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Xylogone ganodermophthora sp. nov., an ascomycetous pathogen causing yellow rot on cultivated mushroom *Ganoderma lucidum* in Korea

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Abstract: Yellow rot, caused by an ascomycetous fungus having a distinctive arthroconidial anamorph, is the most destructive disease of cultivated *Ganoderma lucidum* in Korea, but the identity of the yellow rot pathogen (YRP) remains uncertain. Isolates have been identified as *Xylogone sphaerospora* (with putative anamorph *Sporendonema purpurascens*) or as *Arthrographis cuboidea*. Therefore we used morphological features, pathogenicity tests and phylogenetic analyses of DNA sequences from the nuclear ribosomal genes, including partial small subunit and internal transcribed spacer regions, and from the gene encoding RNA polymerase second largest subunit to evaluate the relationship between YRP isolates and these species. YRP isolates formed a distinct subgroup within a clade that included *X. sphaerospora*, *A. cuboidea* and *Scytalidium lignicola*, the type species of *Scytalidium*, but the disposition of the clade within the Leotiomycetes was uncertain. We describe *Xylogone ganodermophthora* sp. nov. and *Scytalidium ganodermophthorum* sp. nov. for the teleomorph and anamorph of YRP respectively. *Arthrographis cuboidea* is reclassified as *Scytalidium cuboideum* comb. nov., and the anamorph of *X. sphaerospora* is named *Scytalidium sphaerosporum* sp. nov. In pathogenicity tests only *X. ganodermophthora* caused disease in *Ganoderma lucidum*. Amplified fragment length polymorphism analyses showed that *X. ganodermophthora* populations from diseased fruiting bodies or from oak wood in Korea consisted of two clonal groups.

Key words: *Arthrographis cuboidea*, *Neoscytalidium dimidiatum*, *Scytalidium cuboideum*, *Scytalidium ganodermophthorum*, *Scytalidium lignicola*, *Sporendonema purpurascens*, yellow rot pathogen

INTRODUCTION

Ganoderma lucidum (Curtis) P. Karst. is a basidiomycetous fungus (family Ganodermataceae) distributed in the temperate zone of the northern hemisphere including Europe and Asia (Kim 2000, Oh et al. 1998). It is a mushroom used as traditional medicine in Asia and is called *Yeongji* in Korean, *Lingzhi* in Chinese and *Reishi* in Japanese. The mushroom also is consumed worldwide as health food because it has pharmacological functions (Hong and Jung 2004). *Ganoderma lucidum* grows at the base and stumps of deciduous trees in nature. It has been cultivated with oak (*Quercus* species) logs in greenhouses since the 1980s, and cultivation areas have increased in Korea (Choi et al. 1998, 2001).

Yellow rot, caused by an ascomycete fungus having a distinctive arthroconidial anamorph, is the major destructive disease occurring in the *G. lucidum* cultivation areas of Korea (Lee et al. 1996, Oh et al. 1998). The disease causes severe yield loss and limits continuous cultivation of the mushroom in the same place. The identity of the yellow rot pathogen (YRP) remains uncertain. Lee et al. (1996) first identified the YRP as *Xylogone sphaerospora* Arx & T. Nilsson, but Oh et al. (1998) later determined the causal agent as *Arthrographis cuboidea* (Sacc. & Ellis) Sigler. *Xylogone* Arx & T. Nilsson is a genus of cleistothecial ascomycetes comprising the single species, *X. sphaerospora*, described originally from wood chips of *Pinus sylvestris*, *Picea* sp. and *Betula* sp. in Sweden and from stored pulp chips of trees in Australia (von Arx and Nilsson 1969). However the pathogenicity of *X. sphaerospora* for *G. lucidum* is unknown and the

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TABLE I. Strains examined including their original and current names, provenance and sequence numbers

Original determination	Final determination (if different)	Isolate number	Source	Location	GenBank number		
					ITS	RPB2	SSU
YRP	*Xylogone ganodermophthora*	H1	Diseased fruit body of *G. lucidum*	Hongcheon, Gangwon, Korea	GQ272612	GQ290116	GQ280394
YRP	*X. ganodermophthora*	H2	Diseased fruit body of *G. lucidum*	Hongcheon, Gangwon, Korea	GQ272613	GQ290117	GQ280395
YRP	*X. ganodermophthora*	H9	Diseased fruit body of *G. lucidum*	Hongcheon, Gangwon, Korea	GQ272614	GQ290118	GQ280396
YRP	*X. ganodermophthora*	H10	Diseased fruit body of *G. lucidum*	Hongcheon, Gangwon, Korea	GQ272615	GQ290119	GQ280397
YRP	*X. ganodermophthora*	H20	Diseased log used for *G. lucidum* cultivation	Wonju, Gangwon Korea	GQ272616	GQ290120	GQ280398
YRP	*X. ganodermophthora* (T)	H55 (= UAMH 10320)	Diseased fruit body of *G. lucidum*	Yangpyeong, Gyeonggi, Korea	GQ272617	GQ290121	GQ280399
YRP	*X. ganodermophthora*	H88	Diseased log used for *G. lucidum* cultivation	Boeun, Chungbuk, Korea	GQ272618	GQ290122	GQ280400
YRP	*X. ganodermophthora*	H110	Diseased log used for *G. lucidum* cultivation	Hongsung, Chungnam, Korea	GQ272619	GQ290123	GQ280401
YRP	*X. ganodermophthora*	H123	Diseased fruit body of *G. lucidum*	Mungyeong, Gyeongbuk, Korea	GQ272620	GQ290124	GQ280402
YRP	*X. ganodermophthora*	H142	Diseased fruit body of *G. lucidum*	Gangjin, Jeonnam, Korea	GQ272621	GQ290125	GQ280403
Arthrographis cuboidea	*Scytalidium cuboideum*	UAMH 676	Timber, *Betula*	Canada	GQ272627	GQ290131	GQ280408
Arthrographis cuboidea (T, *Geotrichum microspermum*)	*S. cuboideum*	KACC 41223 (CBS 241.62 = UAMH 3101)	Rotten timber	South Africa	GQ272628	GQ290132	GQ280409
Arthrographis cuboidea	*S. cuboideum*	KACC 41224 (CBS 409.84)	Air	France	GQ272630	GQ290133	GQ280410
Arthrographis cuboidea	*S. cuboideum*	KACC 41290 (CBS 192.80)	Unknown	France	GQ272629	GQ290134	GQ280411
Arthrographis cuboidea	*S. cuboideum*	UAMH 3792	Mushroom	Japan	GQ503338		GQ280412
Arthrographis cuboidea	*S. cuboideum*	UAMH 4040	Decayed timber	South Africa	GQ503339		GQ280413
Arthrographis cuboidea	*S. cuboideum*	UAMH 4802	Stained wood, *Quercus rubra*	USA	GQ503340		GQ280414
Arthrographis cuboidea	*S. cuboideum*	UAMH 5643	Stained wood, *Pinus strobus*	USA	GQ503341		GQ280415
Arthrographis kalrae (T)		UAMH 3616	Human, sputum	India	AB116529		
Pithoascus langeronii (T) (anamorph *Arthrographis kalrae*)		KACC 41232 (CBS 203.78)	Herbivore dung	India	GQ272638		GQ280421

TABLE I. Continued

Original determination	Final determination (if different)	Isolate number	Source	Location	GenBank number		
					ITS	RPB2	SSU
Arthrographis lignicola		UAMH 4095 (CBS 689.83)	Wood chips and bark	Canada	GQ272633		
Scytalidium lignicola (T)		KACC 41228 (CBS 233.57)	Decaying wood, *Platanus*	Italy	GQ272634		GQ280419
Sporendonema purpurascens (NT)		UAMH 1497 (CBS 425.51)	Soil, mushroom bed	United Kingdom	GQ272631	GQ290135	GQ280417
Sporendonema purpurascens		KACC 41227 (CBS 406.63)	Compost	France	GQ272632	GQ290136	GQ280418
Xylogone sphaerospora	*X. ganodermophthora*	TPML 97003 (= UAMH 10321)	Diseased log used for *G. lucidum* cultivation	Shintanjin, Daejeon, Korea	GQ272622	GQ290126	GQ280404
Xylogone sphaerospora (T)		ATCC 34392 (UAMH 10841; CBS 186.69)	Wood chip, *Pinus sylvestris*	Sweden	GQ272624	GQ290127	GQ280405
Xylogone sphaerospora		KACC 41220 (CBS 187.69)	Wood chip, *Betula*	Sweden	GQ272623	GQ290128	GQ280406
Xylogone sphaerospora		KACC 41221 (CBS 188.69)	Wood chip, *Picea*	Sweden	GQ272625	GQ290129	GQ280407
Xylogone sphaerospora		KACC 41222 (CBS 385.84)	Preservative-treated timber	Australia	GQ272626	GQ290130	GQ280422

Abbreviations: YRP – yellow rot pathogen; T – type strain; NT – neotype strain.

