Emendation of the genus *Tricharina* (*Pezizales*) based on phylogenetic, morphological and ecological data. Part 3. New type studies and description of two new species

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Abstract: The types of *Tricharina groenlandica* and *T. ochroleuca* are examined and documented. The species complex of *T. hiemalis* is discussed, and *T. subhiemalis* is proposed based on morphological and phylogenetic data. *T. tophiseda* which was currently only known from the type locality is also described and illustrated based on new collections. A new species from Greece, *T. gilvoides*, is presented in detail. Finally, a new dichotomous key to the genus *Tricharina* and allied genera is proposed.

Keywords: *Ascomycota*, distribution, ecology, endophyte, key, *Pyronemataceae*, ribosomal DNA, taxonomy, *Tricharina gilvoides* sp. nov., *T. groenlandica*, *T. hiemalis*, *T. ochroleuca*, *T. subhiemalis* sp. nov., *T. tophiseda*, *Tricharinopsis herinkii*.

In memoriam Christian Lechat, founder of the website AscoFrance, which improved and promoted international research and communication on ascomycetes in many ways.
Thank you, Christian!

Introduction

In our previous studies (Van Vooren *et al.*, 2017, 2019), we focused on the morphological and molecular identity of eight taxa that are assigned to the genus *Tricharina* Eckblad. In this third part, we report the results of the morphological and phylogenetic analyses of two further types: *Tricharina groenlandica* Dissing, Chin S. Yang & Korf and *T. ochroleuca* Eckblad. In addition, we will discuss the species complex of *T. hiemalis* Chin S. Yang & Korf and propose a new taxon to accommodate one of the two phylogenetic clades. Furthermore, we present and document four collections that we assign to *T. tophiseda* Matočec & I. Kušan, which is so far only known from the type locality in Croatia (Kušan *et al.*, 2015). We also propose a new taxon that shares many features with *T. gilva* (Boud. ex Cooke) Eckblad, type of the genus *Tricharina*, but distinguished both genetically and morphologically. Last but not least, we report two new collections of *Tricharinopsis herinkii* (Svrček) U. Lindem., Van Vooren & Healy from Germany and Spain. A new dichotomous key to the genus *Tricharina* and allied genera is proposed at the end of this document.

Material and methods

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

DNA extraction, amplification and sequencing. — 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. Sequences were corrected to remove reading errors in chromatograms.

Sequences obtained during this study were deposited in GenBank under the accession numbers listed in Table 1.

Nomenclature, terminology. — The taxonomic novelties were registered in the MycoBank database (www.mycobank.org). The nomenclature follows the current version of ICN (Turland *et al.*, 2018). The herbarium acronyms are in conformity with the Index Herbariorum (http://sweetgum.nybg.org/science/ih/). For specimens housed in personal herbarium, the terms "pers. herb." are used, followed by the author's reference.

Locations. — Locations of the studied collections are given by countries, in alphabetical order, then region (or province or department), town, more precise location (in native language). The coordinates are given in decimal WGS84 format.

Phylogenetic analysis. — We used the BLAST search algorithm to find the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public database (Cochrane *et al.*, 2011), including those published in our previous articles (Van Vooren *et al.*, 2017, 2019; Lindemann *et al.*, 2022). Selected sequences were aligned with the MUSCLE algorithm (Edgar, 2004) implemented in the software MEGA X (Kumar *et al.*, 2018) with the default execution parameters.

The first analysis included the region ITS of 94 samples (627 positions), whose 68 of *Tricharina* s. str., the others of tricharinoid species belonging to close genera. *Sepultariella semi-immersa* has been chosen as outgroup.

As the ITS region was highly variable and could not be unambiguously aligned, the second analysis included 76 sequences of the LSU region (811 positions), whose 51 of *Tricharina* s. str. and 23 of tricharinoid species belonging to close genera. Two sequences of *Ascobolus denudatus* have been chosen as outgroup because they belong to another family, *Ascobolaceae*.

Both evolutionary analyses were conducted in MEGA X by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2000) with 500 bootstrap iterations. The ITS tree with the highest log likelihood (-4859.45) is shown. The same was done for the LSU tree (-2829.14). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution (4 categories) was used to model evolutionary rate differences among sites.

The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. They were graphically edited with the online tool iTOL v.6 available at https://itol.embl.de (Letunic & Bork, 2021).

Phylogenetic results

Our phylogenetic results are consistent with the previous phylogenies published in Van Vooren *et al.* (2017, 2019). The topology of

Table 1 – List of collections of *Tricharina*, *Tricharinopsis* and *Cupulina* sequenced during this study

Name	Coll. Number	Country	Collector(s)	GenBank Number	
				ITS	28S
Cupulina ascophanoides	LY NV 2022.09.21	Italy	N. Van Vooren	OP747487	OP730569
Tricharina groenlandica	C F-52717 (Gr.83.003) HOLOTYPE	Greenland	H. Dissing	OP747473	–
Tricharina gilva	LY NV 2022.06.06	France	N. Van Vooren	OP747485	OP730555
Tricharina gilva	LY NV 2022.09.10	Italy	N. Van Vooren	OP747486	OP730556
Tricharina aethiopica agg.	MSTR:P-04826	Spain	F.J. Valencia	OP747471	OP730558
Tricharina tophiseda	MSTR:P-04827	Spain	F.J. Valencia	OP747480	OP730564
Tricharina tophiseda	MSTR:P-04828	Spain	F.J. Valencia, M. Vega	OP747481	OP730563
Tricharina tophiseda	MSTR:P-04829	Serbia	D. Savić	OP747479	OP730565
Tricharina tophiseda	MSTR:P-04835	Portugal	M. Vega	OP747482	OP730566
Tricharina hiemalis	C 54666 (Rana 79.076) NEOTYPE	Norway	H. Dissing	OP747484	OP730559
Tricharina aethiopica agg.	CUP MM-000440	Spain (Tenerife)	R.P. Korf *et al.*	OP747472	OP730557
Tricharina gilvoides	MSTR:P-04834	Greece	V. Kaounas	OP747477	OP730560
Tricharina gilvoides	MSTR:P-04830	Greece	V. Kaounas	OP747478	OP730561
Tricharina gilvoides	MSTR:P-04831 HOLOTYPE	Greece	V. Kaounas	OP747476	OP730562
Tricharina striispora	MSTR:P-04836	Spain	F.J. Valencia	OP747483	–
Tricharinopsis herinkii	MSTR:P-04832	Germany	J. Girwert	OP747474	OP730568
Tricharinopsis herinkii	MSTR:P-04833	Spain	G. Muñoz	OP747475	OP730567

the ITS tree (Fig. 1) is similar to the LSU tree (Fig. 2). The new sequences inserted in this phylogeny allow us to isolate three strongly supported clades, two considered as new species, i.e. *Tricharina gilvoides* and *T. subhiemalis* (see Taxonomy), and one considered to represent *T. tophiseda*, a species we did not treat in previous articles.

Sequences extracted from apothecia of the type collections of *Tricharina groenlendica* (holotype) and *T. ochroleuca* (neotype) allow us to delineate these taxa better phylogenetically and highlighted some incongruences with some strains previously used to get sequences of asexual morphs, i.e. *Ascorhizoctonia* spp. Indeed, the ITS sequence of the type strain of *A. gilva* (CBS 236.85) appears to belong to *Tricharina hiemalis*, whereas the sequence obtained from one of the duplicates (TRH 11443) of specimens used to get this culture is nested in the *T. gilva* complex (Fig. 1). A similar case exists with the ITS sequence of the strain based on the neotype of *T. ochroleuca* (CBS 238.85) which appears to be *T. groenlandica*. Further information on these problems is given in the chapter Taxonomy.

Among the new sequences, two of them (coll. CUP:MM-000440, under *T. pallidisetosa*, and MSTR:P-04826, under *T.* cf. *hiemalis*) cannot be clearly assigned to a known species but are considered to belong to the *Tricharina aethiopica* aggregate, a species complex that requires further investigation.

Taxonomy

1. Type studies

Re-examination of type-material of *Tricharina groenlandica*

Tricharina groenlandica is typified by the collection C-F-52717 (ex Dissing Gr.83.003). It contains four dried apothecia measuring 1.5–3.5 mm diam. and several other fruitbody fragments in good condition. The apothecia are cupulate to discoid. The receptacle surface is smooth. The margin has no visible hairs. The colour of the hymenium is yellowish brown in the dried state. The receptacle surface is dark brown. Some of the apothecia are attached to sandy soil. The label indicates: "*Tricharina groenlandica* Dissing, Korf & Yang. Loc.: Sdr. Strømfjord. UTM.: [no data]. Coord.: 67° 01′ N, 50° 43′ W. Distr.: CWm. Alt.: [no data]. Date: 20 July 1983. Leg.: H. Dissing. Det.: H. Dissing. Hab.: Along the road under *Salix* in clay soil. TYPUS."

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

Excipulum composed of two layers: **Medullary excipulum** of *textura intricata*, composed of hyaline, thin-walled cells, up to 5 µm broad; **ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells, up to 20 µm diam., but the outermost layer consists in yellowish brown, slightly thick-walled cells, up to 15 µm diam. **Hairs** are neither observed on the outside of the ectal excipulum nor at the margin, but the margin is made of brown club-shaped and thick-walled cells, up to 35 × 15 µm, arranged like a palisade. **Asci** 170–185 × 10–11 µm, operculate, cylindrical, 8-spored, with crozier, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without inclusions, 2.8–5 µm broad, slightly widened at the apex, up to 6.5 µm. **Ascospores** 16–18.3 (18.8) × 8.8–10.5 µm, X=16.9 × 9.9 µm, Q=1.6–1.9, Qm=1.7 (in water); (16) 16.5–18.8 × (9.1) 9.5–10.4 (10.8) µm, X=17.4 × 9.8 µm, Q=1.7–2.1, Qm=1.8 (in 3% KOH), uniseriate, ellipsoid with somewhat truncate ends, thin-walled, hyaline, smooth, often with granular content, some also with polar inclusions but without oil drops.

Comments: The observations made during the re-examination of the holotype showed a high similarity with the features reported in the protologue of Yang & Korf (1985b: 494). However, some minor differences should be noted. First, the spore size given by Yang & Korf, 13.9–17.6 × 7.5–9.2 (10.2) µm, is somewhat smaller than in our measurement, 16–18.3 (18.8) × 8.8–10.5 µm (in water) and (16) 16.5–18.8 × (9.1) 9.5–10.4 (10.8) µm (in 3% KOH), but such a difference is not significant in our opinion. Second, we did not observe thick-walled (anchor) hyphae on the outside of the receptacle surface, although we do not think that it is an essential feature of the species. *T. groenlandica* is well characterised by the absence of marginal hairs, as well as the large ascospores.

In GenBank database, there exist sequences of the type of *T. groenlandica*, originating from the strain CBS 237.85 isolated by C.S. Yang (cf. CBS strain database). They are stored under GenBank accession numbers U38576 (LSU) and NR_145377 resp. MH861873 (ITS). These sequences are 100% identical with the following sequences: JQ836561 (LSU) and MH861874 resp. JQ824126 (ITS). These latter sequences came from the strain CBS 238.85, also isolated by C.S. Yang, based on the collection CUP 61607 designated by Yang & Korf (1985a: 500) as the neotype of *Tricharina ochroleuca*.

