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Scutellinia jejuensis (*Pezizales*), a new species from Korea

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Abstract — A new species of *Scutellinia* discovered in Jeju, Korea, *Scutellinia jejuensis*, is formally introduced. A combination of morphological characteristics and sequence analysis of the partial LSU rDNA demonstrates that the fungus represents a species distinct from all other subglobose to globose-spored *Scutellinia* species.

Key words — aculeolate-reticulate, Jeju Island, soil-inhabiting, subglobose ascospores

Introduction

The cosmopolitan genus *Scutellinia* (Cooke) Lambotte forms a well-defined group within the family *Pyronemataceae* (*Pezizales*), which contains a group of fungi characterized by a red or orange colored apothecial ascoma, clothed with stiff, brownish or black hairs along the apothecial rim (Schumacher 1990). They are presumed to be saprobic on wood and humus. Of approximately 50 species recognized in the genus, only ten are characterized by subglobose or globose ascospores, and these are all humus saprotrophs (Schumacher 1990, Yao & Spooner 1995, Liu & Peng 1996, Matočec 2000). During research on cup fungi in Korea, we found a soil-inhabiting ascomycete at Mt. Halla in Jeju Island. Based on a careful macro- and micro- observation, the fungus unequivocally belonged to *Scutellinia* and was close to *S. barlae* (Boud.) Maire 1933, *S. minor* (Velen.) Svrček 1971, *S. rotundisperma* Donadini 1983, and *S. trechispora* (Berk. & Broome) Lambotte 1887 judging by its subglobose to globose ascospores and

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aculeolate or reticulate wall sculpturing. The Korean material, however, differs from them in several aspects. We formally describe this fungus as a new species of *Scutellinia* based on morphological characteristics and sequences analysis of the D1/D2 region of LSU rDNA.

Materials and methods

Free-hand sections of the fresh materials were mounted in distilled water, lactic acid, lacto-cotton blue, and Lugol's reagent (IKI). These preparations were examined in brightfield- and DIC- light microscopy, using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for observations and measurements and a Zeiss AX10 microscope (Carl Zeiss, Göttingen, Germany) mainly for photographs. Measurements were performed at 1000× for ascospores and at 100–400× for other structures; they are reported as follows; minimum-maximum (length) × minimum-maximum (width) [mean length ± standard deviation × mean width ± standard deviation, Q (l/w ratio) = average ± SD].

Genomic DNA was extracted directly from the matured apothecia by the methodology described in Lee and Taylor (1990). To raise the efficiency of extraction, the apothecia were pounded using a sterilized glass rod in the cell lysis step. Primers LR0R and LR5 (Moncalvo et al. 2000) were used for the amplification of D1/D2 region of 28S rDNA. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced on an automatic sequencer (ABI Prism TM 377 DNA Sequencer), using the BigDye™ (Applied Biosystems, Foster City, CA, USA) Cycle Sequencing Kit, version 3.1, with primers identical to those used for amplifications. Sequences were edited with the DNASTAR computer package (DNAStar, Inc., Madison, Wis.), version 5.05, and aligned using CLUSTAL X (Thompson et al. 1997). Phylogenetic trees were obtained from the data using Maximum Likelihood (ML) and Maximum Parsimony (MP). For ML inference, RAxML version 7.0.3 (Stamatakis 2006) was used with all parameters set to default values, using the GTRCAT variant. MP analysis was done using MEGA 4.0 (Tamura et al. 2007), with the default settings of the program, for which 1000 bootstrapping replicates were performed. We selected all the available sequences of *Scutellinia,* and used *Octospora leucoloma* Hedw. (DQ220380) as outgroup taxon according to the result of recent phylogenetic analysis (Perry et al. 2007).

Results

Taxonomic description

Scutellinia jejuensis J.G. Han, Y.J. Choi & H.D. Shin, **sp. nov.** FIGURE 1

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Ascosporae subglobosa cum ornamentum aculeolatum-reticulatum. Scutellinia minor similis, sed in sporis ornamentis non reticulatus et minusculus differt.

