Comparative Phylogeography and species delimitation of the Arabian Peninsula lizards

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Abstract

The Arabian Peninsula is an interesting area for research. This area has a complex geological history, a range of habitats and a number of known endemic species. However, to date, this area and its associated biodiversity has been poorly studied. The aims of this study were to investigate the phylogeography and to determine the species delimitation of a selected number of lizard species from the Arabian Peninsula.

The phylogeography of fourteen co-distributed lizard species occurring within the Arabian Peninsula was investigated using a multispecies tree in STAR BEAST (*BEAST) to determine the divergence times and spatial patterns of the co-distributed species. Several common spatial and temporal patterns were identified among the different Arabian Peninsula species. The common patterns indicated close phylogeographic relationships between different regions and species. Importantly, these common patterns also corresponded to historical biogeographic processes. A wide range of ecological habitats was also detected for these groups of lizards and this was assumed to play a major role in establishing the current diversity and distribution patterns. In addition to detecting common patterns, this study also provided valuable information about the unique phylogeographical patterns shown by some of the studied species. Finally, this study also revealed patterns that provided strong evidence for the presence of multiple cryptic species within a species complex.

Species delimitation methods were subsequently applied to two species that had previously demonstrated the potential for cryptic species within *Acanthodactylus boskianus* and *A. opheodurus*. Using a combined approach of genetic distance, allele networks, and Bayesian Phylogenetic and Phylogeography (BPP) analysis, this study was able to identify candidate species within *A. boskianus* and *A. opheodurus*. The mitochondrial DNA tree revealed potential candidate clades within these two species. These candidate species clades were then further examined at two nuclear loci and congruence was observed between the two markers for these clades. This congruence between mitochondrial and nuclear loci strongly indicates the discovery of several new species within *A. boskianus* and *A. opheodurus*, however further research is needed to confirm this discovery.

In conclusion, this study provides the most detailed insight - to date - on the phylogeography and species delimitation of Arabian Peninsula lizards and provides the most up to date assessment of the diversity of the lizards in this important region.

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1. General introduction

1.1 Phylogeography and Species Delimitation

The term 'Phylogeography' was first coined in 1987 (Avise et al., 1987). Phylogeography is a relatively recent field of study which seeks to explain the history of organisms over both space and time using genetic information, traditionally mitochondrial DNA gene trees (Avise et al., 1987). Single-species phylogeography can be utilised to reconstruct the biogeographical history of a single species, using molecular techniques (Edwards and Beerli, 2000). Comparative phylogeography seeks to determine historical patterns in selected groups of co-distributed taxa under study and to identify the events that may have shaped their current distribution over both space and time (Hickerson et al., 2010).

Phylogeographic approaches can identify the distinct gene lineages that might represent a species. Statistical methods for species delimitation can then be applied to these identified groups. Thus, phylogeography can be used to identify potential candidate species which can subsequently be tested by species delimitation methods (Leavitt et al., 2007, Sites and Marshall, 2004, Morando et al., 2003). In particular, phylogeographic approaches can reveal the likely presence of cryptic species or more than one distinct lineage within species, based on the structure of the geographical distribution patterns of lineages (Riddle et al., 2008).

Species are regarded as a fundamental unit of biology (De Queiroz, 2007), however much controversy surrounds the definition of the species category, Many different criteria, or so called 'species concepts' , have been utilised to define the species category, these range from biological (e.g. interbreeding) and ecological (e.g. niche adaptation) to genetic species concepts such as the phylogenetic species concepts (De Queiroz, 2007). Species delimitation is the process of inferring boundaries and numbers of species (De Queiroz, 2007) and usually focuses on identifying distinct species within any group under study. Unlike the relatively recent field of phylogeography, species delimitation has been an ongoing field of endeavour since Darwinian times and before, possibly dating back to the origins of human evolution. Traditionally, species delimitation has been based largely on morphological

differences (Arnold, 1986b) however, advances in molecular and genetic techniques have improved species delimitation methods (e.g. allozyme markers (Porter et al., 1997), mitochondrial DNA (Lumley and Sperling, 2010), and microsatellites (Vanhaecke et al., 2012). In addition, statistical or empirical methods can now be applied to differentiate species based upon divergence times from specific gene lineages (Yang and Rannala, 2010).

The identification of cryptic species complexes across many different genera and the application of advanced, molecular species delimitation methods have identified many new species. In Europe, the application of molecular techniques have almost doubled the known number of amphibian species and in Neotropical regions amphibian species diversity is still heavily underestimated (Fouquet et al., 2007, Veith, 1996). In Australia, a recent study has suggested that the species diversity of Australian geckos has more than doubled in the previous two decades, from 13 to 29 species (Oliver et al., 2009). Recent studies on the Arabian Peninsula lizards have identified 3 new species (Nazarov et al., 2013) and 8 new species of geckos (Carranza and Arnold, 2012), and one species of agamid (Melnikov and Pierson, 2012). These studies highlight the importance of continuing species assessment, including perceived, well defined species in highly industrialised countries (Oliver et al., 2009). For example, in 2012 a new species of leopard frog was discovered in New York City (Newman et al., 2012). In addition, species biodiversity is of great interest in species of conservation concern. For example, the spotted eagle ray (*Aetobatus narinari*) was previously thought to only represent one species, however, recent studies have shown that there may be more than two geographically separate species (Richards et al., 2009). Likewise, the recent analysis of global manta ray (*Manta birostris*) populations has identified two separate species, whereas previously it was thought that only one existed (Marshall et al., 2009). These findings will have important implications for global conservation efforts. Obtaining accurate species biodiversity is therefore essential in order to predict how species will respond to both localised and global change e.g. climate change (Thuiller, 2007) and is essential for species of conservation interest or concern.

The potential applications of both phylogeography and species delimitation are of global importance. Phylogeography is not just for systematists to identify the previously recognised history at the population level within species (Vences and Wake, 2007), it also

has the potential to identify geographical areas of high intraspecific genetic diversity which may support conservation efforts, with particular regard to threatened or endangered species (Newton et al., 1999). In addition, phylogeography and species delimitation are both vital tools that can be applied to the advancement of taxonomy. Taxonomic advances in species description and the application of accurate species limits are of great importance in assisting conservation efforts particularly with regard to threatened or endangered species (Mace, 2004).

1.2 Methodological aspects of phylogeography and species delimitation

Mitochondrial DNA (mtDNA) has long been the traditional marker in phylogeography (Avise, 2009). In many studies, it has been used as an initial indication for the presence of cryptic species. However, despite the utility of mtDNA due to its rapid evolution, the lack of recombination and conservative arrangement of its genes (Hickerson et al., 2010), the fact that it represents a single linkage that is inherited maternally (Avise, 2009), means that mtDNA patterns tell only a part of the historical story of genetic variation. As a result, there has been a considerable reduction in its use as a sole marker either for phylogeography or systematic applications over the last two decades. Thus, the proportion of studies that depend on mitochondrial DNA alone in animals or chloroplast DNA in plants, especially in phylogeography, has declined from 90% to 62% over the last ten years, whereas the use of nuclear markers (nDNA) has increased exponentially (Beheregaray, 2008). While mitochondrial DNA markers still provide useful information and remain a powerful tool for providing initial indications of the phylogeographic structure of the populations under study (Joseph and Omland, 2009), these patterns can be interpreted more completely by incorporating nuclear markers (Hare, 2001).

Technological advances have allowed nuclear DNA markers to become increasingly widely used over the last 20-30 years, starting with mini and microsatellites in the early 1990s (Beheregaray, 2008). Single copy nuclear gene sequences have only become established more recently as more suitable genes have been identified (e.g.Townsend et al., 2008). Unlike mtDNA, nuclear markers are particularly helpful when investigating aspects such as

hybrid zones, incomplete lineage sorting, and the extent of gene flow across potential species borders (Wiens and Penkrot, 2002). Consequently, combining mtDNA and nDNA is increasingly becoming a common approach in phylogeography and in systematic studies.

The increasing focus on multilocus approaches in phylogeography has led to the development of appropriate and more advanced methods of analysis for increasingly comlex datasets. Originally, the most frequently used approach for phylogeographic research was the single gene tree (Beheregaray, 2008). Such gene trees have allowed major advances in the field of molecular ecology, but disadvantages such as the incomplete outcome of the gene tree, the discrepancy with species trees, conflicting topology, and the occurrence of incomplete lineage sorting, limit their usefulness. The increasing use of multilocus sequence data has led to the development of novel analytical approaches, in particular the multispecies or species tree inference (Brito and Edwards, 2009). The elucidation of the species tree becomes the target rather than the single gene tree derived from a single locus, and as a result, single locus approaches are now increasingly uncommon (Dolman and Moritz, 2006). Combining genetic data with species tree inferences yields the multispecies coalescent model. Incongruence in gene trees that results in incomplete lineage sorting can be taken into account if the gene lineage evolution is modelled as a coalescent process that is influenced by population size and mutation rate (Heled and Drummond, 2010, Liu, 2008).

Many studies have attempted to understand and resolve the problems that result in discordance between a gene tree and a species tree by incorporating data from multiple loci (Edwards and Beerli, 2000, Carstens and Knowles, 2007). The basic idea underlying the coalescent theory is that when individuals from one or more populations have been sampled, the tracking of the historical lineages of these individuals ends where they coalesce at one point called the common ancestor. This method of tracking can reflect the genetic diversity of the population from the past and also show the present situation (Joseph and Omland, 2009). However, according to the multiple coalescent theory, every gene shows its relationship with orthologous genes in a small sample of organisms taken from a multi- species population. It is assumed that they did not reveal horizontal gene flow or admixture between them (Heled and Drummond, 2010). Such species tree information

can be obtained from the branching order of the taxa through time (Heled and Drummond, 2010). The species tree contains multiple gene trees and is based on a stochastic coalescent process—the so-called multiple species coalescent (Heled and Drummond, 2010, Rannala and Yang, 2003).

Estimating divergence time is considered to be an important role of the coalescent approach with a suitable model for the type of dataset investigated (Knowles, 2004), and novel analytical methods have expanded the utility of divergence time of groups of organisms for phylogeographic purposes (Kumar, 2005). Estimation of divergence times allows the detection of common patterns in time as well as space, and thus the formulation of hypotheses about the phylogeographic history of co-distributed species. Newly developed divergence time methods have been intensively used, and these methods have the ability to estimate divergence time by calibration of the evolutionary rates of molecular mutation across a population. Consequently, these methods provide valuable information regarding the diversity and the history of the divergence time of species (Kumar, 2005).

Estimates of divergence times remain critically dependent on calibration points. Combinations of paleoclimatic and paleogeographic data and the age of fossil events constitute the key types of calibration points that are used to estimate and construct divergence time on trees (Mantooth and Riddle, 2011).

As with phylogeographic methods, species delimitation methods have also shifted towards a more molecular based approach. Whilst previous species delimitation applications relied on the traditional morphological approach, which is still, one of the important taxonomic tools, the revolution in molecular methods has shifted species delimitation approaches towards these molecular techniques (as described in section 1.1). This coupled with the availability of analytical methods for species delimitation, especially with molecular genetic data, are numerous and they are a promising area for scientific research (Fujita et al., 2012, Leaché and Fujita, 2010, Wiens, 2007, Wilms and Schmitz, 2007).

The Bayesian Phylogenetics and Phylogeography (BPP) program (Yang and Rannala, 2010) is an example of an analytical method that has been applied and used frequently in species

delimitation. Despite the necessities of guide trees and the need to assign candidate species, this coalescence method can provide an appropriate model to investigate and determine separate lineages from within species complexes of species delimitation.

Many computer programs have been applied to these approaches in phylogeography, such as the BEAST software program (Drummond and Rambaut, 2007). BEAST is one of the most popular and powerful programs for studying an organism's evolution and calculating molecular sequence variations among groups of organisms; it also includes variable models and strong statistical methods (Drummond and Rambaut, 2007). The BEAST program can be run in STAR BEAST (*BEAST) mode which implements the multispecies coalescent model (Heled and Drummond, 2010).

1.3 Arabian Peninsula geography and topography

The Arabian Peninsula is located in the southwest of the Asian continent. The Arabian Peninsula incorporates seven countries: the Kingdom of Saudi Arabia, Yemen, Oman, United Arab Emirates, Qatar, Bahrain and Kuwait. The Peninsula is characterised as one of the harshest environments and is one of the most hostile places in the world (Böer, 1997). It is bordered by the Red Sea and Gulf of Aqaba in the west, the Gulf of Aden and the Arabian Sea to the south, and the Arabian Gulf and the Gulf of the Oman in the east (Parker and Rose, 2008) (Fig. 1.1).

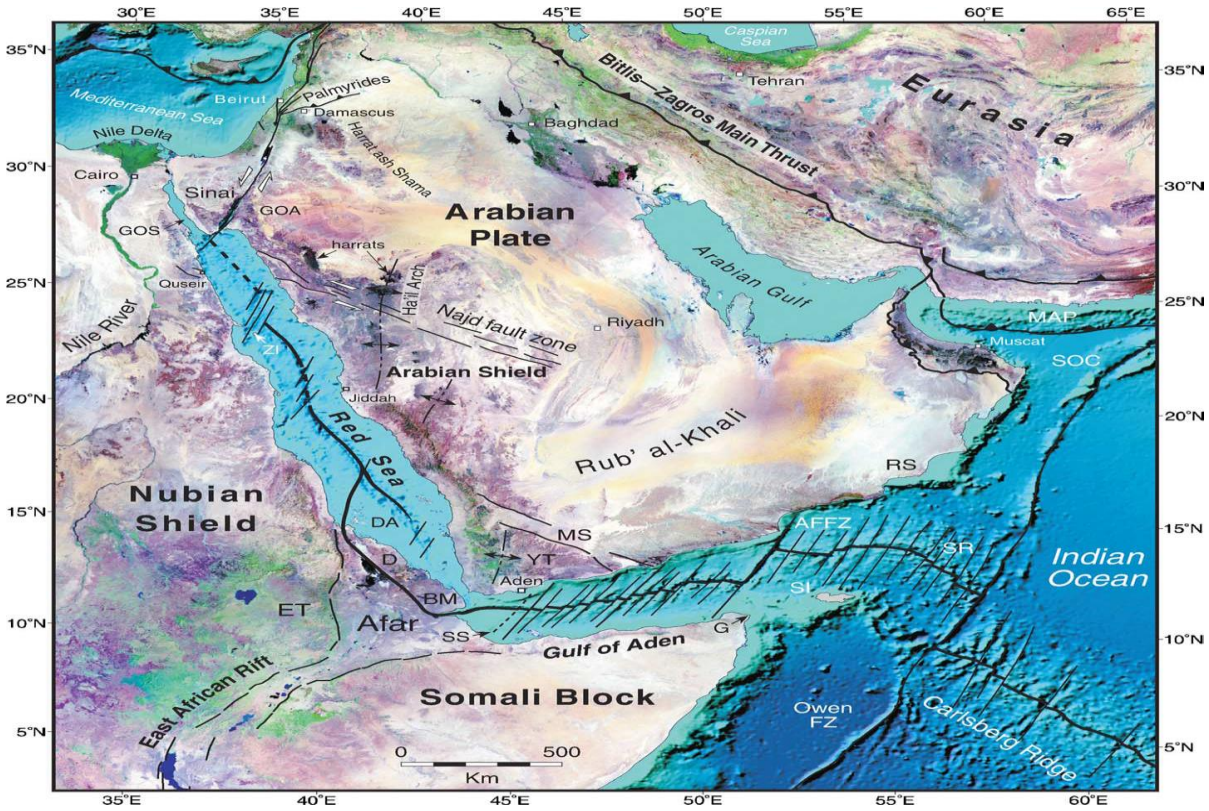


Fig. 1.1. The Arabian Peninsula, geological and geographical map. Adapted from (Bosworth et al., 2005).

The Arabian Peninsula has a long coastline in the west that is dominated by the parallel Hejaz (western mountains) and Asir mountains, which extend about 2100 Km from the farthest points in the northwest to the southern edge of the Red Sea, and are nearly 2000 km wide, or the width of the Arabian Peninsula from western Yemen until the easternmost portion of Oman (Parker and Rose, 2008). The temperature and rainfall data clearly show that the area is dry for the most part of the year. However, not all areas experience this pattern; some biogeographic provinces in the South and South West have higher annual precipitation when compared to other areas. Indeed, the Arabian Peninsula is made up of different topographies and unique ecosystems.

Geographically, the Arabian Peninsula has been divided into several sub-regions for study purposes, and this classification has been much debated among many authors. Geographically, the Arabian Peninsula is characterised by two main geological structures: the Arabian Shield and the Arabian Shelf (Al-Nafie, 2008). The Arabian Shield is an ancient land mass that dates back to the Cambrian age; it extends from the west and covers central Arabia. The surface elements of this Shield are volcanic or basaltic rocks that resulted from

volcanic activity during the Mid-tertiary period. The Arabian Shelf extends to the east of Arabian Shield and comprises geological elements of sedimentary rocks resulting from shallow marine waters that are thought to date from the Cambrian to the Pliocene (Al-Nafie, 2008). Climatically, on the other hand, Moore (1986) has divided the Arabian Peninsula into seven different zones characterised by different ecosystem habitats: the coast of the Red Sea, the features of high mountains of Asir, Yemen and the Akhdar in Oman, the central and north-central arid regions, the elevation of northwest regions and the semi-arid of northern region; the coastline of the Arabian and Oman Gulf; the Al Rub Al Khali desert; and the Mountains of Qara in Oman (Fig.1.2). In addition, the Arabian Peninsula is considered one of the world's driest places; it is dominated by four sand massifs that form about 27% of the Arabian Peninsula. One desert is known as Al Rub Al Khali, the second is the Great Nafud, and these two bodies are connected by the large Ad Dahna sand belt, which extends about 1200 Km from south east of the Great Nafud to the northern part of Al Rub Al Khali Arabia; this sand belt is located between central and eastern Arabia (Fig.1.3). The fourth is A Sharqiyah sands (formerly Wahiba sands) in Oman (Al-Nafie, 2008, Parker and Rose, 2008). These sand and gravel deserts characterise the central part of the Arabian Peninsula and are crossed by several shallow wadis. The Arabian Peninsula vegetation is generally widely dispersed, particularly in the central and northwest part of the Peninsula.

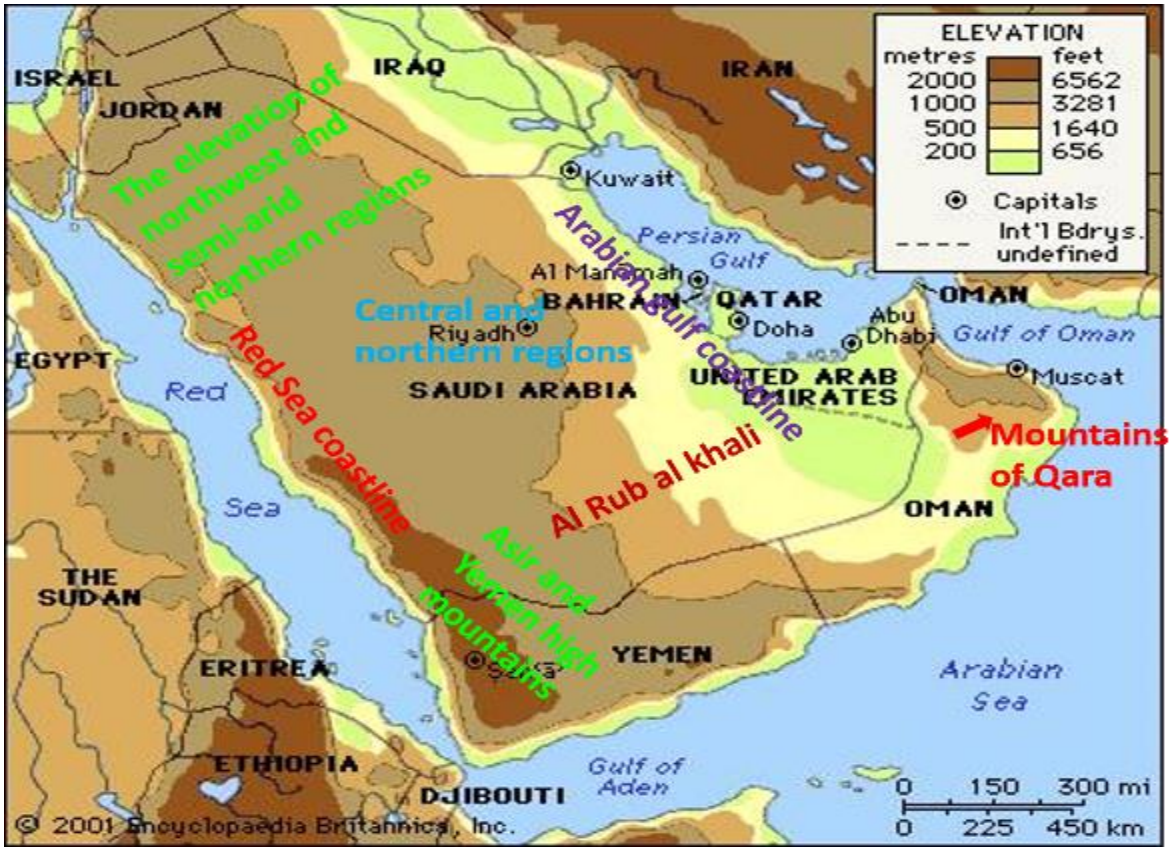


Fig.1.2. Topographical map of the Arabian Peninsula, with political boundaries represented by solid black lines. Ecosystem classifications, in accordance with Moore (1986), are displayed.

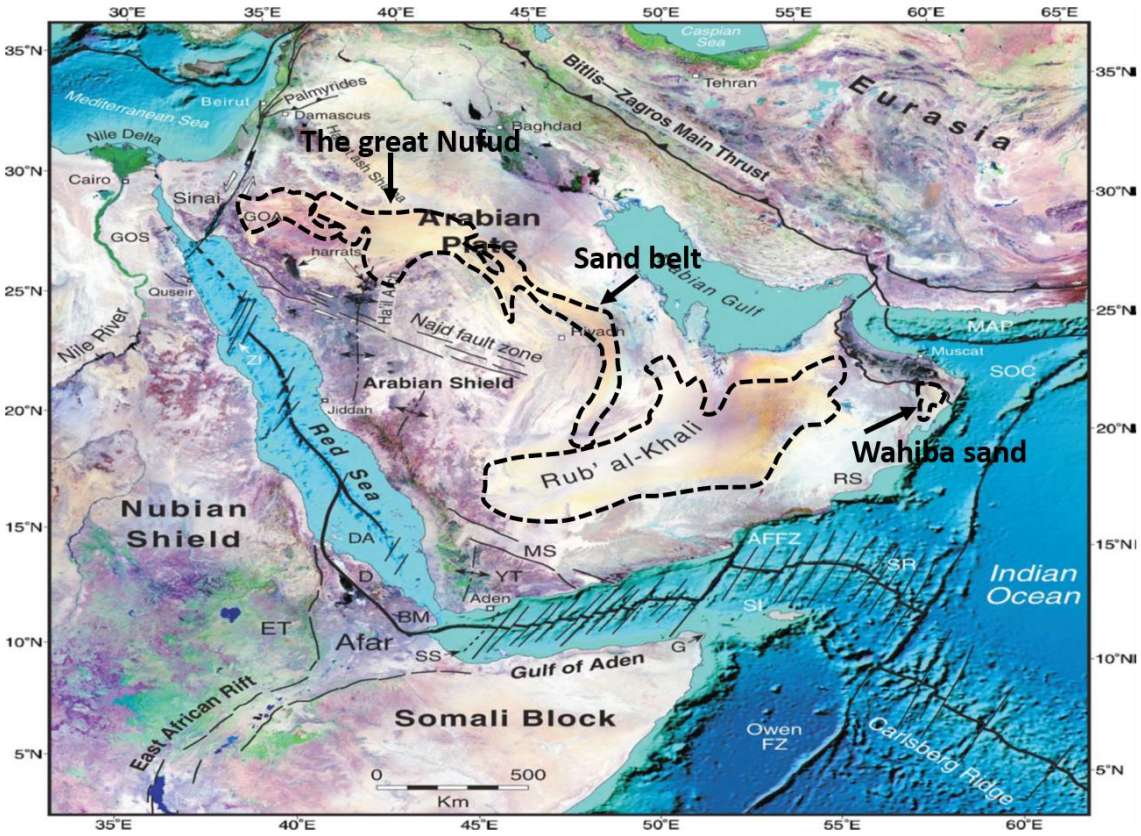


Fig.1.3. Physical map of the Arabian Peninsula showing the location and features of the desert ecosystems. Map modified from (Bosworth et al., 2005)

The Arabian Peninsula has a complex geological history. The Arabian plate is bordered by the Eurasian plate and the African plate. Approximately 25 million years ago (Mya) tectonic activity caused the separation of the Arabian Peninsula from Africa (Thompson, 2000). Possible reasons for the detachment of the Arabian Peninsula could be tectonic activities produced by the Red Sea rifting during the initial Miocene era (Bosworth et al., 2005). The Mediterranean began to link with the Red Sea when the rifting process continued and ended approximately 23 Mya (Hughes et al., 1991). The Zagros mountain range in Iran was an outcome of this rifting process, as were the mountains in the Eurasian plate, which appeared following the collision between the Arabian Peninsula and Eurasia during the early or mid-Miocene era (22-15 Mya) (Thompson, 2000, Bosworth et al., 2005, Harzhauser et al., 2007). In addition, approximately 10 Mya a combination of a large halite deposition coupled with a eustatic sea level decline created the closure of Bab el Mandeb. The width of Bab el Mandeb was reported to be just 5km across at the lowest sea level 10-5.3 Mya ago. Currently, it is measured at 30km wide (Bosworth et al., 2005).

During the Pliocene era (5 Mya), the next rifting phase initiated, separating the Red Sea from the Mediterranean Sea through formation of the Isthmus of Suez. At the same time, the Straits of Mandeb and Gulf of Aden sank into the sea, thus permitting the Indian Ocean and Red Sea to form water channels between them. The Hajar and Dhofar mountain elevation was increased during the last 4-6 Mya due to the opening of the Gulf of Aden; this is reported as the last geographical event in the region's history (Bosworth et al., 2005, Klütsch, 2006).

The climate of the Arabian Peninsula has also been affected by global climate change since the Plio-Pleistocene era and these changes will have impacted on the geology of the region. Since the last glacial period or so called 'Ice Age', this region has experienced an inter-glacial period which has been defined by fluctuations in the localised climate from wet to hyper-arid conditions (Parker, 2010). During 'wet' phases in the Pleistocene the levels of precipitation during the south-west monsoon may have been up to 50% more than the present levels and monsoon winds reaching up to 15 m/sec. The hyper-arid phases experienced during this time resulted in the formation of sand dunes which may have dominated the landscape of the Arabian Peninsula. The levels of moisture content during these hyper-arid phases are of interest as to whether they were sufficient enough to facilitate human occupation (Parker and Rose, 2008). Glacial retreat has also resulted in the formation of several geological features such as the Gulf of Aden. Compared to current levels, sea level was approximately 120m lower and the whole Arabian Gulf region was dry. Reductions in sea level supported the formation of land bridges, such as the Iran and Oman connection, that joined the continents. Recent geographic features of the Arabian Gulf appeared when the Tigris and Euphrates rivers met the Iranian coast and flowed across the Straits of Hormuz (Thompson, 2000, Klütsch, 2006).

1.4 The status of the Arabian Peninsula lizards

The Arabian Peninsula lies at the crossroads of three major zoogeographical realms due to its location. South western Arabia is affected by the Afro-tropical elements, the Palaearctic region comprises the most northern and north easterly parts of the Peninsula, and the

Oriental zone occurs on the eastern part. These distinct zoogeographical realms result in Arabian reptiles reflecting different histories of evolution, geography, and ecology (AbuZinada et al., 2003).

Cox et al., (2012) documented 120 species of lizards occurring in the Arabian Peninsula; these lizards belonged to the following different families: Lacertidae (wall lizards, 27 species), Gekkonidae (geckos, 28 species), Sphaerodactylidae (semaphore geckos, 22 species; with 17 species endemic to the Arabian Peninsula), Agamidae (agamas, 17 species), Phyllodactylidae (leaf toed geckos, 9 species; 6 endemic), Varanidae (2 species, 1 endemic), Scincidae (13 species; 5 endemic), Chamaeleonidae (4species; 3 endemic) and Trogonophidae (3 species; 2 endemic).

Lizard richness in the Arabian Peninsula varies from region to region. For example, south western Saudi Arabia and Yemen, southern Oman (Dhofar), the Hadramout region (southern Yemen), the Hajar mountains that lie between northern Oman and United Arab Emirates, south eastern Oman, and northern and northeast Saudi Arabia all show high diversity, whereas the central regions of the Arabian Peninsula and the empty Quarter have lower diversity (Cox et al., 2012). Currently, the number of Arabian lizard species has increased due to recent discoveries; the total number of documented Arabian lizard species is presently 134 (Carranza and Arnold, 2012, Cox et al., 2012, Melnikov and Pierson, 2012, Nazarov et al., 2013).

Previous studies of the Arabian herpetofauna, including both reptiles and amphibians, focused on distribution and description of the biodiversity. The Royal Danish expedition explored the region's reptiles between 1762 and 1763, collecting and documenting scientific specimens from Egypt, Yemen, and the coastal areas along the Red Sea (Forskal, 1775). Subsequently, several studies have been done on the snakes, lizards and amphibians of the Arabian Peninsula.

Studies conducted in the 1980's focused largely on the biodiversity of the Arabian Peninsula herpetofauna, these studies fully described *Acanthodactylus opheodurus* in addition to four previously undescribed species from the Arabian Peninsula (Arnold, 1980a). In a subsequent

study (Arnold, 1986b) generated a geographical distribution, a key and an annotated checklist of 96 lizard species and another 6 subspecies. In 1988, three species of the genus *Mesalina* were recorded as sympatric species from Eastern Saudi Arabia (Ross, 1988). Schatti and Gasperetti (1994) added more information about the herpetofauna of Southwest Arabia and presented a systematic distribution of 61 taxa of reptiles and amphibians from the Asir, South Tihama and the Yemen Highlands. Crucially, these studies have all focused on very few taxa, with identification based solely on morphological characteristics. However, despite these limitations each study has successfully been able to identify potential new species.

Recently, elements of molecular ecology have been incorporated into these types of studies. However, the lizards of Arabia remain one component of the Arabian fauna where phylogeographic and molecular systematic studies are largely lacking. A basic taxonomic and phylogenetic study of the genus *Uromastyx* from the Arabian Peninsula was conducted by (Wilms et al., 2009) This study forms the basis for the present research on morphology and molecular genetics and resulted in a revision of the taxonomy of the genus *Uromastyx* within its area of distribution and provided an assessment of the taxonomic relationships of this genus using both morphological and genetic methods.

The combined application of molecular techniques with morphological approaches has led to the dramatic increase in the number of species described from the Arabian Peninsula. Three species and one subspecies of *Hemidactylus* from the interior of Yemen were described in 2011 (Busais and Joger, 2011). In addition, eight new species of the genus *Hemidactylus* from the Arabian Peninsula were also described in 2012 (Carranza and Arnold, 2012).

Although the location of the Arabian Peninsula is important, studies of its phylogeographical patterns remain few and far between. Studies on both Arabian reptiles and other Arabian fauna have attempted to investigate the patterns based on disjunction species that occur on both sides of the Arabian Peninsula and also in Africa. At present, no work has been conducted using a comparative phylogeographical approach to study diversity of the local fauna within the Arabian Peninsula. Most of the studies that have been implemented have

attempted to determine phylogeographical patterns between two different continents (e.g., between the Arabian Peninsula and continental Africa) (e.g. Pook et al., 2009, Metallinou et al., 2012) with a view to examining the factors that have previously played a major role in shaping the current distribution status of this group of organisms. However, the congruence between the patterns for the Arabian Peninsula and Africa has received more attention in recent years. For example, Gvoždík et al. (2010) studied the evolutionary relationships between tree frogs of the Middle East with an approach based on phylogeography. The findings from this study indicated a connection between the southern Levant and South Western Arabia, highlighting the significance of geographical barriers to speciation in these regions.

The phylogeographic studies on the Arabian Peninsula have mostly included species that have been affected by historical climate changes over the region. These changes have presumably led to the emergence of a large mammalian biodiversity in Arabia that owes its origins to influences from Africa. Delany (1989) reviewed the mammalian fauna of the Arabian Peninsula and examined the climatic history of this region over the previous 100,000 years. Firstly, this study assigned the mammalian groups to different zoogeographical regions within the wider Arabian region and secondly, determined that for the majority of the previous 100, 000 years the region under study had experienced considerable aridity, with the exception of 35 000 – 17 000 and 11 000 – 6 000 years ago. In addition, world sea levels have also fluctuated with historical levels estimated to be between -105 and -175m below present day levels. It is hypothesised that this reduction in water levels may have created either narrow water barriers or land connections between Africa and Arabia thus allowing mammalian population and reptiles migration from Africa to Arabia (Derricourt, 2005, Pook et al., 2009, Portik and Papenfuss, 2012). However, this theory is contested by some scientists (Fernandes et al., 2006).

Phylogeographical studies focusing on the colonization of Arabia by Pan African -Arabian species have noted significant time discrepancies between different taxa which may be due to different colonisation routes related to the formation of the historical land bridges between the two continents. For example, Winney et al. (2004) studied the phylogeography of Hamadryas baboons (*Papio hamadryas hamadryas*) and found that the Arabian

Hamadryas baboons may have colonized Arabia some 10,000 years ago. This study demonstrates the more recent colonisation of Arabia by mammals compared to reptiles such as *Hemidactylus* geckos, which are reported to have colonised Arabia much earlier at approximately 15 Mya (Carranza and Arnold, 2006). Portik and Papenfuss (2012) also reported that the monitor lizard *Varanus yemenensis* were found to be much closer to the African *V. albigularis,* which confirmed that the Africa was the place of origin for *V. yemenensis.* Therefore, it is hypothesised that this species were dispersed to the Arabian Peninsula through the southern land bridge that may have existed at that time or by dispersal over water approximately 5.3 Mya.

Recent phylogeographical studies have reported similar divergence time patterns for many reptile species in the African – Arabian region. Pook et al., (2009) examined the phylogenetic relationships of African *Echis* and determined that the divergence time patterns of this genus were closely linked to, or appeared to coincide with, the collision between Eurasia and the Afro-Arabian blocks, approximately 22-21 Mya. However the same study also determined more recent divergence times between Arabian and African populations of species such as *Bitis arietans* (4 Mya) and *Naja haje* (1.75Mya), suggesting that a different dispersal event may have been responsible for these species. A later study on *Stenodactylus* lizards from both Africa and Arabia also reported similar deep divergence times (dating back to the Miocene period) which were attributed to geographical and climate changes during the Pliocene and Pleistocene (Fujita and Leaché, 2011). A further study on the *Stenodactylus* lizards (Metallinou et al., 2012) examined the historical events which have influenced the present day distributions of this genus, namely the opening of the Red Sea and climatic changes occurring during the Miocene period. Examining the gecko genus *Hemidactylus* across some regions of the Arabian Peninsula, the Horn of Africa, Levant, and Iran, Šmíd et al. (2013) also reported on deep divergence times within this genus, coinciding with the opening of the Red Sea (approximately 31-23 Mya).

Despite the earlier studies mentioned above, most of the Arabian Peninsula lizards have not attracted much attention (especially those of the interior and northwest regions such as *Acanthodactylus, Ptyodactylus, Bunopus*, and *Pseudotrapelus*) since 1986 (Arnold, 1986b), particularly in terms of molecular systematic methods or phylogeographic studies. Applying

the phylogeographic and species delimitation approaches to the Arabian Peninsula lizards has substantial utility. The discovery of numerous cryptic species and old intraspecific lineages in the minority of species that have been investigated suggests the possibility that many other lizard species actually constitute complexes of multiple cryptic species. Moreover, the fact that many additional species or species complexes co-occur as sympatric species allows us to test for common phylogeographical patterns in space and time across multiple species complexes. The diversity of the group in this study includes different species that inhabit different ecological niches. From ground dwelling to rock dwelling, nocturnal to diurnal, these species will provide an excellent models for the study and the subsequent re-assessment and re-valuation of the biodiversity of the Arabian Peninsula's lizards. As mentioned earlier, the Arabian Peninsula reflects the influence of different biogeographical regions and diversity of habitats. Consequently, there is a strong expectation of the discovery of cryptic species. It is worth noting that some of these habitats remain largely unexplored until now.

This thesis will focus on fourteen lizard species from the Arabian Peninsula. These species are widely distributed throughout the Arabian Peninsula and have been collected from most of its range, encompassing a comprehensive number of both samples and regions. These study species are *Acanthodactylus boskianus, A. opheodurus* and *A. schmidti,* and *Mesalina guttulata, M. adramitana and M. brevirostris*, belonging to the family Lacertidae; *Stenodactylus slevini, S. doriae*, *S. arabicus*, *S. leptocosymbotus*, *Cyrtopodion scabrum*, and *Bunopus tuberculatus* belonging to the family of Gekkonidae; *Ptyodactylus hasselquistii* complex belonging to the family Phyllodactylidae; and *Pseudotrapelus sinaitus,* belonging to the family Agamidae.

1.5 The aims of this thesis

The two main aims of this thesis are to conduct phylogeographic investigations and to establish the species delimitation of lizards from the Arabian Peninsula:

- 1. To conduct a comprehensive study of the phylogeography of co-distributed species of Arabian Peninsula lizards.

The analysis of DNA sequences from fourteen species of lizards from the Arabian Peninsula was used to investigate phylogeographic patterns of these groups. Markers of mtDNA (three genes) and nDNA (two genes) were used. A multispecies approach, phylogenetic analysis utilising the *BEAST program to simultaneously estimate the species tree and species divergence times within the studied Arabian Peninsula lizard groups. To test the hypothesis that co-distributed lizard species may display common patterns and to determine the spatial and temporal divergence times between the lizard groups. Results are presented in Chapter 2. Common patterns and biological factors that may be responsible for diversification and speciation of these groups are also discussed.

- 2. To investigate the occurrence of cryptic species within *Acanthodactylus boskianus* and *A. opheodurus* from the Arabian Peninsula.

Results from nuclear and mitochondrial DNA sequence data were analysed for *Acanthodactylus opheodurus* and *A. boskianus* from the range of these species, from twelve localities within the Arabian Peninsula. Using a candidate species approach, based only on genetic evidence, species delimitation studies were conducted. Mitochondrial clades that represent distinct and highly divergent clades were further analysed to reveal candidate species. The evidence from nuclear DNA data was then used to identify these mitochondrial clades which may potentially represent separately evolving species that show evidence of nuclear divergence. Results are presented in Chapter 3.

2. Comparative phylogeography of the Arabian Peninsula lizards

Abstract

The Arabian Peninsula has been affected by historical events at different temporal scales, which makes its biota interesting for research. Nevertheless, this region remains poorly studied and in particular, there is a lack of phylogeographic studies of Arabian species, resulting in very incomplete knowledge on the genetic structuring of widespread Arabian species. Therefore, the aim of this study was to investigate the comparative phylogeography of selected groups of lizards from the Arabian Peninsula, with a view to determining the presence of any common patterns across multiple species, co-distributed across different regions. DNA samples from fourteen lizard groups, distributed across seven regions of the Arabian Peninsula, were collected and analysed using a multispecies coalescence approach, to obtain a species tree using STAR BEAST (*BEAST) software. This analysis was based on three mitochondrial (Cytochrome b, 12S ribosomal RNA and 16S ribosomal RNA) markers and two nuclear (neurotrophin 3 [NTF-3] and fingerprint protein 35 [R35] markers). Clear phylogeographic structure was observed for most of the studied lizard species and evidence of older divergence times were also observed. In addition, the findings from this study show that of the fourteen lizard species studied, eight showed evidence of either spatial or temporal common patterns between different regions. Common sister group relationships and common ages of divergence (0 – 1 Mya) observed between eastern and central Saudi Arabia for *Acanthodactylus boskianus, A. opheodurus, Mesalina guttulata*, and *Bunopus tuberculatus*. Northwest and eastern Saudi Arabia showed common phylogeographic sister group relationships, but with differing ages of divergence between populations of *Mesalina brevirostris* (1.8 Mya) and *Stenodactylus doriae* (4.3 Mya). Evidence was obtained from southern and northwest Saudi Arabia for common phylogeographic sister group relationships, with differing ages of divergence, between *Pseudotrapelus sinaitus* (6.12 Mya) and *Mesalina guttulata* (11.35 Mya). Similar phylogeographic sister group relationships and similar ages of divergence (3.5 – 3.8 Mya) were also detected between southern and western Saudi Arabia for *Acanthodactylus boskianus* and *Ptyodactylus hasselquistii.* In addition to common patterns, several species also displayed unique phylogeographic

patterns; with older divergence times observed in *Pseudotrapelus sinaitus* (Central Saudi Arabia), *Acanthodactylus boskianus* (Northwest Saudi Arabia and Southern Oman), *Pytodactylus hasselquistii* (Northwest and Central Saudi Arabia; UAE; Southern Oman), *Acanthodactylus opheodurus* (Northwest Saudi Arabia and the clade comprising Southern Saudi Arabia and Southern Oman), *Stenodactylus slevini* also revealed unique patterns in most of its clades especially in eastern Saudi Arabia

This study has provided the first detailed insights into the biogeography of the Arabian Peninsula lizards and the findings from this study have shown clear phylogeographic patterns for most of the studied species and provides fundamental information for future studies in this region. Finally, the results from this study suggest the presence of cryptic species. However, further research is required in this area.

2.1 Introduction

Comparative phylogeography investigates the spatial and temporal processes shaping the distributions of multiple co-distributed species and searches for common patterns in space and time. Phylogenetic trees, along with strong inferences of divergence time, can help determine the historical diversity in species and also explain the distribution patterns of multiple co-distributed lineages (Castoe et al., 2009). This allows us to explore the historical events that resulted in the formation of common, shared distribution patterns observed today (Hickerson et al., 2010).

While phylogeographic studies of single species can only provide historical data on that single species, studying the phylogeography of multiple species from different regions can generate robust general patterns. Therefore, comparisons of co-distributed species are important (Zink, 1996). The importance of detecting similar patterns across multiple co-distributed species in a single area is that it allows the inference that similar genetic structures of species are responses to the same biogeographical and environmental events (Carstens and Richards, 2007). Consequently, events such as the occurrence of barriers, environmental events (e.g. global climate change), and the role of ecological factors (e.g. niche adaptation), can be inferred as a result of multiple species displaying congruent spatial and temporal phylogeographic patterns (Riddle et al., 2008). Therefore, the interpretation of the biotic history of an interesting region(s) can be clarified from comparative phylogeography. Common patterns revealed by group of species, can provide strong evidence of co-association between these groups and regions (Zink, 1996) and may facilitate the process of finding compatible phylogeographical scenarios for given co-distributed lineages (Arbogast and Kenagy, 2001, Avise, 2000, Bermingham and Martin, 1998, Bermingham and Moritz, 1998, Castoe et al., 2009, Lapointe and Rissler, 2005). Such information provides a foundation for researching the complete details regarding the evolutionary history of lineages and also facilitating the mapping of biogeographic relationships between multiple organisms over varying geographical areas over given periods of time. The process of speciation can be unravelled using a combination of molecular data and biogeographical history of all the co-distributed species. In addition, the

relationship between speciation and evolution can be identified (Lamm and Redelings, 2009).

Previous phylogeographical approaches focused on the use of a single locus, mitochondrial DNA (mtDNA), however, currently a multilocus based approach utilising both mtDNA and nuclear DNA (nDNA) are now commonly used in phylogeographic studies for estimating the species tree as opposed to the gene tree. Therefore, not only providing much larger molecular data sets, but also circumventing the biases associated with sole usage of mtDNA such as matrilineal-only transmission, with which to answer complex population history, speciation and demographic questions (Brito and Edwards, 2009).

The multispecies coalescent model has been proposed to integrate population genetic theory with species tree inferences. Special correlation within a given population can be estimated using independent gene trees though partial lineage sorting. This creates incongruence in gene trees, which can be avoided if gene lineage evolution is modelled as a coalescent process that is influenced by population size and mutation rate (Heled and Drummond, 2010, Liu, 2008). The theory underlying the multispecies coalescent model states that every gene shows its relation with orthologous genes in a small sample of organisms taken from a multiple species population (Heled and Drummond, 2010). The species tree which is the so-called multiple species coalescent, contains multiple internal gene trees and uses a coalescent process (Heled and Drummond, 2010, Rannala and Yang, 2003).

At present, the majority of phylogeographic studies have focused either on developed countries in the Northern Hemisphere (Beheregaray, 2008) or on species that are either charismatic such as birds or mammals (Winney et al., 2004), or of conservation importance (Mace, 2004) or of commercial importance (Fernández et al., 2013). Therefore, phylogeographic studies in the Southern hemisphere and in developing countries within the Northern hemisphere are urgently required to redress this bias (Beheregaray, 2008).
The Arabian Peninsula is a region where few phylogeographic studies have been conducted. Studies show that the Arabian Peninsula is the newest lithospheric plate in Earth's crust (Stern and Johnson, 2010). Historical examination explains the complex geological history of

this region and indicates that the Arabian Peninsula was separated from Africa some 50 million years ago (Thompson, 2000). The rifting of the Red Sea (27 Mya) and the opening of the Gulf of Aden (4-6Mya) Bosworth et al., (2005) provided potential vicariance events. In addition, fluctuating sea levels represent significant geological events in the history of this region which may have acted as drivers for speciation. For example; the width of Bab el Mandeb was reported to be 5km when the sea level reached its lowest point in the nearby area at 10-5.3 Mya ago, which is much narrower than at present (30km) (Bosworth et al., 2005).

Climate changes in the Arabian Peninsula region, attributed to geological events in the Quaternary period, produced changes in the aridity of the region which may also have acted as drivers for speciation events. These speciation events had a long term impact on the evolution of species inhabiting this region.

Reptiles are often important components of the fauna of arid areas, and as such they can be used as a significant means of exploring diversity in arid regions (Metallinou et al., 2012). In this study, the comparative phylogeography of a selected group of lizards from the Arabian Peninsula is investigated with the aim of testing for the presence of common patterns across multiple species that are co-distributed across different regions of the Arabian Peninsula. The fourteen species, belonging to four families were broadly categorised based on biological aspects. These aspects were divided into sand dwelling, ground dwelling and rock dwelling groups.

