

Hemileucoglossum pusillum, an earthtongue new to Norway

Per Fadnes¹, Anna G. Fedosova², Viktor Kučera³

¹Faculty of Education, Science and Sports, Institute for Sport, Nutrition and Natural Sciences, Western Norway University of Applied Sciences, campus Stord.

²Laboratory of Systematics and Geography of Fungi,
Komarov Botanical Institute of the Russian Academy of Sciences, RU-197376,
2 Prof Popov Str., Saint Petersburg, Russia.

³Plant Science and Biodiversity Center, Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovakia.

Corresponding author:
per.fadnes@hvl.no

Norsk tittel: *Hemileucoglossum pusillum*, en ny jordtunge for Norge

Fadnes P, Fedosova AG, Kučera V, 2021. *Hemileucoglossum pusillum*, an earthtongue new to Norway. *Agarica* 2021, vol. 42: 65-73

KEYWORDS

Geoglossaceae, *Hemileucoglossum*, earth-tongues, Norway

NØKKELOORD

Geoglossaceae, *Hemileucoglossum*, jordtunger, Norge

SAMMENDRAG

Hemileucoglossum pusillum ble første gang beskrevet fra Slovakia i 2017, og den er ellers kjent fra Spania. Første funn i Norge ble gjort allerede i 2010, og er seinere gjenfunnet de fleste år frem til i dag på samme lokalitet. På den tiden var det gjort lite med revisjon av slektene innenfor Geoglossaceae, og funnet forble ubestemt helt frem til i dag. Ved hjelp av mikroskopi og DNA analyser er det nå bekreftet at arten er *Hemileucoglossum pusillum*, som er første registrering av denne arten i Norge. *Pusillum* betyr liten og puslete, så et forslag til norsk navn kan være puslejordtunge.

ABSTRACT

Hemileucoglossum pusillum was first described from Slovakia in 2017 and is also known from Spain. The first record in Norway was collected as early as 2010 and is later found almost every year since on the same spot. At that time, little was done concerning revision of the genera in Geoglossaceae, and the fungus has remained undetermined until today. By microscopy and molecular studies, it was confirmed that the species was *Hemileucoglossum pusillum*, which is the first record of this species in Norway.

INTRODUCTION

The family Geoglossaceae (*sensu lato*) is very well represented in unfertilized grassland in the Southwestern part of Norway. At least 15 different species are known from the area, from the genera *Geoglossum* (*sensu lato*), *Trichoglossum* and *Microglossum* (Fadnes 2011) (*Microglossum* is now excluded from Geoglossaceae (*sensu stricto*)). The family Geoglossaceae is currently undergoing great changes, demonstrated by different molecular studies (Hustad et al. 2011 and 2013, Arauzo & Iglesias, 2014, Fedosova et al. 2018). New genera have been proposed like *Glutinoglossum* including the former *Geoglossum glutinosum*, *Sabuloglossum* including *Thuemenidium* (*Geoglossum*) *arenarium* (Hustad et al. 2013), and *Hemileucoglossum* to accommodate the

species *Geoglossum littorale* and allies (Arauzo & Iglesias 2014). Before these revisions took place, already in 2010, a new unknown earth-tongue was found in SW-Norway. With available keys, it was not easy to determine. However, there were some similarities with *Geoglossum lineare* described by Hakelien (1967). Especially the form of the paraphyses was similar as well as the spores. However, *G. lineare* should have viscid stipe, and pale brown spores, while the stipe of this species was squamulose and the spores are hyaline. It therefore until now remained an unknown species.

The article by Arauzo & Iglesias (2014) brought new information on the revision of the Geoglossaceae, but an exact determination requires molecular studies.

After ten years of uncertainty, it was microscopically identified by one of the authors (VK) and later confirmed by molecular studies. According to DNA (ITS and LSU) this was *H. pusillum*, and therefore a new species of earthtongues in Norway. This taxon is previously only known from Spain (five locations) and Slovakia (two locations).

MATERIAL AND METHODS

Fresh material of the ascocarp was studied microscopically after soaking in water using a LEICA DM750 microscope and LEICA EZ4W stereo binocular. Spores, asci, paraphyses and setae hair were photographed by an integrated microscope-camera, LEICA ICC50 W and measured by LEICA application Suite (LAS) EZ software.

