

# Exploring the European *Trichophaea*-like discomycetes (*Pezizales*) using morphological, ecological and molecular data.

## Part 3: New discoveries in *Perilachnea*

Nicolas VAN VOOREN  
Francisco Javier VALENCIA  
Matteo CARBONE  
Marcel VEGA

Ascomycete.org, 14 (1) : 7–17  
Mise en ligne le 28/02/2022  
doi 10.25664/ART-0342



**Abstract:** New morphological and phylogenetic studies conducted on new Spanish collections of *Trichophaea*-like discomycetes allowed the description and illustration of three new species of *Perilachnea*: *P. fallax*, very close to *P. flavobrunnea*, distinguished by its ascospore characters, *P. humarioides*, looking like *Humaria hemisphaerica* but with pluriguttulate and smooth ascospores, and *P. verrucispora*, characterised by its verrucose ascospores. The lectotypification of *P. hemisphaerioides* is also provided. An updated key to *Perilachnea* is provided.

**Keywords:** Ascomycota, Pyronemataceae, rDNA, phylogeny, taxonomy, 3 new species.

### Introduction

In the first part of our study on *Trichophaea*-like species, we proposed three new genera, including *Perilachnea* Van Vooren, mainly characterised by its deeply cupulate apothecia — with a *Humaria*-like appearance —, and microscopically by bi- or pluriguttulate ascospores, paraphyses containing small oil bodies, and asci with free croziers. All known species are considered as saprobic. During the publishing process of the first part, three new collections of species resembling *Perilachnea* were detected by one of us (FJV) in the area of Ronda (Andalusia, Southern Spain) that, for the time being, can be considered as a hotspot for this genus. Both morphological and molecular studies of these collections proved they represent three new species which are described and illustrated herein.

### Material and methods

**Morphological study.** — Methods are identical to those defined in the first part of this paper (VAN VOOREN *et al.*, 2021).

**DNA extraction, amplification and sequencing.** — Methods are identical to those defined in VAN VOOREN *et al.* (2021).

**Phylogenetic analyses.** — The new sequences were added in the dataset of *Perilachnea* species previously built (VAN VOOREN *et al.*, 2021). Sequences have been firstly aligned in MEGA X (KUMAR *et al.*, 2018) software with its MUSCLE application (EDGAR, 2004) and then corrected manually. Evolutionary analyses were conducted in MEGA X with 13 nucleotide sequences for the combination of ITS and LSU (1564 positions). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible (GTR) model (NEI & KUMAR, 2000) with 1000 bootstrap iterations. The ITS+LSU tree with the highest log likelihood (-5075.29) is shown. 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 was used to model evolutionary rate differences among sites.

The sequences generated during this study were deposited in GenBank and are listed in the Table 1.

### Molecular results

Our phylogeny confirms the monophyly of the genus *Perilachnea*, a result that was not fully obtained in VAN VOOREN *et al.* (2021) based on a LSU-tef1-rpb2 combination. The genus is divided into two well-supported subclades but we did not find any characters that would enable us to separate species of these two groups from a morphological point of view. However, we can note that *P. verrucispora* sp. nov. is a sister species of *P. hemisphaerioides* (Mouton) Van Vooren and similarly has biguttulate ascospores. Likewise, *P. fallax* sp. nov. which strongly resembles *P. flavobrunnea* (Richon) Van Vooren, M. Carbone & Valencia groups in the same clade (see Taxonomy chapter).

### Taxonomy

***Perilachnea fallax*** M. Carbone, Valencia, Tello & Van Vooren, sp. nov. – MB 839601 – Pl. 1–2

**Diagnosis:** Besides its different genetic profile, differs from *T. flavobrunnea* by ascospores with a more elongated shape, containing more homogeneous small lipid droplets, and a more heterogeneous ecology.

**Type:** coll. TUR-A 208921 (holotype).

Table 1 – Collections sequenced for this study

Name	Collection Ref.	Collector	Country	GenBank Accession numbers	
				ITS	LSU
<i>Perilachnea fallax</i>	TUR-A 208921	V. Avola	Italy	MW546593	MW546559
<i>Perilachnea fallax</i>	JA-CUSSTA 9583	F.J. Valencia	Spain	OM109673	OM109689
<i>Perilachnea fallax</i>	LY NV 2016.03.08	S. Tello	Spain	OM109672	OM109688
<i>Perilachnea humarioides</i>	JA-CUSSTA 9584	F.J. Valencia	Spain	OM109675	OM109691
<i>Perilachnea verrucispora</i>	JA-CUSSTA 9585	F.J. Valencia	Spain	OM109674	OM109690



**Fig. 1** – Phylogram of maximum likelihood (ML) of *Perilachnea* based on the combination of ITS and LSU regions, rooted with *Humaria hemisphaerica*. Nodes with  $\geq 70\%$  ML bootstrap support are annotated with their support values. Terminals that represent sequences obtained during this study are in bold.

**Etymology:** From Latin *fallax* meaning “misleading, deceptive”, because of its similarity with *P. flavobrunnea*.

**Ascomata** solitary or gregarious. **Apothecia** up to 12 mm diam., sessile, cupuliform, spreading with age, hymenium whitish to pearl-grey; external surface whitish to pale yellowish, covered with short brown hairs. **Margin** densely covered with dark brown hairs.

