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Babesia capensis sp. nov. – Description of a Babesia species causing disease in domestic cats from South Africa

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Abstract

Introduction:

A large-form piroplasm that caused disease in domestic cats in the Western Cape Province in South Africa was provisionally termed *Babesia* sp. Western Cape. The aim of this study was to characterize and formally name this novel piroplasm.

Methods

Blood samples from infected domestic cats were evaluated for piroplasmid infection by microscopy of stained blood smears and by PCR for three piroplasm genes using five different PCR protocols. DNA amplified by PCR was sequenced and analyzed phylogenetically. DNA extracted from blood of caracals was also tested by PCR. Clinical history from infected cats was obtained and response to treatment was recorded.

Results

Microscopic evaluation of cat blood smears revealed infection with a large-form *Babesia* sp. with merozoites measuring 2.11 x 1.41 µm, within the size range of other feline *Babesia* sensu stricto species, and larger than small-form species of other piroplasm clades. Analysis of *CytB*, *HSP70* and *18S rRNA* gene sequences from infected cats indicated that they had identical *CytB* sequences, almost identical (> 99.6%) *HSP70* sequences, and their *18S rRNA* had > 99.06% identity to *Babesia* sp. Western Cape. Phylogenetic analyses showed that sequences of these genes clustered together with strong support and away from other species within *Babesia* s.s. Based on these findings we suggest to erect a new taxon and name the previously provisionally termed *Babesia* sp. Western Cape as *Babesia capensis* sp. nov. *18S rRNA* sequences from the cats were 100% identical to GenBank MK616205.1 of a *Babesia* sp. from a caracal identified in an earlier study as infected with *Babesia* sp. Western Cape. Two caracals tested in the current study were positive for the *CytB* gene with sequences that were 98.24–98.6% identical to the sequences amplified from the *B. capensis* sp. nov.-infected cats. Cats infected with *B. capensis* sp. nov. had fever, lethargy and in some cases were anemic or icteric. They responded with clinical improvement to treatment with diminazene aceturate at 2 mg/kg intramuscularly.

Conclusions

Babesia capensis sp. nov., a piroplasmid sp. causing disease in cats, is herein described as a new taxon based on morphology, genetic and phylogenetic characterization.

Background

As diagnostic procedures become more sophisticated, an increasing number of *Babesia* species is recorded from domestic cats. In some instances, the findings are incidental, with cats showing no clinical signs, but some infections are associated with severe clinical manifestations. Furthermore, there is mounting evidence that *Babesia* spp. infecting felids are not necessarily host-specific and may infect multiple vertebrate hosts [1]. With indigenous felid species occurring on all continents, except Australia and Antarctica, more novel *Babesia* spp. infecting domestic cats will probably be discovered and described in due course.

Nine *Babesia* spp. infecting domestic cats were documented in a review published in 2020 [2], comprising four felid-associated species (*Babesia felis*, *Babesia hongkongensis*, *Babesia lengau* and *Babesia leo*), three dog-associated species (*Babesia canis* s.s., *Babesia gibsoni* and *Babesia vogeli*) and three others (*Babesia lohae*, *Babesia microti* and *Babesia vulpes*). The subspecies *B. canis presentii* [3], which was associated with clinical signs in domestic cats, was also mentioned. Two early attempts to infect domestic cats with *Babesia rossi*, considered the most virulent canine *Babesia* species, were unsuccessful [4, 5], as was a subsequent attempt with splenectomised cats [6]. In recent years, two further novel species associated with clinical signs have been described from domestic cats: *Babesia panickeri* [7] in India and *Babesia gallileei* [8] in Israel. This increases the tally of *Babesia* spp. known to infect domestic cats to eleven.

The vast majority of clinical cases of feline babesiosis in South Africa is caused by small piroplasms, especially *B. felis*, although *B. lengau* and *B. leo* have also been incriminated [1, 2, 9]. A large *Babesia* causing disease in a cat was reported from suburban Harare, Zimbabwe [10]. Veterinarians practising in South Africa, especially in the Western Cape Province, occasionally encounter clinical cases in cats infected with large *Babesia* piroplasms. Blood specimens submitted for laboratory diagnosis confirmed the presence of a hitherto unknown *Babesia* species. As no blood smears were available to serve as type specimens, it was not described and named formally, but tentatively referred to as *Babesia* sp. Western Cape [9]. Except for one cat from KwaZulu-Natal, all positive cats were from Cape Town and surrounding areas.

An appeal to practitioners in the Western Cape Province to be on the lookout for these sporadic cases bore fruit. More specimens, both blood in EDTA and blood smears, have recently become available. This paved the way for more in-depth molecular characterisation, morphological description and formal naming of the novel species.

Associated with the findings from domestic cats in the Western Cape Province, some caracals (*Caracal caracal*) living in human-altered landscapes in the Western and Northern Cape Provinces were found to be infected with a *Babesia* that did not match any of the known species [11]. Blood specimens from this study were available for further analysis and comparison with the novel species in domestic cats. Based on the findings from cats and caracals on what appeared to be a distinct parasite, the aim of this study was to morphologically describe, genetically characterize and record clinical findings associated with this piroplasm.

Methods

Animal samples

Samples of DNA extracted from blood (n=4), blood spotted on filter paper (n=8), or blood smear (n=1), were used for genetic characterization. The samples originated from domestic cats (n=8) and caracals (n=5) suspected or confirmed previously of piroplasm infection by observation of blood smears and/or PCR. The project and exportation of samples were carried out with approval of the University of Pretoria Animal Ethics Committee (reference REC018-25) and an import permit from the Israeli Ministry of Agriculture (no. 9/2025). The South African Department of Agriculture, Land Reform and Rural Development granted permission for this research in terms of the Animal Diseases Act (Act no 35 of 1984), reference 12/11/1/1/8 (17249AC).

Piroplasm parasites were detected in erythrocytes by light microscopy in blood smears stained by Romanowsky-type staining solutions in two domestic cats with clinical babesiosis from specimens submitted to the Faculty of Veterinary Science, University of Pretoria, for diagnostic purposes. These blood smears were used for the measurement and description of the parasite's life stages. Blood smears were visualized by oil immersion microscopy at 500× and 1000× magnifications and parasite sizes were measured using a micrometer.

Molecular detection of Babesia in animal samples

DNA Extraction

DNA was extracted from blood specimens using the QIAamp® DNA Mini Kit (Qiagen, Whitehead Scientific, South Africa) and from dried blood spots using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's protocol for dried blood spots. Three pieces of 3 mm diameter each from dried blood spots were placed in 1.5 ml microcentrifuge tubes with 180 µl Buffer ATL and incubated at 85°C for 10 min after which 20 µl of proteinase K was added and mixture was incubated at 56°C for 1 hour. Protocol was continued according to standard protocol for DNA extraction from tissue. In one case, DNA was extracted from a blood smear on a glass slide using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA) according to Vince et al. 1998 [12].

