



# Phylogeny and phylogeography of chelonians from sub-Saharan Africa—A review of current knowledge in tribute to Margaretha D. Hofmeyr

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## Abstract

Species-level phylogeny and especially phylogeography of African chelonians is a comparatively under-studied field of research. We review the current knowledge of phylogeny and phylogeography, highlight congruence of spatial phylogeographic patterns amongst chelonians and other taxa and suggest future research directions to address gaps in knowledge. Our review shows that phylogeographic and phylogenetic investigations have led to unexpected findings. For example, for *Pelomedusa*, a putatively wide-ranging monotypic terrapin genus, cryptic diversity was revealed, with more than ten species being uncovered. The formerly recognized tortoise genus *Homopus* sensu lato was found to be paraphyletic with respect to *Chersina*. To resolve this situation, *Homopus* was restricted to the four-toed species *H. areolatus* and *H. femoralis* and the genus *Chersobius* was resurrected for the five-toed species *C. boulengeri*, *C. signatus*, and *C. solus*. Three previously recognized taxa were shown to be invalid, viz. the putatively extinct terrapin species *Pelusios seychellensis* and the tortoise subspecies *Chersobius signatus cafer* and *Stigmochelys pardalis babcocki*. Together with taxonomy, the knowledge of phylogeographic structuring sets a solid foundation for conservation measures and allows the identification of Management and Conservation Units. However, the current legislation, in particular the enforcement of the Nagoya Protocol under the Convention of Biological Diversity (CBD), has largely halted research on widely distributed taxa and turned the well-intended concept of Access and Benefit Sharing into a major impediment for conservation and research. The current situation leads for many species to a continued usage of outdated and incorrect taxonomic classifications resulting in an error cascade of conservation decisions. This is counterproductive to the aims of the CBD, that is, the protection of biodiversity. Sequencing historical DNA from museum specimens using aDNA approaches could be a short-term approach to mitigate, but not solve, this impediment.

## Keywords

CBD, Nagoya Protocol, Pelomedusidae, Reptilia, systematics, taxonomy, terrapin, Testudines, Testudinidae, tortoise, Trionychidae, turtle

## Introduction

Sub-Saharan Africa, in particular southern Africa (the continental region south of the Kunene, Okavango, and Zambezi rivers), is renowned for its diversity in tortoises

(Testudinidae). Out of 52 currently recognized extant or recently extinct tortoise species, not fewer than 32 occur in Africa and 30 of these in sub-Saharan Africa (includ-

**Table 1.** Phylogeographic and phylogenetic publications on African chelonians co-authored by Margaretha D. Hofmeyr.

Family	Taxon	Reference
Pelomedusidae	<i>Pelomedusa</i>	Vargas-Ramírez et al. (2010), Fritz et al. (2014, 2015), Petzold et al. (2014), Vamberger et al. (2018)
Pelomedusidae	<i>Pelomedusa</i> , <i>Pelusios</i>	Fritz et al. (2011)
Pelomedusidae	<i>Pelusios sinuatus</i>	Vamberger et al. (2019b)
Testudinidae	<i>Chersina angulata</i>	Daniels et al. (2007), Spitzweg et al. (2020)
Testudinidae	<i>Chersobius signatus</i>	Daniels et al. (2010)
Testudinidae	<i>Cylindraspis</i>	Kehlmaier et al. (2019a)
Testudinidae	<i>Homopus areolatus</i>	Hofmeyr et al. (2020)
Testudinidae	<i>Kinixys</i>	Kindler et al. (2012)
Testudinidae	<i>Psammobates tentorius</i>	Zhao et al. (2020a, 2020b, 2021a)
Testudinidae	<i>Stigmochelys pardalis</i>	Fritz et al. (2010), Spitzweg et al. (2019), Dajčman et al. (2021)
Testudinidae	Testudinidae	Hofmeyr et al. (2017)

ing Madagascar and islands of the western Indian Ocean). Five of these species became extinct since the 18<sup>th</sup> century (*Cylindraspis* spp., Mascarene Islands) due to targeted collection and hunting by humans. Nearly all species living in Africa are endemic to this continent (29), and of these, 14 occur in southern Africa, with 11 species confined to this region. In addition to this unique diversity of tortoises, sub-Saharan Africa and the adjacent Arabian Peninsula are home to five soft-shelled turtles (Trionychidae) and the endemic terrapin family Pelomedusidae with currently 27 recognized species. In addition, Madagascar is home to the only extant Old World representative (*Erymnochelys madagascariensis*) of the Podocnemididae, a family of side-necked turtles now otherwise confined to South America (TTWG 2021).

The past twenty years have seen a steady increase in the knowledge of the phylogenetic relationships and the phylogeography of African tortoises and freshwater turtles. The late Margaretha D. Hofmeyr (1950–2020) was, besides the late William R. Branch (1946–2018), an outstanding promoter of this research, as reflected by their co-authorships in a myriad of studies on the topic (Table 1). While Bill was driven by his genuine interest in taxonomy, Retha's tireless and always enthusiastic engagement bore fruits through her keen insights into the natural history of chelonians. This knowledge base was enhanced by her studies of their genetic diversity and has provided the scientific and conservation community with a strong foundation upon which to base conservation actions. The collections that she made during her life-long work with African tortoises (Fig. 1) are now housed in the Museum of Zoology (Senckenberg Dresden, Germany) and will serve for future phylogeographic and population genetic investigations. In recognition of her life and work, we review publications on the molecular phylogeny and phylogeography of tortoises and freshwater turtles from sub-Saharan Africa with a focus on phylogeography. We compare these studies with other African taxa and suggest further research directions. Regrettably, marine turtles nesting or occurring along African coasts and taxa endemic to Madagascar and islands in the western Indian Ocean have not been included in this review given the mainly global distributions of the former, and

**Figure 1.** Retha Hofmeyr with juvenile *Psammobates geometriacus* during fieldwork in 2015 (photo: Melita Vamberger).

the extremely narrow distributions of the latter that do not include continental Africa.

## Pelomedusidae

### Phylogeny

The Pelomedusidae, endemic to sub-Saharan Africa and the southwestern Arabian Peninsula, are the sister group of the Podocnemididae, extant in South America and

Madagascar. Together with their successive sister group, the Chelidae from South America, Australia, and New Guinea, they constitute the suborder Pleurodira (side-necked turtles; TTWG 2021), which originated on Gondwana (de la Fuente et al. 2014; Pereira et al. 2017). Pelomedusidae evolved in Africa as the result of the breakup of Gondwana (de Lapparent de Broin 2000; de la Fuente et al. 2014; Ferreira et al. 2018). Thus, their occurrence on this continent is not, as for the other turtle groups, the result of dispersal to Africa. The situation is different with respect to the only Old World representative of the Podocnemididae, *Erymnochelys madagascariensis*, from Madagascar. Since Podocnemididae are otherwise confined to South America (TTWG 2021), it could be speculated that their presence in Madagascar also results from vicariance of an old Gondwana taxon (cf. Noonan and Chippendale 2006a, 2006b). However, this is not the case. Podocnemididae dispersed only in the Late Cretaceous to Africa and from there to Madagascar. Closely related turtles even spread to Europe, where they became later extinct, as on the African continent (Pérez-García et al. 2017; Ferreira et al. 2018; de Lapparent de Broin et al. 2020; Pérez-García 2022).

With the seminal papers by Vargas-Ramírez et al. (2010), Wong et al. (2010), and Fritz et al. (2011), the knowledge of the diversity and the phylogenetic relationships of the two pelomedusid genera *Pelomedusa* and *Pelusios* received a considerable boost. Previously, it was believed that *Pelusios* is a speciose genus of 18 species, whereas *Pelomedusa* was thought to be monotypic, with *Pelomedusa subrufa* as the only species (Fritz and Havaš 2007; TTWG 2009). However, two independent studies revealed that *Pelomedusa* comprises deeply divergent genealogical lineages (Vargas-Ramírez et al. 2010; Wong et al. 2010). Ten of these lineages were later formally recognized as distinct species (Fritz et al. 2014; Petzold et al. 2014) and several additional lineages most likely represent additional undescribed species (Fig. 2; Petzold et al. 2014; Fritz et al. 2015; Nagy et al. 2015; Vargas-Ramírez et al. 2016; Vamberger et al. 2018). Within *Pelomedusa*, a southern species group contains two formally recognized species (*Pelomedusa galeata*, *P. subrufa* sensu stricto) plus one unnamed candidate species (see below), whereas the remaining taxa and lineages belong to the northern species group (Vargas-Ramírez et al. 2010; Petzold et al. 2014). The genetic divergences of the individual *Pelomedusa* species equal or exceed those between distinct *Pelusios* species (Fritz et al. 2011).

Within *Pelusios*, the phylogenetic relationships of individual species are more entangled than in *Pelomedusa*, with several species groups and some phylogenetically unique and divergent species (*Pelusios gabonensis*, *P. marani*, *P. nanus*; see Fritz et al. 2011, 2012; Thomson et al. 2021). However, one previously recognized and putatively extinct species (*Pelusios seychellensis*) turned out to be invalid as the original species description had been based on mislabeled museum material. According to its genetic identity, *Pelusios seychellensis* represents a junior synonym of the West and Central African species *P. cas-*

*taneus* (Stuckas et al. 2013; Kindler et al. 2016). Furthermore, the phylogenetic relationships within and between the morphologically distinctive *Pelusios carinatus* and *P. rhodesianus* were unexpected. Both nuclear (nDNA) and mitochondrial DNA (mtDNA) phylogenies suggest that the two taxa are not reciprocally monophyletic (Kindler et al. 2016; see also below). On the other hand, the genetic distinctness of the remaining 15 *Pelusios* species was confirmed (Fritz et al. 2011, 2012; Thomson et al. 2021), even though uncorrected *p* distances of mtDNA were found to be very low between two species pairs (*P. bechuanicus* vs. *P. upembae* and *P. castaneus* vs. *P. chapini*; Petzold et al. 2014; for *P. castaneus* and *P. chapini*, see also below).

