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Cerrena aurantiopora sp. nov. (Polyporaceae) from eastern Asia

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Abstract: A new species of polypore in genus *Cerrena* was discovered in Kangwon Province, Korea. The resupinate basidiocarp and light orange, poroid hymenophore were sufficiently different to be distinguished from previously recorded species of *Cerrena*, *C. consors*, *C. cystidiata*, *C. sclerodepsis* and *C. unicolor*. Based on the results of morphological and phylogenetic analyses, we propose this new polypore as *Cerrena aurantiopora* sp. nov.

Key words: Asia, Polyporales, RPB2, taxonomy, wood-rotting fungi

INTRODUCTION

Genus *Cerrena* is characterized by dimitic or trimitic hyphal systems, nonamyloid, hyaline basidiospores and white rot habit (Ryvarden 1991). Corner (1989) suggested that *Cerrena* be treated as a synonym of *Trametes* based on the hyphal system. Although it is obvious that *Cerrena* is closely related to *Trametes*, as pointed out by Gilbertson and Ryvarden (1986), Cunningham (1965), Corner (1989) and Westhuizen (1963), the bipolar mating type and sclerified generative hyphae of *Cerrena* set it apart as a separate genus (Ryvarden 1991). Recent phylogenetic studies with mitochondrial small subunit ribosomal RNA (mtSSU) and nuclear large subunit ribosomal RNA (nLSU) sequences have suggested that *Cerrena* bears a closer relationship to *Antrodiella*, *Rigidoporus*, *Junghuhnia*, *Spongipellis* and *Steccherinum* (Ko and Jung 1999a, b; Lee 2006) than to *Trametes*.

Genus *Cerrena* typified by *C. unicolor* (Bull.: Fr.) Murrill originally was described by Murrill (1903), and four *Cerrena* species, *C. consors*, *C. cystidiata*, *C. sclerodepsis* and *C. unicolor*, have been accepted so far. Among them *C. cystidiata*, *C. sclerodepsis* and *C. unicolor* are listed at Index Fungorum (http://www.indexfungorum.org/Names/Names.asp). *Cerrena consors* recently was transferred to the genus from *Trametes* by phylogenetic study (Ko and Jung 1999b). *Cerrena cystidiata* has pileate basidiocarps and poroid-daedaleoid hymenophore and was described from Brazil (Rajchenberg and de Meijer 1990). *Cerrena sclerodepsis* has pileate basidiocarps and irregular poroid hymenophore and is distributed in South America (Ryvarden 1984). *Cerrena unicolor* has several infraspecific taxa described, which suggests that this species can be variable.

The national biological inventory in Korea, which was organized by the National Institute of Biological Resources (http://www.nibr.go.kr/english/collect/collect_02.jsp), is currently under way and has yielded numerous collections of fungi from Korea. During this investigation of fungal specimens for NIBR we observed a previously unknown species of *Cerrena*. Its basidiocarp color and microscopic features were similar to *C. consors*. However this species was distinctive in that it had a fully resupinate basidiocarp and poroid hymenophore. Therefore we compared the morphological and molecular features of this unknown species with those of other *Cerrena* species and describe it here as new.

MATERIALS AND METHODS

Morphological comparison.—Specimens and isolates in this study are provided (TABLE I). Macroscopic and microscopic characteristics were based on specimens deposited at National Biological Resources Center (KB) and Seoul National University Herbarium (SNU). Measurements and drawings were made on slide preparations mounted in 3% KOH (Largent et al 1977) with a Nikon Eclipse 80i light microscope. A total of 20 basidiospores and basidia were measured for each collection.

DNA extraction, PCR and sequencing.—Total DNA was extracted from dried specimens or mycelial cultures with an AccuPrep Genomic DNA Extraction Kit (Bioneer, Korea). The internal transcribed spacer (ITS) region and partial nuclear LSU rDNA were amplified with primers ITS5 (White et al 1990) and LR5 (Vilgalys and Hester 1990), and the region between conserved domains six and seven of RPB2 were amplified with primers RPB2-6F1 and RPB2-7R1 (Liu et al 1999). PCR amplification was performed as described by Lee and Jung (2008). Amplified products were viewed by electrophoresis on 1% agarose gels and purified with a PCR purification kit (Bioneer). Sequencing was performed with the primers described above on an ABI 3730XL automated sequencer (Applied Biosystems, USA). Two additional primers, ITS4B (Gardes and Bruns 1993) and LR0R (Rehner and Samuels 1994), were used for sequencing ITS regions and partial nLSU rDNA. The nucleotide sequences have been deposited in GenBank. Accession numbers are provided (TABLE I). Sequences were proofread, edited and aligned with the jPHYDIT program

