

Establishment of a PCR Assay for the Detection and Discrimination of Authentic Cordyceps and Adulterant Species in Food and Herbal Medicines

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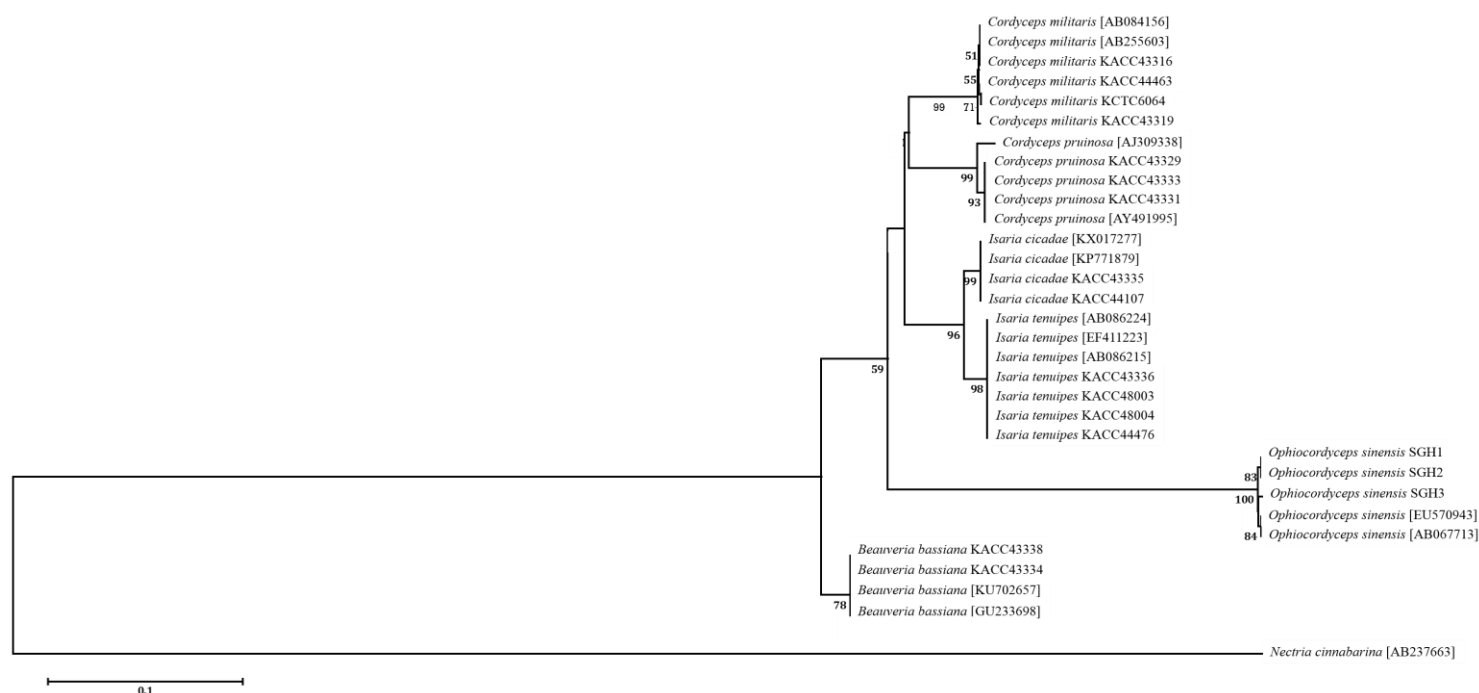


Figure S1. Phylogenetic relationships between six fungal species (Cordyceps and related species, and a closely related medicinal fungus) based on their nrDNA-ITS sequences. Numbers at the nodes are the bootstrap values from 1000 replicates. The nrDNA-ITS sequence of *Nectria cinnabarina* (AB237663) was used as an outgroup.

