

ms.r13 →

Pj_NC_020331	674	AAGCATTATTTCTA	GGTAACGACTGACATTGAGGTACGTA	GGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	760
Pmac_MIT726214	591	AAGCATTATTTCTA	AGTACGACTGACATTGAGGTACGTA	AGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	677
Pcan_CK1_MIT726215	598	AAGCATTATTTCTA	ATTAACGACTGACATTAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	684	
Pcan_CK2_MIT726216	599	AAGCATTATTTCTA	AGTACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	685	
Pory_MIT726213	612	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	698	
Pc_JX499145	580	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	666	
Pw_MIT726211	741	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	827	
Pm_JX499144	549	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	635	
Ppdog_MIT726212	589	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	675	
Psquirrel	596	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	682	
Phare_v1	587	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	673	
Phare_v2	588	AAGCATTATTTCTA	ATTAACGACTGACATTGAGGTACGTAAGCATAAGTAGCGAAAAGGATTAGATACCCCTTGTAAGTTTATGCTGTAA	674	

Pj_NC_020331	761	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	837
Pmac_MIT726214	678	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	753
Pcan_CK1_MIT726215	685	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	763
Pcan_CK2_MIT726216	686	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	764
Pory_MIT726213	699	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	773
Pc_JX499145	667	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	742
Pw_MIT726211	828	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	904
Pm_JX499144	636	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	712
Ppdog_MIT726212	676	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	752
Psquirrel	683	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	764
Phare_v1	674	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	748
Phare_v2	675	ACGATGAATGCTAGAGGTTGAGATATTTTATTTTGGTCTT	TAA	GTGAAGATTTTAAGCATTCCACCTGAGAAGTA	749

pAZ112-10F/R1 → pAZ112-13/R1 →

Pj_NC_020331	838	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	924
Pmac_MIT726214	754	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	840
Pcan_CK1_MIT726215	764	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	850
Pcan_CK2_MIT726216	765	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	851
Pory_MIT726213	774	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	860
Pc_JX499145	743	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	829
Pw_MIT726211	905	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	991
Pm_JX499144	713	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	799
Ppdog_MIT726212	753	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	839
Psquirrel	765	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	851
Phare_v1	749	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	835
Phare_v2	750	CTGTCGCAAGGCTGAAACTCAAACAT	TAGACGGTACAGAGATCAGCAGTGAAGCATGTTGTTTAAATTCGATAACCCAGAT	836

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Pj_NC_020331	925	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	938
Pmac_MIT726214	841	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	888
Pcan_CK1_MIT726215	851	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	880
Pcan_CK2_MIT726216	852	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	888
Pory_MIT726213	861	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	895
Pc_JX499145	830	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	916
Pw_MIT726211	992	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	1034
Pm_JX499144	800	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	824
Ppdog_MIT726212	840	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	871
Psquirrel	852	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	887
Phare_v1	836	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	866
Phare_v2	837	CTTACCACCTCTTG	CTTACCACCTCTTG	CTTACCACCTCTTG	867

This portion (corresponding to nucleotides 939 to 1070 in Pj) is omitted to reduced the space

SSU.r1 →

Pj_NC_020331	1071	TAGATGAATCAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1157
Pmac_MIT726214	1071	TAGATGAATCAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1187
Pcan_CK1_MIT726215	1106	AAGAGTAATTTAGGATTAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1102
Pcan_CK2_MIT726216	1104	AAGAGTAATTTAGGATTAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1134
Pory_MIT726213	1042	AAGAGTAATTTAGGATTAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1128
Pc_JX499145	1295	AGAAATTAATTTAGGCTGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1381
Pw_MIT726211	1355	AGAAATTAATTTAGGCTGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1441
Pm_JX499144	947	AGAAATTAATTTAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1033
Ppdog_MIT726212	995	AGAAATTAATTTAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1081
Psquirrel	1039	AAGAGTAATTTAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1125
Phare_v1	1008	AAGATGAATAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1094
Phare_v2	1011	AAGATGAATAGGATGAAGACAAGTCTCATGACCCCTATG	AGTGGGCTACAGACGTGCTG	CAAAATTTTCTACAATGGGATGCA	1097