## Phylogeography

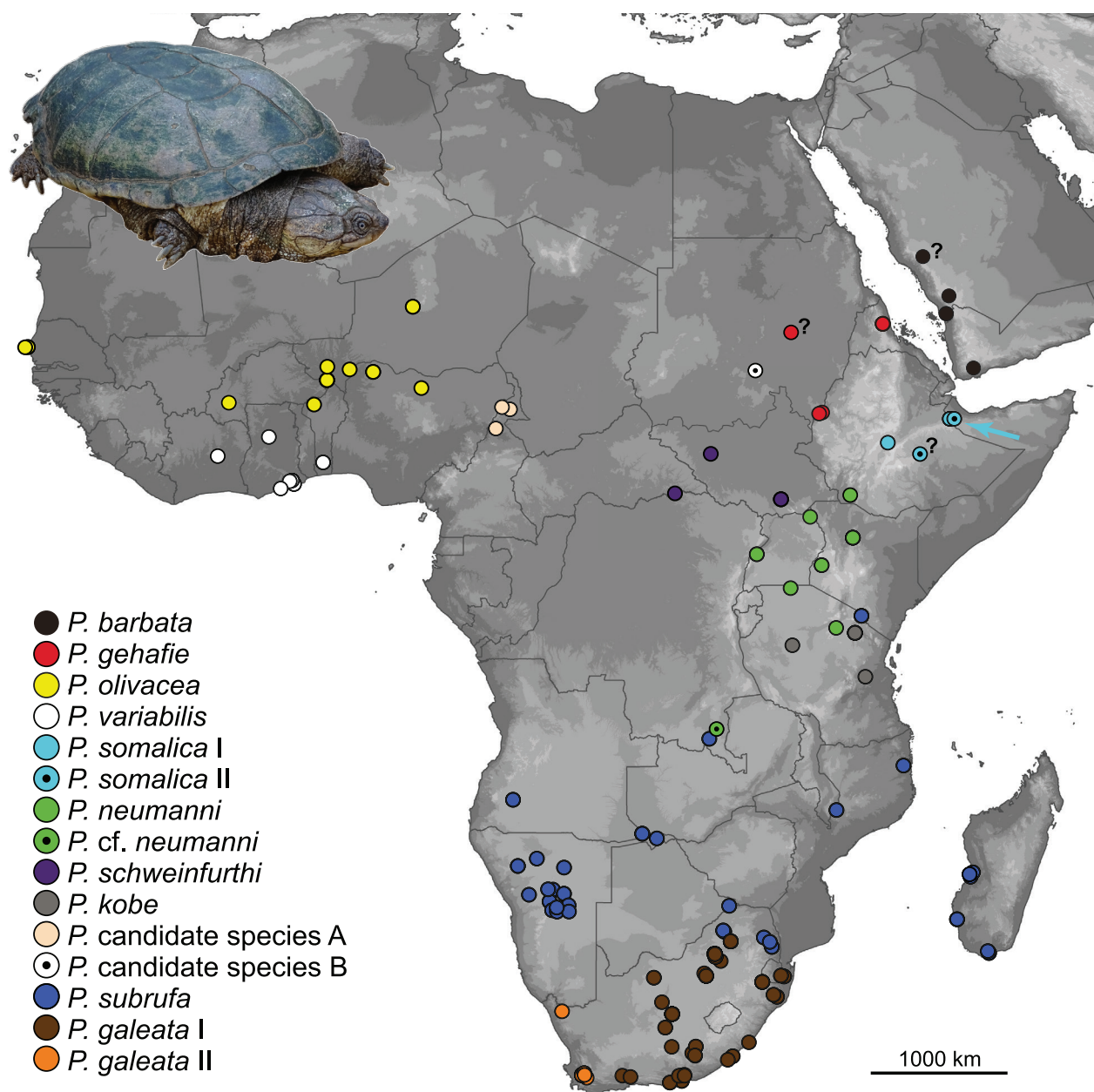
The phylogeographic patterns of the majority of pelomedusid terrapins remain unstudied. For *Pelomedusa*, only species from southern Africa have been examined in detail (Vamberger et al. 2018), and ongoing investigations by Melita Vamberger promise additional insights into fine-scale spatial patterns of genetic diversity.

*Pelomedusa subrufa* sensu stricto is widespread in southern Africa, ranging from southern Angola and Namibia eastward to Mozambique. The species also occurs beyond southern Africa in East Africa and has been introduced in Madagascar (Vargas-Ramírez et al. 2010; Wong et al. 2010; TTWG 2021). In southern Africa, its range marginally extends into northeastern South Africa, and in Limpopo Province, it occurs in close proximity to the morphologically similar species *P. galeata* (Vamberger et al. 2018).

Using 1,850 bp mtDNA and two nuclear loci (1,840 bp), Vamberger et al. (2018) revealed that *P. subrufa* did not show phylogeographic structure, despite its wide distribution range. The lack of structure is likely due to stability of its range throughout the Pleistocene. Similarly, co-distributed snake species (e.g., *Bitis arietans*, *Psammophis leightoni*) do not show notable phylogeographic structure within the central arid regions of southern Africa, and this also has been attributed to climatic stability (Barlow et al. 2013; Taft et al. 2022). In addition, genetic clades of the puff adder (*B. arietans*) show a contact zone in the Limpopo area (Barlow et al. 2013) where the two *Pelomedusa* species also make geographic contact. The spatial parallels of genetic clades between these disparate taxa suggest that climatic stability within the central part of southern Africa has provided opportunity for persistence of gene flow across this arid expanse generally. In contrast, the eastern areas, where clades meet, may have been climatically more dynamic.

Unlike *P. subrufa*, its South African congener *P. galeata* shows pronounced genetic differentiation with two deeply divergent genealogical lineages (Petzold et al. 2014), which most likely represent two distinct species (Vamberger et al. 2018). *Pelomedusa galeata* does not occur in the stable arid climate of central southern Africa or is, at least in the south of the putative distribution gap, very rare (Vamberger et al. 2019a). It is mainly dis-



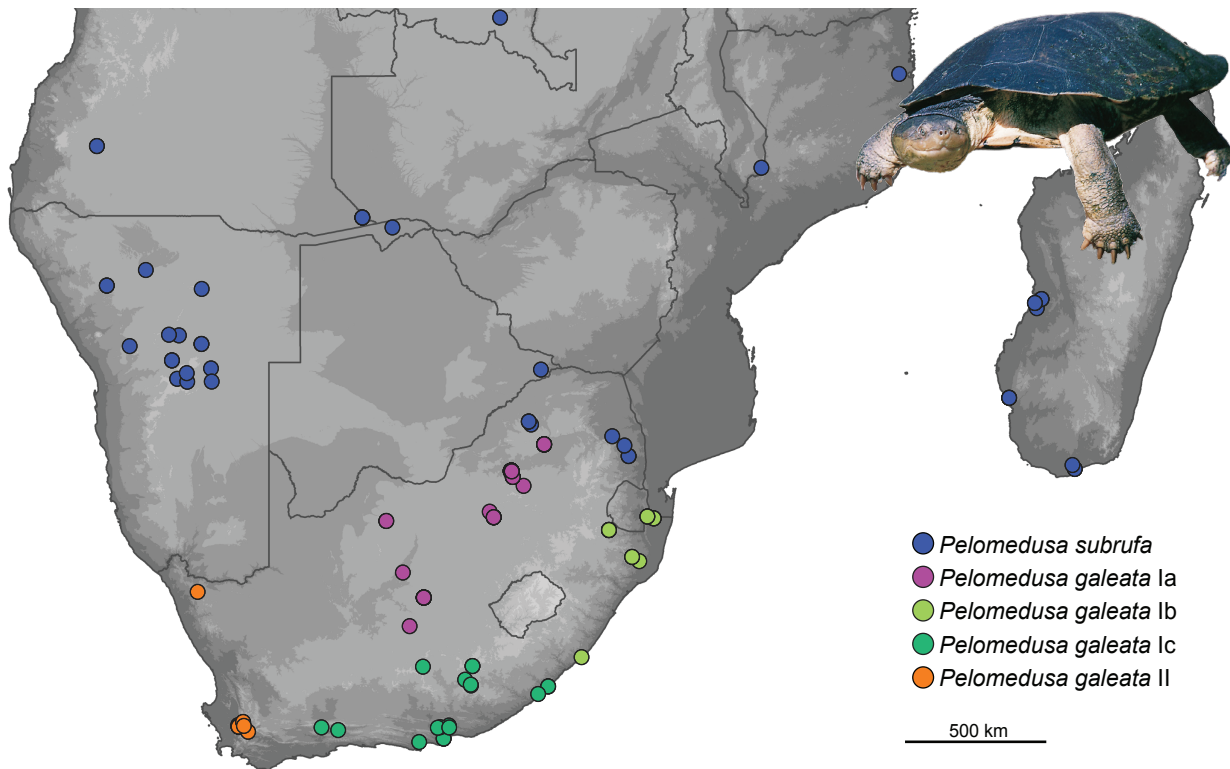


**Figure 2.** Genetically verified records of *Pelomedusa* species (combined from Petzold et al. 2014; Fritz et al. 2015; Vamberger et al. 2018; TTWG 2021). The arrow highlights records of the two lineages of *P. somalica* in only 40 km distance. Inset: *Pelomedusa neumanni*, Ethiopia (photo: Tomáš Mazuch).

tributed across the more mesic regions of South Africa, where there are two deeply divergent genealogical lineages, namely (1) from the western winter-rainfall region of South Africa (southwestern Cape) into the arid north-west of South Africa and (2) from the south coast into the eastern Karoo and savannah regions to the north (Figs 2 and 3). A genetic division between the extreme west and the more eastern regions has been also noted for a wide variety of species, including reptiles, amphibians, mammals, and birds (Tolley et al. 2014). The phylogeographic structure observed in these species has been attributed to the formation of several persistent refugia within South Africa. Disparate refugia were located in the south (Fynbos biome) and west (Succulent Karoo biome), with the central (Nama Karoo biome) region being climatically unstable over the Pleistocene, providing the necessary

drivers for causing, or reinforcing, genetic divergence between populations that have been relegated to the diverse refugia during range contractions.

