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Understanding Speciation in Southern Africa: A comparative approach using a broadly distributed squamate radiation.

Introduction – The extensive geological history of Southern Africa (SA; Fig. 1) has produced hyper-diverse floral and faunal assemblages[1], including one of the richest reptile faunas in the world[2]. As a result of modern, molecular phylogenetic methods, discovery of novel and/or cryptic forms continues unabated. However, molecular data are not always unambiguously illuminating. Within some SA taxa, substantially divergent genetic clades show little morphological differentiation[3,4]; while others display incredible morphological segregation with little genetic difference[5,6]. Recent phylogeographic work suggests lizards of the genus *Agama* may fall into former scenario[7,8].

Although restricted to Africa, the genus *Agama* is one of the most diverse and widespread of the continent's terrestrial vertebrate groups[9]. Southern Africa has 12 recognized species that can be grouped into one of three ecotypes: "rock hoppers" – *A. atra,* (*A. knobelli*), *A. anchietae, A. kirkii, A. planiceps*; "ground dwellers" – *A. hispida,* (*A. etoshae, A. makarikarica*)*, A. aculeata,* (*A. distanti*, *A. armata*); and one "tree-loving" form – *A. mossambica* (taxa in parantheses are at times considered subsp. of the preceding species)[2]. A recent mtDNA-based analysis of the genus *Agama* suggests that the SA species, with the exception of *A. planiceps*, form a well-supported monophyletic radiation, sister to the West African *A. weidholzi*[9]. Within the SA radiation, two well-supported clades were retrieved, suggesting a reciprocally monophyletic relationship between two of the three aforementioned ecomorphs (*A. mossambica* was not included). However, due to limited taxon sampling (5 of 12 sp.), relationships among SA's *Agama* are still poorly understood. In fact, this has been an enduring problem since the early 20th century. Boulenger and Power[10] recognized no other SA group that was in greater need of revision, calling it an "intricate zoogeographical problem"; a sentiment echoed in subsequent works[11–13]. Currently, however, it is still difficult to definitively identify species using the products of those revisions and numerous morphologically aberrant populations have since been identified (Bates, Bauer, Branch, Marais, pers. comm.), none of which have been systematically evaluated.

The recently completed South African Reptile Conservation Assessment (SARCA; sarca.adu.org.za) produced an updated estimate of the geographic ranges of each South African reptile species based on reevaluations of museum records and additional field collections. There were some particularly interesting results for the current distributions of SA *Agama*. First, a substantial zone of sympatry exists between closely related *A. atra*, *A. knobelli*, and *A. anchietae*, stretching from Namibia through to south-central South Africa (Fig. 1; red, pink and green circles). Second, there were abrupt longitudinal zones of parapatric turnover between the three species of the *A. aculeata* group (Fig. 1; white, dashed lines). The implications of these distributions leads to interesting biogeographic possibilities about the direction of the radiation(s), the role climatological and geological events have played in shaping distributions, and the timing of diversification. Currently, however, there is insufficient molecular data to test how these factors and events may have influenced evolution in this radiation.

Objectives – **1**) To use modern molecular phylogenetic methods coupled with a thorough sampling regime to determine species limits, uncover cryptic diversity and test biogeographic hypotheses. **2**) To use comparative phylogeographic and population

genetic tools to investigate gene structure/flow between sister clades/species, to explore the contemporary distribution of genetic diversity, and to produce realistic estimations of each species' geographic range. **3**) To use corroborative molecular and morphological evidence to produce a comprehensive taxonomic revision and an updated species key.

Methods: *Sampling* – I aim to include 25–75 individuals (pending total geographic range) from each putative/recognized species, from a broad sampling of populations throughout each species' range. I currently lack samples for two described species (*A. etoshae* and *A. makarikarica*; Fig. 1), although efforts to obtain material from collaborators is underway. In order to gain the level of sampling required for a comprehensive, comparative phylogeographic analysis, additional fieldwork is required. Provincial permits have been secured for South Africa and my immediate future collecting trips will be focused there (Fig. 1; white, dashed circle).

Molecular Phylogenetics – Of the >200 available tissue samples, DNA has been extracted for >130. I will use a combination of standard nuclear (RAG1) and mitochondrial (ND2, 16S) markers, in addition to 1–2 putatively "faster evolving" nuclear genes[14]. Optimization of primer pairs and cycling conditions is currently underway. I will perform both parsimony and likelihood analyses and I will also investigate any gene vs. species tree discordance using coalescent-based methods[15,16]. I will follow a lineage-based, phylogenetic species concept[17].

Population Genetics – Utilizing 454 pyrosequencing technology, I recently created a library of >30k reads for *A. atra* in order to develop single-copy anonymous nuclear loci (ANL). From those 30k reads, I will select five ANL that amplify across all species. Using these in concert with mtDNA, I will measure population genetic diversity and structure in ARLEQUIN v. 3.1[18]. Coalescent analyses using IMa[19] will determine whether gene flow persists between populations and sister-species. STRUCTURE v2.0[20] and GENELAND[21] will be used to determine the number of populations within a species and the genetic diversity within individuals of populations, with the latter also providing a visual, geographic representation of population (or species) boundaries. LaGrange[22] and MAXENT 2.3[23] will be used to explore any historical changes in geographic ranges as well as to predict current species distributions, which can then be compared with the results from GENELAND.

