Paratricharina multiguttulata sp. nov. – A new species of the *Tricharina*-group with subspherical spores

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Abstract: *Paratricharina multiguttulata* is described as a new species based on collections from Germany, Hungary and Norway. It is unique among the species of the *Tricharina*-group due to its subglobose ascospores. The morphological features are compared with the most closely related species *Paratricharina poiraultii*. A phylogenetic analysis based on 28 nrDNA, rpb2 and tef1 is presented.
Keywords: *Ascomycota*, morphology, *Paratricharina*, *Pezizales*, *Pyronemataceae*, taxonomy.

Zusammenfassung: Auf Basis dreier Aufsammlungen aus Deutschland, Ungarn und Norwegen wird *Paratricharina multiguttulata* als neue Art beschrieben. Aufgrund ihrer subglobosen Ascosporen ist die Art einzigartig unter den Taxa aus der Gruppe der Gattungen rund um *Tricharina*. Die morphologischen Merkmale werden mit der am nächsten verwandten Art *Paratricharina poiraultii* verglichen. Zudem wird eine phylogenetische Analyse auf der Grundlage von 28 nrDNA, rpb2 und tef1 durchgeführt.
Schlagworte: *Ascomycota*, Morphologie, *Paratricharina*, *Pezizales*, *Pyronemataceae*, Taxonomie.

Introduction

Several new species have been described in *Tricharina* Eckblad (syn. *Tricharia* Boud., *nom. illeg.*) and related genera in recent years. Supposedly forgotten species were brought back into awareness and described species, which for many years were only known from the type collections, could be confirmed and molecularly barcoded by newer collections (Kušan *et al.*, 2015; Lindemann, 2017; Lindemann & Böhning, 2016; Van Vooren & Vega, 2018; Van Vooren *et al.*, 2015a, 2015b, 2017, 2019). This was possible by the extensive collecting activities of field mycologists specialized in *Pezizales* who combined detailed morphological studies with modern molecular methods.

In July 2018, the second author found a small inconspicuous discomycete in central Thuringia (Germany), which could be macroscopically assigned to this group of tricharinoid fungi. Microscopically however, the ascospores were quite conspicuous and showed a similarity to those of the genus *Pulvinula* Boud.: subspherical to broadly ellipsoid, more or less thick-walled, filled with medium sized oil droplets.

In November 2019, the same species was found in Hungary by the third author, with a nearly spherical spore-shape. Further research revealed that the fourth author had already collected this species in Norway in 2014. The sequencing of the three finds confirmed that all collections shared the same ITS sequence.

A genus assignment was initially not possible based on morphological characters. Only molecular diagnostics revealed the generic affiliation. The molecular analyses indicate their affiliation with *Paratricharina* (Boud.) Van Vooren, U. Lindem., M. Vega, Ribes, Illescas & Matočec (Van Vooren *et al.*, 2015a), and subsequent morphological analyses support this placement.

The results of the morphological and phylogenetic analysis indicated that there is neither any taxon known which shares the features of the collected species, nor are there any matching sequences in public gene databases. Therefore, this species is here described as new to science.

Material and methods

Morphology. — The methods of observation are described in detail in Lindemann & Böhning (2016). The specimens were studied in fresh state and later from rehydrated exsiccates. All measurements were made in water in fresh state if not stated otherwise. The content of fresh spores has been characterised by an oil content index (OCI) as defined by Baral & Marson (2005).

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. Chromatograms were checked searching for putative reading errors, and these were corrected.

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

Phylogenetic analyses. — BLAST (Altschul *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC, Cochrane *et al.*, 2011) public database. Sequences came mainly from Hansen & Pfister (2006), Hansen *et al.* (2013), Van Vooren *et al.* (2017), Alvarado *et al.* (2018) and Lindemann *et al.* (2019). Sequences first were aligned in MEGA 5.0 (Tamura *et al.*, 2011) software with its Clustal W application and then corrected manually. The final alignment included 370/763 (28S, 84 sequences), 342/865 (tef1, 56 sequences) and 322/589 (rpb2, 55 sequences) variable/total sites. Aligned loci were loaded in MrBayes 3.2.6 (Ronquist *et al.*, 2012), where a Bayesian analysis was performed (three partitions: 28S/tef1-exons/rpb2-exons, GTR+G+I model, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.39 M generations, standard deviation having fallen below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis, 2006) using the standard search algorithm (data partitioned, GTRGAMMA model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Nomenclature. — The registration of the new species was done in the MycoBank Database.

Geodata. — The coordinates are given in decimal WGS84 format.

