

## *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms

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**Abstract:** *Agaricus subrufescens* Peck was cultivated first in the late 1800s in eastern North America. The type consists partly of cultivated material and partly of field-collected specimens. Once a popular market mushroom, the species faded from commerce in the early 20th century. More recently, a mushroom species growing wild in Brazil has been introduced into cultivation in Brazil, Japan and elsewhere. This Brazilian mushroom has been referred to by various names, most commonly as *A. blazei* Murrill (*sensu* Heinemann) and most recently as *A. brasiliensis* Wasser et al. The author first cultivated *A. subrufescens* in 1981 and has grown and studied Brazilian isolates since 1992. The species has an amphithallic pattern of reproduction. Based on DNA sequence analysis of the rDNA ITS region and on mating studies and genetic analysis of hybrid progeny, there is a strong case for conspecificity of the Brazilian mushrooms with *A. subrufescens*. Based on a study of the type and other data, the recent lectotypification of *A. subrufescens* is accepted. Data are presented on mushrooms of diverse geographical origins, including *A. rufotegulis* Nauta from western Europe, another apparent conspecific. A possible role for interpopulational hybridization in current populations of *A. subrufescens* is proposed. The agronomic history of the species is reviewed.

**Key words:** ABM, Cogumelo do Sol, Himematsutake, interpopulational hybridization, phylogeography, Royal Sun Agaricus

### INTRODUCTION

*Agaricus subrufescens* Peck was first described in 1893 by C.H. Peck, the New York state botanist, from two collections. The first was of two mushrooms from a crop being cultivated at “Dosoris” (Glen Cove), New York (sent 15 Oct 1892). Because these arrived in poor condition, additional specimens found growing

“in our [W. Falconer’s] leaf pile in old leaf mold” then were sent (apparently on 24 Oct 1892) (Peck 1893; Falconer to Peck, in lit. [NYS]). The mushroom, called the “almond mushroom” or “almond-flavored mushroom” due to its fragrance and taste, was widely cultivated, sold and eaten in the Atlantic states of the United States from at least Massachusetts to Washington D.C., from the late 19th century into the 20th (Falconer 1894a, b; Anon. 1904; Anonymous 1909; Kauffman 1918). Spawn (inoculum culture for farming) of *A. subrufescens* was even offered for sale (Falconer 1894a, b; Anonymous 1909). As late as 1918 Kauffman (1918) reported it to be in cultivation. Commercial production of *A. subrufescens* subsequently declined as market trends changed; soon the related “button mushroom” species *Agaricus bisporus* (J.E. Lange) Imbach appears to have been the only mushroom species being regularly cultivated in the United States (see Duggar 1920, Charles 1931).

*Agaricus subrufescens* often occurs in domesticated or semidisturbed habitats, including leaf piles. It has been recognized occasionally growing “wild” outside northeastern North America, for example in California (Kerrigan 1982, 1983a, 1986; Kerrigan and Ross 1987a), Israel (R. Kenneth, personal communication 1984–85), Taiwan (Tu and Lin 1981) and Hawaii, where it grows under forest trees (Peterson et al 2000). Brazilian examples in the past three decades have entered commerce and (not having been recognized as *A. subrufescens*) raised questions, discussed below, about nomenclature and identity. In addition, the recently described *A. rufotegulis* Nauta from the Netherlands, the United Kingdom and Portugal (Nauta 1999, Hausknecht 2002) is also extremely similar and considered here to be conspecific with *A. subrufescens*.

The history of *A. subrufescens* was reviewed by Kerrigan (1983a); updated and extended information follows. A discussion of its properties as an easily cultivated mushroom was presented by Kerrigan (1983a, 1984); notably, very similar outdoor methods are typically employed in Brazil today. A culture (RWK 1185; voucher at SFSU) of *A. subrufescens* was isolated by me from basidiomata growing in rich compost covered with sandy soil for raspberry culture in California in 1981. This culture was sold commercially to hobbyist mushroom growers (it recently has been cul-

tivated at commercial scale in the United States and, based on sequence and other data, abroad (see below)). Reproductive micromorphology and genetic behavior of this strain were investigated by Kerrigan and Ross (1987a, b, unpublished).

Mushrooms originating in the Atlantic region of Brazil and agreeing closely with *Agaricus subrufescens* have begun in recent decades to be cultivated on a broad scale as “medicinal mushrooms” that are marketed primarily in Japan (Kerrigan 1983a, Wasser et al 2002). However, in this context the mushroom typically is referred to (incorrectly) as *A. blazei* Murrill, “*A. blazei* ss Heinem.” (see Wasser et al [2002]) or, in some commercial literature and product packaging, “*A. sylvaticus*”.

The conventional account of the origin of ‘*Agaricus blazei* Murrill sensu Heinemann’ and its arrival in Japan is related by Wasser et al (2002). It is consistent with the account given to me by Mr Shusuke Minoura in Hiroshima, Japan, in 1981. His account and my conclusion that the Brazilian *Agaricus* then cultivated in Japan was “almost certainly” *A. subrufescens* were published shortly thereafter with one of Minoura’s photographs (Kerrigan 1983a). The conventional history also agrees with a specific and plausible account by Mr. B.-A. Eckart (personal communication) of Brazil and Germany, related to him by Mr Ernesto Noburo, which attributes the discovery of the Brazilian mushroom and its distribution to Japanese researchers to the late Mr Takatoshi Furumoto, an immigrant from Japan to Piedade, Brazil. Details in the report of Heinemann (1993) indicating that a culture was isolated from a collection made at São Paulo, Piedade, Brazil, in Feb 1973, and cultivated in Japan by Iwade, from which specimens (at BR) were preserved by Hongo, are also concordant. See also Souza Dias et al (2004).

Controversy has existed regarding the correct name of the Brazilian species. A search of the World Wide Web and a review of diverse commercial product literature indicated that association of the name *A. blazei* with the Brazilian mushroom is attributed to P. Heinemann. Minoura used this name in 1981; the published taxonomic determination appears in Heinemann (1993). The name *A. sylvaticus* Schaeff. sometimes is associated with the species, and this is said to have resulted from a determination of Brazilian cultivated material made by a European mycologist at the request of a Brazilian producer (R. Maziero personal communication). *Agaricus sylvaticus* customarily is placed in section *Sanguinolenti* (F.H. Møller & Jul. Schäffer) Singer, while *A. subrufescens* (under any name and including Brazilian material) is certainly a member of section *Arvenses* Konrad & Maubl. (Kerrigan 1982, 1986).

Wasser et al (2002) rejected the name *A. blazei* for the Brazilian mushroom, correctly in this author’s opinion, and recognized the latter as a new species, *A. brasiliensis* Wasser et al. Wasser et al also rejected conspecificity between Brazilian mushrooms and *A. subrufescens*, based on features of spores and cheilocystidia, although they wrote that the two species seemed to be each other’s closest relatives.

However, new data presented below indicate that the medicinal mushroom from Brazil and Japan is biologically and phylogenetically the same species as *A. subrufescens* from North America. I will review features that have been emphasized in the literature on the taxa involved. Because Peck’s name *A. subrufescens* is older than *A. brasiliensis*, it has priority and therefore is the correct name of the species. *A. rufotegulis* Nauta equally belongs to *A. subrufescens*, based on overall morphological and sequence criteria. The rDNA ITS1+2 sequences from Hawaiian specimens of *A. subrufescens* are the most divergent of those studied.

