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Important new records of *Pelomedusa* species for South Africa and Ethiopia

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Abstract

Because of a recent taxonomic revision, the species identity of helmeted terrapins (*Pelomedusa*) became unclear in many regions of their wide distribution range. Based on mtDNA sequence data, here we present the first record of *Pelomedusa subrufa* sensu stricto for the South African province of Mpumalanga. In South Africa, this species was previously known only from a single record in the province of Limpopo. In addition, we provide evidence for the occurrence of at least two distinct *Pelomedusa* species in Ethiopia. A sample from southern Ethiopia (Omo Region) turned out as *P. neumanni*, while another sample from Koka Lake (Oromia Region, central Ethiopia) represents *P. somalica*. Also a historical museum specimen from Ethiopia, most likely collected south of the Shebelle River (Oromia Region), belongs to *P. somalica*. However, these two Ethiopian specimens of *P. somalica* represent highly distinct genetic lineages, which may actually correspond to two different species.

Key words

Pelomedusa neumanni; *Pelomedusa somalica*; *Pelomedusa subrufa*; Pelomedusidae; Reptilia; Testudines.

Introduction

Several papers provided evidence that the widely distributed sub-Saharan helmeted terrapins of the genus *Pelomedusa* represent a species complex (Vargas-Ramírez *et al.* 2010; Wong *et al.* 2010; Fritz *et al.* 2011, 2014; Petzold *et al.* 2014; Nagy *et al.* 2015) and not a single species as thought before (e.g. Wermuth & Mertens 1977; Fritz & Havaš 2007; van Dijk *et al.* 2014). The recent revision by Petzold *et al.* (2014) formally recognized ten distinct *Pelomedusa* species. However, five other genetically distinct lineages have been identified, which may correspond to additional species (Petzold *et al.* 2014; Nagy *et al.* 2015). While some of these species and genetic lineages are morphologically distinctive, others are difficult to tell apart using external morphology alone (Fritz *et al.* 2014, 2015; Petzold *et al.* 2014). Thus, in many cases, helmeted terrapins need to be studied genetically for reliable species determination. This leads to the uncomfortable situation that many of the old records across Africa (cf. Iverson 1992) cannot be assigned to a certain *Pelomedusa* species.

In Ethiopia, the species identity of all *Pelomedusa* records has become unclear. Helmeted terrapins are widely distributed in this country, with records along the southern border, in the Great Rift Valley, in the Blue

