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Morphological and molecular characterization of two novel species of *Agaricus* section *Xanthodermatei*

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Abstract: *Agaricus* specimens collected in France belong to two novel entities resembling small forms of *A. moelleri* and *A. xanthodermus,* two common species in section *Xanthodermatei*. Molecular (IT1+ITS2 DNA sequence) and morphological comparisons between eight presumed similar taxa of the section support the elevation of both entities to species rank. The new entities are described as *A. parvitigrinus* and *A. xanthodermulus*. They form a group with *A. laskibarii,* a rare species also recently described from France, and *A. californicus,* a North-American species. The well known *A. moelleri* and *A. xanthodermus* are the most related species among the studied sample. Like other species of the section, both new species have a phenolic odor and are probably toxic.

Key words: *Basidiomycetes, Agaricus,* section *Xanthodermatei,* ITS, systematics

INTRODUCTION

Within the framework of the phylogenetic analysis of the genus *Agaricus,* section *Duploannulati* Wasser ex Wasser *sensu restr.* Kerrigan, Challen et Callac had been proposed (Challen et al 2003) and an analysis of the section *Xanthodermatei* Singer is in preparation. With this aim, during the past four years, we focussed collecting in the field species of section *Xanthodermatei*. Thus we collected several times in western France specimens belonging to two entities resembling respectively *A. moelleri* Wasser (= *A. praeclaresquamosus* A.E. Freeman) and *A. xanthodermus* Genev., but much smaller. Both names represent taxa belonging to section *Xanthodermatei* and are among the most abundant species of *Agaricus* in France. The little sporophores we collected clearly are related to section *Xanthodermatei*, with their phenolic odor and yellow discoloration on handling, but the small cap diameter up to 3 or 4 cm is unusual in this section. Such specimens must have be taken for *A. moelleri* or *A. xanthodermus* or for specimens of *A. pseudopratensis* (Bohus) Wasser, which is the only small species of section *Xantodermatei* known in Europe except the tropical species *A. endoxanthus* Berk. et Br. that recently was found in Spain (Parra et al 2002) and the not well known *Psalliota nigricans* Velen. We compared morphological traits and sequences of the internal transcribed spacers (ITS1 and ITS2) of the nuclear rDNA of these small specimens with a group that included (i) three taxa morphologically similar: *A. moelleri, A. xanthodermus,* and *A. xanthodermus* var. *macrosporus* Aparici et Mahiques; (ii) the typically small sized species *A. pseudopratensis*; and (iii) two small to medium size species: *A. laskibarii* L.A. Parra et Arrillaga and *A. californicus* Peck. *Agaricus laskibarii* is a medium size species recently described from western France (Parra and Arrillaga 2002, Arrillaga Anabitarte 2004); *A. californicus* is a North American species that can be small (Peck 1895, Kerrigan 1986). Of the about 20 taxa of section *Xanthodermatei* sequenced to date that will be treated separately in a further phylogenetic analysis of the section, only *A. laskibarii* and *A. californicus* have a sequence highly similar to those of the new entities. In addition to their small to medium size, this represents another reason to include them in the present study. We conclude that our small specimens represent collections of two novel species described here as *A. parvitigrinus* and *A. xanthodermulus*.

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MATERIALS AND METHODS

Specimens and sequencing.—We used 18 specimens belonging to eight taxa of section *Xanthodermatei,* and one specimen of *A. bisporus* (J.E. Lange) Imbach (section *Duploannulati*) as outgroup. The origin of the samples included in this analysis and their GenBank accession numbers are listed (TABLE I). DNA sequences of *A. bisporus* RWK 1885 and *A. californicus* ecv 2139_CA already were available in GENBANK. The other specimens, including *A. laskibarii* LAPAG 115 and *A. xanthodermus* var. *macrosporus* MES 1577 (both provided by L.A. Parra), were examined at INRA Bordeaux. Except for LAPAG 115 (sequence provided by R.W. Kerrigan), sequences were obtained at INRA as follows: Tissue cultures were performed on compost-extract agar medium. DNA extraction was made from lyophilized mycelium or pieces of cap with the RPN8510 Nucleon Phytopure plant DNA extraction kit (Amersham Pharmacia Biotech). To

