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Short Communication

Phylogenetic and morphological investigation of the *Mochlus afer-sundevallii* species complex (Squamata: Scincidae) across the arid corridor of sub-Saharan Africa

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ABSTRACT

The aridification of Africa resulted in the fragmentation of forests and the expansion of an arid corridor stretching from the northeast to southwest portion of sub-Saharan Africa, but the role this corridor has had in species-level diversification of southern African vertebrates is poorly understood. The skink species *Mochlus afer* and *M. sundevallii* inhabit wide areas of the arid corridor and are therefore an ideal species pair for studying patterns of genetic and phenotypic diversity associated with this landscape. However, species boundaries between these taxa have been controversial. Using multi-locus molecular and morphological datasets, we investigate diversification patterns of the *M. afer-sundevallii* Species Complex across the arid corridor. Although analyses of genetic data reveals some genetic structure among geographic populations, results of phylogenetic and morphological analyses provide little support for two distinct evolutionary lineages, suggesting that populations previously referred to as *M. afer* and *M. sundevallii* represent a single species, *Mochlus sundevallii*. Genetic diversity is unequally distributed across the arid corridor, with observed patterns consistent with aridification-facilitated diversification southward across southern Africa. Additional geographic and population-level sampling is necessary before more conclusive inferences can be drawn about the role historical climate transitions have played in skink diversification patterns across southern Africa.

1. Introduction

Large-scale geographic processes alter habitats and influence species diversification (van Zinderen Bakker and Mercer, 1986). Therefore, recognizing the distribution of and boundaries between populations or closely related taxa allows for a better understanding of how these processes affect the biodiversity of a region (e.g., Barlow et al., 2013). Throughout sub-Saharan Africa, the diversification and distributions of species have been shaped by a number of physical factors including complex orographic and climatic changes (e.g., Barlow et al., 2013; Portik et al., 2011). Aridification of the continent began approximately 25 million years ago, and resulted in the expansion of open woodland, grassland, and desert habitats and the fragmentation and contraction of lowland forests (Bobe, 2006; van Zinderen Bakker and Mercer, 1986). During Pliocene and Pleistocene glacial cycles, periods of extreme aridity resulted in the formation of a continuous arid corridor across the central and southern part of the continent, which stretched from the Horn of Africa in the northeast to Angola and Namibia in the southwest (Van Zinderen Bakker and Mercer, 1986; Verdcourt, 1969; Fig. 1A). This arid corridor is hypothesized to have contributed to the spread of xeric-adapted plants (Verdcourt, 1969) and mammals (Bobe, 2006) across southern Africa. However, species-level diversification patterns for reptiles distributed across the arid corridor remain understudied (but see Wüster et al., 2007).

The *Mochlus afer-sundevallii* Species Complex is a group of two semi-fossorial skink species that are distributed broadly throughout savannah, woodland, and semi-desert habitat in central and southern Africa with distributions that coincide with the arid corridor (Fig. 1A). Ranges of these species overlap with each other across much of eastern central Africa, with *M. sundevallii* also found in central and western southern Africa (Broadley, 1966). Because of their overlapping distributions and their close phylogenetic relationship (hypothesized by

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Broadley, 1966; Greer 1977), *M. afer* and *M. sundevallii* are an appropriate species pair for investigating the impact of the formation of the arid corridor on population diversification across the region. However, their similar morphology and ecology have made the boundary between the species difficult to discern, and the validity of both taxa has been debated for decades (e.g., Broadley, 1966). Through detailed morphological investigation, Broadley (1966) found that the only consistent phenotypic characters distinguishing these species were size

