

# Phylogenetic analysis, species identification and delimitation of New Caledonian geckos and skinks using DNA barcoding

Justin Bernstein<sup>1</sup>, Aaron M. Bauer<sup>1</sup>, Todd R. Jackman<sup>1</sup>, and Yunyu Wang<sup>2</sup>

<sup>1</sup>Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, Pennsylvania 19085, USA.

<sup>2</sup>South China DNA Barcoding Center, State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, CAS Kunming, China 650223

## Background

As one of the world's biodiversity hotspots, New Caledonia harbors thousands of endemic species (Myers et al. 2000). Due to its unique geological history and in situ speciation, New Caledonia has an extraordinary level of endemism of scincid and gekkotan lizards, many of which remain undescribed and are threatened by anthropogenic activity. Many of these taxa are morphologically similar and display high levels of cryptic diversity. Furthermore, as many of these species are morphologically similar, genetic data is helpful in identifying and delimiting these cryptic taxa such as in the dipodactylid genus *Bavayia*. These geckos contains high levels of cryptic diversity and, as shown by ND2 data, may be represented by more species than are described (Bauer and Jackman 2006).

DNA barcoding has frequently been implemented to identify and delimit described and undescribed species (Hebert et al. 2003). The standardized gene used for animal taxa species delimitation is the mitochondrial gene cytochrome c oxidase subunit 1 (CO1). DNA barcoding using CO1 has been used in many studies to discover and identify animal taxa, commonly using 2% genetic divergence as a threshold to delimit species. DNA barcoding has not been used to identify or delineate New Caledonian vertebrates.

## This Study

A 650 bp region of mitochondrial CO1 was used as a DNA barcode to test the phylogenetic informativeness of CO1 on the following genera: *Bavayia*, *Dierogecko*, *Caledoniscincus*, *Marmorosphax*, *Nannoscincus*, and *Sigaloseps*. The phylogenetic trees produced from the CO1 barcode data were compared to the respective trees for each genus produced from analysis of the mitochondrial gene ND2, which has proved to be effective at resolving species level relationships in New Caledonian lizard taxa. Comparisons were made to determine if the trees from the barcode data recovered the described and putative species supported by ND2 and morphology and to determine if CO1 could retrieve any supraspecific patterns within these taxa. All barcode data will be contributed to Project Cold Code, an initiative to DNA barcode amphibians and nonavian reptiles (Murphy et al. 2013).