#### MATERIAL AND METHODS

All cultural and analytical methods were routine and have been described in earlier publications (Challen et al 2003, Kerrigan et al 1996). Samples evaluated are given (TABLE I). For microscopy, excised dried material was wetted in 95% ethanol, then mounted and measured in 3% KOH; spore length excludes the apiculus. Cultures of commercial samples of “medicinal agaricus” of Chinese and Japanese origin were obtained from spores aseptically washed from lamellae of dried mushrooms, in the form of single spore isolates (SSIs). The karyotic status ( $n$  versus  $n + n$ ) of each SSI was determined from potato-dextrose yeast broth (PDYB) grown samples using allozyme markers including peptidase and esterase. Heteroallelic SSIs were assumed to be heterokaryotic, while homoallelic SSIs were of uncertain status. Crosses were attempted between homoallelic SSIs on PDA, transferred to grain spawn medium, then compost (for cropping); successful hybrid cultures were isolated and cultured in PDYB for genetic analysis.

Some samples (*A. rufotegulis*, *A. brasiliensis* [Isotype], *A. subrufescens* DEH 1073, 513; KRP 070) were available only as herbarium specimens, from which DNA was isolated using the DNAEasy Plant Miniprep Kit (Quiagen). Living cultures were grown in PDYB, harvested, lyophilized and extracted for DNA using the CTAB miniprep procedure (Zolan and Pukkila 1986).

Amplification of the ITS1+2 region of the rDNA, sequencing of the products and subsequent alignment and analysis was done as described in Challen et al 2003. Normally primers ITS1 and ITS4 (White et al 1990) were used to produce a full-length ITS1+2 PCR product; however, some herbarium material provided DNA that only produced shorter PCR products, using primers ITS1 and ITS2, or ITS3 and ITS4. In this project it was routine in sequenc-

TABLE I. Samples evaluated in molecular or cultural studies

ID	Origin	Source	GenBank accession
DEH 513	Pahoa, Hawaii, USA 27 July 1994	SFSU	
DEH 1073	Leilani Estate, Hawaii, USA 26 Mar 1996	SFSU	
KRP 070	Hilo (Bayfront), Hawaii, USA 20 Aug 1996	SFSU	
L 0341732 ( <i>A. rufotegulis</i> )	Sheen Common, Kew, Surry, U.K.	M. M. Nauta (L)	
<i>A. brasiliensis</i> Isotype; No. 0978	Embrapa Florestas, Colombo, Paraná State, Brazil 22 Jan 2001	S. Wasser (HAI)	
BS1 (SSI culture)	Spore print, field cultivated material, near Piedade, Brazil 1993	R. Samp	
RM-1	Cultivated, São Paulo State, Brazil ca. 1994	R. Maziero <sup>1</sup>	
RM-4	Cultivated, São Paulo State, Brazil ca. 1994	R. Maziero <sup>1</sup>	
RM-Af	Cultivated, São Paulo State, Brazil ca. 1994	R. Maziero <sup>1</sup>	
I-101-s1 (SSI culture)	Commercial product, Japan	Commercial source	
HIX1 (Hybrid culture)	Sylvan proprietary hybrid	Sylvan research	
SBRFG-s1 (SSI culture)	Sporeprint of SBRFG, Santa Cruz Co., California, USA, 1991	RWK	
SBRFG (tissue culture)	Subculture of SBRF, Santa Cruz Co., California, USA, 1991	RWK	
SBRF (tissue culture)	RWK 1185, Aptos, Santa Cruz Co., California, USA, 1991	RWK <sup>1</sup>	
CS4 (SSI culture)	Commercial product, China	Commercial source	

<sup>1</sup> The vouchers for RM-1, RM-4, RM-AF, and RWK 1185 are at SFSU

ing full-length products to use internal primers ITS2 and ITS3 in addition to terminal primers ITS1 and ITS4 due to length heterogeneity which often was present in both ITS segments 1 and 2 in this species. The sequence analyzed begins with ggaaggat in the 18S gene and ends with gaacttaa in the 25–28S gene. Sequencing was performed on the most current equipment available at the time at either the Pennsylvania State University or at the University of Pittsburgh. Output was ABI trace files; these were inspected, corrected and assembled using the Seqman module of the Lasergene version 5 package (Dnastar). A sequence from *A. urinascens* (Jul. Schäffer & F.H. Møller) Singer (= *A. macrosporus* [F.H. Møller & Jul. Schäffer] Pilát, nom. illeg.; non *A. macrosporus* Mont. [1837], per Nauta [2000]) (GenBank AF432878) was used as outgroup. Alignment was done using the Clustal W algorithm of the Megalign module of Lasergene, followed by inspection and manual correction. All sequences comprise data from both strands, although in the 18S, 5.8S and 25–28S genes short regions of single strand data are present. A few 5.8S sequences (which are almost invariant within *Agaricus*) are incomplete. Sequences were deposited in GenBank (AY818646-AY818660).

It was apparent that the distance matrix generated from the alignment by Megalign was treating heteromorphic characters as ambiguous data, which then were excluded from similarity calculations. The DNADIST program of PHYLIP also ignores heteromorphic data. Consequently, I manually constructed a pairwise distance matrix in which

each pair of identical characters was scored as 0.0, each pair of fully dissimilar characters was scored, as 1/n, where n = character positions scored, and a pair consisting of one heteromorphic character and one of its constituent characters was scored as 0.5/n. Both character and length heteromorphisms were scored in this way. Total pairwise distance was the sum of scores over all (nominally 711) positions. The matrix file was evaluated using both FITCH and the UPGMA method in NEIGHBOR (PHYLIP; Felsenstein 2004) to produce a graphical representation of similarity, rather than a phylogenetic hypothesis, because the presence of numerous heteromorphisms and the hypothesis of population-level hybridization in this species (see below) are not compatible with the usual assumptions of character state evolution and radiating phylogenetic lineages.

To evaluate the phylogenetic unity of *A. subrufescens* and putatively conspecific taxa, the sequences described above were compared to others of species in several sections of *Agaricus*, primarily section *Arvenses*. Sequences from GenBank included AB113576.1, AF161013, AJ131126.1 and AY484697.1 (as *A. blazei*, deposited by four research groups), AY484671.1, AY474672.1 (as *A. augustus*), AY484690.1 (as *A. arvensis*), AF482834.1 (as *E. depressum*, = *A. inapertus*), AY484670.1 (as *A. nivescens*), AY 484686.1 (as *A. macrocarpus*), and AY484675.1 (as *A. albolutescens*), all in section *Arvenses*. Names associated with GenBank deposits were accepted provisionally. Other sections were represented as follows: *Agaricus: A. campestris*: WIH (M. Chal-

len, HRI: includes AJ418775); *Xanthodermatei*: *A. xanthoder-mus* W3I (M. Challen, HRI: includes AJ418776); *Duploan-nulati*: *A. bisporus* RWK 1885 (AF432886); *Sanguinolenti*: *A. pattersonae* RWK 1415 (includes AJ418715); other: *A. sub-rufescens* (in or near section *Spissicaules*) RWK 1940. The alignment file was prepared as described above and was evaluated under maximum parsimony using PAUP\* version 4.0b8 (Swofford 2000).

