

Pseudotrifarina intermedia (Pezizales), a new genus and a new species discovered in the Mediterranean area

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Ascomycete.org, 7 (6) : 341–346.
Novembre 2015
Mise en ligne le 30/11/2015



Summary: Three collections of a cup-fungus found in the Mediterranean basin in three different localities (southern Spain, southern Italy and Crete) are presented as a new genus and a new species of *Pezizales*. Its morphology is similar to that of *Trifarina* species, but some of its microscopic characters, i.e. ascospores structure, disagree with the usual concept of this genus. Morphological study and phylogenetic analysis have been conducted to determine the correct position within the *Pyronemataceae*.

Keywords: *Pyronemataceae*, *Geopora* clade, *Trifarina*, *Trichophaea*, Mediterranean mycobiota, taxonomy, phylogeny.

Résumé : Trois récoltes d'un discomycète trouvé dans le bassin méditerranéen dans trois localités différentes (sud de l'Espagne, sud de l'Italie et Crète) sont présentées comme nouveau genre et nouvelle espèce de *Pezizales*. Sa morphologie est similaire à celle d'espèces du genre *Trifarina*, mais certains de ses caractères microscopiques, c'est-à-dire la structure des ascospores, ne concordent pas avec le concept habituel de ce genre. Une étude morphologique et une analyse phylogénétique ont été réalisées pour déterminer la position exacte dans les *Pyronemataceae*.

Mots-clés : *Pyronemataceae*, clade *Geopora*, *Trifarina*, *Trichophaea*, fonge méditerranéenne, taxinomie, phylogénie.

Introduction

The richness of mycobiota from the Mediterranean basin is probably underestimated, due to different factors, e.g. climatic conditions that induce erratic growths, adoption of unsuitable names of fungi known from Northern Europe, etc. The small discomycetes make no exception to this fact. DONADINI (1976) was one of the first authors to propose an important catalogue of operculate discomycetes from the French area of Provence, providing data on specific Mediterranean taxa. He described several new species from this region and (re)activated the interest of studying such fungi in the Mediterranean basin. Since this period, numerous publications enriched this catalogue. Recently the first author had the opportunity to conduct a study on the cup-fungus, *Paratrifarina poiraultii* (Boud.) Van Vooren, U. Lindemann, M. Vega, Ribes, Illescas & Matočec (VAN VOOREN *et al.*, 2015), a "lost" species which perfectly illustrates the interest in prospecting this area. Herewith, we present the new species *Pseudotrifarina intermedia* extending the list of fungi specific of the Mediterranean mycobiota. Having been found in southern Italy and Crete (Greece), it has recently been discovered in the south of Spain.

Material and methods

Morphology, cytology and cytochemistry. — The observations were made on fresh and dried material using the methods of "vital taxonomy" (BARAL, 1992). For dried samples, some small pieces of specimen were rehydrated for about twelve hours in tap water. The following mounts were used to observe microscopic characters: tap water, Cotton Blue (CB) both in lactophenol and lactic acid, and Lugol's solution (IKI) to test the amyloid reaction. An acetocarmine solution was used for staining the nuclei. Measurements were made on ≥ 30 ascospores from each collection mounted in water, under the 100 \times oil immersion lens of transmission light microscopes, excluding the ornamentation. X represents the average value of spore dimensions, and Q the ratio between spore length and width, the value in italics represents the average value of this ratio. Macrographs were made in situ using digital cameras, while micrographs were taken using digital cameras mounted directly on microscopes. Line drawings were made freehand to scale.

Abbreviations: * = from fresh material, \diamond = from rehydrated material.

DNA extraction, amplification and sequencing. — DNA was extracted using the same method as described in VAN VOOREN *et al.* (2015). Sequences generated during this study were deposited in Genbank under the accession numbers listed in Table 1.

Taxonomy

Description

Apothecia 3–6 mm in diam., sessile, first globose, more or less buried, then deeply cupuliform, becoming discoid at the end; hymenium greyish beige, nut-brown to pale orange or ochraceous; outside surface ochraceous or dull orange, rough or pustulate, with brownish warts, hairy near the margin; margin irregular, more or less tooth-like, darker than the hymenium, distinctly and densely hairy, with hairs organized in small clusters.

