Emendation of the genus *Tricharina* (*Pezizales*) based on phylogenetic, morphological and ecological data. Part 2

Nicolas VAN VOOREN
Uwe LINDEMANN
Rosanne HEALY

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Abstract: A second series of species of the genus *Tricharina* is reviewed based on morphological and phylogenetic data. The examination of *Tricharina cretea* in the sense of Thind & Waraitch resulted in its description as a new species, published herein as *T. indica*. *T. hiemalis*, *T. japonica*, and *T. striispora* are described and illustrated with new collections, as well as *T. glabra*. *Tricharina herinkii* is redescribed based on the type-collection and on a recent collection. Results from these analyses support the erection of the new genus *Tricharinopsis*, to accommodate this species. The type collection of *Humaria flava* (syn. *Tricharina flava*) was examined and compared to *T. herinkii*, but the lack of molecular data of the former preclude a formal conclusion. The examination of the type collection of the tricharinoid *Leucoscypha subimmersa* gave us the opportunity to transfer it to the genus *Pseudotricharina* because of its microscopic characters. Finally, the revision of the type-collection of *Tricharina praecox* var. *intermedia* was determined to be the same as *Ascorhizoctonia praecox*.
Keywords: distribution, ecology, *Pseudotricharina*, *Pyronemataceae*, ribosomal DNA, *Scutellinia-Trichophaea* lineage, taxonomy, *Tricharinopsis*.

Introduction

In a previous study, we focused on the morphological and molecular identity of the type species of *Tricharina*, *T. gilva* (Boud.) Eckblad, and two other *Tricharina* taxa that were consequently reassigned to other genera: *T. ascophanoides* (Boud.) Chin S. Yang & Korf to *Cupulina* Dougoud, Van Vooren & M. Vega and *T. praecox* (P. Karst.) Dennis to *Ascorhizoctonia* Chin S. Yang & Korf (Van Vooren *et al*., 2017). Here we report the results of morphological and phylogenetic analyses of six additional species of *Tricharina s. lato*. These are mostly rarely collected species. Our report is based on type studies and recent findings of *Tricharina cretea* (Cooke) K.S. Thind & Waraitch, *T. flava* (Fuckel) J. Moravec, *T. herinkii* (Svrček) Benkert, *T. hiemalis* Chin S. Yang & Korf, *T. japonica* Chin S. Yang & Korf, and *T. striispora* Rifai, Chin S. Yang & Korf. In addition, we present a type revision of the tricharinoid species *Leucoscypha subimmersa* K.S. Thind & S.C. Kaushal (Thind & Kaushal, 1979a) and of *Tricharina praecox* var. *intermedia* Egger, Chin S. Yang & Korf (Yang & Korf, 1985). Finally, we report a new collection of *T. glabra* from the Netherlands — a species that was only known from the type location in Northern Germany.

Material and methods

Morphology and cytology. — The methods of observation are detailed in Van Vooren *et al*. (2017).

DNA extraction, amplification and sequencing. — DNA was extracted using the same method as described in Van Vooren *et al.* (2015a).

Phylogenetic analyses. — The analyses were done using the same methods detailed in Van Vooren *et al.* (2017). New sequences were deposited in GenBank under the numbers MN385960–MN386018 listed in Table 1.

Nomenclature. — All the references to articles of ICN come from the Shenzhen Code (Turland *et al.*, 2018). Novelties were registered in the MycoBank Database.

Phylogenetic analysis

There was no discrepancy between the topology resulting from Bayesian analysis and ML analysis for supported nodes. One taxon, *Paratricharina* sp. MSTR 19992, was unstable between the ITS and LSU analyses, clustering without support in the *Pseudotricharina* clade in the LSU analysis (Fig 1), and with moderately high ML support in the *Paratricharina* clade in the ITS analysis (Fig. 2). The LSU analysis resulted in moderately to strongly supported nodes for *Geopora*, *Hoffmannoscypha*, *Ascorhizoctonia*, *Sepultariella*, *Cupulina*, and *Lasiocupulina*. The deeper nodes lacked support (Fig. 2). The ITS analyses (Fig. 2) resulted in a strongly supported node for the *Tricharina gilva* species complex that included collections from Europe and Japan, as well as endophytes from North America, China, and Iran. Several of the collections were from burn sites. *Tricharina hiemalis*, from Europe, was moderately well supported, and included an orchid mycorrhiza. *Tricharina striispora* was well supported, and included collections from Europe and Argentina. *Tricharina japonica* was well supported and included specimens from Japan and Switzerland. A specimen originally identified as *Tricharina cretea* was resolved as sister to *T. japonica*, and is here described as the new species *T. indica*. *Tricharina glabra* from Europe is well supported, and an endophyte from China clusters with this species. *Tricharina groenlandica* from Europe is well supported and includes a specimen from China. *Ascorhizoctonia*, *Sepultariella*, *Cupulina*, and *Lasiocupulina* are strongly supported in one well resolved clade. All ascomata collections of *Ascorhizoctonia* were made on burn sites, and one sequence came from an endophyte. The holotype of *Sepultaria herinkii* appears to belong within the lineage of *Tricharina* and allied genera, rather than *Sepultaria*, but its placement within the phylogeny is not supported in either LSU or ITS analyses. It is treated here in the newly erected genus *Tricharinopsis*.

The sequences of two *Tricharina* taxa genetically close to *T. aethiopica* were not supported as conspecific with *T. aethiopica*, and were morphologically distinct from that species. One collection originates from South Africa. Its habit, the shape and size of the ascospores are similar to species of the *T. gilva* complex. The Spanish collection, growing on wood, is macroscopically reminiscent of *T. japonica* and has unusually long marginal hairs. It will be treated in a forthcoming article. For now these two taxa are referred to as *Tricharina* sp.

The species of *Tricharina sensu lato*

In this chapter, we report the results from our examination of the type specimens of four taxa assigned to *Tricharina s. lato*: *Humaria flava* Fuckel, *T. hiemalis*, *T. japonica*, and *Sepultaria herinkii* Svrček. We also report on recent collections of *T. striispora* from France, Greece, Italy, and Spain, a new collection of *T. glabra* from the Netherlands, and an older collection of *T. cretea* from India. The morphological re-examination of the Indian collection reveals that it is not *Tricharina praecox* var. *cretea* in the sense of Yang & Korf (1985).

The recently described species of *Tricharina s. str.* — *T. aethiopica*, *T. glabra*, and *T. tophiseda* — are well documented and the type descriptions were based on the methods of vital taxonomy (cf. Kušan *et al.*, 2015; Lindemann & Böhning, 2016; Lindemann, 2017). Although no sequence of *T. tophiseda* exists yet, the morphology of the species clearly indicates its classification as a *Tricharina*. Due to the good documentation of *T. aethiopica*, *T. glabra*, and *T. tophiseda*, we will not reproduce here the descriptions of these species again.

Table 1 – List of collections of *Tricharina* and other genera sequenced during this study

Original name (identified as…)	Correct name (if different)	Coll. Number	Country	Collector	GenBank Number	
					ITS	LSU
Byssonectria semi-immersa	*Sepultariella semi-immersa*	M.V. 20131025-02	Germany	M. Vega	MN385986	MN386013
Leucoscypha patavina	*Sepultariella* sp.	M.V. 20140124-06	Portugal	M. Vega	MN385988	MN386014
Paratricharina sp.		MSTR 19992	Germany	D. Wieschollek	MN385983	MN386010
Sepultaria herinkii (holotype)	*Tricharinopsis herinkii*	PRM 772405	Czech Rep.	J.A. Herink	MN385989	MN386012
Sepultariella sp.	*Sepultariella semi-immersa*	M.V. 20140222-11	Spain	M. Vega	MN385987	MN386015
Tricharina cf. *gilva*	*Tricharina hiemalis*	TRH 7821	Norway	S. Sivertsen	MN385973	MN386001
Tricharina cretea	*Tricharina indica* sp. nov.	BPI 571761	India	K.S. Waraitch	MN385976	MN386004
Tricharina gilva		TRH 11443	Greenland	H. Dissing	MN385964	MN385996
Tricharina gilva		TRH 6031	Norway	H. Dissing & S. Sivertsen	MN385971	MN386000
Tricharina gilva		N.V. 2017.06.17	Spain	N. Van Vooren	MN385968	MN385998
Tricharina gilva		M.V. 180516-01	France	M. Vega	MN385967	MN385997
Tricharina gilva		U.L. 317	Portugal	U. Lindemann	MN385965	MN386016
Tricharina glabra		G.J. C170 5778	Netherlands	M. van der Vegte	MN385975	MN386003
Tricharina hiemalis		C.V.L. 200718 (1)	Spain	F.J. Valencia Lopez	MN385974	MN386002
Tricharina japonica		M.K. 2016-181	Japan	M. Kutsuma	MN385960	MN385991
Tricharina japonica		M.K. 2016-214	Japan	M. Kutsuma	MN385963	MN385995
Tricharina japonica		R.D. 31.01.245.11	Switzerland	R. Dougoud	MN385962	MN385993
Tricharina japonica		M.O. 673	Japan	M. Nakajima & M. Ohmae	MN385961	MN385992
Tricharina japonica (holotype)		CUP JA-000286	Japan	S. Imai et al.	MN385990	MN385994
Tricharina ochroleuca	*Tricharina gilva*	U.L. 241	Czech Rep.	Z. Egertová	MN385972	–
Tricharina praecox	*Ascorhizoctonia praecox*	M.K. 2013-30	Japan	M. Kutsuma	MN385982	–
T. praecox var. intermedia (isotype)	*Ascorhizoctonia praecox*	CUP 61648	Canada	K.N. Egger	MN385981	–
Tricharina sp.	*Tricharinopsis herinkii*	G.C. 17102501	France	G. Corriol	MN385985	MN386011
Tricharina sp.	*Tricharina gilva*	C.V.L. 190418 (1)	Spain	F.J. Valencia Lopez	MN385966	–
Tricharina sp.	*Paratricharina poiraultii*	C.V.L. 120318 (1)	Spain	F.J. Valencia Lopez	MN385984	–
Tricharina sp.	*Tricharina* cf. *gilva*	M.K. 2005-56	Japan	M. Kutsuma	MN385970	MN385999
Tricharina sp.		C.V.L. 280518 (2)	Spain	F.J. Valencia Lopez	–	MN386006
Tricharina sp.		Z.E. 54-18	South Africa	Z. Egertová & M. Sochor	MN385977	MN386005
Tricharina sp.	*Tricharina* cf. *gilva*	N.V. 2017.04.10	Spain	F.J. Valencia Lopez	MN385969	MN386017
Tricharina striispora		M.V. 20140222-03	Spain	M. Vega	MN385979	MN386007

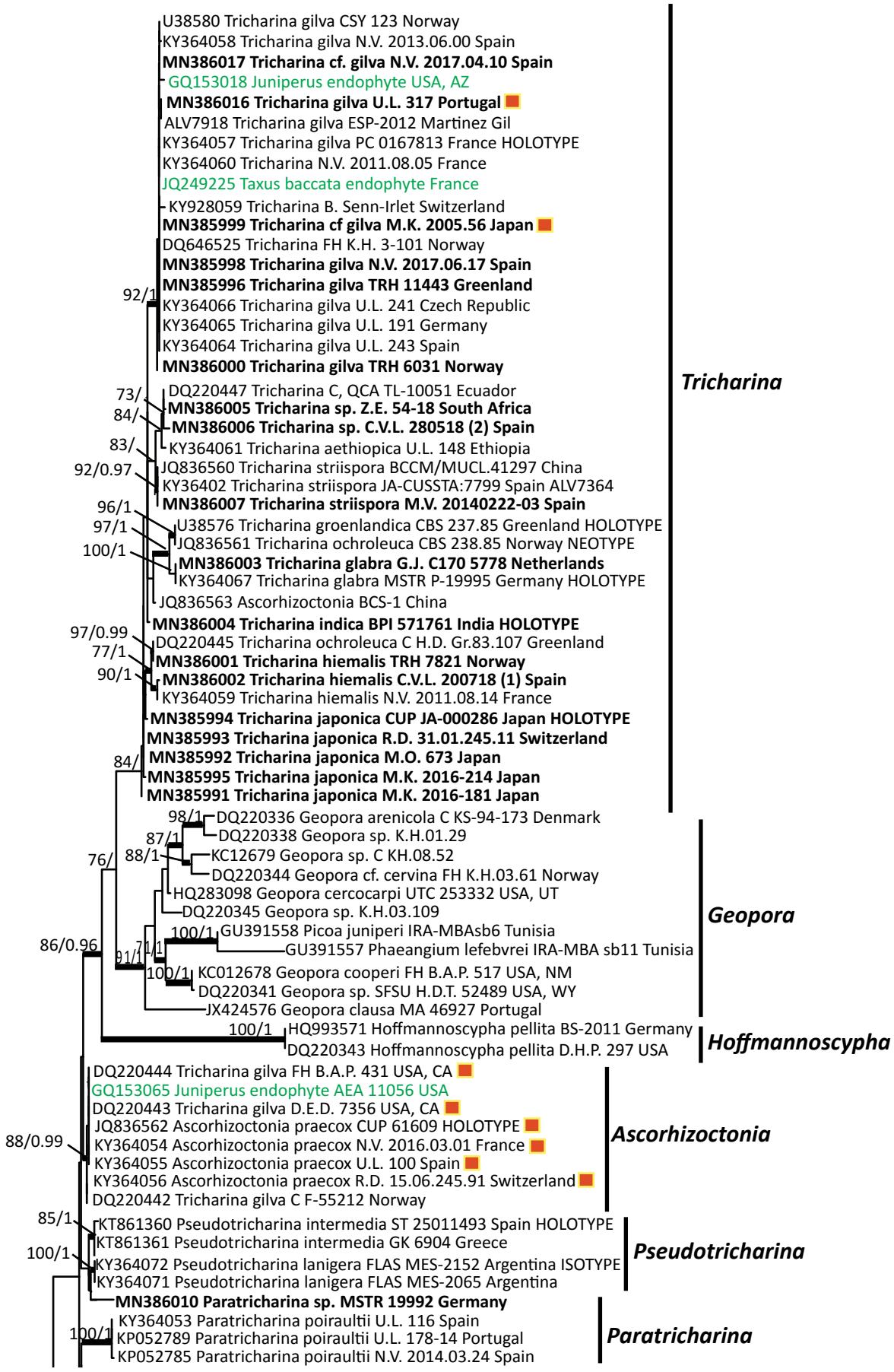

Fig. 1 – RAxML consensus tree based on LSU alignment. Significant support is shown at the nodes, with maximum likelihood bootstrap ≥ 70% on left and Bayesian probability ≥ 0.95 on the right. Where both are significant, the branch is thickened. Newly generated sequences are in bold. Color coding of leaves represents origin of sample sequenced: ascomatal sequences as black, endophytes are green, ectomycorrhizal root (ECM) tips are brown, orchid mycorrhizal root (ORM) tips are purple. Collections from burned habitats are indicated by red boxes framed in yellow.

