An Asian commercial strain of Agrocybe chaxingu and a European wild strain of Agrocybe cylindracea exhibiting morphological difference and high genetic divergence are interfertile

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AN ASIAN COMMERCIAL STRAIN OF *AGROCYBE CHAXINGU* AND A EUROPEAN WILD STRAIN OF *AGROCYBE CYLINDRACEA* EXHIBITING MORPHOLOGICAL DIFFERENCE AND HIGH GENETIC DIVERGENCE ARE INTERFERTILE

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ABSTRACT

Three molecular markers were compared between a cultivated strain of *Agrocybe chaxingu* from South-Eastern Asia (SM960903) and a French wild strain of *Agrocybe cylindracea* (SM47): (i) the nuclear ribosomal unit, (ii) the mitochondrial SSU-rDNA and (iii) the mitochondrial *cob* gene. All markers reveal extensive variations: the ribosomal units possess up to 67 polymorphic sites (89 % of nt identity), the variable domains of the mitochondrial SSU-rDNA differ by large indel sequences and the *cob* gene molecular organization is characterized by non-orthologous group I introns. In fruiting tests, sporophores of the two strains were macroscopically distinguishable, especially by a thicker and stronger annulus in *A. chaxingu*. In inter-stock mating tests, hyphal clamp connections were detected in mycelia of the junction lines for all the confrontations. The dikaryotic status of the presumed hybrids was confirmed by the presence of both *A. chaxingu* and *A. cylindracea* nuclear ribosomal units. Dikaryotization was also observed in distal regions from the junction lines, indicating that nuclei migration occurred in these matings. Four selected hybrids containing each of the four *A. cylindracea* mating type genotypes were put in fruiting conditions. These hybrid sporophores were molecularly verified and shown to be fertile based on the presence of mature basidiospores. Despite extensive variations at the molecular and phenotypic levels, the complete inter-fertility observed between the Asian *A. chaxingu* and the European *A. cylindracea* strains strongly argues for con-specificity of both taxa. *A. chaxingu* would be a variety of the *A. cylindracea* species which was firstly described.

Keywords: *Agrocybe chaxingu*, *Agrocybe cylindracea*, interfertility, sporophores, molecular markers

INTRODUCTION

Agrocybe cylindracea (DC.: Fr) Singer, syn. *A. aegerita* (Brig.) Singer (Basidiomycota, Agaricales) is a species of mushroom cultivated in Europe since antiquity, but sporadically cultivated today. In contrast, in Asia, mushrooms called *A. cylindracea* but also *A. chaxingu* Huang are largely cultivated today. As published in the Edible Fungal Flora of China [1], the diagnosis of *A. chaxingu* does not allow a clear distinction between this species and *A. cylindracea*.

During the last thirty years, based on its ability to easily achieve a complete life-cycle in laboratory conditions, *A. cylindracea* was largely studied as a laboratory model of mushroom-producing Basidiomycete. Numerous reports on its genetics and molecular biology were produced. Particularly, several reports have focused on the mitochondrial genes and genome of *A. cylindracea* wild-type strains. In 1997, the sequence and secondary structure of the *A. cylindracea* mitochondrial SSU-rDNA was described [2]. This led to the characterization of variable domains (V4, V6 and V9) of the SSU-rRNA showing species-specific length variations within the genus *Agrocybe*, including European wild-type strains of *A. cylindracea* and Asian cultivated strains of *A. chaxingu* [3]. In the same way, a comparative analysis of the mitochondrial *cob* gene of *A. cylindracea* and *A. chaxingu* strains revealed important genetic variations between the two species and the presence of non-orthologous group I introns in this gene [4, 5, 6]. More recently, the study of Uhart et al. [7], mainly based on comparison of mitochondrial ribosomal SSU-rDNA variable domains, confirmed the strong genetic divergence between the European wild specimens of *A. cylindracea* and strains cultivated in Asia under the name *A. chaxingu*. At that time, these two entities were considered as belonging to a species complex but, taking in account some inter-sterility (not reported in details) between the two entities, the authors suggested that these strains could finally belong to two distinct species. However, in another paper, based on morphological comparisons, Uhart & Alberto [8] suggested that these two species should be finally synonymised but that the type specimen would have to be examined first.

