A phylogeny of the genus *Chamaeleo* with investigation of cryptic speciation

By

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“Real knowledge is to know the extent of one’s ignorance.”- Confucius
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Declaration:

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Acknowledgments:

This dissertation represents the product of many hours of research, and indeed frustration, trying to disentangle the evolutionary intricacies of this unique clade of reptiles. I consider it a great privilege to have been given the opportunity to work on such fascinating and enigmatic creatures, and even more so to have been afforded the chance to work with such experts in the field.

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Chapter 1

General Introduction:

The stochastic nature of the evolution of sexually reproducing organisms has long presented a challenge to the categorization and subsequent documentation of the planet’s biodiversity (De Queiroz 2007; Avise and Robinson 2008; Carstens et al. 2013). Consequently, a plethora of approaches to this conundrum have been proposed, many of which are borne on the notion that ‘species’ form axiomatic entities of life (De Queiroz 2005). This dogma originates from the work of the Swedish botanist Carolus Linnaeus who envisaged a classificatory system of binomial nomenclature which posited that all organisms could be classified into distinct ‘species’ which form basal units within a greater taxonomic hierarchy (Linnaeus 1735). Linnaeus’ taxonomic framework was based for the most part on morphological similarities between conspecifics. Common understanding at the time was that ‘species’ were unchangeable entities of life, this simplified their categorization. Darwin (1859), in his pioneering “On the origin of the species by means of natural selection”, was one of the first people to challenge the idea that species were unchangeable. He hinted at all species having a transient nature on an evolutionary timescale and originating from a single common ancestor (Brooks and McLennan 1999).

In suggesting an evolutionary ancestry for species, Darwin essentially sparked the beginning of a new biological field, that of evolutionary biology (De Queiroz 2005). Ironically, the field of evolutionary biology further complicated the species problem. Instead of recognizing species as mere units of systematic classification, evolutionary biologists see species as units of evolutionary change (Brooks and McLennan 1999). Since Darwin, taxonomists and evolutionary biologists alike have grappled with the task of proposing a universally true
definition for species. The mid 20th century saw a boom in biological research punctuated by
the assimilation of Darwin’s theory of evolution by natural selection, and Gregor Mendel’s
Mendelian genetics, in a movement known as the Modern (Evolutionary) Synthesis (Mallet
1995; De Queiroz 2005; Rose and Oakley 2007). Ernst Mayr was a prominent figure of the
Modern Synthesis; he proposed what is popularly known as the biological species concept
(Mayr 1942; De Queiroz 2005). Mayr defined species as populations of interbreeding
organisms (or those that could potentially interbreed) that were reproductively isolated from
one another (Mayr 1942). This definition attracted much praise (and criticism), but it also laid
the foundation for other species definitions, many of which were based on criteria applicable
only to the fields of expertise of their proposers. For instance, ecologists made use of an
ecological species concept and geneticists advanced the phylogenetic species concept (De
Queiroz 2005). The stochasticity of natural selection and the mechanisms of inheritance have
made it essentially impossible to provide a single species concept that is explicitly true for all
life forms and it might even be argued that the notion of species is a fictitious human construct
(Carstens et al. 2013; Mishler and Wilkins 2018; Zachos 2018). That said, the concept of
species is unlikely to be disregarded anytime in the near future and it may indeed be very
valuable to conservation, be it a fictitious construct or not (De Queiroz 2007; Zachos et al.

Just as species themselves have evolved over time, the concept of biological species has
evolved significantly since its inception in Linnaean taxonomy. Recent advances in molecular
biology have initiated a general change in opinion among taxonomists from the biological
species concept initially proposed by Mayr to an array of phylogenetic species concepts (Isaac
et al. 2004; Zachos et al. 2013). However, the general consensus among biologists in the 21st
century is that it is best to make use of integrative taxonomy which assimilates multiple species
concepts and views congruency between them as a requirement for the characterization of new species (Fujita et al. 2012; Puillandre et al. 2012).

A positive consequence of the inclusion of molecular techniques in the integrative taxonomy approach is the recognition of cryptic diversity – multiple species that were previously described under the heading of a single species using morphological criteria; this was the norm prior to the Modern Synthesis (Bickford et al. 2007). Morphological criteria alone often either fail to recognize recent adaptive variation, or, morphological variation is present but this variation is not reflected in the genetics of the radiation. Such discordance could result from a variety of phenomena that may include phenotypic plasticity, hybridization, and incomplete lineage sorting (Seehausen 2004; Lefebure et al. 2006; Mossel and Roch 2007; Degnan and Rosenberg 2009; Pfennig et al. 2010). These phenomena are evident in the recent adaptive radiations of African cichlids, Darwin’s finches, and Anolis lizards to mention but a few (see e.g. Losos et al. 1998; Seehausen 2006; Losos and Ricklefs 2009).

The incorporation of molecular techniques in taxonomy can sometimes result in taxonomic inflation (Isaac et al. 2004). Taxonomic inflation is the artefactual increase in biodiversity not due to the discovery of new species but rather due to seemingly arbitrary changes in the way organisms are classified. Such changes are sometimes prompted by subspecific differences that exceed the controversial yet popularly accepted ‘barcode gap’. The barcode gap is a widely accepted notion which postulates that interspecific genetic differences must exceed those found within lineages by an order of magnitude (the so-called 10x rule) (Lefebure et al. 2006; Monaghan et al. 2006; Bickford et al. 2007). Due to the heterogeneity of the rate of molecular evolution across the kingdoms of life, certain groups do not have a valid barcoding gap. In some cases the barcoding gap may be present for a few taxa in a group and this may give the artificial impression that it exists for all taxa, in such cases, an unrealistically low interspecific
genetic distance threshold might be proposed and therefore subspecies might be artificially
elevated to species.

Both taxonomic inflation and the identification of cryptic species can potentially have far-reaching outcomes as many conservation initiatives quantify biodiversity in terms of ‘species diversity’ and hence funding is generally directed at the conservation of listed species (Isaac et al. 2004). It may be argued then that taxonomic inflation and the discovery of cryptic species should benefit the conservation of the planet’s biodiversity. Unfortunately, conservation resources are limited to the point that a concept of conservation triage, akin to the triage used by paramedics, has been proposed to help prioritize conservation efforts (Bottrill et al. 2008).

So, attempting to conserve all species on earth is probably too audacious a goal. With that said, identifying what truly warrants conserving is a task that deserves much attention. While the notion of species may be a controversial one, evolutionary biologists and ecologists recognize that discrete evolutionary lineages hold adaptive evolutionary potential (Moritz 1994; Crandall et al. 2000). In acknowledging this it is evident that while attempting to conserve all listed species might be impractical, conserving lineages that have evolutionary significance, typically mediated by historical geographic barriers or corridors might hold promise for the preservation of distinct adaptive evolutionary potential. The recognition of lineages with independent evolutionary trajectories has hence become a central theme in many conservation initiatives – these lineages have been referred to as evolutionarily significant units (hereafter ESUs) (Moritz 1994; Crandall et al. 2000; Fujita et al. 2012). Akin to ESUs is De Queiroz (2007)’s widely accepted recognition of species forming independently evolving metapopulation lineages (i.e. the General Lineage Concept of Species). De Queiroz (2007) has made a distinction between the problem of ‘species concepts’ and ‘species delimitation’ strategies. While conflicting theories exist pertaining to the mechanisms behind speciation, De Queiroz
(2007) argues that the resulting outcome of these mechanisms is ultimately something that can be unanimously agreed upon: molecular partitioning on an evolutionary timescale. Quantifying the degree of isolation between metapopulation lineages, and when this isolation may be considered taxonomically significant, is something that has attracted much recent attention among taxonomists and molecular systematists (Reid and Carstens 2012; Carstens et al. 2013; Yang and Rannala 2014; Kapli et al. 2017).

Moritz (1995) advocates the use of molecular phylogenies in unraveling taxonomic relationships and identifying ESUs. In the 21st century, molecular phylogenies have become the norm in inferring taxonomic relationships and species delimitation, however, the parameters by which these phylogenies are carried out and interpreted are a source of much debate (Monaghan et al. 2006; Edwards 2009; Fujita et al. 2012; Yang and Rannala 2012). A requirement for a lineage to be considered as evolutionarily significant according to Moritz (1994) is reciprocal monophyly, at least for mitochondrial markers – this is a consensus accepted by many (Fujita et al. 2012; Puillandre et al. 2012). Crandall et al. (2000), however, argue that while reciprocally monophyletic lineages may be of evolutionary significance, this criterion fails to recognize recent adaptive divergence which could hold much evolutionary potential as in the case of African cichlid and Darwin’s finch radiations (Grant et al. 2004; Seehausen 2006; Wagner et al. 2012).

New coalescent phylogenetic approaches circumvent the problems introduced by Moritz’s (1994) requirement of reciprocal monophyly by using multilocus data and in so doing, recognize the evolutionary potential of recent adaptive variation (Degnan and Rosenberg 2009; Yang and Rannala 2010; Fujita et al. 2012). Different regions of the genome are under different selective pressures (although debated, some appear to be under no selection at all) and therefore different genes evolve at different rates. This can result in different gene fragments producing
conflicting trees which may in turn be in conflict with the actual species tree. The Multispecies Coalescent Model (MSC) uses multilocus data to model different evolutionary scenarios and thereby account for discordance between gene trees and species trees as well as ancestral polymorphism (Rannala and Yang 2003; Xu and Yang 2016). Since the derivation of the MSC, software packages that operate on the basis of the MSC, such as Bayesian Phylogenetics and Phylogeography (BPP), have been developed to help delimit species with conflicting gene trees and ancestral polymorphism (Yang 2015; Leaché et al. 2018). In some cases, multilocus data are a luxury that cannot, for financial or practical reasons, be acquired. In such cases, other species delimitation techniques such as the Bayesian Generalized-Mixed Yule Coalescent (hereafter bGMYC) model (Pons et al. 2006; Reid and Carstens 2012) and Multi-rate Poisson tree processes (hereafter mPTP) (Kapli et al. 2017) can be used in coalescent approaches that do not require multilocus data.

