

## Contributions to the knowledge of mycosphaerellaceous genera and species

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*Ramularia lapponica* is neotypified, redescribed, illustrated, and discussed. A key to *Ramularia* species on *Ranunculus* spp. is provided. *Ramularia ragnhildianicola* sp. nov. is a new hyperparasitic species described from Germany on colonies of *Ragnhildiana ferruginea* causing leaf spots on *Artemisia vulgaris*. *Filiella pastinacae* on *Trinia glauca* and *Neopseudocercospora capsellae* on *Cardamine occulta* are reported for the first time, including descriptions and illustrations.

**Zusammenfassung:** Braun, U. & Kruse, J. 2021: Beiträge zur Kenntnis von Gattungen und Arten der Mycosphaerellaceen. *Schlechtendalia* **38**: 163–168.

*Ramularia lapponica* wird neotypisiert, neu beschrieben, abgebildet und diskutiert. Ein Bestimmungsschlüssel für *Ramularia*-Arten auf *Ranunculus* spp. wird zur Verfügung gestellt. *Ramularia ragnhildianicola* sp. nov. ist ein neuer hyperparasitischer Hyphomyzete, der aus Deutschland auf Kolonien von *Ragnhildiana ferruginea*, einem auf *Artemisia vulgaris* vorkommenden Blattfleckenpilz, beschrieben wird. *Filiella pastinacae* auf *Trinia glauca* und *Neopseudocercospora capsellae* auf *Cardamine occulta* sind Erstnachweise, ergänzt durch Beschreibungen und Abbildungen.

**Key words:** *Filiella*, *Neopseudocercospora*, *Ramularia*, Lindroth, neotype, key.

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Braun (1995, 1998) published taxonomic monographs of *Pseudocercospora*, *Ramularia* and allied genera (*Mycosphaerellaceae*). The species treated in these works were described and illustrated on the basis of morphological examinations of numerous fresh as well as herbarium specimens, including type material. First phylogenetic analyses of species of the genera treated in Braun (1995, 1998) led to a reclassification of *Pseudocercospora* Deighton s. lat. and to reassessments of several broadly circumscribed *Ramularia* species, such as *R. inaequalis* (Preuss) U. Braun (Videira et al. 2016). The present additions to *Filiella*, *Neopseudocercospora*, and *Ramularia* are contributions to the host range and taxonomy of *Mycosphaerellaceae*.

### (1) *Ramularia lapponica*

Lindroth (1902) described numerous *Ramularia* species, including *R. lapponica* on *Ranunculus lapponicus*. Braun (1998: 300) listed this species under “Excluded and doubtful species”. Lindroth (l.c.) cited two specimens, one from Finland and one from Svalbard. However, these syntypes could not be traced and other collections were also not available for examination. Braun (l.c.) assumed that this species might be a synonym of *Ramularia didyma* Unger or even an asexual morph of *Entyloma* (*Entylomella*). Braun & Salo (2019) re-examined numerous type collections of *Ramularia* species described by P. A. Karsten and J. I. Lindroth, which are now deposited at the Herbarium of the Finnish Museum of Natural History, University of Helsinki, Finland (H), and designated lectotypes when necessary. These specimens were previously housed in the Herbarium of the Department of Plant Pathology, Helsinki University (HPP), and not available during the preparation of the monographic treatment of the genus *Ramularia* published by Braun (1998). However, the two syntypes of *Ramularia lapponica*, cited in Lindroth (1902) have not been traced, i.e., they are still missing and probably not preserved. Among numerous recently examined *Ramularia* collections from the herbarium in Helsinki, there was a single authentic specimen collected and identified by Lindroth. This sample is an excellent candidate to serve as neotype and allows a redescription of this species and a discussion on its taxonomic status:

