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## Phylogeny and species diversity of the genus *Helvella* with emphasis on eighteen new species from China

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**Abstract:** *Helvella* is a widespread, frequently encountered fungal group appearing in forests, but the species diversity and molecular phylogeny of *Helvella* in China remains incompletely understood. In this work, we performed comprehensive phylogenetic analyses using multilocus sequence data. Six datasets were employed, including a five-locus concatenated dataset (ITS, nrLSU, *tef1-α*, *rpb2*, *hsp*), a two-locus concatenated dataset (ITS, nrLSU), and four single-locus datasets (ITS) that were divided based on the four different phylogenetic clades of *Helvella* recognized in this study. A total of 1 946 sequences were used, of which 713 were newly generated, including 170 sequences of ITS, 174 sequences of nrLSU, 131 sequences of *tef1-α*, 107 sequences of *rpb2* and 131 sequences of *hsp*. The phylogeny based on the five-locus concatenated dataset revealed that *Helvella s. str.* is monophyletic and four phylogenetic clades are clearly recognized, *i.e.*, *Acetabulum* clade, *Crispa* clade, *Elastica* clade, and *Lacunosa* clade. A total of 24 lineages or subclades were recognized, 11 of which were new, the remaining 13 corresponding with previous studies. Chinese *Helvella* species are distributed in 22 lineages across four clades. Phylogenetic analyses based on the two-locus concatenated dataset and four single-locus datasets confirmed the presence of at least 93 phylogenetic species in China. Among them, 58 are identified as known species, including a species with a newly designated lectotype and epitype, 18 are newly described in this paper, and the remaining 17 taxa are putatively new to science but remain unnamed due to the paucity or absence of ascomatal materials. In addition, the *Helvella* species previously recorded in China are discussed. A list of 76 confirmed species, including newly proposed species, is provided. The occurrence of *H. crispa* and *H. elastica* are not confirmed although both are commonly recorded in China.

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## INTRODUCTION

*Helvella* (1753) is one of the most conspicuous members of *Helvellaceae*. Almost all *Helvella* species have saddle-shaped ascocarps with cupulate or lobed caps on a distinct stipe, and ellipsoid ascospores with a large central oil drop with the exception of the *H. macropus* complex which has subfusoid ascospores. *Helvella* species are extraordinarily common and widely distributed in terrestrial biomes of the Northern and Southern Hemispheres (Skrede *et al.* 2017, 2020). Some of them have great dietary and health value, such as, *H. bachu*, *H. lacunosa* and *H. taiyuanensis* (Liu *et al.* 1985, Shameem *et al.* 2016, Zhao *et al.* 2016a). Ecologically, some species in this genus form ectomycorrhizal associations with host plants (*Pinaceae* and *Fagaceae*) (Maia *et al.* 1996, Tedersoo *et al.* 2006, Hwang *et al.* 2015).

As a frequently encountered and important fungal group, the systematics and species diversity of *Helvella* have been widely studied by mycologists from different parts of the world, especially from Europe and North America (Dissing 1966a, b,

Korf 1972, Weber 1972, 1975, Harmaja 1979, Häffner 1987, Abbott & Currah 1997, Landeros *et al.* 2012, 2015, Nguyen *et al.* 2013, Skrede *et al.* 2017, 2020, 2023, Hansen *et al.* 2019, Løken *et al.* 2019). The ascoma shape and colour, pubescence on the apothecium receptacle surface, ascospore size and shape are highlighted in the identification of *Helvella* species. However, it's difficult to discriminate the difference between *Helvella* species with only morphology. Molecular systematics and DNA barcode sequences as a robust tool to identify fungal species have been applied to taxonomic studies of *Helvella*, which are useful for establishing the species concepts of *Helvella* (Landvik *et al.* 1999, Hansen & Pfister 2006, Tedersoo *et al.* 2006, Laessle & Hansen 2007, Nguyen *et al.* 2013, Landeros *et al.* 2015, Skrede *et al.* 2017, 2020, 2023, Hansen *et al.* 2019, Wang *et al.* 2019, 2023a, b, Lu *et al.* 2023, Yu *et al.* 2023). For example, Landeros *et al.* (2015) revealed *Helvella* is monophyletic by analysing partial nrLSU data. Skrede *et al.* (2017) assessed species boundaries within *Helvella* in Europe by using morphology and phylogenetic evidence from four loci – heat shock protein 90 (*hsp*), translation elongation factor alpha (*tef1-α*), RNA polymerase II second

largest subunit (*rpb2*) and the nuclear large subunit ribosomal DNA (nrLSU), in which a total of 55 species in Europe were identified, 30 species were designated neo- and epitypes, and seven new species were described.

In China, based on morphology, or both morphology and molecular data, a lot of research has been focused on the taxonomy of *Helvella* (Teng 1963, Liu *et al.* 1985, Cao 1988, Liu & Cao 1988, Cao & Liu 1990, Cao *et al.* 1990, Zhuang 1995, 1996, 1997, 1998a, b, Zhuang & Wang 1998, Wang & Chen 2002, Xu 2002, Zhuang 2004, Zhuang & Yang 2008, Ariyawansa *et al.* 2015, Zhao *et al.* 2015, Hyde *et al.* 2016, Wang *et al.* 2016, 2019, 2023a, b, Zhao *et al.* 2016a, b, Tibpromma *et al.* 2017, Xu *et al.* 2022, Yu *et al.* 2023). Recently, 16 new species and two new Chinese records were determined based on morphology and molecular phylogenies inferred from ITS, LSU, *tef1-α*, and *hsp* (Wang *et al.* 2023a). Presently, a total of 90 species are recorded from China, but molecular evidence lack for 37 species. Moreover, many newly introduced collections did not match to previously known species either in morphology or molecular analysis. It is therefore necessary to thoroughly revise records of Chinese *Helvella* spp. In the present study, five highly informative genetic markers (ITS, nrLSU, *tef1-α*, *rpb2*, *hsp*) were used in our molecular analyses. Morphological features and DNA-based molecular analyses were conducted based on the specimens gathered from many parts of China, combined with existing type specimens and sequences available in the GenBank database. Our aims were to: 1) readdress the infrageneric group limits and relationships within *Helvella*; 2) clarify the species diversity and phylogenetic relationships of *Helvella* in China, and 3) describe and illustrate new species found in China based on both molecular and morphological data.

## MATERIALS AND METHODS

### Sample collections

Samples collected in China were examined. Voucher specimens were accessioned in the Herbarium Biology Department at Capital Normal University (BJTC). Additional specimens on loan from other fungaria were also studied. The principal fungaria were the Herbarium Mycologicum Academiae Sinicae at the Institute of Microbiology, Chinese Academy of Sciences (HMAS) and the Herbarium of Cryptogams at the Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Herbarium of Mycology of Jilin Agricultural University (HMJAU), and the Herbarium of Shanxi Institute for Functional Foods, Shanxi Agricultural University (HSA). Macroscopic characters were described from fresh and

dried material. Microscopic characteristics were observed in sections obtained from dried specimens mounted in 5 % KOH, Congo Red, cotton blue, Melzer's reagent, or water.

### DNA extraction, PCR amplification, sequencing and nucleotide alignment

Dried specimens were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 mL tube together with one 3-mm-diam tungsten carbide ball, and total genomic DNA was extracted using the E.Z.N.A. Fungal DNA kit (Omega Bio-Tek Inc. Norcross, Georgia, USA) following the manufacturer's protocol. Polymerase chain reactions (PCR) were performed to amplify partial sequences from the five partial genes (ITS, nrLSU, *tef1-α*, *rpb2*, *hsp*) with previously published primer sets. The details of primer combinations, optimized PCR annealing temperatures and references for published primers are provided (Table 1).

Amplification reactions were performed in 25 µL reaction volumes containing 2 µL DNA template, 1 µL per primer (10 µM), 12.5 µL 2× Master Mix (TiangenBiotech Co., Beijing, China), and 8.5 µL ddH<sub>2</sub>O.

Amplification reactions were performed as follows: for the ITS or ITS2 gene: initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 53 or 55 °C for 45 s, 72 °C for 55 s, and a final extension at 72 °C for 10 min; for the nrLSU gene: initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min; for the *tef1-α* gene: initial denaturation at 94 °C for 3 min, followed by 11 cycles including denaturation at 94 °C for 30 s, annealing temperature started at 64 °C (decreased by 1 °C per cycle, until to 54 °C) for 45 s and extension at 72 °C for 1 min, then followed by 30 cycles at 94 °C for 35 s, 56 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min; for the *rpb2* gene: initial denaturation at 94 °C for 3 min, followed by 11 cycles including denaturation at 94 °C for 30 s, annealing temperature started at 62 °C (decreased by 1 °C per cycle, until to 52 °C) for 45 s and extension at 72 °C for 1 min, then followed by 30 cycles at 94 °C for 35 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min; for the *hsp* gene: initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Beijing Zhongkexilin Biotechnology Co. Ltd. (Beijing, China) for purification and sequencing. The newly generated sequences were assembled and edited using SeqMan (DNA STAR package; DNASTar Inc., Madison, WI, USA) with generic-level identities for sequences confirmed via BLAST queries of GenBank.

**Table 1.** Sequences of primers, citations for previously published primers and annealing temperature used for PCR amplification of sequences generated for this study.

Gene region	Forward primer	Primer sequence (5'–3')	Reverse primer	Primer sequence (5'–3')	PCR annealing temp (°C)	References
ITS	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	ITS4	TCCTCCGCTTATTGATATGC	53 or 55	White <i>et al.</i> (1990)
ITS2	ITS3	GCATCGATGAAGAACGCAGC	ITS4	(see above)	55	White <i>et al.</i> (1990)
nrLSU	LROR	ACCCGCTGAACTTAAGC	LR5	TCCTGAGGGAAACTTCG	55	Vilgalys & Hester (1990)
<i>tef1-α</i>	EF1-983F	GCYCCYGGHCAYCGTGAYTTYAT	EF1-2218R	ATGACACCRACRCRACRGTYTG	touchdown	Rehner & Buckley (2005)
<i>rpb2</i>	fRPB2-7cF	ATGGGYAARCAAGCYATGGG	fRPB2-11aR	GCRTGGATCTTRTCRTCSACC	touchdown	Liu <i>et al.</i> (1999)
<i>hsp</i>	H_hspf	CRGGCATCCGGGTGACGTAAT	H_hspr	AGGGKGTGTGCGACTCCGAGG	58	Skrede <i>et al.</i> (2017)

A total of 713 *Helvella* sequences from our 186 collections were used in the molecular phylogenetic analyses, of which 170 in ITS, 174 in nrLSU, 131 in *tef1- $\alpha$* , 107 in *rpb2*, and 131 in *hsp*. Sequences of all DNA regions generated in this study were deposited in GenBank. Other sequences of *Helvella* and related species obtained from GenBank are based on published literature (Nguyen *et al.* 2013, Landeros *et al.* 2012, 2015, Zhao *et al.* 2015, Hyde *et al.* 2016, Wang *et al.* 2016, 2019, 2023a, Zhao *et al.* 2016a, b, Skrede *et al.* 2017, 2020, 2023, Hansen *et al.* 2019, Løken *et al.* 2019), or selected by using the BLASTn search function of the NCBI database to find similar matches with taxa in *Helvella*. For ITS sequence data, the genus search tool in GenBank database was used for retrieving *Helvella*-related ITS sequences. The accession numbers of new and downloaded sequences stored in the NCBI GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov/>) are provided in Supplementary Tables S1–S6.

Six molecular datasets were assembled for this study. Dataset I was comprised of five gene fragments (ITS/nrLSU/*tef1- $\alpha$* /*rpb2*/*hsp*) and contained all the *Helvella* species with available DNA data worldwide. The phylogenetic trees derived from the single-gene dataset (including ITS, nrLSU, *tef1- $\alpha$* , *rpb2* and *hsp*) did not exhibit any incongruence because no well-supported (BS > 70 %) conflict was detected among the topologies of the five genes. The concatenated five-gene dataset was used to infer the phylogenetic placement of *Helvella* species and to assess subgeneric limits and relationships within *Helvella*. *Dissingia oblongispora* and *Pindara terrestris* were used as outgroup taxa. Datasets II–V were comprised of single gene fragment (ITS), which respectively corresponded to four subgeneric phylogenetic clades of *Helvella* that were recognized in this study based on the phylogenetic analyses for Dataset I, *i.e.*, *Acetabulum* clade (Datasets II), *Crispa* clade (Datasets III), *Elastica* clade (Datasets IV), and *Lacunosa* clade (Datasets V). Each dataset contained all corresponded and qualified ITS sequences that were newly provided in this study and downloaded from GenBank. The four single-gene datasets were used to delimit and detect *Helvella* species and assess the species diversity. Dataset VI was comprised of two gene fragments (ITS/nrLSU) and contained all Chinese *Helvella* species recognized in this study except for the species lacking available molecular data. This concatenated two-gene dataset was used to show the species diversity of *Helvella* in China excluding the species lacking DNA data, and to infer the phylogenetic relationships between Chinese *Helvella* species.

Sequences were aligned and edited in MAFFT (Katoh & Frith 2012) under default parameters, and manually adjusted for maximum sequence similarity in Se-Al v. 2.03a (Rambaut 2000). Ambiguously aligned regions and gaps in the alignment were excluded before the analyses. For the concatenated dataset (Datasets I, VI), alignments were constructed separately for each of the gene fragments using MAFFT (Katoh & Frith 2012), optimized using BioEdit v. 7.0.9 (Hall 1999), then concatenated using SequenceMatrix v. 1.7.8 (Vaidya *et al.* 2011). Unsourced gene regions were coded as missing data and the intron regions of protein-coding genes were retained in the final analyses. Poorly aligned sites were identified by Gblocks v. 0.91b ([http://www.phylogeny.fr/one\\_task.cgi?task\\_type=gblocks&tab\\_index=2](http://www.phylogeny.fr/one_task.cgi?task_type=gblocks&tab_index=2); Castresana 2000; using default options except ALLOWED GAP POSITIONS 5 half) with default parameters. All identified ambiguous sites were excluded before the analyses. Alignments of all datasets used in this study were submitted to TreeBASE (No. 30611).

## Phylogenetic analyses

Maximum likelihood (ML) analyses on all six datasets in this study were conducted with RAxML v. 8.0.14 (Stamatakis *et al.* 2014) and the GTRGAMMAI substitution model with parameters unlinked. The ML bootstrap replicates (1 000) were computed in RAxML with a rapid bootstrap analysis and search for the best-scoring ML tree.

Bayesian inference (BI) was performed with MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003) as an additional method for determining branch support. The best substitution model that fit the data at each locus was evaluated using MrModeltest v. 2.3 (Nylander 2004). For the concatenated analyses (Datasets I and VI), each locus was considered a partition and assigned its own best-fitting substitution model, that was GTR+I+G for ITS, nrLSU, *rpb2* and *hsp*, and SYM+I+G for *tef1- $\alpha$* . We used two independent runs with four Markov chains Monte Carlo (MCMC) for 5 155 000 (Dataset I) and 4 420 000 (Dataset VI) generations under the default settings. For the ITS analysis (Datasets II–V), the best substitution model for each dataset respectively was GTR+I+G (Datasets II, III), GTR+G (Datasets IV, V). The MCMC analysis were run for 825 000 (Dataset II), 1 430 000 (Dataset III), 2 330 000 (Dataset IV) and 3 010 000 (Dataset V) generations under the default settings. Average standard deviations of split frequency (ASDSF) values were far lower than 0.01 at the end of the generations. Trees were sampled every 100 generations after burn-in (25 % of trees were discarded as the burn-in phase of the analyses, set up well after convergence), and 50 % majority-rule consensus trees were constructed.

Clades with bootstrap support (BS)  $\geq$  70 % and Bayesian posterior probability (PP)  $\geq$  0.95 were considered as significantly supported (Hillis & Bull 1993, Alfaro *et al.* 2003). All phylogenetic trees were viewed with TreeView (Page 2001).

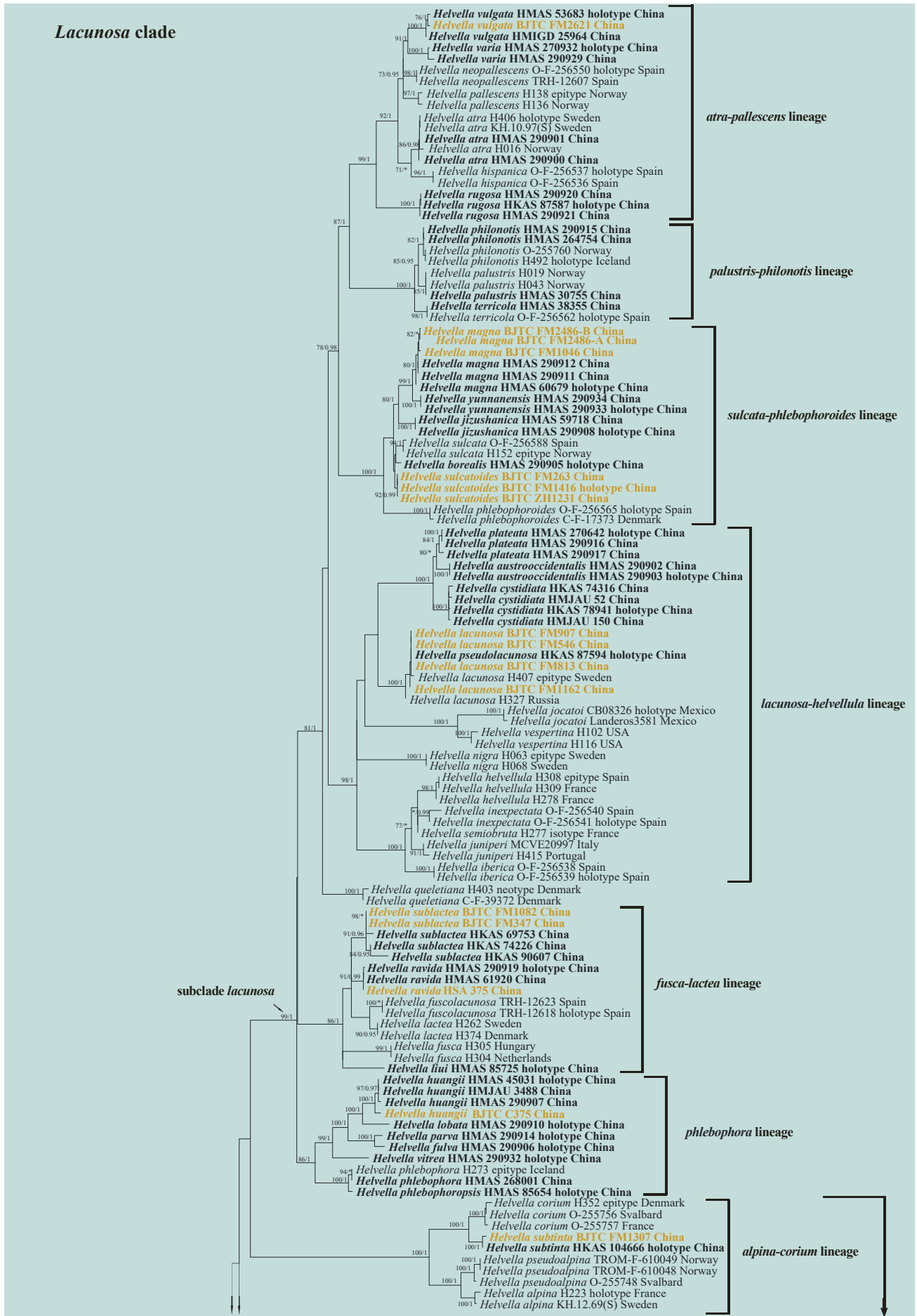
## RESULTS

### Phylogenetic analyses

#### *Combined five-gene phylogeny of Helvella*

Dataset I (ITS/nrLSU/*tef1- $\alpha$* /*rpb2*/*hsp*) contained 1 348 sequences from 136 species, including 629 sequences newly generated from Chinese collections. The length of the aligned dataset was 2 726 bp after exclusion of poorly aligned sites (all in ITS), with 565 bp for ITS, 666 bp for nrLSU, 908 bp for *tef1- $\alpha$* , 346 bp for *rpb2*, and 241 bp for *hsp*. Maximum likelihood and BI analyses yielded similar tree topologies and only the tree inferred from the ML analysis is illustrated with strong statistical bootstrap from ML and posterior probability from BI support values shown (Fig. 1). Our present analysis revealed that the genus *Helvella* is monophyletic, and four distinct clades in *Helvella* can be recognized (Fig. 1), *i.e.*, *Acetabulum* clade, *Crispa* clade, *Elastica* clade, and *Lacunosa* clade.

Within the *Acetabulum* clade, two lineages were furthermore recognized, *i.e.*, the *acetabulum* lineage and *solitaria* lineage. Within the *Crispa* clade, *H. crispa* and its allies consisted of a well-supported lineage which was designated as *crispa* lineage hereafter, and the other two species, *H. maculata* and *H. maculatoides*, both outside *crispa* lineage, formed their own independent branches. Within the *Elastica* clade, eight individual evolving lineages were recognized, *i.e.*, *bicolor-elastica* lineage, *capucina-danica* lineage, *carnosa* lineage, *corbierei-stevensii* lineage, *fallax*-



**Fig. 1.** Phylogeny of Chinese *Helvella* inferred from the Dataset I (ITS/nrLSU/*tef1- $\alpha$* /*rpb2/hsp*) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. Chinese *Helvella* species are printed in bold. New species and new sequences are printed in golden font.

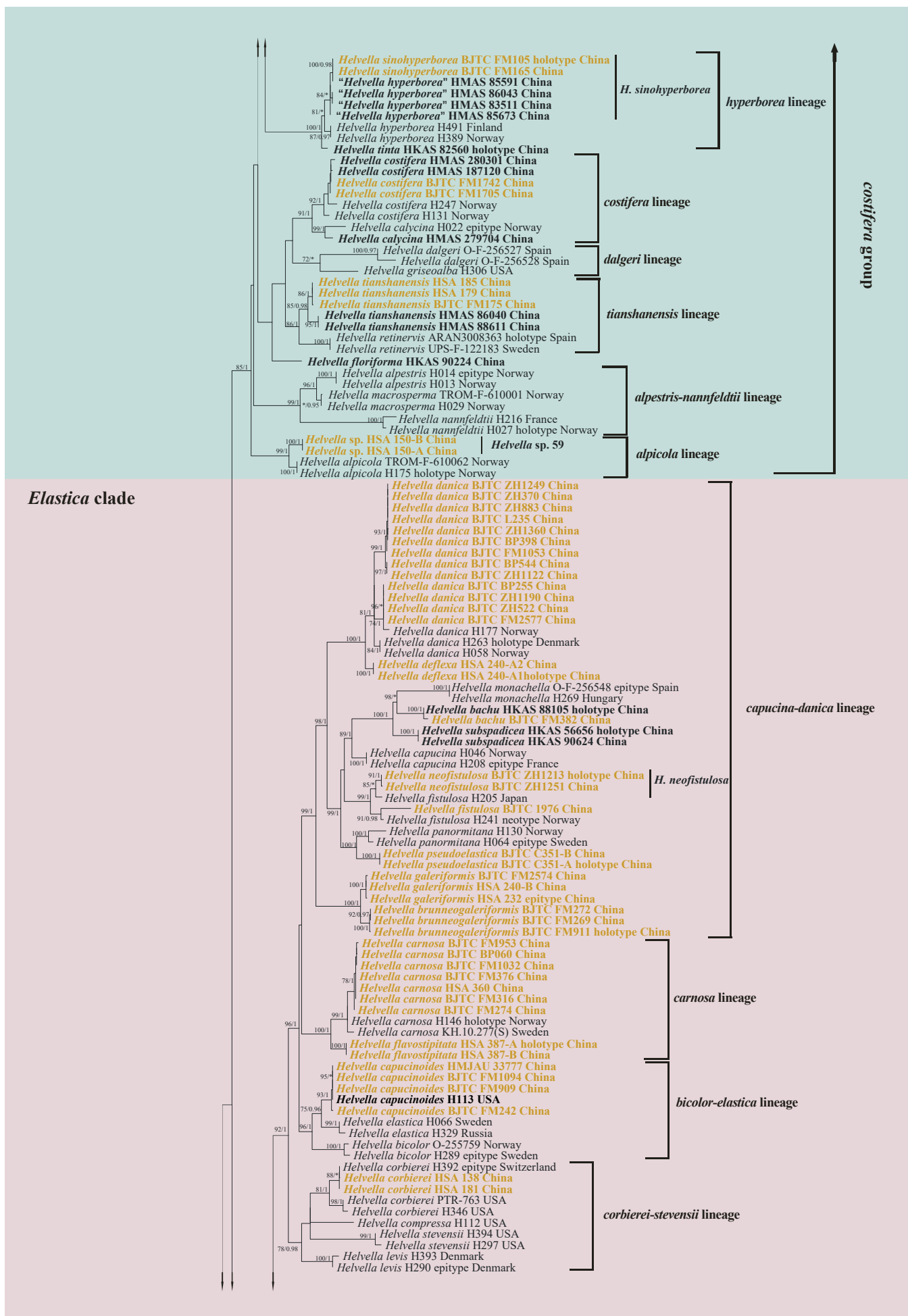


Fig. 1. (Continued).



