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Molecular phylogeny of African hinged and helmeted terrapins (Testudines: Pelomedusidae: *Pelusios* and *Pelomedusa*)

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With 18 currently recognised species, *Pelusios* is one of the most speciose chelonian genera worldwide, even though the taxonomy of some species is contentious. Recent investigations suggested that the closely related, but morphologically distinct genus *Pelomedusa* is paraphyletic with respect to *Pelusios*, and that *Pelomedusa* consists of nine deeply divergent lineages. Using three mitochondrial and three nuclear DNA fragments (2054 bp mtDNA, 2025 bp nDNA), we examined for the first time the phylogeny of *Pelusios* by molecular means. Our analyses included all *Pelusios* species, except the probably extinct *P. seychellensis*, as well as the nine *Pelomedusa* lineages. The results showed that *Pelusios* and *Pelomedusa* are reciprocally monophyletic. Limited sampling of *Pelusios* species and homoplasy introduced by remote outgroups most likely explain the paraphyly of *Pelomedusa* in previous studies. The distinctiveness of most *Pelusios* species was confirmed, but none of the currently recognised species groups within *Pelusios* was monophyletic. In *Pelusios rhodesianus* and *P. sinuatus* distinct genetic lineages were discovered, suggestive of cryptic taxa. In contrast, the recognition of the weakly differentiated *P. castaneus* and *P. chapini* as full species is doubtful, as is the validity of the Malagasy and Seychellois subspecies of *P. castanoides*. GenBank sequences of *P. williamsi* were nested within *P. castaneus*, but the morphological distinctiveness of the two species makes it likely that the GenBank sequences (derived from a turtle from the pet trade) are misidentified. Divergence among the distinct genetic lineages of *Pelomedusa* equals or exceeds the differences among *Pelusios* species, supporting the view that *Pelomedusa* is a species complex.

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Introduction

With 18 extant species, African hinged terrapins (*Pelusios* Wagler, 1830) represent one of the world's most speciose chelonian genera. With the exception of American mud turtles (*Kinosternon* Spix, 1824; 18 species), all other chelonian genera comprise distinctly fewer species. *Pelusios* species occur throughout sub-Saharan Africa, on Madagascar, the Seychelles, and São Tomé, with an apparently introduced population of *P. castaneus* on Guadeloupe, Lesser Antilles (Fritz & Havaš 2007; compare also the distribution maps for most species in Iverson 1992). All species are highly aquatic terrapins that live in many different freshwater habitats, from closed rainforest to open savannah. Certain species also enter brackish waters, whilst others burrow into loose soil or mud to aestivate during the dry season. Most species are medium-sized with shell lengths of 20–30 cm; however, the smallest (*P. nanus*) reaches only 12 cm and the largest (*P. sinuatus*) may exceed 46 cm. The characteristic morphological peculiarity from which *Pelusios* species derive their common name is the movable plastral fore lobe. A hinge between hypoplastral and mesoplastral bones allows a more or less complete closure of the anterior part of the shell (Bramble & Hutchison 1981; Ernst *et al.* 2000; Branch 2008). This hinge is well-developed in nearly all species. However, one species (*P. broadleyi*) has a quite rigid plastron (Bour 1986).

Pelusios, together with the African genus *Pelomedusa*, constitutes the family Pelomedusidae that is sister to the species-poor South American-Malagasy river turtles (Podocnemididae). The two families are the last survivors of a highly diverse group of turtles, the Pelomedusoides, comprising many extinct species. During the Cretaceous and Paleogene, these turtles occurred in freshwater and littoral habitats of all landmasses except Central Asia, Antarctica and Australia. Pelomedusidae, however, are a purely African radiation (Gaffney *et al.* 2006). Pelomedusoides represent together with the South American-Australian Chelidae one of the two major groups of extant chelonians, the side-necked turtles (Pleurodira), usually referred to as one of the two chelonian suborders (Wermuth & Mertens 1961, 1977; Gaffney & Meylan 1988; Ernst *et al.* 2000; Fritz & Havaš 2007; Georges & Thomson 2010).

Among other characters, *Pelomedusa* differs from *Pelusios* by its completely rigid plastron, without any trace of a hinge (Bour 1986; Ernst *et al.* 2000; Boycott & Bourquin 2008; Branch 2008; Fig. 1). *Pelomedusa* was traditionally thought to contain only one species (Wermuth & Mertens 1961, 1977; Gasperetti *et al.* 1993; Ernst *et al.* 2000; Fritz & Havaš 2007; Boycott & Bourquin 2008; Branch 2008). However, two independent recent investigations using mitochondrial and nuclear DNA discovered deeply divergent lineages within *Pelomedusa* and suggested that it could consist of up to nine distinct species (Vargas-Ramírez *et al.* 2010; Wong *et al.* 2010). Moreover, it turned out that *Pelomedusa* could be paraphyletic with respect to *Pelusios*, but in each study only one species of *Pelusios* (*P. sinuatus* or *P. castaneus*) was compared with *Pelomedusa*.

The genus *Pelusios* was never studied comprehensively by molecular means, even though the taxonomy of some species is contentious (Wermuth & Mertens 1961, 1977; Broadley 1981, 1983; Bour 1983, 1986; Ernst *et al.* 2000). Previous molecular studies of a broader phylogenetic scope or a focus on other chelonian genera used only a single *Pelusios* species as a representative of this genus (*P. castaneus*: Wong *et al.* 2010; *P. gabonensis*: Noonan & Chippindale 2006; *P. sinuatus*: Seddon *et al.* 1997; Georges *et al.* 1998; Vargas-Ramírez *et al.* 2010; *P. subniger*: Hedges & Poling 1999; Vidal & Hedges 2004; *P. williamsi*: Shaffer *et al.* 1997; Fujita *et al.* 2004; Krenz *et al.* 2005). The only article using sequence data of more than one species examined the mitochondrial phylogeography of *P. castanoides* and *P. subniger* in the Seychelles (Silva *et al.* 2011), but did not include further species.