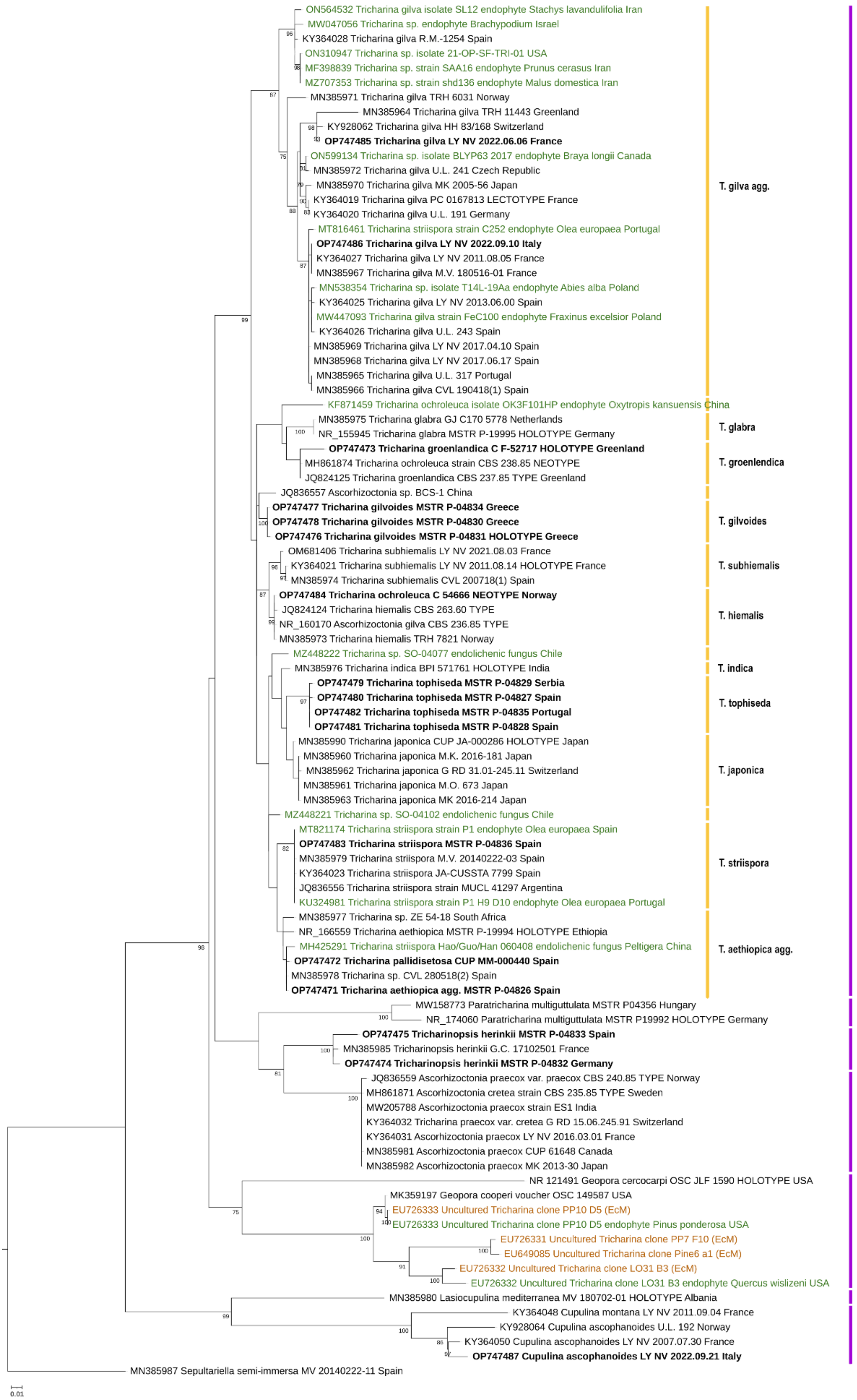


Fig. 1 – Phylogram of *Tricharina* and some allied tricharinoid genera inferred from maximum likelihood (ML) analysis of the ITS region, rooted with *Sepultariella semi-immersa*. Nodes with ≥ 70% ML bootstrap support are annotated with their support values. Sequences generated in this study are in bold. Colour coding of leaves represents origin of sample sequenced: ascomatal sequences as black, endophytes or endolichenic fungi are green, and ectomycorrhizal root (EcM) tips are brown.

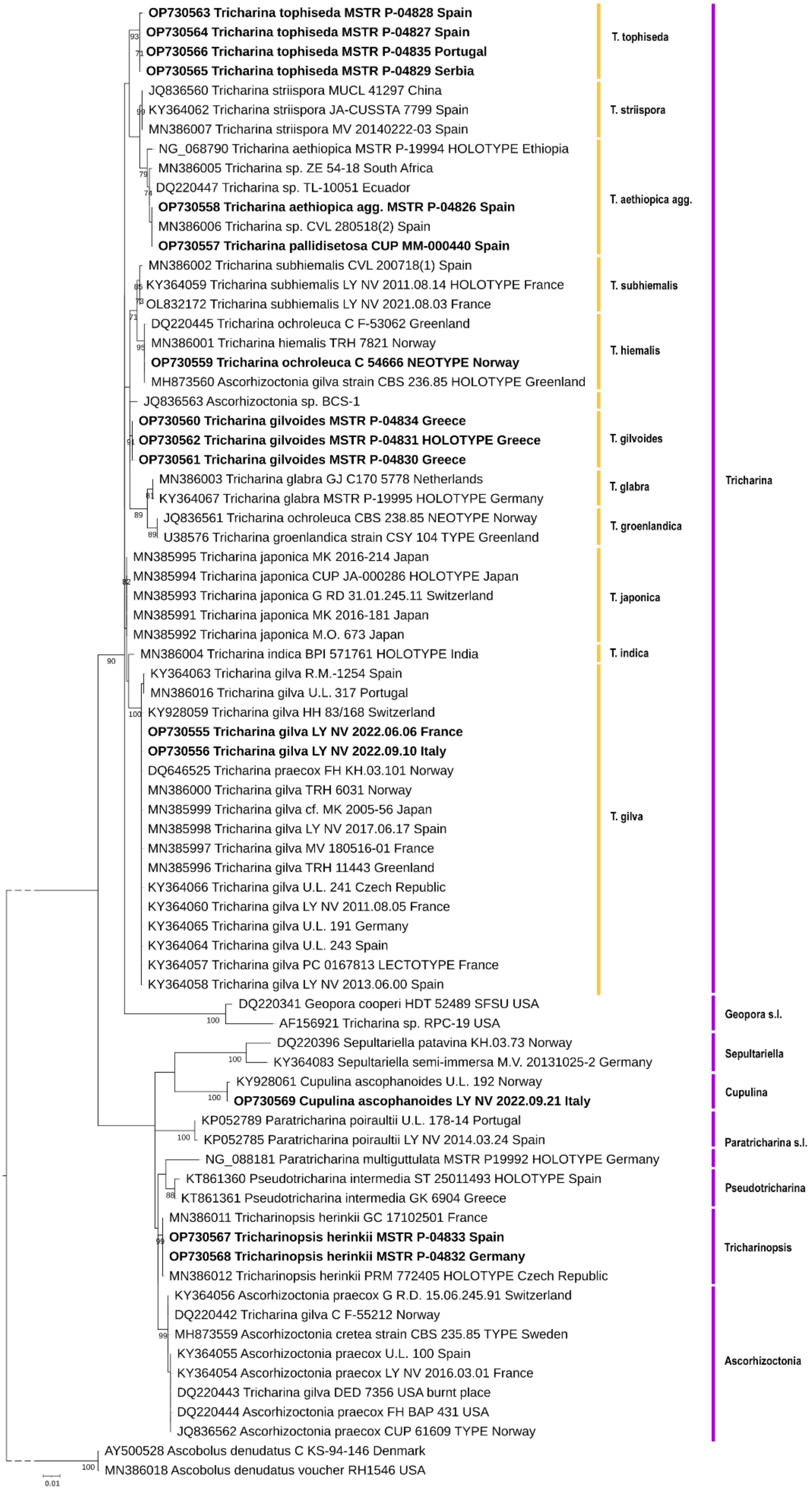


Fig. 2 – Phylogram of *Tricharina* and some allied tricharinoid genera inferred from maximum likelihood (ML) analysis of the LSU region, rooted with *Ascobolus denudatus*. Nodes with ≥ 70% ML bootstrap support are annotated with their support values. Sequences generated in this study are in bold.

We had the opportunity to examine apothecia of the neotype of *T. ochroleuca* (see next chapter). This neotype is morphologically clearly different from the type of *T. groenlandica*, but the sequence coming from the culture of the neotype of *T. ochroleuca* is 100% identical to that of *T. groenlandica*. Furthermore, the newly obtained sequence from the teleomorph of this neotype clusters with sequences belonging to the *T. hiemalis* species complex (see Fig. 1). Based on these data, the conclusion must be that the strain CBS 238.85 cannot come from a culture based on the teleomorph of the neotype of *T. ochroleuca*. Something must have gone wrong or been confused in the isolation of the cultures (see also the comment in the next chapter). We note that this is not the first time that a sequence obtained from a strain isolated by C.S. Yang contradicts the sequence obtained from apothecia of the teleomorph. We have already highlighted the same problem with the anamorph and teleomorph sequences of a collection of "*Tricharina*" *ascophanoides* (Van Vooren *et al.*, 2017: 114).

Re-examination of the neotype of *Tricharina ochroleuca*

Tricharina ochroleuca is typified by the collection C-F-54666, ex Rana 79.076 (Yang & Korf, 1985b). It contains more than fifty apothecia, measuring 1–4 mm diam., in good condition. The apothecia are deeply cupulate, mostly sunken in the sandy soil, growing mostly gregariously, seldom singly. The receptacle surface is hairy. At the margin, the brown hairs are arranged in fascicles. The colour of the hymenium is yellowish brown in dried state. The receptacle surface is dark brown. The label indicates: "*Tricharina* ~~*sp.*~~ *ochroleuca*. UTM.: [no data], Coord.: [no data]. TBU.: [no data]. Alt.: [no data]. Loc.: Nordl., Rana, near the air place, 10 km NW of Mo i Rana. Date: 19 August 1979. Leg.: H. Dissing. Det.: H. Dissing. Hab.: On moist sand. Neotype!"

The package contains furthermore (1) a culture, labelled MO 79.76 ex C and dated Dec. 1984, (2) an annotation slip of the Plant pathology herbarium of Cornell University (CUP): "Specimen No. 79.76 designated as neotype of *Tricharina ochroleuca* (Bres.) Eckblad, see Mycotaxon 24. 1985. A small piece is kept at CUP61601 (isoneotype) ascospores germinated on water agar; a culture is designated as holotype of the anamorphic species *Ascorhizoctonia ochroeluca* [*sic!*]. Annotated by Chin S. Yang. Date June 21, 1985", (3) a handwritten note "Rana 79.076 / *T. ochroleuca* / Neotype / sampled DNA by K. Hansen 2013" without any indication whether the sequence has been obtained from the culture or the teleomorph, and (4) a handwritten note (by H. Dissing) in Danish. English translation: "Occurs solitary or gregariously, 2–4 mm broad, distinctly shallowly saucer-shaped, irregular margin, caramel. Hairs short, blunt, colourless. Spores smooth, colourless, one oil drop. 15 × 7 micron; on sandy soil."

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

Excipulum composed of two layers: **Medullary excipulum** of *textura intricata*, composed of hyaline, thin-walled cells, up to 5 µm broad; **ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells, but the outermost layer consists in yellowish brown, slightly thick-walled cells, up to 25 µm diam. **Marginal hairs** in fascicles, up to 230 µm long, brown or hyaline, straight, thick-walled, sharp, septate, with a basal cell not widened. **Excipular hairs** thick-walled, hyaline, more or less corrugated. **Asci** 150–170 × 11–12 µm (in 3% KOH), operculate, cylindrical, 8-spored, with crozier, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without inclusions, 2.5–3 µm broad, not or slightly widened at the apices, up to 4 µm. **Ascospores** (13.2) 13.6–14.9 (15.9) × (6.7) 7.3–9 µm, X=14.3 × 8.2 µm, Q=1.6–2.1; Qm=1.7 (in water), (13.5) 14–15.6 (16.2) × (9.1) 9.5–10.4 (10.8) µm, X=14.7 × 8.7 µm, Q=1.6–1.9, Qm=1.7 (in 3% KOH), uniseriate, ellipsoid with somewhat tapered ends, thin-walled, hyaline, smooth, with inclusions at the poles but without oil drops.

