

Type study of *Helvella pedunculata* (Ascomycota, Pezizales), a synonym of *Dissingia confusa*

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Abstract: The holotype of *Helvella pedunculata*, housed in the herbarium of the University of Oulu (OULU, Finland), was examined along with other non-type specimens stored under that name. Morphological studies and genetic evidence from 28S rDNA confirm that *H. pedunculata* is a synonym of *Dissingia confusa*. Colour pictures of dried and fresh specimens, as well as images of microscopical characters are provided.

Keywords: *Helvellaceae*, phylogeny, taxonomy, type studies.

Riassunto: L'holotipus di *Helvella pedunculata*, conservato nell'erbario dell'Università di Oulu (Finlandia) viene esaminato insieme ad altri campioni depositati sotto a quel binomio. I risultati morfologici e quelli genetici basati sul 28S rDNA confermano essere sinonimo di *Dissingia confusa*. Vengono allegate anche foto a colori di materiale secco/fresco e del quadro microscopico.

Parole chiave: filogenesi, *Helvellaceae*, studio dei tipi, tassonomia.

Introduction

After the publication of the monographic study on the genus *Helvella* L. (SKREDE *et al.*, 2017), some questions still remain unanswered. One of these is the status of *Helvella pedunculata* Harmaja (1978) and its putative synonymy with *Dissingia confusa* (Harmaja) K. Hansen & X.H. Wang [= *Helvella confusa* Harmaja (1977)] suggested by SKREDE *et al.* (2017). Nothing regarding this issue was reported in HANSEN *et al.* (2019).

Helvella pedunculata was published with a short diagnosis (see below) by HARMAJA (1978) to accommodate dried material coming from Finland and housed in the herbarium of the University of Oulu (OULU). Shortly after, HARMAJA (1979) suggested that this species is present also in Norway and compared it to the closest taxa viz. *Helvella leucomelaena* (Pers.) Nannf., *H. confusa* and *H. oblongispora* Harmaja. The known distribution of *H. pedunculata* was later expanded to Canada (Quebec) by HARMAJA (1981).

After the examination of the holotype, ABBOTT & CURRAH (1997) concluded that *H. pedunculata* should be considered a later synonym of *H. leucomelaena* because of the very small morphological differences among these species. ABBOTT & CURRAH (1997) also considered *H. confusa* as synonym of *H. leucomelaena*. A similar conclusion was reached by LANDEROS *et al.* (2015) after studying the holotype of *H. confusa* and several specimens identified as *H. pedunculata* by Harmaja. However, BAIANO *et al.* (2000), after conducting an extensive morphological study on *H. leucomelaena* and *H. confusa*, concluded that the species are morphologically very different, a decision later supported by the genetic results obtained by SKREDE *et al.* (2017) and HANSEN *et al.* (2019).

Besides the original paper, the only published study of the type of *H. pedunculata* appears to be that of ABBOTT & CURRAH (1997). The aim of the present study is to conduct a new study of this collection and figure out the most probable taxonomic status of *H. pedunculata* after comparing evidence from molecular and morphological data.

Material and methods

Morphological study. — The microscopic studies were based on dried specimens. Two trinocular optical microscopes, Olympus CX41 and Optika B353, with plan-achromatic objectives 10×, 40×, 60× and 100× oil immersion, were used. The following reagents were used: Melzer's reagent, cotton blue (lactophenol and acid lactic), ammoniacal Congo red, 5% KOH. Water mounts were used for the observation of the pigmentation and measurements. At least 25 ascospores were measured from each mature collection.

Phylogenetic study. — Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY & THOMPSON (1980). PCR reactions (MULLIS & FALOONA, 1987) included 35 cycles with an annealing temperature of 54 °C. Primers ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) were employed to amplify the ITS rDNA region, while LR0R and LR5 (VILGALYS & HESTER, 1990; CUBETA *et al.*, 1991) were used for the 28S rDNA region. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected. BLAST (ALTSCHUL *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. Sequences came mainly from SKREDE *et al.* (2017) and HANSEN *et al.* (2019). Sequences were initially aligned in MEGA 5.0 (TAMURA *et al.*, 2011) software with its Clustal W application and then corrected manually. The aligned dataset was loaded in MrBayes 3.2.6 (RONQUIST & HUELSENBECK, 2003), where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.16 M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAXML 8.2.12 (STAMATAKIS, 2014) using the standard search algorithm (GTRCAT model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Studied collections