TABLE I. Origin and GenBank accession number of the 19 analyses collections

N° collection	Origin area	Data	Collector[a]	Habitat	Isolation[b]	GenBank No.
Section *Xanthodermatei*						
A. californicus						
eev 213_CA	USA, CA, Alameda Co.	08/10/2000	ECV	—	VO (USB)	AF482830
CA 155	Mexico, Tlaxcala, Cuapiaxtla	19/07/2001	PC-GM	*Cupressus benthamii*	TC	AY899265
A. laskibarii						
LAPAG 115	FR, Seignosse-40 (Isotypus)	27/10/2000	AL	Dune	VO	AY943975
A. moelleri						
CA 31	FR, Labrède-33	01/10/1990	JG	Road side	TC	AY899263
CA 156	FR, St-Selve-33	06/10/2001	JG	*Alnus, Salix, Fraxinus*	TC	AY899264
A. parvitigrinus						
CA 157	FR, Villandraut-Préchac-33	07/10/2001	JG	*Robinia, Sambucus, Euonymus*	TC	AY899266
CA 158	FR, Villandraut-Préchac-33	07/10/2001	JG	*Robinia, Sambucus, Euonymus*	TC	AY899267
CA 176	FR, Villandraut-Préchac-33	27/10/2002	JG	*Robinia, Sambucus, Euonymus*	TC	AY899268
CA 212	FR, Villandraut-Préchac-33	15/09/2003	PC-JG-RWK	*Robinia, Sambucus, Euonymus*	VO	AY899269
CA 277	FR, Villandraut-Préchac-33	21/20/2000	JG	*Robinia, Sambucus, Euonymus*	VO	—[c]
A. pseudopratensis						
CA 139	Greece, Kalabaka	18/10/2000	PC-RWK-IT	Fold	TC	AY899270
A. xanthodermus						
CA 15	FR, Lommoye-78	13/11/1989	PC	Meadow	TC	AY899271
CA 161	FR, Villenave d'Ornon-33	08/10/2001	PC	Grass, *Quercus rubra*	TC	AY899272
A. xanthodermus var. *macrosporus*						
MES 1577	Spain, Valencia	12/11/1991	RM	*Quercetea ilicis*	VO	—[c]
A. xanthodermulus						
CA 160	FR, Villandraut-Préchac-33	07/10/2001	JG	*Robinia, Sambucus, Euonymus*	TC	AY899273
CA 174	FR, Ile d'Oléron-17	21/10/2002	GD	Grass	TC	AY899274
CA 188	Fr, Le Verdon-33	09/11/2002	JG	*Quercus ilex, Pinus pinaster*	TC	AY899275
CA 204	FR, Ile d'Oléron-17	12/11/2002	GD	Grass	TC	AY899276
Section *Duploannulati*						
A. bisporus						
RWK 1885	DK, Copenhagen	01/10/1992	RWK	Soil and horse manure	TC (Sylvan)	AF432886

[a] PC, P. Callac; GD, G. Dupuy; JG, J. Guinberteau; AL, A. Leiza; GM, Gerardo Mata; RM, R. Mahiques; RWK, R. W. Kerrigan; IT, I. Theochari; ECV, E.C. Vellinga.
[b] TC = tissue culture; VO = voucher.
[c] ITS1 and ITS2 sequences were not obtained.

TABLE II. Morphological comparison between *A. parvitigrinus*, *A. xanthodermulus*, and six other taxa of section *Xanthodermatei*. Morphological traits are taken from our own observations, from *Agaricus* monographs (Cappelli 1984, Kerrigan 1986, Nauta 2001, Wasser 2002), or from the original description of the species (Genevier 1876, Peck 1895, Aparici et Mahiques 1996, Parra et Arrillaga 2002)

Taxon	Sporophore cap diameter	Spore		Pileipellis squamules
		Spore length	Spore width	
A. parvitigrinus	1.6–5 (6) cm	5.5–6.7 μm	3.4–4.4 μm	yes (dark grey)
A. xanthodermulus	1.5–6 (6) cm	6.6–7.8 μm	4.5–5.5 μm	no
A. pseudopratensis	2.5–5 (7) cm	5–7 μm	4–5 μm	yes (brown grey)/no[a]
A. californicus	3–9 (11) cm	5.1–6.4 μm	4.1–5.1 μm	no (or few)
A. laskibarii	5.5–9 cm	5.8–7 μm	4.2–5 μm	no
A. moelleri	6–12 cm	4.5–6 μm	3–4 μm	yes
A. xanthodermus	7–15 cm	5–6.5 μm	3.5–4.5 μm	no
A. xanthodermus var. *macrosporus*	8–12 cm	5–8 μm	4–5 μm	no

[a] For *A. pseudopratensis* var. *niveus* Bohus.

characterize the strains with ITS1 and ITS2 sequences of the nuclear rDNA, a single product was amplified with ITS4 and ITS5 primers (White et al 1990). PCR products were sequenced directly with big dye-terminator chemistry on ABI Prism (Applied Biosystems) DNA sequencers. The region used in comparisons was confirmed by double-stranded sequence; it started 5′-TTGAATTATG, finished 5′-YTT GAATGYT and spanned a maximum length of 655 bases that included the ITS1, the 5.8S rDNA gene and the ITS2.