Molecular studies have been performed, and the fungus has been found identical with the type material. The DNA extraction, amplification and sequencing of the internal transcribed spacer region of the nuclear ribosomal DNA (nrITS1–5.8S–ITS2, ITS) and the 28S nuclear ribosomal large subunit region (nrLSU, LSU) were provided on commercial base in Alvalab (Spain).

Newly generated sequences were submitted in NCBI GenBank (<http://www.ncbi.nlm.nih.gov>, accession numbers: MW295710, MW295713)

Individual ITS and LSU datasets were created in MEGA7 (Kumar et al. 2016) and then were aligned in MAFFT v.7 Web tool (Katoh et al. 2019). Ambiguous regions were eliminated from individual alignments using TrimAl v.1.2b (Capella-Gutiérrez et al. 2009). The best-fit AICc-selected model of evolution (SYM+I+G for ITS and GTR+I+G for LSU) was calculated by PartitionFinder 2 (Lanfear et al. 2017).

The maximum likelihood (ML) phylogenetic analysis was run in RAxML v.7.2.6 (Stamatakis 2006). The Bayesian analyses (BA) was performed using MrBayes v.3.2.7 (Ronquist et al. 2012). Four independent chains were run one million generations with trees sampled every 100 generations. To evaluate the quality of a sample from the posterior and the continuous parameters, effective sample size (ESS) was estimated in Tracer v.1.7.1 (Rambaut et al. 2018). The clades with bootstrap support (BS) value $\geq 80\%$ for ML analysis and posterior probability (PP) value > 0.95 for BA analysis were considered significant.

Individual alignments of ITS and LSU were concatenated into a single dataset. Further ML and BA analyses were performed on the combined dataset as described above except for four independent chains were run ten million generations. Alignments with obtained phylogenies were deposited in TreeBASE (<http://treebase.org>) under the submission ID 27328.

TAXONOMICAL PART

Original description

The original description is cited from Crous, PW, et al. (2017).

Ascocarps scattered to gregarious, clavate, stipitate, 0.8–3.5 cm tall, 0.1–0.5 cm wide, black throughout. Ascigenous part clavate,

broadly clavate or compressed, c. 1/4–1/2 of the total ascocarp length, 0.2–1.1 cm long, black, concolorous with the stipe, compressed or oval in cross section, sharply delimited from the stipe, smooth both in fresh and dry conditions. Stipe terete, cylindrical, oval in cross section, slender to robust, conspicuously hairy with dark brown setose hairs in tufts in upper part of the stipe when fresh, rough to squamulose when dry.

Asci clavate to broadly clavate, (135–)141.5–181.5(–187) \times (14–)15.5–23.5(–25) μm (measured in water), $Q = (6.3\text{--})6.8\text{--}8.7(9.5)$, 8-spored, with euamyloid apical ring and inamyloid wall in MLZ and IKI.

Ascospores elongate-clavate, subfusiform to fusiform, narrowed to the base, sometimes slightly curved, (41–)50–76.5(–82) \times (5–)5.5–7.5(–8) μm (in water), $Q = (8.5\text{--})12.6(15.2)$, hyaline, finally in some asci becoming brown, predominantly 3–4-septate, rarely with 0–5 (–6) septa. Ascoconidia not observed.

Paraphyses cylindrical, sparsely septate, 2–3 μm diam, straight to slightly curved and inflated at the apex, hyaline at basal part to pale brown at the apex, embedded in a dense brown amorphous matter, extending beyond the asci. Apical cells usually inflated and constricted or pyriform, sometimes proliferating (12.5–)18.5–46(–54) \times (4.5–)6–8.5(–11) μm .

Stipe surface squamulose of protruding paraphysal elements forming scales and with tufts of dark brown setose septate hairs (85–)90–120(–144) μm long, straight, moderately septate, basal cell usually inflated, (7–)10–13 (–17.5) μm , medial part (4.5–)5.5–7(–9.5) μm and apical part (2–)3 μm with rounded apex.

Description of the Norwegian material

Specimen molecular studied:

Location: Hovaneset, Stord municipality, Vestland county.