**Subhymenium** very thin, composed of small subglobular hyaline cells. **Medullary excipulum** ~110–120  $\mu\text{m}$  thick, of *textura intricata*, with hyaline hyphae, 3–7 (10)  $\mu\text{m}$  wide, mixed with some subglobose or clavate cells, 8–21  $\times$  7–17  $\mu\text{m}$ . **Ectal excipulum** ~200–220  $\mu\text{m}$  thick, of *textura globulosa/subangularis* with hyaline cells, 10–35  $\mu\text{m}$  diam. or (8) 13–43 (45)  $\times$  (8) 11–29 (33)  $\mu\text{m}$ , with a thick and refractive wall. **Marginal cells** made of globular or subglobular cells, 10–26  $\times$  9–19  $\mu\text{m}$ , clavate in the outermost part. **Excipular hairs** (38) 50–740  $\times$  5–9  $\mu\text{m}$ , superficial, brown, septate, 1–1.5  $\mu\text{m}$  thick-walled, obtuse or slightly sharp at the top, with a simple enlarged or bulbous base. **Marginal hairs** similar but longer and more densely set, 140–1300  $\times$  7–13  $\mu\text{m}$ , superficial, septate, straight or slightly flexuous, with a simple base, sharp at the top, 1.5–2  $\mu\text{m}$  thick-walled. **Anchoring hyphae** present, hyaline, 4–7  $\mu\text{m}$  wide. **Ascospores** uniseriate, ellipsoid but rather elongated to oblong with tapered ends, (16) 18–22.6 (25.2)  $\times$  (8.6) 9–11.4 (11.8)  $\mu\text{m}$ ,  $X = 21.1 \times 10.1 \mu\text{m}$ ,  $Q = 1.7–2.4$ ,  $Q_m = 2.1$ , hyaline, smooth, rather thick-walled (0.5–0.7  $\mu\text{m}$ ), containing several small lipid droplets in living state, sometimes with 2–3 drops larger than the others, up to 5  $\mu\text{m}$  diam., merging into a large single guttule in rehydrated material. **Asci** cylindrical, (260) 290–325 (330)  $\times$  (11) 13.5–18.5 (19)  $\mu\text{m}$ , arising from free croziers, operculate, 8-spored. **Paraphyses** filiform, hyaline, septate, straight, 2.5–4.5 (5)  $\mu\text{m}$  diam., not or slightly enlarged at the top, apical cell 19–51  $\times$  2.5–5  $\mu\text{m}$ , containing numerous small lipid bodies in the upper part, not staining in CRB.

line, septate, straight, 2.5–4.5 (5)  $\mu\text{m}$  diam., not or slightly enlarged at the top, apical cell 19–51  $\times$  2.5–5  $\mu\text{m}$ , containing numerous small lipid bodies in the upper part, not staining in CRB.

**Studied collections:** ITALY. Sicily, Siracusa, Buccheri, Bosco della Contessa, 37.109156° N 14.857673° E, 910 m a.s.l., on wood of *Cupressus sempervirens*, 18 Jan. 2020, leg. V. Avola, det. M. Carbone, herb. TUR-A 208921 (holotype); GenBank ITS MW546593, LSU MW546559, RPB2 MW544620, TEF1 MW544635. SPAIN. Málaga, Ronda, Ronda’s old fairgrounds, 36.74887° N 5.14996° W, 709 m a.s.l., on bark of dead twigs of *Cupressus sempervirens*, 26 Dec. 2020, leg. F.J. Valencia, pers. herb. CVL261220(1). Ronda, Jardines de la Escuela municipal de música y danza de Ronda, 36.752778° N 5.170306° W, 728 m a.s.l., in the litter of *Cedrus*, 15 Jan. 2021, leg. F.J. Valencia, herb. JA-CUSSTA 9583; GenBank ITS OM109673, LSU OM109689. Córdoba, Priego de Córdoba, near San Juan river, 37.5623° N 4.1637° W, 387 m a.s.l., in decaying litter of *Pinus halepensis*, 22 Mar. 2016, leg. S. Tello, det. N. Van Vooren, pers. herb. S.T. 22031601, duplicate LY NV 2016.03.08; GenBank ITS OM109672, LSU OM109688.

**Distribution:** Known from Southern Italy (Sicily) and Southern Spain (Andalusia).

**Comments:** In the comments about *Perilachnea flavobrunnea*, VAN VOOREN *et al.* (2021) highlighted a Sicilian collection found on dead wood of *Cupressus* (voucher TUR-A 208921), morphologically identical to this species but phylogenetically different. Two new collections, from various substrates, were studied and analysed. These Spanish collections, JA-CUSSTA 9583 on *Cedrus* litter and S.T.

**Table 2** – Comparison of ascospore dimensions between *P. flavobrunnea* and *P. fallax*

Species	Collection Ref.	Ascospores dimensions ( $\mu\text{m}$ )	Mean ( $\mu\text{m}$ )	Q ratio	Mean Q ratio
<i>P. flavobrunnea</i>	LY NV 2006.12.08	19–21 (21.5) $\times$ 9.5–10.5 (11)	19.9 $\times$ 10.0	1.8–2.1	2.0
<i>P. flavobrunnea</i>	CVL180121(2)	18.9–21.8 (24.1) $\times$ 10.2–11.6 (12.5)	20.4 $\times$ 10.9	1.7–2.0	1.9
<i>P. fallax</i>	CVL261220(1)	19.2–22.5 (25.2) $\times$ 9.8–11.4 (11.8)	21.0 $\times$ 10.6	1.8–2.2	2.0
<i>P. fallax</i>	JA-CUSSTA 9583	20.2–22.6 (23.8) $\times$ 10.1–11.2 (11.7)	21.4 $\times$ 10.7	1.8–2.1	2.0
<i>P. fallax</i>	LY NV 2021.01.03	18–20 (21) $\times$ 9–10	19.1 $\times$ 9.4	1.9–2.3	2.0
<i>P. fallax</i>	S.T. 22031601	19.2–22.5 (23.8) $\times$ 8.6–10 (11.1)	20.9 $\times$ 9.3	2.0–2.4	2.2
<i>P. fallax</i>	TUR-A 208921	(16) 17.8–20 (20.5) $\times$ (9) 9.4–11 (11.5)	19 $\times$ 10.2	1.7–2.0	1.9

