Lamprospora pseudoarvensis sp. nov. (*Pezizales*) – a lookalike tracked down

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Introduction

This article is a sequel to VEGA *et al.* (2013) which dealt with collections of *Lamprospora arvensis* (Velen.) Svrček. VEGA *et al.* (2013: 506) already discussed collections from Spain and Portugal which differed from the species concept of *L. arvensis* not only by infecting another host — *Pleuridium acuminatum* Lindb. instead of *Ceratodon purpureus* (Hedw.) Brid. — but also by having bigger ascospores with a different ornamentation. Having studied further collections, and with results from sequencing obtained, we are now in a position to summarize the differences between these collections and subsequently describe the new species *Lamprospora pseudoarvensis* on *P. acuminatum*.

HILL *et al.* (2006) was used for the taxonomy of mosses referred to in the paper.

Material and methods

The description of *L. pseudoarvensis* is based on the results of the examination of vital as well as rehydrated collections from several localities in Europe. Most observations were made in tap water. Ascospore ornamentation was also studied after staining with Lactophenol Cotton Blue (LPCB), aqueous Brilliant Cresyl Blue (CRB) or aqueous Methylene Blue (MB), and the absence of the iodine reaction of the asci was checked with Lugol's solution (IKI). Ascospore size was measured from free ascospores. Additional measurements from rehydrated material and are therefore not stated separately. Macrographs were made with digital cameras. Micrographs were taken either in tap water, LPCB or CRB, using digital cameras mounted on microscopes and software for stacking pictures. Scanning electron micrographs (SEM) were taken with a LEO-438 machine from air dryed samples.

DNA extraction, amplification and sequencing

Total DNA was extracted from dry and CTAB-preserved samples employing a modified protocol based on MURRAY & THOMPSON (1980). A portion of each sample was blended with the aid of a micropestle in 600 μ L CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.020 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform: isoamilalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifugated for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifugated again for 2 min and dried.

Abstract: *Lamprospora pseudoarvensis* is described as a new species based on ecology, morphology and phylogenetic analyses. Differences from other bryophilous *Pezizales* occuring on species of *Pleuridium* are discussed as well as differences from morphologically similar bryophilous *Pezizales*. New statements on *L. dicranellae* are reported. A dichotomous key to *L. pseudoarvensis* and similar species is given. **Keywords:** Bryophilous *Pezizales, Ditrichum heteromallum, Ditrichum pusillum*, dichotomous key,

Keywords: Bryophilous Pezizales, Ditrichum heteromallum, Ditrichum pusillum, dichotomous key, Lamprospora dicranellae, Pleuridium acuminatum, Pyronemataceae, taxonomy.

DNA was finally resuspended in 200 μ L ddH₂O. PCR amplification was performed with the primers LROR and LR5 (VILGALYS & HESTER, 1990; CUBETA *et al.*, 1991) to amplify the 28S rDNA region, and primers ITS1F and ITS4 were used to amplify the ITS region. 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 for 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked for putative reading errors, and these were corrected.

Phylogenetic analysis

BLAST was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. The selected sequences came from PERRY *et al.* (2007), HANSEN *et al.* (2013), LINDEMANN *et al.* (2014, 2015) and VAN VOOREN *et al.* (2015), including those of *Paratricharina poiraultii* (Boud.) Van Vooren, U. Lindemann, M. Vega, Ribes, Illescas & Matočec and *Byssonectria deformis* (P. Karst.) U. Lindem. & M. Vega, chosen as outgroup.

Sequences of the ITS and LSU regions were aligned with the Q-INS-i option of MAFFT v7 (KATOH & STANDLEY, 2013), which considers rRNA secondary structure. ITS was aligned using the 200PAM / κ =2 scoring matrix, while 2PAM / κ =2 was used for LSU. Otherwise standard parameters were used. For truncation of the sequences and alignment control, Bioedit 7.2.5 (HALL, 1999) was used. Phylogenetic reconstruction was performed using MrBayes v3.1.2 (RON-QUIST & HUELSENBECK, 2003). For Bayesian analysis, GTR+G was assumed as substitution model for all partitions. For both datasets, two runs with each 2,000,000 generations in four chains were performed, sampling every 100 generations, and with a burn-in of 10 percent. Results were evaluated with Tracer v1.6 (RAMBAUT *et al.*, 2014), all analyses had log likelihood ESS values above 200. For visualisation of phylogenetic trees, FigTree v1.4.3 (RAMBAUT, 2016) was used.

Taxonomy

Lamprospora pseudoarvensis M. Vega, Eckstein, Friebes & R. Tena, *sp. nov.* — MycoBank 822693.

Diagnosis: Differs from other species of *Lamprospora* by its perfectly globose ascospores, 19–24 μ m in diameter, ornamented with both a regular net and occasional stains or warts within the meshes together with an infection on the rhizoids of its host *Pleuridium acuminatum*.

Holotype: The holotype of *Lamprospora pseudoarvensis* is deposited in the Herbarium Hamburgense of the University of Hamburg (HBG–*holotypus*, HBG-024462). The isotype of *L. pseudoarvensis*

is filed under MV20151121-03 in the personal herbarium of Marcel Vega.

Etymology: *pseudoarvensis* = refering to the resemblance with *Lamprospora arvensis*.

Macroscopic features (Fig. 1, c–f)

Apothecia mostly scattered, sometimes gregarious on soil between shoots of *Pleuridium acuminatum*; 0.5–3 mm in diam., first spherical, becoming saucer-shaped, finally thick and discoid with a flat hymenium, sessile; with hyaline septate anchoring hyphae; mostly with a low and narrow fimbriate margin; hymenium light orange to orange, margin and outer surface slightly paler than the hymenium.