In addition to the larger scale patterns, the eastern lineage of *P. galeata* shows further phylogeographic differentiation, with three distinct mitochondrial clades (Fig. 3). One clade occurs in the interior of South Africa, at high elevations above the Great Escarpment where summer-rainfall prevails (Free State, Gauteng, Limpopo, North West, Northern Cape provinces). A second clade has been recorded from the subtropical (low-elevation) summer-rainfall region along the northeast coast of South Africa (Eastern Cape, KwaZulu-Natal) and the third from the south coast and adjacent inland regions, mostly below the Great Escarpment, with all-year (aseasonal) rain (Eastern Cape, Western Cape).



**Figure 3.** Genetically verified records for *Pelomedusa subrufa* and the genetic lineages of *P. galeata* in Southern Africa (redrawn from Vamberger et al. 2018, with new records from TTWG 2021). Inset: *P. galeata*, Eastern Cape (photo: Bill Branch).

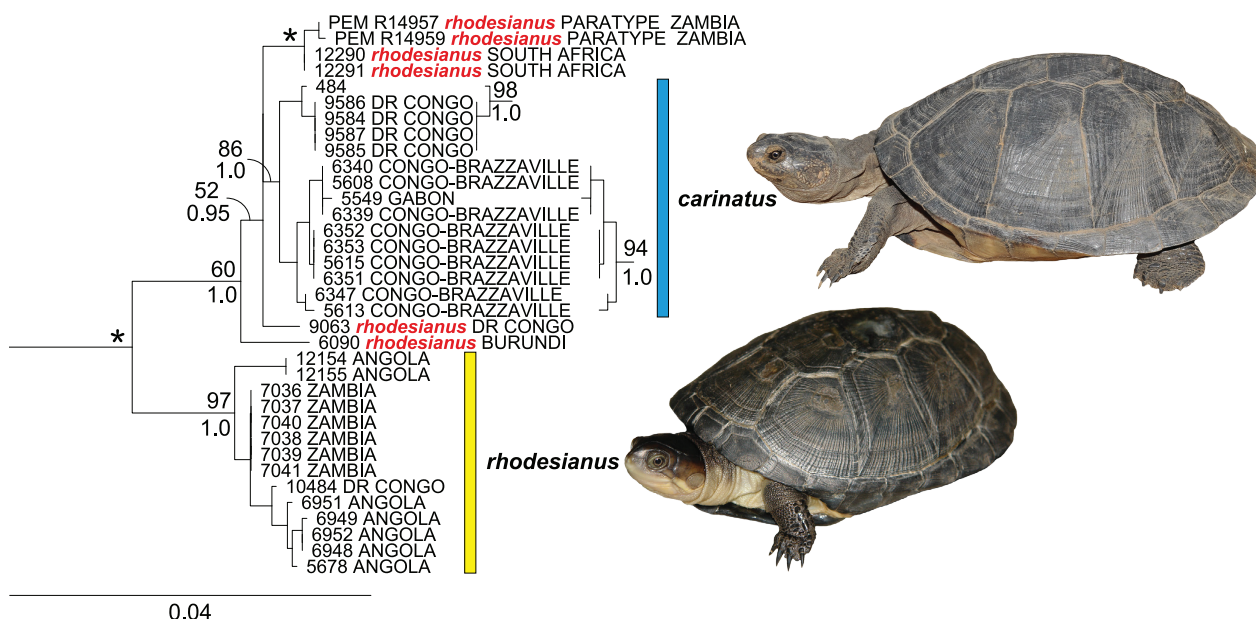
The few data available for other *Pelomedusa* species indicate that phylogeographic structure is also present within *P. neumanni* and *P. somalica*, each of which contains two deeply divergent mitochondrial lineages. In *P. neumanni*, one of these lineages has been recorded from the Omo River region in southernmost Ethiopia and Marsabit County, Kenya. The other lineage of *P. neumanni* has been found in Kakamega County, Kenya, in the Lake Victoria Region, and the Tanzanian Manyara Region. In *P. somalica*, one lineage has been recorded from Lake Koka in Ethiopia (Oromia Region) and the Somali Awdal Region. The other lineage was found in close proximity in the Awdal Region and most likely also in the Shebelle River region (Oromia, Ethiopia; Vargas-Ramírez et al. 2010; Petzold et al. 2014; Fritz et al. 2015). The occurrence of the two lineages in the Awdal Region only 40 km apart suggests that they represent distinct parapatric or even sympatric species (Vargas-Ramírez et al. 2010; Petzold et al. 2014).

Using 2,036 bp of mtDNA, Fritz et al. (2013) examined the phylogeography of the East African species *Pelusios castanoides* and *P. subniger*, with a focus on Madagascar and the Seychelles. Differentiation among the studied populations of *P. subniger* from mainland Africa, the Seychelles, and Madagascar was completely absent. This strongly suggests that the populations on the Seychelles and Madagascar were introduced. Remarkably, there was evidence for an overlooked, but distinct lineage within *P. subniger* from the Democratic Republic of the Congo. In contrast, for *P. castanoides* the material from the Seychelles and Madagascar was weakly, but consistently, differentiated from mainland African samples. Malagasy

and Seychellois samples were reciprocally monophyletic in phylogenetic analyses. However, sampling from mainland Africa was very patchy and most of the species' mainland distribution range was not studied. Therefore, the possibility cannot be excluded that the populations on Madagascar and the Seychelles were introduced long ago from unstudied source regions on the African continent.

For another East African *Pelusios* species (*P. sinuatus*), Vamberger et al. (2019b) examined phylogeographic differentiation using 2,180 bp of mtDNA and three nuclear loci (2,132 bp). These authors revealed two distinct genealogical lineages, one in the north and the other in the south of the distribution. The two lineages meet in the border region of Botswana, South Africa, and Zimbabwe, where they hybridize. The northern lineage corresponds to large-bodied terrapins with carapace lengths up to 55 cm, whereas terrapins belonging to the southern lineage only reach reported maximum sizes of 35 cm. Given this morphological and genetic differentiation, Vamberger et al. (2019b) identified the northern and southern lineages as different subspecies, *P. s. bottegi* and *P. s. sinuatus*.

Using 2,117 bp mtDNA and three nuclear loci (2,012 bp), Kindler et al. (2016) compared the phylogeographic differentiation of six *Pelusios* species, among them two savannah species, two species living in more mesic habitats, and two species being more generalist, occurring in both habitats (termed “intermediate habitats” in Kindler et al. 2016). There was no obvious correlation between the phylogeographic patterns and habitat occurrence. Of the two savannah species, *P. rhodesianus* showed phylogeographic structure, whereas *P. nanus* did not. Also, one species inhabiting forests and mesic savannahs (*P. cari-*



**Figure 4.** Paraphyly of *Pelusios rhodesianus* with respect to *P. carinatus*. Detail of maximum likelihood tree using up to 2,117 bp of mtDNA from Kindler et al. (2016). Numbers above branches are bootstrap values and below are posterior probabilities from a Bayesian analysis of the same dataset. Asterisks indicate maximally supported nodes under both approaches. Insets: *P. carinatus*, Democratic Republic of the Congo (photo: Václav Gvoždík); *P. rhodesianus*, Angola (photo: Uwe Fritz).

*natus*) displayed phylogeographic structure, the other (*P. gabonensis*) not, and one of the species occurring in both habitats (*P. castaneus*) had a pronounced phylogeographic structure, the other (*P. chapini*) not.

An unexpected finding was the relationship of *P. rhodesianus* to the morphologically highly distinctive *P. carinatus*. *Pelusios rhodesianus* was found to comprise two deeply divergent mitochondrial clades (from the center and west versus from the center and east of the distribution range), which were not differentiated with respect to nuclear DNA. The mitochondrial clade from the center and east, and the nuclear sequences of all *P. rhodesianus*, were paraphyletic with respect to *P. carinatus*, which constituted a well-supported clade nested in the paraphyletic *P. rhodesianus*. Within *P. carinatus* two subclades were found, one from the northwest of the Democratic Republic of the Congo and the other from Congo-Brazzaville and Gabon (Fig. 4).

The phylogeographic structure of *P. castaneus* with distinct clades in (1) Cameroon, (2) Nigeria, Ivory Coast plus São Tomé, and (3) Congo-Brazzaville could reflect Pleistocene range interruptions correlated with the fluctuating forest cover in West and Central Africa (Kindler et al. 2016). Similarly, a major discontinuity for amphibians appears to coincide with the Cross River in southeastern Nigeria (Penner et al. 2011), possibly corresponding with the Nigeria/Ivory Coast phylogeographic break. Further material of *P. castaneus* without known provenance and samples from an introduced population on Guadeloupe were also distinct, indicating that there is more variation in other regions (Kindler et al. 2016).

With respect to *P. chapini*, phylogenetic analyses of mtDNA suggested weak differentiation from *P. castaneus* (Fritz et al. 2011; Kindler et al. 2016), whereas those of nuclear loci indicated their distinctness (Kindler et al.

2016), a finding supported by another study (Thomson et al. 2021) using 15 nuclear loci (13,559 bp).

Further research should be conducted to examine the phylogeography of these and other widely distributed *Pelusios* species and to close the sampling gaps in *Pelomedusa* north of southern Africa (see Petzold et al. 2014; Vargas-Ramírez et al. 2016). It is not unlikely that further unrecognized taxa will be identified during such investigations, in particular in *Pelomedusa* and in *Pelusios adansonii*, a freshwater turtle distributed across a patchy range in the Sahel Region (TTWG 2021).

