Crassisporus gen. nov. (Polyporaceae, Basidiomycota) evidenced by morphological characters and phylogenetic analyses with descriptions of four new species

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Abstract

A new poroid wood-inhabiting fungal genus, Crassisporus gen. nov., is proposed on the basis of morphological characters and molecular evidence. The genus is characterized by an annual growth habit, effused-reflexed to pileate basidiocarps with pale yellowish brown to yellowish brown, concentrically zonate or sulcate, and velutinate pileal surface, a trimitic hyphal system with clamped generative hyphae, tissues turning to dark in KOH, oblong to broadly ellipsoid, hyaline, smooth, and slightly thick-walled basidiospores. Phylogenetic analysis based on ITS+nLSU sequences indicate that Crassisporus belongs to the core polyporoid clade. The combined ITS+nLSU+mtSSU+EF1-α+RPB2 sequences dataset of representative taxa in the Polyporaceae demonstrate that Crassisporus is grouped with Haploporus but forms a monophyletic lineage. In addition, four new species of Crassisporus, C. imbricatus, C. leucoporus, C. macroporus, and C. microsporus are described.

Keywords
core polyporoid clade, molecular phylogeny, polypore, taxonomy, wood-decaying fungi

Introduction

Polyporales is one of the most diverse orders of Basidiomycota including more than 1800 described species in 216 genera and 13 families (Kirk et al. 2008). In the last 10 years, many new genera in Polyporales have been established, such as Datroniella B.K.
During the investigations of species diversity and phylogeny of Polyporales, four new species were found that did not belong to any known genus, for which reason, a new genus is established to accommodate them. Morphologically, these four taxa do not fit any of the known polypore taxa. To confirm the position of the new genus, phylogenetic analyses of the new genus and related taxa within Polyporales were carried out based on the internal transcribed spacer (ITS) regions, the large subunit nuclear ribosomal RNA gene (nLSU), the small subunit mitochondrial rRNA gene sequences (mtSSU), the translation elongation factor 1-α gene (EF1-α), and the second largest subunit of RNA polymerase II (RPB2).

**Materials and methods**

**Morphological studies**

The studied specimens were deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions are based on the field notes and measurements of herbarium specimens. Color terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens and observed under a light microscope following Shen et al. (2019). Sections were studied at magnifications up to 1000x using a Nikon E801 microscope and phase contrast illumination (Nikon, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with cotton blue and Melzer’s reagent. Spores were measured from sections cut from the tubes. In presenting the variation of spore size, 5% of measurements were excluded from each end of the range, and were given in parentheses. The following abbreviations were used: KOH, 5% potassium hydroxide; IKI, Melzer’s reagent; IKI-, neither amyloid nor dextrinoid; CB, cotton blue; CB+, cyanophilous; CB-, acyanophilous; L, mean spore length (arithmetic average of all spores); W, mean spore width (arithmetic average of all spores); Q, variation in the L/W ratios between the specimens studied; n (a/b), number of spores (a) measured from given number (b) of specimens.

**DNA extraction and sequencing**

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co. Ltd, Beijing) was used to extract total genomic DNA from dried specimens, and performed the polymerase chain reaction (PCR) according to the manufacturer’s instructions with
Crassisporus gen. nov.

The ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). The nLSU region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The mtSSU region was amplified with primer pairs MS1 and MS2 (White et al. 1990). Part of EF1-α was amplified with primer pairs EF1-983F and EF1-1567R (Rehner and Buckley 2005). RPB2 was amplified with primer pairs fRPB2-5F and fRPB2-7cR or bRPB2-6F and bRPB2-7R (Liu et al. 1999; Matheny 2005). The PCR procedure for ITS, mtSSU and EF1-α was as follows: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, 54 °C for ITS and mtSSU, 54–56 °C for EF1-α for 45 s, 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR procedure for RPB2 was as follows: initial denaturation at 94 °C for 2 min, followed by 10 cycles at 94 °C for 40 s, 60 °C for 40 s and 72 °C for 2 min, then followed by 37 cycles at 94 °C for 45 s, 55–57 °C for 1.5 min and 72 °C for 2 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (Table 1).

