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Molecular data from contemporary and historical collections reveal a complex story of cryptic diversification in the *Varanus (Polydaedalus) niloticus* Species Group

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Abstract

Previous studies of color pattern, tongue pigmentation, and scale counts have been used to distinguish two species of semiaquatic varanids in Africa, but these findings have yet to be tested with molecular data. The *Varanus (Polydaedalus) niloticus* Species Group is comprised of the Nile monitor (*V. niloticus*) and the Ornate monitor (*V. ornatus*). Due to the high rate of exploitation of both species for bushmeat, the leather industry, and the pet trade, a clear understanding of the taxonomy and genetic partitioning is necessary for effective management. Here we utilize a multilocus approach, consisting of mitochondrial and nuclear markers, totaling 4,251 bp, as well as microsatellite loci to assess the taxonomic validity and intraspecific evolutionary patterns within the *V. niloticus* Species Group. By incorporating historical specimens from museum collections as well as contemporary samples, we obtained range-wide coverage for both species across Africa. Concordant results from various approaches all suggest that *V. ornatus* does not represent a distinct monophyletic group. Our analyses recovered three genetic clades within *V. niloticus*, representing western, northern, and southern lineages. The western clade was found to diverge first, around 7.7 mya (95% HPD: 4.6–11.0 mya) and exhibits 8.4% and 8.7% uncorrected sequence divergence between the northern and southern *V. niloticus* clades, respectively. This geographically separate lineage is consistent with previous descriptions of *Tupinambis stellatus* (Daudin, 1802). These findings not only call for taxonomic revision of this species group, but also shed light on the biogeographic history of Africa as well as aid in the management planning of varanids and other co-distributed African species.

Keywords: Nile monitor; Ornate monitor; *V. ornatus*; *V. stellatus*; African biogeography; museum collections

1. Introduction

The effects of past environmental processes are often "written" in the evolutionary relationships and distributional patterns among and within species (Avise, 2000; Haffer, 1969; Mayr and O'Hara, 1986). Historically, evolutionary inferences and taxonomic classifications have been largely based on phenotypic characters (Merrell, 1981). Relying solely on morphology for species delineation can produce misleading assumptions and hinder our ability to accurately resolve patterns of biodiversity (Bickford et al., 2007). Although morphological examinations are useful for identifying species in the field, genetic tools can reveal the underlying patterns of connectivity and provide a more detailed understanding of the biogeographic history of the region (Avise, 2000). Frequent discoveries of cryptic lineages within superficially indistinguishable taxa highlight the utility of molecular analyses in describing biodiversity (Hekkala et al., 2011; Stuart et al., 2006; Welton et al., 2013). Recent molecular studies have uncovered a wealth of information on African biogeography, revealing largely congruent genealogical patterns across species that are consistent with historic climatic oscillations (Alpers et al., 2004; Brown et al., 2007; Flagstad et al., 2001; Greenbaum et al., 2015; Lorenzen et al., 2012; Moodley and Bruford, 2007; Smitz et al., 2013). By performing continent-wide genetic assessments across diverse taxonomic groups, we can build a library of information to aid in preserving African biodiversity, as well as increase our understanding of its evolutionary origins.

The *Varanus (Polydaedalus) niloticus* Species Group, consisting of the Nile monitor (*Varanus niloticus*) and the Ornate monitor (*V. ornatus*) (Böhme and Ziegler, 1997), represents an interesting model to examine taxonomic hypotheses and biogeographic patterns across Africa. Of the six African varanids, *V. niloticus* exhibits the largest distribution across the continent,

spanning most of sub-Saharan Africa and extending northward along the Nile River into Egypt (Lenz, 2004). Due to its semi-aquatic nature, *V. niloticus* is largely dependent on permanent bodies of water throughout its range rather than on specific habitat types (Bayless, 1997; Lenz, 2004). As such, *V. niloticus* inhabits a broad diversity of vegetation zones, from tropical forests to semi-arid savannahs (Bayless, 1997). Like *V. niloticus*, *V. ornatus* is also semi-aquatic (Böhme and Ziegler, 2004), and together they represent the only African varanids with habitat requirements closely tied to watercourses (Bayless, 1997). Although these two species overlap in their distributions, *V. ornatus* is restricted to dense rainforests of western and central Africa (Bayless, 2002; Böhme and Ziegler, 2004).

Information concerning the evolutionary relationships and genetic structure of *Varanus niloticus* and *V. ornatus* could aid in the management of these species, both currently listed on Appendix II of the Convention on International Trade of Endangered Species of Fauna and Flora, CITES (http://www.cites.org/). Although considered common due to its widespread distribution, *V. niloticus* has a long history of commercial exploitation for the leather industry with approximately 500,000 skins exported annually from the Sahelian region, including Chad, Nigeria, Mali, Cameroon, and Sudan (de Buffrenil, 1995; de Buffrénil and Castanet, 2000; De Lisle, 1996; Jenkins and Broad, 1994). Currently, the two highest *V. niloticus* skin exporters, Mali and Chad, report an annual average of approximately 47,000 and 30,000 exported skins respectively (http://trade.cites.org). Additionally, *V. niloticus* is regularly found in the pet trade (Pernetta, 2009; Robinson et al., 2015), with Benin and Togo among the top exporting countries, each reporting approximately 4,000 live *V. niloticus* individuals exported annually (http://trade.cites.org). Accounting for undocumented harvesting and exports, studies suggest

that some *V. niloticus* populations are in danger of extirpation (de Buffrenil, 1995; De Lisle, 1996). Because *V. ornatus* was only recently elevated to full species level (Böhme and Ziegler, 1997), the high harvest rates for *V. niloticus* can likely be applied to *V. ornatus* as well. Range-wide genetic assessments of both species can prioritize conservation efforts as well as allow for genetic sourcing of illegal shipments.

Taxonomic status plays a major role in affording legal protection to organisms (Dubois, 2003; May, 1990). Until recently, *Varanus ornatus* was considered a subspecies of *V. niloticus* (Mertens, 1942). However, Böhme and Ziegler (1997) elevated the status of *V. ornatus* to full species level based on differing pigmentation patterns, tongue coloration, genital morphology, and slight differences in scale counts. Many naturalists of the 19th century also described *V. ornatus* as a distinct entity, mainly noting the more elaborate pigmentation patterns (Daudin, 1803; Schlegel, 1844). Böhme and Ziegler (1997) suggested that the coloration patterns of *V. ornatus* represent an ancestral state compared to those of *V. niloticus*; however, the evolutionary relationship between these two morphologically similar species has yet to be genetically confirmed.

The taxonomic history of *Varanus niloticus* is much more complex than that of *V. ornatus*, with seven recognized synonyms (Böhme, 2003). Several of the original species descriptions partitioned *V. niloticus* into two or more separate species or subspecies. Daudin (1802) described a monitor species in Senegal (*Tupinambis stellatus*) as morphologically distinct from that found in Egypt (*Tupinambis niloticus*). Similarly, Schlegel (1844) described three separate subspecies, inhabiting the Nile River, Senegal, and South Africa, in addition to the Ornate monitor of the

93 Congo. In contrast, Mertens (1942) attributed these varying traits to ontogenetic differences
94 rather than geographically distinct forms.
95
96 By utilizing mitochondrial and nuclear markers, we examine the taxonomic status and
97 evolutionary relationships within the *Varanus niloticus* Species Group, as well as the timing of
98 divergence events. Specifically, our analyses examine whether there is genetic evidence to
99 support a species-level classification of *V. ornatus*. Additionally, we assess the hypothesis of
100 independently evolving geographic lineages within *V. niloticus* against the null hypothesis of
101 panmixia across their range. By utilizing historical collections to supplement our contemporary
102 samples, we were able to achieve a continent-wide genetic assessment. These historical samples
103 include 59 dried and ethanol-preserved specimens collected up to 130 years ago.
104
105 **2. Materials and methods**
106 *2.1. Contemporary sample collection and laboratory protocols*
107 We collected 57 *Varanus niloticus* and nine *V. ornatus* contemporary samples from across both
108 species' ranges (**Table 1**). Samples consisted of either muscle tissue or fresh blood dried on
109 Whatman filter paper. For an outgroup comparison, we also collected samples from two other
110 African varanids, *V. exanthematicus* and *V. albigularis*.
111
112 Genomic DNA was extracted using the DNeasy Blood &Tissue Kit (Qiagen), following the
113 manufacturer's protocol for either tissue or blood. We examined a total of 4,251 bp from four
114 mitochondrial (2,096 bp) and three nuclear (2,157 bp) gene regions as follows: 12S rRNA (293
115 bp), *ND1* (457 bp), *ND2* (528 bp), *ND4* (816 bp), *RAG-1* (781 bp), *KIAA1217* (595 bp), and

