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Lichenological Notes 8: *Acarospora fusca*

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ABSTRACT. – The monographer A.H. Magnusson considered *Acarospora fusca* to be a member of the morphologically defined *A. smaragdula* group, most of whose members are now recognized in the phylogenetically circumscribed genus *Myriospora*. Recently *A. fusca* has been considered a synonym of *M. rufescens*. This study presents newly generated ITS, mtSSU and LSU sequences from the neotype of *A. fusca* that show the species does not match *M. rufescens* or *Myriospora*. Instead, the data support that *A. fusca* belongs to *Acarospora* and Magnusson's interpretation that *A. fusca* is distinct from *M. rufescens*. The newly generated sequences of *A. fusca* were identical to those generated from two specimens identified as *A. anomala* and collected on a wood fence in Sweden.

KEYWORDS. – Acarosporaceae, *Acarospora sinopica,* lignicolous lichens.

INTRODUCTION

During our studies, we often discover new data that do not fit into the main papers we are working on. In Lichenological Notes we publish these random discoveries, lectotypifications, and nomenclatural novelties and make them available to current and future researchers. This is the eighth installment of this series.

MATERIALS AND METHODS

Herbarium study. – The morphology of specimens was studied with dissecting microscopes. At 1000x power with compound microscopes the anatomy of hand sections was examined and measured in water from specimens deposited in NY, PRM, and the private herbaria Jana Kocourková and Kerry Knudsen (hb. K&K), Jirka Malíček (hb. Malíček) and Ulf Schiefelbein (hb. Schiefelbein). The amyloid reaction of the hymenial gel and subhymenium was tested with fresh undiluted IKI (Merck's Lugol) (see protocol in Knudsen & Kocourková 2018). Secondary metabolites were studied with Thin Layer Chromatography using solvent C and following Orange et al. (2001). The description of the variability of *A. fusca* has been slightly revised based on study of new collections (Knudsen et al. 2017).

Photographs. – Macrophotographs of *Acarospora fusca* were taken with the digital camera Olympus DP72 mounted on Olympus SZX 7 Stereomicroscope equipped with PRO-SZM1-Focus Drive

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Motorization using Promicra QuickPhoto Camera 3.0 software and stacked using Olympus DeepFocus 3.1. module. The microphotographs were taken with a digital camera Olympus DP74 mounted on an Olympus BX51 Light Microscope fitted with Nomarski interference contrast and using Promicra QuickPhoto Camera 3.2 software. The figure plate was processed with the same software fitted with Promicra Figure Maker mo.

DNA extraction, amplification and sequencing. – DNA was extracted from the dried neotype of *Acarospora fusca*. Genomic DNA was extracted via the Invisorb® Spin Plant Mini Kit, according to the manufacturer's protocol with slight modifications (i.e., eluted in 60 µL of DNA, instead of 100 µL, and incubated in buffer for 15 minutes before final centrifuging). Total extracted DNA was stored at -20 °C. The quality and yield of DNA isolated was checked on a 1% agarose gel and DNA concentration and purity were then measured precisely using a UVS-99 spectrophotometer (ACTGene). The selected markers (Appendix I) for this study were the internal transcribed spacer complete repeat (ITS; White et al. 1990), the large subunit of the nuclear ribosomal DNA (nLSU; Vilgalys & Hester 1990) and the small subunit of the mitochondrial ribosomal DNA (mtSSU; Zoller et al. 1999). The ITS, nLSU and mtSSU regions were amplified via polymerase chain reaction (PCR). Each reaction contained 1 µL (20-25 ng) of extracted genomic DNA, 10 µL of 2x MyTaq™ HS Red Mix (Bioline, UK), 10 µL of water, 0.4 µM of forward/reverse primer (10 µM) for a total reaction volume of 20 µl. Conditions for ITS, mtSSU rDNA: initial denaturation 95 °C for 5 min, followed by five cycles (95 °C for 33 s, 56 °C for 30 s, and 72 °C for 30 s), than ten cycles (95 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s), and twenty cycles (95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s) with a final extension 72 °C for 10 min; with the following settings for the nLSU: initial denaturation 95 °C for 1 min, followed by five cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s) and finally 30 cycles (95 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s), with a final extension 72 °C for 7 min.

