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

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- molecular analysis
- new taxa
- Pezizales
- phylogeny
- saddle fungi
- taxonomy

**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, both are commonly recorded in China.

**INTRODUCTION**

*Helvella* (1753) is one of the most conspicuous members of *Helvellaceae*. Almost all *Helvella* species have saddle-shaped ascomycors with cupulate or lobed caps on a distinct stipe, and ellipsoid ascosporae with a large central oil drop with the exception of the *H. macropus* complex which has subfusoid ascosporae. *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, Laessoe & 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 2x Master Mix (TiangenBiotech Co., Beijing, China), and 8.5 μL ddH₂O.

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. 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.

<table>
<thead>
<tr>
<th>Gene region</th>
<th>Forward primer</th>
<th>Primer sequence (5’–3’)</th>
<th>Reverse primer</th>
<th>Primer sequence (5’–3’)</th>
<th>PCR annealing temp (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS</td>
<td>ITS1-F</td>
<td>CTTGGTCTATTAGAGGAGGAA</td>
<td>ITS4</td>
<td>TCCTCAGCCTATTTGATGCG</td>
<td>53 or 55</td>
<td>White et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>ITS2</td>
<td>GATCGATGAAAGACGAGCG</td>
<td></td>
<td></td>
<td>55</td>
<td>White et al. (1990)</td>
</tr>
<tr>
<td>nrLSU</td>
<td>LROR</td>
<td>ACCGCGTACTAAGTC</td>
<td>LR5</td>
<td>TCCCTGGAGGAACCTCG</td>
<td>55</td>
<td>Vilgalys &amp; Hester (1990)</td>
</tr>
<tr>
<td>tef1-α</td>
<td>EF1-983F</td>
<td>GCCYCGGCHGCGGCTTGTYTAT</td>
<td>EF1-2218R</td>
<td>ATGACCACRACRGCRACGRTYG</td>
<td>touchdown</td>
<td>Rehner &amp; Buckley (2005)</td>
</tr>
<tr>
<td>rpb2</td>
<td>RPB2-7cF</td>
<td>ATGGGGAARACGGCAGTGGG</td>
<td>RPB2-11aR</td>
<td>GCGTGGATCTTTRCCTCSACC</td>
<td>touchdown</td>
<td>Liu et al. (1999)</td>
</tr>
<tr>
<td>hsp</td>
<td>H_hspf</td>
<td>CRGGCATCAGGGGTGACGTAAT</td>
<td>H_hsp</td>
<td>AGGGKGTGTGTCGACTCCAGG</td>
<td>58</td>
<td>Skrede et al. (2017)</td>
</tr>
</tbody>
</table>
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-α, 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 comprised of five gene fragments (ITS/nrLSU/tef1-α/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-α, 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 Pindarda 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 Lacunosana 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). Unsampled 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; 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-α. 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 (ASDF) 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) ≥ 70 % and Bayesian posterior probability (PP) ≥ 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-α/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-α, 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 *Lacunosana* 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. maculotoideus*, both outside *crispa* lineage, formed their own independent branches. Within the *Elastica* clade, eight individual evolving lineages were recognized, *i.e.*, *bicolor-elastica* lineage, *copucina-danica* lineage, *carnosa* lineage, *corbieri-stevensii* lineage, *fallax-
Fig. 1. Phylogeny of Chinese Helvella inferred from the Dataset I (ITS/nr LSU)/efl-1-α/rib2/hsp) using the maximum likelihood (ML) analysis. Numbers representing likelihood bootstrap support values (MLBS ≥ 70 %, left) and significant Bayesian posterior probability (BPP ≥ 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.
Diversity and phylogeny of Chinese *Helvella*

Fig. 1. (Continued).
Fig. 1. (Continued).
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. maculatoiades*, *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. ephippiformis*, *H. fistulosa*, *H. galeriformis*, *H. guttata*, *H. macroopus*, *H. nordlandica*, *H. orentiomentosa*, *H. pubescens*, *H. scyphoides*, and *H. subpadicea*. 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 m 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. austroaccidentalis*, *H. borealis*, *H. calycina*, *H. costifera*, *H. cystidita*, *H. fulva*, *H. huangii*, *H. jizushanica*, *H. lacunosa*, *H. lului*, *H. lobata*, *H. magna*, *H. parva*, *H. philonotis*, *H. phlebophora*, *H. phlebophoropsis*, *H. plateata*, *H. ravida*, *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). Dissidia oblongispore 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 (Ariyawan 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, Zhaung 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. fistulosas* (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 & YY. Xu, sp. nov. MycoBank MB 849609. Fig. 3.