116 *KIAA1549* (781 bp). Gene regions were selected for analysis based on previous studies showing
117 sequence variation at similar taxonomic levels within *Varanus* (Fitch et al., 2006; Portik and
118 Papenfuss, 2012; Portik et al., 2012; Smissen et al., 2013; Smith et al., 2007; Welton et al.,
119 2013). PCR conditions were optimized for each gene region (**Supplementary Tables S1 and**
120 **S2**) and were carried out in 21 μL total volume reactions. After successful amplification,
121 confirmed on a 1.5% agarose gel at 100 volts, PCR products were sequenced using the Sanger
122 method at Macrogen (New York, NY). Chromatograms were edited and assembled into contigs
123 in Sequencher v4.1 (Gene Codes Corp.). All DNA sequence data generated for this study were
124 deposited in GenBank under the accession numbers JN673347, JN673350–JN673352,
125 JN673363–JN673365, and KT720497–KT721289.
126
127 We amplified 11 microsatellite loci originally developed for the congener species *Varanus*
128 *komodoensis* (Ciofi and Bruford, 1998; Ciofi et al., 2011), *V. salvator* (Fu et al., 2011), and *V.*
129 *acanthurus* (Fitch et al., 2005) following the modifications of Dowell et al. (2015). Fragment
130 analysis was carried out on an ABI 3100 Genetic Analyzer (Applied Biosystems, Inc.) with
131 GeneScan 500 LIZ size standard and genotypes were scored using GeneMarker (SoftGenetics).
132
133 *2.2. Historic sample collection and laboratory modifications*
134 We utilized tissue samples from 59 dried and ethanol-preserved museum specimens housed in
135 the collections of nine institutions (**Table 1**). We processed all historic samples in a clean room
136 facility, separate from contemporary samples and post-PCR products. Samples were soaked in a
137 1x phosphate buffered saline (PBS) solution for six days, changing the buffer every 48 hours.
138 DNA extraction then was performed with the DNeasy Blood and Tissue Kit (Qiagen) with the

following modifications. During the initial tissue digestion step, an additional 20 µL of proteinase K was added after 24 h and samples were incubated at 56°C for a total of 48 h. In the final DNA elution step, we used 75 µL of elution buffer warmed to 56°C and incubated the samples at room temperature for 15 min prior to centrifugation. The elution step was repeated, producing a final extract volume of 150 µL. During all processing steps, samples were mixed by hand to avoid shearing the DNA, as can occur with vortexing.

For mitochondrial genes, we designed internal primers to amplify 200–300 bp overlapping fragments covering all regions. Nuclear primers were also designed to amplify short fragments (125–225 bp) covering variable sites identified in the modern samples (**Supplementary Table S1**). All PCR reactions contained multiple negative controls to ensure that the reagents were not contaminated. Successfully amplified DNA fragments were sequenced using the Sanger method at Macrogen (New York, NY).

For the microsatellite analysis, all samples were genotyped two independent times at each locus to examine the allelic dropout rate. Homozygotes and individuals showing discrepancies between allele calls were genotyped a third time. For the loci that could be consistently amplified, we used the frequency-based assignment test (Paetkau et al., 1995) implemented in GeneClass2.0 (Piry et al., 2004) to calculate the genetic cluster assignments.

2.3. Phylogenetic analyses

All DNA sequences were aligned using Clustal-W (Thompson et al., 1994) implemented in MEGA v6 (Tamura et al., 2013). Haplotype reconstruction of nuclear gene regions was carried

out using PHASE (Stephens and Donnelly, 2003; Stephens et al., 2001) and FastPHASE (Scheet and Stephens, 2006) as implemented in DnaSP v5.10.1 (Librado and Rozas, 2009).

We analyzed the full concatenated dataset, consisting of mitochondrial (2,094 bp) and nuclear (2,157 bp) sequence data, using maximum likelihood (ML) as the phylogenetic optimality criterion with RAxML v8.1.15 (Stamatakis, 2014). The data were analyzed under four partitioning schemes: (i) unpartitioned (one partition), (ii) mitochondrial and nuclear loci (two partitions), (iii) concatenated mitochondrial loci and three individual nuclear loci (four partitions), and (iv) all loci separately (seven partitions). All partitions were analyzed under the general time-reversible (GTR) substitution model (Lanave et al., 1984) with rate heterogeneity across sites modeled by a Γ distribution and four discrete rate categories (Yang, 1994). The GTR+ Γ was selected *a priori* for all analyses as it is the most complex and encompassing model implemented in RAxML (Stamatakis, 2014). We performed 1,000 ML tree searches starting each time with a random stepwise-addition maximum parsimony tree. Node support was assessed using 1,000 bootstrap pseudoreplicates (Felsenstein, 1985). To confirm that the number of bootstraps was sufficient to achieve stable support values, we conducted *a posteriori* bootstrap convergence tests using the frequency-based and majority-rule consensus criteria implemented in RAxML (Pattengale et al., 2010).

In order to account for the potential of incomplete lineage sorting, we estimated the species tree using the Bayesian multigene coalescent-based approach in *BEAST v1.8.1 (Heled and Drummond, 2010). This method incorporates incomplete lineage sorting, and the presence and segregation of ancestral polymorphism into the phylogenetic estimation, making it more robust

against misleading results associated with gene trees (Heled and Drummond, 2010). Individuals were assigned to a taxon based on the major clades inferred from the ML inference. Gene regions were analyzed under a GTR+Γ substitution model. We used an uncorrelated lognormal relaxed clock (Drummond et al., 2006) with a lognormal prior distribution (ucld.mean = 1.0; ucld.stdev = 0.33). The species tree was analyzed with both the Yule and the birth-death speciation process as priors and the piecewise linear and constant root population size model. For each analysis, three independent Markov chain Monte Carlo (MCMC) searches were carried out for 50 million generations, with trees and parameters sampled every 1,000 steps and a burn-in of 5 million steps. All results were examined with Tracer v1.6 (Rambaut et al., 2014) to assess whether the chains had reached stationarity, noting if the effective sample size (ESS) values were sufficient (> 200). Additionally, separate runs were analyzed with AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004) to assess chain convergence. The post-burn-in samples from independent runs were combined with LogCombiner v1.8.1, part of the BEAST package, and the topologies were visualized with DensiTree v2.2.1 (Bouckaert, 2010). Separate models were compared based on Akaike's information criterion (AIC) analogue (Baele et al., 2012) and log marginal likelihood (Newton and Raftery, 1994; Suchard et al., 2001), implemented in Tracer v1.6.

2.4. Phylogenetic hypothesis testing

To test our taxonomic hypothesis, we used the expected likelihood weights test (ELW) (Strimmer and Rambaut, 2002) as well as the Shimodaira–Hasegawa test (SH test) (Shimodaira and Hasegawa, 1999), both implemented in RAxML, to examine the best ML tree against constrained tree topologies created in TreeView v1.6.6 (Page, 2001). To test our hypothesis of geographically separated lineages, we performed a cladistics haplotype aggregation analysis

(CHA) by constructing minimum-spanning haplotype networks in TCS v1.21 (Clement et al., 2000). This approach evaluates the connectivity level within the dataset by grouping haplotypes based on the maximum parsimony criterion (Templeton et al., 1992). Groups connected to one another by a single branch can be considered monophyletic (Brower, 1999). We used the default threshold of 95% as the connection limit to assess the distinctiveness of the lineages for the mitochondrial and nuclear datasets respectively.

2.5. Divergence dating

Lineage divergence times were inferred with BEAST v1.8.1 (Drummond et al., 2012) using the *ND1*, *ND2*, and *RAG-1* dataset due to availability of additional outgroup sequences. We incorporated additional sequences available on GenBank from *Heloderma suspectum* (*ND1* and *ND2*—AB167711, *RAG-1*—AY662606), *Shinisaurus crocodilurus* (*ND1* and *ND2*—AB080274.1, *RAG-1*—AY662610.1), and *Lanthanotus borneensis* (*ND1* and *ND2*—AF407541.1, *RAG-1*—AY662609.1), as well as other varanids, including *V. salvator* (*ND2*—JN673353.1, *RAG-1*— JN673366.1), *V. bengalensis* (*ND2*— JN673345.1, *RAG-1*—JN673358.1), *V. griseus* (*ND2*—JN673348.1, *RAG-1*— JN673361.1), and *V. yemenensis* (*ND2*—JN673354.1, *RAG-1*—JN673367.1). The molecular clock model was calibrated with three points that have been used previously for varanids (Portik and Papenfuss, 2012; Vidal et al., 2012). We incorporated dates for the oldest known, unambiguously dated fossil remains attributed to the genus *Varanus* at 37 mya (Holmes et al., 2010; Smith et al., 2008), the fossil date of *Palaeosaniwa canadensis*, the most recent common ancestor of *Shinisaurus* + *Lanthanotus* + *Varanus* at 70.6 mya (Bryant, 1989; Estes, 1983), and results from previous molecular dating estimates of the full anguimorph clade, the root of our phylogenetic tree, that ranged from 105.5 to 157.0 mya (Douglas et al., 2010; Hugall et al., 2007; Vidal and Hedges,

2005; Wiens et al., 2006). To examine the consistency across calibration methods, divergence dates were inferred using all three calibration points, as well as only fossil-derived dates, and only molecular-derived dates (See **Supplementary Tables S3 and S4** for details on priors).

We analyzed the *ND1*, *ND2*, and *RAG-1* dataset using two separate approaches: (1) including all individuals with the constant size, coalescent tree prior (Kingman, 1982) to account for the high degree of intraspecific relationships, and (2) selecting one individual per subclade with the Yule process speciation prior (Gernhard, 2008) due to the majority of relationships considered interspecific in this scenario. Each scenario was examined under multiple partitioning schemes: (i) unpartitioned (one partition), (ii) mitochondrial and nuclear loci (two partitions), and (iii) all loci separately (three partitions). All partitions were analyzed under the GTR+Γ_4 substitution model, and we assumed an uncorrelated relaxed lognormal clock model to account for rate heterogeneity across lineages (ucld.mean = 1.0; ucld.stdev = 0.33; lognormal distribution). For each method, we carried out three independent MCMC searches for 50 million generations, sampling trees and parameters every 1,000 steps, and discarding the first 5 million generations as burn-in. The results from independent runs were combined with LogCombiner v1.8.1, and analyzed with Tracer v1.6 (Rambaut et al., 2014) and AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004) to confirm proper mixing and convergence. Partitioning schemes were compared based on the AIC analogue (Baele et al., 2012) and log marginal likelihood (Newton and Raftery, 1994; Suchard et al., 2001), as implemented in Tracer v1.6. TreeAnnotator v1.8.1 was then used to build a maximum clade credibility tree that was visualized with FigTree v1.4.2 (Rambaut, 2014).

