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Genetic variation and relationships in *Laetiporus sulphureus* s. *lat.*, as determined by ITS rDNA sequences and *in vitro* growth rate

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ABSTRACT

The aim of this study was to characterise the genetic variation and molecular relationships of the brown rot polypore, *Laetiporus sulphureus s. lat.*, from Europe, South America, Africa, and Asia, using ITS sequences of the nu-rDNA and by comparing the growth rate *in vitro*. In a NJ analysis of the sequences of 130 individuals of *L. sulphureus s. lat.*, eight distinct clusters emerged, supported by BS values of 70–100 %. Within each cluster, the ITS rDNA sequence variation was below 3 %. The sequences were also analysed together with *Laetiporus* sequences available from GenBank. Results demonstrated the possible presence of *L. huroniensis* in Europe (invalidly named *L. montanus*) and *L. gilbertsonii* in South America, and provided more information on the Pan-American and European distribution of one of the clades, currently known in North America as *L. sulphureus*. *L. conifericola* formed a separate distinct clade. Moreover, the analysis revealed two unknown *Laetiporus* taxa in Korea, one in South Africa, and one in Europe. As *L. sulphureus* is described from Europe (France), and we show that more than one taxon exist here, it is presently not possible to define *L. sulphureus s. str.* Certain biological differences between some of the clades (*in vitro* growth rates, chemical composition, and pigmentation) were demonstrated and discussed.

Introduction

Laetiporus sulphureus s. lat. includes cosmopolitan polypore fungi that cause brown rot in stems of mature and overmature old-growth trees in forests and urban areas. In Eurasia, the fungus has been reported as a destructive pathogen of trees throughout Europe, the Ural Mountains, Russian Far East, India, China, and Japan (Bakshi 1950; Domanski *et al.* 1967; Stepanova-Kartavenko 1967; Granatov 1973; Lyubarsky & Vasilyeva 1975; Burdekin 1979; Gibbs & Greig 1990; Ohsawa *et al.* 1994; Dai *et al.* 2007). In North America, *L. sulphureus s. lat.* has a wide distribution and is known as the common cause of

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butt and trunk rots in forests and parks of Canada and the United States, including Alaska and Hawaii (Boyce 1961; Hepting 1971; Berry & Lombard 1978; Bega 1979; Scharpf 1993; Allen *et al.* 1996; Holsten *et al.* 2001; Sinclair & Lyon 2005). The fungus has also been reported as the cause of heart rot in living trees in South America and South Africa (Westhuizen 1959; May 1963; Vizcarra-Sanchez & Deschamps 1985). Following the death of a tree, *L. sulphureus s. lat.* continues to decompose the dead standing and fallen trunks and stumps (Gilbertson & Ryvarden 1986; Ryvarden & Gilbertson 1993).

L. sulphureus s. lat. has a broad host range. For example, in Sweden the fungus has been recorded on 23 different genera of woody plants (Olofsson 1996), and in Czechia and Slovakia on 20 genera, representing 51 different species of trees and shrubs (Kotlaba 1984). From North America it has been reported from 27 tree genera (Sinclair & Lyon 2005). However, there appears to be a certain geographical pattern in host specialisation. In Europe and central North America, the fungus attacks mainly angiosperm trees (in particular *Quercus*) (Gilbertson & Ryvarden 1986; Ryvarden & Gilbertson 1993), whereas in northern Asia (Ural Mountains, China, Russian, Far East, and Japan) and north-western North America (Pacific Coast, British Columbia, Alaska) it also occurs frequently on gymnosperms (e.g. *Abies*, *Picea*, *Larix*) (Stepanova-Kartavenko 1967; Lyubarsky & Vasilyeva 1975; Scharpf 1993; Allen *et al.* 1996; Holsten *et al.* 2001). In the Southern Hemisphere, it is found on *Eucalyptus* and on other angiosperms (Westhuizen 1959; May 1963; Vizcarra-Sanchez & Deschamps 1985).

A characteristic feature of *L. sulphureus s. lat.* is a considerable variation in morphology of its fruiting bodies (colour, pore layer, cap form, trama consistency), which in previous studies resulted in the distinction of up to seven varieties (Rosen 1927; Bondartsev 1953; Domanski *et al.* 1967). In North America, seemingly host-specific morphotypes were examined using mating compatibility tests, allozyme analysis, and RFLPs of the nu-rDNA (Banik *et al.* 1998; Banik & Burdsall 1999, 2000). Following these studies six *Laetiporus* species were recognised, three of which were associated with angiosperm and three with gymnosperm trees (Burdsall & Banik 2001). Between certain species, clear differences were observed in growth rate *in vitro* (Banik *et al.* 2001). A recent molecular analyses of North American *Laetiporus*, using ITS, nuLSU, and mtSSU rDNA sequences, largely confirms the above cited studies Described North American species grouped into five well-supported clusters, representing *L. huroniensis*, *L. conifericola*, *L. gilbertsonii*, *L. cincinnatus*, and *L. sulphureus s. str.*, while only *L. persicinus* fell outside the *Laetiporus* clade (Lindner & Banik 2008). In this, and in other related phylogenetic studies on wood-decay polypores (Wang *et al.* 2004; Tomsovsky *et al.* 2006), the ITS sequence data correlated well with other molecular markers.

