

**ITS1.F**

P.jirovecii	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57
P.macacae	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	87
P.canis	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	76
P.carinii	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	76
P.wakefieldiae	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57
P.murina	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	77
P.squirrel	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57
P.oryctolagi	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57
Phare_v1	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57
Phare_v2	1	TTGCTGGAAAGTTGATCAAATTTGGTCATT	57

P.jirovecii	58	TTCTCGCC	174
P.macacae	88	TTCTCGCC	155
P.canis	77	TTCTCGCC	162
P.carinii	77	TTCTCGCC	57
P.wakefieldiae	58	TTCTCGCC	158
P.murina	78	TTCTCGCC	57
P.squirrel	58	TTCTCGCC	57
P.oryctolagi	58	TTCTCGCC	57
Phare_v1	58	TTCTCGCC	57
Phare_v2	58	TTCTCGCC	57

P.jirovecii	58	TTAGCAGGCA	260
P.macacae	175	TTAGCAGGCA	241
P.canis	156	TTAGCAGGCA	249
P.carinii	163	TTAGCAGGCA	57
P.wakefieldiae	58	TTAGCAGGCA	245
P.murina	159	TTAGCAGGCA	57
P.squirrel	58	TTAGCAGGCA	57
P.oryctolagi	58	TTAGCAGGCA	57
Phare_v1	58	TTAGCAGGCA	57
Phare_v2	58	TTAGCAGGCA	57

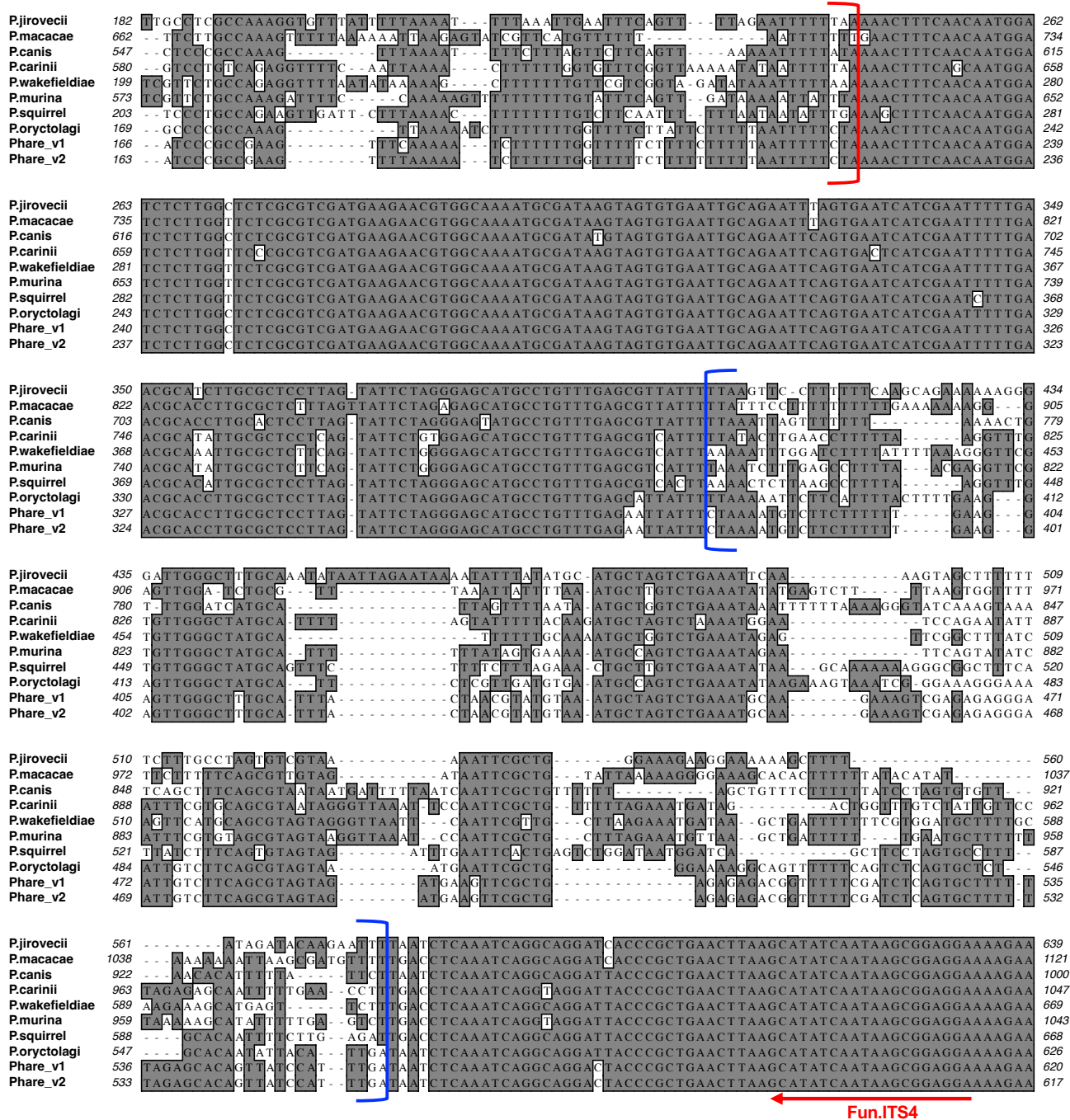
P.jirovecii	58	TAGCCCTGGGTATAGTAACAATG	346
P.macacae	261	TAGCCCTGGGTATAGTAACAATG	315
P.canis	242	TAGCCCTGGGTATAGTAACAATG	334
P.carinii	250	TAGCCCTGGGTATAGTAACAATG	57
P.wakefieldiae	58	TAGCCCTGGGTATAGTAACAATG	332
P.murina	246	TAGCCCTGGGTATAGTAACAATG	57
P.squirrel	58	TAGCCCTGGGTATAGTAACAATG	57
P.oryctolagi	58	TAGCCCTGGGTATAGTAACAATG	57
Phare_v1	58	TAGCCCTGGGTATAGTAACAATG	57
Phare_v2	58	TAGCCCTGGGTATAGTAACAATG	57