Medullary excipulum of *textura intricata*, with hyaline hyphae, 7–10 μm wide. **Ectal excipulum** of *textura subglobulosa* to *globulosa*, composed by hyaline cells up to 45 μm wide, mixed with some clavate cells; anchor hyphae emerging from the base of the apothecium. **External hairs** scattered, superficial, septate, 210–460 \times 7–9 μm , hyaline, obtuse or sharp, more or less flexuous, thick-walled, \times 1–1.2 μm , with a simple, bulbous base. **Marginal hairs** similar to external ones, 62–310 \times 9–20 μm , straight or flexuous, light-colored, pale yellowish brown. **Anchor hyphae** present, with a bulbous base, flexuous, septate, hyaline or light yellowish. **Asci** cylindrical, 165–195 \times 10–12 μm , operculate, arising from croziers, 8-spored, inamyloid. **Paraphyses** cylindrical, not exceeding the asci, not or rarely enlarged at the top, \times 4–6 μm , sometimes forked, hyaline, without vacuole bodies, but filled with low refractive, globose SCBs (soluble cytoplasmic bodies), more visible in Lugol's solution; nuclei do not stain in acetocarmine solution. **Ascospores** uniseriate, fusoid, sub-fusoid to amygdaliform, predominantly equilateral, *(20.2) 20.5–23 \times 10–12 μm [X = 21.8 \times 10.8 μm , Q=1.8–2.0–2.3], \diamond (20.5) 21–24 (25) \times 11–12.5 (13) μm [X = 22.5 \times 11.7 μm , Q=1.7–1.9–2.2], hyaline, relatively thick-walled, containing two oil drops, one bigger than the other, generally with only one large drop and sometimes with a De Barry bubble on rehydrated material, distinctly warted with rather dense, isolated, cyanophilous warts, more or less rounded, angular, sometimes a bit elongated, more rarely coalescent, up to 2 μm in



Plate 1 – *Pseudotrifarina intermedia*, macroscopic aspect

A, C. Collection from Spain, ST 25011493. B. Collection from Italy, A. Baglivo. D. Collection from Greece, GK 6904. Photos: A, C= S. Tello; B= A. Baglivo; D= G. Konstantinides.

diam. (face view) and up to 0.7 µm in height (side view), totally or partially dissolved in 5% KOH; overmature ascospores germinating.

Studied collections: SPAIN. Jaén, Martos, 490 m a.s.l., 37.70666° N, -4.048775° E, on naked soil, under *Olea europaea*, leg. S. Tello, 3 Jan. 2013, herb. ST 03011322. Jaén, Fuensanta de Martos, 755 m a.s.l., 37.6028° N, -3.913347° E, on naked soil, under *Olea europaea*, leg. S. Tello, 25 Jan. 2014, herb. ST 25011493 [**holotype**]; duplicata NV 2014.01.04. ITALY. Lecce, Lizzanello, on soil, leg. A. Baglivo, Apr. 2007, herb. AB (without number). GREECE. Crete, Zaros, 600 m a.s.l., 35.135884° N, 24.912479° E, on soil, on an agricultural road, among mosses and plants, leg. G. Konstantinides, 29 Dec. 2013, herb. GK 6904.

Discussion

Macroscopically, the young specimens of *Pseudotrifarina intermedia* may look like some species of the genus *Sepultaria* Boud.

(= *Geopora s. auct.*). They also share some microscopic characters such as thick-walled, guttulate ascospores, superficial hairs and a bilayered excipulum, but *Sepultaria* species are in most cases larger, possess smooth ascospores and brown hairs. Although *P. intermedia* falls in a clade including *Geopora sensu lato*, its branch is not nested in this genus. Regarding the microscopic features, this species presents some similarities with species of *Trichophaea* Boud. having ornamented ascospores: a bilayered excipulum, superficial hairs with a simple base, guttulate ascospores, and cyanophilic ornaments. However *Trichophaea* species possess brown and straight hairs, they are rarely deeply cupuliform and are whitish, pale grey to grey-coloured (for those with ornamented ascospores). To our knowledge, only *Trichophaea geoporoides* Korf & Zhuang (KORF & ZHUANG, 1985) presents cross characters between *Trichophaea sensu lato* and *Sepultaria*, but it differs from *Pseudotrifarina intermedia* by its whitish hymenium, the presence of incrustated hairs and smooth ascospores.