Microscopic features (Fig. 2)

Asci (200) 240–300 (320) \times (18) 20–32 (36) μ m, cylindrical, 8spored, operculate, inamyloid, shortly bifurcate at the base, arising from perforated croziers. Ascospores [only free ascospores considered] (18) 19–24 (25) µm (ornamentation included), hyaline, globose, always with a large lipid drop, diameter (11) 12–16 μ m, uniseriate. Immature ascospores within the ascus sometimes have several small lipid drops of different sizes. The ascospore ornamentation consists of a regular polygonal net of ridges that are mostly (1) 2–3 (4) μ m broad and 1.5–3 (4) μ m high and often meet in clear angles of 60-120°, ridges are not or only slightly widened at meeting points. When stained the ridges of immature ascospores show very tiny cavities and the "rims" of the ridges appear to be crenulate which could be compared to the rim of a jam jar which is marked with traces of jam (see Fig. 2d). The net's meshes are 3-7 (11) μ m broad, within them subtle interconnections can be observed as well as stains or low warts of irregular shape that can be up to 2 μ m broad and 1 µm high, the stains or warts are sometimes anastomosing. Paraphyses filiform, containing, at least in the the upper half, many vacuoles 1–4 μ m diam., stainable with CRB; distinctive carotenoid pigment turning cyan to olivaceous in Lugol's solution; straight, pluriseptate, apically not or only slightly inflated, terminal cell 45–80 (100) \times 3–5 (6) μ m, sometimes clavate. Medullary excip**ulum** of *textura angularis* with cells measuring $10-35 \times 6-24 \mu m$. Ectal excipulum of textura intricata of elongated thick-walled cells, $12-26 \times 3-11 \mu m$. **Margo** of *textura porrecta*, of elongated hyphae, septate, 70–100 \times 5–11 μ m.

Infection

Lamprospora pseudoarvensis infects the rhizoids of the moss Pleuridium acuminatum. The infection structure consists of appressoria, infection peg and haustoria. The (2-)3-celled, thick-walled appressorium, $30-35 \times 20-25 \mu m$ in side view, sits on a large rhizoid cell and is surrounded by a dense layer of accompanying hyphae,

10–15 µm wide. From the central cell of the appressorium an infection peg penetrates the rhizoid's cell wall. The infection peg is surrounded by a tube of host cell material through which the haustorium grows into the rhizoid cells. Haustoria are thin-walled and strongly curled and ramified and fill the host cell up to 2/3 of its volume. No septa were seen within the haustoria and they do not grow through cross walls of rhizoid cells. However, the observation of internal details of the infection structure was very difficult and hindered by the covering layer of accompanying hyphae. The infection does not weaken the host discernably.

The observed infection structure of *L. pseudoarvensis* is typical for many species of *Lamprospora*. At its site in Germany *L. pseudoarvensis* was accompanied not only by its host *Pleuridium acuminatum* but also by *Ceratodon purpureus*, the host of *L. arvensis*, but only *P. acuminatum* was infected.

Phylogenetic analysis

The morphological evidence for L. pseudoarvensis as a distinct species is well supported by molecular data. Three analysed samples from Spain, Austria and France are clearly resolved as monophyletic (PP = 1) in the LSU tree (fig. 5). The sister group of *L. pseudoarvensis* is L. dicranellae Benkert (PP = 0.82). Lamprospora arvensis branches more basally and forms a well-supported clade (PP = 0.89) with the L. pseudoarvensis / L. dicranellae group as well as L. ascoboloides Seaver and an unidentified Lamprospora sequence, possibly also L. ascoboloides. All newly generated and most deposited Lamprospora sequences were resolved as monophyletic clade (PP = 1) with Octospora lilacina (Seaver) Svrček & Kubička as next relative. Only L. norvegica Benkert, Aas & R. Kristiansen KC012684 was found to cluster with Octosporopsis. This latter result has to be taken with caution, since the long branch of *L. norvegica* suggests possible long branch attraction effects, and thus the sequence might in fact belong elsewhere.

Sequencing of the ITS region was only partly successful and the data has to be interpreted with caution. Nevertheless, phylogenetic reconstruction of the more variable ITS region (fig. 6) shows a similar picture as for the LSU data.

Habitat and occurrence (Fig. 1, a, b)

Like its host *P. acuminatum* the new *Lamprospora* can occur in many different habitats from sea level to the montane level. So far, the highest record is at 1700 metres asl from Bronchales (Teruel) in Spain (VEGA *et al.*, 2013: 498).

Pleuridium acuminatum is a pioneer species of bare soil. It usually occurs on disturbed ground in more or less acidic situations. Therefore, it is extremely rare or lacking in calcareous areas. The morphologically similar *P. subulatum* (Hedw.) Rabenh. has a broader ecological amplitude and also occurs in less acidic environments.