Ground and sand dwelling group

Acanthodactylus species (*A. boskianus* and *A. opheodurus*) are common spiny-footed lizards that occur in sandy arid areas and gravelly soils and belong to the family Lacertidae. The genus *Mesalina* is the sister genus to *Acanthodactylus* (Arnold et al., 2007). Three species of *Mesalina* are described in the current study, *M. guttulata, M. adramitana*, and *M. brevirostris*. *M. guttulata* tends to occur on and between distributed rocks and hard substrata with sporadic vegetation, a gravel plain is the preferred habitat of this species (Disi et al., 2001). *M. brevirostris* occupies hard gravel plains and occurs in the peripheral wadis that containing abundant vegetation (Disi et al., 2001) *M.adramitana* has been found to inhabit hard, dry, and gravel plains that contain scarce vegetation (Arnold, 1980a). *Bunopus*

tuberculatus is one of four species from the genus *Bunopus* that belong to the Palearctic naked-toed geckos (Bauer et al., 2013). *B. tuberculatus* has been known to inhabit various habitats from hard surfaces and fossil dunes to loose Aeolian sand (Arnold, 1980a). The genus *Stenodactylus* constitutes part of the fauna of the arid and hyper-arid regions of Arabia and North Africa (Arnold, 1980b). *S. doriae* and *S. arabicus*, are sand dwelling geckos (Metallinou et al., 2012) whist *S. slevini S. leptocosymbotes* are found to occupy habitat that is characterized by granular sandy planes, sandy and hard ground substrata (Arnold, 1980b, Arnold, 1984).

Rock dwelling group

Psuedotrapelus sinaitus is a strictly diurnal species that occurs mainly on rocky, open habitat. This species favours flatter surfaces and open gravel slopes and can be found climbing and foraging on some plants such as *Acacia ehrenbergiana,* and *A. tortilis* (Arnold, 1980a, Schätti and Gasperetti, 1994). *Ptyodactylus hasselquistii* is a complex member of the genus *Ptyodactylus*. This genus is considered to be a characteristic genera of geckos (Perera and Harris, 2010). *Cytropodion scabrum* belongs to the group of Palaearctic naked-toed geckos (Bauer et al., 2013). Similar to *Ptyodactylus hasselquistii, C. scabrum* is nocturnal and inhabits rocky habitats and can also be found in the walls of buildings (Disi et al., 2001).

The aim of this study

To the best of my knowledge, no previous study has focused on the comparative phylogeographical analysis of Arabian lizards. Very little work has been conducted on the biogeography of the Arabian reptiles or other taxa in general, and much of the previous work has focused on investigating the exchanges and geographical distribution patterns of many organisms that exhibited congruent patterns between Africa and Arabia or have simply described new species from the region (Amer and Kumazawa, 2005, Arnold, 2009, Arnold et al., 2009, Busais and Joger, 2011, Carranza and Arnold, 2012, Fujita and Papenfuss, 2011, Gómez-díaz et al., 2012, Gvoždík et al., 2010, Metallinou et al., 2012, Newman et al., 2004, Pook et al., 2009, Portik and Papenfuss, 2012, Šmíd et al., 2013, Wilms and Böhme, 2007, Winney et al., 2004, Zinner et al., 2009). Consequently, there remains a very considerable knowledge gap on patterns of genetic structure of widespread reptile species within the Arabian Peninsula.

This present study attempts to address this knowledge gap through the comprehensive analysis of phylogeographic patterns of 14 co-distributed lizard species from the Arabian Peninsula. The main hypothesis was to determine any spatial and temporal common patterns of the selected species by estimation of divergence time, using samples collected from different regions of the Arabian Peninsula.

2.2 Materials and Methods

Sampling collection

This study was based on 144 specimens of lizards and 29 specimens of snakes. Lizard samples represented 14 species: *Acanthodactylus boskianus, A. opheodurus, A. schmidti*; *Mesalina guttulata, M. brevirostris, M. adramitana; Stenodactylus doriae, S. slevini, S. leptocosymbotus, S. arabicus; Bunopus tuberculatus; Cyrtopodion scabrum; Ptyodactylus hasselquistii* complex (Nazarov et al., 2013)*;* and *Pseudotrapelus sinaitus.* Snake samples also represented 14 species; *Naja kaouthia, N. naja, N. nivea, N. nigricollis, Porthidium arcosae, P. lansbergii rozei, Bothrops asper, Daboia siamensis, Daboia mauritanica, Echis coloratus, E. carinatus sochureki, E. omanensis* and *E. pyramidium*. The snake samples were used primarily to produce calibration points to calculate divergence times in this chapter. Mitochondrial DNA sequences of snakes were provided by Dr. W. Wüster (Pook et al., 2009). Some snake samples were amplified for nuclear DNA genes, whereas other sequences were obtained from GenBank. The samples of lizards and snakes as well as sequences obtained from GeneBank and their accession numbers and localities are listed in Appendix 1.

Lizards were caught by hand. When live lizards were caught, the tail tips were collected and stored in absolute ethanol, and the animals released again in the wild. Live animals from Saudi Arabia only were collected in bags euthanized and deposited in the zoological department at King Saud University in Riyadh for future use as voucher specimens. These lizards were collected during two field work trips during 2010 and 2011. Each trip was 6-8 weeks in duration (see Appendix 5). Lizards were collected from twelve localities across the Arabian Peninsula: Northwest Saudi Arabia (three localities), Western Saudi Arabia (two

localities), Southern Saudi Arabia (two localities), Central Saudi Arabia, Eastern Saudi Arabia, Northern Oman, Southern Oman and United Arab Emirates.

DNA extraction, amplification and sequencing

The total genomic DNA from the tail tip was extracted using a Qiagen DNeasy™ Tissue Kit. Three mitochondrial DNA genes; cytochrome b (CYTB), the ribosomal 16S rRNA (16S) and the ribosomal 12S rRNA (12S) and two nuclear genes, the fingerprint protein 35 (R35) and the neurotrophin-3 (NTF-3) were amplified.

Primers used to amplify these fragments of genes are listed in Table 1. For mitochondrial DNA genes, the total volume of the polymerase chain reaction (PCR) was carried out with 0.3µl of each primer, 0.8µl of sample (template) DNA and primarily 9.6 µl of Abgene 1.1x ReddyMix™, which consisted of 1.25 units Thermo prime plus DNA polymerase; 75mM Tris-HCl pH8.8; 20mM $(NH_4)_2SO_4$; 1.5mM $MgCl_2$; 0.01% (v/v) Tween®20; 0.2mM of each dNTP; and a precipitant red dye for electrophoresis leaving the total volume of 11 µl. The volume of the PCR reactions for nuclear DNA was 15 µl, increasing the concentration of ReddyMix to 13µl, 0.4µl for each primer and 1.2µl of DNA template.

The PCR products were obtained following a 15-minute incubation at 37°C and a 15 minute incubation at 74°C. This was achieved by adding the enzyme Exonuclease I and Shrimp Alkaline Phosphate (Werle et al., 1994), which cleaned up the PCR products prior to sequencing. The PCR products were visualised on 0.5% to 1% agarose gel containing 5µl to 10µl ethidium bromide. The PCR products were sent to Macrogen in Korea (Seoul, S. Korea—http://dna.macrogen.com) for sequencing.

Table 2.1: Primers used to amplify Mitochondrial DNA and nuclear genes. [a](Kumazawa and Endo, 2004); [b]this study; [c](Palumbi, 1996); [d](Fu, 2000); [e](Palumbi et al., 1991); [f](Kocher et al., 1989); [g](Townsend et al., 2008); [H](Leaché, 2009).

Table 2.1. Primer sequences and PCR conditions			
Primer	**Sequence**	**Cycles**	**Annealing**
CYTB			
Gludge-L[c]	TGACTTGAARAACCAYCGTTG	40	48°C
H15488[d]	TTG CTG GGG TGA AGT TTT CTG GGT C		
Gludge-L[c]	TGACTTGAARAACCAYCGTTG	40	48°C
H15149[f]	GCCCCTCAGAATGATATTTGTCCTCA		
L14841[f]	CCATCCAACATCTCAGCATGATGAAA	40	40°C
rctyb-1H[a]	TGAGGACAAATATCMTTCTGAGG		
L14841[f]	CCATCCAACATCTCAGCATGATGAAA	40	40°C
H15488[d]	TTG CTG GGG TGA AGT TTT CTG GGT C		
rcytb-2L[a]	GCGTAGGCRAATAGGAAGTATCA	35	50°C
rctyb-1H[a]	TGAGGACAAATATCMTTCTGAGG		
F-doriae26[b]	AACTCCTTCATCGACCTTCC	35	50°C
R-doriae701[b]	GGCGAAAATAGTGCTAGGTG		
F-bonop83[b]	GCTCACTATTAGGGCTCTGC	35	50°C
R-bonop650[b]	GGCGTCTTTGTAGGTGAAGT		
hasselq83[b]	ACGGCTGACTTATCCGAAAC	40	40°C
hasselq-R[b]	TCCCAGGAGATAGGGGTTTA		
16S[e]			
16SL	CGCCTGTTTATCAAAAACAT	30	50°C
16SR	CCGGTCTGAACTCAGATCACGT		
12S[f]			
L1091	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT	35	43°C
H1478	TGACTGCAGAGGGTGACGGGCGGTGTGT3		
NTF-3[g]			
NTF3-f1	ATGTCCATCTTGTTTTATGTGATATTT	35	(L) 50°C
NTF3-r1	ACRAGTTTRTTGTTYTCTGAAGTC	35	(L) 48°C
		35	(S) 45°C
R35[H]			
R35-f	GACTGTGGAYGAYCTGATCAGTGTGGTGCC	35	(L) 60°C
R35-R	GCCAAAATGAGSGAGAARCGCTTCTGAGC	35	(L) 48°C
		35	(S) 45°C

Each reaction was initiated with a 2 minute denaturing cycle at 94°C, and terminated with 5 minutes at 72°C as a final extension. All reactions were denatured at 94°C for 30 seconds, and extended at 72°C for 1 minute. Annealing cycles were 30 seconds long. Locus specific annealing temperatures and number of cycles are indicated above. (L)=Lizards, (S)=snakes

Phylogenetic analysis

CodonCode Aligner was used (v.3.5.6 CodonCode Corp.) to assemble sequencing and editing contigs. Each set of data for each gene were aligned and combined using the Muscle program (Edgar, 2004). Further adjustment alignments were made by eye. The translation of protein-coding genes into amino acid sequences was conducted in CodonCode aligner to check if stop codons existed. To determine all heterozygous positions for each nuclear gene, an input file using SeqPHASE (Flot, 2010) was generated. This file was input into the program PHASE (v 2.1.1) to construct the phased haplotypes from the diploid genotype in an organism (Stephens et al., 2001, Stephens and Scheet, 2005). Phase analyses were implemented separately for each species in order to calculate haplotypes within the population for each species. These analyses involved two runs with different, randomly selected starting seeds, each consisting of 1,000 generations with a thinning interval of 10 and preceded by a burn-in of 100 generations.

Species tree and gene tree

The evolutionary relationships between co-distributed groups of lizards and phylogeographic patterns were inferred under a multispecies coalescent model using the program *BEAST (Heled and Drummond, 2010).

Phased haplotype sequences (as described in section 1.3.3) from the two nuclear loci (R35 and NTF-3) and three mitochondrial genes (cytb, 12S and 16S) were used for this analysis. Species trees were estimated under a Yule speciation tree and piecewise constant population size model. Unlinked substitution and molecular clock models were assigned to each mitochondrial gene and each nuclear locus. Individual gene trees were estimated for each nuclear locus, whilst a single mitochondrial gene tree topology was linked and generated from the cytb 12S and 16S dataset, since these genes represent a single locus. The best-fit substitution model of the dataset was inferred using PartitionFinder (Lanfear et al., 2012). The highest score based on corrected Akaike Information Criterion (AICc) was selected as optimal. Where the model selected by PartitionFinder was unavailable in BEAUti or if parameters did not converge for that specific model after tens or even hundreds of millions of generations, then another was chosen according to the second-best AICc score. A preliminary *BEAST analysis indicated that uncorrelated lognormal relaxed molecular clock

failed to converge. Therefore, a strict molecular clock was used for the final analysis. The final analysis consisted of two independent runs, one of 150 million generations and one of 250 million generations, each sampling the MCMC chain every 10,000 generations. Convergence of analysis was based on the effective sample size (ESS) values, those that were above 200 were checked using Tracer, then 10% as a burn-in was removed. These two runs were combined in Logcombiner to give a final posterior. The first 10% of trees, based on Tracer, were removed from each run as a burn-in, and the remainder was combined and re-sampled in Logcombiner. The final tree was annotated by TreeAnnotator from 35,001 species trees to provide the maximum clade credibility tree and posterior clade probabilities.

Molecular dating

Evolutionary history that leads to splitting events for a group of species with their divergence time is usually represented as a time tree (Hedges and Kumar, 2004). Molecular dating that seeks to estimate the divergence time of clades has recently been considered one of the fundamental aspects of molecular ecology (Bromham and Penny, 2003, Rutschmann, 2006, Yang and Rannala, 2006) .

Fossil evidence and geological events are the most useful sources of prior information that can be used to place informative priors on nodes and hence, provide information to help to explain, divergence times. The lack of material and incomplete information from fossil evidence usually creates misleading estimates of the divergence time for particular cladogenesis events. In fact, fossil calibration may provide an approximate minimum age for the presence of a clade, but it is difficult to provide the true maximum age, except in the cases of oceanic islands where speciation events cannot be older than the islands themselves (Hedges and Kumar, 2004). For this reason, the development of molecular statistical methods such as algorithms which employ priors, typically utilise maximum constraints with soft bounds and minimum constraints with hard bounds. These allow either lognormal or normal population distribution probabilities to be optimised and may most accurately model the likely divergence time of a group of species (Yang and Rannala, 2006).
The Order Squamata comprises the lizards, snakes and amphisbaenians which form a monophyletic group of scaly reptiles in most phylogenetic trees (Vidal and Hedges, 2009).

There is evidence that the diversification of the Squamata group initiated in the Jurassic and Cretaceous periods approximately, 260 million years ago (Vidal and Hedges, 2009). Estimating the divergence time for a group lacking calibrations by utilising calibration points for other groups under specific assumptions and conditions (Hedges and Kumar, 2004). Unfortunately, no fossils belonging to the sampled lizards were available. Therefore two fossil calibrations from snakes and one geological event were used to estimate the divergence time of Arabian Peninsula lizards (Pook et al., 2009, Vidal and Hedges, 2005).

1. Vidal and Hedges (2005) used five fossils and nine nuclear DNA genes, one of which has been used in this study., Their main dates for the divergence of the crown clade Squamata fall well within 251 Mya to 221 Mya. Based on this work, we constrained the root age of the species tree as a normal distribution with a mean of 240 and a standard deviation of 10.

2. *Porthidium:* This study used a normal distribution with a mean of 3.5 Mya and a standard deviation of 0.51 Mya, since the first divergence time between three populations of the South American Neotropical pit viper genus *Porthidium* appear to coincide with the uplift of the Isthmus of Panama, approximately 3.5 Mya (Wüster et al., 2002).

3. *Echis*: The basal cladogenesis in *Echis* (Pook et al., 2009) is dated to 22 Mya. In this study, the split between *E. coloratus* from United Arab Emirates and *E. omanensis* from southern Oman took place approximately 8.1 Mya. Therefore, a normal distribution with a mean of 22 Mya and standard deviation of 1 was used to constrain the basal cladogenesis of *Echis.*

4. *Naja*: A lognormal distribution with a 16 Mya zero offset and standard deviation of 1 was used for this node. According to Szyndlar and Rage (1990) and Wüster et al. (2007) the split between the Asian *Naja* clade and its African sister clade dates back to a minimum age of 16 Mya, based on the fossil evidence for species with African and Asian affinities within this genus.

2.3 Results

DNA sequences

A dataset of five genes comprising three mitochondrial DNA genes (cytb, 12S, and 16S) and two nuclear loci (R35 and NTF-3) were used in this study. In the final alignment, 642 base pairs (bp) of cytb; 540 bp of 16S; and 392 bp of 12S, and 635 bp of R35 and 653 bp of NTF-3 were aligned for each individual gene. No stop codons were found for the coding genes cytb, NTF-3, and R35. Table 2.2 illustrates the characteristics of the DNA data set used in this study.

Table 2.2. DNA characteristics including genes, length sequences (LS), variable sites (v.s.), parsimony informative sites (P.S.) and models used in this study.

Gene	LS	P.S.	V.S.	Model
12S	392	237	250	GTR+I+G
16S	540	313	332	GTR+I+G
CYTB	642	427	524	TVM+G
NTF-3	653	310	320	K80+I+G
R35	635	341	345	K81+G

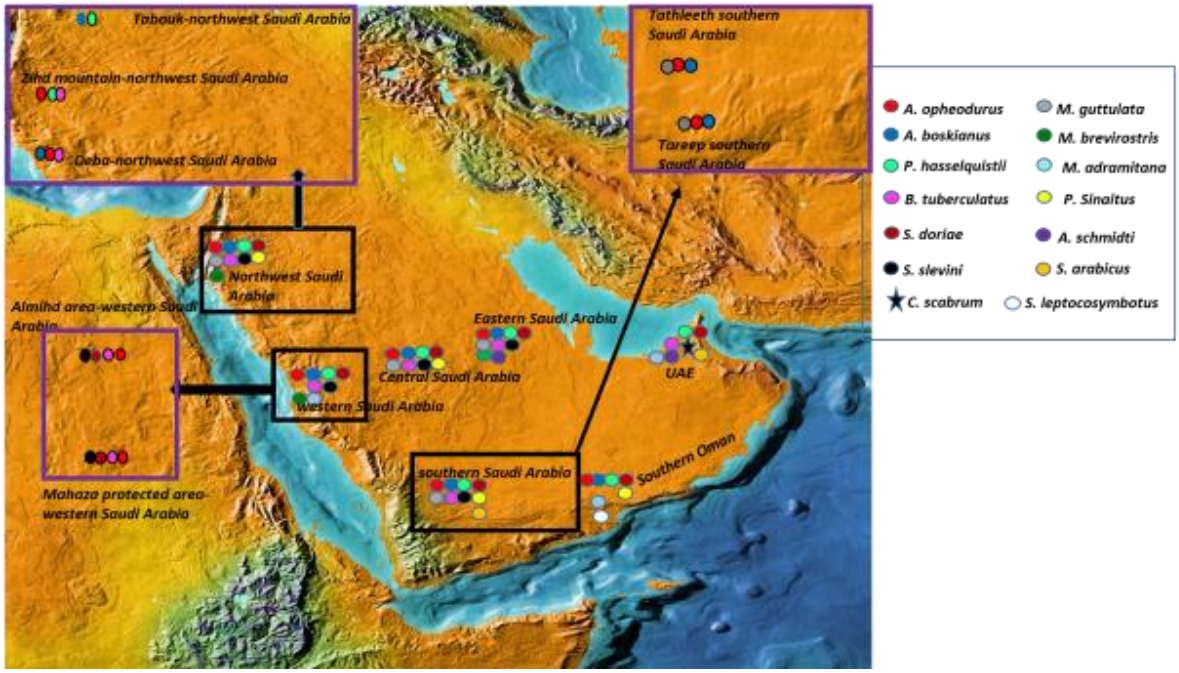


Fig 2.1. Distribution map of lizard localities from the Arabian Peninsula used in this study. Colours correspond to different species. Magnified boxes indicate localities from Southern, Western, and Northwest Saudi Arabia where some species were collected.

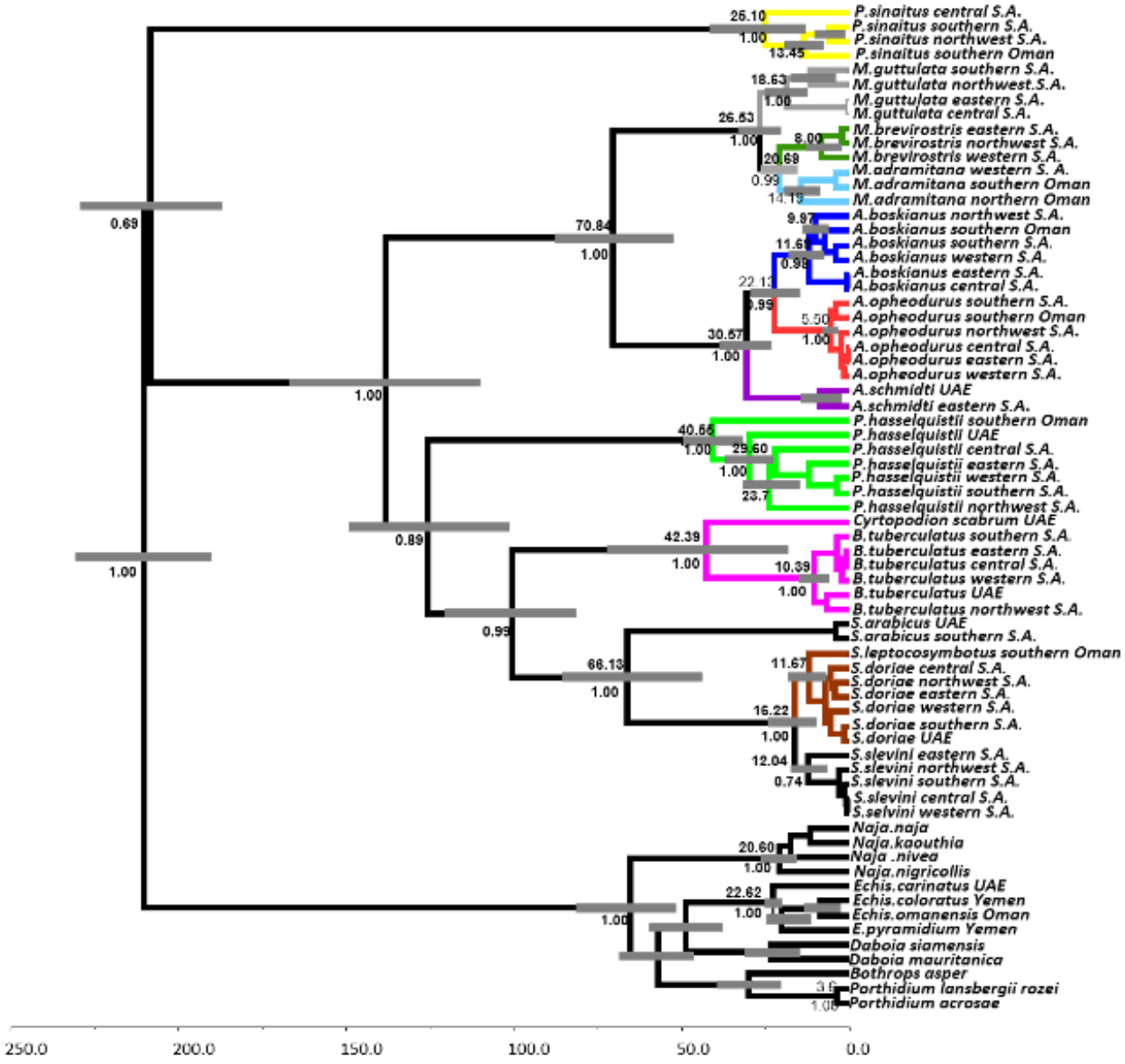
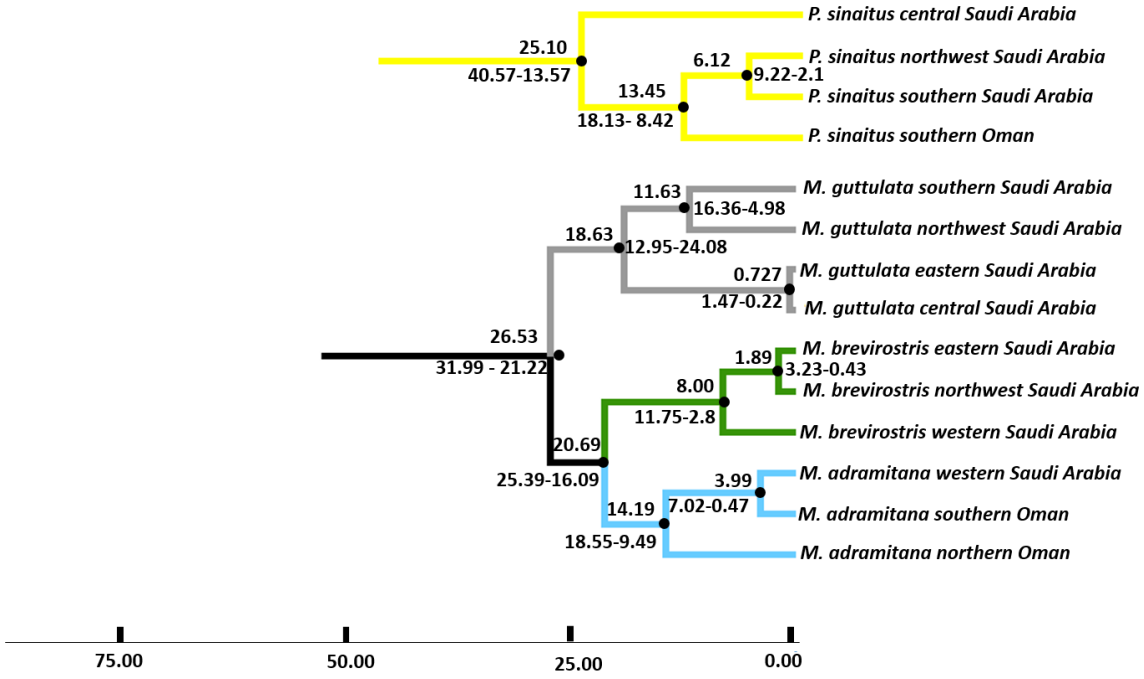
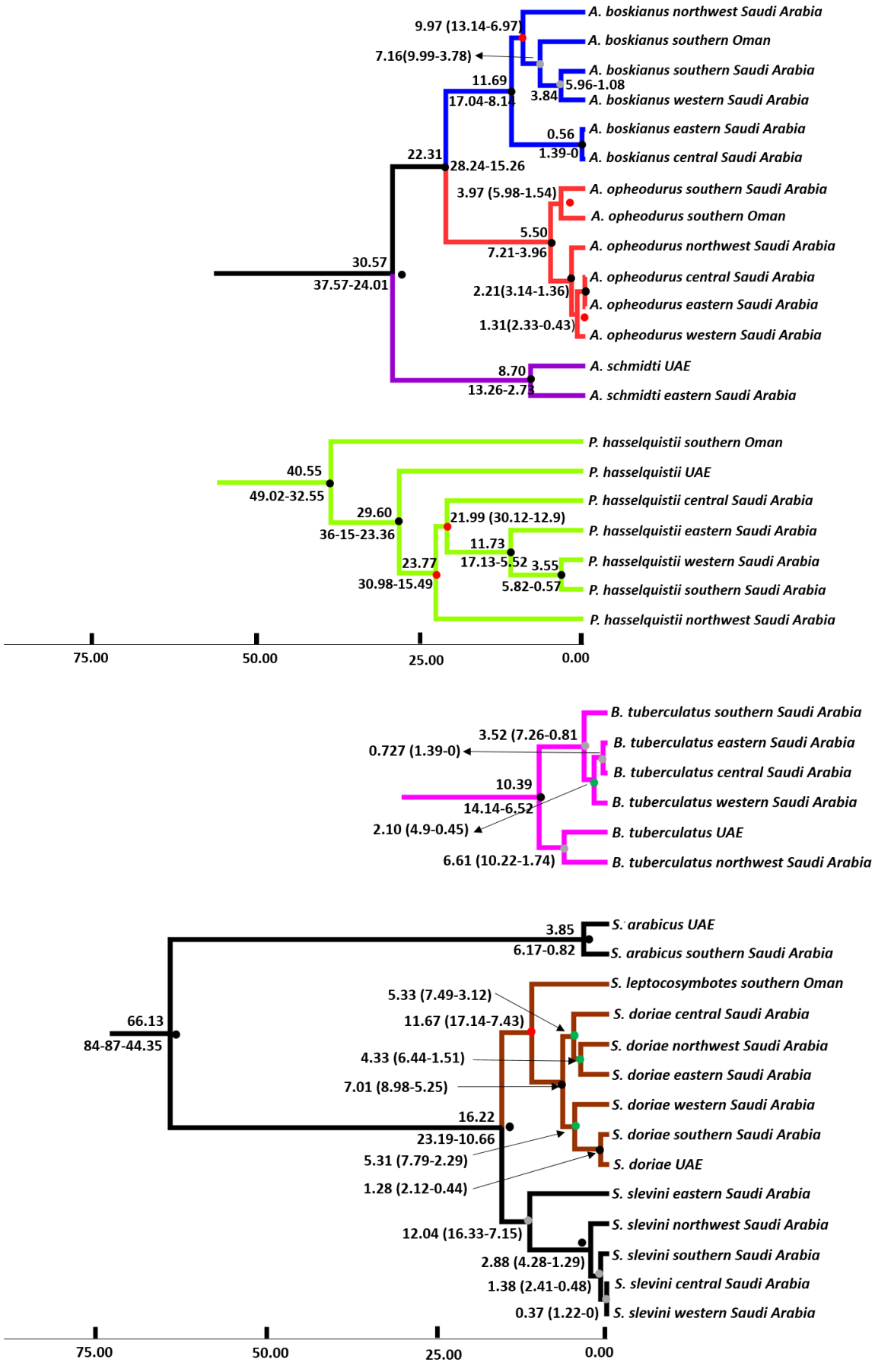


Fig. 2.2. Multilocus species tree of lizards from the Arabian Peninsula inferred using *BEAST from three mtDNA genes (cytb, 12S, 16S) and two nuclear loci (R35 and NTF-3). Grey bars are the 95% highest probability density (HPD) confidence intervals. Numbers below the nodes are posterior probability support values. Scale times in millions of years are indicated at the bottom of the tree.


P. sinaitus central Saudi Arabia
25.10
40.57-13.57
6.12
9.22-2.1
P. sinaitus northwest Saudi Arabia
P. sinaitus southern Saudi Arabia
13.45
18.13- 8.42
P. sinaitus southern Oman
11.63
16.36-4.98
M. guttulata southern Saudi Arabia
M. guttulata northwest Saudi Arabia
18.63
12.95-24.08
0.727
1.47-0.22
M. guttulata eastern Saudi Arabia
M. guttulata central Saudi Arabia
26.53
31.99 - 21.22
1.89
3.23-0.43
M. brevirostris eastern Saudi Arabia
8.00
11.75-2.8
M. brevirostris northwest Saudi Arabia
M. brevirostris western Saudi Arabia
20.69
25.39-16.09
3.99
7.02-0.47
M. adramitana western Saudi Arabia
M. adramitana southern Oman
14.19
18.55-9.49
M. adramitana northern Oman
75.00
50.00
25.00
0.00


A. boskianus northwest Saudi Arabia
9.97 (13.14-6.97)
A. boskianus southern Oman
7.16(9.99-3.78)
A. boskianus southern Saudi Arabia
11.69
5.96-1.08
17.04-8.14
3.84
A. boskianus western Saudi Arabia
A. boskianus eastern Saudi Arabia
0.56
1.39-0
A. boskianus central Saudi Arabia
22.31
28.24-15.26
A. opheodurus southern Saudi Arabia
3.97 (5.98-1.54)
A. opheodurus southern Oman
5.50
A. opheodurus northwest Saudi Arabia
7.21-3.96
30.57
37.57-24.01
A. opheodurus central Saudi Arabia
2.21(3.14-1.36)
A. opheodurus eastern Saudi Arabia
1.31(2.33-0.43)
A. opheodurus western Saudi Arabia
A. schmidti UAE
8.70
13.26-2.73
A. schmidti eastern Saudi Arabia
P. hasselquistii southern Oman
40.55
P. hasselquistii UAE
49.02-32.55
P. hasselquistii central Saudi Arabia
29.60
21.99 (30.12-12.9)
36-15-23.36
P. hasselquistii eastern Saudi Arabia
11.73
17.13-5.52
23.77
3.55
P. hasselquistii western Saudi Arabia
30.98-15.49
5.82-0.57
P. hasselquistii southern Saudi Arabia
P. hasselquistii northwest Saudi Arabia
75.00
50.00
25.00
0.00
B. tuberculatus southern Saudi Arabia
3.52 (7.26-0.81
B. tuberculatus eastern Saudi Arabia
0.727 (1.39-0)
B. tuberculatus central Saudi Arabia
10.39
B. tuberculatus western Saudi Arabia
14.14-6.52
2.10 (4.9-0.45)
B. tuberculatus UAE
6.61 (10.22-1.74)
B. tuberculatus northwest Saudi Arabia
3.85
S. arabicus UAE
6.17-0.82
S. arabicus southern Saudi Arabia
S. leptocosymbotes southern Oman
5.33 (7.49-3.12)
S. doriae central Saudi Arabia
66.13
11.67 (17.14-7.43)
84-87-44.35
S. doriae northwest Saudi Arabia
4.33 (6.44-1.51)
S. doriae eastern Saudi Arabia
7.01 (8.98-5.25)
S. doriae western Saudi Arabia
16.22
S. doriae southern Saudi Arabia
23.19-10.66
5.31 (7.79-2.29)
S. doriae UAE
1.28 (2.12-0.44)
S. slevini eastern Saudi Arabia
S. slevini northwest Saudi Arabia
12.04 (16.33-7.15)
S. slevini southern Saudi Arabia
2.88 (4.28-1.29)
S. slevini central Saudi Arabia
1.38 (2.41-0.48)
0.37 (1.22-0)
S. slevini western Saudi Arabia
75.00
50.00
25.00
0.00

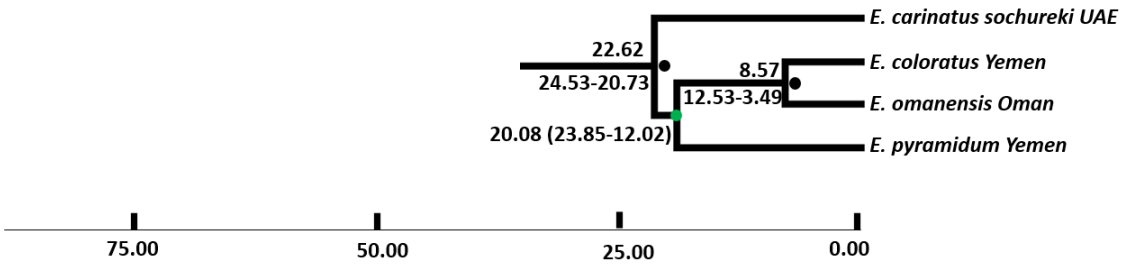


Fig 2.3. The multilocus tree in figure 2.2 has been divided into sub trees for each species (or each genus, in some cases to compare divergence time easily). Colours around the nodes indicate the posterior probability support value: the black circles are over 0.95; grey circles are between 0.70-0.95; Red circles are between 0.50-0.70, and the green circles are values less than 0.50.

Phylogenetic analysis

A multilocus species tree formed using *BEAST generated the four monophyletic groups of lizards and snakes, with weakly supported posterior probability (0.69) that the snakes are placed outside the remaining lizard tree. Well-supported nodes were shown for most clades among groups (Figs. 2.2 and 2.3). The squamata tree presented in this study is not a fully comprehensive assessment of the Arabian Peninsula lizards. Another important limitation of the squamata tree is that it only provides patchy coverage of the squamata clade. Only four gecko genera (*Ptyodactyllus, Stenodactylus, Bunopus and cyrtopodion)* which belong to two families (Gekkonidea and Ptyodactylidae) and are represented as a monophyletic clade. Two genera (*Acanthodactylus* and *Mesalina*) from the family Lacertidae also formed a monophyletic clade. One species (*Pseudotrapelus sinaitus*) from Agamidae family was placed as a sister clade for the remaining lizard groups. Another main monophyletic clade was that of snake group. The findings of this study broadly consist with recently published squamata trees, with one exception. These trees showed the snakes to be sister species to the Agamids (Townsend et al., 2004, Vidal and Hedges, 2005, Wiens et al., 2006, Wiens et al., 2010).

The basal divergence within the *P. sinaitus* complex and the divergence between the *Mesalina* groups took place at approximately 25-26 Mya, and the divergence time of the split between *Acanthodactylus opheodurus* and *A. boskianus*, was dated at approximately

22 Mya (Figs. 2.2 and 2.3). The divergence time for the split of *M. guttulata* from the two *Mesalina* species (*M. brevirostris* and *M. adramitana*) was 26.54 Mya.

The analysis of the species tree indicates that *Ptyodactylus hasselquistii* from southern Oman constitutes the sister species of the rest of the *P. hasselquistii* group, from which it separated at approximately 40.56 Mya. The United Arab Emirates (UAE) *Cyrtopodion scabrum* formed a sister species of *B. tuberculatus* with an estimated divergence time between two species at approximately 42.39 Mya. The *Stenodactylus* genus, which is represented here by four species (*S. leptocosymbotus*, *S. arabicus, S. doriae,* and *S. slevini),* formed four distinct clades for each species. For the genus *Echis,* the monophyletic clade consists of *E. carinatus* from the UAE and the clades formed by *E. omanensis* from Oman and the clade formed by *E. pyramidum* from Yemen. *E. carinatus* separated from these clades at approximately 22 Mya.

Phylogeographic Patterns

The results from this study reveal common phylogeographic patterns among groups of lizard species. Very recent sister group relationships were observed between eastern and central Saudi Arabia for *Acanthodactylus boskianus*, *A. opheodurus, Mesalina guttulata* and *Bunopus tuberculatus*. These regions revealed a shallower structure of phylogeographic patterns across all regions. Interestingly, the divergence time of these patterns seem to coincide with each other, as the estimation of 95% highest posterior density [HPD] intervals for this divergence ranged from 0–1.47 Mya (Fig 2.3). Common spatial and temporal patterns were also seen at the clade level for all four species (Fig 2.4; Fig 2.5).

Close sister group relationships were also seen between northwest and southern Saudi Arabia for two species, *Pseudotrapelus sinaitus* and *Mesalina guttulata*. The divergence of *P. sinaitus* between northwest and southern Saudi Arabia took place at approximately 6 Mya while *M. guttulata* diverged at approximately 13 Mya. Another close sister group relationship were found between western and southern Saudi Arabia for *Acanthodactylus boskianus* and *Ptyodactylus hasselquistii*. The divergence times for the two species, estimated at 3.84 Mya and 3.55 Mya respectively, closely matched. Northwest and eastern Saudi Arabia showed similar phylogeographic patterns for two species, *Mesalina brevirostris* and *Stenodactylus doriae*. The separation between northwest and eastern Saudi Arabia for

M. brevirostris took place at approximately 1.89 Mya whereas for *S. doriae* is estimated at 4.3 Mya. Similar phylogeographic patterns were also seen at the clade levels for the two species (Fig 2.4). The clades consisting of central, eastern, and western Saudi Arabian species were similar for *Acanthodactylus opheodurus* and *Bunopus tuberculatus* with divergence times estimated at 1-3 Mya (Fig 2.5.).

Common clade divergence times between 10 – 12 Mya were observed between clades of *M. guttaluta* (southern and northwestern Saudi Arabia), *A. boskianus (*eastern and central Saudi Arabia vs all other clades), *P. hasselquistii* (eastern versus southern and western Saudi Arabia), *S. slevini (*eastern Saudi Arabia versus all other clades) and *Bunopus tuberculatus* (UAE and northwestern Saudi Arabia versus all other clades. (Fig 2.4; Fig 2.5)). Common clade divergence times between 5-7 Mya were observed between clades of *P. sinaitus* (northwest and southern Saudi Arabia versus southern Oman), *A. boskianus* (northwest and southern Saudi Arabia versus southern Oman) and *Bunopus tuberculatus* (UAE and northwest Saudi Arabia against all the other clades). Common clade divergence times between 3-5 Mya were observed between clades of *P. hasselquistii* (southern and western Saudi Arabia), *A. opheodurus* (southern Saudi Arabia and southern Oman and also between northwestern Saudi Arabia against central, eastern and western Saudi Arabia), *S. doriae* (western Saudi Arabia versus southern Saudi Arabia and UAE and between central Saudi Arabia versus eastern and northwestern Saudi Arabia and between northwest and eastern Saudi Arabia).

Conversely, this study also showed that different phylogeographic patterns occurred in many cases among different regions and species. Fig 2.6 and Tables 2.3, 2.4, and 2.5 provide more information about these common and different phylogeographic patterns. Notably, this study revealed important and very restricted distribution patterns within the Arabian Peninsula among different species. For example, central Saudi Arabia showed a deep divergence and unique pattern for *P. sinaitus* and *P. hasselquistii*. *A. boskianus* was restricted to northwest Saudi Arabia and *P. hasselquistii* and *S. slevini* were restricted to eastern Saudi Arabia.

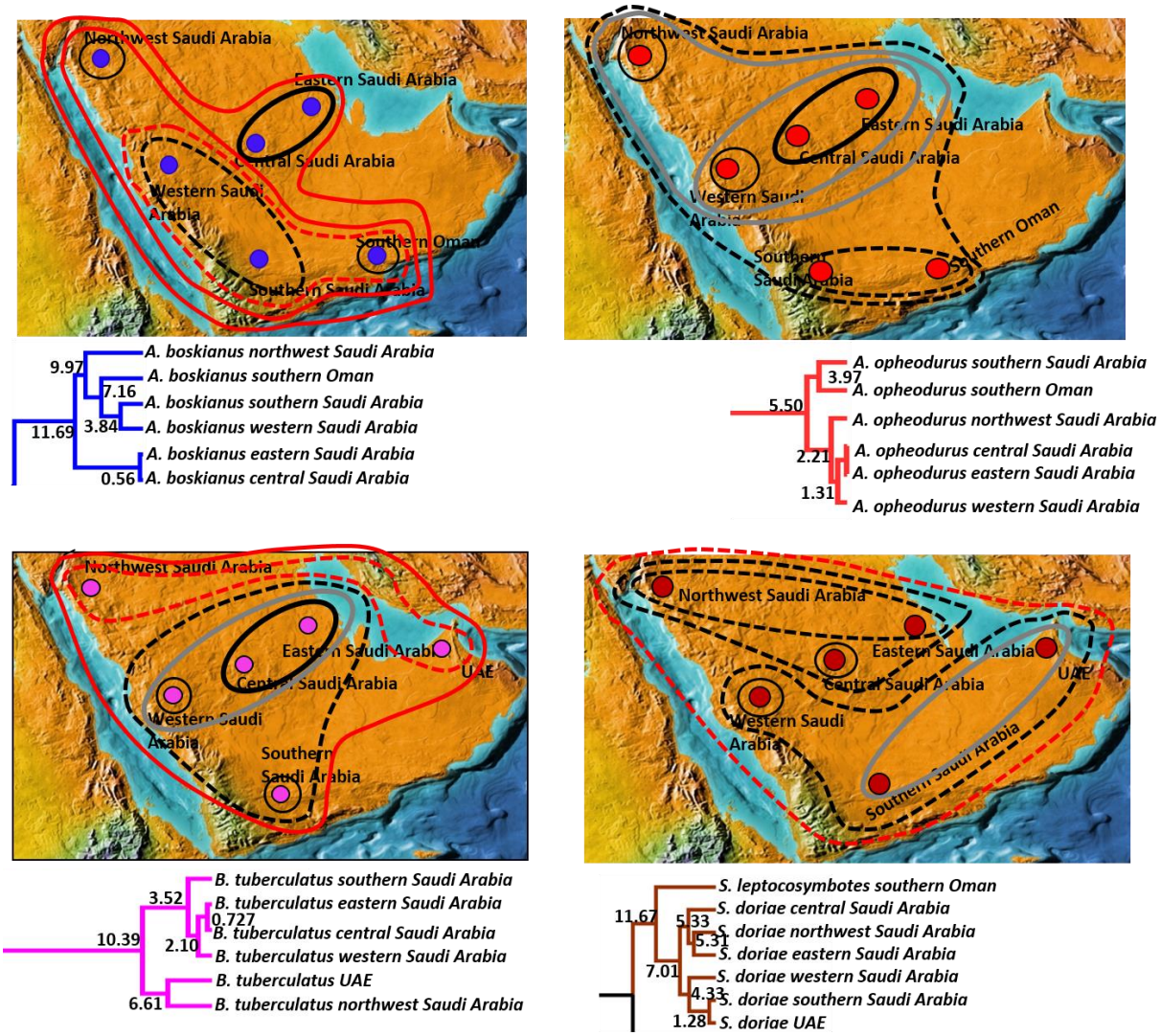


Fig 2.4. Clade maps depicting common spatial and temporal patterns for four lizard species across the Arabian Peninsula.

