Epitypification of *Lamprospora rehmii* Benkert (*Pezizales*)

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Abstract: *Lamprospora rehmii* Benkert, a species of bryophilous *Pezizales* infecting the moss *Pleuridium acuminatum* Lindb., is presented from recent collections, one of which is designated as epitype. An illustration from the protologue is designated as lectotype. Sequence data from the LSU and ITS regions have been deposited in GenBank.
Keywords: Ascomycota, bryophilous *Pezizales*, *Pleuridium acuminatum*, *Pyronemataceae*.

Introduction

Finding and studying a rarely reported species always evokes a tingling sensation and we were excited when we got hold of the almost forgotten *Lamprospora rehmii*, which was described by Benkert (1987) on the basis of one historical collection — from Rehm from 1869 — then completed by another one from Gams from 1951 (Benkert, 1994).

Regrettably we learnt that there was no type material left in S (Stockholm, Sweden); therefore we designate the illustration of the protologue (Benkert, 1987: 262) as lectotype and a recent collection from La Rioja, Spain, as epitype. We obtained sequences and take now the opportunity to present *L. rehmii* with a description and with photos.

Methods

The description of *L. rehmii* is based on the results of the examination of vital collections of apothecia from three localities in France and Spain. Observations were made in tap water, ascospore ornamentation was also studied after staining with Lactophenol Cotton Blue (LPCB) and the absence of the iodine reaction of the asci was checked with Lugol's solution (IKI). Ascospore size was measured from ascospore prints as well as from free ascospores from squash mounts. Macrographs were made with a digital camera, micrographs were taken either in tap water or in LPCB, using digital cameras mounted on microscopes. Scanning electron microscopy (SEM) was performed on a JEOL-6380 LV microscope. A fragment of a mature apothecium was fixed in osmium tetroxide vapors 2 wk at 5–10 °C and goldcoated in Bal-Tec SCD 050 sputter coater.

DNA extraction and analysis

DNA was extracted from dried or CTAB-stored apothecia using the Zymo Research Fungal/Bacterial kit (Zymo Research, Orange, USA). Nuclear rDNA region was amplified with the primer pairs NL1, NL4 for LSU (O'Donnell, 1993) and ITS1F, ITS4; ITS1F, ITS2; ITS3, ITS4 for ITS (White *et al.*, 1990; Gardes & Bruns, 1993). The PCR reactions were performed with the following program: 5 min at 95 °C for initial denaturation, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 53 °C and 60 s extension at 72 °C, followed by a final extension for 10 min at 72 °C. The length and quality of the PCR products were checked by gel electrophoresis (1% agarose). Positive PCR reactions were purified with a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taipei, Taiwan) and sequenced using both PCR primers in the sequencing laboratory of the Faculty of Science, Charles University, Prague, Czech Republic. Sequences generated in this study were deposited in Genbank under the accession numbers listed in Table 1.

Sequences of ribosomal DNA (ITS and LSU) of *L. rehmii* together with other *Lamprospora* and *Octosporopsis* sequences from GenBank were aligned with MAFFT (online version 7) using the E-INS settings (Katoh *et al.*, 2017), with a gap opening penalty of 1.0 and an offset value of 0.1. Maximum likelihood searches were performed in PhyML 3.0 (Guindon *et al.*, 2010) via the Montpellier online server (http://www.atgc-montpellier.fr/phyml/) with 1000 bootstrap replicates. Bayesian searches were conducted in MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). Two independent runs of two million generations sampled every 100th generation with the first 25% of samples discarded as burn-in. Parameters for the analyses were estimated using the SMS (Lefort *et al.*, 2017), which selected the general time reversible substitution model (GTR + G + I) as best fitting.

Description

Lamprospora rehmii Benkert, *Z. Mykol.*, 53 (2): 239 (1987).

Macroscopic features (Fig. 1 c, d, e):
Apothecia scattered, sometimes gregarious on soil between shoots of *Pleuridium acuminatum*; 0.5–2 mm in diam., first spherical, becoming saucer-shaped, finally discoid, sessile; with septate anchoring hyphae; mostly with a narrow fimbriate margin; hymenium yellow-orange to orange, appearing rough in mature apothecia due to protruding asci, margin and outer surface slightly paler than the hymenium; outer surface downy.