Phylogenetic analyses

Sequences used for phylogenetic analyses in this study are listed in Table 1. Sequences of ITS, nLSU, mtSSU, EF1-α, and RPB2 were aligned initially in MAFFT 7 (Katoh and Standley 2013; http://mafft.cbrc.jp/alignment/server/) and then manually adjusted in BioEdit (Hall 1999). Finally, these gene fragments were concatenated with Mesquite 3.2 (Maddison and Maddison 2017) for further phylogenetic analyses. Phylogenies were inferred from the combined 2-gene dataset (ITS+nLSU) and 5-gene dataset (ITS+nLSU+mtSSU+EF1-α+RPB2). *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. obtained from GenBank were used as outgroups to root trees in the 2-gene based analysis. *Laetiporus montanus* Černý ex Tomšovský & Jankovský and *L. sulphureus* (Bull.) Murrill were selected as outgroups to root trees in the 5-gene based analysis. The final concatenated sequence alignments were deposited in TreeBase (https://treebase.org/treebase-web/home.html; submission ID 23521).

Phylogenetic analyses used in this study followed the approach of Zhu et al. (2019) and Song and Cui (2017). Maximum parsimony (MP) analysis was performed in PAUP* v. 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates.
| Species             | Sample no. | Locality            | GenBank accessions |
|---------------------|------------|---------------------|--------------------|
| **Aboritiporus biennis** | EL 65-03  | Sweden              | JN649325           |
| **Abundiporus**      | Cui 10950  | China               | KA456254           |
| **fuscoenteres**     |            |                     | KA456256           |
| **A. puberatus**     | Dai 11310  | China               | KC787568           |
| **A. sclerotatus**   | Dai 11927  | China               | KC787569           |
| **A. violaceus**     | Ryvarden 32807 | Finland         | KF018127           |
| **Arthrodia albida** | CBS 308.82 | USA                 | DQ491414           |
| **A. macra**         | MUAF 887  | Czech Republic      | EU340898           |
| **Bjerkandera adusta** | NBRC 4983 | Unknown             | AB733156           |
| **Cineroremyces lintilladis** | KHL 12078 | Norway             | FN907906           |
| **Climacocystis borealis** | KHL 13318 | Estonia            | JQ031126           |
| **Coriolopsis brunneolens** | Cui 13911 | China              | MK116480*          |
| **C. brunneolens**   | Dai 12180  | China               | KC867414           |
| **C. polyzona**      | BKW004     | Ghana               | JN164978           |
| **C. retropicola**   | Cui 13849  | China               | MK116481*          |
| **C. rutilus**       | Dai 10788  | China               | KC867350           |
| **C. leucopus**      | Cui 16801  | Australia           | MK116488*          |
| **C. macrosporus**   | Cui 14465  | China               | MK116485*          |
| **C. microsporus**   | Dai 16221  | China               | MK116486*          |
| **Daedaleopsis confusa** | Cui 14030 | China              | MK116482*          |
| **D. haianensis**    | Cui 5178   | China               | MK116487*          |
| **Dinia mollis**     | RLG6304sp  | USA                 | JN165002           |
| **Earliella scabrosa** | PR1209    | Puerto Rico        | JN165009           |
| **Fomes fomentarius** | ES 2008-3 | Sweden             | JX109860           |
| **Fomitellia simpina** | JVO610    | Guatemala           |KF274645           |
| **F. simpina**       | Ryvarden 39027 | Puerto Rico |KF274643            |
| **Fomitopsis betulinia** | Dai 11449 | China            | KR605798           |
| **F. pinicola**      | Cui 10405  | China               | KC844852           |
| **Fragilioripia fragilis** | Dai 13080 | China             | KJ734260           |
| **F. fragilis**      | Dai 13559  | China               | KJ734261           |
| **Yuan 5516**        | Dai 10977  | China               | KJ734263           |
| **Funalia gallica**  | Dai 10565  | China               | KJ637388           |
| **F. trogii**        | RLG42865p  | USA                 | JN164993           |
| **Gelatosporia subvermispora** | BRNU 592909 | Czech Republic | FJ496694           |
| **Grammatopeltops subtrigona** | Cui 9035  | China              | JQ845094           |
| **Haploporus latisporus** | Dai 11873 | China          | KU941847           |
| **H. latisporus**    | Dai 10562  | China               | KU941848           |
| **H. odorus**        | Yuan 2365  | China               | KU941846           |
| **H. subtrigona**    | Dai 4222   | China               | KU941849           |
| **Heterobasidion annosum** | PFC 5327  | Greece             | KC492915           |
| **Hexagonia apiaria** | Cui 6447  | China               | KC867362           |
| **H. apiaria**       | Dai 10784  | China               | KX900635           |
| **H. birta**         | Dai 5081   | China               | KX900636           |
| **Hornodoriporus latisporus** | Dai 12054 | China           | KX900639           |