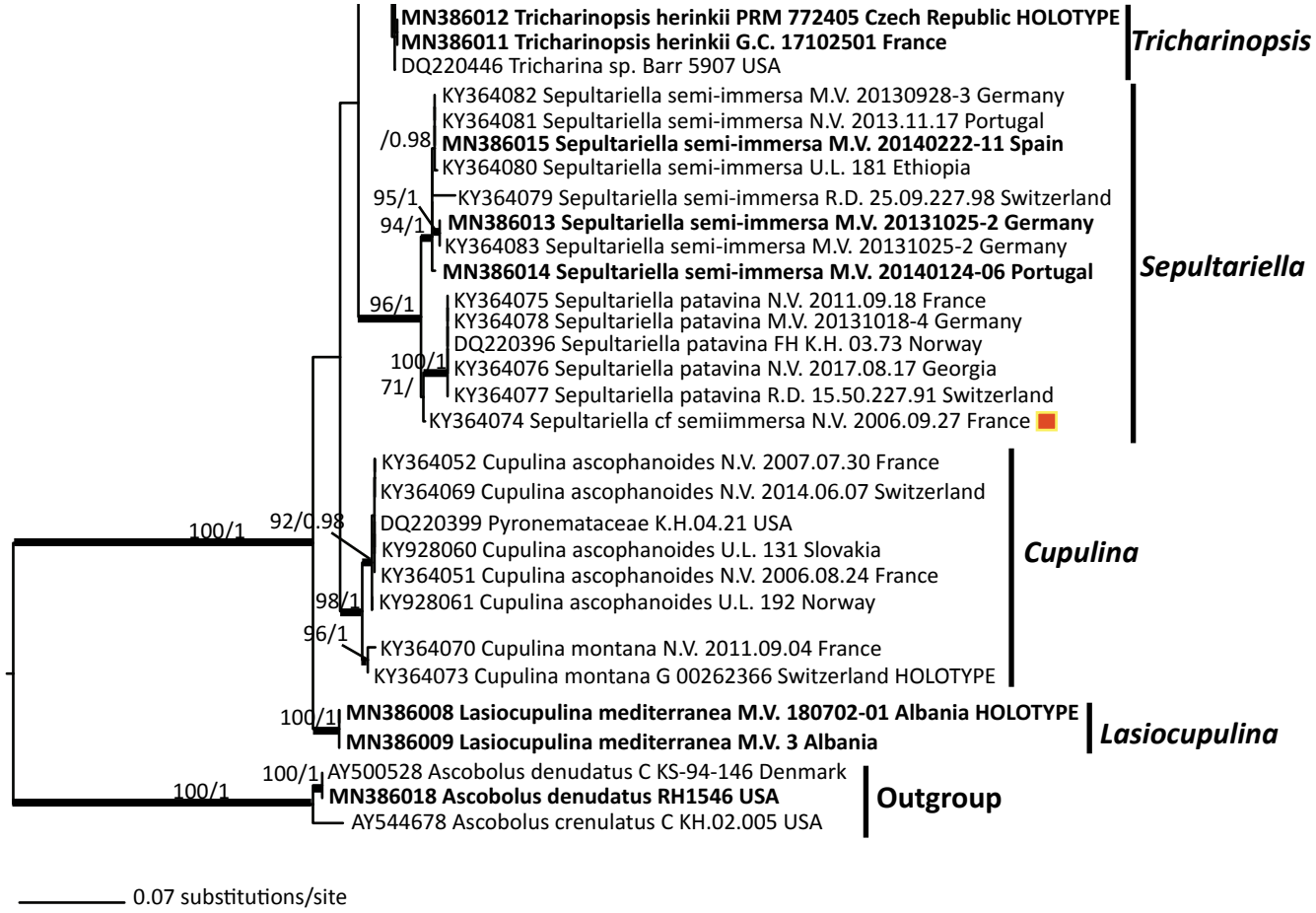

Fig. 1 – RAxML consensus tree based on LSU alignment *(continued)*.

Tricharina cretea

Peziza cretea Cooke, a species described in 1878 from the plaster walls and ceiling of a building in Great Britain whose roof had burned, was recombined as *Tricharina cretea* by Thind & Waraitch (1971) based on an Indian collection from the state of Jammu and Kashmir, although the authors admitted some differences from the type specimen of *P. cretea*. Yang & Korf (1985) also recombined *P. cretea* as a *Tricharina*, but as a variety of *T. praecox*. Their work was based on the type specimen of *P. cretea* and numerous other collections from Europe. After comparing the description by Thind and Waraitch with the redescription from the type of *P. cretea* by Yang & Korf, we doubt that the Indian collection corresponds to *Tricharina praecox* var. *cretea* in the sense of Yang & Korf (1985), i.e. a taxon of the genus *Ascorhizoctonia* as redefined by us (Van Vooren *et al.*, 2017). Our study of the Indian taxon treated as *T. cretea* by Thind and Waraitch (1971), housed in the BPI herbarium, confirmed that this taxon is different from *T. praecox* var. *cretea*.

Re-examination of *Tricharina cretea* in the sense of Thind & Waraitch

Description (†) of microscopic characters (Plate 1):

Excipulum composed of two layers: **Medullary excipulum** rather thin, about 80–100 µm wide, of *textura intricata*, with hyaline hyphae; **Ectal excipulum** thin, less than 50 µm wide, of *textura angularis/subglobulosa*, composed of hyaline or yellowish cells (up to 10 µm diam.), becoming brown-yellowish in the outermost part. **Anchor hyphae** not seen. **Excipular hairs** superficial, rather dense especially near the margin, more or less flexuous, 140–300 × 6–9 µm, obtuse or pointed at the top, with a simple enlarged to bulbous base (up to 30 µm wide), pale brownish, septate, wall 0.8–2 µm thick, mixed with some other that are rigid, a bit shorter, 70–210 × 7–12 µm. **Marginal hairs** similar but straight and not bulbous, 38–230 × 8–13 µm, often clustered, mostly pointed at the top, brownish, few septate. **Asci** operculate, cylindrical, about 150 µm in length, 8-spored, inamyloid, probably with crozier (hard to see because asci are collapsed). **Paraphyses** filiform, hyaline, without vacuole nor guttules, not enlarged at the apices, 3–4 µm wide. **Ascospores** uniseriate, ellipsoid-fusoid or ellipsoid with tapering ends, sometimes asymmetrical, (14.5) 15–17 × (7.7) 8–9 (9.5) µm [X = 15.8 × 8.5 µm, n = 31], Q = 1.7–2.0 [Qm = 1.9], hyaline, without oil drops or polar granules, smooth, rather thick-walled, wall often refractive in CB.

Studied collection: India – Jammu and Kashmir, Doda, Bhadarwah, on wet soil, in a mixed forest, 30 Sept. 1967, *leg*. K.S. Waraitch, *ex* coll. 2163, BPI accession number 571761 (isotype).

Comments: Our observations agree with the description and illustrations of Thind & Waraitch (1971), with minor differences in some measurements that can be explained by our examination on rehydrated material, but we note that hairs are up to 395 µm in length in the original description (vs. 300 µm in our examination). This species is similar to *Tricharina japonica*, in the fusoid, often inequilateral spore shape, but the spore length and the Q ratio are less. The hairs are also clearly shorter than those of *T. japonica* (see description below). From *T. praecox* var. *cretea* in the sense of Yang & Korf (1985), it differs by commonly inequilateral, smooth ascospores and the absence of bipolar granules in the ascospores[1], from *T. tophiseda*, it is distinguished by shorter hairs, the different shape

[1] Although no information is given concerning the spore ornamentation in the original descriptions of *Peziza cretea* (Cooke, 1877, 1878), Yang & Korf (1985) demonstrated verrucose ascospores on this species.

of the ascospores and the absence of bipolar spore granules (Kušan *et al.*, 2015). We disagree with the opinion of Yang & Korf (1985: 499) who assigned this collection to *Tricharina ochroleuca* (Bres.) Eckblad, for two reasons: first because we demonstrated the latter is an ambiguous name based on a heterogeneous species concept (Van Vooren *et al.*, 2017), and second because the spore shape and content do not agree with *T. ochroleuca* in the sense of Yang & Korf (1985).

Finally, our phylogenetic results demonstrate the isolated position of this collection within the *Tricharina* genus. Since there are no published descriptions that match this species molecularly or morphologically, we publish it as a new species.

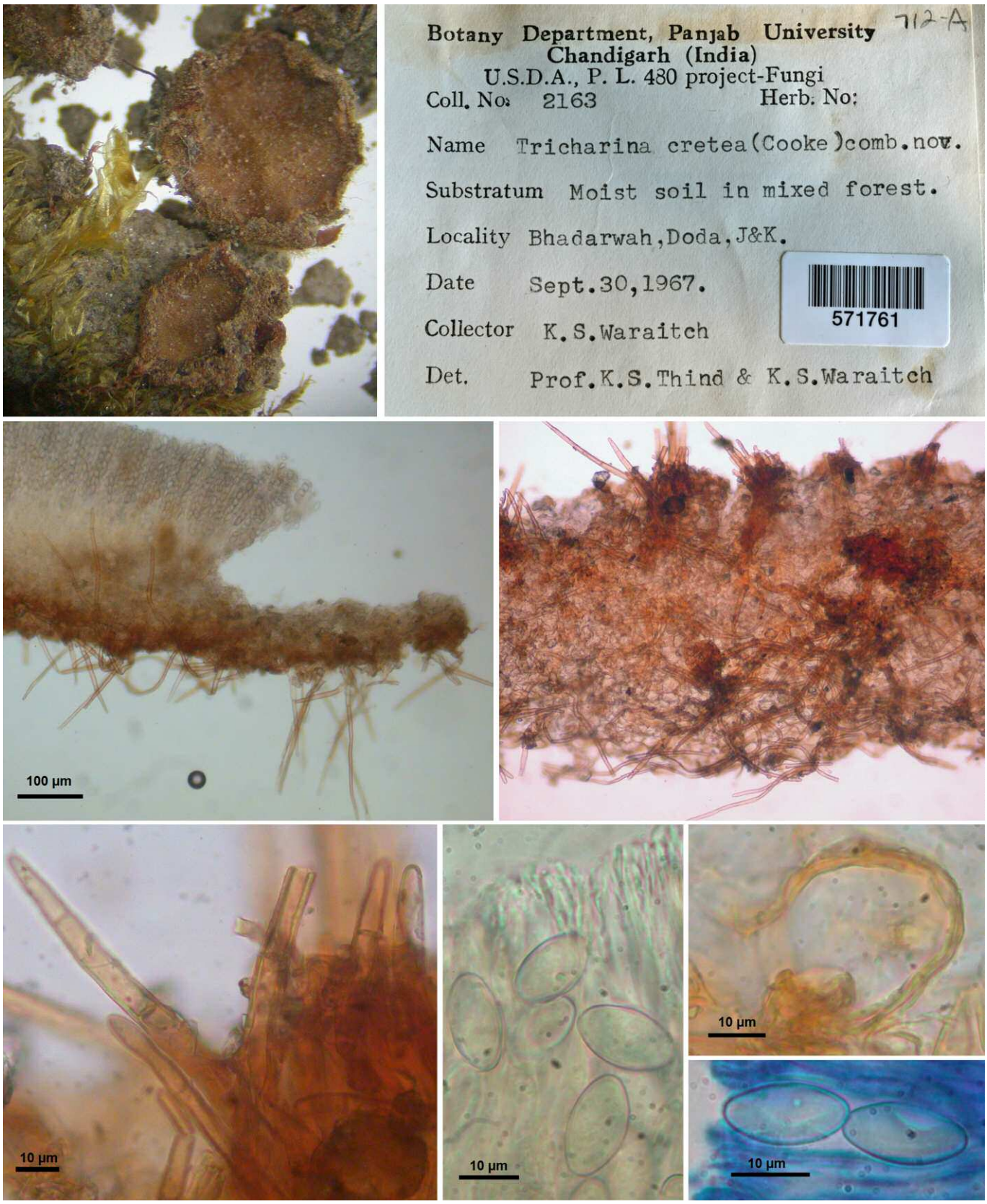

Plate 1 – *Tricharina cretea sensu* Thind & Waraitch. Coll. BPI 571761.
A. Dried apothecia. B. The label of the collection. C. Section of an apothecium, showing hairs. D. Ectal excipulum with hairs. E. Marginal hairs. F. Ascospores (dead) in water. G. Bulbous base of an excipular hair. H. Ascospores in CB. All photos by N. Van Vooren.

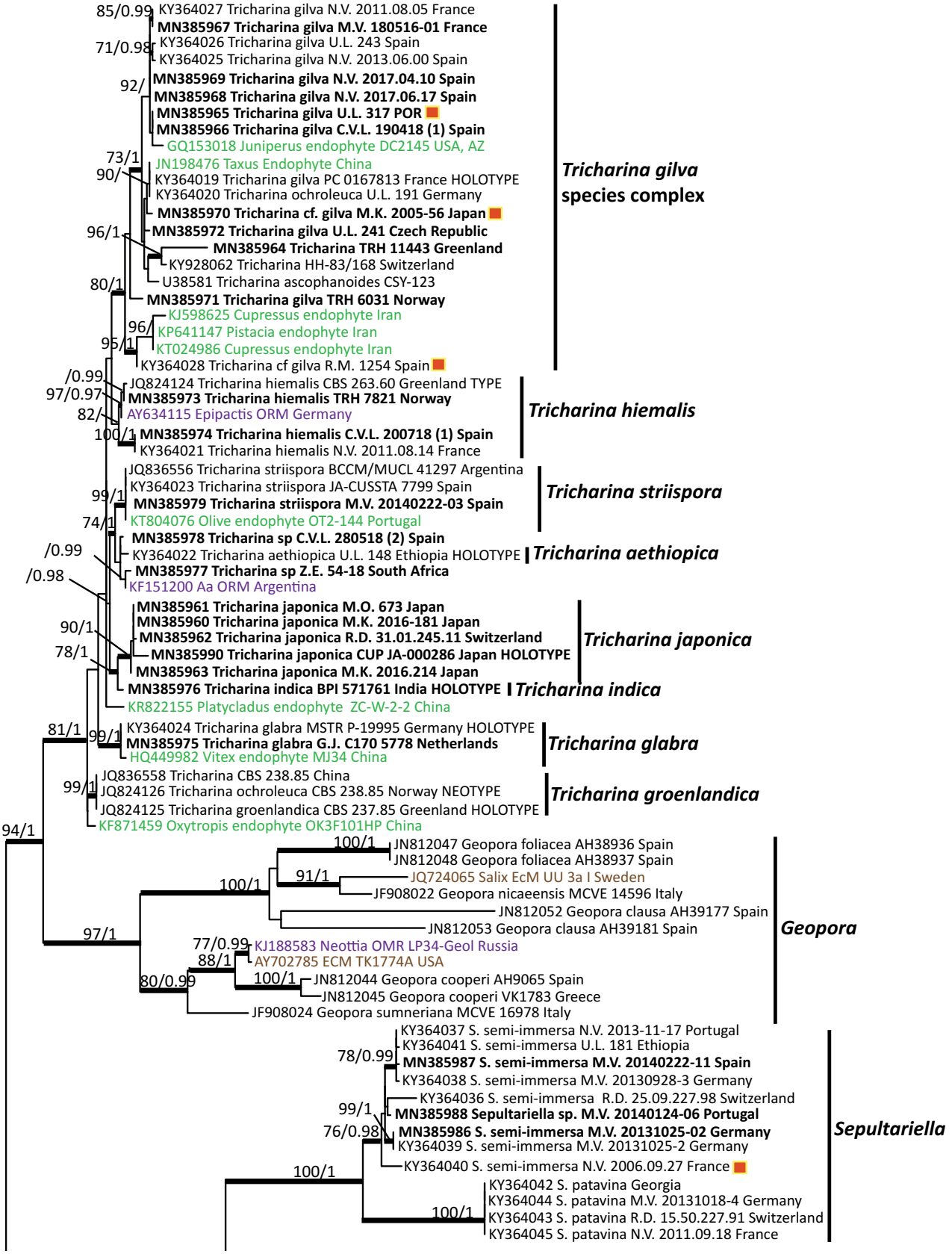

Fig. 2 – RAxML consensus tree based on ITS alignment. Significant support is shown at the nodes, with maximum likelihood bootstrap ≥ 70% on left and Bayesian probability ≥ 0.95 on the right. Where both are significant, the branch is thickened. Newly generated sequences are in bold. Color coding of leaves represents origin of sample sequenced: ascomatal sequences as black, endophytes are green, ectomycorrhizal root (ECM) tips are brown, orchid mycorrhizal root (ORM) tips are purple. Collections from burned habitats are indicated by red boxes framed in yellow.