Underlying the diversification and spatio-temporal distribution of biological organisms is a complex array of historical geological and climatic events. The study of biogeography lies at this interface between biology and geography (Cracraft 1975; Nelson 1985; Smith 1989; Yu et al. 2015). In addition to providing frameworks from which species can be delimited from one another, molecular phylogenies have become fundamental in disentangling biogeographic relationships between taxa (Moritz 1995; Ree et al. 2005). The use of fossil data and molecular clocks allow for phylogenetic approximations of divergence times which can then be correlated with the timing of carbon-dated geo-climatic events. Indeed, many phylogenetic species delimitation studies have made use of time-calibrated phylogenies in a geological context to better understand the factors driving interspecific diversification (Kuriyama et al. 2011; Giribet et al. 2012; Ceccarelli et al. 2014; Jongsma et al. 2018). Not only does phylogenetic biogeographic inference allow for correlations to be made between evolutionary diversification of species and historical geography, it also allows for the reconstruction of ancestral ecosystem
compositions through the identification of patterns of shared biogeographic affinities among diverse biological taxa (Morley 2007; Linder et al. 2012). In addition to seeking to understand the geological and climatic factors that underlie the evolutionary history of species in a phylogeny, many studies make use of ecological niche modelling in an attempt to identify unique bioclimatic preferences among closely related taxa which may have resulted in ecological partitioning and subsequently speciation between taxa (Raxworthy et al. 2007; Blair et al. 2013; da Silva and Tolley 2017). This approach has grown in popularity with the implementation of the programme MaxEnt, which is presumably able to reliably model suitable climate while making use of presence-only data (Phillips and Dudík 2008; Elith et al. 2011).

Cryptic diversity appears ubiquitous across the kingdoms of life with studies revealing its persistence in crustaceans, spiders, bats, moths, and beetles among others (Mayer and von Helversen 2001; Hendrixson and Bond 2005; Monaghan et al. 2005; Lefèbure et al. 2006; Dumas et al. 2015). The herpetofauna (reptiles and amphibians) are no exception, with much documented cryptic diversity (see e.g. Shaffer et al. 2015; Vasconcelos et al. 2016). Within the reptiles, the Chamaeleonidae are a relatively young lineage (ca. 90 my old) compared to other squamate families. They are widespread across Africa and Madagascar, with a few species in the Middle East, southern Europe, and southern Asia (Vidal and Hedges 2005; Wiens et al. 2012; Tolley et al. 2013). Previous studies have shown members of this family to exhibit cryptic diversity, with documented examples in *Rhampholeon*, *Bradypodion* and *Calumma* (Tolley et al. 2004; Gehring et al. 2012; Branch et al. 2014; Prötzel et al. 2015).

The genus *Chamaeleo* is a pan-African genus of chameleons that also includes all European, Middle Eastern, and Asian species and was first described by Linneaus in 1758 (Tilbury 2018). While the taxonomy within the *Bradypodion*, *Rhampholeon* and *Calumma* genera has been somewhat resolved, for the most part through molecular means (Tolley et al. 2004; Branch et
al. 2014; Prötzel et al. 2015, 2017), there remains a paucity of phylogenetic work on the genus *Chamaeleo*. As far as African chameleon genera are concerned, *Chamaeleo* tend to occupy more xeric environments; most species prefer savannah and savannah-woodland, with a few species tolerating semi-desert and one species (*C. namaquensis*) occupying true desert (Tilbury 2018). While a few *Chamaeleo* species will venture into dense forest, the genus does not appear to have a clear affinity for forest and the early Eocene presence of forest (Morley 2007; Kissling et al. 2012) more likely fragmented, rather than connected, their populations. Where other chameleon genera may have experienced episodes of habitat fragmentation during arid periods of the Miocene (i.e. *Trioceros* – see Ceccarelli et al. 2014), these episodes of aridity would have likely reduced forest to savannah and thereby facilitated connectivity for *Chamaeleo*. The recent taxonomic elevation of the sub-genus *Trioceros* (previously classified under *Chamaeleo*) to genus level hints at the lack of evolutionary clarity within these chameleon taxa (Tilbury and Tolley 2009).

A case in hand may be the common flap-necked chameleon (*Chamaeleo dilepis* Leach 1819). This species is a large, typical chameleon widespread throughout sub-Saharan Africa (Largen and Spawls 2006; Reaney et al. 2012; Tilbury 2018). The large range of this species (arguably the largest of any chameleon species), taken with the confounding phenotypic variation that exists across its range, renders the current taxonomy questionable (Largen and Spawls 2006; Tilbury 2018). To further add to the taxonomic confusion surrounding this species, a recent preliminary study found potential species level genetic distances between populations within this species and suggested that the species may in fact be a complex of at least two distinct species (Main et al. 2018). However, Main and co-workers only sampled populations within South Africa so it is possible that there may be even more distinct lineages within this species across its entire range. Clearly, an in-depth phylogeny of this group, placed within the context of the entire genus, is needed if its taxonomy is to be resolved. Such would allow for the
implementation of conservation management actions that preserve all lineages and thereby
prevent a loss of unrecognized genetic diversity.

Here, in chapter two I use two mitochondrial (ND4 and 16S) and two nuclear (PRLR and
RAG1) markers to construct a comprehensive phylogeny of the genus *Chamaeleo* to address
the taxonomic ambiguity that currently plagues the group. Furthermore, I make use of various
species delimitation models to identify potential cryptic speciation within the genus. In chapter
three, I estimate a time-calibrated phylogeny using a secondary calibration point inferred from
Tolley et al. (2013)’s large-scale fossil-calibrated phylogeny of the Chamaeleonidae and using
this time-calibrated phylogeny I carry out an ancestral area reconstruction of the group
according to the dispersal-extinction cladogenesis (DEC) model implemented in RASP in order
to test whether *Chamaeleo* has Southern African origins. In addition, I perform ecological
niche modelling on different cryptic species within the *C. dilepis* complex determined in
Chapter 2 to examine whether there exists ecological support for these species.

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ABSTRACT:
The availability of new methods with which to delimit species is fast uncovering cryptic diversity throughout the kingdoms of life, and repeatedly highlighting the gaps in traditional morphological taxonomy. An unfortunate consequence of the limited reliability of morphological taxonomy is that many cryptic species go unrecognised. *Chamaeleo* is a genus of chameleons comprising 14 species with large, overlapping distributions and characterized by considerable phenotypic variation. It is thought to have substantial cryptic diversity and has long been in need of a taxonomic revision informed by molecular data. Accordingly, the aims of the present study were to carry out a comprehensive phylogeny of the genus, accompanied by species delimitation methods. Two mitochondrial (ND4 and 16S) and two nuclear (RAG1 and PRLR) markers were sequenced for representative individuals of every known species in the genus, spanning their natural ranges where possible. Bayesian and maximum likelihood analyses were carried out using the mitochondrial dataset, as well as the combined data. Species delimitation was investigated using the Bayesian General Mixed Yule-Coalescent model, distance-based DNA barcoding, and the Bayesian Phylogenetics and Phylogeography programme. The analyses suggested the presence of 18 species within *Chamaeleo*, splitting *C. dilepis* and *C. gracilis* into three candidate species each, and *C. anchietae* into two candidate species. Additionally, the results suggest that *C. necasi* should be synonymised with one of the candidate species of *C. gracilis*. These findings lay a phylogenetic foundation for a taxonomic re-evaluation of the genus and hold implications for the conservation of the group at large.
INTRODUCTION:

Both the concept of species, and how they are delineated, have evolved considerably over the past century with a general change in opinion from species being immutable units of classification to species being transient, spatio-temporal units of evolutionary change (Brooks and McLennan 1999; De Queiroz 2005, 2007). Notwithstanding, the species conundrum still attracts much debate, with some even suggesting that species concepts should be done away with altogether (Mishler and Wilkins 2018; Zachos 2018). While this debate pertains to the processes underlying speciation events and the exact definition of species, in line with the general lineage concept of species, there is a prevailing consensus that isolated metapopulation lineages accumulate genetic differences that are evolutionarily tangible (De Queiroz 2007).

Given the evolutionary timescale of speciation events, most modern species delimitation strategies make inferences on the basis of phylogenetic relationships between taxa and attempt to account for lineage-specific divergence rates and molecular clock-informed divergence time estimates (Moritz 1994; Crandall et al. 2000; Yang and Rannala 2010; Fujita et al. 2012).

Due to its ease of availability, single locus data such as mitochondrial DNA (mtDNA) is often used to delimit species, this initially being used as the benchmark marker through distance-based DNA barcoding (Lefèbure et al. 2006; Puillandre et al. 2012). DNA barcoding has since uncovered many cryptic lineages within single ‘morphospecies’ (see e.g. BinaPerl et al. 2014; Vasconcelos et al. 2016; Chambers and Hebert 2016). However, there are shortfalls associated with delimiting species purely on the basis of the genetic distances that separate taxa. In particular, barcoding (or the distance-based approach underpinning barcoding) does not operate within an evolutionary framework, and the rate of mtDNA evolution is heterogeneous across the kingdoms of life – so in some taxa barcoding gaps are not effective for delimiting species (Galtier et al. 2009; Puillandre et al. 2012). Many studies have also uncovered cryptic diversity using evolutionary relationships inferred from actual phylogenies and coupled these
findings with distance-based thresholds (Hamilton et al. 2011; Main et al. 2018; Engelbrecht et al. 2019). Given these criticisms, species delimitation methods for single-locus markers have been developed that operate in a coalescent framework, and model changes in branching rates that may indicate changes from a coalescent process to a Yule process (essentially identifying changes between intra-specific and inter-specific processes). These include the General Mixed Yule-Coalescent (GMYC) model (Pons et al. 2006; Fujisawa and Barraclough 2013) and its Bayesian implementation bGMYC (Reid and Carstens 2012) as well as Multi-Rate Poisson Tree Processes mPTP (Kapli et al. 2017). When only single-locus data are available, these methods may be seen as the gold standard in species delimitation, however, when using only a single locus to infer species, ancestral polymorphism, incomplete lineage sorting, and species-tree-gene-tree discordance can all potentially confound results. To account for these factors, methods that make use of the Multispecies Coalescent (MSC) on multilocus data, such as the programme Bayesian Phylogenetics and Phylogeography (BPP), are able to account for gene tree uncertainty by inferring a species tree from multiple gene trees and exploring different tree topologies in a Bayesian reversible jump Markov Chain Monte Carlo (rjMCMC) framework (Yang and Rannala 2014; Yang 2015).

Chamaeleo is a pan-African genus of chameleons with some members also occurring in Southern Europe, Southern Asia and the Middle East (Tilbury 2018). This genus is comprised of 14 species (Uetz et al. 2019), however, many of these species have large, overlapping distributions with confusing phenotypic variation within and between them rendering their current taxonomy somewhat questionable (Largen and Spawls 2006; Main et al. 2018; Tilbury 2018). Indeed, until 2009 the genus included the sub-genus Trioceros which was subsequently elevated to its own genus, highlighting the need for a taxonomic revision of the group with possible conservation implications (Tilbury and Tolley 2009). Certain species of Chamaeleo in particular, which all broadly resemble one another, present a real quandary to taxonomy: C.
anchietae, C. dilepis, C. gracilis, C. laevigatus, C. necasi and C. senegalensis. *Chamaeleo anchietae* encompasses three subspecies, each with small, isolated distributions (Table 1 and Fig 1; also see Tilbury 2018 and Uetz et al. 2019).