Table 1. Species, specimens and GenBank accession numbers of sequences used in this study.
| Species                  | Sample no. | Locality | GenBank accessions |
|-------------------------|------------|----------|--------------------|
| **Crassisporus gen. nov.** |            |          |                    |
| *H. martius*            | MUCL 41677 | Argentina| FJ411092 FJ393859  |
|                         | MUCL 41678 | Argentina| FJ411093 FJ393860  |
| *Hydopelyssporus fimбриatus* | LR 40855 | Puerto Rico| JN649347 JN649347  |
| *L. sulphureus*          | Cui 12388  | China    | KR187105 KR354486  |
| *L. sulphureus*          | Cui 10011  | China    | JX124704 JX124704  |
| *Lotus setalina*        | HHB9942Sp  | USA      | JN164983 JN164979  |
| *M. majus*              | Cui 10253  | China    | JQ314366 JQ80437   |
| *M. majus*              | Cui 10745  | China    | MK116484 MK116493  |
| *M. subcavernulab*      | Cui 14247  | China    | MG487213 MG487422  |
| *M. versicpes*          | Cui 9283   | China    | KX880618 KX880658  |
| *P. hainaniana*         | Cui 6364   | China    | JQ861744 JX559283  |
| *P. medulla-panis*      | MUCL 49581 | Poland   | FJ411088 FJ393876  |
| *P. substraminea*       | Cui 10177  | China    | JQ001852 JQ001844  |
| *P. substraminea*       | Cui 1091   | China    | JQ001853 JQ001845  |
| *P. subtratamoeba*      | MUCL 47648 | Argentina| FJ411084 FJ393856  |
| *P. micropanis*         | MUCL 43581 | Cuba     | FJ411086 FJ393858  |
| *P. neofulva*           | MUCL 45091 | Cuba     | FJ411080 FJ393852  |
| *P. pendula*            | MUCL 46034 | Cuba     | FJ411081 FJ393853  |
| *Phanerochaete chrysoporium* | BKMF-1767 | USSR     | HQL848566 GQ470643 |
| *Plebius unica*         | KHL 11786  | Sweden   | EU118657 EU118657  |
| *Pycnoporus cinnabarinus* | Dai 14386 | China    | KX880629 KX880667  |
| *Skeletotusis amorpha*  | Miettinen 11038 | Finland | FJ907913 FJ907913  |
| *Stereum bimucron*      | NBRC 6520  | Unknown  | AB733150 AB733325  |
| *Trametes conchifer*    | FP106793Sp | USA      | JN164924 JN164797  |
| *T. pubescens*          | FP101414Sp | USA      | JN164963 JN164811  |
| *T. tephroleuca*        | Cui 7987   | China    | KC848293 KC848378  |
| *T. versicolor*         | FP135156Sp | USA      | JN164919 JN164809  |
| *Truncospora detrita*   | MUCL 42649 | French Guyana | FJ411099 FJ393866 |
| *T. macrospora*         | Cui 8106   | China    | JX941573 JX941597  |
| *T. ochroleuca*         | Cui 5671   | China    | JX941584 JX941602  |
| *T. ochroleuca*         | MUCL 39726 | China Taiwan | FJ411098 FJ393865 |
| *T. ochroleuca*         | Cui 5671   | China    | JX941584 JX941602  |
| *T. ochroleuca*         | Cui 10225  | China    | JX941584 JX941602  |
| *T. ochroleuca*         | Cui 7987   | China    | JX941573 JX941597  |
| *V. rugosiorientalis*   | Cui 5644   | China    | JQ786609 JF706342  |
| *V. rugosiorientalis*   | Cui 7144   | China    | JQ786608 JF706341  |
| *V. rugosiorientalis*   | Cui 10739  | China    | JX941573 JX941597  |
| *V. rugosiorientalis*   | Cui 10739  | China    | JX941573 JX941597  |
| *V. rugosiorientalis*   | Cui 10739  | China    | JX941573 JX941597  |

a Newly generated sequences for this study
(Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each maximum parsimonious tree generated.