Comments: The examination of the neotype of *T. ochroleuca* showed a high similarity with the features reported in Yang & Korf (1985b: 500) but we noted three differences: First, the dried apothecia of this collection are only up to 4 mm diameter, while Yang & Korf noted that the apothecia measured 2.5–12 mm diameter. In Dissing's handwritten note, the apothecia are given with a diameter of 2–4 mm in agreement with our own observation. Yang & Korf (1985b) seem to have described not only the neotype collection, but obviously included other finds in their description. Second, the hair length was reported with a length between 220 and 330 µm, while we could only find hairs with a length up to 230 µm. Third, according to Yang & Korf (1985b), the species possesses ascospores with "polar granules" which we also detected. In contrast, Dissing's handwritten note states that the ascospores contain "one oil drop". This must be a mistake, because the ascospores of *Tricharina* s. str. never have oil drops, possibly bipolar spore granules.

Besides the hair length, the ascospore shape plays a decisive role in the identification of *Tricharina* species. Comparing the ascospore shape of the neotype of *T. ochroleuca* with the other known *Tricharina* taxa, this collection belongs morphologically to the *T. hiemalis* species complex (Van Vooren *et al.*, 2019: 158ff.). As noted above, this opinion is also confirmed by the phylogenetic results.

The sequences obtained from the teleomorph of the neotype contradict those deposited in GenBank under accession number JQ824126 based on a strain deposited in the CBS-KNAW collections (CBS 238.85, ex CSY 102) and isolated by C.S. Yang. Contrary to Dissing's handwritten note accompanying the neotype, both the CBS database and Yang & Korf (1985b: 475) list "S. Silvertsen and H. Dissing" as collectors. In addition, the collection date is different: 18 Aug. 1979 (*vide* CBS-KNAW and Yang & Korf, 1985a, 1985b) while 19 Aug. 1979 is given in the *Tricharina* monograph by Yang & Korf (1985b: 500). It seems that something has been mixed up in the laboratory despite other dates agreeing.

With regard to the taxonomical status of the neotype of *T. ochroleuca*, Van Vooren *et al.* (2017: 105) re-examined the type collection of *Peziza ochroleuca* Bres. (S-F190915) and revealed that Bresadola had mixed two species (as already suggested by Yang & Korf, 1985b). We also explained that the procedure used to designate a neotype was contrary to ICN rules, because a lectotype was eligible. For these reasons, *P. ochroleuca* Bres. must be considered a *nomen dubium*. Moreover, we showed that all previous finds identified as *T. ochroleuca* could be assigned either to the *T. gilva* species complex or to the *T. hiemalis* species complex. This result was supported by molecular data (Van Vooren *et al.* 2017, 2019).

2. Reconsideration of the *Tricharina hiemalis* species complex

As stated in Van Vooren *et al.* (2019) and confirmed herein by new phylogenetic analyses (Fig. 1 and 2), the *Tricharina hiemalis* species complex is divided into two well-supported clades. Based on this result, we reevaluated the morphology of the collections assigned to the two lineages. It turned out that the size of the ascospores of the collections in the two lineages does not differ significantly, but their shape does: on the one hand, the ascospores are narrow ellipsoid with tapered poles, on the other hand, they are medium ellipsoid with tapered poles. This gives a mean Q ratio of 1.7 for *T. hiemalis* s. str. and 1.5–1.6 for the second lineage (cf. Plate 4). We also noted, comparing the way of growth, that *T. hiemalis* s. str. grows predominantly sunken into the (sandy) soil, while the other species seems to prefer a superficial growth. Finally, *Tricharina hiemalis* has probably a more northern distribution than the other species. We hope that future collections of both species could confirm this hypothesis.

To accommodate the second lineage, we propose the following species:

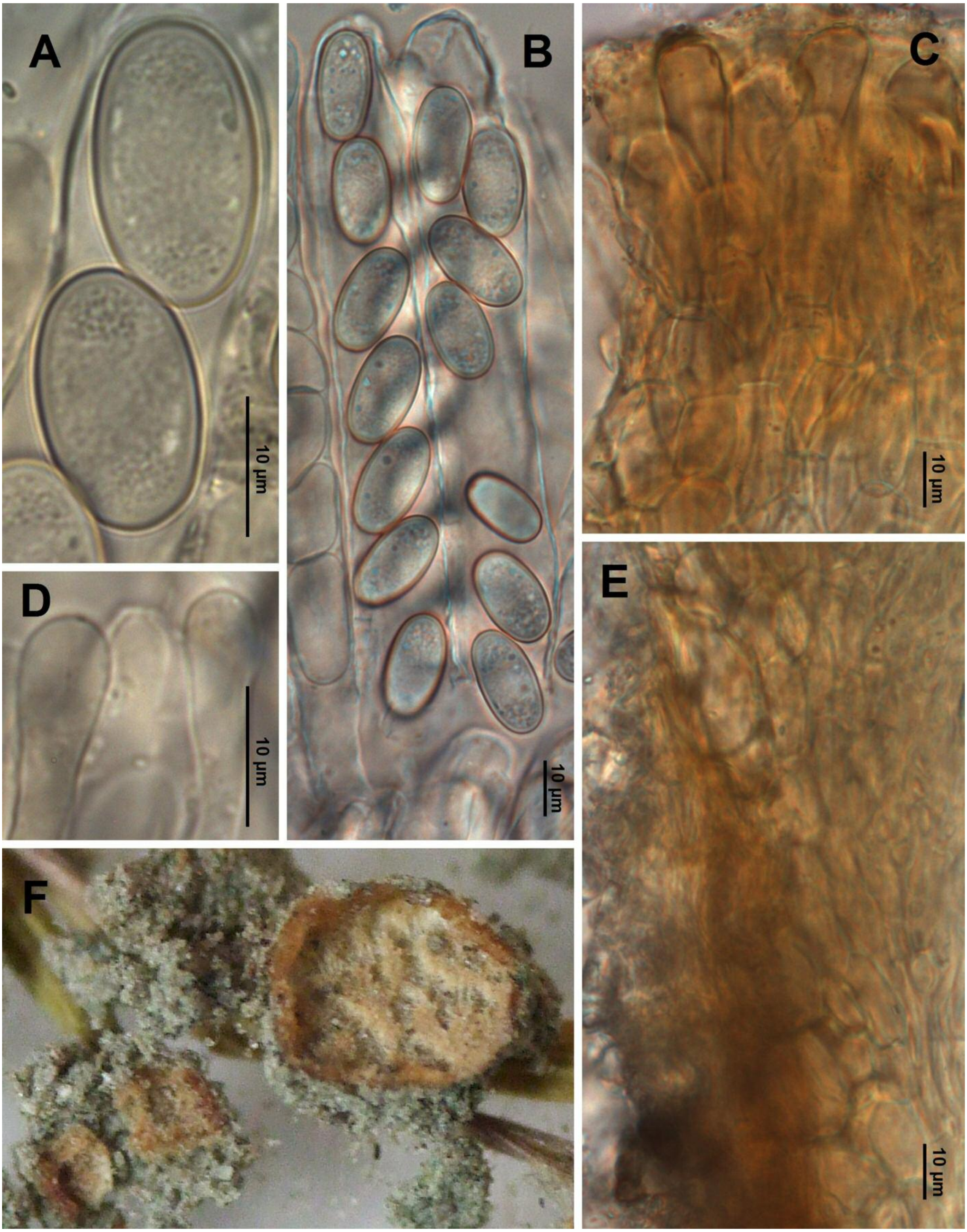
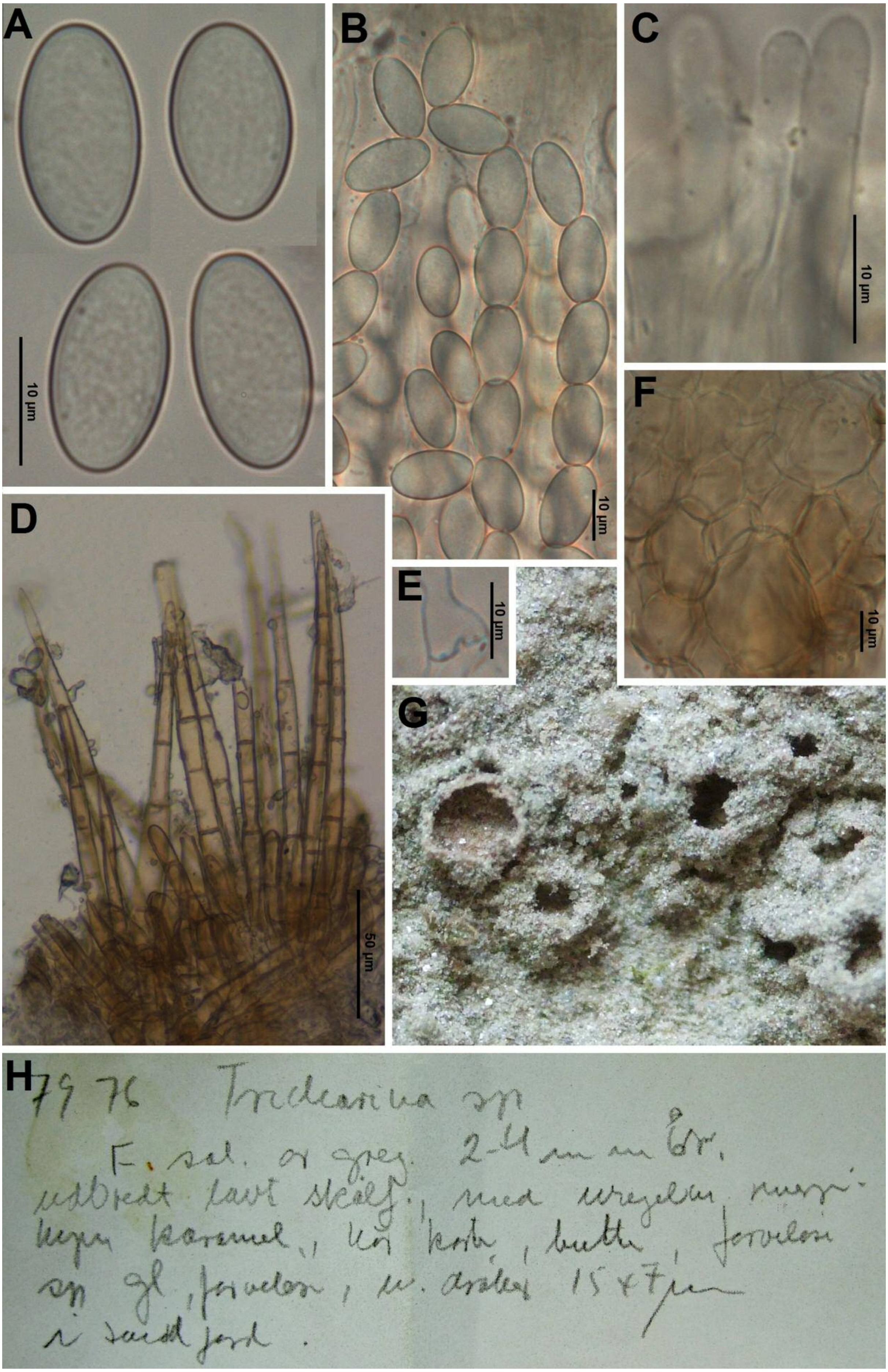


Plate 1 – *Tricharina groenlandica* (holotype, C-F-52717).
A: Ascospores (dead) in water. B: Asci (dead) in 3% KOH. C: Marginal cells (front view). D: Paraphyses (dead) in 3% KOH. E: Ectal excipulum, outer layer. F: Dried apothecia. All photos by U. Lindemann.



7976 Tricharina sp

F. sol. el greg. 2-4 mm in br.
udbredt lavt skålf., med uregelm. [illegible]
karamel., hår korte, butte, farveløse
sp gl., farveløse, m. dråber 15×7 µm
i sandet jord.

Plate 2 – *Tricharina ochroleuca* (neotype, C-F-54666).
A: Ascospores (rehydrated) in water. B. Ascospores (dead) in 3% KOH. C: Paraphyses (dead) in 3% KOH. D: Marginal hairs in a bundle. E: Ascus base with crozier (dead) in 3% KOH. F: Ectal excipulum of *textura globulosa*. G: Ascomata sunken into sandy soil. H: Handwritten note by H. Dissing. All photos by U. Lindemann.