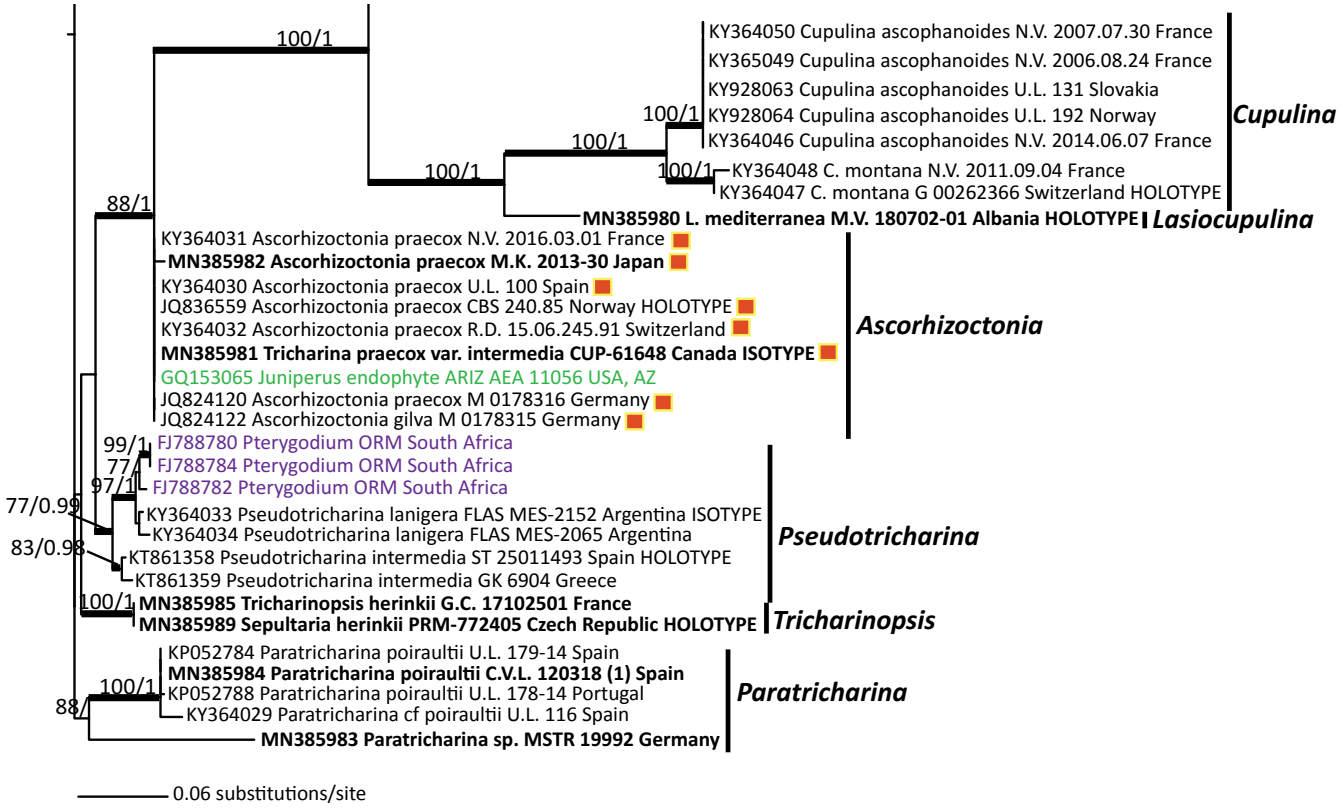

Fig. 2 – RAxML consensus tree based on ITS alignment (*continued*).

Tricharina indica Van Vooren & U. Lindemann, *sp. nov.* – MB 828454.

Diagnosis: Differs from *Tricharina japonica* by its shorter ascospores, shorter marginal hairs and bulbous excipular hairs, and from *T. pallidisetosa* by shorter ascospores without bipolar granules.

Holotype: BPI 571761; GenBank ITS MN385976, LSU MN386004.

Etymology: From Latin, *indica*, after the country where the species was collected first.

Misapplication: *Tricharina cretea* (Cooke) K.S. Thind & Waraitch, *Res. Bull. Panjab Univ.*, 21 (1-2): 154 (1971).

Tricharina flava

In the course of his taxonomic revision of the genus *Cheilymenia*, J. Moravec re-examined the type of *Humaria flava* considered as a *Cheilymenia* by Boudier (1907). He excluded the taxon from *Cheilymenia* and proposed a new combination in *Tricharina* (Moravec, 1990).

Re-examination of type-material of *Humaria flava*

Humaria flava is typified by the collection G 00127716 (*ex* herb. Barbey-Boissier, *ex* herb. Fuckel 1894) housed in the herbarium of Conservatoire et Jardin botaniques de la Ville de Genève (Switzerland). The type contains one complete apothecium of 2 mm diam. and seven fruitbody fragments; the biggest one measures 5 mm diam. The complete apothecium is deeply cupulate and smooth on the outside; the margin is without any hairs. The colour of the hymenium is brownish-yellowish (dried), the outside has the same colour. The hymenium of the fruitbody fragments has the same colour, but the outside is darker through remnants of soil. The complete apothecium and one of the fruitbody fragments are glued to a small carton. The other fragments are loose in a separate paper bag. The substrate on which the species grew is also in a separate paper bag.

A drawing by L. Fuckel and three handwritten notes accompany the samples: one by A. Raitviir who examined the type material in December 1981 and two other by J. Moravec who examined it in March 1990. There is also a copy of the printed original description (Fuckel, 1870). According to the original description, the type collection was found in autumn (the year is not mentioned) on naked muddy soil at the "Altrhein" near Hattenheim in Germany (Hessen).

The re-examination of the complete apothecium revealed that it was immature (no asci, ascospores or paraphyses could be observed). In contrast, the hymenial elements of the fruitbody fragments were fully developed. The following description is based upon observations made on the fruitbody fragments.

Description (†) of microscopic characters (Plate 2–3):

Excipulum composed of two layers: **Medullary excipulum** of *textura prismatica*; **Ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells (up to 29 µm diam.), the outermost layer consists of yellowish brown, slightly thick-walled cells. **Excipular hairs** superficial, densely interwoven, up to 7.6 µm wide, hyaline, smooth, multiseptate, thick-walled (wall refractive), up to 1.0 µm wide. All observed hairs are broken. Therefore it was not possible to measure their length or to describe their apices. No **marginal true hairs**, but club-shaped cells present at the margin, hyaline, rather thick-walled. **Asci** 140–160 × 14–15 µm, operculate, cylindrical, 8-spored, base with crozier, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without content, 3.8–5.2 µm wide, not or slightly enlarged at the apices. **Ascospores** uniseriate, ellipsoid with tapered ends to subfusoid, thin-walled, hyaline or slightly brownish, smooth, without oil drops but containing some bipolar granules, measuring in H_2O: 18.6–20.3 (21.4) × 9.7–12.7 µm [X = 19.2 × 11.2 µm, n = 21], Q = 1.6–2.0 [Qm = 1.7]; in 3% KOH: 19.9–21.5 (25.6) × 10–11.8 µm [X = 21 × 11.1 µm, n = 11], Q = 1.7–2.0 [Qm = 1.9].

Comments: The observations made during the re-examination of the holotype material are widely consistent with the protologue of Fuckel (1870: 322) and Moravec's handwritten comments (see Plate 2). However, some minor differences should be noted:

Firstly, the spore size given by Fuckel is 22 × 10 µm whereas Moravec noted 16.5–22.5 × 8.5–10.8 (12) µm. In our examination, the spore length is somewhat smaller than Fuckel's data but in the range of what Moravec measured; the spore width is slightly larger than the measurements of Fuckel and Moravec but not significant in our opinion.

Secondly, the shape of the ascospores is described in the protologue as "*oblongo-ovatis*" which means elongated ovoid; Fuckel's drawing shows ascospores that are ellipsoid and not ovoid (cf. Plate 3). Moravec drew an ascospore with a fusoid-trapezoidal shape. In our review, we also saw some deformed ascospores like Moravec's drawing but mostly they were ellipsoid with tapered ends to subfusoid (cf. Plate 3).

Thirdly, whereas Fuckel in the protologue noted that the asci have a length of 280 µm, we measured on dead asci only 140–160 µm. Even if one considered the shrinking effect between vital and dead material, which results in differences of 20–30% (cf. Baral, 1992), the length of the asci is significantly shorter.

Fourthly, contrary to the assertion of Fuckel and Moravec, the ascospores are not eguttulate but contain some inclusions at the

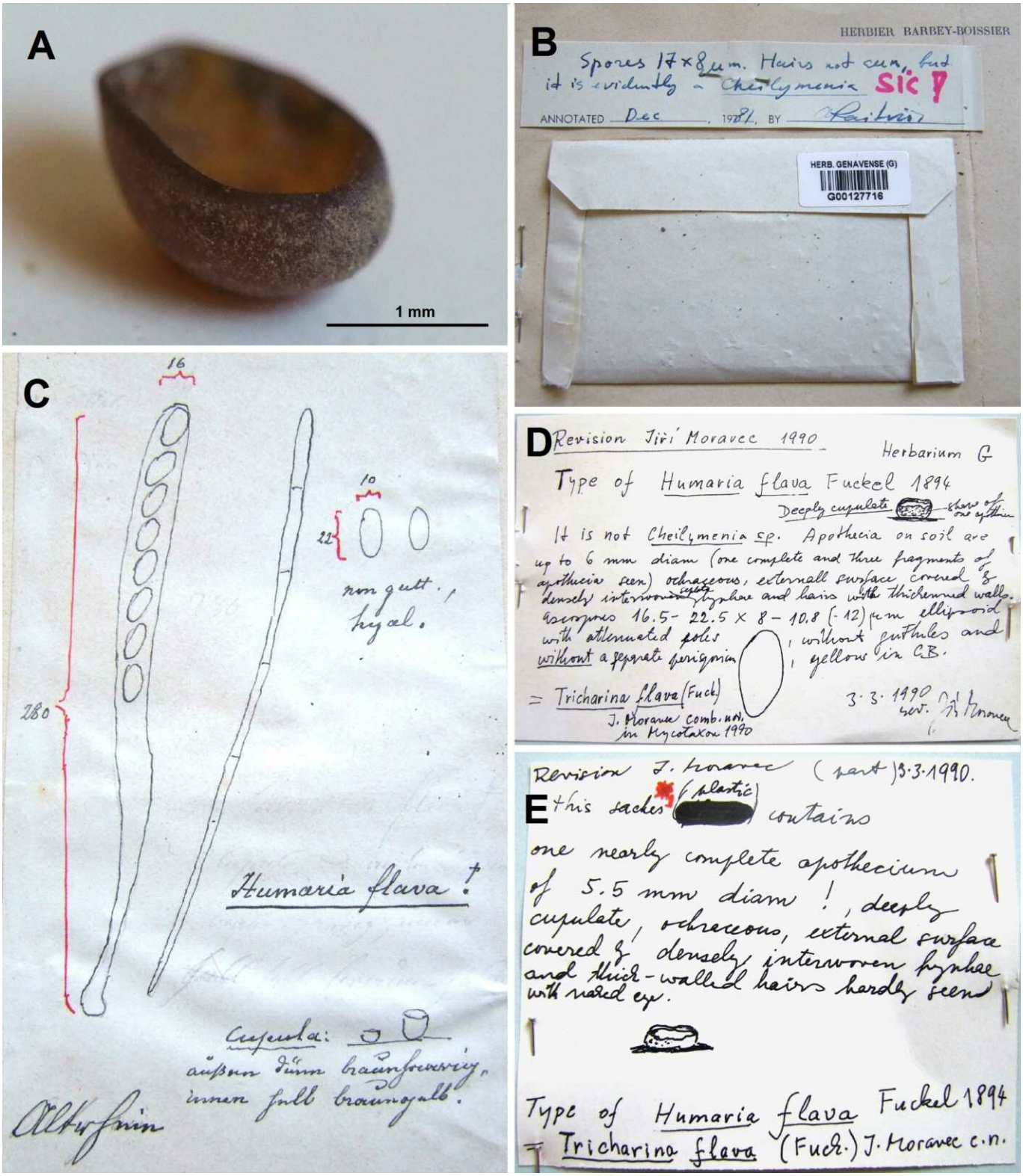

Plate 2 – *Humaria flava* (holotype, G 00127716)
A: Complete apothecium in dry state (immature). B: Raitviir's handwritten note together with the paper bag of the type with substrate. C: Fuckel's drawing with handwritten notes and data. D-E: Moravec's handwritten notes. All photos by U. Lindemann.

poles. The cell plasma of the ascospores is slightly brownish in H_2O, but hyaline in 3% KOH.

Furthermore, Fuckel (1870) wrote in his diagnosis that the excipulum is covered with brown hairs. He also noted in the German description: "Der *H. tenuis* steht sie sehr nahe, unterscheidet sich aber, durch die angegebenen Merkmale, bestimmt von derselben." which means "Similar to *H.* [*Geopora*] *tenuis*, but regarding the morphological features clearly different from this species." This suggests that *H. flava* looks like a *Geopora*. This cannot be verified because the apothecium is fully hairless. Concerning the excipular hairs observed on the fruitbody fragments, it is not clear from which part of the apothecium the fragments originated. Are they from the flanks or from the bottom of the apothecium?

Although this collection presents eguttulate ascospores, inamyloid asci and paraphyses without content, the general habit of the species as well as the hairless margin suggest a different genus than *Tricharina* (cf. below the discussion about *Tricharina herinkii*, a morphologically very similar species to *H. flava*). Because the apothecium is immature, it is not possible to say whether it is an early stage of development or another species that was accidentally mixed with

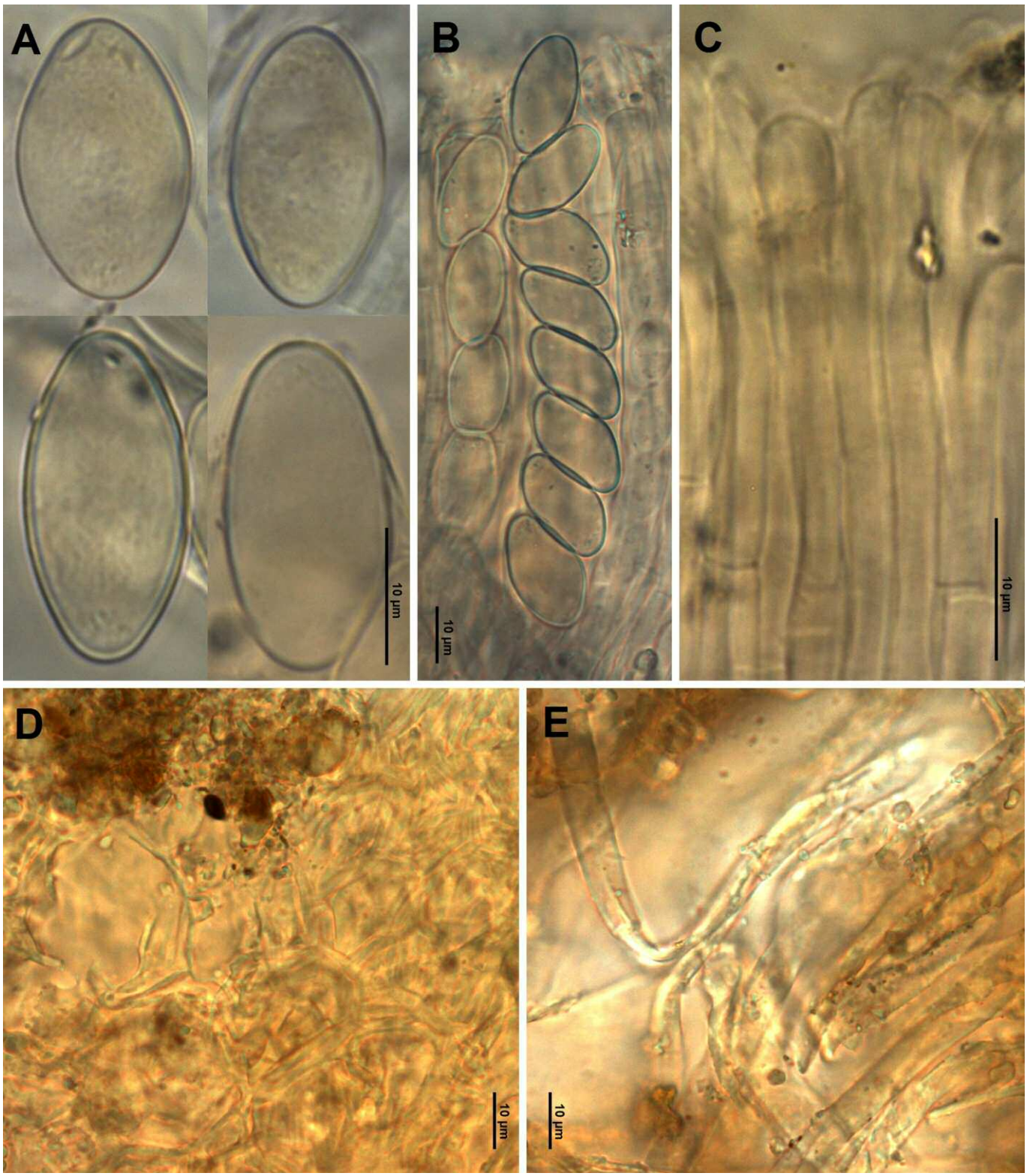

Plate 3 – *Humaria flava* (holotype, G 00127716)
A: Ascospores (dead) in H_2O (above) and 3% KOH (below). B: Asci (dead) in 3% KOH. C: Paraphyses (dead) in 3% KOH. D: Ectal excipulum in 3% KOH: Excipular hairs of the flanks (detail) in 3% KOH. All photos by U. Lindemann.

the type material. In this case, the type material of *T. flava* would contain two different taxa.