Microscopic features (Figs. 2–3):
Asci 200–320 (500) × 22–29 (36) µm, cylindrical, 8-spored (occasionally with four mature and four immature ascospores), operculate, inamyloid, shortly bifurcate at the base, arising from perforated croziers. **Ascospores** (20.4) 21–25 (25.6) µm (ornamentation included), hyaline, globose, with a large lipid drop, diam. 11–15 (15.4) µm, uniseriate. The ascospore ornamentation consists of prominent warts and tubercles of very different shape — from spherical to bulbous (sometimes elongated) warts to allantoid (sausage-like) or bone-shaped tubercles — all varying in size. The warts and tubercles are between 2–4–8 µm broad and 2–4 µm high and nearly cover the entire ascospore surface of mature ascospores whereas there is more free space between the ornamentation in immature ones. Occasionally there are tiny warts between the larger ones and the tubercles (see Figs. 2 a-c). **Paraphyses** filiform, containing, at least in the upper half, numerous VBs 1–2.8 µm diam., distinctive carotenoid pigment turning olivaceous in Lugol's solution; straight, rarely bent, pluriseptate, apically slightly inflated, terminal cell 25–60 × 4–8 µm, at times clavate. **Medulla** of *textura angularis-*

Table 1 – Sequenced collections of *Lamprospora rehmii* and sequences acquired from GenBank with accession numbers and voucher information.

Species	Herbarium #	Geographic origin, collector	Host	LSU	ITS
Lamprospora rehmii	F317032	Spain, R. Martínez	*Pleuridium acuminatum*	MH087070	MH087068
Lamprospora rehmii	RM-1306	Spain, R. Martínez	*Pleuridium acuminatum*	MH087069	MH087067
Lamprospora arvensis	RM-2399	Spain, R. Martínez	*Ceratodon purpureus*	KY858948	KY858958
Lamprospora arvensis	JE-43560	Germany, J. Eckstein	*Ceratodon purpureus*	KY858949	KY858959
Lamprospora arvensis	HBG-024465	Germany, M. Vega	*Ceratodon purpureus*	KY858950	KY858960
Lamprospora arvensis	HBG-024466	Germany, J. Siembida	*Ceratodon purpureus*	KY858951	KY858961
Lamprospora dicranellae	HBG-024467	Portugal, M. Vega	*Ditrichum heteromallum*	KY858952	-
Lamprospora dicranellae	HBG-024468	France, M. Vega	*Ditrichum heteromallum*	KY858953	KY858962
Lamprospora dicranellae	HBG-024469	Germany, M. Vega	*Ditrichum heteromallum*	KY858954	KY858963
Lamprospora dicranellae	HBG-024470	Germany, M. Vega	*Ditrichum pusillum*	KY858955	-
Lamprospora ditrichi	TRH:F-10629	Norway, S. Sivertsen	*Flexitrichum flexicaule*	MG949140	-
Lamprospora pseudoarvensis	HBG-024462	Spain, M. Vega	*Pleuridium acuminatum*	KY858945	KY858956
Lamprospora pseudoarvensis	HBG-024463	Austria, G. Friebes	*Pleuridium acuminatum*	KY858947	KY858957
Lamprospora pseudoarvensis	HBG-024464	France, B. Jeannerot	*Pleuridium acuminatum*	KY858946	-
Lamprospora spitsbergensis	TRH:8581	Norway, H. Dissing, S. Sivertsen	*Hennediella heimii* var. *arctica*	MG949137	-
Lamprospora sp.	KH.03.131	Norway, K. Hansen	-	DQ220361	-
Lamprospora sp.	KH.03.150	Norway, K. Hansen	-	DQ220362	-
Lamprospora sp.	TL-2012	Finland, A. Lesonen	-	EU940123	EU940199
Lamprospora sp.	TL-11703	Ecuador, K. Hansen *et al.*	-	KC012685	-
Lamprospora sp.	TL-11753	Ecuador, K. Hansen, T. Læssøe	-	KC012686	-
Lamprospora sp.	TRH:9458	Norway, H. Dissing, S. Sivertsen	*Aongstroemia longipes*	MG949141	-
Lamprospora sp.	TRH:9665	Norway, H. Dissing, S. Sivertsen	*Bryoerythrophyllum recurvirostrum*	MG949139	-
Lamprospora sp.	TRH:F-10708	Norway, H. Dissing, S. Sivertsen	*Distichium* sp.	MG949138	-
Lamprospora sp.	TRH:F-10783	Norway, S. Sivertsen	*Trichostomum crispulum*	MG949136	-
Octosporopsis nicolai	UL152-13	Portugal, M. Vega	*Lunularia cruciata*	KF771034	KF771044

intricata with elongated hyphae 5–10 µm wide and thin cell walls up to 1 µm. **Ectal excipulum** of *textura angularis-prismatica* with cells measuring 10–30 µm as well as more cylindrical cells measuring 20–40 µm × 5–15 µm, with thick cell walls of 1–3 µm. **Margo** of *textura porrecta*, elongated hyphae with carotenoid pigment, 15–35 × 6–10 µm, cell walls of 1–2 µm, at the margin about 20–50 × 7–14 µm.

Infection: Appressoria of *L. rehmii* on the rhizoids of *P. acuminatum* are mostly 2-celled, approx. 26 × 18 µm in side view and covered by a thin layer of accompanying hyphae.

