Comparative Immunology, Microbiology and Infectious Diseases 55 (2017) 30–41

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Full Length Article

Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil

Karin Werther[a], Mayara de Cássia Luzzi[a], Luiz Ricardo Gonçalves[a], Juliana Paula de Oliveira[a], José Roberto Ferreira Alves Junior[b], Rosangela Zacarias Machado[a], Marcos Rogério André[a,*]

[a] Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias/ Universidade Estadual Paulista (FCAV/UNESP), Jaboticabal, SP, Brazil
[b] Instituto Federal Goiano/ IF Goiano, Urutaí, GO, Brazil

ARTICLE INFO

Keywords:
Anaplasma
Ehrlichia
Babesia
Haemoproteus
Plasmodium
Orinoco goose

ABSTRACT

Although Orinoco goose (*Neochen jubata*) is an anatid species widely distributed in South America, scarce are the reports on the occurrence of arthropod-borne pathogens in this avian species. The present work aimed to verify, by serological and molecular methods, the occurrence of haemosporida piroplasmids and Anaplasmataceae agents in wild Orinoco geese captured in Brazil. Between 2010 and 2014, 62 blood samples were collected from free-living geese captured in the Araguaia River, Goiás State, Brazil. Six geese (10%) were seropositive for *Anaplasma phagocytophilum,* showing titers ranging from 40 and 80. Twenty out of 62 blood samples (32.25%) were positive in nested PCR for hemosporidia (*cytochrome b* gene). Fifteen and five sequences shared identity with *Haemoproteus* and *Plasmodium*, respectively. Six out of 62 blood samples (9.68%) were positive in nested PCR for *Babesia* spp. (*18S rRNA* gene); one sequence showed to be closely related to *Babesia vogeli*. Thirty (48.38%) out of 62 Orinoco geese blood samples were positive in nested cPCR assays for Anaplasmataceae agents (16S rRNA gene): three for *Anaplasma* spp. and 27 for *Ehrlichia*. Six geese were simultaneously positive to *Haemoproteus* and *Ehrlichia*; three animals were co-positive to different *Ehrlichia* species/genotypes; and one goose sample was positive for both *Anaplasma* and *Ehrlichia*. The present work showed the occurrence of *Ehrlichia, Anaplasma, Babesia*, *Plasmodium*, and *Haemoproteus* species in free-living *N. jubata* in Brazil. The threat of these arthropod-borne pathogens in Orinoco goose's fitness, especially during the breading season, should be assessed in the future.

1. Introduction

Orinoco goose (*Neochen jubata*) is an anatid species widely distributed in South America, where it occurs in central and Amazonic regions of Brazil. This anatid species is a terrestrial grazer and nests in large tree cavities, usually made by other birds (such as wood-peekers) [1–3]. Adults have nearly 60 cm of length and 1.200 g of weight, brown back and abdomen, yellow head and chest, black wing, red beak and feet [4,5]. Orinoco geese live in pairs or in families, joining big groups during the molt. They migrate in an expressive longitudinal direction [6]. Although its population is estimated at 10,000–25,000 individuals, it is classified as a Near Threatened species, because its population is in an expressive and continuous reduction in some places, owing to hunting pressure and habitat loss for livestock and husbandry [7].

Among the threats to avian species, haemosporidian protozoa (*Leucocytozoon* spp., *Plasmodium* spp. and *Haemoproteus* spp.) are related to reduction of life span, lifetime number and quality of offspring in birds [8]. Additionally, piroplasmids belonging to genus *Babesia* spp. comprises another group of protozoa associated with performance decreasing in birds [9–12].

Although the occurrence of haemosporidian parasites has been well documented in birds around the world, few are the reports on bacterial hemoparasites in these animals. Recently, the role of avian species as reservoirs or carriers of vector-borne bacterial pathogens has been investigated [13,14]. Anaplasmataceae agents (*Ehrlichia* spp. and *Anaplasma* spp.) comprise a group of tick-borne Gram-negative bacteria that can cause disease in human and animals [15]. *Ehrlichia* spp. and *Anaplasma* spp. has been already detected in ticks collected on birds from Sweden [16], Latvia [17], USA [18] and avian's blood samples from Spain [19], Brazil [20] and Europe [21]. The present work aimed to verify, by serological and molecular methods, the occurrence of haemosporida (*Leucocytozoon* spp., *Plasmodium* spp. and *Haemoproteus* spp.), piroplasmids (*Babesia* spp.) and Anaplasmataceae agents (*Anaplasma* spp. and *Ehrlichia* spp.) in wild Orinoco geese (*Neochen jubata*) captured in the Araguaia River, state of Goiás, Brazil.

* Corresponding author at: Laboratório de Imunoparasitologia, Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias Júlio de Mesquita Filho (UNESP), Campus de Jaboticabal, Via de Acesso Prof. Paulo Donato Castellane, s/n, Zona Rural, CEP: 14884-900, Jaboticabal, São Paulo, Brazil.
E-mail addresses: marcos_andre@fcav.unesp.br, marcosandre.fcav@gmail.com (M.R. André).

http://dx.doi.org/10.1016/j.cimid.2017.09.003
Received 22 June 2017; Received in revised form 15 September 2017; Accepted 15 September 2017

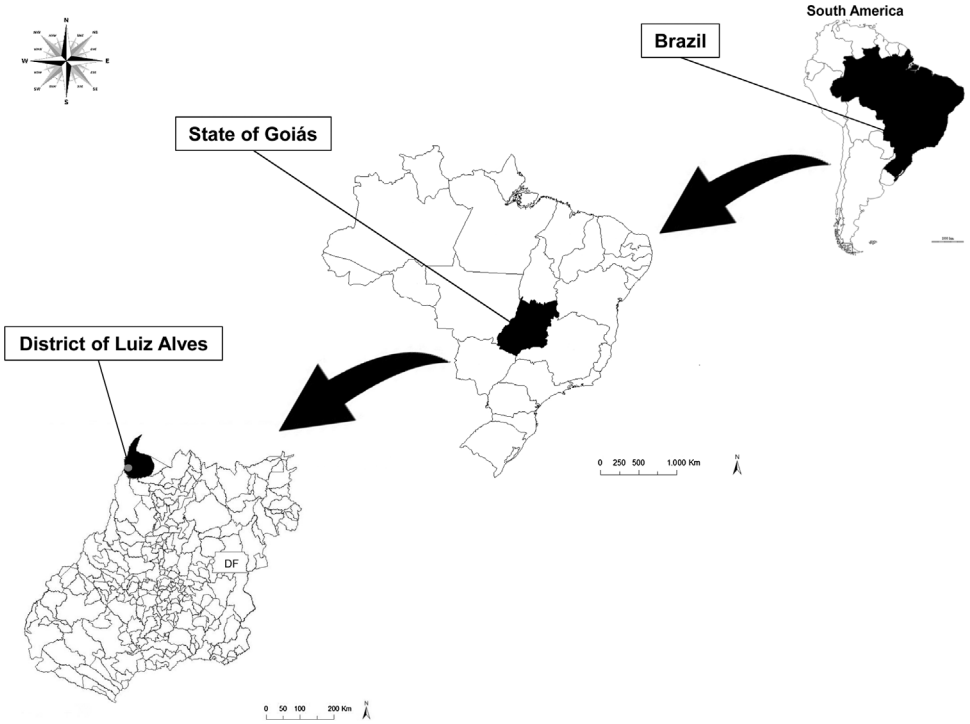

Fig. 1. Capture site. Map of Goiás, central-western Brazil, showing the locality where Orinoco geese were sampled in the present study.

2. Material and methods

2.1. Animals and studied area

During the molting period, when geese became flightless, sixty-two free-living Orinoco geese were hand-caught. Using the manual restraint, whole blood samples were collected by puncture of ulnar vein from animals captured in the Araguaia River, state of Goiás, Brazil (13°13′02.1"S 50°34′37.8"W) (Fig. 1**).** During the year of 2010, 21 animals were caught; however, the sexing procedure of captured animals was not performed. On the other hand, molecular sexing (Unigen® – Biologia pelo DNA) was performed in animals captured during the years of 2013 and 2014. Among 41 animals caught, 20 were males and 21 females. Additionally, ectoparasites inspection was performed at the time of blood collection. Finally, the serum and EDTA-blood samples from Orinoco geese were stored at −20 °C for further analysis. Blood smears were made from each goose blood sample, fixed with methanol, and stained with Giemsa (TRALL, 2006). The slides were examined under an optical microscope Olympus B-50 and the images were analyzed with CellSens Standard 1.8.This project was approved by the university's Ethics Committee under the protocol number 012273/11 and ICMBio – SISBIO* (Brazilian Governments permission for research in wildlife) permission number 21650-4. *SISBIO is a remote-access system that allows researchers to request authorizations for the collection of biological material and for research with wild animals in Brazil.

2.2. Serology for babesia vogeli, anaplasma phagocytophilum, ehrlichia chaffeensis and ehrlichia canis

Sixty geese serum samples were submitted to Indirect Fluorescent Antibody Test (IFAT) in order to detect IgG antibodies against *Babesia vogeli*, *A. phagocytophilum*, *E. chaffeensis* and *E. canis*.

Babesia spp. antigen was prepared by intravenously inoculation of *B. vogeli* (Jaboticabal strain) into a splenectomized three month-old dog, negative for hemoparasites by PCR and serology [22]. Blood smears were performed twice a day to check for the presence of parasites in microscopic examination of Giemsa-stained blood smears. After observing parasitaemia peak on the fifth day after inoculation, infected blood was collected with Alsever solution (113.7 mM glucose, 27.2 mM sodium citrate, 71.8 mM sodium cloride). *E.canis* antigen was obtained from *E. canis* (Jaboticabal strain)-infected DH82 cells maintained in culture in the Immunoparasitology Laboratory, UNESP, Jaboticabal, São Paulo [23].

Slides containing air-dried fixed *B. vogeli* trophozoite-infected blood and *E. canis*-infected DH82 cells were used in IFAT as previously described [24,25]. Commercial slides (Focus Diagnostics, Cypress, CA, USA) coated with *E. chaffeensis*-infected DH82 cells and *A. phagocytophilum*-infected HL-60-infected were also used as antigens in order to detect exposure to Anaplasmataceae agents in Orinoco geese following manufactureŕs instructions.