PCR

Babesia spp. molecular characterization was done using five different PCR protocols with different primers and conditions (Table 1). PCR was performed in a total volume of 25 µl using the PCR-ready High Specificity mix (Syntezza Bioscience, Jerusalem, Israel) with 4 µl of DNA template, 400 nM of each primer and sterile DNase/RNase-free water (Sigma, St. Loius, MO, USA). Amplification was performed by a programmable conventional thermocycler (Biometra, Goettingen, Germany). Initial denaturation at 95°C for 5 min, was followed by 35-45 cycles of denaturation at 95°C for 30 s, annealing and extension conditions (Table1) and final extension at 72°C for 30 s, unless detailed otherwise. The extension step was continued, after the last cycle, the for an additional 5 min. PCR products were electrophoresed on

1.5% agarose gels stained using Red-Safe (Intron Biotechnology, Korea) and evaluated for the size of amplified fragments under UV light by comparison to a 100 bp DNA molecular weight marker. Positive DNA controls from dogs naturally infected with *B. vogeli* were run with each corresponding reaction. Non-template control (NTC) reactions were performed by using the same reagents and procedures described above but without DNA added to the reaction to rule out contaminations.

DNA from all the positive PCR products was sequenced at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel, using the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

PCR was performed to amplify DNA of three different piroplasmids genes including the *Heat shock protein 70 (HSP70*), *Cytochrome b* (*Cytb*), and the *18S rRNA* (Table 1). Segments of the 18S *rRNA* gene were amplified using three protocols and primer sets because some of the samples could only be amplified by certain protocols and not by others. The near full-length of the piroplasma *18S rRNA* gene was sequenced in parts using 3.2 pmol of the following primers: Nbab1F (5'-AAGCCATGCATGTCTAAGTATAAGCTTTT-3'), TB Rev (5'-AATAATTCACCGGATCACTCG-3'), BT 2R (5'-CCCGTGTTGAGTCAAATTAAGCCG-3'), BT 3F (5'-GGGCATTCGTATTTAACTGTCAGAGG-3'), Nbab 4F (5'-CCGTTAACGGAACGAGACCTTAACC-3') and Nbab 4R (5'-GGTAGGCCAATACCCTACCG-3') [11]. Sequences were then reconstructed using MEGA version X (http://www.megasoftware.net) [14]. The resulting sequences were evaluated using the ChromasPro software version 2. 1.1 (Technelysium Pty Ltd., Australia) and compared for similarity with sequences available in GenBank, using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). New DNA sequences from *Babesia*-infected cats in this study were deposited as new accessions in GenBank.

Distance matrix and consensus sequence comparing variable regions were compared using Geneious 7.1.9 (https://www.geneious.com) and DNA polymorphism analysis was calculated using DNasp v6 [15].

Phylogenetic analysis

Phylogenetic trees were constructed based on marker gene sequences determined in this study and corresponding relevant sequences of other *Babesia* spp. deposited previously in the GenBank database. Sequences were analyzed using the MEGA version X [12] followed by nucleotide sequence alignment using MUSCLE. Phylogenetic relationships were inferred using IQ-TREE 3.0.1 (http://www.iqtree.org) [16, 17]. Substitution model of each tree was selected via ModelFinder based on the Bayesian Information Criterion (BIC) [18]. Branch support was assessed using 10,000 ultrafast bootstrap replicates.

Results

Family Babesiidae Poche, 1913

Genus *Babesia* Starcovici, 1893

***Babesia capensis* sp. nov.**

Type-host: Domestic cat *Felis catus* Linnaeus, 1758 (Mammalia: Felidae).

Other hosts: Caracal *Caracal caracal* Schreber, 1776 (Mammalia: Felidae).

Type-locality: The village of Jacobsbaai (32.9724 °S, 17.8913°E), Western Cape Province, South Africa (Fig. 1).

Other localities: Domestic cats from St Helena Bay (32.75°S, 18.02°E) and the Cederberg Region (32.07°S, 17.96°E and 32.54°S, 17.28°E), Western Cape Province, as well as caracals from the Central Karoo region (32.66°S, 22.25°E) and Namaqualand (30.12°S, 17.84°E) in the Western and Northern Cape Provinces of South Africa, respectively (Fig. 1).

***Type-material*:** A stained thin blood smear from a 3-year-old neutered male domestic cat (Jessie) containing the holotype (Fig. 2g) was deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession number NMB P 1256. An isotype (stained blood smear) from the same cat was also deposited in the same collection under accession number NMB P 1257, as were paratypes (stained glass smears) from a different cat (Choko) under accession numbers NMB P 1321 and NMB P 1322.

In addition, three genomic DNA samples extracted from the blood of infected cats have been deposited at the Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel as Jessie, RLB 21/124 and RLB 21/125.

Vector: Unknown.

***Representative DNA sequences*:** Present study (GenBank: PX661654-PX661656 PX795690 PX795691, 18S *rRNA*; PX686955-PX686958, *CytB*; PX686951- PX686954, HSP 70).

***ZooBank registration*:** To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN) [19], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:744D3327-D23D-4421-9FBD-C80A6082C3B2. The LSID for the new-name *Babesia capensis* is urn:lsid:zoobank.org:act:5519F6DF-37F4-46D7-9AAE-550200C1093B.

Etymology: The species is named after the Western Cape Province in South Africa, where the first cats infected with this parasite were detected.

Description

All measurements, in micrometers ± standard error, were carried out on parasites within erythrocytes from the blood smears of two cats from the Western Cape Province of South Africa (Jessie and Choko), of which one (Jessie) was also designated as the holotype sample.

Trophozoites [Measurements based on 46 parasites; see Figs. 2a-2e]. Round to elliptical forms measuring 1.45-2.96 µm (2.09 ± 0.37) in length and 1.08-2.04 µm (1.57 ± 0.24) in width (*n* = 46). Trophozoites enter erythrocytes as elongated and slim forms (Fig. 2a) and then transform into round forms (Fig. 2b) which gradually enlarge and become ellipsoidal (Figs. 2c-2d). They then become wider in one pole and start dividing into two merozoites producing a triangular fan-shaped form (Fig. 2e) and later fully dividing and separating into adjacent pear-shaped merozoites (Fig. 2f).

Merozoites

Merozoite sizes were measured separately in erythrocytes where only a single pair of merozoites was present and in erythrocytes where two pairs (four parasites) were detected, as merozoites in erythrocytes with two pairs tended to be smaller than those found in cells harboring four parasites.

Merozoites in erythrocytes containing two parasites measured 1.48-2.96 µm (2.11 ± 0.38) in length and 0.74-1.85 µm (1.41 ± 0.29) in width (*n* = 45) while merozoites in erythrocytes containing four parasites measured 1.25-2.59 µm (2.09 ± 0.35) in length and 0.74-2.22 µm (1.12 ± 0.32) in width (*n* = 32). The length and width of merozoites in erythrocytes containing two parasites were significantly larger than those of merozoites in erythrocytes with four parasites (length: t=3.036, df=75, P=0.003, width: t=4.247, df=75, P<0.001). Pear-shaped merozoites initially attached to each other (Fig. 2f) widen and in some cases appear more spheric (Fig. 2h) and then begin to divide (Fig. 2i) until four separate intracellular merozoites are evident (Fig. 2j), which in some cases form a sea star shape with four merozoites opposing each other (Fig. 2k). These four merozoites may enlarge to take up most of the infected erythrocyte volume (Fig. 2l). Merozoites and trophozoites are frequently found in the same microscopic field in cats with high parasitemia (Fig. 3).