Phylogenetic analysis: Two analysed samples from Spain have identical LSU and ITS sequences. Based on the phylogram from rDNA (LSU, ITS) sequences (see Fig. 7) the closest relative of *Lamprospora rehmii* is *L. pseudoarvensis* M. Vega, Eckstein, Friebes & R. Tena — a species associated with the same host bryophyte. Both species belong to a well-supported clade together with *L. arvensis* Benkert and *L. dicranellae* Benkert.

Amplification of the ITS region of *L. rehmii* showed that this species possesses a very long ITS1 domain, therefore its sequences cannot be well aligned with those of other species, with exception of the closest sequenced relative *Lamprospora pseudoarvensis*. This is likely a result not only of the accumulation of insertions but also due to a very fast mutation rate in this group of fungi and particular region in general. ITS thus only has a restricted use for the phylogenetic analysis of bryophilous *Pezizales*, being suitable mostly for the investigation of closely related species groups or as a barcoding gene. However, use of this region in those types of studies is also problematic due to its frequently increased length resulting in common amplification of the shorter fragments belonging to contaminants.

Habitat and occurrence: Concerning the habitat and occurence of the host *Pleuridium acuminatum* we refer to Vega *et al.* (2017: 140 & 143).

Specimens examined:

1. France, Pyrénées-Atlantiques, Sedzère, 355 metres asl, slope above a roadside ditch, *leg*. Beñat Jeannerot, January 25th 2014. Host: *P. acuminatum*. Soc.: *Lamprospora annulata* Seaver, *L. pseudoarvensis*. Pers. herb. GM 20140104.

2. Spain, La Rioja, Lumbreras, Parque Natural de la Sierra de Cebollera, 42° 2′ 51″ N, 2° 38′ 47″ W, 1470 metres asl, turning area of a forest road, *leg*. R. Martínez-Gil, October 17th 2015. Host: *P. acuminatum*, accompanying mosses: *Bryum* sp. Pers. herb. RM-1306, duplicate pers. herb. MV20151029-01. *Ibidem*, October 24th 2015, pers. herb. RM-2366.

3. Spain, La Rioja, Villoslada, Parque Natural de la Sierra de Cebollera, 42° 3′ 13″ N, 2° 38′ 54″ W, 1360 metres asl, humid slope besides a forest road, *leg*. R. Martínez-Gil, October 24th 2015. Host: *P. acuminatum*, accompanying mosses: *Bryum* sp., *Polytrichastrum formosum* (Hedw.) G.L.Sm. Soc.: *L. annulata*. Pers. herb. RM-2367, additional collection: October 26th 2017, RM-2400, Stockholm accession number F317032 (EPITYPE).

Fig. 1 – *Lamprospora rehmii*. a: Villoslada, habitat of epitype F317032 & RM-2367. b: Lumbreras, habitat of RM-1306 & RM-2366. c–e: apothecia with the host *Pleuridium acuminatum*, c: F317032. d: RM-2367. e: RM-2366. Photos: Rubén Martínez-Gil.

