



Short Note

Phylogenetic relationships of the West African mud turtle (*Pelusios castaneus*) on the islands of São Tomé and Príncipe, West Central Africa

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Abstract. On the island nation of São Tomé and Príncipe, West Africa, only one species of terrapin has been recorded: the West African mud turtle, *Pelusios castaneus*. Here, we use a mtDNA phylogenetic approach to shed light on the geographical origin of *Pelusios castaneus* on both islands. Our results indicate several independent colonisations from different African mainland regions (Nigeria, Ivory Coast, Sierra Leone and Congo). However, it is still unclear if the species arrived on the islands by natural dispersal (e.g., through vegetation rafts) or by human agency (e.g., as a food source). Our work provides important insights into the origins of *P. castaneus* in São Tomé and Príncipe, but a more in-depth study is needed to fully understand the origins and evolutionary histories of these populations.

Keywords: biological invasions, chelonian dispersal, conservation, Gulf of Guinea, mtDNA, phylogenetic analysis.

São Tomé and Príncipe is an island nation located in the Gulf of Guinea off West Africa. It comprises of two main islands – São Tomé (854 km²) and Príncipe (142 km²) – located approximately 150 km apart and respectively, ca. 250 and 225 km off continental Africa (fig. 1). Both islands were uninhabited before their discovery in the 15th century. Following

human arrival a diverse range of species was introduced, which now contribute to the singular biota of these islands (Ceríaco et al., 2022a). São Tomé and Príncipe host a disproportionately large number of endemic vertebrates, and in recent years multiple studies have tried to unveil their origin and dispersal mechanisms (see e.g., Bell et al., 2022; Ceríaco et al., 2022b).



Figure 1. Location of São Tomé and Príncipe in relation to mainland Africa. The map displays the main rivers that flow into the Gulf of Guinea (Niger, Ogooué and Congo) and the main ocean currents: Benguela Coastal Current and Guinea Current.

The West African mud turtle, *Pelusios castaneus* (Schweigger, 1812), is the only documented terrapin on São Tomé and Príncipe (Ceríaco et al., 2022b). This species has a wide but disjunctive native distribution with three unconnected populations in western Africa, extending from Senegal to Angola (Bour et al., 2016) and has also been introduced in Guadelupe, Lesser Antilles (Fritz et al., 2011). Three 19th century specimens initially believed to be from the island of Mahé, Seychelles, likely had their origin misidentified, casting doubt on the presence of the species in the Seychelles (Stuckas et al., 2013). In both São Tomé and Príncipe, *P. castaneus* it is known locally as “*bencú*” and has been present at least since the late 1800’s (Ceríaco et al., 2022b). According to local folklore, when a turtle hatches facing the ocean it becomes a sea

turtle, while if towards the forest it becomes a “*bencú*”.

To our knowledge, there are no studies exclusively focused on the terrapins of São Tomé and Príncipe (Ceríaco et al., 2022b). In 2011, Fritz et al. uncovered genetic similarities between one *P. castaneus* from São Tomé and another from the Ivory Coast, suggesting a recent colonization from mainland Africa. Subsequently, Kindler et al. (2016) confirmed that this same specimen clustered with the Ivory Coast and Nigeria clade, distinct from the terrapins from Congo-Brazzaville and Guadelupe. So far, Kindler et al. (2016) remains the most comprehensive mtDNA phylogenetic study on *P. castaneus* with the identification of three distinct evolutionary units: one from the coastal Republic of Congo; another from eastern Cameroon; and a third clade from the Ivory Coast and Nigeria, including the specimen

from São Tomé. Hence, all previous phylogenetic studies contained only a single specimen from São Tomé and, so far, no specimens of *P. castaneus* from Príncipe have been analysed. Here, we provide the first molecular assessment of the phylogenetic affinities of *P. castaneus* from Príncipe, as well as new molecular data for the São Tomé population.