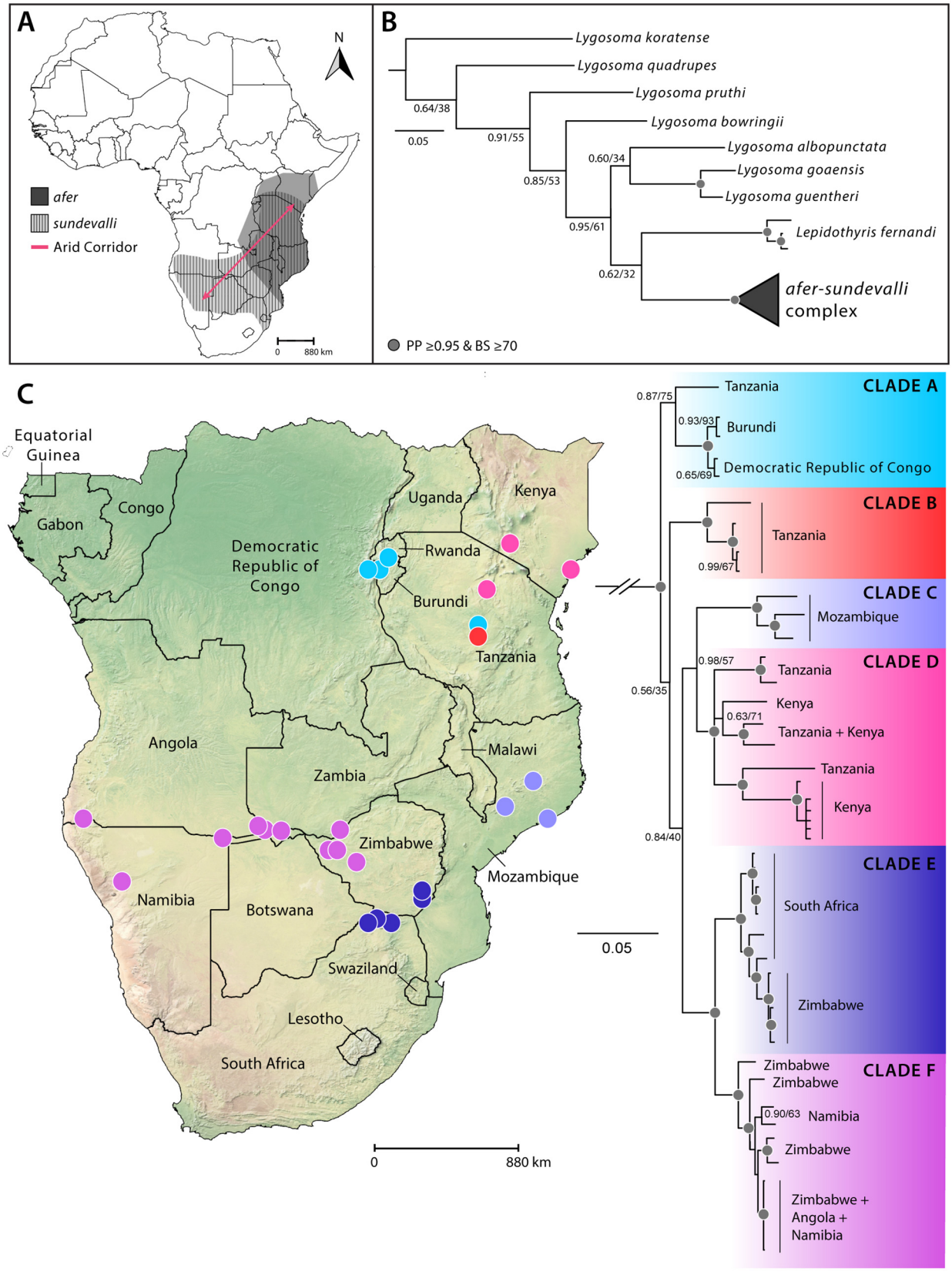

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Fig. 1. (A) Hypothesized species ranges for *M. afer* and *M. sundevallii* across the arid corridor in southern Africa shown as maximum inclusion polygons drawn from all known georeferenced locality data available from vouchered collections at CAS, CM, MCZ, MVZ, PEM, SARCA, TCWC, TMP, USNM, and YPM museums (for abbreviation definitions see Sabaj Pérez, 2014) and from Broadley (1966). (B) Relationship of the *M. afer-sundevallii* Species Complex in relation to outgroup taxa incorporated into phylogenetic analyses, illustrated by the maximum clade credibility tree resulting from Bayesian analyses of concatenated analyses. Nodes supported by ≥95% Bayesian posterior probability and ≥70% Maximum likelihood bootstrap support were considered significantly supported and are marked with gray circles. (C) Expanded view of the *M. afer-sundevallii* Species Complex and topographic map of southern Africa, showing hypothesized relationships among, and geographic distributions of, sampled populations. Terminals are labeled with general geographic localities; subclade labels refer to focal clades referenced in the Results and Discussion.

(snout–vent length [SVL]: *M. afer*: 80–140 mm; *M. sundevallii*: 60–85 mm) and pigmentation pattern (*M. afer*: irregularly speckled dorsum; *M. sundevallii*: uniform or regularly spotted dorsum). As a result of this lack of diagnostic characters between the two species, their taxonomic history is complex.

Mochlus sundevallii was described as *Eumices* [sic] (*Riopa*) *sunderallii* [sic] from South Africa by Smith (1849) [the specific epithet was corrected to *sundevallii* in an errata slip published with the book] and *M. afer* was described shortly thereafter from Mozambique Island, Mozambique by Peters (1854) as *Eumeces afer*. Günther (1864) designated a new genus, *Mochlus*, based on a specimen he described as a new species, *Mochlus punctulatus*, collected from either modern day Malawi or Mozambique during the 1858–1864 Zambesi Expedition. Subsequently, Bocage (1867) regarded all three species as *M. afer*. Boulenger (1887) agreed with Bocage's revision but resurrected the name *sundevallii* for the species and placed it in the genus *Lygosoma.* Additionally, Günther (1880) described a new species, *Sepacontias modestus,* which was recognized by subsequent authors as distinct from *Lygosoma sundevallii* (e.g. Boulenger, 1887; Mittleman, 1952) until Broadley (1966) synonymized *modestus* with *Riopa* (*Lygosoma*) *sundevallii* and resurrected the name *afer* for larger-bodied individuals in the complex. Most recently, Wagner et al. (2009) included samples referred to as *M. afer* and *M. sundevallii* from Kenya in their phylogenetic study of the *Lepidothyris fernandi* Species Complex, and recovered the samples as part of a clade that had nearly identical sequences between individuals, suggesting that these individuals were not distinct species.

