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FULL PAPER

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Relationships among three Japanese *Laetiporus* taxa based on phylogenetic analysis and incompatibility tests

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Abstract Relationships among three Japanese *Laetiporus* taxa ("*L. sulphureus* var. *sulphureus*" auct. jap., *L. sulphureus* var. *miniatus*, and *L. versisporus*) were assessed with phylogenetic analysis and incompatibility tests. Gene phylogenies inferred from the internal transcribed spacer region of nuclear ribosomal DNA, elongation factor 1α, and β-tubulin gene regions suggested that Japanese *Laetiporus* was divided into four groups: the yellow pore form of *L. sulphureus* var. *miniatus*, the white pore form of *L. sulphureus* var. *miniatus*, and two "*L. sulphureus* var. *sulphureus*"/*L. versisporus* groups. A morphologically distinct species, *Laetiporus versisporus*, sharing a clade with "*L. sulphureus* var. *sulphureus*" auct. jap., was proved to be an anamorphic form of "*L. sulphureus* var. *sulphureus*" auct. jap. The "*sulphureus/versisporus*" isolates showed two divergent sequence types in each region. Some isolates had intraindividual polymorphism assigned to both sequence types. This finding suggests that speciation via hybridization is ongoing in the "*sulphureus/versisporus*" group. Single spore isolates from the "*sulphureus/versisporus*" group, white pore group, and yellow pore group were incompatible with each other. Our results provided strong support for the new recognition of three *Laetiporus* taxa in Japan.

Key words Anamorph · β-tubulin · EF1α · Hybridization · Internal transcribed spacer · Intraindividual polymorphism

Introduction

Laetiporus spp. occur worldwide from boreal to tropical zones and cause red-brown cubical heart-rot in the wood of many deciduous and coniferous trees. *Laetiporus* spp. also include edible mushrooms with a long history of consumption, especially in North America (Gilbertson and Ryvarden 1986) and Japan (Imazeki et al. 1988). This genus is easily distinguished by the bright orange or yellow color of its basidiocarps. However, much variation in the color of the basidiocarps and poor microscopic characteristics make it difficult to define the intrageneric taxa.

Incompatibility tests and restriction fragment length polymorphisms in the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA have shown that *Laetiporus sulphureus* (Bull.: Fr.) Murrill sensu lato in North America is a complex of six taxa (Banik and Burdsall 1999, 2000; Banik et al. 1998). The differences in morphological and ecological characteristics have supported for their delimitation. The taxa were described as *L. cincinnatus* (Morgan) Burds., Banik & Volk, *L. conifericola* Burds. & Banik, *L. gilbertsonii* Burds., *L. gilbertsonii* Burds. var. *pallidus* Burds., and *L. huroniensis* Burds. and Banik, in addition to *L. sulphureus* (Burdsall and Banik 2001). In Europe, phylogenetic analysis based on the ITS region indicates that *L. sulphureus* may be separated into two taxa depending on the host type (Rogers et al. 1999).

In Japan, two species and one variety of *Laetiporus* have been reported based on their morphology and color (Imazeki and Hongo 1989). The color of "*Laetiporus sulphureus* var. *sulphureus*" auct. jap. is similar to that of the European *L. sulphureus*, which has a yellow pore surface and a yellow pileus surface. However, it is distinguished from the European form by its nonimbricated pilei and occurrence that is restricted to warm temperate and subtropical zones.

Laetiporus sulphureus var. *miniatus* (Jungh.) Imazeki has a wide distribution from cool temperate to boreal areas of Japan and a wide host preference, from hardwoods to conifers. It has an orange pileus surface, and the color of its pore surface can vary from white to lemon yellow. In addition, basidiocarps with an orange pileus surface and a yellow pore surface have been reported in Kagoshima Prefecture in warm temperate areas on hardwoods (Imazeki and Hongo 1989). The affiliation of this type remains unknown.

Laetiporus versisporus (Lloyd) Imazeki has completely distinct morphological characteristics. Matured fruit bodies

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are semiglobose and consist of abundant chlamydospores in the context without producing a hymenophore. Fruit bodies are at first lemon yellow then turn white to brown. This species occurs mostly in the southern part of Japan. Sometimes it produces deficient tubes and basidiospores, which seems to make it an intermediate form between "*L. sulphureus*" and *L. versisporus*.

The objective of this study was to define the intrageneric taxa of Japanese *Laetiporus* based on DNA analysis and incompatibility tests using isolates collected throughout Japan. Three regions (ITS region of the nuclear ribosomal DNA, the partial elongation factor 1α and β-tubulin gene regions) were analyzed for phylogenetic relationships among Japanese taxa.

Materials and methods

Fungal materials and DNA manipulations

More than 150 specimens were collected throughout Japan, mostly from 2000 to 2006. The morphological characteristics and color of basidiocarps, hosts, and origins were recorded. Single spore isolates were obtained using a medium extracted from oak wood or larch wood (500 ml wood extract, 500 ml water, 20 g agar) using the modified methods described by Banik and Burdsall (1999). The wood extract was prepared by autoclaving approximately 50 g sawdust (*Quercus serrata* or *Larix kaempferi*) in 1 l water for 30 min. Some specimens produced chlamydospores inside and pore-like pits on the lower side of the basidiocarps (the intermediate form). From basidia produced on the pore-like pits, some single spore isolates were obtained from an intermediate form. A total of 68 isolates were selected and used in this study (Table 1). All specimens examined were deposited at the mycological herbarium of Forestry and Forest Product Research Institute (TFM), and Japanese isolates were deposited at the culture bank of Forestry and Forest Product Research Institute.

