

# Deciphering Evolutionary Ancestry in African Tree Frogs: Genus *Leptopelis*

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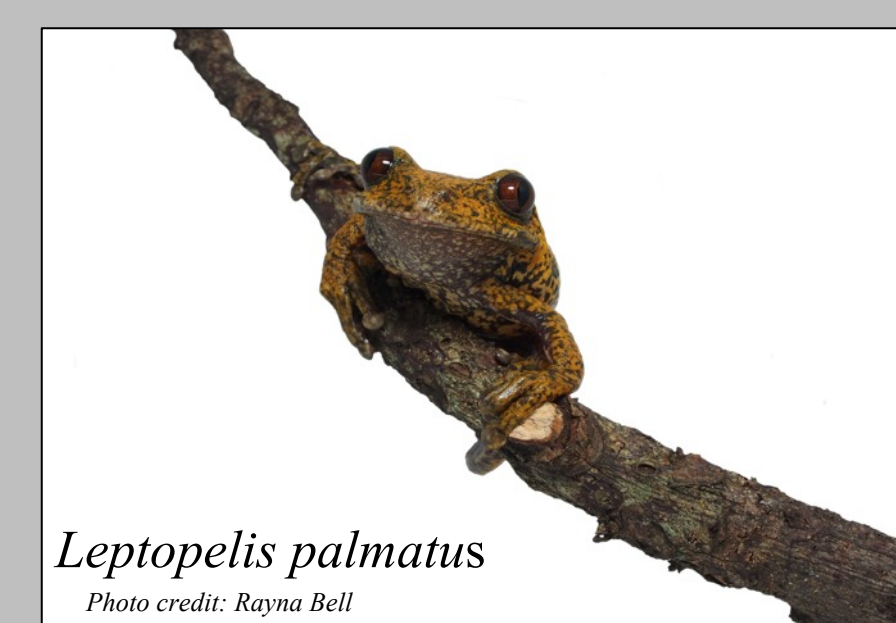
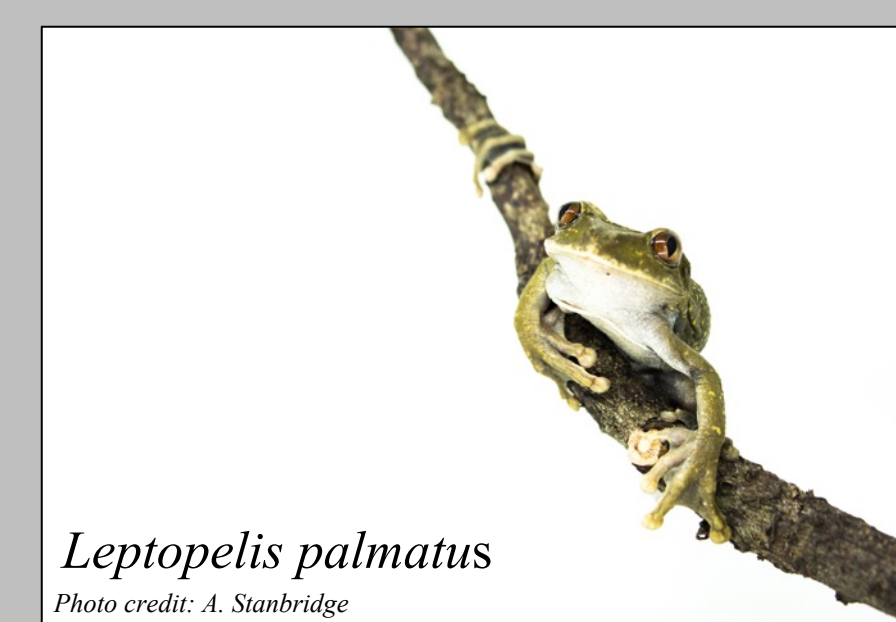


## Introduction

The genus *Leptopelis* is a group of African Tree Frogs that have a poorly understood taxonomic history, with 53 currently accepted species (Frost 2017). These species are closely related and difficult to distinguish by morphological features alone, often times being misidentified in the field. *Leptopelis palmatus* is an endemic species to Príncipe Island, one of four islands in the Gulf of Guinea archipelago (Jones 1994). Reconstructing its colonization history and subsequent speciation is key to understanding the biogeography of this archipelago.