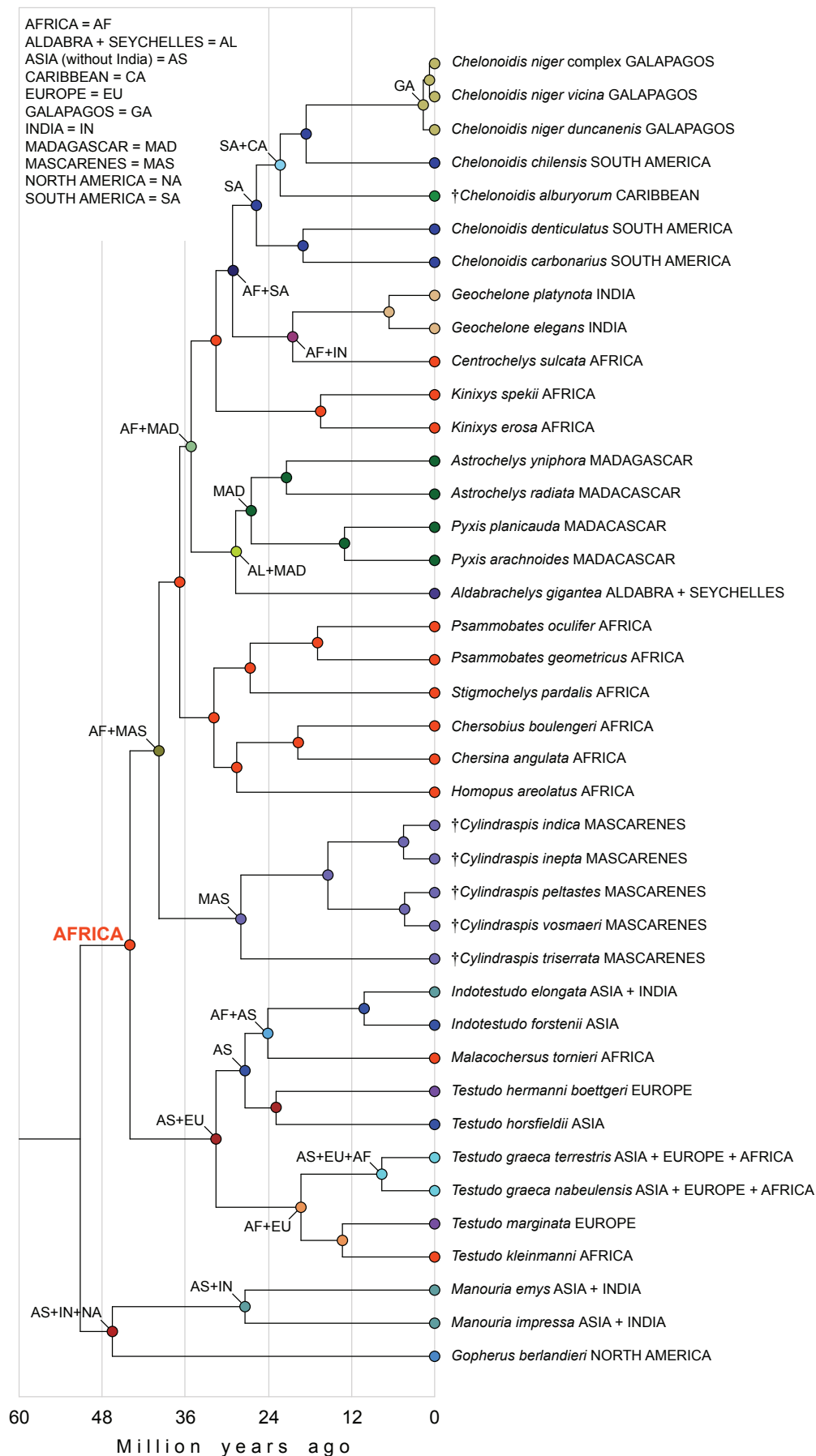
## Testudinidae

### Phylogeny

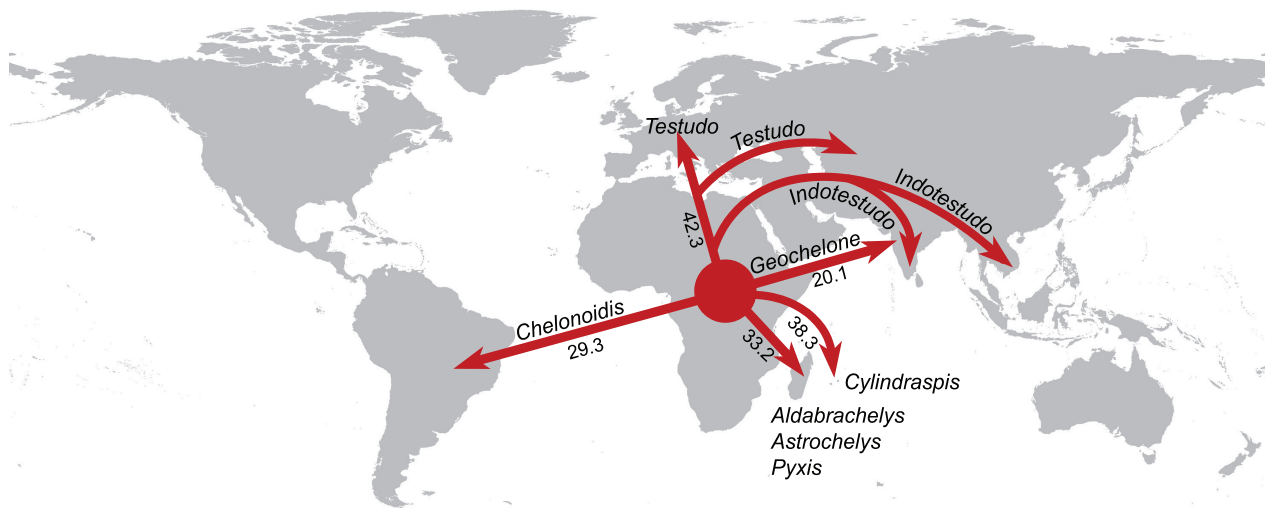
The family of land tortoises, or simply tortoises (Testudinidae), is the sister group of the Old World pond turtles (Geoemydidae) and represents, like the Trionychidae (see below), a clade that originated on Laurasia—the Cryptodira or hidden-necked turtles (de la Fuente 2014; Pereira et al. 2017). Most likely, tortoises spread during the Paleocene or Eocene via the Arabian Plate into Africa (Georgalis et al. 2021) where they diversified considerably.

Several studies have examined the phylogeny of tortoises using molecular markers, among them Le et al. (2006), Parham et al. (2006), Fritz and Bininda-Emonds (2007), Kindler et al. (2012), Hofmeyr et al. (2017), Kehlmaier et al. (2017, 2019a, 2021a), and—as part of an investigation covering most chelonians—Thomson et al. (2021). The most important insight derived from these investigations is that Africa is the cradle of the majority of extant testudinids, except for the Asian genus *Manouria*





**Figure 5.** Simplified ancestral range analysis for tortoises (Testudinidae) based on mitochondrial genomes (15,510 bp; modified from Kehlmaier et al. 2019a). The nodes show only the most likely ancestral ranges coded by colors; the ancestral range for the most basal node could not be resolved. For details, see Kehlmaier et al. (2019a). Note that the ancestral range ‘Africa’ for *Testudo* concerns regions north of the Sahara.



**Figure 6.** The multiple out-of-Africa dispersals of testudinid lineages. Numbers at arrows are inferred divergence times (million years) for the mitochondrial genomes of the respective taxa from their African sister lineages (from Kehlmaier et al. 2021a). For the *Indotestudo-Malacochersus-Testudo* clade it cannot be excluded that *Malacochersus* results from a secondary reinvasion of Africa (inferred split age of *Malacochersus* and its non-African sister: 22.5 million years). Here, the divergence time of the last common ancestor of the whole *Indotestudo-Malacochersus-Testudo* clade and its African sister branch is shown.

and the Nearctic genus *Gopherus*, which obviously represent relics of an older Laurasian radiation (Figs 5 and 6).

