Lasiocupulina mediterranea (*Pezizales*), a new genus and species from Albania

Nicolas VAN VOOREN
Marcel VEGA

Ascomycete.org, 10 (6) : 221–227
Mise en ligne le 30/12/2018
10.25664/ART-0246

Abstract: A description and illustration is provided of the new genus *Lasiocupulina* and new species, *L. mediterranea*, recently discovered in a canyon during a field trip in Albania. The fringed margin is the most remarkable character of this small discomycete. The results of a rDNA analysis are also provided and confirm the novelty and phylogenetic position of this taxon.
Keywords: *Cupulina*, molecular phylogeny, *Pyronemataceae*, taxonomy, *Tricharina*.

Résumé : description et illustration d'un nouveau genre et d'une nouvelle espèce, *Lasiocupulina mediterranea*, découverte récemment dans un canyon lors d'une excursion en Albanie. La marge frangée est le caractère le plus remarquable de ce petit discomycète. Une analyse ADN est également fournie et confirme l'originalité de ce taxon.
Mots-clés : *Cupulina*, phylogénie moléculaire, *Pyronemataceae*, taxinomie, *Tricharina*.

Introduction

During a recent field trip in Albania, the second author collected a small discomycete resembling *Cupulina ascophanoides* (Boud.) Van Vooren. The microscopical examination and a phylogenetic analysis revealed that the two collections represent a new genus and a new species, close to *Cupulina* (Dougoud *et al*., 2015). We present the results of our study herein.

Materials and methods

Morphology, cytology and cytochemistry. — The observations were made on living and dried material, rehydrated for about two hours in tap water. The microscopic characters were observed and measured in tap water and Lugol's solution (IKI) was used to test the amyloid reaction of the ascus. Measurements were made on 30 ascospores floating freely in our preparation in water (rehydrated material), under the 100× oil immersion lens of a transmission light microscope Olympus CX-31. 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 a digital camera, while micrographs were taken using digital camera mounted directly on the microscope. Line drawings were made freehand to scale.

DNA extraction, amplification and sequencing. — DNA was extracted using the same method as described in Van Vooren *et al.* (2015), except for evolutionary analyses which were conducted in MEGA X (Kumar *et al*., 2018) with 90 nucleotide sequences for the LSU locus (845 sites) and 86 for the ITS (469 sites). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2000) with 1000 bootstrap iterations. The LSU tree with the highest log likelihood (-5200.76) is shown; the same was done for the ITS tree (-6116.38). The percentage of trees in which the associated taxa clustered together is shown next to the branches. 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 [5 categories (+G, parameter = 0.3255)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 32.78% sites). The trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

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

Table 1: Collections of *Lasiocupulina mediterranea* sequenced and used in the molecular phylogenetic study, with voucher information and GenBank accession numbers

Species	Herbarium #	Collector	28S LSU	ITS
Lasiocupulina mediterranea	MV180702-01	M. Vega	MK238281	MK238279
Lasiocupulina mediterranea	N.V. 2018.07.01	M. Vega	MK238282	MK238280

Taxonomy

Lasiocupulina Van Vooren & M. Vega, *gen. nov.* – MB 828903

Diagnosis: Differs from *Cupulina* by the presence of a hairy margin and its molecular data.

Type species: *Lasiocupulina mediterranea* Van Vooren & M. Vega

Etymology: From Greek, *lasios* (λάσιος), which means shaggy or wooly, and *Cupulina*, referring to its similarity to that genus.

Lasiocupulina mediterranea Van Vooren & M. Vega, *sp. nov.* – MB 828904

Diagnosis: Differs morphologically from the most similar *Cupulina ascophanoides* by its smaller ascospores, its fringed margin made of short, hyaline to light brown-yellow hairs, and its growth at a lower elevation. Holotype – Coll. MV180702-01, deposited in HBG.

Etymology: Derived from the Mediterranean Sea, referring to the region in which the species was collected.

Description (Pl. 1–3)

Apothecia sessile, discoid, 1.5–2 mm diam., fully pale orange, with a fringed margin made of short, hyaline hairs.