Tricharina glabra

On 4 Nov. 2017, two years after the collection of the type of *T. glabra* on the German island of Fehmarn in the Baltic Sea (cf. Lindemann & Böhning, 2016), a new collection of this species has been made in the vicinity of Renesse (Zeeland) on the Dutch coast of the North Sea. The morphological characteristics, as well as the ecological conditions of the Dutch collection correspond to the German type to a high degree. The Dutch find of *T. glabra* was made on a sandbank between green algae and ephemeral mosses in the outer coastal dunes which is occasionally flooded (Plate 4). The spore size of this collection fits well with the type: 20.5–26 × 13–18.5 µm [X = 23.1 × 15.7 µm], Q = 1.3–1.6 [Qm = 1.5] *vide* Gerrit Jansen. The only difference between the type and this Dutch collection concerns the paraphyses: the yellow pigment was not observed in the Dutch collection.

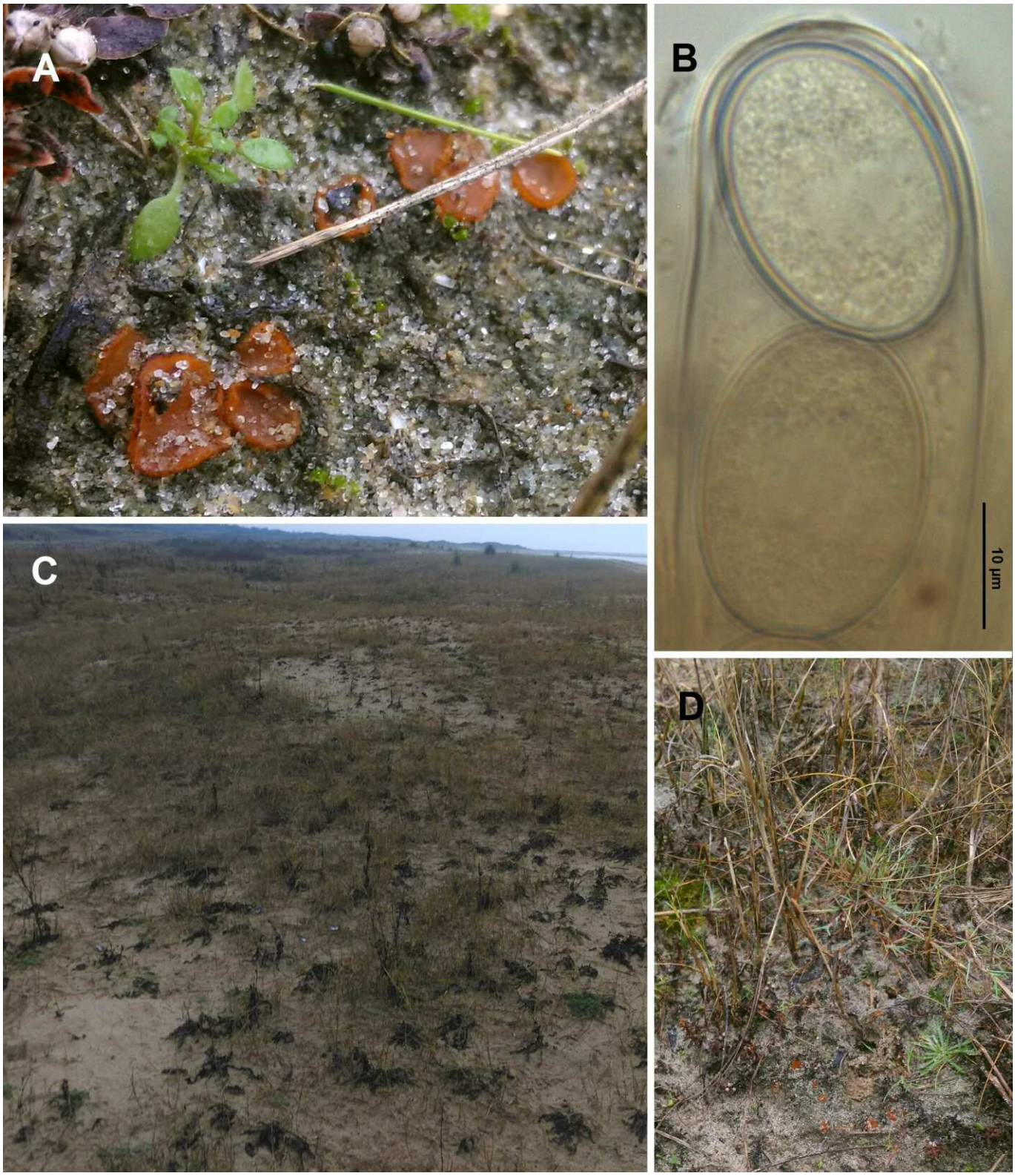

Plate 4 – *Tricharina glabra* (G.J. C170 5778)
A: Apothecia in fresh state *in situ*. B: Ascospores in the living state. C: Location at the outer dunes near Renesse (Zeeland). D: Habitat of the collection. Photos A, C + D by I. Wind, B by U. Lindemann.

Collection data: Netherlands – Province Zeeland, Renesse, N 51.734527°, E 3.733806°, alt. 5 m, on sandy soil in the outer dunes, 4 Nov. 2017, *leg*. Marjon van der Vegte, pers. herb. G.J. C170 5778, GenBank accession numbers ITS MN385975, LSU MN386003.

Tricharina herinkii

Re-examination of type-material of *Sepultaria herinkii*

Sepultaria herinkii is typified by the collection PRM 772405 (*ex* herb. J. A. Herink, 1026/43) of which we have examined only a part[2]. This part contains seven dried apothecia ranging in size from 4–8 mm diam., in good condition. The apothecia are cupulate to disc-shaped and densely hairy on the outside; the margin is without any hairs. The colour of the hymenium is brownish-yellowish in dried state, the outside is darker brown. The apothecia are partly sunken in sandy soil. Svrček's label indicates "*Sepultaria Herinki* Svrček, *sp. nov.* Praha XIX. – Králov. obora, ad terr. humosam sub arb. frond. diver. (*Q.*, *Acer*, *Til*. etc.). *Legit* J. A. Herink & M. Svrček. *Determ*. M. Svrček. 25. IX. 1943. Photo No. 1132."

Description (†) of microscopic characters (Plate 5):

Excipulum composed of two layers: **Medullary excipulum** of *textura prismatica* intermingled with globose cells; **Ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells (up to 25 µm diam.), the outmost layer consists of brown-yellowish, slightly thick-walled cells from which brown or hyaline thick-walled anchor hyphae arise (which are up to 11 µm broad). **Hairs** on the whole outside the of the ectal excipulum, superficial, densely interwoven, up to 600 µm, 5–9.5 µm broad, brownish, towards the apices often hyaline, smooth to (sometimes) finely verrucose, slightly to strongly flexuous, only a few hairs at the margin, which are more or less straight, obtuse, multiseptate, thick-walled (walls refractive), 0.5–1.0 µm broad, arising from a bulbous base, walls of the hairs not staining in CB. **Asci** operculate, cylindrical, 215–220 × 15–17 µm (only few intact asci observed), 8-spored, base with crozier, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without content, 2.5–4.5 µm broad, not or (rarely) slightly enlarged at the apices. **Ascospores** uniseriate, ellipsoid with tapered ends to subfusoid (fusoid only if overmature), thin-walled, hyaline, smooth, without oil drops but containing some bipolar spore granules at the poles, (18) 18.5–22.5 × (9.5) 10–11.5 (12.5) µm [X = 20 × 10.8 µm, n = 45], Q = 1.7–2.1 [Qm = 1.8].

Comments: The observations made during this re-examination are broadly consistent with the description of Svrček (1949: 86). However, some minor differences should be noted:

First, the spore size given by Svrček, i.e. 16–20 × 9–10 µm, is somewhat smaller than in your examination, about 2 µm in length and about 1.5 µm in width. In contrast, Benkert (2010: 52) gave (16) 17–21 (22) × (9) 10–11 (11.5) µm as spore size for his own collections of *S. herinkii*. This corresponds well to our current data.

Second, the shape of the ascospores is described in the protologue as "*longe fusoideo-ellipsoideae*"; on table VII, fig. 20, Svrček drew two ascospores with a fusoid-trapezoidal shape. In our observation, we saw this shape only for those ascospores that were obviously overmature or deformed by rehydration (cf. Plate 5). In contrast to this, the ascospores of the type of *S. herinkii* are mainly ellipsoid with tapered ends to subfusoid depending on the degree of maturity. This is in conformity with Benkert (2010: 3) who also examined the holotype.

Another collection identified as *Geopora herinkii* in Senn-Irlet (1989) was investigated. The ascospores of Senn-Irlet's collection are distinctly different in size and shape from those of the holotype of *S. herinkii*. In addition, at the margin of Senn-Irlet's collection straight brown hairs organized in fascicles are present. *S. herinkii* lacks this conspicuous feature. The analysis of the rDNA sequences of this collection were congruent with the morphological analysis in separating this taxon from *Geopora* (see Van Vooren *et al.*, 2017, Fig. 1 and 2).

Due to the features of the ascospores, which differ significantly from those of *Geopora* species, Benkert (2010: 52) suggested a classification of *S. herinkii* in *Tricharina* and proposed a new combination. After our emendation of *Tricharina* and allied genera (Van Vooren *et al*., 2017), *Tricharina* is now restricted to species which share the following features: eguttulate ascospores, paraphyses without true pigments, more or less pointed, straight, clustered marginal hairs and only few excipular hairs similar to the marginal ones. Although the macroscopic habit of *S. herinkii* is very similar to a *Tricharina* species, the absence of clustered hairs at the margin and the outer surface which is densely covered with long hyaline to brown flexuous hairs (reminiscent of a *Geopora* species) help to distinguish this species. Our phylogenetic analyses position it in a distinct clade, outside the *Tricharina* genus, close to the genera *Ascorhizoctonia* and *Pseudotricharina*.

Humaria flava (see above) and *S. herinkii* appear to be similar, especially in the size and shape of ascospores and the *Geopora*-like habit. It is possible that these two species are synonymous, but the type material of *H. flava* is very old and too scanty for destructive sampling, as well as a poor candidate for sequencing. We therefore consider *H. flava* to be a putative synonym of *S. herinkii* but wait for new collections from the same geographic area of the original collection to be sequenced.

Considering the results of the morphological investigation and phylogenetic analysis of *S. herinkii*, we propose a new genus to accommodate this particular species:

Tricharinopsis U. Lindemann, Van Vooren & Healy, *gen. nov*. – MB 828455.

Diagnosis: Differs from *Tricharina* Eckblad by the absence of clustered hairs at the margin and by an outer surface densely covered with long hyaline to brown flexuous hairs (reminiscent of a *Geopora* species).

Type species: *Sepultaria herinkii* Svrček

Etymology: Looks like a *Tricharina* species (Latin: *-opsis* = looking like).

Tricharinopsis herinkii (Svrček) U. Lindemann, Van Vooren & Healy, *comb. nov.* – MB 828456.

Basionym: *Sepultaria herinkii* Svrček, *Acta Mus. Nat. Prag., 4B*, 6 (*Bot*. 1): 86 (1948).

Homotypic synonyms: *Geopora herinkii* (Svrček) Senn-Irlet, *Beitr. Kenntn. Pilze Mitteleur.*, 5: 200 (1989); *Tricharina herinkii* (Svrček) Benkert, *Z. Mykol.*, 76 (1): 52 (2010).

Type: PRM 772405 – holotype; GenBank accession numbers ITS MN385989, LSU MN386012.

Other synonyms:

? = *Humaria flava* Fuckel, *Jb. nassau. Ver. Naturk*., 23-24: 322 (1870).

≡ *Lachnea flava* (Fuckel) Sacc., *Syll. fung*., 8: 179 (1889); *Cheilymenia flava* (Fuckel) Boud., *Hist. Class. discom. Eur.*: 63 (1907); *Tricharina flava* (Fuckel) J. Moravec, *Mycotaxon*, 38: 481 (1990).

Type: G 00127716, *ex* herb. Fuckel 1894 – holotype [examined].

Taxonomy

Tricharinopsis herinkii (Svrček) U. Lindemann, Van Vooren & Healy – Plate 6.

Apothecia up to 10 mm diam., sessile, cupulate, partly buried in the substrate, a bit spread at the end, with a hymenium beige or pale grayish beige; external surface concolorous or slightly darker on the young specimens, partially covered by scattered, hyaline small hairs. **Margin** eroded or torn, without true hairs.

[2] Table XI in Svrček (1949) shows thirteen apothecia of the holotype collection.

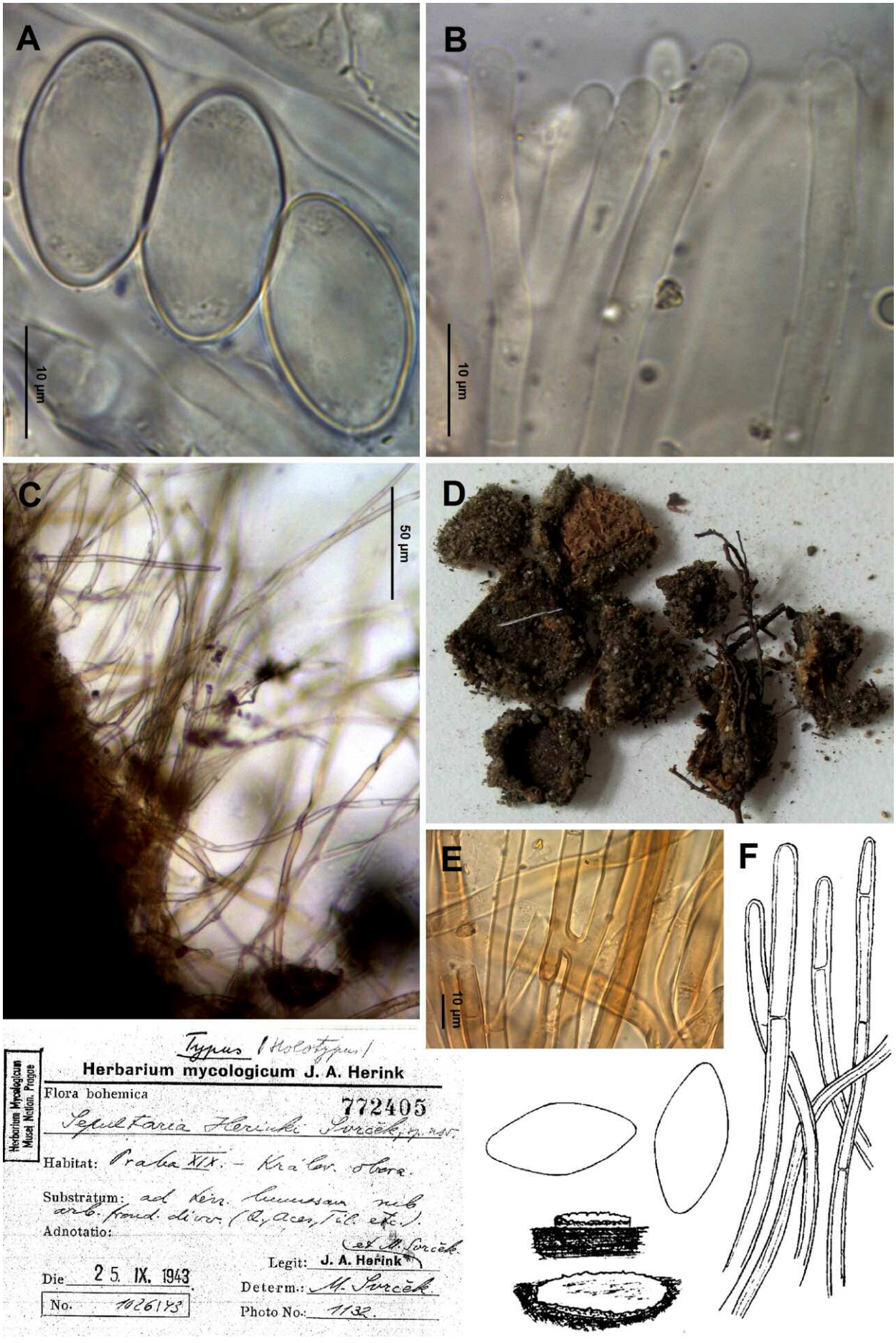

Plate 5 – *Sepultaria herinkii* (holotype, PRM 772405)
A: Ascospores (dead) in 3% KOH. B: Paraphyses (dead) in 3% KOH. C: Hairs of the flanks in H_2O. D: Apothecia of the holotype (dried), E: Excipular hairs (detail) in H_2O. F: Drawing of the features of the holotype by Svrček (1949): Tab. VII (re-arranged). All photos by U. Lindemann.