Ota & Hattori (2003) used the ITS region to define intra-specific taxa of *L. sulphureus s. lat.* from Japan. NJ analysis of the sequences revealed five well-supported clusters. Two clusters comprised Japanese specimens from gymnosperm and another two Japanese specimens from angiosperm trees. The fifth cluster consisted exclusively of five European strains, four of which were collected from angiosperm hosts and one from yew (*Taxus baccata*). Another published ITS rDNA sequence analysis of five *L. sulphureus s. lat.* European strains was in agreement with Ota & Hattori (2003), as the specimens collected from angiosperm and gymnosperm hosts clustered separately, and the gymnosperm types clustered together with a specimen originating from Siberian larch (*Larix sibirica*) in Siberia (Rogers *et al.* 1999).

Different species and varieties of *Laetiporus* may differ in pure culture morphology (Chi *et al.* 1999; Banik *et al.* 2001). Recent studies have also revealed significant differences in composition and concentration of volatile compounds from fruiting bodies of *Laetiporus* of different origin (Rapior *et al.* 2000; Davoli *et al.* 2005; Wu *et al.* 2005).

To date, the *Laetiporus* species complex has been relatively well studied in North America and to some extent in Japan, whereas available data on European populations of these fungi are scarce and fragmented. Moreover, little is known of the genetic structure of *Laetiporus* populations from the other parts of the world, and their relationships with already studied populations. In the study of Lindner & Banik (2008), two *Laetiporus* samples from Hawaii and another two from the Caribbean grouped into two separate well-supported clusters within the *Laetiporus* clade, and were classified as two unknown species. The aim of the present work was to investigate the genetic variation and molecular relationships of *Laetiporus* spp. from Europe, South America, Africa, and mainland Asia, using ITS sequences of the nu-rDNA and by comparing growth rates *in vitro*. The generated ITS rDNA sequences were analysed together with those already present in GenBank.

Materials and methods

Material studied

Samples of the fruit bodies of 130 *Laetiporus* specimens were collected, each originating from a different tree (resource unit). From 82 of those, pure cultures were isolated by cutting out a piece of trama, surface sterilizing it by flame, and placing it onto 2 % malt agar medium in a Petri dish. The remaining part of each specimen was dried and stored as voucher specimens. Geographic origin, hosts, and GenBank accession numbers are presented in Table 1. Voucher number 1 is deposited at Upper Austrian State Museums, Linz (LI); numbers 6 and 7 are deposited at the Department of Forest Protection and Wildlife Management, Mendel University of Agriculture and Forestry, Brno (BRNL); numbers 3, 4, 6–8, 13 at the Moravian Museum, Brno (BRNM); number 5 at the Czech National Museum, Prague (PRM); 71–109 at the College of Natural Science, Seoul National University (SNU); remaining vouchers were deposited at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala.

DNA extraction, PCR, sequencing, and sequence analyses

The extraction of DNA (either from a pure culture or a piece of dried fruiting body), PCR amplification and DNA sequencing procedures followed the methods of Kårén *et al.* (1997). The ITS rDNA region of all the strains was amplified and sequenced in both directions using the primers ITS1 and ITS4 (White *et al.* 1990). All sequencing was carried out on an

Table 1 – Fungal specimens used in this study

Specimen No.	Specimen type	Collection ID	Geographic origin	Host tree	GenBank accession no.
Europe					
1	FB[a]	L17-LI	Austria	*Larix decidua*	EU840553
2	PC[b]	UOS-CZ	Czechia	*Fraxinus excelsior*	EU840554
3	FB	L14-706686	Czechia	*L. decidua*	EU840555
4	FB	L16-706697	Czechia	*Picea abies*	EU840556
5	FB	L6-PR897053	Czechia	*P. abies*	EU840557
6	FC[c]	L12-706688	Czechia	*P. abies*	EU840558
7	FC	L8-706687	Czechia	*Prunus domestica*	EU840559
8	FB	L10-686283	Czechia	Unknown	EU840560
9	PC	SLYV-CZ	Czechia	*Prunus* sp.	EU840561
10	PC	Q1-CZ	Czechia	*Quercus* sp.	EU840562
11	PC	Q2-CZ	Czechia	*Quercus* sp.	EU840563
12	PC	Q3-CZ	Czechia	*Quercus* sp.	EU840564
13	FB	L13-706696	Czechia	*Sorbus aucuparia*	EU840565
14	PC	OLRIM1025	Denmark	*Q. robur*	EU840566
15	PC	OLRIM1026	Denmark	*Q. robur*	EU840567
16	PC	OLRIM1027	Denmark	*Q. robur*	EU840568
17	FB	RV-RIGA	Latvia	*Cladrastis kentukea*	EU840569
18	PC	OLRIM1028	Lithuania	*F. excelsior*	EU840570
19	PC	OLRIM1035	Lithuania	*Populus tremula*	EU840571
20	FB	PYR-KRUO	Lithuania	*Pyrus* sp.	EU840572
21	FB	PYR-KAUN	Lithuania	*Pyrus* sp.	EU840573
22	PC	OLRIM117	Lithuania	*Q. robur*	EU840574
23	PC	OLRIM118	Lithuania	*Q. robur*	EU840575
24	PC	OLRIM119	Lithuania	*Q. robur*	EU840576
25	PC	OLRIM583	Lithuania	*Q. robur*	EU840577
26	PC	OLRIM584	Lithuania	*Q. robur*	EU840578
27	PC	OLRIM585	Lithuania	*Q. robur*	EU840579
28	PC	OLRIM586	Lithuania	*Q. robur*	EU840580
29	PC	OLRIM588	Lithuania	*Q. robur*	EU840581
30	PC	OLRIM590	Lithuania	*Q. robur*	EU840582
31	PC	OLRIM591	Lithuania	*Q. robur*	EU840583
32	PC	OLRIM597	Lithuania	*Q. robur*	EU840584
33	PC	OLRIM598	Lithuania	*Q. robur*	EU840585
34	PC	OLRIM599	Lithuania	*Q. robur*	EU840586
35	PC	OLRIM600	Lithuania	*Q. robur*	EU840587
36	PC	RVG1	Lithuania	*Q. robur*	EU840588
37	PC	RVS1	Lithuania	*Q. robur*	EU840589
38	PC	RVS2	Lithuania	*Q. robur*	EU840590
39	PC	RVS3	Lithuania	*Q. robur*	EU840591
40	PC	RVS4	Lithuania	*Q. robur*	EU840592
41	PC	RVS5	Lithuania	*Q. robur*	EU840593
42	PC	RVS6	Lithuania	*Q. robur*	EU840594
43	PC	RVS7	Lithuania	*Q. robur*	EU840595
44	PC	RVP1	Lithuania	*Q. robur*	EU840596
45	PC	RVP2	Lithuania	*Q. robur*	EU840597
46	PC	RVP3	Lithuania	*Q. robur*	EU840598
47	PC	RVP4	Lithuania	*Q. robur*	EU840599
48	PC	VITTOR-SP	Spain	*Q. robur*	EU840600
49	PC	OLRIM1029	Sweden	*F. excelsior*	EU840601
50	PC	OLRIM1034	Sweden	*F. excelsior*	EU840602
51	PC	FR-SIG	Sweden	*F. excelsior*	EU840603
52	PC	OLRIM1092	Sweden	*Juglans regia*	EU840604
53	PC	KATRIN-1	Sweden	*Salix alba*	EU840605
54	PC	KATRIN-2	Sweden	*S. alba*	EU840606
55	PC	KATRIN-3	Sweden	*S. alba*	EU840607
56	PC	OLRIM587	Sweden	*Salix* sp.	EU840608
57	PC	OLRIM1036	Sweden	*Salix* sp.	EU840609
58	FB	OLRIM1100	Sweden	*Salix* sp.	EU840610
59	PC	OLRIM1030	Sweden	*Q. robur*	EU840611
60	PC	OLRIM1031	Sweden	*Q. robur*	EU840612
61	PC	OLRIM1032	Sweden	*Q. robur*	EU840613
62	PC	OLRIM1033	Sweden	*Q. robur*	EU840614

