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Comparative phylogeographies of six species of hinged terrapins (*Pelusios* spp.) reveal discordant patterns and unexpected differentiation in the *P. castaneus*/*P. chapini* complex and *P. rhodesianus*

CAROLIN KINDLER[1,a], MAGGIE MOOSIG[1,a], WILLIAM R. BRANCH[2,3], JAMES HARVEY[4], CHRISTIAN KEHLMAIER[1], ZOLTÁN T. NAGY[5], HYNEK PROKOP[6], PAVEL ŠIROKÝ[7] and UWE FRITZ[1]*

[1]*Museum of Zoology, Senckenberg Dresden, A. B. Meyer Building, Dresden, 01109, Germany*
[2]*Department of Herpetology, Port Elizabeth Museum, P.O. Box 13147, Humewood, 6013, South Africa*
[3]*Department of Zoology, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth, 6031, South Africa*
[4]*35 Carbis Road, Pietermaritzburg, 3201, South Africa*
[5]*Royal Belgian Institute of Natural Sciences, JEMU, Rue Vautier 29, Brussels, 1000, Belgium*
[6]*U Zámečku 459, Pardubice, 53003, Czech Republic*
[7]*Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, Brno, 612 42, Czech Republic*

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Using up to 2117 bp of mitochondrial DNA and up to 2012 bp of nuclear DNA, we analysed phylogeographic differentiation of six widely distributed species of African hinged terrapins (*Pelusios* spp.) representing different habitat types. Two taxa each live in savannahs or in forests and mesic savannahs, respectively, and the remaining two species occur in intermediate habitats. The species living in forests and mesic savannahs do not enter dry savannahs, whereas the savannah species may occur in dry and wet savannahs and even in semi-arid steppe regions. We found no obvious correlation between habitat type and phylogeographic pattern: one savannah species (*P. rhodesianus*) shows phylogeographic structure, i.e. pronounced genetic differences among geographically distinct populations, and the other (*P. nanus*) not. One species inhabiting forests and mesic savannahs (*P. carinatus*) has phylogeographic structure, the other (*P. gabonensis*) not. The same pattern is true for the two ecologically intermediate species, with phylogeographic structure present in *P. castaneus* and absent in *P. chapini*. Nuclear evidence suggests that the latter two taxa with abutting and partially overlapping ranges are distinct, while mtDNA is only weakly differentiated. *Pelusios castaneus* shows pronounced phylogeographic structure, which could reflect Pleistocene range interruptions correlated with the fluctuating forest cover in West and Central Africa. Our results do not support the recognition of an extinct subspecies of *P. castaneus* for the Seychelles. *Pelusios carinatus* contains two well supported clades, which are separated by the Congo River. This species is closely related to *P. rhodesianus*, a taxon consisting of two deeply divergent mitochondrial clades. One of these clades is paraphyletic with respect to *P. carinatus*, but the two clades of *P. rhodesianus* are not differentiated in the studied nuclear markers and, again, paraphyletic with respect to *P. carinatus*. Using mtDNA sequences from the type material of *P. rhodesianus*, we were able to allocate this name to one of the two clades. However, owing to the confusing relationships of *P. rhodesianus* and *P. carinatus*, we refrain from taxonomic decisions. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **117**, 305–321.

ADDITIONAL KEYWORDS: Africa – Pelomedusidae – *Pelusios carinatus* – *Pelusios gabonensis* – *Pelusios nanus* – *Pelusios seychellensis* – taxonomy.

*Corresponding author. E-mail: uwe.fritz@senckenberg.de
[a]Both authors contributed equally.

INTRODUCTION

The African turtle genus *Pelusios* comprises 17 currently recognized species of hinged terrapins and belongs to the most speciose genera among turtles (Fritz *et al.*, 2011; Stuckas, Gemel & Fritz, 2013; van Dijk *et al.*, 2014). Hinged terrapins are generally darkly coloured, small- to large-sized freshwater turtles with shell lengths between 12 and 48.5 cm (Ernst, Altenburg & Barbour, 2000; Branch, 2008). The common name of these terrapins was coined in reference to a shared morphological peculiarity, the cartilaginous hinge that connects the plastral forelobe to the shell, allowing the closure of the anterior shell (Bramble & Hutchison, 1981; Ernst *et al.*, 2000; Branch, 2008). *Pelusios* species are widely distributed across sub-Saharan Africa, in Madagascar and in the Seychelles (Ernst *et al.*, 2000; Branch, 2008). Introduced populations exist on Guadeloupe, Lesser Antilles (*P. castaneus*: Bour, 1983; Ernst *et al.*, 2000; Fritz *et al.*, 2011), Madagascar and the Seychelles (*P. subniger*: Fritz *et al.*, 2013), and also for the second species of Madagascar and the Seychelles (*P. castanoides*) an anthropogenic origin cannot be excluded (Fritz *et al.*, 2013).

For a long time, the systematics and taxonomy of *Pelusios* species were based on morphology alone (e.g., Williams, 1954; Laurent, 1965; Bour, 1978, 1983, 1986, 2000; Broadley, 1981, 1983; Bour & Maran, 2003) and there was pronounced disagreement on the status of several taxa among different authors (see the review in Ernst *et al.*, 2000). Molecular phylogenetic investigations using three mitochondrial genes and three nuclear loci supported the distinctness of 16 or 17 of the 18 previously recognized species (Fritz *et al.*, 2011, 2013; Fritz, Vargas-Ramírez & Široký, 2012a; Stuckas *et al.*, 2013). For *P. castaneus* and *P. chapini* from West and Central Africa, Fritz *et al.* (2011) found weak genetic differentiation suggestive of conspecificity. Conversely, the same authors highlighted that genetically distinct lineages currently identified with *P. rhodesianus* and *P. sinuatus* could represent additional unnamed species. Using mtDNA sequences from the lectotype of *P. seychellensis*, Stuckas *et al.* (2013) synonymized this allegedly extinct species with *P. castaneus*, which does not occur in the Seychelles. After its description, *P. seychellensis* has been never found again, and this species was most likely founded on mislabelled museum specimens bearing incorrect locality data. Finally, Fritz *et al.* (2013) assessed the phylogeography of the widely distributed species *P. castanoides* and *P. subniger*. Samples of *P. subniger* from the Democratic Republic of the Congo turned out to be genetically distinct and could represent an undescribed species. Yet, the phylogeography of most other species of *Pelusios* remains unstudied, leaving open the possibility that further unrecognized species exist.

Some species of *Pelusios* are endemic to restricted regions (*P. broadleyi*, *P. marani*, *P. upembae*, *P. williamsi*; cf. van Dijk *et al.*, 2014), and for these taxa no or no pronounced phylogeographic variation is expected. Among the remaining species, we were able to obtain broad sampling for six species (*P. carinatus*, *P. castaneus*, *P. chapini*, *P. gabonensis*, *P. nanus*, *P. rhodesianus*; Fig. 1), allowing an assessment of their phylogeography. Thus, together with the previously published data for *P. castanoides* and *P. subniger* (Fritz *et al.*, 2013), the phylogeographies of eight out of the 13 widely distributed species could be assessed. This is exceptional for wide-ranging African reptile species (cf. Barlow *et al.*, 2013).

The examined six *Pelusios* species represent different distribution patterns and they occur in different habitat types. Two species (*P. carinatus*, *P. gabonensis*) inhabit waters in forests and mesic savannahs of Central Africa, while two other species (*P. nanus*, *P. rhodesianus*) live in savannah habitats in the northern part of southern Africa. Ecologically, the remaining two species (*P. castaneus* from West Africa and *P. chapini* from Central Africa) occupy an intermediate niche and occur in a variety of habitats, ranging from savannah to forest waters, but both species seem to avoid closed forests (Ernst *et al.*, 2000; Maran & Pauwels, 2005, 2007, 2009; Branch, 2008; Maran, 2009, 2010). *Pelusios* species living in forests and mesic savannahs do not enter dry savannahs, whereas savannah species may be found in dry and wet savannahs and even semi-arid steppe regions (e.g., Maran & Pauwels, 2007; for the definition of dry and wet savannahs, see Scholes, 1990).

In the present study, we particularly focus on the differentiation and relationships of *P. castaneus*, *P. chapini* and *P. rhodesianus*. In the latter species, considerable phylogeographic variation was expected. Its distribution range is disjunct, with apparently isolated populations in KwaZulu-Natal, South Africa. There is also geographical variation regarding coloration, with a northern and a southern morphotype (Broadley, 1981). These unnamed coloration varieties could correspond to the two genetically distinct lineages of *P. rhodesianus* identified by Fritz *et al.* (2011). These authors studied two samples of *P. rhodesianus*, one from Burundi and one from Angola, that constituted in phylogenetic analyses successive sisters of *P. carinatus*. Fritz *et al.* (2011) tentatively identified these genetic lineages with Broadley's coloration types and speculated that both could represent distinct species. Thus, our present paper aims also at clarifying the taxonomy of *P. rhodesianus*.

