Rept. Tottori Mycol. Inst. (菌蕈研究所研究報告) **28**: 143-150, 1990.

A revision of the taxonomic status of *Pleurotus citrinopileatus**

Ikuo OHIRA

Abstract

Morphologically, *Pleurotus citrinopileatus* is distinguishable from *P. cornucopiae* by having a yellow pileus and smaller basidiospores. However, the interfertility test between the two species showed that they were fully compatible, and the crosses between them produced normal fruitbodies with yellow pilei. Basidiospores from these fruitbodies were more or less intermediate in size between those of the parents. Based on these results, it is proposed that *P. citrinopileatus* should be reduced to varietal status under *P. cornucopiae*.

Key Words: *Pleurotus citrinopileatus, P. cornucopiae,* morphological variation, interfertility test, Tamogitake.

Introduction

Pleurotus citrinopileatus Singer, originally described by Singer (1943) from Khabarovsk, USSR, is an agaric fungus so far known only from eastern Asia, including far eastern USSR (Vassillieva, 1973; Parmasto, 1987), northern China (Tai, 1979), and Japan (Yokoyama, 1985). It is characterized by a clear yellow, infundibuliform pileus, well-developed, often branched, central stipe, and its preferable occurrence on *Ulmus* spp. In Japan, the fungus is well known as an excellent edible mushroom in the northern regions (Tohoku and Hokkaido) under the Japanese name Tamogitake. *Pleurotus citrinopileatus* is very similar to the European species *P. cornucopiae* (Paul.) Rolland in its habit and habitat, although *P. cornucopiae* has a pale creamy to pale gray-brown or pale ochre-brown pileus, a less branched stipe, and somewhat larger basidiospores (Pilāt, 1935; Romagnesii, 1969; Phillips, 1981; Hilber, 1982). Imai (1935) applied the name *P. cornucopiae* to this yellow-capped fungus, but Corner (1961), who examined the Japanese material, questioned the application of the name *P. cornucopiae* to the yellow-capped fungus in Japan, treating it as *P.* aff. *cornucopiae*. This study was initiated to clarify the taxonomic status of *P. citrinopileatus* by means of interfertility tests between *P. citrinopileatus* and *P. cornucopiae*.

* Contribution No. 244 from the Tottori Mycological Institute, Kokoge 211, Tottori, 689-11 Japan. A part of this report was presented at the 30th Annual Meeting of the Mycological Society of Japan at Aomori, June 1986.

Materials and Methods

Six strains of *P. citrinopileatus* from Japan and three strains of *P. cornucopiae* from Europe were used (Table 1). Identifications of the strains were reconfirmed by examining fruitbodies obtained by culturing these strains on sawdust - rice bran medium (5 : 1, v/v) at 20°C. The dried specimen of *P. citrinopileatus* from USSR (TAA-103774) was also examined. Monokaryotic isolates were obtained from the cultivated fruitbodies and mated in the interfertility test through the methods of Miles et al. (1966) and Ohira (1977), respectively. Dikaryosis and common-*B* heterokaryosis were judged by the occurrence of clamp connections and pseudoclamps, respectively. For scanning electron microscopy of hymenial structures, samples were prepared by the method of Nakai and Ushiyama (1974) and examined with a JEOL JSM-U3 scanning electron microscope at 15 kV. The strains used are deposited in the Tottori Mycological Institute Culture Collection.

Results

Morphological comparisons. The two species differed in color of the pileus and shape of the stipe. *Pleurotus citrinopileatus* strains had bright yellow pilei, whereas those of *P. cornucopiae* were pale brown. The stipes were branched more often in *P. citrinopileatus*. In both species, a floccose-membranous veil was observed on fruitbodies only when they were very young (Fig. 1). At first, the veil entirely covered the primordium, but disappeared as the fruitbody grew. The veilar hyphae were 5-12.5 μm in diam., smooth, hyaline, thin-walled, and clamped (Fig. 1, inset). The size of basidiospores from the cultivated fruitbodies of *P. citrinopileatus* was 6-9$\times$2-3.5 μm. These were shorter and narrower by about 1 μm on the average than those of *P. cornucopiae*, measuring 7-11$\times$3-5 μm (Table 2 and Fig. 3). The cheilocystidia had one or (rarely) two filiform, thin-walled appendages in the sterile gill-edge of *P. citrinopileatus* (Fig. 2), and also in *P.*