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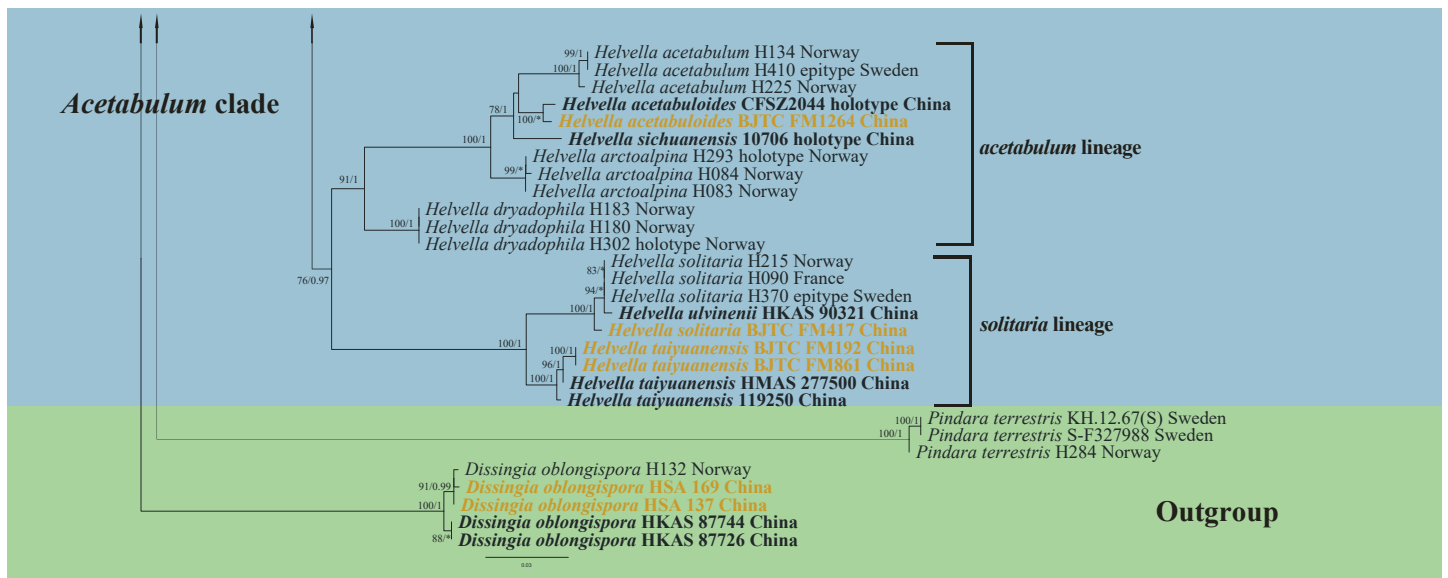


Fig. 1. (Continued).

pezoides lineage, hypocrateriformis lineage, macropus lineage, and rivularis-sublicia lineage. The species *H. atroides*, *H. fibrosa*, *H. japonica* and *H. orientitomentosa* are outside the macropus lineages and formed their own independent branches. Within the Lacunosa clade, 13 lineages were recognized, i.e., *alpestris-nannfeldtii* lineage, *alpicola* lineage, *alpina-corium* lineage, *atrapallescens* lineage, *costifera* lineage, *dalgeri* lineage, *fusca-lactea* lineage, *hyperborea* lineage, *lacunosa-helvellula* lineage, *palustris-philonotis* lineage, *phlebophora* lineage, *sulcata-phlebophoroides* lineage, and *tianshanensis* lineage. Both *H. floriforma* and *H. queletiana* are outside all lineages and formed their own independent branches. Furthermore, six of the 13 lineages (*atrapallescens* lineage, *fusca-lactea* lineage, *lacunosa-helvellula* lineage, *palustris-philonotis* lineage, *phlebophora* lineage and *sulcata-phlebophoroides* lineage) and the species *H. queletiana* gathered into a distinct and strong supported group, which was recognized as the *lacunosa* subclade. The remaining seven lineages and *H. floriforma* in the *Lacunosa* clade were scattered outside the *lacunosa* subclade. For improving the discussion, they were designated as a group, i.e., *costifera* group (Fig. 1).

#### ITS-based phylogenetic analysis

The ITS dataset was employed to delimit and detect the Chinese *Helvella* species diversity in this study. ITS sequence is considered a universal barcode for fungi (Schoch *et al.* 2012) and helpful for analyses of *Helvella* groups from ectomycorrhizal root tips. A total of 715 ITS sequences, including 168 novel sequences from Chinese collections, were obtained for this study. Our multigene phylogenetic analysis (Fig. 1) revealed that Chinese *Helvella* collections were classified in four clades, therefore these ITS sequences were accordingly split into four parts (Datasets II–V) that represented four clades (*Acetabulum* clade, *Crispa* clade, *Elastica* clade and *Lacunosa* clade). This parsing of species facilitates the molecular phylogenetic analyses for delimiting many Chinese *Helvella* species and detecting the phylogenetic relationships between these *Helvella* species. The phylogenetic analyses for all four clades are documented below.

**Acetabulum clade:** Dataset II (ITS) was used to delimit and detect the *Helvella* species of the *Acetabulum* clade. This dataset comprised 30 sequences of the *Acetabulum* clade, in which eight sequences were isolated from our *Helvella* specimens. The length of the dataset was 321 bp after alignment and exclusion of poorly aligned sites. Maximum likelihood and BI analyses yielded identical tree topologies; only the tree inferred from the ML analysis is shown (Supplementary Fig. S1). This ITS-based phylogenetic analysis resolved seven clusters from this clade. Of these, five clusters were identified as known species because each cluster contains the authentic sequence(s) of these species, i.e., *H. acetabulum*, *H. acetabuloides*, *H. solitaria*, *H. sichuanensis*, and *H. taiyuanensis*. They all occur in China. The remaining two clusters represent two putatively unknown species, marked as *Helvella* sp. 1, 2, which are respectively from Iran or an unknown country (See Supplementary Fig. S1, Supplementary Table S3).

**Crispa clade:** Dataset III (ITS) was used to delimit and detect the *Helvella* species of the *Crispa* clade. This dataset comprised 126 sequences from the *Crispa* clade, in which 35 sequences were isolated from our *Helvella* specimens. The length of the dataset was 794 bp after alignment and exclusion of poorly aligned sites. Maximum likelihood and BI analyses yielded identical tree topologies; only the tree inferred from the ML analysis is shown (Supplementary Fig. S2). A total of 13 clusters with strong support were recognized in this clade. Of these, six clusters were identified as known species, i.e., *H. crispoides*, *H. involuta*, *H. maculatoides*, *H. orienticrispa*, *H. pseudoreflexa*, and *H. zhongtiaoensis*, and they all occur in China. Two clusters composed of Chinese collections were new to science, which are described as new species in this study (see Taxonomy). The remaining five clusters were suggested as putatively unknown species, marked as *Helvella* sp. 3–7. Notably, *Helvella* sp. 5 occurs in China and an unknown country. The other four species are respectively from France, Germany or the United States (See Supplementary Fig. S2, Supplementary Table S4).

**Elastica clade:** Dataset IV (ITS) was used to delimit and detect the *Helvella* species of the *Elastica* clade. This dataset comprised 318 sequences, including 102 sequences newly isolated from our *Helvella* specimens. The length of the dataset was 578 bp after alignment and exclusion of poorly aligned sites. Maximum likelihood and BI analyses yielded identical tree topologies; only the tree inferred from the ML analysis is shown (Supplementary Fig. S3). A total of 66 strong support clusters corresponding to 66 *Helvella* species were resolved in this clade. Of these, 26 clusters corresponded to known species because each cluster contains the authentic sequence(s) of these species. Sixteen species of them occur in China, *i.e.*, *H. atroides*, *H. bachu*, *H. capucinoides*, *H. carnosa*, *H. corbierei*, *H. danica*, *H. ephippioides*, *H. fistulosa*, *H. galeriformis*, *H. guttata*, *H. macropus*, *H. nordlandica*, *H. orentitomentosa*, *H. pubescens*, *H. scyphoides*, and *H. subspadicea*. Fourteen clusters composed of Chinese collection(s) are treated in this study (see Taxonomy). The other 26 clusters were suggested as putatively new species as currently no sporocarps are available for these species, or we were not able to examine the specimens in this study. They are accordingly marked as *Helvella* sp. 8–33. Notably, 14 species occur in China, *i.e.*, *Helvella* sp. 14–15, *Helvella* sp. 18, *Helvella* sp. 20, *Helvella* sp. 23–32. The others are from France, Germany, Italy, Japan, United States, South Korea, Spain, Switzerland, etc. (See Supplementary Fig. S3, Supplementary Table S5).

**Lacunosa clade:** Dataset V (ITS) was used to delimit and detect the *Helvella* species of the *Lacunosa* clade. This dataset comprised 247 sequences, including 29 sequences newly isolated from our *Helvella* specimens. The length of the dataset was 517 bp after alignment and exclusion of poorly aligned sites. Maximum likelihood and BI analyses yielded identical tree topologies; only the tree inferred from the ML analysis is shown (Supplementary Fig. S4). A total of 66 strongly supported clusters corresponding to 66 *Helvella* species were resolved in this clade. Of these, 39 clusters corresponded to known species because each cluster contains the authentic sequence(s) of these species. Twenty-nine species occur in China, *i.e.*, *H. atra*, *H. austrooccidentalis*, *H. borealis*, *H. calycina*, *H. costifera*, *H. cystidiata*, *H. fulva*, *H. huangii*, *H. jizushanica*, *H. lacunosa*, *H. liui*, *H. lobata*, *H. magna*, *H. parva*, *H. philonotis*, *H. phlebophora*, *H. phlebophoropsis*, *H. plateata*, *H. ravidata*, *H. rugosa*, *H. sublactea*, *H. subtinta*, *H. terricola*, *H. tianshanensis*, *H. tinta*, *H. varia*, *H. vitrea*, *H. vulgata* and *H. yunnanensis*. Two clusters composed of Chinese collections are described as new species in this study (see Taxonomy). The other 25 clusters were suggested as putatively new species as currently no sporocarps are available for these species, or we were not able to examine the specimens in this study. They are accordingly marked as *Helvella* sp. 34–58. Notably, one of these species occur in China, *i.e.*, *Helvella* sp. 42. The others are respectively from Denmark, Germany, Hungary, Mexico, Norway, Sweden, United States, etc. (See Supplementary Fig. S4, Supplementary Table S6).

### Species diversity of *Helvella* in China

Dataset VI (ITS/nrLSU) comprised 581 sequences with 574 sequences from Chinese samples, in which all Chinese *Helvella* species determined based on the phylogenetic analyses for Datasets I–V outlined above were included except for *H. palustris* (only having *hsp*) (Fig. 1, Supplementary Figs S1–S4). *Dissingia oblongispora* was selected as the outgroup. The length of the aligned dataset was 1 238 bp after exclusion of poorly aligned

sites, with 529 bp for ITS and 709 bp for nrLSU. Maximum likelihood and BI analyses yielded identical tree topologies; only the tree inferred from the ML analysis is shown (Fig. 2).

Our phylogenetic analyses revealed 92 phylogenetic species from the Chinese collections, which were further clustered in four *Helvella* clades (Fig. 2). Of these, 57 clusters correspond to 57 species known from China (Ariyawansa et al. 2015, Zhao et al. 2015, Hyde et al. 2016, Wang et al. 2016, 2019, 2022, 2023a, Zhao et al. 2016a, b, Tibpromma et al. 2017, Zhuang et al. 2018, Xu et al. 2022, Lu et al. 2023, Yu et al. 2023), including five species new to China, *i.e.*, *H. carnosa* (Fig. 2, Supplementary Fig. S3), *H. corbierei* (Fig. 2, Supplementary Fig. S3), *H. fistulosa* (Fig. 2, Supplementary Fig. S3), *H. nordlandica* (Fig. 2, Supplementary Fig. S3) and *H. scyphoides* (Fig. 2, Supplementary Fig. S3). Of the remaining 35 species, 18 species are described as new species (see Taxonomy section in this paper), and 17 are suggested as potentially undescribed species (marked as *Helvella* sp. 5 in Fig. 2 & Supplementary Fig. S2; *Helvella* sp. 14–15, *Helvella* sp. 18, *Helvella* sp. 20, *Helvella* sp. 23–32 in Figs 2 & S3; *Helvella* sp. 42. in Figs 2 & S4; *Helvella* sp. 59 in Figs 1 & 2). Notably, *H. floriforma* (Figs 1, 2, Datasets I, VI), *H. palustris* (Fig. 1, Dataset I), and *Helvella* sp. 59 (Figs 1, 2, Datasets I, VI) are not represented in the ITS dataset because there were no available ITS sequence of the three species.

## TAXONOMY

Based on our phylogenetic and morphological data, 18 new species are described and illustrated, an epitype is designated for one species, and five species are newly recorded from China.

***Helvella albopatella*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849609. Fig. 3.

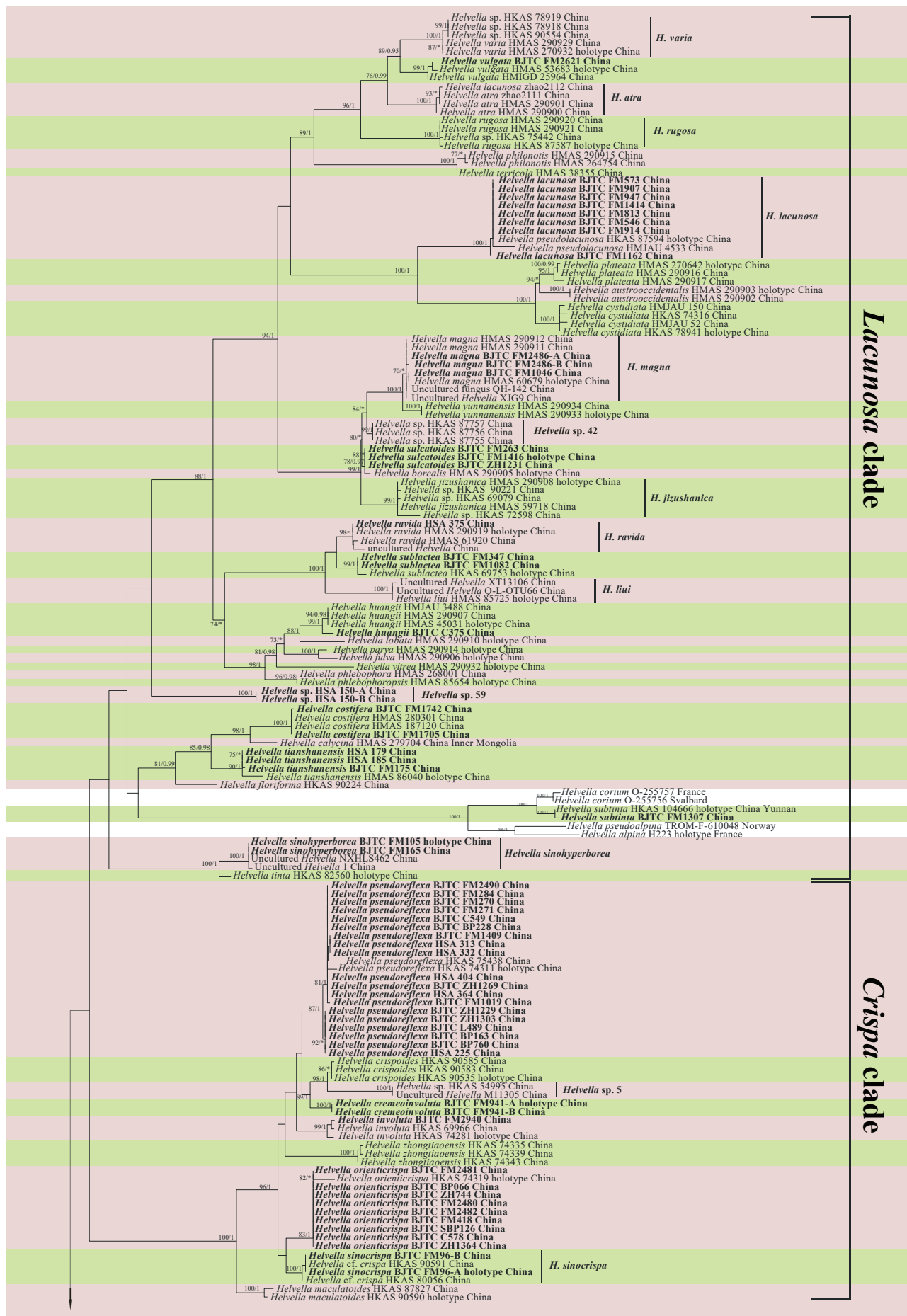
**Etymology:** *albopatella*, *albo-*, white, *patella*, disc, referring to the white and disc-like cap of this species.

**Apothecia** stipitate-cupulate; cap regularly or irregularly discoid, slightly cumulate, or occasionally recurved, 0.4–0.7 cm high, 0.9–2.0 cm broad; hymenium white, grey white to pale white, becoming dark brown when dry; receptacle surface pubescent, concolourous with the disc, becoming yellowish when dry; stipe terete, 1.0–3.0 cm high, 0.15–0.3 cm broad, solid, greyish white to white, becoming yellowish when dry. **Ectal excipulum** 75–100 µm broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 15–35 × 11–16 µm. **Medullary excipulum** 225–300 µm broad, of interwoven *textura intricata*, hyphae 2.0–6.0 µm broad. **Asci** pleurohynchous, tapering towards the base, 8-spored, 225–265 × 13–17 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 16–18 × 11–12.5 µm, [Lm × Wm = 16.7 × 11.7 µm, Q = 1.32–1.56, Q<sub>av</sub> = 1.42 ± 0.07]. **Paraphyses** filiform, septate, pale brown, 6.0–10.0 µm wide at clavate to subcapitate tips and 2.0–4.0 µm below.

**Habitat:** Scattered on the ground in broadleaf forest and coniferous forest.

**Distribution:** Known from Shanxi Province, Northern China.





**Fig. 2.** Phylogeny of Chinese *Helvella* species inferred from the Dataset VI (ITS/nrLSU) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. New species are printed in **bold**.

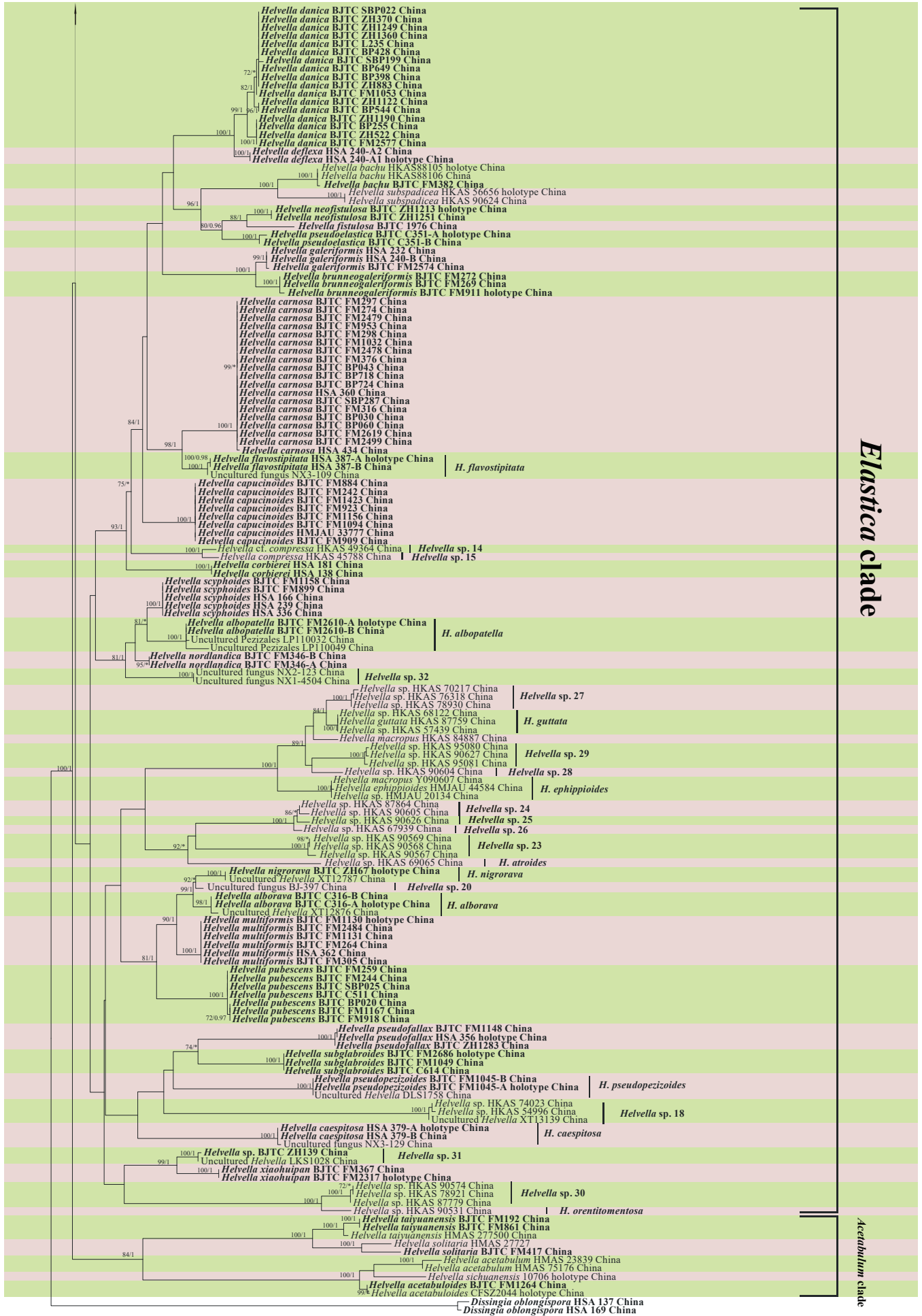
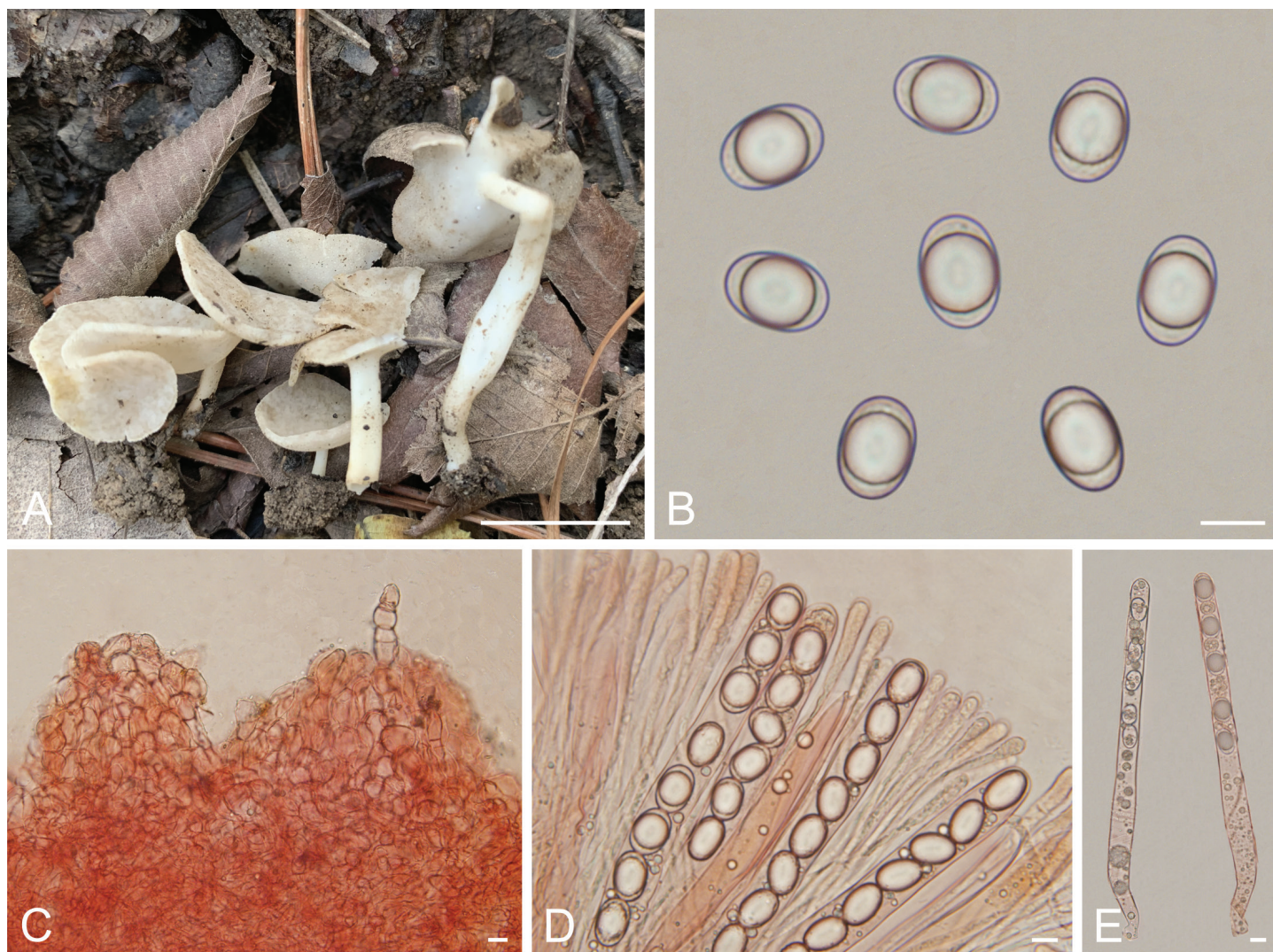


Fig. 2. (Continued).



