

The *Helvella corium* species complex

Phylogeny, species delimitation and distribution in the
Nordic countries

Solveig Bua Løken



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Department of Biosciences
Faculty of Science and Mathematics
University of Oslo
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Author: Solveig Bua Løken

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“... Most practicing taxonomists take for granted that species can be recognised by inspection, though inspection sometimes has to be extremely close.”

Andersson L (1990) The driving force: species concepts and ecology. *Taxon*, 375.

Forord

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Abstract

This project represents an in-depth systematic study of a pseudo-cryptic species complex, all species formerly nested under the name *Helvella corium*. Species limits and evolutionary relationships of this morphospecies complex were re-assessed, using an extended set of genetic markers (LSU, HSP, RPB2, EF-1 α and 5.8S for 47 specimens). Following a multispecies coalescent approach, the newly developed software STACEY was implemented to delineate evolutionary species. Considerable interspecific differences in evolutionary rates were observed in ribosomal loci, suggesting careful use of these regions in delimitation of *Helvella* species. All identified species were supported with morphological characters to aid in non-molecular species discrimination. In order to assess the species' distribution in the Nordic countries, all specimens stored under the name *Helvella corium* in the Nordic herbaria were examined and barcoded with HSP. This was supplemented with fresh collections from fieldwork in primarily under-studied alpine regions of Norway in 2015-2017. Altogether, 469 out of 496 specimens were successfully barcoded.

The *Helvella corium* morphospecies complex consists of seven phylogenetically distinct species, nested in two divergent and strongly supported evolutionary lineages. In the alpina-corium lineage we find *H. corium*, *H. alpina* and *H. pseudoalpina* sp. nov. The alpestris-nannfeldtii lineage consists of *H. alpestris*, *H. macrosperma* and *H. nannfeldtii*. *Helvella alpicola* is supported as sister to the alpestris-nannfeldtii lineage. *Helvella corium* is the only species that occur in temperate, boreal and arctic-alpine biomes. The other six species seem restricted to the arctic-alpine biome where they occupy different but overlapping habitats.

This project highlights how modern biosystematics, with the use of molecular markers and advanced statistical tools, can extend our knowledge of fungal diversity and distribution patterns. In addition, molecular systematics has implications for other fields of biological sciences, such as ecology and conservation biology. By re-examining this species complex in a genealogical framework, we can begin to understand their specific ecological roles in natural ecosystems and evaluate their needs for conservation.

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1 Introduction

Helvella is a species-rich genus of ascomycetous fungi (Pezizales, Helvellaceae). The genus comprises considerable morphological variation in apothecial shapes, and such variation has been interpreted differently over time. Traditionally, *Helvella* was restricted to stipitate, capitate (i.e. non-cupulate) taxa. Cupulate species, which today is included in *Helvella*, were assigned to *Peziza* and/or *Acetabula* (Pezizaceae) (Fries 1822; Fuckel 1870). Several authors have contributed with comprehensive reviews of the genus, including Boudier (1910), Nannfeldt (1932; 1937), Dissing (1966a; b), Weber (1972; 1975), Harmaja (1979), Häffner (1987), Abbott & Currah (1997), Landeros *et al.* (2015) and Skrede *et al.* (2017). Quélet (1873), and later Nannfeldt (1937) and Dissing (1966a; b) considered apothecial morphology as a trait too variable and adaptive to be useful in genus level delineation. In their view, microanatomy was more useful in delineation of genera. Species with similar microanatomy, the capitate as well as the cupulate, were thus included in *Helvella*. Still, apothecial macromorphology was retained as important in delineation of subgroups (sections) within the genus (Boudier 1907; Dissing 1964; 1966b). *Helvella* species are widespread in both the Northern and Southern hemispheres, and presumably represent both mycorrhizal and saprotrophic species. Although the genus includes many of the most conspicuous species of ascomycetes, its infrageneric phylogeny and species limits were for long poorly understood.

Helvella corium, first described as *Peziza corium* by Weberbauer (1873), is a relatively small, cupulate-stipitate and black species. The species has seen many revisions both before and after it was recombined in *Helvella* (Massée 1895), and has been referred to as e.g. *Cyathipodia* and *Leptopodia* (Boudier 1907; 1910). Dissing (1966a; b) adopted a broad concept of *H. corium*, while other authors have introduced a few additional species closely related to *H. corium*. *Helvella alpestris* was described from alpine areas of France (Boudier 1894; 1895), and *H. arctica* (Nannfeldt 1937) as yet another alpine relative from Abisko in Northern Sweden. The latter species was distinguished from *H. corium* by its white crystalline deposits on the marginal hairs of the receptacle (Nannfeldt 1937). Favre (1955)

proposed a variety of *H. arctica*, i.e. *Helvella arctica* var. *macrosperma*, with smaller ascocarps and larger ascospores. This variety was later given specific rank as *Helvella macrosperma* by Fellner & Landa (1991), and has been recognized as a specific taxon by subsequent authors (Van Vooren 2014; 2015; Skrede *et al.* 2017).

Historically, fungal species have been recognized based on morphological characters and/or mating behavioural traits. Since many macromorphological traits in fungi show a high degree of environmental plasticity (Valladares *et al.* 2007), it has been challenging for taxonomists to interpret and understand patterns of fungal diversity. Over the years, there has been a hard debate on how to define a species. This is especially challenging in mycology where knowledge of reproductive barriers may not be easily obtained and morphology is plastic. Theoretically, the evolutionary species concept has often been favoured by fungal taxonomists, i.e.: “...a lineage of ancestral descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate” (Simpson 1951; Wiley 1978). Thus, when phylogenetic analysis of sequence data was introduced as a tool in delimitation of evolutionary units, it was therefore warmly embraced by most fungal biosystematists (Taylor *et al.* 2000). Today, phylogenetic species recognition¹ (Taylor *et al.* 2000; Dettman *et al.* 2003) has completely outperformed the traditional methods in recognizing evolutionary fungal species. This is because genetic differentiation often occurs before morphological differentiation and/or reproductive isolation. Thus, numerous cryptic and pseudo-cryptic fungal species have been, and still are being discovered (e.g Vilgalys & Sun 1994; Rokas *et al.* 2003; Dettman *et al.* 2003; Kauserud *et al.* 2007; Nguyen *et al.* 2013). Cryptic species are here defined as “morphologically indiscernible biological/phylogenetic units present within taxonomic species” (Knowlton 1993; Balasundaram *et al.* 2015), and pseudo-cryptic species as “species that are readily distinguished morphologically once the appropriate characters² are considered” (Knowlton 1993).

Present day molecular markers used to infer fungal phylogenies and phylogenetic species in fungi are diverse, including both RNA-coding, non-coding and protein

¹ i.e. recognition of species based on congruent gene trees across multiple un-linked loci

² e.g. molecular systematic data

coding loci. The small (SSU) and large (LSU) ribosomal subunits are commonly used to infer deep branching patterns in Ascomycota (Spatafora *et al.* 2006; Schoch *et al.* 2009; Berbee *et al.* 2000). The internal transcribed spacer (ITS) region is selected as the primary barcode region in fungi (Schoch *et al.* 2012). However, it has also been used for species level phylogenetic inference, including in *Helvella* (Nguyen *et al.* 2013; Zhao *et al.* 2015). This non-coding region is subject to less stabilizing selection than coding regions, something that results in it showing high sequence variability even between closely related species (Schoch *et al.* 2012; Yahr *et al.* 2016). The region is therefore too variable to align across large genera, and is thus unreliable as a phylogenetic marker (Landvik *et al.* 1999; Nilsson *et al.* 2008; Harder *et al.* 2013; Balasundaram *et al.* 2015).

Several studies have pointed out that single-copy, protein-coding markers may outcompete multi-copy ribosomal genes and the ITS region in delimiting fungal species (Raja *et al.* 2011; Stielow *et al.* 2015; Hansen & Olariaga 2015). Commonly used protein-coding markers in molecular phylogenies include β -tubulin2 (TUB2) and the large subunit RNA polymerase II (RPB1 and RPB2), which have proven useful in resolution of deep as well as shallow clades among ascomycetes (Spatafora *et al.* 2006; Hofstetter *et al.* 2007; Schoch *et al.* 2009). The translation elongation factor 1- α (TEF) has also shown great ability to resolve even closely related species, and is increasingly used as a phylogenetic marker (Hansen *et al.* 2013; Balasundaram *et al.* 2015; Stielow *et al.* 2015; Hansen & Olariaga 2015). Recently, the minichromosome maintenance complex component 7 (MCM7) and the ribosome biogenesis protein TSR1 have been introduced as valuable markers for resolving higher as well as lower level taxonomic units in Ascomycota (Aguileta *et al.* 2008; Raja *et al.* 2011; Mark 2016). Moreover, a small portion of the heat shock protein 90 (HSP) has recently been shown to give good resolution at species level in *Helvella* (Skrede *et al.* 2017). HSP is successfully amplified even from old specimens and it was argued that this may partially be due to its short length, i.e. 272 bp, resulting in even degraded DNA being amplified (Skrede *et al.* 2017). It was therefore introduced as a useful secondary barcode marker in *Helvella*.

In the study by Skrede et al. (2017), species limits, phylogeny and taxonomy within *Helvella* were assessed, using a multilocus genealogical approach. They found that the traditional morphospecies concept of *H. corium* in fact represented a pseudo-cryptic species complex that comprised five phylogenetic species nested in two divergent evolutionary lineages: the alpina-corium lineage with *H. corium* and *H. alpina*, and the alpestris-nannfeldtii lineage with *H. alpestris*, *H. macrosperma* and *H. nannfeldtii*. However, the phylogenetic relationships within and between these lineages were not fully resolved. It was suggested that a sixth species, i.e. *H. alpicola*, might as well belong to this species complex, but its phylogenetic placement within the genus was unresolved (Skrede et al. 2017). The pseudo-cryptic species of the *Helvella corium* morphospecies complex are morphologically very similar and thus difficult to distinguish in the field. They occupy overlapping habitats of the alpine region of Northern Europe, and are often found in close proximity to *Dryas octopetala* and *Salix* spp., yet have never been confirmed ectomycorrhizal (Weidemann 1998).

In recent years, new methods and software for delimiting species based on multilocus data have become available (e.g. BPP (Yang & Rannala 2010), Structurama (Huelsenbeck et al. 2011), PTP (Zhang et al. 2013), DISSECT (Jones et al. 2015)). Based on DISSECT, STACEY (Jones 2017) was introduced in 2015, as a package implemented in BEAST2 (Bouckaert et al. 2014). This analytical tool is based on multispecies coalescent theory, and utilizes multilocus data and Bayesian inference to estimate gene trees, the species tree and species delimitations simultaneously. In contrast to most species delimitation software, it does not require an input guide tree. Two important priors are introduced in STACEY: collapseHeight that regulates the node height and collapseWeight that regulates the number of clusters. If clusters are separated by node heights so small that they approach zero, these nodes are assumed as representing coalescent events. Because multispecies coalescent theory assumes that all individuals affected by the same coalescent event belong to the same species, individuals within such clusters are thought to represent a single species (Jones et al. 2015; Toprak et al. 2016).

Against this background, the aims of this thesis were to: (1) better resolve the phylogenetic relationships within the alpina–corium and alpestris–nannfeldtii evolutionary lineages of *Helvella*, using an extended set of genetic markers; (2) implement the newly developed software STACEY to delineate phylogenetic species of the complex; (3) re-evaluate morphological characters to be used in species discrimination; and (4) assess the distribution of these species in the Nordic countries, based on newly collected specimens and old fungarium collections.

2 Materials and methods

2.1 Sampling

This study is based on freshly collected specimens of the *Helvella corium* morphospecies complex from alpine areas of Norway, and dried specimens deposited in the Nordic University fungaria. All specimens under the name *H. corium* in the fungaria were sampled, barcoded and used for distribution mapping (see Table S1) of the individual species. A subset of this sample was chosen to infer phylogeny and to aid in molecular species delimitation (see Table 1).

2.1.1 Fieldwork collections

Fieldwork was conducted in primarily under-studied alpine regions of Norway and Svalbard during August 2015, 2016 and 2017 (Table 2). The localities were chosen according to known habitat preferences of the *Helvella* species in question (T. Schumacher, pers. comm.). The fieldwork provided in total 68 new collections thought to belong to the *Helvella corium* morphospecies complex. Ascocarps were collected and dried at 30°C for 24h. Upon drying, a small (sesame seed sized) piece of the stipe was put directly in CTAB - Lysis buffer *BioChemica* (Panreac AppliChem) for subsequent DNA extraction.

2.1.2 Fungarium collections

Samples were obtained from the following University fungaria: O, TRH, BG, TROM, S, UPS, GB, UME, and C. Specimens from fungaria in Finland and Iceland were requested, but not obtained. All fungarium specimens stored under the name *Helvella corium* (496 in total) were inspected and sampled for barcoding purposes (see Table 3 and Table S1). A small piece of the stipe of a dried specimen was cut off and put directly in CTAB Lysis buffer (AppliChem Panreac).

Table 1: Overview of *Helvella* specimens included in phylogenetic analyses of this study. Specimens included in the final analysis are marked with an asterix. All specimens with fungarial number “O-XXXXXX” are new fieldwork collections not yet deposited in a herbarium. Type specimens are written in bold. Shaded squares represent obtained sequences

Species	Sample ID, fungaria	Locality	Collection year	Sequences obtained					
				HSP	RPB2	TEF	LSU	ITS	
<i>H. corium</i>	H2184, O-XXXXXX*	Svalbard. Longyearbyen	2017						
	H547, O-XXXXXX*	France. Savoie	1992						
	H436, O-253281	Svalbard. Spitsbergen. Kongsfjorden	1988						
	H950, O-XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016						
	H956, O-XXXXXX	Norway. Hedmark. Folldal. Einunndalen	2016						
	H957, O-XXXXXX	Norway. Sør-Trøndelag. Oppdal. Vinstradalen	2016						
	H1958, O-XXXXXX	Norway. Troms. Salangen	2017						
	H248, O-253277	Norway. Hordaland. Ulvik. Finse	1996						
	H1088, C-F-86904	Greenland. Thule Airbase	1994						
	H1089, C-F-63828	Greenland. Mestervig. Ochenpas	1968						
	H294, C-F-16568	Russia. North Ural mountains	1990						
	<i>H. alpina</i>	H2106, O-XXXXXX*	Norway. Oppland. Dovre. Grimdalen	2017					
		H1124, C-F-55730*	Greenland	1987					
H223, O-253228*		France. Savoie	1992						
H336, O-253227		Canada. British Colombia. Whistler	1994						
H1159, C-F-54601		Norway. Nordland. Rana. Virvassdalen	1979						
H540, C-F-34420		Russia. Khatanga airport	1993						
H1095, C-F-50287		Greenland. S Strømfjord	1982						
<i>H. pseudoalpina</i>	H498, O-XXXXXX*	Svalbard. Longyearbyen	2015						
	H1965, O-XXXXXX*	Norway. Troms. Balsfjord	2017						
	H1966, O-XXXXXX*	Norway. Troms. Balsfjord	2017						
<i>H. alpicola</i>	H2278, TRH-F-20631	Norway. Nordland, Saltdal, Junkerdalen. Bibeldalen	1988						
	H231, O-253226*	Switzerland. Graubunden	1984						
	H952, O-XXXXXX*	Norway. Nordland. Saltdal. Junkerdalen	2016						

<i>H. macrosperma</i>	H029, O-253328*	Norway. Oppland. Dovre. Grimsdalen	2007					
	H047, O-253329*	Norway. Oppland. Dovre. Grimsdalen	2009					
	H1982, O-XXXXXX*	Norway. Troms. Målselv	2017					
	H050, O-253330*	Norway. Oppland. Dovre. Grimsdalen	2009					
	H053, O-253331*	Norway. Oppland. Dovre. Grimsdalen	2009					
	H1997, O-XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017					
<i>H. arctoalpina</i>	H033, O-253237*	Norway. Oppland. Dovre. Grimsdalen	2009					
<i>H. acetabulum</i>	H225, O-253212*	Norway. Oppland. Dovre. Grimsdalen	1984					
<i>H. subilica</i>	H148, O-70080*	Norway. Akershus. Asker	1994					
<i>H. rivularis</i>	H1978, O-XXXXXX*	Norway. Troms. Balsfjord	2017					
<i>H. fallax</i>	H018, O-253351*	Norway. Oppland. Dovre. Grimsdalen	2009					
<i>H. pezizoides</i>	H061, O-253366*	Sweden. Halmstad	2009					
<i>H. scyphoides</i>	H140, O-65348*	Norway. Hedmark. Åmot	2002					
<i>H. sp2</i>	H1983, O-XXXXXX*	Norway. Troms. Målselv	2017					
<i>H. macropus</i>	H238, O-291425*	Norway. Rogaland	2009					
<i>H. fibrosa</i>	H240, O-291352*	Norway. Sør-Trøndelag	2008					
<i>H. lacunosa</i>	H1041, O-XXXXXX*	Norway. Nordland. Saltdal. Junkerdalen	2016					
<i>H. atra</i>	H1055, O-XXXXXX*	Norway. Hedmark. Kvikne	2016					
<i>H. philonotis</i>	H2110, O-XXXXXX*	Norway. Oppland. Dovre. Grimsdalen	2017					
<i>H. calycina</i>	H022, O-253255*	Norway. Oppland. Dovre. Grimsdalen	2009					
<i>H. costifera</i>	H247, O-253283*	Norway. Oppland. Vågå	1998					
<i>H. crispa</i>	H235, O-360158	Norway. Nordland. Andøy	2005					
<i>H. hyperborea</i>	H1309, C-F-55004	Norway. Nordland. Rana	1981					
<i>H. hypocrateriformis</i>	H275, C-F-57126	Switzerland. Graubünden	1982					
<i>H. pulla</i>	H149, O-069282	Norway. Møre og Romsdal. Nesset	2008					
<i>H. bicolor</i>	H1033, O-XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016					
<i>H. capucina</i>	H1051, O-XXXXXX	Norway. Hedmark. Kvikne. Innerdalsvatnet	2016					

Table 2: Overview of localities where species of the *Helvella corium* complex were found during fieldwork August 2015-2017

Date	County	Locality
12.08.15		Svalbard, Longyearbyen
10.08.16	Nordland	Junker dalen, along E77
11.08.16	Nordland	Junker dalsura
14.08.16	Hedmark	Gløta, Einundalen
14.08.16	Sør-Trøndelag	Vinstradalen, Oppdal
15-16.08.16	Oppland	Grimsdalen
23.07.17		Svalbard, Longyearbyen
30.07.17	Hordaland	Finse
08.08.17	Nordland	Gratangen
08.08.17	Nordland	Laberget, Salangen
09.08.17	Troms	Finnsnes
12.08.17	Finnmark	Talvik, along E6
13.08.17	Troms	Kåfjord dalen, Guolasjárvi
14.08.17	Troms	Middagsnes, Balsfjord
16.08.17	Troms	Frø kentindskaret, Målselv
19.08.17	Troms	Iselvdalen, Målselv
20.08.17	Troms	Laksebukta, Balsfjord
23.08.17	Oppland	Grimsdalen

Table 3: Overview of *Helvella* specimens sequenced in this study. Number of barcodes refers to successfully sequenced specimens for the partial heat shock protein 90 (HSP)

Source	Number of specimens	Number of barcodes
O	97	91
TRH	17	17
BG	17	14
TROM	32	31
C	134	123
S	51	49
UPS	54	51
GBG	12	12
UME	9	8
Field	73	73
Total	496	469

2.2 Laboratory work

2.2.1 DNA extraction

Samples were processed prior to DNA extraction. One tungsten bead was added to each sample, i.e. specimens in CTAB Lysis buffer (AppliChem Panreac). Samples were first frozen, then incubated at 65 °C on heating block for 30 min, and subsequently vortexed thoroughly. Samples were frozen a second time, and allowed to thaw at room temperature before DNA extraction. DNA was extracted using E.Z.N.A.®HP Fungal DNA Kit (Omega Biotek D3195), following a slightly modified version of the manufacturer's protocol for dried samples. The optional step of adding 10 µL 2-mercaptoethanol was undertaken, as well as the protocol for column equilibration with NaOH. In the elution step, 50 µL elution buffer was used to increase DNA concentration.

2.2.2 Molecular markers and specimen selections

Efforts were made to PCR amplify the selected partial heat shock protein 90 (HSP) region for all sampled specimens. This marker was used for barcoding purposes.

A subset of 80 specimens, of which 21 represented outgroup taxa, were selected to infer multilocus phylogenies and delimit phylogenetic species of the *Helvella corium* morphospecies complex (see Table 1). Efforts were made to sample broadly, as for the dataset to represent as much genetic diversity as possible. Thus a few specimens from other arctic-alpine regions outside of the Nordic countries were included. For some species, it was difficult to sample broadly due to few recorded findings (i.e. *H. alpicola*, *H. macrosperma*). Five putatively unlinked loci, were targeted to infer multilocus phylogenies and to delineate phylogenetic species: a 272 bp region of the protein-coding gene HSP, a 356 bp region of the protein-coding gene RPB2, a 571 bp region of the protein-coding gene TEF, a 743 bp region of the large ribosomal subunit LSU (including the D1 and D2 regions), and the whole internal transcribed spacer region (ITS1, 5.8S, ITS2). Only the highly conserved 160 bp region of the 5.8S ribosomal RNA region was further used in the phylogenetic analyses. The protein-coding genes MCM7 and TSR1 were

targeted in initial studies, but the available universal primers failed to amplify these regions in *Helvella*. The primers used are shown in Table 4.