![Map of Africa with type localities of Chamaeleo species and subspecies](Image)

**Figure 1:** Type localities (inferred from vague locality names) of the species and subspecies of *Chamaeleo* that closely resemble one another morphologically and have overlapping distributions (from Table 1).

The taxonomy of *C. dilepis* has repeatedly confused taxonomists since the species was first described by Leach in 1819 (see Tilbury 2018), probably because its range is larger than that of any other chameleon species and it exhibits perplexing phenotypic variation across this range. It currently comprises eight subspecies, each of which have vaguely understood distributions and in some cases imprecise type localities with substantial distributional overlap between them (Table 1 and Fig 1). Additionally, phenotypic differences between subspecies are not always obvious (see Tilbury 2018). *Chamaeleo gracilis* is a tropical savannah inhabitant with one subspecies described in addition to the type species; these two variants of *C. gracilis*
have overlapping distributions with subspecies of *C. dilepis*, *C. senegalensis* and *C. necasi.* 

*Chamaeleo laevigatus* is a species whose taxonomy has also presented a challenge, with debate as to whether it is in fact its own species or whether it is just a variant of *Chamaeleo senegalensis* (Joger 1990; Chirio and LeBreton 2007; Trape et al 2012; but see Tilbury 2018 for a review). *Chamaeleo necasi* is a recently described species (Ullenbruch, Krause and Bohme, 2007) with a vague type locality and limited information pertaining to the extent of its geographical range (Tilbury 2018). Additionally, *C. necasi* resembles *C. gracilis* morphologically and while the type locality is vague for *C. necasi* (Table 1), it appears likely that its range overlaps both that of *C. gracilis* and *C. senegalensis*. *Chamaeleo senegalensis* has been suggested to include *C. laevigatus*, its range also overlaps with both that of *C. gracilis* and *C. necasi* and it morphologically resembles that of *C. gracilis* (Tilbury 2018). It is clear that the taxonomy of the abovementioned species is in need of a phylogenetically informed revision, given that morphology appears confounding within this genus.

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<td></td>
<td>roperi</td>
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<td>Kilifi, north of Mombasa, Kenya</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ruppoli</td>
<td>Boulenger 1893</td>
<td>Ogaden, Somaliland</td>
</tr>
<tr>
<td><em>Chamaeleo gracilis</em></td>
<td>Hallowell 1844</td>
<td>gracilis</td>
<td>Hallowell 1844</td>
<td>Liberia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>etienne</td>
<td>Schmidt 1919</td>
<td>Banana, DRC</td>
</tr>
<tr>
<td><em>Chamaeleo necasi</em></td>
<td>Ullenbruch, Krause and Bohme 2007</td>
<td>NA</td>
<td>NA</td>
<td>Togo</td>
</tr>
<tr>
<td><em>Chamaeleo laevigatus</em></td>
<td>Gray 1863</td>
<td>NA</td>
<td>NA</td>
<td>500 miles south of Khartoum, Sudan</td>
</tr>
<tr>
<td><em>Chamaeleo senegalensis</em></td>
<td>Daudin 1802</td>
<td>NA</td>
<td>NA</td>
<td>Senegal</td>
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</tbody>
</table>
While 30% of all chameleon species are currently listed as threatened by the International Union for Conservation of Nature (IUCN), no Chamaeleo species fall within threatened categories (IUCN, 2017). The majority of threatened chameleon taxa are narrow endemics and depend heavily on intact forest, making deforestation a very real risk to their status in the wild. In contrast, the affinity of most Chamaeleo species for savannah-type habitats, coupled with their moderate to large distributions and tolerance of modified habitats, might mean that the threat of habitat loss is less pervasive to them than other chameleon species. In contrast, Chamaeleo species make up some of the most heavily exported species for the pet trade (Jenkins et al. 2014). The Convention for International Trade in Endangered Species of Fauna and Flora (CITES) provides lists of species for which international trade is permitted at regulated levels, and a list of species whose trade is prohibited; the assignment of species to these lists is in part dependent on their threat status. Because CITES export quotas are set per species, the future of potential unrecognised cryptic species of Chamaeleo hinges on an accurate species inventory of the genus. If species that are currently understood to have widespread distributions are found to comprise multiple species with narrow distributions then the current export quota for these species might be unsustainable.

Given that a preliminary study with limited sampling pointed to cryptic diversity and potentially multiple species within Chamaeleo dilepis (Main et al. 2018), cryptic lineages are expected in the widespread species of Chamaeleo. To examine this, a comprehensive phylogeny of the genus Chamaeleo was constructed, including multiple samples from all known species. Species delimitation methods were used to examine the potential for unrecognised cryptic diversity which point to candidate species, and to examine the validity of existing species.
MATERIALS AND METHODS:

Laboratory Protocols:

Multiple samples representing all currently known species of *Chamaeleo* were acquired from the South African National Biodiversity Institute (SANBI) tissue bank. In some cases, multiple samples were available from across the range of species. Sequence data for *Chamaeleo* specimens of known provenance were also downloaded from GenBank, including multiple sequences of *C. dilepis* from Main et al. (2018). In addition, sequence data for a representative of an outgroup genus (*Trioceros*) were downloaded, with the final dataset comprising 56 individuals (Appendix I).

Figure 2: Sampling localities for all *Chamaeleo* taxa in this study.
Total genomic DNA was extracted from tail clips or liver samples following standard salt extraction (Aljanabi and Martinez 1997) or Cetyl Trimethyl Ammonium Bromide (CTAB) (Allen et al. 2006) extraction protocols. The mitochondrial NADH subunit 4 gene (ND4), 16S ribosomal subunit (16S), nuclear recombination activating gene 1 (RAG1) and nuclear prolactin receptor gene (PRLR) were PCR amplified using the primers F3 and R4 for ND4 (Raxworthy et al. 2002), L2510 and H3080 for 16S (Romano and Palumbi 1997), F118 and R1067 for RAG1 (Matthee et al. 2004), and ChamF1 and ChamR1 for PRLR (Tolley et al. 2013). Fragment amplification was carried out in 30 µL reaction volumes with 25-60 ng µL\(^{-1}\) genomic DNA, 10x thermophilic buffer (20 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.1mM EDTA, 1mM DTT), 2.5 mM MgCl\(_2\), 0.25 µM of each primer, 0.2 mM dNTPs, and 1 Unit Super-Therm Taq DNA polymerase. The thermocycling profile followed 4 minutes of initial denaturation at 95 ºC followed by 38 cycles for ND4 and 35 cycles for 16S, RAG1 and PRLR of 45 seconds at 94 ºC, 45 seconds for primer specific annealing, and 1 minute extension at 72 ºC, with a final extension of 10 minutes at 72 ºC. Annealing temperatures were 52-55 ºC for ND4, 48-52 ºC for 16S and 57 ºC for RAG1 and PRLR. Amplifications were performed on a GeneAmp PCR 2720 system (Applied Biosystems, Foster City, CA, USA). After verifying successful amplifications on a 1% agarose gel, amplicons were cycle sequenced using Big Dye chemistry and analysed on an ABI3730xl automated sequencer (Applied Biosystems). Sequences were manually verified in Geneious 8.19 (Kearse et al. 2012) and aligned using the Muscle plug-in in Geneious.

**Phylogenetic Analyses:**

Phylogenetic analyses were run for the mitochondrial only dataset and for the combined mt and nuclear datasets (mt–ND4: 856 bp, 16S: 494 bp, nuclear–PRLR: 595 bp, RAG1: 943 bp). The analyses were also run on separate gene trees (total mitochondrial, PRLR and RAG-1).

The genus *Chamaeleo* forms a polytomy with six other extant arboreal chameleon genera.
(Tolley et al. 2013). Given this, a representative from one genus (*Trioceros*) was used as an outgroup taxon (Appendix I). Bayesian analyses were implemented in MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001) on the mitochondrial dataset and the combined dataset. For the latter, a partition homogeneity test was carried out in PAUP 4.0a164 (Swofford 2003) that confirmed topological congruence between gene trees (p=0.41) and suggested that the gene fragments could be combined without conflict. The combined dataset was partitioned by marker and the optimal model of evolution determined for each marker using jModelTest (Darriba et al. 2012), and incorporated into the analyses (16S: GTR; ND4 GTR + I + G; PRLR and RAG1: HKY + G). The mitochondrial only dataset was also partitioned according to the same scheme as above.

Both analyses were run with a random starting tree and four independent chains of 50 million generations, sampling every 1000 generations. Convergence and adequate mixing of chains was assessed in Tracer 1.7 (Rambaut et al. 2018) and convergence was considered adequate when ESS ≥ 200 for all the parameters. The first 10% of trees were discarded as burn-in. Nodes with posterior probabilities (pp) ≥ 0.90 were considered moderately-supported, while those with ≥ 0.95 pp were considered well-supported. In addition to the Bayesian analysis, a maximum likelihood (ML) analysis was carried out using RaxML (Stamatakis 2006) on the same two datasets, implementing the same partitioning scheme as in the Bayesian analysis with the GTRGAMMA model of evolution and 1000 bootstrap replicates. Here, nodes that received ≥ 75% were considered supported. All phylogenetic analyses were run on the CIPRES Science Gateway (Miller et al. 2010; http://www.phylo.org/sub_sections/portal/).

