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

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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 gekkonid 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 as such in the diploid/diploidy genus Bavoyia. These geckos contain 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 standard gene used for animal taxon species delimitation is the mitochondrial gene cytochrome c oxidase subunit I (CO1). DNA barcoding using CO1 has been used in many studies to identify and discover 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: Bavoyia, Droserogekko, 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 Caledonia 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 represented in that dataset.

Methodology

A total of 505 specimens from the genera Bavoyia, Droserogekko, 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 DNAasy (Qiagen) and salt extraction protocols.

Primers were used to amplify the 650 bp region at the 5’ and 3’ end of mitochondrial CO1 using PCR.

The designated barcode region of CO1 was sequenced using an ABI 3730x1 prism automated sequence (Applied Biosystems) and edited and aligned manually (Genewise v.1.7). These CO1 sequences were then aligned using CLUSTAL W. The best models of substitution for each species were determined using the Akaike Information Criterion and model selection was performed with MEGA 7. The concatenated CO1 dataset was then edited and corrected using MEGA 7.

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Figure 1. Marmorosphax

Figure 2. Sigaloseps

Figure 3. Dierogekko

Figure 4. Nannoscincus

Figure 5. Caledoniscincus

Figure 6. Bavoyia

Results and Discussion

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 live and preserved specimens from an introduced mammal 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.

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.

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Supraspecific divergence

Immediate 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 Bavoyia 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 Bavoyia specimen from a putative species clade (“cyclus” sp. 17) being 1.91% divergent from a B. robusta specimen.

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 Bavoyia, Dierogekko, Caledoniscincus, Marmorosphax, Nannoscincus, and Sigaloseps.

<table>
<thead>
<tr>
<th>Species</th>
<th>% of ND2 species recovered by CO1</th>
<th>Intraspecific Range of CO1</th>
<th>Interspecific Range of CO1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bavoyia</td>
<td>100</td>
<td>0–7.52</td>
<td>1.92–10.91</td>
</tr>
<tr>
<td>Dierogekko</td>
<td>114</td>
<td>0.12–10.26</td>
<td>5.33–18.40</td>
</tr>
<tr>
<td>Marmorosphax</td>
<td>100</td>
<td>0–7.02</td>
<td>9.72–17.84</td>
</tr>
<tr>
<td>Nannoscincus</td>
<td>88</td>
<td>0–10.81</td>
<td>3.5–16.48</td>
</tr>
<tr>
<td>Sigaloseps</td>
<td>100</td>
<td>0–6.35</td>
<td>10.53–16.17</td>
</tr>
</tbody>
</table>

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

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Literature Cited


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