In August 2022, tissue samples from three individuals were collected from Santo António city (1.64037, 7.42125) in Príncipe, and from three individuals from São Tomé city (0.3386, 6.7312) in São Tomé (fig. 1). All individuals were collected from nearby streams by locals which kept them as pets. Muscle tissue samples were collected and preserved in 96% ethanol. Genomic DNA was extracted using the E.Z.N.A Tissue DNA Kit (Mag-Bind→). A fragment of the 12S rRNA gene was amplified by PCR using the primers L1091 and H1478 published by Kocher et al. (1989), and following the thermocycling conditions described in Fritz et al. (2011). Amplification of the 12S mtDNA fragment was conducted in a 10 μ l volume, comprised of 5 μ L of QUIAGEN Multiplex PCR Master Mix (Quiagen, Crawley, UK), 0.3 μ L of each primer, 3.4 μ L of ultra-pure water, and 2 μ L of DNA extract. All amplified fragments were sequenced in a Sanger sequencer and deposited in GenBank (accession numbers OQ296941–OQ296946). A total of 284 12S sequences of *Pelusios* sp. were retrieved from GenBank and added to the dataset. The obtained sequences were imported into Geneious Prime® (v.2022.2.2 Biomatters Ltd.) where the alignment was performed using MAFFT v.7.490 (Katoh et al., 2002; Katoh and Standley, 2013), under default parameters. Phylogenetic analysis based on the 12S mitochondrial fragment was performed under a Bayesian Inference (BI) method, using *Pelomedusa subrufa* as the outgroup (following Fritz et al., 2011). To determine the best fitting nucleotide model, we used ModelFinder (Kalyaanamoorthy et al., 2017) from the IQ-TREE Web server (Trifinopou-

los et al., 2016). BEAST v.2.6.6 (Bouckaert et al., 2019) was used for the BI genealogy. Analyses were run twice for 10^6 generations with a sampling frequency of 1000. Models and prior specifications applied were as follows (otherwise by default): Strict Clock, Coalescent with Constant Population Size, and the JC69 nucleotide model based on ModelFinder. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v.1.7.1 (Rambaut et al., 2018) after obtaining an effective sample size (ESS) > 200. The initial 10% of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator. Trees were visualized using FigTree v.1.4.4 (Rambaut, 2009).

The obtained 12S fragments from the newly sequenced specimens had a length of 383bp. According to the obtained genealogy, all collected individuals cluster with others assigned as *P. castaneus* with high posterior probability (fig. 2c). Moreover, within *P. castaneus* we can identify four major clades, with the individuals from São Tomé spread across three of them and Príncipe present in two. For one of the clades, the geographic origin of the samples is unknown, but the remaining three comprise specimens from distinct African countries, namely, the Democratic Republic of Congo, Nigeria, Ivory Coast and Sierra Leone.

The sequence BO1 from Príncipe had an 100% nucleotide pairwise match with BO5 from São Tomé, as well as with the 12S sequence from the Ivory Coast (LN833904.1). However, the Bayesian phylogenetic analysis clusters BO1 and BO2 from Príncipe with the individuals from the Ivory Coast and Sierra Leone. This result indicates that, in the absence of human-mediated colonization, São Tomé was unlikely to act as a stepping stone between