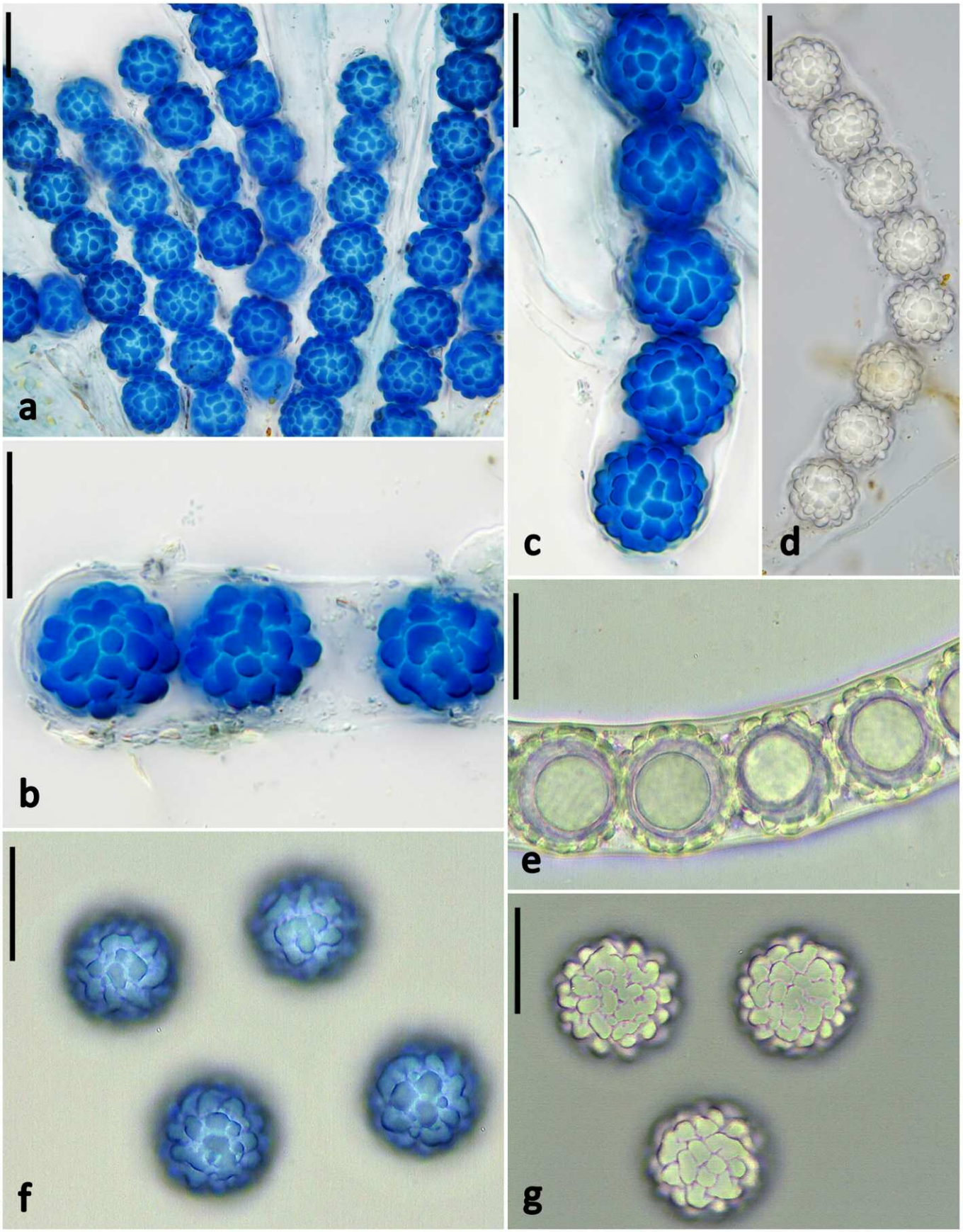

Fig. 2 – *Lamprospora rehmii*. a–c: asci in LPCB. d–e: ascus in tap water. f: free ascospores in LPCB. g: free ascospores in tap water. Scale bar = 20 µm for all photos. a–g: F317032. Photos: Lukáš Janošík (a–e) and Rubén Martínez-Gil (f–g).