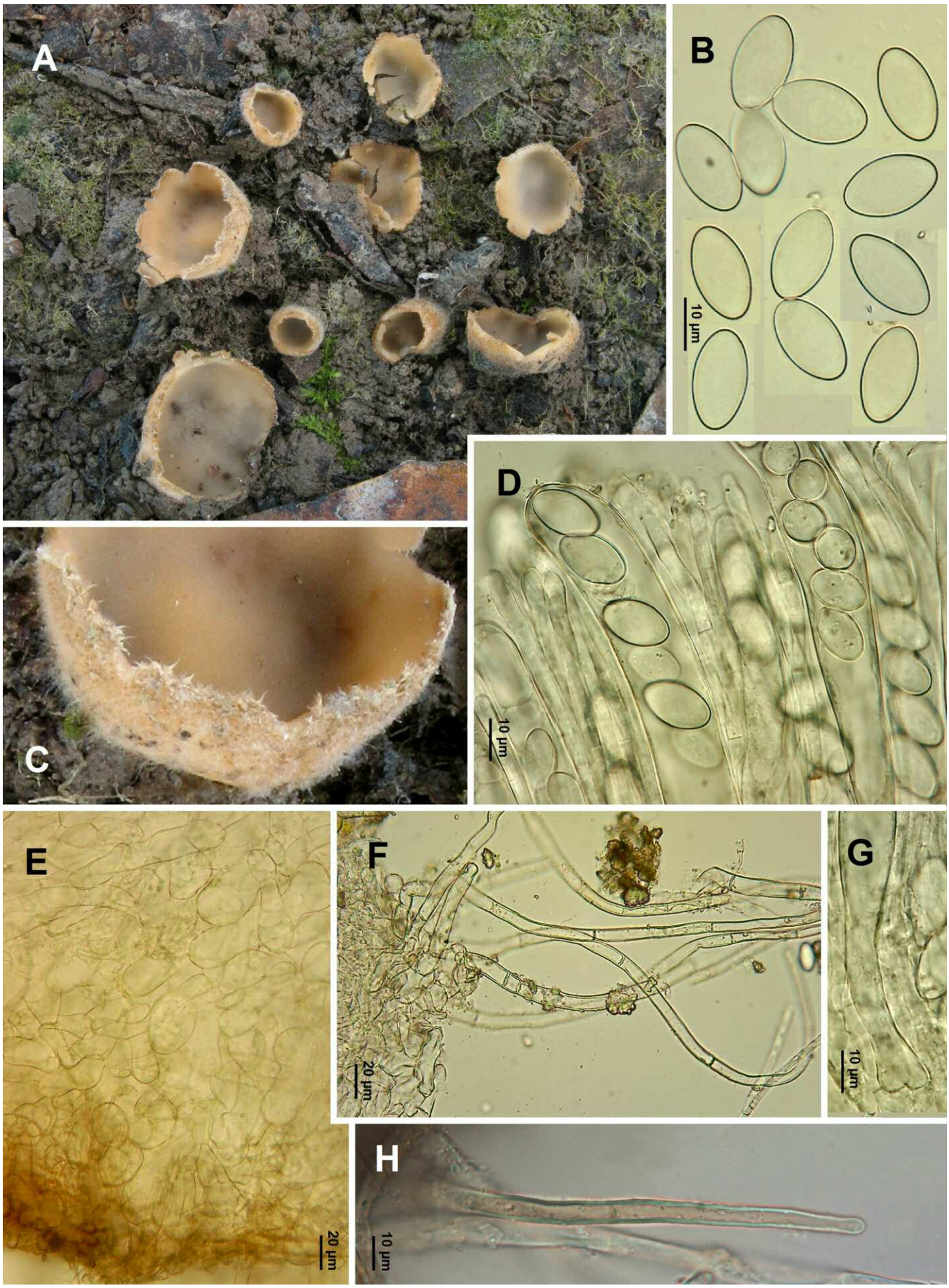

Plate 6 – *Tricharinopsis herinkii* (G.C. 17102501)

A: Apothecia in fresh state *in situ*. B: Ascospores in the living state in H_2O. C: Marginal zone and outer excipulum of an apothecium in fresh state (detail). D: Asci and paraphyses in living state in H_2O. E. Medullary (upper part) and ectal excipulum (lower part) in H_2O. F: Flexuous excipular hairs in H_2O. G. Ascus base in H_2O. H: Straight excipular hair in H_2O. Photos A–G by G. Corriol, H by U. Lindemann.

Subhymenium thin, of *textura intricata*, with hyaline hyphae. **Medullary excipulum** of *textura* ± *intricata* or *subprismatica*, hyaline, composed of elongated hyphae mixed with clavate cells. **Ectal excipulum** of *textura subglobulosa*, rather thin, with cells 14–45 µm wide, slightly brown-colored. **Anchor hyphae** present, hyaline, reaching 600 µm in length, 5–6 µm wide, mixed with hairs; they are difficult to be separated from some flexuous hairs, but the latter are light-colored. **Marginal hairs** rather short, up to about 200 µm, 7–12 µm wide, straight, obtuse, hyaline to brown, septate, wall up to 1 µm thick. **Excipular hairs** denser, reaching 460 µm in length, 5.5–8 µm wide, flexuous, brown-colored, septate, sometimes anastomosing, wall thinner, about 0.5–0.6 µm thick. **Asci** cylindrical, 185–200 × 13–15 µm, shortened at the base, with crozier, 8-spored, inamyloid. **Paraphyses** filiform, not widened at the top, Ø 4–6 µm, hyaline, nuclei not stained in acetic carmine. **Ascospores** (16.5) 17–20.5 (21.5) × 9–11 (12) µm [X = 18.4 × 10.5 µm, n = 21], Q = 1.5–1.9 [Qm = 1.8], 16–20 × 9–10 µm in the Svrček's protologue, ellipsoid with tapered ends or subfusoid, hyaline, smooth, very thin-walled, without oil-drops, but sometimes with small polar granules on dead rehydrated spores.

Studied collections: France – Alpes-de-Hautes-Provence, Manosque, close to "Plan d'eau des Vannades", along a small artificial channel, N 43.831456° E 5.836631°, alt. 300 m, on soil, numerous specimens, 25 Oct. 2017, *leg*. G. Corriol, pers. herb. G.C. 17102501 and N.V. 2017.10.01. Czech Republic – Prague, Královská obora, on wet soil, "under" broad-leaved trees (*Quercus*, *Acer*, *Tilia*, etc.), 25 Oct. 1943, *leg*. J.A. Herink, *det*. M. Svrček, PRM 772405 [holotype]. Germany – Thüringen, Weimarer Land, Mönchenholzhausen, N 50.926546° E 11.155685°, alt. 426 m, on soil, 2 Aug. 2019, *leg*. J. Girwert, *det*. U. Lindemann, pers. herb. U.L. 324.

***Tricharina* sp. B – Barr 5907 (FH)**

The sequence from the collection "*Tricharina* sp. B" Barr 5907 (FH) USA, Massachusetts 1971, collected by M. E. Barr (GenBank LSU DQ220446), from Perry *et al.* (2007) falls within the *Tricharinopsis* clade. Unfortunately, the sample cannot be found at FH. *Tricharina* sp. B is phylogenetically congeneric, maybe even conspecific with *T. herinkii*, but we know nothing about its morphology except that it is similar to a *Tricharina* species as the provisional name indicates. We hope that this species may be recollected, so that the ITS may be obtained and the morphology compared with the European collections.

With the publication of this new genus, we think it is useful to propose a comparative table of the main characters of *Tricharina* and allied genera (see Table 2 on the next page). A determination key will be provided in the next part of our work.

Tricharina hiemalis

T. hiemalis Chin S. Yang & Korf is a widespread but rare species, known from North America (Whitney & Parmeter, 1964) and Europe (Yang & Korf, 1985b; our data). It is very close to *T. gilva* but the ascospores possess a slightly different spore shape, the marginal hairs are distinctly longer than *T. gilva* (reaching up to 460 µm) whereas the hairs of the latter are never longer than 300 µm. In general, the apothecia of *T. hiemalis* are a bit hairier than of *T. gilva*.

According to Yang & Korf (1985), the type collection was based on the "perfect state of *Rhizoctonia hiemalis* developed in culture by P.H.B. Talbot […] June 1964 (K)." Unfortunately, we were unable to locate this collection in the mycological collections at Kew (Bond, pers. comm.) and we suppose that it is lost. The paratype CUP-061631 was examined, but it is so scanty that it is difficult to correctly evaluate all the features of this species.

Re-examination of the type-material of *Tricharina hiemalis* (paratype)

Description (†) of microscopic characters:

Excipulum composed of two layers: **Medullary excipulum** of *textura intricata*; **Ectal excipulum** of *textura globulosa*, composed of hyaline or yellowish, thick-walled cells (up to 33 µm diam.), yellowish-brown in the outermost part. **Excipular hairs** present on the whole outside of the ectal excipulum, superficial, up to 225 µm, 9–12 µm wide, very pale brownish, septate, with a refractive wall, 1.5–2 µm thick. **Marginal hairs** sparse, similar but straighter, hyaline, broken at the top, arising from a simple and slightly enlarged base. **Asci** operculate, cylindrical, 8-spored. **Paraphyses** filiform, hyaline, not enlarged at the top. **Ascospores** uniseriate, ellipsoid with tapered ends, 16–18 (18.2) × 9.5–11 µm [X = 17.1 × 10.1 µm, n = 30], Q = 1.6–1.8 [Qm = 1.7], hyaline, smooth, without oil drops but sometimes containing a small polar granule.

Comments: This collection presents the largest ascospores of all the studied *T. hiemalis* samples, but their shape conforms with the latter. Another character that deviates from the other collections is the color of marginal hairs which is here fully hyaline. Such discoloration is unusual in *Tricharina* although we observe hyaline apex on some hairs of *T. hiemalis*. A similar phenomenon is rather frequent in *Scutellinia* species, especially in mountainous areas, probably due to external factors (humidity, temperature, light, etc.) and is not considered as a valuable taxonomical character.

Taxonomy

Tricharina hiemalis Chin S. Yang & Korf, *Mycotaxon*, 24: 494 (1985) – Plates 7-8.

Apothecia sessile, 3–5 (11) mm diam., at first cupulate then spreading out, with a white to pale beige hymenium, ochraceous beige at the end; outside subconcolorous, a bit darker due to the dense small brownish hairs. **Margin** densely hairy, with dark brown hairs, organized in small clusters.

Excipulum composed of two layers, a **medullary excipulum** rather thin, of *textura intricata*, with hyaline hyphae, and an **ectal excipulum** of *textura globulosa*/*subglobulosa*, with hyaline cells, 12–40 µm diam., but with colored cells in the outer part. **Marginal cells** more elongated or clavate. **Marginal hairs** superficial, straight, 110–450 × 7–25 µm, dense, more or less fasciculate, septate, × 1–2 µm thick-walled, brown but often paler at the top, often very pointed at the top, with a simple base, enlarged to subbulbous, mixed with shorter ones. **Asci** cylindrical, 180–200 × 12–14 µm, with crozier, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, without droplets, not enlarged at the top, 3.5–5 µm diam. **Ascospores** ellipsoid, a bit tapering at the ends, (14) 15–16 (16.5) × 9.5–10.5 µm [X = 15.6 × 10.2 µm], Q = 1.4–1.7 [Qm = 1.5], but see the other spore dimensions from rehydrated material in Table 3, smooth, hyaline, without guttules, sometimes containing few inclusions at the poles, more or less refractive in CB.

Studied collections: Czech Republic – Bohemia, Mladá Boleslav, on soil, 18 Oct. 1967, *leg*. J. Moravec, *ex* herb. Moravec CUP-061631 [paratype]. France – Doubs, Amondans, Moulin Saillard, N 47.065167° E 6.04306°, alt. 510 m, on soil, on tuf, 17 Aug. 2011, *leg*. G. Moyne & N. Van Vooren, pers. herb. N.V. 2011.08.14. Norway – Nordland, Rana, Plurdalen, Toftlia-Sarkdet, N 66.3225825° E 14.6485791°, alt. 360 m, on soil mixed with ashes, 6 Sept. 1968, *leg*. S. Siversten, TRN 7821 [as *Tricharina* cf. *gilva*]. Spain – Málaga, Ronda, Zona del arroyo del Cupil, N 36.78171°, E -5.249°, alt. 630 m, on wet soil between stones in a rivulet, 20 July 2018, *leg*. F.J. Valencia López, pers. herb. C.V.L. 200718(1).

Comments: It is interesting to note the heterogeneity in spore size in the different collections we studied in comparison to the type collection measurements (*fide* Yang & Korf, 1985: 496). The new data

Table 2 – Morphological differences between *Tricharina s. str.* and related genera

	Tricharina	*Tricharinopsis*	*Ascorhizoctonia*	*Cupulina*	*Lasiocupulina* *	*Pseudotricharina*	*Paratricharina*
Ascospores	Smooth or striately warted in *T. striispora*, ellipsoid to subfusoid / trapezoid	Smooth, ellipsoid with tapered ends to subfusoid	Smooth to verrucose, ellipsoid with tapered ends to subfusoid	Smooth, ellipsoid to fusoid	Smooth, ellipsoid	Warted, ellipsoid with tapered ends to subfusoid	Smooth to finely verrucose, ellipsoid, slightly thick-walled, encapsulated by a delicate sheath
Spore content in living stage	Eguttulate or filled with numerous LBs in *T. glabra*	Eguttulate	With bipolar spore granules	With bipolar spore granules or filled with numerous small oil drops	With few bipolar spore granules	With one or two large oil drops	With some little lipid bodies
Paraphyses	Without pigments, except in *T. glabra* with yellowish pigment (not constant)	Without pigments	Filled with yellowish granular pigments	Without pigments	Without pigments	With a light yellow granular cytoplasm in *P. lanigera*	With granular pigments in VBs
Hairs	Hyaline to brown	Hyaline to light brown	Hyaline to light brown	Hyaline	Hyaline to light brown	Hyaline to light brown	Hyaline to dark brown
Margin hairs clustering	+	-	+	-	-	+	-
Flanks	Sparsely covered with some ± straight, hyaline to brown hairs, similar to the marginal hairs	Densely covered with long, hyaline to brown, flexuous hairs (reminiscent of ding a *Geopora* species)	Sparsely covered with some ± straight, hyaline to brown hairs, similar to the marginal hairs	Sparsely covered with some ± straight or flexuous, hyaline hairs	Sparsely covered with some flexuous, hyaline anchor hyphae	Covered with straight, hyaline to brown hairs, similar to the marginal hairs	Densely covered with short, brown, ± flexuous hairs
Ecology	Terrestrial (occasionally on burnt ground)	Terrestrial	Strictly pyrophilous	Terrestrial	Terrestrial	Terrestrial	Terrestrial

* See Van Vooren & Vega (2018) for this new genus.