Here, we intend to complete previous studies about the genetic divergence between the two entities and to test the inter-fertility between two strains already studied in the papers cited above: a European wild specimen of *A. cylindracea* and a strain cultivated in Asia under the name *A. chaxingu.* We expect to clarify the situation not only at the taxonomic level but also to obtain a better understanding of species circumscription and of their geographical range in order to help manage their genetic resources and future breeding programs.

MATERIALS AND METHODS

Strains. The European wild–type strain *Agrocybe cylindracea* (= *Agrocybe aegerita*) SM47 from South-West of France and the industrially cultivated *Agrocybe chaxingu* SM960903 from Eastern Asia were previously described [3-7]. Both strains are permanently deposited in the collection of the Laboratory (INRA UR 1264, Villenave d'Ornon, France).

In vitro DNA manipulation and sequencing. Sequences used as molecular markers (nuclear ribosomal unit, mitochondrial *cob* gene) were obtained by conventional procedures from cloned PCR products.

Total DNA of fungal strains were extracted from 0.1 g of dried carpophores or 0.2g of fresh mycelium, after grinding in liquid nitrogen to obtain a fine powder. Nucleic acids were extracted according to the *N*-cethyl-*NNN*-trimethyl ammonium bromide (CTAB) procedure adapted for small quantities of basidiomycete mycelia [9]. DNA (OD_{260}) was quantified using a NanoDrop spectrophotometer (NanoDrop ND-1000, Nanodrop technologie, DE, USA), diluted in deionized sterilized Milli-Q water (Milli-Q water system production, Millipore, Saint-Quentin en Yveline, France) and stored at -20°C.

PCR amplifications were carried out using the Go *Taq* polymerase from Promega Corp. (Madison, Wis, USA) and respective primer pairs synthesized by Eurofins MWG Operon (Germany).

The primers used to amplify the nuclear ribosomal unit were the conventional ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'GGAAGTAAAAGTCGTAACAAGG3')

described by [10]. A CAPS test was developed by using the *Hae*II restriction enzyme from Promega Corp. (Madison, Wis, USA), according to the manufacturer's recommendations.

The primer pairs used to verify, by PCR, the presence of the *A. chaxingu* (E1C/E2C) and *A. cylindracea* (EU1A/ER1A and EU2A/ER2A) introns have been previously described [5].

E1C (nt532 5′CGTAATTACTAATTTATTATC3′ nt552);
E2C (nt1811 5′ACAAAAGGTAATAAATAGTG3′ nt1792);
EU1A (nt267 5′ACCAAATGTAGATTTTGCA3′ nt285);
ER1A (nt2976 5′CTTCCAATATGTATATATAC3′ nt2957);
EU2A (nt3448 5′TTATATTCCAGCAGATCC3′ nt3465);
ER2A (nt4675 5′TAGTTTGTTAGGAATAGATC3′ nt4656).

The sizes of the V4, V6 and V9 variable domains of the mitochondrial SSU-rDNA were verified by electrophoresis analyses of the specific PCR products in 1.2 % agarose gels. Specific primers were as previously described [3, 7].

PCR were performed in a Programmable Thermal Cycler PTC 200 (MJ Research Inc., Watertown, Mass., USA). Each reaction contained 10 to 100 ng of fungal genomic DNA, 1 µM of both primers, 200 µM of each dNTP, 1 unit of *Taq* DNA polymerase, in a final volume of 50 µl of the appropriate buffer. Reactions were run for 30 cycles at 95 ° C for 30s, then two degrees below the lowest Tm of both oligonucleotides for 30s, 72° C for 1 to 2 min, and one final cycle at 72° C for 5 min. An aliquot of 10 µl of each PCR product was analysed by agarose (1%, w/v) gel electrophoresis containing 200ng/ml of ethidium bromide, in TEB buffer [11].

For DNA sequencing, PCR products were purified with the Wizard SV gel and PCR Clean-Up System (Promega Corp.Madison, WI, USA) before they were sequenced by the primer walking methods using the Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Courtaboeuf, France) and the corresponding primers used for the PCR reaction. Sequence reactions were carried out according to the supplier's recommendations, in a final volume of 10 µl containing 100 ng of PCR product and 0.5 µM of the specific primer. Sequence reactions were carried out in a thermocycler by applying an initial denaturation step at 95°C for 1 minute; 27 cycles with each composed of the three steps: 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. The reaction products were ethanol precipitated, dried, and then separated by capillary electrophoresis (on an automated sequencer ABI 3130x1, ABI Prism Corp., France) at the genomic platform of the University Bordeaux Segalen (France). Sequencing profiles were edited and corrected using the BioEdit sequence alignment editor v7.0.9 free software (Ibis Biosciences Carlsbad, CA, USA).