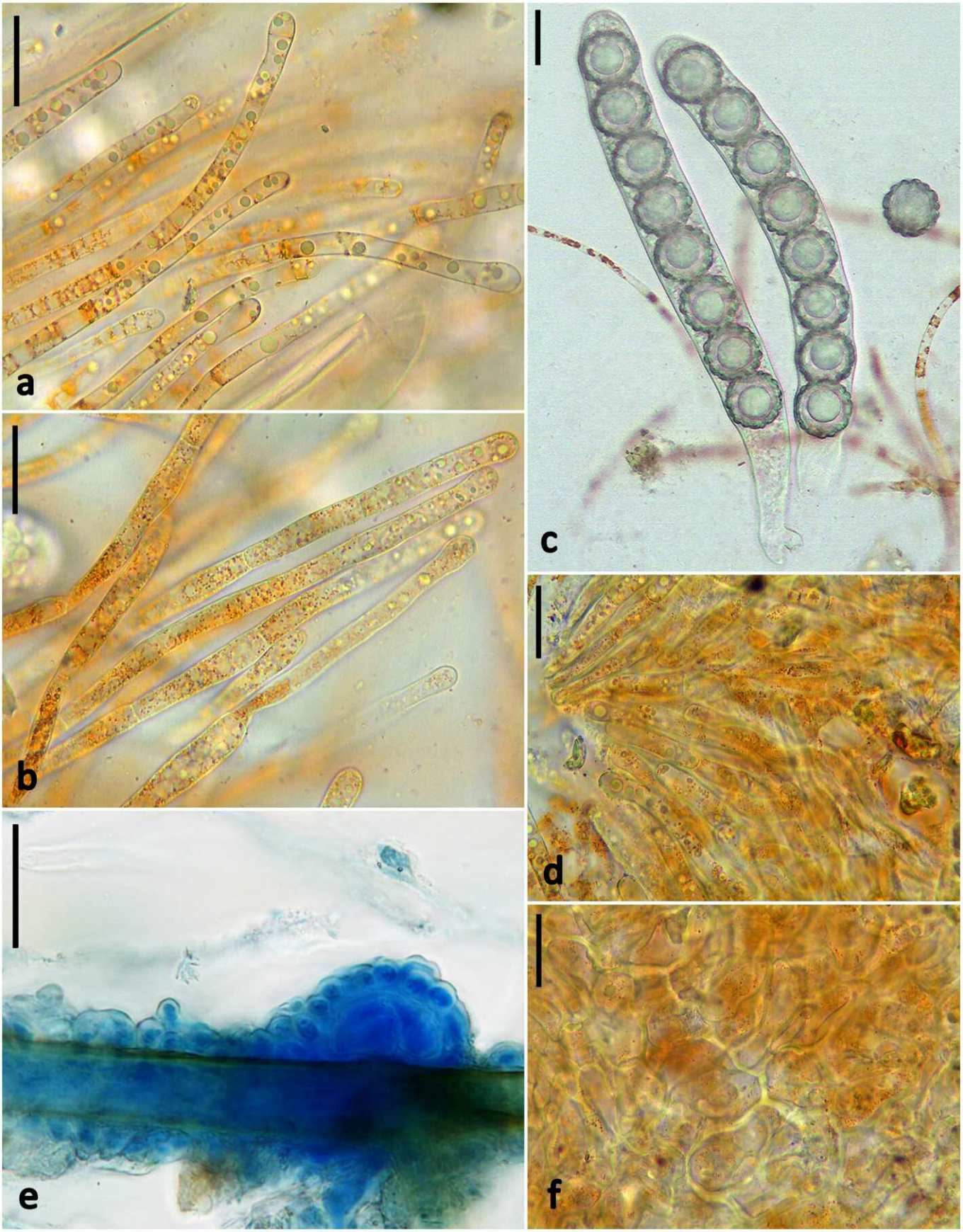

Fig. 3 – *Lamprospora rehmii*. a–b: paraphyses in tab water. c: asci with crozier in tab water. d: margin in tap water. e: infection peg in LPCB. f: ectal excipulum in tap water. Scale bar = 20 µm for all photos. a–f: F317032. Photos: Rubén Martínez-Gil (a-d, f) and Lukáš Janošík (e).

