A MOLECULAR GENETIC PERSPECTIVE ON THE EVOLUTION OF *HYALOMMA* TICKS WITH EMPHASIS ON THE PHYLOGEOGRAPHY OF *H. TRUNCATUM*

Arthur Francis Sands

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Supervisors:

Prof Conrad A. Matthee

Prof Dmitry A. Apanaskevich

Dr Sonja Matthee

Faculty of Science

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DECLARATION

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Some of the contents contained in this thesis (Chapters 2-3) are taken directly from manuscripts submitted or drafted for publication in primary scientific literature. This has resulted in some overlap in content between chapters.

Arthur Francis Sands

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ABSTRACT

The tick genus *Hyalomma* is spread throughout the old world and species in this genus are vectors of a number of harmful pathogens. This makes them of key veterinary and medical importance, yet their systematics, and the factors giving rise to their diversity, remain largely unknown. As different species, and even different lineages, can vary in vector potential and level of acaricide resistance, it is thus of particular importance to resolve the systematics of the genus. To resolve the systematics of the genus and to obtain better insights into the mechanisms that play a role in tick evolution, the present study used both a phylogeographic and phylogenetic approach. The aims of the study were: 1) to provide a phylogeographic perspective for *H. truncatum* using the mtDNA COI and nDNA H3 and CRT gene regions, and 2) to create a comprehensive phylogeny for all the described extant *Hyalomma* species by using morphological and molecular data, derived from multiple nuclear and mitochondrial genetic markers (COI, 16S, 28S, ITS II and H3). By making use of phylogeographic networks, AMOVA analyses and Bayesian analyses, 186 *H. truncatum* specimens could be divided into two lineages across Africa (with a northern and southern clade). Historical demographic population analyses suggest that the two clades have different evolutionary histories, and support the notion that they have been isolated for a prolonged period time. On a regional scale, the northern clade showed higher levels of substructure with five COI phylogroups over the sampled region. The geographical positioning of these phylogroups aligns with those seen in multiple species of ungulates, primates and rodents, and it is argued that they have formed as a result of glacial cycles that caused shifts in the distribution of host species. The southern clade lacked substructure (probably due to the lack of geographic barriers to gene flow in the region). A COI sequence distance of 9.88% (SD ± 0.40%) and significant population differentiation at nuclear DNA level suggest that the two continental lineages probably represent separate species. To gain further insights into the status of *H. albiparmatum* and *H. nitidum*, and the species status of the two *H. truncatum* clades, a higher level systematic study was conducted on 82 specimens inclusive of all recognised *Hyalomma* species. Three nDNA markers (ITS II, 28S and H3), two mtDNA markers (COI and 16S) and 47 morphological characters were used to resolve relationships among *Hyalomma*

species. Parsimony and Bayesian analyses were performed and a dated phylogeny was also constructed using available fossil data. The data suggests that the first diversification within *Hyalomma* began around 36.25 Mya (95% HPD 34.75-39.80 Mya) and thereafter later divergences gave rise to five groups. Since *Hyalomma* have limited dispersal capabilities off the host, it is likely that mechanisms responsible for speciation events are more than likely coupled to vicariance events separating multiple hosts. Certainly several faunal exchanges between zoogeographic regions such as those associated with the African-Eurasian land bridge across the Arabian plate 16-20 Mya can be correlated to speciation in *Hyalomma*. Furthermore sea-level oscillations of the Mediterranean Sea degradation of the Paratethys Sea, and the development of the Himalayan mountainous belt and the East African Rift Valley have been proposed as mechanisms driving speciation in a number of host such as ungulates and rodents. Although these events likely played a role in early *Hyalomma* evolution, substantiating the mechanisms involved in the recent divergences of many extant *Hyalomma* species remains difficult. The latter is mainly due to the availability of limited knowledge on the exact ranges and host associations of several taxa. Nevertheless, the results documented in this thesis propose a number of changes to the current taxonomy of the genus: 1) Ticks recognized as *H. truncatum* comprised of two distinct species. Following conventional zoological nomenclature, we propose the southern clade of *H. truncatum* to likely represent a novel species (*H. species nova*). 2) *Hyalomma marginatum* and *H. turanicum* should be regarded as synonyms. 3) *Hyalomma nitidum* should be synonymized with the *H. truncatum* clade found in north Africa, while *H. albiparmatum* should remain a distinct species entity.

OPSOMMING

Die bosluis genus *Hyalomma* se verspreiding strek oor die hele Ou Wêreld en spesies in die genus is draers van 'n aantal skadelike patogene. Dus is hulle van veeartsenykundige en mediese belang, maar hul sistematiek asook die faktore wat aanleiding gee tot hul diversiteit bly grootliks onbekend. Aangesien verskillende spesies, en selfs verskillende genetiese groepe binne spesies, verskillende vlakke van akarisidiese weerstand en patogeen potensiaal kan hê, is dit van besondere belang om die sistematiek van die genus te bepaal. Ten einde die sistematiek van die genus te bepaal en beter insig tot die maganismes betrokke by bosluis evolusie te bekom, het die huidige studie beide ‘n filogeografiese en filogenetiese benadering gevolg. Die doelwitte van die studie was as volg: 1) om ‘n filogoegrafiese perspektief vir *H. truncatum* te verskaf deur gebruik te maak van die mitokondriale (mtDNA) COI en nukluêre (nDNA) H3 en CRT geen merkers, en 2) om ‘n omvattende filogenie vir alle beskryfde ekstante *Hyalomma* spesies daar te stel deur beide morfologiese en molekulêre data, afkomstig van verskeie nukluêre en mitokondriale genetiese merkers (COI, 16S, 28S, ITS II en H3), te gebruik. Filogoegrafiese netwerke, AMOVA ontledings, en die bou van 'n Bayesiaanse topologie, het aangedui dat 186 *H. truncatum* monsters, onderverdeel kan word in twee genetiese groepe binne Afrika (met ‘n noordelike en suidelike klade). Historiese demografiese bevolkings-resultate dui daarop dat die twee groepe se evolusionêre geskiedenis van mekaar verskil, wat moontlik ondersteunig verleen aan die idee dat hulle vir 'n lang tyd reeds van mekaar geïsoleer is. Op die streek vlak is ‘n hoër graad van substruktuur, met onderverdeling tot vyf filo-groepe regoor die studie gebied, binne die noordelike klade gevind. Die geografiese plasing van hierdie filogroepe stem ooreen met dié wat reeds in verskeie ander spesies van hoefdiere, primate en knaagdiere beskryf is. Dit word aangevoer dat hierdie patrone weens gletser siklusse, wat verskuiwings van gasheer spesies se verspreidings versoorsaak het, tot stand gebring is. Daarteen het die suidelike klade geen substruktuur getoon nie (waarskynlik as gevolg van die gebrek aan geografiese hindernisse tot geenvloei binne die verspreiding). ‘n COI volgorde afstand van 9,88% (SD% ± 0,40) gekoppel met beduidende populasie differensiasie op die nukluêre DNS vlak, stel voor dat die twee kontinentale

genetiese groepe heel moontlik as twee aparte spesies beskou kan word. Om verder insigte rondom die status van *H. albiparmatum* en *H. nitidum,* asook die spesie status van die twee genetiese groepe binne *H. truncatum*, te bekom, is 'n hoër-vlak sistematieke studie met 82 monsters van alle erkende *Hyalomma* spesies onderneem. Drie nDNA merkers (ITS II, 28S en H3), twee mtDNA merkers (COI en 16S) en 47 morfologiese karakters is gebruik om verwantskappe tussen *Hyalomma* spesies op te los. Parsimoniese en Bayesiaanse ontledings is uitgevoer en 'n gedateerde filogenie, wat gebruik maak van beskikbare fossiel data, is ook gebou. Die data dui daarop dat die eerste diversifikasie binne *Hyalomma* rondom 36.25 Mjg (miljoen jaar gelede) begin het (95% HPD 34.75-39.80 Mjg) en daarna met gereelde tussenposes plaasgevind het, en sodoende gelei het tot die ontstaan van vyf groepe. Aangesien *Hyalomma* se verspreidings-vermoë af van die gasheer beperk is, is dit waarskynlik dat meganismes verantwoordelik vir spesiasie gekoppel was aan vikariansie gebeurtenisse binne ‘n verskeindenheid van gashere. Verskeie fauna-uitwisselings tussen zoo-grafieses streke, soos dié wat verband hou met die Afrika-Eurasiese landbrug oor die Arabiese plaat 16-20 Mjg, korreleer met spesiasie binne *Hyalomma*. Verder is die ossillasie van watervlakke in Parathetys see asook die ontwikkeling van die Himalaja-gordel en Oos-Afrika Rift Vallei voorgestel as meganismes wat spesiasie binne verskeie gashere, soos hoefdiere en knaagdiere, gedryf het. Alhoewel hierdie gebeurtenisse waarskynlik ‘n rol gespeel het by vroeë *Hyalloma* evolusie, is dit steeds moeilik om die meganismes betrokke by die onlangse divergensies van verskeie *Hyalomma* spesies te staaf, weens beperkte kennis rondom die presiese omvang van verspreidings en gasheer assosiasies van verskeie taksa. Ongeag hiervan, dui die resultate van hierdie tesis op 'n aantal voorgestelde veranderinge aan die huidige taksonomie van die genus: 1) Die tans erkende *H. truncatum* bestaan uit twee afsonderlike spesies. Op grond van konvensionele dierkundige nomenklatuur stel ons voor dat die suidelike klade van *H. truncatum* waarskynlik 'n nuwe spesie is (*H. spesies* nova). 2) *Hyalomma marginatum* en *H. turanicum* moet beskou word as sinonieme. 3) *Hyalomma nitidum* moet verklaar word as ‘n sinoniem tot die *H. truncatum* klade wat binne noord-Afrika voorkom, terwyl *H. albiparmatum* 'n afsonderlike spesie entiteit behoort te bly.

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CHAPTER 1

GENERAL INTRODUCTION

1. Life Histories of Ticks

The origin of ticks as haematophagus ectoparasites dates back to more than 65 Mya (de la Fuente 2003; Mans and Neitz 2003), and as such they have had ample time to specialize and adapt to their hosts and the changing environments. It is therefore not surprising that the life history of extant parasitic ticks is diverse. In particular, species differ in the extent of host association (i.e. time spent on the host), host range, and mode of transmission (Walker and Bouattour 2003).

Ticks (Acari) fall into three families and can act as vectors of a number of pathogens. Their ability to transmit diseases makes them of key importance (Nieberding and Olivieri 2007). At present it is widely believed that there are around 900 species of ticks globally which are represented by 19 genera. Of these, Ixodidae, or hard ticks, represent 14 genera which include around 700 of the total species. Following the egg-stage, hard ticks have three distinct life stages: larva, nymph and adult. However, in general genera differ in the number of hosts that are required to complete their life cycles and also the number of life stages that are free-living in the external environment. At the congeneric level, differences are mainly confined to the number of host species that are used (Walker and Bouattour 2003). Ixodid ticks can therefore be grouped into one-, two- or three-host ticks, of which the latter two types are more commonly found. One-host life cycle ticks are associated with a single host for their entire life and do not re-enter the open environment after attaching at the larval stage (except to lay eggs in the environment at the end of their adult lives; Walker and Bouattour 2003). Two-host life cycle ticks follow a similar process, however, they drop off the primary host when moulting from nymphs into adults and seek new hosts when the process is complete (Walker and Bouattour 2003). Generally this allows individuals to switch hosts. For instance, larval and nymphal stages associate commonly with smaller hosts, while adults generally seek larger hosts. The three-host life cycle follows much the same trend as the two-host life cycle, except of an extra period of detachment and entry into the external environment between the larval and nymphal stages (Walker and Bouattour 2003).

Given the variation in time spent on the host, and the number and type of hosts that are used, it is to be expected that the life cycle and specifically the life history of hard

ticks play an important role in their evolutionary history. In addition, external environmental conditions commonly influence the survival of free-living stages, which in turn may add complexity to evolutionary predictions. Indeed, studies investigating the genetic structure of ticks have revealed contrasting results ranging from a complete lack of genetic structure to strong genetic structure (see McCoy, Boulinier, and Tirard 2005; Kempf et al. 2009; Noureddine, Chauvin, and Plantard 2011; Cangi et al. 2013). A few cases as examples include Cangi et al. (2013), who suggested a genetic break in southern Africa for *Hyalomma rufipes*, and they attributed this to juvenile survival in the environment and competition with other ticks (probably *Hyalomma truncatum*). Additionally Noureddine, Chauvin, and Plantard (2011) argued that the significant genetic disparity observed between Palearctic populations of *Ixodes ricinus* (which has a three-host life cycle and associates with a large diversity of hosts including mammals, birds and reptiles) in Europe and North Africa was likely a result of different life history traits such as seasonal activity, pathogenic potential and varying host associations causing isolation. However the effect of spacial scale on genetic patterns should also not be ignored. Biogeography and past paleoclimatic events have driven speciation in a number of fauna and these have also been used to describe evolutionary patterns in a number of tick species (Qiu et al. 2002; Kemp et al. 2009; Beati et al. 2012; Beati et al. 2013). For instance, *Ixodes ricinus* populations within Europe show a lack of genetic structure. This is possibly due to a high level of connectedness between populations as a result of passive dispersal (dispersal of the parasite via its host without any need to actively move from one location to the next), and a recent population expansion (Noureddine, Chauvin, and Plantard 2011). Furthermore, Kemp et al. (2009) studied *Ixodes uriae* and has shown substantial population structure between different islands due to associations with specific sea bird colonies (not specific seabird species).

2. Phylogenetics & Phylogeography

Phylogenetics aims to describe the evolutionary relationship among organisms (Nixon and Wheeler 1990). It can look at the higher level evolutionary relationships of distinct clades and species, and on a finer scale, can be used to describe the historical processes involved in the geographical distribution of individuals (Avise

and Wollenberg 1997; Avise 2000). The outcome of the latter research (phylogeography), documents the geographic distribution of genetic variation and is much needed to describe the processes involved in shaping the evolutionary history of species (Avise and Wollenberg 1997; Avise 2000). In most instances, phylogeography often only incorporates intra-species structure, while phylogenetics usually includes an inter-species approach focussing on higher taxonomic rankings (Hickerson et al. 2010).

2.1 The use of morphological data in phylogenetics

Morphological data in phylogenetics incorporate unique physical characteristics of phenotypes of one taxon and compares them to those of another. This can help to elucidate evolutionary divergence at the phenotypic level (Patterson 1982). Many studies have investigated the utility of morphological data in phylogenies especially since the emergence of more advanced molecular techniques (Hillis 1987; Patterson 1988; Scotland, Olmstead, and Bennett 2003). Through these, a number of broad scale advantages and disadvantages between the two data sets have been noted. Advantages of morphological data include: 1) The divergence time of lineages can be determined using fossil records, especially given the difficulties in using ancient DNA, and this may provide a more accurate ancestral evolutionary history of lineages; 2) quantifying ancestral states using morphological data allows us to speculate about the ecology of extinct taxa more accurately; 3) morphological data are not prone to problems associated with contamination (Hillis 1987; Smith 1994). On the other hand, there are also a number of disadvantages when morphological characters are considered: 1) It often expresses convergent evolution and thus homoplasy is rarely quantified (Hillis 1987; Scotland and Pennington 2000; Scotland, Olmstead, and Bennett 2003); 2) patterns of inheritance are not always clear and the lack of fossil data makes it difficult to elucidate ancestral states (Hillis 1987; Scotland, Olmstead, and Bennett 2003); 3) there are far less characters to compare than in molecular studies; 4) human bias and interpretation can be a primary concern since characters are often continiuous and the results could become subjective to the researcher's perception (Smith 1994; Scotland and Pennington 2000; Scotland, Olmstead, and Bennett 2003); 5) different methods of character coding contain varying problems (see Archie 1985; Pimentcl and Riggins 1987; Bryant 1989; Nelson 1994; Pleijel 1995; Brower and Schawaroch 1996; Hawkins, Hughes, and Scotland

1997; Scotland and Pennington 2000). It is nowadays widely accepted, that if correctly treated, a combination of morphological and molecular data would include more informative data points, and thus should increase phylogenetic resolution (Hillis 1987; Wiens 2004; Lopardo, Giribet, and Hormiga 2011; Del Rosario Castañeda and De Queiroz 2013).

2.2 The use of DNA sequence data in phylogenetics

Due to heritability, detecting changes in DNA sequences remains arguably a more accurate way of determining the evolutionary relatedness of individuals (Cavalli-Sforza 1998). DNA sequences, through shared genetic similarities, cannot only provide us with a better understanding of evolutionary history but can also inform us of current population structure. In addition, intra- and inter-specific divergences among lineages can form an important component towards an accurate taxonomy. Intraspecifically, DNA sequence data can provide us with an idea of which populations are interacting and which are more isolated (Cavalli-Sforza 1998). However DNA is also not without error. For example, good knowledge of the mutation rate of DNA is needed since many parts of the genome of species are either mutating too fast or too slow to detect appropriate levels of divergence (Slowinski and Page 1999; Zhang and Hewitt 2003; Hurst and Jiggins 2005; Degnan and Rosenberg 2009). There may also be conflicts among gene trees, due to independent lineage sorting (Degnan and Rosenberg 2009; Kutschera et al. 2014; Szöllősi et al. 2015).

The mitochondrial genome provides a useful evolutionary marker in most animals and it is generally strictly inherited through female lineages. The cytochrome c oxidase sub-unit I region (COI) of the mitochondrial genome has frequently been used in bar-coding and phylogeographic studies (see Ruvolo et al. 1994; Yokobori et al. 1994; Hebert, Ratnasingham, and deWaard 2003; Mousson et al. 2005; Lopes and de Freitas 2012) and ticks are no exception (see Murrell, Campbell, and Barker 2000; Cruickshank 2002; Noureddine, Chauvin, and Plantard 2011; Cangi et al. 2013; Zhang and Zhang 2014). The COI region is generally conserved among conspecifics, yet at the same time the mutation rate is fast enough to document intraspecific divergences (Cruickshank 2002; Hebert, Ratnasingham, and deWaard 2003; Zhang and Zhang 2014). Additional ribosomal DNA (rDNA) mitochondrial

genes also prove useful segments to analyse (Hillis and Dixon 1991; Mueller 2006). For ticks, the 16S rRNA region has been shown to be informative at establishing evolutionary relationships among species (see Black and Piesman 1994; Cruickshank 2002). Mitochondrial markers however are also not without limitations. Their biggest advantages can also be their biggest limitation. For instance, they do not account for the exchange of nuclear DNA and are therefore biased towards only the maternal evolutionary history (Zhang and Hewitt 1996). In addition their fast pace of evolution can lead to a loss of phylogenetic signal through homoplasy, particularly when distant evolutionary events are studied. Finally, since all genes on the mtDNA are linked, only a single frame of evolution (one gene tree) is being investigated (Zhang and Hewitt 2003).

Nuclear DNA markers avoid many of the problems associated with mitochondrial DNA (mtDNA), but are not without limitations themselves. Nuclear DNA (nDNA) is most often less homoplasic in relation to mtDNA (Zhang and Hewitt 2003). This is due to primarily slower rates of evolution of nDNA genes, which in turn can reduce their informative potential for distinguishing recent divergence events (Zhang and Hewitt 2003). Furthermore, recombined nuclear genes are known to be difficult to sequence as a result of the montage forms they may take on (Zhang and Hewitt 2003). Although SNP's and certain introns can circumnavigate some of these problems, the availability of primers to amplify variable regions, especially among ticks, remains sparse (Cruickshank 2002).

A combination of mtDNA and nDNA markers is advantageous to overcome some of the problems highlighted above (Zhang and Hewitt 1996; Rubinoff and Holland 2005; de Queiroz and Gatesy 2007). Nuclear rDNA markers, like 28S proves to be useful in substantiating mtDNA gene trees (Cruickshank 2002). Like the COI and 16S, 28S has suitable mutation rates for interspecific investigations and thus have been widely used in phylogenetic studies of invertebrates (see Hillis and Dixon 1991; Crampton, McKay, and Barker 1996; Klompen et al. 1996; Whiting et al. 1997; Cruickshank and Thomas 1999; Giribet et al. 1999; Mallatt, Garey, and Shultz 2004). Additionally other nDNA regions such as the Calreticulin gene region (CRT), Histone *3* (H3) and Internal Transcribed Spacer region *2* (ITS II) have also been shown to be variable enough to show adequate resolution (see Wesson et al. 1993; Kuraku et al. 1999;

Marko 2002; Svenson and Whiting 2004; Xu et al. 2004; Xu et al. 2005; Buhay et al. 2007; Yeo et al. 2007; Colgan, Hutchings, and Beacham 2008; Schultz and Wolf 2009; Agnarsson 2010; Goto et al. 2012; Assunção et al. 2013; Cangi et al. 2013; Wielstra, Baird, and Arntzen 2013).

3. Old World Biogeography & Paleoecology

Throughout the Old World (Africa, Asia and Europe), a number of major geographic structures and climatic variables (past and present) have facilitated genetic breaks, habitat shifts and speciation events in a broad range of terrestrial fauna.

3.1 African biogeography

In Africa historical geological and climatic factors have been implicated in causing genetic discontinuity of many species that can act as hosts for ticks. For example, Moodley and Bruford (2007) studied the highly mobile bushbuck antelope, *Tragelaphus scriptus,* and proposed 26 key biogeographical regions within sub-Saharan Africa. Colangelo et al. (2013) investigated the phylogeography of the rodent, *Mastomys natalensis,* over most of sub-Saharan Africa and described the existence of 6 distinct phylogroups. From these studies and others (see Freitag and Robinson 1993; Grubb et al. 1999; Lehmann et al. 1999; Barnett et al. 2006; Nicolas et al. 2008; Brouat et al. 2009; Evans et al. 2011; Lorenzen, Heller, and Siegismund 2012), the East African Rift Valley system is often implicated as a vicariant barrier causing speciation. The Rift Valley runs from as far north as Ethiopia to as far south as Malawi (Roberts et al. 2012). Although rifting dates back to at least 25-30 million years ago (Mya), major uplift of the rift also occurred between 13.5-15 Mya, 3-5 Mya, and it reached its present day extent approximately 1.6-2 Mya (Denys, Chorowicz, and Tiercelin 1986; Leakey and Harris 1987; Wichura et al 2010; Roberts et al. 2012). Colangelo et al. (2013) also proposed that large historical lakes that formed as part of rifting (Trauth et al. 2005; Trauth et al. 2007; Salzburger, Van Bocxlaer, and Cohen 2014), could be implicated as possible contributors to the formation of 2 of the 3 phylogroups in *M. natalensis*. Other mountain ranges have also been indicated to have prevented gene flow over the continent such as the Cameroon Volcanic Line (see Hassanin et al. 2007; Moodley and Bruford 2007; Fjeldså and

Bowie 2008; Colangelo et al. 2013), the Zimbabwe Highlands (see Loader, Poynton, and Mariaux 2004) and the Drakensberg (see Schwab et al. 2012). Dense forests and wooded areas have also been suggested as possible barriers to gene flow to some species. The most pertinent of these has been the equatorial forests of Guinea-Congo, Cameroonian Highland forests, East African forests and the *Brachystegia* woodland (see Coe and Skinner 1993; Freitag and Robinson 1993; Lovett and Wasser 1993; Matthee and Robinson 1997; van Alphen-Stahl, Bloomer, and Crowe 2005; Colangelo et al. 2013). Large rivers and their systems such as the Congo, Sanaga, Niger, Volta, Nile, Orange and the Zambezi have also been key obstacles to gene flow (see Morales and Melnick 1997; Eriksson et al. 2004; Van Daele et al. 2004; Dobigny et al. 2005; Sole, Scholtz, and Bastos 2005; Anthony et al. 2007; Nicolas et al. 2008; Brouat et al. 2009). Arid areas in Africa such as the Kalahari (Kalahari sand flows) and Sahara are also no exception (see Werger 1978; Carnahan et al. 2002; Matthee and Flemming 2002; Hassain et al. 2007).

3.2 Asian biogeography

In Asia, similar to Africa, a number of geographic and climatic variables have been proposed to restrict ranges and dispersal of numerous species. The most obvious of these are the mountain ranges: The extreme altitudes of the Himalayas, the Kunlun and Tien Shan, the Tanggula Mountains, the Altai Mountains, Anatoli, Caucasus and Zagros belts, the Kopet-Dagh Mountains, and the Ural Mountains have all been suggested to restrict gene flow (see Kurup 1974; Sanmartín 2003; Dennell 2004; Guo and Wang 2007; Melville et al. 2009). Interestingly most of the uplift around central Asia occurred in three main steps around 20 Mya, 8 Mya and 3.6 Mya (Harrison et al. 1992; Harrison et al. 1995; Cui et al. 1996; Li et al. 1996; Voelker 1999). Large water bodies and rivers such as the Caspian Sea, Amu Darya River, Red Sea and those around the Isthmus of Suez, Black Sea, the Yenisei River and Persian Gulf have been proposed to restrict the movement of terrestrial animals from crossing from Asia into and out of Africa and Europe, while also restricting movement within Asia (see Dumont 1998; Sanmartín 2003; Melville et al. 2009; Graham, Oláh-Hemmings, and Fet 2012). The Arabian, Syrian and Thar deserts have all been linked to the restriction of movement of animals (see Kurup 1974; Sanmartín 2003). In addition, certain areas of isolation such as the Tarim Basin, Tibetan Plateau, Junggar Basin, Alashan Plateau have all been suggested as areas

of high endemism (see Guo and Wang 2007; Melville et al. 2009). In south-east Asia the Brahmaputra and Salween rivers and the mountain ranges in Myanmar (Burma), have been implicated in preventing dispersal between the Indian and south-east Asian regions (see Takacs et al. 2005; Su et al. 2007; Veron et al. 2007; Patou et al. 2010). Finally the Isthmus of Kra, near the Thai–Malaysian border, represents a limit between the Indo-Chinese and Sundaic subregions (see Corbet and Hill 1992; Hughes, Round, and Woodruff 2003; Woodruff and Turner 2009; Patou et al. 2010). During high-sea-level periods, a restriction in the land region around the Isthmus of Kra caused faunal compressions north and south of the isthmus (Woodruff and Turner 2009).

3.3 European biogeography

While studies exploring European biogeography are dominated by the extreme altitudes of the Alps and temporal variation north and south of them (see Boccaletti, Elter, and Guazzone 1971; Taberlet et al. 1998; Bilton et al. 1998; Hewitt 1999; Schmitt, Gießl, and Seitz 2002; Melis et al. 2006; Heikinheimo et al. 2007), others have also been noted. Mountain ranges like the Carpathian and Pyrenees have been suggested to play dominant rolls in the population dynamics over the region (see Schmitt, Gießl, and Seitz 2002; Dennell 2004; Heikinheimo et al. 2007; Braaker and Heckel 2009). Internal water bodies such as the Adriatic Sea (see Sanmartín 2003; Dennell 2004; Heikinheimo et al. 2007), and external, between Europe, Africa and Asia (the Aegean and Bosporus, the Black and the Mediterranean seas) have all been shown to be major mechanisms of isolation for terrestrial species (see Sanmartín 2003; Dennell 2004).

3.4 Old World paleoclimates and habitat shifts

Above geology and current habitat constraints, paleoclimates and habitat shifts associated with glacial cycles have been noted as major contributors to speciation events and current phylogeographic patterning of species (see Taberlet et al. 1998; Hewitt 2004; Dubey et al. 2006; Weiss and Ferrand 2007; Zhang et al. 2008; Lorenzen, Heller, and Siegismund 2012). Among these, glacial cycles from the Miocene right through to the Pleistocene played a role (see Kukla and Cílek 1996; Reed 1997; Potts 1998; Taberlet et al. 1998; Hewitt 1999; Nichol 1999; Dynesius and Jansson 2000; Hewitt 2000; Zachos et al. 2001; Mercer and Roth 2003;

deMenocal 2004; Hewitt 2004; Chase and Meadows 2007; Potts 2007; Weiss and Ferrand 2007; Zhang et al. 2008; Lorenzen et al. 2010). These have caused favourable habitats to shift, and the development of grasslands was observed from the end of Eocene, 40 Mya (Retallack 2001). Although grasslands had an early origin, their expanse and shift towards becoming a dominant biome in the Old World seems to be closer tied to climatic events post the mid-Miocene (see Cerling 1992; Cerling et al. 1997; Retallack 2001; Mercer and Roth 2003; deMenocal 2004; Dennell 2004; Jacobs 2004).

4. The Study Taxon

4.1 Hyalomma

The genus *Hyalomma* (Koch 1844) refers to a group of ticks with defining characteristics such as scutums / conscutums that are dark reddish-brown to near black, protruding eyes and the appearance of striped ligaments. There are currently 27 recognized species found throughout Asia, Europe and Africa (Guglielmone and Nava 2014). Most members of this genus have a three-host life cycle, but exceptions exist: *H. dromedarii*, *H. schulzei, H. truncatum, H. nitidum* and *H. albiparmatum* are confined to one or two hosts (see Murrell, Campbell, and Barker 2001; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Schuster, and Horak 2008).

Initial studies proposed *Hyalomma* to have an Asian origin (Balashov 1994). Murrell, Campbell, and Barker (2001), using a total evidence approach, proposed that the lineage containing *Nosomma–Hyalomma* likely evolved from an Oriental common ancestor that lived in the region around 19 Mya. They suggested that if this holds, dispersal occurred from this point into Africa and Eurasia around 14 Mya (Murrell, Campbell, and Barker 2001). Subsequent to this, de la Fuente's (2003) described a *Hyalomma* amber fossil, dated to the Eocene (35-50 Mya) and from the Baltic region, suggesting that the age and origin of the genus could be substantially older and further west than initially thought.

Taxonomic uncertainties are rife within the *Hyalomma* genus. At the higher taxonomic level a number of recent changes have been proposed and include those by Camicas et al. (1998); Murrell, Campbell, and Barker (2001), Rees, Dioli, and Kirkendall (2003), Barker and Murrell (2004), Apanaskevich and Horak (2006), Apanaskevich and Horak (2008a), Apanaskevich and Horak (2008b), Apanaskevich, Schuster, and Horak (2008), Apanaskevich, Filippova, and Horak (2010), Guglielmone and Nava (2014), and Zhang and Zhang (2014). Most of these were however based on morphological differentiation which is often very difficult to interpret (see Camicas et al. 1998; Murrell, Campbell, and Barker 2001; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Schuster, and Horak 2008; Apanaskevich, Filippova, and Horak 2010; Guglielmone and Nava 2014). The lack of a clear phylogenetic understanding within the genus is also perplexed by a number of unresolved species complexes. In addition, the virtual absence of genetic data is best exemplified by a “GenBank” search where sequences of only ten members of the genus are available (www.ncbi.nlm.nih.gov/). Furthermore, it is believed that the number of species may be confounded due to initial misidentification of specimens used for sequencing (Zhang and Zhang 2014). There is thus a critical need to compare morphologically identified species against molecular data in order to provide more robust evidence. The phylogeny, in turn, can provide valuable taxonomic insights and can also be useful to ascertain which factors were more than likely responsible for generating the taxonomic diversity in the genus.

4.2 Species complexes

The genus *Hyalomma* is characterized by several species complexes. Resolving these are important in modern conservation and evolutionary biology (Donoghue 1985; Bickford et al. 2007). A species complex represents a group of possible closely related species, where the exact recognition of status between species is cryptic or unresolved (Bickford et al. 2007). This stems from conflicting characteristics among members in relation to varying species concepts (Meier 2000; Bickford et al. 2007). Species complexes are taxonomically very difficult to unravel since members belonging to these complexes can often also hybridize, morphological characters can lead to misinterpretation (due to convergent evolution),

and the degree of geographic isolation between members are not constant (Donoghue 1985; Bickford et al. 2007).