*L. macrotis*, *L. millsoni*, and *L. rufus* form a species complex that occurs across West and Central Africa, as well as on Bioko Island, a continental land-bridge system. These species have been hypothesized to be the closest ancestors of *L. palmatus* due to their large size and proximity to Príncipe (Schiotz 1999). In addition, their distribution across continental Africa and Bioko Island make them an ideal study system for investigating speciation across a range of environments. In this project, we investigate questions of phylogeography and ancestral history in order to learn more about the evolutionary relationships amongst the organisms in this island system.

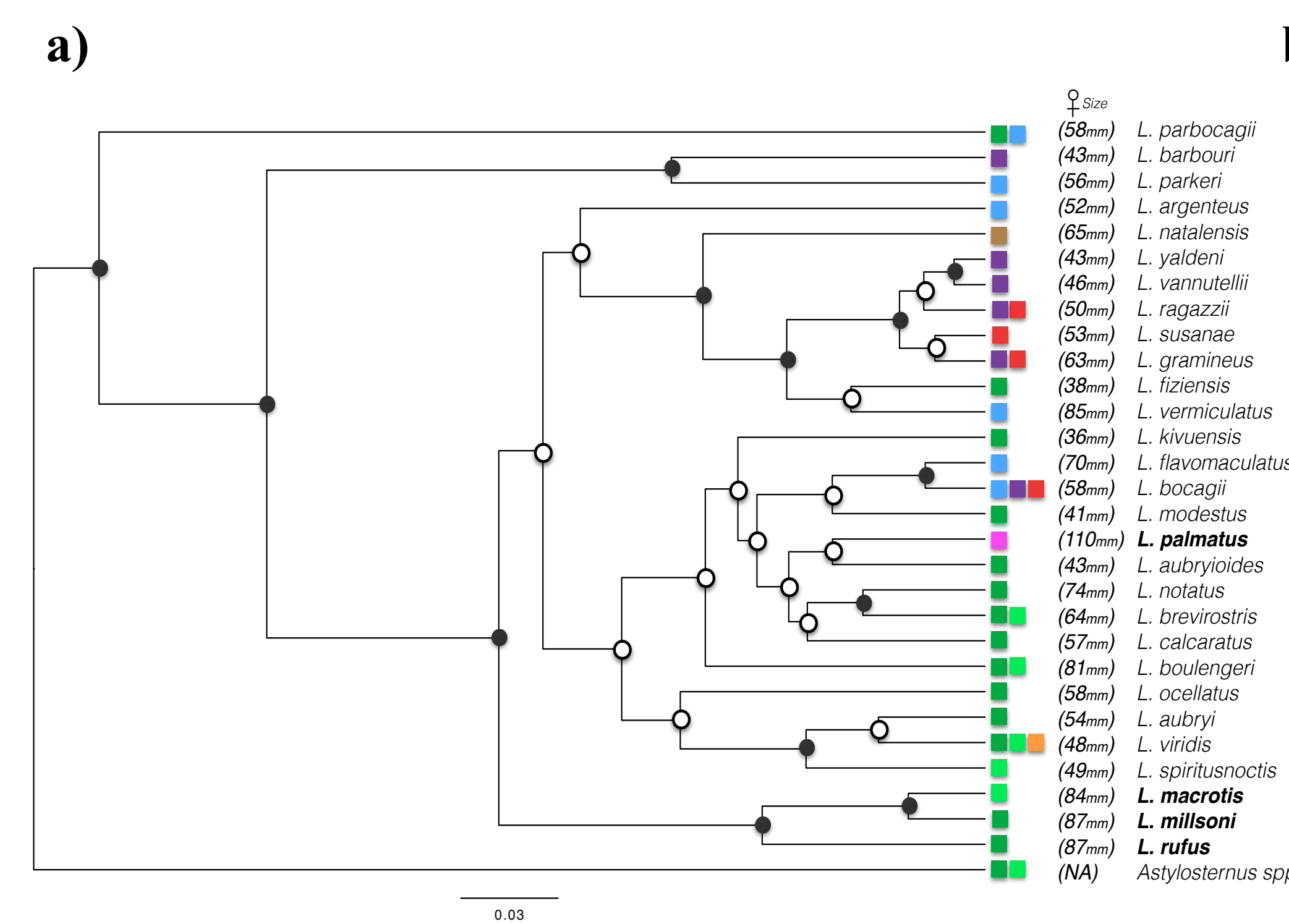
## Variation in *Leptopelis* Species Complex