Species	Voucher no. (herbarium)	Geographical origin, year and collector	LSU	ITS
L. pseudoarvensis	HBG-024462	Spain, 2015, M. Vega	KY858945	KY858956
L. pseudoarvensis	HBG-024463	Austria, 2015, G. Friebes	KY858947	KY858957
L. pseudoarvensis	HBG-024464	France, 2014, B. Jeannerot	KY858946	
L. arvensis	RM-2399	Spain, 2016, R. Martínez	KY858948	KY858958
L. arvensis	43560 (JE)	Germany, 2015, J. Eckstein	KY858949	KY858959
L. arvensis	HBG-024465	Germany, 2015, M. Vega	KY858950	KY858960
L. arvensis	HBG-024466	Germany, 2015, J. Simbieda	KY858951	KY858961
L. dicranellae	HBG-024467	Portugal, 2014, M. Vega	KY858952	
L. dicranellae	HBG-024468	France, 2013, M. Vega	KY858953	KY858962
L. dicranellae	HBG-024469	Germany, 2013, M. Vega	KY858954	KY858963
L. dicranellae	HBG-024470	Germany, 2013, M. Vega	KY858955	

Table 1 - Sequenced collections with voucher information and GenBank accession numbers



Fig. 1 – Lamprospora pseudoarvensis

a: habitat of the Austrian collections (GF20150005 and GF20160003). b: habitat of the holotype (HBG-024462), arrow indicating the exact position where the apothecia were found. c–f: apothecia with the host *Pleuridium acuminatum*. c: MV20151121-03. d, e: collections from Nantes, France (*leg*. P. Ribollet, not documented by voucher). f: GF20150005. Photos: a: M. Friebes. b, c: M. Vega. d, e: P. Ribollet. f: G. Friebes.

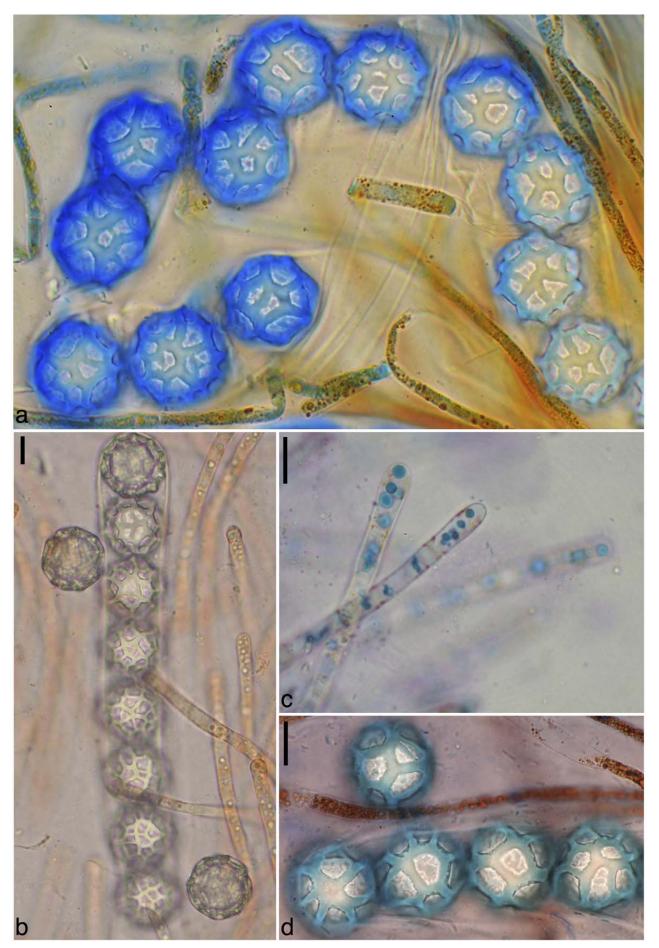


Fig. 2 – Lamprospora pseudoarvensis

a: ascospores in LPCB. b: asci and paraphyses in water. c: paraphyses in CRB and tap water. d: immature ascospores in aqueous MB. Scale bar = 10 µm for all photos. a: BJ-2013-21. b, c: MH20315. d: RT10053001 (collection from Bronchales, Spain, by Raúl Tena Lahoz, see VEGA *et al.*, 2013). Photos: a: B. Jeannerot. b, c: M. Hairaud. d: R. Tena Lahoz.

However, only *P. acuminatum* was observed as a host of *L. pseudoarvensis* so far. *P. acuminatum* has a European temperate distribution with further occurences around the Mediterranian Sea, Macaronesia, Caucasus, China and North America (SMITH, 2004: 148).

RIBOLLET (2016: 9) reports frequent finds of "*L. arvensis* f. on *Pleurid-ium*" around Nantes in the département Loire-Atlantique, France, upon request he named localities such as a canal slope, a side of a well-tended alley on a cemetery, a mound of earth piled up recently, unbuilt land, a lawn which is frequently crossed by pedestrians and a recently tilled soil at the edge of a path (pers. comm.).

Beñat Jeannerot (pers. comm.) considers *L. pseudoarvensis* to be the most frequent species of *Lamprospora* in the département Pyrénées-Atlantiques (France) in winter. There, landscaping includes cutting the vegetation on the roadside in late summer and is mainly done by local farmers who use heavy devices and thus tear out the plants rather than cutting them off. In the following months, pioneer mosses such as *P. acuminatum* invade the bare spots.

RUBIO *et al.* (2002) do not give any details on the habitats of their collections filed under *L. arvensis*. The Asturian ones reported from *P. acuminatum* (RUBIO *et al.*, 2002: 68-69) undoubtedly belong to *L. pseudoarvensis* whereas the collections on *Ceratodon purpureus* from Valladolid and Segovia fit better with *L. arvensis*.

The earliest record of *L. pseudoarvensis* known to us so far dates from 1989: During the revision of the herbarium of Antoine Ayel in 2014 Nicolas Van Vooren stumbled over material of a *Lamprospora* found on January 18th 1989 in Poncins (département Loire, France) and determined by Ayel as *Octospora areolata* (Seaver) Caillet & Moyne. Van Vooren's documentation and the identification of the moss as *P. acuminatum* proved the *Lamprospora* to be *L. pseudoarvensis*.

So far the new species is known from France, Spain, Portugal (specimen 5 in VEGA *et al.*, 2013: 498), Austria and Germany. Although the information about the distribution of the new species is sparse, the data indicates its occurence at least throughout western and central Europe.