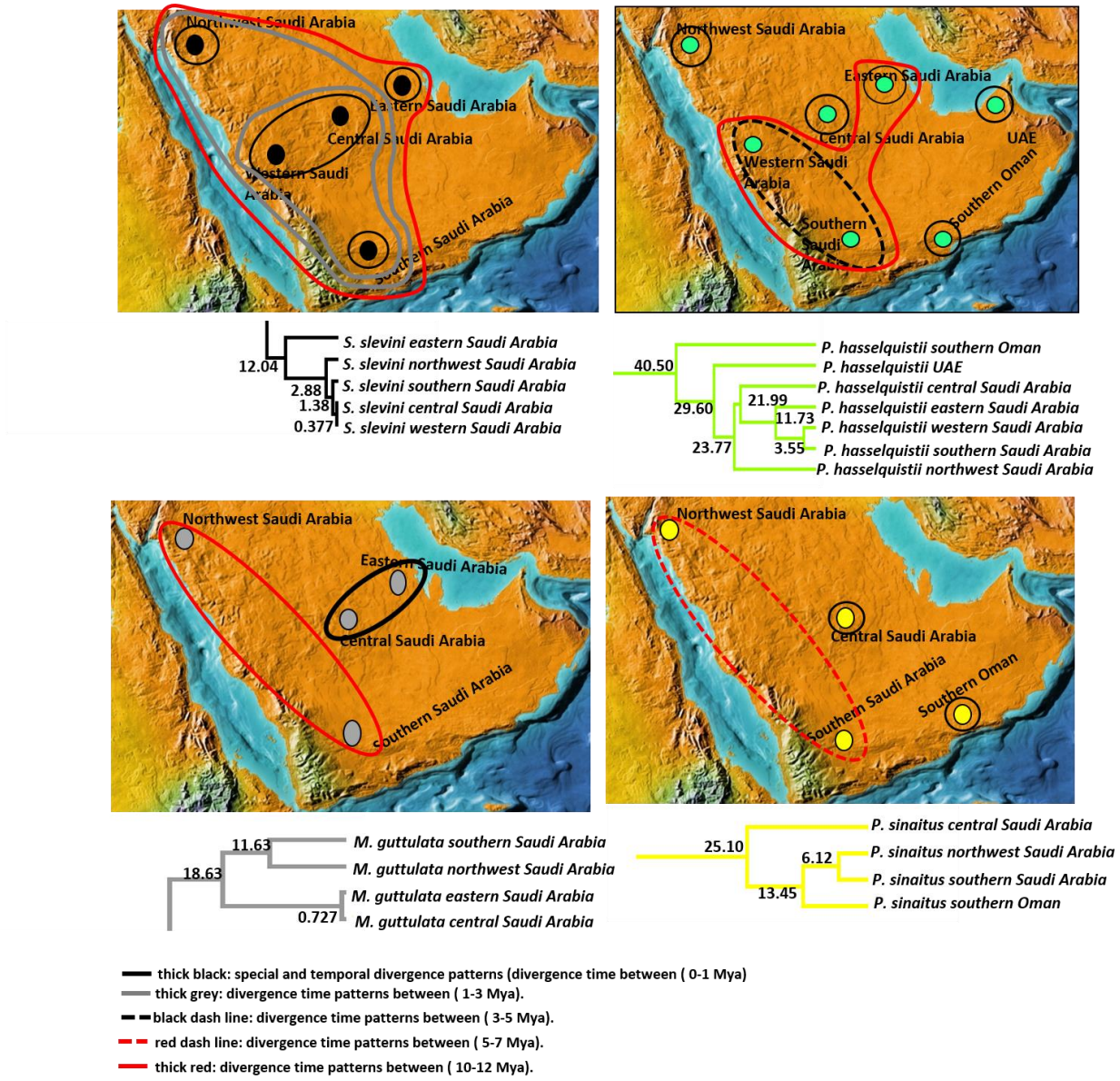


Fig 2.5. Clade maps depicting common spatial and temporal patterns for four lizard species across the Arabian Peninsula.

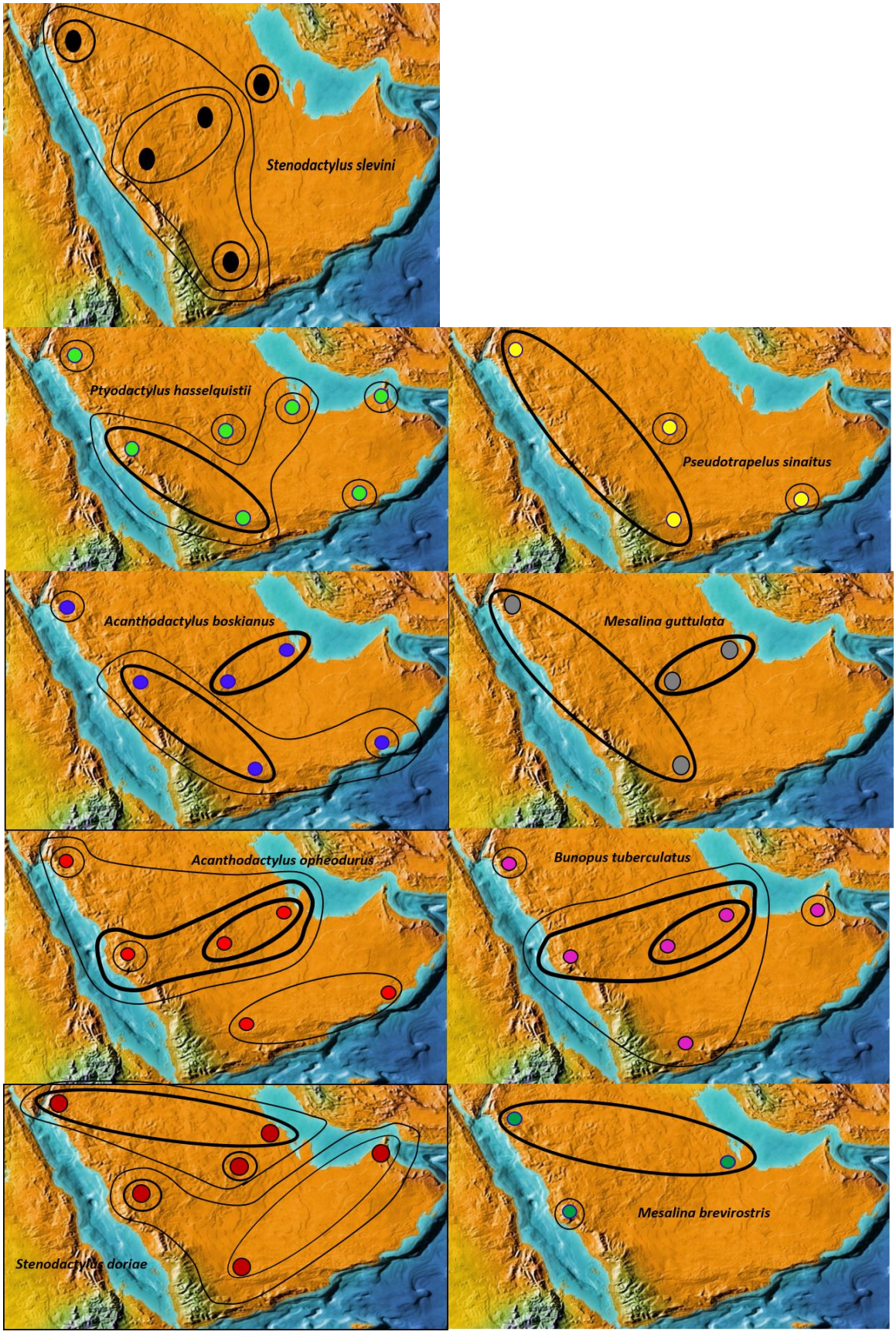


Fig 2.6. Clade maps depicting the generalised, geographical common patterns by species as described in Table 2.3 and 2.4.

Table 2.3. Common phylogeographic patterns illustrating close phylogeographic sister group relationships between groups of lizards and snakes from the Arabian Peninsula (derived from Fig. 2.3). NWSA=northwest Saudi Arabia; WSA= western Saudi Arabia; SSA= southern Saudi Arabia; ESA= eastern Saudi Arabia; CSA= central Saudi Arabia; SO= southern Oman; NO= northern Oman, and UAE= United Arab Emirates. Individual species are represented here by different colours. Numbers after the species names represent the divergence time, and numbers between parentheses represent the interval value.

Common Patterns	NWSA	WSA	CSA	SSA	ESA	SO
P. sinaitus 6.12(9.22-2.1)						
M. guttulata 11.35 (16.36-4.98)						
A. boskianus 0.56 (1.39-0)						
A. opheodurus 0.179 (0.179-0)						
B. tuberculatus 0.727 (1.39-0)						
M. guttulata 0.727 (1.47-0.22)						
P. hasselquistii 3.55 (5.82-0.57)						
A. boskianus 3.84 (5.96-1.08)						
S. doriae 4.33 (6.44-1.51)						
M. brevirostris 1.89 (3.23-0.43)						

Table 2.4. Close phylogeographic relationships in the context of overall sampling and distribution. Red = unique geographic patterns among species, Blue = close phylogeographic relationships between eastern and central Saudi Arabia; green = northwest and eastern Saudi Arabia; gold = northwest and southern Saudi Arabia; yellow = southern and western Saudi Arabia Pink = southern Saudi Arabia, southern Oman and Yemen. Grey sections represents clades with different spatial patterns. All species were sampled from the regions that are represented in this table. For example, *M. adramitana* were sampled from southern, northern Oman and western Saudi Arabia only.

P. sinaitus	SO	NWSA+SSA			CSA	
M. guttulata		NWSA+SSA		ESA+CSA		
M. adramitana	SO+WSA	NO				
M. brevirostris		NWSA+ESA			WSA	
A. boskianus	SO	NWSA	SSA+WSA	ESA+CSA		
A. opheodurus	SO+SSA	NWSA		ESA+CSA	WSA	
P. hasselquistii	SO	NWSA	SSA+WSA	ESA	CSA	UAE
B. tuberculatus		NWSA+UAE	SSA	ESA+CSA	WSA	
S. doriae		NWSA+ESA	SSA+UAE	CSA	WSA	
S. slevini		NWSA	SSA	ESA	CSA+WSA	
Echis coloratus group	SO+Yemen					

Table 2.5. Basal lineages of each species vs. the remaining sister clades from different geographical regions. Colours are representative of the individual species as shown in the species tree in Figures 2.2 and 2.3.

Species	Basal lineages	Sister clades				
P. sinaitus	CSA		NWSA+SSA			SO
M. guttulata	CSA+ESA		NWSA+SSA			
M.brevirostris	WSA		ESA+NWSA			
M. adramitana	NO		WSA+SO			
A. boskianus	ESA+CSA		SSA+WSA		NWSA	SO
A. opheodurus	SSA+SO		CSA+ESA+WSA		NWSA	
P. hasselquistii	SO	UAE	NWSA	CSA	ESA	WSA+SSA
B. tuberculatus	UAE+NWSA		ESA+CSA+WSA		SSA	
S. doriae	CSA		NWSA+ESA	WSA	SSA+UAE	
S. slevini	ESA		SSA+CSA+WSA	NWSA		

2.4 Discussion

This study has revealed the phylogeographic patterns of fourteen co-distributed lizard species found on the Arabian Peninsula. In so doing, it provides the first molecular and historical biogeographical investigation of these groups and provides fundamental knowledge on the current distribution patterns of the Arabian Peninsula fauna. Primarily, this study focused on the estimation of spatial relationships and divergence times between groups and within lizard species. This focus allowed us to examine the past geographical and geological events that may have played a major role in shaping the current distribution patterns of lizards and snakes on the Arabian Peninsula. The results from this study reveal common patterns of close phylogeographical relationships among several species. These shared close spatial relationships were found between northwest and southern Saudi Arabia in *Pseudotrapelus sinaitus* and *Mesalina guttulata*; between eastern and central Saudi Arabia in *Acanthodactylus boskianus, A. opheodurus, Bunopus tuberculatus,* and *Mesalina guttulata.* Northwest and eastern Saudi Arabia showed similar sister group relationships in populations of *Mesalina brevirostris* and *Stenodactylus doriae,* and western and southern Saudi Arabia in *Acanthodactylus boskianus* and *Ptyodactylus hasselquistii* complex.

Phylogeographic relationships between northwest and southern Saudi Arabia

Close sister group relationships were observed between northwest and southern Saudi Arabia for two of the studied lizard species; *Mesalina guttulata* and *Pseudotrapelus sinaitus.* The split between *Mesalina guttulata* and the clades of its sister species, formed by *M. brevirostris* and *M. adramitana*, was estimated to have occurred approximately 26 Mya ago [HPD 21.31-31.91], and the split of central Saudi Arabian *P. sinaitus* from the monophyletic clades comprising *P. sinaitus* from southern Oman and southern and northwest Saudi Arabia, its sister lineages, occurred approximately 25 Mya ago [HPD 13.57- 40.57]. These common patterns match very well with geological events documented for this region as approximately 27 Mya the Afro-Arabian continents separated, forming the Red Sea and the gulf of Aden (Bosworth et al., 2005). The findings from this study suggest that this historical event could be responsible for the subsequent species diversification observed in *Mesalina guttulata* and *Pseudotrapelus sinaitus* between different regions.

Published estimates of the divergence time between *Mesalina guttulata* and *Mesalina brevirostris* largely support the findings of this study. Smid and Frynta (2012) estimated the divergence time between *M. watsonami* from Iran and other *Mesalina* species including *M. guttulata* and *M. brevirostris* species from North Africa and the Middle East at approximately 15.9 Mya [HPD 25.6-7.8].

Both *Mesalina guttulata* and *Pseudotrapelus sinaitus* display similar sister group relationships between populations from northwest and southern Saudi Arabia. The Northwest *P. sinaitus* group seemed to have split from southern Saudi Arabia later, at approximately 6 Mya, compared to *M. guttulata,* at 11 Mya from the same regions, but the confidence intervals overlap substantially. The clade of *Mesalina guttulata* comprising northwest and southern Saudi Arabia split around 18 Mya from its eastern and central Saudi Arabian groups, whereas the same clades of *P. sinaitus* diverged from southern Oman lineages slightly later, at 13 Mya.

However, contrasting divergence times have also been reported for *Mesalina* species. This may be due to differing methods used to establish the calibration points in addition to differing species distributions within regions. Smid and Frynta (2012) measured the rates of change in a single locus (cytochrome b [cytb] to determine the divergence time between M*. watsonana* from Iran and other *Mesalina* species including *M. guttulata* and *M. brevirostris* species from north Africa and Middle East at approximately 15.9 Mya with the 95% HPD confidence Intervals as (25.6-7.8 Mya), whereas the divergence time estimation between *M guttulata* and *M. brevirostris* was 9.5 Mya with (15.5-4.6 Mya) of the HPD. These findings are consistent with the current study findings. In contrast, Kapli et al. (2008) used 16S and cytb genes and estimated the time split at approximately 7 Mya (± 0.8).

Using a broader range of samples and a multilocus approach (two mtDNA and one nDNA genes), Kapli et al. (2014) estimated the initial divergence of the genus *Mesalina,* including three of the species represented in this study, at approximately 22 Mya. They estimated the divergence time between the *Mesalina guttulata* complex and *M. brevirostris* and *M. adramitana* within the Arabian Peninsula at approximately 16 Mya, which is consistent with the findings of this study. This suggests that this genus arose in the Middle East in the early Miocene and subsequently moved to the Arabian Peninsula. Consequently, these authors

attributed the split between Arabian and African *Mesalina* to vicariance events and speciation events due to climate change since the Miocene.

The distributions are generally sympatric but the two species are not syntopic: *P. sinaitus* is a rock-dwelling lizard, while *M. guttulata* is ground dwelling species that occurs in plains characterised by hard substrata (Ross, 1988). Despite the fact that these two species were sampled from central Saudi Arabia, close and recent sister group relationships appear between southern and northwest Saudi Arabia populations compared to central populations. Lineages of *P. sinaitus* and *M. guttulata* from southern and northwest Saudi Arabia were separated by long distances, including different ecological habitats that varied from mountainous terrain to sandy habitats and extended more than 1500 Km. Thus, this distance could have isolated both lineages of the two species from each other, leading to the conclusion that these two forms shared a historical event that shaped their current-day distribution. In addition, these species are strictly diurnal and they inhabit different ecological niches.

The distribution and range of these species from Saudi Arabia is poorly understood. Therefore, despite the extensive survey conducted, *Mesalina guttulata* was not found in western Saudi Arabia, despite being found in eastern, southern and central Saudi Arabia. *Pseudotrapelus sinaitus* was not found in eastern Saudi Arabia, despite being found in southern and northwesten Saudi Arabia. The former seems to be replaced by *M. brevirostris* in western Saudi Arabia and *M. adramitana* and the latter replaced by *Trapelus pallidus* and *T. flavimaculatus* in eastern Saudi Arabia (Arnold, 1980a, Arnold, 1984).

Phylogeographic relationships between central and eastern Saudi Arabia

Close sister group relationships were seen between eastern and central Saudi Arabia in three species of lacertids and one gecko species. These species were: *Acanthodactylus boskianus, A. opheodurus, Mesalina guttulata* and *Bunopus tuberculatus* The divergence times overlapped in all cases, with the HPD ranging from 1.47- 0 Mya between lineages from eastern and central Saudi Arabia. This recent divergence indicates that these distributions until recently were contiguous or remain so to this day.

These shared spatial and temporal patterns can be explained by the existence of continuous habitat types between these regions. All four species are ground dwellers as opposed to rock dwellers. Interestingly, *A. boskianus, A. opheodurus* and *M. guttulata* are diurnally active whilst B*. tuberculatus* is nocturnally active. *A. boskianus* and *A. opheodurus* were found to be sympatric species, with similar morphological forms (Arnold, 1989, Arnold, 1980a).

The observed shallow divergence patterns may have resulted from incomplete lineage sorting or from gene flow. Between both regions (central and eastern Saudi Arabia) the habitat type is continuous (sandy habitat); therefore, it is likely that gene flow is the most likely explanation for the observed shallow divergence patterns. The recent divergence times and common patterns for this group of species indicate that dispersal events could have happened between these regions and consequently excludes the possibility of the occurrence of vicariance.

Therefore, the sand belt corridor known as the Ad-Dahna desert, which connects the Rub Al Khali (the empty quarter) with the A Nafud Al Kabir desert in the Al Jouf province of northern Arabian Peninsula (Fig. 2.7) may not have served as a barrier between the eastern and central Arabian Peninsula for *A. boskianus, A. opheodurus, M. guttulata and B. tuberculatus.*

In marked contrast, however, other species such as *Ptyodactylus hasselquistii* and *Stenodactylus slevini* reveal distinct clades from the eastern Saudi Arabia region. *Ptyodactylus. hasselquistii* tends to conceal cryptic species based on its ancient divergence times and both species differ in their habitat use. *P. hasselquistii* can be found in rocky habitats, whilst *S. slevini* can be found in sandy habitats and gravel plains. In both regions, these species show distant separation from their sister groups.

In the case of *S. slevini,* the sister group of the central Saudi Arabia population is from western Saudi Arabia, from which it shows more recent divergence (1.22-0 Mya), than the distinct clade from eastern Saudi Arabia (16.33-7.15 Mya). Sandy dwellers *Stenodactylus*

doriae from eastern Saudi Arabia also display close phylogeographic affinities to northwest Saudi Arabian populations rather than those from central Saudi Arabian populations.

Phylogeographic relationships between eastern and northwest Saudi Arabia

Similar sister group relationships from eastern and northwest Saudi Arabia for *Mesalina brevirostris* and *Stenodactylus doriae* were determined. The divergence time between the two regions for *Mesalina brevirostris* was estimated at approximately 1.89 Mya and at 4.33 Mya for *Stenodactylus doriae*. The close phylogeographic relationships for both species suggest that their distributions were contiguous until relatively recently and that a shared biogeographical process may have played a role in shaping their current distribution patterns. The divergence time for both species from both regions are older compared to the divergence time for the four sand-dwelling species from eastern and central Saudi Arabia. However, this finding may support the hypothesis that suitable habitats in eastern and central Saudi Arabia remained continuous or had diversified from each other more recently than habitat types between eastern and northwest Saudi Arabia. Interestingly, the biological factors and ecological habitats that may have affected diversification of both species are extremely different. *Stenocatylus doriae* is a nocturnal, sand-dwelling species (Metallinou et al., 2012), whereas *M. brevirostris* is a diurnal ground-dwelling species that can occupy hard gravel plains and occurs at the edges of wadis containing abundant vegetation (Disi et al., 2001). The findings from this study indicate that the northwestern and eastern Saudi Arabian populations of both species were connected and isolated from each other during the period spanning the upper Pliocene to the Pleistocene. These isolations can be explained by the long distance between these areas (1500km). Whilst the sand belt (Fig 2.8) does not serve to produce vicariance in the four studied lizard species between eastern and central Saudi Arabia, it was hypothesised to produce vicariance between lizard species from eastern and northwest Saudi Arabia.

Phylogeographic relationships between western and southern Saudi Arabia

Close sister group relationships were revealed by this study between western and southern Saudi Arabia for *Acanthodactylus boskianus* and *Ptyodactylus hasselquistii.* The divergence time between the two regions for *A. boskianus* and *P. hasselquistii* lineages took place at 3.5 Mya and 3.84 Mya respectively. This might suggest that common biogeographical events

may have played a role in shaping the current distribution patterns of these species. However, even though the divergence times between these species match very closely, the habitat occupied by these two forms and biological aspect are distinctly different. *Acanthodactylus boskianus* is diurnal and ground dwelling and typically inhabits areas containing hard substrata or more stable sand in valley beds and bottoms (Arnold et al., 2007, Disi et al., 2001). In contrast, *P. hasselquistii* is a generally nocturnal but occasionally diurnal (Nazarov et al., 2013) rock dweller found mainly between rocks in limestone or sandstone areas (Carillo de Espinoza et al., 1990). The evidence presented here indicates that the divergence between ancestors of *A. boskianus* and *P. hasselquistii* from western and southern Saudi Arabia occurred at approximately 3.5 Mya. Ecological changes in the climate and vegetation during the Pliocene-Pleistocene (Gómez-díaz et al., 2012) , and the four humid periods spanning from the Miocene until Pleistocene (Le Houérou, 1996) probably allowed these two forms to specialize and diversify in these regions when these two areas were associated and then isolated. Although the western and southern Saudi Arabian regions revealed common patterns, the distribution between these different forms indicated a separation of more than 800 Km. Therefore, long distances characterised by several ecological habitats, may have allowed for species dispersal.

Affinities of *Pseudotrapelus sinaitus* from Central Saudi Arabia

This study, in addition to interpreting the common patterns of the Arabian Peninsula lizards, has provided worthwhile information regarding the possibility of unique patterns and potential for distinct cryptic species. The central Saudi Arabian *P. sinaitus* formed a distinct clade from the remaining Arabian Peninsula *P. sinaitus.* The divergence time estimated from the species tree between central Saudi Arabia *P. sinaitus* and the clade formed by samples from southern Oman, southern and northwestern Saudi Arabia took place is approximately 25 Mya [HPD 40.5-13.5]. The ancestor of southern Oman lineages separated from a clade comprising southern and northwest Saudi Arabia at 13.45 Mya [HPD 18.13-8.42], subsequently followed by divergence between the populations from northwest and southern Saudi Arabia at 6.12 Mya [HPD 9.22-2.1]. The Southern Oman lineage has recently been described as a separate species, *P. dhofarensis* (Melnikov and Pierson 2012), which supports the idea of multiple cryptic species. However, this clade appears to be a geographically distant sister to the northwest and southern Saudi Arabia clade. The ancient

divergence time of the central Arabian population of *P. sinaitus* and the paraphyly of *P. sinaitus* in relation to *P. dhofarensis* strongly suggest the existence of additional cryptic species within this complex. At present, only two species are considered valid in the genus *Pseudotrapelus* (Melnikov et al., 2012); namely, *P. sinaitus* and *P. aqabensis*. The latter species has been recently described as a new species from Al Aqapah, southern Jordan (Melnikov et al., 2012). This study states that *P. aqabensis* was recorded in northwest Saudi Arabia, whereas *P. sinaitus* is distributed across the Arabian Peninsula, Syria, Sinai, Jordan, Israel, and northeastern Africa (Melnikov et al., 2012). In addition, *P. sinaitus werneri* is considered to be a subspecies of *P. sinaitus,* which is distributed in the Basalt Desert of Syria and Jordan (Melnikov et al., 2012). The possibility of two lineages of *P. sinaitus* in northwestern Saudi Arabia has been proposed (Sindaco and Jeremčenko, 2008).

Samples of *P. sinaitus* were collected from central (Fig 2.1) southern and northwestern Saudi Arabia and from southern Oman only. No samples were collected, nor specimens seen, in western and eastern Saudi Arabian regions. No literature exists on the specific distribution range of this species from the Arabian Peninsula; it is generally stated in the literature as having a general distribution across the whole Arabian Peninsula. (Arnold, 1986b, Schatti and Gasperetti, 1994). However, since we did not see the species in western and eastern Saudi Arabia, it may well be absent in the region, or, if it is present, it is extremely rare.

The Central Saudi Arabian population may have been separated from those of other regions due to vicariance events. Despite the fact that aridification had increased and existed for a prolonged period, geological deposit evidence indicates that many river systems were common in the interior of the Arabian Peninsula (Huang et al., 2007). One of these river systems is the Wadi Birk that crosses the Tuwayq escarpment (Figs 2.7; 2.8) in central Saudi Arabia, where *P. sinaitus* was collected, and where the escarpment rises to an elevation of about 1,100 meters above sea level (Habibi, 1994, Friend, 1999). The geological (rock) features of the Tuwayq escarpment are considered to date back to the upper Jurassic (Al-Nafie, 2008). The morphological characteristics within the genus of *Pseudotrapelus* (Melnikov et al., 2012) and the old divergences between populations of this species

provided by this study from the Arabian Peninsula suggest the existence of additional cryptic species of *Pseudotrapelus* that warrant further investigation.

Fig 2.7. Tuwayq escarpment habitat from central Saudi Arabia where *P. sinaitus* collected.

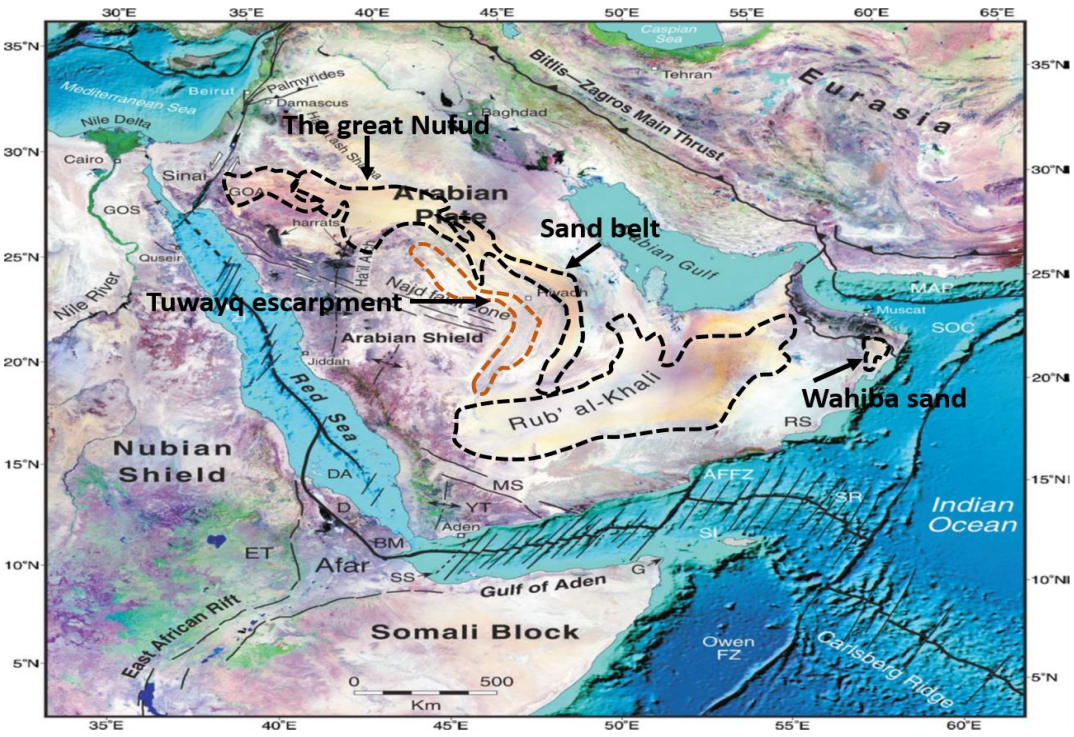


Fig 2.8. Physical map of the Arabian Peninsula illustrating the geographical features of the sand belt, the great Al Nufud, and the Tuwayq escarpment. Modified from (Bosworth et al., 2005).

Phylogeographic patterns in the *Ptyodactylus hasselquistii* complex in the Arabian Peninsula

This study has also provided novel results regarding the high level of genetic divergence within the *Ptyodactylus hasselquistii* complex within the Arabian Peninsula.

A recent study (Nazarov et al., 2013) described three new species of *P. hasselquistii* from the Middle East. These new species are; *P. dhofarensis* from southern Oman, *P. orlovi* from northern Oman, and *P. ananjeva* from southern Jordan. Based on genetic data (COI mtDNA gene) and morphological variations the authors found *P. hasselquistti* to be a species complex. This finding is in accordance with the results from this study, which demonstrate ancient divergence times for the *P. hasselquistii* species complex. However, the findings from this study also indicate other distinct lineages from within this species complex, which may represent additional cryptic species.

Ptyodactylus hasselquistii occupies rocky habitat, is nocturnal and often associated with human buildings (Disi et al., 2001). An indication for cryptic diversity for *P. hasselquistii* species can be concluded as a result of ancient and deep divergences within the species.

In the present study, *P. hasselquistii* (*P. dhofarensis*) from southern Oman appeared to have been separated for a long time from the rest of the Arabian populations and represented a distinct clade, and formed the basal lineages of the Arabian Peninsula groups. Deep divergence times estimated for these lineages were dated at approximately 40 Mya [HPD 49-32] (Fig. 2.3).

The southern Oman *P. hasselquistii* (*P. dhofarensis*) appears to be a sister species of the clades formed by the United Arab Emirates lineages and the monophyletic group of populations from Saudi Arabia. The UAE clade diverged from the other regions at approximately 29 Mya [HPD 36-23] followed by the divergence of the northwest Saudi Arabian clade from the remaining regions at 23 Mya [HPD 30-15]. Based on the geographical distribution, morphological characteristics and genetics, nine species are now recognized as belonging to the genus *Ptyodactylus* (Nazarov et al., 2013).

Variation in morphological features of *Ptyodactylus* between Arabian regions was documented in 1986 (Arnold, 1986b). More recent studies on the phylogenetics of this genus have also found high genetic variability within *P. hasselquistii* from Oman and between *P. hasselquistii* and *P. oudrii* form North Africa (Perera and Harris, 2010) . According to this study, the uncorrected genetic distance of the 12S gene was extremely high between unknown localities of the Oman samples and was supported by nuclear marker variations. The findings of the present study of deep divergence of *P. hasselquistii* complex, which resulted in genetic variability in groups from southern Oman, UAE, and northwest Saudi Arabia, are consistent with these findings.

The southern Oman lineages separated from the other Arabian Peninsula regions with highest probability density ranging from 49-32 Mya and with strong support evident for posterior probability[1.00] (Fig. 2.2). This was subsequently followed by separation of the UAE lineage at 29 Mya [HPD: 36-23]. These dates coincide with the collision of Arabia and Asia that resulted in the formation of the Zagros mountains about 50 million years ago; and the emergence of the southern Oman mountains (Thompson, 2000).

The estimated divergence time of southern Oman , the UAE and northwest Saudi Arabia lineages in this study resembled the estimated dates of the origin of the genus *Stenodactylus,* from Arabia approximately 30 Mya during the separation of the Arabian Peninsula from the African continents as a result of the rifting and formation of the Red Sea and the Gulf of Aden (Metallinou et al., 2012). Similarly, the divergence estimated between two species from the genus *Uromastyx* from Asia and Africa took place between 29-25 Mya (Amer and Kumazawa, 2005). This suggests that the common patterns of vicariance may have led to the present day distribution patterns of *P. hasselquistii* in these regions. Following these patterns, the diversification of central, eastern, and western and southern Saudi Arabian *P. hasselquistii* took place at approximately 21 Mya onwards. Central Saudi Arabian lineages formed as distinct lineages, leaving the others as monophyletic clades.

The divergence time for the central Saudi Arabia lineage is in concordance with *Pseudotrapelus sinaitus* from the same region. The divergence time for these species overlapped and were closely matched (30-12 Mya). This suggested that a common

biogeographical pattern triggered the isolation of the species in this area. The onset of diversification events for eastern Saudi Arabia and clades formed by southern and western Saudi Arabia seem to have happened more recently, probably as a result of speciation events in these regions that coincided with adaptations and the stabilization of environmental conditions.

Phylogeographic patterns within the genus *Stenodactylus* from the Arabian Peninsula

Four species of the genus *Stenodactylus* were represented in this study: *S. doriae*, *S. slevini*, *S. leptocosymbotus* and *S. arabicus.* Previous research (Metallinou et al., 2012) confirmed that the genus *Stenodactylus* originated in Arabia at about 30 Mya, with an estimated divergence time between *S. doriae* and *S. slevini* of 11Mya [HPD: 15-7.4], and an estimated divergence time between *S. doriae* and *S. leptocosymbotus* at 7 Mya [HPD 10-4.2]. In this study, species tree coalescence provided a divergence time estimation between *S. doriae* and *S. slevini* of approximately 16 Mya [HPD: 23.19-10.66], while the divergence time between *S. leptocosymbotus and S. doriae* is estimated at 11.67 Mya [HPD 17.14-7.43] (Fig.2.3).

The divergence time between *S. slevini* and *S. doriae* in this study predates the previous estimate (Metallinou et al., 2012) by about 6 Mya, but the [HPD] values overlapped. Interestingly, the present study indicated that *S. leptocosymbotus* from southern Oman and *S. slevini* from central Saudi Arabia appear to show similar divergence times. The former diverged from the monophyletic *S. doriae* clades formed by the remaining Arabian Peninsula populations at 11.97 Mya (Fig 2.3). Similar divergence patterns are seen for *S. slevini*, where the central Saudi Arabian population diverged from other *S. slevini* groups approximately 12 Mya. These common time patterns for these two species in different regions might have been affected by Arabian Peninsula- wide events. However, *S. leptocosymbotus* and *S. slevini* are endemic to the Arabian Peninsula (Arnold, 1980b), and they are adapted to occupy sandy substrata, hard ground and sandy plains, whereas *S. doriae* are found in sandy habitats (Metallinou et al., 2012). Metallinou et al. (2012) also explained the patterns of the split and diversification for both species as a Northern and Southern ancestor respectively,

from Arabian and Saharan regions with common patterns demonstrating rapid range expansion.

Detection of a highly distinct, older *S. slevini* clade from eastern Saudi Arabia in this study suggests that hitherto unsuspected intraspecific variation exists in this species, and that the long separation for S*. slevini* in eastern Saudi Arabia may have led to this lineage becoming an independent evolutionary lineage. This may be due to the formation of a sand belt extending from south to northwest Saudi Arabia, separating eastern Saudi Arabia from the other regions.

The findings from this study suggest that the current distribution patterns of the Arabian Peninsula lizards were primarily determined by historical events that began within the Miocene – Pliocene time period and demonstrates that the current distribution patterns of Arabian Peninsula lizards match very closely with known historical and climatic events. This study represents the most detailed study to date, on the phylogeography of the Arabian Peninsula lizards and provides the first comprehensive analysis into the spatial and temporal distributions of the lizard species within this region. Therefore, the findings from this study are not only critical in understanding the systematics and taxonomy of the studied species, but are also of vital importance for the application of conservation efforts within this region.

Conclusions

By reconstructing the historical biogeography at various time scales using a multispecies tree approach, based on three mitochondrial genes and two nuclear genes, this study has provided the first detailed insights into the biogeography of the Arabian Peninsula lizards. This study identified evidence for some similar phylogeographic patterns among different groups of lizards. Close sister group relationships were observed between eastern and central Saudi Arabia for *Acanthodactylus boskianus, A. opheodurus, Mesalina guttulata*, and *Bunopus tuberculatus*, northwest and eastern Saudi Arabia of *Mesalina brevirostris* and *Stenodactylus doriae*, southern and northwest Saudi Arabia of *Pseudotrapelus sinaitus* and *Mesalina guttulata*, and close sister group relationships of western Saudi Arabia *Acanthodactylus boskianus* and *Ptyodactylus hasselquistii.* These common phylogeogaphical patterns indicate that biogeographical processes and ecological factors have played major

roles in establishing the current distribution and diversification patterns seen in the Arabian Peninsula lizards. These groups of lizards revealed highly specialized adaptation to specific habitats, which has led to their success in their current distributions. However, the data indicate that the diversification of the Arabian Peninsula lizards was a quite recent event.

Conversely, this study also showed many unique spatial and temporal patterns and cryptic species among the different regions and species studied. Notably, these findings revealed important and very restricted distribution patterns within the Arabian Peninsula among different species. For example, central Saudi Arabia showed a deep divergence and unique pattern for *P. sinaitus* whilst *P. hasselquistii* showed unique divergence patterns across most of the geographical regions studied, except eastern, western and southern Saudi Arabia. In the case of *A. boskianus* unique phylogeographic patterns were observed in northwest Saudi Arabia. *Stenodactylus slevini* demonstrated unique phylogeographic patterns across most of the regions studied, but especially in eastern Saudi Arabia.

Interestingly, the findings from this study demonstrate that across all the regions and species studied, no single common phylogeographical pattern was identified. Thus, all the species studied appeared to demonstrate distinct individual histories. As such, much more detailed or species-specific investigations are required across the different regions to accurately determine the historical biogeography of each species. Importantly, we must also note the limited range sampling for many of the species; for example many of the studied species also are known to exist in areas outside the geographical areas noted in this study (both within and outside the Arabian Peninsula). These extended ranges would also need to be incorporated into future phylogeographic studies to enable better understanding of the entire biogeography of these species.

3. Species delimitation in *Acanthodactylus boskianus* and *A. opheodurus* from the Arabian Peninsula

Abstract

The species unit is the fundamental unit of biological classification. Therefore, accurate determination of the boundaries between different species, or so called “species limits”, is a fundamental requirement for future biological research. The aim of this chapter was to test for the presence of cryptic species within two species of the genus *Acanthodactylus* from the Arabian Peninsula using multilocus data from three mitochondrial (cytb, 12S and 16S) and two nuclear (NTF3 and R35) DNA genes. Poor prior information on geographic distribution as well as ecological and morphological aspects suggested the presence of cryptic species within *Acanthodactylus boskianus* and *A. opheodurus* in the Arabian Peninsula. Mitochondrial data revealed the monophyletic candidate species *A. boskianus* from northwest and southern Saudi Arabia and a candidate species was identified within *A. opheodurus* from northwest Saudi Arabia. Based on these mitochondrial clades, nuclear DNA genes were investigated using Bayesian Phylogenetic and Phylogeography (BPP), allele networks, multilocus networks, and genetic distance methods to clarify these cryptic species. The congruence between the two data sets permitted recognition of *A. boskianus* from northwest and southern Saudi Arabia and *A. opheodurus* from northwest Saudi Arabia as likely confirmed candidate species.

3.1 Introduction

The species unit is the basic unit of biological classification. An understanding of species limits is important to research in ecology (Bortolus, 2008), evolutionary biology (Fujita et al., 2012), clinical research (e.g.,Wüster, 1996) and conservation biology (Mace, 2004). Identifying species borders, revising taxonomic units, and discovering new species are considered processes of species delimitation. These processes can be derived from multiple sources of evidence, such as morphology, physiology, genetics, geography, or other sources of biological information (Bauer et al., 2011, Leaché and Fujita, 2010, Padial et al., 2010, Zhang et al., 2011). Historically, until the recent development of a variety of genetic and molecular approaches, species delimitation was based largely on the morphological characteristics of organisms (Arnold, 1986a, Wiens and Servedio, 2000).

Recent decades have seen an increased emphasis on the use of molecular techniques such as the use of mitochondrial and nuclear DNA to identify species boundaries (Padial et al., 2010). The widespread utilisation of these molecular methods has produced tremendous advances in the field of species delimitation (Fujita et al., 2012). Despite this, controversy over the sole use of molecular methods for species delimitation exists. Concerns over the accuracy of molecular methods used to delimit species are well documented (DeSalle et al., 2005, Lefébure et al., 2006, Will and Rubinoff, 2004). In addition, characterising species on the basis of genetic information only requires extensive field sampling and the collection of a large number of sample organisms in addition to the analysis of DNA sequences (Bauer et al., 2011). This requirement can complicate and restrict both conservation attempts and future studies. Therefore, in order to improve the accuracy and viability of species delimitation a combination of both morphological and molecular approaches is generally preferable (Lefébure et al., 2006, Wiens, 2007).

Despite the fact that the practice of determining species limits with various different genetic approaches has been much debated, genetic approaches to species delimitation have considerable advantages over morphological techniques. Primarily, morphological identification of species limits is highly subjective and is subject to observer interpretation and bias. Conversely, genetic data is more objective and allows for comparisons between

different taxa (Fujita et al., 2012). In addition, analysis of genetic data may provide information on the evolutionary history of organisms and reveal hidden or 'cryptic species' that could not be detected using morphological techniques (Fujita et al., 2012).

The integrated species concept defines species as separately evolving meta-population lineages (De Queiroz, 2007). A number of properties, including reproductive isolation, gene tree monophyly, or morphological divergence, can be used as operational criteria to identify these meta-population lineages. However, these concepts require methods that can provide accurate diagnoses of either new formal species or reproductively isolated lineages (Padial et al., 2010). Sites and Marshall (2004) described 12 methods for delimiting species or reproductively isolated lineages. These methods were classified into two main groups; tree-based and non-tree-based. Genetic data obtained from selective sequencing of DNA may provide evidence for different groups within species by using non-tree-based methods. However, results that are extracted from-tree-based methods can be used more frequently, because they aim to find monophyletic groups that can represent new species which are subsequently referred to as 'candidate species' (Sites and Marshall, 2004).

Deducing species limits from gene sequences requires overcoming a number of challenges. Ancestral allelic variation in parent species gives rise to incomplete lineage sorting among sister species. Alleles of parent species are subsequently transferred to daughter species after a speciation phenomenon. The persistence of these allelic lineages causes non-monophyly of alleles for either one or both of the sister species, since alleles within a species may share their latest common ancestry with homologous alleles from sister species, instead of sharing with other alleles found within the same species (Avise, 2000, Avise, 2009). In contrast, preservation of ancestral haplotypes is also caused by incomplete lineage sorting due to insufficient time between divergent groups. Moreover, due to a distinct historical genealogy of each gene locus, the association among or between species can be unclear or misrepresentative, so that the gene tree is unrepresentative of the species tree (Maddison and Knowles, 2006). Patterns of genetic differentiation among recently diverged taxa can thus be due to preserved ancestral polymorphism, leading to a deficiency of phylogenetic resolution and misleadingly high estimates of gene flow. This is due to these populations not having had enough time after divergence from one another to achieve the

effective separation that can cause fixed genetic differences. Therefore, in order to accurately infer species limits, gene tree methods must either account for incomplete lineage sorting or alternative methods must be developed (Fujita et al., 2012).

The advent of coalescent-based methods of species delimitation has started to address these problems and has greatly facilitated the use of multilocus gene sequences in species delimitation. A number different methods of coalescent species delimitation have been developed. Maximum likelihood, genetic distance and coalescent species delimitation methods are often commonly used to describe species boundaries (Fujita et al., 2012, Guindon et al., 2010, Sites Jr and Marshall, 2003). These methods have been shown to be useful, in particular, in the analysis of gene trees for recently diverged species, where gene tree estimates are not fully resolved (Rannala and Yang, 2003). The method most commonly utilised in recent years is Bayesian Phylogenetics and Phylogeography (BPP) (Yang and Rannala, 2010). BPP analysis requires the creation of a guide tree which infers the relationships among the species studied, in addition to the assignment of individuals to candidate species. BPP analysis is subsequently run utilising different scenarios (speciation events versus no speciation events). Output from the analysis is in the form of individual gene trees for each species estimated within a Bayesian framework using prior probabilities assigned to population size and divergence times. Reversible jump Markov chain Monte Carlo (rjMCMC) sampling is used to generate the posterior distribution of speciation models. The utilisation of a Bayesian framework enables BPP analysis to estimate speciation probabilities within this context. In comparison to maximum likelihood, BPP analysis has been shown to be the most accurate (Camargo et al., 2012).

Whilst these methods represent significant advances in the field of species delimitation, it is also important to acknowledge their limitations. A fundamental limitation of these methods is the requirement of a prior assignment of individuals into species and a specification of the relationship among these candidate species. This typically relies on prior information gained from taxonomy or morphological characteristics. For groups where this prior information is lacking, obtaining accurate prior assignments of individuals to species may prove difficult thereby compromising the accuracy of the analysis.

Several studies have attempted to circumvent the problem of obtaining initial guide tree accuracy prior to subsequent testing by BPP analysis. Mitochondrial phylogeny is typically used as a guide tree for previously described species (Fuchs et al., 2011, Yang and Rannala, 2010), but is often supplemented by evidence from morphological characteristics (Camargo et al., 2012). Where no prior taxonomic studies have been conducted, genetic evidence may be the only solution to specifying species limits (Leaché and Fujita, 2010). In their study, genetic evidence suggested the presence of four cryptic species of forest geckos (*Hemidactylus*), however subsequent modification of the guide tree resulted in significantly higher numbers of inferred cryptic species. Therefore, whilst coalescent species delimitation methods remain a useful tool for describing species boundaries, the results must be interpreted with caution, bearing in mind the limitations of the selected methodology.

The Arabian Peninsula has 27 described species of Lacertidae, of which 14 species are endemic (Cox et al., 2012). First reviewed in 1986 (Arnold, 1986b), these species have subsequently been poorly studied, possibly due, in part, to the Arabian Peninsula as a region being poorly studied. Therefore, there is huge scope for the potential discovery of new species within this region especially amongst the lacertid lizards.

The spiny-toed lizards (*Acanthodactylus*) are a genus belonging to the Lacertidae family that occurs mainly on sandy ground in arid areas. They are an Old World clade, widely distributed from the Middle East, where they originated, to India and North Africa (Harris and Arnold, 2000). To date, 41 species have been described (Uetz, 2010). Originally the taxonomy of this genus was described using only morphological methods (Arnold, 1983, Salvador, 1982). However, the application of molecular methods has led to subsequent revisions within this genus (Fonseca et al., 2009, Harris and Arnold, 2000). At present the taxomony of this genus is still being studied using a combination of molecular and morphological evidence and incongruence between these two methods may lead to further taxonomic revisions (Crochet et al., 2003, Fonseca et al., 2009, Fonseca et al., 2008, Harris et al., 2004).

Hence, the genus *Acanthodactylus* is taxonomically confusing, with species often being at least superficially similar but also quite variable. Some forms that are externally very alike

are distinguishable by essential differences in the male intromittent organ, the hemipenis, and its supporting armature (Arnold, 1986a, Arnold, 1983). Species boundaries and species groups within *Acanthodactylus* have been discussed by Salvador (1982) and Arnold (1983). Arnold (1983), based on morphological characters, recognosed eight groups within the genus. Among these groups are the *A. boskianus* group, which includes *A. boskianus*, *A. grandis* and *A. schreiberi*; the *A. opheodurus* group, which includes *A. felicis, A. masirae, A. opheodurus,* and *A. yemenicus;* and the *A. cantoris* group, which includes *A. arabicus, A. blanfordii, A. cantoris, A. gongrorhynchatus, A. haasi, A. schmidti,* and *A. tilburyi.*, A recent phylogeographic study focusing on the lacertidae lizards of the Arabian Peninsula (Chapter 2) has identified the potential presence of cryptic species within two groups of lizards (*A. boskianus* and *A. opheodurus*). This discovery highlights the potential for new lizard species to be discovered in this genus and promotes the need for an extensive taxonomic review.