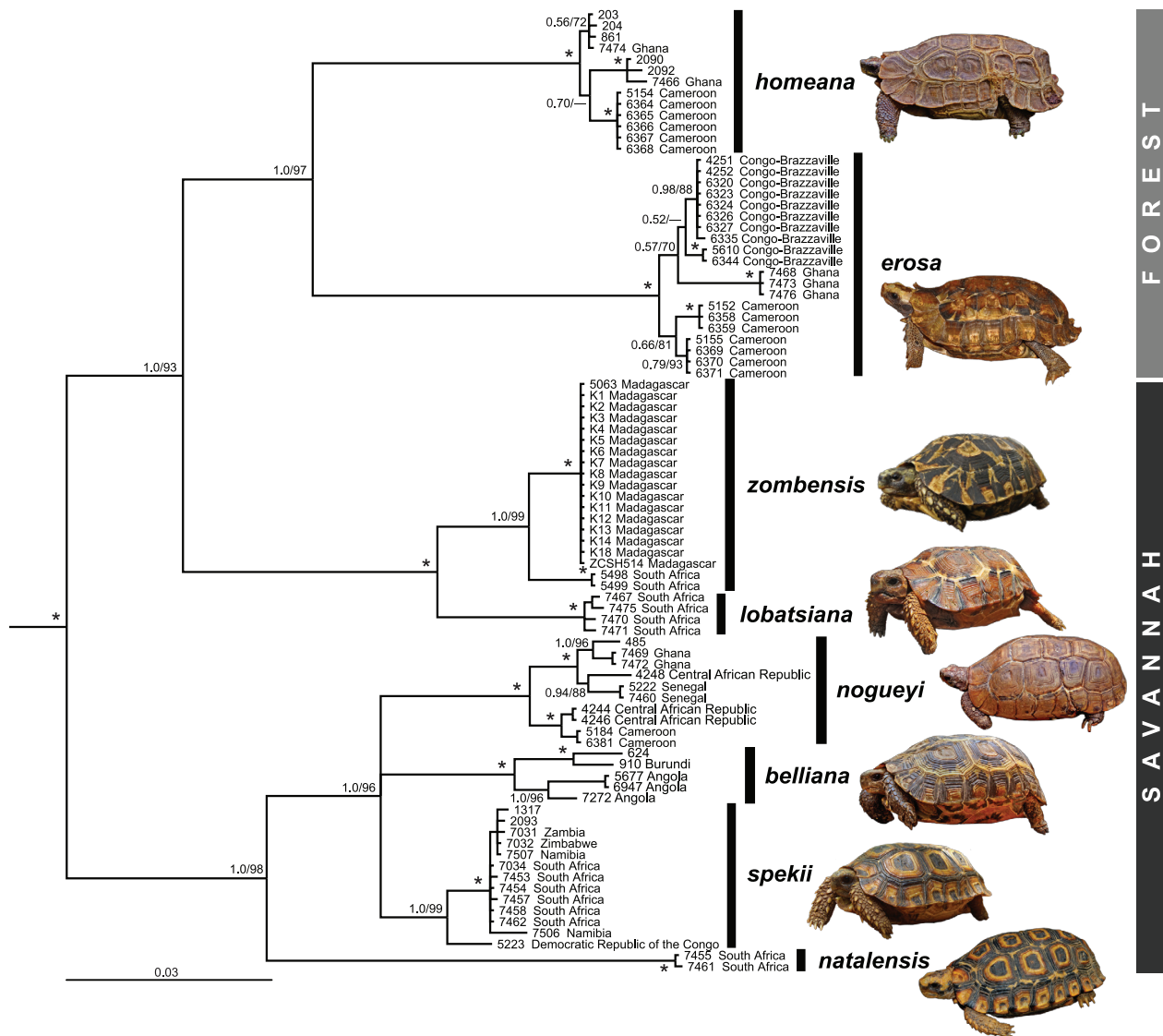
Among the 52 extant or recently extinct tortoise species (including the five recently extinct *Cylindraspis* species), 44 (85%) have their phylogenetic roots in Africa or live in Africa. Three out-of-Africa dispersal waves led to (1) the extant South American tortoise radiation (*Chelonoidis* spp., i.e., including the extant and extinct tortoises from Galápagos and the Bahamas), (2) the western and central Palearctic plus Southeast and South Asian tortoise radiation (*Indotestudo*, *Testudo*—including *Malacochersus* from sub-Saharan Africa), and (3) the South Asian radiation (*Geochelone*; Le et al. 2006; Kehlmaier et al. 2017, 2019a, 2021a). Also, the extinct Mascarene giant tortoises (*Cylindraspis*) and the tortoises from Madagascar (*Astrochelys*, *Pyxis*) and Aldabra (*Aldabrachelys*, extinct also on Madagascar) result from two further independent out-of-Africa dispersals (Crotini et al. 2012; Samonds et al. 2012; Kehlmaier et al. 2019a; Fig. 6). Overseas dispersal has played a key role in the colonization of South America and the mentioned oceanic islands (Le et al. 2006; Kehlmaier et al. 2017, 2019a, 2021a) including Madagascar (Crotini et al. 2012; Samonds et al. 2012). The same is true for the volcanic Canary and Cape Verde Islands, from where three extinct tortoise species have been described. They have been tentatively assigned to the African genus *Centrochelys* in the most recent review, even though the supporting evidence for this assignment is weak (Georgalis et al. 2021). Kehlmaier et al. (2021b) radiocarbon-dated one type specimen of the Cape Verde species *C. atlantica* (Sal Island) and succeeded in sequencing the mitochondrial genomes of all three type specimens. It turned out that the species description was based on bones from a single South American red-footed tortoise (*Chelonoidis carbonarius*) that was still alive between 1962 and 1974. The circumstances of this taxonomic confusion currently

cannot be disentangled and the phylogenetic placement of the original subfossil material from Sal Island remains unclear.

Regarding southern African tortoise species, Hofmeyr et al. (2017) provided firm evidence for the paraphyly of the formerly recognized genus *Homopus* with respect to *Chersina angulata*. These authors resolved this situation by resurrecting the genus *Chersobius* for the five-toed species of *Homopus* (*Chersobius boulengeri*, *C. signatus*, *C. solus*), with *Homopus* being restricted to the four-toed species *H. areolatus* and *H. femoralis* (see also Hofmeyr and Branch 2018). This suggestion found wide support (TTWG 2021), even though Thomson et al. (2021) proposed instead lumping together these three morphologically distinct genera under the oldest available name, *Chersina*. In a similar vein, Le et al. (2006) and Thomson et al. (2021) recommended to lump together the large-bodied *Stigmochelys pardalis* and the three small-bodied *Psammobates* species under *Psammobates*, in contrast to Fritz and Bininda-Emonds (2007) who emphasized their morphological distinctness and argued for a continued use of *Stigmochelys* sensu stricto for *S. pardalis* and *Psammobates* for the three small species. Recently, the checklist of the Turtle Taxonomy Working Group of the IUCN SSC Tortoise and Freshwater Turtle Specialist Group (TTWG 2021) did not endorse lumping together *Stigmochelys* and *Psammobates*.

A study on the borderline between phylogeography and phylogeny focused on the relationships within the hinged-back tortoise genus *Kinixys* (Kindler et al. 2012). An unexpected finding was that the savannah species of *Kinixys* were polyphyletic in that two taxa (*domerguei*, *zombensis*) previously regarded as subspecies of the savannah species *K. belliana* represent, together with the savannah species *K. lobatsiana*, the sister group of the forest species *K. erosa* and *K. homeana* (Fig. 7). Using mtDNA, this clade of savannah and forest species was





**Figure 7.** Mitochondrial phylogeny of *Kinixys* species based on a 2,273-bp-long alignment of mtDNA (Bayesian analysis). Numbers at nodes are posterior probabilities and bootstrap values from a maximum likelihood analysis; asterisks indicate maximum support under both approaches (redrawn from Kindler et al. 2012; for sample codes, see there). For the widely distributed species (*Kinixys homeana*, *K. erosa*, *K. zombensis*, *K. nogueyi*, *K. belliana*, *K. spekii*), the internal branching structure shows phylogeographic variation is present in the clades. Insets: *K. homeana*, pet trade (photo: Pavel Široký); *K. erosa*, Democratic Republic of the Congo (photo: Václav Gvoždík); *K. zombensis*, South Africa (photo: Flora Ihlow); *K. lobatsiana*, South Africa (photo: Flora Ihlow); *K. nogueyi*, pet trade (photo: Pavel Široký); *K. belliana*, Angola (photo: Luis Ceriaco); *K. spekii*, South Africa (photo: Flora Ihlow); *K. natalensis*, South Africa (photo: James Harvey).

sister to the remaining savannah taxa, including *K. belliana* sensu stricto. To resolve the polyphyly of the formerly recognized species *K. belliana* sensu lato, Kindler et al. (2012) elevated *K. zombensis* to species level but were reluctant to accept the genetically similar population from Madagascar (previously recognized as *K. b. domerguei*) as a valid subspecies of *K. zombensis* (see below under phylogeography). Within the savannah clade, another taxon formerly regarded as conspecific with *K. belliana* was revealed to be deeply divergent, necessitating it being recognized as another distinct species (*K. nogueyi*) in a clade with *K. belliana* sensu stricto and *K. spekii*. These taxonomic adjustments informed by phylogenetics brought the tally of *Kinixys* species to eight.

## Phylogeography

Phylogeographic variation has been studied in several testudinid species from sub-Saharan Africa, even though for many species only superficial data are available. In this review, the taxa will be presented in sequence roughly from north to south.

Livoreil and van der Kuyl (2005) examined the widely distributed *Centrochelys sulcata* using 409 bp of the rapidly evolving mitochondrial control region. This species is native to the Sahel Region and the adjacent southernmost Sahara, from the Atlantic coast across the Red Sea to the southwestern Arabian Peninsula (Petrozzi et al. 2020; TTWG 2021), i.e., across a region spanning the west–east taxis for approximately 6,400 km. Unfortunately, Li-

voreil and van der Kuyl (2005) had only limited material available (samples from Sudan, Senegal, and Mali). They found very little variation, with three haplotypes that differed by one to two mutations. One haplotype was unique to individuals from Senegal, whereas a second haplotype occurred exclusively in Sudan. The third haplotype was more widespread, recorded in individuals from Sudan, Senegal, and Mali. Given that this species is considered Endangered due to a suspected population reduction of more than 50% (Petrozzi et al. 2021), a more comprehensive investigation that incorporates additional genetic markers might be useful to identify important Conservation Units.

Compared to *C. sulcata*, the phylogeography of the second widely distributed African testudinid, *Stigmochelys pardalis*, has been well studied (Fritz et al. 2010; Spitzweg et al. 2019; Dajčman et al. 2021). This species is distributed in East Africa from the Horn of Africa in Ethiopia southwest to Angola and through southern Africa to the southernmost extent of the continent in the Cape of Good Hope in South Africa (TTWG 2021). Using 1,938 bp of mtDNA, Fritz et al. (2010) examined phylogeographic variation across most of the species' range. They found several haplotype clusters, but these do not appear to be due to environmental barriers causing vicariance, but are the result of the accumulation of local genetic variation through limited geographic dispersal, i.e., isolation by distance. Fritz et al. (2010) revealed that mtDNA sequences that were previously identified with the distinct subspecies *S. p. babcocki* represent nuclear mitochondrial insertions (numts), whereas they found no evidence for the validity of this subspecies and recommended to synonymize it into the nominotypical subspecies. Building on these results, two later studies (Spitzweg et al. 2019; Dajčman et al. 2021) focused on the southern part of the distribution of *S. pardalis*. Using mtDNA sequences (1,136 bp) and 14 microsatellite loci, Spitzweg et al. (2019) identified two major genetic clusters: A southern cluster from southern coastal South Africa and a northern cluster, distributed from Namibia across northern South Africa to adjacent Mozambique. Microsatellite data indicated further substructure and greater genetic diversity within the northern cluster. This pattern was interpreted as resulting from Pleistocene range disjunctions with subsequent admixture, mainly in the north. The distribution of one of the northern subclusters matches with a well-known biodiversity hotspot (Maputaland-Pondoland-Albany hotspot). Dajčman et al. (2021), having a denser sampling from the north of South Africa, added another northern subcluster and suggested that the identified clusters and subclusters be recognized as Management Units.