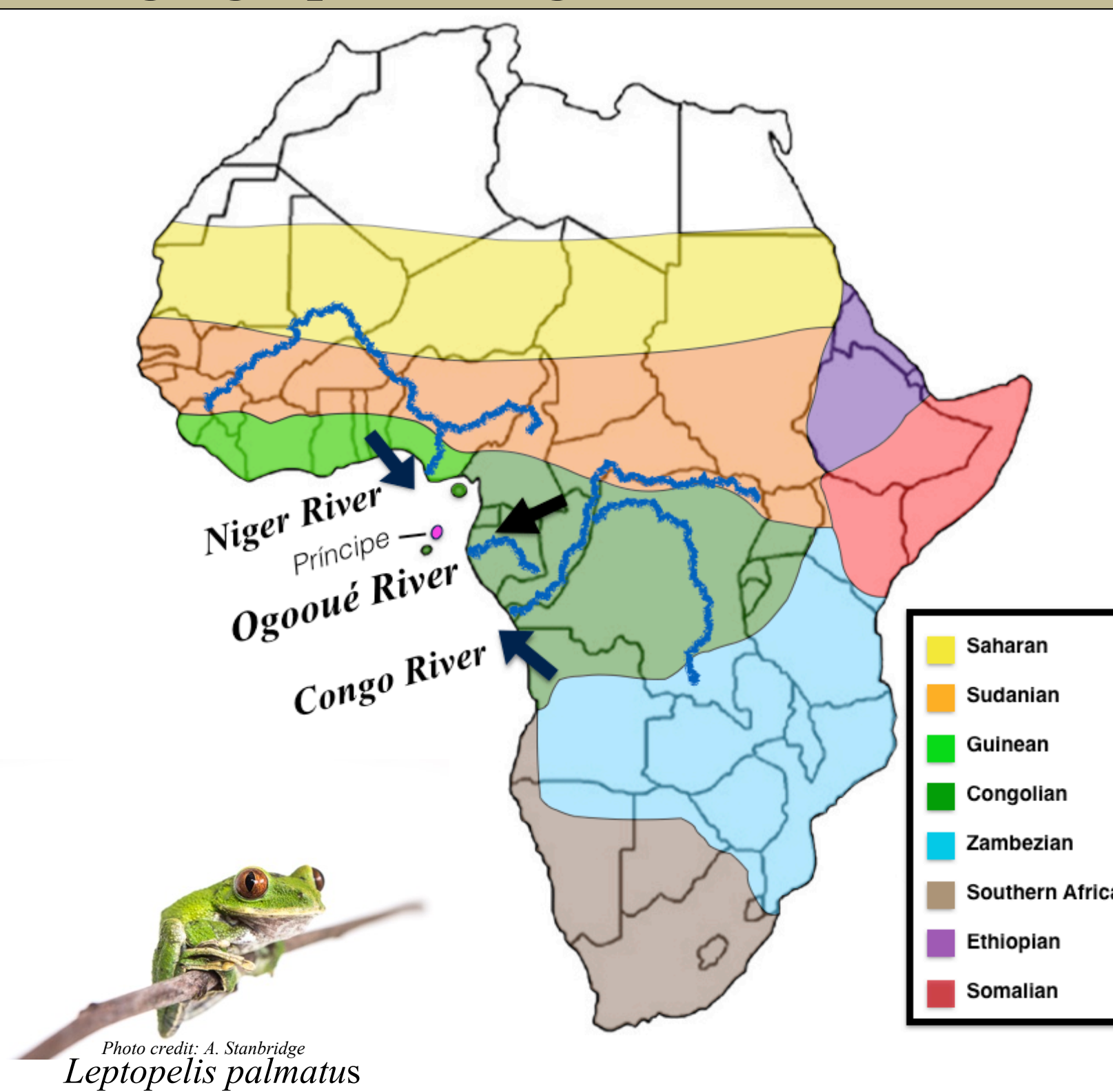
## Methods

We collected 16s mitochondrial gene sequences for 33 species of *Leptopelis* and 146 individuals for our four target species. To determine whether *L. palmatus* is closely related to the *L. rufus*/*L. millsoni*/*L. macrotis* complex we generated a phylogeny using BEAST v 1.8.4 including one representative sequence for each species, with *Astylosternus* as an out-group. We assessed phylogeographic structure across the *L. rufus*/*L. millsoni*/*L. macrotis* complex with more than 100 specimens from 19 localities amongst the three species. We took 14 standard morphological measurements on more than 30 specimens to evaluate field identifications and search for more reliable morphological characters for species identification.