Specimens examined

Besides the collections we studied for VEGA *et al.* (2013) we examined the following specimens of *L. pseudoarvensis* and *L. arvensis*.

L. pseudoarvensis

1 Spain, Barranco de Guarimar, Pajaritos (La Gomera, Canary Islands), 28°6'19.8" N, 17°14'32.4" W, 1324 m asl, slope besides a forest road, *leg*. Rubén Negrín, Domingo Chávez & Miguel Ángel Ribes, December 27th 2013. Bryophyte host: *Pleuridium* sp., pers. herb. MR 271213 10.

2 France, Sedzère (Pyrénées-Atlantiques), 355 m asl, slope above a roadside ditch, *leg*. Beñat Jeannerot, January 25th 2014. Host: *P. acuminatum*, HBG-024464, duplicates pers. herb. BJ-2013-21, pers. herb. MV20140201-01.

3 Germany, Duderstadt (Thuringia), 51°29'21.7" N, 10°16'27.3" E, 231 m asl, base-poor grassland with some *Calluna*, *leg*. Jan Eckstein, January 18th 2015. Host: *P. acuminatum*, no. 38073 (JE).

4 Austria, Gleichenberger Kogel, Bad Gleichenberg (Styria), 46°53'13" N, 15°54'50" E, 440 m asl, bank besides a forest, *leg*. Gernot Friebes, February 8th 2015. Host: *P. acuminatum*, HBG-024463, duplicate pers. herb. GF20150005.

Additional collection at this site: February 13th 2016, pers. herb. GF20160003.

5 France, Penvenan, Queffioec (Côtes-d'Armor), 48°49'31.7" N, 3°17'17.7" W, 26 m asl, on a slope, *leg*. Michel Hairaud & Brigitte Capoen, March 4th 2015. Host: *P. acuminatum*, soc.: *Lamprospora annulata* Seaver; pers. herb. MH20315.

6 Spain, Sierra de Guadarrama, Valle de Lozoya (Madrid), 40°50'12.1" N, 3°54'48.3" E, 1470 m asl, besides a trail next to a tree stump, *leg*. Marcel Vega, Miguel Ángel Ribes & Fermín Pancorbo, November 21st 2015. Host: *P. acuminatum*; Holotype (HBG-024462), Isotype: pers. herb. MV20151121-03.

L. arvensis

7 Germany, Berschweiler, nature reserve Hosenbachtal (Rhineland-Palatinate), 411 m asl, grassland, *leg.* Joschi Simbieda, *det.* Marcel Vega, November 2nd 2013. Host: *Ceratodon purpureus*, HBG-024466, duplicate pers. herb. MV20131114-01.

Additional collections at this site: April 4th 2015, pers. herb. MV20150405-02 and October 22nd 2015, MV20151027-01.

8 Germany, Harzungen (Thuringia), 51°32′58.7″ N, 10°49′3.7″ E, 260 m asl, dry grassland with some heath vegetation over gypsum soil in slightly northern exposition, *leg.* Jan & Günter Eckstein, December 25th 2013. Host: *C. purpureus*, no. 34579 (JE). Additional collection at this site December 5th 2015. Host: *C. purpureus*, no. 3560 (JE).

9 Spain, Entrena "La Rad" (Rioja), 42°22'54" N, 2°29'28" W, 546 m asl, animal's crossing, *leg*. Rubén Martínez Gil, *det*. Marcel Vega, February 22nd 2015. Host: *C. purpureus*, pers. herb. RM-2368, duplicate pers. herb. MV20151231-02.

Additional collections at this site: January 2nd 2016, pers. herb. RM-2399 and March 19th, 2016, pers. herb. RM-2341.

10 Germany, Sterley-Pipersee (Schleswig-Holstein), 53°37'50.37" N, 10°50'55.69" E, 50 m asl, sand pit, *leg*. Marcel Vega, December 30th 2015. Host: *C. purpureus*, HBG-024465.

L. dicranellae

Besides the collections we studied for ECKSTEIN *et al.* (2014) we examined the following specimens of *L. dicranellae*:

11 France, Aime-la-Plagne (Savoy), Cormet d'Arêches, 45°36'46.36" N, 6°36'8.92" E, 2160 m asl, meadow, *leg*. Marcel Vega, August 28th 2013 (alpine foray Ascomycete.org). Host: *Ditrichum heteromallum* (Hedw.) E. Britton, HBG-024468, pers. herb. MV20130828-01.

12 Germany, Friedhof Stellingen (Hamburg), 53°35′24.88″ N, 9°55′17.05″ E, 24 m asl, old grave, *leg*. Marcel Vega, October 6th 2013. Host: *Ditrichum pusillum* (Hedw.) Hampe, pers. herb. MV20131006-01. Additional collections at this site: October 21st, November 8th and November 24th 2013, HBG-024470.

13 Germany, Höltigbaum nature reserve (Hamburg), 53°37′29.63″ N, 10°11′54.64″ E, 40 m asl, grassland, *leg*. Marcel Vega, November 16th 2013. Host: *D. heteromallum*, HBG-024469.

14 France, Lanans (Doubs), approx. 500 m asl, grain field, *leg*. Pierre Chaillet, November 16th 2013. Host: *D. pusillum*, pers. herb. MV20131116-07.

15 Portugal, Fanal (Madeira), 32°48'40.82" N, 17°8'30.23" W, 1152 m asl, slope besides a willow, *leg*. Marcel Vega, December 25th 2014. Host: *D. heteromallum*, HBG-024467, duplicate pers. herb. MV20141225-06.

