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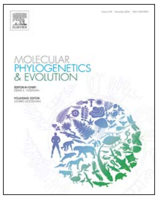

Cryptic diversity in *Rhampholeon boulengeri* (Sauria: Chamaeleonidae), a pygmy chameleon from the Albertine Rift biodiversity hotspot

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ABSTRACT

Several biogeographic barriers in the Central African highlands have reduced gene flow among populations of many terrestrial species in predictable ways. Yet, a comprehensive understanding of mechanisms underlying species divergence in the Afrotropics can be obscured by unrecognized levels of cryptic diversity, particularly in widespread species. We implemented a multilocus phylogeographic approach to examine diversity within the widely distributed Central African pygmy chameleon, *Rhampholeon boulengeri*. Gene-tree analyses coupled with a comparative coalescent-based species delimitation framework revealed *R. boulengeri* as a complex of at least six genetically distinct species. The spatiotemporal speciation patterns for these cryptic species conform to general biogeographic hypotheses supporting vicariance as the main factor behind patterns of divergence in the Albertine Rift, a biodiversity hotspot in Central Africa. However, we found that parapatric species and sister species inhabited adjacent habitats, but were found in largely non-overlapping elevational ranges in the Albertine Rift, suggesting that differentiation in elevation was also an important mode of divergence. The phylogeographic patterns recovered for the genus-level phylogeny provide additional evidence for speciation by isolation in forest refugia, and dating estimates indicated that the Miocene was a significant period for this diversification. Our results highlight the importance of investigating cryptic diversity in widespread species to improve understanding of diversification patterns in environmentally diverse regions such as the montane Afrotropics.

1. Introduction

The East African Rift valley system started to form in the early Oligocene from hot mantle plumes causing up-lift of the African plate resulting in rifting, the formation of horst and grabens, and associated volcanic activity (Chorowicz, 2005; Paul et al., 2014). The Albertine Rift (AR) portion in Central Africa was initiated in the late Oligocene (Roberts et al., 2012) and increased geophysical rifting in the AR occurred during the Miocene (Macgregor, 2015). Rifting oscillations influenced forest environments in the AR, largely through uplift events that altered climate and drainage patterns across the region (Sepulchre et al., 2006). Miocene volcanism has also contributed to the age and distribution of AR forests (Griffiths, 1993). The paleoclimate of the AR was generally stable through the Cretaceous (Maley, 1996), during which tropical Africa was dominated by a nearly continuous rainforest block. African rainforests began to decline in extent throughout the Cenozoic, with a pronounced increase in forest losses after the mid-Miocene (Kissling et al., 2012). Altered precipitation patterns across East Africa, driven by global cooling, contributed to the decline of the African tropical forest ecosystem in the Miocene (Zachos et al., 2001).

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Decreased Miocene rainfall is linked to the expansion of grass-dominated savannas across East Africa (Jacobs et al., 1999), and as grasslands expanded, forests contracted, and thereby forest connectivity was greatly reduced during this period (Kissling et al., 2012). These ancient geologically and climatically induced forest dynamics during the Miocene have left a profound legacy on the geographic distribution of genetic diversity in forest-distributed fauna in the AR (e.g., Tolley et al., 2011), and may have left a greater genetic imprint than Quaternary ice ages (Hewitt, 2000).

The proposed timing and mechanisms that underlie the remarkably high biodiversity in forests of the AR are not conclusive. One line of evidence supports recent species divergence within Pleistocene (upper limit ca. 1.8 Mya) refugial habitats (i.e., Pleistocene Forest Refuge Hypothesis [Mayr and O'Hara, 1986]), whereas another suggests that divergence occurred before Pleistocene climatic changes and species have been maintained as paleoendemics since the Miocene (ca. 5–23 Mya) (i.e., Evolutionary Museum Hypothesis, a derivative of the Montane Speciation Hypothesis [Fjeldså and Lovett, 1997]). Both of these hypotheses are based on allopatric models of speciation from isolation in forest refugia, but they differ greatly in their timing of diversification events. Speciation in forest refugia that formed in response to Pleistocene climatic changes have been implicated as biogeographic drivers among small mammals (Demos et al., 2014, 2015), land snails (Boxnick et al., 2015; Wronski and Hausdorf, 2008), and birds (Bowie et al., 2006; Voelker et al., 2013). However, frogs (Larson et al., 2016; Portillo et al., 2015), chameleons (Hughes et al., 2017; Tolley et al., 2011), and snakes (Greenbaum et al., 2015; Menegon et al., 2014) likely diversified prior to Pleistocene biogeographic events, such as the reduction of forests in response to global cooling in the Miocene. Afromontane forests have functioned as stable refugia during ancient climate changes and thereby promoted vicariance-driven diversification in some AR taxa (e.g., Hughes et al., 2017); however, this model does not fully account for the lack of genetic structure found in some widespread AR species (e.g., Greenbaum et al., 2013, 2015). Several physical biogeographic barriers have been identified in the AR, including the Virunga volcanoes that have been active from the Plio–Pleistocene to the present (Ebinger and Furman, 2003), and the uplift of the Rwenzori mountains that occurred around the Plio–Pleistocene boundary (ca. 3–2 Mya [Kaufmann et al., 2015]). These physical features have influenced patterns of gene flow for taxa between various highland areas of the AR (e.g., Huhndorf et al., 2007). However, genetic patterns for some AR taxa are not congruent with respect to identified barriers, and thus species-specific responses have been frequently detected. Much of the AR is ancient and several of its prominent geological features emerged before Pleistocene aridification pulses altered African ecosystems (e.g., deMenocal, 1995), and as a result, the AR represents an ideal region to test several biogeographic hypotheses regarding the timing and environmental mechanisms of biotic evolution.

