Two superficially similar lichen crusts, *Gregorella humida* and *Moelleropsis nebulosa*, and a description of the new lichenicolous fungus *Llimoniella gregorellae*

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Abstract: Vondrák, J., Palice, Z., Mareš, J. & Kocourková, J. 2013. Two superficially similar lichen crusts, *Gregorella humida* and *Moelleropsis nebulosa*, and a description of the new lichenicolous fungus *Llimoniella gregorellae*. – Herzogia **26**: 31–48.

Although some characters distinguishing *Gregorella humida* and *Moelleropsis nebulosa* were previously known, sterile specimens and specimens with poorly-developed apothecia are often difficult to separate. We provide morphological and anatomical characters that will allow reliable determination of such difficult collections. The most important character for determination of sterile thalli is the shape of the mycobiont cells in the thallus granules. A key summarizes the diagnostic characters of *G. humida* and *M. nebulosa* (and some similar species). The *Nostoc* photobiont in *G. humida* is morphologically similar to *Nostoc* from *M. nebulosa* but the two are not closely related within the genus. The ecology of both lichen species is similar, but there are differences in the preference for differently acidic substrates and in co-occurring bryophytes and lichens. In Central Europe, *M. nebulosa* was frequently collected in the first half of the 20th century, but there are few recent records, whereas *G. humida* was only occasionally collected before the last two decades, but is now regularly collected. *Moelleropsis nebulosa* rarely hosts lichenicolous fungi, though we have seen *Lichenochora mediterranae* (previously known only on *Fuscopannaria*) and *Sarcopyrenia* sp. on it. *Gregorella humida* rarely hosts a single lichenicolous fungus, described here as *Llimoniella gregorellae*, spec. nova, which causes obvious harm to host thalli; ITS sequences indicate that it belongs in Leotiomycetes.

Zusammenfassung: Vondrák, J., Palice, Z., Mareš, J. & Kocourková, J. 2013. Die beiden oberflächlich ähnlichen Krustenflechten *Gregorella humida* und *Moelleropsis nebulosa* und die Beschreibung des neuen lichenicolen Pilzes *Llimoniella gregorellae*. – Herzogia **26**: 31–48.

Obgleich einige Trennmerkmale zwischen *Gregorella humida* und *Moelleropsis nebulosa* schon bekannt waren, sind sterile Proben und Proben mit schlecht entwickelten Apothecien oft schwer zu unterscheiden. Wir liefern morphologische und anatomische Merkmale, die eine zuverlässige Bestimmung solch schwieriger Aufsammlungen erlauben. Das wichtigste Merkmal für die Bestimmung steriler Thalli ist die Form der Mykobiontenzellen in den Thalluskörnchen. Ein Schlüssel wird präsentiert, der die diagnostisch bedeutsamen Merkmale von *G. humida* und *M. nebulosa* (und einiger ähnlicher Arten) zusammenfasst. Der *Nostoc*-Photobiont von *G. humida* ähnelt morphologisch dem von *M. nebulosa*, aber die beiden sind innerhalb der Gattung nicht näher miteinander verwandt. Die Ökologie der beiden Flechtenarten ist ähnlich, aber es gibt Unterschiede in der Präferenz für unterschiedlich saure Substrate und in den als Begleitarten auftretenden Moosen und Flechten. In Mitteleuropa wurde *M. nebulosa* häufig in der ersten Hälfte des 20. Jahrhunderts gesammelt, aber es gibt wenige aktuelle Funde. Dagegen wurde *G. humida* vor den letzten zwei Jahrzehnten nur gelegentlich gesammelt, wird heute aber regelmäßig gefunden. *Moelleropsis nebulosa* wird selten von lichenicolen Pilzen besiedelt; wir haben auf dieser Art *Lichenochora mediterranae* (zuvor nur von *Fuscopannaria* bekannt) und *Sarcopyrenia* sp. gefunden. Auf *G. humida* kommt selten ein lichenicoler Pilz vor, der hier als *Llimoniella gregorellae* beschrieben wird. ITS-Sequenzen weisen darauf hin, dass er zu den Leotiomycetes gehört.

Keywords: Arctomiaceae, cyanolichens, Leotiomycetes, lichenicolous fungi, *Nostoc*, Pannariaceae, photobiont.

Introduction

The recent placements of *Moelleropsis nebulosa* and *M. humida*, now *Gregorella humida*, into two distant families (Lumbsch & Huhndorf 2010) has greatly improved our understanding of these two species, but some problems remain. There is the practical problem of actually determining collections, as fertile specimens of both species may be very similar and sterile specimens even more so. There is the theoretical problem that although the species have similar ecology (epigeic r-strategists), their population histories are very different.

Originally, we merely sought diagnostic characters in apothecia that would distinguish samples of *M. nebulosa* with small immarginate apothecia from *G. humida*, but we also found characters in the vegetative thallus that distinguish sterile populations of both species. Photobionts, ecology and distribution of both species were then also studied to find additional differences.

Our attention was drawn to the need to undertake this work when we encountered a sterile crust that appeared to be morphologically identical to thalli of *M. nebulosa* but which proved to have an ITS sequence (GB KC806067; CBFS JV6995) 100 % similar to the one *G. humida* sequence in the GenBank, and unrelated to sequences of true *M. nebulosa*. This result was surprising to us, as we had previously known *G. humida* only with a less developed, brownish thallus. As we were aware of frequent records of sterile crustose cyanolichens morphologically identical to *M. nebulosa* it was clear that the question of how to separate the two species needed to be addressed.

Previous research on *Moelleropsis* and *Gregorella*

Although described in 1871 (as *Biatora humida* Kullh.; combined into *Moelleropsis* by Coppins & P.M.Jørg. in 1993; and into *Gregorella* by Lumbsch in 2005), *G. humida* was rarely collected until Cezanne et al. (2003) published many new records of this taxon from Germany. Subsequently, it has been collected from many other sites (e.g. Czarnota 2003, Jørgensen 2007a, Woods 2009, Zimmermann et al. 2011). Ekman & Jørgensen (2002) published the first molecular data from the species, which revealed that it is not related to the type species of *Moelleropsis*, *M. nebulosa* (Hoffm.) Gyeln., and even falls far outside the family Pannariaceae. Lumbsch et al. (2005) found that it belongs in Arctomiaceae, close to *Arctomia* and *Wawea*, and transferred it into the new monotypic genus *Gregorella*.

Gregorella humida has been increasingly recorded during the last two decades, but *M. nebulosa* seems to be almost a forgotten species in most regions of Europe, and there are few recent records (Ekman et al. 2000). It was not always so. There were many reports since 1794 when it was first described (as *Patellaria nebulosa*) until quite recently. Between 1900 and 1940, for example, it was commonly collected and repeatedly published from the Czech Republic (cf. Vězda & Liška 1999).

Some recent authors have provided data on characters of *M. nebulosa* and *G. humida*. Selected characters observed in material from Nothern Europe, the British Isles, North America and Central Europe are given in Table 1 (with references). The descriptions of characters presented by these authors are surprisingly similar but they sometimes differ from our own descriptions.

Materials and methods

Our morphological data are generated from our own collections and herbarium material from Central Europe (mainly the Czech Republic) housed in PRA-V, PRC and PRM. We used the following methods throughout (including for the new lichenicolous fungus). Sizes of apothe-

cia, thallus granules and squamules were measured in the dry state under the stereomicroscope. All other characters were assessed microscopically (1000× magnified) in water, without any chemical pretreatment. Measurements are accurate to 0.5 µm (ascospores, vegetative fungal cells), 1 µm (e.g. vegetative diaspores, cyanobacterial colonies) or 10 µm (larger structures, e.g. hymenium height and width of exciples). All measurements of cells (ascospores, paraphyses) include their walls. Measurements are given as (min.–) X1–X2–X3 (–max.), where min. / max. are the extremes from all measurements, X1 is the lowest specimen arithmetic mean observed, X2 is arithmetic mean of all observations, X3 is the highest specimen arithmetic mean observed. Ten measurements per specimen were always done in each assessed sample. Total numbers of assessed samples and standard deviations from all measurements are given for the individual characters in square parentheses [n; SD]. In some characters (e.g. hymenium width), only the min.–max. span is provided; these characters are considered less crucial and were assessed less precisely. Morphological terminology follows Smith et al. (2009). Images from SEM microscopy were used to show differences in surface structures of thalli of both species. Methods for high-performance liquid chromatography (HPLC) followed Søchting (1997). Four samples were analyzed: *G. humida* "PRA, ZP1858" (apothecia + thallus), "CBFS JV6984" (thallus); *M. nebulosa* "PRM 633660" (apothecia + thallus), "PRM832462" (thallus).

