Sarcogyne (Acarosporaceae) on calcareous rock in Europe and North America

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DOI:
https://doi.org/10.13158/heia.36.1.2023.52

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Abstract: Knudsen, K., van Zon, S., Tsurykau, A., Kocourková, J., Hodková, E., Huereca, A. & Malíček, J. 2023. *Sarcogyne* (Acarosporaceae) on calcareous rock in Europe and North America – Herzogia **36**: 52–71.

Sarcogyne pruinosa and *S. regularis* are revised and lectotypes selected. *Sarcogyne pruinosa* is recognized as oldest name for the species and *S. regularis* as a synonym. The description of the species is revised. *Sarcogyne pruinosa* does not occur in North America. Two new species are described, *Sarcogyne nimisii* from Italy and *Sarcogyne belarusensis* from Belarus, Germany and Italy. *Sarcogyne platycarpoides* is lectotypified and *S. melaniza* is recognized as its synonym. New records are reported of *S. distinguenda* and *S. nivea* from the Czech Republic and Italy and *S. fallax* from the Czech Republic. A key is supplied for 14 species of *Sarcogyne* on calcareous rock in Europe.

Zusammenfassung: Knudsen, K., van Zon, S., Tsurykau, A., Kocourková, J., Hodková, E., Huereca, A. & Malíček, J. 2023. *Sarcogyne* (Acarosporaceae) auf Kalkgestein in Europa und Nordamerika – Herzogia **36**: 52–71.

Sarcogyne pruinosa und *S. regularis* wurden revidiert und Lectotypen wurden ausgewählt. *Sarcogyne pruinosa* ist der älteste Name für die Art und *S. regularis* ist ein Synonym. Die Beschreibung der Art wurde überarbeitet. *Sarcogyne pruinosa* kommt nicht in Nordamerika vor. Zwei Arten wurden beschrieben, *Sarcogyne nimisii* aus Italien and *Sarcogyne belarusensis* aus Weißrussland, Deutschland und Italien. *Sarcogyne platycarpoides* wurde lectotypifiziert und *S. melaniza* ist ein Synonym. Neue Funde werden für *S. distinguenda* und *S. nivea* aus Tschechien und Italien gemeldet, und *S. fallax* aus Tschechien. Ein Bestimmungsschlüssel ist beigefügt für 14 Arten von *Sarcogyne*, die in Europa auf Kalkfelsen vorkommen.

Key words: Carbonized margins, convex apothecia, lecideine apothecia, nomenclature, *Sarcogyne coeruleonigrans*.

Introduction

For the last four years (2019–2022) we have been studying the Acarosporaceae of the Chihuahuan Desert in New Mexico (Knudsen et al. 2020, 2021a, 2023). Any lecideine *Sarcogyne* with a black margin on calcareous rock in North America is usually identified as *S. regularis* Körb. (Knudsen & Stanley 2007). On the webpage of the Consortium of North American Lichen Herbaria, 1154 specimens of *S. regularis* are listed for North America (Canada, Mexico, United States) (CNALH 2022). *Sarcogyne regularis* was originally reported as *Sarcogyne pruinosa* auct. for North America (Magnusson 1935a). From New Mexico Magnusson reported one specimen of *S. pruinosa*. The specimens we collected in New Mexico had a wide range of margin width, either amyloid or hemiamyloid hymenial gel, and occasionally convex apothecia. To revise taxa from North America, we needed to know the identity of *Sarcogyne pruinosa* (Schaer.) A.Massal. and *S. regularis*.

Materials and Methods

We studied specimens from FR, HBG, L, OTB, PRM, SBBG (UCR lichen herbarium transferred to SBBG in 2022 and 2023), STU, TSB, and the private herbaria of Jana Kocourková and Kerry Knudsen (hb. K&K) and Jiří Malíček (hb. Malíček). The morphology of specimens was examined with dissecting microscopes. The anatomy of hand sections was examined and measured in water at 1000× magnification with compound microscopes. Mature ascospores of *S. pruinosa* were measured 40 times and *S. belarusensis* K.Knudsen, Tsurykau, Kocourk. & Hodková was measured 80 times. Atypical ascospores were also taken into account when measuring. The length, width and length/width ratio (l/w) of the ascospores are given as (min–)($\bar{x}$ − SD)–$\bar{x}$–($\bar{x}$ + SD)(–max), where 'min' and 'max' are the extreme values observed, $\bar{x}$ the arithmetic mean and SD the corresponding standard deviation. Only 10 ascospores outside asci were measured for *S. nimisii* Knudsen, Kocourk. & Hodková to preserve the holotype and only specimen. For other species discussed, measurements in the literature were found adequate and used. The margin was measured from the edge of hymenium to outside of the margin in water. Using the width of margin can be problematic especially because of buildup of melanin on the outside of some apothecia. Our measurements of the margin are based on sections of apothecia without thick buildups of melanin. The amyloid reaction of the hymenial gel and subhymenium was tested with fresh undiluted IKI (Merck's Lugol) (see protocol in Knudsen & Kocourková 2018a). The ascus stain was studied in IKI (Hafellner 1993). No secondary metabolites were detected with TLC (Orange et al. 2001).

Molecular phylogeny

DNA was extracted from 29 dried herbarium specimens (Table 1). Genomic DNA was extracted from lichenized thalli including apothecia via the Invisorb® Spin Plant Mini Kit, according to the manufacturer's protocol with slight modifications (i.e. eluted in 55 µL of buffer, 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 for this study were the internal transcribed spacer complete repeat (ITS) (White et al. 1990), the large subunit of the nuclear ribosomal DNA (nrLSU) (Vilgalys & Hester 1990), and the small subunit of the mitochondrial ribosomal DNA (mtSSU) (Zoller et al. 1999).

The ITS, nrLSU, 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 Red DNA Polymerase (Bioline), 8.2 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), then 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 15 min. Conditions 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 10 min. Before sequencing, the PCR products were purified using the enzymatic method ExoSap-ITTM Express Reagent 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 20 min. Purified PCR products, water, and forward primer (8 µL in total volume) were sent to BIOCEV, Vestec, CZE.

Table 1. A list of sampled specimens included in the molecular phylogeny. Newly produced sequences are shown in bold.

Species	Location	Voucher ID	Accession ITS	Accession mt SSU	Accession LSU
Pleopsidium flavum	Czech Republic, Prague, Divoká Šárka	Malíček	OK142757	OK032142	-
Sarcogyne arenosa	U.S.A., California, Los Angeles Co.	Knudsen 11102 & Sagar (S)	LN810851	LN810977	LN810851
Sarcogyne arenosa	U.S.A., California, San Luis Obispo Co.	Dart 1340	**OQ171124**	**OQ184835**	**OQ195884**
Sarcogyne belarusensis	Belarus, Grodno region, Kostenevo	Golubkov 80	**OQ171089**	**OQ184801**	**OQ195852**
Sarcogyne belarusensis	Belarus, Grodno region, Kostenevo	Golubkov 81	**OQ171090**	**OQ184802**	**OQ195853**
Sarcogyne belarusensis	Belarus, Grodno region, Lunno	Golubkov 78	**OQ171091**	**OQ184803**	**OQ195854**
Sarcogyne belarusensis	Belarus, Vitebsk region, Gomel	Golubkov 73	**OQ171092**	**OQ184804**	**OQ195855**
Sarcogyne belarusensis	Belarus, Vitebsk region, Ruba	Vynaev 76	**OQ171093**	**OQ184805**	**OQ195856**
Sarcogyne belarusensis	Belarus, Grodno region, Krasnoselsk	Leonova 85	**OQ171094**	**OQ184806**	**OQ195857**
Sarcogyne belarusensis	Belarus, Vitebsk region, Osetische	Bely 32	**OQ171095**	**OQ184807**	**OQ195858**
Sarcogyne coeruleonigrans	Mexico, Galeana, Nuevo Leon	A. Huereca 876	ON794191	ON787673	ON964965
Sarcogyne coeruleonigrans	Mexico, Saltillo, Coahuila, El Cercado	A. Huereca 882	ON794188	ON787670	ON964962
Sarcogyne coeruleonigrans	Mexico, Galeana, Nuevo Leon	A. Huereca 885	ON794189	ON787671	ON964963
Sarcogyne coeruleonigrans	Mexico, Saltillo, Coahuila	A. Huereca 869	ON794187	ON787669	ON964961
Sarcogyne coeruleonigrans	U.S.A., Oscura, Malpais	Kocourková 10969	**OQ171111**	**OQ184822**	**OQ195872**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Brokeoff Mountains	Kocourková 10873.2	**OQ171112**	**OQ184823**	**OQ195873**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Brokeoff Mountains	Kocourková 10899	**OQ171118**	**OQ184829**	**OQ195878**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Carlsbad Cavern area	Kocourková 10869	**OQ171119**	**OQ184830**	**OQ195879**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Brokeoff Mountains	Kocourková 10893	**OQ171120**	**OQ184831**	**OQ195880**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Otero Co.	Kocourková 10625	**OQ171104**	**OQ184816**	**OQ195866**
Sarcogyne coeruleonigrans	U.S.A., New Mexico, Dona Ana Co.	Schultz 16242	**OQ171108**	**OQ184820**	**OQ195870**
Sarcogyne distinguenda	Sweden, Jamtland	Westberg 08–305 (S F120452)	LN810854	LN810979	LN810854
Sarcogyne distinguenda	Norway, Hedmark	Haugan H3852 (O L17425)	LN810855	LN810980	MZ262744
Sarcogyne fallax	Portugal, Papagovas, Loininhá	Zaca 2347	MZ262722	MZ262734	MZ262744
Sarcogyne magnussonii	Canada, Saskatchewan	Freebury 829	MW715694	MW715738	MW715727
Sarcogyne malpaiensis	U.S.A., New Mexico, Corrizozo Malpais	Knudsen 19317.1	OK142764	OK032149	-