Tricharina subhiemalis Van Vooren, U. Lindemann, Moyne & Valencia, *sp. nov.* – MB 846758 – Plate 3

Diagnosis: Differs from *Tricharina hiemalis* by wider ascospores with a mean Q ratio < 1.7, by ascomata growing superficially on soil, and its genetic profile.

Holotype: France – Doubs, Amondans, Moulin Saillard, 47.065167° N, 6.04306° E, elev. 510 m, on tuf, 17 Aug. 2011, *leg.* G. Moyne & N. Van Vooren, LY NV 2011.08.14. – GenBank: KY364021 (ITS), KY364059 (28S).

Etymology: From Latin "*hiemalis*", meaning "in winter", and the prefix "*sub-*", meaning "below, underneath" because the species is very closely related to its sibling species *Tricharina hiemalis*.

Description

Apothecia sessile, 2–5 (11) mm diam., at first deeply 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 light brownish hairs. Margin densely hairy, with light and dark brown hairs, arranged in small clusters.

Excipulum composed of two layers, a **medullary excipulum** rather thin, about 50–60 µm, of *textura intricata*, with hyaline hyphae, 5–10 (11) µm wide, and an **ectal excipulum** of *textura globulosa/subglobulosa*, 120–140 µm thick, with hyaline cells, 10–45 µm diam., but with coloured cells in the outer part. **Marginal cells** brown, thick-walled, more elongated or clavate. **Marginal hairs** superficial, straight, 110–450 (460) × 6–25 µm, dense, more or less fasciculate, septate, × 1–2 µm thick-walled (wall refractive), brown but often paler at the top, often very pointed at the top, with a simple base, widened to subbulbous, up to 35 µm. **Asci** cylindrical, 180–200 × 12–15 µm, with croziers, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, without droplets, not widened at the top, 3.5–5 µm diam. **Ascospores** ellipsoid, slightly tapered at the poles, (13.5) 14–16.5 (17.9) × (8.5) 9–11.1 (11.6) µm, X=15.6 × 10.3 µm, Q=1.4–1.7, Qm=1.5, smooth, hyaline, without guttules, more or less refractive in CB.

Additional collections examined: France – Savoie, Val-Cenis, Sollières, along the river Arc, 45.243228° N 6.792555° E, elev. 1230 m, on sandy soil, under *Salix* sp., 30 Aug. 2021, *leg*. N. Van Vooren, herb. LY NV 2021.08.03; GenBank OM681406 (ITS), OL832172 (LSU). Spain – Málaga, Ronda, Zona del arroyo del Cupil, 36.781710° N, 5.249000° W, elev. 630 m, on wet soil between stones in a rivulet, 20 July 2018, *leg*. F.J. Valencia, *rev.* U. Lindemann, pers. herb. C.V.L. 200718(1) and MSTR:P-04826 (cf. Plate 8, A–D in Van Vooren *et al.*, 2019); GenBank MN385974 (ITS), MN386002 (LSU).

3. *Tricharina gilvoides* – a new taxon different from the *Tricharina gilva* species complex

Recently, we studied some collections morphologically similar to *T. gilva*, but phylogenetically belonging to an independent lineage (see Fig. 1). Although morphologically similar, we observed some differences, mainly related to the spore size and shape. We propose to describe these collections as a new species.

Tricharina gilvoides U. Lindemann, Van Vooren & Kaounas, *sp. nov.* – MB 846759 – Plate 5

Diagnosis: Differs from Tricharina gilva by shorter broad ellipsoid ascospores, by smaller ascomata, and its genetic profile.

Holotype: Greece – Attica, Artemida, 37.968294° N, 23.994972° E, elev. 40 m, on soil under *Cupressus sempervirens*, near a forest with *Pinus halepensis*, 5 Feb. 2019, *leg*. V. Kaounas, *rev*. U. Lindemann, herb. MSTR:P-04831 (ex pers. herb. V.K. 5542) – GenBank: OP747476 (ITS), OP730562 (LSU).

Etymology: From Latin "*gilva*", meaning yellow, and the suffix "*-oides*", meaning "similar to" because the species can be easily confused with *Tricharina gilva*.

Description

Apothecia sessile, superficial, 2–5 mm diam., cupulate, with an ochre-brown to greyish-white hymenium, outside subconcolorous, densely hairy. Margin densely hairy, with light brown to whitish-hyaline hairs, organised in small bundles.

Excipulum composed of two layers: **medullary excipulum** of *textura intricata*, with hyaline hyphae; **ectal excipulum** of *textura globulosa/angularis*, with hyaline cells, 15–30 µm diam., but, in the outermost part, with brown-coloured, slightly thick-walled cells. **Marginal hairs** superficial, 200–250 × 7–10 µm, with a simple base, straight or slightly curved, dense, more or less fasciculate, septate, hyaline or brown with hyaline ends, wall 1–1.5 µm thick, the shortest hairs rounded at the top, the longest hairs often very sharp. **Asci** cylindrical, 190–225 × 12–15 µm, with croziers, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, without droplets, 2.5–3.5 µm diam., often slightly widened at the top, 5–6.5 µm diam. **Ascospores** broadly ellipsoid with truncate poles, (11.8) 13–16 (16.5) × (8.5) 9.3–10.5 (11.7) µm, X=14.5 × 10 µm, Q=(1.3) 1.4–1.5 (1.6), Qm=1.45 (in water), smooth, hyaline, uninucleate, thin-walled.

Table 2 – Comparison of the ascospore size and shape of *Tricharina hiemalis* and *T. subhiemalis* (the main difference marked in bold)

	Tricharina hiemalis				***Tricharina subhiemalis***		
Coll. Num.	CUP 061707	CUP 061631 (paratype)	TRN 7821	C 54666*	LY NV 2011.08.14	C.V.L. 200718(1)	LY NV 2021.08.03
Sequenced	Y	N	Y	Y	Y	Y	Y
Spore size (µm)	(11.7) 13.2–15.4 × 7.3–8.8	16–18 (18.2) × 9.5–11	14–16.2 (17) × 7.7–9	(13.2) 13.6–14.9 (15.9) × (6.7) 7.3–9	(14.3) 15–16.5 × 9.1–10.5	(13) 13.8–15.7 (16) × (9.6) 9.9–10.6	(3) 13.5–16 × 8.5–9.5 (10)
Mean spore size (µm)	~14.3 × 8	17.1 × 10.1	15 × 8.5	14.3 × 8.2	15.5 × 10.1	14.9 × 10.2	14.6 × 9.2
Q ratio	–	1.6–1.8	1.8–2.0	1.6–2.1	1.5–1.6	1.3–1.6	1.4–1.7
Mean Q ratio	**~1.7**	**1.7**	**1.8**	**1.7**	**1.5**	**1.5**	**1.6**
Marginal hairs size (µm)	220–300 (450)	up to 225	up to 400	up to 230	up to 350	up to 320	up to 240
Location	Canada	Czech Rep.	Norway	Norway	France	Spain	France
Substrate	isolated from soil	on soil	sunken in sandy soil	sunken in sandy soil	superficial, on tuf	superficial, on a stone with soil	superficial, on sandy soil

* neotype of *T. ochroleuca*

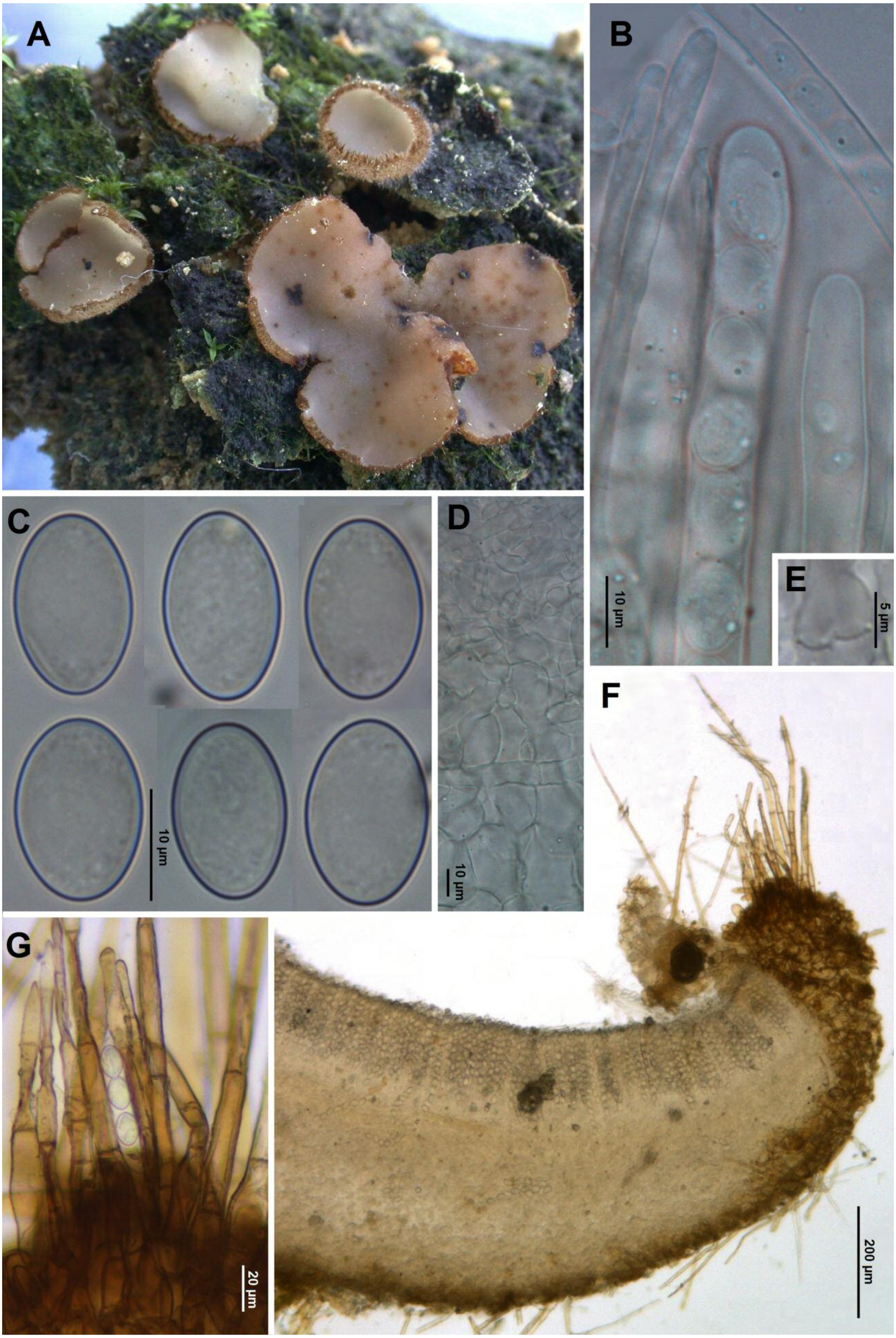


Plate 3 – *Tricharina subhiemalis* (holotype, LY NV 2011.08.14)
A: Apothecia in fresh state. B: Asci and paraphyses (dead) in water. C: Ascospores in the living state in water. D: Medullary (upper part) and ectal excipulum (lower part) in 3% KOH. E: Ascus base in H_2O. F: Cross cut through the apothecium. G. Marginal hairs (base) in water. Photo A by N. Van Vooren, photos B–G by U. Lindemann.