Fifteen samples of *G. humida* and eighteen specimens of *M. nebulosa* were used for pH measurements of the substrate. The pH was measured in well-mixed suspension of c. 1 ml of dry soil below thalli and 10 ml of distilled water after 15 min. of intensive shaking. The pH of paper sheets and glue used for fixing old lichen samples was also examined, but their possible influence on pH of fixed herbarium samples was found to be negligible. Co-occurring bryophyte species were identified from 12 specimens of *G. humida* and 22 specimens of *M. nebulosa*.

The ITS sequences of mycobiont and lichenicolous fungus were generated by direct PCR (e.g. Arup 2006) with PCR cycling parameters following Ekman (2001). Partial 16S rRNA gene and adjacent ITS region of cyanobacterial photobionts were amplified following Boyer et al. (2001), using 10–20 ng of template DNA isolated directly from the lichen thalli by modified XS extraction protocol (Yilmaz et al. 2009). The PCR products were cloned using the standard pGEM®-T Easy vector system, and sequenced on the ABI PRISM 3130xl automated sequencer. Obtained sequences were aligned by MAFFT v. 6 (Katoh et. al. 2009) together with 47 *Nostoc* OTUs, representing the whole known variability of the genus in GenBank, and two outgroup taxa. A Bayesian tree was generated in MrBayes v. 3.2 (Ronquist & Huelsenbeck 2003) by running two independent runs of 4 Markov chains for 20 million generations with sampling frequency of 100, and subsequent majority consensus from the last 75 % of the sampled trees. The node bootstrap supports were calculated using maximum likelihood via phyML v. 3.0 (GTR+G+I likelihood model, 1000 replicates; Guindon & Gascuel 2003) and maximum parsimony via PAUP v.4.10b (100 random-addition heuristic searches with TBR branch swapping, 1000 non-parametric bootstrap replicates; Swofford 2002).

Samples investigated

***Gregorella humida*: Czech Republic.** Central Bohemia. Příbram, Lešetice, SE foot of discharge hopper, c. 0.7 km W of village, alt. 550 m, 49°38'48"N/14°0'37"E, 26 May 2008, J. Malíček & J. Vondrák (CBFS JV6861, 6866; herb. Malíček 1260); Ibid.: 11 Feb. 2011 (CBFS JV8370; herb. Malíček 3311); Sedlčany, sand-pit on S edge of town, alt. 375 m, 49°38'58"N/14°25'28"E, on sandy soil, 14 May 2011, J. Malíček (herb. Malíček 3464). East Bohemia. Krkonoše Mts, Černý Důl: limestone mining area W of the village, a heaped plateau S of the quarry, N50°37,98'/E015°42,29', alt. 680–690 m, on loamy soil, 10 Jun 2005, J. Liška, Z. Palice & Š. Slavíková (PRA, Palice 8904); Hanušovice, Chrastice, at village, alt. c. 550 m, 2 Oct. 2008, J. Vondrák (CBFS JV6694). North Bohemia. Jizerské hory Mts, Janov, Hrabětice, alt. 740 m, 25 Jul 2011 & 29 Apr 2012, Z. Palice (PRA, Palice 14802, 15157). South Bohemia. České Budějovice, Staré Hodějovice, 1.3 km NW of village (at settling-pit), alt. c. 420 m, 48°57'22.362"N/

14°30'37.976"E, 2 Feb. 2011, J. Vondrák (CBFS JV8448); Jindřichův Hradec, Kamenice nad Lipou, „Hutě" settlement close to vil. Bohdalín, alt. c. 600 m, 49°17'42.96"N/15°0'30.05"E, 1 Sep. 2007, J. Vondrák (CBFS JV5627); Novohradské hory Mts, Pohorská Ves: open place in managed spruce forest along forestry road at W-facing slope at foothill of the crest of Mt Stubenberg [A], 2 km SE of Žofín settlement, 48°39'36.5"N/14°42'37.2"E, alt. 805 m, 14 Oct. 2010, Z. Palice (PRA Palice 14284); Prachatice, Husinec, Výrov, in industrial zone at main road between Husinec and Těšovice, alt. c. 500 m, 26 Nov. 2006, J. Vondrák (CBFS JV4901); Ibid.: 23 Sept. 2007, J. Malíček & J. Vondrák (herb. Malíček 1011); Ibid.: 13 Apr. 2009 (CBFS JV6986); Týn nad Vltavou, Temelín, at railway between Temelín and nuclear power-plant „Temelín", alt. 490 m, 49°11'19"N/14°21'57"E, 4 Apr. 2009, J. Vondrák (CBFS JV6984, 6985, 6992, 6995, 6998, dupl. in Herb. Palice); Ibid.: 16 March 2011 (CBFS JV8445); Volary, c. 2 km S of Černý Kříž, in sand quarry on bare soil, alt. 775 m, 25 Apr. 1996, Z. Palice (PRA-V 3439; herb. Palice); Ibid.: 17 June 1996 (PRA Palice 1858). **Poland.** Gorce Mts: Poręba Wielka, alt. 610 m, 2000, P. Czarnota (PRA Palice, s.n., dupl. ex GPN). **Russia.** Southern Urals: Orenburg region, Tyul'gan district, vill. Tashla, *Tilia cordata-Acer platanoides-Quercus robur-Ulmus laevis* forest in uppermost stream of brook Kuplya, alt. 400–480 m, 52°28'21"N/56°16'36"E, on soil at forest gravel road, 2011, J. Vondrák (CBFS JV9960).