Briefly, antigen slides were removed from storage (−20 °C) and allowed to thaw at room temperature for 30 min. Ten microliters of two fold dilutions of sera (cut-off of 1:40) were placed in wells on antigen slides. Known positive serum samples for the studied agents were obtained from naturally infected deer [26] and dogs [27] from Brazil. Negative serum samples were obtained from wild deer captured in Brazil [26], and dogs maintained in the kennel of the Department of Veterinary Pathology, UNESP, Jaboticabal, São Paulo, Brazil, that had not been exposed to these agents, according to negative PCR and IFAT results. Slides were incubated at 37 °C in a moist chamber for 45 min, washed 3 times in PBS (pH 7.2) for 5 min, and air dried at room temperature. FITC-labeled anti-dog, deer and chicken IgG conjugates (Sigma–Aldrich®, St. Louis, MO, USA) were diluted according to the manufacturer (dilution of 1:32 for anti-dog, 1:10 for anti-deer and 1:10 for anti-bird conjugates) and then added to each well. These slides were incubated again at 37 °C, washed 3 times in PBS, once more in distilled water, and air dried at room temperature. Next, slides were coversliped, and examined under a fluorescence microscope.

2.3. DNA extraction

DNA was extracted from 10 μL of each goose EDTA-whole blood sample using the QIAamp DNA blood mini-kit (QIAGEN®, Valencia, California, USA), in accordance with the manufacturer's instructions. DNA concentration and quality was measured using absorbance ratio

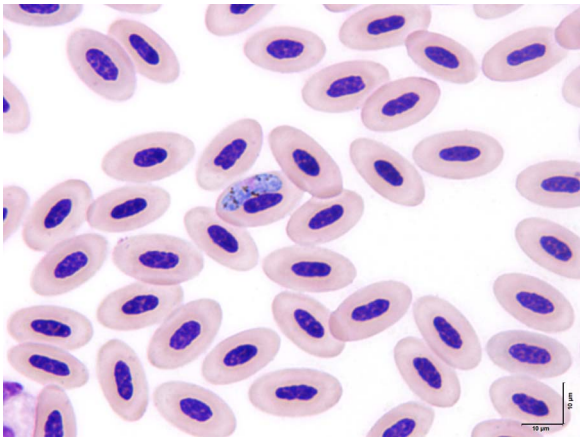

Fig. 2. *Plasmodium* evolutive form in a goose Giemsa-stained blood smear. 1000X.

between 260/280 nm (Nanodrop, Term Scientific, USA). Then, DNA samples were submitted to conventional PCR (cPCR for hemosporida, piroplasmids and Anaplasmataceae agents) and real time PCR (qPCR for *A. phagocytophilum*, *E. chaffeensis* and *E. canis*) assays.

2.4. cPCR for haemosporidia, piroplasmids and anaplasmataceae agents

Each sample of extracted DNA was used as a template in cytochrome b-based nested PCR assays for *Leucocytozoon* spp., *Plasmodium* spp. and *Haemoproteus* spp. [28–30]. Positive and negative DNA controls for *Plasmodium* sp. and *Haemoproteus* sp. were used in the cPCR reaction (Table S1).

Previously described PCR protocols based on 18S rRNA gene [12,31–33] were used for *Babesia* spp. DNA amplification (Table S1). *Babesia* sp. DNA samples obtained from naturally infected wild felids were also used as positive controls [34]. Ultra-pure sterile water was used as negative control in all PCR assays described above. In each set of reactions, five tubes containing ultra-pure water were used as controls. In order to prevent PCR contamination, DNA extraction, reaction setup, PCR amplification and electrophoresis were performed in separated rooms.

16S rRNA-based nested PCR assays were used for amplify *Ehrlichia* spp. [35] and *Anaplasma* spp. [36] DNA (Table S1). *Anaplasma phagocytophilum* and *E. chaffeensis* DNA samples were kindly supplied by Prof. Dr. John Stephen Dumler (University of Maryland, Baltimore, MD, USA). *E. canis* DNA from DH82 cells infected with the Jaboticabal strain of *E. canis* [37] was also used as positive control.

Positive samples to 16S rRNA Anaplasmataceae cPCR protocols were submitted to additional molecular characterization using nested PCR protocols based on *omp-1* [38], *dsb* [39] and *groESL* [40–42] genes (Table S1).

The reaction products were purified using Silica Bead DNA Gel Extraction Kit (Fermentas, São Paulo, SP, Brazil). Purified amplified DNA fragments from positive samples were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser – Applied Byosystem/Perkin Elmer). Consensus sequences were obtained through the analysis of the sense and antisense sequences using the CAP3 program (http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py). Comparisons with sequences deposited in GenBank were done using the basic local alignment search tool (BLAST). The sequences were aligned with sequences published in GenBank using Clustal W in Bioedit v. 7.0.5.3 [43]. The alignment sequences of the genes amplified in the present study were utilized to identify the different genotypes using DnaSP (v. 5.10) [44] (using default parameters). Phylogenetic inference was based on maximum likelihood (ML) and performed with RAxML-HPC BlackBox 7.6.3 [45] (which includes an estimation of bootstrap node support) through the CIPRES Science Gateway [46]. Akaike information criterion available on MEGA 5.05 [47] was applied to identify the most appropriate model of nucleotide substitution.

2.5. qPCR assays for A. phagocytophilum, E. chaffeensis and E. canis

Each 62 samples was used as a template in 10 μL qPCR assay aiming to detect *msp2-A. phagocytophilum*, *vlpt-E.chaffeensis* and *dsb-E.canis* fragments (Table S2). The mixtures contained 5 μL of Buffer (GoTaq® qPCR Master Mix, Promega, Wis., USA,) a final concentration of 1 μM of each primer and TaqMan-probe (Integrated DNA Technologies, Coralville, IA, USA) and 1 μL of DNA sample. qPCR amplifications were performed in a thermal cycler (CFX96 Thermal Cycler, Bio-Rad, Hercules, CA, USA). The amplification conditions were 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s and 55 °C for 30 seg. The number of copies was determined according to the formula (X g/μL DNA/[fragment length in bp × 660]) × 6.022 × 10^{23} × copies/μL. *A. phagocytophilum* and *E. chaffeensis* DNA positive controls were kindly supplied by Prof. Dr. John Stephen Dumler (University of Maryland, Baltimore, MD, USA). *E. canis* DNA from DH82 cells infected with the Jaboticabal strain of *E. canis* [37] was also used as positive control. Ultra-pure sterile water was used as negative control in all qPCR reactions.

3. Results

3.1. Blood smears

Suggestive structure of *Plasmodium* spp. was observed in blood smears of one animal (Fig. 2). The goose was positive in the nested PCR for *Plasmodium* spp., based on *cytochrome b* gene. No suggestive inclusions of piroplasmids and Anaplasmataceae agents were found in the sampled birds blood smears.

3.2. Serological assays (IFAT)

Out of 60 serum samples of Orinoco geese analyzed by IFAT, six (10%) were positive for *A. phagocytophilum,* showing titers of 40 and 80. One of them was also positive in the cPCR for *Anaplasma* spp. based on *16S rRNA* gene. None was seropositive for *E. chaffeensis*, *E. canis* or *Babesia vogeli*.

3.3. PCR assays, identity by BLAST analysis and phylogenetic assessment

3.3.1. Haemosporidian agents

Sixteen out of 62 blood samples (25.8%) were positive in the nested PCR for *Haemoproteus* spp./*Plasmodium* spp. based on the *cytochrome b* gene [28,29]. Among these 16 positive samples, 14 sequences were obtained and submitted to BLAST and, posteriorly, to ML analysis. The BLAST analysis showed that 12 sequences shared identity with *Haemoproteus* spp. detected in birds from South America. Additionally, the sequences amplified in the present study were divided into two genotypes. The genotype #1 consisted of 11 sequences (MF061993-MF061996; MF061998-MF062004) that shared identity ranging from 98% to 99% with *H. macrovacuolatus* (KJ499987; KJ592828; KJ175078) detected in black-bellied whistling duck (*Dendrocygna autumnalis* – Anatidae) from South America. The genotype #2 comprise only one sequence (MF061997) sharing 99% identity with *H. macrovacuolatus* (KF175078) identified in *D. autumnalis* from Colombia. Moreover, other two sequences (MF043229 and MF043230) shared 100% identity with *Plasmodium* sp. detected in a black-crowned night heron (*Nycticorax nycticorax* – Ardeidae [KU057967]), and in a wedge-billed woodcreeper (*Glyphorynchus spirurus* – Furnariidae [KU562663]), sampled in Brazil (Table 1). Lastly, five out of these 16 positive samples were positive (three for *Haemoproteus* spp. and two for *Plasmodium* spp.) when submitted to additional cPCR assays (Table S1). However, these

Table 1
Co-positivity and maximum identity of arthropods-borne pathogens sequences amplified in goose from Brazil by BLAST analysis.