2.2.3 Polymerase Chain Reaction (PCR) and Sanger sequencing

For all PCR reactions, PuReTaq Ready-To-Go PCR Beads (GEhealthcare, Waukesha, WI) were used in 25 μ L reactions. The following PCR protocols were used to amplify the five loci: 4 min at 95 $^{\circ}$ C, 40 (50 for LSU) cycles of 25 sec (30 sec for HSP and LSU) at 95 $^{\circ}$ C, 30 sec at 53 $^{\circ}$ C (58 $^{\circ}$ C for HSP, 52 $^{\circ}$ C for LSU) and 60 sec at 72 $^{\circ}$ C, followed by a 10 min extension at 72 $^{\circ}$ C and an indefinite hold at 10 $^{\circ}$ C. Amplified PCR products were visualized with electrophoresis on 1% agarose gels. For PCR reactions that yielded product, 5 μ L PCR product was purified with 0.2 μ L ExoSAP-IT (GEhealthcare) and 1.8 μ L H₂O. Samples were then run on a thermocycler at 37 $^{\circ}$ C for 15 min, followed by 80 $^{\circ}$ C for 15 min. Cleaned PCR product was diluted with 45 μ L water per sample. 5 μ L PCR product and 5 μ L primer was added to clean tubes and labelled before sequencing. Sanger sequencing was performed by GATC Biotech (Constance, Germany).

Table 4: Overview of PCR and sequencing primers used to amplify the *Helvella corium* morphospecies complex and relevant outgroup taxa in this study

Locus ¹	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
HSP	H_hspf ⁴ : CRGGCATCCGGGTGACGTAAT	H_hspr ⁴ : AGGGKGTGTGCGACTCCGAGG
RPB2	H_rpb2r ² : TCCACAATCTGCATCCCGATTC	H_rpb2f ⁴ : CCAGACATGGACAGAAGGTTGAG
TEF	EF595F ³ : CGTGACTTCATCAAGAACATG	EF1160R ³ : CCGATCTTG TAGACGTCCTG
LSU	H_LSUf ¹ ² : AGCGGAGGAAAGAAACCAAC	H_LSUR ² : TCCCAACAGCTATGCTCCTAC
ITS	ITS5 ⁵ : GGAAGTAAAAGTCGTAACAAGG	ITS4 ⁵ : TCCTCCGCTTATTGATATGC
ITS2	ITS3 ⁵ : GCATCGATGAAGAACGCAGC	ITS4 ⁵ : TCCTCCGCTTATTGATATGC

¹ LSU: 28s large subunit ribosomal RNA, domains D1\D2; RPB2: RNA polymerase II; HSP: heat shock protein 90;

TEF: translation elongation factor 1-alpha; ITS: The internal transcribed spacer region (ITS1, 5.8S and ITS2).

² From Landeros *et al.* (2015), modified in Skrede *et al.* (2017).

³ From Kausserud & Schumacher (2001)

⁴ From Skrede *et al.* (2017)

⁵ From White *et al.* (1990)

2.3 Analyses

2.3.1 Assembly and alignment

Sequence assembly and editing was done using Geneious version 9.1.6 (<http://www.geneious.com>; (Kearse *et al.* 2012)). All sequences were manually inspected and edited.

Multiple sequence alignments were assembled and analysed, both with (a) and without (b) missing data: (1) an HSP alignment with 542 accessions representing all successfully barcoded specimens and reference sequences from Skrede *et al.* (2017). This alignment was mainly used to infer taxonomic placement of the samples; (2a) a subset of alignment 1 with 80 HSP accessions, and (2b) with 47 HSP accessions; (3a) a RPB2 alignment with 72 accessions, and (3b) with 47 RPB2 accessions; (4) a TEF alignment with 47 accessions; (5a) an LSU alignment with 73 accessions, and (5b) with 47 LSU accessions; (6) a 5.8S alignment with 29 accessions; and (7) a concatenation of alignments 2b, 3b, 4b, 5 and 6. This resulted in an alignment of 47 accessions, of which 15 represented outgroup taxa of *Helvella* species outside the lineages in question. The complete ITS region proved difficult to align across the whole species complex, thus ITS sequences from the alpina-corium lineage and the alpestris-nannfeldtii lineage were aligned separately (Figures S1 and S2).

Preliminary alignments were made using MAFFT version 7.309 (Katoh & Standley 2013) within Geneious 9.1.6, under default parameter settings. All alignments were inspected and manually adjusted when necessary.

2.3.2 Phylogenetic inference and species delimitation

Substitution models for each locus were determined based on the AICc model selection criterion (small-sample-size corrected version of Akaike information criterion) as implemented in PartitionFinder v. 1.1.1 (Lanfear *et al.* 2017). Search was set to “greedy” and branch lengths set to “linked”.

Molecular species delimitation was performed based on altogether 32 individuals of the *Helvella corium* species complex (plus 15 individuals representing outgroup taxa) using STACEY as implemented in Beast2 (Jones *et al.* 2015; Jones 2017). STACEY uses a multi-species coalescent approach (Rannala & Yang 2003; Degnan & Rosenberg 2009; Yang & Rannala 2010) to infer gene trees and the species tree simultaneously. It does not require an *a priori* assignment of individuals to species, nor guide trees. All possible species combinations were assessed, treating each individual as a hypothetical species (Heled & Drummond 2010; Jones *et al.* 2015). In the resulting maximum clade credibility tree, each cluster represents a putative species under the multispecies coalescent model.

Alignments 2-6 were used as input files for species tree analyses. Separate runs were done using alignments with (a) and without (b) missing data. Input xml files were prepared in BEAUti v2.4.7 and the corresponding substitution models were set according to results from PartitionFinder. Bayesian posterior probabilities of different species scenarios were estimated using a strict clock model for all loci. All partitions were kept unlinked. The following priors were adjusted: PopPriorScale was given a lognormal distribution with M=-7 and S=2, and the CollapseWeight a beta distribution with alpha=1 and beta=1. The collapse height was set to 0.00001. All other priors were accepted as the defaults in BEAUti. The analyses were run for 115 million generations and sampled at every 5000th. The output file was inspected in Tracer v1.6.0 (Rambaut *et al.* 2018) to ensure convergence of the MCMC chains (ESS values > 200). Output trees were processed in TreeAnnotator v2.4.7 (supplied with the BEAST package), where burnin was set to 10%, and a Maximum Clade Credibility tree was produced. The tree was displayed with FigTree v1.4.3 (tree.bio.ed.ac.uk/software/figtree/). The final visualization was done using iTOL (Letunic & Bork 2016) and edited in Adobe Photoshop.

Cluster analyses were performed using SpeciesDelimitationAnalyser (Jones *et al.* 2015), where burnin was set to 10%, collapseheight to 0.001 and simcutoff to 0.95. Visualization was done in RStudio (RStudio Team 2016) using

PlotSimMatrix (script provided in the supplementary information for DISSECT (Jones *et al.* 2015)).

In addition, maximum likelihood (ML) analyses were performed. Alignments 2-6 were analysed individually using RAxML 7.2.8 (Stamatakis 2006; 2014) as implemented in Geneious. Alignment 7 was used to infer multilocus maximum likelihood phylogeny. For these analyses, Rapid Bootstrapping and search for best-scoring ML tree algorithms were used. Bootstrap analyses were performed with 1000 pseudoreplicates.

2.3.3 Morphological examinations

Due to great morphological similarity within the group, definite species identification in the field proved to be challenging. Microanatomical examinations and morphological species delimitation were therefore carried out after molecular delimitation had been undertaken. For microanatomical description of the species, microscopical examination was carried out on a selection of four *Helvella corium*, three *H. alpina*, three *H. pseudoalpina*, four *H. alpestris*, four *H. macrosperma*, four *H. nannfeldtii*, and three *H. alpicola* specimens.

Slides were prepared as manually cut sections and squash preparations of rehydrated apothecia observed in distilled water and lactophenol cotton blue (LCB) media. Microanatomical characters of hymenium and receptacle, i.e. reproductive structures and hairs of the outer exipulum, were studied in greater detail. For each specimen, 10 replicates were made for all measurements and 20 ascospores were measured in each specimen.

In addition, macro- and microanatomy of freshly collected specimens collected in 2017 was studied in detail. Macromorphological traits of cup and stipe as well as ecological traits were noted on site during the fieldwork.

2.3.4 Distribution and ecology

Distribution maps were made using coordinate data for the specimens barcoded with HSP (see Table S1). An interactive map was created in R Studio (RStudio Team 2016) using the packages plotly (Sievert *et al.* 2017) and ©Mapbox. Static figures were drawn by hand using the output from the interactive map. The package maps (Becker *et al.* 2017) was used to draw height gradient maps of Norway.

Weidemann (1998) sequenced root tips of *Dryas octopetala* and *Salix reticulata* for the 5.8S and ITS2 regions. Some of the genotypes from *S. reticulata* (i.e. S1 and S2) were annotated *Helvella* sp., as they did not match any of the reference sequences of *Helvella* species that were available at the time. These sequences were examined and compared to the new ITS sequences obtained in this study. Alignment was done following the same procedure as described under 2.3.1.

3 Results

3.1 Sequence amplification

Of 469 obtained barcode sequences, 281 represented *Helvella corium*, 12 *H. alpina*, nine *H. pseudoalpina* sp nov., nine *H. alpicola*, 69 *H. nannfeldtii*, 37 *H. alpestris*, and five *H. macrosperma*. Forty-seven additional sequences deposited in the University fungaria as *Helvella corium* were shown to belong to *Helvella* species outside the two lineages in question. This, along with additional HSP sequences obtained from Skrede *et al.* (2017), became the basis of the species distribution mapping (see Table S1).

Amplicons were not produced for all individuals targeted for multilocus phylogenies. Of 80 initial individuals, 80 HSP, 72 RPB2, 47 TEF, 73 LSU and 29 5.8S sequences were obtained (see Table 1). The TEF and ITS regions proved especially difficult to amplify for old, dried fungarium material, possibly due to some degree of primer mismatch. The final phylogenetic analyses were therefore based on 47 specimens; those specimens where amplicons for HSP, RPB2, LSU and TEF sequences were successfully obtained. Twenty-nine 5.8S sequences were also included.

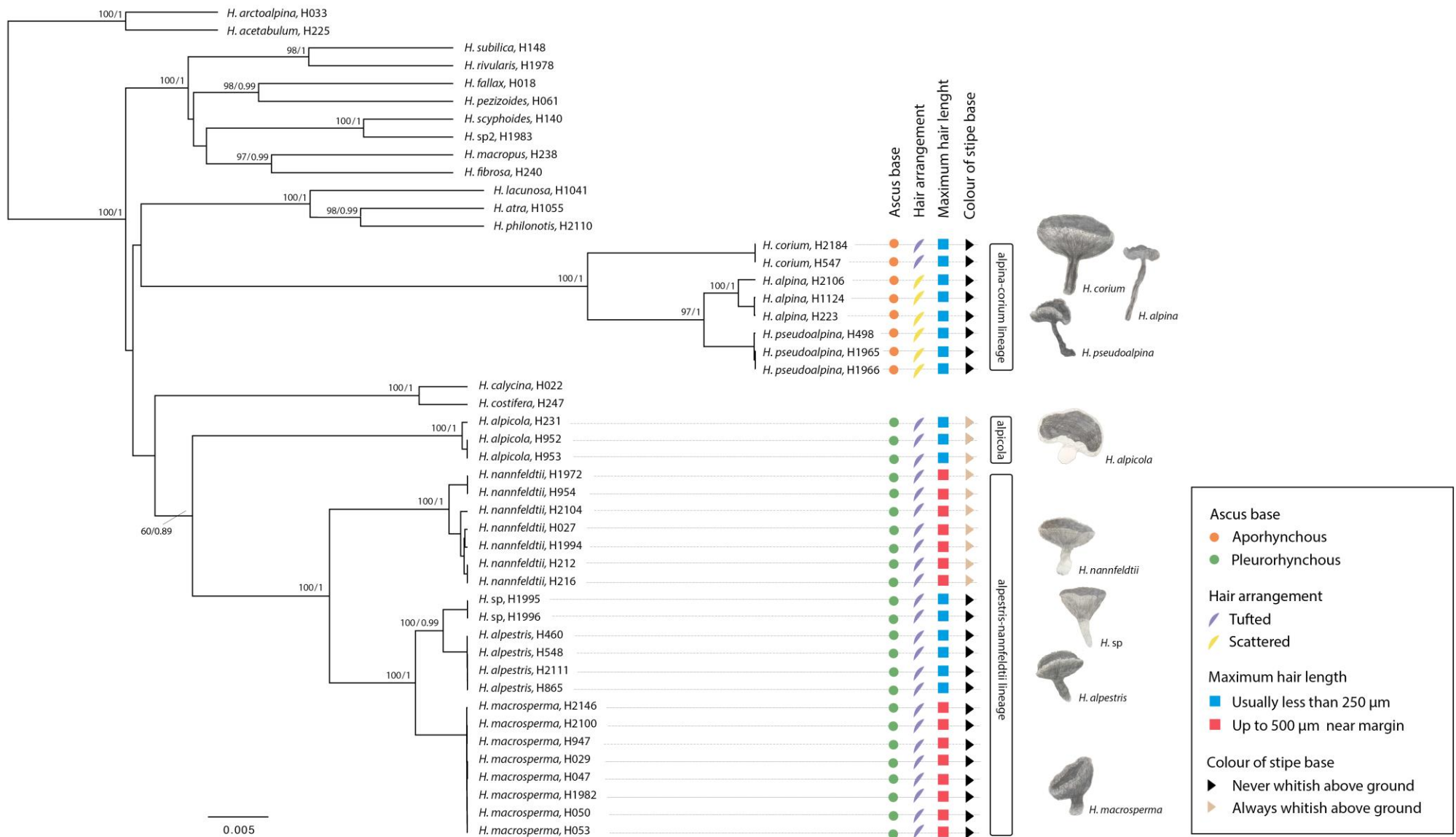


Figure 1: Maximum clade credibility tree of the *Helvella corium* morphospecies complex, along with outgroup taxa, resulting from the STACEY analysis (Beast2). The analysis is based on partial sequences of the heat shock protein 90 (HSP), the nuclear large ribosomal subunit (LSU, including D1–D2 domains), the large subunit of RNA polymerase II (RPB2), the translation elongation factor 1- α (TEF) and the complete 5.8S ribosomal RNA. RaxML maximum likelihood bootstrap values (added manually) > 60 and Bayesian posterior probability (BPP) > 0.8 are shown above nodes. Selected morphological character states are mapped on the tree, as explained in detail in the results section. The scale bar reflects the number of substitutions per site. Drawings: S. B. Løken

3.2 Phylogenetic inference and species delimitation

The STACEY run converged with ESS values > 200 for all parameters. The resulting maximum clade credibility tree (based on 21 600 sampled trees, 2 400 trees were discarded as burn-in) is shown in Figure 1. Bayesian posterior probabilities (BBP) are shown above branches. For simplicity, maximum likelihood RAxML bootstrap values (MLB) were also added to the tree, as the topologies resulting from the two multilocus analyses were congruent.

STACEY supported seven distinct clades within the *Helvella corium* morphospecies complex (see Figure 1), distributed in three evolutionary divergent lineages: (1) the alpina-corium lineage (BBP 1.0, 1.0 MLB) with *H. corium*, *H. alpina* and *H. pseudoalpina* sp. nov; (2) the alpestris-nannfeldtii lineage (BBP 1.0, 1.0 MLB) with *H. alpestris*, *H. macrosperma* and *H. nannfeldtii*; and (3) *H. alpicola*. *Helvella pseudoalpina* is strongly supported as sister to *H. alpina* (1.0 BPP, 1.0 MLB), and *H. corium* as sister to this clade (1.0 BPP, 1.0 MLB). *Helvella alpestris* and *H. macrosperma* are supported as sister species (1.0 BPP, 1.0 MLB), and *H. nannfeldtii* is placed as sister to this clade (1.0 BPP, 1.0 MLB). The two specimens denoted “*H. sp*” is separated as a distinct clade from *H. alpestris* (1.0 BPP, 0.99 MLB), yet with a short branch length. *Helvella alpicola* was recovered as sister to the alpestris-nannfeldtii lineage with moderate support (BBP 0.89, 0.60 MLB). The average distance between sister species varied from 0.005 to 0.015 substitutions per site in the species tree. According to this phylogeny, the *Helvella corium* morphospecies complex represents a paraphyletic group, although with low support for basal nodes (see Figure 1). Even though the final analysis was based on only 47 individuals (i.e. alignments 2b-6 with no missing data), a run based on the full phylogenetic dataset (i.e. alignment 2a-6 with missing data and 80 individuals) produced the same topology and delimitation of species.

For the seven clades in the *Helvella corium* morphospecies complex, Bayesian inference with STACEY and ML analyses showed a high degree of congruence. Resolution was poor outside these clades, and the majority of the basal nodes had very low posterior probabilities (as well as bootstrap support). Phylogenetic placement of the lineages within the genus *Helvella* was therefore difficult based

on the sampling done in this study. STACEY showed topological congruence between all gene trees. However, gene trees produced by the ML analysis showed incongruence regarding basal nodes, as well as regarding placement of specimens denoted "*H. sp.*". These are nested among *H. alpestris* in the ML gene tree for HSP, and among *H. macrosperma* in the ML gene tree for RPB2. For the other markers, maximum likelihood analyses placed it as sister to *H. alpestris*, as in the multilocus ML tree and in the STACEY output.

The cluster analysis performed with SpeciesDelimitationAnalyser produced a similarity matrix shown in Figure 2. This also illustrated that the species complex can be separated into seven distinct clusters with strong support within (red colour) and low support between (white colour). The individuals within the clusters have zero posterior probability of belonging to a different cluster, as indicated by the white colour separating clusters. The singletons in-between represent outgroup taxa of *Helvella* species outside the species complex in question. However, some clusters exhibit considerable intraspecific genetic structure. In *H. alpestris* there is a clear separation of the specimens denoted "*H. sp.*" This might represent yet another species within the *alpestris-nannfeldtii* lineage, but STACEY does not completely separate it from *H. alpestris*. This clade is so far only represented with the two collections included in this analysis, collected by the author in Northern Norway in 2017. *Helvella nannfeldtii* also exhibits extensive intraspecific variation, but the STACEY analysis still accepts all individuals as belonging to the same species.

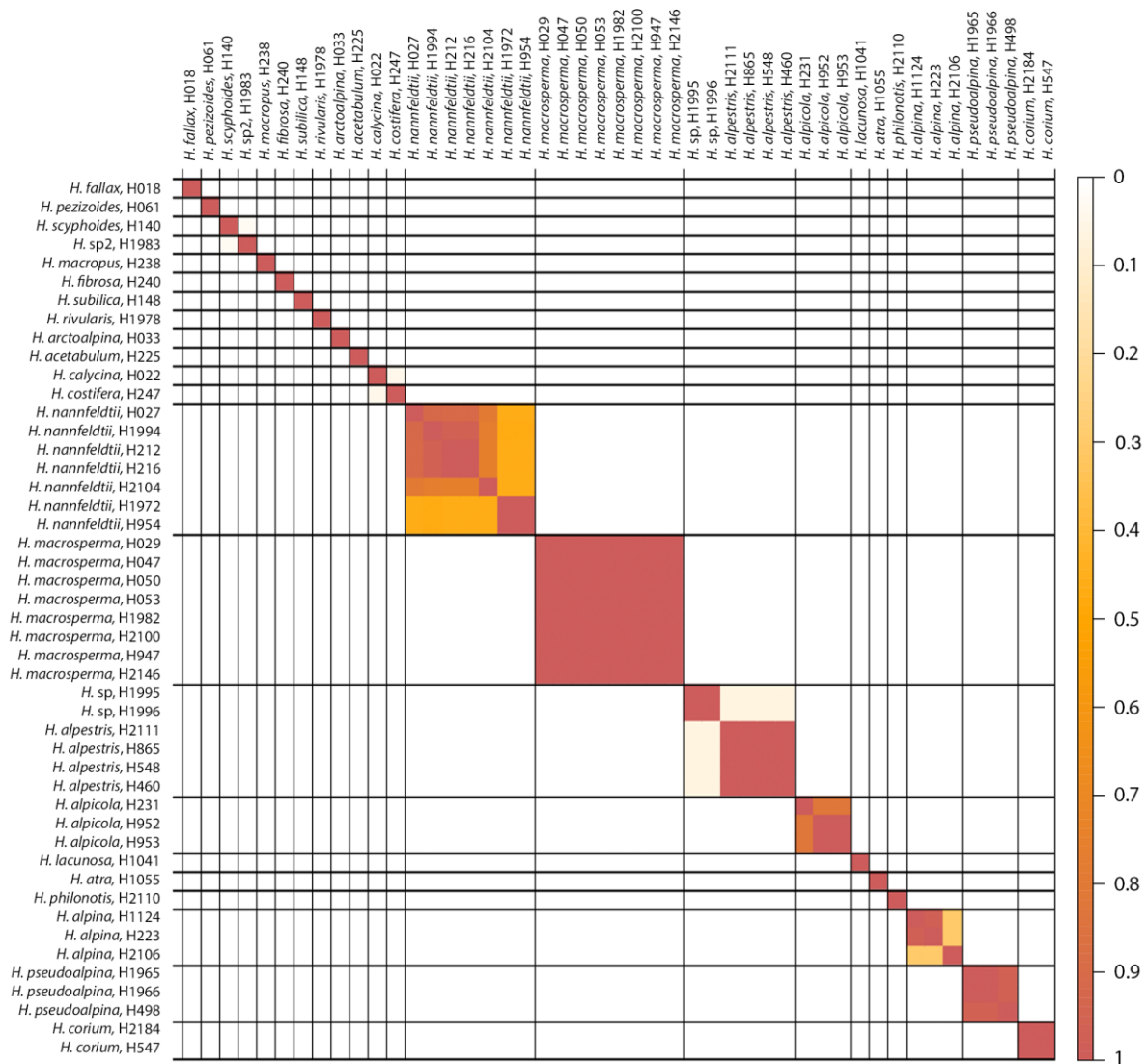


Figure 2: Similarity matrix showing Bayesian posterior probabilities (BPP) for pairs of individuals from the *Helvella corium* morphospecies complex and outgroup taxa belonging to the same cluster from the STACEY analysis. Red means 1.0 BPP, white means 0.0 BPP. For this analysis, collapseheight was set to 0.001 and simcutoff to 0.95.