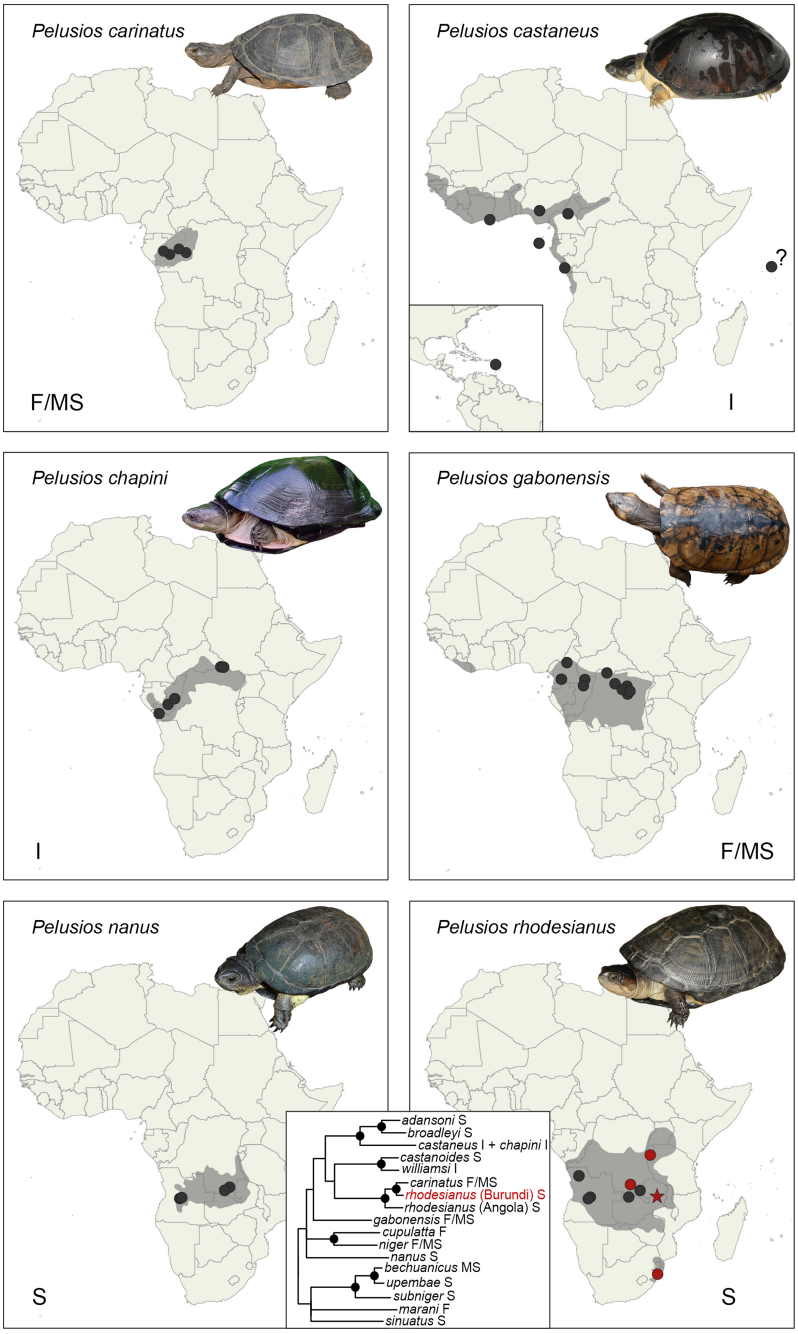

Figure 1. Geographical distribution of the six studied species of *Pelusios* and sampling sites. Habitat preferences are indicated (F/MS – forest/mesic savannah; S – savannah; I – intermediate). Shown are projected historical distribution ranges according to van Dijk *et al.* (2014), see there for further explanation and distributions of the remaining *Pelusios* species. In the map for *P. castaneus*, the doubtful geographical origin of the lectotype of *P. seychellensis* (Mahé, Seychelles) is highlighted by a question mark, see Discussion. The inset shows the introduced population on Guadeloupe, Lesser Antilles. For *P. gabonensis*, the isolated distribution range in West Africa requires confirmation because the underlying old records could refer to the superficially similar *P. cupulatta*. This species was described only in 2003 (Bour & Maran, 2003); *P. cupulatta* and *P. gabonensis* are not sister species (Fritz *et al.*, 2011). For *P. rhodesianus*, the red symbols represent the mitochondrial haplotypes clustering with *P. carinatus* (red star: type locality). At the bottom of the figure, a simplified mitochondrial phylogeny is shown for all *Pelusios* species (modified from Stuckas *et al.*, 2013). Nodes with black circles are supported in Bayesian and Maximum Likelihood analyses by posterior probabilities and bootstrap values ≥ 0.95 and 80.

For the present study, we considerably expand the data set of Fritz *et al.* (2011, 2012a) and Stuckas *et al.* (2013) and use phylogenetic analyses of the same molecular markers (three mitochondrial and three nuclear DNA fragments, together up to 4129 bp) for inferring phylogeographic structure. Among the studied specimens are the holotype and two paratypes of *P. rhodesianus* from the collection of the Port Elizabeth Museum, South Africa.

MATERIAL AND METHODS

Sampling

Blood or tissue samples of 114 hinged terrapins were studied, corresponding to 15 *Pelusios carinatus*, 19 *P. castaneus*, 9 *P. chapini*, 24 *P. gabonensis*, 26 *P. nanus*, and 21 *P. rhodesianus* (Table S1). In addition to new material (90 samples), previously published DNA sequences (Fritz *et al.*, 2011, 2012a; Stuckas *et al.*, 2013) from 24 terrapins were included. For 12 of these samples, missing sequence data were completed for the present study. Among the previously published data were mtDNA sequences of the lectotype of *P. seychellensis* from Stuckas *et al.* (2013), and among the terrapins studied for the present paper were the holotype (PEM R12373) and two paratypes of *P. rhodesianus* (PEM R14957, PEM R14959) from the collection of the Port Elizabeth Museum, South Africa. These specimens have been collected in the early 20th century (Hewitt, 1927). From each of the dry type specimens (shells with attached skin), a small piece of skin was removed for study. All other samples were ethanol-preserved.

DNA Extraction, PCR and Sequencing

The same mitochondrial and nuclear DNA fragments were targeted as in Fritz *et al.* (2011). For historical samples, only three mitochondrial genes were studied (12S, cyt *b*, ND4). These genes were also sequenced for fresh material, but the ND4 fragment comprised then also adjacent DNA coding for tRNAs. In addition, two protein-coding nuclear genes (C-mos, Rag2) and the intron 1 of the nuclear R35 gene were sequenced for fresh samples. Details of DNA isolation, PCR and sequencing are described in the Supporting Information (see also Tables S2–S5). For fresh material, the obtained 12S fragments were up to 393 bp long; cyt *b* fragments, up to 875 bp; and mtDNA fragments comprising the partial ND4 gene plus adjacent DNA coding for tRNAs were up to 843 bp. All studied nuclear DNA blocks could be sequenced directly. C-mos sequences had a length of up to 324 bp; R35 sequences, up to 1030 bp; and Rag2 sequences, up to 658 bp.

For the samples of the historical type specimens, all necessary precautions for ancient DNA work were taken (see details in Supporting Information), and short overlapping mtDNA fragments were sequenced to reconstruct longer contigs. The resulting contig for the 12S gene was of the same length as the sequences of fresh material (393 bp), and the contigs for the cyt *b* and ND4 genes had 567 bp and 532 bp length, respectively. For three fresh samples of *P. rhodesianus* (6948, 7038, 12154), cyt *b* and ND4 sequences were repeated using the primers for museum specimens.

Alignment, Uncorrected *P* Distances and Haplotype Networks

Sequences were aligned and inspected using BIOEDIT 7.0.5.2 (Hall, 1999) and MEGA 6.0.5 (Tamura *et al.*, 2013). All sequences aligned perfectly and gaps occurred only in non-protein-coding sequence blocks. Among the nuclear loci, a few mixed bases indicated heterozygosity (Table S6), but a heterozygous length polymorphism occurred only once. For the R35 intron of *Pelusios castaneus* two different length variants were found. One of these is characterized by a 16-bp-long deletion. In one sample, the two alleles represented both variants of this length polymorphism (sample 63; Table S1). Gametic haplotypes of heterozygous samples were estimated using DNASP 5.10.01 (Librado & Rozas, 2009) and the phase option. For the phased nuclear sequences, haplotype networks were constructed using TCS 1.21 (Clement, Posada & Crandall, 2000) and the default 95% connection limit; gaps were coded as fifth character state.

