

Discovering the Origin of the Introduced Malagasy Giant Chameleon (*Furcifer oustaleti*) in Florida

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Abstract

The Malagasy Giant Chameleon (*Furcifer oustaleti*) is a CITES protected species indigenous to Madagascar. It has been introduced to South Florida where it has established a population, and could potentially be an invasive species, but this has never been before studied. The purpose of this experiment is to pinpoint where these Florida chameleons originated from in Madagascar. We accomplished this by creating locality-specific mitochondrial barcodes for *F. oustaleti* in their native range. We then compared the mitochondrial DNA of Florida chameleons to the mitochondrial barcodes of the Madagascar chameleons. We were unable to pinpoint one exact location that they could be coming from, but all 9 matching localities were in southern Madagascar. Knowing where the chameleons come from is the first step to helping prevent them from harming any environment which they are being introduced to through the pet trade.



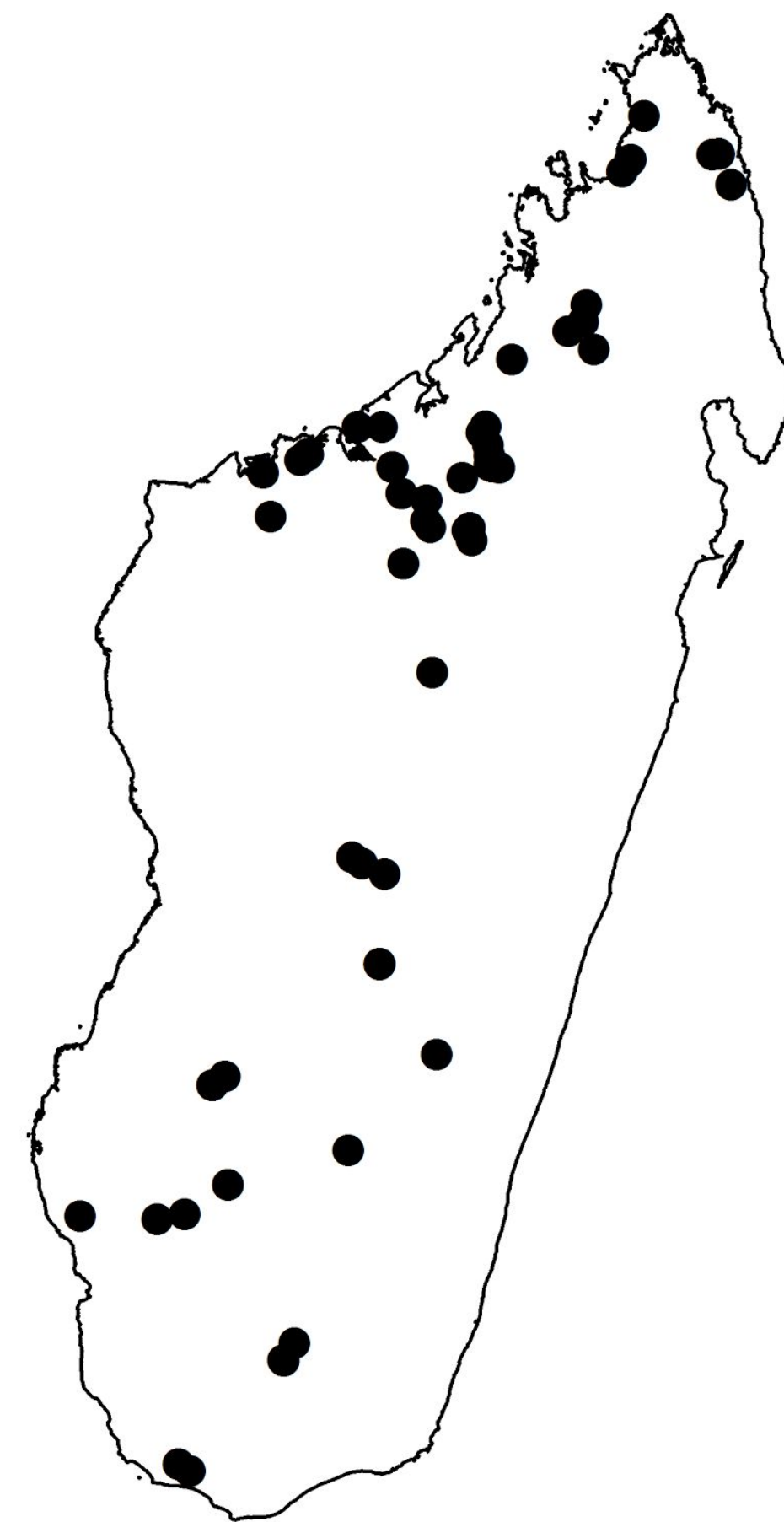
Furcifer oustaleti (male)