Phylogenetic analysis

The 3-gene phylogeny was mainly focused on the */Scutellinia-Trichophaea* lineages as delineated by Hansen *et al.* (2013). The analysed specimens are molecularly well supported as a new species. However, the new species clades together with *Paratricharina poiraultii*, but this is not supported by the analyses of our multi-

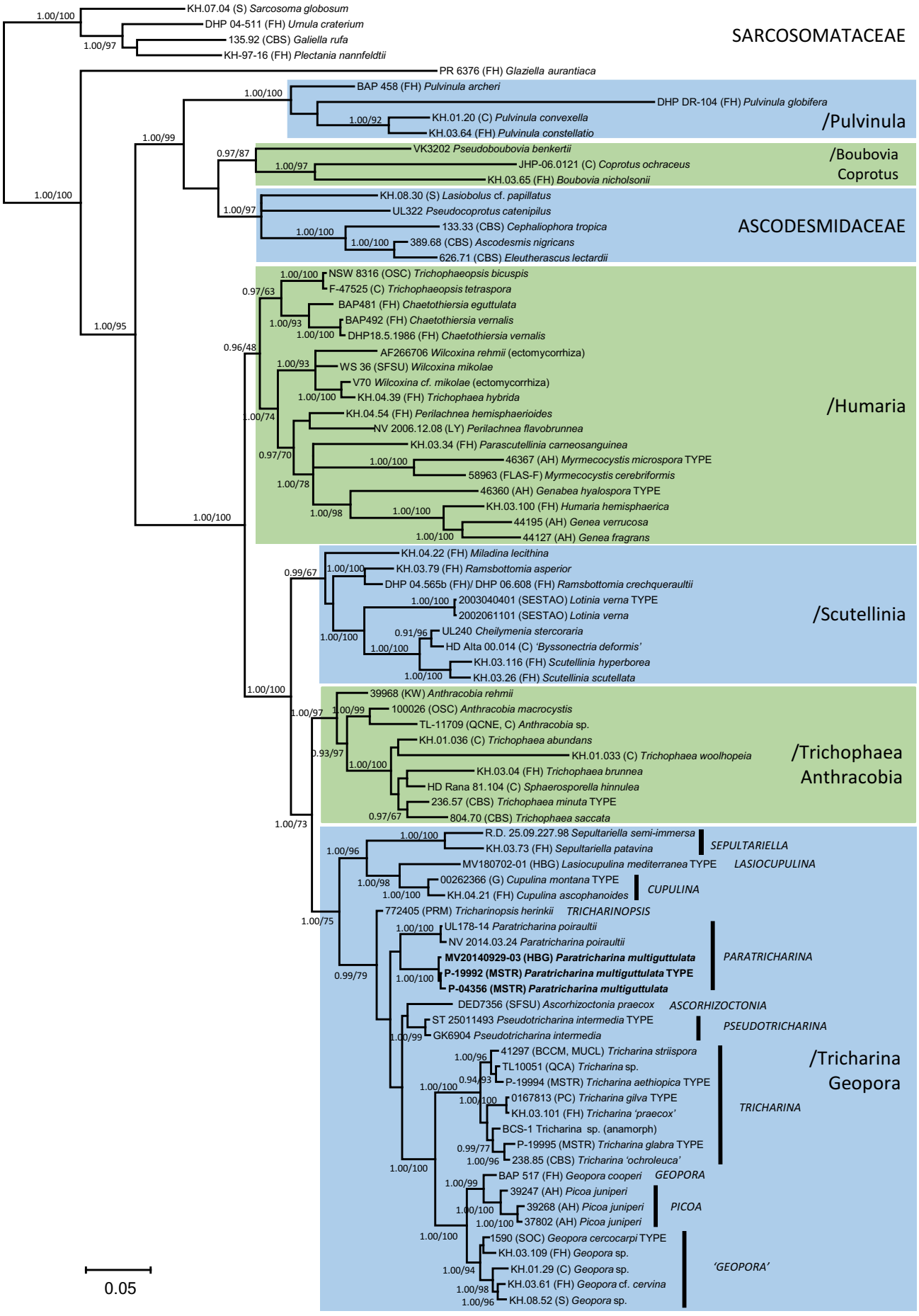

Fig.1 – 50% majority rule 28S rDNA-tef1-rpb2 consensus phylogram of selected clades in the family *Pyronemataceae* (*Pezizales*) and some closely related lineages (with family *Sarcosomataceae* as outgroup), obtained in MrBayes from 2925 sampled trees. Nodes were annotated if supported by ≥ 0.95 Bayesian PP (left) or ≥ 70% ML BP (right).

Table 1 – List of collections of *Paratricharina multiguttulata* sequenced for this study

Collection Number	Strain Number (ALVALAB)	Country	Collector	GenBank Accession Number			
				ITS	28S	rpb2	tef1
P-19992 (MSTR) – TYPE	ALV 17143	Germany	D. Wieschollek & J. Girwert	MW158772	MW158782	MW161232	MW161230
P-04356 (MSTR)	ALV 22768	Hungary	Z. Sochorová & M. Sochor	MW158773	MW158783	MW161233	MW161231
MV20140929-03 (HBG)	ALV 22769	Norway	M. Vega	MW158774	MW158784	–	–

Table 2 – Specimens included in the phylogenetic analysis (3-gene: 28S rDNA, RBP2, TEF1).
Taxon names on phylotree terminals reflect the phylogenetically supported name. Names changed from assignation in GenBank are designated with an asterisk. Names in apostrophes such as '*Byssonectria 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.

