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Scleroderma yunnanense Y. Wang : a Gastroid Fungi New to Nepal

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Abstract

A new species of *Scleroderma* having large sized edible fruiting body, thick peridium and echinulate-spiny spores is identified as *Scleroderma yunnanense* Y. Wang (Sclerodermataceae, Boletales, Basidiomycota) based on both morphological and Internal Transcribed Spacer (ITS) of nuclear ribosomal DNA sequence analysis. The specimen collected from *Pinus patula* plantation, in Thaha Municipality of Makawanpur district, Nepal has been described with a discussion on phylogenetic affinities, the global distribution, ecology and ethnomycology.

Key words: Fungal diversity, Earthball, Gastromycete, Nepal

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Introduction

The genus *Scleroderma* Pers. is a gasteroid basidiomycetes of mostly epigenous habit, globose to subglobose basidiospores with typical reticulate-echinulate ornamentation [1, 2] and distributed worldwide in the tropical-temperate ecosystems. *Scleroderma* was initially described by Persoon in 1801 [3]. The name comes from the Greek *sclera* meaning *hard* and *derma* meaning *skin*. This genus is systematically placed under Sclerodermataceae family, suborder Sclerodermatineae within the Boletales [4]. Studies and new reports based on morphology and DNA analysis are imparting lights on taxonomy and phylogeny of *Scleroderma* [5, 6]. As a result, new findings are consolidated in the systematic evaluations of the genus through emendation of *Scleroderma* by Guzmán et *al.* [7]. Nepal host high diversity of mycoflora from its tropical low land to high elevation mountains and a large proportion is still waiting to be documented [8-9]. However, some studies have contributed substantially to enrich the species abundance of certain group like *Scleroderma* [9-15]. Recently Adhikari [16] has updated the checklist and reported 11 species of *Scleroderma* from Nepal. Molecular techniques have been extensively used to supplement morphology based identification of the fungi including *Scleroderma* [5, 15, 17]. Therefore, in this study we have described a new addition to *Scleroderma* genus for Nepal *i.e. Scleroderma yunnanense* Y. Wang, from Chitlang, Makawanpur district, Bagmati province, Nepal using both morphological and molecular evidences.

Materials and Methods

In course of investigation of the fungi of Nepal, the specimen was found in October 2022 growing on the ground in a *Pinus patula* plantation (exotic pine) in Chitlang Ward no. 9 in Thaha Nagarpalika Municipality, Makawanpur District, Nepal. The photographs were taken immediately *in situ*. The microscopic examinations were done using Olympus microscope (10 x 100). The section of specimen was mounted on lactophenol with cotton blue. The basal well developed rhizomorphs were also examined. DNA extraction was performed on Silica-gel dried inner part of the fruiting body using commercially available Qiagen DNeasy Plant Mini Kit. PCR reaction was performed in 10µL reaction volume, each containing 5µL master mix (1x), 0.5µL forward primer (5u/µL), 0.5 µL reverse primer (5u/µL), 1-1.5µL template and nuclease free water. The Internal Transcribed Spacer (ITS) locus was amplified using primer pairs ITS1 and ITS4 [18] in Applied Biosystem Veriti 96 well Thermal Cycler programmed with an initial cycle of 3 min at 94^{0}C followed by 35 cycles of 30s at 94^{0}C, 30s at 50^{0}C and 1.1 min at 72^{0}C. The final extension cycle was run for 7 min at 72^{0}C. Amplified PCR product was purified using SapExo (Jena Bioscience Inc, UK). Sequencing was performed in the Genetic Analyzer 3500XL (Applied Biosystems) at the Molecular Biotechnology Laboratory of NAST. Sequence was edited and contig was made in Bioedit 7.2 software [19]. The basic alignment search tool was used to find homologous sequences in an online GenBank Database and the top ten hits including outgroup sequences were downloaded for phylogenetic

Figure 1: Fruiting body

Figure 2: Exposing inner portion of peridium

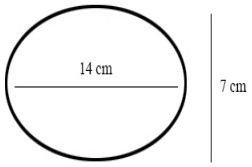

Figure 3: Size of fruit body

Figure 4: Upper view of fruiting body after drying

Figure 5 : Lower view of fruiting body with rhizoidal strands.