## RESULTS

*Marker-assisted analysis of reproduction.*—Single spores were isolated and germinated from several samples of *A. subrufescens*, including I-101, a strain developed from Brazilian germ plasm and cultivated and sold commercially in Japan (as 'Iwade 101', per the provider of the sample) and from SBRFG, a sub-culture of the RWK 1185 isolate made by Kerrigan in California in 1981. These SSIs then were propagated in broth and subjected to allozyme analysis (Kerrigan et al 1992). Segregation of alleles in offspring occurred at the PEP1 and PEP2 loci (Kerrigan et al 1996, Royse and May 1982) in progeny of I-101 and at an esterase locus in SBRFG. This demonstrates that meiosis, recombination and partitioning of recombinant nuclei into spores is occurring in these isolates of *A. subrufescens*, therefore the species is not homothallic. Furthermore, some SSIs had heteroallelic genotypes, proving that multiple nuclei were present in heterokaryotic spores, while other spores were homoallelic, implying that they could be homokaryotic. All these observations are consistent with the presence of a basic system of amphithallic reproduction in which both uniparental reproduction (via intramixis) and outcrossing (heteromixis) is possible. The tendency of at least some strains of *A. subrufescens* to produce substantial numbers of bi- and tri-spore basidia under some conditions also is consistent with the presence of an amphithallic life cycle (Kerrigan and Ross 1987a, see also comments in Heinemann 1993).

An interesting property of SSIs of SBRFG is that they varied with respect to reproductive ability (as expected among amphithallic offspring). Although the SBRFG parent had a pigmented pileus, some fertile SSI offspring produced basidiomata with white pilei. The latter observation could be explained by the presence of a Mendelian determinant for pileus color located at sufficient distance from the centromere to allow frequent crossing over, and the presence of one recessive allele in the parent, leading to a minority of homoallelic recessive heterokaryotic offspring (cf. Kerrigan et al 1993). These observations were first noted in work at UCSB in 1986 (Ker-

rigan and Ross unpublished). Some SSIs of I-101 (e.g., -s1) also were fertile.

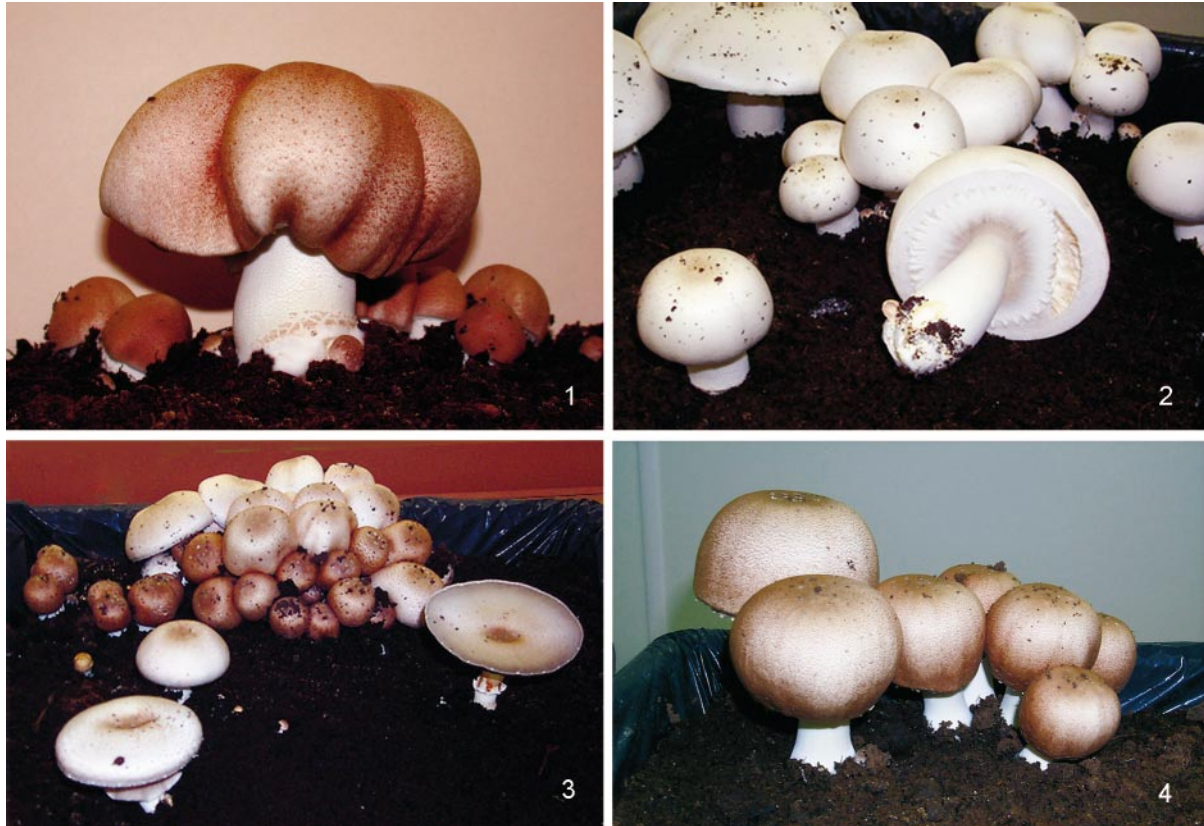
*Interfertility and hybrid analysis.*—All tested SSIs from the BS1 (Brazilian commercial) and CS4 (Chinese commercial) samples were heteroallelic in allozyme analysis, so no crosses from these SSIs were attempted. Homoallelic SSIs from the I-101 and SBRFG stocks were selected to be the progenitors of a series of hybrids. Hybrid cultures were isolated for each pairing. The hybrid between SBRFG-s1 and I-101-s1, called H1X1, will serve as an example.

The hybrid status of the putative new hybrids was verified by allozyme analysis. In the case of the hybrid H1X1, the progenitor I-101-s1 carries allele *Pep2-s*, while progenitor SBRFG-s1 carries allele *Pep2-f*. Both SSIs are fertile, therefore either or both might be heterokaryotic. The hybrid H1×1 between these two SSIs has the expected genotype *Pep2-s/f*, demonstrating that this isolate incorporates DNA from each parent. The simplest and most conventional explanation is that H1×1 received one nucleus from I-101-s1 and another nucleus from SBRFG-s1.

A set of 25 *A. subrufescens* hybrids between parents I-101 and SBRFG were grown on small containers of compost under standard conditions, with the SBRFG parent also present as a control. The phenotype of H1×1 serves as an example of how morphological and cultural traits may be inherited from the progenitors of such a hybrid.

Parent SBRFG had a thick-fleshed, wavy pileus with a brown pigmented surface and robust basidiocarps (FIG. 1). SSI SBRFG-s1, progenitor of the hybrid, was similar but white and fruited a few days later than its parent (FIG. 2). SSI I-101-s1 had a thin-fleshed, narrowly convex pileus with a brown pigmented surface and gracile basidiocarps that fruited about 10 d later than SBRFG (FIG. 3). H1×1 produced robust mushrooms that fruited concurrently with those of SBRFG, with brown-pigmented pilei that were convex and not (or only obscurely) wavy (FIG. 4). The several traits described here were inherited and expressed in different modes from the two SSIs; for example the pileus color trait exhibited classic Mendelian dominant/recessive behavior.