Microscopy and Schaeffer reaction.—Spores, cystidia, and basidia were measured in 3% KOH by light microscopy with an ocular micrometer and 100× oil-immersion objective. The cross-marked reaction (or Schaeffer reaction) was performed on cap of dry specimens by drawing cross lines respectively with aniline and nitric acid. The reaction is positive when the point of intersection becomes orange or fire red (Cappelli 1984).

Dissimilarity analysis.—Analyses were performed with the tools available in Infobiogen (http://www.infobiogen.fr/index.html). Alignment was performed with version 1.8 of Clustal W (Higgins and Sharp 1988). The distance matrix and the phenogram were performed with the DNADIST program (with the F84 model) and the Fitch program of the PHYLIP software package (Felsenstein 1993).

RESULTS

Phenotype analysis.—Sporophore height (cap diameter smaller or larger than 5.5 cm), spore size (on average shorter or longer than 6.5 μm) and pileipellis aspect (squamules) are the most distinctive traits separating the taxa of section *Xanthodermatei* considered here, except *A. californicus* that can be intermediary. Morphological traits are reported (TABLE II). Cap diameter is not indicated in the original diagnosis (Genevier 1876) for *A. xanthodermus,* and we retained the size generally given in the literature. We must note that Wasser (2002) indicates 2–12 cm; we have never seen such small specimens of *A. xanthodermus* except those we describe here as *A. xanthodermulus*. *A. xanthodermulus* appears well characterized by its small cap diameter, long spores and the lack of squamules (TABLE II). *Agaricus parvitigrinus* is characterized by its small cap diameter and its dark gray squamules; these traits make this species easy to distinguish from all the other taxa except *A. pseudopratensis,* which differs by its brown squamules and the frequently rufescent flesh. More subtle traits must be examined to differentiate the latter two species (i.e., the sporophore silhouette which is spindly for *A. parvitigrinus* and squat for *A. pseudopratensis*).

Molecular analysis.—We obtained ITS1 and ITS2 sequences for all studied specimens except CA 277 (*A. parvitigrinus*) and MES 1577 (*A. xanthodermus* var. *macrosporus*). The four specimens of *A. parvitigrinus* collected in the same area had exactly the same sequences. The four specimens of *A. xanthodermulus* also shared identical sequences, although they were collected in sites 200 km apart. The alignment spanned 663 bases. Heteromorphic sequence sites, possibly due to heterozygosity, are uncommon but not rare in *Agaricus* sequences; among the present sample, heteromorphisms were found only at four positions in the single sequence of *A. laskibarii*. The Fitch phenogram (FIG. 2) based on the ITS1 and ITS2 DNA sequences shows that these two new species clearly differ from their two look-alike taxa (*A. moelleri* and *A. xanthodermus*). They also clearly differ from *A. pseudopratensis*. In contrast they form a group with *A. californicus* and *A. laskibarii*. *Agaricus xanthodermulus* is close to *A. laskibarii* because they have only three different bases at positions 146, 228 and 266 in the alignment. However it was possible to characterize these two species in the studied sample: *A. xanthodermulus* is characterized by a specific polymorphism at position 222 of its own sequence (tatgttTttcatt = position 228: tatgt [y]Ttt [t/-]ca[y]); and *A. laskibarii* is characterized by two specific polymorphisms respectively at position 141 of its own se-

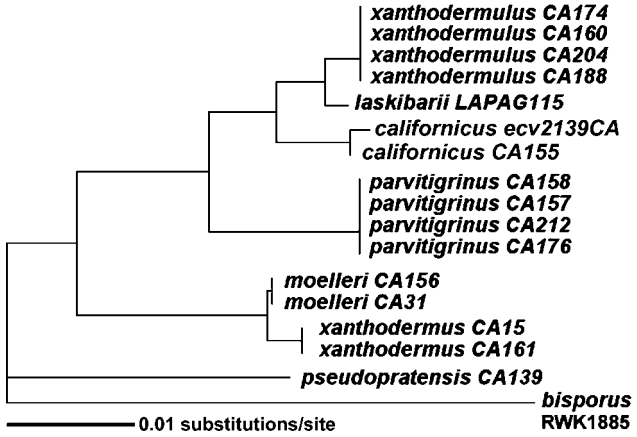


FIG. 1. Fitch phenogram for ITS1 and ITS2 sequences of 16 specimens of *Agaricus* belonging to seven species of section *Xanthodermatei,* including two new species *A. xanthodermulus* and *A. parvitigrinus,* and of one specimen of *A. bisporus* used as outgroup. The tree is unrooted.