Subhymenium of *textura intricata*, with orange-coloured hyphae. **Medullary excipulum** ~50–70 µm thick, of *textura intricata*, made of hyaline hyphae. **Ectal excipulum** ~50–60 µm thick, of *textura globulosa/angularis*, with hyaline cells, 10–25 µm wide, becoming a *textura prismatica* in the marginal area. **Marginal hairs** 45–100 × 6–12 µm, hyaline or light brown-yellow coloured, superficial, dense, obtuse, septate, with a slightly enlarged single base, 1–1.2 µm thick-walled, mixed with hyaline, clavate cells. **Anchor hyphae** present but scattered at the outer surface base, 4–6 µm wide, hyaline, with a bulbous base, 15–20 µm wide. **Asci** cylindrical, 160–190 × 13–16 µm, 8-spored, tapering at the base, with croziers, inamyloid. **Paraphyses** cylindrical, not enlarged at the top, 3–4 µm diam., hyaline. **Ascospores** ellipsoid with tapered ends, (16) 17–19 × 9–11 µm [X = 17.7 × 10.2 µm], Q = 1.6–1.7–1.9, hyaline, smooth, thin-walled, eguttulate, sometimes with a few bipolar spore granules (BSG); overmature spores guttulate, germinating outside the asci.

Studied collections: Albania – Vuno, Kanioni i Gjipesë, 40.130069° N, 19.673491° E, 151 m a.s.l., approx. 700 m from the shores of the Mediterranean Sea, on bare soil, in a dried stream, *leg*. M. Vega, 2 Jul. 2018, M.V. 180702-01 (holotype, HBG). Same locality, on soil, between small mosses and liverworts, 40.131736° N 19.674066° E, 177 m a.s.l., 2 Jul. 2018, ex herb. M.V. 180702-03, N.V. 2018.07.01 (LY).

Discussion

Morphologically *Lasiocupulina mediterranea* is rather similar to *Cupulina ascophanoides* but there is a striking difference when studied under the binocular hand-lens: the margin is distinctly fringed due to the presence of numerous short hairs (Pl. 1, fig. C–E). Although *C. ascophanoides* possesses some scattered marginal hy-

Plate 1 – *Lasiocupulina mediterranea*
A. Locality of the collections. B. Type of habitat. C-D: Coll. N.V. 2018.07.01 *in situ*. E: Specimen rehydrated from coll. N.V. 2018.07.01. F: Coll. MV180702-01 (holotype) *in situ*. All photos by M. Vega, except E by N. Van Vooren.