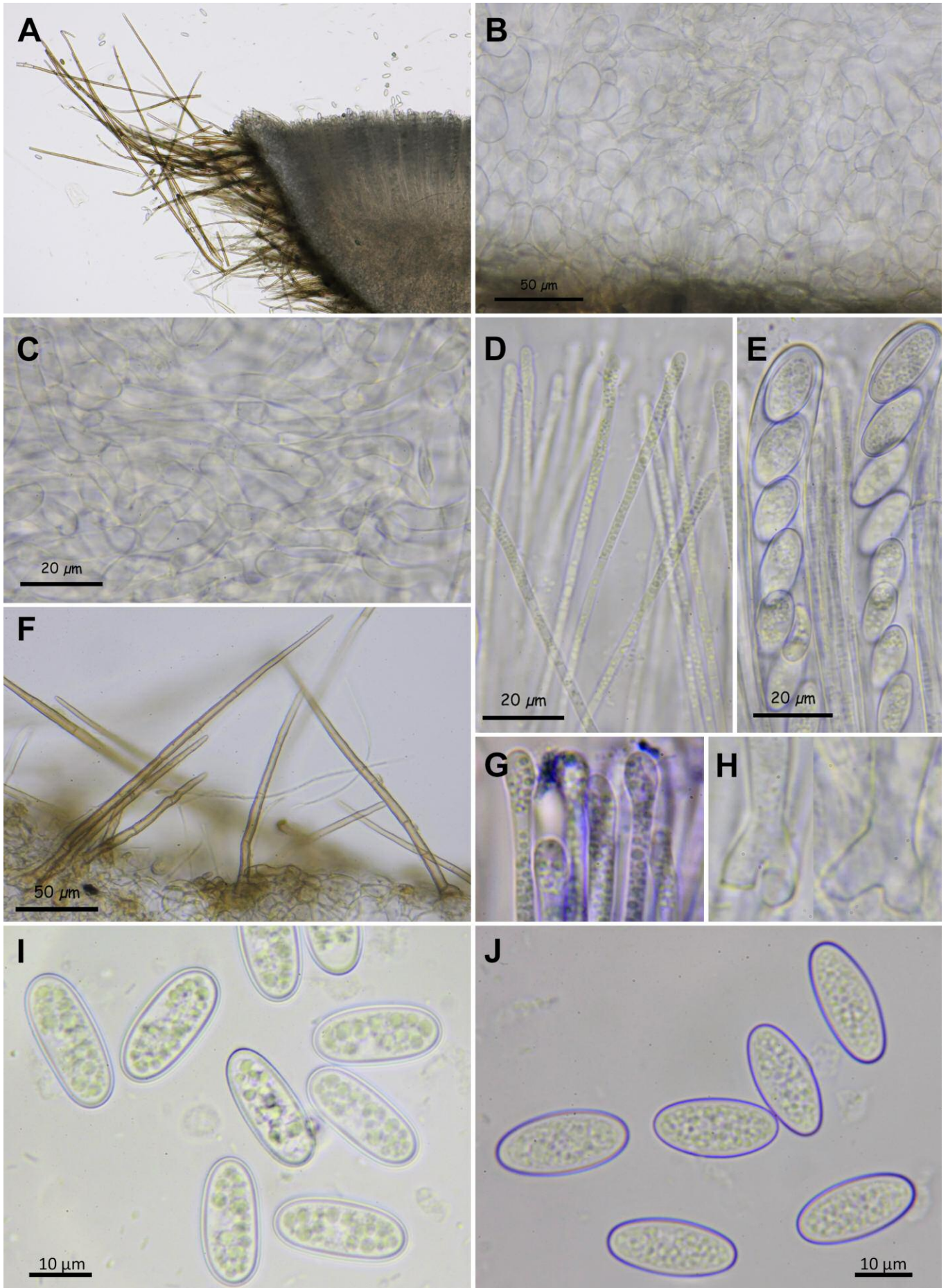




**Plate 1 – *Perilachnea fallax* (in situ)**

A–B. Coll. JA-CUSSTA 9583, photos F.J. Valencia. C. Coll. TUR-A 208921, photo V. Avola. D–E. Coll. ST 22031601, photos S. Tello.