Date of collecting: 10.09.2017

Coordinates: 32V LM 05902,34580

Collector: Per Fadnes

Herbarium number: O-F-257329.

Macro- and micromorphological studies are in addition based on several collections from the same spot from different years. These collections are stored in a private herbarium by one of the authors (PF).

Locality

Specimens of *H. pusillum* were found during several years from 2010 to 2020. The locality is a semi natural calcareous grassland (grazed by sheep) in a peninsula (Hovaneset) in Stord municipality, SW-Norway. Grassland fungi on this locality have been studied since 2003, the last 11 years weekly during the season. Results from the 11 first years were published in 2014 (Fadnes 2014), and a number of totally 71 different grassland fungi, among them ten earthtongues have been found in the locality. During the last seven years, the number has raised to 90 different species, making it the most species rich locality of grassland fungi in Norway known today. *H. pusillum* grows in a north facing hill only a few meters from the seashore. It is heavily embedded in a dense moss-carpet, and due to its very small size it can be difficult to spot. However, it has been found eight times during the last 11 years, appearing on the same spot (Fig. 1).

Macromorphology

Ascocarps are black throughout, mostly scattered, clavate, stipitate, 1.0–3.5 cm tall – they vary in size but are relatively small compared to other earthtongues (Fig. 2). Ascigenous part is clavate, sometimes compressed, twisted and folded, spatuliform, sometimes irregularly lobed and sometimes with longitudinal groove(s), 0.4–0.8 cm wide, 0.6–1.0 cm long, smooth, round to oval, normally sharply delimited from the stipe.

Stipe cylindrical sometimes curved, slender to robust depending on size of the ascocarp,



Figure 1. Locality where *H. pusillum* grows in Hovaneset, Stord municipality, SW-Norway. Photo: PF

1–2.3 cm long, 0.13–0.17 cm wide. Squamulose, rough due to tufts of setose hair especially in the upper part.

Micromorphology

Asci clavate to broadly clavate, (127–)140–180(–220) × 17–22 μm, Q = 7.2–9.0 (–9.5) (measured in water), eight spored (Figure 3).

Ascospores elongate, clavate, fusiform often tapering to one base, often curved, normally hyaline, 0–4 septate. Septa often difficult to spot, seldom more than four septa, (41–) 52–70 (–75) × (4.5–) 5.5–7.5 (–8.2) μm, Q = 7.0–12.2. (Fig. 4A and B)

Paraphyses cylindrical, sparsely septate, 2–4 μm in diameter, up to 7–8 μm at the terminal cell, which is some inflated, constricted or pyriform at the apex. Paraphyses are hyaline but brown at apex, embedded in a dense brown amorphous matter and extending beyond the asci (Fig. 3).

Stipe surface squamulose due to tufts of brown septate setae hairs extending from the surface. Basal cell normally inflated, broader than the rest of the setae hair. Size of hair (70–) 90–140 (–160) μm with rounded apex (Fig. 5).



