

A new lichen species, *Scoliciosporum micareae* (lichenized Ascomycota, Scoliciosporaceae), occurring in western Europe

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Abstract

Scoliciosporum micareae sp. nov. is described as new to science. The study is based on the DNA sequence of the mitochondrial ribosomal locus, as well as morpho-anatomical and chemical analyses. This species is distinguishable from others in the genus by its well-defined thallus warts or granules of 0.03–0.2 mm wide, and slightly curved, (1–)3-septate ascospores of 15–20(–22) × (2.0–)2.5–3.0 μm. So far, this new species is known to grow on tree trunks in Belgium, Germany and the Netherlands.

Key words: Biodiversity, Ecology, Lecanorales, Taxonomy,

Introduction

An extensive inventory of lichens and lichenicolous fungi in the area of Eindhoven and surrounds (The Netherlands), led to the discovery of several new novelties (van den Boom 2004, 2015, van den Boom & van den Boom 2009, van den Boom & Alvarado 2021, Suija & van den Boom 2023). During the starting point, in the eighties, many specimens of *Scoliciosporum* (Scoliciosporaceae) has been collected, including from the very common *Scoliciosporum umbrinum* (Ach.) Arnold (Arnold 1871:50), the two rare species *S. chlorococcum* (Graewe ex Stenh.) Vězda (Vězda 1978:414) and *S. galluræ* Vězda & Poelt (Nimis & Poelt 1987:221). One species deviated from these known species was collected

several times, even in Belgium it was collected a few times. In that country even further *Scoliciosporum* species, *S. curvatum* Sérus. (1993:458), *S. pruinosum* (P. James) Vězda (1978:414), and *S. sarothamni* (Vain.) Vězda (1978:411) are known. The only known German collection from Brandenburg suggests that the new species must have a rather wide distribution area. At the type locality of the new species, it grows on a trunk of *Quercus robur*, close to young *Picea*, on which has been found *Fellhanera bouteillei* (Desm.) Vězda growing exceptionally on needles and twigs (Fig.1).



Fig. 1. Type locality with *Quercus robur* and young *Picea*

Materials and methods

Morphological and anatomical analysis

Hand-cut apothecial sections and squashed thallus preparations were examined with a compound microscope (Olympus BX2). Ascospores and other anatomical details were studied and measured in water or in 10% potassium hydroxide (K) if features were otherwise unseparated. In each collection (~5 well-developed ascospores representing the observed variation of size and shape), conidia and paraphyses were measured to 0.5 μm accuracy. Chemical spot tests were performed under a compound microscope using sodium hypochlorite

**A new lichen species, *Scoliciosporum micareae* (lichenized Ascomycota,
Scoliciosporaceae), occurring in western Europe**

(C) and K (Orange *et al.* 2001). Pigments were determined following the system of Meyer & Printzen (2000). The crystals from all studied specimens were investigated in tissue sections by using a compound microscope with polarization filters.

DNA extraction and sequencing

Extraction of DNA from several specimens including van den Boom 61312 followed the protocol of Cubero *et al.* (1999). A PCR was performed to amplify the mitochondrial ribosomal locus mtSSU, using primers SSU1 and SSU3R (Zoller *et al.* 1999), following PCR conditions described in Zoller *et al.* (1999), using GoTaq G2 (Promega, Leiden, The Netherlands). PCR products were cleaned using EXOCLEANUP FAST (Vwr International, Radnor, PA, USA). Sanger sequencing was performed at MacroGenEurope (Amsterdam, The Netherlands).