Based mainly on shell morphology, many authors recognised two species groups within *Pelusios* (Williams 1954; de Broin 1969; Auffenberg 1981; Broadley 1981, 1983; Bour 1986, 2000; Ernst *et al.* 2000). The *adansonii* group, sometimes also called the *gabonensis* group or *adansonii-gabonensis* section, was thought to include *P. adansonii*, *P. broadleyi*, *P. gabonensis*, *P. marani*, and *P. nanus*. Bour (1986) suggested that *P. williamsi* could also belong in the *adansonii* group, but at the same time questioned whether the traditionally recognised species groups represented monophyletic units. Species of the *adansonii* group are

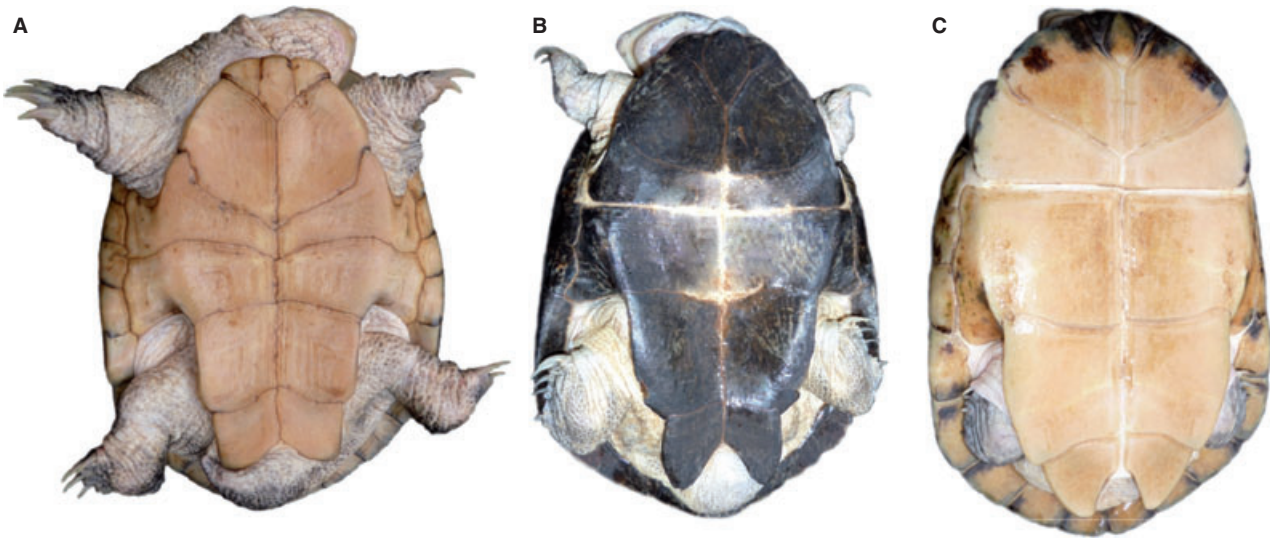

Fig. 1 Plastral view of typical representatives of *Pelomedusa* and *Pelusios*. —A. *Pelomedusa* lineage V, South Horr, Kenya. —B. *Pelusios broadleyi* (*adansonii* species group), Loyangalani, Marsabit Province, Kenya. —C. *Pelusios carinatus* (*subniger* species group), Gabon. Note the hinge in the *Pelusios* species (wide white seam at the base of the plastral fore lobe).

characterised by short abdominal scutes relative to the elongate anterior plastral lobe, and also have a short bridge between the carapace and plastron (Fig. 1). Their bony mesoplastra are more or less tapered towards the plastral midseam. The remaining species, characterised by relatively longer abdominal scutes and a longer bridge (Fig. 1), were placed in the *subniger* group. The mesoplastra of these species do not narrow medially and have parallel transverse contacts with the hyo- and hypoplastra. The plastral fore lobe has a greater mobility than in species of the *adansonii* group.

Based on three mitochondrial and three nuclear DNA fragments (2054 bp mtDNA, 2025 bp nDNA), we present here a molecular phylogeny of all *Pelusios* species except the probably extinct *P. seychellensis* (Ernst *et al.* 2000), and also include in our analyses all nine *Pelomedusa* lineages identified by Vargas-Ramírez *et al.* (2010). In doing so, we aim to clarify phylogenetic relationships between and within the genera *Pelusios* and *Pelomedusa*. Furthermore, we include in our analyses representatives of *Pelusios castaneus* from the African continent, from São Tomé and Guadeloupe and of *P. castanoides* from South Africa, Madagascar and the Seychelles to assess whether geographic differentiation exists in these species.

Materials and methods

Gene selection and taxon sampling

Three mitochondrial (12S rRNA, cyt *b*, ND4) and three nuclear DNA fragments (C-mos, R35, Rag2) previously shown to reveal phylogenetic relationships of and differences among terminal chelonian taxa (e.g. Georges *et al.* 1998; Le *et al.* 2006; Vargas-Ramírez *et al.* 2008, 2010; Fritz *et al.* 2010) were chosen. Ethanol-preserved blood or tissue samples of 51 individuals representing 16 species of *Pelusios* were studied (Table S1). Most samples originated from field-collected terrapins, and some have been described and figured in Bour & Maran (2003), Maran & Pauwels (2007, 2009), and Maran (2009a,b). Homologous sequences of *Pelusios williamsi* (Shaffer *et al.* 1997; Fujita *et al.* 2004; Krenz *et al.* 2005), of each of the nine *Pelomedusa* lineages of Vargas-Ramírez *et al.* (2010), of all podocnemidid species (Vargas-Ramírez *et al.* 2008), and of *Trachemys scripta elegans* were downloaded from GenBank for comparison (Table S1). For the *Pelomedusa* samples of Vargas-Ramírez *et al.* (2010), 12S rRNA and C-mos had not been sequenced before, which is why such sequences were generated for each *Pelomedusa* lineage. Remaining samples and DNA are stored at −80 °C in the tissue sample collection of the Museum of Zoology, Dresden.

Laboratory procedures

Total genomic DNA was extracted from fresh tissue or blood samples using the innuPREP DNA Mini Kit for tissues and the innuPREP Blood DNA Mini Kit (both Analytik Jena AG, Jena, Germany) for blood samples. DNA fragments were amplified using an array of previously published and newly designed primer sets (Table S2). PCR was performed in a 25 μL volume, using 10 pmol of the respective forward and reverse primer (20 pmol for newly

designed primers), 5 pmol dNTPs (Fermentas, St. Leon-Rot, Germany), one unit of *Taq* DNA polymerase (Bioron, Ludwigshafen, Germany), and the corresponding complete buffer. PCR temperature profiles are summarised in Table S3. Product purification followed Fritz *et al.* (2010). All fragments were sequenced in both directions on an ABI 3130xl sequencer (Applied Biosystems, Foster City, CA, USA), except for 12S that was only sequenced forward using the primer L1091 because of short fragment length and very high sequence quality. Cycle sequencing products were purified by salt/ethanol precipitation as in Fritz *et al.* (2010) or by using Sephadex (GE Healthcare, Munich, Germany). For cycle sequencing conditions and GenBank accession numbers of sequences produced in the present study, see Tables S1 and S3.

Alignment, substitution model, partitioning and phylogenetic analyses

All sequences aligned easily in BIOEDIT 7.0.5.2 (Hall 1999). After concatenating the three mtDNA fragments, an alignment of 2054 bp (including gaps) was obtained. The partial 12S rRNA gene contributed 391 bp, the cyt *b* fragment 795 bp, and the partial ND4 gene 673 bp. The latter DNA fragment also comprised the flanking DNA, coding for tRNA-His (74 bp) and tRNA-Leu (41 bp). Eighty base pairs (including an insertion of 18 bp in one sample of *Pelusios castanoides*, MTD T 5497) between tRNA-His and tRNA-Leu correspond to what is annotated as one of the two sequences coding for tRNA-Ser in the complete mitochondrial genome of *Pelomedusa subrufa* (GenBank accession number AF039066; Zardoya & Meyer 1998a,b). However, using the tRNASCAN-SE search server (http://selab.janelia.org/tRNAscan-SE/; setting source: mito/chloroplast) its secondary structure did not yield a tRNA, which is why we refrain from annotating this block of sequence data. Concatenating the three nuclear fragments resulted in an alignment of 2025 bp (including gaps) of which 324 bp corresponded to the C-mos gene, 1043 bp to the intron region of the R35 gene, and 658 bp to the Rag2 gene. Each combined data set contained only sequences from samples of which none or, in rare cases, one of the DNA fragments was missing (Table S1). For total evidence analyses, the mtDNA and nDNA data sets were merged, resulting in an alignment of 4079 bp length. For total evidence analyses only samples with at least three DNA fragments were used.