RAxML v. 7.2.6 (Stamatakis 2006) was used to perform maximum likelihood (ML) analysis involved 200 ML searches under the GTR+GAMMA model and only the best tree from all searches was kept. In addition, 200 rapid bootstrap replicates were run with the GTR+CAT model to assess the reliability of the nodes.

MrModeltest v. 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best fit evolution model for the combined multi-gene dataset for Bayesian inference (BI). Bayesian inference was calculated with MrBayes v. 3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 2 million generations (ITS+nLSU), for 5 million generations (ITS+nLSU+mtSSU+EF1-α+RPB2) until the split deviation frequency value <0.01, and trees were sampled every 100 generation. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Phylogenetic trees were viewed using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Branches that received bootstrap support for maximum parsimony (MP), maximum likelihood (ML) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP and ML) and 0.95 (BPP) were considered as significantly supported, respectively.

**Results**

**Molecular phylogeny**

The combined 2-gene dataset included sequences from 68 fungal samples representing 59 taxa. The dataset had an aligned length of 2111 characters, of which 1249 characters were constant, 196 were variable and parsimony-uninformative, and 666 were parsimony-informative. MP analysis yielded 37 equally parsimonious trees (TL = 4143, CI = 0.345, RI = 0.617, RC = 0.213, HI = 0.655). Best model for the combined 2-gene dataset estimated and applied in the BI was GTR+I+G, lset nst = 6, rates = in-vgamma; prset statefreqpr = dirichlet (1,1,1,1). MP, ML and BI analyses yielded similar tree topologies with an average standard deviation of split frequencies = 0.006293 (BI), and the ML topology is shown in Figure 1. The phylogeny (Fig. 1) inferred from the combined ITS+nLSU sequences demonstrated seven major clades for 59 species of the Polyporales. The new genus *Crassisporus* embed in the core polyporoid clade and grouped with *Haploporus* Bondartsev & Singer.

The combined 5-gene (ITS, nLSU, mtSSU, EF1-α, RPB2) dataset included sequences of 82 fungal samples representing 57 taxa. The dataset had an aligned
Crassisporus gen. nov.

Figure 1. Phylogeny of *Crassisporus* and related genera in Polyporales based on combined ITS and nLSU sequences. Topology is from ML analysis with parsimony bootstrap support values (≥50 %), maximum likelihood bootstrap support values (≥50 %) and Bayesian posterior probability values (≥0.95).

length of 4306 characters, of which 2521 characters were constant, 258 were variable and parsimony-uninformative, and 1527 were parsimony-informative. MP analysis yielded 1 equally parsimonious tree (TL = 8989, CI = 0.339, RI = 0.620, RC = 0.210, HI = 0.661). Bayesian and ML analyses resulted in a similar topology as the MP analysis, with an average standard deviation of split frequencies = 0.006328 (BI); and the ML topology is shown in Figure 2. A further phylogeny (Fig. 2) inferred from the combined 5-gene dataset was obtained for more representative taxa in the Polyporaceae and showed that the new genus grouped with *Haploporus* clade but distinctly formed a monophyletic lineage.
Figure 2. Phylogeny of *Crassisporus* and related species obtained for more representative taxa in the Polyporaceae based on combined sequences dataset of ITS+nLSU+mtSSU+EF1-α+RPB2. Topology is from ML analysis with parsimony bootstrap support values (≥50%), maximum likelihood bootstrap support values (≥50%), and Bayesian posterior probability values (≥0.95).
Taxonomy

**Crassisporus B.K. Cui & Xing Ji, gen. nov.**
MycoBank: MB 828486

**Notes.** Differs from other genera by the combination of effused-reflexed to pileate basidiocarps, pale yellowish brown to yellowish brown, concentrically zonate or sulcate, velutinate pileal surface, a trimitic hyphal system with clamped generative hyphae, tissues darkening in KOH, and oblong to broadly ellipsoid, hyaline, smooth and slightly thick-walled basidiospores.

**Etymology.** *Crassisporus* (Lat.): referring to thick-walled basidiospores.

**Type species.** *Crassisporus macroporus* B.K. Cui & Xing Ji.

Basidiocarps annual, effused-reflexed to pileate. Pileal surface pale yellowish brown, yellowish brown to umber-brown when dry, concentrically zonate or sulcate, velutinate. Pore surface usually white, cream buff to cinnamon-buff when fresh, buff, pale yellowish brown to yellowish brown when dry. Context pale yellowish brown to yellowish brown, leathery to corky when dry. Hyphal system trimitic with clamped generative hyphae, skeletal hyphae hyaline to pale yellowish brown, binding hyphae hyaline to pale yellowish brown, tissues turning to black in KOH. Cystidia absent, thin-walled cystidioles usually present. Basidiospores oblong to broadly ellipsoid, hyaline, smooth, slightly thick-walled, IKI-, CB-. Causing a white rot.