Figure 2. Ascocarps of *H. pusillum* from Hovaneset, SW-Norway. Photo: PF

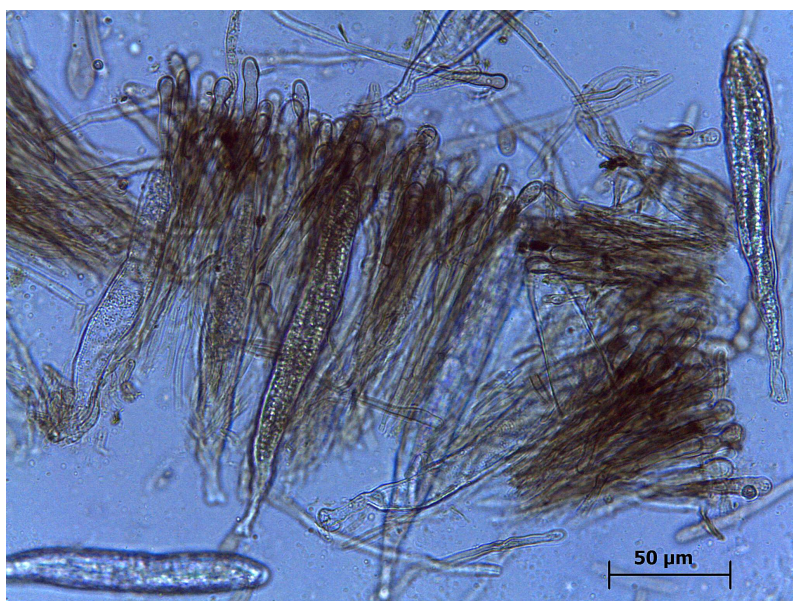


Figure 3. Asci and paraphyses of *H. pusillum* with dense brown amorphous matter from Hovaneset, SW-Norway. Photo: PF

Molecular study

Table 1 represents 25 specimens involved in the phylogenetic analysis. Five type specimens including holotype of *H. pusillum* were used in the analyses. *Graddonina coracina* (Bres.) Dennis was taken as an outgroup. Two newly generated sequences (one ITS and one LSU) were obtained for this study. In total 25 ITS

and 23 LSU sequences were analyzed.

The concatenated ITS-LSU data matrix had an aligned length 1521 bp, which was reduced to 1321 bp after elimination of 200 bp by TrimAl. To remove the prestationary posterior probability distribution burn-in of 19% (ESS = 10672.1) was estimated with Tracer to be sufficient. The most likely tree topology produced by the ML analysis of the combined ITS-LSU dataset is illustrated (Fig. 6).

The phylogenetic analyses confirmed identity of the Norwegian specimen and specimens of *H. pusillum* from Slovakia and Spain.

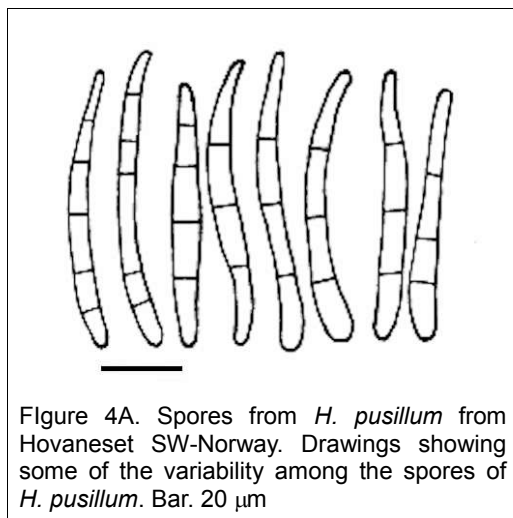


Figure 4A. Spores from *H. pusillum* from Hovaneset SW-Norway. Drawings showing some of the variability among the spores of *H. pusillum*. Bar. 20 µm