Collection acronyms: ATCC – American Type Culture Collection, Manassas, Virginia; CBS – Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; KACC – Korean Agricultural Culture Collection, Suwon, Korea; TPML – Tree Pathology and Mycology Lab, Kangwon National University, Chuncheon, Korea; UAMH – University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada.

taxonomic position of the genus *Xylogone* also is uncertain. *Arthrographis* G. Cochet ex Sigler & J.W. Carmich. is an anamorphic genus of arthroconidial fungi in which narrow, hyaline, fertile hyphae are borne on simple conidiophores (Sigler and Carmichael 1976). The type species, *Arthrographis kalrae* (R.P. Tewari & Macph.) Sigler & J.W. Carmich., is associated with soil and human infections, but *Arthrographis cuboidea* is a soft rot fungus associated with pink- or blue-stained wood including oak (Sigler and Carmichael 1976, 1983; Wang and Zabel 1990). Although *A. cuboidea* has not been reported as a pathogen of *G. lucidum*, it was recorded from diseased logs used for cultivation of shiitake mushroom, *Lentinus edodes* (Berk.) Singer, in Japan (Uchida et al. 1993). *Sporendonema purpurascens* (Bonord.) E.W. Mason & S. Hughes differs from *Arthrographis* by presence of separating cells between arthroconidia (Sigler and Carmichael 1976). *Sporendonema purpurascens*, known as ''red *Geotrichum*'' and ''lipstick mold'', has been reported to cause diseases of the cultivated mushroom (Wood 1957), but its pathogenicity on *G. lucidum* is unknown. Lee et al. (1996) considered *S. purpurascens* as the putative anamorph of *X. sphaerospora*; however this was not the view of the original authors who described the latter as distinct from all known anamorphic genera (von Arx and Nilsson 1969). *Scytalidium lignicola* Pesante, the type species of *Scytalidium* Pesante, is another arthroconidial fungus causing soft rot decay and stain in wood (Wang and Zabel 1999). This species also has been isolated from diseased logs in Korea (H.J. Kang unpubl).

The objectives of this study were to assess the prevalence of YRP disease in Korea, to identify the YRP isolates by morphological and molecular comparison to reference isolates of the aforementioned species, to assess potential pathogenicity of some of these fungi for *G. lucidum* and to evaluate YRP population diversity in Korea. Partial sequences of the small subunit ribosomal RNA gene (SSU rDNA), internal transcribed spacer regions (ITS) and partial sequences of RNA polymerase second largest subunit (RPB2) were obtained and compared with sequences from GenBank. Evidence from morphological and molecular analyses provides support for the description of a new *Xylogone* species to accommodate the YRP isolates.

MATERIALS AND METHODS

Disease assessment, fungal isolation and culture.—Presence of yellow rot was evaluated in 17 cultivation houses of *G. lucidum* in six provinces of Korea 2001–2003. Twenty-five samples of *G. lucidum* fruiting bodies with yellow rot symptoms were collected. Ascocarps (cleisothecia) were detached from the diseased tissue of fruiting bodies or from oak logs and rinsed with 1% NaOCl. Each ascocarp was broken between two layers of flame-sterilized cover glass on potato dextrose agar (PDA, Difco), and then the lower cover glass was placed upside down on PDA and incubated 4–7 d in the dark at 28 C. The mycelial tips growing beyond the cover glasses were transferred to fresh PDA. After 14 d incubation for sporulation conidia were spread on the surface of 2% water agar and each germinating conidium was picked under a light microscope (150×). Single-conidial isolates were transferred to PDA slants for short term use. For long term storage each isolate was grown on PDA 7 d, and mycelial plugs were stored in 15% glycerol at −80 C.

Ten isolates of YRP representing all six Korean provinces (Gangwon, Gyeonggi, Chungbuk, Chungnam, Gyeongbuk, Jeonnam) and a Korean isolate (TPML 97003) that had been identified as *X. sphaerospora* (Lee et al. 1996) were included in the cultural and phylogenetic analyses (TABLE I). Other reference isolates examined are provided (TABLE I). For examination of cultural characteristics isolates were grown on PDA in the dark at 25–26 C and examined after 72 h, 7 and 14 d for observation of conidia. Plates were observed up to 5 wk for presence of ascomata. Selected YRP and reference isolates were grown also on cereal agar or on oatmeal salts agar (prepared in-house, recipes online at http://www.devonian2.ualberta.ca/uamh/Misc_PDF/Media.pdf)

Pathogenicity test.—Both in vitro and in vivo approaches were used to test pathogenicity of YRP isolates on *G. lucidum*. For the in vitro test colonies of *G. lucidum* (cultivar Yeongji 2) were grown on PDA slants 14 d at 28 C. Test isolates included 17 YRP strains and reference isolates *X. sphaerospora* (ATCC 34392, ex-type culture), *A. cuboidea* (UAMH 676) and *S. pupurascens* (UAMH 1497, neotype culture). These were grown on PDA 7 d at 26 C. A mycelial plug of each test isolate was placed on the mycelium of *G. lucidum*. After 14 d incubation mycelium of *G. lucidum* from below the test plug was subcultured to fresh PDA to determine viability. Subcultures were examined after 14 d incubation and plates held 1 mo.

For the in vivo test a single YRP isolate H55 was used. Twenty grams of *G. lucidum* grown on sawdust medium (Lee et al. 1996) were inserted into a hole drilled into a sterile oak log. At 0, 4, 6, 8 and 10 wk post inoculation, 1 mL arthroconidium suspension (1×10^4 conidia/mL) was sprayed on the log. Five logs were inoculated per treatment, including untreated control. The number of primordia and dry weight of *G. lucidum* fruiting bodies were measured 9 mo after inoculation.

Sequencing and analysis.—YRP and other isolates (TABLE I) were grown in 50 mL potato dextrose broth 5 d at 28 C. Fungal genomic DNA was extracted following the methods of Cubero et al. (1999) and Chi et al. (2009). The primer pairs used for the amplification of ITS, RBP2 and SSUrDNA regions included ITS1 and ITS4 (White et al. 1990), fRPB2-5F (5′-GAYGAYMGWGATCAYTTYGG-3′) and fRPB2-7cR (5′-CCCATRGCTTGYTTRCCCAT-3′), fRPB2-7cF (5′-

ATGGGYAARCAAGCYATGGG-3′) and f2RPB2-11aR (5′-GCRTGGATCTTRTCRTCSACC-3′) (Liu et al. 1999), SR1R and SR2, SR9R and SR6 (Vilgalys et al. 1994). All primers were synthesized by Bioneer Corp. (Daejeon, Korea). PCR was conducted in 50 μL reaction volumes. Each reaction tube contained 50 ng template DNA, 1× *rTaq* PCR buffer (Takara Biomedicals, Shiga, Japan), dNTP at 0.2 mM each, primers at 10 μM, and 1.25 U *rTaq* polymerase (Takara Biomedicals). PCR reactions were performed in a thermal cycler (PTC-200, MJ Research, Waltham, Massachusetts) with initial denaturation step at 94 C for 2 min, 30 cycles of 94 C (1 min)/50 C (1 min)/72 C (2 min) and a final extension step at 72 C for 10 min. PCR products were sequenced directly with an ABI3700 DNA analyzer (Applied Biosystems, Foster City, California) at the National Instrumentation Center for Environmental Management (Seoul National University, Seoul, Korea) after purification with a PCR clean-up system (Promega, Madison, Wisconsin). Sequences were edited with Lasergene 6.0 (DNASTAR, Inc., Madison, Wisconsin) and aligned with sequences retrieved from GenBank with Clustal W (Thompson et al. 1994) or Se-Al 2.0a11 (Rambaut 2002). Introns in the SSU rDNA sequences were excluded before the analysis. Maximum parsimony (MP) analyses were performed with PAUP 4.0b10 (Swofford 2002). The robustness of trees was determined with the full heuristic search option for 100 bootstrap replications. Gaps were treated as missing data and unalignable positions excluded. A Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck et al. 2001), based on Markov chain Monte Carlo method (Huelsenbeck and Ronquist 2001). The data matrix was analyzed with the general time-reversible substitution model including estimation of invariant sites and assuming a discrete gamma distribution (GTR + I + G) with six rate categories provided for the nucleotide substitution model. The nucleotide substitution model was chosen by analyzing the data matrix with the Akaike information criterion of Modeltest 3.7 to choose the best model to fit the data (Posada and Crandall 1998). Four Markov chains were run simultaneously, and trees were sampled every 100 generations for 1 000 000 generations, with the first 2500 trees deleted as burn-in. Inferences of posterior probabilities (PP) were calculated from 7501 trees and only clades with posterior probability values greater than or equal to 95% were considered phylogenetically informative. The resultant consensus tree was viewed with PAUP. GenBank accession numbers for isolates newly sequenced are provided (TABLE I).

Amplified fragment length polymorphism (AFLP).—AFLP were performed on 60 YRP isolates with the protocol of Vos et al. (1995) as modified by Leslie and Summerbell (2006). Six primer pair combinations (EAA/MAT, EGA/MTC, ECG/MGC, ETG/MTT, ETA/MCA, ECC/MCG) were used, and the EcoRI primers in the final specific amplification reactions were 5′ end labeled with [γ^{33}P]ATP. Dried gels were exposed to X-rays (Classic Blue Sensitive, Molecular Technologies, St Louis, Missouri) 2–5 h at −70 C to identify DNA bands. We manually scored the presence or absence of polymorphic AFLP bands representing DNA fragments 200–800 base pairs (bp) long and recorded the allele data (presence or absence) in a binary format. Bands of the same size were presumed to be homologous. We estimated molecular weights of AFLP fragments by comparison with the Low Mass Ladder™ (Life Technologies, Bethesda, Maryland) DNA standard that was 5′ end labeled with [γ^{33}P]ATP.