The pygmy chameleon genus *Rhampholeon* currently contains 19 described taxa that are largely restricted to sub-montane and montane forests distributed across West, Central, and East Africa (Uetz et al., 2017). Many species of *Rhampholeon* are endemic to small forest fragments that face immediate threats of deforestation, and thus nine species are currently considered Endangered or Critically Endangered (IUCN, 2017). The Eastern Arc Mountains and Southern Rift Highlands, stretching from Kenya south to Tanzania, Malawi, and Mozambique, represent the highest regional concentration of species diversity for *Rhampholeon* with 16 species (Tolley and Herrel, 2013). The only pygmy chameleon species in West Africa is *R. spectrum* and its distribution extends from Nigeria and Central African Republic south to Gabon (Tilbury, 2010). *Rhampholeon hattinghi* and *R. boulengeri* occur allopatrically in the AR highlands of Central Africa. The recently described *R. hattinghi* is a Critically Endangered pygmy chameleon endemic to Mount Nzawa, Democratic Republic of the Congo (DRC), a massif in the southern AR (Tolley and Tilbury, 2015). *Rhampholeon boulengeri* is currently assessed as Least Concern, because it has a relatively large distribution in forest habitats across the AR, west into the Congo Basin (DRC) and east to Kakamega Forest (Kenya), and much of this forest is still relatively intact (Tolley and Plumptre, 2014). In addition to having one of the largest geographic distributions of any *Rhampholeon* species, *R. boulengeri* also occurs in forests across a remarkably wide range of elevations from 500 m to nearly 2300 m (Tilbury, 2010). *Rhampholeon hattinghi* is similar in appearance to *R. boulengeri*, and thus was initially considered to be a disjunct population of the more widespread species (e.g., Tilbury, 2010); however, genetic data revealed it as an independently evolving lineage (Tilbury and Tolley, 2015). Steindachner (1911) described *R. boulengeri* from a series of specimens collected by Rudolf Grauer in 1908. However, the type locality was imprecisely given as “forest beyond the sand hills on the north-western shores of Lake Tanganyika”. Tilbury and Tolley (2015) considered the Itombwe Plateau as the type locality because Rudolf Grauer collected specimens in 1908 from forests of the Itombwe Plateau, which is located to the northwest of Lake Tanganyika, however, Grauer did not write books about his travels and the precise localities where he collected in the plateau are unknown (Greenbaum, 2017).

In general, *Rhampholeon* are considered forest specialists with low vagility (Branch et al., 2014), and are thus unlikely to disperse over long distances regardless of suitable habitat corridors (Matthee et al., 2004). As a result, most pygmy chameleon species are endemic to the montane localities from where they were originally described (Uetz et al., 2017). The morphology of *Rhampholeon* is considered highly conservative (Branch et al., 2014), from which a potential for cryptic species results (Bickford et al., 2007), particularly in geographic regions that have received only cursory attention to the biota, such as the AR (Greenbaum, 2017). Moreover, several recent accounts have drawn attention to the likelihood that *R. boulengeri* represents a species complex (Tilbury, 2010; Tolley and Plumptre, 2014; Tilbury and Tolley, 2015). Therefore, *R. boulengeri* is an excellent model for investigating how diverse landscapes with complex histories of geomorphological and climatic changes have influenced the distribution of genetic diversity. In this study, we investigated the evolutionary history of *R. boulengeri* with a statistical framework to test three hypotheses related to cryptic diversity. We use a multilocus gene-tree and a comparative approach with four coalescent-based species-tree estimations to test whether *R. boulengeri* represents a single widespread species in the AR or a complex of genetically distinct species. We assess phylogeographic patterns and compare species distributions to test whether allopatric speciation driven by forest fragmentation underlies the diversification in the *R. boulengeri* species complex and for the genus in general. We implement fossil-calibrated Bayesian methods on a large-scale phylogeny to determine whether the timing of diversification in *Rhampholeon* follows a single break-up of African forests followed by isolation, or multiple forest fragmentations and reconnections over time.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

Forty-six samples of *R. boulengeri* were collected during field surveys in various forests across four Central African countries of the AR from 2008 to 2016, including Burundi, DRC, Rwanda, and Uganda (Table 1). Two additional samples were collected from the Yala Nature Reserve, Kakamega Forest, western Kenya. We also included additional sequences in our analyses that were not generated for this study: ND2 fragments and one RAG1 fragment for two individuals from Bwindi Impenetrable National Park, Uganda (CAS 201681–82); a 16S fragment for an individual from Irangi (near Kahuzi-Biega National Park), DRC (ZFMK 47571); and a 16S fragment for an individual from Cyamudongo Forest, Rwanda (ZFMK 55104) (Fisseha et al., 2013). For phylogenetic analyses, we included 18 of the 19 currently recognized *Rhampholeon* species and three species of *Rieppeleon* as outgroups (Branch et al.,