Goose ID and GenBank access numbers	*Haemoproteus* spp. cytochrome b	*Plasmodium* spp. cytochrome b	*Babesia* spp. 18S rRNA	*Anaplasma* spp. 16S rRNA	*Ehrlichia* spp. 16S rRNA and (omp-1)[1]
G1 (KX898559)	–	+[a]	–	–	(100%) – *E. chaffensis* (CP007480)
G8	–	+[a]	–	–	–
G10 (KX898136)	–	–	–	–	(100%) – *E. canis* (KX818219)
G11	–	–	–	–	+[a]
G12	–	–	–	+[a]	–
G13 (MF062001) (KX898560)	(99%) – *H. macrovacuolatus* (KJ499987)	–	–	–	(100%) – *E. chaffensis* (CP007480)
G14 (KX886807) (KX898561)	–	–	–	(100%) – *A. phagocytophilum* (KY458571)	(100%) – *E. chaffensis* (CP007480)
G15 (KX886808)	–	–	–	(100%) – *A. phagocytophilum* (KY458571)	–
G16 (MF062002)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	–
G18 (MF043229)	–	(100%) *Plasmodium* sp. (KU057967)	–	–	–
G19 (MF062003)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	–
G20 (MF062004) (KX898562)	(98%) – *H. macrovacuolatus* (KJ499987)	–	–	–	(100%)*E. chaffensis* (CP007479)
G21 (KX898137)	–	–	–	–	(99%) – *E. canis* (KX818219)
M1		–	+[a]		–
M2 (MF061993)	(99%) – *H. macrovacuolatus* (KJ175078)	–	–	–	–
M3 (MF061994)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	–
M4 (MF034729)	–	–	(99%) – *Babesia* sp. (KY450742)	–	–
M5 (KX898563) (KX833247)	–	–	–	–	(100%) – *E. chaffensis* (CP007480); *Ehrlichia* sp. (JN217097) [1]
M8 (MF061995) (KX898564)	(99%) – *H. macrovacuolatus* (KJ175078)	–	–	–	(100%) – *E. chaffensis* (CP007478)
M13 (KX898565)	–	–	–	–	(100%) – *E. chaffensis* (KY644145)
M14 (KX898566)	–	–	+[a]	–	(100%) – *E. chaffensis* (CP007480)
M15 (KX898584) (KX898567)	–	–	–	–	(99%) – *E. canis* (KX818219); (100%) – *E. chaffensis* (CP007480)
M16 (KX 898568)	–	–	–	–	(100%) – *E. chaffensis* (CP007480)
M17 (MF061996) (KX891551)	(99%) – *H. macrovacuolatus* (KJ175078)	–	–	–	(100%) – *E. canis* (KX818219)
M18 (KX891552)	–	–	–	–	(100%) – *E. canis* (KF972447)
M19 (KX891553) (KX898569)	–	–	–	–	(99%) – *Ehrlichia* sp. (JQ260854) (100%) *E. chaffensis* (KY644145)
M21 (KX898570)	–	–	+[a]	–	(100%) *E. chaffensis* (KY644145)
M23 (MF061997) (KX898571)	(99%) – *H. macrovacuolatus* (KJ175078)	–	–	–	(100%) – *E. chaffensis* (CP007480)
M24 (KX898572)	–	–	–	–	(100%) – *E. chaffensis* (CP007480)
M25 (MF061998)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	–
M26 (KX898573)	–	–	–	–	(100%) – *E. chaffensis* (CP007480)
M27 (KX898574)	–	–	–	–	(100%) – *E. chaffensis* (CP007479)
M29	–	–	+[a]	–	–
M31	–	–	+[a]	–	–
M32 (MF043230)	–	(100%) *Plasmodium* sp. (KU562663)	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Goose ID and GenBank access numbers	*Haemoproteus* spp. cytochrome b	*Plasmodium* spp. cytochrome b	*Babesia* spp. 18S rRNA	*Anaplasma* spp. 16S rRNA	*Ehrlichia* spp. 16S rRNA and (omp-1)[1]
M33 (MF061999) (KX898575)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	(100%) – *E. chaffensis* (CP007480)
M36	–	–	–	–	+[a]
M40 (MF062000)	(99%) – *H. macrovacuolatus* (KJ592828)	–	–	–	+[a]

sequences showed poor quality and were not deposited in GenBank. No goose sampled showed positivity for *Leucocytozoon* spp.

Concerning to ML analysis, the genotypes #1 and #2 identified among the *Haemoproteus* sequences, according to BLAST analysis, were positioned near to *H. macrovacuolatus* and supported by a high bootstrap value (95%) (Fig. 3**)**. Furthermore, also according to BLAST results, the *Plasmodium* sequences clustered together with sequences that shared highest identity, supported by a high bootstrap value (100%) and near to *P. gallinaceum* sequences (Fig. 4**)**. All haemoporidian sequences amplified in the present study showed query coverage of 100% when compared to sequences previously deposited in GenBank. All the sequences submitted to BLAST and phylogenetic analyses were deposited in GenBank under the access numbers: *Haemoproteus* sp. (MF061993-MF062004); *Plasmodium* sp. (MF043229–MF043230).

3.3.2. Piroplasmids agents

Six out of 62 blood samples (9.68%) were positive in the nested PCR for *Babesia* spp. based on the *18S rRNA* gene, using the protocol previously described [33]. None of the samples was positive in the other protocols presented in Table S1. Due to the low intensity of bands, only one amplicon was purified and submitted to sequencing. The BLAST analysis showed 99% identity to *Babesia* sp. detected in a specimen of wild rodent (*Thrichomys fosteri*) sampled in Brazil (KY450742) (Table 1**)**. Additionally, the ML analysis, supported by high bootstrap value (86%), positioned the sequence amplified in the present study near to *B. vogeli* identified in dogs and cats from different Brazilian states, as well as near to the sequence identified in the wild rodent (Fig. 5**)**. The 18S rRNA *Babesia* sequence showed query coverage of 100% with sequences previously deposited in GenBank and was deposited in GenBank under the access number MF034729.

3.3.3. Anaplasmataceae agents

Twenty seven (43.55%) out of 62 Orinoco geese blood samples were positive in nested cPCR assays for Anaplasmataceae agents based on 16S rRNA gene: three for *Anaplasma* spp. and 24 for *Ehrlichia* spp. Among the positive samples to *Anaplasma*, only two sequences were obtained and submitted to BLAST and ML analyses. The amplified sequences showed 100% identity to *A. phagocytophylum* detected in a raccon dog from South Korea (KY458571). Also, the sequences, when submitted to ML analysis, clustered with other *Anaplasma* spp. sequences identified in wild mammals and ticks in several regions around the world, including an *Anaplasma* sequence originated from a Brazilian vulture (JN217095) (Fig. 6**)**. On the other hand, among the 24 positive samples for *Ehrlichia* spp. based on 16S rRNA gene, 23 sequences were obtained and analyzed. These 23 sequences were divided into four genotypes. The genotype #1 consisted of five sequences that shared 100% identity with several *E. canis* sequences detected around the world (Table 1**)**. The genotype #2 was composed by only one sequence showing 99% identity with *E. canis* amplified from a dog sampled in India (KX818219). Additionally, the genotype #3 consisted of 16 sequences that shared 100% identity with several *E. chaffeensis* sequences, including the *E. chaffeensis* strain Arkansas (NR074500). Finally, the genotype #4 was composed by only one sequence sharing 99% identity with *E. chaffensis* str. West Paces (CP007480) (Table 1). The ML analysis, in accordance with the results found in BLAST analysis, clustered the sequences belonging to genotypes #1 and #2 with *E. canis* sequences detected in dogs and ticks and with *Ehrlichia* sp. detected in wild felids from Brazil, supported by a high bootstrap value (100%). On the other hand, the genotypes #3 and #4 were positioned nearest to *E. chaffeensis* (Fig. 7**)**. Finally, among the 27 positive samples for *Ehrlichia* spp. in nested PCR assays based on 16S rRNA gene, seven showed to be positive in a nested cPCR based on *omp-1* gene. However, due to low bands intensity, only one sequence was obtained. The sequence shared 100% identity with *Ehrlichia* sp. (JN217097) and *E. canis* (EF014897) detected in a goose and in a dog, respectively, in Brazil (Table 1). However, the *omp-1* sequence, when submitted to ML analysis, was

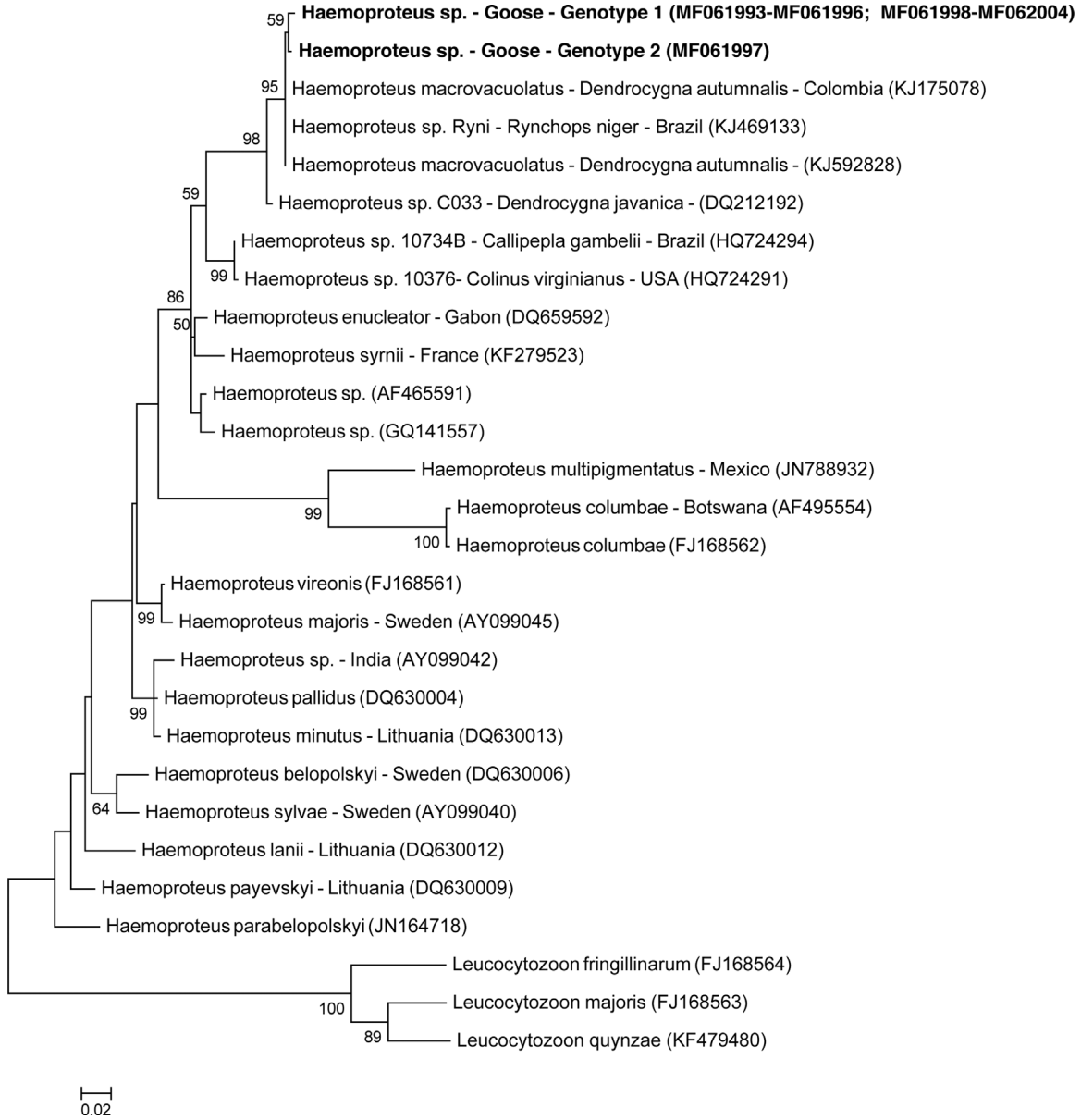

Fig. 3. Phylogenetic relationships within the *Haemoproteus* genus based on *cytochrome b* gene. The tree was inferred by using the Maximum Likelihood (ML) with the GTR + G + I model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Leucocytozoon*species were used as outgroup.

