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Phylogeny of the genus *Agama* based on mitochondrial DNA sequence data

Adam D. LEACHE^{1,2}, Rebecca A. CHONG¹, Theodore J. PAPPENFUSS¹, Philipp WAGNER³, Wolfgang BÖHME³,
Andreas SCHMITZ⁴, Mark-Oliver RÖDEL⁵, Matthew LEBRETON⁶, Ivan INEICH⁶, Laurent CHIRIO⁶, Aaron
BAUER⁷, Edem A. ENIANG⁸, & Sherif BAHHA EL DIN⁹

¹ Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA

² E-Mail: aleache@ucdavis.edu

³ Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

⁴ Muséum d'histoire naturelle, Department of Herpetology and Ichthyology, Geneva 6, Switzerland

⁵ Museum für Naturkunde at the Humboldt University Berlin, Germany

⁶ Muséum national d'Histoire naturelle, Département Systématique et Evolution (Reptiles), Paris, France

⁷ Department of Biology, Villanova University, Villanova, PA, USA

⁸ Department of Forestry and Wildlife, University of Uyo, Akwa Ibom State, Nigeria

⁹ 3 Abdala El Katib St, Dokki, Cairo, Egypt

Abstract. We present a preliminary phylogeny for 19 species of African *Agama* lizards based on a maximum likelihood phylogenetic analysis of 1,181 bp of mitochondrial DNA sequence data. Monophyletic radiations of species in East, South and West Africa are supported, as well as a clade containing two species (*A. doriae* and *A. sankaranica*) distributed across the Sahel region. West African populations of *A. agama* are paraphyletic with respect to *A. finchi* from westernmost Kenya, providing further evidence for a biogeographic corridor between West and East Africa. Populations of *A. agama* form four phylogeographic groups, which suggests that *A. agama* may be composed of multiple independent evolutionary lineages.

Key words. Africa, phylogeography, Systematics, biogeography, *Agama agama*.

INTRODUCTION

African lizards in the genus *Agama* are among the most diverse and widespread terrestrial squamates in Africa, making them an ideal group for testing biogeographic hypotheses and conducting comparative ecological and evolutionary studies. However, the robust phylogenetic framework for *Agama* that is needed to meet these goals is currently lacking. Constructing a phylogeny for *Agama* requires detailed investigations of phylogenetic relationships across multiple spatial and temporal scales. In general, the higher-level relationships within the Agamidae are poorly understood (MACEY et al. 2006), and dense sampling within *Agama* and other African and Asian taxa is necessary for testing the monophyly of and identifying the sister taxon to *Agama*. At the population level, several widespread and polytypic species, such as *Agama agama* in West Africa and *A. lionotus* in East Africa, have unclear species limits (BÖHME et al. 2005) and may be composed of multiple independent evolutionary lineages.

These broadly distributed taxa pose some difficult species delimitation problems (WAGNER 2007; WAGNER et al. 2008a, WAGNER et al. 2008b) and require detailed phylogeographic investigations.

The goals of our collaborative research on *Agama* lizards are to combine our sampling efforts from separate biodiversity fieldwork programs in different countries across Africa to 1) infer a comprehensive molecular phylogeny for the genus *Agama*, 2) resolve species limits in widespread, non-exclusive taxa, 3) conduct phylogeographic investigations of the *A. agama* and *A. lionotus* complexes, and 4) describe new species, where warranted. In this paper, we present a preliminary phylogeny for the genus *Agama* based on an analysis of mitochondrial DNA sequence data (mtDNA). In addition, we investigate the phylogeography of *A. agama* across West Africa.

MATERIAL & METHODS

A total of 19 species of *Agama* were included in the phylogenetic analyses (Table 1). We included 15 specimens of *A. agama* from 10 countries across West Africa to investigate the phylogeographic relationships among populations. We selected four species from the Agaminae to represent outgroup taxa, including *Acanthocercus atricollis*, *Laudakia stellio*, *Trapelus mutabilis*, and *Xenagama taylori*. We rooted our phylogeny with *Laudakia stellio*, although we note that the higher-level relationships within the Agaminae are poorly understood (MACEY et al. 2000; 2006).