Phylogenetic analysis

A BLASTn search (Wheeler *et al.* 2007) was conducted on the GenBank website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify potential sequences of high similarity. All available mtSSU sequences from *Scoliciosporum* were downloaded from GenBank, for a total of 12 sequences. Twelve additional sequences from Lecanorales were downloaded. Sequences were aligned with MAFFT version 7.453 (Katoh and Standley 2013). The alignment was adjusted manually and ambiguous regions were delimited using Mesquite version 3.6 (Maddison & Maddison 2015). The final matrix consisted of 25 sequences and 665 characters (Table 1). A ML search was performed using RAxML-HPC2 version 8.2.12 (Stamatakis 2006, Stamatakis *et al.* 2008) as implemented on the CIPRES portal (Miller *et al.* 2010), with the GTRGAMMA model, looking for the best ML tree and performing 1000 bootstrap pseudoreplicates within the same run. Rooting of the tree followed Miadlikowska *et al.* (2014).

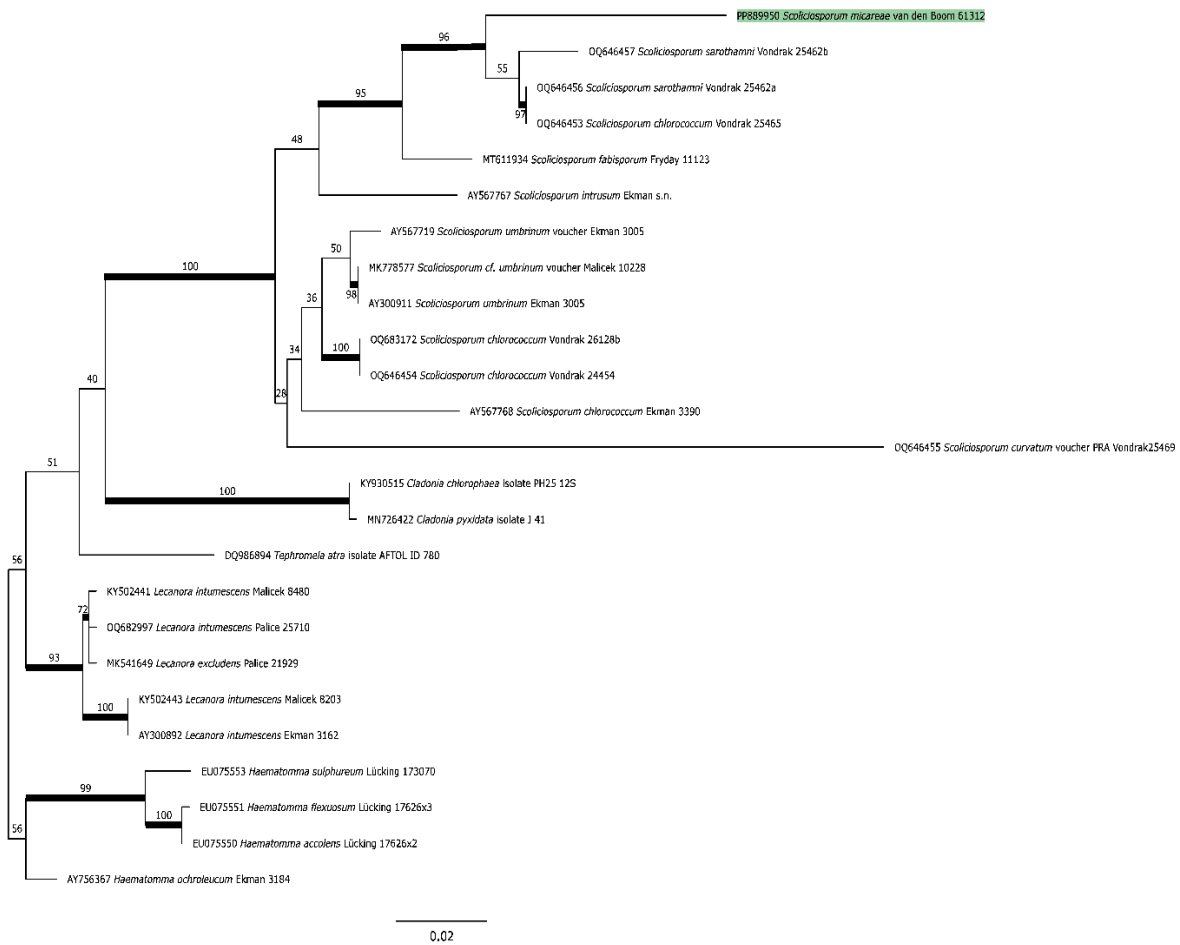


Fig. 2. Best ML tree resulted from the RaXML analysis on mtSSU sequences of representatives of Lecanorales showing the position of *Scoliciosporum micareae* highlighted in green. Values above branch represent bootstrap support, and thick branches have bootstrap support superior or equal to 70. Rooting follows Miadlikowska et al. (2014).

Results