16 Germany, Segrahner Forst (Schleswig-Holstein), 53°31'9.678" N, 10°45'39.192" E, 43 m asl, in a rut on a forest track, *leg*. Marcel Vega, October 16th 2016. Host: *D. heteromallum*, pers. herb. MV20161016-01.

Discussion

To compare the new *L. pseudoarvensis* with similar species of bryophilous *Pezizales*, we consider here a) bryophilous *Pezizales* known or suspected to parasitise species of the moss genus *Pleurid-ium* on the one hand and b) bryophilous *Pezizales* with ascospores ornamented with both a more or less regular net of rather thick ridges (2 µm and wider) and more or less distinctive warts or stains on the other hand.

In a) there are *L. annulata* Seaver, *L. rehmii* Benkert, *L. tuberculata* Seaver, *Octospora lilacina* and *O. phagospora* (Flageolet & Lorton) Dennis & Itzerott, and in b) *L. dicranellae* Benkert, *L. bavarica* Benkert and *L. arvensis*.

a) Bryophilous *Pezizales* known or suspected to parasitise species of the moss genus *Pleuridium*

Lamprospora annulata is known to parasitise Pleuridium sp. and P. subulatum (Hedw.) Rabenh. as well as Ephemerum minutissimum

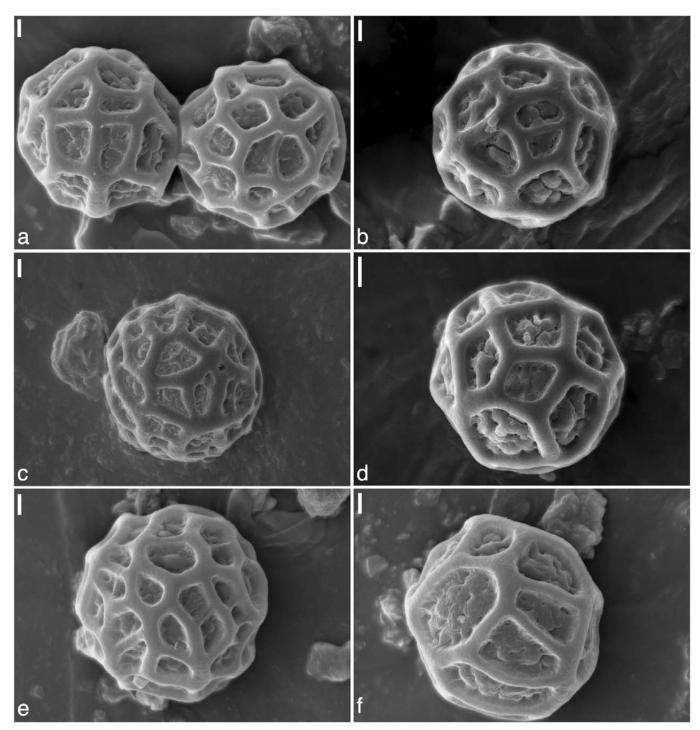


Fig. 3 – SEM photographs of ascospores of *Lamprospora pseudoarvensis*. a, e, f: BJ-2013-21. b, d: GF20150005. c: JE 38073. Scale bar = 2 µm for all photos. Photos: J. Eckstein.

Lindb. (ECKSTEIN & ECKSTEIN, 2013: 57). Its ascospores are smaller than the ones of *L. pseudoarvensis* and their ornamentation is characterised by ridges and prominent warts. The ridges often form circles but never connect in a way to form a reticulate pattern (fig. 1 in KRISTIANSEN, 2006: 70).

Prominent warts or tubercles cover the entire surface of the ascospores of *L. rehmii* (Fig.1 in BENKERT, 1994: 142). They therefore differ entirely from the reticulate ascospores of *L. pseudoarvensis*. *Lamprospora rehmii* is reported from *Pleuridium* sp. without any further details on the species in BENKERT (1994: 140), whereas in BENKERT (2009: 59) the author repeats his result from BENKERT (1987: 239) that the host could be either *Dicranella* sp. or *Ditrichum* sp., which presumably is a careless mistake.

Lamprospora tuberculata occurs both on *P. acuminatum* and *P. subulatum* (BENKERT, 2009: 60). Its ascospore ornamentation con-

sists only of large tubercles and occasional small warts (fig. 6 in Eck-STEIN *et al.*, 2014: 20).

BENKERT (1998: 59, 2009: 64) reports *Pleuridium* sp. and/or *Pohlia lutescens* (Limpr.) H. Lindb. as possible hosts of *Octospora phagospora* and *O. lilacina*. However, our own unpublished data indicate *Dicranella heteromalla* (Hedw.) Schimp. as host of *O. lilacina* and *Pohlia lutescens* as host of *O. phagospora*. Both species have ellipsoid spores.

Therefore neither the three species of *Lamprospora* nor the two species of *Octospora* occuring or possibly occuring on *Pleuridium* can be confused with the new *L. pseudoarvensis*.

b) Bryophilous Pezizales with similar ascospores

We consider species with globose ascospores and an ornamentation consisting of a more or less regular net of rather thick ridges