Figure 6: Rhizoid piece

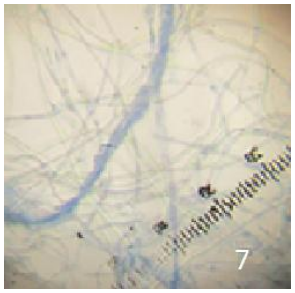

Figure 7: Rhizoidal mycelium

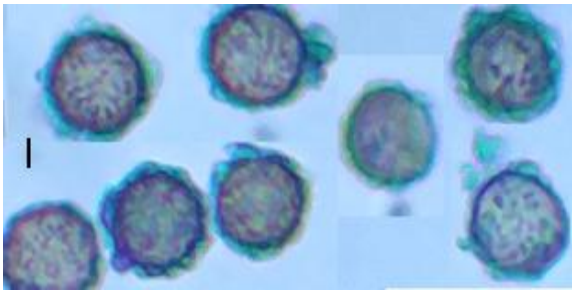

Figure 8: Reticulate spores showing winged types (Scale bar =10 µm).

analysis. Neighbor-joining phylogenetic tree was constructed in MEGA software [20].The specimen was dried and is deposited in the National Herbarium and Plant Laboratories (KATH), Godawari, Nepal while the DNA sequence was deposited in GenBank and the accession number PQ757097 was obtained.

Result

Morphological examinations

Morphologically our specimen match with the *S. yunnanense* particularly the diagnostic features: 2-5 mm thick scaly peridium and the coarsely echinulate globose to subglobose basidiospores.

Taxonomy

Scleroderma yunnanense Y. Wang, Mycotaxon 125: 195 (2013).

Fruit body subhypogeous, solitary, hard, ellipsoid, height of 7 cm and breadth of 14 cm (Figure 1-3), pale yellowish brown, the upper portion covered with large, flattened cracks, coarse warts with minute warts scattered slightly below, on drying very minute warts are seen (Figure 4), very finely hairy with fine rhizoidal strands. Rhizoide mycelium hyaline, thin walled, up to 2.5 – 3.5 µm broad, hyphae branching, clamp connections are also seen. Peridium (skin) firm and 2 to 5 mm thick, creamy white, opening through apical pore. Mycelium thin walled, hyaline, 2.5 – 3.5 µm broad. Peridium is ruptured to larger areas of scales. Flesh white at first becoming pale yellow and finally purplish brown. Spores purplish brown in mass, dark brown in KOH and water, globose to subglobose, echinulate with coarse warts (3.5) 6.5 - 8.7 (11) µm. No pseudostipe. Rhizoidal strands at base. Pleasant odor. (Nepal specimen and morphological examinations, Figure 1-8)

Ethnomycology: It is said to be edible by local inhabitants, cooking with potato, roasted in fire, Pleasant odor.

Specimen examined: Growing under *Pinus patula* forest (exotic pine: planted). Nepal, Bagmati Province, Makawanpur District,, Thaha Nagarpalika (municipality), Ward no 9, Chitlang, Makawanpur, 27'63"35° N, 85'16"46° E, Date 2079/7/5 (2022/10/22), M. K. Adhikari no. 20797 National Herbarium and Plant Laboratories (KATH), Godawari, Nepal

Habitat : Ecologically in Nepal it is associated with exotic pine, *Pinus patula*

Distribution: China, Japan and now Nepal. This species has a subtropical to tropical distribution in Asia.

Common names – Nepali: Alu Chyau (potato mushroom). English: Earthball.