*Etymology*: *albopatella*, 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 hyphid 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** pleurorhynchous, 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, Qw = 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.
Diversity and phylogeny of Chinese Helvella

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 ≥ 70 %, left) and significant Bayesian posterior probability (BPP ≥ 0.95, right) are indicated above the nodes. New species are printed in **bold**.
Fig. 2. (Continued).
Specimens examined: China, Shanxi Province, Linfen City, Pu County, Wulushan mountains, on the ground in broadleaf forest dominated by Quercus sp., 36°56ʹ23ʹʹN, 111°23ʹ46ʹʹE, alt. 1 644.6 m, 26 Aug. 2022, N. Mao (holotype BJTC FM2610-A, GenBank Acc. No.: ITS = OR355082, nrLSU = OR355252, tef1-α = 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-α 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 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, 13–30 × 8–16 μm. Medullary excipulum 175–250 μm broad, of interwoven textura intricata, hyphae 2.0–4.0 μm broad. Asci pleurorhynchous, tapering, 8-spored, 215–275 × 13–19 μm. Ascospores ellipsoid, with one large oil drop when mature, 17–19 × 10–12 μm, [Lm × Wm = 17.9 × 10.9 μm,
Q = 1.45–1.81, Qₙ = 1.64 ± 0.09]. Paraphyses filiform, septate, pale brown, 4.0–6.0 μm wide at clavate to subcapitate tips and 2.0–3.5 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 mixed broadleaf forest, alt. 898 m, 25 Aug. 2020, G.Q. Chen (holotype BJTC C316-A, GenBank Acc. No.: ITS = OR355106, nrLSU = OR355276, tef-1α = OR359033, rp2b = 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.* *Helvella 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 × Wm = 19.1 × 11.9 μm); *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 rp2b, five nrLSU, and 26 tef1-α substitutions separated *H. alborava* from *Helvella multiformis*; seven hsp, nine rp2b, 16 nrLSU substitutions and 41 tef1-α 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, 0.5–2.2 cm high, 1.0–2.7 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 when dry; receptacle surface glabrous, white, becoming yellow when dry; stipe terete 2.5–7.5 cm high, 0.3–0.7 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* 80–130 μm broad, of *textura angularis,* cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 23–56 × 13–29 μm. *Medullary excipulum* 150–230 μm broad, of interwoven *textura intricata,* hyphae 2.5–6.5 μm broad. *Asci* pleurorhynchous, tapering, 8-spored, 260–330 × 15–20 μm. *Ascosporas* ellipsoid, with one large oil drop when mature, 17–20.5 × 10.5–12.5 μm, [Lm × Wm = 19 × 11.5 μm, Q = 1.5–1.8, Qₙ = 1.65 ± 0.07]. Paraphyses filiform, 2.5–4.5 μm below, pale yellow to pale brown, septate, clavate to subcapitate, 6–13(–15) μm 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, 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, tef-1α = OR359000, rp2b = OR359124, hsp = OR366062); ibid., Lviang 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 (330–360 × 15–17 μm) and relatively longer ascospores (19–22 × 10.6–12.2 μm) (Skrede et al. 2017). Helvella carnosa has narrower asci (270–300 × 13–15 μm) and relatively narrower paraphyses at the apex (5–8 μm) (Skrede et al. 2017). In addition, six hsp, 13 rp2b, 16 nrLSU, and 20 tef1-α substitutions separated it from *H. elastica*; five hsp, 11 rp2b, 17 nrLSU substitutions, and 32 tef1-α 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, 0.4–1.0 cm high, 0.5–1.8 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, 1.5–3.0 cm high, 0.2–0.3 cm broad, pale grey, becoming greyish brown when dry, base often with white mycelia. *Ectal excipulum* 75–150 μm broad, of *textura angularis,* cells hyaline to pale brown, outermost cells club-shaped, 16–31 × 8–14 μm. *Medullary excipulum* 150–300 μm broad, of interwoven *textura intricata,* hyphae 2.0–5.5 μm broad. *Asci* pleurorhynchous, tapering, 8-spored, 210–300 × 14–21 μm. *Ascosporas* ellipsoid, with one large oil drop when mature, 16.5–20 × 10–12 μm, [Lm × Wm = 18.5 × 11.1 μm, Q = 1.50–1.80, Qₙ = 1.66 ± 0.07]. Paraphyses filiform, septate, pale brown, 5.0–9.0 μm wide at clavate to subcapitate tips and 1.5–3.5 μm below.