Table 1. Fungal strains employed

Fungus	Strain No.	Locality or source
Pleurotus citrinopileatus	TMI-30150	Hokkaido, Tokoro-gun, Saroma-cho
	TMI-30151	from Hokkaido Forest Products Research Inst.
	TMI-30152	from Hokkaido Forest Products Research Inst.
	TMI-30153	from Hokkaido Forest Products Research Inst.
	TMI-30154	from Hokkaido Forest Products Research Inst.
	TMI-30155	from Hokkaido Forest Products Research Inst.
Pleurotus cornucopiae[1)]	CCBAS-463	Moravia, Czechoslovakia
	CCBAS-464	Rkutino, Bulgaria
	CCBAS-465	Primorsko, Bulgaria

[1)] These strains were provided by Institute of Microbiology, Czekoslovak Academy of Sciences.

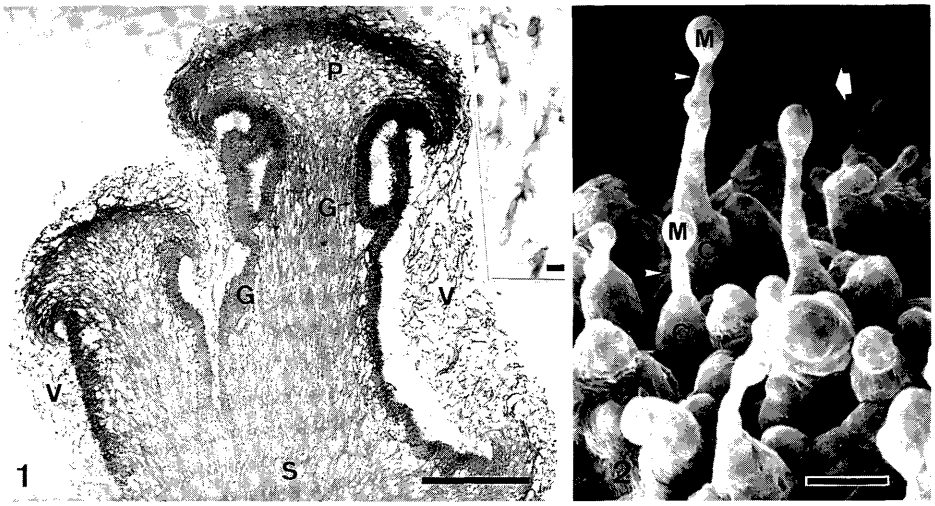

FIG. 1. Longitudinal section of fruitbodies at early stages in *Pleurotus citrinopileatus* (stained with fast green). Immature gills (G) were covered with a floccose-membranous veil (V) that was continuous from pileus (P) to stipe (S). Scale bar=0.5 mm. The inset shows veil hyphae at high magnification. Scale bar=10 μm.

FIG. 2. Scanning electron micrograph showing cheilocystidia (c) with a filiform appendage (arrow heads) bearing a mucilagenous globule (M) in the gill-edge of *Pleurotus citrinopileatus*. A basidium (arrow) with four sterigmata is also seen. Scale bar=5 μm.

Table 2. The size of basidiospores from each strain of *Pleurotus citrinopileatus* and *P. cornucopiae*

Fungus	Strain No.	Size of basidiospores (average)[1] μm
Pleurotus citrinopileatus	TMI-30150	6-9×2-3.5 (7.4×2.8)
	TMI-30151	6-9×2-3.5 (7.4×3.0)
	TMI-30152	6-9×2-3.5 (7.7×3.2)
	TMI-30153	6-9×2-3.5 (7.0×2.6)
	TMI-30154	6-9×2-3.5 (7.2×2.6)
	Total	6-9×2-3.5 (7.4×2.8)
Pleurotus cornucopiae	CCBAS-463	7-11×3-5.0 (8.5×3.7)
	CCBAS-464	7-10×3-4.5 (7.9×3.7)
	CCBAS-465	7-10×3-5.0 (8.2×4.0)
	Total	7-11×3-5.0 (8.2×3.8)

[1] 100 basidiospores per strain were measured.