***Moelleropsis nebulosa*: Austria.** Oberösterreich. Schärding, alt. 540 m, 2001, F. Berger (PRA, Palice 12169). **Czech Republic.** Central Bohemia. Beroun, Karlštejn, alt. c. 400 m, 5 June 1933, J. Suza (PRM 633652); Beroun, Karlštejn, loc. Velká hora, 16 Apr. 1926, J. Klika & A. Hilitzer (PRM 832466). East Bohemia. Vápenný Podol, 1910, V. Kuťák (PRM 832473); Chotěboř, at railway embankment in forest near the town game-keeper house, 1889, C. Bayer (PRC). North Bohemia. Česká Lípa, Provodín, loc. Provodínské kameny [Meichelsberg], without date, J. Anders (PRM 832475); Louny, Počerady, village Třískolupy [Schiessglock] destroyed by coal mining, loc. "Hora" [probably the hill Zvonice, 275 m], without date, J. Anders (PRM 832470). West Bohemia. Kdyně, at pathway to Rýzmberk, 6 Sep. 1936, A. Hilitzer (PRM 832465); Šumava Mts, Čeňkova Pila, alt. 720 m, 23 May 1995, Z. Palice 14890 (PRA). South Moravia. Blansko, Rájec, 350 m, Apr. 1913, J. Suza (PRM 633662); Boskovice, Borotín, alt. 430 m, on soil at road, Jan. 1934, J. Dyr [Crypt. Čechosl. Exs. 146] (PRA-V 3433, PRC, PRM 633660, 789732, 832460, 865673); Brno, Jundrov, at hill Holedná, alt. c. 350 m, 38 March 1918, J. Suza (PRM 633655); Brno, Hády, A. Vězda (PRA-V 3438); Brno, Jehnice, alt. 300 m, 3 March 1920, J. Suza (PRM 633666); Dukovany, loc. Skryjský mlýn, alt. 280 m, 5 March 1920, J. Suza (PRM 633653); Tišnov, in valley of river Loučka, loc. Bruhalův mlýn, 1921, J. Suza (PRM 633664); Třebíč, at village Kracovice, alt. 500 m, 19 Aug. 1918, J. Suza (PRM 633661); [Velké Meziříčí], beneath the village Milasín, 1907, M. Servít (PRC); [Velké Meziříčí], Strážek, valley of river Bobrůvka, Feb. 1909, M. Servít (PRC); [Velké Meziříčí], Strážek, a mill beneath Mitrov settlement, Aug. 1909, M. Servít (PRC); [Velké Meziříčí], Křižanov, beneath loc. "Sv. Hora", at road cutting, (?)1909, M. Servít (PRC) [Servít 1910: 49]; Velké Meziříčí, Horní Bory, alt. 500 m, 1921, J. Suza (PRM 633667); Náměšť nad Oslavou, in forest near Koroslepy (Kuroslepy) SE of Náměšť nad Oslavou, 1909, M.Servít (PRC); Znojmo, at ruin of Nový Hrádek, on soil, 28 March 1932, J. Suza (PRM 633663). **Germany.** Sächs. Schweiz: ad terram arenosam muscosam, L. Rabenhorst [Rabenhorst, Lichenes Europaei Exs. 967] (PRC). **Hungary.** Bükk Mts, at village Mályinka, alt. 580 m, 27 Aug. 1937, F. Fóriss (Lich. Bükk. Exs. 6; PRM 832462). **Slovakia.** Piešťany, at village Hradok, alt. 300 m, Apr. 1933, J. Suza (PRM 633656); Považský Inovec Mts, Vozokany, loc. „Marhat", Aug. 1930, J. Suza (PRM 633657); Slovenské Rudohoří Mts, Košické Hamry, alt. c. 400 m, 27 July 1937, J. Suza (PRM 633659, 832461).

Protopannaria pezizoides – samples incorrectly identified as *Pannaria* (*=Moelleropsis*) *nebulosa*: **Czech Republic.** Central Bohemia. Slapy, loc. Svatojánské proudy, 4 Nov. 1934, A. Hilitzer (PRM 832463); Štěchovice, loc. Červený vrch, May 1935, Nebeský & A. Hilitzer (PRM 832464). East Bohemia. Krkonoše, loc. Kotelní jáma, 9 Sep. 1923, A. Hilitzer (PRM 832477). North Bohemia. Warnsdorf, 12 July 1923, J. Anders (PRM 832478). South Moravia. Blansko, in valley of brook Křtinský potok, 10 Oct. 1955, A. Vězda (PRA-V 3436); Velká Bíteš, in valley of river Bitýška, at village Svatoňov, alt. 450 m, 29 Sept. 1963, A. Vězda (Lich. Sel. Exs. 280; PRA-V 5880).

Results and discussion

Characters of the apothecia

***Gregorella humida*: Apothecia** brown to dark brown, flat or ± convex, (0.2–)0.30–0.32–0.34 (–0.5) mm diam. [3; 0.09]. **Thalline exciple** absent. **True exciple** thin, inconspicuous (Fig. 1E) or absent. **Hypothecium** pale brown or colourless. **Hymenium** c. 75–100 µm high, without pigments, but **epithecium** brownish. **Asci** cylindrical, with amyloid external sheath but with KI– tholus (Fig. 2A). **Paraphyses** not numerous, conglutinated (individual paraphyses visible after treatment with KOH), anastomosed and branched, c. 1.5–3.0 µm wide, with tips sometimes widened up to 4.0–4.5 µm. **Ascospores** (Fig. 2C) uniseriate or biseriate, colourless, often with 1–2 large oil drops, (8.5–)12.4–14.5–16.1(–19.0) × (5.0–)7.1–8.0–9.8(–15.0) µm

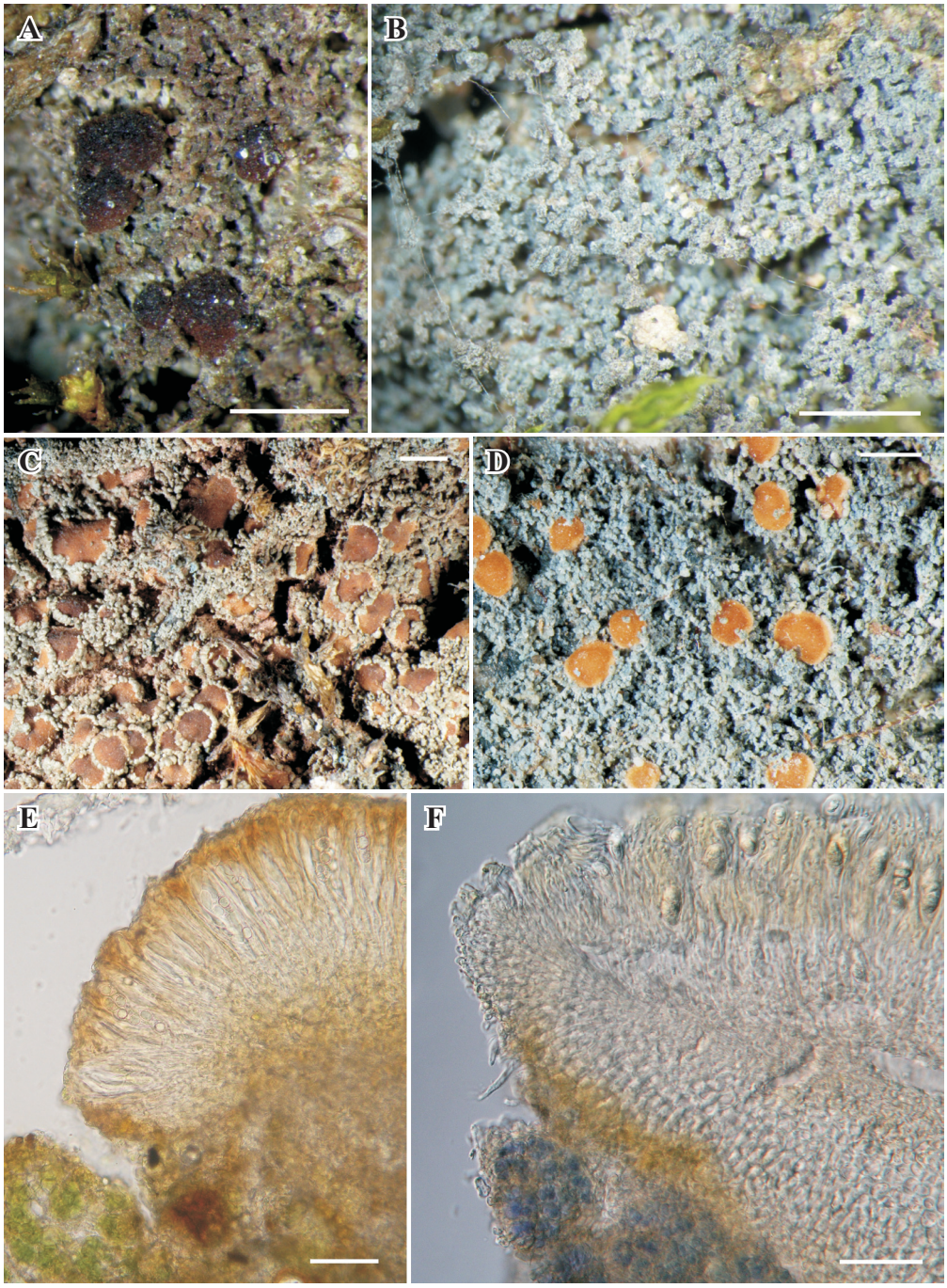

Fig. 1: *Gregorella* and *Moelleropsis*; **A** – fertile *Gregorella*, CBFS JV6985; **B** – sterile *Gregorella*, CBFS JV8370; **C** – apothecia of *Moelleropsis* with well-developed thalline exciple, PRM 633660; **D** – apothecia of *Moelleropsis* with indistinct thalline exciple, PRM 633655; **E** – *Gregorella*, vertical section in apothecium, CBFS JV6985 (in water); **F** – *Moelleropsis*, vertical section in apothecium, PRM 633655 (in water). Scales: A–D = 1 mm; E, F = 50 µm.