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TABLE I. *Cerrena* species sequenced for this study

Species	Voucher, strain number	Locality	GenBank accession number		
			ITS	LSU rDNA	RPB2
Cerrena aurantiopora	NIBRFG0000102423[a]	Korea	FJ821532	FJ821521	FJ821546
J.S. Lee & Y.W. Lim	NIBRFG0000102428	Korea	FJ821533	FJ821522	FJ821547
	SNU-m 03110102[b]	Korea	FJ821531	FJ821520	—
Cerrena consors (Berk.)	F20080702KCM29	Korea	FJ821527	FJ821516	FJ821542
K.S. Ko & H.S. Jung	F20080208LYW10	Korea	FJ821528	FJ821517	FJ821543
	F20080405CGY02	Korea	FJ821529	FJ821518	FJ821544
	F20080628LYW14	Korea	FJ821530	FJ821519	FJ821545
Cerrena unicolor (Bull.: Fr.)	F20080329LYW07	Korea	FJ821537	FJ821526	FJ821541
Murrill	F1 (isolate)	Finland	FJ821534	FJ821524	FJ821538
	M32 (isolate)	Sweden	FJ821535	FJ821525	FJ821539
	CU2 (isolate)	Czech Republic	FJ821536	FJ821513	FJ821540

[a] NIBR, National Institute of Biological Resources, Incheon, Korea, holotype.
[b] SNU, Seoul National University Herbarium, Seoul, Korea.

(Jeon et al 2005), and aligned sequence datasets were deposited in TreeBase (S2399 and M4543~4546).

Phylogenetic analyses.—Phylogenetic trees were inferred with neighbor joining (NJ), equally weighted maximum parsimony (MP), and maximum likelihood (ML) methods implemented in PAUP 4.0b10 (Swofford 2002). All gaps were scored as missing data. In the NJ analyses rates for variable sites were assumed equal and no sites were assumed invariable. Data matrices were repaired with a Jukes-Cantor correction. The robustness of inferred NJ topologies was tested by 1000 bootstrap replicates. MP analyses were conducted with a heuristic search using a random stepwise addition of 100 replicates and tree bisection reconnection (TBR) branch swapping and MAXTREES set to auto-increase. A bootstrap analysis was performed with 1000 replicates, with 10 random taxon addition sequences, MAXTREES set to auto-increase and TBR branch swapping to default (Felsenstein 1985). Likelihood settings were parameterized with the best-fit models (TVM + I for ITS, TIM + I for LSU rDNA and GTR + I for RPB2) by AIC in Modeltest 3.7 (Posada and Crandall 1998). ML analyses were conducted with a heuristic search method and ''as is'' taxon addition sequence with TBR branch swapping. Branch support was assessed by bootstrapping with simple taxon addition, 100 replicates and five trees held at each step (Salamin et al 2003). The trees were rooted with the sequences that showed the highest similarity to those of *Cerrena* in GenBank and supported as a sister group of *Cerrena* by a preliminary nLSU phylogenetic analysis of a wide range of Polyporales.

TAXONOMY

Cerrena aurantiopora J.S. Lee & Y.W. Lim, sp. nov. FIG. 1A

MycoBank MB 513165

Basidiocarpus resupinatus, aurantiacus, poris rotundus vel angulatus. Holotypus in NIBR (NIBRFG0000102423).

Etymology. aurantiopora refers to its light orange, poroid hymenophore.

Basidiocarp annual, fully resupinate, attached tightly to the substrate, soft when fresh, hard and brittle when dry, several centimeters to decimeters in extent; hymenophore poroid, pores rounded to somewhat angular, oblong to labyrinthine on vertical substrata, cream to salmon-pink, 3–4 pores per mm, tube length up to 4 mm. *Hyphal system* dimitic; generative hyphae with clamps, thin- to thick-walled, 2.8–3.5 μm diam; skeletal hyphae 3.4–4.5 μm diam. *Basidia* broadly clavate to ovoid, 4 sterigmata, 12.5–14.2 × 4.5–6.0 μm, with a basal clamp. *Basidiospores* ellipsoid, hyaline, 5.0–5.8 × 3.0–3.5 μm, inamyloid. Causing white rot.