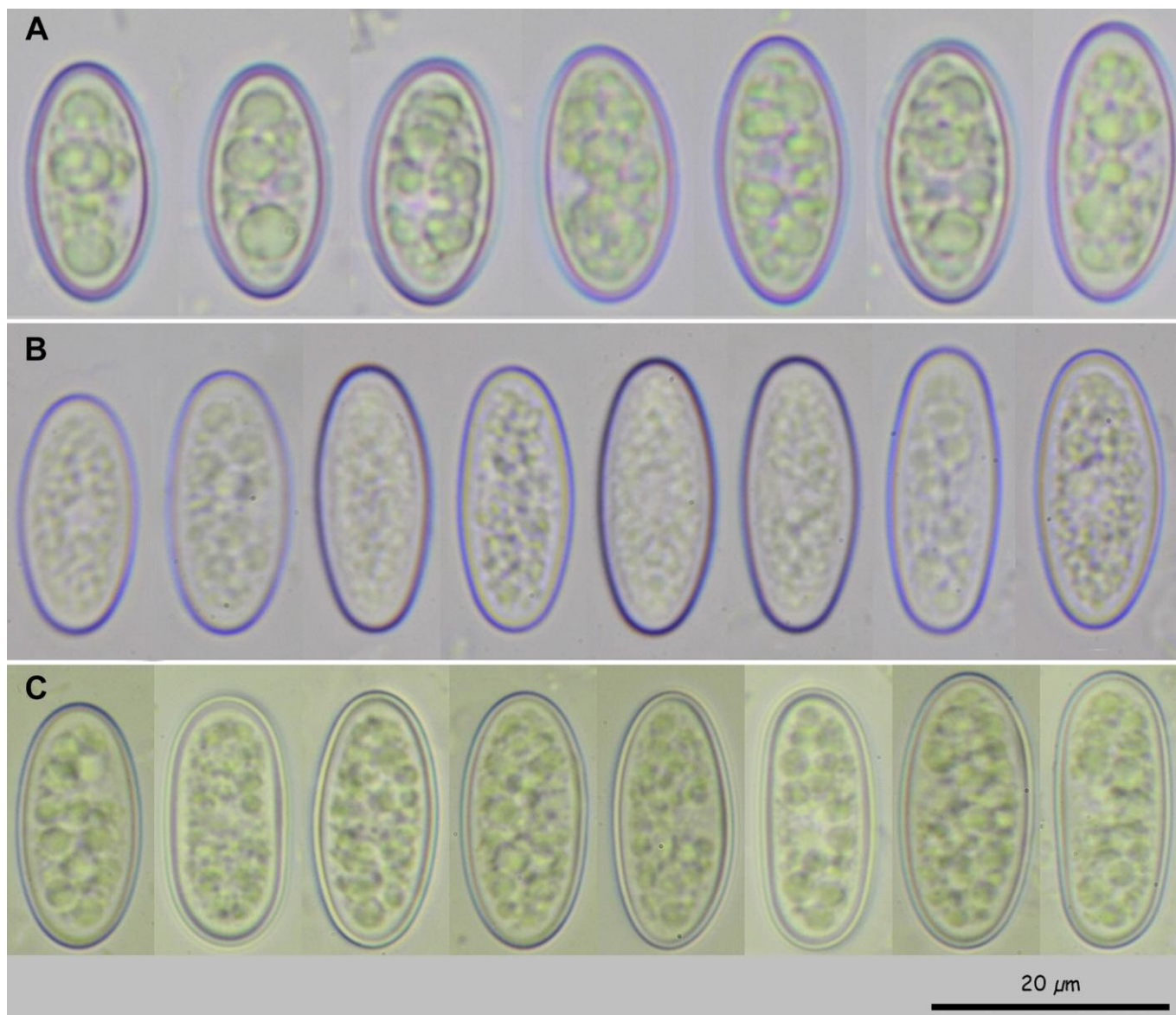




**Plate 2 – *Perilachnea fallax*. Microscopical characters**

A. Section of an apothecium. B. Ectal excipulum. C. Medullary excipulum. D. Paraphyses. E. Top of asci. F. Hairs. G. Top of paraphyses in aqueous Cresyl blue. H. Base of asci. I. Ascospores (coll. JA-CUSSTA 9583). J. Ascospores (coll. S.T. 22031601). All photos in water, except G. All photos by S. Tello, except I by F.J. Valencia.





**Fig. 2** – Sporogram comparing ascospores of *P. flavobrunnea* and *P. fallax*. A. *P. flavobrunnea*, coll. CVL140117(1). B. *P. fallax*, coll. S.T. 22031601. C. *P. fallax*, coll. JA-CUSSTA 9583.

22031601 on *Pinus halepensis* litter, were phylogenetically compared with the Italian one. Using the BLAST® algorithm (ALTSCHUL *et al.*, 1990), they both obtained 99.88% of identity with the LSU region, and 97.85% of similarity with the ITS region. Our ITS+LSU phylogeny (Fig. 1) confirms that the three collections group into a strongly supported clade.

Apart from a more diverse ecology, *P. fallax* mainly differs from *P. flavobrunnea* by its spore shape, often more elongated or oblong, although we can see that the Q ratio is variable in the studied collections (Table 2 and fig. 2). The ascospore content is also slightly different, with larger lipid bodies present in *P. flavobrunnea*. In any case, a careful examination of fresh material and of naturally-ejected mature ascospores is required to separate these two species, especially when both collections are growing on *Cupressus*.

***Perilachnea humarioides*** Valencia, M. Vega & Van Vooren, *sp. nov.*  
– MB 839602 – Pl. 3–4

**Diagnosis:** Differs from other *Perilachnea* species by its growth in association with woody debris of deciduous trees, and microscopically by smaller ascospores and Q ratio, in mean, than those of *P. flavobrunnea* and *P. fallax*.

**Type:** coll. JA-CUSSTA 9584 (holotype).

**Etymology:** *humarioides* because of its striking macroscopical resemblance with *Humaria hemisphaerica*.

**Ascomata** solitary or gregarious. **Apothecia** 7–17 mm diam., up to 8.5 mm high, sessile, deeply cupuliform, spreading with age, hymenium whitish, pearl-grey, yellowish cream at the end; external surface yellowish cream, covered with short appressed brownish to ochre hairs. **Margin** hairy, with chesnut to dark brown hairs, ± curved, organised in small pyramidal tufts.

**Subhymenium** and **medullary excipulum** not distinguished, thin, ~110–120 µm thick, of *textura intricata*, with hyaline hyphae, 3.5–7 µm wide, mixed with some inflated or clavate cells, 8–25.5 (28) × 7–20.5 µm. **Ectal excipulum** ~250–300 µm thick, of *textura globulosa/angularis* with hyaline cells, (6.5) 8–35 µm diam., having a thick refractive wall. **Marginal cells** comprising a *textura subprimastica*, hyaline, the terminal cell being clavate, 11.5–37 × 8.5–26.5 µm. **Marginal hairs** superficial, of two types: first, short, 30–140 (210) × 8–17 µm, light brown, straight, with a simple base, septate, obtuse at the top, often mixed with clavate cells; second, long, 260–1030 (1300) × 8–12 (16) µm, brown, straight or slightly flexuous, with a simple base, enlarged or bulbous up to 27 µm, septate, sharp at the top or sometimes roundish, 1–1.2 (2.6) µm thick-walled. **Excipular**