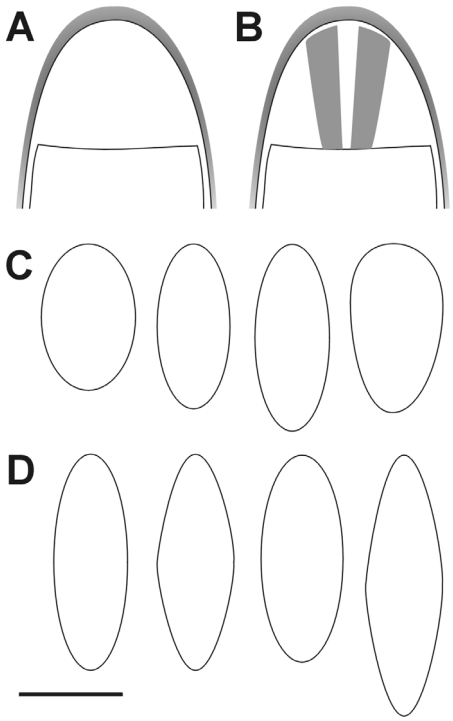

Fig. 2: *Gregorella humida* and *Moelleropsis nebulosa*. **A** – ascus apex in *Gregorella*; **B** – ascus apex in *Moelleropsis*; **C** – ascospore variability in *Gregorella*; **D** – ascospore variability in *Moelleropsis*. Scale = 10 µm.

[7; 2.4 (length); 1.7 (width)]; ellipsoid, tear-shaped, subglobose or rarely narrowly ellipsoid; length/width ratio (1.0–)1.7–1.9–2.1 (–2.8) [7; 0.6]. Yellow-brown pigment in epihymenium ± intensifying in KOH and HNO_3. HPLC did not reveal any substances soluble in acetone.

***Moelleropsis nebulosa*: Apothecia** pale brown to dark brown, flat, (0.3–)0.6–0.8–1.1 (–1.6) mm diam. [3; 0.4]. **Thalline exciple** absent in young apothecia but distinct in older apothecia, c. 40–170 µm thick, formed of granules c. 60–170 µm diam. **True exciple** well-developed in young apothecia (Fig. 1F; with occasional single-celled colourless setae, up to about 50 µm long), but reduced in older apothecia, c. 30–100 µm thick, without pigmentation in section; its internal part formed of prosoplectenchymatic hyphae c. 1.5–3 µm thick, external part ± paraplectenchymatous, of cells c. 3–7 × 4–11 µm. **Hypothecium** yellow-brown or colourless. **Hymenium** c. 80–120 µm high, without pigments, but **epithecium** brownish. **Asci** cylindrical, with amyloid external sheath and with amyloid tholus similar to the *Porpidia*-type (Fig. 2B). **Paraphyses** numerous, not conglutinated (individual paraphyses well visible in sections), ± anastomosed and branched, c. 2.0–3.0 µm wide, with tips not widened. **Ascospores** (Fig. 2D) uniseriate or biseriate, colourless, rarely with oil drops, (12.5–)14.8–18.5–21.8(–25.5) × (5.0–)6.1–7.0–7.9(–9.0) µm [10; 3.1 (length); 1.0 (width)]; ± uniform in shape: ellipsoid to narrowly ellipsoid; length/width ratio (1.8–)2.6–2.7–2.8(–4.0) [10; 0.4]. Yellow-brown pigment in epihymenium ± intensifying in KOH and HNO_3. HPLC did not reveal any substances soluble in acetone.

Moelleropsis nebulosa is rather variable in the apothecial size and in the degree of development of a thalline exciple. While its phenotypes with large apothecia having a well-developed thalline exciple are easily separated from *Gregorella humida*, specimens with small apothecia (0.3–0.5 mm diam.), without a thalline exciple and with an indistinct true exciple (Fig. 1D) are macroscopically very similar to *G. humida*.

The colour of apothecia varies in both species. In dry conditions pale brown apothecia are often encountered in *M. nebulosa* but are very rare in *G. humida*, while dark brown apothecia are common in *G. humida* but rather rare in *M. nebulosa*.

Ascospore shapes and sizes are variable in both species; differences between extreme width and length values as well as the span of lowest and highest specimen arithmetic means are great: 3.7 µm (span of specimen means) and 10.5 µm (min.–max. span) in the ascospore length of *G. humida* and 7.0 µm (span of specimen means) and 13.0 µm (min.–max. span) in the as-

Table 1: Characters of *Gregorella humida* and *Moelleropsis* nebulosa in various literature sources and in this study.

Gregorella humida	Jørgensen (2007a) (Nothern Europe)	Woods (2009a) (Great Britain)	Wirth (1995) (Central Europe)	**This study (Central Europe)**
Thallus granules/ goniocysts	30–60 µm diam.	30–60 µm diam.	thinly granular	(30–)61–69–81 (–130) µm diam. [4; 23]
Exciple	true exciple soon excluded; thalline exciple absent	no margin or margin soon excluded	not visible	true exciple inconspicuous or absent; thalline exciple absent
Asci	ascus apex I–	KI+ ascus wall, KI– tholus	not considered	with amyloid external sheath but with KI– tholus
Ascospores	14–19(–25) × 7–10 µm	12.5–19(–24) × 6.5–9.5 µm	14–19 × 7–9.5 µm	(8.5–)12.4–14.5–16.1(–19.0) × (5.0–)7.1–8.0–9.8 (–15.0) µm [7; 2.4 (lenght); 1.7 (width)]
Chemistry	no secondary substances (by TLC)	no secondary substances (by TLC)	not considered	Yellow-brown pigment in epihymenium and goniocysts (K+, N+ intensif.), N± fleetingly pink; no substances (by HPLC)
Moelleropsis nebulosa	Jørgensen (2007b) (Nothern Europe)	Woods (2009b) (Great Britain)	Jørgensen (2002a) (North America)	**This study (Central Europe)**
Thallus granules/ goniocysts	to 100 µm diam.	30–100 µm diam.	30–100 µm diam.	(40–)61–84–110 (–170) µm diam. [4; 35]
Exciple	true exciple present, up to 100 µm thick; often with thalline grains	true exciple to 100 µm thick, paraplectenchy-matous; often with granular thalline exciple	true exciple present, paraplectenchy-matous; thalline exciple present	true exciple usually well-developed; thalline exciple may be present in older apothecia (other details in the text)
Asci	with apical amyloid structures	apex with KI+ blue tholus	apex with I+ blue apical dome	with amyloid external sheath and with amyloid tholus similar to the *Porpidia*-type
Ascospores	10–15 × 5–8 µm	(11–)13–15(–20) × 6–8 µm	10–15(–20) × 5–8 µm	(12.5–)14.8–18.5–21.8(–25.5) × (5.0–)6.1–7.0–7.9 (–9.0) µm [10; 3.1 (length); 1.0 (width)]
Chemistry	no secondary substances (by TLC)	no secondary substances (by TLC)	secondary metabolites not detected	as in *Gregorella humida*

cospore length of *M. nebulosa*. The ascospore size differs considerably between the species. The ascospore shape is rather uniformly narrowly ellipsoid in *M. nebulosa*, as shown by the length / width ratio: 2.6–2.8 (span of specimen means). In *G. humida*, the ascospore shape varies from ellipsoid to subglobose; the length / width ratio of 1.7–2.1 (span of specimen means). The considerable variation in published information for ascospore sizes (Table 1) probably reflects the variation between specimens (and insufficient sampling and averaging).

