Paratricharina confusa sp. nov. and *Hellenicoscyphus hyalotrichus* gen. and sp. nov., two new tricharinoid discomycetes (*Pezizales*) from the Mediterranean basin

Uwe LINDEMANN
Francisco Javier VALENCIA
Vasileios KAOUNAS
Volker KUMMER
Nicolas VAN VOOREN

Abstract: Two new tricharinoid species, *Paratricharina confusa* and *Hellenicoscyphus hyalotrichus*, are described and illustrated based on collections from Spain and Greece. The morphological features are compared with the most closely related species, especially *Paratricharina poiraultii*. A multigene analysis showing their taxonomic position is presented and discussed.
Keywords: *Ascomycota*, morphology, *Pyronemataceae*, taxonomy, *Tricharina*.

Ascomycete.org, 14 (2) : 67–79
Mise en ligne le 25/04/2022
10.25664/ART-0347

Introduction

The genus *Paratricharina* Van Vooren, U. Lindem., M. Vega, Ribes, Illescas & Matočec was published as a new genus with *P. poiraultii* (Boud.) Van Vooren, U. Lindem., M. Vega, Ribes, Illescas & Matočec (basionym: *Lachnea poiraultii* Boud.) as type. This fungus is a small discomycete with densely interwoven dark brown marginal and excipular hairs and an orange-brown hymenium (Van Vooren *et al.*, 2015a). It seems that this species has not been found for a hundred years, but after its "rediscovery" in 2015 it turned out that the species is not so rare in the Mediterranean basin. Last year, a second species was introduced in this genus: *Paratricharina multiguttulata* U. Lindem., Wieschollek, Sochorová & M. Vega (Lindemann *et al.*, 2021), characterised by subglobose, thick-walled ascospores, filled with many little oil drops — a unique feature in the *Tricharina*-like discomycetes. In this article, we describe a third species of the genus *Paratricharina* found in southern Spain and Greece. It shares many features with the type, *P. poiraultii*, but can nevertheless be morphologically distinguished. Furthermore, we describe a second tricharinoid discomycete collected in Greece that also shares many morphological features with *P. poiraultii*. Despite these similarities, the molecular data revealed that this fungus does not belong to *Paratricharina* nor any other known tricharinoid genus (Van Vooren *et al.*, 2015a, 2015b, 2017, 2018, 2019; Table 3 in this paper).

Material and methods

Morphological study. — The microscopic studies were based on both fresh and dried specimens, and made with optical microscopes with plan-achromatic objectives. For dried specimens, a small piece was rehydrated in water for about two hours before the observation. The following main reagents were used: iodine solution (Melzer's reagent or Lugol's solution), Cotton Blue in lactic acid (CB), Cresyl Blue (CRB) and 3% KOH. Water mounts were used for the observation of the pigmentation and measurements. Ascospore measurements are given including ornamentation.

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, EF1-728F, EF1-983F and EF1-2218R (Carbone & Kohn, 1999; Rehner & Buckley, 2005) for the translation elongation factor 1α (tef1) gene, and bRPB2-6F2 (reverse of bRPB2-6R2), and bRPB2-7R2 for the RNA polymerase II second largest subunit (rpb2) gene (Matheny *et al.*, 2007). 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.

Phylogenetic analysis. — Two different datasets were assembled: 1) LSU, tef1 (exons) and rpb2 (exons) sequences of selected species of the family *Pyronemataceae* and the most closely related clades (with family *Sarcosomataceae* and genus *Glaziella* Berk. as outgroups), and 2) ITS, LSU, tef1 (exons and introns) and rpb2 (exons and introns) of the genus *Paratricharina* (with *Pseudotricharina intermedia* as outgroup). BLASTn (Altschul *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC, Cochrane *et al.*, 2011). The sequences retrieved were mainly from studies conducted by Hansen *et al.* (2013), Perry *et al.* (2007) and Van Vooren *et al.* (2017). Sequences first were aligned in MEGA 5.0 software (Tamura *et al.*, 2011) with its Clustal W application and then realigned manually as needed to establish positional homology. Aligned loci were loaded in MrBayes 3.2.6 (Ronquist *et al.*, 2012), where a Bayesian analysis was performed (data partitioned [3: LSU, tef1, rpb2 for *Pyronemataceae* and 4: ITS, LSU, tef1, rpb2 for *Paratricharina*]; two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation, burn-in 25%) until the average split frequencies between the simultaneous runs fell below 0.01 after 0.45 M generations (*Pyronemataceae*) and 0.07 M (*Paratricharina*). Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis, 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

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 a personal herbarium, the terms "pers. herb." are used, followed by the author's reference.

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

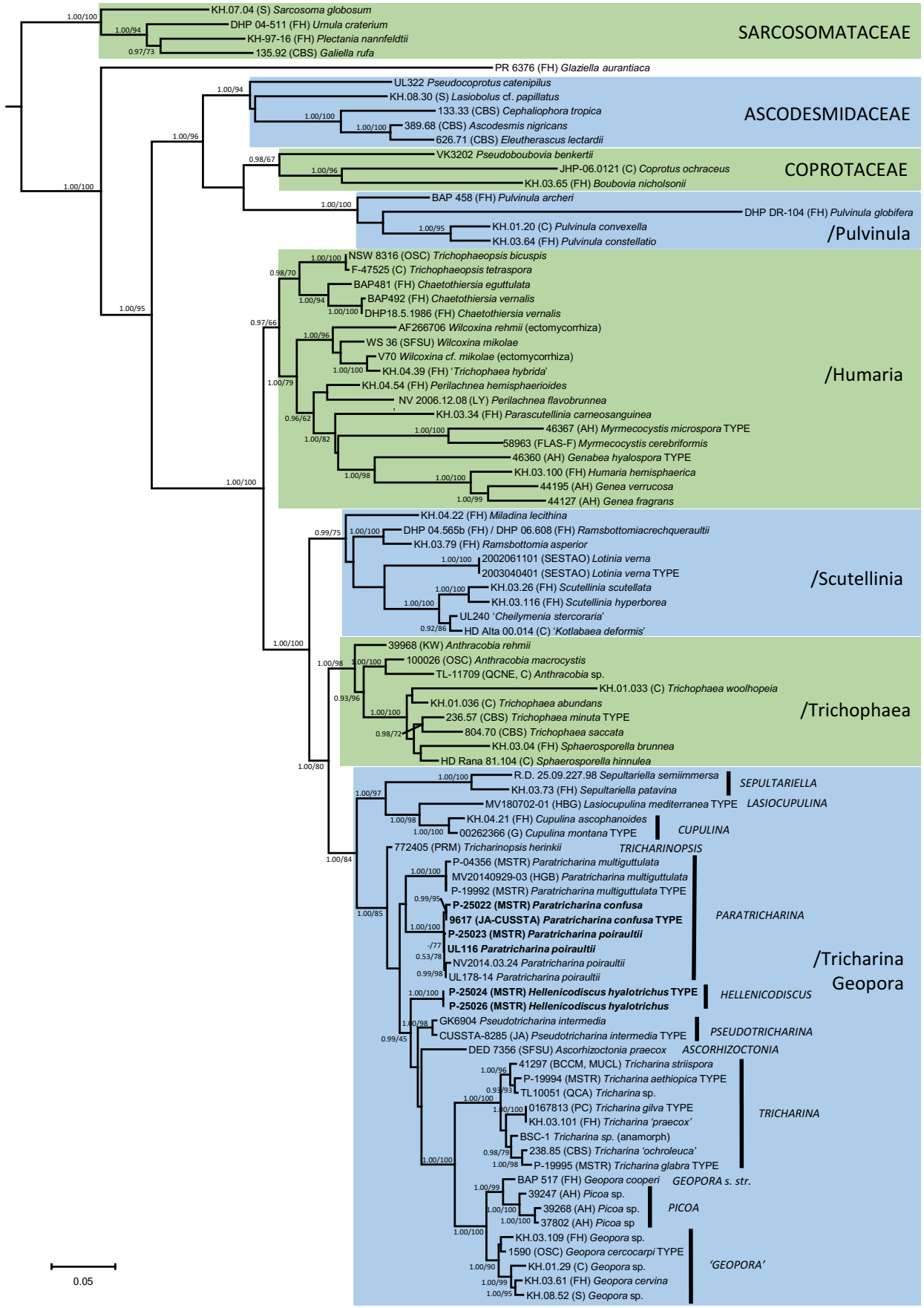

Fig. 1 – Best scoring 28S rDNA – tef1 – rpb2 phylogram of the family *Pyronemataceae* (*Pezizales*) (with the family *Sarcosomataceae* and the genus *Glaziella* as outgroups) obtained using RAxML. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Nonsignificant support values are exceptionally represented inside parentheses. Sequences newly generated in this study are in bold. Taxon names on phylotree terminals reflect the phylogenetically supported name. Names in apostrophes such as '*Kotlabaea deformis*' indicate that the collections from which the sequences in GenBank were obtained were incorrectly identified, but it has not yet been possible to clarify to which species they can be correctly assigned (cf. Lindemann *et al*., 2015; Lindemann & Alvarado, 2017).