**Fig. 3.** *Helvella albopatella* (BJTC FM2610-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B–E = 10  $\mu$ m.

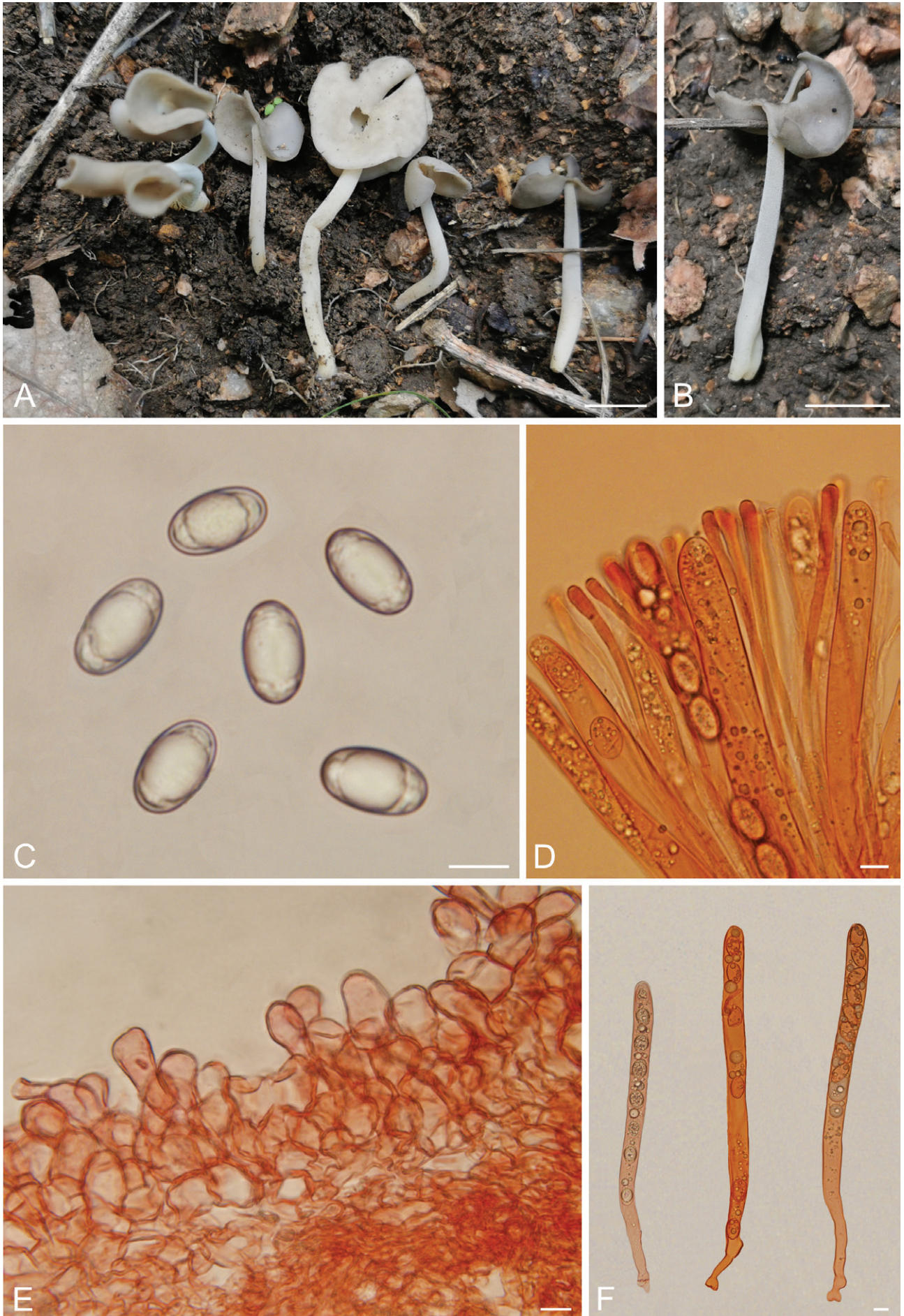
*Specimens examined:* **China**, Shanxi Province, Linfen City, Pu County, Wulushan mountains, on the ground in broadleaf forest dominated by *Quercus* sp., 36°56'23"N, 111°23'46"E, alt. 1 644.6 m, 26 Aug. 2022, N. Mao (**holotype** BJTC FM2610-A, GenBank Acc. No.: ITS = OR355082, nrLSU = OR355252, *tef1- $\alpha$*  = OR359014, *rpb2* = OR359137, *hsp* = OR366079); *ibid.*, (BJTC FM2610-B).

*Notes:* *Helvella albopatella* is recognized by its disc-like cap and overall whitish ascomata with terete stipe. It is placed in the *hypocrateriformis* lineage, *Elastica* clade, sister to *H. nordlandica*, in our phylogenetic tree (Fig. 1). Morphologically, *H. albopatella* is distinguished from all other species in this lineage (including *H. nordlandica*) by its overall whitish ascomata with disc-like cap and a terete stipe. Molecularly, there are one *hsp*, three *rpb2*, five nrLSU, and 12 *tef1- $\alpha$*  substitutions between *H. albopatella* and *H. nordlandica*. Four ITS sequences downloaded from GenBank matched *H. albopatella* in our analysis (Fig. S3). These sequences are respectively extracted from ascomata or ectomycorrhizal root tips of *Quercus liaotungensis* and *Quercus* sp., from China. These results implied that *H. albopatella* is also occurs in another region of China and can form ectomycorrhizas with *Quercus* species. *Helvella albopatella* shares less than 94.87 % similarity in ITS region with other *Helvella* species.

*Helvella alborava* L. Fan, N. Mao & H. Zhou, *sp. nov.* MycoBank MB 849610. Fig. 4.

*Etymology:* *alborava*, *albo-*, means white, *rava*, means grey, referring to grey whitish ascomata.

*Apothecia* stipitate-capitate; cap regularly or irregularly saddle-shaped, sometimes regularly or irregularly cup-like, 0.6–2.0 cm high, 1.0–1.9 cm broad, at first margin adhered to stipe, then reflexed with age; hymenium greyish white to greyish, becoming brown to dark brown when dry; receptacle surface fine pubescent, concolourous with hymenium, becoming pale yellow to greyish brown when dry; stipe glabrous to fine pubescent, terete, 2.1–4.2 cm high, 0.2–0.5 cm broad, solid or hollow, greyish white, becoming pale brown to greyish brown when dry, towards base with inconspicuous grooves, base slightly enlarged. *Ectal excipulum* 80–120  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 13–30  $\times$  8–16  $\mu$ m. *Medullary excipulum* 175–250  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.0–4.0  $\mu$ m broad. *Asci* pleurorhynchous, tapering, 8-spored, 215–275  $\times$  13–19  $\mu$ m. *Ascospores* ellipsoidal, with one large oil drop when mature, 17–19  $\times$  10–12  $\mu$ m, [Lm  $\times$  Wm = 17.9  $\times$  10.9  $\mu$ m,



**Fig. 4.** *Helvella alborava* (BJTC C316-A, holotype). **A, B.** Apothecia. **C.** Ascospores in water. **D.** Asci and paraphyses in Congo Red. **E.** Ectal excipulum in Congo Red. **F.** Immature asci in Congo Red. Scale bars: A = 1 cm; B–F = 10  $\mu$ m.

$Q = 1.45\text{--}1.81$ ,  $Q_{av} = 1.64 \pm 0.09$ ]. *Paraphyses* filiform, septate, pale brown,  $4.0\text{--}6.0$   $\mu\text{m}$  wide at clavate to subcapitate tips and  $2.0\text{--}3.5$   $\mu\text{m}$  below.

**Habitat:** Scattered on the ground in broadleaf forest.

**Distribution:** Known from Hebei Province, Northern China.

**Specimens examined:** **China**, Hebei Province, Xinglong County, on the ground in mixed broadleaf forest, alt. 898 m, 25 Aug. 2020, G.Q. Chen (**holotype** BJTC C316-A, GenBank Acc. No.: ITS = OR355106, nrLSU = OR355276, *tef1- $\alpha$*  = OR359033, *rpb2* = OR359155, *hsp* = OR366098), *ibid.*, (BJTC C316-B).

**Notes:** *Helvella alborava* is phylogenetically nested in the *rivularis-sublicia* lineage, *Elastica* clade (Fig. 1). There are three other species in this lineage from China, *i.e.*, *H. multiformis*, *H. nigrorava* and *H. pubescens*. *Helvella multiformis* is distinguished from *H. alborava* by its shape and colour of cap (cup to saddle, grey to black) and large spores ( $Lm \times Wm = 19.1 \times 11.9$   $\mu\text{m}$ ); *Helvella nigrorava* by its dark grey to greyish black and saddle-shaped caps, and distinct pubescence on the surface of the receptacle and stipe (also see the comments on *H. nigrorava* in this paper); *Helvella pubescens* by its cup-like cap and stipe densely covered with pyramidal ‘warts’ (Skrede *et al.* 2017). In addition, seven *hsp*, three *rpb2*, five nrLSU, and 26 *tef1- $\alpha$*  substitutions separated *H. alborava* from *H. multiformis*; seven *hsp*, nine *rpb2*, 16 nrLSU substitutions and 41 *tef1- $\alpha$*  substitutions from *H. pubescens*. Two ITS ECM-sequences match *H. alborava* in our analysis (Fig. S3). They are extracted from the ectomycorrhizal root tips of *Quercus variabilis*, and *Q. liaotungensis* from Shanxi Province and Beijing, Northern China. These indicate that *H. alborava* could form ectomycorrhizas with the two plant species. *Helvella alborava* shares less than 97.6 % similarity in ITS region with other *Helvella* species.

***Helvella brunneogaleriformis*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849611. Fig. 5.

**Etymology:** *brunneogaleriformis*, because this species is phylogenetically closely related to *H. galeriformis* but with a brown cap.

**Apothecia** stipitate-capitate; cap irregularly umbrella-type, hemispheric to irregularly saddle-shaped,  $0.5\text{--}2.2$  cm high,  $1.0\text{--}2.7$  cm broad, at first margin adhered to stipe, then irregularly folded and free from stipe with age; hymenium ochre to brown when fresh, becoming dark brown to black brown when dry; receptacle surface glabrous, white, becoming yellow when dry; stipe terete  $2.5\text{--}7.5$  cm high,  $0.3\text{--}0.7$  cm broad, solid or hollow, white, or gradually becoming pale yellow brown to brown towards the base, becoming yellow when dry, base slightly enlarged, occasionally with a few shallow grooves. *Ectal excipulum*  $80\text{--}130$   $\mu\text{m}$  broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate,  $23\text{--}56 \times 13\text{--}29$   $\mu\text{m}$ . *Medullary excipulum*  $150\text{--}230$   $\mu\text{m}$  broad, of interwoven *textura intricata*, hyphae  $2.5\text{--}6.5$   $\mu\text{m}$  broad. *Asci* pleurorhynchous, tapering, 8-spored,  $260\text{--}330 \times 15\text{--}20$   $\mu\text{m}$ . *Ascospores* ellipsoid, with one large oil drop when mature,  $17\text{--}20.5 \times 10.5\text{--}12.5$   $\mu\text{m}$ , [ $Lm \times Wm = 19 \times 11.5$   $\mu\text{m}$ ,  $Q = 1.5\text{--}1.8$ ,  $Q_{av} = 1.65 \pm 0.07$ ]. *Paraphyses* filiform,  $2.5\text{--}4.5$   $\mu\text{m}$  below, pale yellow to pale

brown, septate, clavate to subcapitate,  $6\text{--}13(15)$   $\mu\text{m}$  wide at tips.

**Habitat:** Scattered on the ground in coniferous forest.

**Distribution:** Known only from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Xinzhou City, Wutai County, Wutaishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*, alt. 1 222 m, 27 Aug. 2019, C. Yang (**holotype** BJTC FM911, GenBank Acc. No.: ITS = OR355065, nrLSU = OR355235, *tef1- $\alpha$*  = OR359000, *rpb2* = OR359124, *hsp* = OR366062); *ibid.*, Lvliang City, Jiaocheng County, Guandishan mountains, on the ground in coniferous forest dominated by *L. principis-rupprechtii* and *P. asperata*, alt. 2 003 m, 7 Sep. 2017, Y.Y. Xu (BJTC FM269); *ibid.*, (BJTC FM272).

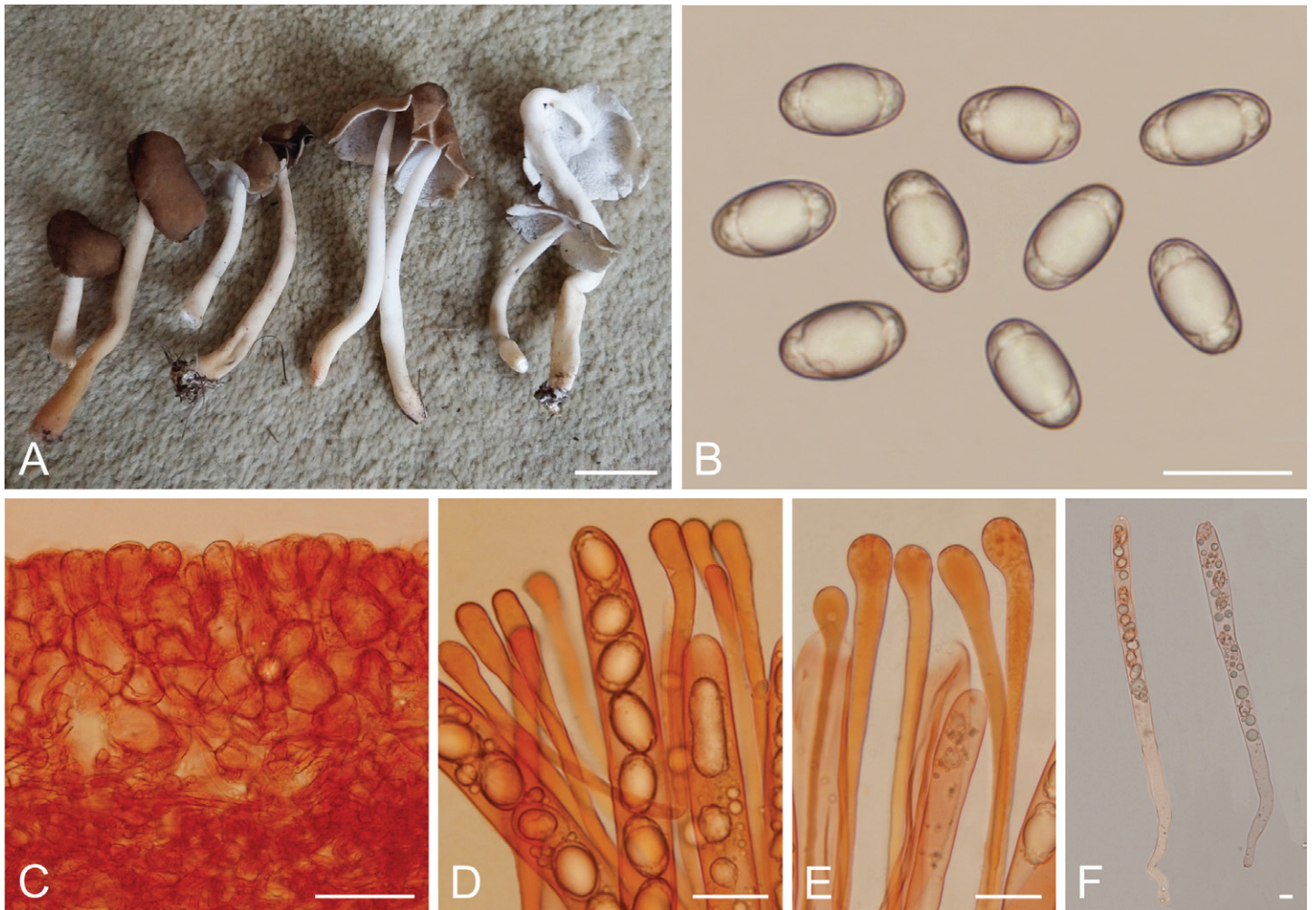
**Notes:** *Helvella brunneogaleriformis* is sister to *H. galeriformis* in the *capucina-danica* lineage, *Elastica* clade, as revealed in present analysis (Fig. 1). Morphologically, both species are similar in cap shape. However, the two species can be separated by the hymenium colour, which is ochre to brown in *H. brunneogaleriformis*, whereas grey blackish in *H. galeriformis*. *Helvella brunneogaleriformis* is similar to *H. elastica* and *H. carnosa* in its brown hymenium, and yellowish stipe. However, *H. elastica* and *H. carnosa* typically have a saddle-like cap, and *H. elastica* has larger asci ( $330\text{--}360 \times 15\text{--}17$   $\mu\text{m}$ ) and relatively longer ascospores ( $19\text{--}22 \times 10.6\text{--}12.2$   $\mu\text{m}$ ) (Skrede *et al.* 2017). *Helvella carnosa* has narrower asci ( $270\text{--}300 \times 13\text{--}15$   $\mu\text{m}$ ) and relatively narrower paraphyses at the apex ( $5\text{--}8$   $\mu\text{m}$ ) (Skrede *et al.* 2017). In addition, six *hsp*, 13 *rpb2*, 16 nrLSU, and 20 *tef1- $\alpha$*  substitutions separated it from *H. elastica*; five *hsp*, 11 *rpb2*, 17 nrLSU substitutions, and 32 *tef1- $\alpha$*  substitutions from *H. carnosa*. *Helvella brunneogaleriformis* shares less than 97.8 % similarity in ITS region with other *Helvella* species.

***Helvella caespitosa*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849612. Fig. 6.

**Etymology:** *caespitosa*, referring to its habit of growing in a cluster.

**Apothecia** stipitate-capitate; cap saddle-shaped, margin strongly deflexed,  $0.4\text{--}1.0$  cm high,  $0.5\text{--}1.8$  cm broad; hymenium dark grey to grey blackish, becoming greyish black when dry; receptacle surface pale grey, becoming greyish brown when dry; stipe pubescent, terete,  $1.5\text{--}3.0$  cm high,  $0.2\text{--}0.3$  cm broad, solid, pale grey, becoming greyish brown when dry, base often with white mycelia. *Ectal excipulum*  $75\text{--}150$   $\mu\text{m}$  broad, of *textura angularis*, cells hyaline to pale brown, outermost cells club-shaped,  $16\text{--}31 \times 8\text{--}14$   $\mu\text{m}$ . *Medullary excipulum*  $150\text{--}300$   $\mu\text{m}$  broad, of interwoven *textura intricata*, hyphae  $2.0\text{--}5.5$   $\mu\text{m}$  broad. *Asci* pleurorhynchous, tapering, 8-spored,  $210\text{--}300 \times 14\text{--}21$   $\mu\text{m}$ . *Ascospores* ellipsoid, with one large oil drop when mature,  $16.5\text{--}20 \times 10\text{--}12$   $\mu\text{m}$ , [ $Lm \times Wm = 18.5 \times 11.1$   $\mu\text{m}$ ,  $Q = 1.50\text{--}1.80$ ,  $Q_{av} = 1.66 \pm 0.07$ ]. *Paraphyses* filiform, septate, pale brown,  $5.0\text{--}9.0$   $\mu\text{m}$  wide at clavate to subcapitate tips and  $1.5\text{--}3.5$   $\mu\text{m}$  below.

**Habitat:** Clustered or grouped on the ground in coniferous and broadleaf mixed forest dominated by *Betula* sp., *Pinus tabuliformis*, *Populus* sp., *Quercus wutaishansea*.



**Fig. 5.** *Helvella brunneogaleriformis* (BJTC FM911, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Paraphyses in Congo Red. **F.** Immature asci in Congo Red. Scale bars: A = 2 cm; B, D, E = 20 µm; C = 50 µm; F = 10 µm.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Lvliang City, Xing County, Heichashan mountains, on the ground in coniferous and broadleaf mixed forest dominated by *Betula* sp., *Pinus tabuliformis*, *Populus* sp., *Quercus wutaishansea*, alt. 1 590 m, 6 Sep. 2018, J.Z. Cao (**holotype** HSA 379-A, GenBank Acc. No.: ITS = OR355091, nrLSU = OR355261, *tef1-α* = OR359022, *hsp* = OR366087); *ibid.*, (HSA 379-B).

**Notes:** *Helvella caespitosa* is diagnosed by its grey blackish saddle-shaped cap with strongly deflexed margin, dark grey stipe with pubescent surface, and habit of growing in cluster. Our present analysis places *H. caespitosa* in the *fallax-pezizoides* lineage in the *Elastica* clade and sister to *H. bresadolae* and *H. pezizoides* (Fig. 1). *Helvella bresadolae* is easily confused with *H. caespitosa* in appearance of its cap. However, *H. bresadolae* is distinguished from *H. caespitosa* by its stipe base without white mycelia and relatively smaller asci (200–240 × 14–17 µm). The difference between *H. pezizoides* and *H. caespitosa* is in the cap margin of ascomata, which is inrolled in *H. pezizoides* but deflexed in *H. caespitosa* (Dissing 1966b). In addition, one *hsp* and 13 nrLSU substitutions separated it from *H. bresadolae*; 10 *hsp*, 13 nrLSU, and 19 *tef1-α* substitutions from *H. pezizoides*. Three ITS sequences downloaded from GenBank matched *H. caespitosa* in our analysis (Fig. S3). These sequences are respectively extracted from ectomycorrhizal

root tips of *Picea crassifolia*, *Pinus tabuliformis*, and *Quercus liaotungensis*, from Ningxia Hui Autonomous Region, Northern China. These indicated that *H. caespitosa* also occurs in other regions of China, and has a wide host range. *Helvella caespitosa* shares less than 89.94 % similarity in ITS region with other *Helvella* species.

***Helvella cremeoinvoluta*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849613. Fig. 7.

**Etymology:** *cremeoinvoluta*, referring to its high similarity to *H. involuta* in appearance of ascoma but pale grey-white to pale cream receptacle.

**Apothecia** stipitate-capitate; cap saddle-shaped to irregularly lobate, 0.5–1.3 cm high, 1.1–2.3 cm broad, margin strongly rolled upwards and completely wrapped the hymenium at first, then unrolled with age; hymenium glabrous, garish white to cream, becoming pale brown to yellowish brown when dry; receptacle surface subpubescent, pale grey white to pale cream, becoming yellowish when dry; stipe 2.6–4.8 cm high, 0.7–1.7 cm broad, white to yellowish white, becoming pale yellow to greyish yellow when dry, ribbed, blunt-edged, partly anastomosing and double-edged, with lacunae between ribs, chambered inside. **Ectal excipulum** 125–190 µm broad, of *textura angularis*, cells hyaline to pale brown, outermost cells catenuliform in long fascicled



**Fig. 6.** *Helvella caespitosa* (HSA 379-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Asci in Congo Red. Scale bars: A = 1 cm; B, E = 10 µm; C, D = 20 µm.

tufts, with cylindrical to subclavate cells of  $18\text{--}37 \times 11\text{--}18 \mu\text{m}$ . *Medullary excipulum* 240–330 µm broad, of interwoven *textura intricata*, hyphae 3–7 µm broad. *Asci* pleurorhynchous, tapering, 8-spored,  $225\text{--}275 \times 13\text{--}18 \mu\text{m}$ . *Ascospores* ellipsoid, with one large oil drop when mature,  $17\text{--}18.5 \times 9.5\text{--}11 \mu\text{m}$ , [Lm  $\times$  Wm =  $17.6 \times 10.1 \mu\text{m}$ , Q = 1.60–1.85,  $Q_{av} = 1.74 \pm 0.10$ ]. *Paraphyses* filiform, septate, hyaline to pale brown, 4–6 µm wide at tips and 2–4 µm below.

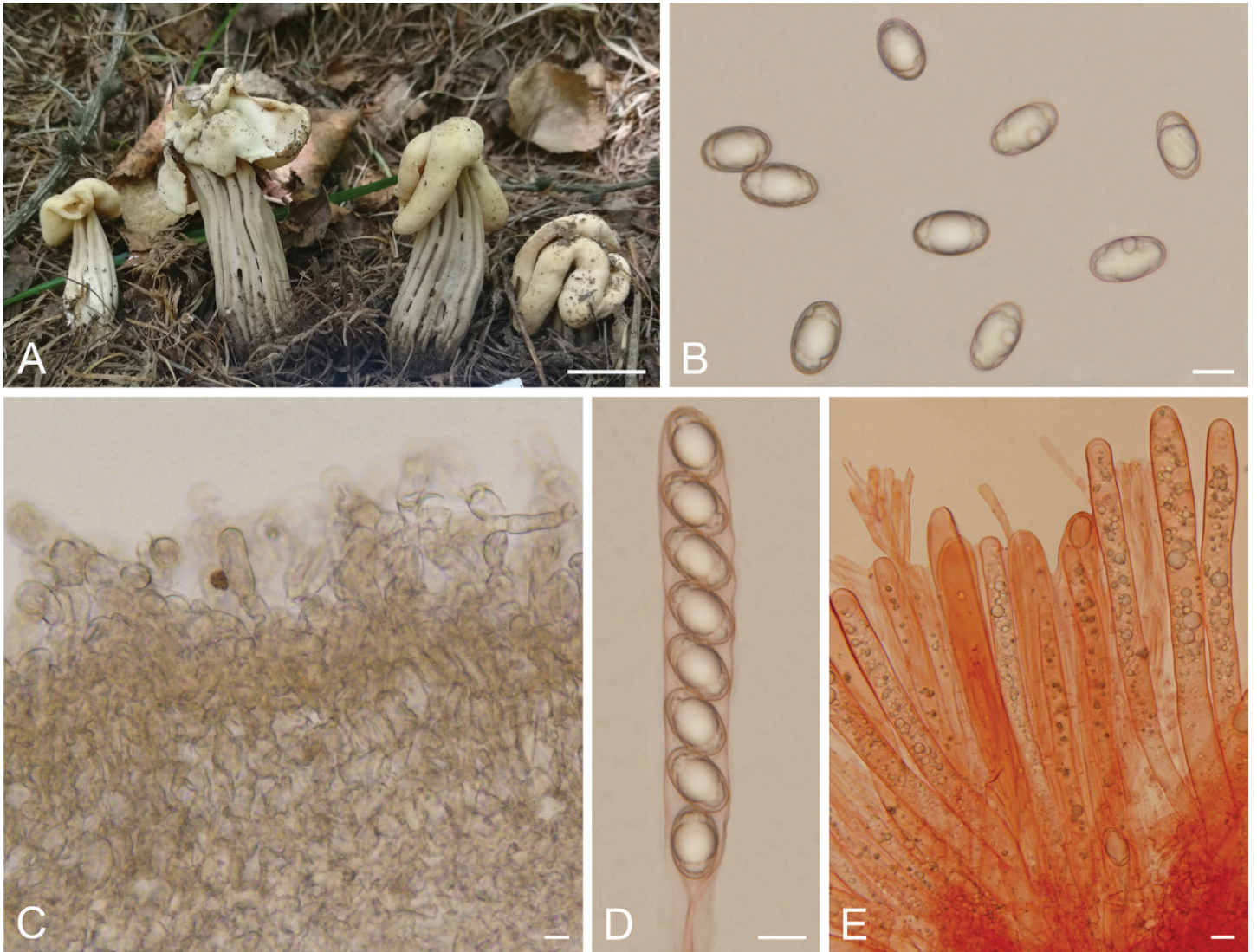
**Habitat:** Scattered to gregarious on the ground in coniferous forest dominated by *Larix principis-rupprechtii*.