Species	Voucher ID	GenBank accession number		
		28S	rpb2	tef1
Anthracobia macrocystis	100026 (OSC)	AY544660	FJ238343	FJ238388
Anthracobia rehmii	39968 (KW)	KC201236	–	–
Anthracobia sp.	TL-11079 (QCNE)	KC012664	JX943778	KC109219
Ascodesmis nigricans	389.68 (CBS)	DQ168335	JX943761	KC109221
*Ascorhizoctonia praecox**	DED 7356 (SFSU)	DQ220443	JX943790	KC109297
Boubovia nicholsonii	KH.03.65 (FH)	DQ220395	JX943755	KC109222
'Byssonectria deformis'	HD Alta 00.014 (C)	DQ220356	JX943795	KC109245
Cephaliophora tropica	133.33 (CBS)	KC012669	JX943763	KC109224
*Chaetothiersia eguttulata**	BAP 481 (FH)	DQ220402	JX943820	KC109267
Chaetothiersia vernalis	BAP 492 (FH)	DQ220403	–	–
Chaetothiersia vernalis	DHP18.5.1986 (FH)	DQ220401	–	–
Cheilymenia stercoraria	UL240	KX592807	–	–
Coprotus ochraceus	JHP-06.121 (C)	KC012673	JX943764	KC109229
*Cupulina ascophanoides**	KH.04.21 (FH)	DQ220399	JX943791	KC109277
Cupulina montana	00262366 (G)	KY364073	–	–
Eleutherascus lectardii	626.71 (CBS)	MH872042	EU360913	KC109230
Galiella rufa	135.92 (CBS)	FJ176869	FJ238352	FJ238401
Genabea hyalospora	46360 (AH)	MG019781	MG004577	–
Genea fragrans	44127 (AH)	KJ938735	–	KJ938962
Genea verrucosa	44195 (AH)	MZ127810	MZ153112	MZ153113
Geopora cercocarpi	1590 (SOC)	HQ283091	–	–
Geopora cf. cervina	KH.03.61 (FH)	DQ220344	JX943785	KC109235
Geopora cooperi	BAP 517 (FH)	KC012678	JX943787	KC109236
Geopora sp.	KH.03.109 (FH)	DQ220345	JX943786	KC109238
Geopora sp.	KH.01.29 (C)	DQ220338	JX943783	KC109237
Geopora sp.	KH.08.52	KC012679	JX943784	KC109239
Glaziella aurantiaca	PR 6376 (FH)	KC012681	JX943754	KC109242
Humaria hemisphaerica	KH.03.100 (FH)	–	JX943824	KC109244
Lasiobolus cf. *papillatus*	KH.08.30 (S)	KC012687	JX943758	KC109314
Lasiocupulina mediterranea	MV180702-01	MN386008	–	–
Lotinia verna	2003040401 (SESTAO)	KP195729	–	KP195727
Lotinia verna	2002061101 (SESTAO)	KP195728	–	KP195726
Miladina lecithina	KH.04.22 (FH)	DQ220372	JX943793	KC109255
Myrmecocystis cerebriformis	58963 (FLAS-F)	MG019803	–	MG004568
Myrmecocystis microspora	46367 (AH)	MG019812	–	MG004575
Parascutellinia carneosanguinea	KH.03.34 (FH)	DQ220388	JX943823	KC109265
Paratricharina poiraultii	UL 178-14	KP052789	KP052791	KP052790
Paratricharina poiraultii	NV 2014.03.24 (LY)	KP052785	KP052787	KP052786
*Perilachnea hemisphaerioides**	KH.04.54 (FH)	KC012710	JX943821	KC109303
*Perilachnea flavobrunnea**	NV 2006.12.08 (LY)	KY024698	–	–
Picoa juniperi	39247 (AH)	JN392186	KT350971	–

Table 2 – (continued)

Species	Voucher ID	GenBank accession number		
		28S	rpb2	tef1
Picoa juniperi	39268 (AH)	JN392194	KT350978	–
Picoa juniperi	37802 (AH)	JN392192	–	–
Plectania nannfeldtii	KH-97-16 (FH)	AY945853	DQ017592	KC109214
Pseudoboubovia benkertii	VK3202	KP309872	KP309877	–
Pseudocoprotus catenipilus	UL322	MH846258	MH844626	–
Pseudotricharina intermedia	ST 25011493	KT861360	KT861364	KT861362
Pseudotricharina intermedia	GK6904	KT861361	KT861365	KT861363
Pulvinula archeri	BAP458 (FH)	DQ220392	JX943771	KC109270
Pulvinula constellatio	KH.03.64 (FH)	DQ062987	JX943773	KC109271
Pulvinula convexella	KH.01.020 (C)	DQ062986	JX943772	KC109272
Pulvinula globifera	DHP DR-104 (FH)	DQ220393	JX943774	KC109274
Ramsbottomia asperior	KH.03.79 (FH)	DQ220408	JX943796	KC109278
Ramsbottomia crechqueraultii	DHP 04.565b (FH) / DHP 06.608 (FH)	KC012698	JX943692	KC109279
Sarcosoma globosum	KH.07.04 (S)	–	JX943753	KC109215
Scutellinia hyperborea	KH.03.116 (FH)	KC012702	JX943801	KC109284
Scutellinia scutellata	KH.03.26 (FH)	KC012703	JX943800	KC109285
*Sepultariella patavina**	KH.03.73 (FH)	DQ220396	–	KC109275
Sepultariella semi-immersa	R.D. 25.09.227.98 (G)	KY364079	–	–
Sphaerosporella hinnulea	HD Rana 81.104	DQ220431	–	–
Tricharina aethiopica	P-19994 (MSTR)	NG_068790	–	–
Tricharina gilva	0167813 (PC)	KY364057	–	–
Tricharina glabra	P-19995 (MSTR)	NG_060015	–	–
Tricharina 'ochroleuca'	238.85 (CBS)	JQ836561	–	–
Tricharina 'praecox'	KH.03.101	DQ646525	JX943788	KC109298
Tricharina sp.	TL-10051 (QCA)	DQ220447	JX943789	KC109299
Tricharina sp.* (anamorph)	BCS-1	JQ836563	JQ836565	–
Tricharina striispora	41297 (MUCL)	JQ836560	JQ836564	–
Tricharinopsis herinkii	772405 (PRM)	MN386012	–	–
Trichophaea abundans	KH.01.036 (C)	DQ220449	JX943780	KC109300
*Trichophaea brunnea**	KH.03.04 (FH)	DQ220433	JX943779	KC109302
Trichophaea hybrida	KH.04.39 (FH)	DQ220454	JX943822	KC109304
Trichophaea minuta	236.57 (CBS)	DQ220452	JX943781	KC109305
Trichophaea saccata	804.70 (CBS)	DQ220451	JX943782	KC109306
Trichophaea woolhopeia	KH.01.033 (C)	DQ220460	–	KC109307
Trichophaeopsis bicuspis	NSW 8316 (OSC)	DQ220461	–	–
Trichophaeopsis tetraspora	F-47525 (C)	DQ220463	–	–
Urnula craterium	DHP 04-511 (FH)	AY945851	DQ017595	KC109216
Wilcoxina cf. *mikolae* (ectomycorrhiza)	V70	AF430285	–	–
Wilcoxina mikolae	WS 36 (SFSU)	DQ220468	–	–
Wilcoxina rehmii (ectomycorrhiza)	AF266706	AF266706	–	–