Table 1 – (*continued*)

Specimen No.	Specimen type	Collection ID	Geographic origin	Host tree	GenBank accession no.
63	PC	OLRIM1037	Sweden	*Q. robur*	EU840615
64	PC	VARD-OAK	Sweden	*Q. robur*	EU840616
65	PC	SJ1-R1	Sweden	*Q. robur*	EU840617
66	FB	OLRIM1040	Sweden	*Q. robur*	EU840618
67	FB	OLRIM1041	Sweden	*Q. robur*	EU840619
68	FB	OLRIM1042	Sweden	*Q. robur*	EU840620
69	FB	OLRIM1043	Sweden	*Q. robur*	EU840621
70	FB	OLRIM1044	Sweden	*Q. robur*	EU840622
Asia					
71	FB	11208A	South Korea	*Abies holophylla*	EU840623
72	FB	KR960611-13	South Korea	*Carpinus laxiflora*	EU840624
73	FB	3296 G	South Korea	*C. laxiflora*	EU840625
74	FB	7133A	South Korea	*Castanea crenata*	EU840626
75	FB	BT980722-16	South Korea	*Celtis sinensis*	EU840627
76	FB	JR040721-24	South Korea	*Q. mongolica*	EU840628
77	FB	JR040825-45	South Korea	*Q. mongolica*	EU840629
78	FB	JR040923-05	South Korea	*Q. mongolica*	EU840630
79	FB	11280A	South Korea	*Q. variabilis*	EU840631
80	PC	mkacc50048	South Korea	*Quercus* sp.	EU840632
81	PC	mkacc53979	South Korea	*Quercus* sp.	EU840633
82	PC	mkacc53788	South Korea	*Quercus* sp.	EU840634
83	PC	mkacc53886	South Korea	*Quercus* sp.	EU840635
84	FB	11039A	South Korea	*Quercus* sp.	EU840636
85	FB	1594 G	South Korea	*Quercus* sp.	EU840637
86	FB	9858A	South Korea	*Quercus* sp.	EU840638
87	FB	2501 G	South Korea	*Quercus* sp.	EU840639
88	FB	10259A	South Korea	*Quercus* sp.	EU840640
89	FB	8801A	South Korea	*Tamarix chinensis*	EU840641
90	FB	KR010710-07	South Korea	*Zelkova serrata*	EU840642
91	PC	mkacc50003	South Korea	Unknown angiosperm	EU840643
92	PC	mkacc54164	South Korea	Unknown angiosperm	EU840644
93	PC	SN020620-02	South Korea	Unknown angiosperm	EU840645
94	PC	SN020620-01	South Korea	Unknown angiosperm	EU840646
95	FB	CR980531-26	South Korea	Unknown angiosperm	EU840647
96	FB	JR040825-55	South Korea	Unknown angiosperm	EU840648
97	FB	OY041031-11	South Korea	Unknown angiosperm	EU840649
98	FB	SB060809-21	South Korea	Unknown angiosperm	EU840650
99	FB	3341 G	South Korea	Unknown angiosperm	EU840651
100	FB	11072A	South Korea	Unknown angiosperm	EU840652
101	FB	4628-1A	South Korea	Unknown angiosperm	EU840653
102	FB	10118A	South Korea	Unknown angiosperm	EU840654
103	FB	1453 G	South Korea	Unknown angiosperm	EU840655
104	FB	1915 G	South Korea	Unknown angiosperm	EU840656
105	FB	9178A	South Korea	Unknown angiosperm	EU840657
106	FB	6326A	South Korea	Unknown angiosperm	EU840658
107	FB	11976A	South Korea	Unknown angiosperm	EU840659
108	FB	12028A	South Korea	Unknown angiosperm	EU840660
109	FB	7244A	South Korea	Unknown angiosperm	EU840661
Africa					
110	FB	RV4A	South Africa	*Eucalyptus* sp.	EU840662
111	FB	RV5A	South Africa	*Eucalyptus* sp.	EU840663
112	PC	RV2A	South Africa	*Eucalyptus* sp.	EU840664
113	PC	RV3A	South Africa	*Eucalyptus* sp.	EU840665
North America					
114	PC	OLRIM1038	Canada	*Pseudotsuga menziesii*	EU840666
115	PC	OLRIM1099	Canada	*P. menziesii*	EU840667
South America					
116	PC	5032	Uruguay	*Eucalyptus* sp.	EU840668
117	PC	5055	Uruguay	*Eucalyptus* sp.	EU840669
118	PC	5067	Uruguay	*Eucalyptus* sp.	EU840670
119	PC	5564	Uruguay	*Eucalyptus* sp.	EU840671
120	PC	6674	Uruguay	*Eucalyptus* sp.	EU840672