HOLOTYPE – on damp soil, Mulchat-oreum, Mt. Halla National Park, Jeju, Korea, 33°25'21.42"N 126°37'18.11"E, alt. 610 m, 5 XI 2008, J.G. Han, Y.J. Choi and H.D. Shin (*KUS-F52411*). Sequence ex-type: GU361609 for D1/D2 region of 28S rDNA.

ETYMOLOGY – the specific epithet refers to the Jeju Island of Korea where the fungus was first collected.

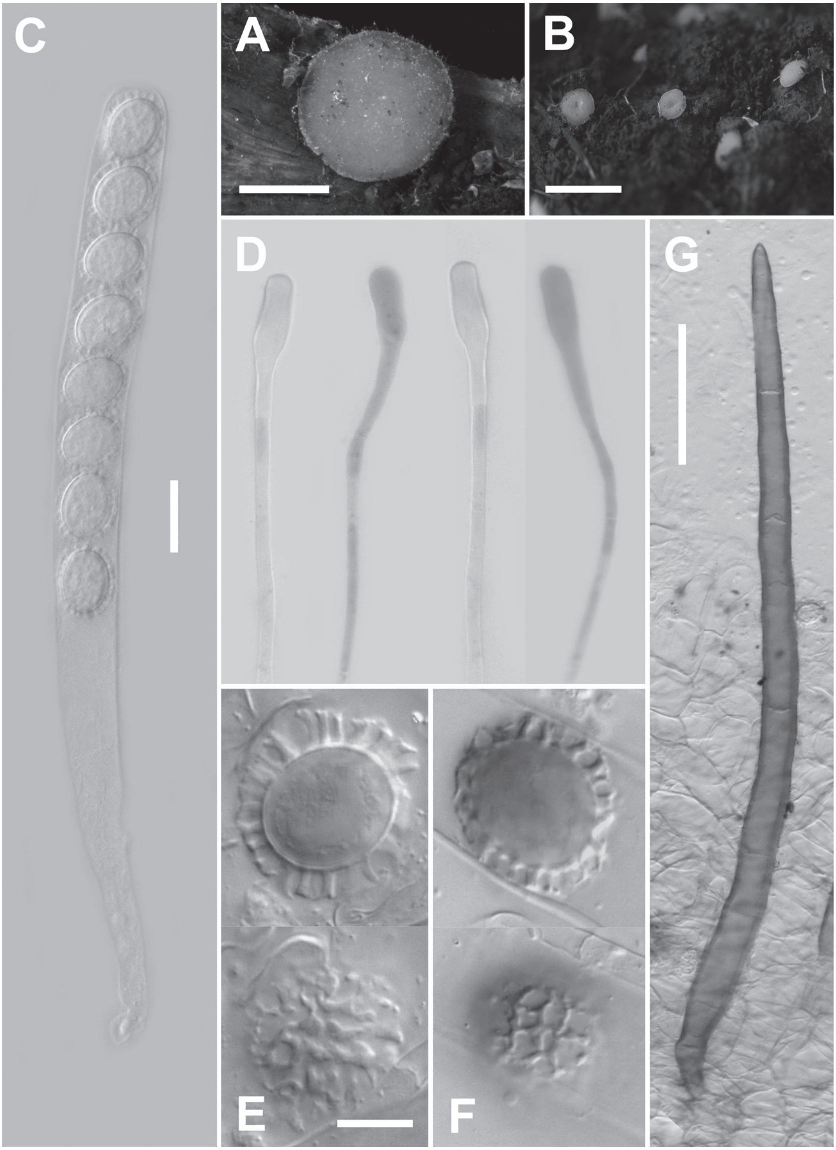

Figure 1. *Scutellinia jejuensis* (holotype *KUS-F52411*). A–B: flesh apothecia on damp soil, C: ascus, apical pore not blued in IKI, D: paraphyses, E–F: subglobose ascospores sculpturing aculeolate ornamentations, note on their interconnections, G: acuminate, thick-walled hair oriented from globose ectal cells.

Scale bars = 2 mm for A, 1 cm for B, 20 µm for C–D, 10 µm for E–F, and 100 µm for G.