Table 1 – List of collections sequenced for this study. All other specimens included in the current phylogenetic analysis (see fig. 1) are listed in Lindemann *et al.* (2021, table 2).

Name	Coll. Number	Country	Collector	GenBank Accession Number			
				ITS	28S	rpb2	tef1
Paratricharina confusa	9617 (JA-CUSTA) TYPE	Spain	F. J. Valencia	OM971819	OM971815	OM974176	OM974182
Paratricharina confusa	P-25022 (MSTR)	Greece	V. Kummer	OM971820	OM971816	OM974177	OM974183
Paratricharina poiraultii	P-25023 (MSTR)	Portugal	U. Lindemann	OM971821	OM971817	OM974178	OM974184
Paratricharina poiraultii	U.L. 116 (priv. herb. U. Lindemann)	Spain	F. Hampe & J. Kleine	KY364029	KY364053	OM974179	OM974185
Hellenicoscyphus hyalotrichus	P-25024 (MSTR) TYPE	Greece	V. Kaounas	OM971822	OM971818	OM974180	OM974186
Hellenicoscyphus hyalotrichus	P-25026 (MSTR)	Greece	V. Kaounas	OM971823	–	OM974181	–

Results

The 3-gene phylogeny (fig.1) based on a combination of LSU, tef1 (exons) and rpb2 (exons) was mainly focused on the /*Scutellinia-Trichophaea* lineages as delineated by Hansen *et al.* (2013). The new *Paratricharina* species forms a non-significant clade together with *Paratricharina poiraultii* (PP 0.64, ML 38). This result could be caused by an insufficient phylogenetic signal obtained from the markers analysed or the noise introduced by the other clades. However, a polytomy at the root of the *Tricharina* clade could also be interpreted as an ancient diversification stage that produced the known lineages more or less at the same time, followed by an accelerated diversification in the clades of *Tricharina s. str*. and the clade of *Geopora* Harkn. and *Picoa* Vittad. The second new described species forms an independent lineage apparently not related to *Paratricharina* nor *Pseudotricharina* Van Vooren, Tello & M. Vega.

The special *Paratricharina* tree (fig. 2) is based on ITS, LSU, tef1-exons and introns, and rpb2-exons. Compared to the 3-gene phylogeny, the additional variability introduced by introns and ITS, plus the removal of phylogenetic noise from other genera in the first dataset explain the different support obtained in some clades. On the one hand, the tree shows reciprocal support for three different lineages within the *P. poiraultii* clade, and two lineages within *P. multiguttulata*. While the new *Paratricharina* species is morphologically distinguishable from *P. poiraultii*, this is not the case for the other lineages found within *P. multiguttulata* and *P. poiraultii*. These lineages may represent species of their own but must be considered as cryptic at the current state of knowledge.

Taxonomy

Paratricharina confusa U. Lindemann, Valencia, Van Vooren & V. Kummer *sp. nov.* – MB 842904

Diagnosis: Differs from *Paratricharina poiraultii* by the hairless margin, the larger ascospores, the light brownish hymenium, and its genetic profile.

Holotype: Spain, Málaga, Ronda, Partido Rural Navares/Tejares, 36.736691° N, 5.1483607° W, elev. 730 m, on the riverbanks of Guadalevín river, between small bryophytes on soil, with *Salix alba* and *Equisetum ramosissimum*, 2 May 2020, *leg*. F. J. Valencia, JA-CUSSTA 9617 – GenBank: OM971819 (ITS); OM971815 (28S), OM974176 (rpb2), OM974182 (tef1).

Etymology: From Latin "*confusus*", meaning confused, because the species can be easily confused with its sibling species *Paratricharina poiraultii*.

Description: Apothecia 2–5.5 mm diam., brownish ochre or salmon-ochre, sessile, first cupulate, then spreading; receptacle concolorous or slightly lighter than the hymenium, covered with brown granulations or pustules; margin slightly enrolled when young, then crenulate and slightly velvety.

Microscopical features: Medullary excipulum of *textura intricata*, composed of narrow, hyaline, septate hyphae, (5.3) 6.3–8.8 (9.5) µm wide. **Subhymenium** not distinguished from medulla. **Ectal excipulum** 116–224 µm thick, of *textura globosa/angularis* with some globose and subglobose hyaline cells (15.9) 17.3–39.6 (50) × (13.4) 14.4–26.3 (29.2) µm. **Margin** of *textura globulosa/angu-*

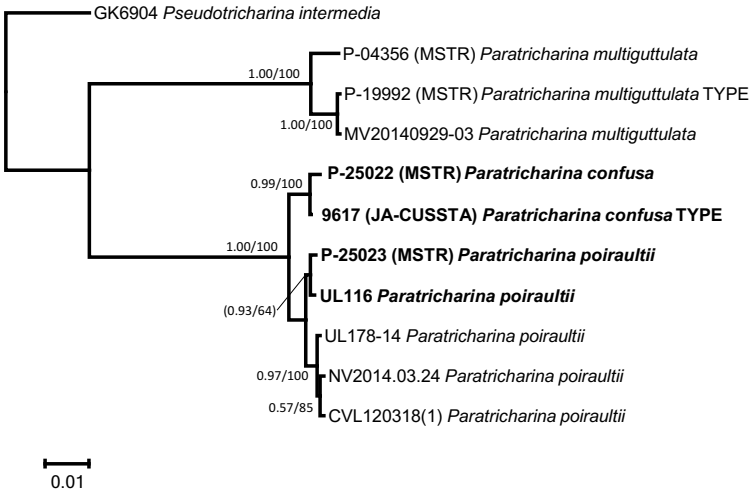

Fig. 2 – Best scoring ITS – 28S rDNA – tef1 – rpb2 phylogram of the genus *Paratricharina* (*Pyronemataceae*) (with *Pseudotricharina intermedia* as outgroup) obtained using RAxML. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Nonsignificant support values are exceptionally represented inside brackets. Sequences newly generated in this study are in bold.