Habitat and distribution. KOREA: Gangwon Province, Mount Chiak, on the base of the trunk of live *Celtis sinensis*, 15 Mar 2008, leg. *Y.W. Lim* (HOLOTYPE NIBRFG0000102423); Gangwon Province, Mount Seorak, on stump of *Acer mono*, 19 Mar 2008, *Y.W. Lim* (NIBRFG0000102428); Jeonbuk Province, Mount Deogyu, on trunk of dead *Quercus*, 1 Nov 2003, *J.S. Lee, C. Kim, J.Y. Park* and *K.M. Kim* (SNU-m 03110102).

Notes. Basidiocarp morphology of *C. aurantiopora* is similar to *Oligoporus placentus* in the shape of basidiocarps and hymenophoral configuration, such as the fully resupinate, cream to salmon-pink poroid hymenophore. However microscopic features and host trees distinguish the two. *Oligoporus placentus* has a monomitic hyphal system and cylindrical basidiospores. Hosts for *C. aurantiopora* include deciduous trees, whereas that for *Oligoporus placentus* is dead conifers.

KEY TO SPECIES OF *CERRENA*

1. Paleotropical or temperate-boreal species 2
1. Neotropical (South American) species 4
   2. Basidiocarp resupinate; hymenophore poroid; pore surface light orange . . . . . *C. aurantiopora* J.S. Lee & Y.W. Lim