For further analyses, each data set was collapsed into haplotypes using BIOEDIT and identical sequences were removed from the alignments. The best evolutionary model for each coding or non-coding DNA fragment was determined using JMODELTEST 0.1.1 (Posada 2008) and the Bayesian Information Criterion (Table S4).

Phylogeny of *Pelomedusa* and *Pelusios* species was inferred using probabilistic and parsimony approaches for each data set (mtDNA, nDNA, total evidence). All trees were rooted with *Podocnemis expansa*, representing a species of the sister group (Podocnemididae) of Pelomedusidae. Bayesian reconstructions partitioned by DNA fragment were performed with MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). Evolutionary models were defined without priors and two runs of four chains for each data set conducted (temp = 0.01 for mtDNA and total evidence partitions, temp = 0.1 for nDNA) until the likelihood values of the two runs had reached a stationary plateau and converged. Maximum Likelihood analyses using the same partition scheme were conducted using RAxML (Stamatakis 2006), via the RAxML graphical interface (RAXMLGUI 0.9b2; Silvestro & Michalak 2010). The programme was run using PYTHON 2.7 (http://www.python.org/download/releases/2.7/) with settings of 100 runs and 1000 thorough bootstrap replicates. Maximum Parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford 2002) with the settings 'gapmode = new' and 'add = cl' (no limit of maximum number of trees saved); bootstrap support (1000 replicates) was calculated with the additional setting 'maxtre = 1'. For parsimony statistics, consistency and retention indices, see Table S5.

In order to explore the influence of taxon sampling and outgroups, additional RAxML analyses were run for the mtDNA, nDNA and total evidence data sets. Analyses with expanded taxon sampling included, in addition to those sequences run previously, the other seven podocnemidid species and as outgroup the red-eared slider (*Trachemys scripta elegans*), a distantly related species representing the other suborder of extant chelonians, the hidden-necked turtles (Cryptodira). Two sets of analyses with reduced taxon sampling were run. These included the nine *Pelomedusa* lineages, either 5 or 10 *Pelusios* species (one sequence each) and *Podocnemis expansa* as outgroup. Unrooted RAxML trees including all *Pelusios* and *Pelomedusa* sequences, but without outgroup were also computed.

Results

With respect to the trees derived from the mitochondrial and nuclear data sets, it was obvious that the terminal clades were generally well-resolved in the mitochondrial trees, but the deeper branching patterns were in part contradictory and received only weak support. By contrast, some deeper nodes of the nuclear trees had distinctly higher support values, but the terminal clades were often weakly resolved (Figs S1–S3). The trees based on the total evidence data set combined the phylogenetic resolution of the mitochondrial and nuclear data partitions, and slightly improved the evidence for the placement of some *Pelusios*

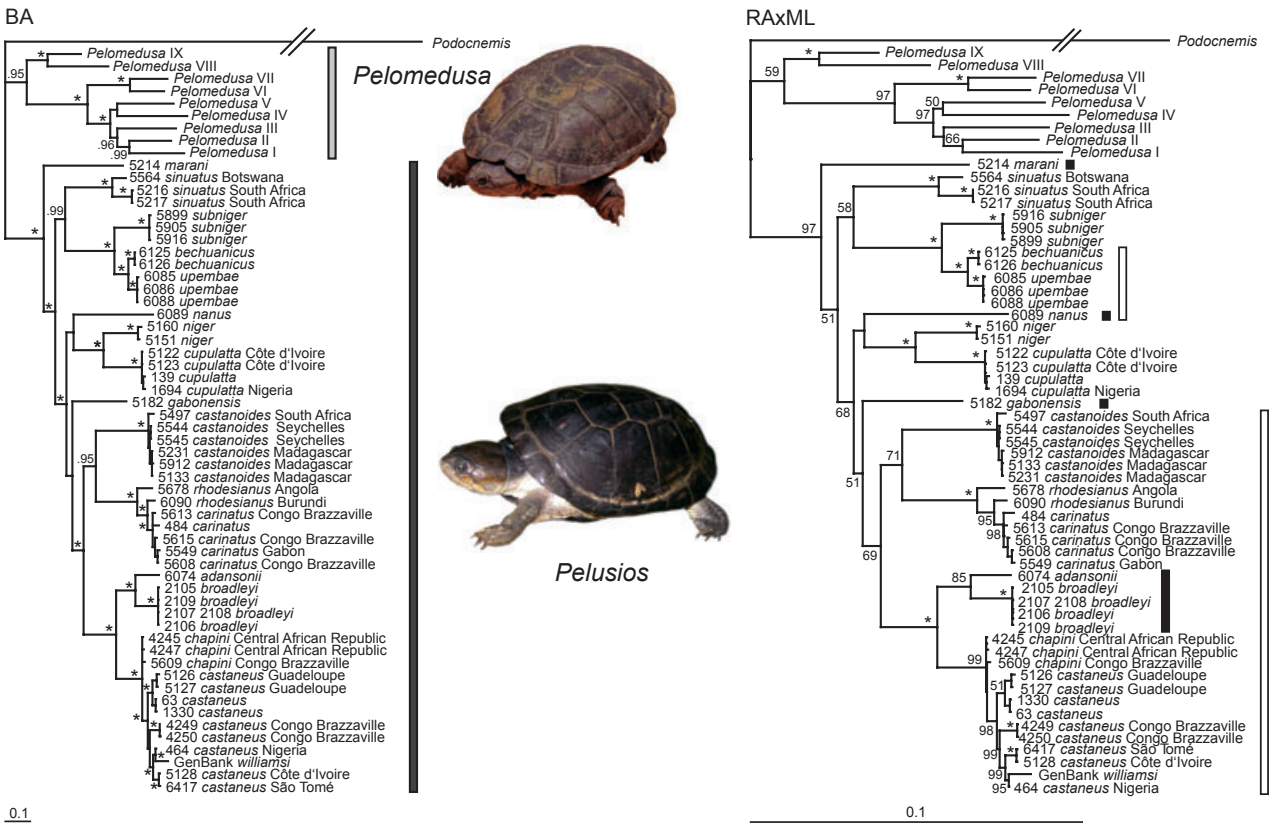

Fig. 2 Phylogeny of *Pelomedusa* and *Pelusios* as inferred by Bayesian and Maximum Likelihood approaches, rooted with *Podocnemis expansa*. Numbers along branches are posterior probabilities or bootstrap values equal to or greater than 0.95 or 50, respectively; asterisks indicate maximum support. Numbers preceding taxon names are MTD T numbers (Table S1). The black bars in the right tree indicate species traditionally placed in the *adansonii* group; the remaining species correspond to the *subniger* group. The white bars symbolise the species group proposed by Bour (1986).

species. All well-supported clades and branching patterns found in the mtDNA and nDNA analyses were confirmed, with reciprocally monophyletic clades corresponding to *Pelusios* and *Pelomedusa.* The well-supported clades and topologies of the RAxML trees (mtDNA, nDNA and total evidence) using only *Podocnemis expansa* as outgroup did not differ from the RAxML trees that were rooted with *Trachemys scripta elegans* and included sequences of all eight podocnemidid species. In the latter analyses, Pelomedusidae always constituted a clade with maximum support that was the sister group of Podocnemididae (not shown).