Acanthodactylus boskianus (Daudin, 1802) is a widely distributed species among its genus. Its range extends across Arabia, Egypt, North Africa, Palestine, Jordan, Iraq, Syria, and adjoining Turkey (Arnold, 1986b, Schleich et al., 1996). The main habitat for this species is gravelly soil in arid and semi-arid regions, usually with sparse and low vegetation, avoiding the hyper-arid areas (Arnold, 1989, Arnold, 1983, Disi et al., 2001). Identifications for *A. boskianus* by Arnold (1983), and Salvador (1982) and Boulenger (1921) indicated the presence of three sub-species within the Arabian Peninsula, based solely on morphological characteristics which vary from region to region. However, these putative sub-species were not confirmed, as morphological differences were attributed to ecological niche adaptations (Arnold, 1986b, Arnold, 1983).

Acanthodactylus opheodurus is found in southern Oman and was first described in 1980 (Arnold, 1980a). No sub-species has been determined, to date, for *A. opheodurus*, but because this species occurs sympatrically with the similar *A. boskianus*, it has been overlooked for a long time, which may have led to the mis-identification of these two forms (Disi et al., 2001). In southern Arabia, where these two forms coexist in similar niches as sympatric species, *A. boskianus* tends to be larger than *A. opheodurus*. In that area, *A. opheodurus* displaced *A. boskianus* to be restricted to specific narrower niches (Arnold,

1980a). However*, A. opheodurus* is distributed across approximately the entire Arabian Peninsula, Jordan, Syria, and south-western Iraq (Arnold, 1986b).

The aim of this study was to investigate cryptic species within these two species of *Acanthodactylus* in the Arabian Peninsula using multilocus data. Information on geographic distribution, ecology and morphology for this genus, that could be used to evaluate species limits are rare. Therefore, the hypothesis applied in this study is a candidate species based approach, based only on genetic evidence. The evidence from two nuclear loci data was used to identify mitochondrial clades (hereinafter referred to as clades) that may potentially represent separately evolving species (hereinafter referred to as candidate species) that show evidence of nuclear divergence and may represent new candidate species.

3.2 Material and Methods

Sampling

Tissue samples from tail tips were collected from specimens from throughout the Arabian range of *Acanthodactylus boskianus, A. opheodurus,* and *A. schmidti*. Tissue samples were preserved in 95% ethanol. Eleven localities across the Arabian Peninsula were targeted. These localities are: southern Saudi Arabia (two localities); northwest Saudi Arabia (three localities); western Saudi Arabia (two localities); central Saudi Arabia; eastern Saudi Arabia; southern Oman and United Arab Emirates (Fig 3.1; Fig 3.2). One hundred and fifteen individuals were sampled in total (N = 81, 30, and 4 for *A. opheodurus; A. boskianus*, and *A. schmidti* respectively; Appendix 2).

DNA extraction and sequence amplification

Entire genomic DNA was extracted using a Qiagen DNeasy™ Tissue Kit (catalogue no. 69506). Partial fragments from the following three mitochondrial genes were amplified using PCR and sequenced: 12S ribosomal RNA (rRNA) [426 base pairs (bp)], 16S rRNA (620 bp), and 655 base pairs of cytochrome b (CYT). In addition, two nuclear loci [fingerprint protein 35 [R35] (646 bp) and neurotrophin-3 [NTF-3] (656bp)], were sequenced for the

105 *Acanthodactylus opheodurus, A. boskianus,* and *A. schmidti* individuals (Appendix 2-Table 1).

Mitochondrial gene fragments were amplified in 11 µl (total volume), each containing 9.6 µl of Abgene 1.1x ReddyMix™ (1.25 units Thermo prime Plus DNA polymerase; 75mM Tris-HCl pH8.8; 20mM $(NH_4)_2SO_4$; 1.5mM $MgCl_2$; 0.01% (v/v) Tween®20; 0.2mM of each dNTP; and a precipitant red dye for electrophoresis), 0.3µl of each primer, and 0.8µl of sample (template) DNA. Nuclear DNA was amplified in 15 µl total volume reactions consisting of 13µl of ReddyMix, 0.4µl for each primer, and 1.1µl of DNA template. Exonuclease 1 and thermo-sensitive alkaline phosphatase enzymes were used to clean up all PCR products. Bi-directional direct sequencing was performed for nuclear loci, using the same forward and reverse primers described in Table 3.1. Single direction sequencing using the forward primer only was used for mitochondrial fragments (Table 3.1). Sequencing was carried out by Macrogen Inc. (dna.macrogen.com).

Table 3.1. Primer sequences and PCR conditions

Primer	Sequence	Cycles	Annealing
CYTB			
L14841	CCATCCAACATCTCAGCATGATGAAA	40	40°C
rctyb-1H	GCGTAGGCRAATAGGAAGTATCA		
16S			
16SL	CGCCTGTTTATCAAAAACAT	30	50°C
16SR	CCGGTCTGAACTCAGATCACGT		
12S			
L1091	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT	35	43°C
H1478	TGACTGCAGAGGGTGACGGGCGGTGTGT3		
NTF-3			
NTF3-f1	ATGTCCATCTTGTTTTATGTGATATTT	35	50°C
NTF3-r1	ACRAGTTTRTTGTTYTCTGAAGTC	35	48°C
		40	55°C
R35			
R35-f	GACTGTGGAYGAYCTGATCAGTGTGGTGCC	35	60°C
R35-R	GCCAAAATGAGSGAGAARCGCTTCTGAGC	35	55°C

Each reaction was initiated with a 2 minute denaturing cycle at 94°C, and terminated with a 5 minute 72°C final extension. All reactions denatured at 94°C for 30 seconds, and extended at 72°C for 1 minute. Annealing cycles were 30 seconds long. Locus specific annealing temperatures and number of cycles are indicated above.

L14841= (Fu, 2000), rctyb-1H= (Kumazawa and Endo, 2004), 16SL and 16SR= (Palumbi et al., 1991) L1091 and H1478= (Kocher et al., 1989), NTF3-f1 and NTF3-r1= (Townsend et al., 2008), and R35-f and R35-R= (Leaché, 2009).

Phylogenetic analysis

CodonCode Aligner (v.3.5.6 CodonCode Corp.) was used to assemble sequences and to edit contigs. Gene fragment sequences were aligned using Muscle (Edgar, 2004) and additional adjustments were made by eye. Protein-coding genes were translated into amino acid

sequences (in CodonCode Aligner) to check for stop codons. The presence of double peaks at single nucleotide sites indicated heterozygosity at nuclear loci (R35 and NTF-3). SeqPHASE (Flot, 2010) was used to create input files for PHASE v. 2.1.1., which was used to construct the phased haplotypes from the diploid data (Stephens et al., 2001, Stephens and Scheet, 2005). PHASE analyses were implemented separately for each species. These analyses involved two independent runs with different randomly selected starting seeds, each consisting of 1000 generations with a thinning interval of 10, and preceded by a burn-in of 100 generations.

For subsequent phylogenetic analyses, the datasets were partitioned by genes and the best-fit evolution model for the whole datasets (cytb, 12S, and 16S combined), and for each gene was assessed and selected according to the Akaike Information Criterion (AIC) as implemented in PartitionFinder (Lanfear et al., 2012).

The maximum likelihood rooting trees for cytochrome b, 16S, and 12S, were created by Raxml v.7.3.1 (Stamatakis, 2006), implemented through the CIPRES portal (Miller et al., 2010), using the GTR+Gamma model, with 500 bootstrap replicates to assess branch support. The corresponding sequence of *Mesalina guttulata* from southern Arabia was used to root the mtDNA trees as a closely related outgroup (Arnold, 1989).

Comparisons between *A. boskianus* from the Arabian Peninsula and *A. boskianus* from Egypt were conducted using cytb and 12S gene sequences from GeneBank. In addition, sequences of 12S gene of *A. boskianus* taken from (Harris and Arnold, 2000) and (Khannoon et al., 2013), as well as sequences from Israel (see Appendix 4 for their accession numbers), and the current study, were aligned to construct a Maximum likelihood tree in RAMXL (Figs. 3.17; 3.18).

Moreover, to test whether *A. boskianus* and *A. opheodurus* represent a monophyletic or paraphyletic group with other *Acanthodactylus* species, maximum likelihood trees were constructed for representative species from the genus of *Acanthodactylus*. This was achieved using available sequences in GenBank for two mitochondrial genes (12s and 16s). Selected sequences from GenBank comprised either one or both 12s and 16s genes. These sequences were aligned with Arabian Peninsula *A. boskianus* and *A. opheoduras* sequences.

The maximum likelihood tree (Fig. 3.19) was constructed as described above. (See Appendix 3).

Nuclear data analysis

Three methods based on nuclear genes were conducted. First, networks of nuclear alleles were generated for each single gene using the median-joining method implemented in NETWORK v.4.6 (fluxus-engineering.com). Second, patterns of nuclear genetic variation in the NTF-3 and R35 genes were assessed for the 105 specimens of *Acanthodactylus opheodurus, A. boskianus*, and *A. schmidti*. Allele distance matrices were generated for each locus under the Kimura two-parameter model –K2P (Kimura, 1980) in MEGA 4 (Tamura et al., 2007). These were then converted into a matrix of standardised between-specimen distance across both loci using the software POFAD (Phylogeny of Organisms From Allelic Data) (Joly and Bruneau, 2006). The resulting matrix of standardised multilocus distances between individuals was then converted into a two-dimensional ordination of individuals using a Principal co-ordinates analysis (PCoA) using MVSP v.3.13n (www.kovkomp.com).

Finally, the output of the standardised multilocus distance matrix of the three species of *Acanthodactylus* from POFAD were used to construct distance network using the NeighborNet algorithm which is implemented in Split Tree 4 v4.12.8 (Huson and Bryant, 2006).

Coalescent species delimitation

Bayesian phylogenetic and phylogeography (BPP) analysis was implemented to investigate species limits of *Acanthodactylus boskianus* and *A. opheodurus* (Yang and Rannala, 2010). A maximum likelihood tree, of mtDNA, was used to determine potential candidate species and was subsequently used as a guide tree in this analysis. The analysis was run twice for algorithm 0 and twice for algorithm 1 in order to calculate the mean posterior probabilities for each algorithm.

The parameters used in this analysis followed the methodology of Barlow (2012). Briefly, equal prior speciation probabilities on all nodes of the guide tree were specified for

algorithm 0 using a fine tuning parameter of ϵ = 15 . Gamma (α,β) distributed priors were assigned to population size parameters (Θs) and the root age of the species tree (τ0), whilst all other divergence time parameters were assigned a Dirichlet prior. To represent large ancestral population sizes and shallow divergence times; The prior G(1,10) was specified for Θs and for τ0, G(2,2000) was specified. A random rates model was assigned to evolutionary rates to allow the evolutionary rate to vary amongst loci in accordance with a Dirichlet *D*(α) prior distribution. Assuming even rates of evolution amongst loci, an α value of 1.5 and m value of 1 were assigned to the Dirichlet prior for algorithm 1. Automatic adjustments of the step lengths used in the rjMCMC algorithm were utilised to achieve appropriate acceptance proportions. Following a burn in phase of 10,000 iterations, the rjMCMC chain was sampled every five iterations for a total of 100,000 samples of the posterior distribution. To check consistancy between runs, each analysis was run twice with different starting trees and different randomly selected starting seeds. The output files from BPP were used to verify both convergence and the effective sampling of parameters.

3.3 Results

Sequence data

A total of 114 individuals from the genus *Acanthodactylus* (*A*. *opheodurus,* N = 82; *A. boskianus,* N = 28; *and A. schmidti,* N = 4) were identified based primarily on diagnostic morphological characteristics in addition to using comparison sequences available at the National centre for Biotechnology Information (NCBI). Samples were collected from eleven localities across the Arabian Peninsula (Fig. 3.1). Samples were sequenced at three mitochondrial gene locations (Cytochrome b, 12S, and 16S) and two nuclear loci (R35, NTF-3).

The total resulting combined sequences of three mtDNA genes were 1623 bp in length. The informative parsimony, variable sites, long sequences and model selection for each gene are provided in the following table (Table 3.2).

Genes	Length (bp)	Parsimony-informative sites	Variable sites	Model
mtDNA combined	1623	404	524	GTR+I+G
cytb	732	257	279	HKY+I+G
16S	504	86	379	TVM+I+G
12S	387	58	94	GTR+I+G
R35	679	53	57	TrN+I+G
NTF-3	619	24	32	TrN+I

Table 3.2. Variability and DNA dataset characteristics.

MtDNA phylogeny

Uncorrected genetic distance variation (P-distance) values are displayed in Appendix 3.

Maximum likelihood analysis of the combined mitochondrial genes resulted in three reciprocally monophyletic groups corresponding to the three conventional species, *A. opheodurus, A. boskianus* and *A. schmidti*. All three species represent significantly supported clades (Fig. 3.2). *A. schmidti* was sampled from only two areas, eastern Saudi Arabia and the United Arab Emirates. A well-supported clade was formed by this species.

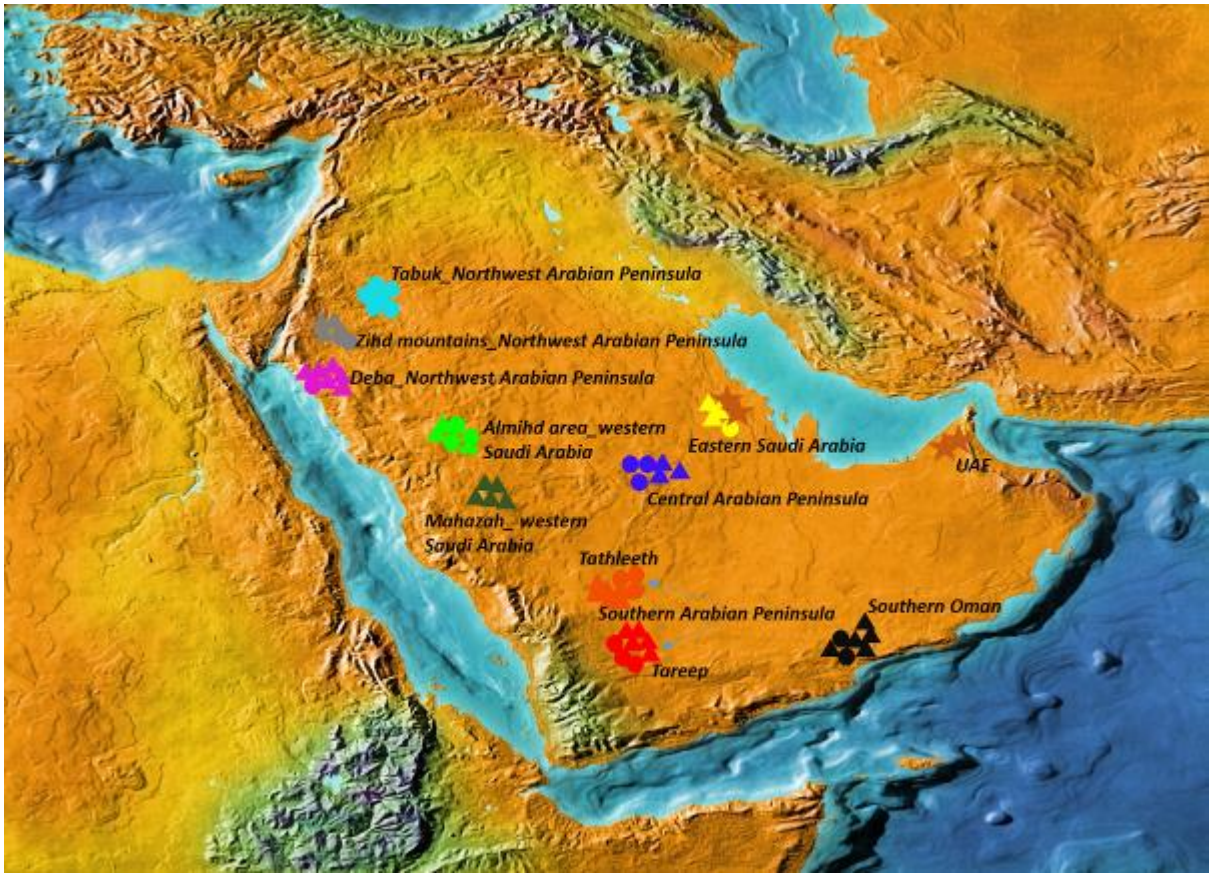


Fig. 3.1. Sampling localities of *Acanthodactylus opheodurus* (triangles); *A. boskianus* (circles); and *A. schmidti* (stars) on the Arabian Peninsula. Colours refer to localities and represent *mtDNA* clades.

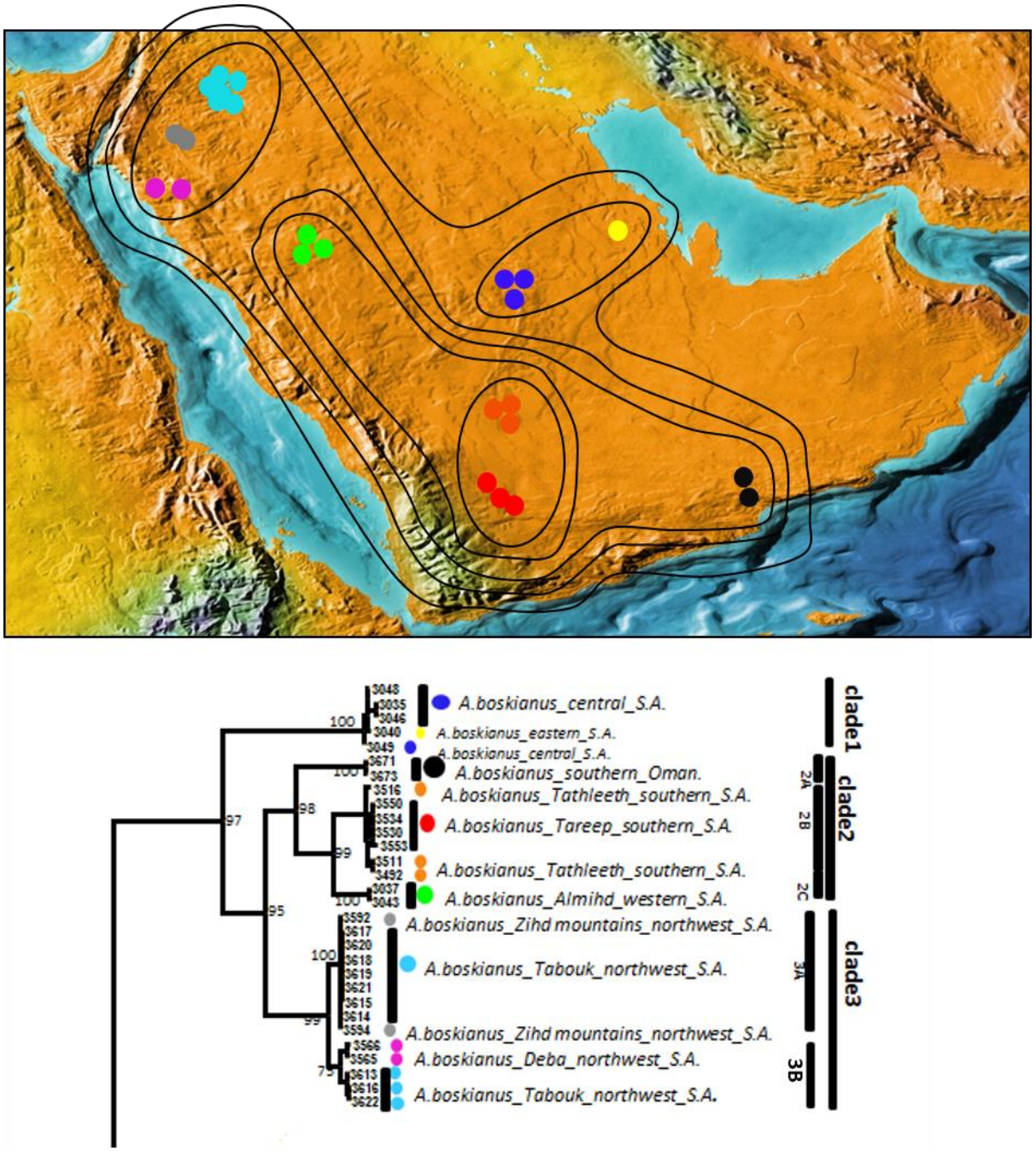


Fig 3.2. Clade map for *Acanthodactylus boskianus* from the Arabian Peninsula.

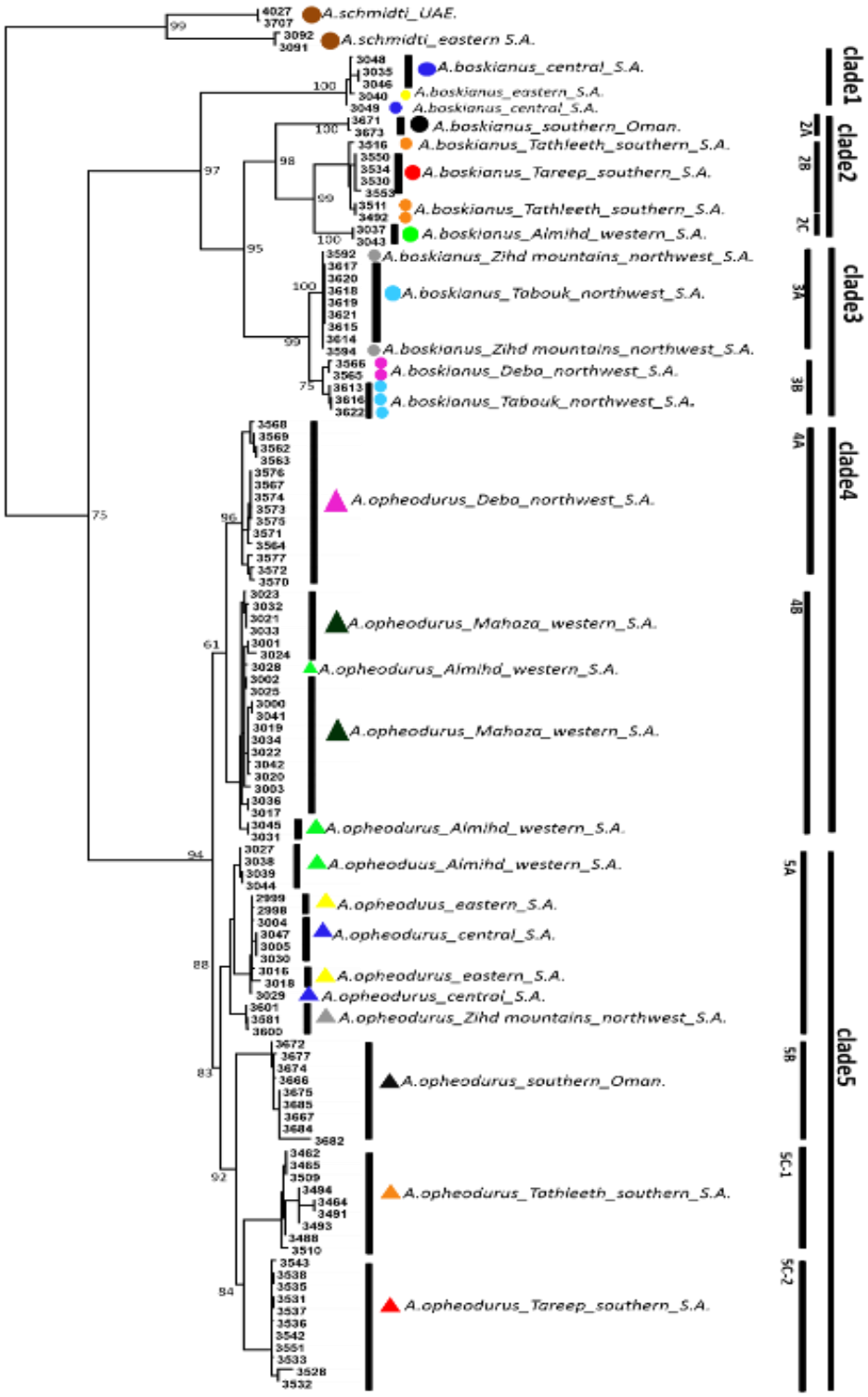


Fig. 3.3. Maximum Likelihood (ML) tree derived from the combined data of three mtDNA genes (12S, 16S and cytb). The numbers close to the nodes are bootstrap values. The tree was rooted using *Mesalina guttulata* as a closely related outgroup.

The monophyletic *A*. *boskianus* group is comprised of three deeply divergent clades (Figs. 3.2, 3.3). Clade 1 comprises haplotypes from eastern and central Saudi Arabia, which are highly divergent from the other *boskianus* geographical lineages. This clade clusters strongly with a highly significant bootstrap support value, but lacks clear internal phylogeographic structure. Clade 2 is formed by haplotypes from southern Oman, southern Saudi Arabia, and western Saudi Arabia. This clade is further divided into three sub-clades. Sub-clade 2A consists of haplotypes unique to southern Oman, sub-clade 2B consists of haplotypes unique to southern Saudi Arabia and sub-clade 2C consists of haplotypes unique to the Almihd area of western Saudi Arabia. Clade 3 consists of haplotypes found in northwest Saudi Arabia. This clade is further divided into two deeply divergent sub-clades; sub-clade 3A is found in the Deba region on the Red Sea coast around Tabuk, and sub-clade 3B is found in the Zihd Mountains.

A. opheodurus mtDNA

Acanthodactylus opheodurus forms a strongly supported monophyletic group (Figs. 3.3, 3.4). This group is divided into two main haplotype clades. Clade 4 is formed by tightly clustered haplotypes from the Deba (northwest), Mahaza, and Almihd regions (western Saudi Arabia). Clade 5 comprises haplotypes from central, eastern, Almihd (western), the Zihd Mountains (northwest), southern Saudi Arabia and southern Oman. However, both these two main clades are subdivided into several sub-clades. Clade 4 comprises two distinct genetic sub-clades. Sub-clade 4A comprises samples taken from Deba (the northwest part of the Arabian Peninsula only). Sub-clade 4B comprises samples taken from the Mahaza protected area and the Almihd area (both in western Saudi Arabia). Clade 5 is divided into four sub-clades. Sub-clade 5A comprises samples collected from the Almihd region (western Saudi Arabia), central and eastern Saudi Arabia, and the Zihd Mountains (the northwest region of the Arabian Peninsula). Sub-clade 5B consists of lineages from southern Oman. Sub-clade 5C consists of two deeply divergent sub-clades (5C-1 and 5C-2) within haplotypes from southern Saudi Arabia. These genetic lineages were sampled from two southern Arabian geographical regions (Tathleeth and Tareep).

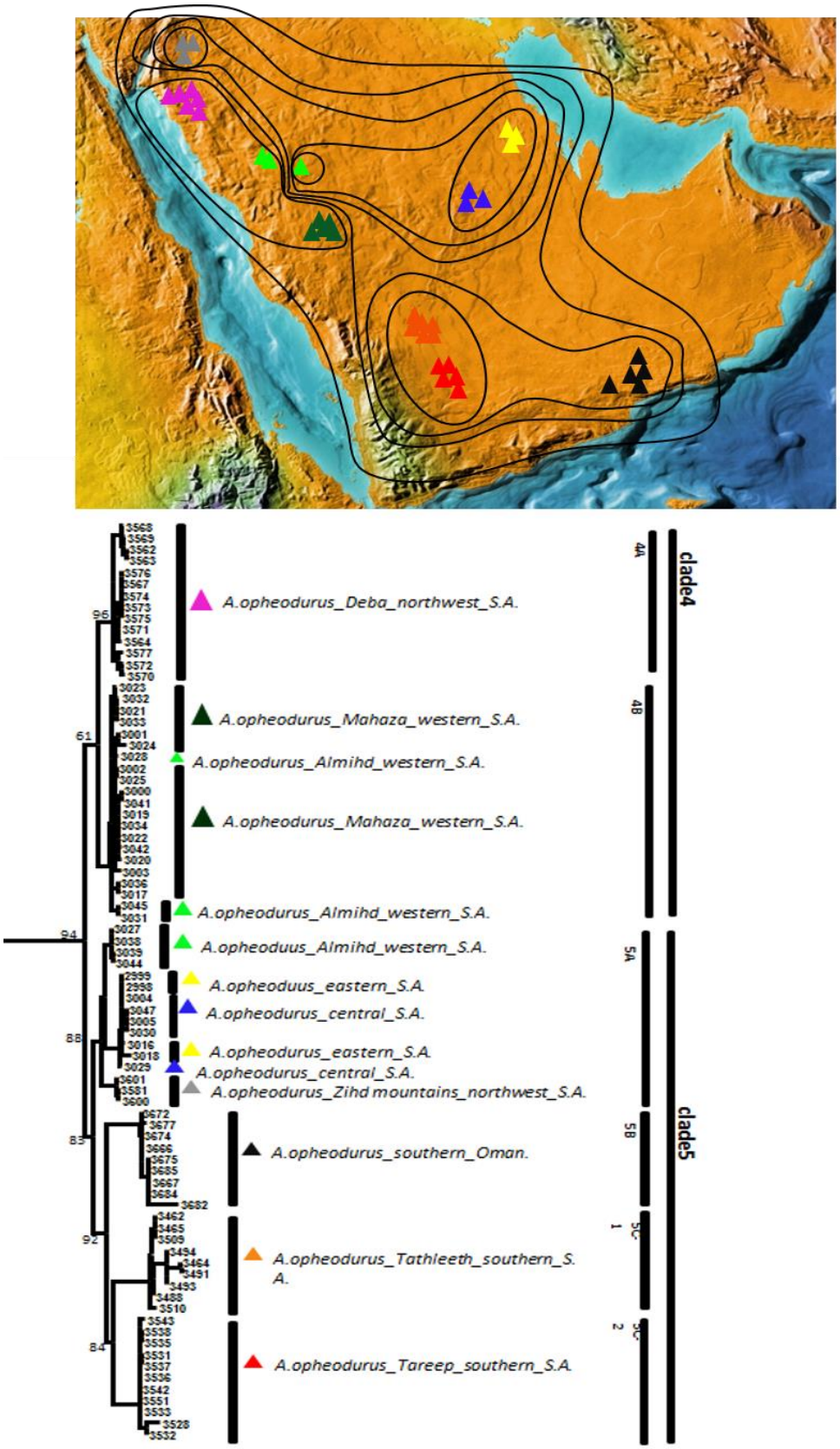
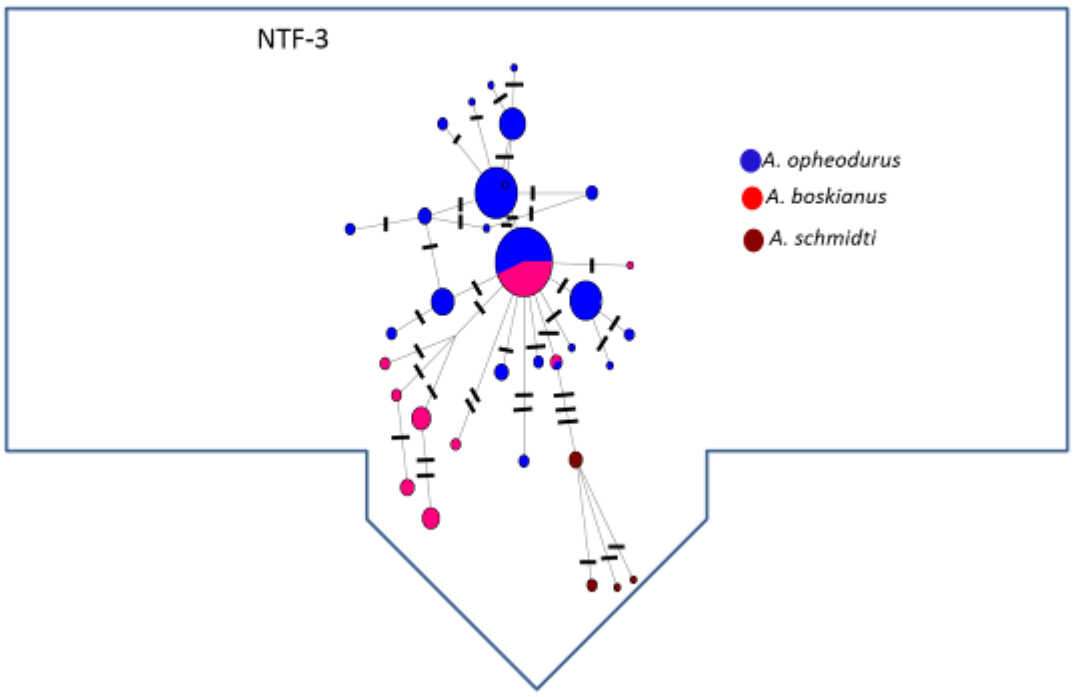


Fig. 3.4. Clade map of *A. opheodurus* from the Arabian Peninsula.

Nuclear DNA sequence patterns

Acanthodactylus group

Allele networks for R35 (Fig. 3.6) revealed a similar pattern to the mtDNA tree and clearly distinguished between species. However, the allele network for the NTF-3 gene (Fig. 3.5) indicated the sharing of alleles between *A. opheodurus* and *A. boskianus*. Individuals of *A. boskianus* from central, southern, western, northwest Saudi Arabia and southern Oman cluster together in the NTF-3 allele network with some individuals of *A. opheodurus* from eastern Saudi Arabia, western Saudi Arabia (Mahaza and Almihd), southern Saudi Arabia (Tathleeth), and the Zihd Mountains (the northwest part of the Arabian Peninsula).



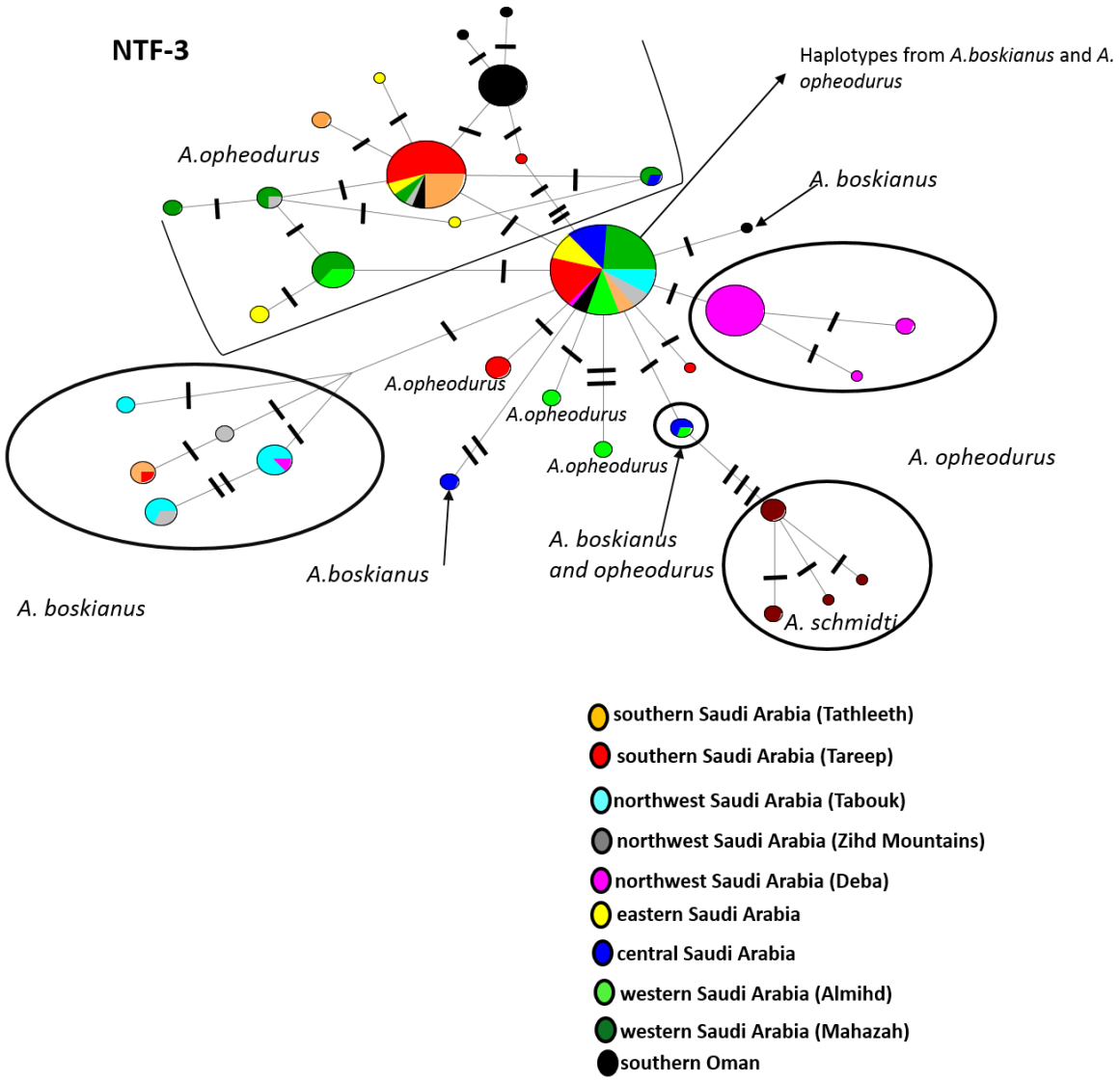


Fig. 3.5. Median-joining allele network for nuclear locus NTF-3 for *Acanthodactylus opheodurus, A. boskianus, and A. schmidti*. Nodes are coloured according to mitochondrial clades/localities. Node size is proportional to allele frequencies. Black bars indicate mutation points.

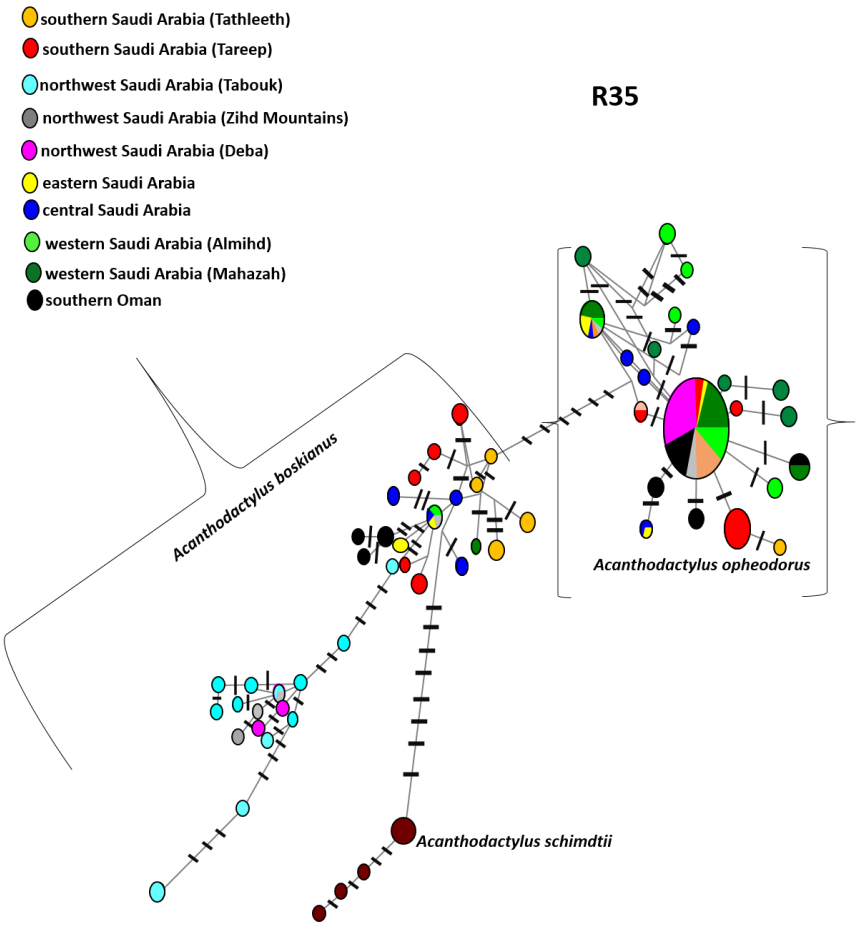


Fig. 3.6. Median-joining allele network for nuclear locus R35 for *Acanthodactylus opheodurus, A. boskianus, and A. schmidti*. Nodes are coloured according to mitochondrial clades/localities. Node size is proportional to allele frequencies. Black bars indicate mutation points.

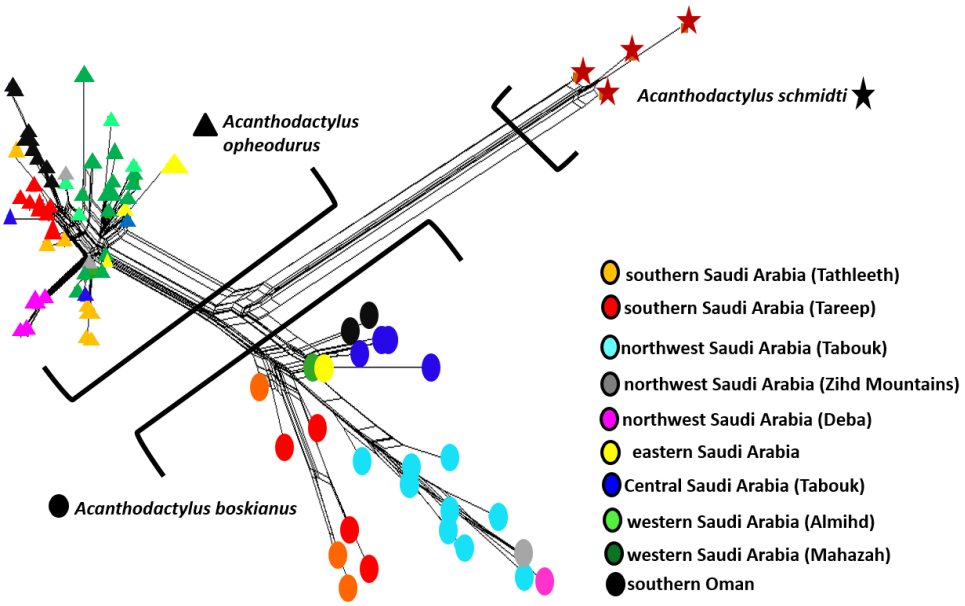


Fig. 3.7. Multilocus nuclear distance network for the *Acanthodactylus* group. Individuals are coloured according to mitochondrial clade assignment, consistent with Fig. 3.1.

The ordination of individuals along the first and second principal co-ordinates of the principal co-ordinate analysis (PCoA) are displayed in Fig. 3.8. The nuclear genetic distance of the three focal *Acanthodactylus* species revealed distinct clusters for each species (Fig. 3.8). The multilocus nuclear network (Fig. 3.7) also showed clear, distinct patterns for the three species. *A. boskianus* individuals from northwest Saudi Arabia are clearly distinct and are strongly resolved by a split on the multilocus nuclear network. Similar distinct patterns have been revealed in *A. opheodurus* individuals from northwest Saudi Arabia.

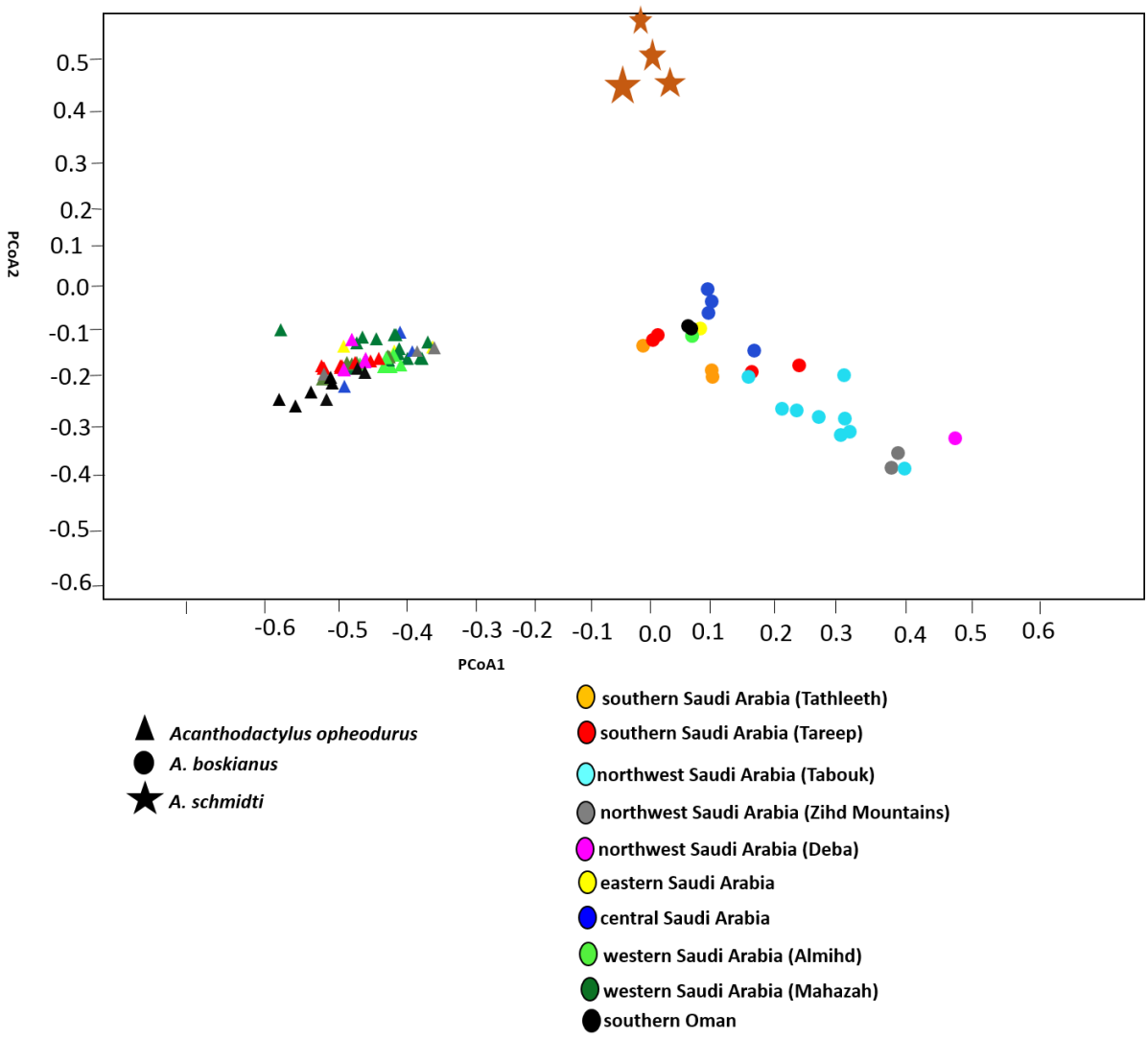


Fig. 3.8. Ordination of *Acanthodactylus* group along the first and second principal co-ordinates of a PCoA of standardised multilocus nuclear genetic distance. Total genetic variation was 54.44% and 29% for PCoA1 and PCoA2, respectively. Circles = *A. boskianus;* triangles = *A. opheodurus* and the brown star is *A. schmidti*. Individuals are coloured according to mitochondrial clade assignment, consistent with Fig. 3.1.