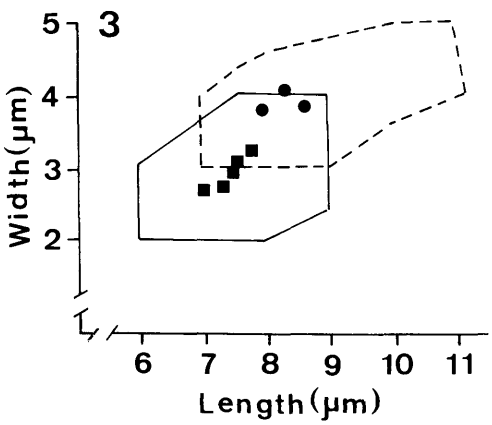

FIG. 3. Dispersion patterns of basidiospore size of *Pleurotus citrinopileatus* (full line) and *P. cornucopiae* (dotted line). The average of each strain is shown by ■ (*P. citrinopileatus*) and ● (*P. cornucopiae*).

cornucopiae.

The morphological characteristics observed agreed well with those of *P. citrinopileatus* (Singer, 1943; Vassilieva, 1973; Parmasto, 1987) and *P. cornucopiae* (Pilát, 1935; Romagnessi, 1969; Phillips, 1981; Hilber, 1982) previously described in the literature. Thus it was possible to distinguish the two taxa on the basis of the difference in the pileus color and the basidiospore size.

Interfertility tests. Both *P. citrinopileatus* and *P. cornucopiae* were tetrapolar, as shown in Tables 3 and 4. The recombination of incompatibility factor *B* was recognized in *P. cornucopiae* (Table 4), and also in *P. citrinopileatus* (data not shown). Interstrain pairings among monokaryotic isolates derived from four dikaryotic strains of *P. citrinopileatus* and three dikaryotic strains of *P. cornucopiae* were carried out (Table 5). The dikaryotic strains of *P. citrinopileatus* were compatible with each other and with those

Table 3. Results of pairings among 10 monokaryotic isolates of *Pleurotus citrinopileatus* (TMI-30150) in all possible combinations

		A1B1				*A2B2*			*A1B2*		*A2B1*
		1	5	6	7	2	8	9	3	10	4
A1B1	1	−[1]	−	−	−	+	+	+	−	−	−
	5	−	−	−	−	+	+	+	−	−	−
	6	−	−	−	−	+	+	+	−	−	−
	7	−	−	−	−	+	+	+	−	−	−
A2B2	2	+	+	+	+	−	−	−	−	−	−
	8	+	+	+	+	−	−	−	(+)	−	−
	9	+	+	+	+	−	−	−	−	−	−
A1B2	3	−	−	−	−	−	(+)	−	−	−	+
	10	−	−	−	−	−	−	−	−	−	+
A2B1	4	−	−	−	−	−	−	−	+	+	−

[1] +, bilateral dikaryosis; (+), common-*B* heterokaryosis; −, no clamps.

Table 4. Results of pairings among 12 monokaryotic isolates of *Pleurotus cornucopiae* (CCBAS-465) in all possible combinations

		A13B12		*A8 B13*		*A13B13*			*A8 B12*		*A13B*rec[1]		
		1	5	2	6	3	7	9	4	8	10	11	12
A13B12	1	−[2]	−	+	+	−	−	−	−	−	−	−	−
	5	−	−	+	+	−	−	−	(+)	(+)	−	−	−
A8 B13	2	+	+	−	−	−	−	−	−	−	+	+	+
	6	+	+	−	−	−	−	−	−	−	+	+	+
A13B13	3	−	−	−	−	−	−	−	+	+	−	−	−
	7	−	−	−	−	−	−	−	+	+	−	−	−
	9	−	−	−	−	−	−	−	+	+	−	−	−
A8 B12	4	−	(+)	−	−	+	+	+	−	−	+	+	+
	8	−	(+)	−	−	+	+	+	−	−	+	+	+
*A13B*rec	10	−	−	+	+	−	−	−	+	+	−	−	−
	11	−	−	+	+	−	−	−	+	+	−	−	−
	12	−	−	+	+	−	−	−	+	+	−	−	−