Furcifer oustaleti (female)

Introduction

- The Malagasy giant chameleon (*Furcifer oustaleti*) is a widespread species complex native to Madagascar (see distribution map at right). Previous studies have shown that *F. oustaleti* is highly variable in mitochondrial DNA between localities (Florio, unpublished data).
- *F. oustaleti* has been recently introduced in Florida at a single locality (Gillette et al., 2010).
- The introduced *F. oustaleti* chameleons inhabiting Florida are potentially invasive species.
- Invasive species may have harmful effects on the environment, native species, and could possibly transmit disease and pathogens.
- To investigate exactly where the Florida chameleons originate from in Madagascar, we created locality-specific mitochondrial barcodes for *F. oustaleti* in their native range. We then compared the DNA of Florida chameleons to the DNA sequences of Madagascar chameleons to find exact matches.
- It is important to do this because the first step to assessing the effect of an introduced species on an environment is to know where the species originated from in its native range.



Map showing localities in Madagascar where *F. oustaleti* is found. We created locality-specific barcodes for these *F. oustaleti* samples.

Materials and Methods

1. We obtained 17 samples of the introduced *F. oustaleti* in Florida from the Florida Museum of Natural History.
2. We extracted DNA from the tissues using the Qiagen DNeasy Blood and Tissue Kit, and performed a polymerase chain reaction (PCR) to amplify the mitochondrial CO1 and ND4 genes of these samples.
3. We confirmed the success of the PCR in amplifying the mitochondrial genes through gel electrophoresis. For the successful reactions, we used ExoSap to remove unwanted DNTP's and primers from the PCR products.
4. Afterward, we ran a cycle-sequence reaction, and then cleaned the samples again using 70% ethanol. The purified DNA was sequenced in both directions using Big Dye on an ABI 3730 Automated DNA Sequencer.
5. After obtaining the DNA sequences, we used the Geneious program to clean and edit the sequences and created an alignment between Madagascar and Florida *F. oustaleti* samples by using the program MUSCLE.
6. We created a pairwise distance chart to identify identical sequences between Florida and Madagascar *F. oustaleti* samples.