**Habitat:** Clustered or grouped on the ground in coniferous and broadleaf mixed forest dominated by *Betula* sp., *Pinus tabuliformis,* *Populus* sp., *Quercus wutaishansea*.
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). 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
tufts, with cylindrical to subclavate cells of 18–37 × 11–18 µm. Medullary excipulum 240–330 µm broad, of interwoven textura intricata, hyphae 3–7 µm broad. Asci pleurorhynchous, tapering, 8-spored, 225–275 × 13–18 µm. Ascospores ellipsoid, with one large oil drop when mature, 17–18.5 ×9.5–11 µm, [Lm × Wm = 17.6 × 10.1 µm, Q = 1.60–1.85, Qav = 1.74 ± 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°55ʹ39ʹʹN, 113°35ʹ43ʹʹE, alt. 1 543 m, 28 Aug. 2019, Y. Shen (holotype BJTC FM941-A, GenBank Acc. No.: ITS = OR355153, nrLSU = OR355324, tef1-α = OR359066, rpb2 = OR359178, hsp = OR366131); ibid., 38°55ʹ39ʹʹN, 113°35ʹ40ʹʹE, 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 (15–17 × 9.5–11.5 µm), and broader paraphyses at the apex (6–7.5 µm) (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.
**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 µm 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 × 10–20 µm. *Medullary excipulum* 100–125 µm broad, of interwoven *textura intricata*, hyphae 2.0–6.0 µm broad. *Asci* pleuromorphous, tapering, 8-spored, 290–325 × 15–23 µm. *Ascospores* ellipsoid and with slightly narrow ends, with one large central oil drop and a few of small ones when mature, 19.5–23 × 12–14.5 µm, [Lm × Wm = 21.0 × 13.1 µm, Q = 1.46–1.81, Qav = 1.60 ± 0.08]. *Paraphyses* filiform, septate, subhyaline to pale brown, 6.0–11.0 µm wide at clavate tips and 2.0–4.0 µm 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, Luliang City, Jiaocheng County, Pangguangou, 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-α = 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-α substitutions separated *H. deflexa* from *H. danica*. *Helvella deflexa* shares less than 96.16 % similarity in ITS region with other *Helvella* species.
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 µm broad, of textura angularis, cells hyaline to pale brown, arranged in rows turning out perpendicular to receptacle surface, outermost cells clavate, 18–43 × 9–21 µm. Medullary excipulum 150–250 µm broad, of interwoven textura intricata, hyphae 2.0–7.0 µm broad. Asci pleurorhynchous, tapering, 8-spored, 250–310 × 14–20 µm. Ascospores ellipsoid, with one large oil drop when mature, 18–20.5 × 10.5–12.5 µm, [Lm × Wm = 19.3 × 11.5 µm, Q = 1.53–1.84, Qav = 1.67 ± 0.08]. Paraphyses filiform, septate, pale yellow to pale brown, 5.5–9 µm wide at clavate to subcapitate tips and 2.0–4.0 µm 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, Pangquangou, 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-α = 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...
with delicate pubescence and relatively narrower asci (13–15 µm) (Skrede et al. 2017), whereas *H. flavostipitata* has a glabrous receptacle surface and wider asci (14–20 µm diam). Molecularly, *H. flavostipitata* deviates consistently in one *hsp*, five nrLSU, and nine *tef1*-α 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 brown when dry, occasionally with grooves, base slightly enlarged. **Ectal excipulum** 120–200 µm broad, of *textura angularis*, cells hyaline to pale brown, sometimes extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multiseptated, cells elongate club-shaped, 16–40 × 8–21 µm. **Medullary excipulum** 300–360 µm broad, of interwoven *textura intricata*, hyphae 3.0–6.0 µm broad. **Asci** pleurorhynchous, tapering, 8-spored, 250–300 × 17–20 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 18–20.5 × 11–13 µm, [Lm × Wm = 19.1 × 11.9 µm, Q = 1.44–1.81, Qav = 1.61 ± 0.09]. **Paraphyses** filiform, septate, subhyaline to pale...
brown, 4.5–7.0 µm wide at clavate to subcapitate tips and 2–4 µm 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. 1 675 m, 27 Aug. 2020, **N. Mao** (holotype) BJTC FM1130, GenBank Acc. No.: ITS = OR355100, nrLSU = OR355270, tef1-α = 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.**, Luliang City, Jiaocheng County, Guandishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*, alt. 2 003 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). Moleurally, five hsp and two rpb2 substitutions separate this new species from *H. poculiformis*; five hsp, six rpb2, five nrLSU substitutions, and 11 *tef1-α* 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 *Helvella* species, supporting it as a distinct species.