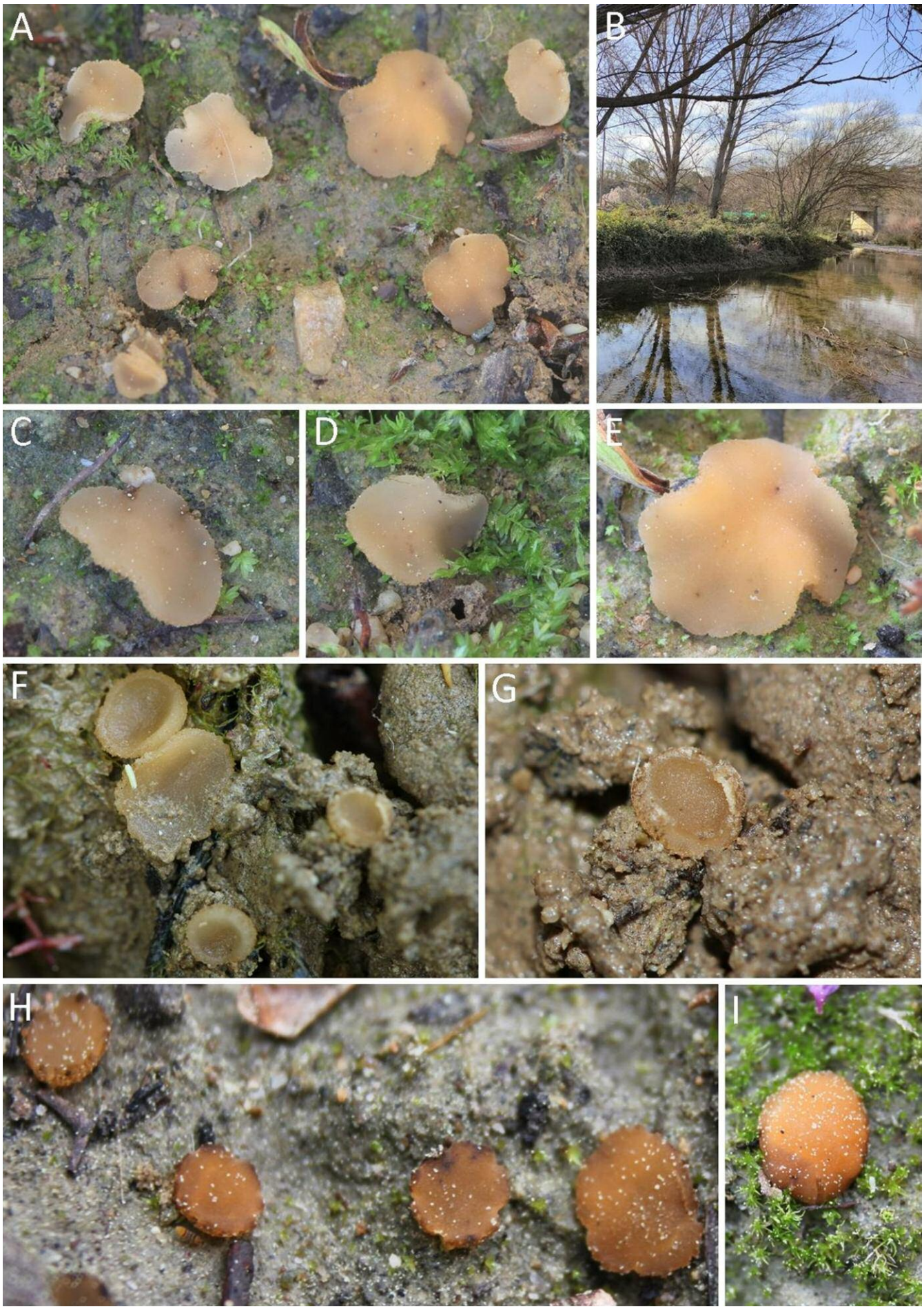

Plate 1 – *Paratricharina confusa*. Ascomata *in situ*
A, C–E: Coll. JA-CUSSTA 9617 (holotype), F–G: 2. Coll. JA-CUSSTA 9618 (topotype), B: Habitat of the type collection, H–I: Coll. MSTR P-25022. Photos by F. J. Valencia (A–G) and V. Kummer (H–I).

laris with two types of apical cells: the first ones are clavate or ovoid, thin-walled, hyaline, containing large non-refractive vacuoles, 30–115 × 10.1–41.4 µm; the second ones are hair-like hyphae, thick-walled, (0.9-1.2 µm thick), hyaline, septate, with an obtuse apex and enlarged base, sometimes bifurcate, sometimes encrusted, (21.3) 35.4–83.6 (92.2) × (9.8) 10.1–13.6 (15.6) µm. **Crystals** present at the whole margin. **Excipular hairs** scattered, single or in bunches, (53.6) 54.6–116.8 (153.6) × (5.8) 7–13.9 (17.3) µm, hyaline to light brown, thick-walled, up to 2.0 µm, obtuse, septate, with an enlarged base, with incrustations on the outer walls, usually short. **Anchor hyphae**, present, hyaline with yellowish walls, septate, up to 360 µm in length, with a widened base, 7.5–12.3 µm. **Asci** 8-spored, cylindrical or slightly curved, 213–302 × 11.6–21.3 µm, inamyloid, with croziers. **Paraphyses** cylindrical, sometimes bifurcate near apex, with non-refractive vacuoles, visible in CRB, septate; near the margin some paraphyses show moniliform cells below apex; terminal above the last septum 32–104 × 4.7–8.3 µm. **Ascospores** ellipsoid, biseriate at maturity, hyaline, containing bipolar small lipid granules which merge in Lugol and stain red (because of glycogen content), thick-walled, up to 0.8 µm, smooth in water and Lugol, very finely warted in CB, uninucleate, with a thin sheath around free ascospores: (17.1) 17.9–20.7 (21.6) × (10.3) 10.8–11.8 (12.4) µm, Q = 1.5 -1.8, Me = 19.1 × 11.1 µm; Qe = 1.69 (N = 60); rehydrated in KOH: 16.5–19.9 (21.7) × (9.8) 10.4–11.5 (11.8) µm; Q = 1.5–1.8; Me = 18.2 × 10.9 µm ; Qe = 1,68 (N = 25).

Additional specimens examined: Greece. Rhodes, Malonas, approx. 1.8 km SSE of the monastery of Kamyri, in the gorge of Skoutouljaris, 36.172639° N, 28.042861° E, elev. 60 m, on clay deposits at the edge of the river, 5 March 2013, *leg*. V. Kummer, MSTR P-25021; Rhodes, Malonas, approx. 1.8 km SSE of the monastery of Kamyri, in the gorge of Skoutouljaris, 36.172222° N, 28.0425° E, elev. 60 m, on clay deposits at the edge of the river, 26 March 2015, *leg*. V. Kummer, MSTR P-25022. Spain. Málaga, Ronda, Partido Rural Navares/Tejares, 36.736524, -5.1472074, elev. 730 m, on the riverbanks of Guadalevín river, on wet soil, left by the water when the flow decreases, with *Salix alba*, *Populus alba* and *Rubus ulmifolius*, accompanied by *Pulvinula* sp. and *Peziza celtica*, 19 July 2021, *leg*. F.J. Valencia, JACUSSTA 9618 (topotype).

Discussion: *Paratricharina confusa* can easily be confused with *P. poiraultii* because it shares most of its morphological characteristics. Nevertheless, there exist clear distinguishing features (see Table 2). First, the apothecia of *P. poiraultii* are much larger than those of *P. confusa* which seems to be a tiny species. Second, the colour of the hymenium of the two species is different. Third, the margin of *P. confusa* apothecia has only short, hair-like hyphae in contrast to the densely interwoven brown hairs at the margin and outside of the *P. poiraultii* ascomata, and the excipular hairs in *P. confusa* are only scattered. Fourth, the ascospores in the living state are somewhat larger than those of *P. poiraultii*. Finally, *P. confusa* seems to prefer wet sites. The known collections come from (recently flooded) riversides, whereas *P. poiraultii*, as far as is known, does not need such moist conditions (Van Vooren *et al.*, 2015a).