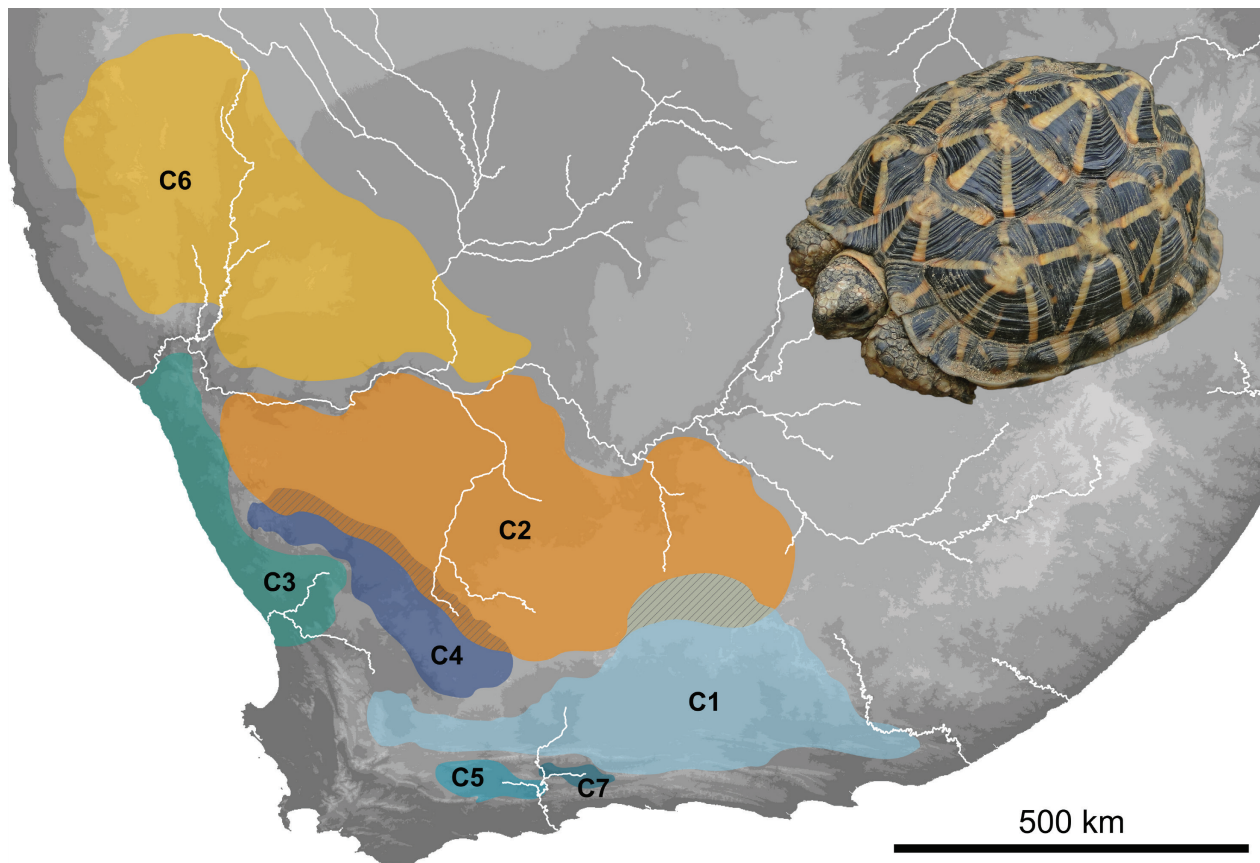
Populations of *S. pardalis* beyond South Africa and Namibia are poorly studied and a rangewide investigation could yield valuable insights in population structuring and the correlation of morphological traits and genetic differentiation. For instance, it is well known that *S. pardalis* displays extreme differences in body size across its range (Fritz et al. 2010), with reported maximum sizes of 75–85 cm in the northernmost and southernmost popu-

lations (from the Horn of Africa and southernmost South Africa, respectively) and much smaller sizes in geographically intermediate populations (30–50 cm). It remains largely unknown whether this variation results from environmental factors, as suggested by Lambert (1995), or correlates with genetic differentiation. For southern Africa it was confirmed that the two major genetic clusters revealed by microsatellite loci match different classes of average body size (Dajčman et al. 2021), suggesting that a genetic component plays a certain role at least.

One of the most promising genera for future phylogeographic investigation is *Kinixys* with six poorly studied but widely distributed species (*K. belliana*, *K. erosa*, *K. homeana*, *K. nogueyi*, *K. spekii*, *K. zombensis*) and two range-restricted species (*K. lobatsiana*, *K. natalensis*). Despite highly patchy sampling and small sample sizes, the pioneering study by Kindler et al. (2012) revealed phylogeographic structuring for all six widely distributed species. These authors used 2,273 bp of mtDNA for their investigation and added for a smaller subset three nuclear loci (2,569 bp).

Kindler et al. (2012) suggested that the observed differentiation of the two forest species (*K. erosa*, *K. homeana*) correlates with late Pleistocene forest refugia. Using the mtDNA dataset (Fig. 7), they found within *K. homeana* three moderately to well-supported clades. Two clades contained mainly material from the pet trade plus one sample each from Ghana; a third clade comprised sequences from Cameroon. In *K. erosa*, some phylogeographic structure was also apparent, and in particular there was a clade that appears to occur exclusively in Ghana that is notably divergent from samples collected in Congo-Brazzaville and Cameroon. For *K. zombensis* 17 individuals from Madagascar and two from northeastern South Africa were studied, with material from each region corresponding to a distinct clade. Since it is likely that the Malagasy hinged-back tortoises were introduced 1,500–1,000 years ago (Raselimanana and Vences 2003), this finding was thought to reflect that the continental African source population is genetically distinct from South African *K. zombensis* (Kindler et al. 2012).

The phylogenetic analyses (Fig. 7) also provided support to elevate the former subspecies *K. belliana nogueyi* to a full species (*K. nogueyi*), and there was further evidence of phylogeographic structure within this species (Kindler et al. 2012). One well-supported clade contained material from Ghana, the Central African Republic, and Senegal and another well-supported clade comprised samples from the Central African Republic and Cameroon. Kindler et al. (2012) also showed that the number of toes is not diagnostic for *K. nogueyi* because four-toed and five-toed populations exist. These traits were formerly believed to be diagnostic for what is now *K. nogueyi* and *K. belliana* sensu stricto. Kindler et al. (2012) had very few samples available for *K. belliana* sensu stricto, but these formed two separate clades, each having some structure within. One clade contained a tortoise from Burundi plus one sample of unknown provenance and the other clade, three tortoises from Angola. For *K. spekii*, a single sample from the Democratic Republic of the Con-



**Figure 8.** Distribution of the seven mitochondrial clades (C1–C7) of *Psammobates tentorius* (redrawn from Zhao et al. 2021b). Note the overlap between C1 and C2 and between C2 and C4. Inset: *Psammobates tentorius verroxii*, Namibia (photo: U. Fritz).

go was clearly distinct from material from Zambia, Zimbabwe, Namibia, and South Africa.

Current research by Flora Ihlow using fresh samples and historical DNA from collection material aims at clarifying the phylogeographic differentiation and distribution of several *Kinixys* species.

A series of four papers by Zhao et al. (2020a, 2020b, 2021a, 2021b) examined the phylogeography of the *Psammobates tentorius* complex using up to 2,428 bp of mtDNA, one nuclear locus of 406 bp and 14 microsatellite loci. They discovered a complex spatial pattern of seven mitochondrial clades (Fig. 8), which neither completely matched the differentiation revealed by microsatellite loci nor the three recognized subspecies. Despite clear mitochondrial differences and some congruence with microsatellite clusters, broad admixture occurred between geographically adjacent microsatellite clusters (Zhao et al. 2020a), suggestive of their conspecificity. Nevertheless, using different analytical approaches, Zhao et al. (2021b) concluded that a four species scheme best represents the observed genetic differentiation, with two species corresponding to the recognized subspecies *P. t. tentorius* and *P. t. trimeni* but with the subspecies *P. t. verroxii* split into two distinct species separated by the Orange River. Pending further study and the clarification of nomenclatural issues, the checklist of the Turtle Taxonomy Working Group of the IUCN SSC Tortoise and Freshwater Turtle Specialist Group (TTWG 2021) provisionally refrained from the formal recognition of these four species.

It is clear that additional research is required to clarify the species number in the *P. tentorius* complex. For this, the application of an explicit species concept is a necessary prerequisite. Furthermore, cline analyses across contact zones of distinct genetic clusters would be promising to elucidate genetic break zones, especially if making use of variable nuclear markers, such as microsatellites or SNPs, in combination with information from mtDNA (compare Kindler et al. 2017 for an example of this approach). While integration of different data types has been carried out for *P. tentorius* to assess species level diversity (Zhao et al. 2021b), it is also imperative that a species delimitation approach within a full phylogenetic framework is applied (Reid and Carstens 2012; Carstens et al. 2013) that includes congeners. For example, confusing taxonomy and lack of an explicit species concept had plagued taxonomic assessments of some reptiles in southern Africa, but teasing out species can be accomplished when a clear concept is circumscribed in advance, and a phylogenetic approach is used to assess species (e.g., Taft et al. 2022; Tolley et al. 2022a, 2022b).

An additional challenge in the *P. tentorius* complex is the confusing nomenclatural history. No less than 26 species group names are synonymized under the three currently recognized subspecies (TTWG 2021). These names need to be matched with genetic clades and clusters before the taxonomy can be revised. Second generation sequencing combined with approaches developed for ancient DNA (aDNA) is a powerful tool to overcome



this challenge by sequencing the name-bearing type material (compare for instance the cutting edge approach described in Kehlmaier et al. 2019b for other chelonian type material; see also Raxworthy and Smith 2021).