2.6. Population-level analyses

257 Genetic diversity estimates were calculated for the mtDNA dataset ($N = 125$) with Arlequin
258 v3.5.1 (Excoffier and Lischer, 2010) with a 10% limit for missing data, grouped into populations
259 based on results from the phylogenetic analyses. Within-population diversity measures included
260 the number of polymorphic sites (*S*), number of haplotypes (*h*), haplotype diversity (*H)*, mean
261 number of pairwise nucleotide differences (*k*), and mean number of pairwise nucleotide
262 differences per site (π). Between-group mean distances (i.e. the number of pairwise nucleotide
263 differences per site) were calculated in MEGA v6 (Tamura et al., 2013). We also estimated the
264 level of genetic differentiation between groups using the Φ_{ST} fixation index in Arlequin, with
265 statistical significance assessed using 10,100 permutations.
266
267 Neutrality tests to evaluate traces of demographic changes were performed on the mitochondrial
268 dataset for contemporary samples ($N = 69$) grouped into populations. Tajima's *D* (Tajima, 1989),
269 Fu's *F* (Fu, 1997), and R_2 (Ramos-Onsins and Rozas, 2002) statistics were calculated in DnaSP
270 v5.10.1 (Librado and Rozas, 2009) and significance values were based on 10,000 coalescent-
271 based simulations.
272
273 *2.7. Microsatellite analysis*
274 We used MICRO-CHECKER (Van Oosterhout et al., 2004) to test for the presence of mis-
275 scoring and null alleles. To further examine the genetic partitioning of *Varanus niloticus* and *V.*
276 *ornatus* populations throughout their range, we used STRUCTURE v2.3.3 (Pritchard et al.,
277 2000), which groups individuals into populations based on Hardy-Weinberg and linkage
278 equilibrium. We examined up to 15 genetic clusters, one greater than the number of grouped
279 localities, using the admixture model with correlated allele frequencies. For each *K* value, we
280 performed 10 runs of 10 million MCMC iterations each, with an additional burn-in period of one

281 million steps. The appropriate *K* value was inferred by examining the Δ*K* measure (Evanno et al.,
282 2005), as calculated with the web-based program Structure Harvester (Earl and vonHoldt, 2012).
283 Based on the results from Structure Harvester (see Results section), we additionally analyzed the
284 western individuals (1–5 genetic clusters) separately from the rest of the continent (1–12 genetic
285 clusters) with the same parameters as detailed above. Results were averaged across runs using
286 CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007) and visualized with DISTRUCT v1.1
287 (Rosenberg, 2004). The geographic distribution of genetic cluster assignments was plotted on a
288 map using ArcMap v10.1 (ESRI, 2012).
289
290 **3. Results**
291 *3.1. Phylogenetic analyses*
292 Our concatenated mitochondrial and nuclear dataset consisted of 4,251 bp and included 108
293 *Varanus niloticus* and 17 *V. ornatus* individuals (**Table 1**). All partitioning schemes produced
294 concordant tree topologies; however, partitioning by the individual loci yielded the highest
295 likelihood score, and we therefore only present the result of this analysis (**Supplementary Files**
296 **S1–S4; Supplementary Table S5**). The ML tree topology uncovered three major geographically
297 structured groups with high bootstrap support (**Fig. 1; Supplementary File S1**). All individuals
298 identified as *V. ornatus* were recovered within the *V. niloticus* clades and did not form a separate
299 monophyletic group. Individuals from West Africa formed the first-diverging clade, while the
300 remaining individuals (referred to hereafter as the continental clade) were additionally divided
301 into a northern and a southern group. The three major lineages were further partitioned into sub-
302 groups that had high bootstrap support.
303

304 The *BEAST analyses using both the Yule and birth-death species tree priors yielded concordant
305 tree topologies for all gene regions (**Supplementary Files S5–S9**), with a slightly better model
306 fit using the birth-death prior (**Supplementary Table S6**). We therefore present the results of the
307 analysis under the birth-death speciation prior. The tree topologies were generally consistent
308 across gene regions, with a few exceptions (**Fig. 2**). The *RAG-1* gene produced a topology in
309 which the southern clade diverged first and is recovered as sister to the remaining clades.
310 Although all loci recovered three *V. niloticus* clades that were generally segregated into western,
311 northern, and southern regions, *KIAA1549* and the mtDNA loci formed more clearly resolved
312 clades than did *RAG-1* and *KIAA1217*. However, the resulting coalescent-based species tree
313 estimation displayed a tree topology congruent with our ML results.
314
315 *3.2. Phylogenetic hypothesis testing*
316 To assess the taxonomic validity of *Varanus ornatus* based on phylogenetic criteria, we
317 compared our optimal ML tree (partitioned by locus) to a constrained tree with *V. ornatus*
318 individuals comprising a monophyletic group separate from *V. niloticus*. Although this
319 hypothesis could not be rejected based on the SH test (difference in likelihood = 0.54, sd = 0.77),
320 the constrained tree produced a lower posterior probability with the ELW test (0.477) compared
321 to the best ML tree (0.523).
322
323 We constructed a cladistic haplotype aggregation analysis (CHA) to test our hypothesis of
324 geographically separated lineages within *Varanus niloticus* against the null hypothesis of
325 panmixia across their distribution. This test was performed separately for the concatenated
326 mitochondrial (2,094 bp; $N = 125$) and nuclear (2,157 bp; $N = 156$ haplotypes) datasets. The

nuclear dataset, showing less overall variation, produced a single connected haplotype network under the default threshold of 95% parsimony, with western haplotypes forming a discrete cluster (**Fig. 3**). For the mitochondrial dataset, TCS recovered two unconnected networks under the 95% threshold, with the western group forming a separate haplotype network. To produce a single minimum-spanning network, we manually increased the number of mutational steps. Individuals within each regional group formed separate clusters in the network and were connected to other such groups by a single branch, implying monophyly (Brower, 1999). This result supported our hypothesis of genetic subdivision within *V. niloticus*.

3.3. Divergence dating

We estimated divergence dates based on the *ND1*, *ND2*, and *RAG-1* dataset with two approaches – including all ingroup individuals with a coalescent prior and including one individual per subclade with a Yule speciation prior. Both approaches yielded tree topologies congruent with the ML and *BEAST analyses (**Supplementary Fig. S1 and S2**) and divergence estimates between the methods exhibited a linear relationship with a slope near 1.0 (**Supplementary Fig. S3; Supplementary Tables S3 and S4**). Calibrating with only fossil-derived dates or only molecular-derived dates produced more recent divergence estimates than both calibration methods combined (**Supplementary Fig. S3**). The Yule speciation prior with all three calibration points produced divergence dates that were more consistent with previously published studies (Portik and Papenfuss, 2012; Vidal et al., 2012). Additionally, all partitioning schemes resulted in similar divergence times, with partitioning by individual loci showing the best model fit for the Yule speciation prior (**Supplementary Tables S6–S8**). We therefore only discuss the results from this approach; however, the results from all methods are reported (**Supplementary Tables S3, S4, S7, and S8**).

Our analysis indicated that the time to the most recent common ancestor of the western *Varanus niloticus* clade and the continental clade is approximately 7.7 mya (95% HPD: 4.6–11.0 mya), which is followed by the split between the northern and southern groups around 4.7 mya (95% HPD: 2.7–6.9 mya). Additionally, the southern coastal subclade, occupying parts of Cameroon, Bioko Island, and Ivory Coast, diverged from the rest of the southern clade around 3.2 mya (95% HPD: 1.7–5.0 mya). More recent divergence occurred between the western subclades, around 1.7 mya (95% HPD: 0.7–2.8 mya), and the northern subclades around 0.5 mya (95% HPD: 0.1–1.0 mya).

3.4. Population-level analyses

Across calculations, intraclade diversity was highest for the southern clade, followed by the western clade, then the northern clade (**Table 2**). Intraclade sequence divergence values (highest = 1.7% for the southern clade) did not overlap with the interclade divergence values (> 4.2%). Additionally, the genetic differentiation measures between clades, as assessed with Φ_{ST} fixation indices, were statistically significant for all pairwise comparisons (**Table 3**). Divergence values for comparisons involving the western clade were consistently higher than distances between the northern and southern clades. However, the tests for demographic changes produced non-significant results for all populations (**Supplementary Table S9**), suggesting long-term stability in population sizes.

3.5. Microsatellite analysis

For the historical museum samples, five microsatellite loci amplified consistently, which included *K7*, *K11*, *VARSA10*, *VA38*, and *VA74*. These loci exhibited sufficient variation to

successfully assign 87.9% ($\alpha = 0.05$) of contemporary individuals to the correct genetic cluster. The error rate for museum samples, calculated as the proportion of individuals showing allele discrepancies across separate amplifications, was determined to be 0.119 across loci. In all cases of discrepancies, the third amplification confirmed a previous allele call. Although our MICRO-CHECKER results indicated an excess of homozygosity for a few loci in select populations, it did not detect allelic dropout of large alleles, a concern when amplifying degraded DNA (Allentoft et al., 2011; Navidi et al., 1992; Taberlet et al., 1996; Tvedebrink et al., 2009).