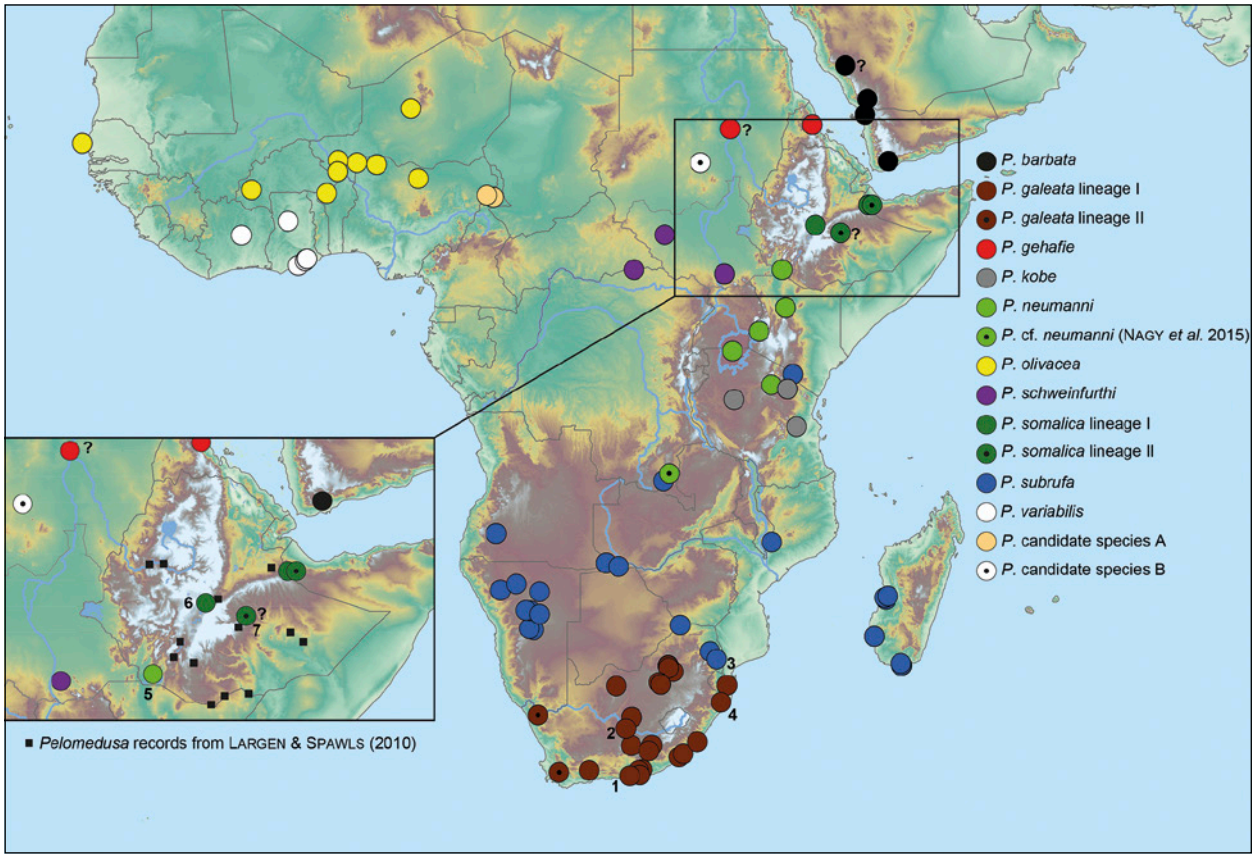

Fig. 1. Genetically verified records of species, candidate species and genetic lineages of helmeted terrapins (*Pelomedusa*). *Pelomedusa* cf. *neumanni* and the distinct genetic lineages of *P. galeata* and *P. somalica* represent additional candidate species. The populations on Madagascar are thought to be introduced. Modified from PETZOLD *et al.* (2014) and NAGY *et al.* (2015). For *P. galeata*, the locality Swellendam (Western Cape, South Africa) from PETZOLD *et al.* (2014) is not shown because the sequence data of this terrapin were chimerical and composed of both lineages of *P. galeata* (for details, see PETZOLD *et al.* 2014). New records: (1) north of Humansdorp, Eastern Cape, South Africa; (2) Rooipoort Guest Farm, Northern Cape, South Africa; (3) Sabie Park, close to Paul Kruger Gate, Mpumalanga, South Africa; (4) Bekaphanzi Pan, Hluhluwe-iMfolozi Game Reserve, KwaZulu-Natal, South Africa; (5) near Buska Lodge, 3 km E Turmi, Omo Region, Ethiopia; (6) Koka Lake, Oromia Region, Ethiopia. The possible record south of the Shebelle River, Oromia Region, Ethiopia (7) bears a question mark, like the questionable records from PETZOLD *et al*. (2014). Inset shows enlarged map of Ethiopia, including records (squares) from LARGEN & SPAWLS (2010).

Nile drainage system, and in the east of Ethiopia in the Ogaden Region (LARGEN & SPAWLS 2010). However, two genetically verified museum specimens collected in 'Abyssinia' raised the possibility that two distinct species of *Pelomedusa*, *P. gehafie* and *P. somalica*, might occur in Ethiopia (PETZOLD *et al.* 2014). These museum specimens from the late 19th and early 20th centuries lacked exact locality data, and when the specimens were collected, Abyssinia was often identified with present-day Ethiopia and Eritrea. Hence, the two terrapins could also have originated from the latter country, where *P. gehafie* occurs (FRITZ *et al.* 2014; PETZOLD *et al.* 2014). Besides Eritrea, there exists only one somewhat questionable record for *P. gehafie*, from the region of Khartoum, Sudan (PETZOLD *et al.* 2014). Genetically verified records of *P. somalica* are only known from the Awdal Region, Somalia. There, two highly distinct lineages have been recorded approximately 40 km apart (Fig. 1), suggestive of two parapatric or even sympatric species (VARGAS-RAMÍREZ at al. 2010; PETZOLD *et al.* 2014).

For South Africa, the situation is different. There are many genetically verified records, providing evidence for the occurrence of at least two distinct *Pelomedusa* species, *P. galeata* and *P. subrufa* sensu stricto. Like *P. somalica*, *P. galeata* is comprised of two deeply divergent genetic lineages, which may actually correspond to two distinct species (PETZOLD *et al.* 2014). One of these lineages is widely distributed over most of South Africa, while there are only two sites known for the other one, both in the westernmost range of *P. galeata* (Northern Cape and Western Cape provinces; Fig. 1). *Pelomedusa subrufa* sensu stricto, widely distributed from southern Angola and Namibia to East Africa and introduced in Madagascar, has been recorded until now only from the South African province of Limpopo (Hoedspruit, western border region of Kruger Park; PETZOLD *et al.* 2014).