quence (ctttcgTtggata = position 146 in the alignment: t[c/-]tt[y/-]gTtggatr) and at position 259 of its own sequence (gtctttGcatggg = position 266 in the alignment: gt[y]tttGcatggg). We must note that, at the four heteromorphic positions of the sequence of *A. laskibari* (53: R, 192: Y, 582: Y and 626: Y), the sequences of *A. xanthodermulus* are homomorphic and in all the cases have one of the two bases of *A. laskibarii* (respectively G, C, C and T). These differences between the two species do not affect the distance between them (0.0046) but modify their relative branch lengths in the Fitch phenogram. *Agaricus xanthodermulus* is also close to *A. californicus* but to a lesser extent because they differs at eight positions. We note incidentally that *A. moelleri* and *A. xanthodermus* form another group and that they differ from each other only by two bases. In conclusion, except *A. laskibarii* and *A. californicus,* which are close to *A. xanthodermulus,* the two novel species are relatively distant from all the examined taxa; they differ from each of them at least at 25 positions and from each other at 20 positions.

TAXONOMY

Agaricus parvitigrinus Guinberteau et Callac, sp. nov. FIGS. 2–4 and 8

Habitus parvus gracilisque. Pileus 1.6–5(6) cm primum convexo-trapezoides, deinde truncoconicus, tandem convexo-planus, complanatus in disco permanens, nonnumquam umbonatus. Lamellae diluto roseo, deinde theobromino griseo. Pileipellis constanter fuliginea tamquam sepia in disco, dissiliens paulatim exilibus squamulis innatis saturato griseo, in albo fundamento disseminatis. Stipes subaequalis, saepe curvus circa basim bulbosam constantem, nonnumquam abrupte; 4–6(10) cm × 3–5 mm (6–10 mm in bulbo). Annulus crassus, membranaceus descendensque, infero latere squamis ornato, a stipite facile solvitur. Cum raduntur pileus stipesque diluto flavo, deinde ferrugineo fusco colorantur. A stipite facillime pileus solvitur. Caro brevi diluto flavo fit cum secatur, nullo vestigio relicto. C6H5OH olet. Sporae cylindro-ellipsoideae (4.6–)5.5–6.7(–7.4) × 3.4–4.4(–5.2) μm. Basidia tetrasporigera vel bisporigera claviformiaque (18–25 × 7–9 μm). Cheilocystidia claviformia, forte sphaero-pedunculata (11–21 × 5–10 μm). Silvam frondosis arboribus mixtis colit in arenoso solo; in Franciae occidentali parte. Holotypus "France, 07 October 2001, CA 158" in herbario LIP depositur.

Pileus at first trapezoid-convex with the cap margin often pleated before the dehiscence, becoming truncately conical, later expanding to plano-convex with the discal part remaining flat, sometimes with low umbo; 1.6–5(6) cm diam and 4–5 mm thick; pileipellis uniformly bistre sepia at the center, gradually breaking up into appressed fibrillose squamules of less than 1 mm of width or length. These dark grayish squamules are scattered and well distinct from the white background. At maturity the center remains always bistre sepia, while the cap margin sometimes becomes completely white. On handling surface becomes yellow then ferruginous. Margin slightly exceeding the lamellae.

Lamellae free, ca. 14/cm at 1 cm from stipe, at first light pink, later grayish chocolate, margin not distinctive.

