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Addition of *Helvella involuta* (Helvellaceae; Pezizales) to Indian Mycobiota from North-West Himalaya

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ABSTRACT

While conducting macrofungal survey across different temperate mixed forests of district Chamoli, located in Uttarakhand, India, we came across fascinating discovery within the genus *Helvella*. Through extensive literature review, detailed morphological examinations and phylogenetic analysis, the specimen was identified as *Helvella involuta*. This finding marks the first documentation of the species from India.

Keywords: Macrofungi, Phylogeny, Uttarakhand, nrITS, Taxonomy.

INTRODUCTION

Linnaeus (1737, 1753) initially suggested the genus *Elvela* (= *Helvella*, orth. var.) for *Elvela mitra* (=*Helvella crispa* ss. Fries (1823)). Fries (1823) then established the family *Elvellaceae* which was later renamed as *Helvellaceae* (Corda, 1842) and includes species produced both above-ground (apothecial forms) and below-ground (hypothecial form) ascomata. The classification of the morphologically diverse genus *Helvella* has undergone several modifications over time. Boudier (1907) identified 5 genera namely, *Acetabula, Cyathipodia, Helvella sensu stricto*, *Leptopodia*, *Macropodia*, and which were later merged into a broader concept of *Helvella* (Abbott and Currah, 1997).

The widely spread *Helvella* L., a type of saddle-shaped mushroom belonging to the family *Helvellaceae* within the Sub-Division Pezizomycotina of the Ascomycota, can be found in both deciduous and coniferous forests. This taxon is distinguished by its light-coloured, bi- to tri-lobed apothecia, featuring a hairy to densely hairy surface on the receptacle and a margin that curls inward when young, along with a white ridged stalk (Weber, 1972). In the macrofungal exploration through the Bedni-Bugyal trek, a large number of wild macro-fungi specimens were collected. Thorough macro- and micro-morphological examination of our collections revealed one taxon to be a first record for the Indian mycobiota. The nrITS sequences justified this taxon to be *Helvella involuta* Q. Zhao, Zhu L. Yang & K.D. Hyde.

MATERIAL AND METHODS

Macro- and Micromorphology

Fresh ascomata were gathered and photographed on-site using a Nikon D5300 camera. Different macroscopic features were documented directly from the fresh fruiting bodies in their natural environment, including details about the habitat and the host plants they were associated with (Vellinga, 1988). Colours were coded as per Kornerup and Wanscher (1978). Microscopic details were examined from hand-cut sections of dried specimens (Largent *et al.*, 1977). These sections were prepared by mounting them in a solution composed of 5% KOH, 1% Phloxine, and 1% Congo red, then observed using a compound microscope (Olympus CH20i). Line drawings were done using Camera Lucida at 1000 X magnification. Microscopic photographs were taken with Olympus BX43 compound microscope. Ascospores were measured and recorded as: minimum – mean - maximum.

DNA Extraction, PCR Amplification, and Sequencing

Fungal genomic DNA was extracted from 100 mg of dried fruit bodies using a fungal genomic DNA Mini Kit (RGCB, Thiruvananthapuram, India). Using primer pairs ITS1 and ITS4, the ITS region of the nuclear ribosomal DNA gene was amplified (White *et al.*, 1990). PCR amplification was conducted in a 20 µL reaction volume containing Phire PCR buffer, dNTPs, DNA template, Phire Hotstart II DNA polymerase enzyme, BSA, DMSO, Betaine, and forward and reverse primers.

The PCR program included initial denaturation at 96°C for 2 min, followed by 30 cycles of denaturation at 96°C for 30 sec, annealing at 50°C for 40 sec, and extension at 60°C for 4 min. Purification of PCR products was done using the QIAquick Gel Extraction Kit (QIAGEN, Germany), followed by Sanger sequencing using the same primers in an automated DNA sequencer. The obtained sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov), and accession numbers for two collections are provided in **Figure 1.**