Species of the genus *Jafnea* Korf (KORF, 1960, emend. RIFAI, 1968) are deeply cup-shaped, hairy, possess fusoid and ornamented

Table 1 – Collections of *Pseudotrifarina intermedia* sequenced and used in the molecular phylogenetic study, with voucher information and GenBank accession numbers.

Species	Herbarium #	Collector	LSU	ITS	tef-1α	rpb2
<i>Pseudotrifarina intermedia</i>	ST 25011493	S. Tello	KT861360	KT861358	KT861362	KT861364
<i>Pseudotrifarina intermedia</i>	GK 6904	G. Konstantinides	KT861361	KT861359	KT861363	KT861365

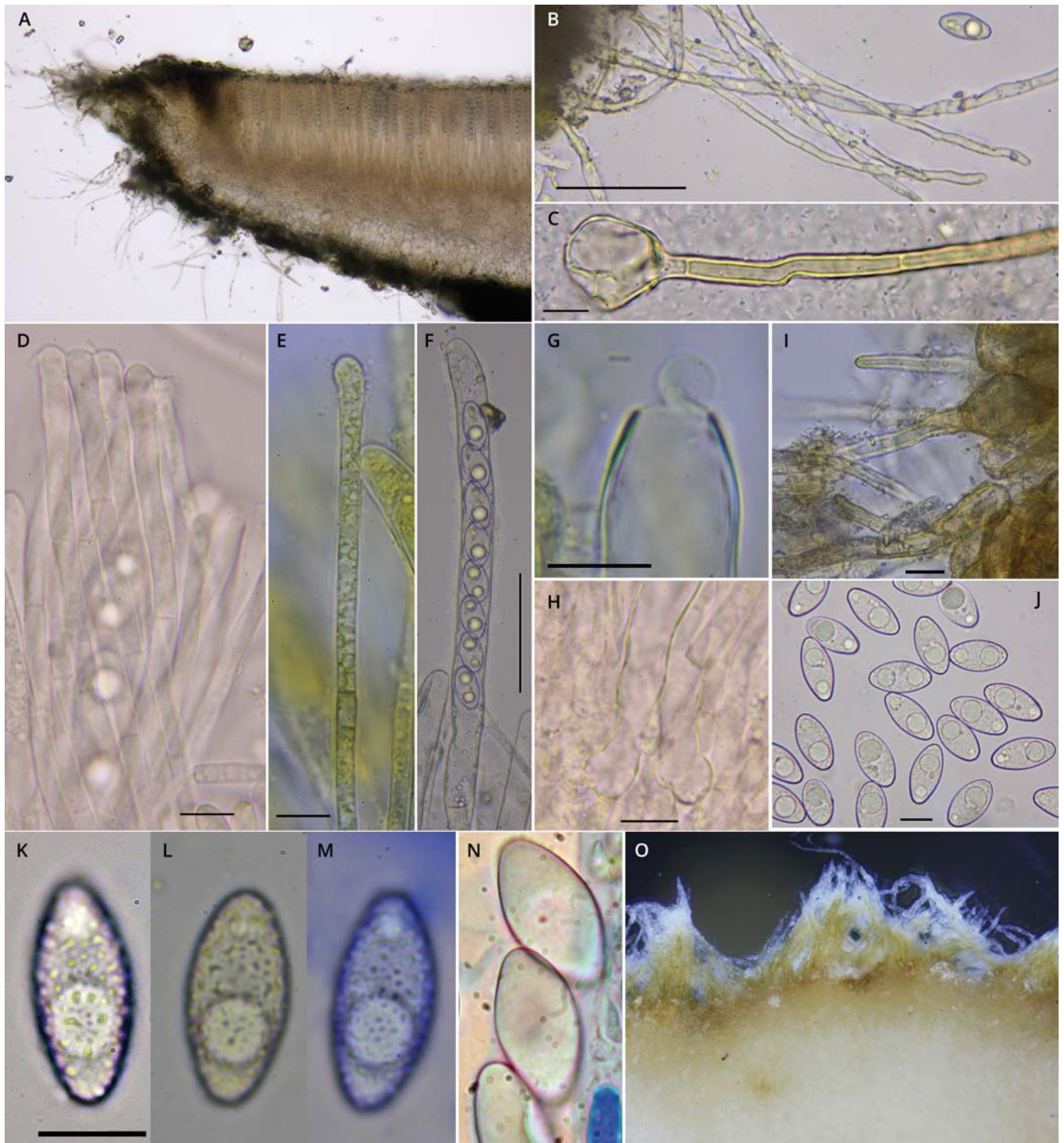


Plate 1 – *Pseudotrifarina intermedia*, microscopic features (all in tap water if no mention).