Species Delimitation:

Due to the uncertainty of species delimitation protocols in accurately accounting for evolutionary process, several analyses were carried out. Firstly, species were inferred using a
Bayesian implementation of the General Mixed Yule-Coalescent (bGMYC) model (Reid and Carstens 2012), which allows for objective delimitation as it does not require *a priori* species assignment. The GMYC, first implemented by Pons et al. (2006), lies at the interface between population genetics and phylogenetics and attempts to determine the point of departure at which coalescent processes (intraspecific) become Yule processes (interspecific). Pons et al. (2006)’s implementation of the GMYC makes use of a single ultrametric tree, with the shortcoming that it is unable to account for uncertainty in tree topology or phylogenetic error. Reid and Carstens (2012)’s Bayesian implementation of the GMYC allows for integration over multiple trees in an MCMC analysis, thereby accounting for both phylogenetic error and uncertainty in tree topology. Bayesian GMYC (bGMYC) analyses were carried out in R using the package bGMYC 3.0.1 (Reid and Carstens 2012) on the mtDNA and the combined datasets, separately. For each dataset, BEAST 2 (Bouckaert et al. 2014) was first used to generate ultrametric trees. For the combined dataset, the same partitioning scheme and substitution models were used in BEAST as those used in the Bayesian and maximum likelihood analyses. For each dataset, two independent runs of 100 million generations were sampled every 1000 generations and assessed in Tracer for adequate convergence and mixing (ESS ≥ 200). After removing 50% burn-in, the trees from each of the runs were combined in LogCombiner (Bouckaert et al. 2014) and the last 500 trees were saved for the bGMYC analysis. In the bGMYC package, a random sample of 100 trees from the 500 trees generated with BEAST was obtained and pruned to remove outgroup taxa. On this random sample of 100 trees, a bGMYC Markov Chain Monte Carlo (MCMC) of 50000 generations was run with a burn-in of 40000 generations and thinning set to every 100 to produce 1000 samples. Default priors were used for the Yule and coalescent rate change parameters, the upper threshold was set to 30 and the starting value of the threshold parameter was set to 23.
The bGMYC method of species delimitation was developed for the use of single-locus data and although it performs well for some multi-locus studies (Ceccarelli et al. 2014; Engelbrecht et al. 2019), it has also been found to over-split lineages (Carstens et al. 2013). Two factors that may confound the inference of species from single-locus data are ancestral polymorphism and incomplete lineage sorting which may result in discordance between species trees and gene trees. Therefore, candidate species were also inferred using the programme Bayesian Phylogenetics and Phylogeography (BPP 3.4 - Yang 2015). BPP implements a Bayesian multispecies coalescent approach on multilocus data to infer species. BPP was run on the combined dataset using the joint species-delimitation and species-tree inference option (A11 – Yang, 2015). This option uses the nearest neighbour interchange (NNI) and subtree pruning and regrafting (SPR) algorithms to adjust tree topology and to explore species tree scenarios given multiple gene trees, in so doing it does not rely on a fixed, user-specified guide tree and is able to account for uncertainty among gene trees (Yang and Rannala 2014). Option A11 of BPP may lump two species together, but it will not split pre-defined species. Species delimitation with BPP is heavily dependent on the selection of priors. Two priors of interest when running BPP are the ancestral population size ($\theta$) and the root age ($t_0$). To ensure the robustness of species inferences, multiple different combinations were tested for these priors, modelling large and small ancestral population sizes as $\theta \sim I(3, 0.2)$ and $\theta \sim I(3, 0.002)$ respectively and deep and shallow root ages as $t_0 \sim I(3,0.2)$ and $t_0 \sim I(3,0.002)$ respectively (Yang and Rannala 2010, 2014; but also see Wüster et al. 2018). All BPP runs were carried out for 100000 generations with a burn-in of 20000 generations, sampling every 2 generations.

Finally, a DNA barcoding method was used to test species inferences. DNA barcoding is a distance-based approach to species identification that relies on the premise that between-species genetic distances exceed within-species genetic distances by an order of magnitude. This genetic distance threshold is known as a barcoding gap (Lefèbure et al. 2006; Puillandre...
SpeciesIdentifier 1.8 (Meier et al. 2006) was used to infer a sequence divergence threshold for species from frequency distributions of inter and intraspecific distances within the Chamaeleo genus. Given that the 16S dataset was the most complete, and the only dataset encompassing the full species complement for the genus, this dataset was used to infer species with SpeciesIdentifier. The results from the two model based species delimitation analyses were used to guide input for SpeciesIdentifier, in order to estimate genetic distances between the candidate species inferred by those methods.

RESULTS:

Phylogenetic analyses:

Topologies of the mitochondrial and the combined dataset were not in conflict for either the Bayesian or maximum likelihood analyses. The combined dataset resulted in higher nodal support than the mitochondrial dataset for both analyses (Fig. 3; Fig. 1, Appendix II). Chamaeleo forms a well-supported, monophyletic clade with C. namaquensis as the sister taxon to all other Chamaeleo species. In general, deep nodes were well supported, as were the nodes defining most currently described species. The split between C. dilepis/gracilis and C. laevigatus/senegalensis clades has low bootstrap support (60%) but is supported by Bayesian posterior probability. A number of the shallow nodes do not show full support by both analyses, but these are all within the C. dilepis clade. Although most of the currently described species are supported (i.e. 12 species), C. gracilis is paraphyletic and C. necasi is embedded within a clade of C. gracilis.
Figure 3: A Bayesian consensus tree for *Chamaeleo*. Node support is indicated for both Bayesian and likelihood analyses.
Species delimitation:
The bGMYC and BPP analyses yielded identical results, both of which retrieved 18 species within *Chamaeleo*, splitting the *C. dilepis* into three separate species, *C. anchietae* into two species, and *C. gracilis* into three species (Fig. 4). Furthermore, *C. necasi* and *C. gracilis* clade 3 were retrieved as a single species. The results were the same for the mtDNA (not shown) and the combined datasets, and the BPP yielded the same delimitations and posterior probability support regardless of ancestral population size and root age prior.

**Figure 4:** Species delimitation results for three different methods (bGMYC, BPP, and genetic distance-based DNA barcoding) for the Bayesian phylogeny produced from the combined dataset. Breaks in coloured bars indicate interspecific splits inferred by each method. *Chamaeleo necasi* is indicated in bold and with a dark yellow bar nested within the light yellow *Chamaeleo gracilis* 3; all methods lumped these two species together.
The DNA barcoding approach was largely congruent with the other methods, with the exception of splitting the *C. dilepis* complex into five (rather than three) species (Fig. 5; those to the right of the barcode code gap shown in grey), with agreement between all methods for the presence of *C. dilepis* 1 and 2 as species, but barcoding split *C. dilepis* 3 into three species.

**Figure 5**: Frequency histogram of pairwise differences between and within candidate species of *Chamaeleo* for 16S. Blue bars denote intraspecific distances, and yellow indicate interspecific distances. The barcode gap is shown in grey. Intraspecific distances that exceeded the barcode gap were for individuals within the *Chamaeleo dilepis* 3 clade (Fig. 4).

*Chamaeleo dilepis* 1 with a central African sampling distribution best matches the type locality of *C. dilepis dilepis* (“Congo” which is most likely either the Democratic Republic of Congo, or the Republic of Congo) and the clade is therefore considered to represent the nominate species. The sampling area for the *Chamaeleo dilepis* 2 clade covers a large area in Southern Africa whereas, the samples making up the *C. dilepis* 3 clade were all from East Africa (Fig. 6).
Figure 6: The presumed range of each *Chamaeleo dilepis* candidate species (coloured polygons) revealed by species delimitation as well as all records of *C. dilepis sensu lato* (black dots) and sample sites included in the phylogeny (colour symbols). The dark grey polygon represents the presumed distribution of *C. dilepis* (adapted from Tolley 2014).

For *C. anchietae*, the sampling locality of *C. anchietae* 1 (Huila Province, Angola) best matches the type locality of the nominate species of *C. anchietae* (also Huila Province, Angola) and the sampling locality of *C. anchietae* 2 (Kundelungu National Park, DRC) is closest the type locality of *C. anchietae vinckei* (Katanga, DRC; Table 1, Fig. 7). *Chamaeleo anchietae* has also been recorded from Malawi, and although samples from that area were not included in the phylogeny, the Malawian records are presumably from clade 2. In addition, there are a number of *C. anchietae* records from Rwanda, however, given that the one Rwandan “*C. anchietae*” included in the phylogeny was in the *C. dilepis* 3 clade, this puts the Rwandan records in doubt (Fig. 3).
The sampling localities of the three *C. gracilis* clades are separated by large geographic distances, particularly as compared to its currently known distribution (Fig. 8). However, the area covered by *C. gracilis* 3 is the closest match to the type locality of nominate *C. gracilis* (“Liberia”; Fig. 8) with one sample originating in that country (Appendix I). The single sample of *C. necasi* is embedded within *C. gracilis* 3 clade.
Figure 8: The presumed range of each *Chamaeleo gracilis* candidate species (coloured polygons) revealed by species delimitation as well as all records from across the known range of *C. gracilis* (black dots) and sample sites included in the phylogeny (colour symbols). The dark grey polygon represents the currently known distribution of *C. gracilis* (adapted from Tolley et al. 2014).

*Chamaeleo laevigatus* and *C. senegalensis* are supported as separate species by each of the delimitation methods (Fig. 3). Each of the sampling sites fall within the currently known distribution of these two species (Fig. 9).
Figure 9: Sampling localities (coloured symbols) of *Chamaeleo senegalensis* and *C. laevigatus* used in this study as well as all known records of *C. senegalensis* (white dots) and *C. laevigatus* (black dots). The currently known distribution for *C. senegalensis* (dark grey polygon) and *C. laevigatus* (light grey polygon) are also shown (Tolley and Trape 2014; Wilms et al. 2013).

**DISCUSSION:**

The phylogenetic analysis complemented by model based species delimitation shows that *Chamaeleo* likely comprises 18 species in contrast to the currently recognised 14 species. There is cryptic diversity present in three of the currently recognised species, *C. dilepis*, *C. gracilis*, and *C. anchietae*, while *C. necasi* does not appear to be a valid species (Fig. 3). In general, other described species are well supported and monophyletic (Fig. 2).

Large genetic differences within *C. dilepis* were found across a limited area of its range in South Africa, suggestive of species level differentiation (Main et al. 2018). However, that study...
was not comprehensive, lacking most species and covering only a limited geographic range, which prevented conclusions regarding species delimitation. In the present study, this constraint was overcome by covering a greater range of the potential diversity within *Chamaeleo* by including all species, and multiple samples from across the range of the more widespread species. Indeed, cryptic diversity is certainly present within *C. dilepis* pointing to three candidate species delimited here by several model based delimitation methods. These candidate species are logical in terms of geography, with *C. dilepis* 1 found in Central Africa, *C. dilepis* 2 for the most part found in Southern Africa and *C. dilepis* 3 found in East Africa (Fig. 6). However, *C. dilepis* 1 and *C. dilepis* 2 appear to be parapatric near southern Angola, southern DRC and northern Zambia. In the present phylogeny sampling is not comprehensive enough in this region to discern where the transitions between these clades are on the landscape.

*Chamaeleo dilepis* 1 was inferred from multiple individuals from three African countries (DRC, Republic of Congo, and Angola). Given that these individuals were sampled relatively far apart from one another (approximately 1800 km between each sampling locality), more comprehensive sampling along intermediate points between the sampling localities used in the present study might improve our understanding of relationships within this candidate species.

