Erioscyphella lunata (*Lachnaceae*), a rare discomycete collected in Spain

Salvador TELLO
Hans-Otto BARAL

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Abstract: The rare discomycete *Erioscyphella lunata*, inhabiting coniferous leaves and originally described from China, is re-described from a recent collection from southern Spain, which is the first report from Europe. Its molecular data are compared to the closest species and confirm its distinctness from the recently described smaller-spored *E. curvispora*.
Keywords: Andalucía, Ascomycota, conifer needles, *Lachnum lunatum*, *Pinus nigra*, vital taxonomy.

Resumen: El raro discomycete *Erioscyphella lunata*, que habita en las hojas de coníferas descrito originalmente de China, es re-descrito de unas colecciones recogidas en el sur de España, que es el primer informe para Europa. Sus datos moleculares se comparan con las especies más cercanas y confirman su distinción de la recientemente descrita *E. curvispora*, de esporas más pequeñas.
Palabras clave: Acículas de coníferas, Andalucía, Ascomycota, *Lachnum lunatum*, *Pinus nigra*, taxonomía vital.

Introduction

During an excursion In early spring 2016, prospecting the Ascomycetes fungi growing on different species of *Pinus* (*Pinaceae*) in the south of the province of Jaén (Spain) on chalky soil at altitudes between 1000 and 1700 m, a small yellow discomycete growing on pine needles fallen from the tree *Pinus nigra* was collected. The fungus reminded of *Erioscyphella curvispora* Perić & Baral, recently described from Montenegro (Perić & Baral, 2015), but deviated by much larger asci and ascospores. Its characters appeared instead to concur well with the protologue of the Chinese *E. lunata* (W.Y. Zhuang & Spooner) Perić & Baral which was described in Zhuang (2000) from needles of *Pinus ?armandii*, as *Lachnum lunatum* W.Y. Zhuang & Spooner. A thorough morphological analysis revealed no major differences to that species. A molecular comparison was not possible since no data were available for the type collection from China, but the high molecular distance to *E. curvispora* confirms independence of the two species. Collections from the Netherlands on *P. nigra* studied by Rommelaars (2013) and from England on *P. sylvestris* studied by P. Thompson (ined.) are somewhat intermediate between both species.

Materials and methods

The Spanish collections of *Erioscyphella lunata* were examined in the fresh, living state in tap water based on methods of "vital taxonomy" (see Baral, 1992), using a Canon EOS 40D camera mounted on a microscope Optika B-350, with a LED lamp for illumination in the case of macro photography (S.T.), and a Nikon Coolpix 4500 held free-hand to a Zeiss Standard 14 (H.B.). The following media were used for microscopic observation: tap water, 5% KOH, potassium iodide solution 3% with 1% iodine (IKI), aqueous cresyl blue ~0.5%, and aqueous Congo Red. Measurements were performed in tap water for living cells and separately in KOH for dead cells, partly by adding Congo Red. Cresyl Blue (CRB) was used to test cell wall surfaces of hairs, ascospores etc. Measurements for living (*) and dead elements (†, usually in KOH) are given separately. Specimens are deposited in the private herbarium of S. Tello (S.T.) and H.O. Baral (H.B.), and the herbarium of JACUSSTA (Junta de Andalucía, La Trufa, Zagrilla, Priego de Córdoba). The rDNA sequence of *E. lunata* gained by P. Alvarado (Alvalab) comprises the ITS1-5.8S-ITS2 and 28S D1–D4 region and was deposited in GenBank.

Taxonomy

Erioscyphella lunata (W.Y. Zhuang & Spooner) Perić & Baral, *in* Perić, *Mycol. Monten.*, 17: 103 (2015).

≡ *Lachnum lunatum* W.Y. Zhuang & Spooner, *in* Zhuang, *Mycologia*, 92 (3): 594 (2000).

Description of Spanish collections

Ascomata gregarious or solitary 0.2–0.7 mm, with a short stout stipe, superficial, hymenium flat, smooth, bright (egg-)yellow, exterior of receptacle and stipe covered with white hairs. **Stipe** 0.1–0.2 × 0.1–0.2 mm cylindrical, wider at the top.