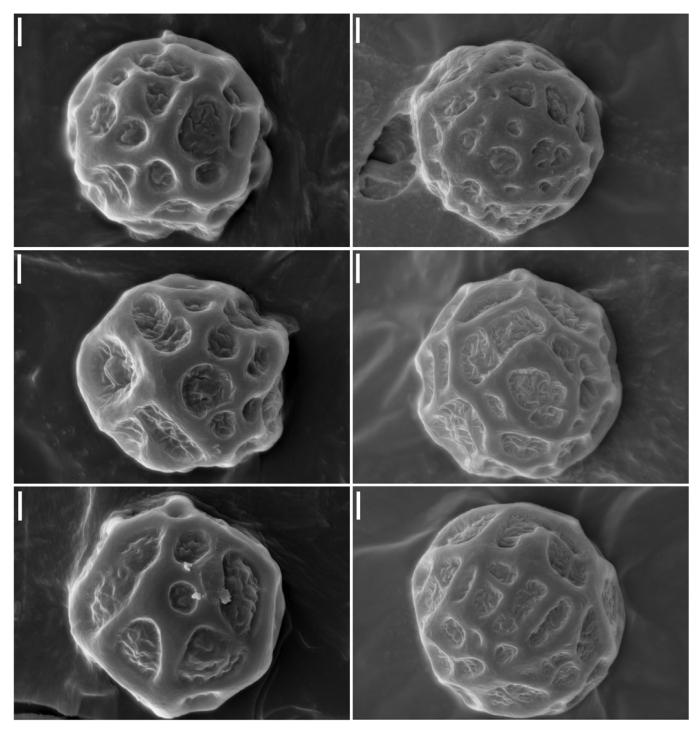


Fig. 4 – SEM photographs of ascospores of *Lamprospora arvensis*. Photographs on the left: MV20131114-01; photographs on the right: JE 34579. Photos: J. Eckstein.

and stains or warts within the meshes of the net as similar to *L. pseudoarvensis*. There are three such species and none of their hosts are closely related to the genus *Pleuridium*.

L. dicranellae

It should be mentioned first that the identification of *L. dicranellae* can be a test of patience as its ascospore ornamentation is in some cases difficult to stain (no matter which dye is used and no matter if ascospores are immature in the ascus, free, or studied from a spore print). Ascospores of *L. dicranellae* measure (13) 14–17 (18) µm (ECK-STEIN *et al.*, 2014: 19, pers. observation) and their ornamentation consists of numerous prominent warts and rather coarse and irregular ridges anastomosing at times and thus forming a kind of rudimentary reticulum which can resemble an ornamentation of the *seaveri*type.

Not only are the ascospores of *L. pseudoarvensis* considerably larger but their ornamentation is characterised by fairly regular ridges which mostly meet in angles and low warts or merely stains and/or thin and low ridges in the meshes, thus a mix-up of the two species is hardly conceivable.

The only host of *L. dicranellae* that had previously been proven is *Ditrichum heteromallum* (Hedw.) E. Britton (ECKSTEIN *et al.*, 2014: 19); the collections from Hamburg and Besançon stated above occured on *Ditrichum pusillum*, a host not reported until now. It should be added here that the collections of *L. dicranellae* from Hamburg and Schleswig-Holstein show that this species is not restricted to mountainous regions, as previously stated in ECKSTEIN *et al.* (2014: 19).

For the time being it remains unclear whether a species of the genus *Dicranella* too can serve as a host for *L. dicranellae* as assumed in the original description (BENKERT, 1987: 217–219).

Although the genera *Ditrichum* and *Pleuridium* both belong to the *Ditrichaceae*, a family within the order *Dicranales*, they are not closely related. *Dicranella* belongs to the *Dicranellaceae*, yet another family in the *Dicranales*.

L. bavarica

This species was described based on a single collection, which might have been partly immature (BENKERT, 2011: 150). While its ascospores, which measure (20) 22–24 (25) μ m (BENKERT, 2011: 150), are in the same range as the ones of *L. pseudoarvensis*, their ornamentation is much more similar to *L. arvensis* than to *L. pseudoarvensis* (see BENKERT, 2011: 151, Plate 1). Its very broad ridges cover the ascospore's surface extensively and the angles of the reticulum's comparatively small meshes are rather rounded and reminiscent of a bull's eye like in *L. arvensis* while they are mostly right-angled in *L. pseudoarvensis*.

Its host *Paraleucobryum longifolium* (Hedw.) Loeske occurs on rotten wood and thus has a very different ecology to *P. acuminatum*, the host of *L. pseudoarvensis*, which grows on bare, acidic soil.

Although further collections of *L. bavarica* may still widen the current species concept, it is unlikely that this species could be mistaken for *L. pseudoarvensis*.

L. arvensis

Since this species was presented in detail in VEGA *et al.* (2013) we focus here on the differences from *L. pseudoarvensis* which are given in the following table.

Table 2 – Comparison of macroscopic and microscopic features of

 L. pseudoarvensis and *L. arvensis*

Feature	L. pseudoarvensis	L. arvensis		
Apothecia	1.5–3 mm	(1) 2–4 mm		
Margin	low, slightly fimbriate	raised, prominent		
Ascospore size	(18) 19–24 (25) μm	(16) 18–20 (22) µm		
Ornamentation				
- ridges	± regular	very variable in width and height		
- meeting points of the ridges	hardly thickened	thickened/knotty		
- angles of the net's meshes	clearly angled	rather rounded meshes often reminiscent of a bull's eye		
Host	Pleuridium acuminatum	Ceratodon purpureus		

The SEM photographs (Fig. 3 and 4) show the rather marked and regular net of *L. pseudoarvensis* in contrast to the one of *L. arvensis* with both ridges of inconsistent breadth and height and nodular thickenings, whereas the angles of the meshes of the two species do not appear as diverse as perceived under a light microscope.

There are also other bryophilous *Pezizales* which have features that can be observed more easily under a light microscope, e.g. the foamy content of the ascospore tubercles of *L. maireana* Seaver which is very obvious when using a light microscope but cannot be seen at all with a SEM.