A mtSSU sequence was recovered from a specimen of *Scoliciosporum micareae* (van den Boom 61312). Sequencing attempts on other specimens failed. The sequence was of moderate quality but still exploitable for phylogenetic analyses. The best ML tree recovered the genus *Scoliciosporum* as monophyletic with high support (BS=100), and the sequence of *Scoliciosporum micareae* is unambiguously nested within this clade, confirming it belongs to *Scoliciosporum*. It is not highly similar to any sequence on GenBank (max similarity 85.7% to *Scoliciosporum sarothamni* OQ646456). It forms a well-supported clade with sequences of

A new lichen species, *Scoliciosporum micareae* (lichenized Ascomycota,
Scoliciosporaceae), occurring in western Europe

Scoliciosporum sarothamni (BS=96, Figure 3). Relationships within families, as well as most relationships within the genus *Scoliciosporum*, were not strongly supported by our analysis.

Taxonomy, ecology

Scoliciosporum micareae van den Boom, E. Lebreton & Magain sp. nov. (Fig. 3,4) MycoBank No.: MB854433

Diagnose: Thallus containing warts or granules, without chemical compounds.

Apothecia subglobose, often shiny, ascospores often slightly curved (falcate), (1–)3-septate, mesopycnidia and micropycnidia present.

Type:—NETHERLANDS, prov. Noord Brabant, SW of Eersel, SW of Cartierheide, Kempen, Bladel, edge of small *Calluna* heathland with some *Betula*, *Quercus robur* and dead standing trunk, grid ref.: 57-12-51, 51°19'N, 5°15'E, 1 April 2020, P. & B. van den Boom 61476 (holotype LG!, isotype hb. van den Boom!). Genbank Accession: PP889950.



Fig. 3. *Scoliciosporum micareae*, habitus, warted thallus and apothecia. Scale = 0.2 mm.