A. Vertical section of an apothecium. B. Anchor hyphae. C. Bulbous base of an anchor hypha. D. Top of paraphyses. E. Paraphyse in Lugol's solution showing the small SCBs. F. Ascus. G. Top of an ascus with the operculum. H. Bases of asci. I. Marginal hairs. J. Ascospores. K. Detail of spore ornamentation. L. Detail of spore ornamentation seen in IKI. M. Detail of spore ornamentation seen in CB. N. Ascospores in CB after a pretreatment in 5% KOH. O. Detail of margin showing clusters of hairs. Photos: S. Tello, except N by N. Van Vooren. Scale bars=10 μ m, except B, F and G=50 μ m.

ascospores, and have a bilayered excipulum but the external hairs are short, brownish, the ascospores are larger, and the nuclei of their cells are stained by acetocarmine.

Coloured species of *Leucoscypha* Boud. like *L. semi-immersa* (P. Karst.) Svrček can also be cited as similar cup-fungi. They also possess hairs with a bulbous base, fusoid ascospores, but the latter are smooth.

Finally, the morphology of this cup-fungus points to the genus *Trifarina* Eckblad in the sense of BOUDIER (1885, under *Tricharia*), ECKBLAD (1968), YANG & KORF (1985) and LINDEMANN (2013), and more particularly to *T. japonica* Chin S. Yang & Korf (YANG & KORF, 1985; DOUGOUD & DE MARCHI, 2012; KUŠAN *et al.*, 2015) which presents fusoid ascospores, but the latter has smooth, eguttulate¹ and thin-walled spores, less than 21 μ m in length. From a general point of view, the

¹ *Trifarina* species only present some small granules of low refractivity at their poles.