**Distribution:** Known only from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Xinzhou City, Wutai County, Wutaishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii*,  $38^{\circ}55'39''\text{N}$ ,  $113^{\circ}35'43''\text{E}$ , alt.

1 543 m, 28 Aug. 2019, Y. Shen (**holotype** BJTC FM941-A, GenBank Acc. No.: ITS = OR355153, nrLSU = OR355324, *tef1- $\alpha$*  = OR359066, *rpb2* = OR359178, *hsp* = OR366131); *ibid.*,  $38^{\circ}55'39''\text{N}$ ,  $113^{\circ}35'40''\text{E}$ , alt. 1 543 m, 28 Aug. 2019, Y. Shen (BJTC FM941-B).

**Notes:** *Helvella cremeoinvoluta* and *H. crispoides* are closely related to each other in our phylogenetic analyses (Figs 1, 2). Morphologically, *H. crispoides* differs from *H. cremeoinvoluta* by its grey receptacle surface, white stipe, relatively shorter ascospores ( $15\text{--}17 \times 9.5\text{--}11.5 \mu\text{m}$ ), and broader paraphyses at the apex ( $6\text{--}7.5 \mu\text{m}$ ) (Tibpromma *et al.* 2017). *Helvella involuta* is similar to *H. cremeoinvoluta* in cap shape and ascospores size, but the colour of its receptacle surface is clearly yellowish (Zhao *et al.* 2015), that is completely different from the present species (grey-white to pale cream). *Helvella cremeoinvoluta* shares less than 96.1 % ITS similarity with other *Helvella* species, supporting it is a distinct species.



**Fig. 7.** *Helvella cremeinvoluta* (BJTC FM941-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in water. **D.** Asci in Congo Red. **E.** Immature asci and paraphyses in Congo Red. Scale bars: A = 1 cm; B–E = 10  $\mu$ m.

***Helvella deflexa*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849614. Fig. 8.

**Etymology:** *deflexa*, referring to the strongly deflexed margin of the cap.

**Apothecia** stipitate-capitate; cap saddle-shaped, 0.7–1.1 cm high, 2.0–3.0 cm broad, with a strongly deflexed margin; hymenium brown to dark brown; receptacle surface yellowish white; stipe terete, 1.5–3.0 cm high, 0.3–0.5 cm broad, solid or hollow, white to cream. **Ectal excipulum** 75–100  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells elongate club-shaped, 21–50  $\times$  10–20  $\mu$ m. **Medullary excipulum** 100–125  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.0–6.0  $\mu$ m broad. **Asci** pleurorhynchous, tapering, 8-spored, 290–325  $\times$  15–23  $\mu$ m. **Ascospores** ellipsoid and with slightly narrow ends, with one large central oil drop and a few of small ones when mature, 19.5–23  $\times$  12–14.5  $\mu$ m, [Lm  $\times$  Wm = 21.0  $\times$  13.1  $\mu$ m, Q = 1.46–1.81, Q<sub>sv</sub> = 1.60  $\pm$  0.08]. **Paraphyses** filiform, septate, subhyaline to pale brown, 6.0–11.0  $\mu$ m wide at clavate to subcapitate tips and 2.0–4.0  $\mu$ m below.

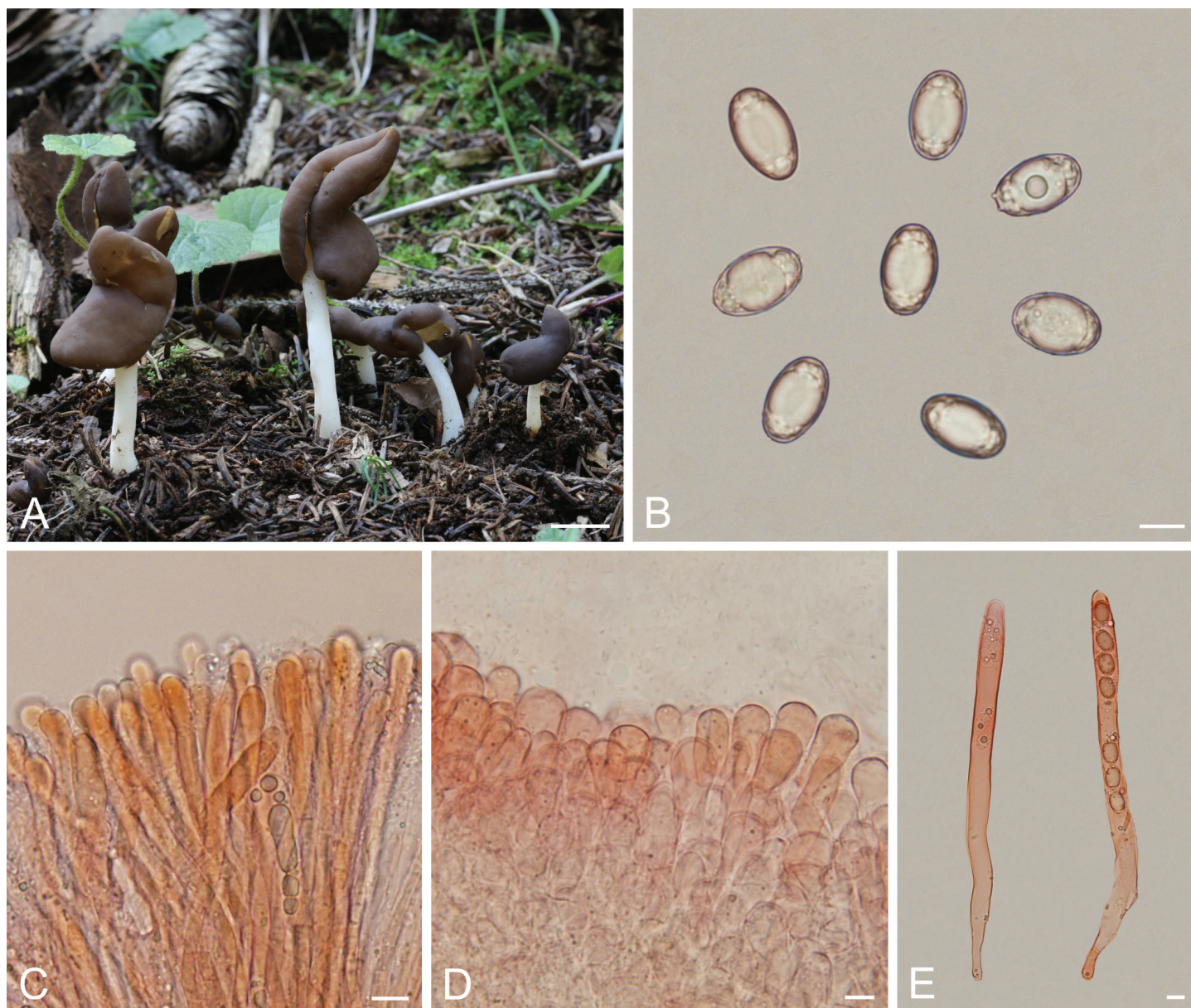
**Habitat:** Scattered on the ground in coniferous and broadleaf mixed forest dominated by *Betula* sp. and *Larix principis-rupprechtii*.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Lvliang City, Jiaocheng County, Pangquangou, on the ground in coniferous and broadleaf mixed forest dominated by *Betula* sp. and *Larix principis-rupprechtii*, alt. 2 160 m, 28 Aug. 2018, L.J. Guo (**holotype** HSA 240-A1, GenBank Acc. No.: ITS = OR355069, nrLSU = OR355239, *tef1- $\alpha$*  = OR359004, *rpb2* = OR359127, *hsp* = OR366066); *ibid.*, (HSA 240-A2).

**Notes:** *Helvella deflexa* is recognized by its saddle-shaped cap with strongly deflexed margin, a long and white terete stipe and ascospores with slightly narrow ends. It is phylogenetically nested in the *capucina-danica* lineage of *Elastica* clade, closely related to *H. danica* (Fig. 1). *Helvella danica* differs from *H. deflexa* by its campanulate cap and clustered habit (Skrede et al. 2017). Molecularly, one *hsp*, three *rpb2*, two nrLSU and seven *tef1- $\alpha$*  substitutions separated *H. deflexa* from *H. danica*. *Helvella deflexa* shares less than 96.16 % similarity in ITS region with other *Helvella* species.





**Fig. 8.** *Helvella deflexa* (HSA 240-A1, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Paraphyses in Congo Red. **D.** Ectal excipulum in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B–D = 10  $\mu$ m.

***Helvella flavostipitata*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.*  
Mycobank MB 849615. Fig. 9.

**Etymology:** *flavostipitata*, *flavo-*, yellow, referring to the yellow stalk of this saddle fungus.

**Apothecia** stipitate-capitate; cap saddle-shaped, 2–2.4 cm high, 1.6–2.4 cm broad, margin adhered to stipe; hymenium yellowish brown to brown, becoming dark brown when dry; receptacle surface glabrous, pale grey to greyish yellow, becoming yellow when dry; stipe terete, 3.5–5.0 cm high, 0.3–0.4 cm broad, hollow, yellow, not change colour when dry, base often with white mycelia. **Ectal excipulum** 80–120  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 18–43  $\times$  9–21  $\mu$ m. **Medullary excipulum** 150–250  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.0–7.0  $\mu$ m broad. **Asci** pleurorhynchous, tapering, 8-spored, 250–310  $\times$  14–20  $\mu$ m. **Ascospores** ellipsoid, with one large oil drop when mature, 18–

20.5  $\times$  10.5–12.5  $\mu$ m, [Lm  $\times$  Wm = 19.3  $\times$  11.5  $\mu$ m, Q = 1.53–1.84, Q<sub>av</sub> = 1.67  $\pm$  0.08]. **Paraphyses** filiform, septate, pale yellow to pale brown, 5.5–9  $\mu$ m wide at clavate to subcapitate tips and 2.0–4.0  $\mu$ m below.

**Habitat:** Scattered on the ground in coniferous forest.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Lvliang City, Jiaocheng County, Panguangou, on the ground in coniferous forest dominated by *Picea* sp., alt. 1 860 m, 6 Sep. 2018, *H. Liu* (**holotype** HSA 387-A, GenBank Acc. No.: ITS = OR355035, nrLSU = OR355205, *tef1- $\alpha$*  = OR358976, *rpb2* = OR359103); *ibid.*, (HSA 387-B).

**Notes:** *Helvella flavostipitata* is phylogenetically sister to *H. carnosa* in the *carnosa* lineage, *Elastica* clade (Fig. 1). Morphologically, both species have regularly saddle-shaped and brown caps, but *H. carnosa* has a receptacle surface



**Fig. 9.** *Helvella flavostipitata* (HSA 387-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B, E = 10  $\mu$ m; C, D = 20  $\mu$ m.

with delicate pubescence and relatively narrower asci (13–15  $\mu$ m) (Skrede *et al.* 2017), whereas *H. flavostipitata* has a glabrous receptacle surface and wider asci (14–20  $\mu$ m diam). Molecularly, *H. flavostipitata* deviates consistently in one *hsp*, five nrLSU, and nine *tef1- $\alpha$*  substitutions. Five ITS sequences from GenBank matched *H. flavostipitata* in our analysis (Fig. S3). These sequences are respectively extracted from ascomata or ectomycorrhizal root tips of *Pinus wallichiana* and *P. tabuliformis*, from China or Pakistan. These results implied that *H. flavostipitata* can form ectomycorrhizas with *Pinus* species. *Helvella flavostipitata* shares less than 91.5 % similarity in ITS region with other *Helvella* species.

***Helvella multiformis*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849616. Fig. 10.

**Etymology:** *multiformis*, referring to the cap shape that includes cup and saddle.

**Apothecia** stipitate-cupulate; cap regular cupulate to saucer-shaped at first, then expanding to saddle-shaped, occasionally applanate, 0.7–2.2 cm high, 1.5–3.7 cm broad; hymenium, greyish black, dark brown, blackish to black, becoming dark brown to black when dry; receptacle surface pubescent, greyish to dark grey, becoming brown when dry; stipe terete, 2.0–5.0 cm high, 0.3–0.7 cm broad, solid or hollow, greyish white to greyish yellow, becoming greyish brown when dry, occasionally with grooves, base slightly enlarged. **Ectal excipulum** 120–200  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, sometimes extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptate, cells elongate club-shaped, 16–40  $\times$  8–21  $\mu$ m. **Medullary excipulum** 300–360  $\mu$ m broad, of interwoven *textura intricata*, hyphae 3.0–6.0  $\mu$ m broad. **Asci** pleurohynchous, tapering, 8-spored, 250–300  $\times$  17–20  $\mu$ m. **Ascospores** ellipsoid, with one large oil drop when mature, 18–20.5  $\times$  11–13  $\mu$ m, [Lm  $\times$  Wm = 19.1  $\times$  11.9  $\mu$ m, Q = 1.44–1.81, Q<sub>av</sub> = 1.61  $\pm$  0.09]. **Paraphyses** filiform, septate, subhyaline to pale



**Fig. 10.** *Helvella multififormis* (A, C, E–H. BJTC FM1130, holotype. B. BJTC FM1131. D. BJTC FM2484). **A–D.** Apothecia. **E.** Ascospores in water. **F.** Asci and paraphyses in Congo Red. **G.** Ectal excipulum in Congo Red. **H.** Immature asci in Congo Red. Scale bars: A–D = 1 cm; E–H = 10  $\mu$ m.

brown, 4.5–7.0  $\mu\text{m}$  wide at clavate to subcapitate tips and 2–4  $\mu\text{m}$  below.

**Habitat:** Scattered on the ground in broadleaf forest and coniferous forest.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Jincheng City, Qinshui County, Lishan mountains, on the ground in broadleaf forest dominated by *Quercus* sp., 35°29'4"N, 112°1'25"E, alt. 1 675m, 27 Aug. 2020, *N. Mao* (**holotype** BJTC FM1130, GenBank Acc. No.: ITS = OR355100, nrLSU = OR355270, *tef1- $\alpha$*  = OR359027, *rpb2* = OR359149, *hsp* = OR366092); *ibid.*, (BJTC FM1131); *ibid.*, Linfen City, Pu County, Wulushan mountains, on the ground in coniferous forest dominated by *Pinus* sp., 10 Sep. 2017, *X.Y. Yan* (BJTC FM305); *ibid.*, Lvliang City, Jiaocheng County, Guandishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*, alt. 2 003 m, 7 Sep. 2017, *J.Z. Cao* (BJTC FM264); *ibid.*, Lvliang City, Xing County, Heicha mountains, on the ground in broadleaf forest dominated by *Populus* sp., alt. 1 590 m, 5 Sep. 2018, *L.J. Guo* (HSA 362); *ibid.*, Taiyuan City, Loufan County, Yundingshan mountains, on the ground in coniferous forest dominated by *Pinus* sp., alt. 1 642 m, 23 Aug. 2022, *N. Mao* (BJTC FM2484).

**Notes:** *Helvella multiformis* is diagnosed by its variation of cap shape and colour (cup to saddle, grey to black). Phylogenetically it is nested in the *rivularis-sublicia* lineage, *Elastica* clade, and closely related to *H. poculiformis* and *H. sublicia* (Fig. 1). *Helvella poculiformis* is differentiated from *H. multiformis* by its cup-like and grey or grey-brown cap (never saddle-shaped and black), *H. sublicia* (syn. *Helvella ephippium*) by its whitish, greyish to dark grey cap (lacking black tints) (Skrede *et al.* 2020). Molecularly, five *hsp* and two *rpb2* substitutions separate this new species from *H. poculiformis*; five *hsp*, six *rpb2*, five nrLSU substitutions, and 11 *tef1- $\alpha$*  substitutions from *H. sublicia*. Four ITS sequences from GenBank matched *H. multiformis* in our analysis (Fig. S3). These sequences are respectively extracted from ascomata or ectomycorrhizal root tips of *Epipactis helleborine*, and *Pinus tabuliformis*, from Shanxi Province, Ningxia Hui Autonomous Region. These indicate that *H. multiformis* also occurs in other regions of China, and could form ectomycorrhiza with *E. helleborine* and *P. tabuliformis*. *Helvella multiformis* shares less than 95.35 % ITS similarity with other *Hevelia* species, supporting it as a distinct species.

***Helvella neofistulosa*** L. Fan, N. Mao & C.L. Hou, *sp. nov.* MycoBank MB 849617. Fig. 11.

**Etymology:** *neofistulosa*, *neo-*, new, because this species is phylogenetically closely related to *H. fistulosa*.

**Apothecia** stipitate-capitate; cap saddle-shaped, bi- to trilobate, or irregularly lobate, 0.6–2.5 cm high, 1.3–2.5 cm broad, at first margin adhered to stipe, then reflexed with age; hymenium grey to greyish yellow, becoming ochre to dark brown when dry; receptacle surface subpubescent, greyish white, becoming yellow when dry; stipe 1.3–5.3 cm high, 0.3–0.7 cm broad, solid or hollow, greyish white to cream, becoming yellow when dry, base slightly enlarged. **Ectal excipulum** 70–120  $\mu\text{m}$  broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 22–50  $\times$  10–23  $\mu\text{m}$ . **Medullary excipulum** 160–250

$\mu\text{m}$  broad, of interwoven *textura intricata*, hyphae 2.3–4.5  $\mu\text{m}$  broad. **Asci** pleurorhynchous, tapering, 8-spored, 250–300  $\times$  14–21  $\mu\text{m}$ . **Ascospores** ellipsoid, with one large oil drop when mature, 18–22.5  $\times$  10.5–12.5  $\mu\text{m}$ , [Lm  $\times$  Wm = 20  $\times$  11.6  $\mu\text{m}$ , Q = 1.58–1.89, Q<sub>av</sub> = 1.72  $\pm$  0.08]. **Paraphyses** filiform, 2.5–4.5  $\mu\text{m}$  below, septate, clavate to subcapitate, 6–11  $\mu\text{m}$  wide at tips.

**Habitat:** Scattered on the ground in mixed broadleaf forest.

**Distribution:** Known from Beijing and Hebei Province, Northern China.

**Specimens examined:** **China**, Beijing, Huairou District, Labagoumen, on the ground in mixed broadleaf forest, alt. 1 250 m, 25 Aug. 2020, *H. Zhou* (**holotype** BJTC ZH1213, GenBank Acc. No.: ITS = OR355059, nrLSU = OR355229, *tef1- $\alpha$*  = OR358994, *rpb2* = OR359118, *hsp* = OR366056); Hebei Province, Chicheng county, Dahaituo, on the ground in mixed broadleaf forest, alt. 1 267 m, 25 Aug. 2020, *H. Zhou* (BJTC FM1251).

**Notes:** *Helvella neofistulosa* is placed in a position sister to *H. fistulosa* in the *capucina-danica* lineage, *Elastica* clade (Fig. 1). Morphologically, the two species can be separated by the cap colour, which is grey to greyish yellow in *H. neofistulosa* but brown in *H. fistulosa*. Molecularly, there are two *hsp*, one *rpb2*, three nrLSU, and 22 *tef1- $\alpha$*  substitutions between the two species (Skrede *et al.* 2017). Two ITS sequences downloaded from GenBank matched *H. neofistulosa* in our analysis (Fig. S3). The two sequences are respectively extracted from ascomata or ectomycorrhizal root tips of broadleaved trees, from China or Japan, implying that *H. neofistulosa* also occurs in Japan and can form ectomycorrhizas with broadleaved trees. *Helvella neofistulosa* shares less than 95.08 % ITS similarity with other *Hevelia* species, supporting it is a distinct species.

***Helvella nigrorava*** L. Fan, Y.Y. Xu & C.L. Hou, *sp. nov.* MycoBank MB 849618. Fig. 12.

**Etymology:** *nigrorava*, *nigro-*, means black, *rava*, means grey, referring to the blackish grey cap of this species.

**Apothecium** stipitate-capitate; cap saddle-shaped, 1.5 cm high, 1.2 cm broad; hymenium dark grey to greyish black, becoming black brown to black when dry; receptacle surface pubescent, concolourous with hymenium, becoming dark brown to black brown when dry; stipe pubescent, terete, 1.7 cm high, 0.2–0.35 cm broad, solid or hollow, greyish white to grey, becoming greyish brown to brown when dry, base slightly enlarged. **Ectal excipulum** 75–115  $\mu\text{m}$  broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 11–37  $\times$  9–16  $\mu\text{m}$ . **Medullary excipulum** 135–200  $\mu\text{m}$  broad, of interwoven *textura intricata*, hyphae 2.0–5.0  $\mu\text{m}$  broad. **Asci** pleurorhynchous, tapering, 8-spored, 235–265  $\times$  15–20  $\mu\text{m}$ . **Ascospores** ellipsoid, with one large oil drop when mature, 16.5–18.5  $\times$  11–12.5  $\mu\text{m}$ , [Lm  $\times$  Wm = 17.5  $\times$  11.8  $\mu\text{m}$ , Q = 1.40–1.63, Q<sub>av</sub> = 1.49  $\pm$  0.07]. **Paraphyses** filiform, septate, pale yellow to pale brown, 4.5–6.0  $\mu\text{m}$  wide at clavate to subcapitate tips and 2.5–4.0  $\mu\text{m}$  below.

**Habitat:** Scattered on the ground in broadleaf forest.

**Distribution:** Known from Beijing, Northern China.



**Fig. 11.** *Helvella neofistulosa* (BJTC ZH1213, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in water. **D.** Asci and paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B, E = 10  $\mu$ m; C, D = 20  $\mu$ m.

*Specimen examined:* **China**, Beijing, Changping District, Tiebiyinshan Mountains, on the ground in broadleaf forest, alt. 362 m, 14 Aug. 2019, *H. Zhou* (**holotype** BJTC ZH67, GenBank Acc. No.: ITS = OR355105, nrLSU = OR355275, *tef1*- $\alpha$  = OR359032, *rpb2* = OR359154, *hsp* = OR366097).