Mycelia for DNA extraction were grown on a liquid MYG medium [2% (w/v) malt extract, 0.2% (w/v) yeast extract, 2% (w/v) glucose] at 25°C in the dark and harvested 10 days after inoculation. DNA was extracted using a DNeasy extraction kit (Qiagen, Valencia, CA, USA). The ITS region of nrDNA and the fragments of β-tubulin and EF1α genes were used as molecular markers. The oligonucleotide primers used this study were ITS4 and ITS5 for ITS (White et al. 1990), EF1-526F and EF1-1567R for EF1α (http://ocid.nacse.org/research/deephyphae/EF1primer.pdf), and B-TUB-1F and B-TUB-1R for β-tubulin (Oda et al. 2004).

Each polymerase chain reaction (PCR) contained approximately 10 ng template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 (0.1) μM of each primer, 2.5 mM (2 mM) of each dNTP, and Takara taq (0.5 U) (Takara, Tokyo, Japan) in a total volume of 20 μl. PCR amplification was performed using an Applied Biosystems Perkin-Elmer DNA thermal cycler (9800) or a Bio-Rad iCycler, under the following conditions: 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at 52°–56°C, and 30 s at 72°C, with a final extension of 7 min at 72°C. PCR products were purified with MicroSpin Columns and Sephacryl S-300 (GE Healthcare, Piscataway, NJ, USA).

Direct sequencing of PCR products was conducted for both strands using a BigDye Terminator cycle sequencing Ready Reaction Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) with an Applied Biosystems 3100 sequencer. Some of the PCR products that showed evidence of heterozygosity in the sequencing chromatograms (i.e., base calls were ambiguous) were cloned into a pGEM-easy vector (Promega, Madison, WI, USA), and at least eight clones from each isolate were sequenced. All sequences were determined in both directions.

Data analyses

Representative DNA sequences of amplified fragments were deposited in GenBank (AB308135-308267). Sequences were aligned using Clustal X (Jeanmougin et al. 1998) and deposited in TreeBase as submission no. S2052. The sequences that included only a few transversions were used directly for the phylogenetic analyses: T/C indicated as Y, A/G indicated as R. Multiple sequences derived from one isolate were included in the analyses and are shown as -a, -b, and -c in the trees. Only unambiguous alignments were used in the phylogenetic analysis.

The phylogenetic analysis of the aligned sequences was performed using distance and parsimony methods in PAUP ver.4.0b (Swofford 2001). For the distance analysis, the neighbor-joining method generated from HKY 85 distances was used. The maximum-parsimony trees were generated by heuristic searches with TBR (tree bisection reconnection) branch swapping and MulTrees in effect. Starting trees were obtained via stepwise addition with 100 taxa addition sequences. MaxTrees was initially set to 2000, and zero-length branches were collapsed. All characters were of equal weight and unordered. The gaps were treated as fifth characters. The strength of the internal branches of the resulting trees was statistically tested by bootstrap analysis (Felsenstein 1985) from 1000 bootstrap replications.

Incompatibility tests

Pairings were conducted by the method described by Banik and Burdsall (1999). Two 5-mm-diameter mycelium plugs, one from each of the two isolates, were placed in contact with each other in the center of a plate containing PDA medium (Nissui, Tokyo, Japan), and a second inoculum of each isolate was placed 2 cm away from the first inoculum and incubated for 7 to 10 days at 25°C.

The mating types of single spore isolates from each basidiocarp were determined by paring 12 single spore isolates in all combinations. One to three tester strains from each basidiocarp were paired with those of the other basidiocarps in all combinations to determine the incompatibility

Table 1. The Japanese *Laetiporus* isolates used in this study

Current species name	Isolate no.	Color		Accession no.			Origin	Host	Collection date	NIAS MAFF no.	TFM Specimen no.
		Upper surface	Pore surface	ITS	β-tubulin	ef1α					
"*L. sulphureus* var. *sulphureus*"	WD820	Yellow	Yellow	AB308135	AB308197	AB308219	Ogasawara Is. Tokyo	Hardwood	25-Nov-90		F-15910
	WD1991	Unknown	Unknown	AB308136		AB308220	Kouchi	Unknown	17-Oct-96	420687	
	WD2099	Unknown	Unknown	AB308137		AB308221	Yamaguchi	Unknown	31-Oct-99		F-19337
	WD2024	Unknown	Unknown	AB308138		AB308222	Ehime	Unknown	7-Nov-96		
	L32	Yellow	Yellow	AB308139		AB308223	Shiga	Unknown	8-Oct-00		F-19717
	L35	Orange	Yellow	AB308140		AB308224, AB308225	Kumamoto	*Quercus* sp.	16-Oct-00		F-19720
	L47 (WD2437)	White to pale yellow	Yellow	AB308141		AB308226	Miyazaki	Hardwood	23-Oct-00		F-19732
	L48 (WD2320)	Pale orange to yellow	Yellow	AB308142		AB308227	Miyazaki	Unknown	26-Oct-00	420795	F-19733
	L51	Orange	White to pale cream	AB308143, AB308144, AB308145, AB308146, AB308147		AB308228	Miyazaki	Hardwood	27-Oct-00		F-19736
	L54-7 (WD2311)* L54-10 (WD2312)*	Yellow	Yellow	AB308148	AB308198	AB308229	Kagoshima	Unknown	27-Oct-00	420786	F-19739
	L68-6 (WD2442)* L68-7* L68-9*	Yellow to orange	Yellow	AB308149	AB308199	AB308230	Kyoto	Unknown	12-Oct-01		F-21721
	L50-5 (WD2443)* L50-6* L50-1*	Orange	Yellow	AB308150	AB308200	AB308231	Miyazaki	Hardwood	28-Oct-00		F-19735
	L55-6 (WD2444)* L55-2* L55-7*	Pale orange	Pale cream	AB308151		AB308232	Kagoshima	Unknown	27-Oct-00		F-19740
	L90-3 (WD2314)*	Orange	Yellow	AB308152	AB308201	AB308233	Ibaraki	*Castanopsis cuspidata* var. *sieboldii*	23-Oct-02	420789	F-21743
Intermediate type**	L46-20*	Pale orange	Yellow	AB308153		AB308234	Kumamoto	Unknown	22-Oct-02		F-19731
	L85-1 (WD1213)* L85-6*	Orange	Yellow	AB308154	AB308202	AB308235	Tokyo	*Castanea crenata*	16-Oct-02	420788	F-21738
	L97 (WD2315)	Orange	Pale orange	AB308155, AB308156	AB308203, AB308204	AB308236, AB308237	Mie	Unknown	13-Nov-02	420790	F-21750
L. versisporus	WD153	Unknown	Unknown	AB308157, AB308158	AB308205	AB308238	Chiba	*Castanopsis cuspidata* var. *sieboldii*	4-Aug-87	420098	F-14748
	L5	Cream	Yellow	AB308159, AB308160	AB308206	AB308239	Nara	Unknown	2-Jul-02		F-19592
	WD2073	Unknown	Unknown	AB308161		AB308240	Kagoshima	Unknown	28-Oct-98		F-19234
	L49	Pale brown	Pale brown	AB308162		AB308241	Miyazaki	Hardwood	27-Oct-02		F-19734
	PRC17	Unknown	Unknown	AB308163			Mt. Baishanzu, China	Unknown	22-Jul-94		