All trees based on the nuclear and total evidence data partitions found *Pelomedusa* and *Pelusios* reciprocally monophyletic (Figs 2, 3; Figs S1–S3). The *Pelusios* clade received unambiguously high support under each method, whereas the *Pelomedusa* clade was only moderately to weakly supported. The highest support was obtained under MP for the nuclear partition (bootstrap value of 79). The major topologies of the RAxML trees with a reduced number of *Pelusios* species (5 or 10 species) did not differ from the ones with all sequences included. However, in the total evidence trees support values for the reciprocal monophyly of *Pelusios* and *Pelomedusa* increased with the number of included *Pelusios* species, whereas such an effect did not occur in the respective nDNA trees.

All trees based on the mitochondrial partition found the relationships of *Pelomedusa* and *Pelusios* weakly resolved, suggesting either the genera were reciprocally monophyletic but with low support (BA), or a paraphyletic *Pelomedusa* with respect to *Pelusios* (MP) or *vice versa* (RAxML; Figs S1–S3; trees with reduced taxon sampling not shown).

Pelomedusa consisted in all analyses of two well-supported clades, one comprising lineages I–VII and the other lineages VIII + IX. The degree of differentiation among the nine *Pelomedusa* lineages equalled or exceeded that among all *Pelusios* species.

Based on the mitochondrial (Figs S1–S3) and total evidence partitions (Figs 2, 3) of the complete data set, all tree-building methods found each *Pelusios* species clearly

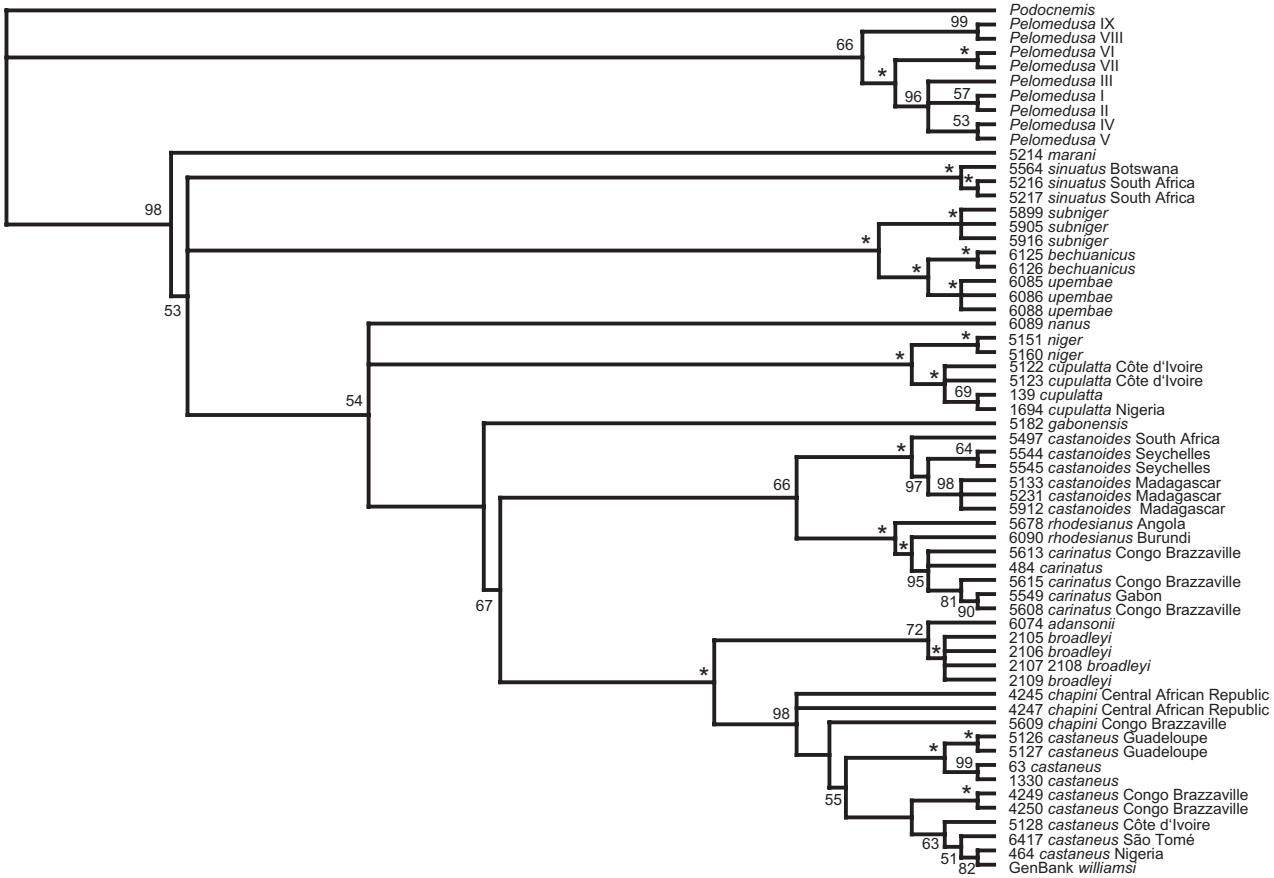

Fig. 3 Strict consensus of 108 equally parsimonious trees (3329 steps; CI = 0.4126, RI = 0.7783) for *Pelomedusa* and *Pelusios* species, rooted with *Podocnemis expansa*. The 50% majority rule tree was identical. Numbers along branches are bootstrap values greater than 50. Asterisks indicate maximum support. Numbers preceding taxon names are MTD T numbers (Table S1).

