

Capitotricha attenuans comb. nov. (Lachnaceae, Helotiales), the story of a forgotten fungus

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Ascomycete.org, 12 (5): 205–213

Mise en ligne le 31/10/2020

doi 10.25664/ART-0308



Abstract: *Capitotricha attenuans* is reported on twigs of the type substrate *Rumex suffruticosus* in a subalpine area near the type locality in the Cantabrian Mountains about 175 years after it was distributed as an exsiccatum by M.C. Durieu under the name *Peziza bicolor* (\equiv *Capitotricha bicolor*). In the 19th century the species was given two different names, *Peziza attenuans* and *Lachnella hispanica*, and in the 20th century R.W.G. Dennis referred it to synonymy of *C. bicolor*. We here transfer its oldest legitimate name to *Capitotricha* because the species is sufficiently distinct from *C. bicolor* in having asci arising from croziers and larger ascospores, besides deviating ITS and LSU rDNA data. An epitype is designated based on the recent sample with a sequence.

Keywords: Ascomycetes, Asturias, croziers, drought tolerance, host specificity, rDNA, taxonomy.

Introduction

In 1835, the French soldier and botanist Michel Charles Durieu de Maisonneuve (1796-1878) made a journey of botanical exploration throughout the Cantabrian territories of the Iberian Peninsula, focusing particularly on Asturias. What is known about this trip is based on the account written by GAY (1836), whose Spanish translation was published more than a century later with numerous side-notes by JÁUREGUI (1958), and later on recapitulated by FERNÁNDEZ PRIETO & VÁZQUEZ (2007).

In those years, there were several botany enthusiasts among the military, such as Bory de Saint Vincent and Léon Dufour, who took advantage of their stay in the Iberian Peninsula during the Franco-Spanish war to study some of its peculiar flora. It was probably the former who, thanks to his naturalistic and cartographic knowledge, arranged Durieu's trip across Asturias (FERNÁNDEZ PRIETO & VÁZQUEZ, *op. cit.*).

Durieu, who had taken part in the French expedition in 1823 with the army of the Hundred Thousand Sons of Saint Louis and who knew the Spanish language and traditions, had to finance the 1835 expedition by selling to several herbariums the specimens he considered new or more interesting, at a modest price. After a complicated journey and entry into Spain, he finally arrived in Gijón, and later on, he made it to Oviedo. Throughout his journey, he kept recording everything he could get his hands on and considered interesting, constantly paying attention to those ethnographic details that struck him.

On June 5, after having spent some time in jail due to some administrative issues, and once he had retired, Durieu entered Grado and ventured inland until reaching Cangas del Narcea, a small town in the southwest of the region; there, he produced a detailed description of the Asturian flora. On July 5, at the pass of Leitariegos, which connects Asturias with the neighboring province of León, he reached Cueto de Arbas, an elevated schistose zone in the Asturian mountains, which reach a height of 2108 m and on whose exposed slopes *Rumex suffruticosus* J. Gay ex Willk. (*Polygonaceae*) grows. This plant is endemic to the high siliceous mountains of the center, north and northwest of the Iberian Peninsula.

Thus, during the months of July and August 1835, Durieu collected some small ascomata growing on that plant, which would end up in the hands of Cooke, after a failed sale of this record to the Kew herbarium (K). Cooke, together with W. Phillips, published it as *Lachnella hispanica*, to be later recombined by SACCARDO (1892) in the genus *Dasyscypha*. Years later DENNIS (1963) offered a brief review of this fungus, this time along with some drawings, in which

he wrongly stated that “the fungus is the typical *Dasyscyphus bicolor* (Bull. ex St. Amans) Fuckel” (\equiv *Capitotricha bicolor* (Bull.) Baral).

The fungus presented here is very common in the *Rumex suffruticosus* populations of the pass of Leitariegos, according to various occasions when it was seen in the field, between May and September. Possibly it occurs also in the colder season but no excursions have been done to verify this, and the frequent snow cover and avalanches would make it difficult. The species grows in lignified dead basal parts of the plant, developing a great number of small ascomata with yellowish hymenium and conspicuous snow-white hairs. These ascomata, which have adapted to the sudden changes in humidity and temperature in the region, are able to shield themselves by folding the hairs inwards, thus maintaining the necessary humidity to ensure their survival. A detailed study of the fruit bodies and the corresponding genetic studies suggest it is necessary to combine this fungus into the genus *Capitotricha* (Raitv.) Baral, where it clusters distant from other species of the genus, including the heterogeneous *C. bicolor*.

Materials and methods

The two here reported collections of *Capitotricha attenuans* were predominantly studied in the living state. The preparation methods follow BARAL (1992). Specimens were deposited in the private herbarium of the first author (E.R.D.). rDNA sequences were obtained from apothecia in the laboratory of Pablo Alvarado (alvalab.es) with the following protocol: DNA extraction, amplification and sequencing: Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY & THOMPSON (1980). PCR reactions (MULLIS & FALOONA, 1987) included 35 cycles with an annealing temperature of 54 °C. Primers ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) were employed to amplify the ITS rDNA region. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

ITS rDNA sequences were aligned with Muscle in the free software MEGA7 (KUMAR *et al.*, 2016). A Maximum likelihood phylogenetic analysis was made on a reduced dataset taken from RUBIO *et al.* (2019) with the settings Kimura-2, gamma-distributed, use all sites, with 500 bootstrap replicates, by using the “Find Best DNA Model” option. Distance analysis was done with MEGA7 with the settings p-distances, transitions + transversions, uniform rates, pairwise deletion. Two included CBS sequences and that of H.B. 6923a are unofficial.



