



Cryptic Species of Pacific Skinks (Emoia): Further Support from Mitochondrial DNA Sequences

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CRYPTIC SPECIES OF PACIFIC SKINKS (EMOIA): FURTHER SUPPORT FROM MITOCHONDRIAL DNA SEQUENCES.—The recent advances in molecular biology are applicable to a wide variety of taxonomic ques-

tions. One use of these techniques is the identification of sibling and cryptic species, which often have broad overlap in diagnostic characters (e.g., Hutchinson et al., 1990; Hickson et al., 1992; Austin, 1995). A longstanding example of this is found in the scincid genus *Emoia*, where the high degree of morphological similarity between two sibling species has led to almost a century of confusion. Werner (1898) first described *Emoia impar* from the Bismarck Archipelago and distinguished it from *Emoia cyanura* by two characters: the presence of mid-dorsal scale fusion and absence of a parietal eye. However, the distinction between the two species was never widely accepted, and most literature this century has followed Burt and Burt (1932) in not using the name *E. impar*. During the 1970s, T. P. Webster noted that there were two distinct morphotypes in what was formally recognized as *E. cyanura* (notes in bottles of *E. cyanura* at the United States National Museum of Natural History identifying specimens as *E. impar* are initialed with TPWIII). Webster never redescribed this taxon, and both morphotypes continued to be referred to as *E. cyanura*. Ineich (1987) suggested that *E. cyanura* was actually two cryptic but distinct species that were sympatric throughout their distribution, and he described one morphotype as *Emoia pheonura*. This description was synonymized by Brown (1991), however, who believed that the evidence presented by Ineich was unconvincing and that Crombie and Steadman (1986) had shown that these characters were unable to resolve the species. After examining over 1200 museum specimens, Ineich and Zug (1991) re-evaluated the description *E. pheonura* and concluded that the two morphotypes represented the two distinct species, *E. cyanura* and *E. impar*. They also provided synonymies for each species and descriptions of five morphological characters that can be used to differentiate between the two species: presence or absence of an epiphetal eye, presence or absence of middorsal scale fusion, underbelly color, tail hue, and differences in anterior loreal scale height and length. The presence of sympatric populations of both morphs with minimal overlap in these character states was interpreted as evidence that the morphotypes represented distinct, reproductively isolated species. We used mitochondrial DNA (mtDNA) sequences to determine the degree of sequence divergence within and between the two morphs. Divergence between the morphs, along with similarity of sequence within a morphotype, would support the hypothesis that the two morphotypes are separate species.

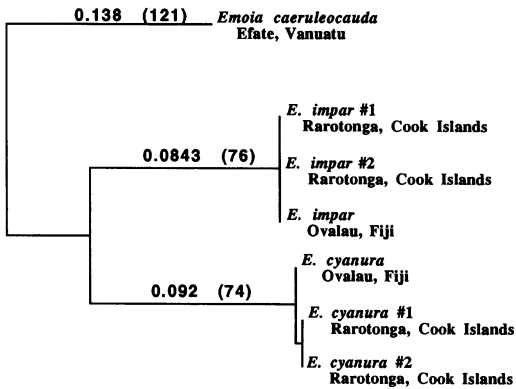


Fig. 1. Neighbor-joining tree depicting relationships among *Emoia impar* and *E. cyanura* from the Cook Islands and Fiji and *E. caeruleocauda* from Vanuatu. Branch numbers indicate the expected number of nucleotide substitutions per site on that branch; absolute number of changes are in parentheses. Vertical branches represent zero distance.

Methods and materials.—The amount of sequence divergence between the two species was estimated by comparing 935 base pairs of the mitochondrially encoded cytochrome *b* (*cyt b*) gene from individuals of both putative species collected from two islands where the two morphs occur in sympatry: Ovalau, Fiji; and Rarotonga, Cook Islands. Another similar species, *Emoia caeruleocauda*, was also sequenced. DNA was isolated from liver using sodium chloride extraction (Miller et al., 1988). The *cyt b* gene was amplified with the polymerase chain reaction (PCR) using published primers (L14724 and H15560 from Palumbi et al., 1991; H15752 from Richman and Price, 1992; L14817 and H15175 from Kocher et al., 1989), as well as one developed in our laboratory (Radtkey et al., 1995). Double-stranded DNA was amplified using the procedure of Innis et al. (1990); and from the products obtained, a small aliquot (5 λ of the original 50- λ reaction) was carried over to a second PCR reaction to which only one primer was added. This produced a single strand of DNA which was purified with microcentrifuge filtration units. Single-stranded DNA was produced for both strands of DNA. Purified single-stranded DNA was used as a template for sequencing using the chain termination method (Sanger et al., 1977). Both strands were sequenced to reduce sequencing error.

In addition to counting the number of fixed differences between the species, we estimated the genetic distance between morphotypes by using the Jukes and Cantor (1969) correction. These distances were then used to create a tree

with the neighbor-joining method of Saitou and Nei (1987). The software package PHYLIP (Felsenstein, 1989) was used to conduct all analyses. All sequences are available in Genbank, and the accession numbers are included in Materials Examined.

Results and discussion.—Sequence divergence between *E. cyanura* and *E. impar* was 17–19%, with *E. caeruleocauda* differing from *E. cyanura* by 23% and from *E. impar* by 22–23%. Intra-specific divergence between populations of *E. cyanura* from Rarotonga and Ovalau was 0–0.002%. No sequence differences were found between *E. impar* from Ovalau and Rarotonga (Fig. 1). There were 150 base pair changes between *E. impar* and *E. cyanura*, 197 between *E. impar* and *E. caeruleocauda*, and 195 between *E. cyanura* and *E. caeruleocauda*.

The two species are clearly distinct, because the amount of genetic divergence between *E. cyanura* and *E. impar* is comparable to that found between either species and *E. caeruleocauda*. Furthermore, the difference between individuals of the same species from different islands was less than 0.5%. This consistency corroborates previously demonstrated differences in morphology, strengthening the hypothesis that the two represent distinct species.

The level of divergence among species of *Emoia* is similar to that found between other closely related lizard species. Hedges et al. (1991) used *cyt b* sequences to reconstruct a phylogeny of xantusiid lizards. They found that the amount of divergence between four closely related xantusiid species ranged from 13–19.6%. Thorpe et al. (1994) found that three species of *Gallotia* differed by 7.9–11.3%.

Emoia cyanura was previously believed to be the most widespread and abundant skink in the Pacific (Ineich and Zug, 1991; Zug, 1991). No ecological studies have been conducted on *E. cyanura* since its revision, although Ineich (1987) suggested that *E. cyanura* was found mainly in open habitats whereas *E. impar* preferred forests. A posteriori analysis of voucher samples and field notes (Zug, 1991) seems to support these conclusions, although at least one author has found both species present in the same microhabitat (Gill, 1993). Since both species are similar in coloration and morphology, careful ecological and behavioral studies are required to clarify their ecological relationships. Ongoing studies also will clarify the biogeographic and evolutionary history of these distinct species.

Materials examined.—Cook Islands: *Emoia cyanura* (#1) CAS 183331, *E. cyanura* (#2) CAS

196383, *E. impar* (#1) CAS 183334, *E. impar* (#2) CAS 196382; Fiji: *E. cyanura* CAS 196381, *E. impar* CAS-196380; Vanuatu: *Emoia caeruleocauda* USNM 333959. The Genbank accession numbers are U20451-U20457.

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