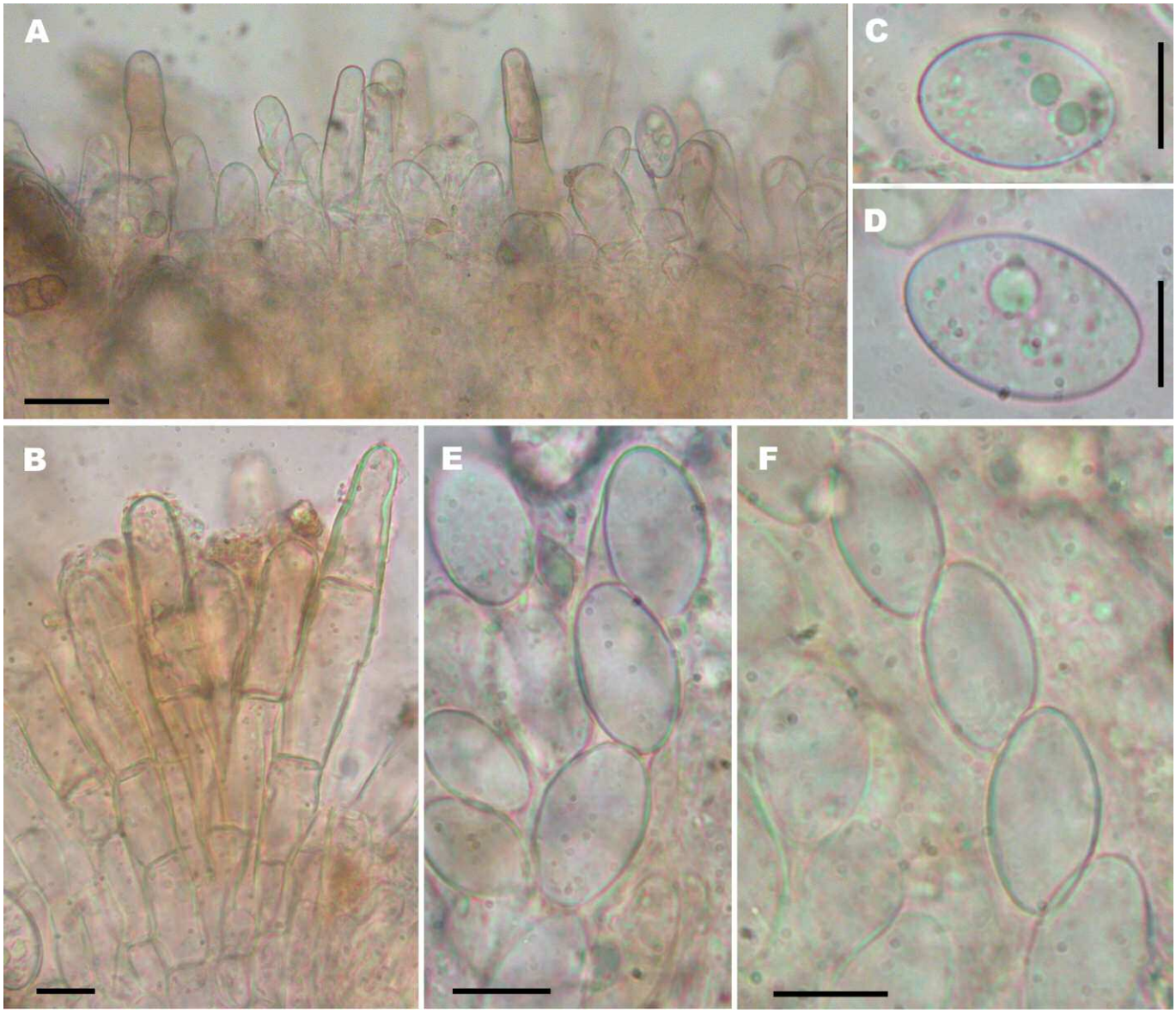


Plate 2 – *Lasiocupulina mediterranea* – Microscopic characters in water.
A–B: Marginal hairs. C–D: Overmature ascospores. E–F: Ascopores. Scale bars = 10 µm. All photos by N. Van Vooren.

phoid hairs (Dougoud, 2002; Van Vooren *et al.*, 2017) mixed with emerging cells, here we can observe true hyaline or light-coloured hairs in the prolongation of the marginal cells. The ascospores of *L. mediterranea* differ as well, they are a bit shorter on average, reaching 19 µm in maximum length (vs. 24 µm for *C. ascophanoides*), and the ascospore shape is different due to its tapered ends. The bipolar spore granules also seem to be less numerous compared to our own observations of *C. ascophanoides* or are absent, but further collections of *L. mediterranea* are needed to confirm that. *Cupulina montana*, the type-species of *Cupulina*, has very different and much longer ascospores (Dougoud *et al.*, 2015) and thus cannot be confused with this new species. Ecologically it is important to also note that *L. mediterranea* was growing at a lower elevation than the two species mentioned (between 750 and 2520 m a.s.l. for *C. ascophanoides*[1], 1600 to 2040 m a.s.l. for *C. montana*).

The presence of coloured hairs and eguttulate ascospores could also suggest the genus *Tricharina* Eckblad emend. Van Vooren *et al.* (2017). However, the marginal hairs are organized in clusters and are generally longer, except in *T. glabra* U. Lindemann & Böhning, the other characters of which are very different though (Lindemann & Böhning, 2016). *Pseudaleuria fibrillosa* (Massee) J. Moravec possesses eguttulate ascospores and similar marginal hairs but its ascospores are ellipsoid with rounded ends, the paraphyses contain carotenoids in lipid bodies, and numerous flexuous excipular hairs are present.

The molecular data based on the 28S LSU locus (Fig. 4) and on ITS (Fig. 5) exclude a relationship with the genus *Tricharina* and place the new species into a sister clade of *Cupulina*. The estimates of evolutionary divergence between sequences of *Lasiocupulina mediterranea* and those of *Cupulina ascophanoides* show a difference of 3.9 to 4.1% on the 845 bp of the LSU alignment and about 2.5% on the 469 bp on the ITS alignment. We are convinced this justifies the proposal of a new genus.