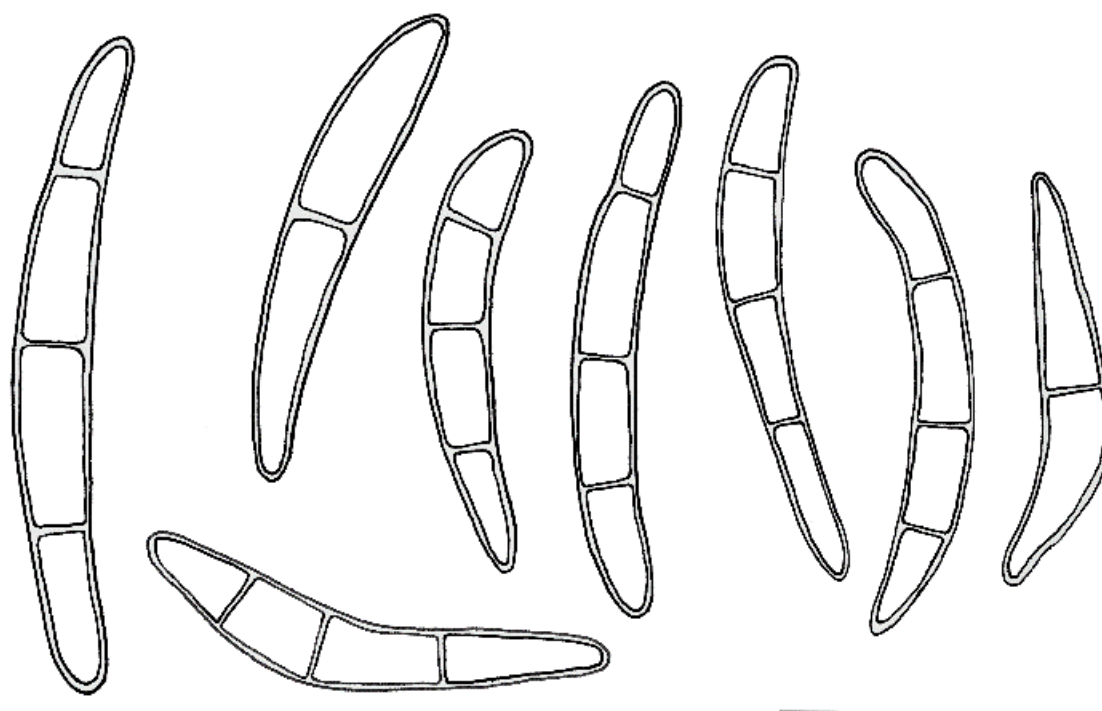


Fig. 4. Ascospores of *Scoliciosporum micareae*. Scale = 10 μm .

Thallus corticolous, crustose, greyish to greenish or pale brownish, sometimes irregularly cracked areolate, rather variable, consisting of smaller warts or granules (0.03–0.2 mm wide), roundish to irregular, often coalesce, ecorticate, upper part without crystals; algae chlorococcoid, 6–15 μm diam. Prothallus not detected. **Apothecia** often abundantly present, to c. 0.15–0.20 mm wide, at first slightly immersed in thallus warts, becoming sessile, without a margin, strongly convex, seemingly micareoid, pale to dark reddish brown, to brownish or blackish, never real black, often shiny. **Hymenium** 25–45 μm ; **excipulum** (parathecium) without algae, with interwoven thin hyphae; **paraphyses** abundantly present, c. 1.5–2 μm wide (in water), towards the top densely branched and clearly sinuous; **epithecium** violacea grey (K+ violet); **hypotheicum** hyaline. **Asci** *Lecanora*-type, slightly clavate, 25–35 \times 12–15 μm , 8-spored. **Ascospores** straight to slightly curved (falcate), 15–20(–22) \times (2.0–)2.5–3.0 μm , (1–)3-septate. **Mesopycnidia** sometimes present, usually very inconspicuous, immersed, 30–60 μm wide, hyaline, or brownish pigmented at the top; **mesoconidia** c. 5–6 \times 2–2.5 μm .

Micropycnidia rarely present, usually very inconspicuous, immersed, 30–40 μm wide, hyaline, or brownish pigmented at the top; **microconidia** c. 3–4.5 \times 1–1.5 μm . **Macropycnidia** not

observed.

Chemistry:—no chemicals compound detected by TLC.

Etymology:—The epithet refers to the similarity in habitus with a *Micarea* species.

Ecology:—mainly on trunks of young or medium size *Quercus robur* trees in rather open places in forests, or at forest edges. It has been found in *Quercus* or *Pinus* forests or in mixed forests with *Betula*, *Larix*, *Picea* etc. Belgian specimens are from *Fraxinus* and *Salix*. In Germany it is associated with *Populus*. Accompanying species in the type locality are *Coenogonium pineti* (Schrad. ex Ach.) Lücking & Lumbsch and *Lepraria finkii* (B. de Lesd.) R.C. Harris, growing on the same tree, very close to the new species. The neighbouring tree, an young *Picea*, hosted a very special lichen community, with *Fellhanera bouteillei* abundantly present, with many apothecia on needles and twigs, *Byssoloma diderichii* Sérus. was also present on needles and *Fellhanera viridisoediata* Aptroot, A.M. Brand & Spier and *Fellhaneropsis myrtillicola* (Erichsen) Sérus. & Coppins, on twigs only. That spot counted more than 40 lichens and lichenicolous fungi. In one collection (61473) *Micarea micrococca* could be found. The most important Belgian spot (van den Boom 18891) hosted the recently described *Catinaria occidentalis* van den Boom, together with *Chaenotheca stemonea* (Ach.) Müll.Arg. and *Opegrapha zonata* Körb. In the area of the German record was found the rare *Arthonia coronata* Etayo.