(*continued on next page*)

Table 1 – (continued)

Specimen No.	Specimen type	Collection ID	Geographic origin	Host tree	GenBank accession no.
121	PC	6676	Uruguay	*Eucalyptus* sp.	EU840673
122	PC	6677	Uruguay	*Eucalyptus* sp.	EU840674
123	PC	6688	Uruguay	*Eucalyptus* sp.	EU840675
124	PC	6689	Uruguay	*Eucalyptus* sp.	EU840676
125	PC	6692	Uruguay	*Eucalyptus* sp.	EU840677
126	PC	6693	Uruguay	*Eucalyptus* sp.	EU840678
127	PC	6694	Uruguay	*Eucalyptus* sp.	EU840679
128	PC	6695	Uruguay	*Eucalyptus* sp.	EU840680
129	PC	6730	Uruguay	*Eucalyptus* sp.	EU840681
130	PC	5179	Uruguay	*Prunus* sp.	EU840682

a Fruit body.
b Pure culture.
c Fruit body and pure culture.

Applied Biosystems 310 automated DNA sequencer with the Big-dye Ready-Reaction kit (PE Applied Biosystems, Foster City, CA, USA). Sequences were aligned using the Clustal algorithm of MegAlign from the Lasergene Package (version 3.08, DNASTAR, Madison, WI) and adjusted manually using Sequence Editor Se-Al (version 1.0a1) (Rambaut 1996). Sequence analyses were performed in two steps. First, we analysed ITS rDNA sequence-based relationships among all 130 specimens collected during this study. Then, three to four representative sequences from each well-supported cluster were analysed together with *Laetiporus* sequences in GenBank of appreciable quality (over 500 bp in size and few missing characters). For the analyses, a NJ similarity tree was constructed in PAUP 4.0b10 (Swofford 2002) using the Hasegawa–Kishino–Yano (HKY85) model (Hasegawa *et al.* 1985). BS analysis consisted of 1 K replicates.

In vitro *growth tests*

In vitro growth tests included six isolates (Table 1; numbers 56, 65, 116, 120, 124, and 129). Each strain was tested for radial growth rate and dry mass accumulation under three temperature regimes (20, 24, and 28 °C), on each of the seven following media (dry mass accumulation tests were performed on liquid media, similar to those described below except that they did not contain agar): malt extract agar medium (MEA; 20 g malt extract, 15 g agar); potato dextrose agar medium (PDA; 300 g potatoes, 20 g glucose, 15 g agar); potato–carrot-10 agar medium (PC10; prepared from 300 g potatoes, 10 g carrots that were boiled and filtered off, 15 g agar); potato–carrot-100 agar medium (PC100; 300 g potatoes, 100 g carrots, 15 g agar, prepared as the PC10 medium); peptone–corn–glucose agar medium (PCG; 5 g corn steep liquor, 5 g NaCl, 5 g peptone, 0.5 g $CaCl_2 \cdot 2H_2O$, 2 g glucose, 15 g agar); peptone–corn–malt extract–glucose agar medium (PCMG; 5 g corn steep liquor, 5 g NaCl, 5 g peptone, 0.5 g $CaCl_2 \cdot 2H_2O$, 5 g malt extract, 2 g glucose, 15 g agar); juice agar medium (V8; 200 ml canned eight-vegetables juice mix, 4 g $CaCO_3$, 15 g agar). All media were prepared according to Atlas & Parks (1997).

Tests for radial growth rate were conducted on 9 cm Petri dishes containing 20 ml of the respective agar medium. A 5 mm agar plug taken from the edge of an actively growing colony was placed at the centre of the dish. Three replicates were made for each medium and strain ($3 \times 7 \times 6$; 126 cultures in total), and incubated in the dark at 20, 24, and 28 °C. Both minimal and maximal diameters of each colony were measured after a week, the mean of which was scored as the radial growth rate of the strain. The tests for dry mass accumulation were conducted in 100 ml Erlenmeyer flasks containing 50 ml of the respective liquid medium. The inoculations, experimental design, and incubation were similar to the growth rate tests described above. One week after inoculation, fungal mycelia from each flask were filtered, dried at 60 °C for 3 d, and weighed.