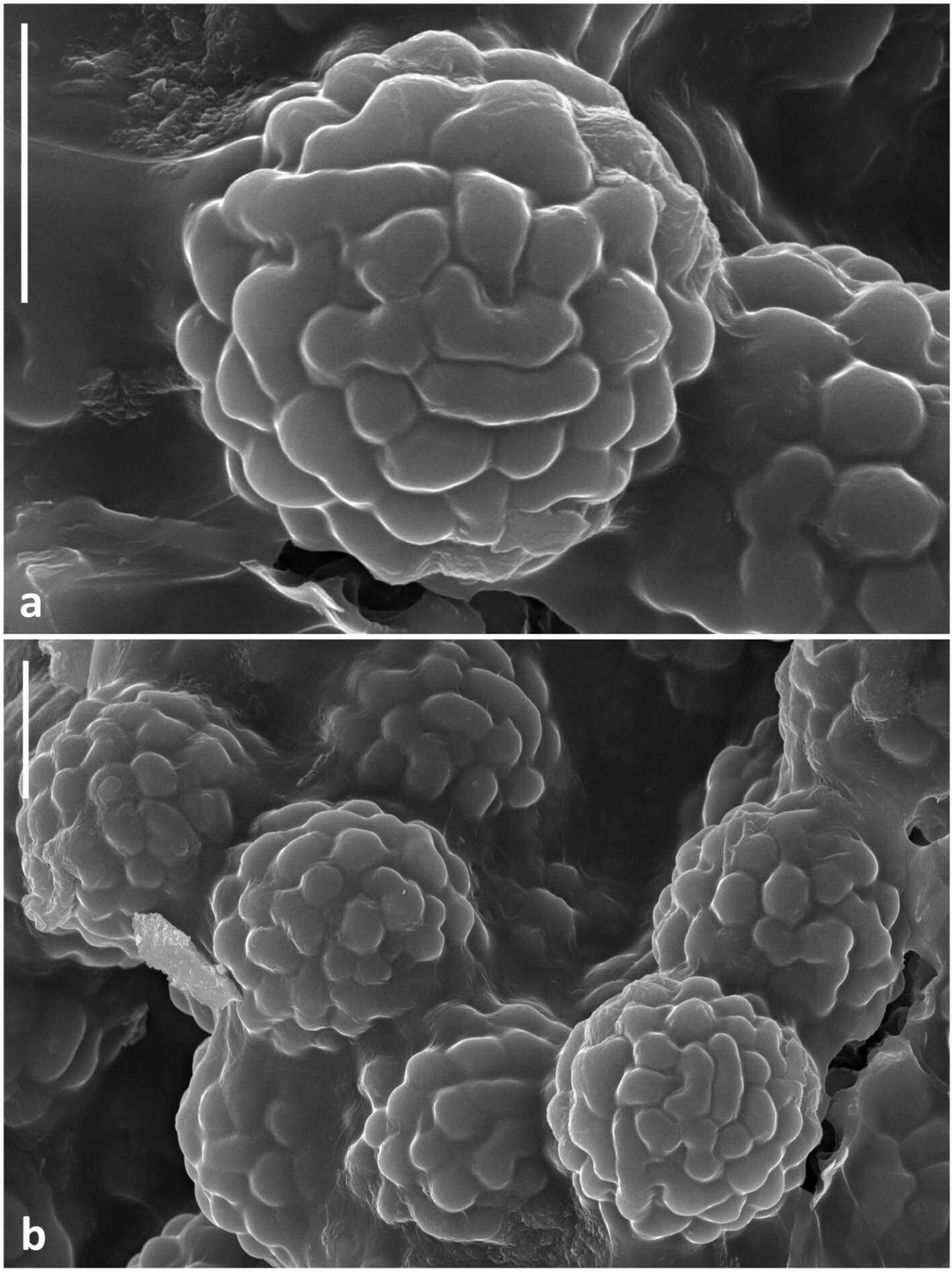

Fig. 4 – *Lamprospora rehmii*. SEM photos of ascospores. RM-2367. Scale bar = 10 µm for both photos. Photos: Lukáš Janošík.

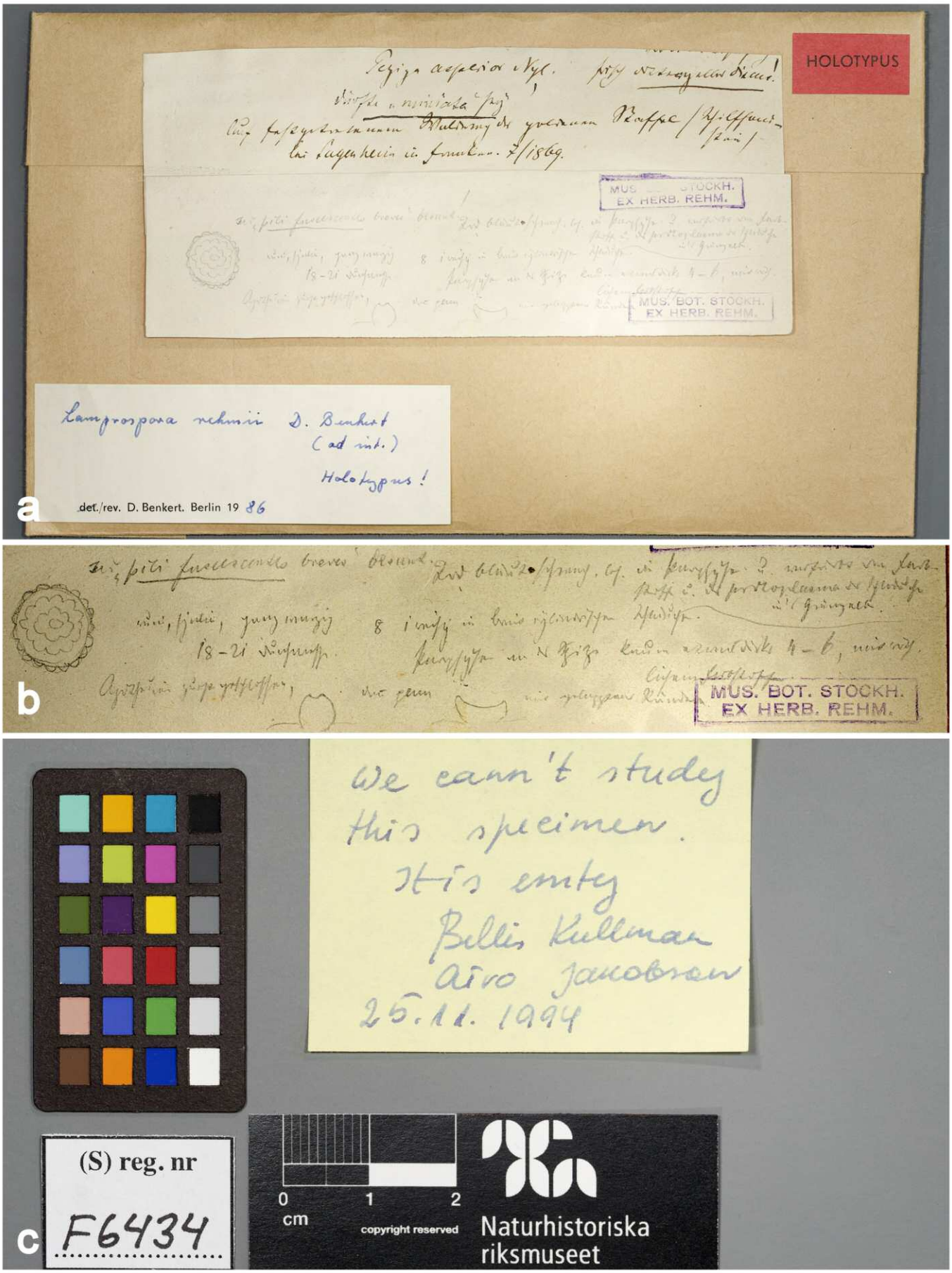

Fig. 5 – Photos of the voucher (a–b) of Rehm's collection of *Lamprospora rehmii* from Sugenheim deposited at Stockholm and the note by Kullmann & Jakobson. Copyright: Naturhistoriska riksmuseet, Stockholm.