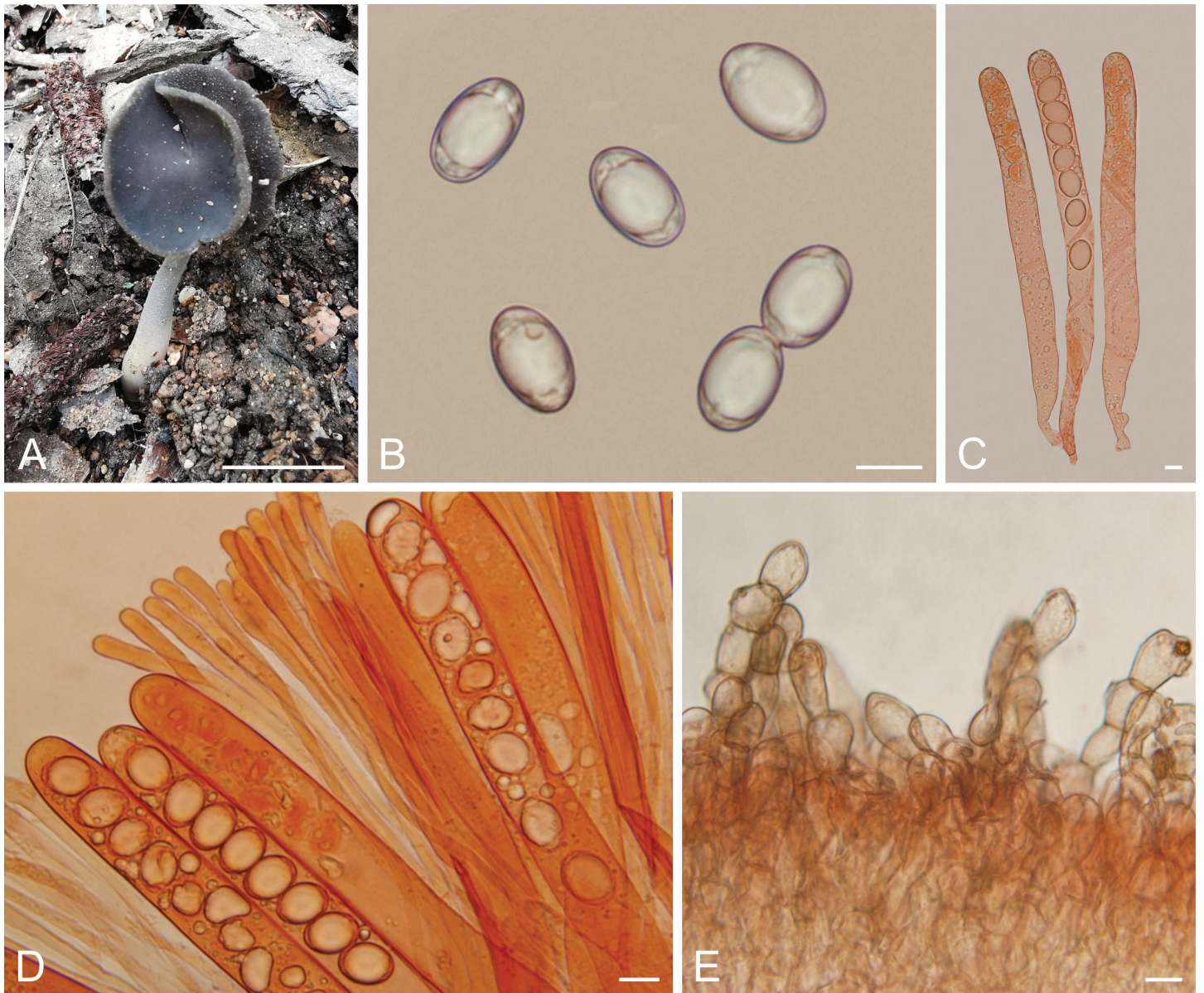
*Notes:* *Helvella nigrorava* was phylogenetically sister to *H. alborava*, a new species described in this study, in the *rivularis-sublicia* lineage, *Elastica* clade (Fig. 1). The two species can be separated by the cap shape and colour, which is saddle-shaped or irregularly cup-like and grey to greyish white in *H. alborava*, but regularly saddle-shaped and dark grey to greyish black in *H. nigrorava*. Also, *H. nigrorava* is more distinct pubescent on both of receptacle and stipe than that in *H. alborava*. Molecularly, there were four *hsp*, three *rpb2*, two nrLSU, and 20 *tef1*- $\alpha$  substitutions between the two species. One ITS sequence (GenBank MF405780) extracted from ectomycorrhizal

root tip of *Quercus variabilis* in Shanxi Province, Northern China matched this species (Fig. S3), indicating that *H. nigrorava* can form ectomycorrhizas with this plant species. *Helvella nigrorava* shares less than 95.9% similarity in ITS region with other *Helvella* species.

***Helvella pseudoelastica*** L. Fan, Y.Y. Xu & H. Zhou, *sp. nov.* MycoBank MB 849619. Fig. 13.

*Etymology:* *pseudoelastica*, referring to the similarity to *H. elastica* in appearance of ascomata.

*Apothecia* stipitate-capitate; cap saddle-shaped to bilobate, 0.7–1.2 cm high, 1.2–1.9 cm broad; hymenium greyish white, becoming brown when dry; receptacle surface greyish white, becoming yellowish when dry; stipe terete, 2.8–4.3 cm high, 0.2–0.4 cm broad, solid or hollow, white to yellowish white



**Fig. 12.** *Helvella nigrorava* (BJTC ZH67, holotype). **A.** Apothecium. **B.** Ascospores in water. **C.** Asci in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Ectal excipulum in Congo Red. Scale bars: A = 1 cm; B–E = 10 µm.

with distinct tints of pinkish especially near the base, becoming yellowish brown when dry. *Ectal excipulum* 85–115 µm broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells elongate club-shaped, 15–40 × 8–20 µm. *Medullary excipulum* 125–150 µm broad, of interwoven *textura intricata*, hyphae 2.0–5.0 µm broad. *Asci* pleurorhynchous, tapering, 8-spored, 260–290 × 16–21 µm. *Ascospores* ellipsoid, with one large oil drop when mature, 20–23 × 11–13 µm, [Lm × Wm = 21.5 × 12.1 µm, Q = 1.57–1.91, Q<sub>av</sub> = 1.77 ± 0.11]. *Paraphyses* filiform, septate, pale brown, 5.0–8.0 µm wide at clavate to subcapitate tips and 2.0–4.0 µm below.

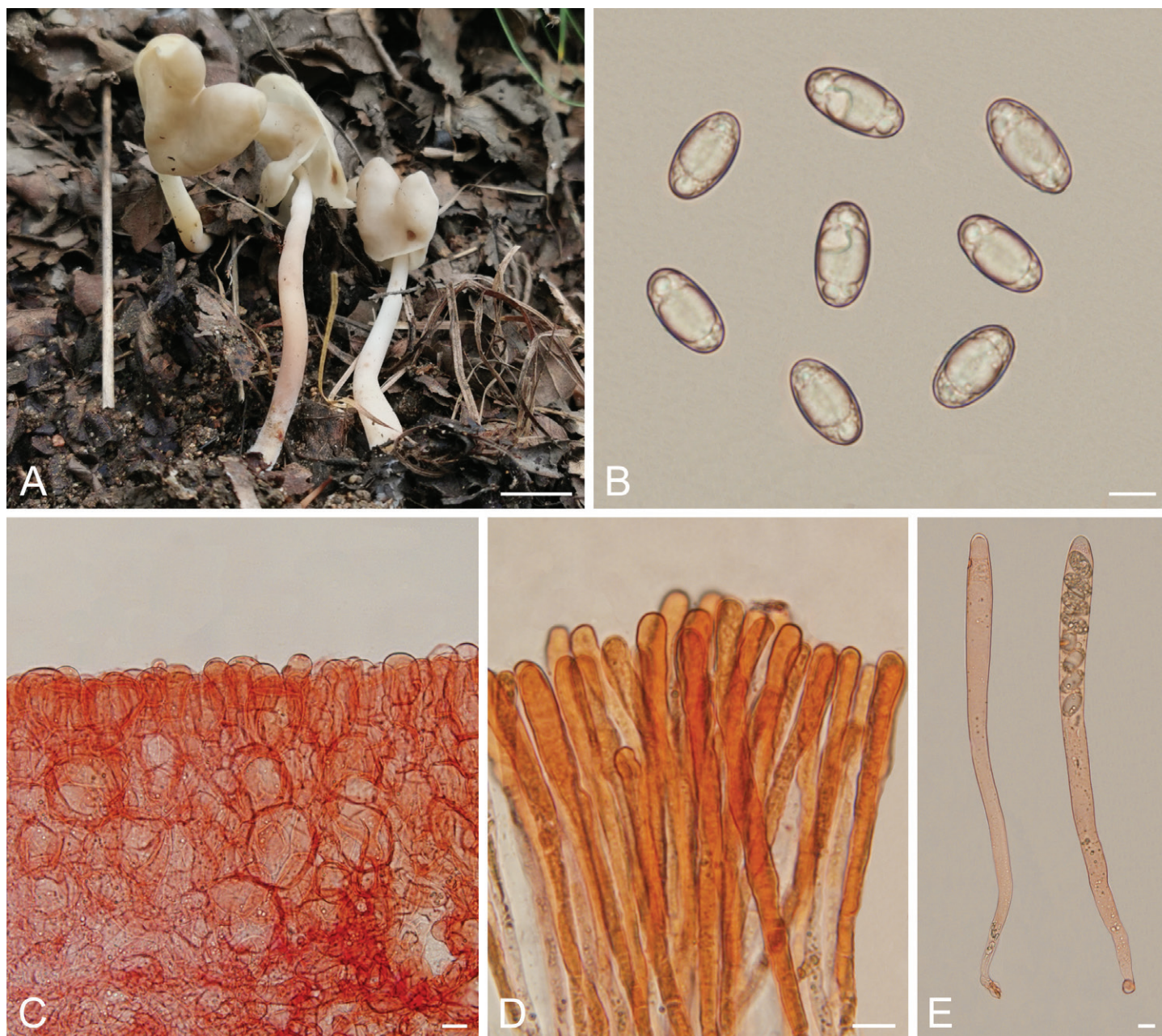
**Habitat:** Scattered on the ground in broadleaf forest.

**Distribution:** Known from Hebei Province, Northern China.

**Specimens examined:** **China**, Hebei Province, Xinglong County, on the ground in broadleaf forest, alt. 946 m, 22 Aug. 2020, G.Q. Cheng (**holotype** BJTC C351-A, GenBank Acc. No.: ITS = OR355170, nrLSU =

OR355341, *tef1-α* = OR359078, *rpb2* = OR359189, *hsp* = OR366143); *ibid.*, (BJTC C351-B).

**Notes:** *Helvella pseudoelastica* was nested in the *capucina-danica* lineage, *Elastica* clade (Fig. 1). Morphologically, it is difficult to separate *H. pseudoelastica* from the species of *capucina-danica* lineage, *bicolor-elastica* lineage and *carnosa* lineage in the *Elastica* clade by morphology alone. Most of these specimens had been misidentified as *H. elastica* during past decades. The stipe with distinct pinkish tint may be unique for this new species, but more specimens are needed to confirm this observation. *Helvella pseudoelastica* is closely related to *H. panormitana*, but there are two *hsp*, six *rpb2*, five nrLSU, and 12 *tef1-α* substitutions between them. *Helvella pseudoelastica* shares less than 93.08 % similarity in the ITS region with other *Helvella* species. An ITS specimen-sequence (GenBank KR673633) from South Korea matched *H. pseudoelastica* in our phylogenetic analysis (Fig. S3), which indicated that *H. pseudoelastica* also occurs in South Korea.



**Fig. 13.** *Helvella pseudoelastica* (BJTC C351-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B–E = 10  $\mu$ m.

***Helvella pseudofallax*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849620. Fig. 14.

**Etymology:** *pseudofallax*, referring to its morphological similarity to *H. fallax*.

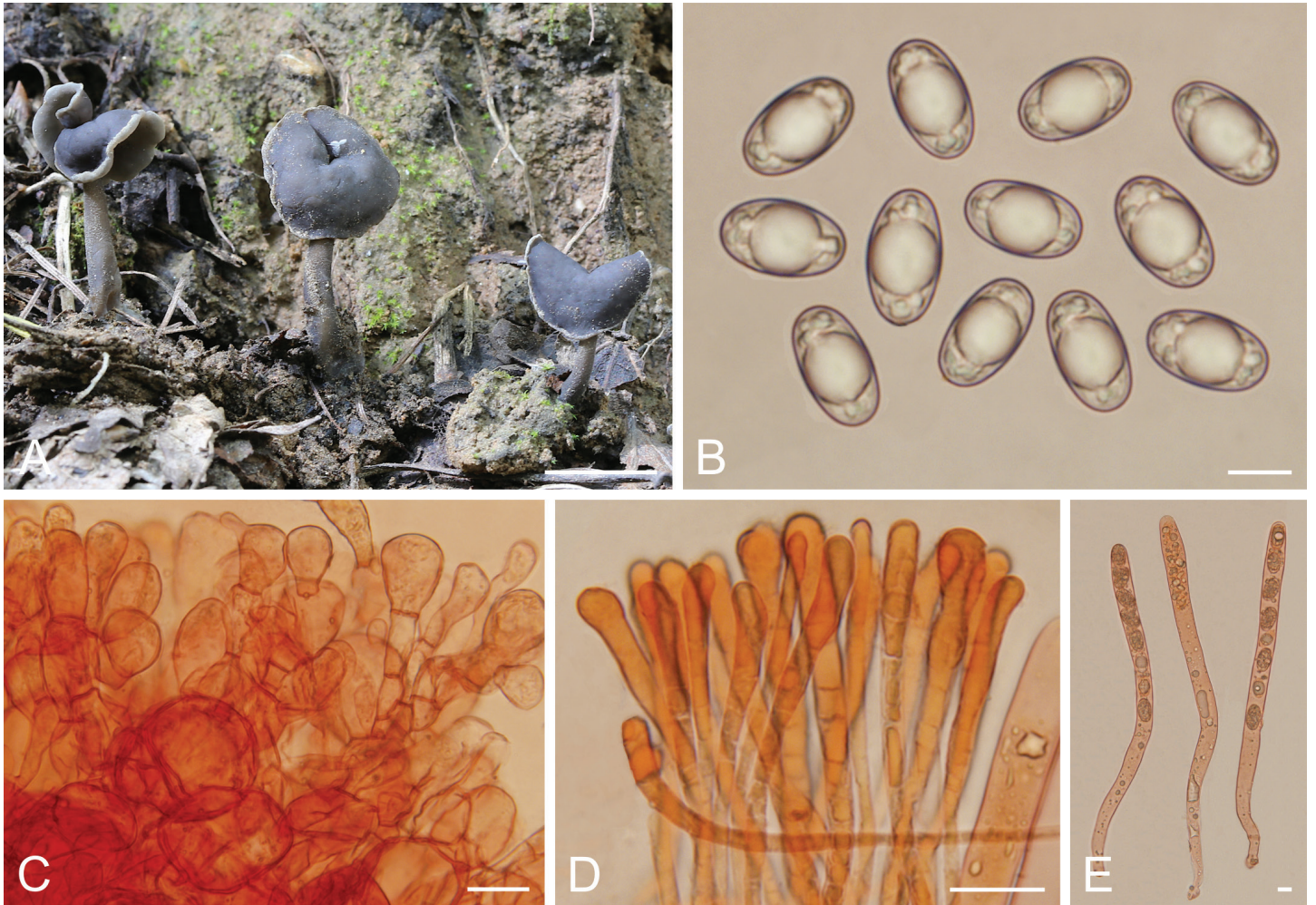
**Apothecia** stipitate-capitate; cap saddle-shaped or irregularly lobed, 0.6–1.0 cm high, 0.7–1.8 cm broad, at first margin adhered to stipe, then occasionally irregularly folded and free from stipe with age; hymenium grey, dark grey to blackish, becoming greyish black when dry; receptacle surface subpubescent, pale grey, becoming greyish black to black brown when dry; stipe subpubescent, terete, 1.5–5.0 cm high, 0.2–0.6 cm broad, solid or hollow, grey, becoming greyish black when dry. **Ectal excipulum** 150–200  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-

shaped, 20–45  $\times$  9–20  $\mu$ m. **Medullary excipulum** of interwoven *textura intricata*, 200–350  $\mu$ m thick, hyphae, 2.0–6.5  $\mu$ m broad. **Asci** pleurorhynchous, tapering, 8-spored, 230–300  $\times$  13–19  $\mu$ m. **Ascospores** ellipsoid, with one large oil drop when mature, 15.5–19.5  $\times$  9.5–11  $\mu$ m, [Lm  $\times$  Wm = 17.5  $\times$  10.3  $\mu$ m, Q = 1.55–1.90, Q<sub>av</sub> = 1.70  $\pm$  0.08]. **Paraphyses** filiform, septate, pale brown, 6.0–10.0  $\mu$ m wide at clavate to subcapitate tips and 2.0–4.5  $\mu$ m below.

**Habitat:** Scattered on the ground in broadleaf forest dominated by *Populus* sp.

**Distribution:** Known from Hebei and Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Lvliang City, Xing County, Heichashan mountains, on the ground in broadleaf forest dominated



**Fig. 14.** *Helvella pseudofallax* (HSA 356, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B, E = 10 µm; C, D = 20 µm.

by *Populus* sp., alt. 1 590 m, 5 Sep. 2018, J.Z. Cao (**holotype** HSA 356, GenBank Acc. No.: ITS = OR355083, nrLSU = OR355253, *tef1-α* = OR359015, *rpb2* = OR359138, *hsp* = OR366080); *ibid.*, Xinzhou City, Wutai County, Wutaishan mountains, on the ground in broadleaf forest dominated by *Populus* sp., alt. 1 910 m, 3 Sep. 2020, J.Z. Cao (BJTC FM1148); Hebei Province, Zhangjiakou City, Chicheng County, on the ground in broadleaf forest, alt. 1 314 m, 26 Aug. 2020, H. Zhou (BJTC ZH1283).

**Notes:** *Helvella pseudofallax* was phylogenetically placed in the *fallax-pezizoides* lineage of the *Elastica* clade (Fig. 1). *Helvella fallax* and *H. pulla* are highly similar to the present species in morphology, but the pubescent receptacle and slightly reflexed cap margin separate it from the two former species (Skrede *et al.* 2017, 2020). In addition, six *hsp*, nine *rpb2*, 19 LSU, and 20 *tef1-α* substitutions separated this new species from *H. fallax*; 12 *hsp*, nine *rpb2*, and 21 LSU substitutions from *H. pulla*. *Helvella pseudofallax* shares less than 93.74 % similarity in the ITS region with other *Helvella* species.

***Helvella pseudopezizoides*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849621. Fig. 15.

**Etymology:** *pseudopezizoides*, referring to the similarity to *H. pezizoides*.

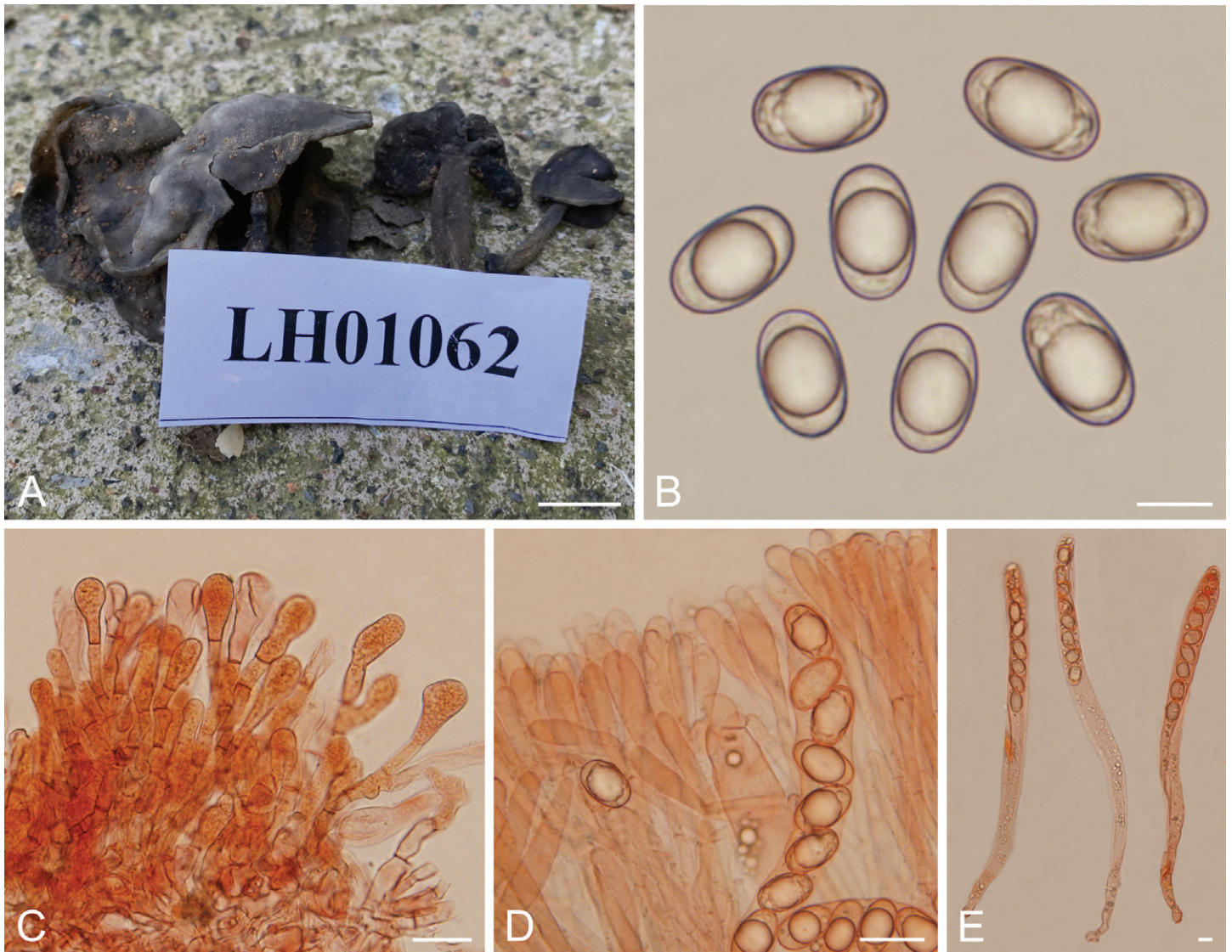
**Apothecia** stipitate-capitate; cap saddle-shaped, or irregularly lobed, margin adnate to stipe, 0.9–2.0 cm high, 0.7–2.5 cm broad; hymenium grey, dark grey to grey blackish, becoming black when dry, slightly wrinkled with age; receptacle surface pubescent to villose, greyish black, becoming black when dry; stipe terete, 1.5–3.0 cm high, 0.2–0.7 cm broad, solid or hollow, concolourous with receptacle surface, becoming black when dry, occasionally with grooves. **Ectal excipulum** 100–200 µm broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 20–45 × 8–16 µm. **Medullary excipulum** 300–400 µm broad, of interwoven *textura intricata*, hyphae 2.0–6.0 µm broad. **Asci** pleurorhynchous, tapering, 8-spored, 230–300 × 13–18 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 16–19.5 × 10.5–12 µm, [Lm × Wm = 18.1 × 11.2 µm, Q = 1.50–1.76, Q<sub>av</sub> = 1.62 ± 0.06]. **Paraphyses** filiform, septate, pale brown, 6.0–11.0 µm wide at clavate to subcapitate tips and 2.0–4.5 µm below.

**Habitat:** Scattered on the ground in forest dominated by *Quercus* sp.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Jingcheng City, Qinshui County, Shangwoquan Village, on the ground in broadleaf forest





**Fig. 15.** *Helvella pseudopezizoides* (BJTC FM1045-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Asci in Congo Red. Scale bars: A = 1 cm; B, E = 10 µm; C, D = 20 µm.

dominated by *Quercus* sp., alt. 1 170 m, 25 Aug. 2020, *H. Liu* (**holotype** BJTC FM1045-A, GenBank Acc. No.: ITS = OR355089, nrLSU = OR355259, *tef1-α* = OR359020, *rpb2* = OR359143, *hsp* = OR366085); *ibid.*, (BJTC FM1045-B).

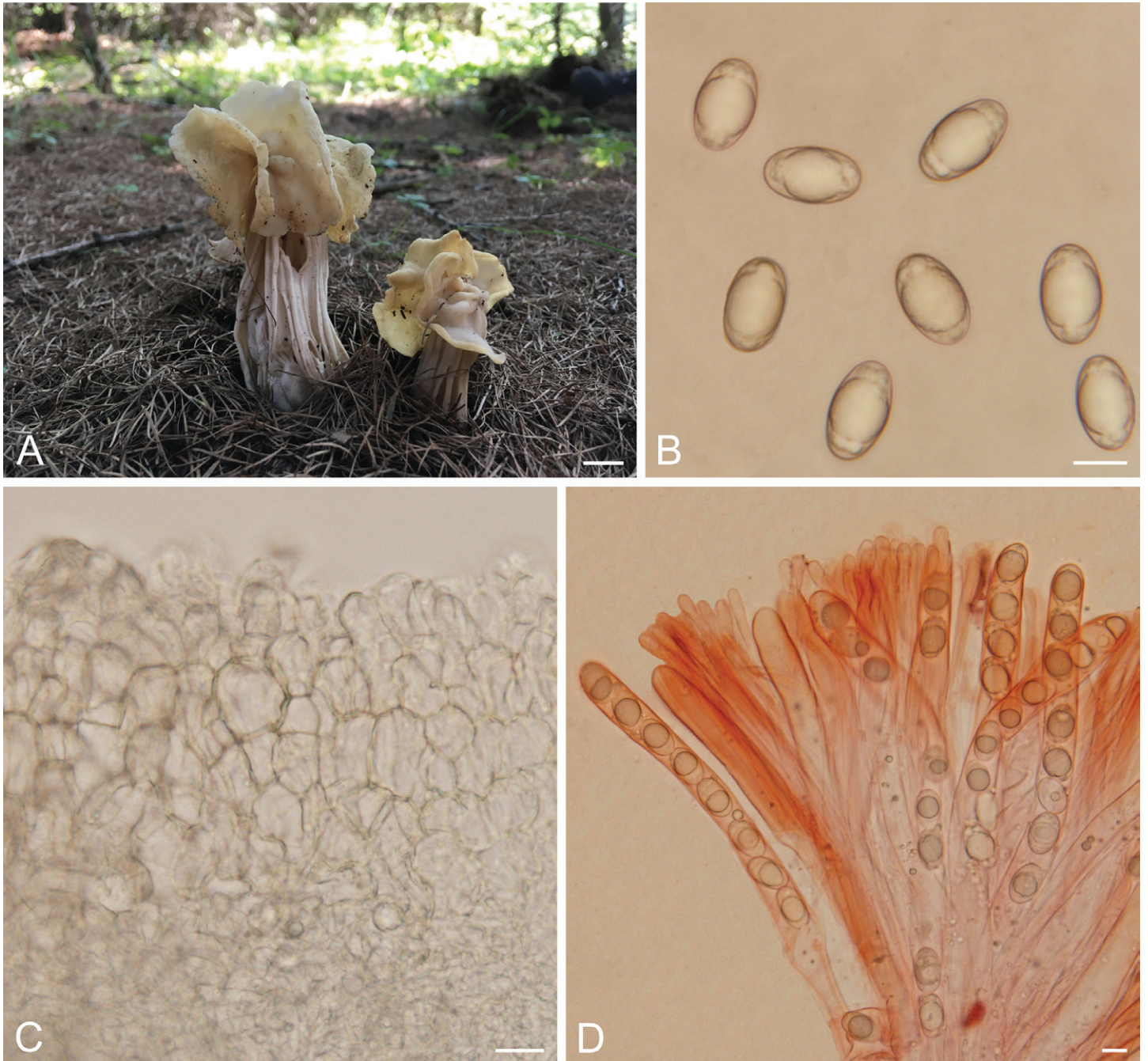
**Notes:** *Helvella pseudopezizoides* is diagnosed by its overall dark grey to blackish ascomata with saddle-shaped or irregularly lobed cap and thin terete stipe. It was phylogenetically nested in the *fallax-pezizoides* lineage of the *Elastica* clade (Fig. 1). *Helvella pezizoides* is highly similar to the present species, but it differs by its somewhat inrolled cap margin, and smaller ascospores (14.5–16.5 × 8.5–9.6 µm) (Skrede *et al.* 2020). Other species in the *fallax-pezizoides* lineage are also somewhat similar in morphology, including *H. fallax*, *H. caespitosa*, *H. pseudofallax*, *H. pulla*, *H. subglabra*, *H. subglabroides*, but the cap colour in all of them is grey or dark grey, not blackish to black, then the stipe is pale at least near base (Skrede *et al.* 2017, 2020, the present study). In addition, three *hsp*, three *rpb2*, 20 nrLSU, and 16 *tef1-α* substitutions separated this new species from *H. fallax*; 14 *hsp*, and 26 nrLSU substitutions from *H. caespitosa*; 15 *hsp*, 11 *rpb2*, 28 nrLSU, and 26 *tef1-α* substitutions from *H. pezizoides*; 12 *hsp*, eight *rpb2*, 23 nrLSU, and 40 *tef1-α* substitutions from *H.*

*pseudofallax*; seven *hsp*, three *rpb2*, and 23 nrLSU from *H. pulla*; four *hsp*, and five *rpb2* from *H. subglabra*; five *hsp*, three *rpb2*, 24 nrLSU, and 21 *tef1-α* substitutions from *H. subglabroides*. An ITS sequence (GenBank KX444420) from an ECM root tip of *Quercus liaotungensis* in Beijing matched this species (Fig. S3), indicating that this species could form ectomycorrhiza with this host. *Helvella pseudopezizoides* shares less than 89.95 % similarity in the ITS region with other *Helvella* species.

