Bushmeat Barcoding with COX1

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INTRODUCTION

Bushmeat is meat from wildlife species hunted for human consumption. Bushmeat is a critical nutritional and economic resource, however over exploitation has lead to sharp declines in the populations of many endangered reptile and mammal species (Fig 1.). The international bushmeat trade is estimated to be worth over $60 billion dollars/year ($15 billion in illegal trade). Bushmeat regulation depends on the ability to identify morphologically ambiguous animal products.
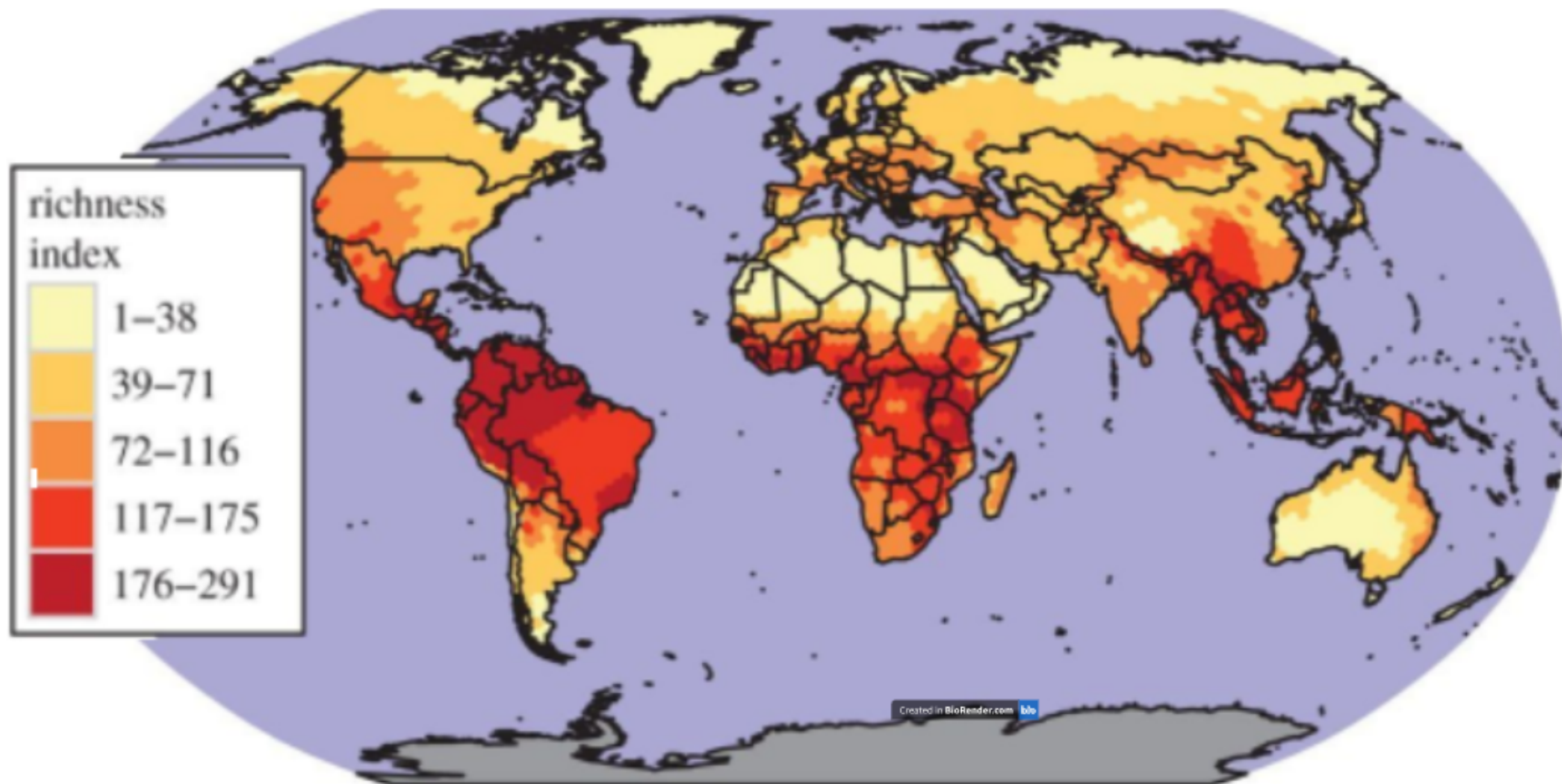

Figure 1: Species richness map for terrestrial mamals endangered by bushmeat hunting
Ripple, William & Abernethy, Katharine & Betts, Matthew & Chapron, Guillaume & Dirzo, Rodolfo & Galetti, Mauro & Levi, Taal & Lindsey, P. & Macdonald, Thomas & Peres, Carlos & Wallach, Arian & Wolf. (2016). Bushmeat hunting and extinction risk to the world's mammals. 10.6084/m9.figshare.4056588.v3.

The COX1 gene is an established standard for DNA barcoding with relialble applications for species identification. A 2010 study by Eaton et al. collected tissue samples from 7 different crocodile species in various South American and Central African locations and sequenced the COX1 gene. The study built a tree for the COX1 genes from the different crocodile species.

Figure 2: *M.cataphractus* (left) *O.osborni* (right)

GOALS & HYPOTHESIS

Goals

Use the crocodile COX1 sequences Eaton et al. depostied on NCBI to recreate their phylogenetic tree using different programs for comparison.

Add the NCBI refrence COX1 genes for each different crocodile species to see where they fall on the tree.

Hypothesis

Because Eaton et al. achieved high confidence for their species identifications, the NCBI refrence COX1 genes will fall within the expected species.