The clustering analysis, performed with 11 loci for modern samples ($N = 67$) and five loci for museum samples ($N = 42$), produced a hierarchical pattern of structuring across the distribution of *Varanus niloticus*. The optimal K value for the full dataset, inferred from the highest ΔK (Evanno et al., 2005) occurred at $K = 2$ (**Supplementary Fig. S4**), grouping the western individuals into a separate genetic cluster. However, analyzing the western and continental groups separately resulted in additional partitioning for the continental group ($K = 2$) and a single genetic cluster for the western group (**Supplementary Fig. S4**).

The genetic cluster assignments were closely aligned with the phylogenetic clades, with the exception of localities near the contact zone between the northern and southern groups (**Fig. 4**). In the more coastal areas of Cameroon and Tanzania, we found evidence of extensive admixture. The Uele population in Democratic Republic of the Congo, while a member of the northern phylogenetic clade, displayed alleles consistent with the southern clade.

4. Discussion

Rather than basing taxonomic decisions on morphology alone, incorporating genetic approaches allowed us to uncover patterns of biodiversity that were not immediately recognizable. Utilizing multiple independent genetic loci to reconstruct species trees guards against the misleading results that can be associated with single-gene assessments (Zhang and Hewitt, 2003). Our analysis of 4,251 bp of both mitochondrial and nuclear sequences, combined with our microsatellite data, did not support a species-level classification for *Varanus ornatus,* but rather revealed cryptic evolutionary lineages within *V. niloticus*. These findings not only clarify the taxonomy of the species group, but shed light on the biogeographic history of Africa as well as aid in the management of these varanids and other African species.

4.1. Ornate monitor taxonomic status

Although *Varanus ornatus* has been considered a distinct entity since 1803 (Daudin, 1803), our results show that the *V. ornatus* individuals included in this study are genetically indistinguishable from *V. niloticus*. Both museum-vouchered and contemporary specimens identified as *V. ornatus* were interspersed throughout the *V. niloticus* clades, recovered in both the southern and western lineages. The type locality of *V. ornatus* is Malimbe, Cameroon (Böhme and Ziegler, 2004), and the eight *V. ornatus* collected from Cameroon were recovered in both subclades of the southern lineage. The phylogenetic position of the *V. ornatus* individuals within the tree is consistent with the geographic localities, not the species identification, and the constrained tree which placed *V. ornatus* in a separate monophyletic group produced a lower posterior probability than our best ML estimation. Eleven of the 17 *V. ornatus* individuals were re-examined and all displayed the coloration patterns described by Böhme and Ziegler (1997).

Although Böhme and Ziegler (1997) reported morphological differences between these two forms, many of the characters they presented had overlapping ranges. These characters included the number of light ocelli cross-bands (6–11 versus 4–6 on the dorsal surface and 10–18 versus 9–12 on the tail), scale counts (128–183 versus 146–175 scales around the midbody), and the number of paryphasmata on the outer genital organs (5–10 versus 8–12) in *Varanus niloticus* and *V. ornatus* respectively (Böhme and Ziegler, 1997). Other varying traits included the tongue coloration, described as pigmented (blueish-black) in *V. niloticus* and light (whitish-pink) in *V. ornatus* (Böhme and Ziegler, 1997). Differences in head proportions were also speculated, but small samples sizes prevented an in-depth analysis (Böhme and Ziegler, 1997).

Although the Ornate form is found in higher frequency in the forested regions of western and central Africa, Böhme and Ziegler (1997) report occurrences of this form outside of the typical distribution (Lake Chad and "Kilwa E. Africa"), although they attributed these occurrences to incorrect locality information. Rare occurrences of deviating tongue color and cross-band patterns were also noted in five individuals (Böhme and Ziegler, 1997). Phenotypic morphs have been documented for many herpetofauna, with the retention of the morphological varieties attributed to multiple evolutionary mechanisms (Huyghe et al., 2007; King and Lawson, 1995; Martin, 2002; Meyers et al., 2006; Sinervo and Lively, 1996). Thus, the coloration patterns attributed to *Varanus ornatus* likely represent a polymorphism within the species that might be selectively favored in the dense forested regions, producing higher frequencies. The ecological context of this coloration polymorphism is poorly understood, and additional study is required to address this hypothesis.

444 *4.2. Nile monitor diversification*

445 Our continent-wide genetic analysis uncovered cryptic evolutionary lineages within *Varanus*
446 *niloticus*. Evidence from our phylogenetic analyses (ML and BI), cladistic haplotype analysis
447 (CHA), genetic distance calculations, and Bayesian clustering methods all support deep
448 evolutionary divergence between the western clade and the rest of the continent. Comparing
449 levels of sequence divergence exhibited by the *V. niloticus* clades to levels among currently
450 recognized varanid species offers a basis for designating the western lineage as a distinct species.
451 The western group, which was consistently recovered as sister to the continental clade, exhibited
452 levels of sequence divergence above that typically observed between sister varanid species,
453 showing 8.4% and 8.7% uncorrected sequence divergence between the northern and southern
454 groups, respectively, based on the mitochondrial dataset. Welton et al. (2014) found that, within
455 the *V. salvator* Complex, taxa currently recognized as separate species differed from each other
456 by 1.0% to 3.5% uncorrected sequence divergence based on *ND1* and *ND2* sequence data. Portik
457 and Papenfuss (2012) demonstrated *ND2* sequence divergence between the sister taxa *V.*
458 *albigularis* and *V. yemenensis* is 9.6%, with levels of divergence across other African species
459 ranging from 9% to 23.9%. Other studies have found divergence levels ranging from 3.4% to
460 27.7% (applying the Jukes and Cantor correction) between sister varanid species in Australia
461 based on *ND4* (Fitch et al., 2006).
462
463 The continental clade showed additional partitioning into northern and southern lineages, evident
464 in the phylogenetic analyses and by the interclade distance of 4.2% based on the mitochondrial
465 dataset. Although this level of sequence divergence is within the range observed between sister
466 varanid species, results from other analyses suggest the groups are not as well resolved as the

western lineage, making their taxonomic status questionable. Both the mitochondrial and nuclear CHA tests recovered the northern and southern groups within a single connected network under the 95% parsimony threshold. Although the mitochondrial network connected the northern and southern clusters by a single branch indicating monophyly (Brower, 1999), the nuclear network showed lower resolution with intermixed haplotypes from northern and southern localities. Our microsatellite analysis also revealed a considerable degree of admixture near the contact zone of the northern and southern groups. These results suggest gene flow or the retention of ancestral polymorphisms. Future studies assessing the degree of contemporary gene flow among the northern and southern lineages could help clarify the taxonomic status of these populations.

Although our analysis did not include a morphological component, the original descriptions of Nile monitors parallel the phylogeographic patterns uncovered in our study. Daudin (1802) described a Senegal form, *Tupinambis stellatus*, as a separate species from an Egypt form, *T. niloticus*. Although acknowledging their similarities, Daudin reported the Senegal form as having a long tail with a double-keel, similar to a longitudinal fold, while the Egypt form lacked this structure. Additionally, he noted *T. stellatus* as having black pigmentation on the dorsal side with a transverse pattern of white ocellated markings. In contrast, *T. niloticus* was described as having a dirty gray or light yellowish appearance with irregularly positioned white ocellated markings. Similarly, Schlegel (1844) described three forms of the Nile monitor: one inhabiting the Nile River (*Monitor elegans niloticus*), one found in Senegal (*M. elegans senegalensis*), and one found near the Cape of Good Hope in South Africa (*M. elegans capensis*). Schlegel (1844) also noted differences in the base color of the three forms, displaying olive-tinged brown, dark brown, and black, respectively. As mentioned previously, Mertens (1942) discounted these

differences as ontogenetic variation; however, the partitioning described by Daudin (1802) and Schlegel (1844) reflect the geographic partitioning revealed by our analysis. Future studies should be aimed at clarifying these morphological differences to facilitate field identification of the genetic lineages.

4.3. Biogeographic history

The divergence times we recovered are congruent with those previously published for other taxa (Portik and Papenfuss, 2012; Vidal et al., 2012) and mirror the climatic events occurring across the African continent. Our divergence estimate for the most recent common ancestor of the western and continental *Varanus niloticus* lineages suggests that diversification took place in the Miocene. This time period marked the onset of drastic climatic events across the continent, transitioning from a warm, humid climate with vast expanses of rainforest cover to a climate similar to that of present-day Africa (Axelrod and Raven, 1978; Coetzee, 1993; Plana, 2004). By the Late Miocene, global cooling and aridification had reduced forested regions to small, isolated patches (Maley, 1996; Plana, 2004). Requiring permanent bodies of water, Nile monitor populations were likely isolated by the drought-like conditions occurring during this period.