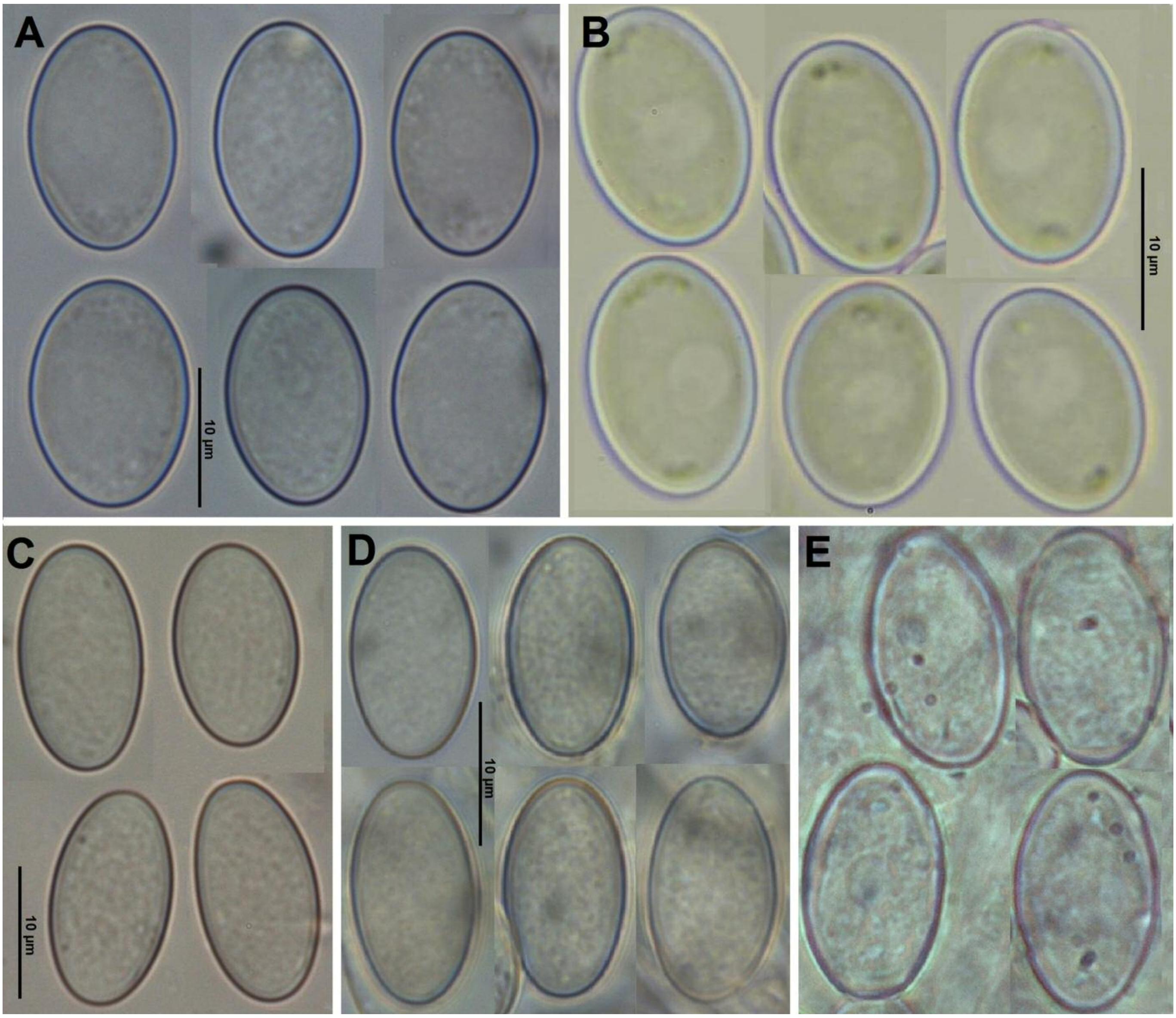


Plate 4 – Sporogram of *Tricharina subhiemalis* (upper row) and *T. hiemalis* (lower row)
A: *Tricharina subhiemalis* (holotype, Coll. LY NV 2011.08.14). B: *T. subhiemalis* (paratype, Coll. C.V.L. 200718[1]). C: Neotype of *T. ochroleuca* (Coll. C 54666). D: *T. hiemalis* (Coll. TRN 7821). E: *T. hiemalis* (Paratype, coll. CUP 061631). Photos A, C & D by U. Lindemann, B by F.J. Valencia, E by N. Van Vooren.

Additional examined collections: Same location as the type: 29 Jan. 2019, *leg*. V. Kaounas, herb. MSTR:P-04830 (ex pers. herb. V.K.5520), GenBank: OP747478 (ITS), OP730561 (LSU); 25 Jan. 2021, *leg*. V. Kaounas, herb. MSTR:P-04834 (ex pers. herb. V.K.6162), GenBank: OP747477 (ITS), OP730560 (LSU).

Comments: *T. gilvoides* is morphologically quite similar with *T. gilva* and can easily confused with it, but it differs from *T. gilva* by shorter broad ellipsoid ascospores (*T. gilva*: 14–18 × 8.5–11 µm [X = 16 × 10 µm], Qm=1.6 vs. *T. gilvoides*: 13–16 × 9.5–10.5 µm [X = 14.5 × 10 µm], Qm=1.45), and by smaller ascomata (*T. gilva*: 4–6.5 mm vs. *T. gilvoides* 2–5 mm). Furthermore, the hairs of *T. gilvoides* are often hyaline or brown with hyaline ends, a feature which is rarely observed in collections of *T. gilva*. The fruitbodies of *T. gilva* in the young state are deeply cupulate, reminiscent of a *Geopora* species. This is not the case in *T. gilvoides*. Regarding the genetics, we are aware that *T. gilva* is a species aggregate, but, contrary to its genetic variability, the morphological differences between the different clades are so small that it is not yet possible to identify different (sub-)species.

The occurrence of *Tricharina gilvoides* on the eastern coast of Greece, at a low elevation, suggests this taxon could be a species of the Mediterranean mycobiota. Mycologists collecting discomycetes in the Mediterranean areas should be careful of *Tricharina* collections looking like *T. gilva*.

4. New records of *Tricharina tophiseda*

Tricharina tophiseda was described from Croatia and extensively documented (Kušan *et al.*, 2015), but not sequenced. Three *Tricharina* collections came recently to our attention that show a great morphological similarity with *T. tophiseda*. These collections have been sequenced and form an independent clade within the *Tricharina* lineage (see Fig. 1). Even though no sequence of the type of *T. tophiseda* is available, we are convinced that the recent collections from Portugal, Spain and Serbia can be assigned to *T. tophiseda*. These collections will be documented in the following.

Description (Plate 7-8)

Apothecia sessile, superficial, 3–7 mm diam., at first cupulate then spreading out, with the marginal zone splitting, with a greyish

Plate 5 – *Tricharina gilvoides*

A: Ascospores in water. B: Paraphyses in water. C: Marginal hairs. D: Ascus base with crozier. E: Ascomata *in situ* (holotype: MSTR:P-04831). F: Ectal excipulum in 3% KOH. G: Ascomata *in situ* (topotype: MSTR:P-04830). H: Apothecial margin (close-up view) I: Medullary excipulum in 3% KOH. Photos A–D, F + I by U. Lindemann, E, G + H by V. Kaounas. [All photos of the microscopic features are from the type collection]

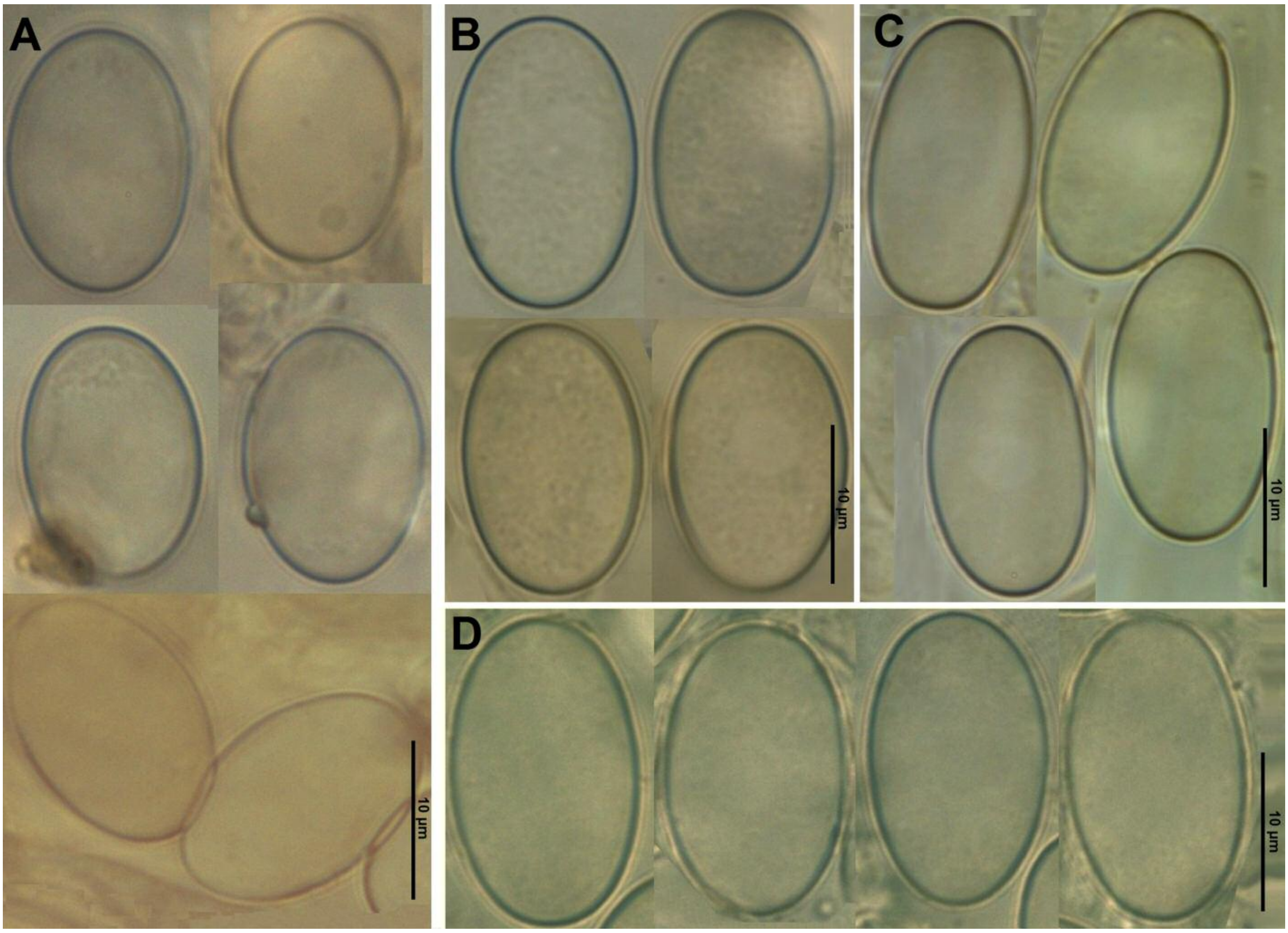


Plate 6 – Sporogram of *Tricharina gilvoides* (left side: A) and *T. gilva* (right side: B–D)
A: *Tricharina gilvoides* (holotype MSTR:P-04831). B: *Tricharina gilva*: Czech Republic, Prague, *leg*. Z. Egertova, 17 Jul. 2014 (U.L. 241). C: *T. gilva*: Germany, Erbach/Donau, *leg*. R. Seibert, 26 Apr. 2014 (U.L. 191). D: *T. gilva*: Spain, La Rioja, Sojuela, *leg*. R. Martinéz Gil, 23 Apr. 2016 (U.L. 243). All photos by U. Lindemann.

white to salmon ochre hymenium, outside subconcolorous, with brown hairy spots. Margin with scattered dark brown hairs, arranged in small bundles.

Excipulum composed of two layers: **medullary excipulum** of *textura intricata*, with hyaline hyphae; **ectal excipulum** of *textura globulosa/angularis*, with hyaline cells, 15–35 µm diam., but, in the outermost layer, with brown-coloured, slightly thick-walled cells. **Marginal hairs** superficial, straight or slightly curved, dense, more or less fasciculate, septate, often slightly bent down at the septae and with quite a lot small irregularities at the walls, up to 360 µm long, up to 10 µm wide, wall 1–1.5 µm thick, always rounded at the top, never sharp, with a simple bulbous base. **Asci** cylindrical, 200–240 × 13–19.5 µm, with croziers, 8-spored, inamyloid. **Paraphyses** cylindrical, septate, hyaline, with non-refractive vacuoles, not or slightly widened at the top, 5.5–7.5 µm diam. **Ascospores** ellipsoid to ellipsoid-oculiform, (14.1) 15.3–18.9 (20.4) × (8.5) 9–10.5 µm, X=16.5 × 10 µm, Q=1.5–1.8, Qm=1.65, up to 22.5 × 13 µm (just before germination), smooth, hyaline, uninucleate, slightly thick-walled, with bipolar spore granules and/or few small guttules at the poles.