Results and discussion

Amplification and sequencing the ITS rDNA from all 130 strains gave sequences of 504–650 bp in length. The NJ analysis of the sequences grouped the strains into eight distinct clusters, supported by BS values of 70–100 % (Fig 1). The within-cluster sequence variation was 0 in clusters A and B, less than 1 % in clusters E–H, less than 2 % in cluster C, and less than 3 % in cluster D (Fig 1). The observed nucleotide sequence variation was within the range reported for many biological species of wood-inhabiting basidiomycetes (Anderson & Stasovski 1992; Farnet *et al.* 1999; Vasiliauskas *et al.* 1999; Isikhuemhen *et al.* 2000; James *et al.* 2001; Lickey *et al.* 2002; De Koker *et al.* 2003; Zervakis *et al.* 2004; Tomsovsky *et al.* 2006) and ascomycetes (e.g. Menkis *et al.* 2004 and references therein). Our results indicate that each resolved cluster represents a distinct taxon within the *Laetiporus sulphureus* complex. The results from the second analysis, with GenBank data included, support this conclusion, as the representatives of four clusters (A, B, E and F) grouped together with four well-characterised North American *Laetiporus* species, whereas the other four (C, D, G and H) formed distinct, well-supported (93–100 %) groups within the *Laetiporus* clade (Fig 2).

Cluster A (Fig 1) included four individuals of *Laetiporus* originating from the mountains of Central Europe and collected from coniferous trees. When analysed together with GenBank data, representatives of this group formed a well-supported (80 %) cluster with North American *L. huroniensis*, which is also found exclusively on wood of conifers in America (Burdsall & Banik 2001; Lindner & Banik 2008). This

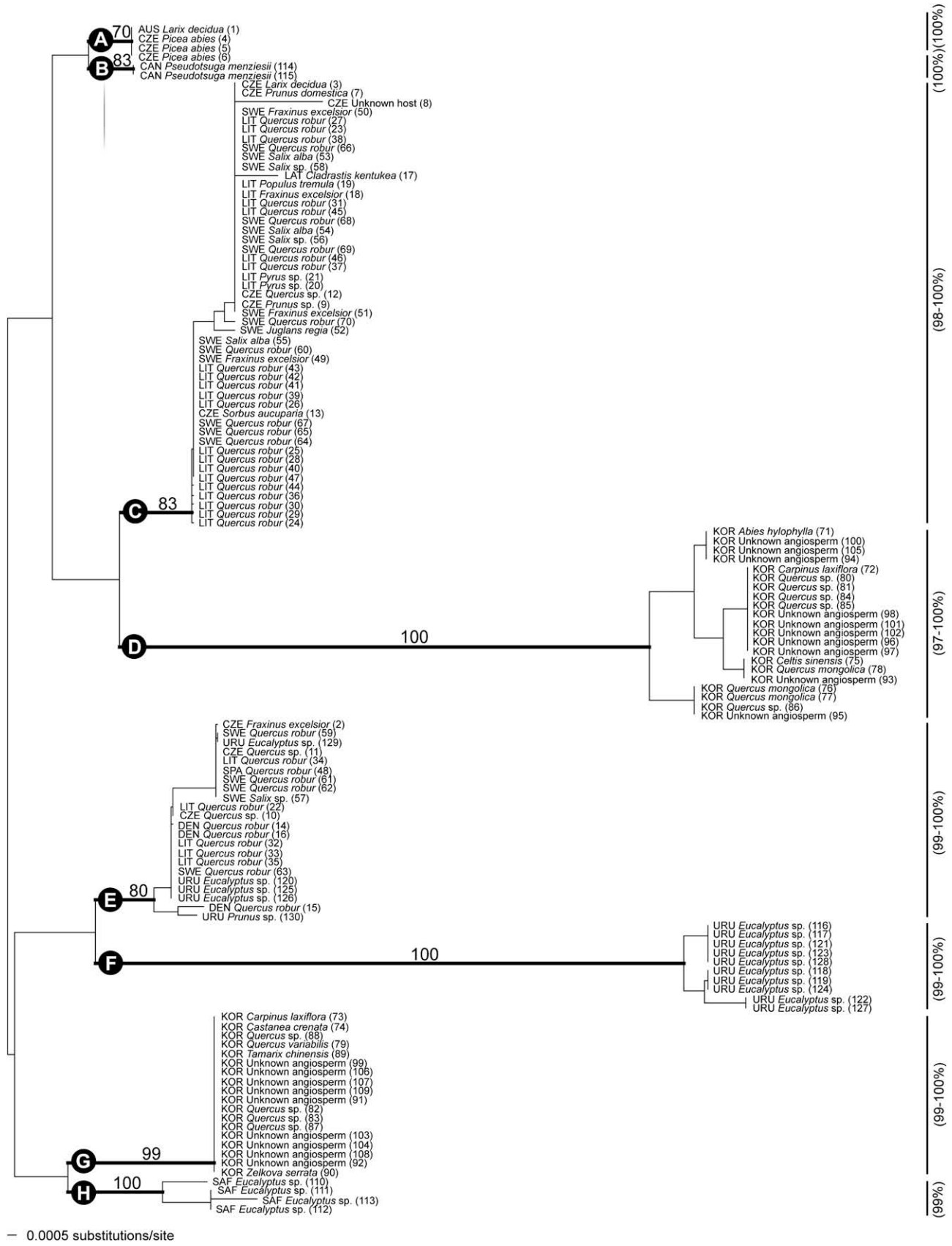

Fig 1 – NJ topology (unrooted) of ITS rDNA sequences of *Laetiporus sulphureus s. lat.* from our collection. For each specimen, information on geographic origin (country abbreviations: AUS, Austria; CAN, Canada; CZE, Czechia; DEN, Denmark; KOR, South Korea; LAT, Latvia; LIT, Lithuania; SAF, South Africa; SPA, Spain; SWE, Sweden; URU, Uruguay), host tree, and its number in Table 1 are given. Percentages in brackets on the right indicate sequence similarity observed within the sequenced sample of the respective cluster. BS values of 70 % or higher, based on 1 K replicates, are indicated above branches.