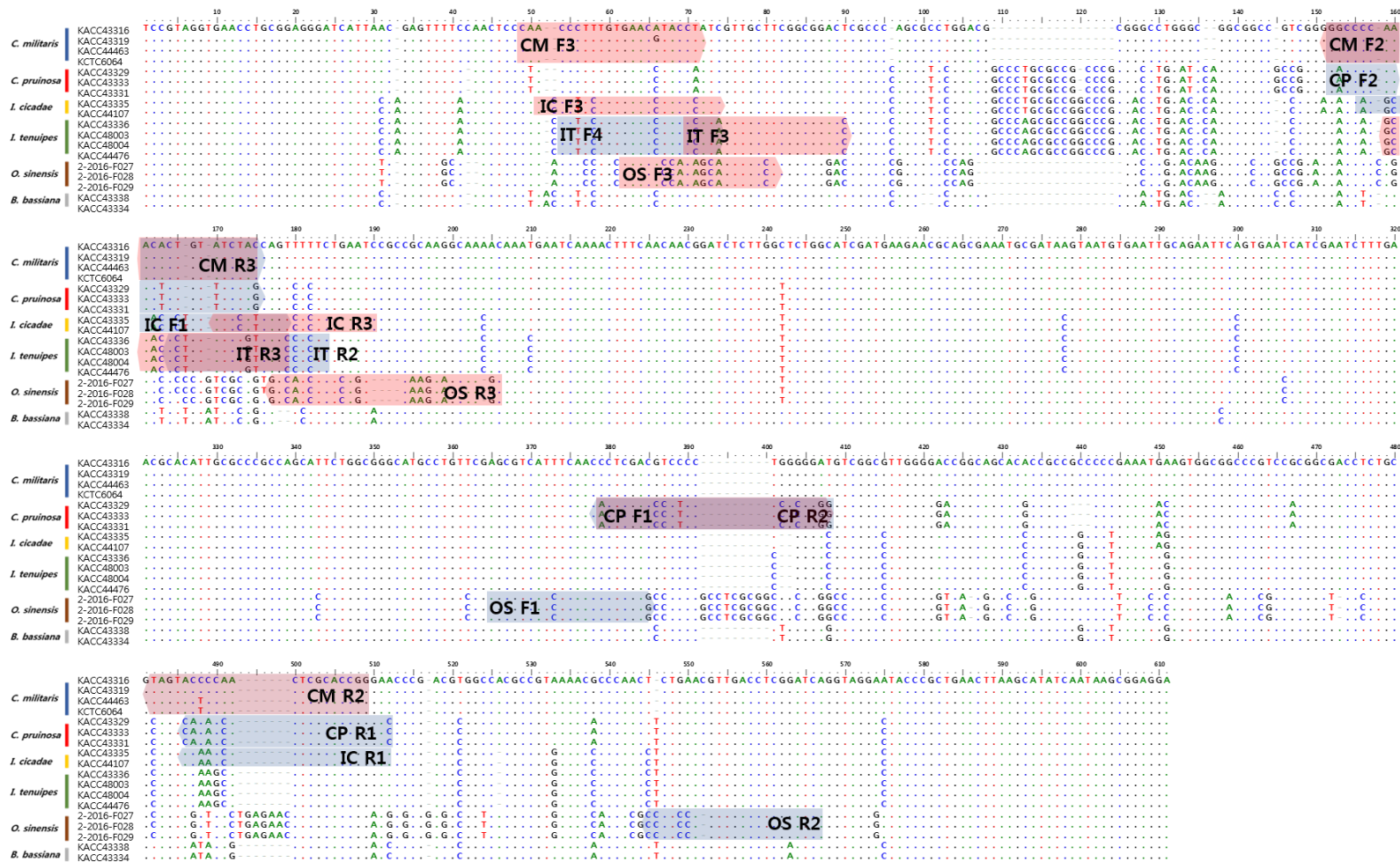
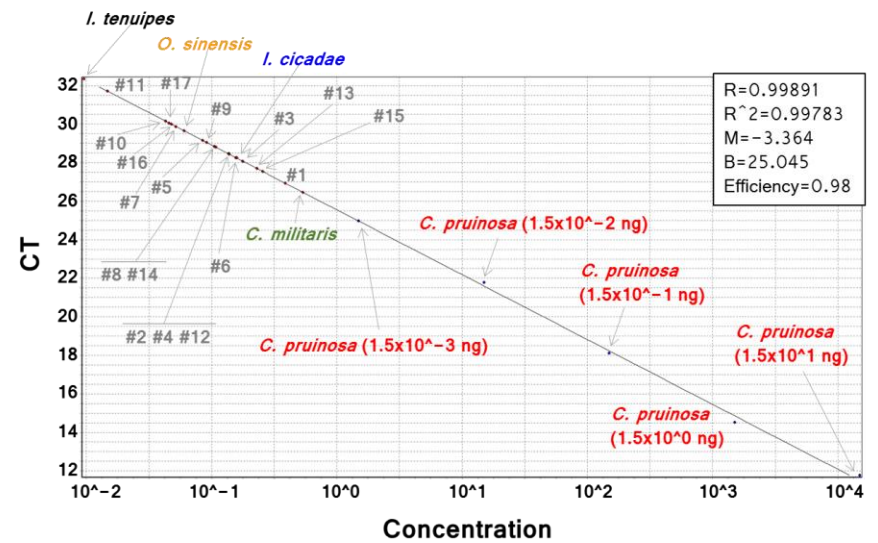
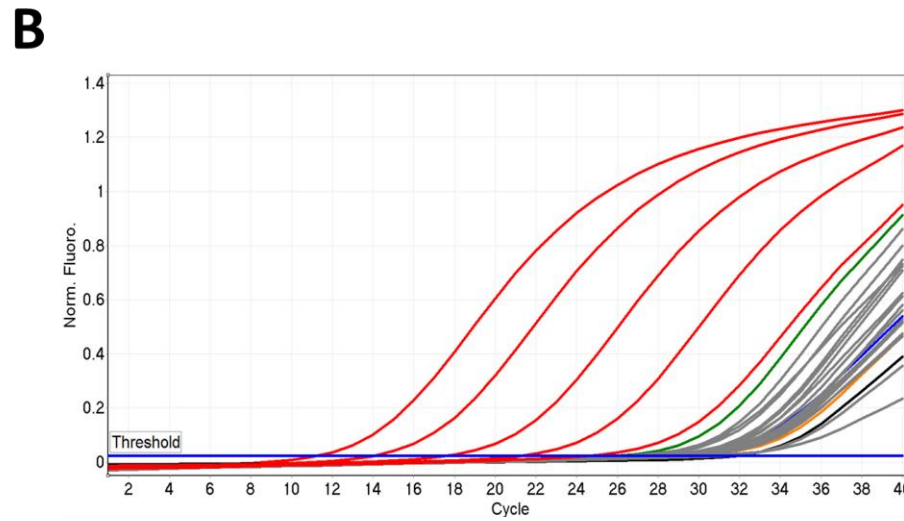
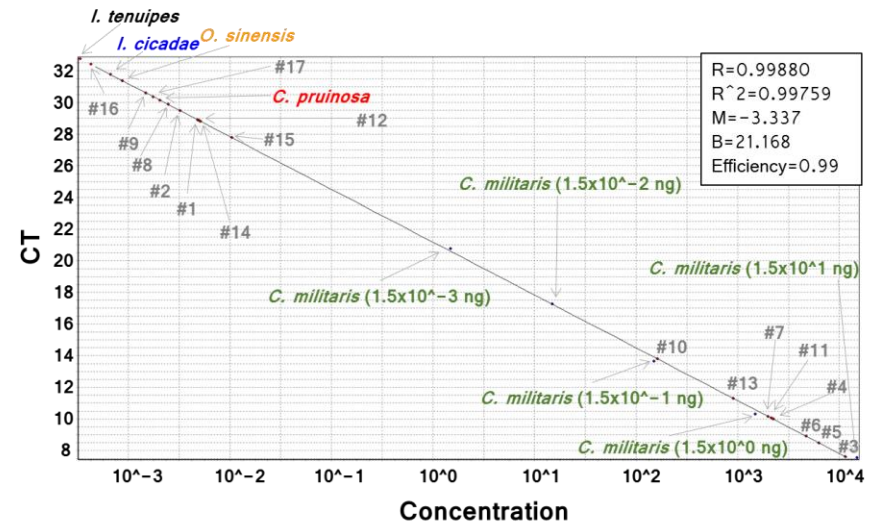
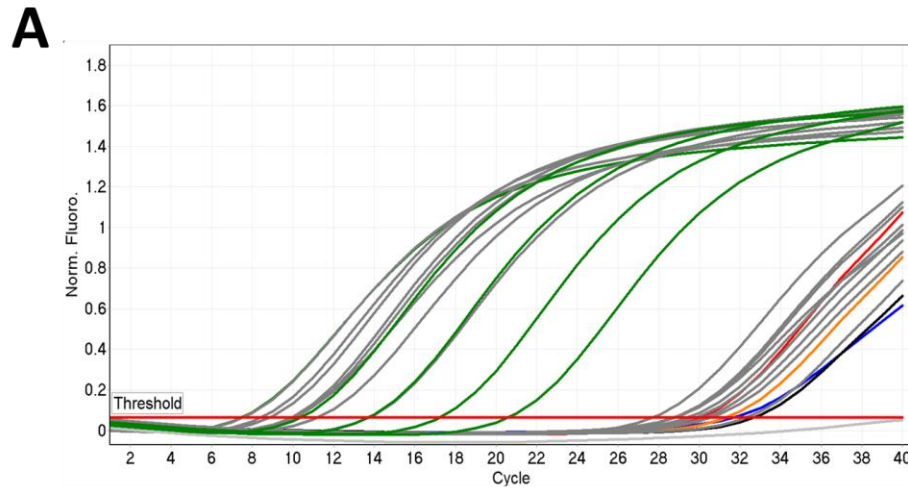
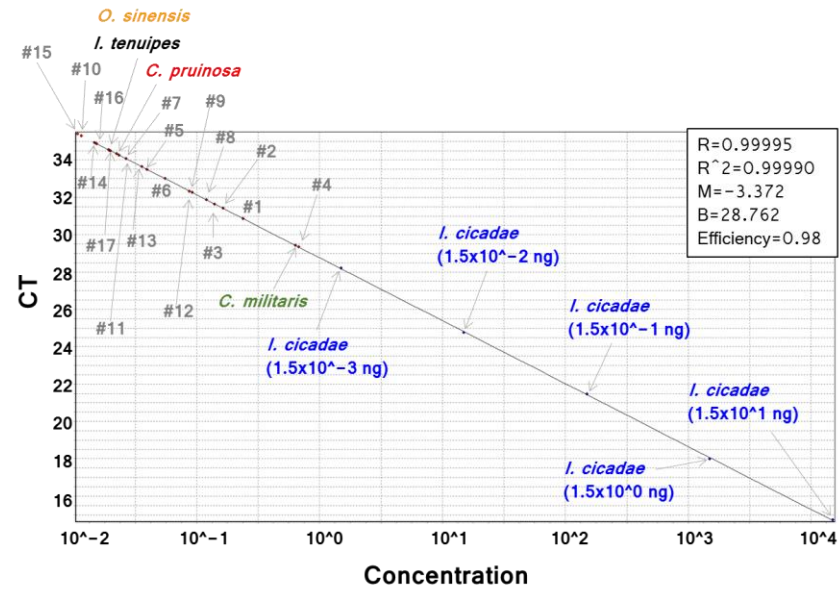
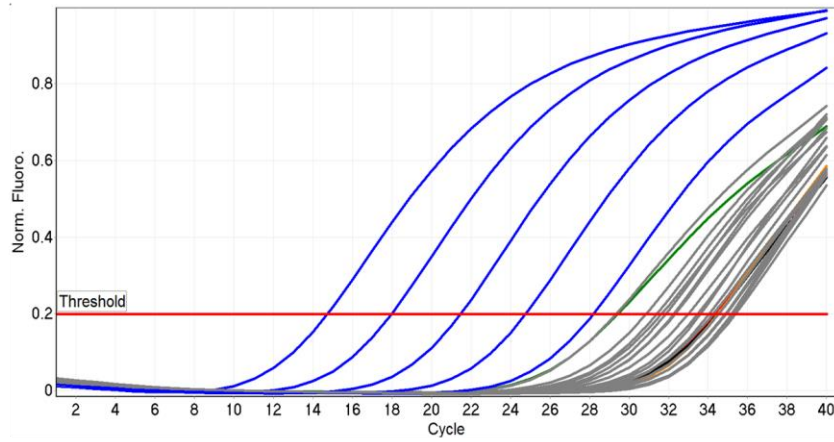