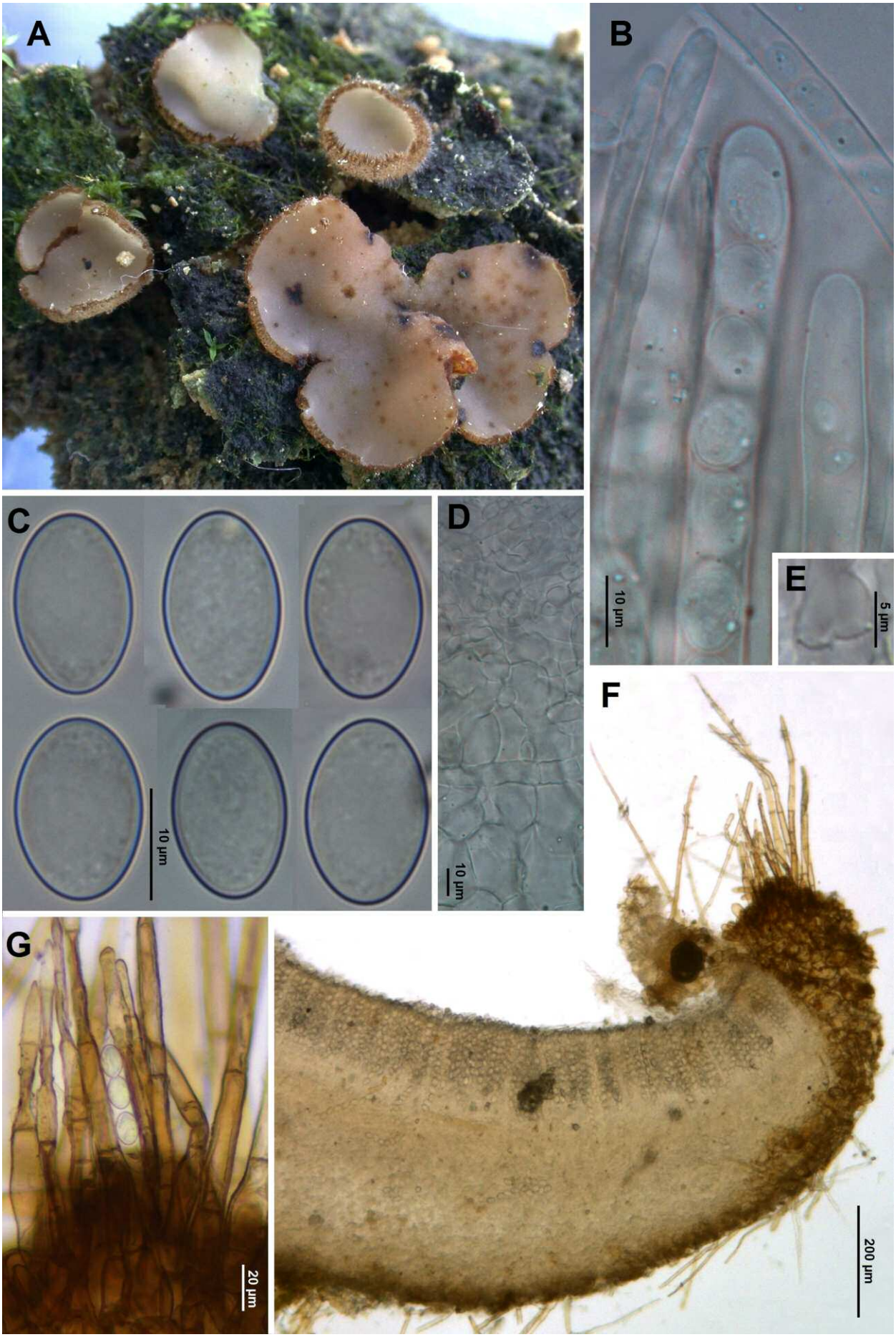

Plate 7 – *Tricharina hiemalis* (N.V. 2011.08.14)

A: Apothecia in fresh stage. B: Asci and paraphyses (dead) in H_2O. C: Ascospores in the living state in H_2O. D: Medullary (upper part) and ectal excipulum (lower part) in 3% KOH. E: Ascus base in H_2O. F: Vertical cut through the apothecium. G. Marginal hairs (base) in H_2O. Photo A by N. Van Vooren, photos B–G by U. Lindemann.

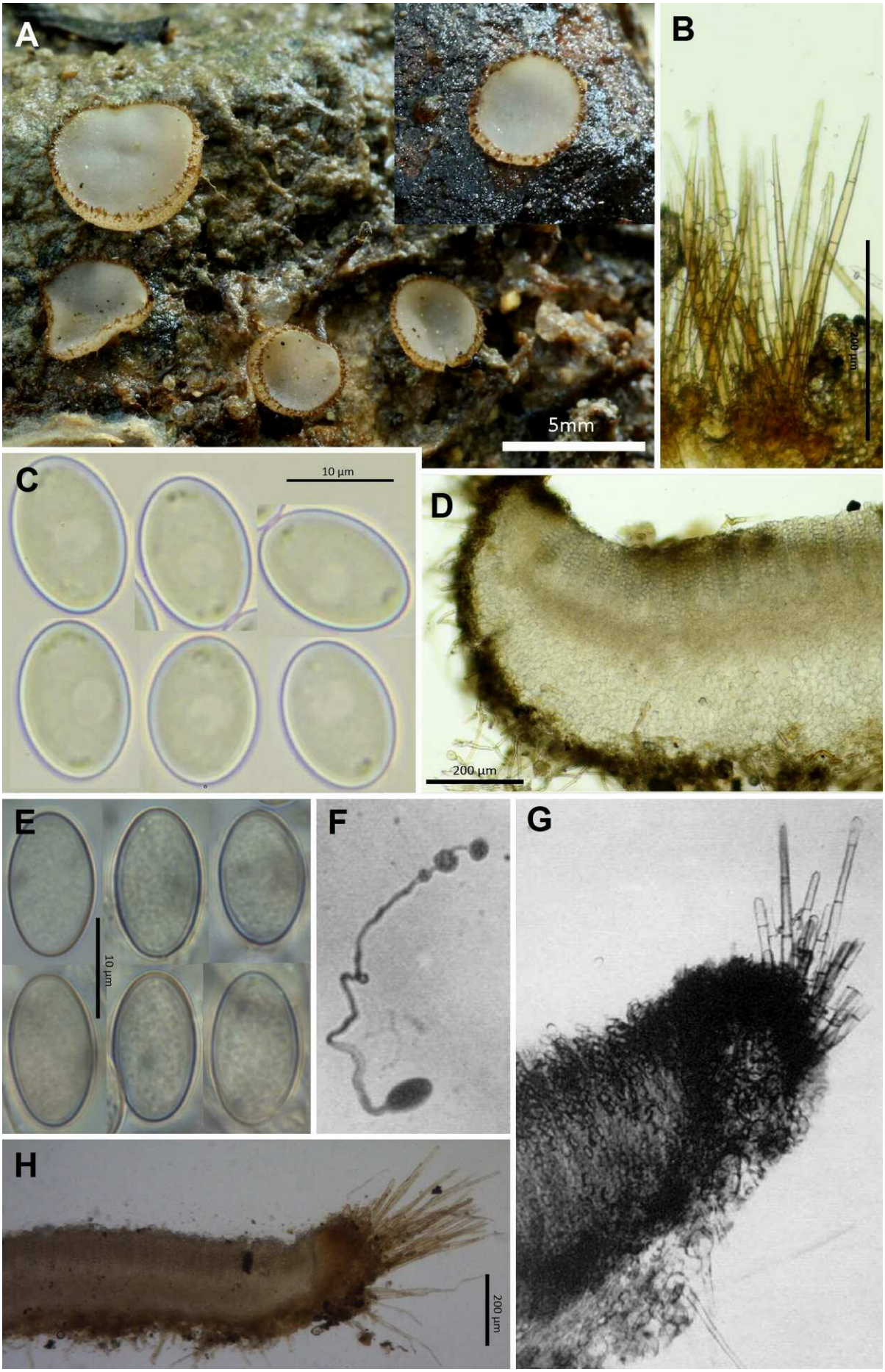

Plate 8 – *Tricharina hiemalis* (A-D from coll. C.V.L. 200718[1], E + H from coll. TRN 7821, F + G from the type) A: Apothecia in fresh state. B: Marginal hairs in H_2O. C: Ascospores in the living state in H_2O. D: Vertical cut of an apothecium. E: Dead ascospores in H_2O. F: Germinating ascospore. G: Vertical cut of an apothecium. H: Vertical cut of an apothecium. Photos A-D by F.J. Valencia Lopez, E + H by U. Lindemann, F + G from Whitney & Parmeter, 1964: 115, Fig. 1 + 3 (re-arranged).

Table 3 – The range of spore size and marginal hair size of the collections of *T. hiemalis* we studied and from the holotype

Coll. Number	Spore size (µm)	Mean spore size (µm)	Q value	Mean Q value	Marginal hair size (µm)
Talbot, June 1964 (K, holotype) *vide* Yang & Korf (1985)	(11.7) 13.2–15.4 × 7.3–8.8	?	?	?	220–300 (450)
CUP-061631 (paratype)	16–18 (18.2) × 9.5–11	17.1 × 10.1	1.6–1.8	1.7	up to 225
C.V.L. 200718(1)	(13) 13.8–15.7 (16) × (9.6) 9.9–10.6 (10.9)	14.9 × 10.2	1.3–1.6	1.5	up to 320
N.V. 2011.08.14	(14.3) 15–16.5 × 9.1–10.5	15.5 × 10.1	1.5–1.6	1.5	up to 350
TRN 7821	14–16.2 (17) × 7.7–9	15.0 × 8.5	1.8–2.0	1.8	up to 400

changes the Q value for the species. This could represent a difference in maturity; so additional fresh collections are required to confirm this hypothesis. Because we suppose the holotype lost, the question of typification of *T. hiemalis* is open. As an ITS sequence from the holotype exists, we could consider not necessary the designation of a "new" type. Nevertheless, we think that a physical type is required for morphological exams and for exploring another locus. Unfortunately, the two paratypes cited by Yang & Korf (1985) are poorly workable, first because one of them (coll. Hariot in PC, not seen) is probably too old for sequencing, second because Moravec's collection is too scanty and unrepresentative (see re-examination above). The collection TRN 7821, N.V. 2011.08.14 or C.V.L. 200718(1) could serve for an epitypification but we hesitate to use this procedure because a sequence already exists from the type. The phylogenetic results on ITS and LSU loci show that the species could be split into two different taxa but there is no real phenotypic basis for that, unless it may reflect the distribution of these taxa. Therefore we advocate waiting for new collections that will permit species hypothesis testing, and to treat *T. hiemalis* as a species complex in the meantime.

Tricharina japonica

T. japonica Chin S. Yang & Korf is a unique species in the genus *Tricharina*. It combines some very distinctive morphological features, especially its ascospores and marginal hairs.

Re-examination of the type-material of *Tricharina japonica*

Tricharina japonica is typified by the collection CUP-K-(JA-000286). It contains seven dried apothecia in the size of 4–7 mm diam., in good condition, and several fragments of broken apothecia. The dried apothecia are cupulate and densely hairy on the outside, especially at the margin where long brown hairs are growing in fascicles. In dried state, the color of the hymenium is light orange-brown in contrast to the outside, which is dark brown. The label of the type indicates "Fungi of Japan No. 286, *Tricharina* on soil, Mt. Tachibana, near Fukuoka Pref., Fukuoka, Kyushu. Coll. by S. Imai, H. Yoshii, R. P. Korf, *et al*. Det. W.C. Denison, 28 X 1957."

The holotype of *T. japonica* was recently re-examined by Kušan *et al.* (2015: 42). The revision contains a very detailed description of the type as well as a plate with macro- and microphotos of main morphological features (*loc. cit*., Fig. 3, M-W). We have nothing to add to this description and illustration.

Taxonomy

Tricharina japonica Chin S. Yang & Korf, *Mycotaxon*, 24: 497 (1985) – Plates 9-10.

Apothecia 5–10 (15) mm diam., sessile, at first cupulate then discoid or spread out, with a white to greyish hymenium; outside concolorous to pale brown. **Margin** densely hairy, with dark brown hairs, organized in small clusters. **Outer surface** sparsely covered with dark brown hairs similar to the marginal ones.

Excipulum composed of two layers, a **medullary excipulum** rather thin, of *textura intricata*, with hyaline hyphae, intermixed with few globose cells, and an **ectal excipulum** of *textura globulosa/angularis*, with hyaline cells, 15–40 (50) µm diam., but colored in the outer part. **Marginal cells** more elongated or clavate. **Marginal hairs** superficial, straight, up to 820 µm, dense, more or less fasciculate, septate, × 1–2 µm thick-walled, dark brown, pointed, with a simple enlarged to bulbous base. **Asci** cylindrical, 200–245 × 13–15.5 µm, with crozier, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, without droplets, not or only slightly enlarged at the top, up to 5–7 µm diam. **Ascospores** smooth, hyaline, in front view fusoid, slightly asymmetrical, in lateral view trapezoid, without oil drops, sometimes containing few inclusions at the poles, (15.5) 16.5–21.5 (23) × (7.5) 8.5–10 (10.5) µm [X = 19 × 9.2 µm], Q = 1.7–2.6 [Qm = 2.1].

Studied collections: Japan – Fukuoka Pref., Mt. Tachibana, on soil, 28 Oct. 1957, *leg*. S. Imai, H. Yoshii, R.P. Korf *et al.*, *ex* Fungi of Japan no. 286, CUP-JA-000286 [holotype]. Fukuoka Pref., Kyoto, Kibune, on forest ground, 29 Oct. 2016, *leg*. M. Kutsuma, pers. herb. M.K. 2016-181. Fukuoka Pref., Kyoto, Mt. Daimonji, on sandy ground, 18 Dec. 2016, *leg.* M. Kutsuma, pers. herb. M.K. 2016-214. Kanawaga Pref., Kamakura, Hiromachi ryokuchi, 23 Nov. 2016, *leg*. M. Nakajima, pers. herb. M.O. 668. Kanawaga Pref., Kamakura, Hiromachi ryokuchi, 25 Dec. 2016, *leg*. M. Nakajima & M. Ohmae, pers. herb. M.O. 673.