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**Crassisporus imbricatus** B.K. Cui & Xing Ji, sp. nov.
MycoBank: MB 828487
Figs 3, 4

**Notes.** *Crassisporus imbricatus* is characterized by imbricate basidiocarps, pale greyish-brown pore surface when dry, round to angular pores (3–5 per mm), and oblong ellipsoid basidiospores (10–14 × 4.5–6.2 μm).

**Holotype.** CHINA. Hainan Province, Changjiang County, Bawangling Nature Reserve, on dead angiosperm tree, 9 May 2009, Dai 10788 (BJFC).

**Etymology.** *Imbricatus* (Lat.): referring to the imbricate basidiocarps.

**Description.** Fruitbody: Basidiocarps annual, effused-reflexed to pileate, imbricate, soft corky, without odor or taste when fresh, leathery to corky upon drying. Pilei semicircular or elongated, projecting up to 1.5 cm, 3.5 cm wide, and 2.5 mm thick at base. Pileal surface yellowish brown, velutinate, concentrically zonate. Pore surface buff when fresh, becoming pale greyish brown when dry; sterile margin indistinct, pores round to angular, 3–5 per mm; dissepiments slightly thick, entire to slightly lacerate.
Context yellowish brown, leathery, up to 2.5 mm thick. Tubes concolorous with context, corky, up to 1.5 mm long.

Hyphal structure: Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI-, CB-; tissues turning to black in KOH.

Context: Generative hyphae infrequent, hyaline, thin-walled, unbranched, 2–3.5 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, rarely branched, straight, interwoven, occasionally simple-septate, 2.5–5.5 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 1.2–2.5 μm in diam.

Tubes: Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 1.5–3 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled, occasionally branched, strongly interwoven, rarely simple-septate, 1.5–3.5 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 1–2 μm in diam. Cystidia and cystidioles absent. Basidia clavate, bearing four sterigmata and a basal clamp connection, 19–32 × 9–12 μm; basidioles dominant, in shape similar to basidia, but distinctly smaller.

Spores: Basidiospores oblong ellipsoid, hyaline, slightly thick-walled, smooth, IKI-, CB-, 10–14(−15) × 4.5–6.2(−6.6) μm, L = 12.33 μm, W = 5.34 μm, Q = 2.27–2.36 (n = 60/2).

Type of rot. White rot.

Additional specimen (paratype) examined. CHINA. Hainan Province, Changjiang County, Bawangling Nature Reserve, on fallen branch of *Pinus lateri*, 10 May 2009, Cui 6556 (BJFC).
Figure 4. Microscopic structures of *Crassiporus imbricatus* (drawn from the holotype) **A** basidiospores **B** basidia and basidioles **C** hyphae from trama **D** hyphae from context.
**Crassisporus leucoporus** B.K. Cui & Xing Ji, sp. nov.
Mycobank: MB 828488
Figs 5, 6

**Notes.** *Crassisporus leucoporus* is characterized by a white pore surface when fresh, round to angular pores (3–4 per mm) and oblong ellipsoid basidiospores (8.4–11.2 × 4.2–5.4 μm).

**Holotype.** AUSTRALIA. Queensland, Cairns, Roadside of Mount Whitfield Park, on fallen angiosperm branch, 18 May 2018, Cui 16801 (BJFC).

**Etymology.** *Leucoporus* (Lat.): referring to the white pore surface when fresh.

**Description.** Fruitbody: Basidiocarps annual, effused-reflexed to pileate, corky, without odor or taste when fresh, soft leathery to corky upon drying. Pilei semicircular or elongated, projecting up to 1.5 cm, 3 cm wide, and 6 mm thick at base. Pileal surface yellowish brown to umber-brown, finely velutinate, concentrically sulcate. Pore surface white when fresh, becoming cream, clay buff to pale yellowish brown when dry; sterile margin distinct, cream to pale yellowish brown, up to 1.5 mm wide; pores round to angular, 3–4 per mm; dissepiments slightly thick, entire. Context pale yellowish brown to fulvous, leathery, up to 3 mm thick. Tubes pale yellowish brown, corky, up to 2.5 mm long.