Collections examined: Portugal – Lisbon, Jardim Botânico, 38.719039° N, -9.150387° E, elev. 80 m, on the ground, 5 Febr. 2013, *leg*. M. Vega, herb. MSTR:P-04835 [ex pers. herb. U.L. 90], GenBank: OP747482 (ITS), OP730566 (LSU). Serbia – Vojvodina, Fruška Gora National Park, monastery Novo Hopovo, 45.128078° N, 19.847097° E, elev. 260 m, on the ground, 22 Oct. 2021, *leg*. D. Savić, herb. MSTR:P-04829, GenBank: OP747479 (ITS), OP730565 (LSU). Spain – Málaga, Ronda, Dehesa del Mercadillo, 36.762944° N, 5.175611° W, elev. 567 m, on the soil of a wet slope with quince trees (*Cydonia oblonga*), 8 Jan. 2020, *leg*. F.J. Valencia, herb. MSTR:P-04827 and pers. herb. C.V.L. 080120(1), GenBank: OP747480 (ITS), OP730564 (LSU). Málaga, La Concepción Jardín Botánico-Histórico, 36.761467° N, 4.426917° W, elev. 90 m, on soil between small bryophytes, 27 Jan. 2019, *leg*. M. Vega & F.J. Valencia, herb. MSTR:P-04828 [ex pers. herb. M.V.190127-02], GenBank: OP747481 (ITS), OP730563 (LSU).

Comments: According to the protologue (Kušan *et al.*, 2015), *Tricharina tophiseda* is morphologically characterised by the following features: hymenium light orange, orange grey in places, rarely with a pinkish white tinge, very long marginal hairs (up to 932 µm) and ellipsoid-limoniform to narrowly ellipsoid-oculiform ascospores that are ± radially symmetrical, containing bipolar spore granules.

Based on the collections presented herein, the shape of the ascospores, their content as well as their dimensions correspond very well with the protologue. In addition, the crozier at the ascus base, and finally the structure of the ascomata fit well. The main differences between these new collections and the type are the colour of the hymenium and the very long hairs. In none of our studied specimens could such an intense colouration be observed. Regarding the hymenium, we know that at different stages of maturity the hymenium colour of *Tricharina* species can change significantly. About the hairs length, we already observed the variability of this character. For example, the hairs of *Tricharina japonica* Chin S. Yang

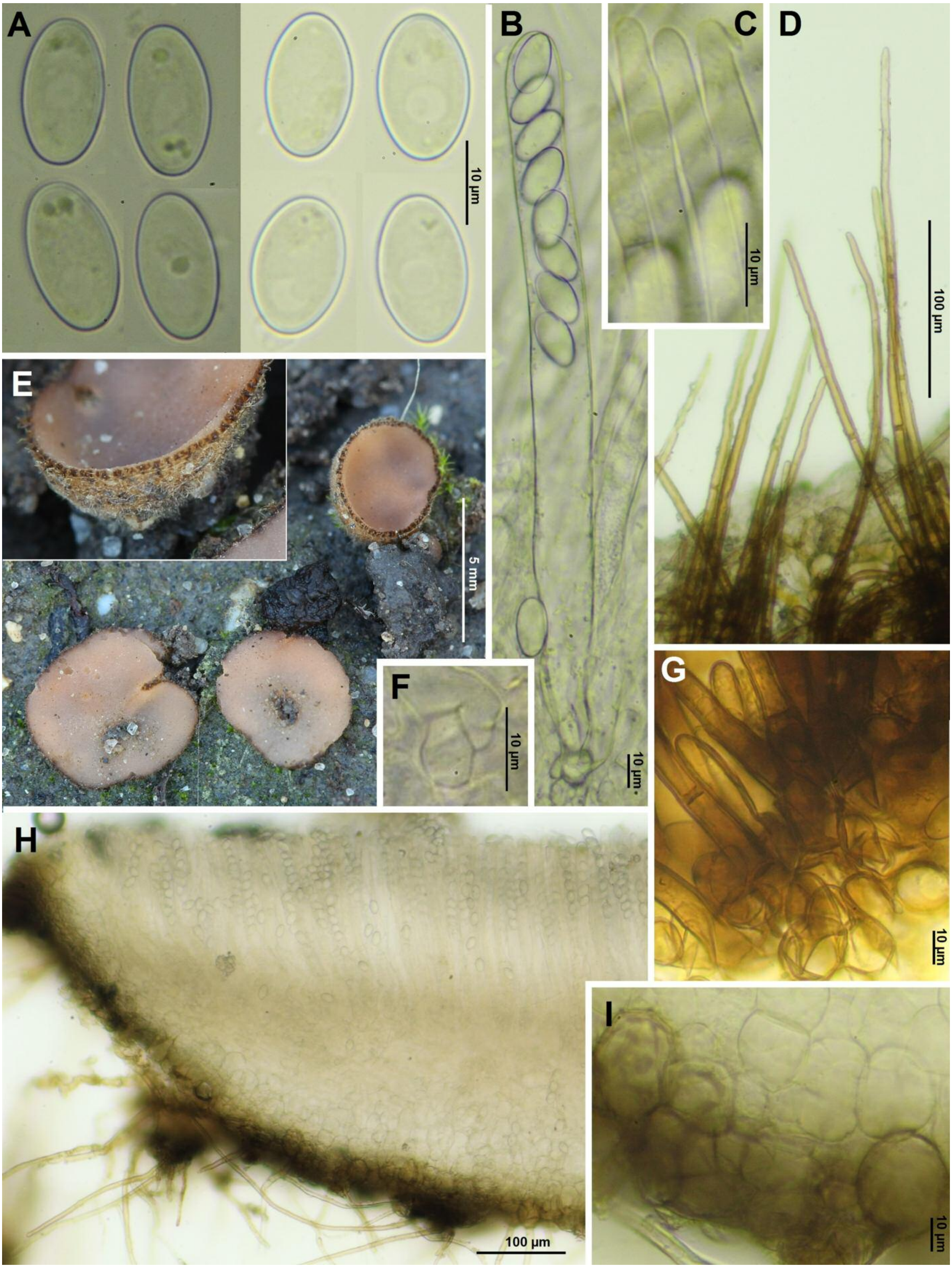


Plate 7 – *Tricharina tophiseda* (MSTR:P-04827)
A: Ascospores in water. B: Ascus in water. C: Paraphyses in water. D: Marginal hairs. E: Apothecia in situ. F: Ascus base with crozier. G: Marginal hairs, basal cells. H: Cross cut through the apothecium. H-I: Ectal excipulum in water. All photos by F.J. Valencia.

Plate 8 – *Tricharina tophiseda* (MSTR:P-04828)

A: Ascospores and ascus apex in water. B: Paraphyses in water. C: Marginal hairs. D: Apothecia *in situ*. E: Apothecial margin (close-up view) F: Cross cut through the apothecium. G: Ectal excipulum in water. H: Ascus base with crozier. Photos A–C & E–H by U. Lindemann, D by M. Vega.

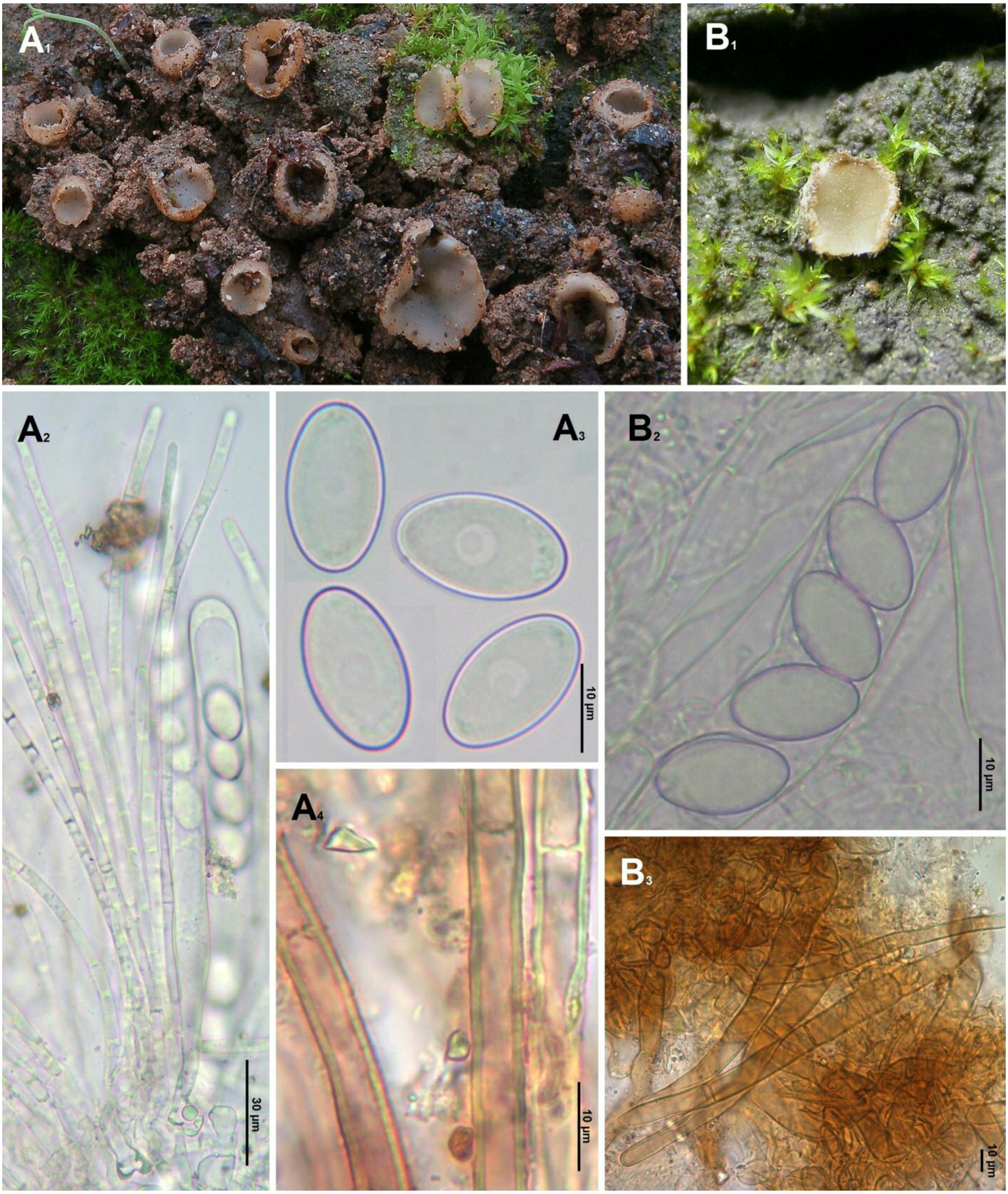


Plate 9 – *Tricharinopsis herinkii* (A1-4: Coll. MSTR:P-04833 [Spain], B1-3: MSTR:P-04832 [Germany])
A1: Ascomata *in situ*. A2: Paraphyses, ascus + ascus base with crozier, A3: Ascospores in water. A4: Hairs in Congo red. B1: Ascomata *in situ*. B2: Ascospores in water. B3: Excipular hairs. Photos A1-4 by G. Muñoz, B1-2 by D. Wieschollek, B3 by U. Lindemann.

& Korf are up to 820 µm long in the type collection, whereas, in none of the later studied collections of this species, could such long hairs be found. In the only known European collection of *T. japonica*, the hairs are only 400 µm long (Dougoud & De Marchi, 2012; Van Vooren *et al.*, 2019, Table 4). Neither the colour of the hymenium nor the hairs length can be individually used as a clear discriminating feature, even though it must be admitted that, in some *Tricharina* species, the hair length does not exceed a certain value and, in combination with other features, must be considered as a good taxonomic character.