3.3 Molecular markers

For the protein-coding loci (HSP, RPB2 and TEF), very low levels of intraspecific genetic variation were observed. The three markers provided good phylogenetic signal for species level delimitation. The LSU region clearly separated six of seven species, but the marker displayed slightly more intraspecific genetic variation than the protein-coding loci.

Complete ITS sequences (ITS1, 5.8S and ITS2) from six out of seven species of the *Helvella corium* morphospecies complex are provided in this study (only ITS2 and parts of 5.8S were successfully obtained from *H. alpicola*). Between lineages, the ITS region is very divergent, and objective alignment of the whole region across the morphospecies complex was not achievable. Unexpectedly, the 5.8S gene provided valuable phylogenetic signal in this species complex. The 5.8S gene, excluding the ITS1 and ITS2 regions, was thus included in the phylogenetic analyses. A table showing pairwise similarity in % identity across the ITS1 and ITS2 regions is presented in Table 5. ITS-alignments of the alpina-corium- and the alpestris-nannfeldtii lineages separately, are presented in Tables S1 and S2 (supplementary).

High levels of intraspecific genetic variation were found in ribosomal regions (LSU and ITS) of *H. nannfeldtii*. Two distinct genetic groups were identified in the LSU region (H1972 and H954 vs. all other individuals of *H. nannfeldtii* included in the phylogeny), with a considerable number of substitutions separating them. Genetic variation was also found within these groups. These genetic groups of LSU partly correspond to the genetic groups found in the ITS region for *H. nannfeldtii*. Between two and 43 substitutions in the LSU region separate specimens of *H. nannfeldtii* that are included in this study. For comparison, *H. corium* and *H. alpina* are separated by 25 LSU substitutions, and *H. macrosperma* and *H. alpestris* by seven LSU substitutions. Little or no intraspecific variation was found in the LSU and ITS regions of *H. corium*, *H. alpina*, *H. pseudoalpina*, *H. alpicola*, *H. alpestris* and *H. macrosperma*.

Table 5: Pairwise % identity in the ITS-region (ITS1/ITS2) between species of the *Helvella corium* morphospecies complex. Individuals of *H. nannfeldtii* used in this table represent one of the genetic groupings within the species. The "NA" means no ITS1 sequences were obtained for *H. alpicola*

	<i>H. corium</i>	<i>H. alpina</i>	<i>H. pseudoalpina</i>	<i>H. alpicola</i>	<i>H. nannfeldtii</i>	<i>H. alpestris</i>	<i>H. macrosperma</i>
<i>H. corium</i>	100/100	86/81-84	85/80-82	NA/67	57-60/61	61-63/68-71	59-62/65-71
<i>H. alpina</i>	86/81-84	96-100/ 96-100	91-92/92-93	NA/61-64	59-60/56-60	62-63/68	60-62/65-69
<i>H. pseudoalpina</i>	85/80-82	91-92/92-93	100/100	NA/63	58/58-61	60/68-71	60/67-71
<i>H. alpicola</i>	NA/67	NA/61-64	NA/63	NA/100	NA/76	NA/83-85	NA/78-84
<i>H. nannfeldtii</i>	57-60/ 61	59-60/56-60	58/58-61	NA/76	100/100	81-82/83	80-81/81-85
<i>H. alpestris</i>	61-63/68-71	62-63/67-69	60/68-71	NA/83-85	81-82/82-84	100/100	94/97-98
<i>H. macrosperma</i>	59-62/65-71	60-62/65-69	60/67-71	NA/78-84	80-81/81-85	94/97-98	100/100

3.4 Morphology and taxonomy

Morphological characters are found to correspond to the clusterings resulting from STACEY (Figure 1 and 2). The seven clusters are therefore acknowledged as species. No morphology was found to correspond to the genetic groupings of *H. nannfeldtii*. The two specimens (*H. sp*) that are separated from *H. alpestris* show some degree of macro-morphological differentiation, but the sampled material is too insufficient to described it as a new species to science at this point.

In the following, a synoptic key to the discerned phylogenetic species of the *Helvella corium* morphospecies complex is presented, as well as emended taxonomic descriptions of the individual species. The species are presented in the following order: The alpina-corium lineage, *H. alpicola* and the alpestris-nannfeldtii lineage.

3.4.1 Synoptic key

1. *H. corium*
2. *H. alpina*
3. *H. pseudoalpina* spec. nov
4. *H. alpicola*
5. *H. nannfeldtii*
6. *H. alpestris*
7. *H. macrosperma*

Apothecium

- a. Receptacle and hymenium blackish to black all over **1, 2, 3, 5, 6, 7**
- b. Receptacle brownish, hymenium blackish **4**
- c. Cup up to 3.5cm in diameter **1**
- d. Cup always less than 3.5cm in diameter **2, 3, 4, 5, 6, 7**
- e. Receptacle surface little to moderately pubescent, with scattered, short-celled, hyphoid hairs **2, 3**
- f. Receptacle moderately pubescent, with tufts of hyphoid hairs usually less than 250µm in length **1, 4, 6**
- g. Heavily pubescent, with tufts of hyphoid hairs usually more than 250µm in length; at margin approaching 500µm **5, 7**

Stipe

- a. Slender, length more than two times longer than apothecium width **2, 3**
- b. Thick, length equal to or less than apothecium width **1, 4, 5, 6, 7**
- c. Brown-blackish above, whitish below **4, 5**
- d. Black above, whitish only below ground **1, 6, 2, 3, 7**
- e. Terete (cylindrical) without grooves **4, 5, 6, 7**
- f. +/- Grooved **1, 2, 3**

Ascus type

- a. Aporhynchous **1, 2, 3**
- b. Pleurorhynchous **4, 5, 6, 7**

Ascospores

- a. Predominantly more than 20µm long **1, 4, 6**
- b. Predominantly less than 20µm long **2, 3, 5, 7**
- c. With one large oil drop or empty **1, 3, 4, 5, 6, 7**
- d. With one large oil drop and several small drops towards the poles **2**

3.4.2 Descriptions of species



Figure 3: Photos of fresh (a-d, g) and dried (e-f) apothecia of the alpina-corium lineage and *H. alpicola*. a. *Helvella corium* b. *H. corium* (H2184); c. *H. corium* (H1958); d. *H. corium* (H1998); e. *H. alpina* (H336); f. *H. pseudoalpina* (H2278) g. *H. alpicola* (H552). Photos: b–e: S.B. Løken; a and g: T. Schumacher.

Helvella corium (O. Weberb.) Masseur. Brit. Fungus-Fl. 4: 463. 1895 — Fig. 3 a-d.

Emended description (from Nannfeldt 1937: 56, 60, as *H. arctica*).

Apothecium regularly to somewhat irregularly cupulate, stipitate, black all over, in arctic/alpine areas often with white crystals at margin, cup up to 3.5 cm across. Stipe black all over, never whitish above ground, predominantly with longitudinal grooves that never continue onto receptacle. Receptacle surface densely pubescent with brown-walled

hyphoid hairs forming hyphal fascicles 150-360 µm long, gradually increasing in length towards margin where they form distinct triangular tufts, individual hair cells up to 21 µm broad. Asci aporhynchous, 210-340 x 11.3-18.8 µm. Ascospores ellipsoid, with one large oil droplet or empty, 16.3-22.5 x 10-13.8 µm. Paraphyses brownish along the whole length, gradually increasing in pigmentation towards tip, 2.5 µm broad below, gradually enlarged to 3.8-7.0 µm at the subcapitate tips.

Specimens used for macro- and microanatomical examinations: FRANCE, Savoie. T. Schumacher [H547] (O-). –SWEDEN, Torne Lappmark, Jukkasjärvi, E of Abiskoajok. Aug. to Sept. 1928, J.A. Nannfeldt [H292] (C-F-92111-Fung. Exs. Suec. 369 isotype of *H. arctica* Nannf.). –NORWAY, Troms, Salangen. 8 August 2017, S. B. Løken & T. Schumacher [H1958] (O-); Finnmark, Alta, Talvik. 12 August 2017, S. B. Løken & T. Schumacher [H1970] (O-); Troms, Balsfjord, Lakselvbukt. 20 August 2017, S. B. Løken & T. Schumacher [H1998, H1999, H2101] (O-, O-, O-); Nordland, Saltdal, Junkerdalen. August 2016, S. B. Løken & T. Schumacher [H950] (O-); Hedmark, Folldal, Einunndalen. August 2016, S. B. Løken & T. Schumacher [H955] (O-); Sør-Trøndelag, Oppdal, Vinstradalen. August 2016, S. B. Løken & T. Schumacher [H957] (O-). –SVALBARD, Longyeardalen, Longyearbyen. 20 July 2017, S. B. Løken & B. A. Granbo [H2184] (O-).

Notes – *Helvella corium* is by far the most common species of this morphospecies complex and is also the only species that thrives in temperate as well as boreal and arctic/alpine biomes. *Helvella corium* forms a well-supported lineage with *H. alpina* and *H. pseudoalpina* spec. nov. Eleven HSP, 10 RPB2, 18 TEF and 25 LSU substitutions separate *H. corium* and *H. alpina*. Ten HSP, nine RPB2, 16 TEF and 26 LSU substitutions separate *H. corium* and *H. pseudoalpina*.

Helvella alpina Skrede, T. Carlsen & T. Schumach. Persoonia. 39: 19. 2017 — Fig. 3 e.

Emended description (from Skrede *et al.* 2017: 19)

Apothecia regularly cupulate, stipitate, black all over, occasionally with white crystals at margin, cup 0.8– 2.0 cm across. Stipe slender, solid to hollow, black all over, never whitish above ground, occasionally with a few longitudinal grooves, 0.2–0.3 cm thick, 1.0–3.5 cm long. Medullary excipulum of textura intricata, hyphae 2–5 µm broad, hyaline. Outer excipulum of textura angularis, cells 10–25 µm diam, intermixed with subhyaline to brown-walled hyphae, turned perpendicularly to receptacle surface. Receptacle surface subpubescent with scattered, brown-walled hyphoid hairs, not tufted, 60-200 (occasionally up to 350) µm long, individual cells ovoid to subglobose, up to 20 µm broad.

Asci aporhynchous, 230-340 x 12,5-15 µm. Ascospores ellipsoid, with one large oil droplet and several smaller ones towards poles, 16,3-21,3 x 10-13,8 µm. Paraphyses 2.0–2.8 µm broad below, septate, brownish along the whole length, gradually enlarged to 4.0–6.5 µm at the subcapitate tips.

Specimens used for macro- and microanatomical examinations: CANADA, British Columbia, Whistler National Park. 13 August 1994, T. Schumacher [H336] (O-253227). – FRANCE, Savoie, Plan des Evettes. 26 August 1992, T. Schumacher [H223] (O-253226 holotype). – RUSSIA, Khatanga Air Port. H. F. Gøtzsche [H540] (C-F-34420). – SWEDEN, Torne Lappmark, Jukkasjärvi. 1945, G. Degelius [H711] (UPS-F-145392). – NORWAY, Nordland, Ballangen, Langvatn. 8 August 1970, O. Skifte [H921] (TROM-F-41055); Oppland, Dovre, Grimsdalen. 23 August 2017 [H2106] (O-); Troms, Tromsø, ca. 2 km W of Breivikeidet. 29 August 1954, F.E. Eckblad [H2162] (O-F-174772).

Notes – *Helvella alpina* is sister species to *H. pseudoalpina* spec. nov., from which it diverges in one substitution in HSP, one substitution in RPB2, six substitutions in TEF and 10 substitutions in LSU.

Helvella pseudoalpina S. B. Løken, Skrede & T. Schumach. spec. nov. — MycoBank MBXXXXXX — Fig. 3 f.

Etymology: From Greek ‘false’ and Latin ‘occurring in mountainous regions’, referring to morphological resemblance to *H. alpina*.

Holotype: Norway, Nordland, Saltdal, Junkerdalen, Bibeldalen. 28 August 1988, L. Ryvar den [H2278] (TRH-F-20631)

Apothecia regularly cupulate, stipitate, black all over, never whitish above ground, cup 0.8–1.5 cm across. Stipe slender, solid, black all over, occasionally with a few longitudinal grooves, 0.2–0.3 cm thick, 1.0–3.5 cm long. Receptacle surface subpubescent with scattered, brown-walled hyphoid hairs, not tufted, 90-320 (occasionally up to 350) µm long, individual cells 12.5-30 x 7.5-22.5 µm. Asci aporhynchous, 250-330 x 12.5-17.5 µm. Ascospores ellipsoid, with one large, round oil droplet, 15-21.3 x 10-13.8 µm. Paraphyses 2.5 µm broad below, septate, light brownish along the whole length, gradually enlarged to 5.0–8.8 µm at the subcapitate tips.

Specimens used for macro- and microanatomical examinations: GREENLAND, Qeqertarsuaq (Godhavn). 11 August 1977, P.M. Petersen [H349] (C-F-63820). – NORWAY, Troms, Tromsø, Tromsdalen. 27 August 1961, O. Skifte [H941, H942, H943] (TROM-F-11412, TROM-F-11405,

TROM-F-11404); Troms, Balsfjord, Mestervik. 9 August 2017, S.B. Løken & T. Schumacher [H1965, H1966, H1967] (O-, O-, O-); Nordland, Saltdal, Junkerdalen, Bibeldalen. 28 August 1988, L. Ryvarden [H2278] (TRH-F-20631 holotype). – SVALBARD, Longyeardalen, Longyearbyen. August 2015, S. Svantesson [H498] (O-).

Notes – *Helvella pseudoalpina* is sister species to *H. alpina* from which it diverges in one substitution in HSP, one substitution in RPB2 six substitutions in TEF and 10 substitutions in LSU.

Helvella alpicola Skrede, T. Carlsen & T. Schumacher. *Persoonia*. 39: 19. 2017 — Fig. 3 g.
Emended description (from Skrede *et al.* 2017: 19)

Apothecium cupulate, stipitate, receptacle dark greyish, hymenium greyish black, cup 0.5–1.5 cm broad. Stipe terete, greyish to whitish below, 0.2–0.3 cm broad, 0.5–1.8 cm high, with 2–3 shallow grooves at base. Receptacle surface densely pubescent with multiseptate, subhyaline, hyphoid hairs forming hyphal fascicles near margin, 60–350 µm long, individual hair cells 10–40 x 7.5–25 µm with conspicuous brown pigments at septa. Medullary excipulum of loose textura intricata, hyphae 2–5 µm broad, hyaline. Outer excipulum of textura angularis, cells 10–27 µm diam. Asci pleurorhynchous, 250–400 x 12.5–18 µm. Ascospores ellipsoid, empty, 18–22.5 x 11–15 µm. Paraphyses 2.5–3.8 µm below, light brownish, septate, gradually enlarged to 5–10 µm at the clavate tips.

Specimens used for macro- and microanatomical examination: SWITZERLAND, Graubünden, Inn at Resgia. 26 August 1984, H. Dissing [H231] (O-253226). – NORWAY, Nordland, Saltdal, Junkerdalsura. 27 August 1988, A.E. Torkelsen [H175] (O-185924 holotype); Nordland, Saltdal, Junkerdalsura. 11 August 2016, S. B Løken & T. Schumacher [H552, H952, H953] (O-, O-, O-).

Notes – Based on molecular data, *H. alpicola* represents a divergent sister lineage to the *alpestris-nannfeldtii* lineage. It diverges from *H. nannfeldtii* in four substitution in HSP, 11 substitution in RPB2, 12 substitutions in TEF and 52 substitutions in LSU.



Figure 4: Photos of fresh apothecia from the alpestris-nannfeldtii lineage. a. *Helvella nannfeldtii* (H1971) b. *H. nannfeldtii* (H2104); c. *H. nannfeldtii* (H2104); d. *H. alpestris* (H2111); e. *H. alpestris* (H2102); f. *H. alpestris* (H2111) g. *H. macrosperma* (H1982) h. *H. macrosperma* (H2100) i. *H. sp.* (H1996). Photos: a–e: S.B. Løken.

Helvella nannfeldtii Skrede, T. Carlsen & T. Schumach Persoonia. 39: 33. 2017 — Fig. 4 a-c.

Emended description (from Skrede *et al.* 2017: 33)

Apothecium cupulate, short-stipitate, receptacle very dark brown to black, hymenium black, rarely with white crystals at margin, 1–3 cm broad, 1–3 cm high. Stipe terete, solid, greyish black above, always whitish below also above ground, 0.2–0.3 cm broad, 0.5–2 cm

long. Receptacle surface pubescent with subhyaline to light brown-walled hyphoid hairs forming hyphal fascicles 190-500 µm long, gradually increasing in length towards margin forming distinct triangular tufts, individual hair irregular in shape and generally much constricted at septa. Medullary excipulum of loosely interwoven textura intricata, hyphae 3–5 µm broad. Outermost excipulum of textura angularis, cells dark brown, thick-walled, 15–30 µm diam, intermixed with broad short-segmented brownish hyphae forming a textura intricata. Asci pleurorhynchous, 250-340 x 13.75-20 µm. Ascospores ellipsoid, with one large oil droplet or empty, 15-22.5 x 10-15 µm. Paraphyses dark brown along upper two thirds, gradually increasing in pigmentation towards tip, 2.5-3.8 µm broad below, septate, gradually enlarged to 5-8.8 µm at the subcapitate tips.

Specimens used for macro- and microanatomical examinations: FRANCE, Savoie, Val d'Isere, Gorges du Mal. 31 August 1992, T. Schumacher [H212] (O-253332); Savoie, Bon Valle, Sur Arc. 2 September 1992, T. Schumacher [H216] (O-253333). – NORWAY, Oppland, Dovre, Grimsdalen, Veslegrimsa. 8 August 2009, T. Carlsen, T. Schumacher & I. Skrede [H027] (O-253338 holotype); Hedmark, Follidal, Einunndalen. August 2016, S.B. Løken & T. Schumacher [H954] (O-); Troms, Kåfjord, Guolasjärvi. 13 August 2017, S.B. Løken & T. Schumacher [H1971, H1972] (O-, O-); Troms, Målselv, Frøkentindelva. 16 August 2017, S.B. Løken & T. Schumacher [H1984, H1985] (O-, O-); Troms, Målselv, Iselvdalen. 19 August 2017, S.B. Løken & T. Schumacher [H1991, H1992, H1993, H1994] (O-, O-, O-, O-); Oppland, Dovre, Grimsdalen. 23 August 2017, S.B. Løken & T. Schumacher [H2103, H2104] (O-, O-).

Notes – *Helvella nannfeldtii* forms a well supported lineage with *H. alpestris* and *H. macrosperma*. Four HSP, two RPB2, seven TEF and 47 LSU substitutions separate *H. nannfeldtii* from *H. alpestris*. Three HSP, one RPB2, five TEF and 43 LSU substitutions separate *H. nannfeldtii* from *H. macrosperma*.

Helvella alpestris Boud., Bull. Soc. Bot. France 41: CCXL. 1894 — Fig. 4 d-f.