The phylogenetic analysis has shown that *P. poiraultii* splits into two lineages. However, the sequenced collections of both lineages are morphologically very similar. To clarify this point, further collections need to be documented in the fresh state and subsequently

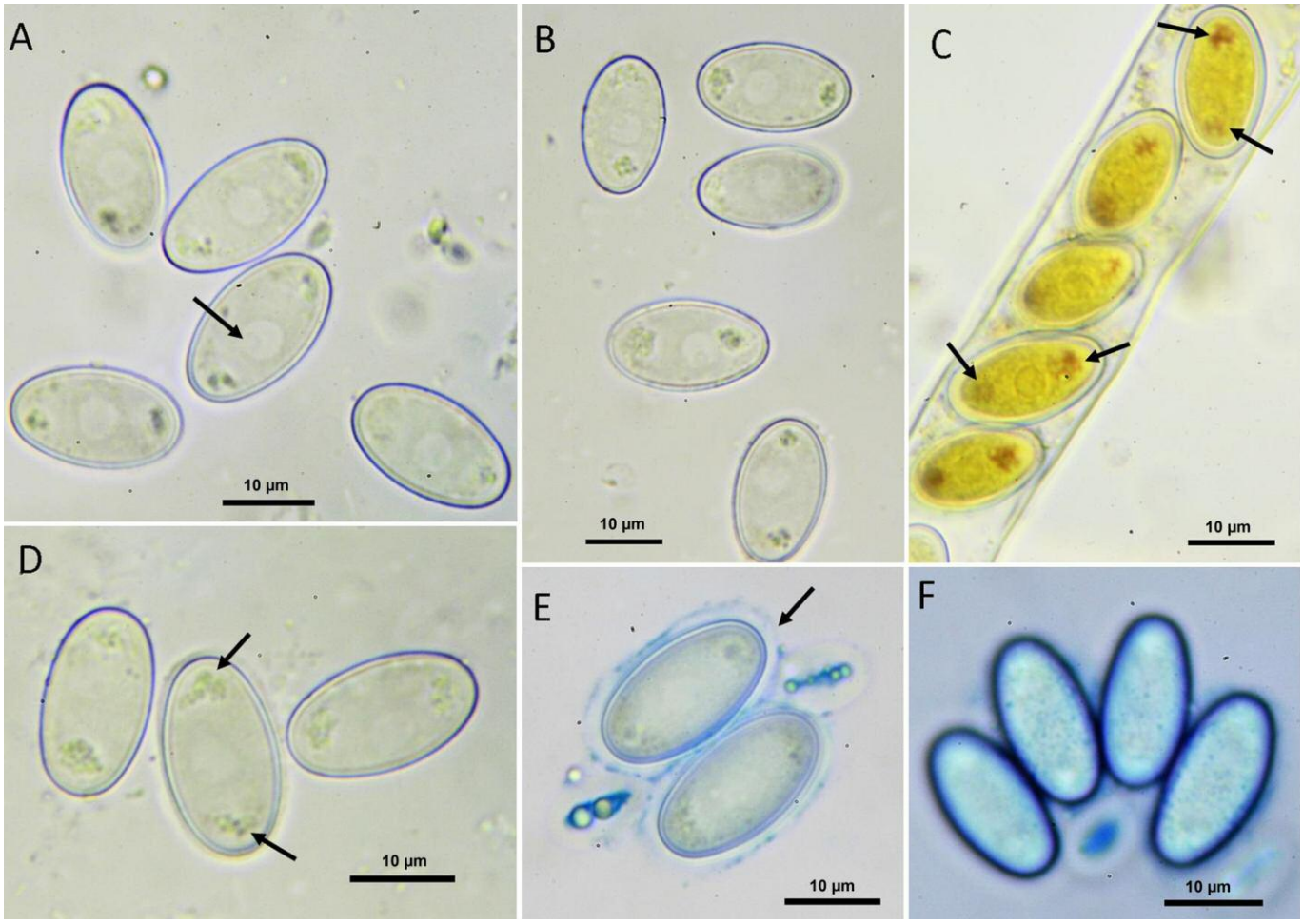

Plate 2 – *Paratricharina confusa*. Ascospores.
A, B & D: Ascospores in water, showing nuclei and bipolar lipid granules (arrows). C: Ascospores in Lugol's solution, showing the glycogen reaction near the bipolar lipid granules. E: Ascospores in CB, showing the sheath (arrow) around freshly ejected ascospores. F: Ascospore in CB, showing the finely verrucose ornamentation. All photos by F. J. Valencia.

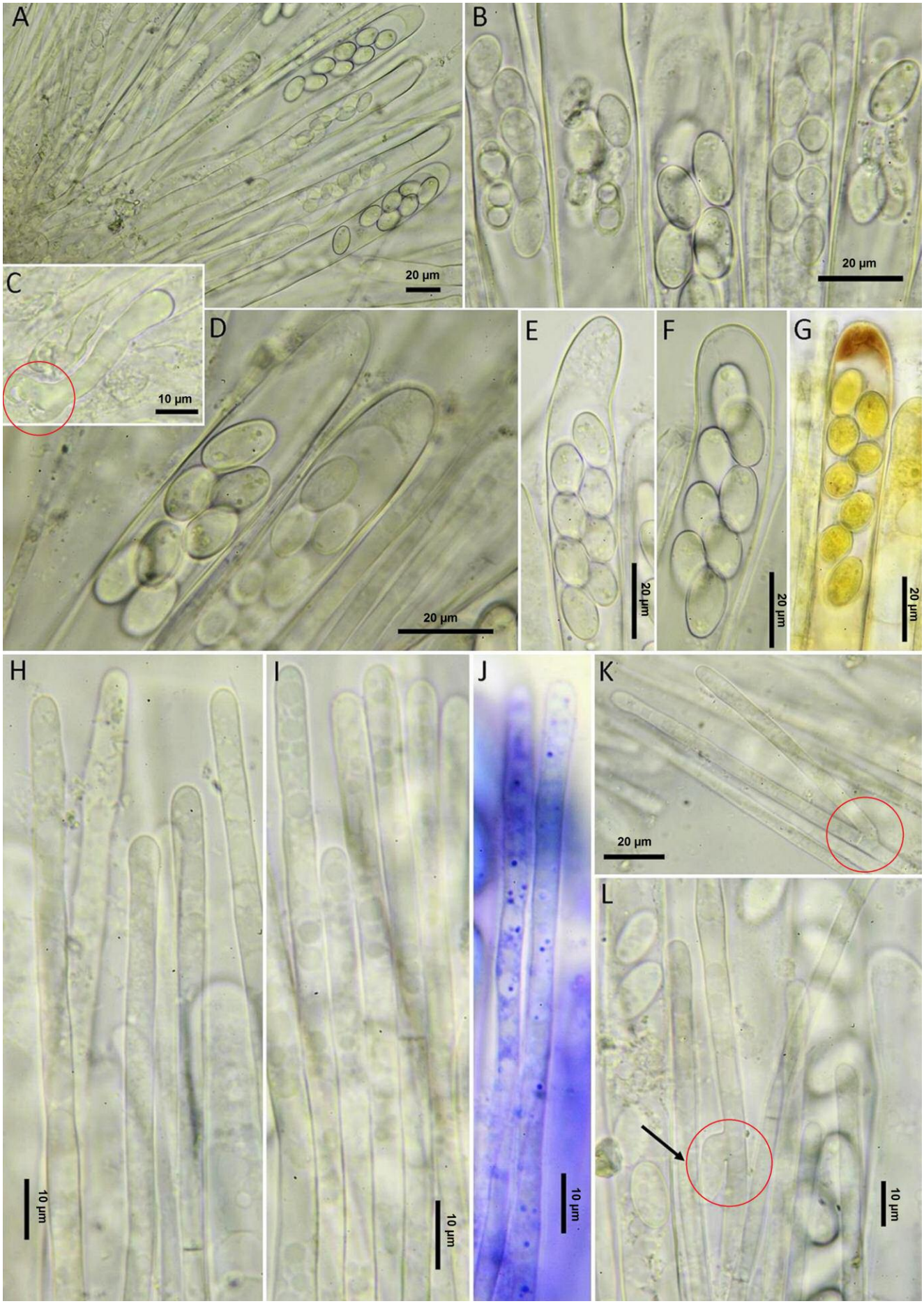

Plate 3 – *Paratricharina confusa*. Asci and paraphyses.

A, B & D–F: Asci in water, showing biseriate ascospores. C: Ascus base with crozier. G: Asci in in Lugol's solution. H–I: Paraphyses in water, showing vacuolar content. J: Paraphyses in CRB, colouring the vacuolar and lipid content. K–L: Paraphyses in water, with bifurcations. All photos by F. J. Valencia.