Species	Location	Voucher ID	Accession ITS	Accession mt SSU	Accession LSU
Sarcogyne nimisii	Italy, Trieste Coast	Nimis 35083	**OQ171133**	**OQ184844**	**OQ195892**
Sarcogyne nivea	Czech Republic, Eastern Bohemia, Police nad Metují	Malíček 12679	**OQ171100**	**OQ184812**	**OQ195863**
Sarcogyne nivea	Czech Republic, Northern Bohemia, Karlov pod Ještědem	Malíček 14530	**OQ171085**	**OQ184797**	**OQ195848**
Sarcogyne pruinosa	Italy, Campania, Prov. Salerno	Nimis 31664	**OQ171124**	**OQ184835**	**OQ195884**
Sarcogyne pruinosa	France, Corsica	Kocourková 10986	**OQ171138**	**OQ184848**	-
Sarcogyne pruinosa	Italy, Abruzzi, Cesano	Nimis 26982	**OQ171127**	**OQ184838**	**OQ195887**
Sarcogyne pruinosa	Italy, Puglia, Prov. Foggia	Nimis 29960	**OQ171128**	**OQ184839**	**OQ195888**
Sarcogyne pruinosa	Italy, Marche, Prov. Macerata, Lago di Fiastra	Nimis 31152	**OQ171129**	**OQ184840**	**OQ195889**
Sarcogyne pruinosa	Italy, Abruzzi, Prov. Teramo, S. Stefano	Nimis 24913	**OQ171130**	**OQ184841**	**OQ195890**
Sarcogyne pruinosa	Czech Republic, Central Bohemia, Týřovice	Malíček 12613	ON794203	ON787687	ON964977
Sarcogyne pruinosa	Czech Republic, Džbán, Milská stráň	Kocourková 10458	OK142765	OK032150	-
Sarcogyne pruinosa	Czech Republic, Džbán	Kocourková 10458.2	ON794204	ON787688	ON964978
Sarcogyne pruinosa	Romania, South Carpathians, Piatra Craiului Mts., Zarnesti	Malíček 15314	**OQ171090**	**OQ184802**	**OQ195853**
Sarcogyne pruinosa	Czech Republic, Southern Moravia, Netčiny	Malíček 2560	**OQ171097**	**OQ184809**	**OQ195860**
Sarcogyne pruinosa	Macedonia, Varda River valley, Negotino	Malíček 7975	**OQ171086**	**OQ184798**	**OQ195849**
Sarcogyne pruinosa	Czech Republic, Central Bohemia, Prague, Prokopské valley	Kocourková 7422	**OQ171087**	**OQ184799**	**OQ195850**
Sarcogyne pruinosa	Czech Republic, Central Bohemia, Český Kras	Kocourková 10752	**OQ171088**	**OQ184800**	**OQ195851**
Sarcogyne pruinosa	Italy, Puglia, Prov. Bari, Spinazzola	Nimis 22919	**OQ171086**	**OQ184798**	**OQ195849**

Sequences were checked against the UNITE database and NCBI database for contamination. All newly generated sequences were deposited in GenBank (Table 1). The sequences were proofread and concatenated manually into a single data set using SEQUENCHER® version 5.4.6 (GeneCodes). Sequences were aligned using the multiple sequence alignment online service MAFFT version 7 with 'G-INS-1' strategy (KATOH & TOH 2008). Indels longer than 1 bp were coded by the simple gap coding method (SIMMONS & OCHOTERENA 2000) as implemented in SEQSTATE 1.4.1 (MÜLLER 2005). A partition homogeneity test (ILD) with heuristic search was performed under one thousand replicates between the nrITS, nrLSU, and mtSSU sequences by PAUP* version 4.0a169 (SWOFFORD 2002) to determine whether the partitions were homogeneous. For phylogenetic analyses (i.e. ITS + mtSSU + nrLSU data sets), the GTR+I model was selected as the best-fitting model of nucleotide substitution based on the Akaike Information Criterion using JMODELTEST 2.1.10 for each gene (DARRIBA et al. 2012). Phylogenetic trees were constructed using MRBAYES 3.2.2

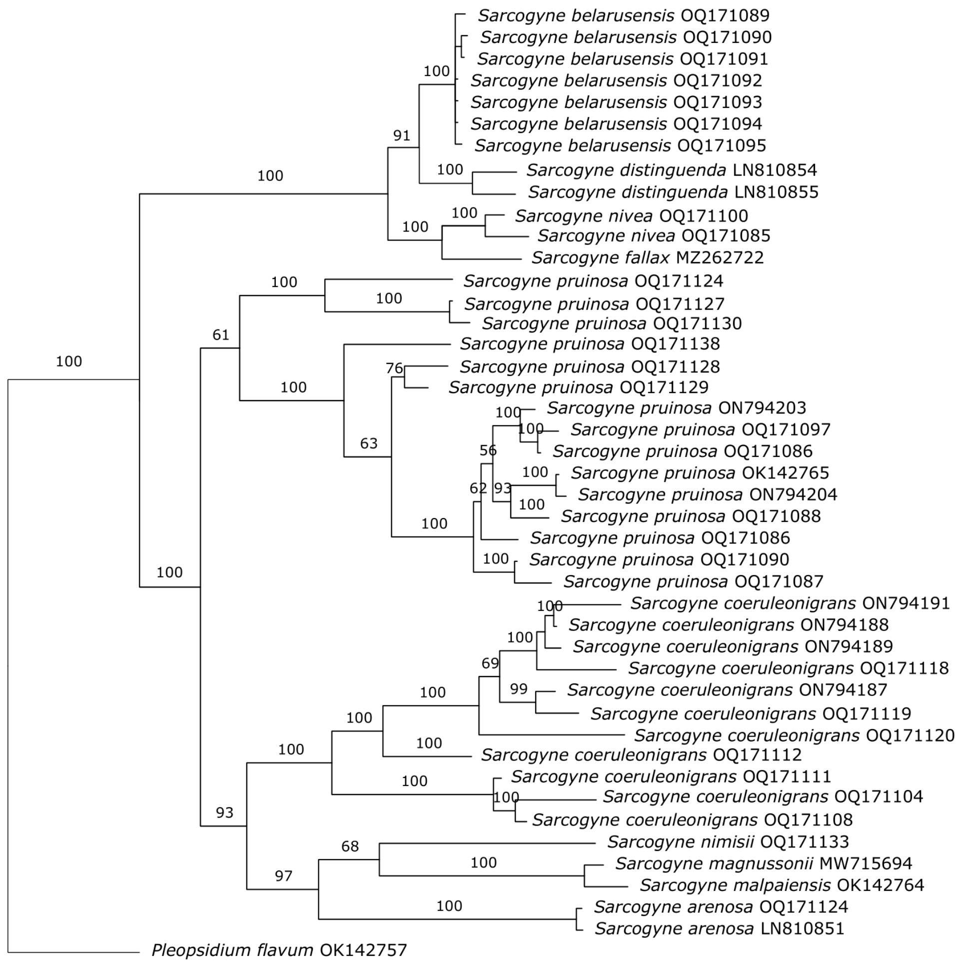

Fig. 1. Bayesian inference tree obtained by phylogenetic analysis using combined nrITS, nrLSU and mtSSU sequences, including 29 newly sequenced specimens of *Sarcogyne*.