## Phylogeny of 30 *Leptopelis* Species



## Biogeographical Regions of Africa



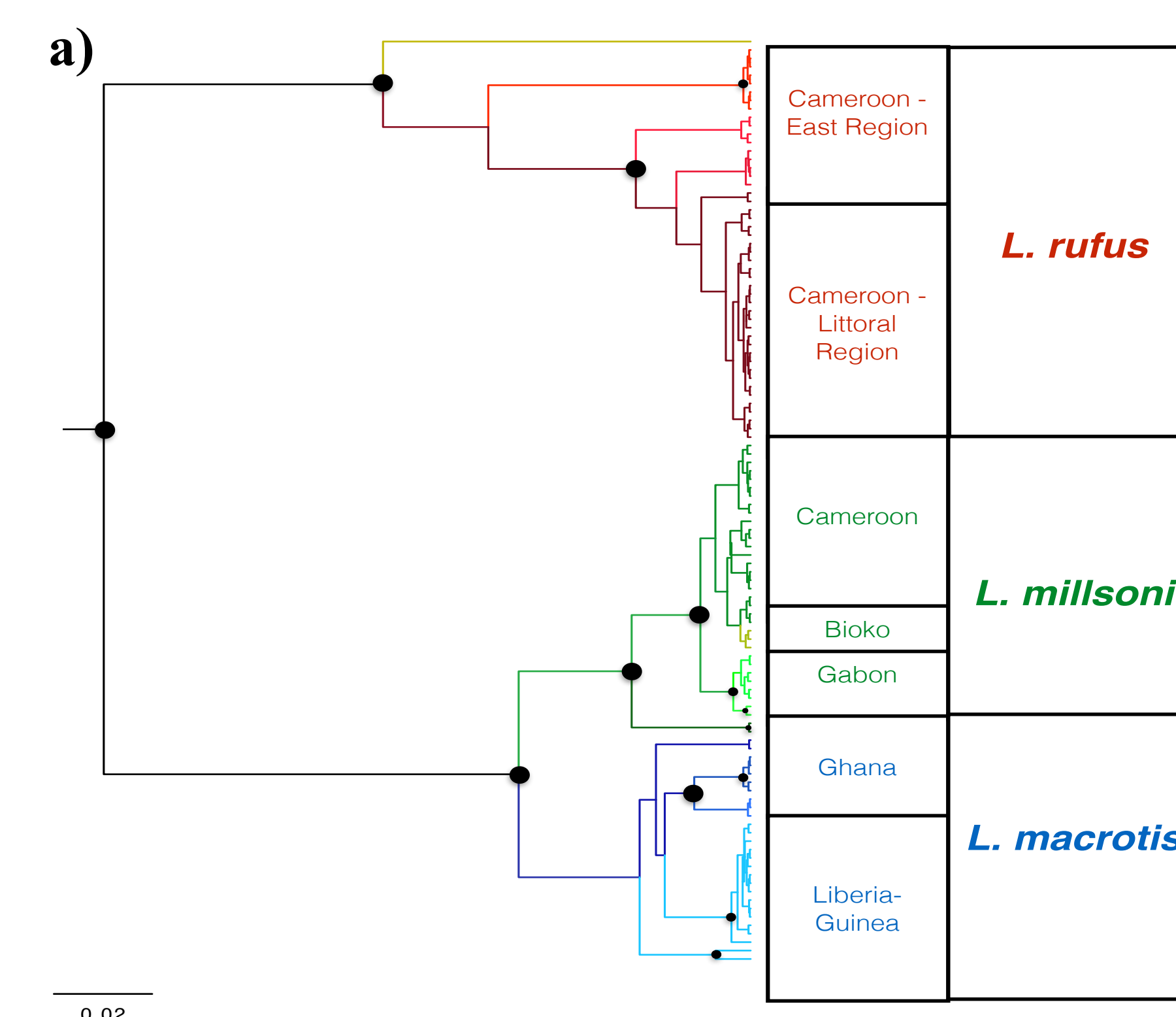
**Figure 1** – (a) Phylogenetic tree of 16s mitochondrial gene. Includes 30 *Leptopelis* species with geographic distribution by color and maximum female size. Closed circles at nodes represent (>95% CI). Female size and distribution from (Schiotz et al 1999). (b) Biogeographical map of African ecosystems partitioned into 8 areas; Partitioned areas from (Linder et al. 2012).