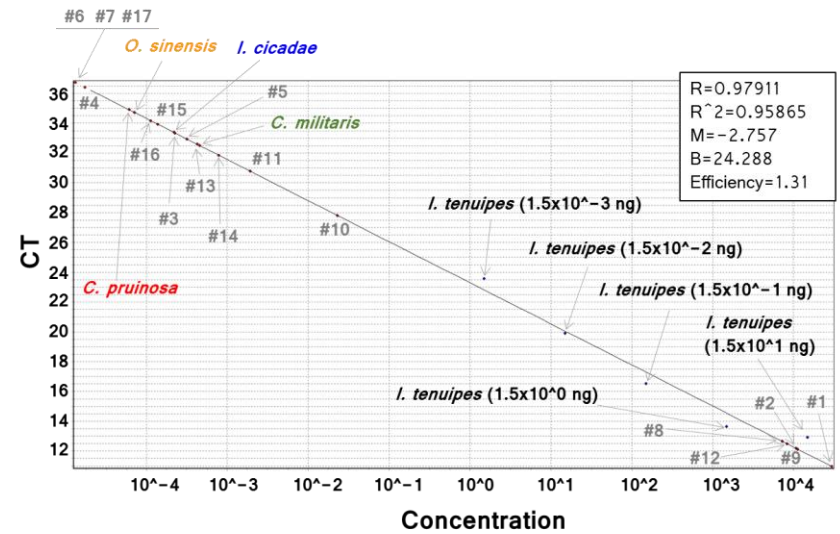
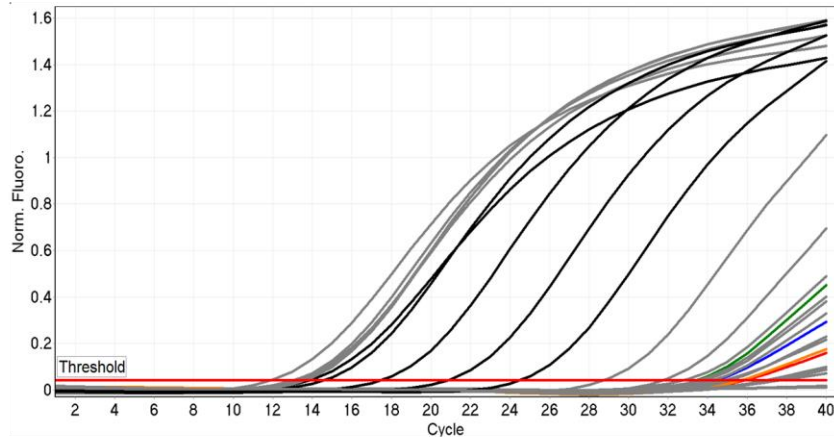
Figure S2. Comparison of nrDNA-ITS sequences and positions of species-specific primers for the development of SCAR markers. Sequences were aligned using the ClustalW alignment tool of the BioEdit program (version 7.2.6). The arrow boxes indicate primer sequences and their polarities. The primer names are given in the boxes. Dots (.) and dashes (-) indicate identical nucleotides and gaps, respectively, when compared with *C. militaris* KACC 43316, to maximize sequence alignment presentation.



C



D



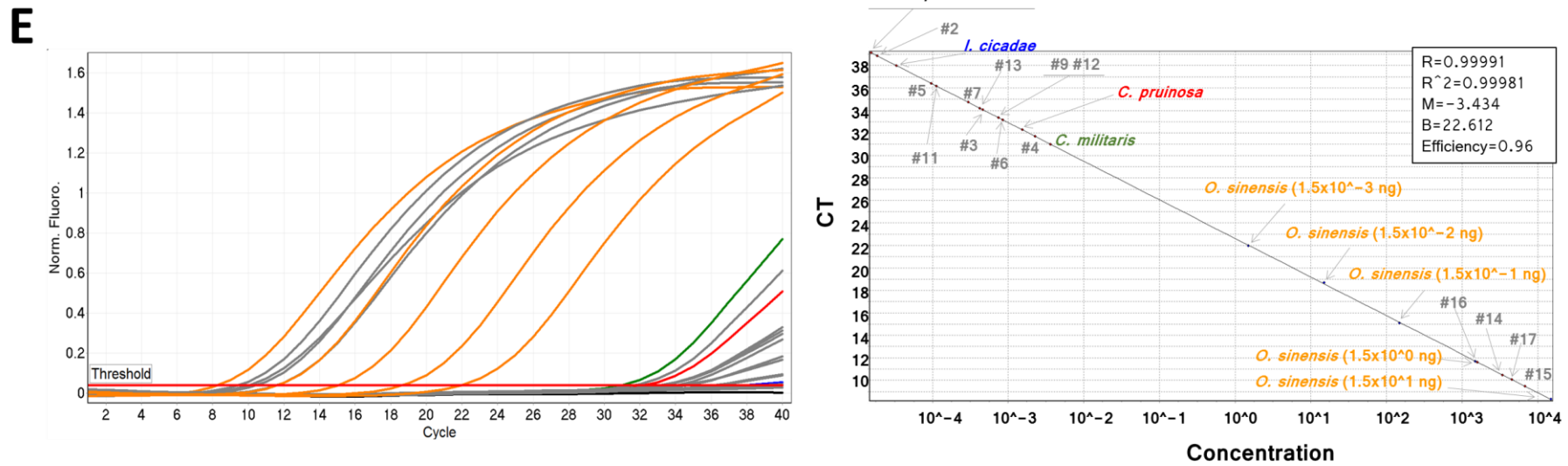


Figure S3. Establishment of the real-time PCR assay for distinguishing Cordyceps and the related species, and the results from real-time PCR assays of commercial products. (A) *C. militaris*, (B) *C. pruinosa*, (C) *I. cicadae*, (D) *I. tenuipes*, and (E) *O. sinensis*. Right panels: Real-time PCR data for five serial dilutions of the control genomic DNA template (15 ng, 1.5 ng, 150 pg, 15 pg, and 1.5 pg) and commercial products. Left panels: Quantification data for five serial dilutions of the control genomic DNA and commercial product templates.