DNA was extracted from tissues following the PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc). Two portions of the mitochondrial genome were PCR amplified and sequenced, including a small fragment of the 16S rRNA gene (16S) and a portion of the ND4 protein coding gene (ND4) and the adjacent histidine, serine, and leucine tRNA genes. Oligonucleotide primers used for PCR and sequencing are provided in Table 2. The 16S rRNA gene was PCR amplified for 30 cycles (95°C 30s, 58°C 30s, 72°C 50s) and the ND4 was amplified for 35 cycles (95°C 30s, 55°C 40s, 72°C 1 min). PCR products were purified using ExoSAP-IT (USB Corp.). Cycle sequencing products were ethanol precipitated, and then sequenced using an ABI 3730 automated sequencer.

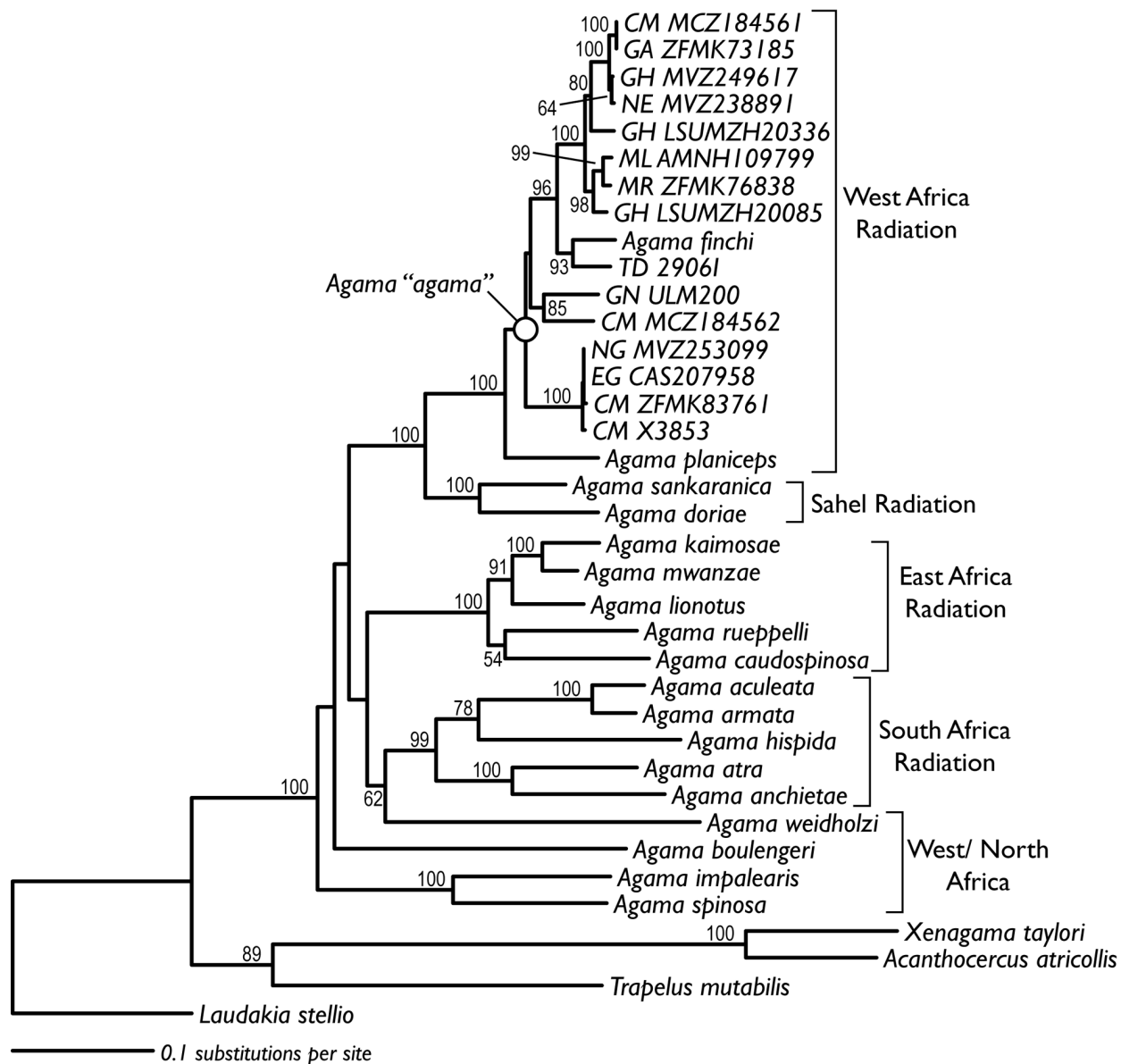


Fig. 1. Phylogenetic relationships within the genus *Agama* based on a maximum likelihood analysis of mtDNA sequence data using the GTR+ Γ model of nucleotide substitution. Maximum likelihood bootstrap values $\geq 50\%$ are shown.

Table 1. Voucher numbers and locality data for specimens used in the study. All sequences are deposited in GenBank (Accession Nos. GU128430 – GU128502). Voucher abbreviations are as follows: CAS, California Academy of Sciences; MVZ, Museum of Vertebrate Zoology; ZFMK, Zoologisches Forschungsinstitut und Museum; MCZ, Museum of Comparative Zoology; LSUMZ, Louisiana State University Museum of Natural Science; ULM, University of Louisiana at Monroe Museum of Natural History; AMNH, American Museum of Natural History; AMB, Aaron M. Bauer personal collection; specimen numbers beginning or ending with “X”, “I”, or “TR”, personal collector numbers for authors from the Muséum national d’Histoire naturelle.

| Species | Locality | Voucher |
|---------------------------------|---|--------------|
| <i>Agama aculeata</i> | Botswana, Kgalagadi District | MVZ 198076 |
| <i>Agama agama</i> | Cameroon | ZFMK 83761 |
| <i>Agama agama</i> | Cameroon, Mende, monts Takamanda | X 3853 |
| <i>Agama agama</i> | Cameroon, Yaounde | MCZ 184561 |