**Helvella neofistulosa** L. Fan, N. Mao & C.L. Hou, **sp. nov.** MycoBank MB 849618. 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 µm broad, of interwoven *textura intricata*, hyphae 2.3–4.5 µm broad. Asci pleuronsynchous, tapering, 8-spored, 250–300 × 14–21 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 18–22.5 × 10.5–12.5 µm, [Lm × Wm = 20 × 11.6 µm, Qv = 1.58–1.89, Qav = 1.72 ± 0.08]. **Paraphyses** filiform, 2.5–4.5 µm below, septate, clavate to subcapitate, 6–11 µm 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-α = OR358994, rpb2 = OR359118, hsp = OR366056; Hebei Province, Chicheng county, Dahuai town, 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*. Moleurally, there are two hsp, one rpb2, three nrLSU substitutions, and 22 *tef1-α* 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 *Helvella* 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 µm broad, of *textura angularis*, cells hyaline to pale brown, extending into fascicled hyphoid hairs in outer ectal excipulum, hairs multisepateated, cells elongate club-shaped, 11–37 × 9–16 µm. **Medullary excipulum** 135–200 µm broad, of interwoven *textura intricata*, hyphae 2.0–5.0 µm broad. Asci pleuronsynchous, tapering, 8-spored, 235–265 × 15–20 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 16.5–18.5 × 11–12.5 µm, [Lm × Wm = 17.5 × 11.8 µm, Qv = 1.40–1.63, Qav = 1.49 ± 0.07]. **Paraphyses** filiform, septate, pale yellow to pale brown, 4.5–6.0 µm wide at clavate to subcapitate tips and 2.5–4.0 µm below.

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

**Distribution**: Known from Beijing, Northern China.
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-α = 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-α 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, YY. 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.
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, \( L_m \times W_m = 21.5 \times 12.1 \mu m \), \( Q = 1.57–1.91, Q_{av} = 1.77 \pm 0.11 \). **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.
**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 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 × 9–20 µm. **Medullary excipulum** of interwoven *textura intricata*, 200–350 µm thick, hyphae, 2.0–6.5 µm broad. **Asci** pleurorhynchous, tapering, 8-spored, 230–300 × 13–19 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 15.5–19.5 × 9.5–11 µm, [Lm × Wm = 17.5 × 10.3 µm, Q = 1.55–1.90, Q_{av} = 1.70 ± 0.08]. **Paraphyses** filiform, septate, pale brown, 6.0–10.0 µm wide at clavate to subcapitate tips and 2.0–4.5 µm 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.
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, \[Lm \times Wm = 18.1 \times 11.2 \, \mu m, \, Q = 1.50–1.76, \, Q _{av} = 1.62 \pm 0.06\]. **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...
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*; 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 greyish.
whitish, becoming pale yellow when dried, ribbed, blunt-edged, partly anastomosing and double-edged, with lacunae between ribs, chambered inside. **Ectal excipulum** 80–180 µm broad, of *textura angularis*, cells hyaline to pale brown, outermost cells catenuliform in long fascicled tufts, with cylindrical to subclavate, end cells 15–37 × 8.5–15 µm. **Medullary excipulum** 190–270 µm broad, of interwoven *textura intricata*, hyphae 2.5–5 µm broad.