Finally, the results obtained suggest that the full diversity in this group of tricharinoid species is still to be discovered.

[1] With the exclusion of the Norwegian collections, located at a lower elevation (but at a higher latitude).

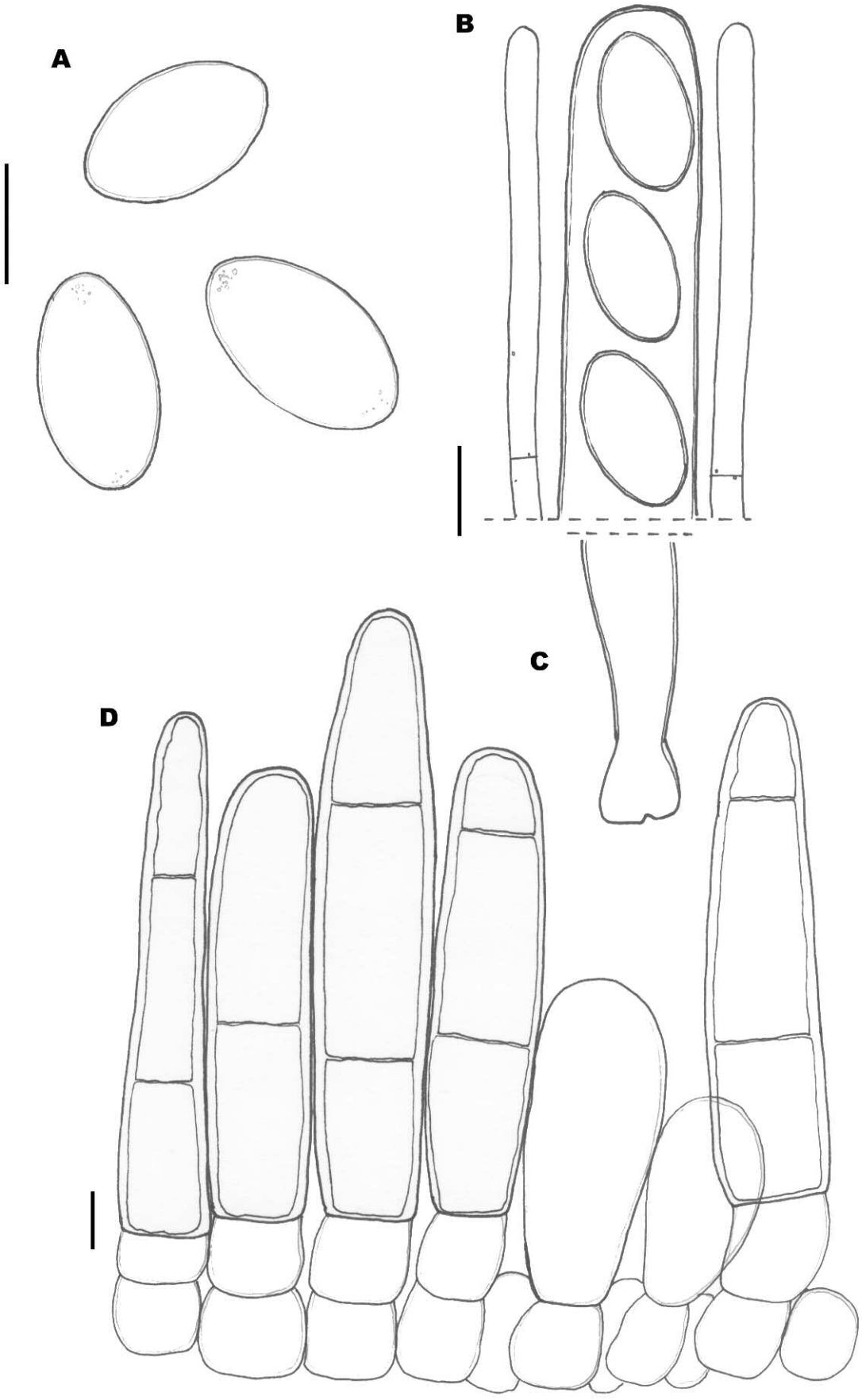


Plate 3 – *Lasiocupulina mediterranea* – Microscopic characters in water.
A: Ascospores. B: Top of paraphyses and ascus. C: Ascus base. D: Marginal hairs. Scale bars = 10 µm. Drawing by N. Van Vooren.