[1] Recombinant *B* factors.
[2] +, bilateral dikaryosis; (+), common-*B* heterokaryosis; −, no clamps.

of *P. cornucopiae* from Europe. Further, a common *A* factor was recognized in TMI-30153 (*P. citrinopileatus*) and CCBAS-465 (*P. cornucopiae*).

Fruitbodies produced by the crosses. The crosses between *P. citrinopileatus* and *P. cornucopiae* fruited normally on sawdust - rice bran medium at 20°C. These fruitbodies had yellow pilei like those of *P. citrinopileatus*, and the size of their basidiospores was more or less intermediate between those of the parents (Figs. 4, 5; Table 6). The average basidiospore size of the crosses was closer to that of *P. citrinopileatus*. Basidiospores of the crosses readily germinated on agar medium and formed monokaryotic mycelia. Dikaryons resulting from matings between compatible monokaryons produced fruitbodies whose pileus color was either yellow or pale brown.

Discussion and Conclusion

Morphological characters such as the color of pileus and the size of basidiospores have been considered to be significant in separating *P. citrinopileatus* from *P. cornucopiae* (Singer, 1943, 1986; Hilber, 1982). However, the strains of the two species used in this study were fully compatible with each other, and a common *A* factor was recognized in *P. citrinopileatus* (TMI-30153) and *P. cornucopiae* (CCBAS-465). Furthermore, the resulting crosses formed normal fruitbodies bearing basidiospores which were able to germinate. These results indicate that the two species are conspecific. Thus the morphological characters considered to separate the two species may be genetically controlled and have no value at specific rank. The author considers the morphological differences between the two

Table 5. Results of interstrain pairings of monokaryotic isolates from four strains of *Pleurotus citrinopileatus* and three strains of *P. cornucopiae*

Fungus				*P. citrinopileatus*								*P. cornucopiae*											
Strain No.				TMI-30155				TMI-30152				TMI-30153				CCBAS-463				CCBAS-464				CCBAS-465			
Incomp. factors				*A3 B3*	*A4 B4*	*A3 B4*	*A4 B3*	*A5 B3*	*A6 B5*	*A5 B5*	*A6 B3*	*A7 B6*	*A8 B7*	*A7 B7*		*A9 B8*	*A10B9*	*A9 B9*	*A10B8*	*A11B10*	*A12B11*	*A11B11*	*A12B10*	*A8 B12*	*A13B13*	*A8 B13*	*A13B12*
TMI-30150	*A1 B1*			+[1]	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A2 B2			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A1 B2			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A2 B1			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
TMI-30155	*A3 B3*			−	+	+	−	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A4 B4			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A3 B4			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	A4 B3			(+)	+	+	−	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
TMI-30152		*A5 B3*		+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
		A6 B5		+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
		A5 B5		+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
		A6 B3		+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
TMI-30153			*A7 B6*	+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
			A8 B7	+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	−	+	−	+
			A7 B7	+	+	+		+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
CCBAS-463	*A9 B8*															+	+	+	+	+	+	+	+	+	+	+	+
	A10B9															+	+	+	+	+	+	+	+	+	+	+	+
	A9 B9															+	+	+	+	+	+	+	+	+	+	+	+
	A10B8															+	+	+	+	+	+	+	+	+	+	+	+
CCBAS-464	*A11B10*																			+	+	+	+	+	+	+	+
	A12B11																			+	+	+	+	+	+	+	+
	A11B11																			+	+	+	+	+	+	+	+
	A12B10																			+	+	+	+	+	+	+	+

P. citrinopileatus

P. cornucopiae

[1] +, bilateral dikaryosis; (+), common-*B* heterokaryosis; −, no clamps.

taxa to be significant at the varietal rank. Because the name *P. cornucopiae* has nomenclatural priority, *P. citrinopileatus* should be treated as *P. cornucopiae* var. *citrinopileatus*. The varietal name has been published formerly by me as *P. cornucopiae* (Paul.) Roll. var. *citrinopileatus* (Sing.) Ohira (in Nagasawa, 1987).

