Wildlife health in human-modified landscapes: epidemiology of tick-borne pathogens affecting black-backed jackals and caracals

Storme Viljoen

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Supervisors: Dr Jacqueline Bishop and Prof Justin O'Riain

Declaration

I hereby declare that all the work presented in this thesis is my own, and is presented as original research undertaken for the purpose of fulfilling an MSc degree. This work has not been submitted for any other degree. All contributions from other persons have been duly acknowledged in the text.

Signed by candidate

Storme Viljoen

Prof M. Justin O'Riain

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Table of Contents

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Abstract

Despite the importance of disease as a wildlife management challenge, baseline research on the epidemiology of pathogens occurring in wildlife populations within both rural and urban landscapes has received little attention to date. The aim of this study was to improve our understanding of wildlife health in human-modified landscapes in South Africa, by providing comparisons of body condition, host-attached tick diversity and tick-borne pathogen (TBP) epidemiology of two common mesocarnivore species, the black-backed jackal (*Canis mesomelas*) and caracal (*Caracal caracal*). Jackals (n=46) and caracals (n=27) were sampled from small livestock farmlands in the Central Karoo region, in addition to caracals from farmlands in Namaqualand (n=14), and the urban matrix of the Cape Peninsula (n=16). Body condition was evaluated using both ratio (Body Mass Index) and residual (Ordinary Least Squares) methods, and morphometry was compared with historical datasets for each species. There was no apparent effect of sex, age class or location on body condition of jackals or caracals. Host-attached tick diversity was highest in urban caracals compared with the two other caracal populations, possibly indicating that they are exposed to a greater diversity of potential tick vectors. Molecular methods (mPCR/RLB; conventional PCR screening and phylogenetic analysis) used to screen for selected pathogens of veterinary and/or zoonotic concern, including *Ehrlichia, Anaplasma, Babesia* and *Theileria* species, revealed that Central Karoo jackals exhibited a lower prevalence of TBPs, compared with sympatric caracals. *Hepatozoon canis*, a ubiquitous pathogen of domestic and wild canids globally, was observed in 46.5% of jackals. *Theileria ovis,* a piroplasm of small livestock, was found in 4.7% of jackals. Jackals and caracals appear to be distinct in their TBP epidemiological roles, despite sharing similar tick communities. Pathogens found in caracals include *Hepatozoon felis*, *Babesia felis*, *Babesia leo* and a potentially undescribed *Babesia* species, genetically similar to *B. venatorum*, an emerging zoonosis. An *Anaplasma* species previously described in South African domestic dogs was also found in the urban caracals. All caracals were infected with at least one TBP. Together, these findings suggest that land use does not significantly influence the body condition of these adaptable predators, but that there is a health cost associated with living in the urban space. Cape Peninsula caracals show substantially higher rates of TBP co-infection (81% versus 14.8% and 0% in farmlands) and greater pathogen diversity compared to farmland caracals. The findings of this study include numerous examples of previously undescribed genetic diversity of tick-borne pathogens infecting South African mesocarnivores living in transformed landscapes. This work adds to our understanding of wildlife health within the 'One Health' framework and represents the first detailed examination of TBPs in jackals on farmlands and is also the first work that focuses specifically on TPBs in caracals anywhere in the world.

List of figures

Chapter 2. Methods and Materials

Chapter 3. Results

List of tables

Chapter 2. Methods and Materials

Chapter 3. Results

Chapter 1. General Introduction

Anthropogenic changes associated with increasing human population size are adversely influencing the integrity and functionality of natural ecosystems around the globe. The resulting loss of biodiversity and diminished ecosystem function (Vitousek *et al.*, 1997; Harrison & Bruna, 1999; Tilman *et al.*, 2001), together with the widespread translocation of both domestic and wild species, has significant consequences for the health of wildlife in natural systems (Harvell *et al.,* 2002; Patz *et al.,* 2004; Acevedo-Whitehouse & Duffus, 2009; Martin *et al.,* 2010; Dhama *et al.,* 2013; Romanelli *et al.,* 2014). Essentially, human-induced changes in wildlife disease ecology promote a destabilization of long-established host-pathogen dynamics (Norval *et al.*, 1992), which is what, in extreme cases, drives the emergence of disease epidemics with which conservationists, wildlife managers, farmers and public health experts are united in their concern.

Disease is the most obvious manifestation of poor health and both infectious and non-infectious diseases are currently acknowledged as important emerging conservation issues (Meffe, 1999; Daszak *et al.*, 2001; Deem *et al.*, 2001; Aguirre *et al.*, 2002; Kalema-Zikusoka, 2005). Infectious disease, i.e. the clinical manifestation of symptoms resulting from infection by a pathogen (i.e. an infectious disease-causing agent), is distinguishable from non-infectious disease, which is caused by genetic or environmental factors. While the prevalence and effects of both are exacerbated by both natural and anthropogenic environmental perturbations, this review focuses exclusively on the ecology of infectious disease and it relates to wildlife health.

The ecology of disease in wildlife

Wobeser (1981) describes disease in free-ranging populations as “any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxins, climate change, and infectious agents” (from Deem *et al.*, 2001). These impairments act to negatively affect long-term population persistence, but more immediately, they may reduce the ability of individuals of an otherwise healthy population to fulfil their ecological roles (Deem *et al.*, 2001). It is important to recognise that while disease in contemporary systems is considered a severe threat to biodiversity, mainly due to the negative influence of anthropogenic change, disease is fundamentally a natural phenomenon (Pedersen *et al.*, 2007; Aguirre & Tabor, 2008; Smith *et al.*, 2009).

Naturally-occurring diseases, which include those caused by infectious agents and genetic or environmental causes, shape the behaviour and ecology of wildlife (Altizer *et al*., 2006; Tompkins *et al*., 2011) as well as being significant drivers of evolutionary change (Smith *et al*., 2009a; Hawley & Altizer, 2011). Pathogens, including bacteria, protozoans, fungi and viruses, comprise a significant and often neglected proportion of the biodiversity of an ecosystem (East *et al.*, 2011). Wildlife host-pathogen dynamics have evolved under strong selective pressure over ecological and evolutionary time scales, resulting in stable biological communities, characterised by few to no severe clinical manifestations of disease (Kock, 2005; Munson *et al.,* 2010). In addition to contributing to immunologically competent host populations, endemic diseases play important regulatory roles in population size dynamics and composition (Grenfell & Dobson, 1995). Altered dynamics associated with infectious disease are among some of the most prominent concerns of wildlife managers, livestock farmers, veterinarians and human public health professionals. This is due to the magnitude of the effects that infectious disease can have on human, domestic animal and wildlife populations, and which range from mild behavioural changes and reduced fitness, to massive population die-off during epidemic disease events (Kennedy *et al.*, 2000; Munson *et al*., 2008; Goller *et al.,* 2010;).

Trends in wildlife disease research

Wildlife disease research is largely driven by anthropocentric interests (Thompson *et al*., 2010). Much research continues to be focused on zoonotic diseases (disease which can be passed from animals to humans) or diseases which can be transmitted to livestock (e.g. McCallum & Dobson, 1995; Holmes, 1996; Daszak *et al*., 2000; Rhyan & Spraker, 2010), while work on wildlife disease in natural populations has focused almost exclusively on direct threats of disease to the persistence of endangered species (Cleaveland *et al*., 2007). Given that more than 75% of human diseases are zoonotic, with links to either wildlife or domestic animals (Taylor *et al*., 2001), it is unsurprising that there has been a push to incorporate animal health into mainstream public health planning and research (Kahn *et al*., 2009; Romanelli *et al*., 2014). Wildlife species that share the landscape with humans and domestic animals, including companion animals and livestock, may have a critical role to play in the epidemiological dynamics of relevant pathogens (Michel *et al*., 2006; Siembieda *et al*., 2011; Otranto *et al*., 2015), and currently we lack a breadth of research to understand these roles in many contemporary systems.

In recent years, issues arising as a consequence of global change have helped establish a platform of shared risk between animals and humans (Rabinowitz *et al*., 2005; Zinsstag *et al*., 2009). Researchers

have reported numerous instances where disease has directly contributed to substantial population die-off (Roelke-Parker *et al*., 1996; Pounds *et al*., 2006), but also to more subtle effects, whereby diminished functioning of the affected individual may lead to reduced reproductive fitness and altered behaviour (Daszak *et al*., 2000). On a broader scale, these effects on the individual and population can impact greatly on the biological communities in which they occur, and on associated environmental and ecosystem integrity. In order to address what Travis *et al*. (2014) call a "critical global challenge", researchers are confronted with characterising the health consequences of the interconnection of humans, livestock, and wildlife within their respective socio-ecological contexts.

The 'One Health' paradigm

Recognition of how disease and health contribute to the complexity of biological systems is the impetus behind the development of the 'One Health' paradigm (Kahn *et al*., 2009; Mazet *et al*., 2009; Travis *et al*., 2014). Central to this thinking is the acknowledgement of the interconnectedness of human, animal and environmental health within their shared socio-ecological context (Kock, 1996). The concept of 'One Health' moves beyond considering health as individual clinical issues, to include epidemiological interactions, ecology and public health thinking for populations, communities and ecosystems (Zinsstag *et al*., 2011). This paradigm is particularly valuable for applied research into wildlife health as it attempts to account for complexity through a systems approach. In practice, 'One health' research incorporates the study of ecosystem health, agricultural ecosystems research, adaptive management, resilience, and sustainability (Zinsstag *et al*., 2011). Although there has been criticism of this concept in that it can appear to be too holistic in scope, Zinsstag *et al*. (2009) propose ways in which this can be translated into practically useful fields. Integrated disease surveillance and monitoring, collaborative research in human-animal epidemiology and health services development are highlighted as essential foci, with particular relevance for developing countries, where human, wildlife and livestock communities are strongly interactive.

Applied research in health, at its most elementary level of study, needs to include health and pathogen surveillance. Perhaps the greatest challenge for the study of disease in wildlife is the paucity of information on the diversity and ecology of pathogens that exist in natural systems (Colwell *et al*., 2009; Mathews, 2009). Despite recognition of the important role of pathogens as components of biodiversity and in the conservation of ecosystem integrity, the study of pathogens in wildlife populations is often neglected (Thompson *et al*., 2010). There is a critical need for accurate and relevant data on the diversity and prevalence of potentially important pathogens if we are to

understand the role of diseases in threatening wildlife, associated communities and ecological processes (Smith *et al.* 2009). In particular, research efforts need to focus on documenting pathogen diversity, range and baseline prevalence in native wildlife in order to establish their potential importance as etiological role-players, and to identify predictors of disease occurrence (Smith *et al*., 2009a).

The use of body condition indices in wildlife populations

The persistence and viability of populations is dependent on the health of the individuals that make up that population. In order to examine health at the scale of the individual, conservation scientists and managers typically start by quantifying the body condition of individuals. Individuals with greater energy stores are assumed to be in better condition, and thus are better able to cope with external stressors and are more likely to successfully reproduce. Although there are several types of body condition measures, morphological condition indices are among the most widely used. Morphological indices use body mass in relation to some body size measure to quantify condition, based on the assumption that structural mass is constant and proportional to body size, but total mass would indicate any deviation from the standard, "healthy" shape (Stevenson and Woods, 2006). At a population level, individual body condition is used to inform and complement other population level health assessments (Stevenson and Woods, 2006) and to infer the health of a population relative to other populations.

There are however concerns with this approach, most of which stem from the lack of knowledge around what we term, "healthy", the lack of standardized procedures to establish this, and no unifying approach to measuring morphological condition, even for similar taxa (Marker and Dickman, 2003; de Waal *et al*. 2004; Boast *et al*. 2013). Despite this, body condition indices are still broadly applied to humans and many other animal species including reptiles, amphibians, fish and insects (see Stevenson and Woods, 2006 (Table 1)). As a consequence of these challenges, numerous techniques exist for quantifying body condition, including linear morphometric measurements, skin-fold thickness and both body mass and hydrostatic weighing. Other techniques involve measuring body composition using chemical techniques, such as isotope or gas dilution, or the four-part molecular model technique, whereby scientists are able to separate and individually quantify the four major tissue components of an animal (organic matter, inorganic matter, water and fat) (Wang *et al*., 1992; Reynolds and Kutz, 2001; Speakman, 2001). Methods such as total body electrical conductivity and bioelectrical impedance, as well as scanning techniques (ultrasound, x-ray absorptiometry, magnetic resonance imaging), have also been trialled, but as with the physical techniques, each comes with a

suite of limitations and challenges that has to date precluded the adoption of single method for estimating body condition in animals.

Currently, the greatest difficulty in selecting an appropriate technique for estimating body condition is in determining how well the data generated can be applied to the sample population, within the context of when and how the data are being collected. Typically, for animals over a certain size threshold, chemical techniques become impractical. In many cases, body condition assessments need to be performed in the field, which limits the scope of choices for such evaluations. Electrical, chemical and scanning techniques often require the use of costly equipment, which may not be portable and which could require training in their use. All of these factors need to be considered when formulating a research plan.

The Kidney Fat Index (KFI, also referred to as the renal fat index, RFI) appears to be the preferred method for estimating body condition in medium-sized canids, like the black-backed jackal (*Canis mesomelas*), and is thought to be the best single index for predicting total body fat (Winstanley *et al*., 1998). Unfortunately, the invasiveness of the KFI method makes this approach one that can seldom be considered, unless animals are already dead. This poses a problem when comparing body condition across populations, and when individuals need to be sampled while living. Thus, the scope of condition index methods is limited to morphometric and electrical (bioelectrical impedance) techniques.

Morphological condition indices therefore remain the easiest method to use across a range of animal sizes and field circumstances and consequently, it is the most widely applied measures of animal health (Stevenson and Woods, 2006; Pieg and Green, 2009). Indices derived from animal morphology generally fall into three categories: mass to length ratios, e.g. a body mass index, residuals, which represents the deviation of measured mass values to predicted values based on a statistical formula (e.g. Ordinary Least Squares, Reduced Major Axis residuals), or a dimensionless measure derived from mass divided by density (1 $g.cm^{-3}$). Each of these morphological condition indices receives preferential use for the different taxa. In humans, the vast majority of studies use body mass index (BMI) as the standard measure of body condition. In contrast, very few studies of other mammals and other taxonomic classes utilise BMI. It appears that the most commonly used morphological condition index for carnivores is based on mass residuals, although the preferred method of condition estimation is still a topic of debate (Green, 2001; Schulte-Holstedde *et al*., 2005; Moya-Larano *et al*., 2008; Pieg & Green, 2009; 2010). Another popular index for assessing the condition of wild-caught animals is

derived from the use of Ordinary Least Squares (OLS) residuals of measured weights and those predicted based on the modelling of linear body size measures (Schulte-Hostedde *et al*., 2005).

Identifying and characterising pathogens using molecular methods

Pathogen detection has traditionally been carried out through microscopic examination of blood smears in cases where pathogens are visible, such as for piroplasms and some rickettsias (e.g. erythrocyte-associated *Anaplasmas* and certain *Ehrlichia* species). While this is undoubtedly the quickest way to determine the presence of infection in blood, the method generally lacks the sensitivity to be able to distinguish species (Shaw *et al*., 2001) and is heavily reliant on the competence and experience of the observer. Serological assays which detect antibodies to specific infections are more sensitive, but these too are limited in that they can only detect exposure within the lifetime of the host and not confirm active infection, which can be problematic in areas of endemic disease (Waner *et al.*, 2001; Harrus *et al.*, 2002; Mylonakis *et al.*, 2003). Currently, the gold standard of pathogen detection and characterization is molecular genetic screening. Genetic techniques are indicative of active pathogen infection in the host, as opposed to being limited to evidence of previous exposure.

Advances in molecular genetic tools that allow the amplification of minute amounts of pathogen DNA collected from a variety of host tissues (via Polymerase Chain Reaction (PCR)), including exudates and scats (e.g. Bodewes *et al*., 2014), has greatly increased the breadth of potential sources for diagnosing the presence of pathogens. The advantages of genetic techniques in disease surveillance are numerous (Archie *et al*., 2009; Benton *et al*., 2014) and evidenced by their increasing use in wildlife infectious disease studies (e.g. Duarte *et al*., 2013; Kelly *et al*., 2014; Williams *et al*., 2014; Zanet *et al*., 2014). Genetic surveillance requires small amounts of biological material, which can be accessed non-invasively if necessary (e.g. viral genomics in carnivore scats, (Bodewes *et al*., 2014)), and which can originate from a wide variety of tissues, including blood, organs and urine. Genetic testing via PCR has the added benefit of yielding a DNA product which can be sequenced and compared across samples. The advent of open access genetic databases, such as GenBank™, has allowed for unprecedented comparative work to take place across disease systems; this is critical when one considers the ubiquity of the pathogen strains that have been anthropogenically transported across the globe. Importantly, PCR is safe, relatively cost-effective, and can be conducted with minimal experience, which makes it useful for large-scale application across vast areas.

At a minimum, molecular tools complement traditional epidemiological approaches (Archie *et al*., 2009) but can also be used to vastly improve the resolution of investigations and address new and relevant questions. Advances in the field of molecular epidemiology are providing valuable tools for understanding disease ecology (Biek *et al*., 2006; Blanchong *et al*., 2008; Archie *et al*., 2009; Artois *et al*., 2009). These include the application of genomic sequencing, PCR and bioinformatics methods that provide valuable insight into pathogen transmission routes, origins of emergence, host-pathogen dynamics and the identification of reservoirs (Archie *et al*., 2009; Benton *et al*., 2014). By applying the tools of population and landscape geneticists, these methods can be applied to pathogens in order to reconstruct epidemiological histories, comment on how disease spreads in an ecosystem, model risk of emerging infectious disease outbreaks and identify the physiological, environmental and landscape drivers of disease dynamics (Archie *et al*., 2009). Techniques taken from spatial and statistical modelling, e.g. using Bayesian theory, have also been integrated into disease ecology studies with great promise (Manel *et al*., 2005; Excoffier and Heckel, 2006; Waples and Gaggiotti, 2006; Faubet *et al*., 2007).

The Human-Wildlife-Livestock (HWL) interface in Africa

The risk of pathogen spill-over between humans, wildlife, and domestic animal populations is substantial, most notably so at the HWL interface (Cleaveland *et al*., 2005; Kock, 2005; Caron *et al*., 2012). Occurrence of these cross-species interactions is an ever-increasing outcome of human population growth and the associated encroachment into wild spaces, enhancing the risk of (i) humans contracting zoonotic disease (Tampieri *et al*., 2008); (ii) livestock contracting diseases from local wildlife (Cleaveland *et al*., 2001) and (iii) wildlife populations being exposed to diseases from both domesticated animals and humans. For example, domestic dogs acting as disease reservoirs for the pathogens such as rabies and Canine Distemper Virus, which can be transmitted to wild carnivores (Alexander and Appel, 1994; Butler *et al*., 2004; Lembo *et al*., 2008).

The HWL interface is particularly important when examining disease within the One Health paradigm. Interactions at this interface are multi-faceted and relate to health issues as well as conservation and environmental issues, human social dynamics, culture and economics (Kock, 2005). The One Health approach to addressing infectious disease questions is most important in regions where humans, wildlife and livestock are frequently interacting (Osofsky *et al*., 2005), such as in the pastoral communities of southern Africa. Africa more broadly provides a good example of where assessment of the HWL interface is critically important, as the continent epitomises the concept of the direct

dependence of human communities on natural systems. Africa represents the best example of a continent that still maintains vast areas of intact natural habitat, whilst maintaining a heavy reliance on livestock economies, particularly in East and southern Africa (Cleaveland *et al.,* 2005; Kock, 2005). The human-wildlife-livestock (HWL) interface is a critical area of interest in the study of disease dynamics, due to the complex interactions that characterise the ecology of these systems. These interactions frequently have serious consequences for human and animal public health, biodiversity conservation, livestock and wildlife animal-based economies, all of which are of critical concern in the developing countries of Africa in general, and southern Africa specifically.

Globally, Africa maintains some of the richest biodiversity and within the rural context, particularly its iconic dryland pastoral systems, humans and associated livestock share an interface with a diverse array of wild rodents, ungulates and most notably, carnivores (Bengis *et al*., 2002). Within the Carnivora, members of the Felidae and Canidae families, are particularly threatened by cross-over pathogens of all mammalian species (Murray *et al*., 1999; Pedersen *et al*., 2007). An important reason for the vulnerability of these wild carnivores to disease threats in domestic animal species is their close phylogenetic relationship with domestic dogs and cats (Millán *et al*., 2009). In conjunction, carnivore species interact with human-dominated landscapes, where human population expansion, habitat destruction and climate change act to destabilize established host-pathogen equilibria and introduce highly virulent pathogens into naïve systems (Smith *et al*., 2009a; Munson *et al*., 2010;). Southern Africa provides excellent opportunities for research by wildlife disease ecologists as it is comprised of countries, e.g. Namibia, Botswana and South Africa, that are amongst the most developed on the continent, while still having a strong dependency on livestock farming, large rural human populations and a growing wildlife ranching and protected area tourism industry. With their regionally and globally important emerging economies emphasis needs to be placed on finding ways to optimise the use of resources to inform public health professionals, livestock owners and wildlife managers to adequately manage these risks and develop strategies to mitigate disease threats.

Ticks: a model for examining vector-borne diseases at the HWL interface

Many of the most relevant diseases to livestock managements and owners of companion animals are those that are spread by predominantly arthropod vectors. Disease vectors are defined as living organisms that are able to transmit infectious pathogens from one host species to another (WHO, 2016). Together, mosquitos, sand flies, fleas, and ticks make up the group of haematophagous

arthropods that constitute the most important disease vectors worldwide (Jongejan and Uilenberg, 2004; Pfaffle *et al*., 2013).

Ticks (Acari: Ixodidae), in particular, are considered important vectors of diseases that infect livestock species across the globe (Jongejan and Uilenberg, 2004). Ticks also transmit the greatest variety of pathogenic micro-organisms of any of the arthropod vectors (Durden, 2006), and are relevant to the spread of notable diseases for not only livestock, but also humans and companion animals. Pathogens spread by ticks, commonly referred to as Tick-Borne Pathogens (TBPs), are the causative agents for many of the most important diseases affecting humans (e.g. Lyme disease and Tick-bite fever), companion animals (e.g. Babesiosis, Ehlichiosis and Anaplasmosis), and livestock (e.g. Red water disease, Heartwater, Gallsickness). In addition, tick infestation can lead to an array of other health impacts including, allergies, paralysis, and toxicosis. These health impacts along with tick-borne diseases combine to make ticks a significant concern to professionals who manage health in the farming, veterinary or public sector. Indeed, in developing countries like those of southern Africa, tick-borne diseases have notable impacts on the farming communities that are the most resource-limited (Perry *et al*., 2002; Minjauw and McLeod, 2003)

Tick ecology

Ticks comprise three families, the Argastidae (soft ticks), Ixodidae (hard ticks) and Nuttalliellidae, characterized by only one known species (Guglielmone *et al*., 2010). Ixodidae make up approximately 80% of all tick species (Horak *et al.*, 2002) and are responsible for the transmission of the vast majority of TBPs infecting mammalian hosts (Jongejan and Uilenberg, 2004), hence this review focusses exclusively on the hard ticks, or Ixodidae. Ixodid ticks have either a one, two or three host life cycle. For a one-host life cycle, the tick could go through all three life stages (larvae, nymph and adult) on the same host, before finding a mate and reproducing. In a two or three-host life cycle, the tick would drop off the host at critical stages in order to moult or reproduce. Ixodids can have a maximum of three different hosts during their life cycle (Oliver, 1989), and these need not be the same species, as host preference can vary greatly in the different tick stages (e.g. Horak *et al*., 2006).

Ticks can be highly selective of their hosts, or employ a more generalist approach to host selection. In addition, a single tick can be co-infected with several pathogens at any one time (Milutinovic *et al*., 2008; Nicholson *et al*., 2010). Ticks, however, only act as effective vectors of a pathogen if the following criteria are met: the tick selects to attach to an infectious host; is able to take a blood meal containing the pathogen; can then maintain the pathogen through at least one moult or reproductive

life stage (known as transstadial and transovarial transmission, respectively), and finally, can then transmit the pathogen to a new, competent host (Kahl *et al*., 2002). Given these criteria, it becomes clear that pathogens are dependent on the development, reproduction and survival of their tick vectors in order to persist (Randolph *et al.*, 1998).

Tick-borne pathogens and associated disease

Ticks represent an important ecological connection between their vertebrate host species. As such, many of the pathogenic agents carried by ticks, including viruses, bacteria and protozoa, can infect a combination of humans, domestic animals and wildlife. Species of the genera *Ehrlichia*, *Anaplasma*, *Babesia*, *Theileria* and *Hepatozoon* are examples of generalist pathogens that have been studied in domestic animals and humans. In some instances, these species have also been surveyed in wildlife populations.

Pathogen species of the family Anaplasmataceae, which include *Anaplasma* and *Ehrlichia* species, are well-studied in human and domestic animal hosts. Anaplasmataceae (Order: Rickettsiales) are obligate intracellular, Gram-negative bacteria (Dumler *et al*., 2001, 2005) that are recognised as causative agents of a number of emerging infectious diseases (Dugan *et al*., 2005). For example, *Ehrlichia* species are important pathogens in canine veterinary practice. *Ehrlichia canis*, the etiological agent of Canine Monocytic Ehrlichiosis elicits clinical signs which vary from subclinical infection to severe illness characterised by fever, lethargy, anaemia, weight loss and other febrile disease symptoms (Harrus *et al*., 1997; Neer *et al,*. 2002). Zoonotic capacity has also been noted for *E. canis* in Venezuela (Perez *et al*., 2006) and *E. chaffeensis*, which affects white-tailed deer (*Odocoileus virginianus*) in North America (Dumler *et al*., 2001). *Ehrlichia canis* has also been found to infect domestic cats (Maia *et al*. 2014) and wild canids (Fishman *et al.*, 2004; Almeida *et al.*, 2013) In South Africa, *Ehrlichia* infections have been examined in humans (Pretorius *et al*., 1999), domestic dogs (Pretorius and Kelly, 1998; Matjila *et al*., 2008) and in a wild canid, the African wild dog (*Lycaon pictus*) (Matjila *et al*., 2008), however, no wild dogs were infected with *Ehrlichia*. While *Ehrlichia* infections appear to be prevalent in domestic dogs, they have yet to be reported in wild canid populations in South Africa, although jackals and African wild dogs have been artificially infected, demonstrating their competence as potential hosts (van Heerden, 1979).

Similarly, Matjila *et al*. (2008) found no evidence of *Anaplasma* infection in African wild dogs, nor have other species been reported in wild felid populations. Within the *Anaplasma* genus, species differ with respect to their target cells; *A. marginale*, *A. centrale*, and *A. ovis* infect erythrocytes ('Erythrocytic

Anaplasma'), while *A. phagocytophilum* and *A. bovis* infect leucocytes and macrophages and *A. platys* infects platelets (Dumler *et al*., 2001). Erythrocytic *Anaplasma* species do not show zoonotic potential, however, they can be important diseases of domestic and wild ruminants (Aubry & Geale, 2001; Garcia-Perez *et al*., 2016). The clinical manifestation of *Anaplasma* species can vary greatly from subclinical to severe, and manifest as non-specific febrile disease symptoms (Garcia-Perez *et al*., 2016). Of great concern is *A. phagocytophilum* which can be transmitted to humans, causing Human Granulocytic Anaplasmosis, and is the causative agent of Tick-Borne fever in ruminants. *Anaplasma phagocytophilum* has a worldwide distribution, and is thought to be maintained by natural enzootic cycles between *Ixodes sp.* ticks and wildlife hosts. In Europe, *A. phagocytophilum* has been identified in red foxes from numerous countries (Hodzic *et al*., 2015).

Another group of TBPs that have been the focus of extensive research are protozoans within the Phylum Apicomplexa, specifically the piroplasms (*Babesia* and *Theileria* species) and those of the genus *Hepatozoon*. Species of *Babesia* and *Theileria* (Order: Piroplasmida) are spread through tick saliva during a blood meal, where the pathogen infects the host erythrocytes and/or leucocytes. As with Anaplasmataceae, clinical manifestations of disease can vary from subclinical to acute infection with the vertebrate host presenting with fever, lethargy, malaise, jaundice and anorexia. Infection may be fatal in some instances. While chronic infection is generally subclinical (Penzhorn, 2006; Schnittger *et al*., 2012; Maia *et al*., 2014), severe infection with *Babesia* can be linked with host stress factors, age, immunological status and concomitant infections (Schnittger *et al*., 2012).

Some species of *Babesia* have demonstrated zoonotic potential (e.g. *B. divergens* (Malandrin *et al*., 2010), *B. microti* (Anderson *et al*., 1991), *B. venatorum* (Herwaldt *et al*., 2003)), while others are important disease-causing agents in domestic dogs, e.g. *B. canis* throughout Europe, *B. gibsoni* worldwide (Kledmanee *et al*., 2009). *Babesia* infections have been noted in both domestic and wild animals in many parts of the world (Penzhorn, 2006; Criado- Fornelio *et al*., 2009; Williams *et al*., 2014; Zanet *et al*., 2014), and are considered to be the second most common blood parasite after the parasitic protozoan, *Trypanosoma* (Yabsley & Shock, 2013). *Babesia* infections in wildlife have been comparatively well-researched (Penzhorn, 2006; Yabsley *et al*., 2006; Schnittger *et al*., 2012; Yabsley and Shock, 2013). In South Africa specifically, *Babesia* infections in wildlife populations have been studied extensively (Penzhorn *et al*., 1999; Bosman *et al*., 2007; Matjila *et al*., 2008; LeClaire *et al*., 2014), particularly in wild felids (Penzhorn *et al*., 2001; Penzhorn *et al*., 2004; Bosman *et al*., 2007; Bosman *et al*., 2010).

In the genus *Theileria*, transmission and clinical manifestation is akin to that of *Babesia*. Unlike *Babesia*, however, there is no evidence of zoonotic potential of *Theileria* species (Yabsley & Shock, 2013). Some *Theileria* species, e.g. *T. parva* and *T. annulata*, can cause severe disease and fatality in domestic animals (Gitau *et al*., 1999). Like *Babesia*, some *Theileria* infections are predominantly benign, but may lead to acute infection when the host is under stress, e.g. during translocation or when co-infected with other pathogens (Kocan & Kocan, 1991; Hofle *et al*., 2004; Sawczuk *et al*., 2008).

Hepatozoon species (Order: Eucoccidia) are another Apicomplexan parasite of veterinary concern worldwide. The *Hepatozoon* genus includes species that infect a wide variety of animals, including birds, herpetofauna and mammals (Smith, 1996). *Hepatozoon* is highly unusual among TBPs in that it is spread by direct ingestion of the entire tick vector, through grooming for example, which contains mature oocysts of the *Hepatozoon* parasite. Other modes of transmission have also been proposed, which includes vertical transmission through the intrauterine route, observed in domestic dogs (Murata *et al*., 1993) or through the ingestion of prey tissue that contains *Hepatozoon* cysts (McCully *et al*., 1975; Smith *et al*., 1996). In carnivores, *Hepatozoon sp*. appear to have a worldwide distribution, and have been found in numerous species of wild canids and felids in the areas that have been surveyed (Gianitti *et al*., 2012; Almeida *et al*., 2013; Starkey *et al*., 2013; Farkas *et al*., 2014; Maia *et al*., 2014; Barandika *et al*., 2016).

Hepatozoonosis, the disease caused by species of *Hepatozoon*, is considered an emergent disease in felid species (Criado-Fornelio *et al*., 2003) and likely in other carnivore species as well. Although infections with *Hepatozoon* species are often subclinical (MCully *et al*., 1975; Averbeck *et al.,* 1990; Mercer *et al*., 1998; Kocan *et al*., 2000; Metzger *et al.,* 2008), some instances can manifest where the vertebrate host shows generalised symptoms including weight loss, lethargy, diarrhoea, polyuria, and fever (Kocan *et al*., 2000, Garret *et al*., 2005). Young canids appear to be particularly vulnerable to manifesting clinical symptoms (Kocan *et al*., 2000; Garret *et al*., 2015). Animals with concomitant infections are also susceptible to *Hepatozoon* infection that manifests clinically (Baneth *et al*., 1998; Kubo *et al*., 2006). In rare instances, hepatozoonosis can be fatal, as was seen in spotted hyenas (*Crocuta crocuta*) in the Serengeti (East *et al*., 2008).

The use of sentinel species for disease surveillance and monitoring

Disease surveillance in wild and domestic animal populations is critical for early detection of disease outbreaks. Assessing the health status and infection prevalence in wildlife populations has often been neglected due to the inherent difficulty of accessing wild individuals, which is logistically demanding and financially costly. As a result, poor baseline information is available, compounded by the uncertainty regarding wildlife behaviour and ecology in locally understudied species (Delahay *et al*., 2009). Disease surveillance in wildlife is in its infancy with regard to diagnostic protocols, the vast majority of which have been developed exclusively for humans and livestock (Artois *et al*., 2009). There remains a pressing need to adopt systematic surveillance of wildlife, which prioritises disease screening for important pathogens and makes use of sentinel species or sentinel locations, which are appropriate to the system of study (Kuiken *et al*., 2005; Aguirre and Tabor, 2008; Aguirre, 2009; Grogan *et al*., 2014).

One of the greatest challenges in trying to incorporate wildlife into a One Health framework, or indeed to begin to address epidemiological questions that involve wildlife species, is the ecological scope of the roles that these species play. The diversity that encompasses "wildlife" involves numerous taxonomic levels that may function in entirely unique ways. As a way of ameliorating this challenge of scope, researchers select sentinel species for study. The use of sentinel species is an important tool for gaining insight into the wider health of an ecosystem (Aguirre, 2009), depending on the suitability of the species selection for addressing the question of interest. A sentinel species is thus one that is selected based on its utility in detecting a perceived stress to the ecosystem (e.g. pathogens, toxins, environmental degradation) and for its vulnerability to that stress.

The use of species as sentinels of broader problems affecting the landscape has received considerable attention in recent years, particularly for monitoring ecosystem health for both contaminants, such as in the quintessential "canary in the mine shaft" example, and more recently for monitoring infectious disease (Aguirre, 2009; Grogan *et al*., 2014). Animal sentinels are distinguished by having qualities that either enhance the detectability of a disease or of some relevant aspect of disease dynamics, and which should be relatively cost-effective to survey (McCluskey, 2003). A sentinel population should either have a more easily detectable response to a pathogen, or should be more likely to have exposure to that pathogen. Importantly, these sentinels should have obvious ecological relationships with other species in the ecosystem, be they behavioural, spatial, or epidemiological, as this allows for extrapolation of surveillance data to the wider community (Halliday *et al*., 2007).

Carnivores exemplify many of these criteria and are thus a good choice for sentinels of infectious disease (Clifford *et al*., 2006; Halliday *et al*., 2007; Aguirre, 2009). As keystone species, apex carnivores have an importance to the functioning of ecological systems in the landscape that is disproportionate to their population size (Mills *et al*., 1993; Estes, 1996; Noss *et al*., 1996). Being at the top of trophic webs, carnivores overlap spatially with the prey base species and will also experience interspecific overlap with other predators, as well as having sexual interactions or territorial disputes within their own species. As such, carnivores may be exposed to pathogens through numerous modes of transmission including ingestion of infected prey, from pathogens that persist in the landscape, vector transmission, and aerosol transmission through sniffing faeces, urine or during territorial fighting and mating.

Mesocarnivores are a division of carnivores that have additional value as health sentinels, particularly when conventional large apex predators have been removed from a system. Unlike their larger counterparts, mesocarnivores have survived in increasingly disturbed landscapes (Roemer *et al*., 2009; Verdade *et al*., 2011). Their adaptive nature means that they may tolerate pathogen exposure and moderate urbanisation (Verdade *et al*., 2011), allowing them to function as disease reservoirs for other animal populations that may be far removed from pathogen exposure otherwise (Guerra *et al*., 2003; Sabeta *et al*., 2007). Mesocarnivores hold promise for infectious disease surveillance due to their relative ubiquity both within and outside protected areas and higher abundance compared to their larger counterparts (Roemer *et al.*, 2009). Additionally, their adaptability and tolerance for humans and human-associated landscapes lend to their importance in disease systems that are associated with changes in land-use from natural to urban and agricultural systems (Ditchkoff *et al*., 2006; Šálek *et al*., 2014). Further, human-mediated extirpation of large carnivores worldwide, has been implicated in the phenomenon of "mesocarnivore release", which is the increase in mesocarnivore populations and their occupation of the role of apex predator in the system (for review see Ritchie & Johnson, 2009). Sport-hunting, fur trading and retaliatory culling, are used worldwide in an attempt to manage mesocarnivore numbers. While these practices impact on the demography and stability of mesocarnivore populations and may manifest in further trophic cascades (Prugh *et al*., 2009), they also provide researchers with the opportunity for routine sampling of large numbers of individuals at the landscape level, thus increasing their value as sentinel species.

Introduction to this study

In South Africa, common mesocarnivore species that are found in human-modified landscapes are the black-backed jackal and the caracal (*Caracal caracal*). The drylands of Namibia and South Africa represent ecological strongholds for jackals and caracals, where they occur at comparatively high densities (Walton and Joly, 2003; Avgan *et al*., 2016). Despite being classified as mesocarnivores, black-backed jackals, hereafter referred to as "jackals", and caracals often function as the top carnivores outside of protected areas where lions (*Panthera leo*), leopards (*Panthera pardus*) and spotted hyenas have largely been eradicated. Co-ordinated culling of jackals and caracals is routine across the farmlands of South Africa, and particularly in the agricultural lands of the Groot Karoo region (Nattrass and Conradie, 2015). In the south Western Cape region, the mountain network comprising the Cape Fold Mountains, provides a refuge for mountain-dwelling species such as the caracal. As a result, caracal persist through these areas and into urban metropoles of the region, including the city of Cape Town where they are now the apex predator.

Both jackals and caracals are keystone species in the systems in which they persist, and are of economic importance as a result of their affinity for depredation of small livestock on farmlands across southern Africa (Beinart, 1998; Bergman *et al*., 2013; Nattrass and Conradie, 2015). Knowledge of the ecology of these two species is critical to our understanding of ecology in human-modified landscapes. Furthermore, an understanding of how these wildlife species contribute to the epidemiological dynamics of a system, or whether they may be vulnerable to the health impacts associated with living in proximity to humans is critical to the management of human and domestic animal health, and of how we choose to manage land use in the future. In light of this challenge, this work takes the first step in attempting to create a baseline for research on tick-borne pathogens affecting mesocarnivores in human-modified landscapes.

Infectious disease in black-backed Jackals

Jackals are common across their range in east and southern Africa (MacDonald *et al*. 2004) and exhibit impressive adaptability to modified landscapes, bringing them into close contact with humans, their domestic animals and livestock (Loveridge and MacDonald, 2001; 2003; MacDonald *et al*., 2004). Given their relatively high population densities, large dispersal distances and wide species range the epidemiological role of jackals in the spread and maintenance of relevant diseases warrants consideration. Nevertheless, evidence of how jackals contribute to the transmission of multi-host

carnivore pathogens, and whether humans, livestock and other wildlife species are influenced by jackal disease dynamics remains scarce (Bellan *et al*., 2012).

Owing to their importance as rabies vectors, disease in black-backed jackals has been the focus of previous studies (Courtin *et al*., 2000; Bellan *et al*., 2012), however little research on tick-borne pathogens has been carried out in the species. Jackals have been surveyed for certain pathogens of zoonotic or conservation interest, particularly those found in co-occurring domestic dog populations (Alexander *et al*., 1994; Bellan *et al.,* 2012). In particular, the jackal has been noted as an important rabies vector, contributing, in some cases, to epidemic status (McKenzie, 1993; Swanepoel *et al*., 1993; Bingham *et al*. 1999a,b; Courtin *et al*. 2000). This species may also host canine adenovirus (Spencer *et al*., 1999), canine distemper virus, canine parvovirus, African horse sickness (Binepal *et al*., 1992; Alexander *et al*., 1994), and anthrax (Lindeque and Turnbull, 1994). Other parasites include various trematodes, cestodes (see Walton & Joly, 2003) and protozoans, such *Babesia rossi* (previously referred to as *B. canis* or *B. canis rossi*), an Anaplasmataceae species speculated to be *Ehrlichia canis* (Alexander *et al*., 1994), *Hepatozoon canis*, *Sarcocystis* sp. and *Toxoplasma gondii* (see Walton & Joly, 2003). Sarcoptic mange is also found in this species, and like CDV and rabies, it may have contributed to local population declines in jackals (Keep, 1970; McKenzie, 1997; Rowe-Rowe, 1992).

Infectious disease in caracals

To date, comparatively little research on pathogen diversity in wild caracals has been undertaken. Thus, their epidemiological importance is largely unknown. There has been no extensive work on the epidemiology of pathogens or parasites infecting caracals, other than incidental sampling of individuals during larger studies on other felids (Bosman *et al*., 2007; Thalwitzer *et al*., 2010; André *et al*., 2012). For example, in a recent study focused on cheetahs (*Acinonyx jubatus*) in Namibia, Thalwitzer *et al*. (2010) showed seropositivity in Namibian caracals to a number of economically and ecologically important viruses, including feline herpes virus, feline calicivirus, feline parvovirus, feline coronavirus, canine distemper virus and puma lentivirus. None of the sampled individuals, however, showed any clinical manifestations of disease or any evidence of exposure to feline leukaemia virus or feline immunodeficiency virus. An important limitation of this study is that only three individuals were tested. Limited sample size is common to all of the existing literature on caracal pathogens, e.g. Kennedy *et al*. (2003) and Bosman *et al*. (2007) both report negative results feline coronavirus and *Babesia* infection based on samples sizes of two individuals. Given their ecological and economic importance in the semi-arid farmland ecosystems, together with their persistence in peri-urban environments, the paucity of research on caracal epidemiology clearly requires urgent remediation.

Aims and objectives

Fundamental to research on wildlife health is a grasp of the pathogen communities that exist within those systems. The lack of baseline pathogen surveillance for caracals, as keystone species on South African and Namibian farmlands is a strong impetus for a large scale study into caracal disease ecology. Additionally, jackals present themselves as useful sentinels of wider ecosystem health, considering the potential for comparison with extensive existing literature on their ecological equivalents from other parts of the globe, the red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) (Alexander *et al*., 1994; Wolfe *et al*., 2001; Duscher *et al*., 2013; Farkas *et al*., 2014; Cardoso *et al*., 2015).

In light of the paucity of research on carnivore health outside of protected areas in South Africa, the aim of this study was to use jackals and caracals as sentinels to study and characterize the health of carnivore populations living in human-modified landscapes. I examine body size and condition of jackals in farmlands and caracals from various land-use types, making comparisons both with historical datasets and among study sites (where data are available).

Ticks are one of the most important arthropod disease vectors globally, and transmit a greater diversity of pathogens than any other arthropod vector (Madder *et al*., 2013). In South Africa, extensive research has been conducted on tick ecology (Horak and Fourie, 1986; Horak *et al*., 1987; Walker *et al*., 2000; Horak and Matthee, 2003; Golezardy and Horak, 2007; Tonetti *et al*., 2009; Horak *et al*., 2010; Matthee *et al*., 2010; Golezardy *et al*., 2016), thus providing an excellent framework for comparison with new research. Host-attached tick diversity is evaluated for each population and considered as it relates to the presence of important tick-borne pathogens (TPBs) present in these mesocarnivore populations.

Finally, in order to establish a baseline dataset of TBP epidemiology in these host populations, this study uses a combination of molecular techniques to determine pathogen prevalence and diversity in human-modified landscapes. Detection procedures (mPCR/RLB and conventional PCR screening) for pathogens of zoonotic and/or veterinary concern have already been developed for use in closely-related host species (see Bosman *et al*., 2007; Matjila *et al*., 2008), and while there is an established literature on these pathogens in wildlife populations in other parts of the globe, critical gaps in our knowledge of the epidemiology of these pathogens remains, particularly in the role of wildlife hosts persisting in the non-protected areas of South Africa.

The specific objectives of this study are as follows: firstly, to determine whether body size and condition in the populations under study are within the range for their species. Additionally, for caracals, to determine whether there are differences in body condition associated with land-use type (rural vs urban). Mesocarnivores living on semi-arid farmlands have access to small stock in addition to resident wildlife and I thus predicted that their body condition would be better than urban conspecifics which are marginalised by extensive modification of the landscape to small, isolated patches of natural habitat.

The second objective is to examine and compare tick diversity on jackals and caracals. The tick species present in each system would have important consequences for which pathogens are circulating in the respective host populations (Pfaffle *et al*., 2013). Habitat suitability for ticks is driven by landscape factors, such as vegetation structure, as well as by climatic conditions and host availability (Estrada-Pena, 2001; Jore *et al*., 2014). I predicted that tick diversity would be higher in the more mesic urban areas (Randolph, 2010; Dantas-Torres and Otranto, 2013) and that tick communities would further differ with land use given the different host communities in rural versus urban areas.

Pathogen prevalence and diversity should be somewhat reflective of tick communities, given that pathogens often require specific species or genera of ticks in order to complete their lifecycle. Thus, the third objective of this study is to determine the presence and prevalence of tick-borne pathogens circulating in jackals and caracals, and finally, to examine the diversity of these tick-borne pathogens and contextualize these findings within the global body of work on blood pathogens in wild carnivores generally, and specifically in those persisting in transformed landscapes. I hypothesize that tick-borne pathogen prevalence and diversity will vary according the land-use type, and predict that Cape Peninsula caracals, living in very close proximity to the urban space will suffer from higher pathogen prevalence rates, of a greater diversity of TBPs (which they may share with sympatric domestic host species) and will have a higher incidence of co-infections, ultimately suggesting a poorer state of health in urban caracals.

This thesis is presented as a single body of work, with sub-sections that address each of the stated objectives. Initially, it deals with questions of jackal and caracal population demographics and morphometry and then tackles an assessment of host body condition. This is followed by an investigation into the tick species present on jackals and caracals, which were collected during host sampling and subsequently identified to species level. The majority of the thesis deals with tick-borne pathogen prevalence, which was investigated with the use of various molecular techniques, including

Reverse Line Blot (RLB) hybridization and conventional polymerase chain reaction (PCR). Subsequent to this, the diversity of tick-borne pathogens circulating in the jackal and caracal hosts is examined using a phylogenetic tree-building framework.

Given the paucity of research into jackal and caracal ecology in human-modified systems, specifically the epidemiology of their pathogens, this work proposes to contribute to the understanding of the roles that mesocarnivore species play in the epidemiological landscape of the modified ecosystems in which they are able to persist. Furthermore, this work adds to the literature on tick-borne pathogens (TBPs) in South African wildlife populations. It represents the first detailed examination of TBPs in jackals on farmlands and is also the first work that focuses specifically on TPBs in caracals anywhere in the world.

Chapter 2. Methods and materials

A. Study sites

This study makes use of caracal samples collected from independent study sites across three regions viz., the Central Karoo, Namaqualand and the Cape Peninsula within South Africa (Figure 2.1) and jackals collected only from the Central Karoo. Jackal samples were only collected from one of the three study sites as they do not occur on the Cape Peninsula, and occur at very low densities in Namaqualand (B. Cristescu and K. T. Teichman, *pers. comm.*). Sites vary from being predominately urban (Cape Peninsula) to rural, small-stock farmland (Central Karoo and Namaqualand) with small isolated protected areas located within all three regions. Each study site is described in detail below.