In *Hyalomma,* recent morphological studies addressed the species status of certain taxa by indicating possible cryptic species or complexes (see Apanaskevich and Horak 2006; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Guglielmone and Nava 2014). The proposed possible hybridization ability between well-defined *Hyalomma* species implies that species complexes in the genus could be more difficult to resolve taxonomically than previously thought (see Rees, Dioli, and Kirkendall 2003; Dalal, Kumar, and Gupta 2007; Zhang and Zhang 2014). Hybridization has been suggested between *H. truncatum* / *H. rufipes* / *H. dromedarii* (Rees, Dioli, and Kirkendall 2003) and *H. dromedarii* / *H. anatolicum* (Dalal, Kumar, and Gupta 2007). Furthermore, based on a database review, *H. truncatum* / *H. marginatum* / *H. dromedarii* have also been suggested to be in a complex relationship (Zhang and Zhang 2014).

Hybridization among *H. truncatum, H. nitidum* and *H. albiparmatum* (that form the focus of the present study) is not well studied, adding further difficulties in deciphering the validity of yet another species complex in the genus. Due to the wide distribution of members of the *H. (Euhyalomma) truncatum* complex (Koch 1844) the complex was regarded as valid with three species (see Feldman-Muhsam 1962; Apanaskevich and Horak 2008b). It was suggested that the extensive distribution range of the species is the reason for the wide range of morphological variability present in this taxon (Apanaskevich and Horak 2008b). It is thus not surprising that in the early 1900's Schulze described a number of species close to *H. truncatum*. Many of these, including *H. nitidum* (Schulze 1919) and *H. albiparmatum* (Schulze 1919) were later suggested to be synonyms of *H. truncatum* (see Feldman-Muhsam 1962; Camicas et al. 1998). However Hoogstraal (1956) and Walker (1974) examined a large number of *H. albiparmatum* from Kenya and Tanzania, and concluded that they are not a synonym of *H. truncatum*, but a separate species. This decision, however, was based only on a single morphological character in male specimens (Hoogstraal 1956; Walker 1974; Apanaskevich and Horak 2008b). Male *H. albiparmatum* can only be distinguished from *H. truncatum* by an ivory-coloured parma (Apanaskevich and Horak 2008b). It is impossible to distinguish between the

females and immature stages of these species (Apanaskevich and Horak 2008b). Hoogstraal (1979) also proposed the re-instating of *H. nitidum* as a separate species and indicated that a description of this species would be published in a future communication. Unfortunately the formal description never materialized but the species name *H. nitidum* now appears in the literature to describe west and central African *H. truncatum* specimens. More recently Tomassone et al. (2005) published a paper on the discriminating characters, distribution and host-parasite records of *H. nitidum*. He suggested two criteria for distinguishing *H. nitidum* from *H. truncatum*: The reduction in clarity of ivory-coloured bands on the segments of the legs (both sexes), and the external cuticular preatrial fold of the genital operculum that differs in shape (females only) (Tomassone et al. 2005).

The current debate persists surrounding the taxonomic uncertainty of the members of the *H. (E.) truncatum* complex. Do these members represent a single species, namely *H. truncatum* or are *H. albiparmatum* and *H. nitidum* also valid entities (Hoogstraal 1956; Feldman-Muhsam 1962; Walker 1974; Hoogstraal 1979; Camicas et al. 1998; Tomassone et al. 2005; Apanaskevich and Horak 2008b)?

4.3 Hyalomma truncatum, H. nitidum and *H. albiparmatum life history and distribution*

Hyalomma truncatum has a two-host life cycle (Apanaskevich and Horak 2008b). While adults can be commonly found on a large spectrum of hosts from medium sized mammals, reptiles and even birds, they are primarily found on larger domestic and wild ungulates of the orders Cetartiodactyla and Perissodactyla (see Apanaskevich and Horak 2008b and references therein). Juvenile ticks infest and parasitize small mammals such as rodents and hares, and are usually excluded from larger mammals and birds (see Apanaskevich and Horak 2008b and references therein). *Hyalomma truncatum* has been noted throughout Africa; this range extends the full breadth of the continent, while occurring from as far north as southern Egypt to South Africa in the south (Hoogstraal 1956; Theiler 1962; Kolonin 1982; Apanaskevich and Horak 2008b) (Fig 1.1). The species has been noted as a vector of viruses (e.g. Crimean-Congo haemorrhagic fever), protozoa (e.g. Equine piroplasmosis) and toxins causing harmful effects to their hosts (e.g. sweating

sickness) (see Bezuidenhout and Malherbe 1981; Hoogstraal, Wassef, and Buttiker 1981; De Waal 1990; De Waal 1992).

Hyalomma albiparmatum also maintain a two-host life cycle (Apanaskevich and Horak 2008b). Adults have been found on a range of medium-large domestic and wild mammalian fauna, while their presence on birds should be neglected (see Apanaskevich and Horak 2008b). Juveniles have only been noted on *Lepus capensis* (Cape Hare) although they likely could also be found on a variety of similar sized mammalian fauna (Hoogstraal 1956; Walker 1974; Apanaskevich and Horak 2008b). The species is confined to east Africa where valid records of the species have been recorded in Kenya and Tanzania (Yeoman et al. 1967; Walker 1974; Apanaskevich and Horak 2008b) (Fig 1.1). Disease relationships are much the same as for *H. truncatum*, however they have also been recorded as vectors of *Rickettsia conorii,* which are known to cause Kenya Tick Typhus (also known as Tick Bite Fever in humans) (see Heisch et al. 1962).

Hyalomma nitidum, as with *H. albiparmatum* and *H. truncatum*, also maintains a two-host life cycle, with much the same adult and juvenile hosts as the other two species (see Apanaskevich and Horak 2008b). They can be found in the humid regions of west and central Africa from Senegal in the north-west to the Central African Republic in the south-east (Tomassone et al. 2005; Apanaskevich and Horak 2008b) (Fig 1.1). Our knowledge of disease relationships with *H. nitidum* is very limited. To date, only Crimean-Congo haemorrhagic fever has been associated with this species (Sureau et al. 1976; Tomassone et al. 2005; Apanaskevich and Horak 2008b).

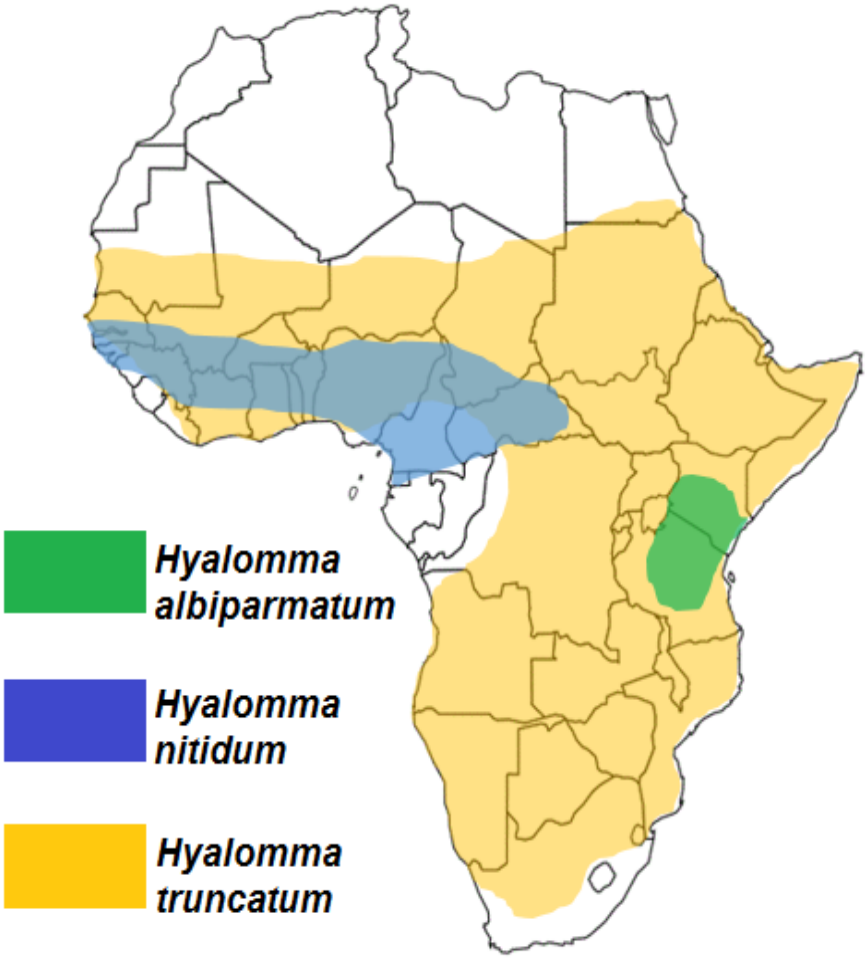


Figure 1.1: *The approximate distribution of Hyalomma albiparmatum, Hyalomma nitidum and Hyalomma truncatum throughout Africa.*

5. Scope & Aims of The Study

5.1 Scope of the study

The overall aim of the MSc study was to investigate systematic questions relating to the medically important tick genus, *Hyalomma*. The detection of phylogeographic pattern/s of the widely distributed *H. truncatum* should increase our understanding of genetic barriers to gene flow at the continental scale and the data could furthermore provide insights into the mechanisms involved in the evolution of ticks (Apanaskevich 2004; Apanaskevich and Horak 2008b). In addition, understanding the mechanisms responsible for the dispersal of ticks across the landscape can aid in predicting the

spread of diseases. For example, the vector potential of *Hyalomma* ticks may vary between different genetic lineages, species, or assemblages (see Hoogstraal and Aeschlimann 1982; Swanepoel and Burt 2004; Shyma et al. 2012; Gou et al. 2013). In addition, it has been documented that distinct genetic lineages can show different levels of acaricide resistance (Criscione, Poulin, and Blouin 2005), which in turn may directly impact on the health of local livestock and even humans (Hornok and Horváth 2012; Bente et al. 2013; Nyangiwe et al. 2013). Finally, the study aims to provide a molecular perspective on the complex relationship of *H. truncatum,* particularly to *H. nitidum* and *H. albiparmatum* (Apanaskevich and Horak 2008b). Given the importance to decipher species complexes, further emphasis was placed on molecularly quantifying conflicting taxonomic descriptions based on morphology alone. Murrell, Campbell, and Barker (2001) already highlighted the need for such a revision. To assist in this goal a higher-level phylogeny of *Hyalomma* was also constructed. Intensive global sampling was performed with the objective to obtain all recognised *Hyalomma* species from as many locations as possible. Deciphering the evolutionary relationships among species within *Hyalomma* will provide significant taxonomic advances in this field.

5.2 Aims

The aims of the present study were:

1. To provide a phylogeographic perspective for *H. truncatum* using the mtDNA COI and, nDNA H3 and CRT gene regions.

2. To create a comprehensive phylogeny for all the described extant *Hyalomma* species by using morphological data and molecular data, derived from multiple nuclear and mitochondrial genetic markers (COI, 16S, 28S, ITS II and H3).

CHAPTER 2

THE CONTINENTAL PHYLOGEOGRAPHY OF *HYALOMMA TRUNCATUM* (Ixodida: Ixodidae: Hylomminae)

1. Introduction

Hyalomma truncatum (Koch 1844) is suggested to be one of the most widely distributed ixodid ticks within the Afrotropical zoogeographic region. It is present from southern Egypt in the north to South Africa in the south, and its range spans the full breadth of the continent from Senegal to Somalia (Fig 1.1). They are only absent in certain areas of the Afrotropical Zoogeographic Region, where extreme levels of humidity and moisture prevails, and in the extreme arid conditions of the Sahara in north Africa (Fig 1.1) (Apanaskevich and Horak 2008b). Furthermore they display a two-host life cycle (Apanaskevich and Horak 2008b). Adults are primarily found on larger domestic and wild ungulates of the orders Cetartiodactyla and Perissodactyla, but can also be found on other medium sized mammals, reptiles and even birds (see Apanaskevich and Horak 2008b and references therein). Juvenile ticks parasitize small mammals such as rodents and hares, and can be excluded from larger mammals and birds (see Apanaskevich and Horak 2008b and references therein).

The phylogeographic patterns of other tick species in the Afrotropical Zoogeographic region (e.g. *H. rufipes*, *I. ricinus* and *Amblyomma variegatum*) depict substantial genetic structure (Noureddine, Chauvin, and Plantard 2011; Beati et al. 2012; Cangi et al. 2013) that may suggest that *H. truncatum* may also be similarly structured. Furthermore, morphological variation within *H. truncatum,* and the large extent of its range, lead Schulze (1919) and Schulze and Schlottke (1930) to describe a number of species within *H. truncatum*, but these were later synonymized (Camicas et al. 1998; Apanaskevich and Horak 2008b).

To the best of our knowledge, no phylogeographic study on *H. truncatum* has taken place to date and thus the mechanisms responsible for possible diversification are not yet known. Looking at the phylogeographic patterns for other ixodid tick species, Noureddine, Chauvin and Plantard (2011), suggested that latitudinal temporal variation caused shifts in reproduction stages between European and African populations of *I. ricinus* and this limitations to gene flow between the continental populations. Beati et al. (2012) suggested host selection rather than isolation by distance (IBD) was the most probable cause of phylogeographic structure in *A. variegatum* across north Africa (also see Madhav et al. 2004). Interestingly Cangi et

al. (2013) proposed that the phylogeographic structure of *H. rufipes* was likely due to life history variables associated with interspecific competition (possibly with *H. truncatum*) and consequently, juvenile survival in the environment.

Apart from life history characteristics, we can expect that biogeography and paleoecology implicated in host diversification, may also have an effect on the genetic structure of *H. truncatum*. The most pertinent of these are glacial cycles and habitat shifts over the Plio-Pleistocene boundary (see Potts 1998; Dynesuis and Jansson 2000; Hewitt 2000; Zachos et al. 2001; deMenocal 2004; Chase and Meadows 2007). For example, the East African Rift Valley and associated lakes have often been implicated in east-west and east-south divergences within and between species that can act as hosts for the ticks (see Freitag and Robinson 1993; Hassain et al. 2007; Moodley and Bruford 2007; Nicolas et al. 2008; Lorenzen, Heller, and Siegismund 2012; Colangelo et al. 2013). Additionally other mountain ranges and lakes, (Cameroon Volcanic Line, Lake Chad), rivers (Congo, Niger, Volta and Zambezi), deserts (Kalahari and Sahara), and even woodlands and forests (Cameroonian Highland, Guinea-Congo and *Brachystegia*) have been proposed as barriers to gene flow (see Matthee and Robinson 1997; Girman et al. 2001; Eriksson et al. 2004; Hassanin et al. 2007; Moodley and Bruford 2007; Nicolas et al. 2008; Lorenzen, Heller, and Siegismund 2012; Colangelo et al. 2013).

We hypothesize that *H. truncatum* populations will show high levels of geneflow at the smaller geographic scale (due to their occurrance on large ungulates) but at the larger geographic scale, the pattern will more reflect host vicariance (multiple hosts species show similar phylogeographic patterns across the continent). If this holds, the east African rift system and moist forests of central African may pose barriers to dispersal of this species.

2. Materials and Methods

2.1 Sampling design

Hyalomma truncatum specimens were collected from 22 localities across southern, eastern and western Africa (Fig 2.1). While attempts were made to sample *H.*

truncatum from five localities in the central African region (in Burundi and the Democratic Republic of the Congo), our sampling recorded only *H. rufipes* across these sites. Nevertheless specimens were sampled according to protocols described by Cangi et al. (2013) and for this study were collected from cattle (*Bos taurus*), horse (*Equus caballus*), pig (*Sus domesticus*), sheep (*Ovis aries*) and goat (*Capra aegagrus*). Ticks were placed in 100% ethanol until further analysis. Special note was made of the sampling locality through Global Positioning System (GPS) data. During sampling it was aimed to collect at least 15 tick specimens from each location (Sup. Table 1). To expand our sampling, sequences from one locality in Ethiopia were drawn from GenBank (AJ437084.1; AJ437085.1; AJ437086.1; AJ437087.1). In total, 186 individuals from 11 countries were included (Fig 2.1).

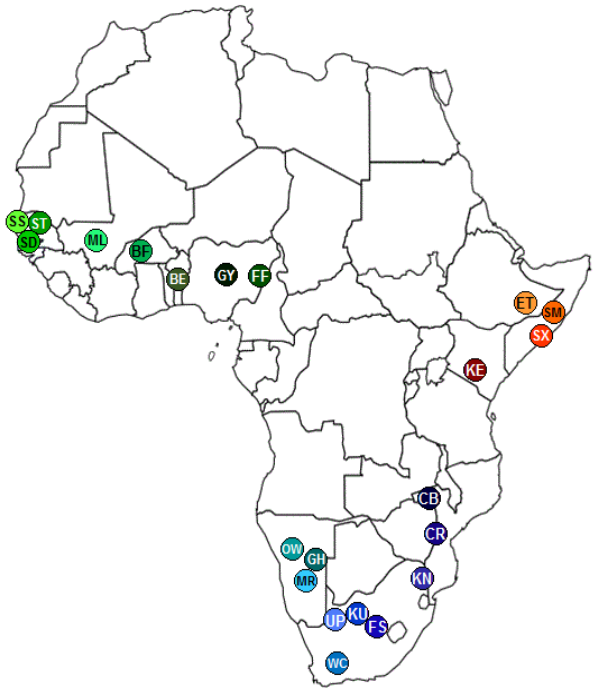


Figure 2.1: *The 22 Hyalomma truncatum sampling localities, through 11 countries across Africa. Locality codes correspond to those available in Sup. Table 2.*

2.2 Validation of H. truncatum

Morphological validation of *Hyalomma* species remains difficult due to the cryptic nature of their morphology. All identification of *H. truncatum* specimens used in this study were carried out by D.A. Apanaskevich (National Tick Collection, Georgia Southern University) and I.G. Horak (Department of Veterinary Tropical Diseases, University of Pretoria) who compared all specimens against known voucher representatives of *H. truncatum.* Diagnostic morphological characters compared

included: 1) the width and shape of the genital operculum in *H. truncatum* females which is represented by a wide, high “U”-shaped arc; 2) the narrowing of the conscutum in an area of spiracular plates among males (which is greater than any other *Hyalomma* species); 3) generally smoother conscutums in relation to other common *Hyalomma* species found over the sampled range (for example *H. glabrum* (Apanaskevich and Horak 2006), *H. rufipes* (Apanaskevich and Horak 2008a) and *H. somalicum* (Apanaskevich and Horak 2009)). To distinguish between more closely related morpho-species, such as *H. albiparmatum* and *H. nitidum* we further inspected individuals believed to overlap in range for further morphological differences: Male *H. albiparmatum* can only be distinguished from *H. truncatum* by an ivory-coloured parma in the male (Apanaskevich and Horak 2008b). It is impossible to distinguish between the females and immature stages of *H. albiparmatum* and *H. truncatum*, and for this reason, only male *H. truncatum* from overlapping areas (Kenya) were identified and included. While Tomassone et al. (2005) suggested two criteria for distinguishing *H. nitidum* from *H. truncatum*: 1) the reduction in clarity of ivory-coloured bands on the segments of the legs (both sexes); 2) the external cuticular preatrial fold of the genital operculum that differs in shape, being concave in *H. truncatum* and convex in *H. nitidum* (females only). Finally validation of specimens was also conducted by making use of GenBank blast searches (http://www.ncbi.nlm.nih.gov/). Although the pitfalls and limitations of using DNA barcodes in species identifications have been widely discussed (Yassin et al. 2010; Goldstein and DeSalle 2011; Zhang and Zhang 2014), similar phylogeny based studies of ticks show high levels of success (Klompen et al. 2000; Murrell, Campbell, and Barker 2001; Barker and Murrell 2004).

2.3 Molecular techniques

Total genomic DNA was isolated using a CTAB manual extraction technique (Winnepenninckx, Backeljau, and De Wachter 1993) with minor modifications. The extraction utilized the complete animal which was left in the buffer in a heat block for 48 hours to digest at 55ºC. Exoskeletons of digested specimens were removed and stored in 100% ethanol for future reference.

DNA sequences were generated for the mtDNA Cytochrome c oxidase I (COI) gene and two nDNA genes, Histone 3 (H3) and Calreticulin (CRT). The primers used in

the amplification were either taken from published sources or designed specific to *Hyalomma* using the NCBI's Primer 3 software (www.ncbi.nlm.nih.gov/tools/primer-blast) (Table 2.1). This was done via blast searches and aligning the targeted region against a range of available *Hyalomma* sequences on Genbank.

Amplifications were performed in a GeneAmp PCR 2700 thermal cycler (Applied Biosystems) (Sup Table 1). PCR cycling conditions followed those described in Sands et al. (2015) although annealing varied according to the primer pair and locus selected (Table 2.1). Aliquots of PCR products were separated by 1% agarose gel electrophoresis. In certain instances PCR products were excised from the gel and purified using a BioFlux, Biospin Gel Extraction Kit (Bioer Technology Co., Ltd.). Finally, all sequencing of PCR products was performed by the University of Stellenbosch Sequencing Facility using BigDye Chemistry and an ABI 3730 XL DNA Analyzer (Applied Biosystems).

Table 2.1: *Gene regions, primer names, primer sequence and the edited sequence length of the amplified product used in this chapter. The optimal annealing temperatures of the primer pairs and the sources of the primers used are also indicated.*

Region	Gene	F/R	Primer name		Edited sequence length (BP)	Optimal annealing temperature	Source
mtDNA	COI	Forward	AR-U-COIa	5'-AAACTRTKTRCCTTCAAAG-3'	666	45^0C	Cangi et al. 2013
		Reverse	AR-L-COIa	5'-GTRTTAAARTTTCGATCSGTTA-3'			Cangi et al. 2013
nDNA	CRT	Forward	HyCRT+F1	5'-GAGTCBACGAAAGGCGACAA-3'	516	60^0C	Designed for present study
		Reverse	HyCRT-R1	5'-CSTCSGGGTCCTTGATCTTC-3'			Designed for present study
	H3	Forward	HyH3F	5'-GTGGATGGCRCAMARGTTGG-3'	267	56.5^0C	Designed for present study
		Reverse	HyH3R	5'-GCAAGAGYACCGGWGGVAAR-3'			Designed for present study

2.4 Sequence editing and alignment

Sequences were visually inspected and edited using the program Geneious R7.1 (Biomatters Ltd.). Sequence ambiguities associated with heterozygote states in the nDNA sequences were resolved by determining the gametic phase of alleles via PHASE 2.1 (Stephens, Smith, and Donnelly 2001; Stephens and Donnelly 2003) algorithms in DnaSP 5.10.01 (Librado and Rozas 2009). MCMC simulations were run for 100,000 generations with a thinning interval of 1 in every 10,000 generations discarded as burn-in. A probability threshold of 0.9 was considered for the differentiation between all phases. To optimize sequence quality and to limit missing

data, ends were trimmed. All sequences were aligned by the CrustalW Multiple Alignment tool (Thompson, Higgins, and Gibson 1994) in BioEdit 7.1.3.0 (Hall 1999).

2.5 Phylogeographic analyses

Evolutionary relationships among DNA haplotypes were established in TCS 1.21 (Clement, Posada, and Crandall 2000) which generates statistical haplotype networks with 95% confidence connections. To further test for differentiation among geographical populations (containing more than ten specimens) the groups identified by the TCS haplotype networks were used as priors in analyses of molecular variance (AMOVAs) (Excoffier, Smouse, and Quattro 1992). The latter were executed in Arlequin 3.5.1.2 (Excoffier and Lischer 2010), where calculated Φst values act to indicate the level of genetic variation between preassigned populations. To limit error associated with multiple pairwise comparisons, p-values of Φst values were subjected to Holm's sequential Bonferroni corrections (Dunn 1961; Holm 1979). Additionally Arlequin 3.5.1.2 (Excoffier and Lischer 2010) was also used to calculate: 1) Haplotypic diversity (*h*) and nucleotide diversity (π); 2) Fu's Fs (Fu 1997) and mismatch distribution (Harpending et al. 1998), using a 1,000 replicates of the parametric bootstrap method (Schneider and Excoffier 1999). Furthermore, isolation by distance (IBD) was calculated using GenAlEx 6.5 (Peakall and Smouse 2012) to ascertain the level of genetic variation attributed to geographic distance.

SplitsTree 4.13.1 (Huson and Bryant 2006) was used to calculate COI sequence distance between samples and create Neighbour-Net (Bryant and Moulton 2004) phylogenetic networks. Furthermore MrBayes 3.2.5 (Ronquist et al. 2012) was incorporated to construct a Bayesian topology for the COI data. jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008) and the Akaike Information Criterion (AIC) (Akaike 1973) was used to determine the best-fit model for the COI data to define as the prior (Posada and Buckley 2004). Bayesian inference MCMC chains ran for 5,000,000 generations, saving one tree every 1,000 generations. Validation of convergence and mixing was assessed in Tracer 1.5 (Rambaut and Drummond 2007) to ensure that all effective sample size (ESS) values were > 200. TreeAnnotator 1.8.2 (Drummond et al. 2012) was used to summarize trees, after discarding 2,000 trees as burn-in. Furthermore BASP 6.0 (Corander et al. 2008) was used to investigate posterior coalescent clusterings. Clustering of linked loci was

selected and the codon linkage model selected. The analysis was run 5 times each for K 1-22 as the assumed maximum number of populations present.

To test for common evolutionary history among lineages, lineages with notable differentiation on the topology and average COI sequence distances > 2% (that are proposed to be out of the bounds of normal intraspecific structure for Ixodid ticks (Zhang and Zhang 2014)) were pooled and subjected to temporal population demographic analyses. Bayesian Skyline Plots (Drummond et al. 2005) were generated through BEAST 1.8.2 (Drummond et al. 2012) where MCMC simulation ran for 100,000,000 generations, sampling every 10,000 generations. The COI codon position was established in MacClade 4.0 (Maddison and Maddison 2000) and the data was subsequently partitioned into 1st + 2nd and 3rd codon positions in the input file (BEAuti 1.8.2 (Drummond et al. 2012)). jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008), using the Akaike Information Criterion (AIC) (Akaike 1992), was used to select HKY model for sequence evolution and the construction of the plots was completed through Tracer 1.5 (Rambaut and Drummond 2007), as well as to assure that ESS values were > 200. Dating of the events were done using a COI rate of evolution of 1.5% per million years. This was established through divergence dating via fossil calibration of the genus (see Chapter 3).

3. Results

3.1 Phylogeographic networks and analysis of molecular variance (AMOVA)

The mtDNA COI gene, representing 666 bp of the mtDNA genome, revealed 118 unique haplotypes (GenBank accession numbers: KT999398-KT999579). TCS analysis showed four distinct groups that could not be connected with 95% certainty (Fig 2.2). These groups corresponded to geographic regions (southern African, western African, central-eastern African and eastern African) and there was no haplotype sharing among them. Within the groups, haplotype sharing was more common among geographic sampling localities especially in the southern African clade and the western African clade (Fig 2.2). There is no clear visual indication of geographic population substructure within the southern and eastern African clades, but some substructure was evident across the western African region where three

subclusters can be visualized (Figs 2.2 and 2.3). These were partly confirmed by BAPS analysis, which supported the presence of a single group within the southern African region, three groups within the west African region, but a single group in the east African region (D; Fig 2.3).

Haplotypic diversity (*h*) within these geographic regions was remarkably similar: southern, 0.97 (SD ± 0.09); eastern, 0.96 (SD ± 0.03); western, 0.97 (SD ± 0.01). *Hyalomma truncatum* nucleotide diversity (π) was found to be 0.056% (SD ± 0.026%) overall, while within regions similar low diversity were again found (although moderately higher in western Africa): southern, 0.007% (SD ± 0.004%); eastern 0.007% (SD ± 0.004%); western, 0.014% (SD ± 0.007%). AMOVA analyses indicated strong levels of regional population differentiation with the highest level of differentiation between the southern African population and the remainder of the populations in eastern and western Africa (Table 2.2).

Table 2.2: *mtDNA: Cytochrome c oxidase 1 AMOVA results showing the pairwise Φst values with p-values (below) for regional population comparisons (significant p ≤ 0.05, based on corrected p-values, are highlighted in* ***bold****). The central-east African clade was omitted due to limited samples.*

COI	southern Africa	western Africa	eastern Africa
southern Africa	X	na	na
	X	na	na
western Africa	**0.899**	X	na
	p≤0.05 (+)	X	na
eastern Africa	**0.925**	**0.557**	X
	p≤0.05 (+)	**p≤0.05 (+)**	X

The Bayesian topology (Fig 2.3) indicates *H. truncatum* is divided into two well supported lineages, one representing northern sampling localities and the other representing southern African localities (Fig 2.3). Furthermore, five phylogroups with mostly weak posterior probabilities were identified across northern Africa, while the southern African lineage showed no such substructure. Additionally the Neighbour-Net (Bryant and Moulton 2004) phylogenic network, using COI sequence data, clearly depicts a large differentiation between the northern sampling regions (western, central-eastern, eastern) and the southern region (southern) (Fig 2.3). This mtDNA COI sequence divergence reflected by these long branches is 9.88% (SD ± 0.40%), while within the two groups an average sequence diversity of 1.84% (SD ± 1.00%) is present for *H. truncatum* across northern Africa and 0.73% (SD ± 0.30%) for southern African *H. truncatum* (Fig 2.3).