Characters of the thallus

***Gregorella humida*: Thallus** (Figs 1A, B) blue-grey, brown-grey or olive-grey, granulate or formed of goniocysts. Granules / goniocysts (30–)61–69–81(–130) µm diam. [4; 23]. Squamules occasionally present, c. 130–430 µm diam. Granules / goniocysts formed of paraplectenchymatous fungal tissue (Fig. 3A) with colourless, mainly ±isodiametric cells, (3.0–)5.6–5.8–6.0(–8.25) µm diam. [3; 1.4]. Shapes of mycobiont cells globose or variously irregular, but rarely strongly elongated in one direction (Fig. 3C). Paraplectenchymatous tissue fully covers the surface of granules. **Photobiont** is the cyanobacterium *Nostoc* sp. in clusters enclosed by mucilaginous envelopes. Cyanobacterial colonies (12–)17–19–21(–27) µm diam. [3; 5.2]. Yellow-brown pigment often conspicuous, intensifying in KOH and HNO_3; after HNO_3 fleetingly pink. HPLC did not reveal any substances soluble in acetone.

Thallus may be infected by a lichenicolous member of the Leotiomycetes, which is described below and placed in *Llimoniella*. As the species of *Llimoniella* are narrowly host specific (Diederich et al. 2010), we consider infections of this fungus as an additional character for the identification of sterile *G. humida* thalli.

***Moelleropsis nebulosa*: Thallus** (Figs 1C, D) blue-grey or brown-grey, granulate or formed of goniocysts. Granules / goniocysts (40–)61–84–110(–170) µm diam. [4; 35]. Squamules occasionally present, c. 200–330 µm diam. Granules / goniocysts formed of prosoplectenchymatous fungal tissue (Figs 3B, D) with variously curved and branched, colourless short hyphae, (4.25–)6.2–6.8–7.6(–10.0) × (1.75–)2.8–3.1–3.4(–5.0) µm [3; 1.7 (length); 0.9 (width)]. Prosoplectenchymatous tissue usually not covering the whole surface of the granules; but photobiont sheaths partly forming the granule surface. **Photobiont** is the cyanobacterium *Nostoc* sp. in clusters enclosed by mucilaginous envelopes. Cyanobacterial colonies (11–)24–26–27(–49) µm diam. [2; 10]. Yellow-brown pigment not always conspicuous, but intensifying in KOH and HNO_3; after HNO_3 fleetingly pink. HPLC did not reveal any substances soluble in acetone.

Thallus may be infected by the following lichenicolous fungi. (1) *Lichenochora mediterraneae* Calat., Nav.-Ros. & E.Calvo (observed in sample "PRC: Strážek, 1909, M. Servít"), characterized by forming small faded galls on host; black, partly to fully immersed perithecia with paraplectenchmatous wall; c. 30–50 × 4.5–6.5 µm, 3-septate ascospores in 2-spored, unitunicate asci; hamathecium of short indistinct chains of ±isodiametric cells. This fungus is known from *Fuscopannaria* (e.g. Calatayud et al. 2000) and we suspect it may be host specific to *Fuscopannaria* s.lat. including *Moelleropsis*. (2) *Sarcopyrenia* sp. (observed in sample "PRM832462: 1939, F. Fóriss"), characterized by sessile black perithecia with wall consisting of dark outer part and colourless inner part, formed of large isodiametric cells (c. 10–25 µm diam.); ascospores are acicular, c. 20–30 × 1.5–2.0 µm, with rounded ends, arranged in bundle in unitunicate asci. Host range for this fungus is uncertain; a similar undescribed *Sarcopyrenia* is reported from another cyanolichen, *Lichinella* sp. (Tretiach & Navarro-Rosinés 1996).

Both *G. humida* and *M. nebulosa* vary a lot in the size of the granules that form the thallus; they may have large granules of > 100 µm diam. (more common in *Moelleropsis nebulosa*),

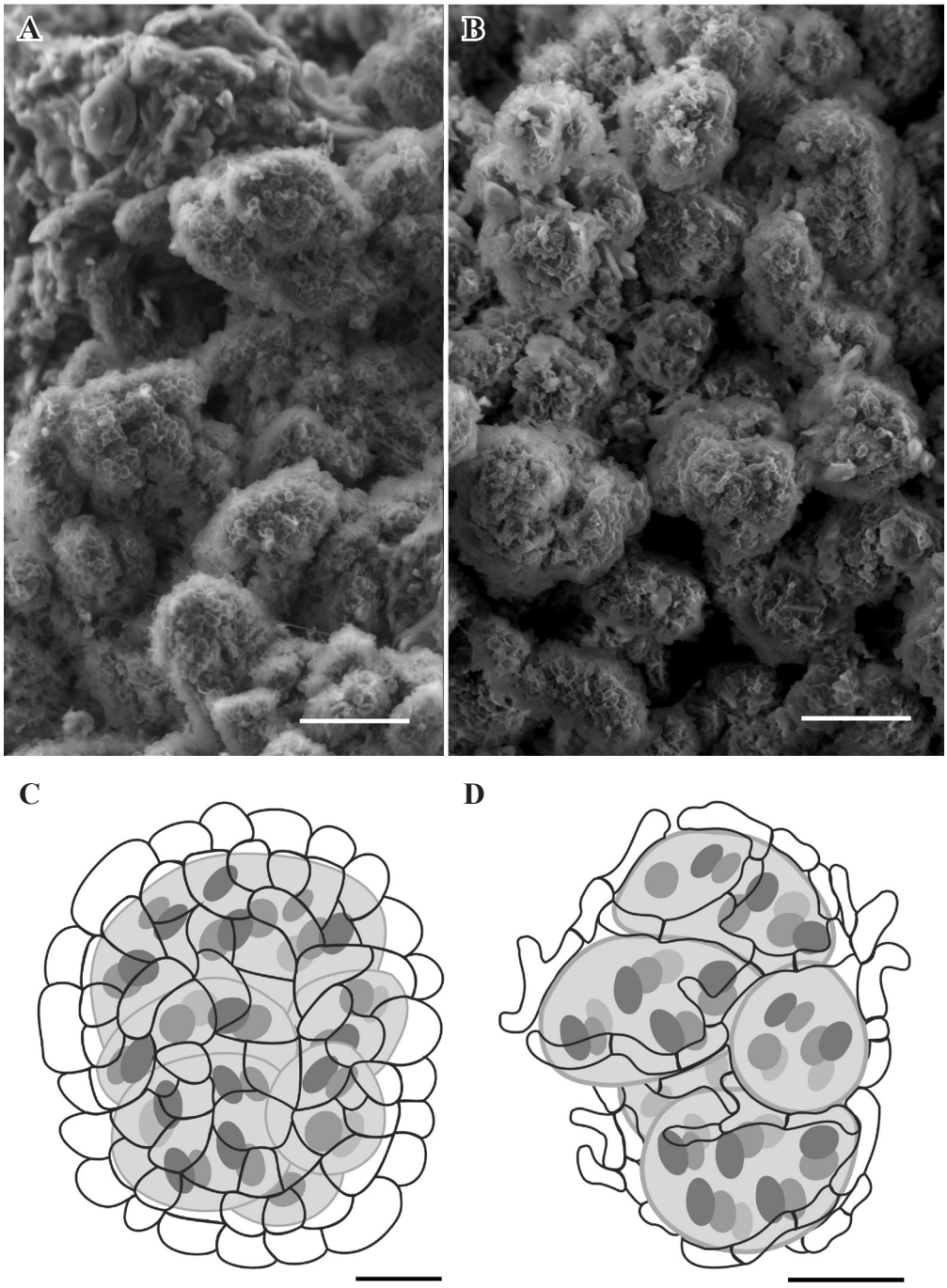

Fig. 3: *Gregorella humida* and *Moelleropsis nebulosa*. **A**, **C** – thallus granules in *Gregorella*; **B**, **D** – thallus granules in *Moelleropsis*. Scales: A, B = 100 µm; C, D = 10 µm.

but also small goniocysts of c. 30–80 µm diam. (common in both species). Squamules are ± larger and more frequently observed in *M. nebulosa* than in *G. humida.* The colour of the thalli is also variable; ± brownish (more common in *Gregorella*) or ± greyish (common in both species). According to our observations, sterile specimens of both species sometimes cannot be separated in the field.