The Central Karoo

The primary study site for this investigation was farmland near Rietbron (-32.666667, 22.250000) in the semi-desert Central Karoo region of the Western Cape Province, South Africa (Figure 2.1, Figure 2.2a). This area falls within the Nama Karoo biome, more specifically in the Lower Karoo (NKi 1, Gamka Karoo area, Mucina and Rutherford, 2006); one of the more arid units of the biome. Altitude for this area ranges from 500-1100m above sea level and is characterised by a continental climate, with highly variable annual rainfall patterns (Mucina *et al*., 2006). Most of the rain falls in the late summer, peaking in March. Mean annual precipitation (MAP) ranges from 50-240mm with a maximum of up to 750mm on the top of mountain ranges (Potgeiter and Du Plessis, 1972; Mucina *et al*., 2006). Average temperatures range from -5°C to 17 °C in winter and 15°C to 43°C in summer. Drought in this region is common and may persist for prolonged periods (Booysen and Rowswell, 1983).

The vegetation of the region is dominated by early succession asteraceous shrubland (Cowling *et al*., 1994; Mucina *et al*., 2006) along with a low diversity of succulent, grass, geophyte and forb species (Mucina *et al*., 2006). Rocky outcrops, drainage lines and river systems, most of which are non-perennial, may host small trees. Sandy bottomlands tend to be covered by dense grass stand after extended rains. The fauna of this region include wildlife species that have historically occurred in the Nama Karoo, such as tortoises (*Stigmochelys pardalis, Homopus* sp. *Psammobates* sp.), ostriches (*Struthio camelus*) and large migratory herds of springbok (*Antidorcas marsupialis*). Large predators, such as lions and spotted hyenas have been extirpated from farmland areas following extensive human occupation post-1850 (Skead, 1980; Mills *et al*., 1985), and only persist in fenced game farms

and nature reserves (Acocks, 1979; Dean and Milton, 2003). Smaller carnivores, such as genets (*Genetta* sp.*)*, Cape and bat-eared foxes (*Vulpes chama, Otocyon megalotis*), African wild cat (*Felis silvestris*), striped polecat (*Ictonyx striatus*), and several mongoose species still persist on farmlands together with caracals and black-backed jackals that have assumed the role of apex predators. Other cosmopolitan mammal species include Greater kudu (*Tragelaphus strepsiceros*), springbok (*Antidorcas marsupialis*), steenbok (*Raphicerus camprestis*), common duiker (*Sylvicapra grimmia*), klipspringer (*Oreotragus oreotragus*), suricates (*Suricata suricatta*) and scrub hares (*Lepus saxatilis*).

Permanent human settlement in the Central Karoo began in the mid-1700s when the 'trekboers' began establishing farms (Schoeman, 2013). Currently, this region is dominated by privately-owned small stock farms, most of which farm Angora goats for mohair production. Merino and Dorper sheep are also common livestock species farmed for both wool and meat (Hoffman *et al*., 1999). Farmlands are typically fenced with a combination of mesh and barbed wire, so-called "jackal-proof fencing", and may be further divided into fenced 'camps'. In addition to the extirpation of large predators, other landscape modifications to allow for settled farming include the provision of permanent water from boreholes, and crops to provide supplementary livestock feed (Mucina and Rutherford, 2006). Game farms and protected areas are also present and are becoming more common (Reed and Kleynhans, 2009; Pasquini *et al*., 2010).

Namaqualand

This study site is in the Namakwa District of the Northern Cape Province (Figure 2.1, Figure 2.2b), and forms part of the Succulent Karoo biome. This study site is on the Western foothills of the Kamiesberg Bioregion, within the Namaqualand Hardeveld unit (Mucina and Rutherford, 2006). Elevation varies from 300-750m (van Deventer and Nel, 2006; Desmet, 2007). The main distinction between the Succulent and Nama Karoo biomes is the degree of exposure to coastal climate influences, which gives the Succulent Karoo its Mediterranean climate, characterised by winter rainfall (Cowling *et al*., 1999). Namaqualand is also a semi-desert region, but has greater climatic stability because of its more predictable rainfall patterns (Hoffman and Cowling, 1987). Most rainfall occurs between May and September, with the peak in June (Desmet, 2007). MAP is approximately 160 mm, although some years receive less than 100mm. In contrast to the Nama Karoo biome, rainfall events are mild and widespread when they occur (Schulze, 1997). Temperatures range from 7°C to 17°C in winter and 15°C to 30°C in summer. This predictability and rarity of drought events preserves a greater degree of species diversity, particularly of succulent species (Cowling *et al*., 1999).

The vegetation of this area is dominated by Namaqualand klipkoppe shrubland, which is open shrubland dominated by low-growing (<1m) shrubs. During the winter and spring season, annuals from the Asteraceae family bloom, drawing a massive seasonal influx of tourism to the area. Mammal diversity in Namaqualand is very similar to that of the Central Karoo.

The Cape Peninsula

While the Central Karoo and Namaqualand sites are similar in terms vegetation structure, land use and aridity, the Cape Peninsula represents a highly contrasting site. This study site is part of the Cape Town Metropolitan area, located within the Western Cape Province of South Africa and is the southwestern most tip of the African continent. The Cape Peninsula is a mixed-use landscape, characterised by protected areas that are surrounded by a mosaic of urban, rural and industrial land uses (Figure 2.1, Figure 2.2c). The climate is classified as Mediterranean with hot, dry summers and cold, wet winters. Rainfall in this region is abundant, with a MAP of 480-540mm. Average minimum and maximum temperatures are 7°C to 18°C in winter and 16°C to 26°C in the summer months. Most of the rain falls in the month of June. The Cape Peninsula falls within South Africa's most species rich endemic biome, Fynbos, which has vegetation characterised by fine-leaved, evergreen shrubs (Rebelo *et al*., 2006) whose regeneration is fire-driven.

Table Mountain National Park is a dominant feature of the site, and provides a topographical ecotone. Afromontane forests persist in incised valleys on the steep mountain slopes but extensive areas have been planted with commercial crops including vineyards, commercial pine and gum tree plantations and large areas have been invaded by invasive plant species, most notably members of the *Acacia* genus. Table Mountain National Park (TMNP) is an open access, unfenced protected area and is readily accessed by the public and their domestic animals. The park is utilised by tourists and local residents for various activities, such as mountain biking, hiking and to walk their domestic dogs (*Canis familiaris*). Surrounding TMNP are densely populated areas, used for a mix of rural, residential and industrial land uses. Substantial traffic volumes occur near park boundaries (*pers. obs.)*. Within the Peninsula, caracals appear to spend most of their time within the national park and urban protected areas and green belts they are also recorded within urban and agricultural areas (L. Serieys *pers comm*.), where they are encounter exotic prey species (e.g. rats (*Rattus rattus*), domestic cats (*Felis catus*) and Eastern grey squirrels (*Sciurus carolinensis*). Within TMNP, the following mammal species are found: eland *(Taurotragus oryx*), red hartebeest (*Alcelaphus buselaphus*), bontebok (*Damaliscus dorcas dorcas*), grysbok (*Raphicerus melanotis*), common duiker (*Sylvicapra grimmia*), grey rhebok (*Pelea capreolus*) and steenbok, Cape Mountain zebra (*Equus zebra zebra*), large-spotted genet (*Genneta tigrina*),

common genet (*Genneta genetta*), porcupine (*Hystrix africaeaustralis*), rock hyrax (*Procavia capensis*), chacma baboons (*Papio ursinus*), Cape clawless otter (*Aonyx capensis*), water mongoose (*Atilax paludinosus*), small grey mongoose (*Galerella purverulenta*) and the Cape fox.

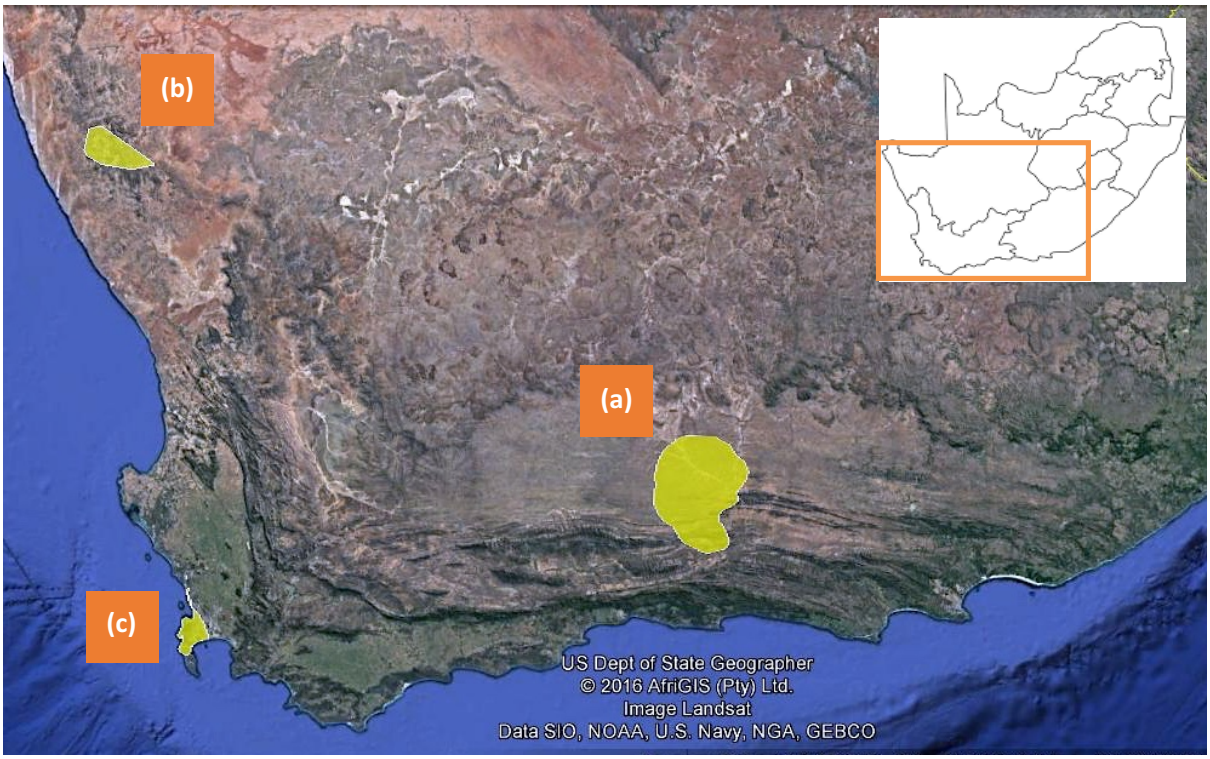

Figure 2.1: Map of study sites (shaded in yellow) in the Western and Northern Cape Provinces of South Africa. Sites are indicates as (a) the Central Karoo, (b) Namaqualand and (c) the Cape Peninsula. Caracals (*Caracal caracal*) were sampled from all three sites, while black-backed jackals (*Canis mesomelas*) were only sampled in the Central Karoo. Provincial boundaries of South Africa are indicated in the top right-hand corner

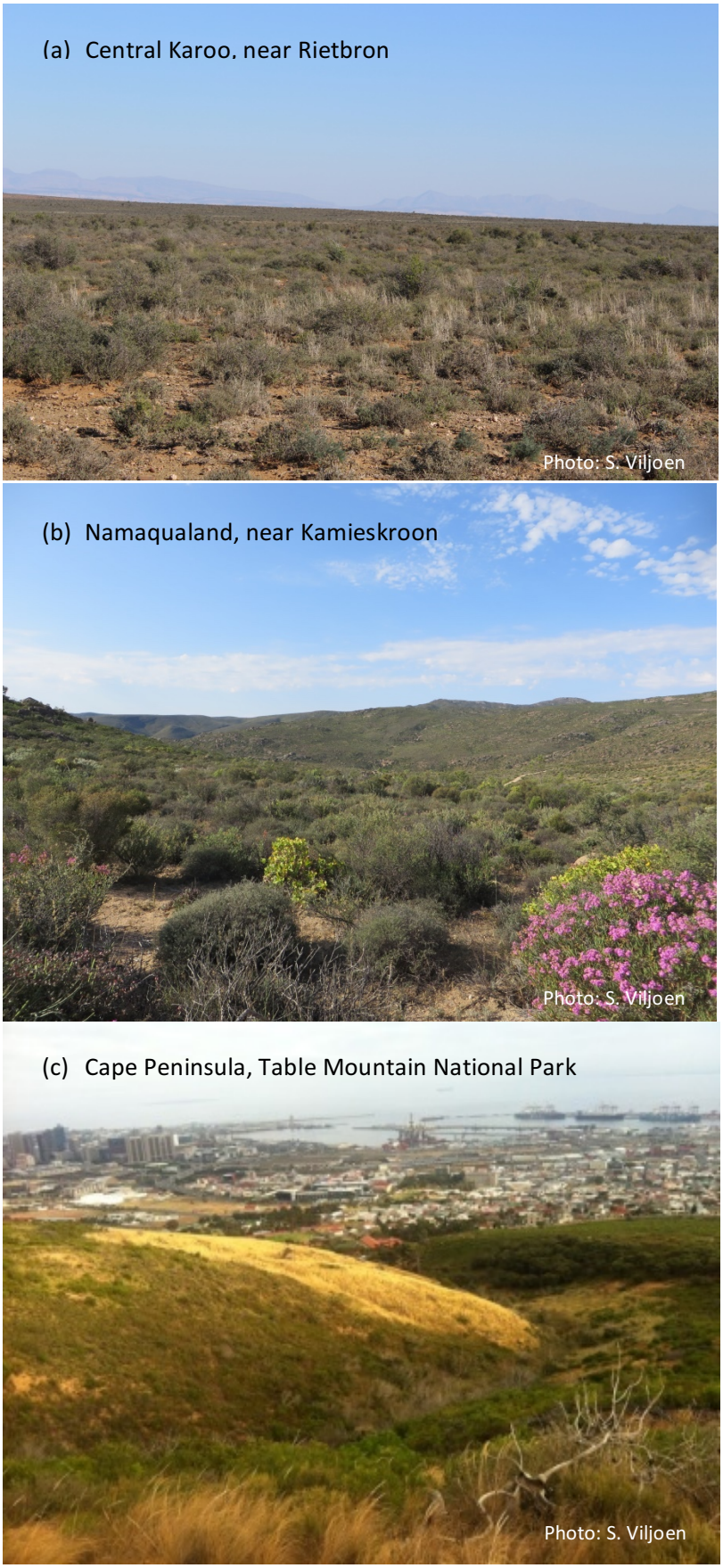

Figure 2.2: Typical landscapes of the three study sites included in this study, including (a) the Central Karoo, (b) Namaqualand and (c) the Cape Peninsula, South Africa

B. Study animals and sampling protocols

Sampling in the Central Karoo region took place over seven days in late April 2015, whereas that of both Namaqualand and the Cape Peninsula took place over 12- 16 months, spanning all seasons during 2014/2015. Aligned to this, the method of sampling in the Central Karoo yielded carcasses, whereas all of the Namaqualand individuals and the vast majority of those from the Cape Peninsula were processed while under sedation. Differences in sampling method and sample size across study sites are detailed in Table 2.1.

Table 2.1: Sample method and sample sizes for black-backed jackals (*Canis mesomelas*) and caracals (*Caracal caracal*) from three study sites in South Africa

	Central Karoo	Namaqualand	Cape Peninsula
Species examined	jackals; caracals	caracals	caracals
Sampling period	April 2015	2014/2015	2014/2015
Sampling method	post-mortem	live-capture	live-capture; post-mortem
No. of caracals sampled for morphometrics	27	16	15
No. of caracal blood samples	27	14	16
No. of jackals sampled for morphometrics	46	-	-
No. of jackal blood samples	43	-	-

All samples in the Central Karoo were collected during post-mortems performed on jackals and caracals that were culled during annual predator control operations on 19 privately owned farms covering an area of 310 774 hectares in the Central Karoo. All post-mortem examinations were performed within 24 hours of death (usually within 6-8 hours). Both target species were killed by professional hunters in accordance with the regulations of the local conservation authority (Cape Nature permit number AAA007-00161-0056). Carcasses were subsequently made available to the project by the Western Cape Nature Conservation Board (Permit no. 0056-AAA007-00161). No ethical clearance was required by the Science Faculty Animal Ethics Committee as the animals were not killed for research purposes. Animals were killed by firstly using remote call-ups with commercially available audio lures (FOXPRO Inc., Pennsylvania, United States) and a spotlight, followed by a fatal shot using a 0.243 calibre rifle or equivalent. The geographical location (GPS) of each carcass was recorded by

each hunter. Carcasses were processed at a temporary field laboratory located within the boundaries of the block hunt area over eight successive nights, from 15-23 April 2015 (austral autumn). These samples were analysed together with three individuals obtained in March 2015, originating from hunts in the nearby area of Merweville (32.666667, 21.516667) and included as part of the Central Karoo biome.

Whole blood, morphometric measurements, and ticks were collected from 14 live-captured caracals from Namaqualand and 13 live-captured caracals from the Cape Peninsula, as well as three Cape Peninsula caracals killed in road accidents. Samples were collected while individual were anaesthetized and under veterinary supervision. Ethical clearance for the capture and processing of live caracals in Namaqualand (PEACE Project) was obtained through the University of Cape Town (2013/V30/BC) with permits for the use of animals issued from the national (South African National Parks: CRC-2013/029-2014) and provincial authorities (Northern Cape Department of Environment and Nature Conservation: FAUNA 1157/2013; FAUNA 1158/2013). Ethical clearance for sample collection in the Cape Peninsula (Urban Caracal Project) was obtained through the University of Cape Town (2014/V20/LS), and animal use permits were issued by South African National Parks (2014/CRC/2014-017, 2015/CRC/2014-017) and CapeNature (AAA007-00147-0056).

Animal necropsies and the collection of morphometric data

All animals were sexed and assigned to an age class based on tooth characteristics such as wear and size. Caracal age classes included kitten (all deciduous teeth), sub-adult (permanent teeth emerging but deciduous teeth still present, or permanent teeth not fully emerged but no deciduous teeth), and adult (permanent teeth fully emerged). For jackals, an age chart was used which uses tooth shape and wear to determine approximate age (Figure 2.3).

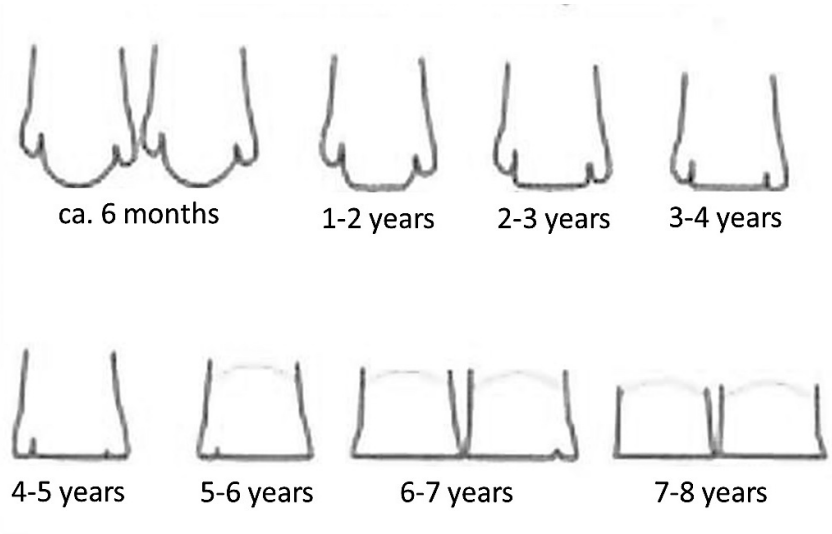

Figure 2.3: Aging chart for black-backed jackals (*Canis mesomelas*) using incisor eruption and wear characteristics (Source: Mr Taffie Mulder, adapted from Lombaard 1971)

Determining mesocarnivore body condition using morphometry

Morphometric measurements at all three study sites were taken in accordance with standard protocols for carnivores (Boitani and Powell, 2012). Measurements included body mass (kg), measured with a hanging scale, accurate to 0.1 kg. Total body length (from the tip of the nose to the end of the last caudal vertebra), body length (tip of the nose to base notch of the sacrum), tail length (sacrum to the end of the last caudal vertebra), shoulder height (from longest toe to top of scapula on left foreleg; leg extended to full length), chest girth (taken directly behind the forelegs) and neck girth (taken directly behind the ears) were all measured using a soft tape measure, accurate to 1 mm.

Biological tissue sampling for pathogen screening

Blood samples were collected for screening of tick-borne pathogens in the both jackals and caracals. In all Central Karoo animals and three Cape Peninsula caracals, blood sampling was performed post-mortem. Upon opening a carcass, blood from the right ventricle of the heart was drawn using a 21-gauge, sterile needle, and stored in an EDTA-coated 10ml Vacutainer™ tube to prevent blood clotting. Whole blood samples were stored frozen at -20ºC. All necropsy procedures were undertaken using aseptic technique as far as possible, with instruments and surfaces being chemically sterilized or replaced between each necropsy to prevent cross-contamination of samples. For live-trapped animals in Namaqualand and the Cape Peninsula, whole blood samples were drawn from the saphenous vein of anaesthetised animals. All samples were returned to the laboratory and stored at -20ºC (Central

Karoo and Namaqualand samples) or -80°C (Cape Peninsula samples) for a maximum of 12 months before processing.

Collection of ticks for assessment of tick vector diversity

During external examination of both dead and anaesthetised animals, attached ticks were removed from individuals and stored in 70-96% alcohol for subsequent identification to species level. All ticks were photographed using a Leica EZ4D stereo-microscope (Leica Microsystems, Germany). Where possible, each tick was identified to species level and life stage (larva, nymph, adult) using a combination of guides (Latif and Walker, 2004; Walker *et al*., 2010, 2014). Sex was also recorded for adult ticks. Species identifications were subsequently confirmed by Prof. Ivan Horak, a recognised expert in African tick identification.

C. Detection of pathogens in animal host blood

The presence of a range of blood pathogens was tested for using a number of complementary approaches. Whole blood samples were obtained for animals from each of the three sites. Animals for which insufficient blood was available (caracal n=1; jackals n=4) were excluded from blood pathogen testing.

Pathogen detection using blood smears

A visual survey for the presence of blood pathogens was carried out using Giemsa-stained blood smears (Giemsa's azure eosin methylene blue, Cat. No. 109204, Merck Millipore Corporation, MA 01821, USA). Staining was performed to differentiate between blood cells (erythrocytes, lymphocytes) and parasites, as each of these has a differential staining capacity. To make a thin smear, a small drop of blood from the right ventricle of the heart was smeared onto glass slides. Blood smears were performed in triplicate for each individual, however, only the best smear from each animal was used for staining. After air drying for two hours under a protective cover (to limit ingress of dust and access by insects), each slide was fixed in 100% methanol for 30 seconds and left to air dry. Fixed slides were taken back to the University of Cape Town for staining within two weeks of collection. Staining was performed using 5% Giemsa solution in phosphate buffer solution at pH 7.2 (Buffer tablets Cat. No. 109468, Merck Millipore, USA). Stain was applied to each slide for 20 minutes by submersion in a stain-filled coplin jar. Excess stain was removed by thoroughly rinsing under running water and then air dried. Slides were viewed under oil immersion using a clean coverslip, at 1000 magnification, with

a Nikon Eclipse 50i Compound Microscope (Nikon Instruments Europe B.V., Netherlands). Each slide was examined from the top, left hand corner and 10 non-overlapping fields of view (FOVs) were examined. The presence of any blood parasites was noted.

Molecular characterisation of blood pathogens

Total DNA was extracted from whole blood using the QIAamp® DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA extracts were stored at 4ºC during processing and at -20ºC for long-term storage.

Pathogen detection by Reverse Line Blot Hybridisation Assay

Multiplex PCR-based Reverse Line Blot Hybridisation (RLB) (Kong and Gilbert, 2007) was used to diagnose infection for a wide range of blood parasites (Table 2.2). This technique involves a hybridisation assay, whereby oligonucleotide probes corresponding to the pathogens being tested for, are covalently bound to a nylon membrane (Figure 2.4a). This membrane is then exposed to the amplified, denatured DNA from a host species, which is labelled with biotin from the primers used in the PCR amplification (Figure 2.4b), thus enabling the DNA to bind to Streptavidin-Peroxidase (Figure 2.4c). Only when biotin-labelled, denatured DNA has bound to the probe (indicating that host blood contains the pathogen DNA for a specific probe, e.g. *Theileria annae*), is the Streptavidin-Peroxidase able to bind. It is this chemical that reacts to a chemiluminescent agent that is detectable when exposed to an X-ray film (Figure 2.4d). The prepared membrane is placed inside a mini-blotter apparatus, which has lanes arranged so that they run perpendicular to the probe lanes. This arrangement ensures that each lane of denatured DNA is exposed to all of the probes of the membrane (Figure 2.5).

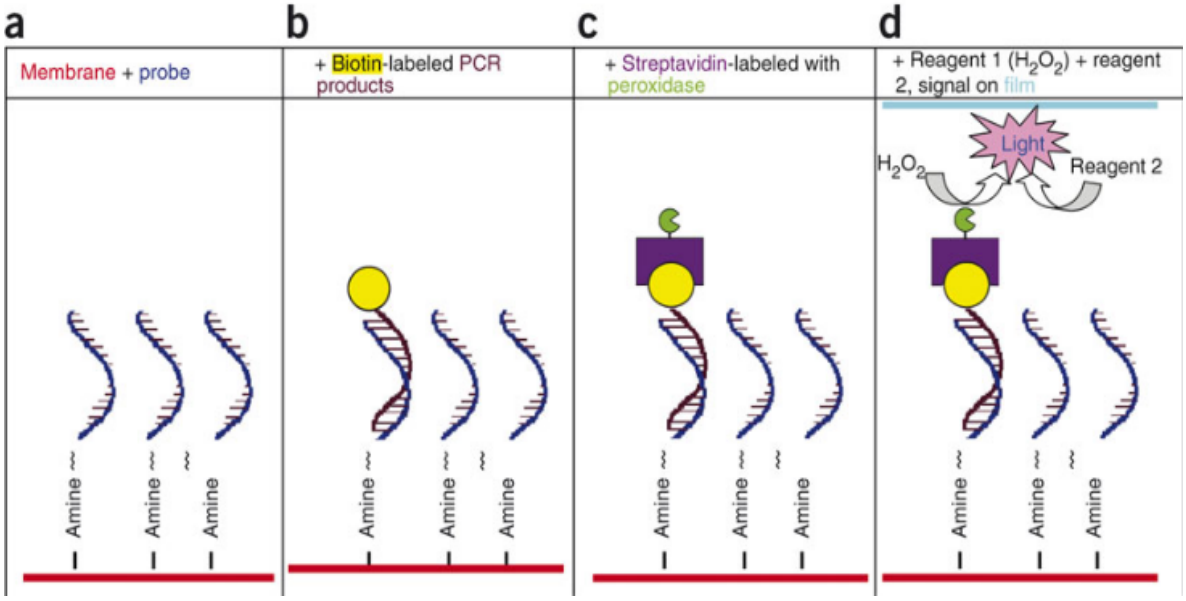

Figure 2.4: Membrane design for oligonucleotide probe binding and pathogen DNA detection using the reverse line blot hybridisation technique. The order of events is noted as a, b, c and d (from Kong and Gilbert, 2007)

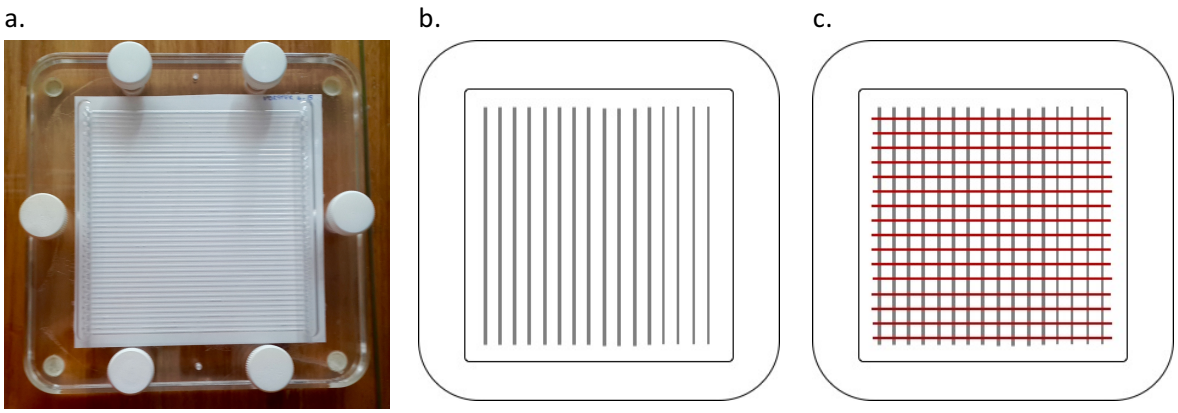

Figure 2.5: Application of probes that target pathogen DNA during a Reverse Line Blot (RLB) hybridisation assay, (a) RLB mini-blotter apparatus with prepared membrane inside, (b) schematic of prepared membrane with vertical lanes where pathogen-specific probes are covalently bound and (c) horizontal lanes in which amplified DNA samples are applied, allowing exposure to each of the vertical probe lanes

To avoid cross-reaction of primers during PCR, amplification was performed separately for *Babesia/Theileria* species and *Ehrlichia/Anaplasma* species. The *Babesia/Theileria* PCR was carried out using primers, RLB-F2 (5'-GAC ACA GGG AGG TAG TGA CAA G-3') and RLB-R2 (biotin-5'-CTA AGA ATT TCA CCT CTG ACA GT-3') which amplify the 18S rRNA gene, spanning the V4 region from 460-540 bp (Gubbels *et al.,* 1999; Matjila *et al.,* 2004; Matjila *et al.,* 2008). For the *Ehrlichia/Anaplasma* PCR, primers Ehr-F (5'-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3') and Ehr-R (5'-Biotin-CGG GAT CCC

GAG TTT GCC GGGACT TYT TCT-3') were used to amplify the 460-520 bp of the V1 hypervariable region in the 16S SSU rRNA gene (Bekker *et al, .*2002; Matjila *et al.,* 2008). Nuclease-free water was included as a negative control, and either *Babesia bovis* or *Anaplasma centrale* were used as positive controls.

Reaction mixtures (25 µl total volume) for both PCR reactions consisted of 12.5 µl of Quantitative PCR Supermix-UDG (containing 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 6 mM MgCl2, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 800 µM dUTP, 40 units/ml UDG, 60 units/ml Platinum® Taq DNA Polymerase and stabilizers (ThermoFisher Scientific, South Africa)); 0.25 µl of 100µM stock of each forward and reverse primer; 7 µl of nuclease-free water and up to 5 µl of DNA template. The UDG PCR master-mix is used to avoid carry-over contamination from previous reactions, by acting to remove uracil bases from the phosphodiester structure of DNA, thus acting to block replication of these sites (Longo *et al*., 1990).

The conditions of each PCR reaction started with an initial step of 3 min at 37ºC, which acts to activate the UDG (uracil DNA glycosylase), followed by a 10 minute step at 94ºC to inactivate the UDG and activate the Taq polymerase. This is followed by 2 cycles at 94ºC (20 seconds), 67ºC (30 seconds) and 72ºC (30 seconds), which is repeated with the annealing temperature decreased by 2ºC until the annealing reaches 59ºC (touchdown PCR). Thereafter, there are 40 cycles of 94ºC (20 seconds), 57º (30 seconds) and 72ºC (30 seconds) before a final extension step at 72º for 7 minutes.

Following PCR, amplified products for the *Babesia*/*Theileria* and *Ehrlichia/Anaplasma* reactions were combined and diluted with 130 µl of 2X SSPE/0.1% SDS buffer. The reverse Line Blot hybridisation protocol, as described by Gubbels *et al.* (1999), with modification from Matjila *et al.* (2004), was conducted on these diluted PCR products. However, instead of ECL hyperfilm, X-ray film was used and exposed to the chemiluminescent membrane for 1-3 seconds, followed by X-ray development and fixation. The list of oligonucleotide probes used is listed in Table 2.2.

The probes designed for this assay were selected to detect vector-borne haemoprotozoan DNA extracted from host blood. Both genus specific ("Catch all") probes and species specific probes are included on the membrane so in the event of a genus-specific reaction, it can either be identified to species level if its species probe is present, or can be sequenced to determine its identity. In this way, RLB assays allow for the detection of potentially new species of parasite. Two different probes are included as *Babesia* genus-specific probes (*Babesia* catch-all 1, *Babesia* catch-all 2) in order to account for the large variation in the *Babesia* 18S rRNA gene region.

Table 2.2: Oligonucleotide probes bound to the membrane used for reverse line blot hybridisation using PCR products from black-backed jackal (*Canis mesomelas*) and caracal (*Caracal caracal*) blood samples

Oligonucleotide Probe	5'-3' sequence	Reference
Rickettsial bacteria		
Ehrlichia/Anaplasma catch all	GGG GGA AAG ATT TAT CGC TA	Matjila *et al.,* 2008
Ehrlichia canis	TCT GGC TAT AGG AAA TTG TTA	Schouls *et al.,* 1999
Ehrlichia ruminantium	AGT ATC TGT TAG TGG CAG	Matjila *et al*., 2008
Anaplasma bovis	GTA GCT TGC TAT GRG AAC A	Bekker *et al.,* 2002
Anaplasma centrale	TCG AAC GGA CCA TAC GC	Matjila *et al.,* 2008
Anaplasma chaffiensis	ACC TTT TGG TTA TAA ATA ATT GTT	Matjila *et al*., 2008
Anaplasma marginale	GAC CGT ATA CGC AGC TTG	Matjila *et al.,* 2008
Anaplasma phagocytophilum	TTG CTA TAA AGA ATA ATT AGT GG	Bekker *et al.,* 2002
Anaplasma sp. omajienne	CGG ATT TTT ATC ATA GCT TGC GCT	Matjila *et al.,* 2008
Piroplasms		
Theileria/Babesia catch all	ATT AGA GTG TTT CAA GCA GAC	Nijhof (unpublished)
Theileria catch all	ATT AGA GTG CTC AAA GCA GGC	Matjila *et al.,* 2008
Theileria annae	CCG AAC GTA ATT TTA TTG ATT G	Yisaschar-Mekuzas *et al.,* 2013
Theileria annulata	CCT CTG GGG TCT GTG CA	Gubbels *et al.,* 1999
Theileria bicornis	GCG TTG TGG CTT TTT TCT G	Nijhof *et al.,* 2003
Theileria buffeli	GGC TTA TTT CGG WTT GAT TTT	Gubbels *et al.,* 2000
Theileria equi	TTC GTT GAC TGC GYT TGG	Butler *et al.,* 2008
Theileria lestoquardi	CTT GTG TCC CTC CGG G	Schnittger *et al.,* 2004
Theileria mutans	CTT GCG TCT CCG AAT GTT	Gubbels *et al*., 1999
Theileria ovis	TGC GCG CGG CCT TTG CGT T	Bekker *et al.,* 2002
Theileria parva	GGA CGG AGT TCG CTT TG	Gubbels *et al.,* 1999
Theileria separata	GGT CGT GGT TTT CCT CGT	Schnittger *et al.,* 2004
Theileria sp. buffalo	CAG ACG GAG TTT ACT TTG T	Oura *et al.,* 2004
Theileria sp. kudu	CTC CAT TGT TTC TTT CCT TTG	Nijhof *et al*., 2005
Theileria sp. sable	GCT GCA TTG CCT TTT CTC C	Nijhof *et al*., 2005
Theileria taurotragi	TCT TGG CAC GTG GCT TTT	Gubbels *et al*., 1999
Theileria velifera	CCT ATT CTC CTT TAC GAG T	Gubbels *et al.,* 1999
Babesia catch all 1	ATT AGA GTG CTC AAA GCA GGC	Nijhof (unpublished)
Babesia catch all 2	ACT AGA GTG TTT CAA ACA GGC	Nijhof (unpublished)
Babesia bicornis	TTG GTA AAT CGC CTT GGT	Nijhof *et al.,* 2003
Babesia bigemina	CGT TTT TTC CCT TTT GTT GG	Gubbels *et al.,* 1999
Babesia bovis	CAG GTT TCG CCT GTA TAA TTG AG	Gubbels *et al.,* 1999
Babesia caballi	GTT GCG TTK TTC TTG CTT TT	Govender *et al.,* 2011

Oligonucleotide Probe	5'-3' sequence	Reference
Babesia canis	TGC GTT GAC GGT TTG AC	Govender *et al.,* 2011
Babesia divergens	ACT RAT ATC GAG ATT GCA C	Govender *et al.,* 2011
Babesia felis	TTA TGC GTT TTC CGA CTG GC	Bosman *et al.,* 2007
Babesia gibsoni	TAC TTG CCT TGT CTG GTT T	Yisaschar-Mekuzas *et al.,* 2013
Babesia lengau	CTC CTG ATA GCA TTC	Bosman *et al.,* 2010
Babesia leo	TTA TGC TTT TCC GAC TGG C	Bosman *et al.,* 2007
Babesia microti	GRC TTG GCA TWC TCT GGA	Govender *et al.,* 2011
Babesia occultans	CCT CTT TTG GCC CAT CTC GTC	He *et al.,* 2012
Babesia rossi	CGG TTT GTT GCC TTT GTG	Govender *et al.,* 2011
Babesia sable	GCG TTG ACT TTG TGT CTT TAG C	Oosthuizen *et al.,* 2008
Babesia vogeli	AGC GTG TTC GAG TTT GCC	Matjila *et al.,* 2004

Conventional PCR for the pathogen isolation and sequencing

Using primers that target large DNA regions (~1000bp), conventional PCR amplification and sequencing was performed. Samples were selected for PCR and sequencing based on positive results from the RLB assay. The set of BTF1 and BTR2 primers were also used as a diagnostic tool for comparison with the results of the RLB assay, and thus were applied to all samples. These BT primers (BTF1; BTR2) target an ~848 bp site of the 18S rRNA region of apicomplexan parasites, including *Babesia sp.*, *Theileria sp.* and *Cytauxzoon sp.* (Kubelová *et al*., 2011; Kelly *et al*., 2014; Terao *et al*., 2015).

Reactions using BT primers (Jefferies *et al*., 2007a) were carried out in volumes of 10µl for diagnostics, followed by a proportional 25µl reaction for certain positive samples selected for sequencing. Reactions (10µl) consisted of 0.4µl extracted DNA, 5µl DreamTaq Green PCR Master Mix (2X) (ThermoScientific ™, South Africa), 4.1µl ddH_2O, and 0.5µl each of 50µM primers, BTF1 (forward; 5'-GGCTCATTACAACAGTTATAG-3') and BTR2 (reverse; 5'- GGACTACGACGGTATCTGATCG-3'). Cycling conditions for caracal samples followed Jefferies *et al.* (2007) (primary amplification only). For jackal samples, the annealing temperature was reduced to 50º Celsius in order to optimize the PCR reaction. Sequences and references for all primer sets used in this study are shown in Table 2.3.

An ultrapure water (ddH_2O) sample was included in each PCR reaction to monitor for cross-contamination. PCR reactions were carried out using an Applied Biosystems 2720 96-Well Thermal Cycler and amplified products were visualised by ethidium bromide staining on 1% agarose gel by

electrophoresis (SeaKem® LE Agarose, Whitehead Scientific (Pty) Ltd). Samples were assigned a positive diagnosis if they presented a band at the expected size targeted by the primer set. Representative samples showing positive results were randomly selected for sequencing. After separation using gel electrophoresis, bands were excised and cleaned using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI, USA).

Table 2.3: Primers used in the reverse line blot (RLB) hybridisation assay and in conventional PCR screening and sequencing

Target	Primer name	5'-3' sequences	References
Babesia/Theileria species 18S rRNA	RLB-F2	GAC ACA GGG AGG TAG TGA CAA G	Gubbels *et al.,* 1999
	RLB-R2	CTA AGA ATT TCA CCT CTG ACA GT	
Ehrlichia/*Anaplasma* 16S rRNA	Ehr-F	GGA ATT CAG AGT TGG ATC MTG GYT CAG	Bekker *et al.,* 2002; Matjila *et al.,* 2008
	Ehr-R	CGG GAT CCC GAG TTT GCC GGGACT TYT TCT	
Babesia/Theileria/Hepatozoon 18S rRNA	BTF1	GGC TCA TTA CAA CAG TTA TAG	Jefferies *et al.,* 2007
	BTR2	GGA CTA CGA CGG TAT CTG ATC G	
Babesia/Theileria 18S rRNA	NBab 1F	AAG CCA TGC ATG TCT AAG TAT AAG CTT TT	Oosthuizen *et al.,* 2008
	TB-Rev	AATAAT TCA CCG GAT CAC TCG	

Sequencing of pathogen DNA

Direct sequencing was performed on selected samples that reacted with generic catch-all probes on the RLB assay, targeting a partial 18S rRNA gene of *Babesia* and *Theileria* species (ca. 1800 bp). Reactions were performed in a volume of 25µl, consisting of 2.5 µl extracted DNA, 12.5µl DreamTaq Green PCR Master Mix (2X) (ThermoScientific™, South Africa), 8µl ddH_2O, and 1µl each of 10µM primers Nbab 1F (5'-AAG CCA TGC ATG TCT AAG TAT AAG CTT TT-3') and TB Rev (5'-AATAAT TCA CCG GAT CAC TCG-3'). Cycling conditions were as follows: 2 min at 95°C, 30 seconds each at 95°C, 31°C, 72°C (35 cycles), and a final extension step of 10 minutes at 72°C. Samples suspected of being infected with *Theileria* species were amplified by Ms Ilse Vorster, using this protocol at the University of Pretoria by Ms Ilse Vorster and sequencing of *Theileria* amplicons was performed by Inqaba Biotech (Pretoria, South Africa). For those samples which could not be amplified using the sequencing primers,

Nbab 1F and TB Rev, I used the original primers and PCR conditions from the RLB assay, RLB F and RLB R, to amplify shorter gene regions. Shorter amplifications could then be sent for direct sequencing or used in cloning reactions.

A separate reaction was carried out using primers which target a partial 16S rRNA gene *Ehrlichia* and *Anaplasma* species (ca. 1500bp). Reactions were performed in a volume of 25µl, consisting of 2µl extracted DNA, 12.5µl DreamTaq Green PCR Master Mix (2X) (ThermoScientific ™, South Africa), 9.5µl ddH_2O, and 0.5µl each of 10µM primers fD1 (5'- AGA GTT TGA TCC TGG CTC AG-3') and rP2 (5'-ACG GCT ACC TTG TTA CGA CTT-3') (Kubo *et al*., 2015).

Where direct sequencing yielded a chromatogram with multiple peaks at the same positions, suggesting infection with multiple species of the same genus, samples were cloned using the pGEM-T Easy Vector System I (Promega, Madison, USA), with vector cells, and JM109 competent cells (Promega, Madison, USA). Blue-white screening was used to distinguish positive clones. The transformed cell culture was plated onto Luria Broth (LB) agar plates, treated with ampicillin (100mg/mL), 100mM IPTG and 3% X-gal in dimethylformamide. Cloning was performed according to the manufacturer's instructions and each reaction was plated in duplicate for each individual. A minimum of five positive colonies, along with a negative colony, were selected for a colony PCR amplification using the plasmid specific primers, which amplify the section of the vector in which the target insertion occurs.

Colony PCR used the following cycling conditions: 2 minutes at 95°C, 35 cycles of 30 seconds at 95°C, 20 seconds at 50°C, 40 seconds at 72°C and a final step for 10 minutes at 72°C. Each 20µl reaction consisted of 10µl DreamTaq Green PCR Master Mix (2X) (ThermoScientific™, South Africa), 8µl ddH_2O, and 1µl each of primers commercially available primers M13-F (5'-GTA AAA CGA CGG CCA GT- 3') and M13-R (5'-CAG GAA ACA GCT ATG AC-3') (ThermoScientific™, South Africa) at a concentration of 0.1 $pmol.\mu l^{-1}$. Amplified PCR products were gel purified, using a Wizard SV gel and PCR clean-up system (Promega, Madison, WI, USA) and sequenced using the M13-F primer.

All DNA sequencing was done using the Big Dye Terminator Cycle Sequencing Kit version 3.1 (ABI, Darmstadt, Germany) on an ABI3730xl Genetic Analyser (Applied Biosystems, USA). Sequencing was performed at the Central Analytical Facility, University of Stellenbosch, South Africa. Sequence chromatograms were visualised and manually edited in BioEdit Sequence Alignment Editor v. 7.2.5 (Hall, 1999).

D. Data analyses

Determination of body condition using morphometry

The sex ratios of caracal and black-backed jackal populations were based on all of the age classes, and were calculated separately for adults only. Chi-squared tests of independence were used to test for significant differences (α=0.05) in the proportion of males and females sampled in the population. Only adults were included in morphometric analyses with the means and standard deviations calculated for the following body measurements: total length, body length, tail length, weight, shoulder height, neck girth, chest girth and Body Mass Index (BMI). Body Mass Index was calculated using the following equation:

$$BMI = {weight\ (kg)}\Big/{body\ length\ (m)^2}$$

The data for each of the measurements were tested for normality of distribution using the Shapiro-Wilkes test, with a p-value threshold for normality of 0.05. For non-parametric data, I used a Mann-Whitney U-Test to test for differences between males and females for each measurement. In the case of normally distributed data, an F-test for equal variances was used prior to a Student's T-test.

To examine body condition indices across study sites, a linear model was constructed using the Ordinary Least Squares (OLS) regression (Schulte-Hostedde *et al*., 2005). Residuals were based on mass-body size scaling and were used as a proxy of body condition. Predictor variables were selected from the pool of body size measurement(s) using an ANOVA framework. For caracal populations, shoulder height and chest girth were used as combined predictors of weight, while for the jackal populations, body length, chest girth and neck girth were used as the combined predictors. These models were selected as being the best at predicting animal weight, according the Akaike Information Criterion (AIC) value. The model with the lowest AIC value was selected and used to calculate the OLS residuals.

Phylogenetic analysis to determine pathogen identity and diversity

Using the nucleotide Basic Local Alignment Search Tool (BLASTn) DNA sequences from all sampled were compared with those in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Sequence identity was verified according to a sequence identity threshold of 97%. Highly similar sequences from the

same host species and study site, or those obtained as clones from the same individual, were used to create a consensus sequence, using the consensus sequence function in BioEdit version 7.2.5 (Hall *et al.,* 1999). These consensus sequences represent all of the variation within the original sequences. Reference sequences of known identity were selected from the Genbank database and used in conjunction with sequences from this study to create an alignment dataset, using the ClustalW (Thompson *et al*., 1994) multiple sequence alignment accessory application in BioEdit. Final alignments were trimmed to the length of the shortest sequence and analysed in MEGA v6.0. I (Tamura *et al*., 2013).

Separate phylogenetic trees were generated for *Babesia/Theileria* species, *Hepatozoon* species, and *Ehrlichia Anaplasma* species. In each case, both the Neighbour-Joining and Maximum Likelihood methods of tree construction were used. Using the model selection application in MEGA, the appropriate substitution model was selected according to which yielded the lowest AICc value based on the multiple sequence alignment being used. Values for model estimation were calculated in MEGA v6.0.1. For each tree, node support was evaluated with 1000 bootstrap replicates. Appropriate outgroups were selected based on existing literature.