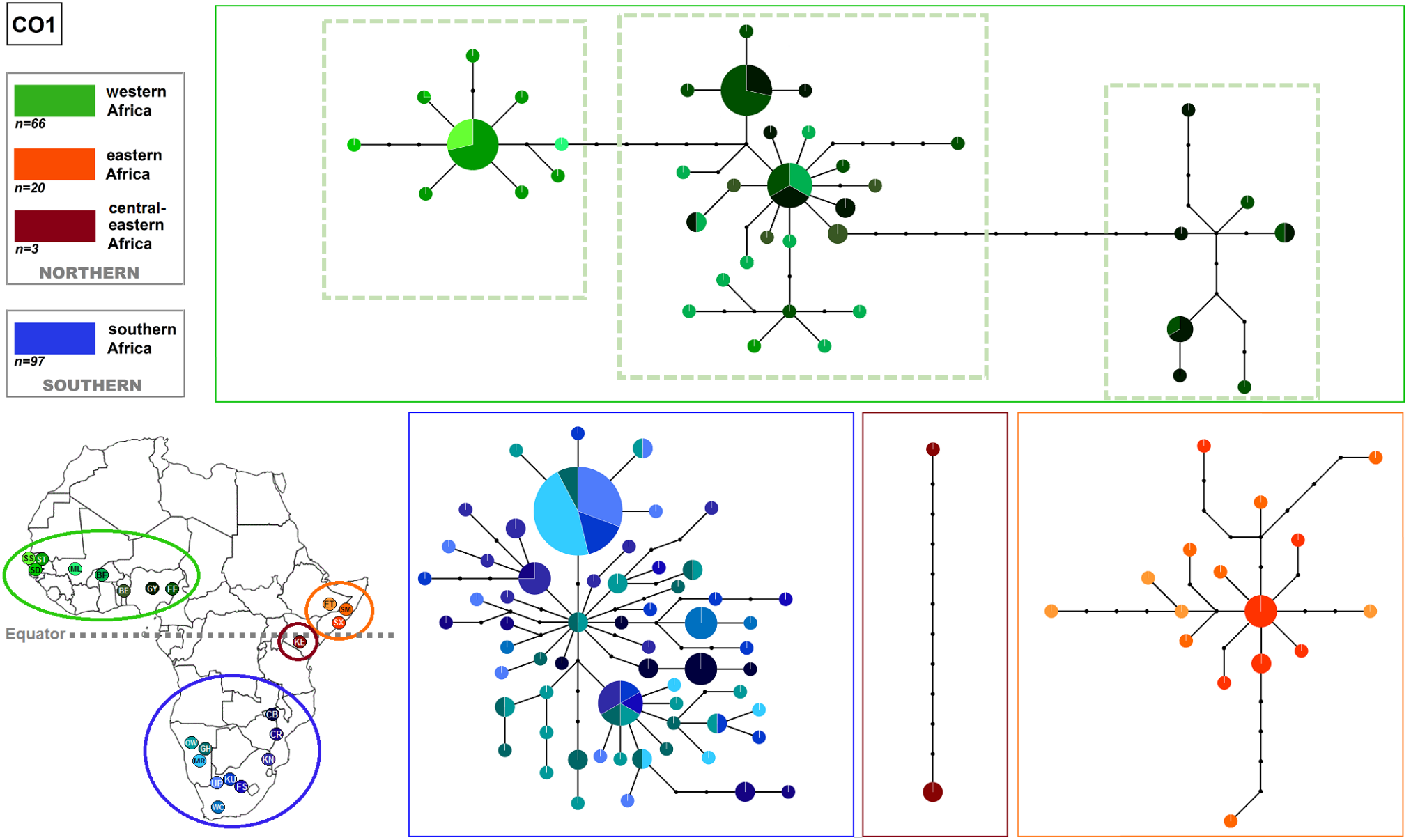


Figure 2.2: *Cytochrome c oxidase I statistical parsimony network where circle sizes represent relative frequencies of haplotypes and the number of site changes are indicated by solid dots. Colours correspond to those on the map.*

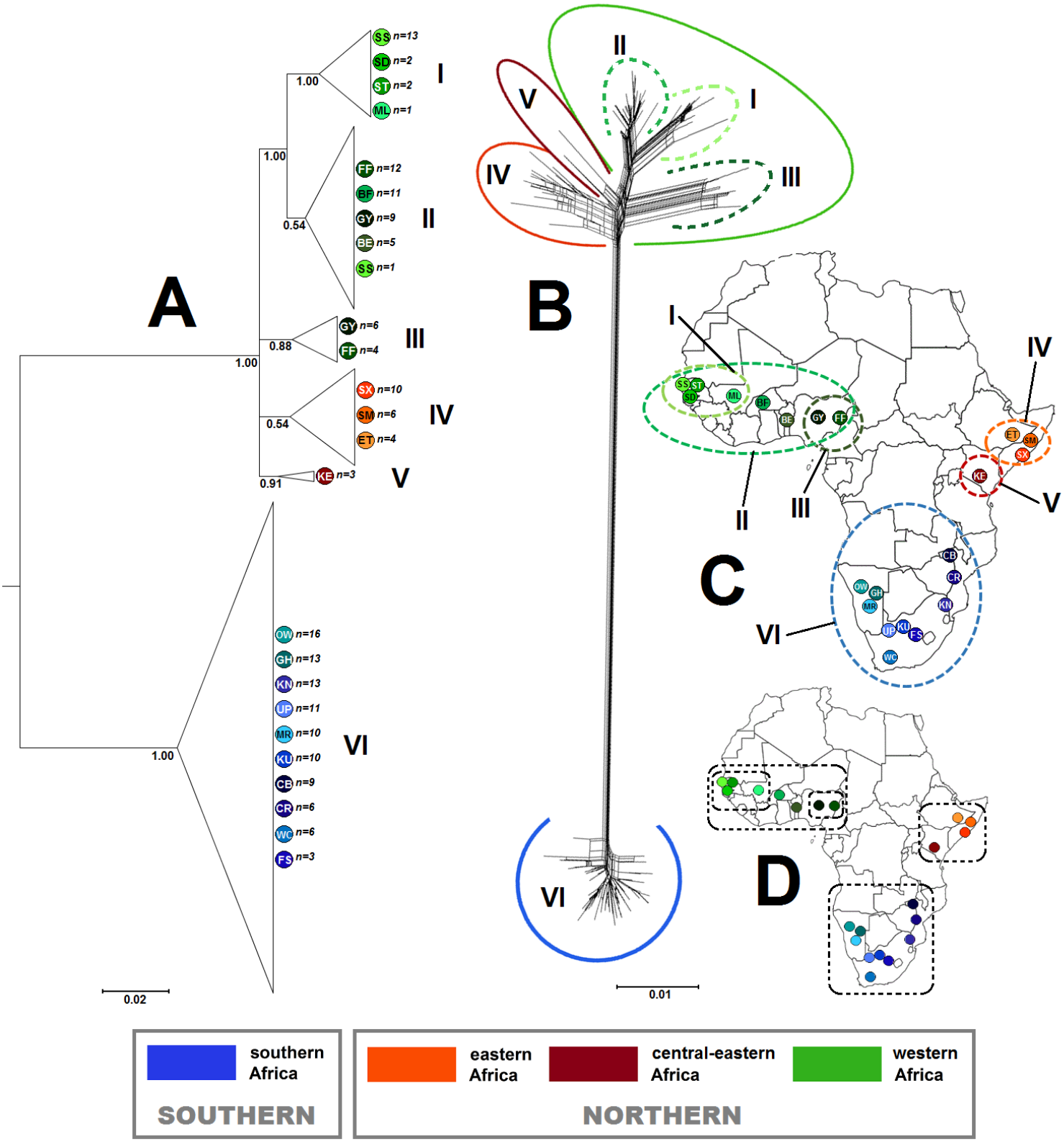


Figure 2.3: A: *Bayesian topology based on the COI gene region. Bayesian posterior- probabilities are indicated below the branches. Colour circles are indicative of the sampling locality (Fig 2.1) and "n" the number of haplotypes within the phylogroup. Phylogroups are listed "I-VI" and correspond to those seen in the Neighbour-Net phylogenetic network and map.* ***B:*** *COI Neighbour-Net phylogenetic network for H. truncatum showing strong disparity between northern and southern regions/lineages. Multiple connections represent possible conflict due to ambiguous signals in the data. Haplotype groupings are labelled according to the results of the statistical parsimony network (see Fig 3.2).* ***C:*** *Map of Africa indicating the locations of the six phylogroups as found by haplotype networks and the Bayesian topology.* ***D:*** *Map of Africa indicating the locations of the five groups as established by BAPS.*

Due to the strong inter-regional structure, yet weak intra-regional structure, a subset of samples used for regional COI analyses were investigated at the nDNA level (Sup. Table 1). A 267 bp potion of the H3 gene, representing 72 individuals (144 Phased alleles) revealed 57 unique haplotypes (GenBank accession numbers: KT999646-KT999717). Unlike the COI data, unique haplotypes did not confirm the geographic genetic structure and 19 haplotypes were shared among the three mtDNA clades considered. Similar to COI, haplotypic diversity (*h*) for the H3 gene was congruent among sampling regions: southern, 0.967 (SD ± 0.013); eastern, 0.966 (SD ± 0.018); western, 0.959 (SD ± 0.010). H3 nucleotide diversity (π) within *H. truncatum* across Africa was found to be 0.020% (SD ± 0.011%), while intra-regionally this was: southern 0.018% (SD ± 0.010%); eastern, 0.021% (SD ± 0.011%); western, 0.019% (SD ± 0.010%).

A 516 bp portion of the CRT gene region, for 66 individuals (132 Phased alleles), revealed 53 unique haplotypes (GenBank accession numbers: KT999580-KT999645). Very much the same as for H3, CRT haplotypes were not restricted to regions, and seven haplotypes were shared between regions. Intra-regional CRT haplotypic diversity (*h*) was found to be: southern, 0.955 (SD ± 0.013); eastern, 0.942 (SD ± 0.037); western, 0.865 (SD ± 0.029). Overall nucleotide diversity (π) for *H. truncatum* across Africa was noted at 0.007% (SD ± 0.004%) and intra-regional: southern, 0.006% (SD ± 0.004%); eastern, 0.008% (SD ± 0.005%); western, 0.005% (SD ± 0.003%).

When the nuclear DNA data was a-priori assigned to the same geographic regions than that obtained for the COI data both nDNA genes' AMOVA results indicated non-significant Φst ($p > 0.05$) values of population differentiation between the western and eastern African regions (Table 2.3). However these two regions showed significant Φst differentiation from southern Africa ($p \leq 0.5$) (Table 2.3). Interestingly TCS haplotype networks based on H3 and CRT regions, showed very little structure with regard to haplotype portioning between sampling regions, although greater haplotype sharing seem to be evident between western and eastern Africa (Figs 2.4 and 2.5): Equally weighted to account for differences in population size, 65.12% of the H3 haplotypes between western Africa and eastern Africa are shared. Southern Africa and western Africa only share 39.39% of their haplotypes and southern Africa

and eastern Africa share 33.75% of their haplotypes. Similarly, with CRT, 43.79% of haplotypes between western Africa and eastern Africa are shared, while again those between southern Africa and western Africa equates to 29.61% and 29.04% respectively.

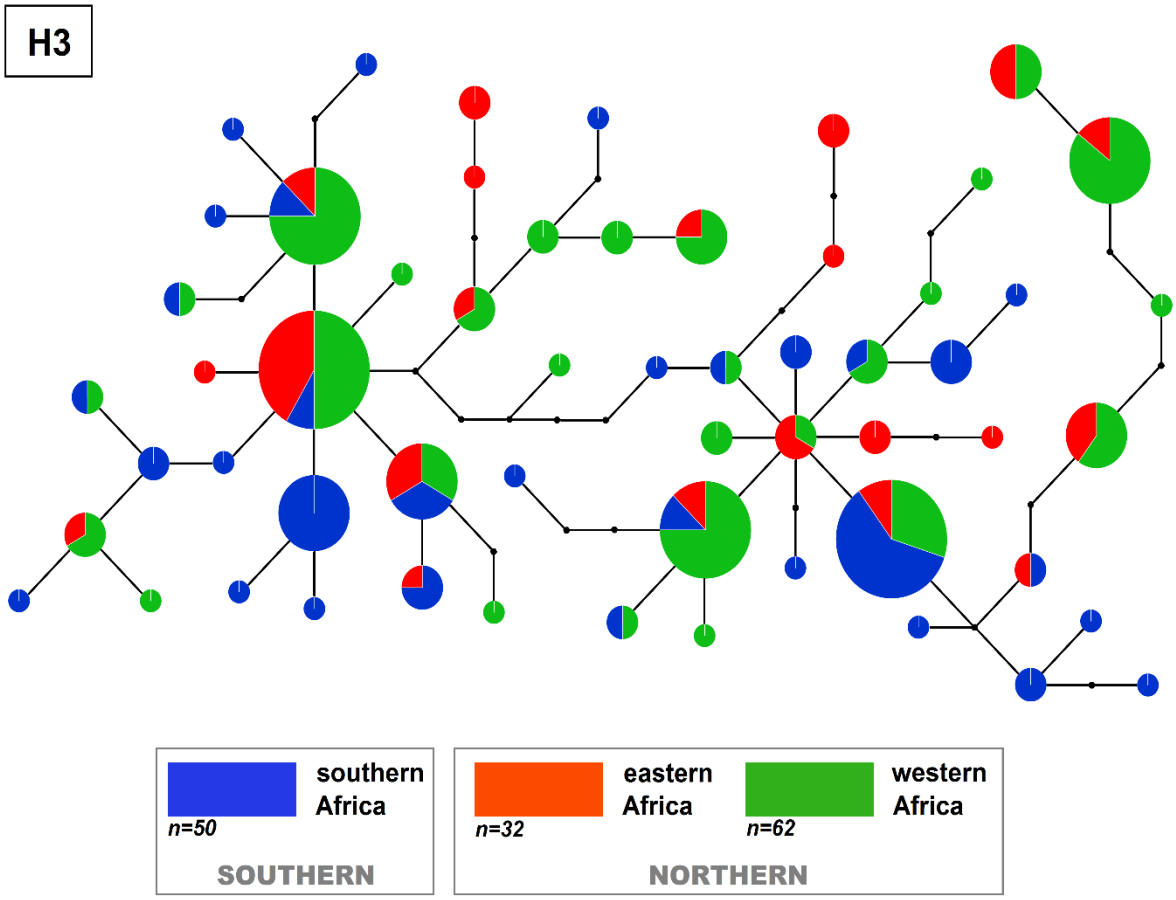


Figure 2.4: *Histone 3 statistical parsimony network where circle sizes represent relative frequencies of haplotypes and the number of site changes are indicated by solid dots. Colours correspond to those on the map (see Fig 2.2).*

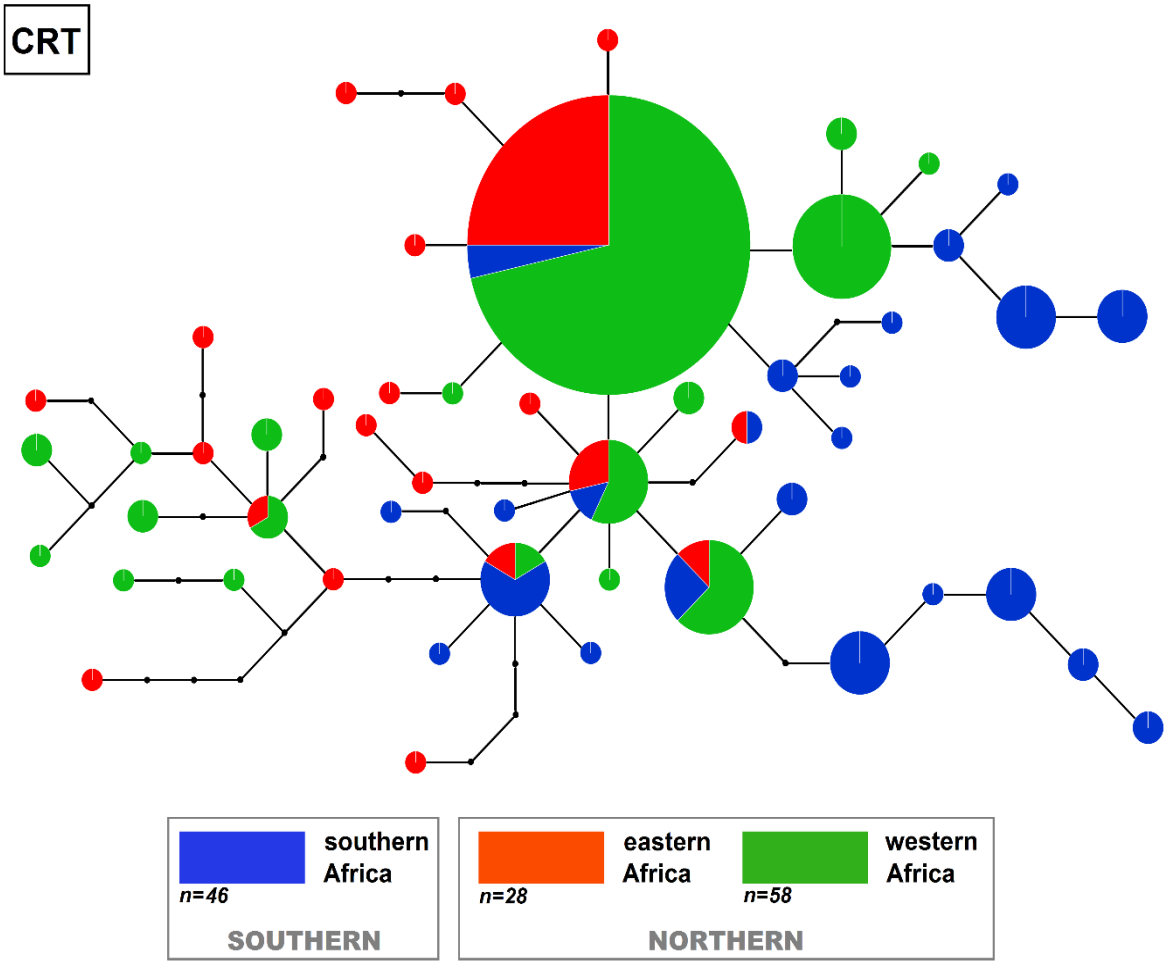


Figure 2.5: *Calreticulin statistical parsimony network where circle sizes represent relative frequencies of haplotypes and the number of site changes are indicated by solid dots. Colours correspond to those on the map (see Fig 2.2).*

Table 2.3: *nDNA: Histone 3 (Above the diagonal) and Calreticulin (Below the diagonal) AMOVA results showing the pairwise Φst values with p-values (below) for regional populations comparisons (significant p ≤ 0.05, based on corrected p-values, are highlighted in **bold**). The central-east African clade was omitted due to limited samples.*

CRT & H3	southern Africa	western Africa	eastern Africa
southern Africa	X	**0.092**	**0.086**
	X	**p≤0.05 (+)**	**p≤0.05 (+)**
western Africa	**0.222**	X	0.003
	p≤0.05 (+)	X	p>0.05 (-)
eastern Africa	**0.187**	0.035	X
	p≤0.05 (+)	p>0.05 (-)	X

3.2 Ancestral population demographics

Based on high COI sequence distances differentiating the two lineages, it was necessary to test whether the two genetic assemblages (northern and southern Africa) experienced the same population demographics. Interestingly there is a significant and strong indication of IBD for northern African *H. truncatum* (R^2 = 0.418, p ≤ 0.05) (Sup. Fig 1), while the southern African lineage of *H. truncatum* projects a significant, but weak indication of IBD (R^2 = 0.028, p ≤ 0.05) (Sup. Fig 2).

The wide occurrence of a common COI haplotypes among sampling localities within the individual phylogroups (regions), and shared H3 and CRT haplotypes between phylogroups would suggest possible recent historical expansion events. This is supported by negative and significant Fu's Fs values for both species (northern: COI Fu's Fs = -24.30, p ≤ 0.05: H3 Fu's Fs = -18.58, p ≤ 0.05: CRT Fu's Fs = -26.18, p ≤ 0.05) (southern: COI Fu's Fs = -25.53, p ≤ 0.05: H3 Fu's Fs = -23.62, p ≤ 0.05: CRT Fu's Fs = -14.45, p ≤ 0.05) coupled to the majority of mismatch distribution results for each set of DNA sequences (northern: COI SSD = 0.01, p > 0.05: H3 SSD = 0.00, p > 0.05: CRT SSD = 0.21, p ≤ 0.05) (southern: COI SSD = 0.01, p ≤ 0.05: H3 SSD = 0.00, p > 0.05: CRT SSD = 0.00, p > 0.05). Bayesian Skyline Plots, using the optimal model (HKY + G) indicates noticeable population expansions for both lineages (Fig 2.6): In southern Africa expansion seems to have been more gradual with a smaller expansion event around 100 thousand years ago (Kya), while in northern Africa, a clear expansion event likely occurred just over 200 Kya (Fig 2.6).

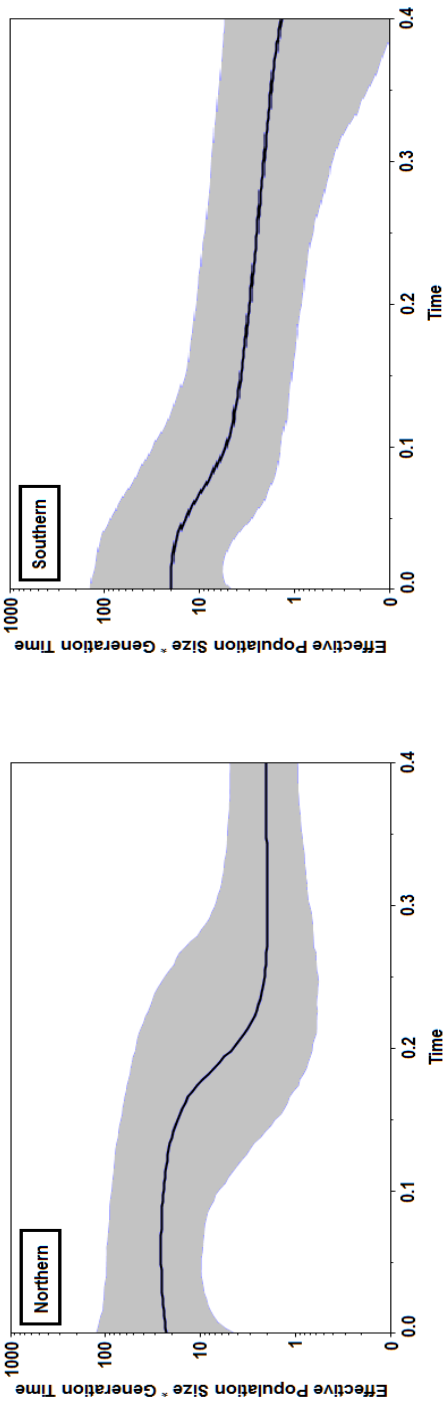


Figure 2.6: *Bayesian Skyline Plots indicating demographic population expansions for the northern (left) and southern (right) lineage of H. truncatum. Time is indicated in millions of years ago (Mya).*

4. Discussion

Evident from the study is that *H. truncatum* can be divided into two major geographically distinct lineages that also do not share similar population demographics through mtDNA analyses (Figs 2.3 and 2.6). These two major lineages are also supported by with nDNA AMOVA analyses. Additionally, mtDNA COI analyses, at the finer scale, identified four major groups, some of which there is further evidence for substructure. Life history characteristics of *H. truncatum* are unlikely drivers of the observed phylogeographic structure: at least when considering the marked differences in the phylogeography when compared to *H. rufipes* (see Cangi et al. 2013). For one, *H. truncatum* does not display a similar genetic break within southern Africa (see Cangi et al. 2013). In fact, it is more likely that climatic effects and biogeographic barriers have contributed to the structure observed in *H. truncatum*. The dispersal capabilities of ticks off the host is often limited (Randolf 1998; Anderson and Magnarelli 2008; Cangi et al. 2013) and it is thus reasonable to suggest that the dispersal of the ticks can be attributed to long distance host dispersal. Indeed, dispersal within geographic clades seems to be high since haplotypes are shared among localities within geographic regions (despite sometimes being isolated by large distances). At the continental scale, the species show a pattern more congruent with vicariant barriers known to effect the distribution of multiple host species (Matthee and Robinson 1997; van Alphen-Stahl, Bloomer, and Crowe 2005; Moodley and Bruford 2007; Lorenzen, Heller, and Siegismund 2012; Colangelo et al. 2013;).

In the northern *H. truncatum* lineage substructure shows congruence with specific geographic areas. Up to five phylogroups can be recognized across northern Africa and the genetic pattern obtained here can be coupled to significant indications of isolation by distance ($R^2 = 0.418$, $p \leq 0.05$). The formation of most of Africa's mountain ranges and drainage systems are probably too old to have played a major role in the phylogeographic structure observed across northern Africa (Halliday et al. 1988; Burke 2001; Stankiewicz and Wit 2006; Moore et al. 2007; Bryja et al. 2010; Wichura et al. 2010). Rather the phylogeographic structure is more likely a direct cause of more relatively recent glacial cycles, restricting host movement. In west Africa, Booth (1958) already hypothesized that three areas of faunal refugia exist

and their contemporary boundaries are the Volta and Niger rivers. The refugia are, west of the Volta River, another east of the Niger River, and a third between the Niger and Volta rivers. Interestingly, a number of host species such as rodents (Nicolas et al. 2008; Brouat et al. 2009; Bryja et al. 2010; Colangelo et al 2013), primates (Harcourt 2012; Zinner et al. 2013), ungulates (Moodley and Burford 2007) and even birds (Fuch and Bowie 2015) support this pattern and show similar breaks according to the rivers. It is thus likely that the phylogeographic signature depicted by *H. truncatum* over western Africa is the effect of the isolation of host species. If this holds, the Volta river, which runs from Burkina Faso to the Atlantic Ocean through Ghana, has likely acted as the contemporary barrier between phylogroups I and II. The Niger River likely has a similar effect between phylogroups II and III. Similarly, the eastern African phylogroup (IV) and the central-eastern African phylogroup (V) were probably also driven and maintained by host divergence in the region, caused by habitat shifts around the East African Rift Valley and associated lakes (see Arctander, Johansen, and Coutellec-Vreto 1999; Girman et al. 2001; Colangelo et al. 2013). However, it is important to note that the presence of phylogroup (V) is disputable according to BAPS analyses (D; Fig 2.3), this is probably due to the limited sampling in the region, and thus conclusions based on the divergence of these two east African phylogroups should be treated cautiously. Limited sampling over central-northern Africa, means it is difficult to conclude on specific barriers to gene flow between west and east parts of the continent, however host gene flow between west and east Africa have been largely governed by three major barriers across the region: 1) Guineo-Congolian and Cameroonian highland forests; 2) the Cameroon volcanic line; 3) the East African Rift Valley and associated lakes (see Moodley and Bruford 2007; Lorenzen, Heller, and Siegismund 2012; Colangelo et al. 2013 and references therein). These may have thus acted as similar contemporary barriers to gene flow between *H. truncatum* phylogroups in western Africa (I, II and III) and those on the eastern side (IV and V) (Fig 2.3).

Unlike northern Africa, in southern Africa, there are limited indications of geographical partitioning among *H. truncatum* haplotypes. Instead, southern Africa is characterized by weak IBD ($R^2 = 0.028$, $p \leq 0.05$), more individuals sharing common haplotypes, and common haplotypes are found at distant localities within the region (Fig 2.2). Although this supports the hypothesis that the phylogeography of *H.*

truncatum in southern Africa may also have been greatly affected by glacial cycles and habitat oscillations over the last 400,000 years (van Zinderen Bakker 1978; Partridge 1997; Dynesius and Jansson 2000; deMenocal 2004), the data suggest that these events are more recent. The lack of structure suggest that the vicariant barriers documented for this region (Matthee and Flemming 2002; Moodley and Buford 2009; du Toit et al. 20012; Schwab et al. 2012) did not affect *H. truncatum* in the same way as in north Africa. Interestingly, despite the well documented barriers to dispersal for some host species, exceptions exist in multimammate mice, *Mastomys* spp. (Sands et al. 2015), springhare, *Pedetes capensis* (Matthee and Robinson 1997), yellow mongoose, *Cynictis penicillata* (van Vuuren and Robinson 1997), cape buffalo, *Syncerus caffer* (Van Hooft, Groen, and Prins 2002), cheetah, *Acinonyx jubatus* (Charruau and Fernandes 2011) and several other savannah ungulates (Lorenzen, Heller, and Siegismund 2012) occuring in this region. Additionally Bayesian Skyline Plots of demographic population expansion events for *H. truncatum* also support a recent range expansion that postdate the vicariance events documented (Matthee and Flemming 2002; du Toit et al. 20012; Schwab et al. 2012).

An interesting phenomenon to also consider is that the demographic expansion detected in southern Africa also correlates with more recent expanses of grasslands and the subseqeunt movement of domesticated agricultural livestock breeds (Fig 2.6) (see van Zinderen Bakker 1978; Bradley et al. 1996; Luikart et al. 2001; Bruford, Bradley, and Luikart 2003; Jacobs 2004; Relethford 2008; Kalinowski 2011). The fact that ticks were collected solely from domestic animals may account for passive dispersal of ticks in the southern African region and thus also contribute towards the lack of phylogenetic patterning observed. However similar effects would then also be expected for northern Africa and thus it would not account for the more pronounced phylogeographic structure observed for *H. truncatum* across northern Africa, nor explain the strong phylogenetic disparity between the two lineages of *H. truncatum* (northern and southern).

The far higher level of divergence between the northern African phylogroups (I, II, III, IV and V) and the single southern African phylogroup (VI) in *H. truncatum* is noteworthy and potentially points to the existence of two species. Seasonal shifts in

the activity of *I. ricinus* populations in Europe and north Africa have been suggested as the major driver of genetic diversity in this tick species (Noureddine, Chauvin, and Plantard 2011). In addition, Ogden, Mechai and Margos (2013) modelled climate as a major factor affecting the ranges of ticks due to their effects on life cycles. As in the case of *Hyalomma,* it has been noted that adult activity in the genus is more prevalent during the warmer months (Yousfi-Monod and Aeschlimann 1986; Boulkaboul 2003) which is also supported by the higher prevalence for Crimean-Congo haemorrhagic fever during these times (Bente et al. 2013). Since *H. truncatum* occurs in two different hemispheres, they may have developed different breeding cycles and this would further contribute as a potential postmating isolating mechanism. Irrespective, Zhang and Zhang (2014), proposed that the COI gene may be very useful in distinguishing ixodid tick species and further suggested that the average interspecific sequence distance among recognised species is around 8%, while intraspecifically this is generally regarded as < 2%. If this holds, the current recognition of *H. truncatum* as single species is incorrect. The 9.88% sequence distance between the northern and southern lineages and the < 2% seen intraspecifically in each lineage would be far better compared to values obtained between and within well-established species. This is further supported by our nDNA AMOVA results (Table 2.3). CRT and H3 AMOVA analyses indicate no population differentiation among northern populations, while strong differentiation to the southern lineage (Table 2.3). However, at this stage the taxonomy of *H. truncatum* is complicated. The taxon forms part of a species complex and shares a close morphological relationships with *H. albiparmatum* and *H. nitidum* (see Apanaskevich 2008b and references therein). It is thus critically important that a molecular review of the genus take place to resolve the taxonomy of *H. truncatum*.

This study could also have importance for animal health. The close evolutionary relationships among all *H. truncatum* in northern Africa (≤ 1.84% COI sequence distance) might mean that ticks in central-eastern and eastern Africa may be equally likely to be potential vectors of pathogens associated with *H. truncatum* in western Africa and vice versa. The viruses, such as Bhanja (a *Phlebovirus* implicated in Meningoencephalitis and partial paralysis), Dugbe (a *Nairovirus*) and Jos (a *Thogotovirus* implicated in cell necrosis), and causative organism of Q-fever, Kenyan tick typhus, as well as certain *Theileria* species have all been suggested to be

carried by *H. truncatum* across north Africa (Heisch et al. 1962; Hoogstraal, Wassef, and Buttiker 1981). Additionally a number of pathogens have been attributed to *H. truncatum* in southern Africa; sweating sickness (Bezuidenhout and Malherbe 1981) and *Babesia caballi*, that causes equine piroplasmosis (De Waal 1990) and it is not ascertained whether the genetically distinct lineages in fact both harbour and spread all these diseases.

CHAPTER 3

SYSTEMATICS OF THE PARASITIC TICK GENUS, *HYALOMMA* (Ixodida: Ixodidae: Hylomminae), USING A MULTI-DISCIPLINARY APPROACH

1. Introduction

Ticks within the genus *Hyalomma* are obligate haematophagus ectoparasites of many wild and domesticated animals, including humans. Members of the genus are of immense medical and veterinary importance (see Heisch et al. 1962; Taboada and Merchant 1991; Aktas, Dumanli, and Angin 2004; Norval et al. 2004; Formosinho and Santos-Silva 2006; Bente et al. 2013) and different species and lineages of *Hyalomma* have been shown to vary in acaricidal resistance and vector potential (see Hoogstraal and Aeschlimann 1982; Swanepoel and Burt 2004; Shyma et al. 2012; Gou et al. 2013).

The taxonomic description of *Hyalomma* is based mainly on morphology (see Apanaskevich and Horak 2005; Apanaskevich and Horak 2006; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Santos-Silva, and Horak 2008; Apanaskevich, Schuster, and Horak 2008; Apanaskevich and Horak 2009; Apanaskevich, Filippova, and Horak 2010; Apanaskevich and Horak 2010), with genetic investigations primarily focussing at the relationships among tick genera (see Black and Piesman 1994; Black, Klompen, and Keirans 1997; Mangold, Bargues, and Mas-Coma 1997; Mangold, Bargues, and Mas-Coma 1998; Klompen et al. 2000; Murrell, Campbell, and Barker 2001; Barker and Murrell 2004). In fact, to date the most comprehensive phylogeny of the genus only includes five representatives of *Hyalomma* (see Barker and Murrell 2004; Zhang and Zhang 2014).

The generally low levels of interspecific morphological differentiation, coupled to high levels of intraspecific variation in *Hyalomma* (see Howell 1978; Walker 1991; Pretorius and Clarke 2000), are causing significant vaccilations in the taxonomy of the group (Camicas et al. 1998; Murrell, Campbell, and Barker 2001; Rees, Dioli, and Kirkendall 2003; Barker and Murrell 2004; Apanaskevich and Horak 2006; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Schuster, and Horak 2008; Apanaskevich, Filippova, and Horak 2010; Guglielmone and Nava 2014). There are furthermore clear indications of hybridization between recognized morpho-species (Rees, Dioli, and Kirkendall 2003; Dalal, Kumar, and Gupta 2007). However using morphological characters to identify the species may

be less fruitful than previously thought: Lv et al. (2014) suggested engorgement of ticks may cause structural changes that could complicate species assignment, and other anomalies such as gynandromorphism (where both male and female characteristics are simultaneously displayed in an organism) has been noted in a wide range of *Hyalomma* (see Buczek, Bartosik, and Buczek 2014; Chen et al. 2015; Kar et al. 2015; Keskin, Bursali, and Tekin 2012). In addition, problems may exist on reference databases such as GenBank due to the incorrect identification of *Hyalomma* species in previous literature (Zhang and Zhang 2014). Finally, the genus is characterized by several species complexes and species with wide ranges that may show cryptic lineages (Chapter 2; Cangi et al. 2013; Zhang and Zhang 2014).