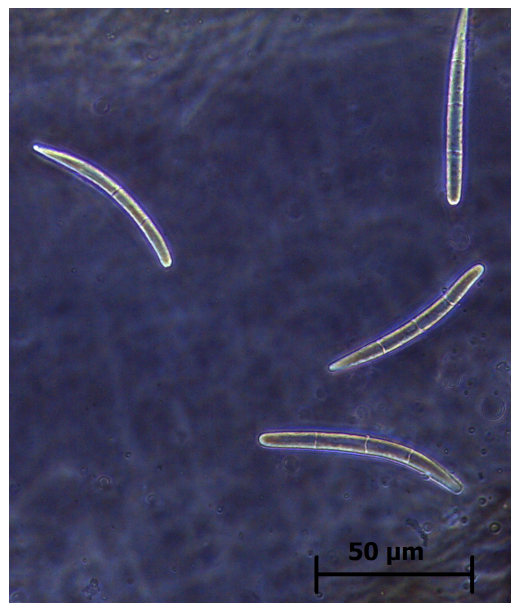


Figure 4B. Spores from *H. pusillum* from Hovaneset, SW-Norway. Photo: PF

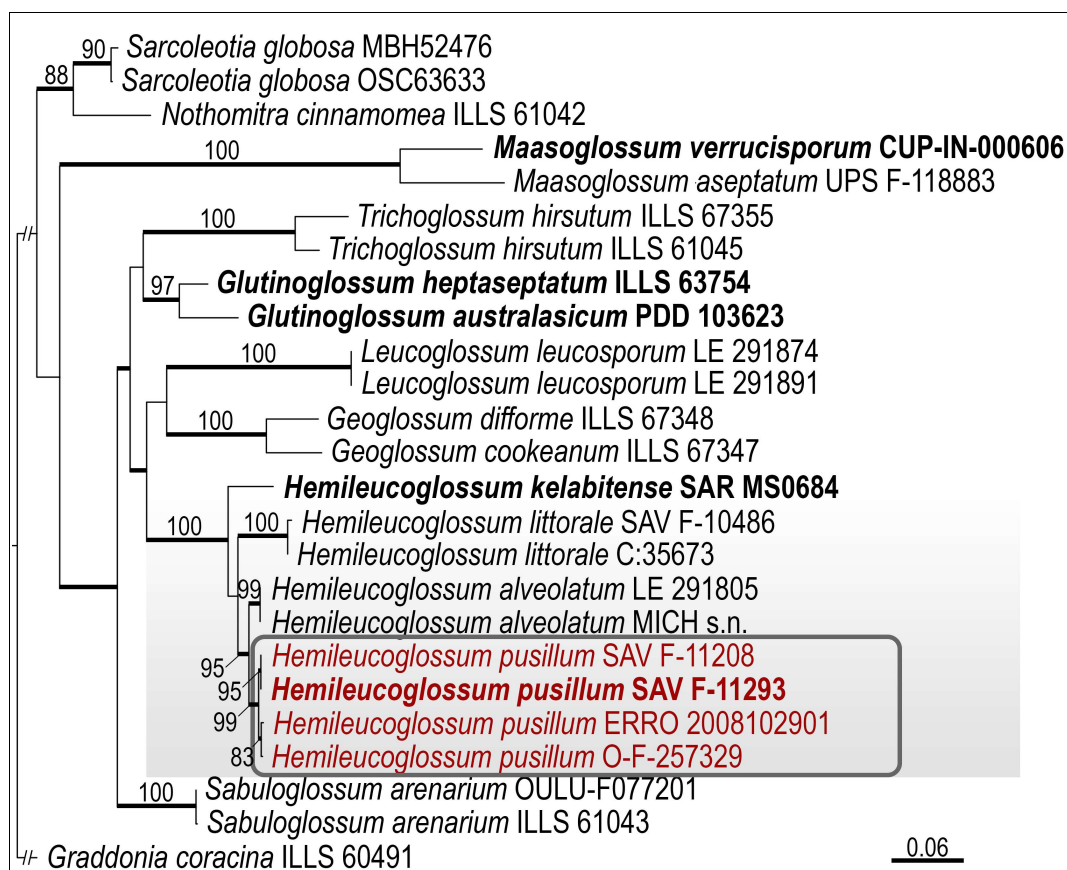


Figure 6. Phylogenetic tree generated from maximum likelihood analysis based on ITS-LSU sequence data. Numbers above branches indicate BS values > 80%, thickened branches indicate PP value > 0.95. Holotypes and isotype are in bold. The clade *H. pusillum* is indicated with box and color. Affiliation to the genus *Hemileucoglossum* is highlighted. Scale bar represents the number of nucleotide changes per site.

Sequences from all these specimens formed a strongly supported clade of *H. pusillum* (BS=99%, PP=1). A sister clade to *H. pusillum* was *H. alveolatum*.

COMMENTS

The taxonomy of Geoglossaceae has historically been difficult due to lack of good morphological characters to distinguish the different species. The group includes species mostly with black or blackish color and generally stipitate, claviform or capitate form. The ascocarps of most earthtongues genera and species

show a great variation both in size and in form, and microscopic characters are therefore important. The genus *Trichoglossum* is the easiest one, because it is densely covered with long setae hairs all over the ascocarp, and in Norway, we have until now, only recognized three different species. The genus *Geoglossum* (sensu lato) is the most difficult genus and have recently been revised by many authors. Schoch et. al. (2009) reduced the family only to include the genera *Sarcoleotia*, *Geoglossum* and *Trichoglossum*. *Microglossum*, which has earlier been included in

Table 1. Specimens and NCBI GenBank accession numbers of DNA sequences used in phylogenetic analysis.