Fruiting test, spore germination, and monospore culture. Polypropylene bags containing 250 g of chopped humidified wheat straw + 25 g of oat flour + 50 ml of glucose solution (15g / l), were sterilized in an autoclave. Malt agar medium colonized by mycelium was used to inoculate the bags: the culture medium of two Petri dishes of 9 cm in diameter were deposited at the top of each bag. Incubation lasted 15 days at 25 ° C; then, the temperature was lowered to 18 ° C. After 20 days, bags were opened and fruiting began after one week.

Sporeprints were recovered from mature sporocarps on tracing paper. Spore suspensions at different dilution were spread on complete yeast medium [12]. Eight days later, spores began to give rise to young mycelia that were isolated. Single spore isolates (SSIs) were subcultivated individualy on malt agar medium.

Mating tests. Small pieces of agar culture of two different SSIs were placed at about 2 cm from each other in Petri dish containing malt agar medium. The inoculated mycelia were allowed to grow together at 25°C. Positive mating was indicated by the formation of aerial fluffy mycelium at the junction line between two monokaryons. The fluffy mycelium appearing at the junction line is a presumed dikaryon resulting from a plasmogamy between the two sexually compatible

monokaryotic SSIs. For each confrontation two replicates were made. The fluffy mycelium was isolated and subcultured on malt agar medium.

Cytological method for positive mating confirmation. A simple method was used to confirm the positive mating. SSIs of *Agrocybe* are monokaryotic and without clamped hyphae, while fertile dikaryons exhibit clamp connections. For each positive mating, a fragment of mycelium from the junction zone of any replicate was examined under microscope (x100, congo red staining). Observation of clamp connections confirmed the dikaryotic status of the mated mycelium.

Figure 1. Sporophores of the parental strains differentiated in cultivation.
1a: *Agrocybe chaxingu* strain SM960903, cap after opening; 1b: *Agrocybe chaxingu* strain SM960903 cap before opening; 1c. *Agrocybe cylindracea* strain SM 47 at various stages of maturation.

RESULTS

Morphological comparison of the parental strains. Ten culture bags were performed for each strain. Strain SM47 of *A. cylindracea* began to fruit 35 days after the inoculation of the substrate, while strain SM960903 of *A. chaxingu* began one week later in all replicates. Morphological differences between the sporophores of the two strains were noticeable (Fig.1). These differences include cap color that was much more palish for SM47, or the longer stipe of SM960903.

However, these differences likely characterize the strains and not the species in which these criteria have shown to be highly variable and also sensitive to environmental conditions. However differences were also noted for two other traits that may be taxonomically pertinent: the color of the stipe and the aspect and behavior of the partial veil: (i) the color of the stipe was light brown in *A. chaxingu* SM960903 as in other strains of *A. chaxingu* that we have cultivated (data not reported here) along with the white color of tiny scales that make them more visible. In *A. cylindracea*, the stipe was almost uniformly white; (ii) the partial veil is larger and thicker in SM960903 with the underside layer breaking of in crown wheel before it comes off the cap. Moreover, it fits in the upper stipe; the hymenial cavity is very narrow; the cap opening occurs lately and often after spore maturity. At the opening of the cap, we put some bags on the outside and saw the large membranous ring floating in the wind, likely facilitating the spread of spores that had settled on it. In *A. cylindracea*, the cap opened earlier and the partial veil contracted, forming a shorter and thicker annulus that was not covered by spores at this time. In other respects, we did not observe significant difference in spore sizes between the two specimens. They were on average 10.2 × 5.9 µm (n = 30) for SM47 and 10.4 × 5.9 (n = 30) for SM 960903.