Despite this longstanding taxonomic debate over the identity and validity of *M. afer* and *M. sundevallii*, there have been no molecular phylogenetic studies attempting to clarify the boundaries between these species. However, due to the broad distribution of this species complex, it is possible that multiple morphologically cryptic lineages exist, as seen in previous studies of African squamates (e.g. Barlow et al., 2013; Engelbrecht et al., 2013). Recognizing distinct lineages is important to understanding the biogeographic and evolutionary history of a group and therefore, in this study, we generate a molecular phylogeny of the *Mochlus afer-sundevallii* Species Complex to: (i) investigate support for species-level boundaries among sampled populations across the range of both species, (ii) assess patterns of genetic structure across the landscape, (iii) investigate whether the currently recognized diagnostic morphological characters are good predictors of species' boundaries, and (iv) explore whether inferred genetic and morphological boundaries for both species conform to the patterns of historical aridification-driven diversification in southern Africa.

2. Materials and methods

2.1. Taxon sampling, gene sampling, and sequencing

Ingroup sampling consisted of 57 individuals of the *Mochlus afer-sundevallii* Species Complex including eight individuals identified by the collectors and/or curators as *M. afer*, 42 individuals identified as *M. sundevallii*, and seven unidentified individuals of *Mochlus.* The *a priori* identification of specimens was based on geography and morphology. Outgroup sampling included nine species from the genera *Lepidothyris*, *Lygosoma*, and *Trachylepis* following results from recent phylogenetic studies of the group (Pyron et al., 2013; Wagner et al., 2009). A full list of specimens, their localities, and gene coverage is shown in Table S1.

Genomic DNA from liver, tail, or muscle tissue preserved in 95% ethanol was extracted using Qiagen DNeasy Tissue Kits (Qiagen). We sequenced the nuclear locus Recombination Activating Gene 1 (*RAG1*) and the mitochondrial genes NADH Dehydrogenase Subunit 2 (*ND2*) and 12S Ribosomal RNA (*12S*). Primers, annealing temperatures, and published sources for each gene are shown in Table S2. PCR products were sequenced with a BigDye® Terminator 3.1 kit (ThermoFisher) and were visualized on an ABI 3730xl DNA visualizer (ThermoFisher).

2.2. Sequence alignment and phylogenetic analysis

Sequences were aligned in Geneious v9.0.4 (Biomatters, Ltd.), with alignments checked by eye. Coding regions for *RAG1* and *ND2* were placed into the correct reading frame and examined for amino acid mismatches and stop codons. We generated Maximum Likelihood (ML) trees separately for nuclear (*RAG1*) and mitochondrial (*ND2* & *12S*) loci (Data Dryad: https://doi.org//10.5061/dryad.6180s6f) in RAxML v8.0.0 (Stamatakis, 2014) to examine datasets for gene tree discordance. Following the observation of congruent topologies among mitochondrial and nuclear datasets with no well-supported discordance, we conducted all final phylogenetic analyses using the concatenated dataset. Additionally, we explored the impact of missing data on the results by conducting ML analyses on concatenated datasets excluding markers and individuals for which there was incomplete data. These analyses resulted in consistent topologies, and therefore, we felt justified in including all sequence data in our final analyses.

The combined dataset was partitioned by gene and codon position, and we performed partitioned ML analyses employing the GTR + Γ substitution for each partition. Branch support was assessed with 1,000 bootstrap replicates. We also ran partitioned Bayesian Inference (BI) analysis on our dataset with MrBayes v3.2.6 (Ronquist & Huelsenbeck, 2003), and chose best substitution model for each partition using the Akaike Information Criterion implemented in jModelTest v2.1.8 (Darriba et al., 2012; Table 1). MrBayes was run for 50,000,000 generations, sampling every 5000 generations. Convergence between chains was confirmed in TRACER v 1.6 (Rambaut et al., 2014) and the first ten percent of trees were discarded as burnin. The concatenated alignment and all topologies are deposited in Dryad (https://doi.org//10.5061/dryad.6180s6f).

Table 1
Models of evolution selected by AIC (as implemented in jModelTest) for mitochondrial (*ND2*, *12S*) and nuclear (*RAG1*) data partitions.