Our results also indicate that the northern and southern clades shared a common ancestor during the Early Pliocene. In contrast to the events of the Miocene, this time period was characterized by increasing humidity with the accompanying expansion and reconnection of the rainforest (Coetzee, 1993; Plana, 2004). Congruent north-south genetic breaks have been observed in numerous savannah-dwelling species and are largely attributed to the dense rainforest belt that extended across the continent during humid periods, dividing the savannah to the north and south

513 (Lorenzen et al., 2012). Further divergence within the southern clade continued into the Pliocene,
514 producing a separate lineage in coastal Cameroon and possibly extending into West Africa (one
515 occurrence in Ivory Coast). This pattern, which is consistent with those found in dwarf
516 crocodiles (Smolensky et al., 2015) and chimpanzees (Mitchell et al., 2015), reflects major
517 watershed boundaries separating the Congo Basin from the more coastal drainage basins to the
518 west as well as the convergence of the Gulf of Guinea rainforest and the Congolian rainforest
519 biomes.
520
521 Within-lineage partitioning provides a more detailed understanding of the genetic connectivity
522 and dispersal patterns of *Varanus niloticus*. The western lineage is subdivided into sister groups,
523 dating to 1.7 mya (95% HPD: 0.7–2.8 mya), which were likely shaped by historic and present-
524 day drainage patterns and possibly forest refugia. One subclade consists of localities throughout
525 the Senegal basin and extending into the Niger basin, reflecting the ancient connection between
526 these two systems that lasted until the end of the Pleistocene (Drake et al., 2011). The other
527 subclade within the western lineage is comprised of coastal localities from Liberia to Cameroon,
528 occupying river drainages emptying into the Gulf of Guinea. The isolation between these coastal
529 watersheds and those further inland was likely magnified by the arid periods that punctuated the
530 Pleistocene (deMenocal, 2004). Similarly, the genetic partitioning within the northern clade
531 mirrors the hydrological patterns of the region. Localities in the Nile River drainage basin were
532 recovered in a separate subclade from those in the Niger and Lake Chad basins, with divergence
533 estimates around 0.5 mya (95% HPD: 0.1–1.0 mya). The placement of individuals from the Uele
534 region of northern Democratic Republic of the Congo in the northern clade is indicative of the

535 past and possibly continuing intermittent connections between the Nile and Congo basins,
536 reflected in the genealogical patterns of other fauna as well (Gidis et al., 2011).
537
538 The continent-wide genetic patterns uncovered by our analyses represent a small piece of
539 Africa's biogeographic puzzle. Congruent patterns across diverse taxonomic groups aid in
540 resolving the larger picture surrounding the processes shaping biodiversity. Genetic breaks
541 separating populations in West Africa have been observed in a diverse array of taxa (Alpers et
542 al., 2004; Arctander et al., 1999; Brouat et al., 2009; Bryja et al., 2010; Dobigny et al., 2013;
543 Eaton et al., 2009; Flagstad et al., 2001; Smolensky et al., 2015), emphasizing the role of West
544 Africa as a center for biodiversity and endemism. Additionally, divergence dating analyses are
545 increasingly finding the Miocene to be a crucial time period in shaping patterns of African
546 biodiversity (Diedericks and Daniels, 2014; Furman et al., 2015; Hekkala et al., 2011; Oaks,
547 2011).
548
549 **5. Conclusions**
550 Although a majority of monitors are relatively conspicuous, reaching large sizes and occupying a
551 top position in the food chain, many of these species still lack thorough scientific inquiries. All
552 varanids are managed under CITES, yet limited information exits on the conservation status of
553 most *Varanus* species, despite their high rate of exploitation (Mendyk et al., 2014). *Varanus*
554 *niloticus* along with a large proportion of varanids, have yet to be assessed for their conservation
555 status by the International Union for Conservation of Nature (IUCN; http://www.iucnredlist.org).
556 Genetic analyses are, therefore, essential tools for informing management decisions and setting
557 conservation priorities. In addition to delineating the genetic partitioning within *Varanus*

niloticus, this study provides a vouchered database of genetic variation across their range. This information can be used to monitor the geographic origins of *V. niloticus* shipments in the skin and pet trade, and to genetically identify populations under heavy harvest pressures.

The combined evidence from our range-wide multilocus assessment of the *Varanus niloticus* Species Group along with the original morphological descriptions, calls for urgent taxonomic revisions in this group. Our results indicate that *V. ornatus* likely represents a phenotypic morph of *V. niloticus*, and therefore, should not be recognized as a distinct species. Here, we recognize *V. ornatus* as a synonym of *V. niloticus*. Additionally, the cryptic western lineage within *V. niloticus* exhibits genetic differentiation levels above those typically found between sister varanid species. In a fine-scale genetic analysis, Dowell et al. (2015) similarly found a sharp genetic break between *V. niloticus* populations in western Mali and those farther east, with very little evidence of admixture despite no obvious current geographic barrier. The same study also found reduced genetic diversity levels within the western lineage compared to neighboring *V. niloticus* populations (Dowell et al., 2015). Due to the intense harvest rate of *V. niloticus* throughout West Africa and the Sahel, and the relative rarity of this lineage with respect to range size, consideration for a separate species classification under the name *V. stellatus* Daudin (1802), the oldest available synonym for that population, would greatly enhance conservation efforts. We therefore tentatively recognize the western lineage as *Varanus stellatus*, and propose a thorough morphological investigation using these proposed species boundaries to further test this classification.

This study also emphasizes the utility of historical collections, acting as repositories of genetic diversity information (Paknia et al., 2015; Turney et al., 2015). Museums provide a window into the past and allow a direct comparison of historic and contemporary genetic diversity levels (Ramakrishnan and Hadly, 2009). Archival specimens often represent localities where species are no longer found, as in the case of *Crocodylus suchus* (Hekkala et al., 2011). Additionally, natural history collections house specimens from presently inaccessible localities, either due to remoteness or political instability. By incorporating historical museum specimens into our analysis, we were able to provide a more complete picture of the biodiversity patterns across the African continent.

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932

933 **Figure Legends**

934

935 **Fig. 1** Maximum likelihood tree of the *Varanus (Polydaedalus) niloticus* Species Group. The
936 scale bar denotes 0.03 substitutions/site. Values at major nodes indicate bootstrap support (%).
937 Tips marked by a star represent specimens originally identified as *V. ornatus*. Colored bars
938 denote the major clades (West, North, and South) and subclades (W1, W2, N1, N2, S1, and S2).
939 Maps showing the locality of individuals belonging to each of the major clades and subclades are
940 also depicted, showing *V. ornatus* localities as squares and *V. niloticus* localities as circles.

941

Fig. 2 Results from the multilocus coalescent-based analysis with *BEAST. This shows the phylogenetic tree topology for unlinked gene regions and the combined species tree. The colored bars identify individuals from each of the major clades: green = western, blue = northern, and orange = southern. Trees from the MCMC sampling procedure are depicted, resampled at a frequency of 100,000, and a burn-in of 10%. Higher levels of confidence are represented by higher tree density and the consensus tree is delineated by the dark blue line.

Fig. 3 Minimum-spanning networks created using the mitochondrial (mtDNA) and nuclear (nucDNA) datasets. Circle sizes are proportional to the number of individuals and the colors are consistent with the subclade designation shown in **Fig. 1**. Mutational steps >5 are denoted and the dashed line indicates disjoint subnetworks (< 95% parsimony threshold). Black circles indicate hypothetical haplotypes.

Fig. 4 Geographical distribution of genetic clusters. The bold horizontal bars delineate the three major clades inferred from the phylogenetic analyses. The map shows the locality and the proportion of genetic membership to each of the clusters (continental: $K = 2$, western: $K = 1$) for the individuals used in the analysis ($N = 109$).

Table 1 Contemporary samples and museum specimens of *Varanus niloticus* and *V. ornatus* utilized in this study. The specimen ID refers to the field number (contemporary) or museum accession number (archival specimens). Museum acronyms: AMNH–American Museum of Natural History (New York, NY, USA), CAS–California Academy of Sciences (San Francisco, CA, USA), FLMNH–Florida Museum of Natural History (Gainesville, FL, USA), MCZ–Museum of Comparative Zoology, Harvard (Cambridge, MA, USA), MNHN-RA– Muséum national d'Histoire naturelle (Paris, France), MVZ–Museum of Vertebrate Zoology (Berkeley, CA, USA), NHM–Natural History Museum (London, UK), PEM–Port Elizabeth Museum, Bayworld (Port Elizabeth, South Africa), UMMZ–University of Michigan Museum of Zoology (Ann Arbor, MI, USA), USNM–National Museum of Natural History, Smithsonian Institution (Washington, DC, USA) , UTEP–University of Texas at El Paso (El Paso, TX, USA), UWBM–Burke Museum of Natural History and Culture (Seattle, WA, USA), SANBI–South Africa National Biodiversity Institute (Silverton, South Africa).

Country	Locality	Collector	Museum	Specimen ID	Year
Species Identification: *Varanus niloticus*					
Cameroon	Gamba	I. Ineich	N/A	Ineich80	2001
Nigeria	Lagos	?	NHM	1893.1.11.10–11	1893
Nigeria	Jebba	?	NHM	1899.8.23.8–9	1899
Benin	Country Only	?	MNHN-RA	1894.0259–0260	1894
Togo	Country Only	J. Beraducci	FLMNH	56590, 60998, 61009, 61037,61468, 63940*	1984
Ghana	Volta Region	A. Leache, R. Diaz, M. Fujita	MVZ	245359	2004
Ghana	Ahato Region	A.Leaché & D. Portik	UWBM	6058	2011
Ghana	Brong-Ahato Region	A.Leaché & D. Portik	UWBM	6095, 6060	2011
Burkina Faso	Arly	?	USNM	223945**	1968
Liberia	Country Only	J. Beraducci	FLMNH	61065	1984
Sierra Leone	Country Only	W. Branch	PEM	R19410	2012
Sierra Leone	Newton Colony	T.S. Jones	MCZ	53577	1953
Sierra Leone	Mussia	T.S Jones	MCZ	53578	1953
Guinea	Killissi	I. Ineich	N/A	Ineich550	2004
Guinea	Djakka	I. Ineich	N/A	Ineich554	2004
Guinea	Siguiri	I. Ineich	N/A	Ineich565	2004
Senegal	Country Only	?	NHM	1920.1.20.66	1920
Mali	Flabougou	V. de Buffrénil	N/A	FLA22–25	2008
Mali	Niono	V. de Buffrénil	N/A	NIONO4–7	2008
Mali	Mopti	V. de Buffrénil	N/A	MOP96_20, 66, 78, 79	1996