"*L. sulphureus* var. *miniatus*"	WD3	Unknown	Unknown	AB308164, AB308165		AB308242	Hokkaido	Unknown	24-Sep-84	420096	F-11746
	WD723	Unknown	Unknown	AB308166, AB308167, AB308168		AB308243	Hokkaido	Unknown	15-Sep-90		F-14769
	WD728	Unknown	Unknown	AB308169		AB308244	Hokkaido	Unknown	16-Nov-90		F-15776
	WD1061	Orange	Salmon	AB308170		AB308245	Yamanashi	*Quercus mongolica* var. *crispula*	10-Jun-50		
	FH1122	Unknown	Unknown	AB308171	AB308207	AB308246	Hokkaido	*Prunus mume*	28-Sep-89		
	FH1259	Unknown	Unknown	AB308172		AB308247	Hokkaido	*Prunus sargentii*	3-Oct-94		F-24240
	FH1292	Unknown	Unknown	AB308173		AB308248	Hokkaido	*Quercus mongolica*	6-Jun-96		F-24241
	FH1343	Unknown	Unknown	AB308174		AB308249	Hokkaido	*Prunus salicina*	1-Jun-97		F-24242
	FH1387	Unknown	Unknown	AB308175		AB308250	Hokkaido	*Taxus cuspidate*	1-Sep-96		
	L24 (WD2317)	Orange	Cream	AB308176	AB308208	AB308251	Shizuoka	*Quercus mongolica*	24-Sep-00	420792	F-19710
	L34-2 (WD2306)*	Orange	Pale orange to cream	AB308177	AB308209	AB308252	Miyagi	*Quercus* sp.	8-Oct-00	420781	F-19719
	L34-4*										
	L42-9 (WD2307)*	Orange	White	AB308178		AB308253	Kumamoto	Hardwood	21-Oct-00	420782	F-19727
	L42-15*										
	L43-4 (WD2308)*	Orange	White	AB308179	AB308210	AB308254	Kumamoto	Hardwood	22-Oct-00	420783	F-19723
	L43-6*	Orange	White								
	L39 (WD2319)	Orange	White	AB308180		AB308255	Ehime	Unknown	18-Oct-00	420794	F-19724
	L44	Orange	White	AB308181			Kumamoto	Unknown	22-Oct-00		
	L67-5 (WD2309)*	Orange	Pale orange to cream	AB308182	AB308211	AB308256	Ibaraki	*Quercus mongolica*	28-Sep-01	420784	F-21720
	L67-9*										
	L72-1 (WD2310)*	Orange	Pale cream	AB308183	AB308212	AB308257	Aomori	Unknown	23-Jun-02	420785	F-21725
	L72-5*										
	WD1058	Unknown	Unknown	AB308184	AB308213	AB308258	Chiba	*Abies firma*	1946	420012	
	WD2090	Orange	Yellow	AB308185		AB308259	Yamanashi	Unknown	29-Sep-99		F-19322
	WD1062	Orange	Yellow	AB308186		AB308260	Yamanashi	Unknown	10-Jun-50		
	WD1063	Unknown	Unknown	AB308187		AB308261	Aomori	Unknown	22-Sep-52		
	FH1019	Unknown	Unknown	AB308188	AB308214		Hokkaido	*Picea glehnii*	1-Oct-87		
	FH1108	Unknown	Unknown	AB308189		AB308262	Hokkaido	*Picea glehnii*	27-Jul-89		
	FH1206	Unknown	Unknown	AB308190	AB308215	AB308263	Hokkaido	*Picea glehnii*	15-Jun-92		
	FH1379	Unknown	Unknown	AB308191		AB308264	Hokkaido	*Abies sachalinensis*	19-Jul-98		
	L66	Orange	Pale orange to yellow	AB308192			Gumma	Conifer	6-Sep-01		F-21719
	L13	Orange	Yellow	AB308193			Yamanashi	*Abies* sp.	27-Aug-00		F-19600
	L23-4 (WD2303)*	Orange	Yellow	AB308194	AB308216	AB308265	Yamanashi	Conifer	23-Sep-00	420776	F-19709
	L23-6*										
	L27-4 (WD2304)*	Orange	Yellow	AB308195	AB308217	AB308266	Yamanashi	Unknown	4-Oct-00	420779	F-19713
	L27-3*										
	L114-1 (WD2305)*	Orange	Yellow	AB308196	AB308218	AB308267	Yamanashi	Unknown	29-Sep-03	420780	F-21767
	L114-5*										