Results

Florida *F. oustaleti* chameleons

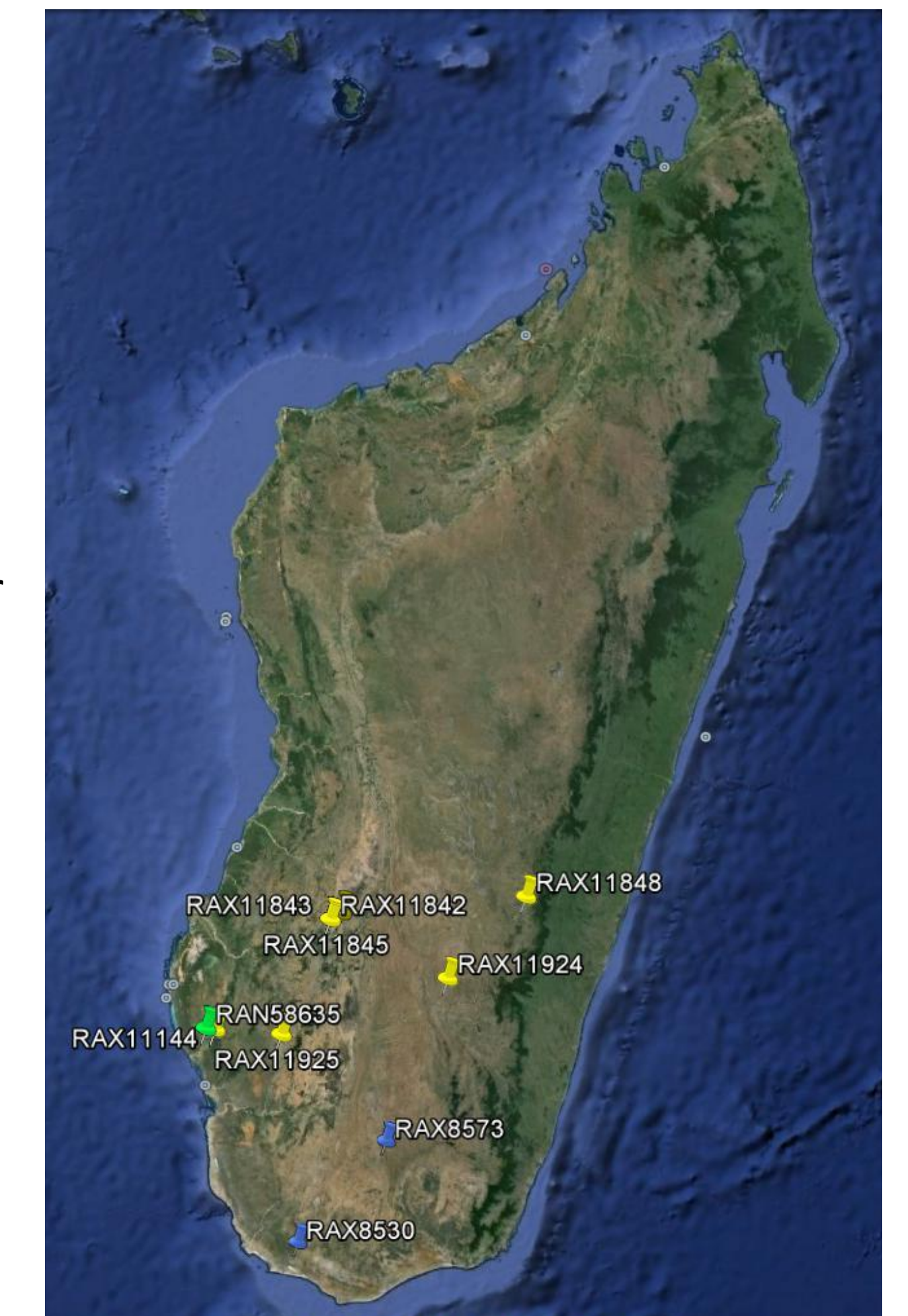
- 16/17 samples sequenced for ND4, and 15/17 samples for CO1.
- All Florida samples had identical sequences (identical haplotypes) for both ND4 and COI.

Madagascar *F. oustaleti* chameleons

- 129 Madagascar *F. oustaleti* chameleon samples were already sequenced for ND4 (Florio, unpublished data), so we used those for comparison with our Florida samples.
- We chose 43 samples that represented 43 localities in Madagascar. Of these samples, we could only obtain COI sequences for 26/43 samples.
- Then, we created a pairwise distance chart (with results up to 9 decimal points) to see which Madagascar samples were identical to Florida samples.

Florida vs. Madagascar *F. oustaleti* chameleons

- Using the pairwise distance chart, we identified 9 localities in Madagascar where the ND4 and COI Florida *F. oustaleti* sequences were exact matches (see map on the right), all of which were located in southern Madagascar.



Map showing the localities in Madagascar with identical sequence matches to the 17 Florida sample sequences. Pinpoint colors are as follows: yellow = identical with ND4; blue = identical with COI; green = identical with both ND4 and COI

Discussion



Furcifer oustaleti found roosting in an avocado grove in Southern Florida (source: Gillette et al., 2010)

- While we can't pinpoint one locality, we can definitely conclude the Florida chameleons originated from southern Madagascar.
- All the Florida *F. oustaleti* samples had identical sequences for both ND4 and COI, suggesting there may have been a single introduction..
- The matching localities in Madagascar were near several major cities, which may be because they are easily accessible by car & plane.
- Previous studies indicate that *F. oustaleti* has changed its diet to survive in Florida, and they also have been breeding in Florida. This suggests *F. oustaleti* has the potential to be an invasive species.
- Our results provide the foundation for future studies of this introduced chameleon species by telling us where they came from in Madagascar.

Acknowledgements

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