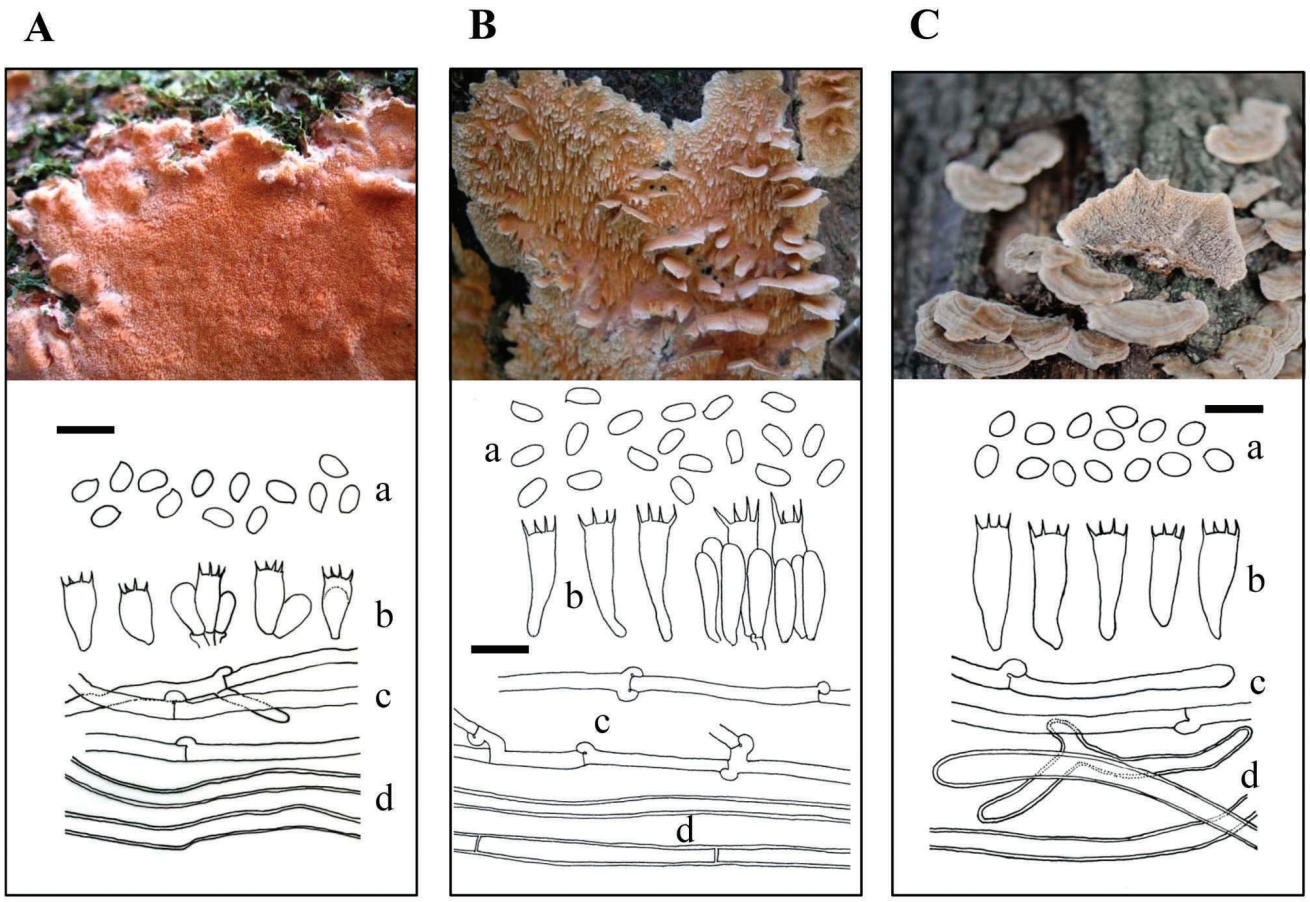

FIG. 1. Basidiocarps and microscopic features of *Cerrena* species. A. *Cerrena aurantiopora* (holotype), B. *C. consors*, and C. *C. unicolor*. a. basidiospores; b. basidia; c. generative hyphae; d. skeletal hyphae. Bars = 10 μm.

2. Basidiocarp effused-reflexed to pileate; hymenophore irpicoid, hydnoid 3
3. Pore surface reddish orange . . . . . *C. consors* (Berk.) K.S. Ko & H.S. Jung
3. Pore surface white to cream . . . . . *C. unicolor* (Bull.: Fr.) Murrill
4. Gloeocystidia present; basidiocarp effused-reflexed to pileate; hymenophore irpicoid, hydnoid . . . . . *C. cystidiata* Rajchenb. & de Meijer
4. Gloeocystidia absent; basidiocarp effused-reflexed to pileate; hymenophore poroid to sinuate . . . . . *C. sclerodepsis* (Berk.) Ryvarden

RESULTS AND DISCUSSION

Cerrena aurantiopora is characterized by its fully resupinate basidiocarp and poroid hymenophore that is cream to light orange. It is found frequently at the base of the trunk of live *Celtis sinensis* (Ulmaceae, Japanese or Chinese hackberry) and/or recently dead wood of *Acer mono* (Aceraceae, painted maple). Basidiocarp morphology of *C. aurantiopora* is similar to that of *Oligoporus placentus*. However *Oligoporus placentus* has a monomitic hyphal system, cylindrical basidiospores, colonizes dead wood of conifers (Gilbertson and Ryvarden 1986) and is phylogenetically distant from the group that includes *Cerrena* species (FIG. 2A). To date four *Cerrena* species, *C. consors*, *C. cystidiata*, *C. sclerodepsis* and *C. unicolor*, have been accepted. *Cerrena aurantiopora* differs from existing *Cerrena* species in the shape of basidiocarps and hymenophoral morphology. All four previous *Cerrena* species are characterized by a toothed hymenophoral configuration, effused-reflexed, dimitic or trimitic hyphal systems, nonamyloid, hyaline basidiospores and white rot physiology. The hyphal system of *C. unicolor* is reported as trimitic (Gilbertson and Ryvarden 1986), however we could not observe binding hyphae from Korean material of *C. unicolor*. In Korea only three species, *C. aurantiopora*, *C. consors* and *C. unicolor*, are known. Microscopic features of the three Korean *Cerrena* species are similar (FIG. 1, TABLE II). All share ellipsoid basidiospores, clavate basidia and a dimitic hyphal system. However the small basidia (12.5–14.2 × 4.5–6.0 μm) of *C. aurantiopora* are clearly distinguished from those of *C. unicolor* (19.4–25.0 × 5.1–6.2 μm) and *C.*