closely related to *Ehrlichia* sp. detected in wild animals sampled in Brazil, supported by a high bootstrap value (100%) and phylogenetically distinct from *E. canis* sequences (Fig. 8**)**. All the Anaplasmataceae sequences showed query coverage ranging from 99% to 100% with sequences previously deposited in Genbank, and were deposited under the access numbers: *Anaplasma* sp. (KX886807–KX886808); *Ehrlichia* spp. ([16S RNA – KX898136–KX898137; KX891551–KX891553; KX898559–KX898575; KX898584] [*omp1* – KX833247]).

No sample was positive for *Ehrlichia ewingii* neither for the other genes tested (*groEL* and *dsb*) by cPCR assays. All samples were negative in qPCR assays for *msp2-A. phagocytophilum*, *vlpt-E.chaffeensis* and *dsb-E.canis*.

3.3.4. Co-positivity by arthropod-borne pathogens

Among the 62 geese blood samples analyzed by cPCR, eleven showed co-infection (Table 1). Seven were simultaneously positive to *Haemoproteus* and *Ehrlichia* species. Furthermore, three samples were co-positive to different *Ehrlichia* species/genotypes. Additionally, two samples were co-positivity to *Babesia* and *Ehrlichia* species, one for *Plasmodium* and *Ehrlichia* species and one goose sample was positive for both *Anaplasma* sp. and *Ehrlichia* sp. (Table 1).

4. Discussion

In this work it was demonstrated, through cPCR, serology and blood smears, those Orinoco geese are exposed to Anaplasmataceae agents, haemosporidian and piroplasmids. Even though suggestive structures of hemosporidian protozoan were observed in blood smears, it is well referenced in the literature that this technique shows a low sensitivity in detecting hemoparasites when compared to molecular and serological tests [24], mainly in low parasitemia infections.

Recently, the description of piroplasmids in avian species around the world has been raised [9–12,48–52]. According to Chavatte et al. [53] avian piroplasmids form a poliphyletic group that is currently divided into three clades: the first one is related to *Babesia duncani* and protheilerids and formed *by B. ardeae* from *Ardea cinerea, B. poelea* and

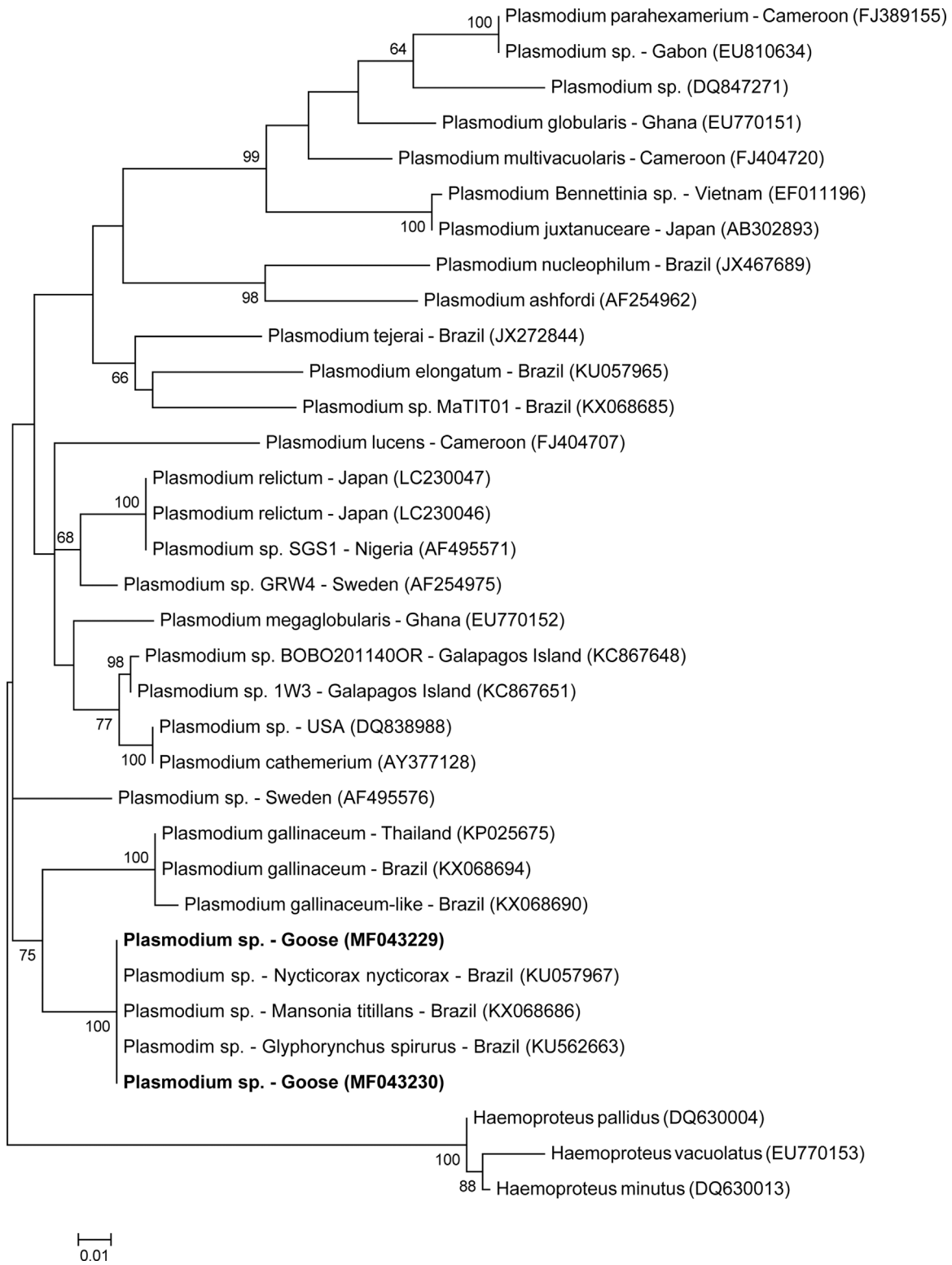

Fig. 4. Phylogenetic relationships within the *Plasmodium* genus based on *cytochrome b* gene. The tree was inferred by using the Maximum Likelihood (ML) with the GTR model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Haemoproteus* species were used as outgroup.

Babesia sp. *1 JM-2013* from *Sula leucogaster, B. uriae* from *Uria aalge*, and *Babesia* sp. TAS117 and TAS124 from *Eudyptula minor*; the second one formed by *Babesia kiwiensis* from *Apteryx mantelli* and *B. kiwiensis*-like from *Turdus falklandii* positioned in a small clade within the babesids; and the third one formed only by *Babesia bennetti* from *Larus cachinnans* that was positioned near to *B. bovis* and *B. ovis* within the babesids [49–52,54]. The present work showed a possible novel clade, positioning the piroplasmid detected in *N. jubata* in *B. vogeli* clade. The availability of other avian piroplasm sequencesis much needed in order to allow comparisons and a better understanding of their evolution and phylogenetic relationship with piroplasms circulating in mammals and other birds [53,55]. Additionally, the use of additional fast-evolving

