Lachnea poiraultii (*Pezizales*), rediscovered after more than one hundred years

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Summary: Recent collections of a cup-fungus identified as *Lachnea poiraultii* Boud. from the Iberian and Istrian (Croatia) peninsulas provided the opportunity to investigate its modern taxonomic position. An examination of the type specimen of this taxon confirmed the determination of Croatian, Portuguese and Spanish collections. Based on the results of multi-gene phylogenetic analyses which placed *L. poiraultii* within the *Pyronemataceae* with no support of affiliation with another genus, and the study of microscopic features in the living state, the new genus *Paratricharina* is proposed to accommodate *L. poiraultii*. The morphological similarities and differences from the most closely related species are discussed. A full description and illustrations of *P. poiraultii* are also provided.
Keywords: Ascomycota, *Pyronemataceae*, *Tricharina*, *Paratricharina*, taxonomy, phylogeny.

Résumé : de récentes récoltes d'un discomycète identifié comme *Lachnea poiraultii* Boud. en provenance des péninsules ibériques et istriennes (Croatie) ont fourni l'opportunité d'évaluer son placement taxinomique moderne. Un examen de spécimens type de ce taxon a confirmé la détermination des récoltes croates, portugaises et espagnoles. Sur la base des résultats d'une analyse phylogénétique multigènes qui ont placé *L. poiraultii* au sein des *Pyronemataceae* sans support d'un rattachement à un autre genre, et l'étude des caractères microscopiques à l'état vivant, le nouveau genre *Paratricharina* est proposé pour accommoder *L. poiraultii*. Les similitudes morphologiques et les différences avec les espèces les plus proches sont discutées. Une description complète et des illustrations de *P. poiraultii* sont également fournies.
Mots-clés : Ascomycota, *Pyronemataceae*, *Tricharina*, *Paratricharina*, taxinomie, phylogénie.

Introduction

Lachnea poiraultii was published by Boudier (1901) to accommodate an operculate discomycete with an orange hymenium, hairy outer surface and microscopic characters similar to those of the genus *Tricharina* Eckblad. Boudier included it in the genus *Lachnea* in his own sense (Boudier, 1885, 1907), i.e. a group of species in the *Pezizales* with stiff brown hairs, longer at the margin, and having smooth or more rarely verrucose ascospores containing guttules.

No other collection of this species has been reported since its first description, for more than one hundred years. In the revision of Boudier's Icones by Korf (1985), this taxon was considered as a putative *Geopora* Harkn. or *Humaria* Fuckel species.

Recent collections made in Croatia, Portugal and Spain, the examination of type specimens, and phylogenetic analyses of multiple DNA loci facilitated a re-circumscription of this species and a proposal for its taxonomic placement.

Materials and Methods

Morphology, cytology and cytochemistry

The observations were made on fresh and dried material using methods of "vital taxonomy" (Baral, 1992) supplemented with cytological and cytochemical tests using lethal media applied directly to the living cells; some small pieces of dried specimens were rehydrated during about twelve hours in water. The following mounts were used to observe microscopic characters: water, 0.5 and 5% KOH, iodine reagents (Lugol's solution, Melzer's reagent), Methyl (Cotton) Blue both in lactophenol and lactic acid, and 1% aqueous solution of CRB (Cresyl Blue Brilliant), Congo Red (CR, after Pfister *et al.*, 2009). Measurements were made on ≥ 20 ascospores mounted in water from each collection, except on Croatian material where 50 living ascospores mounted in tap water were measured and 50 dead ascospores mounted in Methyl blue in lactic acid (in two Croatian collections, both from spore deposits) to test the shrinkage of dead spores. Spores are measured under the 100× oil immersion lens of transmission light microscopes, excluding the ornamentation. X represents the mean value of spore dimensions, and Q the ratio between spore length and width, the value in italics represents the mean value of this ratio.

The terminology and abbreviations of cytological elements (cell inclusions and exudates) related to the living cells are used after Baral (1992). Spore shape is given after Kušan *et al.* (2014).

Macrophotographs were made *in situ* using digital cameras, while microphotographs have been taken using digital cameras mounted directly on microscopes. Line drawings were made freehand to scale.

DNA extraction, amplification and sequencing

Total DNA was extracted from dry specimens blending a portion of them with the aid of a micropestle in 600 µl CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform: isoamyl alcohol (24:1) was added and carefully mixed with the samples and emulsified. It was then centrifuged for 10 min at 13,000 g, and the DNA in the supernatant was precipitated with a 1/1 volume of isopropanol. After a second centrifugation for 15 min at the same speed, the pellet was washed in cold 70% ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µl of ddH2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS, LR0R and LR5 (Vilgalys & Hester, 1990) were used to amplify the 28S nLSU region, EF1-983F and EF1-1567R (Rehner & Buckley, 2005) for the translation elongation factor 1α tef1 gene, and reverse of bRPB2-6R2 (Matheny *et al.*, 2007) and a modified version of fRPB2-7.1R (Matheny, 2005), fRPB2-7.1R2 (5′ – CCCATNGCYTGYTTVCCCATDGC – 3′) for the RNA polymerase II second largest subunit, rpb2. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 72 °C step 10 min. PCR products were checked on 1% agarose gels (visualized with GelRed dye), and positive reactions were sequenced with one or both of the sequencing primers. Chromatograms were checked in MEGA 5.0 (Tamura *et al.*, 2011) for putative reading errors, and these were corrected.

Phylogenetic analyses

BLAST was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. The selected sequences came mainly from Hansen *et al.* (2005a), Hansen & Pfister (2006), Perry *et al.* (2007), Hansen *et al.* (2013), and Stielow *et al.* (2013), including those of *Glaziella aurantiaca* (Berk. & M.A. Curtis) Sacc., chosen as outgroup. Introns were removed from tef1 and rpb2 data sets. Sequences were first aligned in MEGA 5.0 (Tamura *et al*., 2011) software with its Clustal W application and then corrected manually. The final alignment included 331/848 (28S nLSU), 355/867 (tef1) and 311/597 (rpb2) variable sites. The concatenated alignment was loaded in PAUP* 4.0b10 (Swofford, 2001) and each locus was subjected to MrModeltest 2.3 (Nylander, 2004). The Model GTR+Γ+I was selected for all partitions, and implemented in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003), where a Bayesian analysis was performed (LSU-tef1-rpb2 data partitioned, two simultaneous runs, six chains, with temperature set to 0.2, and sampling every 100th generation) until convergence parameters were met after about 260,000 generations at which point the standard deviation had fallen below 0.01. The initial 25% of sampled trees were discarded as burn-in. In addition to Bayesian analysis, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis, 2006) using the standard search algorithm (LSU-tef1-rpb2 data partitioned), the same model of nucleotide substitution, and node support evaluated by 2,000 bootstrap replications. Significance threshold was set at or above 0.95 for posterior probability (PP) and 65% bootstrap proportions (BP). Sequences generated during this study were deposited in Genbank under the accession numbers listed in Table 1.