Calculation of pathogen prevalence

The term 'prevalence' is used to refer to the observed prevalence of infection in the sampled population. Prevalence was calculated as the proportion of the total sample population that showed a positive result for a pathogen or group of pathogens. A true positive result was evaluated for each individual based on the outcome of the RLB hybridisation assay, the conventional PCR (BTF1/BTR2 primers) and the results of the BLASTn analysis and phylogenetic evaluation of the amplified sequences. Clopper-Pearson confidence intervals for binomial response data were calculated using the 'exactci' function in the 'PropCIs' package in R (Scherer, 2014). Chi-squared tests of homogeneity were used to test for differences in prevalence rates among groups. For multiple comparisons, p-values were subjected to Benjamini-Hochberg correction using the 'fifer' package in R (Fife, 2017). All statistical analyses, unless otherwise specified, were carried out in R for Windows v 3.2.2 (R Core Development Team 2015).

Chapter 3. Results

Assessing body size and condition in jackals

A total of 46 jackals were sampled from the Central Karoo ranging in age from six months to approximately seven years with the majority of jackals classified as adults (69%, n=31) and the remaining 31% (n=14) classified as sub-adult. The sex ratio of all jackals sampled is fairly even (χ^2 = 1.09; df = 1, p = 0.3), and close to parity when comparing adults only (χ^2 = 0.29; df = 1, p = 0.6) (Table 3.1).

Table 3.1: Population demographic structure and sex ratios of the black-backed jackal (*Canis mesomelas*) population samples obtained from the Central Karoo, South Africa

	All individuals			
n	M	F	Ratio (M:F)	Chi-squared analysis
45	26 (57.8%)	19 (42.2%)	1.37	χ^2 = 1.09; df = 1, p = 0.3
	Adults Only			
n	M	F	Ratio (M:F)	Chi-squared analysis
31	17 (54.8%)	14 (45.2%)	1.21	χ^2 = 0.29; df = 1, p = 0.6

Size measurements from this study are similar to those for published literature on black-backed jackals in southern Africa (Table 3.2). Standard deviation values are often not reported in historical literature, which prevents statistical comparison. The mean weight of jackals in this study appears to be small, while the range of measures is close to those of published studies for male jackals. Female weight range is smaller than reported in other studies however the mean weight of female is intermediate among the four studies compared. A similar trend is observed for body length, with the size ranges in the Central Karoo jackals being within the range of those previously reported.

Table 3.2: Body measurements for adult black-backed jackals (*Canis mesomelas*) in this study and for other studies in Southern Africa

a. Weight (kg)

Male			Female				
n	Range	Mean	n	Range	Mean	Reference	Region
39	6.8-9.5	7.9	52	5.5-10	6.6	National Museum records (from (Walton and Joly, 2003)	Zimbabwe
59	5.9-12	8.2	42	6.2-9.9	7.4	(Stuart, 1981)	Cape Province
123	6.4-11.4	8.4	84	5.9-10	7.7	(Rowe-Rowe, 1978)	KwaZulu-Natal
17	6.8-9	7.8	14	4.6-8.2	6.9	*This study*	Central Karoo

b. Head-Body length (mm)

Male			Female				
n	Range	Mean	n	Range	Mean	Reference	Region
65	690-900	785	42	650-850	745	Stuart 1981	Cape Province
4	711-812	768	5	673-711	688	Rowe-Rowe 1978	KwaZulu-Natal
17	730-830	782	14	660-790	730	*This study*	Central Karoo

Using a body condition index based on residuals, jackal weight was best predicted by body length ($t_{(37)}$= 5.463, p < 0.001), chest girth ($t_{(37)}$= 3.046, p = 0.004) and neck girth ($t_{(37)}$= 2.076, p = 0.04) (Table 3.3). Linear model fitting was performed using all three variables to predict jackal weight ($F_{(3,37)}$=23.91, p<0.001), yielding a multiple R-squared of 0.66 (adjusted R- squared = 0.63). The model is thus able to predict 63% of the variation in jackal weight, using body length, chest girth and neck girth (Table 3.3). The ordinary least square residuals of actual weight to predicted weight were used as body condition indices (BCIs). Body condition index values for jackals ranged from -1.13 to 1.18, with a median value of -0.07.

Table 3.3: The different models for predicting the Body Condition Index in black-backed jackal (*Canis mesomelas*) sampled in the Central Karoo. Model selection was based on the lowest Akaike Information Criterion (AIC) value

Model	AIC	ΔAIC	No. of parameters
Weight ~ Body length + Chest girth + Neck girth	-44.052	1.532	3
Weight ~ body length + shoulder height + chest girth + neck girth	-42.52	0	4
Weight ~ body length + shoulder height + neck girth	-40.671	-1.849	3
Weight ~ body length + shoulder height + chest girth	-39.552	-2.968	3
Weight ~ shoulder height + chest girth + neck girth	-24.872	-17.648	3

There was no significant difference in jackal BMI between sexes ($F_{(1,41)}$ = 0.00, p=0.9961) or age classes ($F_{(1,41)}$ = 0.2054, p=0.6528), nor was the combined effect of sex and age class significant ($F_{(1,41)}$ = 1.1171, p=0.2967) (Figure 3.1). The same outcome was found using BCI differences among jackals, as there was no apparent effect of sex or age class on body condition of jackals ($F_{(1,37)}$ = 0.12, p=0.73 and $F_{(1,37}$ = 1.52, p=0.23, respectively) (Figure 3.2). The interaction between sex and age class does not show significant differentiation among groups ($F_{(1,37)}$ = 0.35, p=0.56).

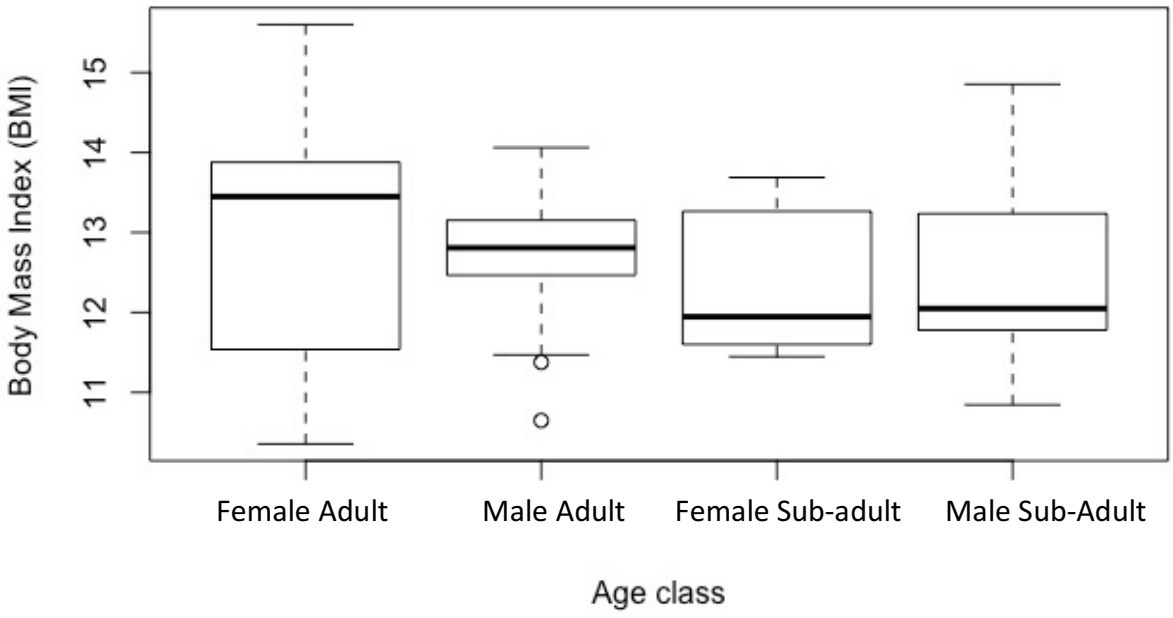

Figure 3.1: Box and whisker plots indicating median values for the Body Mass Index (BMI) of black-backed jackals (*Canis mesomelas*) sampled in the Central Karoo farmlands, South Africa. Solid bold lines represent the median, boxes represent the interquartile range (IQR), whiskers are the reasonable limits of the data (1.5*IQR). Jackals are grouped by sex (male and female) and age class (adult and sub-adult), outliers are shown as white circles

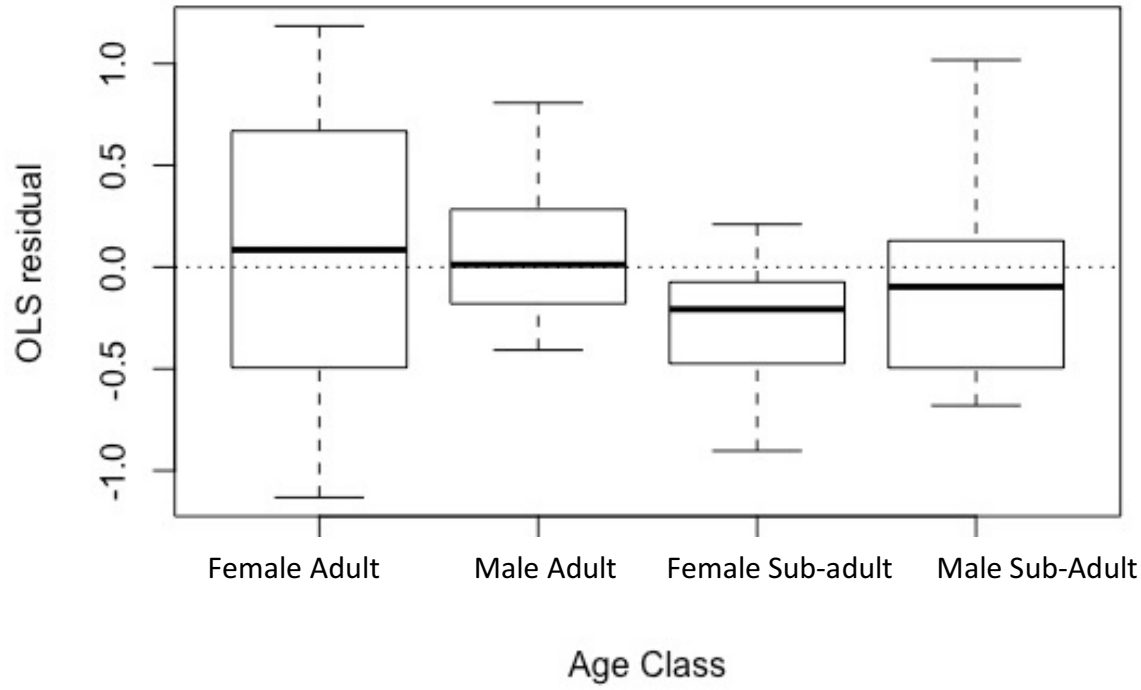

Figure 3.2: Box and whisker plot indicating median body condition indices based on Ordinary Least Squares (OLS) residuals for weight, based on a linear model of body length, chest girth and neck girth, for black-backed jackals (*Canis mesomelas*) from the Central Karoo, South Africa. Solid bold lines represent the median, boxes represent the interquartile range (IQR), whiskers are the reasonable limits of the data (1.5*IQR). Jackals are grouped by sex (male and female) and age class (adult and sub-adult)

Assessing body size and condition in caracals

A total of 27 caracals, consisting of 12 adults, 13 sub-adults and 2 juveniles were sampled in the Central Karoo. Sixteen individuals, including 11 adults and 5 sub-adults were sampled in Namaqualand, and a further 15 were sampled from the Cape Peninsula, of which 10 were adults and 5 were sub-adults. The total sample of caracals used in the morphometric analysis was 58 individuals, across all sites. The Central Karoo caracal sample had an even sex ratio ($\chi^2 = 0.93$; df = 1, p = 0.34) with a slight bias towards females. When comparing only adults there was parity in the sex ratio ($\chi^2 = 0.0$; df = 1, p = 1.0). Similarly, the Cape Peninsula population exhibited sex ratios that did not differ significantly from parity (all individuals: $\chi^2 = 1.67$; df = 1, p = 0.2; adults only: $\chi^2 = 0.4$; df = 1, p = 0.53). The Namaqualand sample was significantly male biased (3:1, $\chi^2 = 4.00$; df = 1, $p < 0.05$) but when comparing adults only the sex-ratio is approximately equal (1.75:1, $\chi^2 = 0.82$; df = 1, p = 0.37) (Table 3.4).

Table 3.4: Sex ratios of caracal (*Caracal caracal*) populations sampled in South Africa. Sample site, total number of caracals sampled, total number (and %) of males and females across all age classes, sex ratio and χ^2 test to determine whether sex ratio differs from parity

Site	All individuals				
	n	Male	Female	Ratio (M:F)	Chi-squared analysis
Central Karoo	27	11 (40.7%)	16 (59.3%)	0.69	$\chi^2 = 0.93$; df = 1, p = 0.34
Namaqualand	16	12 (75%)	4 (25%)	3	$\chi^2 = 4.00$; df = 1, p < 0.05
Cape Peninsula	15	10 (67%)	5 (33%)	2	$\chi^2 = 1.67$; df = 1, p = 0.2
			Adults Only		
	n	Male	Female	Ratio (M:F)	Chi-squared analysis
Central Karoo	12	6 (50%)	6 (50%)	1.0	$\chi^2 = 0.0$; df = 1, p = 1.0
Namaqualand	11	7 (64%)	4 (57%)	1.75	$\chi^2 = 0.82$; df = 1, p = 0.37
Cape Peninsula	10	6 (60%)	4 (40%)	1.5	$\chi^2 = 0.4$; df = 1, p = 0.53

The caracals sampled in this study appear to be morphologically similar to those in previous studies in southern Africa (Table 3.5) and the mean weight of caracal in the three study sites were not significantly different (Table 3.6). Body length of caracals is also similar to historical datasets and does not differ between study sites (Table 3.6). Chest girth and BMI also did not differ between sites (Table 3.6). With the exception of BMI males were on average longer, heavier and had bigger chest circumference than females. No combination of caracal sex and site of origin differed substantially from any other.

Table 3.5: Body measurements for adult caracals (*Caracal caracal*) in this study and for other studies in Southern Africa

a. Weight (kg)

Male			Female				
n	Range	mean	n	Range	mean	Reference	Region
46	8.6 - 20.0	14.53	32	8.6 – 14.5	10.98	(Pringle and Pringle, 1979)	Eastern Cape
61	8.0 – 18.1	12.7	40	7.0 - 15,9	10.1	(Stuart, 1982)	Southern Africa
6	10.4- 13.1	12	4	7.8 – 8.9	8.3	*This study*	Cape Peninsula
7	10.6 – 15.0	12.9	4	7.6 – 8.8	8.2	*This study*	Namaqualand
6	7.5 – 16.1	11.1	6	7.2 – 10.9	9.1	*This study*	Central Karoo

b. Head-Body length (mm)

Male			Female				
n	Range	mean	n	Range	mean	Reference	Region
65	750-1057	881	40	710 - 1029	834	(Stuart, 1982)	Eastern Cape
not specified	850 - 1080	870	not specified	710 - 1030	820	Smithers' Mammals of Southern Africa (Apps, 2008)	Southern Africa
6	762 - 888	849	3	755 - 830	797	*This study*	Cape Peninsula
7	656 -915	818	4	560 -810	682	*This study*	Namaqualand
6	771 - 925	824	6	715 - 857	772	*This study*	Central Karoo

Table 3.6: Morphometric variable statistical comparisons of the mean body length, weight, chest girth and Body Mass Index (BMI) for adult male and female caracals (*Caracal caracal*) in the different study sites. Significant differences at the p<0.05 level are indicated in bold

Morphometric variable	Sample sites	Sex	Sites + Sex
Body length (cm)	H=2.09, df = 2, p = 0.35	**W= 82.5, df = 1, p < 0.01**	$F_{(2,26)}$ = 0.945, p = 0.0412
Weight (kg)	F=0.46, df = 2, p = 0.64	**W= 18, df=1, p <0.001**	$F_{(2,27)}$ = 1.885, p = 0.1713
Chest girth (cm)	F=1.35, df = 2, p = 0.28	**T = -5.58, df = -27.75, p < 0.01**	$F_{(2,27)}$ = 0.493, p = 0.6163
BMI	H=2.67, df = 2, p = 0.26	W=82.9, df=1, p = 0.12	$F_{(2,26)}$ = 0.395, p = 0.6775

'H' denotes Kruskal-Wallis test for non-parametric data with more than two groups
'F' denotes F-test for parametric data with more than two groups;
'W' denotes Wilcoxon test for non-parametric data with only two groups
'T' denotes Students T-test for parametric data with only two samples

Both shoulder height ($t_{(30)}$=2.195, p =0.04) and chest girth ($t_{(30)}$=5.234, p < 0.001) were found to be important for predicting caracal weight. A model including these variables ($F_{(2,30)}$= 33.75, p<0.001) was selected as the best model because it had the lowest AIC among all the models tested (Table 3.7). This model explains 67% of the variation in caracal body weight (Adjusted R^2 = 0.9718; Multiple R^2 = 0.6923). The OLS residuals of this model (a proxy for caracal BCI) were similar across study sites ($F_{(2,30)}$= 0.37, p = 0.69). There was no effect of sex on BCI of caracals ($F_{(1,35)}$=1.39, p=0.25), nor was there any significant interaction between study site and sex ($F_{(2,30)}$=0.20, df=2, p=0.82) (Figure 3.3).

Table 3.7: The different models for predicting the Body Condition Index in caracals (*Caracal caracal*) sampled from multiple populations in South Africa. Model selection was based on the lowest Akaike Information Criterion (AIC) value

Model	AIC	ΔAIC	No. of parameters
Weight ~ shoulder height + chest girth	21.912	1.472	2
Weight ~ body length + shoulder height + chest girth	23.384	0	3
Weight ~ body length + chest girth	24.858	-1.474	2
Weight ~ body length + shoulder height	40.077	-16.693	2

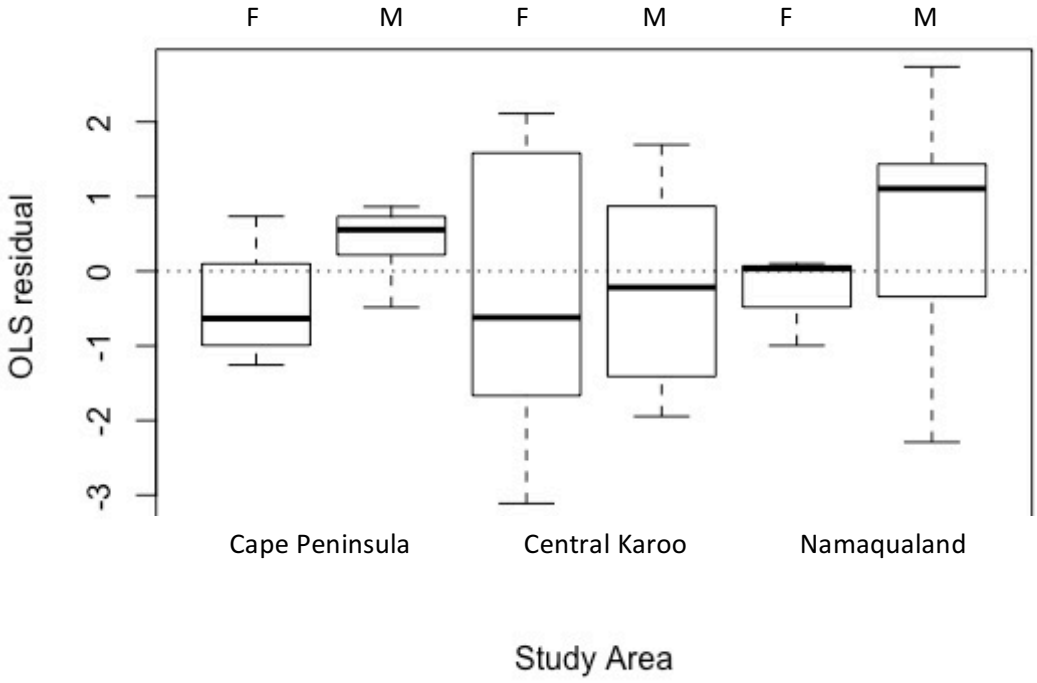

Figure 3.3: Boxplot indicating Ordinary Least Squares (OLS) residuals, used as a proxy for body condition, for populations of caracal in the Cape Peninsula, Central Karoo and Namaqualand. Sexes are separated within populations. Solid bold lines represent the median, boxes represent the interquartile range (IQR), whiskers are the reasonable limits of the data (1.5*IQR)

Tick diversity on black-backed jackals and caracals

A total of 96 ticks of the Ixodidae family were collected from 13 jackals and seven caracals from the Central Karoo, 11 caracals from Namaqualand and 13 caracals from the Cape Peninsula (Table 3.8). These included representatives of four genera (*Amblyomma*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus*) and at least six species (Table 3.9). Only three tick species were recorded for jackals sampled in the Central Karoo, all of which were common to caracal from the same area. These were *Amblyomma marmoreum*, *Haemaphysalis elliptica/zumpti* and *Ixodes rubicundus*. Central Karoo caracals were host to adult ticks of the *Haemophysalis elliptica/zumpti* group. Assignment to species level based on external morphology was not possible due to the morphological similarity among the members of this group (I. Horak *pers. comm.*). Adult females of *Ixodes rubicundus*, and the larvae and nymph stages of *Amblyomma marmoreum* were recorded. The larvae of *A. marmoreum* were the most common tick recorded on Central Karoo caracals (57% of caracals with attached ticks), while *Ixodes rubicundus* was more common on jackals.

Namaqualand caracals were also host to *Haemophysalis elliptica/zumpti* adults (although only females were observed), and adults of both sexes for *Ixodes rubicundus*. However, no *Amblyomma marmoreum* specimens were recorded and females of *Rhipicephalus gertrudae* were common. *I. rubicundus* was the most common tick (27% of all caracals) at this site. Tick species recorded on Cape Peninsula caracals had little overlap with those found in the Central Karoo, with *Haemophysalis elliptica/zumpti* being the only species common to all sites. Both sexes of adult *H. zumpti* were recorded on Cape Peninsula caracals. Unlike either the Central Karoo or Namaqualand caracals, the *Ixodes* species observed was not *I. rubicundus*, but rather *I. pilosis*, although only female specimens were recorded. Two species of *Rhipicephalus* were recorded, *R. gertrudae*, which was also observed on Namaqualand caracals, and *R. capensis* which was unique to the Cape Peninsula.

Table 3.8: Tick species found on caracals (*Caracal caracal*) from three study sites in South Africa, and the pathogens of which these ticks are vectors. Suspected, but unconfirmed, vector competence is indicated with an asterisk '*'

Dorsal image of tick	**Tick Species**	**Caracal host population(s)**	**Pathogens and/or disease associated with tick vector**
	*Amblyomma marmoreum* (larva)	Central Karoo	*Ehrlichia ruminantium* (causes Heartwater in runinants) (Jongejan and Uilenberg, 2004; Walker and Olwage, 1987)
	*Ixodes pilosis*	Cape Peninsula	unknown
	*Ixodes rubicundus*	Central Karoo & Namaqualand	Karoo paralysis disease (tick toxicosis) (Horak *et al.*, 1987)
	*Rhipicephalus capensis*	Cape Peninsula	unknown
	*Rhipicephalus gertrudae*	Namaqualand & Cape Peninsula	*Anaplasma marginale** (causes Gallsickness in ruminants) (Berggoetz *et al.*, 2014)
	*Haemaphysalis elliptica/zumpti*	Central Karoo, Namaqualand & Cape Peninsula	*Babesia rossi* *Babesia felis** (Horak et al., 2010; Solano-gallego and Baneth, 2011)

all photographs are from this study (Storme Viljoen)

Table 3.9: Species diversity of ticks collected from jackals (*Canis mesomelas*) and caracals (*Caracal caracal*) across three study sites in South Africa. Confidence intervals (95% CI) are calculated according to the Clopper-Pearson method

	Number of ticks collected				Abundance	Prevalence % (95 % CI)
	Immature	Male	Female	Total		
Central Karoo jackals (n=13)						
Amblyomma marmoreum	6			6	4	31 (9-61%)
Haemophysalis elliptica/zumpti		5	1	6	5	38 (14-68%)
Ixodes rubicundus		2	6	8	4	31 (9-61%)
Central Karoo caracals (n=7)						
Amblyomma marmoreum	13	0	0	13	4	57 (18-90%)
Haemophysalis elliptica/zumpti		2	2	4	3	43 (10-82%)
Haemophysalis zumpti		1		1	1	14 (0-58%)
Ixodes rubicundus			2	2	1	14 (0-58%)
Namaqualand caracals (n = 11)						
Haemophysalis elliptica/zumpti			3	3	3	27 (6-61%)
Ixodes rubicundus		2	22	24	3	27 (6-61%)
Rhipicephalus gertrudae			1	1	1	9 (0-41%)
Cape Peninsula caracals (n=13)						
Haemophysalis elliptica/zumpti			3	3	3	23 (5-54%)
Haemophysalis elliptica		2	1		3	23 (5-54%)
Haemophysalis zumpti		4	3		5	38 (14-68%)
Haemaphysalis sp.	1			1	1	8 (0-36%)
Ixodes pilosus			3	3	2	15 (2-45%)
Rhipicephalus gertrudae		1	2	3	1	8 (0-36%)
Rhipicephalus capensis		2	3	5	4	31 (9-61%)

Diversity and prevalence of tick-borne pathogens

Diversity and prevalence of TBPs was evaluated using a combination of detection techniques. Giemsa-stained blood slides were not of sufficiently good quality for pathogen identification, and ultimately, these were merely used as exploratory examination of potential prevalence rates.

Reverse Line Blot screening to determine pathogen prevalence

In total, 100 blood samples were screened for the presence of *Ehrlichia*, *Anaplasma*, *Theileria* and *Babesia* pathogens using a reverse line blot hybridization assay (Figure 3.4). Of the 100 samples, 43 were from jackals in the Central Karoo, 27 were caracals also originating from the Central Karoo, along with 14 from caracals in Namaqualand and 16 from caracals in the Cape Peninsula. Nineteen jackals (44%) tested positive for any of the TBPs examined, i.e. showed hybridisation to a specific probe (Figure 3.4). Six samples (14%) hybridised to the *Ehrlichia/Anaplasma* genus-specific probe but none to *Ehrlichia/Anaplasma* species-specific probes. Two samples (5%) hybridised to the genus-specific probes for *Theileria/Babesia*, and *Theileria*, and to the species-specific probes for *Thelieria* sp. (Sable) and *T. ovis*. Fourteen individuals (33%) hybridised to the *Babesia* 1 probe, while six of these (14%) also reacted to the *Babesia* 2 probe. No jackal samples hybridised to any of the *Babesia* species-specific probes.

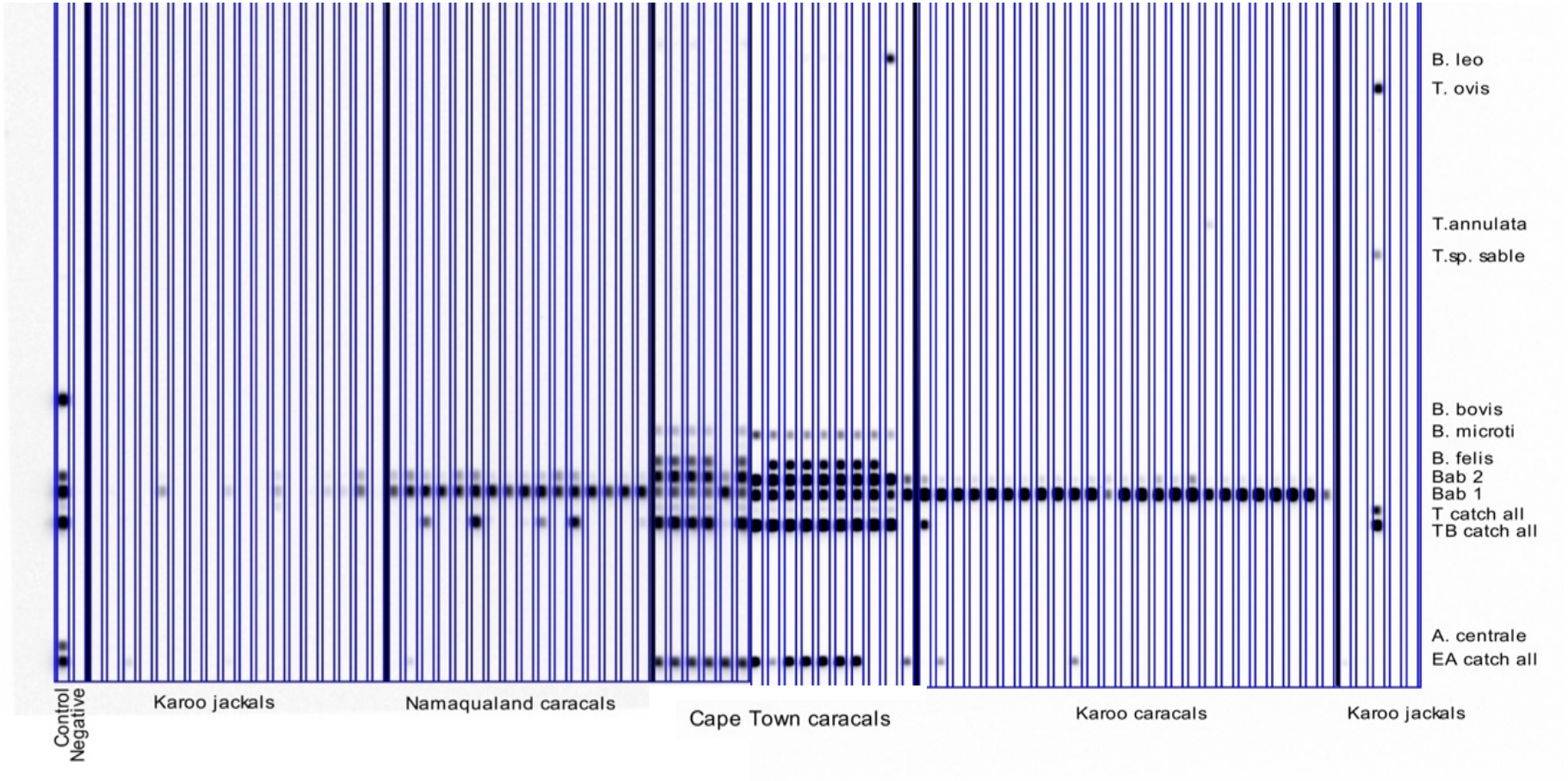

Figure 3.4: Reverse line blot assay of PCR amplificons from jackals (*Canis mesomelas*) and caracals (*Caracal caracal*) from three study sites in South Africa. Columns represent individual animals, while rows represent oligonucleotide probes. Probes for which there were positive results are indicated on the right-hand side

All caracals, from all three sites, tested positive for the *Babesia* 1 genus-specific probe, while 96% (n=55) also tested positive for the *Babesia* 2 probe (Table 3.10). Both Babesia 1 and Babesia 2 are specific only to genus level, but differ in the regions they target. 25% (n=14) of caracals had *Babesia* species-specific binding, all of which were from the Cape Peninsula. Two of the sixteen (12.5%) Cape Peninsula caracals indicated no *Babesia* species-specific binding. According to the RLB, hybridization to multiple species-specific probes, including *B. felis*, *B. microti* and *B. leo* occurred among Cape Peninsula caracals, with 9/16 individuals (56%) hybridising to all three, 3/16 (19%) hybridising to *B. felis* and *B. microti* and one individual (6%) hybridising to *B. microti* and *B. leo*. One caracal showed hybridization only to *B. microti*, which was not observed for either *B. felis* or *B. leo* (Figure 3.4).

Table 3.10: Prevalence of infection by tick-borne pathogens in jackals (*Canis mesomelas*) and caracals (*Caracal caracal*) living in the Central Karoo, as well as caracals from Namaqualand and the Cape Peninsula, South Africa. Confidence intervals (95% CI) are calculated according to the Clopper-Pearson method

	Prevalence (%) (95% CI)			
	Jackals	Caracals		
Tick-borne Pathogen	Central Karoo (n=43)	Central Karoo (n=27)	Namaqualand (n=14)	Cape Peninsula (n=16)
Positive	44 (40-71%)	100 (87-100%)	100 (77-100%)	100 (79-100%)
Catch-all probes				
Ehrlichia/Anaplasma sp.	14 (5-28%)	11 (2-29%)	0 (0-23%)	88 (62-98%)
Theileria/Babesia sp.	5 (0-16%)	4 (1-19%)	43 (18-71%)	88 (62-98%)
Theileria sp.	5 (0-16%)	0 (0-13%)	0 (0-23%)	31 (11-59%)
Babesia sp. 1	33 (19-49%)	100 (87-100%)	100 (77-100%)	100 (79-100%)
Babesia sp. 2	14 (5-28%)	93 (76-99%)	100 (77-100%)	100 (79-100%)
Species-specific binding				
Babesia felis	0 (0-8%)	0 (0-13%)	0 (0-23%)	75 (48-93%)
Babesia microti	0 (0-8%)	0 (0-13%)	0 (0-23%)	88 (62-98%)
Babesia leo	0 (0-8%)	0 (0-13%)	0 (0-23%)	63 (35-85%)
Theileria annulata	0 (0-8%)	4 (1-19%)	0 (0-23%)	0 (0-21%)
Theileria ovis	5 (0-16%)	0 (0-13%)	0 (0-23%)	0 (0-21%)
Theileria sp. (Sable)	5 (0-16%)	0 (0-13%)	0 (0-23%)	0 (0-21%)

Analysis of *Ehrlichia* and *Anaplasma* species in jackal and caracal populations

Based on the outcome of the RLB analysis, pathogen isolates were selected for sequencing and subjected to both BLAST and phylogenetic analysis in order to accurately determine their identity and genetic relationship to related species. Two of the jackal samples that reacted to the EA catch-all probe (JR8 and JR16) were sequenced using the fD1 and rP2 primers, which amplify a partial 16S rRNA gene region. When subjected to a BLASTn search in GenBank, these sequences identified as being most similar to *Clostridium* species. The sequence for JR16 has 99% similarity to *Clostridium perfringens* (KP944158.1). The other sequence, JR8, was most similar to *C. noyvi* from pigs (*Sus scrofa*) in Japan (AB857215.1) and to a *C. haemolyticum* clinical isolate (NR_113381.1).

According to the RLB assay, the prevalence of *Ehrlichia/Anaplasma* species in the Central Karoo caracals was similar to that of the jackals from the same area. However, according to BLASTn analysis, these were found all determined to be *Clostridium* species. Of the three *Ehrlichia/Anaplasma* positive sequences from the Central Karoo caracals, only one was successfully sequenced. A partial 16S rRNA sequence isolated from this caracal had 99% sequence similarity with *Clostridium perfringens* isolated from infected humans in China (KP944158.1, KP944156.1) (Figure 3.5). In all cases, neighbour joining tree topology was identical to that of the maximum likelihood (ML) trees, and thus only ML trees are presented. None of the Namaqualand caracals appear to be infected with any *Ehrlichia/Anaplasma* bacteria, while the Cape Peninsula caracals showed an 88% (CI: 62-98%) prevalence rate of infection of these species.

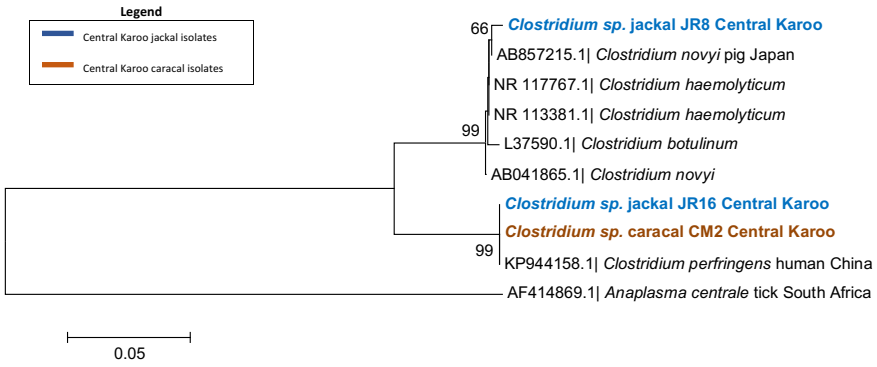

Figure 3.5: Maximum Likelihood phylogenetic tree of partial 16S rRNA gene sequence (860 bp) for parasites within the *Clostridium* genus. Sequences from this study are indicated in bold. Bootstrap values based on 1000 replicates are indicated at branch nodes. Substitution model used is a Tamura-3-parameter model with Gamma distribution (G=0.36). Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

Sequences from Cape Peninsula caracals identified with 99% similarity to an *Anaplasma phagocytophilum*-like species isolated from domestic dogs in South Africa (AY570539.1, AY570538.1) and from a Mongolian gazelle (*Procapra gutturosa*) in China (KM186950.1). Sequences amplified using universal primers for *Ehrlichia* and *Anaplasma* species (fD1 and rP2) are represented in a Maximum-Likelihood phylogenetic tree (Figure 3.6), rooted with *Wolbachia pipientis* (Inokuma *et al*., 2005). There are two distinct and well-support clades, each representing species from the *Anaplasma* and *Ehrlichia* genera respectively. None of sequences isolated in this study can be assigned to the genus, *Ehrlichia*. Within the *Anaplasma* clade, the erythrocytic *Anaplasma* species (*A. marginale*, *A. centrale* and *A. ovis*) are distinct from the leucocytic *Anaplasma* species (*A. phagocytophilum*, *A. platys* and *A. bovis*), which is in agreement with the accepted 16S rRNA phylogeny of the Anaplasmataceae (Inokuma *et al*., 2005; Tateno *et al*., 2013; Garcia-Perez *et al*., 2016). The *A. phagocytophilum* group contains sequences that are allocated to *A. phagocytophilum* and A*. phagocytophilum*-like species, such as sequences isolated from domestic dogs in South Africa (AY570538.1, AY570539.1, AY570540.1). Isolates from Cape Peninsula caracals cluster with '*Anaplasma* sp. Dog South Africa' sequences (89% bootstrap support).

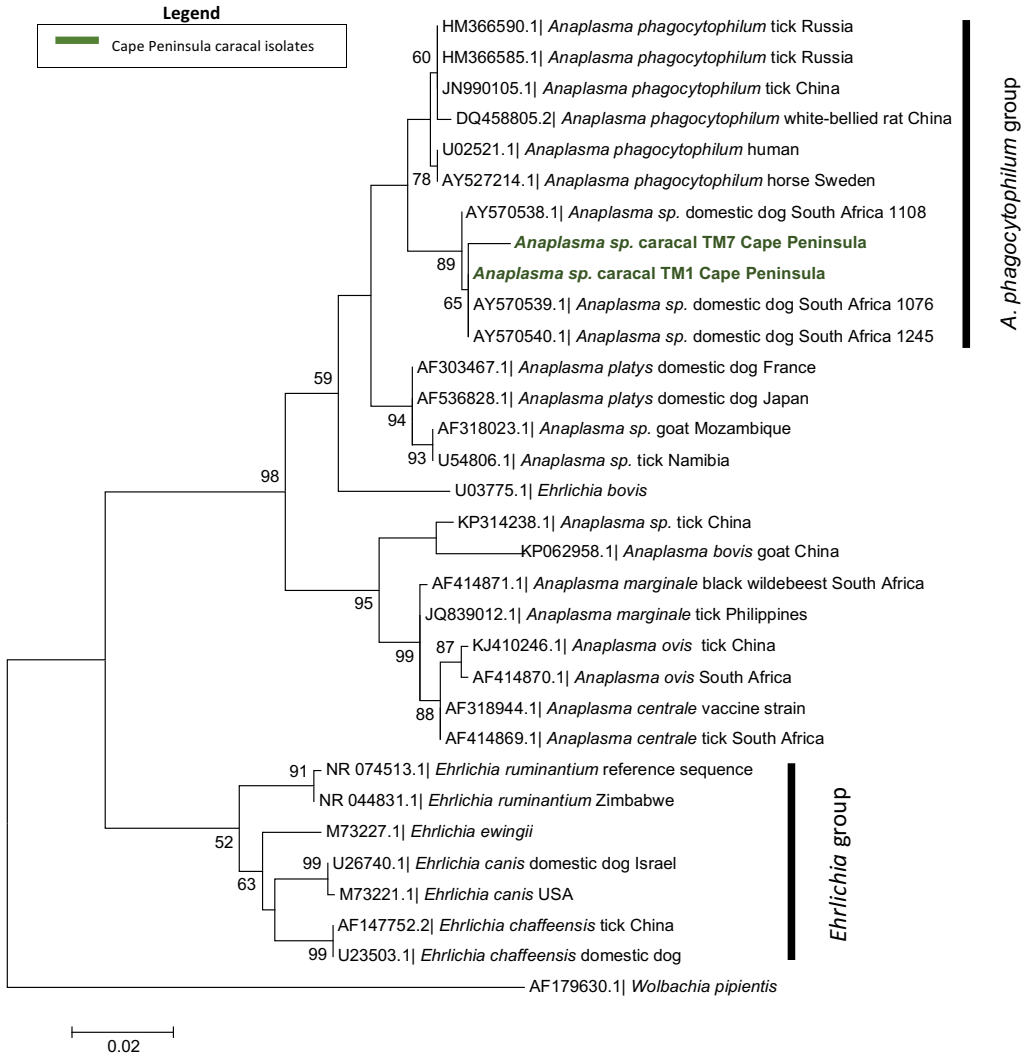

Figure 3.6: Maximum Likelihood phylogenetic tree of partial 16S rRNA gene sequence (851 bp) for parasites within the *Ehrlichia* and *Anaplasma* genera. Sequences from this study are indicated in bold. Bootstrap values based on 1000 replicates are indicated at branch nodes. Substitution model used is a Kimura-2-parameter model with Gamma distribution. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

Analysis of *Babesia, Theileria* and *Hepatozoon* species in jackal and caracal populations

Amplifications from five jackals were selected for sequencing. All five sequences were identified as 99-100% identical to *Hepatozoon canis* sequences isolated from a domestic dog in Sudan (DQ111754.1) and domestic cats (*Felis catus*) from Israel (KC138532.2, KC138531.2). Using the general *Babesia/Theileria* 18S rRNA primers, an additional PCR band was observed for two jackals (JR7 and JR8). These bands were also sequenced and identified as being most similar to the apicomplexan protozoan *Sarcocystis taeniata* (95% identity for a 99% query cover). According to the RLB, two jackals were positive for *Theileria ovis. T. ovis* isolates from jackals were 99% similar (only 89% query cover) to *T. ovis* from sheep in China (FJ603460.1) and Spain (AY533144.1), and to a Tanzanian goat (AY260173.1). Jackal isolates could only be successfully sequenced from one individual; these cluster in a strongly supported clade (97% bootstrap support) of *T. ovis* sequences (Figure 3.7)

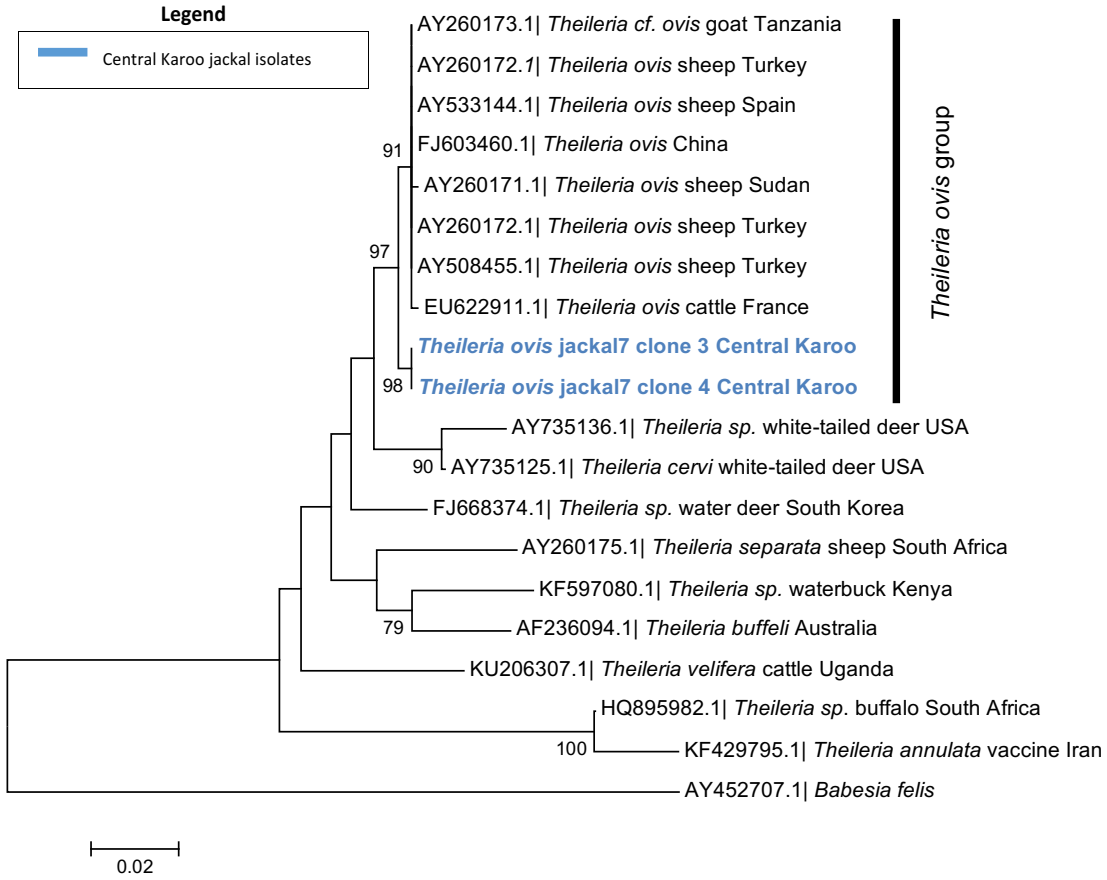

Figure 3.7: Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequence (748 bp) for parasites within the *Theileria* genus. Sequences from this study are indicated in bold. Bootstrap values based on 1000 replicates are indicated at branch nodes. Substitution model used is a Tamura-3-parameter model with Gamma distribution and invariant sites. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

In caracals from all three sites, partial 18S rRNA DNA sequences corresponded to *Hepatozoon felis*, with a 99-100% sequence identity when compared to existing GenBank sequences. The most similar sequences were from *Hepatozoon sp*. isolated from a lion in Zambia (KF270665.1; KF270668.1) and *H. felis* from domestic cats in Spain (AY628681.1.) and Israel (KC138533.1) and wild felids from India, including Indian leopards (*Panthera pardus fusca*) and the endangered Asiatic lion (*Panthera leo persica*) (HQ829439.1, HQ829438.1, HQ829444.1). Another *H. felis* sequence was obtained from a Namaqualand caracal and showed 99-100% sequence identity to *H. felis* isolated from various tick species infecting wild cats (*Prionailurus bengalensis euptilura*) in Japan (AB983435.1, AB983434.1, AB983420.1). Using the TB_RLB primers, a sequence identified as *H. felis* was isolated from a Cape Peninsula caracal. This sequence showed 99% sequence similarity to *H. felis* from domestic cats in Israel (KC138534.1, KC138533.1) and Spain (AY628681.1).