Genetic divergence between the parental strains of *A. cylindracea* SM47 and *A. chaxingu* SM960903. The genetic variation between the parental strain *A. cylindracea* SM47 and *A. chaxingu* SM960903 was studied using three different molecular markers: (i) the nuclear ribosomal unit, (ii) the variable domains V4, V6 and V9 of the mitochondrial SSU-rDNA encoding the 16S RNA of the small sub-unit of the mito-ribosome, and (iii) the molecular organization of the *cob* gene which encodes the unique mitochondrial protein of the Complex III of the respiratory chain. All these markers revealed high divergence between the two strains and it is to be noticed that the specific feature of each strains for all these markers were shared with all the studied European *A. cylindracea* strains (10 different strains) for *A. cylindracea* SM47 and with three additional Asian *A. chaxingu* strains for A. chaxingu SM960903 (data not shown). In details, the nuclear ribosomal region of the *A. cylindracea* SM47 strain, corresponding to the ITS1/ITS4 PCR product had a size of 637 nt, while that of *A. chaxingu* SM 960903 had a size of 652 nt. When aligned (Clustal W), the two sequences possessed 67 polymorphic sites (distributed in 39 non contiguous loci), i.e. showing a percentage of nt identity slightly lower than 90 %.

When cleaved by the restriction endonuclease *Hae*III, the ITS1/ITS4 PCR product of *A. cylindracea* generates three fragments of size 341 nt, 180 nt and 116 nt. On the contrary, the *Hae*III restriction profile of the ITS1/ITS4 PCR product of *A. chaxingu* is constituted by four fragments of size 341 nt, 201 nt, 84 nt and a small one of 26 nt not detectable in agarose gel electrophoresis. From this, the use of the *Hae*III restriction endonuclease allowed the development of a CAPS test to easily discriminate both strains by agarose (1.2 %) gel electrophoresis analysis of the *Hae*III-digested PCR product generated by the ITS1/IIT4 couple of primers. The amplified nuclear ribosomal region *A. cylindracea* strain had a profile with 3 fragments (341, 180 and 116 nt), the *A. chaxingu* one possess the same large fragment of 341 nt accompanied by two fragment of 201 nt and 84 nt. The length variations between the two polymorphic fragments of *A. cylindracea* (180 nt and 116 nt) and *A. chaxingu* (201 nt and 84 nt) were easily detectable by agarose gel electrophoresis analysis.

As previously reported, there were also important genetic variations between mitochondrial genes of *A. aegerita* SM47 and *A. chaxingu* SM960903 strains.

The length of the V4 domain varied from 170 nt in *A. cylindracea* to 281 nt in *A. chaxingu*; the length of the V6 domain varied from 172 nt in *A. cylindracea* to 158 nt in *A. chaxingu* and the length of the V9 domain varied from 221 nt in *A. cylindracea* to 246 nt in *A. chaxingu*. These size variations were mainly due to insertion/deletions events of large sequences occurring in these variables domains [3].

In the same way, the previously established [5] molecular organization of the *cob* gene, shown in figure 2, was verified in the parental *A. cylindracea* SM47 and *A. chaxingu* SM960903 strains by PCR with specific primer pairs located in the flanking exon sequences of each gene. As shown in Figure 2, the two strains differed by the presence/absence of three non-orthologous large group I introns: two in *A. cylindracea* and one in *A. chaxingu*.

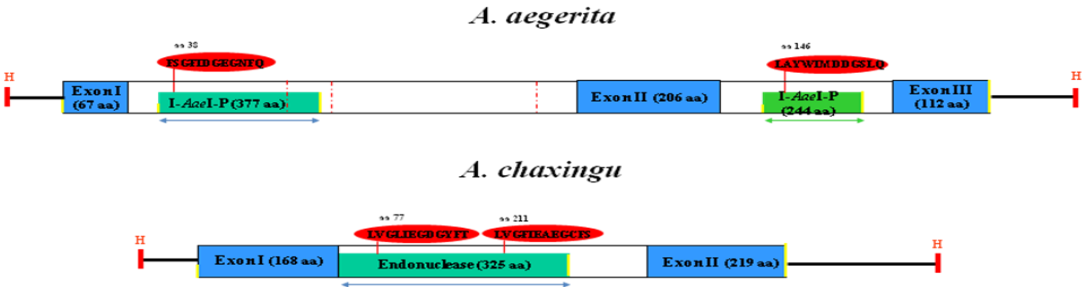

Figure 2: Comparison of the molecular organization of the *cob* gene in *A. chaxingu* (GenBank Acc. N° AY772389) and *A. aegerita* (GenBank Acc. N° AY781064). The *cob* exonic sequences and the intronic *heg* are in blue and green boxes, respectively. The typical LAGLIDADG motif of each HE are indicated. From [5].