locus dataset. The deeper level node that includes *Ascorhizoctonia* Chin S. Yang & Korf, *Geopora* Harkn., *Paratricharina*, *Pseudotricharina* Van Vooren, Tello & M. Vega, *Tricharina*, and *Tricharinopsis* U. Lindem., Van Vooren & Healy is moderately supported, and the *Cupulina* Dougoud, Van Vooren & M. Vega, *Lasiocupulina* Van Vooren & M. Vega, *Sepultariella* Van Vooren, U. Lindem. & Healy lineage is strongly supported as sister to these (Fig. 1).

Although the new species and *Paratricharina poiraultii* are not supported with a phylogenetic analysis of available molecular data, we prefer to conservatively ascribe the new species to *Paratricharina*, rather than to a new genus, because the morphology fits very well, and the molecular topology fits too. We think that the lack of statistical support may be due to missing intermediate taxa within *Paratricharina*, and hope that our study inspires others to search for more species in this small, poorly known genus.

Due to the hypervariability of the ITS rDNA region, we did not include ITS sequences in the phylogenetic analysis of the generic affiliation since the results would not be robust.

Taxonomy

Paratricharina multiguttulata U. Lindemann, Wieschollek, Sochorová & M. Vega, *sp. nov.* – MB 837788 – Pl. 1–4

Diagnosis: Differs from *Paratricharina poiraultii* by smaller apothecia, larger subspherical to broadly ellipsoid ascospores filled with small oil drops, a hairless margin and an inconspicuously hairy receptaculum and its genetic profile.

Holotype: Germany, Thuringia, southwest of Hayn (MTB 5032/434), 50.927685° N, 11.157536° E, elev. 410 m, on soil in mossy fairway over limestone in oak-hornbeam forest, 17 July 2018, *leg*. D. Wieschollek & J. Girwert, MSTR P-19992; GenBank: MW158772 (ITS); MW158782 (28S), MW161230 (tef1), MW 161232 (rpb2).

Etymology: The epithet refers to the multiguttulate ascospores.

Description

Macroscopical features: Apothecia 1–4.5 mm diam., brownish-ochre, sessile, first cupulate, later spread out, margin hairless but external surface somewhat granulated, towards the base with a hyaline hairy fluff.

Microscopical features: Subhymenium and **medullary excipulum** not clearly distinguished, of *textura intricata*, composed of irregular, slender, sometimes slightly inflated, septate hyphae, 2–13 µm wide. **Ectal excipulum** 170–210 µm thick at base, 75–170 µm at the flanks, of *textura globulosa-angularis*, composed of thin-walled hyaline cells, 13–44 × 10–35 µm. The outermost layer consists of hyaline to brown, thick-walled cells, walls up to 1 µm. **Margin** of *textura globulosa/angularis*, hairless, composed of hyaline cells. **Hairs** of the receptacular surface superficial, hyaline to light brown, thick-walled (wall 0.4–0.9 µm wide), septate, straight to flexuous, obtuse, short, up to 100 µm in length, 4–7 µm wide, arising from the globose cells of ectal excipulum. **Asci** cylindrical, unitunicate, operculate, inamyloid, 8-spored, 200–220 × 15–18 µm (rehydrated), tapered towards the base, base forked, arising from croziers. **Ascospores** uniseriate, subglobose to broadly ellipsoid, (17.7) 18.6–22.3 × 14.8–18.6 µm, X = 19.8 × 16.4 µm, Q = 1.1–1.4, Qm = 1.2 (n = 37; holotype coll.), smooth to finely verrucose (in the Hungarian collection; warts 0.3–0.9 µm broad, observed in CB), hyaline, thick-walled (0.8–1.2 µm), filled with many small to middle-sized oil drops which merge together to one big oil drop after dehydration; no ascospore sheath observed. **Paraphyses** cylindrical, straight, septate, hyaline, 3.5–4.3 µm wide, ± enlarged at the top, up to 7.5 µm, terminal cell 43–100 µm long, sometimes containing low refractive vacuoles, having tiny hyaline bodies close to the septa.