The geographic range covered by the sampling of *C. dilepis* 2 is comparatively large, encompassing much of South Africa and also extending into Mozambique, Malawi, Namibia, Zambia, Zimbabwe, Botswana, southern Angola, and southern Tanzania. Main et al. (2018) found relatively deep divergences within this same clade, and suggested that the differences could be at the species level, although cautioned that firm conclusions could not be made due to limited sampling. Here, the comprehensive species sampling and delimitation methods instead indicate that differences within *C. dilepis* 2 are not at the species level. The lack of shared haplotypes and large sequence divergences observed by Main et al. (2018) therefore
point to strong population level structure within *C. dilepis* 2. This new finding highlights the importance of comprehensive sampling in inferring species from phylogenies as limited sampling can confound such inferences.

*Chamaeleo dilepis* 3 is an East African clade, which includes individuals from Tanzania (including Zanzibar), Kenya, and Rwanda. This is the only *C. dilepis* clade for which congruency in species delimitation was not observed between all three methods. Distance-based barcoding splits this clade into three separate species, however, both BPP and bGMYC support this clade as a single species. Taking a conservative approach, guided by the congruency between the more advanced delimitation methods, point to this clade comprising a single species.

In *Chamaeleo*, cryptic diversity is not unique to *C. dilepis* with two candidate species consistently delineated for *C. anchietae* across all methods. The distance separating *C. anchietae* 1 from the most westerly sampled *C. anchietae* 2 is relatively large, in excess of 1500 km, and there are no additional records of *C. anchietae* in this region suggesting that the gap is genuine. That is, *C. anchietae* 1 is found in Angola, whereas samples of *C. anchietae* 2 are from South-Eastern DRC and Tanzania. There is also a record of this species in northern Malawi (Tilbury 2018), which is essentially midway between the two samples included in clade 2, suggesting that populations from Malawi should be assigned to clade 2. Furthermore, the two areas have different climates and habitats, separated by an arid corridor (Beck et al. 2018), and this could have promoted their diversification. However, these inferences are preliminary as sample sizes are small and geographic coverage is incomplete. Furthermore, confirmation that the intermediate area of the arid corridor does not contain populations of *C. anchietae sensu lato* would be useful to understand these distributions.
Interestingly, an individual identified as *C. anchietae* (ZMFK 54962) from Rwanda was found to group within the *C. dilepis* 3 clade (Fig. 6 and 7). This clarifies some taxonomic confusion over the potential Rwandan distribution of this species that stemmed from the misidentification of this specimen (Tilbury 2018). Given that this Rwandan individual is within the *C. dilepis* 3 clade, it seems likely that other *C. anchietae* records from Rwanda (Fig. 7) are actually *C. dilepis*. However, more comprehensive sampling of chameleons identified as *C. anchietae* from Rwanda would help clarify this situation.

*Chamaeleo anchietae* is morphologically similar to *C. laevigatus* and they are presumed to be closely related (Tilbury 2018). However, this was not reflected in the phylogeny. Indeed, *C. anchietae* is deeply divergent from most other *Chamaeleo* species. This is not a unique case of discordance between morphology and genetics, in fact, many other studies have illustrated that this is a common phenomenon (e.g. Graham et al. 1998; Hamidy et al. 2011; DeBiasse and Hellberg 2015; Sharma et al. 2015). While morphology was once the sole basis for species identification, it has become clear that when used in concert with other data (e.g. molecular, ecological, physiological, behavioural etc.), there is greater confidence for inferences pertaining to species delimitation.

Challenges with *Chamaeleo* classification are also evident within *C. gracilis* where three candidate species have been identified. These results appear to make geographic sense, although samples included were limited in geographic coverage, with *C. gracilis* 1 occurring in a high elevation region of Tanzania, *C. gracilis* 2 in western Chad, and *C. gracilis* 3 across Guinea, Sierra Leone and Liberia in West Africa. The sampling regions are separated by more than 3000 km and have vastly different climates (Beck et al. 2018) suggesting that diversification between the three clades could have been related to environmental drivers. However, there are many records of *C. gracilis* from the intervening areas so it would be
important to examine this further through the inclusion of additional representative specimens covering a broader geographic range. In addition to unveiling cryptic diversity in three species of *Chamaeleo*, all delimitation methods support *C. laevigatus* and *C. senegalensis* as sister species, but clearly separate. This is an important finding as Chirio and LeBreton (2007) (cited by Tilbury, 2018) do not recognise *C. laevigatus* but rather consider it as variant of *C. senegalensis*. Furthermore, the divergence between *C. senegalensis* and *C. laevigatus* is deeper than that between many other sister species and this lends even more to the support of *C. laevigatus* and *C. senegalensis* as separate species. A clear mismatch exists between distribution records for *C. senegalensis* and *C. laevigatus* and their respective IUCN distribution polygons (Fig. 9). A *C. laevigatus* sample from the present study (taken from southern Chad) supports the IUCN distribution polygon for *C. laevigatus*, however, if the records for *C. senegalensis* are valid, then there is extensive geographic overlap between *C. laevigatus* and *C. senegalensis* which has probably perpetuated the confusion surrounding the validity of these species. The IUCN presumed distribution polygon (Wilms et al. 2013) for *C. senegalensis* is in need of a revision if the eastern-most records for *C. senegalensis* are accurate.

**Taxonomy**

The *C. dilepis* complex consists of three species-level clades which, pending a morphological reassessment of specimens from these areas, should each be described as their own species. Given that *Chamaeleo dilepis* 1 was sampled from across central Africa, and the type locality is “Congo” (Table 1; Fig. 1), this clade probably represents the nominate species and would retain the name *Chamaeleo dilepis*.

For both *C. anchietae* and *C. gracilis* there is cryptic diversity that is at odds with current subspecific assignments. *Chamaeleo anchietae* 1 was sampled from Huila Province in Angola, matching the type locality of the nominate species, making it possible to assign *C. anchietae* 1
to *C. anchietae anchietae* (Table 1; Fig 1). *Chamaeleo anchietae* 2 was sampled from both the DRC and Tanzania and the individual from the DRC was sampled in Kundelungu National Park which is near the type locality of *C. anchietae vinckei* (Katanga, DRC) (Table 1; Figs 1 & 7). Assuming that this pattern holds given an updated phylogeny with additional geographic coverage, both subspecies should be elevated to specific status, and the subspecies rankings should be abandoned. In this case, *C. anchietae anchietae* would retain the nominate name. Given that Malawian chameleons identified as *C. anchietae* (see Tilbury 2018; Fig. 7) from the Nyika Plateau fall geographically between both *C. anchietae* 2 sample sites from the present study (Fig. 7), it seems plausible that populations from Malawi are also part of the *C. anchietae* 2 clade. However, inclusion of Malawian *C. anchietae* samples in a phylogeny would confirm this.

In the case of *C. gracilis*, the present study adds two potential candidate species. *Chamaeleo gracilis* 3 from Liberia, Sierra Leone and Guinea represents *C. gracilis* given type locality is “Liberia” (Figs 1 & 8). Additionally, given the nesting of *C. necasi* within this clade, *C. necasi* should be synonymised with *C. gracilis*. *Chamaeleo gracilis* 1 and 2, while inferred from very limited sampling, strongly suggest the presence of at least two additional species within *C. gracilis*. Indeed, additional sampling with greater geographic coverage, might unveil additional species, or at least lend stronger support to the presence of these two candidate species. As well as being at the species level, the three clades do not match the subspecies distributions. This suggests that the subspecies concept should be abandoned for this group.

**CONCLUSION:**

The results presented here provide strong evidence for the presence of cryptic lineages within *Chamaeleo*, some of which can be considered candidate species. In particular, *C. dilepis* comprises three species, *C. gracilis* three species, and *C. anchietae* two species. Additionally,
the results suggest that *C. necasi* should be synonymised with the nominate species of *C. gracilis* (*C. gracilis* 3). *Chamaeleo laevigatus* and *C. senegalensis* are separate species. These findings suggest a revision of the taxonomy of *Chamaeleo* is needed incorporating morphological assessment. Some species were poorly sampled (*C. gracilis* 1 and 2; *C. anchietae* 2) and it is possible that more extensive sampling would reveal additional cryptic taxa, or at a minimum would lend support toward their description as separate species and a better understanding of their distributions. Given that CITES regulations and the issuing of export permits serve as a measure to ensure that exports are not detrimental to wild populations, an accurate understanding of the taxonomy of *Chamaeleo* may be fundamental in ensuring that species are not overexploited.

REFERENCES:


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Reid NM, Carstens BC. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC evolutionary biology 12: 196.


### Table 1: Species, field numbers, GenBank accession numbers for four genes and sampling localities of *Chamaeleo* used in the study, as well as outgroup taxa. TBA indicates sequence data that will be deposited in GenBank upon submission of a manuscript to a peer-reviewed journal. NA indicates not available.

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*Corresponding museum specimen catalogued as C. anchietae
Figure 1: A Bayesian consensus tree for the mitochondrial dataset for *Chamaeleo*. Node support is indicated for Bayesian and likelihood analyses.
Chapter 3

Biogeography of *Chamaeleo* and ecological partitioning of *Chamaeleo dilepis*

ABSTRACT:

*Chamaeleo* is a pan-African genus of chameleons that also extends into southern Europe, southern Asia, and the Middle East, with much geographic overlap and confusing phenotypic variation between its taxa. The large, overlapping ranges of members of *Chamaeleo* present an interesting but complex case for biogeographic ancestral area reconstruction. A previous study (Chapter 2) found strong evidence for the presence of cryptic species within the *Chamaeleo dilepis* complex. The aims of the present study were twofold: firstly, to reconstruct the ancestral biogeographic history of the genus *Chamaeleo* and secondly, to investigate whether there is evidence for niche partitioning between the three cryptic species within the *C. dilepis* complex. A time-calibrated phylogenetic analysis was run on a dataset encompassing all known species of *Chamaeleo*. Dispersal-Extinction-Cladogenesis (DEC) was used on this time-calibrated phylogeny to reconstruct the ancestral biogeography of *Chamaeleo*. Additionally, Maxent was used to carry out ecological niche modelling on the three clades within *C. dilepis*. The common ancestor of *Chamaeleo* emerged during the Eocene, most likely in the Zambezian region. *Chamaeleo* probably exploited mesic corridors that opened up during forest contractions of the Oligocene and Miocene to move into North Africa and Eurasia. Niche partitioning is evident between *C. dilepis* clades and only the suitable niche of *C. dilepis* 2 appears relatively stable since the late Pleistocene. These results provide a glimpse at the evolutionary biogeographic history of *Chamaeleo* and could aid future revisions of distribution maps pertaining to the *C. dilepis* complex.
INTRODUCTION:

Climate change is at the helm of evolutionary diversification on earth. As climate shifts, certain phenotypes will not be able to adapt to the new conditions, particularly if they have narrow thermal or moisture tolerances. Climate is also the basis for the prevailing vegetation and consequently the prevailing biome in a region (Pörtner 2001, 2002; Morley 2007; Somero 2010; Kissling et al. 2012). Together with geological change, climate change has played a pivotal role in the present day distribution of the planet’s fauna and flora. The study of biogeography is tasked with correlating the evolutionary history of different organisms with the timing of known geological and climatic changes (Cracraft 1975; Nelson 1985). Recent advances in molecular techniques have prompted a number of studies correlating a molecularly informed evolutionary history with known geological and climatic events (Tolley et al. 2006, 2011, 2013; Kuriyama et al. 2011; Measey and Tolley 2011; Barlow et al. 2013; da Silva and Tolley 2017; Jongsma et al. 2018).