(Ronquist & Huelsenbeck 2003). Input data was formatted for MRBAYES via the FABOX (Villesen 2007) with slight modification. Sequences of *Pleopsidium flavum* were included as an outgroup. Three replicate analyses with four chains each were computed 30,000,000 generations, sampling every 1000th generation. After this number of runs, the average standard deviation of split frequencies reached a value lower than 0.01, indicating that convergence was reached. The Bayesian phylogenetic tree with bootstrap approximation (1000 replicates) was visualized using Fig-TREE v1.4.4 (Rambaut 2012) and rooted with *Pleopsidium flavum*.

Results

Sequences of nrITS, mtSSU and nrLSU were deposited in GenBank (Supplemental Table S1). The final alignment contained 1844 concatenated characters, consisting of 1–507 (ITS), 508–1209 (nrLSU), 1210–1844 (mtSSU) nucleotide positions. Of these characters, 1451 were constant, 122 were variable and parsimony-uninformative and 271 were parsimony-informative and the sequence alignments are accessible at: https://doi.org/10.5281/zenodo.7558669. Tree (Fig. 1).

In Knudsen et al. (2023) a *Sarcogyne regularis* clade was recovered as the sister of an *Acarospora glaucocarpa* clade in the non-monophyletic *Sarcogyne* group. We based our tree in this paper on this *Sarcogyne regularis* clade. We included specimens from North America (Mexico, U.S.A.) and a selection of specimens identified as *Sarcogyne regularis* from Belarus, the Czech Republic, France (Corsica), Italy, Macedonia, and Romania. We recovered three clades: the *Sarcogyne nivea* clade, the *Sarcogyne pruinosa* clade, and a North American clade of calciphytes which included a new species from Italy, *S. nimisii.* In the *Sarcogyne nivea* clade we recovered *S. distinguenda* H.Magn., *S. fallax* H.Magn., *S. nivea* Kremp. and a new species common in Belarus, *S. belarusensis.* These species had hemiamyloid hymenial gel and all had been misdetermined as *S. regularis* by collectors. The second clade recovered *S. pruinosa* (syn. *S. regularis*). The North American clade circumscribed *Sarcogyne coeruleonigrans* (Knudsen et al. 2023). To our surprise, the new species *Sarcogyne nimisii* from Trieste in Italy was recovered in a clade with *S. malpaiensis* from New Mexico and the neotype of the *S. magnussonii* B.de Lesd. from Canada and originally described from New Mexico (Knudsen & Kocourková 2012, Knudsen et al. 2023). This clade included *Sarcogyne arenosa* (Herre) K.Knudsen & Standley, a species Magnusson included in Magnusson's heterogeneous species concept of North American *Sarcogyne pruinosa* auct. (Magnusson 1935a, Knudsen & Standley 2007).

Nomenclature

A pruinose calciphyte was described originally as *Lichen pruinosus* Ach. (Acharius 1799). The name was invalid because *Lichen pruinosus* Humb. had priority (von Humboldt 1793). Schaerer published *Lecidea immersa* var. *pruinosa* Schaer. (Schaerer 1833). In 1854, *Sarcogyne pruinosa* (Schaer.) A.Massal. was published (Massalongo 1854). Körber recognized *S. pruinosa* as a different taxon from *S. regularis* Körb., his new species. It differed from S. *pruinosa* in having epruinose apothecia becoming hemispheric and eventually excluding a thin margin (Körber 1855). During the 19th century, the two species were identified primarily by whether their apothecia had pruina or not. By the time Magnusson began his studies of Acarosporaceae in the 1920s, there was uncertainty about the two species and no clear diagnostic difference was recognized. Magnusson did not recover the species level difference. After examining many specimens including type material, Magnusson stated he could not tell the difference between *S. pruinosa* and *S. regularis* (Magnusson 1935b). In his taxonomy, *Sarcogyne pruinosa* (Sm.) Körb. nom. illegit., became the name and authority for both taxa and *S. regularis* was reduced to *S. pruinosa* var. *regularis* (Körb.) H.Magn. In his key, variety *regularis* can be identified as having 1 mm wide, sessile, black and epruinose apothecia. In the description of the variety, Magnusson states that they are sometimes convex, but based on the anatomy of the apothecia, it is the same species as *S. pruinosa*. In the description of *S. pruinosa* (which includes all the varieties and forms), Magnusson states that *S. pruinosa* had an apothecial disc which was flat and only somewhat convex. Peter James reversed this nomenclature in 1965 when he rejected the name *Sarcogyne pruinosa* (Sm.) Körb. as invalid and based on Acharius having illegitimately published *Lichen pruinosus* (James 1965). Following Magnusson's taxonomy that *regularis* and *pruinosa* were the same species, James applied the name *S. regularis* as the replacement name for *S. pruinosa*. Quickly on checklists (for instance Santesson 1993), *S. regularis* replaced *S. pruinosa.* In the 21st century in Europe, *S. pruinosa* is a barely remembered name. We have found that *Sarcogyne pruinosa* is the oldest name for the species and *S. regularis* is synonymized with it.

Nomenclature for *S. pruinosa* is based on Index Fungorum and the work of the Kew Mycology in 2018 (Index Fungorum 2022).

Taxonomy

Sarcogyne pruinosa (Schaer.) A.Massal., Geneac. lich. (Verona), 10 (1854). Fig. 2 and Fig. 3

≡*Lecidea immersa* var. *pruinosa* Schaer., Lich. helv. spicil. 4–5: 158 (1833). **type:** Schaerer, Lich. Helv. Exs. Ed. I, 202, [Switzerland ?] (SMNS-STU-F-0006365, STU, MBT 616774, lectotype designated here).

=*Biatorella latericola* J.Steiner, Ann. Naturhist. Mus. Wien 23: 226 (1909); **type:** Austria, Carinthia, ad tegulas prope Krumpendorf ad lacum Wörther See, on brick, J. Steiner, Arnold Exsicc. 1727 (PRM, MBT 10011326, lectotype designated here), syn. nov.

= *Sarcogyne regularis* Körb., Syst. lich. germ. (Breslau), 267 (1855); **type:** Sudeten, Körber, no date, 'Typenherbar' (L0837140, MBT 10011318, lectotype designated here).

Description. Thallus endolithic, algae mostly 10 µm wide scattered in substrate between and below apothecia, occasionally, especially in soft crumbling limestone, developing a white epilithic or chasmolithic ecorticate thallus, farinose with clusters of algae, and on brick or hard rock forming an ecorticate non-farinose epilithic thallus. Mycelial base of apothecia in substrate, sometimes forming a stipe but remaining immersed, hyphae mostly 2–3 µm wide often encrusted with crystals and obscure from gelatinization, the vertical hyphae continuous with the medulla of the apothecia and with the endolithic thallus. **Apothecia** lecideine, black, pruinose or epruinose, 0.2–0.5–1.0(–1.5) mm wide, 0.1–0.4(–0.7) mm thick, immersed or not, usually circular, usually with a dispersed pattern, not forming conspicuous clusters through replication. **Disc** pruinose or epruinose, black, flat and slightly below margin, or convex, or hemispheric excluding the margin. **Margin** carbonized, usually epruinose or lightly pruinose, thin or thick, sometimes rough or flexuous with a buildup of melanin, but not curling inward over the disc or becoming segmented. **Parathecium** 30–110 µm wide, formed of radiating, 1 µm wide hyphae, outer layer black 15–80 µm wide, inner layer reddish, *c.* 20–30 µm wide along edge of hymenium, widths variable. **Hymenium** usually 90–100(–130) µm tall, **epihymenium** dark 10–15 µm tall, **paraphyses** 1.5–2 µm wide at midlevel, the apices expanded up to 3 µm or unexpanded, often in dark pigment caps, hymenial gel IKI+ dark blue, euamyloid. **Asci** mostly 70–80 ×10–20 µm, cylindrical or clavate, **ascospores** variable (2.0–)3.1–4.0–4.9(–6.0) × (1.0–)1.3–1.7–2.1(–2.5) µm, l/b=2.5. **Subhymenium** 30–50 µm tall, IKI+ dark blue. **Hypothecium** 20 µm thick distinct from lower vertical hyphae of the medulla. **Medulla** 0.2–0.5 mm thick, continuous with mycelial base. **Pycnidia** not observed. Lacking secondary metabolites.

Ecology and distribution. *Sarcogyne pruinosa* grows on calcareous rock and anthropogenic substrates in full sun or shade at a variety of elevations and is expected throughout Europe. We do not recognize *S. pruinosa* as occurring in North America (Knudsen & Standley 2007; see discussion in *Sarcogyne coeruleonigrans*). Specimens of *S. regularis* need to be revised from outside Europe.