Emended description (from Skrede *et al.* 2017: 17, 18)

Apothecium cupulate to discoid, stipitate, black all over, predominantly with white crystals at margin, cup 0.5-1.8 cm across. Stipe terete, solid, black all over, never whitish above ground, c. 0.2–0.4 cm broad, 0.4–1.5 cm high. Medullary excipulum of textura intricata, hyphae 2–5 µm broad. Outer excipulum of brown-walled globose to angular cells, 10–20 µm diam. Receptacle surface densely pubescent with dark brown-walled hyphoid hairs forming hyphal fascicles 50-380 µm long, gradually increasing in length

towards margin where they form distinct triangular tufts, individual hair cells 10-40 x 7.5-17.5 µm. Asci pleurorhynchous, 230-350 x 12-17.5 µm. Ascospores ellipsoid, with one large oil droplet or empty, 16-22.5 x 10-13.8 µm. Paraphyses brownish along the whole length, gradually increasing in pigmentation towards tip, septate, 2.5-3.75 µm broad below, gradually enlarged to 4.5-8.8 µm at the gnarled, clavate tips.

Specimens used for macro- and microanatomical examinations: CANADA, Nunavut, Kitikmeot Region, above Bloody Falls. 19 July 2014, J.M. Saarela, P.C. Sokoloff, R.D. Bull [H460] (O ex DAOM-574891). – FRANCE, Savoie, Val'dIsere. T. Schumacher [H548] (O-). – NORWAY, Troms, Tromsø, Tromsø museum. 10 July 1980, S. Sivertsen & O. Skifte [H928] (TROM-F-11403); Oppland, Dovre, Grimsdalen, Jegerhøi. 23 August 2017, S.B. Løken & T. Schumacher [H2111] (O-); Finnmark, Porsanger, Lakselv. 16 July 1961, F.E. Eckblad [H2154] (O-F-174783).

Notes – All examined specimens of *H. alpestris* are found on almost bare silt or slate grounds, in close proximity of *Salix reticulata*, as opposed to *H. corium* and *H. macrosperma* that can be found also on plant covered grounds. Based on molecular data, *H. alpestris* is sister species to *H. macrosperma* from which it is separated by one substitution in HSP, one substitution in RPB2, four substitutions in TEF and seven substitutions in LSU.

Helvella macrosperma (J. Favre) R. Fellner & Landa. Česka Mykol. 45 (1-2): 35. 1991 — Fig. 4 g-h.

Emended description (from Skrede *et al.* 2017: 32)

Apothecium regularly cupulate, short-stipitate, black all over, rarely with white crystals at margin, cup 0.5-2 cm diam, 0.5-1.5 cm high. Stipe terete, solid, black all over, never whitish above ground, 0.4-1.5 cm high, 0.2-0.4 mm broad. Receptacle and stipe surface densely pubescent with dark brown-walled hyphoid hairs forming hyphal fascicles 180-500 µm long, gradually increasing in length towards margin forming distinct triangular tufts all over; individual hair cells 10-40 x 10-20 µm, constricted at septa with conspicuous deposits of incrusting pigments on the interior of the cell walls. Medullary excipulum of loosely interwoven *textura intricata*, hyphae 3-5 µm broad, shortcelled; outer excipulum of brown-walled globose to angular cells, 10-20 µm diam, gradually arranged perpendicularly to the surface. Asci pleurorhynchous, 240-330 x 10-20 µm. Ascospores broadly ellipsoid, with one large oil droplet or empty, 17.5-22.5 x 11.3-13.8 µm. Paraphyses brownish along the whole length, gradually increasing in pigmentation

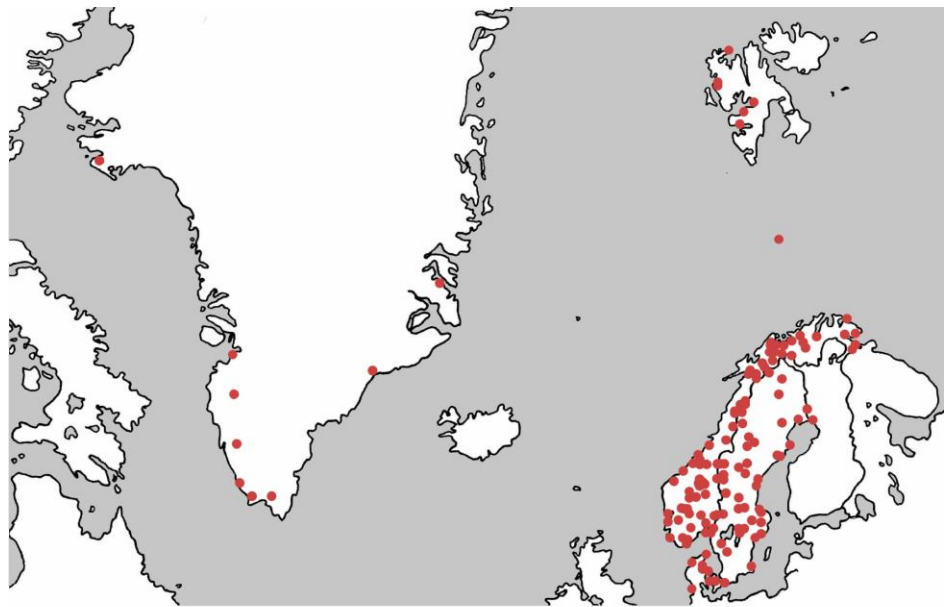
towards tip, septate, 2.5-3.75 μm broad below, gradually enlarged to 5-7.5 μm at the gnarled, clavate tips.

Specimens used for macro- and microanatomical examinations: NORWAY, Nordland, Saltdal, Junkerdalen. August 2016, S.B. Løken & T. Schumacher [H947] (O-); Troms, Målselv, Hååkåfjellet. 16 August 2017, S.B. Løken & T. Schumacher [H1982] (O-); Troms, Balsfjord, Lakselvbukt. 20 August 2017, S.B. Løken & T. Schumacher [H1997, H2100] (O-, O-); Oppland, Lom, Høyrokampen by Bøvertun. 31 August 2007, A.K. Wollan [H2146] (O-); Oppland, Dovre, Grimsdalen Veslegrimsa. 2007, Master Fieldcourse [H029] (O-).

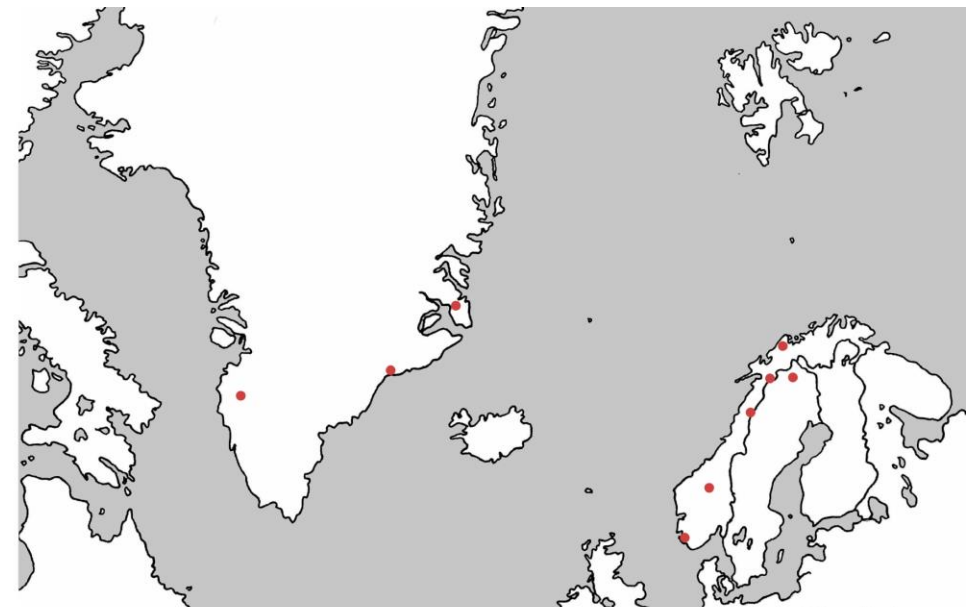
Notes –Based on molecular data, *H. macrosperma* is sister species to *H. alpestris* from which it diverges in one substitution in HSP, one substitution in RPB2, four substitutions in TEF and seven substitutions in LSU.

3.5 Distribution and ecology

Altogether, 469 specimens were successfully DNA barcoded for parts of the heat shock protein 90 (HSP). This material provided a decent basis for inference of species distributions of the *Helvella corium* complex in the Nordic countries. Species distribution maps are shown in Figure 5a and b. *Helvella corium* is found all over the Nordic countries and is the only species with a wide temporal, boreal and arctic-alpine distribution. The other six species seem restricted to the arctic-alpine biome: *Helvella alpina* is found in Northern Norway, Southern Norway, Sweden, Greenland, Canada, Russian Siberia, and the French Alps. *Helvella pseudoalpina* is here recorded from Northern Norway, Svalbard and Greenland. *Helvella alpicola* is so far recorded from Northern Norway and the Swiss Alps. *Helvella nannfeldtii* is found in Northern Norway, Southern Norway, Sweden, Iceland, Greenland, and the French and Austrian Alps. *Helvella alpestris* is here reported from Northern Norway, Southern Norway, Sweden, Greenland and Canada.



H. corium



H. alpina

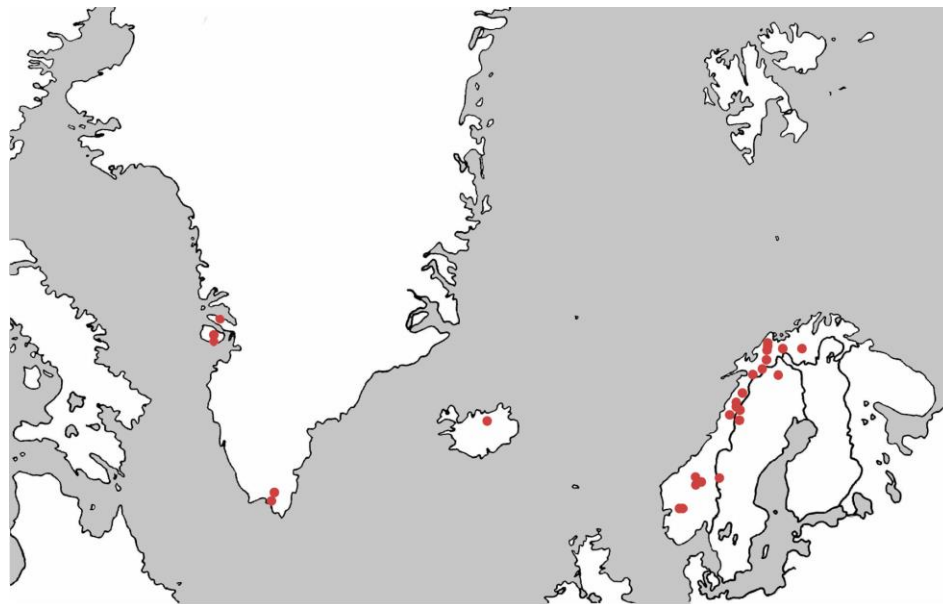


H. pseudoalpina

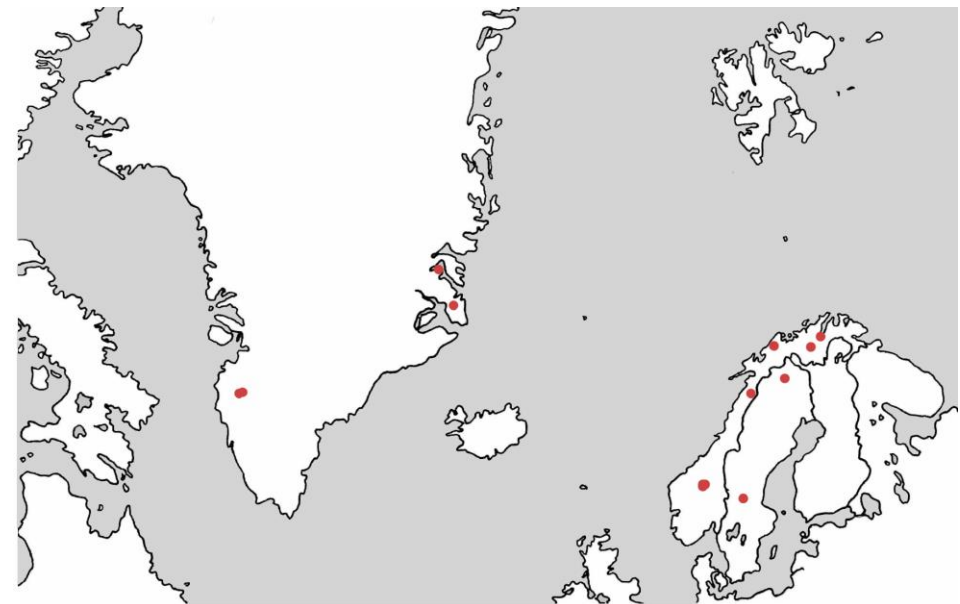


H. alpicola

Figure 5a: Species distribution in the Nordic countries of *Helvella corium*, *H. alpina*, *H. pseudoalpina* and *H. alpicola*. Distribution maps are based on coordinate data shown in Table S1 (supplementary).



H. nannfeldtii



H. alpestris



H. macrosperma



Helvella sp.

Figure 5b: Species distribution in the Nordic countries of *Helvella nannfeldtii*, *H. alpestris*, *H. macrosperma* and *Helvella* sp. Distribution maps are based on coordinate data shown in Table S1 (supplementary).

In this sample, *H. macrosperma* is only recorded from Norway, but has recently been reported from alpine areas of France, Italy, Switzerland and the Czech Republic as well (Van Vooren 2014; 2015). The consistent differences in distribution along an elevation gradient are illustrated by *H. corium* and *H. nannfeldtii* (see Figure 6).

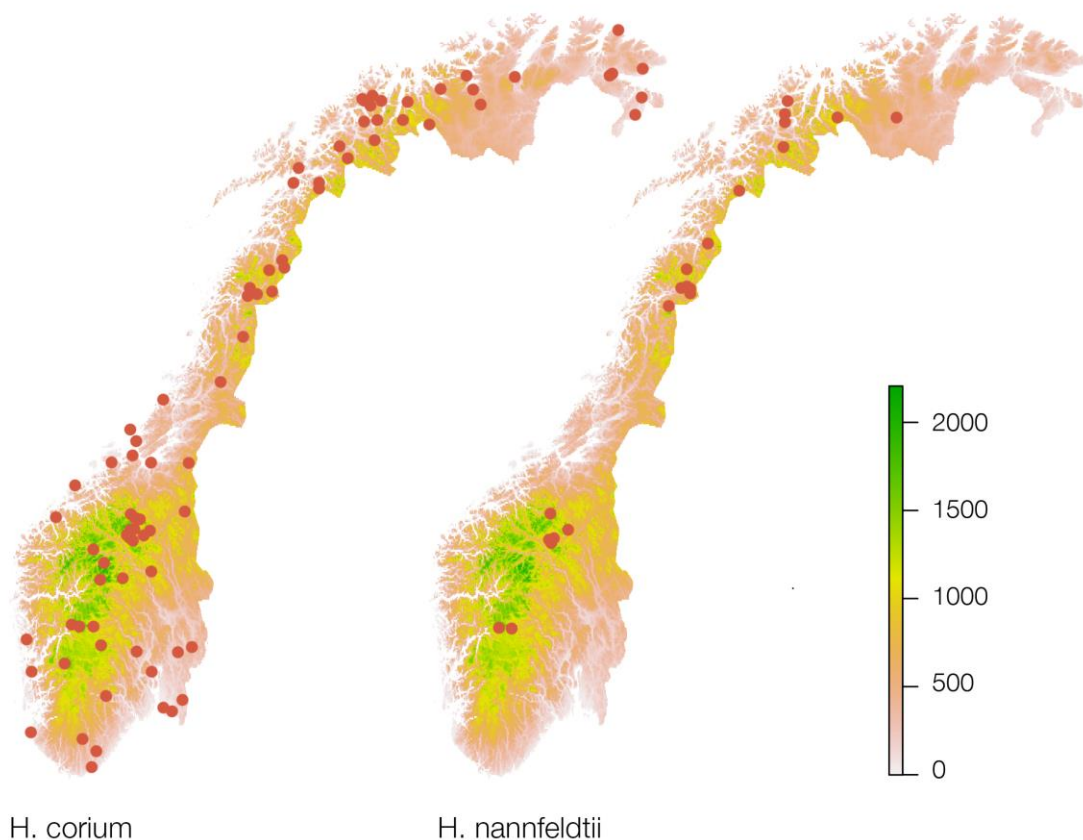


Figure 6: Distribution of *Helvella corium* and *H. nannfeldtii* along an elevation gradient (meter above sea level) in Norway. *Helvella corium* is found in alpine, as well as in boreal and temperate regions. *Helvella nannfeldtii* is restricted to the arctic-alpine biome (see also Figure 5b).

One of the genotypes for 5.8S and ITS2 (i.e. S1) found in root tips of *Salix reticulata*, as obtained from Weidemann (1998), proved identical to the genotype of *H. alpestris* produced in the present study.

4 Discussion

4.1 Molecular markers

Development of next generation sequencing techniques has made enormous amounts of genetic information available to infer molecular phylogenies (Lemmon & Lemmon 2013; Nagy & Szöllősi 2017). Still, Sanger sequencing of a few selected, phylogenetically highly informative loci are still preferred by most systematists when inferring phylogenies and delimiting species in fungi.

Indeed, a larger amount of sequence data is not necessarily associated with better phylogenetic signal and resolution (Lemmon & Lemmon 2013). Large, multilocus phylogenies based on phylogenomic data can include extensive non-phylogenetic signal and produce conflicting, yet highly supported, phylogenetic trees (Philippe *et al.* 2011). The number of phylogenetic markers necessary to infer reliable evolutionary histories has been a matter of debate (Rokas *et al.* 2003; Aguileta *et al.* 2008; Balasundaram *et al.* 2015). Moreover, recent studies point out that the choice of genetic markers is more important than the number of markers and their length (Aguileta *et al.* 2008; Balasundaram *et al.* 2015; Stielow *et al.* 2015). Relying on comparison of orthologous genes, as well as improved models of sequence evolution, is advised (Philippe *et al.* 2011).

In this study, five markers (HSP, RPB2, TEF, LSU and 5.8S) were enough to get a stable support for species and shallow clades, but not sufficient to infer topology of the deep branches of the tree (Figure 1). The high informativeness of single-copy protein-coding loci when delimiting species was confirmed by this study. Three protein-coding loci (HSP, RPB2 and TEF) known to perform well in species level delimitations in other ascomycete groups (Hansen *et al.* 2013; Stielow *et al.* 2015; Skrede *et al.* 2017), in addition to the LSU and 5.8S regions of the nuclear ribosomal DNA, were utilized. For improving support for basal nodes in the tree, one might have included sequence data from additional and highly conserved loci. An example is the SSU ribosomal RNA, which is proposed as a good marker for resolving deep branching patterns in Ascomycota (Berbee *et al.* 2000; Spatafora *et al.* 2006; Schoch *et al.* 2009). However, since priority was given to infer the

number of phylogenetic species as well as topology of shallow clades, this locus was not included in the present study. Including the MCM7 and TSR1 loci could also have been profitable, as these loci have shown to reveal higher-level relationships in other ascomycete genera (Aguileta *et al.* 2008; Raja *et al.* 2011; Zhao *et al.* 2015; Mark 2016). Unfortunately, in this study, universal MCM7 and TSR1 primers failed to amplify DNA in *Helvella* species, even after repeated changes in PCR conditions. Amplification also failed for specimens of *Helvella crispa*, even though Zhao *et al.* (2015) recently published MCM7 sequences from this species. It is assumed that design of specific *Helvella* primers is needed if these markers are to be used in further phylogenetic studies of *Helvella*. It would probably also be favourable to design *Helvella*-specific primers for TEF, as the amplification success of this locus is low in this genus.

The ribosomal loci, i.e. LSU and ITS, were informative in delimiting six of seven species in the *Helvella corium* morphospecies complex (Figure 2). *Helvella nannfeldtii* represented the seventh exceptional species that showed remarkable levels of divergence in these two ribosomal regions. Conflicting gene trees, as observed between individuals of *H. nannfeldtii* in LSU in this study, might result from intraspecific recombination. This implies that the two clusters of *H. nannfeldtii* are not reproductively isolated (Taylor *et al.* 2000). Based on this inconsistency, the divergent clusters in this species are not considered to represent two cryptic lineages. This is further demonstrated by the output of the STACEY analysis (see Figure 2), where all individuals of *H. nannfeldtii* are regarded as belonging to the same species.

Both the LSU and the ITS regions are present in numerous copies in fungal genomes, thereby forming gene families. Gene families are expected to be subject to concerted evolution (Arnheim *et al.* 1980; Strausbaugh 2001), where homogenization of paralogous genes is achieved through homologous recombination. However, exceptions do occur, resulting in heterogeneous copies of the same locus within a genome and apparent heterozygosity (Selosse *et al.* 2016). Substantial intragenomic heterozygosity of ribosomal DNA is thought to be infrequent in the fungal kingdom (Thiéry *et al.* 2016), but is observed (Stensrud

et al. 2007; Nilsson *et al.* 2008; Lindner *et al.* 2013; Harder *et al.* 2013). Thus, if the primers used in this study are amplifying paralogs of LSU and ITS in *H. nannfeldtii*, one might be comparing variable non-homologous sequences. As the same level of intraspecific variation is not found in any other species of the *Helvella corium* morphospecies complex, the ribosomal DNA of *H. nannfeldtii* is evidently evolving at a different rate compared to even its closest relatives. Thus, LSU and ITS sequences are not suitable for species-level delimitation in *H. nannfeldtii*, although they separate other species of the complex well. Moreover, the ITS region in *Helvella* shows extremely high interspecific variability (see Table 5) compared to related fungal genera (Olariaga *et al.* 2015; Taşkın *et al.* 2017). For this reason, it is difficult to construct objective alignments across lineages which makes ITS unsuitable as a phylogenetic marker across the genetically divergent *Helvella* genus (Landvik *et al.* 1999).