Partition	Number of characters (bp)	AIC model
RAG1, 1st codon	373	GTR + Γ
RAG1, 2nd codon	373	GTR + Γ
RAG1, 3rd codon	373	GTR + Γ
ND2, 1st codon	342	GTR + Γ
ND2, 2nd codon	341	HKY + Γ
ND2, 3rd codon	341	GTR + Γ
tRNAs[a]	312	GTR + Γ
12S	475	GTR + Γ

[a] $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$.

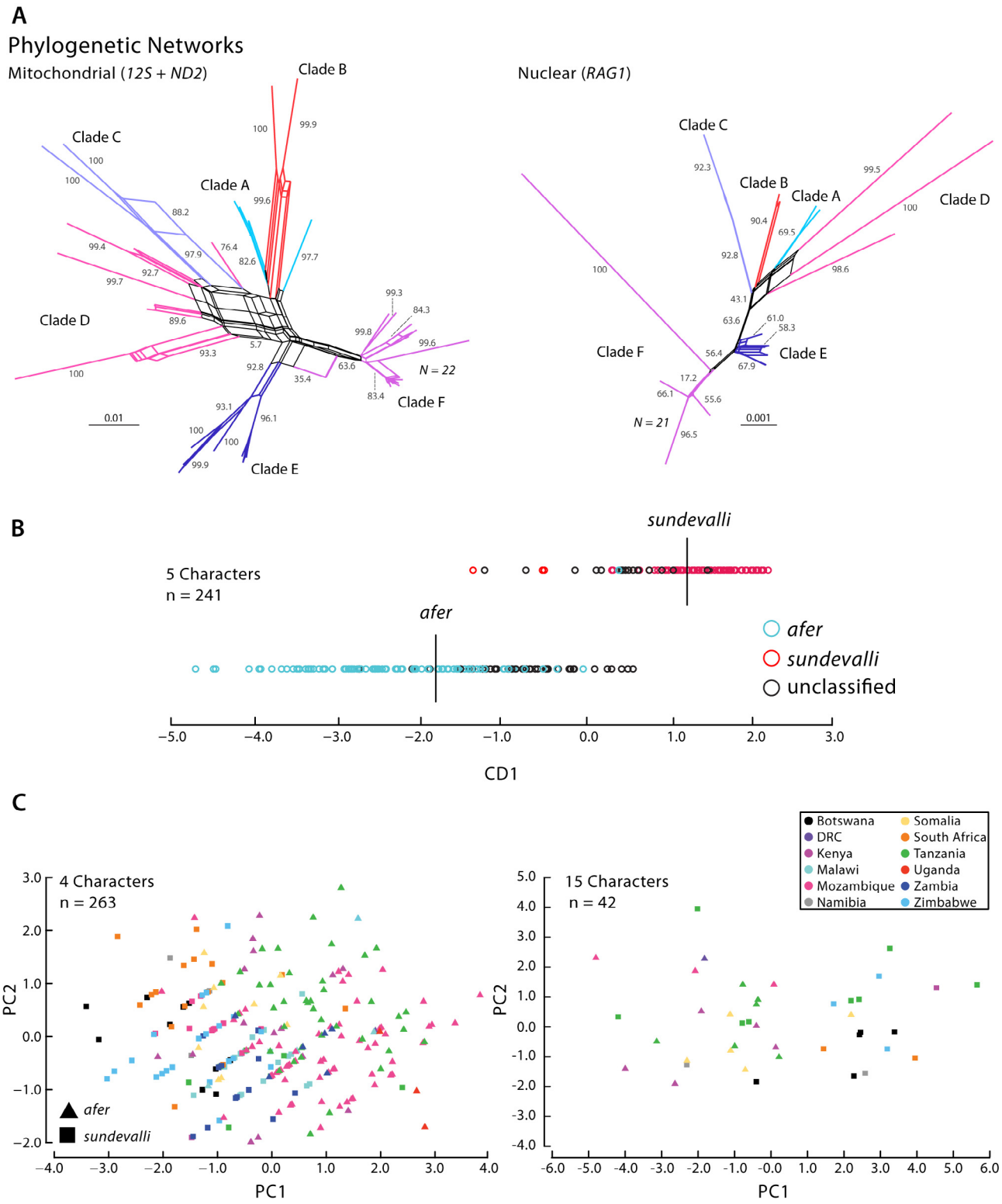

Fig. 2. (A) SplitsTree networks (Huson and Bryant, 2006) for the concatenated mitochondrial (*12S* + *ND2*) and nuclear (*RAG1*) datasets. Numbers match terminal labels in Fig. 1. (B) Linear discriminant function analysis of the DGB + ESF morphological dataset. Vertical lines through each scatterplot represent the mean canonical discriminant score for each species and the colors represent the predicted species association of each individual based on 95% posterior probability. (C) Results of principle component analyses of the DGB + ESF morphological dataset including SVL, MBSRC, SC, and Toe4Lam (left) and the ESF extended morphological dataset including AGD, MBW, MBD, TW, TD, HL, HW, HD, ED, END, SNL, IND, MBSRC, Toe4Lam, and SC (right).