Taxonomy

Description of recent collections

Apothecia 5–25 (40) mm in diameter, sessile, slightly cupulate with inrolled margin, expanded at the end in Iberian collections, first with a dull orange hymenium, then becoming brick-coloured, but occasionally slightly yellowish or cream-coloured (the pigment seems to be soluble in water); outer surface covered by dense brown-red hairs, often agglutinated to form a brown pustulate pattern, sometimes appressed, but mostly straight and projecting outwards. **Subhymenium** thin, 50–70 µm thick, of short-celled *textura intricata* to *intricata-epidermoidea*, with pale orange hyphae. **Medullary excipulum** 200–250 µm thick, of *textura intricata*, with hyaline and thin-walled hyphae, predominantly horizontal in upper portion, gradually changing its orientation towards the ectal excipulum. **Ectal excipulum** 230–290 µm thick, of *textura prismatica*, with predominantly vertically oriented cells in upper portion, 7–25 µm wide, hyaline, becoming a *textura globulosa-subangularis* in the outermost part, with brownish cells, measuring (16) 20–41 (52) µm wide. **Marginal hairs** organized in more or less triangular bundles; hairs of two types: short, 30–70 × 8–10 µm, obtuse; or long, 95–270 × 7–12 µm, obtuse or almost pointed at the top; all of them are pale brown, more or less sinuous, septate, with wall 1.8–2.2 µm thick, and with a simple base arising from the globose cells of the outer layer of the ectal excipulum. **Excipular hairs** similar to those on the margin, but longer, reaching 450 µm in length, more or less flexuous. **Asci** cylindrical, 300–350 × (11) 14–17.5 (19) µm, 335–382 × 17.3–19.8 µm when fully turgescent (living state), shortly bifurcate at the base, arising from perforated croziers, inamyloid, 8-spored. **Paraphyses** cylindrical, 4–7 µm at the top, without reaction in Lugol's solution or Melzer's reagent; in the living state apical cell contains large hyaline refractive globular to oblong KOH-soluble cytoplasmic bodies, large non-refractive vacuoles that may contain single or few minute brick-orange carotenoid granule(s) mixed with hyaline, minute weakly refractive vacuolar bodies. **Ascospores** narrowly ellipsoid, (13.5) 15–19.8 (20.1) × 9–11 (11.3) µm [X=17.8 × 10.2 µm, Q=1.4–*1.8*–2.0] when freshly ejected in living state and (15.0) 15.8–17.5 (18.2) × (8.1) 8.8–9.4 (10.3) µm [X=16.6 × 9.2 µm, Q=1.6–*1.8*–2.0] in dead state from Methyl Blue/lactic acid mount, hyaline, equatorially uninucleate, appearing non-guttulate in dead state (lipid bodies masked due to increased refractivity of collapsed sporoplasm) but with minute bipolar dispersed lipid aggregations that rapidly coalesce to form biguttulate lipid pattern in living state, more or less thick-walled, smooth observed in water and Cotton Blue, but with a rough to finely verrucose perispore when mounted in Lugol's solution, entirely encapsulated by delicate but persistent sticky sheath best visible in CR mount.

Studied collections: CROATIA. Brtonigla-Nova Vas (Istra County), N 45.372144°, E 13.632355°, 112 m asl, 30 March 2013, *leg*. M.J. Šimić, I. Kušan & N. Matočec, CNF-2/9332 and 27 February 2014, *leg*. M.J. Šimić, CNF-2/9560, both collected on sandy moist soil densely beset by mosses along small stream in degraded thermophilic deciduous forest of *Ulmus canescens*, *Prunus spinosa* and *Rubus* sp. PORTUGAL. Serra de Montejunto, N 39.18061°, E -9.02545°, 188 m asl, 24 January 2014, *leg*. M. Vega, on bare ground, on a narrow footpath; pers. herb. M.V. 20140124-04, U.L. 178-14. SPAIN. Jaén, Aldeaquemada, Río Guarrizas, N 38.40786°, E -3.383139°, 700 m asl, 16 March 2014, *leg*. T. Illescas, M. Á. Ribes *et al.*, on naked soil, with some little mosses, close to a river; pers. herb. N.V. 2014.03.24, U.L. 179-14. Málaga, Jardín Botánico-Histórico La Concepción, N 36.76587°, E -4.42523°, 90 m asl, 24 January 2015, *leg*. and det. M. Vega, conf. U. Lindemann, on bare ground below *Olea* sp. between mosses (*Barbula* sp., *Dicranella* sp., *Bryum* sp., *Tortula* sp.); pers. herb. M.V. 20150124-01. Same locality, N 36.76608°, E -4.42456°, 86 m asl, 24 January 2015, *leg*. and det. M. Vega, conf. U. Lindemann, next to a small track on soil, between mosses (*Barbula* sp., *Dicranella* sp.); pers. herb. M.V.20150124-02.

Other collection: SPAIN. Barcelona, Les Fonts de Terrassa, Can Fonellet, N 41.51940°, E 2.03369°, 172 m asl, 29 January 2015, *leg*. and det. J. Bometón, conf. M.Á. Ribes, on a slope, on soil between mosses, with *Cupressus sempervirens*, *Ulmus minor* and *Prunus* sp.

Type material revision

There are three collections named *Lachnea poiraultii* in Boudier's herbarium housed in Muséum national d'histoire naturelle (PC); it seems that they were all collected in the same locality in southern France, although one of them is provided without location or date:

- PC0738324: *Lachnea Poiraulti* Boud. Icon. mycol. n° 568, Antibes *ad terram Februario* 1900 *misit* D. Poirault;
- PC0738328: *Lachnea Poiraulti* n° 17, no date, no location, accompanied by a drawing of spores;
- PC0738329: *Lachnea Poiraulti* Boud., Antibes, Villa Thuret, Février 1900 *misit* D. Poirault.

Following the original description by Boudier (1901: 199-200), this species has been found "à Antibes, dans le parc de la Villa Thuret [...] par notre collègue et ami M. G. Poirault." The diagnosis indicates "*Februario* 1900" as the date. The collections PC0738324 and PC0738329 agree with these data, but it is not possible to ascertain whether they are duplicates or two different collections from the same locality. We propose to designate the collection PC0738324 as the lectotype.