Additional specimens examined: Germany, Thuringia, southwest of Hayn (MTB 5032/434), 50.927685° N, 11.157536° E, elev. 410 m, single and very scattered on soil in mossy fairway over limestone in oak-hornbeam forest, 7 July 2019, *leg*. D. Wieschollek, MSTR P-25017 (topotype). Hungary, northeast of Sopron, 47.7021944° N, 16.6686944° E, elev. 115 m, on soil in a field, 2 November 2019, *leg*. Z. Sochorová and M. Sochor, MSTR P-04356. Norway, Oslo, Etterstadkroken 7 E, 59.908954° N, 10.808220° E, elev. 76 m, on soil between grass in a front yard, 29 September 2014, *leg*. M. Vega, HBG MV 20140929-03.

Discussion

The results of the phylogenetic analysis were quite surprising when it became clear that the new species is most likely to be assigned to *Paratricharina*, because the type species, *Paratricharina poiraultii*, seems to be quite different at first sight. A closer study, however, revealed numerous similarities (Table 3). Morphologically this taxon is justifiably assigned to *Paratricharina*.

Table 3 – Morphological comparison between *Paratricharina poiraultii* and *P. multiguttulata*

	P. poiraultii	***P. multiguttulata***
Apothecia		
- diameter	5–25 (40) mm	1–4.5 mm
Structure of the excipulum		
- medullary excipulum	*textura intricata*	*textura intricata*
- ectal excipulum	*textura prismatica/angularis* and *globulosa/angularis*	*textura globulosa/angularis*
- outermost layer of the ectal excipulum	*textura globulosa-subangularis*, consisting of brown thick-walled cells	*textura globulosa-subangularis*, consisting of brown thick-walled cells
Hairs		
- shape and colour	brown, thick-walled, septate, slightly pointed	hyaline to light brown, thick-walled, septate, slightly pointed
- arising from	globose cells of the *textura angularis*	small cells of the *textura angularis*, sometimes also globose cells (cf. plate 2 F, G and plate 3 D)
- rooting	no	no
Paraphyses		
- shape	cylindrical, not or only slightly enlarged at the apex	cylindrical, ± enlarged at the apex
- colour	hyaline	hyaline
- content	non-refractive vacuoles	non-refractive vacuoles
Ascospores		
- shape	ellipsoid to narrowly ellipsoid (Q = 1.4–2)	subglobose to broadly ellipsoid (Q = 1.1–1.4)
- size	(13.5) 15–19.8 (20.1) × 9–11 (11.3) µm	(17.7) 18.6–22.3 (25.7) × 14.8–18.6 (20) µm
- arrangment in asci	uniseriate	uniseriate
- colour	hyaline	hyaline
- thick-walled	yes	yes
- content	small polar oil drops (OCI = 2)	filled with many small to middle sized oil drops (OCI = 4–5)

Plate 1 – *Paratricharina multiguttulata*

A + D: MSTR P-19992 (holotype, Germany). B: MSTR P-25017 (topotype). C + E: MSTR P-04356 (paratype, Hungary). F: HBG MV20140929-03 (paratype, Norway). Photos by D. Wieschollek (A, B + D), C + E by Z. Sochorová, F by M. Vega.