*ITS1+2 DNA sequences.*—For available isolates and specimens (TABLE I), the sequences of the ITS1+2 regions of the nuclear rDNA were aligned and compared. The samples studied had an rDNA ITS1+2 sequence with nominal length of 711 nt; however, due to frequent length polymorphisms at either of two positions, the actual sequence lengths ranged from 710 to 712 or 713 nt and a majority of samples yielded sequences of more than one length. These length polymorphisms occurred at positions 49–51



FIGS. 1–4. *Agaricus subrufescens* basidiomata in culture. 1. SBRFG,  $\times 0.4$ . 2. SBRFG-s1,  $\times 0.3$ . 3. I-101-s1,  $\times 0.3$ . 4. H1X1,  $\times 0.35$ . The pleats in the pileus of SBRFG are a fairly extreme example.

(ttt versus tttt) and at or near position 485 (tttt versus tttttt) in the nominal 711 nt sequence. They were commonly heteromorphic within single isolates (downstream from the indel, two distinct peaks were superimposed at each position when the output trace file was examined), which interfered with sequencing and necessitated bidirectional sequencing using four primers. The sequences were otherwise identical except at 16 other variable positions (TABLE II).

At a number of positions (ranging from four to nine in single sequences), nucleotide heteromorphisms always were present in North and South American samples and these pairs always corresponded to alternate nucleotides seen in some samples (e.g., a versus g versus r at position 601) (TABLE II). The term heteromorphism should not be equated automatically with heteroallelism (because the ITS region is moderately or highly repeated), although equivalence in this instance would be the simplest possibility and is supported by the sequence of H1X1.

The sample from the UK, identified by M.M. Nauta as *A. rufotegulis*, fits this sequence pattern, although no heteromorphisms were present. The three suc-

cessfully amplified sequences from Hawaii (DEH 1073, 513; KRP 070) were identical among themselves and were similar to non-Hawaiian sequences but had slightly different sequence characteristics. Like *A. rufotegulis*, Hawaiian sequences lacked both compositional and length heteromorphisms. They had two sequence characters, at positions 281 and 478, not found in any of the other non-Hawaiian sequences. Finally, their lengths were one nucleotide shorter than all other “nominal” sequences, at a third position ( $\sim 485$ ), where other sequences either were nominal or one nucleotide longer. DNA extracts from three other Hawaiian specimens of *A. subrufescens* (DEH 337, 527, 1452 [SFSU]) unfortunately failed to amplify.

Excluding several GenBank sequences identified as *Agaricus blazei*, mainly deposited by laboratories in Asia, the public sequences with the greatest affinity to the *A. subrufescens* samples all belong to taxa in section *Arvenses*. One of these is GenBank AF432878, deposited as *A. macrosporus* RWK 1925 but correctly named *A. urinascens*. The *A. urinascens* sequence was 2.9–3.2% divergent from the *A. subrufescens* samples (using Megalign), while distances among *A. subrufescens* samples ranged from 0 to 0.27% (hand calculat-

TABLE II. Variable positions within the nominal<sup>1</sup> ITS1+2 rDNA sequence of *A. subrufescens*

Sample	Origin	Position																			N.H.
		33	~50	134	142	157	158	166	180	202	212	258	281	418	478	~485	601	653	695		
DEH 513	Hawaii	A	—	G	G	A	T	C	G	C	C	A	A	C	C	—	G	T	C	0,0	
DEH 1073	Hawaii	A	—	G	G	A	T	C	G	C	C	A	A	C	C	—	G	T	C	0,0	
KRP 070	Hawaii	A	—	G	G	A	T	C	G	C	C	A	A	C	C	—	G	T	C	0,0	
<i>A. rufotegulis</i>	UK	A	—	G	G	A	T	C	G	C	C	A	A	C	C	—	G	T	C	0,0	
<i>A. brasiliensis</i>	Brazil	A	—	G	G	A	T	C	G	C	C	A	A	C	C	—	G	T	C	4,0	
BS1	Brazil	A	T	R	R	R	W	Y	Y	Y	A	R	A	C	—	R	T	T	C	7,1	
RM-1	Brazil	A	T	R	R	R	W	Y	Y	Y	A	R	A	C	—	R	T	T	C	6,1	
RM-4	Brazil	A	T	R	R	R	W	Y	Y	Y	A	R	A	C	—	R	T	T	C	9,1	
RM'A. f.	Brazil	A	T	R	R	R	W	Y	Y	Y	A	R	A	C	—	R	T	T	C	9,1	
I-101-s1	[Japan]	R	—	G	G	A	T	Y	G	C	A	G	G	Y	—	R	K	Y	Y	6,0	
H1X1	Hybrid	A	T	G	G	A	T	Y	G	C	Y	A	G	C	T	T	R	T	Y	4,2	
SBRFG-s1	California	R	T	R	R	R	W	C	G	C	Y	A	G	Y	T	A	K	Y	Y	9,2	
SBRFG	California	R	T	R	R	R	W	C	G	C	Y	A	G	Y	T	A	K	Y	Y	9,2	
CS4	[China]	R	T	R	R	R	W	C	G	C	Y	A	G	Y	T	A	K	Y	Y	9,2	

<sup>1</sup> The nominal sequence length is 711 nt. N.H. = number of heteromorphisms (character, length). Origin data in brackets indicates a physical origin not believed to correspond to biogeographical origin. Heteromorphic codes: R = A+G; W = A+T; Y = C+T; K = G+T; — = no nucleotide present. In all cases where a T is present at pos. ~50 or ~485, the putatively heterokaryotic sequence is length-heteromorphic. The nominally 692 other positions are invariant.

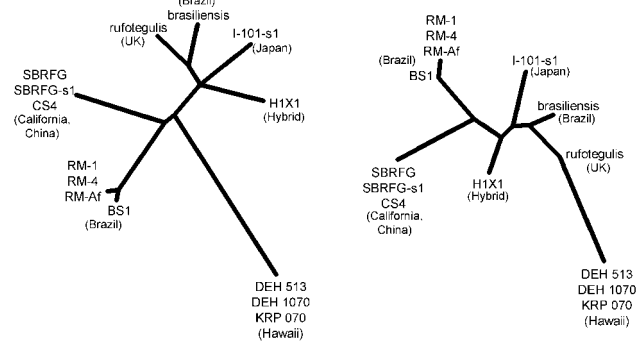


FIG. 5. UPGMA (from NEIGHBOR) (left) and FITCH (right) phenograms produced from the hand-calculated distance matrix of eight unique sequences based on Clustal W alignment file of 15 studied sequences. Sequence IDs refer to TABLES I and II.

ed), with Hawaiian isolates having the highest divergence scores.

In the FITCH and UPGMA trees derived from the distance matrix, Brazilian sequences always were interspersed with those from other regions (FIG. 5). No phylogenetic signals associated either with geographical regions (other than Hawaii) or with recently proposed taxa were evident. In contrast, in an MP analysis of *A. subrufescens* sequences within *Agaricus*, particularly within section *Arvenses*, all *A. subrufescens* sequences (including several deposited in GenBank as ‘*A. blazei*’) formed a monophyletic unit within *Arvenses* (FIG. 6). Bootstrap values for the *A. subrufescens* clade and the *Arvenses* sectional clade were both 100%. Within *A. subrufescens*, a clade formed by the three identical Hawaiian sequences received 88% bootstrap support. Taken together results from both distance and parsimony analyses indicate the presence of a single phylogenetic species.