C

	10	20	30	
C. pruinosa_CP F1	CAAGACTCGA	CCC	CTCCTCGCGGGG	TCGG
O. sinensis 2-2016-F027	...C...G...	CCCGC...	...CGGC...	
C. militaris_KACC43316	...C...GT...	...G.G...	AT...	
I. cicadae_KACC43335	...C...GT...	...G...	AC...	
I. tenuipes_KACC43336	...C...GT...	...CG...	AC...	
B. bassiana_KACC43338	...C...T...	...T.G.A...		
O. acicularis_JN049820	...C...G.A...	CG...GG		
O. agriotidis_AB968403	...C...GG...	...CTAG...	CC.C...	
O. appendiculata_JN943325	...C...G...	CCCC.AG.C.CG	...CGG...	
O. arborescens_AB968398	...C...G...	CGGCT		
O. australis_KF937349	CT.TA.T.TA	TTAATATTAAGTATA	T.TT.GA.A	
O. clavata_JN943327	...G...GG.G...	CC...C.A...	CC.C...	
O. coccidiicola_AB968404	...C...G...	CGCCTCGG		
O. coenomyia_AB968396	...G...C...G...	...C...C...	...C...	
O. communis_GU723768	...C...CG...GT	...C.TC.AG...	...CC...	
C. dipterigena_AY245629	A.C...TA.G...C	TGT.G...TAG	...CC...	
C. entomorrhiza_AJ786560	...G...C...GG...	...C...CTCGC...	...CC...	
O. ferruginosa_AB968405	...C...AGG...	CCGCGAC...CA...TC	CC...	
O. gracilis_HM142942	...G...C...G...	...C...CA...	...C...	
O. heteropoda_FJ765027	...C...G...G...	...C...C.G...C...	...CC...	
O. irangiensis_AY646400	...G...AA.GA	GCTATATGG...T.TC	T.TT.GA...	
O. lloydii_KP200892	T.CCTG.TG	TG...CGGTGAT...	C.CG...T	
O. longissimi_AB968406	...T.C...G...	GCCGCGCG.C	CTCCTCC.CC	
O. macroacicularis_KR005829	...C...G...	CCCTC.G...C.CG	...CGGC...	
O. myrmecophila_MH028143	...CT...A.CA	G.AGTCTA	T.TT.GA.C	
O. nigrella_JN049853	...G...C...G...	...CCGCG...		
O. nutans_AB176463	...A.G.ATTTATACCT	GTATAGT.TT	GA.A	
O. rhizoidea_GU723769	...C...G...	CGGCT.GG		
C. robertsii_AJ309335	...C...G...	CCCTC.GG.C.CG	...CGGT...	
O. rubiginosiperithecata_JN94	...C...G...	...C...C...	...C...	
O. sobolifera_AB968409	...T...C...G...	CG.GAG.C.C	...C...	
O. sphecocephala_JN943350	...GT...A.GG	TGCTATAGTAGGGT	TT.ATAGTAA	
O. stylophora_JN049828	...C...G...	CCCCA.C...C.CG		
C. tricentri_AJ309364	...GT...AA.GA	ACTATATGTG...	TT-TATT.GA.A	
O. unilateralis_GU723756	...C...GG.G...	GCGCCG...C.CGTC	CGG...	
O. xuefengensis_KY429942	...C...G...	CCGC...CG.CA	CCTCC...	
C. tuberculata_GQ250005	...C...G...	...C.G.G...	...CC...	
C. spgazzinii_HQ248195	...C...GT...	...C...CG...	...AT...	
C. scarabaeicola_AY245639	...C...TT...	...C.T.TAG...	...A...	
C. roseostromata_AY245637	...C...G...	...G.G...	...AT...	
C. pseudomilitaris_JN943305	...C...G.T...	...C...T.G.A...	...CC...	
C. pleuricapitata_JN943304	...C...AGG...	...C...C.CG...	...CTT...	
C. ninchukispora_AY245642	...C...G...	...C...G...	...CC...	
C. memorabilis_JN116706	...C...T...	CGGCTT.T.T	...AG...	
C. hawkesii_AJ309341	...C...AGG.A...	CCCCCG...GGG	CT.TGGC...	
C. gunnii_AJ309340	...C...AGGTA...	CCCCCG...GGG	CT.TGGC...	
C. fraticida_JQ349068	...C...A.G.G...	TG.G.T.G		
C. cylindrica_GU980041	...G...C...GG.G...	CC...C...	...CC.C...	
C. coccidioperithecata_JN0498	...C...G...T...	...C.TC.G.A...	...CC...	
C. cardinalis_KF937320	...C...G...T...	...C...T.G.A...	...CC...	
C. brongniartii_AB100036	...C...G...T...	...C...T.T.G.AA...	...AC...	
C. bifusispora_AY245627	...C...G...T...	...C...CG...	...AC...	
I. japonica_AF200370	...C...G...T...	...C...CG...	...AC...	
P. hepiali_HM135170	...C...G...T...	...C.T.T.G.AAA...	...AA...	
M. anisopliae_AB071711	GC...A.GT...	...C.TGT...	AC.T...	