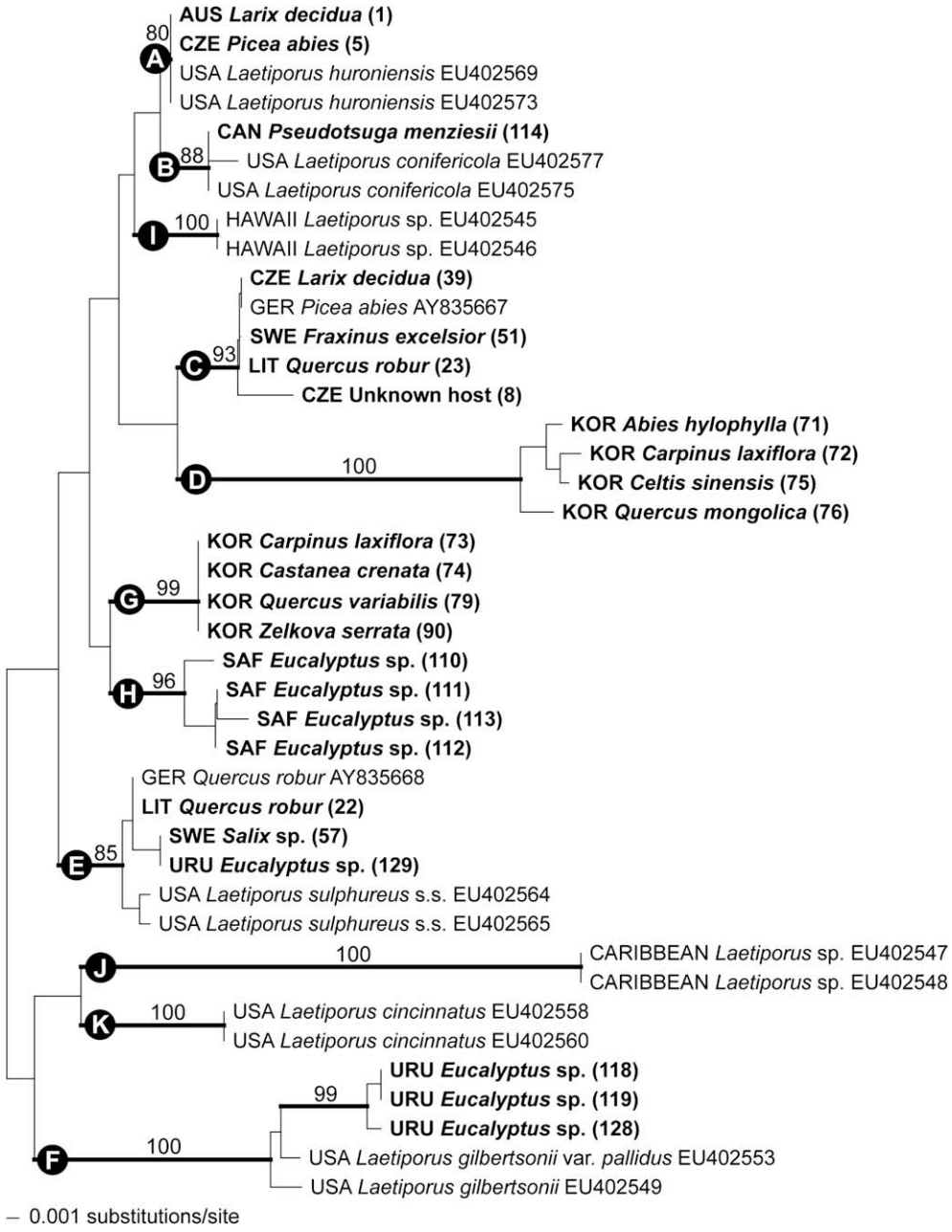

Fig 2 – NJ topology (unrooted) of ITS rDNA sequences of *Laetiporus sulphureus s. lat.* including cluster representatives defined in our material (bold), and sequences of the fungi available in GenBank. For each specimen, information on geographic origin (country abbreviations: AUS, Austria; CAN, Canada; CZE, Czechia; GER, Germany, KOR, South Korea; LIT, Lithuania; SAF, South Africa; SWE, Sweden; URU, Uruguay; USA, United States of America), host tree, and its number in Table 1 are given. BS values of 70 % or higher, based on 1 K replicates, are indicated above branches.

strongly suggests that the distribution range of *L. huroniensis* does include Europe. The European specimens of cluster A used in our work have been described as *L. montanus* Cerny (Cerny 1989). However, this name is invalid according to the nomenclatural rules in ICBN because no Latin diagnosis was provided and no holotype mentioned. The ITS sequences of the European (*L. montanus*) specimens differ from *L. huroniensis* in length, due to presence of a seven base deletion in the American sequences published by Lindner & Banik (2008). Interfertility tests between North American and European individuals of cluster A should be performed to evaluate the taxonomical importance of this sequence variation.