*Study of the type of A. subrufescens.*—The material of the holotype is now more fragmented than it appeared to be in the photograph of Didukh et al (2003). Referring to their photograph, I was able to segregate and label specimens and fragments assigned by Didukh et al as “type A” and “type B”, with an additional upper stipe fragment now assigned to element B. Element A comprises two basidiomata, in agreement with Falconer’s letter to Peck (21 Oct 1892 [NYS]), which are relatively gracile and have dark brown pileus pigment. Lamellae of these basidiomata are fused and their cellular structure is collapsed and indistinct. Element B comprises four basidiomata, based on stipe apices, not two as reported in Didukh et al (2003), in fragments. The B specimen fragments are in better condition and have a slightly more robust aspect and less pronounced pileus pigmentation, relative to the A specimens. Spores of the

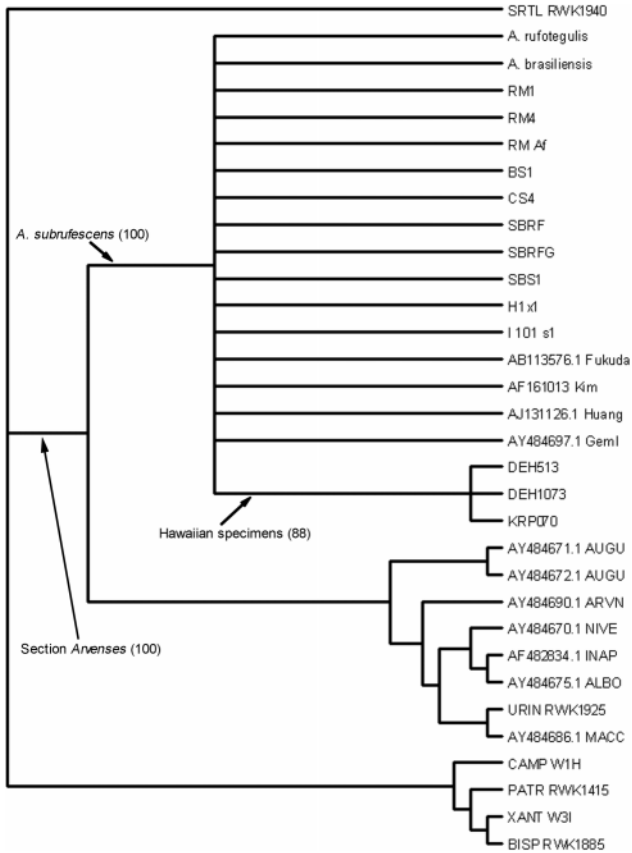


FIG. 6. Strict consensus tree of 22 919 most parsimonious trees (out of first 100 000 random addition replicates; length = 227 steps). Clades of interest and bootstrap values (from 1000 replicates) are indicated. Taxon abbreviations: AUGU = *A. augustus*, ARVN = *A. arvensis*, NIVE = *A. nivescens*, INAP = *A. inapertus*, ALBO = *A. albolutescens*, URIN = *A. urinascens*, MACC = *A. macrocarpus*, CAMP = *A. campestris*, PATR = *A. pattersonae*, XANT = *A. xanthodermus*, BISP = *A. bisporus*, SRTL = *A. subrutilescens*.

B element are larger, on average, than those of A (TABLE III), as reported by Didukh et al (2003). However, in both elements the spore lengths span similarly broad ranges (5.1–7.5 or –8.3  $\mu\text{m}$ ). The distribution of lengths in element A is clearly bimodal, and it appears to be bimodal or multimodal in element B (FIG. 7). Element B has a greater proportion of large spores (ca. 50%) relative to A ( $\leq 10\%$ ). Developing spores were observed on the hymenium of the B element; while spore tetrads were most common; triads, diads and occasional monads also were observed at progressively lower frequencies. Cheilocystidia could not be observed in element A, as expected given the condition of the lamellae. Irregular to semiglobose, catenulate cheilocystidia ca. 7.5–13.5  $\mu\text{m}$  broad were observed in element B. Inflated elongate cells and occasional subglobose elements 10–26  $\mu\text{m}$  broad were observed among narrower hyphae of

the pileal disc of element B. No velar material could be identified with certainty in any part of the holotype. One loose lamellar fragment that fell from one of the basidiomata of element A had a spore size distribution like that of element B, indicating at least minor cross-contamination of the two formerly mixed elements due to the large number of loose fragments present.

#### DISCUSSION

This study demonstrates interfertility between North American *A. subrufescens* and a “medicinal *Agaricus*”, an isolate made from cultivated Japanese material but believed to be of Brazilian origin, enabling the production of a first interpopulational hybrid generation. The presence in hybrids of genetic material from the two progenitors, and of novel phenotypes, was documented. This indicates that members of these geographically distant mushroom populations might constitute a single “biological species”. The reproductive system is believed, based on a concordance of early data, to be amphithallic with heterothallic and pseudohomothallic components.

The data presented here further show that the DNA sequences of the ITS1+2 regions of geographically diverse members of this species are very similar. Three Hawaiian samples had no heteromorphisms but three otherwise unique polymorphisms, while one UK sample also had no heteromorphisms. Heteromorphisms always are present and frequent in the North and South American isolates and specimens studied to date. These data suggest an interpretation, developed below, based on the possibility of interpopulational hybridization; this hypothesis is speculative but useful in providing guidance for further studies.

No geographical population (with the exception of the Hawaiian samples) could be distinguished uniquely by sequence characters; the data provide no justification for recognizing distinct North American, South American or European taxa (some heteromorphisms were unique to the California + “Chinese” samples, but because only a single genotype might be represented, the significance of those characters cannot be assessed). FITCH and UPGMA phenograms derived from the distance matrix showed American, Brazilian and European sequences intermingled, while the Hawaiian sequences formed a distinct branch (FIG. 5).

With respect to Hawaii, the question of how levels of sequence divergence relate to taxonomic rank-relationships arises. Some rough benchmarks for taxonomic rank relationships in *Agaricus*, appropriate to various degrees of ITS1+2 sequence divergence, are beginning to emerge. Two varieties of *A. bisporus*,

TABLE III. Comparison of spore sizes reported for *A. subrufescens* and synonymized taxa