Niger	Niamey	V. de Buffrénil	N/A	NIA34–35	2009
Niger	Niamey	T.J. Papenfuss	MVZ	238936	2002
Niger	Diffa	V. de Buffrénil	N/A	DIF47, 48, 51, 53	2009
Chad	Dassoulom	I. Ineich	N/A	Ineich603	2004
Chad	Am N'Guitey	V. de Buffrénil	N/A	AMNG6–7	2002
Chad	Abba Liman	V. de Buffrénil	N/A	ABLIM1–2	2002
Chad	Lake Chad	V. de Buffrénil	N/A	LCDJA151-152	1997
Chad	Lake Fitri	V. de Buffrénil	N/A	LFKOUR239, 243	1997
Chad	Lake Lere	V. de Buffrénil	N/A	LLKD260–261	1997
Egypt	Luxor	J. Anderson	NHM	1897.10.28.224	1897
Egypt	Luxor	?	NHM	1906.7.25.3	1902
Sudan	White Nile	d'Arnaud	NHM	6526	?
Ethiopia	Awharra, Mullka	?	NHM	1902.12.13.38	1902
Ethiopia	Barro River (Sobat)	?	NHM	1905.10.16.5	1905
Ethiopia	Ogaden Region	?	AMNH	19445***	1920
Unknown	Unknown	Lang-Chapin	AMNH	74604	?
DRC	Uele	Lang-Chapin	AMNH	10512, 10517, 10519	1911–1913
DRC	Ituri	Lang-Chapin	AMNH	10500–10502	1910
DRC	Stanleyville	Lang-Chapin	AMNH	10085	1912
DRC	Tandala	?	USNM	216247	1979
DRC	Kyolo, Katanga	E. Greenbaum	UTEP	21180	2010
Uganda	Rhino Camp	Smithsonian Exp.	USNM	42260	1910
Kenya	Fort Hall	?	NHM	1906.8.25.1	1906
Tanzania	Tanganyika	C. Christy	NHM	1927.5.27.10–11	1927
Tanzania	Shinyanga	Chrysler Expedition	USNM	72028	1926
Tanzania	Siga Caves	A. Loveridge	MCZ	47377	1939
Tanzania	Magrotto Mtn.	A. Loveridge	MCZ	47378	1939
Tanzania	Country Only	Mission du St Esprit	MNHN-RA	5610	?
Tanzania	Morogoro	?	UMMZ	61412	1921
Burundi	Rumonge	E. Greenbaum	UTEP	21181	
Angola	Cunga	?	NHM	1904.5.2.27	1904
Malawi	Fort Johnston	C. Christy	NHM	1926.5.8.14	1926
Mozambique	Tete	R. Fergusson	N/A	MZ01–07	2014
Mozambique	Nhica do Rovuma	I. Ineich	N/A	Ineich371	

Mozambique	Beira	C. Grant	NHM	1907.4.29.32	1907
South Africa	North of Humansdorp	M. Burger	SANBI	410	2003
South Africa	Country Only	E. Stanley	SANBI	3483	2008
South Africa	Wilgepark	M. Cunningham	SANBI	3697	2008
South Africa	Edendale	M. Burger	SANBI	5308	2007
South Africa	Phalaborwa	M. Burger	SANBI	5456	2007
South Africa	Limpopo	D. Portik	MVZ	267341	2011
South Africa	Cookhouse	W. Conradie	PEM	WC10-180	2011
South Africa	Brown's Vale	W. Conradie	PEM	WC10-194	2011
South Africa	Nelspruit	L. La Grange	N/A	SA01–03	2012
South Africa	Ngoye Hills, Zuzuland	Grant	NHM	1905.3.7.102–103	1905
South Africa	East London	?	MNHN-RA	1896.0374–0375	1896
Species Identification: *Varanus ornatus*					
Nigeria	Igbo-Ora	?	USNM	194862	1967
Ivory Coast	Elima	Chaper	MNHN-RA	6452	1882
Eq. Guinea	Bioko	L.G. Henwood and J.V. Vindum	CAS	207622	1998
Cameroon	Metet	?	AMNH	36473	1923
Cameroon	Yaounde	I. Ineich	N/A	Ineich81, 765	2001
Cameroon	West of Edea	D. Portik	Pending	519	2011
Cameroon	Doume	Marche	MNHN-RA	5281, 2007.2424	1878
Gabon	Country Only	A. Leconte	MNHN-RA	8265, 2007.2425	?
DRC	Kaputo, South Kivu	E. Greenbaum	UTEP	21182–21183	2012
DRC	Lake Tumba	E. Greenbaum	UTEP	21184	2013
DRC	North of Kutu	E. Greenbaum	UTEP	21185	2013
DRC	Lac Mai-Ndomb	E. Greenbaum	UTEP	21186	2013
DRC	Equateur: Efofa	E. Greenbaum	UTEP	21187	2013

*Locality either Togo or Liberia

**Originally mis-identified as *Varanus exanthematicus*

***Identified as *Varanus ornatus*, but not within currently recognized species distribution

Table 2 Genetic diversity estimates for the three *Varanus niloticus* clades

Group	*N*	*S*	*h*	*H* (SD)	*k* (SD)	π (SD)
West	34	77	26	0.984 (0.011)	21.695 (9.806)	0.010 (0.005)
North	32	38	22	0.964 (0.019)	7.111 (3.426)	0.004 (0.002)
South	59	196	52	0.995 (0.004)	21.591 (9.656)	0.017 (0.008)

N, number of individuals; *S*, number of polymorphic sites; *h*, number of haplotypes; *H*, haplotype diversity; *k*, mean uncorrected number of pairwise nucleotide differences; π, mean uncorrected number of pairwise nucleotide differences per site; SD, standard deviation.

Table 3 Pairwise genetic distances between the three *Varanus niloticus* clades. Above the diagonal are the number of pairwise nucleotide differences per site (uncorrected) and below the diagonal are the Φ_{ST} fixation indices. All Φ_{ST} values are statistically significant ($P < 0.001$).

	West	**North**	**South**
West		0.084	0.087
North	0.926		0.042
South	0.840	0.709	

Figure 1

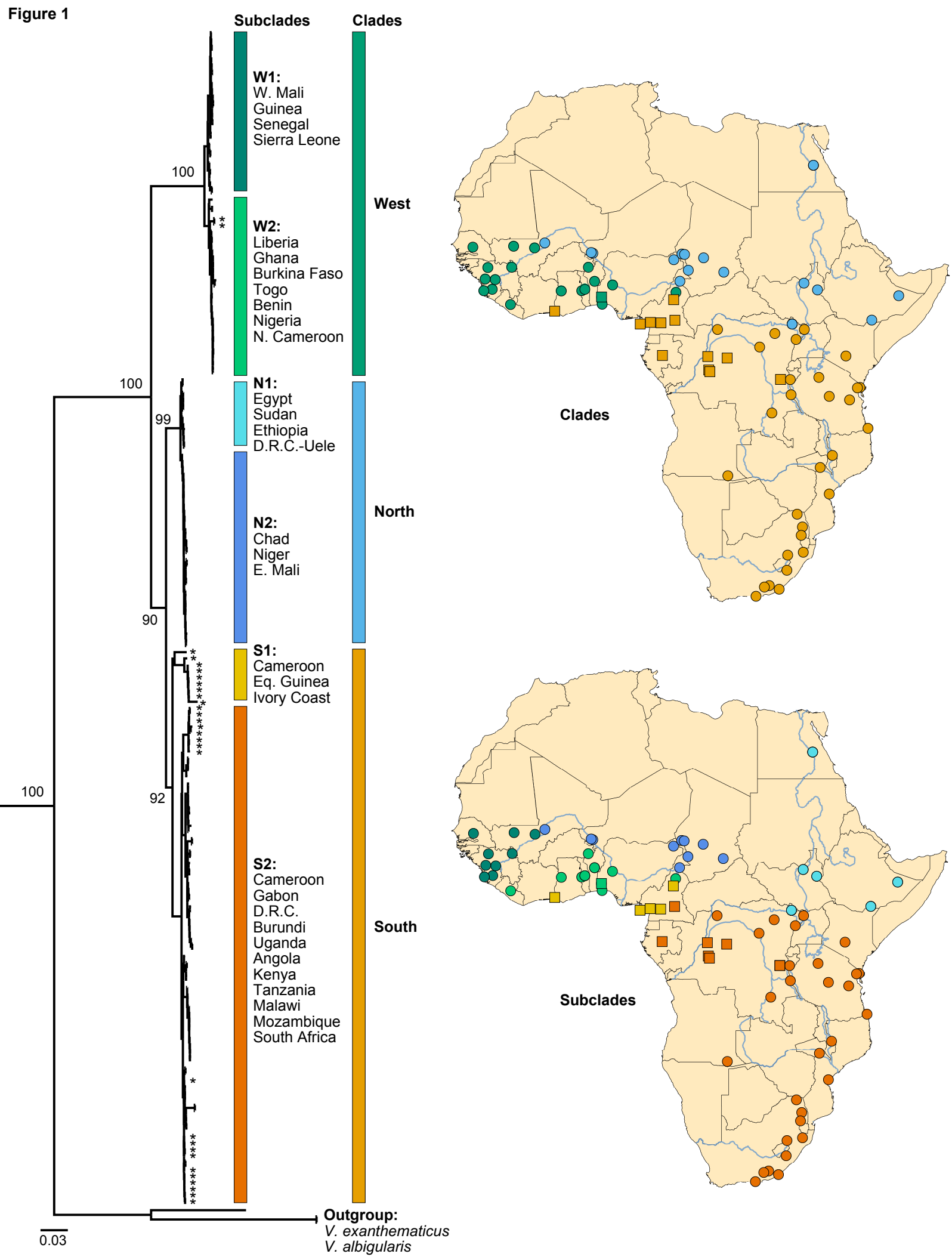

Subclades
Clades
W1:
W. Mali
Guinea
Senegal
Sierra Leone
W2:
Liberia
Ghana
Burkina Faso
Togo
Benin
Nigeria
N. Cameroon
West
N1:
Egypt
Sudan
Ethiopia
D.R.C.-Uele
N2:
Chad
Niger
E. Mali
North
S1:
Cameroon
Eq. Guinea
Ivory Coast
S2:
Cameroon
Gabon
D.R.C.
Burundi
Uganda
Angola
Kenya
Tanzania
Malawi
Mozambique
South Africa
South
100
100
99
90
100
92
Outgroup:
V. exanthematicus
V. albigularis
0.03
Clades
Subclades