4.2 Phylogenetic inference and species delimitation

The STACEY analysis suggested that there are seven well-supported clusters in the *Helvella corium* morphospecies complex (Figure 2). STACEY may overestimate the number of clusters when a limited number of individuals and loci are used, as may be the case in this study (Toprak *et al.* 2016; Sukumaran & Knowles 2017). However, as morphological investigations also support this classification, we feel confident that the seven clusters represent well-defined species.

As STACEY is based on the multispecies coalescent model, it assumes random mating and that no gene flow occurs after speciation (Leaché *et al.* 2014; Xu & Yang 2016). Restricted gene flow between sister species will not have severe effects on the species tree topology (Heled & Drummond 2010; Toprak *et al.* 2016). However gene flow between non-sister species may lead to incongruence between gene trees and species trees, as hybrid individuals should cluster with different species in different gene trees (Taylor *et al.* 2000). This, if hybrid individuals are present in the dataset, there is a risk of obtaining inaccurate species tree topologies (Leaché & Fujita 2010). Because speciation seldom is instantaneous in nature, some violations to the model might have occurred in this dataset. In this study, the Bayesian inference shows no topological incongruence

across loci or between gene and species trees, but the ML analyses raises uncertainty regarding the placement of the possibly new, but unnamed species, *H. sp.* It is not fully understood how well STACEY handles the inclusion of hybrid individuals (Wagner *et al.* 2017). Still, if *H. sp.* should indeed represent hybrid individuals, it is a matter of hybridization between sister species (i.e. *H. alpestris* and *H. macrosperma*). As mentioned earlier, this would not have severe effect on the overall tree topology. The material used here is far too scarce to conclude on this matter.

4.3 Morphology and taxonomy

Using a molecular genealogical approach as basis for classification has allowed us to get a better understanding of the taxonomical value of different morphological characters in *Helvella*. Although several authors have recognized the morphological variation within *Helvella corium* (Boudier 1907; Nannfeldt 1937; Favre 1955; Dissing 1964; 1966a; b), accurate species delimitations have not existed before molecular data was introduced (Skrede *et al.* 2017).

In Dissing's (1966a; b) comprehensive review of *Helvella* in Europe, he proposed his new section Macropodes to include all *Helvella* species with cupulate apothecia, pubescent to villous receptacle, a distinct solid stipe with or without furrows, and furrows of the stipe (if present) not extending onto the receptacle. This included *Helvella corium*, as well as *Helvella macropus*, *H. villosa*, *H. cupuliformis*, and *H. queletii*. Yet, these macromorphological characters, as shown by Skrede *et al.* (2017), and further supported by this study, are not valuable for intrageneric classifications. Indeed, cupulate-stipitate species are found scattered across the genus.

This study of the alpina-corium and alpestris-nannfeldtii lineages of *Helvella* further elaborates on the morphological differentiation between the species. The species display considerable phenotypical plasticity, and definite species identification is still challenging without barcoding. However, some informative characters have been found to discriminate between them.

Ascus development, i.e. aporhynchous or pleurorhynchous asci (Chadefaud 1943; Berthet 1964), has been considered a valuable taxonomic character for defining sections of *Helvella* (Weber 1972). *Helvella corium* is recognized as having aporhynchous asci (Häffner 1987) and later *H. macrosperma* was found to have pleurorhynchous ascus development (Van Vooren 2014; 2015; Skrede *et al.* 2017). Indeed, aporhynchous asci represent a synapomorphy for the alpina-corium lineage. The alpestris-nannfeldtii lineage has pleurorhynchous asci, as is the most common form of ascus development in *Helvella*. Skrede *et al.* (2017) acknowledged the diagnostic value of the arrangement of hyphoid hairs of the receptacle in these lineages. This is confirmed in this study: hairs are scattered in *H. alpina* and *H. pseudoalpina*, and tufted in *H. corium*, *H. nannfeldtii*, *H. alpestris*, *H. macrosperma* and *H. alpicola*.

Additional +/- informative characters in species discrimination within the complex include: (1) colour of stipe basis (base always white above ground in *H. nannfeldtii* and *H. alpicola*); (2) hair length (hairs approaching 500µm near margin in *H. nannfeldtii* and *H. macrosperma*); (3) spore size (predominantly more than 20µm in *H. corium*, *H. alpicola* and *H. alpestris* vs. less than 20µm in *H. alpina*, *H. pseudoalpina*, *H. nannfeldtii* and *H. macrosperma*); (4) spore morphology (one large oil drop or none in *H. corium*, *H. pseudoalpina*, *H. alpicola*, *H. alpestris*, *H. macrosperma*, and *H. nannfeldtii* vs. one large oil drop and several smaller drops near the poles in *H. alpina*); and (5) paraphysis shape (clavate in the alpestris-nannfeldtii lineage vs. subcapitate in the alpina-corium lineage).

In describing *H. arctica*, Nannfeldt (1937) paid much attention to the external, white crystals observed on the marginal hairs of the receptacle. This was used as a discriminating morphological character against *H. corium*. Later, Dissing (1966b) concluded that this trait represented a mere adaptation to alpine environments, and synonymized *H. arctica* and *H. corium*. Based on a molecular approach, Skrede *et al.* (2017) also found good reasons to place *H. arctica* in synonymy with *H. corium*. The white crystalline deposits around the apothecial margin is observed in all species of the *Helvella corium* morphospecies complex under alpine conditions, but are most prominent in *H. alpestris* and in alpine

specimens of *H. corium*. Häffner (1987), and later Landeros *et al.* (2015), apparently misapplied *H. alpestris* sensu Boudier (1894; 1895) for *H. philonotis*

Even though the *Helvella corium* morphospecies complex represents a paraphyletic group, the species share numerous macromorphological traits. Evidently, considerable genetic differentiation has occurred independently of morphological differentiation among these evolutionary lineages. The phenotypical similarity might represent morphological stasis. It has been argued that morphological stasis can result from strong stabilizing selection for adaptation to harsh environments e.g. (Nevo 2001; Lumbsch & Leavitt 2011). In fact, many cryptic and pseudo-cryptic plant species are found in harsh alpine and Arctic regions (Grundt *et al.* 2006; Skrede *et al.* 2008; Brochmann & Brysting 2010). Similarly, strong morphological similarity in non-sister species might be due to strong adaptive value of certain morphological traits (Bickford *et al.* 2007). This might explain the pseudo-cryptic nature of the *Helvella corium* morphospecies complex.

4.4 Distribution and ecology

Evidently, *Helvella corium* has a broader distribution than the other species. *Helvella corium* is found in temperate, as well as boreal and arctic-alpine regions, and is by far the most common of the species (see Figure 5a). The other six species (*H. alpina*, *H. pseudoalpina*, *H. alpicola*, *H. alpestris*, *H. macrosperma* and *H. nannfeldtii*) are all restricted to the arctic-alpine biome where they occupy different, but overlapping habitats (see Figure 5 and 6). *Helvella nannfeldtii* seems to be the most common of the arctic-alpine species, and probably has a circumpolar distribution. The other arctic-alpine species (*H. alpina*, *H. pseudoalpina*, *H. alpicola*, *H. alpestris*, *H. macrosperma*) represent rare species, although they could be underrepresented in fungarium collections due to a lack of a broad sampling effort. Fungi with small ascocarps are difficult to find, and these species are found in rugged, alpine areas where few people venture.

Arctic-alpine species commonly have disjunct distributions in mountainous areas of the alpine zone, a zone that is stretching down towards sea level as one moves

above the Arctic Circle. This also seems to be true for most species of this complex. An explanation might be that they are adapted to harsh climates, or that the species have limited ability to disperse to the temperate zone due to e.g. competitive disadvantage e.g. (Birks 2008).

The overlapping species distributions further support the existence of some form of reproductive barriers, and thus the reality of the seven different species. It is important to note that the focus of this study was on the Nordic countries, and inferences of e.g. pan-Arctic distributions will require a broader sampling. Further investigations of fungal specimens outside of Scandinavia, as well as a broader sampling in the field, are necessary to get a more complete picture of these species' distributions.

The species of the *Helvella corium* morphospecies complex show a high degree of niche conservatism. They all favour calcareous soils, and are often found in close proximity of *Dryas octopetala* and/or *Salix* spp. No evidence has previously been provided regarding mycorrhizal associations of these species (Weidemann 1998). As cultivation of the species has not yet been achieved, clear evidence is hard to obtain from laboratory experiments. However, this study provides an additional finding; a presumable mycorrhizal habit of *H. alpestris* with *Salix reticulata*. This can be inferred as *H. alpestris* is found associated with ectomycorrhizal root tips of *S. reticulata*, following up the data from Weidemann (1998). Furthermore, because amplification of the ITS region has proven challenging with universal sequencing primers, root tips of candidate plant species could be sequenced with *Helvella*-specific HSP and/or RPB2 primers in order to settle the ecological niche of the other species of the complex.

This study demonstrates the suitability of a genealogical approach to interpret and understand patterns of biological diversity in fungi. It further illustrates the importance and implications of biosystematics in relation to other fields of biological sciences. With the data available at the time, Weidemann (1998) could not support the hypothesis that *Helvella corium* forms ectomycorrhizal associations with *Salix reticulata*. However now, 20 years later, the evolutionary

species limits of the *Helvella corium* morphospecies complex have been resolved. This led to the realization that a relative of *H. corium* (i.e. *H. alpestris*) was indeed represented in the dataset of Weidemann (1998) as an associate of ectomycorrhizal root tips of *S. reticulata*! This nicely illustrates that until we can adequately describe the species that actually exist in nature, we can neither understand the ecological roles nor the conservational needs of the pertinent species in natural ecosystems.

References

- Abbott SP, Currah RS (1997) The Helvellaceae: Systematic revision and occurrence in northern and northwestern North America. *62*, 1–125.
- Aguileta G, Marthey S, Chiapello H *et al.* (2008) Assessing the performance of single-copy genes for recovering robust phylogenies. *Systematic Biology*, **57**, 613–627.
- Arnheim N, Krystal M, Schmickel R *et al.* (1980) Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. *Proceedings of the National Academy of Sciences*, **77**, 7323–7327.
- Balasundaram SV, Engh IB, Skrede I, Kauserud H (2015) How many DNA markers are needed to reveal cryptic fungal species? *Fungal biology*, **119**, 940–945.
- Becker RA, Deckmyn A, Minka TP, Brownrigg R, Wilks A (2017) maps: Draw Geographical Maps. R package version 3.2.0. <https://CRAN.R-project.org/package=maps>.
- Berbee ML, Carmean DA, Winka K (2000) Ribosomal DNA and resolution of branching order among the ascomycota: how many nucleotides are enough? *Molecular Phylogenetics and Evolution*, **17**, 337–344.
- Berthet P (1964) *Essai biotaxinomique sur les Discomycètes*. Thesis, Lyon.
- Bickford D, Lohman DJ, Sodhi NS *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**, 148–155.
- Birks HH (2008) The Late-Quaternary history of arctic and alpine plants. *Plant Ecology & Diversity*, **1**, 135–146.
- Bouckaert R, Heled J, Kühnert D *et al.* (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **10**.
- Boudier J, Fischer ED (1894) Rapport sur les espèces de champignons trouvées pendant l'assemblée à Genève et les excursions faites en Valais, par les Sociétés de Botanique de France et de Suisse, du 5 au 15 Aout 1894. *Bulletin de la Société Botanique de France*, **41**, 7.
- Boudier J (1895). Description de quelques espèces récoltées en août 1894 dans les régions élevées des Alpes du Valais. *Bulletin de la Société Mycologique de France*, **11**, 27–30.
- Boudier J (1907) *Histoire et classification des Discomycètes d'Europe*. Librairie des Sciences Naturelles, Paris, France.
- Boudier J (1910) *Icones mycologicae ou iconographie des champignons de France*. Librairie des Sciences Naturelles, Paris, France.
- Brochmann C, Brysting AK (2010) The Arctic – an evolutionary freezer? *Plant Ecology & Diversity*, **1**, 181–195.
- Chadefaud M (1943) Sur les divers types d'éléments dangeardiens chez les Ascomycètes. *Revue Scientifique*, **81**, 77–80.
- Degnan JH, Rosenberg NA (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution*, **24**, 332–340.
- Dettman JR, Jacobson DJ, Taylor JW (2003) A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution*, **57**, 2703–2720.
- Dissing H (1964) Studies in Arctic and Subarctic Discomycetes. I. The genus *Helvella*. *Botanisk Tidsskrift*, **60**, 108–128.

- Dissing H (1966a) A revision of collections of the genus *Helvella* L. ex St-Amans emend. Nannf. in the Boudier Herbarium. *Revue de Mycologie*, **31**, 189–224.
- Dissing H (1966b) The genus *Helvella* in Europe with special emphasis on species found in Norden. *Dansk Botanisk Arkiv*, **25**, 1–172.
- Favre J (1955) Les champignons supérieurs de la zone alpine du Parc National Suisse. *Ergebnisse der wissenschaftlichen Untersuchungen des schweizerischen Nationalparks*, **33**, 1–112.
- Fries EM (1822) *Systema Mycologicum. Vol. 2 (1)*. Officina Berlingiana, Lund, Sweden.
- Fuckel L (1870) Symbolae mycologicae. Beiträge zur Kenntnis der rheinischen Pilze. In: *Jahrbucher des Nassauischen Vereins für Naturkunde* **23-24**, 1–459.
- Grundt HH, Kjølnner S, Borgen L, Rieseberg LH, Brochmann C (2006) High biological species diversity in the arctic flora. *Proceedings of the National Academy of Sciences*, **103**, 972–975.
- Hansen K, Olariaga I (2015) Species limits and relationships within *Otidea* inferred from multiple gene phylogenies. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, **35**, 148–165.
- Hansen K, Perry BA, Dranginis AW, Pfister DH (2013) A phylogeny of the highly diverse cup-fungus family Pyronemataceae (Pezizomycetes, Ascomycota) clarifies relationships and evolution of selected life history traits. *Molecular Phylogenetics and Evolution*, **67**, 311–335.
- Harder CB, Læssøe T, Frøslev TG *et al.* (2013) A three-gene phylogeny of the *Mycena pura* complex reveals 11 phylogenetic species and shows ITS to be unreliable for species identification. *Fungal biology*, **117**, 764–775.
- Harmaja H (1979) Studies on cupulate species of *Helvella*. *Karstenia*, **19**, 33–45.
- Häffner J (1987) Die Gattung *Helvella*, morphologie und taxonomie. *Beihefte zur Zeitschrift für Mykologie*, **7**, 1–165.
- Heled J, Drummond AJ (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.
- Hofstetter V, Miądlukowska J, Kauff F, Lutzoni F (2007) Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the Lecanoromycetes (Ascomycota). *Molecular Phylogenetics and Evolution*, **44**, 412–426.
- Huelsenbeck JP, Andolfatto P, Huelsenbeck ET (2011) Structurama: bayesian inference of population structure. *Evolutionary Bioinformatics Online*, **7**, 55–59.
- Jones G (2017) Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology*, **74**, 447–467.
- Jones G, Aydin Z, Oxelman B (2015) DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. *Bioinformatics*, **31**, 991–998.
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.
- Kausserud H, Schumacher T (2001) Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (Basidiomycota). *Mycological research*, **105**, 676–683.
- Kausserud H, Shalchian-Tabrizi K, Decock C (2007) Multilocus sequencing reveals

- multiple geographically structured lineages of *Coniophora arida* and *C. olivacea* (Boletales) in North America. *Mycologia*, **99**, 705–713.
- Kearse M, Moir R, Wilson A *et al.* (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.
- Knowlton N (1993) Sibling species in the sea. *Annual Review of Ecology and Systematics*, **24**, 189–216.
- Landeros F, Iturriaga T, Rodríguez A, Vargas-Amado G, Guzmán-Dávalos L (2015) Advances in the phylogeny of *Helvella* (Fungi: Ascomycota), inferred from nuclear ribosomal LSU sequences and morphological data. *Revista Mexicana de Biodiversidad*, **86**, 856–871.
- Landvik S, Kristiansen R, Schumacher T (1999) *Pindara*: a miniature *Helvella*. *Mycologia*, **91**, 278.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**, 772–773.
- Leaché AD, Fujita MK (2010) Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings. Biological Sciences*, **277**, 3071–3077.
- Leaché AD, Harris RB, Rannala B, Yang Z (2014) The influence of gene flow on species tree estimation: a simulation study. *Systematic Biology*, **63**, 17–30.
- Lemmon EM, Lemmon AR (2013) High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 99–121.
- Letunic I, Bork P (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic acids research*, **44**, W242–5.
- Lindner DL, Carlsen T, Henrik Nilsson R *et al.* (2013) Employing 454 amplicon pyrosequencing to reveal intragenomic divergence in the internal transcribed spacer rDNA region in fungi. *Ecology and Evolution*, **3**, 1751–1764.
- Lumbsch HT, Leavitt SD (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity*, **50**, 59–72.
- Mark K (2016) *Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota)*. Thesis, University of Tartu Press.
- Massée G (1895) *British Fungus-flora 4*. Bell & sons, London, Great Britain.
- Nagy LG, Szöllősi G (2017) Fungal phylogeny in the age of genomics: insights into phylogenetic inference from genome-scale datasets. *Advances in Genetics*, **100**, 49–72.
- Nannfeldt J (1937) Contributions to the mycoflora of Sweden. 4. On some species of *Helvella*, together with a discussion of the natural affinities within Helvellaceae and Pezizaceae trib. Acetabuleae. *Svensk Botanisk Tidsskrift*, **31**, 47–66.
- Nannfeldt JA (1932) Bleka Stenmurklan, *Gyromitra gigas* (Krombh.) Cke *Friesia*, **1**, 34–45.
- Nevo E (2001) Evolution of genome-phenome diversity under environmental stress. *Proceedings of the National Academy of Sciences*, **98**, 6233–6240.