Here is the microscopic description of the revised collection:

Medullary excipulum of *textura intricata*. **Ectal excipulum** of *textura angularis*, with cells up to 40 µm wide, with some compact brown cells in the outermost part. **Excipular hairs** rather dense, 90–420 × 7–12.5 µm, obtuse or slightly pointed, brown, septate, with wall 1.5–3 µm wide, partially enlarged at the base which is simple. **Anchoring hyphae** present at the base of the apothecia, up to 750 µm in length. **Marginal hairs** present but broken and hard to evaluate. **Asci** cylindrical, about 300 µm in length and 10–11 µm wide, with crozier, inamyloid, 8-spored. **Paraphyses** cylindrical,

Plate 1 — *Paratricharina poiraultii*

A-B. Collection from Jaén (Spain). C. Collection from Serra de Montejunto (Portugal). D. Lectotype of *Lachnea poiraultii*. E-F. Collections from Málaga (Spain). G. Habitat of the collections from Jardín Botánico-Histórico La Concepción at Málaga. Photos: A-B: M.A. Ribes; C, E-G: M. Vega; D: N. Van Vooren (with the agreement of MNHN).

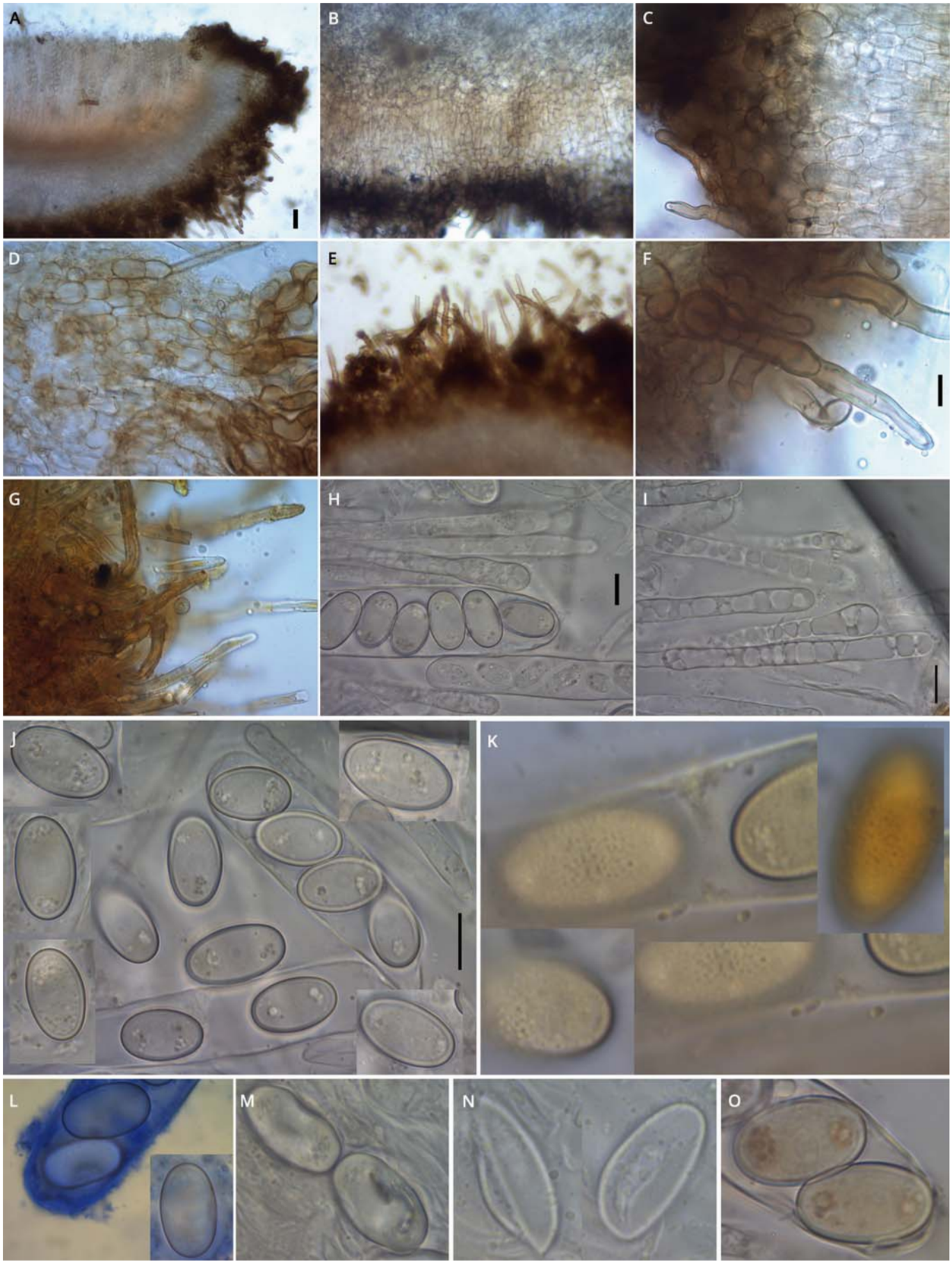

Plate 2 — *Paratricharina poiraultii*. Microscopic features

A. Vertical section of an apothecium. B-C. Excipulum showing a *textura prismatica*. D. Cells close the margin. E-G. External and marginal hairs. H. Asci and paraphyses. I. Content of paraphyses. J. Living ascospores in water. K. Ascospores in Lugol's solution, showing an ornamented perispore. L. Ascospores in Cotton Blue. M. Ascospores showing a refractive layer. N. Perispores. O. Ascospores in Lugol's solution. A, E, G, K, M and N from U.L. 178-14; B-D, F, H-J, L and O from U.L. 179-14. Scale bars = 10 µm. Photos: U. Lindemann.