Differentiation. *Sarcogyne pruinosa* differs especially from the European calciphyte *S. praetermissa* in not having a margin higher than the disc and curling inward above the disc (Knudsen & Kocourková 2018b). The calciphytes *S. distinguenda* and *S. nivea* produce epilithic or chasmolithic thalli that are farinose and ecorticate in soft limestone. *Sarcogyne pruinosa* can also produce a farinose thallus in soft limestone. Like *S. nivea* it can also produce a non-farinose thallus on hard rock. *Sarcogyne pruinosa* differs from these two species in having dark euamyloid IKI+ hymenal gel vs. hymenial gel that is blue becoming red. *Sarcogyne distinguenda* and *S. fallax* also differ in having a higher hymenium. *Sarcogyne platycarpoides* was often mistaken for *S. pruinosa* because of the pruinose disc, euamyloid hymenial gel and occurrence on calcareous rock. It differs from *S. pruinosa* in having a usually wider margin (100–150 vs. 30–110 µm) and sometimes algae in the lower part of apothecia (Knudsen et al. 2022).

Discussion. *Sarcogyne pruinosa* usually has flat discs with occasional convex apothecia. Rarely the discs become hemispheric and exclude the margin. Apothecia can be epruinose or pruinose. Körber described *S. regularis* as having hemispheric discs. These are rare.

Selected specimens examined. Albania. Northern Albania: Shkodër distr., Prokletije Mountains, 1650 m, on low outcrops of limestone with layers of argillaceous shale, 1650 m, 14 Aug. 2007, L. Muggia (TSB41703). **Austria.** Auf Alpenkalk am [Hochkar?] bei Lassing im Niederösterreich, June 1857, I. S. Poetsch (L); 'Auf Alpenkalk am Gamstein in Niederösterreich und Steiermark' 21 Aug. 1860, I. S. Poetsch (L); Klamm, Kärnten, Weißenbachklamm, 29 July 1994, S. Bakker (L-0828635); Styria, Graz, Steirisches Randgebirge, Grazer Bergland, Sommeralm, N side of Plankogel, in meadow, 1270 m, on calcareous rocks, 13 Nov. 2010, L. Muggia & J. Hafellner (TSB41702). **Belarus.** Grodno region, Grodno Distr., close to Kemenka village, 6th fort of the Grodno fortress, 160 m, on calcium carbonate crust on granite pebble, 24 July 2013, A. J. Leonova (hb. K&K, GSU); the city of Grodno, Rumlevo park, on sandstone, 23 Aug. 2008, V. Golubkov (SBBG, GSU). **Belgium.** Le Diable Chateau, 2 km NE of La Roche, 28 Apr. 1990, L. Spier 1660 (L-0752743). **Croatia.** Dalmatien, Gravosa, leg. [illegible] (L), Ragusa, on kalk, 150 m, 1907, Dr. Latzel (PRM 955949). **Czech Republic.** Central Bohemia, Roudnice nad Labem, Říp, 250 m, 1932, J. Podzimek (PRM 955947, det. by Magnusson as *S. pruinosa* var. *intermedia*); Distr. Mladá Boleslav, Benátky and Jizerou, Mladá National Nature Reserve, pasture for herbivores, 220 m, on calcareous pebbles (marlstones) 10 Oct. 2022, J. Malíček (hb. Malíček); Praha, Pražská plošina plateau, Prokopské údolí Nature Reserve, Hemrovy rocks, on west-facing slope, 265 m, on diabase, 7 June 2009, J. Kocourková 7422 (hb K&K), west-exposed rocky diabase slopes, W of Děvín hill, 300–310 m, on diabase rock, 6 May 2009, J. Malíček 1680 (hb. Malíček); Distr. Praha-západ, Český kras Protected Landscape Area, 2 km SE of Loděnice, Branžovy old limestone quarry, 417 m, in abandoned quarry at base of south-east exposed wall, 19 Sep. 2009, J. Kocourková 10493 (hb. K&K); Distr. Příbram, Středočeská pahorkatina hilly country, Bytíz, old uranium mine heap north of 11A, 545 m, on pebbles, 18 Sep. 2020, F. Bouda 2280 (PRM 955382). Northern Bohemia, Distr. Liberec, Jizerské hory Mts. (Protected Landscape Area), Raspenava, Vápenný vrch reserve, 385 m, on limestone, 5 Sep. 2011, F. Bouda (PRM 860043). Silesia, Distr. Frýdek-Místek, Beskydy Protected Landscape Area, Horní Lomná, Velký Polom Reserve, rock above the road on WNW-facing slope of mount, 905 m, on flysh ("calcareous sandstone"), 3 June 2021, J. Malíček 14509 & Z. Sejfová (hb. Malíček); Distr. Nový Jičín, Šramberk Nature Monument, limestone outcrops on SW-facing slope under Štramberská trúba, 400–440 m, on limestone, 30 Oct. 2020, J. Malíček 14373 (hb. Malíček). Eastern Bohemia, Hlinsko, Medkovy kopce, 650 m, on calcareous rock, Nádvorník (PRM 955945, det. by Magnusson as *S. pruinosa* var. *intermedia*). Northern Bohemia, Distr. Trutnov, Krkonoše Mts., Pec pod Sněžkou, Sněžka Mt., Rudný brook valley, high wall of flat rhyolite outcrop at the brook enriched with heavy metals (copper ores and arsenopyrite) and calcareous sediments, 13 Aug. 2016, J. Kocourková 9259 (hb. K&K). Southern Moravia, Distr. Brno-country, Moravský kras Landscape Protected Area, 1 km S of Ochoz u Brna, Valley of Říčka River, Lysá hora hill, above scree forest opened calcareous outcrops on S-facing slope, 337 m, on calcareous rock, 25 June 2016, J. Kocourková 10616 & K. Knudsen (hb. K&K); Distr. Hodonín, Ježov, S border of Ježový Iom Nature Monument, 240 m, on semi-shady calcareous sandstone rock, 9 Apr. 2010, J. Malíček 2560 (hb. Malíček); Distr. Znojmo, Podyjí National Park, Hardegg, S-facing rock, 335 m, on limestone, 10 Aug. 2022, J. Malíček 15434 (hb. Malíček). **France.** Chaux de Crotenay (Jura), muur in 't dorp ri. Pont de Chaux.' 24 July 1986, A. J. de Bakker (L); 'Op beton van brug'. Auvergne, Cantal La Plaine Mary, 2 km NW of Puy Mary, 1140 m, 3 Aug. 1998, L. Spier 8877 (L-0752742); Corsica, North Corsica, Saint Florent, E, road D81, near the camping d' Olzo, calcareous outcrops with thin shrubby vegetation, on calcareous stones, 13 m, 7 Sep. 2022, J. Kocourková 10986 (hb. K&K). **Germany.** Baden-Württenberg, Lauterbachtal (Marbach), 24 Sep. 1989, H. Schöller 431 (FR-0264666). Sachsen-Anhalt, Unterharz, southeastern slope, 430–470 m, on limestone boulders in meadow, 22 July 2000, A. P. Dornes & G. Helms (FR 0262372). Rhineland-Pfalz, Oberrin-Ebe Mainz Mombach, Bagge 2234 (FR 58935); Hessen, Westl. Hintertaunus, Dörsheid cemetery wall, south side, 350 m, 24 Aug. 1991, H. Schöller 1.070 (FR). Rhein-Main lowland, S of Falkenberg, former sand pit, 10 Oct. 1994, H. Schöller 427 (FR 58870); Frankfurt, 1862, Metzler 2235 (FR 58934); Thüringen, Der Ringgau, 320–400 m, on limestones in old landslide, 7 May 1994, A. P. Dornes (FR). **Italy.** Abruzzi, Prov. L'Aquila, Sulmona, Eremo di M. Morrone, S-exposed calcareous rock, 500 m, 5 Apr. 1997, P. L. Nimis & M. Tretiach (TSB26898); Friuli-Venezia Giulia, Prov. Trieste, Santa Croce near Trieste, c. 90 m, immersed in limestone, 1 June 1985, P. L. Nimis (TSB5704); Prov. Udine, Prealpi Giulie, Alta del Torre, 700 m, on calcareous rock, 27 Sep. 1987, P. L. Nimis & M. Tretiach (TSB9826); Marche, Prov. Macerata, M.ti Sibillini, Bolognola, 1350 m, on limestone, 6 June 1999, M. Tretiach (TSB30979); Puglia, Prov. Bari, Spinazzola, SS 97 between Masseria Calderoni and Poggiorsini, 455 m, on cement wall, 7 Apr. 1996, P. L. Nimis & M. Tretiach (TSB22919); Prov. Foggia, Lago di Occhito below Cerenza, 245 m, 13 Apr. 1989, P. L.Nimis & M. Tretiach (TSB30071); Sardegna, Prov. Nuoro, Arcu Genna Cruxi, 1000 m, on calcareous rock, 3 Sep. 1989, P. L. Nimis, C. Roux, M. Tretiach, A. Vězda (TSB); **North Macedonia.** Vardear River Valley, Negotino-Krivolak,