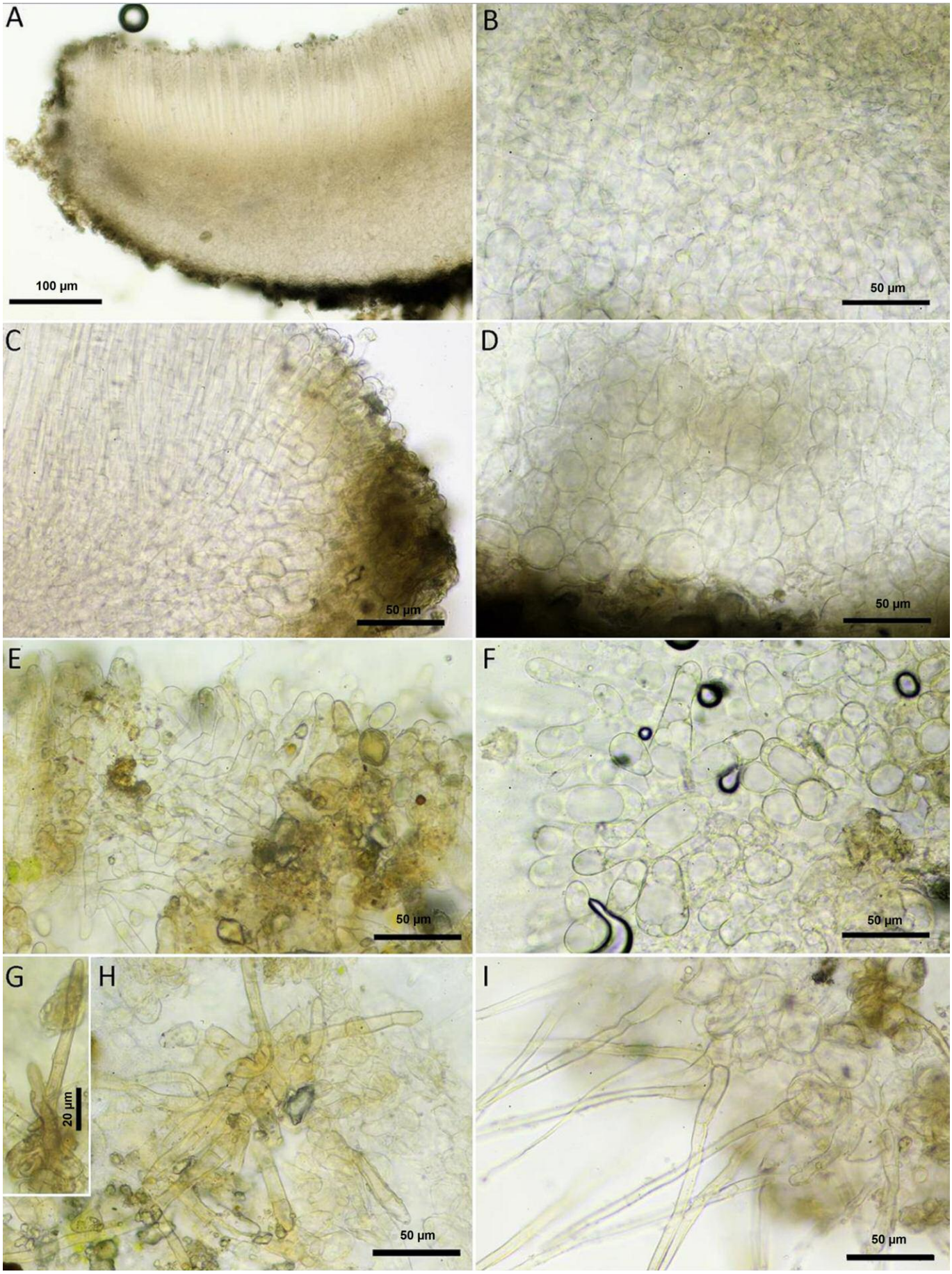

Plate 4 – *Paratricharina confusa*. Excipular structure, hairs and hair-like hyphae.
A: Cross section of an apothecium. B: Medullary excipulum of *textura intricata*. C: Marginal zone. D: Ectal excipulum of *textura globosa*/*angularis*. E: Hair-like hyphae at the margin. F: Marginal cells. G–H: Excipular hairs. I: Anchor hyphae. All photos by F. J. Valencia.

Table 2 – Morphological comparison between *Paratricharina poiraultii* and *P. confusa* (the main differences marked in bold)

	P. poiraultii	***P. confusa***
Apothecia		
- diameter	**5–25 (40) mm**	**2–5.5 mm**
- colour of the hymenium	**orange-brown (when fresh)**	**brownish-ochre or salmon-ochre**
- margin	**enrolled, with densely interwoven brown hairs**	**smooth to crenulate, only slightly velvety**
Excipulum structure		
- medullary excipulum	*textura intricata*	*textura intricata*
- ectal excipulum	*textura prismatica/angularis* and *globulosa/angularis*	*textura angularis/globulosa*
- outermost layer of the ectal excipulum	*textura globulosa-subangularis*, **made of brown thick-walled cells**	*textura globulosa-subangularis*, **consisting of hyaline to light brown thick-walled cells**
- margin	**with true hairs**	**without true hairs, but with hair-like hyphae (see plate 4E)**
Hairs		
- shape and colour	**brown, thick-walled, septate, slightly pointed, densely interwoven**	**scattered, hyaline to light brown, thick-walled, septate, slightly pointed**
- arising from	small cells of the *textura angularis*	small cells of the *textura angularis*
- rooting	no	no
Paraphyses		
- shape	cylindrical, not or only slightly enlarged at the apex	cylindrical, not or only slightly enlarged at the apex
- colour	hyaline	hyaline
- content	non-refractive vacuoles	non-refractive vacuoles
Ascospores		
- shape	ellipsoid to narrowly ellipsoid (Q = 1.4–2.0)	ellipsoid to narrowly ellipsoid (Q = 1.6–2.0)
- size	**15–20 × 9–11 µm**	**17-21.5 × 10–12 µm**
- arrangement in asci	**uniseriate**	**biseriate at maturity**
- colour	hyaline	hyaline
- wall thickness	thick-walled	thick-walled
- content	small polar oil drops	small polar oil drops

sequenced. For the time being, *P. poiraultii* must be considered as a cryptic species.

Finally, as *P. confusa* is the third described species of the genus *Paratricharina*, we propose an amended description of the latter: Ascomata epigeous. Apothecia, sessile, slightly cupulate with a ± inrolled margin, then discoid; outer surface covered with hyaline to brown hairs. Margin with or without hairs. Excipulum two-layered: medullary excipulum of *textura intricata*, with thin-walled hyphae; ectal excipulum of vertically oriented *textura prismatica*, with thin-walled cells, becoming a *textura globulosa/angularis* in the outermost part, made of thick-walled brownish cells. Hairs superficial, hyaline to pale brown, more or less sinuous, septate, obtuse or sharp, with a simple base arising from globose cells of the outermost part of the ectal excipulum. Asci cylindrical, with forked base, arising from perforated croziers, inamyloid, 8-spored. Paraphyses cylindrical, rounded and slightly widened at the apex, with vacuolar content, without reaction in Lugol's solution. Ascospores subglobose to ellipsoid, hyaline, with small polar aggregates of minute lipid bodies, rapidly merged into two small polar guttules or with medium sized guttules (in living state), more or less thick-walled, smooth, but partly with a rough to finely verrucose perispore if mounted in Lugol's solution, often entirely encapsulated with a delicate persistent sheath. Hyaline anchoring hyphae present.

Hellenicoscyphus U. Lindemann, Van Vooren & Kaounas *gen. nov.* – MB 843256

Diagnosis: Differs from *Paratricharina* by its hyaline marginal hairs organised in small bundles, the lack of excipular hairs and from *Tricharina* by its hyaline marginal hairs, the lack of excipular hairs and its bipolar spore granules, and from both genera by its genetic profile.