Ectal excipulum composed of hyaline prismatic or almost globular cells, which decrease in size towards margin, near base of receptacle *(6–) 9–16 (–22) × (5.5–) 7–13 (–18) µm, N = 40, Me = 11.3 × 8.8 µm, †6.5–17.5 x 5.5–11 µm, distinctly thick-walled also in the living state, not thicker in KOH (*/†0.5–1 µm). **Medullary excipulum** composed of elongated hyaline cells, sometimes bifurcate or branched (4–) 4.7–8 (–9) µm wide, Me = 6.4 µm, forming a network of intricate texture. **Hairs** *(51.5–) 57.5–80 (–84) × 4.7–6.2 (–6.8) µm, N = 33, Me = 66.3 × 5.3 µm, cylindrical, apex rounded to obtuse, often somewhat spathulate to lageniform or sublanceolate, hyaline, thin-walled (†0.3 µm), emerging from cylindrical cortical cells, with 1–3 septa, terminal cell 30–38 µm long, lower cells always shorter, entirely densely covered with small prominent warts, partly immersed in a kind of yellowish resinous mass, wall surface and warts stained blue-violet in CRB. **Asci** *(79–) 83–97 (–101) × (6–) 6.5–8 (–8.3) µm, N = 37+11, Me = 88.8–89.8 × 7.2–7.3 µm, †62–80 × (4.5–) 5–5.5 (–6.2) µm, cylindrical, with a short and wide stipe; 8-spored, *pars sporifera* *73–84 µm long, †50–75 µm, subbiseriate in living asci, fitting together by pointing with the convex side inwards; apex conical, with slightly hemiamyloid apical ring (IKI blue then dirty reddish-grey, type rB), *Calycina* type; without croziers at the base. **Ascospores** *(12.5–) 14–16.5 (–17.8) × (2.5–) 2.7–3.6 (–4) µm, †(5.5–) 9–14 × 2.2–3 µm [direct distance from end to end], Q = *(3.7–) 4–5 (–5.6), N = 63, Me = *15.2 × 3.5 µm, Qe = *4.4; *(14–) 15–18 (–20) µm [measured along the curved axis], Q = *(3.9–) 4.4–5.7 (–7.5), N = 67, Me = *16.3–17.7 × 3.1–3.3 µm, Qe = *5–5.8; curved like a boomerang, gradually tapering at both ends, smooth, hyaline, non-septate; containing quite a few small oil droplets (LBs) grouped in each half, these sometimes confluent after rehydration; wall surface unstained in CRB. **Paraphyses** not exceeding the living asci, cylindrical to very slightly lanceolate, septate below, upper cell *40–59 × (2.2–) 2.8–4 (–4.7) µm, lower cells *9–13 × 2.5–3 µm; containing some minute yellow oil drops along the lateral wall and between the vacuoles.

Habitat (Spanish collections): on fallen needles of *Pinus nigra*. Altitude: 1085–1350 m. Desiccation tolerance: ectal excipular cells, hairs, paraphyses and many mature asci still alive after 3 and 6 weeks in the herbarium.

Distribution: Ningxia (central northern China), Andalucía (southern Spain).

Material studied: España, Andalucía, 21 km S of Jaén, 1 km E of Valdepeñas de Jaén, La Solana, 30SVG 28885 60623, 37° 35′ 24″ N, 3° 48′ 20″ W, 1085 m, on fallen needles of *Pinus nigra*, 13/02/2016, *leg*. S. Tello (ex S.T. 13021602, JA-CUSSTA 8292, GenBank KX501132 ITS, KX501133 LSU). *Ibid*., 20/04/2016, *leg*. S. Tello (S.T. 20041601). *Ibid*., 1.3 km E of Valdepeñas de Jaén, La Solana, 30SVG 29141 60971, 37° 35′ 35″ N, 3° 48′ 9″ W, 1155 m, on fallen needles of *Pinus nigra*, 12/05/2016, *leg*. S. Tello (ex S.T. 12051601, H.B. 10005). 19 km S of Jaén, 5.5 km ENE of Valdepeñas de Jaén, Puerto de las Coberteras, 30SVG 32838 63220, 37° 36′ 49″ N, 3° 45′ 40″ W, 1350 m, on fallen needles of *Pinus nigra*, 8/03/2016, *leg*. S. Tello (ex S.T. 08031602, JA-CUSSTA: 8293, H.B. 9987).

Observations: The here presented Spanish collections identified as *Erioscyphella lunata* concur profoundly with the original description of the type collection of *Lachnum lunatum* from China, for which reason we believe that they are conspecific. Size and shape of the dead ascospores (†9–14 × 2.2–3 µm) correspond well to the protologue (†12–13 × 2.5–2.8 µm) when comparing the straight distance from end to end, also ascus size (†62–80 × 4.5–6.2 µm) matches almost perfectly the holotype description (†60–70 × 5–6 µm). Even the characteristic spore arrangement, reported in the present paper, can tentatively be recognized in the dead ascus figured in the protologue. The contents of the ascospores in the protologue are given as "2 large and several small guttules", which appears to refer to rehydrated living spores as here shown on Fig. 3a,