Another argument in favour of our determination under *T. tophiseda* is that there exists only one *Tricharina* and two other *Tricharina*-like species that have a comparable ascospore shape: 1) *T. japonica* has similar ascospores but they are significantly larger than those of *T. tophiseda*, and also have a different shape (Kušan *et al.*, 2015, Fig. 3; Van Vooren *et al.*, 2019, Plate 10). 2) *Tricharinopsis herinkii* (Svrček) U. Lindem., Van Vooren & Healy and *Ascorhizoctonia praecox*, but both species are morphologically well distinguishable from *T. tophiseda* (Van Vooren *et al.*, 2017, 2019).

5. New records of *Tricharinopsis herinkii*

Since our taxonomic emendation of *Tricharinopsis herinkii* (Van Vooren *et al.*, 2019), we received two new collections of this species. The first collection from Spain is very typical and is reminiscent of a *Geopora* species rather than a *Tricharina*, while the second collection from Germany looks more like a *Tricharina* species. However, microscopically, these collections could be assigned to *T. herinkii*. Sequences taken from both collections confirmed the morphological identification. The species is thus recorded in five European countries: Czech Republic (type), France, Germany, Spain, and Slovakia (V. Halasů, pers. comm.).

Description of the Spanish collection (Plate 9) in fresh state (*fide* G. Muñoz)

Apothecia sessile, superficial, 3–6 mm diam., at first cupulate then spreading out, with the marginal zone splitting, with a greyish white hymenium, outside subconcolorous, with hyaline to light brown hairy spots. Margin with scattered hyaline to light brown hairs.

Excipulum composed of two layers: **medullary excipulum** of *textura intricata*, composed of hyaline, thin-walled cells, up to 5 µm broad; **ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells. **Marginal hairs** up to 200 µm, hyaline to brown, straight, thick-walled, septate, with a basal cell not widened. **Excipular hairs** thick-walled, hyaline, more or less corrugated. **Asci** operculate, cylindrical, 8-spored, with croziers, inamyloid. **Paraphyses** filiform, multiseptate, hyaline, without inclusions, not widened at the apices. **Ascospores** 17.6–20.1 × 10.1–11.6 µm, X=18.9 × 10.9 µm, Q=1.6–1.9, Qm=1,74, uniseriate, ellipsoid with somewhat tapered ends to subfusoid, thin-walled, hyaline, smooth, no inclusions or oil drops.

Collections examined: Germany – Thüringen, Weimarer Land, Mönchenholzhausen, 50.926546483° N, 11.15568595° E, elev. 420 m, on the soil of an earth track with packed vegetation-free lanes and grassy-mossy central strip, oak-hornbeam forest with lime trees, 2 Aug. 2019, *leg.* J. Girwert, herb. MSTR:P-04832, GenBank: OP747474 (ITS), OP730568 (LSU). Spain – Zaragoza, Parque Grande José Antonio Labordeta, 41.631472° N, -0.898222° E, elev. 220 m, on the banks of the river Huerva, terrestrial growth, under diverse riverside trees (*Populus alba, P. nigra, Fraxinus* sp., etc.), 23 Nov. 2019, *leg.* G. Muñoz, herb. MSTR:P-04833 and pers. herb. G.M.3533, GenBank: OP747475 (ITS), OP730567 (LSU).

Conclusion and outlook

In our work on the genus *Tricharina*, we have been able to revise most of the type collections, and obtained, in most cases, molecular data, which has allowed us to clearly delineate the generic relationships within this genus. In addition, we were able to exclude some species previously assigned to *Tricharina* and, in some cases, had to propose new genera to accommodate them.

Unfortunately, we did not succeed in borrowing the types of some taxa, i.e. *T. tophiseda*, *T. praecox* (P. Karst.) Dennis var. *praecox*, and *T. pallidisetosa* (E.K. Cash) K.S. Thind & S.C. Kaushal. Therefore, we cannot fully complete the revision of the types assigned to the genus *Tricharina*, but we are hoping that this will be possible in the future. Secondly, the *Tricharina aethiopica* species complex is the least clarified so far. Although we received some new collections that are phylogenetically close to *T. aethiopica* (see Fig. 1 and 2), it is still not possible to morphologically separate the phylo-species. And, last but not least, there are still many collections housed in various herbaria for which a revision, based on the current knowledge, is also necessary. More undescribed species are possibly hidden there, still waiting to be discovered.

Key to the species of *Tricharina* and allied genera

Preliminary note: The key refers to what we call "tricharinoid" species, i.e. small brown, inconspicuous discomycetes (mostly measuring between 2–10 mm diam.) with a greyish-white, light brown, salmon-brown or orange-brown hymenium. Many species of this group have conspicuous marginal hairs, often arranged in bundles. Microscopically, most species have smooth ascospores, paraphyses without coloured contents and non-rooting hairs. The hairs mostly arise from the brown, thick-walled marginal or excipular cells. Ecologically, none of these species grows on dung. Usually, they grow on soil and only exceptionally on wood (*Tricharina striispora*). One species is strictly pyrophilous (*Ascorhizoctonia praecox*), some others can occasionally grow on old fire-sites (especially *Tricharina gilva*). As far as we know, several tricharinoid species have an endophytic life cycle (Van Vooren *et al.*, 2019). Species that form ectomycorrhizae have not yet been found.

Macroscopically, *Trichophaea*-like discomycetes can be very similar, although there is often a strong colour contrast between the black-brown, often long marginal hairs and a whitish or pale greyish hymenium (Van Vooren *et al.*, 2020, introduction). Furthermore, many *Trichophaea*-like species have guttulate ascospores and/or possess a more or less distinct spore ornamentation (Bronckers, 2003; Van Vooren *et al.*, 2020, 2022; Van Vooren & Lindemann, 2021). Such a pronounced ascospore ornamentation is very rarely found in tricharinoid species, except for the genus *Pseudotricharina* where the ascospores have large oil drops and are strongly ornamented. For this reason, this genus is not included in the key (for more information on this genus, see Van Vooren *et al.*, 2015; Healy *et al.*, 2017). The ascospores of *Cheilymenia* species also have eguttulate ascospores, whose ornamentation is not always immediately recognisable. However, they can be quickly distinguished from tricharinoid species by the usually bright yellow, orange or reddish ascomata.

Although not genetically closely related, *Pseudaleuria fibrillosa* (Massee) J. Moravec and *Lotinia verna* Pérez-Butrón, Fern.-Vic. & P. Alvarado are included in the key, because these species can easily be confused with tricharinoid species in the field. Furthermore, *P. fibrillosa* was classified in *Tricharina* for a long time. This means that the species and genera listed in the key are mostly, but not necessarily closely genetically related.

Regarding the practical usability of the key, no attempt was made to map true phylogenetic or generic relationships. This means that the species are listed according to their morphological characteristics and not according to their generic affiliation. The following genera are included in the key: *Ascorhizoctonia* (1 species), *Cupulina* (2 species), *Hellenicoscyphus* (1 species), *Lasiocupulina* (1 species), *Lotinia* (1 species), *Paratricharina* (3 species), *Pseudaleuria* (with 1 species of 2), *Sepultariella* (2 species), *Tricharina* (11 species, including 2 new published in this paper), and *Tricharinopsis* (1 species).

Preconditions for the use of the key: The observation of the following morphological features (in the fresh state in water!) are necessary to successfully use this dichotomous key:

Ascospores: size, mean length/width ratio (abbr.: Qm), shape (subglobose, ellipsoid with truncate poles, ellipsoid with tapered poles, fusoid, ellipsoid-oculiform [= shape like an eye] or lentiform [= lens-shaped]), equilateral or not, content (without content, with bipolar spore granules and/or with small to medium oil drops), thick-walled or thin-walled, ornamented or not.

Paraphyses: content (coloured or not), apex (swollen or not).

Hairs and hair-like hyphae: colour (hyaline and/or brown), length, excipular and/or marginal, in bundles at the margin or not, thin- or thick-walled.

Hymenium: colour (greyish-white, light brown, orange-brown).

Apothecia: diameter in millimetres.

Ecology: pyrophilous or not.

1 Apothecia with macroscopically visible, marginal, true hairs (at least with a 10× magnifying glass) **9**

1* Apothecia without visible marginal hairs **2**

2 Ascospores smooth (in water) and thin-walled **3**

2* Ascospores smooth (in water) and thick-walled........ **7**

3 Ascospores ellipsoid-oculiform to fusoid........ **4**

3* Ascospores ellipsoid **6**

4 Ascospores filled with many small oil drops .... **(1)** ***Cupulina montana***
Apothecia 2–4 mm diam., cupulate, yellow-ochre-brown to orange-brown, margin crenulate, without true marginal hairs but with hair-like hyphae. Ascospores fusoid, 27–35 × 12–14 µm, smooth, thin-walled, filled with small oil drops. On soil, in mountainous areas. Lit.: Dougoud *et al.* (2015).

4* Ascospores filled with two medium-sized oil drops and some small oil drops **5**

5 Hymenium light brown, ascomata: mostly 1–3 mm diameter........ **(2)** ***Sepultariella semi-immersa***
Apothecia 1–3 (7) mm diam., cupulate, light brown to brown, margin slightly crenulate, without true marginal hairs but with hair-like hyphae at the margin. Ascospores 19–23 × 9–11 µm, small ellipsoid to subfusoid, smooth, thin-walled, with two medium-sized oil drops and some small oil drops. On soil, from lowlands to low mountain range. Lit.: Dougoud (2002).

5* Hymenium orange or orange-brown, ascomata: 5–10 mm diameter........ **(3)** ***Sepultariella patavina***
Apothecia 5–10 mm diam., cupulate, orange or orange-brown, margin slightly crenulate, with very small, hyaline, thick-walled marginal hairs (up to 60 µm). Ascospores fusoid to subfusoid, 20.5–27(30) × 10–12 µm, smooth, with two medium- to large-sized oil drops and some small droplets. On soil, from lowlands to subalpine stage. Lit.: Dougoud (2002).

6 Ascospores small ellipsoid, with few granules at the poles .... **(4)** ***Tricharina groenlandica***
Apothecia 2.5–4 mm diam., first cupulate, later spreading out, hymenium orange-brown, margin dentate, marginal cells brown, thick-walled, in a row. Ascospores ellipsoid, 16–18.5 × 9–10.5 µm, smooth, slightly thick-walled, eguttulate but with few granules at the poles. On clay soil. Known only from the type locality in Greenland. Lit.: Yang & Korf (1985b); this paper (type revision).

6* Ascospores broadly ellipsoid, with many minute inclusions in the whole ascospore........ **(5)** ***Tricharina glabra***
Apothecia 2–4 mm diam., first cupulate, later spreading out, hymenium orange-brown, margin dentate, marginal cells brown, thick-walled. Ascospores broadly ellipsoid, 19–23.5 × 15–17 µm, smooth, slightly thick-walled, with many inclusions, merging to small oil drops when old. Paraphyses with orange-ochre droplets (not constant), greenish in Lugol's solution. On sandy soil, near the coast (lowlands). Lit.: Lindemann & Böhning (2016).

7 Ascospores subglobose to broadly ellipsoid, with small to medium sized oil drops .... **(6)** ***Paratricharina multiguttulata***
Apothecia 1–4.5 mm diam., brownish-ochre, sessile, first cupulate, later spreading out, margin hairless, crenulate, marginal cells hyaline. Ascospores subglobose to broadly ellipsoid, 18.5–22.5 × 14.5–18.5 µm, smooth, slightly thick-walled, with several small to medium-sized oil drops. Paraphyses with low refractive vacuoles. On soil from the lowlands to low mountain range. Lit.: Lindemann *et al.* (2021).

7* Ascospores ellipsoid **8**

8 Hymenium light brown, with short, thick-walled brown excipular hairs and hair-like hyphae at the margin .... **(7)** ***Paratricharina confusa***
Apothecia 2–5.5 mm diam., brownish ochre or salmon-ochre, first cupulate, later spreading out, more or less crenulate, margin hairless but with hair-like hyphae. Ascospores ellipsoid to narrowly ellipsoid, 18–21 × 10.5–12 µm, thick-walled, smooth (finely verrucose in CB), with bipolar spore granules. Paraphyses with non-refractive vacuoles. On wet soil from the lowlands to low mountain range. Lit.: Lindemann *et al.* (2022).