For the cyt *b* gene, uncorrected *P* distances were obtained using MEGA and the pairwise deletion option. For calculating uncorrected *P* distances, sequence data of the focal species of this study and all remaining *Pelusios* species from Fritz *et al.* (2011, 2012a, 2013) and Stuckas *et al.* (2013) were included; the data set was trimmed to the length of the previously published sequences (795 bp).

Phylogenetic Analyses

For phylogenetic analyses, our new sequences were merged with previously published data for *P. carinatus*, *P. castaneus*, *P. chapini*, *P. gabonensis*, *P. nanus* and *P. rhodesianus* from Fritz *et al.* (2011, 2012a) and Stuckas *et al.* (2013). Homologous sequences of *Pelusios marani* and *Pelomedusa variabilis* were added as outgroups (Table S1). The genus *Pelomedusa* represents the sister group of *Pelusios*, and *P. marani* is likely to be the sister taxon of all other *Pelusios* species (Fritz *et al.*, 2011).

Mitochondrial and nuclear DNA fragments were concatenated, respectively, resulting in an mtDNA alignment of 2117 bp length and an nDNA data set of 2012 bp length, including gaps in the non-protein-coding sequence blocks. A third alignment of 4129 bp consisted of the concatenated mitochondrial and nuclear data sets. For the above mentioned heterozygous individual of *P. castaneus* with a length polymorphism of the R35 intron, the two alleles were concatenated with the other sequence data of this sample and both variants were included in phylogenetic analyses. Additional single heterozygous positions (Table S6) of other samples were coded for phylogenetic analyses as ambiguities.

Best-fit substitution models and the optimal partitioning scheme were assessed for each alignment using the software PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012) and the Bayesian Information Criterion. Three different partition schemes were examined: (a) unpartitioned, (b) partitioned by gene, with DNA coding for tRNAs merged in one partition, and (c) maximum partitioning, i.e. using each codon position of protein-coding genes, the merged DNA coding for tRNAs and non-protein-coding sequence blocks (12S, R35) as a distinct partition. While PARTITIONFINDER selected the unpartitioned scheme as the best one for the nuclear data set, the maximum partitioning scheme was found as the best solution for the mitochondrial data set and the concatenated mitochondrial and nuclear sequences.

Phylogenetic relationships were examined for each of the three data sets. The mtDNA data set included 113 ingroup sequences (the holotype of *P. rhodesianus* failed to amplify, see Results). In the calculations for the concatenated nuclear DNA only data of those 71 terrapins were included for which sequences of all three loci were available. However, for the concatenated mitochondrial and nuclear sequences, two different analyses were run. One included only those 71 terrapins for which all mitochondrial and all nuclear loci were available, while the other included also eight additional terrapins for which only one or two nuclear loci were available.

For calculating phylogenetic trees, a Bayesian and a Maximum Likelihood approach were used. Bayesian trees were obtained with MRBAYES 3.2.3 (Ronquist *et al.*, 2012) using the partition schemes and evolutionary models of Table S7 and default parameters. Two parallel runs, each with four chains, were conducted. The chains ran for 10 million generations with every 500th generation sampled. The calculation parameters were analysed using a burn-in of 2.5 million generations to assure that both runs converged. Subsequently, only the plateau of the remaining trees was sampled using the same burn-in, and a 50% majority rule consensus tree was generated. The posterior probability of any individual clade in this consensus tree corresponds to the percentage of all trees containing that clade, and is a measure of clade frequency and credibility.

Maximum Likelihood trees were computed using the software RAxML 7.2.8 (Stamatakis, 2006) and the default GTR + G model across all partitions. Five independent ML searches were performed with different starting conditions and the rapid bootstrap algorithm to explore the robustness of the branching patterns by comparing the best trees. Then, 1000 non-parametric thorough bootstrap values were calculated and plotted against the best tree.

RESULTS

For most samples, sequences of all three mtDNA fragments were obtained, and the missing data from previous papers (Fritz *et al.*, 2011, 2013) could be completed for the respective samples. Only for one *Pelusios chapini*, the cyt *b* gene could not be sequenced because the sample was used up. However, sequences of the three nuclear loci could not be generated for all terrapins. Thus, a complete nuclear data set was available only for 71 samples. For another eight samples, one or two nuclear loci could be obtained. For the two paratypes of *P. rhodesianus*, all three mtDNA fragments could be generated. In contrast, no DNA sequences could be obtained for the holotype, despite repeated efforts. For accession numbers of new and previously published sequences, see Table S1. The cyt *b* and ND4 sequences for three samples of *P. rhodesianus* (6948, 7038, 12154) obtained with primer combinations designed for historical material were consistent with the sequences amplified with primers for fresh material (see Discussion).

Phylogenetic Analyses

In the mitochondrial trees, all terminal clades were well supported, but not all of these clades matched completely with the currently recognized *Pelusios* species (Fig. 2). *Pelusios castaneus* and *P. chapini* were only weakly divergent, and sequences of these two species were placed in one well supported clade. Only the Maximum Likelihood analyses suggested, with weak support, the monophyly of *P. castaneus*. However, *P. chapini* was then paraphyletic with respect to *P. castaneus*. Bayesian inference placed the sequences of the two species in an unresolved polytomy. Deep branching patterns were generally weakly resolved, with low nodal support. Accordingly, the topologies of the Maximum Likelihood and Bayesian trees differed for most deep nodes, and only