In VEGA et al. (2013: 507) we reported the discrepancies concerning the holotype and lectotype of L. arvensis. With ascospores measuring up to 25 µm (VELENOVSKÝ, 1934: 324) on the one hand and a drawing depicting an ascospore with a simple reticulate ornamentation (SVRČEK, 1979: 189, Tab. III, 1) on the other, a mix-up of specimens might have happened. BENKERT (1987: 208) re-examined the lectotype (PRM 150329) and reported a spore size of (16) 18–20 (22) μ m and Ceratodon purpureus as the accompanying moss, which is in accordance with the current concept of L. arvensis presented here. Furthermore, a different specimen by VELENOVSKÝ (PRM 149874) was accompanied by C. purpureus and Polytrichum piliferum Hedw. (BENKERT, 1987: 209), the latter of which is not a suitable host for L. arvensis. Therefore, the name L. arvensis (Velen.) Svrček applies to the species with C. purpureus as host moss. Leaving the inconsistencies of typification aside, we would like to indicate that the notes on the distribution of *L. arvensis* in Europe given in VEGA *et al.* (2013: 507) need to be seen in a new light now as there might have been collections attributed to L. arvensis in the past which could represent L. pseudoarvensis instead.

Key

With available keys being outdated, another new partial key (after the one given in VEGA *et al.*, 2016) is provided to facilitate identification of bryophilous *Pezizales* with globose ascospores ornamented with a more or less regular net of rather thick ridges and occasional warts or stains.

In addition to the species already mentioned, the key also includes *Lamprospora moynei* Benkert and *L. cailletii* Benkert, both of which have an ascospore ornamentation showing 1) warts and 2) ridges anastomosing at times. Specimens of all species mentioned were studied by at least one of the authors except for *L. bavarica*, which, as stated above, is currently known only from the type. Additional information has been gathered from BENKERT (1987, 2009, 2011), ECKSTEIN & ECKSTEIN (2013), ECKSTEIN *et al.* (2014), VEGA *et al.* (2013) and VEGA *et al.* (2015).

Key to bryophilous Pezizales with globose ascospores and an ornamentation of both rather thick ridges and stains or warts

1	Ascospores with ridges not forming a net but anastomosing at times
	Ascospores with ridges forming a net
2	Ascospores with ridges often forming circles, on Pleuridium and Ephemerum
2	Ascospores with thick-walled hollow ridges never forming circles, on Ephemerum
3	Ascospores with ridges mostly <1.5 µm wide forming an irregular alveolate reticulum accompanied by a faint secondary
	reticulum and occasional warts, on Tortella L. cailletii
3	Ascospores with ridges often >1.5 µm wide and no secondary reticulum but warts, different hosts
4	Ascospores with long curved ridges outlining an alveolate, often rudimentary reticulum and rather prominent warts,
	on Ditrichum
4	Ascospores with ridges forming an areolate reticulum and without warts or only with low warts or stains
5	Ascospores with \pm regular ridges not or scarcely widened at crossings forming a \pm regular reticulum with angular meshes,
	ascospores (18) 19–24 (25) μm, on Pleuridium
5	Ascospores with ridges variable in width and height, often considerably widened at crossings resulting in meshes with
	rounded angles often giving the impression of a bull's eye, different hosts
6	Ascospores 18–20 (22) µm, on Ceratodon L. arvensis
6	Ascospores (20) 22–24 (25) μm, on Paraleucobryum L. bavarica

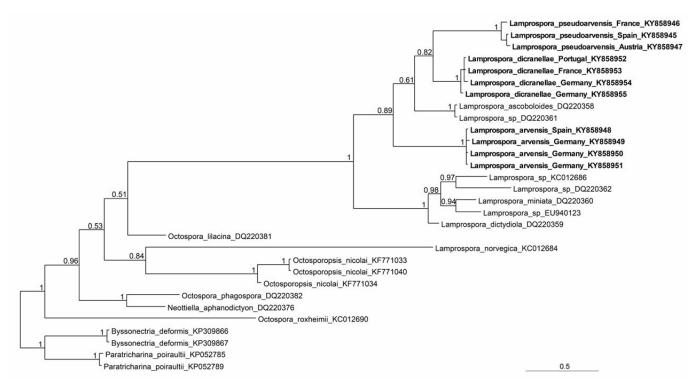


Fig. 5 – Bayesian phylogram of *Lamprospora* and selected relatives based on LSU data. Bayesian posterior probability values are indicated above corresponding branches. NCBI GenBank accession numbers of the corresponding entries is given after the species binomial. Bolded terminals are newly generated sequences.

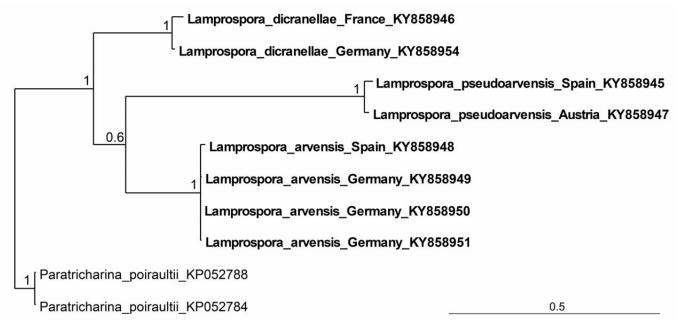


Fig. 6 – Bayesian phylogram of *Lamprospora* and selected relatives based on ITS data. Bayesian posterior probability values are indicated above corresponding branches. NCBI GenBank accession numbers of the corresponding entries is given after the species binomial.

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References

- BENKERT D. 1987. Beiträge zur Taxonomie der Gattung Lamprospora (Pezizales). Zeitschrift für Mykologie, 53 (2): 195-270.
- BENKERT D. 1994. Beiträge zur Kenntnis bryophiler *Pezizales*-Arten.
 3. *Lamprospora rehmii. Beiträge zur Kenntnis der Pilze Mitteleuropas*, 9: 139-142.