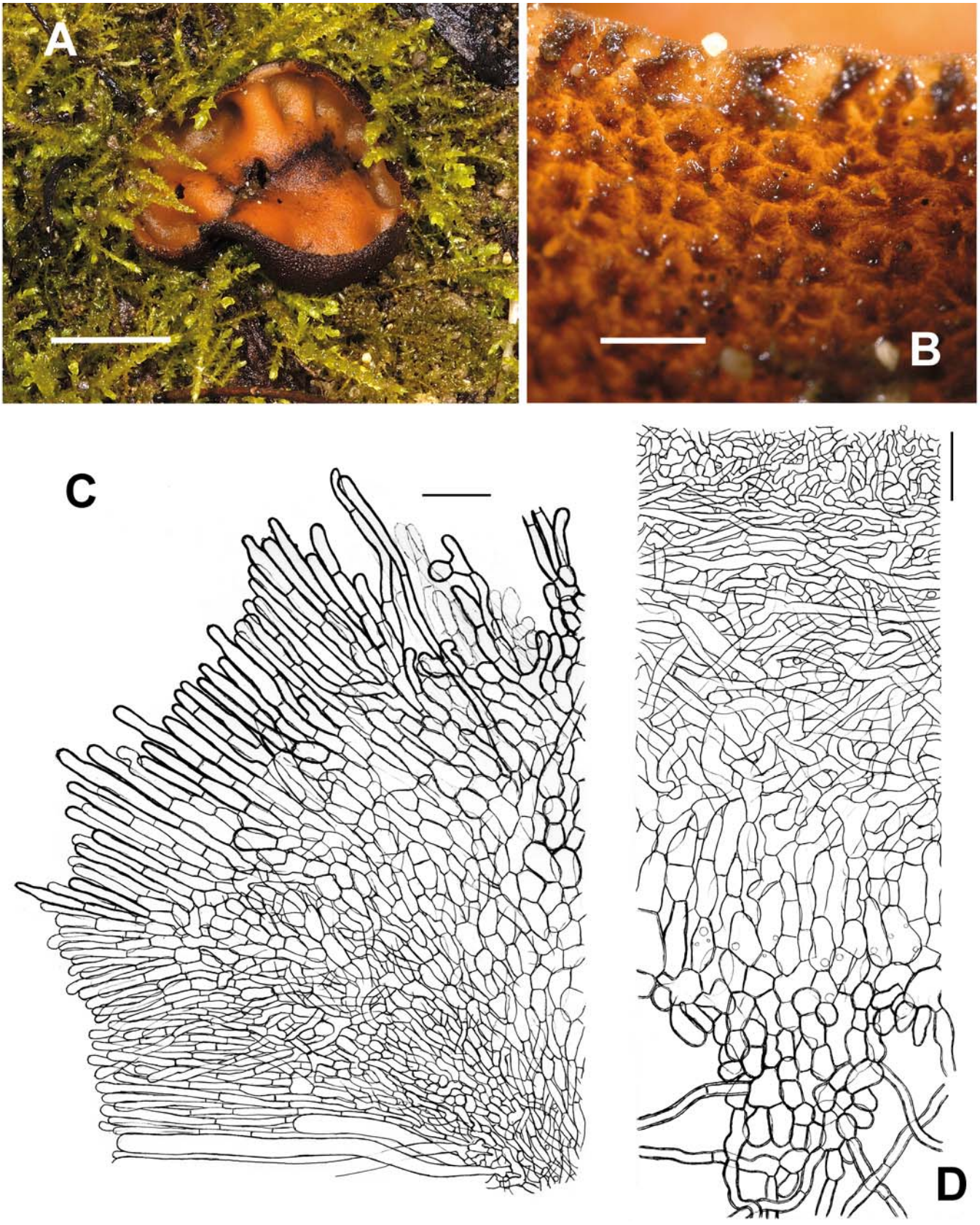

Plate 3 — *Paratricharina poiraultii*. Apothecium and texture

A: Apothecium *in situ* (bar = 1cm). B: Apothecial vestiture (bar = 200 µm). C: Marginal texture in original spatial position (bar = 50 µm). D: Excipular texture at middle flank (bar = 50 µm). All from CNF 2/9332. Photos and drawings: N. Matočec.

about 3 µm wide at the apex, hyaline. **Ascospores** ellipsoid, (16) 17–20 (21) × (9.2) 9.5–10.5 (11) µm [X = 18.8 × 10.1 µm, Q=1.7–*1.9*–2.0], hyaline, smooth, rather thick-walled, with some polar inclusions that can merge and produce two polar guttules.

Nomenclatural considerations

The genus *Lachnea* (Fr.) Boud. is illegitimate because *Lachnea* L. (1753) has priority. As typified by Boudier (1885) and confirmed by Eckblad (1968: 158), the genus falls in synonymy with *Humaria* Fuckel, with *Peziza hemisphaerica* Hoffm. as type-species — which binomial is thus synonymized as *Humaria hemisphaerica* (Hoffm.) Fuckel. The phylogenetic and morphological evidence presented here do not support *L. poiraultii* as a *Humaria*.

As stated in the discussion and in the phylogenetic analyses, both microscopic and macroscopic characters of *Lachnea poiraultii* counter a classification of this fungus in the genus *Tricharina* in the sense of Eckblad (1968) and Yang & Korf (1985). Furthermore, *Tricharina* appears to be paraphyletic and requires additional investigation to clarify the systematic position of its type-species, *T. gilva* (Boud.) Eckblad, within the *Geopora* clade.

Based on our evidences (see Discussion), we propose the new genus *Paratricharina*:

Paratricharina Van Vooren, U. Lindemann, M. Vega, Ribes, Illescas & Matočec, *gen. nov.* — MB 812169

Description: Apothecia about 10–20 mm in diameter or less, sessile, slightly cupulate with inrolled margin, expanded when old; outer surface covered by dense brown-red hairs, sometimes appres-