Remarks:—The thallus warts or granules in this species has never been found in the genus *Scoliciosporum*. In most species thallus is not well definite, it is usually not well developed and looks as from green algae or sorediate-like. Ascospores in the new species are rather different from other species in the genus, mostly slightly curved, 3-septate (falcate) and attenuated ends.

The new species is most similar to the following species.

Scoliciosporum sarothamni (Vain) Vězda, according to sequencing, this species is close to the new species, but it is sorediate, has gyrophoric acid and the ascospores are spirally arranged in the ascus.

Scoliciosporum gallurae Vězda & Poelt has a leprose, green thallus and wider ascospores of 2.5–3.5(–4) μm , apothecia are much less abundantly present and slightly immersed.

Scoliciosporum perpusillum J. Lahm ex Körb. has grey to greenish sorediate thallus, ascospores 1.5–2 μm wide.

A new lichen species, *Scoliciosporum micareae* (lichenized Ascomycota,
Scoliciosporaceae), occurring in western Europe

Specimens examined:—**Netherlands**, prov. Noord Brabant, W of Eersel, Kempen, just S of De Goorloop, N rim along field on *Quercus robur* trees, grid ref. 57-21-13, 5°14'N, 51°18.5'E, 35 m, 29-4-2020, P. & B. van den Boom, 59758, 59763 (hb. v.d. Boom); W of Eersel, Kempen, just N edge of De Goorloop, along trail, small group, of *Quercus robur*, on *Q. robur*, grid ref. 57-11-55, 51°19.5'N, 5°13.9'E, 35 m, 6-5-2020, P. & B. van den Boom 59776 (hb. v.d. Boom); W of Eersel, Kempen, S edge of reserve De Goorloop, a very small with *Betula*, *Quercus robur* trees, including a few dead standing trees, grid. ref. 57-21-15, 51°15.0'N, 5°19.3'E, 35 m, 6-5-2020, P. & B. van den Boom 59769 (hb. v.d. Boom); SW of Eersel, SW of Cartierheide, Kempen, Bladel, edge of small *Calluna* heathland with some *Betula*, *Quercus robur* and dead standing trunk, grid ref. 57-12-51, 51°19.5'N, 5°15.1'E, 35 m, 1-4-2020, P. & B. van den Boom 59681 (hb. v.d. Boom); SW of Eersel, SW of Cartierheide, Kempen, mixed forest with young *Picea*, young *Larix* and medium size *Quercus robur*, grid 57-12-51, 51°19.3'N, 5°15.0'E, 35 m, 1-4-2020, P. & B. van den Boom 59681 (hb. v.d. Boom); NE of Budel, S of Cranendonck, between Cranendonckse bos and Rister, mixed forest, with several fallen trees, grid ref. 57-26-35, 51°17.7'N, 5°35.5'E, 30 m, 27-10-2021, P. & B. van den Boom 60916 (hb. v.d. Boom); SW of Eersel, W of Cartierheide, Hapertse Heide (W), west side of *Calluna* heathland along trail along forest, some medium size *Quercus robur* trees, on *Q. robur*, grid ref. 57-12-41, 51°19.9'N, 5°15.2'E, 35 m, 27-10-2022, P. & B. van den Boom 61473 (hb. v.d. Boom); N of De Barrière, N of road to Bergeijk, edge of *Pinus* forest with some young *Quercus* trees, on *Q. robur*, grid ref. 57-24-51, 51°16.5'N, 5°23.9'E, 35 m, 22-4-2020, P. & B. van den Boom 59745 (hb. v.d. Boom).—**Belgium**, prov. Luxembourg, W of Houffalize, W of Engreux, E of La Penne, along Ourthe Occidentale, east bank, *Quercus* wood with shaded schist outcrop, IFBL J7.25, 50°07'N, 5°48.5'E, 300 m, 31-5-1997, P. & B. van den Boom 18891 (hb. v.d. Boom); NW of Bouillon, 2 km SSW of Ucimont, Moulin des Rivages, E exposed schist outcrops in E sloping forest, fence post, E side of Semois, on *Fraxinus*, IFBL L6.11, 49°49.0'N, 5°02.4'E, 230 m, 3-4-1999, P. & B. van den Boom 21920 (hb. v.d. Boom); 3.1 km SSE of Herbeumont, Car, opposite of the crossing to the road to Ste. Cécile, *Salix* and stones at bank of trail, IFBL L6.35, 49°45.3'N, 5°15.1'E, 365 m, 16-10-2006, P. & B. van den Boom, 37093 (hb. v.d. Boom).—**Germany**, Brandenburg, west von Templin, Beutel, c. 1,2 km W, lockere Laubmischwald-Sukzessionsfläche mit Birken, Eichen, Pappeln und offenen Heideflächen, on dead branch of *Populus*, MTB 2846/3, 53°07,0'N, 13°24,0'E, 60 m, 18-7-2012, P. & B. van den Boom 48175 (hb. v.d. Boom).