*Chamaeleo* is a pan-African genus of chameleons with uncharacteristically (for chameleons) large ranges with overlap between species. The genus currently comprises 14 species, however, with large intraspecific variation, this number is likely an underestimate of the actual species diversity within the genus (Chapter 2). In Chapter 2, three species-level clades were revealed within the *Chamaeleo dilepis* complex. One of these clades was sampled from near the type locality (“Congo”) of typical *C. dilepis* and this clade therefore represents *C. dilepis* while the other two clades are additional candidate species within the complex. In contrast to the forest affinity of most other chameleon genera, *Chamaeleo* tends to occupy more mesic or xeric regions, with an affinity for savanna, grassland (in the case of *C. anchietae*) and even desert (Tilbury 2018). It is therefore possible that the genus originated in Southern Africa, coinciding with cooler and drier climate that prevailed in that region during the Oligocene and Miocene (Morley 2007; Feakins and deMenocal 2010). Given that multiple species delimitation methods...
suggested that the three *C. dilepis* clades each represent full species (Chapter 2), it is expected
that the bioclimatic preferences of these clades will be distinct from one another and that they
will show minimal overlap in suitable climatic extent through time. Such a finding would lend
support to the clades representing different species.

While species of the *Chamaeleo* genus tend to occupy large ranges in the present day, and they
appear to be mesic habitat generalists, it is still possible that their suitable climate space
contracted during paleoclimatic cycling of the Pleistocene. The Last Interglacial (LIG) covers
a period from 129 000-116 000 years ago during which some of the hottest African climates of
the Pleistocene were experienced (Partridge et al. 1997; Govin et al. 2014; Maslin et al 2014;
Tierney et al. 2017). Precipitation during this period varied considerably with latitude;
Southern Africa experienced a largely arid climate during this period while the central
equatorial African climate was wet and monsoonal. In contrast, the Last Glacial Maximum
(LGM), which occurred approximately 20 000 years ago, represents the last period of extensive
 glaciation at northern hemispheric latitudes and consequently resulted in cooler temperatures
in equatorial Africa (though no glaciation there). However, Southern Africa actually saw a
warming climate during the LGM (Wu et al. 2007). Given this, it is expected that, in line with
the apparently mesic bioclimatic preference of the *Chamaeleo* genus, the LIG and LGM would
have, in general, provided smaller suitable climatic envelopes than the present day for *C.
dilepis*. In particular, *C. dilepis* 1 and *C. dilepis* 3 are both centred more toward the lower,
tropical latitudes (Fig. 1) and so the monsoonal climate during the LIG would have presumably
contracted their suitable climate space.
Figure 1: Sampling points used for the Maxent ecological niche modelling analysis. *Chamaeleo dilepis* clades are colour coded: *C. dilepis* 1 = green; *C. dilepis* 2 = blue; *C. dilepis* 3 = purple.

The widespread distribution of *C. dilepis* 2 is centred more toward the higher latitudes reaching into the subtropics (Fig. 1) and this would be expected to favour the stability of this species’ suitable climate space through time, especially because the effects of the LIG and LGM were presumably more moderate toward the south.

To examine whether *Chamaeleo* did indeed originate in Southern Africa, the biogeographic history of *Chamaeleo* was evaluated using an ancestral area reconstruction carried out on a time-calibrated phylogeny of the genus. Additionally, to test whether there exists ecological support for *C. dilepis* species delimited in Chapter 2, suitable climatic space for each *C. dilepis* clade was modelled using the programme Maxent for the current day, the LIG and the LGM.
MATERIALS AND METHODS:

Biogeographic history

Using the combined four-gene dataset from Chapter 2, a time-calibrated phylogeny was estimated in BEAST 2 (Bouckaert et al. 2014). With a paucity of fossil data for *Chamaeleo* and much uncertainty as to the taxonomy of the few fossils available (Bolet & Evans 2014), it was decided that a secondary calibration point would be incorporated in lieu of fossil calibrations. The large-scale fossil-calibrated phylogeny of the Chamaeleonidae family was used as a source for the secondary calibration point for the most recent common ancestor of *Chamaeleo* (Tolley et al. 2013). A lognormal distribution with an offset mean age of 43 million years ago (mya) and a standard deviation of 5 mya was applied and this produced a distribution that closely reflected Tolley et al. (2013)’s 95% HPD confidence intervals for the same node. The origin of the family has been shown to be African (Tolley et al. 2013), but that analysis included all chameleon genera as well as a sister family (Agamidae). The current analysis was restricted to understanding the history within *Chamaeleo*, not the entire family. Therefore, the inclusion of other genera and sister families would produce a time-tree with deep nodes, that are not necessary for the present analysis, and could potentially bias the dates for the target clade backwards. Therefore, one outgroup genus was included and the root of the tree was coded as having an African origin, as in Tolley et al. (2013).

The dataset was partitioned by marker, applying optimal substitution models inferred using jModelTest 2 (Darriba et al. 2012 - 16S: GTR; ND4 GTR + I + G; PRLR and RAG1: HKY + G). PRLR and RAG1 were unlinked for substitution models and clock models. Given that they form part of the same locus, the mitochondrial markers (16S and ND4) were linked across substitution models and clock models. Trees were linked across all markers. A lognormal relaxed molecular clock (Drummond et al. 2006) was set for all markers and a Birth-Death tree prior was used. Two independent runs were carried out with the analysis set to 200 million
generations, sampling every 5,000 trees. Convergence was assessed for each run in Tracer 1.7 (Rambaut et al. 2018) and determined adequate when the ESS for each parameter ≥ 200. The last 10,000 trees of both runs were then combined in LogCombiner 2.5.1 (Bouckaert et al. 2014) and a maximum clade credibility tree with mean tree heights was determined in TreeAnnotator 2.5.1 (Bouckaert et al. 2014). All BEAST analyses were run on the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal/).

An ancestral area reconstruction for the genus *Chamaeleo* was made using the time-calibrated phylogeny, with terminal tips coded to the following biogeographic regions: A = Eurasia (Europe, Asia and the Middle-East), B = North Africa, C = Congolian, D = Sudanian, E = Ethiopian-Somalian (includes Ethiopian and Somalian sub-regions), F = Zambezian, G = Southern African (see Fig. 3 inset). These areas were chosen on the basis of biogeographic partitioning of the African continent (Linder et al. 2012), and were broad enough to prevent overparameterization whilst still allowing for the hypothesis that *Chamaeleo* had Southern African origins to be tested. The Dispersal-Extinction-Cladogenesis (DEC) reconstruction approach implemented in the programme Reconstruct Ancestral State in Phylogenies (RASP: Yu et al. 2015) was used to carry out an ancestral area reconstruction on the time-calibrated phylogeny. Dispersal probabilities between regions were set according to geographic proximity of regions. That is, regions not bordering each other were given low probability of dispersal between them. Time-dependent dispersal constraints between regions were also set, based on paleo-vegetation maps of Morley (2007) and Kissling et al. (2012). Given the relatively xeric tolerability and savannah affinity of most *Chamaeleo* species (Tilbury 2018), the late Eocene climatic optimum of approximately 50 mya that promoted forest expansion was presumed unfavourable to *Chamaeleo* diversification and dispersal. Conversely, arid episodes of the Miocene (ca. 20-13 mya) were assumed to have favoured *Chamaeleo* dispersal and diversification and dispersal probabilities were set to account for these assumptions (Morley...
Geographically adjacent regions were given a dispersal probability of 1.0 between them during favourable climatic dispersal conditions and this was reduced to 0.5 during unfavourable dispersal conditions. Regions not geographically adjacent to one another were given dispersal probabilities corresponding to their proximity to one another, regions with another biogeographic region in between them were given a dispersal probability of 0.25 during favourable conditions which was reduced to 0.1 during unfavourable conditions (Table 1, Appendix IV). For regions with two or more biogeographic regions between them, dispersal was constrained to a probability of 0.

**Niche modelling**

Current climatic niche

Two new candidate species were revealed for *Chamaeleo dilepis* in Chapter 2 (Clades 2 and 3). To investigate potential climatic partitioning between these candidate species and the nominate species (Clade 1), niche modelling was carried out in the programme Maxent 3.1.4 (Phillips and Dudík 2008; Elith et al. 2011). There are many records for *C. dilepis* that were not used in Chapter 2’s phylogeny, therefore, the general location of each *C. dilepis* clade from the phylogeny was used to guide the assignment of additional records to clades. These records were taken from Tolley et al. (2016)’s species occurrence dataset assimilated from various sources (GBIF; HerpNet; Natural History Museum London; Bates et al. 2014). This increased the sampling complement to 80 individuals for *C. dilepis* 1, 1062 individuals for *C. dilepis* 2, and 80 individuals for *C. dilepis* 3 (Fig. 1). A total of 19 bioclimatic variables were downloaded from WorldClim (Hijmans et al. 2005; Warren et al. 2008). These variables are averaged over 50 years and include temperature and precipitation data. Each clade was analysed separately in Maxent and duplicate points were removed from the analysis. In each case, the most suitable of the 19 bioclimatic variables were determined by running preliminary models. These models were run under the default programme settings except the maximum number of iterations was...
set to 5000 to allow for the models to converge. For each model, ten replicates were run, each
with a random subsample of 25% used for testing so that the dependence of the models on
presence data could be determined. For each clade, the averaged results from the 10 runs were
examined and in each case the area under the curve (AUC) and the receiver operating
characteristic (ROC) plots were noted. This information, together with the three jackknife tests
(AUC, test gain, and regularized training gain) was used to refine the bioclimatic variable
dataset, omitting variables that did not contribute more than 1% to the model or permutation
importance. Additionally, any variable whose presence hampered the model’s predictive
performance was also excluded. The model was then re-run on this refined dataset and the
above process was repeated again until all variables used contributed favourably to the model’s
predictive performance.