FINLAND. Koillismaa. Kuusamo, Oulanka National Park, South slope of Ampumavaara, North of the Biological Station, at spring brook, among fen mosses, 11.VII.1968, A.-M. Jakkula (OULU F026213, originally labelled as *Helvella pedunculata* by H. Harmaja, then revised as *H. leucomelaena* by F. Landeros). *Ibidem*, on mosses on calcareous spring fen, 24.VIII.1979, T. Ulvinen (OULU F026214, originally labelled as *Helvella pedunculata* by H. Harmaja, then revised as *H. leucomelaena* by F. Landeros). Kuusamo, Oulanka National Park, Liikasenavaara, bottom part of Murmisaarenrinne, about 350 m NW from the end of the new forest road, on a mossy slope close to a brook, 17.VII.1991, M. Ohenoja & E. Ohenoja (OULU F023450, originally labeled as *Helvella* then *H. pedunculata* by V. Haikonen, then revised as *H. leucomelaena* by F. Landeros). Oulun Pohjanmaa. Kiiminki, limestone area of Keskikylä, north margin of Isonhalmeenmaa, about 50 m west of the springs, among bryophytes at steepy margin of eutrophic bog, 02.VII.1975, E. & M. Ohenoja, *det.* H. Harmaja in 1978 (OULU F026201, holotype of *H. pedunculata*). *Ibidem*, on bare ground under a big boulder south of the communal pipeline, 08.VIII.1990, M. Kaukonen (OULU F033196, originally labeled as *H. pedunculata*, then revised as *H. leucomelaena* by F. Landeros). *Ibi-*

Table 1 – Samples newly generated for the present study and GenBank codes

Species	Herbarium voucher	GenBank Accession numbers	
		ITS	28S nrLSU
<i>Helvella pedunculata</i> (Holotype)	OULU F026201	MW751460	MW752882
<i>Helvella pedunculata</i>	OULU F026207	–	MW752883
<i>Dissingia confusa</i>	TUR-A 208512	–	MW752884
<i>Dissingia confusa</i>	TUR-A 208514	–	MW752885
<i>Dissingia confusa</i>	TUR-A 208515	–	MW752886
<i>Dissingia confusa</i>	OULU F050783	–	MW752887
<i>Dissingia confusa</i>	TUR-A 208513	–	MW752888
<i>Dissingia leucomelaena</i>	TUR-A 208517	–	MW752889
<i>Dissingia leucomelaena</i>	TUR-A 208516	–	MW752890