## Methodology

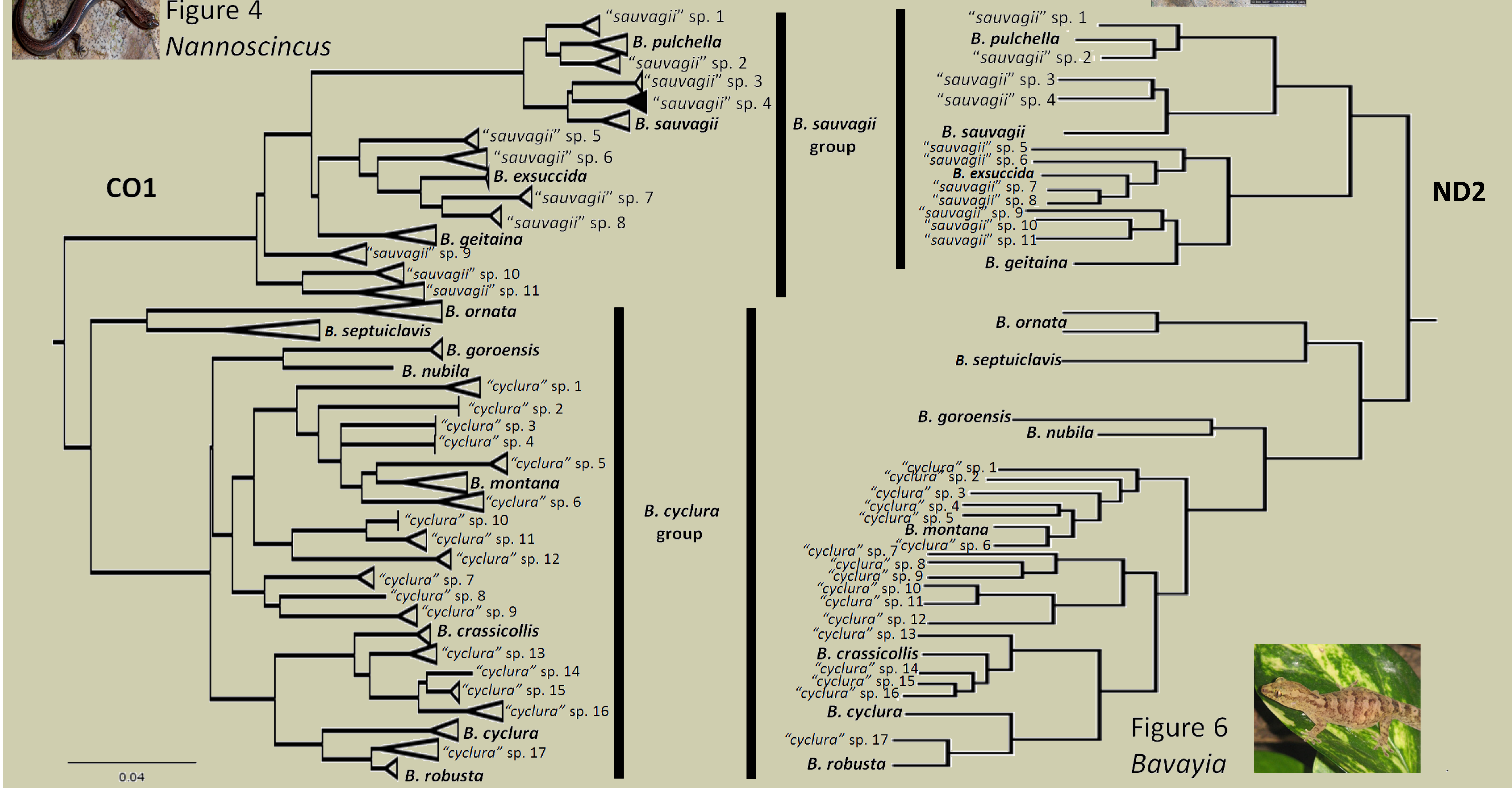
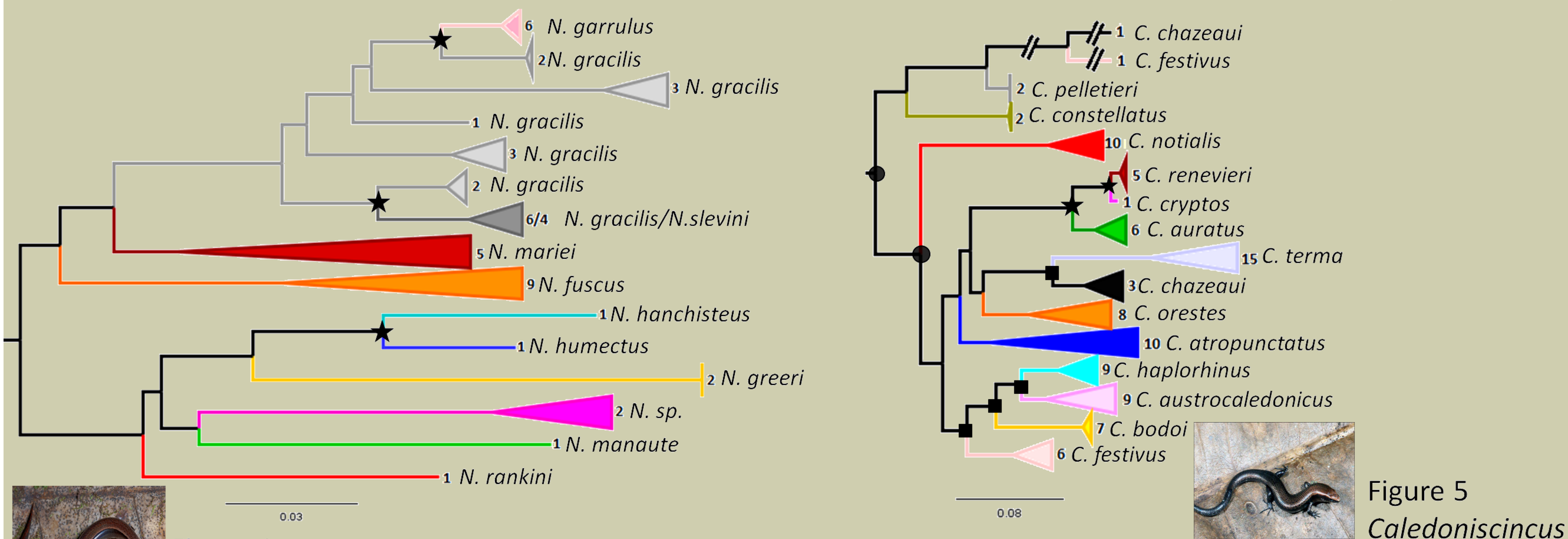
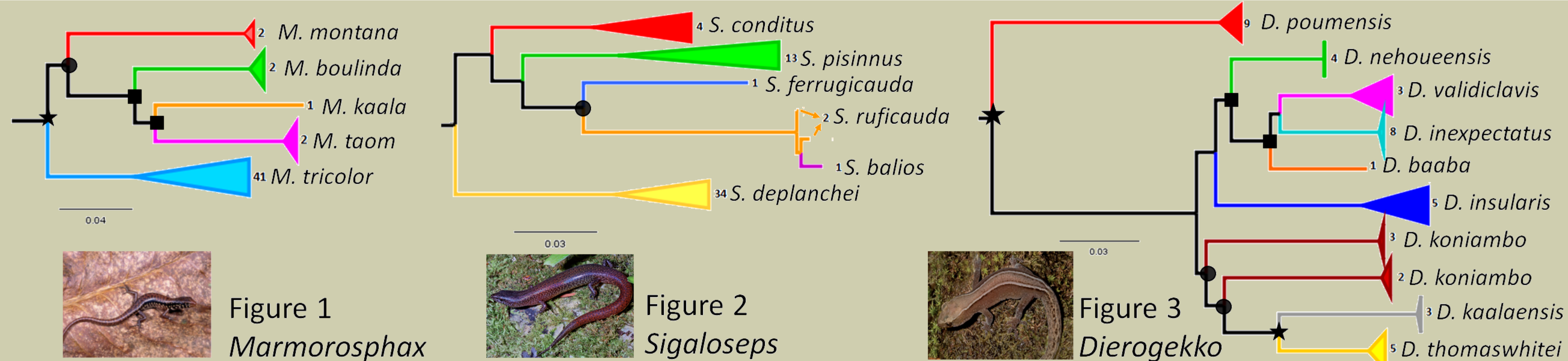
A total of 505 specimens from the genera *Bavayia*, *Dierogecko*, *Caledoniscincus*, *Marmorosphax*, *Nannoscincus*, and *Sigaloseps* were chosen from previous studies done at Villanova University. Individuals represent specimens from throughout the known ranges of each genus and from all described species and putative clades.