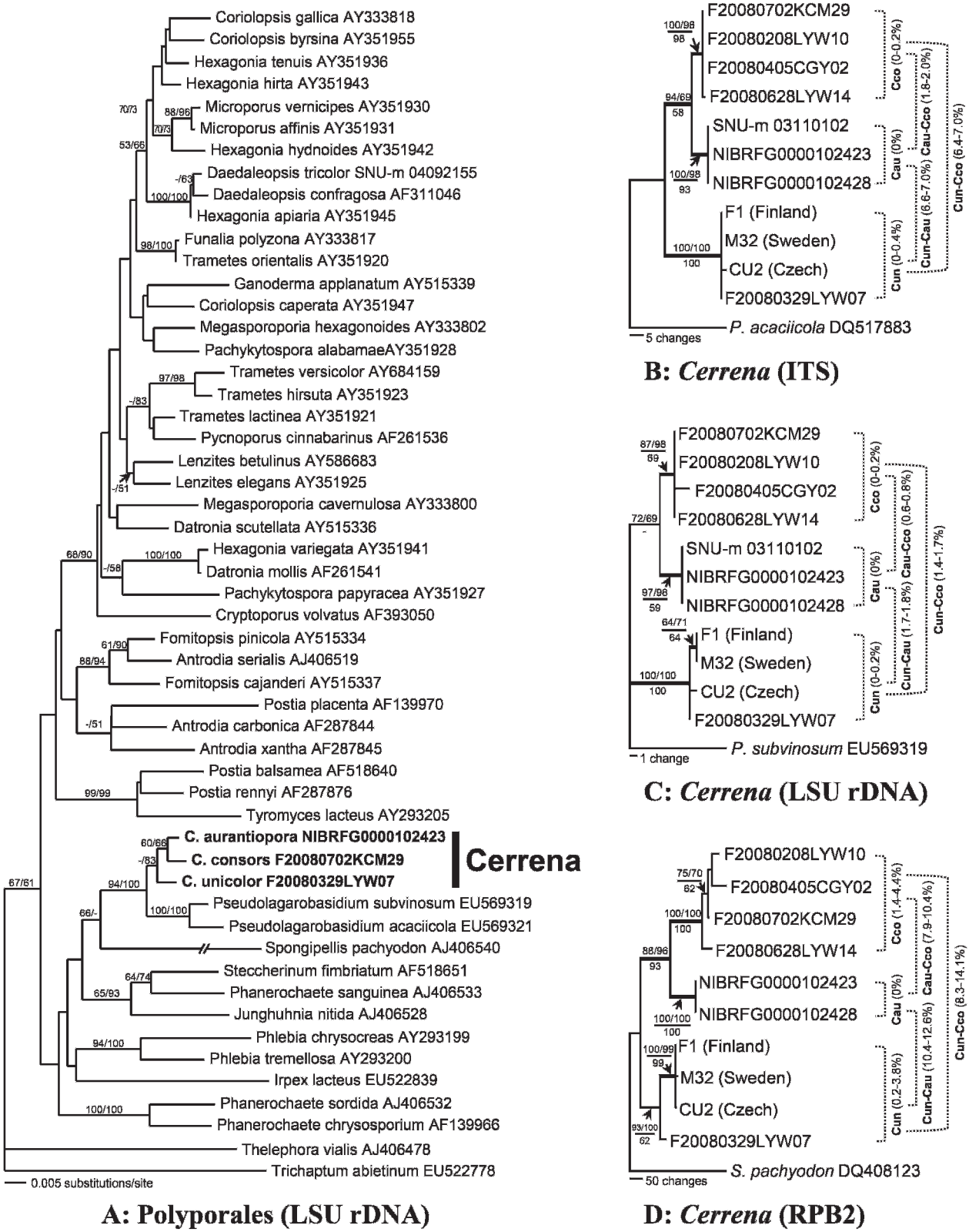

FIG. 2. Phylogenetic position of *Cerrena* within the order Polyporales (A) and relationships of *Cerrena* species (B, C and D). A. NJ tree of *Polyporales* based on sequence data of the nLSU rDNA. *Thelephora vialis* (AJ406478) and *Trichaptum abietinum* (EU522778) were used as outgroup. MP trees of *Cerrena* species based on sequence data of the ITS regions (B), nLSU rDNA (C), and RPB2 gene (D). *Pseudolagarobasidium acaciicola* (DQ517883), *P. subvinosum* (EU569319) and *Spongipellis pachyodon*