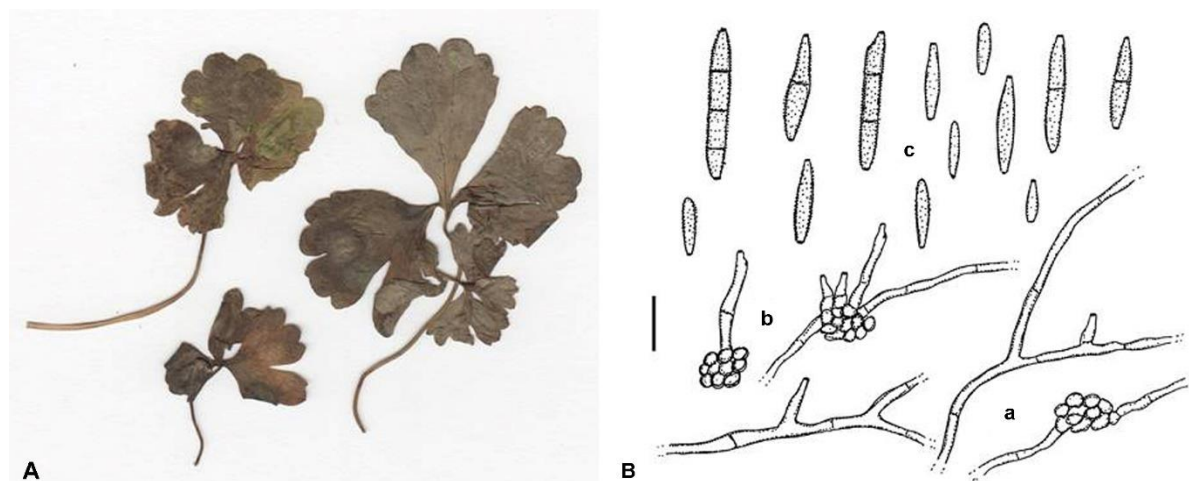
***Ramularia lapponica*** Lindr., Acta Soc. Fauna Flora Fenn. **23**(3): 14, 1902

Fig. 1

Neotype (designated here, MycoBank, MBT10000451): Finland, Lapland, Portimojärvi, on *Ranunculus lapponicus*, Jul. 1928, J. Liro, ex herb. HPP (H 6038179).

Leaf spots amphigenous, almost lacking to pale, whitish-greyish, grey to dark brown, 3–20 mm diam., margin indefinite or darker, but not very distinct, lesions finally becoming necrotic. Colonies amphigenous, whitish, effuse. Primary mycelium immersed; secondary mycelium superficial;

superficial hyphae emerging through stomata, branched, septate, hyaline, thin-walled, smooth, 1–2.5  $\mu\text{m}$  wide. Stromata immersed, substomatal to intraepidermal, 10–45  $\mu\text{m}$  diam., composed of swollen hyphal cells, circular to somewhat irregular in outline, 2–5  $\mu\text{m}$  diam. Conidiophores solitary, arising from superficial hyphae, or solitary to loosely aggregated, arising from stromata, through stomata or erumpent, erect, straight to curved-sinuuous, unbranched, subcylindrical or width somewhat narrowed from base to top, 4–30  $\times$  1–3.5  $\mu\text{m}$ , 0–2-septate, hyaline, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 4–22  $\mu\text{m}$  long, with 1–3 conidiogenous loci, somewhat thickened and darkened, 0.5–1.5  $\mu\text{m}$  wide. Conidia in simple or branched chains, ellipsoid, ovoid, fusiform, cylindrical, 5–28  $\times$  (1–)1.5–4.5  $\mu\text{m}$ , 0–3-septate, hyaline, thin-walled, verruculose, ends short obconically truncated, with conspicuous hila, 0.5–1.5  $\mu\text{m}$  wide, slightly thickened and darkened-refractive.



**Fig. 1:** *Ramularia lapponica*. A. Symptoms on leaves (neotype). B. Superficial hyphae, with and without solitary conidiophores (a); conidiophores arising from stomata (b); conidia (c) [drawing by U. Braun].