Hyphal structure: Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI-, CB-; tissues turning to black in KOH.

Context: Generative hyphae infrequent, hyaline, thin-walled, 1.1–2.6 μm in diam.; skeletal hyphae in context dominant, pale yellowish brown, thick-walled with a narrow to wide lumen, unbranched, straight, interwoven, occasionally simple-septate, 1.8–3.9 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 0.7–2.2 μm in diam.

Tubes: Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 1–2.8 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled with a narrow to wide lumen, occasionally branched, more or less straight, strongly interwoven, 0.9–3.3 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 0.8–2.1 μm in diam. Cystidia absent, cystidioles fusoid, sometimes septate at the tips, hyaline, thin-walled, 16.7–28.1 × 5.1–6.3 μm. Basidia clavate, bearing four sterigmata and a basal clamp connection, 18.1–29.2 × 6.4–9.8 μm; basidioles dominant, in shape similar to basidia, but smaller.

Spores: Basidiospores oblong ellipsoid, hyaline, smooth, slightly thick-walled, IKI-, CB-, (7.9–)8.4–11.2(–11.5) × (4–)4.2–5.4(–5.7) μm, L = 9.49 μm, W = 4.79 μm, Q = 1.99 (n = 60/1).

**Type of rot.** White rot.
**Crassisporus gen. nov.**

**Figure 5.** Basidiocarps of *Crassisporus leucoporus*. Scale bars: 1 cm (A); 2 cm (B).

*Crassisporus macroporus* B.K. Cui & Xing Ji, sp. nov.  
MycoBank: MB 828489  
Figs 7, 8

**Notes.** *Crassisporus macroporus* is characterized by cream-buff to cinnamon-buff colored pore surface with distinct sterile margin when fresh, large pores (2–3 per mm) with thin dissepiments, a trimitic hyphal system with cyanophilous skeletal hyphae, the presence of fusoid cystidioles, and oblong ellipsoid basidiospores (9.5–13.2 × 4–6.2 μm).
Figure 6. Microscopic structures of *Crassisporus leucoporus* (drawn from the holotype) A basidiospores B basidia and basidioles C cystidioles D hyphae from trama E hyphae from context.
Crassisporus gen. nov.

Figure 7. Basidiocarps of *Crassisporus macroporus*. Scale bars: 2 cm.

Holotype. CHINA. Guangxi Autonomous Region, Huanjiang County, Mulun Nature Reserve, on fallen angiosperm branch, 10 July 2017, Cui 14468 (BJFC).

Etymology. *Macroporus* (Lat.): referring to the large pores.

Description. Fruitbody: Basidiocarps annual, effused-reflexed to pileate, corky to leathery, without odor or taste when fresh, soft leathery upon drying. Pilei flabelliform, semicircular or elongated, projecting up to 1.5 cm, 4 cm wide and 5 mm thick at base; resupinate part up to 7 cm long, 4 cm wide, and 5 mm thick at center. Pileal surface buff to yellowish brown when fresh, becoming yellowish brown upon drying, finely velutinate, concentrically sulcate. Pore surface cream, buff to cinnamon-buff when fresh, becoming buff, pale yellowish brown to yellowish brown when dry; sterile margin distinct, buff to pale yellowish brown, up to 2 mm wide; pores round to angular,
Figure 8. Microscopic structures of *Crassisporus macroporus* (drawn from the holotype) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hyphae from trama **E** hyphae from context.
Crassisporus gen. nov.

2–3 per mm; dissepiments thin, entire to lacerate. Context yellowish brown to pale yellowish brown, leathery, up to 1.5 mm thick. Tubes pale yellowish brown, corky, up to 2 mm long.

Hyphal structure: Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI-, CB+; tissues turning to black in KOH.

Context: Generative hyphae infrequent, hyaline, thin-walled, unbranched, 1.5–3.5 μm in diam.; skeletal hyphae dominant, pale yellowish brown, thick-walled with a narrow lumen to subsolid, unbranched, more or less straight, interwoven, occasionally simple-septate, 2–5.5 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 1–3 μm in diam.

Tubes: Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 1–2 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, occasionally branched, more or less straight, strongly interwoven, 1.5–3 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 0.8–2 μm in diam. Cystidia absent, cystidioles fusoid, hyaline, thin-walled, 13–20 × 4.5–6 μm. Basidia clavate, bearing four sterigmata and a basal clamp connection, 17–28 × 7–8 μm; basidioles dominant, in shape similar to basidia, but smaller.