D

	10	20	30	40
C. pruinosa_CP R1	CGGGTCCCG	GTGCGAC	TGGT	GTCAGTGGCGAG
O. sinensis 2-2016-F027	...C.C.TT.CG...	...G.GT.CT	CAGT	GA.CT...
C. militaris_KACC43316	...T...G...	...T.G...	GT...	A...
I. cicadae_KACC43335	...T...G...	...G...	TGT	
I. tenuipes_KACC43336	...T...G...	...C...	TGT	
B. bassiana_KACC43338	...T...G...	CT...T	A.T...	A...
O. acicularis_JN049820	...C.C...G.CGA...	...G.GT.CT	CGGAGA	CT...
O. agriotidis_AB968403	...C.CGT...CG...	...G.GT.TC	CGGGA	CT...
O. appendiculata_JN943325	...C.C...CG...	...G.GT.CT	CAGG	GA.CT...
O. arborescens_AB968398	...C.C.TT.CG...	...G.GT.CT	CTGG	GA.CT...
O. australis_KF937349	...CA...TTAAAAA...	...CTTCCAATTATG	...T.TTAA	T...
O. clavata_JN943327	...CCG...CG...	...G.C.T.C		
O. coccidiicola_AB968404	...CTC.TT.CG...	...G.GT.CT	CTGG	GA.CT...
O. coenomyia_AB968396	...C.C...T.CA...	...C.CTGT	...CT...	T...
O. communis_GU723768	...CCT...CGTA...	...G.CGT.C	...T...	T...
C. dipterigena_AY245629	...A...C.G...G...	CT.AACG	AACT	...A...CT
C. entomorrhiza_AJ786560	...C.C...T.CG...	...C.AAG	CT	...C...
O. ferruginosa_AB968405	...CC.CG...CG...	...GCTT.CC	TAGCGCT	...T...C
O. gracilis_HM142942	...C.C...T.CA...	...C.CTGT	...CT...	T...
O. heteropoda_FJ765027	...C.C...T.CG...	...G.GTGT	...G	CT...A...
O. irangiensis_AY646400	T.CTAGT.TTCCAA	TTGCAA.TAAA	TCT	...TTA...
O. lloydii_KP200892	...G.G.CCTCC...	...G	ATCAGT	...G...
O. longissimi_AB968406	G.C.CC...CGA...	...GCGT	T.CT	...C...
O. macroacicularis_KR005829	...C.C.TT.CG...	...G.GT.CT	CTGG	GA.CT...
O. myrmecophila_MH028143	...CC.A.TATAA...	...A.TGCAA	TAGT	...T...TTA...A
O. nigrella_JN049853	-----Noninformation-----			
O. nutans_AB176463	...CGA.TTCT...	...ATTGTA.C.TT	...T...	CTA...A
O. rhizoidea_GU723769	...C.C...TC...	...G	C.AA	TCT...G
C. robertsii_AJ309335	...C.C.TT.CG...	...G.GT.CT	CTGG	GA.CT...
O. rubiginosiperithecata_JN94	...C.C...G.CG...	...G.GT.CT	CAGG	GA.CT...
O. sobolifera_AB968409	...C.CC...CGA...	...GCGT	TC.T	...T...
O. sphecocephala_JN943350	T.CTAAAT.TCCA	ATGGCGA.TAAAA	GTCT	...TGT...
O. stylophora_JN049828	...C.C...G.CG...	...G.GT.CT	CTGG	GA.CT...
C. tricentri_AJ309364	...CTAAAT.TTCCA	TTGTGAAT.AT	...T...	T...A...A
O. unilateralis_GU723756	...CCCGT...CGTC...	...GA	GGTCGT	...G...
O. xuefengensis_KY429942	...C.C.TT.CG...	...G.GT.CT	CTGG	GA.CT...
C. tuberculata_GQ250005	...T.TT.A...	...G	T.G	T...CT
C. spgazzinii_HQ248195	...T...T...A...	...G	T.G	AGT...
C. scarabaeicola_AY245639	...T...T...	...G	T.G	A.T...A...
C. roseostromata_AY245637	...T...T...	...G	T.G	GT...A...
C. pseudomilitaris_JN943305	...T...T...	...G	GT.AT	G...T...A...
C. pleuricapitata_JN943304	...C.C.T...CGA...	...G.GT.CT	CGTG	CT.CT...
C. ninchukispora_AY245642	...TG...	...G		...C...
C. memorabilis_JN116706	...CT...GC...	...G	GT.ATTT	ACT...A...
C. hawkesii_AJ309341	...C...T...	...G	GT.TGT	CT...A...
C. gunnii_AJ309340	...C...T...	...G	GT.TGT	CT...A...
C. fraticida_JQ349068	...C.C...T.GT...	...G	GT.GTAT	...T...A...A
C. cylindrica_GU980041	...C.CCT...CG...	...G	GT.T.CT	...T...
C. coccidioperithecata_JN0498	...T...T...	...G	T.G	ACT...
C. cardinalis_KF937320	...TCT...A...	...G	GC.A	T...CT...A...
C. brongniartii_AB100036	...T...T...	...G	T.G	A.T...A...
C. bifusispora_AY245627	...T...T...	...G		TGT...
I. japonica_AF200370	...T...T...	...G	...C...	TGT...
P. hepiali_HM135170	...T...T...	...G	T.G	A.T...A...
M. anisopliae_AB071711	...C...T...T...	...G	T.CTTT	ACT...

E

	10	20	30	40	50
I. cicadae IC F3	ACTCCCA	A CCCTTCTGTGAACC	TACGC AT	CTTGCTTCGG	
O. sinensis 2-2016-F027	CTC...	A...CCTG C...A CC...AGCAC...		
C. militaris KACC43316	C.T...A...CT		
C. pruinosa KACC43329	CTC..T.	...C.T.....CA		
I. tenuipes KACC43336C...	A.....		
B. bassiana KACC43338	CTC..T.CT		
O. acicularis JN049820	.ACT..C	A...CCTG C...G...CACTG.AC...		
O. agriotidis AB968403	G.....	A...CCTG C...T...C C ACGC...		
O. appendiculata JN943325	CTC..G.	A...CCTG C...C...CACC...		
O. arborescens AB968398	A...CCTG...T...CACAGC...		
O. australis KF937349T.C	TAA.CCT.....TT A.TATCTT.A	TTAC.....		
O. clavata JN943327	CTC..AC	C...CGTG C...G.GAC.CCCGGAAGC...		
O. coccidiicola AB968404	A...CC...C...G...CAC ACAC...		
O. coenomyia AB968396	GA...CCTG C...G...CGCGC...		
O. communis GU723768	A...CA.....TACAGC GC...		
C. dipterigena AY245629	GA.AA.CCA.....A...C ATACC...		
C. entomorrhiza AJ786560	A...CA...CC.....T...C		
O. ferruginosa AB968405	A...CC...A.AG...A A...C CTAC	TA.....C...		
O. gracilis HM142942	AA...CA...C...G...C CAGCC...		
O. heteropoda FJ765027	A...ACTG...T...CTCAC...		
O. irangiensis AY646400	T...AA...CA.....CA...CTATA	TA.....C...		
O. lloydii KP200892	Noinformation			
O. longissimi AB968406	GA...CC.....A T...CACA CC...		
O. macroacicularis KR005829	A...CCTG C...T...CAC ACGC...		
O. macrocephala MH028143	..G.GGG	A.T...C.GA.ACC...GT.AACCTACCTATC...		
O. nigrella JN049853	A...CG.G C.C...GC.TA.CGTCC...		
O. nutans AB176463T.C	AATTC.....A.GTATAT...TTTC	C...C...		
O. rhizoidea GU723769	A...ACTG...A...CCCAAGC...		
C. robertsii AJ309335	CTC...	A...CCTG...C...CTCACC...		
O. rubiginosiperitheciata JN94	A...CGTG...G...CTC GC...		
O. sobolifera AB968409	A...CC...T...CGCAACC...		
O. sphecocephala JN943350	T...AA...CA.....A...CTATA	TC...C...		
O. stylophora JN049828	A...CCTG...G...C CAGCC...		
C. tricentri AJ309364	.TCT..C	AA...CA.....T G.C.CTAT	F.....C...		
O. unilateralis GU723756	..G...A	A...CA.GAGA.C...CGTCGGA AACGAC...		
O. xuefengensis KY429942	A...CC...C...A...CAC ACGC...		
C. tuberculata GQ250005	A...C.G...A...C G		
C. spegazzinii HQ248195A...CT...A		
C. scarabaeicola AY245639	CTC..A.CT...C T		
C. roseostromata AY245637C.T.....G...CT		
C. pseudomilitaris JN943305	A...AAA.....A...CTC TC A		
C. pleuricapitata JN943304	A...CCTGTGA.C.G...C GC...		
C. cinchukispora AY245642	CTC..T.	...C.T...CA C		
C. memorabilis JN116706	A...C.G...AA...A...TTA C T		
C. hawkesii AJ309341	A...C.G...AACTTA...CTATA C TA...		
C. gunnii AJ309340	A...C.G...AACTTA...CTATA C T		
C. fraticida JQ349068	A...C.G...AACTTA...CAC A		
C. cylindrica GU980041	A...CCTGAC...G...CAC AC...		
C. coccidioperitheciata JN0498	T.C.TT.	...C.TCTGTG.A...CT C		
C. cardinalis KF937320	A...AAA.....A...CT TC A		
C. brongniartii AB100036	CTC..T.A.....CT TC A		
C. bifusispora AY245627A...C...		
I. japonica AF200370C...A		
P. hepiali HM135170	A...T...A...CT		
M. anisopliae AB071711C.G...AA TTA...CTTTA...T		