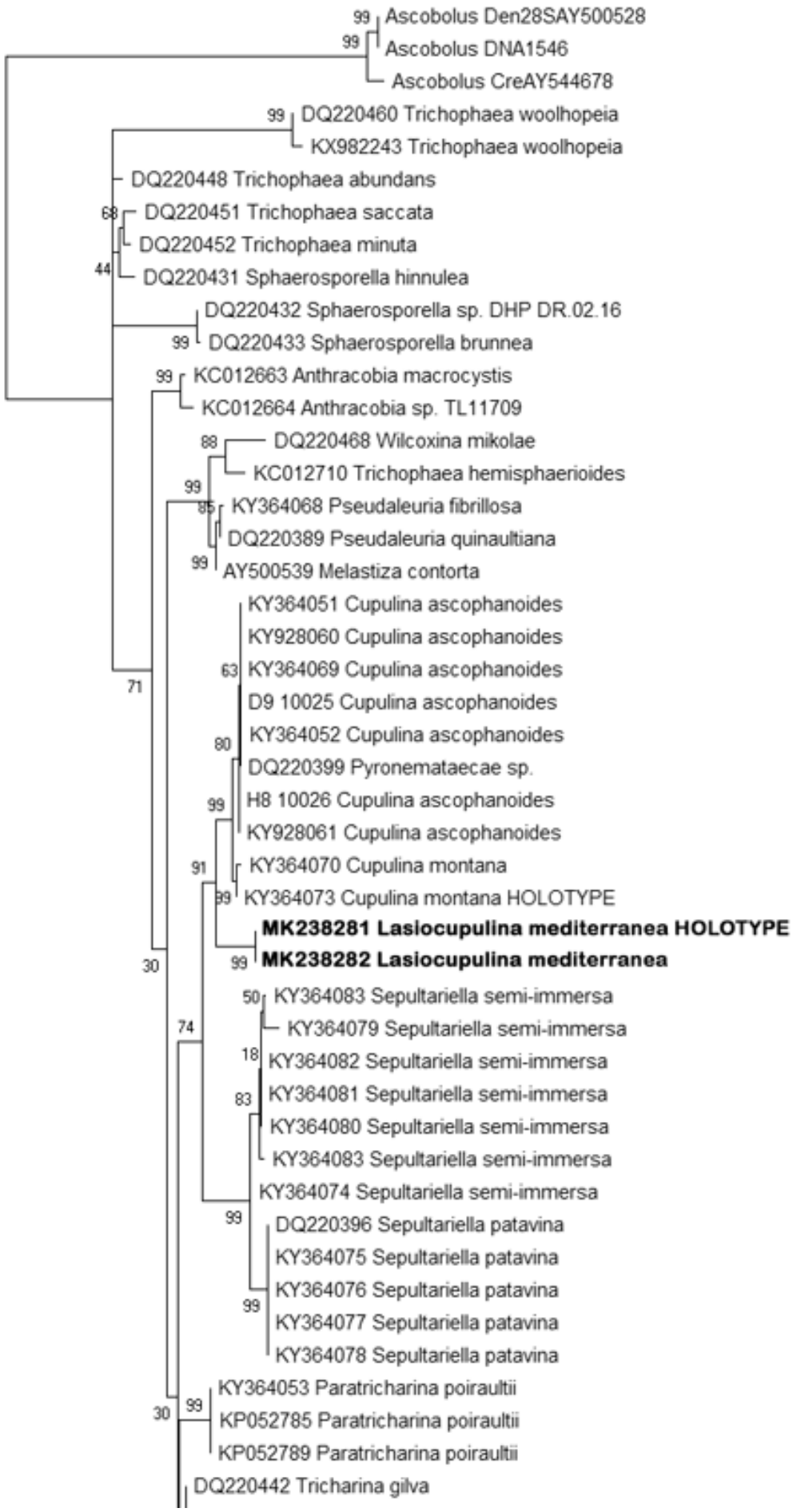


Plate 4 – Molecular phylogenetic analysis by Maximum Likelihood method of LSU alignment of *Cupulina* and allies, including the new *Lasiocupulina*, rooted by *Ascobolus* species.