Spores: Basidiospores oblong ellipsoid, hyaline, smooth, slightly thick-walled, IKI-, CB-, 9.5–13.2(–14) × 4–6.2(–6.5) μm, L = 11.24 μm, W = 4.96 μm, Q = 2.26–2.31 (n = 60/2).

Type of rot. White rot.

Additional specimen (paratype) examined. CHINA. Guangxi Autonomous Region, Huanjiang County, Mulun Nature Reserve, on dead angiosperm tree, 10 July 2017, Cui 14465 (BJFC).

Crassisporus microsporus B.K. Cui & Xing Ji, sp. nov.

MycoBank: MB 828514

Figs 9, 10

Notes. Crassisporus microsporus is characterized by pileate basidiocarps, small pores (5–7 per mm), and small, broadly ellipsoid basidiospores (4–5 × 3–3.7 μm).

Holotype. CHINA. Yunnan Province, Ruili, Mori Tropical Rainforest Park, on living angiosperm tree, 17 September 2017, Cui 16221 (BJFC).

Etymology. Microsporus (Lat.): referring to the small basidiospores.

Description. Fruitbody: Basidiocarps annual, pileate, sessile, corky, without odor or taste when fresh, soft leathery to corky upon drying. Pilei semicircular, projecting up to 2 cm, 4 cm wide, and 4.5 mm thick at base. Pileal surface pale yellowish brown to yellowish brown, finely velutinate, concentrically sulcate. Pore surface cream, buff to cinnamon-buff when fresh, buff, pale yellowish brown to yellowish brown when dry; sterile margin distinct, buff, up to 1 mm wide; pores round to angular, 5–7 per mm;
Figure 9. Basidiocarps of *Crassisporus microsporus*. Scale bars: 1 cm.

dissepiments slightly thick, entire. Context pale yellowish brown to yellowish brown, leathery to corky when dry, up to 1.5 mm thick. Tubes concolorous with context, soft corky to corky, up to 3 mm long.

Hyphal structure: Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI-, CB-; tissues turning to deep brown in KOH.

Context: Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 1.2–3.5 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled with a narrow lumen, rarely branched, straight, interwoven, occasionally simple-septate, 2.5–6 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 0.8–2.5 μm in diam.
Figure 10. Microscopic structures of *Crassisporus microsporus* (drawn from the holotype) A basidiospores B basidia and basidioles C cystidioles D hyphae from trama E hyphae from context.
Tubes: Generative hyphae infrequent, hyaline, thin-walled, rarely branched, 1.2–3 μm in diam.; skeletal hyphae dominant, hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, moderately branched, more or less straight, strongly interwoven, 1.5–3 μm in diam.; binding hyphae hyaline to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 0.8–2.5 μm in diam. Cystidia absent, cystidioles fusoid, hyaline, thin-walled, 12.5–18 × 4–5.5 μm. Basidia clavate, bearing four sterigmata and a basal clamp connection, 14–21 × 4.5–6 μm; basidioles in shape similar to basidia, but distinctly smaller.

Spores: Basidiospores broadly ellipsoid, hyaline, smooth, slightly thick-walled, IKI-, CB-, 4–5(−5.2) × (−2.8)3–3.7(−3.9) μm, L = 4.5 μm, W =3.23 μm, Q = 1.4 (n = 60/1).

Type of rot. White rot.

Discussion

In the present study, *Crassisporus* is proposed based on morphological characters and phylogenetic analyses. In the ITS+nLSU analysis, *Crassisporus* was nested in the core polyporoid clade with strong support (100% MP, 100% ML, 1.00 BPP; Fig. 1). A further study based on combined ITS+nLSU+mtSSU+EF1-α+RPB2 sequences data indicated that *Crassisporus* grouped with *Haploporus* with low support, but formed a monophyletic lineage with a strong support (100% MP, 100% ML, 1.00 BPP; Fig. 2). Morphologically, *Crassisporus* is characterized by the combination of an annual growth habit, effused-reflexed to pileate basidiocarps, pale yellowish brown to yellowish brown, concentrically zonate or sulcate pilei, velutinate pileal surface, a trimitic hyphal system with clamped generative hyphae, tissues turning to dark in KOH, oblong to broadly ellipsoid, hyaline, smooth and slightly thick-walled basidiospores.