Based on a morphological review of the genus, a number of close relationships among species have been proposed (see Delpy 1949; Tendeiro 1955; Hoogstraal 1956; Hoogstraal and Kaiser 1959; Hoogstraal, Wassef, and Buttiker 1981; Camicas et al. 1998; Apanaskevich 2004; Apanaskevich and Horak 2005; Apanaskevich and Horak 2006; Apanaskevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Santos-Silva, and Horak 2008; Apanaskevich, Schuster, and Horak 2008; Apanaskevich and Horak 2009; Apanaskevich, Filippova, and Horak 2010). For instance, Delpy (1949) and Tendeiro (1955) suggested *H. somalicum* and *H. excavatum* to be closely related species, however a review by Hoogstraal and Kaiser (1959) suggests *H. somalicum* likely represent a subspecies of *H. impeltatum*. This was further corroborated by Apanaskevich and Horak (2009) and Apanaskevich, Schuster and Horak (2008) who suggested that the species should remain distinct entities, and that they likely form a close relationship within the group of species containing *H. asiaticum*, *H. dromedarii*, and *H. schulzei*. Additionally Filippova (2003) indicated that *H. scupense* may be closely related to *H. marginatum* (at the time considered to be in a complex with *H. glabrum*, *H. isaaci*, *H. rufipes* and *H. turanicum*, which were later re-instated as valid species; see Apanaskevich and Horak 2006; Apanaskevich and Horak 2008a). Complex relationships are not limited to the aforementioned examples. *Hyalomma lusitanicum* has been considered either synonyms or subspecies of both *H. aegypticum* and *H. excavatum* (Neumann 1899; Deply 1949; Hoogstraal 1956). Interestingly, *H. franchinii* has also been synonymised under the latter species (Delpy 1949; Tenderio 1955). But finally, Hoogstraal and Kaiser (1959) afforded *H. excavatum*, *H. franchinii* and *H.*

lusitanicum individual species status and proposed that they likely form a close relationship within a group further consisting of *H. anatolicum*. This hypothesis has been recently corroborated by Apanaskevich and Horak (2005) and Apanaskevich, Santos-Silva and Horak (2008). Lastly, *H. albiparmatum*, *H. impressum*, *H. nitidum* and *H. truncatum* have been proposed to be in a very close relationship due to the lack of morphological disparity between species (Apanaskevich and Horak 2008b). In fact, the individual species status of *H. albiparmatum*, *H. nitidum* and *H. truncatum* have been subjected to much debate in the literature (see Hoogstraal 1956; Feldman-Muhsam 1962; Walker 1974; Hoogstraal 1979; Camicas et al. 1998; Tomassone et al. 2005; Apanaskevich and Horak 2008b). A recent phylogeographic study on *H. truncatum* (see chapter 2) observed two lineages of *H. truncatum* which have a similar sequence divergence between them than that reported for other recognized species. Resolving this issue is particularly pertinent since the species validity of *H. albiparmatum*, *H. nitidum* and, to a lesser extent, *H. impressum* (based on morphology) have recently been questioned (see Apanaskevich and Horak 2008b).

Irrespective ofthe problems associated with the taxonomy of *Hyalomma*, at present it is believed that the genus comprises 27 recognized species (Table 3.1). Members of the genus are geographically widespread across the Afrotropical, Palearctic and Oriental Zoogeographic Regions (Kolonin 1982). It has been suggested that the genus originated in the Oriental region 19 Mya (Balashov 1994; Murrell, Campbell, and Barker 2001) but a later discovery of an older fossil in the Baltic area dates the genus back to at least 35-50 Mya (de la Fuente 2003). What gave rise to the diversity of species characterising *Hyalomma* is completely unknown at present.

Since members of the genus are mostly generalist ectoparasites occurring on a multitude of host species during different life stages (Apanaskevich 2004) the role of host diversification within this genus is probably negligible (Casati et al. 2008; Kempf et al. 2009; Noureddine, Chauvin, and Plantard 2011; Beati et al. 2013; Cangi et al. 2013; van der Mescht, Matthee, and Matthee 2015). Instead, we propose that large scale abiotic changes that will influence the majority of host species in the same way (vicariance), will more than likely pose barriers to dispersal to multiple hosts and this in turn may result in allopatric speciation processes within the genus *Hyalomma*

(Matthee et al. 2004; Heikinheimo et al. 2007; Kempf et al. 2009; Lorenzen, Heller, and Siegismund 2012).

The tectonic uplift due to colliding plates and varying sea levels around the continental meeting points of Africa, Asia and Europe have often been recorded in early faunal exchange events, it is thus likely that changes in the Mediterranean Sea and other water bodies surrounding the Arabian peninsula could have acted as early mechanisms for allopatric speciation, especially between zoogeographic regions (see Rögl 1999; Matthee et al. 2004; Koufos, Kostopoulos, and Vlachou 2005; Gaubert and Cordeiro-Estrela 2006; Harzhauser et al. 2007; Sen 2013). While within regions, the formation of mountains (such as with the Himalayas, East African Rift Valley and those around the Baltic region; and the development of lakes and drainage systems associated with the uplift) may have played key roles in restructuring the landscape and driving diversification among a variety of taxa post the Oligocene (Matthee and Robinson 1997; Sanmartin 2003; Lou et al. 2004; Moodley and Burford 2007; Pisano et al 2015). Additionally, it has been suggested that seasonal changes across the equator may have also acted as a contemporary barrier within *Hyalomma* species through impacts on breeding cycles (see chapter 2 and references therein). Importantly, the influences of further climatic changes (Randolph 1997; Beati et al. 2013; Medlock et al. 2013; Ogden, Mechai, and Margos 2013) and other life history traits, such as the availability of suitable hosts in the environment and competition (Kempf et al. 2009; Cangi et al. 2013; Ogden, Mechai, and Margos 2013), should not be ignored as part of the mechanisms responsible for tick speciation, but it is proposed that these events are effecting the more recent intraspecific divergences within species (see Chapter 2).

To address the mechanisms involved in shaping the evolution of *Hyalomma,* and to test the various taxonomic hypotheses proposed for the genus, we constructed a phylogeny based on comprehensive taxonomic sampling. A combination of data derived from morphological, mtDNA and nDNA markers were used. We hypothesize that species complexes may be fairly common within the genus, particularly among members with low levels of morphological variation (*H. truncatum*, *H. albiparmatum* and *H. nitidum*; Apanaskevich and Horak 2008b), and those suggested to be able to hybridise (*H. truncatum, H. rufipes, H. dromedarii* and *H. anatolicum* (Dalal, Kumar,

and Gupta 2007; Rees, Dioli, and Kirkendall 2003). We also hypothesize that *Hyalomma* likely evolved in the Oriental region and diversified outwards from the Palearctic region. If the latter holds, the pattern and dates of the divergences will co-inside with large scale abiotic changes affecting a large number of host species that showed pulses of intercontinental exchanges (for example see Matthee et al. 2004 and references therein). It is proposed that the outcome of this study will significantly improve our current understanding of the number of species within the genus (which is also important for disease ecology and control) and by including a molecular clock, we may be able to provide additional evidence to explain the mechanisms involved in tick evolution in general.

2. Materials & Methods

2.1 Sampling design

Hyalomma specimens were collected from the same hosts as described in Chapter 2 and also from domestic, buffalo (*Bubalus bubalis*) and wild one and two humped camels (*Camelus sp.*), Arabian oryx (*Oryx leucoryx*), European hedgehog (*Erinaceus sp*.), white rhinoceros (*Ceratotherium simum*), and the spur-thighed tortoise (*Testudo graeca*) among others. Eighty-two *Hyalomma* specimens, representing all 27 known species from 26 different countries were included (Table 3.1). It was aimed to include at least three representatives of each of the recognised species covering as much of the geographic variation as possible for each species (Table 3.1). All freshly collected specimens were placed in 100% ethanol for further analysis. Outgroups (*Amblyomma variegatum* and *Nossoma monstrosum*) were chosen based on their published close taxonomic relationships with *Hyalomma* (Murrell, Cambell, and Barker 2000; Murrell, Cambell, and Barker 2001). Mitochondrial DNA GenBank sequences for eight *Hyalomma* species were also available (Sup. Table 2). These sequences formed part of previous studies and were included to cross reference the sampled members of the *Hyalomma* genus.

Table 3.1: *Sampling localities for Hyalomma and respective outgroups included in the present study. The letters "a", "b" or "c" following the country of origin are indicative of the collaborating authority responsible for providing the sample: (a) Dmitry A. Apanaskevich, United States National Tick Collection, Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia, 30460-8056, USA. (b) Ivan G. Horak, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa. (Current genus scrutiny: Hyalomma). (c) Sonja Matthee, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Stelenbosch, 7602, South Africa.*

No.	Species	Country of origin	GenBank accession no.
	Hyalomma		
1	*H. aegyptium*	Israel (a)	KU130407, KU130490, KU130573, KU130656, KU130737
2	*H. aegyptium*	Israel (a)	KU130408, KU130491, KU130574, KU130657, KU130738
3	*H. aegyptium*	Israel (a)	KU130409, KU130492, KU130575, KU130658, KU130739
1	*H. albiparmatum*	Kenya (a)	KU130410, KU130493, KU130576, KU130659, KU130740
2	*H. albiparmatum*	Kenya (a)	KU130411, KU130494, KU130577, KU130660, KU130741
3	*H. albiparmatum*	Kenya (a)	KU130412, KU130495, KU130578, KU130661, KU130742
1	*H. anatolicum*	Iraq (a)	KU130413, KU130496, KU130579, KU130662, KU130743
2	*H. anatolicum*	Pakistan (a)	KU130414, KU130497, KU130580, KU130663, KU130744
3	*H. anatolicum*	Pakistan (a)	KU130415, KU130498, KU130581, KU130664, KU130745
1	*H. arabica*	Saudi Arabia (a)	KU130416, KU130499, KU130582, KU130665, KU130746
2	*H. arabica*	Saudi Arabia (a)	KU130417, KU130500, KU130583, KU130666, KU130747
3	*H. arabica*	Saudi Arabia (a)	KU130418, KU130501, KU130584, KU130667, KU130748
1	*H. asiaticum*	Turkmenistan (a)	KU130419, KU130502, KU130585, KU130668, KU130749
2	*H. asiaticum*	Turkmenistan (a)	KU130420, KU130503, KU130586, KU130669, KU130750
3	*H. asiaticum*	Turkmenistan (a)	KU130421, KU130504, KU130587, KU130670, KU130751
1	*H. brevipunctatum*	India (a)	Morphological data only
1	*H. dromedarii*	Iraq (a)	KU130422, KU130505, KU130588, KU130671, KU130752
2	*H. dromedarii*	Pakistan (a)	KU130423, KU130506, KU130589, KU130672, KU130753
3	*H. dromedarii*	Saudi Arabia (a)	KU130424, KU130507, KU130590, KU130673, KU130754
4	*H. dromedarii*	Senegal (a)	KU130425, KU130508, KU130591, KU130674, KU130755
1	*H. excavatum*	Israel (a)	KU130426, KU130509, KU130592, KU130675, KU130755
2	*H. excavatum*	Israel (a)	KU130427, KU130510, KU130593, KU130676, KU130756
3	*H. excavatum*	Tunisia (a)	KU130428, KU130511, KU130594, KU130677, KU130757
4	*H. excavatum*	Israel (a)	KU130429, KU130512, KU130595, KU130678, KU130758
1	*H. franchinii*	Egypt (a)	Morphological data only
1	*H. glabrum*	South Africa (a)	KU130430, KU130513, KU130596, KU130679, KU130759
2	*H. glabrum*	South Africa (a)	KU130431, KU130514, KU130597, KU130680, KU130760
3	*H. glabrum*	South Africa (c)	KU130432, KU130515, KU130598, KU130681, KU130761
1	*H. hussaini*	Pakistan (a)	KU130433, KU130516, KU130682, KU130762
1	*H. hystricis*	India (a)	Morphological data only
1	*H. impeltatum*	Senegal (a)	KU130434, KU130517, KU130599, KU130683, KU130763
2	*H. impeltatum*	Saudi Arabia (a)	KU130435, KU130518, KU130600, KU130684, KU130764
3	*H. impeltatum*	Senegal (a)	KU130436, KU130519, KU130601, KU130685, KU130765
1	*H. impressum*	Benin (a)	KU130437, KU130520, KU130602, KU130686, KU130766
2	*H. impressum*	Benin (a)	KU130438, KU130521, KU130603, KU130687, KU130767
1	*H. isaaci*	Pakistan (a)	KU130439, KU130522, KU130604, KU130688, KU130768
2	*H. isaaci*	Sri Lanka (a)	KU130440, KU130523, KU130605, KU130689, KU130769
3	*H. isaaci*	Pakistan (a)	KU130441, KU130524, KU130606, KU130690, KU130770
1	*H. kumari*	Pakistan (a)	KU130442, KU130525, KU130607, KU130691, KU130771
2	*H. kumari*	Pakistan (a)	KU130443, KU130526, KU130608, KU130692, KU130772
1	*H. lusitanicum*	Portugal (a)	KU130444, KU130527, KU130609, KU130693, KU130773
2	*H. lusitanicum*	Italy (a)	KU130445, KU130694, KU130774
1	*H. marginatum*	Ukraine (a)	KU130446, KU130528, KU130610, KU130695, KU130775
2	*H. marginatum*	Portugal (a)	KU130447, KU130529, KU130611, KU130696, KU130776
3	*H. marginatum*	Russia (a)	KU130448, KU130530, KU130612, KU130697, KU130777
1	*H. nitidum*	Benin (a)	KU130449, KU130531, KU130613, KU130698, KU130778
2	*H. nitidum*	Benin (a)	KU130450, KU130532, KU130614, KU130699, KU130779
3	*H. nitidum*	Benin (a)	KU130451, KU130533, KU130615, KU130700, KU130780
1	*H. punt*	Somalia (b)	KU130452, KU130534, KU130616, KU130701, KU130781
2	*H. punt*	Somalia (b)	KU130453, KU130535, KU130617, KU130702, KU130782
3	*H. punt*	Somalia (b)	KU130454, KU130536, KU130618, KU130703, KU130783
1	*H. rhipicephaloides*	Israel (a)	KU130455, KU130537, KU130619, KU130704, KU130784
2	*H. rhipicephaloides*	Israel (a)	KU130456, KU130538, KU130620, KU130705, KU130785
1	*H. rufipes*	Senegal (a)	KU130457, KU130539, KU130621, KU130706, KU130786
2	*H. rufipes*	Senegal (a)	KU130458, KU130540, KU130622, KU130707, KU130787
3	*H. rufipes*	Nigeria (a)	KU130459, KU130541, KU130623, KU130708, KU130788
4	*H. rufipes*	Burkina Fuso (a)	KU130460, KU130542, KU130624, KU130709, KU130789
5	*H. rufipes*	Somalia (b)	KU130543, KU130625, KU130710, KU130790
6	*H. rufipes*	Namibia (c)	KU130461, KU130544, KU130626, KU130711, KU130791
7	*H. rufipes*	Namibia (c)	KU130462, KU130545, KU130627, KU130712, KU130792
8	*H. rufipes*	Mozambique (c)	KU130463, KU130546, KU130628, KU130713, KU130793
9	*H. rufipes*	Mozambique (c)	KU130464, KU130547, KU130629, KU130714, KU130794
10	*H. rufipes*	South Africa (c)	KU130465, KU130548, KU130630, KU130715, KU130795
1	*H. schulzei*	Iraq (a)	KU130466, KU130549, KU130631, KU130716, KU130796
2	*H. schulzei*	Iraq (a)	KU130467, KU130550, KU130632, KU130717, KU130797
1	*H. scupense*	Russia (a)	KU130468, KU130551, KU130633, KU130718, KU130798
2	*H. scupense*	Pakistan (a)	KU130469, KU130552, KU130634, KU130719, KU130799
3	*H. scupense*	Tunisia (a)	KU130470, KU130553, KU130635, KU130720, KU130800
4	*H. scupense*	Iran (a)	KU130471, KU130554, KU130636, KU130721, KU130801
1	*H. somalicum*	Somalia (b)	KU130472, KU130555, KU130637, KU130722, KU130802
2	*H. somalicum*	Somalia (b)	KU130473, KU130556, KU130638, KU130723, KU130803
1	*H. truncatum*	Benin (a)	KU130474, KU130557, KU130639, KU130724, KU130804
2	*H. truncatum*	Kenya (a)	KU130475, KU130558, KU130640, KU130725, KU130805
3	*H. truncatum*	Senegal (a)	KU130476, KU130559, KU130641, KU130726, KU130806
4	*H. truncatum*	Mali (a)	KU130477, KU130560, KU130642, KU130727, KU130807
5	*H. truncatum*	South Africa (a)	KU130478, KU130561, KU130643, KU130728, KU130808
6	*H. truncatum*	Namibia (a)	KU130479, KU130562, KU130644, KU130729, KU130809
7	*H. truncatum*	Somalia (b)	KU130563, KU130645, KU130730, KU130810
1	*H. turanicum*	Iraq (a)	KU130480, KU130564, KU130646, KU130731, KU130811
2	*H. turanicum*	Iraq (a)	KU130481, KU130565, KU130647, KU130732, KU130812
3	*H. turanicum*	Iraq (a)	KU130482, KU130566, KU130648, KU130733, KU130813
4	*H. turanicum*	Pakistan (a)	KU130483, KU130649, KU130734, KU130814
	Amblyomma		
1	*A. variegatum*	Senegal (a)	KU130401, KU130484, KU130567, KU130650
2	*A. variegatum*	Senegal (a)	KU130402, KU130485, KU130568, KU130651
3	*A. variegatum*	Nigeria (a)	KU130403, KU130486, KU130569, KU130652
4	*A. variegatum*	Nigeria (a)	KU130404, KU130487, KU130570, KU130653
	Nosomma		
1	*N. monstrosum*	Sri Lanka (a)	KU130405, KU130488, KU130571, KU130654, KU130735
2	*N. monstrosum*	Sri Lanka (a)	KU130406, KU130489, KU130572, KU130655, KU130736

2.2 Morphological data matrix

Hyalomma are easily distinguished from other genera. Defining characteristics such as scutums / conscutums that are dark reddish-brown to near black, protruding eyes and the appearance of striped ligaments, makes them easy to separate from closely related genera, such as *Nossoma, Rhipicephalus* and *Amblyomma*. Intraspecifically morphological differentiation among *Hyalomma* species remains difficult due to the cryptic nature of external morphology. The majority of the 47 morphological characters incorporated in this study were collected by Apanaskevich and partners during a recent morphological review of the genus (see Apanaskevich 2003; Apanaskevich and Horak 2005; Apanaskevich and Horak 2006; Apanaskevich and Horak 2007; Apanakevich and Horak 2008a; Apanaskevich and Horak 2008b; Apanaskevich, Santos-Silva, and Horak 2008; Apanaskevich, Schuster, and Horak 2008; Apanaskevich and Horak 2009; Apanaskevich, Filippova, and Horak 2010; Apanaskevich and Horak 2010) (Table 3.2). In summary, stereoscopic microscope comparisons of representatives of each of the 27 species at larval, nymph and adult life stages were compared against known vouchers or descriptions thereof (Table 3.2). In most instances the specimens studied were either collected in the field or laboratory reared and are housed in various collections worldwide (see references above). Where data was not available or the character state could not be established, we treated the data as missing (Table 3.2). Similar to Beati and Keirans (2001), we assumed morphological characters are independent.

Table 3.2: *The 47 morphological character states incorporated in this study for each Hyalomma species.*

					HYALOMMA SPECIES																										OUTGROUP
	CHARACTER	**0**	**1**	**2**	***H. aegyptium***	***H. albiparmatum***	***H. anatolicum***	***H. arabica***	***H. asiaticum***	***H. brevipunctata***	***H. dromedarii***	***H. excavatum***	***H. franchinii***	***H. glabrum***	***H. hussaini***	***H. hystricis***	***H. impeltatum***	***H. impressum***	***H. isaaci***	***H. kumari***	***H. lusitanicum***	***H. marginatum***	***H. nitidum***	***H. punt***	***H. rhipicephaloides***	***H. rufipes***	***H. schulzei***	***H. scupense***	***H. somalicum***	***H. truncatum***	***H. turanicum***	***Amblyomma hebraeum***
1	Shape of eyes (all stages)	*Roundish*	*Oval*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	Shape of eyes (adults)	*Flat*	*Spherical*		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
3	Position of eyes (adults)	*Marginal*	*Orbital*		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
4	Pigmentation on scutum or conscutum (adults)	*Intensive*	*Marbled*	*Absent*	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	0
5	Number of ventro-median setae on I segment of palpi (adults)	*< 5*	*5 <*		1	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0
6	Spurs of coxa I (adults)	*Separated*	*Juxtaposed*		0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
7	Median spur of coxa I (adults)	*Absent*	*Present*		1	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0
8	Coloration of leg segments (adults)	*Uniformly brown*	*Ivory-coloured pigment diffused*	*Ivory pigment forming rings and strips*	0	2	1	0	2	0	2	1	1	2	0	0	2	2	2	0	1	2	2	2	0	2	2	2	2	2	2	2
9	Number of anal shields (male)	*2 pairs*	*3 pairs*	*Absent*	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	-
10	Ratoi length : width of adanal schield (male)	*< 1.5*	*1.5 <*		0	1	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	-
11	Ventral lateral projections of basis capituli (male)	*Absent*	*Present*		0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
12	Festoons (male)	*11*	*4 or 5*		0	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0
13	Caudal field (male)	*Not pronounced*	*Pronounced*		0	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0
14	Cervical grooves (male)	*Pit-like*	*Furrow-like*		0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
15	Cervical grooves (male)	*Short (distinct in 1/3 of conscutum or less)*	*Long (distinct in 1/2 of conscutum or more)*		0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
16	Lateral grooves (male)	*Long (reach midlength of conscutum)*	*Short (don"t reach midlength of conscutum)*		-	0	1	1	1	0	1	1	1	0	0	-	1	0	0	0	1	0	0	1	1	0	1	1	1	0	0	1
17	Posterio-median margin of adanal shields (male)	*Straight*	*Curved*		0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	-
18	Parma (male)	*Always absent*	*Present (at least sometimes)*		0	1	1	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0
19	Median projection of adanal shields (male)	*Poor developed*	*Absent*	*Well developed*	1	2	2	1	2	1	2	2	2	2	1	1	2	2	2	1	2	2	2	0	1	2	2	2	2	2	2	-
20	Ratio I : w of basis capituli (male)	*< 1.7*	*1.9 <*		0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
21	Marginal grooves (male)	*Present*	*Absent*		1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

22	**Narrowing of conscutum in area of spiracular plates (male)**	***Not clear***	***Clear***		**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
23	Ventral lateral projections of basis capituli (female)	*Absent*	*Present*		0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
24	Genital operculum (female)	*Wide*	*Narrow*		0	0	0	0	1	1	1	0	0	0	0	1	1	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1
25	Genital operculum (female)	*High arc (U)*	*V-shaped (high)*	*Low arc*	0	0	2	0	0	0	1	2	2	?	0	0	0	0	2	0	2	2	0	0	0	2	0	0	1	0	2	1
26	Vestibular part of vagina (female)	*Swollen*	*Not swollen*		0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
27	Ratio l : w of basis capituli (female)	*Approximately ≤2*	*Approximately 2.5*		0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
28	Ratio l : w of gnathosoma (adults)	*l > w*	*l = w*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	Ratio l of palpi (II and III articles) : l of basis capituli (adults)	*Palpi > basis capituli*	*Palpi <= basis capituli*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	Ratio l : w of gnathosoma (female)	*l > w in 2 times and more*	*l < w*	*l > w in 1.3 to 1.6 times*	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
31	Lateral projections of basis capituli (female)	*Absent*	*Almost not pronounce from lateral edge*	*Clearly pronounce from the lateral adge*	1	1	1	2	1	2	1	1	1	1	2	1	1	1	1	2	1	1	1	1	2	1	1	1	1	1	1	0
32	Position of lateral projections of basis capituli (female)	*Absent*	*Anteriorly*	*Middle or posterior*	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0
33	Base of capituli (nymph)	*Hexagonal*	*Triangular*	*Rectangular*	0	0	0	0	0	1	0	0	0	0	1	?	0	0	0	1	0	0	0	?	0	0	0	0	0	0	0	0
34	Lateral projections of basis capituli (nymph)	*Midlength*	*Posterior*		1	1	1	1	1	1	1	1	1	1	1	?	1	1	1	1	1	1	1	?	1	1	1	1	1	1	1	0
35	Hypostome (nymph)	*Strong dentated*	*Poorely dentated*		1	0	1	1	1	1	1	1	1	0	1	?	1	0	0	1	1	0	0	?	1	0	1	1	1	0	0	1
36	Scutum ratio l : w (nymph)	*l ≥ w*	*l < w*		1	1	1	0	1	0	1	1	1	1	0	?	1	1	1	0	1	1	1	?	0	1	1	1	1	1	1	0
37	Setae of alloscutum (nymph)	*With dentation*	*Without dentation*		0	0	1	?	1	0	1	1	1	1	0	?	1	0	1	0	0	1	0	?	1	1	1	1	1	0	1	0
38	Spurs of caxae II-IV (nymph)	*Moderate*	*Large*	*Reduced (fold-like)*	0	0	0	0	0	0	0	0	0	1	0	?	0	0	1	0	0	1	0	?	0	1	0	2	0	0	1	1
39	Coxal pore (nymph)	*Present*	*Absent*		0	0	1	?	0	0	1	1	0	1	0	?	0	0	1	0	0	1	0	?	1	1	1	1	0	0	1	0
40	Ratio II : III segments of palpi (nymph)	*II < III more than 2 times*	*II < III less than 2 times*		0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0
41	Spurs of coxae I (nymph)	*Separated*	*Close together*		0	0	0	0	0	1	0	0	0	0	1	?	0	0	0	1	0	0	0	?	0	0	0	0	0	0	0	0
42	Spurs of coxae I (nymph)	*Narrow*	*Broad*		0	0	0	0	0	0	0	0	0	1	0	?	0	0	0	0	1	1	0	?	0	1	0	0	0	0	1	0
43	Posteromedian spur of coxae Ish	*Shorter than posterolateral*	*Subequal to posterolateral*		0	1	1	1	1	1	1	1	0	1	1	?	1	1	1	1	1	1	1	?	0	1	1	0	1	1	1	0
44	Scutum (larva)	*Short (portion of scutum posterior to eyes 1/4 or less of scutal length)*	*Moderately long (portion of scutum posterior to eyes ca 1/3 of scutal length)*	*Long (portion of scutum posterior to eyes ca 1/2 of scutal length)*	2	2	1	0	0	0	0	1	2	2	0	?	0	1	2	0	2	2	2	?	0	2	0	2	0	2	2	2
45	I segment of palpi (larva)	*Present*	*Reduced*		0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0
46	Eyes (larva)	*Undivided*	*Divided*		1	1	1	1	1	1	1	1	1	1	1	?	1	1	1	1	1	1	1	?	1	1	1	1	1	1	1	0

2.3 Molecular data generation

DNA extraction, primer design, amplification, sequencing and validation of DNA products, via GenBank blasting, were performed as described in Chapter 2 (see Chapter 2: 2). For this study DNA sequences were generated for two mitochondrial genes (mtDNA), Cytochrome c oxidase I (COI) and 16S rRNA (16S), and three nuclear genes (nDNA), Histone 3 (H3), 28S rRNA (28S) and the Internal Transcribed Spacer 2 region (ITS II) (Table 3.3).

2.4 Sequence editing and alignment

Sequences were visually inspected and edited using the program BioEdit 7.1.3.0 (Hall 1999). Once confirmed, sequences were aligned by the CrustalW Multiple Alignment (Thompson, Higgins, and Gibson 1994) in BioEdit 7.1.3.0 (Hall 1999). To limit missing data, ends of all genes were trimmed, and gaps were inserted in 16S, 28S and ITS II to best align hypervariable regions associated with loops in the secondary structures. Furthermore, 32 bp of the 16S hypervariable region (between positions 152 bp and 186 bp) were removed due to ambiguous sequence alignment.

Table 3.3: *Gene regions, primer names, primer sequence and the edited sequence length of the amplified product used in this chapter. The optimal annealing temperatures of the primer pairs and the sources of the primers used are also indicated.*

REGION	GENE	F/R	PRIMER NAME		EDITED SEQUENCE LENGTH (bp)	OPTIMAL ANNEALING TEMPERATURE	SOURCE
mtDNA	16S	Forward	16+1	5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3'	378	52^{0}C	Black and Piesman 1994
		Reverse	16S-1	5'-CCGGTCTGAACTCAGATCAAGT-3'			Black and Piesman 1994
	COI	Forward	AR-U-COIa	5'-AAACTRTKTRCCTTCAAAG-3'	664	45^{0}C	Cangi *et al.* 2013
		Reverse	AR-L-COIa	5'-GTRTTAAARTTTCGATCSGTTA-3'			Cangi *et al.* 2013
nDNA	ITS II	Forward	RIB-8	5'-GTCGTAGTCCGCCGTC-3'	273	62^{0}C	Rees, Dioli and Kirkendall 2003
		Reverse	RIB-11	5'-GAGTACGACGCCCTACC-3			Rees, Dioli and Kirkendall 2003
	28S	Forward	28v	5'-AAGGTAGCCAAATGCCTCG-3'	632	55^{0}C	Hillis and Dixon 1991
		Reverse	28x	5'-GTGAATTCTGCTTCACAATGATAGGA-3'			Hillis and Dixon 1991
	H3	Forward	HyH3F	5'-GTGGATGGCRCAMARGTTGG-3'	268	56.5^{0}C	Designed for present study
		Reverse	HyH3R	5'-GCAAGAGYACCGGWGGVAAR-3'			Designed for present study

2.5 Phylogenetic reconstruction and divergence dating

Trees were generated for each data type separately to explore potential conflict among phylogenies. In contrast to the morphological phylogeny containing all currently recognised species, the individual gene trees only contained 24 of the 27 recognised species (Figs 3.1 and 3.2; Sup. Figs 4 and 5). Since combined analyses of nDNA, mtDNA and morphological character fragments have been shown to generally increase resolution for closely related lineages (Klompen et al. 2000; Murrell, Campbell, and Barker 2001; Cruickshank 2002; Matthee et al. 2004; de Queiroz and Gatesy 2007), the phylogenetic relationships were also derived from a combined matrix of all available data (COI, 16S, 28S, H3, ITS II and 47 distinct morphological characters).

All phylogenetic reconstructions were based on Parsimony (MP) and Bayesian inference (BI). The MP analysis was performed in PAUP 4.0b10 (Swofford 2001), using the heuristic search option, with TBR branch swopping and random taxon addition. In instances where multiple equally parsimonious trees were retrieved, only 1000 equally parsimonious trees were saved during each replicate. Nucleotide substitutions were unweighted and the robustness of nodes was assessed by the bootstrap method using a 1,000 replicates (Felsenstein 1985). Nodal bootstrap values > 70% were considered well supported. For the Bayesian analyses, jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008) and the Akaike Information Criterion (AIC) (Akaike 1973) was used to determine the best-fit model to define as the priors for each gene fragment separately (Posada and Buckley 2004). The Standard Discrete Model was used for the morphological dataset (Ronquist and Huelsenbeck 2003). To determine the posterior probability (PP) of associations, we used MrBayes 3.2.5 (Ronquist et al. 2012). These probabilities were generated in two parallel Markov Chain Monte Carlo (MCMC) simulations using five chains for 5,000,000 generations, saving one tree in every 1,000 generations. Burnin of 10% of the total generations was determined via parameter convergence in Tracer 1.5 (Rambaut and Drummond 2007) after standard deviation (SD) of split frequencies had reached stationarity.