RESULTS

Occurrence and symptoms of yellow rot in G. lucidum *cultivation houses.*—One hundred forty-five YRP isolates were obtained from diseased fruiting bodies of *G. lucidum* or logs with yellow rot symptoms in 17 sampling sites. In YRP-free cultivation houses fruiting bodies of *G. lucidum* formed on oak logs were healthy and the pilei expanded into a kidney shape (FIG. 1a, b). In YRP-infected houses pilei formed on only a few logs and were malformed (FIG. 1c, d). In YRP-free areas the base of the stipe of *G. lucidum* was growing actively, producing a typical white mycelial mat (FIG. 1e), while the stipe infected by YRP was discolored and the mycelial mat was not observed (FIG. 1f). The inner tissue of oak logs producing healthy *G. lucidum* contained white mycelium (FIG. 1g), whereas the wood of contaminated logs was discolored greenish yellow (FIG. 1h).

Pathogenicity of YRP isolates on G. lucidum.—In the in vitro experiment all 17 YRP isolates successfully colonized the PDA slants in which *G. lucidum* was growing; however *X. sphaerospora*, *A. cuboidea* and *S. pupurascens* failed to colonize the slants. YRP-inoculated slants developed a yellow-brown diffusing pigment, whereas slants inoculated with other fungi remained clear (FIG. 2). When mycelium from under the YRP-inoculated plugs was transferred onto new PDA plates only YRP isolates were recovered, suggesting that YRP isolates were pathogenic to *G. lucidum*. In the cases of inoculations with other tested species only *G. lucidum* was recovered from subcultured PDA plates.

In the in vivo test, in which a spore suspension of YRP isolate H55 was sprayed on *G. lucidum*-inoculated oak logs at 0, 4, 6, 8 and 10 wk post inoculation, the number of primordia and dry weight of fruiting bodies decreased according to time post inoculation. The most severe effects occurred after 0 and 4 wk when the YRP inoculation inhibited the growth of *G. lucidum* (FIG. 3). After 1 y we re-isolated YRP having similar morphology to the H55 isolate from the oak logs (data not shown), which confirmed Koch's postulates.

AFLP data.—In the AFLP analysis six primer pair combinations tested on 60 YRP isolates resulted in 217 AFLP bands, of which 11 (5%) were polymorphic.

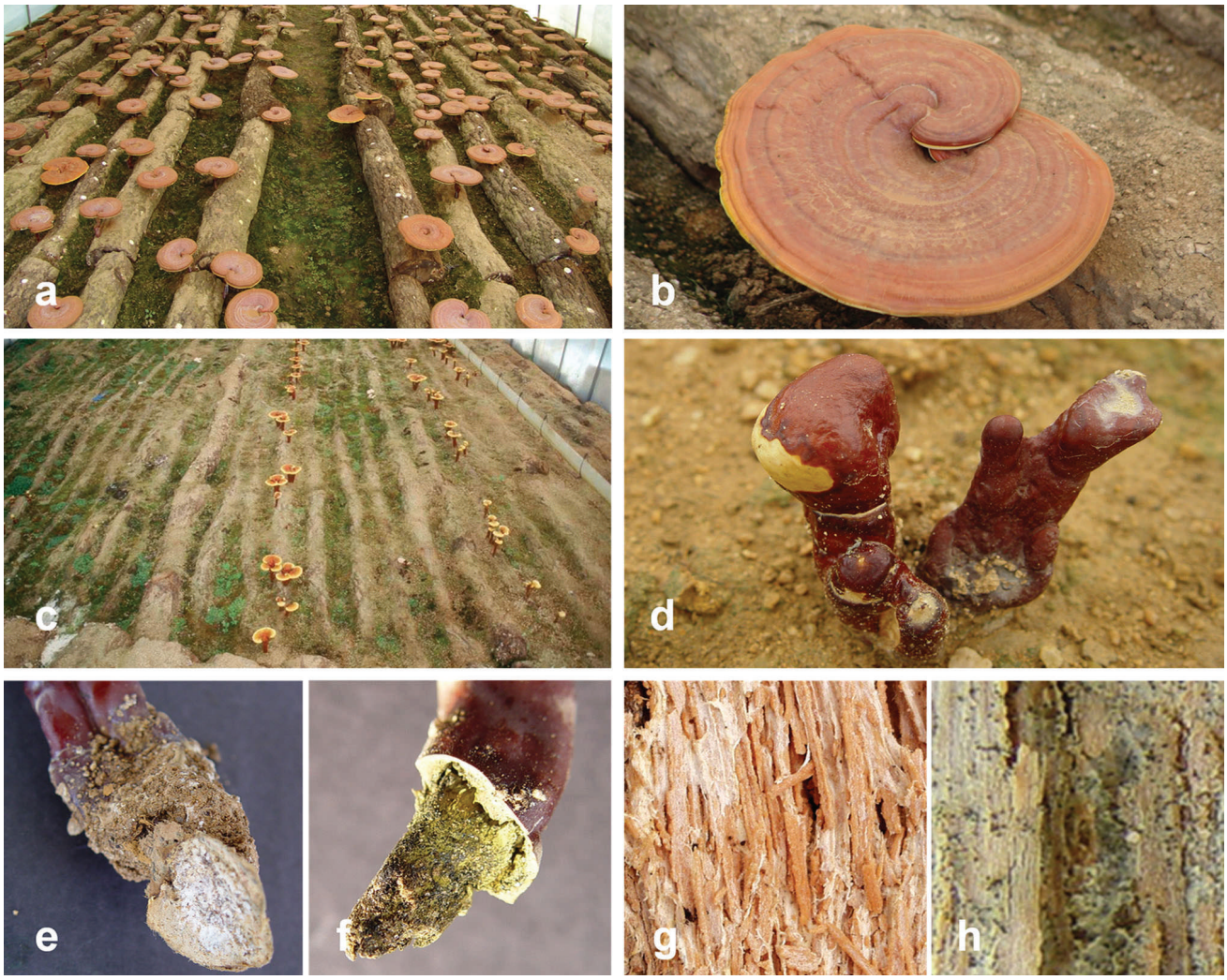

FIG. 1. Morphology of healthy and YRP-infected *G. lucidum*. a, b. Healthy fruiting bodies on long logs and on an oak log cultivated in soil. c. Yellow rot infection showing fruiting bodies formed on only a few logs. d. Malformed pileus caused by yellow rot. e. Actively growing white mycelium at the base of a healthy mushroom. f. Greenish yellow discoloration of inner tissue at the base of a diseased mushroom. g. Inner tissue of an uninfected oak log showing white mycelial mat of *G. lucidum* on the surface. h. Inner tissue of diseased oak logs showing greenish yellow discoloration. Black specks are ascomata.

The AFLP data divided the YRP population into two clonal groups, in which the proportion of the two groups was 56 to 4. The four clonal isolates were from one restricted region of Jeonnam Province, and we could not find any hybrid isolates between the two groups.

Phylogenetic analyses.—In SSU, ITS and RPB2 analyses the YRP isolates grouped with *Xylogone sphaerospora*, *Arthrographis cuboidea* and *Scytalidium lignicola* in a strongly supported clade. The SSU dataset included 43 taxa and comprised 1699 characters of which 1250 were constant, 226 were parsimony informative and 223 parsimony uninformative. The tree is one of 705 equally parsimonious trees (FIG. 4). YRP, *X. sphaerospora* and most *A. cuboidea* isolates were placed in three strongly supported subclades (98%, 95% and 98% BS respectively). The maximum a posteriori tree from the Bayesian analysis showed similar topology to the MP tree and support for the three subclades was higher at 100% PP. *Sporendonema purpurascens* and *Pithoascus langeronii* Arx (anamorph *Arthrographis kalrae*) were excluded from the clade and grouped with some Pezizales. The 45-taxon ITS dataset had an aligned length of 690 characters, of which 259 were constant, 357 informative and 74 uninformative; the tree was one of 100 equally parsimonious trees (FIG. 5). The RPB2 analysis yielded a single tree (FIG. 6) from a dataset comprising 26 taxa with an aligned length of 2235 characters, including 154 uninformative positions and 826 parsimony informative positions. The ITS and RPB2

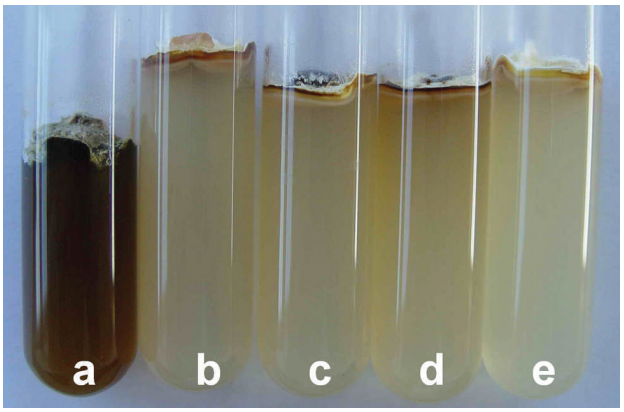

FIG. 2. In vitro pathogenicity test on *G. lucidum*. Yellow-brown PDA slant (a) shows successful colonization of YRP isolate H55 on *G. lucidum*. Other test isolates, *Xylogone sphaerospora* ATCC 34392 (b), *Sporendonema purpurascens* UAMH 1497 (c), *Arthrographis cuboidea* UAMH 676 (d), failed to colonize the *Ganoderma* mycelium, resulting in no color change of PDA slants. In mock control (e) only agar plug of *Ganoderma* was inoculated on PDA.