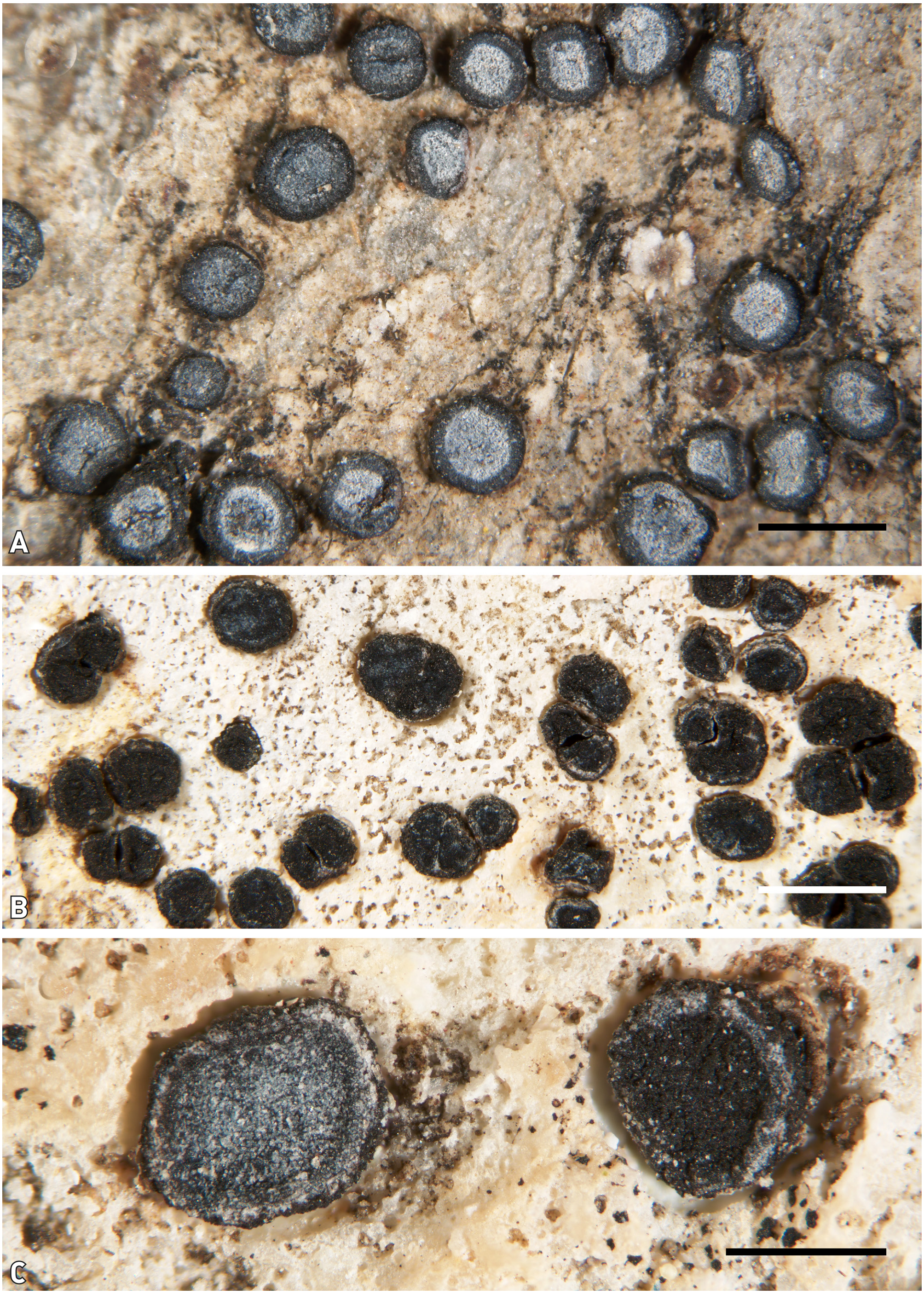

Fig. 2. *Sarcogyne pruinosa.* **A** – Nimis (TBS 33625); **B** & **C** – Nimis (TBS 29960) **A** – Pruinose apothecia. **B** – Non-pruinose apothecia. **C** – Apothecia in pits. Scales A, B = 1 mm, C = 500 µm.

Fig. 3. *Sarcogyne pruinosa*, **A** – Metzler 2235 (FR). **A** – Convex to hemisphaeric apothecia. **B** – Section of convex apothecium. Scales A=1 mm, B= 500 µm.

steppe grasslands at right bank of the river, NE from village, 120–160 m, on calcareous stone, 13 June 2014, J. Malíček 7995 (hb. Malíček); **Slovakia.** Považsky Inovec, Priešťany, Lúka, ruin of castle Tematín c. 5 km NE of village, 470 m, on calcite, 22 Apr. 2006, F. Bouda 157 (PRM 925578).

Sarcogyne coeruleonigrans K.Knudsen, Hodková & Kocourk., Western North American Naturalist 83 (2023). Page number unassigned at time of publication. See Mycobank.

Type: U.S.A. New Mexico: Otero Co., Sacramento Mountains, La Luz, near Highway 82, W of Tunnel Vista Observation Site, below dirt road parallel to the Steep Hill Rd, thin chaparral vegetation with *Juniperus* and *Opuntia*, 32.9514 -105.8815, 1729 m, on loose pebbles covered with thin limestone crusts, 15 March 2020, J. Kocourková 10625 & K. Knudsen (PRM! holotype, hb. K&K! isotypes).

Description. For full description and image see Knudsen et al. (2023). The most important character of *Sarcogyne coeruleonigrans* is its phenotypic variability. The apothecia vary from 0.2–0.5 mm in exposed locations in the lower Chihuahuan Desert like Bishop's Cap or the Tularosa basin to 1.2 mm wide in the pinyon juniper belt of the Brokedown Mountains and Carlsbad Caverns National Park, and at high elevations in Mexico. The width of the margin is (35–)50–90(–120) µm, the lower and higher widths depending on microhabitat conditions and size of apothecia. The largest apothecia become convex but do not exclude their margins. Using our protocol, IKI reactions of hymenial gel, a species level character, is usually stable, either euamyloid or hemiamyloid. The hymenial reactions of *S. coeruleonigrans* are unstable and can vary from dark to pale blue, or blue turning red, or red, and in squash preparations can be blue and red with red dominant or turn completely red. Thus it could be observed as being euamyloid with a narrow margin (for instance by Magnusson 1935a).

Ecology and distribution. On limestone and calcareous and calciferous sandstone in the Chihuahuan and Sonoran Desert in U.S.A. (Arizona, New Mexico) and Mexico (Garcia, Nuevo Leön, Satillo) at elevations from 130–2134 m. It is expected in the Guadalupe Mountains in Texas. It could extend into California, Nevada, and Utah in the Mojave and Great Basin Deserts as well as into Kansas or even Montana. Not reported yet from concrete.

Differentiation. See Knudsen et al. (2023).

Discussion. Magnusson's description of *Sarcogyne pruinosa* in North America was based on 13 specimens from North America, one from Mexico (Mexico, Aquacaliente; a Hasse collection from 1910, probably from near Palm Springs in California and not Mexico), and 12 from the United States (California, Colorado, Iowa, Michigan, New Mexico, Nebraska, Ohio, South Carolina, Vermont) (Magnusson 1935a). His description of *S. pruinosa* in America is not the same as his description of the species in Europe (Magnusson 1935a). He states that *S. pruinosa* in North America is not as variable as in Europe. The margin of *S. pruinosa* in Europe is "30–50(80–100)" µm wide (Magnusson 1935b) while the North American *pruinosa* has a 30–50 µm wide margin. He notes that one specimen from Iowa has a 50–100 µm wide margin. The North American taxon cannot be *S. pruinosa* because this species has a 30–110 µm wide margin. The American taxon has apothecia that only "finally become a little convex" and do not become hemispheric and exclude the margin. *Sarcogyne coeruleonigrans* can become convex but not hemispheric and does not exclude its margin. Magnusson also includes in his concept of *S. pruinosa* in North America *S. arenosa* (Herre) K.Knudsen & Standley, a common Californian species, which has been sequenced and is not *S. pruinosa* (Knudsen & Standley 2007, Fig. 1). *Sarcogyne coeruleonigrans* was probably at least the specimen Magnusson identified from New Mexico.