2.3. Analysis of population genetic structure

To inspect genetic structure among sampled populations of the *M. afer-sundevallii* Species Complex, we generated phylogenetic networks for the concatenated mitochondrial (MtDNA) and nuclear (NuDNA) datasets, separately. Networks were created in the program SplitsTree v4.14.2 (Huson and Bryant, 2006; Fig. 2A), with support for inferred splits assessed by a bootstrap analysis with 1000 pseudoreplicates.

Table 2
Uncorrected pairwise sequence divergence (%) for mitochondrial (*12S*) data (below diagonal, white) and nuclear (*RAG1*) data (above diagonal, light gray) for the six subclades recovered in phylogenetic analyses (Fig. 1). Percentages on the diagonal represent intraspecific genetic diversity for mitochondrial data.

	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F
Clade A	**0.0–4.1**	0.5–1.0	0.5–0.7	0.6–0.8	0.1–0.5	0.1–0.8
Clade B	4.8–5.2	**0.0–2.6**	0.5–1.3	0.7–1.3	0.5–1.0	0.5–1.3
Clade C	6.1–6.8	4.8–6.8	**2.4–4.1**	0.7–1.2	0.5–0.7	0.5–1.0
Clade D	5.4–7.6	5.3–8.3	4.8–8.1	**0.0–6.7**	0.7–1.0	0.7–1.3
Clade E	4.8–6.5	4.1–6.8	5.2–7.6	4.8–8.1	**0.0–1.5**	0.3–0.5
Clade F	4.6–5.7	4.4–6.1	5.0–7.0	4.3–8.1	2.6–4.6	**0.0–2.1**

Additionally, we calculated the minimum and maximum uncorrected pairwise distances separately for *RAG1* and *12S* (*ND2* excluded due to missing data, Table 2).

2.4. Morphological analysis

We examined fluid-preserved specimens for variation in meristic, mensural, and qualitative characters (Data Dryad: https://doi.org//10.5061/dryad.6180s6f). We measured or scored 18 morphological characters from 23 and 22 specimens of *M. afer* and *M. sundevallii*, respectively, based on the definitions of Siler et al. (2010) and Broadley (1966): snout–vent length (SVL), axilla–groin distance (AGD), midbody width (MBW), midbody depth (MBD), tail length (TL), tail width (TW), tail depth (TD), head length (HL), head width (HW), head depth (HD), eye diameter (ED), eye–nares distance (END), snout length (SNL), internarial distance (IND), midbody scale row count (MBSRC), supraciliary count (SC), Toe IV lamellae count (Toe4Lam), and pigmentation pattern (Table S2). For morphological analysis, we coded pigmentation patterns as follows: 1 (uniformly colored dorsum); 2 (irregularly, lightly speckled dorsum); 3 (spotted dorsum). All measurements were taken by ESF with digital calipers accurate to 0.01 mm. Because the majority of specimens had missing or regenerated tails, we excluded TL from final analyses. Five of these characters (SVL, MBSRC, SC, Toe4Lam, and pigmentation pattern) were chosen to match a large morphological dataset collected previously (1960s) by DGB on specimens within the complex.

Previous taxonomic accounts of *M. afer* and *M. sundevallii* have proposed that species in the complex can be distinguished by dorsal pigmentation patterns and a tendency towards disparate body sizes. In an effort to test whether these published diagnostic characters support distinct species boundaries for *M. afer* and *M. sundevallii*, we conducted a linear discriminant analysis (DA) on the five-character morphological dataset as an exploratory technique to investigate whether species membership could be determined on the basis of published diagnostic characteristics alone. Because species were identified historically *a posteriori* based on pigmentation patterns and coloration, we expected high separation between groups. However, we employed DA, which maximizes variance between groups, to examine whether currently recognized diagnostic characters for species in the complex recover the putative species with high statistical support.

After excluding juveniles, identified as individuals with SVL < 80 mm for specimens identified as *M. afer* and SVL < 60 mm for specimens identified as *M. sundevallii* (Broadley, 1966), and individuals with missing data, the final, pooled dataset (DGB + ESF) included 241 individuals (*M. afer*: 135; *M. sundevallii*: 106). Prior to conducting the analysis, we calculated the Z scores for each variable using the scale function in R to standardized the variance for each variable.

