OPHIOCORDYCEPS ROBERTSII (OPHIOCORDYCIPITACEAE: ASCOMYCETES) FROM KUMAUN HIMALAYA, UTTARAKHAND, INDIA

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ABSTRACT

Ophiocordyceps robertsii (Hook.) G.H. Sung *et al*. is morphologically very similar to *Ophiocordyceps sinensis* (Berk.) G.H. Sung *et al*., and hence is the most common adulterant species in the caterpillar-mushroom trade. Nonetheless, at present, *O. robertsii* itself is traded, fetching around 80,000 to 100,000 rupees per kg. Comparative anti-thrombotic and anti-dyslipidemic activities of *O. robertsii* and *O. sinensis* were studied, revealing no significant effect of the species *O. robertsii* on any of the two parameters. There are no records of any pharmacological or indigenous use of the species from the region. For the generation of prospective molecular signatures related to species- identity, the *ITS* amplicon was sequenced. The NCBI-BLAST of the sequencing data also validates the identity of the specimen as *Ophiocordyceps robertsii.* This is the first reported specimen of the species from the country.

Keywords: DNA isolation, PCR amplification, *Ophiocordyceps robertsii, Ophiocordyceps sinensis.*

INTRODUCTION

Ophiocordyceps robertsii (Hook.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora was first described from New Zealand (Hooker, 1855). This fungus is mainly found in forest areas and high altitude agricultural fields, where it infects the larvae of Hepialidae moths (Dugdale, 1994). This fungus closely resembles *Ophiocordyceps sinensis* (Berk.) G.H. Sung *et al*. referred to as Yartsa Gumbu or caterpillar mushroom. *O. robertsii*, for its close resemblance with *O. sinensis,* is used as an adulterant with the harvested lot of Yartsa Gunbu, even though, the harvesters, as well as the middlemen or traders can easily distinguish the same by means of the reddish or dark brownish colour of the caterpillar. The fruiting (fungal component) stroma, too is quite easily distinguished from that of *O. sinensis* (plates 1 and 2) and thus is clipped half way down, to resemble the dried stroma of the latter, i.e., *O. sinensis*, as also mentioned by Winkler (2009). Very little is known about the biology of *O. robertsii* and its interactions with hepialid hosts (Casonato

& Hill, 2021). Attempts have been made for sustainable growth and harvesting of *O. robertsii* by artificial culturing of the asexual stages of this fungus (Hooker, 1855). Beattie *et al*. (2011) have shown the ethanol, cold, and hot water extracts of *O. robertsii* exhibit significant cytotoxic activity against one or two cancer cell lines tested. The fungus *Ophiocordyceps robertsii* has to date, not been reported from India. This is the first record of the fungus from Kumaun Himalaya, India.

Even though specimens reported from, say, agricultural fields appear similar morphologically; however, the mature head of the stroma (stroma is the mushroom part of the specimen, the reproductive structure, which again is divided into stipe, or stem and the head-bearing the ascospores). It would thus be safe to denote forests, located on an average between 2200-2500 meters amsl are the prime habitat of *O. robertsii.* Further, such forests could be characterized as being dominated by the herbaceous *Lecanthus peduncularis* (Royle) Wedd. on the ground, which could again be the host plant of Hepialid larvae, with the tree species dominated by *Aesculus indica* (Wall. ex Cambess) Hook., *Acer sterculiaceum* Wall., *Neolitsea frondosa* (Nees) Gamble, *Juglans regia* L., and *Persea odoratissima* (Nees) Kosterm (Plate 3).

METHODOLOGY

(i) *DNA isolation from fungal parts*- The fungal part of the specimen was mechanically separated and processed using a commercially available Fungal DNA purification kit, according to the manufacturers' instructions (Himedia Labs Pvt. Ltd.) to obtain pure genomic DNA. Extracted DNA was then stored at -80°C for subsequent use.

(ii) *PCR amplification of barcoding amplicons*- For the fungal part, PCR protocol was based on the amplification of internal transcribed spacer (ITS) from the fungal genomic DNA using specific primers (Kelly *et al.*, 2011; Larena *et al.*, 1999). The PCR product was then electrophoresed in 1 percent agarose gel and analyzed using a trans-illuminator/Gel-documentation system.