## Results

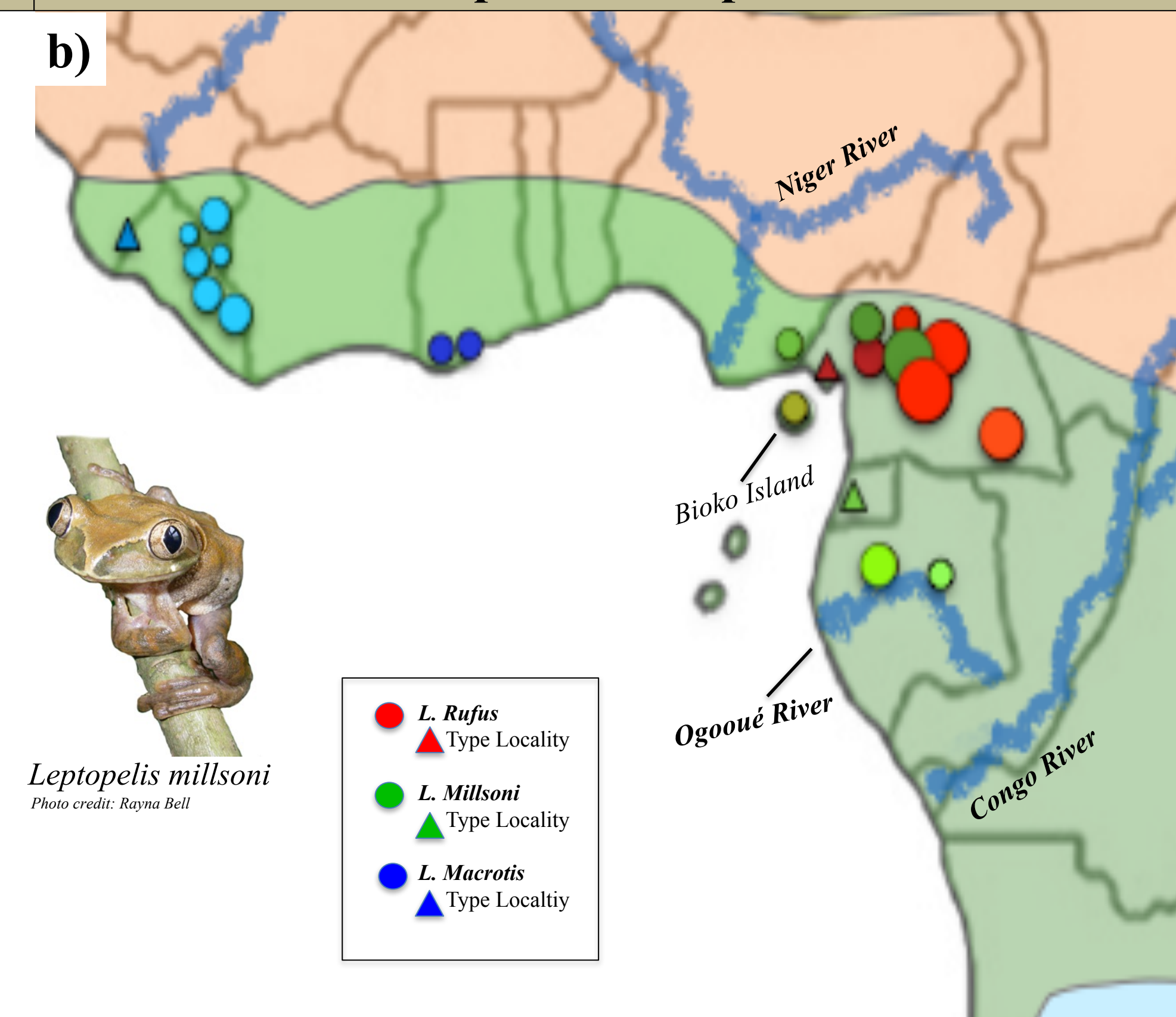
In **figure 1 (a)**, *Leptopelis palmatus* is clearly separated from the *L. macrotis*/*L. millsoni*/*L. rufus* complex with strong support (>95% CI). There is not strong support for anything beyond the divergence of these groups, although it appears that there is a closer relationship with species of the Guinean-Congolian distribution.