Apothecia gregarious, almost sessile. Receptacle at first globose, then becoming shallowly cupulate to discoid, light red, externally covered with short dark brown hairs. Margins concolorous with the receptacle, surrounded by dark brown hairs. Disc up to 6 mm diam., plano-convex, reddish orange to scarlet when fresh, turning yellowish orange when dry. Ectal excipulum hyaline to yellowish, composed of textura globulosa to angularis, thin-walled, cells 43–100 × 28–95 µm. Marginal hairs not differentiated from lateral hairs, cylindric-conical, gradually narrowed to the apex, ventricose, thick-walled, walls 4–6 µm wide, brown to dark brown, with uni- or bi-furcate base, 3–11-septate, 190–640 × 17–30 µm. Asci cylindric, hyaline, 8-spored, walls not becoming blue in IKI without KOH pretreatment, 255–380 × 20–29 µm (318.8 ± 29.7 × 23.9 ± 2.7 µm, n = 26). Ascospores subglobose to globose but rarely broadly ellipsoidal when immature, hyaline, mature spores covered with ornamentations, aculeolate-reticulate, truncate-conical warts, commonly forming sinuate ridges which partly interconnect to a reticum below, 2.5–3 µm high, 0.5–1 µm wide, uniseriate, occupying upper 1/2 of the entire ascus length, 16–23 × 13–19 µm (18.7 ± 1.4 × 15.2 ± 1.1 µm, Q = 1.23 ± 0.08, n = 100) (not including the ornamentation). Paraphyses cylindric, hyaline, septate, unbranched, 3.5–4 µm, apical cells clavate, 37–69 × 6–9 µm (54.0 ± 9.7 × 8.1 ± 0.8 µm, n = 24), not exceeding the asci.

Phylogenetic analysis

The phylogenetic relationship among *Scutellinia* species was inferred from ML and MP analyses of the aligned sequences of the D1/D2 LSU rDNA. The result of the phylogenetic reconstructions by ML inference is shown in Figure 2. In the D1/D2 alignment, 86 of the 880 characters were parsimony-informative, and the parsimony analysis produced eight most parsimonious trees of 279 steps, with a CI and RI of 0.7380 and 0.6409, respectively. Since no differences were found between the tree topologies of the ML and MP analyses, only the ML tree is shown in Figure 2, with the addition of the support values of the MP analysis. In the phylogenetic tree, *S. jejuensis* occupied an independent branch within the genus *Scutellinia* and further formed a well-supported clade with *S. barlae*, *S. hyperborea*, and *S. trechispora* with high supporting values of 97 and 93 in ML and MP, respectively. However, sequence distances among the three species were considerable; 1.7% (15 of 880 nucleotide characters were different) to *S. barlae* and 1.6% (14 of 850) to *S. hyperborea* and *S. trechispora*.

Discussion

Up to now, ten *Scutellinia* species have been known to possess globose or subglobose ascospores, and all are found on soil (Schumacher 1990, Yao & Spooner 1995, Liu & Peng 1996, Matočec 2000). Among them, four species

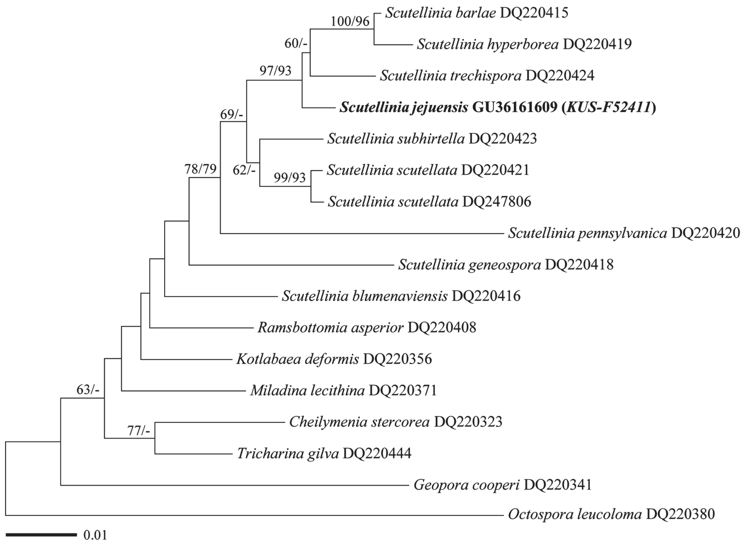

Figure 2. Phylogenetic tree inferred from ML analysis of the partial D1/D2 region of rDNA. Support values (ML BS/MP BS) above 50% are given above the branches. The number of nucleotide changes between taxa is represented by branch length. The scale bar equals the number of nucleotide substitutions per site. *Scutellinia jejuensis* sequence is shown in bold.