Species	Country	Voucher No.	GenBank accession No.		Notes
			ITS	LSU	
<i>Geoglossum cookeanum</i>	Czech Republic	ILLS 67347	KC222122	KC222135	
<i>G. difforme</i>	USA	ILLS 67348	KC222123	KC222136	
<i>Glutinoglossum australasicum</i>	New Zealand	PDD 103623	KP690088	KP690100	holotype
<i>G. heptaseptatum</i>	Czech Republic	ILLS 63754	KC222130	KC222143	holotype
<i>Graddonina coracina</i>	USA	ILLS 60491	JQ256423	JN012009	outgroup
<i>Hemileucoglossum alveolatum</i>	USA	MICH s.n.	KP657560	KP657565	
<i>H. alveolatum</i>	Russia	LE 291805	MF353087	—	
<i>H. littorale</i>	Denmark	C:35673	KP657561	KP657566	
<i>H. littorale</i>	Slovakia	SAV F-10486	MF353089	MF353092	
<i>H. kelabitense</i>	Borneo	SAR MS0684	MT021979	MT021912	holotype
<i>H. pusillum</i>	Norway	O-F-257329	MW295710*	MW295713*	
<i>H. pusillum</i>	Slovakia	SAV F-11293	MF353090	MF353093	holotype
<i>H. pusillum</i>	Slovakia	SAV F-11208	MF353088	MF353091	
<i>H. pusillum</i> (as <i>H. littorale</i>)	Spain	ERRO 2008102901	KP144108	—	
<i>L. leucosporum</i>	Russia	LE 291891	KP272112	KP272113	
<i>L. leucosporum</i>	Russia	LE 291874	KP272114	KP272115	
<i>Maasoglossum aseptatum</i>	Sweden	UPS F-118883	KP657562	KP657567	
<i>M. verrucisporum</i>	Bhutan	CUP-IN-000606	KP657563	KP657568	isotype
<i>Nothomitra cinnamomea</i>	France	ILLS 61042	JQ256424	JQ256439	
<i>Sabuloglossum arenarium</i> (as <i>Thuemenidium arenarium</i>)	Netherlands	ILLS 61043	JQ256426	JQ256440	
<i>S. arenarium</i> (as <i>T. arenarium</i>)	Finland	OULU-F077201	GU324765	GU324764	
<i>Sarcoleotia globosa</i>		OSC63633	AY789410	AY789409	
<i>S. globosa</i>		MBH52476	AY789429	AY789428	
<i>Trichoglossum hirsutum</i>	Czech Republic	ILLS 61045	JQ256428	JQ256442	
<i>T. hirsutum</i>	USA	ILLS 67355	KC222132	KC222145	

* – sequences obtained in this study.

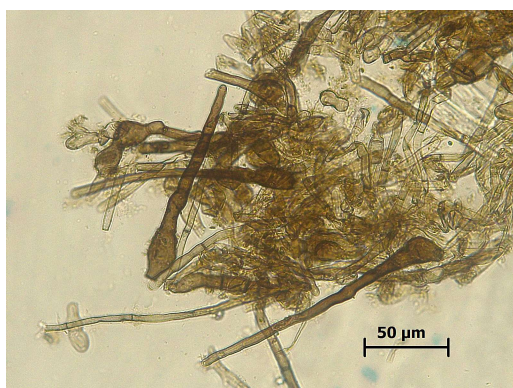


Figure 5. Setose hairs from stipe of *H. pusillum* from Hovaneset, SW-Norway. Photo: PF

Geoglossaceae, is now excluded, and shown to be very distant related based on molecular methods (Schoch et al 2009, Sandnes 2006). Hustad et al. (2011 and 2013) also included the genus *Nothomitra* in Geoglossaceae and created a new genus *Sabuloglossum* (including the former *Geoglossum arenarium*) and *Glutinoglossum* (including the former *Geoglossum glutinosum*). Studies of Fedosova et al. (2018) have later proposed 13 species in the genus *Glutinoglossum* by molecular studies, where seven species are known from Europe.

The studies of Arauzo & Iglesias (2014) confirmed the genus *Leucoglossum* proposed by Imai in 1942. They also proposed a new genus *Hemileucoglossum*, which includes species with hyaline spores and setae on the stipe showing resemblance with those of the genus *Trichoglossum*.