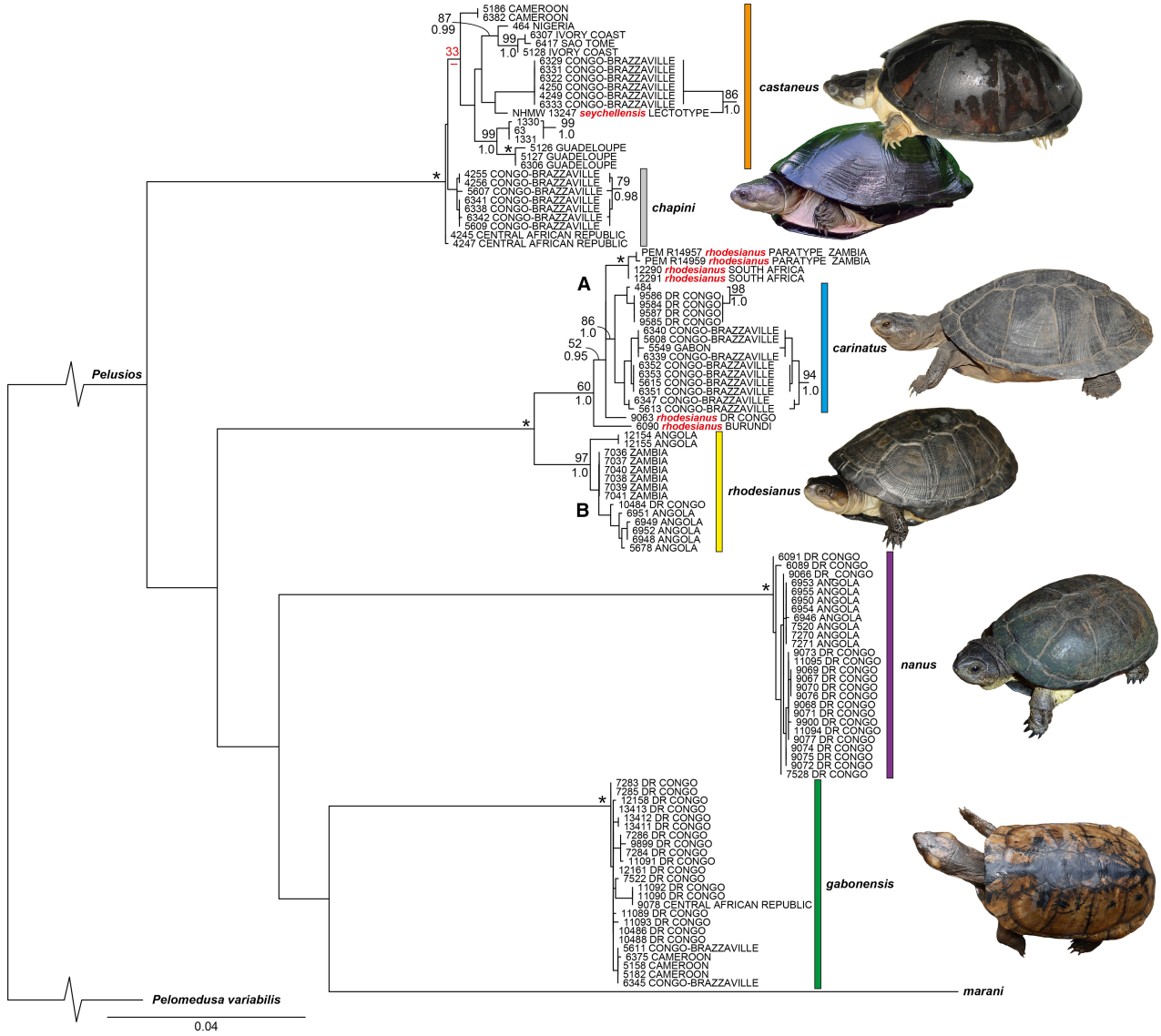

Figure 2. Maximum Likelihood tree using up to 2117 bp of mtDNA (12S, cyt *b*, ND4 + tRNAs) for the six studied *Pelusios* species plus *P. marani*, rooted with *Pelomedusa variabilis*. Root length shortened by 80%. A and B designate the two clades in which sequences of *P. rhodesianus* cluster (see text). Numbers preceding country names are lab codes or voucher numbers; for further explanation, see Table S1. Except for *P. castaneus* and terminal clades with short branch lengths, support values equal to or greater than 50 (bootstrap) and 0.95 (Bayesian posterior probabilities) are shown at nodes. Maximum support under both methods is symbolized by asterisks. For space reasons, support values for some clades shown at mirrored branches (right). Note the weak support for monophyly of *P. castaneus* (red support value, the minus symbol indicates that this branch was not found by the Bayesian 50% majority rule tree). *Pelusios rhodesianus* clustering with *P. carinatus*, and placement of the lectotype of *P. seychellensis*, highlighted in red.

the close relationship of *P. carinatus* and *P. rhodesianus* was well supported under both approaches. Maximum Likelihood suggested that *P. castaneus* and *P. chapini* together represent the sister clade of the remaining *Pelusios* species under study (Fig. 2). In contrast, Bayesian inference favoured a weakly supported sister group relationship (posterior probability: 0.94) of *P. castaneus* and *P. chapini* and the well supported clade containing *P. carinatus* and *P. rhodesianus*; *P. gabonensis*, *P. nanus* and *P. marani* were then the successive sister taxa.

Within *P. chapini*, *P. nanus* and *P. gabonensis* only shallow divergences were found, while *P. castaneus*, *P. carinatus* and *P. rhodesianus* (Fig. 2) showed pronounced differences suggestive of phylogeographic structuring. In the paraphyletic clade

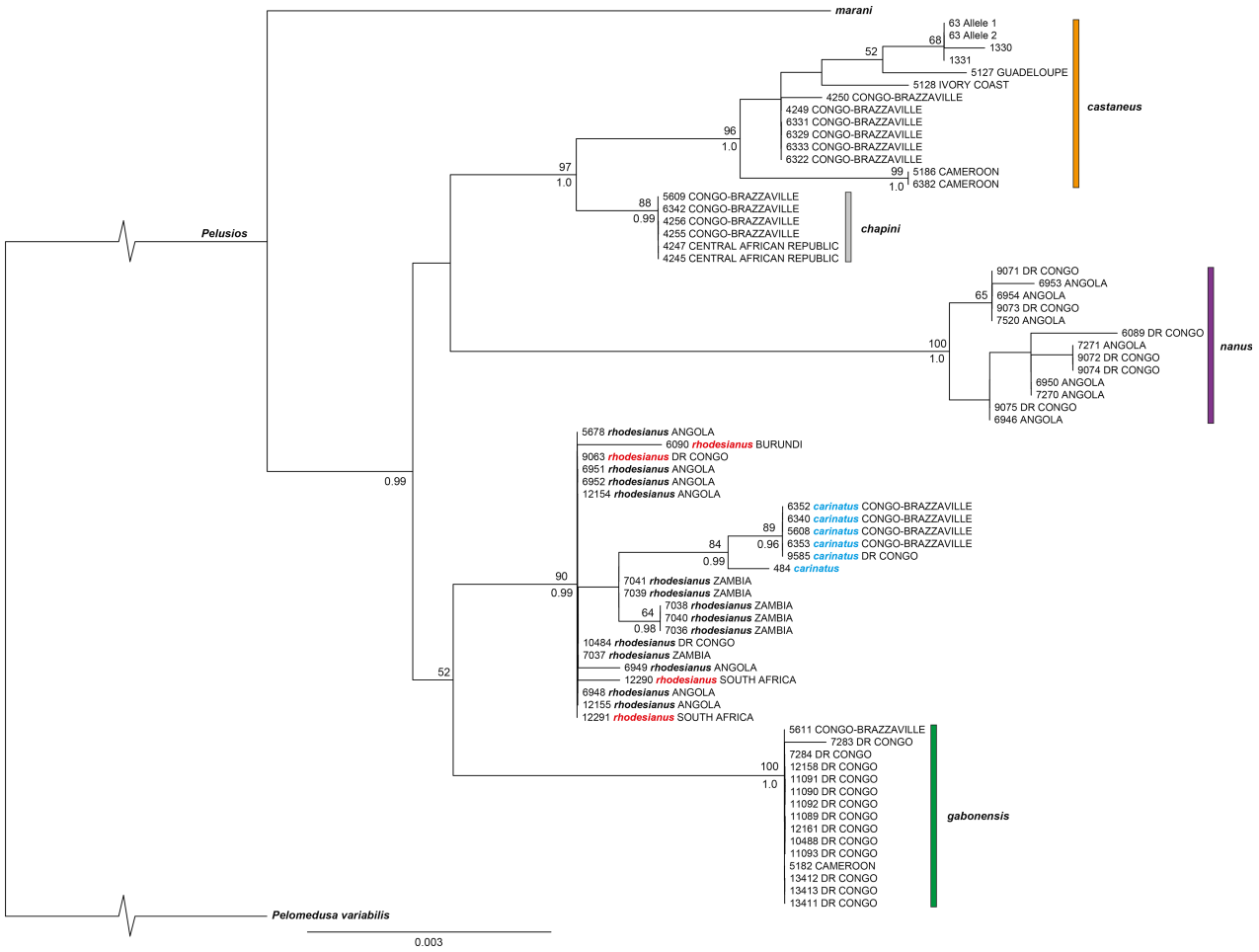

Figure 3. Maximum Likelihood tree using up to 2012 bp of nDNA (C-mos, R35, Rag2) for the six studied *Pelusios* species plus *P. marani*, rooted with *Pelomedusa variabilis* (based on samples for which all three nuclear loci were available). Root length is shortened by 50%. The topology of the Bayesian 50% majority rule tree was identical. The *Pelusios rhodesianus* samples in red correspond to those mitochondrial sequences clustering with *P. carinatus* (blue). For further explanation, see Fig. 2.

comprised of *P. castaneus* and *P. chapini*, the divergences within *P. castaneus* exceeded the difference between *P. castaneus* and *P. chapini*. The concatenated sequences of the lectotype of *P. seychellensis* were always embedded within *P. castaneus*, supporting the synonymy of the two species (Stuckas *et al.*, 2013). Sequences of two *P. castaneus* from Cameroon were distinct from the remaining *P. castaneus* and came out as the sister group of these in Maximum Likelihood analyses. The remaining *P. castaneus* corresponded to several well supported clades. One clade contained terrapins from West Africa (Ivory Coast, Nigeria) and São Tomé, and another one corresponded to all sequences from Congo-Brazzaville (which represent the same haplotype). The terrapins from Congo-Brazzaville were, with high support, sister to the lectotype of *P. seychellensis*, and three samples from terrapins from the pet trade represented the sister clade of another three terrapins from the introduced population of Guadeloupe, Lesser Antilles.

The more inclusive clade comprised of sequences of *P. castaneus* and *P. chapini* received maximum support under both tree-building methods, as did the clades corresponding to sequences of *P. nanus* and *P. gabonensis*.