distinct, except *P. castaneus*, *P. chapini* and *P. williamsi*. The GenBank sequences of *P. williamsi* were consistently embedded within *P. castaneus*, and the three sequences of *P. chapini* occurred basally in the same clade and were only weakly distinct from *P. castaneus*. All total evidence trees, with high support, suggested *P. adansonii* and *P. broadleyi* as sister species and the paraphyletic *P. castaneus-chapini* group as their sister. The sister group of this clade was consistently formed by another major clade comprising the species *P. castanoides*, *P. rhodesianus* and *P. carinatus*. The latter two species were always placed in a well-supported clade being, with moderate support, the sister group of *P. castanoides*. However, *P. rhodesianus* consisted of two quite distinct lineages from Burundi and Angola that constituted the successive sisters of *P. carinatus*. All methods proposed *P. gabonensis* as sister taxon to the more inclusive clade comprising all aforementioned *Pelusios* species, albeit with weak support. BA and RAxML revealed *P. nanus* + (*P. cupulatta* + *P. niger*) as the successive sister group. However, while the clade comprising *P. cupulatta* and *P. niger* received high support under all methods, the position of *P. nanus* remained weakly supported. Correspondingly, both MP consensus trees placed *P. nanus* and (*P. cupulatta* + *P. niger*) separately in a basal multifurcation with the other clade. According to the RAxML and BA trees, a weakly or well-supported clade consisting of *P. sinuatus* + (*P. subniger* + (*P. bechuanicus* + *P. upembae*)) is the sister group to the major clade including all the other above-mentioned taxa. While the clade *P. subniger* + (*P. bechuanicus* + *P. upembae*) is well-supported under both methods and confirmed by MP analysis, the position of *P. sinuatus* remains controversial, as reflected by the two MP consensus trees which place *P. sinuatus* as a distinct lineage in a polytomy together with the clade *P. subniger* + (*P. bechuanicus* + *P. upembae*) and the mentioned major clade. In all trees, *P. marani* constituted the sister taxon to all other *Pelusios* species.

It is noteworthy that sequences of *P. sinuatus* were clearly divergent, with one sample from Botswana being distinct from two South African terrapins. The degree of divergence

between the two lineages of *P. sinuatus* exceeds the differences between *P. bechuanicus* and *P. upembae*. By contrast, sequences of *P. castanoides*, although from three biogeographically highly distinct sites (South Africa, Madagascar, Seychelles), were only slightly differentiated. Structure within *P. castaneus* is somewhat more pronounced, although the distribution ranges of *P. castaneus* and *P. castanoides* are of similar size (Iverson 1992). However, the sequence of a sample of *P. castaneus* from São Tomé closely resembles that of a terrapin from the Côte d'Ivoire.

Discussion

We generated and analysed for the present study mitochondrial and nuclear DNA sequences of 16 out of the 18 currently recognised species of *Pelusios*, and also included in our phylogenetic calculations nine deeply divergent lineages of the closely related genus *Pelomedusa*. Altogether, we analysed 2054 bp of mitochondrial and 2025 bp of nuclear DNA, each of these partitions representing three distinct DNA fragments. Sequences from a further *Pelusios* species (*P. williamsi*) from the same lab (Shaffer *et al.* 1997; Fujita *et al.* 2004; Krenz *et al.* 2005) were available via GenBank (but see below), so that we could include all nominal *Pelusios* species in the present study, except the probably extinct *P. seychellensis*.

Two previous studies using mitochondrial and nuclear DNA sequences found evidence for a possible paraphyly of *Pelomedusa* with respect to the only studied *Pelusios* species (Vargas-Ramírez *et al.* 2010: *P. sinuatus*; Wong *et al.* 2010: *P. castaneus*). However, in the study of Vargas-Ramírez *et al.* (2010), *Pelomedusa* was consistently monophyletic when only nuclear DNA was used for tree reconstruction, implying that the phylogenetic signal responsible for the paraphyly was caused by mtDNA. This is confirmed by our present results based on the same lineages of *Pelomedusa* and nearly all *Pelusios* species.

Our RAxML and MP analyses of mtDNA sequences always resulted in a paraphyletic assemblage, and only BA found the two genera reciprocally monophyletic, but with extremely low support values of 0.78 and 0.73 (Figs S1–S3). Paraphyletic mtDNA topologies were also obtained when only 5 or 10 *Pelusios* species were included in RAxML calculations. In contrast, all analyses based on the total evidence data set found *Pelomedusa* and *Pelusios* reciprocally monophyletic (Figs 2, 3). Here, by increasing the number of included *Pelusios* species (5, 10 or all species available), the RAxML trees showed increasing bootstrap values for the monophyly of the two genera. This suggests an impact of taxon sampling on the total evidence analyses, but not on the analyses of mtDNA. It seems likely that the remote outgroup introduced homoplasy in the mitochondrial data. We used *Podocnemis expansa*, one of the eight species of the Podocnemididae, for rooting most of our trees. Podocnemidids are the closest extant relatives of pelomedusids (Gaffney & Meylan 1988; Shaffer *et al.* 1997; Georges *et al.* 1998; Fujita *et al.* 2004). However, their ancestors separated already in the Lower Cretaceous (Gaffney *et al.* 2006; Noonan & Chippindale 2006; Vargas-Ramírez *et al.* 2008), resulting in highly divergent DNA sequences. Correspondingly, we obtained no better or worse resolution of the deep nodes when using an even more remote outgroup (*Trachemys scripta elegans*). This rooting problem affected only the fast-evolving mtDNA sequences that show under the parsimony criterion low consistency and retention indices (CI = 0.3858, RI = 0.4437; Table S5), suggestive of homoplasy, whereas the nuclear data set has much higher values (CI = 0.8763, RI = 0.7419; Table S5). Indeed, analyses of nuclear data consistently found, with reasonable support, that the two genera are monophyletic (Figs S1–S3), and this phylogenetic signal is also responsible for the same topology in the total evidence analyses (Figs 2, 3). Further support for homoplasy in the mtDNA data set delivers an unrooted RAxML tree (without outgroups; not shown). Here, the long branch separating *Pelomedusa* and *Pelusios* is supported by a bootstrap value of 100.

At the alpha-taxonomic level, our results confirm the distinctiveness of most of the currently recognised *Pelusios* species and suggest that the diversity of *Pelusios* could be even greater. The differences between the lineages of *Pelomedusa* resemble or exceed the divergence of distinct *Pelusios* species, implying that *Pelomedusa* represents a species complex (Vargas-Ramírez *et al.* 2010) and not a single species as was traditionally assumed. We discovered clearly divergent lineages within *P. rhodesianus* and *P. sinuatus*, even though only a few samples of each species were studied. Samples of *P. rhodesianus*, one from Burundi and the other from Angola, were quite distinct and in phylogenetic analyses successively sister to *P. carinatus*, but did not constitute a monophyletic clade as expected. Since *P. carinatus* and *P. rhodesianus* occur in sympatry in the catchment system of the Congo River (Iverson 1992), their species status is not challenged by this phylogenetic placement. Laurent (1956, 1964) and Broadley (1981) reported coloration differences between northern and southern representatives of *P. rhodesianus*, and noted that Angolan terrapins are the northernmost representatives of the southern morph. Our two samples of *P. rhodesianus* may therefore represent both of the morphologically distinctive forms. A sample of *P. sinuatus* from Botswana differed significantly from two South African samples from KwaZulu Natal, perhaps due to an association with west- vs. east-flowing river systems.