Hepatozoon phylogenies are often rooted with *Babesia* species (e.g. Metzger *et al.,* 2008; Giannitti *et al.,* 2012; Demoner *et al.,* 2016) and hence *Babesia felis* was selected as the outgroup. In the Maximum-Likelihood phylogenetic tree of *Hepatozoon* species (Figure 3.8), there is very strong bootstrap support for the *H. canis* clade (99%), with the Central Karoo jackal *Hepatozoon sp*. isolates clustering with *H. canis* from a domestic dog in Sudan (DQ111754.1). There are two distinctive clusters of *Hepatozoon sp*. from caracals in the present study. The *H. felis* clade contains both caracal clusters, but these are polyphyletic within this clade.

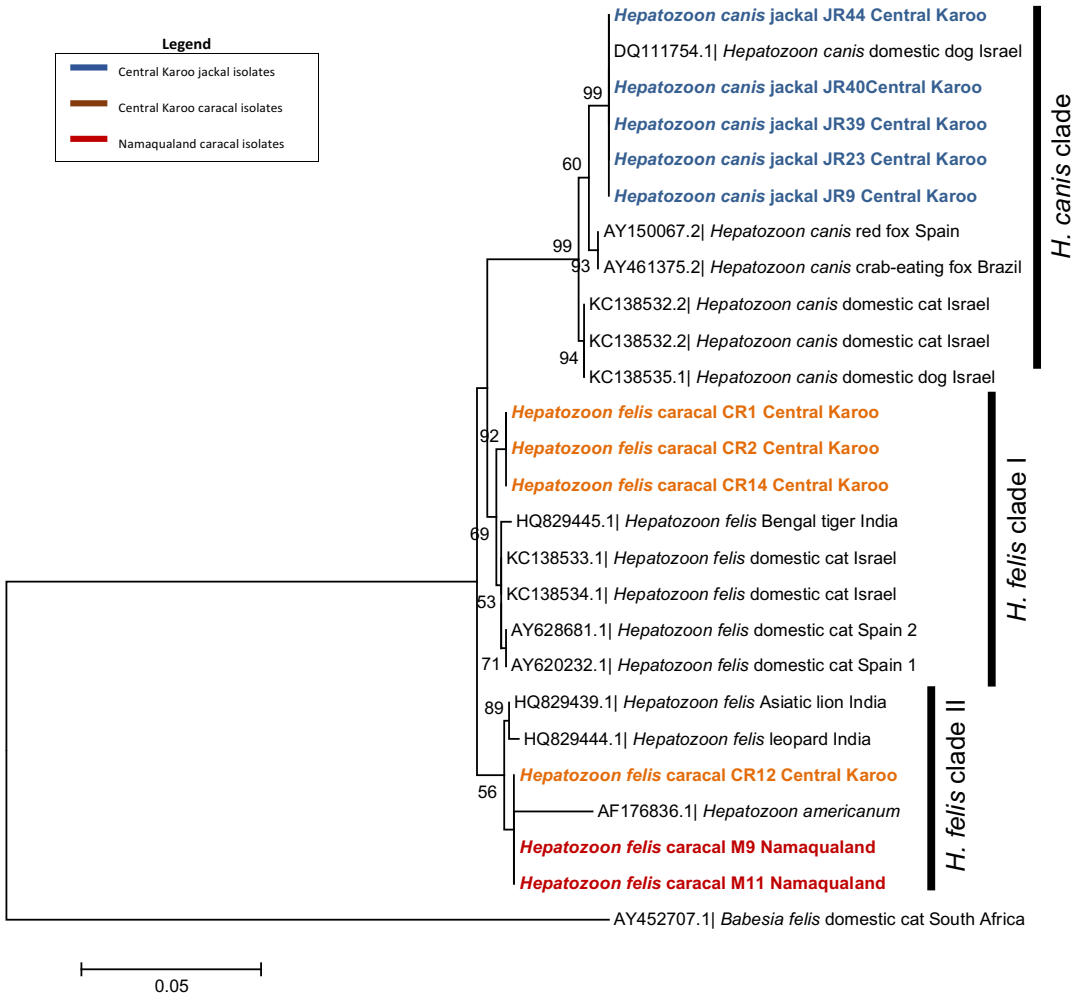

Figure 3.8: Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequence (863 bp) for parasites within the genus, *Hepatozoon*. Sequences from this study are shown in bold. Bootstrap values based on 1000 replicates and > 50% are indicated at branch nodes. Substitution model used is a Tamura-3-parameter model with Gamma distribution. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

When additional, shorter sequences from this study were included in the phylogeny (Figure 3.9), support for the branches decreased but the placement of many isolates remained unchanged. These additional sequences include an isolate from a Cape Peninsula caracal, which clusters with the majority of the Central Karoo caracal isolates. Sister to the *H. felis* group containing isolates from this study (*H. felis* I), is a clade containing *H. felis* sequences isolated from Asian felids and their ticks, as well as *Hepatozoon* species isolated from caracals in Namaqualand and from a Central Karoo caracal (*H. felis* II). These sequences cluster together (57% bootstrap support), and are sister to *H. americanum*. Once again, isolates from the Central Karoo jackals all cluster within the *H. canis* group.

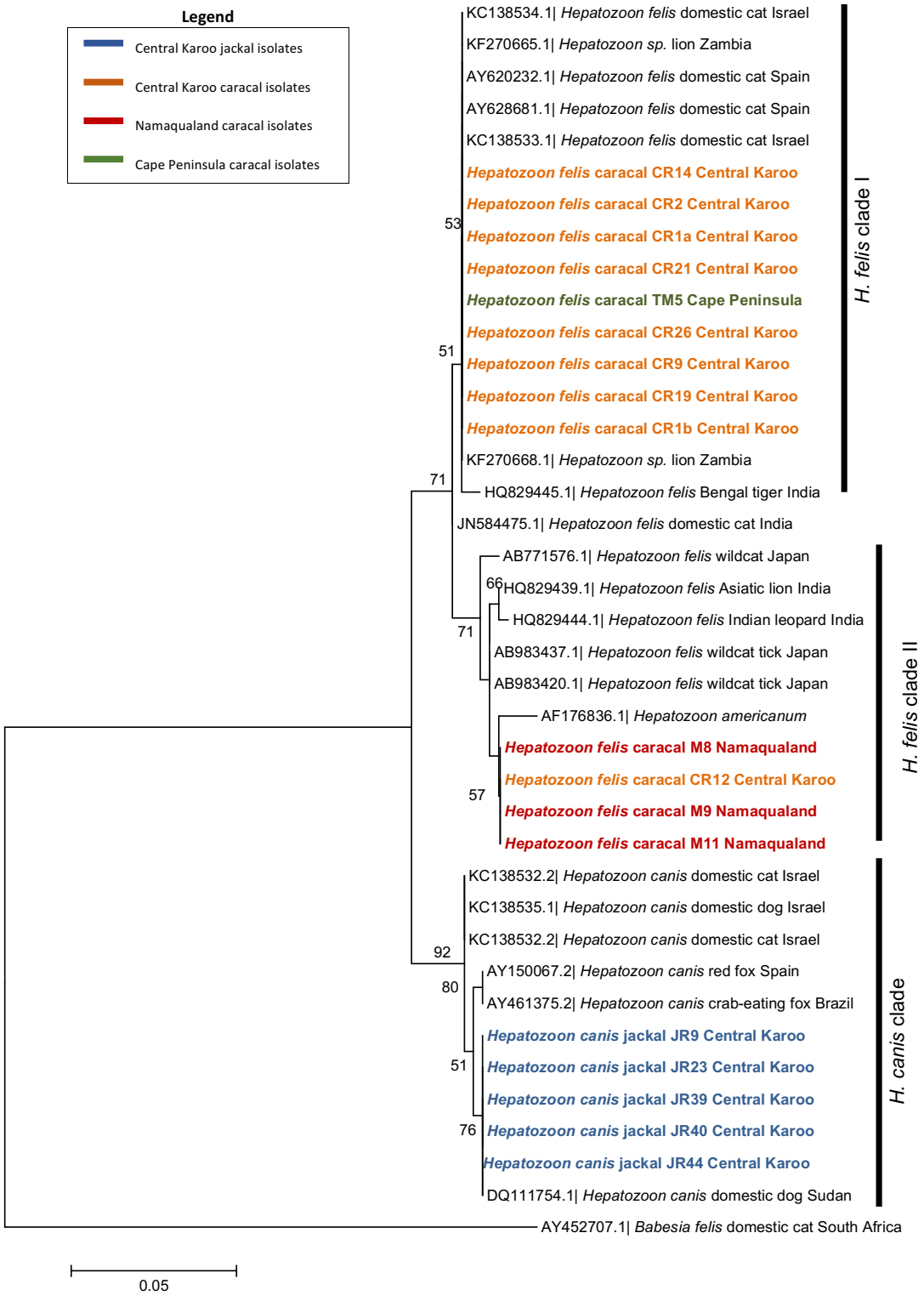

Figure 3.9: Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequence (440 bp) for parasites within the genus, *Hepatozoon*. Sequences from this study are shown in bold. Bootstrap values based on 1000 replicates and > 50% are indicated at branch nodes. Substitution model used is a Tamura-3-parameter model with Gamma distribution. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

Using both the RLB and conventional PCR, no *Babesia* species were found in any of the jackal sampled in this study. *Babesia* species were, however, found in all of the caracal populations. Using the Nbab/TB-1F primer set, which is the preferred primer set for phylogenetic comparisons as it targets a larger gene region, only one of the partial 18S rRNA DNA isolates from Central Karoo caracals could be amplified. The amplified sequence was most similar to the *Babesia* sp. BF472 sequence in the GenBank database (KC790442.1), for which no host information is provided. However, query cover is only 68% with 99% sequence identity. This Central Karoo caracal *Babesia* sp. was also similar (68% query cover, 95% identity) to a *B.* sp. isolated from a captive cheetah from Zimbabwe (KJ598882.1), and *B. venatorum* sequences from *Ixodes* ticks in Mongolia (LC005776.1). Using the Nbab/TB-1F primer set, sequences from two Namaqualand caracals showed 99% identity with *Babesia* sp. 472 (KC790442.1). These sequences are also highly similar to *B. venatorum*, isolated from humans in China (LC005775.1, KM244044.1), with a 99% sequence identity, although in all cases with these sequences, there is only a 90-91% query cover.

Cape peninsula caracals are infected with numerous *Babesia* species. *Babesia felis* was identified in two urban caracals, with sequences showing 98-99% sequence similarity with *Babesia* sp. identified in caracals from Kruger National Park, South Africa (AF244914.1, AF244913.1) and *B. felis* from a domestic cat in Port Elizabeth, South Africa (AY452699.1). *Babesia leo* was identified in three of the urban caracals, with 97-100% sequence similarity to *B. leo* from a lion in Kruger National Park (AF244911.1) and domestic cat in Port Elizabeth, South Africa (AY452708.1). However, for one of these sequences, the query cover was only 73%, and it had similarity with a *Babesia* sp. isolated from a Pampas cat (*Leopardus pajeros*) in Brazil (HQ187781.1). This sequence was isolated using the TB_RLB primers, which only target a ~500bp gene region, and not the Nbab primer set.

Phylogenetic trees for Babesia species are rooted on the apicomplexan *Cardiosporidium cionae* (Schnittger *et al*., 2012; Baneth *et al*., 2015). The *Babesia* sp. phylogeny, as estimated by Maximum-Likelihood (Figure 3.10), depicts a separation into three main clades. These are indicated as Clades I, II and III, and all have moderate to strong bootstrap support (>50%). Clade I contains isolates in the *Babesia felis*, *B. leo* and *B. microti/vulpes/rodhaini* groups. There is strong support for the independence of Clade I (94%). All of the *Babesia* isolates from the Cape Peninsula caracals are found within Clade I, with the majority assigned to the *B. felis* group. Also located with the *B. felis* group is one of the isolate clones from a Central Karoo caracal. None of the Namaqualand isolates were found with the *B. felis* group. While there is distinct and well-supported clustering of the *B. felis* group, there is fine structuring of the tree near the terminal nodes, suggesting some differences among isolates of

the *B. felis* species, although these have low bootstrap support. Isolates from urban and Central Karoo caracals fall within a distinct clade of *B. felis* (73% bootstrap support), while other urban caracal isolates are sister to this group. Urban caracal isolates cluster with the *B. felis* reference sequences from a lion in Kruger National Park, South Africa and a domestic cat from coastal South Africa (Port Elizabeth), and *Babesia* sp. isolated from a caracal. Also within Clade I, is the *B. leo* group, which contains several original isolates from the Cape Peninsula.

Clade II is a well-supported group (65%) containing the *Babesia sensu stricto* species (*B. ovis*, B. *canis*, *B. gibsoni*, *B. odocoilei*, *B. venatorum*). Within Clade II are sequences from Namaqualand caracals, which are very closely related to one another, but are distinct from the most closely identified species found in GenBank, viz., *B. venatorum* and *B. odocoilei*. Another clone from the Central Karoo caracal was also in Clade II, but was sister to the clade containing *B. gibsoni*, *B. canis* and *B. venatorum*.

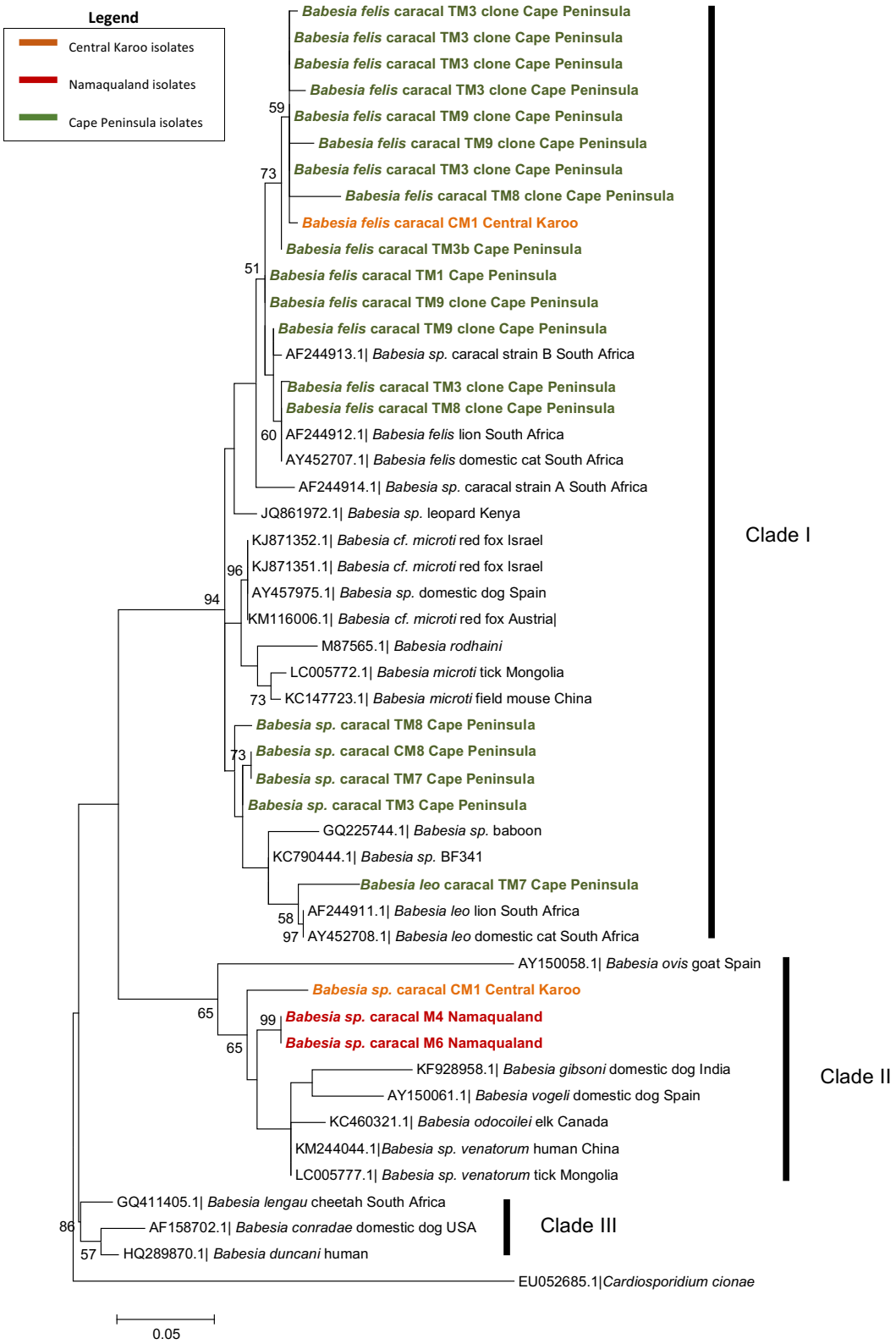

Figure 3.10: Maximum Likelihood phylogenetic tree of partial 18S rRNA gene sequence (446 bp) for parasites within the genus, *Babesia*. Sequences from this study are shown in bold. Substitution model used is a Kimura-2-parameter model with Gamma distribution. Evolutionary distance is determined using a scale bar representing the number of base substitutions per site

Pathogen prevalence in jackals and caracals

Based on the combined outcome of the RLB assay and phylogenetic analysis of pathogen identity, the prevalence of TBPs and incidental pathogen findings are presented in Appendix 3. Of the pathogens examined in this study, Central Karoo jackals are host to only two species, *Hepatozoon canis* and *Theileria ovis*, with *H. canis* observed at a prevalence rate of 46,5% (CI: 31.6-62.3%) and *T. ovis* being confirmed in 4.7% of jackals (CI: 0.6-15.8%). Incidental findings of *Clostridium* sp. were noted in 6 jackals (14%; CI: 5.3-27.9%). Additionally, an undescribed *Sarcocystis* sp. was observed in 2.3% (CI:0-12.3%) of jackals. Mixed infections were observed at a prevalence rate of 11.6% (CI: 3.9-25.1%).

In Central Karoo caracals, three TBPs were confirmed in the population. *Hepatozoon felis* was observed at a prevalence rate of 92.6% (CI:75.7-99.1%), *Babesia felis* at 7,4% (CI:0.9-24.3%) and an unknown *Babesia* sp. was observed in a single caracal (3,7%; CI:0-19%). As in the jackal population, *Clostridium* sp. (*C. perfringens* in one instance) were identified in Central Karoo caracals at a prevalence of 11.1% (CI:2.4-29.1%). Mixed infections were noted in 4 individuals (14.8%; CI:4.2-33.7%). Namaqualand caracals showed similar prevalence patterns, with *H. felis* observed in 85.7% (CI:57.2-98.2%) of individuals. However, the only *Babesia* species observed appears to be an unknown species, closely related to *B. venatorum*, and occurring at a prevalence rate of 14.3% (CI: 1.8-42.8%). *Clostridium* species were not seen in Namaqualand caracals, and no mixed infections were noted.

Pathogen prevalence in caracals from the Cape Peninsula contrasted with that of caracals from both the Central Karoo and Namaqualand. Urban caracals show evidence of infection with *H. felis*, *B. felis*, *B. leo* and an *Anaplasma phagocytophilum*-like species. Farmland caracal populations (Central Karoo and Namaqualand) are similar in their *H. felis* and *B. felis* prevalence rates, while the prevalence of *Hepatozoon felis* (6.3%; CI:0.2-30.2%) (Table 3.11) was lower for the Cape Peninsula caracals (χ^2 = 74.864, df = 2, p-value < 0,001). Prevalence rates of *B. felis* (75%; CI:47.6-92.7%) was higher in the Cape Peninsula versus the Central Karoo or Namaqualand caracal populations (χ^2 = 124,39, df = 2, p-value < 0,001). *B. leo* was only observed in the Cape Peninsula caracal, and was observed in 68% of the sampled population (CI:41.3-89%). The Cape Peninsula is the only population in which an *Ehrlichia* or *Anaplasma* species was observed, with *A. phagocytophilum*-like species, most similar to *Anaplasma* isolates from South African dogs, occurring in 14 caracals (87.5%; CI:61.6-98.4%). The rate of mixed infections was also higher in the Cape Peninsula caracals (χ^2 = 116.98, df = 2, p-value < 0.001), occurring in 81.3% (CI:54.4-96%) of the sampled population.

Table 3.11: Raw and adjusted (Benjamini-Hochberg corrected) p-values for Chi-squared test comparisons of tick-borne pathogen prevalence in caracal (*Caracal caracal*) populations from the Central Karoo (n=27), Namaqualand (n=14) and Cape Peninsula (n=16), South Africa. Significant (p<0.05) values are indicated in bold

	Hepatozoon felis		*Babesia felis*		Mixed infection	
Caracal populations	raw p	adjusted p	raw p	adjusted p	raw p	adjusted p
Central Karoo vs. Namaqualand	0.5956	0.5956	0.539	0.539	0.2802	0.2802
Central Karoo vs. Cape Peninsula	**0**	**0**	**0**	**0**	**0**	**0.0001**
Namaqualand vs. Cape Peninsula	**0**	**0**	**0**	**0**	**0**	**0**

Chapter 4. Discussion

This study represents the first assessment of the health in the two most commercially important mesocarnivores within farmlands of the Central Karoo region and is also the first study to assess health in caracals across different land uses and aridity in South Africa. Health in jackal and caracal populations was described using body condition indices, and by screening for tick-borne pathogens of veterinary relevance. By examining the diversity of host-attached ticks on jackal and caracal, the study also contributes to our current knowledge and understanding of potential tick vectors in human modified landscapes in southern Africa.

Morphometrics and body condition of mesocarnivores in human-modified landscapes

Both jackals and caracals were similar in size and weight to conspecifics in southern Africa (Rowe-Rowe, 1978; Pringle and Pringle, 1979; Stuart, 1981, 1982; Walton and Joly, 2003; Apps, 2008), suggesting that the individuals in this study are typical of their species, and that size and weight is unlikely to confound the interpretation of body condition or pathogen prevalence. There was no suggestion that body condition varied between sexes or age classes for jackals or caracals, or that caracal body condition varied with land use. Farmland jackals and caracals host similar tick communities, while urban caracals (from a more mesic climate) host greater tick diversity. Trends in tick-borne pathogen prevalence and diversity reflect a similar pattern, with the two farmland caracal populations being highly similar, while the urban caracals have a more distinctive pathogen profile.

Central Karoo jackals are morphologically similar to conspecifics in southern Africa

After controlling for sexual dimorphism, jackals sampled in this study are similar in weight and size to jackals sampled in other parts of the Karoo and other regions of southern Africa (Rowe-Rowe, 1978; Stuart, 1981). While the Karoo is naturally limited in surface water and wild prey biomass, the artificial provisioning of both groundwater and small domestic livestock might explain why Central Karoo jackals are not smaller than conspecifics from more mesic, productive regions of southern Africa.

The even sex ratio of jackals sampled in this study is was similar to previous findings on both farms and neighbouring protected areas in this region (Minnie *et al*., 2015), suggesting that living within a human-modified landscape characterised by extensive small-livestock farming and sustained human persecution has not disrupted key life history traits of jackals. While low productivity habitats, such as

arid areas, are expected to host animals with comparatively larger home ranges and smaller body size (the 'resource rule', McNab 2010), the transformations of these landscapes into small-stock farmland has essentially improved the productivity of the landscape in ways that would unintentionally benefit local wild carnivores. Investigations into body size of wild canids, mostly red foxes, living in transformed landscapes in other parts of the globe suggest that for adaptable species that are able to take advantage of the increased abundance of resources that humans bring, proximity to humans can lead to increased body size and weight (Cypher and Frost, 1999; Gortazar *et al*., 2000; Yom-Tov, 2003).

Although male jackals are larger than females, the body mass indices (BMIs) are similar for a given sex and age class. This suggests that outside of the breeding season, which for jackals is from late May to October (sampling took place in April) neither sex nor age class appears to affect their body condition. A similar trend was observed in free-ranging cheetahs from Namibian small stock farmlands, where no differences in BMI where observed between sexes for adult or sub-adult cheetahs (Marker and Dickman, 2003; Castro-Prieto, 2011). The findings, based on the OLS residuals method for determining body condition index, is in agreement with the findings using BMI as a proxy for condition: neither sex, age class or any interaction between sex and age class were found to affect body condition.

Caracals are morphologically consistent across land use types in South Africa

The caracals sampled in this study are morphologically similar to each other and to previous studies in other regions of southern Africa (Table 3.5; 3.6), suggesting that for caracals, there does not appear to be significant intraspecific variation in body size across sites of varying land use. In addition, both BMI and BCI values for caracals were similar for different sexes and study sites suggesting that neither land use nor aridity has a marked impact on the body condition of male or female caracals.

I had predicted that urban carnivores would be heavier and larger that their rural counterparts, similar to results for kit foxes (*Vulpes macrotis*) in California, and mesocarnivore species in other parts of the world (see Bateman and Fleming, 2012). However, the lack of body size variation in caracals living in different land uses has previously been noted by Yom-Tov (2003), who studies caracals in Israel over 50 years using museum specimens, and proposed that since caracals do not make use of anthropogenic food sources to the same degree as other urban adapted species (e.g. foxes), their size and mass is unlikely to change in response to their proximity to humans. The finding that urban caracals living in a more mesic region are not larger and heavier than those from semi-arid farmlands, indicates that farmland caracals might benefit from access to small livestock, or that there may be more negative consequences (such as pollution impacts or being forced to live in more marginal high

lying areas) associated with living in near urban spaces. Currently it is not possible to disentangle these confounding variables but for the purposes of comparing disease prevalence it is important to note that caracal from all three sites had a similar body condition.

Studies that examine the response of wildlife to urbanisation often focus on changes in life history and behaviour (Fuller *et al*., 2010; Magle *et al*., 2012), with very few focusing on either physiological responses or differences in pathogen prevalence. In the few studies undertaken to date, neither urban nor rural host populations show consistently higher pathogen prevalence rates. For example, Truyen *et al.* (1998) report no differences in the patterns of exposure to viral pathogens between rural and urban red foxes in Germany. However, another study on German red foxes reported a higher prevalence of Canine Distemper Virus (CDV) in urban foxes compared to those in rural areas (Frölich *et al.,* 2000). Urban grey foxes (*Urocyon cinereoargenteus*) and bobcats (*Lynx rufus*) in California, USA, also showed higher prevalence of exposure to CDV and feline calicivirus (FCV), respectively (Riley *et al*., 2004). However, the authors note that for the majority of pathogens for which they tested (including viruses, *Toxoplasma gondii* and *Bartonella henselae*) there was no association between urbanisation and exposure. In this study, caracals living in the urban matrix of the Cape Peninsula had a higher prevalence of *Babesia* species (*B. felis* and *B. leo*), as well as a higher incidence of co-infection by the TBPs. However, prevalence of *Hepatozoon felis* was significantly higher in the farmland caracal populations, indicating that differences in prevalence are pathogen specific and that co-infection is likely the more useful comparison.

Tick diversity on jackals and caracals

All three tick species found in the Central Karoo caracals, *Amblyomma marmoreum*, *Haemaphysalis elliptica/zumpti* and *Ixodes rubicundus*, were also found on the jackals, suggesting that Central Karoo jackals and caracals host similar tick communities. The tick species composition observed on farmland mesocarnivores suggests many of their ticks are generalist species (Espinaze *et al*., 2015), which could have important consequences for pathogen transmission in different host species. The similarity between the Central Karoo and Namaqualand is likely as a result of the similar climate and host communities that exist in both sites (Estrada-Peña, 2005).

Caracals from the Cape Peninsula site differ from farmland caracals in that they appear to host a higher tick diversity. Urban caracals also differed in their tick species composition, whereby *Ixodes rubicundus* is replaced with *I. pilosus*, and both *R. gertrudae* and *R. capensis* are present. The only tick species

common to all three sites, and also one of the most prevalent ticks found in this study is the *H. elliptica/zumpti* group. Horak *et al.* (2010) note that *H. elliptica* is the only tick of veterinary importance that is hosted by both domestic and wild felids. This tick is acknowledged as the vector for *Babesia rossi*, which causes Canine Babesiosis (Lewis *et al*., 1996), and is also suspected of being the vector for *B. felis* and *B. leo* (Horak *et al*., 2010), although this has yet to be demonstrated conclusively. The high prevalence of *B. felis* and *B. leo* in the Cape Peninsula caracal populations provides some support for this hypothesis, but should not be interpreted as evidence of the vector role of *H. elliptica*.

In sub-Saharan Africa, where tick-borne diseases are among the most important threats to livestock and domestic animals, very few tick species have been established as disease vectors as most vectors of TBPs remain unknown (Madder *et al*., 2013). Ticks can affect the health of the host in a myriad of ways. Certainly, blood-feeding by large numbers of ectoparasites can lead to considerable blood loss and anaemia and tick-induced immunosuppression has also been observed, particularly in agricultural landscapes where heavy tick burdens are known to cause production losses (Norval *et al*., 1989; Jonsson, 2006). Of the tick species recorded in this study, *I. rubicundus* is the causative agent of Karoo paralysis, a toxicosis that is caused by a toxin in the saliva of female *I. rubicundus* ticks. Paralysis usually starts with the legs, but is known to spread to the respiratory system and cause death, particularly in young lambs (Latif and Walker, 2004). Peak times for observing paralysis in livestock is in April and May (the sampling period for this study), when it is likely that tick burdens may be highest.

Other than causing biting stress associated with high tick burdens*, Ixodes pilosus*, *Amblyomma marmoreum* and *Rhipicephalus capensis* are not known to transmit any notable pathogens or be responsible for any severe toxicosis. *Rhipicephalus gertrudae* has recently been reported to harbour the rickettsial pathogens, *Anaplasma centrale* and *A. marginale* (Berggoetz *et al*., 2014), both of which are economically important in the livestock industry, and may play a vector role in the transmission of A*. marginale*, the causative agent of gallsickness in cattle.

Below I provide an overview of each of the tick species reported from jackals and caracals in this study, noting tick life cycle, known hosts and relevance for pathogen transmission.

Amblyomma marmoreum

This tick species was observed in Central Karoo caracals and jackals, but was absent from the caracal populations sampled in Namaqualand and the Cape Peninsula. This is a three-host tick, and only the larval and nymph stages were found on Central Karoo predators, which corroborate the findings of

Horak *et al.* (2006), who note that no adult *A. marmoreum* ticks are found on carnivore or herbivore mammalian hosts. *Amblyomma marmoreum* ticks are most commonly found on leopard tortoises, *Stigmochelys paradalis*, (Horak *et al*., 2006), hence their being referred to as the 'South African tortoise tick' (Theiler and Salisbury, 1959). However, while leopard tortoises and other reptiles are the host for adult ticks, the larval and nymph stages may infest other species, including scrub hares, ground bird, domestic and smaller wild ruminants and both domestic and wild carnivores (Horak *et al.,* 1987; Horak *et al.,* 2006; Horak *et al.,* 2010). From work done by Horak *et al.* (1987; 2000), wild felid species, including lions, cheetahs, leopards, domestic cats and caracals appear to be important hosts for the immature stages of *A. marmoreum*.

In the literature on *A. marmoreum*, caracals feature strongly as hosts, but this is likely due to disproportionate sampling of the species as a result of culling for predator control in South Africa over many decades (Beinart, 1998). Black-backed jackals have also been previously noted as hosts of immature ticks, having a 100% prevalence in the animals (n=8) sampled by (Horak *et al*., 2006). The presence of this tick appears contingent on the presence of tortoises and other large reptiles, as these are required as hosts for adult ticks and thus the completion of the tick life cycle (Horak *et al*., 2006). *A. marmoreum* ticks have been found as far south as Stellenbosh and Franschhoek, in the Western Cape, where they were observed on domestic dogs in shelters (Horak and Matthee, 2003).

Ixodes rubicundus

Adult ticks of *Ixodes rubicundus* were found in jackals and caracals from the Central Karoo, as well as caracals from Namaqualand. Commonly known as the 'Karoo paralysis tick', the female adults of this species produce salivary toxins that cause localised paralysis in their hosts. *Ixodes rubicundus* is a three-host tick that has a range limited to South Africa. *Ixodes rubicundus* infests a wide range of host species, with immature stages infesting red rock rabbits (*Pronolagus randensis*) and elephant shrews, and adults being found on goats, sheep, cattle, wild bovids/ungulates and wild felids (Stampa, 1959; Horak *et al.,* 1987; Fourie *et al.,* 1996; Fourie *et al.,* 2005). In particular, caracals are notable hosts for adult ticks, but can also be infested by the immature stages (Horak *et al.,* 1987). Caracals are able to support the entire life cycle of *Ixodes rubicundus* without the presence of any other host species. Domestic dogs and black-backed jackals from the Northern and Western Cape have also been recorded as hosts (Horak and Matthee, 2003; Horak *et al*., 2010; Matthee *et al*., 2010).

Ixodes pilosus

Ixodes pilosus, like *I. rubicundus*, is a three-host tick. This species occurs in the Mediterranean and Savanna climates of South Africa, which may limit its distribution in the semi-arid Karoo and Namaqualand. In warmer regions, the activity of *I. pilosus* is year-round, but is limited to the spring and summer seasons in the cooler coastal regions (Theiler, 1950). Referred to as "The Sourveld tick", this species has a geographic distribution that includes the sourveld region, characterised by coarse, grassy vegetation along the south and south-east parts of South Africa (Theiler, 1950; Horak *et al*., 1987). In this study, *Ixodes pilosus* was only observed on the Cape Peninsula caracals. However, Horak *et al.* (1987) had previously recorded this tick species on caracals in the Eastern Cape, South Africa. This tick species had a wide host range, including rodents and lagomorphs during the immature stages, and antelopes, cattle, domestic dogs and caracals during the adult stages (Horak *et al*., 1987). *Ixodes pilosus* is commonly found on both domestic cats and caracals (Horak *et al.,* 1987; Horak and Matthee, 2003; Horak *et al.,* 2010), suggesting that these may be among the preferred host species for this tick. Black-backed jackals have also been recorded as hosts of *I. pilosus* (Cumming, 1999).

Rhipicephalus gertrudae

Rhipicephalus gertrudae was found in the Namaqualand and Cape Peninsula caracals, as well as the Central Karoo jackals and the single tick from a Namaqualand jackal was identified as *R. gertrudae*. The absence of *R. gertrudae* in the Central Karoo caracals seems to have been a sampling artefact, as the range of this tick species extends over all three study areas, and both caracals and jackals are suitable hosts. Prevalence of this tick was lowest compared to prevalence rates of other tick species observed in this study. This species is only known from South Africa and Namibia (Walker *et al*., 2000). *Rhipicephalus gertrudae* shares many of its preferred hosts with *R. simus*, which it replaces in the Western Cape (Walker *et al*., 2000). It also replaces the brown dog tick, *R. sanguineus*, in the western parts of the Western and Northern Cape (Walker *et al*., 2000). Unlike *R. sanguineus*, which has a very specific host range that is largely limited to domestic dogs, *R. gertrudae* infests a wide variety of hosts, as well as domestic dogs. Immature ticks prefer murid rodents as hosts (Walker *et al*. 2000), while adult stages have a host range that includes sheep, horses, cattle, primates and wild carnivores (Walker *et al*., 2000; Horak *et al*., 2002;). Adult ticks are also known to use humans as hosts (Walker *et al*., 2000). Sheep and domestic dogs are common hosts of *R. gertrudae* in the winter rainfall regions of South Africa (Horak and Fourie, 1992; Horak and Matthee, 2003), such as the Cape Peninsula and Namaqualand study site. Domestic cats may also be infested with *R. gertrudae* (Horak and Matthee, 2003). However, *I. pilosus* and *Haemophysalis elliptica* are more prevalent in the cats on which *R. gertrudae* has been observed (Horak and Matthee, 2003).

Rhipicephalus capensis

The second species of *Rhipicephalus* ticks observed in this study, *R. capensis*, was only observed in the Cape Peninsula caracals. Unlike *R. gertrudae*, *R. capensis* was observed at a relatively high prevalence (31%). Comparatively little is known about this tick species, most likely as a result of its narrow geographical distribution, which is limited to a band along the west coast of the Western and Northern Cape of South Africa (Walker, 1991). This tick has only been recorded once in the Eastern Cape, South Africa, but this is likely to be incidental and not part of the species range of this tick (Walker *et al*., 2000).

There is some confusion among taxonomists working with *R. capensis*, as many have used the term "*R. capensis*-like" when describing this species. Walker (1991) notes that this group includes *R. capensis sensu stricto*, and *R. follis sensu stricto* and *R. gertrudae*. Knowledge of hosts of *R. capensis* is limited, but is known to include cattle and horses, Cape Mountain zebra and eland and gemsbok (*Oryx gazella*). Single records of ticks exist for a Cape fox (Walker *et al*., 2000) and leopard (Horak *et al*., 2010).

Haemaphysalis elliptica/zumpti

Many of the samples collected in this study could only be identified to the level of the *H. elliptica/zumpti* group, although several of the Cape Peninsula specimens could be assigned to species level. This group of ticks was common to all three study sites and was found in both caracals and jackals in the Central Karoo. Prevalence rates for *H. elliptica/zumpti* were among the highest in all host populations examined. This is in agreement with the finding of Horak *et al*., (2010), who observed that *H. elliptica* is the most abundant tick species infesting domestic and wild felids. *H. elliptica*, commonly referred to as the 'yellow dog tick', is one of the most commonly observed ticks on domestic dogs in South Africa and Mozambique (De Matos *et al*., 2008; Horak *et al*., 2010; Matthee *et al*., 2010). It is also a prevalent tick, with the highest abundance, on domestic cats in the Western Cape, South Africa (Horak *et al*., 2000; Horak and Matthee, 2003). Large wild felids are often observed as being infested with *H. elliptica* in South Africa (Horak *et al*., 1987; Horak *et al*., 2000).

Immature stages appear to prefer murid rodents as hosts (Norval 1984; Matthee *et al.,* 2007), but adult stages prefer to utilise other species (Apanaskevich *et al*., 2007), including wild felids, and both wild and domestic canids (Norval, 1984; Horak *et al*., 1987; Horak *et al*., 2000). *Haemaphysalis elliptica* is also found on sheep and cattle, but this is suggested to be most likely as a result of proximity to

domestic dogs (Walker *et al*., 2014). This tick is the only species, other than *R. sanguineous*, to be adapted to parasiting dogs in sub-Saharan Africa (Walker *et al*., 2014).

Haemaphysalis zumpti is morphologically and ecologically similar to *H. elliptica*, with the notable exception that it does not occur on the larger felid species, instead preferring smaller carnivores as hosts (Walker, 1991; Horak *et al*., 2010). This species has also been recorded on domestic dogs in South Africa (Horak and Matthee, 2003; Matthee *et al*., 2010), and both *H. elliptica* and *H. zumpti* have been recorded on caracals (Horak *et al*., 2010).

Blood pathogen diversity and prevalence

This study represents the first molecular survey of tick-borne pathogens in jackals and caracals from farmland and urban areas in South Africa. Jackals in the Central Karoo do not appear to suffer from extensive infection with TBPs of veterinary or public health concern, as the majority of jackals did not show infection with any of the TBPs examined. Using a combination of methods, only two tick-borne pathogens were detected in jackals, *Hepatozoon canis* and *Theileria ovis*. While *H. canis* has previously been reported in jackals from South Africa, based on blood smears (McCully *et al*., 1975), this is the first molecular confirmation of *H. canis* in jackals from anywhere in their range. Furthermore, while *T. ovis* has previously been isolated from a domestic dog in Nigeria (Kamani *et al*., 2013) this is the first report of *T. ovis* in any wild canid worldwide.

In Central Karoo farmlands, it is clear that jackals support a lower prevalence of TBPs in comparison with sympatric caracals. Caracals in the Central Karoo had a 100% infection rate with at least one TBP, a finding common to all three caracal populations examined in this study. Despite sharing many of the same tick species, it might be that jackals are simply less susceptible to infection with TBPs. This is contrary to the findings of Riley *et al*. (2004), who found that grey foxes had higher levels of pathogen exposure than their felid (bobcat) counterparts. These authors speculated that this difference in exposure and reported number of epidemics for canids and felids (see Murray *et al*., 1999), is due to canids generally being more social than felids, which with the exception of lions, tend to be solitary. Thus, felids would be less likely to be exposed to infectious agents spread through direct contact although the spread of tick-borne pathogens is less likely to be as strongly influenced by the sociality of their vertebrate hosts.

By contrast, neither *H. canis*, nor *T. ovis* were detected in Central Karoo caracals suggesting that while these species share the same environment, including tick communities, they appear to have distinct epidemiological roles. The only TBPs observed in the Central Karoo caracal population were *Hepatozoon felis* and two *Babesia* species, *B. felis* and an unknown *Babesia*, most similar to *B. venatorum*. This is the first report of *B. venatorum* in caracals, and indeed in any wild species in Africa and it has recently been considered an emerging zoonosis (Yabsley and Shock, 2013; Jiang *et al*., 2015;). As in the jackals, Central Karoo caracals do not support a wide diversity of TBPs, and the vast majority of the high overall prevalence rate in this population is attributed to widespread infection with *H. felis*. The caracal population in Namaqualand revealed a very similar TBP profile to the Central Karoo population. Given that these populations share many of the same tick species, similar climates

and host communities, it follows that they should be exposed to the same pathogens and exhibit similar prevalence rates.

In these semi-arid jackal and caracal populations, species of the genus *Hepatozoon* are the dominant TBP circulating, although distinct species were found in jackals and caracals without exception. At least two strains of *H. felis* were identified in caracals, one being found exclusively in the Central Karoo and, based on phylogenetic analysis, very similar to *H. felis* isolated from domestic and wild felids across the globe, e.g. lions in Zambia, tigers (*Panthera tigris*) in India and domestic cats from Spain and Israel (Figure 3.8; 3.9). The second *Hepatozoon* strain identified in this study occurred in both the Central Karoo and Namaqualand. Based on phylogenetic analysis, this strain appears to be distinctive from those previously reported for *H. felis*.

Peninsula caracals had very low prevalence of *Hepatozoon* species relative to Namaqualand and Karoo caracals but a greater overall prevalence and diversity of TBPs. Similar to caracals in farmlands, urban caracals have 100% rate of infection with at least one TBP. However, they also demonstrate considerably greater incidence of multiple TBP co-infections. Of particular interest is the finding that Cape Peninsula caracal are infected with Anaplasmataceae, specifically *Anaplasma* sp. SA dog, a TBP similar to *A. phagocytophilum*, but which has only been described once before in a South African domestic dog (Inokuma *et al*., 2005). *Anaplasma phagocytophilum* is known to occur in other domestic animals and wildlife, including wild ruminants, reptiles, rodents and birds (see Stuen *et al*., 2013 for an extensive list of hosts). Having this unusual *Anaplasma* species common to both domestic dogs and caracals in South Africa suggests a possible epidemiological link where these species occur together.

In addition to *Anaplasma* sp. SA dog, Cape Peninsula caracals were also host to *Babesia felis* and *Babesia leo*, which have both previously been reported occurring in caracals (Penzhorn *et al*., 2001). *Hepatozoon felis* was detected in all three caracal populations. To my knowledge, this is the first report of *H. felis* in caracals. Unlike their semi-arid farmland counterparts, urban caracals have a low prevalence of *H. felis,* while prevalence rates of *Babesia* sp. were much higher in urban caracals. Phylogenetic analysis of the *Babesia* species circulating in urban caracals suggests there is a large degree of *B. felis* diversity represented in the Cape Peninsula's caracal population, possibly indicating multiple infections. Given that *B. felis* is described in domestic cats, it follows that being sympatric with domestic cats in the Cape Peninsula could be an important factor determining the prevalence and diversity of *Babesia* infection in caracals.

None of the samples that were positive for *Ehrlichia/Anaplasma* based on the RLB, were found to be *Ehrlichia* species. This finding is not unexpected, as a number of studies that use molecular techniques to detect blood parasites in carnivores report similar findings (Filoni *et al*., 2006; Williams *et al*., 2014). Possible reasons for this could be that *Ehrlichia* parasites only occur at very low prevalence and that the samples size of most studies, including this one, are too low to detect its presence in the population.

Detection of Theileria ovis in farmland jackals

Species in the genus *Theileria* are notable parasites of livestock species throughout the old world (Bishop *et al*., 2004). Many diseases, including East Coast Fever, Corridor Disease (both caused by infection with *T. parva*) and Tropical/Mediterranean Theileriosis (*T. annulata*) are caused by *Theileria* species. *Theileria ovis*, as the name suggests, is commonly found in domestic sheep, but is considered to be non-pathogenic (Razmi *et al*., 2003; Bishop *et al*., 2004) and therefore of little economic relevance to the livestock managers. Its presence in jackals demonstrates an interesting potential epidemiological connection within this system, and suggests that ticks are able to spread pathogens (and thus disease) between these species. This has important implications for livestock management if a pathogenic strain were introduced to small stock populations, as jackals could act as reservoirs or amplifying hosts for these pathogens, and act to undermine disease management efforts if the aim of such efforts was disease eradication. Additionally, novel pathogens in small stock hosts could also be transmitted to immune-naïve wildlife populations (e.g. spillover of *Mycobacterium bovis* to wildlife species, (Palmer *et al*., 2012)). This phenomenon is becoming increasingly important to understand as rapid landscape and climate change are driving changes in disease risk and infectious disease ecology (Altizer *et al*., 2013).

In caracals, two individuals hybridised to the *Theileria* specific probe, with only one instance where a caracal sample indicated species-specific binding for *Theileria*. A caracal from the Central Karoo hybridised to the *T. annulata* probe, however, this finding could not be confirmed with sequencing. The species range of *T. annulata* does not extend into the southern African countries (Jongejan and Uilenberg, 2004), and thus this finding is most likely a cross-reaction.

Detection of multiple Babesia species in caracals

The use of conventional sequencing of samples using the BTF1/BTR2 primers, which are universal primers for *Babesia*, *Theileria* and *Hepatozoon* species, indicated that prevalence rates in caracal varied between 71-85% across the three study sites. However, the difference in prevalence between caracal populations was not statistically significant. The higher prevalence values observed in the RLB are likely due to the sensitivity of the method, specifically its ability to detect the presence of pathogens at low concentrations (Xiong *et al*., 2006; Kong and Gilbert, 2007; Gimenez *et al*., 2009). Sequencing of *Babesia/Theileria* isolates indicated that caracals were infected with *Hepatozoon felis*, *Babesia felis*, *Babesia leo* and a *Babesia* species that was most similar to *B. venatorum*. In the case of the sample that was identified on the RLB assay as *B. microti* (TMC07), sequencing suggests that this isolate represents *B. leo* and highlights the possibility of cross-reactivity of species-specific probes on the RLB and the risk of non-detection of new genetic variants.

Incidental findings: Detection of Hepatozoon, Clostridium and Sarcocystis species in jackals and caracals

Three species of *Babesia* were detected in caracals using the RLB, *B. felis*, *B. microti* and *B. leo*. Based on hybridisation to the *Babesia* 1 probe, prevalence rates across all three sites was 100% for *Babesia* species in caracals. According to the *Babesia* 2 probe, this rate was only 96%, with only 93% of the Central Karoo caracals hybridising to the probe, as opposed to 100% in both Namaqualand and the Cape Peninsula populations. This discrepancy is likely indicative of variation in the nucleotide sequences amplified, which bind to the *Babesia* 1 probe, but not to *Babesia* 2. Although the *Babesia* catch-all probes on the RLB are meant to be specific for *Babesia* species, sequencing of PCR amplicons revealed that some of these were, in fact, *Hepatozoon felis*. This suggests that while the RLB probe is sensitive to detection of *Babesia* species, it is not sufficiently specific to exclude the binding of other closely related Apicomplexans, such as *Hepatozoon* species, and highlights the need for confirmation sequencing following screening techniques.