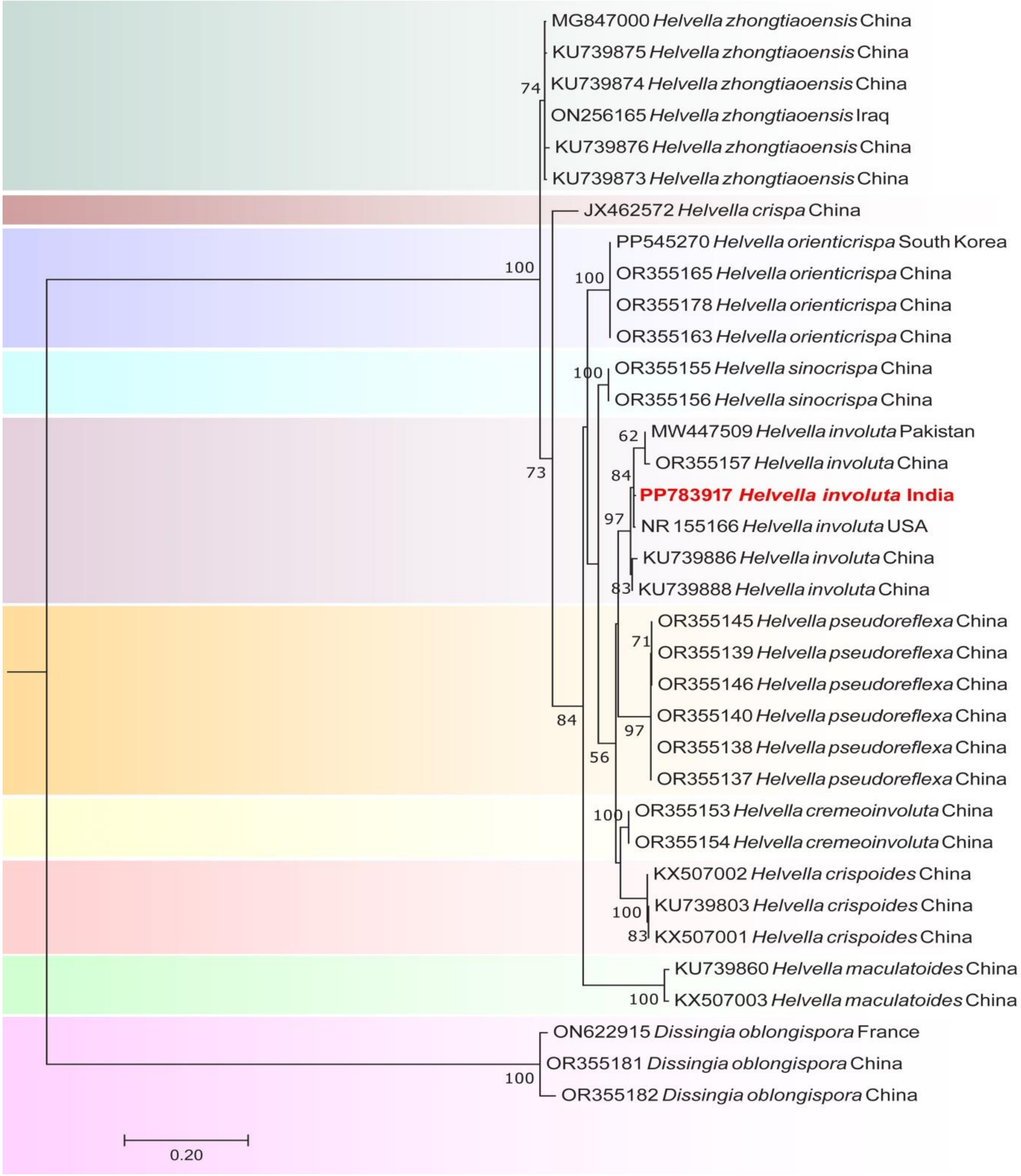

Figure 1: Maximum Likelihood phylogenetic tree inferred from ITS-rDNA sequence data using GTR+GAMMA model of nucleotide evolution constructed in RAxML v.2.0.10. Branches are labelled with ML bootstrap support values (≥50 %). Sequence derived from the ***Helvella involuta*** is shown in bold in the tree.