***Helvella sinocrispa*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849622. Fig. 16.

**Etymology:** *sinocrispa*, referring to its similarity to *H. crispa* in morphology.

**Apothecia** stipitate-capitate; cap saddle-shaped to irregularly lobate, 1.4–2.5 cm high, 2.5–4 cm broad, at first margin rolled to hymenium, then expanded or cracked with age; hymenium glabrous, cream white to whitish, becoming brown to dark brown when dry; receptacle surface subpubescent, pale yellow to greyish yellow, becoming pale brown to yellowish brown when dry; stipe robust, 2.5–5.5 cm high, 1.0–2.6 cm broad, glabrous, white to dirty



**Fig. 16.** *Helvella sinocrispa* (BJTC FM96-A, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in water. **D.** Asci and paraphyses in Congo Red. Scale bars: A = 1 cm; B–D = 10  $\mu$ m.

whitish, becoming pale yellow when dried, ribbed, blunt-edged, partly anastomosing and double-edged, with lacunae between ribs, chambered inside. *Ectal excipulum* 80–180  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, outermost cells catenuliform in long fascicled tufts, with cylindrical to subclavate, end cells 15–37  $\times$  8.5–15  $\mu$ m. *Medullary excipulum* 190–270  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.5–5  $\mu$ m broad. *Asci* pleurorhynchous, tapering, 8-spored, 255–285  $\times$  13–16.5  $\mu$ m. *Ascospores* ellipsoid, with one large oil drop when mature, 18–20  $\times$  10.5–12  $\mu$ m, [Lm  $\times$  Wm = 19.3  $\times$  11.2  $\mu$ m, Q = 1.60–1.90,  $Q_{av}$  = 1.73  $\pm$  0.08]. *Paraphyses* filiform, septate, hyaline to pale brown, 4.5–7  $\mu$ m wide at tips and 2–3.5  $\mu$ m below.

**Habitat:** Solitary or scattered on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*.

**Distribution:** Known only from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Xinzhou City, Ningwu County, Guancenshan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*, alt. 2 230 m, 24 Aug. 2017, M. Chen (**holotype** BJTC FM96-A, GenBank Acc. No.: ITS = OR355155, nrLSU = OR355326, *tef1- $\alpha$*  = OR359068, *rpb2* = OR359180, *hsp* = OR366133); *ibid.*, (BJTC FM96-B).

**Notes:** *Helvella sinocrispa* is recognized by its isolated phylogenetic position in the *Crispa* clade (Fig. 1). Morphologically it is highly similar to three other species of this clade, namely *H. crispa*, *H. orienticrispa*, and *H. pseudoreflexa*. In fact, it is difficult to distinguish these four species by morphology alone. DNA analysis is therefore needed to accurately identify

them. Molecularly, three *hsp*, six *rpb2*, five nrLSU, and 11 *tef1- $\alpha$*  substitutions separated this new species from *H. crispa*; three *hsp*, four *rpb2*, one nrLSU, and 12 *tef1- $\alpha$*  substitutions from *H. orienticrispa*; three *hsp*, four *rpb2*, two nrLSU, and 11 *tef1- $\alpha$*  substitutions from *H. pseudoreflexa*. Four ITS sequences downloaded from GenBank matched *H. sinocrispa* in our analysis (Fig. S2). These sequences are extracted from ascomata and ectomycorrhizal root tips from China, Europe or the USA. These results implied that *H. sinocrispa* is widely distributed in Northern Hemisphere. DNA analysis revealed that *H. sinocrispa* shares less than 94.04 % similarity in ITS region with other *Helvella* species, supporting it as a distinct species.

***Helvella sinohyperborea*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849623. Fig. 17.

**Etymology:** *sinohyperborea*, referring to its close phylogenetic relationship to *H. hyperborea*.

**Apothecia** stipitate-cupulate; cap regular cupulate or irregularly undulate with wrinkled surface, 1.3–2.0 cm high, 2.1–6.0 cm broad; hymenium dark brown to blackish, becoming black when dry; receptacle surface upper part slightly lighter than the hymenium, below gradually white, becoming black when dry; stipe short, 1.0–2.5 cm high, 0.5–0.8 cm broad, hollow, white, becoming pale yellow to pale brown when dry, ribbed, blunt-edged, partly anastomosing and double-edged, occasionally with lacunae between ribs, ribs extending onto the receptacle. **Ectal excipulum** 100–150  $\mu\text{m}$  broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs

in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 15–40  $\times$  11–19  $\mu\text{m}$ . **Medullary excipulum** 400–700  $\mu\text{m}$  broad, of interwoven *textura intricata*, hyphae 2.0–6.0  $\mu\text{m}$  broad. **Asci** pleurorhynchous, tapering, 8-spored, 240–300  $\times$  13–19  $\mu\text{m}$ . **Ascospores** ellipsoid, with one large oil drop when mature, 16–18.5  $\times$  11–12.5  $\mu\text{m}$ , [Lm  $\times$  Wm = 17.5  $\times$  11.8  $\mu\text{m}$ , Q = 1.38–1.58, Q<sub>av</sub> = 1.49  $\pm$  0.05]. **Paraphyses** filiform, septate, pale brown, 4.5–6.0  $\mu\text{m}$  wide at clavate to subcapitate tips and 1.5–3.5  $\mu\text{m}$  below.

**Habitat:** Scattered on the ground in coniferous forest dominated by *Larix principis-rupprechtii*.

**Distribution:** Known from Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Xinzhou City, Ningwu County, Guancen mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii*, alt. 2 220 m, 24 Aug. 2017, X.Y. Yan (**holotype** BJTC FM105, GenBank Acc. No.: ITS = OR355132, nrLSU = OR355303, *tef1- $\alpha$*  = OR359056, *rpb2* = OR359170, *hsp* = OR366121); *ibid.*, alt. 2 099 m, 25 Aug. 2017, X.Y. Yan (BJTC FM165).

**Notes:** *Helvella sinohyperborea* is characterized by the cup-like or undulate cap, the short robust ribbed stipe. Our present analysis places *H. sinohyperborea* in the *hyperborean-tinta* lineage in the *Lacunosa* clade, closely related to *H. hyperborea* and *H. tinta*. *Helvella hyperborea* is a Northern European species. It is differentiated from *H. sinohyperborea* by its regular cup-like cap, long stipe and pale brown hymenium (Skrede *et al.* 2017). *Helvella tinta* is a species known from southwestern China. It



**Fig. 17.** *Helvella sinohyperborea* (A, D–G. BJTC FM105, holotype. B, C. BJTC FM165). **A.** Apothecium. **B, C.** Apothecium infected by *Hypomyces* sp. **D.** Ascospores in water. **E.** Asci and paraphyses in Congo Red. **F.** Ectal excipulum in water. **G.** Immature asci in Congo Red. Scale bars: A–C = 1 cm; D–G = 10  $\mu\text{m}$ .

is distinguished from *H. sinohyperborea* by its cup-like cap of ascomata with a consistently mottled, greyish to dark brown hymenium and receptacle surface (Hyde et al. 2016). Molecularly, two *hsp* and one *rpb2* substitutions separate this new species from *H. hyperborea*; four *hsp*, five nrLSU, and one *tef1- $\alpha$*  substitutions from *H. tinta*. *Helvella sinohyperborea* shared less than 97.83 % similarity in ITS region with other *Helvella* species. Two ITS sequences downloaded from GenBank matched *H. sinohyperborea* in our analysis (Fig. S4). These sequences are both extracted from ectomycorrhizal root tips of *Picea crassifolia*, from Inner Mongolia, Ningxia Hui Autonomous Region. This indicated that *H. sinohyperborea* also occurs in other regions of China, and could form ectomycorrhiza with *P. crassifolia*.

***Helvella subglabroides*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.*  
Mycobank MB 849624. Fig. 18.

**Etymology:** *subglabroides*, referring to its high similarity to *H. subglabra* in appearance of its ascomata.

**Apothecia** stipitate-capitate; cap saddle-shaped, 0.7–2.5 cm high, 0.8–2.0 cm broad, at first margin adhered to stipe, then reflexed with age; hymenium grey to greyish yellow, becoming dark grey to greyish black when dry; receptacle surface glabrous to fine pubescent, concolourous with hymenium, becoming brown when dry; stipe terete, 2.0–5.0 cm high, 0.2–0.4 cm broad, solid, subpubescent, greyish white to grey, becoming dark grey when dry, base occasionally with inconspicuous grooves and white mycelia. **Ectal excipulum** 50–100  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 20–45  $\times$  9–20  $\mu$ m. **Medullary excipulum** 100–200  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.5–6.5  $\mu$ m broad. **Asci** pleurorhynchous, tapering,



**Fig. 18.** *Helvella subglabroides* (BJTC C614). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B, E = 10  $\mu$ m; C, D = 20  $\mu$ m.

8-spored,  $200\text{--}260 \times 12.5\text{--}18.5 \mu\text{m}$ . *Ascospores* ellipsoid, with one large oil drop when mature,  $15\text{--}19.5 \times 9\text{--}11 \mu\text{m}$ , [Lm  $\times$  Wm =  $17.2 \times 10 \mu\text{m}$ , Q = 1.5–2,  $Q_{av} = 1.73 \pm 0.10$ ]. *Paraphyses* filiform, septate, yellowish brown,  $5.5\text{--}11.5 \mu\text{m}$  wide at clavate to subcapitate tips and  $2.5\text{--}4.0 \mu\text{m}$  below.

**Habitat:** Scattered on the ground in mixed coniferous and broadleaf forest.

**Distribution:** Known from Hebei, Shanxi, and Yunnan Province, China.

**Specimens examined:** **China**, Shanxi Province, Changzhi City, Qinyuan County, Lingkong mountains, on the ground in coniferous forest and broadleaf mixed forest, 15 Aug. 2022, *N. Mao* (**holotype** BJTC FM2686, GenBank Acc. No.: ITS = OR355088, nrLSU = OR355258, *tef1- $\alpha$*  = OR359019, *rpb2* = OR359142, *hsp* = OR366084); *ibid.*, Jincheng City, Qinshui County, Shangwoquan Village, on the ground in broadleaf forest dominated by *Quercus* sp.,  $35^{\circ}36'57''\text{N}$ ,  $112^{\circ}3'7''\text{E}$ , alt. 1 170 m, 25 Aug. 2020, *H. Liu* (BJTC FM1049); Hebei Province, Zhangjiakou City, Chicheng County, Dahaituo Township, on the ground in coniferous forest and broadleaf mixed forest, alt. 1 222 m, 26 Aug. 2020, *G.Q.*

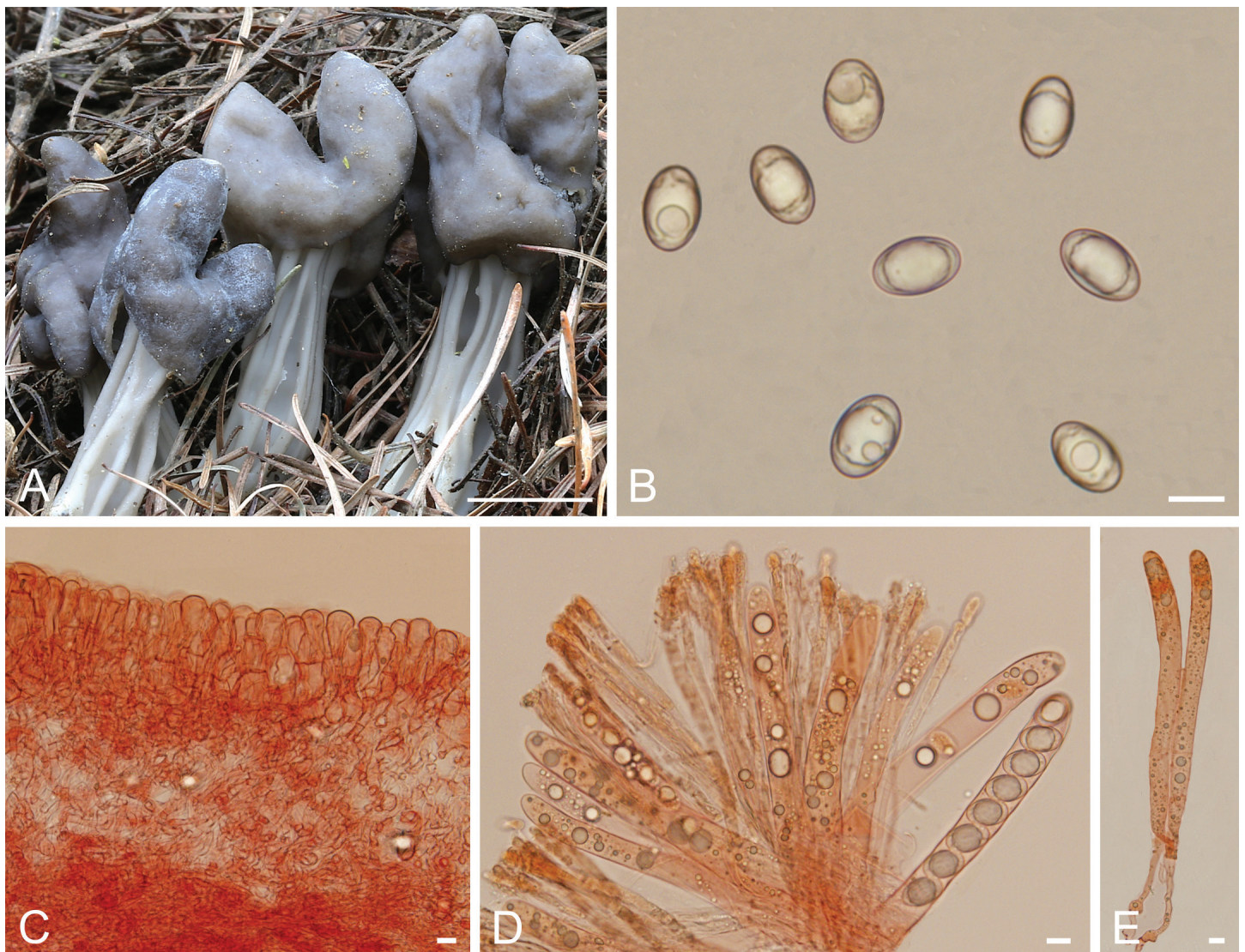
*Cheng* (BJTC C614); Yunnan Province, Lijiang City, Jinshan mountains, 12 Sep. 2012, *Q. Zhao* (HKAS 78939); *ibid.*, (HKAS 78940).

**Notes:** Our present analysis places *H. subglabroides* in the *fallax-peizoides* lineage, *Elastica* clade, and is closely related to the North American *H. subglabra* (Fig. 1). Morphologically, *H. subglabroides* is also similar to *H. subglabra*, both having a saddle-shaped cap. However, the two species can be separated by their hymenium colour, which is grey to greyish yellow in the former but dark grey in the latter (Weber 1972). Molecularly, three *hsp*, four *rpb2*, eight nrLSU substitutions distinguish *H. subglabra* from *H. subglabroides*. *Helvella subglabroides* shares less than 96.15 % similarity in ITS region with other *Helvella* species.

***Helvella sulcatoides*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849625. Fig. 19.

**Etymology:** *sulcatoides*, referring to its similarity to *H. sulcata*.

**Apothecia** stipitate-capitate; cap saddle-shaped, mostly bilobate, 1.3–1.8 cm broad, 1.3–2.0 cm high, margin attached to stipe;



**Fig. 19.** *Helvella sulcatoides* (BJTC FM1416, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Immature asci in Congo Red. Scale bars: A = 1 cm; B–E = 10  $\mu\text{m}$ .

hymenium grey, dark grey to black grey, becoming black when dried, slightly wrinkled; receptacle surface pale grey to greyish brown, becoming greyish brown when dried, smooth, without ribs on receptacle surface; stipe 0.5–0.9 cm broad, 1.2–2.4 cm high, greyish white to black grey, becoming dark brown to black brown when dry, ribbed, longitudinal ribs high and prominent, sharp, occasionally double-edged, sometimes with a few deep ‘holes’ between ribs. *Ectal excipulum* of *textura angularis*, 65–100 µm thick, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells arranged in dense, club-shaped, 13–40 × 7–18 µm. *Medullary excipulum* of interwoven *textura intricata*, 130–200 µm thick, hyphae 2.5–6.5 µm broad. *Asci* pleurorhynchous, tapering, 8-spored, 225–275 × 14–18 µm. *Ascospores* ellipsoid, with one large oil drop when mature, 14.5–16.5 × 9–10.5 µm, [Lm × Wm = 15.9 × 9.9 µm, Q = 1.41–1.74, Q<sub>av</sub> = 1.56 ± 0.08]. *Paraphyses* filiform, septate, hyaline to pale brown, 4–6 µm wide at tips and 2.5–4 µm below.

**Habitat:** Solitary or scattered on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*.

**Distribution:** Known from Beijing and Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Xinzhou City, Wutai County, Wutaishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*, 11 Aug. 2020, H. Liu (**holotype** BJTC FM1416, GenBank Acc. No.: ITS = OR355119, nrLSU = OR355290, *tef1-α* = OR359043, *rpb2* = OR359160, *hsp* = OR366108); *ibid.*, Lvliang City, Jiaocheng County, Guandishan mountains, 37°52′16″N, 111°30′25″E, alt. 2 003 m, 7 Sep. 2017, J.Z. Cao (BJTC FM263); Beijing, Huairou District, Labagoumen, alt. 1 345 m, 25 Aug. 2020, H. Zhou (BJTC ZH1231).

**Notes:** Phylogenetically, *H. sulcatoides* is placed in the *sulcata-phleboforoides* lineage, *Lacunosa* clade (Fig. 1). It is closely related and similar to *H. borealis* and *H. sulcata*, but *H. borealis* is differentiated from *H. sulcatoides* by its grey white hymenium, slightly broader paraphyses at the apex (6.5–10.5 µm) and habit of growing on rotten wood (Wang et al. 2023a); *H. sulcata* by its black hymenium, large asci (290–320 × 13–16 µm), and broad spores (14.8–16.8 × 10.5–13.2 µm) (Skrede et al. 2017). *Helvella phleboforoides*, a species recently described from Europe, is also closely related to *H. sulcatoides*, but can be separated by its irregularly radiating ribs on the receptacle surface, and large asci (280–320 × 12–15 µm) (Skrede et al. 2020). In addition, one *hsp*, five *tef1-α* substitutions separated *H. sulcatoides* from *H. borealis*; one *hsp*, four nrLSU, three *tef1-α* substitutions from *H. sulcata*; four *hsp*, six *rpb2*, and eight nrLSU substitutions from *H. phleboforoides*.

***Helvella xiaohuipan*** L. Fan, N. Mao & Y.Y. Xu, *sp. nov.* MycoBank MB 849626. Fig. 20.

**Etymology:** *xiaohuipan*, Chinese, meaning this *Helvella* is a small-sized and grey species with cup-like cap.

**Apothecia** stipitate-cupulate; regular cupulate to saucer-shaped, 0.3–0.7 cm high, 0.7–2.0 cm broad; hymenium grey to dark grey, becoming brown to black-brown when dry; receptacle surface pubescent, concolourous with hymenium, becoming greyish

brown when dry; stipe terete, slender, 1.2–1.8 cm high, 0.15–0.35 cm broad, solid, yellowish white, becoming yellow when dry. *Ectal excipulum* 150–175 µm broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 12–37 × 10–20 µm. *Medullary excipulum* 250–300 µm broad, of interwoven *textura intricata*, hyphae 2.0–5.0 µm broad. *Asci* pleurorhynchous, tapering, 8-spored, 235–275 × 14–20 µm. *Ascospores* ellipsoid, with one large oil drop when mature, 17–19.5 × 10.5–12 µm, [Lm × Wm = 18 × 11.4 µm, Q = 1.46–1.72, Q<sub>av</sub> = 1.58 ± 0.06]. *Paraphyses* filiform, septate, pale brown, 4.5–6.0 µm wide at clavate to subcapitate tips and 3.5–4.5 µm below.

**Habitat:** Scattered on the ground in broadleaf forest and coniferous forest.

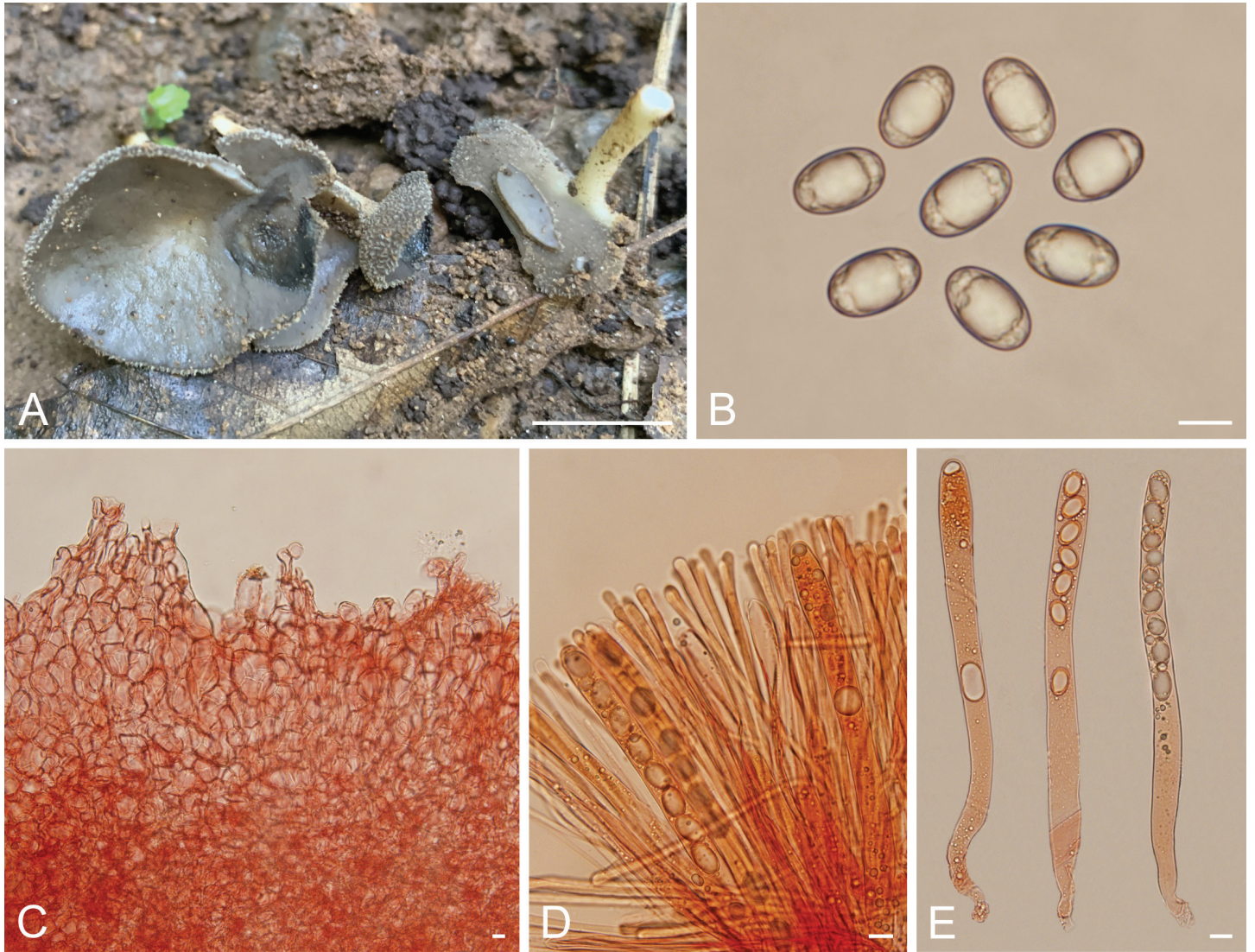
**Distribution:** Known from Hebei and Shanxi Province, Northern China.