Etymology: From the ancient Greek ἑλληνικός (*hellênikós*) meaning "from Greece" because the type was first collected in Greece, and σκύφος (*skúphos*) meaning "cup".

Type: *Hellenicoscyphus hyalotrichus* U. Lindemann, Van Vooren & Kaounas

Key to the genus *Paratricharina*

1 Ascospores globose to subglobose ..... ***P. multiguttulata***
1* Ascospores ellipsoid **2**
2 Marginal hairs present ..... ***P. poiraultii***
2* Marginal hairs absent ..... ***P. confusa***

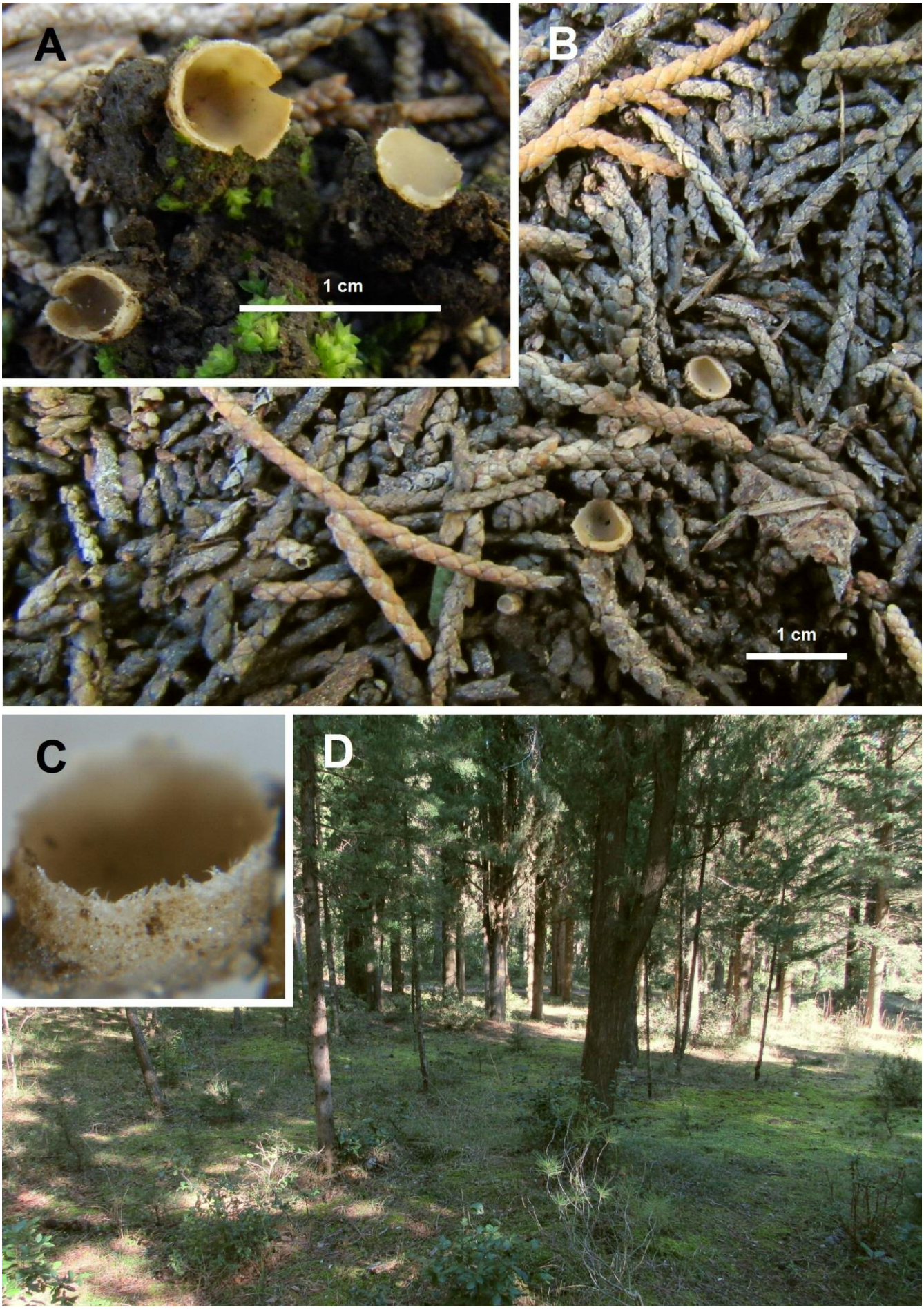

Plate 5 – *Hellenicoscyphus hyalotrichus*. Ascomata and biotope.
A: Coll. MSTR P-25025. B: Coll. MSTR P-25024 (Type, *in situ*). C: Coll. MSTR P-25026 (close-up view). D: Area of coll. P-25024–25026. All photos by V. Kaounas.

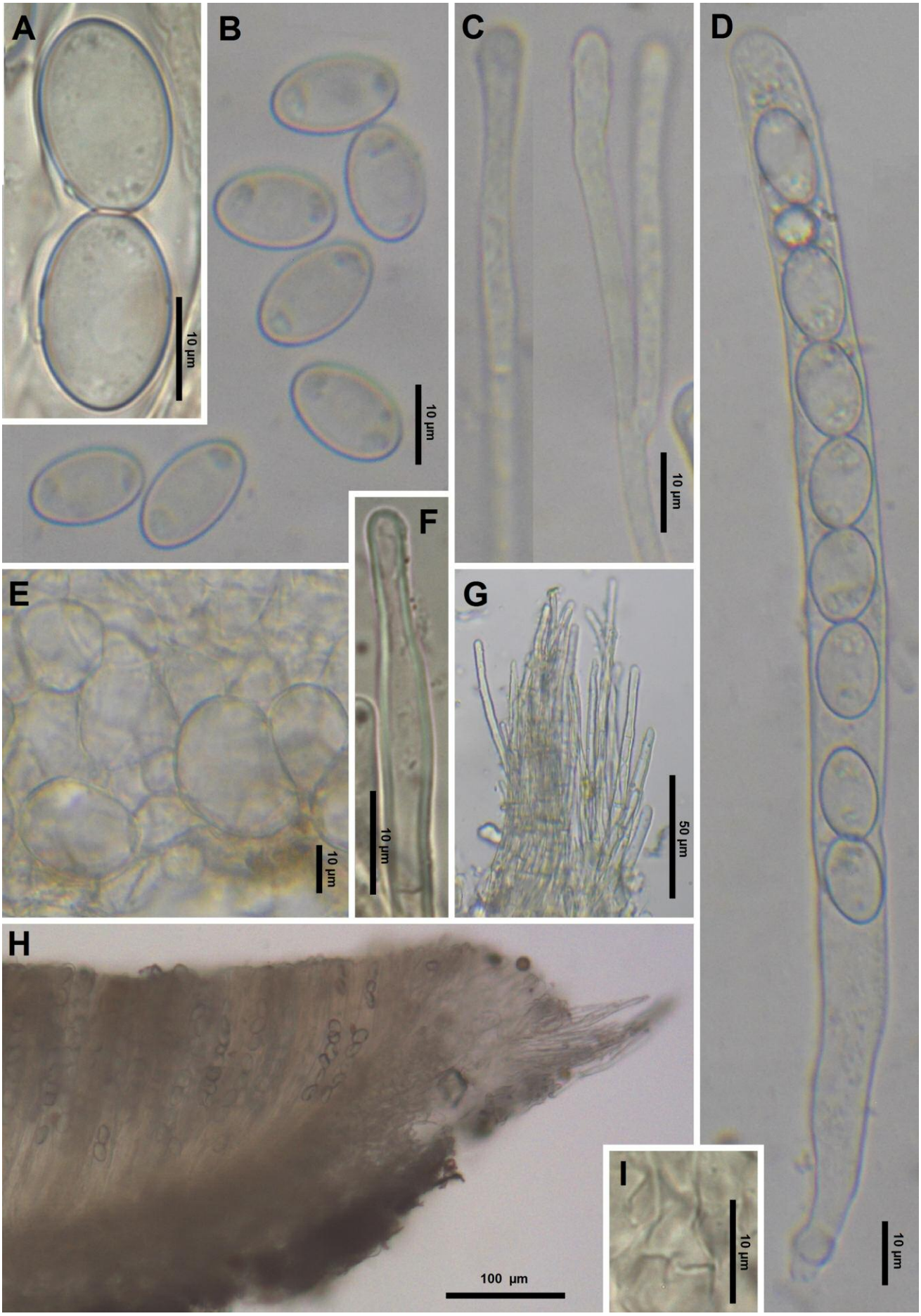

Plate 6 – *Hellenicoscyphus hyalotrichus*. Microfeatures.