Before sequencing, the PCR products were purified using the enzymatic method Exo-Sap-IT™ Express PCR Product Cleanup provided by Thermo Fisher Scientific, Inc. according to the manufacturer's protocol. PCR products were run on a 1.0 % agarose gel via electrophoresis and stained with ethidium bromide for 25 min. Purified PCR products (1 µL), water (6 µL) and forward primer (1 µL) were sent to the BIOCEV (Vestec, CZ). Sequences were checked against the UNITE database and NCBI nr-database for contamination.

RESULTS

Bouly de Lesdain described *Acarospora fusca* from an anthropogenic substrate, tile or steppingstones probably of siliceous rock, among the sand dunes of Dunkerque, France (B. de Lesdain 1914, Magnusson 1929). Magnusson wrote a description of the type material and identified specimens from the Czech Republic, Germany, and the United Kingdom (Magnusson 1929). The type specimens are presumed to have been lost in the bombing of Dunkerque (Abbayes 1999). As was stated by Westberg et al. (2011) "The identity of *A. fusca* is unclear and this problem may never be solved if the type was destroyed in the Second World War". After studying the problem, we designated a neotype, a recent collection from Germany which could be sequenced (Knudsen et al. 2017). We synonymized it with *Myriospora rufescens* (Ach.) Hepp ex Uloth.

We subsequently generated sequences of ITS, mtSSU, and LSU from the neotype of *A. fusca* and compared them with BLASTn to the NCBI reference database GenBank. The BLASTn search results of our newly generated sequences from the neotype of *A. fusca* returned close matches to two specimens identified by Acarosporaceae expert M. Westberg as *A. anomala* and collected on a wood fence in Sweden (Westberg et al. 2015). The percent sequence identity scores with zero E value was used as the metric. The match between the two specimens identified as *A. anomala* and the neotype of *A. fusca* (Appendix 1) was 100.00% for ITS and mtSSU, and for LSU was 100.00% and 99.84% (Appendix III). The new data confirms Magnusson's interpretation that *A. fusca* was a distinct species from *M. rufescens* (Magnusson 1929) and support inclusion of *A. fusca* in *Acarospora* rather than *Myriospora*. It should be noted that another species Magnusson considered a member of the morphological *smaragdula* group, *Acarospora sinopica* (Wahlenb.) Körb., was recovered in *Acarospora* (Crewe et al. 2006, Knudsen et al. 2020, Magnusson 1929).

If the identifications of *A. anomala* vouchers are correct, then *A. anomala* should be treated as a heterotypic synonym of the older name *A. fusca*. Magnusson's description of *A. anomala*, the size, color

and thickness of areoles, the usually small solitary apothecia and ascospore size are similar to *A. fusca* (Magnusson 1929). The thickness of cortex of *A. anomala* is in the lower range of *A. fusca* (10–)40–60 µm vs. 15–22 µm. The hymenium of *A. anomala* is also in the lower range of *A. fusca* 85–90(–100) vs. (85–)100–140(–160) µm. Magnusson (1929) says the parathecium is expanded or not around the disc of |*A. anomala* but does not give measurements while the parathecium of *A. fusca* varies in width, sometimes around same apothecium, from indistinct up to 60 µm wide around the disc, and sometimes the parathecium merges with the cortex. *Acarospora anomala* also occurs on rock (M. Westberg, pers. comm.)

A lectotype is not designated from the syntypes of *A. anomala* (Magnusson 1924) and the species is not synonymized with *A. fusca* because the authors were unable to study the type material at UPS. We assume that Westberg's identification of *A. anomala* is correct. In practice in this paper, we treat *A. anomala* as a synonym of *A. fusca*. Due to confusion in European lichenology about the identity of *A. anomala* one should not automatically assume all reports of the species are *A. fusca* (Nimis 2016).