Asci pleurorhynchous, tapering, 8-spored, 255–285 × 13–16.5 µm. Ascospores ellipsoid, with one large oil drop when mature, 18–20 × 10.5–12 µm, [Lm × Wm = 19.3 × 11.2 µm, Q = 1.60–1.90, Qav = 1.73 ± 0.08]. **Paraphyses** filiform, septate, hyaline to pale brown, 4.5–7 µm wide at tips and 2–3.5 µm 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-α = 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

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**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 µm.
them. Molecularly, three hsp, six rpb2, five nrLSU, and 11 tef1-α substitutions separated this new species from H. crispa; three hsp, four rpb2, one nrLSU, and 12 tef1-α substitutions from H. orienticrispa; three hsp, four rpb2, two nrLSU, and 11 tef1-α 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 & YY. 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, blunted-edged, partly anastomosing and double-edged, occasionally with lacunae between ribs, ribs extending onto the receptacle. **Ectal excipulum** 100–150 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, 15–40 × 11–19 µm. **Medullary excipulum** 400–700 µm broad, of interwoven textura intricata, hyphae 2.0–6.0 µm broad. **Asci** pleurorhynchous, tapering, 8-spored, 240–300 × 13–19 µm. **Ascospores** ellipsoid, with one large oil drop when mature, 16–18.5 × 11–12.5 µm, [Lm × Wm = 17.5 × 11.8 µm, Q = 1.38–1.58, Qav = 1.49 ± 0.05]. **Paraphyses** filiform, septate, pale brown, 4.5–6.0 µm wide at clavate to subcapitate tips and 1.5–3.5 µm 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-α = 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

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

\textbf{Helvella subglabroides} L. Fan, N. Mao & Y.Y. Xu, \textit{sp. nov.} MycoBank MB 849624. Fig. 18.

\textit{Etymology: subglabroides}, referring to its high similarity to \textit{H. subglabra} in appearance of its ascomata.

\textit{Helvella subglabroides} 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. \textit{Ectal excipulum} 50–100 \(\mu\)m broad, of \textit{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. \textit{Medullary excipulum} 100–200 \(\mu\)m broad, of interwoven \textit{textura intricata}, hyphae 2.5–6.5 \(\mu\)m broad. \textit{Asci} pleurorhynchous, tapering.
8-spored, 200–260 × 12.5–18.5 µm. Ascospores ellipsoid, with one large oil drop when mature, 15–19.5 × 9–11 µm, [Lm × Wm = 17.2 × 10 µm, Q = 1.5–2, Qav = 1.73 ± 0.10]. Paraphyses filiform, septate, yellowish brown, 5.5–11.5 µm wide at clavate to subcapitate tips and 2.5–4.0 µm 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-α = OR359019, rpb2 = OR359142, hsp = OR366084); ibid., Jincheng City, Qinshui County, Shangwoquan Village, on the ground in broadleaf forest dominated by Quercus sp., 35°36ʹ57ʹʹN, 112°3ʹ7ʹʹE, 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-pezizoides* 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;
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. 

**Etymology:** Phylogenetically, **H. hsp** is differentiated from **H. borealis** and **H. sulcata** by its grey white hymenium, large asci (290–320 × 13–16 μm), and broad spores (14.8–16.8 × 10.5–13.2 μm) (Skrede et al. 2020). In addition, one **hsp** five **tef1-α** substitutions separated **H. sulcatae** from **H. borealis**; one **hsp**, four **nrLSU**, three **tef1-α** substitutions from **H. sulcata**; four **hsp**, six **rpb2**, and eight **nrLSU** substitutions from **H. phlebophoroides**.