Table 4 – The range of spore size and marginal hairs size of the studied collections of *T. japonica*

Coll. Number	Spore size (µm)	Mean spore size (µm)	Q value	Mean Q value	Marginal hair size (µm)
CUP-JA-000286 (holotype) *vide* Kušan *et al.* (2015)	† (16) 16.4–21.5 (23.2) × (7.4) 7.7–9.8 (10.1), n = 200	19 × 8.7	1.85–2.51	2.18	145–790
R.D. 31.01.245.11 *vide* Dougoud & Marchi (2012)	(15.5) 16.5–19.5 (20.5) × (8.7) 9.5–10 (10.5), n = 25	18.8 × 9.5	?	2.0	120–400
M.O. 673	(16.9) 18.1–21.5 (22.8) × 8.5–10.3, n = 27	19.7 × 9.4	1.9–2.2	2.1	140–560
M.K. 2016-214	(17.6) 18.2–21 (22.7) × (7.5) 8.4–9.3, n = 27	19.7 × 8.9	2.0–2.6	2.2	up to 700
M.K. 2016-181	16.5–19 × 9.1–10.4, n = 31	17.7 × 9.7	1.7–2.0	1.8	up to 500

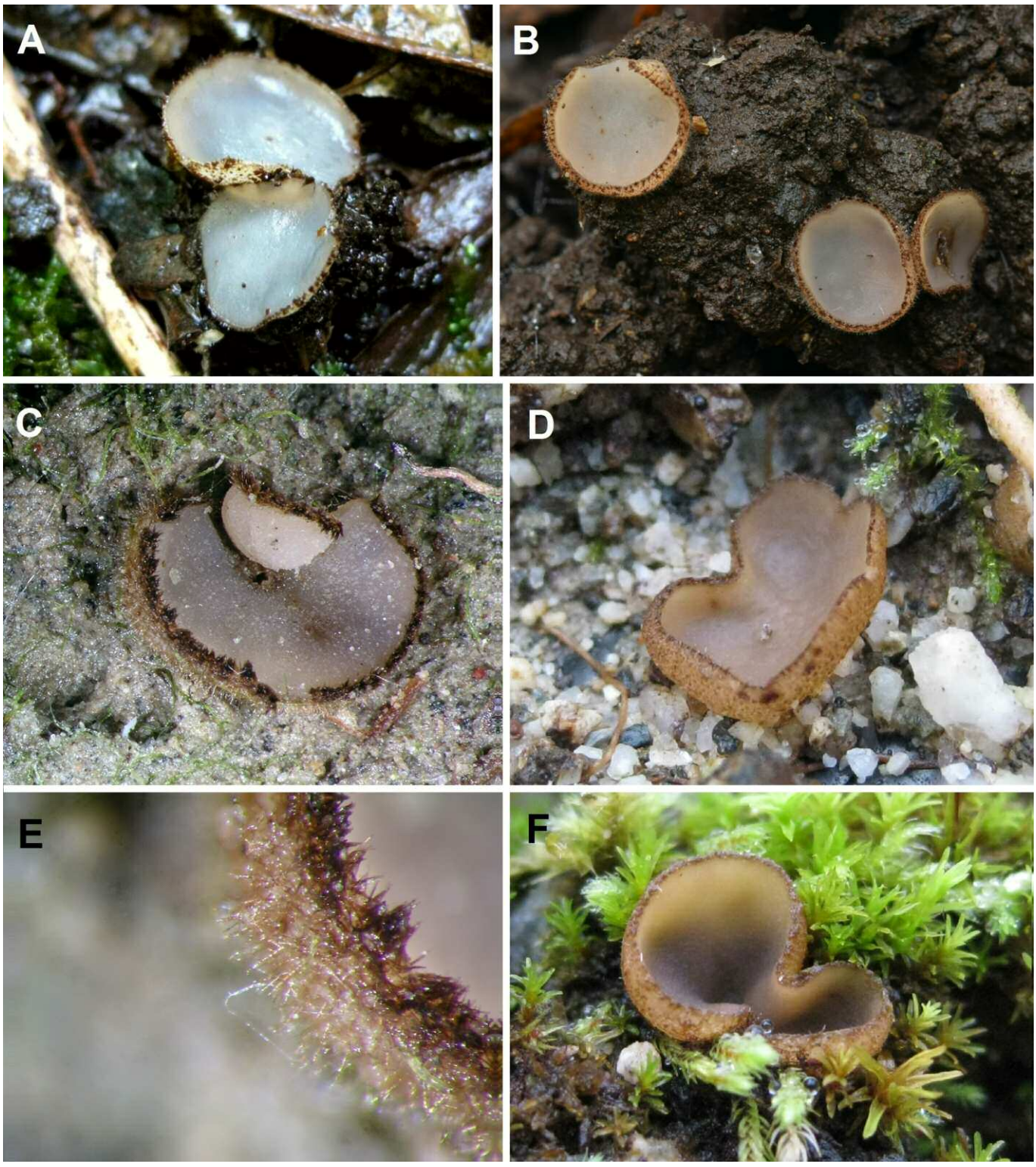

Plate 9 – *Tricharina japonica* (macroscopic habit)
A: Coll. M.K. 2016-214. B: Coll. M.O.-673. C: R.D. 31.01.245.11. D: M.K. 2016-181. E: Detail of R.D. 31.01.245.11. F: M.K. 2016-181 (another apothecium). Photos: A, E + F by M. Kusuma, B by M. Ohmae, C + E by R. Dougoud.

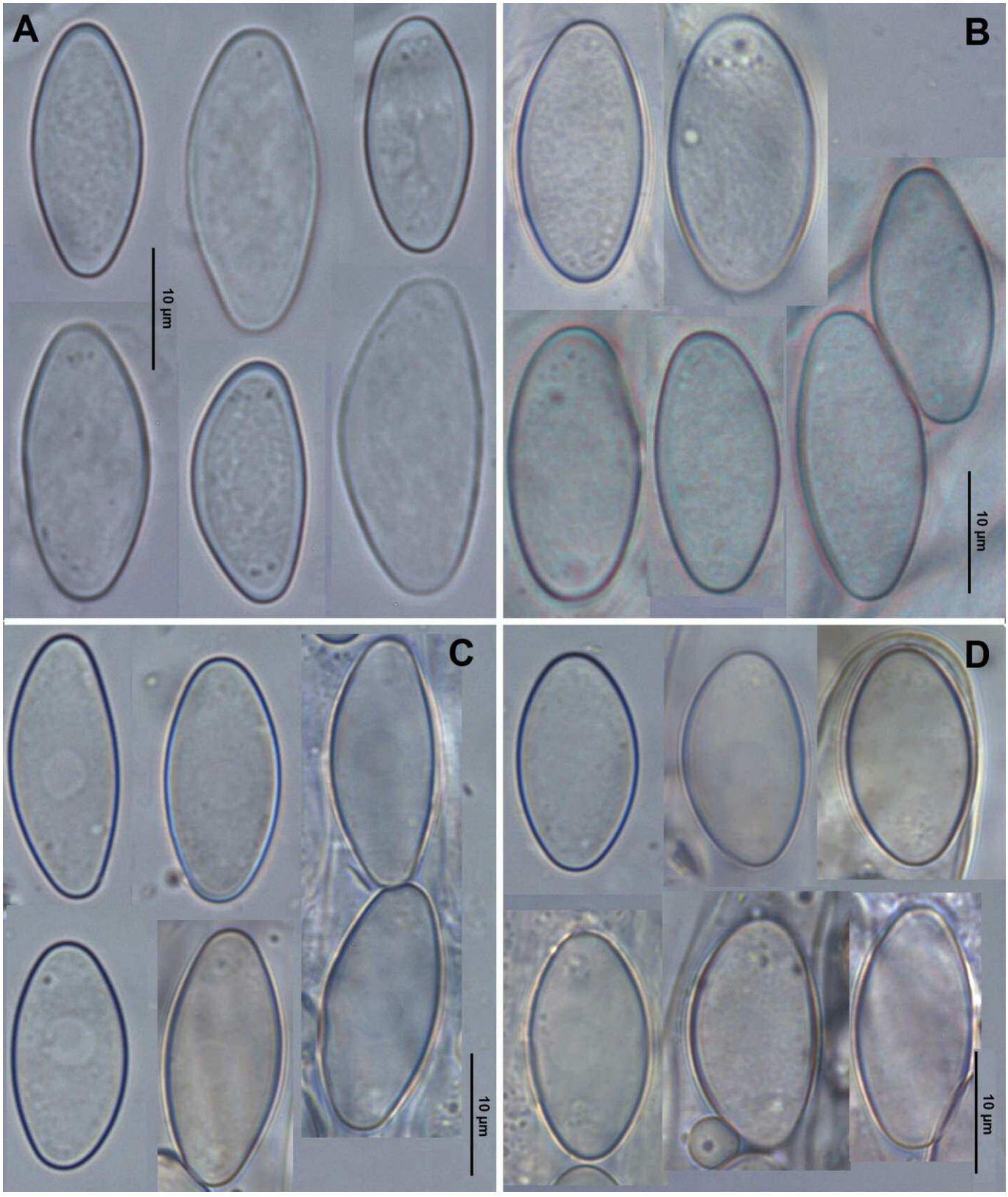

Plate 10 – *Tricharina japonica* (ascospores)
A: Coll. CUP-JA-000286 (holotype). B: Coll. M.O.-673. C: M.K. 2016-181. D: M.K. 2016-214. All photos by U. Lindemann.

Comments: Compared to the other *Tricharina* taxa, the ascospores of *T. japonica* are quite big and have an unusual shape. In front view, they are fusoid with more or less symmetrical sides, but in lateral view, they are trapezoid with distinctly asymmetrical sides. The hairs of *T. japonica* are the longest in the genus. After the type revision of Yang & Korf (1985), the hairs are up to 820 µm, after the type revision of Kušan *et al*. (2015) up to 790 µm. The current data of our own type revision confirm these measurements (Table 4).

Tricharina striispora

Tricharina striispora is a well-defined species, characterized by striate ascospores (Yang & Korf, 1985; Argaud, 2008; Galán *et al*., 2010; Ivaldi & Fouchier, 2014; Sammut, 2016), with stripes visible when observed in CB or in water with a 1000× lens with oil immersion.

Tricharina striispora Rifai, Chin S. Yang & Korf, *Mycotaxon*, 23: 509 (1985) – Plate 11.

Apothecia sessile, 4–6 mm diam., partly buried in soil, cupulate, pale beige to ochraceous beige; outside surface subconcolorous, covered by small reddish brown hairs. **Margin** with brown hairs in fascicles.

Excipulum composed of two layers, a **medullary excipulum** of *textura intricata*, with hyaline hyphae, and an **ectal excipulum** of *textura angularis/subglobulosa*, with yellowish cells, 20–42 µm diam., more deeply yellow-brownish, in the outer part. **Marginal hairs** superficial, straight, 180–550 × 8–14 µm, up to 25 µm in the basal part, septate, × 0.5–1.5 µm thick-walled, pale brown, sharp or obtuse, with a simple enlarged to bulbous base. **Asci** cylindrical, with crozier, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, without droplets, not enlarged at the top, 6–8 µm diam. **Ascospores** ellipsoid with tapered ends or subfusoid, (17) 17.5–19.5 (19.8) × 9–10 µm [X = 18.4 × 9.4 µm], Q = 1.8–2.2 [Qm = 2.0], hyaline, without oil drops but often with small inclusions at the poles, not refractive in CB and showing longitudinal stripes made of very fine, non-cyanophilous, warts.

Studied collections: France – Bouches-du-Rhône, Marseille, Montolivet, N 43.3172776° E 5.4292024°, alt. 148 m, on soil, under *Olea europaea*, 21 Mar. 2014, *leg*. P. Ivaldi, pers. herb. N.V. 2014.03.12. Greece – Lesbos Island, Mytilene, N 39.070555° E 26.57°, alt. 100 m, 17 Nov. 2007, *leg*. G. Konstantinides, pers. herb. G.K. 2724. Italy – Rome, Cimitero Monumentale del Verano, N 41.901944°, E 12.530167°, alt. 41 m, on soil, under *Olea europaea*, 23 Feb. 2015, *leg*. M. Vega, pers. herb. M.V. 20150223-11. Spain – Jaén, Fuensanta de Martos, Sierra de la Grana, N 37.601519° E 3.917626°, alt. 745 m, on soil in an olive tree plantation, 21 Jan. 2014, *leg*. S. Tello, JACUSSTA 7799. Andalusia, Málaga, Jardín Botánico-Histórico La Concepción de Málaga, N 36.766444° E -4.425083°, alt. 95 m, on soil, 22 Feb. 2014, *leg*. M. Vega, pers. herb. M.V. 20140222-03.

Comments: This is a widespread species known from Australia (type locality), Southern Europe (France, Greece, Italy, Malta, Portugal, and Spain) and South America. It is very often found in or near to plantations of *Olea europaea*. Previous phylogenetic analyses suggest that this species exists as an endophyte, though its trophic mode and life cycle are not well-studied (Van Vooren *et al*., 2017, Fig. 2).

Review of *Leucoscypha subimmersa*

Leucoscypha subimmersa K.S. Thind & S.C. Kaushal (Thind & Kaushal, 1979a) is typified by the collection Kaushal 2583 (PAN). We examined an isotype deposited at BRA (No. CR3179, *ex herbarium mycologicum* J. Moravec). It contains four dried apothecia in the size range of 1–2 mm diam., in rather bad condition. The dried apothecia are cupulate and densely hairy on the outside. The color of the hymenium is light brown to dark brown in dried state, the outside is dark brown. The apothecia are sunken in sandy soil. The label of the isotype indicates "*Leucoscypha subimmersa* Thind et Kaushal. Isotype of PAN, No. 2583 (holotype). India, Dehra Dun. On soil in tropical forest, 10. IX. 1973. *Leg*. S.C. Kaushal. *Det*. Thind et S.C. Kaushal. *Rev*. Jiri Moravec."

Description (†) of microscopic characters (Plate 12):

Excipulum indistinguishable but bilayered after the description by Thind & Kaushal (1979a): **Ectal excipulum** of *textura angularis*, **medullary excipulum** of *textura intricata*. **Excipular hairs** hyaline to slightly yellowish, smooth, obtuse at the top, with few septa, straight to more or less flexuous, slightly bulbous at the base, up to 10 µm wide, thick-walled, wall refractive. **Asci** operculate, cylindrical, 8-spored, inamyloid, with crozier. **Paraphyses** filiform, multiseptate, hyaline, without content, 3–4 µm broad, not or slightly enlarged at the apices (up to 5 µm). **Ascospores** uniseriate, ellipsoid to slightly fusoid, 18–19.7 × 9.4–11.8 µm[3], slightly thick-walled, hyaline, roughly warted, containing two oil drops.

Comments: Because of the rather bad condition of the apothecia of this isotype, it was not possible to make a more detailed examination. However, the features which could be observed are consistent with the original description by Thind & Kaushal (1979a: 461; cf. *ibid*. 460, Fig. D–G), but some minor differences should be noted. The spore size given by Thind & Kaushal (*loc. cit*.) differs not in length but somewhat in width. We measure 9.4–11.8 µm whereas Thind & Kaushal note 10–13 (15) µm. Furthermore, the ascospores in the rehydrated sample seem to be more fusoid than those shown on Fig.1, E in Thind & Kaushal (cf. *ibid*. 460).

Two attempts to amplify DNA from the isotype of *Leucoscypha subimmersa* failed, so sequences were not obtained. Nevertheless, we think that the protologue and our re-examination of the type-material are sufficient to circumscribe this species without ambiguity as a member of the genus *Pseudotricharina* Van Vooren, Tello & M. Vega (Van Vooren *et al*., 2015b). The bilayered structure of the excipulum, the size, content and, ornamentation of the ascospores and also the shape of the hairs indicate clearly a taxonomic position in *Pseudotricharina*. From the two other species of the genus, *Pseudotricharina intermedia* Van Vooren, Tello & M. Vega and *P. lanigera* Healy, D. Torres, Pfister & M.E. Sm., *L. subimmersa* differs not only by the size of the ascospores (15–20 × 10–13 (15) µm vs. (20.2) 20.5–23 × 10–12 µm for *P. intermedia* and (18.4) 20.8–23.2 × 11.2–12 (14.4) µm for *P. lanigera*) but also by the more pronounced ornamentation of the ascospores.

We therefore propose a new generic placement of *L. subimmersa* in *Pseudotricharina*:

Pseudotricharina subimmersa (K.S. Thind & S.C. Kaushal) U. Lindemann & Van Vooren, *comb. nov.* – MB 828457.

Basionym: *Leucoscypha subimmersa* K.S. Thind & S.C. Kaushal, *Bot. Notiser*, 132: 461 (1979).

Review of *Tricharina praecox* var. *intermedia*

Re-examination of type-material of *Tricharina praecox* var. *intermedia*

Tricharina praecox var. *intermedia* is typified by the collection DAOM (*ex* herb. K.N. Egger #1132). An isotype is deposited at CUP (No. 61648). It contains thirteen dried apothecia in good condition, measuring 2–5 mm diam. The dried apothecia are cupulate and

[3] In the rehydrated sample there were only a few non-deformed ascospores; therefore, no mean values are given.