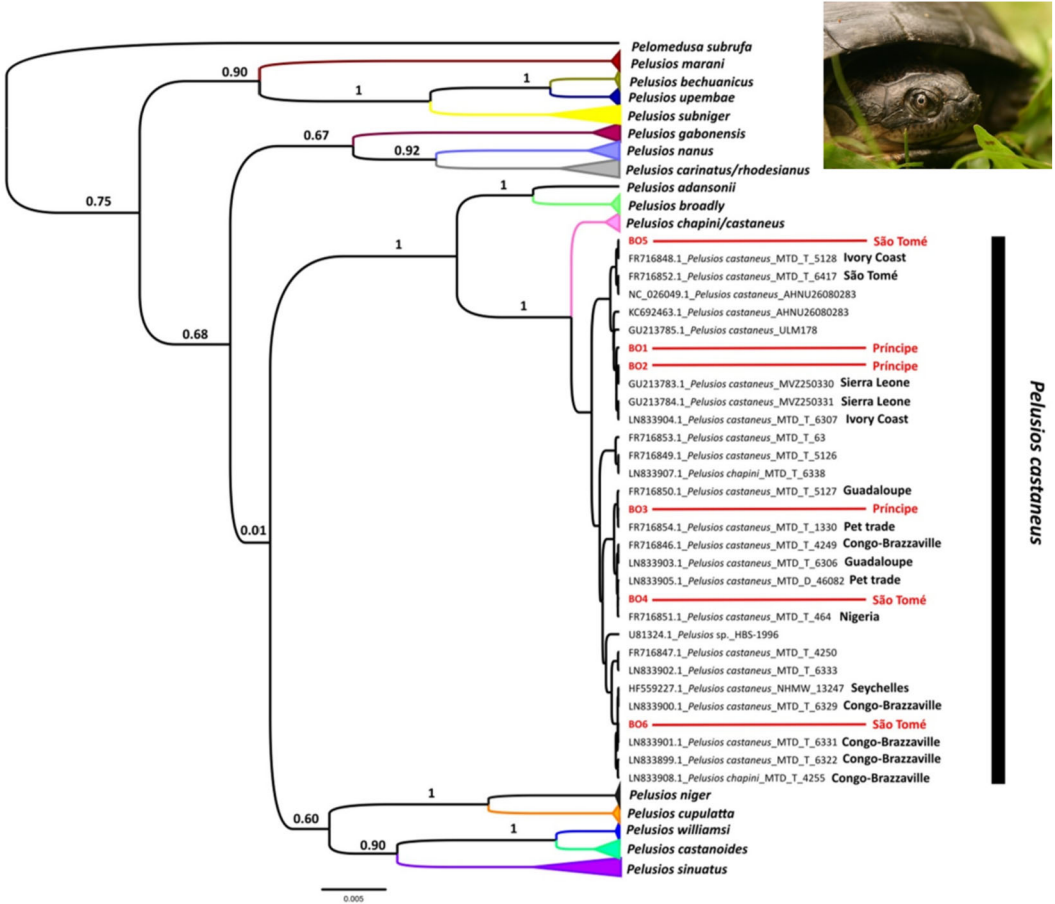


Figure 2. Bayesian phylogenetic tree of *Pelusios* species and placement of the specimens collected in São Tomé and Príncipe among *Pelusios castaneus* (red lines).

mainland Africa and Príncipe. Rather, it suggests that *P. castaneus* colonized Príncipe directly from the mainland. This also seems to be the case for BO3 from Príncipe, which does not cluster with other individuals from São Tomé. Nonetheless, further sampling in additional localities from both São Tomé and Príncipe, as well as from mainland Africa, is paramount to clarify the most likely origin of these insular populations.

Island colonisations can be attributed to either natural dispersal or human introduction, but distinguishing between the two can be challenging (e.g., Ceriaco et al., 2020). While the gecko *Hemidactylus longicephalus* (Bocage, 1873) (Ceriaco et al., 2020) and the

skink *Trachylepis affinis* (Grey, 1838) (Ceriaco et al., 2016) are recognized as recent introductions to São Tomé and Príncipe, their arrival mechanisms remain unclear. However, the introduction of terrestrial mammals, such as feral pigs *Sus scrofa* (Linnaeus, 1758), mona monkeys *Cercopithecus mona* (Schreber, 1774), African civets *Civettictis civetta* (Schreber, 1776) and least weasels *Mustela nivalis* (Linnaeus, 1766), is less doubtful and seems to have been the result of deliberate or accidental introductions by early settlers (Rainho et al., 2022). Similarly, the arrival of *Hemidactylus mabouia* (Moreau de Jonnès, 1818)

was probably the result of human transportation, albeit more recently (Agarwal et al., 2021).

The most widely accepted theory for the natural dispersal of non-volant vertebrates to oceanic islands such as São Tomé and Príncipe is through dispersal on vegetation rafts carried by “freshwater pathways” formed during the heavy discharge of major rivers from the African mainland (Bell et al., 2022). The Niger, the Congo and the Ogooué are the main rivers that flow into the Gulf of Guinea (Ceríaco et al., 2022c) (fig. 1), which might explain the genetic similarity of some of the individuals analysed in this study to the specimens from Nigeria and Congo. Additionally, the dominant currents in the Gulf of Guinea, such as the Guinea current in the north and the Benguela current in the south (Ceríaco et al., 2022c), could have transported rafts from northwestern and southeastern rivers carrying individuals from Sierra Leone, Ivory Coast or Congo to São Tomé and Príncipe (fig. 1).