| <i>Agama agama</i> | Cameroon, Yaounde | MCZ 184562 |
| <i>Agama agama</i> | Equatorial Guinea, Bioko Island, Malabo | CAS 207958 |
| <i>Agama agama</i> | Gabon, Barrage de Tchimbele | ZFMK 73185 |
| <i>Agama agama</i> | Ghana, Greater Accra Region, Tesano | LSUMZ H20336 |
| <i>Agama agama</i> | Ghana, Northern Region, Buiepe | LSUMZ H20085 |
| <i>Agama agama</i> | Ghana, Volta Region | MVZ 249617 |
| <i>Agama agama</i> | Guinea, Diaragbela, Niger River | ULM 200 |
| <i>Agama agama</i> | Mali | AMNH 109799 |
| <i>Agama agama</i> | Mauritania, Selibabi | ZFMK 76838 |
| <i>Agama agama</i> | Niger, Niamey | MVZ 238891 |
| <i>Agama agama</i> | Nigeria, Cross River National Park | MVZ 253099 |
| <i>Agama agama</i> | Tchad, Bol | 2906 I |
| <i>Agama anchietae</i> | Namibia, Khorixas District | AMB 7582 |
| <i>Agama armata</i> | Tanzania | ZFMK 84990 |
| <i>Agama atra</i> | South Africa, Northern Cape Province | CAS 193435 |
| <i>Agama boulengeri</i> | Mauritania, Nouakchott Dist. | MVZ 235764 |
| <i>Agama caudospinosa</i> | Kenya, Naru Moru | ZFMK 83662 |
| <i>Agama doriae</i> | Cameroon, Daré Ville | 4218 X |
| <i>Agama finchi</i> | Kenya, Malaba | ZFMK 83653 |
| <i>Agama hispida</i> | South Africa, Northern Cape Province | AMB 4800 |
| <i>Agama impalearis</i> | Mauritania, Nouakchott District | MVZ 235766 |
| <i>Agama kaimosae</i> | Kenya, Nandi | ZFMK 83660 |
| <i>Agama lionotus</i> | Kenya, Nakuru | ZFMK 83646 |
| <i>Agama mwanzae</i> | Kenya, Mara | ZFMK 82076 |
| <i>Agama planiceps</i> | Namibia, Outjo Dist., Kamanjab | AMB 7638 |
| <i>Agama rueppelli</i> | Somalia, Awdal Region | MVZ 241340 |
| <i>Agama sankaranica</i> | Ghana, Volta Region | MVZ 249656 |
| <i>Agama spinosa</i> | Djibouti, Dikhil | MVZ 236458 |
| <i>Agama weidholzi</i> | Senegal | TR 481 |
| <i>Acanthocercus atricollis</i> | Uganda, Rukungiri District | CAS 201726 |
| <i>Laudakia stellio</i> | Turkey, Antalya Province | MVZ 230213 |
| <i>Trapelus mutabilis</i> | Egypt | ZFMK 64395 |
| <i>Xenagama taylori</i> | Somalia, Galbed Region | MVZ 241356 |