Divergence dating was performed on the recognised 24 species for which comprehensive molecular data were available. A lognormal relaxed molecular clock

approach (Drummond et al. 2006) was utilized in BEAST 1.8.2 (Drummond et al. 2012). Input files were generated in BEAUti 1.8.2 (Drummond et al. 2012) using fossil calibrated divergence dates and the best-fit models as determined by jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008). Early runs were evaluated in Tracer 1.5 (Rambaut and Drummond 2007) to optimise run-time parameters before final analysis. We used exponential priors for the established divergence date calibration. Hard minimum and relaxed maximum bounds were set so that 95% of the probability was contained around the divergence date. The divergence date of the *Nosomma–Hyalomma* lineage from *Rhipicephalus* has been proposed to have occurred 19 Mya (Balashov 1994; Murrell, Campbell, and Barker 2001), but the amber fossil data suggests the genus was present between 35-50 Mya (de la Fuente 2003). The divergence date of the origin of *Hyalomma* was thus set to fall between 35-50 Mya. Data were treated as unpartitioned and the Birth-Death Process was used as tree prior. The consensus topology MCMC simulation ran for 100,000,000 generations, sampling every 10,000 generations. Validation of convergence and mixing was assessed in Tracer 1.5 (Rambaut and Drummond 2007) to ensure that all effective sample size (ESS) values were > 200. TreeAnnotator 1.8.2 (Drummond et al. 2012) was used to summarize trees, after discarding 2,000 trees as burn-in.

The dated consensus tree was then evaluated in RASP 3.2 (Yu et al. 2015), where the current zoogeographic distribution of extant taxa was aligned to plot ancestral distributions at each node. This was done via Statistical Dispersal-Vicariance Analysis (S-DIVA) (Yu, Harris, and He 2010). S-DIVA is an advanced form of DIVA (Ronquist 2001) designed to circumnavigate impossible ranges. The software also models events such as dispersal, extinction, vicariance and duplication, all the while not making assumptions on biogeographic patterns (Ronquist 2001). It can thus be very useful in reconstructing ancestral distributions among organisms with a shared evolutionary history (Ronquist 1997).

3. Results

3.1 Taxonomy of Hyalomma

Among 23 recognised species and for which COI data was available, the average COI sequence divergence between species is 11.46% (SD = 2.5%) and within species the average diversity is 0.50% (SD ± 0.6%) (Table 3.4). In sharp contrast to this general pattern, the sequence divergence between *H. nitidum* and *H. truncatum* from western Africa is 1.12% (SD ± 0.59%), and between *H. marginatum* and *H turanicum* it is 0.44% (SD ± 0.16%) (Table 3.4). On the other side of the spectrum, an intraspecific sequence divergence of 10.22% (SD ± 0.38%) separated northern African *H. truncatum* and southern African *H. truncatum* (Table 3.4).

Table 3.4: *COI sequence distances within and between Hyalomma species. GenBank comparisons are based on sequences provided in Sup. Table 2.*

	SAMPLE SIZE (*N*)	AVERAGE SEQUENCE DISTANCE (%)	STANDARD DEVIATION (SD)	RANGE
Between 24 recognised *Hyalomma* species	84	11.46%	±2.47%	0.44-16.62%
Between 8 GenBank available species of *Hyalomma*	37	10.93%	±2.78%	2.34-15.68%
Within 24 recognised *Hyalomma* species	84	0.50%	±0.57%	0.00-2.096%
Within 8 GenBank available species of *Hyalomma*	37	0.81%	±1.21%	0.00-3.51%
Between *H. truncatum* & *H. spp. n.*	(5 + 2)	10.28%	±0.38%	9.78-10.71%
Within *H. truncatum* (NORTHERN)	5	2.10%	±0.76%	0.31-2.80%
Within *H. spp. n.* (Recognised as *H. truncatum* from SOUTHERN Africa)	2	0.62%	±0.00%	0.62%
Between *H. nitidum* & *H. truncatum* (western)	(3 + 3)	1.12%	±0.59%	0.31-1.71%
Between *H. nitidum* & *H. truncatum* (NORTHERN)	(3 + 5)	1.67%	±0.83%	0.31-2.64%
Between *H. nitidum* & *H. spp. n.*	(3 + 2)	10.04%	±0.08%	9.94-10.09%
Between *H. albipermatum* & *H. truncatum* (NORTHERN)	(3 + 5)	7.30%	±0.35%	6.87-7.76%
Between *H. albipermatum* & *H. spp. n.*	(3 + 2)	10.33%	±0.09%	10.25-10.40%
Between *H. albipermatum* & *H. nitidum*	(3 + 3)	6.63%	±0.28%	6.37-7.14%
Between *H. spp. n.* & *H. impressum*	(2 + 2)	11.34%	±0.18%	11.18-11.49%
Between *H. rufipes* (NORTHERN : SOUTHERN)	(6 + 4)	2.67%	±0.25%	2.17-2.95%
Within *H. rufipes* (NORTHERN)	6	0.35%	±0.27%	0.00-0.78%
Within *H. rufipes* (SOUTHERN)	4	0.41%	±0.21%	0.00-0.62%
Between *H. marginatum* & *H. turanicum*	(3 + 4)	0.44%	±0.16%	0.16-0.62%
Within *H. marginatum*	3	0.10%	±0.09%	0.00-0.16%
Within *H. turanicum*	4	0.31%	±0.34%	0.00-0.62%

3.2 Phylogenetic associations

Individual phylogenetic analyses of mtDNA, nDNA and morphology were not all equally informative in resolving the evolutionary history of *Hyalomma*. The faster evolving COI and 16S mtDNA gene trees separately (data not shown) and combined, and the morphological data set, showed a higher degree of resolution when compared to the individual (data not shown) and combined nDNA data sets (28S, ITS II and H3) (Sup. Fig 5). The different molecular data sets revealed different outcomes, but none of the conflicting nodes had high bootstrap or posterior probability values (Sup. Figs 4 and 5). The combined mtDNA topology showed the most resolution and supported the monophyly of 22 of 25 *Hyalomma* (13 internal nodes had PP and or BS support). On the other hand, the combined nDNA only supported the monophyly of 11 *Hyalomma* species and two internal nodes displayed PP and BS support. The only relationship that was supported by both methods and both data sets was the sister taxon relationship between *H. arabica* and *H. rhipicephaltoidies*. Interestingly a further two nodes are supported by either BS or PP (the node supporting the monophyly of *H. hussaini* and *H. kumari* and the node supporting the monophyly of *H. asiaticum*, *H. dromedarii*, *H. impeltatum*, *H. punt*, *H. schulzei* and *H. somalicum* (although the latter remains unresolved internally). While nDNA may show little congruency with mtDNA data, it is not in conflict with that presented (Sup. Figs 4 and 5). The morphological data alone also showed weak support for many of the relationships (Sup. Fig 3), but also in lieu of no strongly supported conflict. Combining the data in a single supermatrix or “total evidence approach” gave the best resolution (also see Klompen et al. 2000; Murrell, Campbell, and Barker 2001; de Queiroz and Gatesy 2007).

The combined total supermatrix comprised 2,242 characters, for 85 taxa (*H. brevipunctata*, *H. franchinii* and *H. hystricis* were omitted due to a lack of DNA data). The optimal prior model for the BI analysis were nst = 6, rates = gamma for all five gene regions. This same data set revealed 545 parsimony informative characters and the MP analysis saved a 1,000 equally parsimonious trees containing 1,680 steps. The majority of the branch swapping was confined to associations among individuals within the same species. Overall, the MP and BI analyses resulted in a fairly well supported topology where 5 higher level clades supported by both

parsimony and posterior probabilities could be identified (1-5; Fig 3.1). In addition, some substructure was also identified within two of these clades (4 and 5; Fig 3.1)

The monophyly of *Hyalomma* was well supported (BS = 100%, PP = 1.00) (Fig 3.1). Although seven interspecific nodes were weakly supported, five of these were related to species complexes where the species status of these lineages have been questioned: Specifically the nodes reflecting the associations between *H. nitidum, H. truncatum* (northern and southern lineage; Chapter 2) and *H. impressum* and also the node describing the relationships among *H. marginatum* and *H. turanicum* (Fig 3.1). Apart from these, the monophyly of the rest of the *Hyalomma* species is supported by significant PP and BS ranging from 77% for *H. excavatum* to > 87% for the remaining species (Fig 3.1). The combined analysis also increased the support for some associations suggested by the individual data set trees. For example: The monophyly of *H. lusitanicum*, *H. excavatum* and *H. anatolicum* was previously found but not supported and now has stronger support (previously a PP = 0.85; now BS = 86%, PP = 1.00). The placement of *H. aegypticum* is also better supported (BS = 100%, PP = 1.00). More resolution is also obtained regarding the more basal placement of the clade containing *H. arabica* and *H. rhipicephaloides,* and finally the clade containing *H. glabrum*, *H. marginatum*, *H. turanicum* and *H. rufipes* (Fig 3.1).

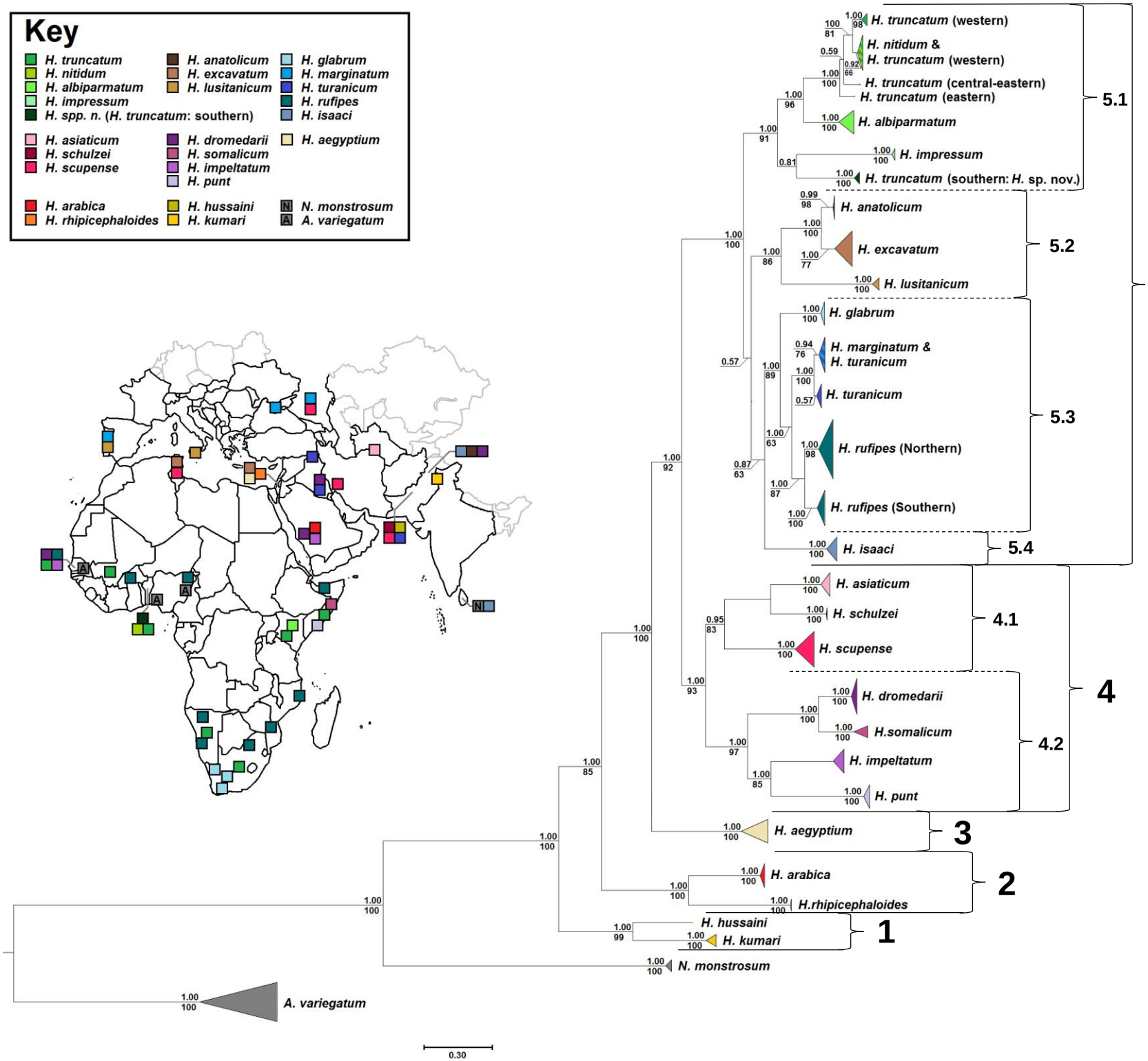


Figure 3.1: *Hyalomma Bayesian tree based on a super-matrix of COI, 16S, 28S, ITS II, H3 gene regions and 47 morphological characters. Maximum parsimony bootstrap values are indicated below and Bayesian posterior- probabilities are above branches. Branch lengths represent the number of base-pair changes. Colour squares on the map indicate the approximate location at which specimens were sampled. The key indicates the respective colours associated to species on the map and tree.*

3.3 Divergence dating

The BEAST topology based on molecular data only (COI, 16S, 28S, H3, ITS II) (Fig 3.2) differs in some respects from the supermatrix topology (Fig 3.1). The first of these differences concerns lineage (5.4), the placement of *H. isaaci*, which moves to a more basal position but still within clade five (Figs 3.1 and 3.2). The second difference concerns the relationship between southern *H. truncatum* and *H. impressum*. Importantly, however, none of these nodes had high support in the combined analysis where all data were used. The exact biogeographic interpretation and dating of these species are thus speculative and will not be discussed as part of this section.

Molecular clock estimates and S-DIVA analysis suggest *Hyalomma* (*senso stricto*) likely had an Oriental origin between 36.25-59.62 Mya (Fig 3.2). The first divergence within the genus likely occurred around 36.25 Mya (95% HPD 34.75-39.80 Mya) with a westward expansion into the Palearctic region (Fig 3.2). Since this point, members of the genus diverged repeatedly at regular intervals giving rise to the present species composition and range (Fig 3.2). From the Palearctic region, the southward expansion into the Afrotropical region likely occurred in two separate events: 22.69 Mya (95% HPD 16.79-25.08 Mya) and 16.23 Mya (95% HPD 12.57-19.40 Mya) and were probably followed by regional vicariant events as suggested by S-DIVA (Yu, Harris, and He 2010), showing a conserved ancestral distribution in in a number of lineages (5.1, 5.2, 4.1 and 4.2) (Fig 3.2). However it would appear a number of dispersal events have reached their peak in more recent times (< 2 Mya). This has led to the Oriental region to be recolonized by species, such as *H. anatolicum*, *H. dromedarii* and *H. isaac*i and *H. scupense* (Fig 3.2). *Hyalomma excavatum* and *H. anatolicum* have dispersed back into the Afrotropical, as the ancestral lineage likely became restricted in the Palearctic region approximately 10.82 Mya (95% HPD 8.03-14.06 Mya) (Fig 3.2). Furthermore, the Palearctic region has been recolonized by *H. dromedarii* and *H. impeltatum* as they have likely dispersed from an Afrotropical origin. There is little indication of direct dispersal from the Afrotropical region to the Oriental region and vice versa. It would appear stepwise dispersal events between these two regions have occurred through, or in conjunction with, the Palearctic region (Fig 3.2).

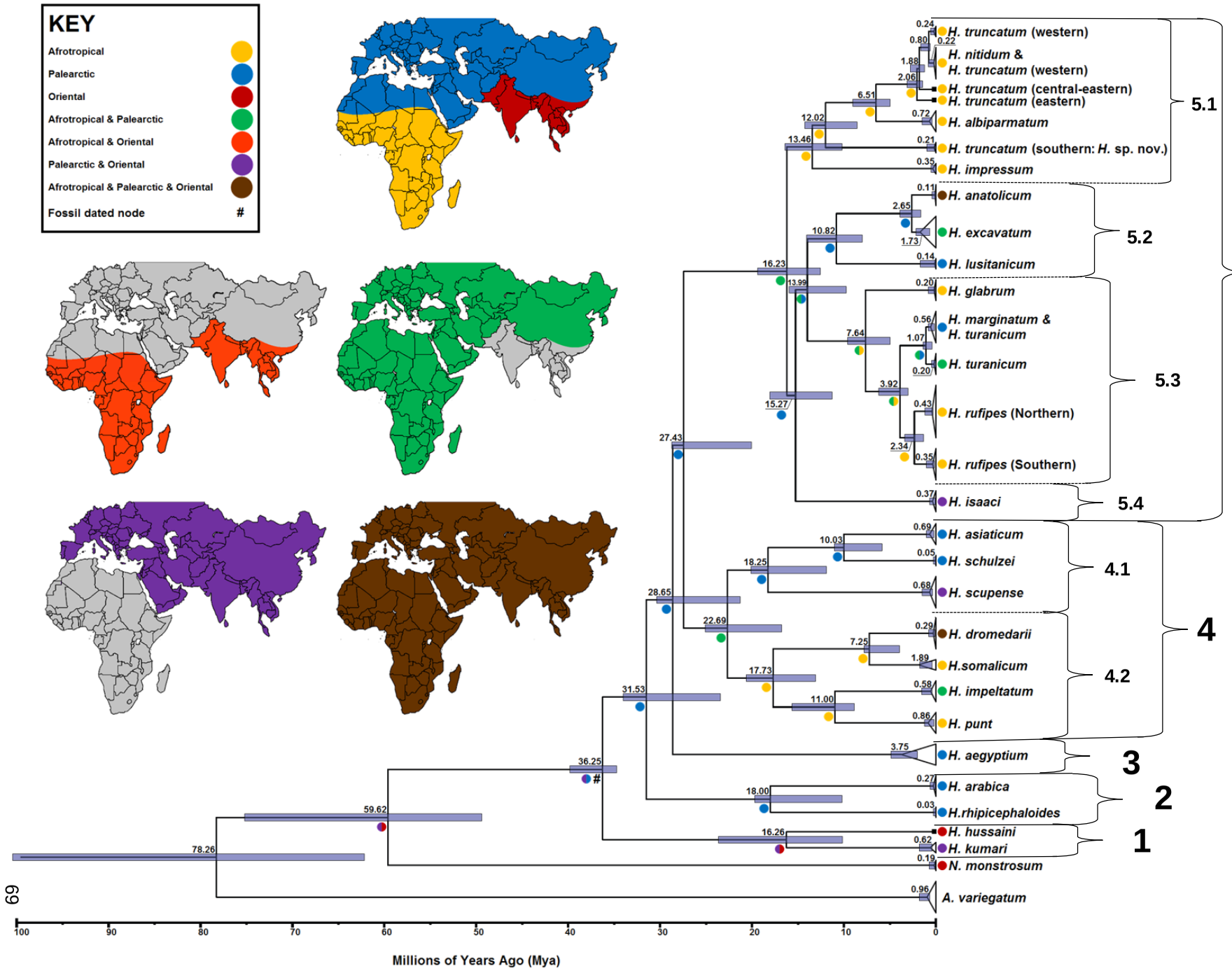


Figure 3.2: *Dated tree for Hyalomma obtained from the fossil-calibrated BEAST analysis (Outgroup taxa from bottom to top: Amblyomma variegatum and Nonsomma monstrosum). The Fossil-calibrated node is indicated by the BLACK "#" (Oldest fossil record of Hyalomma: 35–50 Mya). Values at each node represent dates in millions of years before present. The BLUE bars at each node represent the 95% confidence intervals around divergence dates. Colour pies represent the optimal range of current and ancestral taxa as calculated through S-Diva analysis in RASP.*

4. Discussion

4.1 Phylogenetic assessment

The present study provides strong molecular support for the monophyly of *Hyalomma* (Balashov 1994; Barker and Murrell 2004; Murrell, Campbell, and Barker 2001). Although *H. brevipunctatum*, *H. franchinii*, *H. hystricis* are not incorporated into our complete data matrix, supplementary data based on morphological data alone suggest *H. brevipunctatum* shares a monophyletic origin with *H. hussaini* and *H. kumari*. They share 46 of the 47 characters compared in this study and are distinguished from all other *Hyalomma* by the shape of the basis capituli, which is dorsally triangular and hexagonal in all other *Hyalomma*. Furthermore the three species share similar ranges, and both *H. kumari* and *H. brevipunctatum* are found solely in the Oriental region. *Hyalomma franchinii* is nested in a clade comprising *H. lusitanicum, H. excavatum, H. anatolicum* and this association is supported by 41 of the 46 morphological characters and furthermore, adults of the four species share diffused ivory-coloured pigments on their legs as opposed to other *Hyalomma* which either are more uniformly brown or the ivory-coloured pigments form more distinct rings and stripes. Additionally *H. franchinii* shares similar distribution with these species over north Africa and south-western Asia. Finally, the position of *H. hystricis* is totally unresolved. The lack of characters for *H. hysterics* at laval and nymph stages, means the species appears to share an equal association to a number of different *Hyalomma* species making it difficult to speculate on its position at this point.

Combining the data into a single matrix increases the support for several of the interspecific associations (Fig 3.1; Sup. Figs 3, 4 and 5). The total evidence suggests the existence of at least five well resolved groups within the genus, and within these additional statistical and BS support was recovered for several additional subgroups (Fig 3.1). Despite the reported limitations of morphological data when drawing phylogenenies for ticks (such as structural changes during feeding, Lv et al. 2014; gynandromorphisms, Buczek, Bartosik, and Buczek 2014; hybridization, Rees, Dioli, and Kirkendall 2003), the combined topology presented herein supports many of previous hypotheses based on morphology alone. For example, the relationships among *H. dromedarii*, *H. somalicum*, *H. impeltatum* and *H. punt* (subclade 4.2; Fig

3.1) form part of a larger lineage including *H. asiaticum* and *H. schulzei* (clade 4; Fig 3.1) as previously proposed (see Apanaskevich, Schuster and Horak 2008; Apanaskevich and Horak 2010; Apanaskevich, Filippova and Horak 2010). The close relationships between *H. anatolicum*, *H. excavatum* and *H. lusitanicum* are also corroborated in our study (subclade 5.2; Fig 3.1) (Hoogstraal and Kaiser 1959; Apanaskevich and Horak 2005), and so are the reportedly close relationships between *H. glabrum*, *H. marginatum* and *H. rufipes* (subclade 5.3; Fig 3.1) (Apanaskevich and Horak 2008a; Apanaskevich and Horak 2009). The topology presented herein not only supports most of the previous morphological suggestions but also advanced our understanding on some other phylogenetic relationships proposed in the literature. For example, Hoogstraal and Kaiser (1959) and Apanaskevich and Horak (2009) suggested a close relationship between *H. somalicum* and *H. impeltatum*. Although the data presented herein support a subclade association including both these species (subclade 4.2; Fig 3.1), it advanced our knowledge by strongly suggesting sister taxon relationships between *H. impeltatum* and *H. punt* and also between *H. somalicum* and *H. dromedarii* (subclade 4.2; Figs 3.1 and 3.2).

Despite good total evidence support for the majority of the phylogenetic hypotheses based on morphology alone, the addition of molecular data also provided new insights to help resolve the questionable vacillations in the taxonomy of some species. Extremely low levels of genetic differentiation was detected between *H. marginatum* and *H. turanicum* (at the mtDNA COI level = 0.44% ± 0.16%; Table 3.4). This level of differentiation is much more similar to intraspecific divergences as suggested by Zhang and Zhang (2014), and when considering the paraphyletic clustering in the tree (Fig 3.1), we suggest that *H. turanicum* should be synonymized under *H. marginatum.* The latter is based on priority since *H. turanicum* (Pomerantzev 1946) was first described as a subspecies of *H. marginatum* (Koch 1844). Whether subspecies status should be reinstated, would require a much more detailed investigation at the phylogeographic level. A second point is the relationship between *H. albiparmatum*, *H. impressum*, *H. nitidum*, and *H. truncatum* (sublineage 5.1; Fig 3.1): The validity of *H. nitidum* (Schulze 1919) and *H. albiparmatum* (Schulze 1919) has been subject to much debate (see Hoogstraal 1956; Feldman-Muhsam 1962; Walker 1974; Hoogstraal 1979; Camicas et al. 1998; Tomassone et al. 2005;

Apanaskevich and Horak 2008b). Most recently, Apanaskevich and Horak (2008b) proposed that the phenotypic variation observed between *H. albiparmatum*, *H. nitidum* and *H. truncatum* is simply due to the large distribution range of a single species and the former two should rather be synonymised into *H. truncatum* (which has priority). The molecular tree and sequence distances among these putative species partly support this hypothesis in strongly suggesting that *H. nitidum* should be synonymized with *H. truncatum* (sublineage 5.1; Fig 3.1; Table 3.4). In accordance with the rules of priority of the International Code for Zoological Nomenclature, *H. nitidum* represents a junior synonym of *H. truncatum* (west and eastern African lineages; Fig 3.1; Chapter 2). *Hyalomma truncatum* however are found to be further paraphyletic if *H. albiparmatum* is considered to be a unique species entity (subclade 5.1; Fig 3.1). Based on sequence distances and the topology (Figs 3.1 and 3.2; Table 3.4; Zhang and Zhang 2014), *H. albiparmatum* is unique and genetically diverse from all other taxa and should thus retain its species status. If this is followed, the southern lineage of *H. truncatum* needs to be described as a separate species. Interestingly, *H. truncatum* was first described and named by Koch (1844) and the type specimen is from Senegal. The northern African (eastern and western Africa clades) lineage which incorporates Senegalese specimens should thus retain the original name. However, *H. truncatum* has been described in Africa under a number of synonyms. Many of these were described within narrow margins of the equator and thus could be problematic to assign. The earliest and most reliable synonym that could form part of the southern African lineage is *H. zambesianum,* Schulze and Schlottke (1930). *Hyalomma zambesianum* was collected on the banks of the Zambezi River, although it is unclear as to the exact location or country (Schulze and Schlottke 1930). Depending on the validation of the type specimens to the southern African specimens, a synonym, such as the above, may be favoured or a new description and naming may need to be issued. For the purpose of the rest of this dissertation we refer to the southern lineage of *H. truncatum* as *Hyalomma species nova* (*H.* sp. nov.).

4.2 Divergence time estimates and faunal exchanges

At the larger continental scale the diversification in *Hyalomma*, giving rise to five lineages, likely begun 36.25 Mya (95% HPD 34.75-39.80 Mya). S-DIVA results suggested that the common ancestor was probably widespread over the larger

Palearctic and possibly also the Oriental regions (Fig 3.2). The current species composition and distribution would suggest that regular faunal exchanges occurred in a stepwise fashion between zoogeographic regions followed by subsequent vicariant events (Fig 2.2). These mechanisms of vicariance could have affected the evolution of *Hyalomma* directly (through affecting natural dispersal of ticks) or indirectly (by affecting a broad range of faunal hosts). We propose that host movement is probably more influential in this. Tick dispersal off the host is limited (Randolf 1998; Anderson and Magnarelli 2008; Cangi et al. 2013), and subsequently a number of studies have indicated host selection among ticks is a key factor in their dispersal and range (Scott et al. 2001; Madhav et al. 2004; Kempf et al. 2009; Beati et al. 2012; Cangi et al. 2013).

Hyalomma kumari and *H. hussaini* represent the earliest diverging lineage within the genus (clade 1; Fig 3.2) followed by a lineage containing *H. rhipicephaloides* and *H. arabica* (clade 2; Fig 3.2). The divergence between clades one and two, 36.25 Mya (95% HPD 34.75-39.80 Mya) was likely caused by a vicariant event on the Oriental-Palearctic boundary. Interestingly, approximately 35 Mya, major uplift of the Himalayas has been reported (due to the collision of the Indian and Eurasian Plates) and it is this likely that this event could have contributed towards the early diversification in *Hyalomma* (Molnar and Tapponnier 1977; Najman et al. 1994; Rashid 2014; Tamma and Ramakrishnan 2015). A number of fairly rapid divergences, probably in the central- and western-Palearctic region (due to the distribution of extant taxa and S-DIVA results), followed during the Oligocene between 31.53-27.43 Mya (95% HPD 34.02-20.07 Mya). These finally gave rise to another monophyletic assemblage: *H. aegyptium* (clade 3; Fig 3.2), and the common ancestor of the rest of the genus (clades 4 and 5; Fig 3.2). The divergence of clade three, and occurrence of the common ancestor of clades four and five, correlate to a time period when the eastern Mediterranean and Paratethys seas were undergoing many structural changes (Rögl 1999). Outcrops of land were continuously joining and segregating from mainland Eurasia during this period and it is possible that *Hyalomma* may have become isolated on such land bodies, driving speciation (Rögl 1999). The divergence of the common ancestor of clade four, 22.69 Mya (95% HPD 16.79-25.08 Mya), and the common ancestor of clade five 16.23 Mya (95% HPD 12.57-19.39 Mya), both from a Palearctic-Afrotropical origin, suggests at least two

early dispersal events into the Afrotropical region occurred. The latter date partly overlaps with the formation of the landbridge between Africa and Eurasia 16-20 Mya (Rögl 1999; Cox 2000; Krijgsman 2002; Koufos, Kostopoulos, and Vlachou 2005; Harzhauser et al. 2007; Sen 2013).

The subsequent divergence events in clades and subclades would appear to have evolved independently within specific areas, with only very recent re-colonization events among zoogeographic regions. Certainly *H. albiparmatum*, *H. impressum*, *H. truncatum* and *H.* sp. nov. (subclade 5.1; Fig 3.2), and *H. dromedarii*, *H. impeltatum*, *H. punt* and *H. somalicum* (subclade 4.2; Fig 3.2) have strong Afrotropical origins. Furthermore, similar can be said for *H. arabica* and *H. rhipicephaloides* (clade 3; Fig 3.2), *H. asiaticum*, *H. schulzei* and *H. scupense* (subclade 4.1; Fig 3.2), and *H. anatolicum*, *H. excavatum* and *H. lusitanicum* (subclade 5.2; Fig 3.2) in the Palearctic region, and *H. hussaini* and *H. kumari* (clade 1; Fig 3.2) in the Oriental region. The divergence of *H. glabrum*, *H. rufipes* and *H. marginatum* (subclade 5.3; Fig 3), appears to be the only lineage with mixed zoogeographic origins, probably diversifying on the fringe of the Afrotropical and Palearctic regions. These divergences during the Miocene and Pliocene have occurred at points of huge biogeographic activity, such as shifts in habitats and biomes, and the formation of mountains, drainage systems and lakes, in Africa, Asia and Europe (Csontos et al. 1992; Jolivet and Faccenna 2000; Retallack 2001; Briggs 2003; Sanmartin 2003; Jacobs 2004; Wichura et al 2010; Salzburger, Van Bocxlaer, and Cohen 2014), which have been used to substantiate speciation in a number of taxa (Matthee and Robinson 1997; Sanmartin 2003; Lou et al. 2004; Moodley and Burford 2007; Pisano et al 2015). This would suggest allopatric speciation has been a key driver of many *Hyalomma* species, yet it is difficult to speculate the exact event or formation involved in each of the divergence events. For one, exact ranges among extant *Hyalomma* species is most often very loosely defined to countries and host associations, may be more diverse than recorded (see Apanaskevich and Horak 2005; Apanaskevich and Horak 2008a; Apanaskevich, Santos-Silva, and Horak 2008; Apanaskevich, Schuster, and Horak 2008; Apanaskevich, Filippova, and Horak 2010; Apanaskevich and Horak 2010). The extent of distributions have been quantified on linking morphological identification, with older literature, which may be problematic. This means correlating geographical formations (such as rivers, lakes

and mountains) to specific breaks among many *Hyalomma* species, or ancestral hosts, is a difficult task.

