(Supplemental Information)

Molecular phylogenetic analysis of new *Entoloma rhodopolium*-related species in Japan and its identification method using PCR-RFLP.

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Table 1S Mushroom samples collected in this study.

sample name		location	year	morphologically	Accession #	
					ITS	RPB2
KUB 1	frozen	Yamagata	2010	*Entoloma rhodopolium*	LC088033	LC148032
KUB 2	frozen	Yamagata	2010	*Entoloma rhodopolium*	LC088034	LC148033
KUB 3	freeze-dried	Yamagata	2010	*Entoloma rhodopolium*	LC088035	LC148034
KUB 4	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	—	—
KUB 5	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	LC088036	LC148035
KUB 6	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	LC088037	LC148036
KUB 7	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	LC088038	LC148037
KUB 8	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	—	—
KUB 9	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	LC088039	LC148038
KUB 10	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	LC088040	—
KUB 11	freeze-dried	Shimane	2008	*Entoloma rhodopolium*	—	—
KUB 101	frozen	Niigata	2011	*Entoloma rhodopolium*	LC088041	LC148039
KUB 102	frozen	Niigata	2011	*Entoloma rhodopolium*	LC088042	LC148040
KUB 103	frozen	Hokkaido	2011	*Entoloma rhodopolium*	—	—
KUB 104	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088043	LC148041
KUB 105	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088044	—
KUB 106	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088045	LC148042
KUB 107	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088046	—
KUB 108	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088047	—
KUB 109	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088048	—
KUB 110	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088049	—
KUB 111	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088050	—
KUB 112	frozen	Tokyo	2011	*Entoloma rhodopolium*	—	—
KUB 113	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088051	—
KUB 114	frozen	Tokyo	2011	*Entoloma rhodopolium*	LC088052	—
KUB 121	dried, specimen	Tottori	2007	*Entoloma rhodopolium*	—	—
KUB 122	dried, specimen	Tottori	2007	*Entoloma rhodopolium*	—	—
KUB 123	dried, specimen	Tottori	2007	*Entoloma rhodopolium*	LC088053	—
KUB 124	dried, specimen	Tottori	2007	*Entoloma rhodopolium*	LC088054	—
KUB 125	dried, specimen	Okayama	2008	*Entoloma rhodopolium*	—	—
KUB 126	dried, specimen	Tottori	2008	*Entoloma rhodopolium*	LC088055	—
KUB 127	dried, specimen	Tottori	2008	*Entoloma rhodopolium*	LC088056	—
KUB 128	dried, specimen	Tottori	2008	*Entoloma rhodopolium*	LC088057	—
KUB 129	dried, specimen	Tottori	2008	*Entoloma rhodopolium*	—	—
KUB 130	dried, specimen	Tottori	2010	*Entoloma rhodopolium*	LC088058	LC148043
KUB 131	dried, specimen	Tottori	2010	*Entoloma rhodopolium*	—	—
KUB 132	dried, specimen	Tottori	2008	*Entoloma rhodopolium*	LC088059	LC148044
KUB 133	dried, specimen	Tottori	2008	*Entoloma sarcopum*	LC088060	—
KUB 134	dried, specimen	Tottori	2010	*Entoloma sarcopum*	LC088061	—
KUB 135	dried, specimen	Tottori	2009	*Entoloma sarcopum*	—	—
KUB 136	dried, specimen	Tottori	2008	*Entoloma sarcopum*	LC088062	—
KUB 201	frozen	Yamagata	2012	*Entoloma sarcopum*	LC088063	—
KUB 202	frozen	Yamagata	2012	*Entoloma sarcopum*	LC088064	LC148045
KUB 203	frozen	Fukushima	2012	*Entoloma sarcopum*	LC088065	LC148046
KUB 204	frozen	Tochigi	2010	*Entoloma sarcopum*	LC088066	LC148047
KUB 205	frozen	Tochigi	2010	*Entoloma sarcopum*	LC088067	LC148048
KUB 206	frozen	Yamagata	2012	*Entoloma sinuatum?*	LC088068	—
KUB 207	frozen	Hokaido	2012	*Entoloma sinuatum?*	LC088069	—

(-), sequences were not obtained

Table 2S Datasets used for molecular phylogenic study.