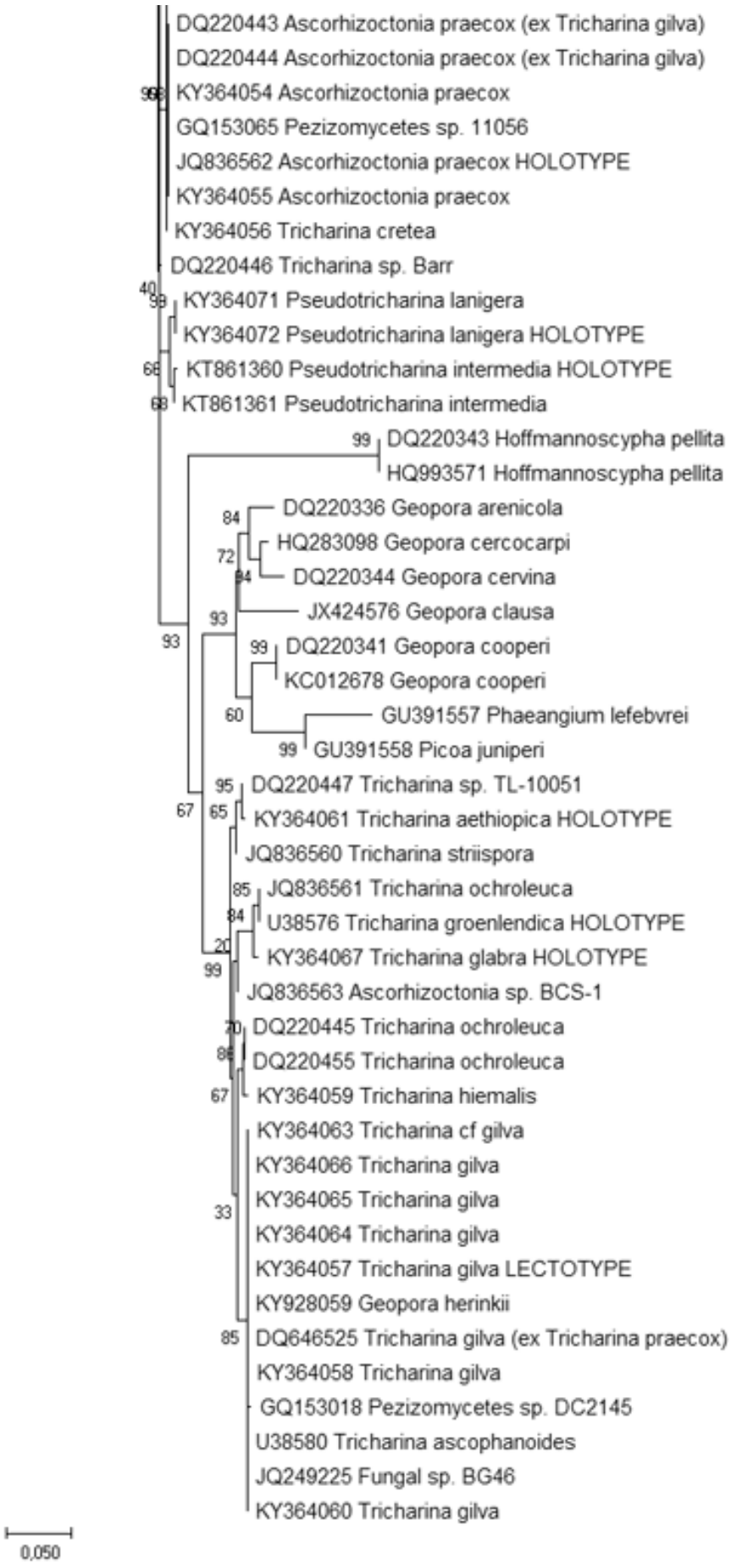


Plate 4 (continued) – Molecular phylogenetic analysis by Maximum Likelihood method of LSU alignment of *Cupulina* and allies, including the new *Lasiocupulina*, rooted by *Ascobolus* species.