METHODS

- Downloaded the Eaton et al.'s 45 crocodile COX1 gene sequences from NCBI along with 5 NCBI refrence COX1 genes for the various crocodile species

- Alignment and phylogenetic reconstructions were performed on CLUSTALW using the function "build" of ETE3 v3.1.1. Alignment visualized with MAFFT Viewer.

- ML tree was inferred using RAxML v8.1.20 ran with model GTRGAMMA and default parameters with *A.mississippiens* as the outgroup. Branch supports were computed out of 100 bootstrapped trees.

CONCLUSIONS AND OUTLOOK

-Discrepancy in *M.cataphractus* placement could be due to differences in agignment/building programs or location of samples. Eaton et al. only collected samples from Gabon and Congo while their natural habitat spans from Senegal to the DRC.

- Could also be due to chronological discrepency; Eaton et al. samples were all collected before 2010 while the NCBI refrence was published in 2021.

- Repeat this analysis for the aligator and deer sequences published in the paper.

RESULTS

- Reconstructed tree showed similar species relationships to tree made by Eaton et al.

- The *M.cataphractus COX1* sequence was the only NCBI refrence to fall in a different clade than the sequences of the same species collected by Eaton et al.
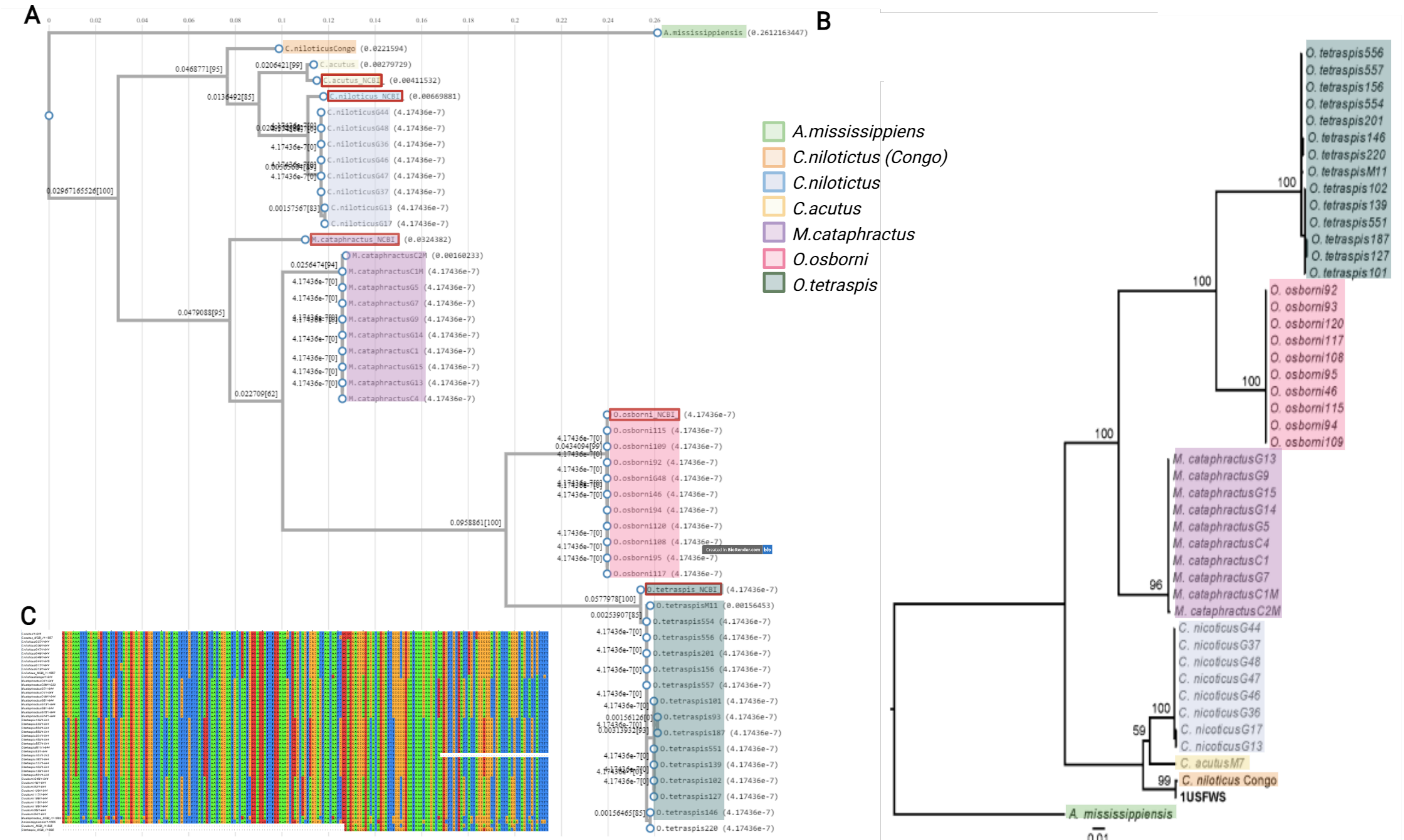

Figure 4. A) Reconstructed crocodile COX1 phylogenetic tree with boostrap values and NCBI COX1 references boxed in red. B) Crocodile COX1 phylogenetic tree from Eaton et al. Adapted from Eaton, M.J., Meyers, G.L., Kolokotronis, SO. et al. Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. Conserv Genet 11, 1389–1404 (2010). https://doi.org/10.1007/s10592-009-9967-0 [2]

Figure 3: A) Reconstructed COX1 tree with added NCBI refrences outlined, B) Eaton et al. COX1 tree, C) Sequence alignment visualization (MAFFT viewer) for reconstructed tree.
Eaton, M.J., Meyers, G.L., Kolokotronis, SO. et al. Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. Conserv Genet 11, 1389–1404 (2010). https://doi.org/10.1007/s10592-009-9967-0