TAXONOMIC SECTION

***Acarospora fusca* B. de Lesd.**, Recherch. Lich. Dunkerque 1(Suppl.): 100. 1914. **TYPE: FRANCE.** Dunkerque, Malo Terminus, on tile on sand, 1910, *B. de Lesdain s.n.* (hb. B. de Lesdain, [n.v.]. presumed lost). GERMANY. MECKLENBURG. West Pomerania, Vorpommern-Greifswald, Greifswald, Koos Island, eastern edge of the island, 54°10′13″N 13°25′19″E, ca. 1 m., on single boulder on boulder beach, 6.viii.2004, *U. Schiefelbein 4446* (NY!, neotype (designated by Knudsen et al. 2017); hb. K&K!, hb. Schiefelbein!, isoneotypes).

FIGURE 1.

DESCRIPTION. – *Hypothallus* endosubstratal, IKI–. *Thallus* of dispersed or contiguous areoles usually covering area of 1 cm or less, areoles verruciform (areoles with an immersed apothecium dilating until the thallus is reduced to a thalline margin around the disc and resembling *Lecanora* apothecia) and/or areoles not verruciform with several apothecia, rounded to angular, 0.2–0.9(–1.5) mm diam., 0.2–0.4 mm thick, usually replicating through division. *Upper surface* dark or light brown, epruinose, smooth to rugulose, flat to somewhat convex. *Lower surface* broadly attached. *Epicortex* lacking or less than 10 µm thick. *Cortex* (10–)40–60 µm thick, individual cells of hyphae, 2–5 µm diam., upper layer brown ca. 10 µm thick, lower layer hyaline. *Algal layer* 50–100 µm thick, even, sometimes thin, rarely interrupted by hyphal bundles less than 10 µm wide, continuous beneath apothecia, algal cells 5–15 µm wide. *Medulla* 100–250 µm thick, continuous with attaching hyphae, mostly 2–3 µm wide, mixed with substrate particles.

Apothecia usually 1 (2–4) per areole, immersed, disc rounded, 0.07–0.25(–0.6) mm diam., reddish brown to blackening, flat, smooth or rough with pigment accretions or ontogenic remnants of the thallus, epruinose. *Parathecium* sometimes uneven in width around same apothecium, sometimes merging with cortex, indistinct or expanding up to 60 µm around the disc, often forming elevated margin concolorous with the thallus. *Hymenium* (85–)100–140(–160) µm tall, epihymenium reddish brown to dark brown, 10 µm high, hymenial gel IKI+ red (hemiamyloid), paraphyses 1.0–1.5 µm wide at midlevel, sparsely branched, tips cylindrical to clavate and widened to 2.5 µm. *Asci* up to 150 × 27 µm, ascospores narrowly ellipsoid to bacilliform, 3–5(–6) × (1–)1.5(–2) µm, several hundred per ascus. *Subhymenium* (20–)40–55 µm tall, oil drops usually rare or lacking, IKI+ blue. *Hypothecium* 10–15 µm tall. *Pycnidia* not observed.

CHEMISTRY. – No substances detected with TLC.

DISTRIBUTION AND ECOLOGY. – Belarus (Knudsen & Kocourková 2020a), Czech Republic (Magnusson 1929), Germany (Knudsen et al. 2017; Magnusson 1929), France (B. de Lesdain 1914; Magnusson 1929), and Sweden (Degelius 1961, det. Magnusson, det. as *Myriospora rufescens* in Westberg et al. 2011), and United Kingdom (Magnusson 1929) at usually low elevations below 500 m on granite, sandstone and schist, or volcanic rock, sometimes on small stones, and on anthropogenic surfaces such as wood and tile. Due to its small size, it can be easily overlooked. As an apparently lowland species, like *Acarospora franconica* H. Magn., this species is possibly rare due to the widespread human development of low elevations in Europe over thousands of years and has survived by its ability to grow on anthropogenic substrates as its natural habitat was reduced (Knudsen & Kocourková 2020b).