We ran the DA using the lda function in the MASS package v7.3-45 (Venables and Ripley, 2002) in R v3.2.1 (R Core Team, 2014). Prior probabilities for assignment to each group was set at 0.5 so that each sample had an equal chance of being assigned to either *M. afer* or *M. sundevallii*. We used the predict function in MASS to obtain posterior probabilities of group assignment. Conservatively, we considered only classifications with posterior probabilities ≥0.95 percent to be correct. We ran additional DAs on the dataset with cross-validation in R by dividing the dataset into two randomly sampled groups, one to train the model and another to test it. This process was repeated 100 times and allowed us to investigate how random subsets of the data affected the results.

Principal components analysis (PCA) was run using the stats package v3.1.2 (R Core Team, 2014) in R on the meristic and mensural data of the combined DGB + ESF dataset to investigate the variation in morphology across the complex. Whereas DA produces linear combinations of variables to maximize group separation, PCA produces linear combinations of variables to capture and minimize variance between groups. Therefore, the PCA allowed us to examine the distribution of examined specimens within the complex in morphospace without correcting for species assignment. Prior to analysis we excluded juveniles, individuals with missing data, and the qualitative character for pigmentation for a total dataset of 263 individuals. We ran the PCA using the correlation matrix.

Understanding the limitations of a four-character dataset, we conducted additional PCA analyses on an expanded dataset of 15 characters for 42 individuals to investigate variation in body size and shape, again without *a priori* assignment to species. We conducted a preliminary PCA on the data using the correlation matrix but without adjusting mensural characteristics for body size. The results of this analysis showed some separation of *afer* and *sundevallii* along PC1 corresponding to traditionally recognized morphological and geographical species boundaries (Fig. S1). To account for the disproportionately large effect of body size on principal components analysis, mensural characters were size corrected with the equation Xadj = X-β(SVL-SVLmean) where X is the original value of the morphological character, Xadj is the size-corrected value of the morphological character, SVLmean is the average SVL for the entire dataset, and β is the unstandardized linear regression coefficient, calculated separately per species (Lleonart et al., 2000). We ran the PCA using the correlation matrix.

3. Results

3.1. Taxon sampling, gene sampling, and sequence alignment

Near complete sequence data was obtained for *RAG1* and *12S*; however, *ND2* sequence data amplified successfully for only 35 of 59 ingroup taxa (Table S1). Sequence lengths and number of variable sites for ingroup sequences are: *RAG1*: 1119 base pairs (bp), 49 variable sites (vs), 20 parsimony informative sites (pi); *ND2* + *tRNAs*: 1336 bp, 369 vs, 237 pi; 12S: 475 bp, 103 vs, 76 pi.

3.2. Phylogenetic analyses

The *Mochlus afer-sundevallii* Species Complex is recovered as monophyletic (PP = 1.0, MLBP = 99; Figs. 1B, C), comprising six well-supported subclades (Fig. 1C, subclades A–F). These groups include three east-central subclades (Figs. 1C, 2A: subclades A, B, D), and three southern subclades (Figs. 1C, 2A: subclades C, E, F). No analysis recovered two reciprocally monophyletic groups corresponding to *Mochlus afer* and *M. sundevallii*. Although support for population structure among subclades was observed, the relationships of these groups relative to each other remain less well resolved. Populations recovered within each subclade are clustered geographically (Fig. 1C). Interestingly, Tanzania and Zimbabwe are observed to possess the greatest subclade diversity, with populations sampled from these countries recovered in three and two distinct subclades, respectively (Fig. 1C).

3.3. Analysis of population genetic structure

Uncorrected pairwise distances among subclades for *12S* and *RAG1*

reveal low genetic diversity overall across the *M. afer-sundevallii* Species Complex. We observe higher mitochondrial genetic diversity among populations sampled from east-central Africa as compared to those sampled from southern Africa (Table 2). Although the distribution of samples recovered in subclade F span the greatest geographic distance, levels of mitochondrial sequence divergence among its members (0.0–2.1%; Table 2.) are similar to divergences observed among individuals of subclade B (0.0–2.6%; Table 2), a single population sampled from one locality (Fig. 1C).

Phylogenetic network analyses provide some support for several of the subclades observed in phylogenetic analyses. The mtDNA (*12S* + *ND2*) network revealed divergent, well supported splits for subclades B, C, and E; however, samples supported to be part of subclades A, D, or F in phylogenetic analyses were not recovered as cohesive genetic clusters (Fig. 2A), with clade A paraphyletic with clade B and clade D paraphyletic to clade C. Northern sublcades (A, B, C, D) did not show any reticulation with southern subclades (E, F). Although the nuDNA (*RAG1*) network revealed genetic structure similar to observed phylogenetic results, low genetic diversity resulted in a poorly resolved network (Fig. 2A).