- Nguyen NH, Landeros F, Garibay-Orijel R, Hansen K, Vellinga EC (2013) The *Helvella lacunosa* species complex in western North America: cryptic species, misapplied names and parasites. *Mycologia*, **105**, 1275–1286.
- Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics Online*, **4**, 193–201.
- Olariaga I, Van Vooren N, Carbone M, Hansen K (2015) A monograph of *Otidea* (Pyronemataceae, Pezizomycetes). *Persoonia - Molecular Phylogeny and Evolution of Fungi*, **35**, 166–229.
- Philippe H, Brinkmann H, Lavrov DV *et al.* (2011) Resolving difficult phylogenetic questions: why more sequences are not enough. *PLoS biology*, **9**, e1000602.
- Quélet L *Enchiridion fungorum in Europa media et praesertim in Gallia vigentium*. Lutetiae, France.
- Raja H, Schoch CL, Hustad V, Shearer C, Miller A (2011) Testing the phylogenetic utility of MCM7 in the Ascomycota. *MycKeys*, **1**, 63–94.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, **22**, 341.
- Rannala B, Yang Z (2003) Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics*, **164**, 1645–1656.
- Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature*, **425**, 798–804.
- RStudio Team (2016) RStudio: Integrated development for R. RStudio, Inc., Boston, MA. <http://www.rstudio.com/>.
- Schoch CL, Seifert KA, Huhndorf S *et al.* (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 6241–6246.
- Schoch CL, Sung G-H, López-Giráldez F *et al.* (2009) The Ascomycota tree of life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological Traits. *Systematic Biology*, **58**, 224–239.
- Selosse M-A, Vincenot L, Öpik M (2016) Data processing can mask biology: towards better reporting of fungal barcoding data? *The New Phytologist*, **210**, 1159–1164.
- Sievert C, Parmer C, Hocking T *et al.* (2017) Create Interactive Web Graphics via “plotly.js” [R package plotly version 4.7.1].
- Simpson GG (1951) The species concept. *Evolution*, **5**, 285.
- Skrede I, Borgen L, Brochmann C (2008) Genetic structuring in three closely related circumpolar plant species: AFLP versus microsatellite markers and high-arctic versus arctic–alpine distributions. *Heredity*, **102**, 293–302.
- Skrede I, Carlsen T, Schumacher T (2017) A synopsis of the saddle fungi (*Helvella*: Ascomycota) in Europe – species delimitation, taxonomy and typification. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, **39**, 201–253.
- Spatafora JW, Sung G-H, Johnson D *et al.* (2006) A five-gene phylogeny of Pezizomycotina. *Mycologia*, **98**, 1018–1028.
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic

- analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.
- Stensrud Ø, Schumacher T, Shalchian-Tabrizi K, Svegård IB, Kauserud H (2007) Accelerated nrDNA evolution and profound AT bias in the medical fungus *Cordyceps sinensis*. *Mycological Research*, **111**, 409–415.
- Stielow JB, Lévesque CA, Seifert KA *et al.* (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, **35**, 242–263.
- Strausbaugh LD (2001) Concerted Evolution. In: *Encyclopedia of Genetics*, pp. 436–441. Elsevier, Amsterdam, Netherlands.
- Sukumaran J, Knowles LL (2017) Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 1607–1612.
- Taşkın H, Büyükalaca S, Hansen K, O'Donnell K (2017) Multilocus phylogenetic analysis of true morels *Morchella* reveals high levels of endemics in Turkey relative to other regions of Europe. *Mycologia*, **104**, 446–461.
- Taylor JW, Jacobson DJ, Kroken S *et al.* (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology*, **31**, 21–32.
- Thiéry O, Vasar M, Jaius T *et al.* (2016) Sequence variation in nuclear ribosomal small subunit, internal transcribed spacer and large subunit regions of *Rhizophagus irregularis* and *Gigaspora margarita* is high and isolate-dependent. *Molecular Ecology*, **25**, 2816–2832.
- Toprak Z, Pfeil BE, Jones G *et al.* (2016) Species delimitation without prior knowledge: DISSECT reveals extensive cryptic speciation in the *Silene aegyptiaca* complex (Caryophyllaceae). *Molecular Phylogenetics and Evolution*, **102**, 1–8.
- Valladares F, Gianoli E, Gómez JM (2007) Ecological limits to plant phenotypic plasticity. *The New Phytologist*, **176**, 749–763.
- Van Vooren N (2014) Notes sur le genre *Helvella* L. (Ascomycota, Pezizales). 2. Les sous-genres Cupuliformes et Macropodes. *Phytotaxa*, **212**, 29–47.
- Van Vooren N (2015) Quelques Pezizales intéressantes recoltées en Vanoise. *Phytotaxa*, **218**, 23–29.
- Vilgalys R, Sun BL (1994) Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. *Proceedings of the National Academy of Sciences*, **91**, 4599–4603.
- Wagner F, Härtl S, Vogt R, Oberprieler C (2017) “Fix Me Another Marguerite!”: Species delimitation in a group of intensively hybridizing lineages of ox-eye daisies (*Leucanthemum* Mill., Compositae-Anthemideae). *Molecular Ecology*, **26**, 4260–4283.
- Weber N (1972) The genus *Helvella* in Michigan. *The Michigan Botanist*, **11**, 147–201.
- Weber NS (1975) Notes on Western species of *Helvella*. I. *Nova hedwigia. Beihefte*, **51**, 25–38.
- Weberbauer O (1873) Die pilze Norddeutschlands mit besonderer berücksichtigung Schlesiens. *J. U. Kern's verlag*, Wrocław, Poland.

- Weidemann HM (1998) *Påvisning av Helvella ektomykorrhiza hos Dryas og Salix ved hjelp av taxon-selektive nrDNA baserte Helvella primere*. Thesis, Department of Biology, University of Oslo, Norway.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols*, pp. 315–322. Elsevier.
- Wiley EO (1978) The evolutionary species concept reconsidered. *Systematic Biology*, **27**, 17–26.
- Xu B, Yang Z (2016) Challenges in species tree estimation under the multispecies coalescent model. *Genetics*, **204**, 1353–1368.
- Yahr R, Schoch CL, Dentinger BTM (2016) Scaling up discovery of hidden diversity in fungi: impacts of barcoding approaches. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **371**, 20150336.
- Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, **107**, 9264–9269.
- Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, **29**, 2869–2876.
- Zhao Q, Tolgor B, Zhao YC, Yang ZL (2015) Species diversity within the *Helvella crispa* group (Ascomycota: Helvellaceae) in China. *Phytotaxa*, **239**, 130–142.

Supplementary information

Table S1: Overview of specimens of the *Helvella corium* morphospecies complex used to assess species distributions. Specimen ID as well as geographical origin (with coordinate data) is shown for each specimen. All specimens with fungarium number “O-XXXXXX” are new fieldwork collections not yet deposited in a herbarium. “NA” means data not annotated. Type specimens are written in bold.

Species	Sample ID, fungarium	Locality	Collection year	Lat	Long
<i>H. alpestris</i>	H013, O-253220	Norway. Oppland. Dovre. Grimsdalen	2007	62.0893084	9.6514889
	H014, O-253221	Norway. Oppland. Dovre. Grimsdalen	2007	62.0631	9.5452
	H031, O-253222	Norway. Oppland. Dovre. Grimsdalen	2009	62.0893084	9.6514889
	H036, O-253223	Norway. Oppland. Dovre. Grimsdalen	2007	62.0893084	9.6514889
	H042, O-253224	Norway. Oppland. Dovre. Grimsdalen	2009	62.0893084	9.6514889
	H246, O-253219	Norway. Oppland. Dovre	1997	62.0349474	9.490123
	H460, DAOM-574891	Canada. Nunavut	2014	67.742478	-115.359312
	H483, O-253225	Norway. Oppland. Dovre	1984	62.0349474	9.490123
	H503, NA	Norway. Oppland. Dovre. Grimsdalen	NA	62.0349474	9.490123
	H506, NA	Norway. Oppland. Dovre. Grimsdalen	NA	62.0349474	9.490123
	H507, NA	Norway. Oppland. Dovre. Grimsdalen	NA	62.0349474	9.490123
	H 548, NA	France. Savoie. Val'dIsere	1992	45.430260	6.981125
	H719, UPS-F-145393	Sweden. Torne Lappmark. Jukkasjärvi	1946	68.03292	20.29801
	H726, UPS-F-145724	Sweden. Dalarna. Orsa	1950	61.131097	14.766762
	H865, S-F-122366	Norway. Oppland. Dovre	1985	62.12133	9.45125
	H914, TROM-F-38205	Norway. Oppland. Dovre. Grimsdalen	1972	62.0631	9.5452
	H915, TROM-F-08580	Norway. Oppland. Dovre. Grimsdalen. Tverrhåi	1985	62.0896	9.6606
	H916, TROM-F-11410	Norway. Nordland. Fauske. Blåmannsisen	1967	67.2451	15.9267
	H928, TROM-F-11403	Norway. Troms. Tromsø. Tromsø museum	1980	69.639	18.9137
	H963, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen, Grimsa	2016	62.044175	9.498163

<i>H. alpestris</i>	H965, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H966, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H967, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H968, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H969, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H970, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H971, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H972, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2016	62.100000	9.634444
	H1098, C-F-50667	Greenland. Ella Island. St. Elvdal	1982	72.873698	-25.099968
	H1103, C-F-86999	Greenland. S Strømfjord	1988	67.022538	-50.665513
	H1115, C-F-86617	Greenland. Jameson Land	1989	71.214487	-23.516480
	H1155, C-F-45367	Greenland. S Strømfjord. Sandflugtdalen	1946	67.067134	-50.333505
	H1190, C-F-45351	Norway. Nordland. Fauske. Blåmannsisen	1967	67.2451	15.9267
	H2102, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen	2017	62.032500	9.496111
	H2111, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen. Tverråi	2017	62.100000	9.634444
	H2145, O-F-174749	Norway. Oppland. Dovre. Grimsdalen. Verkensetter	2013	62.0619	9.5443
	H2154, O-F-174783	Norway. Finnmark. Porsanger. Lakselv	1961	70.0371	24.9329
	H2173, O-F-174774	Norway. Oppland. Dovre. Grimsdalen	1987	62.0619	9.5443
	H2203, UME-31384	Norway. Oppland. Dovre. Grimsdalen	1997	62.12133	9.45125
	H2241, NA	Norway. Oppland. Dovre. Grimsdalen	1981	62.0665936	9.5644765
	H2242, NA	Norway. Oppland. Dovre. Grimsdalen	1982	62.099794	9.636997
	H2244, NA	Norway. Oppland. Dovre. Grimsdalen	1982	62.099794	9.636997
	H2246, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.099794	9.636997
	H2253, NA	Norway. Oppland. Dovre. Grimsdalen	1982	62.0569808	9.5944898
	H2254, NA	Norway. Oppland. Dovre. Grimsdalen	2005	62.091482	9.641733
	H2255, NA	Norway. Oppland. Dovre. Grimsdalen	1975	62.091482	9.641733
	H2256, NA	Norway. Oppland. Dovre. Grimsdalen	1982	61.9631389	9.3113528
	H2262, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.023625	9.4754556

	H2277, TRH-F-21353	Norway. Finmark. Kautokeino. Virdnejávri	1983	69.5624	23.7593
<i>H. alpicola</i>	H175, O-185924	Norway. Nordland. Saltdal	1988	66.822903	15.453428
	H231, O-253226	Switzerland. Graubunden	1984	46.696700	9.585232
	H552, O-XXXXXX	Norway. Saltdal. Junkerdalsura	2016	66.8175979	15.4286945
	H553, O-XXXXXX	Norway. Saltdal. Junkerdalsura	2016	66.8175979	15.4286945
	H554, O-XXXXXX	Norway. Saltdal. Junkerdalsura	2016	66.8175979	15.4286945
	H952, O-XXXXXX	Norway. Nordland. Saltdal. Junkerdalsura	2016	66.8175979	15.4286945
	H953, O-XXXXXX	Norway. Nordland. Saltdal. Junkerdalsura	2016	66.8175979	15.4286945
	H1439, C-F-53856	Norway. Nordland. Rana	1972	66.3354	15.2121
	H2124, O-F-81595	Norway. Nordland. Saltdal. Junkerdalen	1995	66.82145	15.45649
	H1516, C-F-53788	Norway. Nordland. Rana	1972	66.3354	15.2121
	H1520, C-F-86730	Norway. Nordland. Saltdal. Junkerdalen	1993	66.82145	15.45649
	H1534, C-F-103009	Norway. Nordland. Rana	1971	66.3354	15.2121
	H1537, C-F-53934	Norway. Nordland. Rana	1973	66.3354	15.2121
	H1541, C-F-54548	Norway. Nordland. Rana	1976	66.3354	15.2121
<i>H. alpina</i>	H091, O-253227	Canada. British Columbia. Whistler	1994	50.131802	-122.958265
	H223, O-253228	France. Savoie. Plan des evettes	1992	45.353431	7.114937
	H336, O-253227	Canada. British Columbia	1984	50.131802	-122.958265
	H 540, C-F-34420	Russia . Khatanga airport	1993	71.971097	102.446327
	H 546, NA	France. Savoie	1992	45.430260	6.981125
	H711, UPS-F-145392	Sweden. Torne Lappmark. Jukkasjärvi	1945	68.03292	20.29801
	H921, TROM-F-41055	Norway. Nordland. Ballangen. Langvatn	1970	68.1092	17.0793
	H1092, C-F-63730	Greenland. Head of Kangerdlugssuaq. S Strømfjord	1971	68.233596	-32.178284
	H1095, C-F-50287	Greenland. S Strømfjord, road 5 km E of airport	1982	67.022538	-50.665513
	H1100, C-F-86575	Greenland. S Strømfjord, 3 km W of airport	1987	67.001688	-50.788079
	H1113, C-F-86574	Greenland. Std. Strømfjord, 3 km W of airport	1987	67.001688	-50.788079
	H1114, C-F-86623	Greenland. Jameson Land. "Clavicorona ely"	1989	71.214487	-23.516480
	H1124, C-F-55730	Greenland. Sdr. Strømfjord, N of the airport	1987	67.018986	-50.732117
	H1136, C-F-63799	Greenland. Head of Kangerdlugssuaq. SStrømfjord	1973	68.233596	-32.178284

<i>H. alpina</i>	H1159, C-F-54601	Norway. Nordland. Rana. Virvassdalen	1979	66.3354	15.2121
	H2106, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen	2017	62.040833	9.488611
	H2133, O-F-174762	Norway. Rogaland. Sola. Solasanden	1962	58.8978	5.49306
	H2162, O-F-174772	Norway. Troms. Tromsø	1954	69.6975	18.7736
<i>H. corium</i>	H224, NA	Norway. Troms. Tromøya	1989	69.674385	18.925007
	H242, O-362201	Svalbard. Kongsfjorden	1986	78.2163884	15.5957623
	H248, O-253277	Norway. Hordaland. Ulvik	1996	60.598977	7.509829
	H292, C-F-92111	Sweden. Norrbotten	1928	68.349300	18.826976
	H294, C-F-16568	Russia. N Ural Mts	1990	66.657784	66.354505
	H324, C-F-55580	Svalbard. Bunsow Land	1985	78.2163884	15.5957623
	H328, C-F-34476	Russia. Ural Mts	1982	66.766303	65.372774
	H352, C-F-71638	Denmark. Mid Zealand	1984	55.59	11.84
	H434, O-253280	Norway. Troms	1989	69.674385	18.925007
	H435, NA	Norway. Troms	1989	69.674385	18.925007
	H436, O-253281	Norway. Svalbard. Spitsbergen	1988	78.2163884	15.5957623
	H451, O-253278	Norway. Hordaland. Ulvik. Finse	2014	60.598977	7.509829
	H453, O-253279	Norway. Hordaland. Ulvik. Finse	2014	60.598977	7.509829
	H511, NA	Svalbard. Longyearbyen	2015	78.2163884	15.5957623
	H518, NA	Norway. Hordaland. Ulvik. Finse	2015	60.598977	7.509829
	H541, C-F-56264	Alaska. Fairbanks	1996	64.834723	-147.758959
	H543, C-F-86750	Norway. Nordland. Rana	1993	66.3354	15.2121
	H547, NA	France. Savoie	1992	45.430260	6.981125
	H556, NA	Norway. Hordaland. Ulvik. Finse	2016	60.5750468	7.4829638
	H557, NA	Norway. Hordaland. Ulvik. Finse	2016	60.598977	7.509829
	H558, NA	Norway. Hordaland. Ulvik. Finse	2016	60.598977	7.509829
	H559, NA	Norway. Hordaland. Ulvik. Finse	2016	60.598977	7.509829
	H622, UPS-F-145366	Sweden. Öland. Torslunda	1888	56.62943	16.50676
H623, UPS-F-694057	Sweden. Närke. Rinkaby	1984	59.31587	15.31987	
H625, UPS-F-145368	Sweden. Södermanland	1974	58.661734	17.129722	

<i>H. corium</i>	H626, UPS-F-673600	Sweden. Närke. Rinkaby	1984	59.31587	15.31987
	H627, UPS-F-673566	Sweden. Närke. Glanshammar	1983	59.34232	15.41657
	H628, UPS-F-673545	Sweden. Närke. Glanshammar	1983	59.34232	15.41657
	H629, UPS-F-673519	Sweden. Närke. Rinkaby	1983	59.31587	15.31987
	H630, UPS-F-145367	Sweden. Närke. Viby	1974	59.03083	14.85175
	H631, UPS-F-673520	Sweden. Närke. Glanshammar	1983	59.34232	15.41657
	H632, UPS-F-673523	Sweden. Närke. Glanshammar	1983	59.34232	15.41657
	H635, UPS-F-646630	Sweden. Värmland. Östmark	1990	60.31597	12.71603
	H637, UPS-F-145370	Sweden. Uppland	1938	59.815090	17.662529
	H638, UPS-F-145372	Sweden. Uppland	1938	59.815090	17.662529
	H639, UPS-F-145371	Sweden. Uppland	1938	59.815090	17.662529
	H640, UPS-F-585646	Sweden. Uppland. Älvkarleby	2012	60.59905	17.4915
	H641, UPS-F-145374	Sweden. Västmanland. Sala	1942	59.9503	16.56862
	H642, UPS-F-145375	Sweden. Västmanland. Vikers	1960	59.43202	14.82918
	H643, UPS-F-145377	Sweden. Dalarna. Bjursås	1977	60.75952	15.44774
	H644, UPS-F-145376	Sweden. Dalarna. Bjursås	1977	60.75952	15.44774
	H645, UPS-F-145380	Sweden. Medelpad. Timrå	1980	62.51171	17.32118
	H646, UPS-F-002100	Sweden. Härjedalen. Storjö	1986	62.83088	12.84079
	H647, UPS-F-145379	Sweden. Jämtland. Åre	1979	63.41576	12.54533
	H648, UPS-F-145378	Sweden. Jämtland. Åre	1967	63.41576	12.54533
	H649, UPS-F-001766	Sweden. Medelpad. Sundsvall	1984	62.38342	17.31872
	H700, UPS-F-145382	Sweden. Härjedalen. Tännäs	1895	62.4872	12.57119
	H704, UPS-F-145387	Sweden. Jämtland. Nyhem	1975	62.91073	15.6755
	H705, UPS-F-145385	Sweden. Norrbotten. Töre	1959	65.96613	22.73697
	H706, UPS-F-145386	Sweden. Jämtland. Brunflo	1929	63.10963	14.92319
	H707, UPS-F-006293	Sweden. Lule Lappmark. Gällivare	1989	67.28781	20.11498
	H708, UPS-F-145388	Sweden. Åsele Lappmark. Vilhelmina	1943	64.93561	16.01967
	H709, UPS-F-672997	Sweden. Lycksele Lappmark. Tärna	1979	65.6956	15.20619
	H710, UPS-F-683147	Sweden. Åsele Lappmark. Dorotea	1985	64.51985	15.9181

<i>H. corium</i>	H712, UPS-F-145391	Sweden. Torne Lappmark. Jukkasjärvi	1986	68.03292	20.29801
	H713, UPS-F-145389	Sweden. Torne Lappmark. Jukkasjärvi	1983	68.03292	20.29801
	H714, UPS-F-647082	Sweden. Torne Lappmark. Jukkasjärvi	1923	68.03292	20.29801
	H715, UPS-F-145398	Sweden. Torne Lappmark. Jukkasjärvi	1946	68.03292	20.29801
	H718, UPS-F-145396	Sweden. Torne Lappmark. Jukkasjärvi	1927	68.03292	20.29801
	H720, UPS-F-145397	Sweden. Torne Lappmark. Jukkasjärvi	1927	68.03292	20.29801
	H722, UPS-F-178942	Norway. Hedmark. Folldal	1985	62.15349	9.99101
	H725, UPS-F-145402	Denmark. Själland	1943	55.556401	11.699998
	H854, S-F-158220	Norway. Troms. Bardu	2008	68.666667	18.166667
	H855, S-F-122362	Norway. Nord-Trøndelag. Meråker	1981	63.4087	11.82983
	H856, S-F-122351	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H857, S-F-122349	Sweden. Härjedalen. Tännäs	1982	62.4872	12.57119
	H858, S-F-122350	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H859, S-F-122356	Sweden. Dalarna. Leksand	1984	60.71296	14.93161
	H860, S-F-122353	Sweden. Härjedalen	1976	62.4872	12.57119
	H861, S-F-122358	Sweden. Dalarna. Leksand	1987	60.71296	14.93161
	H862, S-F-122360	Sweden. Jämtland. Åre	1981	63.41576	12.54533
	H863, S-F-122364	Sweden. Västergötland. Rångedala	1989	57.78259	13.11947
	H864, S-F-122365	Sweden. Torne Lappmark. Jukkasjärvi	1954	68.03292	20.29801
	H867, S-F-122368	Sweden. Norrbotten	1964	66.582837	23.706130
	H868, S-F-122363	Sweden. Dalarna. Orsa	1984	61.31766	14.7168
	H869, S-F-122369	Sweden. Västerbotten	1943	64.646782	16.613133
	H870, S-F-122386	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H872, S-F-122383	Sweden. Härjedalen	1976	62.4872	12.57119
	H873, S-F-122380	Sweden. Jämtland. Åre	1981	63.41576	12.54533
	H874, S-F-122175	Sweden. Jämtland. Åre	1981	63.41576	12.54533
	H875, S-F-122173	Sweden. Jämtland. Åre	1981	63.41576	12.54533
	H876, S-F-122174	Sweden. Torne Lappmark. Jukkasjärvi	1927	68.03292	20.29801
	H877, S-F-122170	Sweden. Torne Lappmark. Jukkasjärvi	1943	68.03292	20.29801