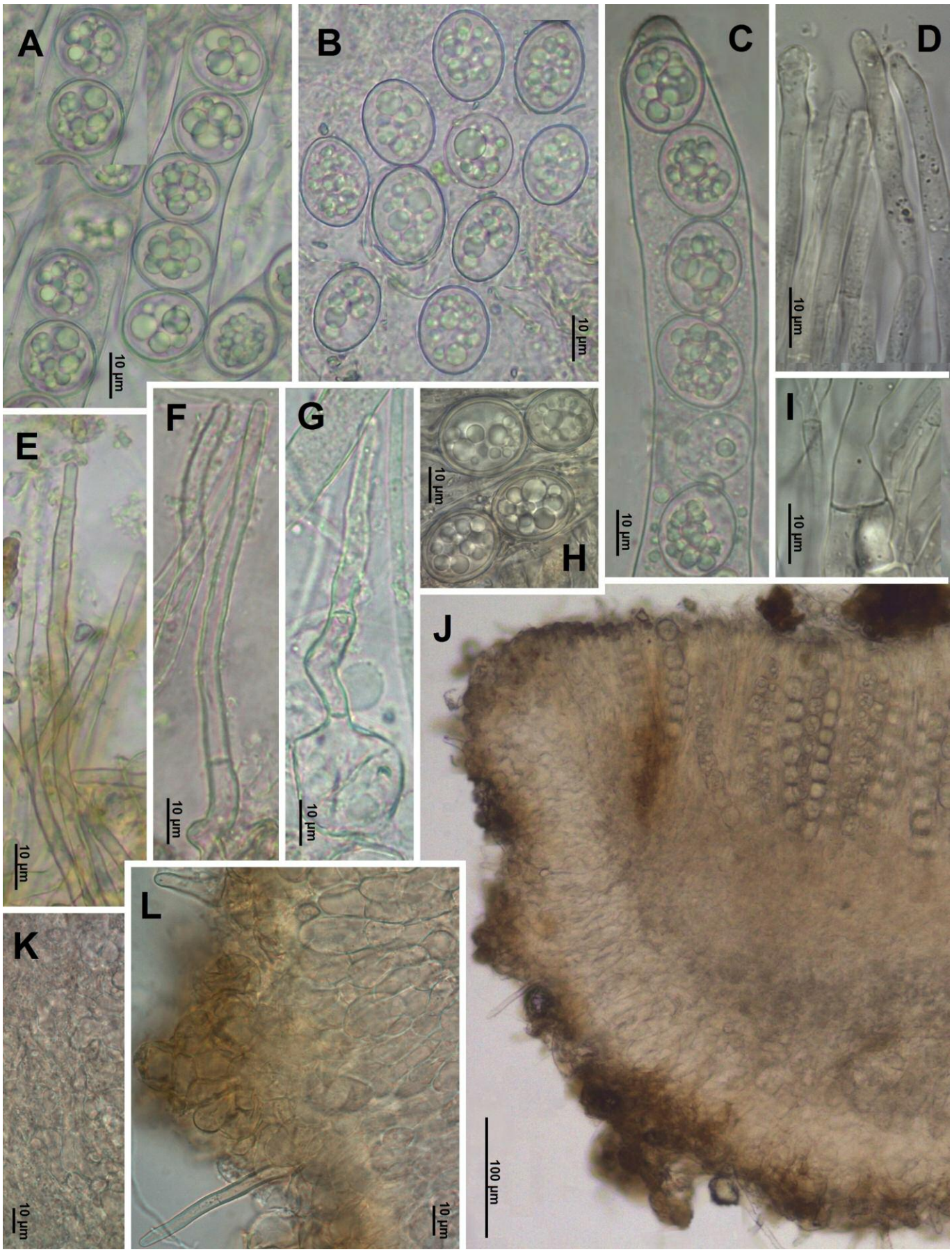

Plate 2 – *Paratricharina multiguttulata* (MSTR P-19992 [Holotype], MSTR P-25017 [Topotype])
A + B: Ascospores. C: Ascus. D: Paraphyses in KOH. E–G: Excipular hairs. H: Ascospores in KOH. I: Ascus base with crozier. J: Vertical cut. K: Medullary excipulum. L: Ectal excipulum with hairs. All pictures from the holotype, except of B (topotype). Photos A–C and E–G by D. Wieschollek, D and H–L by U. Lindemann.