Data name	**Accession #**	
	ITS	RPB2
Entoloma bisporigerum KK76-14	LN850532	—
Entoloma bisporigerum KK1604-12	LN850533	LN850681
Entoloma bisporigerum KK106-02	LN850534	LN850682
Entoloma bisporigerum KK934-03	LN850535	—
Entoloma bisporigerum transvenosum var	LN850538	LN850683
Entoloma borgenii KK135-10	LN850522	—
Entoloma borgenii KK304-06	LN850520	—
Entoloma borgenii KK681-03	LN850521	—
Entoloma borgenii KK307-05	LN850519	LN850680
Entoloma borgenii KK458-07	LN850523	—
Entoloma boreale holoKK106-09	LN850624	LN850697
Entoloma boreale KK257-08	LN850622	—
Entoloma boreale KK158-11	LN850623	—
Entoloma eminens holoKK417-12	LN850584	—
Entoloma eminens KK40-14	LN850583	—
Entoloma griseopruinatum KK295-12	LN850590	—
Entoloma griseopruinatum KK1607-12	LN850588	—
Entoloma insitatum KK3-12	LN850542	—
Entoloma insitatum topoM.Enderle6	LN850543	—
Entoloma lupinum holoKK13-14JVauras	LN850570	LN850695
Entoloma lupinum KK126-05	LN850569	—
Entoloma majaloides KK782-12	LN850478	LN850654
Entoloma majaloides KK887-06JVauras	LN850479	LN850655
Entoloma majaloides KK405-09	LN850482	LN850660
Entoloma majaloides KK54-14	LN850488	LN850667
Entoloma majaloides KK124-08JVauras	LN850483	LN850662
Entoloma nidorosum KK268-08	LN850501	LN850672
Entoloma nidorosum KK419-12	LN850503	LN850673
Entoloma nidorosum KK1670-12	LN850504	LN850671
Entoloma nidorosum KK415-12	LN850502	—
Entoloma nidorosum JHP380	LN850509	—
Entoloma nidorosum KK343b-09	LN850505	LN850675
Entoloma nidorosum KK381-08	LN850506	LN850674
Entoloma nidorosum J.Ateva26	LN850507	LN850676
Entoloma rhodopolium KK157-14	LN850496	—
Entoloma rhodopolium KK1664-12	LN850497	LN850705
Entoloma rhodopolium neoKK213-14	LN850494	LN850669
Entoloma rhodopolium K.Alho24	LN850498	—
Entoloma rhodopolium KK177-14	LN850495	LN850670
Entoloma aff. *rhodopolium* KK24-07	LN850499	—
Entoloma aff. *rhodopolium* PH	LN850500	—
Entoloma rhodopolium Er-1	AB301602	—
Entoloma sarcopum EC-3	AB301603	—
Entoloma sericatum KK101-02	LN850446	LN850637
Entoloma sericatum KK691-06	LN850452	LN850635
Entoloma sericatum KK691-06	LN850452	LN850635
Entoloma sinuatum st45	KC710108	—
Entoloma sinuatum st182	KC710116	—
Entoloma sinuatum H6003960	GU373512	—
Entoloma sinuatum TJB5349	DQ486700	—
Entoloma sp MLS007	GQ397994	—
uncultured ectomycorrhizal fungus	AB587746	—
Clitocybe dealbata 11212	JF907804	DQ825407
Lyophyllum leucopaeatum Hae251-97	AF357032	DQ367434