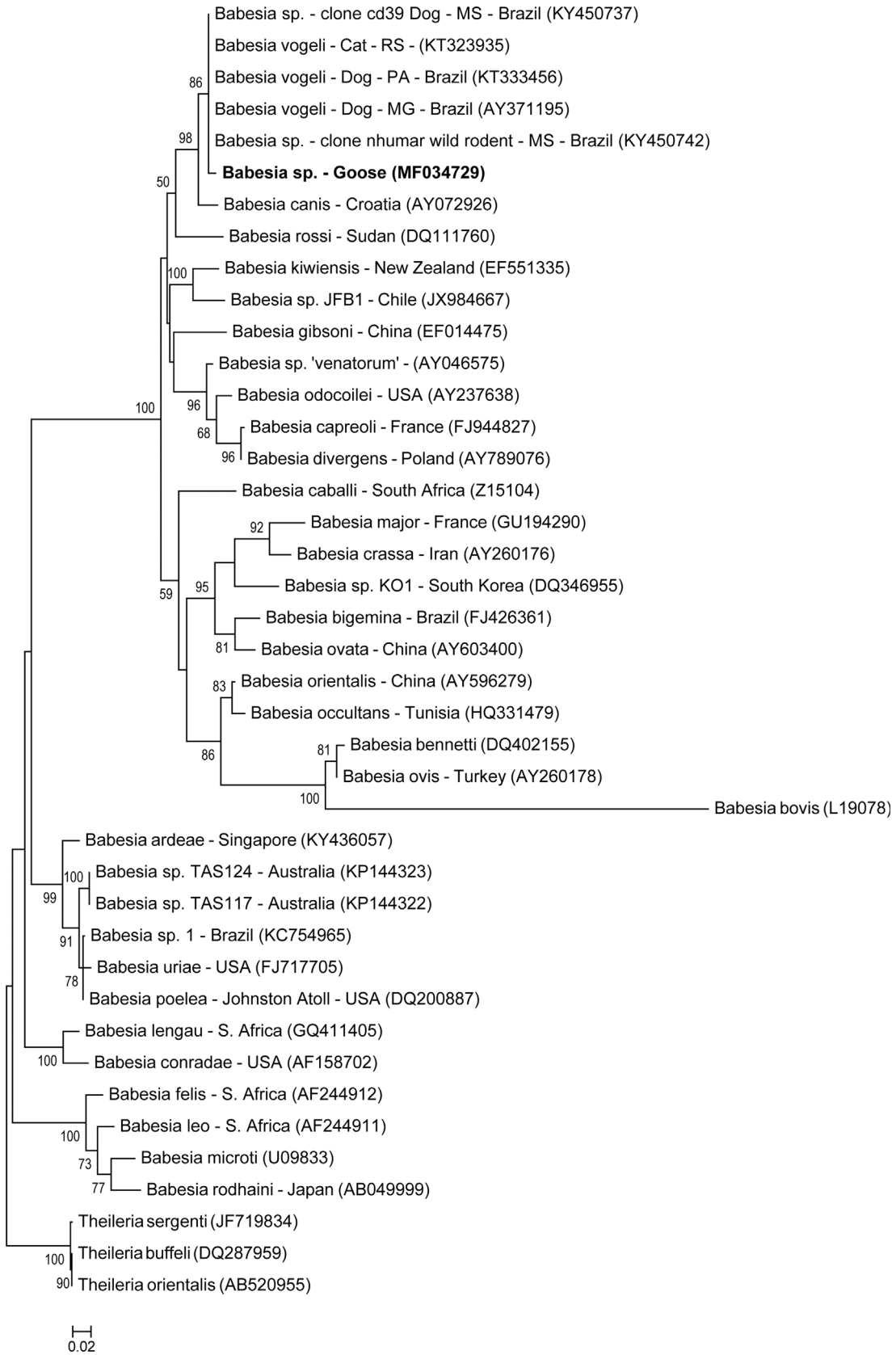

Fig. 5. Phylogenetic relationships within the *Babesia* genus based on 18S rRNA gene. The tree was inferred by using the Maximum Likelihood (ML) with the GTR model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Theileria* species were used as outgroup.

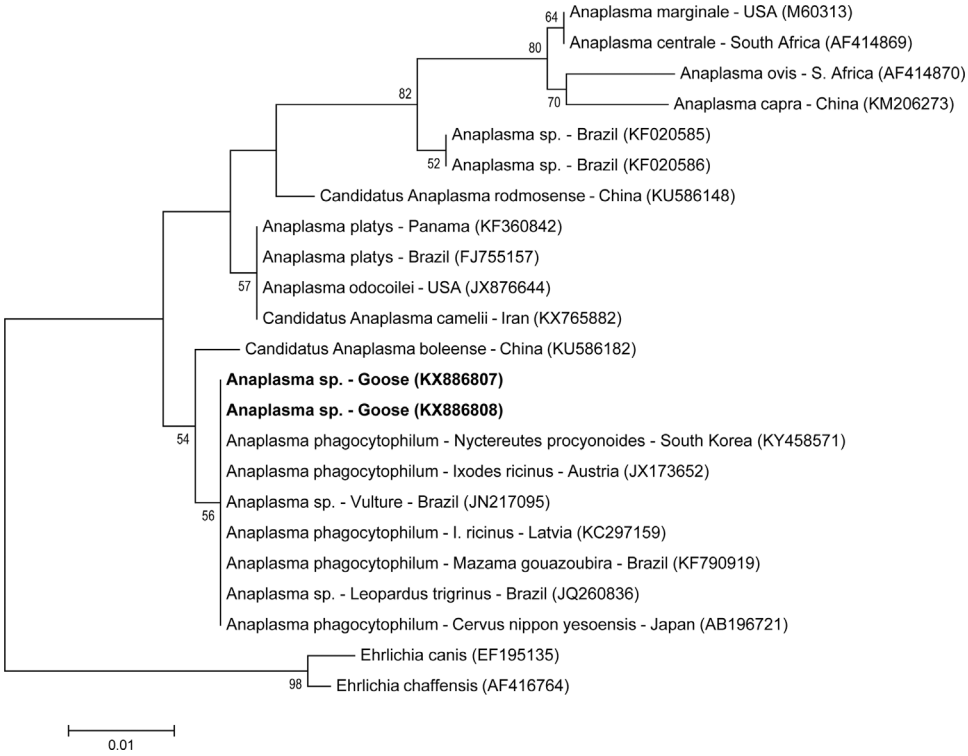

Fig. 6. Phylogenetic relationships within the *Anaplasma* genus based on 16S rRNA gene. The tree was inferred by using the Maximum Likelihood (ML) with the JTT model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Ehrlichia* species were used as outgroup.

target genes, such as mitochondrial gene sequences, is important to resolve the complex taxonomy of piroplasmids [53,55]. Although ixodid ticks has been incriminated as vectors of the avian piroplasms [10,48,49,56,57] and argasids as potential vectors [10,57], no ticks were found attached at the sampled geese at the time of blood samples collection.

In IFAT, six out of 60 animals (10%) presented antibodies to *A. phagocytophilum.* Although the number of seropositive animals for this agent was higher than that obtained by molecular detection in cPCR (two birds), a previous work suggests that IFAT is not a sensitive method for the detection of Anaplasmataceae agents in birds [58]. In addition, it is worth mentioning the existence of cross-reactions between the various agents of the Anaplasmataceae family [59]. Furthermore, the kinetics of antibodies against the pathogens under study should be better studied. This fact can explain, in parts, the lack of concordance between molecular and serological tests. Vitaliano et al. [60] and Furuta et al. [61], when experimentally infected southern western caracara (*Caracara plancus*) and chickens (*Gallus gallus*) with *T.*

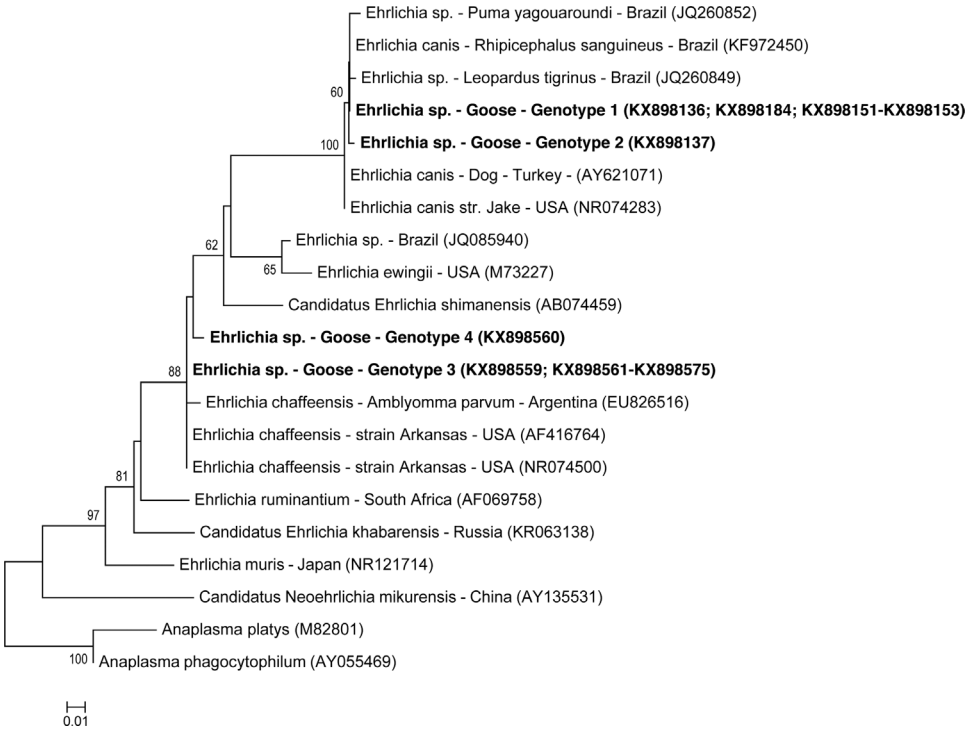

Fig. 7. Phylogenetic relationships within the *Ehrlichia* genus based on 16S rRNA gene. The tree was inferred by using the Maximum Likelihood (ML) with the JTT model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Anaplasma* species were used as outgroup.

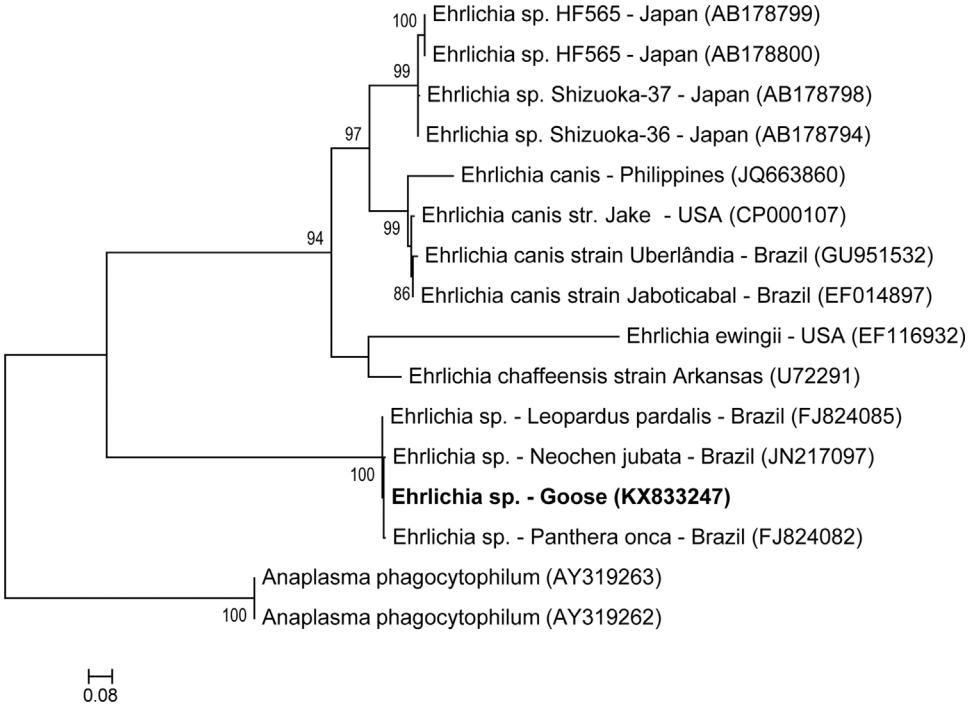

Fig. 8. Phylogenetic relationships within the *Ehrlichia* genus based on *omp-1* gene. The tree was inferred by using the Maximum Likelihood (ML) with the GTR + G + I model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to a bootstrap values higher than 50% accessed with 1000 replicates. *Anaplasma phagocytophilum* was used as outgroup.

gondii and *N. caninum,* respectively, demonstrated that avian species showed the presence of transient detectable antibodies for the studied protozoans, suggesting a different kinetics for the immune-humoral response in birds.