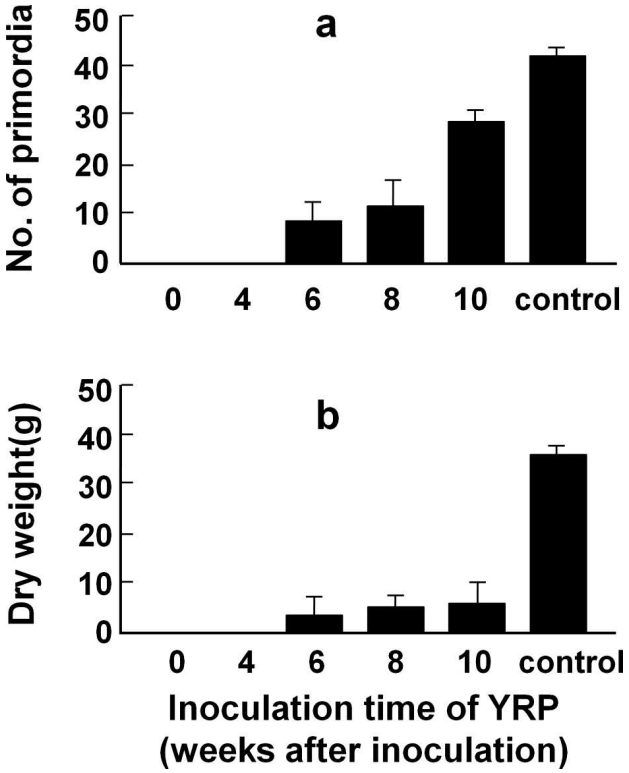

FIG. 3. In vivo pathogenicity test of YRP isolate H55 on *Ganoderm lucidum*-inoculated wood logs. Number of primordia (a) and dry weight of fruiting bodies (b) per wood log inoculated with YRP isolate.

trees showed similar topographies within the YRP-*Xylogone* clade. The 11 YRP isolates formed a monophyletic group (BS 100%) and included the mushroom isolate TPML 97003 that had been identified previously as *X. sphaerospora* (Lee et al. 1996). Isolates of *X. sphaerospora*, *A. cuboidea* and *S. lignicola* each formed their own strongly supported clades (BS 100%). *Arthrographis cuboidea* formed a sister clade with *X. sphaerospora* in both analyses, but a grouping of YRP with *S. lignicola* was weakly supported only in the ITS analysis. *Arthrographis lignicola* Sigler, another wood-associated species, was distinct from both *A. kalrae*, the type species, and *A. cuboidea* in the ITS analysis.

A Group I intron was detected in SSU sequences of all 11 isolates of YRP and in most of the isolates of *A. cuboidea* and removed before analysis. The YRP intron was 554 bp long and corresponded to subunit position 1199 in a sequence of *Escherichia coli* (GenBank J01695, Gargas et al. 1995). The intron in *A. cuboidea* occurred at the same position but was shorter, 432–440 bp long, and it occurred in six of eight isolates.

*Morphological features of the YRP-*Xylogone *clade and* Sporendonema purpurascens.—Members of the YRP-*Xylogone* clade, as distinguished in the phylogenetic analyses, demonstrated common morphological features including fast-growing colonies on PDA, production of strong yellow to golden brown diffusible pigments, vegetative hyphae less than 6 μm wide bearing unbranched fertile hyphae that fragment by schizolytic dehiscence to form narrow arthroconidia up to 3 μm wide. Taxa within the clade differed in colonial pigmentation and texture, arthroconidial features, production of ascomata and chlamydospores. We summarized these morphological features (TABLE II, FIG 7). YRP, represented by isolate H55 (UAMH 10320), produced floccose, yellow to greenish yellow colonies on PDA and yellow pigment diffused into the medium within 3–4 d. Colonies of *X. sphaerospora* and *S. lignicola* were felt-like, flat, appressed to the medium, paler orange white to gray, and darkened to grayish brown with production of ascomata or chlamydospores respectively. Colonies of *A. cuboidea* were flocculent, powdery, producing copious pigment that darkened to bluish-black with age. Both YRP and *X. sphaerospora* produced non-ostiolate ascomata without appendages and smooth, hyaline ascospores, but the ascomata and ascospores of YRP isolates were larger than those of *X. sphaerospora*. Arthroconidial development was similar in YRP, *A. cuboidea* and *X. sphaerospora* in that the lateral fertile hyphae initially were sparingly septate, then became closely septate and fragmented into rectangular to cylindrical arthroconidia. In *A. cuboidea* the fertile hyphae occurred in dense tufts and arthroconidia were frequently wider than long,

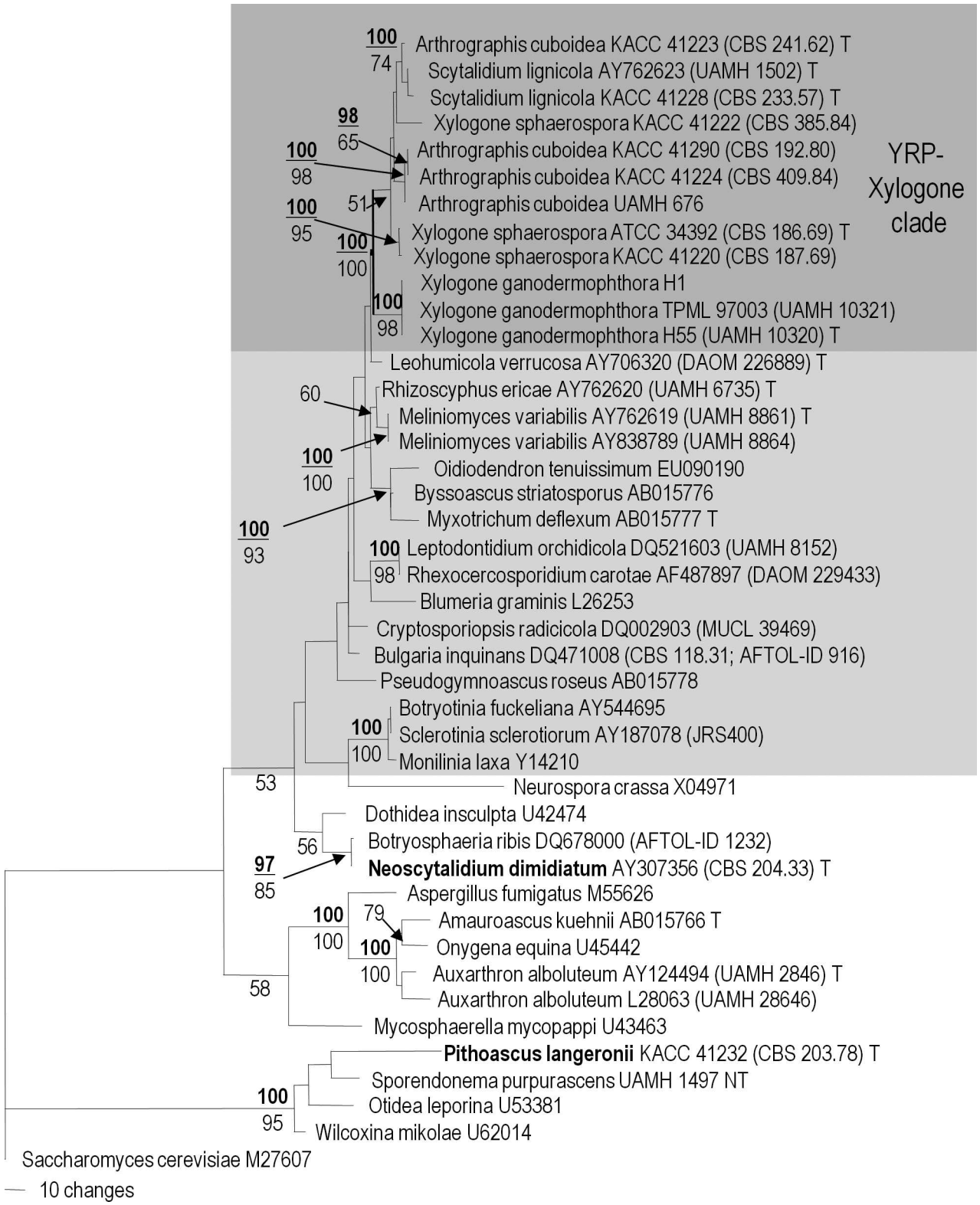

FIG. 4. One of 705 equally parsimonious trees (CI 0.653, RI 0.724, HI 0.347) based on SSU sequences from a 43 taxon dataset showing the placement of the YRP, *Xylogone sphaerospora*, *Arthrographis cuboidea* and *Scytalidium lignicola* among members of the Leotiomycetes (shaded box). PP (≥ 95%, above branches) from Bayesian analysis and BS values (≥ 50%, below the branches) from MP analysis are shown. *Saccharomyces cerevisiae* was outgroup taxon. GenBank accession numbers for isolates newly sequenced are provided (TABLE I). T = ex-type culture.