F

	10	20	30	40
I. cicadae IC R3	TTGGGGCGATTGAGA	GAGACTGATG GATACAGGGTTGGTG		
O. sinensis 2-2016-F027	.GTTCTT..C	G AGA..TG.CAC..C.C.GG...G.CT.G		
C. militaris KACC43316	A.A...G.A...T...TG.G		
C. pruinosa KACC43329C.A...A...A...TG.G		
I. tenuipes KACC43336G...CA		
B. bassiana KACC43338	F.....A...TG...G A...A...A.TT..		
O. acicularis JN049820	TGCTT..F.CG.C...GA..TG.C.C.AC.GF.G.A..CTT.G		
O. agriotidis AB968403	GCCT..C.AC.C	GAT..T.CAC..AAG...AG.CT.G		
O. appendiculata JN943325	CTTT..C..G..G AGAA.TG.CAC..C.C.GGA.AG.C..G		
O. arborescens AB968398	C.TGTTT..C	G.G AGAA.TG.CAC..C.C.GA.AG.CT.G		
O. australis KF937349	AAAAATAA.C	...A...T.A.A.AA ATA.T.TA.CATTTAT		
O. clavata JN943327	..G.CGAC.C	...GGGGCACCG...GG.C.GA.GG		
O. coccidiicola AB968404	...CTT..C	G.G AGCA.TG.CAC..C.C.GGA.AG.CT.G		
O. coenomyia AB968396	AAAA.CA..C	...GGACG.C.CGCCC.CCCC.G.G		
O. communis GU723768	..TGTTTT.C	...G CA.TG.ATCTGC.A.T.CAAGA.TT..		
C. dipterigena AY245629	ACAT...GC	...A...TTC.G...AAC.GGTTT.C.TG		
C. entomorrhiza AJ786560	TC..C...A	...C.TCCG.AGA.G...G.T..G		
O. ferruginosa AB968405	CCT.CAAT.F	C.G.GGGAAACC...ATA.AA.CGAG		
O. gracilis HM142942	G..G...C	...GGACAC.CGCCCC.CCG.G		
O. heteropoda FJ765027	GCCT.TGCA.C	...GGAGAA.TT.C.CC.C.C.GGC.TAATG		
O. irangiensis AY646400	ATAAAATC.C	...A...CTAG.TAA.ATAA.A...T		
O. lloydii KP200892	Noinformation		
O. longissimi AB968406	..CGC..TA	C..G..A GAT.CC..C.CGTG..A..G		
O. macroacicularis KR005829	GTTTTTT..C	G..G AGAA.TG.CAC..C.C.GGA.AG.CT.G		
O. macrocephala MH028143	ATAT.C.C.TA	A.ATG.CTCA.A.A.T...T..T		
O. nigrella JN049853	CCGC..GA	TAATCAGAGG.ATC.C.CCGGC.TCG.G		
O. nutans AB176463	TTTAA.F	C.G...A...TGAA.A...T.C...TTAA		
O. rhizoidea GU723769	TTT.C.C	G..G...CG.CTGGAA.TC...GA.TCCT		
C. robertsii AJ309335	.GTTTCTT..C	G..G AGAA.TG.CAC..C.C.GGA.AG.CT.G		
O. rubiginosiperitheciata JN94	..TAT...C	G...TA..TG.CAC..C.C.GGA..G.CT.G		
O. sobolifera AB968409	A...C..CA	C..G..GTGCT.CC..CATGCG..A...		
O. sphecocephala JN943350	AAAA.C	...A...CTAG.TAT.A.A.GA.T..		
O. stylophora JN049828	..TTTTTT..C	G..G AGAA.TG.CAC..C.C.GA.AG.CT.G		
C. tricentri AJ309364	TAAT.F	C...C.A...G...ATAA.T.AC...T		
O. unilateralis GU723756	TCTT.C	C...G...CTG...CTGCC...AGGCTCAGC.AC.T		
O. xuefengensis KY429942	G.TTTCTT..C	G..G AGA..TG.CAC..C.C.GGA..G.CT.G		
C. tuberculata GQ250005	G..A...A	...TGCTC...A...G.T...		
C. spegazzinii HQ248195T...A...TG.G		
C. scarabaeicola AY245639	A...T...A...A...G.TT.A		
C. roseostromata AY245637	A...A...G.A...T...TG.G		
C. pseudomilitaris JN943305A...G.C.A...AAGA.TT.A		
C. pleuricapitata JN943304	..TA.TCC	C...T GAT.CCG.C.GG.ATGC..GA.TGA.TC..		
C. cinchukispora AY245642C.A...A...A...TG.G		
C. memorabilis JN116706	TTT.F	C...G A.TG.CAC.ATA.C.ATCAAGA.TT..		
C. hawkesii AJ309341	..TTTTGT.C	C...CGGTAT.C.A.ACA.TA.AGAGAAA.ACACGA		
C. gunnii AJ309340	..TTTTGT.C	C...CGGTAT.C.A.ACA.TA.AGAGAAA.ACA.GA		
C. fraticida JQ349068	TAATCC.C	...CATGCAT.CCAGA.T.CA.AA.A.T		
C. cylindrica GU980041	AT..TGAC	C...GAGTA...GC.CC.CAGGGGC.CGATG		
C. coccidioperitheciata JN0498G...A...A...A...TG.G		
C. cardinalis KF937320GAC.A...A.GA.T...		
C. brongniartii AB100036	F.....A...GTG...A...A...A.TT.A		
C. bifusispora AY245627G...CA		
I. japonica AF200370T...G...A...A...TT..		
P. hepiali HM135170TAAACC.C...A GATACT.AT.AAA.A.TTCA.A		
M. anisopliae AB071711			