The isolates beginning with "FH" were provided by Dr. Yamaguchi in FFPRI Hokkaido branch; and those of "L" were newly collected for this study

All specimens and isolates listed here are deposited in FFPRI

*, Single spore isolate. Lxx-1,-2,-3 show single spore isolates derived from a basidiocarp numbered "Lxx"; **, intermediate form with conidiophores in the context and pore layer underside of the pileus

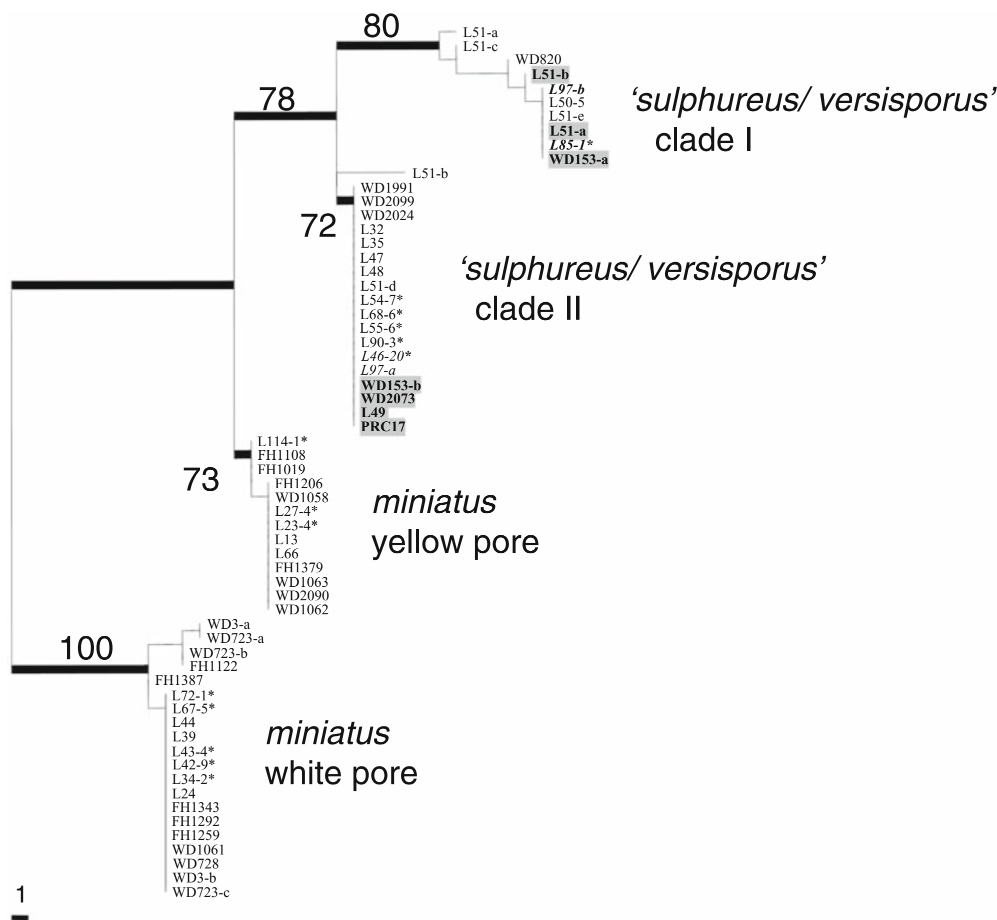

Fig. 1. Neighbor-joining (NJ) tree of Japanese *Laetiporus* spp. based on the sequences of internal transcribed spacer (ITS) region of nrDNA. Bootstrap values are shown *above branches*. *, Single spore isolate. *Light gray shaded*, *L. versisporus*; *italic*, intermediate form; -a, -b, -c, -d, and -e, clone sequence

groups. Individual pairings were performed at least two times.

After incubation for 7 to 10 days at 25°C, compatibility was determined on the basis of the macroscopic mycelial appearance: In pairings between single spore isolates of the same species, increased density and pigmentation in the center area were observed (compatible reaction), as were fusions and separations without a line. In pairings between different species, the formation of a dense line separating the two single spore cultures was observed consistently (incompatible reaction). The incompatibility was evaluated by allozyme analyses following the method of Banik and Burdsall (1999, 2000).

Results

Phylogenies based on ITS, EF1α, and β-tubulin sequences

A total of 54 isolates of Japanese *Laetiporus* spp. were used for analyzing the ITS regions of nrDNA, 47 isolates for EF1α and 20 isolates for β-tubulin. All loci were successfully amplified and sequenced. The topologies of NJ and MP trees based on each region were similar, but the topologies among three regions were incongruent with each other. The NJ tree (Fig. 1) based on the ITS region of nrDNA revealed the white pore form of *L. sulphureus* var. *miniatus* clade (100% bootstrap value), the yellow pore form of *L. sulphureus* var. *miniatus* clade (73% bootstrap value), and a clade consisting of "*L. sulphureus* var. *sulphureus*", *L. versisporus*, and an intermediate form (78% Bootstrap value).

This "*sulphureus/versisporus*" clade was divided into two subclades, clade I (80% bootstrap value) and clade II (72% bootstrap value). Clone sequences of L97, WD153, and L51 were assigned to both clades, and one clone sequence of L51 (L51-c) fell between clade I and II in the tree. The sequences in clade II showed no polymorphism, but those in clade I showed some variations.