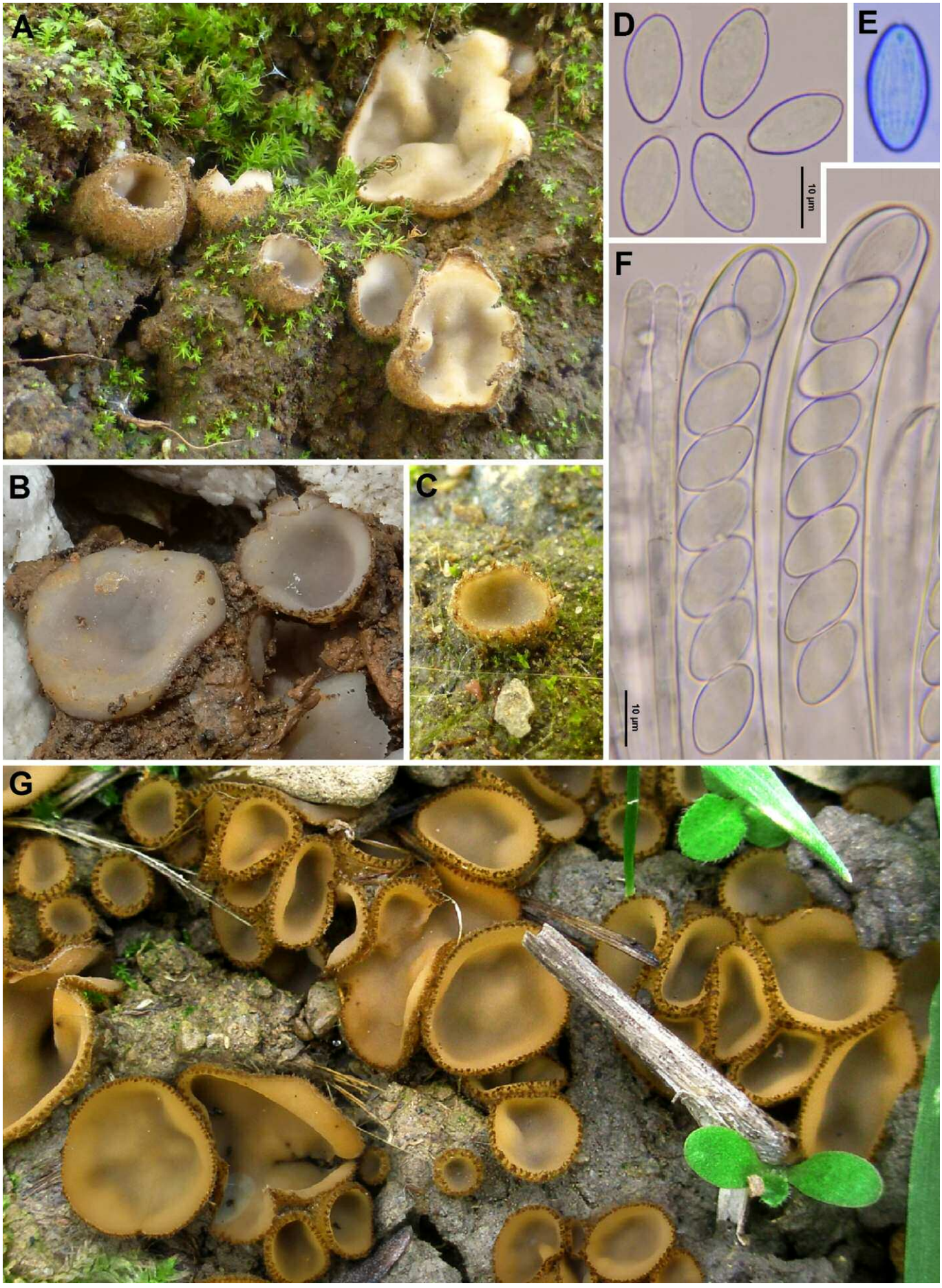

Plate 11 – *Tricharina striispora*

A-C: Apothecia in fresh state, coll. M.V. 20150223-11 (A), coll. JA-CUSSTA 7799 (B), coll. M.V. 20140222-03 (C). D: Ascospores in the living state in H_2O. E: Ascospore in CB. F. Asci and paraphyses in H_2O (D–F from JA-CUSSTA 7799). G: Apothecia in fresh state, coll. G.K. 2724. Photos A + C by M. Vega, photos B + D–F by S. Tello, photo G by G. Konstantinides.

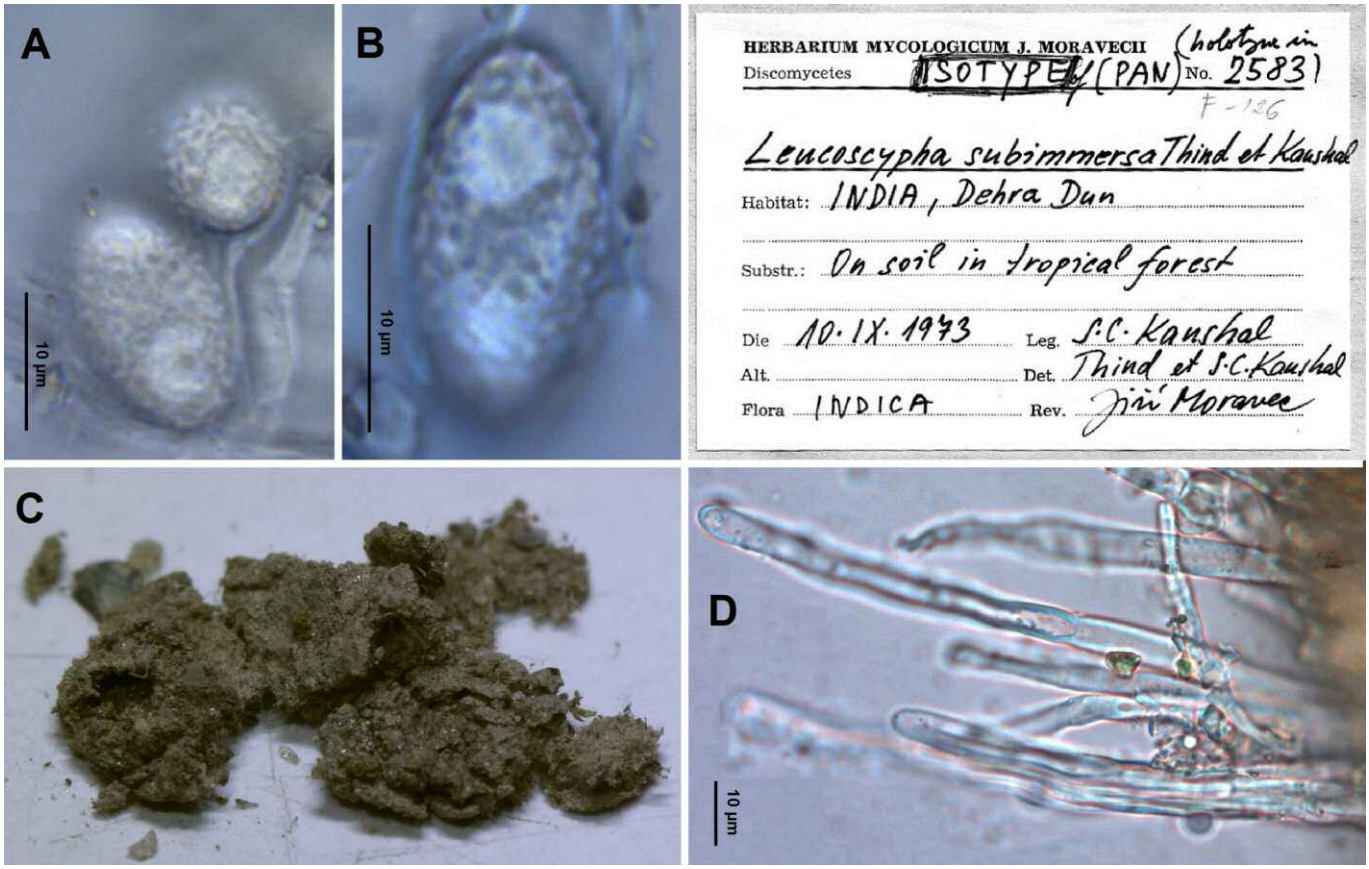

Plate 12 – *Leucoscypha subimmersa* (isotype, BRA CR3179)
A + B: Ascospores (dead) in CB after a pretreatment in 3% KOH. C: Apothecia of the holotype (dried). D: Marginal hairs in H_2O. All photos by U. Lindemann.

densely hairy on the outside, especially at the margin. The colour of the hymenium is light brown in dried state, the outside is dark brown. Growing on an abundant subiculum, the apothecia are partly sunken in sandy soil mixed with small pieces of ash. The label of the isotype indicates "*Tricharina praecox* (Karst.) Dennis var. *intermedia* Egger, Yang & Korf, *var. nov*. Isotype of var. On ash and burnt soil (sandy), in old gravel pit. Christina Lake, British Columbia, Canada, ex. Herb. K.N. Egger. Collected by K.N. Egger. Determined by C.S. Yang. 8 May 1984."

Description (†) of microscopic characters (Plate 13):
Excipulum composed of two layers: **Medullary excipulum** of *textura intricata*; **Ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells (up to 25 µm diam.), the outermost layer consists of brown-yellowish, slightly thick-walled cells from which hyaline, thick-walled, slightly to strongly flexuous anchor hyphae arise. **Marginal hairs** up to 170 µm in length, clustered, hyaline, smooth, thick-walled, wall refractive (wall circa 0.5 µm wide), obtuse or pointed at the top, brownish and few septate only near the base, base not bulbous, up to 10 µm wide. **Asci** 170–180 µm in length, operculate, cylindrical, 8-spored, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without content, 3–4 µm broad, not or slightly enlarged at the apices (up to 5 µm). **Ascospores** (13.6) 14.1–16.3 (16.9) × (8.1) 8.4–10 µm [X = 15.3 × 9 µm, n = 48], Q = 1.6–1.8 [Qm = 1.8], uniseriate, ellipsoid with tapered ends, slightly thick-walled, hyaline, smooth, without oil drops but containing few bipolar spore granules.

Comments: Our observations are broadly consistent with the description of the holotype in Yang & Korf (1985: 509; cf. *ibid*. 503f). However, some minor differences should be noted. The spore size given by Yang & Korf differs in length (about 1.5 µm) and in width (about 0.5 µm): 11.7–15.4 × 7.7–9.5 µm vs. (13.6) 14.1–16.3 (16.9) × (8.1) 8.4–10 µm in our measurement. The illustration of ascospores shown in Yang & Korf (1985: 508) are obviously deformed. We rehydrated the ascospores in 3% KOH. In this medium, we observed only very few deformed or collapsed ascospores. So, the different methods of rehydrating could probably cause the differences in the spore size.

Yang & Korf (1985: 509) suggest two morphological criteria for a delimitation of the variety *intermedia* from variety *praecox*. The ascospores of *intermedia* should have only rarely an ornamentation. We sequenced several collections of *Ascorhizoctonia praecox s. lato*, some with ornamented ascospores, some without. The ITS sequences are always identical. We conclude that the spore ornamentation is not a decisive taxonomical feature. Secondly, the colour of the hymenium should be different after Yang & Korf: The variety *praecox* should have a "brick-colored, grayish-ochraceous, or yellowish, drying gray to grayish brown" hymenium (*ibid*., 503–504), whilst the variety *intermedia* a "pale orange to orange-tinted" one (*ibid*., 509). In our experience, the color of the hymenium of *A. praecox* differs from site to site, depending on external conditions such as the moisture, the sunlight and the period of appearance (cf. Plate 5 in Van Vooren *et al.*, 2017). Finally, we sequenced the type of *Tricharina praecox* var. *intermedia* and compared the sequence to those of the type of var. *praecox*. The ITS sequences are 99% identical. Therefore, we conclude that there is no reason to maintain this variety.

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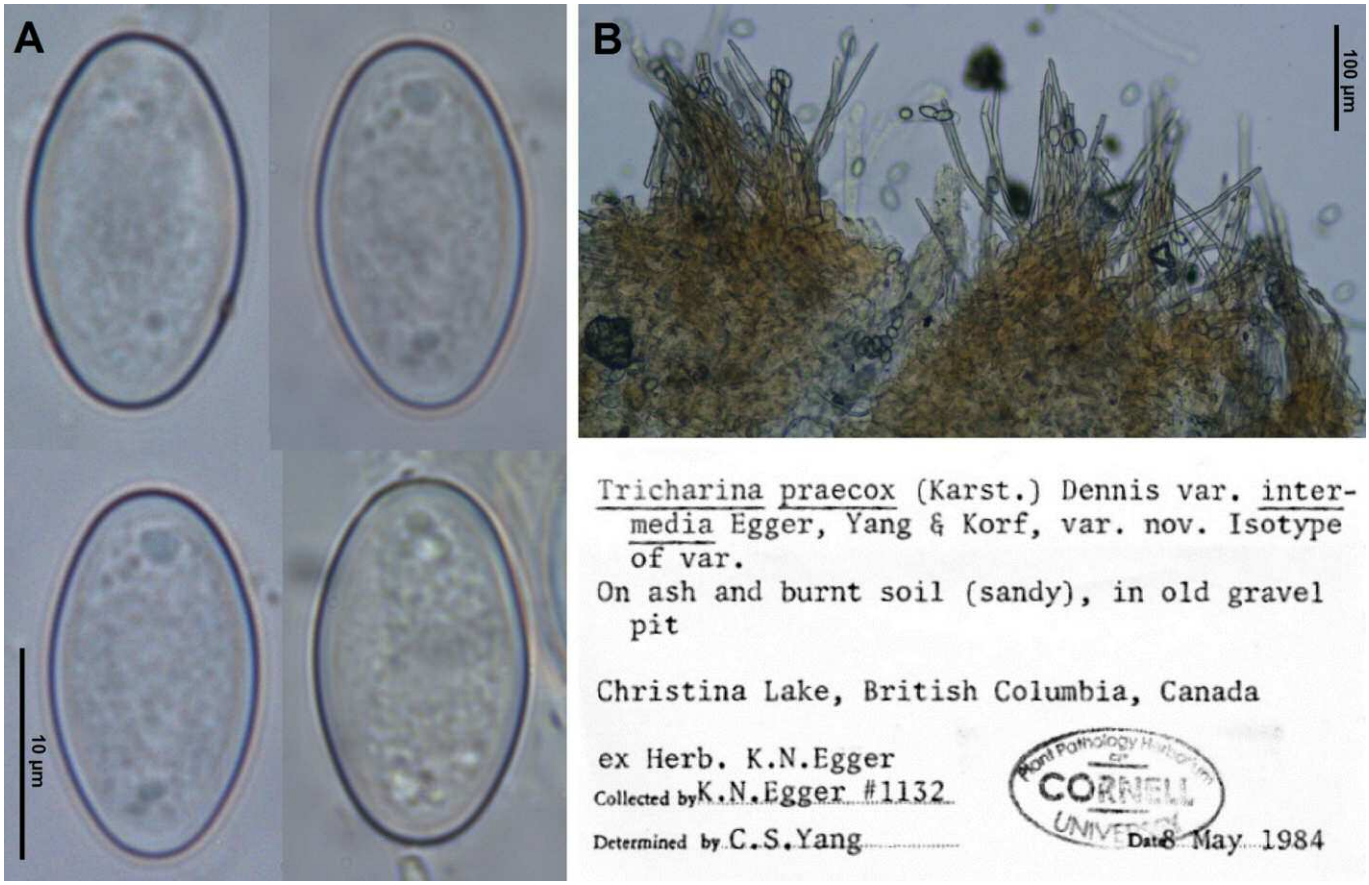

Plate 13 – *Tricharina praecox* var. *intermedia* (isotype, CUP 61648)
A: Dead ascospores in H_2O. B: Marginal hairs. All photos by U. Lindemann.

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1: N. Van Vooren – 36 rue de la Garde, 69005 Lyon, France – nicolas@vanvooren.info

2: U. Lindemann – Pflügerstr. 62, 12047 Berlin, Germany – uwe.lindemann0907@gmail.com

3: R. Healy – Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA – rhealy1@ufl.edu