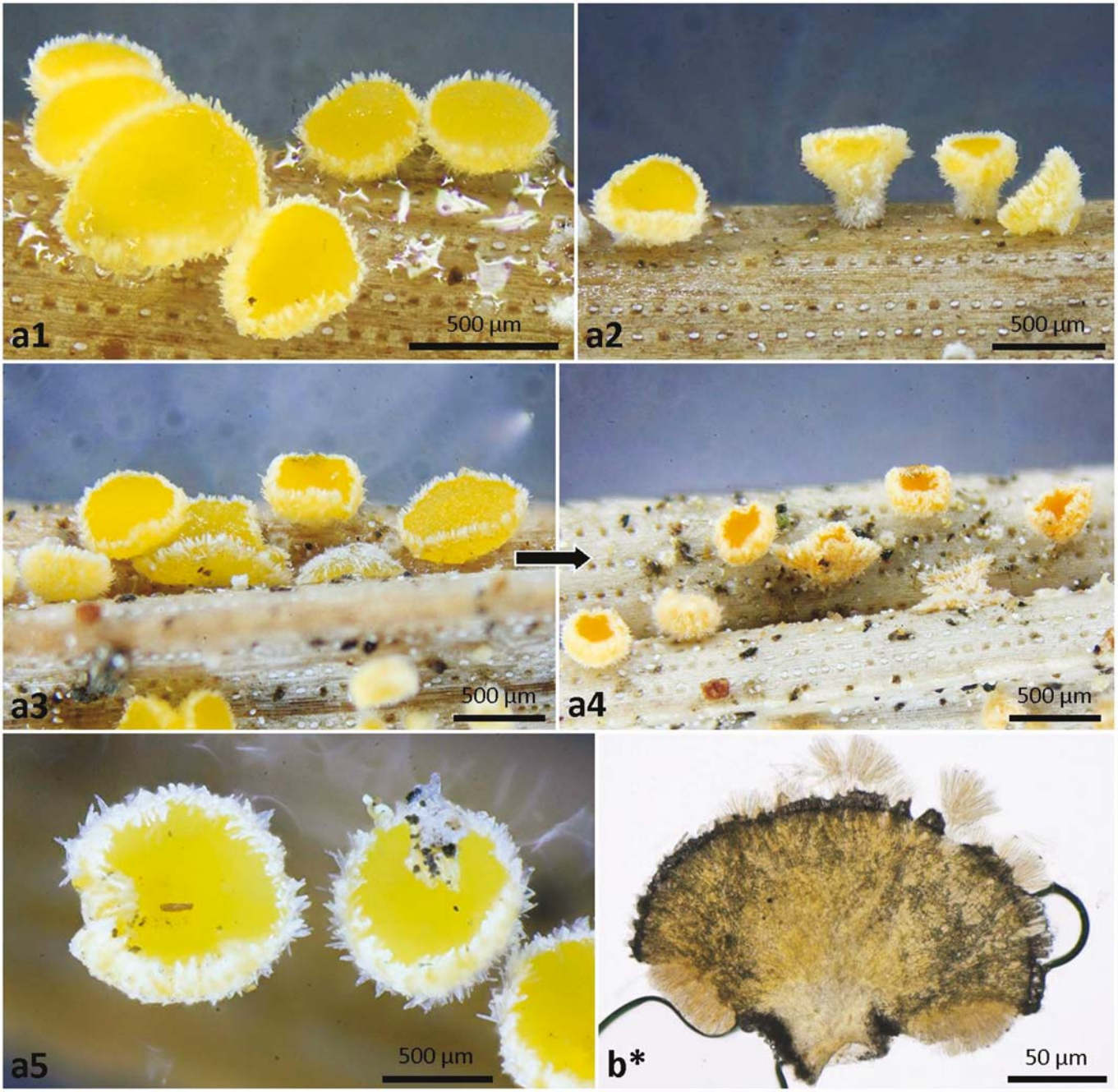

Fig. 1 – Morphology of *Erioscyphella lunata* (a1,2,5 & b, S.T.13021602, a3,4 ex S.T. 12051601, H.B. 10005). a. Fresh and dry (a4) apothecia. b. Exterior of apothecium. Mounting medium: H_2O = b.