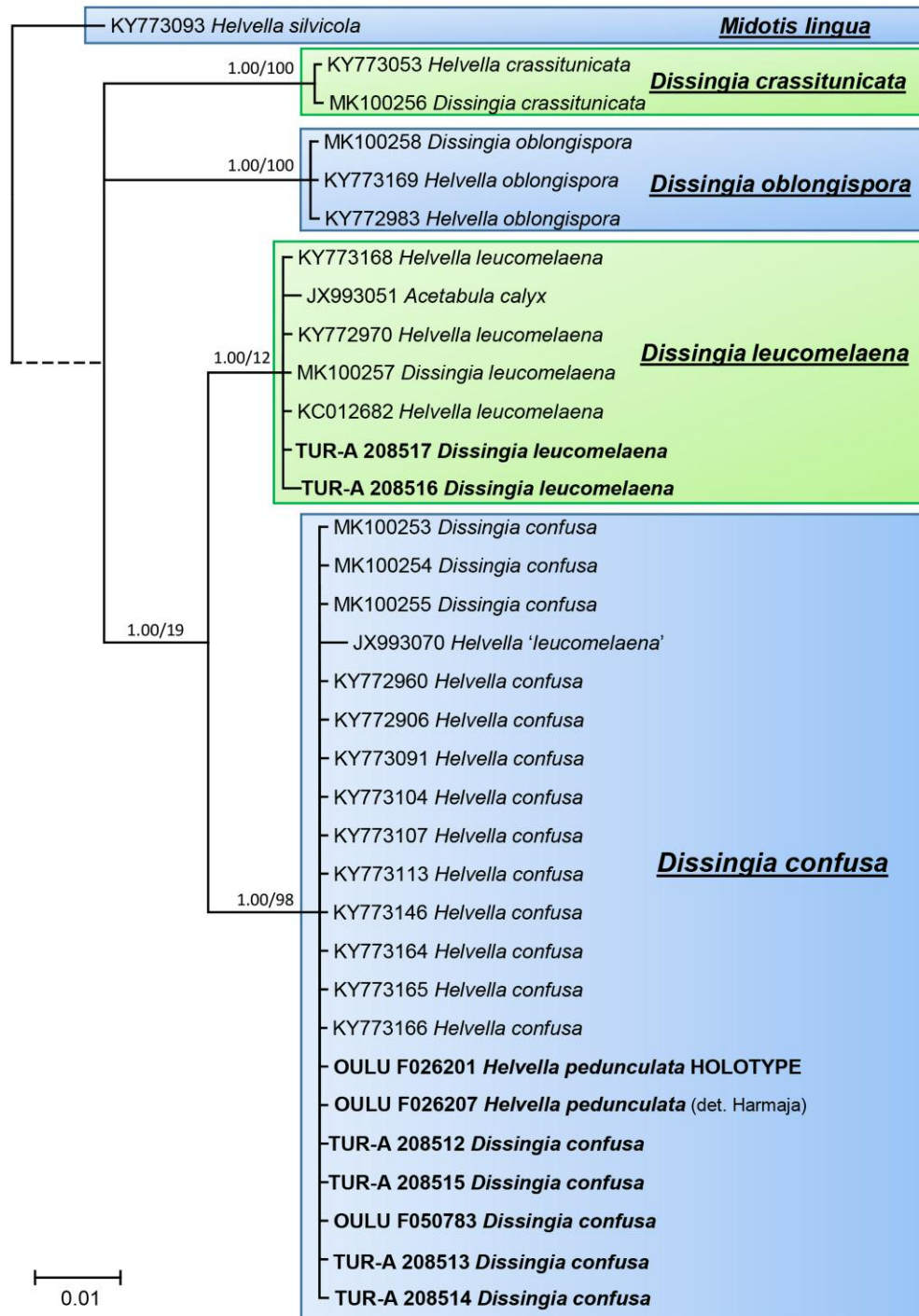


Fig 1 – 50% majority rule 28S rDNA consensus phylogram of the genus *Dissingia* (as defined by HANSEN *et al.*, 2019), obtained in MrBayes from 1200 sampled trees. Nodes were annotated if supported by ≥ 0.95 Bayesian PP (left) or $\geq 70\%$ ML BP (right).

H O L O T Y P

HERBARIUM UNIVERSITATIS OULUENSIS
PLANTAE FENNICAE

Helvella pedunculata Harmaja

Oulun Pohjanmaa. Kiiminki. Limestone area of Kes-
kikylä. N margin of Isonhalmeenmaa some 50 m
W of the springs.

Amongst bryophytes at seepy margin of eutro-
phic bog.

Grid 27°E 7228:446⁰/₂, elev. ca. 45 m a.s.l.
coll. Esteri & Martti Ohenoja
2.7.1975 det. Harri Harmaja 1978

HOLO TYPE

of *Helvella pedunculata* Harmaja,
Karstenia 18: 57, 1978.

=

~~det.~~ det. Harri Harmaja 1978
(publ. in)

University of Helsinki, Finland

vidit Veli Haikonen 1987

M.F.E.O.

Helvella

2.7.75

Kiiminki, Isonhal-
meenmaan N-laita
lähteestä n. 50m W.
Lottoannalatti

Helvella pedunculata Harmaja
OULU - Holotype

DET: S. Abbott

Feb. 1991

Plate 1 – Holotype of *Helvella pedunculata*. Cover and notes. (M. Carbone)

dem, on calcareous sand with *Distichium capillaceum* and *Pohlia cruda*, 14.VII.1989, M. Ohenoja (OULU F033199, originally labeled as *H. pedunculata*, then revised as *H. leucomelaena* by F. Landeros). *Ibidem*, North slope, in shade of a limestone block, on mineral soil. 4.IX.1971, M. Ohenoja (OULU F026210, originally labeled as *H. pedunculata* by H. Harmaja, then revised as *H. leucomelaena* by F. Landeros). *Ibidem*, SW margin, on block with limestone in rich forest with *Picea abies*, 10.VIII.1971, T. Ulvinen (OULU F026212, originally labeled as *H. pedunculata* by H. Harmaja, then revised as *H. leucomelaena* by F. Landeros). Kiiminki, calcareous area of Keskikylä, W margin of Murtoinsaaret, old small forest road in rich forest. 2.VII.1975, E. Ohenoja (OULU F026209, originally labeled as *H. pedunculata* by H. Harmaja, then revised as *H. leucomelaena* by F. Landeros). Kiiminki, calcareous area, SW of Raivio house, near a spring with *Cratoneuron*, among fen mosses, 19.VII.1975, T. Ulvinen