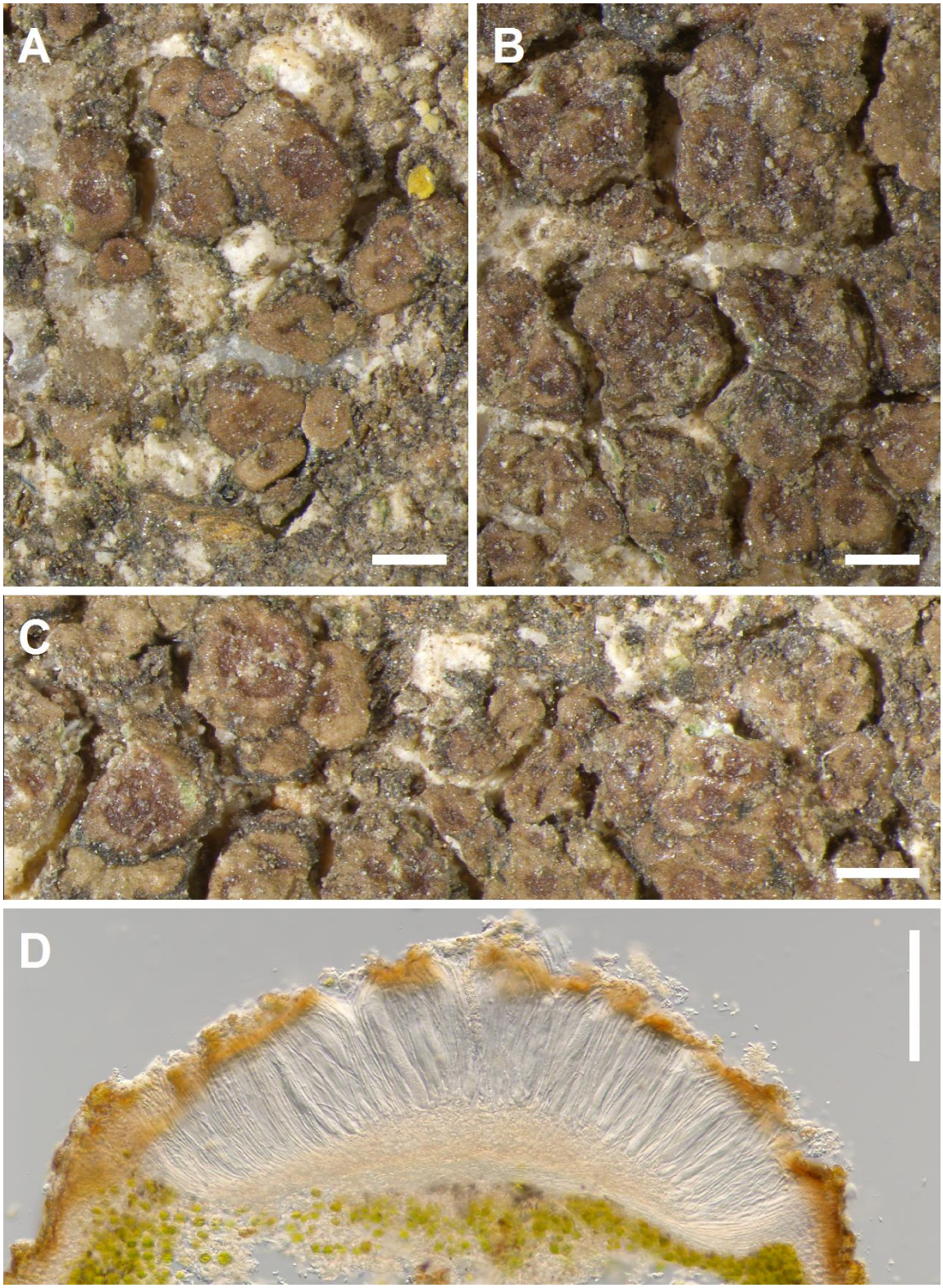

Figure 1. Neotype of *Acarospora fusca* (*Schiefelbein 4446*, NY): **A,** dispersed areoles of the thallus with solitary apothecia. **B,** areoles with mostly solitary apothecia forming a contiguous indeterminate crust. **C,** apothecia 1–(2–4) per areole in contiguous crust. **D,** vertical section of ascoma. Scale bars: A–C = 500 μm; D = 100 μm. (Reproduced from Knudsen et al. 2017, *Mycotaxon* 132: 861).