In contrast, *P. castaneus*, *P. chapini* and *P. williamsi* were not clearly differentiated (Figs 2, 3; Figs S1–S3). *Pelusios*

chapini was originally described as a subspecies of *P. castaneus* (Laurent 1965), but later elevated to a full species by Bour (1983). Morphological differences between the two parapatric taxa refer mainly to maximum body size (*P. castaneus*: 22 cm shell length, *P. chapini*: 38 cm shell length; Ernst *et al.* 2000), but this character is known to vary considerably in some chelonian species (Fritz *et al.* 2005, 2007, 2010), without any taxonomic relevance, and this may be true here. If a taxonomic distinction for *chapini* is still desired, its original classification as a subspecies of *P. castaneus* (Laurent 1965) could be re-instated. Unlike the previous case, *P. williamsi* is a morphologically clearly distinct species that is thought to be allied to *P. castanoides* and not to *P. castaneus* (Bour 1986). Consequently, the phylogenetic position of *P. williamsi* amongst *P. castaneus* and *P. chapini* is unexpected. *Pelusios williamsi* is the only species for which we did not generate sequences but used GenBank data from the same lab, based on a turtle from the pet trade (Shaffer *et al.* 1997; Fujita *et al.* 2004; Krenz *et al.* 2005). It is well known that sequences deposited in GenBank may be taxonomically misidentified (e.g. Vilgalys 2003; Meier *et al.* 2006; Seberg & Petersen 2007; Fritz *et al.* 2010), and this seems also likely for *P. williamsi*. Therefore, we refrain from any taxonomic conclusion. Parenthetically it may be noted that our sequence data of two terrapins from Guadeloupe (Lesser Antilles) confirm that the introduced *Pelusios* species there is without doubt *P. castaneus* (Bour 1983; Iverson 1992; Ernst *et al.* 2000) and not *P. subniger*, as presumed by other authors (Schwartz & Thomas 1975; Broadley 1981; Schwartz & Henderson 1991; Hedges 1996).

Compared to *P. castaneus*, only very shallow divergences occur within *P. castanoides* (Figs 2, 3). This suggests that Madagascar and the Seychelles were only recently colonised and that *P. castanoides* could be taxonomically oversplit. Bour (1978, 1983) described the Malagasy and Seychellois populations as distinct subspecies (*P. c. kapika* and *P. c. intergularis*, respectively), with the nominotypical subspecies occurring in Africa. However, according to our data, the terrapins from Madagascar and the Seychelles are only minimally differentiated from their South African conspecifics. This is in agreement with the findings of Silva *et al.* (2011) who compared sequence variation of the partial cytochrome *b* gene of Seychellois and Malagasy *P. castanoides* and found only slight differences. Likewise, the similarity of the sequences of two *P. castaneus* from São Tomé and the Côte d'Ivoire implies that the islands of São Tomé and Principe were recently reached by this species.

Traditionally, *Pelusios* species were assigned to two groups, the *adansonii* group and the *subniger* group (Williams 1954; de Broin 1969; Auffenberg 1981; Broadley 1981, 1983; Bour 1986, 2000; Ernst *et al.* 2000; Table 1). However, Bour (1986) proposed that the two groups do not represent monophyletic units, and our results support this view (Fig. 2). In addition, Bour (1986) suggested that 12 out of the then 16 recognised *Pelusios* species were very closely allied, and that most of them represent recently diverged species pairs ('superspecies' *sensu* Mayr 1963; Table 1). While our genetic data confirm that most of Bour's (1986) 'superspecies' are sister species, with the possible exception of *P. castanoides* and *P. williamsi* (see above), it is obvious that his more inclusive group of 12 species is at odds with our phylogeny. In particular, *P. bechuanicus* + *P. upembae* are not closely allied to the other species of Bour's (1986) group, but are rather sister to *P. subniger*. We found also no support for a close relationship of *P. nanus* and *P. adansonii*, as suggested by some authors (e.g. Wermuth & Mertens 1961, 1977; Ernst *et al.* 2000).

Table 1 Previously recognised species groups of *Pelusios*. Superspecies *sensu* Bour (1986) are connected by a plus symbol

Pelusios adansonii group	*Pelusios subniger* group	Species group of Bour (1986)*	Isolated *Pelusios* species according to Bour (1986)*
adansonii	*bechuanicus*	*adansonii* + *broadleyi*	*gabonensis*
broadleyi	*carinatus*	*bechuanicus* + *upembae*	*niger*
gabonensis	*castaneus*	*carinatus* + *rhodesianus*	*sinuatus*
marani	*castanoides*	*castaneus* + *chapini*	*subniger*
nanus	*chapini*	*castanoides* + *williamsi*	
	cupulatta	*nanus*	
	niger	*seychellensis*	
	rhodesianus		
	seychellensis		
	sinuatus		
	subniger		
	upembae		
	williamsi		

**Pelusios cupulatta* and *P. marani* were not described when Bour (1986) proposed this classification.

Conclusions

According to our present study, the morphologically well-defined genera *Pelusios* and *Pelomedusa* are reciprocally monophyletic units. The weak support for their monophyly derived from mitochondrial data seems to result from homoplasy introduced by remote outgroups used for tree rooting. The distinctiveness of nearly all currently recognised *Pelusios* species was confirmed by molecular means, but all previously suggested species groups within *Pelusios* are not monophyletic. Divergence between the distinct genetic lineages of *Pelomedusa* equals or exceeds the differences between *Pelusios* species, supporting the view

that *Pelomedusa* is a species complex. However, additional sampling is needed to clarify the situation in *Pelusios castaneus*, *P. chapini*, *P. castanoides*, *P. rhodesianus*, *P. sinuatus* and *P. williamsi*. While we found distinct lineages in *P. rhodesianus* and *P. sinuatus*, suggestive of cryptic taxa, the diversity of *P. castaneus*, *P. chapini* and *P. castanoides* seems to be overestimated. The recognition of *P. castaneus* and *P. chapini* as full species is doubtful, like the validity of the Malagasy and Seychellois subspecies of *P. castanoides*. It is probable that the GenBank sequences of *P. williamsi* are misidentified and the relationships of *P. williamsi* need to be investigated with fresh material.

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References

Auffenberg, W. (1981). The fossil turtles of Olduvai Gorge, Tanzania, Africa. *Copeia*, *1981*, 509–522.

Bour, R. (1978). Les tortues actuelles de Madagascar (République malgache): liste systématique et description de deux sous-espèces nouvelles (Reptilia-Testudines). *Bulletin du Société d'Études scientifique de Anjou*, *Nouvelle Série*, *10*, 141–154.

Bour, R. (1983). Trois populations endémiques du genre *Pelusios* (Reptilia, Chelonii, Pelomedusidae) aux îles Seychelles; relations avec les espèces africaines et malgaches. *Bulletin du Muséum national d'Histoire naturelle*, *4e Série*, *Section A*, *5*, 343–382.