Notes: *Ramularia lapponica* is a well characterised species, in vivo morphologically readily distinguishable from all other *Ramularia* species on *Ranunculus* spp. by having superficial hyphae with solitary conidiophores. Braun (1998) provided a key to *Ramularia* spp. on *Ranunculaceae*, and Pirnia & Braun (2018) published a special key to *Ramularia* spp. on *Ranunculus* spp., including *R. ranunculicola* described in the latter paper. The species concerned can be keyed out as follows:

**Key to *Ramularia* species on *Ranunculus* spp.**

1. Conidia consistently solitary and 5–13  $\mu\text{m}$  wide; on numerous *Ranunculus* spp. .... *Ramularia simplex* Pass.
- 1\* Conidia solitary and catenate, but not consistently solitary, narrower, usually 1.5–7  $\mu\text{m}$  ..... 2
2. Conidia long and slender (*Pseudocercospora*-like), 15–65(–80)  $\times$  1.5–4  $\mu\text{m}$ , 0–6-septate; on *Ranunculus muricatus*, *R. sardous* and *R. trilobus* ..... *Ramularia torrendii* (Bres.) U. Braun
- 2\* Conidia shorter (< 40  $\mu\text{m}$ ) and wider, 2–7(–8)  $\mu\text{m}$ , 0–3-septate ..... 3
3. Stromata well-developed, large, 20–100  $\mu\text{m}$  diam.; conidia often or almost consistently solitary ... 4
- 3\* Stromata almost lacking or small, 10–35  $\mu\text{m}$  diam.; conidia usually catenate; ..... 5
4. Conidia 10–35  $\times$  3–7  $\mu\text{m}$ , usually aseptate; on *Ranunculus muricatus*, Iran, and probably Turkey and Italy ..... *Ramularia ranunculicola* Pirnia & U. Braun
- 4\* Conidia (10–)15–35(–40)  $\times$  4–7(–8)  $\mu\text{m}$ , 0–2(–3)-septate; on *Ranunculus carpaticus* and *R. montanus* ..... *Ramularia ranunculi-montani* (C. Massal.) U. Braun
5. Conidia (3–)4–7(–8)  $\mu\text{m}$  wide, 0–3-septate; on *Ranunculus acris* and various other *Ranunculus* spp. .... *Ramularia acris* Lindr.
- 5\* Conidia narrower, (1–)1.5–5  $\mu\text{m}$  ..... 6
6. With superficial hyphae; conidiophores solitary, arising from superficial hyphae, lateral, and solitary to loosely aggregated, arising from small stromatic hyphal aggregations; on *Ranunculus lapponicus* ..... *Ramularia lapponica* Lindr.
- 6\* Mycelium internal, superficial hyphae lacking; conidiophores fasciculate, arising from substomatal stromata, through stomata (var. *didyma*), or stromata lacking to developed, 10–50  $\mu\text{m}$  diam.,

intraepidermal, rarely substomatal (var. *exigua* (U. Braun) U. Braun); on numerous *Ranunculus* spp. .... *Ramularia didyma* Unger