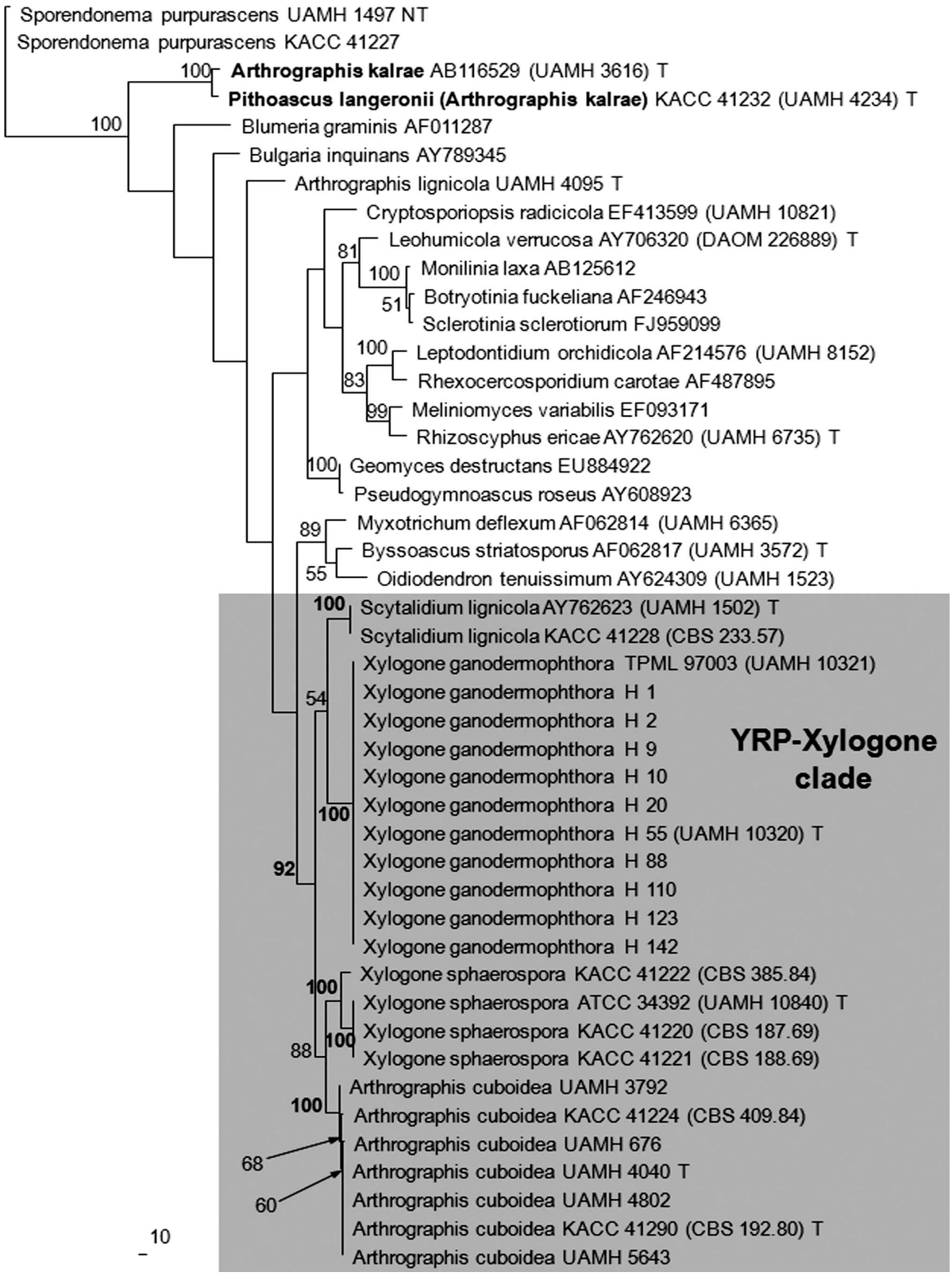

FIG. 5. One of 100 equally parsimonious trees (CI 0.564, RI 0.757, HI 0.436) based on the ITS dataset of 45 strains showing the phylogenetic relationships among YRP, *Xylogone sphaerospora*, *Arthrographis cuboidea* and *Scytalidium lignicola*. Bootstrap values above 50% are shown. *Sporendonema purpurascens* was outgroup taxon. GenBank accession numbers for isolates newly sequenced are provided (TABLE I). T = ex-type culture.

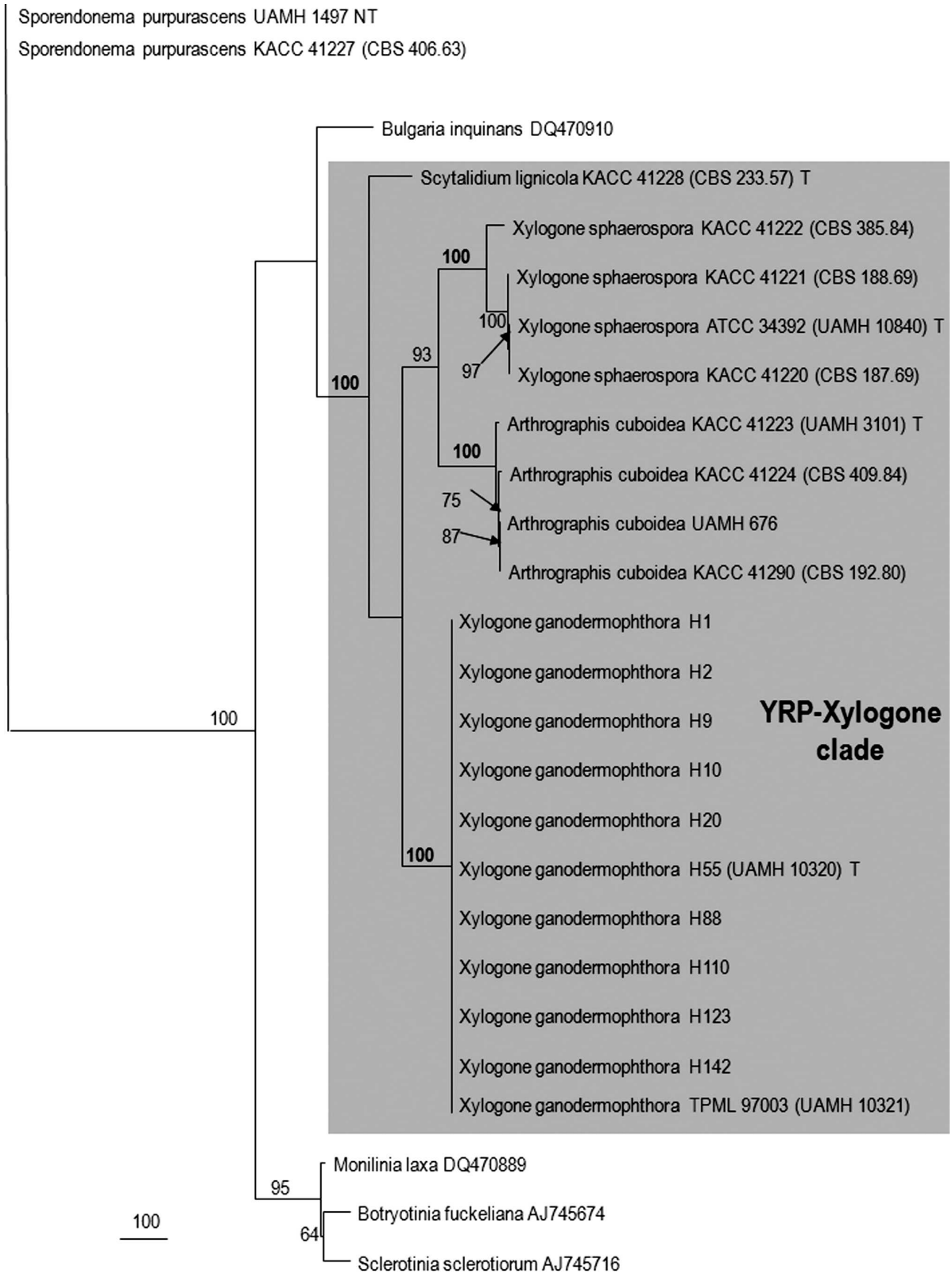

FIG. 6. Single most parsimonious tree (CI 0.754, RI 0.858, HI 0.246) based on RPB2 dataset of 26 strains. Bootstrap values above 50% are shown. *Sporendonema purpurascens* was outgroup taxon. GenBank accession numbers for isolates newly sequenced are provided (TABLE I). T = ex-type culture.