Bour, R. (1986). Note sur *Pelusios adansonii* (Schweigger, 1812) et sur une nouvelle espèce affine du Kenya (Chelonii, Pelomedusidae). *Studia Geologica Salmanticensia*, *Volumen Especial 2 (Studia Palaeocheloniologica II)*, 23–54.

Bour, R. (2000). Une nouvelle espèce de *Pelusios* du Gabon (Reptilia, Chelonii, Pelomedusidae). *Manouria*, *3*(8), 1–32.

Bour, R. & Maran, J. (2003). Une nouvelle espèce de *Pelusios* de Côte d'Ivoire (Reptilia, Chelonii, Pelomedusidae). *Manouria*, *6*(21), 24–43.

Boycott, R. C. & Bourquin, O. (2008). *Pelomedusa subrufa* (Lacépède 1788), helmeted turtle, helmeted terrapin. In A. G. J. Rhodin, P. C. H. Pritchard, P. P. van Dijk, R. A. Saumure, K. A. Buhlmann & J. B. Iverson (Eds) *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group* (pp. 007.1–007.6). Lunenburg, MA: Chelonian Research Foundation (Chelonian Research Monographs No. 5).

Bramble, D. M. & Hutchison, J. H. (1981). A reevaluation of plastral kinesis in African turtles of the genus *Pelusios*. *Herpetologica*, *37*, 205–212.

Branch, B. (2008). *Tortoises, Terrapins and Turtles of Africa*. Cape Town, South Africa: Struik, 128 pp.

Broadley, D. G. (1981). A review of the genus *Pelusios* Wagler in southern Africa (Pleurodira: Pelomedusidae). *Occasional Papers of the National Museums and Monuments, Series B (Natural Sciences)*, *6*, 633–686.

Broadley, D. G. (1983). Neural pattern – a neglected taxonomic character in the genus *Pelusios* Wagler (Pleurodira: Pelomedusidae). In A. G. J. Rhodin & K. Miyata (Eds) *Advances in Herpetology and Evolutionary Biology. Essays in Honor of Ernest E. Williams* (pp. 159–168). Cambridge, MA: Museum of Comparative Zoology.

de Broin, F. (1969). Sur la présence d'une tortue, *Pelusios sinuatus* (A. Smith) au Villafranchien inférieur du Tchad. *Bulletin de la Société Géologique de France*, 7, 909–916.

Ernst, C. H., Altenburg, R. G. M. & Barbour, R. W. (2000). *Turtles of the World*. World Biodiversity Database, CD-ROM Series, Windows, Version 1.2. Amsterdam, The Netherlands: Biodiversity Center of ETI.

Fritz, U. & Havaš, P. (2007). Checklist of chelonians of the world. *Vertebrate Zoology*, *57*, 149–368.

Fritz, U., Široký, P., Kami, H. & Wink, M. (2005). Environmentally caused dwarfism or a valid species – is *Testudo weissingeri* Bour, 1996 a distinct evolutionary lineage? New evidence from mitochondrial and nuclear genomic markers. *Molecular Phylogenetics and Evolution*, *37*, 389–401.

Fritz, U., Hundsdörfer, A. K., Široký, P., Auer, M., Kami, H., Lehmann, J., Mazanaeva, L. F., Türkozan, O. & Wink, M. (2007). Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia*, *28*, 97–121.

Fritz, U., Daniels, S. R., Hofmeyr, M. D., González, J., Barrio-Amorós, C. L., Široký, P., Hundsdörfer, A. K. & Stuckas, H. (2010). Mitochondrial phylogeography and subspecies of the wide-ranging sub-Saharan leopard tortoise *Stigmochelys pardalis* (Testudines: Testudinidae) – a case study for the pitfalls of pseudogenes and GenBank sequences. *Journal of Zoological Systematics and Evolutionary Research*, *48*, 348–359.

Fujita, M. K., Engstrom, T. N., Starkey, D. E. & Shaffer, H. B. (2004). Turtle phylogeny: insights from a novel nuclear intron. *Molecular Phylogenetics and Evolution*, *31*, 1031–1040.

Gaffney, E. S. & Meylan, P. A. (1988). A phylogeny of turtles. In M. J. Benton (Ed.) *The Phylogeny and Classification of the Tetrapods. Vol. 1, Amphibians, Reptiles, Birds* (pp. 157–219). Oxford, UK: Clarendon Press/Systematics Association.

Gaffney, E. S., Tong, H. & Meylan, P. A. (2006). Evolution of the side-necked turtles: the families Bothremydidae, Euraxemydidae, and Araripemydidae. *Bulletin of the American Museum of Natural History*, *300*, 1–700.

Gasperetti, J., Stimson, A. F., Miller, J. D., Ross, J. P. & Gasperetti, P. R. (1993). Turtles of Arabia. *Fauna of Saudi Arabia*, *13*, 170–367.

Georges, A. & Thomson, S. (2010). Diversity of Australasian freshwater turtles, with an annotated synonymy and keys to species. *Zootaxa*, *2496*, 1–37.

Georges, A., Birrel, J., Saint, K. M., McCord, W. P. & Donnellan, S. C. (1998). A phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation. *Biological Journal of the Linnean Society*, *67*, 213–246.

Hall, T. A. (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hedges, S. B. (1996). The origin of West Indian amphibians and reptiles In R. Powell & R. W. Henderson (Eds) *Contributions to West Indian Herpetology: A Tribute to Albert Schwartz* (pp. 95–128). Ithaca, NY: Society for the Study of Amphibians and Reptiles (Contributions to Herpetology, 12).

Hedges, S. B. & Poling, L. L. (1999). A molecular phylogeny of reptiles. *Science*, *283*, 998–1001.

Iverson, J. B. (1992). *A Revised Checklist with Distribution Maps of the Turtles of the World*. Richmond, IN: Privately Printed, 363 pp.

Krenz, J. G., Naylor, G. J. P., Shaffer, H. B. & Janzen, F. J. (2005). Molecular phylogenetics and evolution of turtles. *Molecular Phylogenetics and Evolution*, *37*, 178–191.

Laurent, R. F. (1956). Contribution à l'herpétologie de la région des grands lacs de l'Afrique centrale. *Annales du Musée Royal du Congo Belge*, *Série 8*, *48*, 1–390 + plates i-xxxi.

Laurent, R. F. (1964). Reptiles et amphibiens de l'Angola. *Publicações Culturais da Companhia de Diamantes de Angola*, *67*, 1–165.

Laurent, R. F. (1965). A contribution to the knowledge of the genus *Pelusios* (Wagler). *Annales du Musée Royal du Congo Belge*, *Série 8*, *135*, 1–33 + plates i-iii.

Le, M., Raxworthy, C. J., McCord, W. P. & Mertz, L. (2006). A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, *40*, 517–531.

Maran, J. (2009a). Nouvelles observations sur la distribution des tortues de la République du Bénin, de la Côte d'Ivoire et du Togo. *Chéloniens*, *15*, 12–25.

Maran, J. (2009b). Nouvelles observations sur la distribution des tortues du Cameroun. *Chéloniens*, *16*, 16–32.