(OULU F026211, originally labeled as *H. pedunculata* by H. Harmaja in 1978, then revised as *H. leucomelaena* by F. Landeros). Varsinais-Suomi. Parainen, Skräbböle, north of Sementtitie, small open site in forest with *Betula*, *Pinus sylvestris*, *Picea abies* and *Salix caprea*, on gravel humus soil, human influenced site with limestone dust from the quarry and the road nearby, 05.VII.1998, J. Vauras (OULU F050783, originally labeled as *H. oblongispora*, then revised as *H. pedunculata* by P. Salo in 2005). ITALY. Emilia-Romagna. Zocca (MO), close to the "Museo del castagno e del borlengo", under *Picea abies*, 06.VI.2019, A. Testoni (TUR-A 208513). Lombardia. Vendrogno (LC), Loc. Set, under *Picea abies*, 25.V.2018, A. Mariani (TUR-A 208512). Piemonte. Pontechianale (CN), Fraz. Chianale, under pure *Larix decidua*, 13.VI.2019, M. Carbone (TUR-A 208514). Busca (CN), Santuario di Valmala, under *Picea abies*, 14.VI.2019, M. Carbone (TUR-A 208515). NORWAY. Nordland. Rana, Dunderlandsdalen, Örtfelmoen,

South-East of Ranaelven, brookside bank on calcareous ground, 11.IX.1976, T. Ulvinen & W. Jakowlev (OULU F026207, originally labeled as *H. pedunculata* by H. Harmaja in 1978, then revised as *H. leucomelaena* by F. Landeros). SWITZERLAND. Valais. Entremont District, Liddes, locality Palazuit, under *Picea abies* along a stream, 28.VI.2008, M. Carbone (in M. Filippa herbarium, "short-simple stiped apothecia"). *Ibidem*, 28.VI.2008, M. Carbone (in M. Filippa herbarium, "long-ribbed stiped apothecia").

Dissingia leucomelaena. ITALY. Piemonte. Canosio (CN), under an isolated *Pinus sylvestris*, ca. 1600 m a.s.l., 30.IV.2016, M. Carbone &

M. Manavella (TUR-A 208517). Toscana. Viareggio (PI), at sea level, under *Pinus domestica*, in sandy mossy soil, 02.III.2013, M. Carbone (TUR-A 208516).

Phylogenetic results

Neither Bayesian or Maximum Likelihood analyses support any significant difference between the 28S rDNA sequences obtained from the holotype of *H. pedunculata* and another sample of this