A: Ascospores (rehydrated) in H_2O. B: Ascospores. C: Paraphyses. D: Ascus. E: Ectal excipulum. F: Hair apex. G: Marginal hairs organised in a small bundle. H: Cross section of an apothecium. I: Ascus base with free crozier. Photos A, F, H & I by U. Lindemann, B–E & G by V. Kaounas.

Definition of *Hellenicoscyphus*: Ascomata epigeous, apothecial, sessile, cupuliform, light brown to ochre. Receptacle hairless. Marginal hairs organised in small bundles, superficial, septate, with a simple widened base. Excipulum two-layered: medullary layer of *textura intricata*, and ectal layer of *textura globulosa/angularis*. Asci operculate, narrowing toward base, arising from free croziers, inamyloid, 8-spored. Paraphyses slender, hyaline. Ascospores uniseriate, ellipsoid, hyaline, smooth, containing small bipolar granules. Species probably saprobic. Asexual morph unknown.

Hellenicoscyphus hyalotrichus U. Lindemann, Van Vooren & Kaounas *sp. nov.* – MB 843257

Diagnosis: Differs from *Paratricharina poiraultii* by the shape of the ascospores, the marginal hyaline hairs of the ascomata, the light brownish hymenium, and its genetic profile.

Holotype: Greece, Attica, Acharnes, Varymbombi-Sfendali, 38.161087° N, 23.790450° E, elev. 488 m, on soil between mosses and *Cupressus* litter, under *Cupressus sempervirens*, *Pinus halepensis*, *Quercus ilex* and *Q. coccifera*, 5 Feb. 2021, *leg*. V. Kaounas, MSTR P-25024 (ex V.K. 6181) – GenBank: OM971822 (ITS); OM971818 (28S), OM974180 (rpb2), OM974186 (tef1).

Etymology: From ancient Greek ὕαλος (*hýalos*) meaning "translucent, glassy, clear or transparent", and τριχος (*trichós*) meaning "hair, wool, bristle" because of the conspicuous hyaline marginal hairs of the species.

Description: Apothecia 2–5 mm diam., hymenium light brown to ochre, paling with age, sessile, first cupulate, then spreading while the margin tears; receptacle concolorous, hairless; margin densely covered with macroscopically whitish hairs organised in small bundles.

Microscopical features: Medullary excipulum of *textura intricata*, composed of narrow, hyaline, thin-walled, septate hyphae. **Subhymenium** not distinguished from medulla. **Ectal excipulum** of *textura globulosa/angularis*, composed of hyaline, thin-walled cells, (8.2) 8.9–21.8 (22.1) × (6.7) 8.4–17.5 (18.3) µm. **Margin** of *textura prismatica*, composed of thin-walled, hyaline cells, the upper part made of clavate cells, 22–35 × 7–13 µm. **Marginal** hairs (90) 115–203 (232) × (3.2) 3.4–5.5 (5.7) µm, superficial, hyaline, thick-walled (wall up to 1 µm), multi-septate, straight to slightly curved, obtuse, with a simple basal cell widened up to 16 µm, not rooting. **Asci** (180) 190–235 × 10.5-13.7 µm, 8-spored, cylindrical or slightly curved, inamyloid, narrowing toward base, with free croziers. **Paraphyses** cylindrical, 3.5–4.5 µm wide, apex slightly widened up to 4.9–7.4 µm, containing non-refractive vacuoles, sometimes bi-furcate. **Ascospores** ellipsoid, tapered at the poles, (13.4) 14.8–17 (17.5) × (7.9) 8.7–10.7 (11.0) µm, Me = 16 × 9.8 µm; Q = 1.4–1.9; Qe = 1,64 (N = 90); rehydrated in KOH: (14.7) 15.1–16.5 (17.1) × 9–10.2 µm, Me = 15.9 × 9.7 µm; Q = 1.5–1.8, Qe = 1.65 (N = 35), uniseriate, hyaline, containing bipolar small lipid granules, slightly thick-walled, smooth in water, Lugol's solution and Cotton Blue in lactic acid, uninucleate.

Additional specimens examined: Greece, same location of the type collection, 29 Jan. 2018, *leg*. V. Kaounas, MSTR P-25025 (ex V.K. 5095); same location, 5 Feb. 2018, *leg*. V. Kaounas, MSTR P-25026 (ex V.K. 5120).

Discussion: *Hellenicoscyphus hyalotrichus* shows many morphological similarities with several *Tricharina*-like discomycetes. The

Table 3 – Comparison of main characters of the closest genera from *Hellenicoscyphus*

	Hellenicoscyphus	***Paratricharina***	***Pseudotricharina***	***Tricharina***	***Tricharinopsis***	***Ascorhizoctonia***
Ascospores (shape)	ellipsoid	subglobose to ellipsoid	ellipsoid to subfusoid	ellipsoid to subfusoid / trapezoid	ellipsoid with tapered ends to subfusoid	ellipsoid with tapered ends to subfusoid
Ascospores (ornamentation)	smooth	smooth to finely verrucose	verrucose	smooth, or finely striate in *T. striispora*	smooth	smooth to finely verrucose
Spore content, in living state	bipolar spore granules	bipolar spore granules	oil drops and without granules	eguttulate or filled with numerous minute LBs in *T. glabra*	eguttulate and without granules	bipolar spore granules
Paraphyses content	without pigments	without pigments	without pigments	without pigments, except in *T. glabra* with yellowish pigment (not constant)	without pigments	filled with yellowish granular pigments
Marginal hairs	hyaline	hyaline to dark brown	hyaline to light brown	mainly brown	hyaline to light brown	hyaline to light brown
Marginal hairs clustering	+	-	+	+	–	+
Flanks	devoid of excipular hairs	densely covered with short brown, ± flexuous hairs	covered with straight to flexuous, similar to marginal hairs	sparsely covered with some ± straight, hyaline to brown hairs, similar to the marginal hairs	densely covered with long flexuous hairs (reminiscent of a *Geopora* species)	sparsely covered with some ± straight, similar to the marginal hairs
Ecology	terrestrial	terrestrial	terrestrial	terrestrial (occasionally on burnt ground or decaying wood)	terrestrial	strictly pyrophilous

shape of the ascospores is reminiscent of *Tricharina hiemalis* Chin S. Yang & Korf (Yang & Korf, 1985; Van Vooren *et al.*, 2017) or *Trichar-inopsis herinkii* (Svrček) U. Lindem., Van Vooren & Healy (Van Vooren *et al.*, 2019), but these latter have a different ascospore content. While the ascospores of *H. hyalotrichus* contain bipolar small lipid granules, those of *T. hiemalis* and *T. herinkii* are devoid of such a content. In the case of *T. hiemalis*, brown marginal hairs are present. On the other hand, the ascospores content of *H. hyalotrichus* is very similar to those of *Paratricharina poiraultii* and *P. confusa* but can be distinguished by other features (cf. Diagnosis). The ascospores of *Cupulina ascophanoides* (Boud.) Van Vooren are also quite similar, but they differ in size and shape. Finally, *Ascorhizoctonia praecox* Chin S. Yang & Korf should be mentioned because the ascospores are also quite similar, but this species possesses brown marginal hairs and has a strictly pyrophilic ecology. The hyaline marginal hairs are reminiscent of *Pseudotricharina lanigera* Healy, D. Torres, Pfister & M.E. Sm., but it has warted ascospores, containing a large oil drop. Despite all listed similarities, the combination of its features makes *H. hyalotrichus* clearly distinguishable (see Table 3).