EF1α loci possessed polymorphisms mainly in the intron, but there were also a few polymorphic positions in the coding regions. There were two amino acid changes among the isolates. The NJ tree based on the EF1α gene fragment (Fig. 2) revealed four main clades: the white pore form of *L. sulphureus* var. *miniatus* (61% bootstrap value), the yellow pore form of *L. sulphureus* var. *miniatus* (100% bootstrap value), and two distinct clades that consisted of "*L. sulphureus* var. *sulphureus*", *L. versisporus* and an intermediate form (100% and 76% bootstrap values, respectively). One "*L. sulphureus*" isolate (L35) showed two different sequences. The isolate of L35-a belonged to clade II,

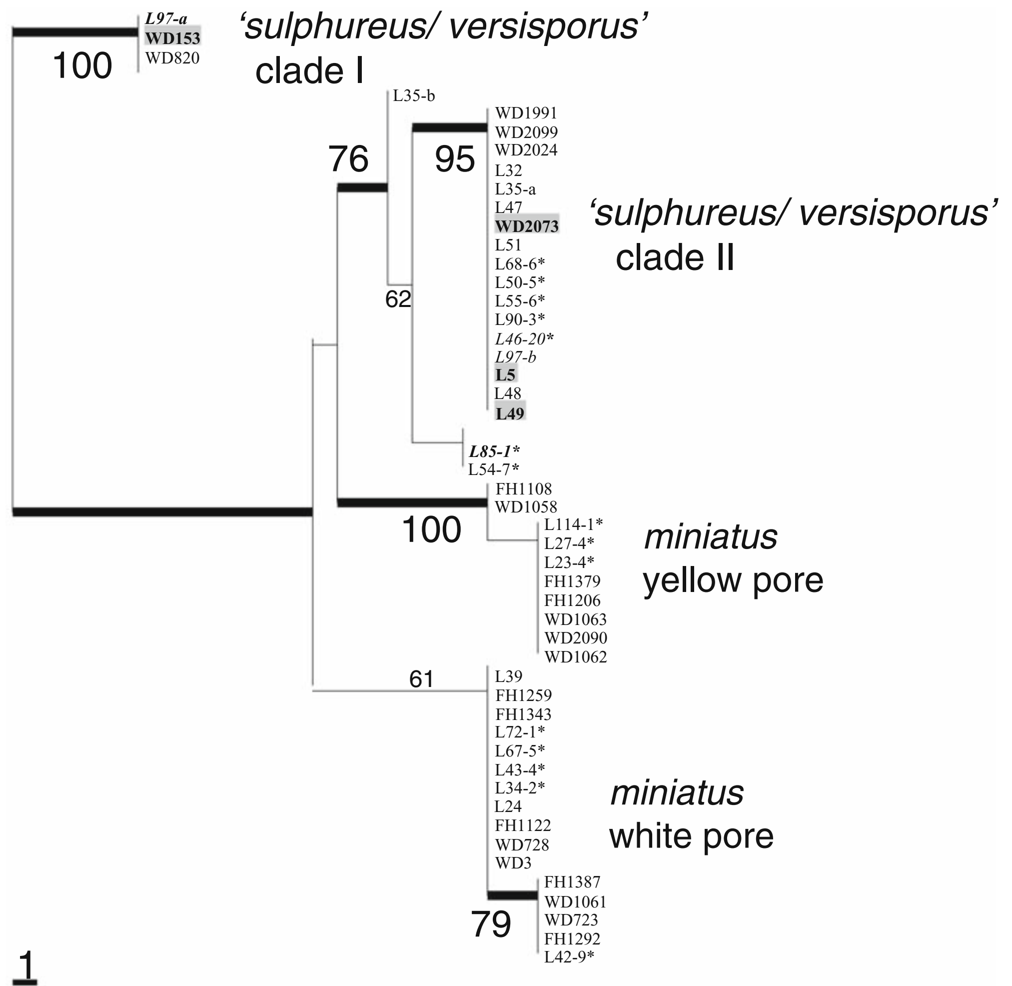

Fig. 2. NJ tree of Japanese *Laetiporus* spp. based on the sequences of EF1α. *, Single spore isolate. *Dark gray shaded*, *L. versisporus*. *Light gray shaded*, intermediate form; -a, -b, -c, -d, and -e, clone sequence

and L35-b showed intermediate sequences between clades I and II. WD1213 and L54-7 also showed intermediate sequences between clades I and II.

The NJ tree based on β-tubulin sequences identified three main clades (Fig. 3): the white pore form of *L. sulphureus* var. *miniatus* (98% bootstrap value) and one strongly supported clade consisting of "*L. sulphureus* var. *sulphureus*," *L. versisporus*, and the intermediate form (*sulphureus/versisporus* clade I), and a clade consisting of "*L. sulphureus* var. *sulphureus*," *L. versisporus*, and the yellow pore form of *L. sulphureus* var. *miniatus* (82% bootstrap value). One isolate of "*L. sulphureus* var. *sulphureus*" (L97) possessed two distinct types of sequences.

Sequence variation of the "*sulphureus/versisporus*" group in the ITS, EF1α and β-tubulin gene regions

The isolates of "*L. sulphureus*," *L. versisporus*, and the intermediate form showed two divergent types of sequences in the ITS (see Fig. 1), EF1α (see Fig. 2), and β-tubulin regions (see Fig. 3). In the ITS region, three dikaryotic isolates gave two ITS variations (WD153-a, -b, L5-a,-b, and L97-a, -b) and one dikaryotic isolate of L51 had five (Fig. 5A). Each clone sequence of WD153-a, L97-b, L51-e and two clone sequences from L5 were assigned to clade I and WD153-b, L97-a and L51-d were assigned to clade II (see Fig. 1). L51-a, -b, and -c showed intermediate sequences between clade I and clade II (Fig. 5A). In the EF1α region, the dikaryotic isolate of L35 and L97 gave two EF1α variations and in the β-tubulin sequence, the dikaryotic isolate of L97 gave two β-tubulin variations (Fig. 5B,C). Compared with three trees, the combination of the isolates in clade I and II were incongruent with each other.