Phylogenetic Analysis

Phylogenetic analysis was conducted utilizing the nrITS dataset, including newly obtained sequence, sequences from nBLAST searches (Altschul *et al.*, 1997) in GenBank (Clark *et al.*, 2016) and previously published phylogenies (Zhao *et al.*, 2015; Kaygusuz *et al.*, 2020; Mao *et al.*, 2024). For this study, a dataset consisting of 34 nrITS sequences of *Helvella* species, including the newly acquired sequence, was employed. The alignment of the nrITS dataset was performed using MAFFT v.7 (Katoh and Standley, 2013). Maximum likelihood (ML) phylogenetic analysis of nrITS sequences was executed using RaxML GUI software (Edler *et al.*, 2021) with 1000 bootstrap (BS) replicates analyzed to ascertain nodal support values. Bootstrap support values (>50%) obtained from the ML analysis are presented above or below the branches at nodes. Species of *Dissingia* were utilized as an outgroup for the current phylogenetic analysis (GenBank no. ON622915, OR355181 and OR355182).

RESULTS

Phylogenetic Inferences

The nrITS sequences obtained in this study have been submitted to GenBank (PP783917). In nBLAST search, the nrITS sequence of the Indian collection (SC/PU/27) displayed 100% identity with *H. involuta* reported from the China. In the nrITS phylogenetic tree, sequence of the present specimen clustered within the clade formed by the sequences of *H. involuta* from the China indicating towards its identity as *Helvella involuta* **(Figure 1).**

Taxonomy

Helvella involuta Q. Zhao, Zhu L. Yang & K.D. Hyde (2015) **(Figure 2, 3)**

GenBank: — PP783917

Figure 2: ***Helvella involuta***.: **a–c,** Fresh ascomata in the field and basecamp; **d**, Asci and paraphyses; **e–g,** Ascospores; **h–I,** Transverse section through receptable surface of pileus. Scale bars: **a–c** = 10 mm; **d–h** = 10 µm.