<i>H. corium</i>	H878, S-F-122205	Sweden. Södermanland. Mörkö	1986	58.94398	17.67944
	H880, S-F-122208	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H882, S-F-026905	Sweden. Härjedalen. Tännäs	1979	62.4872	12.57119
	H883, S-F-122211	Sweden. Jämtland. Borgvattnet	1995	63.40516	15.82549
	H884, S-F-122212	Sweden. Södermanland. Sorunda	1983	58.99894	17.81178
	H885, S-F-122243	Sweden. Torne Lappmark. Jukkasjärvi	1981	68.03292	20.29801
	H887, S-F-122248	Sweden. Härjedalen	1976	62.4872	12.57119
	H888, S F-122247	Sweden. Medelpad. Sundsvall	1987	62.0	17.0
	H889, S-F-122249	Sweden. Härjedalen	1976	62.4872	12.57119
	H890, S-F-122252	Sweden. Härjedalen. Tännäs	1980	62.4872	12.57119
	H892, S-F-122253	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H894, S-F-122409	Denmark. Sjælland	1882	55.652656	11.080520
	H918, TROM-F-11407	Norway. Nordland. Rana. Mo	1988	66.3112	14.2193
	H923, TROM-F-41052	Norway. Nordland. Ballangen. Langvatn	1970	68.1092	17.0793
	H924, TROM-F-41053	Norway. Nordland. Ballangen. Langvatn	1970	68.1092	17.0793
	H925, TROM-F-11408	Norway. Nordland. Ballangen. Geitvatn	1965	68.2307	17.054
	H926, TROM-F-11411	Norway. Troms. Kåfjord. Olderdalen	1967	69.633	20.6138
	H927, TROM-F-11406	Norway. Troms. Tromsø. Tromsø museum	1969	69.6328	18.9273
	H929, TROM-F-38141	Norway. Troms. Tromsø. Hjellnes	2014	69.5531	19.1339
	H930, TROM-F-38139	Norway. Troms. Tromsø. Tønsvika	2000	69.7392	19.177
	H931, TROM-F-41051	Norway. Troms. Tromsø. Breivikeidet	1963	69.656586	19.567328
	H933, TROM-F-30059	Norway. Troms. Balsfjord. Tomasjorddalen	1986	69.3419	19.4125
	H934, TROM-F-11489	Norway. Troms. Tromsø. Tromsøya	1981	69.675339	18.926380
	H935, TROM-F-24980	Norway. Finnmark. Alta. Peska	2010	69.847944	23.248725
	H936, TROM-F-24113	Norway. Bjørnøya. Miseryfjell	1983	74.421923	19.196829
	H937, TROM-F-22883	Svalbard. Vest-Spitsbergen. Woodfjorden	1966	79.794630	13.855047
	H938, TROM-F-13149	Norway. Finnmark. Berlevåg	1987	70.8775	29.0517
	H939, TROM-F-11402	Norway. Finnmark. Porsanger. Lakselv airport	1981	70.0639	24.9632
	H940, TROM-F-21028	Norway. Troms. Balsfjord. Malangen. Nordfjord	2003	69.3023	18.8954

<i>H. corium</i>	H944, TROM-F-06749	Norway. Troms. Storfjord. Skibotndalen	1992	69.3034	20.4296
	H948, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7506545	15.7098901
	H949, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7490875	15.7600545
	H950, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7527372	15.6950236
	H951, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7506388	15.7101029
	H955, O- XXXXXX	Norway. Hedmark. Folldal. Einunndalen	2016	62.2325035	10.2373824
	H956, O- XXXXXX	Norway. Hedmark. Folldal. Einunndalen	2016	62.2325035	10.2373824
	H957, O- XXXXXX	Norway. Sør-Trøndelag. Oppdal. Vinstradalen	2016	62.4554667	9.668675
	H1044, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7506388	15.7101029
	H1045, O- XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.7506388	15.7101029
	H1074, BG-F-11655	Sweden. Torne Lappmark. Abisko	2016	68.354489	18.815750
	H1075, BG-F-11653	Norway. Møre og Romsdal. Eide	1977	63.0312	7.29994
	H1076, BG-F-11654	Norway. Sør-Trøndelag. Oppdal. Kongsvold	1978	62.5367	9.51846
	H1077, BG-F-11652	Norway. Nordland. Saltdal. Junkerdalselva	1977	66.8772	15.6332
	H1078, BG-F-11650	Norway. Nordland. Rana. Selfors	1986	66.4390	14.3492
	H1079, BG-F-11651	Norway. Troms. Tromsø. Skittenev	1987	69.6975	18.7736
	H1080, BG-F-11649	Norway. Nord-Trøndelag. Namsskogan	1983	64.8107	13.0909
	H1081, BG-F-11648	Norway. Hordaland. Odda. Odda smelteverk	1954	59.9661	6.86071
	H1082, BG-F-11647	Norway. Hordaland. Bergen. Åsane	1975	60.3703	5.41776
	H1083, BG-F-11646	Norway. Hordaland. Stord. Sagvåg	1976	59.8258	5.48698
	H1085, BG-F-11644	Norway. Hordaland. Bergen. Breistein	1986	60.3703	5.41776
	H1087, C-F-64529	Greenland. Disko Fjord, near Jakobshavn	1992	69.234574	-51.104537
	H1088, C-F-86904	Greenland. Thule Airbase	1994	76.538424	-68.698537
	H1089, C-F-63828	Greenland. North East Greenland. Mestervig	1968	72.238262	-23.923202
	H1094, C-F-84544	Greenland. Kapisigdlit	1986	64.417166	-50.033291
	H1096, C-F-50733	Greenland. Mestervig, around Blyminen	1982	72.238262	-23.923202
	H1097, C-F-50309	Greenland. Mestervig. Nyhavn (on airport area)	1982	72.238262	-23.923202
	H1099, C-F-53241	Greenland. Mestervig. Minebyen	1983	72.238262	-23.923202
	H1101, C-F-50764	Greenland. Mestervig. Around Blyminen	1982	72.238262	-23.923202

<i>H. corium</i>	H1102, C-F-50709	Greenland. Mestervig	1982	72.238262	-23.923202
	H1104, C-F-87000	Greenland. Thule airbase	1988	76.538424	-68.698537
	H1106, C-F-86891	Greenland. Grønnedal	1991	61.219724	-48.133162
	H1116, C-F-63792	Greenland. Pâmiut. Frederikshåb	1973	61.994092	-49.673776
	H1120, C-F-86856	Greenland. Narssarssuaq	2002	61.163042	-45.428081
	H1121, C-F-86988	Greenland. Paamiut. Frederikshåb	1979	61.994092	-49.673776
	H1122, C-F-86991	Greenland. Sdr. Strømfjord. Sandflugtdalen	1946	67.067134	-50.333505
	H1123, C-F-86989	Greenland. Narssarssuaq	1979	61.163042	-45.428081
	H1125, C-F-55737	Greenland. Thule Airbase	1987	76.538424	-68.698537
	H1126, C-F-50318	Greenland. Mestervig. Blyminen	1982	72.238262	-23.923202
	H1127, C-F-56058	Greenland. Thule. Dundas	1987	76.527907	-68.847853
	H1128, C-F-56039	Greenland. Thule. Dundas	1987	76.527907	-68.847853
	H1129, C-F-53064	Greenland. Mestervig. Minebyen	1983	72.238262	-23.923202
	H1131, C-F-49857	Greenland. Narssarssuaq	1981	61.163042	-45.428081
	H1132, C-F-49994	Greenland. Narssarssuaq	1981	61.163042	-45.428081
	H1133, C-F-63798	Greenland. Head of Kangerdlugssuaq. S Strømfjord	1973	68.233596	-32.178284
	H1134, C-F-64553	Greenland. Narssaq. Ved hotell Arctic	1981	61.158168	-45.422163
	H1135, C-F-49977	Greenland. Narssarssuaq	1981	61.158168	-45.422163
	H1138, C-F-63793	Greenland. Pâmiut. Frederikshåb	1973	61.994092	-49.673776
	H1140, C-F-88836	Greenland. Narssarssuaq	1983	61.163042	-45.428081
	H1141, C-F-88844	Greenland. Narssarssuaq	1984	61.163042	-45.428081
	H1142, C-F-89696	Greenland. Thule Airbase, S of base	1988	76.538424	-68.698537
	H1143, C-F-86905	Greenland. Paamiut. Frederikshåb	1993	61.994092	-49.673776
	H1144, C-F-88847	Greenland. Narssarssuaq	1984	61.163042	-45.428081
	H1146, C-F-92982	Greenland	1998	61.997118	-49.663997
	H1148, C-F-92997	Greenland	1999	72.237424	-23.960967
	H1153, C-F-86997	Greenland. Paamiut. Frederikshåb	1978	61.994092	-49.673776
	H1156, C-F-55701	Svalbard. Bunsowland. Gipsdalselva	1988	78.4703195	16.725582
	H1158, C-F-53886	Norway. Nordland. Rana. Ørtfjellmoen bridge	1973	66.3354	15.2121

<i>H. corium</i>	H1161, C-F-55681	Svalbard. Bröggerhalvøya, near Lovenbreen	1988	78.927916	11.730340
	H1163, C-F-55690	Svalbard. Bröggerhalvøya. Ny Ålesund	1988	78.9711506	12.0393253
	H1165, C-F-102966	Norway. Nordland. Mo i Rana. Ørtfjelmoen	1975	66.3354	15.2121
	H1168, C-F-102969	Norway. Nordland. Mo i Rana. Bleikingen	1975	66.3354	15.2121
	H1169, C-F-55216	Norway. Nordland. Plurdalen. Stillvasstranda	1981	66.3151583	14.6443377
	H1173, C-F-55669	Svalbard. Bröggerhalvøya. Ny Ålesund	1988	78.9711506	12.0393253
	H1176, C-F-54669	Norway. Nordland. Rana	1979	66.3354	15.2121
	H1178, C-F-45363	Sweden. Torne Lappmark. Abisko	1974	68.354489	18.815750
	H1180, C-F-45364	Sweden. Torne Lappmark. Abisko	1974	68.354489	18.815750
	H1181, C-F-45369	Sweden. Torne Lappmark, near Abisko	1951	68.354489	18.815750
	H1183, C-F-46277	Sweden. Skåne. Limhamn. Kalkbrudd	1967	55.568249	12.930664
	H1184, C-F-46275	Sweden. Torne Lappmark. Near Stordalen station	1968	68.354489	18.815750
	H1185, C-F-102963	Sweden. Torne Lappmark. Abisko	1976	68.349284	18.831632
	H1187, C-F-45361	Sweden. Torne Lappmark. Abisko	1974	68.354489	18.815750
	H1188, C-F-45362	Sweden. Torne Lappmark. Abisko	1974	68.354489	18.815750
	H1191, C-F-86750	Norway. Nordland. Rana	1993	66.3354	15.2121
	H1192, C-F-54635	Norway. Nordland. Rana. S Bjöllavatn	1979	66.727065	15.0395302
	H1193, C-F-53756	Norway. Nordland. Rana. Ørtfjellmoen bridge	1972	66.3354	15.2121
	H1194, C-F-56076	Norway. Nordland. Rana	1973	66.3354	15.2121
	H1195, C-F-45354	Svalbard. Ny Ålesund	1966	78.921717	11.900628
	H1197, C-F-55677	Svalbard. Bröggerhalvøya. Ny Ålesund	1988	78.927916	11.730340
	H1198, C-F-45359	Norway. Troms. Tromsø. Near Tromsø museum	1969	69.634897	18.911946
	H1402, C-F-102797	Denmark. Jylland. Gravlev. Thingbæk kalkbrud	1962	56.832778	9.811802
	H1403, C-F-39605	Denmark. Jylland. Cimbria cementwork	1961	56.689999	10.05
	H1404, C-F-102793	Denmark. East Jylland. Grenå plantage	1983	56.412710	10.894662
	H1405, C-F-102794	Denmark. Jylland. Skagen	1983	57.700547	10.513656
	H1406, C-F-102791	Denmark. N W Jylland. Toremål. Tved klitplantage	1980	57.055106	8.635724
	H1407, C-F-102792	Denmark. N W Jylland. Toremål. Tved klitplantage	1980	57.055106	8.635724
	H1408, C-F-44808	Denmark. East Jylland. Spretrup	2006	56.565018	10.047423

<i>H. corium</i>	H1409, C-F-29800	Denmark. East Jylland. Spentrup Kalkbrud	1987	56.549999	10.03
	H1411, C-F-39609	Denmark. Jutland. Hadsund Kalkbrud	1968	56.689999	10.05
	H1413, C-F-11499	Denmark. East Jylland. Spentrup Kalkbrud	1987	56.561421	10.018103
	H1415, C-F-39610	Denmark. Jutland. Rømø. Nørreland	1967	55.16	8.53
	H1958, O- XXXXXX	Norway. Troms. Salangen	2017	68.865418	17.865416
	H1970, O- XXXXXX	Norway. Finnmark. Alta. Talvik	2017	70.087778	22.985278
	H1998, O- XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017	69.291944	19.413889
	H1999, O- XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017	69.291944	19.413889
	H2101, O- XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017	69.291944	19.413889
	H2119, O-F-68006	Norway. Nord-Trøndelag. Flatanger	2005	64.50449	10.79392
	H2121, O-F-174770	Norway. Troms. Kvænangen. Sørfjord	1968	69.8475	21.9192
	H2122, O-F-174784	Norway. Finnmark. Nesseby. Veinesbukten	1970	70.1091	28.8498
	H2125, O-F-174741	Norway. Akershus. Asker. Dikemark	1973	59.805	10.3778
	H2126, O-F-174785	Norway. Finnmark. Sør-Varanger. Kirkenes	1983	69.7194	30.03693
	H2127, O-F-73286	Norway. Finnmark. Sør-Varanger. Melkefoss	2001	69.40371	29.77592
	H2128, O-F-174740	Norway. Østfold. Sarpsborg. Grimsøkilen	1983	59.14336	11.20516
	H2130, O-F-174786	Norway. Finnmark. Vadsø. Vadsø	1972	70.1932	30.0534
	H2131, O-F-72221	Norway. Nordland. Tysfjord	1998	68.25409	16.03158
	H2132, O-F-174759	Norway. Telemark. Kviteseid. Morgedal	1984	59.4112	8.49793
	H2134, O-F-69040	Norway. Nordland. Tjeldsund. Fjeldalsheia	2008	68.5007	16.2970
	H2135, O-F-174787	Finland. Enontekiö. Prorojärvi. Waltijoki	1961	69.238964	21.571668
	H2136, O-F-174771	Norway. Troms. Målselv. Bardufoss. Fosshøgda	1954	68.9738	19.2614
	H2137, O-F-174768	Norway. Sør-Trøndelag. Oppdal. Kongsvoll	1980	62.5367	9.51846
	H2138, O-F-83139	Norway. Vestfold. Tjøme. Havna	1996	59.086	10.4222
	H2139, O-F-174764	Norway. Hordaland. Ulvik. Finse	1957	60.6272	7.19296
	H2140, O-F-174778	Norway. Nordland. Rana	1989	66.4390	14.3492
	H2148, O-F-174754	Norway. Buskerud. Nore og Uvdal. Uvdal	1984	60.2683	8.32541
	H2150, O-F-177882	Norway. Møre og Romsdal. Skodje	2004	62.4871	6.4937

<i>H. corium</i>	H2151, O-F-81427	Norway. Østfold. Rakkestad. Kilebutangen	1995	59.3261	11.5772
	H2153, O-F-174777	Norway. Nordland. Rana. Steinbruddet	1954	66.4390	14.3492
	H2156, O-F-71592	Norway. Buskerud. Os. Ustaoset	1989	60.6107	8.03354
	H2157, O-F-174746	Norway. Oppland. Lom. Høyrokampen. Bøvertun	1956	61.7052	8.43794
	H2159, O-F-174745	Norway. Oppland. Dovre. Grimsdalen	1986	62.0631	9.5745
	H2163, O-F-177853	Norway. Hedmark. Kongsvinger. Varåsen	1993	60.2135	11.9858
	H2164, O-F-174769	Norway. Sør-Trøndelag. Oppdal. Kongsvoll	1952	62.5367	9.51846
	H2165, O-F-174776	Norway. Sør-Trøndelag. Røros	1962	62.5650	11.6868
	H2166, O-F-68742	Norway. Sør-Trøndelag. Bjugn. Duehellaren	1979	63.9796	9.51307
	H2169, O-F-174760	Norway. Vest-Agder. Kristiansand. Strai	1976	58.1821	7.9117
	H2171, O-F-174751	Norway. Oppland. Ringebu. Ringebufljellet	1979	61.5633	10.2990
	H2172, O-F-174748	Norway. Oppland. Fron by Øyangen	1985	61.417796	9.216748
	H2174, O-F-174767	Norway. Hordaland. Odda. Ved smelteverket	1953	59.9661	6.86071
	H2175, O-F-174761	Norway. Rogaland. Klepp. Orre	1990	58.7654	5.54165
	H2176, O-F-174743	Norway. Akershus. Nes. Kvernberget. Vormsund	1951	60.1478	11.4063
	H2178, O-F-174755	Norway. Buskerud. Krådsherad. N Hamremoen	1974	60.1769	9.748
	H2179, O-F-174747	Norway. Oppland. Dovre. Grimsdalen. Tverråi	1956	62.1213	9.45125
	H2181, O-F-157971	Norway. Aust-Agder. Evje og Hornnes. Linddalen	2005	58.6579	7.6076
	H2182, O-F-174766	Norway. Hordaland. Ulvik. Finse. Blåisen	1960	60.6272	7.19296
	H2184, O- XXXXXX	Svalbard. Longyearbyen. Longyeardalen	2017	78.203602	15.611787
	H2185, O- XXXXXX	Svalbard. Longyearbyen. Longyeardalen	2017	78.203602	15.611787
	H2186, O- XXXXXX	Svalbard. Longyearbyen. Longyeardalen	2017	78.203602	15.611787
	H2187, O- XXXXXX	Svalbard. Longyearbyen. Longyeardalen	2017	78.203602	15.611787
	H2188, O-F-174756	Norway. Aust-Agder. Birkenes. Oggevatn	1985	58.4437	8.1128
	H2201, UME-26841	Sweden. Västerbotten. Vännäs	1974	63.978862	19.635679
	H2202, UME-33191	Sweden. Västerbotten. Löfvånger	1983	64.465881	21.580379
	H2205, UME-25412	Sweden. Västerbotten. Umeå	1970	63.871989	20.232382

<i>H. corium</i>	H2207, UME-26840	Sweden. Norrbotten. Älvsby	1973	65.74345	20.65949
	H2208, UME-25418	Sweden. Västerbotten. Umeå	1970	63.880903	20.201515
	H2209, UME-29681	Norway. Hedmark. Folldal	1985	62.15349	9.99101
	H2210, GB-0133953	Sweden. Härjedalen. Tännäs	2011	62.4424	12.6796
	H2211, GB-0063444	Sweden. Dalarna. Särna	2008	61.6941	13.1399
	H2212, GB-0063443	Sweden. Härjedalen. Tännäs	1981	62.4424	12.6796
	H2213, GB-0072565	Sweden. Jämtland. Åre	1976	63.3929	12.9287
	H2214, GB-0063442	Sweden. Västergötland. Trollhättan	1981	58.2788	12.2791
	H2215, GB-0054005	Sweden. Lycksele lappmark. Tärna	1976	65.7102	15.2624
	H2216, GB-0054004	Sweden. Lycksele lappmark. Tärna	1976	65.7102	15.2624
	H2217, GB-0045931	Sweden. Uppland. Österlövsta	1975	60.4365	17.7786
	H2219, GB-0045930	Sweden. Västergötland. Gårdhem	1981	58.2445	12.3344
	H2234, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.5750468	7.4829638
	H2236, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.593716	7.524507
	H2247, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.0626658	9.5976781
	H2248, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.0626658	9.5976781
	H2249, NA	Norway. Oppland. Dovre. Grimsdalen	1983	62.087647	9.6406363
	H2250, NA	Norway. Oppland. Dovre. Grimsdalen	1996	62.091482	9.641733
	H2251, NA	Norway. Oppland. Dovre. Grimsdalen	1989	62.091482	9.641733
	H2257, NA	Norway. Oppland. Vang. Eidsbugarden	1996	61.3756389	8.2996417
	H2258, NA	Norway. Hedmark. Ulvik. Finse	2007	60.593716	7.524507
	H2260, NA	Norway. Oppland. Vang. Eidsbugarden	1996	61.3756389	8.2996417
	H2261, NA	Norway. Hedmark. Ulvik. Finse	2016	60.598977	7.509829
	H2263, TRH-F-8173	Norway. Østfold. Fredrikstad. Lisleby	1981	59.1985	10.8735
	H2264, TRH-F-1574	Norway. Sør-Trøndelag. Trondheim. Bymarka	1980	63.4316	10.2925
	H2265, TRH-F-23623	Norway. Sør-Trøndelag. Oppdal, S of Storvollen	2002	62.422	9.8809
	H2266, TRH-F-9202	Norway. Sør-Trøndelag. Bjugn. Duehellaren	1979	63.7991	9.7104