One of the sampled animals was positive in cPCR for *Anaplasma* sp. and also revealed antibodies against *A. phagocytophilum* antigen in IFAT, demonstrating cross-reaction between the detected genotype and *A. phagocytophilum*. Although the genotype of *Anaplasma* sp. detected in the birds was shown to be phylogenetically close to *A. phagocytophilum*, the geese were negative in qPCR specific for the human granulocytic anaplasmosis agent, based on *msp-2* gene. Genotypes phylogenetically associated with *A. phagocytophilum* but negative in *A. phagocytophilum*-specific qPCR assays based on the *msp-2* gene have been already reported in wild carnivores [35], deer [26,62] and rodents [63] in Brazil, African buffaloes (*Syncerus caffer*) in Mozambique [64] and dogs in Colombia [65]. Additionally, genotypes closely related to *A. phagocytophilum* have also been detected in cats [66], caracaras and vultures (*Coragypus atratus*) [20] in Brazil. Our findings corroborate previous studies conducted in South America and Africa and highlight the hypothesis of the circulation of non-isolated *Anaplasma* spp. genotypes in wild animals.

On the other hand, antibodies to *E. canis* and *E. chaffeensis* were not detected in the serum samples from the sampled Orinoco geese, despite the positivity of some animals for genotypes phylogenetically associated with *E. canis* and *E. chafeensis*. In contrast, antibodies to *Ehrlichia* spp. were detected in deer [26] and wild carnivores [23] harboring new genotypes of *Ehrlichia* spp. in Brazil. However, it is not possible to attest that the birds sampled were free of chronic infection by the agent only relying on serological results. In our study, only peripheral blood samples from the birds were collected. Although tissues, such as spleen and liver, could be used to confirm a chronic infection by the parasites under study, no goose died or was euthanized during the present study. Although the genotypes of *Ehrlichia* spp. detected in geese were shown to be phylogenetically close to *E. canis* and *E. chaffeensis,* the sampled birds were negative in qPCR specific for both agents, based on *dsb* and *vlpt* genes, respectively. Genotypes phylogenetically associated with *E. canis and/or E. chaffeensis* but negative in specific qPCR assays based for these agents have been already reported in wild carnivores [34], deer [26] and rodents [63] in Brazil. Genotypes closely related to *E. canis* have also been detected in wild birds [20] and cats [67]. Interestingly, *Ehrlichia* and *Anaplasma* genotypes detected in the present study grouped with 16S genotypes previously detected in wild animals in Brazil, reinforcing the hypothesis of the circulation of yet non-isolated *Anaplasma* and *Ehrlichia* among the wildlife in Brazil. Indeed, the *omp-1Ehrlichia* sequence amplified from one sampled goose in the present study grouped with genotypes previously detected in wild felids in Brazil [23], separated from *E. canis* and *E. chaffeensis*. Considering that *omp-1 gene* evolves faster than16S rRNA gene, it shows a better phylogenetic discrimination that the last one. The isolation of these agents for a better molecular and antigenic characterization is much needed in order to understand the ecology of ehrlichiosis and anaplasmosis in Brazil. Although there is no molecular confirmation of infection by Anaplasmataceae agents in humans Brazil, serological evidence of exposure to *Ehrlichia chafeensis* and *Anaplasma phagocytophilum* antigens have been reported among human beings in the state of Minas Gerais [68]. In the future, the zoonotical potential of Anaplasmataceae agents circulating in wild animals in Brazil should be further investigated. Considering that Orinoco goose is a migratory species, the occurrence of these new *Ehrlichia* and *Anaplasma* genotypes in this animal's dispersal routes deserves additional attention.

Although several studies have been performed in Brazil regarding the genetic diversity of hemosporidian parasites in birds [69–73], the present work shows the first molecular detection by *Plasmodium* and *Haemoproteus* in *N. jubata*. Recently, birds belonging to Anatidae family represented the group most infected by hemosporidians in a molecular survey performed among 677 captive birds maintained in captivity in São Paulo Zoo, the largest zoo in Latin America [74]. Moreover, coinfection by *Haemoproteus* and *Ehrlichia* has been described for the first time in an avian species. *Plasmodium* genotypes detected in Orinoco geese were related to *Plasmodium* spp. previoysly detected in *Nycticorax nycticorax*, *Glyphorynchus spirurus* and *Mansonia titillans* mosquitoes in Brazil. On the other hand, *Haemoproteus* genotypes (#1 and #2) detected in sampled *N. jubata* were closely related to *H. macrovacuolatus* detected in *Dendrocygna autumnalis*, and *Haemoproteus* genotypes detected in *Rynchops niger*and *Dendrocygna javanica*. While *Haemoproteus* parasites are mainly transmitted by biting midges (Ceratopogonidae) or Hippoboscidae flies [75], *Plasmodium* are transmitted by mosquitoes (Culicidae). Recently, *Mansonia* mosquitoes, namely *M. titillans* and *M.*

pseudotitillans, were incriminated as putative vectors of avian *Plasmodium* lineages in Brazil [76].

It is worth mentioning that at the time of sampling, the geese were in the molting season, suggesting that they were facing a period of physiological stress and great metabolic expenditure. In addition, they were in the reproduction period, directing their energies for taking care of gosling. This physiological distress period may have propitiated the recrudescence of cryptic parasitemia, favoring the detection of the studied agents by molecular techniques. Although detection probability of hemosporidian infection was significantly lower in blood when compared to liver, heart and pectoral muscle tissue types combined in specimens of *Pyriglena leucoptera* [77], it is known that *Plasmodium* relapse has been associated with increased concentrations of corticosterone [78], which is shown to be elevated during the breeding season [79].

Finally, tick-borne diseases (TBDs) may be difficult to control due to their complex epidemiology that may involve different tick vectors and animal hosts [80]. Parasites and parasitism in One Health present potential importance, especially in wildlife [81–83]. An integrative systemic approach towards humans and animal's health, the "health in social-ecological systems", may allow a global comprehension of the inextricable interconnection of humans, pet animals, livestock and wildlife and their social and ecological environment [84]. Human and animal medicine are connected and requires an interdisciplinary team of qualified professionals [85,86], that grow closer together [87] and present bilateral communication, for the control of TBDs, particularly for those of zoonotic concern [80].

5. Conclusions

In summary, the present work showed the occurrence of *Ehrlichia* spp., *Anaplasma* sp., *Babesia* sp., *Plasmodium* sp., and *Haemoproteus* sp. in free-living *N. jubata* in Brazil. The threat of these arthropod-borne pathogens in Orinoco geese fitness, especially during the breading season, should be assessed in the future.

Conflicts of interest

None.

Acknowledgments

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support to Dr. K. Werther (number #2011/11230-1) and Dr. M. R. André (number #2015/14896-1) and M.C. Luzzi' Scientific Initiation scholarship (#2013/15688-8).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cimid.2017.09.003.