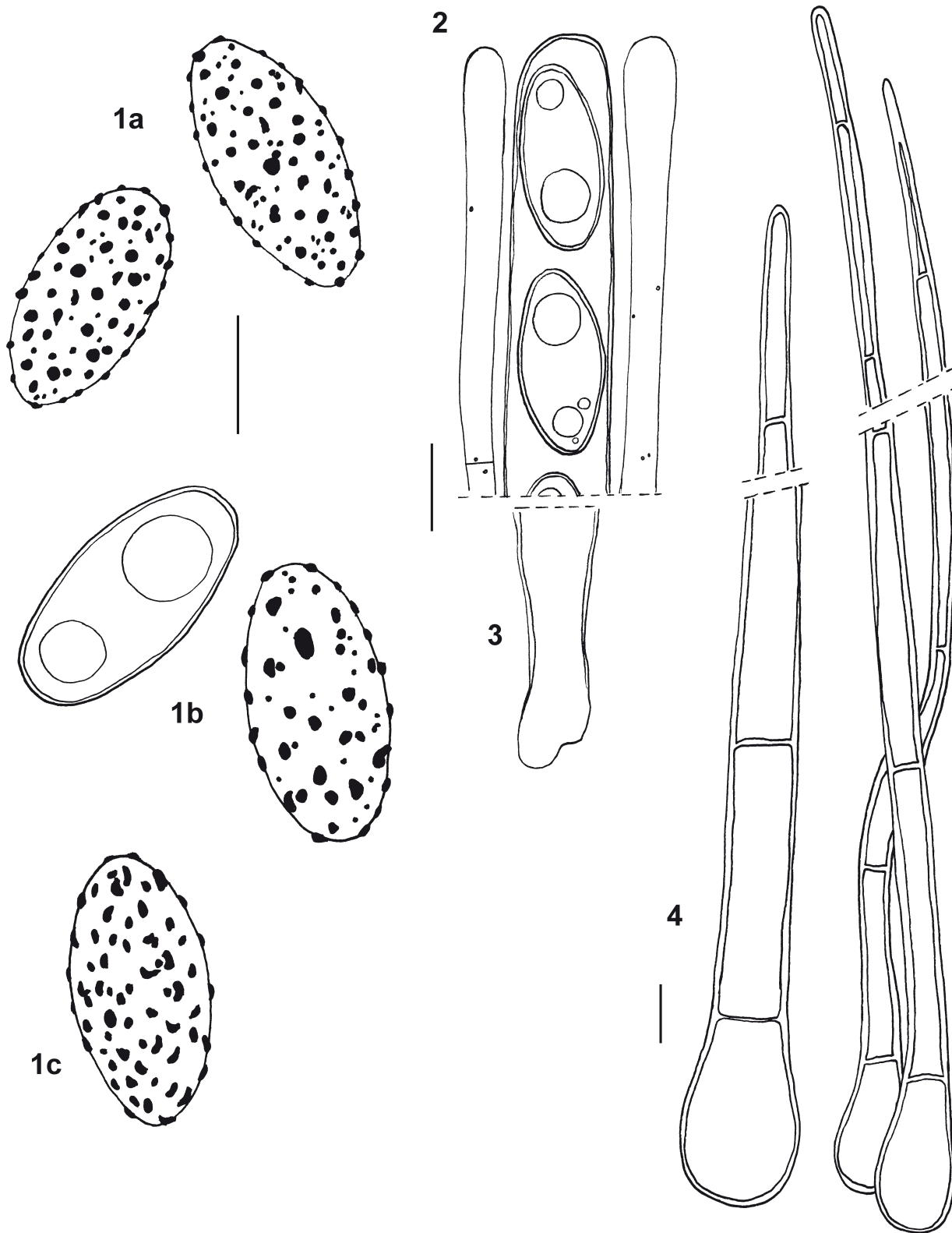


Plate 3 – *Pseudotrifarina intermedia*, microscopic features. Drawing: N. Van Vooren

1. Ascospores, a: coll. NV 2014.01.04, b: coll. Baglivo, c: coll. GK 6904. 2. Top of an ascus and paraphyses. 3. Ascus base. 4. Marginal hairs. Scale bars=10 μ m.