Acanthodactylus boskianus

Western samples of *A. boskianus* originate from the Almihd area only as no samples were detected in the Mahaza protected area. The genetic variation within the *A. boskianus* samples was examined using the allele network for each locus and combined loci in order to conduct a PCoA (Fig. 3.9 and 3.10). In the case of the R35 allele network, northwest Saudi Arabia *A. boskianus* revealed unique alleles. In addition, within these lineages, shared alleles were detected from samples collected from the Zihd Mountains and Tabuk in northwest

Saudi Arabia. However, the lineages from southern Oman and the majority of both geographical lineages from southern Saudi Arabia and some from the central Arabian Peninsula have revealed unique alleles. A nuclear network analysis of an NTF-3 gene fragment indicated allele sharing between individuals from all geographical lineages. The distinctive haplotypes were observed in individuals assigned to the southern and northwest Saudi Arabia lineages (Fig. 3.10). The lineages from central Saudi Arabia and southern Oman also revealed some unique alleles. The ordination of individuals along the first and second principal co-ordinates of the PCoA of nuclear genetic distance revealed a distinct cluster for each geographical region or even within samples from a single region. A PCoA scatter plot showed a distinct cluster of *A. boskianus* lineages from northwest Saudi Arabia. The PCoA revealed differentiation within the lineages from both sites in northwest Saudi Arabia (Tabouk and the Zihd Mountains). Two distinct clusters were revealed in the Tathleeth and Tareep lineages, both from southern Saudi Arabia (Fig. 3.9).

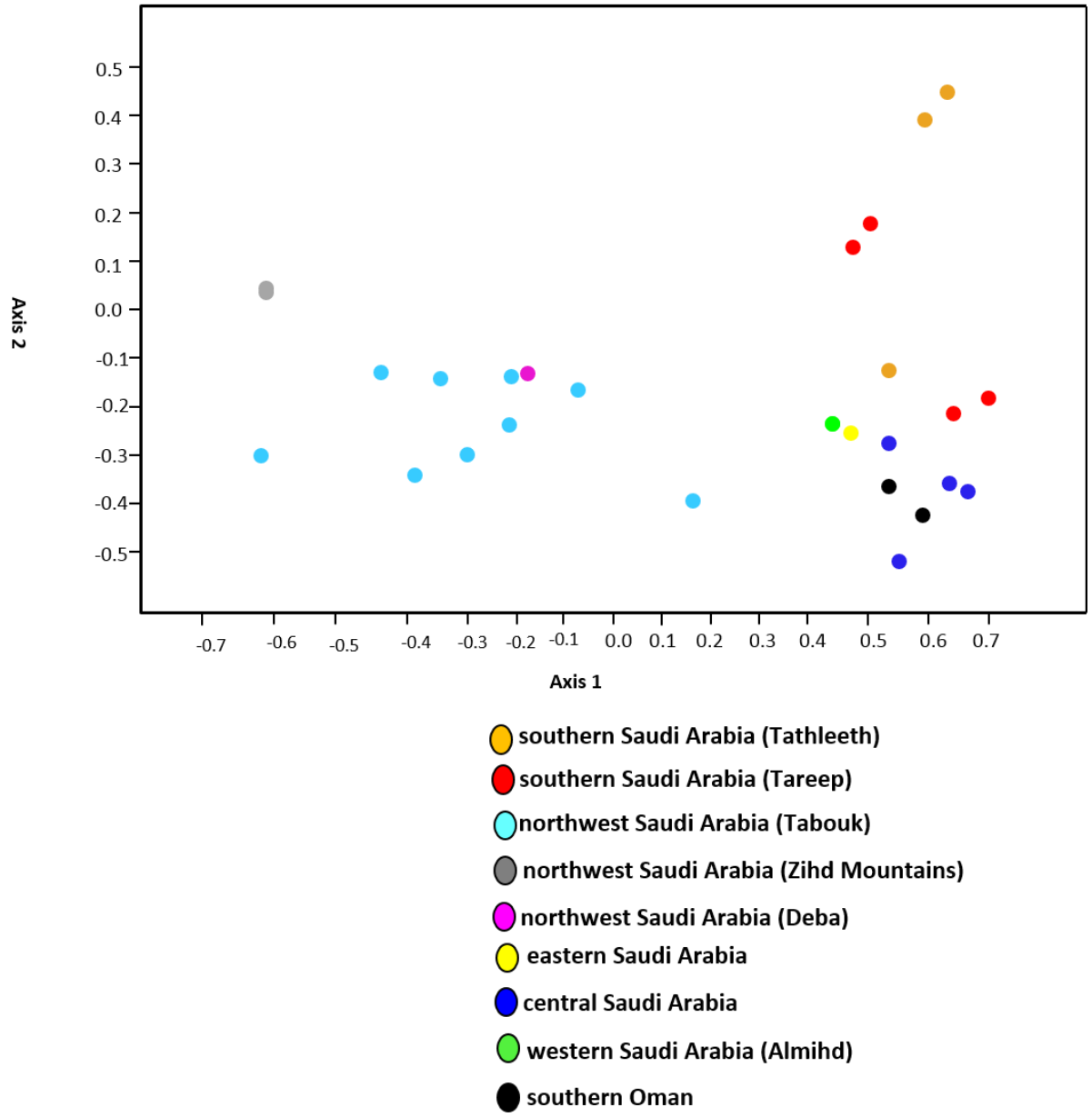


Fig. 3.9. Ordination of *A. boskianus* individuals along the first and second principal co-ordinates of a PCoA of standardised multilocus nuclear genetic distance. Total genetic variation was 61% and 19% for PCoA1 and PCoA2, respectively. Individuals are coloured according to mitochondrial clade assignment, consistent with Fig. 3.1.

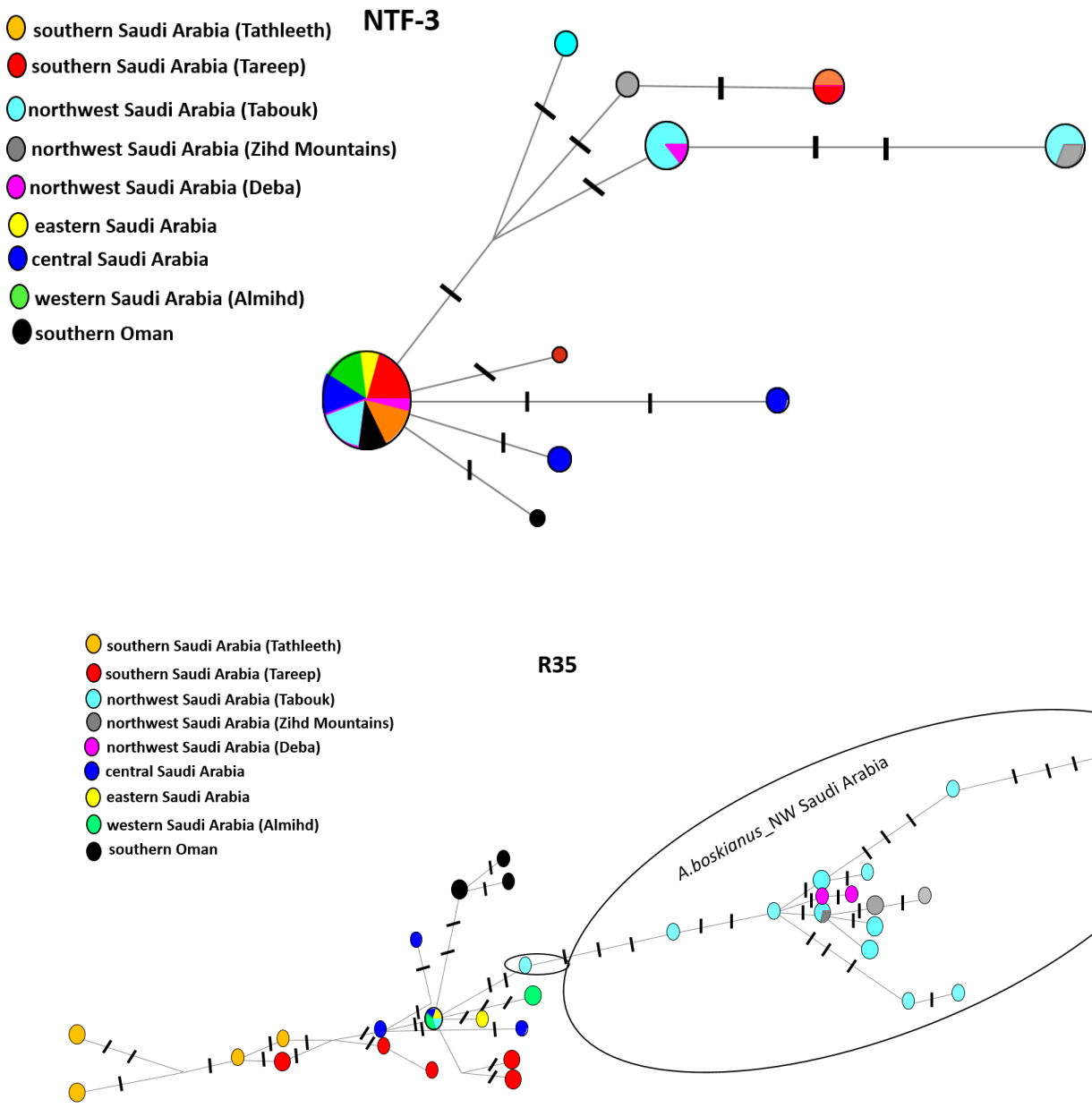


Fig. 3.10. Median-joining allele network for nuclear loci NTF-3 and R35 for *Acanthodactylus boskianus*. Nodes are coloured according to mitochondrial clades. Node size is proportional to allele frequencies. Black bars indicate mutation points.

Acanthodactylus opheodurus

A. opheodurus was sampled from two localities in the northwest only, from the Zihd Mountains and the Daba. The majority of alleles in the R35 locus (Fig. 3.11) are shared across the *A. opheodurus* sample distribution. Unique alleles were found at this locus in individuals from Almihd and Mahaza (western Saudi Arabia). Additional unique alleles were detected in individuals from southern, western and central Saudi Arabia and southern Oman. Network allele analysis of NTF-3 (Fig 3.11) revealed unique alleles in all the Deba (northwest Saudi Arabia) lineages. In addition, the allele network of NTF-3 locus revealed that the majority of individuals assigned to both localities from southern Saudi Arabia share alleles with the remaining regions of *A. opheodurus*.

The ordination of individuals along the first and second principal co-ordinates of the PCoA of nuclear genetic distance (Fig. 3.12) revealed differentiation among all *A. opheodurus* lineages. A PCoA scatter plot revealed a distinct cluster pattern of individuals from Deba (northwest Saudi Arabia). The genetic distance within the southern Oman lineages showed noteworthy differentiation. Further evidence of differentiation in genetic distance was also found within the Almihd and Mahaza (western Saudi Arabia) lineages. The genetic distances of samples assigned to central Arabian Peninsula were differentiated along PCo1 and PCo2 of the PCoA.

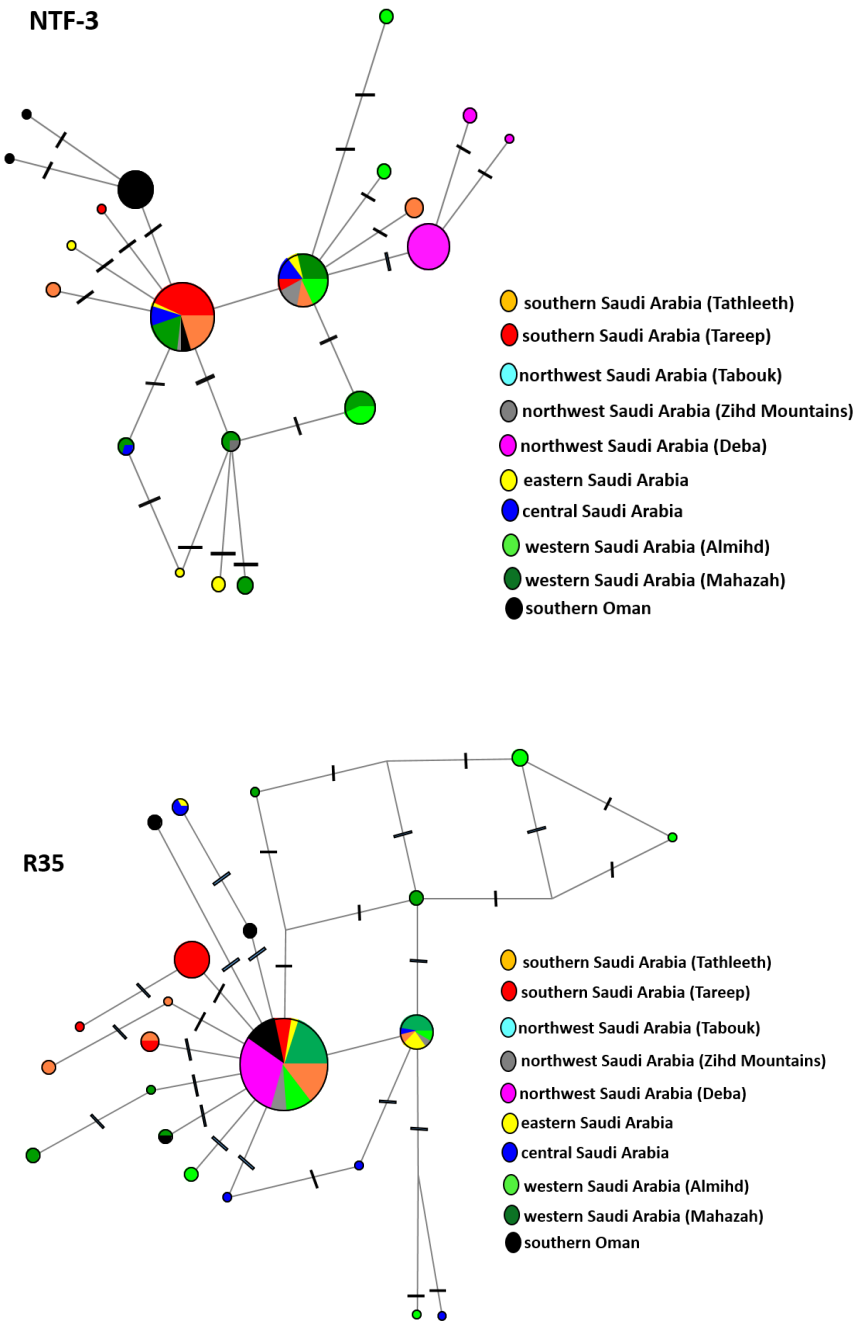


Fig. 3.11. Median-joining allele network for nuclear loci NTF-3 and R35 for *Acanthodactylus opheodurus*. Nodes are coloured according to mitochondrial clades. Node size is proportional to allele frequencies. Black bars indicate mutation points.

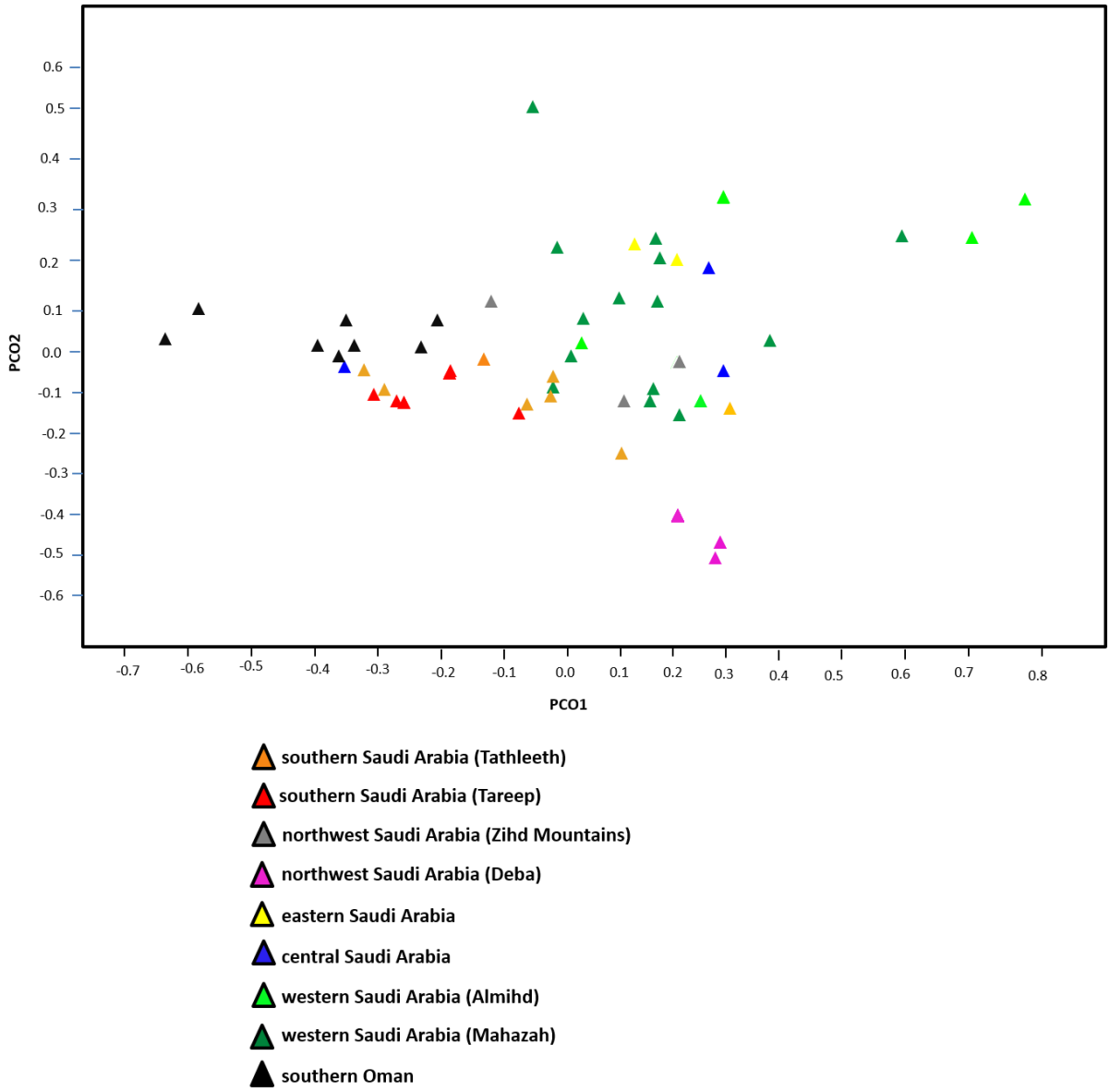


Fig. 3.12. Ordination of *A. opheodurus* individuals along the first (41%) and second (26%) principal co-ordinates of a PCoA of standardised multilocus nuclear genetic distance. Individuals are coloured according to mitochondrial clade assignment, consistent with Fig. 3.1.

Coalescent species delimitation.

Bayesian phylogenetic and phylogeography (BPP v.2.2) were implemented using a mitochondrial tree (Fig 3.4) as a guide tree. Recognized speciation events (showing strong posterior support for the nodes) were apparent at most nodes of *A. boskianus*. (Fig 3.13; 3.14). The populations from eastern and central Saudi Arabia appear to represent one species and populations from northwest Saudi Arabia (Deba and Tabouk) also appear to

represent one species. In contrast, all nodes of *A. opheodurus* resulted in strongly supported posterior probabilities, demonstrating speciation events (Fig 3.15; 3.16).

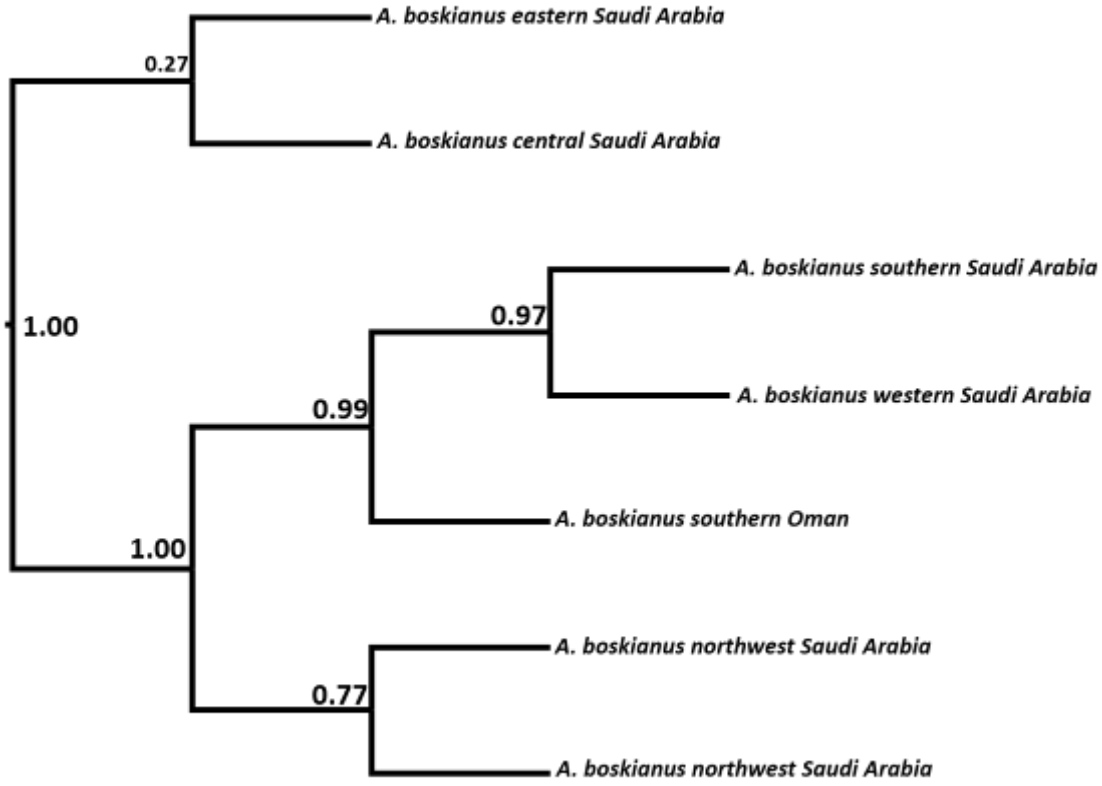


Fig. 3.13. Species tree representing the output from BPP (algorithm 0) analysis showing posterior probability support values for *Acanthodactylus boskianus*.

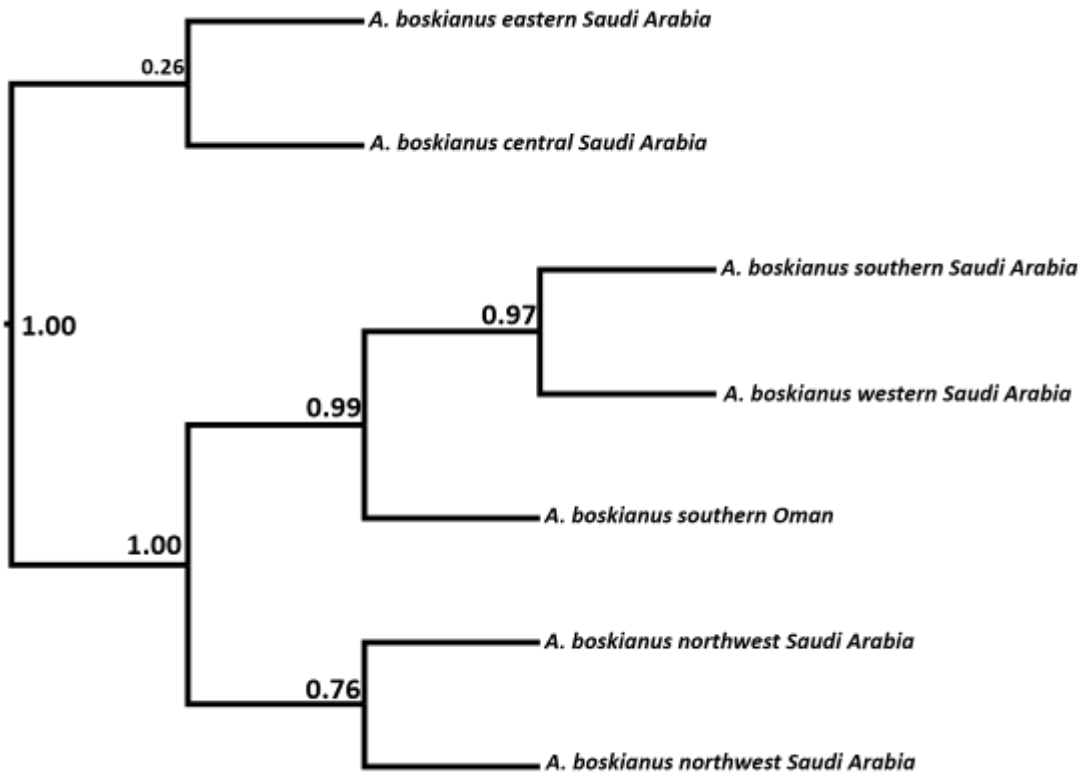


Fig. 3.14. Species tree representing the output from BPP (algorithm 1) analysis showing posterior probability support values for *Acanthodactylus boskianus*.

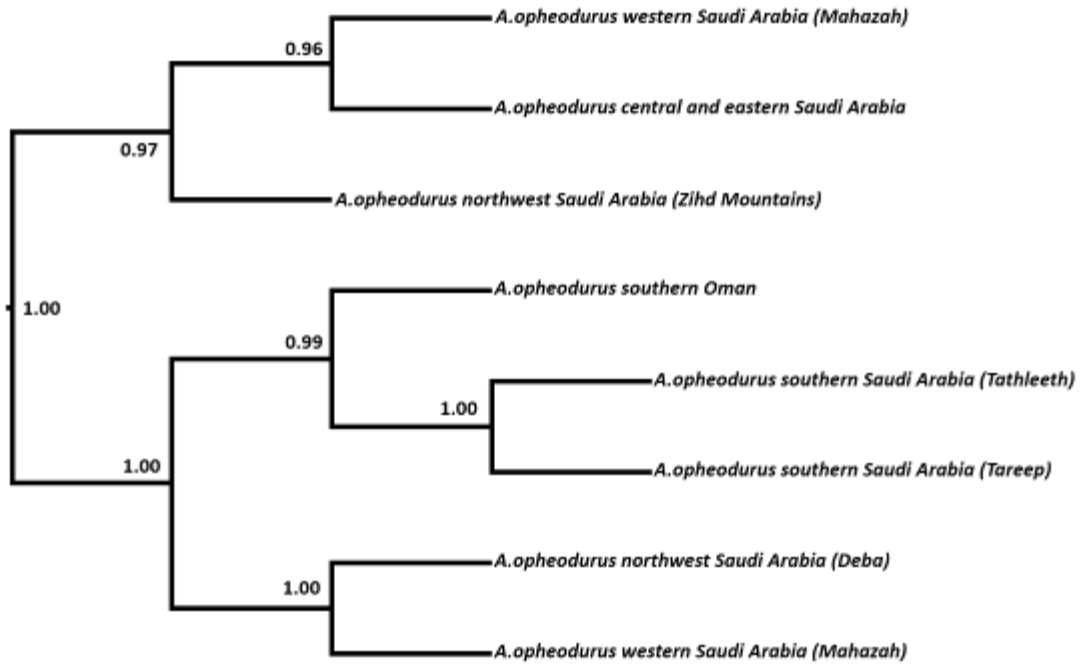


Fig. 3.15. Species tree representing the output from BPP (algorithm 0) analysis showing posterior probability support values for *Acanthodactylus opheodurus*.

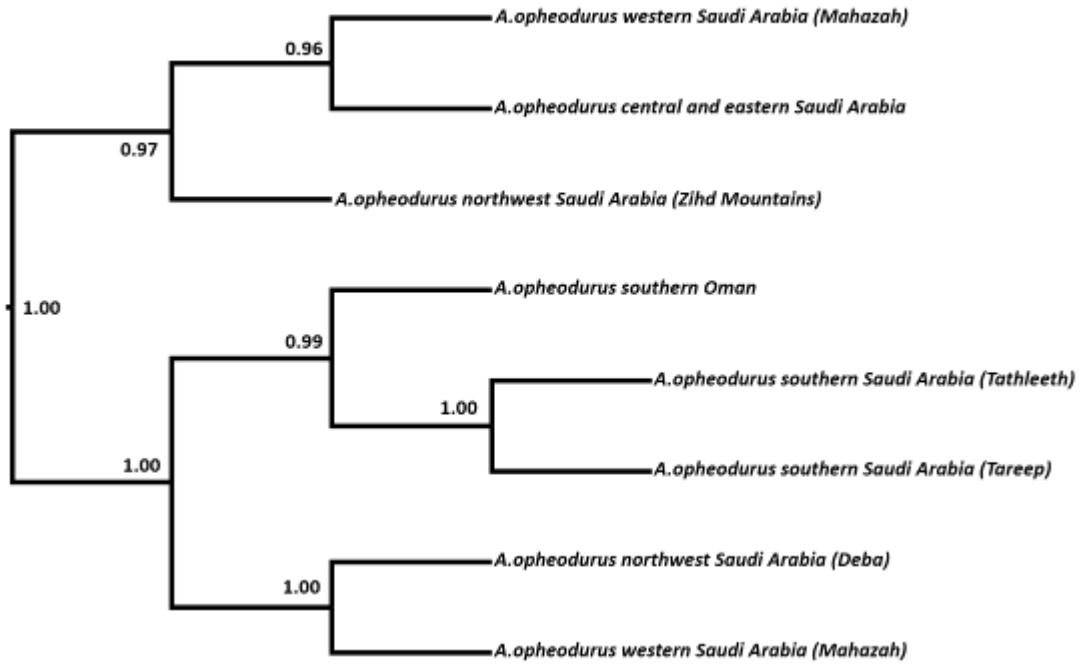


Fig. 3.16. Species tree representing the output from BPP (algorithm 1) analysis showing posterior probability support values for *Acanthodactylus opheodurus*.

3.4 Discussion

Sequence data from three mitochondrial and two nuclear loci have revealed new information about the distribution of genetic variation within two *Acanthodactylus* species and have highlighted potential candidate species. Further analysis of the mitochondrial guide tree, using coalescent species delimitation, assuming large ancestral population sizes and shallow divergence, strongly supports the hypothesis that each of these groups has a distinct evolutionary lineage.

This study confirms that all three species of *Acanthodactylus* represent independent monophyletic clades. Complete lineage sorting was observed for most loci. Only the nuclear locus NTF-3 revealed incomplete lineage sorting, with some *A. opheodurus* and *A. boskianus* individuals sharing haplotypes. An explanation for haplotype sharing between *A*. *boskianus* and *A*. *opheodurus* in NTF3, especially in slowly evolving genes such as NTF-3, may be due to the retention of ancestral haplotypes. This finding is supported by the geographically widespread occurrence of the shared haplotype, which is more suggestive of a retained ancestral haplotype than of haplotype sharing due to ongoing gene flow.

Analysis of mitochondrial DNA clades and nuclear genetic data show high species diversity within *Acanthodactylus boskianus* and *A. opheodurus*. Species delimitation is a hypothesis-testing process which is important for deciding when a new species is recognised from a study group. According to Padial et al., (2010) and Vieites et al. (2009) the process of delimiting candidate species is based on three categories. These categories are: unconfirmed candidate species (UCS), confirmed candidate species (CCS), and deep conspecific lineages (DCL). UCS are single locus genetic clades (e.g., mitochondrial gene tree clades) for which additional evidence of differentiation has not been found. CCS represent the candidate species whose individuals revealed high genetic distance, and whose separate identity is confirmed by other congruent taxonomic characters, such as morphology, independent nuclear markers, or an occurrence of syntopic groups that did not show any interbreeding between them, thus confirming their status as independently evolving lineages. DCL represents populations which show deep genetic distances in a single locus (especially mitochondrial DNA), but additional characters do not show congruent variation,

thus suggesting that the mitochondrial lineages do not denote independently evolving organismal lineages (Padial et al., 2010).

In this study, mitochondrial DNA clades of northwestern and southern Saudi Arabian *Acanthodactylus boskianus* (clades 2C and 3), and northwestern Saudi Arabian *Acanthodactylus opheodurus* (clade 4A) were hypothesised as candidate species. The congruence between mitochondrial DNA and nuclear DNA patterns can be used as strong evidence to indicate that these lineages are different species. Recent work suggests that such concordance between mitochondrial and nuclear loci can be used as identification tools for confirmed candidate species (Fouquet et al., 2007, Tomohiko et al., 2008).

Mitochondrial clades and the NTF-3 allele network (Figs. 3.4, 3.5) show different levels of lineage sorting from different geographical areas, within the Arabian Peninsula. For example, there are nested central and eastern lineages at one clade of both species (*A. opheodurus* and *A. boskianus*), and a nested lineage of *A. opheodurus* from the northwest Arabian Peninsula (Zihd mountain lineages) within western, central, and eastern Saudi Arabian lineages. These results for both the mtDNA tree and nuclear loci suggest that this lack of genetic differentiation is not owing to high levels of current gene flow but is due to recent divergence of these populations and large amounts of shared ancestral variation.

This study provides evidence of different genetic structure between lizard species. *A. boskianus* has a deeper divergence suggests that the current distribution is not recent, possibly due to vicariance events. By contrast, low levels of divergence in *A. opheodurus* suggest more recent or more rapid expansion out of its original location.

Among mitochondrial clades of the northwest Arabian Peninsula, *A. boskianus* (Fig. 3.2) showed highest levels of intraspecific divergence (clades 3A and 3B). These clades had a strongly supported bootstrap value (99%). Northwest *A. boskianus* samples were from the Tabouk locality, except for two individuals collected from the Zihd Mountains and from Deba. No clear phylogeographic pattern is seen within northwest Saudi Arabia *A. boskianus* clades from these regions, which differs from the *A .opheodurus* pattern from the same regions. *A. opheodurus* individuals from Deba (Red Sea coast) comprised as a distinct clade

from the Zihd Mountains. This latter clade appears closer to western, central, and eastern Saudi Arabia than the conspecific samples from Deba site. It is important to note that *A. opheodurus* was not sampled from the Tabouk region.

Species limits within *A. boskianus*

Acanthodactylus boskianus populations from northwest and southern Saudi Arabia, belonging to mitochondrial clades 3A, 3B and clade 2B, respectively, were identified as genetically distinct from the remaining Arabian Peninsula populations. The clusters of these mitochondrial clades were recognised as candidate species, showing high genetic distance and unique alleles in their nuclear DNA. The absence of more evidence from morphology and broader samples keep these lineages classified as candidate species. However, *A. boskianus*, individuals from northwest and southern Saudi Arabia were found to possess unique alleles (Fig. 3.10). In the case of NTF-3 genes, sharing of alleles between all *A. boskianus* regions, including some haplotypes from northwest and southern Saudi Arabia *A. boskianus* was observed. This pattern of haplotype sharing may be the result of incomplete lineage sorting between widespread ancestral haplotypes. Ordination analysis (Fig. 3.9) revealed distinct clusters for northwest and southern Saudi Arabia *A. boskianus*. Clean distinct clusters and high genetic distance for these lineages increases the confidence that these lineages of *A. boskianus* are genetically distinct species. Moreover, a multi-locus nuclear network (Fig. 3.7) of *A. boskianus* individuals from northwest and southern Saudi Arabia are clearly distinct and are strongly resolved from the remaining *A. boskianus* group by a split on the multi-locus nuclear network. This finding was supported by the BPP analysis which showed strong posterior support for all nodes.

According to (Arnold, 1986b), there is considerable geographic variation within *A. boskianus*, mainly expressed by morphological characteristics, notably, body size, which varies with geographical distribution. In addition, divergence patterns within *A. boskianus* have been observed in two populations of *A. boskianus* from eastern Arabia and northwest Africa (Harris and Arnold, 2000). The latter authors determined the species to be paraphyletic, with respect to the Arabian and the Moroccan populations of *A. boskianus* (Harris and Arnold, 2000).

The divergence and variation observed in this study of Arabian Peninsula *A. boskianus* appears consistent with the level of divergence observed in allopatric populations from Egypt. *A. boskianus* populations from the east and west of Egypt revealed diversity in the chemical fingerprints of its femoral gland secretions (Khannoon et al., 2013). In addition, phylogenetic analyses using DNA analysis of mitochondrial genes 12S, ND4, and Cytb showed that the eastern and western Egyptian populations are genetically distinct and that the chemical divergence of these lizards' odour profiles may be an example of signal evolution. These differences suggest the existence of a geographic barrier as the main reason for genetic and chemical divergence of these lizards (Khannoon et al., 2013). Analysis of comparisons between Arabian *A. boskianus* and Egyptian *A. boskianus* revealed that *A. boskianus* mtDNA clades from Arabia are different from *A. boskianus* from Egypt, suggesting that these two forms are different (Fig. 3.17). Harris and Arnold (2000) showed that *Acanthodactylus boskianus* is a paraphyletic species, but not consistent with the findings from this current study. However, the result of the analysis (Fig.3.18) showed that the Arabian Peninsula sequences revealed different clades regardless of the weak support bootstrap, and with the comprehensive manner, both species in this analysis were revealed to be monophyletic species (Fig. 3. 19).

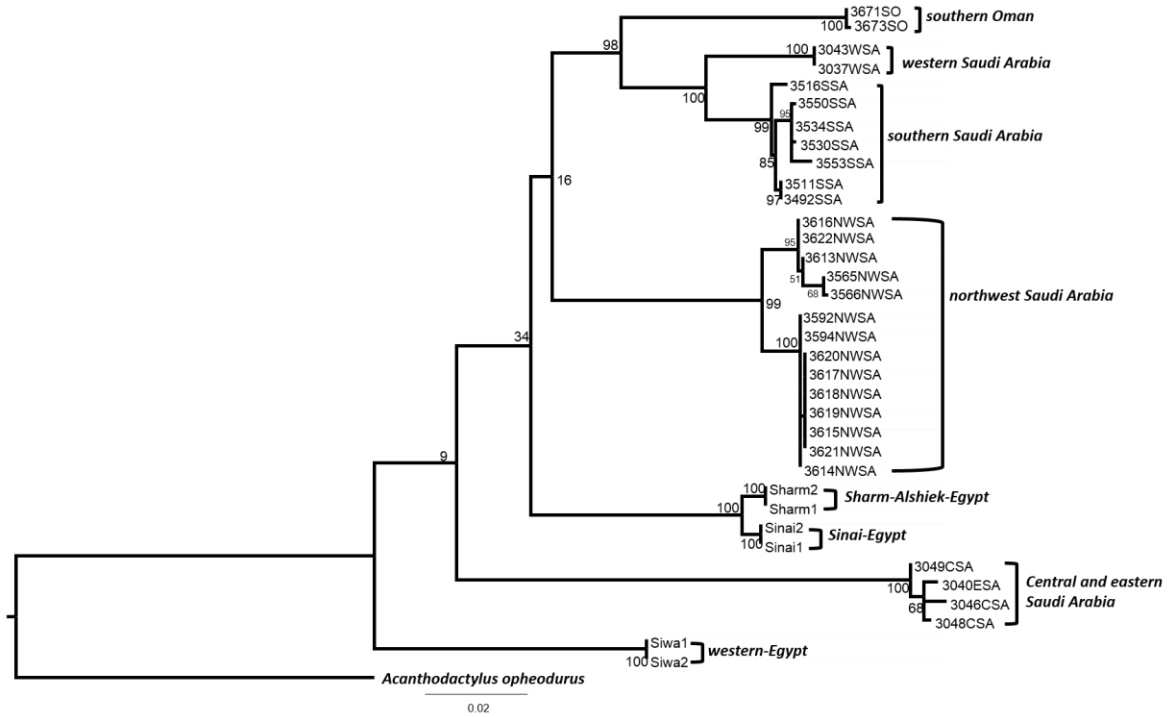


Fig. 3.17. Maximum likelihood tree of the combined mtDNA sequences (12S and cytb gene) of *Acanthodactylus boskianus* from the Arabian Peninsula and Egyptian population. SO= southern Oman, WSA= western Saudi Arabia, SSA= southern Saudi Arabia, NWSA= northwest Saudi Arabia, CSA=central Saudi Arabia, ESA=eastern Saudi Arabia, Sharm= Sharm al shiek, Sinai, Siwa= western Egypt, 3551oph= *A. opheodurus* (out group).

Fig. 3.18 Maximum likelihood tree of *Acanthodactylus boskianus* from the Arabian Peninsula, Egypt, Morocco, and Israel, based on 12S gene sequences.

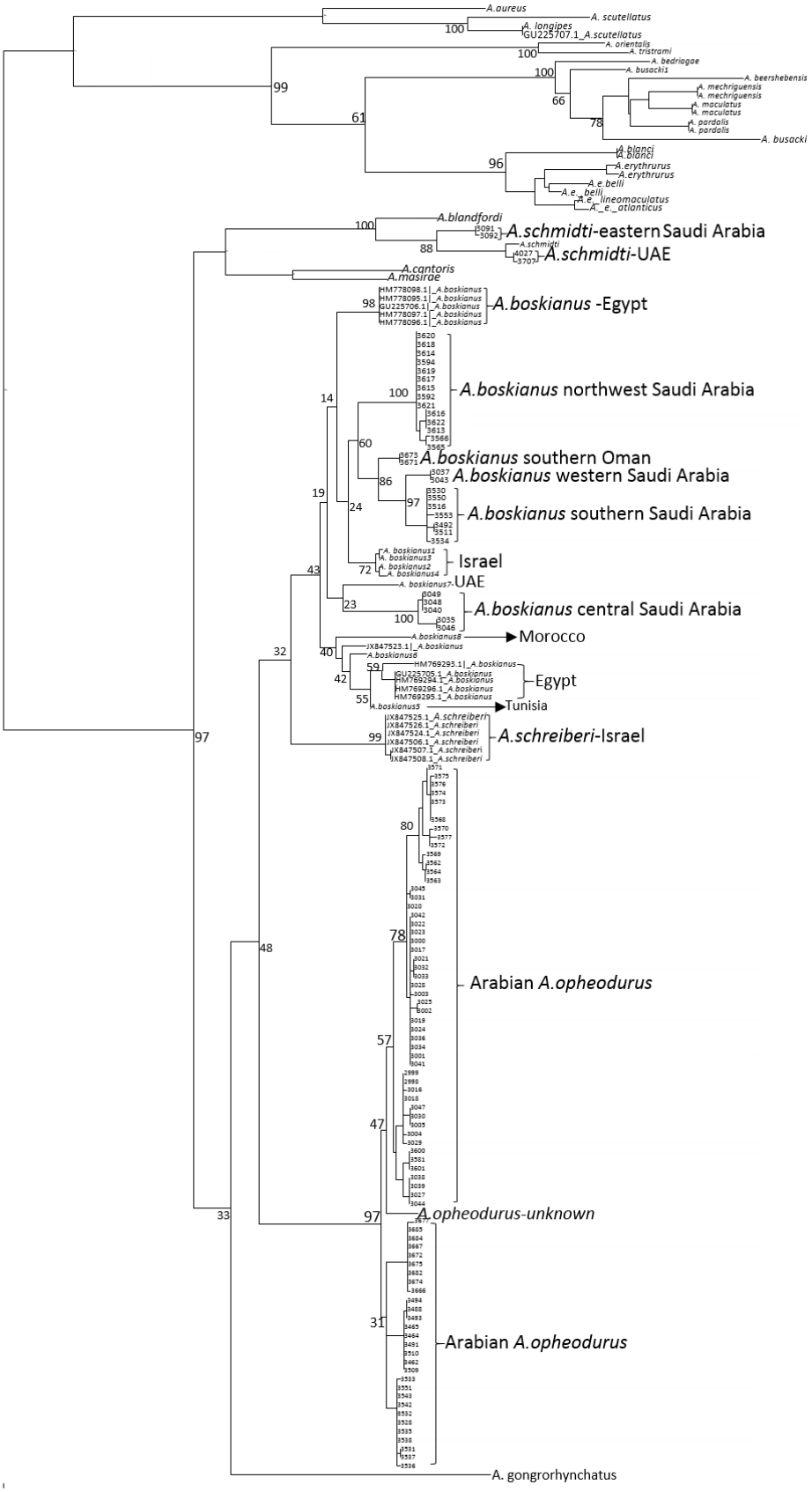


Fig. 3.19. Maximum likelihood tree of mtDNA genes (12S and 16S), for representative species from *Acanthodactylus* genus.

Acanthodactylus boskianus was recorded for the first time from its type locality in Egypt by Daudin (1802). It is currently thought that this species may have three subspecies: *A. b. boskianus*, which occurs mainly in Egypt (the Nile delta area and parts of Sinai); *A. b. euphraticus*, which is distributed throughout Iraq; and *A. b. asper*, which is distributed over the whole of the species range (Arnold, 1983, Boulenger, 1921, Salvador, 1982). However, to date, these sub-species are not confirmed. In addition, the findings from this study do not show any evidence of these sub-species within *A. boskianus*.

Among Arabian Peninsula populations, Arnold (1986b) assigned all *A. boskianus* to one species based on morphological characteristics. Subsequent genetic testing, from the present study, has confirmed that Arabian *A. boskianus* is a different species from Egyptian *A. boskianus*. This difference between two forms could be a result of vicariance events. The physical barrier of the Red Sea may have separated these two forms consequently creating two distinct evolutionary lineages.

Combinations of several taxonomic characters such as morphology and molecular genetics can lead to the discovery or description of new species or genetically distinct lineages (Yang and Rannala, 2010) . Based on the hypothesis that mitochondrial clades represent candidate species, the congruence with nuclear DNA genetic variation, and BPP analysis, lineages of northwest and southern Saudi Arabian *A. boskianus* can be identified as confirmed candidate species. Even with the absence of morphological evidence, the genetic data from unlinked molecular loci data can indicate that they are genetically isolated from each other and thus qualify as a distinct species (Padial et al., 2010). Evidence from molecular data presented in this study can provide information regarding the existence of cryptic species that can be considered as a candidate species, although the combination of molecular data with morphological characters would allow for confirmation that these forms represent distinct species. Moreover, it would clearly be desirable to identify morphological characters that can be used to distinguish these species in the field.