<i>H. corium</i>	H2267, TRH-F-1579	Norway. Sør-Trøndelag. Agdenes	1978	63.5279	9.61812
	H2268, TRH-F-10219	Norway. Finnmark. Kautokeino. Suoluvuobmi	2001	69.5838	23.5307
	H2269, TRH-F-1580	Norway. Finnmark. Nesseby. Ræppen	1978	70.0718	28.7088
	H2270, TRH-F-24593	Norway. Finnmark. Kautokeino. Suoluvuobmi	1984	69.5909	23.5314
	H2271, TRH-F-1576	Norway. Møre og Romsdal. Aure. Kvernavatnet	1993	63.4102	8.7097
	H2272, TRH-F-1583	Norway. Nordland. Hattfjelldal. Elsvatnveien	1971	65.5923	14.0241
	H2273, TRH-F-1582	Norway. Finnmark. Nesseby	1992	70.1038	28.6456
	H2274, TRH-F-1581	Norway. Sør-Trøndelag. Bjugn. Duehellaren	1979	63.7991	9.7104
	H2275, TRH-F-1578	Norway. Sør-Trøndelag. Oppdal. Grønbakken	1975	62.2735	9.6743
	H2279, TRH-F-7095	Norway. Finnmark. Kautokeino. Suoluvuobmi	1996	62.2533	9.4909
	H2281, S-F-XXXXXX	Sweden. Härjedalen. Tännålen. Hamrafjället	2015	62.573413	12.225434
	H2283, S-F-XXXXXX	Norway. Nordland. Rana. Virvassdalen	2003	66.3984484	15.1653978
	H2285, S-F-XXXXXX	Sweden. Lappland. Abisko	2009	68.354489	18.815750
	H2287, S-F-XXXXXX	Sweden. Lappland. Abisko	2009	68.354489	18.815750
	H2288, S-F-XXXXXX	Finland. Perä-Pohjanmaa Prov. Tornio. Kalkkimaa	2013	65.910651	24.469414
	H2289, S-F-XXXXXX	Sweden. Lappland. Abisko	2009	68.354489	18.815750
<i>H. macrosperma</i>	H029, O-253328	Norway. Oppland. Dovre. Grimsdalen	2007	62.0349474	9.490123
	H047, O-253329	Norway. Oppland. Dovre. Grimsdalen	2009	62.0894225	9.7304371
	H050, O-253330	Norway. Oppland. Dovre. Grimsdalen	2009	62.0349474	9.490123
	H053, O-253331	Norway. Oppland. Dovre. Grimsdalen	2009	62.0349474	9.490123
	H947, O-XXXXXX	Norway. Nordland. Saltdal. Junkerdalen	2016	66.824069	15.495279
	H1982, O-XXXXXX -	Norway. Troms. Målselv. Håkkåfjellet N	2017	68.873056	18.852500
	H1997, O-XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017	69.291944	19.413889
	H2100, O-XXXXXX	Norway. Troms. Balsfjord. Lakselvbukt	2017	69.291944	19.413889
	H2146, O-F-285169	Norway. Oppland. Lom. Lom. Høyrokampen	2007	61.6422	8.0541
<i>H. nannfeldtii</i>	H017, O-253337	Norway. Oppland. Dovre. Grimsdalen	2007	62.0349474	9.490123
	H027, O-253338	Norway. Oppland. Dovre. Grimsdalen	2009	62.0349474	9.490123
	H028, O-253339	Norway. Oppland. Dovre. Grimsdalen	2007	62.0349474	9.490123

<i>H. nannfeldtii</i>	H188, O-253340	Norway. Oppland. Dovre	2005	62.0349474	9.490123
	H212, O-253332	France. Savoie, Val d'isere	1992	45.432188	6.982487
	H216, O-253333	France. Savoie. Bon Valle	1992	45.432188	6.982487
	H254, O-253341	Norway. Oppland. Dovre	1996	62.017185	9.469969
	H318, C-F-92123	Norway. Nordland	1974	66.421883	15.136483
	H362, O-253342	Norway. Oppland. Dovre	1996	62.0349474	9.490123
	H367, O-253343	Norway. Oppland. Dovre	1985	62.0349474	9.490123
	H452, O-253334	Norway. Hordaland. Ulvik. Finse	2014	60.598977	7.509829
	H454, O-253335	Norway. Hordaland. Ulvik. Finse	2014	60.598977	7.509829
	H457, O-253336	Norway. Hordaland. Ulvik. Finse	2014	60.598977	7.509829
	H479, O-253344	Norway. Oppland. Dovre	1983	62.0349474	9.490123
	H 542, NA	Norway. Ulvik.Finse.Middalsbreen	2007	60.5731763	7.4735135
	H 544, C-F-85371	Austria. Wattentaler Lizum	1998	47.17250	11.63778
	H 545, NA	Austria. Tirol. Obergurgl	1990	46.871930	11.027065
	H 549, NA	Norway. Oppland. Dovre. Grimsdalen	1981	62.0349474	9.490123
	H 550, NA	Norway. Oppland. Dovre. Sjøberget	1981	62.0713611	9.656
	H564, NA	France. Savoie. Val d'Isere	1992	45.430260	6.981125
	H575, NA	Norway. Oppland.Dovre.Grimsdalen	1982	62.0349474	9.490123
	H717, UPS-F-145390	Sweden. Torne Lappmark. Jukkasjärvi	1960	68.03292	20.29801
	H721, UPS-F-145394	Sweden. Torne Lappmark. Jukkasjärvi	1946	68.03292	20.29801
	H723, UPS-F-145399	Iceland. Nordurland eystra	1971	65.674806	-17.598255
	H866, S-F-122367	Sweden. Torne Lappmark. Jukkasjärvi	1936	68.03292	20.29801
	H886, S-F-122246	Sweden. Härjedalen. Tännäs	1978	62.4872	12.57119
	H891, S-F-122251	Sweden. Härjedalen. Tännäs	1974	62.4872	12.57119
	H917, TROM-F-11413	Norway. Nordland. Fauske. Laksdalen	1967	67.2098	15.8097
	H920, TROM-F-11409	Norway. Nordland. Ballangen. Langvatn	1970	68.1092	17.0793
	H922, TROM-F-41054	Norway. Nordland. Ballangen. Langvatn	1970	68.1092	17.0793
	H954, O- XXXXXX	Norway. Hedmark. Folldal. Einunndalen	2016	62.2577542	10.1932519
	H958, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen, Grimsa	2016	62.044175	9.498163

<i>H. nannfeldtii</i>	H959, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen, Grimsa	2016	62.044175	9.498163
	H960, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen, Grimsa	2016	62.044175	9.498163
	H1056, O- XXXXXX	Norway. Hedmark. Folldal. Einunndalen	2016	62.2325035	10.2373824
	H1090, C-F-63723	Greenland. Oaqortoq area. North of Oanisartut	1971	60.720478	-46.034217
	H1093, C-F-86946	Greenland. Narssarssuaq	1991	61.163042	-45.428081
	H1105, C-F-86947	Greenland. Narssarssuaq	1991	61.163042	-45.428081
	H1137, C-F-63821	Greenland. Oeqertarssuaq. Godhavn	1977	69.244974	-53.541913
	H1152, C-F-86996	Greenland. Disko. Oullissat	1932	69.853047	-53.432315
	H1154, C-F-45368	Greenland. Kutsiaq	1947	70.6667	-52.4500
	H1157, C-F-54968	Norway. Nordland. Rana. Midtistua mountain	1981	66.7607695	15.0129
	H1160, C-F-54599	Norway. Nordland. Rana. Virvassdalen	1979	66.3354	15.2121
	H1162, C-F-54600	Norway. Nordland. Rana. Virvassdalen	1970	66.3354	15.2121
	H1164, C-F-102965	Norway. Nordland. Rana. Virvassdalen	1975	66.3354	15.2121
	H1166, C-F-102967	Norway. Nordland. Rana. Ørtfjellmoen	1974	66.3354	15.2121
	H1167, C-F-102968	Norway. Nordland. Rana. Umskartjønn	1968	66.3354	15.2121
	H1170, C-F-54985	Norway. Nordland. Rana. Midtistua	1981	66.7607695	15.0129
	H1171, C-F-53258	Norway. Finnmark. Biggeluobal. W of Masi	1978	69.366667	23.433333
	H1174, C-F-54447	Norway. Nordland. Rana. Skaret W of Rauvatnet	1975	66.1343887	14.2679386
	H1175, C-F-54598	Norway. Nordland. Rana. Virvassdalen	1979	66.3354	15.2121
	H1177, C-F-54602	Norway. Nordland. Rana. Virvassdalen	1979	66.3354	15.2121
	H1196, C-F-45360	Norway. Troms. Tromsdalen	1969	69.650583	18.995362
	H1962, O- XXXXXX	Norway. Troms. Balsfjord. Stålvikbotn	2017	69.302500	18.893056
	H1971, O- XXXXXX	Norway. Troms. Kåfjord. Guolasjärvi	2017	69.346389	21.058333
	H1972, O- XXXXXX	Norway. Troms. Kåfjord. Guolasjärvi	2017	69.375556	21.069722
	H1976, O- XXXXXX	Norway. Troms. Balsfjord. Middagsnes	2017	69.426944	18.977778
	H1977, O- XXXXXX	Norway. Troms. Balsfjord. Middagsnes	2017	69.426944	18.977778
	H1979, O- XXXXXX	Norway. Troms. Balsfjord. Middagsnes	2017	69.426944	18.977778
	H1984, O- XXXXXX	Norway. Troms. Målselv. Frøkentindelva	2017	68.876944	18.831944
	H1985, O- XXXXXX	Norway. Troms. Målselv. Frøkentindelva	2017	68.876944	18.831944

<i>H. nannfeldtii</i>	H1991, O- XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.850833	18.907222
	H1992, O- XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.850833	18.907222
	H1993, O- XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.850833	18.907222
	H1994, O- XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.850833	18.907222
	H2103, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen	2017	62.032500	9.496111
	H2104, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen	2017	62.032500	9.495833
	H2107, O- XXXXXX	Norway. Oppland. Dovre. Grimsdalen	2017	62.041667	9.488611
	H2123, O-F-174780	Norway. Nordland. Rana. Rauvatn	1975	66.13636	14.27847
	H2158, O-F-174752	Norway. Oppland. Dovre. Grimsdalen. Grimsa	1976	62.0629	9.6026
	H2160, O-F-174781	Norway. Nordland. Rana. Krokstrand. Tørrbekken	1975	66.4701	15.0337
	H2168, O-F-81594	Norway. Sør-Trøndelag. Oppdal. Knutshø	1994	62.5367	9.51846
	H2170, O-F-174753	Norway. Buskerud. Hol. Ustaoset. Djuptjønnhovda	1960	60.54	7.9593
	H2183, O-F-174779	Norway. Nordland. Rana. Virvassdalen	1975	66.3354	15.2121
	H2206, UME-26839	Sweden. Lycksele lappmark. Tärna	1967	65.810719	15.231846
	H2233, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.5750468	7.4829638
	H2235, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.5750468	7.4829638
	H2237, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.5750468	7.4829638
	H2238, O- XXXXXX	Norway. Hedmark. Ulvik. Finse	2017	60.5750468	7.4829638
	H2239, NA	Norway. Oppland. Dovre. Grimsdalen	1982	62.0665936	9.5644765
	H2240, NA	Norway. Oppland. Dovre. Grimsdalen	1983	62.0665936	9.5644765
	H2245, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.099794	9.636997
	H2252, NA	Norway. Oppland. Dovre. Grimsdalen	1984	62.023625	9.4754556
	H2259, NA	Norway. Oppland. Dovre. Grimsdalen	1981	62.0737361	9.5417389
	H1515, C-F-53793	Norway. Nordland. Rana	1972	66.3354	15.2121
	H1542, C-F-86745	Norway. Nordland. Rana	1993	66.3354	15.2121
	H2276, TRH-F-1577	Norway. Nordland. Rana. Virvassdalen. Blerekåga	1979	66.3444	15.2122
	H2282, S-F-XXXXXX	Norway. Nordland. Dunderlandsdalen. Lian	2003	66.4159056	14.8049111
	H2284, S-F-XXXXXX	Norway. Nordland. Dunderlandsdalen. Lian	2003	66.4159056	14.8049111

<i>H. nannfeldtii</i>	H2286, S-F-XXXXXX	Sweden. Lappland. Kiruna. Låktatjåkka	2009	68.423736	18.331525
<i>H. pseudoalpina</i>	H349, C-F-63820	Greenland. Qeqertarsuaq	1977	69.244366	-53.543432
	H498, O-XXXXXX	Norway. Svalbard. Longyearbyen	2015	78.2163884	15.5957623
	H941, TROM-F-11412	Norway. Troms. Tromsø. Tromsdalen	1961	69.6399	18.9909
	H942, TROM-F-11405	Norway. Troms. Tromsø. Tromsdalen	1961	69.6399	18.9909
	H943, TROM-F-11404	Norway. Troms. Tromsø. Tromsdalen	1961	69.6399	18.9909
	H1130, C-F-45366	Greenland. Christianshåb	1969	68.823646	-51.182642
	H1965, O-XXXXXX	Norway. Troms. Meistervik. Middagsnes	2017	69.302222	18.892500
	H1966, O-XXXXXX	Norway. Troms. Meistervik. Middagsnes	2017	69.302222	18.892500
	H1967 O-XXXXXX	Norway. Troms. Meistervik. Middagsnes	2017	69.302222	18.892500
	H1274, C-F-86897	Greenland. Narssaussuaq	1991	61.163042	-45.428081
	H2278, TRH-F-20631	Norway. Nordland. Saltdal. Junkerdalen	1988	66.8193	15.4439
<i>Helvella</i> sp.	H1995, O-XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.854722	18.906944
	H1996, O-XXXXXX	Norway. Troms. Målselv. Iselvdalen	2017	68.854722	18.906944

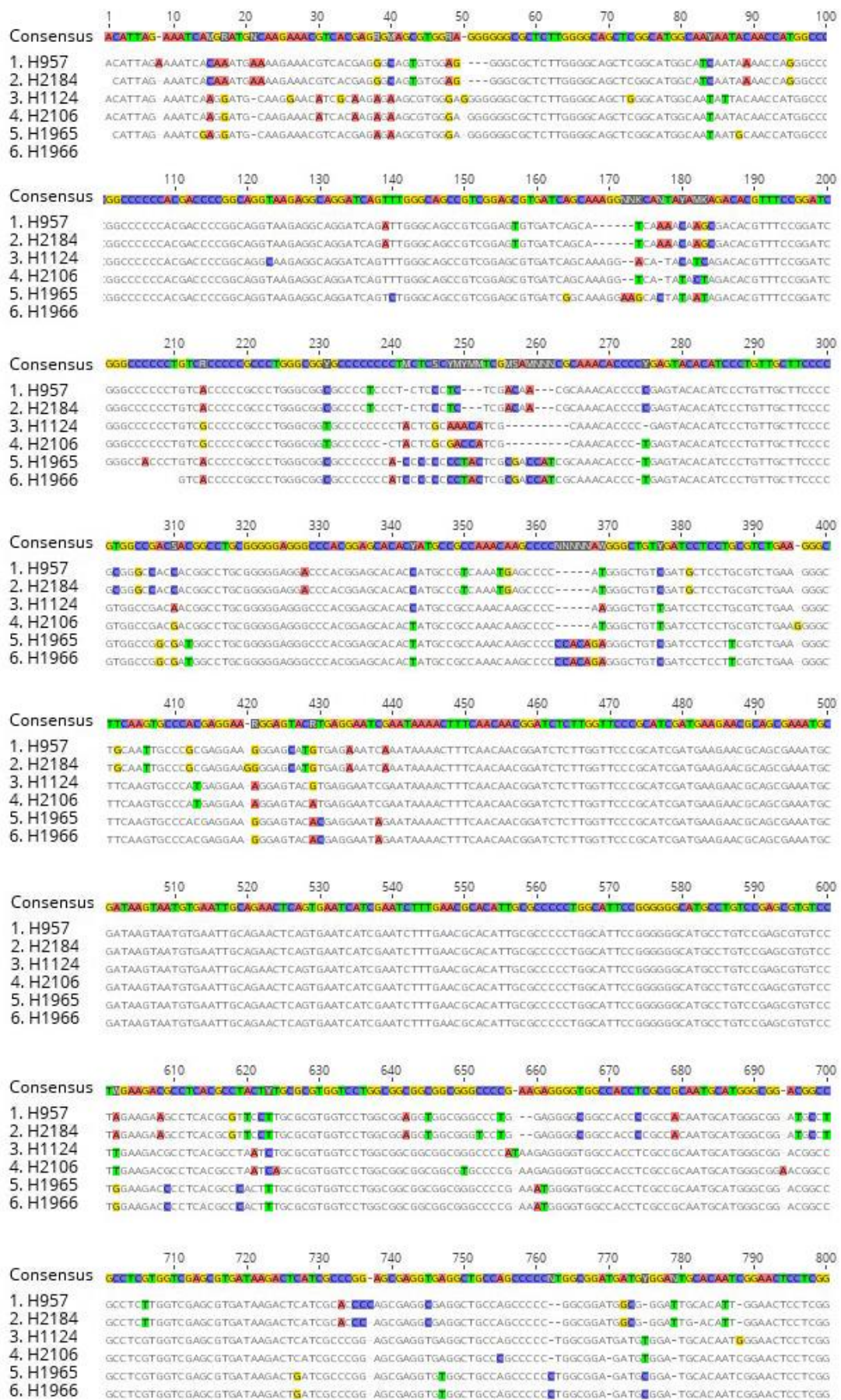


Figure S1: Alignment across the ITS region (ITS1, 5.8S and ITS2) for species of the alpina-corium lineage of the *Helvella corium* morphospecies complex. H957 and H2184 represent *H. corium*, H1124 and H2106 represent *H. alpina*. H1965 and H1966 represent *H. pseudoalpina*.

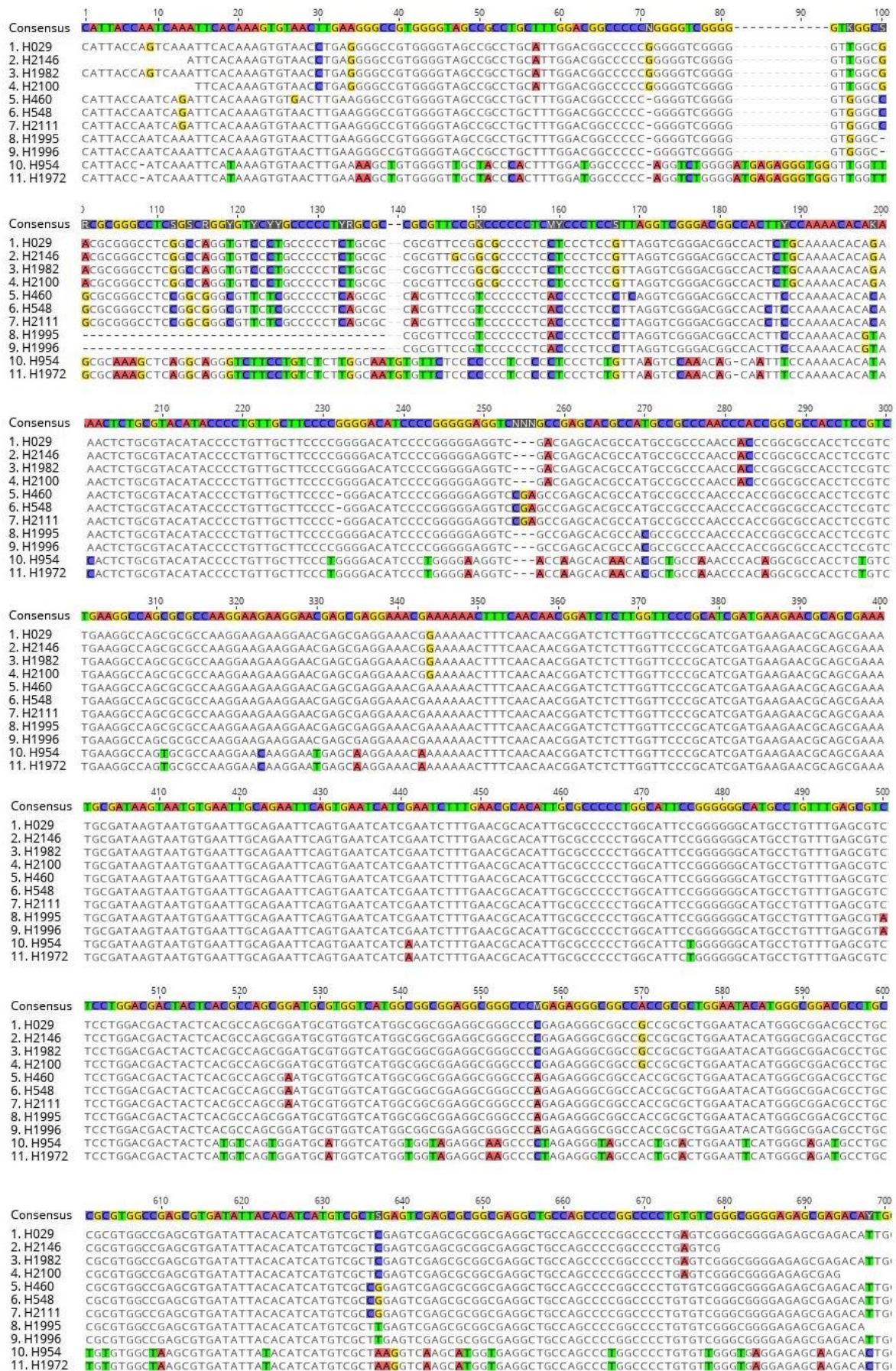


Figure S2: Alignment across the ITS region (ITS1, 5.8S and ITS2) for species of the alpestris-nannfeldtii lineage of the *Helvella corium* morphospecies complex. H029, H2146, H1982 and H2100 represent *H. macrosperma*. H460, H548 and H2111 represent *H. alpestris*. H1995 and H1996 are denoted *Helvella* sp. H954 and H1972 represent *H. nannfeldtii*.