Taxon	Material	Reference	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )
<i>A. subrufescens</i>	Type [NYS]	Peck 1893	6.1–7.1 <sup>1</sup>	4.1–5.1
<i>A. subrufescens</i>	Type [NYS]	Miller (notes)	6.0–8.4	4.1–5.5
<i>A. subrufescens</i>	Type [NYS] (element A)	Wasser et al 2002	(5–) 5.5– <u>6.4</u> <sup>2</sup> –6.6 (–7)	4.0– <u>4.6</u> –5.0 (–5.5)
<i>A. subrufescens</i>	Type [NYS] (element A) from stipe	Kerrigan (notes)	(5.2–) <u>6.0</u> –6.1 (–6.6)	(4.0–) <u>4.3</u> (–4.6–4.9)
<i>A. subrufescens</i>	Type [NYS] (element A) from lamella	Kerrigan (notes)	(5.1–) <u>5.9</u> –6.0 (–6.2–8.3)	(4.0–) 4.2– <u>4.3</u> –4.4 (–4.9–5.2)
<i>A. subrufescens</i>	Type [NYS] (element B)	Wasser et al 2002	6.5– <u>7.2</u> –8.0	4.5– <u>4.8</u> –5.2
<i>A. subrufescens</i>	Type [NYS] (element B) from stipe	Kerrigan (notes)	(5.1–) 6.2– <u>6.5</u> –6.9 (–7.5)	(4.0–) 4.6– <u>4.7</u> (–5.4)
<i>A. subrufescens</i>	Burnham 4 Sep 1911 [NYS]	Kerrigan (notes)	<u>6.9</u>	<u>4.4</u>
<i>A. subrufescens</i>	Burnham Aug 1911 [NYS] <sup>3</sup>	Kerrigan (notes)	<u>5.6</u>	<u>4.4</u>
<i>A. subrufescens</i>	Rives, Wash. D.C. [NYS] <sup>3</sup>	Kerrigan (notes)	<u>7.3</u>	<u>4.8</u>
<i>A. subrufescens</i>	RWK 1185 [SFSU]	Kerrigan 1982, 1986	(5.3–5.6–) <u>6.1</u> (–6.8–7.5)	(4.1–) <u>4.4</u> (–4.5–4.9)
<i>A. subrufescens</i>	RWK 1185 [SFSU] (young)	Kerrigan 1982	(4.9–) <u>5.6</u> (–6.4)	(4.1–) <u>4.5</u> (–4.9)
<i>A. subrufescens</i>	Kauffman Aug 1912 [MICH]	Kerrigan (notes)	6.4– <u>6.5</u>	<u>4.4</u> –4.5
<i>A. subrufescens</i>	Smith 6789 [MICH]	Kerrigan (notes)	<u>7.4</u> –7.5	<u>4.6</u> –4.7
<i>A. subrufescens</i>	[N=12; Hawaii]	Peterson et al 2000	4.5– <u>5.4</u> – <u>6.2</u> –7.2	3.0– <u>4.0</u> – <u>4.6</u> –5.2
<i>A. subrufescens</i>	DEH 513 [SFSU]	Kerrigan (notes)	<u>5.7</u>	<u>4.2</u>
<i>A. subrufescens</i>	DEH 1073 [SFSU]	Kerrigan (notes)	<u>5.7</u>	<u>4.5</u>
<i>A. subrufescens</i>	KRP 070 [SFSU]	Kerrigan (notes)	<u>5.8</u>	<u>4.3</u>
<i>A. subrufescens</i>	[Sterling 226 [NYS]] <sup>4</sup>			
<i>A. brasiliensis</i>	Isoype (HAI 0978)	Wasser et al 2002	5.6–7	3.8–4.6
<i>A. brasiliensis</i>	Isoype (HAI 0978)	Kerrigan (notes)	(5.5–) <u>6.1</u> – <u>6.2</u> (–6.9)	(4.0–) <u>4.3</u> (–4.8)
<i>A. rufotegulis</i>	Type, others [L]	Nauta 1999	(5.0–) <u>5.7</u> – <u>6.1</u> (–6.6)	(3.6–) <u>4.0</u> – <u>4.6</u> (–5.1)
<i>A. rufotegulis</i>	0341732 [L]	Kerrigan (notes)	(5.4–) <u>6.0</u> (–6.9)	(4.1–) <u>4.4</u> – <u>4.5</u> (–4.9)
<i>A. rufotegulis</i>	21737 [WU]	Hausknecht 2002	(5.2–) <u>5.6</u> (–6.4)	(4.0–) <u>4.2</u> (–4.6)
<i>A. blazei</i> Murrill sensu Heinemann	Hongo 5766 [BR]	Heinemann 1993	<u>5.76</u>	<u>4.41</u>
<i>A. blazei</i> Murrill sensu Heinemann	Hongo 5883 [BR]	Heinemann 1993	<u>5.69</u>	<u>4.50</u>
<i>A. blazei</i> Murrill sensu Heinemann	Both collections	Heinemann 1993	(4.9–) <u>5.72</u> (–6.4)	(4.0–) <u>4.45</u> (–4.7)

<sup>1</sup> Peck's data were converted from English to Metric measurements.

<sup>2</sup> Underscores indicate averaged data.

<sup>3</sup> Boxes or box lids of these two specimens may have been switched at an earlier date. The Rives specimens were figured by Peck.

<sup>4</sup> Sterling's notes describe 'slight reddish' to 'blood red' discolorations of the pileus and stipe context, respectively, implying that collection 226 belongs to a different taxon.

distinguished by morphological characters and reproductive behaviors, each exhibited single unique sequence character differences from var. *bisporus* (Callac et al 1993, 2003). An alpine relative of *A. devoniensis* P.D. Orton that has two unique sequence characters currently is considered to be a subspecies (Challen et al 2003, Kerrigan unpublished). The homothallic "highland" and "lowland" entities (Kerrigan et al 1999) of *A. subfloccosus* (J. Lange) Hlaváček s. l., arguably either subspecies or sister species, differ at only one position in the ITS1+2 sequence (Chal-

len et al 2003, Kerrigan unpublished). Other "sister species" have greater ITS1+2 divergence (e.g., about 1.4–1.9% in *A. bisporus* versus *A. subfloccosus*, which corresponds to about 10–14 character differences. However some species in section *Xanthodermatei* Sing. might differ by smaller numbers of ITS1+2 sequence characters (Kerrigan et al unpublished, Callac et al unpublished) By comparison with these observations any taxon that might be proposed for the Hawaiian *A. subrufescens* could be argued to deserve either infraspecific or specific rank, presumably de-

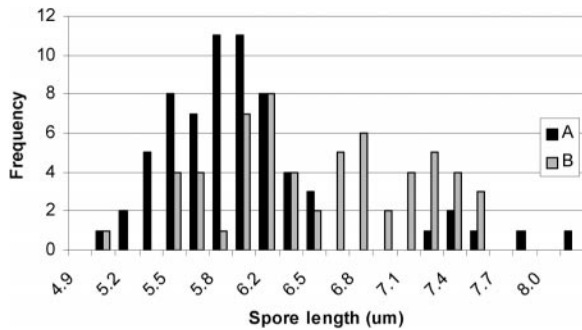


FIG. 7. Histogram of frequency distribution of spore lengths from A and B elements of the type of *A. subrufescens*.

pending upon other observations that hopefully would include interfertility data.

*Phylogeographic structure and dispersal.*—The distribution of *A. subrufescens* is documented only patchily, because the species appears not to have been well recognized in some regions (Europe, South America). However, it appears to have a tropical or subtropical to temperate zone distribution. *Agaricus subrufescens* is the only species of *Agaricus* known to be harmed or killed by prolonged exposure to temperatures of ca. 4 C or lower (Kerrigan unpublished, cf. *A. brasiliensis* in Wasser et al 2002). Wasser et al understandably were unaware of this trait of *A. subrufescens* when they attempted to use it to distinguish *A. subrufescens* from *A. brasiliensis*. It is also remarkably tolerant of high temperatures (Kerrigan 1983a). It is unusually versatile nutritionally, having been observed to have spontaneously colonized a bag of wet sawdust on one occasion (Kerrigan unpublished). The most “natural” habitat reported for the species is piles of leaves.

The Hawaiian Islands are geographically isolated.