The concept of *Sarcogyne regularis* in Knudsen & Standley (2007) included *S. coeruleonigrans* but also included other undescribed taxa from the central California coast, from the San Bernadino Mountains and San Jacinto Mountains in southern California, and the White Mountains east of the Sierra Nevada. But it did not include *Sarcogyne arenosa*. It was a heterogenous circumscription of *Sarcogyne regularis* from a limited sampling of specimens from southwestern North America.

On calcareous rock at least four other undescribed taxa occur in North America based on our current tree of *Sarcogyne* (Kocourková lab, unpublished data) that could be identified as *S. regularis* using these earlier morphological species concepts of *S. pruinosa* (Magnusson 1935a, b) or *S. regularis* (Knudsen & Standley 2007). We do not recognize *S. pruinosa* as occurring in North America or identifications based on these treatments.

Sarcogyne platycarpoides Anzi Fig 4 a, b

Comm. Soc. crittog. Ital. 2(fasc. 1), 19 (1864); **type:** Italy, Lombardy, growing with *Lecidea zeoroides* on the ridge of Mt. Spluga on mica-schist; less abundant near Bormio (Ostèglio), M. Anzi. (TO, MBT 10011319, lectotype designated here).

≡*Sarcogyne pruinosa* var. *platycarpoides* (Anzi) H.Magn., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) **9**(5.1): 94 (1935), syn. nov.

=*Lecanora glaucocarpa* var. *melaniza* Nyl. ex Norrlin, Meddn Soc. Fauna Flora fenn. 2: 28 (1878); **type:** Russia, Republic of Karelia: Ruskeala, on marble, 1874, J. P. Norrlin (H-NYL248892—holotype!), syn. nov.

=*Acarospora glaucocarpa* f. *melaniza* (Nyl.) H.Magn., Göteborg. Vetensk.-och Vitter.-Handl., Ser. 4, 28 (no. 2): 92 (1924), syn. nov.

=*Sarcogyne melaniza* (Nyl.) K.Knudsen, Kocourk. & Hodková, Archive for Lichenology 32(2): 8 (2022), syn. nov.

=*Sarcogyne pruinosa* f. *macroloma* Flörke ex Körb., Parerga lichenol. (Breslau) 3: 236 (1861), **type:** Austria, Salzburg, Gaisberg (hb. Laurer, location of type unknown). For drawing of specimen from Switzerland see Magnusson (1935a), syn. nov.

=*Sarcogyne pruinosa* var. *macroloma* (Flörke) H.Magn., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 9(5.1): 93 (1935), syn. nov.

=*Acarospora cervina* form *pruinosa* Kremp. Denkschr. Kgl. Bayer. Bot. Ges., Abt. 2, 4: 172 (1861); **type:** Germany, Bavaria, Südbay. Alpen, Oberaudorf, on conglomerate rocks, A. von Kremphelhuber (M-0190212, MTB 10011328, lectotype designated here)

=*Acarospora glaucocarpa* form *pruinosa* (Kremp.) Arnold, Flora, Regensburg 67: 314 (1884), syn. nov.

Description. Thallus endolithic, algae usually in clusters in substrate or at base of apothecium, or rarely in lower part of margin forming pockets of algae between the hypothecium and the outer surface, **algae** 8–12 µm wide. **Apothecia** 0.5–2.0 mm wide, mostly 0.2–0.4 mm high, broadly attached when small, eventually forming a mycelial base narrower than the apothecia. **Disc** usually white pruinose, reddish brown when wetted, rarely partially pruinose to epruinose, usually flat in appearance, sometimes convex. **Margin** black, epruinose or lightly pruinose. **Parathecium** 90–150 µm wide, of radiating hyphae 1 µm wide, melanized outer layer 60–100 µm thick, inner layer hyaline, widths variable. **Hymenium** (100–)140–150 µm tall, **epihymenium** 10 µm or less tall, reddish brown, paraphyses mostly 2 µm wide at mid-level, apices barely expanded, hymenial gel IKI+ dark blue, euamyloid. **Asci** 100–120 × 10–20 µm, **ascospores** ellipsoid to narrow ellipsoid, 3.0–5.0 × 1.0–1.5 µm. **Subhymenium** 40–60 µm tall, IKI+ dark blue. **Hypothecium** up to 60 µm thick, inspersed with substrate crystals, continuous with attaching hyphae. **Pycnidia** not observed. No secondary metabolites detected (Knudsen et al. 2022).

Ecology and distribution. In sun or shade, usually in alpine zone, but as low as 290 m in Czech Republic, on mica-schist and calcareous rock and pebbles (Austria, Czech Republic, Germany, Italy, Norway, western Russia near Finland, Switzerland) (Magnusson 1935b, Knudsen et al. 2022).

Differentiation. *Sarcogyne platycarpoides* has been recovered as a distinct species in several published phylogenies as *A. glaucocarpa* s. lat., form or variety *melaniza*, or *Sarcogyne melaniza* (Westberg et al. 2015a, Knudsen et al. 2020, 2021a, 2023).

Though often misidentified as *S. regularis* and *S. pruinosa*, *S. platycarpoides* differs from *S. pruinosa* in having a wider margin (100–150 vs. 30–110 µm) and usually a higher hymenium (100–)140–150 vs. 90–110(–130) µm high. Both species can have convex apothecia. Though sometimes containing algae, it differs from other specimens of *Acarospora glaucocarpa* s. lato in not having a thick algal layer beneath the hymenium and in the upper margin area.

Discussion. Magnusson (1935b) determined *Sarcogyne platycarpoides* when it lacked algae in the apothecia as *Sarcogyne pruinosa* var. *platycarpoides* or *S. pruinosa* var. *macroloma.* When it had algae in the apothecia, Magnusson determined specimens of *S. platycarpoides* as *A. glaucocarpa* f. *melaniza.*

Apothecia of some *Sarcogyne* replicate by division forming clusters, like *Sarcogyne californica* H. Magn. (Knudsen 2022). Replication by division was not seen in specimens of *S. pruinosa*. The apothecia emerged one by one from the endolithic thallus. Though sometimes apothecia were close together without evidence of replication of division. The specimen identified by C. Roux as *S. regularis* var. *macroloma* (Flörke ex Körb.) Golubkova, nom. illegit., is replicating by division and it is not related to *S. pruinosa* or *S. regularis* nor does it represent Magnusson's concept of *macroloma*, which is a synonym for *Sarcogyne platycarpoides* (see drawing in Magnusson 1935b, Roux et al. 2019).

Specimens examined. Czech Republic. Mimoň, Hradčany, 'Hradčanské stěny", 290–350 m a.s.l., on calcareous sandstone, 27 July 2009, J. Malíček 2240 & Z. Palice (hb. Malíček); Western Bohemia: distr, Plzeň, Nečtiny, area of church with cemetery at E. edge of village, 485 m, on monument, 11 Apr. 2014, J. Malíček 7624 (hb. Malíček). **Germany.** Bavaria, (unreadable) nach Hochland, on calcareous rock, 20 July 1941, L. Laven (BONN). **Italy.** Piemonte, Alpi Liguri, Prov. Cuneo, 2200 m, on calcareous rock, 20 July 2000, P. L. Nimis, M. Tretiach & J. Hafellner (TSB39786); Regione Veneto, Prov. Belluno-Dolomiti, Tre Cime di Laredo, dintormi del Rifugio Auronzo, 2300–2400 m, on dolomite, 31 Aug. 1994, P. L. Nimis (TSB15694). **Norway.** Nord-Trøndelag: Snåsa, Bergsåsen, 150 m, on calcareous rock in open pine forest, 6 Aug. 2015, M. Westberg (SBBG).

Sarcogyne belarusensis K.Knudsen, Tsurykau, Kocourk. & Hodková, sp. nov. Fig. 4, c

MB#847325

Holotype: Belarus, Vitebsk region, Polotsk distr., 0.5 NNW of Gomel village, 55°17'N/28°46'E, 170 m, on moss-covered pill box, 29 Aug. 1989, V. Golubkov (GSU, holotype; PRM, isotype).

Diagnosis: Similar to *S. pruinosa* but with hemiamyloid vs. euamyloid hymenial gel.

Etymology. Named for the type area in Belarus.