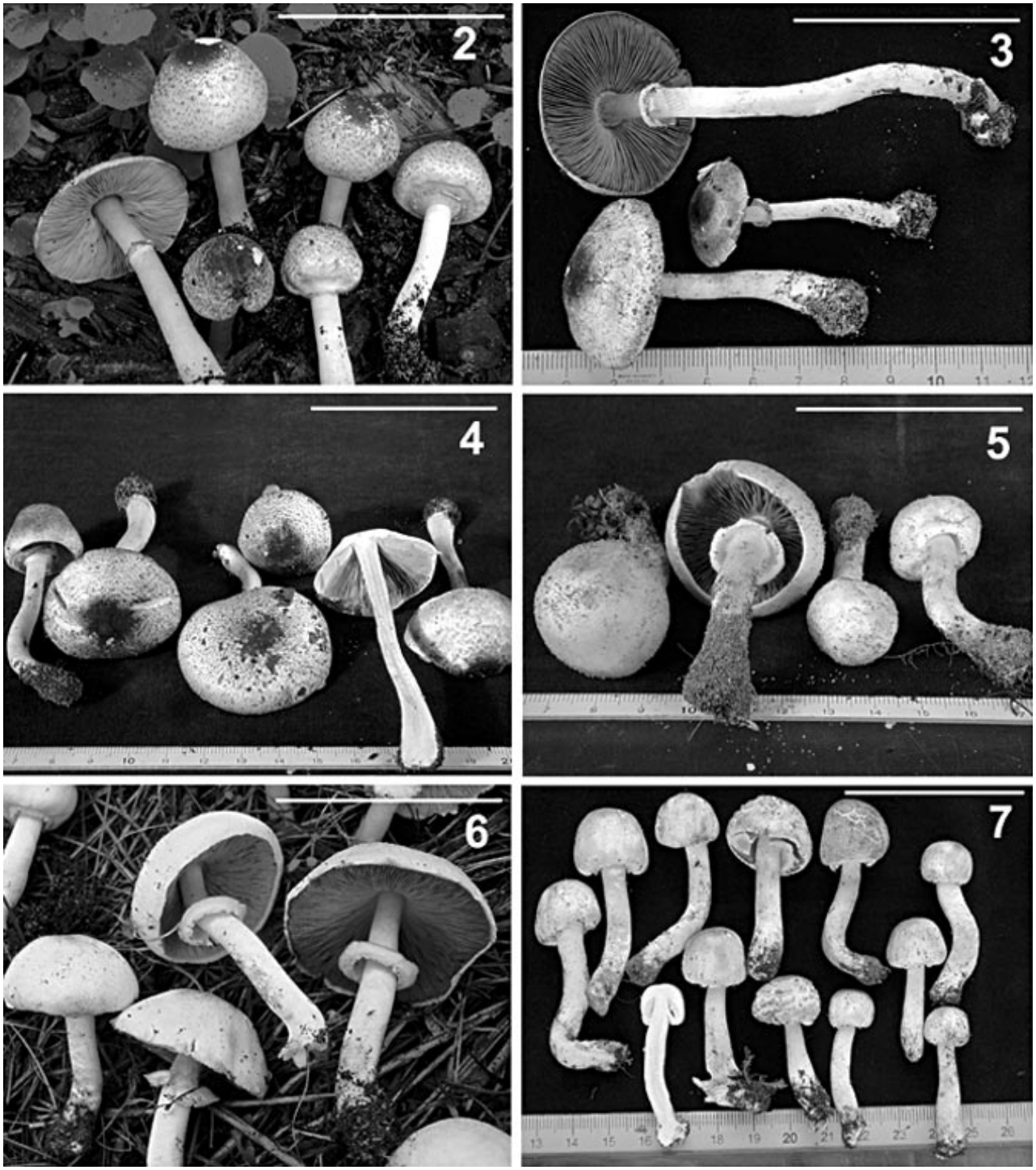
Stipe glabrous, often curved, subequal, progressively thicker toward the base that is bulbous, sometimes abruptly; 4–6(10) cm long × 3–5 mm thick (6–10 mm at the bulb); white becoming yellow then ferrugineous on handling; rhizomorphs sometimes visible.

Veils: universal veil not detected; partial veil thick, membranous, forming a pendant annulus, often with radial squamules on the underside, these occasionally arranged as a cogwheel; annulus sometimes loosened itself from the stipe.

Context white, light yellow when sectioned, fading away soon. The pileus easily breaking free from the stipe. Odor unpleasant, phenolic like ink. Schaeffer reaction negative.

Spores dark brown at maturity, cylindrical-ellipsoid, (4.6–)5.5–6.7(–7.4) × 3.4–4.4(–5.2) μm, mean = 6.0 × 3.8 μm (N = 30 × 4), L/W = 1.61; apiculus visible. *Basidia* tetrasporic (some bisporic), clavate, 18–25 × 7–9 μm; sterigmata 3 μm long. *Cheilocystidia* clavate to sphero-pedunculate, 11–21 × 5–10 μm. Presence of pileipellis hyphae entirely colored by a light brown pigment apparently parietal incrusting; dark brown vacuoles lacking.

Characteristic internal transcriber spacer polymorphism.—The four sequenced specimens of *A. parvitigrinus* (CA 157, CA 158, CA 176 and CA 212) have identical ITS1 and ITS2 sequences. *Agaricus parvitigrinus* can be distinguished from its three neighbor species, *A. laskibarii* L.A. Parra et Arrillaga, *A. cali-*



FIGS. 2–7. Sporophores. 2. *Agaricus parvitigrinus* CA 212 in the field. 3. *Agaricus parvitigrinus* CA 176. 4. *Agaricus parvitigrinus* HOLOTYPE CA 158. 5. *Agaricus xanthodermulus* HOLOTYPE CA 160. 6. *Agaricus xanthodermulus* CA 174 in the field. 7. *Agaricus xanthodermulus* CA 204. Photos J. Guinberteau (2–5 and 7) et G. Dupuy (6). Scale bars: 2–7 = 5 cm.

fornicus Peck and *A. xanthodermulus* sp. nov. (see below), by ITS polymorphisms at positions 36–38, 59, 120, 414 and 550 of its own sequence (respectively: ctggctTtCaggagc, gcctgtTtggact, ggaagcAggtcaa, cttggtGttccga and agaactAtttgcg).