References

[1] W. Endo, T. Haugaasen, C.A. Peres, Seasonal abundance and breeding habitat occupancy of the Orinoco goose (*Neochen jubata)* in western Brazilian Amazonia, Bird Consev. Int. 24 (2014) 518–529.
[2] B.C. Livezey, A phylogenetic analysis of modern sheldgeese and shelducks (Anatidae, Tadornini), Ibis 139 (1997) 51–66.
[3] J. Kear, Cavity-nesting ducks: why woodpeckers matter, Br. Birds 96 (2003) 217–233.
[4] N. Luna, M. Libua, M.O. Gallegos, M. Cueva, L. Rodríguez, Redescubrimiento del ganso de monte *Neochen jubata* (Spix, 1825) para la avifauna Argentina, Nótulas Faunísticas 24 (2008) 1–13.
[5] H. Sick, Ornitologia Brasileira, first ed., Nova Fronteira, Rio de Janeiro, 1997.
[6] L.C. Davenport, I. Nole Bazán, N. Carlos Erazo, East with the night: longitudinal migration of the Orinoco goose (*Neochen jubata*) between Manú National Park, Peru and the Llanos de Moxos, Bolivia, PLoS One 7 (2012) e46886.
[7] BirdLife International (2017) Species factsheet: *Neochen jubata*. Downloaded from http://www.birdlife.org on 13 June 2017.
[8] M. Ashgar, D. Hasselquist, B. Hansson, P. Zehtindjjev, H. Westerdahl, S. Bensch, Chronic infection. Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds, Science 347 (2015) 436–438.
[9] M.J. Yabsley, T.M. Work, R.A. Rameyer, Molecular phylogeny of *Babesia poelea* from brown boobies (*Sula leucogaster*) from Johnston Atoll, central Pacific, J. Parasitol. 92 (2006) 423–425.
[10] M.A. Peirce, N.J. Parson, *Babesia ugwidiensis*, a new species of avian piroplasm from phalacrocoracidae in South Africa, Parasite 19 (2012) 375–379.
[11] A. Paparini, L.M. Mclnnes, D. Di Placido, G. Mackereth, D.M. Tompkins, R. Clough, U.M. Ryan, P.J. Irwin, Piroplasms of New Zealand seabirds, Parasitol. Res. 113 (2014) 4407–4414.
[12] P. Quillfeldt, J. Martínez, L. Bgoni, P.L. Mancini, S. Merino, Blood parasites in noddies and boobies from Brazilian offshore islands −differences between species and influence of nesting habitat, Parasitology 141 (2014) 399–410.
[13] N.H. Ogden, L.R. Lindsay, K. Hanincova, I.K. Barker, M. Bigras-Poulin, D.F. Charron, A. Heagy, C.M. Francis, C.J. O'callaghan, I. Schwartz, R. Thompson, A role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada, Appl. Environ. Microbiol. 74 (2008) 1780–1790.
[14] E. Lommano, C. Dvorák, L. Vallotton, L. Jenni, L. Gern, Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland, Ticks Tick Borne Dis. 5 (2014) 871–882.
[15] Y. Rikihisa, Anaplasma phagocytophilum and Ehrlichia chaffeensis, subversive manipulators of host cells, Nat. Rev. Microbiol. 8 (2011) 328–339.
[16] A. Björsdorff, S. Bergström, R.F. Massung, P.D. Haemig, B. Olsen, *Ehrlichia*-infected ticks on migrating birds, Emerg. Infect. Dis. 7 (2001) 877–879.
[17] V. Capligina, I. Salmane, O. Keišs, K. Vilks, K. Japina, V. Baumanis, R. Ranka, Prevalence of tick-borne pathogens in ticks collected from migratory birds in Latvia, Ticks Tick Borne Dis. 5 (2014) 75–81.
[18] R.J. Dingler, S.A. Wright, A.M. Donohue, P.A. Macedo, J.E. Foley, Surveillance for *Ixodes pacificus* and the tick-borne pathogens *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in birds from California's inner coast range, Ticks Tick Borne Dis. 5 (2014) 436–445.
[19] J. De La Fuente, V. Naranjo, F. Ruiz-Fons, U. Höfle, I.G. Fernández De Mera, D. Villanúa, C. Almazán, A. Torina, S. Caracappa, K.M. Kocan, C. Gortázar, Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain, Vector Borne Zoonotic Dis. 5 (2005) 390–401.
[20] R.Z. Machado, M.R. André, K. Werther, E. de Sousa, F.A. Gavioli, J.R. Alves Júnior, Migratory and carnivorous birds in Brazil: reservoir for *Anaplasma* and *Ehrlichia* species? Vector Borne Zoonotic Dis. 12 (2012) 705–708.
[21] S. Jahfari, E.C. Coipan, M. Fonville, A. Docters van Leeuwen, P. Hengeveld, D. Heylen, P. Heyman, C. Van Maanen, C.M. Butler, G. Földvári, S. Szekeres, G. Van Duijvendijk, W. Tack, J.M. Rijks, J. Van der Giessen, W. Takken, S.E. Van Wieren, H. Takumi, Circulation of four Anaplasma phagocytophilum genotypes in Europe, Parasit Vectors 7 (2014) 365.
[22] M.R. André, C.H. Adania, C.H. Teixeira, S.M. Allegretti, R.Z. Machado, Molecular and serological detection of *Babesia* spp. in neotropical and exotic carnivores in Brazilian zoos, J. Zoo Wildl. Med. 42 (2011) 139–143.
[23] M.R. André, C.H. Adania, R.Z. Machado, S.M. Allegretti, P.A. Felippe, K.F. Silva, A.C. Nakaghi, Molecular and serologic detection of *Ehrlichia* spp. in endangered Brazilian wild captive felids, J. Wildl. Dis. 46 (2010) 1017–1023.
[24] A.C.H. Nakaghi, R.Z. Machado, M.T. Costa, M.R. André, C.D. Baldani, Canine ehrlichiosis: clinical, hematological, serological and molecular aspects, Cienc. Rural 38 (2008) 766–770.
[25] P.I. Furuta, T.M.F.S. Oliveira, M.C.A. Teixeira, A.G. Rocha, R.Z. Machado, M. Tinucci-Costa, Comparison between a soluble antigen based ELISA and IFAT in detecting antibodies against *Babesia canis* in dogs, Rev. Bras. Parasitol. Vet. 18 (2009) 41–45.
[26] A.B. Sachii, J.M. Duarte, M.R. André, R.Z. Machado, Prevalence and molecular characterization of Anaplasmatacea agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*), Comp. Immunol. Microbi. Infect. Dis. 35 (2012) 325–334.
[27] K.C. Sousa, H.M. Herrera, I.H. Domingos, J.B. Campos, I.M. Santos, H.H. Neves, R.Z. Machado, M.R. André, Serological detection of *Toxoplasma gondii*, *Leishmania infantum* and *Neospora caninum* in cats from an area endemic for leishmaniasis in Brazil, Rev. Bras. Parasitol. Vet. 23 (2014) 449–455.
[28] O. Heelgren, J. Waldenström, S. Bensch, A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium* and *Haemoproteus* from avian blood, J. Parasitol. 90 (2004) 797–802.
[29] S. Bensch, M. Stjernman, D. Hasselquist, O. Ostman, B. Hansson, H. Westerdahl, R.T. Pinheiro, Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds, Proc. Biol. Sci. 1452 (2000) 1583–1589.
[30] S.L. Perkins, J.J. Schall, A molecular phylogeny malarial parasites recovered from cytochrome b gene sequences, J. Parasitol. 88 (2002) 972–978.
[31] M.J. Yabsley, W.R. Davidson, D.E. Stallknecht, A.S. Varela, P.K. Swift, J.C.J.R. Devos, S.A. Dubay, Evidence of tick-borne organisms in mule deer (*Odocoileus hemionus*) from the western United States, Vector Borne Zoonotic Dis. 5 (2005) 351–362.
[32] L. Medlin, H.J. Elwood, S. Stickels, M.L. Sogin, The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions, Gene 71 (1998) 491–499.
[33] R. Jefferies, U.M. Ryan, P.J. Irwin, PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies, Vet. Parasitol. 144 (2007) 20–27.