**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 multisepate, 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 pleurorhynchos, tapering, 8-spored, 235–275 × 14–18 μm. **Ascospores** ellipsoid, with one large oil drop when mature, 17–19.5 × 10–12 μm, [Lm × Wm = 15.9 × 9.9 μm, Q = 1.41–1.74, Qm = 1.56 ± 0.08]. **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, Xinzhou City, Wutaishan County, Wutaishan mountains, on the ground in coniferous forest dominated by *Larix principis-rupprechtii* and *Picea asperata*.

**Specimens examined:** *China*, Shanxi Province, Xinzhou City, Wutaishan 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.*, Liulian City, Jiaocheng County, Guandishan mountains, 37°52ʹ16ʹʹN, 117°51ʹ15ʹʹE, alt. 107 m, 17 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. sulcatae* is placed in the *sulcata-phlebophoroides* lineage, *Lacunosa* clade (Fig. 1). It is closely related and similar to *H. borealis* and *H. sulcata*, but *H. borealis* is differentiated from *H. sulcatae* 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 phlebophoroides*, a species recently described from Europe, is also closely related to *H. sulcatae*, 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. sulcatae** from **H. borealis**; one **hsp**, four **nrLSU**, three **tef1-α** substitutions from **H. sulcata**; four **hsp**, six **rpb2**, and eight **nrLSU** substitutions from **H. phlebophoroides**.

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

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**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 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, Y.Y. Yan (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.*, Lviang 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, Y.X. 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 FM274); *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.

**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., Qiuqiangou, on the ground in coniferous and broad-leaved mixed forest, alt. 1 600 m, 4 Aug. 2018, H. Liu (HSA 181).

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

**Helvella fistulosa** Alb. & Schwein., *Conspr. 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, Shuibao 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, Pangguangou, 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 *Lucunosa* 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 *Lucunosa* clades (Fig. 1). Compared to the phylogeny proposed by Skrede et al. (2017), the combination of our *Acetabulum*, *Crispa* and *Lucunosa* clades corresponded to the Clade B suggested by Skrede et al. (2017). Among them, our *Acetabulum* clade corresponded to their *acutum-solitaria* lineage, our *Crispa* clade corresponded to their *crispa* lineage, our *Lucunosa* 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. maculatoideae 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, corbirei-stevensii lineage, fallax-pezizoides 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-nannfeldti 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., atrapallescens lineage, fusca-lactea lineage, lacunosa-helvellula lineage, palustris-philonotis lineage, sulcata-plebophoroides 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, atrapallescens lineage, fusca-lactea lineage, palustris-philonotis lineage, sulcata-plebophoroides lineage. The remaining seven lineages were newly recognized in this study. Both the Lacunosa-nigra lineage and helvellula 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-helvellula 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. helvellula 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. plebophoroides, 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-pezizoides 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. crispa** 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. capucinoides** (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-α/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 %.

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. taiyuensis, 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 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).


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).

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.

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, Karstienia 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).

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. xiaoahuipan*, a new species described in this study (Fig. 53). 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. viliosa* in China.

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. 52). Therefore, *H. crispa* might be a species endemic to Europe.

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).

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. hypocrateriformis* (= *H. hypocrateriformis*, Skrede et al. 2017) in Index Fungorum.

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* 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 species are either introduced as new species, or matched other similar species in the elastico-bicolor lineage, *carniosa* lineage and *capucina-danica* lineage of the *Elastica* clade in this study (Fig. 1).

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

This species is described from Helongiang 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 species 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, Karstienia 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
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This species was described from Jiling Province in north-eastern China based on a specimen (MBNU 0738, holotype) deposited in 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 jiliensis J.Z. Cao et al., Acta Mycol. Sin. 9: 185. 1990. Helvella jiliensis is described from Jiling Province in north-eastern China based on a specimen (MBNU 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 prélim.: 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 re-examined as a synonym of H. levis (Skrede et al. 2017).

Helvella leucomealaena (Pers.) Nannf., in Lundell & Nannfeldt, Fungi Exsicc. Suecici Fasc. 19–20: 21, no. 952. 1941. Helvella leucomealaena 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 leucomealaena has been transferred to Dissingia as D. leucomealaena (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 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, 54) 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 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. subsapadicea 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. 54) 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* 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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**Conflict of interest:** The authors declare that there is no conflict of interest.

**REFERENCES**


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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.