Key to the species

We combine here diagnostic characters found in the apothecia and the thalli of both species. For practical reasons, we also include some similar terricolous species, which might be confused with the studied species; data for the latter were adopted from Jørgensen (2002b, 2007b).

1 Thallus brownish, rarely with a bluish tinge, with abundant squamules; granules may be present, but large, usually >100 µm diam. In undisturbed natural habitats **2**

1* Thallus variously coloured, but often with a bluish tinge; of isidia or granules (goniocysts) c. 50–150 µm diam.; squamules may be present, but not abundant. In various habitats. **4**

2 Squamules up to 1 mm wide; apothecia with entire or indistinctly granulate thalline exciple; asci with amyloid sheets but without internal amyloid structures. ..... ***Protopannaria pezizoides***

2* Squamules 1–3 mm wide, thalline exciple often excluded; asci with apical amyloid ring structures **3**

3 Thallus dark brown, in herbarium often covered by tiny terpenoid crystals (terpenoids and fatty acids detectable by TLC); thallus surface covered by imbricate lobes or granules; in arctic-alpine habitats. ..... ***Fuscopannaria praetermissa***

3* Thallus brown or lead blue, without white crystals on the surface; no secondary compounds by TLC; margin of squamules dissolve in soredia; not known from Europe ..... ***Fuscopannaria cyanolepra***

4(1) Thallus of granules (isidia), 100–150 µm diam., with distinct upper paraplectenchymatous cortex of 1–3 rows of cells. ..... ***Vahliella atlantica***

4* Thallus of non-corticate granules (goniocysts) c. 40–150 µm diam. **5**

5 At least some corticate squamules (c. 1–2 mm in diam.) present in the sorediate crust; not known from Europe (very similar to *Moelleropsis nebulosa*) ..... ***Fuscopannaria cyanolepra***

5* Squamules (if present) smaller, up to 0.5 mm, without distinct cortex. Known from Europe **6**

6 Apothecia flat or convex, c. 0.2–0.5 mm diam., without true and thalline exciples. Ascospores 12–16 × 7–10 µm (span of mean values; 10 measurements per specimen), variable in shape, but mainly broadly ellipsoid; length/width ratio 1.7–2.1 (span of specimen mean values). Paraphyses conglutinated (individual paraphyses well-visible only after treatment with KOH). Asci with amyloid external sheath but without amyloid tholus (Fig. 2A). Granules/goniocysts c. 40–100 µm diam., formed of paraplectenchymatous tissue, fully covering mucilaginous envelopes of *Nostoc* (Figs 3A, C) .... ***Gregorella humida***

6* Apothecia ± flat, c. 0.3–1.6 mm diam., always with true exciple, well visible in section (but less conspicuous in young and old apothecia). Thalline exciple formed of granules, usually absent from young apothecia but present in older apothecia. Ascospores 14.5–22 × 6–8 µm (span of mean values; 10 measurements per specimen), (narrowly) ellipsoid; length/width ratio 2.6–2.8 (span of specimen mean values). Paraphyses not conglutinated (individual paraphyses well-visible in section). Asci with amyloid external sheath and with amyloid tholus similar to the *Porpidia*-type (Fig. 2B). Granules/goniocysts c. 50–150 µm diam., formed by short hyphae c. 5–9 × 2–4 µm, only partly covering mucilaginous envelopes of *Nostoc* (Figs 3B, D) ..... ***Moelleropsis nebulosa***

Photobionts

Morphological appraisals of photobionts from *Gregorella humida* and *Moelleropsis nebulosa* confirmed that they belong to *Nostoc* (irregularly coiled filaments composed of ± identical sub-

spherical vegetative cells and intercalary heterocytes, enclosed in gelatinous envelopes). The observed characters did not allow identification of the *Nostoc* species or clear morhological separation of *G. humida* and *M. nebulosa* photobionts. The 16S rRNA gene sequences of photobionts from both lichens apparently correspond to the core cluster of *Nostoc*, which also contains all sequenced symbiotic members of the genus from lichens and plants (Papaefthimou et al. 2008). Our phylogenetic analysis of *Nostoc* s. str. contains two original sequences of cyanobionts from *G. humida* (JX129884 – Poland "PRA, Palice, s.n."; JX129885 – Czech Republic "PRA, Palice 14802") and two from *M. nebulosa* (JX129887 – Austria "PRA, Palice 12169"; JX129886 – Czech Republic "PRA, Palice 14890"). Sequences of photobionts from *M. nebulosa* clustered tightly together, whereas the sequences from *G. humida* are similar but unresolved within a large subclade. Sequences obtained from *M. nebulosa* are obviously not closely related those from *G. humida* (Fig. 4). Moreover, the similar *Nostoc* sequences for each of the studied lichen species suggest possible stringent host specifity in these two taxa.

Ecology

***Gregorella humida*:** The studied samples were collected from barren soil (sand or clay) with low organic content in e.g. road cuttings, loose grasslands, water ditches, sand-pits and railway embankments. Localities are often in urbanized or agricultural landscapes, but also in open places in managed forests, from lowlands to mountains (up to c. 800 m). Measured pH of its substrate was 4.9–5.9 [data from 15 samples]. Bryophytes present in more than two samples of *G. humida* are *Ceratodon purpureus*, *Pogonatum aloides* and *Bryum* sp. *Gregorella humida* is often accompanied by other inconspicuous crustose pioneer lichens which are sometimes termed short-living or ephemeral lichens (Poelt & Vězda 1990). We have recorded a range of co-occurring lichen species similar to that listed by Cezanne et al. (2003), but we also recorded *Baeomyces rufus*, *Placynthiella uliginosa*, *Scutula dedicata* and *Vezdaea acicularis*.

***Moelleropsis nebulosa*:** The studied samples came from mineral or humus-rich soil (rarely siliceous pebbles and stones) in quarries, road cuttings, forest edges, and railway embankments. This lichen commonly overgrows bryophytes, which suggests fast growth. Localities are situated in agricultural or near-natural woodland sites from lowlands to mountains (up to c. 750 m). Measured pH of its substrate was 4.1–5.3 [data from 18 samples]. Bryophytes found in more than two samples of *M. nebulosa* are *Bartramia pomiformis*, *Brachythecium albicans*, *B. glareosum*, *B. velutinum*, *Bryoerythrophyllum recurvirostrum*, *Bryum* sp., *Ceratodon purpureus*, *Hypnum cupressiforme*, *Racomitrium canescens* and *Tortula subulata*. Other lichens are only occasionally present in herbarium samples of *Moelleropsis*; patches of *Lepraria* and squamules of *Cladonia* were sometimes intermingled.

We expected that pH measurements would show more acidic substrata for *G. humida*, which is only known from siliceous bedrocks, than for *M. nebulosa*, which sometimes grows on soils above calcareous bed rocks (also observed by Pykälä 2007). However, our pH measurements clearly showed the opposite. The presence of different bryophytes with the two lichens suggests a possible explanation. Although some basiphilous bryophytes were found in specimens of *M. nebulosa*, most of the species associated with this lichen are pleurocarpous mosses which tolerate low pH and prefer humus-rich soils that may be acidic even over limestone. *Gregorella humida* usually grows with only a few pioneer moss species on mineral soils, only occasionally with pleurocarpous mosses, indicating more stable habitats with organic soils.