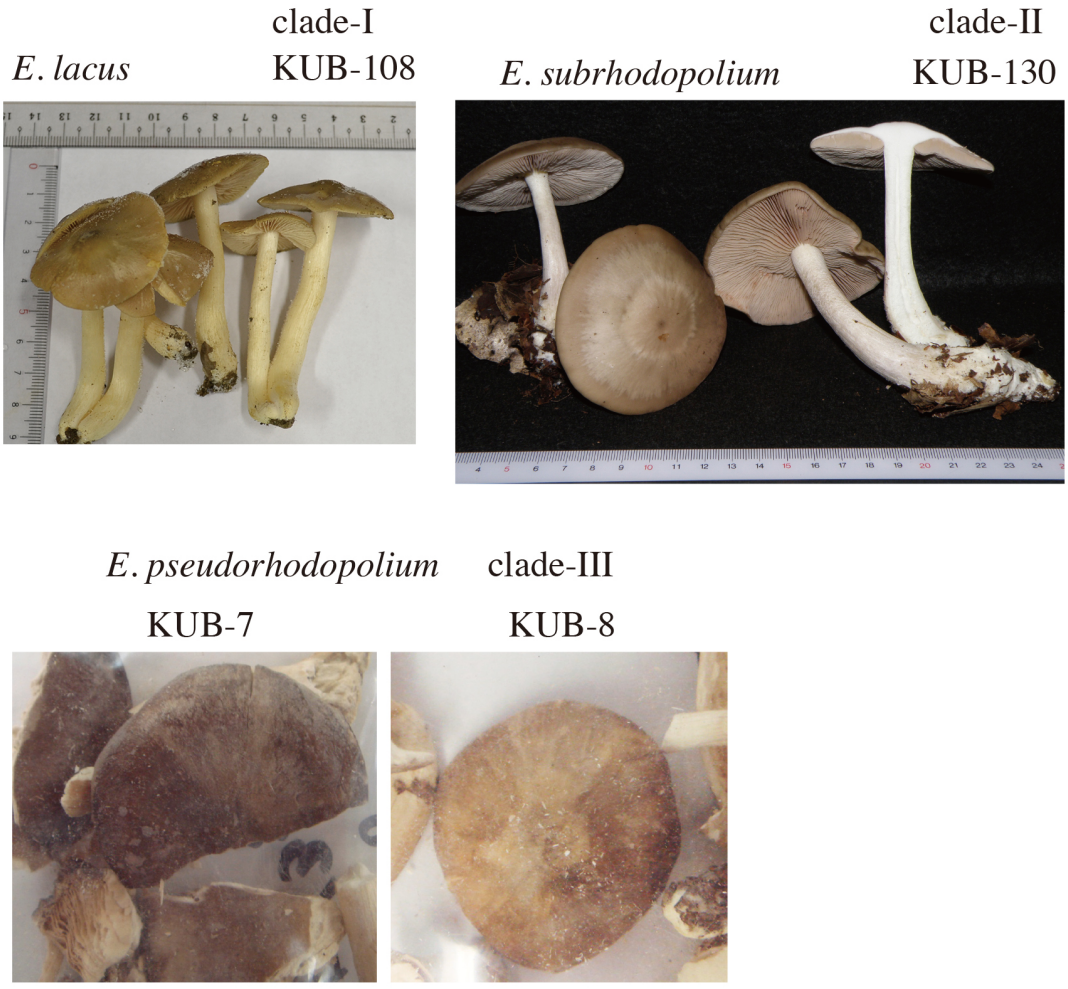


Figure 1S. Photographs of other samples from Japanese Entoloma rhodopolium clades.

