Close relationship of *Plasmodium* sequences detected from South American pampas deer (*Ozotoceros bezoarticus*) to *Plasmodium* spp. in North American white-tailed deer

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**ABSTRACT**

We report, for the first time, the presence of ungulate malaria parasites in South America. We conducted PCR-based surveys of blood samples of multiple deer species and water buffalo from Brazil and detected *Plasmodium* sequences from pampas deer (*Ozotoceros bezoarticus*) samples. Phylogenetic analysis revealed that the obtained sequences are closely related to the *Plasmodium odocoieli* clade 2 sequence from North American white-tailed deer (*Odocoileus virginianus*). Nucleotide differences suggest that malaria parasites in South American pampas deer and North American *P. odocoieli* clade 2 branched more recently than the Great American Interchange.

*Plasmodium* parasites of even-toed ungulates have been reported from Africa ([*Sylvicapra grimmia*], marshbuck [*Tragelaphus speki*], and goat [*Capra aegagrus hircus*]; van den Bergh, 1937; reviewed in Garnham, 1966), Asia (water buffalo [*Bubalus bubalis*] and mouse deer [*Tragus javanicus*]; reviewed in Garnham, 1966), and North America (white-tailed deer [*Odocoileus virginianus*]; Kuttler et al., 1967; Garnham and Kuttler, 1980) based on microscopic observations. In 2016, three groups reported the first molecular analyses of this group of malaria parasites (reviewed in Templeton et al., 2016b). Martinsen et al. (2016) detected two molecular analyses of this group of malaria parasites (reviewed in Templeton et al., 2016b). Martinsen et al. (2016) detected two *Plasmodium* sequences, termed *P. odocoieli* clade 1 and 2 based on the report by Kuttler et al. (1967), from white-tailed deer and *Anopheles* mosquitoes in several locations in the United States of America (Martinsen et al., 2016). Boundenga et al. (2016) reported *Plasmodium* sequences from duiker antelope (*Cephalophus spp.*) in Africa (Boundenga et al., 2016). Thirdly, Templeton et al. (2016a) detected two distinct *Plasmodium* sequences from water buffalo in Thailand and Vietnam (Templeton et al., 2016a), which were provisionally called *Plasmodium bubalis* types I and II based on a report in India (Sheather, 1919); and one sequence from a goat in Zambia, provisionally called *Plasmodium caprae* based upon a report in Africa (de Mello and Paes, 1923). It was shown that DNA sequences from the three studies formed a monophyletic clade within haemosporidian parasites, distinct from a clade containing other mammalian and avian/reptile *Plasmodium* parasites (Templeton et al., 2016a,b).

Based on the divergence of the two groups of *Plasmodium* sequences detected from white-tailed deer and mosquitoes in North America, Martinsen et al. (2016) suggested that they likely represented distinct species and that the ancestor of these parasites migrated from Siberia to North America with their host deer. This model was based on the estimated divergence time of 2.3–6 million years ago (MYA), consistent with the estimated period ancestral deer crossed the Bering Land Bridge to North America 4.2 to 5.7 MYA (Gilbert et al., 2006). During the Great
American Interchange around 3 MYA (Stehli and Webb, 1985; Duarte et al., 2008), deer migrated from North America to South America, and thus it would be expected that *Plasmodium* parasites co-migrated with their deer hosts, dependent on available mosquito vectors. Water buffalo were introduced to the Brazil Amazon Basin as early as 1895 (Sheikh et al., 2006), thus it is possible that *P. bubalis* was also introduced to Brazil along with their host. No ungulate parasites have been reported in South America to date, and therefore we sought to determine the occurrence of South American ungulate malaria parasites by conducting PCR-based screening of archived DNA samples obtained from ungulates in Brazil.