**Specimens examined:** **China**, Shanxi Province, Yuncheng City, Xia County, Sijiao Town, on the ground in broadleaf forest dominated by *Quercus* sp., 35°07′89″N, 111°40′09″E, alt. 970 m, 12 Aug. 2022, N. Mao (**holotype** BJTC FM2317, GenBank Acc. No.: ITS = OR355079, nrLSU = OR355249, *tef1-α* = OR359011, *rpb2* = OR359134, *hsp* = OR366076); Hebei Province, Tangshan City, Zhangzhuangzi Village, on the ground in broadleaf forest dominated by *Castanea mollissima*, 40°14′21″N, 117°51′15″E, alt. 107 m, 17 Sep. 2017, T. Ling (BJTC FM367).

**Notes:** *Helvella xiaohuipan* is characterized by its grey and cup-like cap, pubescent receptacle surface, yellowish white slender terete stipe and ellipsoid ascospores. *Helvella xiaohuipan* was phylogenetically nested in its own lineage (*xiaohuipan* lineage) in the *Elastica* clade, sister to the *fallax-pezizoides* lineage (Fig. 1). Currently, there are only two species recognized in this lineage, both from China. *Helvella fibrosa* (= *H. villosa*) in the *fibrosamacropus* lineage is similar to *H. xiaohuipan* in colour and shape of ascomata (Dissing & Nannfeldt 1966). However, *H. fibrosa* differs from *H. xiaohuipan* by its grey stipe and ascospores longer than 20 µm. *Helvella pubescens* from the *rivularis-sublicia* lineage and *H. scyphoides* from the *hypocrateriformis* lineage also have a disk-like cap, but the colour of the cap is brown (Skrede et al. 2017) rather than grey as in *H. xiaohuipan*. Moreover, species in the *fallax-pezizoides* lineage have saddle-shaped ascocarps that make them easy to distinguish from the new species. An ITS specimen-sequence (GenBank JX462576, labelled as *Helvella cf. corium*) from China matched *H. xiaohuipan* in our phylogenetic analysis (Fig. S1), indicating that *H. xiaohuipan* also occurs in other regions of China. DNA analysis revealed that *H. xiaohuipan* shares less than 92.88 % similarity in ITS region with other *Helvella* species, supporting it as a distinct species.

***Helvella galeriformis*** B. Liu & J.Z. Cao, *Acta Mycol. Sin.* **7**: 199. 1988, L. Fan, N. Mao & Y.Y. Xu, *emend.* Fig. 21.

**Apothecia** stipitate-capitate; cap irregularly hemisphere, irregularly umbrella type to irregularly saddle-shaped, 0.6–2 cm high, 1.5–3.5 cm broad, at first margin adhered to stipe, then more or less deflexed; hymenium greyish black, occasionally with olive green tinge; receptacle surface white, concolourous with stipe; stipe terete, 1.2–5.0 cm high, 0.3–0.7 cm broad, solid or hollow, cream to white, base slightly enlarged, with



**Fig. 20.** *Helvella xiaohuipan* (BJTC FM2317, holotype). **A.** Apothecia. **B.** Ascospores in water. **C.** Ectal excipulum in Congo Red. **D.** Asci and paraphyses in Congo Red. **E.** Asci in Congo Red. Scale bars: A = 1 cm; B–E = 10  $\mu$ m.

shallow grooves. *Ectal excipulum* 90–150  $\mu$ m broad, of *textura angularis*, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 22–56  $\times$  10–25  $\mu$ m. *Medullary excipulum* 150–250  $\mu$ m broad, of interwoven *textura intricata*, hyphae 2.5–6.5  $\mu$ m broad. *Asci* pleurorhynchous, tapering, 8-spored, 300–360  $\times$  15–20  $\mu$ m. *Ascospores* ellipsoid, with one large oil drop when mature, 19–22.5  $\times$  11.5–13.5  $\mu$ m, [Lm  $\times$  Wm = 20.8  $\times$  12.5  $\mu$ m, Q = 1.5–1.8,  $Q_{av}$  = 1.65  $\pm$  0.07]. *Paraphyses* filiform, 2.5–4.5  $\mu$ m below, pale yellow to pale brown, septate, clavate to subcapitate, 6–10  $\mu$ m wide at tips.

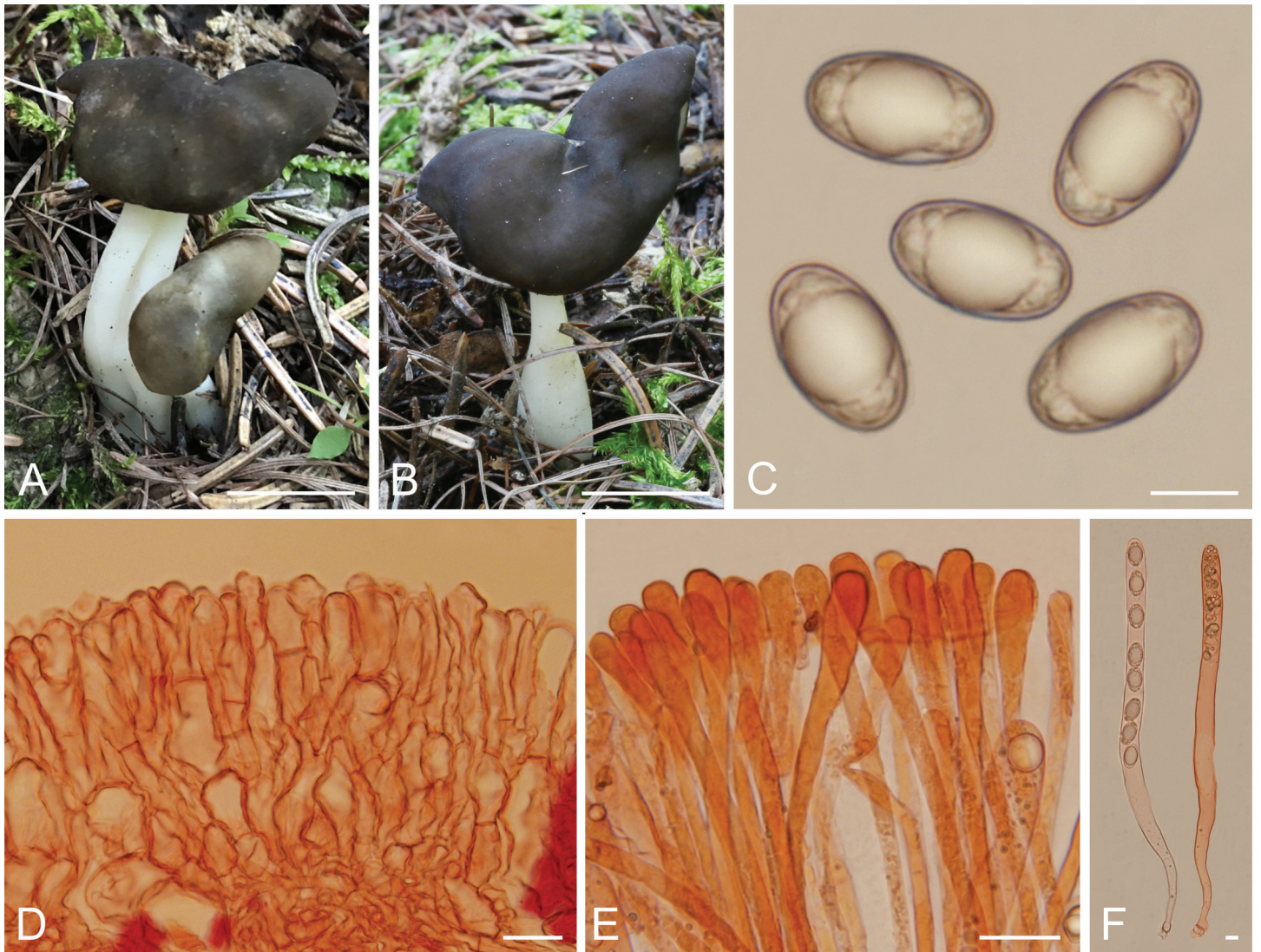
**Habitat:** Scattered on the ground in mixed coniferous and broadleaf forest.

**Distribution:** Known only from Shanxi Province, Northern China.

**Specimens examined:** **Lectotype** here designated, Liu & Cao (1988), *Acta Mycol. Sin.* 7: 199. 1988, fig. 2, MycoBank MBT 10014903. **China**, Shanxi Province, Lvliang City, Jiaocheng County, Guandishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Betula* sp., alt. 2 160 m, 28 Aug. 2018, *H. Liu* (**epitype** here designated HSA 232, MycoBank MBT 10014391, GenBank Acc.

No.: ITS = OR355066, nrLSU = OR355236, *tef1- $\alpha$*  = OR359001, *rpb2* = OR359125, *hsp* = OR366063; *ibid.*, L.J. Guo (HSA 240-B); *ibid.*, on the ground in coniferous forest dominated by *Picea* sp., alt. 1 875 m, 25 Aug. 2022, *N. Mao* (BJTC FM2574).

**Notes:** The holotype of *H. galeriformis* collected from Guancenshan Mountains (cited as ‘Gauinqin Mountain’) in Shanxi Province (MHSU 452) has been lost. However, because Liu & Cao (1988) provided an illustration (Liu & Cao 1988; fig. 2), this is here designated as lectotype, allowing us to introduce an epitype based on recently collected material (HSA 232). Recently, we collected several specimens in the same region of the holotype of *H. galeriformis* (Liu & Cao 1988), but the swollen cells in the medullary excipulum were not observed. However, the diagnosed colour and peculiar shape of ascomata matched well with the original description of *H. galeriformis*. Considering the collecting site, habitat and morphology, we regard these specimens as conspecific to *H. galeriformis*. Therefore, we designate a lectotype and epitype for *H. galeriformis* here. *Helvella galeriformis* is sister to *H. brunneogaleriformis* in our phylogenetic analyses (Figs 1, 2). Morphologically, *H. brunneogaleriformis* differs from *H. galeriformis* by its brown hymenium without blackish tint, smaller ascospores (17–20.5



**Fig. 21.** *Helvella galeriformis* (HSA 232, epitype). **A, B.** Apothecium. **C.** Ascospores in water. **D.** Ectal excipulum in Congo Red. **E.** Paraphyses in Congo Red. **F.** Asci in Congo Red. Scale bars: A, B = 1 cm; C, F = 10 µm; D, E = 20 µm.

× 10.5–12.5 µm), and broader paraphyses at the apex (6–13 µm). Molecularly, *H. brunneogaleriformis* deviates consistently in one *hsp*, one *tef1-α*, and eight nrLSU substitutions from *H. galeriformis*. *Helvella galeriformis* shares less than 97.8 % similarity in the ITS region with *H. brunneogaleriformis*.

***Helvella carnosa*** Skrede et al., *Persoonia* **39**: 237. 2017. Fig. 22.

**Habitat:** Scattered on the ground in coniferous forest or broadleaf forest.

**Distribution:** Known from Northern area in China.

**Specimens examined:** **China**, Beijing, Yanqing County, Songshan Mountains, alt. 812 m, 31 Aug. 2017, C.L. Hou (BJTC BP030); *ibid.*, (BJTC BP043), *ibid.*, alt. 816 m, C.L. Hou (BJTC BP060); *ibid.*, alt. 919 m, 5 Sep. 2018, C.L. Hou (BJTC BP718); *ibid.*, alt. 1 010 m, 5 Sep. 2018, C.L. Hou (BJTC BP724); *ibid.*, Badaling, alt. 608 m, 6 Sep. 2018, X.Y. Shen. (BJTC SBP287); *ibid.*, Shanxi Province, Xing County, Heichashan Mountains, on the ground in broadleaf forest, alt. 1 590 m, 5 Sep. 2018, J.Z. Cao (HSA 360); *ibid.*, Pu County, Wulushan mountains, on the ground in coniferous forest, alt. 1 700 m, 9 Sep. 2018, L.J. Guo (HSA 434); *ibid.*, Pu County,

Wulushan mountains, 10 Sep. 2017, Y.Y. Xu (BJTC FM297); *ibid.*, (BJTC FM298), *ibid.*, J.Z. Cao (BJTC FM316); *ibid.*, on the ground in coniferous forest, alt. 1 739 m, 26 Aug. 2022, N. Mao (BJTC FM2619); *ibid.*, Lvliang City, Jiaocheng County, Guandishan Mountains, on the ground in coniferous forest, alt. 2 003 m, 7 Sep. 2017, T. Li (BJTC FM274); *ibid.*, Ningwu County, Guancenshan Mountains, on the ground in coniferous forest, alt. 1 346 m, 15 Oct. 2017, X.Y. Yan (BJTC FM376), *ibid.*, Wutai County, Wutaishan Mountains, alt. 1 376 m, 29 Aug. 2019, H. Liu (BJTC FM953); *ibid.*, Qinshui County, Lishan mountains, on the ground in coniferous and broad-leaved mixed forest, alt. 1 650 m, 24 Aug. 2020, N. Mao (BJTC FM1032); *ibid.*, Loufan County, Yunding Mountains, on the ground in coniferous forest, alt. 1 639 m, 23 Aug. 2022, N. Mao (BJTC FM2478); *ibid.*, 1 642 m, 23 Aug. 2022, N. Mao (BJTC FM2479), *ibid.*, on the ground in broad-leaved mixed forest, 1 861 m, 23 Aug. 2022, N. Mao (BJTC FM2499).

**Notes:** *Helvella carnosa* is a species recently described from Europe (Skrede et al. 2017). Our present analysis revealed that *H. carnosa* is common in Northern China (Fig. S3), and can form ectomycorrhiza with *Abies grandis* and *Pinus tabuliformis*, *Helvella carnosa* shares less than 91.86 % similarity in the ITS region with other *Helvella* species.





**Fig. 22.** Photographs of apothecia in their natural habitat. **A, B.** *Helvella carnosus* (A. BJTC BP060. B. BJTC FM2619). **C.** *H. corbierei* (HSA 181). **D.** *H. fistulosa* (BJTC 1976). **E.** *H. nordlandica* (BJTC FM346-A). **F.** *H. scyphoides* (HSA 239). Scale bars = 1 cm.

***Helvella corbierei*** (Malençon) Van Vooren & Frund, *Bull. Mycol. Bot. Dauphiné-Savoie* **198**: 9. 2010. Fig. 22.

**Habitat:** Scattered on the ground in coniferous and broad-leaved mixed forest.

**Distribution:** Known from Northern area in China.

**Specimens examined:** **China**, Shanxi Province, Ningwu County, Dashidong, on the ground in coniferous forest, alt. 2 207 m, 2 Aug. 2018, *H. Liu* (HSA 138); *ibid.*, Qiuqiangu, on the ground in coniferous and broad-leaved mixed forest, alt. 1 600 m, 4 Aug. 2018, *H. Liu* (HSA 181).

**Notes:** *Helvella corbierei* is widely distributed in Europe and North America. It is confirmed from China based on morphological and DNA evidence in this study. *Helvella corbierei* shares less than 95.59 % similarity in the ITS region with other *Helvella* species.

***Helvella fistulosa*** Alb. & Schwein., *Consp. Fungorum Lusat.*: 299. 1805. Fig. 22.

**Habitat:** Scattered on the ground in coniferous and broad-leaved mixed forest.

**Distribution:** Known from the Southwest area in China.

**Specimen examined:** **China**, Yunnan Province, Kunming City, Jinning District, Shuibo Villa, on the ground in coniferous and broad-leaved mixed forest, 28 Aug. 2021, *T.H. Li* (BJTC 1976).

**Notes:** *Helvella fistulosa* is widely distributed in Europe, North America and Japan (Skrede et al. 2017). In this study, a specimen from Yunnan Province matched this species, which confirmed the occurrence of *H. fistulosa* in China. *Helvella fistulosa* shares less than 94.51 % similar in the ITS region with other *Helvella* species.

***Helvella nordlandica*** Skrede & T. Schumach. *Fungal Syst. Evol.* **11**: 72. 2023. Fig. 22.

**Habitat:** Scattered on the ground in coniferous and broadleaf mixed forest.

**Distribution:** Known from Northern area in China.

**Specimens examined:** **China**, Shanxi Province, Linfen City, Pu County, Wulushan mountains, on the ground in coniferous and broadleaf mixed forest, 11 Sep. 2017, *T. Li* (BJTC FM346-A); *ibid.*, (BJTC FM346-B).

**Notes:** *Helvella nordlandica* is a species recently described from Europe (Skrede et al. 2023). Our present analysis showed that *H. nordlandica* is a rare *Helvella* species in North China (Fig. 1). *Helvella nordlandica* shares less than 95.86 % similarity in the ITS region with other *Helvella* species.

***Helvella scyphoides*** Skrede et al., *Persoonia* **39**: 237. 2017. Fig. 22.

**Habitat:** Scattered on the ground in coniferous and broad-leaved mixed forest.

**Distribution:** Known from Northern area in China.

**Specimens examined:** **China**, Shanxi Province, Ningwu County, Xiaoshidong, on the ground in coniferous forest, alt. 2 200 m, 3 Aug. 2018, *H. Liu* (HSA 166); *ibid.*, Luyashan mountains, alt. 2 270 m, 4 Sep. 2018, *J.Z. Cao* (HSA 336); *ibid.*, Jiaocheng County, Pangquangu, on the ground in coniferous and broad-leaved mixed forest, alt. 2 160 m, 28 Aug. 2018, *H. Liu* (HSA 239); *ibid.*, Wutai County, Wutaishan mountains, 27 Aug. 2019, *Y. Shen* (BJTC FM899); *ibid.*, on the ground in coniferous forest, alt. 2 040 m, 3 Sep. 2020, *N. Mao* (BJTC FM1158).

**Notes:** *Helvella scyphoides* is a species recently described from Europe (Skrede et al. 2017). Our present analysis revealed this species is common in Northern China (Figs 1, 2, S3). *Helvella scyphoides* shared less than 93.9 % similarity in the ITS region with other *Helvella* species.

## DISCUSSION

### The species diversity of *Helvella* in China

Our present study revealed that China is extremely rich in species of *Helvella*. Chinese species of *Helvella* were nested in all the four clades recognized based on the samplings worldwide, i.e., *Acetabulum* clade, *Crispa* clade, *Elastica* clade and *Lacunosa* clade (Fig. 1). There were 93 clusters, which corresponded to 93 phylogenetic species in China, recognized in our phylogenetic analyses based on five genes (Figs 1, 2, S1–S4). Among them, 58 species were identified as known species including five new to China, 18 species were described as new species in this study, and 17 species were proposed as potentially undescribed species and were not treated taxonomically due to the lack or poor condition of ascocarps. Moreover, there are 34 names of *Helvella* recorded in China before this study, and although their DNA data are unavailable for the time being, three of them at least are morphologically distinct according to our personal observations of specimens or literature (Liu & Cao 1988, Cao et al. 1990). Thus, we infer that the total number of the genus *Helvella* in China could reach to 96 species with the current diversity observed in the present study.

### Phylogeny and morphological correspondence of *Helvella*

Our five-gene-based phylogenetic analysis resolved the species of *Helvella s. str.* (Hansen et al. 2019) into four distinct clades, i.e., *Acetabulum*, *Crispa*, *Elastica*, and *Lacunosa* clades (Fig. 1). Compared to the phylogeny proposed by Skrede et al. (2017), the combination of our *Acetabulum*, *Crispa* and *Lacunosa* clades corresponded to the Clade B suggested by Skrede et al. (2017). Among them, our *Acetabulum* clade corresponded to their *acetabulum-solitaria* lineage, our *Crispa* clade corresponded to their *crispa* lineage, our *Lacunosa* clade contained all the other remaining lineages in their Clade B. Our *Elastica* clade contained the Clade D plus Clade E suggested by Skrede et al. (2017).

The *Acetabulum* clade occupied a basal position in the phylogenetic tree of the genus *Helvella*, and is the least diverse clade in species and lineages. Currently a total of nine species are recognized, five species occur in China, and two lineages, i.e., *acetabulum* lineage and *solitaria* lineage, were recognized (Figs 1, S1). All the species of the *Acetabulum* clade are relatively

uniform in the appearance of the ascocarp that possess a regularly or irregularly cup-like cap with a robust and ribbed stipe. It is interesting that the species, nested in several lineages in the *Lacunosa* clade including the *costifera* lineage, *dalgeri* lineage and *tianshanensis* lineage, evolved highly convergent morphological features in ascocarp appearance with the species of the *Acetabulum* clade. The ascocarps of *H. acetabulum* (*acetabulum* lineage, *Acetabulum* clade), *H. costifera* (*costifera* lineage, *Lacunosa* clade) and *H. hyperborea* (*hyperborea* lineage, *Lacunosa* clade) are very similar so that the three species are often confused with one another (Harmaja 1978, 1979).

The *Crispa* clade is also a less diverse group in species and lineages compared to the *Elastica* and *Lacunosa* clades. A total of 13 species were recognized, including nine from China. The species *H. crispa* and its allies evolved as the core *crispa* lineage (Figs 1, S2). Morphologically, species of the *crispa* lineage in this clade usually have pale ascocarps. The caps of ascocarps are saddle-shaped or irregularly lobed, white, cream white and sometimes grey. The cap margin is completely free from the robust and sometimes chambered stipe. It is notable that some species of the *Lacunosa* clade also have white or cream-coloured ascocarps; for example, the European *H. lactea* and the Asian *H. sublactea* of the *lactea* lineage, or the European *H. pallescens* of the *atra-pallescens* lineage. However, all the white *Helvella* species in the *Lacunosa* clade possess a cap margin attached to the stipe at several points, by which they can be differentiated from the species of the *crispa* lineage in morphology. *Helvella maculata* from North America and *H. maculatoides* from China may represent their own lineage, because they each evolved an independent branch outside the *crispa* lineage (Fig. 1), and the cap surface (hymenium) of their ascocarps is brown or dark, which is quite different from the species of the *crispa* lineage.

The *Elastica* clade is a very diverse group in species and lineages. Our present analysis revealed eight evolving lineages in this clade (Fig. 1). Among them, six lineages, *i.e.*, *bicolor-elastica* lineage, *capucina-danica* lineage, *corbierei-stevensii* lineage, *fallax-peizoides* lineage, *hypocrateriformis* lineage, *rivularis-sublicia* lineage, were suggested in a previous study (Skrede *et al.* 2017). The *carnosa* lineage was newly recognized in this study. The *macropus* lineage was split from the previous *fibrosa-macropus* lineage suggested by Skrede *et al.* (2017). *Helvella fibrosa* and its allies (with ellipsoid ascospores) were not included in the *macropus* lineage (with subfusiform ascospores), and their phylogenetic positions remain unresolved for the time being. Morphologically, the shared features of *Helvella* species in the *Elastica* clade included the terete stipe that is usually thin, solid and definitely lacks ribs and chambers, although a few of grooves can be observed sometimes, and the multiple shaped caps being typical cup-like to saddle-like. Most species in this clade have ellipsoid ascospores, but the species that evolved in the *macropus* lineage have fusiform to subfusiform ascospores, which seem unique for the species evolved in the *macropus* lineage in *Helvella*. The terete stipe is generally clearly thin, but in the European *H. leucopus*, the Chinese *H. bachu* and their allies it is robust. It is notable that the species evolved in the *alpina-corium* lineage, *alpestris-nannfeldtii* lineage and *alpicola* lineage within the *Lacunosa* clade also have a terete stipe, but their stipes are usually clearly shorter, and in that case, their caps are always cup-like and have a dark brown or blackish hymenium. Chinese species evolved in all of the eight lineages mentioned above. There are at least 66 species evolved in this clade based on our analysis (Figs 1, S3), of which 44 occur in

China. Eighteen new species are described in this study, and 14 potentially species were proposed but not taxonomically treated for the time being. The *Elastica* clade is probably the second largest clade in species diversity of *Helvella*.

*Lacunosa* clade is the most diverse group in phylogeny and morphology of *Helvella*. There were at least 13 lineages evolved in this clade worldwide according to Skrede *et al.* (2017) and this study (Fig. 1). Among them, six lineages, *i.e.*, *atra-pallescens* lineage, *fusca-lactea* lineage, *lacunosa-hellvella* lineage, *palustris-philonotis* lineage, *sulcata-phlebophoroides* lineage, *phlebophora* lineage and the species *H. queletiana* were gathered into a strongly supported subclade. To improve the following discussion on this clade, we marked this subclade as subclade *lacunosa*, as in which *H. lacunosa* was included. The remaining seven lineages, *i.e.*, *alpestris-nannfeldtii* lineage, *alpicola* lineage, *alpina-corium* lineage, *costifera* lineage, *dalgeri* lineage, *hyperborea* lineage, *tianshanensis* lineage, and the species *H. floriforma* were treated as a group and marked as “*costifera* group” (Fig. 1). Among the 13 lineages of the *Lacunosa* clade, six lineages were suggested by Skrede *et al.* 2017, including *alpestris-nannfeldtii* lineage, *alpina-corium* lineage, *atra-pallescens* lineage, *fusca-lactea* lineage, *palustris-philonotis* lineage, *sulcata-phlebophoroides* lineage. The remaining seven lineages were newly recognized in this study. Both the *Lacunosa-nigra* lineage and *hellvella* lineage suggested by Skrede *et al.* (2017) merged into a strong support clade in our five-gene-based phylogeny (Fig. 1), which thus was proposed as a new lineage in this study, *i.e.*, *lacunosa-hellvella* lineage.