In the present paper, we report the second record of *P. subrufa* sensu stricto for South Africa and the first unambiguous, genetically verified identifications of helmeted terrapins for Ethiopia.

Table 1. Helmeted terrapins examined for the present study. Abbreviations: HNHM – Hungarian Natural History Museum, Budapest; MTD – Museum of Zoology, Senckenberg Dresden (Tissue Collection).

Sample/Voucher	Collection site	Coordinates	Date
MTD 13906	North of Humansdorp, Eastern Cape, South Africa	S33.95960 E24.80102	7 October 2010
MTD 13904	Rooipoort Guest Farm, Northern Cape, South Africa	S30.32068 E24.49653	30 September 2010
MTD 13593	Bekaphanzi Pan, Hluhluwe-iMfolozi Game Reserve, KwaZulu-Natal, South Africa	S28.27952 E31.81980	30 November 2014
MTD 13589	Sabie Park, close to Paul Kruger Gate, Mpumalanga, South Africa	S24.97766 E31.47960	22 November 2014
MTD 13014	Near Buska Lodge, 3 km E Turmi, Omo Region, Ethiopia	N4.97444 E36.51583	15 July 2014
HNHM 2004.84.1	Koka Lake, Oromia Region, Ethiopia	N8.39053 E39.07989	Early 1960s

Materials and Methods

We sampled four helmeted terrapins from South Africa (one terrapin each from Eastern Cape, Northern Cape, KwaZulu-Natal, and Mpumalanga) and one from southern Ethiopia (Omo Region; see Fig. 1 and Table 1 for exact locality data). Small pieces of skin or tissue were clipped off and preserved in pure ethanol. Using these samples and the laboratory procedures described for fresh material in Fritz *et al.* (2014), we sequenced 343-344 bp of the mitochondrial 12S rRNA gene (12S), 671-674 bp of the cytochrome *b* gene (cyt *b*), and 810-815 bp of the ND4 gene plus adjacent DNA coding for tRNAs. In addition, using the laboratory procedures for museum material of Fritz *et al.* (2014), we generated homologous sequence data for a dry specimen from the collection of the Hungarian Natural History Museum, Budapest (HNHM 2004.84.1). This terrapin was collected in the early 1960s in central Ethiopia (Oromia Region; Fig. 1, Table 1), and is figured and described in detail in Dely (1971). For this specimen, we obtained 252 bp of the 12S gene, 319 bp of the cyt *b* gene, and 435 bp of the ND4 gene. The DNA sequences of each individual were concatenated and merged with the alignment used by Petzold *et al.* (2014) and Nagy *et al.* (2015). Then, Maximum Likelihood analyses were calculated as described in Petzold *et al.* (2014) and the phylogenetic placement of the studied terrapins was used for species identification. GenBank accession numbers of new DNA sequences are LN824005 – LN824022.

Results and Discussion

The concatenated sequences of the helmeted terrapins from KwaZulu-Natal, Eastern and Northern Cape, South Africa, clustered together with the more widely distributed clade of *Pelomedusa galeata*, while the terrapin from Mpumalanga, South Africa, corresponded to *P. subrufa* sensu stricto (Fig. 2). Also the small size and colour pattern of the Mpumalanga terrapin argue for this species (Fig. 3). Moreover, its triangular pectoral scutes do not meet at the midline, a character sometimes found in *P. subrufa* sensu stricto, but never in *P. galeata* (Petzold *et al.* 2014). This represents the second record of *P. subrufa* sensu stricto for South Africa and the first record for the province of Mpumalanga. The collection site is approximately 90 km away from the previous record in Limpopo province (Petzold *et al.* 2014). Both records are in the western border region of the Kruger Park, suggesting a wider distribution of *P. subrufa* sensu stricto in the Kruger Park area.

The museum specimen from central Ethiopia (HNHM 2004.84.1; Fig. 4) was assigned to one of the two distinct lineages of *P. somalica* (Fig. 2). Consequently, it represents the first unambiguous record for *P. somalica* in Ethiopia and outside Somalia. The localities of *P. somalica* in Somalia (Petzold *et al.* 2014) are approximately 480 km away from the site in central Ethiopia. In contrast to this specimen, the helmeted terrapin from southern Ethiopia was revealed as *P. neumanni* (Figs 2 and 5), a species previously only known from Kenya and Tanzania (Petzold *et al.* 2014). The nearest genetically verified locality of *P. neumanni* lies in Kenya (South Horr, Marsabit; Petzold *et al.* 2014), approximately 320 km distant from the record in southern Ethiopia. It seems possible that the ranges of *P. neumanni* and *P. somalica* abut or overlap in the region of the Great Rift Valley lakes of Ethiopia, even though our records of the two species are approximately 470 km apart.