Unexpected variation was found with respect to *P. carinatus* and *P. rhodesianus*. Sequences of *P. carinatus* constituted a well supported clade. However, six sequences of *P. rhodesianus* (highlighted in red in Fig. 2) were only slightly divergent from *P. carinatus* and were paraphyletic to *P. carinatus* (clade A in Fig. 2). Among the *P. rhodesianus* clustering with *P. carinatus* were also the sequences of the two paratypes of *P. rhodesianus*. The remaining sequences of *P. rhodesianus* were placed

in the well supported distinct clade B (Fig. 2), which was sister to clade A. The two distinct mtDNA variants of *P. rhodesianus* occur in Zambia and the southeastern Democratic Republic of the Congo in close proximity. The sampling sites of the terrapins clustering with *P. carinatus* are far away from the distribution range of the latter species and three of them represent our easternmost sites for *P. rhodesianus* (Fig. 1). Within *P. carinatus*, samples from Congo-Brazzaville and Gabon corresponded to a well supported clade, while samples from the westernmost Democratic Republic of the Congo were placed together with a sample of unknown geographical provenance in another well supported clade (Fig. 2).

Unlike the mitochondrial trees, the deep branching patterns were much better resolved in the nDNA trees (Fig. 3). Both tree-building methods delivered identical topologies, and *P. castaneus* and *P. chapini* constituted well supported, reciprocally monophyletic sister taxa. Also *P. nanus* and *P. gabonensis* represented well supported clades, while *P. carinatus* was returned as a well supported clade nested in *P. rhodesianus*, rendering the latter species paraphyletic. The sequences of the *P. rhodesianus* samples clustering for mtDNA with *P. carinatus* (highlighted in red in Fig. 3) were not differentiated from the remaining samples of *P. rhodesianus*. The divergences within this paraphyletic *P. carinatus*/*P. rhodesianus* clade resembled the divergences within *P. castaneus* and *P. nanus*.

When the mitochondrial and nuclear data sets were combined for tree calculation, the trees resulting from the two data sets (71 samples: all three nuclear loci available; 79 samples: including eight additional samples, for which only one or two nuclear loci were available) were virtually identical. Thus, the trees including more samples are presented here (Fig. S1). Generally, the trees based on the combined mitochondrial and nuclear evidence resembled the mtDNA trees in that deep nodes were weakly resolved. However, now *P. castaneus* and *P. chapini* were reciprocally monophyletic, and both clades received high support. As in the mitochondrial trees, *P. carinatus* was nested in some samples of *P. rhodesianus* (clade A) and the remaining samples of *P. rhodesianus* constituted another clade (B) being sister to this paraphyletic clade A. Both clades A and B were well supported.

Haplotype Networks

Under the default 95% connection limit, the parsimony network analyses of phased nuclear sequences (Fig. 4) resulted for the C-mos gene and the intron 1 of the R35 gene in two networks each, while all Rag2 sequences were connected in one network. Compared to the intron, the networks of the two protein-coding genes (C-mos, Rag2) showed distinctly less variation.

For C-mos (Fig. 4: top), the sequences of *P. castaneus* and *P. chapini* corresponded to a disconnected network; all sequences of *P. chapini* represented the same haplotype which differed by one mutation step from the most frequent haplotype of *P. castaneus*. The latter species was represented by five haplotypes that differed in up to three mutation steps. The sequences of the remaining *Pelusios* species represented the other network, with two unique haplotypes for *P. gabonensis* and three unique haplotypes for *P. nanus*. The haplotypes of either species differed from one another by one or two mutation steps and by a minimum of four or five mutations from the haplotypes of the other species in this network. *Pelusios rhodesianus* was also represented by

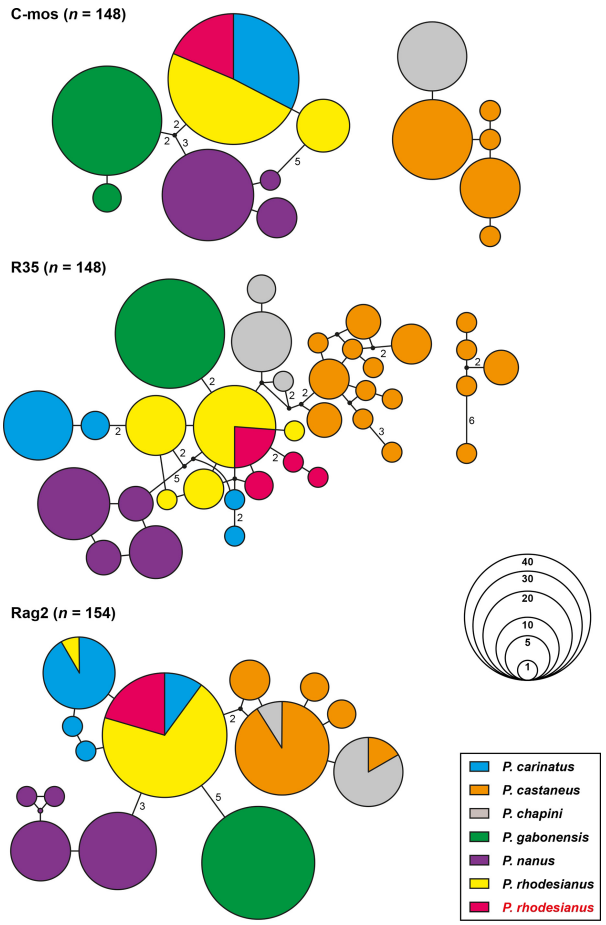

Figure 4. Parsimony networks for phased nDNA sequences. Symbol sizes reflect haplotype frequencies. Small black circles are missing node haplotypes; each line connecting two haplotypes corresponds to one mutation step if not otherwise indicated by numbers.

two haplotypes differing by one step; the more frequent haplotype occurred both in *P. rhodesianus* of clade A and B. All *P. carinatus* shared the same haplotype.

For the intron 1 of the R35 gene (Fig. 4: centre), five haplotypes of *P. castaneus* constituted a network distinct from another one comprised of the remaining haplotypes of *P. castaneus* and all haplotypes of the other studied *Pelusios* species. The disconnected network of *P. castaneus* corresponded to those sequences having the 16-bp-long deletion. In the network of sequences without this deletion, *P. gabonensis* was represented by one haplotype only, while all other species showed more variation. Among different species, no shared haplotypes occurred; however, haplotypes of *P. carinatus* were connected with different haplotypes of *P. rhodesianus*, and the haplotypes of *P. rhodesianus* separated the haplotypes of *P. carinatus* in two groups including tip haplotypes. Sequences of the two genetic forms of *P. rhodesianus* were assigned to a common shared haplotype and four or three unique haplotypes of each form.

For Rag2 (Fig. 4: bottom), *P. gabonensis* and *P. nanus* were represented by unique haplotypes. In contrast, haplotype sharing occurred between *P. castaneus* and *P. chapini* and between *P. carinatus* and *P. rhodesianus*. Most sequences of the two genetic forms of *P. rhodesianus* represented the same haplotype, which occurred also in *P. carinatus*. However, one sequence of *P. rhodesianus* was also assigned to the most frequent haplotype of *P. carinatus*.

Uncorrected *P* Distances

Average uncorrected *P* distances of the cyt *b* gene ranged between *Pelusios* species from 1.38% to 16.54% and within species, values up to 1.50% occurred (Table 1). Among the focal species of the present study, the lowest values were observed between *P. castaneus* and *P. chapini* (4.34%) and between *P. carinatus* and *P. rhodesianus*. The sequences of the *P. rhodesianus* clustering with *P. carinatus* (clade A) differed from *P. carinatus* on average by 2.49%, while the *P. rhodesianus* of clade B differed from *P. carinatus* by 6.22%. Sequences from the *P. rhodesianus* representing the two different clades had an average divergence of 4.04%.

DISCUSSION

With the present investigation, phylogeographic data are available for the majority of the wide-ranging *Pelusios* species. In a previous study, Fritz *et al.* (2013) found for two savannah species from East Africa negligible (*Pelusios castanoides*) or no phylogeographic differentiation (*P. subniger*), suggesting that this could be a general pattern for hinged terrapins from savannah habitats. However, *P. subniger* turned out to consist of two distinct, morphologically cryptic species, and a similar situation could refer to the wide-ranging East African *P. sinuatus*, for which Fritz *et al.* (2011) found two deeply divergent lineages, even though only three samples from Botswana and South Africa were studied. In the present investigation, we found virtually no phylogeographic structuring in *P. chapini*, *P. gabonensis* and *P. nanus*, i.e. for one species each from forest or mesic savannah habitats, from savannah habitats or intermediate habitats, respectively (Fig. 1).

For *P. chapini* and *P. nanus* we have sampled terrapins from only few sites. Yet, these represent populations close to the westernmost and easternmost edges of their distribution ranges (Fig. 1). The westernmost and easternmost sites for *P. chapini* are approximately 1800 km distant, and for *P. nanus*, 1100 km. It would be completely unexpected when the unstudied geographically intermediate populations were genetically differentiated. Thus, we are confident that the observed lacking phylogeographic structure reflects a real pattern and does not result from insufficient sampling.