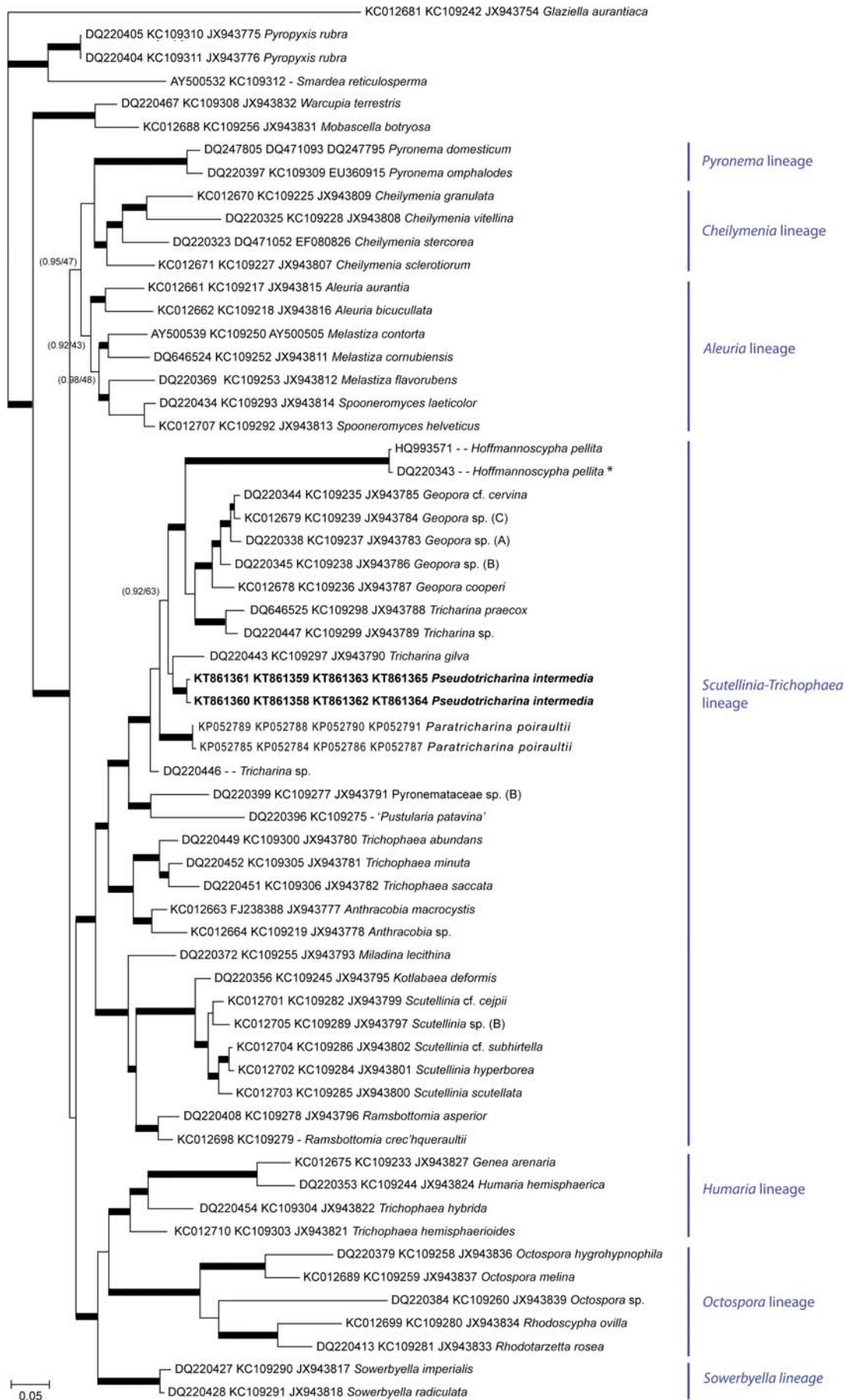


Plate 4 – Consensus 28S-*tef1-rpb2* phylogram of the family *Pyronemataceae* obtained in MrBayes 3.1 from 1,950 sampled trees. Nodes significantly supported by both Bayesian and ML analyses are highlighted with bold bars (0.95 PP, 65 BP). Nodes supported by just one of these inference methods are annotated with labels representing their actual posterior probabilities (left) and ML bootstrap proportions (right). (*) This sequence appears in GenBank as *Geopora pellita*.

presence of guttulate and thick-walled ascospores is in contradiction with the concept of *Tricharina* but, phylogenetically, the paraphyly of this genus (PERRY *et al.*, 2007; VAN VOOREN *et al.*, 2015) urges the mycologists to reconsider the taxonomic value of characters used to define that “genus”. In this context, both the microscopic characters of the fungus — in opposition to its tricharinoid aspect — and its phylogenetic position (Plate 4) led us to propose the new genus *Pseudotracharina*.

Pseudotracharina Van Vooren, Tello & M. Vega, *gen. nov.* — MB 814685

Diagnosis: Differs from the genus *Tricharina sensu* Yang & Korf by the ascospores containing large oil drops, and from *Trichophaea* species by its tricharinoid morphology, marginal hairs more or less flexuous and the rDNA analysis.

Type species: *Pseudotracharina intermedia* Van Vooren, Tello & M. Vega

Etymology: From Greek “*pseudos*”, meaning “lie” or “falsity”, because of the morphological similarity with the genus *Tricharina* contradicted by the microscopic characters.

Pseudotracharina intermedia Van Vooren, Tello & M. Vega, *sp. nov.* — MB 814686

Diagnosis: Differs from the known species of *Tricharina* by its guttulate and roughly warty ascospores, and from *Trichophaea* species by its tricharinoid general morphology. Holotype JA-CUSSTA 8285 deposited in JA herbarium (Consejería de Medio Ambiente y Ordenación del Territorio, Junta de Andalucía). Isotype in S. Tello’s herbarium.

Etymology: From Latin “*intermedi-*”, meaning intermediate, due to the cross characters between the genera *Tricharina sensu lato* and *Trichophaea*.



Plate 5 – Distribution map of *P. intermedia*

Acknowledgements

We acknowledge gratefully George Konstantinides (Greece) and Arturo Baglivo (Italy) for sharing their collections, data and photographs. We also thank Pablo Alvarado for the rDNA sequencing, analysis and Genbank deposit. Finally, Chris Johnson (UK) is thanked for the pre-submission review of the manuscript.

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