Description. Thallus endolithic, algal cells in substrate, sometimes below apothecia. **Apothecia** lecideine, round, 0.2–0.5–0.7(–1.0) mm wide, 0.2–0.4 mm thick, broadly attached, immersed or not, dispersed. **Disc** black to dark brown, pruinose, rarely epruinose, slightly lower than the margin, becoming convex but not excluding margin. **Margin** usually smooth, but sometimes becoming rough with build-up of melanin. **Parathecium** 40–100 µm wide, outer layer black, about half the width, inner layer hyaline, distinguished from adjoining hymenium by the black apices of hyphae and the IKI- reaction. **Hymenium** (80–)90–120 µm tall, **epihymenium** reddish-brown c. 15 µm tall, **paraphyses** 1–1.5 µm wide, apices unexpanded, sometimes in gel cap 2(–4) µm wide, hymenial gel IKI+ dark or pale blue in sections, quickly turning brownish-red or red when squashed. **Asci** 10–22 × 90–100 µm, **ascospores** looking globose in asci, ascospores (3.0–)3.7–4.2–4.7(–5.5) × (1.5–)1.7–2.0–2.2(–2.5) µm, l/b=2.1 (n=80). **Subhymenium** IKI+ blue, 10 µm high. **Hypothecium** indistinct from the medulla, *c.* 100 µm thick, hyphae branching, 1 µm thick, obscure with crystals or not, IKI-, continuous with endolithic thallus. **Pycnidia** not seen. No secondary metabolites detected.

Ecology and distribution. Currently known from Belarus, Germany and Italy on small calcareous stones and concrete, one specimen from calcareous conglomerate sandstone, at low elevations <300 m.

Differentiation. *Sarcogyne belarusensis* is a member of the *Sarcogyne nivea* clade. They all differ from *S. pruinosa* especially in having hemiamyloid vs. euamyloid hymenial gel. *Sarcogyne belarusensis* differs from *S. nivea* and *S. distinguenda* in not having a thallus. *Sarcogyne fallax* has usually a higher hymenium with globose to broadly ellipsoid ascospores.

Discussion. When we were describing *S. belarusensis*, we were speculating it was an eastern European species possibly extending across Russia into northern Asia. We were influenced by the rarity of *S. pruinosa*. We were surprised to find *S. belarusensis* in loans from FR and TSB from Germany and Italy. Calcareous rock is rare in Belarus and the most common rock type are granite boulders deposited by Pleistocene glaciers (TSURYKAU 2018). This is a frequent species in Belarus and occurs on both anthropogenic concrete and often small stones of a conglomerate HCl+ sandstone. It is probably best considered a pioneer species. It may be widespread in Europe like another pioneer species, *A. fusca* B.de Lesd. (syn. *A. anomala* H.Magn.) that grows on wood and often on small silicate stones, and which also occurs in Belarus as well as in Estonia (KNUDSEN et al. 2021c, SUIJA et al. 2021).

Specimens examined. Belarus. Grodno Region, Mosty district, 0.5 km SW of Lunno village, on concrete, 27 June 1994, V. Golubkov (PRM, GSU, MSK); Schuchin district, close to Kostenevo village on stone, 04 July 1999, V. Golubkov (PRM, GSU, MSK); Volkovyssk district, close to Krasnoselsk village, on concrete, 24 July 2014, A. I. Leonova (SBBG, MSK); Vitebsk region, Dokshytsy district, Berezinsky Biosphere Reserve, 1.8 km NNW of Osetische village, on calcareous stone, 13 June 2007, P. Bely (PRM, MSKH 6555); Lepel district, 1.5 km NE of Borovka village, 54°58'N,/28°50'E, on concrete, 19 may 2010, P. Bely (MSKH 6554, GSU); Verhnedvinsk district, on island in Osveja lake close to Sukali village, 13 June 1986, V. Golubkov (MSK-L); Vitebsk district, 2 km SW of Ruba village, alt. c. 170 m, on sandstone, 15 June 1982, G.V. Vynaev (SBBG, GSU, MSK). **Germany.** Rheinland-Pfalz, Kaiserslauterner Vally, Kaiserslautern; University campus, chemical storage building, 270 m, on calcareous stones on a roof, 15 Nov. 1998, A. P. Dornes (FR). **Italy.** Friuli Venezia Giulia, S. Foca, in magredi near Meduna, 150 m, on limestone pebbles, 18 Oct. 1989, M. Tretiach (TSB13966); Sardinia, Prov. Sassari, Nuraghe Santu Antine (ancient megalith), 350 m, on wall of asbestos, May 1986, P. L. Nimis (TSB7499).

Sarcogyne nimisii K.Knudsen, Kocourk. & Hodková, sp. nov. Fig. 4, d

MB#847322.

Holotype: Italy, Friuli Venezia Giulia, Prov. Trieste, coast near Contovello/Kontovel, 13.734°N/ 45.702°E, 230 m, on calcareous rock, 13 May 2002, P. L. Nimis (TSB, holotype).

Diagnosis: Similar to *Sarcogyne platycarpoides* but differing in having both a densely pruinose white margin without melanin, hemiamyloid vs. euamyloid hymenial gel, ascospores broadly ellipsoid 4 × 3 µm to ellipsoid 3–4 × 2 µm vs. 3.0–5.0 × 1.0–1.5 µm, and with a coastal vs. a predominantly alpine distribution.

Etymology. Named for the Italian lichenologist P. L. Nimis of Trieste. He collected the holotype.

Description. Thallus endolithic to chasmolithic, holding together upper surface of rock with densely intricate, mostly 2 µm wide hyphae, forming pale curds of hyphae and crystals, with an associated cyanobacteria with packages of 2–12 cells 1–2 µm wide, green in colorless sheath. Lichenized algal cells rare, not near surface, between apothecia. **Apothecia** round, dispersed, broadly attached, not replicating by division, 0.2–0.8 µm wide. **Disc** convex or level with margin, densely pruinose, red-brown when wetted. **Margin** densely pruinose, white (dark brown when wetted), not incised or rarely scalloped. **Parathecium** formed of radiating hyphae, 1–2 µm wide, apices unexpanded, reddish-brown, inspersed with crystals, (60–)80–120 µm wide. **Hymenium** 120–130(–140) µm high, **epihymenium** <10 µm wide reddish-brown, paraphyses c. 1 µm wide, apices unexpanded, hymenial gel IKI+ red. **Asci** 90–100 × 10–17 µm, ascospores broadly ellipsoid 4 × 3 µm or ellipsoid 3–4 × 2 µm (n=10). **Subhymenium** indistinct, c. 20 µm tall, IKI+ light blue fading to light red. **Hypothecium** merged with medulla, obscure with crystals, hyphae 1–2 µm wide. **Pycnidia** not observed. No secondary metabolites detected.

Ecology and distribution. Known only on calcareous rock from the coast of northeastern Italy with no accompanying lichens.

Differentiation. The mostly likely taxon it can be confused with is the calciphyte *Acarospora glaucocarpa* form *distans* Arnold which has solitary apothecia with a densely pruinose non-carbonized margin and lightly pruinose disc and was reported from Italy (BAGLIETTO & CARESTIA 1880). It is an alpine species and differs especially from *S. nimisii* in having apothecia turning orange when wetted and euamyloid hymenial gel. *Sarcogyne nimisii* with its densely pruinose margin and disc as well as lack of carbonized margin can be easily distinguished from other species in this paper.

In the North American group in our tree, *Sarcogyne magnussonii* and *S. malpaiensis* differ in having carbonized margins and *S. magnussonii* also has a farinose epilithic thallus (KNUDSEN & KOCOURKOVÁ 2012, KNUDSEN et al. 2023). *Sarcogyne arenosa* has non-melanized margins (brown to black) and differs from *S. nimisii* in having apothecia often with a subtending white ring of medullary hyphae and can produce an epilithic or chasmolithic thallus (KNUDSEN & STANLEY 2007).

Fig. 4. *Sarcogyne platycarpoides*, Anzi exiccat. 350 (FR 58946). **A** – Apothecia replicating by division. **B** – Slightly rough pruinose apothecia. **C** – *Sarcogyne belarusensis*, Bely s.n. (PRM, paratype). **D** – *Sarcogyne nimisii*, Nimis (TBS 29960, holotype). Scales A–D = 1 mm.

Species misdetermined as *S. pruinosa.* It is our experience some specimens were not checked for hymenium height and in some cases the epilithic or chasmolithic thallus was overlooked, especially in soft limestone.

Sarcogyne distinguenda Th.Fr.

Bot. Notiser: 153 (1867).

Description. For full description see Magnusson (1935b). Thallus thin, farinose, 40–80 µm thick. Apothecia 0.4–0.5 mm wide. Margin 100–150 µm wide. Hymenium 110–180 µm high, hymenial gel hemiamyloid. Ascospores globose to ovoid, 3–4.5 × 3–3.5 µm. Known from Austria, Germany, Norway, Sweden, and Switzerland. Reported new from Czech Republic and Italy.