Selected specimens examined. – **BELARUS**. **MOGILAV REGION.** CHAUSY DISTRICT, 150 m E of Olkhovka village, 53°49´N, 31°11´E, on granite, 23.iv.1980, *V. Golubkov s.n.* (hb. K&K), ORSHA DISTRICT, 1 km NE of Sarmatsk village, 54°42´N, 30°22´E, on stone, 22.vi.1990, *V. Golubkov s.n.* (hb. K&K). **CZECH REPUBLIC**. **CENTRAL BOHEMIA.** KŘIVOKLÁTSKO, Beroun distr., Broumy, protected area Týřov, SW slope of hill Vápenný vrch, 49.97114°N, 13.79466°E, 300–400 m, on volcanic rock with *A. veronensis*, 12.ix.2019, *J. Vondrák 29065* (PRA). LIBSICE, ca. 160 m. on schist, 1903, *S.J. Podpěra s.n.* (PRM 783166, hb. Servít, det. by A.H. Magnusson in 1929, verified K. Knudsen). **NORTHERN BOHEMIA.** DISTRICT ČESKÁ LÍPA, Doksy, Břehyně-Pecopala National Nature Reserve, trail along drainage ditch E of Břehyňský Rybnik pond, 50°34'51″N, 14°43'45″E, 275 m, on basaltic rocks heaped on a stone bridge, 27.vii.2007, *J. Malíček 2271 & Z. Palice* (hb. Malíček).

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APPENDIX I – PRIMERS USED IN THIS STUDY

Table 1. Primers cited in the methods and used to generate new sequence data reported here.

Gene Name	Primer Name	Primer Sequence (5' to 3')
β-tubulin (Einax & Voigt 2003)	Bt3-LM	GAACGTCTACTTCAACGAG
	Bt10-LM	TCGGAAGCAGCCATCATGTTCTT
nLSU (Vilgalys & Hester 1990)	LROR	ACCCGCTGAACTTAAGC
	LR5	TCCTGAGGGAAACTTCG
ITS (White et al. 1990)	ITS1F	CTTGGTCATTTAGAGGAAGTAA
	ITS4	TCCTCCGCTTATTGATATGC
mtSSU (Zoller et al. 1999)	SSU1	AGCAGTGAGGAATATTGGTC
	SSU3R	ATGTGGCACGTCTATAGCCC

APPENDIX 1I – GENBANK ACCESSION NUMBERS & VOUCHER INFORMATION

Table 2. Voucher metadata and NCBI GenBank accession numbers for newly generated sequences from this study (bold) and existing reference sequences of *Acarospora anomala*.

Species + labcode	Location	Voucher	nITS	nLSU	mrSSU
*Acarospora anomala SAR*136	Sweden, Dalarna	Westberg 10-106 (S)	LN810758	LN810758	LN810883
Acarospora anomala SAR138	Sweden, Dalarna	Westberg 10-108 (S)	LN810759	LN810759	LN810884
***Acarospora fusca* 4446**	**Germany, Mecklenburg**	**Schiefelbein 4446 (Hb. K&K)**	**MT809051**	**MT809053**	**MT809052**

APPENDIX 1II – BLAST RESULTS FOR NEWLY GENERATED SEQUENCES

Table 3. Percent sequence similarity for closest matches with e score of zero to newly generated sequences from the neotype of *Acarospora fusca* based on BLASTn searches of NCBI GenBank.

Query	Marker	Percent Sequence Identity	GenBank No.	Sequence Identification in GenBank
MT809051	ITS	100.00%	LN810758.1	*Acarospora anomala*
MT809051	ITS	100.00%	LN810759.1	*Acarospora anomala*
MT809051	ITS	99.01%	DQ374131.1	*Acarospora nitrophila*
MT809052	SSU	100.00%	LN810884.1	*Acarospora anomala*
MT809052	SSU	100.00%	LN810883.1	*Acarospora anomala*
MT809052	SSU	99.69%	LN810891.1	*Acarospora fuscata*
MT809053	LSU	100.00%	LN810759.1	*Acarospora anomala*
MT809053	LSU	99.84%	LN810758.1	*Acarospora anomala*
MT809053	LSU	97.05%	MT644900.1	*Acarospora fuscata*