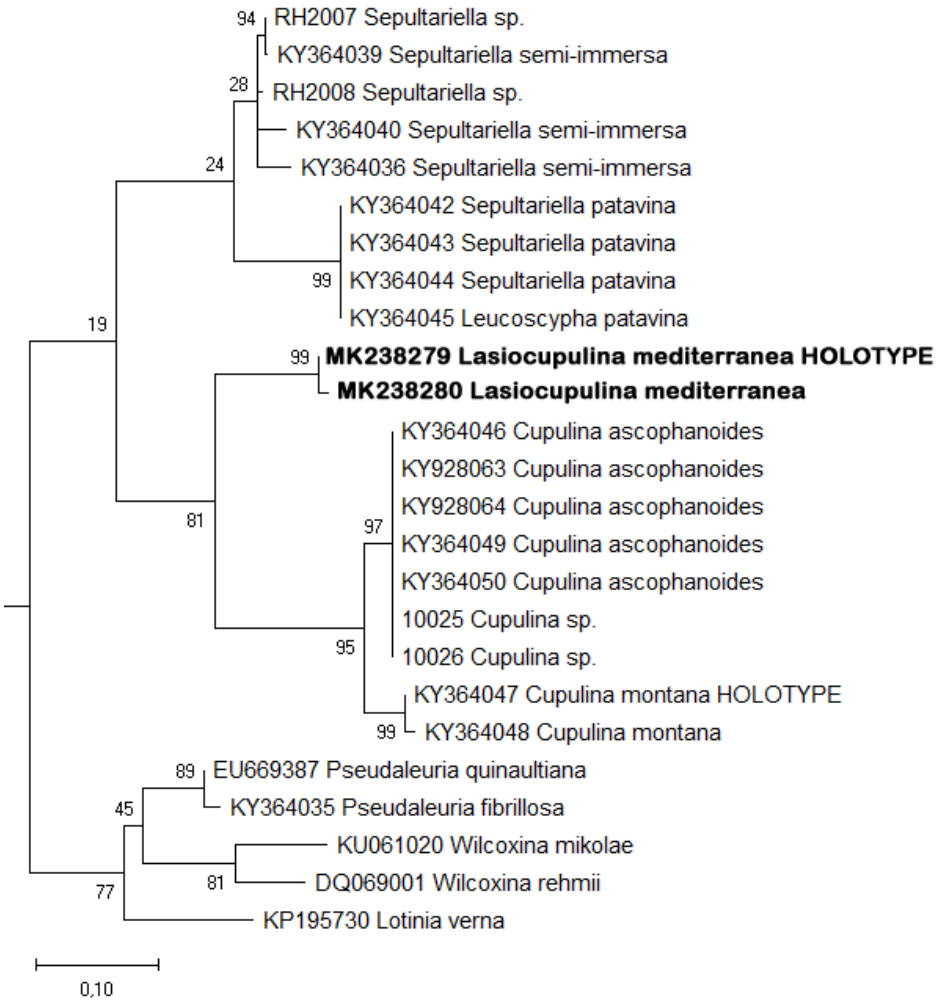


Plate 5 – Extract of the molecular phylogenetic analysis by Maximum Likelihood method of ITS alignment of *Cupulina* and allies, including the new *Lasiocupulina*.

Acknowledgements

We thank Pablo Alvarado (Alvalab, Spain) for the rDNA sequencing. Uwe Lindemann (Germany) and Rosanne Healy (USA) are warmly thanked for the pre-submission review of the manuscript and their valuable suggestions.

References

Dougoud R. 2002. — Contribution à la connaissance de quelques Discomycètes operculés rares ou méconnus. *Fungi non delineati*, 18: 1-69.

Dougoud R., Van Vooren N. & Vega M. 2015. — *Cupulina montana* (*Pezizales*, *Pyronemataceae*), un nouveau genre et une nouvelle espèce des montagnes d'Europe. *Ascomycete.org*, 7 (2): 39–44. doi: 10.25664/art-0126

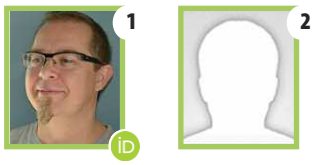
Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. — MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549. doi: 10.1093/molbev/msy096

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

Nei M. & Kumar S. 2000. — *Molecular Evolution and Phylogenetics*. New York, Oxford University Press, 333 p.

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

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



1: N. Van Vooren – 36 rue de la Garde, 69005 Lyon, France – nicolas@vanvooren.info

2: M. Vega – Kohlhoefen 17, 20355 Hamburg, Germany – tomprodukt@web.de