Differential diagnosis

The trophozoite and merozoite stages of *B. capensis* sp. nov. presented in several developmental stages in the erythrocytes of infected cats. After invading erythrocytes, elongated forms developed into rounded forms which enlarged gradually (Figs. 2a-2d) until starting to divide into two merozoites (Fig. 2e). Merozoites that complete division are pear shaped (Fig. 2f) and may initially remain attached to each other by contact of their apical poles (Fig. 2g). They may enlarge and become more spheric (Fig. 2h) and eventually start dividing again (Fig. 2i) forming four merozoites in the same erythrocyte (Fig. 2j). The four merozoites can disperse within the erythrocyte and form a sea star shape with individual merozoites opposing each other (Fig. 2k) and then expand within the erythrocyte (Fig. 2l).

Comparison of the trophozoites and merozoites of *B. capensis* sp. nov. to those of other *Babesia* spp. reported in cats shows similarities and some differences (Table 2). *Babesia felis*, which was described by Davis in 1929 from a Sudanese wild cat (*Felis ocreata* presumably *Felis sylvestris*), has a diameter of about 1.25 µm [20]. The designation *B. felis* was subsequently given to a small-form *Babesia* sp. in South Africa with an average diameter of 1.5 µm that causes severe disease in domestic cats [2, 21]. The South African *B. felis* is likely to be a different species from the piroplasm described by Davis in the Sudan [2],

since the latter was non-pathogenic to domestic cats [20]. This was mentioned by Jackson and Dunning [21], who referred to the pathogenic entity as *Nuttalia* (*sic*) *felis* var *domestica*. The latter appears to be somewhat larger than *B. felis sensu stricto* and is associated with potentially fatal infection related in some cases with icterus, hence termed biliary disease, in cats [2, 21]. The merozoites of *B. capensis* sp. nov. have typical pyriform shapes and present pear-shaped couples similar in form to those seen in large canine *Babesia* spp. Opposingly, *B. felis* infrequently forms pairs of pyriform merozoites and its replication was described as formation of a cruciform shape which includes four small merozoites in a tetrad form [21, 22]. *Babesia leo* is similar morphologically to *B. felis*, and they are also closely related genetically [23]. *Babesia lengau*, also recorded in South African domestic cats, has merozoites measuring on average 1.91 x 1.1 µm and oval trophozoite forms of 1.3 x 1.3 µm and does form pairs of pyriform merozoites [24, 25]. *Babesia panickeri* from a domestic cat in India was described to have pear-shaped merozoites with average sizes of 2.7 x 1.3 µm, hence longer than those of *B. capensis* sp. nov. [7]. *Babesia hongkongensis* from southeast Asia was reported to have round to oval trophozoites with ring forms measuring 1.4 to 1.7 µm resembling small-form *Babesia* spp. with no description of pyriform merozoites [26]. *Babesia canis* subsp. *presenti* reported from Israel was described to form pairs of pyriform merozoites and also rounded trophozoites like *B. capensis* sp. nov., but these forms were larger on average than those measured for *B. capensis* sp. nov. with merozoites of 2.5 x 1.4 µm and trophozoites of 2.7 x 1.7 µm compared to 2.11 x 1.41 and 2.09 x 1.12 µm in *B. capensis* sp. nov. [3]. Conversely, *Babesia galileei* also described in Israeli cats is smaller than *B. capensis* sp. nov. with merozoites of 1.70 x 0.91 µm and trophozoites measuring 1.53 x 1.07 µm [8].

Molecular analysis

Samples from three cats that were analyzed (RLB124, RLB125, and Jessie) produced 1,432bp *18S rRNA* DNA sequences that had 99.72-99.86% identity and 100% cover to *Babesia* sp. Western Cape sequences deposited previously in GenBank (KR611159.1; KR611153.1). Analysis of a shorter *18S rRNA* fragment from the same cats and another cat from the Western Cape (Choko) produced 348-364 bp sequences that had 99.43-100% identity with 100% cover to GenBank KC790442.1 These four cats were considered as being infected by *B. capensis* sp. nov.

The *HSP70* PCR successfully amplified DNA only from the four *B. capensis* sp. nov. cat samples and not from other samples including those of other species (Table 3). The amplified 247 bp sequences were 99.62-100% identical to each other and had 92% identity and 100% cover to *Babesia divergens* GenBank EU185805.1 and 91% identity with 100% cover to *B. galileei* GenBank PP624315.1. Comparable results were also available for 248-312 bp *CytB* sequences amplified from the four cats with *B. capensis* sp. nov. infection that were 100% identical to each other, and only had 90% identity and 99-100% cover to *B. gibsoni* GenBank AB215096.1. These findings indicate that the *HSP70* and *CytB* sequences from cats with *B. capensis* sp. nov infection are unique and different from other described piroplasmid species.

The sample from cat RLB24/032 was submitted to the University of Pretoria by a veterinary practitioner from Rustenburg in the North West Province of South Africa close to Pretoria, and the cat was identified

by PCR at the University of Pretoria laboratory as being infected by *B. felis*. This sample was included in the study in order to attempt amplifying *B. felis HSP70* and *CytB* sequences, which are not available yet in GenBank. Although a 1,572bp sequence of the *18S rRNA* with 99.55% identity and 100% cover to *B. felis* GenBank AF244912.1 was amplified from this cat's sample, we were unable to amplify other genes from it using the *HSP70* and *CytB* primers.

Another cat included in the study (RLB24/033) had an *18S rRNA* sequence length of 1,430 bp that was 97.15% identical with 100% cover to *Babesia* sp. Western Cape GenBank KR611149.1. A shorter 365 bp *18S rRNA* sequence amplified from this cat using a different set of primers indicated that it was 99.73% identical with 100% cover to a sequence from a captive cheetah (*Acinonyx jubatus*) in Harare, Zimbabwe (GenBank KJ598882.1) [27]. The 312 bp *CytB* sequence that was amplified from this cat was closest yet very distant (87.5% identity, 100% cover to GenBank PP215410.1) to *Babesia ailuropodae* of giant pandas (*Ailuropoda melanoleuca*) from China [28]. Distance matrix of 1,430 bp of *18S rRNA* showed that the five sequences of *B. capensis* sp. nov. are closely similar to one another (99.72%-99.86%) and different from the closely-related *Babesia* sp. sequence from cat RLB 24/033 (96.80%-97.01%) (Additional file 1: Additional Figure 1a). The overall nucleotide diversity (pi) for the dataset was 0.01. However, sliding window analysis revealed that this diversity was non-randomly distributed, with significant peaks localized between 450-550 bp and 1100-1200 bp (hypervariable regions). In contrast, the internal diversity among the *B. capensis* sp. nov. sequences remained negligible (pi = 0.00196), indicating that the observed divergence is driven by the unique polymorphic sites found in *Babesia* sp. (Additional file 1: Additional Figure 1b, 1c, 1d). The sequences from cat 24/033 and from the captive cheetah likely represent another piroplasm species circulating in felids in southern Africa, which is not *B. capensis* sp. nov.