In contrast to the three aforementioned species, *P. carinatus*, *P. castaneus* and *P. rhodesianus* show phylogeographic differentiation (Figs 2, 3, and S1). However, there is no obvious correlation between habitat type and phylogeographic pattern, with one savannah species showing phylogeographic structure (*P. rhodesianus*), the other (*P. nanus*) not, and with one species inhabiting forests and mesic savannahs (*P. carinatus*) having phylogeographic structure, the other (*P. gabonensis*) not. The same applies for the two ecologically intermediate species, with phylogeographic structure present in *P. castaneus* and absent in *P. chapini*.

Mitochondrial DNA sequences of *P. carinatus* clustered in two well supported clades, one comprised of samples from Congo-Brazzaville and Gabon and the other contained, beside a sample of unknown origin, samples from the western Democratic Republic of the Congo (Fig. 2). The collection sites of the samples of the two clades are separated by the Congo River, which has in this region a width of approximately 12 km and a strong current. Even though *P. carinatus* is a freshwater turtle, it can be speculated that this wide river constitutes a geographical barrier. However, for *P. gabonensis* we have also studied samples from both sides of the Congo River, and in this species we detected no differentiation paralleling *P. carinatus*. Little information is known about the natural history of most *Pelusios* species (Ernst *et al.*,

Table 1. Average uncorrected *P* distances (percentages) for 795 bp of the cyt *b* gene of *Pelusios* species using data of the present study and Fritz *et al.* (2011, 2012a, 2013) and Stuckas *et al.* (2013). Between-group divergences below diagonal; within-group divergences on the diagonal in boldface; *n* = number of sequences. In *P. subniger* is the putative cryptic species from the Democratic Republic of the Congo (*n* = 2) included. It differs from other *P. subniger* by an average distance of 3.13% (Petzold *et al.*, 2014). For the second genetic lineage of *P. sinuatus*, no cyt *b* data are available (Fritz *et al.*, 2011; Petzold *et al.*, 2014)

	n	*ada*	*bec*	*bro*	*car*	*c'us*	*c'es*	*cha*	*cup*	*gab*	*mar*	*nan*	*nig*	*rho* A	*rho* B	*sin*	*sub*	*upe*	*wil*
adansonii	1	**n/a**																	
bechuanicus	2	11.59	**0**																
broadleyi	7	3.05	13.15	**0**															
carinatus	15	9.92	12.91	11.17	**0.56**														
castaneus	19	6.52	13.70	7.55	11.48	**1.50**													
castanoides	29	9.57	11.50	9.88	10.99	11.17	**0.57**												
chapini	8	6.03	15.91	10.17	13.95	4.34	12.35	**0.23**											
cupulatta	4	10.98	10.79	11.24	11.26	12.14	10.76	15.03	**0.19**										
gabonensis	24	12.73	13.62	13.68	12.84	13.52	12.54	12.42	13.09	**0.88**									
marani	5	12.80	11.21	12.68	13.01	13.57	12.50	15.35	11.82	13.75	**0.05**								
nanus	26	11.59	14.99	15.07	15.31	15.36	12.21	12.94	14.09	12.42	15.25	**0.14**							
niger	2	11.76	13.38	13.90	12.25	15.17	11.85	15.77	9.10	12.73	14.14	13.07	**0**						
rhodesianus A	6	10.81	13.45	11.39	2.49	11.83	11.12	10.92	12.28	11.43	14.19	13.24	12.57	**0.97**					
rhodesianus B	14	7.93	14.23	11.93	6.22	12.39	12.12	10.63	13.44	12.09	14.58	12.41	12.77	4.04	**0.57**				
sinuatus	2	14.02	11.42	13.05	12.52	14.01	13.60	16.32	11.26	13.95	11.02	15.73	14.34	13.92	13.96	**0.13**			
subniger	41	14.57	5.33	12.25	12.70	13.50	11.89	15.28	10.92	13.15	11.21	13.72	11.64	13.50	13.55	10.08	**0.33**		
upembae	3	10.98	1.38	13.15	13.04	14.41	11.50	16.54	10.91	14.01	11.82	14.99	13.56	14.56	13.54	11.80	5.43	**0**	
williamsi	2	9.15	10.91	10.87	10.23	11.03	3.89	15.00	11.01	14.11	11.33	15.73	13.69	13.97	10.77	13.31	12.28	11.31	**0**

2000; Maran & Pauwels, 2005, 2007, 2009; Branch, 2008; Maran, 2009, 2010). Therefore, it cannot be excluded that these phylogeographic differences are associated with different habitat preferences.

Furthermore, samples of *P. castaneus* showed considerable geographical structuring (Figs 2, 3, and S1). Conversely, mtDNA sequences of *P. castaneus* and *P. chapini* were only weakly differentiated. *Pelusios chapini* has originally been described as a subspecies of *P. castaneus* by Laurent (1965) and elevated as a full species by Bour (1983). Both taxa are morphologically similar and differ mainly in maximum size and geographical distribution. While *P. castaneus* has a reported maximum shell length of 28.5 cm (Branch, 2008), *P. chapini* reaches up to 38 cm (Bour, 1983; Ernst *et al.*, 2000; Branch, 2008). The weak mitochondrial differentiation of the two taxa supports at first glance that their original subspecies status should be reinstated (Fritz *et al.*, 2011), with divergences between *P. castaneus* and *P. chapini* resembling the range of variation within *P. castaneus* alone (Fig. 2). However, nuclear genomic evidence suggests that both taxa are distinct. In phylogenetic analyses of nDNA, both taxa are reciprocally monophyletic (Fig. 3) causing also in combined analyses of mtDNA and nDNA the same topology (Fig. S1). For the three studied nuclear DNA blocks, we observed shared haplotypes only for the Rag2 gene (Fig. 4), but it is hard to evaluate whether this reflects incomplete sorting or introgression. However, the abutting and partially overlapping distribution ranges of the two species (Fig. 1) and their similarity in mtDNA support the possibility of introgression. To clarify whether both taxa are reproductively isolated or whether there is ongoing gene flow, further research is needed. The application of additional molecular markers, such as rapidly evolving microsatellite loci or SNPs, and dense sampling across the contact zone would be promising approaches here (cf. Vamberger *et al.*, 2015).

Within *P. castaneus*, a morphologically highly variable species (Maran, 2009; Maran & Pauwels, 2009), we identified several geographically vicariant clades, which are reflected by mitochondrial and nuclear markers (Figs 2, 3). The most distinct samples of *P. castaneus* originate from Cameroon, but also samples from West Africa (Ivory Coast and Nigeria, plus the island of São Tomé), from Congo-Brazzaville and Guadeloupe turned out to be distinct. Another clade corresponds to three samples from the pet trade with unknown geographical provenance, and the concatenated mitochondrial sequences of the lectotype of *P. seychellensis*, generated for a previous paper (Stuckas *et al.*, 2013), represent another distinct branch being sister to the samples of *P. castaneus* from Congo-Brazzaville. This pronounced phylogeographic structuring of *P. castaneus* could be associated with the Pleistocene fluctuations of the forest cover in West and Central Africa (Hamilton & Taylor, 1991; Maley, 1996; Primack & Corlett, 2005), which might have led to the isolation and genetic differentiation of terrapin populations in distinct refugia.

The type sequences of *P. seychellensis* are phylogenetically firmly embedded within *P. castaneus* (Fig. 2), supporting that *P. seychellensis* does not represent an extinct species, as supposed by Bour (1983), but is in fact a junior synonym of *P. castaneus* (Stuckas *et al.*, 2013). In addition, there is no hard evidence that the type series of *P. seychellensis* has been collected on Mahé, Seychelles, as assumed by Bour (1983, 2013), and an erroneous identification of the collection site remains the most likely explanation for associating the type series of *P. seychellensis* with the Seychelles (Stuckas *et al.*, 2013). Nevertheless, the recent turtle checklist of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group recognizes now the alleged Seychelles taxon as an extinct subspecies of *P. castaneus*, for whose unproven former occurrence on the Seychelles human introduction is considered (van Dijk *et al.*, 2014). According to this taxonomic arrangement, *P. castaneus* consists of two subspecies, the extinct *P. c. seychellensis* and the nominotypical subspecies *P. c. castaneus*, which comprises all other populations of *P. castaneus*. However, some of the latter populations are genetically more differentiated than the lectotype of *P. seychellensis* (Fig. 2), and the phylogenetic placement of the alleged Seychelles taxon renders the nominotypical subspecies paraphyletic. This entire situation clearly argues against the recognition of a distinct subspecies *P. c. seychellensis*, and we conclude that *P. castaneus* should be treated as a monotypic species with *P. seychellensis* as a junior synonym.