8* Hymenium yellow to orange-brown, with long, thick-walled, hyaline excipular hairs........ **(8)** ***Cupulina ascophanoides***
Apothecia 1–4 mm, ochraceous beige to orange beige, hemispherical, rarely cupulate, margin hairless, crenulate, marginal cells hyaline, with scattered hyaline hair-like hyphae. Ascospores ellipsoid, sometimes a bit tapered at the poles, 16.5–22.5 × 10–14 µm, smooth, thin- to slightly thick-walled, with bipolar spore granules. On soil from colline to mountainous range. Lit.: Engel & Häffner (1986); Dougoud (2002); Van Vooren *et al.* (2017).

9 Marginal hairs arranged in bundles........ **14**

9* Marginal hairs not in bundles **10**

10 Marginal hairs as hair-like hyphae (short, more or less thin-walled, rounded at the top)........ **11**

10*Marginal hairs not so (usually long, straight, thick-walled and often sharp) **13**

11 Paraphyses with orange pigment, spherical swollen at the apex .... **(9)** ***Pseudaleuria fibrillosa***
Apothecia 5–20 mm, bright orange to orange beige, first cupulate, later spreading out, with a fringed margin, composed of hyaline to light brown hair-like hyphae. Ascospores ellipsoid, 14.5–18.5 × 8–9 µm, smooth, eguttulate. Paraphyses containing orange carotenoid pigments, apex spherical swollen. On soil, from the lowlands to low mountain range. Lit.: Galán & Raitviir (1995); Moravec (2003); Saitta (2019).

11*Paraphyses with hyaline vacuoles, not enlarged at the apex........ **(10)** ***Lasiocupulina mediterranea***
Apothecia 1.5–2 mm diam., pale orange, sessile, discoid, with a fringed margin, composed of short hyaline hair-like hyphae. Ascospores ellipsoid with tapered ends, 17–19 × 9–11 µm, hyaline, smooth, thin-walled, eguttulate, sometimes with a few bipolar spore granules. On soil near the coast (lowlands). Only known from the area of the type collection in Albania. Lit.: Van Vooren & Vega (2018a).

12 Ascospores ellipsoid........ **13**

12*Ascospores ellipsoid-oculiform to fusoid .... **(11)** ***Tricharinopsis herinkii***
Apothecia 5–10 mm, cupulate, partly buried in soil, a bit spread at the end, reminiscent of a *Geopora* species, hymenium beige or pale greyish beige, outside concolorous, covered by scattered, hyaline or brown thick-walled hairs, margin eroded or torn. Ascospores ellipsoid-oculiform to fusoid, 16.5–21.5 × 9–12 µm, smooth, thin-walled, eguttulate. On soil, lowland to low mountain range. Lit.: Svrček (1949); Benkert (2010); Van Vooren *et al.* (2019).

13 Ascospores thick-walled, with bipolar spore granules........ **(12)** ***Paratricharina poiraultii***
Apothecia 5–25 mm diam., hymenium orange-brown when fresh, first cupulate, later spreading out, margin enrolled, with

densely interwoven brown thick-walled hairs. Ascospores ellipsoid to narrowly ellipsoid, 15–20 × 9–11 µm, hyaline, thick-walled, smooth (finely verrucose in Lugol's solution and CB), with bipolar spore granules. Paraphyses with non- refractive vacuoles. On soil from the lowlands to low mountain range. Lit.: Van Vooren *et al.* (2015a).

13*Ascospores thick-walled, without content .....**(13) *Lotinia verna***
Apothecia 1–5 mm diam., sessile, discoid, hymenium chestnut-brown, plane or slightly depressed in the middle, margin with straight or flexuous brown, thick-walled hairs, often forked in the upper third. Ascospores ellipsoid, 20–27 × 16–20 µm, hyaline, thick-walled, smooth, eguttulate. Paraphyses with non-refractive vacuoles and an encrusting but easily detaching brown pigment at the top. On soil, from the lowlands to low mountain range. Lit.: Pérez-Butrón *et al.* (2015); Van Vooren & Vega (2018b).

14 Marginal hairs brown **15**

14*Marginal hairs hyaline ....**(14) *Hellenicoscyphus hyalotrichus***
Apothecia 2–5 mm diam., hymenium light brown to ochre, paling with age, sessile, first cupulate, later spreading while the margin tears, margin with hyaline, thick-walled hairs in bundles, up to 230 µm long. Ascospores ellipsoid, tapered at the poles, 14.5–17.5 × 8.5–11 µm, slightly thick-walled, smooth, with bipolar spore granules. Paraphyses with non-refractive vacuoles. On soil, at low mountain range. Known only from the type locality in Greece. Remark: (16) *Ascorhizoctonia praecox* can have also hyaline marginal hairs as well as (21) *Tricharina gilvoides*. Lit.: Lindemann *et al.* (2022).

15 Ascospores smooth or rarely finely warted **16**

15*Ascospores with a longitudinal striate ornamentation ....**(15) *Tricharina striispora***
Apothecia 4–6 mm diam., sessile, partly buried in soil, cupulate, hymenium greyish white to pale beige, outside darker, margin with straight brown thick-walled hairs in bundles, up to 550 µm long. Ascospores ellipsoid-oculiform to fusoid, 16–19.5 × 8–10 µm, hyaline, thin-walled, smooth with a longitudinal striate ornament, eguttulate. Paraphyses with few non- refractive vacuoles. On soil (exceptionally on wood) from the lowlands to low mountain range. Lit.: Argaud (2008); Galán *et al.* (2010); Ivaldi & Fouchier (2014); Van Vooren *et al.* (2019).

16 Ascospores with bipolar spore granules **17**

16*Ascospores without bipolar spore granules, nor guttules **18**

17 Strictly pyrophilous ....**(16) *Ascorhizoctonia praecox***
Apothecia 2–5 mm diam., sessile, cupulate, later spreading out, partly buried in soil, hymenium first greyish, then ochraceous yellow, sometimes with dull orange tinges or bright yellow, often with some olivaceous tinges, margin with straight (sub-)hyaline to light-brown thick-walled hairs in bundles, up to 270 (330) µm long. Ascospores ellipsoid with tapered poles, 14–18 × 8–11.5 µm, slightly thick-walled, smooth or finely verrucose, eguttulate, with bipolar spore granules. Paraphyses with non-refractive vacuoles, sometimes also with a pale yellowish pigment. From the lowlands to mountainous areas. Lit.: Yang & Korf (1985b); Van Vooren *et al.* (2017).

17*Not so..**(17) *Tricharina tophiseda***
Apothecia 3–7 mm diam., sessile, first cupulate, later spreading out, while marginal zone splitting, hymenium greyish white to salmon ochre, outside subconcolorous with brown hairy spots, margin with straight to slightly curved brown thick-walled hairs in bundles, up to 360 µm (type collection up to 930 µm long). Ascospores ellipsoid-oculiform to fusoid, 15–19 × 9–10.5 µm, hyaline, slightly thick-walled, smooth, eguttulate, with bipolar spore granules and/or small oil drops at the poles. Paraphyses without non-refractive vacuoles. On soil from the lowlands to low mountain range. Lit.: Kušan *et al.* (2015); this paper.

18 Ascospores ellipsoid-oculiform to fusoid **19**

18*Ascospores ellipsoid.. **20**

19 Ascospores 15–17 × 8–9 µm........................**(18) *Tricharina indica***
Apothecia up to 11 mm diam., sessile, first subglobose, later deeply cupulate, hymenium cream coloured, outside brownish, margin with straight brown thick-walled hairs in bundles, up to 395 µm long. Paraphyses hyaline. Ascospores ellipsoid-oculiform to fusoid, 13.5–18.5 × 7.8–9.5 µm, hyaline, thin-walled, smooth, eguttulate. On soil. Lit.: Thind & Waraitch (1971); Van Vooren *et al.* (2019). Known only from the type locality in India.

19*Ascospores 16.5–21.5 × 8.5–10.5 µm...**(19) *Tricharina japonica***
Apothecia 5–10 (15) mm diam., sessile, first cupulate, later spreading out, hymenium white to greyish, outside darker, margin densely hairy, with straight dark-brown thick-walled hairs in bundles, up to 820 µm long (but often shorter). Ascospores in front view fusoid, slightly asymmetrical, in lateral view trapezoid, 16.5–21.5 × 8.5–10.5 µm, thin-walled, eguttulate, sometimes containing few inclusions at the poles. On soil, at low mountain range. Lit.: Yang & Korf (1985b); Dougoud & De Marchi (2012); Van Vooren *et al.* (2019).

20 Hairs shorter than 300 µm.. **21**

20*Hairs longer that 300 µm.. **22**

21 Ascospores 14–18 × 8.5–10 µm, Qm = 1.6 ....**(20) *Tricharina gilva***
Apothecia 5–6.5 mm diam., sessile, partly buried in soil, deeply cupulate, later spreading out, but never applanate, hymenium pale greyish, ochraceous grey to dull orange, outside concolorous or darker, margin with straight brown thick-walled hairs in bundles, up to 260 (300) µm long. Ascospores ellipsoid with somewhat truncate poles, 14–18 × 8.5–10 µm, thin-walled, smooth, eguttulate. Paraphyses hyaline, without vacuoles. On soil, from the lowlands to the alpine stage. Lit.: Yang & Korf (1985b); Van Vooren *et al.* (2017).

21*Ascospores 13–16 × 9.5–10.5 µm, Qm = 1.45 ....**(21) *Tricharina gilvoides***
Apothecia 2–5 mm diam., sessile, cupulate, hymenium greyish white to ochre-brown, outside darker, margin with straight hyaline to light brown thick-walled hairs in bundles, up to 250 µm long. Ascospores broadly ellipsoid with slightly truncate poles, 13–16 × 9.5–10.5 µm, hyaline, thin-walled, smooth, eguttulate. On soil, in the lowlands. Lit.: This paper.

22 Ascospores ellipsoid with tapered poles **23**

22*Ascospores ellipsoid ....**(22) *Tricharina aethiopica***
Apothecia up to 5 mm diam., sessile, first cupulate, later spreading out, hymenium yellowish beige, outside darker, margin with straight brown thick-walled hairs in bundles, up to 500 (600) µm long. Ascospores ellipsoid, 15–17.5 × 8.5–10.5 µm, hyaline, thin-walled, smooth, eguttulate. Paraphyses hyaline, with non-refractive vacuoles. On soil, at afro-mountain range. Lit.: Lindemann (2017).

23 Qm ≥ 1.7..**(23) *Tricharina hiemalis***
Apothecia 2–8 mm diam., sessile, partly buried in sandy soil, cupulate, hymenium pale beige, margin with straight brown thick-walled hairs in bundles, up to 400 µm long. Ascospores ellipsoid-oculiform to fusoid, 14–18 × 7–11 µm, hyaline, thin-walled, smooth, eguttulate, but some inclusions at the poles. On soil, at low mountain range. (Unfortunately, only few data about the macroscopic appearance in the fresh state are available). Lit.: Van Vooren *et al.*, 2019; this paper.

23*Qm < 1.7 ....**(24) *Tricharina subhiemalis***
Apothecia 3–5 (11) mm diam., sessile, first cupulate, later spreading out, hymenium white to pale beige, outside darker, margin densely hairy, with dark brown thick-walled hairs in bundles, up to 450 µm long. Ascospores ellipsoid, a bit tapering at the poles, 14–16.5 × 9.5–11.1 µm, hyaline, smooth, thin-walled, eguttulate. On soil. Lit.: This paper.

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Authors' contribution

U. Lindemann is responsible for the study conception and design, associated to N. Van Vooren. U. Lindemann and N. Van Vooren financially contributed to the generation of rDNA sequences. The morphological analyses were performed by F.J. Valencia, V. Kaounas, N. Van Vooren and U. Lindemann on their collections. The molecular analyses were done by N. Van Vooren. The first draft of the manuscript was written by U. Lindemann and subsequently updated by N. Van Vooren. All plates have been designed by U. Lindemann. All authors read and approved the final manuscript.

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