TABLE II. Morphological features of the YRP-*Xylogone* clade compared with *Sporendonema purpurascens*

	Colony characteristics[b]			Microscopic characteristics						
Species[a]	Colony diam (cm)	Texture and color[c]	Diffusible pigment	Vegetative hyphae (μm)	Arthroconidia[d] (μm)	Dehiscence	Separating cell	Chlamydospores	Ascomata[e] (μm)	Ascospores[c] (μm)
YRP (H55)	8.5	Floccose, dark yellow (4C8)	Brownish yellow (5C8)	Medium (1.2–6)	1.5–4 × 1.5–3	Schizolytic	–	–	45–165	3.3–4.3 × 3–4
Xylogone sphaerospora	8.5	Felt-like, thin, orange white (5A2)	Orange brown (6B/D8)	Medium (1.5–5.5)	1.8–5.5 × 2–3	Schizolytic	–	+ (hyaline, solitary or catenulate)	25–50	4–5.5 × 2.5–4.5
Arthrographis cuboidea	8.5	Flocculent, powdery, orange white (5A3) to brownish orange (6C5)	Reddish golden (6C6)	Medium (3–5.5)	1.8–3.5 × 1.5–2.5	Schizolytic	–	–	–	–
Scytalidium lignicola	7.5	Felt-like, thin, light to grayish orange (6A/B4)	Orange (6B7)	Narrow (1–3)	4–9 × 1.5–2.5	Schizolytic	–	+ (brown, catenulate)	–	–
Sporendonema purpurascens	2 on CER	Floccose, dull red (8B3)	Dull red (8B3)	Broad (5–8)	4–12 × 4–7	Rhexolytic	+	–	–	–

[a] Represented by ex-type cultures.
[b] On PDA after 7d at 25 C except *S. purpurascens* on CER.
[c] Color terms following Kornerup and Wanscher (1978).
[d] On CER in slide culture preparations after 5–7 d at 25 C.
[e] On OAT agar after 7d at 25 C; plates observed 5 wk.

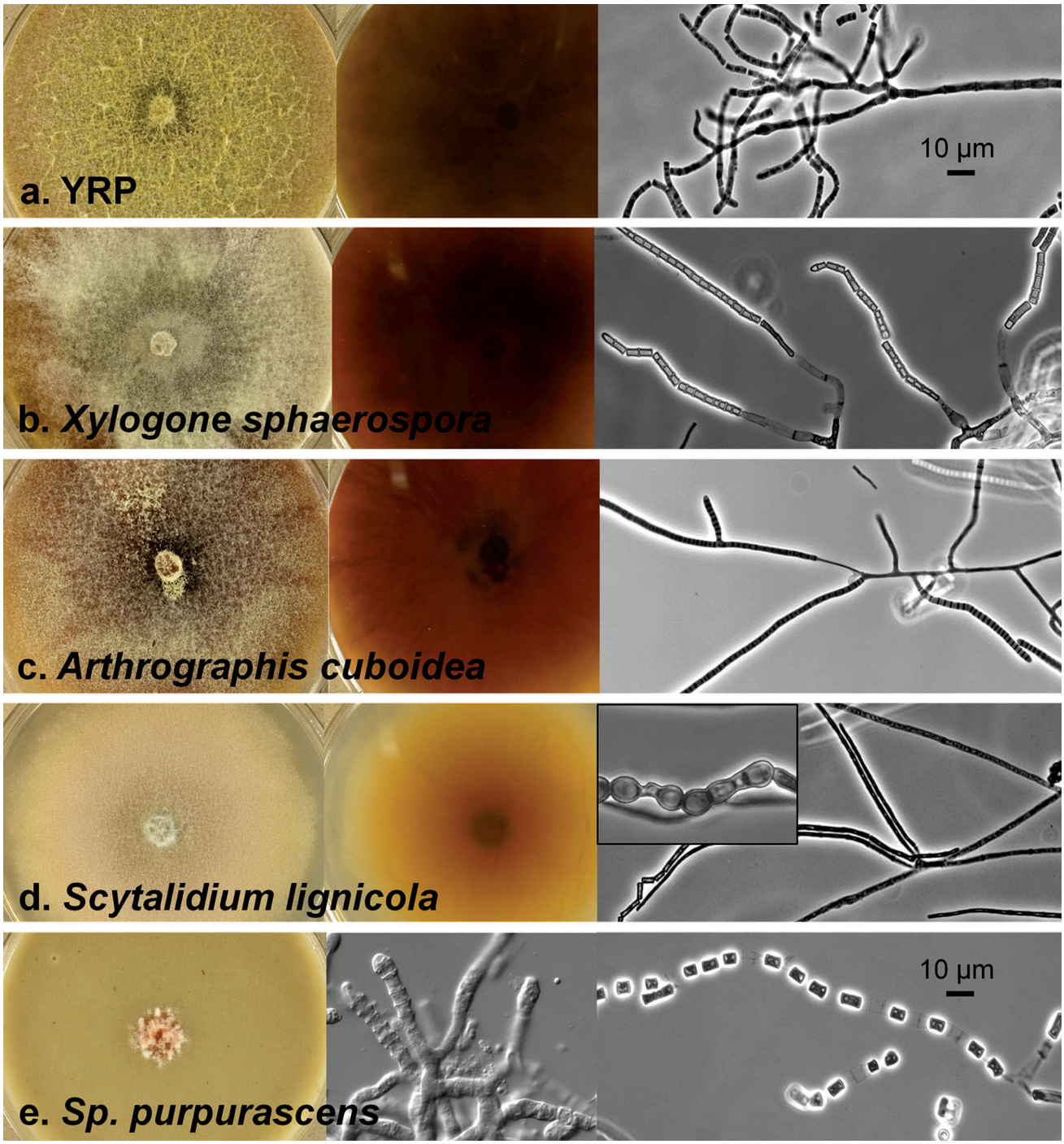

FIG. 7. Morphological features of the YRP-*Xylogone* clade and *Sporendonema purpurascens*. a–d. Comparison of YRP isolate H55 (UAMH 10320) and ex-type cultures of *Xylogone sphaerospora*, *Arthrographis cuboidea* and *Scytalidium lignicola* showing colony obverse and reverse on PDA after 7 d at 25 C and schizolytically dehiscing arthroconidia produced from narrow fertile hyphae. Inset (d) shows catenulate chlamydospores of *S. lignicola*. (e) *Sporendonema purpurascens* showing colony on CER after 7 d at 25 C and rhexolytically dehiscing arthroconidia produced from broad hyphae.

whereas in YRP and *X. sphaerospora* they were less commonly so. In *X. sphaerospora* arthroconidia had double-contoured walls and tended to remain connected in chains of 2–4 cells. A few hyaline to pale brown, solitary to catenulate chlamydospores also were produced. *S. lignicola* differed from all other species by producing abundant brown catenulate chlamydospores and narrower, longer arthroconidia.

In addition to being phylogenetically distant from the YRP-*Xylogone* clade *S. purpurascens* differed morphologically by failing to grow on PDA within 7 d and by having broader hyphae that formed alternate arthroconidia (i.e. conidia separated by one or more cells that undergo lytic dehiscence) (FIG. 7e). Colonies on cereal agar were pink and accompanied by a pink diffusible pigment.

TAXONOMY

Based on multigene phylogenies and morphological similarities, we describe the YRP isolates as *Xylogone ganodermophthora* sp. nov. and classify the arthroconidial anamorphs of both *Xylogone* species in *Scytalidium*. *A. cuboidea* also is reclassified in *Scytalidium* because it is unrelated to *A. kalrae* (Tewari and Macpherson) Sigler and J.W. Carm., the type species of *Arthrographis*.

Xylogone ganodermophthora Kang, Sigler, Lee & Yun, sp. nov. FIGS. 7a, 8, 9a

Mycobank MB515401

Coloniae on PDA ad 25 C, rapide crescentes, floccosae, flavae vel flavoviridae, vel brunneae; cum pigmento diffuso flavo vel flavovirido vel flavobrunneo. Hyphae septatae, ramosae, 1.2–6 μm latae. Ascomata ex hyphis volutis, cleistothecia, nonostiolata, brunnea, cum peridio texturae epidermoideae, appendices absunt, globosa, 45–165 μm diam. Asci per mediam partem dispersi, subglobosi vel globosi, octospori, tenuiter tunicati, rapide evanescentes, 9–11 × 7–10.5 μm. Ascosporae hyalinae, laeves, refractae, subglobosae vel globosae, 3.3–4.3 × 3–4 μm (mediocriter 3.8 × 3.6 μm).

Anamorph: **Scytalidium ganodermophthorum** Kang, Sigler, Lee & Yun, sp. nov.

Mycobank MB515404

Hyphae fertiles e simplicibus conidiophoris, primum cum septis paucis, deinde sensim multiseptatae, denique in arthroconidia fractae. Conidia arthroconidia, hyalina vel flava, cylindrica, 1.5–4 × 1.5–3 μm (mediocriter 2.5 × 2 μm), interdum latiores quam longiores, sine cellulis separandibus vel disiunctoribus.

HOLOTYPE: Korea, Gyeonggi Province, Yangpyeong, from diseased fruit body of cultivated *Ganoderma lucidum*, Oct 2001, H.J. Kang; UAMH 10320 (H55, KACC 93082P), preserved as dried colonies and living culture. PARATYPE: Korea, Daejeon, Shintanjin, from diseased wood log used for *G. lucidum* cultivation, G.K. Lee; UAMH 10321 (TPML 97003), preserved as dried colonies and living culture. (Additional isolates are listed in TABLE I.)