## (2) A new hyperparasitic *Ramularia* species

***Ramularia ragnhildianicola*** J. Kruse & U. Braun, sp. nov.

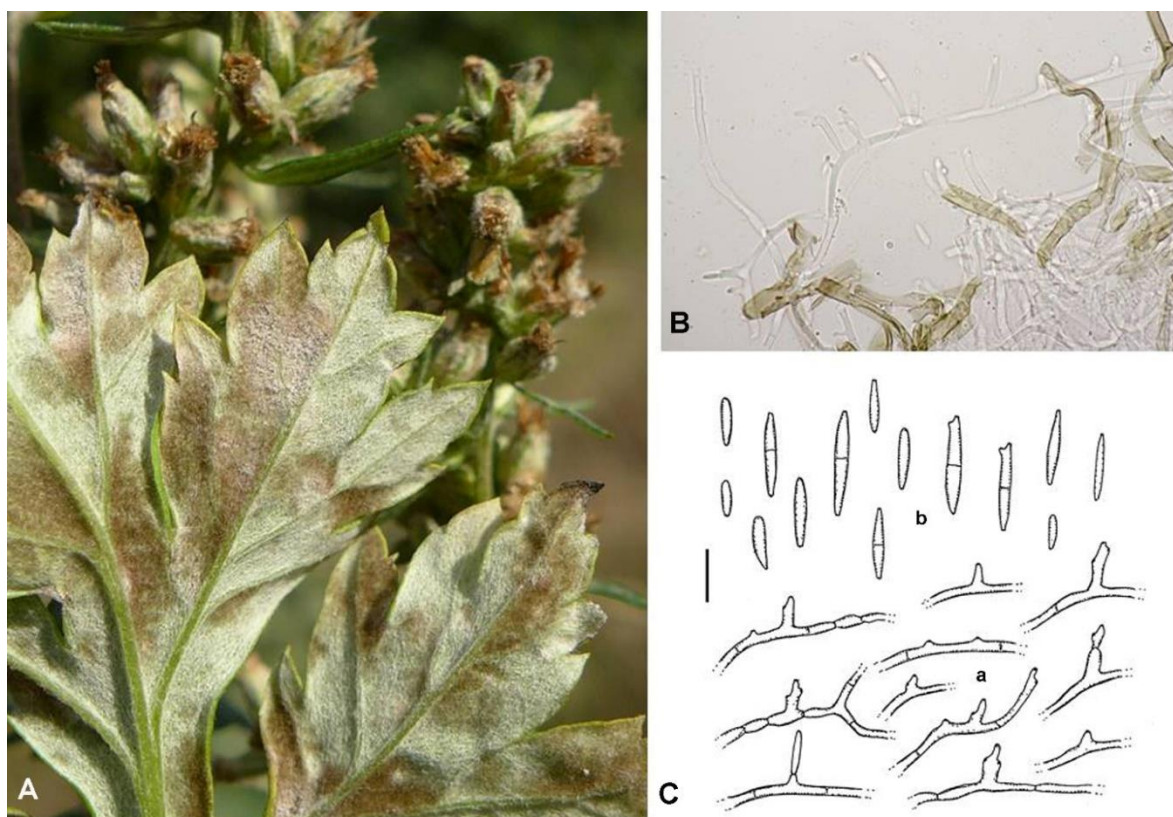
Fig. 2

Mycobank, MB839146.

Etymology: Referring to the genus name of the host fungus, *Ragnhildiana*.

Colonies hyperparasitic on colonies caused by the leaf-spotting *Ragnhildiana ferruginea*, forming diffuse patches or effuse, loose to moderately dense, whitish, overgrowing the brown colonies of the host fungus and immersed. Mycelium colourless, composed of branched hyphae, 1–4  $\mu\text{m}$  wide, hyaline, septate, smooth. Conidiophores solitary, arising from hyphae, lateral, occasionally terminal (at the end of hyphae), erect, conidiophores usually aseptate, i.e., reduced to conidiogenous cells, rarely with a single septum, subcylindrical to usually conical, straight to somewhat geniculate-sinuuous caused by sympodial proliferation, unbranched, 5–18  $\times$  2–4  $\mu\text{m}$ , hyaline, thin-walled, with a single or 2–3 minute conidiogenous loci, sometimes almost denticle-like, slightly thickened and darkened, about 1  $\mu\text{m}$  diam., or conidia arising from minute peg-like protuberances of the hyphae, 1–2  $\mu\text{m}$  long and 0.5–1.5  $\mu\text{m}$  wide, somewhat attenuated towards a truncated tip. Conidia solitary or usually in simple or branched acropetal chains, ellipsoid-ovoid, fusiform, subcylindrical, usually straight, 5–18  $\times$  1.5–3  $\mu\text{m}$ , 0–1-septate, thin-walled, hyaline, smooth or almost so to finely rough-walled, ends rounded to attenuated, with a single basal hilum and 1–2 hila at the apex, minute, 0.4–0.8  $\mu\text{m}$  wide, barely thickened and darkened.