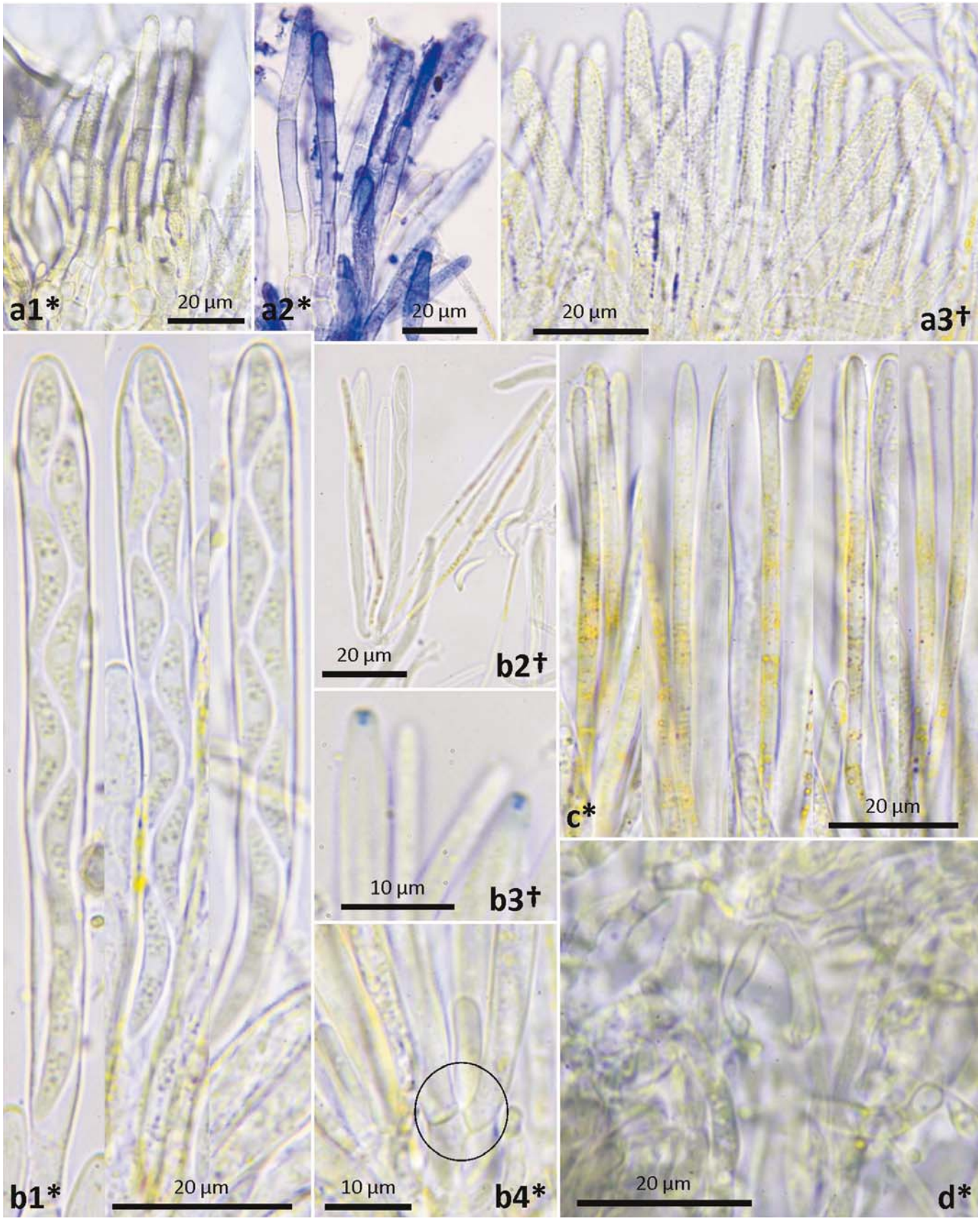

Fig. 2 – Morphology of *Erioscyphella lunata* (all from S.T.13021602). a1-2. Living hairs. a3. Dead hairs. b1. Living asci showing characteristic spore arrangement. b2. Dead asci and paraphyses. b3. Apices of dead immature asci. b4. Base of asci showing absence of croziers, c. Living paraphyses. d. Medullary excipulum. Mounting medium: H_2O = a1, b1, b4, c, d; KOH = a3, b2; KOH + IKI = b3; CRB = a2.

while the holotype drawing shows dead spores with irregularly shaped contents. In the Spanish samples the excipular cells measured †6.5–17.5 × 5.5–11 µm (in KOH + Congo Red) and showed a distinctly thickened wall which, however, was not thinner in the living state. The protologue of *L. lunatum* gives the cells somewhat longer and narrower (9–18 × 3.5–9.5 µm), and thin-walled.

Ascospore arrangement in *E. lunata* is fairly extraordinary within the *Helotiales*. Even at full turgescence the spores reach almost down to the very ascus base. They consistently point with their convex side inwards and lie either in one plane, showing a zigzag line across the asci (Fig. 2 b1), or in different planes. Due to the relative paucity of vacuolar ascus water, ejection is probably less forcible than in *E. curvispora*. Spore ejection from turgescent asci in a water mount was once observed by the first author. Shortly after ejection the spores remain temporarily attached together, apparently by some gel (Fig. 3 c1). Actually, in dead asci containing mature spores a violet stain in CRB is observed which appears to originate from this gel. When adding KOH to living asci, they shrink for around 18% in both length and width (Fig. 3 b1-b2). A comparable shrinkage of ca. 15–25% was observed when adding KOH to living ascospores.

When rehydrating a dried specimen four weeks after collecting (H.B. 9987), many spores were still alive, though with 2–4 large, globose, confluent oil drops (Fig. 3a). However, in the third sample re-studied after 3 (S.T. 12051601) and 6 weeks (H.B. 10005), the living spores contained many small LBs just as they were in the fresh state, and also many mature asci had survived, besides paraphyses, hairs, and excipular cells.