For the species *P. carinatus* and *P. rhodesianus*, our present study revealed a completely unexpected mitochondrial differentiation pattern, with some samples of *P. rhodesianus* clustering with *P. carinatus* (clade A in Fig. 2) and the remaining samples of *P. rhodesianus* (clade B in Fig. 2) representing the deeply divergent sister group of clade A. There are only small genetic divergences between the samples of *P. rhodesianus* and *P. carinatus* in clade A, and these clade A samples of *P. rhodesianus* are paraphyletic with respect to *P. carinatus* (Figs 2 and S1).

To explore this situation in more detail, divergences of the cyt *b* gene of both forms of *P. rhodesianus* can be compared with *P. carinatus* and all other species of *Pelusios* (Table 1). Uncorrected *P* distances of this gene, and of other mitochondrial genes, are often used

as a yardstick to infer taxonomic differentiation of chelonians (e.g., Engstrom, Shaffer & McCord, 2002; Fritz *et al.*, 2008, 2012b; Daniels *et al.*, 2010; Ennen *et al.*, 2010; Vargas-Ramírez *et al.*, 2010; Kindler *et al.*, 2012; Iverson, Le & Ingram, 2013; Martin *et al.*, 2013; Petzold *et al.*, 2014; Thomson *et al.*, 2015) and other reptiles (Torstrom, Pangle & Swanson, 2014), in analogy to the widely used DNA barcoding approach (e.g., Hebert, Ratnasingham & de Waard, 2003). The application of such divergence values is based on the observation that deeply divergent lineages correspond to distinct taxa. However, the opposite is not necessarily true. There are a number of cases known in which the mtDNA of distinct chelonian species differs not or only negligibly, reflecting either recently split species, slow evolutionary rates, mitochondrial introgression or oversplit species (Chelidae: *Mesoclemmys*: Vargas-Ramírez *et al.*, 2012; Emydidae: *Emys*: Fritz *et al.*, 2006; *Graptemys*: Ennen *et al.*, 2010; *Pseudemys*: Spinks *et al.*, 2013; *Trachemys*: Fritz *et al.*, 2012b; Geoemydidae: *Cuora*: Spinks & Shaffer, 2007; *Cyclemys*: Fritz *et al.*, 2008; *Rhinoclemmys*: Vargas-Ramírez, Carr & Fritz, 2013). With keeping all these limitations in mind, comparisons of mtDNA sequence divergences can provide additional insights and 'point to taxa that need additional study' (Shen, Chen & Murphy, 2013).

The samples of *P. rhodesianus* from clade B differ by 4.04% from the *P. rhodesianus* in clade A, and by 6.22% from *P. carinatus*, and these values resemble or exceed the divergences between *P. adansonii* and *P. broadleyi* (3.05%), *P. adansonii* and *P. castaneus* (6.52%), *P. adansonii* and *P. chapini* (6.03%), *P. bechuanicus* and *P. subniger* (5.33%), *P. bechuanicus* and *P. upembae* (1.38%), *P. castaneus* and *P. chapini* (4.34%), *P. castanoides* and *P. williamsi* (3.89%), and between *P. subniger* and *P. upembae* (5.43%). This suggests that the *P. rhodesianus* of clade A and clade B represent distinct species, and that the *P. rhodesianus* of clade B are not conspecific with *P. carinatus*.

The divergence value for *P. carinatus* and the *P. rhodesianus* in clade A is much less pronounced (2.49%) and only between *P. bechuanicus* and *P. upembae* an even lower value was observed (1.38%). The latter two species are allopatrically distributed and *P. upembae* was originally described as a subspecies of *P. bechuanicus* (Broadley, 1981), raising the possibility that their low divergence value reflects rather intraspecific than interspecific variation. Thus, based on sequence divergences, we cannot rule out that *P. carinatus* and the *P. rhodesianus* clustering in clade A are conspecific. However, considering their widely separated distribution ranges (Fig. 1) and their morphological distinctiveness, this hypothesis does not seem very likely.

Pelusios carinatus and *P. rhodesianus* are morphologically easy to tell apart. Both species differ in shell shape, coloration and pattern (Ernst *et al.*, 2000; Branch, 2008). In particular, younger individuals of *P. carinatus* possess a well developed vertebral keel, combined with a characteristic laterally compressed shell, while juveniles and adults of *P. rhodesianus* lack a pronounced vertebral keel and have a relatively flatter, broader shell. All *P. rhodesianus* examined by us conform to this morphological characterization, irrespective of their genetic assignment. Unfortunately, the low number of *P. rhodesianus* specimens from clade A prevents us from an in-depth morphological comparison with terrapins from clade B. Broadley (1981) pointed out that two different types of head coloration exist in *P. rhodesianus*. Accordingly, terrapins from north of the South Equatorial Divide generally have heads with a vermiculated yellow and brown pattern. Terrapins from south of the Divide are thought to be plain-headed, with heads blackish brown above and lighter sides. Based on two genetically distinct samples of *P. rhodesianus*, representing each clade of the present paper, Fritz *et al.* (2011) speculated that the different genetic lineages may correspond to Broadley's coloration types. However, neither the geographical distribution of our new samples nor their morphology corroborated this. Moreover, the studied terrapins for which vouchers or photos were available did match neither with Broadley's scheme nor with the genetic groups of *P. rhodesianus* (Table 2). All *P. rhodesianus* from Angola were plain-headed, including the ones from Uíge province, which should have vermiculated heads, and the terrapins from KwaZulu-Natal, South Africa, which should be plain-headed, had vermiculated heads or an intermediate pattern (Fig. 5). The two head pattern types occurred in both genetic groups of *P. rhodesianus* (Table 2). Thus, the identification of the two genetic groups of *P. rhodesianus* with any of the morphotypes of Broadley (1981) can be rejected. Moreover, our few data on the head pattern conflict with Broadley's delimitation of the distribution ranges of the two morphotypes.

In conclusion, it seems likely that the two genetic groups of *P. rhodesianus* correspond to distinct cryptic species, and one of these is more closely related to *P. carinatus* (Figs 2 and S1). However, our phylogenetic analyses of nuclear markers did not discriminate the two mitochondrial groups of *P. rhodesianus*, and the nuclear sequences of *P. rhodesianus* are paraphyletic with respect to *P. carinatus* (Fig. 3). Yet, the most variable nuclear marker, the intron 1 of the R35 gene, shows private haplotypes for both groups (Fig. 4), which could argue for an incipient differentiation process. With respect to mtDNA

Table 2. Genetic allocation and head pattern of *Pelusios rhodesianus* studied (morphological data are not available for the paratypes, which are shells). For exact locality data and accession numbers, see Table S1

Lab code	Provenance	Clade	Head pattern
6951	Angola: Bié	B	Plain
6952	Angola: Bié	B	Plain
5678	Angola: Bié	B	Plain
12154	Angola: Uíge	B	Plain*
12155	Angola: Uíge	B	Plain*
6090	Burundi	A	Vermiculated
10484	Democratic Republic of the Congo: Katanga	B	Vermiculated
9063	Democratic Republic of the Congo: Katanga	A	Vermiculated
12290	South Africa: KwaZulu-Natal	A	Vermiculated*
12291	South Africa: KwaZulu-Natal	A	Intermediate*
7041	Zambia: Nort-Western Province	B	Plain
7040	Zambia: Nort-Western Province	B	Plain
7036	Zambia: Nort-Western Province	B	Plain
7037	Zambia: Nort-Western Province	B	Plain
7038	Zambia: Nort-Western Province	B	Plain
7039	Zambia: Nort-Western Province	B	Plain

*Conflicting head patterns compared with the scheme of Broadley (1981).

sequences, only the *P. rhodesianus* of clade A are paraphyletic with respect to *P. carinatus*, while clade B containing the remaining sequences of *P. rhodesianus* is sister to the paraphyletic clade A (Fig. 2). Considering the available evidence, we are reluctant to recognize the two clades comprising sequences of *P. rhodesianus* as distinct species.