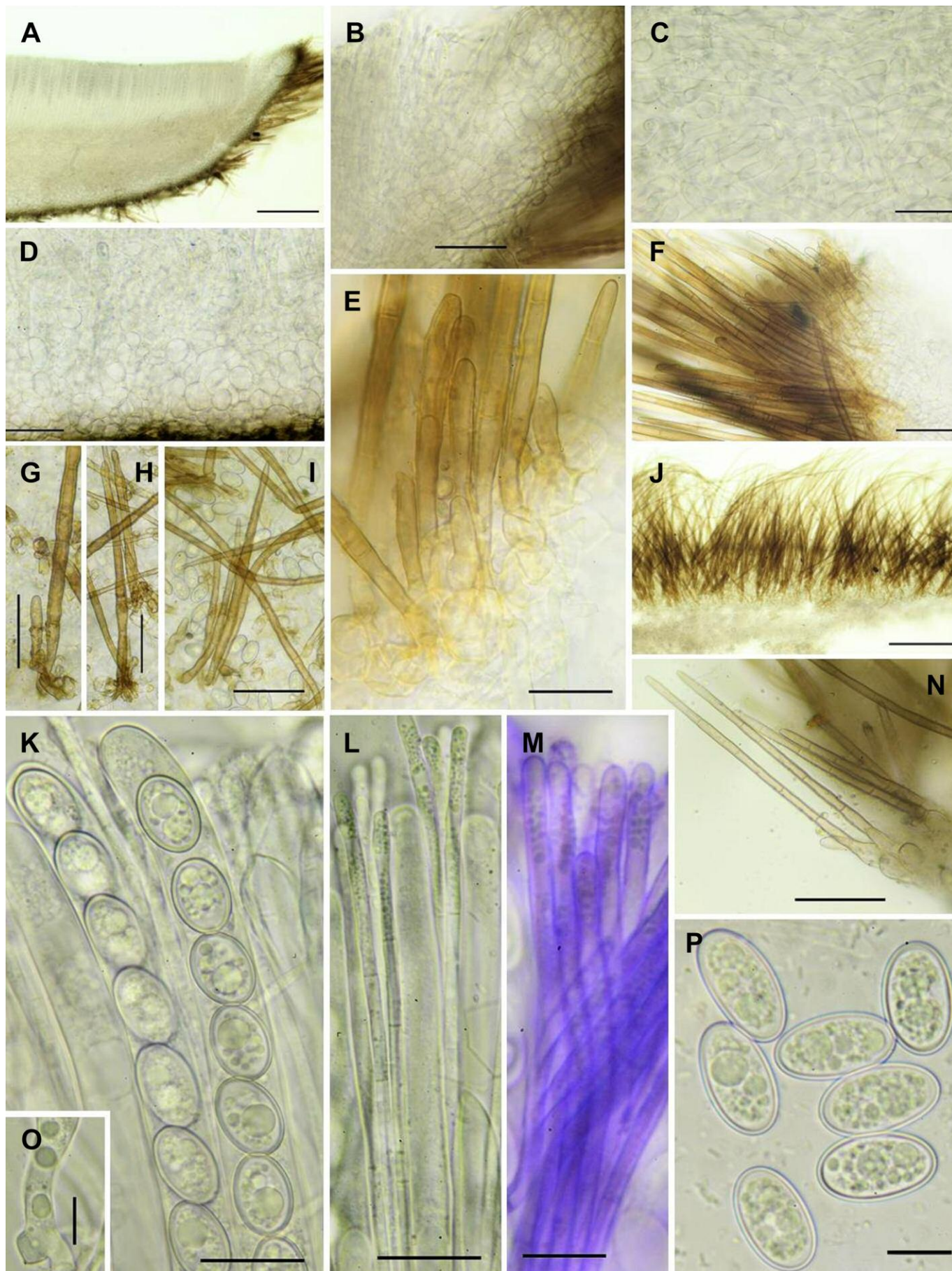




**Plate 3 – *Perilachnea humarioides***

A–E. Coll. JA-CUSSTA 9584: A, B, D–E: Different views *in situ*; C: Habitat. F. Coll. 4 Feb. 2018. All photos by F.J. Valencia.





**Plate 4 – *Perilachnea humarioides*.** Microscopical characters

A. Section of an apothecium. B. Marginal cells. Excipular hairs. C. Medullary excipulum. D. Ectal excipulum. E. Marginal hairs (close-up view). F. Short marginal hairs. G–I. Different types of excipular hairs. J. Marginal hairs. K. Asci. L. Paraphyses in water. M. Paraphyses in aqueous Cresyl blue. N. Marginal hairs. O. Ascus base. P. Ascospores in water. Scale bars: A, J = 200 µm, B, D, F–I = 50 µm, C, E, K, N = 20 µm, M, O, P = 10 µm. All photos by F.J. Valencia.



**hairs** superficial, similar to the short ones, not bulbous, 17.5–650 × 5–12 µm. **Anchoring hyphae** present, hyaline, 4–7 µm wide. **Ascospores** uniseriate, ellipsoid, (15.2) 16.5–19.3 (20) × (9.8) 10.1–11.2 (11.5) µm,  $X = 18.1 \times 10.7$  µm,  $Q (1.4) 1.7–1.9$ ,  $Q_m = 1.7$ , hyaline, smooth, thick-walled, containing several lipid droplets, with 2–3 drops larger than the others, up to 5 µm diam. **Asci** cylindrical, 250–285 (300) × (12) 15–18 µm, arising from free croziers, operculate, 8-spored. **Paraphyses** filiform, hyaline, septate, straight, 3–4 µm diam., not or slightly enlarged at the top, apical cell 30–60 × 4–5 µm, containing small lipid bodies up to 2 µm diam.

**Studied collections:** SPAIN. Málaga, Ronda, near the Club Polideportivo la Torrecilla, 36.754278° N 5.164167° W, 750 m a.s.l., in an abandoned plantation of *Olea europaea* and *Prunus dulcis*, on woody debris of *P. dulcis*, 26 Jan. 2021, *leg.* F.J. Valencia, herb. JA-CUSSTA 9584 (holotype), LY NV 2021.01.02 (isotype); GenBank ITS OM109675, LSU OM109691. Málaga, Ronda, Serranía de Ronda, near the river Setenil, 36.807357° N 5.209772° W, 675 m a.s.l., on dead wood (?*Salix* sp.), 4 Feb. 2018, *leg.* F.J. Valencia, only one apothecium (documented but not conserved).

**Distribution:** Known from Southern Spain (Andalusia).

**Comments:** Macroscopically, this species resembles *Humaria hemisphaerica* (F.H. Wigg.) Fuckel in having deeply cup-shaped apothecia and long marginal hairs. The examination of microscopical characters contradicted this resemblance, revealing smooth pluriguttulate ascospores vs. warted biguttulate ascospores in *H. hemisphaerica*. The molecular data confirmed its originality and positioned it in *Perilachnea*, a result in conformity with the characters of this genus.

Ecologically, *P. humarioides* appears to be the first species of the genus associated with woody debris of deciduous trees, with the exception of *P. hemisphaerioides* which is carbonicolous and can possibly be found on various types of burnt wood.

As one of the studied collections came from dead wood of possibly *Salix* sp., we compared this species with the description of *Trichophaea salicina* (Velen.) Svrček given by VELENOVSKÝ (1934) under *Lachnea salicina*. The latter had been reported having hairs measuring 500–600 × 25–30 µm and smooth subglobose ascospores, about 20 µm wide. This description does not fit with our species.

***Perilachnea verrucispora*** Valencia, M. Vega & Van Vooren, *sp. nov.* – MB 839603 – Pl. 5–6

**Diagnosis:** Differs from *Perilachnea hemisphaerioides* by its larger verrucose ascospores and its growth in the litter of *Cupressus* and other conifers, but not on burnt soil.

**Type:** coll. JA-CUSSTA 9585 (holotype).

**Etymology:** From Latin *verruca* meaning “wart” and ancient Greek σπορά (*spora*) meaning “seed(ing)” = spores, so “with warted spores”.

**Ascomata** solitary or in small groups. **Apothecia** 3–18 mm diam., up to 3 mm high, sessile, cupuliform, then spreading with age, hymenium greyish white to pearl-white; external surface whitish, but densely covered with short light brown hairs. **Margin** hairy, bearing brown to dark brown hairs, ± wavy.

**Subhymenium** and **medullary excipulum** not distinguished, of *textura intricata*, with hyaline hyphae, 3.5–10 µm wide, mixed with some inflated, clavate or subglobose cells, 12–25 × 11–19.5 µm.