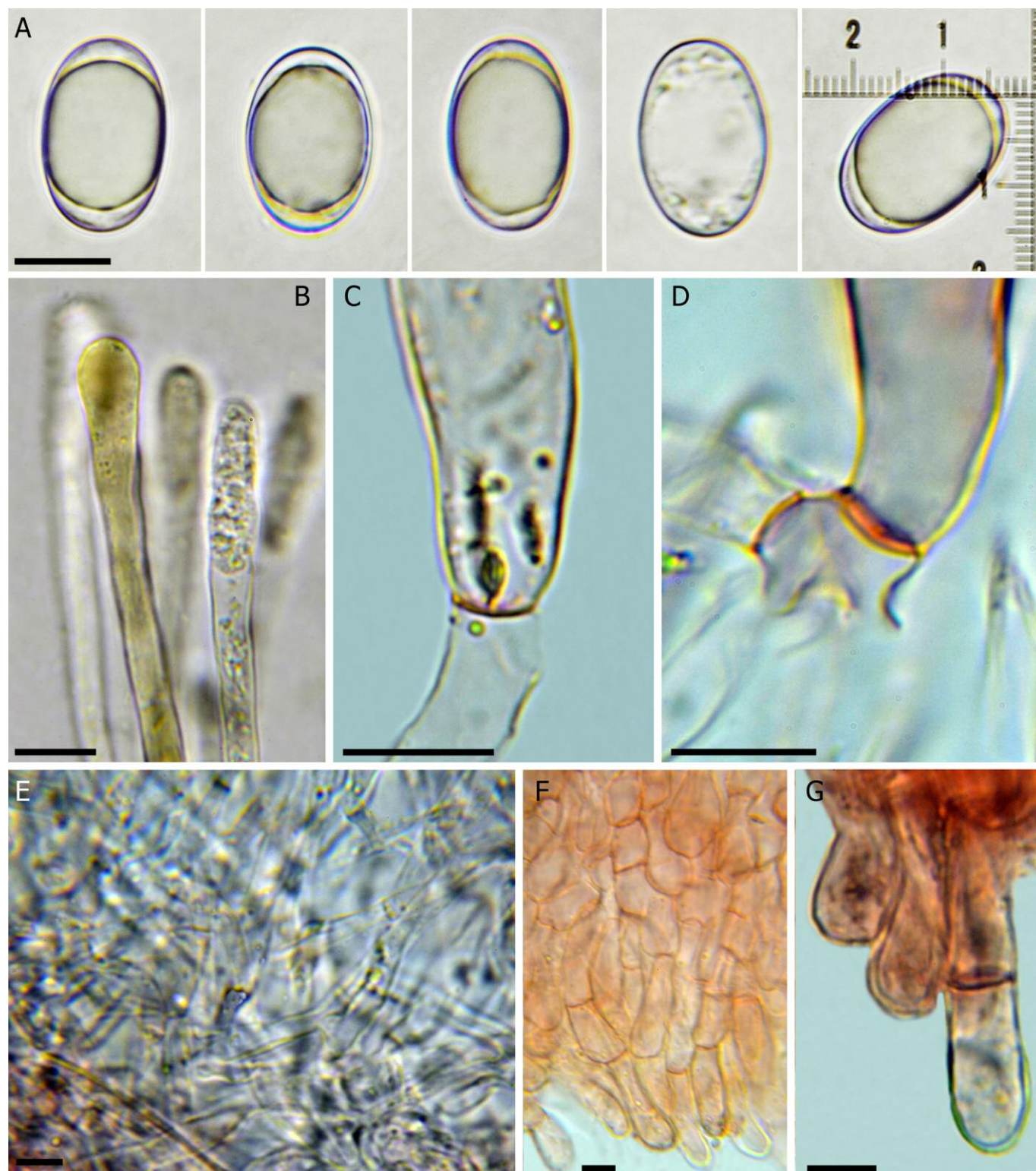


Plate 2 – Holotype of *Helvella pedunculata* (microscopical characters). A: Ascospores in water; B: Paraphyses in water; C–D: Ascus base in Congo red; E: Hyphae of medullary excipulum in Congo red; F: Ectal excipulum in Congo red; G: Terminal cells in Congo red. Scale bars = 10 µm. (C. Agnello).



Plate 3 – A-F: *Dissingia confusa*. A: Holotype of *H. pedunculata* (OULU F026201); B: OULU F050783 (J. Vauras); C-D: TUR-A 208514 (M. Carbone); E: TUR-A 208513 (A. Testoni); F: TUR-A 208512 (A. Mariani). **G-H: *D. leucomelaena*.** G: TUR-A 208516 (M. Carbone); H: TUR-A 208517 (M. Carbone).



Plate 4 – *Dissingia confusa*. Collections of 28.VI.2008 in M. Filippa herbarium (M. Carbone)

species identified by Harmaja, as well as those of *Dissingia confusa* obtained in the present work or retrieved from public databases. On the contrary, samples of *D. leucomelaena* were successfully separated by means of Bayesian analysis, in agreement with the results obtained by SKREDE *et al.* (2017). The synonymy between *H. pedunculata* and *D. confusa* is therefore accepted on the basis of morphological and genetic evidences.

Taxonomy

Helvella pedunculata Harmaja, *Karstenia*, 18 (2): 57 (1978).

Original diagnosis: *A Helvella leucomelaena praecipue differt apotheciis tenuiter pedunculatis stipite costato et sporis brevioribus (longitudine ca. 17.5–20.0 µm).*

Typification (holotype designated by HARMAJA, 1978): FINLAND, prov. Pohjois-Pohjanmaa, par. Kiiminki, Keskikylä, limestone area, north end of Isonhalmeenmaa, 50 m west of the spring, in rich fen among moss, 2.VII.1975, E. & M. Ohenoja (OULU F026201).