Figure 2

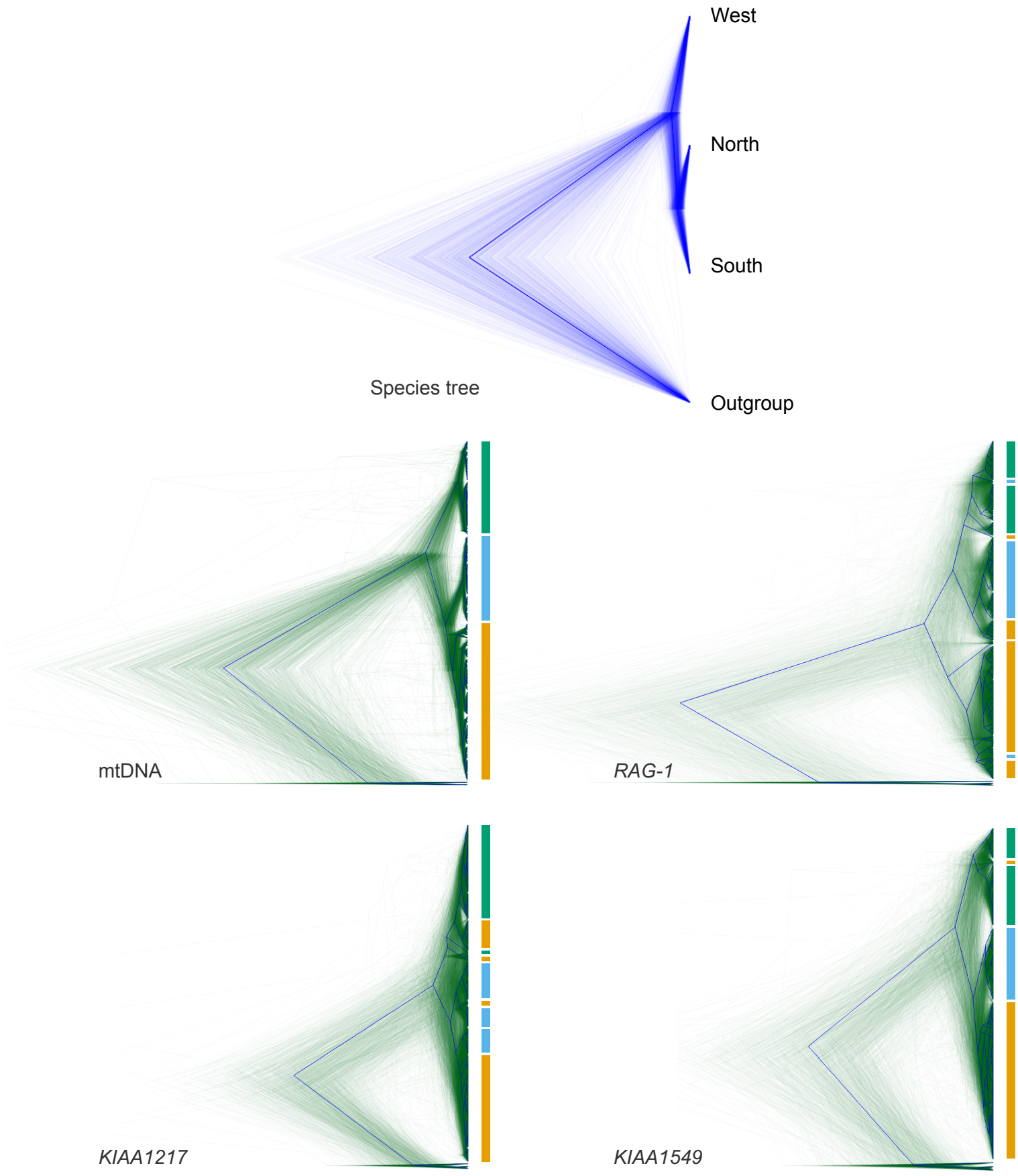

West
North
South
Outgroup
Species tree
mtDNA
RAG-1
KIAA1217
KIAA1549

Figure 3

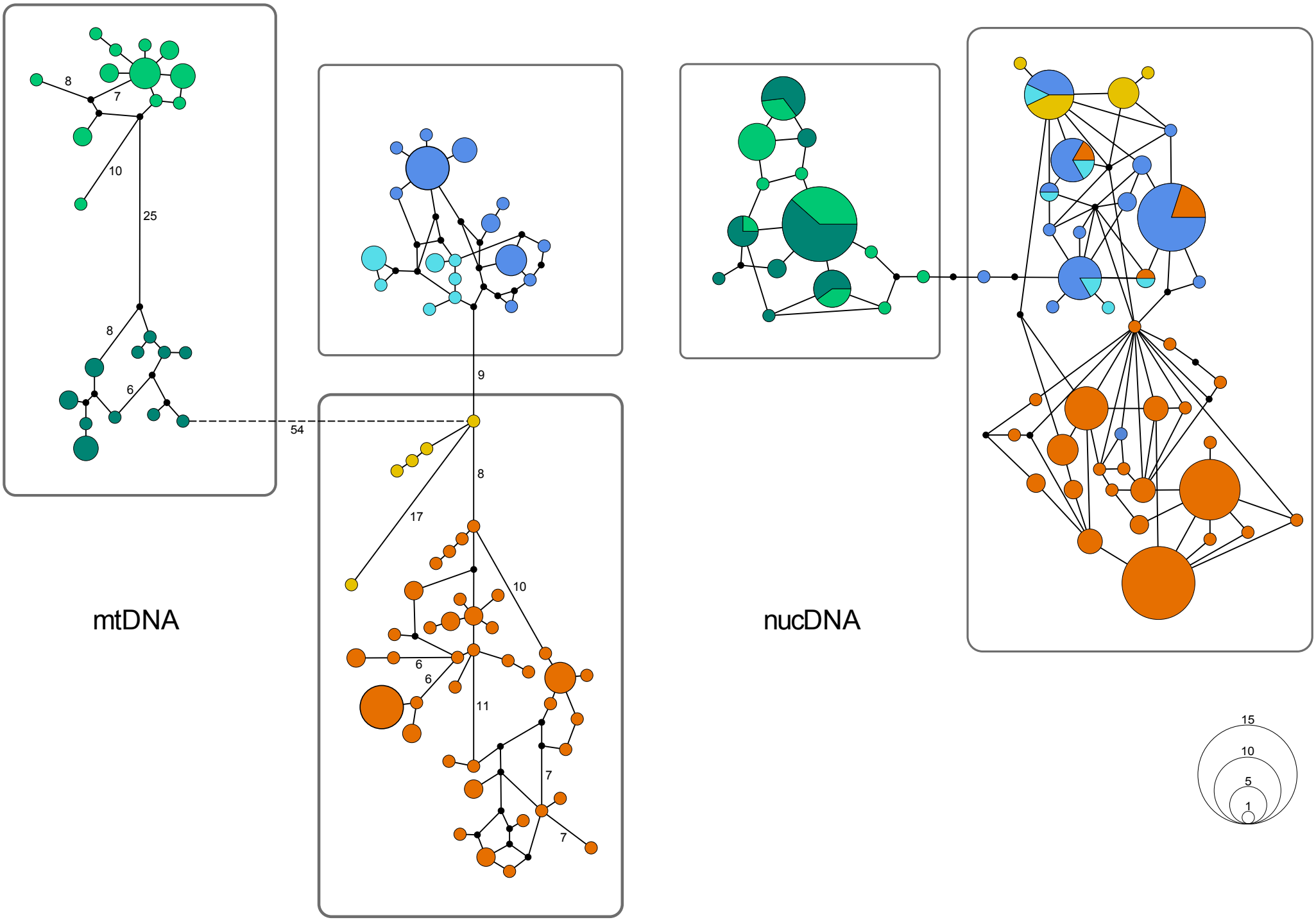
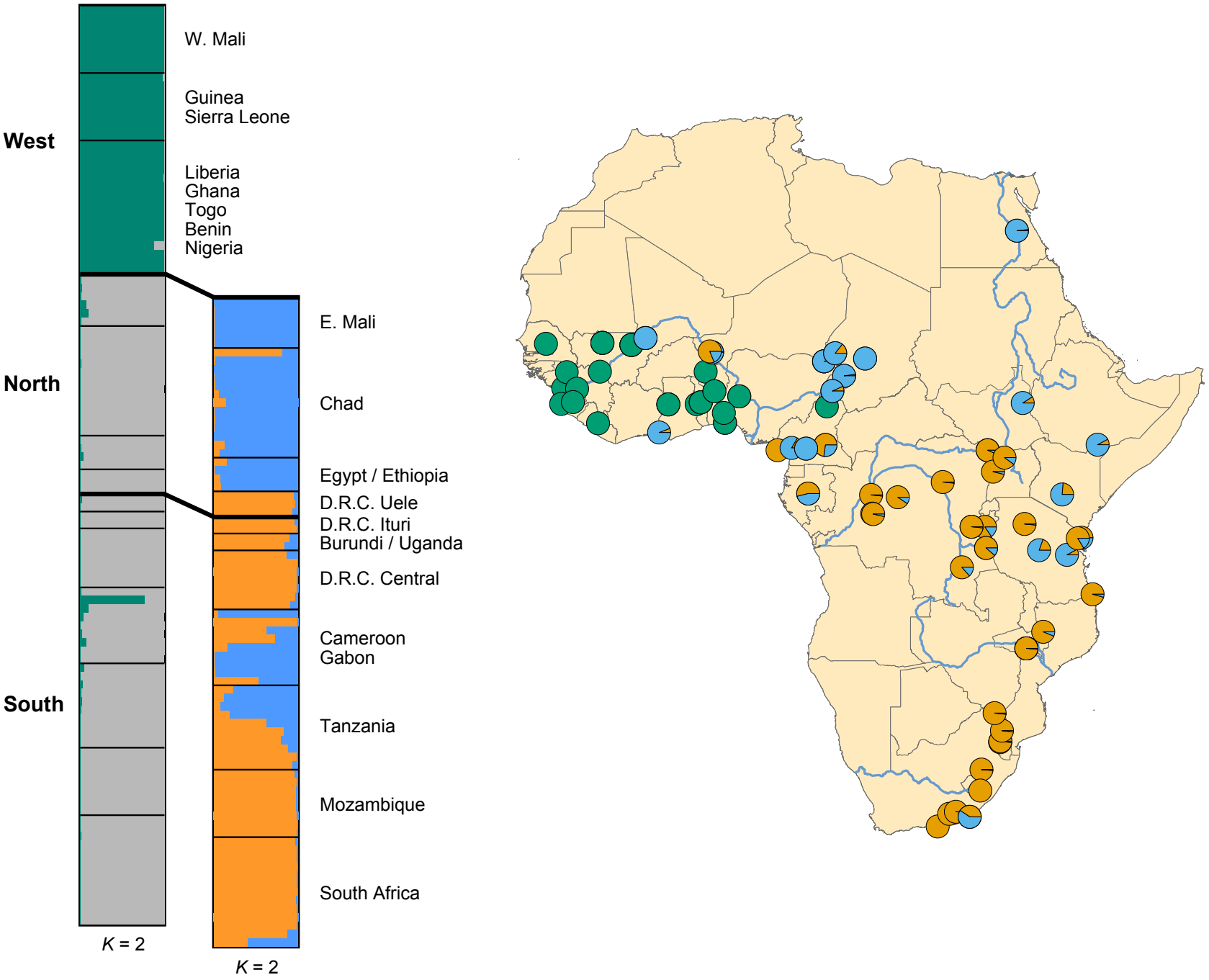
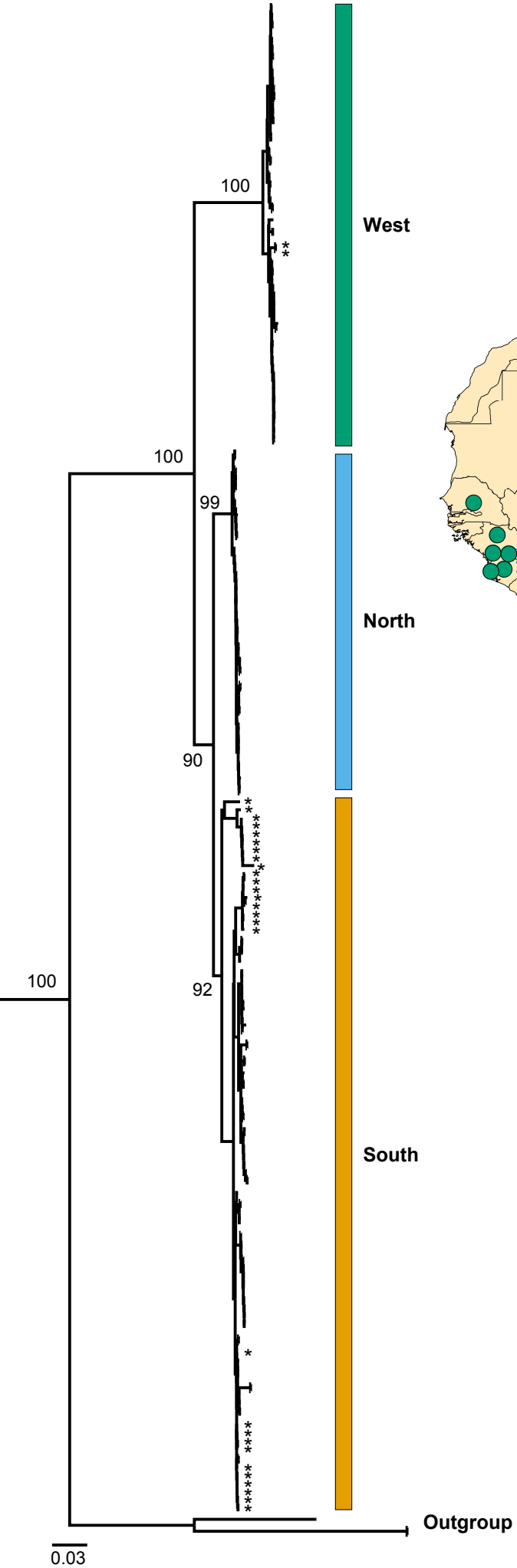
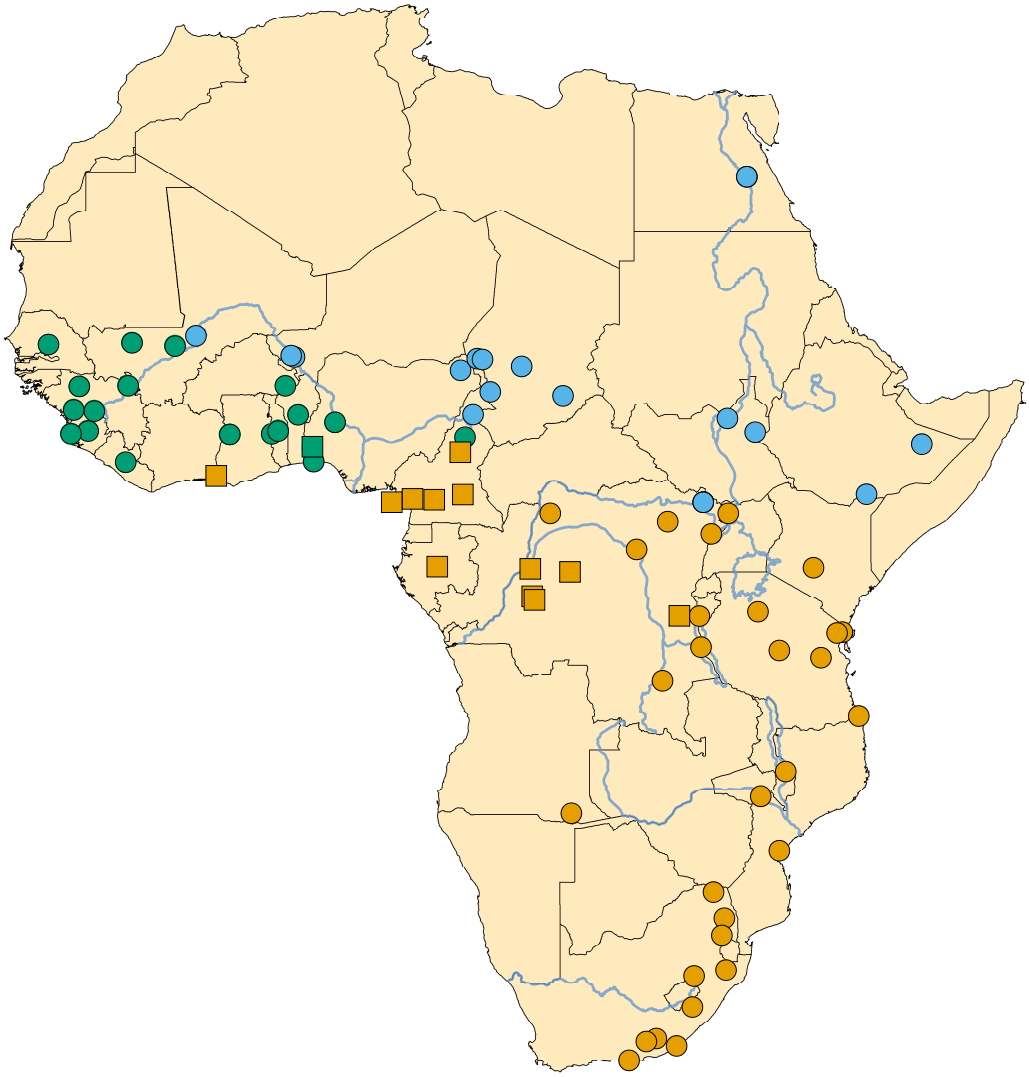


Figure 4


West
North
South
W. Mali
Guinea
Sierra Leone
Liberia
Ghana
Togo
Benin
Nigeria
E. Mali
Chad
Egypt / Ethiopia
D.R.C. Uele
D.R.C. Ituri
Burundi / Uganda
D.R.C. Central
Cameroon
Gabon
Tanzania
Mozambique
South Africa
K = 2
K = 2

*Graphical Abstract





Specimen ID:

- ● *Varanus niloticus*
- ■ *Varanus ornatus* (*)