The *Hepatozoon* species identified in the Central Karoo jackals and caracals are 99-100% similar to known *Hepatozoon* species found in multiple canid and felid species, both domestic and wild, across multiple continents. This trend was also observed by Pawar *et al.* (2012) who investigated *Hepatozoon* species in wild and domestic carnivores in India. As in Pawar *et al.* (2012), *H. felis* was found exclusively in the felid species, while *H. canis* was only found in the canid species. However, these *Hepatozoon* species have previously been reported in the other host group (i.e. *H.canis* found in a domestic cat)

(Criado-Fornelio *et al*., 2009). For wildlife species, it is certainly far more common to observe the *Hepatozoon* species being restricted to one or the other vertebrate host families.

The detection of *Hepatozoon* species when using PCR primers that are designed for *Babesia* species is not uncommon, and has previously been noted by other authors (e.g. Silaghi *et al*., 2012 in bank voles (*Myodes glareolus*) in Germany). Silaghi *et al*. (2012) also report the presence of *Sarcocystis* species in the Eurasian common shrew (*Sorex araneus*) based on PCRs using *Babesia* specific primers. This finding is in line with the incidental finding of *Sarcocystis* species from the blood of two black-backed jackals. *Sarcocystis* is another closely-related protozoan parasite within the phylum, Apicomplexa. Although not vector-borne pathogens, *Sarcocystis* are still pathogens of concern for livestock managers and as obligate two-host parasites whose life cycle depends on both a herbivore intermediate host and a carnivore definitive host (Dubey *et al*., 1989 from Chhabra and Samantaray, 2013) are relevant when examining diseases that move across the HWL interface.

In wildlife species, carnivores including lions and wild dogs have been reported to have very high prevalence of infection (up to 100%) with *Sarcocystis* oocysts during faecal examination (Bjork *et al*., 2000; Flacke *et al*., 2010). *Sarcocystis* is clearly a ubiquitous parasite that is able to infect a great diversity of hosts, and has been highlighted as a new emerging pathogen in humans in Malaysia (Thompson, 2013; Latif and Muslim, 2016). *Sarcocystis* species have a global distribution, although individual species may be limited to specific areas. Often, *Sarcocystis* species have very specific host requirements and cannot be maintained even among closely related intermediate hosts. The close association between intermediate hosts (herbivores, such as sheep, cattle, giraffe (*Giraffa camelopardalis*)) and definitive hosts (carnivores, including domestic dogs and cats, mustelids and humans) are often highly specific. For example, *Sarcocystis capracanis* circulated between sheep and goats, and dogs or red foxes. The *Sarcocystis* species that was found in black-backed jackals did not show similarity above 97% to any existing sequences in GenBank. According to conventional practice (Stackebrandt and Goebel, 1994; Konstantinidis and Tiedje, 2005), this implies that an undescribed *Sarcocystis* species could be circulating in this population.

The phylogenetic analysis indicated that the strain of *Sarcocystis* circulating in jackal were most similar to *S. taeniata*, and *S. tenella*, both dog-transmitted species that infect sheep at very high prevalence rates (Erber, 1982; Prakas *et al*., 2016). It may therefore be likely that this new strain is one which is shared between small stock species and jackals. Further investigation into the identity and

epidemiology of this parasite is required to better understand the epidemiological link between black-backed jackals and domestic animals.

Isolates amplified using Ehrlichia/Anaplasma primers revealed to be Clostridium species

Despite the use of *Ehrlichia* and *Anaplasma* specific PCR primers, isolates from jackals were most similar to *Clostridium* species and not the target pathogens. Based on phylogenetic analysis, there are at least two species of *Clostridium, C. perfringens* and *C. novyi,* circulating in the Central Karoo jackal population. Neither *Ehrlichia* nor *Anaplasma* species were detected in jackals from this study. *Clostridium* species observed are gram-positive anaerobic bacteria and are considered important pathogens in humans and domestic animals. Their importance as pathogens in wildlife populations has received limited attention to date (e.g. free-ranging black bear (*Ursus americanus*), Barnes and Rogers (1980); Siberian tiger (*Panthera tigris altaica*) and a lion, Zhang *et al*. (2012)).

Clostridial species are soil-borne bacteria that comprise a normal part of the enteric microflora of healthy mammals (Jores *et al*., 2008). Disease occurs when toxigenic types proliferate in the gut; often as a result of changes in host factors such as altered diet, environment and when the host sustains external wounds. Virulent, toxogenic clostridial strains produce toxins that cause enteric disease. In domestic and wildlife species, the effects of these toxins manifest as diarrhoea, enterotoxaemia and haemorrhagic gastroenteritis (Hirsh and Biberstein, 2005). Although *C. perfringens* is widely considered as the most important of the pathogenic clostridial enterobacteria, *C. novyi* is of particular importance in environments like the Central Karoo, because farming land-use and an arid climate both promote the emergence of this soil-borne disease (Seifert *et al*., 1996). *Clostridium novyi* type B also interacts with the parasitic trematode liver fluke (*Fasciola hepatica*) to cause necrotic hepatitis ("Black disease") in sheep. Many farmers in the Central Karoo vaccinate against *C. novyi* type B (*pers. obs*.), which makes its presence in the jackal population particularly interesting as this could indicate that jackals are able to maintain the pathogens and contribute to its presence in the environment through the deposition of scat, which contains bacterial spores.

While these pathogens are ubiquitous in the environment, occurring in soil during their spore phase, the sampling of sterile heart blood from carcasses would have reduced the chance of environmental contamination. Clostridial infections are typically diagnosed from faeces or intestinal tissue, where this bacterium usually proliferates. However, the sequences analysed in this study were obtained from DNA extracted from blood, and *Clostridium* sp. were also apparent in the blood slides (Appendix 2). Severe bacteraemia observed on the blood slides also suggests that *Clostridium* was likely to be

present in the circulating blood and not introduced port-mortem. Finally, the *C. perfringens* isolate from Central Karoo caracal was highly similar to that isolated from jackal (Figure 3.5), suggesting that this *Clostridium* strain could be a generalist pathogen among mesocarnivores in the Central Karoo farmlands.

Evaluation of molecular methods to detect blood pathogens

The identities of Apicomplexan isolates from caracal blood based on subsequent sequencing and phylogenetic analysis suggest that the results from studies using only RLB methods should be interpreted with caution. These should always be confirmed with direct sequencing of the amplifications using the same set of primers as used in the initial RLB amplification, as well as appropriate primer sets that are more specific, and are able to amplify longer sequences that will have greater phylogenetic power. For example, the TB-RLB primers, which were used in the initial RLB amplification in this study, only amplify a short sequence (~500 bp) and are designed to be universal. While it is expected that these primers were able to amplify numerous *Babesia* species, it was surprising that they also amplified *Hepatozoon felis* from a Cape Peninsula caracal. The BTF1/BTR2 primers were also able to detect both *Babesia* species and *Hepatozoon felis*, but yielded longer sequences (~800bp) compared to the TB-RLB primers. However, the NBab primer set, which are the longer sequencing primers (~1200+ bp) appear to be more specific as these were only able to amplify *Babesia* species.

In order to confidently determine the identity, prevalence and diversity of Apicomplexan blood pathogens, a multi-step approach is required. While the RLB is a widely-used, sensitive and efficient method for screening for large numbers of samples for relevant pathogens, it should only be used as the first stage of pathogen surveillance. All positive results based on RLB need to be confirmed with direct sequencing, using multiple primer sets and cloning techniques where necessary, in order to determine the true identity of pathogen isolates.

The results of this study indicate that the "catch-all" probes used in this RLB protocol detect a greater diversity of genera than expected. In the case of the *Ehrlichia/Anaplasma* probes, species from the genus, *Clostridium*, were also detected in the Central Karoo jackal and caracal populations. Similarly, for the *Theileria/Babesia* probes, *Hepatozoon* species were detected. However, *Clostridium* and *Hepatozoon* species are closely related to the genera that the probes propose to target, and thus while RLB results should always be interpreted with caution, they are still valuable for pathogen surveillance.

Interestingly, the species-specific probes displayed instances of cross-reactivity, and thereby misdiagnosis of pathogen identity. This finding is perhaps more problematic, as false positive results are difficult to confidently identify when using PCR amplification and sequences. This is because one cannot always determine whether the reason for a lack of amplification of a species is due to its absence, a low parasitaemia, or a PCR that is not adequately optimized for that species (i.e. mismatched primers or sub-optimal annealing temperatures). In these cases, further investigation, using highly specific primers is the logical way forward.

Implications of findings

The findings presented here contribute to the growing body of work on tick-borne pathogens infecting wild carnivores, and specifically to our knowledge of carnivores living in human-modified landscapes. Encouragingly, the field of wildlife pathogen epidemiology is growing, as evidenced by the increasing number of recent scientific papers published on this topic (Kelly *et al*., 2014; Williams *et al*., 2014; Zanet *et al*., 2014; Eygelaar *et al*., 2015; Otranto *et al*., 2015; Alvarado-Rybak *et al*., 2016; Garcia-Perez *et al*., 2016; Khatri-Chhetri *et al*., 2016; Millán *et al*., 2016; Zhang *et al*., 2016). Like many of these studies, the findings presented here represent an exploratory investigation into pathogen diversity and implications for the disease dynamics of a system. Baseline data on pathogen diversity and prevalence is lacking worldwide, even in the systems, such as the human-wildlife-livestock interface, where this knowledge is clearly of economic value. This study is among the more comprehensive investigations on vector-borne pathogens in South Africa undertaken to date. For human-modified landscapes, including urbanized spaces, small-stock farmland and protected area boundaries, it exemplifies the type of work that is increasingly being recognized as critical for the quantification of human impacts on these dynamic ecosystems.

Future research directions

The scope of this investigation greatly benefitted from collaboration with ongoing research projects, where morphological data, ectoparasites and blood samples are routinely collected. Continued collection of routine data and samples is beneficial for collaborative research, however, this is only possible when standardized protocols are applied. Increased emphasis needs to be placed on these 'best practice' methods for which there needs to be consensus in the literature.

For morphometric measures, there is generally consensus for which measurements need to be taken, but less often on exactly how to take these measurements. Subtle differences in measurement

technique may significantly influence the outcome of morphometric comparisons across datasets. Additionally, measurement error is seldom addressed. This error could be partially mitigated by taking the average of multiple values obtained for the same measurement. When handling dangerous animals under sedation, the minimization of time taken to collect samples and data is critical. This aspect of data collection from wildlife needs to be balanced against the desire for useful and accurate data.

Another related issue is how to measure body condition in wildlife species. While it is reassuring that both the ratio and residual proxies for body condition showed similar trends in both jackals and caracals, the degree to which these can be used as reliable and comparable measures of body condition is unclear. Ideally, these and other body condition indices need to be regressed with total body fat if one is to extrapolate to the general condition of the animal. In addition, the lack of a baseline population against which the condition of individuals in this population can be compared makes it difficult to contextualize the findings relative to other populations. Although debate around these techniques has been ongoing, there needs to be consensus on the criteria that researchers should use when selecting the most appropriate methods for the species under investigations (Warton *et al*., 2006; Peig and Green, 2010). This will aid greatly in generating comparative datasets for various taxa. For carcasses, it appears as though the Kidney Fat Index (KFI) is a very good method of estimating nutritional status of an animal (Cavallini, 1996; Eira *et al*., 2006; Minnie *et al*., 2015), and could be used as a direct measure of body condition.

Going forward, studies similar to this one should make use of the KFI when carcasses are available. Furthermore, as long as the persecution of animals is legally taking place, it would be remiss to not make use of the opportunity to collect biological data. The use of material resulting from ongoing block hunts in the Central Karoo has proven extremely valuable in this investigation into the epidemiology of pathogens circulating in wild carnivore populations. These could still be used in other projects which require biological material, including genetic, isotopic, morphometric physiological questions. Body condition data from this study should be used in comparison with jackal populations from other parts of their range in order to examine whether differences in land-use and proximity to humans and domestic dogs affects jackal body condition. This would answer a novel question which has not yet been examined despite the commercial and epidemiological importance of jackals and as farmland pests and disease reservoirs.

Unfortunately, while there was not enough variation in pathogen prevalence rates (zero-inflated data for most pathogens) to examine drivers of infection, it is possible that by acquiring larger sample sizes of host individuals, or by examining additional pathogens, that a Generalized Linear Model (GLM) framework could be employed (e.g. Cottrell, 2011; Duarte *et al*., 2013). The use of logistic regression modelling would be useful is attempting to answer specific questions relating to drivers of pathogen prevalence, which would certainly improve our understanding of epidemiology and disease dynamics.

One of the important next steps in this research is to examine the pathogen communities in the actual ticks found on the individuals whose blood was screened. Pathogen DNA can be extracted from whole ticks, and can thus be used as an indicator of which pathogens these ticks could transmit. It is important to note that finding a pathogen inside a tick is not conclusive evidence of vector ability, as a tick could have ingested infected blood, but not be able to maintain the pathogen life cycle (Millán *et al*., 2016). Thus, while knowledge of which pathogens are present in sympatric ticks is critical when investigating vector ecology, it is merely a first step that should be followed up with experimentation (e.g. Matsumoto *et al*., 2005).

The diversity of pathogen species observed in this relatively small host sample size suggests that there is considerable unexplored diversity in wildlife blood pathogens in these study areas. This new diversity will contribute to the global dataset of genetic sequences for pathogens, and aid in the interpretation of the phylogenetic relationships among these pathogen taxa. Further work needs to be done in characterising these pathogen species, specifically in identifying their morphological characteristics and any variation within these, as well as additional molecular characterization.

Other useful insights into disease dynamics could come from the reporting of clinical disease symptoms in wildlife. These reports could then be used to direct research objectives and aid in the investigation of co-infection dynamics. Identifying clinical disease in wildlife, during periods of endemic stability of a pathogen (i.e. not during periods of disease epidemics), is extremely challenging. Farmers or reserve managers who are more likely to observe wildlife species need to be engaged in reporting their observations. In this way, ecologists and epidemiologists should partner with State Veterinary bodies in order to collaborate on creating research priorities and pursuing a health monitoring agenda. This effort should consider a One Health framework, which facilitates co-operation among the various stake-holders in human, animal and environmental health.

Conclusions

Despite recognition of the important role of pathogens as components of biodiversity and in the conservation of ecosystem integrity, the study of pathogens in wildlife populations is often neglected (Thompson *et al*., 2010). This work contributes to the existing literature on TBPs in wild canids worldwide and to that of health in black-backed jackals in South Africa. A sheep pathogen, *Theileria ovis*, was found in jackals from small-stock farmland, demonstrating that tick borne pathogens may be shared between wildlife and livestock. Similarly, the presence of *Hepatozoon canis* in jackals suggests that they could be a reservoir population for *H. canis* to infect domestic dogs that occur in the area. Based on differences in pathogen prevalence, jackals appear to be less susceptible to infection with TBPs, compared to sympatric caracals, despite sharing many of the same tick species. Possible reasons for this require further investigation.

Caracal populations from arid farmlands are similar in shape and body condition to those living in a mesic, urban environment. In comparison with farmland caracal populations, urban caracals are host to a greater diversity of tick species, suffer from a greater incidence of co-infection with TBPs and exhibit high prevalence rates of TBP genotypes that are known to be pathogenic in domestic animals (e.g. *Babesia felis*, *Anaplasma phagocytophilum*-like). Finding *A. phagocytophilum*-like pathogens (*A. sp* Dog South Africa) suggests that urban caracals are sharing pathogens with the domestic dog population, with the implication that both carnivore populations are vulnerable to changes in the health of the other, with both potentially acting as pathogen reservoirs.

Finally, the findings of this study highlight the paucity of knowledge that exists on tick-borne pathogens in wildlife populations in southern Africa. This work presents numerous examples of hitherto unknown pathogen diversity present in jackals and caracals, and demonstrates the potential for mesocarnivores in human-modified landscapes to have epidemiological links to sympatric species, including economically important domestic species, as well as humans. The extent of the influence that land transformation and human impacts have on health in wild carnivores in South Africa remains to be seen, but extensive screening of relevant pathogens is certainly the first step in improving regional understanding of wildlife disease ecology within the One Health framework.

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Appendices

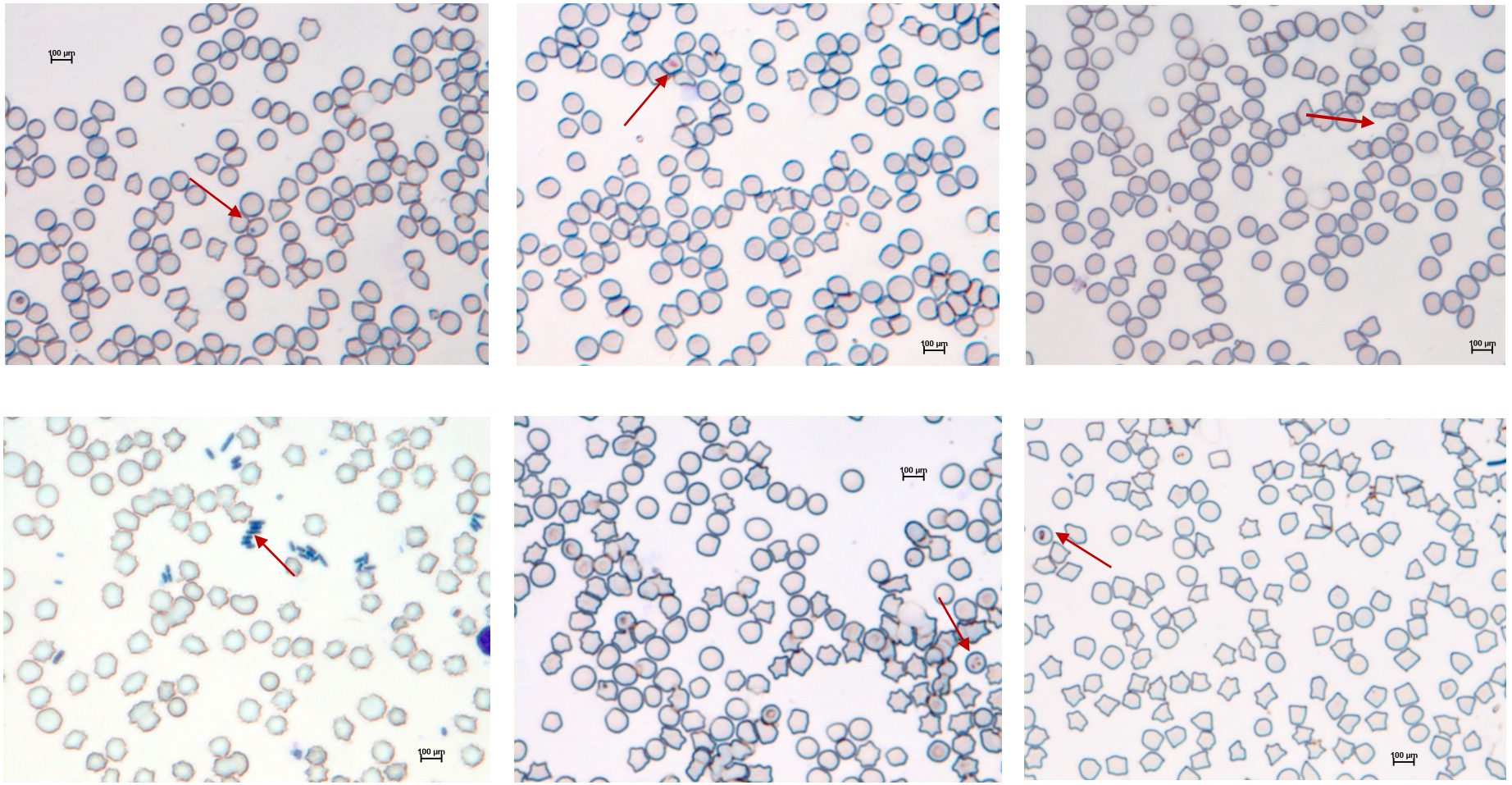
Appendix 1.1: The mean and standard deviation for select morphometric variables for all adult jackals (*Canis mesomelas*) from Central Karoo, separated according to sex. Students T-test with test statistic and probability values for comparison between adult males and females are presented

Variable	All Adults	Males (n=17)	Female (n=14)	t (df)	p-value
Weight (kg)	7,35 ± 0,94	7,8 ± 0,6	6,9 ± 1,0	2,92 (29)	p = 0.007
Total length (cm)	113,4 ± 52,7	116,8 ± 2,6	109,2 ± 4,7	5,38 (19)	p < 0.001[W]
Body length (cm)	75,9 ± 4,1	78,2 ± 2,7	73,0 ± 3,7	4,60 (29)	p < 0.001
Tail length (cm)	37,5 ± 2,0	38,5 ± 1,4	36,3 ± 2,0	3,75 (29)	p < 0.001
Shoulder height (cm)	48,4 ± 2,3	49,5 ± 1,7	46,9 ± 2,0	3,93 (29)	p < 0.001
Chest girth (cm)	42,1 ± 2,4	43,0 ± 2,5	40,9 ± 1,8	2,63 (29)	p = 0.013
Neck girth (cm)	26,4 ± 2,3	26,9 ± 2,0	25,8 ± 2,6	1,27 (27)	p = 0.216
Body Mass Index	12,8 ± 1,3	12,7 ± 0,9	12,9 ± 1,7	-0.42 (19)	p = 0.6778[W]

[W] denoted where a Welch correction was used to account for unequal variances

Appendix 1.2: Body size measurements and calculations for adult caracals (*Caracal caracal*) from the Cape Peninsula, Central Karoo and Namaqualand, South Africa. Values represent Mean ± standard deviation

	Cape Peninsula	Central Karoo	Namaqualand
	mean ± sd	mean ± sd	mean ± sd
All Adults	n = 10	n = 12	n = 11
Body length (cm)	831.67 ± 48.2	798.08 ± 56.83	768.18 ± 130.74
Weight (kg)	10.49 ± 2.08	10.12 ± 2.37	11.16 ± 2.63
Shoulder height (cm)	463.8 ± 48.4	466.08 ± 20.91	438.5 ± 63.1
Chest girth (cm)	395.7 ± 41.2	394.25 ± 55.71	423.1 ± 52.6
Body Mass Index	15.43 ± 2.36	15.79 ± 2.51	19.73 ± 5.74
Adult male	n = 6	n = 6	n = 7
Body length (cm)	848.83 ± 45.45	824.33 ± 52.27	817.71 ± 105.93
Weight (kg)	11.96 ± 1.1	11.13 ± 2.89	12.86 ± 1.47
Shoulder height (cm)	482.17 ± 44.63	473.67 ± 27.78	475.00 ± 45.78
Chest girth (cm)	425.17 ± 15.43	421.83 ± 45.83	454.29 ± 35.4
Body Mass Index	16.63 ± 1.38	16.15 ±2.35	19.94 ± 4.95
Adult female	n = 4	n = 6	n = 4
Body length (cm)	797.33 ± 38.42	771.83 ± 52.15	681.50 ± 137.1
Weight (kg)	8.29 ± 0.48	9.1 ± 1.24	8.2 ± 0.59
Shoulder height (cm)	436.25 ± 44.98	458.5 ± 7.23	374.5 ± 22.61
Chest girth (cm)	351.5 ± 19.12	366.67 ± 53.87	368.5 ± 22.11
Body Mass Index	13.04 ± 2.16	15.43 ± 2.83	19.35 ± 7.78



Appendix 2.1: Giemsa-stained blood smears from caracals (*Caracal caracal*) viewed under oil immersion at x100 magnification. Red arrows indicate the presence of blood pathogens

Appendix 3.3: Pathogen screening results for Caracals (*Caracal caracal*) from the Central Karoo region, South Africa. Highlighted samples are those that have been confirmed by direct sequencing, followed by BLAST analysis and phylogenetic placement

Individual	Sex	Age class	*Hepatozoon felis*	*Babesia felis*	*Babesia leo*	*Babesia sp.* Unknown	*Anaplasma sp.*	*Clostridium sp.*	Mixed Infection
CRTB1	M	Adult	1	0	0	0	0	0	0
CRTB2	F	Adult	1	0	0	0	0	1	1
CTRB3	F	Adult	1	0	0	0	0	0	0
CRTB4	F	Juvenile	1	0	0	0	0	0	0
CRTB6	F	Juvenile	1	0	0	0	0	0	0
CRTB7	M	Sub-adult	1	0	0	0	0	0	0
CRTB8	M	Sub-adult	1	0	0	0	0	0	0
CRTB9	F	Adult	1	0	0	0	0	0	0
CRTB10	M	Adult	1	0	0	0	0	0	0
CRTB11	F	Adult	1	0	0	0	0	1	1
CRTB12	M	Adult	1	0	0	0	0	0	0
CRTB13	F	Adult	1	0	0	0	0	0	0
CRTB14	M	Adult	1	0	0	0	0	0	0
CRTB15	F	Adult	1	0	0	0	0	0	0
CRTB16	F	Sub-adult	1	0	0	0	0	0	0
CRTB17	F	Sub-adult	1	0	0	0	0	0	0
CRTB18	M	Adult	1	0	0	0	0	0	0
CRTB19	M	Sub-adult	1	0	0	0	0	0	0
CRTB20	F	Sub-adult	1	0	0	0	0	0	0
CRTB21	M	Adult	1	0	0	0	0	0	0
CRTB22	F	Sub-adult	1	0	0	0	0	0	0
CRTB23	F	Adult	1	0	0	0	0	0	0
CRTB24	F	Adult	1	0	0	0	0	0	0
CRTB25	F	Sub-adult	1	0	0	0	0	0	0
CRTB26	M	Adult	1	0	0	0	0	0	0
CMER001	F	Adult	0	1	0	1	0	0	1
CMER002	M	Sub-adult	0	1	0	0	0	1	1
Positive			25	2	0	1	0	3	4
Prevalence (%)			92,6	7,4	0,0	3,7	0,0	11,1	14,8

Appendix 3.2: Pathogen screening results for Caracals (*Caracal caracal*) from Namaqualand, South Africa. Highlighted samples are those that have been confirmed by direct sequencing, followed by BLAST analysis and phylogenetic placement.

Individual	Sex	Age class	*Hepatozoon felis*	*Babesia felis*	*Babesia leo*	*Babesia sp.* unknown	*Anaplasma sp.*	*Clostridium sp.*	Mixed Infection
NCM1	M	Sub-adult	1	0	0	0	0	0	0
NCM2	M	Adult	1	0	0	0	0	0	0
NCM3	M	Adult	1	0	0	0	0	0	0
NCM4	M	Adult	0	0	0	1	0	0	0
NCM5	M	Sub-adult	1	0	0	0	0	0	0
NCM6	M	Sub-adult	0	0	0	1	0	0	0
NCM8	M	Adult	1	0	0	0	0	0	0
NCM9	M	Sub-adult	1	0	0	0	0	0	0
NCM10	M	Adult	1	0	0	0	0	0	0
NCM11	M	Adult	1	0	0	0	0	0	0
NCF1	F	Adult	1	0	0	0	0	0	0
NCF2	F	Adult	1	0	0	0	0	0	0
NCF3	F	Adult	1	0	0	0	0	0	0
NCF4	F	Adult	1	0	0	0	0	0	0
Positive			12	0	0	2	0	0	0
Prevalence (%)			85,7	0	0	14,3	0	0	0

Appendix 3.4: Pathogen screening results for Caracals (*Caracal caracal*) from the Cape Peninsula and surrounding area of Cape Town, South Africa. Highlighted samples are those that have been confirmed by direct sequencing, followed by BLAST analysis and phylogenetic placement

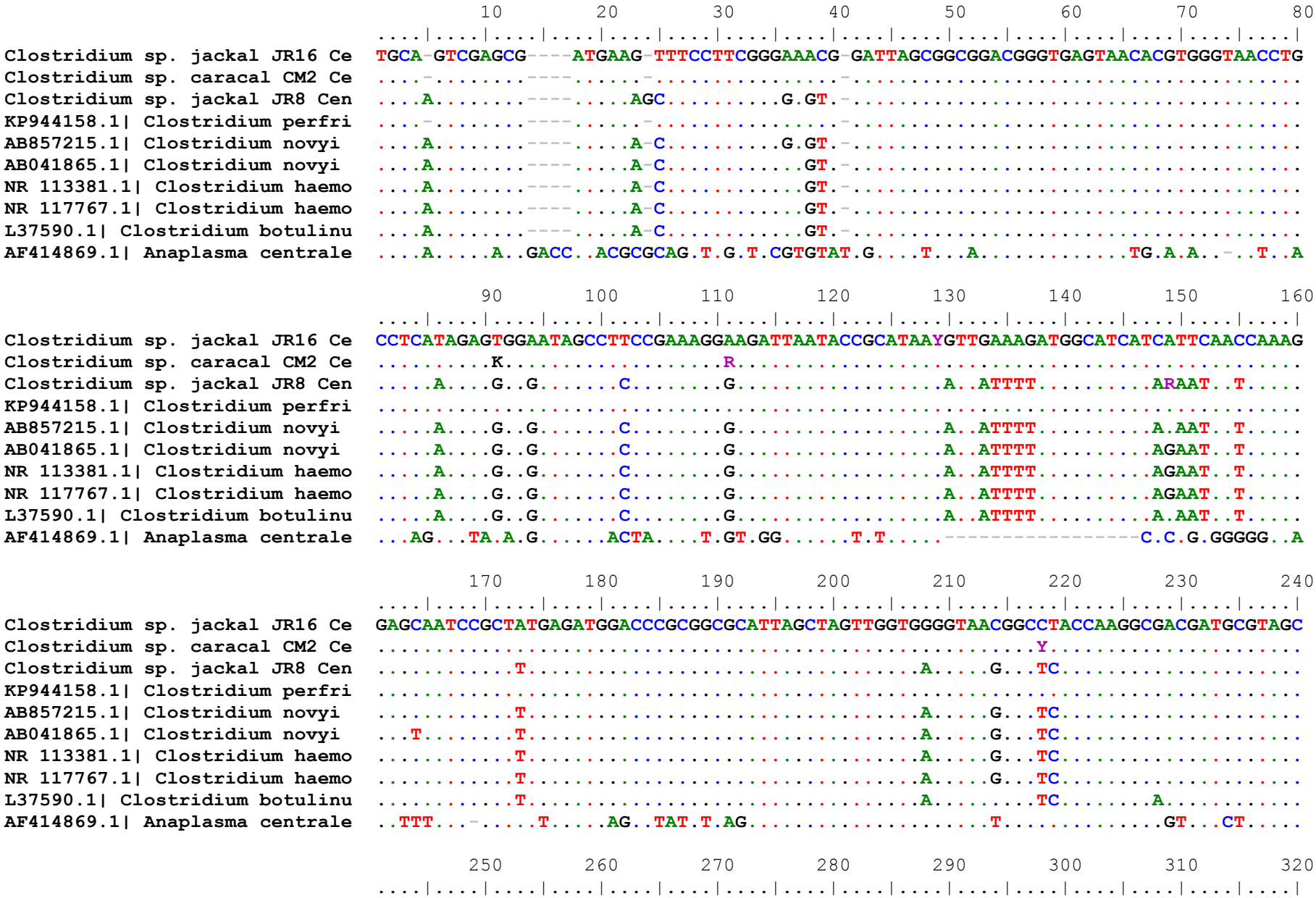
Individual	Sex	Age class	*Hepatozoon felis*	*Babesia felis*	*Babesia leo*	*Babesia sp.* Unknown	*Anaplasma sp.*	*Clostridium sp.*	Mixed Infection
CM05	M	Adult	0	1	0	0	0	0	0
CM08	M	Sub-adult	0	0	1	0	0	0	0
CM09	F	Sub-adult	0	0	0	0	1	0	0
TMC01	M	Adult	0	1	1	0	1	0	1
TMC02	F	Adult	0	1	1	0	1	0	1
TMC03	F	Adult	0	1	1	0	1	0	1
TMC04	M	Adult	0	1	1	0	1	0	1
TMC05	NA	NA	1	0	0	0	1	0	1
TMC06	M	Adult	0	1	1	0	1	0	1
TMC07	M	Sub-adult	0	0	1	0	1	0	1
TMC08	M	Sub-adult	0	1	1	0	1	0	1
TMC09	F	Adult	0	1	0	0	1	0	1
TMC10	M	Adult	0	1	1	0	1	0	1
TMC11	M	Sub-adult	0	1	1	0	1	0	1
TMC12	M	Adult	0	1	1	0	1	0	1
TMC13	F	Adult	0	1	0	0	1	0	1
Positive			1	12	11	0	14	0	13
Prevalence (%)			6,25	75	68,75	0	87,5	0	81,25

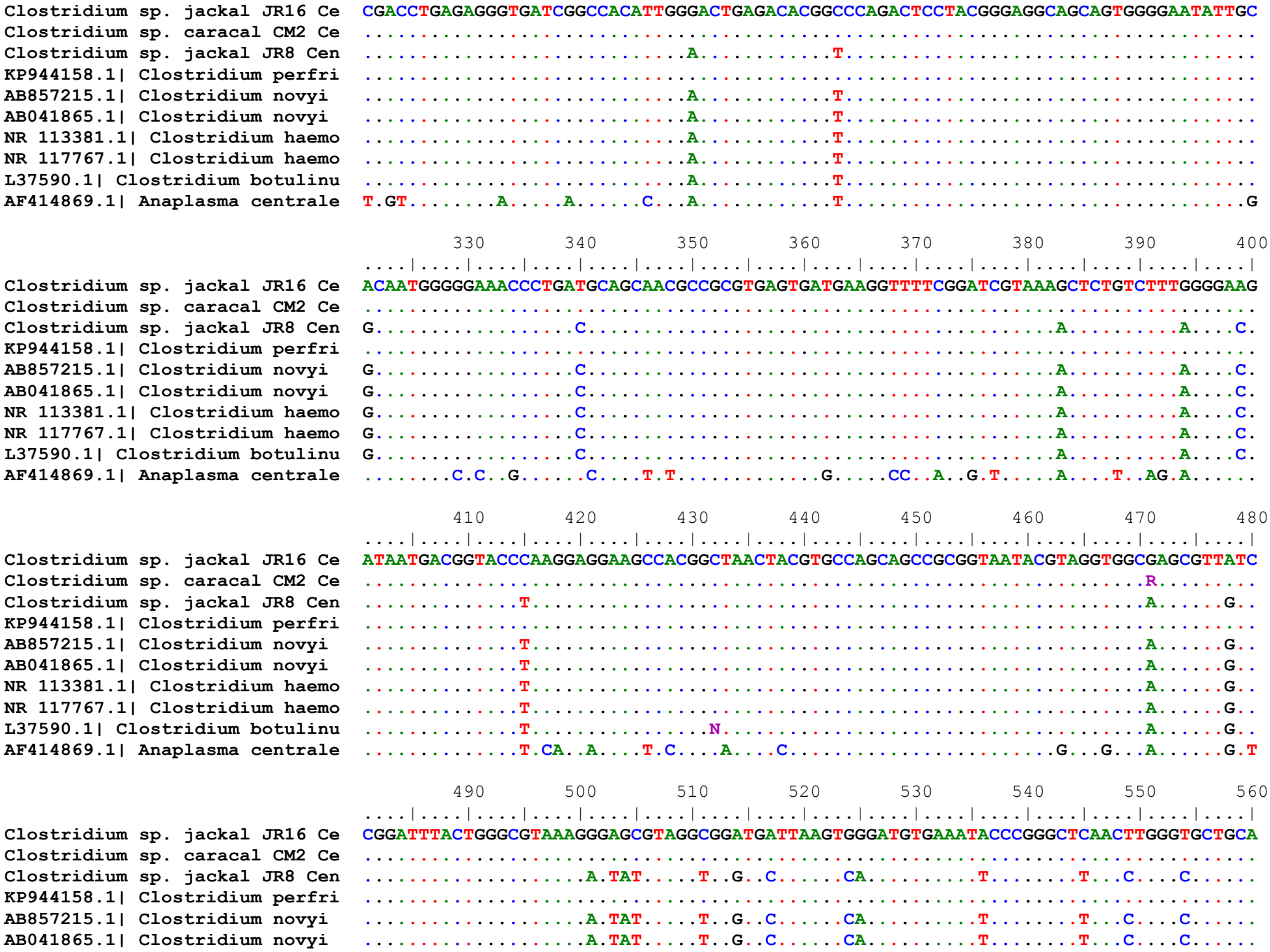
Appendix 3.5: Pathogen screening results for black-backed jackals (*Canis mesomelas*) from the Central Karoo region, South Africa. Highlighted samples are those that have been confirmed by direct sequencing, followed by BLAST analysis and phylogenetic placement

Individual	Sex	Age class	*Clostridium sp.*	*Anaplasma sp.*	*Hepatozoon canis*	*Theileria ovis*	*Sarcocystis sp.*	Mixed Infection
JRTB1	M	Sub-adult	1	0	1	0	0	1
JRTB2	M	Adult	0	0	1	0	0	0
JRTB3	M	Sub-adult	0	0	1	1	0	1
JRTB4	M	Sub-adult	0	0	1	0	0	0
JRTB5	F	Adult	0	0	1	0	0	0
JRTB7	F	Adult	0	0	0	1	1	1
JRTB8	M	Adult	1	0	0	0	0	0
JRTB9	M	Adult	0	0	1	0	0	0
JRTB10	M	Adult	0	0	0	0	0	0
JRTB11	M	Adult	0	0	0	0	0	0
JRTB13	F	Adult	0	0	1	0	0	0
JRTB15	F	Adult	1	0	1	0	0	1
JRTB16	M	Sub-adult	1	0	0	0	0	0
JRTB17	M	Sub-adult	0	0	0	0	0	0
JRTB18	F	Adult	0	0	1	0	0	0
JRTB19	M	Sub-adult	0	0	0	0	0	0
JRTB20	F	Sub-adult	0	0	1	0	0	0
JRTB21	F	Adult	0	0	1	0	0	0
JRTB22	F	Sub-adult	0	0	1	0	0	0
JRTB23	M	Adult	0	0	1	0	0	0
JRTB24	M	Sub-adult	0	0	0	0	0	0
JRTB25	M	Adult	0	0	0	0	0	0
JRTB26	F	Adult	0	0	0	0	0	0
JRTB27	F	Sub-adult	0	0	1	0	0	0
JRTB28	M	Adult	0	0	1	0	0	0
JRTB29	F	Sub-adult	0	0	0	0	0	0
JRTB30	F	Adult	0	0	1	0	0	0
JRTB31	M	Sub-adult	1	0	0	0	0	0
JRTB32	F	Sub-adult	0	0	0	0	0	0

Individual	Sex	Age class	*Clostridium sp.*	*Anaplasma sp.*	*Hepatozoon canis*	*Theileria ovis*	*Sarcocystis sp.*	Mixed Infection
JRTB33	M	Adult	0	0	1	0	0	0
JRTB34	M	Adult	0	0	0	0	0	0
JRTB35	M	Adult	0	0	0	0	0	0
JRTB36	M	Adult	0	0	0	0	0	0
JRTB37	M	Adult	1	0	1	0	0	1
JRTB38	M	Adult	0	0	1	0	0	0
JRTB39	F	Adult	0	0	1	0	0	0
JRTB40	F	Adult	0	0	1	0	0	0
JRTB41	M	Adult	0	0	0	0	0	0
JRTB42	F	Adult	0	0	0	0	0	0
JRTB43	M	Adult	0	0	1	0	0	0
JRTB44	F	Adult	0	0	1	0	0	0
JRTB45	F	Adult	0	0	1	0	0	0
JMER001	M	Adult	0	0	0	0	0	0
Positive			6	0	20	2	1	5
Prevalence (%)			14.0	0.0	46.5	4.7	2.3	11.6

Appendix 4.1: Sequence alignment of 16S rRNA *Clostridium* sequences (859bp)

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Clostridium sp. caracal CM2 Ce
Clostridium sp. jackal JR8 Cen
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AB041865.1| Clostridium novyi
NR 113381.1| Clostridium haemo
NR 117767.1| Clostridium haemo
L37590.1| Clostridium botulinu
AF414869.1| Anaplasma centrale
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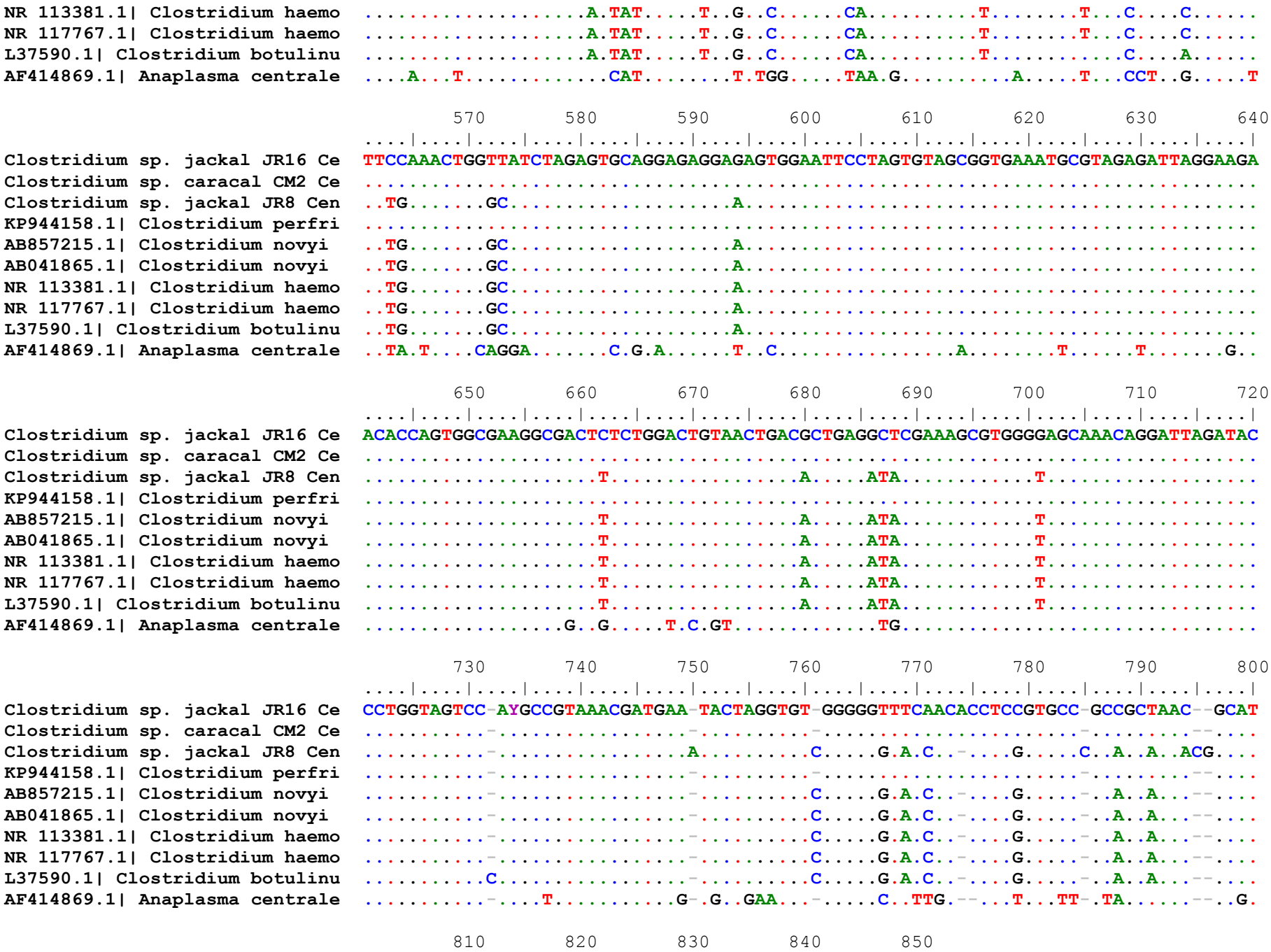
410 420 430 440 450 460 470 480
490 500 510 520 530 540 550 560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NR 117767.1| Clostridium haemo
L37590.1| Clostridium botulinu
AF414869.1| Anaplasma centrale
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Clostridium sp. jackal JR16 Ce
Clostridium sp. caracal CM2 Ce
Clostridium sp. jackal JR8 Cen
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AB857215.1| Clostridium novyi
AB041865.1| Clostridium novyi
NR 113381.1| Clostridium haemo
NR 117767.1| Clostridium haemo
L37590.1| Clostridium botulinu
AF414869.1| Anaplasma centrale
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Clostridium sp. caracal CM2 Ce
Clostridium sp. jackal JR8 Cen
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AB041865.1| Clostridium novyi
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NR 117767.1| Clostridium haemo
L37590.1| Clostridium botulinu
AF414869.1| Anaplasma centrale
730 740 750 760 770 780 790 800
Clostridium sp. jackal JR16 Ce
Clostridium sp. caracal CM2 Ce
Clostridium sp. jackal JR8 Cen
KP944158.1| Clostridium perfri
AB857215.1| Clostridium novyi
AB041865.1| Clostridium novyi
NR 113381.1| Clostridium haemo
NR 117767.1| Clostridium haemo
L37590.1| Clostridium botulinu
AF414869.1| Anaplasma centrale
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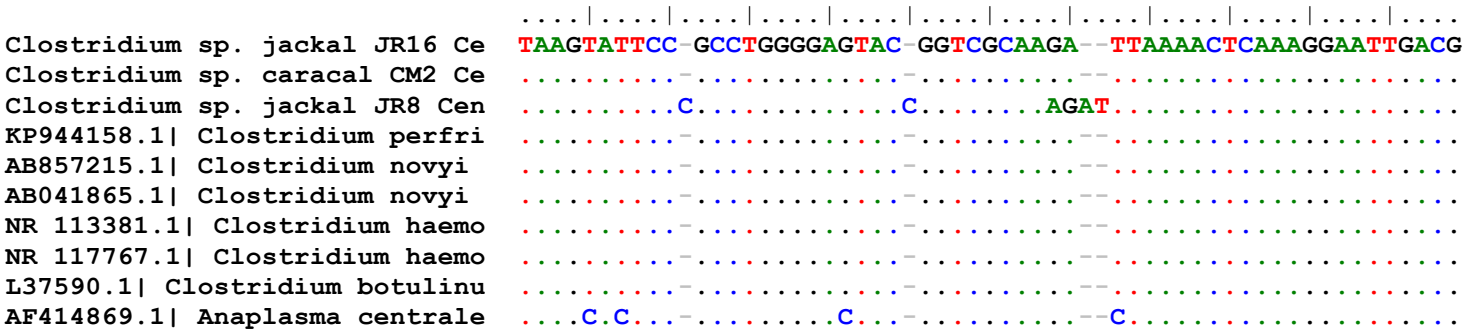
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Clostridium sp. caracal CM2 Ce ..........-..........-..........--....................
Clostridium sp. jackal JR8 Cen ..........C..........C........AGAT....................
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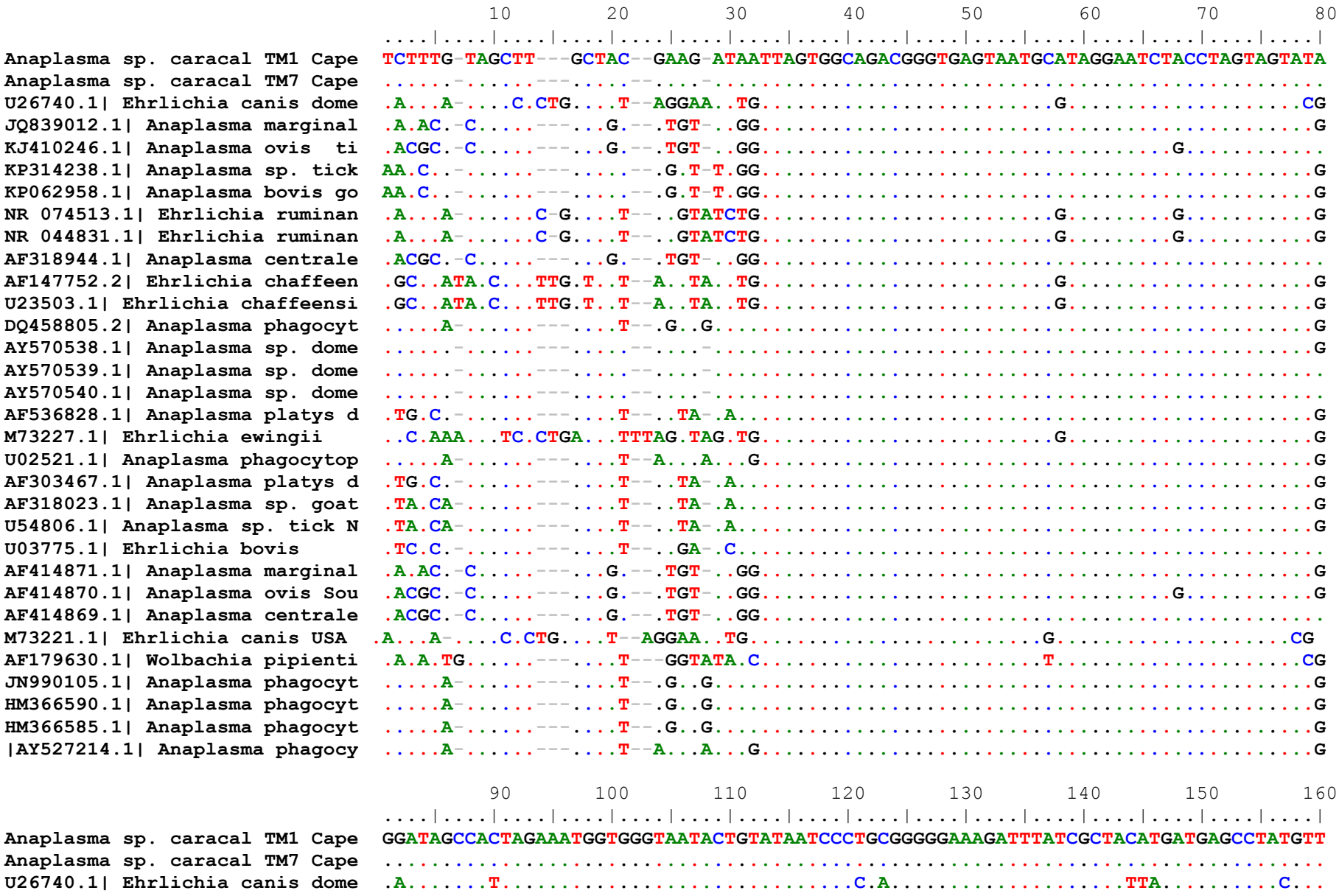
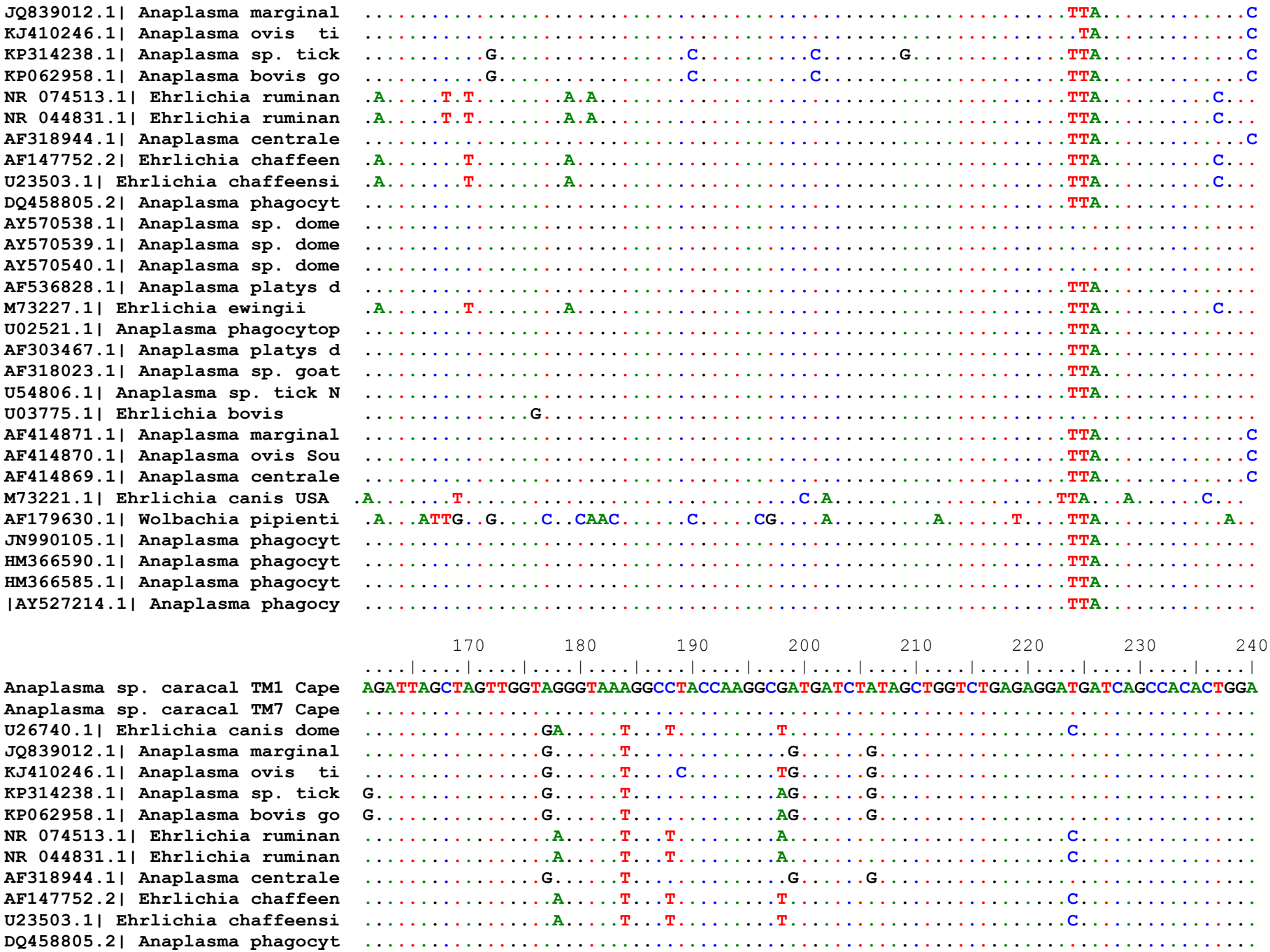
AB857215.1| Clostridium novyi ..........-..........-..........--....................
AB041865.1| Clostridium novyi ..........-..........-..........--....................
NR 113381.1| Clostridium haemo ..........-..........-..........--....................
NR 117767.1| Clostridium haemo ..........-..........-..........--....................
L37590.1| Clostridium botulinu ..........-..........-..........--....................
AF414869.1| Anaplasma centrale ....C.C...-.........C...-..........--C...................