Although terrapins depend on freshwater for their survival, they can endure saltwater crossings. In 2020, in the aftermath of a hurricane, one adult Central American snapping turtle *Chelydra rossignonii*, was reported to arrive to Utila Island from mainland Honduras (Brown et al., 2021). Additionally, in the Seychelles, while the arrival of *Pelusios subniger* (Bonaterre, 1789) is thought to be human-mediated, it is unclear what was the process behind the arrival of *Pelusios castanoides* (Hewitt, 1931) (Fritz et al., 2013). Considering these cases, alongside ample evidence from other taxa arriving to São Tomé and Príncipe islands transported by marine currents (e.g., Bell et al., 2022; Rainho et al., 2022), we cannot dismiss the possibility that *P. castaneus* arrived through a combination of stochastic events associated with river discharges and oceanic currents.

Human introduction is the other possible explanation for the origin of these populations. Mona monkeys, for example, were probably

introduced in Grenada, Caribbean, from populations from São Tomé and Príncipe, most likely during the slave trade period (1500s until mid-1800s) (Glenn and Bensen, 2013). Similarly, it is thought that Grenada populations of *P. castaneus* originated from animals transported for food in the slave trade routes (Lescure, 1983). Given that *P. castaneus* is a part of local cuisine in its natural range in mainland Africa and in São Tomé Island (Luiselli et al., 2013; Ceríaco et al., 2022b), and considering that terrapins and tortoises were frequently used as food source during long overseas voyages (Fritz et al., 2013), it is very possible that the populations of *P. castaneus* in São Tomé and Príncipe originated from individuals transported for human consumption. One specimen from Príncipe (BO3) and one from São Tomé (B04) clustered with individuals from Guadeloupe in our phylogenetic analysis (fig. 2c), indicating that they have close phylogenetic affinities with *P. castaneus* from this Caribbean Island. This suggests that São Tomé and Príncipe might have acted as a stepping stone between continental African and Caribbean populations, which further supports a link to human-mediated colonisation.

Overall, this study provides interesting insights into the origins of *P. castaneus* from São Tomé and Príncipe. However, a more comprehensive study is needed to fully understand the origins and evolutionary histories of these populations, as well as their distribution, demographic trends, ecological requirements, and interspecific interactions. Considering that *P. castaneus* is represented in local folklore, individuals are kept as pets and are often consumed as a delicacy, they would make an excellent subject for ethnobiological study. Such research would provide valuable information about the natural and cultural history of this species on the oceanic islands of the Gulf of Guinea.