Contiguous DNA sequences were aligned and edited using Sequencher v4.8, and multiple sequence alignments were generated using Muscle v3.6 (EDGAR 2004). The open reading frame for the ND4 gene was identified using MacClade v4.08 (MADDISON & MADDISON 2005). The 16S rRNA alignment included indel-rich loop regions that could not be aligned unambiguously and were excluded from the phylogenetic analysis. Phylogenetic relationships were inferred using maximum likelihood using RAxML v7.0.4 (STAMATAKIS 2008), and implemented the GTR+I model of nucleotide substitution. Support values for inferred relationships were estimated from 100 non-parametric bootstrap replicates.

RESULTS

The maximum likelihood analysis of the combined mtDNA sequence data (1,181 characters) supports the monophyly of the genus *Agama* (bootstrap = 100%; Fig. 1). The outgroup taxa *Xenagama taylori* and *Acanthocercus atricollis* form a strongly supported clade (bootstrap = 100%) that is sister to *Trapelus mutabilis*. Within *Agama*, a clade containing *A. impalearis* and *A. spinosa* is sister to all remaining *Agama*, although this relationship is not accompanied by strong support (bootstrap <50%; Fig. 1). Some regional species assemblages are monophyletic, including a clade of Southern African species (*A. aculeata*, *A. an-*

