

Using cryptic variation to explore species limits

Keywords: Cryptic variation, allopatry, speciation, isolation mechanisms, *Xanthomixis zosterops*, Madagascar, conservation

The relatively recent advent of DNA-based phylogenies has widely expanded our taxonomic knowledge of the world's organisms. We are rapidly clarifying genetic relationships among taxa at all levels, from populations to orders. These phylogenies have exposed some surprises, including cryptic taxa that were not recognized in older, morphology-based taxonomies. In most cases, geographic isolation explains the basis for the genetic divergence of these cryptic taxa, if not their lack of morphological differences. If geographic overlap occurs, it is commonly found only at the periphery of the ranges of two taxa. When investigating beyond morphology in these cases, scientists often have discovered ecological or behavioral differences. These differences have provided the mechanisms for continued genetic isolation and the basis for classifying these taxa as cryptic species. Allopatric, genetically-distinct cryptic taxa exhibiting similar differences in ecology or behavior usually are labeled as different species as well. However, despite high genetic divergence, some allopatric taxa have not developed major differences at any phenotypic level. What would happen if these taxa became sympatric? Would minor phenotypic differences or genetic divergence alone serve as an isolation mechanism, or would hybridization meld the distinct lineages into one? Here I propose to use an avian taxon endemic to Madagascar, *Xanthomixis zosterops*, to address these questions concerning cryptic species limits. I expect the answers to help clarify the classification of allopatric cryptic taxa and contribute to conservation decisions concerning these taxa.

Background

Avian evolution and phylogeography has long been a personal interest of mine, and I find Madagascar's avifauna particularly interesting because most of the endemic species show almost no genetic structure (i.e. genetic variation with a geographic component) despite long, independent evolutionary histories. This lack of genetic structure suggests that few barriers to gene flow have been present since the species originated or that the species experienced a recent population bottleneck and then expanded rapidly across the island. *X. zosterops* provides an interesting anomaly, however, because it displays a high level of genetic divergence with deep history in a recent mitochondrial DNA (mtDNA) phylogeny¹.

Part of an endemic radiation of birds unique to Madagascar², the genus *Xanthomixis* comprises three currently-recognized species (a fourth belongs in *Crossleyia*¹). Two species—*X. apperti* and *X. cinereiceps*—show little genetic complexity, but *X. zosterops* contains four distinct haplotype clades with the deepest split occurring not long after the split between *X. apperti* and *X. cinereiceps*, likely in the Pliocene¹ (Figure 1). As one might expect from distinct

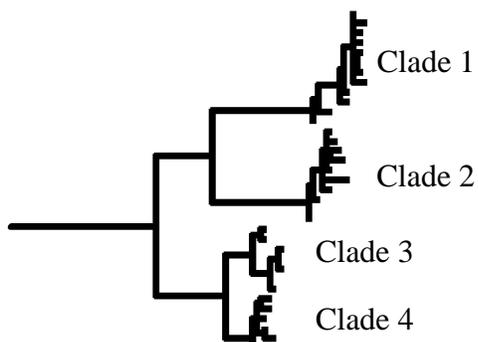


Figure 1. Haplotype relationships of *Xanthomixis zosterops*

histories, *X. apperti* and *X. cinereiceps* show marked morphological differences and are easily identified in the field. Surprisingly, despite relatively similar deep splits, only Clade 1 within *X. zosterops* shows noticeable morphological divergence (personal observation). In birds, song or habitat use often indicate species differences when morphology does not, but *X. zosterops* does not exhibit obvious differences in these categories. Finally, one might assume that the clades reflect allopatric breeding, but this also does not appear to be the case.

Clade 1 is only found in northern Madagascar; however, Clades 2–4 are found together throughout eastern slope humid forests¹. Several locations support individuals from all three of these clades¹. This is an extremely unusual situation for birds and provides a unique opportunity to study the interactions between three sympatric cryptic lineages. Which, if any, *X. zosterops* clades are hybridizing, and to what degree? What phenotypic or microhabitat differences exist between the *X. zosterops* clades that are not apparent at first glance? Will a nuclear DNA phylogeny match the mtDNA tree? These are all questions I will address with this study. I hypothesize that the *X. zosterops* clades developed during a period of allopatry and that Clades 2–4 are now hybridizing in widespread secondary contact, implying that strong mtDNA divergence may not be evidence enough to classify all allopatric cryptic taxa as species. Even if results do not support my hypothesis, they would provide a useful and significant study concerning the possibility of sympatric speciation and the mechanisms that maintain reproductive isolation in such morphologically similar lineages.

Research Plan

Phylogeny construction: To gain a more detailed genetic perspective of *X. zosterops*, I propose to collect additional samples along a transect of the entire species range. Even though over 70 individuals have been genotyped, samples from critical geographic areas have not yet been collected. A sampling transect will allow me to determine the extent and locations of clade overlaps. Using both mitochondrial and nuclear DNA markers, I will construct a detailed phylogeny against which geographic and phenotypic data can be compared to recreate the evolutionary history of the species. Additionally, I will use the nuclear markers—possibly including microsatellites and AFLPs—to determine the extent of hybridization among the clades.

Phenotype differences: To determine possible cryptic species limits in *X. zosterops*, I will look for any differences among the clades in vocalizations, habitat, breeding biology, and molt timing. Using morphometric and colorimetric data, I also will look for shape, size, and plumage differences not readily apparent in the field. I expect to gather these data with extensive field studies involving banding, observation and sound recording of banded birds, and subsequent collecting to determine a bird's MtDNA haplotype clade designation.

Broader Impacts

This research emphasizes the significance of cryptic variation in determining species limits. I will evaluate the relative roles of genetic, ecological, and phenotypic differences as isolation mechanisms. When can allopatric cryptic taxa be considered separate species? I think the answer to this dilemma is largely unresolved despite its importance in our understanding of speciation. I am excited to pursue research that could help to illuminate the answer and that encompasses my passion for all things ornithological.

On an applied level, this research aids in conservation designations and decisions in a critically endangered environment in one of the world's biodiversity hotspots. With current classifications, many cryptic species are lumped under one taxon designation and are not afforded individual conservation concerns. These classifications also blur the lines between endemism regions and downgrade the importance of these regions. Studies that determine cryptic species limits can increase biodiversity estimates, clarify boundaries of endemism regions, and thus aid in conservation decisions. *I attest that this proposal is original.*

Literature Cited

1. Raherilalao, M. J., S. Hackett, J. Bates, and S. Goodman. Phylogeography of “bulbuls” in Madagascar. International Ornithological Conference (2006).
2. Cibois, A., B. Slikas, T. Schulenberg, and E. Pasquet. An endemic radiation of Malagasy songbirds revealed by mitochondrial DNA sequence data. *Evolution* 55, 1198–1206 (2001).