Acknowledgement.

Dr Wolfgang von Brackel is thanked for providing the drawings about the ascospores. Bern van den Boom provided the picture of the type locality.

References

- ARNOLD, F. (1871) Lichenologische Fragmente. XI. – Flora. **54**: 49–56
- CUBERO, O.F., CRESPO, A., FATEHI, J., BRIDGE, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. – Plant Systematics and Evolution **216**: 243–249.
- KATO, K., & STANDLEY, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. – Molecular biology and evolution **30(4)**:772–780.
- MADDISON, W.P. & MADDISON, D.R. (2015) Mesquite: A modular system for evolutionary analysis, version 3.6. <https://www.mesquiteproject.org/>
- MEYER, B. & PRINTZEN, C. (2000) Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. – Lichenologist **32(6)**: 571–583.
- MIADLIKOWSKA, J., KAUFF, F., HÖGNABBA, F., OLIVER, J. C., MOLNÁR, K., FRAKER, E., et al. (2014) A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. – Molecular Phylogenetics and evolution **79**: 132–168.
- MILLER, M.A., PFEIFFER, W., & SCHWARTZ, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. – In 2010 gateway computing environments workshop (GCE) (pp. 1–8).
- ORANGE, A., JAMES, P.W. & WHITE, F.J. (2001) Microchemical Methods for the Identification of Lichens. British Lichen Society. 101 pp.
- NIMIS, P.L. & POELT, J. (1987) The lichens and lichenicolous fungi of Sardinia (Italy). – Studia Geobotanica **7(Suppl.)**: 1–269.
- STAMATAKIS, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – Bioinformatics **22**: 2688–2690.
- STAMATAKIS, A., HOOVER, P., ROUGEMONT, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. – Systematic Biology **57**: 758–771.
- SÉRUSIAUX, E. (1993) New taxa of foliicolous lichens from western Europe and Macaronesia. – Nordic Journal of Botany **13(4)**: 447–461.
- SUIJA, A. & VAN DEN BOOM, P.P.G. (2023) Phylogenetic relationships, taxonomic novelties, and combinations within Stictidaceae (Ostropales, Lecanoromycetes, Ascomycota): Focus on *Absconditella*. – Mycological Progress **22**: 46.
- VĚZDA, A. (1978) Neue oder wenig bekannte Flechten in der Tschechoslowakei. II. – Folia Geobotanica et Phytotaxonomica. **13(4)**: 397–420.
- VAN DEN BOOM, P. (2004) A long-term inventory of lichens and lichenicolous fungi of the Strabrechtse Heide and Lieropse Heide in Noord-Brabant, The Netherlands. – Österreichische Zeitschrift für Pilzkunde **13**: 131–151.
- VAN DEN BOOM, P.P.G. (2015) Lichens and lichenicolous fungi from graveyards of the area of Eindhoven (the Netherlands), with the description of two new species. – Annalen des Naturhistorischen Museums in Wien. Serie B, Botanik und Zoologie **117**: 245–276.
- VAN DEN BOOM, P. & VAN DEN BOOM, B. (2009) Diversity of lichens and lichenicolous fungi in a primeval heathland and adjacent managed forest in southern Netherlands (Groote Heide and 't Leenderbos). – Österreichische Zeitschrift für Pilzkunde **18**: 25–45.