(iii) *Sequencing of PCR products and data analysis*- The PCR product of the desired specificity was up-scaled and Gel-electrophoresed. After electrophoresis, the PCR product was cut out from the gel and gel extraction was carried out. The DNA concentration was estimated using a spectrophotometer/micro-volume spectrophotometer and was dispatched for Sanger sequencing. The electropherogram was manually analyzed for the quality/background noise. FASTA file was generated for the good quality part of the DNA sequence. For sequence similarity determination, DNA sequences of fungal isolates were submitted for BLASTN (or similar algorithms) to determine the close matches in GenBank/other databases (s). Sequences from the different Amplicons were used for identification as per their relative weightages known for species discrimination.

RESULTS

The result shows that the fungus is *Ophiocordyceps robertsii* (Figures 1a and b), although we are unable to isolate the insect DNA.

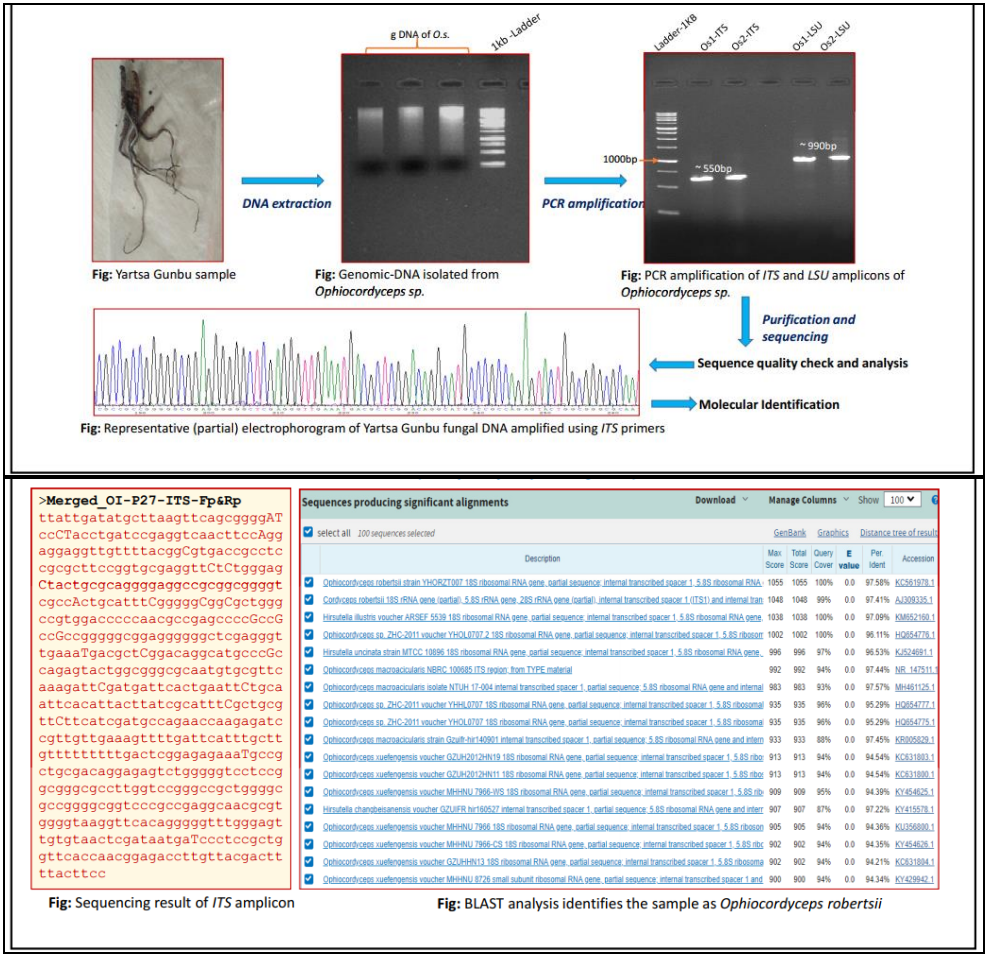

Figure 1: a. DNA- barcoding-based identification of the fungal specimen- DNA Isolation, PCR standardization, and sequencing, b. Analysis of sequencing data.

ACKNOWLEDGMENTS

The authors express their indebtedness to Dr. B.R. Pant, Principal, M.B. Government Postgraduate College, Haldwani (Nainital) for providing the requisite administrative support for the research work.

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Figures 1 and 2: *Ophiocordyceps sinensis* (Berk.) G.H. Sung *et al*. (left) and. *Ophiocordyceps robertsii* (Hook.) G.H. Sung *et al*. (right)

Figure 3- The habitat of *Ophiocordyceps robertsii***3-** The habitat of *Ophiocordyceps robertsii*