Fig. 1 – *Rumex suffruticosus*. Fruiting plants (above), branched woody stems with some dead parts (below). Photos J. Linde.

Taxonomic part

Capitotricha attenuans (Nyl.) E. Rubio, J. Linde & Baral, *comb. nov.*
– Index Fungorum: IF557644 – Fig. 3–7.

Basionym: *Peziza attenuans* Nyl., *Not. Sällsk. Fauna et Fl. Fenn. Förh., Ny Ser.*, 10: 25 (1869).

= *Lachnella hispanica* Cooke & W. Phillips, *Grevillea*, 19 (91): 73 (1891), *nom. illegit.* Art. 52.1 ICN (nomenclaturally superfluous).

Etymology: perhaps named after the attenuated paraphyses or hair tips, but neither of these elements have been described in the protologue.

Syntype: SPAIN, Asturias, Cangas del Narcea, Pico de Cancellas or Pico de Arvas, ~2100 m, twigs of *Rumex suffruticosus*, VII–VIII.1835, *leg.* Durieu (K, *vid.* DENNIS, 1963).

Epitype (designated here): SPAIN, Asturias, Cangas del Narcea, Puerto de Leitariegos, Laguna de Arbas, 1800 m, twigs of *Rumex suffruticosus*, 29.V.2019, *leg.* J. Linde (E.R.D. 7931); MBT 394501.

Apothecia superficial, scattered to gregarious, often aggregated in groups, (0.4–)0.5–1.2(–2.2) mm in diam. (excluding hairs), (0.3–)0.5–1.1(–2) mm high excluding hairs, attached to the substrate by a short stout stipe; disc slightly concave to flat, bright egg-yellow to vividly yellow-orange, margin and entire outside of receptacle concolorous but densely covered by white, long, crystalliferous hairs that slightly agglutinate to form converging fascicles; stipe cylindrical, short to almost absent, 0.1–0.2 (height) × 0.1–0.15 mm, pale yellow, bearing short to long white hairs. Very hygroscopic, hairs extending or converging quickly with changes in air humidity.

Asci *(65–)68–78(–85) × (6–)7–8(–9) μm; cylindrical, sometimes slightly ventricose or narrowed in lower part, *pars sporifera* 24–36 μm long; apex distinctly conical, with an apical ring of the *Calycina*-type reacting deeply blue in IKI (euamyloid); base with a short and very wide stalk arising from croziers. **Ascospores** *(11.5–)14.5–15.7(–18.7) × (2.2–)2.5–3.0(–3.4) μm, average = 15 × 2.7 μm, Q = (4.5–)5.4(–7.2), †8.5–13.5 × 1.7–2.3 μm (KOH), average = †11 × 2 μm, obliquely biseriate, cylindrical-fusoid or sometimes fusoid-clavate, with rounded to obtuse ends, hyaline, smooth, aseptate, uninucleate, eguttulate or usually with a few minute LBs (OCI = 0–0.5), without sheath. **Paraphyses** cylindrical in lower part, cells *10–15 × 3–4.5 μm, unbranched, upper part broadly lanceolate with acute tip, terminal cell *35–46 × (3.5–)4.5–5.3(–6) μm, exceeding the *asci by 10–22 μm. **Subhymenium** not differentiated. **Medullary excipulum** forming a layer of interwoven, *2–3 μm wide hyphae (*textura intricata*), 20–30 μm thick in receptacle. **Ectal excipulum** in receptacle (45–)70–100(–150) μm thick, built up of a *textura prismatica* of more or less rectangular cells of *(5–)12–15(–20) × (4–)5–9(–15) μm, at lower flanks oriented under a 70–80° angle to the surface, at mid flanks under 45° and at margin under 0–10°, *cells thin-walled, without noticeable contents, †cells with thickened, slightly gelified walls. **Excipular hairs** hyaline, straight, cylindrical up to (sub)apex, *100–200 × (3–)4–5(–6) μm, apex either of equal width, rounded to obtuse, or often slightly to strongly tapered, sparsely multiseptate, septa more dense near apex; lateral walls †0.8–1.2 μm thick, overall densely finely warted, warts ~0.3–0.5 μm high, 0.3–0.7 μm wide; bearing druses of refractive crystals mainly at hairs apex and hyaline lumps of resinous substance along lateral walls. **Carotenoids** in LBs abundant in paraphyses and subhymenium/medullary excipulum, especially at septa outside of large non-refractive vacuoles, small or sometimes large (1–2 μm diam.), golden yellow-orange, not dissolving in KOH, staining light green in IKI.

Habitat: on bark of previous year's either still attached or broken, dead but little decayed lignified basal parts of *Rumex suffruticosus*.