Relatively more sequence divergence might be expected within small island populations (Johnson and Seger 2001). The UK is less isolated but might be near the limit of the natural range of the species in the Eastern Hemisphere, which currently remains unclear. The samples from these localities might represent “pure” members of relatively isolated or marginal populations. Their lack of sequence heteromorphism is consistent with this theory. Conversely, samples from Brazil (and Japan by extension) and California are highly heteromorphic. Some sequences in GenBank (e.g., AB113576.1) also exhibit this characteristic. The Americas samples might represent examples of natural or accidental hybrids between formerly “pure” populations, assuming that the dual peaks and sequence length differences arise from allelic variations at the haplotype level (which is supported by the implicit allelic segregation needed to produce the H1×1 sequence) (TABLE II). The observation that heteromorphisms including length polymorphisms are relatively uncommon among species of section *Duploannulati* Wasser emend. Challen et al (2003) supports this hypothesis (TABLE IV). *A. devoniensis* is the exception to this pattern, appearing to exhibit diverse aspects of recent population level hybridization (Callac et al unpublished).

To confirm the potentially allelic nature of the observed heteromorphisms it will be necessary to isolate haplotypes from SSI or deheterokaryotized cultures or to clone PCR products amplified from heterokaryotic genomic DNA. Once haploid sequences are obtained, and if the heteromorphisms are allelic rather than interrepeat variants, it should be possible to construct gene genealogies that might provide better insight into the hypothesized hybridization among populations. This will be a goal of future studies.

Contemporary documents show that spawn of *A.*

TABLE IV. Intraspecific ITS1+2 sequence variation in selected species of *Agaricus*

Species	Samples/sequences	Variable sites	Character heteromorphic sites	Length heteromorphic sites	% affected samples (w/LHS)
<i>A. bisporus</i> var. <i>bisporus</i>	6	4	4	0	0
<i>A. subfloccosus</i> s. l.	2	0 <sup>1</sup>	0	0	0
<i>A. devoniensis</i> s. str.	5	9	2	1	14
<i>A. bitorquis</i>	2	1	0	0	0
<i>A. “vaporarius”</i> <sup>2</sup>	3	0	0	0	0
<i>A. cupressicola</i>	2	0	0	0	0
<i>A. bernardii</i>	5	0	0	0	0
<i>A. subrufescens</i>	12	19	14	2	50

<sup>1</sup> The polymorphism associated with highland vs lowland entities is ignored here.

<sup>2</sup> The name *A. vaporarius* (Persoon) Cappelli, used by Challen et al. (2003), is illegitimate. Some authors regard *A. subperonatus* (J. E. Lange) Singer to be conspecific; if true, that is the correct name of the species. *Agaricus cappellianus* Hlaváček is a homotypic synonym of Cappelli’s name.

*subrufescens* was being sold commercially, sometimes as '*A. fabaceus*', about a century ago (Falconer 1894a, b; Anonymous 1909). At that time the two species names were considered to be synonymous by B.M. Duggar, a USDA BPI collaborator involved with the developing mushroom spawn industry in the USA (Duggar 1905, 1920). *A. fabaceus* is an older American name dating from 1847. It was described having a pileus that was white becoming yellow, and viscid when moist. It appears unlikely to be the same species as *A. subrufescens*. The type of *A. fabaceus* is at Kew. Spawn distribution is a documented route for geographically broad dispersal of *Agaricus* germ plasm into the environment (Kerrigan et al 1999). In addition, any organism that can use composted manure and wood as a substrate, and is thermotolerant, potentially could cross oceans on a traditional wooden sailing ship. When the vigor and amphithallic reproductive versatility (including single-spore fertility) of *A. subrufescens* is factored in, dispersal, establishment and gene flow in this species is not surprising.

A quote from a Boston Mycological Club bulletin (Anonymous 1904) illustrates the reproductive potential of this species: "Its spontaneous appearance in various greenhouses in widely separate localities has brought it to notice at various times because it forced itself so persistently and abundantly on the owners that they have sent in specimens to the Club exhibitions in order to learn whether the visitation was to be regarded as a curse or a blessing. . . . When it appears it comes with a rush, having previously, with its strong growing, strand like mycelium, taken possession of some rich portion of soil, and then it sends its abundant fruits to the light, undaunted by any overlying difficulties."

In Santa Barbara, California, I encountered *A. subrufescens* growing on a lawn about 200 m from a laboratory where I had cultivated it the previous year (Kerrigan and Ross 1987a). The possibility that this represented an escape from cultivation was not contradicted by allozyme data I obtained from both isolates (Kerrigan unpublished). This was the second and only other time I ever encountered the species in nature.

*Morphology of basidiomata.*—Although the present study did not primarily emphasize morphological criteria, a discussion of some points is in order. The basidiomatal morphology of *A. subrufescens* is somewhat variable. This would not be surprising in a situation where populations had diverged in isolation and were now interbreeding. Sporocarps can be robust or gracile, both due to genotype (see above) and environmental influences (Kerrigan unpublished).

Cuticle pigmentation and background yellowing

are also variable (the degree of analogous reddening of the tissues of *A. bisporus* is under genetic control and both traits are managed actively in mushroom breeding programs [Kerrigan 2004, unpublished]). Although Peck noted that the lamellae could be yellowish-white, he reported the flesh to be "white, unchangeable". Wasser et al have emphasized this as a point of distinction between *A. subrufescens* and *A. brasiliensis*. However, this variation does occur naturally within member species of section *Arvenses* (Kerrigan 1982, unpublished). Furthermore, the specimens Peck received had traveled more than 300 km (the straight-line distance between Glen Cove and Albany) in 1892 before he saw them and did not arrive in the best condition—"Dear Sir: I am glad you rec'd the mushrooms, but sorry they were so long delayed and in such poor condition. I sent them to Dr L(-) to forward to you as I did not know your proper address." (Falconer to Peck, 21 Oct 1892 [see also 15 Oct 1892] [NYS]). Even the second shipment apparently suffered. Peck (1897) wrote that "From some of these specimens kindly sent me by the discoverer the original description was derived, but the specimens were not in satisfactory condition to figure." Kauffman (1918) wrote that "The original description was made from old material. . . . Peck's description of this species is, therefore, misleading." Color changes in *Agaricus* diminish in time after harvest as well as with age and deteriorating condition of basidiomata. Finally, the man who obtained and sent the type material of *A. subrufescens* to Peck, Mr W. Falconer (editor of the journal "Gardening" and subsequent author of the 1897 USDA Farmer's Bulletin 53, "How To Grow Mushrooms"), reported that the species had a "lemon-tinted neck", indicating that Peck's description was at least incomplete (Falconer 1894a).

In my experience the cap shape also is variable, ranging from narrowly convex through cuboidal to broadly convex. However, two characters unusual in other species may sometimes be present: (i) a transient concave shoulder or bell-shape to the pileus as expansion in young pilei begins first near the margin; (ii) radially oriented folds or "pleats" of the pileus. Both features are illustrated in Kerrigan (1986). Falconer even included a tiny sketch of a pleated pileus in his first letter to Peck (15 Oct 1892). The elastic veil, which usually remains attached to the pileus margin during expansion, stretching while the lower layer breaks up into a large number of small cottony floccules, is a fairly consistent feature of the species.

*Agaricus subrufescens* is a widely illustrated species. The range of gross morphologies can be seen in these references: Peck 1897; Anonymous 1904; Anonymous 1909; Duggar 1905, 1920 (Pl. VI); Kauffman

1918; Tu and Lin 1981; Kerrigan 1983b, 1984, 1986; Nauta 1999; Hausknecht 2002; Wasser et al 2002; Peterson et al 2000. In its heyday the mushroom was described as “uncouth looking” (Falconer 1894a). The figure and description furnished by Hotson and Stuntz (1938) probably refer to *A. augustus* Fr.