In **figure 2 (a)**, we see clear phylogeographic structure across the species complex with support for distinct lineages of *L. macrotis* in West Africa and *L. millsoni* in Central Africa, distinct lineages of *L. millsoni* on Bioko island, and pronounced phylogeographic structure in *L. rufus* across Cameroon.

## Phylogeographic Tree of West-Central African *Leptopelis* Species Complex

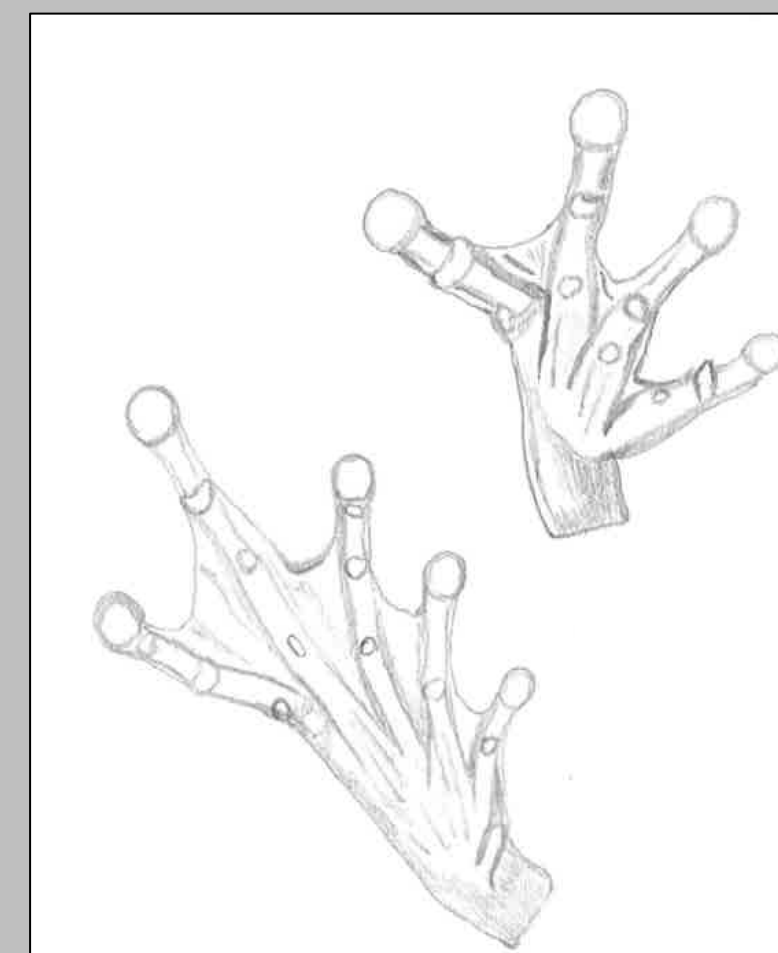


## Sampling Map of West-Central African *Leptopelis* Species Complex

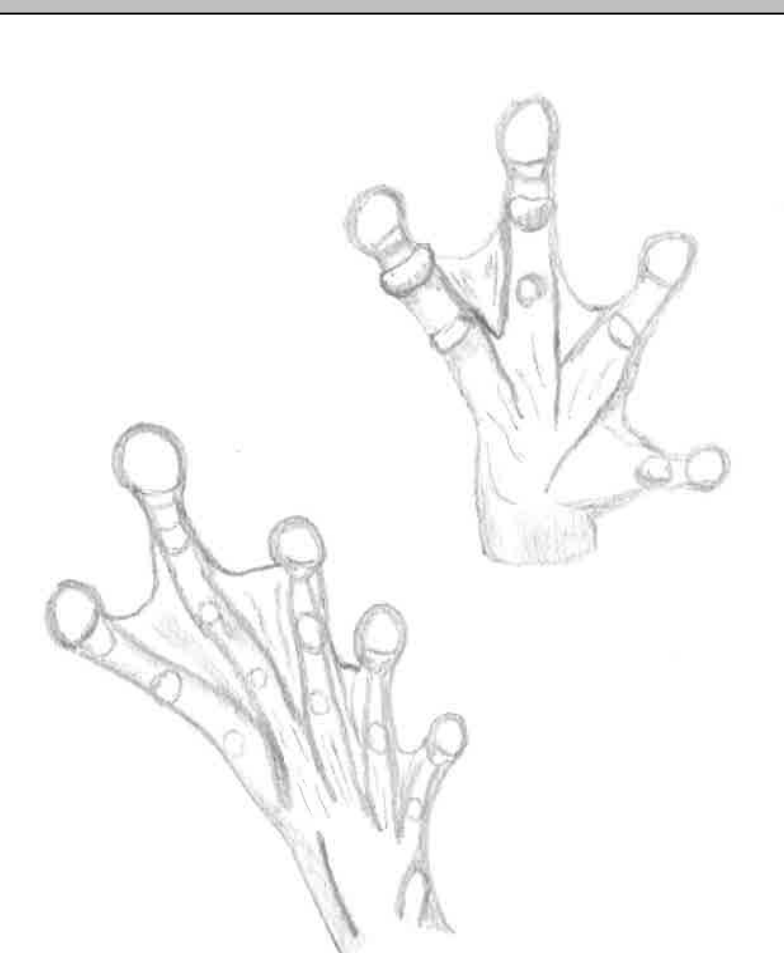


**Figure 2** – (a) Phylogeographic tree of the *L. rufus* (red)/*L. macrotis* (blue)/*L. millsoni* (green) species complex, with geographically sampled and coded lineages. (b) Distribution map of *L. macrotis* (blue), *L. millsoni* (green), and *L. rufus* (red). Circle size is determined by the number of specimens sampled at that location. Triangles represent the type locality of that species.

## Morphological Analyses



*Leptopelis rufus*



*Leptopelis millsoni*

**Figure 3**– Hand sketch of webbing extent in *L. millsoni* and *L. rufus*.

Among other characters, the extent of webbing in feet has been a consistently distinguishable tool for our specimens. We have also found that the ratio of disc (DSC) to Tympanum (TMP) and TMP to head width (HDW) or head length (HDL) is useful in our samples as well. We continue to explore new features that may be incorporated into the literature for future aid in identification.