Appendix 4.2: Sequence alignment of 16S rRNA *Ehrlichia* and *Anaplasma* sequences (850bp)

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KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
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NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
AY570540.1| Anaplasma sp. dome
AF536828.1| Anaplasma platys d
M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
HM366590.1| Anaplasma phagocyt
HM366585.1| Anaplasma phagocyt
|AY527214.1| Anaplasma phagocy
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180
190
200
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Anaplasma sp. caracal TM1 Cape
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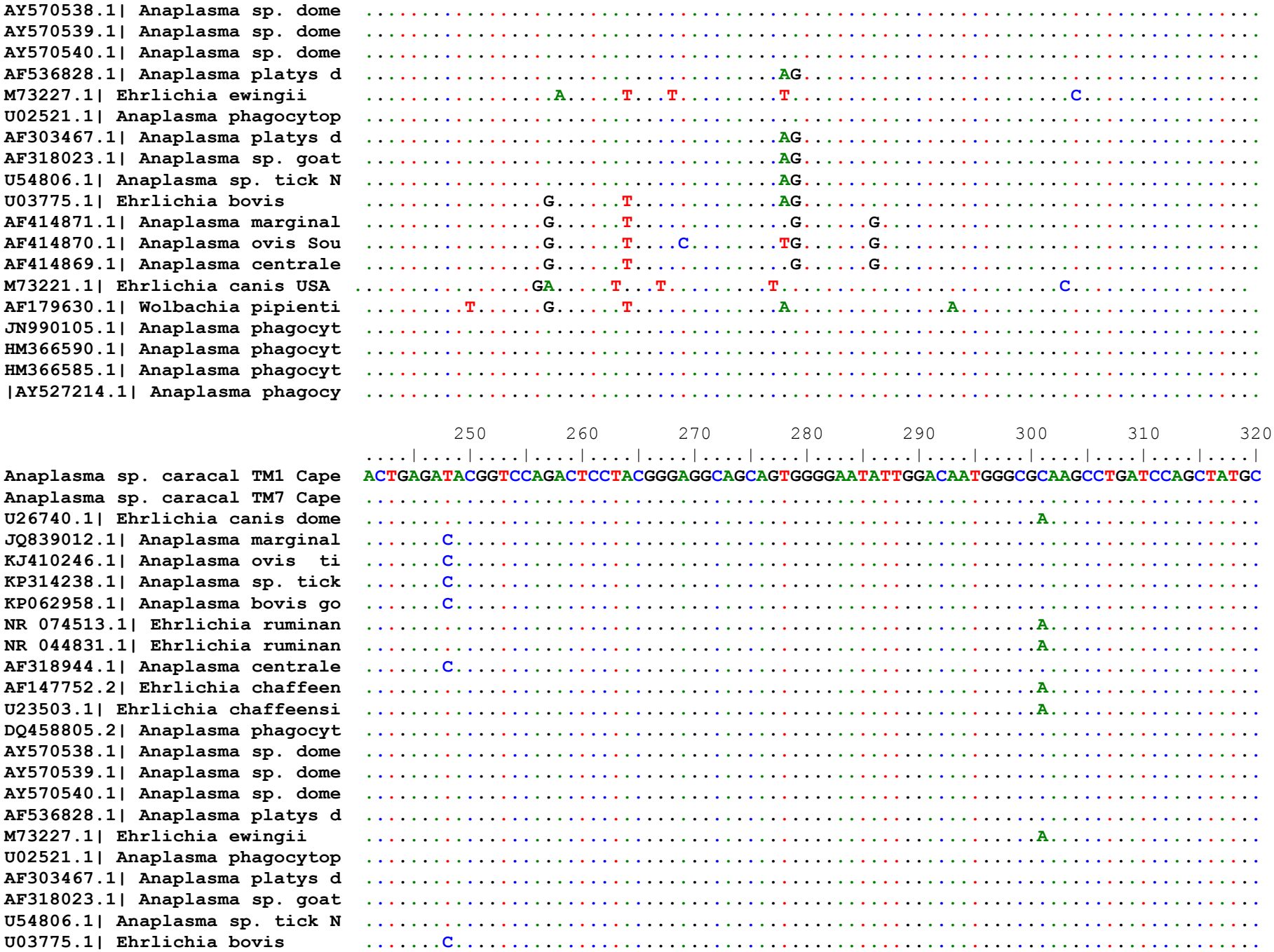
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U26740.1| Ehrlichia canis dome
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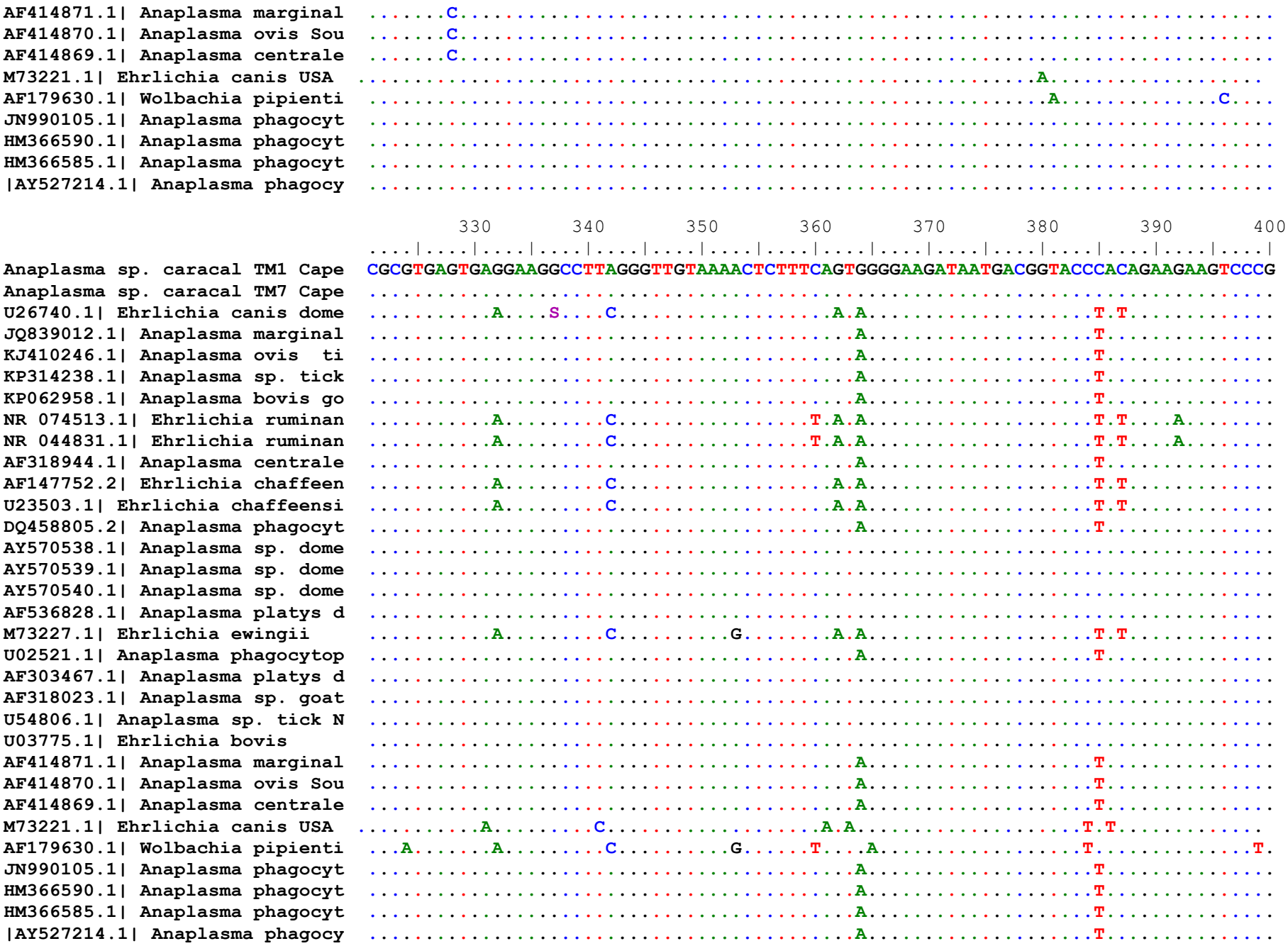
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AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
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AY570539.1| Anaplasma sp. dome
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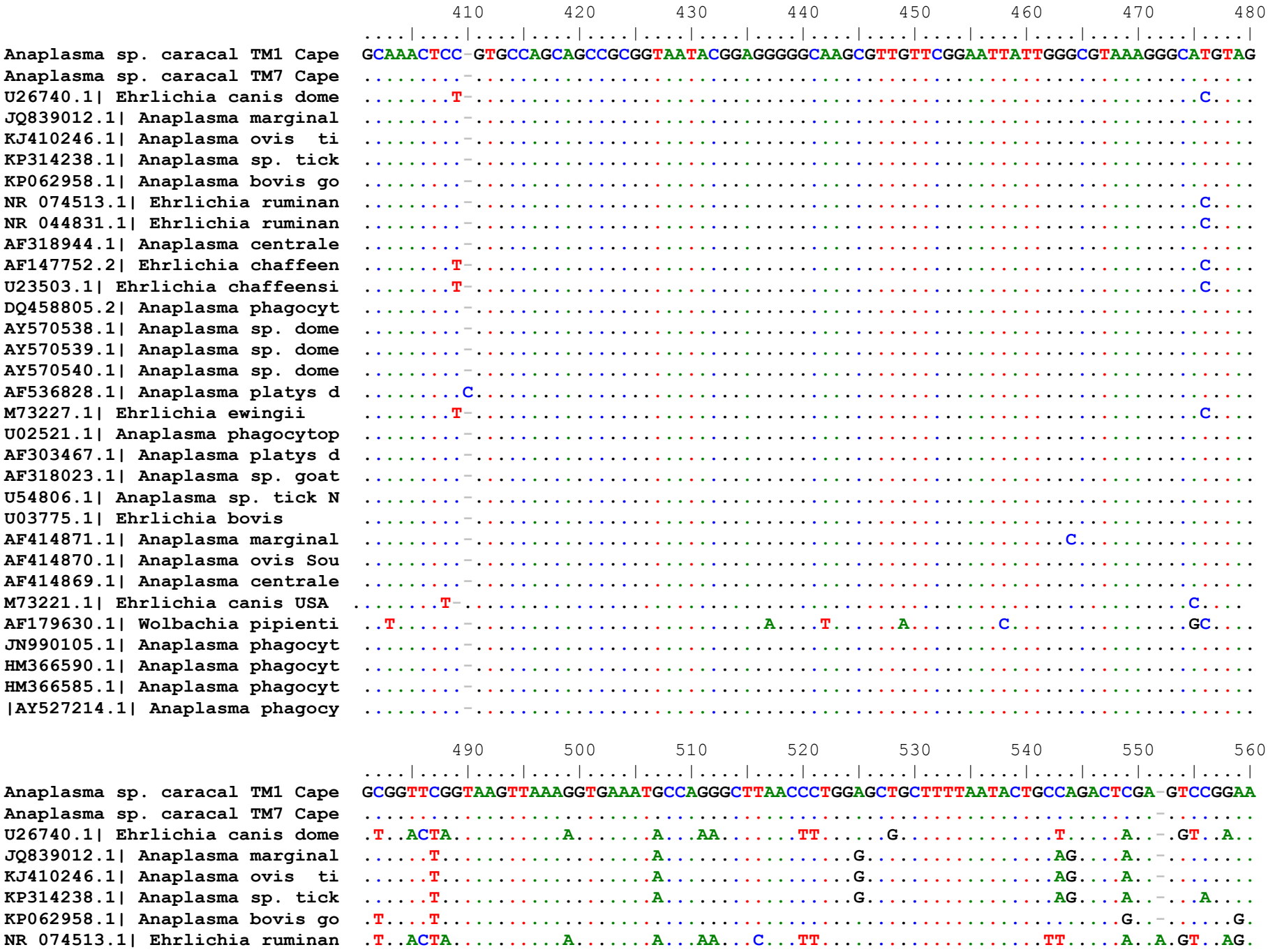
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Anaplasma sp. caracal TM7 Cape
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JQ839012.1| Anaplasma marginal
KJ410246.1| Anaplasma ovis ti
KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
NR 074513.1| Ehrlichia ruminan
NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
AY570540.1| Anaplasma sp. dome
AF536828.1| Anaplasma platys d
M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
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AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
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AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
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|AY527214.1| Anaplasma phagocy
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KP314238.1| Anaplasma sp. tick
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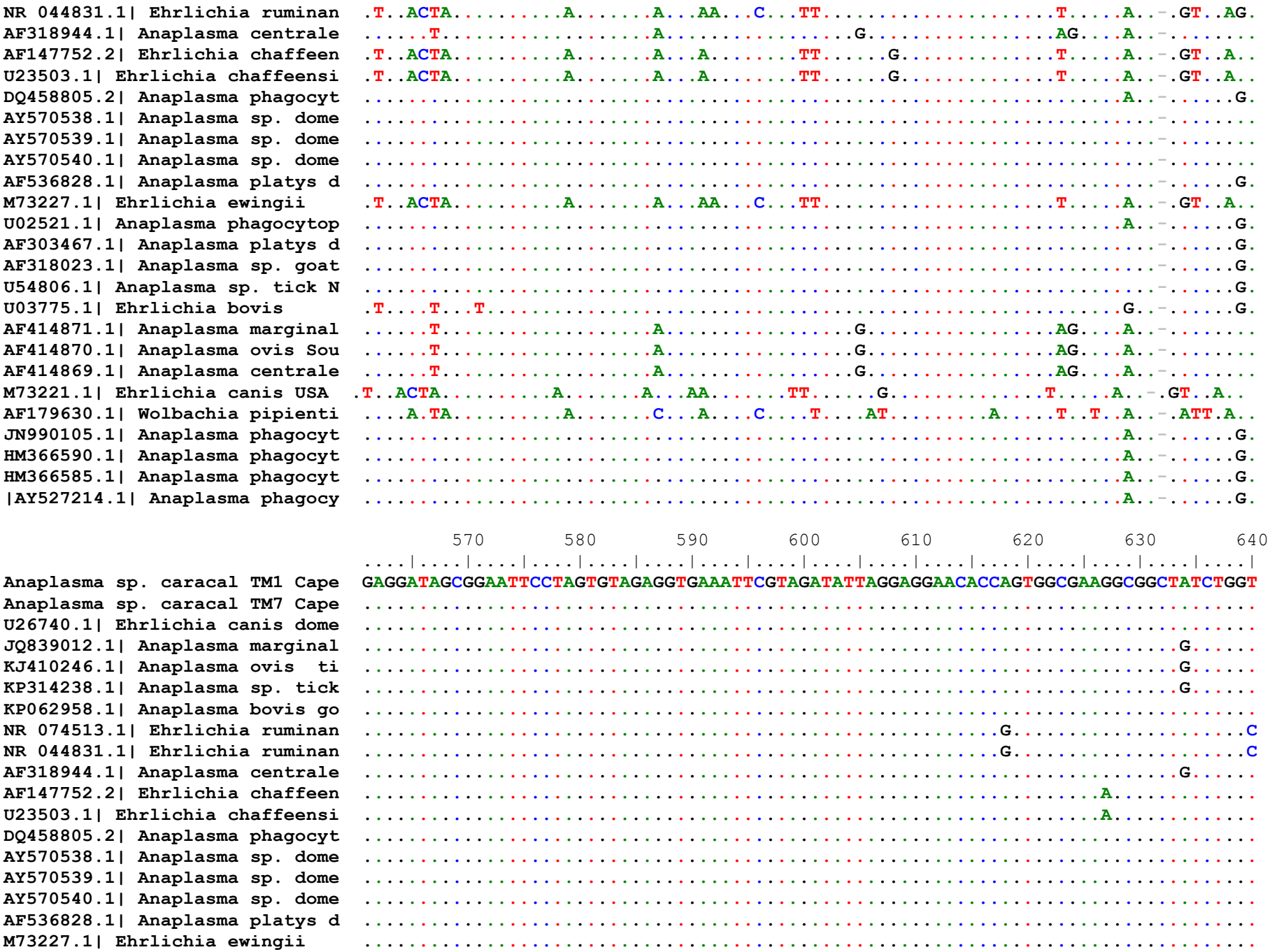
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M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
HM366590.1| Anaplasma phagocyt
HM366585.1| Anaplasma phagocyt
|AY527214.1| Anaplasma phagocy
570
580
590
600
610
620
630
640
Anaplasma sp. caracal TM1 Cape
GAGGATAGCGGAATTCCTAGTGTAGAGGTGAAATTCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTATCTGGT
Anaplasma sp. caracal TM7 Cape
U26740.1| Ehrlichia canis dome
JQ839012.1| Anaplasma marginal
KJ410246.1| Anaplasma ovis ti
KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
NR 074513.1| Ehrlichia ruminan
NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
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AF536828.1| Anaplasma platys d
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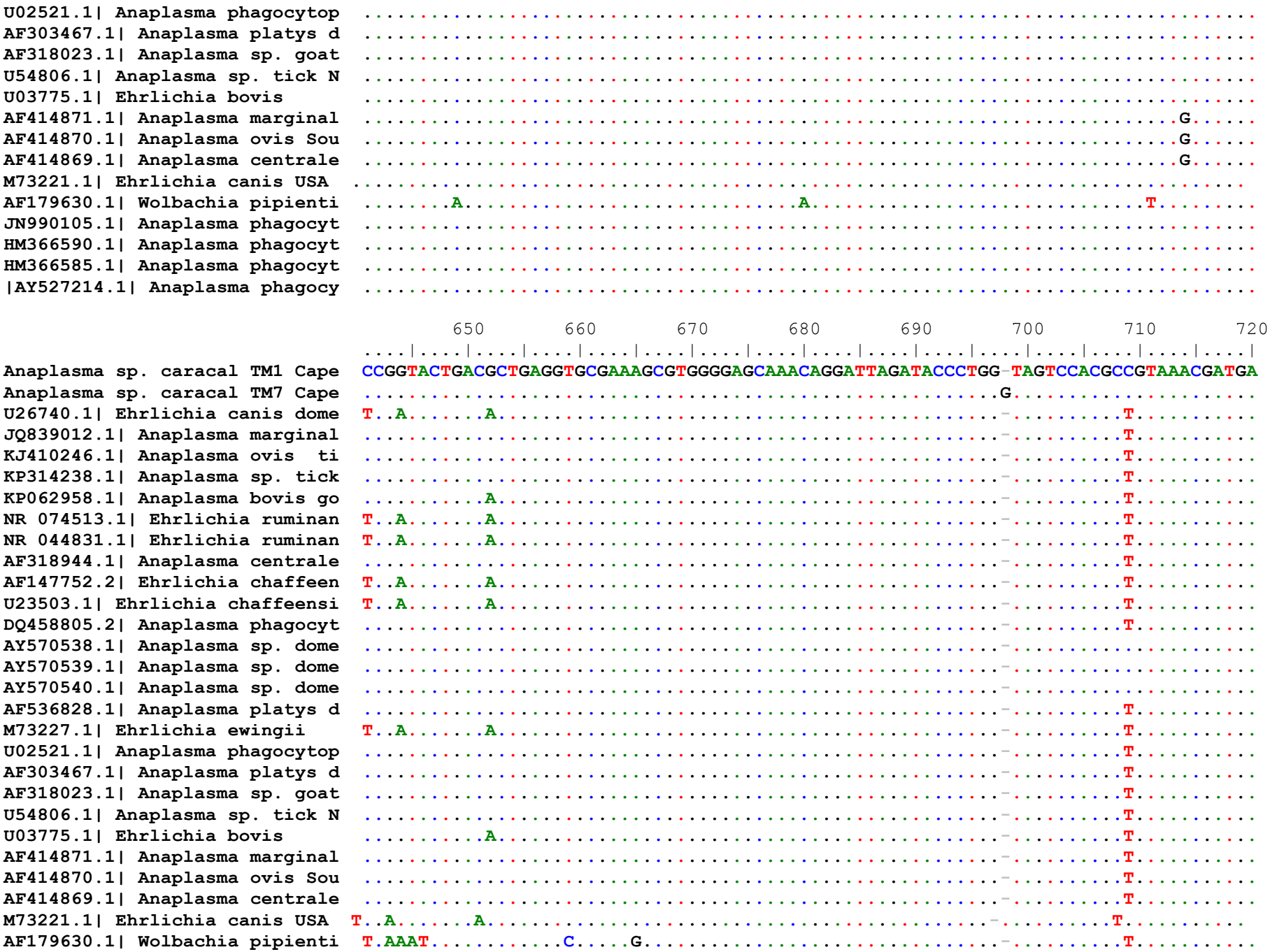
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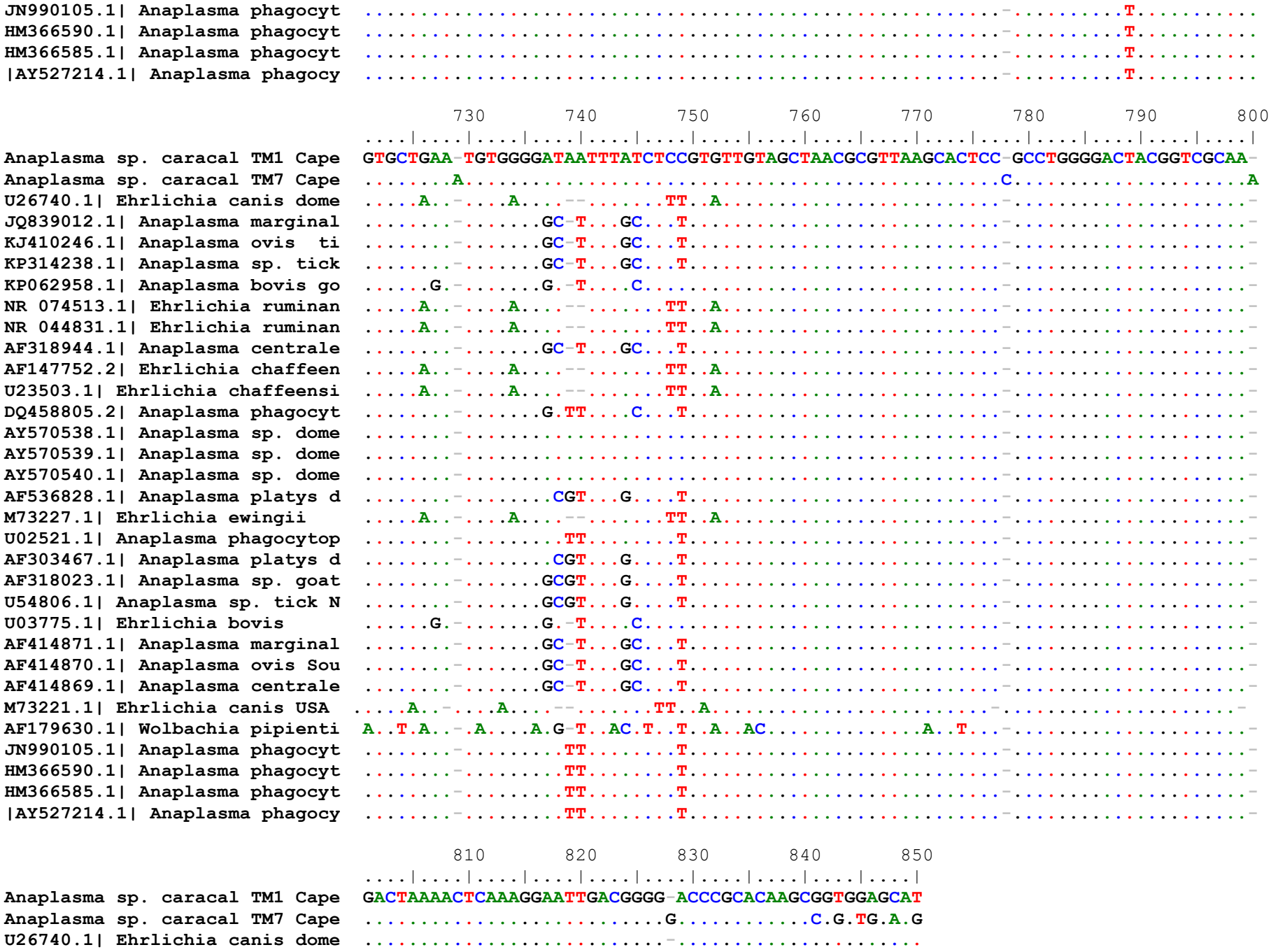
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
HM366590.1| Anaplasma phagocyt
HM366585.1| Anaplasma phagocyt
|AY527214.1| Anaplasma phagocy
650
660
670
680
690
700
710
720
Anaplasma sp. caracal TM1 Cape
Anaplasma sp. caracal TM7 Cape
U26740.1| Ehrlichia canis dome
JQ839012.1| Anaplasma marginal
KJ410246.1| Anaplasma ovis ti
KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
NR 074513.1| Ehrlichia ruminan
NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
AY570540.1| Anaplasma sp. dome
AF536828.1| Anaplasma platys d
M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti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HM366590.1| Anaplasma phagocyt
HM366585.1| Anaplasma phagocyt
|AY527214.1| Anaplasma phagocy
730
740
750
760
770
780
790
800
Anaplasma sp. caracal TM1 Cape
Anaplasma sp. caracal TM7 Cape
U26740.1| Ehrlichia canis dome
JQ839012.1| Anaplasma marginal
KJ410246.1| Anaplasma ovis ti
KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
NR 074513.1| Ehrlichia ruminan
NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
AY570540.1| Anaplasma sp. dome
AF536828.1| Anaplasma platys d
M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
HM366590.1| Anaplasma phagocyt
HM366585.1| Anaplasma phagocyt
|AY527214.1| Anaplasma phagocy
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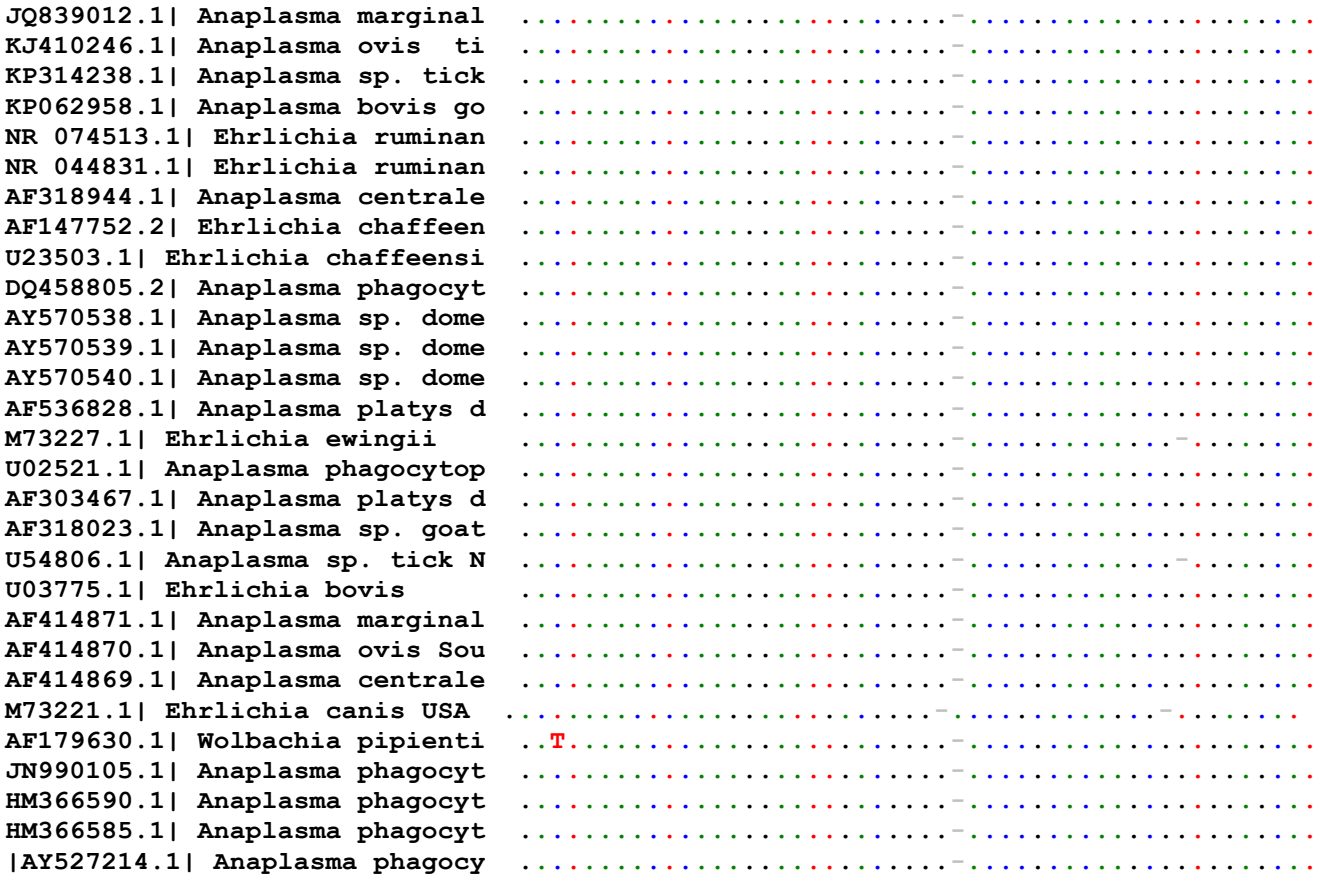
820
830
840
850
Anaplasma sp. caracal TM1 Cape
Anaplasma sp. caracal TM7 Cape
U26740.1| Ehrlichia canis dome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KP314238.1| Anaplasma sp. tick
KP062958.1| Anaplasma bovis go
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NR 044831.1| Ehrlichia ruminan
AF318944.1| Anaplasma centrale
AF147752.2| Ehrlichia chaffeen
U23503.1| Ehrlichia chaffeensi
DQ458805.2| Anaplasma phagocyt
AY570538.1| Anaplasma sp. dome
AY570539.1| Anaplasma sp. dome
AY570540.1| Anaplasma sp. dome
AF536828.1| Anaplasma platys d
M73227.1| Ehrlichia ewingii
U02521.1| Anaplasma phagocytop
AF303467.1| Anaplasma platys d
AF318023.1| Anaplasma sp. goat
U54806.1| Anaplasma sp. tick N
U03775.1| Ehrlichia bovis
AF414871.1| Anaplasma marginal
AF414870.1| Anaplasma ovis Sou
AF414869.1| Anaplasma centrale
M73221.1| Ehrlichia canis USA
AF179630.1| Wolbachia pipienti
JN990105.1| Anaplasma phagocyt
HM366590.1| Anaplasma phagocyt
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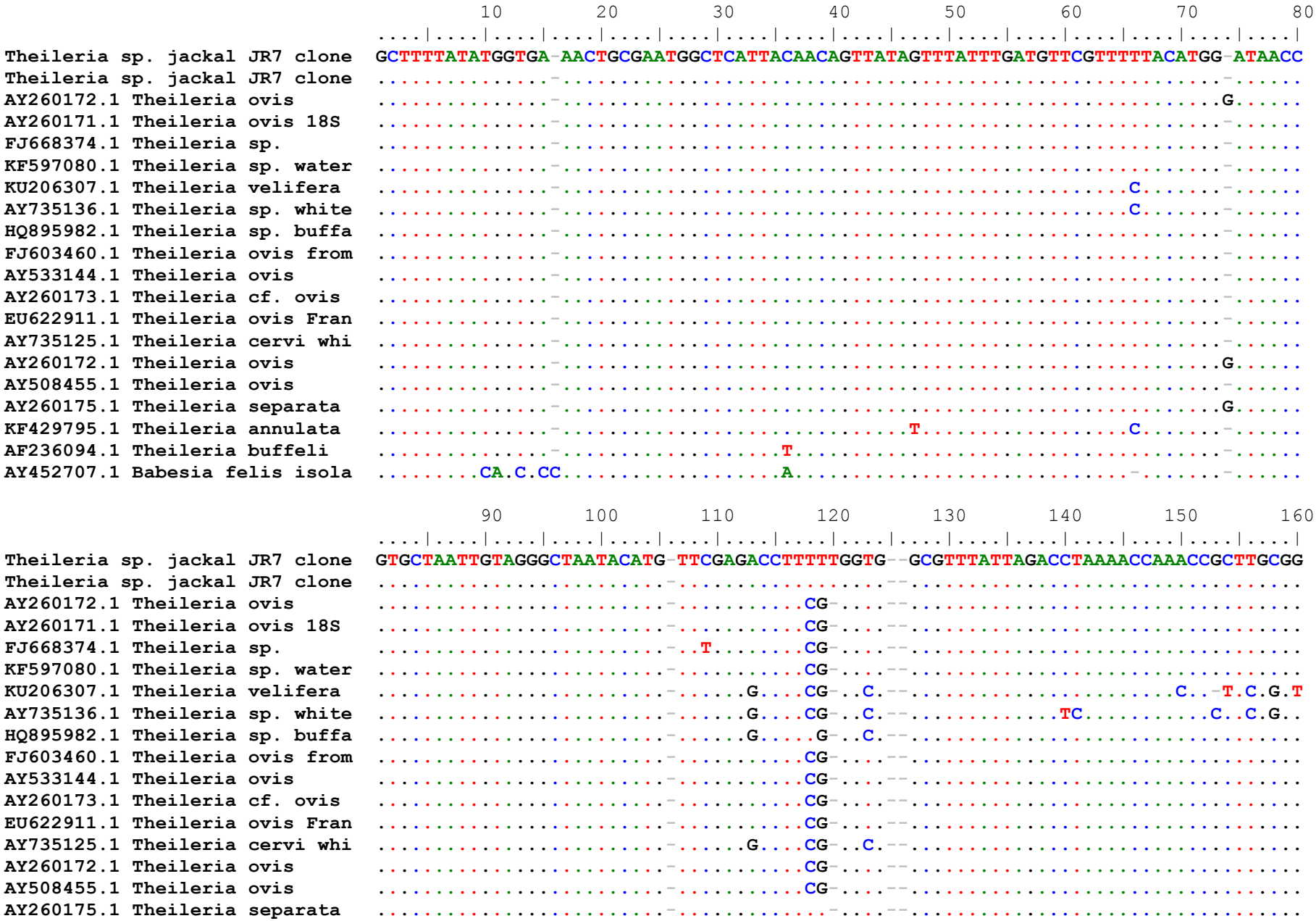
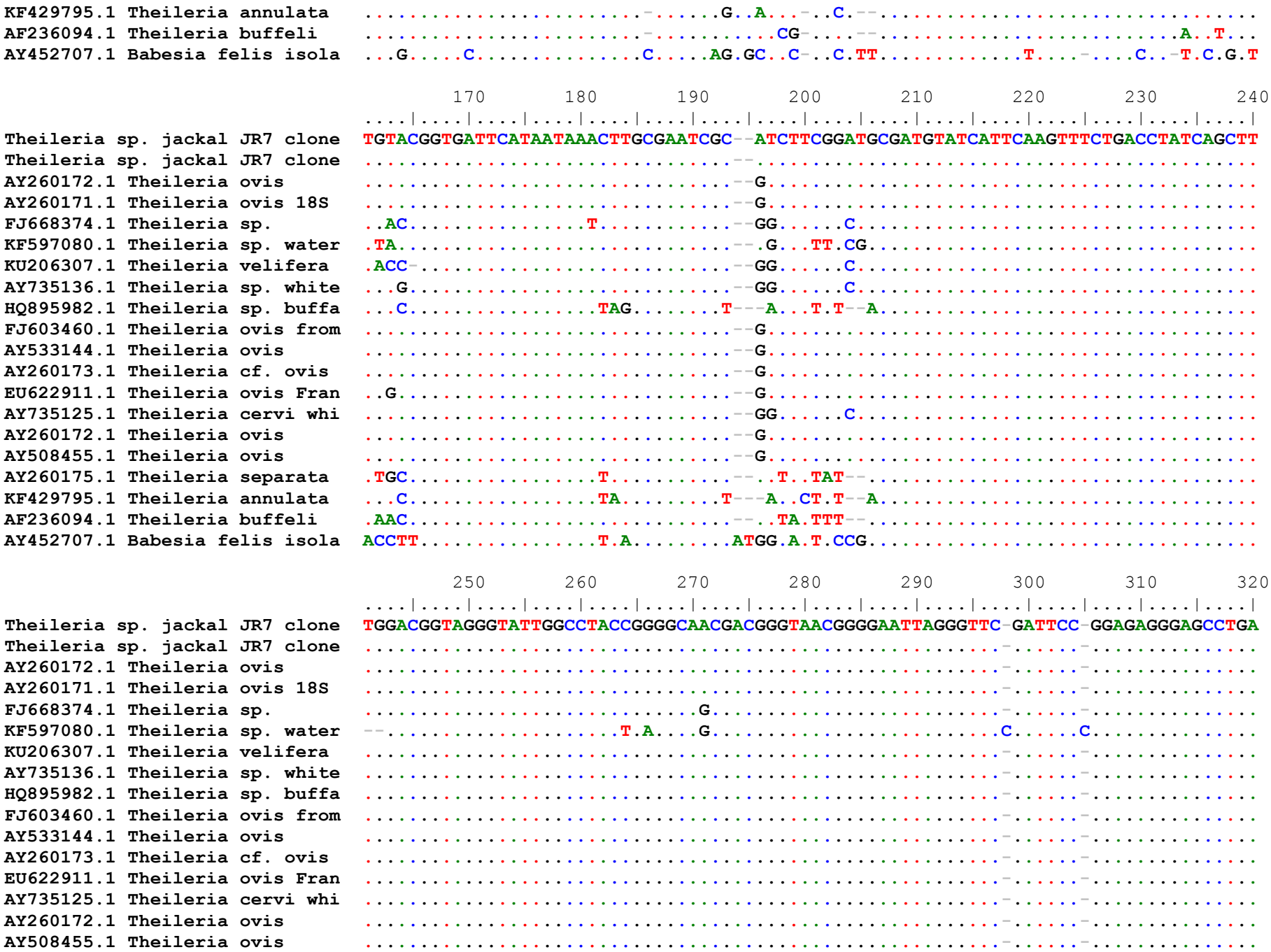
|AY527214.1| Anaplasma phagocy

Appendix 4.3: Sequence alignment of 18S rRNA *Theileria* sequences (748bp)