Species limits in *A. opheodurus*

Mitochondrial *Acanthodactylus opheodurus* clades show shallow patterns compared to the deep divergence patterns that are seen in its sister species, *A. boskianus*. These shallow

patterns may result from recent expansions of this species from its former ranges. According to mitochondrial clades, individuals of *A. opheodurus* from Deba (northwest Arabian Peninsula) formed a distinct sub-clade (Fig. 3.4, clade 4A). Consequently, these lineages from northwest Saudi Arabia represent candidate species among other groups. In addition to distinct mtDNA clades for these lineages, concordant variation in nuclear markers was also observed. That is, these individuals show unique alleles in the NTF-3 gene, but share alleles with other *A. opheodurus* in the R35 gene (Fig.3.11). This pattern may result from the retention of ancestral polymorphism for R35 genes. In addition, all analyses of multi-locus nuclear distances revealed distinct clusters and a clean split of northwest (Deba) lineages of *A. opheodurus* (Figs 3.7, 3.12) from other groups, this supports their recognition as distinct (and undescribed) evolutionary lineages. This finding is supported by the outcome of the BPP analysis. In addition to recognising northwest Saudi Arabia as a distinct lineage by BPP analysis, all *A. opheodurus* nodes demonstrated strong speciation probabilities and supports the notion of candidate species within *A. opheodurus* species complex (Figs 3.15; 3.16). The congruent variation patterns of mitochondrial and nuclear DNA, further increased the confidence in these lineages as candidate species. One of the indicators that these lineages should be recognised as candidate species is that specimens from the Deba (northwest Saudi Arabia), which is considered a candidate species, are different from specimens from the Zihd Mountains (also in northwest Saudi Arabia). The Zihd mountain lineages clustered in its mitochondrial clade with central, eastern, and western Saudi Arabia lineages. These two lineages of *A. opheodurus* from northwest Saudi Arabia show phylogeographic structure, in contrast with *A. boskianus* from the same regions that showed no phylogeographic structure. *A. boskianus* specimens from the Deba and the Zihd Mountains (northwest Saudi Arabia) formed a clade with shallow divergence patterns. However, the cluster of *A. opheodurus* from the Zihd Mountain lineages with central, eastern, and western Saudi Arabia lineages could result in incomplete lineage sorting of mitochondrial DNA due to gene flow between these regions. The same pattern is seen for these groups in the R35 genes.

The findings from this study were also strongly supported by subsequent BPP analysis, demonstrating, in this instance, the concordance between these methods. Given this concordance, the designation of *A. boskianus* from northwest and southern Saudi Arabia

and *A. opheodurus* of northwest Saudi Arabia as potential candidate species appears to be robust. Despite this, it is important to understand the potential limitations of this study and recognise that further work must be undertaken before these candidate species can be presented as novel distinct species. Firstly, this study was based solely on genetic data. Whilst genetic data has shown to be useful in delimiting species, it is also inherently problematic as accurately describing new species based on genetic data alone requires an extensive collection of individuals and DNA sequencing which may impede conservation efforts particularly for vulnerable species (Bauer et al., 2011). Ideally, the incorporation of morphological and geographical data to genetic data sets would provide a more robust assessment of species delimitation (Bauer et al., 2011, Zhang et al., 2011). Secondly, the limitations of the initial sampling must be considered. Whilst coalescent species delimitation methods employed in this study have described potential candidate species, the limitations of the initial sampling on providing a robust assessment of species designation, must be considered. Whilst a large number of individuals were sampled in this study, these individuals were also representative of a large geographical area. Therefore, the relatively sparse geographical sampling of individual clades and in particular, the lack of sampling around clade contact zones, represents a major limitation of this study. However, evidence from previous studies suggests that the number of individuals studied is less important than the number of loci used for coalescent based approaches (Heled and Drummond, 2010, Liu, 2008, Zhang et al., 2011).

The limitations of coalescent based species delimitation approaches must also be considered. Whilst BPP analysis has been shown to provide a robust assessment of species delimitation based only on genetic data, this approach also has fundamental flaws (Yang and Rannala, 2010). A fundamental limitation is that BPP analysis is based upon a guide tree, the construction of this guide tree has a direct impact on the output from this analysis. Therefore, to improve the accuracy of the analysis several different guide trees could be used, based not only on genetic data, but also on geographic, ecological and morphological data (Yang and Rannala, 2010). In addition, coalescent species delimitation, using BPP analysis, does not take into account species migrants, species hybridization, convergence and mixing (Yang and Rannala, 2010).

In light of these limitations, this study indicates that *A. boskianus* of northwest and southern Saudi Arabia and *A. opheodurus* of northwest Saudi Arabia (Deba) are potential genetically distinct species. Defining these species as distinct evolutionary lineages based on the De Queiroz (2007) species concept is an interpretation of the results of this study based soley on genetic data. More substantial evidence for defining these species should be provided by descriptions from many other sources, such as morphology and ecology (Leaché et al., 2009, Padial et al., 2010, Bauer et al., 2011, Zhang et al., 2011). Certain difficulties, for example the acquisition of a representative sample set, are encountered by describing species only on the basis of genetic information and further confirmation is needed (Bauer et al., 2011).

Conclusion

This study provides novel information regarding the species delimitation of *A. boskianus* and *A. opheodurus* from the Arabian Peninsula, the widespread two-sister lizard species, from the genus *Acanthodactylus.* Concordance of patterns of mitochondrial and nuclear DNA variation were used for species delimitation, and the findings confirmed using BPP analysis. This study demonstrates high cryptic diversity within these two species. In the case of *A. boskianus*, candidate species were identified from northwest and southern Saudi Arabia. In the case of *A. opheodurus*, a candidate species has been identified from the Deba region (northwest Saudi Arabia) and other lineages have also been identified as potential candidate species. Despite the fact that this study provides a high genetic distance for these groups, the absence of morphological evidence and the lack of samples from some lineages does not justify the identification of these candidate species as distinct species. Additional evidence combining genetic data with morphology, ecology, and geography is required to robustly support elevation of these candidate groups to species rank. Future study including these characters promises the discovery of cryptic species within *A. boskianus* and *A. opheodurus*.

4. General discussion

This chapter aims to summarise the findings from this thesis with respect to the two main aims of this thesis (outlined in section 1.5). Briefly, the two key aims of this thesis were;

- 1. A comprehensive study of the phylogeography of the co-distributed species of Arabian Peninsula lizards.
- 2. Investigation of the occurrence of cryptic species within *Acanthodactylus boskianus* and *A. opheodurus* from the Arabian Peninsula.

Historical and current connections between biota from different zoographical regions make the Arabian Peninsula an interesting region for testing theories of historical biogeography. This thesis obtained new information regarding the phylogeography and species delimitations of one group of the Arabian Peninsula fauna that has received little attention: the lizards of the Arabian Peninsula (Arnold, 1980a, Arnold, 1980b, Arnold, 1986b). In total, approximately 134 species of lizards are currently recognised from the Arabian Peninsula (Cox et al., 2012, Nazarov et al., 2013, Carranza and Arnold, 2012) and this has been achieved largely through morphological studies only. Therefore, intensive research on the phylogeography of many species and the delimitation of species in widespread species or species complexes is needed to complete the general picture of lizard groups in this area. Information gained from research in these areas will not only advance the taxonomic knowledge of these species but also provide valuable information that can be used to assist conservation efforts by determining lizard biodiversity 'hotspots' which can be incorporated into urban development plans or can be used in the assessment of protected areas. Global information on reptilian species, in general, is lacking. Therefore, studies advancing taxonomic knowledge of reptilian species are urgently required to provide information that can be used to determine the conservation status of different reptilian species (Böhm et al., 2013).

The ecosystem biodiversity of the Arabian Peninsula also provided a unique opportunity to study the habitat preferences among different species of lizards. The group investigated in

this study contained a variety of species that occurred within various ecological habitats. The diversity of these lizards, which contained groups of geckos, lacertids and agamids, allowed to us to explore the different evolutionary history and distribution of these groups. This study also provided information regarding habitat use and ecological adaptation. These groups of lizards showed different types of ecological variation and included three different ecotypes: sand-dwelling, ground-dwelling and rock-dwelling lizards.

This thesis aimed to conduct phylogeographic investigations and to establish the species delimitation of lizards from two species of the genus *Acanthodactylus: A. boskianus* and *A. opheodurus.* This research has led to novel results that pointed to common phylogeographic patterns among co-distributed species of lizards and that also identified cryptic species within these *Acanthodactylus* species. Thus, demonstrating the potential for the discovery of new lizard species within this currently under researched region.

This study represents the most comprehensive phylogeographic analysis of the Arabian Peninsula lizards, to date. In this study, we were able to utilize a multispecies tree approach (Heled and Drummond, 2010) to show spatial and temporal patterns of fourteen co-distributed lizard species within the Arabian Peninsula. Shared common patterns were observed across groups, and evidence was provided for close phylogeographic relationships between these groups and regions.

At present, approximately 134 lizard species have been described from the Arabian Peninsula (Carranza and Arnold, 2012, Cox et al., 2012, Nazarov et al., 2013). The findings from this study suggest that this number may under-represent the total lizard biodiversity within this region. One of the key findings from this study was the identification of the cryptic species complexes within *A. boskianus* and *A. opheodurus*. The subsequent application of species delimitation methods suggested the presence of five new species within *A. boskianus* and 8 new species within *A. opheodurus* species complexes. However, other groups and regions investigated in this study also demonstrated the potential for the discovery of new species within the *Ptyodactylus hasselquistii* species complex across most studied regions and *Pseudotrapelus sinaitus* from central Saudi Arabia and *Stenodactylus*

slevini from Eastern Saudi Arabia. The findings from this study potentially indicate the presence of 43 new species within the 14 species complexes studied. Thirteen of these potential new species, within *Acanthodactylus*, were determined to be candidate species following BPP analysis (Yang and Rannala, 2010). The remaining 30 species were identified using a multispecies coalescence approach (Heled and Drummond, 2010), however these identified species may require further analysis before they can be classified as a new species. As a direct result of this study, it could then be argued that the total number of lizard species within the Arabian Peninsula be increased from 134 to 147. Potential candidate species as identified by the multispecies coalescence approach (species tree) require further validation, however it suggests the possibility of 30 additional species of Arabian Peninsula lizards. Based the findings from these 14 studied lizard species, the total lizard diversity across the entirety of the Arabian Peninsula may be up to three times higher than previously recognised. As a result, one of the priorities for future work should be the application of species delimitation methods to these highlighted groups and regions to determine if they currently contain novel or candidate species.

Our understanding of global biodiversity is in a constant state of flux, with the discovery of new species and the extinction of others. These changes are strongly linked with anthropogenic changes to the environment. As the magnitude of these changes is so large, the assessment of global diversity changes is currently considered to be a research priority in its own right (Sala et al., 2000). Therefore, phylogeographic studies provide vital information on the historical and biogeographical distribution of organisms which can greatly assist in obtaining accurate diversity estimates of organisms or regions (Lee, 2000). Reptiles and amphibians can be used as indicators for environmental change (Grant et al., 1992), therefore, phylogeographic analysis of herpetofauna are of particular importance for both global biodiversity assessments and subsequent conservation efforts. Currently, little research has been conducted on the phylogeography or determination of species limits of the Arabian Peninsula lizards and as such, this study represents the most comprehensive analysis to date. In terms of lizard diversity, the findings from this study are in agreement with global findings on reptilian diversity. Previous studies have indicated that the accepted number of described species dramatically under-represents the actual diversity. Veith (1996) documented the doubling of the known number of amphibian species in Europe,

whilst Oliver et al. (2009) documented the diversity of Australian gecko species more than doubling over two decades. In the previous two years three separate studies have confirmed the existence of twelve new species of gecko and agamids in the Arabian region alone (Carranza and Arnold, 2012, Melnikov and Pierson, 2012, Nazarov et al., 2013) demonstrating the increasing rate of discovery within this group of organisms. Likewise, this study indicates that the diversity of Arabian Peninsula lizards could be three times higher than the previously accepted number of species.

In addition, this study has also identified potential lizard 'biodiversity hotspots' within the Arabian Peninsula, which will be of great importance in conservation efforts within this region. Previous studies have identified areas of high and low lizard diversity (Cox et al., 2012) and the findings from this study agree with these published findings. Areas of high lizard diversity were noted in northwestern and southern Saudi Arabia and southern Oman. These areas also harboured old and basal lineages for most studied species. Areas of low lizard diversity were seen in eastern and central Saudi Arabia. These areas also revealed shallow divergence times, suggesting different historical biogeographical patterns and processes. Lizard diversity within the studied region appeared to be linked with ecological habitats, with high lizard diversity found in areas dominated by mountainous terrain and sandy plains (northwest and southern Saudi Arabia and southern Oman) and areas of low diversity were dominated by gravel plains with poor plant coverage. In addition to the observed species diversity, this study also clearly demonstrated regions of high phylogeographic diversity; these regions contained older and basal lineages which may subsequently represent different species and demonstrated areas of phylogenetic uniqueness within the studied region. These findings will have important implications for conservation management within the region. Despite the fact that the species studied are of little conservation concern directly (as determined by the IUCN: (Baillie et al., 2004)) this study has clearly identified biodiversity hotspots which will be of great significance in conservation planning especially with regard to urban development. In addition, the assessment of lizard diversity within this region has provided important information with regard to lizard biodiversity, which can be used to assess anthropogenic environmental changes.

In general, the fauna of the Arabian Peninsula has been poorly studied. Previous studies documenting the various faunal groups have focused mainly on identification solely by morphological techniques (Arnold, 1977, Arnold, 1980a, Arnold, 1980b, Arnold, 1986b, Arnold, 1986a, Arnold, 1983, Salvador, 1982). However, the application of molecular techniques in combination with morphological techniques has the potential to revolutionise the documentation of global faunal groups (Avise, 2009). The majority of the recent studies concerning the terrestrial Arabian fauna focus on the origins of groups of organisms and focus on the Afro-Arabian exchange of genes and the geological events which may have either enabled this gene flow or acted as a barrier against it (Metallinou et al., 2012, Pook et al., 2009, Portik and Papenfuss, 2012). As such, intra-Arabian terrestrial faunal studies represent an urgent area for future research.

Many of the species complexes examined had not previously been subjected to phylogeographic analysis (*Acanthodactylus boskianus*, *Ptyodactylus hasselquistii*, *Pseudotrapelus sinaitus*, *Bunopus tuberculatus* and *Acanthodactylus opheodurus*). As such, this study documents for the first time the spatial and temporal patterns observed in these species across the Arabian Peninsula. However, due to the lack of other comparative phylogeographic studies on these species within different regions we are unable to compare the findings of this study with any others for these studied species. The historical biogeography of *Stenodactylus* genus has been examined (Metallinou et al., 2012). The divergence times for *Stenodactylus* (*slevini* and *doriae*) as calculated in this study, overlapped with published estimates (Metallinou et al., 2012). Likewise the divergence time estimates for *Messalina guttulata*, *M. brevirostris*, and *M. adramitana* as described in (Kapli et al., 2014) also overlapped with the findings from this study. The lack of other comparable studies on these lizard species either from within the Arabian Peninsula or from other geographical locations makes the findings from this study incomparable to other regions. As far as we are aware, no comparative phylogeographical studies on other taxa within the Arabian Peninsula exists, therefore further highlighting the importance and novelty of this study. Unfortunately, this also precludes the comparison of this study with those of other taxa from within the same region. Future work may therefore include the repetition of this entire study in an alternative geographical location as a collaborative endeavour including DNA sequences of samples from this study or utilising the methodology adopted within this

study to provide a comparative phylogeographical analysis of alternative taxa within the Arabian Peninsula.

Although this study is restricted to the Arabian Peninsula region only, it is represents a good example of a multispecies phylogeographical approach and the methodology utilised within this study can be applied to any other geographical region and / or faunal groups. Previous phylogeographical studies have largely focused on a single species or group which are normally of either commercial or conservational importance (Fernández et al., 2013, Rocha et al., 2007). Whilst the Arabian lizards are not currently known to be of particular commercial or conservational importance (due largely to a lack of information) increasing our knowledge of this region and the species within it may yield important information that may prove to be significant in terms of conservation.

The findings from this project have contributed significantly towards the understanding of the spatial and temporal patterns of the lizard species within the Arabian Peninsula. To date, previous studies on lizard species within this region have been largely restricted to morphological studies only. The application of phylogeographic techniques has led to the discovery of important common spatial and temporal patterns between different lizard species within this region and has provided putative links with environmental niches. As such, this study should be considered as the most comprehensive analysis of Arabian lizards, to date. In addition, this study has been successful in identifying cryptic species within *A. boskianus* and *A. opheodurus* within the Arabian Peninsula, although it is not possible to definitively argue the presence of new species within these groups. Further work incorporating more extensive sampling, alternative methodologies and a more integrated approach may be required before a new species may be officially 'discovered'.

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Appendix 1

Table 1. Sample information for the Arabian Peninsula lizards used in the phylogeographic analysis chapter. UAE = United Arab Emirates.

SPECIES	Locality	Country	field work nos.	Lab codes	cytb	16S	12S	R35	NTF-3
Acanthodactylus opheodurus	east	Eastern Saudi Arabia	5	2998	yes	yes	yes	no	no
A. opheodurus	east	Eastern Saudi Arabia	6	2999	yes	yes	yes	yes	yes
A. opheodurus	mahazah	western Saudi Atabia	17	3001	yes	yes	yes	yes	yes
A. opheodurus	ibex reserve.	central Saudi Arabia	134	3005	yes	yes	yes	yes	yes
A. opheodurus	mahazah	western Saudi Atabia	34	3020	yes	yes	yes	yes	yes
A. opheodurus	Almihd	western Saudi Atabia	90	3027	yes	yes	yes	yes	yes
A. opheodurus	ibex res.	central Saudi Arabia	119	3029	yes	yes	yes	yes	yes
A. opheodurus	Almihd	western Saudi Atabia	14	3031	yes	yes	yes	yes	yes
A. boskianus	ibex res.	central Saudi Arabia	22	3035	yes	yes	no	yes	yes
A. boskianus	Almihd	western Saudi Atabia	49	3037	yes	yes	yes	yes	yes
A. opheodurus	Almihd	western Saudi Atabia	52	3039	yes	yes	yes	yes	yes
A.boskianus	east	Eastern Saudi Arabia	57	3040	yes	yes	yes	yes	yes
A. boskianus	Almihd	western Saudi Atabia	86	3043	yes	yes	yes	yes	yes
A. boskianus	ibex res.	central Saudi Arabia	128	3046	yes	yes	yes	yes	yes
A. opheodurus	*tathleeth*	southern Saudi Arabia	144	3464	yes	yes	yes	yes	yes
A. opheodurus	*tathleeth*	southern Saudi Arabia	145	3465	yes	yes	yes	yes	yes
A. opheodurus	*tathleeth*	southern Saudi Arabia	168	3488	yes	yes	yes	yes	yes
A. opheodurus	*Deba*	Northwest Saudi Arabia	244	3564	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	174	3494	yes	yes	yes	yes	yes
A.boskianus	*tathleeth*	southern Saudi Arabia	191	3511	yes	yes	yes	yes	yes
A.opheodorus	*tareep*	southern Saudi Arabia	211	3531	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	215	3535	yes	yes	yes	yes	yes
A.boskianus	*tareep*	southern Saudi Arabia	230	3550	yes	yes	yes	yes	yes

A.boskianus	*Deba*	Northwest Saudi Arabia	245	3565	yes	yes	yes	yes	yes
A.boskianus	*Deba*	Northwest Saudi Arabia	246	3566	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	248	3568	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	249	3569	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	250	3570	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	252	3572	yes	yes	yes	yes	yes
A.opheodurus	*zihd mountains*	Northwest Saudi Arabia	281	3601	yes	yes	yes	yes	yes
A.boskianus	*tabuk*	Northwest Saudi Arabia	293	3613	yes	yes	yes	yes	yes
A.boskianus	*tabuk*	Northwest Saudi Arabia	296	3616	yes	yes	yes	yes	yes
A.boskianus	*tabuk*	Northwest Saudi Arabia	297	3617	yes	yes	yes	yes	yes
A.boskianus	*tabuk*	Northwest Saudi Arabia	300	3620	yes	yes	yes	yes	yes
A.opheodurus	*Dofar*	Oman	346	3666	yes	yes	yes	yes	yes
A.boskianus	*Dofar*	Oman	351	3671	yes	yes	yes	yes	yes
A.boskianus	*Dofar*	Oman	353	3673	yes	yes	yes	yes	yes
A.opheodurus	*Dofar*	Oman	354	3674	yes	yes	yes	yes	yes
A.opheodurus	*Dofar*	Oman	355	3675	yes	yes	yes	yes	yes
A.opheodurus	*Dofar*	Oman	365	3685	yes	yes	yes	yes	yes
A.schmidti	Al-sharjah	UAE		4027-3457	yes	yes	yes	yes	yes
A.schmidti	albatayeh-Sharjah	UAE		3707	yes	yes	yes	yes	yes
A.schmidti	east	Eastern Saudi Arabia	10	3091	yes	yes	yes	yes	yes
A.schmidti	east	Eastern Saudi Arabia	63	3092	yes	yes	yes	yes	yes
Mesalina guttulata	*tathleeth*	southern Saudi Arabia	140	3460	yes	yes	yes	yes	yes
M.guttulata	*tathleeth*	southern Saudi Arabia	194	3514	yes	yes	yes	yes	yes
M.guttulata	*tareep*	southern Saudi Arabia	228	3548	yes	yes	yes	yes	yes
M.guttulata	*Deba*	Northwest Saudi Arabia	241	3561	yes	yes	yes	yes	yes
M.breviorestis	*tabuk*	Northwest Saudi Arabia	303	3623	yes	yes	yes	yes	yes
M.breviorestis	*tabuk*	Northwest Saudi Arabia	304	3624	yes	yes	yes	yes	yes
M. adramitana	*northoman*	Oman	312	3632	yes	yes	yes	yes	yes
M.adramitana	*northoman*	Oman	314	3634	no	yes	yes	yes	yes
M.adramitana	*northoman*	Oman	316	3636	yes	yes	yes	yes	yes
M.adramitana	*northoman*	Oman	323	3643	yes	yes	yes	yes	yes
M.adramitana	*northoman*	Oman	324	3644	yes	yes	yes	yes	yes

M.adramitana	*Dofar*	Oman	330	3650	yes	yes	yes	yes	yes
M.adramitana	*Dofar*	Oman	342	3662	yes	yes	yes	yes	yes
M.adramitana	*Dofar*	Oman	348	3668	yes	yes	yes	yes	yes
M.adramitana	*Dofar*	Oman	349	3669	yes	yes	yes	yes	yes
M.adramitana	mahazah	western Saudi Atabia	66	3095	yes	yes	yes	yes	yes
M.guttulata	east	Eastern Saudi Arabia	11	4008	yes	yes	yes	yes	yes
M.guttulata	east	Eastern Saudi Arabia	58	4009	yes	yes	yes	yes	yes
M.guttulata	east	Eastern Saudi Arabia	60	4010	yes	yes	yes	yes	yes
M.guttulata	ibex res.	central Saudi Arabia	114	4011	yes	yes	yes	yes	yes
M.guttulata	ibex res.	central Saudi Arabia	122	4012	yes	yes	yes	yes	yes
M.guttulata	ibex res.	central Saudi Arabia	130	4013	yes	yes	yes	yes	yes
M.breviorestis	east	Eastern Saudi Arabia	55	4014	yes	yes	yes	no	no
M.breviorestis	east	Eastern Saudi Arabia	56	4015	yes	yes	yes	yes	yes
M.breviorestis	Almihd	western Saudi Atabia	50	4017	yes	yes	yes	no	yes
M.breviorestis	Almihd	western Saudi Atabia	79	4020	yes	yes	yes	yes	yes
M.breviorestis	Al-sharjah	UAE	4029	3459	yes	yes	yes	yes	yes
Ptyodactylus hasselquistii	*tathleeth*	southern Saudi Arabia	146	3466	yes	yes	yes	yes	yes
P.hasselquistii	*tathleeth*	southern Saudi Arabia	147	3467	yes	yes	yes	yes	yes
P.hasselquistii	*Deba*	Northwest Saudi Arabia	240	3560	yes	yes	yes	yes	yes
P.hasselquistii	*tabuk*	Northwest Saudi Arabia	287	3607	yes	yes	yes	yes	yes
P.hasselquistii	*tabuk*	Northwest Saudi Arabia	288	3608	yes	yes	yes	yes	yes
P.hasselquistii	*tabuk*	Northwest Saudi Arabia	292	3612	yes	yes	yes	yes	yes
P.hasselquistii	*Dofar*	Oman	336	3655	yes	yes	yes	yes	yes
P.hasselquistii	wadi Alhelo-sharjah	UAE		3722	yes	yes	yes	yes	yes
P.hasselquistii	wadi Alhelo-sharjah	UAE		3723	yes	yes	yes	yes	yes
P.hasselquistii	east	Eastern Saudi Arabia	24	3006	yes	yes	yes	yes	yes
P.hasselquistii	mahazah	western Saudi Atabia	38	3008	yes	yes	yes	yes	yes
P.hasselquistii	mahazah	western Saudi Atabia	41	3050	yes	yes	yes	yes	yes
P.hasselquistii	ibex res.	central Saudi Arabia	101	3054	yes	yes	yes	yes	yes

P.hasselquistii	ibex res.	central Saudi Arabia	103	3055	yes	yes	yes	yes	yes
Bunopus tuberculatus	*tathleeth*	southern Saudi Arabia	176	3496	yes	yes	yes	yes	yes
B.tuberculatus	*tathleeth*	southern Saudi Arabia	178	3498	yes	yes	yes	yes	yes
B.tuberculatus	*Deba*	Northwest Saudi Arabia	238	3558	yes	yes	yes	yes	yes
B.tuberculatus	*Deba*	Northwest Saudi Arabia	239	3559	yes	yes	yes	yes	yes
B.tuberculatus	*zihd mountains*	Northwest Saudi Arabia	259	3579	yes	yes	yes	yes	yes
B.tuberculatus	*zihd mountains*	Northwest Saudi Arabia	262	3582	yes	yes	yes	yes	yes
B.tuberculatus	albatayeh-Sharjah	UAE		3709	yes	yes	yes	yes	yes
B.tuberculatus	albatayeh-Sharjah	UAE		3714	yes	yes	yes	yes	yes
Cyrtopodion scabrum	*Alsharjah*	UAE	4026	3456	yes	yes	yes	yes	yes
B.tuberculatus	east	Eastern Saudi Arabia	7	3069	yes	yes	yes	yes	yes
B.tuberculatus	mahazah	western Saudi Atabia	28	3070	yes	yes	yes	yes	yes
B.tuberculatus	east	Eastern Saudi Arabia	36	3072	yes	yes	yes	yes	yes
B.tuberculatus	east	Eastern Saudi Arabia	48	3073	yes	yes	yes	yes	yes
B.tuberculatus	Almihd	western Saudi Atabia	87	3026	yes	yes	yes	yes	yes
B.tuberculatus	ibex res.	central Saudi Arabia	105	3079	yes	yes	yes	yes	yes
B.tuberculatus	ibex res.	central Saudi Arabia	108	3080	yes	yes	yes	yes	yes
Stenodactylus doriae	*tathleeth*	southern Saudi Arabia	151	3471	yes	yes	yes	yes	yes
S.arabicus	*tathleeth*	southern Saudi Arabia	158	3478	yes	yes	yes	yes	yes
S.doriae	*Riyadh*	central Saudi Arabia	164	3484	yes	yes	yes	yes	yes
S.doriae	*Riyadh*	central Saudi Arabia	165	3485	yes	yes	yes	yes	yes
S.doriae	*tathleeth*	southern Saudi Arabia	180	3500	yes	yes	yes	yes	yes
S.doriae	*tathleeth*	southern Saudi Arabia	188	3508	yes	yes	yes	no	yes
S.doriae	*tathleeth*	southern Saudi Arabia	197	3517	yes	yes	yes	yes	yes
S.doriae	*tabuk*	Northwest Saudi Arabia	283	3603	yes	yes	yes	yes	yes
S.doriae	*tabuk*	Northwest Saudi Arabia	284	3604	yes	yes	yes	yes	yes
S.leptocosymbotus	*Dofar*	Oman	340	3660	yes	yes	yes	yes	yes
S.leptocosymbotus	*Dofar*	Oman	359	3679	yes	yes	yes	yes	yes
S.leptocosymbotus	*Dofar*	Oman	360	3680	yes	yes	yes	yes	yes

S.doriae	Al-sharjah	UAE	4023	3453	yes	yes	yes	yes	yes
S.doriae	albatayeh-Sharjah	UAE		3711	yes	yes	yes	yes	yes
S.doriae	albatayeh-Sharjah	UAE		3712	yes	yes	yes	yes	yes
S.slevin	mahazah	western Saudi Arabia	40	3083	yes	yes	yes	yes	yes
S.doriae	east	Eastern Saudi Arabia	47	3085	yes	yes	yes	yes	yes
S.doriae	east	Eastern Saudi Arabia	59	3086	yes	yes	yes	yes	yes
S.doriae	mahazah	western Saudi Arabia	70	3088	yes	yes	yes	yes	yes
S.doriae	madinah	western Saudi Arabia	99	3090	yes	yes	yes	yes	yes
S.slevin	mahazah	western Saudi Arabia	31	3057	yes	yes	yes	yes	yes
S.slevin	mahazah	western Saudi Arabia	39	3059	yes	yes	yes	yes	yes
S.slevin	east	Eastern Saudi Arabia	61	3060	yes	yes	yes	no	no
S.slevin	east	Eastern Saudi Arabia	62	3061	yes	yes	yes	yes	yes
S.slevin	mahazah	western Saudi Arabia	73	3063	yes	yes	yes	yes	yes
S.slevin	Almihd	western Saudi Arabia	92	3064	yes	yes	yes	yes	yes
S.slevin	ibex res.	central Saudi Arabia	118	3067	yes	yes	yes	yes	yes
S.slevin	ibex res.	central Saudi Arabia	120	3068	yes	yes	yes	yes	yes
S.slevin	*tathleeth*	southern Saudi Arabia	154	3474	yes	yes	yes	yes	yes
S.slevin	*tathleeth*	southern Saudi Arabia	155	3475	yes	yes	yes	yes	yes
S.slevin	*Deba*	Northwest Saudi Arabia	235	3555	yes	yes	yes	yes	yes
S.slevin	*Deba*	Northwest Saudi Arabia	236	3556	yes	yes	yes	yes	yes
S.slevin	*Deba*	Northwest Saudi Arabia	237	3557	yes	yes	yes	yes	yes
S.leptocosymbotus	*Dofar*	Oman	343	3663	yes	yes	yes	yes	yes
S.arabicus	albatayeh-Sharjah	UAE		3715	yes	yes	yes	yes	yes
Psuedotrapelus sinaitus	*tathleeth*	southern Saudi Arabia	198	3518	yes	yes	yes	yes	yes
p.sinaitus	*tareep*	southern Saudi Arabia	205	3525	yes	yes	yes	yes	yes
P.sinaitus	*zihd mountains*	Northwest Saudi Arabia	275	3595	Yes	yes	yes	yes	yes
P.sinaitus	*zihd mountains*	Northwest Saudi Arabia	276	3596	No	yes	yes	no	yes
P.sinaitus	*Dofar*	Oman	356	3676	No	yes	yes	yes	Yes
P.sinaitus	*Dofar*	Oman	363	3683	Yes	yes	yes	yes	Yes

P.sinaitus	ibex res.	central Saudi Arabia	109	4004	Yes	yes	yes	yes	Yes
P.sinaitus	ibex res.	central Saudi Arabia	116	4005	yes	yes	yes	yes	yes

Table 2. Sample information for the snake samples used in calibration points in the phylogeograaphic chapter. WW: W Wüster personal collections.

(mtDNA samples)

species	localitiy	cytb/12s/16s (GeneBank Accession)
Naja kaouthia	Thailand	JF357939/JN687924/JF357948
Naja kaouthia	Thailand	FR693728/JF357939/GQ359757
Naja kaouthia	Thailand	AF217835/EU624235/JN687925
Naja naja	unknown	GQ359506/EU547088/GQ359756
Naja naja	Nepal	FR693725/EU624236/EU624270
Naja nivea	S.Africa	FR693729/EU624238/GQ359755
Naja nivea	S.Africa	AF217827/GAP/EU624272
Naja nigricollis	Cameron	GQ359505/EU624237/GQ359505
Daboia(Macrovipera) mauritanica	Morocco	EU624313/EU624261/EU624295
Daboia siamensis	Thailand	DQ305459/AY352773/AY352712
Daboia siamensis		AY165081/DG305413/DQ305436
Porthidium acrosae	Ecuador	AF292575/EU624241/GQ372871
Porthidium lansbergii rozei	Venezuela	AY13375/EU624242/GQ372870
Bothrops asper	Costa Rica	FJ985704/EU624239/GQ372868
Bothrops asper		HE867056/AF057218/AF057265
WW 1612_E_carinatus_sochureki	Sharjah-UAE	GQ359436/GQ359604/GQ359685
WW 1613_E_carinatus_sochureki	Sharjah-UAE	GQ359437/GQ359605/GQ359686
WW 2032_E_pyramidium	Yemen	GQ359480/GQ359645/GQ359729
WW 2031_E_pyramidium	Yemen	GQ359479/GQ359644/GQ359728
WW 2056_E_pyramidium	Saudi Arabia	GQ359486/GQ359651/GQ359735
WW 2055_E_pyramidium	Saudi Arabia	GQ359485/GQ359650/GQ359734
WW 1692_E_coloratus	Thumrait, Oman	GQ359465/GQ359630/GQ359714
WW 1669_E_omanensis	Fujairah-UAE	GQ359489/GQ359654/GQ359738
WW 1670_E_omanensis	Fujairah-UAE	GQ359468/GQ359633/GQ359717
WW1689_E_omanensis	Ar Rustaq, Oman	GQ359472/GQ359637/GQ359721
WW1691_E_omanensis	Ar Rustaq, Oman	GQ359474/GQ359639/GQ359723
WW 1690_E_omanensis	Ar Rustaq, Oman	GQ359473/GQ359638/GQ359722
WW 2029_E_coloratus	Yemen	GQ359477/GQ359642/GQ359726
WW 2030_E_coloratus	Yemen	GQ359478/GQ359643/GQ359727

Table 3. Sample information for the snake samples used in calibration points in the phylogeogrphic chapter.

NTF-3		
Species	**locality**	**samples code and GeneBank Acc.**
Naja naja	Sri lanka	WW580
Naja naja	Nepal	WW595
Naja nivea	S. Africa	WW1482
Naja nivea	S.Africa	WW1295
Naja nivea	S. Africa	WW1906
Naja kaouthia	Burma	WW839
Naja kaouthia	THIALAN	WW585
Naja nigricollis	Mbwewe-Tanzania	WW1405
Naja nigricollis	Angola-WW	WW3160
Naja nigricollis	Cameron	WW1074
Bothrops asper	Belize	WW273
Bothrops asper	Mexico	WW875
Bothrops asper	Costa Rica	WW1318
Bothrops asper		EU390910
Porthidium acrosae	unknwn	WW1017
Porthidium acrosae	Ecuador	WW750
porthidium lancbergi rozei	venezuela	WW787
Daboia siamensis		EU390916
Daboia siamensis		WWA22
Macrovipera mauritania	Morocco	WW1642
2031 Echis pyramidium	Yemen	WW3031
2032 Echis pyramidium	Yemen	WW2032
2055 Echis pyramidium	Saudi Arabia	WW2055
1612_E_carinatus_sochureki	Sharjah-UAE	WW1612
1613_E_carinatus_sochureki	Sharjah-UAE	WW1613
2029 Echis coloratus	Yemen	WW2029
2030 Echis coloratus	Yemen	WW2030
1669 Echis omanensis	Fujairah-UAE	WW1669
1670 Echis omanensis	Fujairah-UAE	WW1670
1689 Echis omanensis	Ar Rustaq, Oman	WW1689
1690 Echis omanensis	Ar Rustaq, Oman	WW1690
1691 Echis omanensis	Ar Rustaq, Oman	WW1691
1692 Echis coloratus	Thumrait, Oman	WW1692
2056 Echis pyramidium	Saudi Arabia	WW2056

Table 4. Sample information for the snake samples used in calibration points in the phylogeogrphic chapter.

R35

species	locality	samples codes and GeneBank Acc.
Naja kaouthia		JN703083
Naja kaouthia	Thailand	WW585
Naja naja	Nepal	WW595
Naja nivea	unknown	WW1295
Naja nigricollis	Cameron	WW1074
Porthidium acrosae	Ecuador	WW750
porthidium lancbergi rozei	Venezuela	WW787
Pothrops asper		JN703092
Bothrops asper	Costa Rica	WW1318
Daboia siamensis		WW-A22
Daboia siamensis (M. mauritanica)	Morocco	WW1642
1669 Echis omanensis	Fujiarah-UAE	WW1669
1670 Echis omanensis	Fujiarah-UAE	WW1670
1692 Echis coloratus	Thumrait-Oman	WW1692
2029 Echis coloratus	Yemen	WW2029
2031 Echis pyramidium	Yemen	WW2031
2032 Echis pyramidium	Yemen	WW2032
2055 Echis pyramidium	Saudi Arabia	WW2055
1612 E carinatus sockureki	sharjah-UAE	WW1612
1613 E.carinatus sochureki	sharjah-UAE	WW1613
1689_E_omanensis	Ar Rustaq,Oman	WW1689
1690_E_omanensis	Ar Rustaq,Oman	WW1690
1691_E_omanensis	Ar Rustaq,Oman	WW1691
2030_E_coloratus	Yemen	WW2030
2056_E_pyramidium	Saudi Arabia	WW2056

Appendix 2

Table 1. Sample information for the *Acanthodctylus* sequences used in the species delimitation chapter.

Yes= amplified, No = did not amplify

SPECIES	LOCALITY		Lap code	cytb	16s	12s	R35	NTF-3
Acanthodactylus opheodurus	east	Eastern Saudi Arabia	2998	yes	yes	yes	yes	yes
A. opheodurus	east	Eastern Saudi Arabia	2999	yes	yes	yes	yes	yes
A.opheodurus	east	Eastern Saudi Arabia	3018	yes	yes	yes	no	yes
A.opheodurus	east	Eastern Saudi Arabia	3016	yes	yes	yes	yes	yes
A. opheodurus	mahazah	western Saudi Arabia	3000	yes	yes	yes	yes	yes
A. opheodurus	mahazah	western Saudi Arabia	3001	yes	yes	yes	yes	yes
A. opheodurus	Almihd	western Saudi Arabia	3002	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3003	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3017	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3019	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3020	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3021	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3022	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3023	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3024	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3025	yes	yes	yes	no	no
A.opheodurus	Almihd	western Saudi Arabia	3027	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3028	yes	yes	yes	yes	yes
A.opheodurus	ibex res.	central Saudi Arabia	3005	yes	yes	yes	yes	yes
A.opheodurus	ibex res.	central Saudi Arabia	3004	yes	yes	yes	yes	yes
A.opheodurus	ibex res.	central Saudi Arabia	3029	yes	yes	yes	yes	yes
A.opheodurus	ibex res.	central Saudi Arabia	3030	yes	yes	yes	no	no
A.opheodurus	*tathleeth*	southern Saudi Arabia	3462	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3464	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3465	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3493	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3494	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3509	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3510	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3531	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3533	yes	yes	yes	yes	yes

A.opheodurus	*tareep*	southern Saudi Arabia	3535	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3537	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3538	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3542	yes	yes	yes	yes	no
A.opheodurus	*tareep*	southern Saudi Arabia	3543	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3551	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3562	yes	yes	yes	no	no
A.opheodurus	*Deba*	Northwest Saudi Arabia	3563	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3564	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3567	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3568	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3569	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3570	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3571	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3572	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3573	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3574	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3575	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3576	yes	yes	yes	yes	yes
A.opheodurus	*Deba*	Northwest Saudi Arabia	3577	yes	yes	yes	no	no
A.opheodurus	*zihd mountains*	Northwest Saudi Arabia	3581	yes	yes	yes	yes	yes
A.opheodurus	*zihd mountains*	Northwest Saudi Arabia	3600	yes	yes	yes	yes	yes
A.opheodurus	*zihd mountains*	Northwest Saudi Arabia	3601	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3651	no	no	no	no	no
A.opheodurus	*Dhofar*	Oman	3666	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3667	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3672	yes	yes	yes	no	yes
A.opheodurus	*Dhofar*	Oman	3674	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3675	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3677	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3682	yes	yes	yes	yes	yes

A.opheodurus	*Dhofar*	Oman	3684	yes	yes	yes	yes	yes
A.opheodurus	*Dhofar*	Oman	3685	yes	yes	yes	yes	yes
A.opheodurus	madinah	western Saudi Arabia	3031	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3032	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3033	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3034	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3036	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3038	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3039	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3041	yes	yes	yes	yes	yes
A.opheodurus	mahazah	western Saudi Arabia	3042	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3044	yes	yes	yes	yes	yes
A.opheodurus	Almihd	western Saudi Arabia	3045	yes	yes	yes	yes	yes
A.opheodurus	ibex res.	central Saudi Arabia	3047	yes	yes	yes	no	yes
A.opheodurus	*Riyadh*	southern Saudi Arabia	3488	yes	yes	yes	yes	yes
A.opheodurus	*tathleeth*	southern Saudi Arabia	3491	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3528	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3532	yes	yes	yes	yes	yes
A.opheodurus	*tareep*	southern Saudi Arabia	3536	yes	yes	yes	yes	yes
A.boskianus	*Deba*	Northwest Saudi Arabia	3565	yes	yes	yes	yes	yes
A.boskianus	*Deba*	Northwest Saudi Arabia	3566	yes	yes	yes	yes	yes
A.boskianus	*zihd mountains*	Northwest Saudi Arabia	3592	yes	yes	yes	yes	yes
A.boskianus	*zihd mountains*	Northwest Saudi Arabia	3594	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3613	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3614	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3615	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3616	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3617	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3618	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3619	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3620	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3621	yes	yes	yes	yes	yes
A.boskianus	*Tabouk*	Northwest Saudi Arabia	3622	yes	yes	yes	yes	yes

A.boskianus	east	Eastern Saudi Arabia	3040	yes	yes	yes	yes	yes
A.boskianus	Almihd	western Saudi Aabia	3037	yes	yes	yes	yes	yes
A.boskianus	Almihd	western Saudi Arabia	3043	yes	yes	yes	yes	yes
A.boskianus	ibex res.	central Saudi Arabia	3035	yes	yes	no	yes	yes
A.boskianus	ibex res.	central Saudi Arabia	3046	yes	yes	yes	yes	yes
A.boskianus	ibex res.	central Saudi Arabia	3048	yes	yes	yes	yes	yes
A.boskianus	ibex res.	central Saudi Arabia	3049	yes	yes	yes	yes	yes
A.boskianus	*tathleeth*	southern Saudi Arabia	3492	yes	yes	yes	yes	yes
A.boskianus	*tathleeth*	southern Saudi Arabia	3516	yes	yes	yes	yes	yes
A.boskianus	*tareep*	southern Saudi Arabia	3530	yes	yes	yes	yes	yes
A.boskianus	*tathleeth*	southern Saudi Arabia	3511	yes	yes	yes	yes	yes
A.boskianus	*tareep*	southern Saudi Arabia	3553	yes	yes	yes	yes	yes
A.boskianus	*tareep*	southern Saudi Arabia	3550	yes	yes	yes	yes	yes
A.boskianus	*tareep*	southern Saudi Arabia	3534	yes	yes	yes	yes	yes
A.boskianus	*Dhofar*	Oman	3673	yes	yes	yes	yes	yes
A.boskianus	*Dhofar*	Oman	3671	yes	yes	yes	yes	yes
A.schmidti	Al-sharjah	UAE	4027	yes	yes	yes	yes	yes
A.schmidti	albatayeh-Sharjah	UAE	3707	yes	yes	yes	yes	yes
A.schmidti	east	Eastern Saudi Arabia	3091	yes	yes	yes	yes	yes
A.schmidti	east	Eastern Saudi Arabia	3092	yes	yes	yes	yes	yes

Appendix 3

Table 1. Uncorrected genetic P-distance (12S) for *Acanthodactylus opheodurus, A. boskianus* and *A. schmidti*.

	1	2	3
(1) *Acanthodactylus opheodurus*			
(2) *Acanthodactylus boskianus*	0.038		
(3) *Acanthodactylus schmidti*	0.040	0.054	

Table 2. Uncorrected genetic P-distance (16S) for *Acanthodactylus opheodurus, A. boskianus*, and *A. schmidti*.

	1	2	3
(1) *Acanthodactylus boskianus*			
(2) *Acanthodactylus opheodurus*	0.065		
(3) *Acanthodactylus schmidti*	0.094	0.091	

Table 3. Uncorrected genetic P-distance (cytb) for *Acanthodactylus opheodurus, A. boskianus* and *A. schmidti.*

	1	2	3
(1) *Acanthodactylus opheodurus*			
(2) *Acanthodactylus boskianus*	0.144		
(3) *Acanthodactylus schmidti*	0.156	0.162	

Table 4. Uncorrected genetic P-distance (12S) for main populations of *A. boskianus* by region.

(1) Western Saudi Arabia
(2) Eastern Saudi Arabia
(3) Central Saudi Arabia
(4) Southern Saudi Arabia (Tathleeth)
(5) Southern Saudi Arabia (Tareep)
(6) Northwest Saudi Arabia (Deba)
(7) Northwest Saudi Arabia (Zihd Mountains)
(8) Northwest Saudi Arabia (Tabouk)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.056								
(3)	0.058	0.002							
(4)	0.007	0.050	0.052						
(5)	0.008	0.051	0.054	0.002					
(6)	0.036	0.046	0.049	0.030	0.031				
(7)	0.030	0.046	0.049	0.023	0.025	0.007			
(8)	0.030	0.047	0.049	0.024	0.025	0.006	0.000		
(9)	0.020	0.043	0.045	0.020	0.022	0.030	0.023	0.024	

Table 5. Uncorrected genetic P-distance (12S) for main populations of *A. opheodurus* by region.