[34] M.R. André, J.S. Dumler, D.G. Scorpio, R.H. Teixeira, S.M. Allegretti, R.Z. Machado, Molecular detection of tick-borne bacterial agents in Brazilian and exotic captive carnivores, Ticks Tick Borne Dis. 3 (2012) 247–253.
[35] G.L. Murphy, S.A. Ewing, L.C. Whitworth, J.C. Fox, A.A. Kocan, A molecular and serologic survey of *Ehrlichia canis*, *E. chaffeensis* and *E. ewingii* in dogs and ticks from Oklahoma, Vet. Parasitol. 79 (1998) 325–339.
[36] R.F. Massung, K. Slater, J.H. Owens, W.L. Nicholson, T.N. Mather, V.B. Solberg, J.G. Olson, Nested PCR assay for detection of granulocytic ehrlichiae, J. Clin. Microbiol. 36 (1998) 1090–1095.
[37] D.M. Aguiar, T.B. Saito, M.K. Hagiwara, R.Z. Machado, M.B. Labruna, Serological diagnosis of canine monocytic ehrlichiosis with Brazilian antigen of Ehrlichia canis, Cienc. Rural 37 (2007) 796–802.
[38] M. Inayoshi, H. Naitou, F. Kawamori, T. Masuzawa, N. Ohashi, Characterization ofehrlichia species from *Ixodes ovatus* ticks at the foot of Mt. Fuji Japan, Microbiol. Immunol. 48 (2004) 737–745.
[39] C.K. Doyle, M.B. Labruna, E.B. Breitschwerdt, Y.M. Tang, R.E. Corstvet, B.C. Hegarty, K.C. Bloch, D.H. Walker, J.W. Mcbride, Detection of medically important *Ehrlichia* by quantitative multicolor Taqman real-time polymerase chain reaction of the dsb gene, J. Mol. Diagn. 7 (2005) 504–510.
[40] J.W. Sumner, W.L. Nicholson, R.F. Massung, PCR amplification and comparasion of nucleotide sequences from the groESL heat shock operon of *Ehrlichia* species, J.Clin. Microbiol. 35 (1997) 2087–2092.
[41] S. Lotric-Furlan, M. Petrovec, T.A. Zupanc, W.L. Nicholson, J.W. Sumner, J.E. Childs, F. Strle, Human granulocytic ehrlichiosis in Europe: clinical and laboratory findings for four patients from Slovenia, Clin. Infect. Dis. 27 (1998) 424–428.
[42] W.L. Nicholson, M.B. Castro, V.L. Kramer, J.W. Sumner, J.E. Childs, Dusky-footed wood rats (*Neotoma fuscipes*) as reservoirs of granulocytic Ehrlichiae (Rickettsiales: ehrlichieae) in northern California, J. Clin. Microbiol. 37 (1999) 3323–3327.
[43] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucl. Acids Symp. Ser. 41 (1999) 95–98.
[44] P. Librado, J. Rozas, DnaSP v5: a software for comprehensive analysis of DNA polymorphism, Bioinformatics 25 (2009) 1451–1452.
[45] A. Stamatakis, P. Hoover, J. Rougemont, A rapid bootstrap algorithmfor the RAxML web servers, Syst. Biol. 57 (2008) 758–771.
[46] M.A. Miller, W. Pfeiffer, T. Schwartz, Creating the CIPRES Science Gateway for inference of large phylogenetic trees, Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, (2010) 1–8, 2010.
[47] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.
[48] M.A. Peirce, R.M. Jakob-Hoff, C. Twentyman, New species of hematozoa from Apterygidae in New Zealand, J. Nat. Hist. 37 (2003) 1797–1804.
[49] M.J. Yabsley, E. Greiner, F.S. Tseng, M.M. Garner, R.W. Nordhausen, M.H. Ziccardi, D.L. Borjesson, S. Zabolotzky, Description of novel *Babesia* species and associated lesions from common murres (*Uria aalge*) from California, J. Parasitol. 95 (2009) 183–1188.
[50] R. Jefferies, J. Down, L. Mclnnes, U. Ryan, H. Robertson, R. Jakob-Hoff, P. Irwin, Molecular characterization of *Babesia kiwiensis* from the brown kiwi (*Apteryx mantelli*), J. Parasitol 94 (2008) 557–560.
[51] J. Martínez, R.A. Vásquez, C. Venegas, S. Merino, Molecular characterization of haemoparasites in forest birds from Robison Crusoe Island: is the Austral thrush a potential threat to endemic birds, Bird Conserv. Int.. 25 (2015) 139–152.
[52] R.E. Vanstreels, E.J. Woehler, V. Ruoppolo, P. Vertigan, N. Carlile, D. Priddel, A. Finger, P. Dann, K.V. Herrin, P. Thompson, F.C. Ferreira Junior, M. É. Braga, R. Hurtado, S. Epiphanio, J.L. Catão-Dias, Epidemiology and molecular phylogeny of Babesia sp. in little penguins Eudyptula minor in Australia, Int. J. Parasitol Parasites Wildl. 4 (2015) 198–205.
[53] J.M. Chavatte, C. Okumura, I. Landau, Redescription of *Babesia ardeae* Toumanoff 1940, a parasite of Ardeidae, including a molecular characterization, Parasitol. Res. 116 (2017) 1089–1097.
[54] J.B. Lack, M.V. Reichard, R.A. Van Den Bussche, Phylogeny and evolution of the Piroplasmida as inferred 18S rRNA sequences, Int. J. Parasitol. 42 (2012) 353–363.
[55] M.E. Schreeg, H.S. Marr, J.L. Tarigo, L.A. Cohn, D.M. Bird, E.H. Scholl, M.G. Levy, B.M. Wiegmann, A.J. Birkenheuer, Mitochondrial genome sequences and structures aid in the resolution of Piroplasmida phylogeny, PLoS One 11 (2016) e0165702.
[56] M.A. Peirce, A taxonomic review of avian piroplasms of the genus *Babesia* Starcovici, 1893 (Apicomplexa: piroplasmorida: Babesiidae), J. Nat. Hist. 34 (2000) 317–332.
[57] S. Merino, M.A. Peirce, M. Fernández, P. Lanzarot, Redescription of *Babesia moshkovskii* (Schurenkova) from the griffon vulture *Gyps fulvus* (Hablizl), J. Nat. Hist. 36 (2002) 1635–1638.
[58] E. Johnston, J.I. Tsao, J.D. Muñoz, J. Owen, *Anaplasma phagocytophilum* infection in American robins and gray catbirds: an assessment of reservoir competence and disease in captive wildlife, J. Med. Entomol. 50 (2013) 163–170.
[59] B. Al-Adhami, W.B. Scandrett, V.A. Lobanov, A.A. Gajadhar, Serological cross-reactivity between *Anaplasma marginale* and *Ehrlichia* species in naturally and experimentally infected cattle, J. Vet. Diagn. Invest. 23 (2011) 1181–1188.
[60] S.N. Vitaliano, T.W. Mineo, M.R. André, R.Z. Machado, J.R. Mineo, K. Werther, Experimental infection of crested caracara (*Caracara plancus*) with *Toxoplasma gondii* simulating a natural conditions, Vet. Parasitol. 172 (2010) 71–75.
[61] P.I. Furuta, T.W. Mineo, A.O. Carrasco, G.S. Godoy, A.A. Pinto, R.Z. Machado, *Neospora caninum* infection in birds:experimental infectios in chikens and embryonated eggs, Parasitology 134 (2007) 1931–1939.
[62] A.C. Mongruel, J.L. Benevenute, M.R. André, A.O. Carrasco, R.Z. Machado, M.C. Seki, Molecular characterization of *Anaplasma* sp. in free-ranging gray brockets (*Mazama gouazoubira*), Vector Borne Zoonotic Dis. 17 (2017) 165–171.
[63] J.L. Benevenute, J.S. Dumler, M. Oqrzewalska, A.L.R. Roque, V.V.C. Mello, K.C.M. de Sousa, P.S. D'Andrea, E.R. de Sampaio Lemos, R.Z. Machado, M.R. André, Assessment of a quantitative 5 (nucleasereal-time polymerase chain reaction using *groEL* gene for *Ehrlichia* and *Anaplasma* species in rodents in Brazil, Ticks Tick Borne Dis. 8 (2017) 646–656.
[64] R.Z. Machado, M.M. Teixeira, A.C. Rodrigues, M.R. André, L.R. Gonçalves, J. Barbosa da Silva, C.L. Pereira, Molecular diagnosis and genetic diversity of tick-borne Anaplasmataceae agents infecting the African buffalo *Syncerus caffer* from Marromeu reserve in Mozambique, Parasit Vectors. 9 (2016) 454.
[65] G. Vargas-Hernandez, M.R. André, D.M. Cendales, K.C. Sousa, L.R. Gonçalves, M.C. Rondelli, R.Z. Machado, M. Tinucci-Costa, Molecular detection of *Anaplasma* species in dogs in Colombia, Rev. Bras. Parasitol. Vet. 25 (2016) 459–464.
[66] M.R. André, N.C. Baccarim Denardi, K.C. Marques de Sousa, L.R. Gonçalves, P.C. Henrique, C.R. de Santis, R.Z. Machado, Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil, Ticks Tick Borne Dis. 5 (2014) 545–551.
[67] M.R. André, H.M. Herrera, S.J. Fernandes, K.C. de Sousa, L.R. Gonçalves, I.H. Domingos, G.C. de Macedo, R.Z. Machado, Tick-borne agents in domesticated and stray cats from the city of Campo Grande state of Mato Grosso do Sul, widwestern Brazil, Ticks Tick Borne Dis. 6 (2015) 779–786.
[68] S.B. Calic, M.A.M. Galvão, F. Bacellar, C.M.B.M. Rocha, C.L. Mafra, R.C. Leite, D.H. Walker, Human ehrlichioses in Brazil: first suspect cases, Braz. J. Infect. Dis. 8 (2004) 259–262.
[69] N.O. Belo, R.T. Pinheiro, E.S. Reis, R.E. Ricklefs, É.M. Braga, Prevalence and lineage diversity of avian haemosporidians from three distinct cerrado habitats in Brazil, PLoS One 6 (2011) e17654.
[70] A. Fecchio, M.R. Lima, M. Svensson-Coelho, M.Â. Marini, R.E. Ricklefs, Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil, Parasitology 140 (2013) 181–192.
[71] G.A. Lacorte, G.M.F. Félix, R.R.B. Pinheiro, A.V. Chaves, G. Almeida-Neto, F.S. Neves, L.O. Leite, F.R. Santos, E.M. Braga, Exploring the diversity and distribution of neotropical avian malaria parasites—a molecular survey from Southeast Brazil, PLoS One 8 (2013) e57770.
[72] C.M. Villar, A.L. Bryan, S.L. Lance, E.M. Braga, C. Congrains, S.N. Del Lama, Blood parasites in nestlings of wood stork populations from three regions of the American continent, J. Parasitol. 99 (2013) 522–527.
[73] F.L. Roos, N.O. Belo, P. Silveira, E.M. Braga, Prevalence and diversity of avian malaria parasites in migratory black skimmers (*Rynchops niger*, Laridae Charadriiformes) from the Brazilian Amazon Basin, Parasitol. Res. 114 (2015) 3903–3911.
[74] C.R.F. Chagas, G. Valkiunas, L.O. Guimarães, E.F. Monteiro, F.J. Vaz Guida, R.F. Simões, P.T. Rodrigues, E.J.A. Luna, K. Kirchgatter, Diversity and distribution of avian malaria and related haemosporidian parasites in captive birds from a Brazilian megalopolis, Malar. J. 16 (2017) 83.
[75] G. Valkiunas, Avian Malaria Parasites and Other Haemosporidia, first ed., CRC Press, Boca Raton, 2005.
[76] F.C. Ferreira Jr., R.A. Rodrigues, Y. Sato, M.A.Z. Borges, É.M. Braga, Searching for putative avian malaria vectors in a seasonally dry tropical forest in Brazil, Parasit. Vectors. 9 (2017) 587.
[77] M. Svensson-Coelho, G.T. Silva, S.S. Santos, L.S. Miranda, L.E. Araújo-Silva, R.E. Ricklefs, C.Y. Miyaki, M. Maldonado-Coelho, Lower detection probability of avian *Plasmodium* in blood compared to other tissues, J. Parasitol. 102 (2016) 559–561.
[78] J. Applegate, Spring relapse of *Plasmodium relictum* infections in an experimental field population of english sparrows (*Passer domesticus*), J. Wildl. Dis. 7 (1971) 37–42.
[79] L.M. Romero, Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates, Gen. Comp. Endocrinol. 128 (2002) 1–24.
[80] F. Dantas-Torres, B.B. Chomel, D. Otranto, Ticks and tick-borne diseases: a one health perspective, Trends Parasitol. 28 (2012) 437–446.
[81] R.C. Thompson, Parasite zoonoses and wildlife: one health, spillover and human activity, Int. J. Parasitol. 43 (2013) 1079–1088.
[82] R.C. Thompson, L. Polley, Parasitology and one health, spillover and human activity, Int. J. Parasitol. 43 (2013) 1079–1088.
[83] E.J. Jenkins, A. Simon, N. Bachand, C. Stephen, Wildlife parasites in a one health world, Trends Parasitol. 31 (2015) 174–180.
[84] J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Tanner, From one medicine to one health and systemic approaches to health and well-being, Prev. Vet. Med. 101 (2011) 148–156.
[85] M. Enserink, Medicine initiative aims to merge animal and human health science to benefit both, Science 316 (2007) 1553.
[86] J.P. Sundberg, P.N. Schofield, One medicine, one pathology, and one health concept, J. Am. Vet. Med. Assoc. 234 (2009) 1530–1531.
[87] L.J. King, L.R. Anderson, C.G. Blackmore, M.J. Blackwell, E.A. Lautner, L.C. Marcus, T.E. Meyer, T.P. Monath, J.E. Nave, J. Ohle, M. Pappaioanou, J. Sobota, W.S. Stokes, R.M. Davis, J.H. Glasser, R.K. Mahr, Executive summary of the AVMA one health initiative task force report, J. Am. Vet. Med. Assoc. 233 (2008) 259–261.