A total of 194 DNA samples were examined from the following animals: 60 free-living pampas deer (*Ozotoceros bezoarticus*) in the Pantanal region, 30 free-living or captive brown brocket deer (*Mazama gouazoubira*) and 4 captive marsh deer (*Blastocerus dichotomus*) in Minas Gerais state, and 100 water buffalo (*Bubalus bubalis*) in Para state in the Amazon region were analyzed. Note that only 3 pampas deer samples were positive (5% positivity).

Sixty pampas deer (*Ozotoceros bezoarticus*) in the Pantanal region, 30 brown brocket deer (*Mazama gouazoubira*) and 4 marsh deer (*Blastocerus dichotomus*) in Minas Gerais state, and 100 water buffalo (*Bubalus bubalis*) in Para state in the Amazon region were analyzed. Note that only 3 pampas deer samples were positive (5% positivity).

**Fig. 1.** Map of Brazil depicting the sampling sites of deer and water buffalo with respective sample size.

**Fig. 2.** Phylogenetic relationships of *Plasmodium* sequences from Brazilian pampas deer within Haemosporidia.

The tree was constructed using ∼3.4 kb of partial mitochondrial nucleotide sequences by the maximum likelihood (ML) method based on the GTR + I + G model. Bootstrap values (BV) for ML with 1000 replicates of ultrafast bootstrap analysis and Bayesian posterior probability (BPP) are indicated for each internal branch. The compositions of collapsed clades are *Leucocytozoon* (L. fringillinarum, L. majoris, and L. sabrusseti); *Haemoproteus* and *Parahaemoproteus*; *Plasmodium* (*P. falciparum* sp. jb1.JA27, *P. falciparum* sp. jb2.SS65141, and *P. falciparum* virions); and bird, lizard and non-ungulate mammalian *Plasmodium* (*P. gallinaceum*, *P. relictum*, *P. juxtnucleare*, *P. latzi*, *P. floridense*, *P. mexicanum*, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. coatneyi*, *P. cynomolgi*, *P. falciparum*, *P. gallinaceum*, *P. relictum*, *P. floridense*, *P. mexicanum*, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. coatneyi*, *P. cynomolgi*, and *P. falciparum*). Mitochondrial DNA sequences (including *cytb* and *cox1*) used in this study were listed in the Supplementary Table S1 of Templeton et al. (2016a). Nucleotide sequences of *Plasmodium* sp. in the North American white-tailed deer was based on Table S4 of Martinsson et al. (2016). Nucleotide positions containing indels or undetermined nucleotides, or those where the alignment was not clearly made were excluded. Nucleotide positions corresponding to the *P. falciparum* mtDNA (NC_002375.1) 974–1502, 1509–1576, 1578–1628, 1637–1678, 1698–1760, 1762–1769, 1774–1800, 1806–1831, 1834–1867, 1870–1909, 1914–2031, 2050–3474, and 3486–4444 were used. The length for the substitutions/site (0.04) is indicated.

GTR + I + G model was superior to other models by both Akaike and Bayesian information criterion (Trifinopoulos et al., 2016). Following model analysis, ML analysis was conducted using IQ-TREE ver. 1.5.5 with 1000 replicates of ultrafast bootstrap analysis. Bayesian posterior probabilities (BPP) were also obtained using MrBayes ver. 3.2 with Metropolis-coupled Markov chain Monte Carlo runs, consisting of one cold and four heated chains with a chain temperature of 0.1, for 10,000,000 generations (Ronquist et al., 2012). Log-likelihood scores and trees with branch lengths were sampled every 1000 generations and the first 2,500,000 generations were excluded as burn-in, and the remaining trees were summarized to obtain BPP. The tree was visualized by FigTree ver.1.4.3. The sequences from Brazilian pampas deer localized within the ungulate malaria parasite clade with the highest ML bootstrap support and BPP (100 and 1.00, respectively, Fig. 2). The tree also indicated that the pampas deer *Plasmodium* sequences form one clade with *P. caprae* and *P. bubalis* Type II sequences (100/1.00) apart from the *P. bubalis* Type I sequence, which also confirmed a phylogenetic relationship reported by Templeton et al. (2016b). *P. odocollei* sequences from white-tailed deer were not included in this analysis, due to the lack of sequence information for the corresponding mtDNA region.

