

Supplementary Table 1. Primers and cycle conditions of conventional PCRs used in this study.

Target group	Target gene	Primer name	Primer sequence (5'-3')	≈ Length of amplicon (bp)	Thermocycling profile	Reference
Ixodidae	cox1	LCO1490	GGTCAACAAATCATAAAGATATTGG	710	95 °C for 5 min; 40× (94 °C for 40 s; 48 °C for 1 min; 72 °C for 1 min); 72 °C for 10 min	Folmer et al. (1994)
		HCO2198	TAAACTTCAGGGTGACCAAAAAATCA			
Ixodidae	16S rRNA	16S+1	CTGCTCAATGATTTTTTAAATTGCTGTGG	460	95 °C for 5 min; 40× (94 °C for 40 s; 51 °C for 1 min; 72 °C for 1 min); 72 °C for 10 min	Black et al. (1994)
		16S-1	CCGGTCTGAACTCAGATCAAGT			
Piroplasms	18S rRNA	BJ1	GTCTTGTAATTGGAATGATGG	500	95 °C for 10 min; 40× (95 °C for 30 s; 54 °C for 30 s; 72 °C for 40 s); 72 °C for 5 min	Casati et al. (2006)
		BN2	TAGTTTATGGTTAGGACTACG			
<i>Hepatozoon</i> spp.	18S rRNA	HepF	ATACATGAGCAAAATCTCAAC	650	95 °C for 5 min; 35× (95 °C for 40 s; 57 °C for 40 s; 72 °C for 60 s); 72 °C for 7 min	Inokuma et al. (2002)
		HepR	CTTATTATTCCATGCTGCAG			
<i>Hepatozoon</i> spp.	18S rRNA	HAM-1F	GCCAGTAGTCATATGCTTGTC	1700	95 °C for 5 min; 40× (95 °C for 30 s; 58 °C for 30 s; 72 °C for 1,5 min); 72 °C for 5 min	Criado-Fornelio et al. (2006); Hodžić et al. (2017)
		HPF-2R	GACTTCTCCTTCGTCTAAG			
<i>Cytauxzoon</i> spp.	cox1*	Th-For2	TGGYTKGCTTATTGGTTTGG	1966	95 °C for 5 min; 40× (94 °C for 20 s; 64 °C for 30 s; 68 °C for 2,5 min); 72 °C for 7 min	Schreeg et al. (2016); Panait et al. (2021)
		Piro_mt_R1	ACTTTGAACACACTGCTCG			
		Th-For2*	TGGYTKGCTTATTGGTTTGG	1656	95 °C for 5 min; 40× (94 °C for 20 s; 60 °C for 30 s; 68 °C for 2 min); 72 °C for 7 min	
		Cytaux_260R*	AATTCCCATCTCGCTATCACTTTC			
<i>Cytauxzoon</i> spp.	cytb*	Cytaux_cytb_F1	CTTAACCCAACTCACGTACC	1434	95 °C for 5 min; 45× (95 °C for 20 s; 53 °C for 30 s; 68 °C for 1,5 min); 68 °C for 7 min	Schreeg et al. (2013); Panait et al. (2021)
		Cytaux_cytb_R3	GGTTAATCTTTCCTATTCTTACG			
		Cytaux_cytb_Finn*	ACCTACTAAACCTTATTCAAGCRTT	1333	95 °C for 5 min; 45× (95 °C for 20 s; 55 °C for 30 s; 68 °C for 1,5 min); 68 °C for 7 min	
		Cytaux_cytb_Rinn*	AGACTCTTAGATGYAAACTTCCC			
<i>Cytauxzoon</i> sp.	18S rRNA*	7549F	GTCAGGATCCTGGGTTGATCCTGCCAG	1726	95 °C for 5 min; 40× (94 °C for 30 s; 65 °C for 30 s; 68 °C for 2 min); 72 °C for 7 min	Millán et al. (2007); Gallusová et al. (2016)
		7548R	GACTGAATTCGACTTCTCCTTCCTTAAG			
		Cyt-SSU_F2*	CATGGATAACCGTGCTAATTG	1335	95 °C for 5 min; 40× (94 °C for 30 s; 53 °C for 45 s; 72 °C for 1,5 min); 72 °C for 5 min	
		Cyt-SSU_R4*	AGGATGAACTCGATGAATGCA			

*nested PCR with inner primers: the second reactions include 2µl template from the first round

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