Geology: on acid schistose rock (boulder, scree). **Vegetation:** open alpine shrubland with *Juniperus alpina*, *Calluna vulgaris*, *Erica* spp., *Vaccinium myrtillus* and *Betula celtiberica*. **Altitude:** 1800–2100 m.

Phenology: V–IX (not tried during winter season).



Fig. 2 – *Capitotricha attenuans* E.R.D.7931. Habitat of the Spanish collection, red arrows indicating the position where the apothecia were found. Photo J. Linde.

Specimens examined: SPAIN: Asturias, 23 km SSE of Cangas del Narcea, 1.2 km SW of Puerto de Leitariegos, near Laguna de Arbas, 42° 59' 25" N 6° 25' 37" W, alt. 1800 m, 6.IX.2018, *leg.* J. Linde, E.R.D. 7929. – *Ibid.*, 29.V.2019, *leg.* J. Linde, E.R.D. 7931 (GenBank: ITS MT370339, LSU MT370355).

Remarks

COOKE & PHILLIPS (in ANON., 1891) proposed the new species *Lachnella hispanica* for a collection on dead stems of *Rumex suffruticosus* from Pico de Cancellas in Spain. The authors compared it with *L. patula* (Pers.) W. Phillips [= *Lachnum patulum* (Pers.) Rehm], a species currently reported from *Quercus* leaves, from which they separated it by rather large ascospores and unusually large paraphyses. The holotype collection to which they refer is part of an exsiccatum distributed by M.C. Durieu in 1856. When DENNIS (1963: 348) re-examined the holotype in K, he placed it in synonymy with *Dasyscyphus bicolor* (Bull.) Fuckel because he saw no morphological differences between the two species. Dennis drew attention to the fact that there exists an earlier name based on the same collection, *Peziza attenuans*. This name was proposed by NYLANDER (1869: 24) in a discussion under *Peziza calycina* Schumach. Although very brief, it represents a valid erection of a new species, as it gives a reference to Durieu's exsiccatum and gives a few characters (hymenial colour, spore size). His phrase "*dici possit haec hispanica P. attenuans*" can be translated as "This Spanish [species] can be called *P. attenuans*". Durieu (Pl. Astur. 46) named his sample "*P. bicolor b. disco luteo*", i.e., an infraspecific taxon of *Peziza bicolor* which has a yellow instead of orange disc.

Our recent records of *Capitotricha attenuans* derive from the same area in Asturias in which Durieu made the type collection. NYLANDER (1869) reported spore measurements of 9–11 × 1.5–2 μm. COOKE & ELLIS (ANON., 1891) found them to be 10–13 × 2–2.5 μm and DENNIS (1963) 9–13 × 1.5–2 μm. The distinctly larger size of the living ascospores reported here are due to the shrinking effect of living cells in herbarium material (BARAL, 1992), as can be seen from our spore measurements in KOH (8.5–13.5 × 1.7–2.3 μm).

Morphologically, *Capitotricha attenuans* is very similar to *C. bicolor*, but the comparatively large ascospores exclude this species when treated in a narrow sense. The ascospores even slightly exceed those of the undescribed "*C. fagiseda*" which is thought to be confined to twigs and cupules of *Fagus* (BARAL, ined.). An important characteristic of *C. attenuans* is the presence of croziers at the ascus base which sharply distinguishes it from *C. bicolor* s.l., *C. fagiseda* *nom. prov.* and