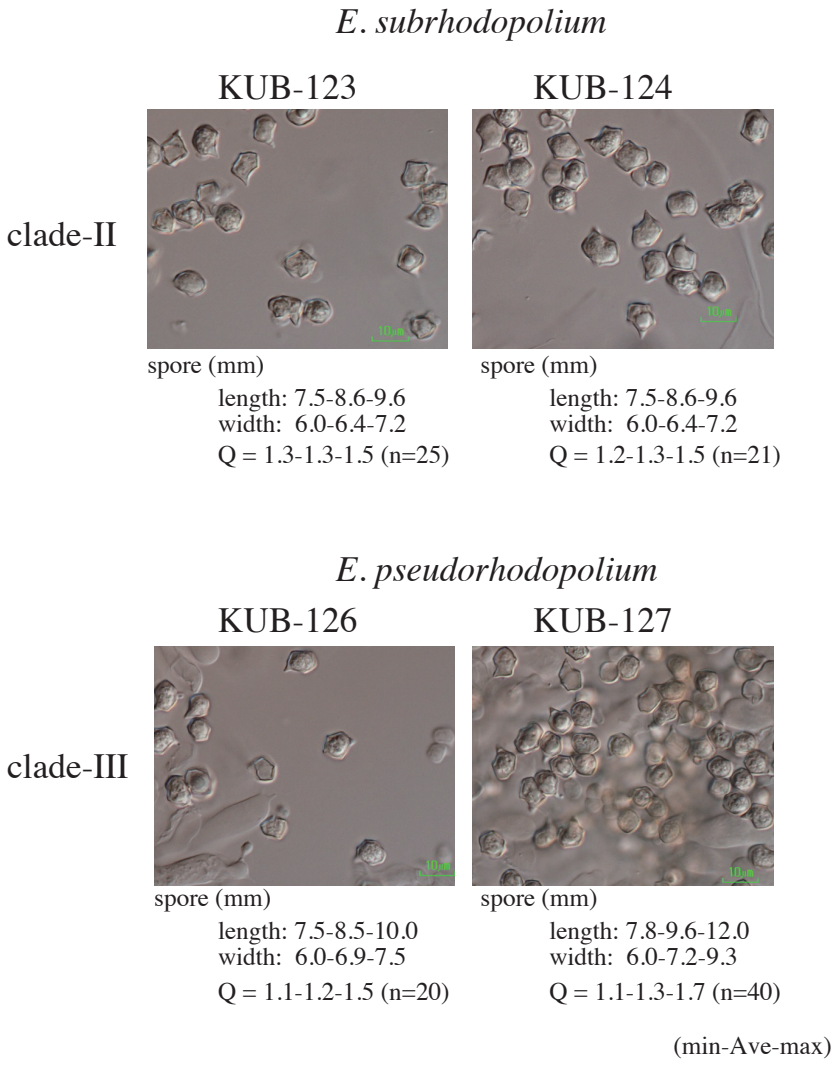


Figure 2S. Photographs of spores from Japanese Entoloma rhodopolium clades-II and III.

p-distance between known species

(a)

		E.sinuatum			*E.subsunuatum*			
		1	2	3	4	5	6	7
1	E_sinuatum_st45							
2	E_sinuatum_st182	0.000						
3	E_sinuatum_H6003960	0.000	0.000					
4	subsinuatum_624	0.037	0.037	0.037				
5	subsinuatum _2269	0.026	0.026	0.026	0.011			
6	subsinuatum_2005100	0.025	0.025	0.025	0.011	0.000		
7	E_subsinuatum_TJB5349	0.029	0.029	0.029	0.014	0.002	0.002	

2.9 ± 0.5 (%)
(sinuatum vs subsinuatum)

		E.nidorosum								*E.rhodopolium*				
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	nidorosum_JAteva26VIII2001													
2	nidorosum_KK343b-09	0.000												
3	nidorosum_KK381-08	0.000	0.000											
4	nidorosum_JHP-380	0.004	0.004	0.004										
5	nidorosum_KK1670-12	0.007	0.007	0.007	0.001									
6	nidorosum_KK415-12	0.005	0.005	0.005	0.000	0.001								
7	nidorosum_KK268-08	0.005	0.005	0.005	0.000	0.001	0.000							
8	nidorosum_KK419-12	0.005	0.005	0.005	0.000	0.001	0.000	0.000						
9	rhodopolium_KK177-14	0.015	0.015	0.015	0.016	0.019	0.017	0.017	0.017					
10	rhodopolium_KAlho24IX1980	0.015	0.015	0.015	0.016	0.019	0.018	0.018	0.018	0.000				
11	rhodopolium_KK157-14	0.015	0.015	0.015	0.016	0.019	0.017	0.017	0.017	0.000	0.000			
12	rhodopolium_KK1664-12	0.015	0.015	0.015	0.016	0.019	0.017	0.017	0.017	0.000	0.000	0.000		
13	rhodopolium_neo-KK213-14	0.015	0.015	0.015	0.016	0.019	0.017	0.017	0.017	0.000	0.000	0.000	0.000	

1.6 ± 0.1 (%)
(nidorosum vs rhodopolium)

Figure 3S. P-distance between the known and new species estimated by MEGA software (*a-d*).
a, p-distance between the known species.

p-distance between *E. lacus* and *E. majaloides*

(b)