Fig. 6 – Photo of the voucher of Rehm's collection of *Ramsbottomia macracantha* from Sugenheim deposited at Stockholm. Copyright: Naturhistoriska riksmuseet, Stockholm.

Discussion

The comprehensive description by Benkert (1987, 1994) combined with ascospore ornamentation makes *L. rehmii* easy to determine. If in doubt we refer to the key to all species of bryophilous *Pezizales* with globose or nearly globose ascospores and an ascospore ornamentation consisting solely of isolated warts in Vega *et al.* (2016: 170).

Nevertheless we would like to mention two features we observed in vital material which complete Benkert's results from studies on exsiccata: As in many other vital collections of bryophilous *Pezizales* we have studied so far, the measurements of ascospores based on sporeprints are at the upper scale or beyond the ones taken from dead material.

Furthermore the quantity and size of the vacuoles within the paraphyses mentioned in our description above — which cannot be studied in exsiccata — can serve as a very important character for determination and should always be noted, a fact completely ignored by the vast majority of our predecessors.

We will come to talk about this and other general results from the study of vital collections of bryophilous *Pezizales* in detail in another paper which will appear soon.

Another observation made is that even though Benkert (1987: 239) claims there are no small warts between the big ones and the tubercles in *L. rehmii*, we must emphasize that whilst there are not many, they are occasionally seen.

Finally we would like to report what we came across during our literature studies: *L. rehmii* was described in Benkert (1987: 239f.), the holotype dating from July 1869 was collected by Heinrich Rehm in Sugenheim (Franconia) in Germany. In Rehm (1887-1896) there are two entries worth a look:

On page 1038 under number 5718 there is a species named *Sphaerospora trechispora* Berk. & Broome — nowadays' *Scutellinia trechispora* (Berk. & Broome) Lambotte. Below Rehm lists *Peziza asperior* Nyl. as one of its synonyms. In the following description Rehm points at a particular collection of *Peziza asperior* Nyl., the apothecia of which have no hairs, thus these specimens have been separated by him and filed under the name *Barlaea asperella* Rehm (see page 932 of the same work).

The description of this *B. asperella* (filed under number 5568), however, rather matches with the features of a species of *Ramsbottomia* but not with the ones of the later *L. rehmii*. The locality given in the description is Sugenheim (Franconia) and thus identical with the one indicated by Benkert for the holotype of *L. rehmii*.

Taking a look at Rehm's herbarium deposited in S unfortunately does not clarify things. There are two vouchers with Sugenheim as locality, the labelling of both is *Peziza asperior* Nyl. — it will remain Rehm's secret why he refrained fom changing the name of one of them to *B. asperella* Rehm.

On the voucher of the exsiccate with the registration number F6436 there is Rehm's stylized sketch — reminiscent of the ascospore ornamentation of *L. tuberculatella* Seaver — that aroused Benkert's interest and induced him to study the exsiccate (Benkert, 1994: 239) which lead to the description of *L. rehmii* (Fig. 5).

The other collection in voucher F189643 was revised by Trond Schumacher as *Ramsbottomia macracantha* (Boud.) Benkert & T. Schumach. in 1985 (Fig. 6).