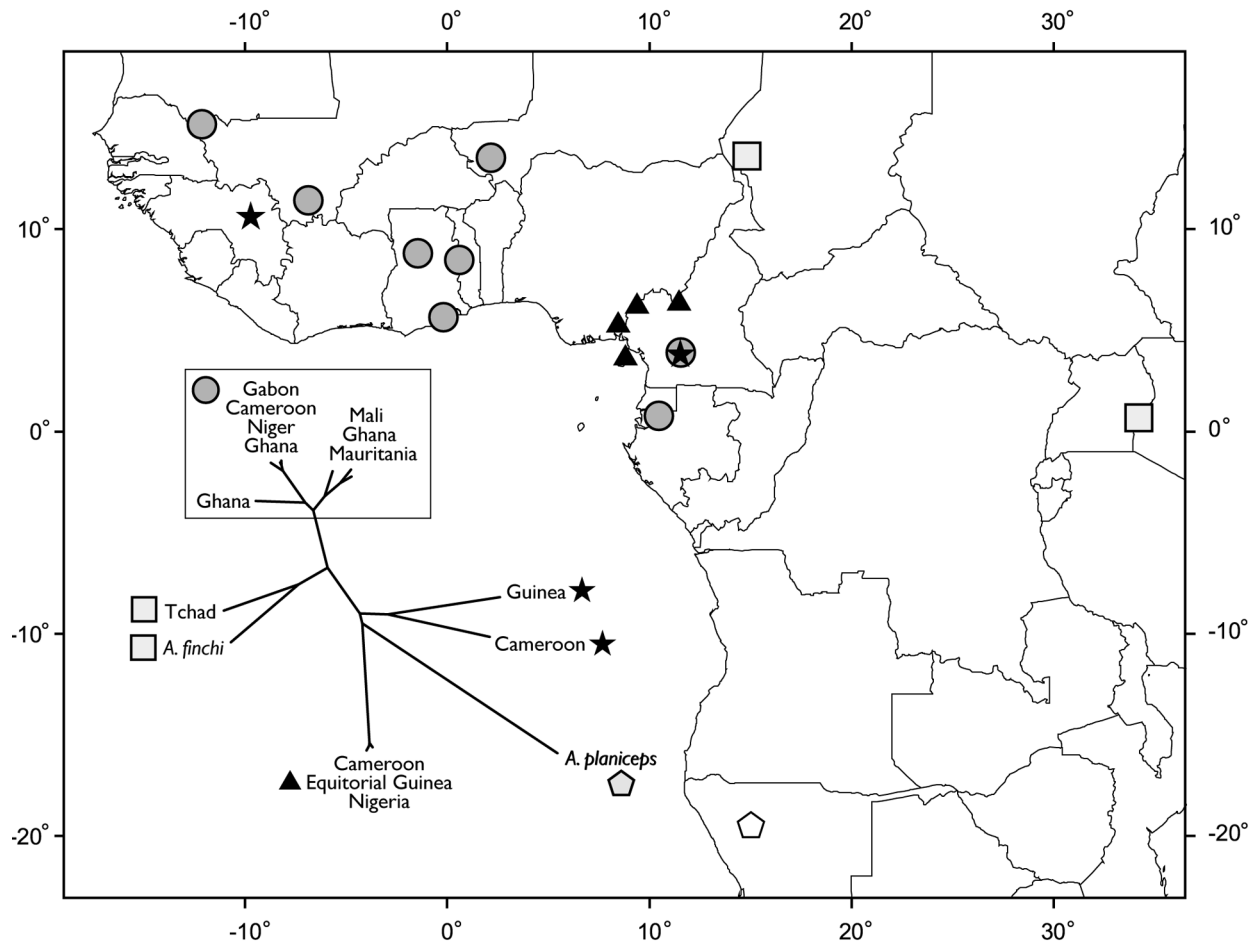


Fig. 2. Phylogeographic structure of *Agama agama* across sub-Saharan Africa. Samples of *Agama agama* are labeled by country in the unrooted phylogeny. The maximum likelihood phylogenetic analysis places *Agama planiceps* sister to *Agama agama*.

chietae, *A. atra*, *A. armata* and *A. hispida*) and a clade of East African species (*A. kaimosae*, *A. mwanzae*, *A. lionotus*, *A. caudospinosa* and *A. rueppelli*), each of which are strongly supported (bootstrap = 99% and 100%, respectively; Fig. 1). Strong support is also provided for a clade of species distributed across the Sahel region of sub-Saharan Africa, including *A. doriae* and *A. sankaranica* (bootstrap = 100%). *Agama planiceps* is sister to a clade containing all 15 specimens of *A. agama* and *A. finchi*, although this relationship is weak (bootstrap < 50%; Fig. 1). The interrelationships among these major clades are not supported by bootstrap values $\geq 50\%$, except for a strongly supported clade (bootstrap = 100%) containing the Sahel species (*A. doriae* and *A. sankaranica*) and *A. planiceps*, *A. agama*, and *A. finchi* (Fig. 1).

Populations of *Agama agama* in West Africa are paraphyletic with respect to *A. finchi* from westernmost Kenya (Fig. 1). Populations of *A. agama* form four phyloge-

graphic groups (Fig. 2). The geographic proximity between samples is a poor predictor of phylogenetic relationships, and three of the four clades overlap spatially (Fig. 2). One of the most well sampled phylogeographic groups within *A. agama* extends across West Africa from Mauritania to Gabon (Fig. 2). Two other clades with broad distributions include a group extending from Guinea to Cameroon and another extending from Tchad to Kenya (*A. finchi*) (Fig. 2). Finally, a clade with a more restricted distribution includes populations from southeastern Nigeria, western Cameroon, and Bioko Island (Equatorial Guinea) (Fig. 2).