		E.lacus										*E.majaloides*				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	KUB106															
2	KUB108	0.000														
3	KUB110	0.000	0.000													
4	KUB113	0.000	0.000	0.000												
5	KUB114	0.000	0.000	0.000	0.000											
6	KUB104	0.001	0.001	0.001	0.001	0.001										
7	KUB107	0.001	0.001	0.001	0.001	0.001	0.000									
8	KUB109	0.001	0.001	0.001	0.001	0.001	0.000	0.000								
9	KUB111	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000							
10	KUB105	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000						
11	majaloides_KK405-09	0.032	0.032	0.032	0.032	0.032	0.033	0.033	0.033	0.033	0.033					
12	majaloides_KK782-12	0.028	0.028	0.028	0.028	0.028	0.030	0.030	0.030	0.030	0.030	0.005				
13	majaloides_KK887-06JVauras	0.030	0.030	0.030	0.030	0.030	0.031	0.031	0.031	0.031	0.031	0.006	0.001			
14	majaloides_KK54-14	0.031	0.031	0.031	0.031	0.031	0.032	0.032	0.032	0.032	0.032	0.015	0.011	0.012		
15	majaloides_KK124-08JVauras	0.030	0.030	0.030	0.030	0.030	0.031	0.031	0.031	0.031	0.031	0.013	0.009	0.010	0.007	

E. lacus

3.1 ± 0.1 (%)

(lacus vs majaloides)

Figure 3S. P-distance between the known and new species estimated by MEGA software (*a-d*).
b, p-distance between *E. lacus* and *E. majaloides*.

p-distance between *E. subrhodopolium, E. sinuatum*, *E. nidorosum*, and *E. rhodopolium*

(c)

E. subrhodopolium

	E.subrhodopolium							
	1	2	3	4	5	6	7	8
KUB3_seq1								
KUB3_seq2	0.001							
KUB123	0.001	0.000						
KUB130	0.001	0.000	0.000					
KUB132	0.004	0.005	0.005	0.005				
KUB1	0.010	0.005	0.005	0.005	0.006			
KUB124	0.010	0.005	0.005	0.005	0.006	0.000		
E_rhodopolium_Er1	0.010	0.005	0.005	0.005	0.006	0.000	0.000	
E_sinuatum_st45	0.097	0.071	0.070	0.070	0.070	0.072	0.073	0.073
E_sinuatum_st182	0.097	0.071	0.070	0.070	0.070	0.072	0.073	0.073
E_sinuatum_H6003960	0.097	0.071	0.070	0.070	0.070	0.072	0.073	0.073
eminens_holoKK417-12	0.078	0.079	0.079	0.079	0.079	0.084	0.084	0.084
eminens_KK40-14	0.076	0.075	0.075	0.075	0.077	0.081	0.081	0.081

7.5 ± 0.9 (%)
(subrhodopolium vs sinuatum)

7.9 ± 0.3 (%)
(subrhodopolium vs eminens)

E. subrhodopolium

	E.nidorosum								*E.rhodopolium*				
	12	13	14	15	16	17	18	19	20	21	22	23	24
KUB3_seq1	0.095	0.093	0.093	0.094	0.098	0.091	0.092	0.092	0.097	0.100	0.097	0.097	0.097
KUB3_seq2	0.092	0.091	0.091	0.090	0.093	0.088	0.088	0.088	0.095	0.097	0.095	0.095	0.095
KUB123	0.092	0.091	0.091	0.090	0.093	0.088	0.088	0.088	0.095	0.097	0.095	0.095	0.095
KUB130	0.092	0.091	0.091	0.090	0.093	0.088	0.088	0.088	0.095	0.097	0.095	0.095	0.095
KUB132	0.099	0.098	0.098	0.099	0.103	0.096	0.096	0.096	0.099	0.101	0.099	0.099	0.099
KUB1	0.095	0.093	0.093	0.093	0.096	0.091	0.091	0.091	0.096	0.098	0.096	0.096	0.096
KUB124	0.095	0.093	0.093	0.093	0.096	0.091	0.091	0.091	0.096	0.098	0.096	0.096	0.096
E_rhodopolium_Er1	0.095	0.093	0.093	0.093	0.096	0.091	0.091	0.091	0.096	0.098	0.096	0.096	0.096
E_sinuatum_st45	0.077	0.075	0.075	0.075	0.070	0.073	0.073	0.073	0.079	0.080	0.079	0.079	0.079
E_sinuatum_st182	0.077	0.075	0.075	0.075	0.070	0.073	0.073	0.073	0.079	0.080	0.079	0.079	0.079
E_sinuatum_H6003960	0.077	0.075	0.075	0.075	0.070	0.073	0.073	0.073	0.079	0.080	0.079	0.079	0.079