Habitat, distribution, occurrence.—Isolated, gregarious or caespitose in mixed deciduous wood (*Carpinus, Robinia, Sambucus, Euonymus*) on sandy alluvial soil. Western France, extent of range unknown, probably limited.

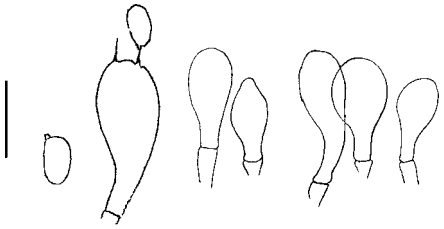


FIG. 8. Spores, basidia, and cheilocystidia of *Agaricus parvitigrinus*. Scale bar = 10 μm.

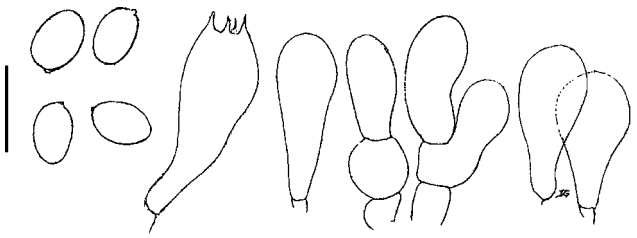


FIG. 9. Spores, basidia, and cheilocystidia of *Agaricus xanthodermulus*. Scale bar = 10 μm.

Specimens examined.—All collections from mixed deciduous wood (*Carpinus, Robinia, Sambucus, Euonymus*), FRANCE, Gironde, Villandraut-Prechac: 21 Oct 2000, CA 277; 7 Oct 2001, CA 157, GENBANK xxx; 7 Oct 2001, CA 158, HOLOTYPE (LIP), GENBANK xxx; 27 Oct 2002, CA 176, GENBANK xxx; 15 Sep 2003, CA 212 GENBANK xxx. Mycelium cultures of CA 157, CA158 and CA 176 are available at INRA Bordeaux. All specimens were collected by J. Guinberteau; specimen CA 212 was collected with P. Callac and R.W. Kerrigan.

Etymology.—The epithet '*parvitigrinus*' is a contraction of '*parvus*' and '*tigrinus*' that respectively refers to the small size of the sporophore and to the spotted aspect of the cap with colored squamules scattered on a white background, as in the case of *Lentinus tigrinus*.

Agaricus xanthodermulus Callac et Guinberteau, sp. nov. FIGS. 5–7 and 9

Habitus parvus gracilisque. Pileus 1.5–5(6) cm primum cuboideus, deinde truncoconicus, forte conico-convexus, tarde inaequaliterque patescens, tandem convexo-planus, complanatus in disco permanens, nonnumquam umbonatus. Lamellae diluto roseo, deinde theobromino griseo. Pileipellis levis vel subglabra, constanter alba vel grisea, propter circumstantia; forte brunneo-grisea a medio ad oram fit, squamis dissilit, vel radiis scinditur, margine excedente. Stipes glaber, tuberosus, politus flexuosusque, saepe curvus circa basim, subaequalis cum basi bulbosa, nonnumquam abrupte; 3–7(10) cm × 3–7 mm (6–10 mm in bulbo). Annulus crassus, membranaceus descendensque, infero latere squamis nonnumquam ornato. Cum raduntur pileus stipesque diluto flavo colorantur. Caro brevi diluto flavo fit cum secatur, nullo vestigio relicto. C6H5OH olet. Sporae ellipsoideae, forte oblongae, (5.7–)6.6–7.8(–8.6) × (4.0)4.5–5.5(–6.3) μm. Basidia tetrasporigera claviformiaque 18–27 × 6–8 μm. Cheilocystidia claviformia, forte sphaero-pedunculata 12–20 × 7–11 μm. Sub frondosis coniferisque arboribus, vel per gramen; in arenoso solo; in Franciae occidentali parte. Holotypus "France, 07 October 2001, CA 160" in herbario LIP depositur.

Pileus at first cuboidal, helmet-shaped, becoming truncately conical to conico-convex, opening slowly and irregularly, finally expanding to plano-convex remaining plane on the disc, sometimes with a low umbo; 1.5–5(6) cm diam, 4–5 mm thick; pileipellis smooth to subglabrous, uniformly white or gray, sensitive to the environment: often discoloring in grayish brown progressively from the top to the edge, sometimes breaking up into squamules and/or radially fissured. On handling surface becomes yellow. Margin exceeding the lamellae.

Lamellae free, ca 17/cm at 1 cm from stipe, at first light pink, later grayish chocolate, margin not distinctive.