Morphometrics – A suite of morphological measurements (following ref. 24) will be taken on both field-collected and museum (including type) specimens. I have measured 73 live and 29 museum specimens (representing 5 sp.). These data have been shown to be useful for delineating "natural" phenotypic groups at both the ecomorphological[5,25] and species[26] levels. To determine which morphometric measures are the most informative, all measurements will be log transformed and regressed against SVL, and a multivariate analysis of the residuals will be run in SPSS v.9.0. Principle components analyses (to distinguish groupings of informative morphometric variables) and discriminant function analyses (to discover if any of the variables can split the individuals into distinct groups) will be performed using R. Before any taxonomic alteration, these data will be compared with traditional diagnostic morphological characters[11–13]. As part of a larger collaborative project, these data will also be compared to performance measures (e.g. diet, sprint speed, bite force) and molecular data to see if the species and/or population-level differences are evidence for an adaptive radiation.

Significance – This research will form the bulk of my Ph.D. dissertation, but more importantly, it will help to resolve the problematic systematics of SA's *Agama*. This will be the first modern study to explicitly address this issue since FitzSimons[11] and the first to employ nuclear data and morphometrics. No SA *Agama* are currently considered threatened or endangered, although a reevaluation of the severely fragmented and range-restricted *A. hispida* group may warrant a change. A thorough understanding of the systematics and phylogeography of each of these species will provide a broader foundation for the study of speciation in SA.

Timeline – I am currently in my second year of my PhD studies at the University of Misssissippi. The summer of 2010 will be spent acquiring sequence data from the samples I already possess (~200). The results of that work will be used as preliminary data for an NSF-DDIG application I plan to submit fall 2010. I plan to return to South Africa January 2011 to fill in current sampling gaps throughout central/eastern South Africa, as well as to extract and sequence those samples. Data analysis will take place 2011–12, and resulting manuscripts will be submitted to Systematic Biology and other relevant journals 2012–13.

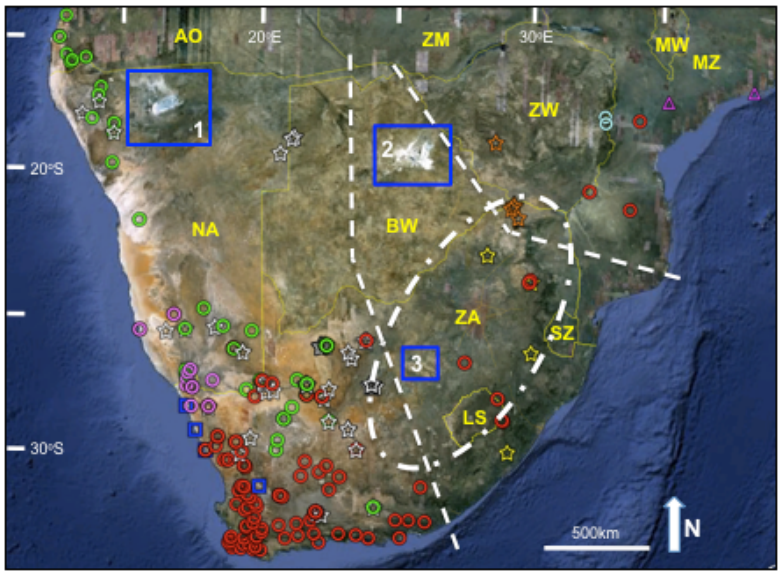

Fig. 1. Map of Southern Africa displaying the current sampling localities for SA *Agama*: *A. anchietae* (○), *A. knobelli* (○), *A. atra* (○), *A. kirkii* (○), *A. aculeata* (☆), *A. distanti* (☆), *A. armata* (☆), *A. hispida* (■) and *A. mossambicus* (Δ). The white, dashed lines indicate zones of parapatry between the *A. aculeata* group. Larger blue boxes represent localities for *A. etoshae* (1), *A. makarikarica* (2) and an *A. hispida*-like population (3). The white, dashed circle illustrates a large sampling gap within South Africa.

Literature Cited

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Budget

Vehicle rental: $0.22/km x 5000km	$1,100.00
Fuel: ~$1.00/liter x 600L	$600.00
Per diem expenses: 2 people x $25/day x 14 days	$700.00
RT domestic airfare: Cape Town to Johannesburg	$250.00
RT domestic airfare: Cape Town to Port Elizabeth	$200.00
RT domestic airfare: Cape Town to Bloemfontein	$250.00
Total:	$3,100.00
Total Requested:	**$2,000.00**