9.3 ± 0.3 (%)
(subrhodopolium vs nidorosum)

9.6 ± 0.2 (%)
(subrhodopolium vs nidorosum)

Figure 3S. P-distance between the known and new species estimated by MEGA software (*a-d*).
c, p-distance between *E. subrhodopolium*, *E. sinuatum*, *E. nidorosum*, and *E. rhodopolium*.

p-distance between *E. pseudorhodopolium, E. lupinum*, and *E. rhodopolium*

(d)

		E.pseudorhodopolium													*E.lupium*		*E.rhodopolium*				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E. pseudorhodopolium	KUB9																0.123	0.121	0.123	0.123	0.123
	KUB10	0.006															0.123	0.124	0.123	0.123	0.123
	KUB7	0.006	0.004														0.119	0.121	0.119	0.119	0.119
	KUB5	0.006	0.004	0.002													0.121	0.123	0.121	0.121	0.121
	KUB6	0.005	0.002	0.001	0.001												0.119	0.121	0.119	0.119	0.119
	KUB101_seq1	0.005	0.002	0.001	0.001	0.000											0.119	0.121	0.119	0.119	0.119
	KUB101_seq2	0.005	0.002	0.001	0.001	0.000	0.000										0.119	0.121	0.119	0.119	0.119
	KUB102	0.005	0.002	0.001	0.001	0.000	0.000	0.000									0.119	0.121	0.119	0.119	0.119
	KUB126	0.005	0.002	0.001	0.001	0.000	0.000	0.000	0.000								0.119	0.121	0.119	0.119	0.119
	KUB127_seq2	0.005	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000							0.119	0.121	0.119	0.119	0.119
	KUB128	0.005	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000						0.119	0.121	0.119	0.119	0.119
	ectomycorrhizal_P09083	0.005	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000					0.119	0.121	0.119	0.119	0.119
	KUB127_seq1	0.007	0.005	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002				0.121	0.123	0.121	0.121	0.121
	lupinum_holoKK13-14JVauras	0.052	0.051	0.047	0.050	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049			0.101	0.103	0.101	0.101	0.101
	lupinum_126-05	0.053	0.053	0.048	0.050	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.001		0.101	0.103	0.101	0.101	0.101

4.9 ± 0.1 (%)
(pseudorhodopolium vs lupinum)

10.1 ± 0.1 (%)
(pseudorhodopolium vs rhodopolium)

Figure 3S. P-distance between the known and new species estimated by MEGA software (*a-d*).
d, p-distance between *E. pseudorhodopolium*, *E. lupinum*, and *E. rhodopolium*.