As outlined above, *P. carinatus* and *P. rhodesianus* are morphologically clearly different (Ernst *et al.*, 2000; Branch, 2008) and their conspecificity has never been considered before. The distribution ranges of the two species overlap in the western Congo region (Fig. 1). Thus, one explanation for the *carinatus*-like haplotypes of *P. rhodesian*us could be hybridization, with mitochondrial introgression from *P. carinatus* into *P. rhodesianus*. However, if so, it would be expected that our western representatives of *P. rhodesianus* cluster with *P. carinatus*, but just the opposite is the case: All seven samples from the western range of *P. rhodesianus* (Angola) represent clade B, which is sister to clade A comprised of *P. carinatus* and *P. rhodesianus*. In contrast, the *P. rhodesianus* samples clustering with *P. carinatus* are from the central and eastern part of the range of *P. rhodesianus* (Figs 2 and S1) and include, besides the paratypes of *P. rhodesianus* from Zambia, samples from the southeastern Democratic Republic of the Congo (Katanga province), Burundi and northeastern South Africa (KwaZulu-Natal). The two genetic groups of *P. rhodesianus* seem to overlap in Katanga and Zambia (Fig. 1). The minimal distance between *P. carinatus* and the *P. rhodesianus* clustering with *P. carinatus* is approximately 1300 km, and the isolated populations of *P. rhodesianus* in KwaZulu-Natal occur approximately 3300 km away from the range of *P. carinatus*. These large distances make it unlikely that introgression caused the observed pattern, and it is also hard to imagine that the eastern representatives of *P. rhodesianus* (clade A) should be conspecific with *P. carinatus*.

One alternative explanation could be incomplete lineage sorting, i.e. ancestral polymorphism. In that case, the sequences of *P. rhodesianus* clustering in clades A and B would represent deeply divergent conspecific mitochondrial lineages. Then, the resemblance of clade A sequences were the heritage of the last common ancestor of *P. rhodesianus* and *P. carinatus*. However, to the best of our knowledge, there is not a single comparable case of ancestral polymorphism with such deeply divergent mitochondrial lineages known among chelonians. Thus, this hypothesis also does not seem to be very likely.

We have also considered the possibility that the sequences of *P. rhodesianus* clustering either in clade A or B do not represent authentic mitochondrial DNA, but instead a numt, i.e. a nuclear genomic insertion of mtDNA. Numts are known to occur in a number of turtle species (Emydidae: Fritz *et al.*, 2012b; Geoemydidae: Stuart & Parham, 2004; Spinks & Shaffer, 2007; Testudinidae: Fritz *et al.*, 2010; Kindler *et al.*, 2012). Yet, all cases known to date concern representatives of the second extant suborder of turtles (Cryptodira), while *Pelusios* belongs to the very distantly related suborder Pleurodira.

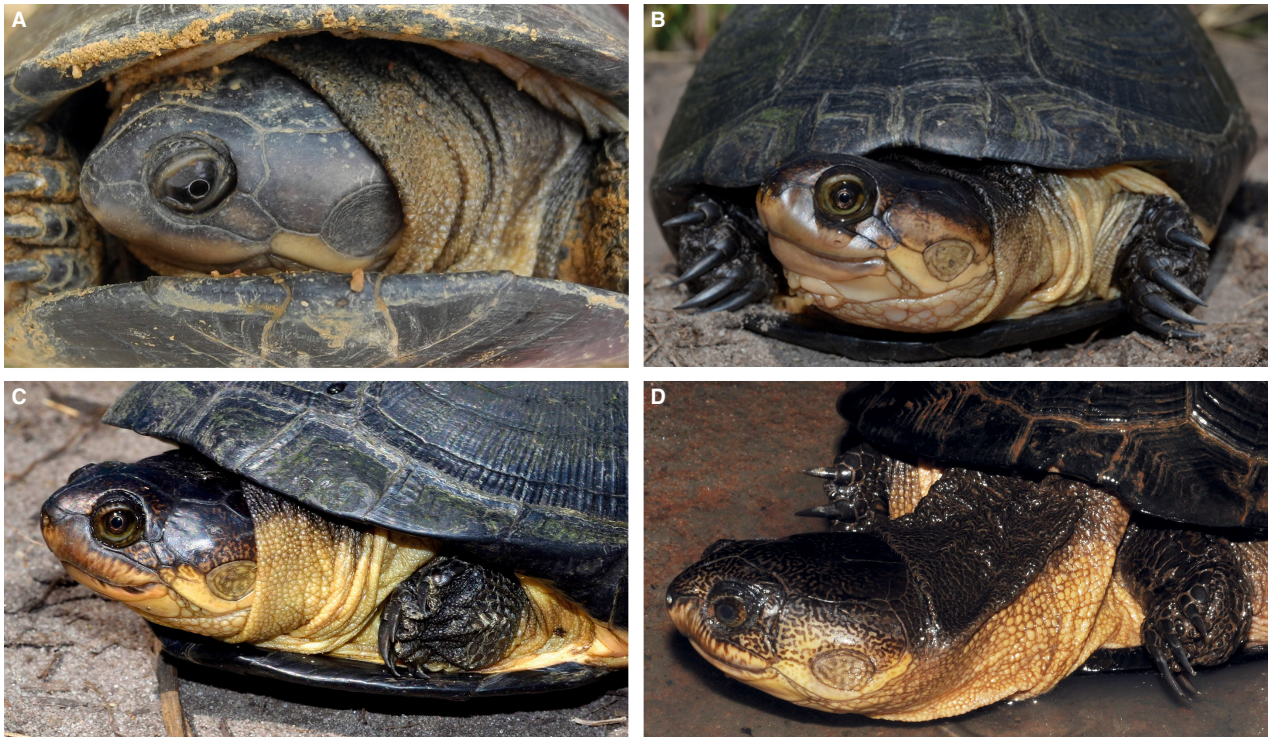

Figure 5. Head patterns in *Pelusios rhodesianus*: (A) plain-headed terrapin from Uíge, Angola, sample 12154, clade B; (B) terrapin with intermediate head pattern and (C) with vermiculated head, both from the vicinity of Mtubatuba, KwaZulu-Natal, South Africa, samples 12290 and 12291, both clade A; (D) terrapin with vermiculated head from Kundelungu National Park, Katanga, Democratic Republic of the Congo, sample 10484, clade B. Photographs: Matthias Nuß (A), James Harvey (B, C), Jos Kielgast (D).

Moreover, we generated for *P. rhodesianus* sequences of both clade A and clade B using different primer combinations, and the resulting sequences were always consistent and showed no abnormalities, such as frame-shift mutations, deletions or stop codons, typical for numts. Therefore, we conclude that we have sequenced authentic mtDNA.

Currently, we cannot resolve this intricate situation and a more comprehensive study using additional nuclear markers and more samples of *P. carinatus* and both groups of *P. rhodesianus* is needed. From a purely nomenclatural point of view it is at least clear that the name *Pelusios rhodesianus* Hewitt, 1927 refers to terrapins from clade A. Even though we did not succeed in sequencing the name-bearing holotype, the two topotypic paratypes yielded sequences of good quality for all three studied mtDNA fragments. This allows for the unambiguous assignment of the name according to the phylogenetic placement of the paratypes (Fig. 2). Thus, if the terrapins of clade B should be deemed as another species in future, they would have to be described as a new species.

Our present study underlines that for resolving such difficult cases mitochondrial markers alone may lead to incomplete or erroneous inferences about phylogeography and taxonomy.

Until now, there are only few phylogeographic studies for African terrapins and tortoises (*Pelusios*: Fritz *et al.*, 2013; this study; *Chersina*: Daniels *et al.*, 2007; *Homopus*: Daniels *et al.*, 2010; *Stigmochelys*: Fritz *et al.*, 2010). However, with data becoming available for more and more species, a future challenge will be to assess the role of different drivers (including ecological traits and strategies, cf. Romiguier *et al.*, 2014) for shaping phylogeographic differentiation of African chelonians, as it has been recently done for tropical anurans (Rodríguez *et al.*, 2015).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Maximum Likelihood tree using up to 2117 bp of mtDNA (12S, cyt *b*, ND4 + tRNAs) and 2012 bp of nDNA (C-mos, R35, Rag2) for the six studied *Pelusios* species plus *P. marani*, rooted with *Pelomedusa variabilis*.
Table S1. Successfully studied *Pelusios* samples and outgroups.
Table S2. PCR and sequencing primers for fresh material.
Table S3. Thermocycling conditions for fresh material.
Table S4. PCR and sequencing primers for the type specimens of *Pelusios rhodesianus*.
Table S5. Thermocycling conditions for the type specimens of *Pelusios rhodesianus*.
Table S6. Heterozygous positions in nDNA sequences.
Table S7. Optimal partition schemes and evolutionary models for MRBAYES calculated with PARTITIONFINDER for alignments including outgroups.
Data S1. Material and Methods.
Data S2. Additional References.