E. curvispora, growing on pine needles of *Pinus heldreichii* in Montenegro, is easily separable by its much smaller, especially shorter asci and ascospores (see Tab. 1), resulting in a lower length/width (Q) quotient (about *4–5 for the ascospores, along curved axis; ca. *4.5–6 in *E. curvispora*). Differences are also seen in the spore arrangement in living asci: in *E. curvispora* the spores are directed with their convex side outwards, resulting in a more compact, biseriate

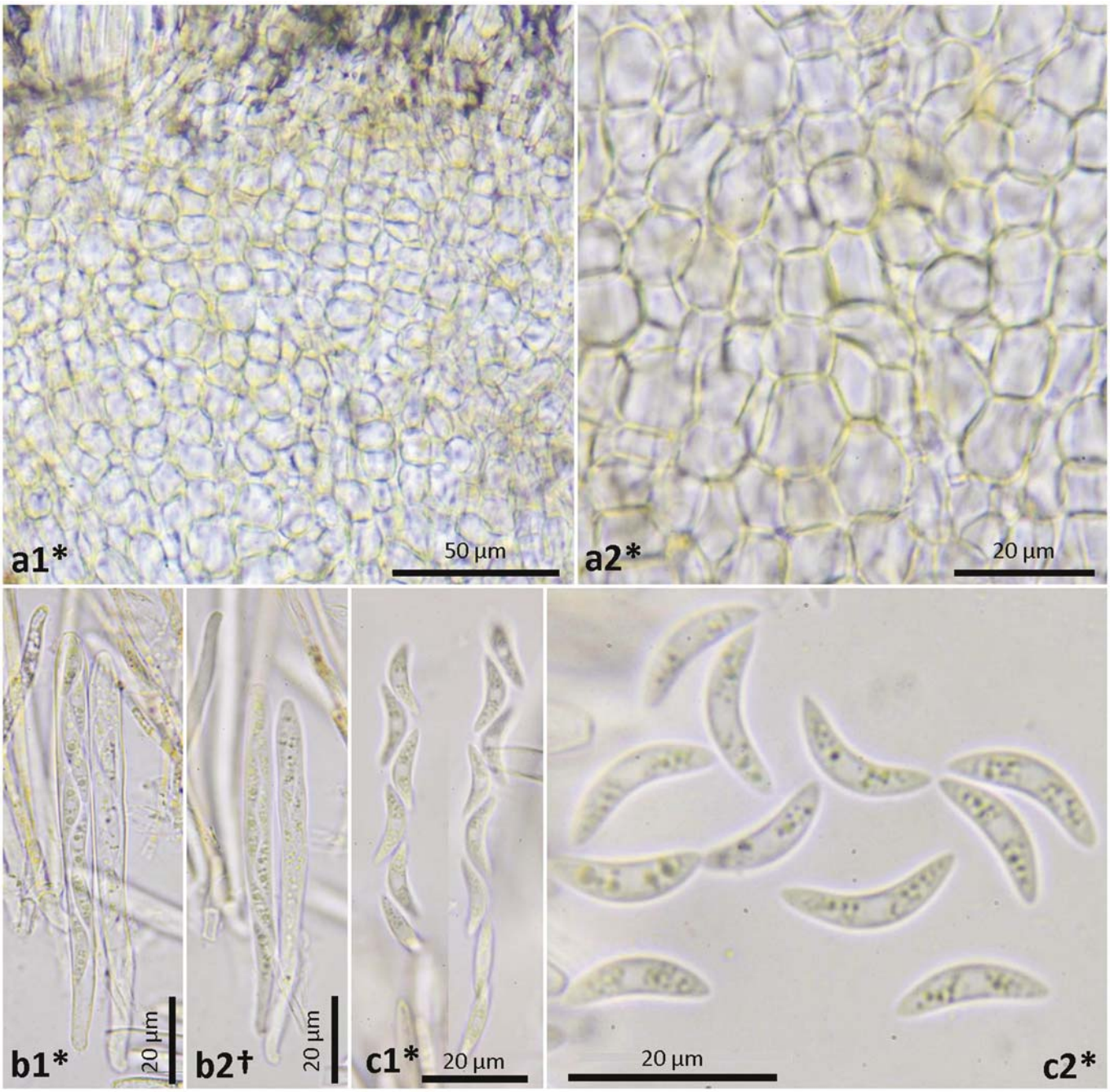

Fig. 3 – Morphology of *Erioscyphella lunata* (a & c2 from S.T. 13021602, b & c1 from S.T. 20041601). a. Ectal excipulum in surface view. b1. Living asci; b2. The same asci in dead shrunken state at the same scale. c. Living ascospores; c1. Ejected ascospores hanging together by some gel; c2. Ejected ascospores separated from each other. Mounting medium: H_2O, except for KOH = b2.