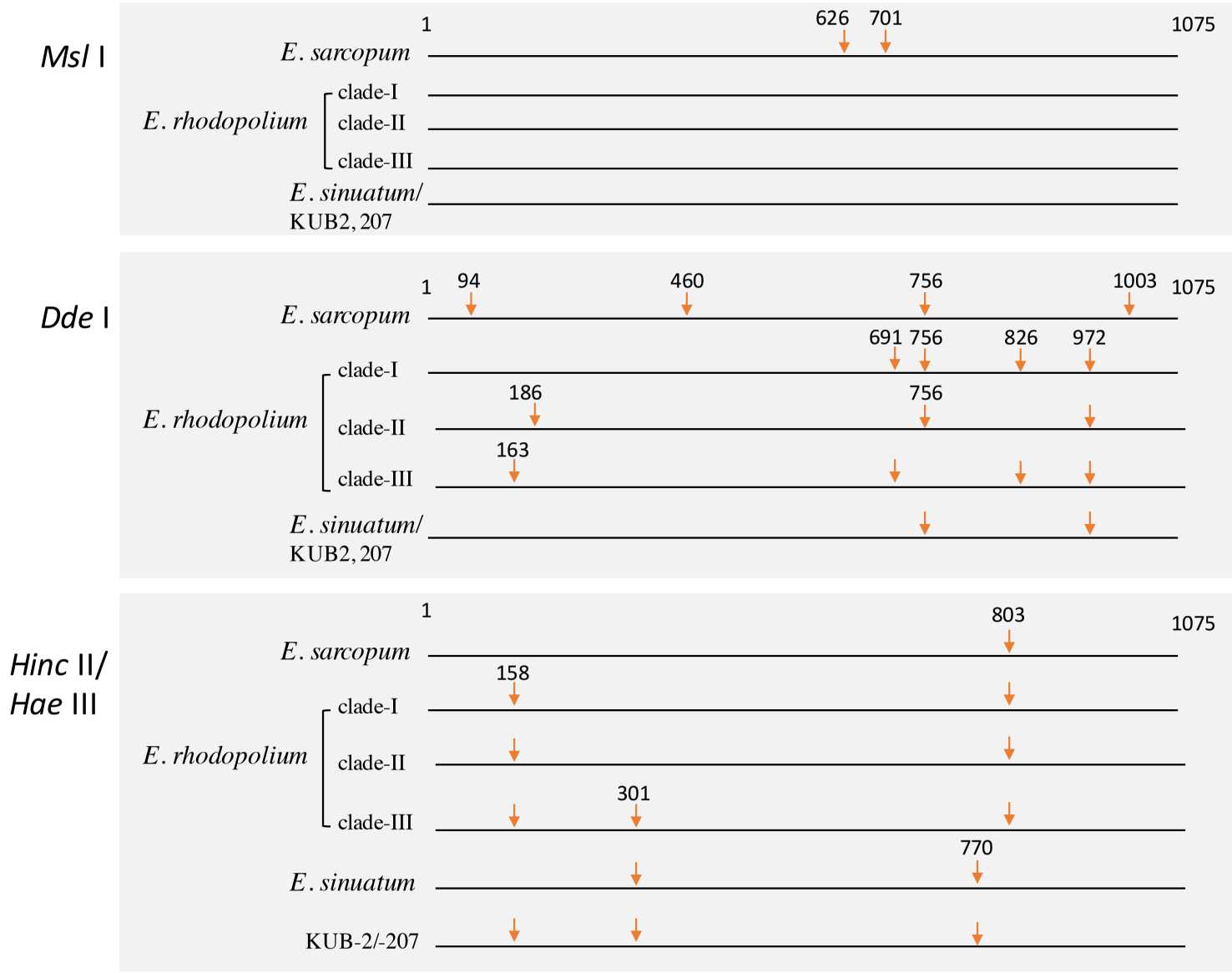


Figure 4S Restriction endonucleases were used for discrimination between edible and poisonous mushrooms and for species classification. MslI digestion revealed the presence of edible E. sarcopum. Other Entoloma species were not digested. DdeI and a combination of HincII/HaeIII double digestion provide different band pattern between three clades of Japanese E. rhodopolium.