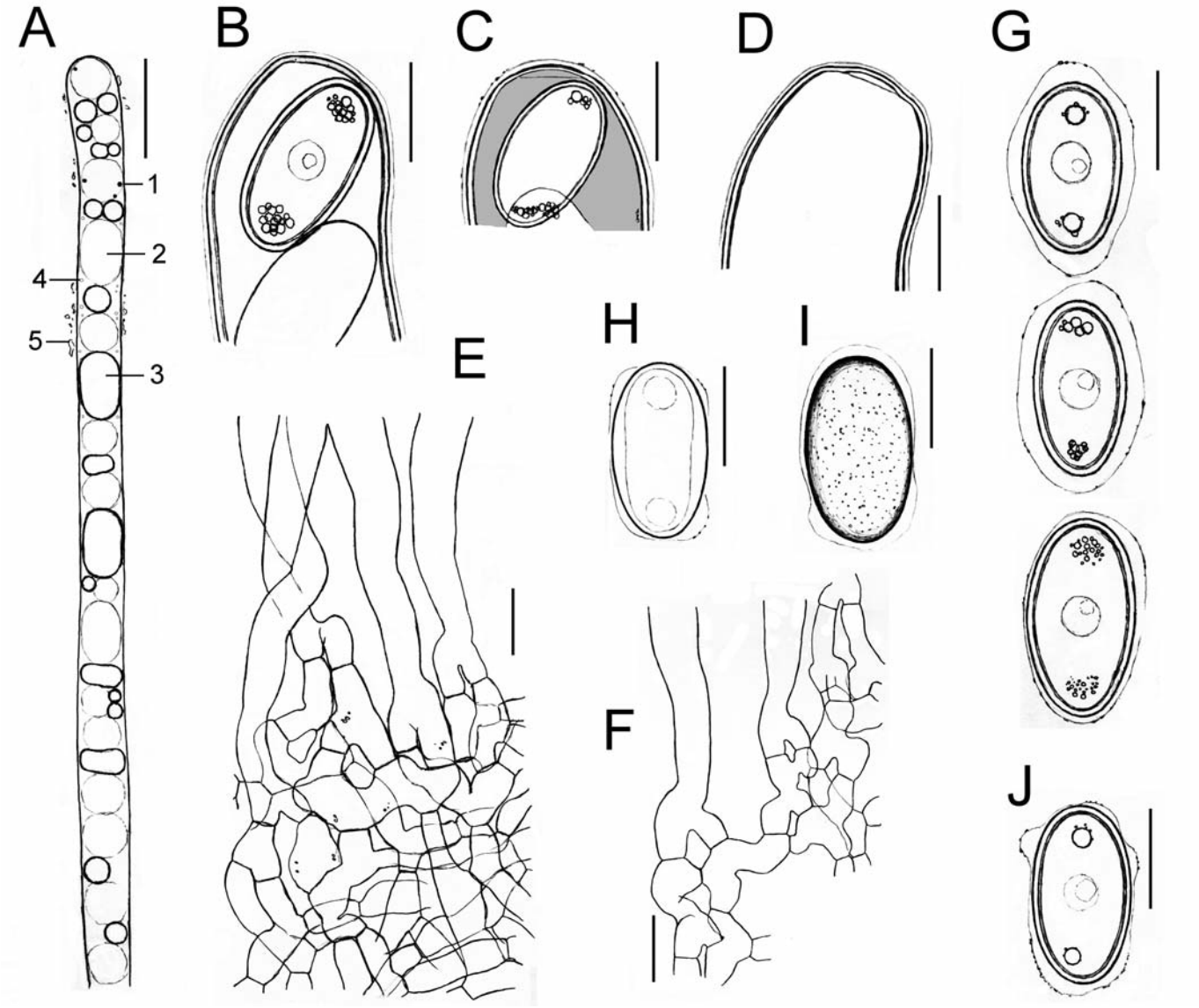

Plate 4 — *Paratricharina poiraultii*. Hymenial elements

A: Paraphysis apical cell (*in statu vivo*); 1- carotenoid granular pigment; 2- non-refractive vacuole; 3- SCB; 4- minute VB; 5- exudate. B: Ascus apex in tap water (*in statu vivo*). C: Ascus apex in CRB (*in statu vivo*). D: Ascus apex in CR aqueous solution (*in statu vivo*, spores not shown). E: Ascal bases with a part of the ascogenous system in tap water (*in statu vivo*). F: Ascal bases with a part of the ascogenous system in Cotton Blue/lactic acid (*in statu emortuo*). G: Three freshly ejected ascospores in tap water (*in statu vivo*); note rapid coalescence of lipid bodies from bipolar dispersed configuration into biguttulate pattern and constant encapsulation by a sheath. H: Ascospore in Cotton Blue/lactic acid (*in statu emortuo*); note almost masked lipid bodies and persistent encapsulation by a sheath. I: Ascospore in Lugol's solution, surface view (*in statu vivo*; lipid bodies not shown); note minute wall ornamentation and persistent encapsulation by a sheath. J: Ascospore from one year old spore print (*in statu sicco et vivo*) rehydrated by 0.1% KOH; note coalesced polar lipid bodies and persistent encapsulation by a sheath. A-G from CNF-2/9332, H-J from CNF-2/9560. Scale bars = 10 µm. Drawing: N. Matočec.

sed and/or forming distinct pustules, but mostly straight and projecting outwards. Subhymenium thin, of *textura intricata*. Medullary excipulum of *textura intricata*, with thin-walled hyphae. Ectal excipulum of vertically oriented *textura prismatica*, with thin-walled cells, becoming a *textura globulosa-subangularis* in the outermost part, then with thick-walled brownish cells. Hairs more or less thick-walled, pale brown, more or less sinuous, septate, obtuse or pointed, with a simple base arising from the globose cells of the outer layer of the ectal excipulum. Asci cylindrical, with forked base, arising from perforated croziers, inamyloid, 8-spored. Paraphyses cylindrical, rounded and slightly enlarged at the apex, with spumous compound KOH soluble content, without reaction in Lugol's solution. Ascospores ellipsoid, hyaline, appearing eguttulate (in dead state) but with small polar aggregations of minute lipid bodies rapidly coalesced into two small polar guttules (in living state), more or less thick-walled, smooth observed in water and Cotton Blue, but with a rough to finely verrucose perispore if mounted in Lugol's solution, entirely encapsulated by a delicate persistent sheath. Base of apothecia covered by hyaline anchoring hyphae.

Type species: *Lachnea poiraultii* Boud.