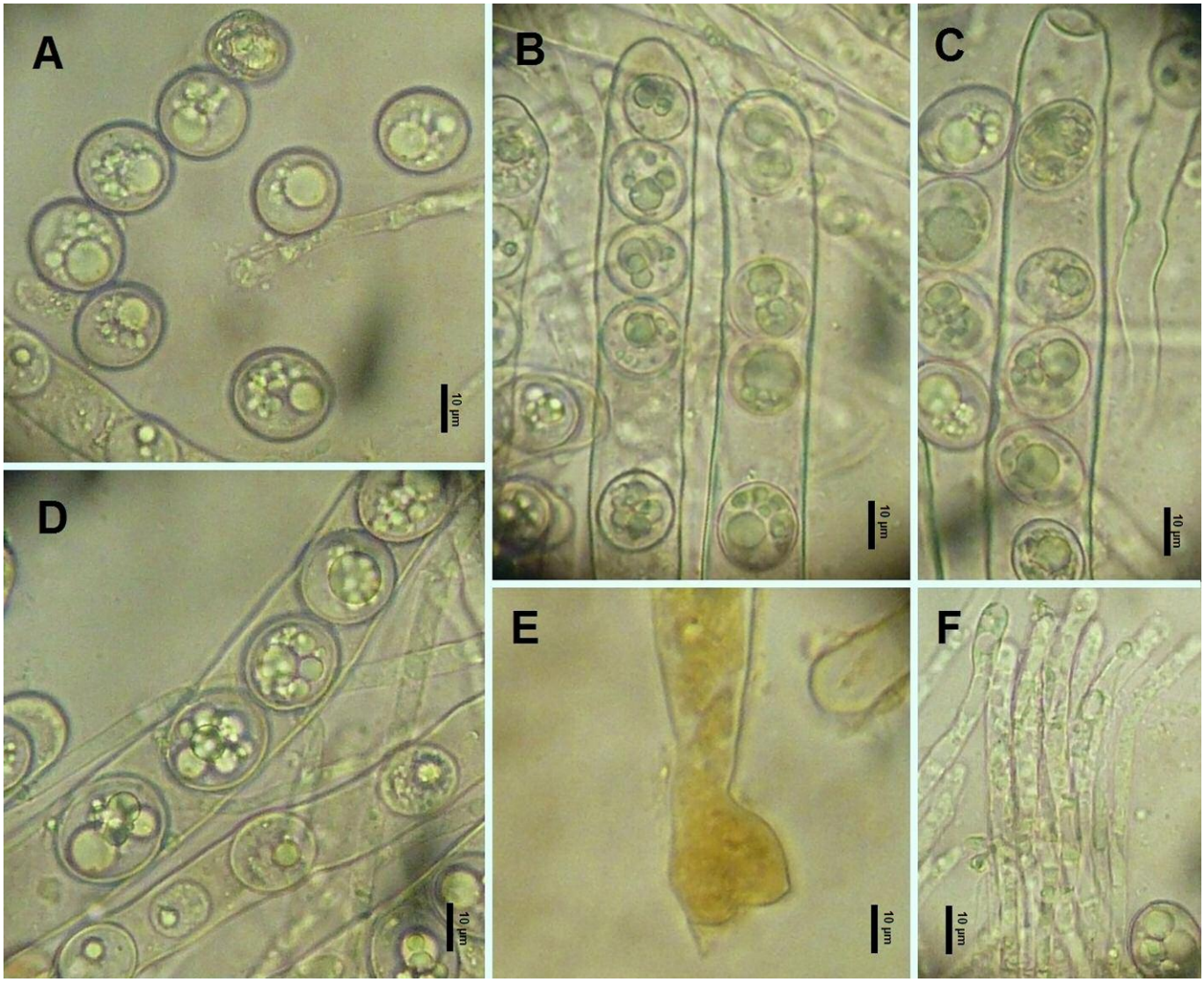

Plate 3 – *Paratricharina multiguttulata* (MV20140929-03)
A: Ascospores. B–C: Asci. D: Ascospores in an ascus. E: Ascus base in Lugol. F: Paraphyses. All photos by M. Vega.

Paratricharina multiguttulata is a distinct species, which is unlikely to be misidentified. The ascospores are reminiscent of species of *Pulvinula*, but these can be easily distinguished due to apothecia coloured in shades of orange, red, pink or white, curved paraphyses which often contain carotenoid pigments and the absence of hairs (Jaklitsch *et al.*, 2016).

Boubovia subprolata (Korf & W.Y. Zhuang) Y.J. Yao & Spooner, formerly also classified to *Pulvinula*, has subspherical ascospores like *P. multiguttulata* but they are much smaller in size (9.5–)10.2–12.4 (–13.2) × (8.8–)9.5–11 µm and lack lipid bodies. It differs also in other characters, e.g. pinkish apothecia without hairs or thinner asci and paraphyses (Korf & Zhuang, 1991).

Cupulina ascophanoides (Boud.) Van Vooren, which had been assigned to *Tricharina* for a long time, is somewhat similar to the new species as well. Not only do they share important features of the morphology (the structure of the excipulum, the paraphyses without VBs, the hyaline to slightly brown hairs originating from a large round excipular cell) but also their macroscopical habit is quite similar. However, *C. ascophanoides* can be easily distinguished by its ellipsoid ascospores with bipolar granules.

Tricharina subglobispora Svrček shares also some features with the new species, especially the subspherical ascospores, although they are distinctly smaller (Svrček, 1974). In their *Tricharina*-monograph, Yang & Korf (1985) re-examined the type collection and detected that it is a mixed collection containing *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička and *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud.

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Authors' contributions

Uwe Lindemann and Dirk Wieschollek were responsible for the study conception and design. They financially contributed to the generation of rDNA sequence. The morphological analyses were performed by the authors on their collections, the molecular analyses were done by Pablo Alvarado (ALVALAB). The first draft of the manuscript was written by Uwe Lindemann and subsequently up-