Two of the five caracal samples tested were negative, and one (RLB 24/035) was positive for *B. leo* with a 392 bp segment of the *18S rRNA* gene that had 100% identity and 100% cover to GenBank PQ867483.1 amplified from a domestic cat in Ethiopia [29]. Two caracals (CMER1 and NCM4) were positive for the *CytB* PCR and 312 bp DNA sequences amplified from them were 99.65% identical to each other had 90% identity and 99% cover to *B. gibsoni* GenBank AB2685197.1. These *CytB* sequences were 98.24-98.6% identical to the sequences amplified from *B. capensis* sp. nov-positive cats. Furthermore, *18S rRNA* sequences from *B. capensis* sp. nov.-positive cats from this study were 100% identical to sequence GenBank MK616205.1 of a *Babesia* sp. from a caracal in South Africa [11]. These findings strongly suggest that caracals in South Africa are infected with *B. capensis* sp. nov.

One of the two *B. capensis* sp. nov. positive caracals in our study (NCM4) was also positive according to a 500 bp sequence of the *18SrRNA* (99.8% identity; 100% cover) to a *Hepatozoon luiperdjie* sequence GenBank MK621317.1, which is a *Hepatozoon* sp. that infects the African leopard (*Panthera pardus pardus*) and domestic cats [29, 30]. Primers that amplify the piroplasmid *18SrRNA* often amplify sequences of the same gene from the genus *Hepatozoon* [31] and therefore, it is likely that this caracal was co-infected with *B. capensis* sp. nov. and *H. luiperdjie*.

Phylogenetic analysis

A phylogenetic analysis of a 1,282 bp fragment of the piroplasmid *18S rRNA* gene (Fig. 4) showed that the *Babesia capensis* sp. nov. sequences clustered together within the *Babesia sensu stricto* clade (clade X), with a strong support (bootstrap 99) separating them from other *Babesia* spp., and sequences of clade X present in GenBank. The phylogenetic tree of the shorter *18S rRNA* fragment (Fig. 5), which was carried out in order to include additional species for which longer partial gene sequences were not available, also demonstrated that *B. capensis* sp. nov. sequences clustered separately from other *Babesia* spp., with strong support (bootstrap 92). The sequence from cat RLB24/033 (deposited in this study as GenBank as PX661657) which proved in the molecular analysis to be different from *B. capensis* sp. nov. and almost identical to a sequence from a captive cheetah from Harare, Zimbabwe (KJ598882.1), branched closely but separately with strong support (bootstraps 100) from *B. capensis* sp. nov. in both *18S rRNA* phylogenetic trees (Figs. 4, 5).

A phylogenetic analysis of *CytB* sequences (Fig. 6) showed that *B. capensis* sp. nov. sequences all clustered identically and were separated with strong support (bootstrap 96) from the sequences of other *Babesia* spp. from different vertebrate hosts and world regions. Finally, a phylogenetic tree of *HSP70* sequences also showed that *B. capensis* sp. nov. sequences aligned identically and clustered together and away from other *Babesia* spp. with strong support (bootstrap 83; Fig. 7).

Some variability in the *18S rRNA* of different *B. capensis* sp. nov. isolates was found in the phylogenetic analysis of the long fragment of this gene (Fig. 4).

Clinical findings and treatment of infected cats

As a consequence of its sporadic and unpredictable occurrence, clinical manifestation of infection with *B. capensis* sp. nov. has not been thoroughly investigated. Reports from veterinary practitioners usually mention that cats are presented due to lethargy and anorexia. Clinical examination usually reveals general signs such as elevated body temperature, rapid breathing and pale and sometimes icteric mucous membranes. After characterizing the pathogen genetically and morphologically, more detailed clinical information and blood samples were obtained from two additional cats that were hospitalized and treated for babesiosis caused by a large *Babesia* sp. PCR was performed on blood of these two cats [13] and following DNA sequencing amplifying 1,432-1,433 bps of the *18S rRNA* gene they were confirmed to be infected with *B. capensis* sp. nov. (case 1 with 99.93% identity and 100% cover, and case 2, with 99.86% identity and 100% cover to GenBank KR611153.1) (Table 3). The *18S rRNA* sequences from these two cats were submitted to GenBank with the following accession numbers (case 1 GenBank PX795690; case 2 GenBank PX795691).

Case 1 (“Luka”)

A 5-year-old neutered male domestic shorthair cat was presented to a veterinary clinic at Clanwilliam, Western Cape Province, South Africa, due to lethargy and loss of appetite. On admittance, it had a fever

of 40°C [reference interval (RI) 37.8-39.5°C]; respiration rate of 30 times/minute (RI 20-30/minute), heart rate of 120 beats/minute (RI 100-120/minute), and capillary refill time of 2 seconds (RI 1-2 seconds). Blood testing and blood smear examination showed a hematocrit of 42% (RI 28-47%), neutrophils with left shift, anisocytosis and large *Babesia* sp. forms which were detected within erythrocytes. The cat was positive by serology for the feline immunodeficiency virus (FIV) using the Idexx snap test, (Idexx, Westbrook, ME, USA). It was treated with intravenous fluids, doxycycline (Kyron Laboratories, Johannesburg, South Africa) (10 mg/kg/day) was administered orally and diminazene aceturate (Intervet, Spartan, South Africa) at 2 mg/kg injected intramuscularly.

Twenty-four hours after its hospitalization, the cat had improved, was bright and alert, had pink mucous membranes and its body temperature had decreased to 37.5°C. On blood examination it had a hematocrit of 32% and microscopy of its stained blood smear still showed the presence of large *Babesia* sp. forms. Treatment with intravenous fluids continued and it received a second dose of oral doxycycline which was continued to be administered daily in the next days. At 48 hours after hospitalization, the cat had regained its appetite, had a body temperature of 37.7°C, a hematocrit of 32%, and its blood smear microscopy showed left shift neutrophilia and large activated macrophages, but no *Babesia* organisms were seen. The cat continued to be stable with no fever 96 and 120 hours after initial hospitalization, and no piroplasms were observed in its blood smear at these times. It received a second diminazene aceturate intramuscular injection of 2 mg/kg at 120 hours, and was discharged the next day (148 hours after admission, day 6) with a continued daily doxycycline oral treatment at home for the next 22 days.

Case 2 (“Mattewis”)

A 10-year-old, neutered male domestic shorthair cat was admitted to the same veterinary clinic in Clanwilliam, Western Cape Province, South Africa, with lethargy and loss of appetite. It had a fever of 42°C and pale mucous membranes. Blood taken on admission had a low hematocrit of 20% and microscopy of a blood smear showed infection of erythrocytes with large *Babesia* sp. and hemotropic *Mycoplasma* sp. The cat was treated with the antimalarial drug primaquine (Medpet, Benrose, South Africa) injected intramuscularly at 2 mg/kg and with oral doxycycline at 10 mg/kg daily for 28 days. Seventy-two hours after being hospitalized, its mucous membranes were icteric, and at 96 hours, a stained blood smear viewed by microscopy indicated an even higher *Babesia* sp. parasitemia than seen on admission, but no hemotropic mycoplasma were observed. The cat was treated at this point with an intramuscular injection of 2 mg/kg of diminazene aceturate which was repeated at the same dose at 192 hours (day 8). Since it appeared clinically normal, the cat was discharged on the 9th day of its hospitalization and was then lost to follow-up.

Discussion

The findings of this study present evidence for the establishment of *B. capensis* sp. nov. as a new taxon fulfilling the requirements of the ICZN guideline for the description of a new species [19].