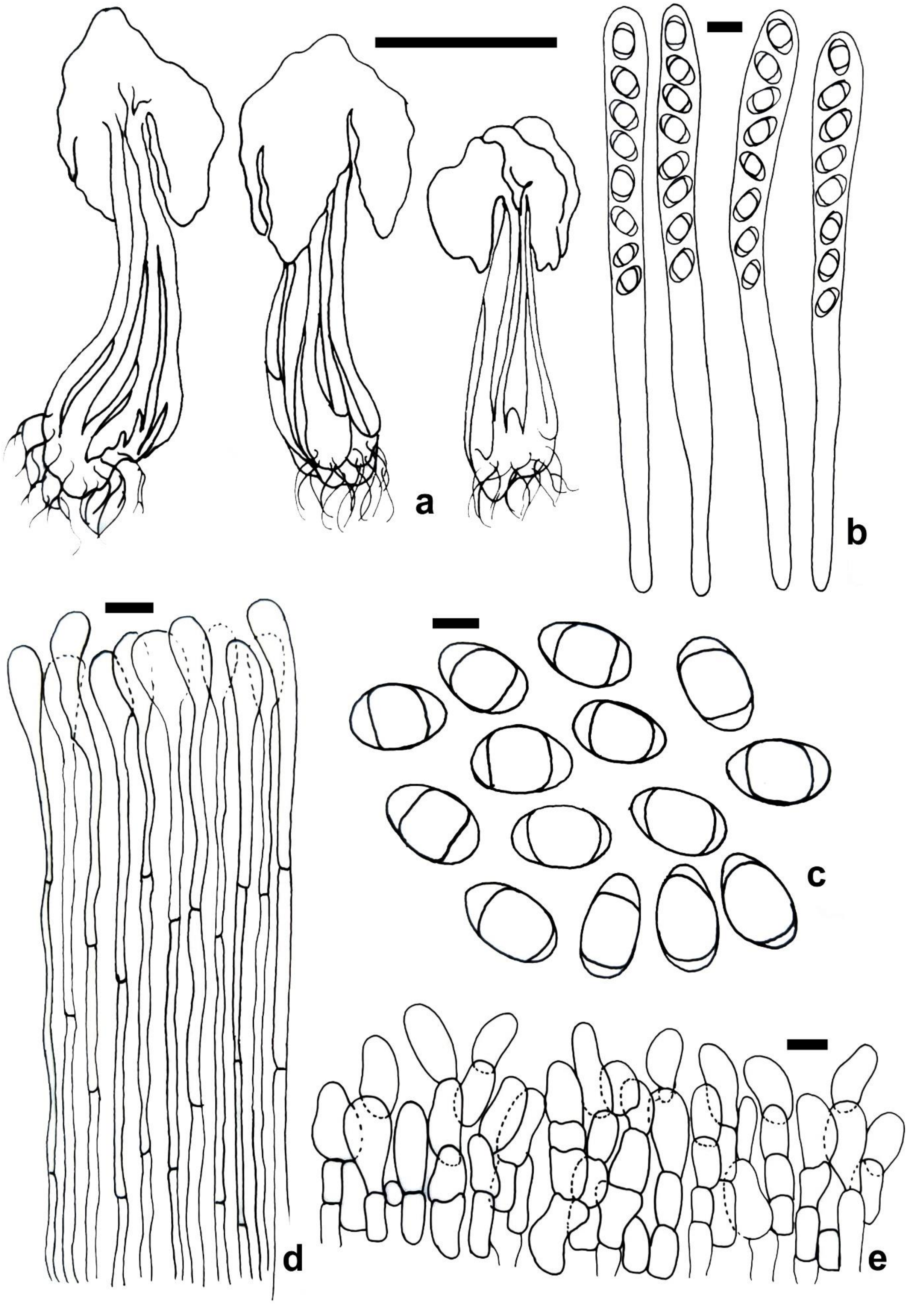

Figure 3: *Helvella involuta*, **a,** Fruiting body; **b,** Asci; **c,** Paraphyses; **d,** Ascospores; **e,** Receptacle surface of pileus showing terminal cells. Scale bars: **a**= 10 mm; **b–e**= 10 µm.

DESCRIPTION

The cap ranges from saddle-shaped to irregularly lobed, measuring 15–35 mm in height. At maturity, the margin is fully curled towards the hymenium. The hymenium appears smooth and whitish, turning cream-yellow (3A2–3A3) coloured when dried. The surface of the receptacle is hairy and yellowish, also becoming yellowish (3B3) when dried, often featuring ribs that are concolorous and blunt, sometimes extending halfway. The stipe measures 20–64 mm in length and 5–2.5 mm in thickness, widening towards the base. It exhibits deep lengthwise grooves and rounded ribs, with a fine layer of hair, ranging from creamy (3A2) to cementious-gray (3A1–3B1) and toughening to a cream (3A2) colour when dried.

Medullary excipulum: ranges from 157.5–235 µm broad, hyaline, composed of thick-walled hyphae 3.39–7.12 µm broad; *Ectal excipulum:* measures 150–215 µm broad, exhibiting angular to prismatic texture, with outermost cells emerging, hyaline, with cylindrical to subclavate, slightly thick-walled end cells 20.88–36.37 × 9.64–13.73 µm**;** *Stipitipellis:* 87–115 µm, hyaline, composed of textura prismatica, terminal cells 22.39–35.1 × 8.09–13.84 µm, clavate; *Asci:* 230–302 µm long, 16.5–17.9 µm at the top, 14.32–18.8 µm wide at the centre and 10.5–12 µm at the base, 8-spored, elongated, subcylindrical to clavate, thin walled; *Ascospores*: (15)16–18(20) × (9)10–12 (13) µm [Q = 1.45–1.80, Q = 1.61 ± 0.09)], oval to ellipsoid, thin walled, smooth under the light microscopy; *Paraphyses*: 5.51–6.75 µm broad, elongated, thin walled, hyaline, septate, filiform, apex slightly enlarged, 5.98–7.63 µm broad.

Habit and habitat: Scattered on the ground, in *Rhododendron arboreum* Sm. forest.

Specimens examined: INDIA. Uttarakhand: district Chamoli, Deval, Lohajung, Dedna trek, Bedni-bugyal, 30°10'02.49"N 79°38'05.98"E, elev. 2499 m, 08 August 2023, *Shikha Choudhary, Priyanka Uniyal* and *Yash Pal Sharma*, SC/PU/23; **HBJU/M/163.**

Distribution: This species was originally described from China (Zhao *et al.*, 2015) and is now known from Himalayan district of Chamoli, Uttarakhand, India.