Past climatic niche

While presence data is only available for the current day, paleoclimatic reconstructions of the
19 bioclimatic variables used above are made available by WorldClim for both the Last
Interglacial (LIG) and the Last Glacial Maximum (LGM) at 30-arc second and 2.5-arc minute
resolution respectively. Although the LIG and LGM both occurred during the Pleistocene,
which is far more recent than the diversification within the C. dilepis clade (see Results; Fig.
2), these two time slices represent the most extreme climates experienced during the
Pleistocene and allow for the reconstruction of more recent climate-driven range expansions
and contractions. For each clade, the current climatic niche modelled above was projected
back onto the LIG and LGM bioclimatic variables after the climatic dataset had been refined
only to include the most informative variables.
RESULTS:

Within *Chamaeleo, C. namaquensis* diverged from other species approximately 40 mya (Fig. 2), followed by the divergence of *Chamaeleo anchietae* during the late Oligocene (*ca.* 29 ± 5 mya). Eurasian taxa split from other *Chamaeleo* during the early Miocene (*ca.* 20 mya) and most species level diversification within the Eurasian clade occurred in the mid-Miocene. Diversification within the *C. dilepis* clade, and for the other African species *C. senegalensis*, *C. gracilis*, and *C. laevigatus*, occurred from the late Miocene to the start of the Pliocene (Fig. 2).
Figure 2: A time-calibrated phylogeny of *Chamaeleo*. 95% High density probability confidence intervals are indicated by blue bars. Black circles represent nodes with ≥ 0.95 posterior probability support. Geologic epochs are indicated.
Biogeographic history

The ancestral area reconstruction suggests that the most recent common ancestor (MRCA) of *Chamaeleo* most likely originated within the Southern African/Zambezian or Southern biogeographic regions (Fig. 3). *Chamaeleo namaquensis*’ sister taxon relationship with the rest of *Chamaeleo*, together with its Southern African distribution suggests that the MRCA of the genus was initially to the south with additional species diversifying to the north. Further support for this northward dispersal and diversification hypothesis is given by the more recent dispersal of *Chamaeleo* out of Africa and into Eurasia in the early Miocene (ca. 23 mya ± 5mya) which suggests their presence in north Africa was recent (Fig. 2 and 3).
Figure 3: A time-calibrated phylogeny of *Chamaeleo* and a representative of its closest related genera showing the results of a DEC ancestral area reconstruction. Coloured doughnut charts represent the proportional likelihood values of each ancestral area. Letters at each terminal tip indicate the input area coding for that taxon. Ancestral areas of negligible proportional likelihood are combined and shown in black.
Niche modelling

The variables that best explained the suitable climatic niches of *C. dilepis* clades were in most cases related to precipitation (Table 2). In particular, precipitation of the driest month (Bio 14) and annual precipitation (Bio 12) were found to be of the three best explaining variables for the *C. dilepis* 1 and *C. dilepis* 3. Temperature appears important in the niche suitability of *C. dilepis* 2 with two of its three best explaining variables pertaining to temperature: temperature seasonality (Bio 4) and mean temperature of the driest quarter (Bio 9).

**Table 2**: The three most important bioclimatic variables for the prediction of suitable niches for three clades of *Chamaeleo dilepis* and their respective contributions to the model.

<table>
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<th>Species</th>
<th>Bioclimatic variable</th>
<th>Detail</th>
<th>% contribution*</th>
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<td></td>
<td>Bio 9</td>
<td>Mean temperature of driest quarter</td>
<td>7.4 (17.1)</td>
</tr>
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<td>Precipitation of driest month</td>
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<tr>
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<td>18.7 (17.4)</td>
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<td>Bio 15</td>
<td>Precipitation seasonality</td>
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*The permutation importance of each variable is indicated in brackets next to the percentage contribution*

The current suitable niches for each of the clades (Fig. 4) are reasonable approximations of the distribution of points that were input, suggesting that the model is not under- or over-predicting (Appendix III, Fig. 1). There may be greater connection between *C. dilepis* 1 and *C. dilepis* 3 in the present day and LGM than during late Pleistocene (LIG). While the inland suitable climate of *C. dilepis* 2 did not extend as far south in the LGM as it does currently, LGM predictions for all *C. dilepis* clades are largely similar to the current day. A slightly larger area of suitable climate space is evident during the LGM than current day for *C. dilepis* 3 and for *C. dilepis* 1 the opposite is true however in both cases these differences are negligible. During the LIG, *C. dilepis* 2 and *C. dilepis* 3 may have experienced a contraction in their suitable
climate space with the suitable climate space of *C. dilepis* 1 shifting northwards, *C. dilepis* 2 contracting slightly shifting westwards, and *C. dilepis* 3 contracting into small pockets.
Figure 4: Predicted suitable climate space of *Chamaeleo dilepis* clades based on a minimum training presence logistic threshold for three time slices (Last Interglacial, Last Glacial Maximum, and Current). Probability of climatic suitability is indicated to the left of the figures for each species. Areas of overlap in suitable climate space between clades are indicated in black.
DISCUSSION:

Biogeographic history:

Initial diversification within the *Chamaeleo* genus coincides with the onset of global cooling during the late Eocene which ended approximately 34 mya. *Chamaeleo namaquensis* and the *C. anchietae* clade showed an early divergence from the rest of the genus, around 40 and 30 mya, respectively. Divergence among the other species occurred primarily in the Miocene, starting around 25 mya. Global retraction in forests (Morley 2007, Kissling et al. 2012) that became prevalent during the Miocene could have made the Congolian and Zambezian regions more penetrable for *Chamaeleo* allowing them to expand northward and diversify as the forests retracted. Diversification within the *Chamaeleo dilepis* clade took place from the late Miocene to the Pliocene (ca. 10-5 mya; Figs 2 & 3).

The early Eocene presence of forest in the tropics would have likely made dispersal through the Congolian and northern Zambezian regions challenging for the apparently more mesic/arid adapted *Chamaeleo* genus. *Chamaeleo namaquensis* is arguably the only chameleon occupant of true desert in the world and currently confined to the Southern African sub-region. This species diverged from the rest of *Chamaeleo* during the mid-Eocene (ca. 42 mya) and the ancestral area reconstruction suggests that it was originally in Southern African/Zambezian region (Fig. 3). This finding supports what was hypothesized at the onset of the study, that *Chamaeleo* originated in Southern Africa. *Chamaeleo namaquensis*, due to its affinity for desert, is in fact more terrestrial than it is arboreal, with shorter limbs and a shorter tail than relative to body size than other arboreal chameleons (Herrel et al. 2013; Tilbury 2018). If the ancestral body type of *C. namaquensis* was similar to that of today, the tropical forests of the Eocene would have probably been impenetrable to a terrestrial chameleon with a phenotype adapted to arid conditions. In the 15 million years following the initial divergence of *C. namaquensis* from the rest of *Chamaeleo*, aridity in southwest Africa (Feakins and deMenocal...
intensified and this probably favoured diversification for other species in the genus. 

*Chamaeleo anchietae* diverged, approximately 29 mya during the Oligocene (Figs 2 & 3). This species currently occupies mesic, high altitude montane shrubland and grassland plateaus and its phenotype appears less arboreally adapted than other species in the genus (Tilbury 2018). The current known distribution of *C. anchietae* is fragmented with pockets in high altitude plateaus of south-western Angola, south-eastern DRC, central Tanzania and the Nyika Plateau in northern Malawi. These pockets seem to track the distribution of an arid corridor connecting southwest Africa with the Horn of Africa which is thought to have formed during the Miocene (Bobe 2006). It makes sense for a species with a propensity for a relatively temperate climate and open habitat to have made use of such a corridor to disperse, especially given that its phenotype might have hindered its dispersal through dense forest. Prior studies have shown this to have been the case for xeric amphibians, large mammals and certain arid flora (Poynton 1995; Bobe 2006; Bellstedt et al. 2012).

During the early Miocene, Eurasian species dispersed out of Africa (*ca.* 23 mya). Globally, the onset of the Miocene brought warmer temperatures which resulted in the expansion of forests in most of the tropics, however, in Africa, this period is thought to have undergone a reduction in forest, with an expansion in grasslands (Morley 2007). This would have opened up corridors (like the arid corridor mentioned above) for *Chamaeleo* species to disperse north of the Congolian region into North Africa and the Ethiopian region and subsequently into Eurasia. Species level diversification appears to have reached a peak from the mid to late Miocene (*ca.* 15-5 mya) during which global cooling and drying favoured the replacement of much African forest with more temperate savannah grassland, opening up more niches for chameleons with less of a propensity for forest (Morley 2007; Kissling et al 2012; but see also Ceccarelli et al. 2014).
The *C. dilepis* clade likely emerged in the Zambezian and Congolian regions approximately 10 million years ago, towards the end of the Miocene and the beginning of the Pliocene. This follows the gradual replacement of forested habitats with grassland in central East Africa, however, the early-to-mid-Pliocene superseding this global cooling and aridification actually resulted in hotter and wetter climates in sub-Saharan Africa (deMenocal 2004; Feakins and deMenocal 2010). This would have increased forest cover, especially in equatorial regions, which might have fragmented areas of suitable habitat for *C. dilepis* and this may have favoured allopatric speciation within the *C. dilepis* complex.

Ecological speciation typically follows populations diverging into distinct ecological niches; this ensures that the two populations evolve independently without any gene flow between them eventually to become separate species (Losos et al. 1998; Losos and Ricklefs 2009). Accordingly then, closely-related species that are parapatric should be expected to inhabit different niches. Therefore, ecological niche modelling is a valuable tool to assist in the validation of species that have been inferred on a phylogenetic basis (Raxworthy et al. 2007; Blair et al. 2013; da Silva and Tolley 2017). While the models used here are based entirely on climate and therefore carry the limitation of not accounting for other influences such as vegetation, elevation, terrain or interspecific competition, they still allow for some general inferences to be made. Firstly, they show support for the presence of distinct, non-overlapping climatic niches for each clade (Fig. 4), with unique bioclimatic preferences among clades (Table 1). This may have promoted the clades being spatially separate. Secondly, the models suggest that there was essentially no overlap between niches during the LIG and LGM and so the spatial separation of the niches appears stable through time, which may maintain their genetic distinctiveness. If anything, the clades are more connected spatially now than they were during the late Pleistocene. In the phylogeny (Fig. 2), *C. dilepis* 3 diverges from the MRCA of the *C. dilepis* complex first and this suggests that *C. dilepis* 1 and 2 were possibly more connected
spatially in the past. This is however not reflected by the models, which indicate greater connectivity between *C. dilepis* 1 and 3 than between *C. dilepis* 1 and 2. This suggests that the more recent (late Pleistocene) paleoclimatic models cannot be used as a proxy to understand diversification that took place in the late Miocene. Instead, something happened in the late Miocene that promoted the divergence of *C. dilepis* 3 from the rest of *C. dilepis*. One possibility is geological influences. The Albertine Rift is the western branch of the East African Rift which runs southward from Lake Albert in Uganda to Lake Malawi in Malawi. This rift is thought to have started forming as a consequence of volcanic activity in the mid-Miocene (13-12 mya) (Harðarson 2014). Given that this rift lies at the interface of the distributions of *C. dilepis* 1 and *C. dilepis* 3 and that the formation of the rift and the divergence of *C. dilepis* 3 from the greater *C. dilepis* clade are almost coeval (*C. dilepis* 3 diverged after the rift started forming, but the rift formed very slowly), it seems plausible that the formation of this rift might have led to the isolation and subsequent divergence of *C. dilepis* 3.