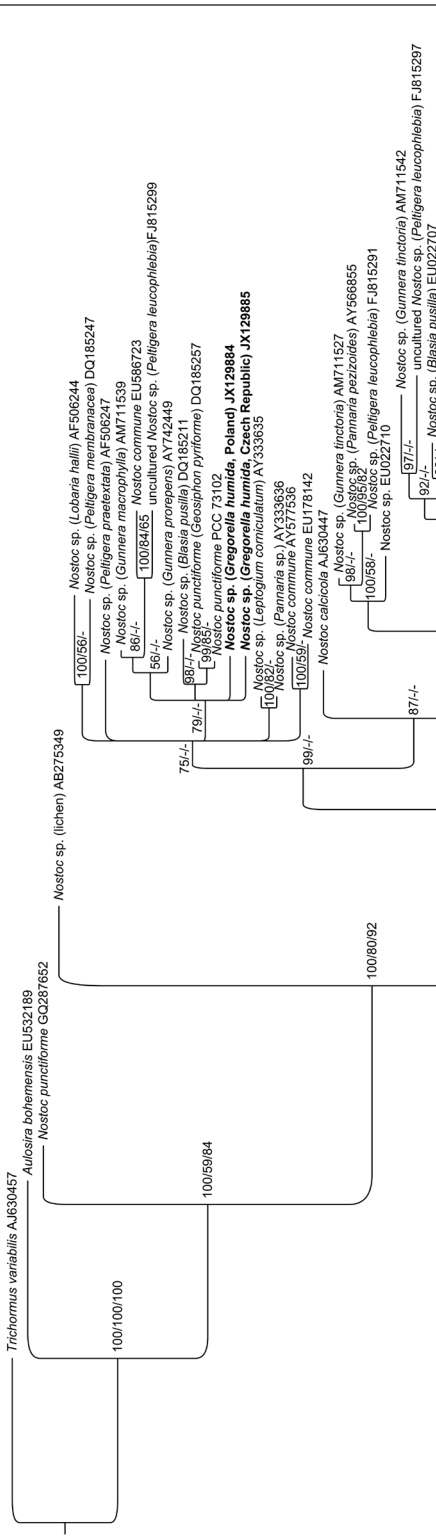

Fig. 4: Placement of symbionts of *Gregorella humida* and *Moelleropsis nebulosa* in the 16S rRNA phylogeny of *Nostoc*.

Past and present distribution in Central Europe

We have extensive floristic data only from the territory of the Czech Republic. *Moelleropsis nebulosa* was collected repeatedly until about 1970, but we know of only one recent record (Fig. 5). *Gregorella humida* was probably not collected from the Czech Republic until 1996 (our data), but the number of known localities has increased in recent years (Fig. 5). Literature data show similar patterns in other Central European countries.

Moelleropsis nebulosa is considered critically endangered in Austria (Türk & Hafellner 1999), extinct in Belgium and Luxembourg (Diederich et al. 2012), extremely rare in Germany (Wirth et al. 2011), critically endangered in Slovakia (Pišút et al. 2001), regionally extinct in Poland (Cieśliński et al. 2006) and vulnerable in Switzerland (Scheidegger et al. 2002). Evidently *M. nebulosa* has become scarce during the 20th century throughout Central Europe.

Gregorella humida is known only from few old records for Central Europe (most of them before 1900; cf. Cezanne et al. 2003). A new attention to *G. humida* was evoked by Poelt & Vězda (1990) and since that time it has been increasingly recorded (e.g. Ernst 1993, Berger & Priemetzhofer 2000, van den Boom 2000, Cezanne et al. 2003, 2008, Czarnota 2003, Sparrius 2003, Zimmermann et al. 2011). This fact does not necessarily mean that *G. humida* was rare in the past, especially as it often grows on even less natural sites than *M. nebulosa* and such urbanized or agricultural habitats were little investigated in the past. Moreover, *G. humida* usually forms less conspicuous crusts than *M. nebulosa*, and such small lichens were often not collected in the past.

We have no data on the population dynamics of either species, but we consider the species to be more or less ephemeral and restricted to human managed habitats at particular stages of succession. These sites are established by random disturbances and their locations change with time. The difference in the recent distribution of the studied lichens in Central Europe may be explained by changing types, strength and frequencies of anthropogenic disturbances in the landscape from one historical period to another. Broadly speaking, slight and small-scale disturbances in the past have been replaced by stronger and more extensive ones in recent times. Possibly *M. nebulosa* cannot tolerate strong disturbance, whereas *G. humida* can and may even prefer it. Another possibility is that the strain of *Nostoc* in *M. nebulosa* might be more sensitive to air pollution. The reductions in range of the related terricolous lichen, *Protopannaria pezizoides*, may have similar causes.

Recent records of abundant *G. humida* may be only snapshots of a temporary phenomenon. When we repeatedly visited two localities in the Czech Republic (May 2008/February 2011, April 2009/March 2011), we observed very different abundances and viabilities of local *G. humida* populations. At the earlier visits, large sterile populations were present, with some apothecia of a lichenicolous fungus. At the later visits, we have seen only unhealthy looking remnants of populations obviously affected by the same lichenicolous fungus. It seems possible that the lichenicolous fungus (perhaps in combination with other factors) may seriously reduce individual populations of *G. humida*.

Description of the Leotiomycete on *Gregorella humida*

Llimoniella gregorellae Kocourk. & Vondrák, **sp. nov.** [MycoBank 801903; ITS sequences of the holotype: JX996120, JX996122]. (Fig. 6)

Diagnosis: Similar to *Llimoniella terricola*, but with larger ascomata, 150–400 µm diam., somewhat thicker paraphyses, 1–1.5 µm in lower part, and longer asci, 55–90 µm. Host is a member of Arctomiaceae.

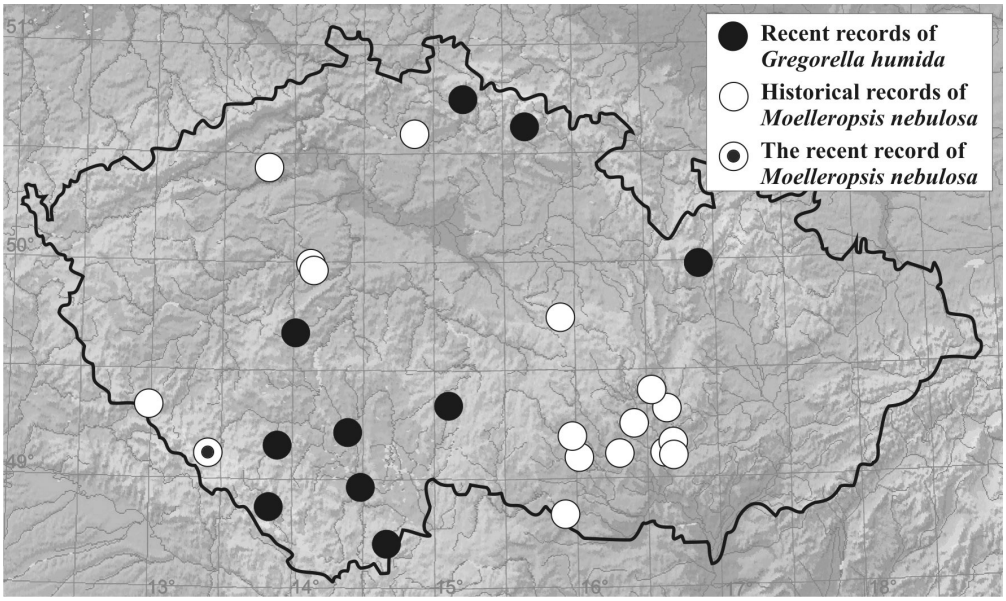

Fig. 5: Distribution of *Gregorella humida* and *Moelleropsis nebulosa* in the Czech Republic.

Typus: Czech Republic. South Bohemia: Týn nad Vltavou, Temelín, at railway between Temelín and nuclear power-plant „Temelín“, alt. 490 m, 49°11'19"N/14°21'57"E, on soil on railway embankment, lichenicolous on *Gregorella humida*, J. Vondrák, 16.3.2011 (CBFS JV9955 – holotype; Hb. myco. K & K – isotype).