(1) Eastern Saudi Arabia
(2) Western Saudi Arabia (MAHAZA)
(3) Western Saudi Arabia (ALMIHD)
(4) Central Saudi Arabia
(5) Southern Saudi Arabia (Tathleeth)
(6) Southern Saudi Arabia (Tareep)
(7) Northwest Saudi Arabia (Deba)
(8) Northwest Saudi Arabia (Zihd Mountains)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.010								
(3)	0.008	0.007							
(4)	0.000	0.010	0.008						
(5)	0.008	0.013	0.013	0.008					
(6)	0.009	0.013	0.013	0.009	0.011				
(7)	0.010	0.006	0.009	0.010	0.013	0.014			
(8)	0.002	0.012	0.009	0.002	0.010	0.010	0.012		
(9)	0.014	0.018	0.017	0.014	0.016	0.011	0.019	0.015	

Table 6. Uncorrected genetic P-distance (16S) for main populations of *A. opheodurus* by region.

(1) Western Saudi Arabia (Mahaza)
(2) Eastern Saudi Arabia
(3) Western Saudi Arabia (Almihd)
(4) Central Saudi Arabia
(5) Southern Saudi Arabia (Tathleeth)
(6) Southern Saudi Arabia (Tareep)
(7) Northwest Saudi Arabia (Deba)
(8) Northwest Saudi Arabia (Zihd Mountains)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.012								
(3)	0.006	0.013							
(4)	0.012	0.007	0.013						
(5)	0.018	0.021	0.016	0.017					
(6)	0.020	0.023	0.018	0.020	0.012				
(7)	0.009	0.020	0.013	0.019	0.022	0.027			
(8)	0.013	0.016	0.010	0.015	0.017	0.019	0.017		
(9)	0.018	0.020	0.015	0.017	0.011	0.013	0.025	0.013	

Table 7 . Uncorrected genetic P-distance (16S) for main populations of *A. boskianus* by region.

(1) Northwest Saudi Arabia (Zihd Mountains)
(2) Southern Saudi Arabia (Tathleeth)
(3) Central Saudi Arabia
(4) Western Saudi Arabia
(5) Eastern Saudi Arabia
(6) Southern Saudi Arabia (Tareep)
(7) Northwest Saudi Arabia (Deba)
(8) Northwest Saudi Arabia (Tabouk)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.040								
(3)	0.039	0.045							
(4)	0.038	0.024	0.041						
(5)	0.038	0.044	0.001	0.040					
(6)	0.037	0.003	0.046	0.021	0.044				
(7)	0.007	0.048	0.046	0.041	0.045	0.044			
(8)	0.002	0.041	0.039	0.038	0.038	0.038	0.008		
(9)	0.027	0.026	0.045	0.027	0.044	0.024	0.030	0.027	

Table 8. Uncorrected genetic P-distance (cytb) for main populations of *A. boskianus* by region.

(1) Central Saudi Arabia
(2) Western Saudi Arabia
(3) Eastern Saudi Arabia
(4) Southern Saudi Arabia (Tathleeth)
(5) Southern Saudi Arabia (Tareep)
(6) Northwest Saudi Arabia (Deba)
(7) Northwest Saudi Arabia (Zihd Mountains)
(8) Northwest Saudi Arabia (Tabouk)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.127								
(3)	0.007	0.130							
(4)	0.122	0.044	0.114						
(5)	0.121	0.048	0.112	0.009					
(6)	0.121	0.081	0.106	0.080	0.082				
(7)	0.122	0.085	0.108	0.082	0.084	0.019			
(8)	0.127	0.086	0.111	0.085	0.086	0.016	0.007		
(9)	0.134	0.082	0.125	0.079	0.083	0.090	0.092	0.093	

Table 9 . Uncorrected genetic P-distance (cytb) for main populations of *A. opheodurus* by region.

(1) Eastern Saudi Arabia
(2) Western Saudi Arabia （Mahaza)
(3) Western Saudi Arabia （Almihd)
(4) Central Saudi Arabia
(5) Southern Saudi Arabia (Tathleeth)
(6) Southern Saudi Arabia (Tareep)
(7) Northwest Saudi Arabia (Deba)
(8) Northwest Saudi Arabia (Zihd Mountains)
(9) Southern Oman

	1	2	3	4	5	6	7	8	9
(1)									
(2)	0.042								
(3)	0.031	0.021							
(4)	0.002	0.043	0.031						
(5)	0.062	0.067	0.062	0.063					
(6)	0.053	0.047	0.048	0.054	0.054				
(7)	0.042	0.032	0.031	0.043	0.065	0.051			
(8)	0.025	0.040	0.031	0.025	0.058	0.047	0.040		
(9)	0.054	0.055	0.053	0.054	0.064	0.055	0.054	0.047	

Appendix 4

Table 1. GeneBank Accession numbers of 12S gene sequences of *Acanthodactylus boskianus* used in the species delimitation chapter.

GeneBank Accession	locality
HM778097	Siwa-western Egypt
HM778098	Siwa-western Egypt
HM778096	Siwa-western Egypt
HM778095	Siwa-western Egypt
HM769296	Sharm El-Sheikh-Egypt
HM769295	Sharm El-Sheikh-Egypt
HM769294	Sharm El-Sheikh-Egypt
HM769293	Sinai-Egypt
HM769292	Sinai-Egypt
HM769291	Sinai-Egypt
HM769288	Sinai-Egypt
GU225704	Abu-Rawash-Egypt
HM596598	Abu-Rawash-Egypt
HM596597	Abu-Rawash-Egypt
AF197499	Eastern Arabia-UAE
AF197483	Morocco
HM778094	Siwa-western Egypt
HM778093	Siwa-western Egypt
HM778092	Siwa-western Egypt
HM778091	Siwa-western Egypt
HM769301	Siwa-western Egypt
HM769300	Siwa-western Egypt
HM769299	Siwa-western Egypt
HM769298	Siwa-western Egypt
HM769297	Siwa-western Egypt
HM769290	Sinai-Egypt
HM769289	Sinai-Egypt
HM749623	Sinai-Egypt
HM749622	Sinai-Egypt

HM749621	Sinai-Egypt
HM749620	Sinai-Egypt
HM749619	Sinai-Egypt
GU225706	Siwa-western Egypt
GU225705	Sinai-Egypt
GU433282	Israel
GU433281	Israel
GU433280	Israel
GU433279	Israel
GU433278	Israel
GU433277	Israel
GU433276	Israel
GU433275	Israel
GU433274	Israel
HM596596	Abu-Rawash-Egypt
HM596595	Abu-Rawash-Egypt
AY633417	Morocco
AY633416	Morocco

Table 2. GeneBank Accession numbers of 12S and cytb gene sequences of *Acanthodactylus boskianus* used in the species delimitation chapter.

GeneBank Accession	Locality
HM778106.1-cytb	Sharm El-Sheikh-Egypt
HM778105.1-cytb	Sharm El-Sheikh-Egypt
HM778104.1-cytb	Sinai-Egypt
HM778103.1-cytb	Sinai-Egypt
HM778108.1-cytb	Siwa-western Egypt
HM778107-cytb	Siwa-western Egypt
HM749619-12S	Sinai-Egypt
HM749620-12S	Sinai-Egypt
HM769295-12S	Sharm El-Sheikh-Egypt
HM769294-12S	Sharm El-Sheikh-Egypt
HM769301-12S	Siwa-western Egypt
HM769300-12S	Siwa-western Egypt

Table 3. GeneBank Accession numbers of 12S and 16S gene sequences of representative species from *Acanthodactylus genus* used in the species delimitation chapter.

species	12s	16s
A.erythrurus lineomaculatus	AY633418	AY633442.1
A.bedriagae	AY633414	AY633438.1
A. blanfordi	AF197481.1	AF197482.1
A.beershebensis	JF912449.1	JF912448.1
A.boskianus	GU433277.1	GU433290.1
A.boskianus	GU433276.1	GU433289.1
A.boskianus	GU433275.1	GU433288.1
A.boskianus	GU433274.1	GU433287.1
A.boskianus	AY633417.1	AY633441.1
A.boskianus	AY633416.1	AY633440.1
A.erythrurus atlanticus	AY633412.1	AY633436.1
A.erythrurus belli	AY633411.1	AY633432.1
A.erythrurus belli	AY633410.1	AY633433.1
A.blanci	AY633407.1	AY633431.1
A.blanci	AY633406.1	AY633430.1
A.erythrurus erythrurus	AY633399.1	AY633423.1
A.erythrurus erythrurus	AY633398.1	AY633422.1
A.maculatus	EU086880.1	EU086907.1
A.maculatus	EU086879.1	EU086906.1
A.pardalis	EU086878.1	EU086905.1
A.pardalis	EU086877.1	EU086904.1
A.busacki	EU086876.1	EU086903.1
A.busacki	EU086869.1	EU086896.1
A.mechriguensis	EU086866.1	EU086893.1
A.mechriguensis	EU086865.1	EU086892.1
A.opheodurus	AF197501.1	AF197502.2
A.boskianus-arabia	AF197499.1	AF197500.1
A.masirae	AF197503.1	AF197504.1
A.tristrami	AF197493.1	AF197494.1
A.orientalis	AF197491.1	AF197492.1
A.longipes	AF197489.1	AF197490.1
A.scutellatus	GU225707	-
A.scutellatus	AF197487.1	AF197488.1
A.aureus	AF197485.1	AF197486.1
A.boskianus-morocco	AF197483.1	AF197484.1
A.gongrorhynchatus	AF080341.1	AF080343.1
A.schmidti	AF080375.1	AF080377.1
A.cantoris	AF080344.1	AF080346.1

A.boskianus-Egypt	HM778098	-
A.boskianus-Egypt	HM778097	-
A.boskianus-Egypt	HM778096	-
A.boskianus-Egypt	HM778095	-
A.boskianus-Egypt	HM769296	-
A.boskianus-Egypt	HM769295	-
A.boskianus-Egypt	HM769294	-
A.boskianus-Egypt	HM769293	-
A.boskianus-Egypt	GU225706	-
A.boskianus-Egypt	GU225705	-
A. schreiberi-Israel	-	JX847526
A. schreiberi-Israel	-	JX847525
A. schreiberi-Israel	-	JX847524
A. schreiberi-Israel	-	JX847523
A. schreiberi-Israel	-	JX847508
A. schreiberi-Israel	-	JX847507
A. schreiberi-Israel	-	JX847506

Appendix 5

Field work

Two field work trips were carried out to the Arabian Peninsula during 2010 and 2011; they were six and eight weeks in duration for first and second trips, respectively. The first trip targeted four regions of the Arabian Peninsula: eastern, central, and two localities (Mahazah and Almihd) in western Saudi Arabia. The second trip was carried out in two localities (Tathleeth and Tareep) in southern Saudi Arabia, and three localities in north western Saudi Arabia (Deba, located on the Red Sea coast, the Zihd Mountains, and Tabouk), and northern and southern Oman. Lizard samples from the United Arab Emirates were kindly donated by, Dr. Wolfgang Wüster. (He provided them from Johannes Els, Breeding Center for Endangered Arabian Wildlife, Sharjah, UAE). Since this thesis is aimed at investigating the phylogeographical patterns and species delimitation of the Arabian Peninsula, the primary goal for these field trips was to collect samples of lizards that were found to be co-distributed across most field locations in the Peninsula. Thus, twelve species that have wide distribution throughout the Arabian Peninsula were identified. Other species have been collected, as well, but from single localities.

DNA samples of tissues from the tail tips for these lizards were preserved in tubes containing 95% alcohol. When live specimens of lizards were caught, tail tips were collected and the animals released again in the wild. In some case, live animals were collected in bags and deposited in the zoological department at King Saud University in Riyadh.

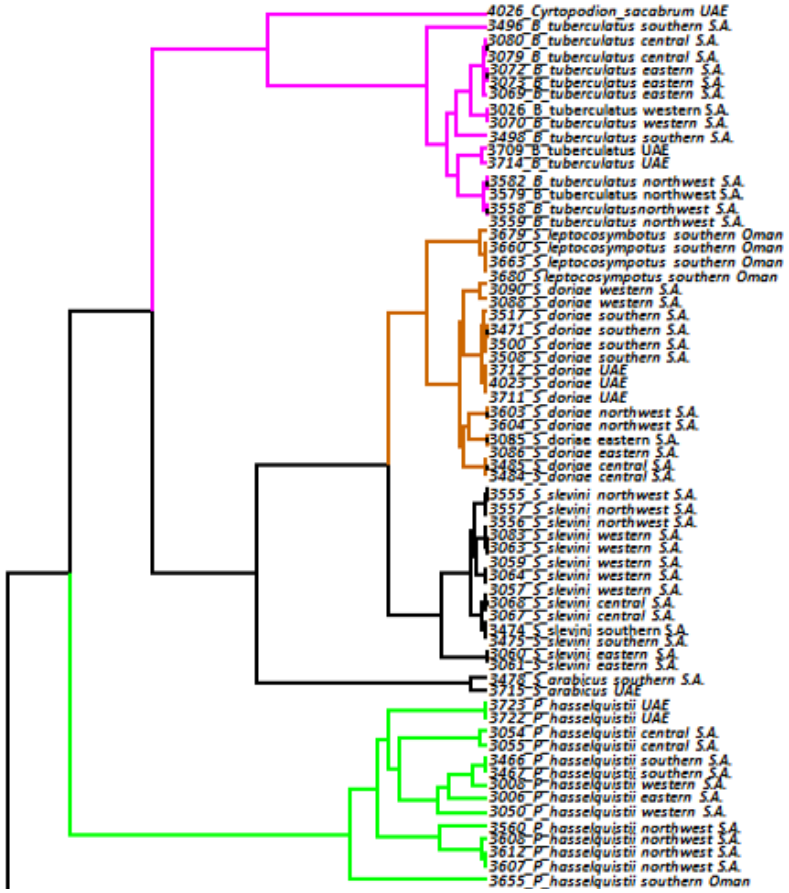
Since the target lizards of this study have different biological activities, they were divided into two groups. The nocturnal group included all gecko species and the diurnal activity group included the lacertid and agamid species. The primary and simplest method to collect these species was to catch them by hand. They were sought out during the night for nocturnal activity species, which could be found by following their tracks and catching them by hand using a night lamp. These species can be found between the rocks in canyons (e.g. *Ptyodactylus hasselquistii*), or under stones and large wooden panels (*Bunopus tuberculatus*), or under small shrubs [e.g. *Stenodactylus slevini* and *Stenodactylus doriae*]. The latter is found to prefer sandy habitats while the former prefers gravel plains. Diurnal

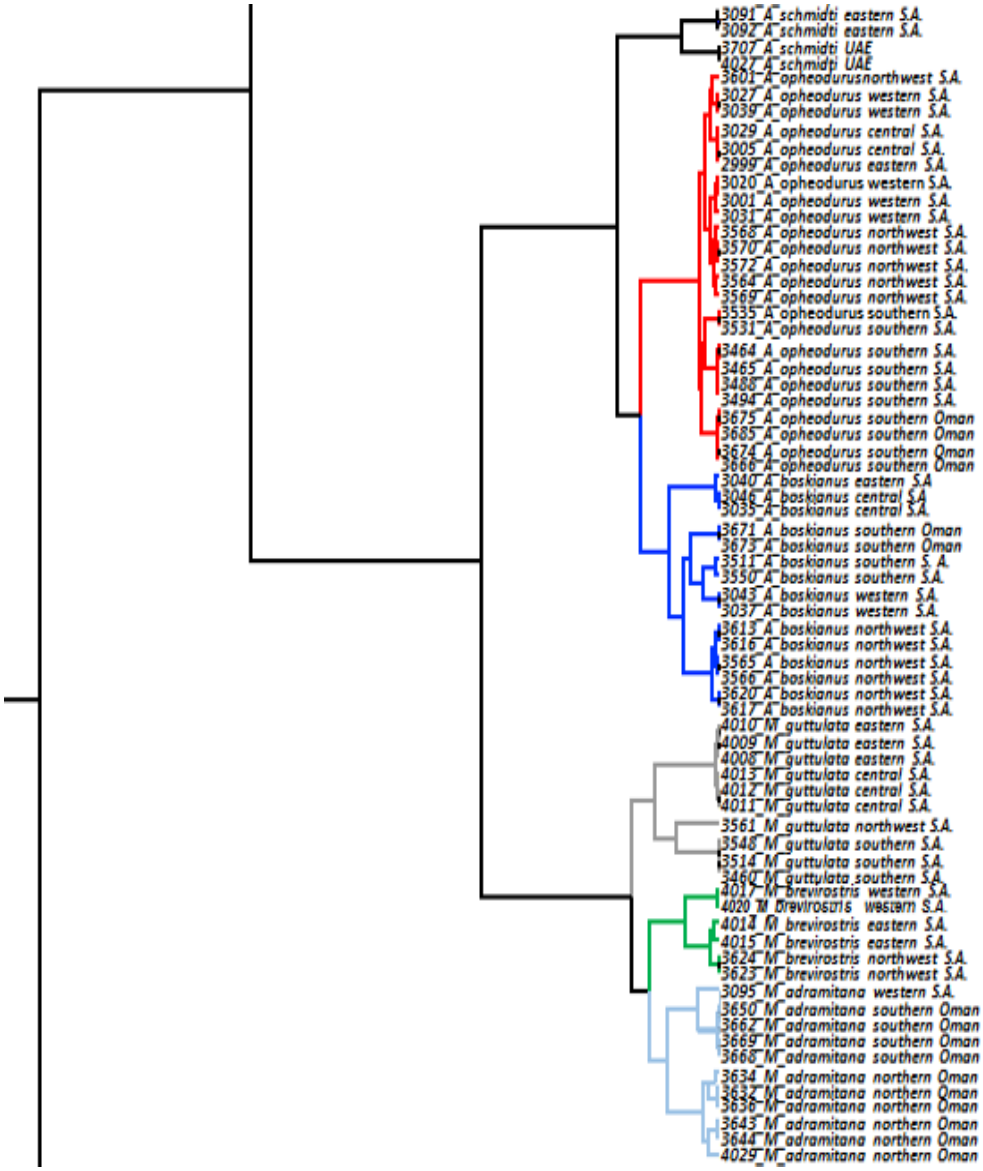
species (Lacertid group, e.g. *Acanthodactylus and Mesalina*) were collected during the morning until 10 or 11 A.M. by hand; they were found near hiding places such as small and large shrubs, or in their burrows. These species ran very fast when they sensed something around them. The strategy to collect these lizards was based on walking by foot through suitable habitats. At mid-day, especially when the sun was vertical and the temperature was extremely high (sometimes reaching 50°C in northern Oman), was the best time to look for the Agamid species (e.g. *Pseudotrapelus sinaitus*). The activity of these species usually started at this time, and they were found in sunny patches at the top of the mountains, or on rocks in high places.

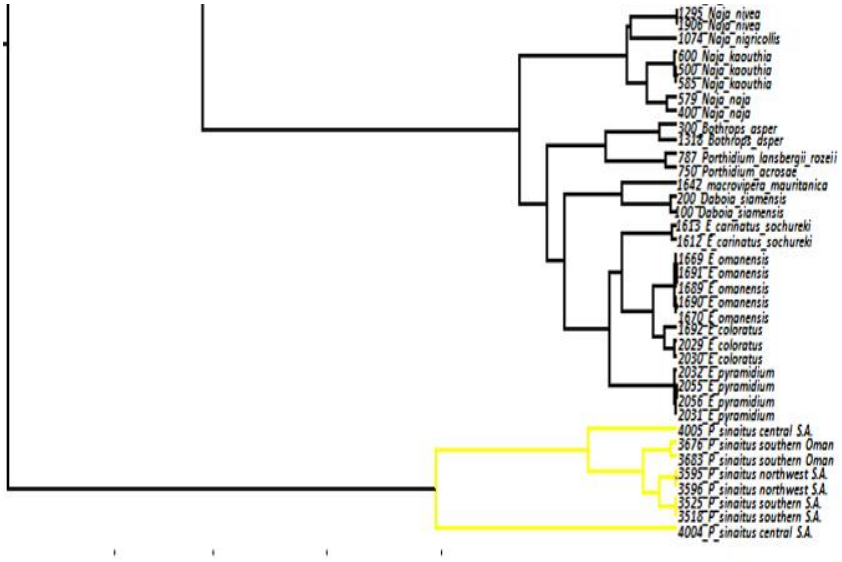
The diversity of species differed between localities, depending on the preferred habitat for a species. For example, during the second trip, northern Oman was a poor locality for sample collection, despite its rocky habitat, and the species found there were *Bunopus spatalarus hajernsis*, and *Mesalina adramitana.* One reason for this was perhaps that the timing of sample collection, in July 2011, was when the temperature was too hot and humid. Another reason was perhaps that the natural area was very difficult to move easily in. The southern and north western regions of Saudi Arabia and southern Oman were more diversified than the central and eastern areas of Saudi Arabia, which is consistent with the recent status reports for Arabian lizard diversity (Cox et al., 2012).

Appendix 6

Fig. 1. mtDNA tree derived from STAR BEAST (*BEAST) analysis used in the phylogeographic chapter. The tree has been divided into sub-trees, due to its large size.

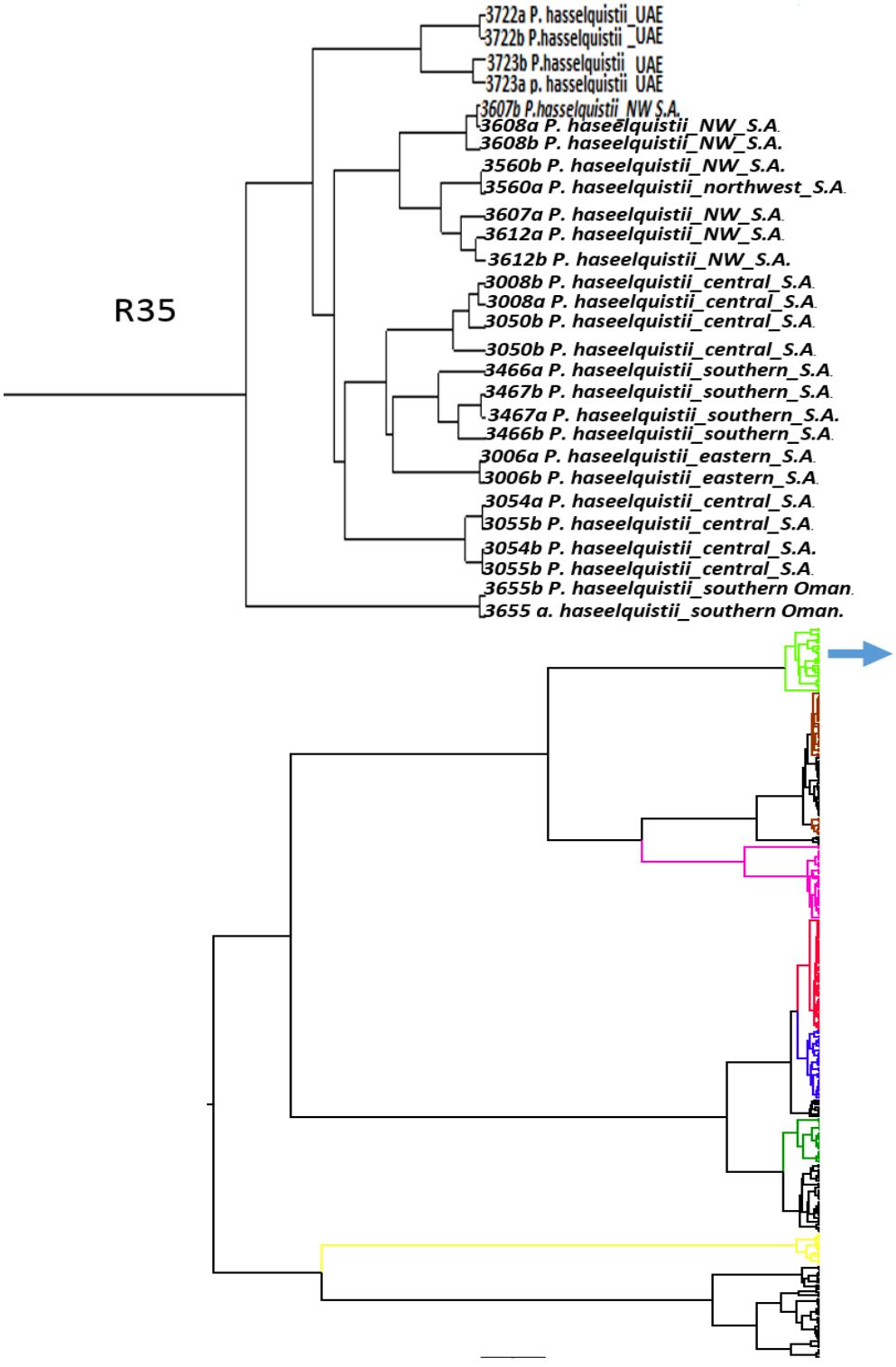


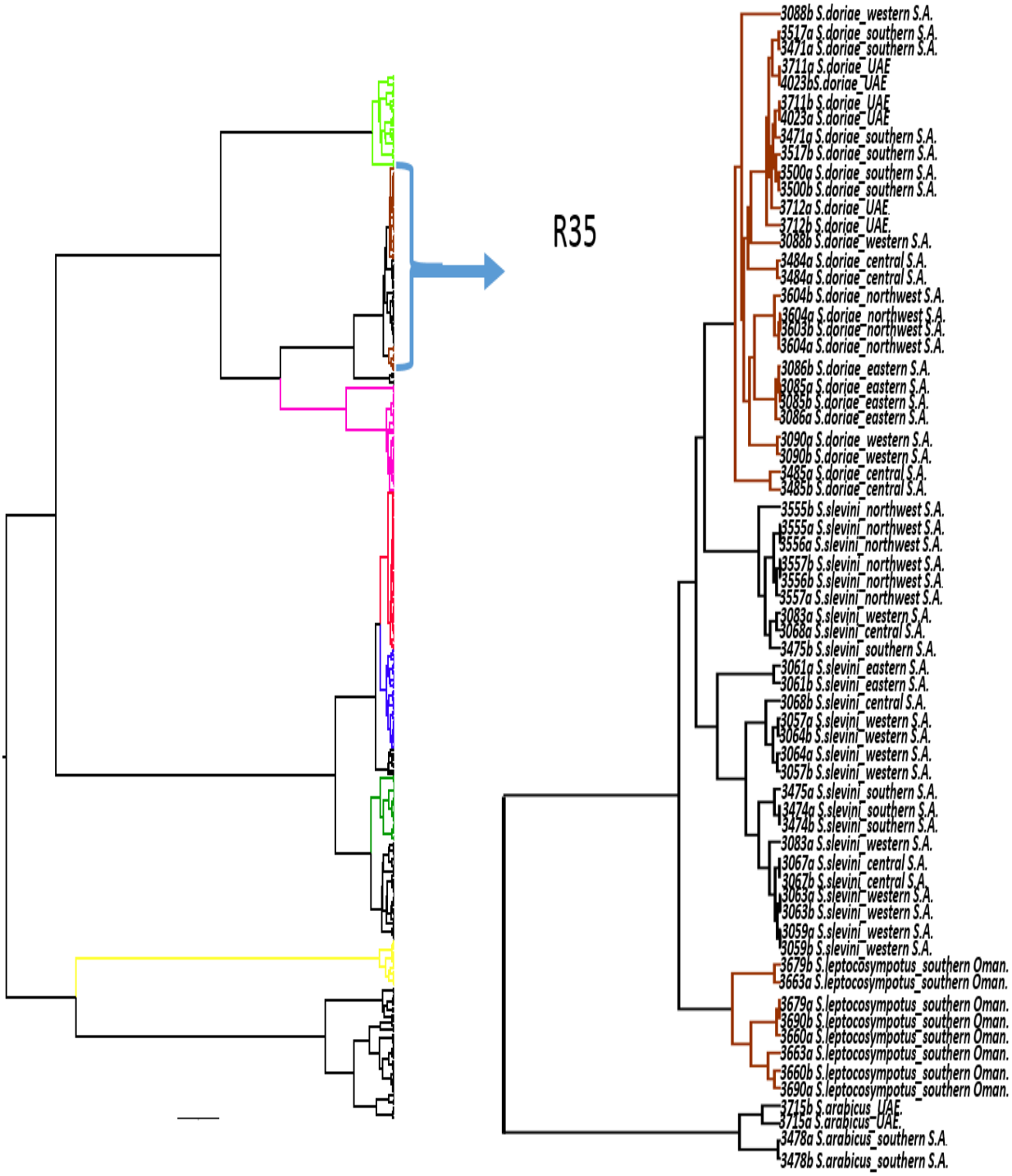

3091_A_schmidti eastern S.A.
3092_A_schmidti eastern S.A.
3707_A_schmidti UAE
4027_A_schmidti UAE
3601_A_opheodurusnorthwest S.A.
3027_A_opheodurus western S.A.
3039_A_opheodurus western S.A.
3029_A_opheodurus central S.A.
3005_A_opheodurus central S.A.
2999_A_opheodurus eastern S.A.
3020_A_opheodurus western S.A.
3001_A_opheodurus western S.A.
3031_A_opheodurus western S.A.
3568_A_opheodurus northwest S.A.
3570_A_opheodurus northwest S.A.
3572_A_opheodurus northwest S.A.
3564_A_opheodurus northwest S.A.
3569_A_opheodurus northwest S.A.
3535_A_opheodurus southern S.A.
3531_A_opheodurus southern S.A.
3464_A_opheodurus southern S.A.
3465_A_opheodurus southern S.A.
3488_A_opheodurus southern S.A.
3494_A_opheodurus southern S.A.
3675_A_opheodurus southern Oman
3685_A_opheodurus southern Oman
3674_A_opheodurus southern Oman
3666_A_opheodurus southern Oman
3040_A_boskianus eastern S.A.
3046_A_boskianus central S.A.
3035_A_boskianus central S.A.
3671_A_boskianus southern Oman
3673_A_boskianus southern Oman
3511_A_boskianus southern S. A.
3550_A_boskianus southern S.A.
3043_A_boskianus western S.A.
3037_A_boskianus western S.A.
3613_A_boskianus northwest S.A.
3616_A_boskianus northwest S.A.
3565_A_boskianus northwest S.A.
3566_A_boskianus northwest S.A.
3620_A_boskianus northwest S.A.
3617_A_boskianus northwest S.A.
4010_M_guttulata eastern S.A.
4009_M_guttulata eastern S.A.
4008_M_guttulata eastern S.A.
4013_M_guttulata central S.A.
4012_M_guttulata central S.A.
4011_M_guttulata central S.A.
3561_M_guttulata northwest S.A.
3548_M_guttulata southern S.A.
3514_M_guttulata southern S.A.
3460_M_guttulata southern S.A.
4017_M_brevirostris western S.A.
4020_M_brevirostris western S.A.
4014_M_brevirostris eastern S.A.
4015_M_brevirostris eastern S.A.
3624_M_brevirostris northwest S.A.
3625_M_brevirostris northwest S.A.
3095_M_adramitana western S.A.
3650_M_adramitana southern Oman
3667_M_adramitana southern Oman
3669_M_adramitana southern Oman
3668_M_adramitana southern Oman
3634_M_adramitana northern Oman
3637_M_adramitana northern Oman
3638_M_adramitana northern Oman
3643_M_adramitana northern Oman
3644_M_adramitana northern Oman
4029_M_adramitana northern Oman


1295_Naja_nivea
1906_Naja_nivea
1074_Naja_nigricollis
600_Naja_kaouthia
500_Naja_kaouthia
585_Naja_kaouthia
579_Naja_naja
400_Naja_naja
300_Bothrops_asper
1318_Bothrops_asper
787_Porthidium_lansbergii_rozeii
750_Porthidium_acrosae
1642_macrovipera_mauritanica
200_Daboia_siamensis
100_Daboia_siamensis
1613_E_carinatus_sochureki
1612_E_carinatus_sochureki
1669_E_omanensis
1691_E_omanensis
1689_E_omanensis
1690_E_omanensis
1670_E_omanensis
1692_E_coloratus
2029_E_coloratus
2030_E_coloratus
2032_E_pyramidium
2055_E_pyramidium
2056_E_pyramidium
2031_E_pyramidium
4005_P_sinaitus central S.A.
3676_P_sinaitus southern Oman
3683_P_sinaitus southern Oman
3595_P_sinaitus northwest S.A.
3596_P_sinaitus northwest S.A.
3525_P_sinaitus southern S.A.
3518_P_sinaitus southern S.A.
4004_P_sinaitus central S.A.

Appendix 7

Fig. 1. R35 gene tree derived from (*BEAST) analysis for the pgylogeographic chapter. The tree has been divided into sub-trees, due to its large size.




R35
3088b S.doriae_western S.A.
3517a S.doriae_southern S.A.
3471a S.doriae_southern S.A.
3711a S.doriae_UAE
4023bS.doriae_UAE
3711b S.doriae_UAE
4023a S.doriae_UAE
3471a S.doriae_southern S.A.
3517b S.doriae_southern S.A.
3500a S.doriae_southern S.A.
3500b S.doriae_southern S.A.
3712a S.doriae_UAE.
3712b S.doriae_UAE.
3088b S.doriae_western S.A.
3484a S.doriae_central S.A.
3484a S.doriae_central S.A.
3604b S.doriae_northwest S.A.
3604a S.doriae_northwest S.A.
3603b S.doriae_northwest S.A.
3604a S.doriae_northwest S.A.
3086b S.doriae_eastern S.A.
3085a S.doriae_eastern S.A.
3085b S.doriae_eastern S.A.
3086a S.doriae_eastern S.A.
3090a S.doriae_western S.A.
3090b S.doriae_western S.A.
3485a S.doriae_central S.A.
3485b S.doriae_central S.A.
3555b S.slevini_northwest S.A.
3555a S.slevini_northwest S.A.
3556a S.slevini_northwest S.A.
3557b S.slevini_northwest S.A.
3556b S.slevini_northwest S.A.
3557a S.slevini_northwest S.A.
3083a S.slevini_western S.A.
3068a S.slevini_central S.A.
3475b S.slevini_southern S.A.
3061a S.slevini_eastern S.A.
3061b S.slevini_eastern S.A.
3068b S.slevini_central S.A.
3057a S.slevini_western S.A.
3064b S.slevini_western S.A.
3064a S.slevini_western S.A.
3057b S.slevini_western S.A.
3475a S.slevini_southern S.A.
3474a S.slevini_southern S.A.
3474b S.slevini_southern S.A.
3083a S.slevini_western S.A.
3067a S.slevini_central S.A.
3067b S.slevini_central S.A.
3063a S.slevini_western S.A.
3063b S.slevini_western S.A.
3059a S.slevini_western S.A.
3059b S.slevini_western S.A.
3679b S.leptocosympotus_southern Oman.
3663a S.leptocosympotus_southern Oman.
3679a S.leptocosympotus_southern Oman.
3690b S.leptocosympotus_southern Oman.
3660a S.leptocosympotus_southern Oman.
3663a S.leptocosympotus_southern Oman.
3660b S.leptocosympotus_southern Oman.
3690a S.leptocosympotus_southern Oman.
3715b S.arabicus_UAE.
3715a S.arabicus_UAE.
3478a S.arabicus_southern S.A.
3478b S.arabicus_southern S.A.


R35
4026a S. scabrum_UAE
4026b S. scabrum_UAE
3579b B. tuberculatus_northwest S.A.
3582b B. tuberculatus_northwest S.A.
3579a B. tuberculatus_northwest S.A.
3582a B. tuberculatus_northwest S.A.
3558a B. tuberculatus_northwest S.A.
3559b B. tuberculatus_northwest S.A.
3559a B. tuberculatus_northwest S.A.
3558b B. tuberculatus_northwest S.A.
3714b B. tuberculatus_UAE
3714a B. tuberculatus_UAE
3709b B. tuberculatus_UAE
3080b B. tuberculatus_central S.A.
3709a B. tuberculatus_UAE
3498b B. tuberculatus_southern S.A.
3496a B. tuberculatus_southern S.A.
3496b B. tuberculatus_southern S.A.
3498a B. tuberculatus_southern S.A.
3072a B. tuberculatus_eastern S.A.
3080a B. tuberculatus_central S.A.
3072a B. tuberculatus_eastern S.A.
3079b B. tuberculatus_central S.A.
3079a B. tuberculatus_central S.A.
3073a B. tuberculatus_eastern S.A.
3069a B. tuberculatus_eastern S.A.
3069a B. tuberculatus_eastern S.A.
3070a B. tuberculatus_western S.A.
3070b B. tuberculatus_western S.A.
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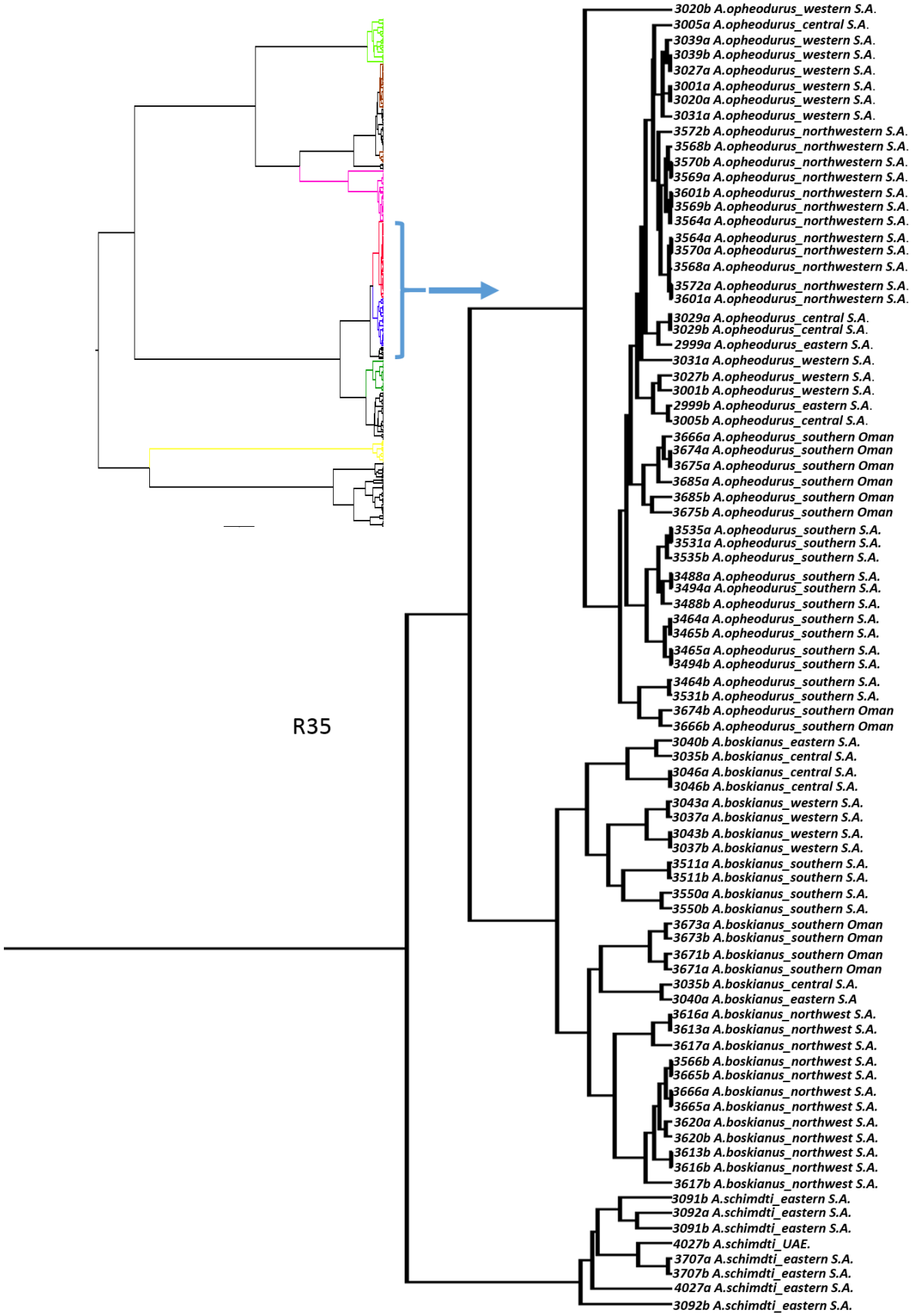
3026b B. tuberculatus_western S.A.
3073b B. tuberculatus_eastern S.A.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3027a A.opheodurus_western S.A.
3001a A.opheodurus_western S.A.
3020a A.opheodurus_western S.A.
3031a A.opheodurus_western S.A.
3572b A.opheodurus_northwestern S.A.
3568b A.opheodurus_northwestern S.A.
3570b A.opheodurus_northwestern S.A.
3569a A.opheodurus_northwestern S.A.
3601b A.opheodurus_northwestern S.A.
3569b A.opheodurus_northwestern S.A.
3564a A.opheodurus_northwestern S.A.
3564a A.opheodurus_northwestern S.A.
3570a A.opheodurus_northwestern S.A.
3568a A.opheodurus_northwestern S.A.
3572a A.opheodurus_northwestern S.A.
3601a A.opheodurus_northwestern S.A.
3029a A.opheodurus_central S.A.
3029b A.opheodurus_central S.A.
2999a A.opheodurus_eastern S.A.
3031a A.opheodurus_western S.A.
3027b A.opheodurus_western S.A.
3001b A.opheodurus_western S.A.
2999b A.opheodurus_eastern S.A.
3005b A.opheodurus_central S.A.
3666a A.opheodurus_southern Oman
3674a A.opheodurus_southern Oman
3675a A.opheodurus_southern Oman
3685a A.opheodurus_southern Oman
3685b A.opheodurus_southern Oman
3675b A.opheodurus_southern Oman
3535a A.opheodurus_southern S.A.
3531a A.opheodurus_southern S.A.
3535b A.opheodurus_southern S.A.
3488a A.opheodurus_southern S.A.
3494a A.opheodurus_southern S.A.
3488b A.opheodurus_southern S.A.
3464a A.opheodurus_southern S.A.
3465b A.opheodurus_southern S.A.
3465a A.opheodurus_southern S.A.
3494b A.opheodurus_southern S.A.
3464b A.opheodurus_southern S.A.
3531b A.opheodurus_southern S.A.
3674b A.opheodurus_southern Oman
3666b A.opheodurus_southern Oman
R35
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3035b A.boskianus_central S.A.
3046a A.boskianus_central S.A.
3046b A.boskianus_central S.A.
3043a A.boskianus_western S.A.
3037a A.boskianus_western S.A.
3043b A.boskianus_western S.A.
3037b A.boskianus_western S.A.
3511a A.boskianus_southern S.A.
3511b A.boskianus_southern S.A.
3550a A.boskianus_southern S.A.
3550b A.boskianus_southern S.A.
3673a A.boskianus_southern Oman
3673b A.boskianus_southern Oman
3671b A.boskianus_southern Oman
3671a A.boskianus_southern Oman
3035b A.boskianus_central S.A.
3040a A.boskianus_eastern S.A
3616a A.boskianus_northwest S.A.
3613a A.boskianus_northwest S.A.
3617a A.boskianus_northwest S.A.
3566b A.boskianus_northwest S.A.
3665b A.boskianus_northwest S.A.
3666a A.boskianus_northwest S.A.
3665a A.boskianus_northwest S.A.
3620a A.boskianus_northwest S.A.
3620b A.boskianus_northwest S.A.
3613b A.boskianus_northwest S.A.
3616b A.boskianus_northwest S.A.
3617b A.boskianus_northwest S.A.
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3092a A.schimdti_eastern S.A.
3091b A.schimdti_eastern S.A.
4027b A.schimdti_UAE.
3707a A.schimdti_eastern S.A.
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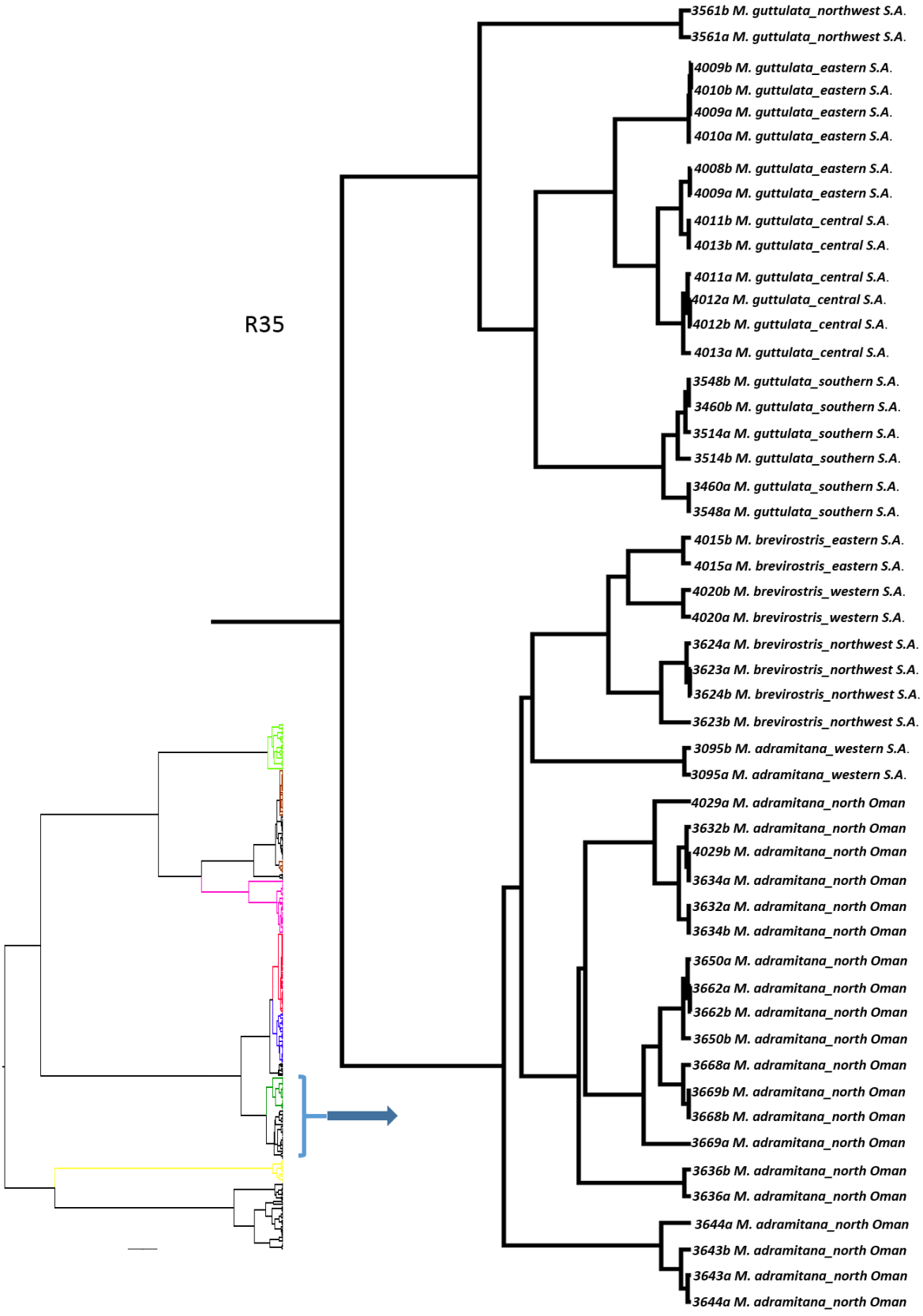
R35
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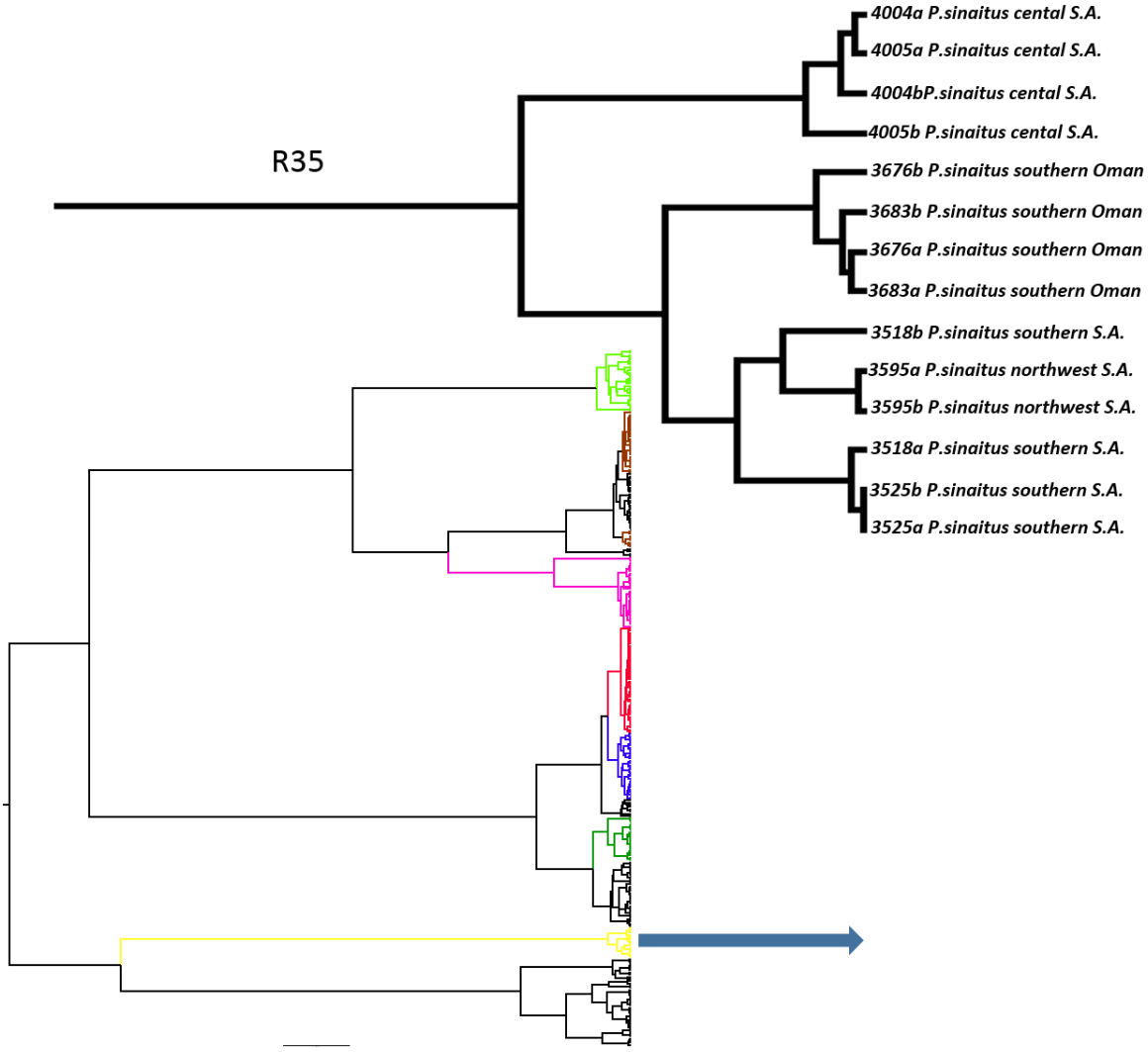
4009a M. guttulata_eastern S.A.
4010a M. guttulata_eastern S.A.
4008b M. guttulata_eastern S.A.
4009a M. guttulata_eastern S.A.
4011b M. guttulata_central S.A.
4013b M. guttulata_central S.A.
4011a M. guttulata_central S.A.
4012a M. guttulata_central S.A.
4012b M. guttulata_central S.A.
4013a M. guttulata_central S.A.
3548b M. guttulata_southern S.A.
3460b M. guttulata_southern S.A.
3514a M. guttulata_southern S.A.
3514b M. guttulata_southern S.A.
3460a M. guttulata_southern S.A.
3548a M. guttulata_southern S.A.
4015b M. brevirostris_eastern S.A.
4015a M. brevirostris_eastern S.A.
4020b M. brevirostris_western S.A.
4020a M. brevirostris_western S.A.
3624a M. brevirostris_northwest S.A.
3623a M. brevirostris_northwest S.A.
3624b M. brevirostris_northwest S.A.
3623b M. brevirostris_northwest S.A.
3095b M. adramitana_western S.A.
3095a M. adramitana_western S.A.
4029a M. adramitana_north Oman
3632b M. adramitana_north Oman
4029b M. adramitana_north Oman
3634a M. adramitana_north Oman
3632a M. adramitana_north Oman
3634b M. adramitana_north Oman
3650a M. adramitana_north Oman
3662a M. adramitana_north Oman
3662b M. adramitana_north Oman
3650b M. adramitana_north Oman
3668a M. adramitana_north Oman
3669b M. adramitana_north Oman
3668b M. adramitana_north Oman
3669a M. adramitana_north Oman
3636b M. adramitana_north Oman
3636a M. adramitana_north Oman
3644a M. adramitana_north Oman
3643b M. adramitana_north Oman
3643a M. adramitana_north Oman
3644a M. adramitana_north Oman