## Conclusion

West and Central African *Leptopelis* species (*L. rufus*, *L. macrotis*, and *L. millsoni*) were previously thought to be the closest ancestors of *L. palmatus* due to both their large size and proximity to the island chain. According to our most recent data, along with more extensive sampling from our collaborators, we find that *L. palmatus* is not closely related to any of the species mentioned above. Our results indicate that *L. palmatus* is within a group of Central African species that are smaller in body size, suggesting that there may be a convergence in gigantism within this genus.

Moving forward, we are going to (1) increase our species and genetic sampling to determine which species of *Leptopelis L. palmatus* is closely related to, and (2) collect ddRADseq data for the *L. macrotis*/*L. millsoni*/*L. rufus* complex and look at more specimens to assess the morphological variation in these populations. These data will provide greater insight into the biogeography of land bridge and oceanic islands in the Gulf of Guinea archipelago and diversification in the Guineo-Congolian forests. In addition, we hope to find additional morphological characteristics to improve field identifications in this challenging species complex.

## Acknowledgments

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## References

Frost, Darrel R. 2017. Amphibian Species of the World: an Online Reference. Version 6.0 (07.24.2017). Electronic Database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA.  
Jones, P.J. 1994. Biodiversity in the Gulf of Guinea: an overview. *Biodiversity and Conservation*. 3:772-784.  
Linder, H.P., K.M. de Klerk, J. Born, N.D. Burgess, J. Fjeldsa, and C. Rahbek. 2012. *Journal of Biogeography*. 10:1365-2699.  
Schiotz A. 1999. Treefrogs of Africa. *Chimaira*. 1:251-258.