We cannot exclude the possibility that more species of *Pelomedusa* occur in Ethiopia. As outlined above, Petzold *et al.* (2014) reported two genetically verified historical museum specimens from 'Abyssinia', one representing *P. somalica* (voucher in the Naturhistorisches Museum Wien, NMW 24449, coll. Carlo von Erlanger 1899-1901) and the other *P. gehafie* (voucher in the Museum für Naturkunde Berlin, ZMB 15693, coll. William Jesse 1868). Abyssinia was identified by many 19th and early 20th century authors with what is now Ethiopia and Eritrea (e.g. Rüppell 1835; Finsch 1869). When the itinerary of Carlo von Erlanger is considered (Pagenstecher 1902; von Erlanger 1904; Sprigade 1904), it is likely that NMW 24449 (*P. somalica*) was collected in present-day Ethiopia, whereas the itinerary of William Jesse (Finsch 1869: plate 23) suggests for ZMB 15693 (*P. gehafie*) an origin in Eritrea. Nevertheless, *P. gehafie* could occur in Ethiopia because helmeted terrapins are known from the region of the Blue Nile (Largen & Spawls 2010; Fig. 1). This river con-

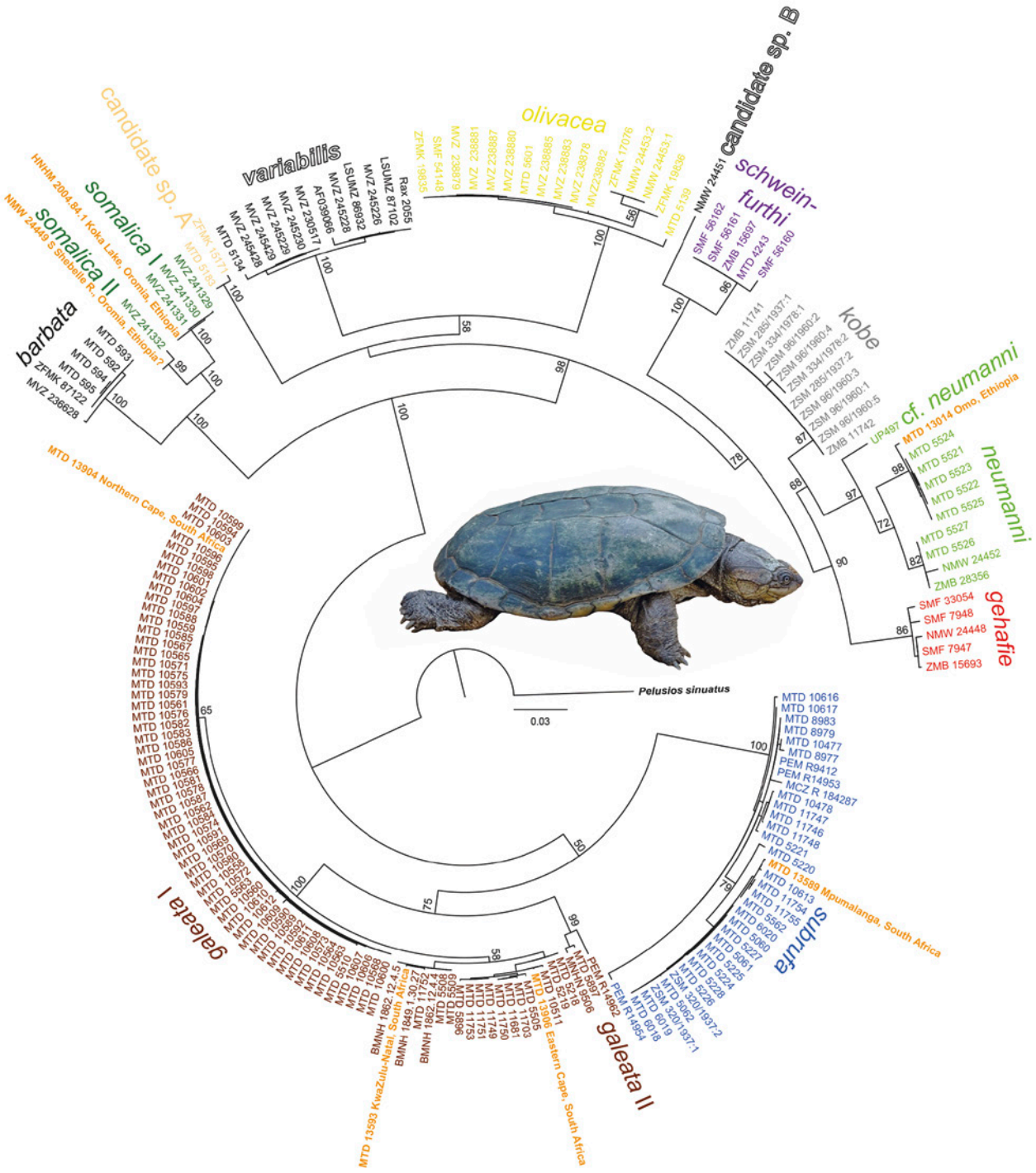

Fig. 2. Maximum Likelihood tree for 189 *Pelomedusa* terrapins, rooted with *Pelusios sinuatus*. The tree is based on the 1848-bp-long alignment of mtDNA used by PETZOLD *et al.* (2014) and NAGY *et al.* (2015) plus sequences from the six terrapins studied for the present paper. Chimerical sequence data from a helmeted terrapin from Swellendam, Western Cape, South Africa, were excluded (see PETZOLD *et al.* 2014). Numbers along branches indicate bootstrap values greater than 50 (not shown for terminal clades with short branch lengths). Terrapins discussed in the present paper are highlighted in orange. For explanation of other sample codes, see PETZOLD *et al.* (2014) and NAGY *et al.* (2015). Inset: *Pelomedusa neumanni*, Omo Region, Ethiopia.

nects Ethiopia with the region of Khartoum, Sudan, from where a questionable record of *P. gehafie* exists (PETZOLD *et al.* 2014).