P.jirovecii	58	AGGAGCTTTT	433
P.macacae	347	AGGAGCTTTT	383
P.canis	316	AGGAGCTTTT	390
P.carinii	335	AGGAGCTTTT	57
P.wakefieldiae	58	AGGAGCTTTT	372
P.murina	333	AGGAGCTTTT	57
P.squirrel	58	AGGAGCTTTT	57
P.oryctolagi	58	AGGAGCTTTT	57
Phare_v1	58	AGGAGCTTTT	57
Phare_v2	58	AGGAGCTTTT	57

P.jirovecii	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	519
P.macacae	434	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	431
P.canis	384	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	443
P.carinii	391	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57
P.wakefieldiae	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	427
P.murina	373	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57
P.squirrel	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57
P.oryctolagi	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57
Phare_v1	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57
Phare_v2	58	GTGAGTTTAGGTTCTAGGCTTTTCTGAAGG	57

P.jirovecii	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	129
P.macacae	520	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	596
P.canis	432	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	499
P.carinii	444	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	525
P.wakefieldiae	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	132
P.murina	428	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	506
P.squirrel	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	131
P.oryctolagi	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	122
Phare_v1	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	123
Phare_v2	58	TTCCGTAGGTGAACCTGCGGAAGGATCATTAG	120

P.jirovecii	130	TCAAACATCTGTGAAC	181
P.macacae	597	TCAAACATCTGTGAAC	661
P.canis	500	TCAAACATCTGTGAAC	546
P.carinii	526	TCAAACATCTGTGAAC	579
P.wakefieldiae	133	TCAAACATCTGTGAAC	198
P.murina	507	TCAAACATCTGTGAAC	572
P.squirrel	132	TCAAACATCTGTGAAC	202
P.oryctolagi	123	TCAAACATCTGTGAAC	168
Phare_v1	124	TCAAACATCTGTGAAC	165
Phare_v2	121	TCAAACATCTGTGAAC	162



**Supplemental Figure S3. Alignment of nuclear internal transcribed spacer 1-5.8S rRNA-internal transcribed spacer 2 (ITS1-5.8S-ITS2) sequences of different *Pneumocystis* species and strains.** Highly conserved sequences are highlighted in dark shadow. Dashes represents gaps introduced to optimize alignment. The ITS1 region is indicated by red square brackets while the ITS2 region is indicated by blue square brackets. The locations of the primer pair ITS1.F-Fun.ITS4, which showed high amplification efficiency (Figure 1D), are indicated by arrows. The sequences of these primers are listed in Table S2. GenBank accession numbers for all sequences are listed in Table S6. P.squirrel, *Pneumocystis* from tree squirrels; Phare\_v1, *P. sp. 'townsendii'* variant 1 from hares; Phare\_v2, *P. sp. 'townsendii'* variant 2 from hares.