The disposition of *B. capensis* sp. nov. in the genus *Babesia* and its segregation in the *Babesia* s.s. (clade X) within this genus is derived from the molecular phylogenetic analysis of the *18S rRNA*, *CytB* and *HSP70* sequences. The *18S rRNA* sequences of the isolates designated as *B. capensis* sp. nov. demonstrate some variability often found intraspecies in other *Babesia* spp. [32, 33], despite the fact that they cluster together and significantly away from other *Babesia* spp. in the phylogenetic analyses and are almost identical as shown by the distance matrix analysis. This variability is attributed mainly to hypervariable region in the gene of this species which we investigated and described. In spite of this variability, these isolates all have identical *HSP70* and *CytB* sequences, and cluster with strong support away from all other piroplasmid species in the phylogenetic analyses. Interestingly, the 18S *rRNA* and *CytB* sequences of cat RLB 24/033 from this study, e.g. GenBank PX661657 and PX686959, respectively, which had an almost identical 18S *rRNA* sequence to that of a captive cheetah from Zimbabwe (GenBank KJ598882.1) and is not considered to be *B. capensis* sp. nov., branched separately with strong support from the *B. capensis* sequences (Figs. 3, 4, 5). This strengthens the idea that *B. capensis* sp. nov. is one species which distinctly differs genetically from other *Babesia* spp. and sequences of felids and other hosts.

The morphology of *B. capensis* sp. nov. merozoites and trophozoites resembles other *Babesia* s.s. species that infect felids in both shape and size, although they are larger than those of *B. galileei*, *B. hongkongensis* and *Babesia* sp. lynx genotype, and their merozoites are smaller than those of *B. panickeri* (Table 2). *Babesia capensis* sp. nov. merozoites are in general smaller than those of canine *Babesia* s.s. species such as *B. vogeli*, *B. canis* and *B. rossi* [34] and also smaller than those of other domestic animal *Babesia* s.s. species including *B. caballi* of equines (length 2.5-3 μm [35]) and *B. bigemina* of cattle (length 2.5-3 μm [34]). On average, cat erythrocytes are smaller than those of dogs and horses, but about the same size as those of cattle [37], therefore the host species erythrocyte size does not appear to explain why *Babesia* s.s. species of cats evolved to be smaller than those of dogs and horses.

In comparison to *Babesia* spp. of felids from other piroplasm clades, such as *B. felis* and *B. leo* of Clade I, and *B. lengau* of clade III (Table 2), *B. capensis* sp. nov. is considerably larger and also forms typical elongated pear-shaped merozoites that oppose each other in couples, unlike small-form felid *Babesia* spp.

We attempted unsuccessfully in this study to amplify additional genes of *B. felis* beyond the *18S rRNA*, which we were able to amplify with a 1,572 bp sequence from a cat (RLB 24/032), and *B. leo* which was amplified with a 1,392 bp sequence from a caracal (RLB 35/035). The new sequences were deposited in GenBank as accession number PX661658 from the cat and PX660498 from the caracal. The fact that we were unable to amplify the *HSP70* and *CytB* from using the PCR primers that worked successfully with *B. capensis* sp. nov. cat samples, strongly suggests that *B. felis* and *B. leo*, which are closely related to each other, possess substantially different DNA sequences at the locations for which the primers of the PCR protocols employed were designed, thus preventing their attachment. This further exemplifies the genetic distance between those species and the felid *Babesia* s.s. such as *B. capensis* sp. nov.

In southern Africa caracals are infected by several piroplasmid species [11]. In this study, we amplified a segment of the *18S rRNA* of *B. leo* from caracal RLB 24/035. We also amplified a part of the *CytB* gene, from two caracals (CMER1 and NCM4), which was only 90% identical to the sequence of this gene from *B. gibsoni*, the closest piroplasmid sp. to this sequence. DNA sequences of the *Cytb* gene with close to the same % identity to *B. gibsoni* were also amplified from the four cats designated as infected with *B. capensis* sp. nov. (RLB124, RLB125, Choko, and Jessie; Table 3). The *Cytb* sequences from the caracals were 98.24–98.6% identical to the sequences amplified from *B. capensis* sp. nov.-positive cats. The fact that *18S rRNA* sequences from *B. capensis* sp. nov.-positive cats from this study were 100% identical to sequences of a *Babesia* sp. from caracals in South Africa described in a previous study [11], and the detection of highly similar *Cytb* sequences in cats and caracals in our study, strongly suggest that caracals are infected with *B. capensis* sp. nov., and they likely constitute a natural reservoir for this pathogen. Other *Babesia* spp. that infect domestic cats in southern Africa have also been detected in wild felids, e.g., *B. felis* and *B. leo* that infect cats and lions [1], and *B. lengau* that infects cheetahs and can cause severe disease in cats [25]. Transmission of *Babesia* spp. from wildlife hosts to domestic cats is likely to take place by sharing the same environment and due to infestation of domestic and wild felids by the same tick vectors. As the tick vector of *B. capensis* sp. nov. as well as vectors of *B. felis* and *B. lengau* have not been identified to date, more research is needed in order to elucidate the life cycle of these pathogens, elucidate transmission patterns, and devise prevention plans.

Hepatozoon luiperdjie, which was described from the African leopard in South Africa and subsequently detected by molecular techniques in cats from Ethiopia [29, 30], has not been reported from caracals before. This study is the first report of this infection in a caracal and there is currently no information regarding the involvement of this parasite in clinical disease in caracals.

The clinical findings described in the cats with *B. capensis* sp. nov. infection include fever, lethargy, and anorexia. As detailed blood tests including a complete blood count (CBC) and complete serum biochemistry panel were not available for the infected cats confirmed to be infected with *B. capensis* sp. nov., it was not possible to characterize the precise hematological changes typical of this infection, although the detection of large-form *Babesia* organisms in erythrocytes was associated with disease. Furthermore, erythrocyte anisocytosis, typical of regenerative anemia, was described in one cat (Cat 1, Luka) and icterus was reported in another cat (Cat 2, Mattewis). Co-infection with other pathogens was also found, with FIV infection in cat 1 and hemotropic *Mycoplasma* sp. infection in cat 2. Co-infection with FIV has been described before in cats with babesiosis, and it is possible that immune-suppression associated with co-infection was involved in the ability of *B. capensis* sp. nov. to induce a more severe disease in these two cats [3, 8]. Cat 1 was treated with diminazene aceturate and doxycycline, with the disappearance of *Babesia* organisms from the blood at 48 hr. after treatment according to blood smear microscopy. Cat 2 was treated initially with primaquine and doxycycline, but 96 hours after the initiation of treatment still had a high parasitemia and received an injection of diminazene aceturate, which was repeated at 192 hours of its hospitalization. No further information was available on its condition, nor whether the infection had been cleared. Although diminazene aceturate is not licensed for treatment of cats and its use was extra-label, experimental treatment of cats with 3.5 mg/kg of this drug did not result

in side effects [38]. As this drug is effective against *Babesia* s.s. species in other animal hosts [39, 40], it is likely that it would also be effective in cats with *B. capensis* sp. nov. However, no PCR testing was performed in the two cases after conclusion of treatment to evaluate whether the treatment was effective in eliminating infection from the cats, and therefore, treatment must be considered cautiously, and perhaps other drugs, such as imidocarb dipropionate, could be useful for cats with *B. capensi*s sp. nov. infection.