3.4. Morphological analysis

As predicted, the results from the linear discriminate function analysis revealed that SVL and pigmentation pattern can be used as characters to identify two separate groups within the *M. afer-sundevallii* Species Complex. However, these characters are not always fully consistent within groupings; individuals were only correctly classified into their *a priori* species with 95% posterior probability as *M. afer* 63.0% of the time (0% classified as *M. sundevallii,* 37.0% not classified into either species) and as *M. sundevallii* 82.1% of the time (0.9% classified as *M. afer*, 17.0% not classified into either species, Fig. 2B). The additional cross-validation DAs produced similar results with SVL and pigmentation pattern as the major discriminating characters between the two groups; 64.5 ± 3.0% of *M. afer* were classified correctly and 97.9 ± 2.2% of *M. sundevallii* were correctly classified.

The results of the principal components analysis for the combined DGB + ESF dataset show substantial overlap between the two putative species in morphospace (Fig. 2C, left). The first two principal components together accounted for 66.6% of the total variance. The first principal component (PC1, 46.3% of variance) loaded roughly equally across all variables, with some additional weight given by MBSRC. The second principal component (PC2, 20.3% of variance) was driven primarily by Toe4Lam. The results of the second PCA on the smaller ESF dataset were similar to those from the larger DGB + ESF dataset (Fig. 2C, right). This analysis was conducted to investigate the distribution of body size and body shape across the *M. afer-sundevallii* Species Complex. The first three principal components together accounted for 73.5% of the total variance. PC1 (53.0% of variance) loaded roughly equally across mensural characters excluding AGD, with some additional weight given by MBW, TD, HW, and HD suggesting that differences in size and body robustness was responsible for roughly half of the variance. The second principle component (PC2; 12.1% of variance) loaded heavily on the meristic characters, whereas PC3 (8.4%) loaded most heavily on AGD, pertaining to the amount of body elongation. However, ordination of the axes of PC1 and PC2 show a single cluster of points indicating that there is no significant separation among samples based on taxonomic assignment or geography (Fig. 2C).

4. Discussion

4.1. Phylogenetic and biogeographic patterns

This study provides the first phylogenetic analysis of the widespread *Mochlus afer-sundevallii* Species Complex. Previous studies of African herpetofauna have revealed considerable levels of cryptic diversity across widespread species resulting from isolation of populations during periods of significant climate change. During periods of harsh climatic conditions, populations were confined to multiple allopatric refugia across southern Africa, resulting in population-level diversification (e.g., *Bitis arietans*, Barlow et al., 2013; *Acontias meleagris* Species Complex, Engelbrecht et al., 2013). Conversely, our results do not suggest that there are deeply divergent putative lineages within the *M. afer-sundevallii* Species Complex; however, we find several interesting patterns of genetic variation across the complex, including clues on how population genetic structure is distributed across the arid corridor.

Phylogenetic analyses do not provide support for a clear separation among sampled populations into two distinct lineages corresponding to each of the species. Although there is support for cohesive genetic structure distributed across southern Africa, the available molecular data and geographic sampling are not sufficient to fully resolve the relationships among these subclades (Figs. 1C, 2). Mitochondrial and nuclear genetic networks reveal three cohesive genetic clusters observed in phylogenetic analyses (Figs. 1C, 2A: subclades B, C, E). However, samples recovered within the remaining three subclades did not form unique, cohesive genetic clusters among networks with strong bootstrap support (Fig. 2A, subclades A, D, F). This lack of high-support in the network and the phylogenetic tree for clade relationships reflects both recent divergences and possibly the effects of missing data in ND2. There appears to be little gene flow between southern and northern subclades, which may be an interesting direction for future research. Overall, although *RAG1* shows lower sequence variation within and among subclades, the phylogenetic network is quite consistent with the mitochondrial network and the results of the phylogenetic analyses (Figs. 1C, 2A).

Of particular note is the observation of greater genetic diversity among populations from the northeastern portions of the arid corridor. Specifically, populations from Tanzania, Burundi, Kenya, and the Democratic Republic of Congo possess the highest levels of inter- and intra-population sequence divergences, despite having fewer distinct populations sampled (Figs. 1C, 2A; Table 2. subclades B, D). In contrast, subclades comprising the southernmost populations sampled possess smaller genetic divergences overall (Figs. 1C, 2A; Table 2: subclades E, F). This includes subclade F from the southwestern regions of the arid corridor (Figs. 1B, C), which displays the lowest levels of sequence divergences (Table 2.), but the broadest geographic distribution among subclades.