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AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
170 180 190 200 210 220 230 240
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Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis
AY508455.1 Theileria ovis
AY260175.1 Theileria separata
KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
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Theileria sp. jackal JR7 clone
Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis
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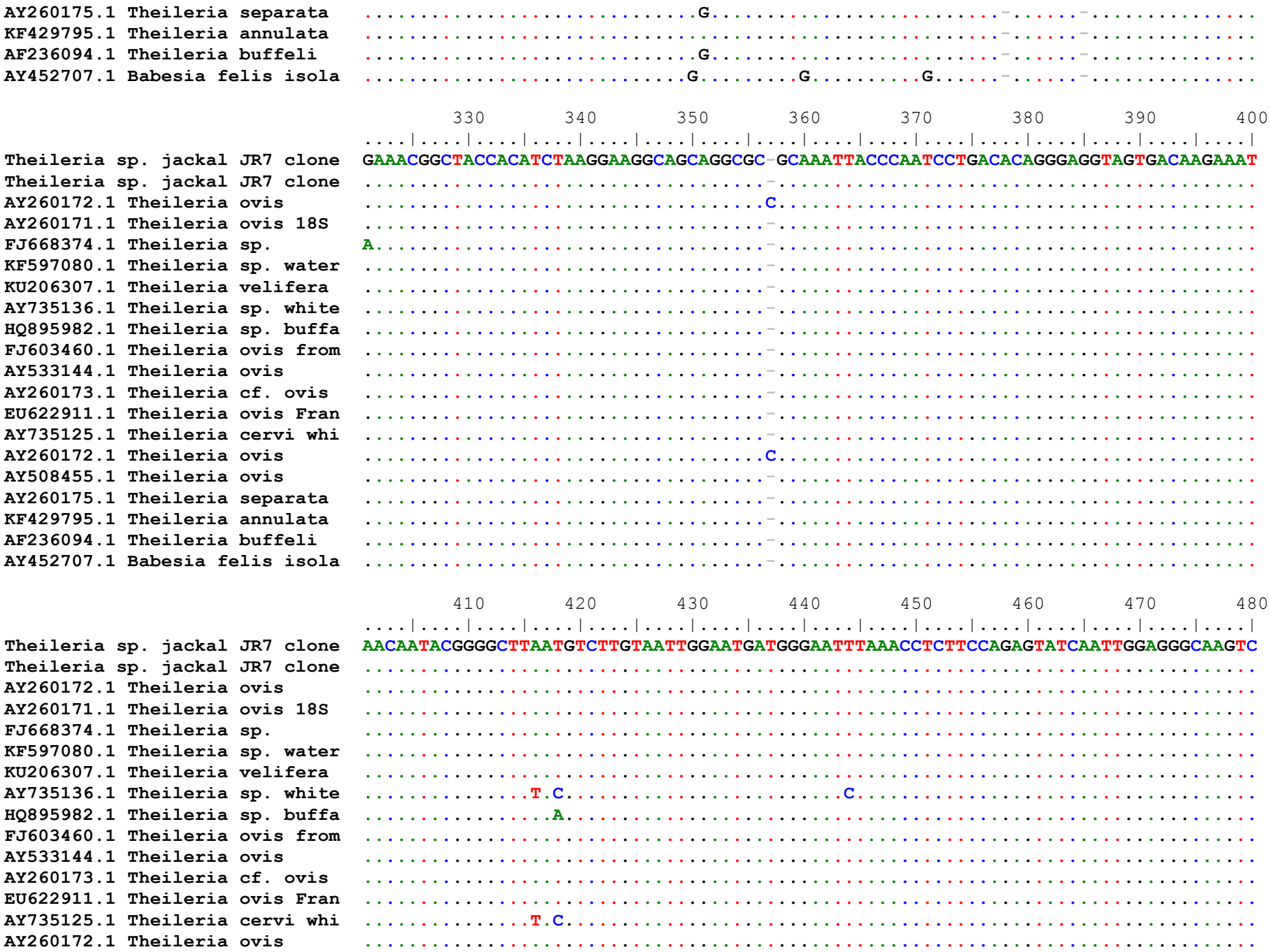
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AY452707.1 Babesia felis isola
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Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis
AY508455.1 Theileria ovis
AY260175.1 Theileria separata
KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
410 420 430 440 450 460 470 480
Theileria sp. jackal JR7 clone AACAATACGGGGCTTAATGTCTTGTAATTGGAATGATGGGAATTTAAACCTCTTCCAGAGTATCAATTGGAGGGCAAGTC
Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
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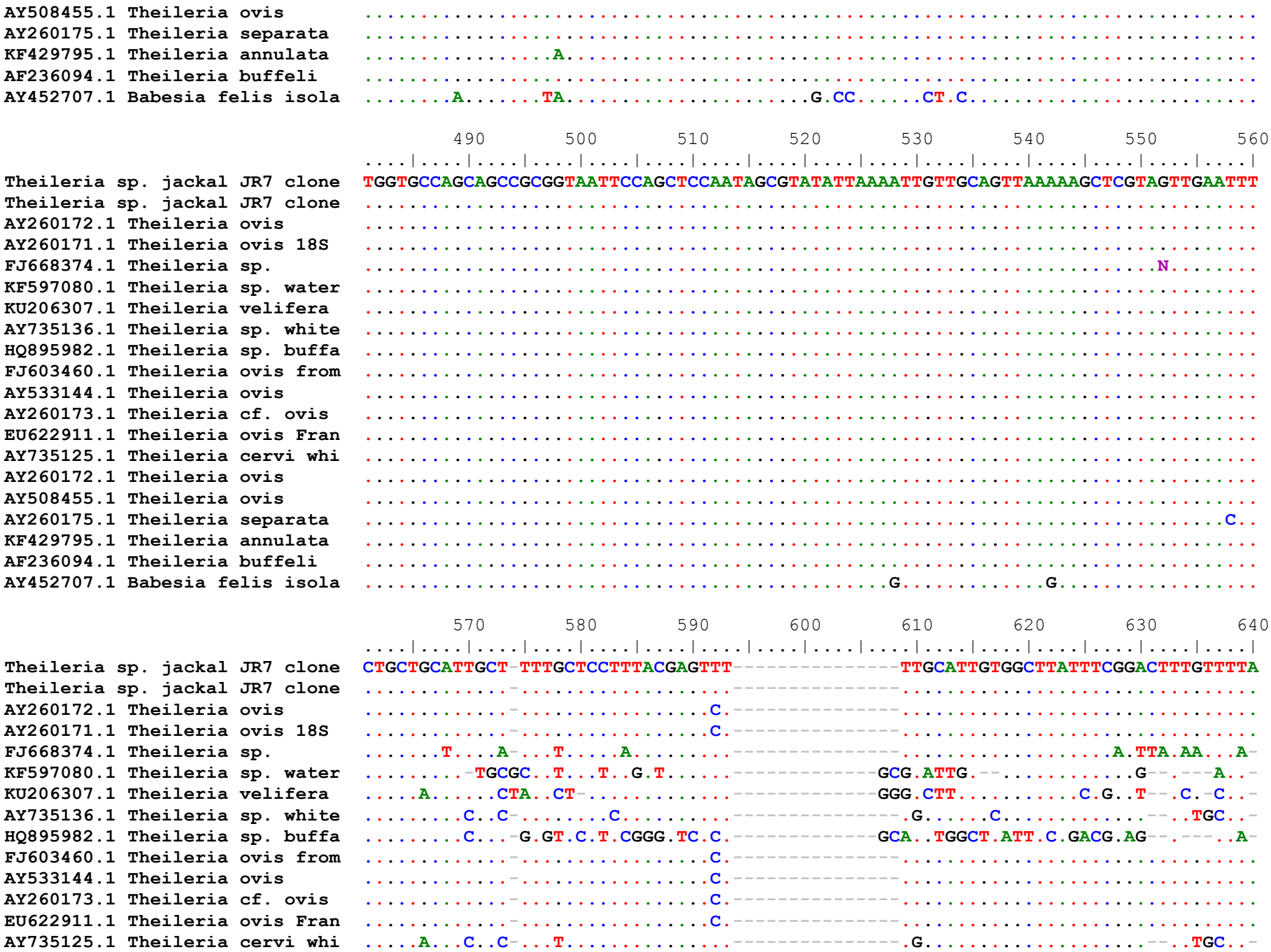
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis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KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
490 500 510 520 530 540 550 560
Theileria sp. jackal JR7 clone TGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAATTGTTGCAGTTAAAAAGCTCGTAGTTGAATTT
Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis
AY508455.1 Theileria ovis
AY260175.1 Theileria separata
KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
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Theileria sp. jackal JR7 clone CTGCTGCATTGCT-TTTGCTCCTTTACGAGTTT----------TTGCATTGTGGCTTATTTCGGACTTTGTTTTA
Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
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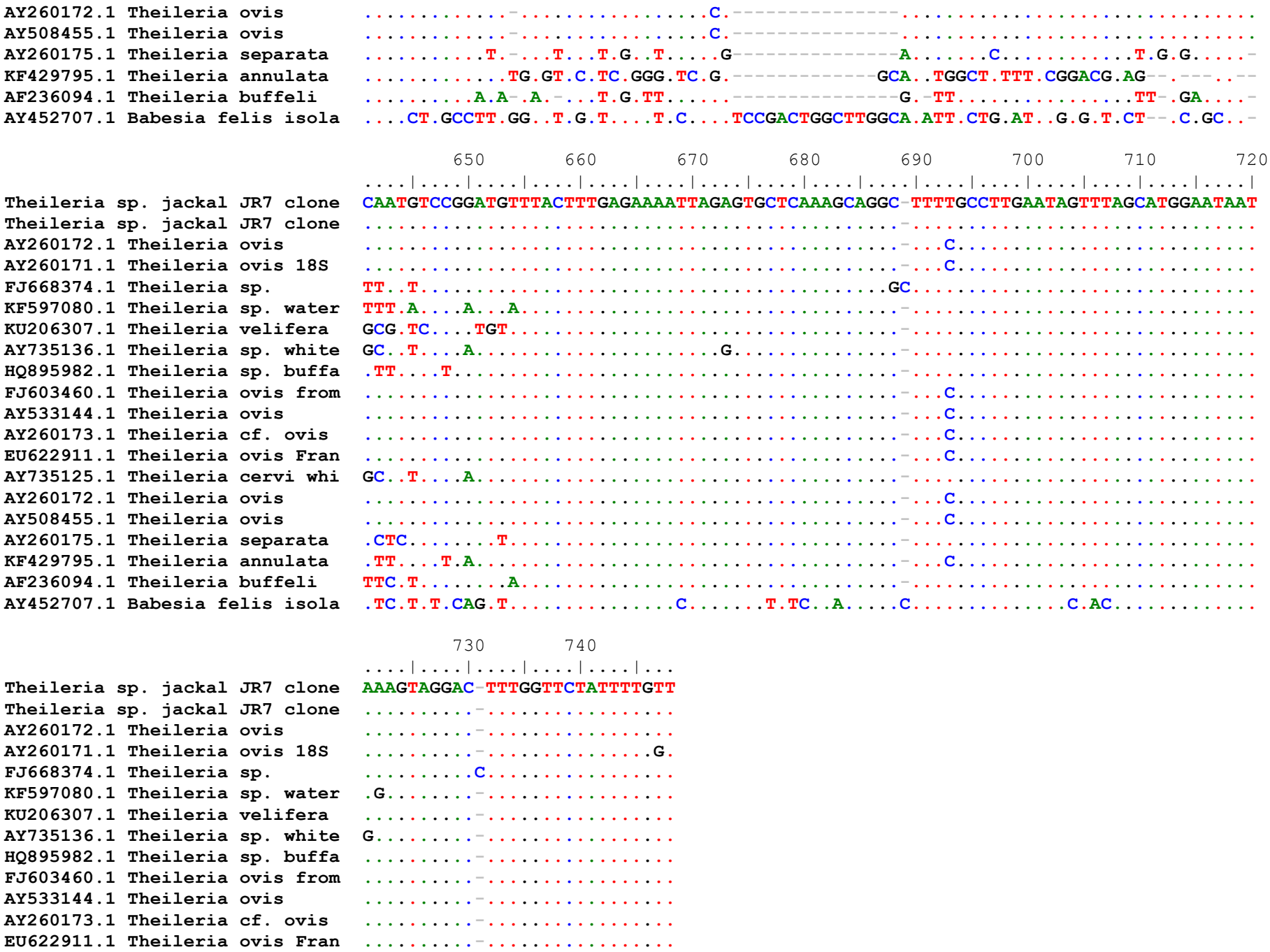
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi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AY508455.1 Theileria ovis
AY260175.1 Theileria separata
KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
650
660
670
680
690
700
710
720
Theileria sp. jackal JR7 clone
Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
FJ668374.1 Theileria sp.
KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
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AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran
AY735125.1 Theileria cervi whi
AY260172.1 Theileria ovis
AY508455.1 Theileria ovis
AY260175.1 Theileria separata
KF429795.1 Theileria annulata
AF236094.1 Theileria buffeli
AY452707.1 Babesia felis isola
730
740
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Theileria sp. jackal JR7 clone
AY260172.1 Theileria ovis
AY260171.1 Theileria ovis 18S
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KF597080.1 Theileria sp. water
KU206307.1 Theileria velifera
AY735136.1 Theileria sp. white
HQ895982.1 Theileria sp. buffa
FJ603460.1 Theileria ovis from
AY533144.1 Theileria ovis
AY260173.1 Theileria cf. ovis
EU622911.1 Theileria ovis Fran

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AY735125.1 Theileria cervi whi  .........-...............
AY260172.1 Theileria ovis       .........-...............
AY508455.1 Theileria ovis       .........-...............
AY260175.1 Theileria separata   G........-...............
KF429795.1 Theileria annulata   .........-...............
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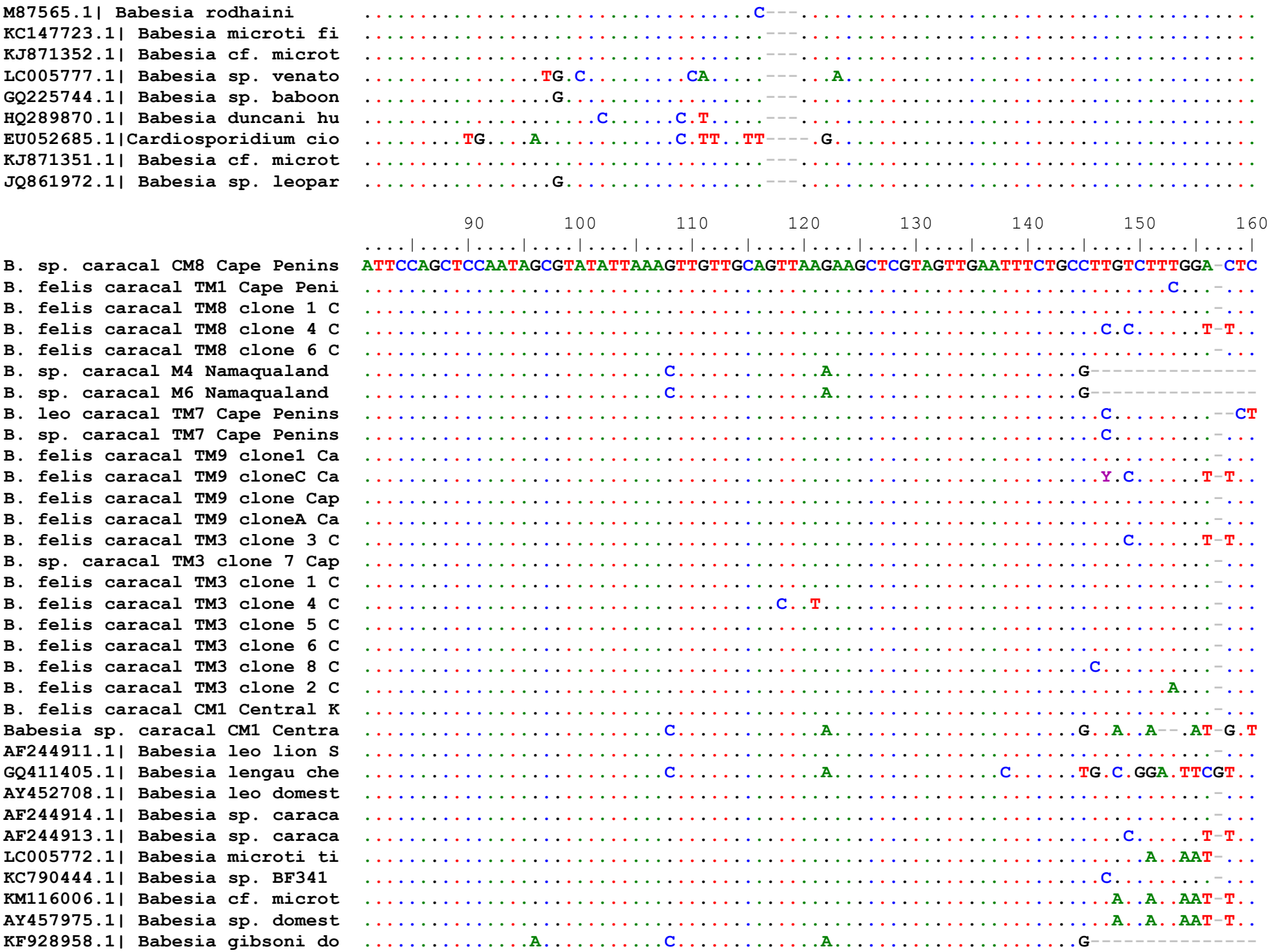
Appendix 4.4: Sequence alignment of 18S rRNA *Babesia* sequences (445bp)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KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar
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100
110
120
130
140
150
160
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B. felis caracal TM1 Cape Peni
B. felis caracal TM8 clone 1 C
B. felis caracal TM8 clone 4 C
B. felis caracal TM8 clone 6 C
B. sp. caracal M4 Namaqualand
B. sp. caracal M6 Namaqualand
B. leo caracal TM7 Cape Penins
B. sp. caracal TM7 Cape Penins
B. felis caracal TM9 clone1 Ca
B. felis caracal TM9 cloneC Ca
B. felis caracal TM9 clone Cap
B. felis caracal TM9 cloneA Ca
B. felis caracal TM3 clone 3 C
B. sp. caracal TM3 clone 7 Cap
B. felis caracal TM3 clone 1 C
B. felis caracal TM3 clone 4 C
B. felis caracal TM3 clone 5 C
B. felis caracal TM3 clone 6 C
B. felis caracal TM3 clone 8 C
B. felis caracal TM3 clone 2 C
B. felis caracal CM1 Central K
Babesia sp. caracal CM1 Centra
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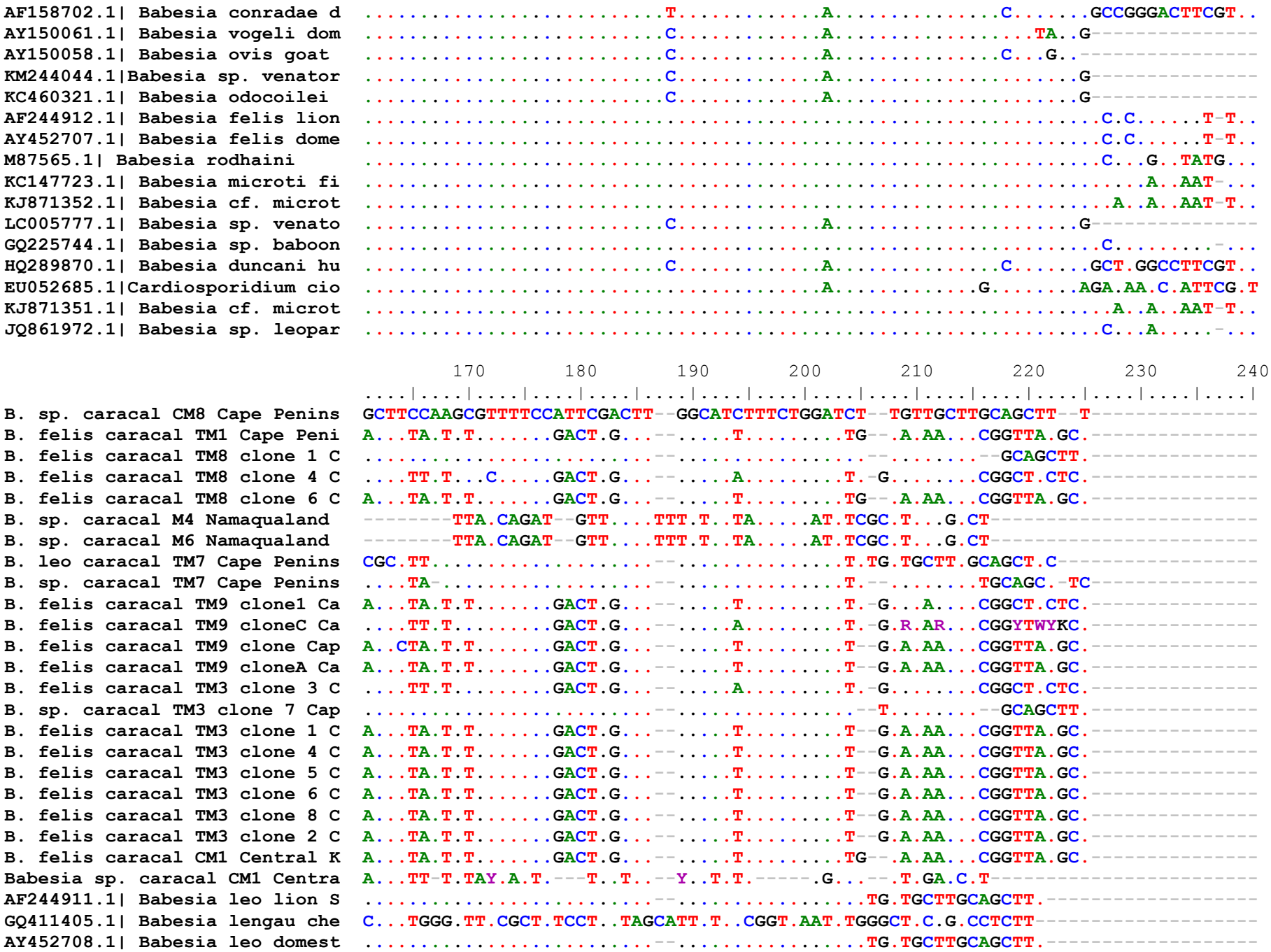
GQ411405.1| Babesia lengau che
AY452708.1| Babesia leo domest
AF244914.1| Babesia sp. caraca
AF244913.1| Babesia sp. caraca
LC005772.1| Babesia microti ti
KC790444.1| Babesia sp. BF341
KM116006.1| Babesia cf. microt
AY457975.1| Babesia sp. domest
KF928958.1| Babesia gibsoni do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AY150058.1| Babesia ovis goat
KM244044.1|Babesia sp. venator
KC460321.1| Babesia odocoilei
AF244912.1| Babesia felis lion
AY452707.1| Babesia felis dome
M87565.1| Babesia rodhaini
KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar
170
180
190
200
210
220
230
240
B. sp. caracal CM8 Cape Penins
B. felis caracal TM1 Cape Peni
B. felis caracal TM8 clone 1 C
B. felis caracal TM8 clone 4 C
B. felis caracal TM8 clone 6 C
B. sp. caracal M4 Namaqualand
B. sp. caracal M6 Namaqualand
B. leo caracal TM7 Cape Penins
B. sp. caracal TM7 Cape Penins
B. felis caracal TM9 clone1 Ca
B. felis caracal TM9 cloneC Ca
B. felis caracal TM9 clone Cap
B. felis caracal TM9 cloneA Ca
B. felis caracal TM3 clone 3 C
B. sp. caracal TM3 clone 7 Cap
B. felis caracal TM3 clone 1 C
B. felis caracal TM3 clone 4 C
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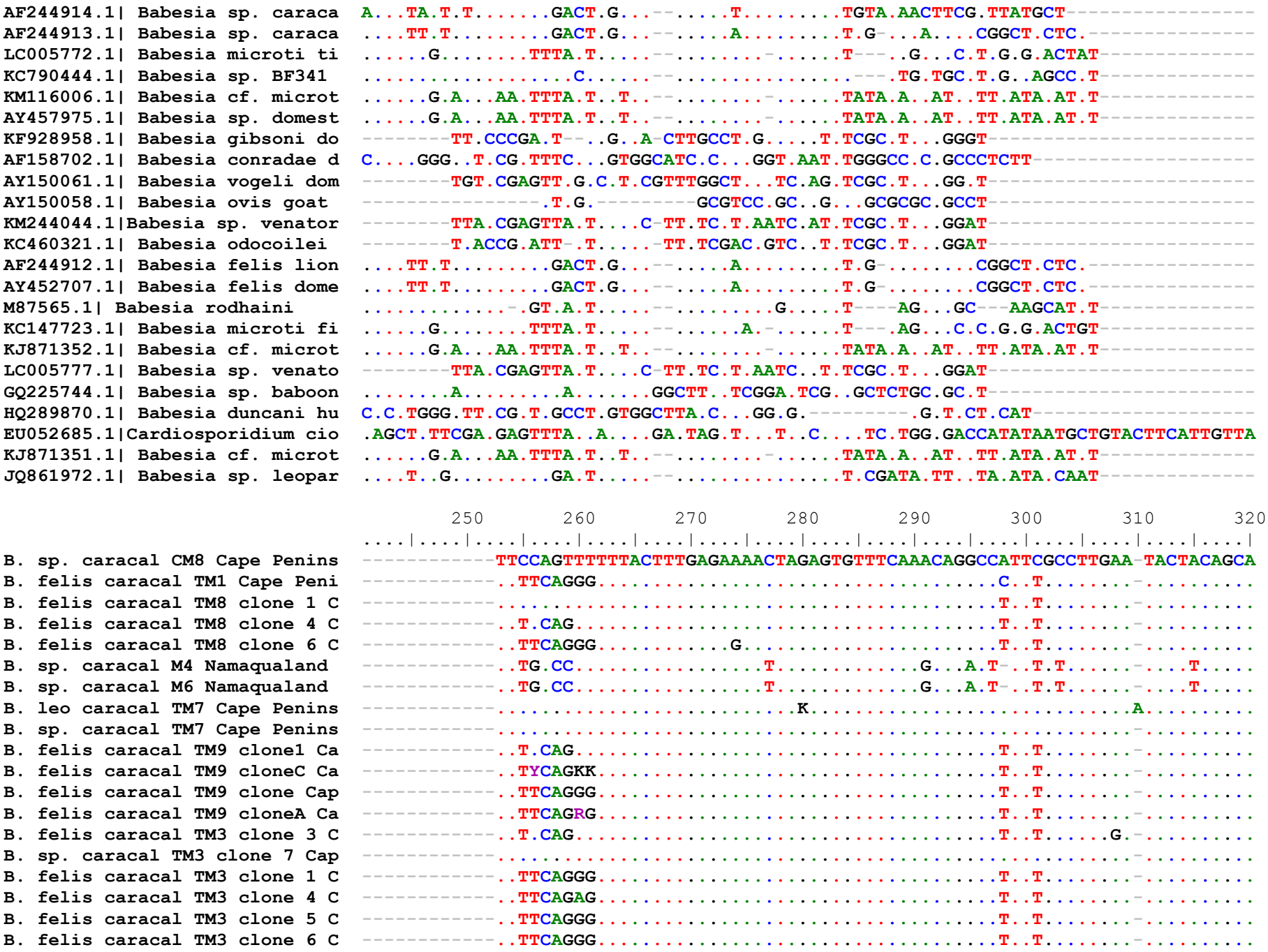
B. felis caracal CM1 Central K
Babesia sp. caracal CM1 Centra
AF244911.1| Babesia leo lion S
GQ411405.1| Babesia lengau che
AY452708.1| Babesia leo domest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LC005772.1| Babesia microti ti
KC790444.1| Babesia sp. BF341
KM116006.1| Babesia cf. microt
AY457975.1| Babesia sp. domest
KF928958.1| Babesia gibsoni do
AF158702.1| Babesia conradae d
AY150061.1| Babesia vogeli dom
AY150058.1| Babesia ovis goat
KM244044.1|Babesia sp. venator
KC460321.1| Babesia odocoilei
AF244912.1| Babesia felis lion
AY452707.1| Babesia felis dome
M87565.1| Babesia rodhaini
KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar
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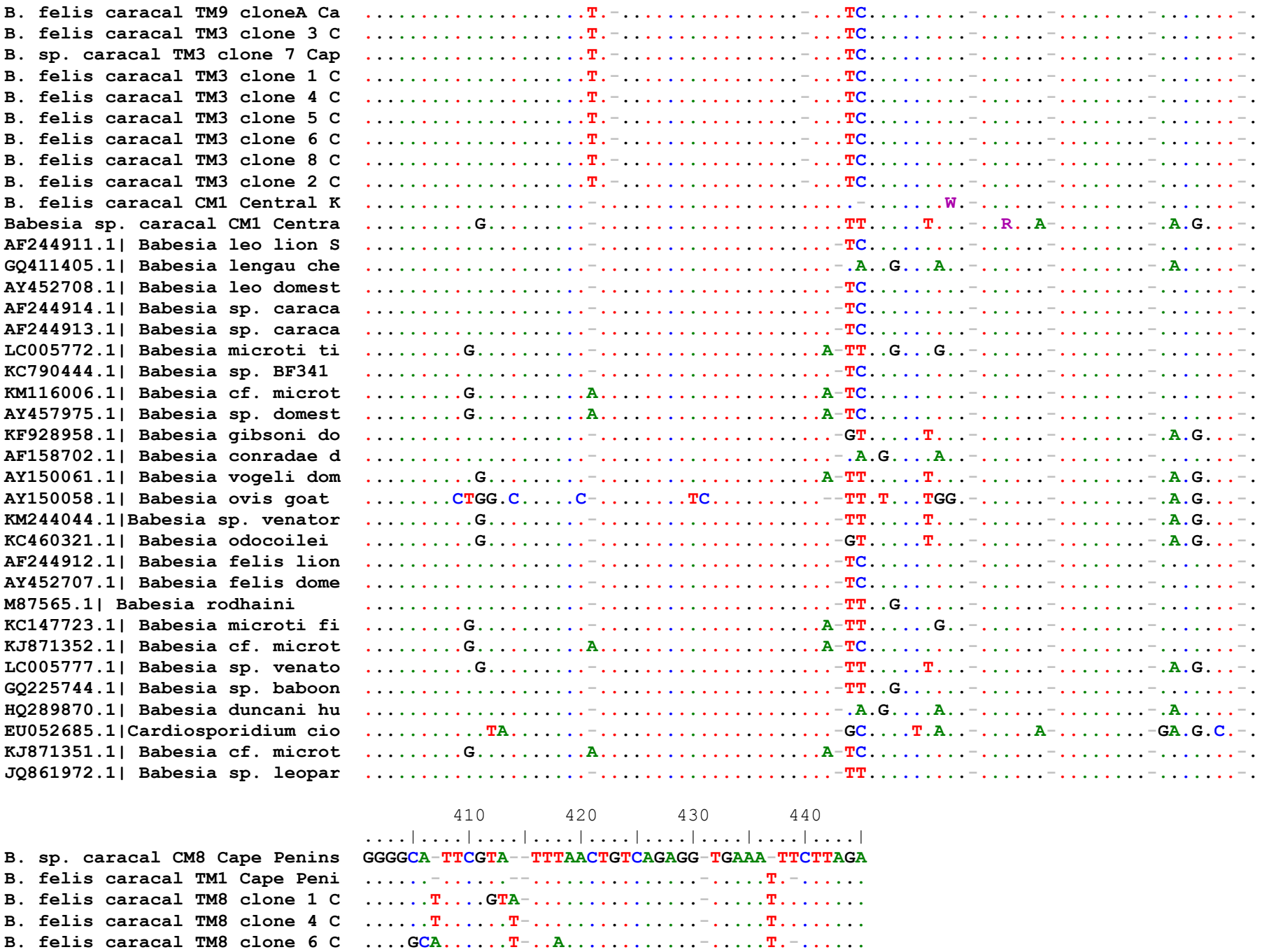
260
270
280
290
300
310
320
B. sp. caracal CM8 Cape Penins
B. felis caracal TM1 Cape Peni
B. felis caracal TM8 clone 1 C
B. felis caracal TM8 clone 4 C
B. felis caracal TM8 clone 6 C
B. sp. caracal M4 Namaqualand
B. sp. caracal M6 Namaqualand
B. leo caracal TM7 Cape Penins
B. sp. caracal TM7 Cape Penins
B. felis caracal TM9 clone1 Ca
B. felis caracal TM9 cloneC Ca
B. felis caracal TM9 clone Cap
B. felis caracal TM9 cloneA Ca
B. felis caracal TM3 clone 3 C
B. sp. caracal TM3 clone 7 Cap
B. felis caracal TM3 clone 1 C
B. felis caracal TM3 clone 4 C
B. felis caracal TM3 clone 5 C
B. felis caracal TM3 clone 6 C


B. felis caracal TM3 clone 8 C
B. felis caracal TM3 clone 2 C
B. felis caracal CM1 Central K
Babesia sp. caracal CM1 Centra
AF244911.1| Babesia leo lion S
GQ411405.1| Babesia lengau che
AY452708.1| Babesia leo domest
AF244914.1| Babesia sp. caraca
AF244913.1| Babesia sp. caraca
LC005772.1| Babesia microti ti
KC790444.1| Babesia sp. BF341
KM116006.1| Babesia cf. microt
AY457975.1| Babesia sp. domest
KF928958.1| Babesia gibsoni do
AF158702.1| Babesia conradae d
AY150061.1| Babesia vogeli dom
AY150058.1| Babesia ovis goat
KM244044.1|Babesia sp. venator
KC460321.1| Babesia odocoilei
AF244912.1| Babesia felis lion
AY452707.1| Babesia felis dome
M87565.1| Babesia rodhaini
KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar
330
340
350
360
370
380
390
400
B. sp. caracal CM8 Cape Penins
B. felis caracal TM1 Cape Peni
B. felis caracal TM8 clone 1 C
B. felis caracal TM8 clone 4 C
B. felis caracal TM8 clone 6 C
B. sp. caracal M4 Namaqualand
B. sp. caracal M6 Namaqualand
B. leo caracal TM7 Cape Penins
B. sp. caracal TM7 Cape Penins
B. felis caracal TM9 clone1 Ca
B. felis caracal TM9 cloneC Ca
B. felis caracal TM9 clone Cap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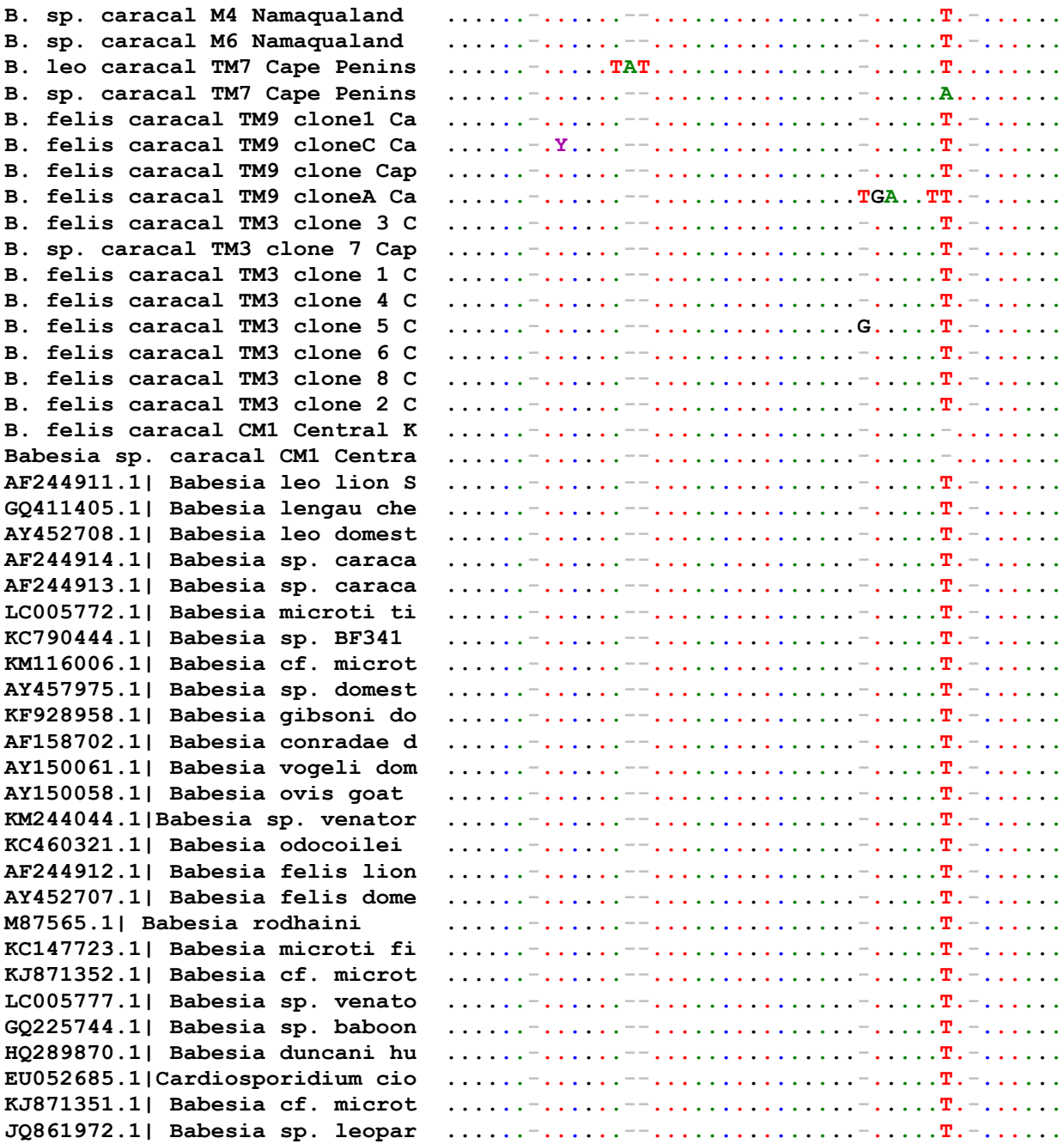
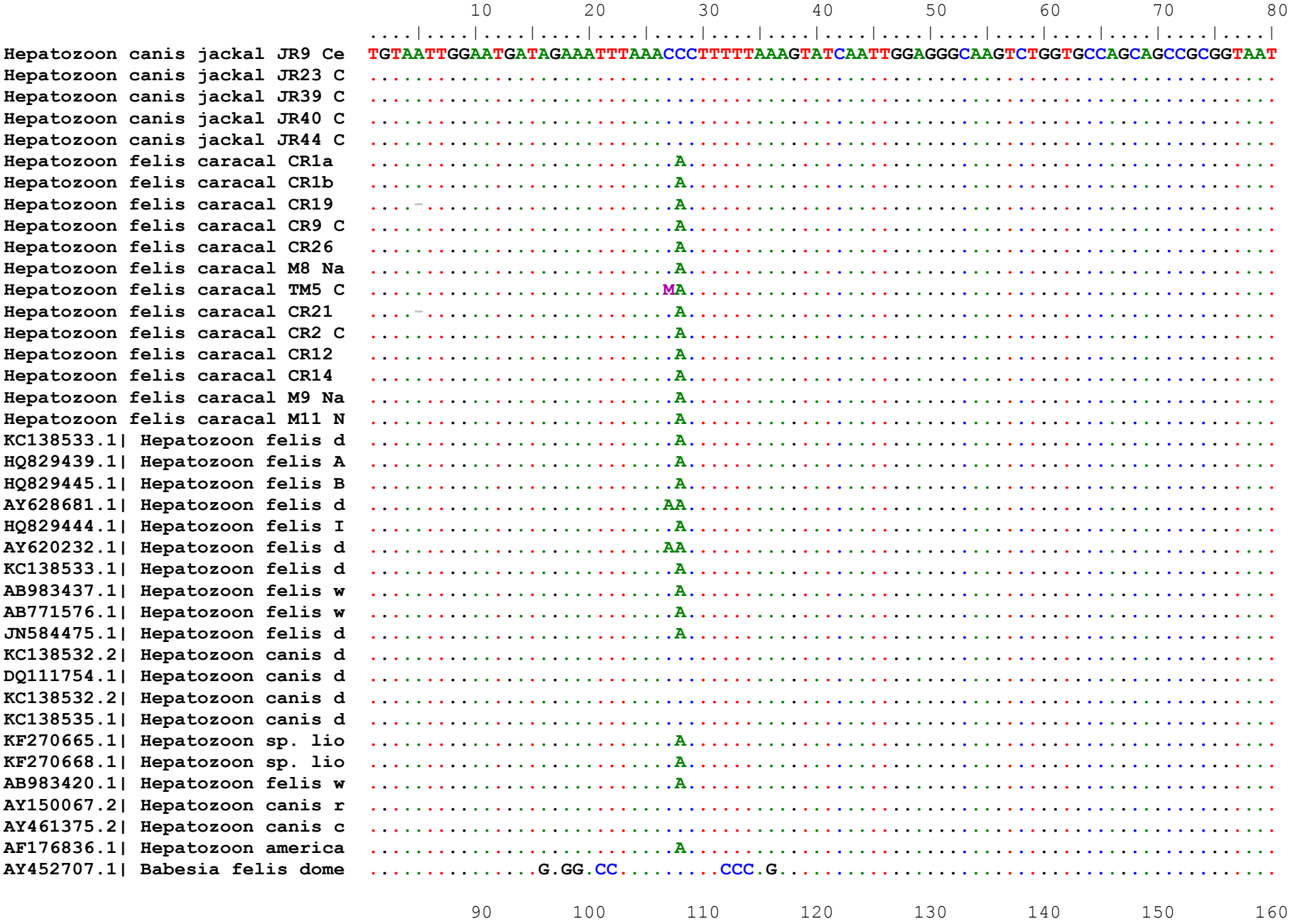
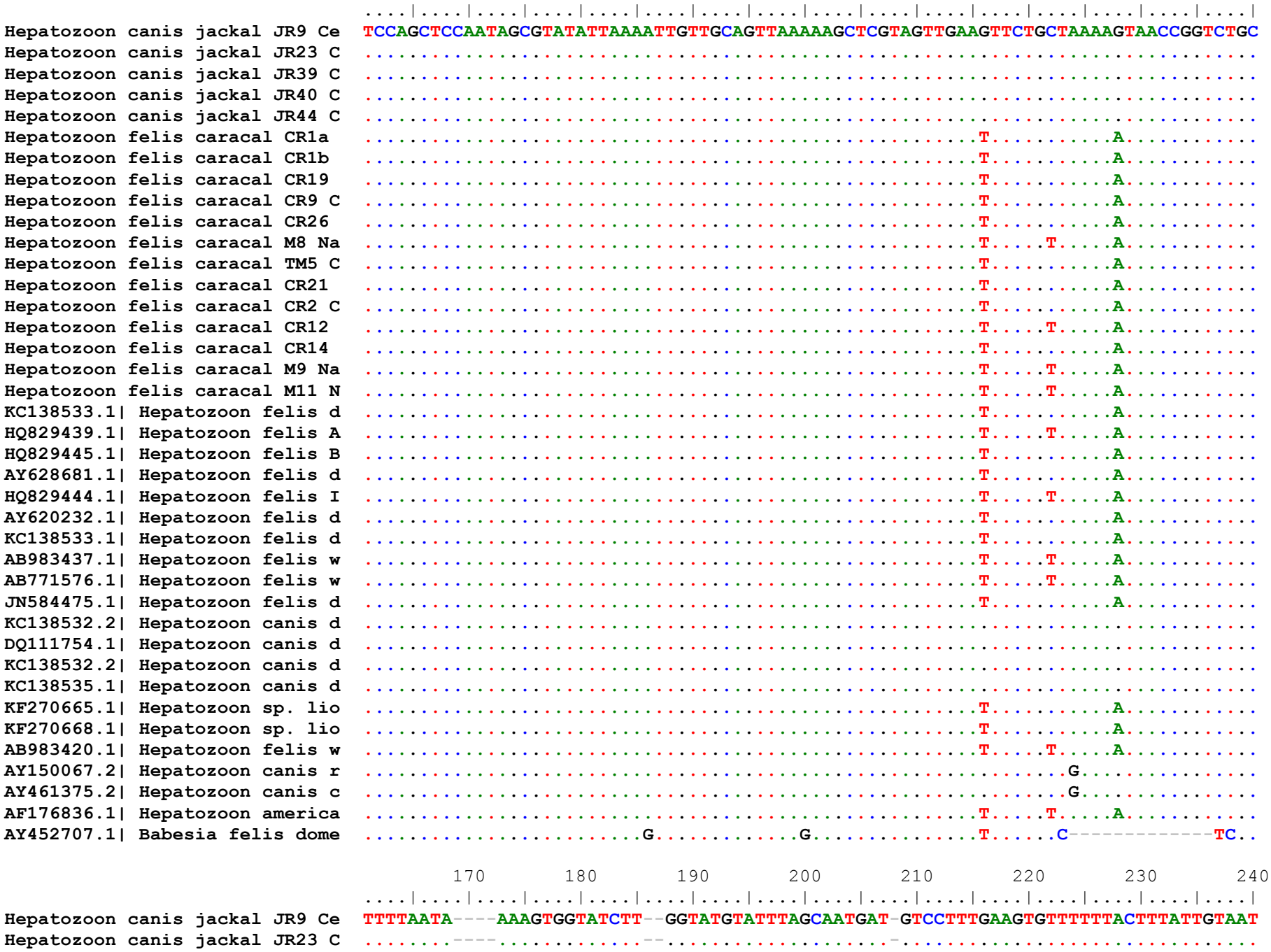
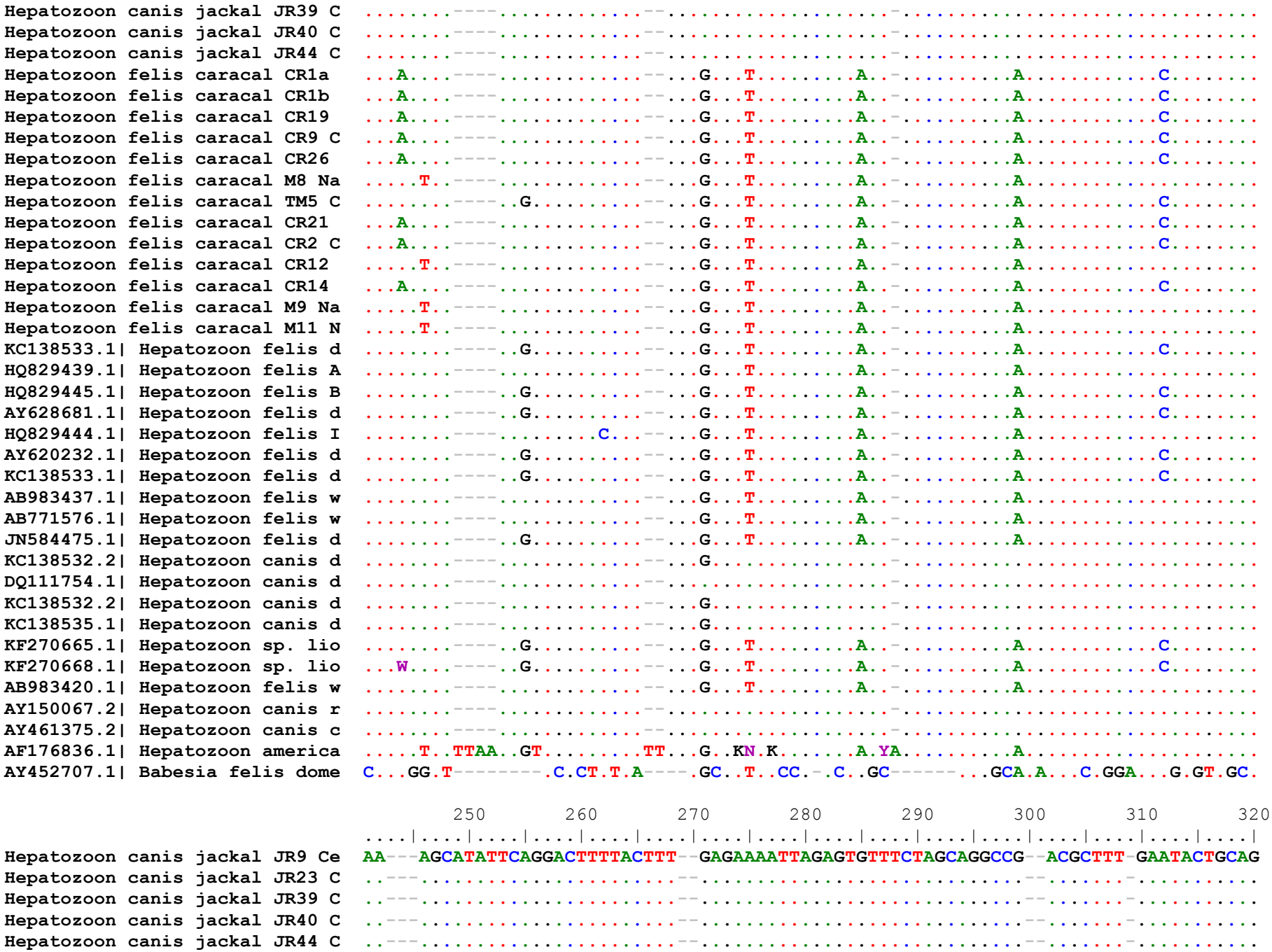
B. felis caracal TM3 clone 3 C
B. sp. caracal TM3 clone 7 Cap
B. felis caracal TM3 clone 1 C
B. felis caracal TM3 clone 4 C
B. felis caracal TM3 clone 5 C
B. felis caracal TM3 clone 6 C
B. felis caracal TM3 clone 8 C
B. felis caracal TM3 clone 2 C
B. felis caracal CM1 Central K
Babesia sp. caracal CM1 Centra
AF244911.1| Babesia leo lion S
GQ411405.1| Babesia lengau che
AY452708.1| Babesia leo domest
AF244914.1| Babesia sp. caraca
AF244913.1| Babesia sp. caraca
LC005772.1| Babesia microti ti
KC790444.1| Babesia sp. BF341
KM116006.1| Babesia cf. microt
AY457975.1| Babesia sp. domest
KF928958.1| Babesia gibsoni do
AF158702.1| Babesia conradae d
AY150061.1| Babesia vogeli dom
AY150058.1| Babesia ovis goat
KM244044.1|Babesia sp. venator
KC460321.1| Babesia odocoilei
AF244912.1| Babesia felis lion
AY452707.1| Babesia felis dome
M87565.1| Babesia rodhaini
KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar
410
420
430
440
B. sp. caracal CM8 Cape Penins
B. felis caracal TM1 Cape Peni
B. felis caracal TM8 clone 1 C
B. felis caracal TM8 clone 4 C
B. felis caracal TM8 clone 6 C