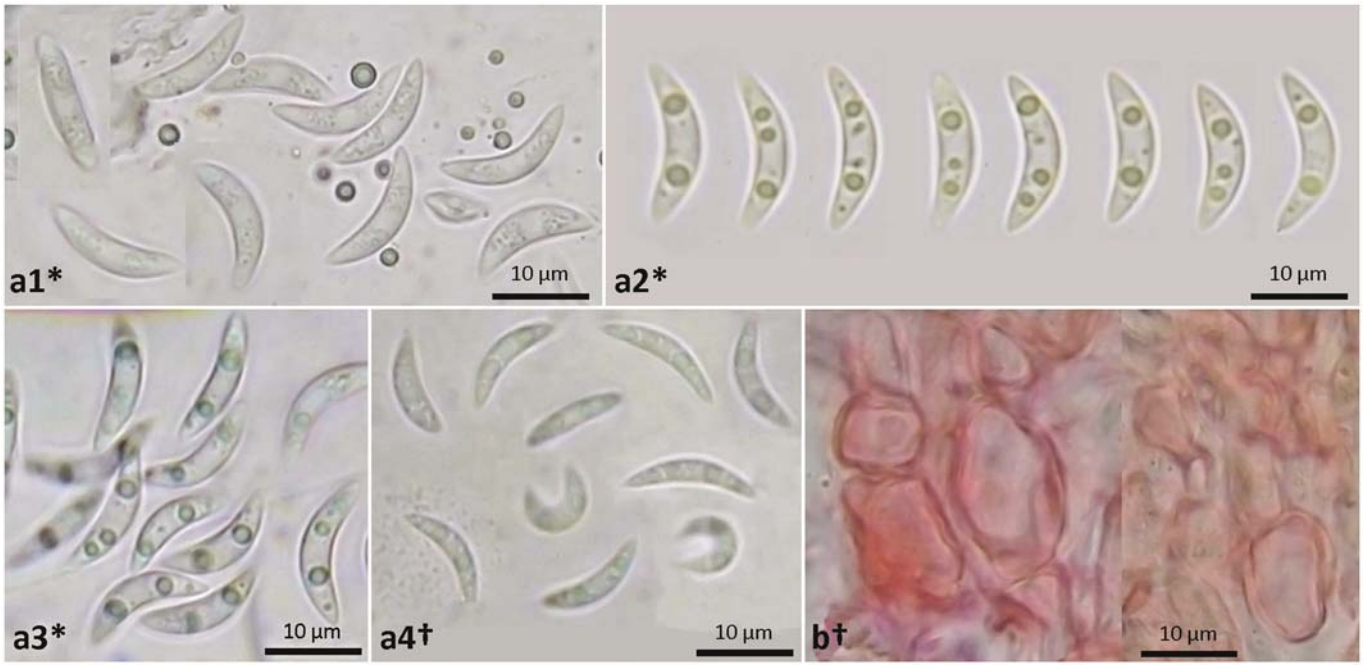

Fig. 4 – (a1 from H.B. 10005, a2-b from S.T. 08031602 = H.B. 9987). a1-a3. Living ascospores after rehydration; a1. showing original pattern of minute guttules (LBs); a2-a3. showing large confluent guttules (LBs). a4. Dead shrunken ascospores at the same scale. b. Dead ectal excipular cells with thick walls. Mounting medium: H_2O = a1-a3, KOH = a4, KOH + Congo Red = b.

spore cluster and, together with a shorter spore length, in a much shorter *pars sporifera*. Also the hymenial colour is much paler yellow to almost white in *E. curvispora*.

Rommelaars (2013) reported *Lachnum lunatum* on needles of *Pinus nigra* from Groningen (Netherlands). The asci (†45–55 × 5–6 µm) are here much shorter than in the Spanish samples, and also the ascospores (*11-15 µm long, probably direct distance from end to end) are smaller. In spore shape and contents made up of groups of small LBs, however, this sample concurs well with Spanish *E. lunata*. Spore arangement in the dead asci appears to concur more with *E. curvispora*, but this species is ruled out due to its much shorter asci and spores and paler hymenial colour. In a sample by P. Thompson (pers. comm.) on needles of *Pinus sylvestris* from Staffordshire (England), the spores as measured *10.3–11.7 × 2.3–2.5 µm (direct distance) and the asci (47.5 × 5 µm), with a spore arrangement unlike that observed in E. lunata. here the spores contained only one minute LB in each half.

Both Zhuang (2000) and Rommelaars (2013) did not specify in their description the presence or absence of croziers at the ascus base. W.Y. Zhuang (pers. comm.) kindly re-examined the holotype of *E. lunata* and found the asci to arise from simple septa. The spores were re-measured as being mainly in the length range of †11–13 µm (direct distance).

According to field notes, the fresh apothecia in the type of *E. lunata* had a size of 0.1–0.3 mm diam. (W.-y. Zhuang, pers. comm.) and a yellow disc. In comparison, the diameter of rehydrated apothecia in H.B. 9987 was 0.22–0.43 mm (excluding hairs), while it was up to 0.7 mm in the first Spanish collection. Also Rommelaars (2013) and P. Thompson (pers. comm.) figured a bright (lemon-)yellow disc on their photos and mentioned a maximum diameter of 0.2 mm or 0.4-0.5 mm, respectively.