DISCUSSION

This study represents the first detailed molecular phylogenetic investigation of the genus *Agama*. Monophyletic radiations of *Agama* lizards occur in East Africa, South

Table 2. Primers used to amplify and sequence mitochondrial DNA.

| Gene | Primers | Reference |
|------|--|-----------------------|
| 16S | 16Sf: CGCCTGTTTAACAAAAACAT 16sR: CCGGTCTGAACTCAGATCACGT | This study |
| ND4 | ND4:CACCTATGACTACCAAAAGCTCATGTAGAAGC LEU: ACCACGTTT TAGGTTTCATTTTCATTAC | ARÉVALO et al. (1994) |

Africa, the Sahel region, and even West Africa (Fig. 1), but reconstructing the biogeographic history of the group is impeded by several factors. First, the inter-relationships among the major lineages of *Agama* inferred by the mtDNA data are not accompanied by strong support, which would make any biogeographic scenarios based on the current phylogeny speculative. Second, our taxonomic coverage is not comprehensive and lacks approximately 10 species that are distributed throughout Africa. Finally, the sister taxon of *Agama* remains unclear. Despite these challenges, there are strong biogeographic signals in the current phylogeny (Fig. 1). For instance, the phylogenetic placement of *A. finchi* from western Kenya within the West African *A. agama* complex provides evidence for a biogeographic corridor between West and East Africa, a result that provides further evidence for a close biogeographic affinity between these regions (WAGNER et al. 2008c). In addition, the close relationship between *A. planiceps* from Namibia and the West African *A. agama* complex provides support for a biogeographic connection between West and South Africa (Figs 1 and 2). This relationship is not surprising given the close similarity in morphology and colour pattern shared between these species (BÖHME et al. 2005).

The intraspecific phylogeny for *Agama agama* highlights the taxonomic problems that this widespread taxon introduces to *Agama* lizard systematics. This species is paraphyletic with respect to *A. finchi* (Figs 1, 2) and appears to be composed of multiple distinct clades. The argument could be made that *A. finchi* should be synonymized with *A. agama* to retain a monophyletic *A. agama*, but *A. finchi* is clearly distinct based on morphology and coloration. The major phylogeographic groups found within *A. agama* based on mtDNA may represent distinct species, and represent a good starting point for testing species boundaries with multiple nuclear markers. Determining if gene flow is absent among these mtDNA groups is an important next step in resolving the systematics of the *A. agama* complex. The paraphyly of *A. agama* also underscores the need for additional detailed comparative morphological investigations that do not rely solely on adult male coloration characteristics (see GRANDISON 1968).

The phylogeographic relationships within the *Agama agama* complex exhibit a surprising amount of spatial overlap between clades. Phylogeographic studies of other *Agama* lizards, including *A. impalearis* (BROWN et al. 2002) and *A. atra* (MATTHEE & FLEMMING 2002) recovered more typical phylogeographic patterns whereby populations formed geographically exclusive clades. The spatial overlap of mtDNA clades in the *A. agama* complex could be evidence of the presence of multiple distinct species, which co-occur throughout West Africa and are as of yet morphologically cryptic. Conversely, this spatial pattern could reflect the recent expansion of distinct lineages that were formerly restricted to exclusive geographic areas. Distinguishing among these different hypotheses of population history await the addition of more specimens from throughout West Africa and the collection of nuclear DNA data. This work is currently underway.

The collaborative nature of this research project is providing an opportunity to conduct detailed investigations of the phylogeny and phylogeography of *Agama* lizards than would have otherwise been impossible. Our current work benefits from having expanded taxonomic sampling and multiple individuals of each species, more detailed outgroup sampling, dense population sampling in *A. agama* and *A. lionotus* species, and the addition of nuclear DNA sequence data.

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