4.3 African biogeography and the evolution of H. truncatum and H. sp. nov.

From a biogeographic viewpoint, it is interesting to see that the same geographic north-south divide present in *H. truncatum-H.* sp. nov. is also present in *H. rufipes* (Fig 3.1). Although the split in *H. rufipes* is also well supported by BS and PP (Fig 3.1), the two *H. rufipes* lineages are monophyletic and the mtDNA COI differentiation is much lower (2.67% (SD ± 0.25%)). More so, the divergence date is relatively recent in *H. rufipes* (2.34 Mya (95% HPD 1.33-3.41 Mya) and much older in *H. truncatum* 12.02 Mya (95% HPD 8.55-14.26 Mya) (Fig 3.2). From this it can be argued that different biogeographic events separated these two lineages. Interestingly, within *H. rufipes* more substructure was also evident within the southern region of the range (Cangi et al. 2013; Chapter 2). The complexity of finding the mechanisms involved in tick speciation are also exemplified by this study where it was suggested that life history traits, particularly juvenile survival and competition with other *Hyalomma* species were probably responsible for the observed fine scale genetic structure. In the case of *H. truncatum* showing a much older isolation, the effects of the development of the *Brachystegia* woodland, the Zambezi River and rifting in east Africa could have also played a major role since multiple potential hosts show similar geographic vicariance (for example see Freitag and Robinson 1993; Matthee and Robinson 1997; Arctander, Johansen, and Coutellec-Vreto 1999; Lehmann et al. 1999; van Alphen-Stahl, Bloomer, and Crowe 2005), but the exact dates do not correlate well. The initiation of the East African Rift Valley probably began 30 Mya, and was a notable geological formation by 15 Mya (subsequently the same time that the drainage pattern of the Zambezi was established; Moore and Larkin 2001), while later uplift and the development of major rift lakes only occurred post 7 Mya (see Chorowicz 2005; Trauth et al. 2005; Wichura et al 2010; Roberts et al. 2012; Salzburger, Van Bocxlaer, and Cohen 2014). The *Brachystegia* woodland has as yet only been suggested to have acted as a key dispersal barrier over more recent times (Matthee and Robinson 1997; Lehmann et al. 1999; van Alphen-Stahl, Bloomer, and Crowe 2005). Probably the most reliable reason for this *Hyalomma* divergence was caused by an isolation event in southern Africa and the development of different breeding cycles that can now act as

contemporary barries between the hemispheres. Adult *Hyalomma* in general have been noted to be more active during warmer months (Yousfi-Monod and Aeschlimann 1986; Boulkaboul 2003; Bente et al. 2013) and thus shifts in reproduction cycles have been used to argue a contemporary barrier in the region for *H. truncatum* and *H.* sp. nov. (see Chapter 2). However, the later divergence between *H. albiparmatum* and *H. truncatum* around 6.51 Mya (95% HPD 4.98-9.04 Mya) aligns better with biogeographic barriers that may have been driven by vicariance among hosts species. The isolation of grassland habitats (Jacobs 2004), rifting in parts of the western branch of the East African Rift Valley around 6 Mya (see Delvaux et al. 1992; Ebinger 1989; Logatchev, Beloussov, and Milanovsky 1972; Partridge, Wood, and deMenocal 1995), and the development of many rift lakes 7-3mya (see Cohen, Soreghan, and Scholz 1993; Stiassny and Meyer 1999) have been suggested as major drivers of speciation in a number of possible host species over this time frame, including springhare, *Padetes* spp. (Matthee and Robinson 1997) and bushbuck, *Tragelaphus* spp. (Moodley and Burford 2007). These may have led to a prolonged period of isolation, enough to have caused the divergence between *H. albiparmatum* and *H. truncatum*.

SUMMARY

Summary

Phylogeographic results indicated that *H. truncatum* comprised two distinct lineages with a southern and a northern African clade. Furthermore, strong regional partitioning was observed for *H. truncatum* across northern Africa lineages, suggesting up to five phylogroups. In southern Africa there was no additional substructure. In northern Africa, we proposed that glacial cycles caused habitat changes that resulted in the disruption of geneflow in host species. The latter probably played a key role in phylogroup formation. In southern Africa, the lack of phylogeographic patterning is likely due to the absence of a single barrier affecting multiple hosts allowing for a high degree of passive dispersal across the region. However, the sequence distance between the northern and southern *H. truncatum* clades is high and more representative of interspecific values within *Hyalomma*. Haplotype networks and ancestral population demographics also suggest the two lineages evolved independently. In concert these data suggest that the two lineages may possibly represent two distinct species, favouring *H. truncatum* for the northern clade. The distinct species status for *H. truncatum* from southern Africa (*H.* sp. nov.), was investigated by conducting full phylogenetic analyses of the genus. The phylogenetic analyses indicated that *Hyalomma* likely arose in the oriental region and begun to diversify 36.25 Mya (95% HPD 34.75-39.80 Mya) from a westward expansion into the Palearctic region. Speciation events occurred at regular intervals, notably with the expanse of grasslands and the formation of putative geological structures, such as rivers and mountains. Additionally, it is possible that climatic oscillations and thus habitat shifts from the Miocene onwards may have also aided in later speciation events. The range of extant taxa suggest multiple faunal exchanges between zoogeographic regions. These exchanges seem to align with both those seen in other species, and points to the importance of tectonic uplift and lowered sea levels around continental margins. In total, there are five monophyletic species groups within *Hyalomma*. The incorporation of molecular data has revealed a number of outcomes for consideration: Firstly, *H. marginatum* and *H. turanicum*, previously believed to be distinct entities, show limited evolutionary partitioning. Secondly, our results corroborate Cangi et al. (2013) and propose two clades for *H. rufipes* across the Afrotropical region that dissect over Mozambique (northern and

southern). Thirdly, we found limited indications of disparity between *H. truncatum* and *H. nitidum*, and thus believe the latter should be considered a synonym of *H. truncatum*. Finally, we find limited support for a hypothesis that *H. albiparmatum* may also represent a synonym of *H. truncatum*. Sequence distance between *H. albiparmatum* and *H. truncatum* correlates closer to that seen between other currently recognised species. The high sequence distance among *H. truncatum* clades and the paraphyletic clustering of these lineages within the genus, provide novel support for the hypothesis that the southern clade be regarded as a new species of *Hyalomma*.

BIBLIOGRAPHY

Agnarsson, I. 2010. "The Utility of ITS2 in Spider Phylogenetics: Notes on prior work and an example from *Anelosimus*." *Journal of Arachnology* 38: 377-382.

Akaike, H. 1973. "Information Theory and an extension of the Maximum Likelihood principle." In: *Sececond international Symposium of Information Theory (Eds. Petrove, P.N. and Csaki, F.)*, 267-281. Akademiai Kiado, Budapest.

Akaike, H. 1992. "Information Theory and an Extension of the Maximum Likelihood Principle." In: *Breakthroughs in Statistics*, 610-624. Springer, New York.

Aktas, M., Dumanli, N. and Angin, M. 2004. "Cattle infestation by *Hyalomma* ticks and prevalence of *Theileria* in *Hyalomma* species in the east of Turkey." *Veterinary Parasitology* 119: 1-8.

Anderson, J.F. and Magnarelli, L.A. 2008. "Biology of ticks." *Infectious disease clinics of North America* 22: 195-215.

Anthony, N.M., Johnson-Bawe, M., Jeffery, K., Clifford, S.L., Abernethy, K.A., Tutin, C.E., Lahm, S.A., White, L.J.T., Utley, J.F. and Wickings, E.J. 2007. "The role of Pleistocene refugia and rivers in shaping Gorilla genetic diversity in central Africa." *Proceedings of the National Academy of Sciences* 104: 20432-20436.

Apanaskevich, D. 2003. "The diagnostics of *Hyalomma* (*Hyalomma*) *aegyptium*." *Parazitologiya* 37: 47-59. (In Russian)

Apanaskevich, D.A. 2004. "Host-parasite relationships of the genus *Hyalomma* Koch, 1844 (Acari, Ixodidae) and their connection with microevolutionary process." *Parazitologiia* 38: 515-523.

Apanaskevich, D.A., Filippova, N.A. and Horak, I.G. 2010. "The genus Hyalomma Koch, 1844. X. Redescription of all parasitic stages of *H.*(*Euhyalomma*) *scupense* Schulze, 1919 (= *H. detritum* Schulze)(Acari: Ixodidae) and notes on its biology." *Folia Parasitologica (Prague)* 57: 69.

Apanaskevich, D.A. and Horak, I.G. 2005. "The genus Hyalomma Koch, 1844. II. The taxonomic status of *H.*(*Euhyalomma*) *anatolicum* Koch, 1844, *H.*(*E.*) *excavatum* Koch, 1844 (Acari: Ixodidae) with redescriptions of all stages." *Acarina* 13: 181-197.

Apanaskevich, DA and Horak, I.G. 2006. "The genus *Hyalomma* Koch, 1844. I. Reinstatement of *Hyalomma* (*Euhyalomma*) *glabrum* Delpy, 1949 (Acari, Ixodidae) as a valid species with a redescription of the adults, the first description of its immature stages and notes on its biology." *Onderstepoort Journal of Veterinary Research* 73: 1-12.

Apanaskevich, D.A. and Horak, I.G. 2007. "The genus *Hyalomma* Koch, 1844. III. Redescription of the adults and larva of *H.*(*Euhyalomma*) *impressum* Koch, 1844 (Acari: Ixodidae) with a first description of its nymph and notes on its biology." *Folia parasitologica* 54: 51.

Apanaskevich, D.A. and Horak, I.G. 2008a. "The genus *Hyalomma* Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the *H.*(*Euhyalomma*) *marginatum* Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology." *International Journal of Acarology* 34: 13-42.

Apanaskevich, D.A. and Horak, I.G. 2008b. "The genus *Hyalomma*. VI. Systematics of *H.*(*Euhyalomma*) *truncatum* and the closely related species, *H.*(*E.*) *albiparmatum* and *H.*(*E.*) *nitidum* (Acari: Ixodidae)." *Experimental and Applied Acarology* 44: 115-136.

Apanaskevich, D.A. and Horak, I.G. 2009. "The genus *Hyalomma* Koch, 1844. IX. Redescription of all parasitic stages of *H.*(*Euhyalomma*) *impeltatum* Schulze & Schlottke, 1930 and *H.*(*E.*) *somalicum* Tonelli Rondelli, 1935 (Acari: Ixodidae)." *Systematic Parasitology* 73: 199-218.

Apanaskevich, D.A. and Horak, I.G. 2010. "The genus *Hyalomma* Koch, 1844. XI. Redescription of all parasitic stages of *H.*(*Euhyalomma*) *asiaticum* (Acari: Ixodidae) and notes on its biology." *Experimental and Applied Acarology* 52: 207-220.

Apanaskevich, D.A., Santos-Silva, M.M. and Horak, I.G. 2008. "The genus *Hyalomma* Koch, 1844. IV. Redescription of all parasitic stages of *H.*(*Euhyalomma*) lusitanicum Koch, 1844 and adults of *H.*(*E.*) *franchinii* Tonelli Rondelli, 1932 (Acari: Ixodidae) with first description of its immature stages." *Folia parasitologica* 55: 61.

Apanaskevich, D.A., Schuster, A.L. and Horak, I.G. 2008. "The genus *Hyalomma*: VII. Redescription of all parasitic stages of *H.*(*Euhyalomma*) *dromedarii* and *H.*(*E.*) *schulzei* (Acari: Ixodidae)." *Journal of Medical Entomology* 45: 817-831.

Archie, J.W. 1985. "Methods for coding variable morphological features for numerical taxonomic analysis." *Systematic Biology* 34: 326-345.

Arctander, P., Johansen, C. and Coutellec-Vreto, M. A. 1999. "Phylogeography of three closely related African Bovids (Tribe Alcelaphini)." *Molecular Biology and Evolution* 16: 1724-1739.

Assunção, P., Jaén-Molina, R., Caujapé-Castells, J., Wolf, M., Buchheim, M.A., Jara, A., Freijanes, K., Carmona, L. and Mendoza, H. 2013. "Phylogenetic analysis of ITS2 sequences suggests the taxonomic re-structuring of *Dunaliella viridis* (Chlorophyceae, Dunaliellales)." *Phycological Research* 61: 81-88.

Avise, J.C. 2000. *Phylogeography: The History and Formation of Species.* Harvard University Press, Cambridge, Massachusetts.

Avise, J.C. and Wollenberg, K. 1997. "Phylogenetics and the origin of species." *Proceedings of the National Academy of Sciences of the United States of America* 94: 7748-7755.

Balashov, Y.S. 1994. "Importance of continental drift in the distribution and evolution of Ixodid ticks." *Entomological Review* 73: 42-50.

Barker, S.C. and Murrell, A. 2004. "Systematics and evolution of ticks with a list of valid genus and species names." *Parasitology* 129: S15-S36.

Barnett, R., Yamaguchi, N., Barnes, I. and Cooper, A. 2006. "The origin, current diversity and future conservation of the modern lion (*Panthera leo*)." *Proceedings of the Royal Society of London B: Biological Sciences* 273: 2119-2125.

Barrett, L.G., Thrall, P.H., Burdon, J.J. and Linde, C.C. 2008. "Life history determines genetic structure and evolutionary potential of host–parasite interactions." *Trends in Ecology & Evolution* 23: 678-685.

Beati, L. and Keirans, J.E. 2001. "Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequence and morphological characters." *Journal of Parasitology* 87: 32-48.

Beati, L., Nava, S., Burkman, E.J., Barros-Battesti, D.M., Labruna, M.D., Guglielmone, A.A., Caceres, A.G., et al. 2013. "*Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne Tick: Phylogeography and evidence for allopatric speciation." BMC Evolutionary Biology 13: 267.

Beati, L., Patel, J., Lucas-Williams, H., Adakal, H., Kanduma, E.G., Temo-Mwase, E., Krecek, R. et al. 2012. "Phylogeography and demographic history of *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae), the Tropical Bont Tick." *Vector-Borne and Zoonotic Diseases* 12: 514-525.

Bente, D.A., Forrester, N.L., Watts, D.M., McAuley, A.J., Whitehouse, C.A. and Bray, M. 2013. "Crimean-Congo Hemorrhagic Fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity." *Antiviral Research* 100: 159-189.

Bezuidenhout, J.D. and Malherbe, A. 1981. "Sweating Sickness: A comparative study of virulent and avirulent strains of *Hyalomma truncatum*." In: *Tick Biology and Control: Proceedings of an International Conference, January 27-29, 1981 (Eds. Whitehead, G.B. and Gibson, J.D.)*. Rhodes University, Grahamstown, South Africa.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. and Das, I. 2007. "Cryptic species as a window on diversity and conservation." *Trends in Ecology & Evolution* 22: 148-155.

Bilton, D.T., Mirol, P.M., Mascheretti, S., Fredga, K., Zima, J. and Searle, J.B. 1998. "Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization." *Proceedings of the Biological Sciences of the Royal Society* 265: 1219-1226.

Black, W.C., Klompen, J.S.H. and Keirans, J.E. 1997. "Phylogenetic relationships among tick subfamilies (Ixodida: Ixodidae: Argasidae) based on the 18S nuclear rDNA Gene." *Molecular Phylogenetics and Evolution* 7: 129-144.

Black, W.C., and Piesman, J.. 1994. "Phylogeny of hard- and soft-Tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences." *Proceedings of the National Academy of Sciences of the United States of America* 91: 10034-10038.

Boccaletti, M., Elter, P. and Guazzone, G. 1971. "Plate tectonic models for the development of the Western Alps and Northern Apennines." *Nature* 234: 108-111.

Booth, A.H. 1958. "The Niger, the Volta and the Dahomey Gap as geographic barriers." *Evolution* 12: 48-62.

Boulkaboul, A. 2003. "Parasitisme des tiques (Ixodidae) des bovins à Tiaret, Algérie." *Revue D'Élevage Et De Médecine Vétérinaire Des Pays Tropicaux* 56: 157-162.

Braaker, S. and Heckel, G. 2009. "Transalpine colonisation and partial phylogeographic erosion by dispersal in the Common Vole (*Microtus arvalis*)." *Molecular Ecology* 18: 2518-2531.

Bradley, D.G., MacHugh, D.E., Cunningham, P. and Loftus, R.T. 1996. "Mitochondrial diversity and the origins of African and European cattle." *Proceedings of the National Academy of Sciences of the United States of America* 93: 5131-5135.

Briggs, J.C. 2003. "The biogeographic and tectonic history of India." *Journal of Biogeography* 30: 381-388.

Brouat, C., Tatard, C., Bâ, K., Cosson, J-F., Dobigny, G., Fichet-Calvet, E., Granjon, L., Lecompte, E., Loiseau, A. and Mouline, K. 2009. "Phylogeography of the Guinea Multimammate Mouse (*Mastomys erythroleucus*): A case study for sahelian species in West Africa." *Journal of Biogeography* 36: 2237-2250.

Brower, A.V.Z. and Schawaroch, V. 1996. "Three steps of homology assessment." *Cladistics* 12: 265-272.

Bruford, M.W., Bradley, D.G. and Luikart, G. 2003. "DNA markers reveal the complexity of livestock domestication." *Nature Reviews Genetics* 4: 900-910.

Bryant, H.N. 1989. "An evaluation of cladistic and character analyses as hypothetico-deductive procedures, and the consequences for character weighting." *Systematic Biology* 38: 214-227.

Bryant, D. and Moulton, V. 2004. "Neighbor-Net: An agglomerative method for the construction of phylogenetic networks." *Molecular Biology and Evolution* 21: 255-265.

Bryja, J., Granjon, L., Dobigny, G., Patzenhauerova, H., Konečný, A., Duplantier, J-M., Gauthier, P., Colyn, M., Durnez, L., and Lalis, A. 2010. "Plio-Pleistocene history of West African sudanian savanna and the phylogeography of the *Praomys daltoni* complex (Rodentia): The environment/geography/genetic interplay." *Molecular Ecology* 19: 4783-4799.

Buczek, A., Bartosik, K. and Buczek, S. 2014. "Four gynandromorphs of *Hyalomma marginatum marginatum* ticks (Acari: Ixodidae) from a laboratory colony." *Journal of Natural History* 48: 1947-1956.

Burger, T.D., Shao, R. and Barker, S.C. 2014. "Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, contains a cryptic species." *Molecular Phylogenetics and Evolution* 76: 241-253.

Buhay, J.E., Moni, G., Mann, N. and Crandall, K.A. 2007. "Molecular Taxonomy in the Dark: Evolutionary history, phylogeography, and diversity of cave crayfish in the subgenus *Aviticambarus*, genus *Cambarus*." *Molecular Phylogenetics and Evolution* 42: 435-448.

Burke, K. 2001. "Origin of the Cameroon line of volcano-capped swells." *The Journal of Geology* 109: 349-362.

Camicas, J-L., Hervy, J-P., Adam, F., and Morel, P-C. 1998. *The Ticks of the World (Acarida, Ixodida): Nomenclature, Described Stages, Hosts, Distribution (Including new species described before 01/01/96.* Éditions de l'Orstom, Paris.

Cangi, N., Horak, I.G., Apanaskevich, D.A., Matthee, S., Das Neves, L.C.B.G., Estrada-Peña, A. and Matthee, C.A. 2013. "The influence of interspecific competition and host preference on the phylogeography of two African Ixodid tick species." *PloS One* 8: e76930.

Carnahan, J., Zheng, L., Taylor, E.E., Toure, Y.T., Norris, D.E., Dolo, G., Diuk-Wasser, M. and Lanzaro, G.C. 2002. "Genetic differentiation of *Anopheles gambiae* s.s. populations in Mali, West Africa, using microsatellite loci." *The Journal of Heredity* 93: 249-253.

Casati, S., Bernasconi, M.V., Gern, L.and Piffaretti, J-C. 2008. "Assessment of intraspecific mtDNA variability of European *Ixodes ricinus* sensu stricto (Acari: Ixodidae)." *Infection, Genetics and Evolution* 8: 152-158.

Cavalli-Sforza, L.L. 1998. "The DNA revolution in population genetics." *Trends in Genetics* 14: 60-65.

Cerling, T.E. 1992. "Development of grasslands and savannas in East Africa during the Neogene." *Palaeogeography, Palaeoclimatology, Palaeoecology* 97: 241-247.

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V. and Ehleringer, J.R. 1997. "Global vegetation change through the Miocene/Pliocene boundary." *Nature* 389: 153-158.

Charleston, M.A. and Robertson, D.L. 2002. "Preferential host switching by primate Lentiviruses can account for phylogenetic similarity with the primate phylogeny." *Systematic Biology* 51: 528-535.

Charruau, P. and Fernandes, C. 2011. "Phylogeography, genetic structure and population divergence time of cheetahs in Africa and Asia: Evidence for long-term geographic isolates. *Molecular Ecology* 20: 706-724.

Chase, B.M. and Meadows, M.E. 2007. "Late Quaternary dynamics of Southern Africa's winter rainfall zone." *Earth-Science Reviews* 84: 103-138.

Chen, Z., Li, Y.Q., Ren, Q.Y., Luo, J., Hu, Y., Li, K., Liu, G.Y., Luo, J.X., Liu, J. and Yin, H. 2015. "Morphological characteristics of normal and gynandromorphic *Hyalomma asiaticum* Schulze and Schlottke, 1930." *The Korean Journal of Parasitology* 53: 361-364.

Chorowicz, J. 2005. "The East African Rift System." *Journal of African Earth Sciences* 43: 379-410.

Clement, M., Posada, D.C.K.A. and Crandall, K.A. 2000. "TCS: A computer program to estimate gene genealogies." *Molecular Ecology* 9: 1657-1659.

Coe, M.J. and Skinner, J.D. 1993. "Connections, disjunctions and endemism in the Eastern and Southern African mammal faunas." *Transactions of the Royal Society of South Africa* 48: 233-255.

Cohen, A.S., Soreghan, M.J. and Scholz, C.A. 1993. "Estimating the Age of Formation of Lakes: An example from Lake Tanganyika, East African Rift System." *Geology* 21: 511-514.

Colangelo, P., Verheyen, E., Leirs, H., Tatard, C., Denys, C., Dobigny, G., Duplantier, J-M., Brouat, C., Granjon, L. and Lecompte, E. 2013. "A Mitochondrial phylogeographic scenario for the most widespread African rodent, *Mastomys natalensis*." *Biological Journal of the Linnean Society* 108: 901-916.

Colgan, D.J., Hutchings, P.A. and Beacham, E. 2008. "Multi-Gene Analyses of the Phylogenetic relationships among the Mollusca, Annelida, and Arthropoda." *Zoological Studies* 47: 338-351.

Corander, J., Marttinen, P., Sirén, J. and Tang, J. 2008. "Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations." *BMC Bioinformatics* 9: 539-553.

Corbet, G.B and Hill, J.E. 1992. *The Mammals of the Indomalayan Region: A Systematic Review.* Oxford University Press, Oxford.

Cox, C.B. 2000. "Plate tectonics, seaways and climate in the historical biogeography of mammals." *Memórias do Instituto Oswaldo Cruz* 95: 509-516.

Crampton, A., McKay, I. and Barker, S.C. 1996. "Phylogeny of ticks (Ixodida) inferred from nuclear ribosomal DNA." *International Journal for Parasitology* 26: 511-517.

Criscione, C.D., Poulin, R. and Blouin, M.S. 2005. "Molecular Ecology of Parasites: Elucidating ecological and microevolutionary processes." *Molecular Ecology* 14: 2247-2257.

Cruickshank, R.H. 2002. "Molecular markers for the phylogenetics of mites and ticks." *Systematic and Applied Acarology* 7: 3-14.

Cruickshank, R.H. and Thomas, R.H. 1999. "Evolution of haplodiploidy in Dermanyssine mites (Acari: Mesostigmata)." *Evolution* 53: 1796-1803.

Csontos, L., Nagymarosy, A., Horváth, F. and Kovác, M. 1992. "Tertiary evolution of the Intra-Carpathian area: A model." *Tectonophysics* 208: 221-241.

Cui, Z.J., Zh Gao, Q., Liu, G.N., Pan, B.T. and Chen, H.L. 1996. "Planation surfaces, palaeokarst and uplift of Xizang (Tibet) Plateau." Science in China (Series D) 26: 378-386.

Dalal, S., Kumar, R. and Gupta, S.K. 2007. "Experimental assortattive interspecies mating between *Hyalomma anatolicum anatolicum* and *Hyalomma dromedarii* on rabbits: Feeding performance of their progeny." *Haryana Veterinarian* 46: 82-84.

de la Fuente, J. 2003. "The fossil record and the origin of ticks (Acari: Parasitiformes: Ixodida)." *Experimental & Applied Acarology* 29: 331-344.

de Queiroz, A. and Gatesy, J. 2007. "The supermatrix approach to systematics." *Trends in Ecology and Evolution* 22: 34-41.

De Waal, D.T. 1992. "Equine Piroplasmosis: A review." *British Veterinary Journal* 148: 6-14.

De Waal, D.T. 1990. "The transovarial transmission of *Babesia caballi* by *Hyalomma truncatum*." *The Onderstepoort Journal of Veterinary Research* 57: 99-100.

Degnan, J.H. and Rosenberg, N.A. 2009. "Gene tree discordance, phylogenetic inference and the multispecies coalescent." *Trends in Ecology and Evolution* 24: 332-340.

Del Rosario Castañeda, M. and De Queiroz, K. 2013. "Phylogeny of the *Dactyloa* clade of Anolis lizards: New insights from combining morphological and molecular data." *Bulletin of the Museum of Comparative Zoology* 160: 345-398.

Delpy, L.P. 1949. "Essai critique de synonymie du genre *Hyalomma* CL Koch 1844 Depuis Linné, 1758." *Annales De Parasitologie Humaine Et Comparée* 24: 464-494.

Delvaux, D., Levi, K., Kajara, R. and Sarota, J. 1992. "Cenozoic paleostress and kinematic evolution of the Rukwa-North Malawi Rift Valley (East African Rift System)." *Bulletin Centres Recherches Exploration Production Elf-Aquitaine* 16: 383-406.

deMenocal, P.B. 2004. "African climate change and faunal evolution during the Pliocene–Pleistocene." *Earth and Planetary Science Letters* 220: 3-24.

Dennell, R. 2004. "Hominid dispersals and Asian biogeography during the lower and early middle Pleistocene, C. 2.0-0.5 Mya." *Asian Perspectives* 43: 205-226.

Denys, C., Chorowicz, J. and Tiercelin, J.J. 1986. "Tectonic and environmental control on rodent diversity in the Plio-Pleistocene sediments of the African Rift System." *Geological Society, London, Special Publications* 25: 363-372.

Dobigny, G., Aniskin, V., Granjon, L., Cornette, R. and Volobouev, V. 2005. "Recent radiation in West African *Taterillus* (Rodentia, Gerbillinae): The concerted role of chromosome and climatic changes." *Heredity* 95: 358-368.

Donoghue, M.J. 1985. "A critique of the Biological Species Concept and recommendations for a phylogenetic alternative." *Bryologist*: 172-181.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. and Rambaut, A. 2006. "Relaxed phylogenetics and dating with confidence." *PLoS Biology* 4: 699.

Drummond, A.J., Rambaut, A., Shapiro, B. and Pybus, O.G. 2005. "Bayesian coalescent inference of past population dynamics from molecular sequences." *Molecular Biology and Evolution* 22: 1185-1192.

Drummond, A.J., Suchard, M.A., Xie, D. and Rambaut, A. 2012. "Bayesian phylogenetics with BEAUti and the BEAST 1.7." *Molecular Biology and Evolution* 29: 1969-1973.

du Toit, N., van Vuuren, B.J., Matthee, S. and Matthee, C.A. 2013. "Biogeography and host-related factors trump parasite life history: Limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts." *Molecular Ecology* 22: 5185-5204.

Dubey, S., Zaitsev, M., Cosson, J-F., Abdukadier, A. and Vogel, P. 2006. "Pliocene and Pleistocene diversification and multiple refugia in a Eurasian shrew (*Crocidura suaveolens* group)." *Molecular Phylogenetics and Evolution* 38: 635-647.

Dumont, H.J. 1998. "The Caspian Lake: History, biota, structure, and function." *Limnology and Oceanography* 43: 44-52.

Dunn, O.J. 1961. "Multiple comparisons among means." *Journal of the American Statistical Association* 56: 52-64.

Dynesius, M. and Jansson, R. 2000. "Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch Climate Oscillations." *Proceedings of the National Academy of Sciences of the United States of America* 97: 9115-9120.

Ebinger, C.J. 1989. "Tectonic development of the Western branch of the East African Rift System." *Geological Society of America Bulletin* 101: 885-903.

Eriksson, J., Hohmann, G., Boesch, C. and Vigilant, L. 2004. "Rivers influence the population genetic structure of Bonobos (*Pan paniscus*)." *Molecular Ecology* 13: 3425-3435.

Evans, B.J., Bliss, S.M., Mendel, S.A. and Tinsley, R.C. 2011. "The Rift Valley is a major barrier to dispersal of African clawed frogs (*Xenopus*) in Ethiopia." Molecular Ecology 20: 4216-4230.

Evans, G.O. and Till, W.M. 1979. "Mesostigmatic mites of Britain and Ireland (Chelicerata: Acari-Parasitiformes): An introduction to their external morphology and classification." *The Transactions of the Zoological Society of London* 35: 139-262.

Excoffier, L. and Lischer, H.E.L. 2010. "Arlequin Suite Ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows." *Molecular Ecology Resources* 10: 564-567.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. "Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data." *Genetics* 131: 479-491.

Feldman-Muhsam, B. 1962. "Revision of the genus *Hyalomma*." *Parasitology* 52: 211-219.

Felsenstein, J. 1985. "Confidence limits on phylogenies: An approach using the bootstrap." *Evolution*: 783-791.

Filippova N.A. 2003: "Re-investigation of type series of *Hyalomma scupense* Schulze, 1918 and *H. detritum* Schulze, 1919 (Acari: Ixodidae) in connection with microevolutionary process within the genus." *Parazitologiya* 37: 455–461.

Fjeldså, J. and Bowie, R.C.K. 2008. "New perspectives on the origin and diversification of Africa's forest avifauna." *African Journal of Ecology* 46: 235-247.

Formosinho, P. and Santos-Silva, M.M. 2006. "Experimental infection of *Hyalomma marginatum* ticks with West Nile Virus." *Acta Virologica* 50: 175-180.

Freitag, S. and Robinson, T.J. 1993. "Phylogeographic patterns in mitochondrial DNA of the Ostrich (*Struthio camelus*)." *The Auk* 110: 614-622.

Fu, Y.X. 1997. "Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection." *Genetics* 147: 915-925.

Fuchs, J. and Bowie, R.C.K. 2015. "Concordant genetic structure in two species of woodpecker distributed across primary West African biogeographic barriers." *Molecular Phylogenetics and Evolution* 88: 64-74

Gaubert, P. and Cordeiro-Estrela, P. 2006. "Phylogenetic systematics and tempo of evolution of Viverrinae (Mammalia, Carnivora, Viverridae) within feliformians: Implications for faunal exchange between Asia and Africa." *Molecular phylogenetics and evolution* 42: 266-278.

Giribet, G., Carranza, S., Riutort, M., Baguna, J. and Ribera, C. 1999. "Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences." *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences* 354: 215-222.

Girman, D.J., Vila, C., Geffen, E., Creel, S., Mills, M.G.L., McNutt, J.W. and Wayne, R.K. 2001. "Patterns of population subdivision, gene flow and genetic variability in the African Wild Dog (*Lycaon pictus*)." *Molecular Ecology* 10: 1703-1723.

Goldstein, P.Z. and DeSalle, R. 2011. "Integrating DNA Barcode Data and Taxonomic Practice: Determination, discovery, and description." *Bioessays* 33: 135-147.

Goto, R., Kawakita, A., Ishikawa, H., Hamamura, Y. and Kato, M. 2012. "Molecular phylogeny of the bivalve superfamily Galeommatoidea (Heterodonta, Veneroida) reveals dynamic evolution of symbiotic lifestyle and interphylum host switching." *BMC Evolutionary Biology* 12: 172-2148-12-172.

Gou, H., Guan, G., Liu, A., Ma, M., Chen, Z., Liu, Z., Ren, Q., Li, Y., Yang, J., Yin, H. and Luo, J. 2013. "Coevolutionary analysis of the relationships between piroplasmids and their hard tick hosts." *Ecology and Evolution* 3: 2985-2993.