Colonies on PDA at 25 C growing rapidly, floccose, yellowish to yellowish green, becoming brown with development of ascomata, producing strong yellow to yellowish green diffusible pigment. *Ascomata* arising from coiled ascogenous hyphae, nonostiolate, initially pale brown, becoming dark brown at maturity with wall of textura epidermoidea, and without appendages, globose, 45–165 μm diam. *Asci* dispersed in centrum, subglobose or globose, thin-walled, quickly evanescent, eight-spored, 9–11 × 7–10.5 μm. *Ascospores* hyaline, smooth, with refractive walls, subglobose to globose, 3.3–4.3 × 3–4 μm (average 3.8 × 3.6 μm). *Fertile hyphae* borne laterally on simple conidiophores, initially sparingly septate, becoming abundantly septate and fragmenting into arthroconidia. *Arthroconidia* hyaline to yellow, rectangular to cylindrical, 1.5–4 × 1.5–3 μm (average 2.5 × 2 μm), sometimes wider than long, lacking separating cells or disjunctors. *Cellulolytic* as determined by ability to degrade a cellophane membrane layered over agar (Sigler and Carmichael 1976).

Xylogone sphaerospora Arx & T. Nilsson, Svensk Bot Tidskr 63:345 FIGS. 7b, 9b

Colonies fast growing, appressed to the medium, initially orange-white, darkening to gray with development of abundant ascomata. *Ascomata* cleistothecia, arising from coiled ascogenous hyphae, initially subhyaline, becoming dark brown at maturity with wall of textura epidermoidea, globose, 25–50 μm diam (50–90 μm diam in original description); lacking setae; *asci* quickly evanescent, 7.5–12 × 6.5–10 μm. *Ascospores* smooth, hyaline, subglobose, 4–5.5 × 2.5–4.5 μm (average 4 × 3.4 μm).

HOLOTYPE: Sweden; Angermanland, örnsköldsvik, *Pinus sylvestris* pulpwood chip, Dec 1966, T. Nilsson, CBS 186.69 (ATCC 34392; UAMH 10840).

Anamorph: **Scytalidium sphaerosporum** Sigler & Kang, sp. nov.

Mycobank MB515402.

Hyphae fertiles e simplicibus conidiophoris, in arthroconidia fractae, saepe in catenis persistentibus. Arthroconidia hyalina vel subhyalina, cylindrica, 1.8–5.5 × 2–3 μm (mediocriter 3.4 × 2.5 μm), raro latiores quam longiores, sine cellulis separantibus vel disiunctoribus.

Fertile hyphae borne laterally on simple conidiophores, fragmenting into arthroconidia, sometimes remaining connected in chains of 2–4. *Arthroconidia* hyaline to subhyaline, cylindrical, 1.8–5 × 2–3 μm (average 3.4 × 2.5 μm), occasionally wider than long, lacking separating cells or disjunctors. *Cellulolytic*.

Scytalidium cuboideum (Sacc. & Ellis) Sigler & Kang, comb. nov. FIG. 7c

Mycobank MB515403

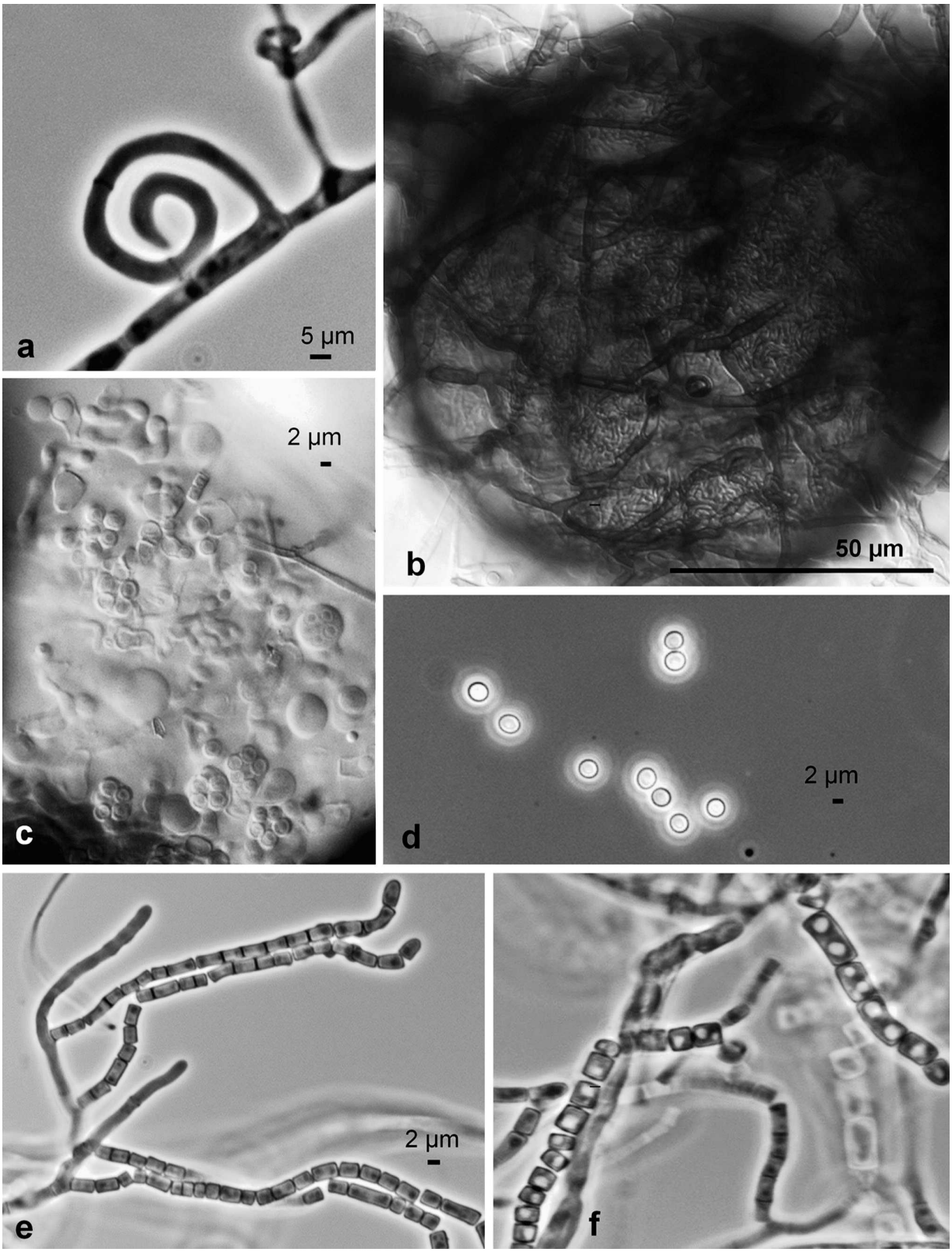

FIG. 8. *Xylogone ganodermophthora* ex-type culture UAMH 10320 (H55) showing (a) coiled ascomatal initial (oil immersion objective), (b) ascocarp with wall of textura epidermoidea, (c) asci, (d) ascospores, (e–f) arthroconidia without disjunctors. (oil immersion objective).