Maran, J. & Pauwels, O. S. G. (2007). Nouvelles observations sur la distribution des tortues de la République de Centrafrique. *Chéloniens*, *8*, 24–38.

Maran, J. & Pauwels, O. S. G. (2009). Nouvelles observations sur la distribution des tortues de la République du Congo. *Chéloniens*, *13*, 12–36.

Mayr, E. (1963). *Animal Species and Evolution*. Cambridge, MA: Belknap Press, 797 pp.

Meier, R., Shiyang, K., Vaidya, G. & Ng, P. K. L. (2006). DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology*, *55*, 715–728.

Noonan, B. P. & Chippindale, P. T. (2006). Vicariant origin of Malagasy reptiles supports Late Cretaceous Antarctic land bridge. *American Naturalist*, *168*, 730–741.

Posada, D. (2008). JMODELTEST: phylogenetic model averaging. *Molecular Biology and Evolution*, *25*, 1253–1256.

Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Schwartz, A. & Henderson, R. W. (1991). *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. Gainesville, FL: University of Florida Press, 714 pp.

Schwartz, A. & Thomas, R. (1975). A check-list of West Indian amphibians and reptiles. *Special Publication, Carnegie Museum of Natural History*, *1*, 1–216.

Seberg, O. & Petersen, G. (2007). Assembling the tree of life: magnitude, shortcuts and pitfalls. In T. R. Hodkinson & J. A. N. Parnell (Eds) *Reconstructing the Tree of Life. Taxonomy and Systematics of Species Rich Taxa* (pp. 33–46). Boca Raton, FL: The Systematics Association.

Seddon, J. M., Georges, A., Baverstock, P. R. & McCord, W. (1997). Phylogenetic relationships of chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA gene sequence variation. *Molecular Phylogenetics and Evolution*, 7, 55–61.

Shaffer, H. B., Meylan, P. & McKnight, M. L. (1997). Tests of turtle phylogeny: molecular, morphological and paleontological approaches. *Systematic Biology*, *46*, 235–268.

Silva, A., Rocha, S., Gerlach, J., Rocamora, G., Dufrenne, A. & Harris, D. J. (2011). Assessment of mtDNA genetic diversity within the terrapins *Pelusios subniger* and *Pelusios castanoides* across the Seychelles islands. *Amphibia-Reptilia*, *32*, 583–588.

Silvestro, D. & Michalak, I. (2010). *Graphical User Interface for RAxML (raxmlGUI)*. Available via http://sourceforge.net/projects/raxmlgui/ [Accessed 15 August 2010].

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.

Swofford, D. L. (2002). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland, MA: Sinauer Associates.

Vargas-Ramírez, M., Castaño-Mora, O. V. & Fritz, U. (2008). Molecular phylogeny and divergence times of ancient South American and Malagasy river turtles (Testudines: Pleurodira: Podocnemididae). *Organisms Diversity Evolution*, *8*, 388–398.

Vargas-Ramírez, M., Vences, M., Branch, W. R., Daniels, S. R., Glaw, F., Hofmeyr, M. D., Kuchling, G., Maran, J., Papenfuss, T. J., Široký, P., Vieites, D. R. & Fritz, U. (2010). Deep genealogical lineages in the widely distributed African helmeted terrapin: evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). *Molecular Phylogenetics and Evolution*, *56*, 428–440.

Vidal, N. & Hedges, S. B. (2004). Molecular evidence for a terrestrial origin of snakes. *Proceedings of the Royal Society of London B*, *271*(Suppl. 4), 226–229.

Vilgalys, R. (2003). Taxonomic misidentification in public DNA databases. *New Phytologist*, *160*, 4–5.

Wermuth, H. & Mertens, R. (1961). *Schildkröten, Krokodile, Brückenechsen*. Jena, Germany: VEB Gustav Fischer, xxvii + 422 pp.

Wermuth, H. & Mertens, R. (1977). Testudines, Crocodylia, Rhynchocephalia. *Das Tierreich*, *100*, i–xxvii + 1-174.

Williams, E. E. (1954). A new Miocene species of *Pelusios* and the evolution of that genus. *Breviora*, *25*, 1–7.

Wong, R. A., Fong, J. J. & Papenfuss, T. J. (2010). Phylogeography of the African helmeted terrapin, *Pelomedusa subrufa*: genetic structure, dispersal, and human introduction. *Proceedings of the California Academy of Sciences*, *61*, 575–585.

Zardoya, R. & Meyer, A. (1998a). Complete mitochondrial genome suggests diapsid affinities of turtles. *Proceedings of the National Academy of Sciences of the USA*, *95*, 14226–14231.

Zardoya, R. & Meyer, A. (1998b). Cloning and characterisation of a microsatellite in the mitochondrial control region of the African side-necked turtle, *Pelomedusa subrufa*. *Gene*, *215*, 149–153.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Bayesian trees for the mitochondrial and nuclear data sets, rooted with *Podocnemis expansa*. Numbers along branches are posterior probabilities ≥0.95, except for the *Pelomedusa* and *Pelusios* clades, where lower values are shown, and for some interior clades with short branch lengths, where values are not presented. Asterisks indicate maximum support. Numbers preceding taxon names are MTD T numbers (Table S1). Note the weakly supported reciprocal monophyly of *Pelomedusa* and *Pelusios* in the mtDNA tree and the well-supported identical topology in the nDNA tree.

Fig. S2 Maximum Likelihood trees for the mitochondrial and nuclear data sets, rooted with *Podocnemis expansa*. Numbers along branches are bootstrap values ≥50. Asterisks indicate maximum support. Numbers preceding taxon names are MTD T numbers (Table S1). Note the paraphyly of *Pelusios* with respect to *Pelomedusa* in the mtDNA tree and the reciprocal monophyly of both in the nDNA tree.

Fig. S3 Maximum parsimony trees for the mitochondrial and nuclear data sets, rooted with *Podocnemis expansa*. The mitochondrial tree corresponds to the strict consensus and 50% majority rule consensus of 24 equally parsimonious trees (2964 steps; CI = 0.3858, RI = 0.4437), the nuclear tree to the 50% majority rule consensus of 4808 equally parsimonious trees (388 steps; CI = 0.8763, RI = 0.7419). Numbers along branches are bootstrap values ≥50. Asterisks indicate maximum support. Numbers preceding taxon names are MTD T numbers (Table S1). Note the paraphyly of *Pelomedusa* with respect to *Pelusios* in the mtDNA tree and the well-supported reciprocal monophyly in the nDNA tree.

Table S1 Samples and GenBank sequences used in the present study. The ND4 fragment comprised also adjacent mtDNA (see Materials and Methods).

Table S2 Primer pairs used in this study.

Table S3 Thermocycling and cycle sequencing conditions.

Table S4 Models selected by the Bayesian Information Criterion using JMODELTEST 0.1.1 (Posada 2008).

Table S5 Parsimony statistics for the three data sets (gaps treated as fifth character state).

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