Graham, M.R., Oláh-Hemmings, V. and Fet, V. 2012. "Phylogeography of co-distributed dune scorpions identifies the Amu Darya River as a long-standing component of central Asian biogeography: (Scorpiones: Buthidae)." *Zoology in the Middle East* 55: 95-110.

Grubb, P., Sandrock, O., Kullmer, O., Kaiser, T.M. and Schrenk, F. 1999. "Relationships between eastern and southern African mammal faunas". In: *African biogeography, climate change, and human evolution (Eds. Bromage, G.T. and Schrenk, F.)*, 253-267. Oxford University Press, Oxford.

Guglielmone, A.A. and Nava, S. 2014. "Names for Ixodidae (Acari: Ixodoidea): Valid, synonyms, Incertae sedis, Nomina dubia, Nomina nuda, Lapsus, incorrect and

suppressed names—with notes on confusions and misidentifications." *Zootaxa* 3767: 1-256.

Guindon, S. and Gascuel, O. 2003. "A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood." *Systematic Biology* 52: 696-704.

Guo, X. and Wang, Y. 2007. "Partitioned Bayesian analyses, Dispersal–vicariance analysis, and the biogeography of Chinese Toad-Headed lizards (Agamidae: *Phrynocephalus*): A re-evaluation." *Molecular Phylogenetics and Evolution* 45: 643-662.

Hafner, M.S. and Nadler, S.A. 1990. "Cospeciation in Host-Parasite Assemblages: Comparative analysis of rates of evolution and timing of cospeciation events." *Systematic Biology* 39: 192-204.

Hafner, M.S. and Page, R.D. 1995. "Molecular Phylogenies and Host-Parasite Cospeciation: Gophers and lice as a model system." *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences* 349: 77-83.

Hall, T.A. 1999. "BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT." *Nucleic Acids Symposium Series* 4: 95-98.

Halliday, A.N., Dickin, A.P., Fallick, A.E. and Fitton, J.G. 1988. "Mantle dynamics: A Nd, Sr, Pb and O isotopic study of the Cameroon line volcanic chain." *Journal of Petrology* 29: 181-211.

Harcourt, A.H. and Wood, M.A. 2012. "Rivers as barriers to primate distributions in Africa." *International Journal of Primatology* 33: 168-183.

Harpending, H.C., Batzer, M.A., Gurven, M., Jorde, L.B., Rogers, A.R. and Sherry, S.T. 1998. "Genetic traces of ancient demography." *Proceedings of the National Academy of Sciences of the United States of America* 95: 1961-1967.

Harrison, T. M., Copeland, P., Kidd, W.S.F. and Lovera, O.M. 1995. "Activation of the Nyainqentanghla Shear Zone: Implications for uplift of the Southern Tibetan Plateau." *Tectonics* 14: 658-676.

Harrison, T.M., Copeland, P., Kidd, W.S.F. and Yin, A. 1992. "Raising Tibet." *Science* 255: 1663-1670.

Hassanin, A., Ropiquet, A., Gourmand, A-L., Chardonnet, B. and Rigoulet, J. 2007. "Mitochondrial DNA Variability in *Giraffa camelopardalis*: Consequences for taxonomy, phylogeography and conservation of giraffes in West and Central Africa." *Comptes Rendus Biologies* 330: 265-274.

Haukisalmi, V., Wickström, L.M., Hantula, J. and Henttonen, H. 2001. "Taxonomy, genetic differentiation and holarctic biogeography of *Paranoplocephala*

spp.(Cestoda: Anoplocephalidae) in Collared Lemmings (Dicrostonyx; Arvicolinae)." *Biological Journal of the Linnean Society* 74: 171-196.

Hawkins, J.A., Hughes, C.E. and Scotland, R.W. 1997. "Primary homology assessment, characters and character states." *Cladistics* 13: 275-283.

Hazhauser, M., Kroh, A., Mandie, O., Piller, W.E., Göhlich, U., Rueter, M. and Berning, B. 2007. "Biogeographic responses to geodynamics: A key study around the Oligo-Miocene Tethyan Seaway." *Zoologischer Anzeiger* 246: 241-256.

Hebert, P.D., Ratnasingham, S. and deWaard, R.J. 2003. "Barcoding Animal Life: Cytochrome C Oxidase Subunit 1 divergences among closely related species." *Proceedings.Biological Sciences / the Royal Society* 270: S96-9.

Heikinheimo, H., Fortelius, M., Eronen, J. and Mannila, H. 2007. "Biogeography of European land mammals shows environmentally distinct and spatially coherent clusters." *Journal of Biogeography* 34: 1053-1064.

Heisch, R.B., Grainger, W.E., Harvey, A.E. and Lister, G. 1962. "Feral aspects of Rickettsial infections in Kenya." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 56: 272-286.

Hewitt, G.M. 1999. "Post-glacial re-colonization of European biota." *Biological Journal of the Linnean Society* 68: 87-112.

Hewitt, G.M. 2000. "The genetic legacy of the Quaternary Ice Ages." *Nature* 405: 907-913.

Hewitt, G.M. 2004. "Genetic consequences of climatic oscillations in the Quaternary." *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences* 359: 183-95.

Hickerson, M.J., Carstens, B.C., Cavender-Bares, J., Crandall, K.A., Graham, C.H., Johnson, J.B., Rissler, L., Victoriano, P.F. and Yoder, A.D. 2010. "Phylogeography's Past, Present, and Future: 10 years after." *Molecular Phylogenetics and Evolution* 54: 291-301.

Hillis, D.M. 1987. "Molecular versus morphological approaches to systematics." *Annual Review of Ecology and Systematics* 18: 23-42.

Hillis, D.M. and Dixon, M.T. 1991. "Ribosomal DNA: Molecular evolution and phylogenetic inference." *Quarterly Review of Biology* 66: 411-453.

Holm, S. 1979. "A simple sequentially rejective multiple test procedure." *Scandinavian Journal of Statistics* 6: 65-70.

Hoogstraal, H. 1956. *African Ixodoidea.VoI.I.Ticks of the Sudan (with Special Reference to Equatoria Province and with Preliminary Reviews of the Genera*

Boophilus, Margaropus, and Hyalomma). Department of the Navy, Bureau of Medicine and Surgery, Washington.

Hoogstraal, H. 1979. "Review Article: The epidemiology of tick-borne Crimean-Congo Hemorrhagic Fever in Asia, Europe, and Africa." *Journal of Medical Entomology* 15: 307-417.

Hoogstraal, H. and Aeschlimann, A. 1982. "Tick–host specificity." *Bulletin de la Socie´te´ Entomologique Suisse* 55: 5-32.

Hoogstraal, H. and Kaiser, M.N. 1959. "Ticks (Ixodoidea) of Arabia: With special reference to the Yemen Chicago Natural History Museum." *Fieldiana Zoology* 39: 297–322.

Hoogstraal, H., Wassef, H.Y. and Buttiker, W. 1981. "Ticks (Acarina) of Saudi Arabia. Fam. Argasidae, Ixodidae." *Fauna of Saudi Arabia* 3: 25-110.

Hornok, S. and Horváth, G. 2012. "First report of adult *Hyalomma marginatum rufipes* (Vector of Crimean-Congo Haemorrhagic Fever virus) on cattle under a continental climate in Hungary." *Parasite Vectors* 5: 170.

Howell, C.J. 1978. *"*Ticks, Mites and Insects Infesting Domestic Animals in South Africa, Part 1." In: *Scientific Bulletin No. 93.* Department of Agricultural Technical Services of the Republic of South Africa, Pretoria.

Hughes, J.B., Round, P.D. and Woodruff, D.S. 2003. "The Indochinese–Sundaic Faunal Transition at the Isthmus of Kra: An analysis of resident forest bird species distributions." *Journal of Biogeography* 30: 569-580.

Hurst, G.D. and Jiggins, F.M. 2005. "Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts." *Proceedings of the Biological Sciences of the Royal Society* 272: 1525-1534.

Huson, D.H. and Bryant, D. 2006. "Application of phylogenetic networks in evolutionary studies." *Molecular Biology and Evolution* 23: 254-267.

Jacobs, B.F. 2004. "Palaeobotanical Studies from Tropical Africa: Relevance to the evolution of forest, woodland and savannah biomes." *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences* 359: 1573-1583.

Jolivet, L. and Faccenna, C. 2000. "Mediterranean extension and the Africa-Eurasia collision." *Tectonics* 19: 1095-1106.

Kalinowski, S.T. 2011. "The computer program STRUCTURE does not reliably identify the main genetic clusters within species: Simulations and implications for human population structure." *Heredity* 106: 625-632.

Kar, S., Akyildiz, G., Yilmazer, N., Shaibi, T., Gargili, A. and Vatansever, Z. 2015. "External morphological anomalies in Ixodid ticks from Thrace, Turkey." *Experimental and Applied Acarology* 67: 457-466.

Kempf, F., Boulinier, T., De Meeûs, T., Arnathau, C., and McCoy, K.D. 2009. "Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*." *Molecular Ecology* 18: 4450-4462.

Keskin, A., Bursali, A. and Tekin, S. 2012. "A case of gynandromorphism in *Hyalomma marginatum* Koch, 1844 (Acari: Ixodidae)." *The Journal of Parasitology* 98: 1271-1272.

Klompen, J.S.H., Black, W.C., Keirans, J.E. and Oliver J.H. 1996. "Evolution of ticks." *Annual Review of Entomology* 41: 141-161.

Klompen, J.S.H., Black, W.C., Keirans, J.E. and Norris, D.E. 2000. "Systematics and biogeography of hard ticks, a total evidence approach." *Cladistics* 16: 79-102.

Koch, C.L. 1844. "Systematische Übersicht über die Ordnung der Zecken." *Archiv für Naturgeschichte* 10: 217-239.

Kolonin, G.V. 1982. *World Distribution of Ixodid Ticks. Genera Hyalomma, Aponomma, Amblyomma.* Publishing House Nauka, Moscow. (In Russian)

Koufos, G.D., Kostopoulos, D.S. and Vlachou, T.D. 2005. "Neogene/Quaternary mammalian migrations in eastern Mediterranean." *Belgian Journal of Zoology* 135: 181.

Krijgsman, W. 2002. "The Mediterranean: Mare Nostrum of Earth sciences." *Earth and Planetary Science Letters* 205: 1-12.

Kukla, G. and Cílek, V. 1996. "Plio-Pleistocene Megacycles: Record of climate and tectonics." *Palaeogeography, Palaeoclimatology, Palaeoecology* 120: 171-194.

Kuraku, S., Hoshiyama, D., Katoh, K., Suga, H. and Miyata, T. 1999. "Monophyly of Lampreys and Hagfishes supported by nuclear DNA–coded Genes." *Journal of Molecular Evolution* 49: 729-735.

Kurup, G.U. 1974. "Mammals of Assam and the mammal-geography of India." In: *Ecology and Biogeography in India*, 585-613: Springer, Netherlands.

Kutschera, V.E., Bidon, T., Hailer, F., Rodi, J.L., Fain, S.R. and Janke, A. 2014. Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow." *Molecular Biology and Evolution* 31: 2004-2017.

Leakey, M.D. and Harris, J.M. 1987. *Laetoli, a Pliocene Site in Northern Tanzania.* Oxford University Press, Oxford.

Lehmann, T., Hawley, W.A., Grebert, H., Danga, M., Atieli, F. and Collins, F.H. 1999. "The Rift Valley complex as a barrier to gene flow for Anopheles gambiae in Kenya." *Journal of Heredity* 90: 613-621.

Li, J., Fang, X., Ma, H., Zhu, J., Pan, B. and Chen, H. 1996. "Geomorphological and environmental evolution in the upper reaches of the Yellow River during the late Cenozoic." *Science in China (Series D)* 39: 380–390.

Librado, P. and Rozas, J. 2009. "DnaSP V5: A software for comprehensive analysis of DNA polymorphism data." *Bioinformatics* 25: 1451-1452.

Loader, S.P., Poynton, J.C. and Mariaux J. 2004. "Herpetofauna of Mahenge Mountain, Tanzania: A window on African biogeography." *African Zoology* 39: 71-76.

Logatchev, N.A., Beloussov, V.V. and Milanovsky, E.E. 1972. "East African Rift development." *Tectonophysics* 15: 71-81.

Lopardo, L., Giribet, G. and Hormiga, G. 2011. "Morphology to the rescue: Molecular data and the signal of morphological characters in combined phylogenetic analyses - a case study from mysmenid spiders (Araneae, Mysmenidae), with comments on the evolution of web architecture." *Cladistics* 27: 278-330.

Lopes, C.M. and de Freitas, T.R. 2012. "Human Impact in Naturally Patched Small Populations: Genetic structure and conservation of the burrowing rodent, Tuco-Tuco (*Ctenomys lami*)." *The Journal of Heredity* 103: 672-681.

Lorenzen, E.D., Heller, R. and Siegismund, H.R. 2012. "Comparative phylogeography of African savannah ungulates." *Molecular Ecology* 21: 3656-3670.

Lorenzen, E.D., Masembe, C., Arctander, P. and Siegismund, H.R. 2010. "A Long-standing Pleistocene refugium in Southern Africa and a mosaic of refugia in East Africa: Insights from mtDNA and the common Eland antelope." *Journal of Biogeography* 37: 571-581.

Lou, J., Yang, D-M., Suzuki, H., Wang, Y-X., Chen, W-Y., Campbell, K.L. and Zhang, Y. 2004. "Molecular phylogeny and biogeography of the Oriental voles: genus *Eothenomys* (Muridae: Mammalia)." *Molecular Phylogenetics and Evolution* 33: 349-362.

Lovett, J.C. and Wasser, S.K. 1993. *Biogeography and Ecology of the Rain Forests of Eastern Africa.* Cambridge University Press, Cambridge.

Luikart, G., Gielly, L., Excoffier, L., Vigne, J.D., Bouvet, J. and Taberlet, P. 2001. "Multiple maternal origins and weak phylogeographic structure in domestic goats." *Proceedings of the National Academy of Sciences of the United States of America* 98: 5927-5932.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C. Yuan, X., Jia, G., Deng, J., Wang, C. and Wang, Q. 2014. "Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida)." *Parasit Vectors* 7: 93.

Maddison, D.R. and Maddison, W.P. 2000. *MacClade 4.0: Analysiof Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, USA.

Madhav, N.K., Brownstein, J.S., Tsao, J.I. and Fish, D. 2004. "A dispersal model for the range expansion of Blacklegged Tick (Acari: Ixodidae)." *Journal of Medical Entomology* 41: 842-852.

Mallatt, J.M., Garey, J.R. and Shultz, J.W. 2004. "Ecdysozoan phylogeny and Bayesian Inference: First use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin." *Molecular Phylogenetics and Evolution* 31: 178-191.

Mangold, A.J., Bargues, M.D. and Mas-Coma, S. 1997. "18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae)." *Parasitology Research* 84: 31-37.

Mangold, A.J., Bargues, M.D. and Mas-Coma, S. 1998. "Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae)." *Parasitology Research* 84: 478-484.

Mans, B.J. and Neitz, A.W.H. 2003. "Adaption of ticks to a blood-feeding enviroment: evolution from a funtional perspective." *Insect Biochemistry and Molecular Biology* 34: 1-17.

Marko, P.B. 2002. "Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama." *Molecular Biology and Evolution* 19: 2005-2021.

Matthee, C.A. and Flemming, F.A. 2002. Population fragmentation in the Southern Rock Agama, *Agama atra*: More evidence for vicariance in southern Africa." *Molecular Ecology* 11: 465-471.

Matthee, C.A. and Robinson, T.J. 1997. "Mitochondrial DNA phylogeography and comparative cytogenetics of the Springhare, *Pedetes capensis* (Mammalia: Rodentia)." *Journal of Mammalian Evolution* 4: 53-73.

Matthee, C.A., van Vuuren, B.J., Bell, D. and Robinson, T.J. 2004. "A Molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene." *Systematic Biology* 53: 433-447.

Matthee, S., Horak, I.G., Beaucournu, J-C., Durden, L.A., Ueckermann, E.A. and McGeoch, M.A. 2007. "Epifaunistic arthropod parasites of the Four-Striped

Mouse, *Rhabdomys pumilio*, in the Western Cape Province, South Africa." *Journal of Parasitology* 93: 47-59.

McCoy, K.D., Boulinier, T. and Tirard, C. 2005. "Comparative host–parasite Population Structures: Disentangling prospecting and dispersal in the Black Legged Kittiwake *Rissa tridactyla*." *Molecular Ecology* 14: 2825-2838.

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J.C., Golovljova, I., et al. 2013. "Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe." Parasites and Vectors 6: 10.1186/1756-3305-6-1.

Meier, R. 2000. *Species Concepts and Phylogenetic Theory: A Debate.* Columbia University Press, New York.

Melis, C., Szafrańska, P.A., Jędrzejewska, B. and Bartoń, K. 2006. "Biogeographical variation in the population density of Wild Boar (*Sus scrofa*) in Western Eurasia." *Journal of Biogeography* 33: 803-811.

Melville, J., Hale, J., Mantziou, G., Ananjeva, N.B., Milto, K. and Clemann, N. 2009. "Historical biogeography, phylogenetic relationships and intraspecific diversity of Agamid Lizards in the Central Asian deserts of Kazakhstan and Uzbekistan." *Molecular Phylogenetics and Evolution* 53: 99-112.

Mercer, J.M. and Roth, V.L. 2003. "The effects of Cenozoic global change on squirrel phylogeny." *Science* 299: 1568-1572.

Molnar, P. and Tapponnier, P. 1977. "The collision between India and Eurasia." *Scientific American* 236: 30-41.

Moodley, Y. and Bruford, W.M. 2007. "Molecular Biogeography: Towards an integrated framework for conserving Pan-African biodiversity." *PloS One* 2: e454.

Moore, A.E., Cotterhill, F.P.D.W., Main, M.P. and Williams, H.B. 2007. "The Zambezi River." In: *Large Rivers: geomorphology and management (Ed. Gupta, A.)*, 311-332. John Wiley & Sons Ltd., USA.

Moore, A.E. and Larkin, P.A. 2001. "Drainage evolution in south-central Africa since the breakup of Gondwana." *South African Journal of Geology* 104: 47-68.

Morales, J.C. and Melnick, D.J. 1997. "A new West African Chimpanzee subspecies?" *Nature* 388: 337.

Moran, N. and Baumann, P. 1994. "Phylogenetics of cytoplasmically inherited microorganisms of arthropods." *Trends in Ecology & Evolution* 9: 15-20.

Mousson, L., Dauga, C., Garrigues, T., Schaffner, F., Vazeille, M. and Failloux, A-B. 2005. "Phylogeography of *Aedes* (*Stegomyia*) *aegypti* (*L.*) and *Aedes*

(*Stegomyia*) *albopictus* (*Skuse*)(Diptera: Culicidae) based on mitochondrial DNA variations." *Genetical Research* 86: 1-11.

Mueller, R.L. 2006. "Evolutionary rates, divergence dates, and the performance of mitochondrial genes in Bayesian phylogenetic analysis." *Systematic Biology* 55: 289-300.

Murrell, A., Campbell, N.J.H. and Barker, S.C. 2000. "Phylogenetic analyses of the Rhipicephaline ticks indicate that the genus *Rhipicephalus* is paraphyletic." *Molecular Phylogenetics and Evolution* 16: 1-7.

Murrell, A., Campbell, N.J.H. and Barker, S.C. 2001. "A total-evidence phylogeny of ticks provides insights into the evolution of life cycles and biogeography." *Molecular Phylogenetics and Evolution* 21: 244-258.

Najman, Y.M.R., Enkin, R.J., Johnson, M.R.W., Robertson, A.H.F. and Baker, J. 1994. "Paleomagnetic dating of earliest continental Himalayan foredeep sediments." *Earth and Planetary Science Letters* 128: 713-718.

Nelson, G. 1994. "Homology and Systematics." In: *Homology: The Hierarchical Basis of Comparative Biology (Ed. Hall, B.K.)*, 101-149. Academic Press, San Diego.

Neumann, G. 1899. "Revision of the family Ixodidae." *Memoirs of the French Zoological Society* 12: 107-294. (In French)

Nichol, J.E. 1999. "Geomorphological evidence and Pleistocene refugia in Africa." *The Geographical Journal* 165: 79-89.

Nicolas, V., Mboumba, J-F., Verheyen, E., Denys, C., Lecompte, E., Olayemi, A., Missoup, A.D., Katuala, P. and Colyn, M. 2008. "Phylogeographic structure and regional history of *Lemniscomys striatus* (Rodentia: Muridae) in tropical Africa." *Journal of Biogeography* 35: 2074-2089.

Nieberding, C.M. and Olivieri, I. 2007. "Parasites: Proxies for host genealogy and ecology?" *Trends in Ecology & Evolution* 22: 156-165.

Nixon, K.C. and Wheeler, Q.D. 1990. "An amplification of the Phylogenetic Species Concept." *Cladistics* 6: 211-223.

Norval, R.A.I., Horak, I.G., Coetzer, J.A.W. and Tustin, R.C. 2004. "Vectors: Ticks." *Infectious Diseases of Livestock* 1: 3-42.

Noureddine, R., Chauvin, A. and Plantard, O. 2011. "Lack of genetic structure among Eurasian populations of the tick *Ixodes ricinus* contrasts with marked divergence from North-African populations." *International Journal for Parasitology* 41: 183-192.

Nyangiwe, N., Matthee, C.A., Horak, I.G. and Matthee, S. 2013. "First record of the pantropical Blue Tick *Rhipicephalus microplus* in Namibia." *Experimental and Applied Acarology* 61: 503-507.

Ogden, N.H., Mechai, S. and Margos, G. 2013. "Changing geographic ranges of ticks and tick-borne pathogens: Drivers, mechanisms and consequences for pathogen diversity." *Frontiers in Cellular and Infection Microbiology* 3: 1-36.

Page, R.D.M. 2003.*Tangled Trees: Phylogeny, cospeciation, and evolution*. University of Chicago Press, Chicago, Illinois.

Partridge, T.C. 1997. "Cainozoic environmental change in Southern Africa, with special emphasis on the last 200 000 years." *Progress in Physical Geography* 21: 3-22.

Partridge, T.C., Wood, B.A. and deMenocal, P.B. 1995. "The Influence of Global Climatic Change and Regional Uplift on Large-Mammalian Evolution in East and Southern Africa." In: *Paleoclimate and Evolution, with Emphasis on Human Origins (Eds. Vrba, E.S., Denton, G.H., Partridge, T.C. and Burckle, L.H.)*, 331-355. Yale University Press, New Haven, Connecticut.

Patou, M-L., Wilting, A., Gaubert, P., Esselstyn, J.A., Cruaud, C., Jennings, A.P., Fickel, J. and Veron, G. 2010. "Evolutionary history of the Paradoxurus Palm Civets–a new model for Asian biogeography." *Journal of Biogeography* 37: 2077-2097.

Patterson, C. 1982. "Morphological characters and homology." *Problems of Phylogenetic Reconstruction* 21: 21-74.

Patterson, C. 1988. "Homology in classical and molecular biology." *Molecular Biology and Evolution* 5: 603-625.

Peakall, R. and Smouse, P.E. 2012. "GenAlEx 6.5: Genetic Analysis in Excel. Population genetic software for teaching and research--An update." *Bioinformatics* 28: 2537-2539.

Pimentcl, R.A. and Riggins, R. 1987. "The Nature of cladistic data." *Cladistics* 3: 201-209.

Pleijel, F. 1995. "On character coding for phylogeny reconstruction." *Cladistics* 11: 309-315.

Pisano, J., Condamine, F.L., Lebedev, V., Bannikova, A., Quéré, J-P., Shenbrot, G.I., et al. 2015. "Out of Himalaya: the impact of past Asian enviromental changes on the evolutionary and biogeographical histroy of Dipodoidea (Rodentia)." *Journal of Biogeography* 42: 856-870.

Pomerantzev, B.I. 1946. "Ticks (Ixodidae) of the USSR and neighbouring countries." *Opredeliteli po Faune SSSR, izdavaemye Zoologicheskim Institutom Akademii*

Nauk SSSR, 26-28. Akademiya Nauk SSSR, Moscow, Leningrad, USSR. (In Russian).

Posada, D. 2008. "jModelTest: Phylogenetic model averaging." *Molecular Biology and Evolution* 25: 1253-1256.

Posada, D. and Buckley, T.R. 2004. "Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian approaches over Likelihood ratio tests." *Systematic Biology* 53: 793-808.

Potts, R. 1998. "Environmental hypotheses of Hominin evolution." *American Journal of Physical Anthropology* 107: 93-136.

Potts, R. 2007. "Environmental hypotheses of Pliocene Human evolution." In: *Hominin Environments in the East African Pliocene: An Assessment of the Faunal Evidence*, 25-49. Springer, Netherlands.

Pretorius, E. and Clarke, F.C. 2000. "Geometric morphometric analyses of the male and female body shape of *Hyalomma truncatum* and *H. marginatum rufipes* (Acari: Ixodidae)." *International Journal of Acarology* 26: 229-238.

Qiu, W-G., Dykhuizen, D.E., Acosta, M.C. and Luft, B.J. 2002. "Geographic uniformity of Lyme Disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the north eastern United States." *Genetics* 160: 833-849.

Radovsky, F.J. 1994. "The evolution of parasitism and the distribution of some Dermanyssoid mites (Mesostigmata) on vertebrate hosts." In: *Mites*, 186-217. Springer.

Rambaut, A. and Drummond, A.J. 2007. "TRACER Version 1.5. Available: http://beast.bio.ed.ac.uk/Tracer

Randolph, S.E. 1997. "Abiotic and biotic determinants of the seasonal dynamics of the tick *Rhipicephalus appendiculatus* in South Africa." *Medical and Veterinary Entomology* 11: 25-37.

Randolph, S.E. 1998. "Ticks are not insects: consequences of contrasting vector biology for transmission potential." *Parasitology Today* 14: 186-192.

Rashid, T. 2014. "Landforms Development in Bangladesh." In: *Holocene Sea-level Scenarios in Bangladesh*, 51-60. Springer, Singapore.

Reed, K.E. 1997. "Early Hominid evolution and ecological change through the African Plio-Pleistocene." *Journal of Human Evolution* 32: 289-322.

Rees, D.J., Dioli, M. and Kirkendall, L.R. 2003. "Molecules and Morphology: Evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae)." *Molecular Phylogenetics and Evolution* 27: 131-142.

Relethford, J.H. 2008. "Genetic evidence and the modern Human origins debate." *Heredity* 100: 555-563.

Retallack, G.J. 2001. "Cenozoic expansion of grasslands and climatic cooling." *The Journal of Geology* 109: 407-426.

Roberts, E.M., Stevens, N.J., O'Connor, P.M., Dirks, P.H.G.M., Gottfried, M.D., Clyde, W.C., Armstrong, R.A., Kemp, A.I.S. and Hemming, S. 2012. "Initiation of the Western Branch of the East African Rift coeval with the Eastern Branch." *Nature Geoscience* 5: 289-294.

Rögl, F. 1999. "Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview)." *Geologica Carpathica* 50: 339-349.

Ronquist, F. 1997. "Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography." *Systematic Biology*: 195-203.

Ronquist, F. 2001. "DIVA version 1.2. Computer program for MacOS and Win32." *Evolutionary Biology Centre, Uppsala University* Available at: http://www.ebc.uu.se/systzoo/research/diva/diva.html.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. 2012. "MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space." *Systematic Biology* 61: 539–542.

Ronquist, F. and Huelsenbeck, J.P. 2003. "MrBayes 3: Bayesian phylogenetic inference under mixed models." *Bioinformatics* 19: 1572-1574.

Rubinoff, D. and Holland, B.S. 2005. "Between Two Extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference." *Systematic Biology* 54: 952-961.

Ruvolo, M., Pan, D., Zehr, S., Goldberg, T., Disotell, T.R. and von Dornum, M. 1994. "Gene trees and hominoid phylogeny." *Proceedings of the National Academy of Sciences of the United States of America* 91: 8900-8904.

Salzburger, W., Van Bocxlaer, B. and Cohen, A.S. 2014 "Ecology and evolution of the African Great Lakes and their faunas." *Annual Review of Ecology, Evolution, and Systematics* 45: 519-545.

Sands, A.F., Matthee, S., Mfune, J.K.E. and Matthee, C.A. 2015. "The influence of life history and climate driven diversification on the mtDNA phylogeographic structures of two Southern African *Mastomys* species (Rodentia: Muridae: Murinae)." *Biological Journal of the Linnean Society* 114: 58-68.

Sanmartín, I. 2003. "Dispersal Vs. Vicariance in the Mediterranean: Historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea)." *Journal of Biogeography* 30: 1883-1897.

Schaffer, W.M. and Rosenzweig, M.L. 1978. "Homage to the Red Queen. I. Coevolution of predators and their victims." *Theoretical Population Biology* 14: 135-157.

Schmitt, T., Gießl, A. and Seitz, A. 2002. "Postglacial colonisation of Western Central Europe by *Polyommatus coridon* (Poda 1761) (Lepidoptera: Lycaenidae): Evidence from population genetics." *Heredity* 88: 26-34.

Schneider, S. and Excoffier, L. 1999. "Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA." *Genetics* 152: 1079-1089.

Schultz, J. and Wolf, M. 2009. "ITS2 Sequence–structure Analysis in Phylogenetics: A how-to manual for molecular systematics." *Molecular Phylogenetics and Evolution* 52: 520-523.

Schulze, P. 1919. "Bestimmungstabelle für das Zeckengenus *Hyalomma* Koch." *Sitzungsber Ges Naturforsch Freunde Berl* 5: 189-196.

Schulze, P. and Schlottke, E. 1930. "Bestimmungstabellen für das Zeckengenus *Hyalomma* Koch *s. str.*" *Sitzungsber Abh Naturforsch Ges Rostock* 2: 32-46.

Schwab, P., Debes, P.V., Witt, T., Hartl, G.B., Hmwe, S.S., Zachos, F.E. and Grobler, J.P. 2012. "Genetic Structure of the Common Impala (*Aepyceros melampus melampus*) in South Africa: Phylogeography and implications for conservation." *Journal of Zoological Systematics and Evolutionary Research* 50: 76-84.

Scotland, R.W., Olmstead, R.G. and Bennett, J.R. 2003. "Phylogeny Reconstruction: The role of morphology." *Systematic Biology* 52: 539-548.

Scotland, R.W. and Pennington, R.T. 2000. *Homology and Systematics: Coding Characters for Phylogenetic Analysis.* Taylor and Francis, London.

Scott, J.D., Fernando, K., Banerjee, S.N., Durden, L.A., Byrne, S.K., Banerjee, M., Mann, R.B. and Morshed, M.G. 2001. " Birds Disperse Ixodid (Acari: Ixodidae) and *Borrelia burgdorferi*-Infected Ticks in Canada." *Journal of Medical Entomology* 38: 493-500.

Sen, S. 2013. "Dispersal of African mammals in Eurasia during the Cenzoic: ways and whys." *Geobios* 46: 159-172.

Shyma, K.P., Kumar, S., Sharma, A.K., Ray, D.D. and Ghosh, S. 2012. "Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*." *Experimental and Applied Acarology* 58: 471-481.

Slowinski, J.B. and Page, R.D.M. 1999. "How should species phylogenies be inferred from sequence data?." *Systematic Biology* 48: 814-825.

Smith, A.B. 1994. *Systematics and the Fossil Record: Documenting Evolutionary Patterns.* Blackwell Scientific, Oxford.

Sole, C.L., Scholtz, C.H. and Bastos, A.D.S. 2005. "Phylogeography of the Namib Desert Dung Beetles *Scarabaeus* (Pachysoma) MacLeay (Coleoptera: Scarabaeidae)." *Journal of Biogeography* 32: 75-84.

Stankiewicz, J. and Wit, M.J. 2006. "A proposed drainage evolution model for Central Africa- Did the Cong flow east?." *Journal of African Earth Science* 44: 75-84.

Stephens, M. and Donnelly, P. 2003. "A comparison of Bayesian methods for haplotype reconstruction from population genotype data." *American Journal of Human Genetics* 73: 1162-1169.