Intra-stock and inter-stock mating tests. Parental strains SM47 of *A. cylindracea* and SM960903 of *A. chaxingu* exhibited hyphal clamp connections, in agreement with their dikaryotic status. Spores from SM47 easily germinated, while only few spores of SM960903 germinated. For SM47, 60 SSIs were isolated, but only 15 SSIs for SM960903. All the SSIs were monokaryotic and usable for intra-stock mating tests, except for two SSIs of SM960903 for which clamped hyphae were detected. For SM47, 12 SSIs chosen at random were mated in all pairwise combinations and four groups of incompatibility were recovered based of the detection of hyphal clamp connexions: all the confrontations between group I (1 SSIs) and group II (4 SSIs), or between group III (2 SSIs) and group IV (5 SSIs) were positive and produced clamped mycelia. These data agreed with the heterothallic life cycle and the bifactorial system of sexual incompatibility already described in *A. cylindracea* [13]. For SM960903, 12 SSIs were confronted in all pairwise combinations but only few positive reactions were observed, so, the inter-compatibility groups remain fully identified.

For the inter-stock mating tests, each strain of the seven SSIs of the SM47 offspring, including the single SSI of the group I and two SSIs of each of the three remaining incompatibility groups, were confronted with each of the 12 SSIs of the SM960903 offspring. Positive reactions were generally not clearly visible but rather ambiguous. After six weeks, mycelia at the junction line were examined and hyphal clamp connections were detected in all 84 (= 7 x 12) confrontations.

Isolation of 56 presumed hybrid mycelia exhibiting clamp connections. For 48 confrontations performed between the 12 SSIs from SM960903 and 4 SSIs from SM47 (SM47-6, SM47-7, SM47-11, SM47-13) that belonged to each of the four incompatibility groups I, II, III and IV, hybrid dikaryotic mycelia were isolated from the junction line, and they were then subcultured for further genetic analyses and fruiting tests. In other respects, in the dishes of confrontation between SSIs from the two parental strains, we also tried to detect hyphal clamp connections in the growing area of each monokaryon: they were almost always detected in the area of monokaryons deriving from strain SM960903, but less frequently in the area of the monokaryons deriving from strain SM47. In four dishes corresponding to four different confrontations, hyphal clamps were detected in the areas of both monokaryons, fragments of

mycelia were isolated from each of the two areas, additionally to the mycelium isolated from the junction line. Finally, a total of 56 hybrid mycelia were isolated.

Genetic confirmation of the hybridization. DNA of the 56 presumed hybrid dikaryotic mycelia (isolated from 48 strains collected on the junction lines and 8 strains coming from regions distant from these lines) were extracted. For each strain, the nuclear ribosomal unit was amplified as described in the Materials and Methods section by using the conventional ITS1/ITS4 couple of primers. The resulting PCR products were digested by *Hae*III and the restriction fragments analysed by agarose gel electrophoresis. As expected, this CAPS marker revealed for each strain, a typical heteroallelic profile. In details, all the 48 presumed hybrids from the junction lines, as well as the 8 strains isolated from the areas of the confronted homokaryons, distant from these junction lines possessed both largest *Hae*III restriction fragments characterizing each parental strain: the 342 nt fragment of *A. Cylindracea* and the 422 nt fragment of *A. Chaxingu*. This pattern confirms in all tested cases the presence of both parental nuclei. All these 56 isolates were therefore assumed to be dicaryotic. The presence of both parental nuclei in the 8 strains coming from regions distant from the junction lines suggests that, as deduced from the presence of clamp connections on these mycelia, a migration of the compatible nuclei has occurred in the recipient homokaryotic hyphae.

Cultivation and morphology of the hybrids. Among the 48 isolated hybrid strains, we chose four representatives as follow: all the parental homokaryons from SM960903 that were different and all the parental homokaryons from SM47 that belonged to the four different incompatibility groups. From the information we had, these four hybrids were potentially the most different that we could find: SM47-13 × SM960903-6, SM47-7 × SM960903-3, SM47-11 × SM960903-12, and SM47-6 × SM960903-2. All of them easily fruited and sporulated. Sporophores of three of them are showed on Fig. 3. The cap color at the disc was dark brown like the *A. chaxingu* parent or palish brown like the *A. cylindracea* parent; however, the cap color at the edges was always palish like the *A. cylindracea* parent. In all cases, the stipe had a white brown color like the *A. chaxingu* parent. The annulus was rather small and did not open lately as for the *A. cylindracea* parent but a crown wheel is more or less visible but less pronounced than in the *A. chaxingu* parent.