Current name: *Dissingia confusa* (Harmaja) K. Hansen & X.H. Wang, in Hansen *et al.*, *Persoonia*, 42: 197 (2019).

≡ *Helvella confusa* Harmaja, *Karstenia*, 17 (1): 43 (1977).

Revision of the holotype

Macroscopical features (Plate 3, fig. A)

The holotype is composed of ca. 11 apothecia in good conditions although the hymenium of almost all apothecia is lacking in some parts. The cups measure up to 2 cm in diameter, hymenium is brownish-black, external surface is minutely pruinose, subconcolorous to the hymenium in the upper part, lighter to whitish toward the stipe. The stipes measure up to ca. 1 cm high, and are whitish and vertically furrowed.

Microscopical features (Plate 2)

Asci subcylindrical, 295–355 × 12.5–14.5 µm, 8-spored, hyaline, inamyloid, operculate, aporynchous. **Ascospores** 19–21.5 × 11.9–13.8 µm, X = 20.1 × 12.7 µm; Q = 1.5–1.7, Q_m = 1.6, hyaline, smooth, ellipsoid, with a big ellipsoid oil drop, less frequently with also very small drops at the poles. **Paraphyses** cylindrical, septate, 2.5–4.5 µm wide, enlarged to 5–7 (–8) µm at tip, often bifurcate in the bottom part, pigmented in the upper cell by small yellowish vacuoles or by a dissolved straw-yellow pigment. **Subhymenium** 40–60 µm thick, composed of a dense *textura intricata* with short hyphae intermixed with polygonal elements. **Medullary excipulum** 300–600 µm thick, composed of a *textura intricata* with septate hyphae, 3–9 µm wide, often larger, inflated and constricted at septa. **Ectal excipulum** 140–220 µm thick, composed by subcylindrical elements, sometimes a bit inflated, arranged like a *textura prismatica* especially towards the surface, hyaline in the inner part, yellow brown towards the margin due to an intracellular pigment, terminal element clavate and up to 14 µm wide.

Discussion

Helvella pedunculata was treated in detail by HARMAJA (1979) who published a table summarising the most important characters differentiating *H. leucomelaena*, *H. confusa*, *H. pedunculata* and *H. oblongispora*. According to HARMAJA (*op. cit.*), the differences between the first three species and *H. oblongispora* are somewhat great. The Finnish author considered that *H. pedunculata* differs macroscopically from the other species because of the well-developed stipe and few other subtle features, and microscopically due to the somewhat smaller ascospores, as well as the larger and more irregular hyphae of the medullary excipulum. Despite the great sim-

ilarities with *H. confusa*, he considered that both names, coined by himself, represent independent species.

Our study of the holotype of *H. pedunculata* confirms the microscopical features reported by HARMAJA (1979), but comparing a larger number of samples of *Dissingia confusa* and original collections of *H. pedunculata* from Harmaja's fungarium (see collections studied), we found a great overlap of the features emphasised by HARMAJA (*op. cit.*) between all of them. *Dissingia confusa* is therefore accepted as a priority synonym of *H. pedunculata* for all collections.

Dissingia confusa is a common species in Italy and frequently collected in association with *Picea abies* from lowland to high elevation in the Alps, from the end of spring to summer (depending on the elevation). Apothecia are very variable in the overall size and morphology of the stipe, which range from small and rather simple (Plate 4, fig. A) to long and ribbed/costate (Plate 4, fig. B); this morphological variability is also visible in Plate 3 (figure B to F). In the last two decades, *D. confusa* has been described in more or less detail by many Italian and French authors; see for example BAIANO *et al.* (2000), FLORIANI (2001), MOYNE (2011) and VAN VOOREN (2014).

In our experience, *D. confusa* seems associated with *Picea* whilst *D. leucomelaena* seems linked to *Pinus*. However, collection TUR-A 208514 (plate 3, figures C–D) was collected in a pure *Larix decidua* forest in the Italian Alps and confirmed in our study to be typical *D. confusa*.