First reports of *Sarcogyne distinguenda.* Czech Republic. Central Bohemia, distr. Kutná Hora, Kutná Hora, Kaňk National Nature Monument, old quarries and cherry orchard, 275–295 m, on calcareous rock, 23 Aug. 2016, J. Malíček 9627 (hb. Malíček). **Italy.** Friuli Venezia Giulia, Trieste, Trieste Karst, Loc. Conconello, on rough calcareous rock, 1978, P. L. Nimis 446 (TSB446).

Sarcogyne nivea Kremp.

Denkschr. Kgl. Bayer. Bot. Ges., Abt. 2.4: 212 (1861).

Description. For full description see Magnusson (1935b). *Sarcogyne nivea* has apothecia 0.3–1.0 mm wide with a hymenium usually about 100 µm high and hemiamyloid hymenial gel. Margin (50–)100–120 µm wide. Ascospores variable from globose to ellipsoid, 3–5 × (1–)2–3 µm. The thallus can be farinose and white on soft limestone. This can be hard to see and one should scratch surface to check for hyphae and lichenized clusters of algae. On hard limestone it can have a non-farinose thallus in dull shades of brown. Known from Germany and France. Reported new from Czech Republic and Italy.

Discussion. The description of this species was based on a 1 cm wide holotype. It looks like *S. pruinosa* which also can have a farinose or non-farinose thallus but differs especially in hymenial gel IKI+ blue turning dirty brown to red (hemiamyloid).

First reports of *Sarcogyne nivea.* Czech Republic. Eastern Bohemia, distr. Náchod, Broumovsko Protected Landscape Area, Police nad Metují, Česká Metuje: Pískovcové sloupy Nature Monument, ENE-facing slope with scree forest and rocky outcrops, 440–470 m, on calcareous rock (opoka), 30 Sep. 2018, J. Malíček 12679 (hb. Malíček); Northern Bohemia: distr. Liberec, Karlov pod Ještědem, Karlovské bučiny National Nature reserve, natural beech forest in S part of protected area, 550 m, on small stone of limestone, 9 June 2021, J. Malíček 14530 (hb. Malíček). **Italy.** Basilicata Potenza: Acqua dei Pastori below Santuario di Madonna di Viggiano, growing with a small patch of *Sarcogyne pruinosa* on calcareous rock, 1996, P. L. Nimis & Tretiach (TSB21956). Friuli Venezia Giulia: Prov. Pordenone, Maniago, on calcareous rock, 280 m, 26 June 1985, (unknown) (TSB5943).

Sarcogyne fallax H.Magn.

Rabenh. Krypt.-Fl., Edn 2 (Leipzig), 9(5.1): 57 (1935).

Description. For full description see Magnusson (1935b). The species looks like *Sarcogyne pruinosa* with apothecia 0.7–1.0 mm wide. It has a carbonized margin 100–150 µm thick, a hymenium 120–150 µm high, usually around 140 µm. The paraphyses are 1–1.5 µm with apices in gel caps with dark pigment mark 2–3 µm wide. Ascospores are globose 3–4 µm wide, or broadly ellipsoid 4–5 × 3–4 µm. The diagnostic characters are an endolithic thallus, a high hymenium with IKI+ hemiamyloid hymenial gel, and globose to broadly ellipsoid ascospores. Known from Germany, France, Italy, Portugal, Slovenia, and Switzerland. Reported new from Czech Republic.

Discussion. The species is probably overlooked because it is similar to *Sarcogyne pruinosa*. The holotype was from Italy (Magnusson 1935a). It was probably lost in WW2 when B. de Lesdain's herbarium was destroyed in the bombing of Dunkirk.

Sarcogyne nimisii has short broadly ellipsoid ascospores like *S. fallax* but they can become ellipsoid and no globose ascospores were observed. It differs from *S. fallax* especially in having a non-melanized margin beneath a heavy white layer of pruina vs. a carbonized margin with or without pruina.

First report of *S. fallax.* Czech Republic. Bohemia septentrionalis, České středohoří, Liběchov, 210 m, on HCl+ rock, 1932, J. Podzimek s.n. (det. *S. pruinosa* v. *intermedia* by Servít) (PRM).

A key to *Sarcogyne* on calcareous rock in Europe

See Knudsen & Kocourková (2018a) for our protocol for IKI reactions of hymenial gel. The test is done on thin sections of apothecia, which is then squashed. All species could have a thin epilithic thallus on very hard rock or when the upper surface of rock is eroding exposing the endolithic thallus. A good example is *Sarcogyne hypophaea* (Nyl.) Arnold which on hard slate can form a thin gray epilithic thallus (Knudsen et al. 2021b). With the exception of *S. pruinosa*, species in our key with an epilithic thallus always have one (Magnusson 1935a).

1 Species with epilithic thallus 2
1* Species without an epilithic thallus 7

2 Hypothecium dark, Italy .... ***Sarcogyne coronata*** (Nimis et al. 2018)
2* Hypothecium not dark 3

3 Epilithic thallus thick (often 0.5–1 mm), Europe .... ***Sarcogyne cretacea*** (see Poelt 1964, photograph in Knudsen et al. 2016, Nimis et al. 2018).
3* Epilithic thallus thin (40 – 500 µm) 4

4 Hymenium 85–130 µm high, ascospores broadly ellipsoid, mostly 7×3 µm, Africa, Spain .... ***Sarcogyne algerica*** (Knudsen & Etayo 2009)
4* Hymenium variable in height, ascospores globose to ellipsoid, less than 7×3 µm 5

5 Hymenial gel IKI+ dark blue, euamyloid .... ***Sarcogyne pruinosa***
5* Hymenial gel IKI+ blue to red or red, hemiamyloid 6

6 Hymenium 85–110 µm high, ascospores globose to broadly ellipsoid, 3–3.5 µm to 4 × 3 µm, not ovoid, Europe .... ***Sarcogyne nivea*** (Magnusson 1935b)
6* Hymenium 110–180 µm high, ascospores globose to broadly ellipsoid, 3.0–4.5 × 3.0–3.5 µm, often ovoid, Europe .... ***Sarcogyne distinguenda*** (Magnusson 1935b)

7 Ascospores globose to 4 × 3 µm .... ***Sarcogyne fallax*** (Magnusson 1935b)
7* Ascospores not globose, sizes variable 8

8 Margin segmented 9
8* Margin not segmented 10

9 Various elevations on calcareous and non-calcareous rock, apothecia angular .... ***Sarcogyne hypophaea*** (Knudsen & Kocourková 2011, Knudsen et al. 2013)
9* Alpine on calcareous schist, apothecia round .... ***Sarcogyne algoviae*** (Westberg et al. 2015b)

10 Hymenial gel IKI+ pale blue to red, or red (hemiamyloid) 11
10* Hymenial gel IKI+ dark blue (euamyloid) 12

11 Margin 30–100 µm wide, disc usually pruinose, margin epruinose or lightly pruinose, not white, melanized .... ***Sarcogyne belarusensis***
11* Margin 120–130(–140) µm wide, disc and margin densely pruinose, white, not melanized .... ***Sarcogyne nimisii***

12 Hymenium <90 µm high, margin curling inward above disc .... ***Sarcogyne praetermissa*** (Knudsen & Kocourková 2018)
12* Hymenium >90 µm high, margin not curling inward over the disc 13

13 Apothecia 0.3–1.5 mm wide, disc convex or not, rarely hemisphaeric and excluding the margin 30–110 µm wide, algae never in apothecia .... ***Sarcogyne pruinosa***

13* Apothecia (0.5–)1–2 mm wide, disc occasionally convex (usually absent), not hemisphaeric excluding the margin, 100–150 µm wide, algae occasionally in apothecia .... ***Sarcogyne platycarpoides***

Acknowledgments

We thank for their help our reviewers and Andreas Beck (M), Konstanz Bensch (Mycobank), František Bouda (PRM), M. Heklau (STU), P. L. Nimis (TBS), H. Thüs (STU) and Christian Printzen (FR). Holger Thüs made a cross-section and Martin Heklau provided photographs of that cross-section of the type material of *S. pruinosa* from STU. We thank Vladimir Golubkov (Grodno) and Pavel Bely (Minsk) for providing us with their collections. Jiří Malíček has been supported by the long-term research development project RVO 67985939. The work of Kerry Knudsen, Jana Kocourková, and Eva Hodková was financially supported by the grant of Ministry of Education, Youth and Sports of the Czech Republic, the program of international cooperation between the Czech Republic and U.S.A. for research, development and innovations INTEREXCELLENCE, INTER-ACTION, no. LTAUSA18188.

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Manuscript accepted: 24 April 2023.

Communicated by: Silke Werth

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