The type species of the genus *Hemileucoglossum*, *H. littorale* differs from *H. pusillum* in having smaller asci and spores, and maybe the best distinguishing character is the presence of long brown branched hyphae on the stipe resembling a mesh. The ecology is also different since *H. littorale* is growing on annually exposed sandy shores of oligotrophic lakes and on soil in a fen-meadow.

There are some small differences in the microscopic data between the Norwegian collect of *H. pusillum* and the holotype, but it

should be within the variation to be expected. Spores are in average a little bit smaller, the asci are in the same range but have a larger maximum size, and the same is for the setose hairs. We have data from the Norwegian species going back ten years in time, and they are all showing the same pattern. Pictures of the ascocarp from all those years shows a great variety both in form and in size, but are all within the stated data, the same is for the microscopic data.

The location where it was found has been surveyed intensively since 2003, so it is no surprise that it was found already in 2010. The area contains at least 10 different earth-tongues from different genera, so it was clear early that this was a new unidentified species.

The article by Arauzo and Iglesias (2014) contains very good pictures and drawings of microscopic characters of *Hemileucoglossum pusillum* (misinterpreted as *H. littorale*) so it is possible to get a long way just by the pictures and illustrations in the article. However, it was first in 2020 that the unnamed earthtongue was identified both microscopically and by molecular investigation. This is so far the only known occurrence of *Hemileucoglossum pusillum* in Norway.

ACKNOWLEDGEMENT

We would like to thank John Bjarne Jordal for reading the manuscript and for his valuable comments.

REFERENCES

- Arauzo S, Iglesias P, 2014. La familia Geoglossaceae en la península Ibérica y Macronesia. *Errotari*. 11: 166-259.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T, 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972-1973.
- Crous PW, Wingfield MJ, Burgess TI, et al. 2017. Fungal Planet description sheets: 625-715 *Persoonia* 39: 270-467.

- Fadnes P, 2011. Earthtongues (Geoglossaceae) in Sunnhordland, Western Norway – Ecological observations, new records and distribution. *Agarica* 30: 47-62
- Fadnes P, 2014. Variations in diversity and fructification of grassland fungi based on 11 years investigation of cultural landscapes in Sunnhordland, Western Norway. *Agarica* 35: 93-105.
- Fedosova AG, Popov ES, Lizoň P, Kučera V, 2018. Towards an understanding of the genus *Glutinoglossum* with emphasis on the *Glutinoglossum glutinosum* species complex (Geoglossaceae, Ascomycota). *Persoonia* 41: 18-38.
- Hakelien N, 1967. Three new Swedish species of *Geoglossum*. *Svensk botanisk tidskrift* 61: 419-424.
- Hustad VP, Miller AN, Moingeon JM, Priou JP, 2011. Inclusion of *Nothomitra* in *Geoglossomycetes*. *Mycosphere* 2(6) :646-654.
- Hustad VP, Miller AN, Dentinger BTM, Cannon PF, 2013. Generic circumscriptions in Geoglossomycetes. *Persoonia* 31: 101-111.
- Imai S, 1942. Contributiones ad studia monographica Geoglossacarum. *Bot. Mag., Tokyo* 56:523-527.
- Kumar S, Stecher G, Tamura K, 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870-1874.
- Katoh K, Rozewicki J, Yamada KD, 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20:1160-1166. <https://doi.org/10.1093/bib/bbx108>
- 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. *Mol Biol Evol* 34: 772-773.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MC, 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst Biol* 67:901-904.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck J, 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Syst Biol* 61:539-542.
- Sandnes ACS, 2006. Phylogenetic relationships among species and genera of Geoglossaceae (Helotiales) based on ITS and LSU nrDNA sequences. *Cand. Scient. Thesis., University of Oslo, Dep. of biology.*
- Schoch CL, Wang Z, Townsend JP, Spatafora JW, 2009. *Geoglossomycetes* cl. nov., *Geoglossales* ord. nov. and taxa above class rank in the *Ascomycota* Tree of Life. *Persoonia* 22: 129-138.
- Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690. <https://doi.org/10.1093/bioinformatics/btl446>