Cluster B (Fig 1) included two individuals of *Laetiporus* originating from British Columbia and collected from Douglas fir. They grouped in a well-supported (88 %) cluster with *L. conifericola* (Fig 2), which is known to occur on conifers in western North America (Burdsall & Banik 2001; Lindner & Banik 2008). Therefore, both samples studied by us represent a previously described species from within its known geographical range.

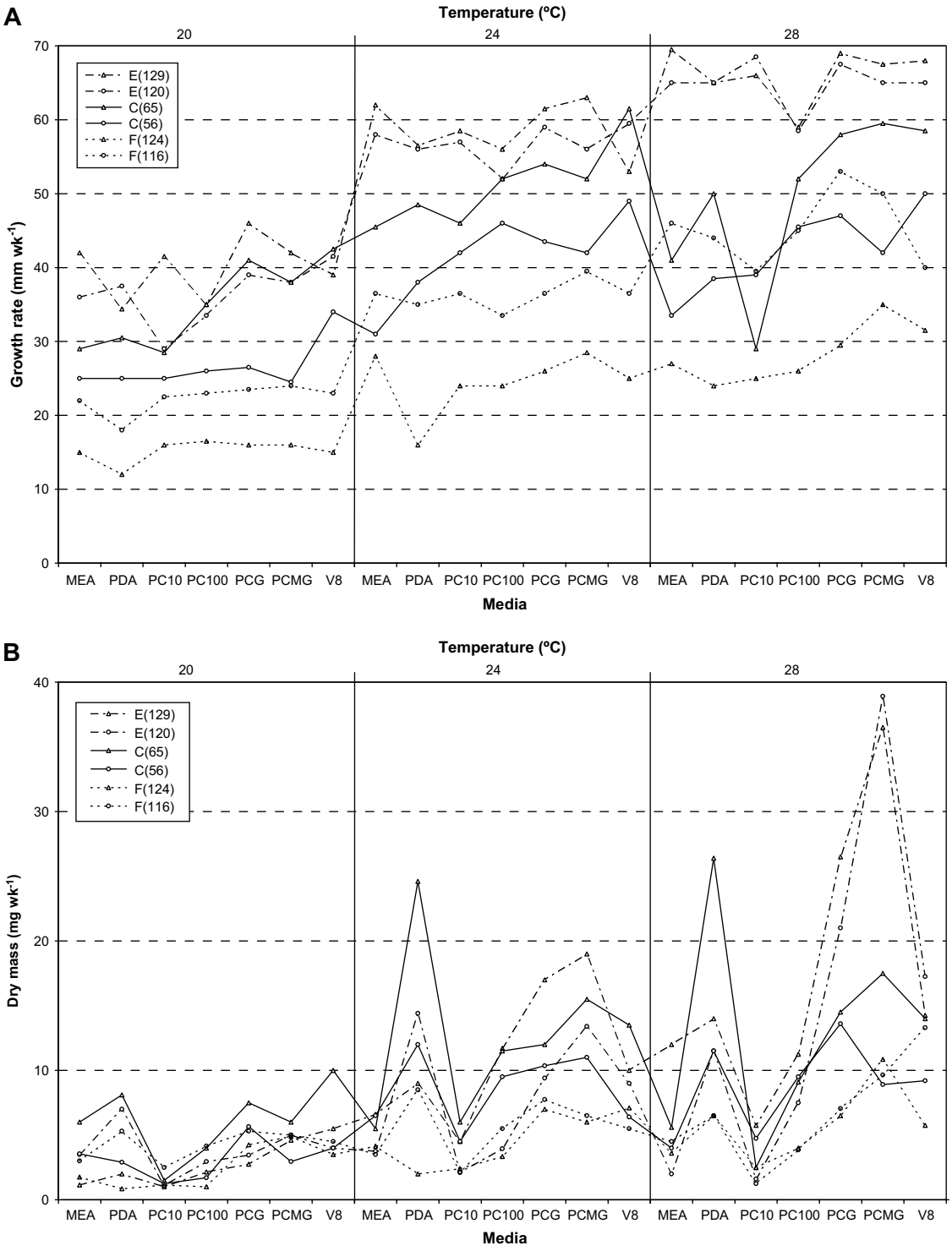

Fig 3 – Diagrams illustrating *in vitro* radial growth rate (A) and dry mass accumulation (B) of six *Laetiporus sulphureus s. lat.* strains under three temperature regimes (20, 24, and 28 °C), and on seven different media: MEA, malt extract; PDA, potato–dextrose; PC10, potato–10 g carrot; PC100, potato–100 g carrot; PCG, peptone–corn–glucose; PCMG, peptone–corn–malt extract–glucose; V8, V8 juice. In the textboxes, tested strains are related to the ITS sequence clusters C, E and F in Fig 1, and with their numbers (in brackets) in Table 1.

Cluster E (Fig 1) included 22 individuals of *Laetiporus* collected from angiosperm hosts. Seventeen of these isolates were from widely distributed sites in Europe (from Spain to Lithuania), and five originated from central South America. Its representative ITS sequences clustered strongly (85 %) with the taxon currently known in North America as *L. sulphureus s. str.* (Fig 2), which occurs exclusively on deciduous trees (particularly on oaks) in America (Burdsall & Banik

2001; Lindner & Banik 2008). Of the 17 individuals from Europe, 15 (88 %) were found on oaks, whereas the other two were from ash and willow (Fig 1). The present study significantly expands the knowledge of the geographic distribution of this taxon in Europe and provides evidence for its occurrence in South America. It is interesting to speculate on the geographic origin of the Uruguayan strains collected from *Eucalyptus* spp. Their ITS sequences were identical to those of European strains originating from broad-leaved trees. As eucalypts were introduced in Uruguay, it might be that *Laetiporus* cluster E also has an anthropogenic origin.