**Ectal excipulum** of *textura angularis/subglobulosa* with hyaline cells, 16–72 × 12–50 µm, becoming light brown in the outermost part.

**Marginal cells** not distinguished from the ectal excipulum, consisting of globose or subglobose cells, 10.5–35 × 9.5–30 µm. **Marginal hairs** superficial, dense, (155) 164–560 (640) × (7.5) 7.9–18 (23.5) µm, light brown, straight, septate, with a simple base, sometimes enlarged, sharp at the top, up to 2.5 µm thick-walled. **Excipular hairs**

superficial, similar to the marginal ones but shorter, (77) 100–333 (390) × (7) 8–14 (19) µm. **Anchoring hyphae** present, long, up to 8 µm wide, pale yellowish, septate, with a non-bulbous base. **Ascospores** uniseriate, ellipsoid, (14.8) 15.5–17.4 (17.8) × (8.7) 8.9–9.8 (10) µm,  $X = 16.6 \times 9.4$  µm,  $Q (1.5) 1.6–1.9 (2.0)$ ,  $Q_m = 1.8$ , hyaline, rather thick-walled, containing two lipid drops, up to 4 µm diam., ornamented with fine isolated warts, 1–2 µm diam. **Asci** cylindrical, (250) 254–320 (325) × (9.1) 10–14.5 (15.5) µm, arising from free croziers, operculate, 8-spored. **Paraphyses** filiform, hyaline, septate, straight, 3–4 µm diam., not or slightly enlarged at the top, apical cell 19–58.5 × 3–5 µm, containing small lipid bodies, not staining in CRB; paraphyses near the margin can show some moniliform cells.

**Studied collection:** SPAIN. Málaga, Ronda, Jardines de la Escuela municipal de música y danza de Ronda, 36.752778° N 5.170417° W, 728 m a.s.l., in litter of *Cupressus*, 18 Jan. 2021, *leg.* F.J. Valencia, herb. JA-CUSSTA 9585; GenBank ITS OM109674, LSU OM109690.

**Distribution:** Known from Southern Spain (Andalusia) and maybe also Turkey (see comments).

**Comments:** *Perilachnea hemisphaerioides* (Mouton) Van Vooren is a well-known species, commonly reported from burnt soils and charcoals, described with smooth biguttulate ascospores (MOUTON, 1897; SVRČEK, 1949; GRADDON, 1960; DENNIS, 1978; VAN VOOREN, 2014), sometimes smooth or rough or finely verrucose (BREITENBACH & KRÄNZLIN, 1981; HUHTINEN, 1984; CETTO, 1989; DOUGOUD, 2001; PERIĆ, 2002; MEDARDI, 2006; BEUG *et al.*, 2014). The discovery of a species having biguttulate ascospores which are distinctly ornamented with small warts, growing in litter, was surprising. The molecular data confirm its originality. Thus ecological, morphological and molecular characters allow us to propose it as a new species.

Although we did not study the corresponding material, we believe that the Turkish collection named *Trichophaea hemisphaerioides* by DOĞAN & AKTAŞ (2010) could represent the same species we describe above, based on the ecology — litter of *Abies cilicica* — and ascospore size.

Among the *Trichophaea*-like species with biguttulate ascospores, we compared *P. verrucispora* with *Trichophaea hazslinskya* (Cooke) Boud. and *T. lecothecioides* (Rehm) Boud. Based on Cooke's original description under *Peziza* “*hazslinskia*”, the latter has an ochraceous hymenium, smooth ascospores measuring 15 × 8 µm and grows on soil (COOKE, 1879, fig. 401). Based on Rehm's original description under *Lachnea lecothecioides*, this discomycete has smooth ascospores measuring 12 × 9 µm, short marginal hairs, up to 90 µm long and grows on burnt soil (REHM, 1896). It could thus be more related to *T. abundans* (P. Karst.) Boud.

## Miscellanea

In VAN VOOREN *et al.* (2021), we failed to correctly designate the lectotype of *Perilachnea hemisphaerioides* under the rules of the Code (ICN Shenzhen).

The lectotype of *Lachnea hemisphaerioides* Mouton is here designated: BR5020088910584, in Meise Botanic Garden (BR) herbarium; MBT 10004926.