Very similar ascospores as in *E. lunata* were drawn by P. Riel (1897 ined., No. 2196) in his unpublished *Dasyscypha echinophila* on spines of fallen involucres of *Castanea sativa* (non *D. echinophila* E.K. Cash).

Ecology: The Spanish samples were made in repopulated orome-diterranean forests of *Pinus nigra* and *P. halepensis*, with scattered native trees and shrubs of *Amelanchier ovalis*, *Crataegus monogyna*, *Pistacia terebinthus*, *Quercus faginea*, *Q. ilex*, and *Sorbus aria*.

The inhabited needles were lying on the damp moist ground, being fallen in the previous year. Only totally 3–4 needles were found to carry apothecia among the many needles on the ground. Their occurrence in early spring suggests that they are adapted to the moist, mediterranean winter climate at mountainous altitude.

The holotype of *E. lunata* was collected in August at 1800 m of altitude in the Liupan Shan mountain range, an area influenced by the seasonal monsoon which is characterised by moist summers and dry winters.

Molecular analysis: *E. lunata* differs in the ITS region from *E. curvispora* by 7.3% and by two inserts of 9 and 1 nucleotides in the ITS1. However, the distances to the two closest species in GenBank, *Erioscyphella abnormis* and *E. sclerotii* (as *Lachnum*), lie at about 6–7%. In a phylogenetic analysis (neighbor-joining) of the ITS region, the two species on pine needles are nested in the moderately supported *Erioscyphella* clade, but they do not cluster together in a subclade. A similar result is obtained in a maximum parsimony analysis.

	***E. curvispora* (Montenegro)**		***E. lunata* (Spain)**	
Asci	*42–53 × 4–6 µm	†35–42 × 4–5 µm	*79–101 × 6–8.3 µm	†62–80 × 4.5–6.2 µm
Pars sporifera	*27.5–33.5 µm	†35–40 µm	*77–84 µm	†50–75 µm
Ascospores[1]	*7.5–11.5 × 2–2.6 µm	†6–9 × 1.5–2 µm	*12.5–19 × 2.5–3.9 µm	†(5.5–) 9–14 × 2.2–3 µm
Hymenium	creamish-white to light yellow-orange		bright egg-yellow	

Tab. 1 – Comparison of ascus and ascospore measurements and hymenial colour between *E. curvispora* and *E. lunata*. ([1] direct distance from end to end)

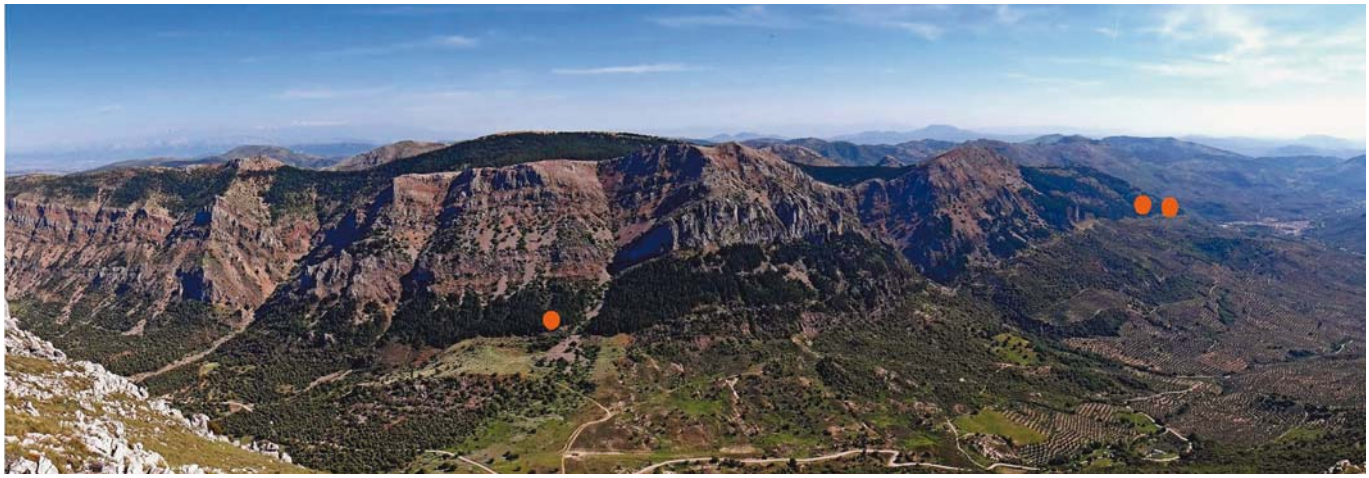

Fig. 5 – Panorama of Sierra de Ventisqueros in Andalucía. Red markings indicate collection sites of *Erioscyphella lunata*.