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References

- ABBOT S.P. & CURRAH R.S. 1997. — The *Helvellaceae*: systematic revision and occurrence in northern and northwestern North America. *Mycotaxon*, 62: 1–125.
- ALTSCHUL S.F., MADDEN T.L., SCHÄFFER A.A., ZHANG J., ZHANG Z., MILLER W. & LIPMAN D.J. 1997. — Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17): 3389–3402. doi: 10.1093/nar/25.17.3389
- BAIANO G., GAROFOLI D. & FILIPPA M. 2000. — Ascomiceti interessanti del Nord Italia. *Fungi non delineati*, 12: 1–74.
- CUBETA M.A., ECHANDI E., ABERNETHY T. & VILGALYS R. 1991. — Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology*, 81: 1395–1400. doi: 10.1094/Phyto-81-1395
- FLORIANI M. 2001. — Funghi di stagione: *Helvella acetabulum*, *Helvella leucomelaena*, *Helvella confusa* e *Helvella solitaria*. *Bollettino del Gruppo Micologico G. Bresadola n.s.*, 44 (1): 18–22.
- GARDES M. & BRUNS T.D. 1993. — ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2 (2): 113–118. doi: 10.1111/j.1365-294x.1993.tb00005.x
- HANSEN K., SCHUMACHER T., SKREDE I., HUHTINEN S. & WANG X.-H. 2019. — *Pindara* revisited – evolution and generic limits in *Helvellaceae*. *Persoonia*, 42: 186–204. doi: 10.3767/persoonia.2019.42.07
- HARMAJA H. 1978. — New species and combination in *Helvella* and *Gyromitra*. *Karstenia*, 18 (2): 57. doi: 10.29203/ka.1978.139
- HARMAJA H. 1979. — Studies on cupulate species of *Helvella*. *Karstenia*, 19 (2): 33–45. doi: 10.29203/ka.1979.184
- HARMAJA H. 1981. — *Helvella hyperborea* and *H. pedunculata* found in North America. *Karstenia*, 21 (2): 49. doi: 10.29203/ka.1981.202

- LANDEROS F., ITURRIAGA T., RODRIGUEZ A., VARGAS-AMADOC 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. doi: [10.1016/j.rmb.2015.09.005](https://doi.org/10.1016/j.rmb.2015.09.005)
- MOYNE G. 2011 [2010]. — *Helvella confusa*, une espèce peu courante. *Mycologia Montenegrina*, 13: 105–108.
- MULLIS K.B. & FALOONA F. 1987. — Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, 155: 335–350. doi: [10.1016/0076-6879\(87\)55023-6](https://doi.org/10.1016/0076-6879(87)55023-6)
- MURRAY M.G. & THOMPSON W.F. 1980. — Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8 (19): 4321–4325. doi: [10.1093/nar/8.19.4321](https://doi.org/10.1093/nar/8.19.4321)
- RONQUIST F. & HUELSENBECK J.P. 2003. — MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12): 1572–1574. doi: [10.1093/bioinformatics/btg180](https://doi.org/10.1093/bioinformatics/btg180)
- SKREDE I., CARLSEN T. & SCHUMACHER T. 2017. — A synopsis of the saddle fungi (*Helvella*: Ascomycota) in Europe – species delimitation, taxonomy and typification. *Persoonia*, 39: 201–253. doi: [10.3767/persoonia.2017.39.09](https://doi.org/10.3767/persoonia.2017.39.09)
- STAMATAKIS A. 2014. — RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies, *Bioinformatics*, 30 (9): 1312–1313. doi: [10.1093/bioinformatics/btu033](https://doi.org/10.1093/bioinformatics/btu033)
- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S. 2011. — MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28 (10): 2731–2739. doi: [10.1093/molbev/msr121](https://doi.org/10.1093/molbev/msr121)
- VAN VOOREN N. 2014. — Contribution à la connaissance des Pézizales (Ascomycota) de Rhône-Alpes – 1^{re} partie. *Cahiers de la FMBDS*, 3: 1–148.
- VILGALYS R. & HESTER M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172: 4238–4246. doi: [10.1128/jb.172.8.4238-4246.1990](https://doi.org/10.1128/jb.172.8.4238-4246.1990)
- WHITE T.J., BRUNS T.D., LEE S. & TAYLOR J.W. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS M.A., GELFAND D.H., SNINSKY J. & WHITE T.J. (eds). *PCR protocols: a guide to methods and applications*. New York, Academic Press: 315–322. doi: [10.1016/B978-0-12-372180-8.50042-1](https://doi.org/10.1016/B978-0-12-372180-8.50042-1)



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