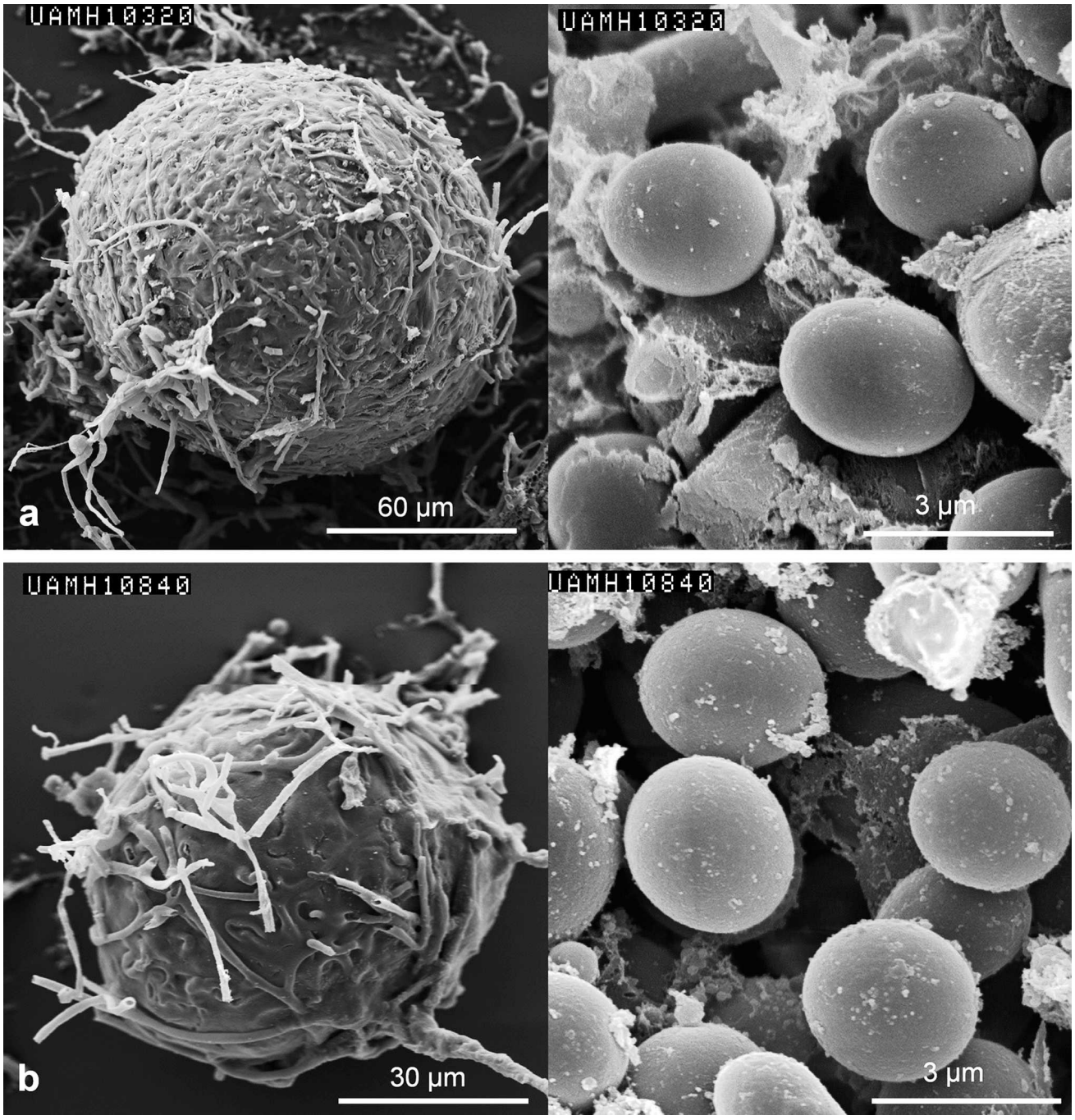

FIG. 9. Scanning electron micrographs of ascocarp and smooth ascospores of (a) *Xylogone ganodermophthora* and (b) *X. sphaerospora* ex-type cultures.

Basionym: *Oospora cuboidea* Sacc. & Ellis, Michelia 2:576, 1882

Synonym: *Arthrographis cuboidea* (Sacc. & Ellis) Sigler, Mycotaxon 4:363, 1976

Geotrichum microsporum Smith, Trans. Br. Mycol. Soc. 45:388, 1962.

Other synonyms and a detailed description are provided in Sigler and Carmichael (1976).

Colonies fast growing, flocculent, powdery, yellowish to brownish orange, front and reverse, developing blue-black diffusible pigments in age. *Unbranched fertile hyphae* borne in dense tufts, fragmenting to form rectangular to cylindrical arthroconidia, 1.8–3.5 × 1.5–2.5 µm (average 2.3 × 2.2 µm), often wider than long. *No teleomorph* known. *Cellulolytic.*

Scytalidium lignicola Pesante 1957 Ann. sper. Agr. n.s. 11:cclxiv. FIG. 7d

Colonies are light to grayish orange darkening to gray or black with development of dark brown, thick-walled, catenulate chlamydospores. *Unbranched fertile lateral branches* fragment to form hyaline, narrow, cylindrical arthroconidia 4–9 × 1.5–2.5 μm, termed ''conidia vera'' by Pesante. Sigler and Carmichael (1976) described in detail the *catenulate chlamydospores* as a second type of arthroconidium, but these are indehiscent structures. *Cellulolytic.*

DISCUSSION

Since it was first reported in the late 1980s yellow rot of *G. lucidum* has become the most destructive fungal disease of cultivated *G. lucidum* in Korea. Despite the importance of YRP in commercial mushroom production, identification and characterization of the fungus has been hampered by its misidentification as *X. sphaerospora* (Lee et al. 1996) and as *A. cuboidea* (Oh et al. 1998). The present study, based on 145 YRP isolates from *G. lucidum* cultivation regions in Korea, provides evidence that YRP constitutes the new species, *X. ganodermophthora*, that groups with *X. sphaerospora*, *Scytalidium cuboidea* and *S. lignicola* within a distinct clade allied with the Leotiomycetes. The pathogenicity tests reported in this study confirm that only *X. ganodermophthora* isolates from Korea cause disease in *G. lucidum*. None of the other species, including *S. purpurascens*, cause any disease in this mushroom. In cultivation sites and in pathogenicity tests *X. ganodermophthora* colonization of *G. lucidum* slants is consistently associated with production of strong yellow pigments that darken to yellowish green or yellowish brown. Oh et al. (1998) suggested that the yellow pigment might be important in host parasitism because the yellow pigment produced on PDA plates inhibited growth of *G. lucidum* by itself. We observed that YRP isolates triggered lysis of the *G. lucidum* cell membrane (H.J. Kang unpubl), but the direct relationship between cell lysis and the yellow pigment still is unknown. If the yellow pigment is a secondary metabolite responsible for host parasitism, then identification and characterization of the pigment might help to understand pathogenicity of YRP. Biochemical and molecular characterization of the yellow pigment is ongoing to determine the potential role of this compound in disease development.

The in vivo pathogenicity test showed that *X. ganodermophthora* can successfully complete its life cycle on *G. lucidum*-inoculated logs but the source of infection is not clear. It seems likely that *X. ganodermophthora* is a wood-associated pathogen because it extensively colonizes and stains the logs (FIG. 1), and the other taxa within the YRP-*Xylogone* clade are found on wood of deciduous and coniferous trees where they produce decay and stain (von Arx and Nilsson 1969, Lee et al. 1996, Sigler and Carmichael 1976, Wang and Zabel 1999). However *X. ganodermophthora* has been recorded occasionally from soil at cultivation areas of *G. lucidum* (Oh et al. 1998). Choi et al. (1998) screened 37 fungicides, including benomyl, and reported that 21 efficiently inhibited growth of *X. ganodermophthora* in vitro but most fungicides also affected the growth of the mushroom. Therefore good hygiene practices including removing contaminated wood and diseased fruiting bodies of *Ganoderma* from the cultivation areas might be the best way to control this disease. The pathogenicity test developed in the present study offers a potential approach to assess biological control methods or efficacy of antifungal chemicals.

To date *X. ganodermophthora* disease of *G. lucidum* is known only from Korea. There have been no reports of similar infection from Japan or other countries that commercially produce this mushroom. AFLP analysis of 60 isolates revealed two clonal groups, the larger of which is widely distributed, and the smaller restricted to the single province of Jeonnam. All isolates within a group were genetically uniform. Only 5% AFLP bands were polymorphic between two groups, and there were no hybrids. The clones could be from asexual reproduction because the germination rate of *X. ganodermophthora* ascospores produced on PDA by self fertilization is low (< 0.1%) (H.J. Kang unpubl) and the fungus produces abundant arthroconidia. Arthroconida might be the major inocula for secondary infection in the *Ganoderma* cultivation farms. However the frequent observation of many ascomata in the diseased logs suggests that the ascospores produced by self cross could be another inoculum source; it is unknown how common sexual recombination by out crossing is in the field.

The four members of the YRP-*Xylogone* clade are united by morphological characteristics including fast-growing colonies, strong diffusible pigments and unbranched fertile hyphae producing narrow, schizolytically dehiscing arthroconidia. Both teleomorphs and anamorphs of *X. ganodermophthora* and *X. sphaer*ospora are strongly similar, which is why isolates of the former have been misidentified as the latter. *X. ganodermophthora* is characterized by presence of a Group I intron, by a fungicolous habit, by yellowish green colonies with diffusible yellow pigments, by larger ascomata, smaller ascospores and by fertile hyphae that fragment readily. *X. sphaerospor*a lacks the Group I intron and is lignicolous, has smaller

ascomata, flat, felt-like brownish colonies and arthroconidia that tend to stay connected in short chains. The arthroconidial development in these species and *S. cuboideum* is strongly similar with fertile hyphae having closely spaced septa and fragmenting into rectangular arthroconidia, but in the latter species fertile hyphae develop in tufts and arthroconidia are commonly broader than long. Arthroconidia of *Scytalidium lignicola* are longer and narrower and are associated with chains of brown chlamydospores.

Hambleton and Sigler (2005) determined that *Scytalidium lignicola* was placed within the Leotiomycetes, but our study is the first to reveal that *S. lignicola* is positioned within a well supported clade distinct from all other Leotiomycetes and that it has affinities with *Xylogone*, also a genus of uncertain disposition. *X. sphaerospora* previously was placed within the *Eurotiales* (von Arx and Nilsson 1969, von Arx 1987) and *Onygenales* (Benny and Kimbrough 1980) but rejected from the latter by Currah (1985) based on the schizolytically dehiscing arthroconidia. Our data (FIG. 4) also confirm the distant relationship between *S. lignicola* and the medically important species, *Neoscytalidium dimidiatum* (Penz.) Crous and Slippers (formerly *Scytalidium dimidiatum* Penz.), which is now classified in the Botryosphaeriaceae (Crous et al. 2006, Tan et al. 2008). These species have been considered mistakenly as synonymous even though they are well differentiated morphologically (Tan et al. 2008).

In conclusion *X. ganodermophthora* is described here based on phylogenetic analyses as well as morphological features, host parasitism and population structure. The recognition of this new taxon will allow further comprehensive study on the biology and development of this destructive pathogen of cultivated *G. lucidum*. Biochemical and molecular characterization of the yellow pigment is ongoing to determine the potential role of this compound in host parasitism.

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