Description: Fungus lichenicolous on the thallus of *Gregorella humida*, at first not causing visible damage to the host (Fig. 6A), but later destroying the thallus (Fig. 6C). *Thallus* inconspicuous; vegetative hyphae indistinguishable within host tissues. **Ascomata** apothecia, (110–)140–150–160(–250) µm diam. [2; 39], immersed to sessile, base broadly attached to the host thallus, roundish, dispersed or in groups, brown to black, matt to slightly shiny, when wet with dark reddish brown disc. Young erumpent apothecia with rough surface, flat or later concave with elevated margin.

Exciple c. 20–50 µm wide, orange-brown in section (Fig. 6B), without hairs, prosoplectenchymatous, composed of thin-walled, branched and anastomosing hyphae, 2–4 µm wide, embedded in dense gel (dissolving in K). Exciple sometimes externally surrounded with thin, subhyaline necrotic layer, especially in lower part, in contact with host thallus. **Basal exciple / hypothecium** up to c. 50 µm high, pale orange brown. **Hymenium** c. 90–110 µm high, colourless to yellowish, ± inspersed, strongly gelatinous. **Epihymenium** pale orange brown. **Paraphyses** numerous, simple or branched in upper part, conglutinated in hymenial gel, which is soluble in KOH. Paraphyses in lower hymenium (1.5–)2.0(–2.5) µm wide [2; 0.3]; paraphyses tips (1.5–)2.5–3.0– 3.5(–4.0) µm wide [2; 0.7; measured after KOH treatment].

Asci (Fig. 6D) cylindrical, apically slightly applanate, thin walled, wall not thickened apically, (40–)52–53–54(–62) × (6.5–)7.4–7.7–8.1(–9.0) µm [2; 6.2 (height); 0.8 (width)].

Ascospores (Fig. 6E) uniseriate, colourless, non-septate, variable in shape, but mostly broadly ellipsoid to almost globose, usually with a single large guttule; (5.0–)7.0–7.3–7.6(–9.5) × (4.0–)

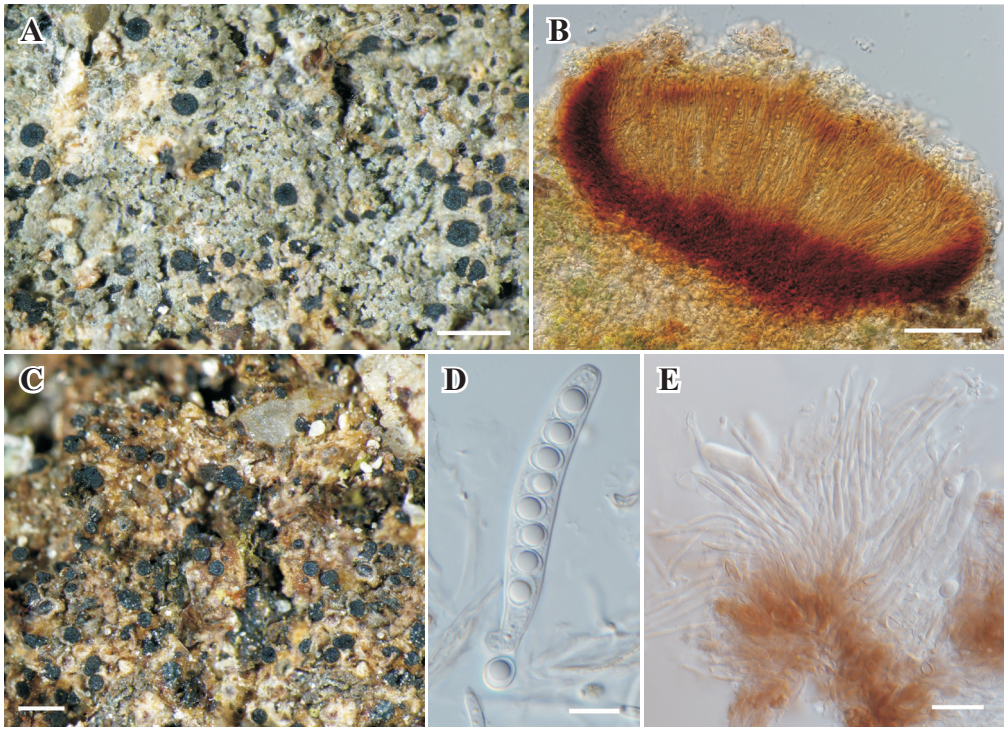

Fig. 6: *Llimoniella gregorellae*. **A** – apothecia on living thallus of *Gregorella humida*, CBFS JV6984; **B** – apothecium, vertical section, isotype; **C** – apothecia on dead host, holotype; **D** – ascus with ascospores, isotype; **E** – paraphyses, isotype (D, E, observed after KOH treatment). Scales: A, C = 0.5 mm, B = 100 µm, D, E = 10 µm.

5.3–5.3–5.4(–7.0) µm [2; 1.2 (length); 0.9 (width)], ascospore length/width ratio (1.1–) 1.3–1.4–1.4(–1.9) [2; 0.2].

Conidiomata not observed, but **conidiogenous cells** originating in basal exciple, ampulliform, orange brown (colour and reactions as for exciple), c. 3–5 × 2–5 µm. **Conidia** enteroblastic, acrogenous, oblong to bacilliform, hyaline, non-septate, about 1.5 µm long. This type of conidiogenesis was observed in several apothecia and we suggest it belongs to *Llimoniella gregorellae*, not to any parasitic hyphomycete.

Reactions: exciple and hypothecium K+ brown-purple (persistent), N+ yellow-orange (persistent); Hymenium and Asci I–.

Phylogeny: We generated three ITS sequences from *Llimoniella gregorellae*; from samples CBFS JV9955 (JX996120, JX996122) and JV8374 (JX996121). These sequences are identical and their closest BLAST results are various unidentified Leotiomycetes (several records with 79 % identity and 99 % coverage). In Myconet (Lumbsch & Huhndorf 2010), *Llimoniella* is placed into Helotiales (Leotiomycetes), genera incertae sedis. Our data are probably the first molecular evidence for placement of *Llimoniella* into Leotiomycetes, because no *Llimoniella* sequences are present in the GenBank.

Remarks: The new fungus very likely belongs to *Llimoniella* s.lat. as understood by Diederich et al. (2010). The species is characterized by following characters: (1) cylindric

thin-walled, non-amyloid asci, without apical chamber, (2) uniseriate, broadly ellipsoid and small ascospores with a large single guttula, (3) prosoplectenchymatous exciple of thin-walled orange-brown hyphae, K+ purple-brown, (4) paraphyses tips not distinctly widened.

Two other species of *Llimoniella* are known from cyanolichens, both without K+ purplish pigment in the exciple; *L. heppiae* (Nav.-Ros., Hladún & Llimona) Diederich & Ertz has also wider paraphyses tips, 5–6 µm, longer ascospores, about 10–13 µm, and darker epihymenium (Diederich et al. 2010), and *L. terricola* (Arnold) M.Schultz, Diederich & Ertz has larger ascomata, 150–400 µm diam., somewhat thicker paraphyses, 1–1.5 µm in lower part, and longer asci, 55–90 µm (Ertz & Diederich 2006). Both fungi are also host specific to lichens of Heppiaceae (Lichinomycetes) and a visible damage of their hosts was not observed (Diederich et al. 2010). We suppose that *L. terricola* is the most similar known species to *L. gregorellae*.

Paratypes: Czech Republic. Central Bohemia: Příbram, Lešetice, SE foot of discharge hopper c. 0.7 km W of village, alt. 550 m, 49°38'48"N/14°0'37"E, on soil at base of hopper, lichenicolous on sterile crust of *Gregorella humida*, J. Vondrák, 11.2.2011 (CBFS JV8374, 9953); South Bohemia: Týn nad Vltavou, Temelín, at railway between Temelín and nuclear power-plant „Temelín", alt. 490 m, 49°11'19"N/14°21'57"E, on soil on railway embankment, lichenicolous on *Gregorella humida*, J. Vondrák, 4.4.2009 (CBFS JV6984, 9954).

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