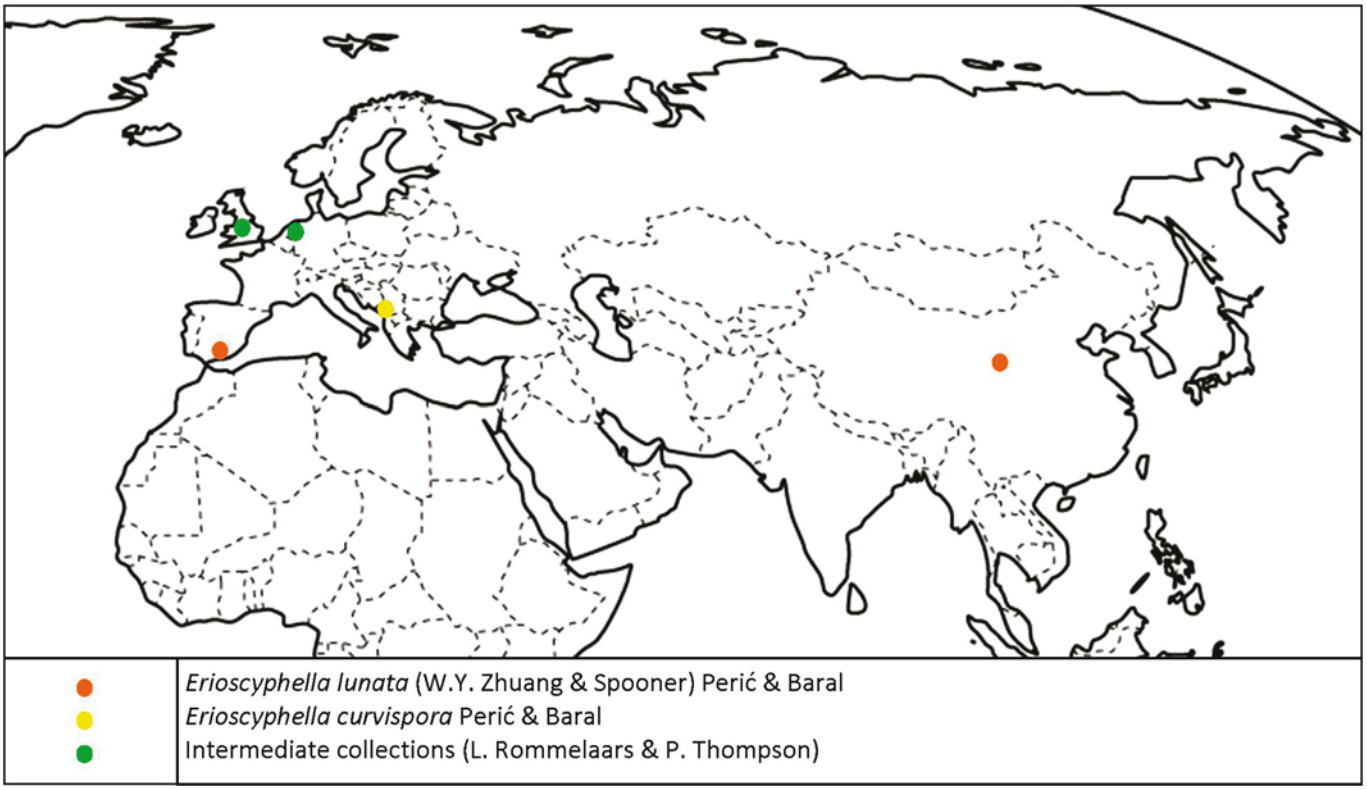

Fig. 6 – Presently known occurrence of *Erioscyphella lunata*, *E. curvispora*, and intermediate collections.

At the 3'-end of the 18S region a large intron of min. 394 nucleotides is present in *E. curvispora* but absent in *E. lunata*. A BLAST search of the 28S region of *E. lunata* in GenBank yields *E. abnormis* as closest match, in the D1-D2 region with a 98% similarity, and in the D3-D4 region with 93%. When analysing the 28S region (NJ), *E. lunata* clusters with high support at the base of the *Erioscyphella* clade which includes *E. abnormis* and some unidentified strains.

Acknowledgements

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References

Baral H.-O. 1992. — Vital versus herbarium taxonomy: morphological differences between living and dead cells of Ascomycetes, and their taxonomic implications. *Mycotaxon*, 44: 333-390.

Perić B & Baral H.-O. 2015 [2014]. — *Erioscyphella curvispora* spec. nov. from Montenegro. *Mycologia Montenegrina*, 17: 89-104.

Rommelaars L. 2013. — Paddenstoelennieuws uit Groningen – II. Één middag Lauwersmeergebied en vijf nieuwe paddenstoelen voor Nederland. *Coolia*, 56 (1): 3–10.

Zhuang W.-Y. 2000. — Hyaloscyphaceous discomycetes from Ningxia Province, China. *Mycologia*, 92 (3): 593-597.

Salvador Tello
Paseo del Obispo 7
23150 Valdepeñas de Jaén
Spain
ajoporros@yahoo.es

Hans-Otto Baral
Blaihofstr. 42
72074 Tübingen
Germany
zotto@arcor.de