Stephens, M., Smith, N.J. and Donnelly, P. 2001. "A new statistical method for haplotype reconstruction from population data." *American Journal of Human Genetics* 68: 978-989.

Stiassny, M.L.J. and Meyer, A. 1999. "Cichlids of the Rift lakes." *Scientific American* 280: 64-69.

Su, Y-C., Chang, Y-H., Lee, S-C. and Tso, I-M. 2007. "Phylogeography of the Giant Wood Spider (*Nephila pilipes*, Araneae) from Asian–Australian regions." *Journal of Biogeography* 34: 177-191.

Sureau, P., Cornet, J.P., Germain, M., Camicas, J.L., and Robin, Y. 1976. "Enquête sur les arbovirus transmis par les tiques en Rêpublique Centrafricaine (1973–1974). Isolement des virus Dugbe,CHF/Congo, Jos et Bhanja." *Bulletin de la Société de pathologie exotique* 69: 27-33.

Svenson, G.J. and Whiting, M.F. 2004. "Phylogeny of Mantodea based on molecular data: Evolution of a charismatic predator." *Systematic Entomology* 29: 359-370.

Swanepoel, R. and Burt, F.J. 2004. "Crimean-Congo haemorrhagic fever." In: *Infectious diseases of livestock (Eds. Coetzer, J.A.W. and Tustin, R.C.)*. Oxford University Press, Cape Town.

Swofford, D.L. 2001. *Paup*: Phylogenetic Analysis using Parsimony (and Other Methods). Version 4.* Sinauer Associates.

Szöllősi, G.J., Tannier, E., Daubin, V. and Boussau, B. 2015. "The inference of gene tress with species trees." *Systematic Biology* 64: e42-e64.

Taberlet, P., Fumagalli, L., Wust-Saucy, A-G. and Cosson, J-F. 1998. "Comparative phylogeography and postglacial colonization routes in Europe." *Molecular Ecology* 7: 453-464.

Taboada, J. and Merchant, S.R. 1991. "Babesiosis of companion animals and man." *Veterinary Clinics of North America: Small Animal Practice* 21: 103-123.

Takacs, Z., Morales, J.C., Geissmann, T. and Melnick, D.J. 2005. "A complete species-level phylogeny of the Hylobatidae based on mitochondrial ND3–ND4 gene sequences." *Molecular Phylogenetics and Evolution* 36: 456-467.

Tamma, K. and Ramakrishnan, U. 2015. "Higher speciation and lower extinction rates influence mammal diversity gradients in Asia." *BMC Evolutionary Biology* 15: 11.

Tendeiro, J. 1955. "Sobre alguns ixodídeos dos géneros *Hyalomma* C.L. Koch 1844 e Aponomma Neumann 1899." *Boletim Cultural da Guiné Portuguesa* 10: 319-461.

Theiler, G. 1962. *The Ixodoidea Parasites of Vertebrates in Africa South of the Sahara (Ethiopian Region).* Report to the Director of Veterinary Services, Onderstepoort, South Africa.

Thomas, F., Verneau, O., de Meeûs, T. and Renaud, F. 1996. "Parasites as to Host Evolutionary Prints: Insights into host evolution from parasitological data." *International Journal for Parasitology* 26: 677-686.

Thompson, J.N. 1994. *The Coevolutionary Process.* University of Chicago Press, Chicago, Illinois.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. "CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." *Nucleic Acids Research* 22: 4673-4680.

Tomassone, L., Camicas, J-L., De Meneghi, D., Di Giulio, A. and Uilenberg, G. 2005. "A note on *Hyalomma nitidum*, its distribution and its hosts." *Experimental & Applied Acarology* 35: 341-355.

Trauth, M.H., Maslin, M.A., Deino, A. and Strecker, M.R. 2005. "Late Cenozoic moisture history of East Africa." *Science* 309: 2051-2053.

Trauth, M.H., Maslin, M.A., Deino, A. and Strecker, M.R., Bergner, A.N.G., Dunford, M. 2007. High- and low-latitude forcing of Plio-Pleistocene East African climate and Human evolution. *Journal of Human Evolution* 53: 475-486.

van Alphen-Stahl, J.D., Bloomer, P. and Crowe, T.M. 2005. "Molecular systematics and phylogeography of the Helmeted Guineafowl (*Numida meleagris*)." Masters dissertation. Unversity of Cape Town, Cape Town.

Van Daele, P.A.A.G., Dammann, P., Meier, J.L., Kawalika, M., Van De Woestijne, C. and Burda, H. 2004. "Chromosomal diversity in mole-rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian Region: With descriptions of new karyotypes." *Journal of Zoology* 264: 317-326.

van der Mescht, L., Matthee, S. and Matthee, C.A. 2015. "Comparative phylogeography between two generalist flea species reveal a complex

interaction between parasite life history and host vicariance: parasite-host association matters." *BMC Evolutionary Biology* 15: 105.

Van Hooft, W.F., Groen, A.F. and Prins, H.H.T. 2002. "Phylogeography of the African Buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape Buffalo subspecies." *Molecular Ecology* 11: 267-279.

Van Valen, Leigh. 1973. "A new evolutionary law." *Evolutionary Theory* 1: 1-30.

van Vuuren, B.J. and Robinson, T.J. 1997. "Genetic population structure in the Yellow Mongoose, *Cynictis penicillata*." *Molecular Ecology* 6: 1147-1153.

van Zinderen Bakker, E.M. 1978. "Quaternary vegetation changes in Southern Africa." In: *Biogeography and Ecology of Southern Africa*, 131-143. Springer, Netherlands.

Verdcourt, B. 1969. "The arid corridor between the north-east and south-west areas of Africa." *Palaeoecology of Africa* 4: 140-144.

Veron, G., Patou, M-L., Pothet, G., Simberloff, D. and Jennings, A.P. 2007. "Systematic status and biogeography of the Javan and Small Indian Mongooses (Herpestidae, Carnivora)." *Zoologica Scripta* 36: 1-10.

Voelker, G. 1999. "Dispersal, Vicariance, and Clocks: Historical biogeography and speciation in a cosmopolitan Passerine genus (*Anthus*: Motacillidae)." *Evolution*: 1536-1552.

Walker, A.R., Bouattour, A., Camicas, J-L., Estrada-Peňa, A., Horak, I.G., Latif, A.A., et al. 2003. *Ticks of domestic animals in Africa: A guide to identification of species*. Bioscience Reports, Edinburgh.

Walker, J.B. 1974. *The ixodid ticks of Kenya: A review of present knowledge of their hosts and distribution*. Commonwealth Institute of Entomology, Eastern Press, London and Reading.

Walker, J.B. 1991. "A review of the Ixodid ticks (Acari, Ixodidae) occurring in Southern Africa." *The Onderstepoort Journal of Veterinary Research* 58: 81-105.

Weiblen, G.D. and Bush, G.L. 2002. "Speciation in fig pollinators and parasites." *Molecular Ecology* 11: 1573-1578.

Weiss, S. and Ferrand, N. (Eds.). 2007. *Phylogeography of Southern European Refugia.* Springer, Dordrecht.

Werger, M.J.A. 1978. *Biogeographical Division of Southern Africa.* Springer, Netherlands.

Wesson, D.M., McLain, D.K., Oliver, J.H., Piesman, J. and Collins, F.H. 1993. "Investigation of the validity of species status of *Ixodes dammini* (Acari:

Ixodidae) using rDNA." *Proceedings of the National Academy of Sciences of the United States of America* 90: 10221-10225.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D. and Wheeler, W.C. 1997. "The Strepsiptera Problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology." *Systematic Biology* 46: 1-68.

Wichura, H., Bousquet, R., Oberhänsli, R., Strecker, M.R. and Trauth, M.H. 2010. "Evidence for middle Miocene uplift of the East African Plateau." *Geology* 28: 543-546.

Wielstra, B., Baird, A.B. and Arntzen, J.W. 2013. "A Multimarker Phylogeography of Crested Newts (*Triturus cristatus* superspecies) reveals cryptic species." *Molecular Phylogenetics and Evolution* 67: 167-175.

Wiens, J. 2004. "The role of morphological data in phylogeny reconstruction." *Systematic Biology* 53: 653-661.

Winnepenninckx, B., Backeljau, T. and De Wachter, R. 1993. "Extraction of high molecular weight DNA from molluscs." *Trends in Genetics : TIG* 9: 407.

Woodruff, D.S. and Turner, L.M. 2009. "The Indochinese–Sundaic Zoogeographic Transition: A description and analysis of terrestrial mammal species distributions." *Journal of Biogeography* 36: 803-821.

Wright, S. 1943. "Isolation by distance." *Genetics* 28: 114-138.

Xu, G., Fang, Q.Q, Keirans, J.E. and Durden, L.A. 2004. "Cloning and sequencing of putative calreticulin complementary DNAs from four hard tick species." *Journal of Parasitology* 90 (1): 73-78.

Xu, G., Fang, Q.Q., Sun, Y., Keirans, J.E. and Durden, L.A. 2005. "Hard tick calreticulin (CRT) gene coding regions have only one intron with conserved positions and variable sizes." *Journal of Parasitology* 91: 1326-1331.

Yassin, A., Markow, T.A., Narechania, A., O'Grady, P.M. and DeSalle, R. 2010. "The genus Drosophila as a model for testing tree-and character-based methods of species identification using DNA barcoding." *Molecular Phylogenetics and Evolution* 57: 509-517.

Yeo, D.C.J., Shih, H-T., Meier, R. and Ng, P.K.L. 2007. "Phylogeny and biogeography of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) from the Malay Peninsula, and the origins of its insular fauna." *Zoologica Scripta* 36: 255-269.

Yeoman, G.H., Walker, J.B., Ross, J.P.J. and Docker, T.M. 1967. *The Ixodid Ticks of Tanzania. A Study of the Zoogeography of the Ixodidae of an East African Country*. Commonwealth Institute of Entomology, Eastern Press, London and Reading.

Yokobori, S-I., Hasegawa, M., Ueda, T., Okada, N., Nishikawa, K. and Watanabe, K. 1994. "Relationship among Coelacanths, Lungfishes, and Tetrapods: A phylogenetic analysis based on mitochondrial Cytochrome Oxidase I gene sequences." *Journal of Molecular Evolution* 38: 602-609.

Yousfi-Monod, R. and Aeschlimann, A. 1986. " Recherches sur les tiques (Acarina, Ixodidae), parasites de bovidés dans l'ouest Algérien. " *Annales De Parasitologie Humaine Et Comparée* 61: 341-358.

Yu, Y., Harris, A.J., He, X.J. 2010. "S-DIVA (statistical dispersal-vicariance analysis): a tool for inferring biogeographic histories." *Molecular Phylogenetics and Evolution* 56: 848-850.

Yu, Y., Harris, A.J., Blair, C. and He, X. 2015. "RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography." *Molecular Phylogenetics and Evolution* 87: 46-49.

Zachos, J., Pagani, M., Sloan, L., Thomas, E. and Billups, K. 2001. "Trends, rhythms, and aberrations in global climate 65 Ma to present." *Science (New York, N.Y.)* 292: 686-693.

Zhang, D-X. and Hewitt, GM. 1996. "Nuclear Integrations: Challenges for mitochondrial DNA markers." *Trends in Ecology & Evolution* 11: 247-251.

Zhang, D-X. and Hewitt, G.M. 2003. "Nuclear DNA Analyses in Genetic Studies of Populations: Practice, problems and prospects." *Molecular Ecology* 12: 563-584.

Zhang, R.L. and Zhang, B. 2014. "Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida)." *Ticks and Tick-borne Diseases* 5: 352-358.

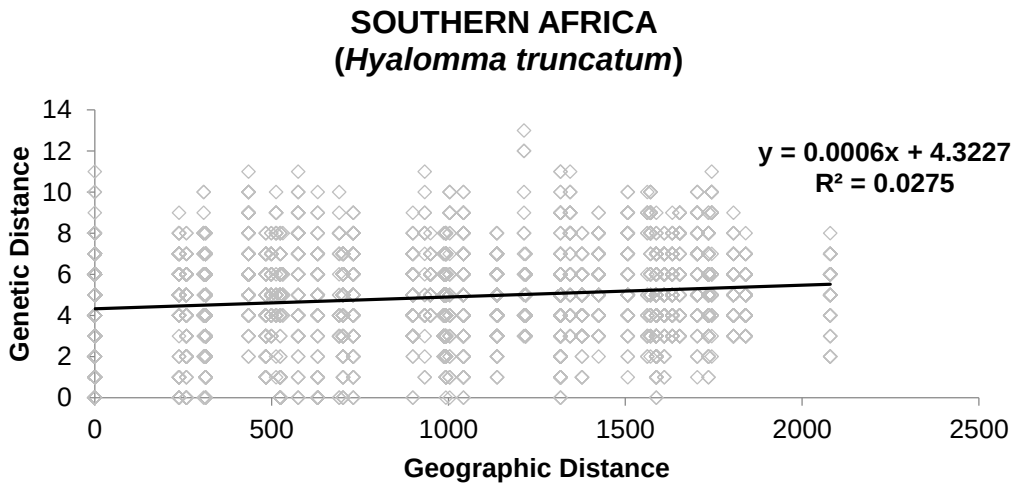
Zhang, Y-J., Stöck, M., Zhang, P., Wang, X-L., Zhou, H. and Qu, L-H. 2008. "Phylogeography of a widespread terrestrial vertebrate in a barely-studied Palearctic region: Green Toads (*Bufo viridis* subgroup) indicate glacial refugia in Eastern Central Asia." *Genetica* 134: 353-365.

Zinner, D., Wertheimer, J., Liedigk, R., Groeneveld, L.F. and Roos, C. 2013. "Baboon phylogeny as inferred from complete mitochondrial genomes." *American Journal of Physical Anthropology* 150: 133-140.

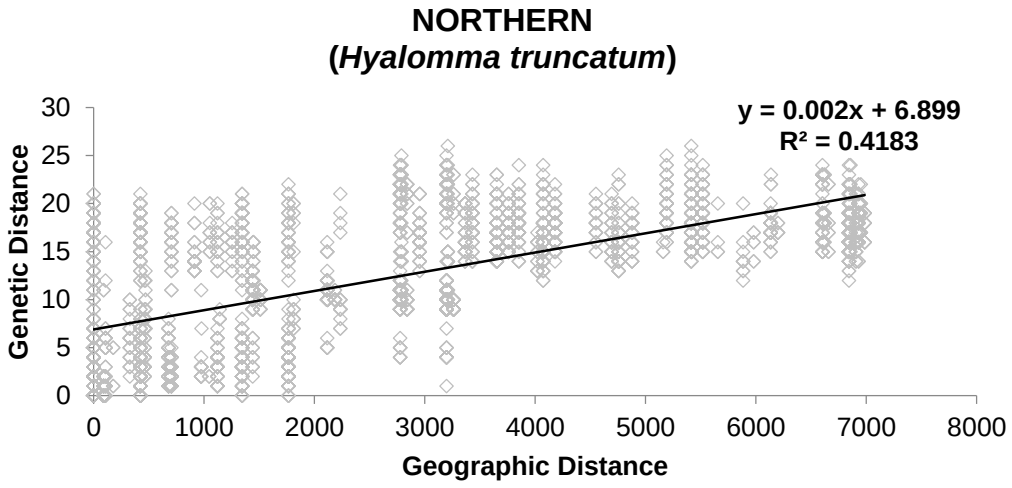
SUPPLEMENTARY INFORMATION

Supplementary Table 1: *Geographic sampling localities and regions for specimens collected and incorporated in Chapter 3.*

				Number of vouchers/sequences				
Country	***Location/Region***	***Location Code***	***GPS***	**Specimens #**	**COI**	**H3 (phased)**	**CRT (phased)**	**GenBank Accession #**
	SOUTHERN AFRICA							
	SOUTHERN AFRICA			**133**	**97**	**50**	**46**	
Mozambique	Cahora Bassa	CB	15.33S 31.52E	11	9	22	22	COI: KT999398-999406 CRT: KT999594-999604 H3: KT999646-999656
	Manica	CR	18.95S 32.79E	17	6			COI: KT999407-999412 CRT: H3:
Namibia	Gobabis	GH	22.42S 18.77E	15	13	28	24	COI: KT999413-999425 CRT: KT999620-999631 H3: KT999673-999686
	Mariental	MR	24.63S 17.97E	10	10			COI: KT999449-999458 CRT: H3:
	Otjiwarongo	OW	20.47S 16.59E	17	16			COI: KT999459-999474 CRT: H3:
South Africa	Upington	UP	28.34S 21.23E	17	11			COI: KT999475-999485 CRT: H3:
	Three Sisters	WC	31.93S 23.00E	6	6			COI: KT999486-999491 CRT: H3:
	Free State	FS	28.20S 26.46E	9	3			COI: KT999492-999494 CRT: H3:
	Kruger National Park	KN	23.99S 31.55E	18	13			COI: KT999426-999438 CRT: H3:
	Kuruman	KU	27.46S 23.43E	13	10			COI: KT999439-999448 CRT: H3:
	NORTHERN AFRICA							
	WESTERN AFRICA			**84**	**66**	**62**	**58**	
Benin	Central Benin	BE	9.33N 2.45W	5	5			COI: KT999574-999578 CRT: H3:
Burkina Faso	Burkina Faso	BF	11.56N 3.32W	15	11			COI: KT999560-999570 CRT: H3:
Mali	Banamba	ML	13.57N 7.14W	1	1			COI: KT999579 CRT: H3:
Nigeria	Fufore	FF	9.14N 12.35E	16	16	32	30	COI: KT999529-999544 CRT: KT999605-999619 H3: KT999657-999672
	Jos	GY	9.81N 8.83E	15	15			COI: KT999545-999559 CRT: H3:
Senegal	Dielmon	SD	13.43N 16.24W	15	2	2	2	COI: KT999497-999498 CRT: KT999580 H3: KT999717
	Sine-Saloum	SS	14.56N 16.25W	15	14	28	26	COI: KT999499-999512 CRT: KT999581-999593 H3: KT999703-999716
	Keur Momar Sarr	ST	15.54N 15.57W	2	2			COI: KT999495-999496 CRT: H3:
	NORTH-EASTERN AFRICA			**16**	**20**	**32**	**28**	
Ethiopia	Gode	ET	5.95N 43.61E	Genbank	4			COI: AJ437084-437087 CRT: H3:
Somalia	Central Somalia	SM	5.41N 46.52E	6	6	12	12	COI: KT999513-999518 CRT: KT999640-999645 H3: KT999696-999702
	Mogadishu	SX	2.01N 44.85E	10	10	20	16	COI: KT999519-999528 CRT: KT999632-999639 H3: KT999687-999695
	EASTERN AFRICA			**3**	**3**	**na**	**na**	
Kenya	Nairobi	KE	1.01S 37.23E	3	3			COI: KT999571-999573 CRT: H3:



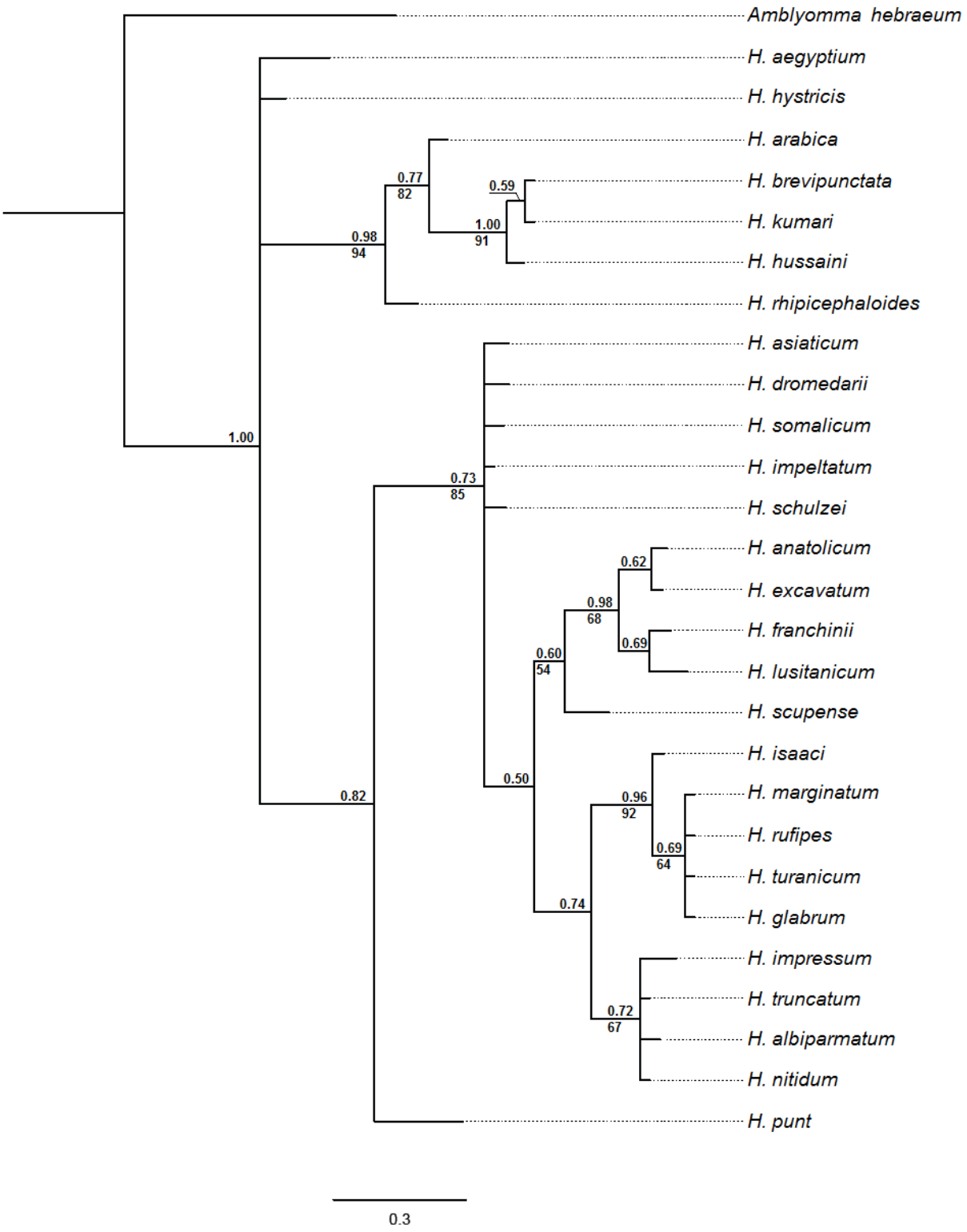
Supplementary Figure 1: *Isolation by Distance; Genetic Distance verse Geographic Distance for H. sp. nov. (H. truncatum from southern Africa).*



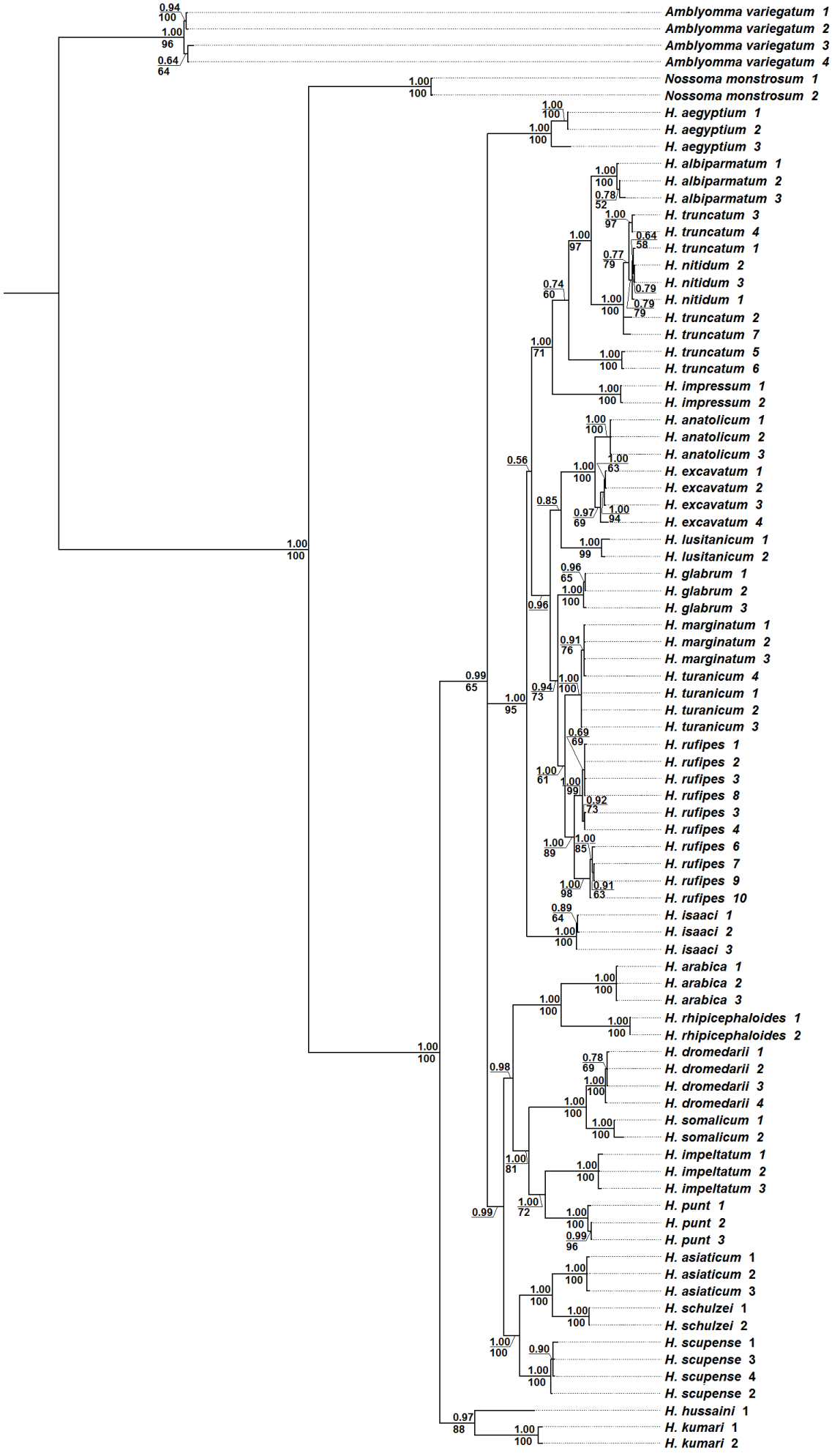
Supplementary Figure 2: *Isolation by Distance; Genetic Distance verse Geographic Distance for H. truncatum (H. truncatum from northern Africa).*

Supplementary Table 2: *GenBank sequences incorporated into the study.*

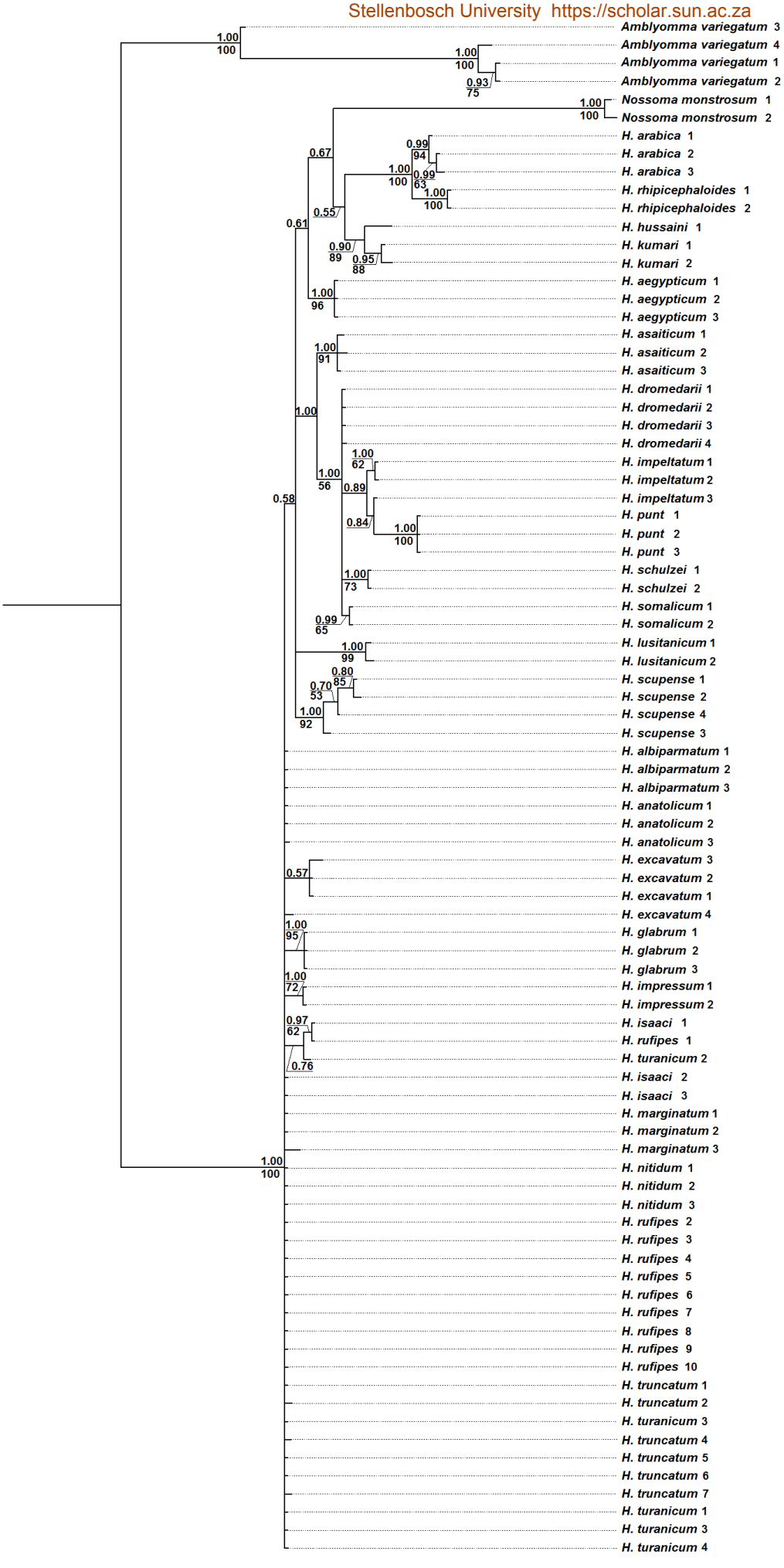
HYALOMMA	NUMBER OF SEQUENCES	GENBANK ACCESSION NUMBERS
H. anatolicum	5	JQ7377067.1, KJ912622.2, KM235710.1, KF583576.1, KC203438.1
H. turanicum	3	KM235709.1, KM235706.1, KM235708.1
H. asiaticum	4	KC203440.1, KF527440.1, JX051147.1, JQ737070.1
H. lusitanicum	7	EU827741.1, EU827719.1, EU027730.1, EU827729.1, EU827712.1, EU827700.1, EU827698.1
H. scupense	8	KM235713.1, KM235714.1, KM235712.1, EU827695.1, KF583581.1, JQ737069.1, KC203435.1, EU827694.1
H. marginatum	2	EU827693.1, EU827692.1
H. dromedarii	3	KM235697.1, AJ437061.1, GQ483461.1
H. aegypticum	5	JX394191.1, JX394190.1, JX394192.1, AF132821.1



Supplementary Figure 3: *Hyalomma Bayesian tree based on 47 morphological characters. Maximum parsimony bootstrap values are indicated below and Bayesian posterior- probabilities are above branches. Branch lengths represent the number of character changes.*



Supplementary Figure 4: *Hyalomma Bayesian tree based on mtDNA data (COI and 16S). Maximum parsimony bootstrap values are indicated below and Bayesian posterior-probabilities are above branches. Branch lengths represent the number of base-pair changes.*



Supplementary Figure 5: *Hyalomma Bayesian tree based on nDNA data (28S, H3 and ITS II). Maximum parsimony bootstrap values are indicated below and Bayesian posterior-probabilities are above branches. Branch lengths represent the number of base-pair changes.*