Stipe glabrous, bumpy, satiny, often flexuous and curved at the base, subequal, progressively thicker toward the bulbous base, sometimes abruptly; 3–7(10) cm long × 3–7 mm thick (6–10 mm at the bulb); white, becoming yellow on handling; rhizomorphs sometimes visible.

Veils: universal veil not detected; partial veil thick, membranous, forming a pendant annulus, sometimes with radial squamules on the underside, these occasionally arranged as a cogwheel.

Context white, light yellow, more strongly so in the bulb when sectioned, soon fading. Odor unpleasant, phenolic, like ink. Schaeffer reaction negative.

Spores dark brown at maturity, ellipsoid to oblong, (5.7–)6.6–7.8(–8.6) × (4.0)4.5–5.5(–6.3) μm, mean = 7.3 × 5.0 μm (N = 30 × 4), L/W = 1.46; apiculus visible. *Basidia* tetrasporic, cavate, 18–27 × 6–8 μm; sterigmata 2.5 μm long. *Cheilocystidia* infrequent, clavate to spheropedunculate, sometimes in chains, 12–20 × 7–11 μm.

Characteristic internal transcriber spacer polymorphism.—The four examined specimens of *A. xanthodermulus* (CA 160, CA 174, CA 188 and CA 204) have identical ITS1 and ITS2 sequences. *Agaricus xanthodermulus* can be distinguished from the three neighbor species, *A. laskibarii* L.A. Parra et Arrillaga, *A. californicus* Peck, and *A. parvitigrinus* sp. nov. (see above), by the characteristic ITS polymorphism tatgttTttcatt at position 222 of its own sequence.

Habitat, distribution, occurrence.—Isolated to gregarious in mixed deciduous wood, in coniferous wood or in rich grassland, on sandy soil. Western France, extent of range unknown, possibly relatively abundant. *Specimens examined*.—FRANCE, Gironde, Villandraut-Pre-

chac, mixed deciduous wood (*Carpinus, Robinia, Sambucus, Euonymus*), 7 Oct 2001, CA 160, *J. Guinberteau,* HOLOTYPE (LIP), GENBANK xxx; FRANCE, Charente Maritime, Oléron Island, rich grassland, 21 Oct 2002, CA 174, *G. Dupuy,* GENBANK xxx; FRANCE, Gironde, Le Verdon, under *Pinus pinaster* and *Quercus ilex,* 9 Nov 2002, CA 188, *J. Guinberteau,* GENBANK xxx; FRANCE, Charente Maritime, Oléron Island, rich grassland, 12 Nov 2002, CA 204, *G. Dupuy,* GENBANK xxx. Mycelium cultures of the four collection are available at INRA Bordeaux.

Etymology.—The '*xanthodermulus*' epithet means 'small *xanthodermus*', indicating that the new species looks like a miniature *A. xanthodermus.*

DISCUSSION

A. parvitigrinus looks like a miniature *A. moelleri* with a more slender silhouette and with the cap squamules more scattered making its cap globally lighter. In the same way *A. xanthodermulus* looks like a miniature *A. xanthodermus* but has significantly larger spores, unless one considers *A. xanthodermus* var. *macrosporus.* This variety differs from *A. xanthodermulus* by its much larger cap and the greater width of its cheilocystidia (6–21 μm vs 6–8 μm). Their truncately conical form, odor of ink and yellow discoloring on handling put all four species together in section *Xanthodermatei.* Within this section the main characteristics of both new species are their small sporophores and their spindly silhouette; moreover, they can be distinguished from each other by their own characteristics: dark grayish, minute squamules for *A. parvitigrinus* and large spores for *A. xanthodermulus.*

The other small species of section *Xanthodermatei* described in Europe are *A. pseudopratensis, A. endoxanthus* and *Psalliota nigricans. Agaricus pseudopratensis* differs from both new species by its ''stature squat, measurements resembling those of *Agaricus campester*'' (Bohus 1971) and by its context often becoming somewhat reddish. It also can be distinguished from *A. xanthodermulus* by its smaller spores. *Agaricus endoxanthus* differs from both new species by the presence of vacuoles containing an abundant dark brown pigment in the pileipellis hyphae; this also characterizes other small tropical species of the *Xanthodermatei*—like *A. rotalis* K.R. Peterson, Desjardin et Hemmes (Peterson et al 2000) and *A. termiticola* Heinem. (Heineman 1980). *Psalliota nigricans* Velen. 1921 (= *Agaricus meleagris* var. *nigricans* [Velen] Pilát 1951) has been described briefly as a small species (cap 3–5 cm diam); the specimen drawn by Velenovsky (1921) looks like *A. parvitigrinus* with a more inflated bulb. However we do not consider *Psalliota nigricans* as a synonym because its spores are significantly shorter (4–5 μm vs 5.5–6.7 μm for *A. parvitigrinus*) and this cannot be verified because the type collection does not exist. We also note its grassy site, while *A. parvitigrinus* was collected in wood. *Agaricus velenovskyi* Pilát 1968 (= *Agaricus meleagris* var. *nigricans* [Velen] Pilát sensu Pilát, 1951, pro parte [quoad descr. & specimina], excl. typo) has a cap 5–11 cm diam and therefore typically is not small. This species that we suspect to be synonym or close to *A. xanthodermus* (see Cappelli 1984) also can be distinguished from *A. parvitigrinus* by its cap, which is white and not ornamented at first, and by its grassy habitat (Pilát 1968).