Acknowledgements

We acknowledge gratefully Marcel Vega for the pre-submission review of the manuscript. Furthermore, we thank Pablo Alvarado (ALVALAB) for having sequenced the material and made the multigene analyses.

Authors' contribution

U. Lindemann is responsible for the study conception and design, associated to N. Van Vooren. U. Lindemann financially contributed to the generation of rDNA sequences. The morphological analyses were performed by F.J. Valencia, V. Kaounas and U. Lindemann. The molecular analyses were done by P. Alvarado (ALVALAB). The first draft of the manuscript was written by U. Lindemann and subsequently updated by all authors. The plates of *Paratricharina confusa* have been designed by F.J. Valencia, those of *Hellenicoscyphus hyalotrichus* by U. Lindemann. All authors read and approved the final manuscript.

References

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. — Basic local alignment search tool. *Journal of Molecular Biology*, 215 (3): 403–410. doi: 10.1016/S0022-2836(05)80360-2

Carbone I. & Kohn L.M. 1999. — A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, 91 (3): 553–556. doi: 10.2307/3761358

Cochrane G., Karsch-Mizrachi I. & Nakamura Y. 2011. — The International Nucleotide Sequence Database Collaboration. *Nucleic Acids Research*, 39: D15–D18. doi: 10.1093/nar/gkq1150

Cubeta M.A., Echandi E., Abernethy T. & Vilgalys R. 1991. — Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology*, 81 (11): 1395–1400. doi: 10.1094/phyto-81-1395

Gardes M. & Bruns T.D. 1993. — ITS primers with enhanced specificity for Basidiomycetes — application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2 (2): 113–118. doi: 10.1111/j.1365-294x.1993.tb00005.x

Hansen K., Perry B.A., Dranginis A.W. & Pfister D.H. 2013. — A phylogeny of the highly diverse cup-fungus family *Pyronemataceae* (*Pezizomycetes*, *Ascomycota*) clarifies relationships and evolution of selected life history traits. *Phylogenetics and Evolution*, 67 (2): 311–335. doi: 10.1016/j.ympev.2013.01.014

Lindemann U. & Alvarado P. 2017. — Revision der Gattung *Kotlabaea*, Teil 2: *K. aurantiaca*, *K. carestiae*, *K. danuviana* und *K. trondii* nebst taxonomischen Bemerkungen zu *Boubovia vermiphila*, *Cheilymenia stercoraria* und zur Gattung *Pseudombrophila*. *Zeitschrift für Mykologie*, 83 (1): 103–126.

Lindemann U., Vega M. & Alvarado P. 2015. — Revision der Gattung *Kotlabaea*: *K. deformis*, *K. delectans* und *K. benkertii*. *Zeitschrift für Mykologie*, 81 (2): 373–402.

Lindemann U., Wieschollek D., Sochorová Z. & Vega M. 2021. — *Paratricharina multiguttulata* sp. nov. – A new species of the *Tricharina*-group with subspherical spores. *Ascomycete.org*, 13 (5): 179–188. doi: 10.25664/art-0335

Matheny P.B., Wang Z., Binder M., Curtis J.M, Lim Y.W., Nilsson R.H., Hughes K.W., Hofstetter V., Ammirati J.F., Schoch C.L., Langer E., Langer G., McLaughlin D.J., Wilson A.W. , Frøslev T., Ge Z.-W., Kerrigan R.W., Slot J.C., Yang Z.-L., Baroni T.J., Fischer M., Hosaka K., Matsuura K., Seidl M.T., Vauras J. & Hibbett D.S. 2007. — Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (*Basidiomycota*, Fungi). *Molecular Phylogenetics and Evolution*, 43 (2): 430–451. doi: 10.1016/j.ympev.2006.08.0244

Mullis K. & Faloona F.A. 1987. — Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, 155: 335–350. doi: 10.1016/0076-6879(87)55023-6

Murray M.G. & Thompson W.F. 1980. — Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8 (19): 4321–4325. doi: 10.1093/nar/8.19.4321

Perry B.A., Hansen K. & Pfister D.H. 2007. — A phylogenetic overview of the family *Pyronemataceae* (*Ascomycota*, *Pezizales*). *Mycological Research*, 111 (5): 549-571. doi: 10.1016/j.mycres.2007.03.014

Rehner S.A. & Buckley E. 2005. — A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, 97 (1): 84–98. doi: 10.3852/mycologia.97.1.84

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. — MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61 (3): 539–542. doi: 10.1093/sysbio/sys029

Stamatakis A. 2014. — RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30 (9): 1312–1313. doi: 10.1093/bioinformatics/btu033

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. — MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28 (10): 2731–2739. doi: 10.1093/molbev/msr121

Turland N., Wiersema J.H., Barrie F.R., Greuter W., Hawksworth D.L., Herendeen P.S., Knapp S., Kusber W.H., Li D.Z., Marhold K., May T.W., McNeill J., Monro A.M., Prado J., Price M.J. & Smith G.F. 2018. — *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code)* adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten, Koeltz Scientific Books, 254 pp.

Van Vooren N., Lindemann U., Vega M., Ribes M.A., Illescas T., Matočec N. & Kušan I. 2015a. — *Lachnea poiraultii* (*Pezizales*), rediscovered after more than one hundred years. *Ascomycete.org*, 7 (3): 105–116. doi: 10.25664/art-0133

Van Vooren N., Lindemann U. & Healy R. 2017. — Emendation of the genus *Tricharina* (*Pezizales*) based on phylogenetic, morphological and ecological data. *Ascomycete.org*, 9 (4): 101–123. doi: 10.25664/art-0204

Van Vooren N., Lindemann U. & Healy R. 2019. — Emendation of the genus *Tricharina* (*Pezizales*) based on phylogenetic, morphological and ecological data. Part 2. *Ascomycete.org*, 11 (5): 145–169. doi: 10.25664/art-0268

Van Vooren N., Tello S. & Vega M. 2015b. — *Pseudotricharina intermedia* (*Pezizales*), a new genus and a new species discovered in the Mediterranean area. *Ascomycete.org*, 7 (6): 341–346. doi: 10.25664/art-0158

Van Vooren N. & Vega M. 2018. — *Lasiocupulina mediterranea* (*Pezizales*), a new genus and species from Albania. *Ascomycete.org*, 10 (6): 221–227. doi: 10.25664/art-0246

Vilgalys R. & Hester M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172 (8): 4238-4246. doi: 10.1128/jb.172.8.4238-4246.1990

White T.J., Bruns T.D., Lee S. & Taylor J.W. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis M.A., Gelfand D.H., Sninsky J. & White T.J. (eds.): *PCR protocols: a guide to methods and applications*. Academic, San Diego, p. 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1

Yang C.S. & Korf R.P. 1985. — A monograph of the genus *Tricharina* and a new, segregate genus, *Wilcoxina* (*Pezizales*). *Mycotaxon*, 24: 467–531.

1: U. Lindemann – Pflügerstrasse 62, 12047 Berlin, Germany – uwe.lindemann0907@gmail.com

2: F.J. Valencia – C/Naranja, N.6, 29400 Ronda, Spain – kurroamanita@gmail.com

3: V. Kaounas – Sokratous 40, TK 19016 Artemis Attiki, Greece – bkaounas@gmail.com

4: V. Kummer – Universität Potsdam, Institut für Biochemie und Biologie, Maulbeerallee 1, 14469 Potsdam, Germany – kummer@uni-potsdam.de

5: N. Van Vooren – 13 chemin du Bois Ponard, 69160 Tassin-la-Demi-Lune, France – nicolas@vanvooren.info