(*S. barlae, S. minor, S. rotundisperma, S. trechispora*) show aculeolate or reticulate sculpturing on ascospore surfaces similar to *S. jejuensis*, while in the other species the surface is tuberculate (*S. citrina* (Massee & Crossl.) Y.J. Yao & Spooner 1995, *S. hyperborea* T. Schumach. 1990, *S. paludicola* (Boud.) Le Gal 1966, *S. sinensis* M.H. Liu 1996, *S. tuberculata* Matočec 2000) or spinulose (*S. legaliae* Lohmeyer & Häffner 1983). Additionaly, *S. jejuensis* differs from *S. hyperborea* in low wart height (0.5–0.8 µm) and from other species with perfectly globose spores.

Scutellinia jejuensis is not likely to be confused with the four species sharing similar spore ornamentation because of its unique morphological and molecular characteristics. The perfectly globose ascospores in *S. barlae, S. rotundisperma,* and *S. trechispora* easily distinguish them from the new subglobose-spored species. In addition, *S. jejuensis* differs from *S. barlae* by having more septa in marginal hairs (3–11 vs 1–4). The present species has shorter (190–640 µm) marginal hairs that are not differentiated from the lateral ones. *Scutellinia rotundisperma* and *S. trechispora* show significantly long hairs (600–1000 and

500–2060 µm, respectively). The morphological separation of *S. jejuensis* from *S. barlae* and *S. trechispora* was also clearly supported by the present phylogenetic analysis of D1/D2 region of LSU rDNA. In overlapping dimensions of marginal hairs and subglobose ascospores, *S. jejuensis* was most similar to *S. minor*. The two species can be, however, easily discriminated by several characters: the wall ornamentation is aculeolate-reticulate with often-connected warts in the new species but aculeolate with isolated warts in *S. minor*. The warts in *S. jejuensis* are larger than those in *S. minor* (2.5–3 × 0.5–1 µm vs 1.0–1.8 × ca. 1.5 µm), and the length/width ratio was somewhat higher. Additionally, *S. minor* shows preference to boreo-polar habitats in Europe (Schumacher 1990, 1993), while *S. jejuensis* was collected in subtropical-warm temperature zone in East Asia.

A boreo-temperate species restricted to Europe, *Scutellinia decipiens* Le Gal 1966, is somewhat closer to *S. jejuensis* in that the ascospores have broadly ellipsoidal to subglobose shape, overlapping dimensions, and somewhat reticulate with partially interconnected warts (Le Gal 1966, Schumacher 1990). However, its longer and wider marginal hairs (400–1500 and 16–35 µm, respectively) and tuberculate sculpturing separates the new species. *Scutellinia kerguelensis* (Berk.) Kuntze 1891 and *S. chiangmaiensis* T. Schumach. 1990 also possess broadly ellipsoidal to subglobose ascospores, but they are easily discriminated from *S. jejuensis* by the smaller (15.3–18.0 × 11.0–13.0 µm) and reticulated ascospores and the larger (21.8–28.2 × 14.4–21.8 µm) and micro-verrucose ones, respectively.

Interestingly *S. jejuensis,* like all known *Scutellinia* species with globose to subglobose ascospores, is found on soil. Other *Scutellinia* species, those with ellipsoid ascospores, occur on well-decayed wood. This suggests that that the substrate may prove to be important in understanding the diversification of *Scutellinia.* In our limited study, the taxa with globose spores also all group together or form a monophyletic group with reasonably high support. Little is known of the evolutionary history of the genus or details of the biology of these species. It might be assumed that there may have been substrate specialization followed by radiation in the evolutionary history of *Scutellinia* species.

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