Table S2. Description of primers, amplicon sizes and thermal conditions used in conventional and nested PCR assays for detection and characterization of *Cytauxzoon* spp.

Agent	Gene/ name primer	Primers sequences	Size (bp)	Thermal sequences	Reference
Endogenous gene	***gapdh*** -GAPDHF -GAPDHR	5′-TCCAACACCACCACTGAGATCGGAC -3′ 5′- GTGAGAAGAAATCGGACTGGCC-3′	400	95°C for 5 min; 35 cycles: 95°C for 15s, 50°C for 30s and 72°C for 30s; 72ºC for 5 min	[44]
***Cytauxzoon* spp. (screening)**	18S rRNA -CytF -CytR	5′-GCGAATCGCATTGCTTTATGCT-3′ 5′-CCAAATGATACTCCGGAAAGAG-3′	300	95°C for 5 min; 40 cycles: 95°C for 45s, 59°C for 45s and 72°C for 1 min; 72°C for 5 min	[45]
Piroplasmids (characterization)	18S rRNA -Nbab_1F -18SApiR	5′-AAGCCATGCATGTCTAAGTATAAGCTTTT-3′ 5′-GGATCACTCGATCGGTAGGAG-3′	1500	95°C for 5 min; 50 cycles: 95°C for 30s, 60°C for 30s and 72°C for 2min; 72°C for 5 min	[48]
Piroplasmids (characterization)	18S rRNA -NBAB1Bab -Hep1615R	5′-GGATAACCGTGC TAATTGT-3′ 5′-AAAGGGCAGGGA CGTAATC-3′	1500	95°C for 10 min; 40 cycles: 95°C for 30s, 55°C for 30s and 72°C for 90s; 72°C for 10 min	[46,47]
***Cytauxzoon* spp. (characterization)**	*cytB* -Cytaux_cytB_F1 -Cytaux_cytb_R3	5′-CTTAACCCAACTCACGTACC-3′ 5′- GGTTAATCTTTCCTATTCCTTACG-3′	1434	95ºC for 3 min; 36 cycles; 95ºC for 30s, 53ºC for30s and 72ºC for 1 min 72ºC for 7 min	[49]
***Cytauxzoon* spp. (characterization-nPCR)**	*cox-1* 1st round -Th-for2 -Piro mt R1 2nd round -Th-For2 -Cytaux 260R	5′- TGGYTKGCTTATTGGTTTGG-3′ 5′- ACTTTGAACACACTGCTCG-3′ 5′- TGGYTKGCTTATTGGTTTGG-3′ 5′-AATTCCCATCTCGCTATCACTTTC-3′	1656	95ºC for 3 min; 36 cycles; 95ºC for 30s, 64ºC for30s and 72ºC for 1 min 72ºC for 7 min Annealing temperature 2nd round = 64.4ºC (ocelot samples) and 66ºC (jaguar samples)	Modified after Gou et al., 2012 [50]; Schreeg et al., 2016 [51]; Panait et al., 2021 [6]
Piroplasmids (characterization)	*cox-3* -Cox3F -Cox3R	5′-ACTGTCAGCTAAAACGTATC-3′ 5′-ACAGGATTAGATACCCTGG-3′	600	94°C for 5 min; 50 cycles: 94°C for 20s, 55°C for 30s and 68°C for 45s; 72ºC for 7 min	[51,52]
Piroplasmids (characterization)	18S – 5.8S rRNA region - Api18S rRNA-1690s - Api5.8SrRNA-20as	5′- CTCCTACCGATCGAGTGATCCGGT -3′ 5′- GCTGCGTCCTTCATCGTTGTGTGAG-3′	600	95ºc for 3 min; 45 cycles: 94ºC for 10s, 66ºC for 15s and 72ºC for 20s; 72ºC for 5 min	[53]

Piroplasmids (characterization)	5.8S – 28S rRNA region - Api5.8SrRNA-20s - Api28SrRNA-1as	5′-CTCACACAACGATGAAGGACGCAGC-3′ 5′-CCGCTTARTTTTATGCTTAAATTCAGCGG-3′	400	95ºc for 3 min; 45 cycles: 94ºC for 10s, 66ºC for 15s and 72ºC for 20s; 72ºC for 5 min	Calchi et al. (2024)