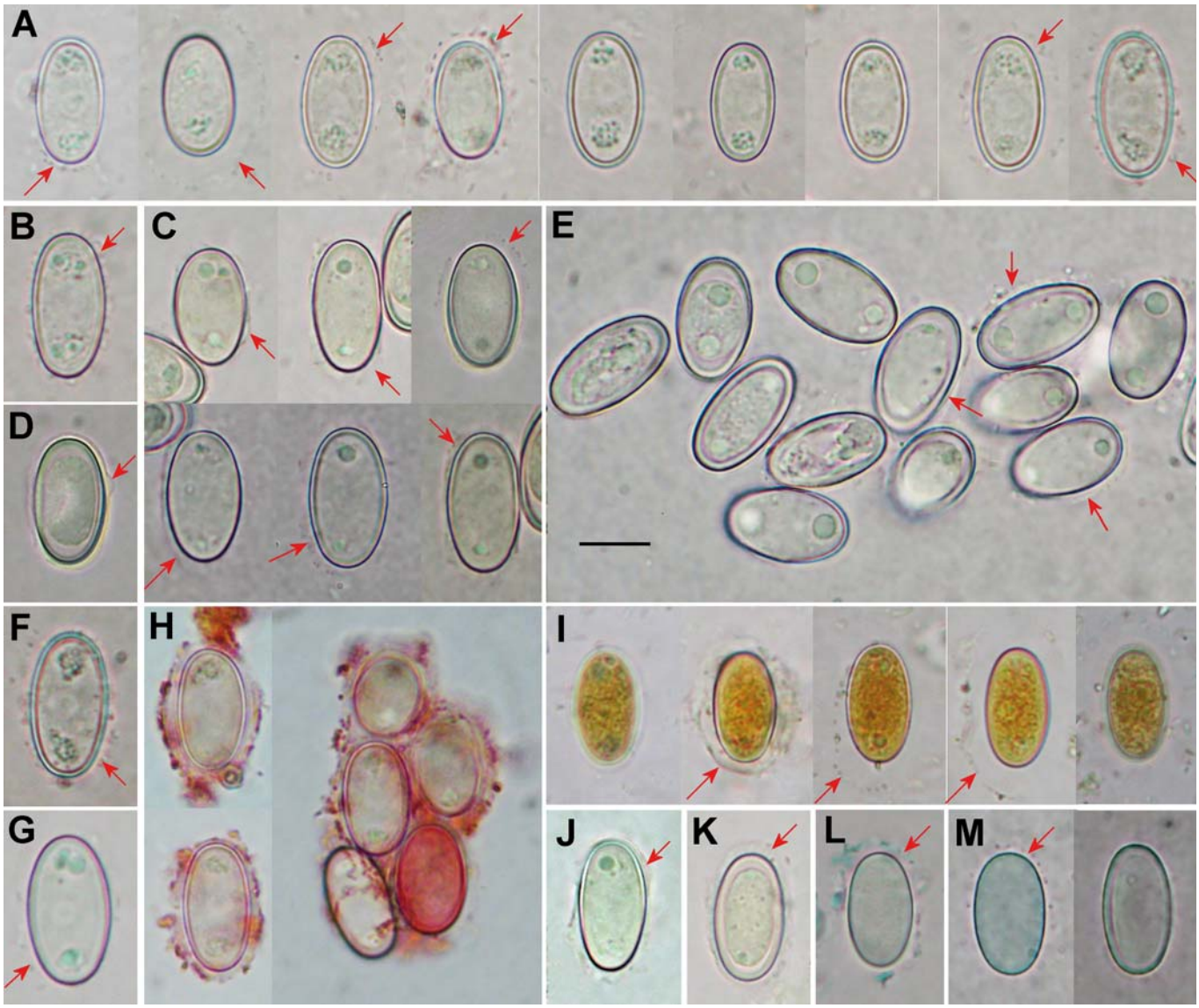

Plate 4 — *Paratricharina poiraultii*. Sporogram

A: Freshly ejected ascospores in tap water immediatelly after discharge (*in statu vivo*); note original unaltered bipolar dispersed lipid bodies; equatorially positioned single nucleus also weakly visible. B: Freshly ejected ascospore in tap water about 30 min after discharge (*in statu vivo*); note bipolar dispersed lipid bodies in a process of coalescence. C: One year old spore print (*in statu sicco et vivo*) rehydrated by 0.1% KOH; note coalesced polar lipid bodies. D: One year old spore print (*in statu sicco et emortuo*) rehydrated by 0.1% KOH; note masked polar lipid bodies. E: A group of ascospores from one year old spore print firmly aggregated by capsulate spore sheath (*in statu sicco*) rehydrated by 0.1% KOH; note that polar lipid bodies are coalesced (*in statu vivo*) or masked (*in statu emortuo*). F: Freshly ejected ascospore in CRB immediatelly after discharge (*in statu vivo*); note original unaltered bipolar dispersed lipid bodies; equatorially positioned single nucleus also weakly visible. G: Freshly ejected ascospore in CRB about 30 min after discharge (*in statu vivo*); note bipolar dispersed lipid bodies in a process of coalescence. H: Freshly ejected ascospores in CR immediatelly after discharge (*in statu vivo*); note original unaltered bipolar dispersed lipid bodies and clearly revealed encapsulated sheath causing ascospores to form firm aggregations. I: Ascospores in Lugol's solution from fresh mount (*in statu vivo*); note coalesced polar lipid bodies. J: Ascospore in Lugol's solution from one year old spore print (*in statu sicco et vivo*); note coalesced polar lipid bodies. K: Ascospore in Lugol's solution from one year old spore print (*in statu sicco et emortuo*); note masked polar lipid bodies. L: Ascospore in Cotton Blue/lactic acid from fresh mount (*in statu emortuo*); note masked polar lipid bodies. M: Ascospores in Cotton Blue/lactic acid from one year old spore print (*in statu sicco et emortuo*); note masked polar lipid bodies. Red arrows indicate capsulate spore sheath. A-B and F-I from CNF-2/9332, C-E and J-M from CNF-2/9560. Bar = 10 µm. Photos: I. Kušan & N. Matočec.

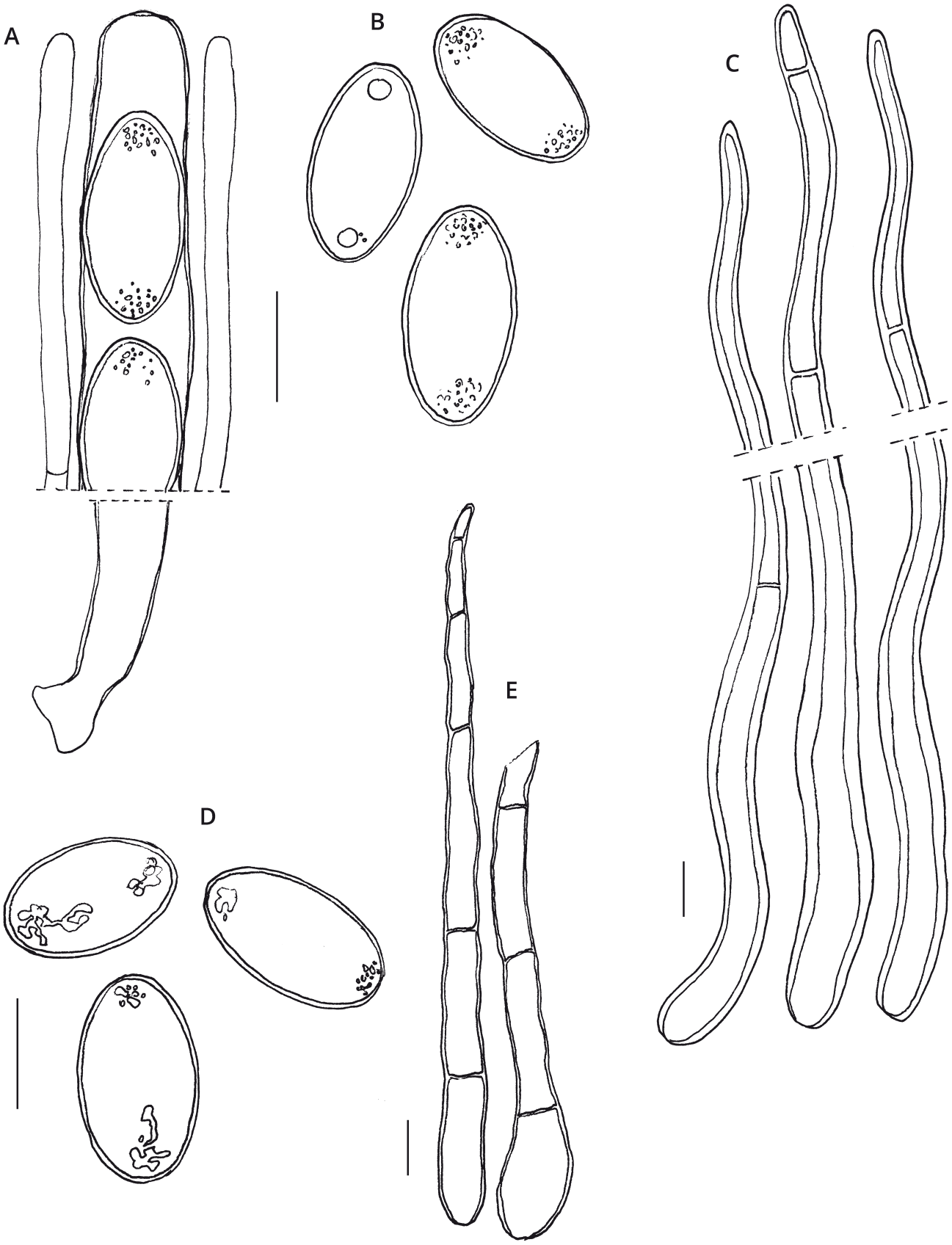

Plate 6 — *Paratricharina poiraultii*. Microscopic features from type-material

A: Ascus and paraphyses. B. Ascospores. C. External hairs. D. Ascospores. E. Marginal hairs. A-C from PC0738324, D-E from PC0738328, all in water mount. Bars = 10 µm. Drawing: N. Van Vooren.