Molecular characterization of the hybrid sporophores. Sporophores produced by the four hybrids were molecularly characterized by nuclear and mitochondrial markers. The CAPS test described above allow to verify that the sporophores differentiated by the four fruiting hybrids possessed the heteroallelic pattern of the nuclear ribosomal units (restriction fragments of both parental strains), and consequently were also dikaryotic.

The molecular organization of the mitochondrial *cob* gene of a sporophore differentiated by each of the four fruiting hybrids was studied by PCR as described in the Materials and Methods section with three primer pairs allowing the evidence of each of the three non-orthologous group I introns to be detected: *iAeI* and *iAeII* carried by the *A. cylindracea* parental *cob* gene and *iAchax* carried by *A. chaxingu*. Interestingly, all the four sporophores possess only the *A. chaxingu iAchax* intron, This suggests that the molecular organization of the mitochondrial *cob* gene present in the differentiated sporophores was that of the parental *A. chaxingu* strain. This was also confirmed by amplification of the V4, V6 and V9 variable domains of the mitochondrial SSU-rDNA whose size corresponded to the *A. chaxingu* SSU-rDNA. As both genes (*cob* and SSU-rDNA) are located in distant regions of the mitochondrial genome (data not shown), the differentiated sporophores were assumed to possess the mitochondria of the parental *A. chaxingu* strain. This is in accordance with the previously reported uniparental heredity of mitochondria observed in sporophores of *A. cylindracea* [14].