Conclusions

This study completes the description of a *Babesia* sp. which infects domestic cats and caracals in southern Africa and had previously provisionally been termed *Babesia* sp. Western Cape. *Babesia capensis* sp. nov. is placed phylogenetically in the *Babesia* s.s. group and is associated with clinical disease in domestic cats. More studies are warranted to find the tick vector of this parasite and find the best treatment against this pathogen.

Abbreviations

HSP: heat shock protein; ICZN: International Code of Zoological Nomenclature; PCR: polymerase chain reaction; s.s.: Sensu stricto

Declarations

Acknowledgments

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Ethics approval and consent to participate

The project and export of the samples were approved by the University of Pretoria Animal Ethics Committee (reference REC018-25).

Availability of data and materials

All data generated or analyzed during this study are included in this published article. Analyzed nucleotide sequences used for pairwise comparisons and tree construction were submitted to the GenBank database under the accession numbers PX660493-PX660498; PX661654-PX661658.1; PCX795690; PX795691(*18S rRNA*), PX686955-PX686961 (*CytB*) and PX686951-PX686954 (*HSP70*). The

holotype was deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession number NMB P 1256. An isotype from the same cat (accession number NMB P 1257) and paratypes from a different cat (accession numbers NMB P 1321 and 1322) were deposited in the same collection.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BP and GB conceived the study and wrote the manuscript. AM coordinated collection of specimens from the Western Cape Province. GB performed the morphologic characterization of parasites. YNB, SMM, IV, MCO and GB analyzed the genetic data. JB and SV provided caracal specimens collecting during a previous study. All authors read and approved the final manuscript.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures



Figure 1

Map of the southwestern part of South Africa indicating the localities of origin of the specimens used in this study. Jessie* indicates the locality of the type specimen.

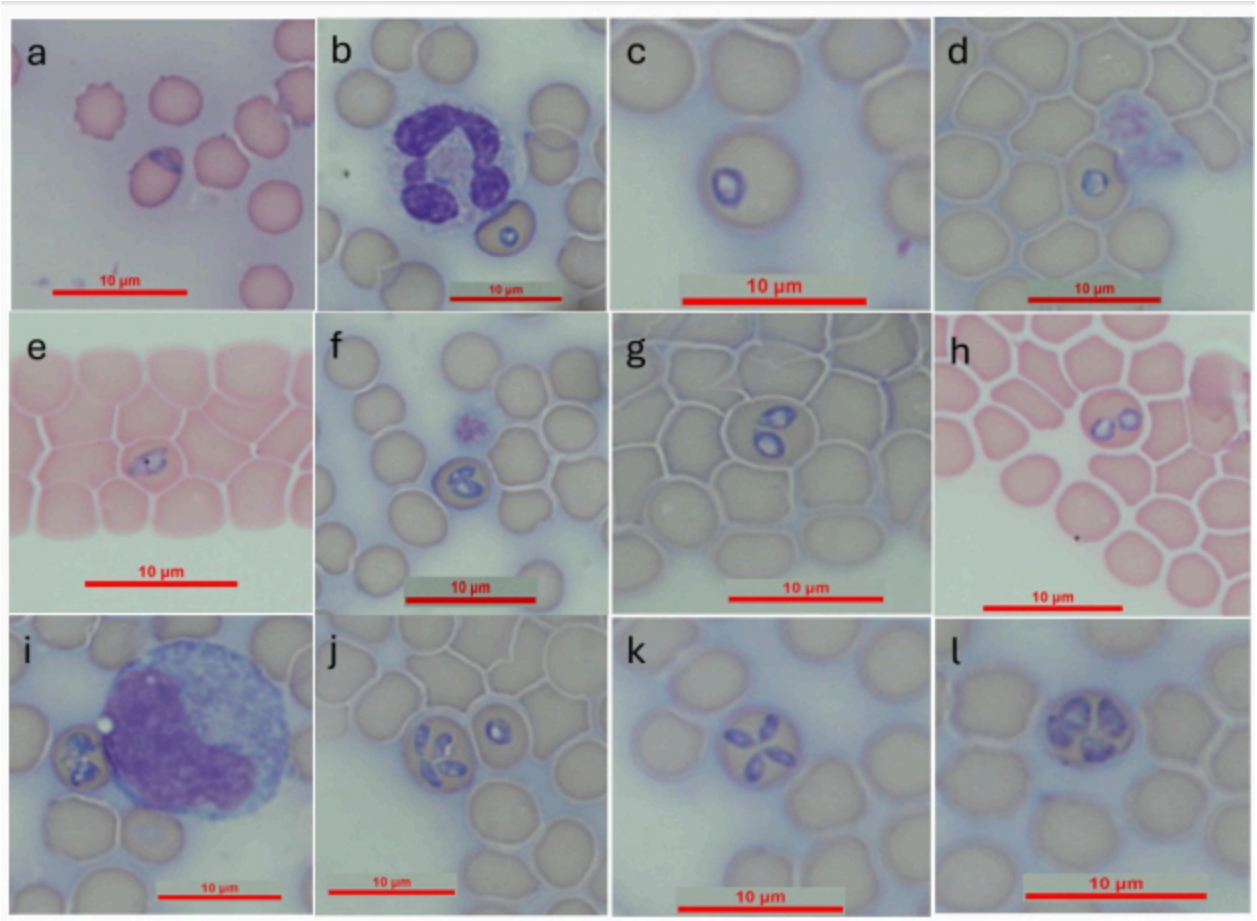


Figure 2

Proposed evolution of *B. capensis* sp. nov. development. Elongated forms shortly after invading erythrocytes (Fig. 2a) transform into rounded trophozoites which enlarge gradually (Figs. 2b-2d) until starting to divide into two merozoites (Fig. 2e). After completing the division, merozoites are pear shaped (Fig. 2f) and initially remain attached to each other through contact of their apical poles (Fig. 2g). Merozoites may enlarge and become more rounded (Fig. 2h) and eventually start dividing again (Fig. 2i) to form four merozoites within the same erythrocyte (Fig. 2j). These four merozoites can disperse within the erythrocyte and form a starfish shape with individual merozoites opposing each other (Fig. 2k) and then expand in size within the erythrocyte (Fig. 2l). Modified Wright's and quick Romanowsky staining.