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References

- Agarwal, I., Ceriáco, L.M.P., Metallinou, M., Jackman, T.R., Bauer, A.M. (2021): How the African house gecko (*Hemidactylus mabouia*) conquered the world. *Royal Society Open Science* **8**: 210749.
- Bell, R.C., Ceriáco, L.M., Scheinberg, L.A., Drewes, R.C. (2022): The amphibians of the Gulf of Guinea oceanic islands. In: *Biodiversity of the Gulf of Guinea Oceanic Islands: Science and Conservation*, p. 479-504. Ceriáco, L.M.P., de Lima, R.F., Melo, M., Bell, R.C., Eds, Springer, Cham.
- Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N. (2019): BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS computational biology* **15**: e1006650.
- Bour, R., Luiselli, L., Petrozzi, F., Segniabeto, G.H., Chirio, L. (2016): *Pelusios castaneus* (Schweigger 1812) – West African mud turtle, swamp terrapin. In: *Conservation Biology of Freshwater Turtles and Tortoises: a Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*, p. 095.1-095.11. Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Iverson, J.B., Eds, Chelonian Research Foundation.
- Brown, T.W., Augustinus, E., Izaguirre, A., Solis, J.M. (2021): Central American snapping turtle (*Chelydridae, Chelydra rossignoni*) on utila island, Honduras, demonstrates hurricanes are a likely past and future oversea dispersal pathway for species introduction in the Caribbean. *Caribbean Journal of Science* **51**: 30-36.
- Ceriáco, L.M., Marques, M.P., Bauer, A.M. (2016): A review of the genus *Trachylepis* (Sauria: Scincidae) from the Gulf of Guinea, with descriptions of two new species in the *Trachylepis maculilabris* (Gray, 1845) species complex. *Zootaxa* **4109**: 284-314.
- Ceriáco, L.M., Agarwal, I., Marques, M.P., Bauer, A.M. (2020): A review of the genus *Hemidactylus* Goldfuss, 1820 (Squamata: Gekkonidae) from Angola, with the description of two new species. *Zootaxa* **4746**: 1-71-1-71.
- Ceriáco, L.M., de Lima, R.F., Bell, R.C., Melo, M. (2022a): Biodiversity in the Gulf of Guinea oceanic islands: a synthesis. In: *Biodiversity of the Gulf of Guinea Oceanic Islands: Science and Conservation*, p. 1-12. Ceriáco, L.M.P., de Lima, R.F., Melo, M., Bell, R.C., Eds, Springer, Cham.
- Ceriáco, L.M., Marques, M.P., Bell, R.C., Bauer, A.M. (2022b): The terrestrial reptiles of the Gulf of Guinea oceanic islands. In: *Biodiversity of the Gulf of Guinea Oceanic Islands: Science and Conservation*, p. 505-534. Ceriáco, L.M.P., de Lima, R.F., Melo, M., Bell, R.C., Eds, Springer, Cham.
- Ceriáco, L.M., Santos, B.S., Lima, R., Bell, R.C., Norder, S., Melo, M. (2022c): Physical geography of the Gulf of Guinea oceanic islands. In: *Biodiversity of the Gulf of Guinea Oceanic Islands: Science and Conservation*, p. 13-36. Ceriáco, L.M.P., de Lima, R.F., Melo, M., Bell, R.C., Eds, Springer, Cham.
- Fritz, U., Branch, W.R., Hofmeyr, M.D., Maran, J., Prokop, H., Schleicher, A., Široký, P., Stuckas, H., Vargas-Ramírez, M., Vences, M. (2011): Molecular phylogeny of African hinged and helmeted terrapins (Testudines: Pelomedusidae: *Pelusios* and *Pelomedusa*). *Zoologica Scripta* **40**: 115-125.
- Fritz, U., Branch, W.R., Gehring, P.-S., Harvey, J., Kindler, C., Meyer, L., Du Preez, L., Široký, P., Vieites, D.R., Vences, M. (2013): Weak divergence among African, Malagasy and Seychellois hinged terrapins (*Pelusios castanoides*, *P. subniger*) and evidence for human-mediated oversea dispersal. *Organisms Diversity & Evolution* **13**: 215-224.
- Glenn, M.E., Bensen, K.J. (2013): The mona monkeys of Grenada, São Tomé and Príncipe: long-term persistence of a guenon in permanent fragments and implications for the survival of forest primates in protected areas. In: *Primates in Fragments: Complexity and Resilience*, p. 413-422. Marsh, L.K., Chapman, C.A., Eds, Springer, New York, NY.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S. (2017): ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587-589.
- Katoh, K., Misawa, K., Kumar, K., Miyata, T. (2002): MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059-3066.
- Katoh, K., Standley, D.M. (2013): MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772-780.
- Kindler, C., Moosig, M., Branch, W.R., Harvey, J., Kehlmaier, C., Nagy, Z.T., Prokop, H., Široký, P., Fritz, U. (2016): 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*. *Biological Journal of the Linnean Society* **117**: 305-321.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA* **86**: 6196-6200.
- Lescure, J. (1983): Introductions passives et actives de reptiles et d'amphibiens dans les Antilles et les Guyanes. *Compte Rendu des Seances de la Societe de Biogeographie* **59**: 59-70.

- Luiselli, L., Petrozzi, F., Akani, G.C. (2013): Long-term comparison reveals trends in turtle trade in bushmeat markets of southern Nigeria. *Herpetozoa* **26**: 57-64.
- Rainho, A., Meyer, C.F., Thorsteinsdóttir, S., Juste, J., Palmeirim, J.M. (2022): Current knowledge and conservation of the wild mammals of the Gulf of Guinea oceanic islands. In: Biodiversity of the Gulf of Guinea Oceanic Islands, p. 593-620. Ceríaco, L.M.P., de Lima, R.F., Melo, M., Bell, R.C., Eds, Springer, Cham.
- Rambaut, A. (2009): Figtree [Computer Software]. <http://tree.bio.ed.ac.uk/software/figtree/>.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A. (2018): Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 177-191. DOI:10.1093/sysbio/syy032.
- Stuckas, H., Gemel, R., Fritz, U. (2013): One extinct turtle species less: *Pelusios seychellensis* is not extinct, it never existed. *PLoS One* **8**: e57116.
- Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A., Minh, B.Q. (2016): W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44**: W232-W235.