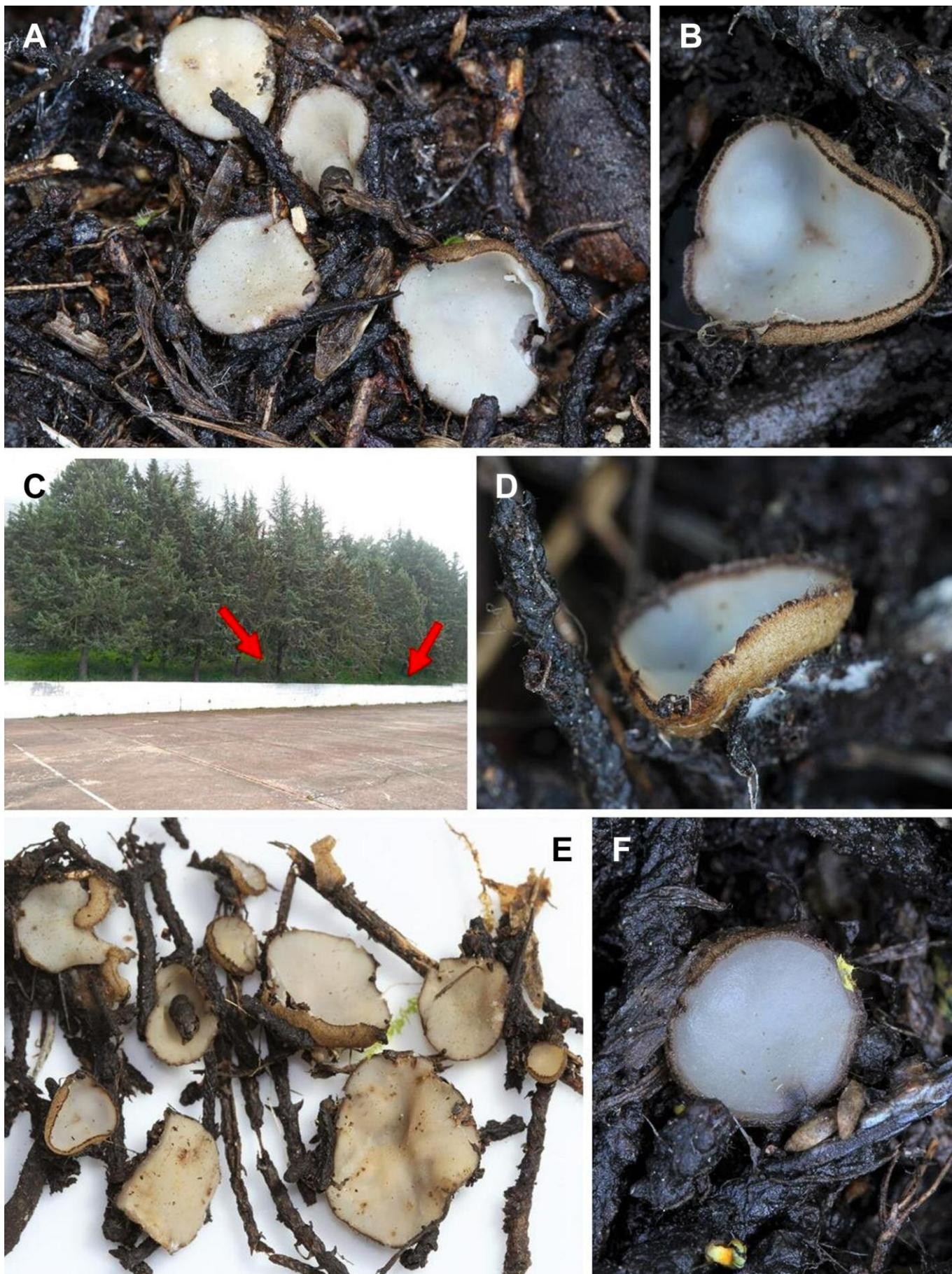
## Acknowledgements

We acknowledge S. Tello (Spain) and V. Avola (Italy) for having shared their collections, photographs and data. Finally, Brian Spooner (UK) is warmly thanked for his presubmission review.

## Authors' contributions

Nicolas Van Vooren was responsible for the study conception and design. Nicolas Van Vooren and Matteo Carbone financially con-

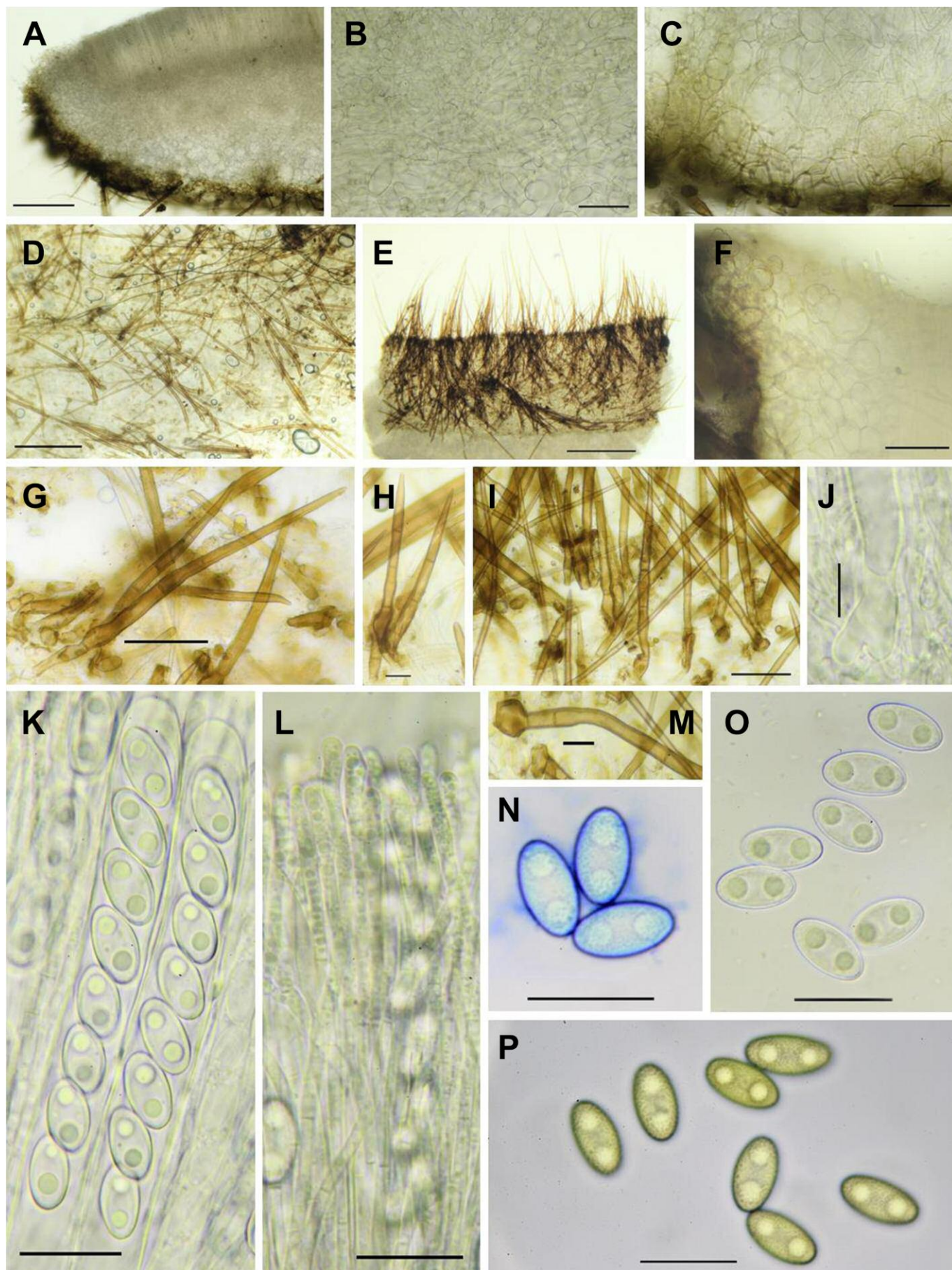




**Plate 5 – *Perilachnea verrucispora***

A–F. Coll. CVL180121(1): A, B, D, F: Different views *in situ*; C: Habitat; E: Assembly of specimens. All photos by F.J. Valencia.