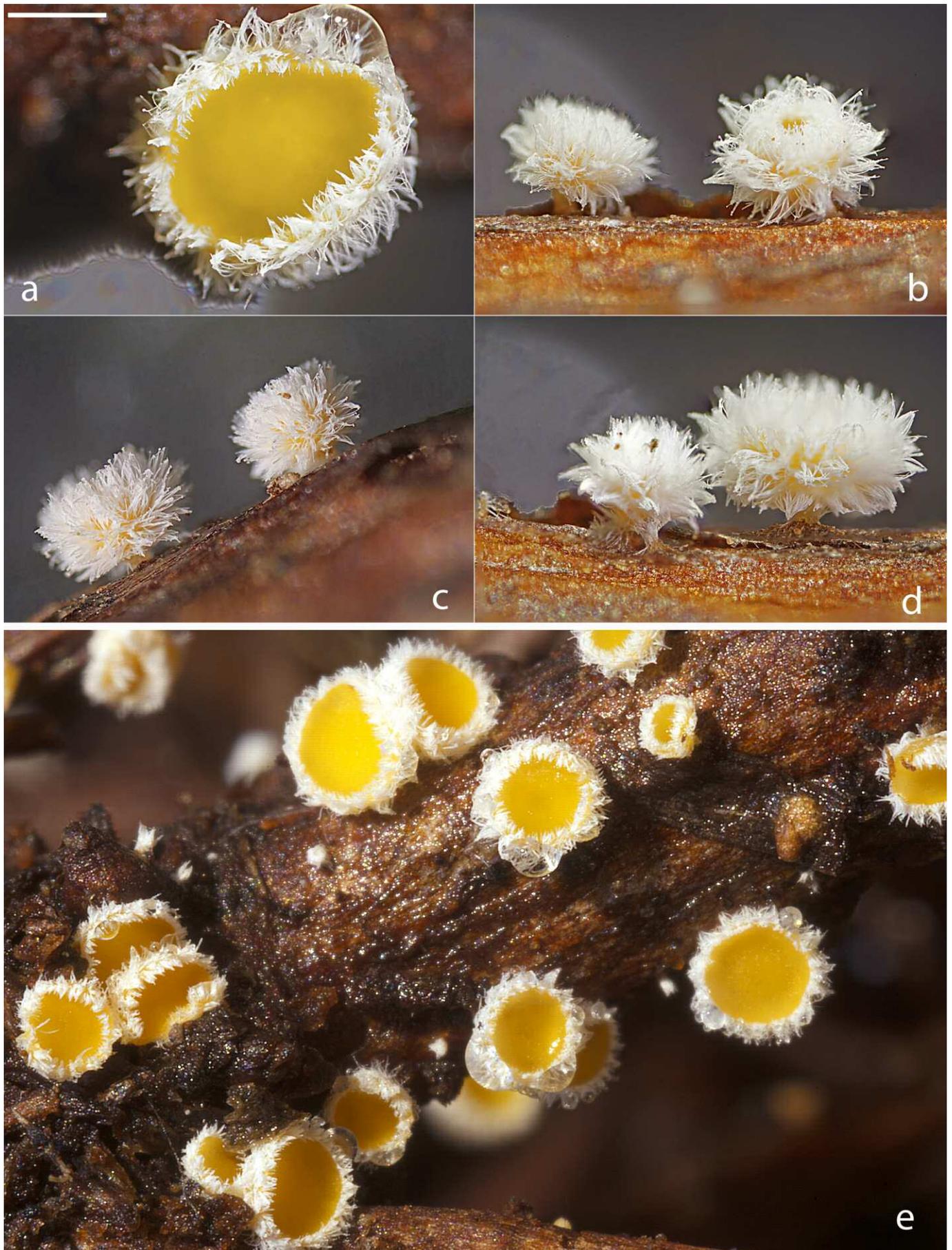


Fig. 3 – *Capitotricha attenuans* E.R.D. 7931. a–e. Fresh apothecia on previous year's stems of *Rumex suffruticosus*. Scale bar (only valid for a) = 0.5 mm. Photos E. Rubio.

C. rubi which all have simple-septate ascus bases. The foliicolous "*Lachnum*" *patulum* s. auct. and caulicolous *C. pterosparti* coincide with *C. attenuans* in having croziers, but the former has much shorter ascospores, *7–11.5 × 2–2.7 μm (BARAL, ined.), and the latter much larger 3-septate ascospores, *15–29.5 × 3.7–5.4 μm (RUBIO et al., 2019).

Phylogenetic comments

When the genus *Capitotricha* was investigated by molecular methods, *C. bicolor* was shown to represent a species complex of closely related, apparently host-specific taxa (ŠANDOVA et al., 2018; RUBIO et al., 2019). Particularly samples on *Quercus* show a consider-

able ITS distance of 4–7% to those growing on other substrates (Fig. 8). According to SUKOVA (2005: 152), "The original substrate of *Peziza bicolor* Bull. illustrated by Bulliard (1789) is possibly a twig of *Quercus*, but this is not unambiguous". Based on this assumption, we have put *C. bicolor* on other hosts in parentheses.

In the present study, sequences were taken from apothecia of the epitype of *C. attenuans*, comprising ITS and LSU D1–D4. In the ITS region it has the closest distance of 0.9–1.3% to *C. fagiseda* and *C. "bicolor"* on *Alnus*, with which it formed an unsupported clade (Fig. 8), 2.5% to *C. "bicolor"* on *Rosaceae*, 2.9% to *C. rubi*, 4.2% to *C. pterosparti*, and 5% to *C. bicolor* on *Quercus*. In the conservative LSU D1–D2 domain the distance is 0% to *C. "bicolor"* on *Alnus*,