G

	10	20	30	40	50
I. tenuipes_KACC43336	ACTCCCAAC	CCTTCTG	TGAAC	CTACCCAT	A GTTGCTCGGGGACC
I. cicadae_KACC43335
O. sinensis_2-2016-F027	CTC..A...	.CC..C...	ACAG CAGA-
C. militaris_KACC43316	A..T..CT
C. pruinosa_KACC43329	CTC..T...	.C.T.....	A..CT
B. bassiana_KACC43338	CTC..T...	T..CT
O. acicularis_JN049820	CTC..A...	.CC..C..C	G...AC.G	ACC...GA-
O. agriotidis_AB968403	CTC..A...	.CC..C..C	T...CA	CDCC...GA-
O. appendiculata_JN943325	CTC..G..A	.CC..C..C	CAC...GA-
O. arborescens_AB968398	CTC..A...	.CC..C..C	T...ACA	CDCC...GA-
O. australis_KF937349	T...T.CTAA	CCT.....	T TATA	T.TTAATT
O. clavata_JN943327	CTC..AC...	.CCG..C...	CGTGA	CC.GGAAGCC...CG.
O. coccidiicola_AB968404	CTC..A...	.CC..C..C	G...ACA	CACC...GA-
O. coenomyia_AB968396	GA..CC..C	G...CGCGCC...G.G
O. communis_GU723768	T...AGCGTC...T
C. dipterigena_AY245629	T..C.G..A	.CCA.....	A...A..AC	GC...A.G..
C. entomorrhiza_AJ786560	CAT.A.C	G.TCA.T.CA
O. ferruginosa_AB968405	AG.AAC.ACTA
O. gracilis_HM142942	CTC..A..A	.CC..C..C	G...CA	CAGCCC...TG-
O. heteropoda_FJ765027	CTC..A..A	.CA.....	C T...TCAC...GAA
O. iranensis_AY646400	CC A...T..A
O. lloydii_KP200892	Noinformation				
O. longissimi_AB968406	GA..CC.....	AT	A.CACCC...G-
O. macroacicularis_KR005829	CTC..A..A	.CC..C..C	T...ACA	CDCC...GA-
O. myrmecophila_MH028143	CTCT..G..A	.C.....	T...C...A.GGG
O. nigrella_JN049853	CCGC.C.C.CG...G
O. nutans_AB176463	T.C.A.TTCA.TGTA
O. rhizoidea_GU723769	CTC..A..A	.ACTG...C	GCAAGCC...GG-
C. robertsii_AJ309335	CTC..A..A	.CC.....	TCAC...GA-
O. rubiginosiperithecata_JN94	CCG..C..CG...GA-
O. sobolifera_AB968409	CTC..A..A	.CC.....	T...GCAACCC...T-
O. sphecocephala_JN943350	T..A..CAC...A..
O. stylophora_JN049828	CC..C..CC...GG-
C. tracentri_AJ309364	TCT..C.ACA..TG
O. unilateralis_GU723756	CGC..A..A	.CCAC..A..C	CGT.GGAAACC...G-
O. xuefengensis_KY429942	CTC..A..A	.CC..C..C	A...ACA	CDCC...GA-
C. tuberculata_GQ250005	CTC..A..A	A...G..CT
C. spgazzinii_HQ248195	A...T..CT
C. scarabaeicola_AY245639	CTC..A..A	T.CT
C. roseostromata_AY245637	G...T..CT
C. pseudomilitaris_JN943305	CTC..A..A	.AAA.....	A...TCCA
C. pleuricapitata_JN943304	A...CCC
C. ninchukispora_AY245642	CTC..T..T	.C.T.....	A..CT
C. memorabilis_JN116706	A...TTAC
C. hawkesii_AJ309341	TT A...T..ACA..GT.
C. gunnii_AJ309340	TT A...T..ACA..GT.
C. fraticida_JQ349068	CCTT
C. cylindrica_GU980041	CTC..A..A	.CC..AC..C	G...ACA	ACC...GGA
C. coccidioperithecata_JN0498	CC.TTA..T..C
C. cardinalis_KF937320	CTC..A..A	.AAA.....	A...T..CT
C. brongniartii_AB100036	CTC..T..T	A...T..TT
C. bifusispora_AY245627	A...T..CT
I. japonica_AF200370	CTC..A..A	.T.....	A...T..CT
P. hepiali_HM135170	TT A...T..AATGA.
M. anisopliae_AB071711	TT A...T..AATGA.

H

	10	20	30	40	50
I. tenuipes_IT R3	CTTGGCGGGATTCA	GA	GCGGCTCACA	GATACAG	GTTGGGTGGTC
I. cicadae_KACC43335
O. sinensis_2-2016-F027	ACT	GAG	.T.CA..GCGC.GG..G.CT.G..G
C. militaris_KACC43316	AAAA..GGTFG.TT.G..C.
C. pruinosa_KACC43329	A..A..GCTA...TT.G..G
B. bassiana_KACC43338	T...A..ATGG A...A
O. acicularis_JN049820	GC.TGTA	CCG	.T..C..G..GTC..AG.CTTG..G
O. agriotidis_AB968403	GCC.ACA	CG	T.GT.CA..G.AGA..AG.CT.G..G
O. appendiculata_JN943325	T...ACT	GAG	A.T..CA..GCGC.GGA..AG.C..G..G
O. arborescens_AB968398	T.G.ACT	GAG	A.T..CA..GCGC.GA..AG.CT.GT..G
O. australis_KF937349	A.AAAAAATAA	C..A	ATAA.A.A.ATATATAGCA
O. clavata_JN943327	A.ACTCAGG...A.CGCT
O. coccidiicola_AB968404	T.G.ACT	GAG	A.T..CA..GCGC.GGA..AA.GCC..G
O. coenomyia_AB968396	AAA..A	GACTCAG	GA..C.GCGCGCCCTCCC.CGC.GC.GAG..CG
O. communis_GU723768	ATGCATCGCA.A...A
C. dipterigena_AY245629	ACATGC...ATT
C. entomorrhiza_AJ786560	CAGAAGACATC
O. ferruginosa_AB968405	AATACTGAGC
O. gracilis_HM142942	G.GGACTCAG
O. heteropoda_FJ765027	T.CACAACTCAG
O. iranensis_AY646400	CAGAA.A.AGGTAA
O. lloydii_KP200892	Noinformation				
O. longissimi_AB968406	A.ACTGAA
O. macroacicularis_KR005829	T...ACT	GAG	A.T..CA..GCGC.GGA..AG.CT.G.T.G
O. myrmecophila_MH028143	ATATTCACT.AAAT.TC
O. nigrella_JN049853	CGAATAATCAGG.TATCGCCCG
O. nutans_AB176463	TTTTTAA.T..GAAA
O. rhizoidea_GU723769	G..CACT	GAGACCTGG
C. robertsii_AJ309335	ACTGAG
O. rubiginosiperithecata_JN94	TCG.ACT	T..T.CA..GCGC.GGA..AG.CT.G..GG.CT.G..G
O. sobolifera_AB968409	CA.ACTGAGT
O. sphecocephala_JN943350	CAGAA.A.AGGTAT
O. stylophora_JN049828	T...ACT	GAG	A.T..CA..GCGC.GA..AG.CT.GT..G
C. tracentri_AJ309364	CA.AA.AGG
O. unilateralis_GU723756	CTTCC..G..T..TGCC
O. xuefengensis_KY429942	ACTGAG
C. tuberculata_GQ250005	G.AA.AT
C. spgazzinii_HQ248195ATA
C. scarabaeicola_AY245639A.AT
C. roseostromata_AY245637AAAA
C. pseudomilitaris_JN943305A.A.A
C. pleuricapitata_JN943304	G.CCACT	A	GATT.C
C. ninchukispora_AY245642A.A
C. memorabilis_JN116706	TTTC..CACT
C. hawkesii_AJ309341CAGACG
C. gunnii_AJ309340CAGACG
C. fraticida_JQ349068CAGA
C. cylindrica_GU980041C.T.A
C. coccidioperithecata_JN0498A.A
C. cardinalis_KF937320A.A.A
C. brongniartii_AB100036T
C. bifusispora_AY245627A.A
I. japonica_AF200370A..G
P. hepiali_HM135170A.ATA
M. anisopliae_AB071711	T..TTAAACC	C..A	ATAT..TA