**Plate 6 – *Perilachnea verrucispora*.** Microscopical characters

A. Section of an apothecium. B. Medullary excipulum. C. Ectal excipulum. D. Excipular hairs. E. Marginal hairs. F. Marginal cells. G–H. Different types of excipular hairs. I. Marginal hairs. J. Ascus base. K. Asci. L. Paraphyses in water. M. Marginal hair with bulbous base. N. Ascospores in Cotton blue. O. Ascospores in water. P. Ascospores in IKI. Scale bars: A, D = 200  $\mu$ m, E = 500  $\mu$ m, B–C, F–G, I = 50  $\mu$ m, H, K–P = 20  $\mu$ m, J = 10  $\mu$ m. All photos by F.J. Valencia.



## Updated key to the species of *Perilachnea*

1. Ascospores biguttulate in living state .....	2
1. Ascospores pluriguttulate .....	3
2. Ascospores smooth; on burnt soil .....	<i>P. hemisphaerioides</i>
2. Ascospores warted; on needle litter .....	<i>P. verrucispora</i>
3. Ascospores $\geq 24 \mu\text{m}$ in length .....	<i>P. ochraceoflava</i>
3. Ascospores $< 24 \mu\text{m}$ in length .....	4
4. On woody debris of deciduous trees .....	<i>P. humarioides</i>
4. On litter or woody debris of conifers .....	5
5. Ascospores ellipsoid, containing small lipid droplets with 2–3 larger drops; on litter of <i>Cupressus</i> or <i>Juniperus</i> .....	<i>P. flavobrunnea</i>
5. Ascospores often more elongated or oblong, containing more homogeneous small lipid droplets; on litter or wood of various conifers .....	<i>P. fallax</i>

tributed to the generation of rDNA sequences. Morphological analyses were performed by the authors on their collections; on other collections, they are mentioned as “rev.”. Molecular analyses were performed by Nicolas Van Vooren, as well as the registration in GenBank. The first draft of the manuscript was written by Nicolas Van Vooren and was subsequently updated by all authors. All plates have been designed by F.J. Valencia, except fig. 2, pl. 1 and 2 by N. Van Vooren. 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](https://doi.org/10.1016/S0022-2836(05)80360-2)
- BEUG M.W., BESSETTE A.E. & BESSETTE A.R. 2014. — *Ascomycete Fungi of North America. A mushroom reference guide*. Austin, University of Texas Press, 488 pp.
- BREITENBACH J. & KRÄNZLIN F. 1981. — *Champignons de Suisse*. Tome 1. Les Ascomycètes. Lucerne, Mykologia, 310 pp.
- CETTO B. 1989. — *I funghi dal vero*. Vol. 6. Trento, Saturnia, 719 pp.
- COOKE M.C. 1879. — *Mycographia, seu Icones fungorum*. Vol. 1, 6th fasc. London, Williams and Norgate, 52 pp. + 13 pl.
- DENNIS R.W.G. 1978. — British Ascomycetes. Vaduz, J. Cramer, 585 p.
- DOĞAN H.H. & AKTAŞ S. 2010. — Two new Ascomycetes records from Mediterranean part of Turkey. *Biological Diversity and Conservation*, 3 (1): 83–86.
- DOUGOUD R. 2001. — Clé des Discomycètes carbonicoles. *Documents mycologiques*, 30 (120): 15–29.
- EDGAR R.C. 2004. — MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32 (5): 1792–1797. doi: [10.1093/nar/gkh340](https://doi.org/10.1093/nar/gkh340)
- GRADDON W.D. 1960. — British records nos. 43–49. *Transactions of the British Mycological Society*, 43 (4): 689–691. doi: [10.1016/S0007-1536\(60\)80061-7](https://doi.org/10.1016/S0007-1536(60)80061-7)
- HUHTINEN S. 1984. — Additions to the ascomycetous flora of the Canadian North. *Karstenia*, 24: 1–11.
- KUMAR S., STECHER G., LI M., KNYAZ C. & TAMURA K. 2018. — MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549. doi: [10.1093/molbev/msy096](https://doi.org/10.1093/molbev/msy096)
- MEDARDI G. 2006. — *Atlante fotografico degli Ascomiceti d'Italia*. Trento, AMB, 454 pp.
- MOUTON V. 1897. — Troisième notice sur des ascomycètes nouveaux ou peu connus. *Bulletin de la Société royale de botanique de Belgique*, 36: 10–21.
- NEI M. & KUMAR S. 2000. — *Molecular Evolution and Phylogenetics*. New York, Oxford University Press, 333 pp.
- PERIĆ B. 2002. — Trois discomycètes nouvelles pour la flore mycologique du Monténégro. *Mycologia Montenegrina*, 5: 93–118.
- REHM H. 1896. — Ascomyceten: Hysteriaceen und Discomyceten. In: Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Die Pilze, 1, III.
- SVRČEK M. 1949 [1948]. — Bohemian species of *Pezizaceae* subf. *Lachneoideae* [České druhy podčeledi *Lachneoideae* (čel. *Pezizaceae*)]. *Sborník Národního Muzea v Praze / Acta Musei Nationalis Pragae*, 4B (6): 3–95.
- VAN VOOREN N. 2014. — Contribution à la connaissance des Pézizales (*Ascomycota*) de Rhône-Alpes – 2<sup>e</sup> partie. *Cahiers de la FMBDS*, 4: 1–172.
- VAN VOOREN N., VALENCIA LÓPEZ F.J., CARBONE M., LINDEMANN U., VEGA M. & VALADE F. 2021. — Exploring the European *Trichophaea*-like discomycetes (*Pezizales*) using morphological, ecological and molecular data. *Ascomycete.org*, 13 (1): 5–48. doi: [10.25664/art-0315](https://doi.org/10.25664/art-0315)
- VELENOVSKÝ J. 1934. — *Monographia Discomycetum Bohemiae*. Pars 1. Prague, 436 pp.



1



2



3



4



1: N. Van Vooren — 13 chemin du Bois Ponard, 69160 Tassin-la-Demi-Lune, France — [nicolas@vanvooren.info](mailto:nicolas@vanvooren.info)

2: F.J. Valencia López — C/Naranja, N.6, 29400 Ronda, Spain — [kurroamanita@gmail.com](mailto:kurroamanita@gmail.com)

3: M. Carbone — Via Don Luigi Sturzo 173, 16148 Genova, Italy — [matteocarb@hotmail.com](mailto:matteocarb@hotmail.com)

4: M. Vega — Kohlhöfen 17, 20355 Hamburg, Germany — [tomprodukt@web.de](mailto:tomprodukt@web.de)