How Frogs are Hiding in Plain Sight: Investigating Myanmar's *Occidozyga* Species Complex

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INTRODUCTION

In the frog genus *Occidozyga*, there is one described species which inhabits Myanmar (fig. 1).

Figure 1 *Occidozyga* frog from Myanmar

However, population estimates for *Occidozyga* within the country may be inaccurate given minimal sampling. The California Academy of Science's (CAS) Herpetofaunal survey has made numerous individuals available for study. We predict that in *Occidozyga*, there are more morphologically identical phenotypes- 'cryptics'- which are actually genetically distinct species.

Using DNA sequences isolated in the lab at USF and measurements from preserved specimens, we've been able to conduct phylogenetic tree analysis. Preliminary results suggest the presence of two new species within the genus. Phylogeographic analysis of our tree also supports the genetic clustering, such that their evolutionary distribution is likely spatially based on the topography of Myanmar.

METHODS & MATERIALS

Morphological Measurements

Morphological features (fig. 2) were measured for 163 frogs. Principal component analysis (PCA) was performed to compare variance in length using BioVinCi version 0.9.1. Qualitative differences, i.e. color, texture or pattern were also noted.

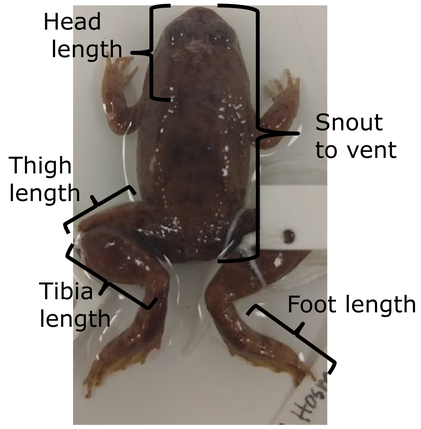

Figure 2 Preserved specimen illustrating approximate measurement method for five of the 12 measurements taken.

METHODS CONT'D

Molecular methods

DNA was isolated from liver tissue collected and preserved in 70% ethanol from 15 individuals. The 16S rRNA gene from mitochondrial DNA (mtDNA) was amplified using primers 16S-AL, and 16S-BH with polymerase chain reaction (PCR), and PCR products were sequenced and then analyzed using Sequencer software.

RESULTS

Quantitative Morphological Measurements

PCA on all twelve measurements (fig. 3) after first separating 163 specimens into groups based on species name listed in CAS catalog.

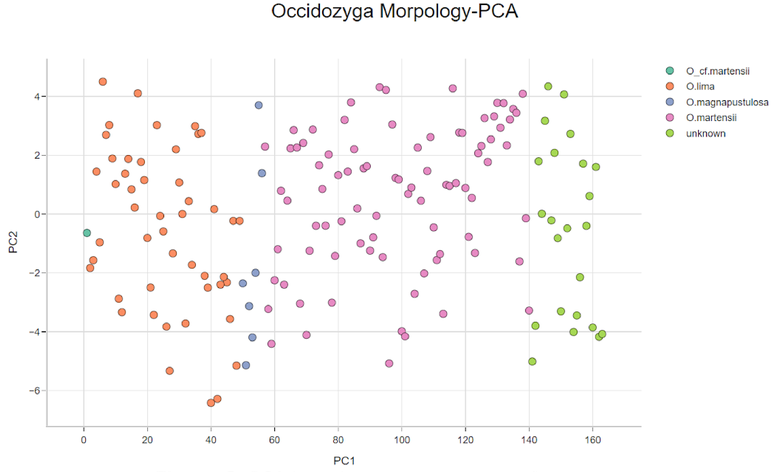

Figure 3 PCA based on measurements of *Occidozyga*. Based on the variation in morphology within and between the surveyed specimens, P.C. 1 (snout- vent length) was the strongest morphological indicator of species.

Sequence and tree statistics

A 500 base pair region length of the 16S from 15 frogs was obtained from CAS. One sample was omitted from further analysis, as its quality score was below 90%. Phylogenetic analysis produced a single tree (fig. 4.), with significantly high support values for all monophyletic clades.

RESULTS CONT' D

All but one previously sequenced and described species from GenBank incorporated in the analysis formed a separate clade from the Mvanmar *Occidozvaa*.

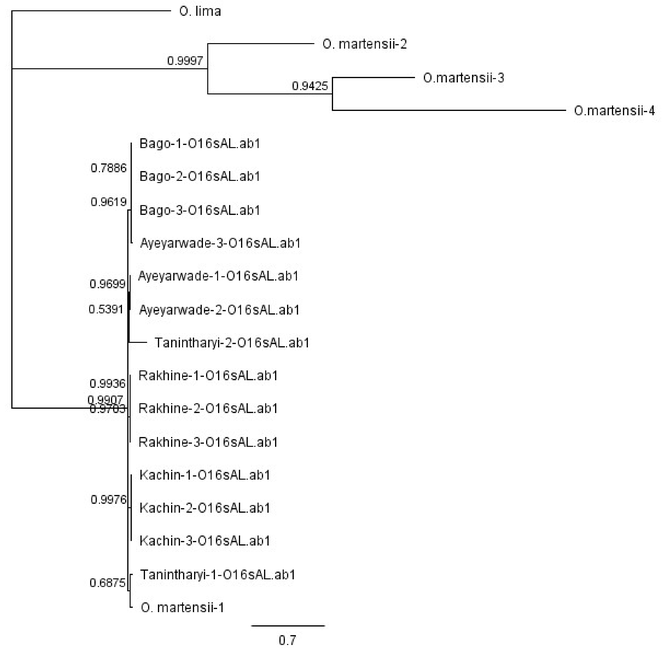

Figure 4 Bayesian evolutionary tree of sequenced *Occidozyga* specimens from Myanmar, compared to GenBank data from known O. *lima* and O. *martensii* individuals. Four unique lineages which largely cluster based on geographical region are highly supported. [Geneious version 11.1 created by Biomatters.]

DISCUSSION

- **Two new candidate species have been identified by this preliminary analysis.**
- **For future work, more specimen samples will be analyzed and incorporated.**
- **From these findings, we expect to continue to gain support for additional undescribed species in Occidozyga.**
- **Myanmar's rich biodiversity has many identified threatened species, but more cryptics may go extinct before identification.**