Acknowledgments: I thank Dr. M. Semerdzieva of Institute of Microbiology, Czekoslovak Academy of Sciences for providing cultures of *P. cornucopiae*, and Dr. E. Parmasto of Institute of Zoology and Botany, Estonian SSR Academy of Sciences, USSR, for the specimen of *P. citrinopileatus*.

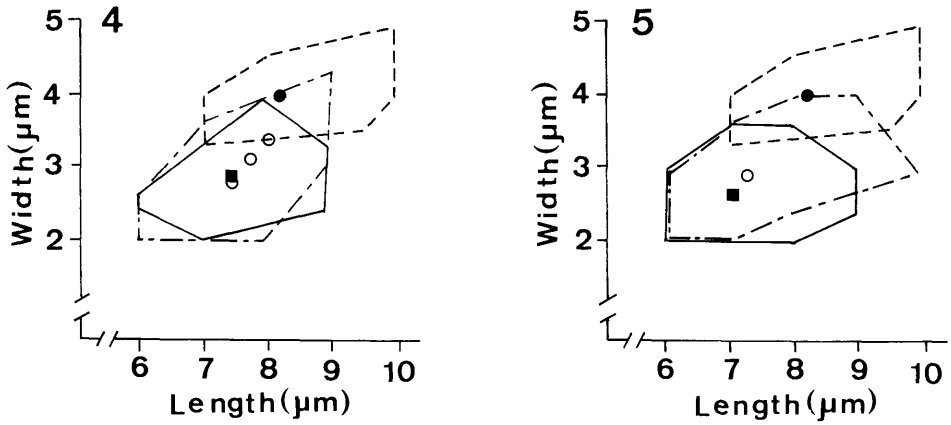

FIG. 4. Dispersion patterns of basidiospore size of TMI-30150 (*Pleurotus citrinopileatus*), CCBAS-465 (*P. cornucopiae*), and the crosses between them : full line and ■ (average)= TMI-30150 ; dotted line and ●=CCBAS-465 ; chain line and ○=crosses.

FIG. 5. Dispersion patterns of basidiospore size of TMI-30153 (*Pleurotus citrinopileatus*), CCBAS-465 (*P. cornucopiae*), and the crosses between them : full line and ■ (average)= TMI-30153 ; dotted line and ●=CCBAS-465 ; chain line and ○=cross.

Table 6. The size of basidiospores of *Pleurotus citrinopileatus*, *P. cornucopiae*, and the crosses between them

Strain No.		Size of basidiospores (average)[1] μm
Parents		
P. citrinopileatus	TMI-30150	6- 9×2-3.5 (7.4×2.8)
	TMI-30153	6- 9×2-3.5 (7.0×2.6)
P. cornucopiae	CCBAS-465	7-10×3-5.0 (8.2×4.0)
Crosses		
TMI-30150(3)×CCBAS-465(4)		6- 9×2-4.0 (7.6×3.1)
TMI-30150(3)×CCBAS-465(3)		6- 9×2-4.0 (7.4×2.8)
TMI-30150(1)×CCBAS-465(3)		6- 9×2-4.5 (7.9×3.4)
TMI-30153(2)×CCBAS-465(4)		6-10×2-4.0 (7.2×2.9)

[1] 100 basidiospores per strain were measured.

References

CORNER, E.J.H. 1981. "The Agaric Genera *Lentinus, Panus, Pleurotus*". Beih. Nova Hedwigia **69**: 1-169.

HILBER, O. 1982. "Die Gattung *Pleurotus* (Fr.) Kummer". J. Cramer, Berlin. 448 p.