DNA was extracted from tail and liver tissues using DNAeasy (Qiagen) and salt extraction protocols.

Primers were used to amplify the 650 bp region at the 5' end of mitochondrial CO1 using PCR.

The designated barcode region of CO1 was sequenced using an ABI 3730xl prism automated sequencer (Applied Biosystems) and edited and aligned manually (Geneious v7.1.7).

Genus level phylogenetic trees of barcode data were estimated using RAxML (v8.1.15; Stamatakis 2014).



## Results and Discussion

**Table 1.** Percent of supraspecific taxa recovered from the ND2 phylogenetic trees by the CO1 phylogenetic trees and intra- and interspecific genetic distances of CO1 of *Bavayia*, *Dierogecko*, *Caledoniscincus*, *Marmorosphax*, *Nannoscincus*, and *Sigaloseps*.

Genus	% of ND2 species recovered by CO1	Intraspecific Range of CO1 Genetic Divergence (%)	Interspecific Range of CO1 Genetic Divergence (%)
<i>Bavayia</i>	100	0–7.52	1.92–10.91
<i>Dierogecko</i>	100	0.12–10.26	5.33–14.80
<i>Caledoniscincus</i>	114*	0.15–13.86	9.72–17.84
<i>Marmorosphax</i>	100	0–7.02	10.87–16.84
<i>Nannoscincus</i>	88	0–10.81	3.5–16.48
<i>Sigaloseps</i>	100	0–6.35	10.53–16.17

\*Percentage greater than 100% indicates more supraspecific taxa in the CO1 tree than the ND2 tree.

The barcode data recovered all monophyletic taxa from the ND2 tree in addition to putative species, with the exception of *Nannoscincus* which had incomplete sampling (See Table 1 and Figures 1–6). The basalmost and distalmost branching patterns of the barcode trees are moderately to well supported. Intermediate branching patterns are unsupported and/or conflict with the ND2 trees. All specimens of *Bavayia* were placed in the correct species groups.

All described and putative species had a genetic divergence greater than 2% from each other, thus adhering to the suggested 2% threshold for species delimitation. One exception is one *Bavayia* specimen from a putative species clade ("cyclura" sp. 17) being 1.91% divergent from a *B. robusta* specimen.

## Conclusions and Conservation

CO1 is a sufficient barcode and accurately places individuals of New Caledonian lizards into their correct species and species groups. It delimits species and exhibits some of the phylogenetic signal of the more informative ND2 gene.

DNA barcoding using CO1 as a standardized gene for species identification and delimitation may prove beneficial to the field of conservation biology in New Caledonia. As many of the species and their corresponding habitats are threatened (IUCN) and protected, the collection of many species is prohibited. The barcoding of tails and partial remains from scats from introduced mammals can allow for the accurate identification of species. This data can provide information on which predators are eating lizards and how much of a threat cats and other predators are to these species.

## Literature Cited

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## Acknowledgements

Many thanks go to the staff and researchers at the South China DNA Barcoding Center at the Kunming Institute of Zoology for assisting Justin Bernstein in generating barcode data for Project Cold Code and for the use of their facilities. Additionally, funding from the National Science Foundation and 6th International Barcode of Life Conference allowed for the generation of more barcode data. Thanks also goes to the graduate students and department of biology at Villanova University for their scientific guidance, intellectual support, and funding. We thank Ross Sadler for the photos.

Figure 1–5. Simplified phylogenetic relationships of *Marmorosphax* (Figure 1), *Sigaloseps* (Figure 2), *Dierogecko* (Figure 3), *Nannoscincus* (Figure 4), and *Caledoniscincus* (Figure 5) estimated from CO1 data using maximum likelihood analyses. Clades of CO1 trees that are identical to the ND2 trees are indicated by circles (●), boxes (■), and stars (★) for low (bootstrap values = 0–69), moderate (bootstrap values = 70–94), and high (bootstrap values = 95–100) support respectively. Numbers at terminal branches represent number of specimens sampled in that clade.

Figure 6. Simplified phylogenetic relationships of *Bavayia* from CO1 data (left) using maximum likelihood analysis in comparison to the ND2 data (right).