Incompatibility tests

The pattern of sexuality for almost all isolates from one basidiocarp was clearly bifactorial. Some monosporous isolates from "*L. sulphureus* var. *sulphureus*" and the intermediate form did not show clear reactions within the sibling pairings. From the results of pairing tests (Fig. 4), Japanese *Laetiporus* isolates were divided into three incompatibility groups: the white pore form of *L. sulphureus* var. *miniatus*, the yellow pore form of *L. sulphureus* var. *miniatus*, and the group containing "*L. sulphureus* var. *sulphureus*" and the intermediate form (Fig. 4).

The white pore form of *L. sulphureus* var. *miniatus* showed clear compatible reactions in all the pairings within

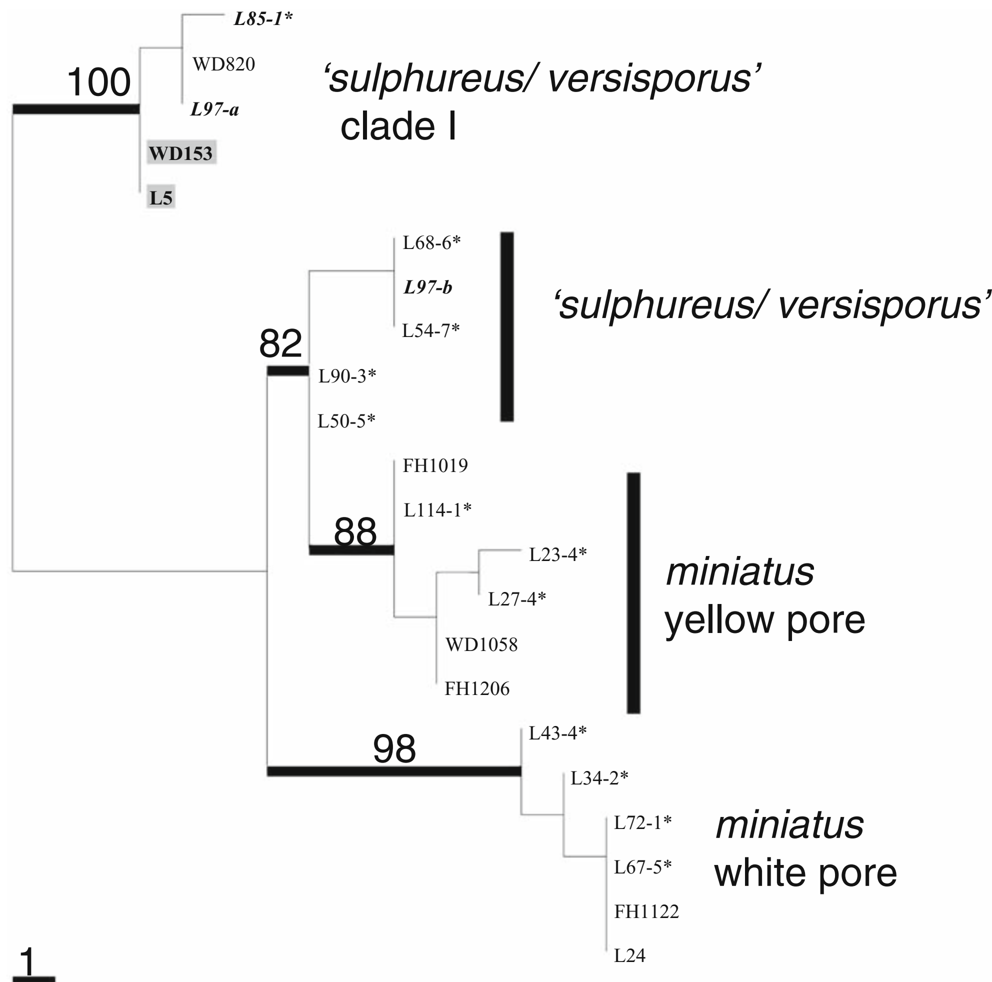

Fig. 3. NJ tree of Japanese *Laetiporus* spp. based on the sequences of β-tubulin. *, Single spore isolate. *Dark gray shaded*, *L. versisporus*. *Light gray shaded*, intermediate form; -a, -b, -c, -d, and -e, clone sequence

the group. The yellow pore form of *L. sulphureus* var. *miniatus* also showed a clear compatible reaction within the group (with a few exceptions), but also showed compatible reactions with some isolates of the "*L. sulphureus* var. *sulphureus*" group. Some pairings between the isolates of "*L. sulphureus* var. *sulphureus*" showed incompatible reactions that formed a clear dense separation line between the cultures.

Discussion

Japanese *Laetiporus* taxa were divided into three groups in this study, but the three groups did not correspond to the taxa widely accepted in Japan. "*Laetiporus sulphureus* var. *miniatus*" was divided into two groups, i.e., the white pore form and the yellow pore form. This result can explain the high variability of "*L. sulphureus* var. *miniatus*" in its color and ecology. The DNA analyses and incompatibility tests showed the white pore form as a clearly distinct group within the Japanese *Laetiporus* groups. This group is distributed widely in the cool temperate and boreal areas from Kyushu to Hokkaido in Japan and occurs mostly on hardwood, for example, *Quercus* spp., *Fagus crenata*, and *Prunus* spp., but also on *Taxus cuspidata* Siebold & Zucc. in Hokkaido. This group is characterized by its pinkish-orange pileus, white pore surface, and imbricated pilei.

