (AC)GG (CT)GG TGT AGA TAA TG	
<i>cyt b</i> fragment 4	PeloCytB_291_for*	CC(AT) GA(AG) AA(CT) TTC A(CT)C CCA GC	88
	PeloCytB_418_rev*	A(CT)(CTA) A(CT)G GCT A(CT)(AG) ACT CC(CT) CC	
<i>cyt b</i> fragment 5	PeloCytB_293_for	AGA (AG)AA CTT CAC CCC AGC TAA C	63
	PeloCytB_400_rev	CCT AAT TTA TTG GGG ATG GAG CG	
<i>cyt b</i> fragment 6	PeloCytB_351_for*	TA(CT) TTC CTC TT(CT) GC(CT) TAC GC	94
	PeloCytB_484_rev*	AA(CT) G(ATG)A G(ATG)T GA(AG) CGT TGT TT	

**TABLE S3 CONTINUED**

Fragment	Primer	Primer sequence (5'–3')	Length of obtained DNA fragment [bp] <sup>1</sup>
ND4 fragment 1	PeloND4_129_for*	CG(CA) CAA ACA GAC CTA AAA TC	100
	PeloND4_268_rev*	GAT GA(AG) GAT AAI CC(CT) TG(CT) GC	
ND4 fragment 2	PeloND4_128_for	ACG (AC)CA AAC AGA CCT AAA ATC AC	75
	PeloND4_248_rev	AAT TAT TAG GAT AGT (CT)GC (AG)CC TG	
ND4 fragment 3	PeloND4_195_for*	GC(AG) (AG)C(CTA) CT(CT) CT(AG ) CAA ACC CC	81
	PeloND4_315_rev*	G(CT)T T(CT)T GGG T(AG)C GTT C(CT)T AG	
ND4 fragment 4	PeloND4_195b_for	GCA GCC CTT CTA CAA ACC CC	81
	PeloND4_319_rev	GTT CG(CT) TT(CT) TGG GT(CT) CGT TC(CT) TAG	
ND4 fragment 5	PeloND4_264_for	TCA TCT ATA CTC TTC TGC (CT)TA GC	94
	PeloND4_403_rev	AAA GCT AT(CT) TTT ATT AAG CTG GC	
ND4 fragment 6	PeloND4_300_for*	GAA CG(AG) ACC CA(CT) AA(CT) CGA AC	109
	PeloND4_448_rev*	GAT GC(CT) AT(CT) ATT GTT AGT TC	
ND4 fragment 7	PeloND4_381_for*	GC(CT) A(AG)C (CT)TA A(CT)A AA(CT) ATA GC	118
	PeloND4_539_rev*	TGT (CT)GT TGA GAA TAT (CT)TG GAG	
ND4 fragment 8	PeloND4_444_for*	GCA TC(CA) (CT)TA TT(CT) A(AG)C TGA GC	122
	PeloND4_606_rev*	TTA A(CT)T GTT CTC GTG T(CT)T GGG	

<sup>1</sup> Length after primer sequences were trimmed



**TABLE S4.** PCR conditions for historical and fresh samples.

mtDNA fragment		ID	C	Thermocycling conditions			
				D	A	E	FE
12S rRNA	Historical samples	94°C, 5 min	40	94°C, 30 s	51°C, 30 s	72°C, 30 s	72°C, 10 min
cyt <i>b</i>	Historical samples	94°C, 5 min	40	94°C, 30 s	52°C, 30 s	72°C, 30 s	72°C, 10 min
ND4	Historical samples	94°C, 5 min	40	94°C, 30 s	52°C, 30 s	72°C, 30 s	72°C, 10 min
12S rRNA	Fresh samples	94°C, 5 min	40	94°C, 30 s	51°C, 30 s	72°C, 45 s	72°C, 10 min
cyt <i>b</i>	Fresh samples	94°C, 5 min	40	94°C, 30 s	50°C, 45 s	72°C, 90 s	72°C, 10 min
ND4	Fresh samples	94°C, 5 min	40	94°C, 45 s	50°C, 30 s	72°C, 60 s	72°C, 10 min

Abbreviations: ID = initial denaturing, C = number of cycles, D = denaturing, A = annealing, E = extension, FE = final extension.

**TABLE S5.** Degree of overlap for individual mtDNA fragments yielding 251–252 bp of the 12S rRNA gene. In brackets are the individual fragment lengths given.

	Fragment 1	Fragment 2	Fragment 3
Fragment 1 (109–112 bp)	—		
Fragment 2 (123–124 bp)	0 bp	—	
Fragment 3 (92 bp)	57 bp	18 bp	—

**TABLE S6.** Degree of overlap for individual mtDNA fragments yielding 319 bp of the *cyt b* gene. For further explanation, see Table S5.

	<b>Fragment 1</b>	<b>Fragment 2</b>	<b>Fragment 3</b>	<b>Fragment 4</b>	<b>Fragment 5</b>	<b>Fragment 6</b>
Fragment 1 (100 bp)	—					
Fragment 2 (115 bp)	19 bp	—				
Fragment 3 (89 bp)	19 bp	89 bp	—			
Fragment 4 (88 bp)	0 bp	31 bp	5 bp	—		
Fragment 5 (63 bp)	0 bp	27 bp	1 bp	63 bp	—	
Fragment 6 (94 bp)	0 bp	0 bp	0 bp	28 bp	7 bp	—

**TABLE S7.** Degree of overlap for individual mtDNA fragments yielding 437 bp of the ND4 gene. For further explanation, see Table S5.

	<b>Fragment 1</b>	<b>Fragment 2</b>	<b>Fragment 3</b>	<b>Fragment 4</b>	<b>Fragment 5</b>	<b>Fragment 6</b>	<b>Fragment 7</b>	<b>Fragment 8</b>
Fragment 1 (100 bp)	—							
Fragment 2 (75 bp)	75 bp	—						
Fragment 3 (81 bp)	34 bp	11 bp	—					
Fragment 4 (81 bp)	34 bp	11 bp	81 bp	—				
Fragment 5 (94 bp)	0 bp	0 bp	9 bp	9 bp	—			
Fragment 6 (109 bp)	0 bp	0 bp	0 bp	0 bp	61 bp	—		
Fragment 7 (118 bp)	0 bp	0 bp	0 bp	0 bp	0 bp	28 bp	—	
Fragment 8 (122 bp)	0 bp	0 bp	0 bp	0 bp	0 bp	0 bp	55 bp	—

**TABLE S8.** Partitioning scheme and models selected by the Akaike Information Criterion in MrMODELTEST 2.3 (Nylander 2004).

Partition	Aligned mtDNA length	Model
12S	358 bp	GTR + I + G
cyt <i>b</i>	674 bp	GTR + I + G
ND4	672 bp	GTR + I + G
tRNAs	144 bp	HKY + G

## References

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