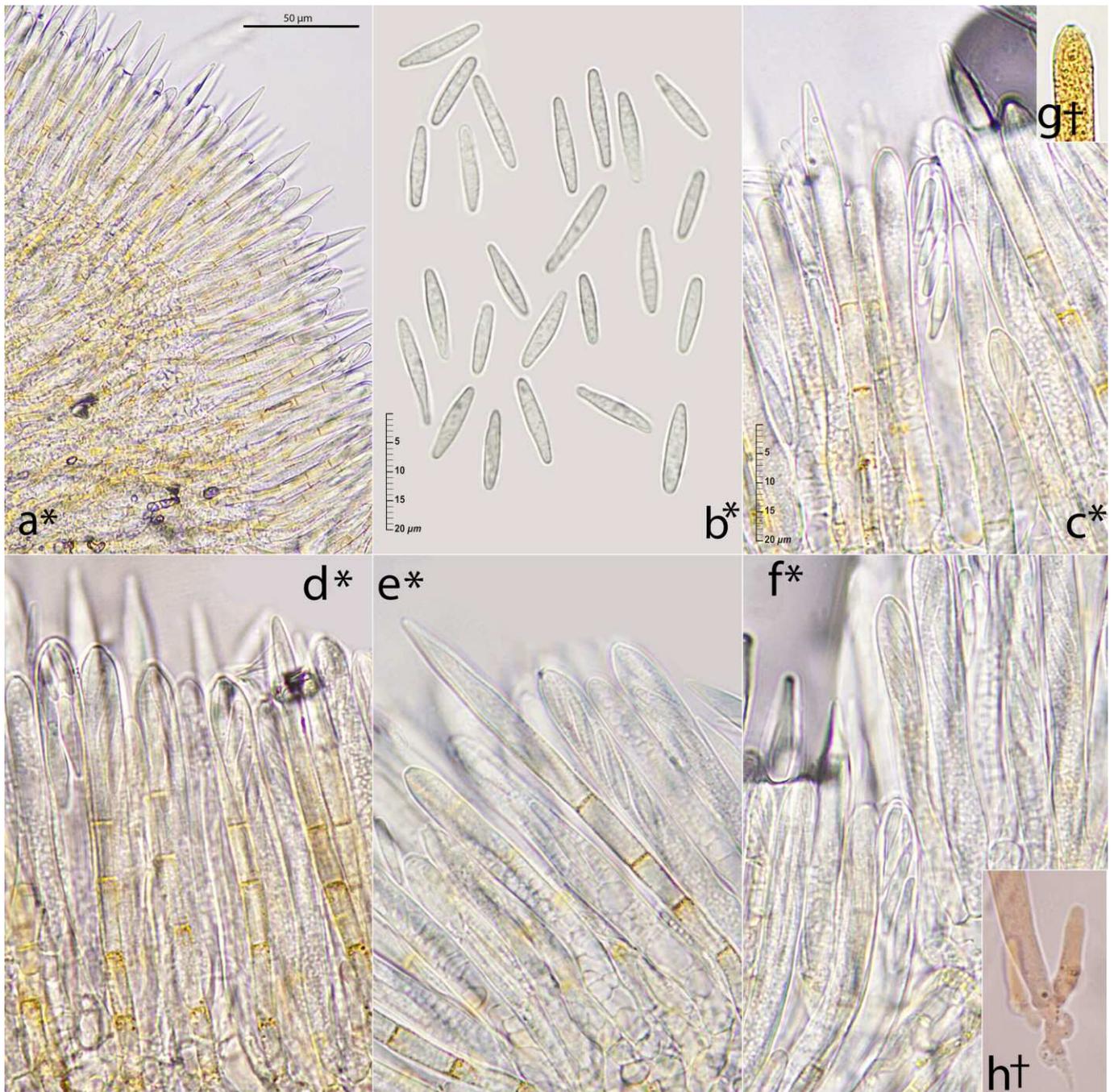


Fig. 4 – *Capitotricha attenuans* E.R.D. 7931. a. Hymenium with lanceolate paraphyses exceeding the asci; b. Free ascospores; c–f. Closeup of hymenial elements showing mature and immature asci and paraphyses; g. Ascus tip in IKI with euamyloid apical ring; h. Ascus base in CR arising from repeated crozier formation. All in living state in water, except for g–h. Scales in b and c apply also to d–h. Photos E. Rubio.