Budget Justification – Numerous critical sampling gaps exist, particularly along corridors of para- and sympatry between species (Fig. 1). I will be returning to South Africa in Jan. 2011 and plan on visiting some of those gaps (Fig. 1; white, dashed circle). While DNA extraction, PCR and sequencing costs will be mostly covered by South Africa National Research Foundation funding to Krystal Tolley (South African National Biodiversity Institute; SANBI) and additional funds from the University of Mississippi to Brice Noonan, money for future field trips does not currently exist. This budget will cover two weeks of fieldwork (South African undergraduate and myself) and two trips to important museums (myself only). Through my collaboration with SANBI, the vehicle cost is subsidized. The fuel cost is estimated from my two 2 weeks of recent fieldwork using the same vehicle. The per diem expenses will cover food and accommodation. I plan to visit the collections of the Transvaal (Johannesburg), Bayworld (Port Elizabeth) and National (Bloemfontein) museums, which currently have the largest and most diverse collections of agamid lizards in South Africa, as well as many of type specimens of the species included in this study. There I plan on collecting morphometric measurements of a subsample of the hundreds of catalogued *Agama* specimens representing all currently described species/subspecies. I plan to stay with the museum curators so no extra accommodation is required for these visits.

Additional Funding Sought Through Other Sources

1. U.S. Department of State – Fulbright Student Fellowship
Will cover RT airfare to South Africa and living expenses while in country. If accepted, I plan on being in South Africa from Jan. to Nov. 2011. This award will not cover research/field costs. *Provisionally accepted by the US Fulbright organization, currently awaiting final South African approval.*

2. National Science Foundation-Doctoral Dissertation Improvement Grant
Will request $12,000.00 to cover molecular costs (e.g. phenol chloroform extractions, PCR reagents, and sequencing costs). *Will submit Nov. 2010.*

3. American Philosophical Society - Lewis and Clark Fund for Exploration and Field Research
Will request $5,000.00 to cover field related expenses for an expedition to Botswana. *Will submit Feb. 2011.*

4. Sigma Xi – Grant-Aid-of-Research
Will request $1,000.00 to cover molecular costs. *Will submit Sept. 2010.*

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Curriculum Vitae

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EDUCATION

Ph.D. in Biology, University of Mississippi (UM), 2008-present (anticipated May 2013)
M.Sc. in Evolutionary Biology, Villanova University (VU), August 2008. GPA: 3.86
B.S. in Integrative Biology, Brigham Young University – Idaho, April 2004. GPA: 3.30

PROFESSIONAL EXPERIENCE

2010–present. Research Assistant, Biology Department, UM.
2009. Curatorial Assistant-in-Training, Peabody Museum, Yale University.
2008–2009. Graduate Teaching Assistant. Biology Department, UM.
2006–2007. Graduate Teaching Assistant**.** Department of Biology, VU.

GRANTS AND AWARDS

2009. ASIH Travel Award, $300.
2008. UM Graduate Student Council Research Grant, $500.
2007. Sigma Xi Grant-In-Aid of Research, $1000.
2006. American Society of Naturalists Travel Award, $800.
2003. BYU-Idaho Department of Biology Academic Scholarship, $1000.

FIELD AND MUSEUM EXPERIENCE

South Africa. 2010. Assisted Dr. K. Tolley (South African National Biodiversity Institute) collecting specimens for the Reptile Speciation Project. Also, personally lead two weeklong collecting expeditions to the Western/Northern Cape provinces. (7 wks.)

Namibia. 2007. Collected reptiles for the Harvard Museum of Comparative Zoology with Dr. A. Bauer. (3.5 wks.)

New Zealand. 2007. Inspected gecko specimens at Te Papa (National Museum of NZ) to identify delimiting morphological features between putatively new species. (1 wk.)

SPECIAL TRAINING

2010. Student Workshop on Descriptive Taxonomy, South African Biosystematics Initiative (SABI). Port Elizabeth, Eastern Cape, ZA.

2009. Estimating Species Trees Workshop, University of Michigan - Museum of Zoology. Ann Arbor, MI, USA.

ORAL PRESENTATIONS

2009. **Nielsen, S.V.** (presenter), A.M. Bauer, T. Jackman and B. Noonan. "Something Old in New Zealand: Dating Suggests Possible Gondwanan Connections for New Zealand's Endemic Geckos." 2009 Joint Meetings of Ichthyologists and Herpetologists.

2008. **Nielsen, S.V.** (presenter), A.M. Bauer and T. Jackman. "Molecular systematics of the geckos of New Zealand." 2008 Joint Meetings of Ichthyologists and Herpetologists.

2007. **Nielsen, S.V.** and E. Snively (presenter). "Phylogenetic inference of extinct geckos using Bayesian analysis." The 2nd University of Alberta Herpetology Symposium.

PUBLICATIONS

_____. **Nielsen, S. V.**, A. M. Bauer, T. J. Jackman and R. A. Hitchmough. Molecular phylogenetic relationships among New Zealand's geckos. **In prep.** Will submit to: *Molecular Phylogenetics and Evolution*

_____. K. L. Krysko, J. Burgess, K. M. Enge, L. A. Somma, M. R. Rochford, and **S. V. Nielsen**. Thirteen new introduced amphibians and reptiles in Florida. **In prep.** Will submit to: *Applied Herpetology*