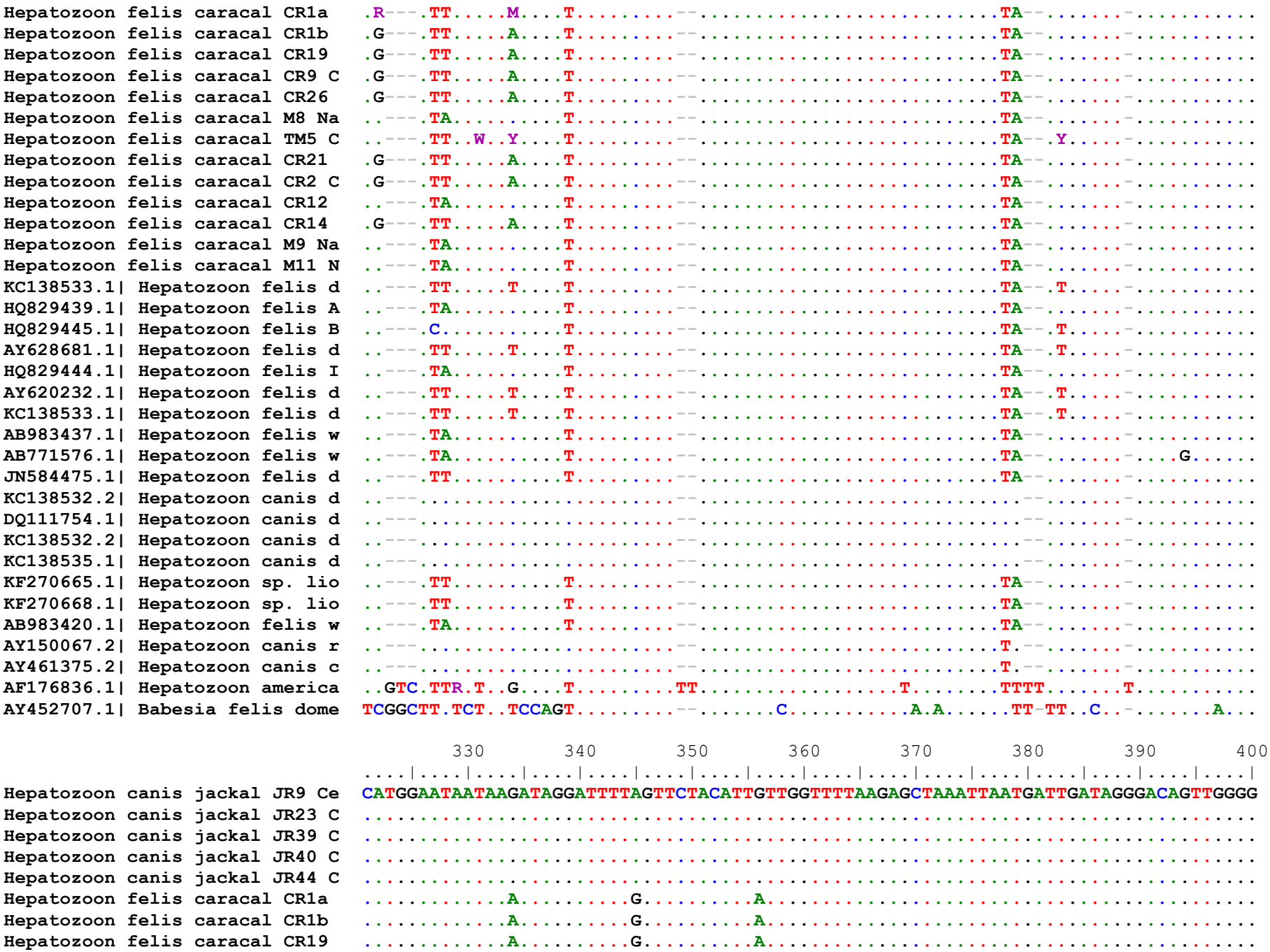

B. sp. caracal M4 Namaqualand
B. sp. caracal M6 Namaqualand
B. leo caracal TM7 Cape Penins
B. sp. caracal TM7 Cape Penins
B. felis caracal TM9 clone1 Ca
B. felis caracal TM9 cloneC Ca
B. felis caracal TM9 clone Cap
B. felis caracal TM9 cloneA Ca
B. felis caracal TM3 clone 3 C
B. sp. caracal TM3 clone 7 Cap
B. felis caracal TM3 clone 1 C
B. felis caracal TM3 clone 4 C
B. felis caracal TM3 clone 5 C
B. felis caracal TM3 clone 6 C
B. felis caracal TM3 clone 8 C
B. felis caracal TM3 clone 2 C
B. felis caracal CM1 Central K
Babesia sp. caracal CM1 Centra
AF244911.1| Babesia leo lion S
GQ411405.1| Babesia lengau che
AY452708.1| Babesia leo domest
AF244914.1| Babesia sp. caraca
AF244913.1| Babesia sp. caraca
LC005772.1| Babesia microti ti
KC790444.1| Babesia sp. BF341
KM116006.1| Babesia cf. microt
AY457975.1| Babesia sp. domest
KF928958.1| Babesia gibsoni do
AF158702.1| Babesia conradae d
AY150061.1| Babesia vogeli dom
AY150058.1| Babesia ovis goat
KM244044.1|Babesia sp. venator
KC460321.1| Babesia odocoilei
AF244912.1| Babesia felis lion
AY452707.1| Babesia felis dome
M87565.1| Babesia rodhaini
KC147723.1| Babesia microti fi
KJ871352.1| Babesia cf. microt
LC005777.1| Babesia sp. venato
GQ225744.1| Babesia sp. baboon
HQ289870.1| Babesia duncani hu
EU052685.1|Cardiosporidium cio
KJ871351.1| Babesia cf. microt
JQ861972.1| Babesia sp. leopar

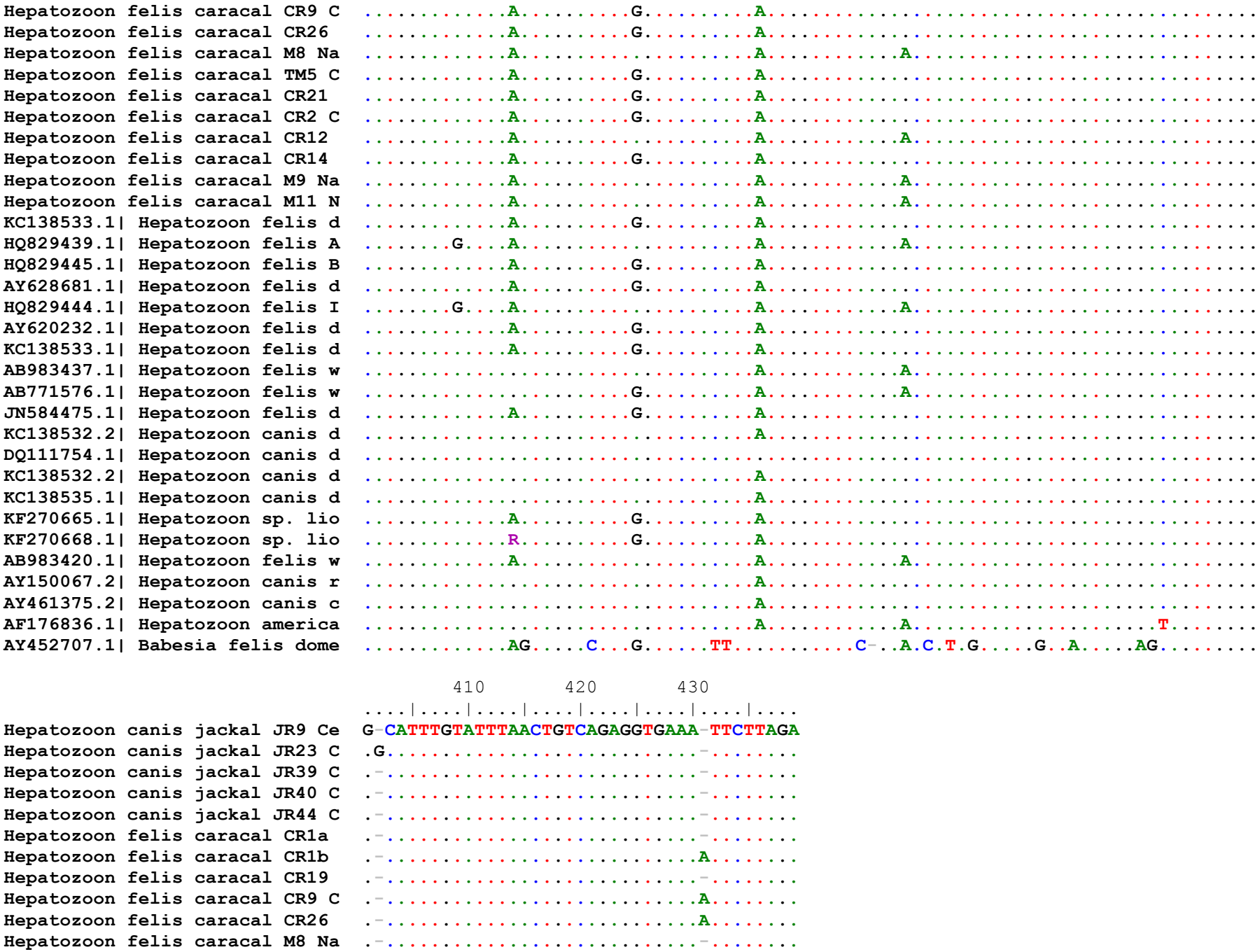
Appendix 4.5: Sequence alignment of 18S rRNA *Hepatozoon* sequences (439bp)

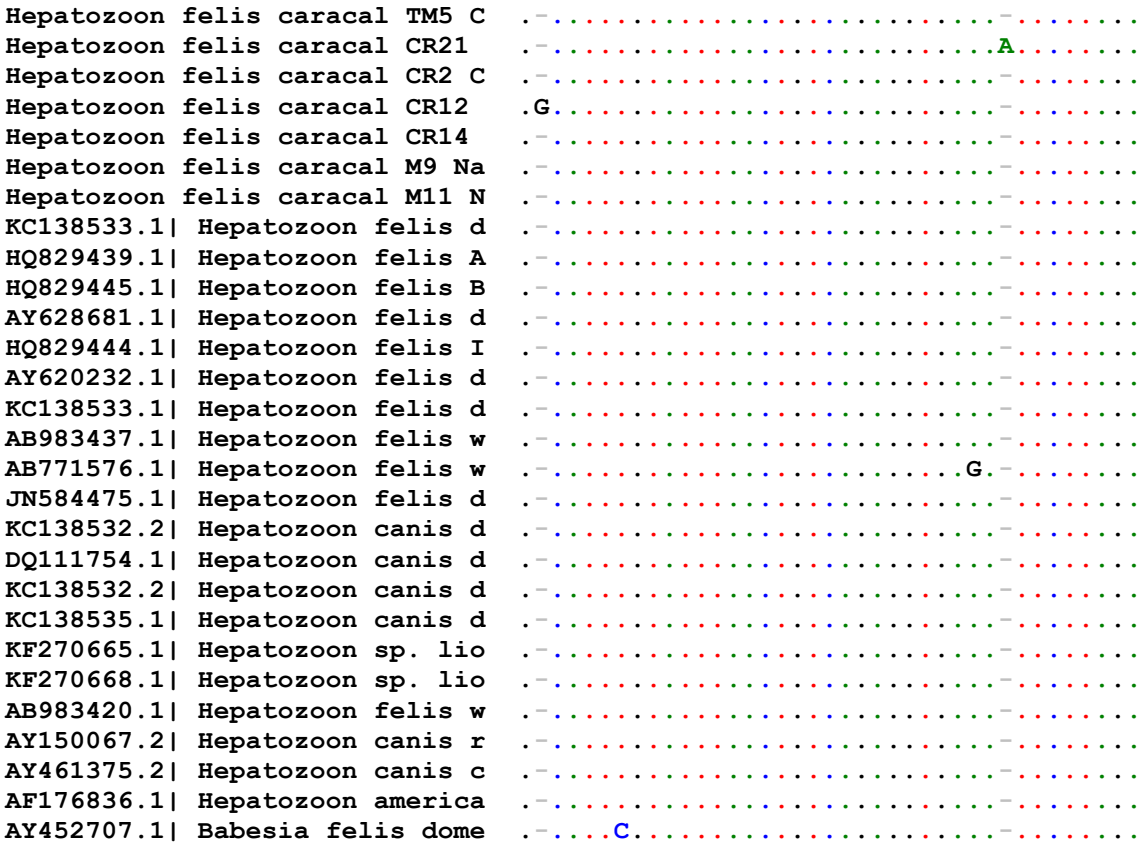



Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR1b
Hepatozoon felis caracal CR19
Hepatozoon felis caracal CR9 C
Hepatozoon felis caracal CR26
Hepatozoon felis caracal M8 Na
Hepatozoon felis caracal TM5 C
Hepatozoon felis caracal CR21
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
AY628681.1| Hepatozoon felis d
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138533.1| Hepatozoon felis d
AB983437.1| Hepatozoon felis w
AB771576.1| Hepatozoon felis w
JN584475.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
KF270665.1| Hepatozoon sp. lio
KF270668.1| Hepatozoon sp. lio
AB983420.1| Hepatozoon felis w
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
TCCAGCTCCAATAGCGTATATTAAAATTGTTGCAGTTAAAAAGCTCGTAGTTGAAGTTCTGCTAAAAGTAACCGGTCTGC
170
180
190
200
210
220
230
240
TTTTAATA----AAAGTGGTATCTT--GGTATGTATTTAGCAATGAT-GTCCTTTGAAGTGTTTTTTACTTTATTGTAAT

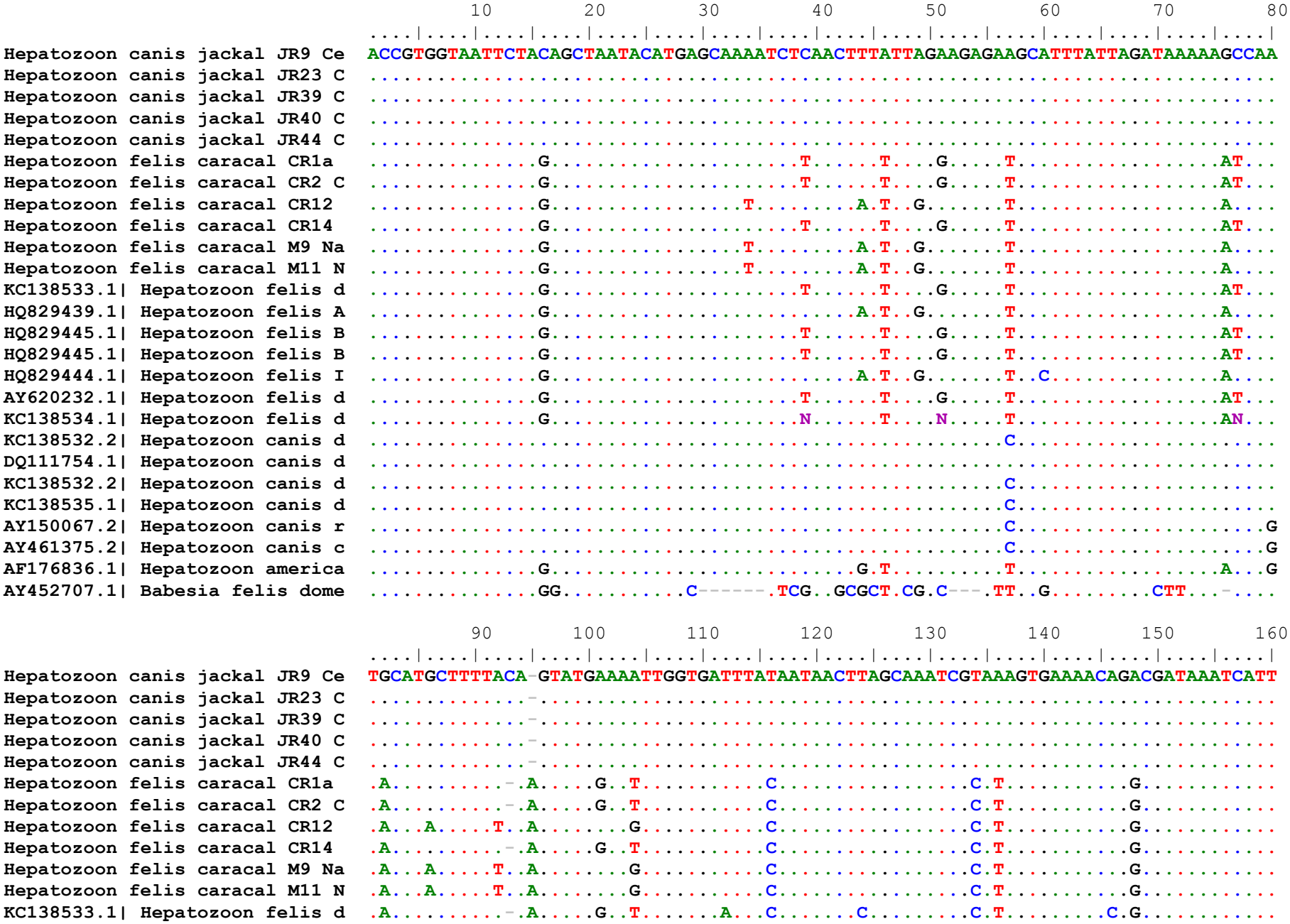

Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR1b
Hepatozoon felis caracal CR19
Hepatozoon felis caracal CR9 C
Hepatozoon felis caracal CR26
Hepatozoon felis caracal M8 Na
Hepatozoon felis caracal TM5 C
Hepatozoon felis caracal CR21
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
AY628681.1| Hepatozoon felis d
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138533.1| Hepatozoon felis d
AB983437.1| Hepatozoon felis w
AB771576.1| Hepatozoon felis w
JN584475.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
KF270665.1| Hepatozoon sp. lio
KF270668.1| Hepatozoon sp. lio
AB983420.1| Hepatozoon felis w
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
250
260
270
280
290
300
310
320
Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C

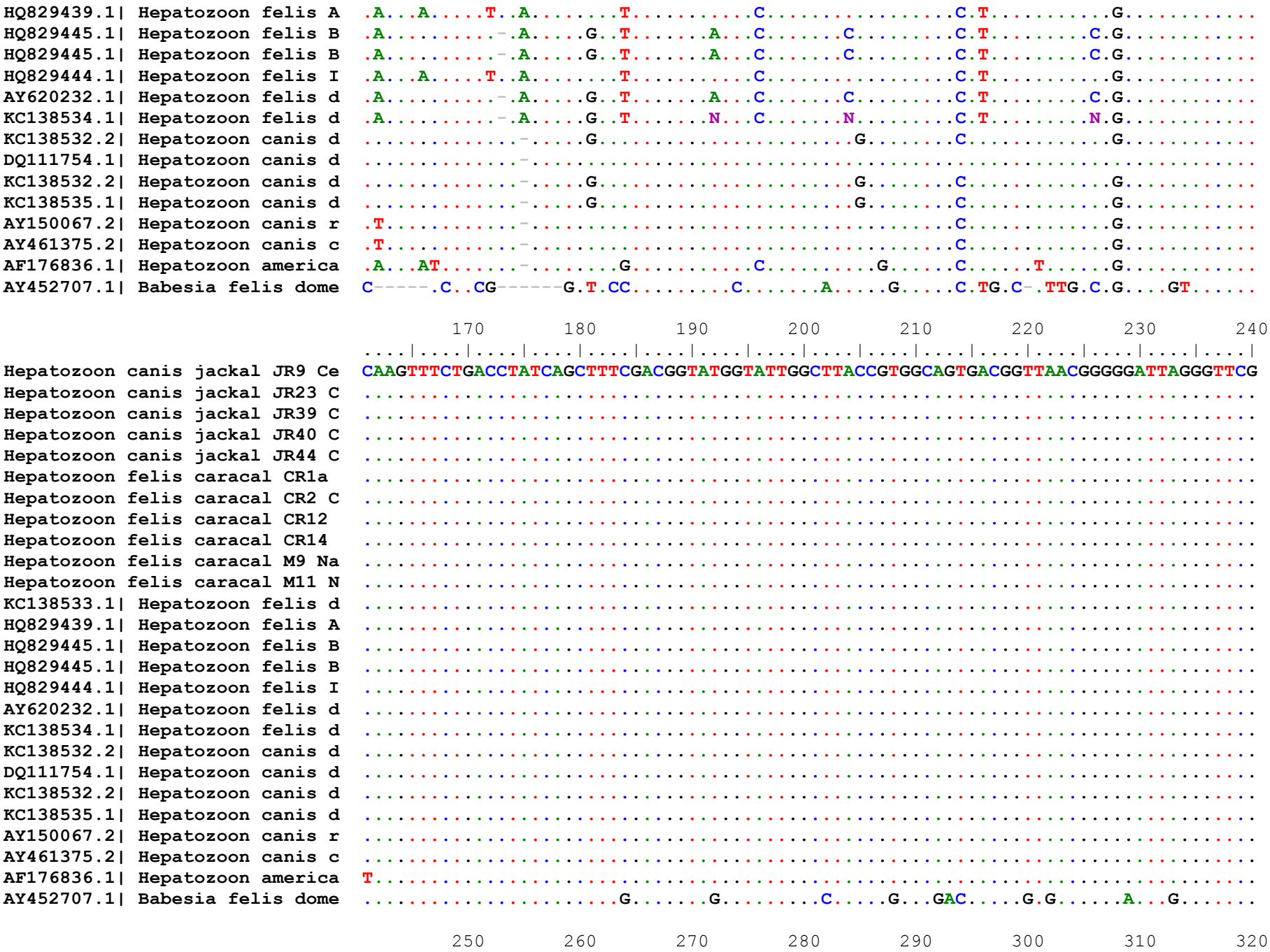

Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR1b
Hepatozoon felis caracal CR19
Hepatozoon felis caracal CR9 C
Hepatozoon felis caracal CR26
Hepatozoon felis caracal M8 Na
Hepatozoon felis caracal TM5 C
Hepatozoon felis caracal CR21
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
AY628681.1| Hepatozoon felis d
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138533.1| Hepatozoon felis d
AB983437.1| Hepatozoon felis w
AB771576.1| Hepatozoon felis w
JN584475.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
KF270665.1| Hepatozoon sp. lio
KF270668.1| Hepatozoon sp. lio
AB983420.1| Hepatozoon felis w
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
330
340
350
360
370
380
390
400
Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR1b
Hepatozoon felis caracal CR19


Hepatozoon felis caracal CR9 C
Hepatozoon felis caracal CR26
Hepatozoon felis caracal M8 Na
Hepatozoon felis caracal TM5 C
Hepatozoon felis caracal CR21
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
AY628681.1| Hepatozoon felis d
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138533.1| Hepatozoon felis d
AB983437.1| Hepatozoon felis w
AB771576.1| Hepatozoon felis w
JN584475.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
KF270665.1| Hepatozoon sp. lio
KF270668.1| Hepatozoon sp. lio
AB983420.1| Hepatozoon felis w
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
410
420
430
Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR1b
Hepatozoon felis caracal CR19
Hepatozoon felis caracal CR9 C
Hepatozoon felis caracal CR26
Hepatozoon felis caracal M8 Na


Hepatozoon felis caracal TM5 C
Hepatozoon felis caracal CR21
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
AY628681.1| Hepatozoon felis d
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138533.1| Hepatozoon felis d
AB983437.1| Hepatozoon felis w
AB771576.1| Hepatozoon felis w
JN584475.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
KF270665.1| Hepatozoon sp. lio
KF270668.1| Hepatozoon sp. lio
AB983420.1| Hepatozoon felis w
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome

Appendix 4.6: Sequence alignment of 18S rRNA *Hepatozoon* sequences (863bp)

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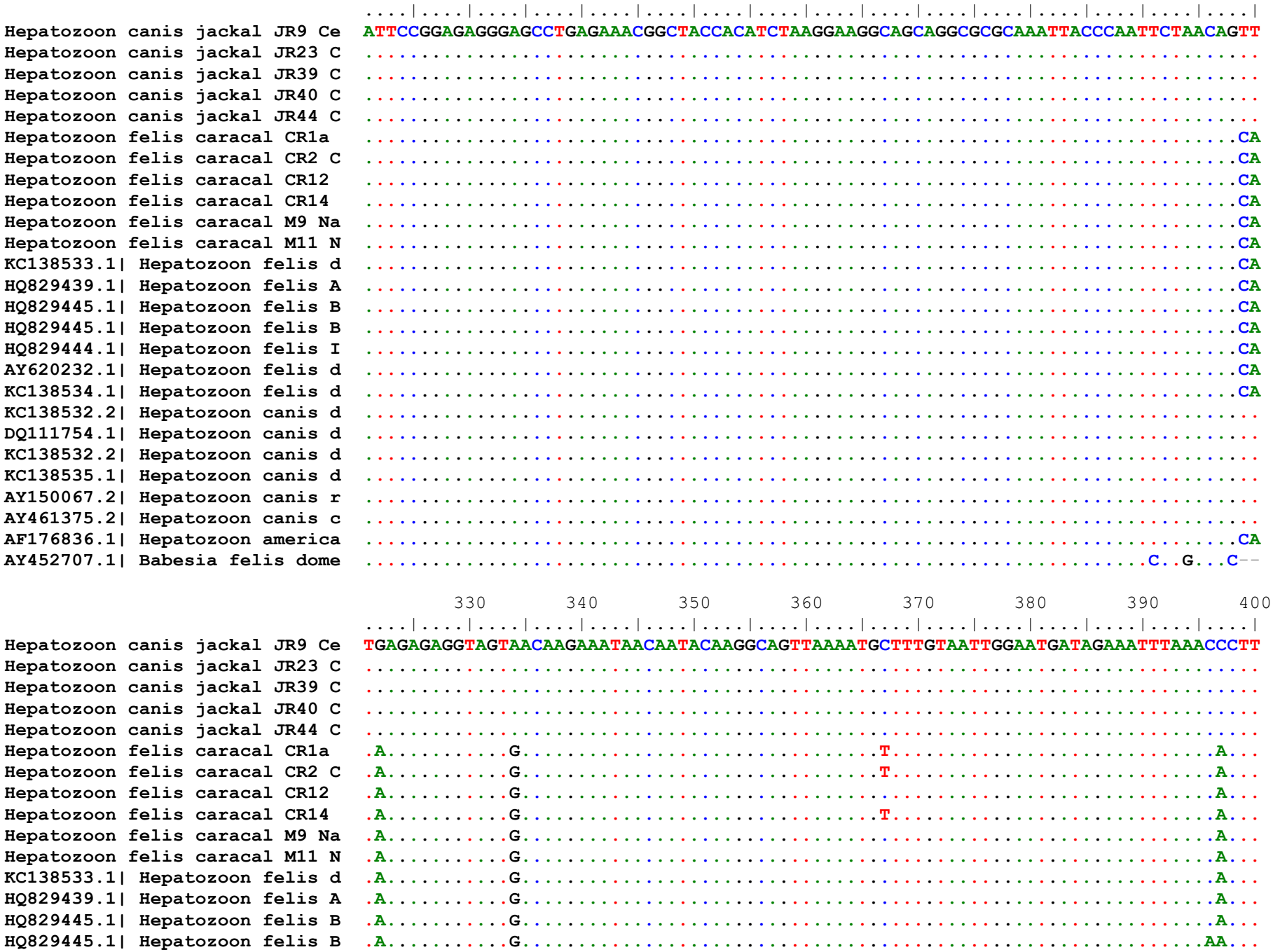
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
170
180
190
200
210
220
230
240
Hepatozoon canis jackal JR9 Ce
CAAGTTTCTGACCTATCAGCTTTCGACGGTATGGTATTGGCTTACCGTGGCAGTGACGGTTAACGGGGGATTAGGGTTCG
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
250
260
270
280
290
300
310
320


Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
330
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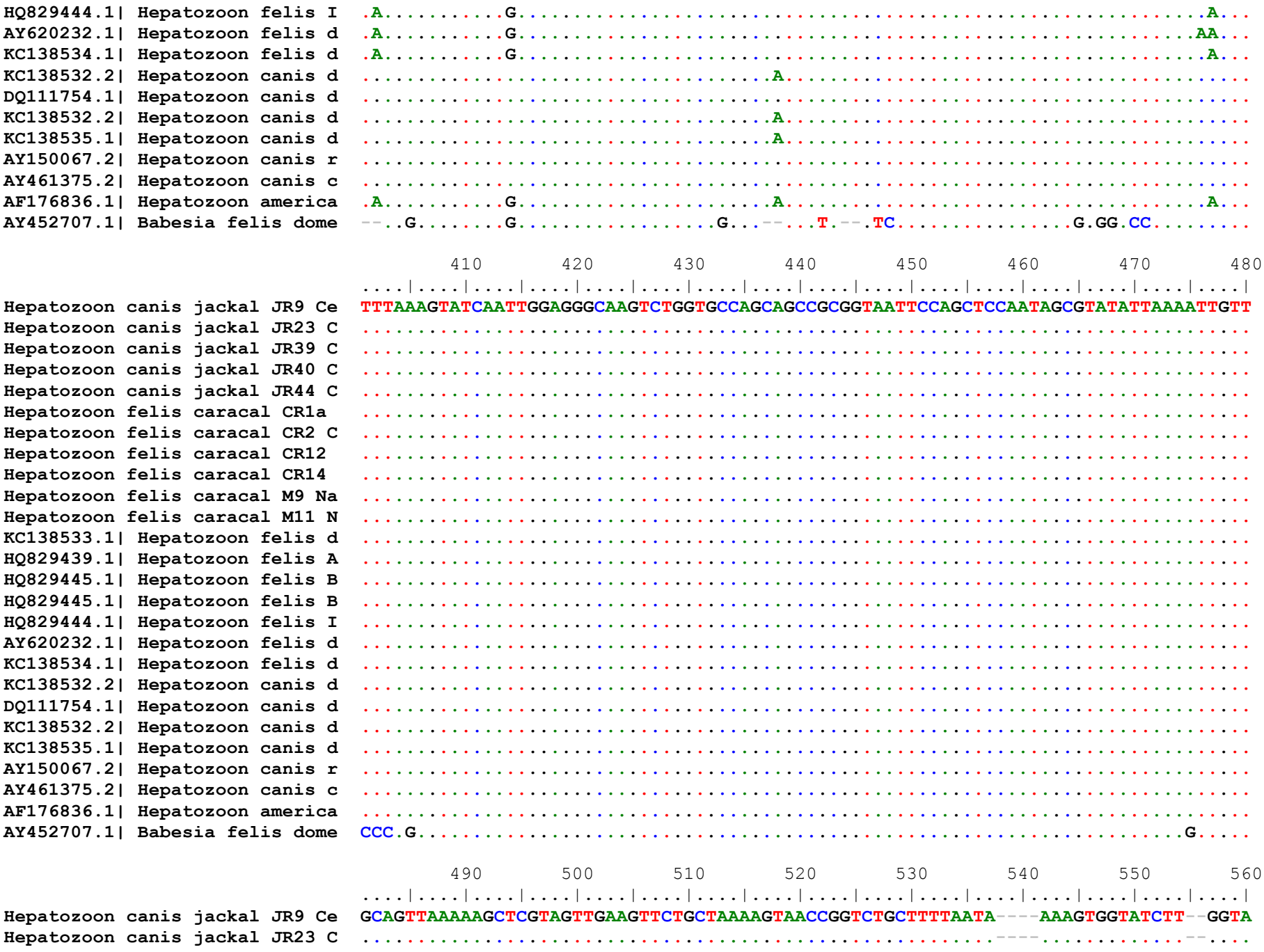
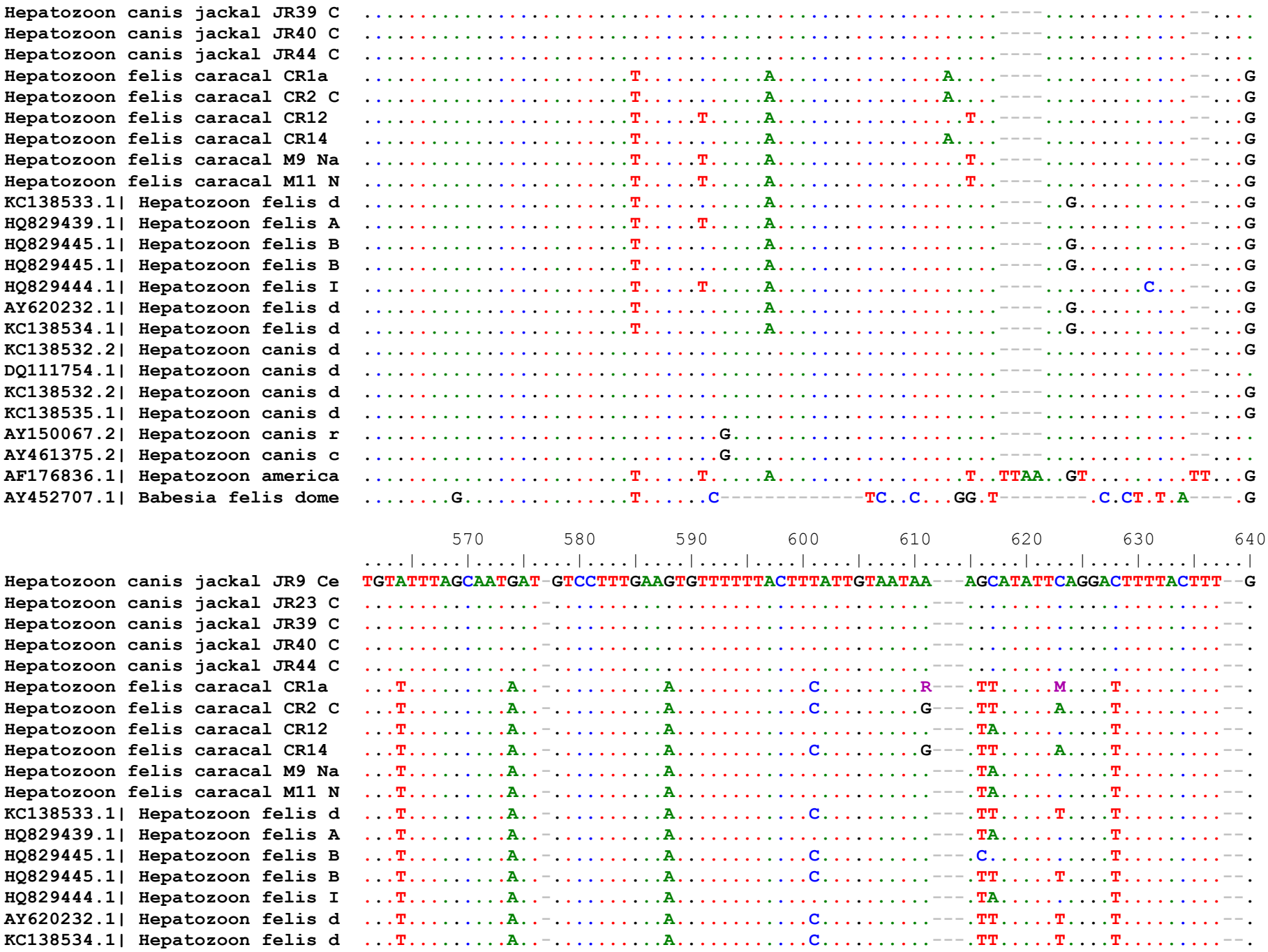
370
380
390
400


HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
410 420 430 440 450 460 470 480
Hepatozoon canis jackal JR9 Ce
TTTAAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAATTGTT
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
490 500 510 520 530 540 550 560
Hepatozoon canis jackal JR9 Ce
GCAGTTAAAAAGCTCGTAGTTGAAGTTCTGCTAAAAGTAACCGGTCTGCTTTTAATA----AAAGTGGTATCTT--GGTA
Hepatozoon canis jackal JR23 C


Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
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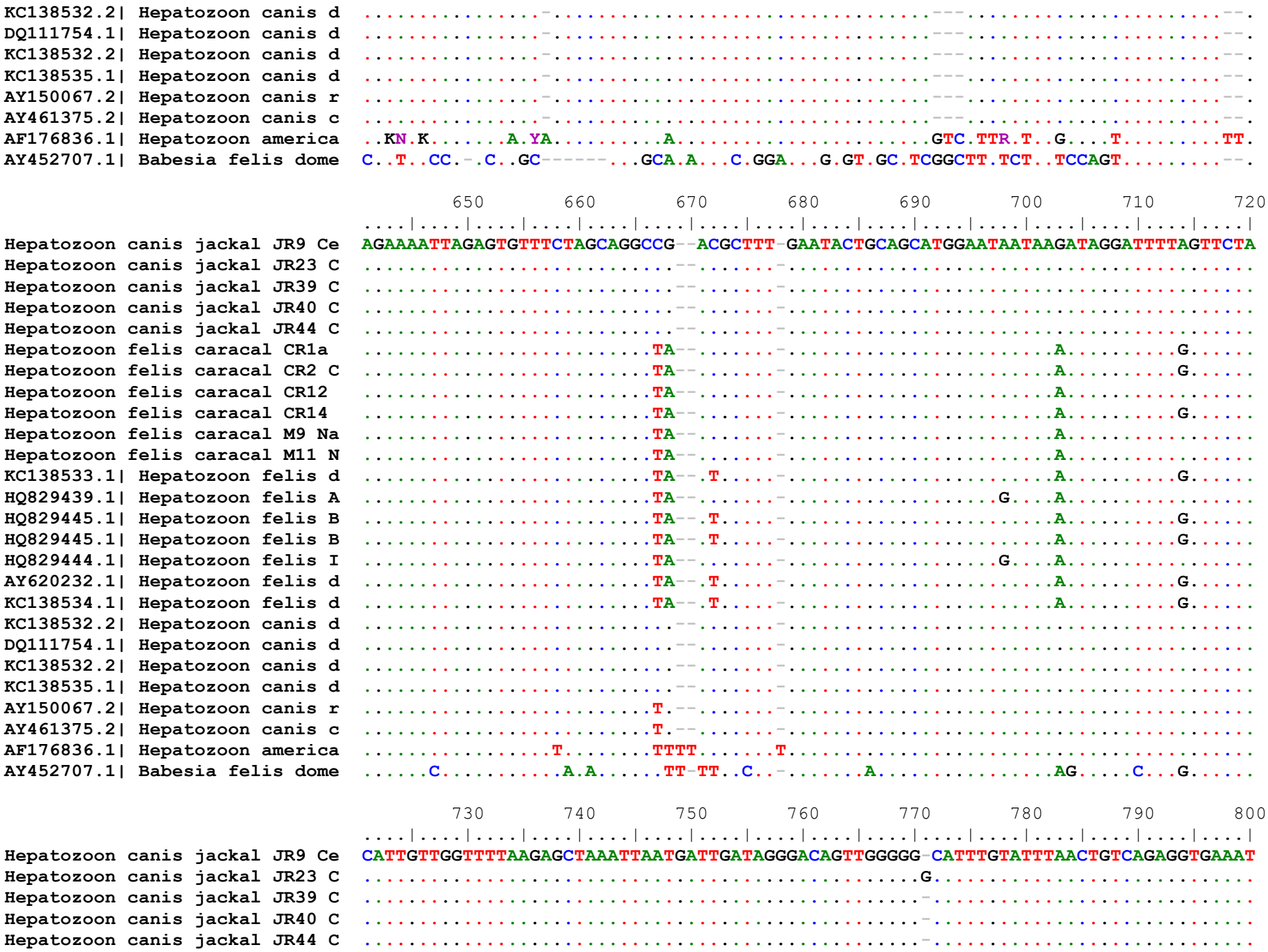
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
570
580
590
600
610
620
630
640
Hepatozoon canis jackal JR9 Ce
TGTATTTAGCAATGAT-GTCCTTTGAAGTGTTTTTTACTTTATTGTAATAA---AGCATATTCAGGACTTTTACTTT--G
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d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DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
650
660
670
680
690
700
710
720
Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
730
740
750
760
770
780
790
800
Hepatozoon canis jackal JR9 Ce
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
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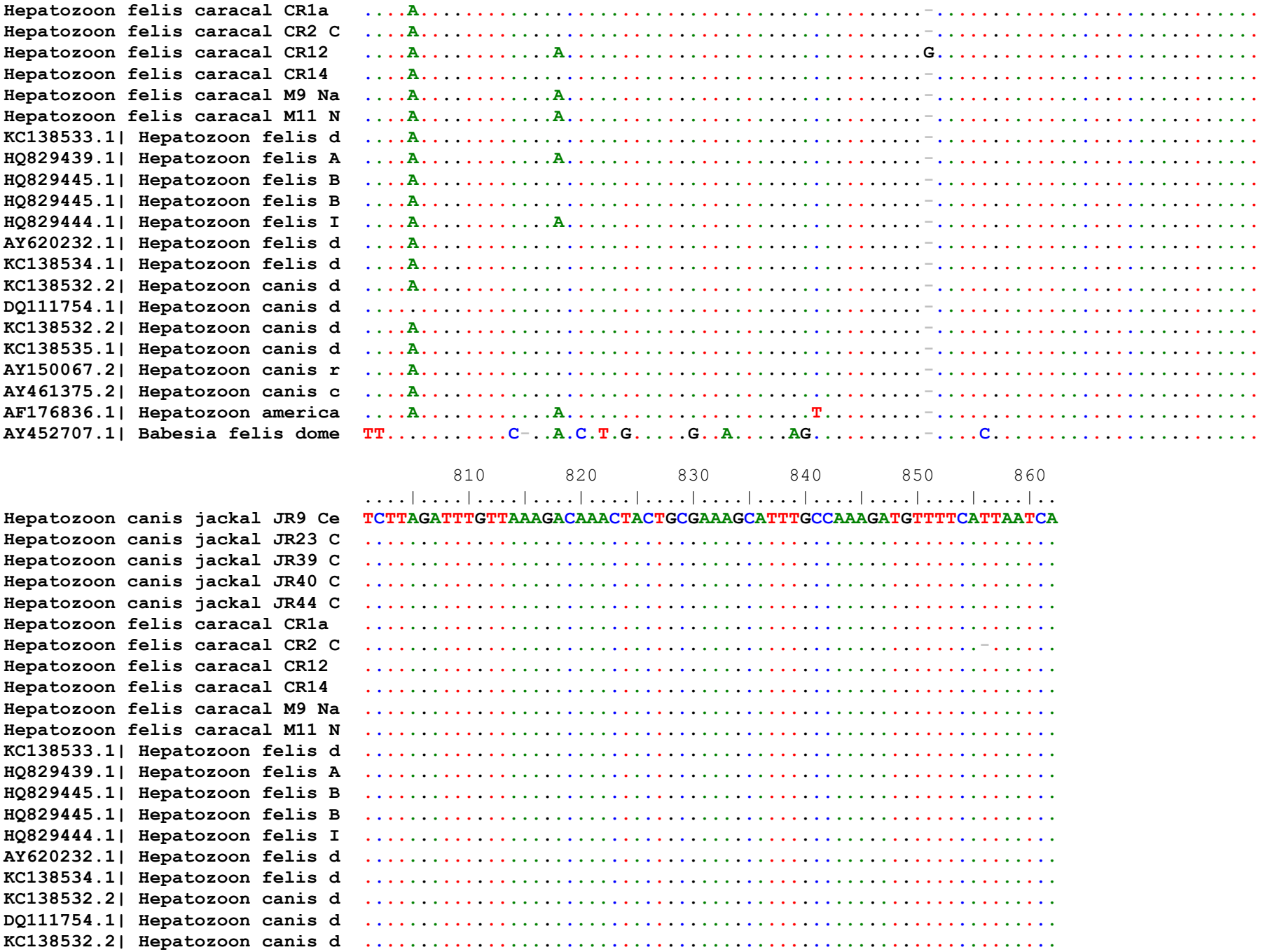
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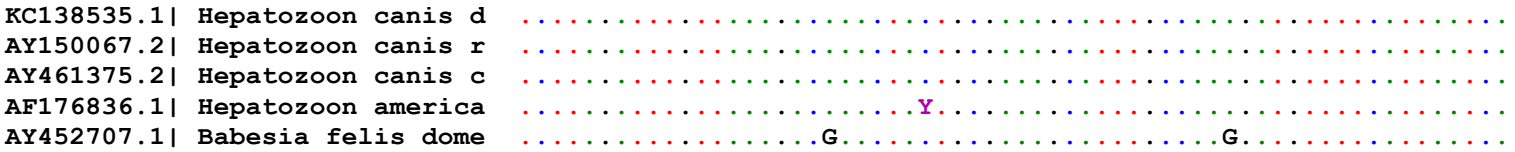
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d
KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
AY461375.2| Hepatozoon canis c
AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome
810
820
830
840
850
860
Hepatozoon canis jackal JR9 Ce
TCTTAGATTTGTTAAAGACAAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCA
Hepatozoon canis jackal JR23 C
Hepatozoon canis jackal JR39 C
Hepatozoon canis jackal JR40 C
Hepatozoon canis jackal JR44 C
Hepatozoon felis caracal CR1a
Hepatozoon felis caracal CR2 C
Hepatozoon felis caracal CR12
Hepatozoon felis caracal CR14
Hepatozoon felis caracal M9 Na
Hepatozoon felis caracal M11 N
KC138533.1| Hepatozoon felis d
HQ829439.1| Hepatozoon felis A
HQ829445.1| Hepatozoon felis B
HQ829445.1| Hepatozoon felis B
HQ829444.1| Hepatozoon felis I
AY620232.1| Hepatozoon felis d
KC138534.1| Hepatozoon felis d
KC138532.2| Hepatozoon canis d
DQ111754.1| Hepatozoon canis d
KC138532.2| Hepatozoon canis d


KC138535.1| Hepatozoon canis d
AY150067.2| Hepatozoon canis r
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AF176836.1| Hepatozoon america
AY452707.1| Babesia felis dome