The species of the *Lacunosa* clade are greatly diverse in ascocarp appearance. They share various types of ascocarps with those species respectively evolved in the *Acetabulum*, *Crispa* and *Elastica* clades. In general, the species that evolved in subclade *lacunosa* possess the saddle-like or irregularly lobbed cap, and the cap margin attached to the stipe at several points except for a few species, such as the European *H. hellvella* and *H. iberica* and their allies, which have a free cap margin. The ascocarp of the species of the *costifera* group is clearly different from those species of the subclade *lacunosa*. Their ascocarp cap is never saddle-like or lobbed, but instead, regular or irregular cup-like. Some species of the *costifera* group have the same ascocarp type as those species in the *Acetabulum* clade. Their ascocarps have a robust and ribbed stipe, for example, the species in the *costifera* lineage, *dalgeri* lineage, *hyperborea* lineage and *tianshanensis* lineage. Some species have ascocarps with thin and terete stipes, which are the same as that in the *Elastica* clade, for example, the species in the *alpestris-nannfeldtii* lineage and *alpina-corium* lineage.

Some species with different phylogenetic positions in the *Lacunosa* clade showed a high morphological convergence. The typical example is *H. queletiana*, *H. phlebophora* and *H. phlebophoroides*, which are morphologically highly similar and easy confused with each other in the field, but are phylogenetically distantly related. Similarly, species of the *alpestris-nannfeldtii* lineage and *alpina-corium* lineage in the *Lacunosa* clade were morphological convergent with the species of the *fallax-peizoides* lineage in the *Elastica* clade.

### White species and white form in *Helvella*

The overall white or cream-coloured species are not very common in *Helvella*. Most of the white species are from the *Crispa* clade, like

*H. crispata* and its allies, or the *fusca-lactea* lineage in the *Lacunosa* clade, like *H. lactea* and *H. sublactea*. However, the white or whitish collections of some non-white species are available in this study, the samples include *H. capucinooides* (BJTC FM1156, BJTC FM1094, Fig. 23D, E) and *H. costifera* (BJTC FM1705, Fig. 23F). Currently, it remains unclear whether the white form of *Helvella* species is common or rare, pending further study.

### Mottled hymenium and parasitism

The mottled hymenium has been checked and used to distinguish species in *Helvella* including *H. maculata* and *H. tinta* (Weber 1975, Hyde et al. 2016). We observed the mottled hymenium in individual apothecia of *H. tianshanensis* and *H. sinohyperborea* in this study (Figs 17, 24H, I). At the same time, the DNA sequences of the mycoparasitic fungi *Hypomyces cervinus* and *Hypomyces* sp. were extracted respectively from these apothecia, but not from the apothecia without mottled hymenia (hymenium colour is uniform). Parasitism is very common in *Helvella*, especially the species of *Hypomyces* (Abbott & Currah 1997, Nguyen et

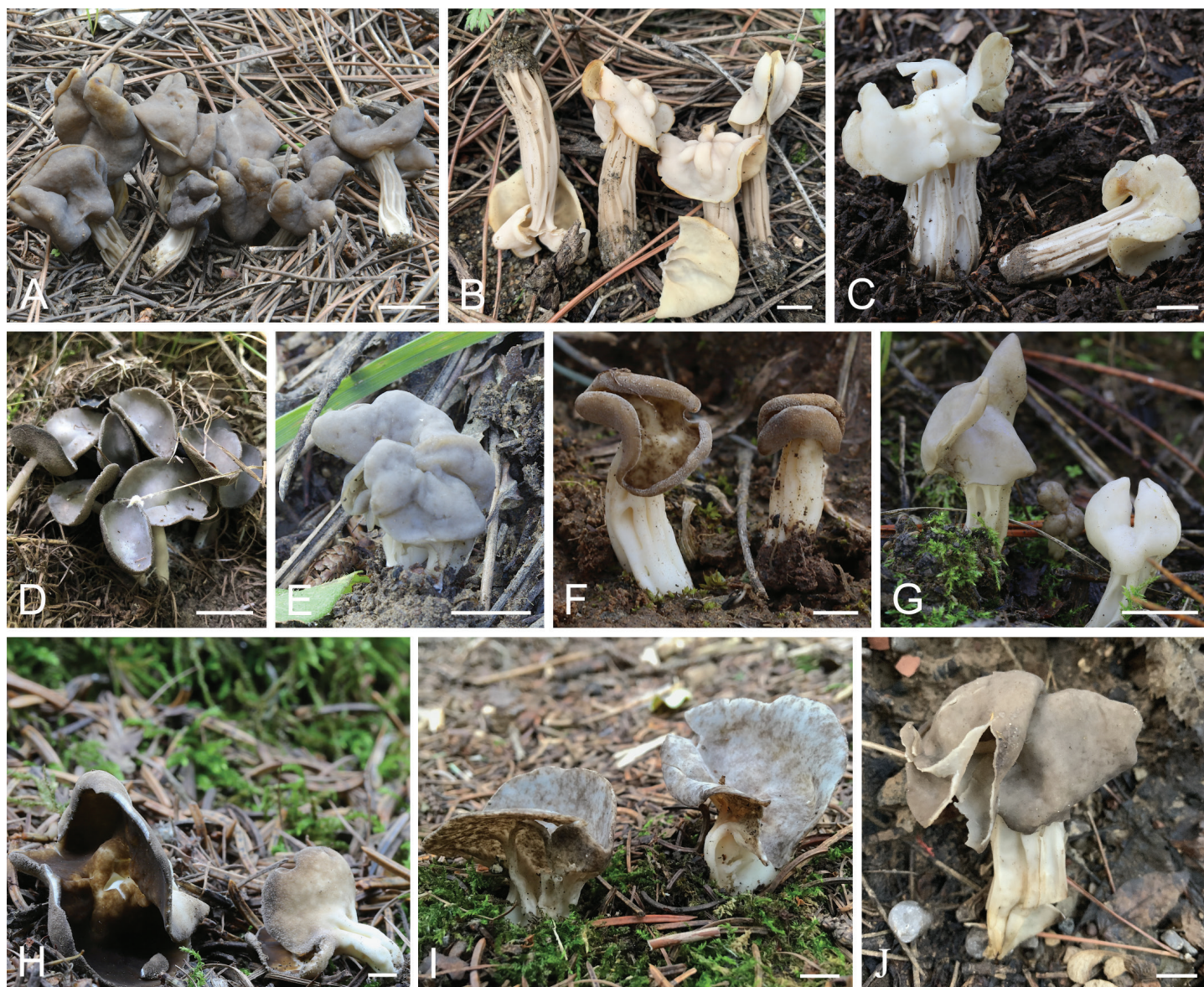
al. 2013). A mottled hymenium could thus be a consequence of such an infection.

### The application of ITS sequence in species identification of *Helvella*

The ITS region is a universal barcode for fungi (Schoch et al. 2012). However, it has not been considered as a good barcode gene for *Helvella* species. One of the reasons may be the difficulty of obtaining ITS sequence data. In this study, we successfully sequenced almost all species recognized from our *Helvella* collections. Our molecular analyses also revealed that the topology of phylogenetic trees generated from ITS is almost identical to that generated from the five-gene-combined dataset (ITS/nrLSU/ *tef1- $\alpha$* /*rpb2/hsp*) at clade level, lineage level and species level (Figs 1, 2, S1–S4). These results showed that the ITS region is effective at least for the delimitation of the Chinese *Helvella* species. In addition, BLASTn analyses for all of *Helvella* species identified in this study revealed that the intraspecific similarity in ITS sequence ranged from 86 % to 98 %.



**Fig. 23.** Photographs of apothecia in their natural habitat. **A.** *Helvella acetabuloides* (BJTC FM1264). **B, C.** *H. capucinooides* (B. BJTC FM242. C. BJTC FM1423). **D, E.** *H. capucinooides* (white form apothecia, D. BJTC FM1156. E. BJTC FM1094). **F.** *H. costifera* (white form apothecia, BJTC FM1705). **G, H.** *H. danica* (G. BJTC ZH883. H. BJTC ZH1190). **I.** *H. lacunosa* (BJTC FM907). Scale bars = 1 cm.



**Fig. 24.** Photographs of apothecia in their natural habitat. **A.** *Helvella magna* (BJTC FM2486-A). **B.** *H. orienticrispa* (BJTC FM2480). **C.** *H. pseudoreflexa* (HSA 332). **D.** *H. pubescens* (BJTC FM918). **E.** *H. ravida* (HSA 375). **F.** *H. solitaria* (BJTC FM417). **G.** *H. sublactea* (BJTC FM1082). **H.** *H. tianshanensis* (HSA 185). **I.** *H. tianshanensis* (apothecia infected by *Hypomyces cervinus*, BJTC FM175). **J.** *H. vulgata* (BJTC FM2621). Scale bars = 1 cm.

Therefore, we infer that 98% ITS identity might be the threshold to delimitate *Helvella* species.

Previous studies suggested that the amplification success rate of ITS sequence is low, especially in old specimens (Skrede *et al.* 2017, Wang *et al.* 2019). Two primer pairs successfully used in our present work are provided in Table 1. We observed that the ITS sequences amplified using the primers of ITS1-F/ITS4 could result in an inaccurate sequence in several *Helvella* species, *i.e.*, *H. solitaria*, *H. taiyuanensis*, and *Helvella* sp. 59. Amplifying the ITS2 sequence using the primers ITS3/ITS4 is, however, effective for these species.

#### Doubtful and excluded species of *Helvella* in China

A total of 90 *Helvella* species have been recorded in China before this study. Of these, 53 species were confirmed in this study. The remaining 37 species are documented as follows.

***Helvella adhaerens*** Peck, *Ann. Rep. Reg. N.Y. St. Mus.* **54**: 956. 1902. [1901].

*Helvella adhaerens* is documented in China (Dai 1979, Zhuang WY 1998b) based on a specimen (HMAS 17262) collected from Beijing in 1955. We re-examined this specimen and identified it as the *H. elastica* complex according to morphological traits. The DNA sequence of this specimen was unsuccessfully sequenced in this study. As it is difficult and even impossible to identify species of the *H. elastica* complex accurately without DNA data according to our experience, the taxonomic treatment of this specimen awaits. *Helvella adhaerens* has been treated as a synonym of *H. elastica* (Dissing 1966b, Skrede *et al.* 2017).

***Helvella albella*** Qué. [as ‘*Elvella*’], *C. r. Assoc. Franç. Avancem. Sci.* **24**: 621. 1896. [1895].

This species was reported based on two specimens deposited in the Mycological Herbarium of Shanxi University (MHSU456, MHSU457) (Liu & Cao 1988), but the specimens got lost according to the authors. *Helvella albella* has been treated as a synonym of *H. bicolor* (Skrede *et al.* 2017). None of Chinese collections matched *H. bicolor* in the present study.

***Helvella albipes*** Fuckel, *Hedwigia* 5: 15. 1866.

This species was reported in Mao's book (1998), but no specimen was cited. Recent work indicated that *H. albipes* is a heterotypic synonym of *H. monachella* (Skrede et al. 2017). *Helvella monachella* is a European species. Its most similar Chinese species is *H. bachu* (Zhao et al. 2016a).

***Helvella chinensis*** (Velen.) Nannf. & L. Holm, in Lundell et al., *Publications from the Herbarium, University of Uppsala, Sweden* 18: 5. 1985.

This name was mentioned in several papers (Lundell et al. 1985, Zhuang 1995, Wang & Chen 2002) but no specimen was cited. No specimens are available for study.

***Helvella compressa*** (Snyder) N.S. Weber, *Beih. Nova Hedwigia* 51: 35. 1975.

Zhuang & Yang (2008) reported this species based on the specimen HKAS 45788, and HMAS 97524 from Tibet of China, but we are not able to re-examine the two specimens in this study.

***Helvella confusa*** Harmaja, *Karstenia* 17: 43. 1977.

This species was reported from China by Zhuang (1998b). This species has been transferred to the genus *Dissingia* as *D. confusa* (Hansen et al. 2019). The occurrence of *Dissingia confusa* in China has been confirmed by Wang et al. (2019).

***Helvella corium*** (O. Weberb.) Masee, *Brit. Fungus-Fl.* 4: 463. 1895.

This is a frequently encountered name of *Helvella* in Chinese papers (Dai 1979, Liu et al. 1985, Zhuang et al. 2018) but no specimen was cited. A Chinese collection (HKAS 69755) labelled as *Helvella cf. corium* matched *H. xiaohuipan*, a new species described in this study (Fig. S3). None of samples from China matched this species in our present molecular analyses. Moreover, according to Cao et al. (1990), the name *H. corium* is a misapplication for *H. rivularis* or *H. villosa* in China.

***Helvella crispa*** (Scop.) Fr., *Syst. Mycol.* 2: 14. 1822.

The name *H. crispa* is one of the commonest *Helvella* species in China. Almost all the white saddle-shaped species with robust ribbed stipe are identified as this species. Recent works revealed that the *H. crispa*-like saddle fungi represented a different phylogenetic species (Zhao et al. 2015, Tibpromma et al. 2017). Our present analysis also recognized two new species from the *H. crispa* complex. *Helvella crispa* is not confirmed in China in this study, but there are at least five species in China that are based on the *H. crispa*-like samples (Zhao et al. 2015, 2016b, this study). Moreover, some sequences from the USA gathered into isolated clusters, indicating the presence of undescribed species, such as *Helvella* sp. 3, *Helvella* sp. 4 (Fig. S2). Therefore, *H. crispa* might be a species endemic to Europe.

***Helvella cupuliformis*** Dissing & Nannf., *Svensk Bot. Tidskr.* 60: 326. 1966.

Zhuang (2004) reported this species based on specimens from Xinjiang of north-western China. No DNA sequences were obtained from these specimens in this study. This species has been treated as a synonym of *Helvella hypocrateriformis* (Skrede et al. 2017).

***Helvella cupuliformis* var. *crassa*** W.Y. Zhuang, *Mycotaxon* 90: 38. 2004.

We checked the type specimens (HMAS 83523, HMAS 83524) of this taxon, but their sequences are unsuccessfully isolated in this study. This name is listed as a synonym of *H. cupuliformis* (= *H. hypocrateriformis*, Skrede et al. 2017) in Index Fungorum.

***Helvella dissingii*** Korf, *Mycotaxon* 31: 381. 1988.

Li et al. (2015) listed this species from China but no specimen was cited. Currently, this species is treated as a synonym of *Helvella fibrosa* (Skrede et al. 2017).

***Helvella elastica*** Bull., *Herb. Fr.* (Paris) 6: tab. 242. 1785.

*Helvella elastica* is one of the most common fungal names in China. However, our present study showed that no Chinese specimens matched to this species phylogenetically (Fig. 1). All the morphologically similar specimens are either introduced as new species, or matched other similar species in the *elastica-bicolor* lineage, *carnosa* lineage and *capucina-danica* lineage of the *Elastica* clade in this study (Fig. 1).

***Helvella ephippium*** Lév., *Ann. Sci. Nat., Bot.*, sér. 2, 16: 240. 1841.

*Helvella ephippium* was reported in China by Cao (1988). Later, it was mentioned by Zhuang (1989, 2004) and Wang & Chen (2002). The specimen cited by Cao (1988) is lost according to the author. Recent work treated *Helvella ephippium* as a synonym of *H. sublicia* (Skrede et al. 2017).

***Helvella fargesii*** Pat., *J. Bot.* 7: 344. 1893.

Dai (1979) reported this species from China but without citing a specimen. Dissing (1966b) treated it as a synonym of *H. elastica*. It is treated as an uncertain species (Skrede et al. 2017).

***Helvella fusca*** Gillet, *Champ. France Discomyc.* 1: 9. 1879.

*Helvella fusca* was reported based on a specimen (HXZE515) from Tibet of China (Xu 2002). We have not re-examined this specimen in this study.

***Helvella glutinosa*** B. Liu & J.Z. Cao, *Acta Mycol. Sin.* 7: 198. 1988.

This species is described from Heilongjiang Province, north-eastern China based on a specimen under conifers (MHSU451, holotype) in September 1986 (Liu & Cao 1988). The only specimen had been lost, but according to the original description, it is a morphologically distinct species, which is diagnosed by the gel cap.

***Helvella helvellula*** (Durieu) Dissing, *Rev. Mycol.* 31: 204. 1966.

This species is recorded from Shanxi Province in Northern China based on a specimen under conifers (MHSU 275) (Liu & Cao 1988). The specimen had been lost, but according to the original description given by Liu & Cao (1988), the cap of this specimen is dark brown rather than black as in *H. helvellula*. It is notable that two specimens newly collected from the same locality are introduced as a new species *H. sinohyperborea* in this study (Fig. 1).

***Helvella hyperborea*** Harmaja, *Karstenia* 18: 57. 1978.

Xu (2002) reported this species from Tibet in China based on a specimen (HXZE1023). Wang et al. (2019) recognized this species again in China based on specimens from Shanxi and Xinjiang Provinces. Our present analysis showed that these

specimens cited by Wang *et al.* (2019) are identical with the newly introduced *H. sinohyperborea* in this study (Fig. 1).

***Helvella jiaohensis*** J.Z. Cao *et al.*, *Acta Mycol. Sin.* **9**: 184. 1990. This species was described from Jiling Province in north-eastern China based on a specimen (HBNU 0700, holotype) deposited in the Northeast Normal University (Cao *et al.* 1990). We were not able to re-examine this specimen in this study. This species has subglobose to very broadly ellipsoid ascospores, which suggests it to be a distinct species (Cao *et al.* 1990).

***Helvella jilinensis*** J.Z. Cao *et al.*, *Acta Mycol. Sin.* **9**: 185. 1990. *Helvella jilinensis* is described from Jiling Province in north-eastern China based on a specimen (HBNU 0738, holotype) deposited in Northeast Normal University (Cao *et al.* 1990). We were not able to re-examine this specimen in this study. According to Cao *et al.* (1990), this species is diagnosed mainly by its thick-walled hyphae in the medullary excipulum. However, whether this feature can be used in species delimitation in *Helvella* is questionable.

***Helvella jimsarica*** W.Y. Zhuang, *Mycotaxon* **90**: 39. 2004. This species was described from Xinjiang Uygur Autonomous Region (Zhuang 2004). No specimen is available for study.

***Helvella lactea*** Boud., *Icon. Mycol., liste pr lim.*: 2. 1904. This species was reported from Shanxi Province, Northern China and Jiling Province, Northeast China based on two specimens (MHSU454, MHSU455) (Liu & Cao 1988). The two specimens had been lost according to one of the authors (Cao, pers. comm.). It is notable that all the *H. lactea*-like specimens newly collected from China matched *H. sublactea* in our phylogenetic analyses in this study.

***Helvella latispora*** Boud., *Bull. Soc. Mycol. France* **14**: 16. 1898. *Helvella latispora* was firstly reported based on a specimen from Tibet in Southwest China (HXZE1440). We were not able to re-examine this specimen in this study. Recently, *Helvella latispora* has been treated as a synonym of *H. levis* (Skrede *et al.* 2017).

***Helvella leucomelaena*** (Pers.) Nannf., in Lundell & Nannfeldt, *Fungi Exsicc. Suecici Fasc.* **19–20**: 21, no. 952. 1941. *Helvella leucomelaena* is documented from China (Teng 1963, Tai 1979, Zhuang 1998b), but it is actually not occurring in China according to Wang *et al.* (2019). *Helvella leucomelaena* has been transferred to *Dissingia* as *D. leucomelaena* (Hansen *et al.* 2019).

***Helvella leucopus*** Pers., *Mycol. Eur.* **1**: 213. 1822. This name in China has been shown to be a misapplication of the Chinese *H. bachu* (Zhao *et al.* 2016a). Currently, *H. leucopus* has been treated as a synonym of *H. monachella* (Skrede *et al.* 2017).

***Helvella maculata*** N.S. Weber, *Beih. Nova Hedwigia* **51**: 27. 1975. This species was reported based on two specimens (MHSU 062, MHSU 063) from Xinjiang in Northwestern China (Liu & Cao 1988). We could not re-examine the two specimens in this study.

***Helvella oblongispora*** Harmaja, *Karstenia* **18**: 57. 1978. This species has been transferred to the genus *Dissingia* as *D. oblongispora* (Hansen *et al.* 2019). The occurrence of *D.*

*oblongispora* in China has been confirmed by Wang *et al.* (2019) and this study.

***Helvella pezizoides*** Afzel., *Kongl. Vetensk. Acad. Nya Handl.* **4**: 308. 1783. Cao (1988) reported this species from China. According to the author, the specimen is probably lost. Our present analysis showed that none of the Chinese specimens matched *H. pezizoides*.

***Helvella pseudolacunosa*** Q. Zhao & K.D. Hyde, *Fungal Diversity* **75**: 142. 2015. This species could be identical to *H. lacunosa* according to our study (Figs 1, S4) and Wang *et al.* (2023a).

***Helvella rivularis*** Dissing & Sivertsen, *Bot. Tidsskr.* **75**: 101. 1980. This species was reported based on a specimen from Shanxi Province in Northern China (Cao *et al.* 1990), but the specimen has been lost according to the author (Cao pers. comm.).

***Helvella sinensis*** B. Liu & J.Z. Cao, *Acta Mycol. Sin.* **4**: 214. 1985. This species is described from Shanxi Province in Northern China (Liu *et al.* 1985). The type specimen is lost according to the author (Cao, pers. comm.). *Helvella sinensis* is a species of the *H. crispa* complex, diagnosed by the red-brown apothecium and broad ascospores. DNA data is lacking for this taxon.

***Helvella spadicea*** Schaeff. [as 'Elvela'], *Fung. bavar. palat. nasc. (Ratisbonae)* **4**: 112. 1774. The name *H. spadicea* is a misapplication for *H. bachu* or *H. subspadicea* in China (Zhao *et al.* 2016a). *Helvella spadicea* is treated as an uncertain species (Skrede *et al.* 2017).

***Helvella subfusispora*** B. Liu & J.Z. Cao, *Acta Mycol. Sin.* **4**: 211. 1985. This species is firstly introduced based on a specimen (HBSU 3319) from the Zhoushan islands of Zhejiang Province in Eastern China (Liu *et al.* 1985). The holotype was destroyed in a fire, thus the specimen HMAS 30483 from Sichuan Province of Southwestern China had been selected as the neotype (Cao *et al.* 1990). Attempts to obtain a sequence from the neotype was unsuccessful in this study. However, according to the original description, this is a morphologically distinct species, which is diagnosed by its saddle-shaped ascocarps with thin and terete stipe, and subfusiform ascospores.

***Helvella subglabra*** N.S. Weber, *Michigan Bot.* **11**: 179. 1972. The North American *H. subglabra* was reported based on five specimens from Southwestern and Northeastern China (Zhao *et al.* 2016b). Our present analysis based on the ITS phylogenetic analysis (Fig. S4) showed these Chinese samples cited by Zhao *et al.* (2016) matched the new species *H. subglabroides* described in this study.

***Helvella ulvinenii*** Harmaja, *Karstenia* **19**: 42. 1979. This species was reported from China based on a specimen from Yunnan (HKAS 90321) (Zhao *et al.* 2016b). *Helvella ulvinenii* has been treated as a synonym of *H. solitaria* (Skrede *et al.* 2017), and the sequence cited for this species by Zhao *et al.* (2016b) also matched *H. solitaria* in the present analysis (Fig. 1).

*Helvella villosa* (Hedw.) Dissing & Nannf., *Svensk bot. Tidskr.* **60**: 330. 1966.

*Helvella villosa* is popularly mentioned in China but without citation of specimens (Cao *et al.* 1990). Recently, *Helvella villosa* has been treated as a synonym of *H. fibrosa* (Skrede *et al.* 2017).

*Helvella xinjiangensis* J.Z. Cao *et al.*, *Acta Mycol. Sin.* **9**: 186. 1990. Attempts to obtain a sequence from the type specimen (HMAS 38353) was unsuccessful in this study. *Helvella xinjiangensis* is treated as a synonym of *H. elastica* (Zhuang 2004).

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#### Supplementary information

**Fig. S1.** Phylogeny of Chinese *Helvella* species of *Acetabulum* clade inferred from the Dataset II (ITS) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. New species are printed in **bold**.

**Fig. S2.** Phylogeny of Chinese *Helvella* species of *Crispa* clade inferred from the Dataset III (ITS) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. New species are printed in **bold**.

**Fig. S3.** Phylogeny of Chinese *Helvella* species of *Elastica* clade inferred from the Dataset IV (ITS) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. New species are printed in **bold**.

**Fig. S4.** Phylogeny of Chinese *Helvella* species of *Lacunosa* clade inferred from the Dataset V (ITS) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS  $\geq 70\%$ , left) and significant Bayesian posterior probability (BPP  $\geq 0.95$ , right) are indicated above the nodes. New species are printed in **bold**.

**Table S1.** Specimens used in the five combined loci (ITS/nrLSU/*tef1- $\alpha$* /*rpb2/hsp*) phylogenetic analysis and their GenBank accession numbers.

Newly generated sequences are in **bold**.

**Table S2.** Specimens used in the two combined loci (ITS/nrLSU) phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in **bold**.

**Table S3.** Specimens used in *Acetabulum* clade ITS phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in **bold**.

**Table S4.** Specimens used in *Crispa* clade ITS phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in **bold**.

**Table S5.** Specimens used in *Elastica* clade ITS phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in **bold**.

**Table S6.** Specimens used in *Lacunosa* clade ITS phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in **bold**.

**Table S7.** The list of *Helvella* species confirmed in China.