Proposed Research   **K.M. Everson**

Introduction and Background. Discovering, documenting, and delimiting species are essential endeavors for a broad range of biological inquiry (e.g., systematics, ecology, and conservation biology). Species delimitation in recently diverged lineages often poses unique challenges: morphological differences may be subtle, absent, or not yet fixed; reproductive barriers may not be fully formed; and lineage sorting of genetic loci may be incomplete.[1] Nonetheless, nascent evolutionary lineages are inordinately informative for understanding the process of diversification.[2] Recent advances in multiple disciples (e.g., genomics, phenomics, and explicit spatial modeling) provide opportunities to synergistically estimate species limits, even in young or cryptic species complexes.

Madagascar is a natural laboratory for studying evolution. After 88 million years of isolation this island continent is now a top biodiversity hotspot with 100% endemism of its 105 native terrestrial mammal species.[3] In 2008 Drs. Link Olson (UAF) and Sharon Jansa (U. Minn.) independently detected a widespread phylogeographic pattern in Madagascar's humid forest small mammals (Fig. 1). Using mitochondrial DNA sequence data, they found three highly divergent, latitudinally demarcated populations in eight endemic species of tenrec (Tenrecidae) and nesomyine rodent (Nesomyinae). The full extent of this pattern, in terms of the number of species exhibiting congruent population structure, is currently unknown.

The historical processes driving *in situ* diversification on Madagascar, and ultimately producing observed phylogeographic patterns, are also poorly understood. Several models have been proposed invoking drivers from climate[4] to rivers[5] to mountains.[6] Each hinges on the climatic and geological conditions of specific epochs throughout Madagascar's natural history, so accurately dating divergences in Malagasy fauna will help pinpoint which mechanisms promoted speciation. Ecological niche modeling (ENM), a tool for estimating past and present species distributions, can also elucidate past drivers of diversification by revealing, for example, past periods of habitat fragmentation, as hypothesized by Wilmé et al. (2006).[5]

Madagascar's endemic tenrecs—one of only four extant lineages of terrestrial mammals occurring on the island—are a classic example of adaptive radiation, with semiaquatic, semifossorial, and scansorial ecomorpholog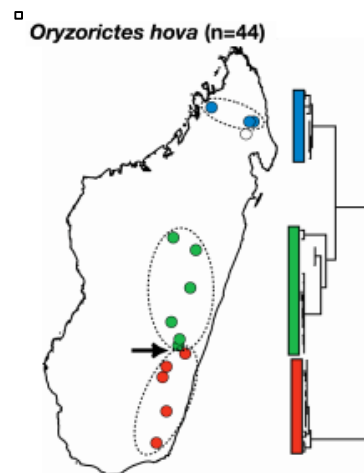ies; velvety pelage to stout, barbed, detachable spines; and body sizes spanning three orders of magnitude (2-2000 grams).[7] Of the 32 currently recognized species, ten have been discovered or taxonomically resurrected since 1992, and two lines of evidence suggest that additional species remain undescribed: 1) Some museum specimens differ both morphologically and genetically from all other recognized tenrecs, and 2) several species contain multiple genetically divergent populations occurring in sympatry, a pattern indicative of cryptic species.[8,9] Quickly and accurately delimiting species boundaries is imperative, as most tenrecs are endemic to Madagascar's humid forest and therefore face conservation threats from anthropogenic habitat loss.

My research will illuminate species limits, species distribution patterns, and historical drivers of speciation by addressing the following objectives:

Objective 1) Evaluate phylogeographic structure in tenrecs. I hypothesize that the phylogeographic pattern identified by

Figure 1. Intraspecific phylogeny of one tenrec species based on mtDNA (right) and each individual's corresponding collection locality (left). Arrow shows a contact zone between two divergent populations.