**A new lichen species, *Scoliciosporum micareae* (lichenized Ascomycota,
Scoliciosporaceae), occurring in western Europe**

- VAN DEN BOOM, P.P.G. & ALVARADO, P. (2021) *Catillaria flexuosa* (Catillariaceae), a new lichen species described from the Netherlands. – *Lichenologist* **53**(2): 193–202.
- WHEELER, D.L., BARRETT, T., BENSON, D.A., BRYANT, S.H., CANESE, K. et al. (2007) Database resources of the national center for biotechnology information. – *Nucleic Acids Res* **35**: D5–D12.
- ZOLLER, S., SCHEIDEGGER, C. & SPERISEN, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. – *Lichenologist* **31**: 511–516.

Table 1. Species, voucher/isolate and mtSSU GenBank ID numbers for taxa used in the phylogenetic analysis

Taxon	voucher/isolate	mtSSU GB number
<i>Cladonia chlorophaea</i>	PH25	KY930515
<i>Cladonia pyxidata</i>	Chesnokov J-41	MN726422
<i>Haematomma accolens</i>	Luecking 17626x2	EU075550
<i>Haematomma flexuosum</i>	Luecking 17626x3	EU075551
<i>Haematomma ochroleucum</i>	Ekman 3184	AY756367
<i>Haematomma sulphureum</i>	Luecking 173070	EU075553
<i>Lecanora excludens</i>	Palice 21929	MK541649
<i>Lecanora intumescens</i>	Malicek 8203	KY502443
<i>Lecanora intumescens</i>	Ekman 3162	AY300892
<i>Lecanora intumescens</i>	Palice 25710	OQ682997
<i>Lecanora intumescens</i>	Malicek 8480	KY502441
<i>Scoliciosporum cf. umbrinum</i>	Malicek 10228	MK778577
<i>Scoliciosporum chlorococcum</i>	Ekman 3390	AY567768
<i>Scoliciosporum chlorococcum</i>	Vondrak24454	OQ646454
<i>Scoliciosporum chlorococcum</i>	Vondrak26128b	OQ683172
<i>Scoliciosporum chlorococcum</i>	Vondrak25465	OQ646453
<i>Scoliciosporum curvatum</i>	Vondrak 25469	OQ646455
<i>Scoliciosporum fabisporum</i>	Fryday 11123	MT611934
<i>Scoliciosporum intrusum</i>	Ekman s.n. (BG)	AY567767
<i>Scoliciosporum micareae</i>	Van den Boom 61312	PP889950
<i>Scoliciosporum sarothamni</i>	Vondrak25462a	OQ646456

<i>Scoliciosporum sarothamni</i>	Vondrak25462b	OQ646457
<i>Scoliciosporum umbrinum</i>	Ekman 3005	AY300911
<i>Scoliciosporum umbrinum</i>	Ekman 3005	AY567719
<i>Tephromela atra</i>	AFTOL-ID 780	DQ986894

Published online 05.11.2024