- BENKERT D. 1998. Beiträge zur Kenntnis bryophiler Pezizales-Arten
 8. Viersporige Taxa der Gattung Octospora. Österreichische Zeitschrift für Pilzkunde, 7: 39-63.
- BENKERT D. 2009. Zwei neue Arten bryophiler *Pezizales* (Ascomycota) aus der Bundesrepublik Deutschland und Auflistung der aus Deutschland bisher nachgewiesenen Arten mit Kurzdiagnostik. *Zeitschrift für Mykologie*, 75 (1): 51-68.
- BENKERT D. 2011. Lamprospora bavarica und L. esterlechnerae (Pezizales), zwei neue Arten aus dem Nationalpark Bayerischer Wald (Deutschland, Bayern). Zeitschrift für Mykologie, 77 (2): 149-155.
- CUBETA M.A., ECHANDI E., ABERNETHY T. & VILGALYS R. 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology*, 81 (11): 1395-1400.
- ECKSTEIN J. & ECKSTEIN G. 2013. Bemerkenswerte Funde bryoparasitischer *Pezizales* (Ascomycota) aus Deutschland. *Boletus*, 34 (2): 55-66.
- ECKSTEIN J., ECKSTEIN G. & VEGA M. 2014. Bemerkenswerte Funde bryoparasitischer *Pezizales* (Ascomycota) aus Deutschland II. *Boletus*, 35 (1): 17-25.
- HALL T.M. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95-98.
- HANSEN K., PERRY B.A., DRANGINIS A.W. & PFISTER D.H. 2013. A phylogeny of the highly diverse cup-fungus family *Pyronemataceae* (*Pezizomycetes*, Ascomycota) clarifies relationships and evolution of selected life history traits. *Molecular Phylogenetics and Evolution*, 67 (2): 311-335.
- HILL M.O., BELL N., BRUGGEMANN-NANNENGA M.A., BRUGUÉS M., CANO M.J., ENROTH J., FLATBERG J.I., FRAHM J.-P., GALLEGO M.T., GARILLETI R., GUERRA J., HEDENÄS L., HOLYOAK D.T., HYVÖNEN J., IGNATOV M.S., LARA F., MAZIM-PAKA V., MUÑOZ J. & SÖDERSTRÖM L. 2006. — Bryological Monograph. An annotated checklist of the mosses of Europe and Macaronesia. *Journal of Bryology*, 28: 198-267.
- KATOH K. & STANDLEY D.M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, 30 (4): 772-780.
- KRISTIANSEN R. 2006. Miniatyrbegersopper nye for Norge. *Agarica*, 26: 69-77.
- LINDEMANN U., VEGA M., RICHTER T. & ALVARADO P. 2014. Octosporopsis nicolai ein rätselhafter Vertreter aus der Familie der Pyronemataceae. Zeitschrift für Mykologie, 80 (2): 565-592.
- LINDEMANN U., VEGA M. & ALVARADO P. 2015. Revision der Gattung Kotlabaea: K. deformis, K. delectans und K. benkertii. Zeitschrift für Mykologie, 81 (2): 373-402.

- MURRAY M.G. & THOMPSON W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8 (19): 4321-4325.
- PERRY B.A., HANSEN K. & PFISTER D.H. 2007. A phylogenetic overview of the family *Pyronemataceae* (Ascomycota, *Pezizales*). *Mycological Research*, 111 (5): 549-571.
- RAMBAUT A. 2016. FigTree v1.4.3. Available from http://tree.bio.ed.ac.uk/software/figtree
- RAMBAUT A., SUCHARD M.A., XIE D. & DRUMMOND A.J. 2014. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer
- RIBOLLET P. 2016. Contribution à la connaissance des genres Octospora Hedw. 1789 et Lamprospora De Not. 1864 en Loire-Atlantique. Seconde partie : le genre Lamprospora De Not. 1864. Cahiers Mycologiques Nantais, 28: 7-13.
- RONQUIST F. & HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12): 1572-1574.
- RUBIO E., SUÁREZ A. & MIRANDA M.A. 2002. Estudios preliminares sobre los géneros *Lamprospora* De Notaris y *Ramsbottomia* W. D. Buckley (Ascomycetes, *Pezizales*) en Asturias. *Boletín de la Sociedad Micológica de Madrid*, 26: 61-82.
- SMITH A.J.E. 2004. *The moss flora of Britain and Ireland*. Cambridge, Cambridge University Press, 1012 p.
- SVRČEK M. 1979. A taxonomic revision of Velenovský's types of operculate discomycetes (*Pezizales*) preserved in National Museum, Prague. Sborník Národního muzea v Praze, 32B: 115-194.
- 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.
- VEGA M., RICHTER T. & SCHUBERT H. 2013. Zur Kenntnis von Lamprospora arvensis (Velen.) Svrček in Europa. Zeitschrift für Mykologie, 79 (2): 497-510.
- VEGA M., ECKSTEIN J., FRIEBES G. & STÖCKLI E. 2015. Lamprospora moynei Benkert – nun auch in Deutschland, Österreich und der Schweiz nachgewiesen. Zeitschrift für Mykologie, 81 (2): 403-416.
- VEGA M., ECKSTEIN J. & VAN DER KOLK H.J. 2016. Lamprospora verrucispora sp. nov. (*Pezizales*). Ascomycete.org, 8 (4): 163-171.
- VELENOVSKÝ J. 1934. Monographia Discomycetum Bohemiae. Vol. 1 and 2. Prague, 436 p. + 31 plates.
- VILGALYS R. & HESTER M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172 (8): 4238-4246.



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