R35
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4005b P.sinaitus cental S.A.
3676b P.sinaitus southern Oman
3683b P.sinaitus southern Oman
3676a P.sinaitus southern Oman
3683a P.sinaitus southern Oman
3518b P.sinaitus southern S.A.
3595a P.sinaitus northwest S.A.
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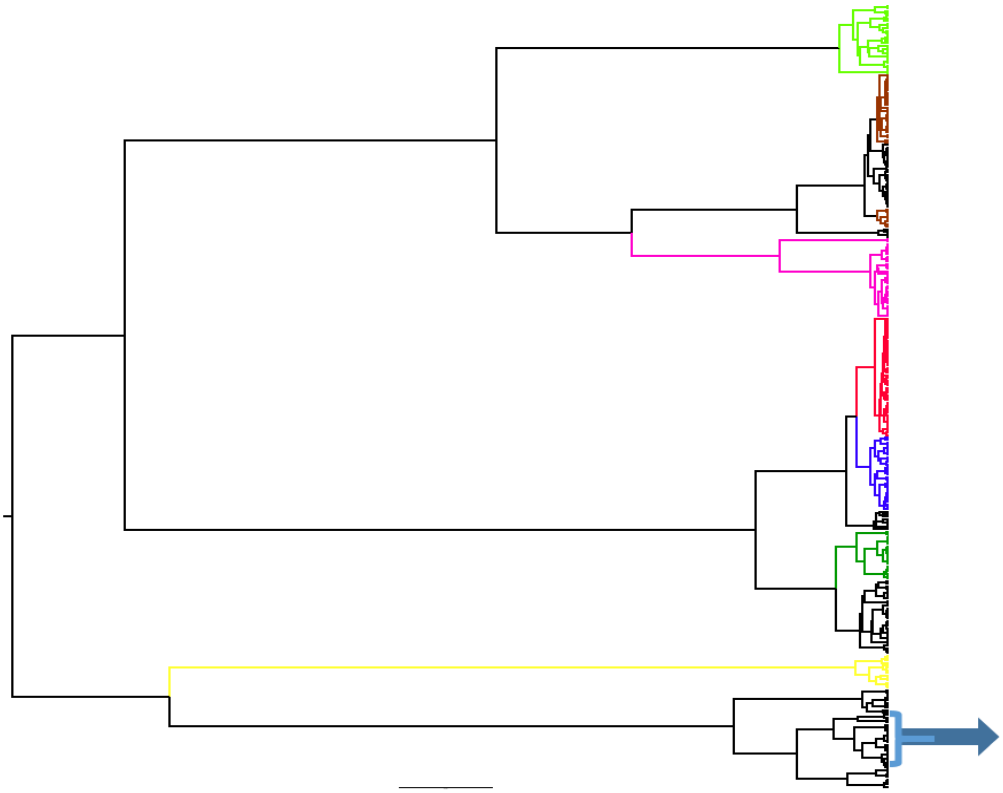
3518a P.sinaitus southern S.A.
3525b P.sinaitus southern S.A.
3525a P.sinaitus southern S.A.



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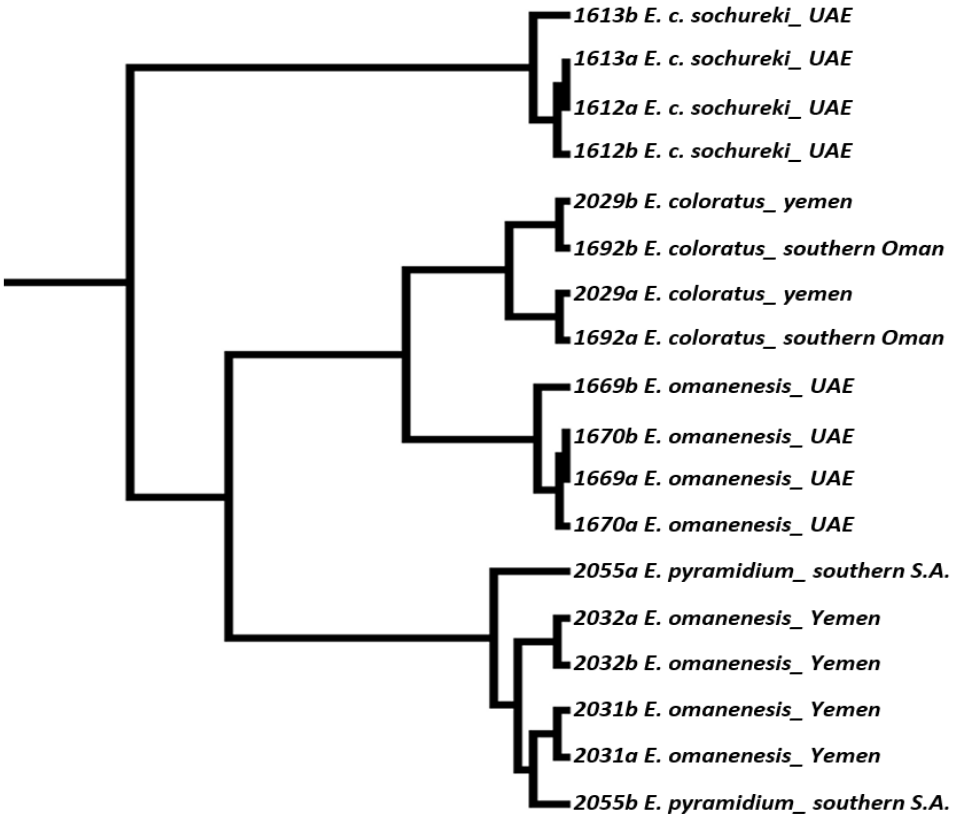
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1612a E. c. sochureki_ UAE
1612b E. c. sochureki_ UAE
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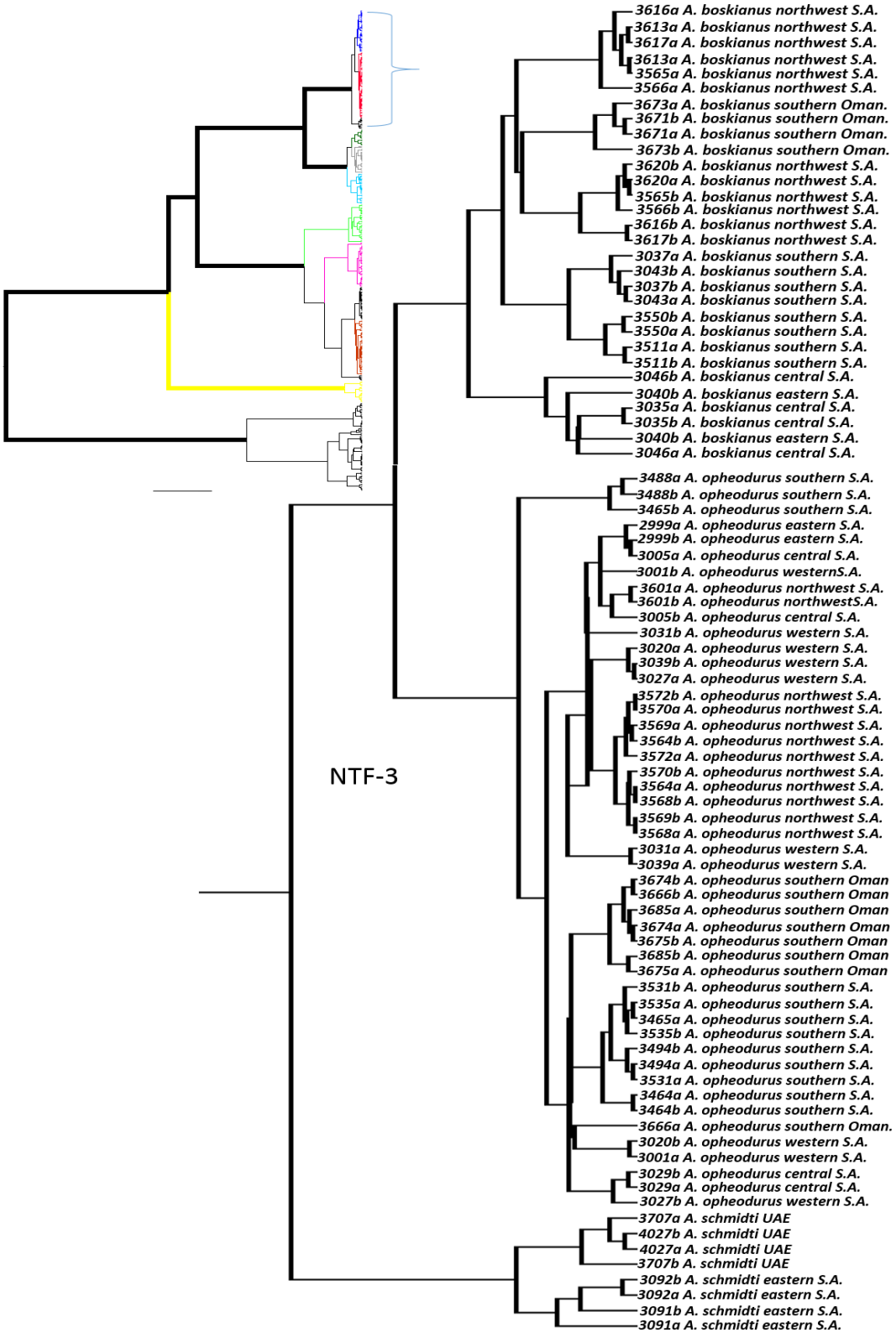
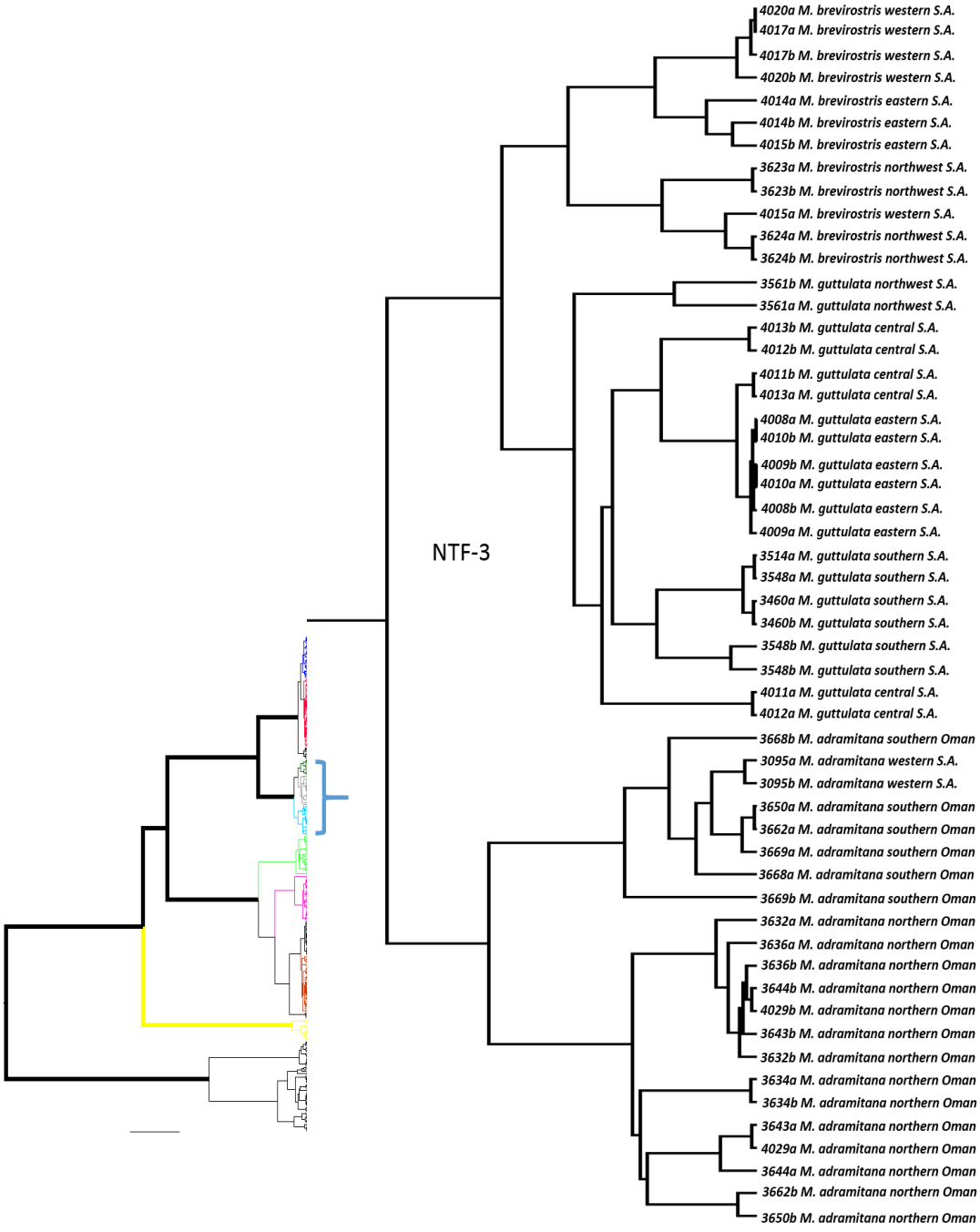
1692b E. coloratus_ southern Oman
2029a E. coloratus_ yemen
1692a E. coloratus_ southern Oman
1669b E. omanenesis_ UAE
1670b E. omanenesis_ UAE
1669a E. omanenesis_ UAE
1670a E. omanenesis_ UAE
2055a E. pyramidium_ southern S.A.
2032a E. omanenesis_ Yemen
2032b E. omanenesis_ Yemen
2031b E. omanenesis_ Yemen
2031a E. omanenesis_ Yemen
2055b E. pyramidium_ southern S.A.

Appendix 8

Fig. 1. NTF-3 gene tree derived from (*BEAST) analysis in the phylogeographic chapter. The tree has been deivided into sub-trees, due to its large size.

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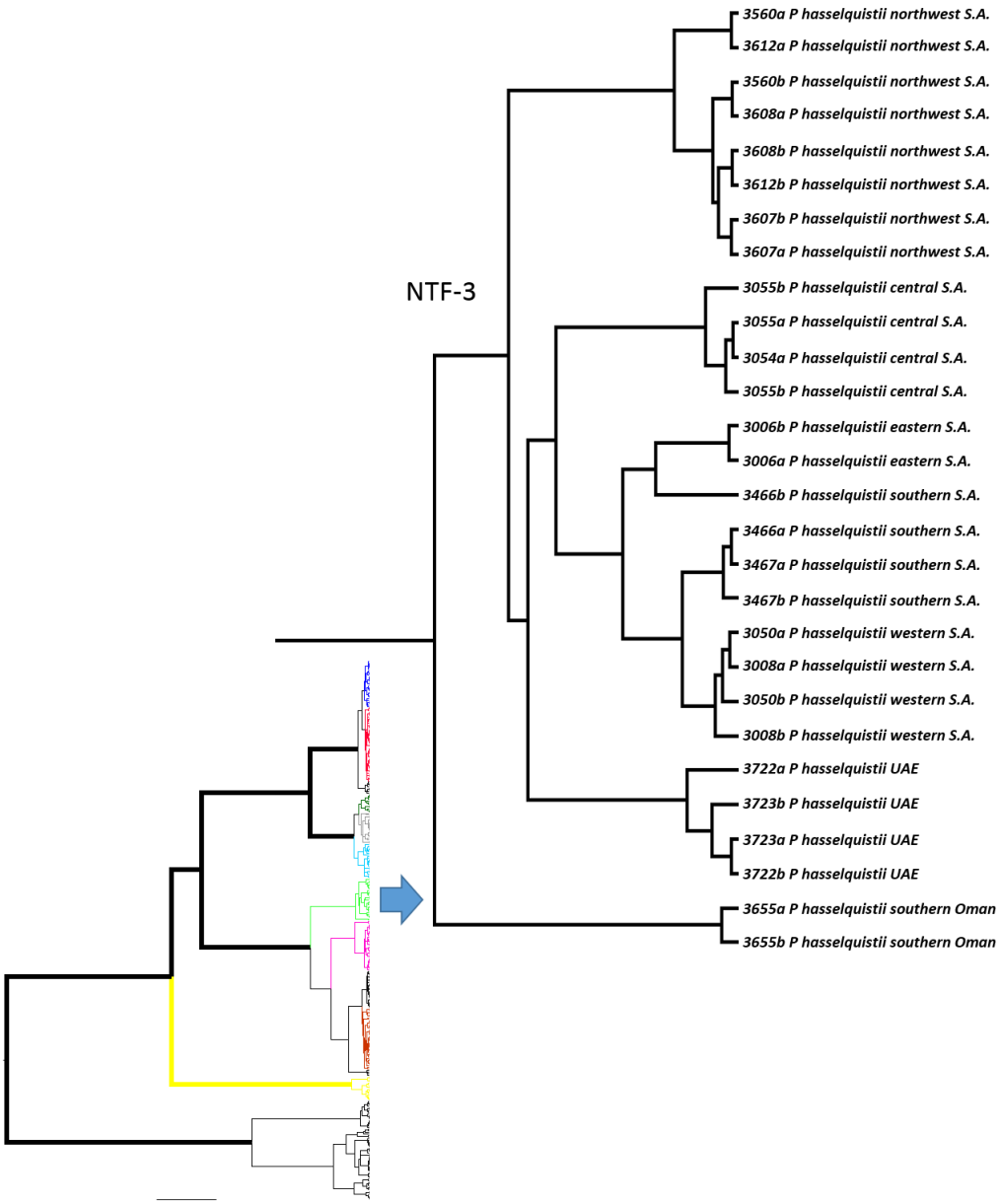
4017a M. brevirostris western S.A.
4017b M. brevirostris western S.A.
4020b M. brevirostris western S.A.
4014a M. brevirostris eastern S.A.
4014b M. brevirostris eastern S.A.
4015b M. brevirostris eastern S.A.
3623a M. brevirostris northwest S.A.
3623b M. brevirostris northwest S.A.
4015a M. brevirostris western S.A.
3624a M. brevirostris northwest S.A.
3624b M. brevirostris northwest S.A.
3561b M. guttulata northwest S.A.
3561a M. guttulata northwest S.A.
4013b M. guttulata central S.A.
4012b M. guttulata central S.A.
4011b M. guttulata central S.A.
4013a M. guttulata central S.A.
4008a M. guttulata eastern S.A.
4010b M. guttulata eastern S.A.
4009b M. guttulata eastern S.A.
4010a M. guttulata eastern S.A.
4008b M. guttulata eastern S.A.
4009a M. guttulata eastern S.A.
NTF-3
3514a M. guttulata southern S.A.
3548a M. guttulata southern S.A.
3460a M. guttulata southern S.A.
3460b M. guttulata southern S.A.
3548b M. guttulata southern S.A.
3548b M. guttulata southern S.A.
4011a M. guttulata central S.A.
4012a M. guttulata central S.A.
3668b M. adramitana southern Oman
3095a M. adramitana western S.A.
3095b M. adramitana western S.A.
3650a M. adramitana southern Oman
3662a M. adramitana southern Oman
3669a M. adramitana southern Oman
3668a M. adramitana southern Oman
3669b M. adramitana southern Oman
3632a M. adramitana northern Oman
3636a M. adramitana northern Oman
3636b M. adramitana northern Oman
3644b M. adramitana northern Oman
4029b M. adramitana northern Oman
3643b M. adramitana northern Oman
3632b M. adramitana northern Oman
3634a M. adramitana northern Oman
3634b M. adramitana northern Oman
3643a M. adramitana northern Oman
4029a M. adramitana northern Oman
3644a M. adramitana northern Oman
3662b M. adramitana northern Oman
3650b M. adramitana northern Oman


NTF-3
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3612a P hasselquistii northwest S.A.
3560b P hasselquistii northwest S.A.
3608a P hasselquistii northwest S.A.
3608b P hasselquistii northwest S.A.
3612b P hasselquistii northwest S.A.
3607b P hasselquistii northwest S.A.
3607a P hasselquistii northwest S.A.
3055b P hasselquistii central S.A.
3055a P hasselquistii central S.A.
3054a P hasselquistii central S.A.
3055b P hasselquistii central S.A.
3006b P hasselquistii eastern S.A.
3006a P hasselquistii eastern S.A.
3466b P hasselquistii southern S.A.
3466a P hasselquistii southern S.A.
3467a P hasselquistii southern S.A.
3467b P hasselquistii southern S.A.
3050a P hasselquistii western S.A.
3008a P hasselquistii western S.A.
3050b P hasselquistii western S.A.
3008b P hasselquistii western S.A.
3722a P hasselquistii UAE
3723b P hasselquistii UAE
3723a P hasselquistii UAE
3722b P hasselquistii UAE
3655a P hasselquistii southern Oman
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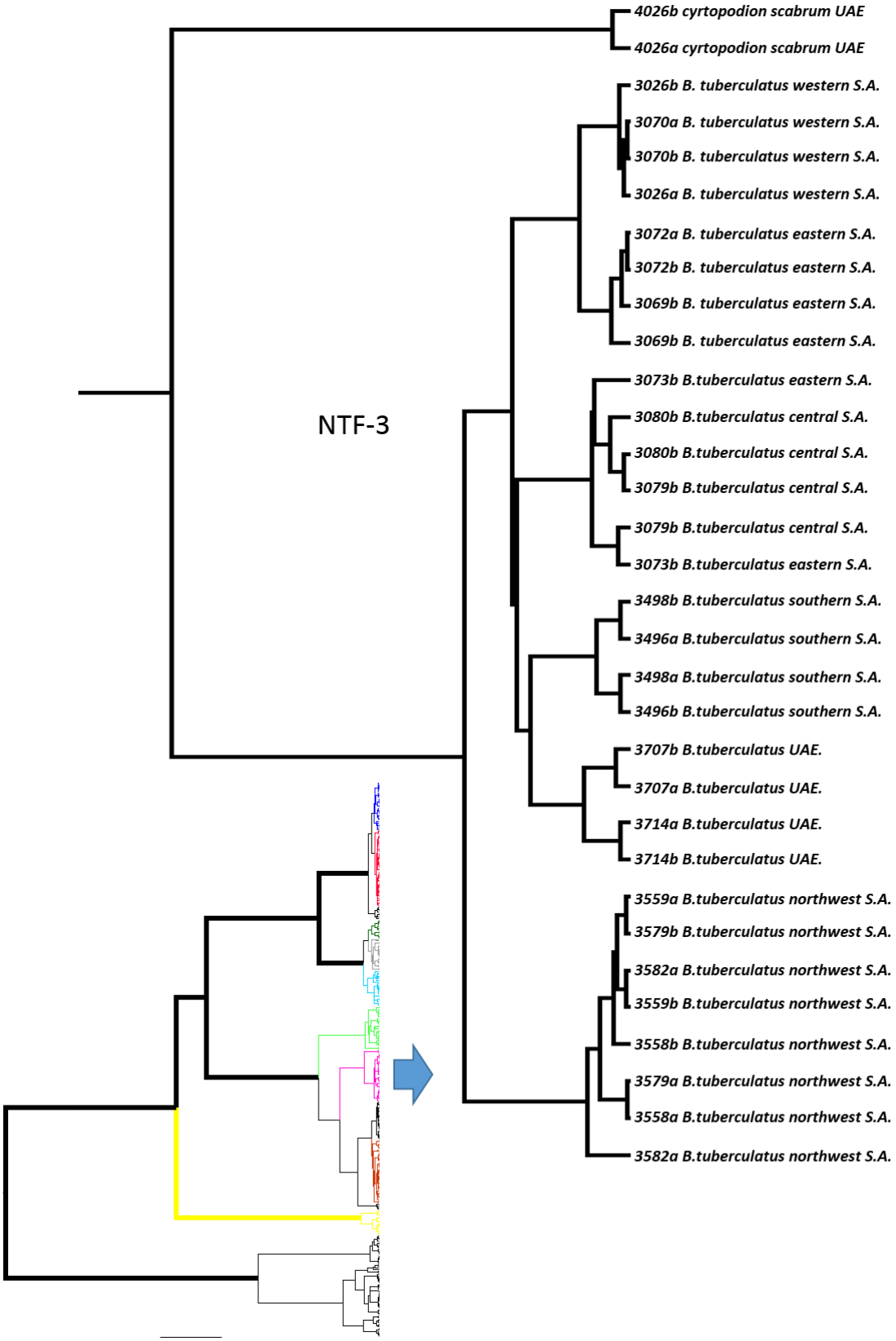
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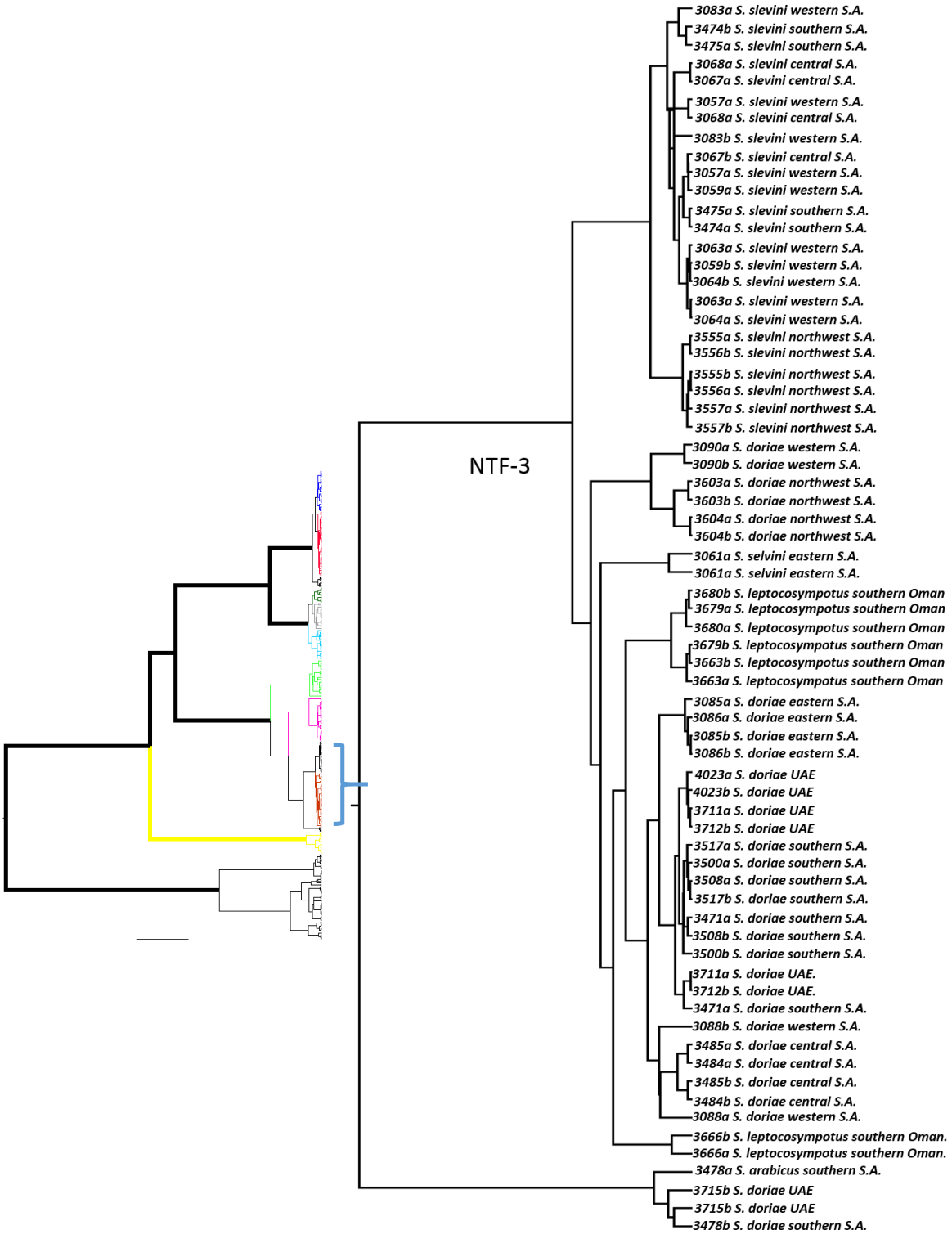
4026a cyrtopodion scabrum UAE
3026b B. tuberculatus western S.A.
3070a B. tuberculatus western S.A.
3070b B. tuberculatus western S.A.
3026a B. tuberculatus western S.A.
3072a B. tuberculatus eastern S.A.
3072b B. tuberculatus eastern S.A.
3069b B. tuberculatus eastern S.A.
3069b B. tuberculatus eastern S.A.
3073b B.tuberculatus eastern S.A.
3080b B.tuberculatus central S.A.
3080b B.tuberculatus central S.A.
3079b B.tuberculatus central S.A.
3079b B.tuberculatus central S.A.
3073b B.tuberculatus eastern S.A.
3498b B.tuberculatus southern S.A.
3496a B.tuberculatus southern S.A.
3498a B.tuberculatus southern S.A.
3496b B.tuberculatus southern S.A.
3707b B.tuberculatus UAE.
3707a B.tuberculatus UAE.
3714a B.tuberculatus UAE.
3714b B.tuberculatus UAE.
3559a B.tuberculatus northwest S.A.
3579b B.tuberculatus northwest S.A.
3582a B.tuberculatus northwest S.A.
3559b B.tuberculatus northwest S.A.
3558b B.tuberculatus northwest S.A.
3579a B.tuberculatus northwest S.A.
3558a B.tuberculatus northwest S.A.
3582a B.tuberculatus northwest S.A.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3474b S. slevini southern S.A.
3475a S. slevini southern S.A.
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3067a S. slevini central S.A.
3057a S. slevini western S.A.
3068a S. slevini central S.A.
3083b S. slevini western S.A.
3067b S. slevini central S.A.
3057a S. slevini western S.A.
3059a S. slevini western S.A.
3475a S. slevini southern S.A.
3474a S. slevini southern S.A.
3063a S. slevini western S.A.
3059b S. slevini western S.A.
3064b S. slevini western S.A.
3063a S. slevini western S.A.
3064a S. slevini western S.A.
3555a S. slevini northwest S.A.
3556b S. slevini northwest S.A.
3555b S. slevini northwest S.A.
3556a S. slevini northwest S.A.
3557a S. slevini northwest S.A.
3557b S. slevini northwest S.A.
3090a S. doriae western S.A.
3090b S. doriae western S.A.
3603a S. doriae northwest S.A.
3603b S. doriae northwest S.A.
3604a S. doriae northwest S.A.
3604b S. doriae northwest S.A.
3061a S. selvini eastern S.A.
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3679a S. leptocosympotus southern Oman
3680a S. leptocosympotus southern Oman
3679b S. leptocosympotus southern Oman
3663b S. leptocosympotus southern Oman
3663a S. leptocosympotus southern Oman
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3086a S. doriae eastern S.A.
3085b S. doriae eastern S.A.
3086b S. doriae eastern S.A.
4023a S. doriae UAE
4023b S. doriae UAE
3711a S. doriae UAE
3712b S. doriae UAE
3517a S. doriae southern S.A.
3500a S. doriae southern S.A.
3508a S. doriae southern S.A.
3517b S. doriae southern S.A.
3471a S. doriae southern S.A.
3508b S. doriae southern S.A.
3500b S. doriae southern S.A.
3711a S. doriae UAE.
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3471a S. doriae southern S.A.
3088b S. doriae western S.A.
3485a S. doriae central S.A.
3484a S. doriae central S.A.
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3484b S. doriae central S.A.
3088a S. doriae western S.A.
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3666a S. leptocosympotus southern Oman.
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3715b S. doriae UAE
3715b S. doriae UAE
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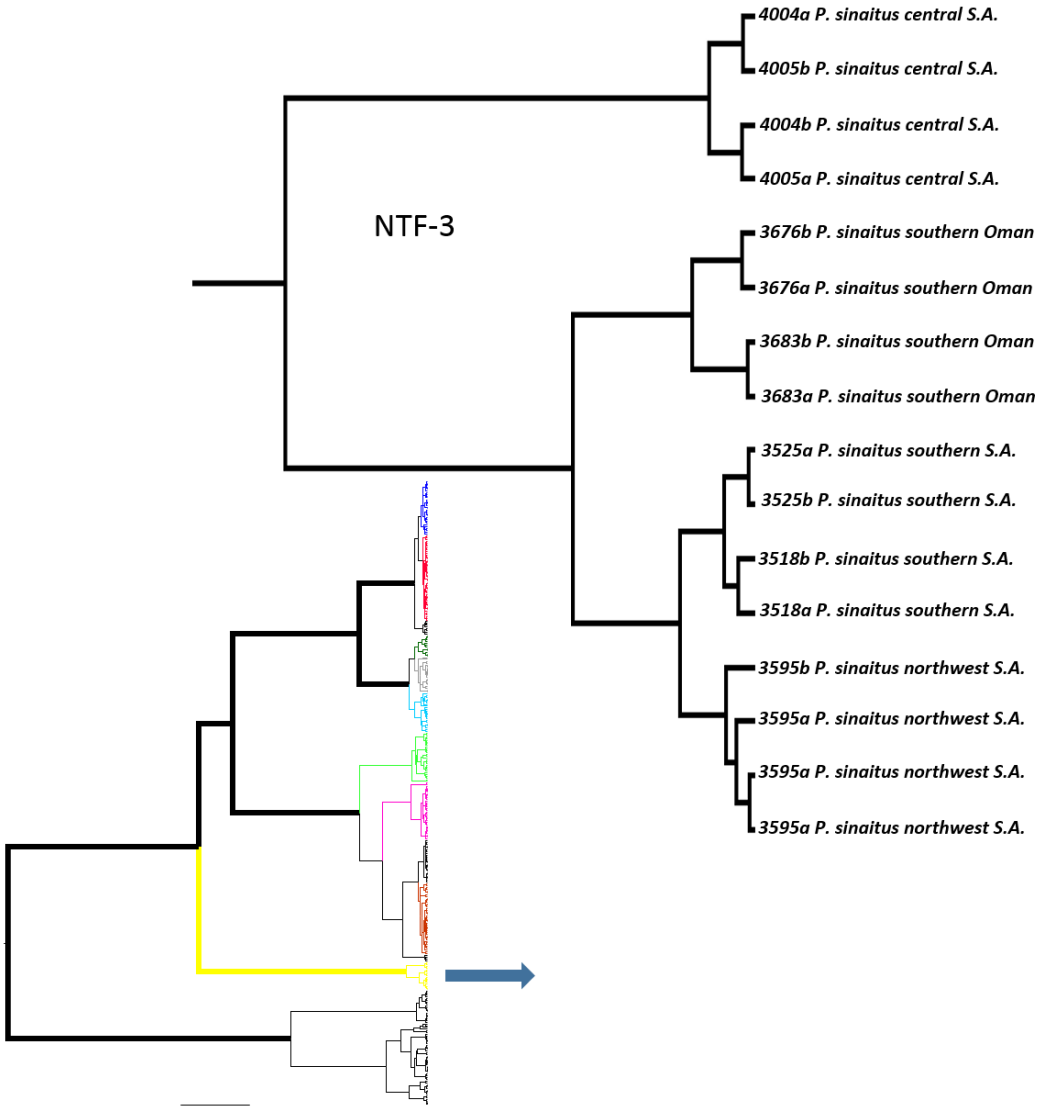
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3676a P. sinaitus southern Oman
3683b P. sinaitus southern Oman
3683a P. sinaitus southern Oman
3525a P. sinaitus southern S.A.
3525b P. sinaitus southern S.A.
3518b P. sinaitus southern S.A.
3518a P. sinaitus southern S.A.
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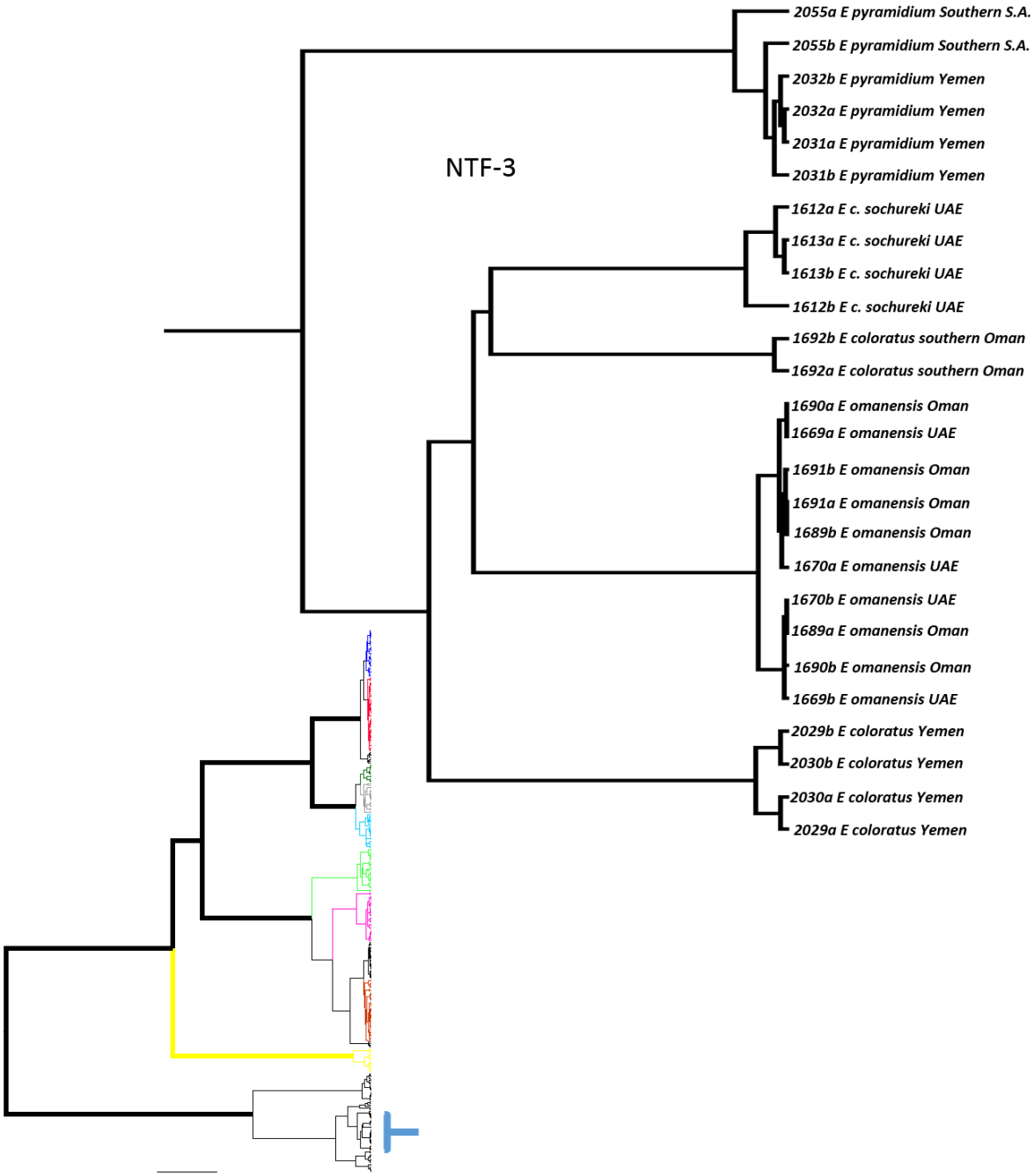
3595a P. sinaitus northwest S.A.
3595a P. sinaitus northwest S.A.
3595a P. sinaitus northwest S.A.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2032b E pyramidium Yemen
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2031a E pyramidium Yemen
2031b E pyramidium Yemen
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1613a E c. sochureki UAE
1613b E c. sochureki UAE
1612b E c. sochureki UAE
1692b E coloratus southern Oman
1692a E coloratus southern Oman
1690a E omanensis Oman
1669a E omanensis UAE
1691b E omanensis Oman
1691a E omanensis Oman
1689b E omanensis Oman
1670a E omanensis UAE
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1689a E omanensis Oman
1690b E omanensis Oman
1669b E omanensis UAE
2029b E coloratus Yemen
2030b E coloratus Yemen
2030a E coloratus Yemen
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