Olson and Jansa will be reflected in all widespread humid-forest tenrecs on Madagascar.
Objective 2) Estimate divergence timing among codistributed taxa. I hypothesize that intraspecific divergence patterns among humid-forest small mammals are temporally congruent and, therefore, likely attributable to a common historical event.
Objective 3) Determine species limits in Malagasy tenrecs.
Methods. Past fieldwork has generated >2000 vouchered tissues for 32 tenrec species across 40 widespread localities. I will use Next-Generation Sequencing to capture genome-wide DNA data for a representative subset of these individuals, following the protocol of Faircloth et al.,[10] which uses ultraconserved genomic elements (UCEs) as molecular markers. These sequences, combined with mtDNA data already developed by Olson's lab, will constitute a large and informative dataset for phylogeographic analysis. I will address **Objective 1** by estimating phylogenies and population structures for all widespread humid-forest tenrecs.

I will address **Objective 2** using the same genomic dataset and msBayes,[11] a program that tests for simultaneous divergence among codistributed species. I will also use time-calibrated phylogenies in mrBayes[12] and BEAST[13] to estimate divergence dates and use ENM (parameterized with climate and georeference data) to model past and present tenrec distributions. These approaches can reveal processes (e.g., historical habitat fragmentation) that may have driven diversification and speciation.

I will estimate species limits (**Objective 3**) using the corroborative approaches of clustering and validation. First, individuals will be assigned to groups via Gaussian clustering in R[14] and Bayesian clustering in Structure.[15] For the validation approach, I will use group assignments as *a priori* partitions in SpedeSTEM[16] and BPP,[17] which use maximum likelihood and Bayesian methods, respectively, to calculate the probability of each grouping given the dataset. Robust evidence is required to delimit species in cryptic lineages, and we expect consistent signal to emerge across both the clustering and validation methods.

Intellectual Merit. The core goals of this study are to diagnose species limits in Malagasy tenrecs—an understudied mammalian lineage—and to identify the patterns and processes behind Madagascar's humid forest small-mammal biodiversity. I will integrate genome-wide sequence data and niche modeling to study driving forces of evolution and responses to past environmental change. This project will reveal new species and areas of cryptic endemism, generate baseline data for conservation in one of the most threatened biodiversity hotspots, and provide insight into the driving forces of speciation that can be extrapolated to other Malagasy taxa as well as other tropical regions worldwide.[18]

Broader Impacts. Over 70% of all tenrecs are endemic to Madagascar's humid forest and therefore face enormous pressure from deforestation and mining. A realistic account of the species diversity and boundaries of tenrecs is fundamental to understanding the evolution of Madagascar's biodiversity and conserving these mammals. Products from this study will include multiple peer-reviewed publications and digital data (DNA sequence data, alignments, tree files, etc.) archived on public online repositories (e.g., GenBank, Dryad). I will conduct this research at the University of Alaska Museum, where I will engage the public in my research during open house events and mentor several undergraduate students in DNA sequencing and systematics.

[1]Raxworthy et al. 2007. *Syst. Biol.* 56:907 [2]Weins. 2004. *Amer. Nat.* 163:914 [3]Goodman & Bernstead. 2005. *Oryx* 39:73 [4]Pearson & Raxworthy. 2009. *Evolution* 63:959 [5]Wilmé et al. 2006. *Science* 312:1063 [6]Wollenberg et al. 2008. *Evolution* 62:1890 [7]Goodman & Benstead. 2003. *The Natural History of Madagascar* [8]Olson et al. 2004. *Biol. J. Linn. Soc.* 83:1 [9]Olson et al. 2009. *J. Mamm.* 90:1095 [10]Faircloth et al. 2012. *Syst. Biol.* 61:717 [11]Hickerson. 2007. *BMC Bio.* 8:268 [12]Huelsenbeck. 2001. *Bioinf.* 17:754 [13]Drummond et al. 2007. *BMC Evol. Biol.* 7:214 [14]Hausdorf & Hennig. 2010. *Syst. Biol.* 59:491 [15]Pritchard et al. 2000. *Genetics* 155:945 [16]Ence & Carstens. 2011. *Mol. Ecol. Res.* 11:473 [17]Yang & Rannala. 2010. *PNAS* 107:9264 [18]Vences et al. 2009. *Trends Ecol. Evol.* 24:456