We next examined the relationship of the *Plasmodium* sequences from Brazilian pampas deer with North American *P. odocollei* sequences. Partial *cytb* (607 bp) and *cox1* (490 bp) sequence regions available for North American *P. odocollei* clade 1 (KU133755 and KU133748 for *cytb*, KU133758 and KU133757 for *cox1*) and clade 2 (KU133751 and KU133759, respectively) were concatenated (total...
1097 bp) and phylogenetic analysis was conducted with related haemosporidian parasites for which the corresponding cytb and cox1 sequences were available. The resulting tree indicated that the 2 sequences from Brazilian pampas deer form one clade with all reported P. odocoilei-type sequences (with ML/BPP of 69/0.81, respectively), and were monophyletic with the P. odocoilei clade 2 with strong support (95/0.93, respectively) (Fig. 3). The difference between Plasmodium sequences from Brazilian pampas deer and P. odocoilei clade 2 sequence (ODVIR02) were 1 nucleotides per 607 bp for cytb and 6 nucleotides per 982 bp for cox1. When a divergence rate of 0.5–1.3% Myr⁻¹, proposed for Plasmodium cytb, was adopted (Ricklefs and Outlaw, 2010; Pacheco et al., 2011) the Plasmodium in Brazilian pampas deer and P. odocoilei clade 2 sequence were estimated to have diverged approximately 0.3–0.9 MYA (0.1–0.3 MYA by cytb and 0.5–1.2 MYA by cox1). This divergence estimate is much more recent than the Great American Interchange, which occurred around 3 MYA (Stehli and Webb, 1985; Duarte et al., 2008). Ancestors of South American pampas deer and North American white-tailed deer are believed to have separated in North America about 5 MYA (Pitra et al., 2004; Gilbert et al., 2006), and then the ancestor of pampas deer migrated to South America and expanded, whereas this lineage became extinct in North America. One evolutionary scenario of Plasmodium in Brazilian pampas deer is that P. odocoilei clades 1 and 2 evolved in North American deer and clade 2 recently migrated to South America (less than 0.9 MYA) and infected local pampas deer. If this is the case, clade 1 P. odocoilei might have also migrated to South America and awaits discovery. An alternative hypothesis is as follows, the estimated divergence time of P. odocoilei clades 1 and 2 was 2.3–6 MYA, and thus divergence of P. odocoilei clades 1 and 2 could have occurred during the Great American Interchange (~3 MYA). Thus, it is possible that the Plasmodium parasite in the ancestor of pampas deer migrated to South America with its host during the Great American Interchange and via geographic isolation diverged to form the clade 2 P. odocoilei malaria parasite in South America and clade 1 P. odocoilei in North America. Following this divergence the clade 2 P. odocoilei then migrated back to North America.

Water buffalo in the Brazilian Amazon are infected with Theileria parasites that harbor similar sequences with Asian Theileria parasites such as T. buffali, T. orientalis or T. sinensis (Silveira et al., 2016). Thus we expected to detect P. bubalis in these water buffalo samples; however, this was not the case and further studies will be necessary to clarify if P. bubalis was introduced to South America.

In conclusion, this is the first report of ungulate malaria parasites in South America. Plasmodium DNA sequences originating from Brazilian pampas deer form a monophyletic group with P. odocoilei clade 2 that infects white-tailed deer in North America. The estimated diverged time of 0.3–0.9 MYA is much more recent than the Great American Interchange. Because our data is solely based on the DNA information, morphological investigations should be performed in the future to confirm that the obtained sequences were derived from Plasmodium parasites actively infecting the pampas deer. Comprehensive population genetic surveys for Plasmodium species infecting South American deer would provide information to clarify the evolutionary history of this group of parasites, which may in-turn shed new light on the migration history of their host deer in American continents.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2018.01.001.

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