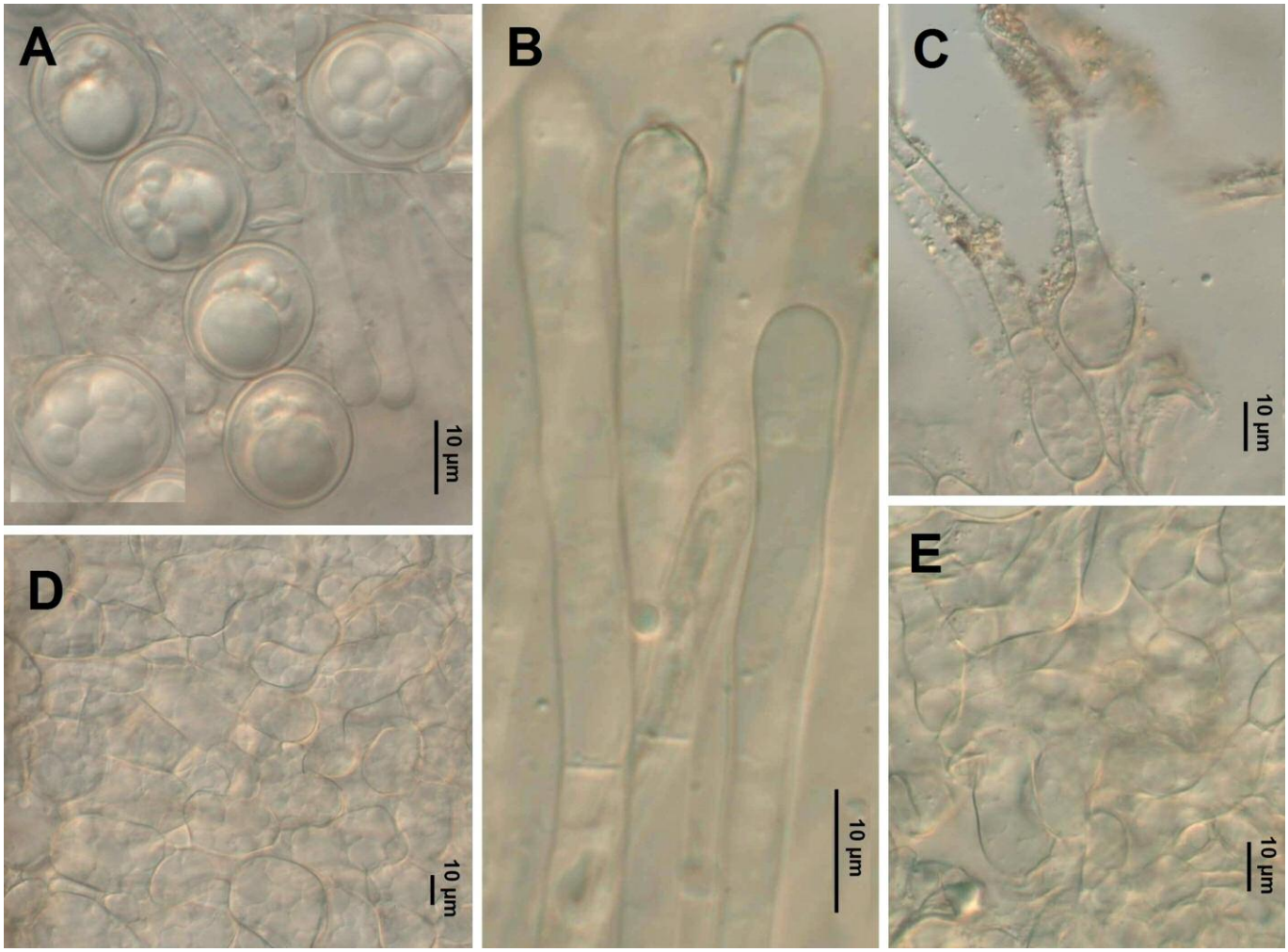

Plate 4 – *Paratricharina multiguttulata* (MSTR P-04356)
A: Ascospores. B: Paraphyses. C: Excipular hairs. D: Ectal excipulum. E: Medullary excipulum. All photos by Z. Sochorová.

dated by all authors. All plates have been designed by Uwe 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

Alvarado P., Healy R., Moreno G., Cabero J., Scholler M., Schneider A., Vizzini A., Kaounas V., Vidal J.M., Hensel G., Rubio E., Mujic A. & Smith M.E. 2018. — Phylogenetic studies in *Genabea*, *Myrmecocystis*, and related genera. *Mycologia*, 110 (2): 401–418. doi: 10.1080/00275514.2018.1451140

Baral H-O. & Marson G. 2005. — *In vivo veritas*. 3rd edition. DVD-ROM

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. *Molecular Phylogenetics and Evolution*, 67 (2): 311–335. doi: 10.1016/j.ympev.2013.01.014

Hansen K. & Pfister D.H. 2006. — Systematics of the *Pezizomycetes* – the operculate discomycetes. *Mycologia*, 98 (6): 1029–1040. doi: 10.1080/15572536.2006.11832631

Jaklitsch W., Baral H.-O., Lücking R. & Lumbsch H.T. 2016. — *Syllabus of Plant Families* - Adolf Engler's Syllabus der Pflanzenfamilien, 13th edition, Part 1/2 Ascomycota. Stuttgart, Borntraeger, 322 pp.

Korf R.P. & Zhuang W.Y. 1991. — A preliminary discomycete flora of Macaronesia: Part 16, *Otideaceae*, *Scutellinioideae*. *Mycotaxon*, 40: 79–106.

Kušan I., Matočec N., Mešić A. & Tkalčec Z. 2015. — *Tricharina tophiseda* – a new species from Croatia, with a revision of *T. japonica* (*Pyronemataceae*, *Pezizales*). *Phytotaxa*, 221 (1): 35–47. doi: 10.11646/phytotaxa.221.1.3

Lindemann U. 2017. — Beiträge zur Erforschung der Pilzflora Äthiopiens. Operculate Discomyceten, Teil 2: *Tricharina aethiopica* sp. nov. *Ascomycete.org*, 9 (3): 63–66. doi: 10.25664/art-0201

Lindemann U. & Böhning T. 2016. — *Tricharina glabra* (*Pezizales*) – eine neue Art in einer schwierigen Gattung. *Zeitschrift für Mykologie*, 82 (2): 449–458.

Lindemann U., Fellmann B. & Castillo J.A. 2019. — *Pseudocoprotus* gen. nov. – eine neue Gattung für *Cheilymenia catenipila* J. Moravec. *Ascomycete.org*, 11 (1): 17–24. doi: 10.25664/art-0253

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.2006.08.024

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

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

Svrček M. 1974. — New or less known Discomycetes. I. *Česká Mykologie*, 28 (3): 129–137.

Stamatakis A. 2006. — RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22 (21): 2688–2690. doi: 10.1093/bioinformatics/btl446

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

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

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., 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., 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

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*. New York, Academic Press: 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.


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