The yellow pore form was shown to be a distinct incompatibility group by the pairing tests, but some single spore isolates of the yellow pore form exhibited unclear reactions with those of "*L. sulphureus* var. *sulphureus*." A close relationship with "*L. sulphureus* var. *sulphureus*" was also suggested from the phylogenetic analyses of the β-tubulin and EF1α regions. This group is restricted to coniferous trees and is mainly distributed in cool temperate to boreal areas, but it has also been collected in Chiba, a warm temperate area. This group is characterized by an orange pileus surface and a lemon-yellow pore surface.

In this study, *L. versisporus* formed the same clade with "*L. sulphureus* var. *sulphureus*" auct. jap. and was never accommodated in the other clades in any of the trees. The results showed that "*L. versisporus*" is the anamorph *of* "*L. sulphureus* var. *sulphureus*," and the other two groups in Japan do not produce anamorphs.

The anamorphic form of *L. sulphureus* was reported as a ptychogasteric form from Europe, North America, and Australia (Stalper 1984), although the anamorphic form is

		L. sulphureus var. *miniatus*																*L. sulphureus* var. *sulphureus*													
		White pore form										Yellow pore form																			
Isolate no.		L34		L42		L43		L67		L72		L23		L27		L114		L46*	L50		L54		L55			L68			L85*		L90
		2	4	9	15	4	6	5	9	1	5	4	6	3	4	1	5	20	1	6	7	10	2	6	7	6	7	9	1	6	3
L34	2		+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4			+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L42	9				+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	15					+	+	+	+	+	+	–	–	–	–	–	–	(–)	–	–	–	–	–	–	–	–	–	–	–	–	–
L43	4						+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6							+	+	+	+	–	–	–	–	–	–	–	(–)	–	–	–	–	–	–	–	–	–	–	–	–
L67	5								+	+	+	–	–	–	–	–	–	(–)	–	–	–	–	–	–	–	–	–	–	–	–	–
	9									+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L72	1										+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5											–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L23	4												(–)	(–)	(–)	+	+	–	(–)	(–)	–	–	–	–	–	–	–	–	–	?	–
	6													+	+	+	+	–	+	–	–	–	+/–	–	–	–	–	–	–	?	–
L27	3														+	+	+	–	?	–	–	–	–	–	–	–	–	–	–	(–)	–
	4															+	+	–	–	–	–	–	–	–	–	–	–	–	–	?	–
L114	1																+	–	?	–	–	–	?	–	–	–	–	–	–	?	–
	5																	–	?	–	–	–	–	–	–	–	–	–	–	?	–
L46*	20																		+/–	?	–	+	+/–	–	?	–	?		?	?	–
L50	1																		–	+	+/–	+	+	+	+	?	?	+	?	?	+/–
	6																				–	+	+	+	+	+	+	+	+		–
L54	7																					+	–	–	+?	+	+	–	–	–	+
	10																						+	–	+/–	+	+	+	–	–	+
L55	2																						–	–	–/+	–	–	–	+	+	?
	6																								+	–	–	–	+	+	?
	7																									–	–	–	+	+	?
L68	6																										–	–	–	–	(–)
	7																											–	–	–	(–)
	9																												–	–	(–)
L85*	1																														(–)
	6																														
L90	3																														

Fig. 4. Pairing tests within Japanese *Laetiporus* taxa. –, Incompatible reaction with dense pigmented demarcation line without change of culture appearance. (–), No pigmented demarcation line without culture appearance change. ?, Unclear reaction. +?, Unclear reaction, but seems to be compatible. +/–, The culture appearance of right column isolate changes and the upper column isolate shows no change. +, Compatible reaction with culture appearance change to dense. *, Intermediate form between *L. sulphureus* and *L. versisporus*

very rare in these areas (Ryvarden and Gilbertson 1993), compared with the frequent occurrence of the form in Japan known as "*L. versisporus*". "*Laetiporus sulphureus* var. *sulphureus*" and its anamorphic form "*L. versisporus*" are common and widely distributed in Japan except on Hokkaido, the northernmost main island. The anamorphic form appears mainly in summer, from June to early September (sometimes in October in the southern part of Japan), whereas the teleomorphic form ("*L. sulphureus* var. *sulphureus*") appears mainly in autumn. Sometimes they occur on the same tree in different seasons, according to our field observations and the herbarium data (data not included in Table 1).

The DNA analyses showed that the "*sulphureus/versisporus*" group had two divergent DNA types of three independent loci: ITS, β-tubulin, and EF1α. Clones from some dikaryon isolates formed two distinct clusters in all three trees, confirming that two types existed in one isolate. In addition, the combination of the "*sulphureus/versisporus*" isolates in clades I and II in each tree were incongruent. The most plausible explanation may be that the two DNA types evolved independently and diverged some time in the past and were later united in a hybridization event. The existence of some haplotypes that are evolutionarily intermediate in sequence composition (see Fig. 5) suggests that recombination occurred between two different DNA types of all three regions in this study.

In ITS regions, natural hybridization events followed by recombination or gene conversion have been reported for some Basidiomycetes, for example, *Melampsora* × *columbiana* G. Newc., a hybrid of *M. medusae* Thüm. and *M. occidentalis* H.S. Jack. of rust fungi (Newcombe et al. 2000), a hybrid of *Flammulina velutipes* (Curtis) Singer and *F. rossica* Redhead & R.H. Petersen (Hughes and Petersen 2001), and a hybrid of the North American pine type and spruce type of *Heterobasidion* Bref. (Garbelotto et al. 1996). These species were previously geographically isolated populations or taxa lacking strong reproductive barriers that have come into contact and share the same niche.