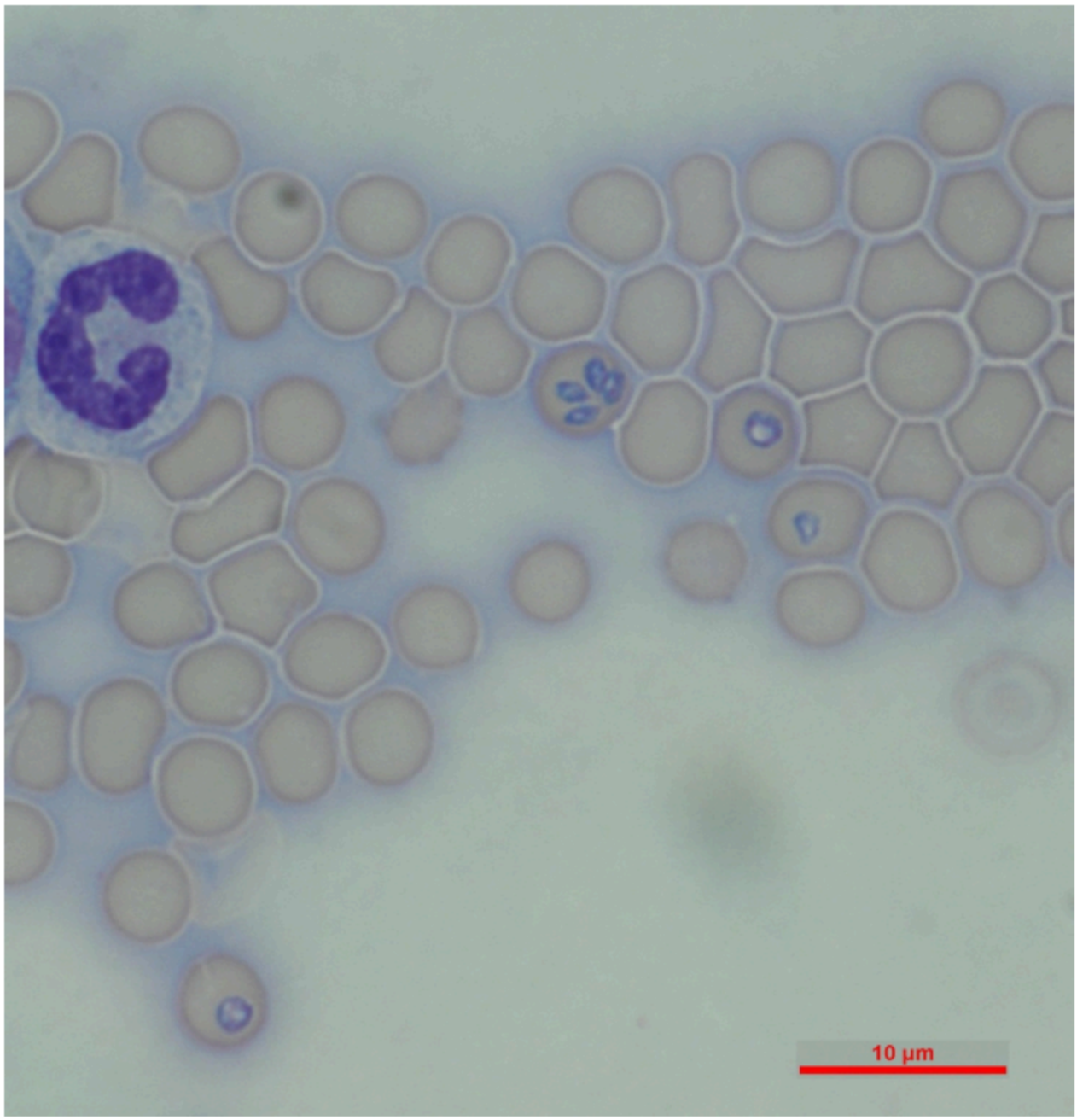


Figure 3

Merozoites and trophozoites of *Babesia capensis* sp. nov., in blood smear of an infected cat. Four merozoites are found within a single erythrocyte and three trophozoites are present as single parasites in erythrocytes. Modified Wright's stain.

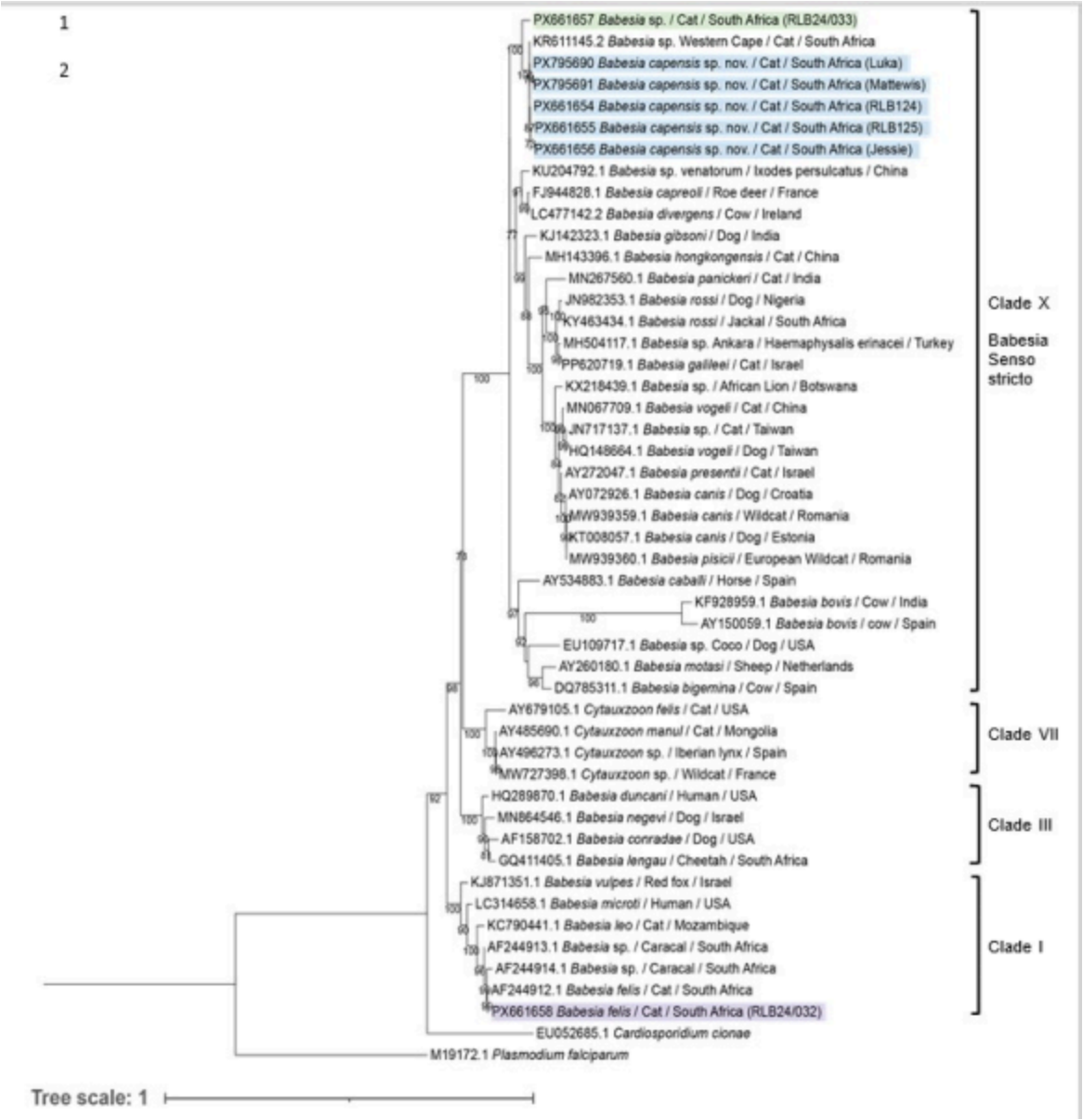


Figure 4

A maximum likelihood phylogenetic tree of nearly complete 18S rRNA gene sequences. Sequences from the cats in this study [Babesia capensis sp. nov. (Blue), Babesia sp. (Green) and Babesia felis (Purple)] compared to other relevant piroplasmid spp. deposited in GenBank. The GenBank accession numbers, host and country of origin are included for each sequence. Clade designation of piroplasmid spp. is shown according to Jalovecka et al., 2019 [41]. The topology was inferred by using the maximum likelihood method and the TIM3+F+I+R3 substitution model. There are 49 sequences in the dataset. The dataset comprised 1,361 nucleotide sites. Plasmodium falciparum 18S rRNA sequence was used as an outgroup. The scale bar represents the evolutionary distance in the units of the number of nucleotide substitutions per site.