TABLE II. Morphological characteristics of *Cerrena* species

Species	*C. aurantiporoides*	*C. consors*	*C. unicolor*
Basidiocarp	Resupinate	Effused-reflexed to pileate	Effused-reflexed to pileate
Hymenophore	Poroid, cream to salmon-pink	Irpicoid, hydnoid, reddish orange	Irpicoid, hydnoid, white to cream
Hyphal system	Dimitic	Dimitic	Dimitic (trimitic)[a]
Basidia	12.5–14.2 × 4.5–6 μm[b]	21–25 × 5–6 μm	19.4–25 × 5.1–6.2 μm
Basidiospores	5–5.8 × 3–3.5 μm	5.7–7 × 2.5–3.3 μm	5.7–6.2 × 3.4–4.2 μm

[a] Gilbertson and Ryvarden (1986) report that *C. unicolor* has a trimitic hyphal system.
[b] Twenty basidia and spores of each specimen were measured.

consors (21.0–25.0 × 5.0–6.0 μm). *Cerrena unicolor* and *C. consors* have similar macro- and microscopic features. *Cerrena aurantiopora* shares common features with *C. consors*, such as basidiocarp color and distribution in only India and eastern Asia, for example Korea and Japan (Imazeki 1943, Ko and Jung 1999b, Núñez and Ryvarden 2001, Ryvarden 1992). *Cerrena unicolor* has white to light cinerous or smoky, flattened teeth. In addition *C. unicolor* is distributed in temperate to boreal zones (Gilbertson and Ryvarden 1986, Palma et al 2005). Basidia (18–20 × 5–6 μm) and ellipsoid basidiospores (5.0–5.8 × 3.2–3.8 μm) reported for South American *C. cystidiata* are similar to those of *C. unicolor* and *C. consors*, but the claviform gloeocystidia distinguish *C. cystidiata* from other *Cerrena* species (Rajchenberg and de Meijer 1990). *Cerrena sclerodepsis* has a trimitic hyphal system, but basidia and basidiospores of the species were not seen (Ryvarden 1984).

To confirm the affinity of genus *Cerrena* and infer the relationships within order Polyporales NJ and MP analyses were carried out based on nLSU rDNA sequences of 52 polyporoid and outgroup taxa, which shows that *Cerrena* has a closer relationship with *Pseudolagarobasidium* and *Spongipellis* than with *Trametes* and *Oligoporus* (FIG. 2A). The alignments of ITS, nLSU rDNA and RPB2 sequences included respectively 558, 862 and 594 nucleotide positions. Little intraspecific sequence variation was observed in the three *Cerrena* species (FIG. 2B–D). However ITS sequence variation among *Cerrena* species was 1.8–7.0%, nLSU rDNA 0.6–1.8%, and RPB2 7.9–14.1%. MP analyses based on sequences of the ITS, nLSU rDNA and RPB2 gene yielded most resolved trees (FIG. 2) (tree length = 93 in ITS, 34 in LSU rDNA and 1126 in RPB2). The three MP trees show that *C. aurantiopora* forms a distinct clade at the 100% confidence level in the ITS tree, 97% in the nLSU rDNA tree and 100% in the RPB2 tree. Three independent lineages that were supported by high bootstrap values in the parsimony analysis were recovered in the NJ and ML analyses. Branches supported in three different analyses are represented as bold branches in all three trees (FIG. 2B–D). All phylogenetic analyses support that *C. aurantiopora* is closer to *C. consors* than to *C. unicolor*. Judging from morphology, geographical differences and molecular analyses, the two Asian *Cerrena* species may form a monophyletic group segregated from the more widely distributed *C. unicolor*.

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(DQ408123) were used as outgroup in each tree. Numbers above branches of all trees before the slash are MP bootstrap proportions, and those after the slash are NJ bootstrap proportions. Values below the branches in B, C and D are ML bootstrap proportions. Branches maintained in three different analyses (MP, ML and NJ analyses) are represented by bold lines. The values in parentheses are the sequence differences within a species and the genetic distances between two species.

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