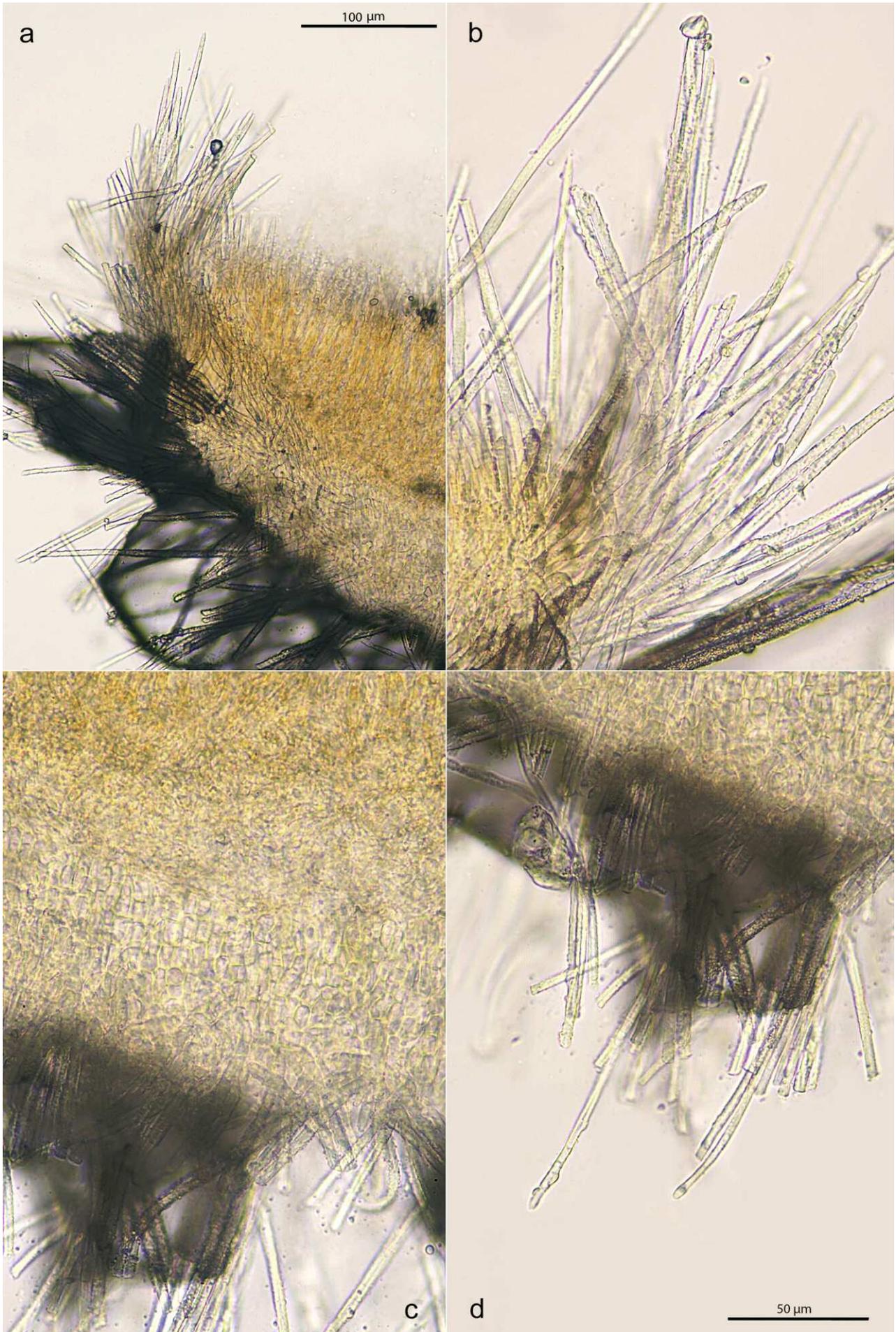


Fig. 5 – *Capitotricha attenuans* E.R.D. 7931. a–d. Section of apothecium showing ectal and medullary excipulum, hairs, and hymenium. All in living state in water. Scale in d applies also to b–c. Photos E. Rubio.