Another *Psammobates* species, *P. geometricus*, belongs to the most threatened tortoise species of the world (Turtle Conservation Coalition 2018). It is endemic to a small region in the Western Cape Province of South Africa and has experienced a population decline of over 80% within the past 100 years, making it Critically Endangered (Hofmeyr and Baard 2018). It occurs as three isolated populations, which are further fragmented due to extreme habitat loss. As a result, it is likely that the metapopulation is no longer intact, and the species is at risk of genetic erosion. Nevertheless, Cunningham et al. (2002) found only negligible differentiation between sample sites using eight microsatellite loci, despite isolation of the sites by the Cape Fold Mountains. Another study currently underway by Melita Vamberger and co-authors will hopefully shed additional light on the genetic structure of this species. Indeed, a comprehensive evaluation of the genetic diversity and whether small fragments are extremely isolated are urgently needed. Early signs of genetic erosion are not usually detectable through declines in genetic diversity, but instead are more easily revealed through strong signatures of genetic isolation indicated by unexpected differences in the allele (or haplotype) frequencies of populations in remaining habitat patches. Allele or haplotype frequency shifts can rapidly occur in such situations due to the effects of genetic drift and stochastic effects in very small populations with reduced adaptive potential (Bijlsma and Loeschcke 2012). For example, the Critically Endangered Chapman's pygmy chameleon (*Rhampholeon chapmanorum*) from Malawi occurs in only five remnant forest patches ranging in size from 1.2 to 16.6 hectares. The populations in these habitat patches are considered isolated and they show signs of strong population genetic structure despite the small distances between them (i.e., a few km), which is interpreted as genetic drift due to small population sizes and is an early sign of genetic erosion (Tolley et al. 2022c). Given the small habitat patches remaining for *P. geometricus* and the suspected population decline, the assessment of genetic erosion is a priority.

The phylogeography of *Chersobius signatus*, endemic to South Africa, was studied by Daniels et al. (2010) using approximately 1,100 bp of mtDNA and one nuclear locus of 390 bp length. Their analyses revealed weak phylogeographic structuring that did not support the two described subspecies (*C. s. signatus*, *C. s. cafer*) but instead suggested that isolation by distance is an important driver in the spatial genetic pattern observed. Although a single individual from the locality of Poffadder was noted as being more divergent than individuals from other sites, re-examination of the original sequence data (ND4 gene, GenBank: GU13922) shows this sample is ca. 2.5% divergent from other samples, as opposed to ca. 1.5% divergence amongst other samples. This very small additional difference is probably not particularly consequential given that the locality is also the most distant from other sites

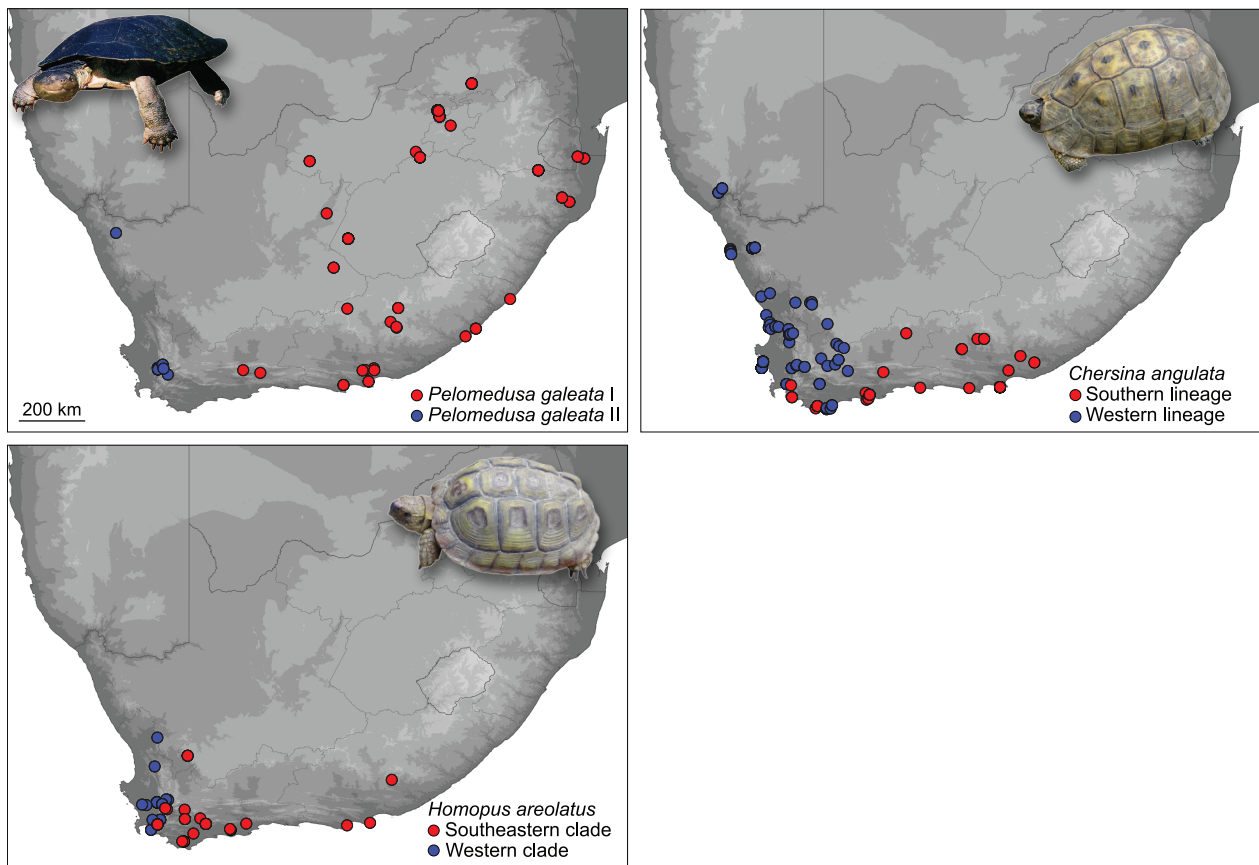
sampled. Other important aspects of this study relate to the speckled color pattern characteristic for the formerly recognized subspecies *C. s. cafer* and this was suggested to be a result of adaptation to the local rock type (granite-gneiss).

Daniels et al. (2007) and Spitzweg et al. (2020) have examined phylogeographic structuring in *Chersina angulata*. Based on sequence variation of three mtDNA fragments (COI: 599 bp, cyt *b*: 320 bp, ND4: 791 bp), Daniels et al. (2007) inferred the existence of three mitochondrial clades in the northwestern, southwestern, and southern part of the South African Cape region that corresponded to two more inclusive clades (western Cape vs. southern Cape), with divergence time estimates for the three clades ranging from 8.4 to 10.4 million years. Daniels et al. (2007) suggested that the two more inclusive clades could represent distinct species. Spitzweg et al. (2020) re-examined the phylogeography of *C. angulata* using range-wide sampling and combined evidence from cyt *b* sequences (1,143 bp), 14 microsatellite loci and species distribution models. For a fossil-calibrated molecular clock calculation using 1,870 bp mtDNA and 1,416 bp nDNA, these authors selected representative samples of *C. angulata* and all other African tortoise lineages. Spitzweg et al. (2020) confirmed the presence of two lineages from the west and south of the range (Fig. 9). In addition, a population from Dassen Island was included in the analysis. The tortoises from Dassen Island are much larger than those from the mainland and reach maximum shell lengths of up to 35 cm, as opposed to 17–19 cm (Hofmeyr 2009). However, the insular population was not genetically differentiated from the western mainland lineage. In contrast to the deep divergences estimated by Daniels et al. (2007), Spitzweg et al. (2020) inferred that the two lineages of *C. angulata* diverged only in the Pliocene (approx. 3.8 million years ago).

Microsatellite analyses indicated broad admixture in the geographic contact zone of the two lineages and supported that they are conspecific under the Biological Species Concept (Mayr 1942). Spitzweg et al. (2020) concluded that each of the two genealogical lineages could be identified with a particular subspecies, but the unclear taxonomic identity of the type specimens of the available early 19<sup>th</sup> century names prevented their naming. This situation might be clarified using aDNA approaches to characterize the type material genetically.

Species distribution models indicated that the ranges of the two lineages have probably not shifted substantially since the Last Glacial Maximum, in accordance with demographic population descriptors suggestive of stationary distributions that did not experience expansion.

The phylogeography of *Homopus areolatus* was studied by Hofmeyr et al. (2020) based on range-wide sampling and a 680-bp-long fragment of the mitochondrial ND4 gene combined with evidence from species distribution modeling. Similar to *Chersina angulata* (Daniels et al. 2007; Spitzweg et al. 2020) and *Pelomedusa galeata* (Vamberger et al. 2018), two clades were identified, one from the west and the other from the south and east of the range. For the two tortoise species, a small over-



**Figure 9.** Records of genetic lineages of *Pelomedusa galeata*, *Chersina angulata*, and *Homopus areolatus* (based on mtDNA; combined from Vamberger et al. 2018; Hofmeyr et al. 2020; Spitzweg et al. 2020). Insets: *P. galeata*, Eastern Cape (photo: Bill Branch); *C. angulata*, Eastern Cape (photo: Uwe Fritz); *H. areolatus*, Western Cape (photo: Uwe Fritz).

lap zone between the clades has been identified (Fig. 9). This overlap zone also appears to be an important area for secondary contact between clades of other reptiles (lizards: Tolley et al. 2009; snakes: Barlow et al. 2013) and mammals (Smit et al. 2007; Willows-Munro and Matthee 2011) and corresponds with a montane region of high annual rainfall at present. This area probably has been dynamic in terms of climate, with the current contact zone possibly being an area into which species have only recently expanded given the higher rainfall. Conversely, the distributions ostensibly contracted during more arid phases into refugia to the west and the east, breaking that contact zone (e.g., Barlow et al. 2013; Tolley et al. 2014). Hofmeyr et al. (2020) inferred that the divergence of the two clades of *H. areolatus* commenced in the late Pliocene (approx. 2.9 million years ago) and as such, it is possible that the clades have experienced repeated range fluctuations through the Pleistocene, with episodes of isolation in disjunct refugia followed by periods of range expansion and secondary contact.

Despite the potential of expansion into secondary contact for the clades, species distribution models revealed that suitable climatic space has contracted overall since the LGM, probably caused by reduced rainfall in the west and higher temperatures in most regions. However, this postulated range contraction appears to be most directly linked to sea level rise since the LGM that has now excluded this species from the former southern extent of the

range. Models also suggested that the two clades may be in greater contact at the zone of sympatry at present than during the LGM.