It is most likely that NMW 24449 is one of the specimens collected by Carlo von Erlanger and Oscar Neumann on 20 June 1900 south of the Shebelle River (= Wabbi or Webbi River, Oromia Region, Ethiopia, approx. N7.80000 E41.00000; NEUMANN 1905; TORNIER 1905). NMW 24449 has on the right side of the carapace an additional fifth costal scute (Fig. 6), exactly as described by TORNIER (1905) as an abnormality for one of the seven specimens from von Erlanger's and Neumann's

Fig. 3. *Pelomedusa subrufa* sensu stricto (adult male, Sabie Park, close to Paul Kruger Gate, Mpumalanga, South Africa; S24.97766 E31.47960), straight carapacial length 11.0 cm. Dorsal and ventral aspect. The black triangles on the submarginals are characteristic for hatchlings of *P. subrufa* (Fritz *et al.* 2015) but often lost during growth. Photos: Uwe Fritz.

Fig. 4. *Pelomedusa somalica* (HNHM 2004.84.1, adult male, Koka Lake, Oromia Region, Ethiopia; N8.39053 E39.07989), straight carapacial length 23.4 cm. Dorsal and ventral aspect. Photos: Judit Vörös.

Fig. 5. *Pelomedusa neumanni* (adult male, near Buska Lodge, 3 km E Turmi, Omo Region, Ethiopia; N4.97444 E36.51583), straight carapacial length approximately 20 cm. Lateral and ventral aspect. Photos: Tomáš Mazuch.

Fig. 6. *Pelomedusa somalica* (NMW 24449, juvenile, most likely collected by Carlo von Erlanger and Oscar Neumann on 20 June 1900 at a site named Oda, south of the Shebelle River, Oromia Region, Ethiopia; approx. N7.80000 E41.00000), straight carapacial length 7.6 cm. Dorsal and ventral aspect. This terrapin shows an abnormal carapacial scutation with five costal scutes on the right side, as described by TORNIER (1905). Photos: Melita Vamberger.

expedition. NMW 24449 represents the same genetic lineage of *P. somalica* as previously identified from a single fresh Somali sample (Rugi, Awdal Region, N9.96980 E43.43250; PETZOLD *et al.* 2014), whereas the specimen from Koka Lake (HNHM 2004.84.1) belongs to the other highly distinct lineage of *P. somalica*. Until now, the latter lineage was only known from three terrapins from the vicinity of Borama (Awdal Region, Somalia, N9.97050 E43.14600; PETZOLD *et al.* 2014). If both lineages should turn out as distinct species, Ethiopia would harbour at least three species of *Pelomedusa*.

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