As hypothesized, suitable climate space for all three clades showed contractions during the LIG. This contraction in suitable climate space was least severe for *C. dilepis* 2 and most notable for *C. dilepis* 3. This was expected given the higher latitude of *C. dilepis* 3 which saw more arid climates during the LIG than the equatorial *C. dilepis* 1 and *C. dilepis* 3 which would have experienced, hot, wet monsoonal climates during this period (Govin et al. 2014). The suitable niche of *C. dilepis* 2 appears the most stable through time, at least during the paleoclimatic cycling of the Pleistocene.

**CONCLUSION:**

The results presented here suggest that *Chamaeleo* originated during the Eocene in the Southern African and Zambezian bioregions and made use of mesic corridors produced from Oligocene climatic cooling and central African forest contractions during the Miocene to
infiltrate central, East and North Africa, Ethiopia and Eurasia. Additionally, there is evidence
for niche partitioning between clades within the *C. dilepis* complex. The climatic niche of *C.
dilepis* 2 appears relatively stable through the late Pleistocene while that of *C. dilepis* 1 and *C.
dilepis* 3 appear sensitive to climatic warming as the models predicted shifts or contractions,
respectively. These results lay a foundation for a reassessment of the current distribution of
taxa within the *C. dilepis* complex. IUCN distribution maps currently exist for the species
complex on the whole, however, given that there appear to be at least three species within this
complex, updated distribution maps will need to reflect this if the taxonomy is revised.

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Figure 1: Current day predictions of suitable climate for three clades of *C. dilepis* with presence records shown.
### Table 1: Dispersal probabilities for different time periods used in the DEC analysis

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1553 1554 1555
Chapter 4

General Conclusion:

The underlying premise of this dissertation was the driving need for a molecularly informed revision of the taxonomy of the *Chamaeleo* genus. Large, overlapping distributions and puzzling phenotypic variation between certain species (*C. dilepis*, *C. gracilis*, *C. anchietae*, *C. necasi*, *C. senegalensis*, and *C. laevigatus* in particular) have confounded the group’s morphological taxonomy. Tilbury and Tolley’s (2009) reappraisal of *Chamaeleo*, which resulted in the elevation of the sub-genus *Trioceros* to its own genus, highlighted a paucity in our understanding of the evolutionary history of these taxa. Prior to this dissertation, few studies had investigated the systematics of members of *Chamaeleo* molecularly, however, many authors had commented on the taxonomic quandary of the *C. dilepis* complex (Klaver and Bohme 1986; Klaver and Bohme 1997; Largen and Spawls 2006), and as Tilbury (2018) put it, “*Chamaeleo dilepis* single-handedly demonstrates the limitations of a taxonomic system which relies heavily on external morphology and the influences on this of ontogenic change, sexual dimorphism, altitudinal effects on phenotype and simple intraspecific variation on morphology.”. Main et al. (2018) found large intraspecific differences between *C. dilepis* clades in South Africa and suggested that there might be cryptic species within the *C. dilepis* complex. Main et al. (2018)’s findings, however, did not allow for conclusions to be made regarding species as the study was preliminary in nature and did not include sampling from across the range of *C. dilepis* and did not include all species in the genus.

Accordingly, the first aim of this dissertation was to carry out a genus-wide molecular phylogeny of *Chamaeleo*, including all species representatives and sampling across the natural ranges of most species in order to disentangle the evolutionary relationships within the group.
Furthermore, a second aim was to investigate cryptic speciation in the genus using two model-based species delimitation methods (bGMYC and BPP) and distance based species delimitation as the larger sampling complement of this genus-wide phylogeny would help overcome the limitations of Main et al. (2018). These two aims underpinned Chapter 2 of this dissertation. Not surprisingly, pervasive cryptic diversity was uncovered, with the phylogenetic analysis revealing 18 species in contrast to the currently recognised 14 species within *Chamaeleo*. In particular, cryptic diversity was evident in three species with *C. anchietae* comprising the nominate species as well as *C. anchietae vinckei* which, pending a morphological re-evaluation, should be elevated to specific status, *C. dilepis* comprising the nominate species as well as two additional candidate species and *C. gracilis* also comprising the nominate species and two additional candidate species. Twelve of the 14 recognised species were shown to be monophyletic, however, *C. necasi* shares a terminal lineage with the nominate form of *C. gracilis*, putting the current taxonomy of *C. necasi* into question and suggesting that it should be synonymised with *C. gracilis*. Furthermore, *C. senegalensis* and *C. laevigatus* were recovered as independent species, putting to rest the debate pertaining to their taxonomic status (Joger 1990; Chirio and LeBreton 2007; Trape et al 2012; Tilbury 2018). *Chamaeleo namaquensis* was recovered as the sister taxon to the rest of *Chamaeleo* and a Eurasian clade of *Chamaeleo* was found to be monophyletic.

The large, imbricating ranges of *Chamaeleo*, together with an apparent tolerance of more arid climates (compared to other chameleon genera) and an affinity for mesic savannah (Tilbury 2018) probably contributed to the widespread cryptic diversity within the group, with prevailing morphological conservatism making it difficult to discern all species morphologically. Understanding phylogenetic relationships and uncovering cryptic diversity on the genus scale were just two pieces of a complex evolutionary puzzle. Accordingly, unravelling the biogeographic history and investigating the geo-climatic drivers behind the
evolution of *Chamaeleo* became a key focus of the third chapter of this dissertation. An ancestral area reconstruction of the genus was made in order to test a hypothesis that the genus originated in Southern Africa. Additionally, ecological niche modelling was implemented for the *C. dilepis* clades delimited in Chapter 2 in order to determine whether there exists ecological support for the clades as distinct species.

*Chamaeleo* was found to have likely diverged from other genera in Southern Africa or Zambezia biogeographic regions approximately 50 mya, a period that corresponds loosely with the early Eocene climatic optimum which was characterised by hotter, wetter climates globally. This supported the hypothesis that *Chamaeleo* originated in Southern Africa. Internal diversification within the genus was shown to have initiated approximately 39 mya, towards the end of the Eocene which coincides broadly with a period of global cooling (Morley 2007; Kissling et al. 2012). *Chamaeleo namaquensis*, the only chameleon occupant of true desert, was found to branch off from the rest of *Chamaeleo* first, with intensified southwest African aridity during the Oligocene and Miocene presumably promoting its independent diversification from other species in the genus. The next species to diverge from the rest of *Chamaeleo* was *C. anchietae*, approximately 29 mya in the Oligocene, and this species likely made use of an arid corridor that opened up during the Miocene (Bobe 2006) to disperse north-eastwards from southwest Africa. Eurasian *Chamaeleo* species were shown to have dispersed out of Africa during the early Miocene, 23 mya. This period coincides with globally warmer temperatures but a replacement of forest with grasslands in East Africa which, due to their mesic habitat preference, would have opened corridors for the dispersal of *Chamaeleo*. Most species-level diversification in *Chamaeleo* was found to have occurred during the mid-to-late Miocene (15-10 mya). *Chamaeleo dilepis* was found to diversify in the late Miocene and early Pliocene, as with the diversification of other species in the genus, this diversification appears to have followed periods of cooling and aridity resulting in the replacement of forest by
grasslands. For *C. dilepis*, the period following its diversification (the mid-Pliocene; 5-3 mya) was hot and wet (deMenocal 2004; Feakins and deMenocal 2010), and would have favoured the expansion of forests. These forests might have fragmented *C. dilepis* and a lack of gene flow between these fragmented populations might have promoted allopatric speciation with the complex. It is also possible that the formation of the Albertine Rift was responsible for the isolation of *C. dilepis* 3 from the rest of the species complex.

Ecological niche modelling revealed that each clade of *C. dilepis* had its own bioclimatic preference and occupied climatically unique areas. Minimal overlap was evident for suitable climate space between clades, even during the LGM and LIG. This supported the status of these clades as independent species. Given that *C. dilepis*, diversified at the end of the Miocene, several million years prior to the LIG and LGM, climate models for these time periods could not be used to unravel finer patterns pertaining to the diversification of *C. dilepis*, however, the suitable climate for these clades appears to have been even less connected during the LIG and LGM than it is now. Because the LIG and LGM represent the most extreme climates experienced during paleoclimatic cycling of the Pleistocene, it seems plausible to suggest that barriers to gene flow have persisted since the LIG. The suitable climatic niche of *C. dilepis* 2 was found to be the most stable through time, while that of *C. dilepis* 3 was found to be the least stable through time, fragmenting into small pockets during the LIG.

The findings of this dissertation have shed light on the evolutionary history and taxonomy of *Chamaeleo*, uncovering pervasive cryptic diversity and suggesting that the group’s diversification tracked historical climatic cooling and aridification in Africa which promoted a contraction of forests and an expansion of savannah and grasslands. Perhaps most importantly, this dissertation provides a foundation from which to re-evaluate both the taxonomy and the known distribution of various members of the genus. One of which, *C. dilepis*, has caused
notable confusion among taxonomists in the past, and these findings will help ameliorate much
of this confusion. This dissertation does however carry some limitations, for certain species (C.
gracilis 1 and 2; C. anchietae 2) there is limited sampling coverage. Additionally, niche
modelling was constrained by the use of only climatic data and thus the inclusion of vegetation,
elevation or terrain data may allow for more confident inferences to be made. These limitations
provide ample scope for further research on this enigmatic genus of reptiles. Greater sampling
coverage in the taxa poorly sampled here might even reveal more cryptic diversity within the
genus.

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