Etymology: *Paratricharina* = composite of Ancient Greek "pará" which means "beside; next to, near" and "*Tricharina*", macro- and microscopically the closest genus to this new one.

In accordance with Art. 55.1 of ICN (Melbourne Code), we propose the following new combination:

Paratricharina poiraultii (Boud.) Van Vooren, U. Lindemann, M. Vega, Ribes, Illescas & Matočec, *comb. nov.* — MB 812170

Basionym: *Lachnea poiraultii* Boud., *Bull. Soc. mycol. Fr.*, 16 (4): 198 (1901).

Lectotype designated here: PC0738324, "*Lachnea Poiraulti*" Boud. Icon. mycol. n° 568, Antibes *ad terram Februario* 1900 *misit* Poirault; MBT 201119.

Phylogenetic analysis

Both inference methods produced very similar topologies, in which the same strongly supported clades were recovered. The topology obtained agreed with that reported for the *Pyronemataceae* by Hansen *et al.* (2013). The deeper nodes were not resolved in these analyses, but the monophyletic status of the following clades was inferred: *Cheilymenia* lineage, *Pyronema* lineage, *Octospora* lineage, *Humaria* lineage, *Sowerbyella* lineage and *Scutellinia–Trichophaea* lineage (Fig. 1). Within the *Scutellinia–Trichophaea* lineage, smaller clades inferred by Hansen *et al.* (2013) were also well supported here: one formed by *Scutellinia*, *Ramsbottomia* and *Miladina*, another composed of *Trichophaea* and *Anthracobia*, and finally the one composed of *Tricharina*, *Geopora*, and the recently proposed genus *Hoffmannoscypha* (Stielow *et al.*, 2013). Samples of *Lachnea poiraultii* sequenced in the present study were well supported within this last clade, but not supported as a member of any of these three genera.

Our results infer that the genus *Tricharina* is paraphyletic (as previously suggested by Perry *et al.*, 2007). As presently circumscribed, *Tricharina* is composed of one lineage within the *Geopora–Hoffmannoscypha* clade and several others of uncertain relationship, including *T. gilva*, the type. Similar paraphyly can be observed in other groups such as the genus *Peziza* Fr., where the creation of several new genera to accommodate the different monophyletic lineages is advocated (Hansen *et al.*, 2005b). Hence, in the present study we choose to propose a new genus for the existing species *Lachnea poiraultii*.

Discussion

Despite remarkable characters, i.e. hairy ascomata, and colour of hymenium, it is striking that this species has not been found again (or reported) since its discovery in 1900. Indeed, plate 355 from Boudier's Icones (Boudier, 1905-1910) is based on collections previously cited. Only one reference has been noted in Donadini (1976: 72) under the name *Mycolachnea poiraultii*, cited after De Crozals (1861-1932), a French mycologist who was living in Toulon, a locality close to Antibes where *Lachnea poiraultii* was first collected. Unfortunately we were unable to find any data on this putative collection in De Crozals' publications.

In our analyses, *Paratricharina poiraultii* was strongly supported in a clade with *Hoffmannoscypha pellita* (Cooke & Peck) Stielow, Hensel, Göker & Klenk [syn. *Geopora pellita* (Cooke & Peck) T. Schumach.], *Geopora* and *Tricharina*. Morphologically, it shares a similar hymenial colour and a similar hairy outer surface with *H. pellita*, but the latter possesses larger guttulate spores (21–27 µm in length). *Humaria solsequia* (Quél.) Van Vooren & Moyne [syn. *Humaria aurantiaca* (Clem.) Häffner, Benkert & Krisai] also has an orange hymenium and external brown hairs, but its spores are narrower and contain larger guttules (Van Vooren & Moyne, 2010). Among *Tricharina* species, those with yellow-orange coloured hymenium and brown marginal hairs like *T. praecox* Chin S. Yang & Korf var. *praecox* or *T. gilva* have some macro- and microscopic similarities with *Paratricharina poiraultii*, but also clear differences. In contrast to *Tricharina* species, the whole outside of the apothecium of *P. poiraultii* is covered by brown hairs. *Tricharina* ascospores contain much smaller amounts of polar granules if any and are devoid of persistent encapsulated sheath. Furthermore, the ectal excipulum of *P. poiraultii* is composed of *textura prismatica* becoming a *textura globulosa-subangularis* of brownish cells at the 1-3 outmost cell layers, whereas the ectal excipulum of *Tricharina* species is composed of *textura globulosa-angularis* (Lindemann, 2013). Only *Tricharina* species with brown marginal hairs have also brownish cells in the ectal excipulum, but these brownish cells are located at the margin whereas the lower flanks of the ectal excipulum are composed of hyaline cells. Finally, the mature spores of *P. poiraultii* are thick-walled whereas the spores of *Tricharina* are thin-walled at maturity.

Distribution of this species, based on our recent findings and the original locality, appears to be in Southern Europe (Fig. 2). Future collections are desirable to confirm this distribution.

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Table 1 — Collections of *Paratricharina poiraultii* sequenced and used in the molecular phylogenetic study, with voucher information and GenBank accession numbers. The abbreviation in the "Herb.-No." indicates the private herbaria of Uwe Lindemann (= U.L.)

Species	Herb.-No.	Country, Collecting Date, Collector	28S	ITS	tef-1α	rpb2
P. poiraultii	U.L.178-14	Portugal, 2014, M. Vega	KP052789	KP052788	KP052790	KP052791
P. poiraultii	U.L.179-14	Spain, 2014, T. Illescas, M. Á. Ribes *et al.*	KP052785	KP052784	KP052786	KP052787