I

	10	20	30	40
O. sinensis OS F1	CATG	CCTGTCCG	AGCGTC	ATCTCAACCCCTCGAGCCCC
C. militaris_KACC43316T.....T.....CPT.....
C. pruinosa_KACC43329T.....T.....	C.A.ACTCGA.....T
I. cicadae_KACC43335T.....T.....CPT.....
I. tenuipes_KACC43336T.....T.....CPT.....
B. bassiana_KACC43338T.....T.....C.T.....
O. acicularis_JN049820T.....T.....A.....
O. agriotidis_AB968403T.....T.....G.....
O. appendiculata_JN943325T.....A.....
O. arborescens_AB968398T.....T.....G.....
O. australis_KF937349T.....	T.T	TACTATA	TTAA
O. clavata_JN943327T.....	GC.C.C.G	ACTCG	G.G.
O. coccidiicola_AB968404T.....	AT.TC	CTCGAG
O. coenomyia_AB968396G.T.....G.....
O. communis_GU723768T.....T.....C.....	GT.....
C. dipterigena_AY245629T.....T.....CTT.....	AG.....
C. entomorrhiza_AJ786560T.....	G.A.GG.....
O. ferruginosa_AB968405A.....	T	TC	CTC
O. gracilis_HM142942T.....T.....G.....
O. heteropoda_FJ765027T.....T.....
O. iranagensis_AY646400A.....	T.AC.G	ACTA	AGA
O. lloydii_KP200892T.....	CTAGT	GCT	C.F
O. longissimi_AB968406T.....	TATC.T	CTCGAGG
O. macroacicularis_KR005829T.....T.....
O. myrmecophila_MH028143T.....	AC	TACTAGA	AG
O. nigrella_JN049853T.....	CGCAG	G	CTCGAG
O. nutans_AB176463	A.TTT.....	G.A.TT	T
O. rhizoidea_GU723769G.....T.....
C. robertsii_AJ309335T.....T.....
O. rubiginosiperitheciata_JN94T.....T.....
O. sobolifera_AB968409T.....	TGTC.T	CTCGAGG
O. sphecocephala_JN943350	A.T	T.AC	GTACTAGAGG	TG
O. stylophora_JN049828T.....T.....
C. tricentri_AJ309364T.....	T.AC	GTACTA	AGA
O. unilateralis_GU723756	C	ATGC	CAC	CTCG
O. xuefengensis_KY429942T.....T.....
C. tuberculata_GQ250005A.....A.....C.....
C. spgazzinii_HQ248195T.....T.....CPT.....
C. scarabaeicola_AY245639T.....T.....CPT.....
C. roseostromata_AY245637T.....T.....CPT.....
C. pseudomilitaris_JN943305T.....T.....T.....
C. pleuricapitata_JN943304T.....	G.C.A	CTC	G
C. ninchukispora_AY245642T.....	T.C.A	CTCGA	T
C. memorabilis_JN116706T.....	T.C.A	CTCGT
C. hawkesii_AJ309341T.....	T	AG	A
C. gunnii_AJ309340T.....T.....	AG	FA
C. fraticida_JQ349068T.....T.....A.....
C. cylindrica_GU980041T.....T.....G.....
C. coccidioperitheciata_JN0498T.....T.....C.T.....
C. cardinalis_KF937320T.....T.....T.....
C. brongniartii_AB100036T.....T.....C.T	T
C. bifusispora_AY245627T.....T.....CPT.....
I. japonica_AF200370T.....T.....CPT.....
P. hepiali_HM135170T.....T.....CPT.....
M. anisopliae_AB071711T.....	TA.GCA.....

J

	10	20	30	40
O. sinensis OS R2	CCTAC	TGATCCGAGG	TCAAC	TGGAG
C. militaris_KACC43316G.TC.....AGT.....GC.....
C. pruinosa_KACC43329G.TC.....AA.....T.....GT.....
I. cicadae_KACC43335G.TC.....GA.....GT.....G.....
I. tenuipes_KACC43336G.TC.....GA.....GT.....G.....
B. bassiana_KACC43338T.....G.TC.....AA.....T.....
O. acicularis_JN049820TCTTGA.....G.....C.....
O. agriotidis_AB968403TCT.TGA.....CT.....G.....C.....
O. appendiculata_JN943325TCTG.....TC.....TG.....
O. arborescens_AB968398TCC.....GA.....GAGA.....T.....
O. australis_KF937349T.....	T.AC	TATAAT
O. clavata_JN943327G.....	GGT.CTG.....
O. coccidiicola_AB968404CTTGA.....A.G.C.....
O. coenomyia_AB968396G.....	C.....	TCCAC	CG.G.....
O. communis_GU723768T.....	CTGAA	AGT	GC
C. dipterigena_AY245629C.TA.....	AAAAT	GT
C. entomorrhiza_AJ786560G.C.TAGA.....GTG.....GC.....
O. ferruginosa_AB968405TCTA.....G.....
O. gracilis_HM142942G.GG.CA.....CT.CAC.....G.....
O. heteropoda_FJ765027TCTA.....AAAGT.....GC.....
O. iranagensis_AY646400A.....	TA	ACTAGAAA
O. lloydii_KP200892G.....	G.....	GC.TCA	FGC.C.C.C
O. longissimi_AB968406CAC.....	AA	CG.C.C.C
O. macroacicularis_KR005829T.CC.....	GA	GAG	TC
O. myrmecophila_MH028143T.....	AC	TA	GT
O. nigrella_JN049853	Noninformation			
O. nutans_AB176463	TAT	T.T	TGT	TA.AA
O. rhizoidea_GU723769TC.TG.....TT.C.....
C. robertsii_AJ309335TCCC.....	GA	GAG	T
O. rubiginosiperitheciata_JN94C.TTG.....GA.....
O. sobolifera_AB968409CAC.....	AAA	FG.C.G
O. sphecocephala_JN943350TA.....	AT	GAA
O. stylophora_JN049828C.TG.....A.G.....
C. tricentri_AJ309364TA.AG.....	T	TTA	G
O. unilateralis_GU723756C.CT.....	P.A.C	C.C.C
O. xuefengensis_KY429942T.CC.....	GA	GAG	T.T
C. tuberculata_GQ250005G.G.TC.....	TA	GT	G.T
C. spgazzinii_HQ248195G.TCT.....	GA	T
C. scarabaeicola_AY245639T.....	G.TC	AA	T
C. roseostromata_AY245637G.TC.....	AA	AGT	GT
C. pseudomilitaris_JN943305G.TC.....	AA	AGT	GT
C. pleuricapitata_JN943304T.....	CGAA	TT	C
C. ninchukispora_AY245642G.TC.....	AA	T	GT
C. memorabilis_JN116706T.....	CA.A	A	AGT
C. hawkesii_AJ309341TT.....	TCTAGAA	AGAA	A.T
C. gunnii_AJ309340T.....	TCTAGAA	AGAA	A.T
C. fraticida_JQ349068T.....	TCTT	GATAGT	GC
C. cylindrica_GU980041TCTAC.....	GA	TT.A	CGTT
C. coccidioperitheciata_JN0498T.....	G.TCG	AA	GT
C. cardinalis_KF937320G.TC.....	AA	GT	GT
C. brongniartii_AB100036T.....	G.TC	AA	T
C. bifusispora_AY245627G.TC.....	GA	GT	G
I. japonica_AF200370G.TC.....	GA	GT	G
P. hepiali_HM135170G.TC.....	GA	GT	G
M. anisopliae_AB071711TATA.....	AA	AGT	G

Figure S4. Confirmation of primer specificity by the alignment of specie-specific SCAR primer regions used for both conventional and real-time PCR assay. **(A)** Primer CM F3, **(B)** Primer CM R3, **(C)** Primer CP F1, **(D)** Primer CP R1, **(E)** Primer IC F3, **(F)** Primer IC R3, **(G)** Primer IT F4, **(H)** Primer IT R3, **(I)** Primer OS F1, and **(J)** Primer OS R2. The boxes indicate primer sequences. Dots (.) and dashes (-) indicate identical nucleotides and gaps, respectively, when compared with corresponding primer region, to maximize sequence alignment presentation.