The dissimilarity analysis shows that both new species are close to *A. laskibarii* and *A. californicus. Agaricus laskibarii* differs by its larger cap and its habitat in the dunes; it differs also from *A. parvitigrinus* by the absence of squamules and from *A. xanthodermulus* by its smaller spores. *Agaricus californicus,* which can have a small cap diameter, differs by a pileus with a grayish brown (or ''brownish-purple'', according to Peck 1895) disk without squamules (or ''somewhat squamulose'', according to Kerrigan 1986) and by fainter phenolic odor and yellow discoloring on handling.

Agaricus pilatianus (Bohus) Bohus that is probably a synonym of *A. iodosmus* Heineman, is a large species distributed in the Mediterranean area, but a small variant was described from the Netherland (Nauta 2001). The cylindrical stipe with a triangular annulus that characterizes this species differs from the generally bulbous stipe with a pendant membranous annulus observed in both new species.

We have known both new entities for several years, and we have observed that mycologists often have mistaken them for small or slender forms of *A. moelleri, A. xanthodermus* or *A. pseudopratensis.* This confusion also exists in the literature (i.e., we consider that the photo 544 p.192 [*A. pseudopratensis* var. *niveus*] in Arrillaga Anabitarte [2004] represents in fact specimens of *A. xanthodermulus*). We primarily hesitated to describe both new entities at the varietal rank. However molecular comparisons show that both new species diverge from these three species. The level of divergence is much greater, for example, than the divergence found between sister species of section *Duploannulati* (Challen et al 2003) like *A. bisporus* and *A. subfloccosus* (J. Lange) Pilát that differ at about 10–14 sequence sites (R.W. Kerrigan pers comm). Finally, all morphological and molecular comparisons reinforce the concepts of these new entities and support their elevation to the species rank.

In the Fitch phenogram *A. xanthodermulus, A. parvitigrinus, A. laskibarii* and *A. californicus* form a group. We note that this group is made up of small

to medium size species. Among them, to our surprise, *A. xanthodermulus* and *A. laskibarii* are close to each other. The slight difference between the sequences of *A. xanthodermulus* and *A. laskibarii* (polymorphisms at three positions) was reinforced by the fact that the specimen of *A. laskibarii* was heteromorphic at four other sequence sites, while the four specimens of *A. xanthodermulus* had identical sequences without any heteromorphism, although the collections came from relatively distant sites in western France. The collection of *A. laskibarii* has been found in the same region (at about 120 km from a site of *A. xanthodermulus*); other collections would be useful to interpret the origin of this heteromorphism, but this species seems particularly rare. We also note that, among the three polymorphic sites for which the two species diverge, two characterize *A. laskibarii* while the remaining one characterizes *A. xanthodermulus* among all the studied species. On the other hand, the morphological differences between these two species are relatively important. They are in fact more pronounced than the differences between the two abundant species *A. moelleri* and *A. xanthodermus,* which mainly differ by the presence/absence of squamules, while their sequences differ by only two polymorphisms. Such a small divergence is of the same order of the maximum difference we observed between specimens belonging to a same species in the analysis of section *Duploannulati* (Challen et al 2003). Pairs of species as close as *A. laskibari*/*A. xanthodermulus* or A. *moelleri*/*A. xanthodermus* possibly reflect relatively recent processes of speciation in the *Xanthodermatei*. The phylogenetic analysis of section *Xanthodermatei* is in progress and will let us compare better the evolutionary processes.

In conclusion, among the species of the *Agaricus* section *Xanthodermatei* there exist now in Europe four species having a cap diameter smaller than 5.5 cm (generally 3–4 cm), *Agaricus pseudopratensis* and *A. endoxanthus,* and two new species, *A. parvitigrinus,* which probably is rare, and *A. xanthodermulus,* which is relatively more abundant than its larger, rare sister species *A. laskibarii*. Phenol, p-quinol and other phenolic metabolites are constituents of *A. moelleri* and *A. xanthodermus* in sufficient quantity to account for their immediate toxicity and their phenolic odor (Gill and Strauch 1984, Wood et al 1998). Both new species have a phenolic odor and probably are toxic.

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