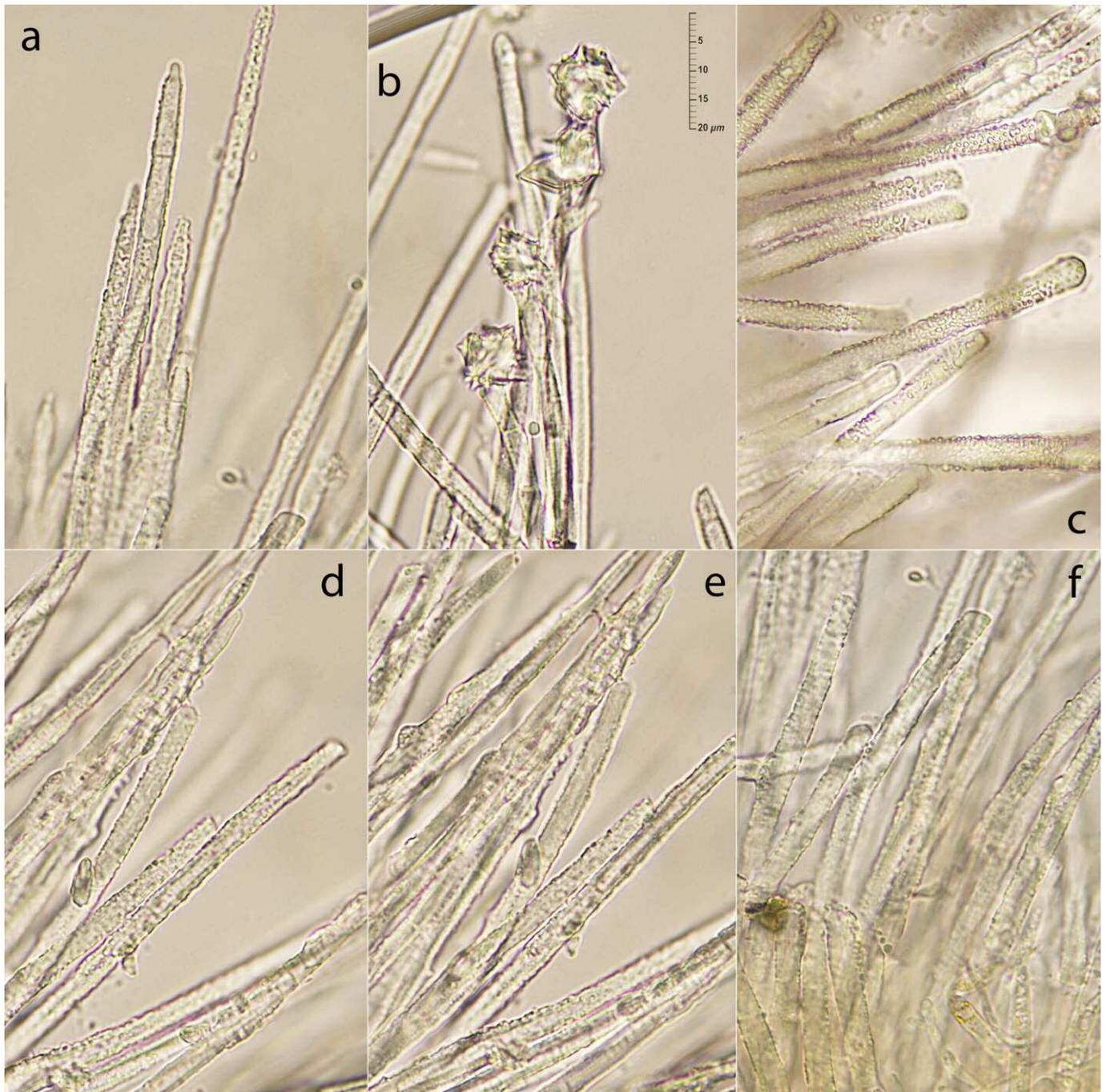


Fig. 6 – *Capitotricha attenuans* E.R.D. 7931. a–c. Upper part of hairs (b with crystal druses); d–f. Middle part of hairs. All in living state in water (with a few dead cells). Scale in b applies to all photos. Photos E. Rubio.

0.2% to *C. "bicolor"* on *Crataegus*, 0.5% to *C. bicolor* on *Quercus*, 0.6% to *C. rubi*, and 0.9% to *C. pterosparti*.

At the 3'-end of SSU the S1506 intron is absent in *C. attenuans*. This intron occurs in *C. bicolor* on *Quercus* with a length of 345 nt. The intron also occurs in *C. pterosparti* (min. 310 nt) and apparently *C. "bicolor"* on *Alnus*, *C. rubi* p.p., and *C. fagiseda*, whereas *C. "bicolor"* on *Rosaceae* and *C. rubi* p.p. (clade with *C. filiformis*) are without it. Between D4 and primer LR5 another intron (L798) is present in all available LSU sequences of *Capitotricha* which cover the D4 domain. Its length is 417 nt in *C. bicolor*, while the quality in *C. attenuans* is not good enough to be sure about this (?418 nt). The distance of *C. attenuans* in the L798 intron is about 3% to *C. "bicolor"* on *Crataegus* and 4% to *C. bicolor* on *Quercus*, but about 30% to *C. pterosparti*.

Acknowledgements

Paul Kirk is thanked for the confirmation that *Peziza attenuans* was a validly published name of Durieu's record. We also thank Luis Carlón for pointing out Durieu's record, and Pablo Alvarado for obtaining sequences of this fungus.

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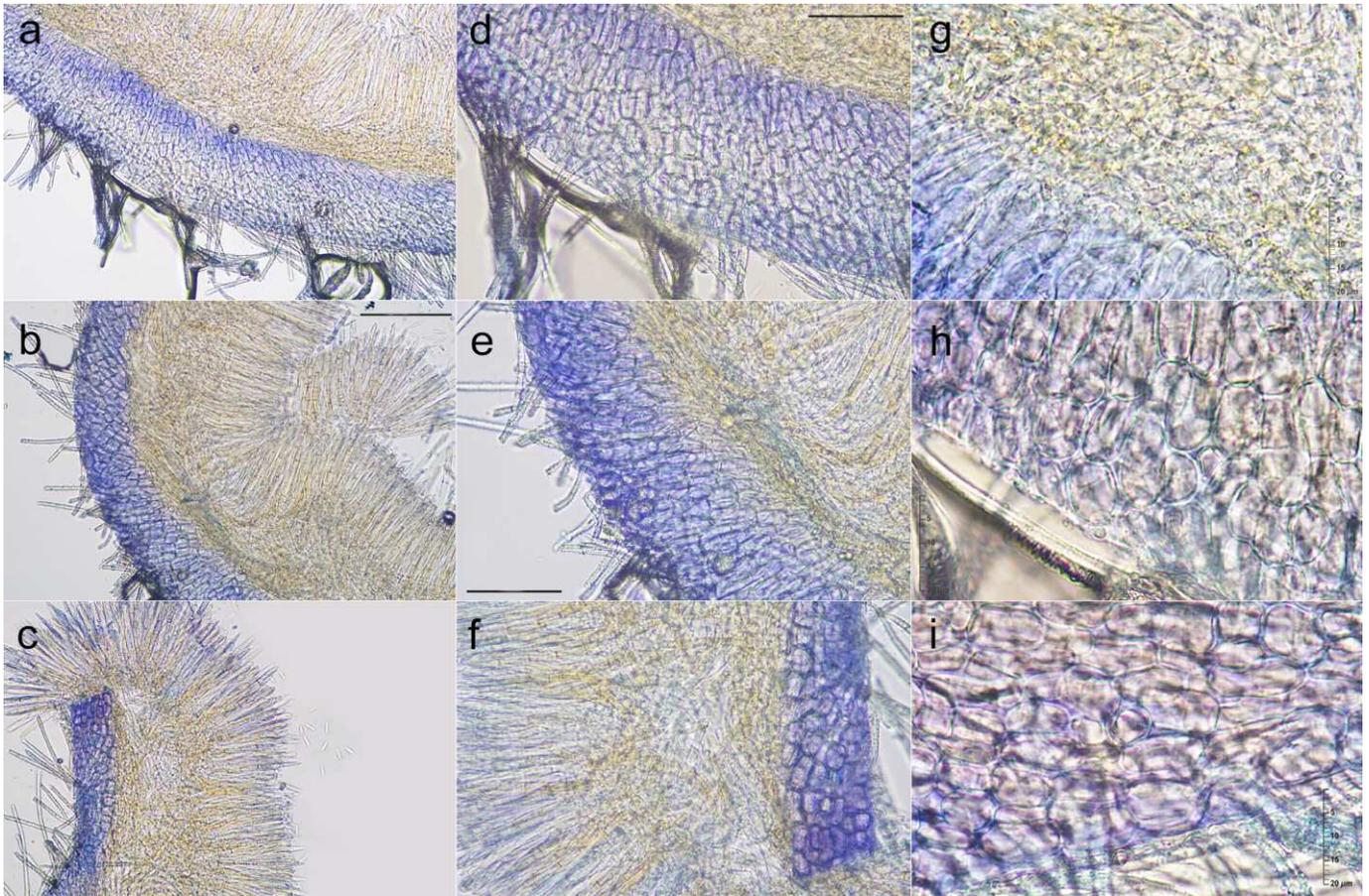