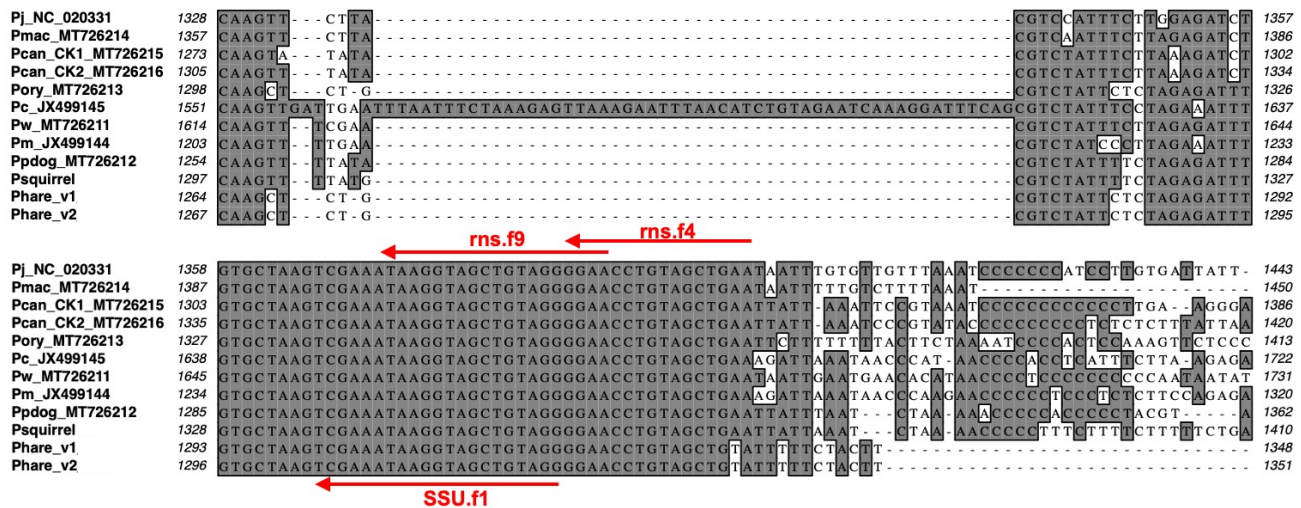
pAZ112-14/R1 ← pAZ112-10R/R1 ←

Pj_NC_020331	1158	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1241
Pmac_MIT726214	1189	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1270
Pcan_CK1_MIT726215	1103	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1186
Pcan_CK2_MIT726216	1135	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1218
Pory_MIT726213	1129	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1211
Pc_JX499145	1382	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1464
Pw_MIT726211	1442	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1526
Pm_JX499144	1034	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1116
Ppdog_MIT726212	1082	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1167
Psquirrel	1126	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1210
Phare_v1	1095	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1177
Phare_v2	1098	TGA	TGGAAGTCTGAGCTAATCCCT	AAAAGATTTT	TAGTCTGGATAAGTGC	CTGGAACCTGCTCTTTGAAGTTGGAATTGCT	1180

pAZ112-10R/R1 ←

Pj_NC_020331	1242	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1327
Pmac_MIT726214	1271	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1356
Pcan_CK1_MIT726215	1187	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1272
Pcan_CK2_MIT726216	1219	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1304
Pory_MIT726213	1212	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1297
Pc_JX499145	1465	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1550
Pw_MIT726211	1527	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1613
Pm_JX499144	1117	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1202
Ppdog_MIT726212	1168	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1253
Psquirrel	1211	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1296
Phare_v1	1178	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1263
Phare_v2	1181	AGTAATCGTCTATCAGCA	GAGACGGTGAATCTTT	TATCTGTGATGTA	CTAAGTCTGCTCAAGCGCGGAATTTT	TAAAGAAATTT	1266

← **rns.f8**



Supplemental Figure S2. Alignment of mitochondrial small-subunit (mtSSU) rRNA sequences of different *Pneumocystis* species and strains. Highly conserved sequences are highlighted in dark shadow. Dashes represents gaps introduced to optimize alignment. New primers designed in this study are indicated in red. The locations of the previously used universal *Pneumocystis* primers [38] were indicated in blue. The sequences of primers pAZ112-10F/R1 and pAZ112-13/R1 are identical to the respective regions in *P. jirovecii* (Pj), primer pAZ112-10R/R1 sequence is identical to the respective regions in *P. carinii* (Pc), and primer pAZ112-14/R1 contains a degenerate nucleotide (Y) at two positions (5'-TTCAAAGAAAYCGAGTTYCAG-3'), which were omitted in the reference [37]. GenBank accession numbers are indicated after the names of *Pneumocystis* species/strains abbreviated as follows: Pj, *P. jirovecii*; Pmac, *P. macacae*; Pcan_CK1, *P. canis* strain CK1; Pcan_CK2, *P. canis* strain CK2; Pory, *P. oryctolagi*; Pc, *P. carinii*; Pw, *P. wakefieldiae*; Pm, *P. murina*, Ppdog, *Pneumocystis ludoviciana* from prairie dogs (Table S6); Psquirrel, *Pneumocystis* from squirrels; Phare_v1, *P. sp. 'townsendii'* variant 1 from hares; Phare_v2, *P. sp. 'townsendii'* variant 2 from hares. GenBank accession numbers for the last 3 sequences are PP484879, PP477339 and PP477340. Numbers on the two ends of each sequence correspond to the nucleotide positions of the full-length mtLSU gene in GenBank.