Microscopic features have been described by Kerrigan (1982, 1986), Heinemann (1993), Nauta (1999), Peterson et al (2000), Hausknecht (2002) and Wasser et al (2002), among others. The type of *A. subrufescens* has been examined and described by Freeman (1979), Wasser et al (2002) and by Smith (1940), who could not “add any information to that found in Kauffman’s account.” Some authors, particularly Wasser et al, have emphasized points of distinction among the entities they recognize. Spore size variability deserves some discussion. Heinemann (1993) found the sizes of spores to be heterogeneous within single collections of cultivated Brazilian stock. He suspected the presence of bisporic basidia among the tetrasporic ones but was unable to verify this. Kerrigan and Ross (1987a, b) did document the presence of dynamically varying numbers of bi-, tri- and tetrasporic basidia in field and cultivated specimens of *A. subrufescens*, leading to an expectation of variation among spore size measurements. Kerrigan (1982, see *A. subrufescens* and *A. augustus*) also presented data on variation in spore size from single sporocarps at different developmental stages. Occasional North American collections of *A. subrufescens* have been observed to have relatively longer spores (e.g., Smith 6789 [MICH], Rives, Washington D.C. [NYS] (TABLE III); the latter specimens were illustrated by Peck [1897]). We should expect considerable variation in spore lengths within and among collections of *A. subrufescens*, given the bimodality or multimodality observed in the distributions of spore sizes in the two elements of the holotype and the known dynamic variability in proportions of n-spored basidia that results from temperature shifts or other diurnal cues.

Cheilocystidia in *A. subrufescens* are usually reported to be variable: cylindrical to clavate or swollen, with few to many catenulate-globose cells (Kerrigan 1982, Heinemann 1993, Nauta 1999, Wasser et al 2002, Hausknecht 2002). However, Wasser et al (2002) did not observe cheilocystidia in element A of the type of *A. subrufescens*. Freeman (1979) also reported cheilocystidia to be absent in the type. By contrast, Kauffman’s (1918) description of Michigan collections noted numerous subcylindrical sterile cells on edges of gills, and Peck authenticated at least some of Kauffman’s *A. subrufescens* material. Typical cheilocystidia are present in element B of the holotype. Unfortunately, given the condition of the ele-

ment A type specimens (discussed above), it is unlikely that cheilocystidia will be observed therein.

Two observations on this species argue against overemphasizing morphological features evaluated from dried specimens. First, observations and experience show that morphology within the interfertile group is rather diverse because of underlying genetic variation and is additionally plastic in response to environmental influences. Second, this is perhaps the easiest species of *Agaricus* to cultivate and, as shown above, is amenable to outcrossing. There are compelling advantages to studying the expression of morphological traits in living material under standard (and possibly nonstandard) conditions and to investigating trait expression when genomes from putatively distinct taxonomic entities share a common cytoplasm. For example, distinctive cells of the veils and/or cuticle of this species have been discussed by Nauta (1999), Heinemann (1993), and Wasser et al (2002). I have observed that SBRFG has sausage-like chains of cells in the universal veil of the annulus, but not in velar patches on the pileus. Similar cells have been observed in *A. subrufescens* from eastern North America (O.K. Miller personal communication). The best way to assess the taxonomic and/or phylogenetic significance of any differences in velar cells among populations would be to study expression of the trait in hybrids and their offspring.

If, as in studies of some other basidiomycetes, barriers to gene flow were discovered within the *A. subrufescens* phylogenetic unit, that might indicate that a more elaborate taxonomic arrangement could be appropriate. For these reasons living cultures from European, Hawaiian and other populations will be of great interest to future studies.

*Other notable reports.*—*Agaricus subrufescens* first was reported from Brazil by J. Rick (1930). However, Rick gave the spore size of material from São Leopoldo as  $5 \times 3 \mu\text{m}$ , so Peck’s name is unlikely to apply to these specimens. Later, Rick (1939) suggested the existence of a ‘varietas microspora’ with spores  $3 \times 2 \mu\text{m}$ ; however this is not a validly published name and is unlikely to be phylogenetically congruent with the species.

In the photographic collection of the late W.F. Isaacs, one transparency of *A. subrufescens* is labeled “from Argentine”. The slide, which shows the mushrooms growing indoors in wooden boxes, was processed in the USA in Mar 1963. This suggests that some aspects of the cultural history of *A. subrufescens* in South America remain incompletely known.

A European culture identified as *A. purpurascens* (Cooke) Pilát (*nom. illeg., non A. purpurascens* Fr.; see *A. porphyrizon* Orton 1960) was cultivated exper-

imentally at INRA Bordeaux, France (Brian et al 1981). Based on experience and on published and unpublished photographs, these mushrooms agree with *A. subrufescens* (J. Guinberteau and P. Callac personal communication). This report indicates that European strains should be amenable to cultural and hybridization studies.

*Nomenclature and typification.*—Peck's publications and correspondence from Falconer to Peck at NYS make clear that the holotype of *A. subrufescens* comprises two separate collections. Under the circumstances lectotypification of *A. subrufescens* is appropriate. Didukh et al (2003) concluded from a study of the type that two different elements were present. They also concluded that two different taxa were present; however I disagree. Elements A and B fall within the range of variation known for *A. subrufescens*. This can be seen in the spore-size data (TABLE III). Didukh et al formally designated element B to be the lectotype. Peck (1897) indicated that the description of the species was based on specimens from Falconer's "compost heap composed chiefly of decaying leaves." Based on the numbers and condition of basidiomata in the two elements of the type, Peck was referring to the B element as the basis for his concept. Although spores from the B element are long relative to Peck's description (and spores from the A element are short), both elements have spore length ranges that bracket Peck's measurement. Didukh et al have selected the best element for the lectotype of *A. subrufescens*. The A element has now been segregated and should be considered an authenticated collection of the species. The synonymy of the species is:

- Agaricus subrufescens* Peck New York State. Mus. Ann. Rep. 46:105. 1893  
 = *Psalliota subrufescens* Kauff. The Agaricaceae of Michigan 239. 1918.  
 = *Agaricus rufotegulis* Nauta Persoonia 17:230. 1999.  
 = *Agaricus brasiliensis* Wasser, Didukh, de Amazonas & Stamets. Int. J. Med. Mush. 4:274. 2002.  
 Misapplied names:  
 – *Agaricus blazei* Murrill *sensu* Heinemann Bull Jard Bot Belgium 62:365–368. 1993.

#### CONCLUSIONS

There has been a tendency to emphasize the morphological variation in *A. subrufescens* and invoke additional taxa to account for this variation. Although the species does present some morphological aspects that are recognizable with experience, sequence data can furnish a more stable character set in such a cir-

cumstance. The sequence data presented here suggest that this is a single phylogenetic unit at the species level, possibly with population level divergence (e.g., in Hawaii) that is being obscured elsewhere with recent re-integration of some populations. The breeding data show that isolates once assigned to different species can interbreed and produce offspring; the biological species concept of *A. subrufescens* therefore appears to be inclusive. The cultural behavior, including responses to high and low temperatures, also supports the shared taxonomic identity of the cultivated strains studied. In my view it is most conservative to unite these entities under the oldest unambiguously associated name, *A. subrufescens* Peck. Future studies might clarify the status of populations and entities, if any, deserving recognition, perhaps at infraspecific rank, in part through expanded interfertility studies, and might clearly document the range of macro- and micromorphologies produced under standard conditions by "wild" isolates and by hybrids among them.

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