Fig. 7 – *Capitotricha attenuans* E.R.D. 7931. a–i. Median section of apothecium, ectal excipulum stained blue-violet in Cresyl Blue (section squashed in a, d, f & i). All in living state. Scale bars : b = 100 μm; d–e = 50 μm. Scales in b, d–e, and i apply to the photos in their respective column. Photos E. Rubio.

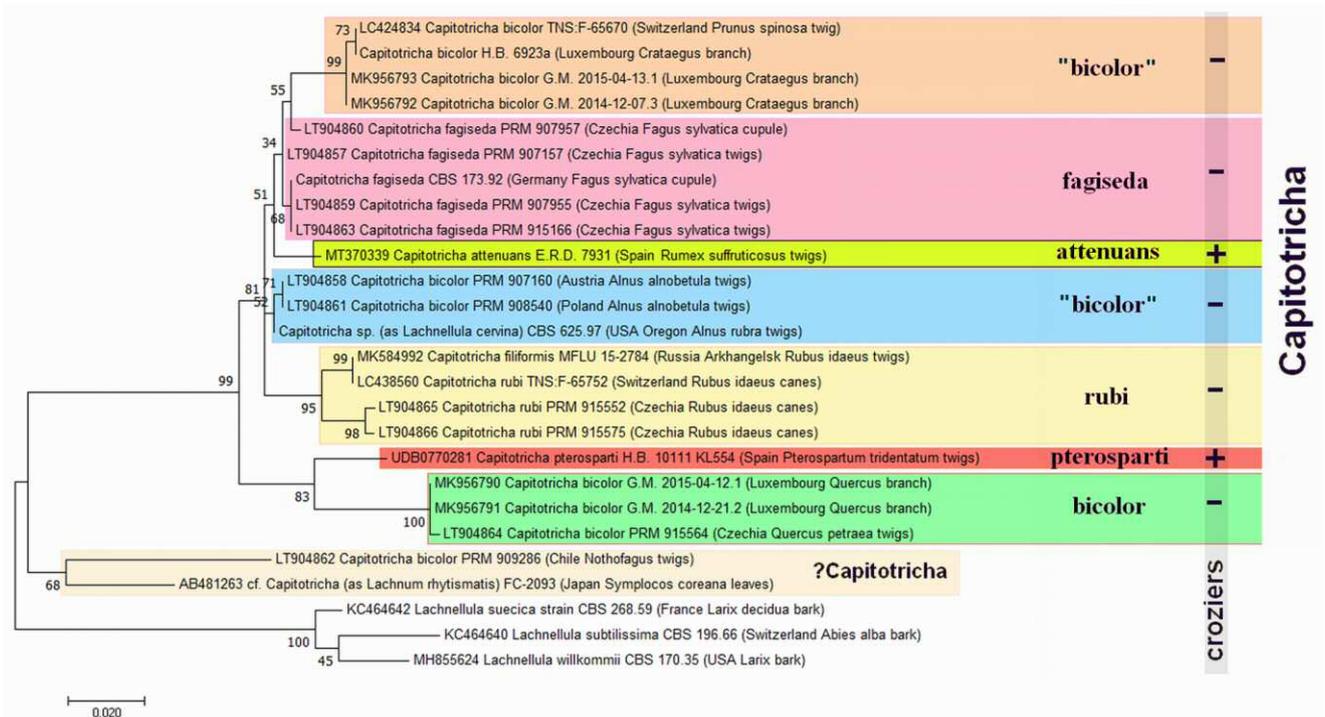


Fig. 8 – Maximum likelihood analysis of ITS1-5.8S-ITS2 rDNA of *Capitotricha* spp., with *Lachnellula* spp. as outgroup.

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