Divergent but homologous sequences from the parental lineages within a species have been reported for *Sclerotium rolfsii* Sacc. [teleomorph *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.], *S. delphinii* Welch (Okabe and Matsumoto 2003), and *Trichaptum abietinum* (Dicks.) Ryvarden (Kauserud and Schumacher 2003). *Sclerotium rolfsii* and *S. delphinii* have intraindividual polymorphism in the ITS region and two divergent ITS types, that is, r-1 and r-2 in *S. rolfsii* and r-2 and r-3 in *S. delphinii*, exist within a species (Okabe and Matsumoto 2003). The dominant ITS type in Japan is r-1 and that in North America is r-2.

Fig. 5. Distribution of sequence polymorphism of "*L. sulphureus*"/*L. versisporus* isolates. Nucleotide positions in each alignment of ITS (contains ITS1, 5.8S rRNA gene and ITS2, 534 bp), EF1α (581 bp) and β –tubulin (419 bp) are noted and gaps indicated by –. *, Monosporous isolates. **, -a, -b, -c, -d, and -e are clone sequences

A

		ITS 1									ITS2			
		15	33	42	43	51	53	61	145	147	341	489	504	516
	WD820	T	–	C	C	A	C	G	G	C	C	C	A	A
	L51-b	T	–	C	–	A	C	G	G	C	C	C	G	A
clade	L5-e	T	A	C	–	A	C	G	G	C	T	C	G	A
I	L97-b**	T	A	C	–	A	C	G	G	C	T	C	G	A
	L85-1*	T	–	C	–	A	C	G	G	C	T	C	G	A
	WD153-a	T	–	C	–	A	C	G	G	C	T	C	G	A
	L5-a	T	–	C	–	A	C	G	G	C	T	C	G	A
	L51-c	T	A	C	–	A	C	G	G	C	C	T	T	G
	L51-a**	C	A	C	–	A	C	G	G	C	T	T	T	G
	L51-b	T	A	–	–	G	T	C	C	T	C	C	G	A
	L51-d	C	–	–	–	G	T	C	C	T	C	T	T	G
clade	L46-20 *	C	–	–	–	G	T	C	C	T	C	T	T	G
II	L97-a	C	–	–	–	G	T	C	C	T	C	T	T	G
	L50-5 *	C	–	–	–	G	T	C	C	T	C	T	T	G
	WD153-b	C	–	–	–	G	T	C	C	T	C	T	T	G

B

			Intron							Intron														
		136	185	195	199	206	211	228	279	367	386	384	385	386	404	415	416	420	443	455	467	473	512	563
clade	WD820	G	C	G	C	C	C	A	A	G	C	C	A	C	G	C	A	T	A	A	T	C	T	T
I	L97-a	G	C	G	C	C	C	A	A	G	C	C	A	C	G	C	A	T	A	A	T	C	T	T
	L35-b	T	T	A	T	T	T	A	C	C	T	T	A	C	A	T	C	C	G	G	T	C	C	C
	L85-1*	T	T	A	T	T	T	G	C	C	T	T	A	C	A	T	C	C	G	G	T	T	C	C
clade	L35-a	T	T	A	T	T	T	G	C	C	T	T	G	G	A	T	C	C	G	G	A	C	C	C
II	L97-b	T	T	A	T	T	T	G	C	C	T	T	G	G	A	T	C	C	G	G	A	C	C	C

C

		6	36	37	40	54	57	68	110	116	203	204	221	240	305
	WD820	G	T	G	C	T	A	A	A	T	G	C	C	A	C
clade	L97-a	G	T	G	C	T	A	A	A	T	G	C	C	A	C
I	L5	G	T	G	C	C	A	A	A	T	G	C	C	A	C
	WD1213	G	T	G	C	T	A	A	A	T	G	C	T	A	C
	L50-5 *	A	T	A	C	C	G	C	C	C	A	T	C	G	T
clade	L97-b	A	C	A	T	C	G	C	C	C	A	T	C	G	T
II	L54-7 *	A	C	A	T	C	G	C	C	C	A	T	C	G	T

Two divergent nrDNA types were also found in *Trichaptum abietinum* in North Europe (Kauserud and Schumacher 2003). The incongruent topologies of the ITS 1 and ITS2 trees suggest that recombination has occurred between different nrDNA lineages. The two North European ITS1 types were similar in length to a North American/Korean type and a unique Korean type, but had unique polymorphisms only in the North European ITS type. This result suggests that the two types have shared a common evolutionary history for some time since the recombination event.

From our results with ITS and EF1α trees, clade I included WD820 from the Ogasawara Islands and WD153 from Chiba, and clade II included many isolates from the southern part of Japan (WD1991, WD2099, WD2024, L47, WD2320, L68S6, L55S6, WD2314, L46S20, WD2073, and L49) and one from central Honshu (WD 2314 from Ibaraki). Two "*sulphureus/versisporus*" types (I and II) are considered to have originated from two lineages. The clade II type seems to be distributed in the southern part of Japan and to be more genetically homogeneous than the other type (clade I), and it is apparently dominant. The high variability in the color of basidiocarps and complicated incompatible reactions within the "*sulphureus/versisporus*" group could be the result of hybridization of two distinct lineages. More study is needed to understand the population structure and the speciation of the "*sulphureus/versisporus*" group.

In this study, Japanese *Laetiporus* taxa were reclassified into three taxa. For nomenclature of the detected species, comparative studies including phylogenetic studies, mating tests, and type studies should be made between Japanese taxa and already-described species from other areas.

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