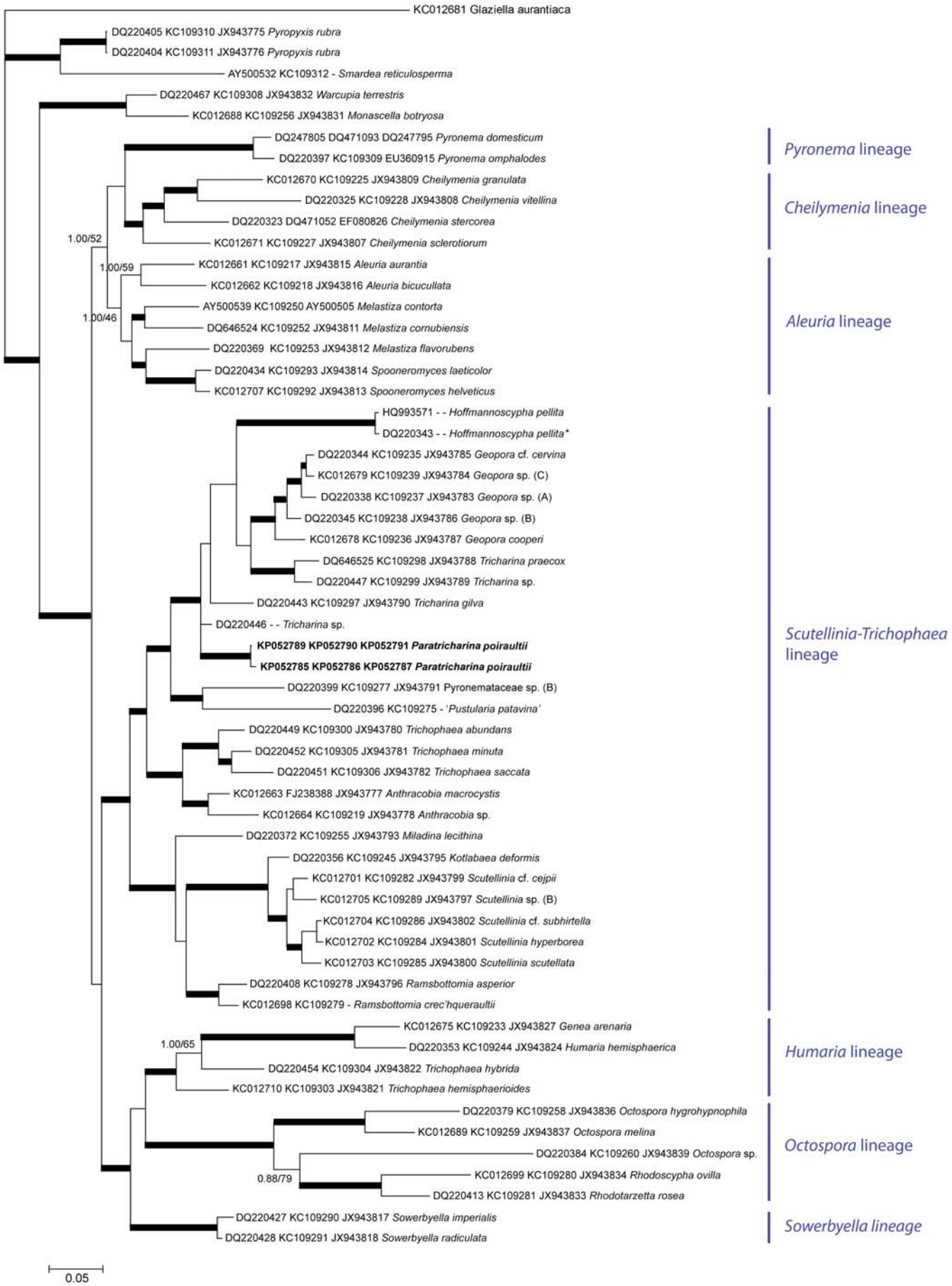

Fig. 1 — Consensus 28S-tef 1α-rpb2 phylogram of the family *Pyronemataceae* obtained in MrBayes 3.1 from 1,950 sampled trees. Nodes significantly supported by both Bayesian and ML analyses are highlighted with bold bars (0.95 PP, 65 BP). Nodes supported by just one of these inference methods are annotated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

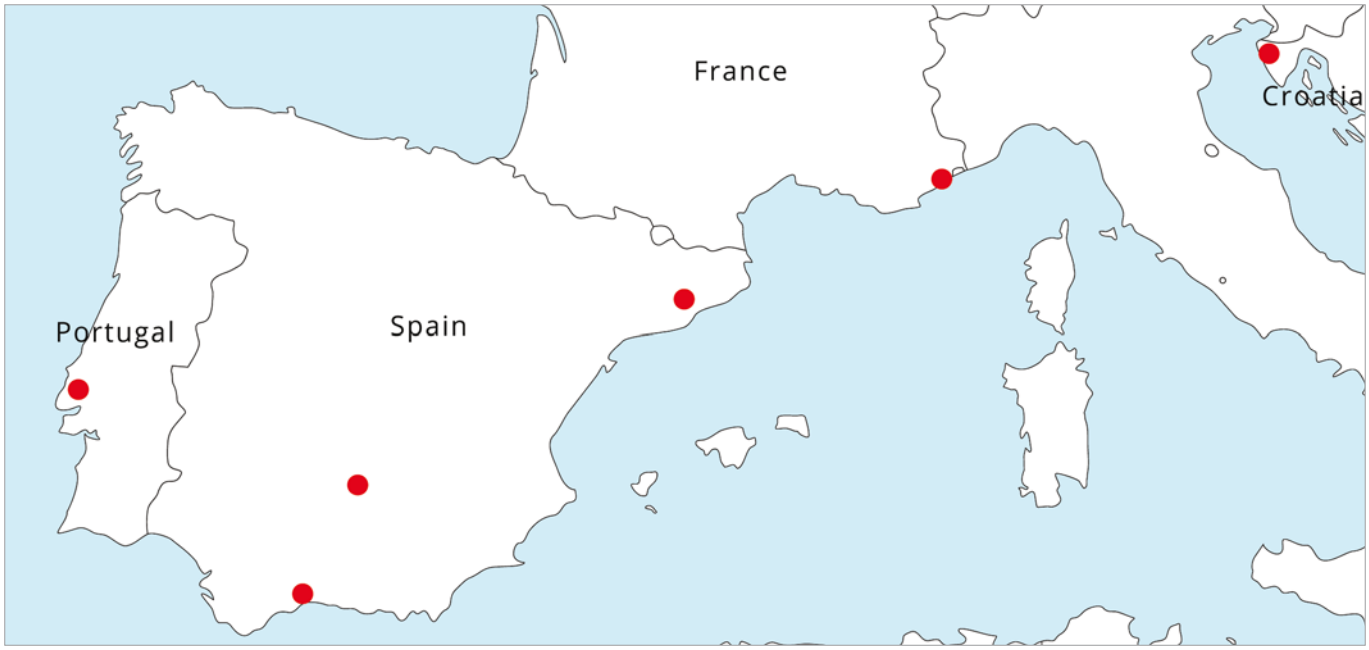

Fig. 2 — Distribution map of known collections of *Paratricharina poiraultii*

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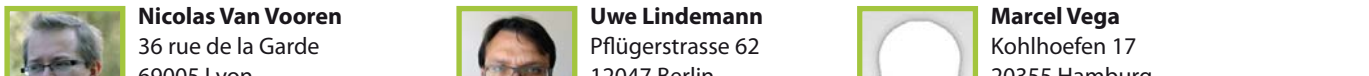

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