IMAI, S. 1936. Tamogitake ni tsuite (on *Pleurotus cornucopiae*). Botany and Zoology **4**: 1131-1132. (in Japanese).

MILES, P.G., TAKEMARU, T. and KIMURA, K. 1966. Incompatibility factors in the natural population

of *Schizophyllum commune*. I. Analysis of the incompatibility factors present in fruit bodies collected within a small area. Bot. Mag. Tokyo **79**: 693-705.
NAGASAWA, E. 1987. Pleurotaceae. In: "Colored Illustrations of Mushrooms of Japan vol. 1", (ed. by IMAZEKI, R. and HONGO, T), pp. 24-35. Hoikusha, Osaka. (in Japanese).
NAKAI, Y. and USHIYAMA, R. 1974. Fine structure of Shiitake, *Lentinus edodes* (Berk.) Sing. I. Scanning electronmicroscopy on basidia and basidiospores. Rept. Tottori Mycol. Inst. **11**: 1-6.
OHIRA, I. 1977. *Pleurotus ostreatus* and its related species. Rept. Tottori Mycol. Inst. **15**: 29-37.
PARMASTO, E. 1987. *Pleurotus citrinopileatus*, one of the favourites. The Mycologist **1**: 106-107.
PHILLIPS, R. 1981. "Mushroom and other fungi of Great Britain and Europe". Pan Books Ltd., London. 288 p.
PILÁT, A. 1935. *Pleurotus* Fries. In "Atlas des Champignons de l'Europe. 2", (ed. by KAVINA, K. and PILÁT, A), Praha. 193 p.
ROMAGNESII, H. 1969. Sur les *Pleurotus* du groupe *ostreatus* (Ostreomyces Pilát). Bull. Soc. Mycol. **85**: 305-314.
SINGER, R. 1943. Das System der Agaricales. III. Ann. Mycol. **41**: 1-189.
SINGER, R. 1986. "The Agaricales in modern Taxonomy", 4th. ed., pp. 174-179. O. Koeltz, Koenigstein.
TAI, F.L. 1979. "Sylloge fungorum Sinicorum". Science Press, Peking. 1527 p. (in Chinese).
VASSILIEVA, L.N. 1973. "Agarikovye Shlyapochnye Griby (por. Agaricales)". Primorskogo Kraya. 331 p.
YOKOYAMA, K. 1985. "A Field Guide to Japanese Mushrooms". JTB Co., LTd., Tokyo. 223 p. (in Japanese).

摘　　要

タモギタケの分類学的検討

大　平　郁　男

東アジアに分布するタモギタケ (*Pleurotus citrinopileatus*) の分類学的検討を目的として，形態および生態的特徴において類似する *P. cornucopiae* との比較検討を行った。子実体の形態比較において，タモギタケおよび *P. cornucopiae* は米ヌカ添加木粉培地上で形成させた子実体を用いた。更にタモギタケについてはソ連産の乾燥標本を比較資料として用いた。その結果，タモギタケと *P. cornucopiae* とは傘の色および担子胞子の大きさにおいて違いが認められた。すなわち，タモギタケの傘は黄色であったが，*P. cornucopiae* は淡褐色であり，黄色の色調は認められなかった。タモギタケの担子胞子は，*P. cornucopiae* のそれに比べ長径，短径ともに約 1 μm 短かったものの，両者の担子胞子サイズの分布は一部重なった。一方，タモギタケと *P. cornucopiae* 間の交配結果は和合性を示し，更にタモギタケ (TMI-30153) と *P. cornucopiae* (CCCBAS-465) の間に共通の *A* 因子が認められた。これらの交配株は黄色い子実体を形成し，交配株の担子胞子は交配親株の中間的なサイズを示した。さらに，交配株の F_1 世代において傘の色が黄色と淡褐色に分かれたことから，傘の色は単なる遺伝形質と考えるべきである。以上の結果より，タモギタケは *P. cornucopiae* の一変種として取り扱うべきとの結論に達した。