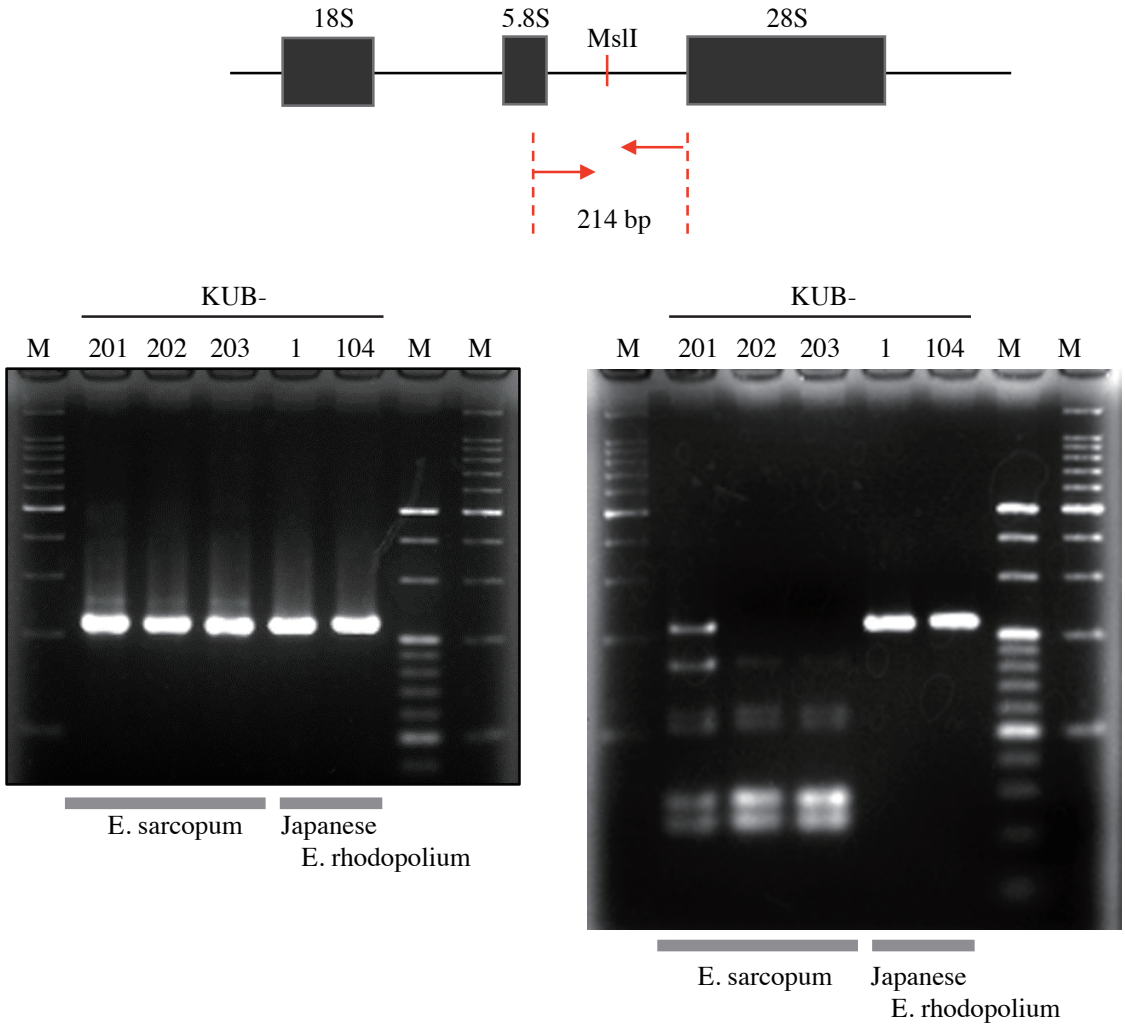
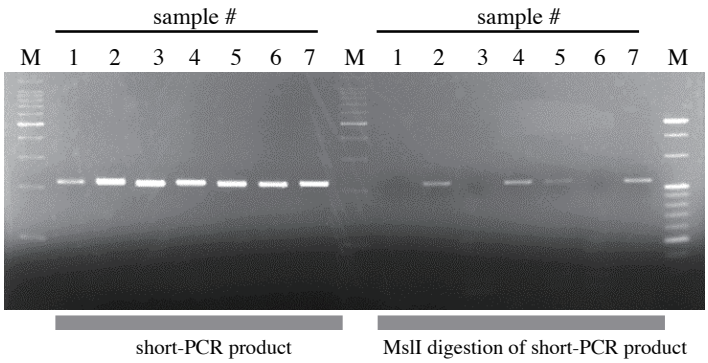


Figure 5S. Restriction endonucleases were used for discrimination between edible and poisonous mushrooms and for species classification. MslI digestion revealed the presence of edible E. sarcopum. Other *Entoloma* species were not digested. DdeI and a combination of HincII/HaeIII double digestion provide different band pattern between three clades of Japanese *E. rhodopolium*.



quasi-mixed mushroom

sample	edible	*E. subrhodopolium*	*E. sarcopum*
1	400	–	–
2	–	200	–
3	–	–	200
4	200	200	–
5	200	100	100
6	200	–	200
7	380	20	–

Figure 6S. Detection of poisonous Japanese *E. rhodopolium* clades in quasi-mixed mushroom samples. A, content of each sample. B, band patterns on 2% agarose gel of samples 1 to 7. PCR products (214 bp) in samples 2, 4, 5, and 7 were left intact, whereas samples 1, 3, and 5 were completely digested. Experimental conditions were the same as that in Figure.6.
Samples 2, 4, 5, and 7 contain poisonous *E. subrhodopolium*.