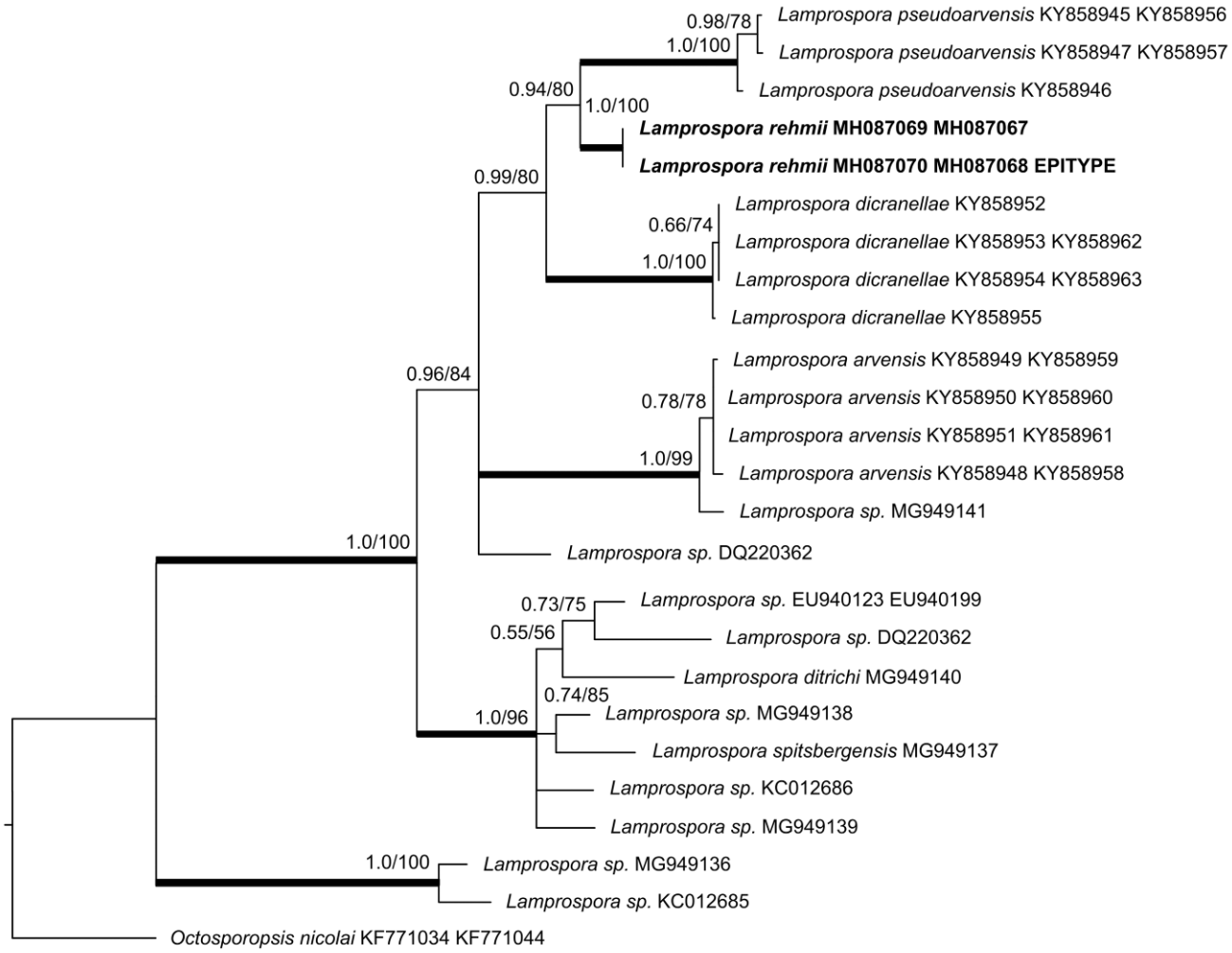

Fig. 7 – Fifty percent majority rule Bayesian phylogram showing phylogenetic relationship of *Lamprospora rehmii* to other related taxa based on ribosomal DNA (ITS, LSU). Numbers above branches represent Bayesian posterior probabilities (BA) and maximum likelihood bootstrap values (ML), respectively. Thickened branches indicate BA ≥0.95 and ML ≥90.

As we were interested in studying both the holotype and Rehm's drawing, we got in touch with Anna-Lena Anderberg from the Swedish Museum of Natural History in Stockholm and thus learnt about the lack of holotype material, a fact which had already been discovered by Bellis Kullman and Aivo Jakobson who wanted to study the type in 1994. When Dagmar Triebel from the Botanische Staatssammlung Muenchen informed us that their collection of *L. rehmii* was in poor condition and not suitable for an epitype we chose the aforementioned collection from La Rioja, Spain. The **epitype** (here designated) is deposited in Stockholm under the accession number F317032, the IF registration number is 554449, the supported **lectotype** (here designated) is the illustration in the protologue (Benkert, 1987: 262, plate 12, fig. 15), the IF registration number is 554448.

Credits

For providing us with samples and information we would like to thank Beñat Jeannerot (France). Jan Eckstein (Germany) kindly examined the infection of the host moss *P. acuminatum* and commented on it, Javier Martínez-Abaigar (Spain) helped with moss determination of the accompanying mosses. We thank the sequencing laboratory of the Faculty of Science, Charles University, Prague, Czech Republic for support. We are grateful to the curators of the following herbaria: Anna-Lena Anderberg and Åsa Dalsätt (Swedish Museum of Natural History, S) and Dagmar Triebel (Botanische Staatssammlung, München, M) for information and documentation. Paul Kirk (UK) kindly helped us out with typification matters and wording. Thanks go to Gernot Friebes (Austria) for reading the manuscript and valuable suggestions and to Carol Hobart (UK) for the English revision.

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