Figure 5

A maximum likelihood phylogenetic tree of partial *18S rRNA*gene sequences. Sequences from this study [*Babesia capensis* sp. nov. (Blue), *Babesia* sp. (Green), ~~and~~ *Babesia felis* (Purple) and *Babesia leo* (Yellow)] compared to other relevant piroplasmid spp. deposited in GenBank. The GenBank accession numbers, host and country of origin are included for each sequence. The topology was inferred by using the maximum likelihood method and HKY+F+I+G4 substitution model.There are 56 sequences in the dataset. The dataset comprised 351 nucleotide sites. *Cardiosporidium cionae 18S rRNA* sequence was used as an outgroup. The scale bar represents the evolutionary distance in the units of the number of nucleotide substitutions per site.

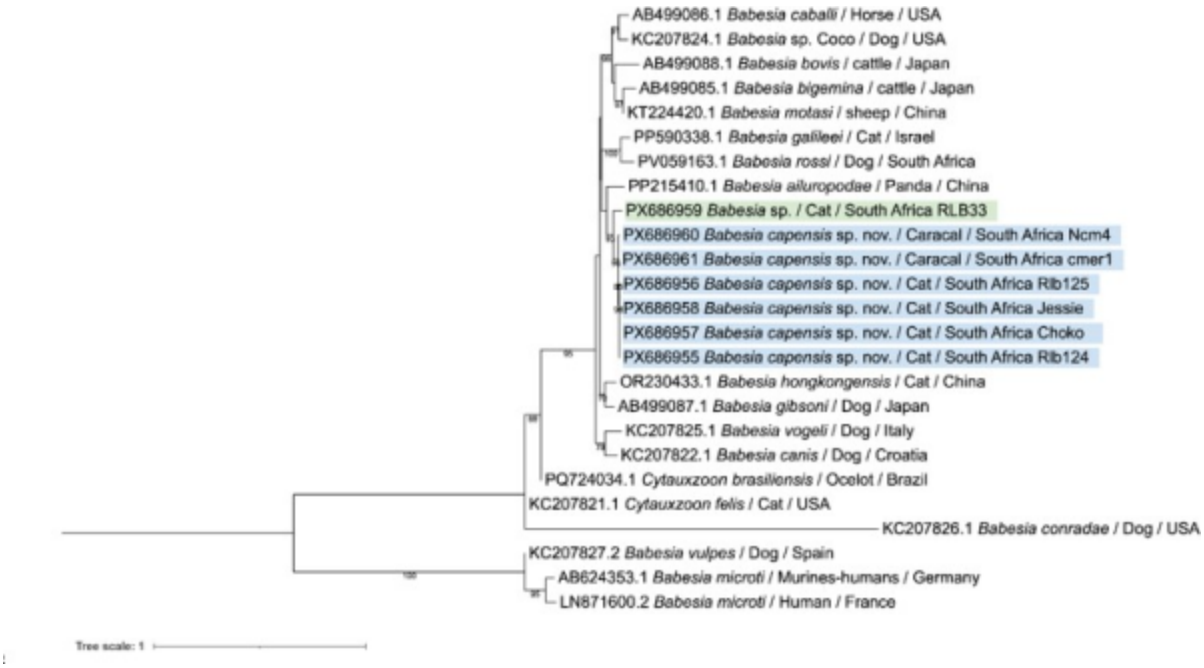


Figure 6

A maximum likelihood phylogenetic tree of *CytB* gene piroplasmid sequences. Sequences from this study [*Babesia capensis* sp. nov. (Blue), *Babesia* sp. (Green)]. are compared to other relevant piroplasmid spp. *CytB* sequences deposited in GenBank. The GenBank accession numbers, host and country of origin are included for each sequence. The topology was inferred by using the maximum likelihood method and TIM2+F+G4 substitution model.There are 25 sequences in the dataset. The dataset comprised 275 nucleotide sites. The scale bar represents the evolutionary distance in the units of the number of nucleotide substitutions per site.

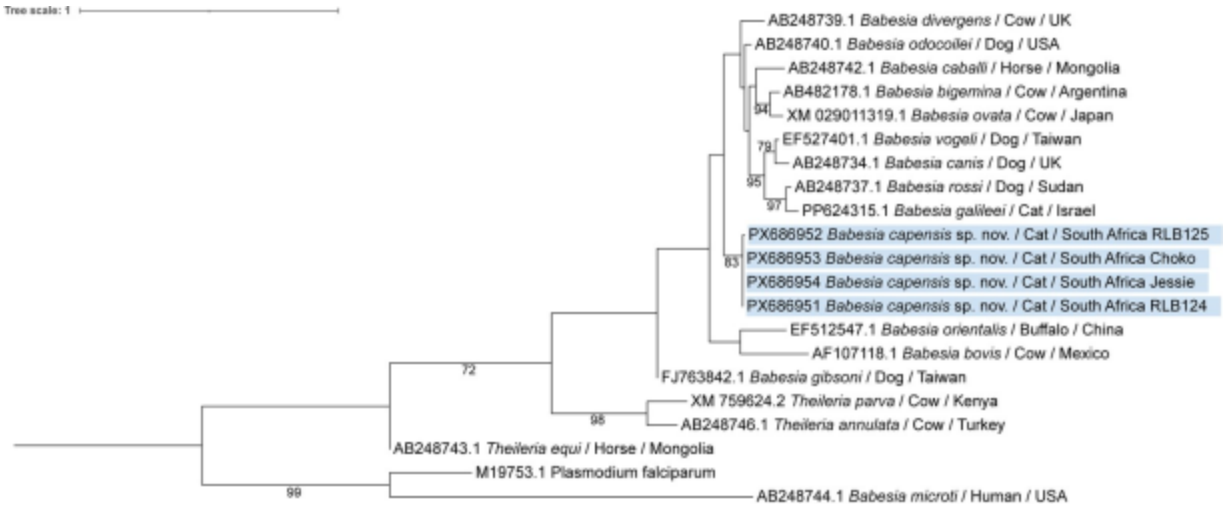


Figure 7

A maximum likelihood phylogenetic tree of HSP70 gene piroplasmid sequences. Sequences from this study [Babesia capensis sp. nov. (Blue)] are compared to other relevant piroplasmid spp. hsp70 sequences deposited in GenBank. The GenBank accession numbers, host and country of origin are

included for each sequence. The topology was inferred by using the maximum likelihood method and HKY+F+I+G4 substitution model. There are 21 sequences in the dataset. The dataset comprised 243 nucleotide sites. The scale bar represents the evolutionary distance in the units of the number of nucleotide substitutions per site.

Supplementary Files

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