Cluster C (Fig 1) was the largest and included 49 individuals of *Laetiporus* originating exclusively from Europe. The samples were collected from nine different genera of deciduous trees, including oaks, and in one case from a conifer, namely a European Larch. Cluster C did not group with any of the *Laetiporus* species defined from North America by Lindner & Banik (2008), but instead, clustered together with one *Laetiporus* sequence from GenBank (*L. sulphureus* AY835667), which was generated from a German specimen collected from Norway Spruce (Davoli *et al.* 2005). As cluster C is clearly distinct from cluster E, this strongly suggests the presence of a second *Laetiporus* species in Europe growing on both deciduous and coniferous trees. Moreover, this taxon seems to be the most common in Europe and occurs on a wide range of host trees. As *L. sulphureus* is described from a specimen growing on oak in France (Bulliard 1789, as *Boletus sulphureus*) the name can be connected to either cluster C or E. Further morphological studies of macro- and microscopic traits and a more detailed knowledge of host range and distribution of both cluster C and E is necessary before a definition of *L. sulphureus s. str.* can be established.

Cluster F (Fig 1) included ten individuals of *Laetiporus*, all originating from central South America (Uruguay) and collected exclusively from eucalypts. These isolates grouped into a well-supported (100 %) cluster with *L. gilbertsonii* (Fig 2), which is also known to occur on eucalypts and oaks in states adjacent to the Mexican border, and north along the Pacific coast of the USA (Burdsall & Banik 2001; Lindner & Banik 2008). The data from the current work, therefore, strongly suggests a pan-American distribution of this taxon.

The well-supported clusters D and G (100 and 99 %, respectively) consisted only of *Laetiporus* individuals originating from Korea. All 18 specimens of cluster G were collected from deciduous trees. Similarly, among the 21 specimens in cluster D, 20 were collected from deciduous trees, and only one was collected from fir (Fig 1). These isolates did not group with any *Laetiporus* species defined by Lindner & Banik (2008) (Fig 2). Their relationships to known species and varieties of the fungus from Japan (*L. versisporus*, *L. sulphureus* var. *sulphureus*, *L sulphureus* var. *miniatus*) (Ota & Hattori 2003), remain to be determined. Similarly, the representatives from Cluster H, originating from eucalypts from South Africa, did not group with any other identified *Laetiporus* cluster (Fig 2). It thus seems likely that *Laetiporus* specimens in South Africa represent a distinct fungal species, characteristic for that geographical area.

The data from *in vitro* growth tests provided some evidence on biological differences between the representatives of *Laetiporus* taxa from cluster E (described in America as *L. sulphureus s. str.*), cluster C and cluster F (*L. gilbertsonii*). Two randomly selected strains from cluster C exhibited rather similar trends in radial growth rates while reacting to change of media and/or temperature. The same was true also for two representatives from each of clusters E and F (Fig 3A). Generally, the strains from cluster E exhibited the fastest growth, strains from the cluster F the slowest, and strains from cluster C intermediate (Fig 3A). The results from dry weight measurements were not so distinctive, but under certain cultivation regimes clear differences between the clusters were observed (Fig 3B). These results are similar to those from Banik *et al.* (2001), who reported differences between *in vitro* growth rates of *L. conifericola* and *L. huroniensis*. The results of our growth tests are also in good agreement with an early North American study, where *L. sulphureus* exhibited the fastest growth at 25–30 °C (Jensen 1969).

Davoli *et al.* (2005) reported two to threefold differences in the two major orange pigments present in *Laetiporus* fruiting bodies collected from Norway spruce and oak. In our study, the Norway Spruce specimen used by Davoli *et al.* (2005) grouped within cluster C (AY835667) whereas the oak specimen grouped in cluster E (AY835668), indicating substantial shifts in fruit body chemical composition between the representatives of those two groups.

Banik *et al.* (2001) reported differences in pure culture pigmentation of *L. huroniensis* and *L. conifericola*. We also observed clear differences in pure culture pigmentation of *Laetiporus* isolates, ranging from almost white to yellow. In a specifically studied group of 14 strains, there was some correlation between colony colour and assignment either to the cluster C or E. For example, of six isolates characterised as white, five (nos 18, 19, 49, 50, 60) grouped within cluster C, and one (no. 22) within cluster E. Conversely, of nine yellow culture isolates, eight (nos 14–16, 57, 59, 61–63) grouped within cluster E, and one (no. 23) within cluster C (Fig 1). The distinction is thus not absolute and more extensive investigation is necessary to establish possible correlations between pure culture colour and molecular data.

In conclusion, the present study showed that *L. huroniensis*, or a closely related taxon, occurs in Europe and that *L. gilbertsonii* is present in South America. The study also provided more information regarding the pan-American and European distribution of the *Laetiporus* taxon currently known as *L. sulphureus s. str.* in North America (cluster E). Surprisingly, a second taxon (cluster C) with a host range and distribution in Europe overlapping that of cluster E was detected. This discovery calls into doubt the propriety to use the European name *L. sulphureus* for the taxon occurring in North America. Moreover, this work revealed two undescribed *Laetiporus* taxa in Korea and one in South Africa. Certain biological differences between different *Laetiporus* clades were demonstrated and discussed. Additional information, based on morphological, ecological, molecular, biological, and compatibility data, is needed to clarify the taxonomic position of these likely new species within the genus *Laetiporus*.

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