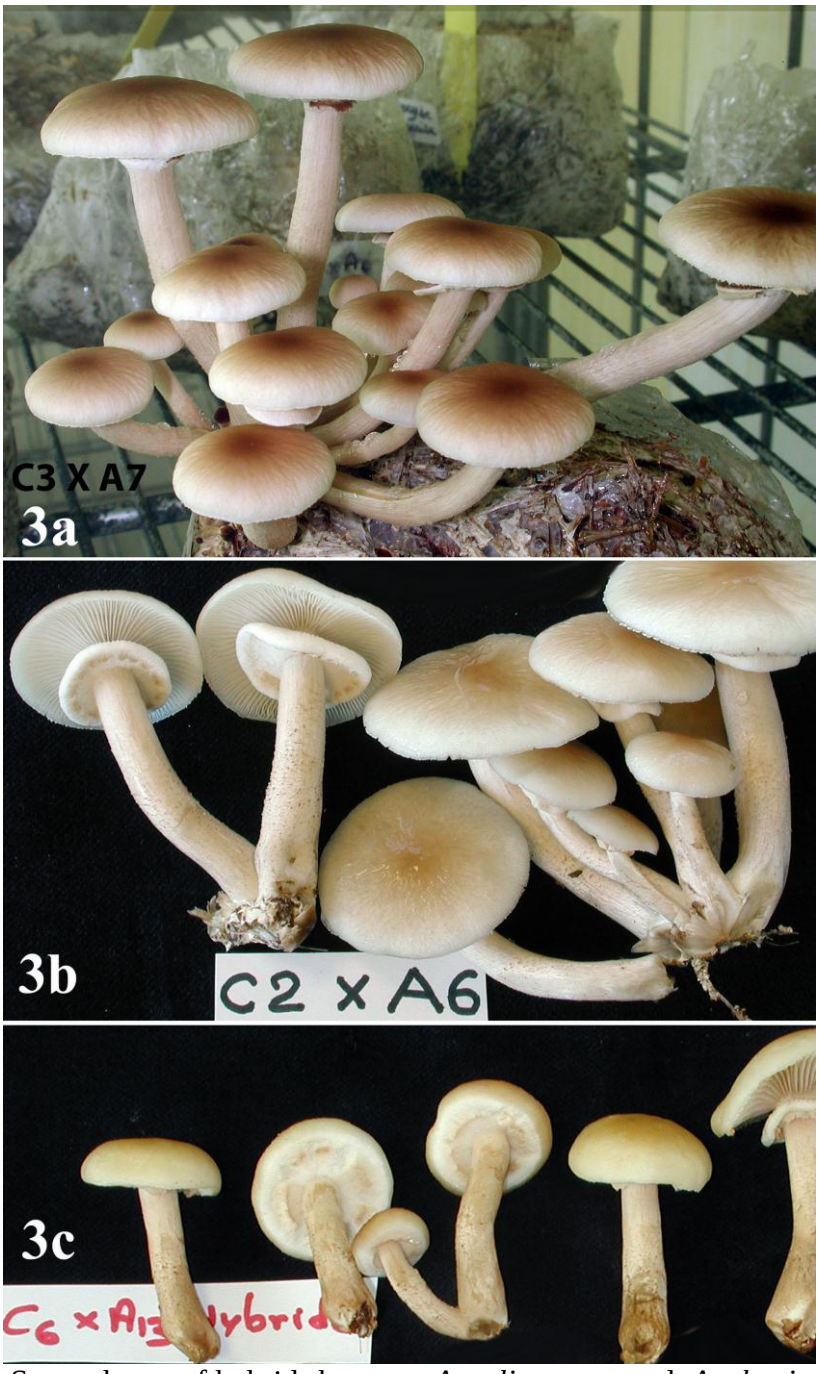

Figure 3. Sporophores of hybrids between *A. cylingracea* and *A. chaxingu*.
3a: SM47-7 × SM960903-3; 3b: SM47-6 × SM960903-2; 3c: SM47-13 × SM960903-6.

DISCUSSION

Although the main difference reported in the literature between *A. cylindracea* and *A. chaxingu* is their natural habitat, our comparison between a wild type strain of *A. cylindracea* and a strain cultivated in Asia under the name *A. chaxingu* showed that the sporophores of the latter had a more colorful stipe and a partial veil that separated later from the cap, forming a wider ring covered with spores. Such differences are not so visible on dried specimens. On the other hand, we are not sure that the habitat is a strong criterion in this complex because, in Europe, *A.*

cylindracea grows easily on various *trees*, including *Acer negundo* which was introduced from North America. Moreover, both strains were able to grow and form fruiting bodies on wheat straw. At the molecular level, the 39 differences between the two parental strains were detected. This is much greater than the number of polymorphic positions that can be found between strains belonging to the same species in the genus *Agaricus*, which rarely exceeds 2 or 3. Such a difference suggests that mutation rate would be higher in the ITS of *Agrocybe* and/or that reproductive barrier more easily takes place in *Agaricus*.

Intra-stock mating tests revealed a bifactorial system of sexual incompatibility for the strain of *A. cylindracea*; although the reproductive system of the other strain was not resolved. In inter-stock mating tests, monokaryons from the two parents were all sexually compatible, indicating that the mating type alleles of tested monokaryons of *A. chaxingu* differ from the alleles of the strain of *A. cylindracea*. Systematic detection of clamped mycelia in the area of the monokaryon of *A. chaxingu* suggests that nuclei from *A. cylindracea* may have migrated, dikaryotizing the monokaryon of *A. chaxingu*. Many hybrid strains were isolated from the interstock mating tests and confirmed cytologically and molecularly. Four representatives of these hybrids were cultivated on fruiting medium: they fruited easily and abundantly. In the four cases, sporophores were fertile with a veil similar to the veil of the *A. cylindracea* parent, but the stipe was colored similarly as *A. chaxingu*. Moreover we showed that the mitochondria were inherited from the *A. chaxingu* strain. Such a uniparental inheritance of the mitochondria is frequent in basidiomycota and especially in *Agrocybe cylindracea* [14]. Since we also noted that nuclei of the monokaryon of *A. cylindracea* apparently easily invaded the monokaryon of *A. chaxingu* and since the putative hybrids were isolated relatively late (6 weeks after the beginning of the co-culture), it is possible that most of the isolates were in fact dikarotized monokaryons of *A. chaxingu*, bearing the mitochondria of *A. chaxingu*. We finally conclude that both entities are completely interfertile although they highly diverge genetically. These data on one hand show that the two entities are conspecific, but, on the other hand, support the concept of a varietal rank for the Asian entity. The type specimen of the latter remains to be studied to determine if the commercial strains named *A. chaxingu* are truly related to this species. Morphological differences being not easy to detect on dried specimens, the best solution should be to compare DNA sequences. Such data would be useful but are not necessary to describe and to name a new variety. To describe such a variety, specimens with morphological and molecular characteristics similar to those of commercial strains used here, but of known origin would be more appropriate.

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