Sequencing and Phylogenetic analysis

The length of raw DNA sequences of the examined specimen obtained from the genetic analyzer was 703 base pairs (bp). When the primer sequences were trimmed from the raw sequences the actual length was 605 base pairs. A phylogenetic tree was constructed using Neighbor joining method with the sequences from Genbank obtained through NCBI blast search. The phylogentic tree thus constructed revealed greater similarity of the specimen from Chitlang, Nepal to the holotype sequences of *S. yunnanense* from Yunnan,

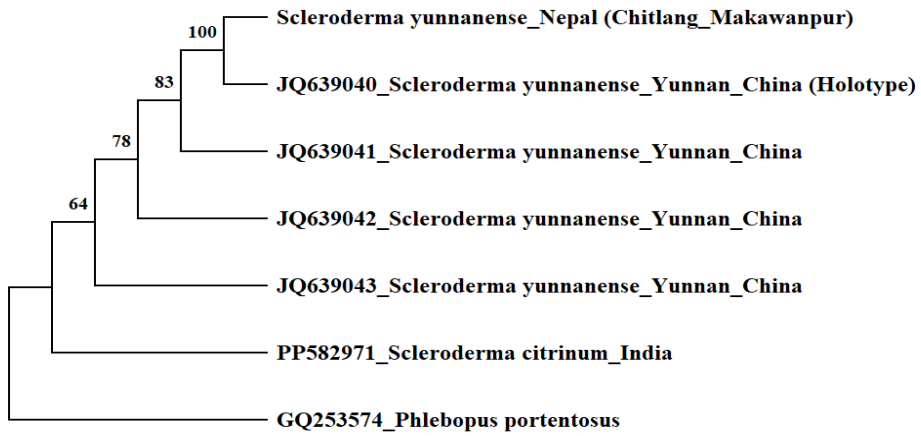

Figure 9. ITS based Neighbor-joining phylogenetic of *Scleroderma* specimen of Chitlang, Makawanpur Nepal showing strong affinity with the holotype of the *S. yunnanense* from Yunnan, China. Number shown at the nodes of the tree represent the bootstrap support for the corresponding clade.

China forming a strong clade with 100 bootstrop support as shown in **Figure 9**.

Discussion

Morphological and molecular evidences strongly suggested that the specimen collected from Chitlang area of Makawanpur district was a new *Scleroderma* species for Nepal, i.e. *Scleroderma yunnanense*. Morphologically the specimen from Nepal had larger sized fruiting body (14 cm in breadth), than the size of 7 cm described for *S. yunnanense* [21], that highlighted the potential of the fruiting body growing larger in different habitat conditions. It has larger scales but not scattered all over body. Zhang *et al.* [21] provided a dichotomous key separating *S. yunnanense* from other *Scleroderma* species known from China. Besides strong phylogenetic affinities, this species has diagnostic morphological features having echinulate-spiny spores and thick peridium. This report extends the known range of *S. yunnanense* from China and Japan to include Nepal.

Like other species of *Scleroderma: S. yunnanense* is believed to be ectomycorrhizal [21, 22]. Apparently, it has a wide host range and reported to associated with Pinus and/or Betula in China [21], Quercus in Japan [22] and Pinus (*Pinus patula*) in Nepal**.** It was found growing after summer season and in the beginning of winter warmer climates. *Scleroderma citrinum* differs from the present specimen, in smaller size of the fruit body (coarsely scaly, yellow to orange) and reticulate spores [10, 16]. The present specimen is quite bigger in size than the previous records of Nepalese *Scleroderma*. It has larger scales but not scattered all over body.

In Nepal both edible and toxic species of *Scleroderma* are reported [10]. This species is also found to be edible as the local inhabitants are consuming it by roasting it in fire along with potatoes. A similar edible use was also documented from Yunnan China [21].

Conclusion

The present study highlights the diversity of mycoflora in Nepal. Morphological examinations supplemented with molecular evidences have contributed substantially to identifying the mushroom specimen collected from Makawanpur as *Scleroderma yunnanense*, a new addition to *Scleroderma* checklist of Nepal. Habitat specificity and its possible influence on the larger fruiting body of *Scleroderma yunnanense* found in Chitlang need to be explored further for their ecological and nutritional significance.

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