Morphologically, the four *Crassisporus* species can be easily distinguished from each other. *Crassisporus microsporus* differs from other species by its small pores (5–7 per mm), and small broadly ellipsoid basidiospores (4–5 × 3–3.7 μm). Except for *C. imbricatus*, *C. leucoporus*, *C. macroporus*, and *C. microsporus*, all have fusoid cystidioles in the hymenium; moreover, *C. imbricatus* produces imbricate basidiocarps. Previously, the type specimen of *C. imbricatus* was identified as *Coriolopsis byrsina* (Mont.) Ryvarden based on morphological characters (Li and Cui 2010). After careful examination of the basidiospores along with DNA sequences analyses, the specimen was found to represent an unknown taxon. Here, we describe it as a new species of *Crassisporus* based on morphological characters and phylogenetic analysis. *Crassisporus macroporus* may be confused with *C. leucoporus* due to their similarity in effused-reflexed to pileate basidiocarps and oblong ellipsoid basidiospores, but *C. leucoporus* is distinguished from *C. macroporus* by its smaller pores (3–4 per mm), white pore surface when fresh, and acyanophilous skeletal and binding hyphae.

Phylogenetically, *Haploporus* groups together with *Crassisporus* (Figs 1, 2), but the former differs by its annual to perennial growth habit, dimitic to trimitic hyphal system, and ornamented, cyanophilous basidiospores (Shen et al. 2016; Cui et al. 2019). *Crassisporus* is similar to *Hexagonia* Fr. and *Neofomitella* Y.C. Dai, Hai J. Li & Vlasák, because these genera share pileate brown basidiocarps, a trimitic hyphal system
with clamped generative hyphae, and tissues becoming dark in KOH. However, Hexagonia is distinguished from Crassiporus by its larger hexagonal pores and thin-walled basidiospores (Núñez and Ryvarden 2001). Neofomitella differs from Crassiporus in having crusted basidiocarps with the cuticle developing from base to margin, and smaller, thin-walled basidiospores (Li et al. 2014b).

Both Perenniporia Murrill and Crassiporus have hyaline and thick-walled basidiospores, but species of Perenniporia have cyanophilous, and variable dextrinoid skeletal hyphae. In addition, Perenniporia usually has truncate basidiospores (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 2001; Zhao et al. 2013; Cui et al. 2019).

Truncospora Pilát is similar to Crassiporus in having pileate basidiocarps and variable presence of cystidioles. However, Truncospora is distinguished from Crassiporus by variable dextrinoid and cyanophilous skeletal hyphae and truncate, strongly dextrinoid basidiospores (Zhao and Cui 2013; Cui et al. 2019).

Abundisporus Ryvarden and Crassiporus share effused-reflexed or pileate basidiocarps, but Abundisporus differs by its pale-umber to deep-purplish-brown or greyish- to umber-brown context, dimitic hyphal system, and pale-yellowish basidiospores (Ryvarden 1998; Zhao et al. 2015; Cui et al. 2019).

Perenniporiella Decock & Ryvarden also has annual, pileate basidiocarps, and hyaline, thick-walled basidiospores, but it differs from Crassiporus in having a dimitic hyphal system (Decock and Ryvarden 2003).

Grammothelopsis Jülich is similar to Crassiporus in having thick-walled basidiospores; however, it differs from Crassiporus in its resupinate to effused basidiocarps with shallow irregular pores, and variable dextrinoid skeletal hyphae (Robledo and Ryvarden 2007; Zhao and Cui 2012).

### Key to species of Crassiporus

1. Cystidioles absent ................................................................. **C. imbricatus**
   – Cystidioles present ............................................................ **2**

2. Basidiospores broadly ellipsoid ............................................. **C. microsporus**
   – Basidiospores oblong ellipsoid ........................................... **3**

3. Pore surface cream, buff to cinnamon-buff when fresh, pores 2–3 per mm...
   ................................................................. **C. macroporus**
   – Pore surface white when fresh, pores 3–4 per mm.............. **C. leucoporus**

### Acknowledgments

We express our gratitude to Drs Tom May (Royal Botanic Gardens Victoria, Australia) and Yu-Cheng Dai (Beijing Forestry University) for arrangement of and assistance during field collections. The research was financed by the National Natural Science Foundation of China (Project Nos. 31670016, 31870008) and Beijing Forestry University Outstanding Young Talent Cultivation Project (No. 2019JQ03016).
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