## Trionychidae

### Phylogeny

Soft-shelled turtles (Trionychidae) are one of the most distinctive and most ancient turtle families dating back to the Early Cretaceous (Brinkman et al. 2017; Georgalis and Joyce 2017), although the oldest record from Africa (Mali) dates only to the Eocene (Georgalis 2021). Both extant subfamilies, Trionychinae and Cyclanorbininae, occur in Africa. Extant Trionychinae are known from North America, Africa, Asia, and New Guinea, extant Cyclanorbininae from sub-Saharan Africa and the Indian subcontinent plus Myanmar (TTWG 2021). The oldest known cyclanorbinine turtle originates from the Late Cretaceous of Mongolia (Danilov et al. 2014; Georgalis and Joyce 2017). Two of the three extant cyclanorbinine genera (*Cyclanorbis* and *Cycloderma*) are endemic to sub-Saharan Africa, each with two species, whereas trionychine soft-shelled turtles are represented in Africa only with one monotypic genus (*Trionyx*) that had prior to the Pleistocene a much wider distribution also including much of the Western Palearctic (TEWG 2015; Geor-

galis and Joyce 2017; TTWG 2021). Outside Africa, *T. triunguis* occurs along the Mediterranean east coast north to southern Turkey (TTWG 2021). The two African cyclanorbine genera are sister taxa; *T. triunguis* belongs to a clade also comprising the Asian giant soft-shelled turtles *Chitra* and *Pelochelys* (Engstrom et al. 2004; Thomson et al. 2021).

## Phylogeography

The phylogeography of African trionychids is poorly studied, even though such information would be of special importance for the Critically Endangered *Cyclanorbis elegans* (Baker et al. 2015; TTWG 2021) to assess the extent of potential Conservation Units for targeted management actions. To date, there is a preliminary assessment of only one cyclanorbine (*Cyclanorbis senegalensis*), which is distributed from Senegal to western Ethiopia. This study was based on just four samples from Benin, Togo, and Ethiopia and an analysis of 2,429 bp of mtDNA (Mazuch et al. 2016). Despite the considerable geographic distance of approximately 3,500 km between the sample sites, little variation was found, with a sequence divergence of only up to 1.9% and a weakly resolved branching pattern in phylogenetic analyses.

Several studies examined the phylogeography of *Trionyx triunguis* (Güçlü et al. 2009, 2011; Gidis et al. 2011; Shanas et al. 2012), but these investigations have focused primarily on the Mediterranean populations from Turkey and Israel. Nevertheless, using different data sets of mtDNA (1,537 bp and 776 bp, respectively), Güçlü et al. (2009, 2011) found clear differences between *T. triunguis* from sub-Saharan Africa and the Mediterranean and very little variation among Mediterranean populations. Güçlü et al. (2009) concluded that the Mediterranean populations are connected through the sea and isolated from the sub-Saharan populations (*T. triunguis* is known to enter the Mediterranean Sea). African samples showed substantially more variation than Mediterranean material.

Güçlü et al. (2011) expanded the initial sampling and used the rapidly evolving mitochondrial control region for comparisons. They found two well-supported African clades. One comprised material from Congo and Gabon; the other contained samples from Ivory Coast and material of unknown provenance. A single sample from the Nile in southern Egypt clustered in a third well-supported clade with the Mediterranean material. Microsatellite analyses of Güçlü et al. (2011) were inconclusive as the loci used were monomorphic or had little variation.

The results from Güçlü et al. (2009, 2011) conflict with those published by Gidis et al. (2011). Using 775 bp of the mitochondrial *cyt b* gene, incorporating GenBank sequences from Güçlü et al. (2009), and including African and Mediterranean *T. triunguis* in their analyses, Gidis et al. (2011) found a weakly resolved phylogenetic branching pattern with African individuals nested in an unresolved polytomy containing sequences from the Mediterranean, Lake Nasser (Egypt), and Cameroon. This result is in contrast to the well-supported African clades found by Güçlü et al. (2009, 2011).

Shanas et al. (2012) compared Israeli and Turkish populations of *T. triunguis* by analyzing 339 anonymous nuclear genomic loci using AFLPs and 500 bp of the *cyt b* gene. Their results supported the absence of population structure across the Mediterranean, and a GenBank sequence (AY259564; Engstrom et al. 2004) from a Liberian *T. triunguis* was found to be identical to the Mediterranean *cyt b* haplotype. However, it is likely that the sample from Engstrom et al. (2004) originated from a pet trade turtle, so that its provenance could be questionable.

## Conclusions

Previous publications suggested for some chelonian taxa from sub-Saharan Africa broad spatial phylogeographic congruence (Fig. 9). In some cases the chelonian lineages and clades were also broadly aligned spatially with those of other vertebrate taxa. For example, there was broad congruency amongst *Pelomedusa subrufa* and *P. galeata* with co-distributed reptiles, amphibians, mammals, and birds, *Pelusios castaneus* with certain amphibians in Central Africa, and there was an overlap zone between clades of *Chersina angulata* and *Homopus areolatus* that corresponds with an overlap zone for a number of other reptiles and mammals. Yet, spatial congruence of clades does not necessarily imply a completely shared evolutionary history, neither in space nor time, even though a shared component is likely to have shaped the current patterns. The reason is that taxa of different phylogenetic age and genetic divergence co-occur in the same region. If they are forced to retreat into the same refugia, each refugium will harbor a subset of the previous community that will further evolve in allopatry. Consequently, old lineages will diverge even more, while previously undifferentiated widely distributed taxa will only start to diverge from their conspecifics in other refugia. Thus, it is not unexpected that a recent analysis of phylogeographic congruence for herpetofauna from South Africa demonstrated that broad spatial congruence of co-distributed taxa is actually uncorrelated (Busschau et al. 2022). We therefore encourage phylogeographic investigators to exercise caution in providing wholesale interpretations regarding congruency of patterns and to give careful attention to timing of diversification events and examine alternatives, and to propose hypotheses that are falsifiable.

Despite notable progress, phylogeny and in particular phylogeography of sub-Saharan chelonians remain poorly studied for the majority of species and offer plenty of future research possibilities. Past investigations contributed to the discovery of previously unknown taxa and genealogical lineages in *Pelomedusa*, *Pelusios*, and *Psammobates* and unexpected phylogenetic relationships within *Kinixys*. Application of phylogenetic methods has also assisted to clarify taxonomy in these groups and in testudinids in general. Some of the more notable discoveries are the recognition of an additional tortoise genus



(*Chersobius*), the unexpected diversity of *Pelomedusa*, and the debunking of *Pelusios seychellensis* and the subspecies of *Chersobius signatus* and *Stigmochelys pardalis*. Further discoveries are expected with additional research.

Cline analyses across contact zones of distinct genetic lineages would be a promising novel tool especially for *Pelomedusa* and the *Psammobates tentorius* complex that could provide insights into the degree of gene flow and taxonomic status. The knowledge of the phylogeographic diversity of sub-Saharan chelonians, which include many imperiled species, would offer a solid foundation for taxonomy and the identification of genetically distinct Management and Conservation Units for targeted conservation actions. Unfortunately, a major impediment to the future of such basic biological research, particularly for widely distributed taxa, is the current local and global legislative quagmire of red tape (Neumann et al. 2018; Prathapan et al. 2018; Britz et al. 2020; Alexander et al. 2021). In particular, the implementation of the Nagoya Protocol (<https://www.cbd.int/abs>) has turned the well-intended concept of Access and Benefit Sharing rooted in the Convention on Biological Diversity (CBD) into a major obstacle for non-commercial research and conservation endeavors.

The Nagoya Protocol was intended to ensure that financial benefits from commercialization of the biological resources of a sovereign nation were realized by the people of that nation. Regrettably, non-commercial research (i.e., academic quest for knowledge) is treated with the same broad brush in terms of Nagoya as that applied to commercial research. In addition, the same rationale now applies to a major emerging debate regarding commercialization of ‘digital sequence information’ or ‘DSI.’ That is, some opponents seek to restrict the use of DNA sequence data stored on global public databases (such as GenBank/NCBI, ENA, and DDBJ), as these sequence data have been perceived as belonging to the nation from which the original DNA samples were sourced. While the application of controls for DSI as applied to commercial ventures has legitimacy, it has been argued that non-commercial research use of DSI should not fall under the same blanket approach (see Hartman Scholz et al. 2022), because otherwise the situation for non-commercial research would be aggravated and the application of research results for conservation further curtailed.

A short-term alternative for the use of fresh genetic samples, and thus a method to circumvent the described Nagoya obstacles, is offered by the application of aDNA approaches for phylogeographic studies using museum material. Sequencing historical DNA using Sanger and Illumina technologies has already contributed to the clarification of the taxonomy, nomenclature, and distribution of extinct and extant African terrapin and tortoise species (*Pelomedusa* spp.: Fritz et al. 2014, 2015; Petzold et al. 2015; *Pelusios rhodesianus*: Kindler et al. 2016; *P. seychellensis*: Stuckas et al. 2013; ‘*Centrochelys*’ *atlantica*: Kehlmaier et al. 2021b; *Psammobates tentorius*: Zhao et al. 2020b). However, such approaches are expensive and

time-consuming and therefore hardly can be used routinely, even though the application of Illumina platforms enhanced the situation considerably.

Essentially, stakeholders and parties to the CBD must hearken back to the real aims of the CBD, first and foremost—the conservation of biodiversity. It is therefore crucial to acknowledge that the CBD targets cannot be met unless there is strong support for, and fostering of, academic-based research including phylogenetic, phylogeographic and taxonomic research. Fair and equitable sharing of resources is certainly imperative in today’s social and economic landscape, but the inclusion of non-commercial, academic research under protocols that were set up for the intended purpose of providing benefit from commercialization of biological resources is detrimental to progress. Without a resolution that allows for academic research, the continued usage of outdated and incorrect taxonomic classifications will result in an error cascade of conservation decisions. This is counter-productive to the aims of the CBD as the discovery and recognition of new species likely would shift conservation aims tremendously (see for instance Nater et al. 2017 and Wich et al. 2019 for a prominent mammalogical example).

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