Hewitt (2004) coined the phrase “southern richness, northern purity” to refer to higher genetic diversity in southern populations of European species compared to northern populations, and suggested that this was a result of species surviving harsh climatic events in moderately hospitable southern climates and then dispersing northwards as conditions improved. The results of this study are consistent with this pattern (although opposite in cardinal direction), particularly when considering what is known of the ecology of the members of the focal species complex. Both putative species prefer semi-arid or well-drained sandy environments throughout tropical sub-Saharan Africa (Broadley, 1966). Therefore, the aridification of Africa is likely to have a considerable impact on the distribution of suitable habitat as well as species-specific dispersal potentials. During the Miocene, open C4 grasslands were established throughout central and southern Africa as a result of aridification throughout the continent (Bobe, 2006). Additionally, climatic oscillations throughout the Miocene, Pliocene, and Pleistocene resulted in periods of increasing and decreasing aridity, causing grasslands to expand and contract (Van Zinderen Bakker and Mercer, 1986). Expansion of the arid corridor, stretching diagonally across southern sub-Saharan Africa, may have facilitated southward expansion of the *M. afer-sundevallii* Species Complex as climatic and habitat conditions in the southern part of the continent became more hospitable. This pattern also has been observed in the skink *Trachylepis sulcata*, with populations in northern Namibia (near the northern extent of the range) were shown to be genetically distinct from populations in

central and southern Namibia and South Africa and harbored higher genetic diversity (Portik et al., 2011). These results were attributed to a more recent southward expansion of *T. sulcata* as a result of increasing aridity in the region (Portik et al., 2011). It is plausible that the *M. afer-sundevallii* Species Complex diversified initially in east-central Africa, and later expanded southward into southwestern Africa during periods of increased aridity and the expansion of the arid corridor. Additionally, habitat in the southern part of the range of the species complex may be less heterogeneous, facilitating ongoing gene flow across larger areas. Given limitations concerning the number of loci incorporated into molecular datasets, and the availability of dense population-level genetic sampling across the complex's range, the observed patterns remain fertile directions for future studies.

4.2. Morphological diversity

The results of our discriminant function analysis and principal components analyses do not reveal a significant morphological distinction between *Mochlus afer* and *M. sundevallii*, despite the availability of a robust dataset that included more than twice the number of specimens and geographic coverage than the available molecular dataset. Traditionally, SVL and pigmentation have been the only two characters used explicitly to distinguish *M. afer* from *M. sundevallii* (Broadley, 1966). The DA was able to accurately separate the species with high probability only 63.0% (*M. afer*) and 82.1% (*M. sundevallii*) of the time, despite individuals being assigned to species *a priori* based on size and pigmentation pattern. To a lesser extent, geographic location has been used to diagnose species, with populations in southern Africa considered to belong to *M. sundevallii* exclusively (Broadley, 1966). However, the results of the PCA do not indicate any morphological separation based on geography, with individuals from southern Africa instead clustering with individuals from central and northern East Africa. This suggests that there is no significant morphological difference between specimens across the range of the *M. afer-sundevallii* Species Complex. Therefore, it is possible that observed morphological variation within the complex, including body size, body shape, and pigmentation patterns are due to heterochronic morphological shifts instead of species boundaries (Fig. 2B). Additional studies on the morphology of this complex may reveal interesting age-related morphological correlations.

4.3. Taxonomic status of the Mochlus afer-sundevallii Species Complex

Due to the limited availability of geographic and population-level sampling for critical regions across southern Africa, the results of this study provide a robust starting point on which to further assess species-level diversity of widespread taxa across southern Africa's arid corridor. Our study provides the first phylogenetic and morphological analysis of the *Mochlus afer-sundevallii* Species Complex. Although our results did not reveal two distinct lineages that each represent putative species, we can relate several subclades to previously identified populations. Based on geography, samples in subclades C and E most likely represent the populations described originally as *M. afer* (Peters, 1854) from coastal and insular Mozambique and *M. sundevallii* (Smith, 1849) from northern South Africa, respectively. Additionally, all samples sequenced in Wagner et al. (2009) fall into subclade D along with several other individuals from Kenya and Tanzania. The remaining 3 subclades (A, B, and F) have never previously been included in phylogenetic or taxonomic studies. To date, no genetic samples are available for populations across much of the northern portions of the complex's distribution, including Uganda, Somalia, Ethiopia, Sudan, and South Sudan. Currently, some of these areas are politically unstable, making fieldwork difficult. Additionally, the collection of additional vouchered specimens and genetic samples from populations in Angola, Botswana, Kenya, Malawi, Mozambique, Mozambique Island, Namibia, and Zambia would add greatly to our understanding of the biogeographic history and landscape genetic diversity of this widespread, burrowing species complex. Increased sampling across several of these regions are of critical importance as they represent type localities of several species for which the genus *Mochlus* was originally described including *M. punctulatus* from Malawi, or possibly Mozambique (see Introduction, synonymized with *M. afer* [Bocage, 1867]) and of *M. afer* (Mozambique Island).

Based on the available DNA sequence and morphological data, the results of this study do not provide conclusive support for the distinction between *Mochlus afer* and *M. sundevallii*. Given the absence of phylogenetic and morphological support for two, distinct and cohesive evolutionary lineages, we propose that the complex represents a single, widespread lineage across sub-Saharan Africa to which the name *Mochlus sundevallii* would apply.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.04.020.

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