DISCUSSION

The combination of characters including irregular or saddle-shaped ascomata, white hymenium turning creamish yellow when dried, yellowish receptacle, concolorous ribs extending halfway, septate paraphyses, and also nrITS based phylogenetic studies confirm our Indian collection as *Helvella involuta*. Macro- and micro-morphological characters of our specimen show similarity with holotype originally reported from China, except the occurrence of the present collection under broadleaf forest in contrast to holotype (under *Pinus yunnanensis)* (Zhao *et al.*, 2015). Morphologically, *Helvella orienticrispa* Q. Zhao, Zhu L. Yang & K.D. Hyde can be confused in the field with the present collection. However, former can be differentiated by its glabrous and pale brown receptacle surface, rolled margin when young and later on expand and split at maturity and occurrence under coniferous forests (Zhao *et al.*, 2015). Phylogenetically, *H. pseudoreflexa* Q. Zhao, Zhu L. Yang & K.D. Hyde, and *H. sinocrispa* L. Fan, N. Mao & Y.Y. Xu related with *H. involuta*. However, *H. pseudoreflexa* differs from the *H. involuta* by its trilobite-shaped to irregularly lobed sporocarp, hymenium undulate-rugose towards centre, cream to grey, becoming yellowish when dried, receptacle surface pubescent with blunt ribs. Similarly, *H. sinocrispa* differs from the *H. involuta* with the combination of characters such as smooth, cream white to whitish hymenium, turns brown to dark brown when dry, outer surface of the cap slightly hairy, pale yellow to greyish yellow and its association in the coniferous forest dominated by *Larix* sp. and *Picea* sp.

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REFERENCES

Abbott, S.P. and Currah, R.S. 1997. The Helvellaceae: systematic revision and occurrence in northern and northwestern North America. *Mycotaxon*, **62**:1-125.

Altschul, S.F., Madden, T.L., Schaffer, A.A., *et al.*, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**:3389-3402.

Boudier, E. 1907. *Histoire et classification des Discomycètes d'Europe*. Paris, France, 234p.

Clark, K., Karsch-Mizrachi, I., Lipman, D.J. 2016. GenBank. (Database issue). *Nucleic Acids Research,* **44(1)**:D67-D72.

Corda, A.C.J. 1842. *Icones fungorum hucusque cognitorum*. Vol. 5, Prague, Czechia.

Edler, D., Klein, J., Antonelli, A. 2021. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution,* **12**:373-377.

Fries, E.M. 1823. *Systema Mycologicum, Vol. 2*. Officina Berlingiana, Lundae, Sweden, 620p.

Katoh, K. and Standley, D.M. 2013. MAFFT multiple sequence alignment software ver. 7: Improvement in performance and usability, *Molecular Biology and Evolution,* **30**:772-780.

Kaygusuz, O., Türkekul, I., Battistin, E. 2020. Some noteworthy records of *Helvella* from Turkey based on morphology and DNA sequence data. *Journal of Fungal Biology,* **10(1)**:290-299.

Kornerup, A. and Wanscher, J.H. 1978. *Methuen Handbook of Colour* (Don Pavey – Translator). 3rd Edition. Methuen Publishing Ltd, London, 256p.

Largent, D., Johnson, D., Watling, R. 1977. *How to identify mushrooms to genus III: microscopic features*. Mad River Press Inc., Eureka, California, 148p.

Linnaeus, C. 1737. *Hortus Cliffortianus*. Amsterdam, Netherlands.

Linnaeus, C. 1753. *Species Plantarum*. Vol. 2. Holmiae, Sweden, 680p.

Mao, N., Xu, Y.Y., Zhang, Y.X., *et al.,* 2024. Phylogeny and species diversity of the genus *Helvella* with emphasis on eighteen new species from China. *Fungal Systematics and Evolution*, **12(1)**:111-152.

Vellinga, E.C. 1988. Glossary. In: *Flora agaricina neerlandica, Vol.1*, (Eds.: Bas,C., Kuijper, Th.W., Norelco's, M.E., *et al.*). A.A. Balkema and Rotterdam, Netherlands, pp.54-64.

Weber, N.S. 1972. The genus *Helvella* in Michigan. *The Michigan Botanist*, **11(4**):147-201.

White, T.J., Bruns, T., Lee, S., *et al.*, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Application,* (Eds.: Innis, M.A., Gelfand, D.H., Sninsky, J.J., *et al.*). Academic press, New York, pp.315-322.

Zhao, O., Zhao, Y., Hyde, K.D. 2015. Species diversity within the *Helvella crispa* group (Ascomycota: Helvellaceae) in China. *Phytotaxa,* **39(2):**130; doi:10.11646/phytotaxa .239.2.2.