**Holotype:** Germany, Rheinland-Pfalz, Landkreis Bad Dürkheim, Birkenheide, 1.6 km northwest of Eysersheimer Hof, meadow, 49°29'42.02"N, 8°15'18.15"E, c. 105 m alt, on *Ragnhildiana ferruginea* (Fuckel) U. Braun, C. Nakash., Videira & Crous parasitic on *Artemisia vulgaris*, 8 Sep. 2020, J. Kruse (POLL 9793). **Isotype:** HAL 3375 F.



**Fig. 2:** *Ramularia ragnhildianicola* sp. nov. A. Symptoms on leaves. B. Micrograph, superficial hyphae with solitary conidiophores and conidia [micrograph by B. Sothmann]. C. Solitary conidiophores arising from superficial hyphae (a); conidia (b) [drawing by U. Braun].

Notes: Attempts to cultivate and sequence this fungus were unfortunately not successful. However, this is a typical *Ramularia* with unique characteristics quite different from all described species of this genus, which warrants the introduction of a new species. *Ramularia ragnhildianicola* is confined to the fructification caused by *Ragnhildiana ferruginea* on leaves of *Artemisia vulgaris*, i.e., this species is undoubtedly hyperparasitic on this leaf-spotting ascomycete. Its hyphae grow superficially on the colonies of the host fungus and are immersed. This is the first hyperparasitic *Ramularia* on a cercosporoid fungus. *Ramularia butomi* Lind, on immature ascomata of various leaf- and stem-inhabiting ascomycetes, has similar conidia, but superficial hyphae and solitary conidiophores arising from hyphae are lacking. The conidiophores are fasciculate and much longer, to 60  $\mu\text{m}$  (Braun 1998). *Ramularia coleosporii* Sacc. on *Chrysomyxa* and *Coleosporium* spp. (rust fungi) may also form superficial hyphae, but the conidiophores are much longer, (20–)30–200(–270)  $\mu\text{m}$ , and the conidia are 3–8  $\mu\text{m}$  wide. *Ramularia uredinis* (W. Voss) Sacc., also known to be hyperparasitic on rust fungi, may also form superficial hyphae, but the conidiophores are fasciculate and not solitary, i.e., they do not arise from superficial hyphae (Braun 1998).

### (3) *Filiella pastinacae* on *Trinia glauca*

A leaf-spotting hyphomycete has recently been found on *Trinia glauca* (*Apiaceae*). The morphology of this collection agrees well with *Pseudocercospora pastinacae* (emend.) as circumscribed in Braun (1995: 139). This species occurs on hosts of various apiaceous genera, including *Angelica*, *Apium*, *Archangelica*, *Astrantia*, *Eremodaucus*, *Heracleum*, *Laserpitium*, *Libanotis*, *Pastinaca*, *Peucedanum*, and *Physospermum*. Several species names previously described from different hosts of the *Apiaceae*, mainly under *Cercospora* Sacc. and *Cylindrosporium* Grev., were reduced to synonymy with *P. pastinacae*. *Trinia glauca* is a new host for this species, which has recently been reallocated to the new genus *Filiella* Videira & Crous, based on phylogenetic analyses of material on the type host of this species, *Pastinaca sativa* (Videira et al. 2016). Collections on hosts belonging to other genera of the *Apiaceae*, including *Trinia*, are morphologically indistinguishable. If they nevertheless belong to different cryptic (phylogenetic) species is still unclear and requires further phylogenetic analyses. For the time being, such collections can only be assigned to *Filiella pastinacae* s. lat. (sensu Braun 1995).



**Fig. 3:** *Filiella pastinacae*. A. Symptoms on leaves. B. Micrograph, stroma with short conidiophores and some conidia [micrograph by B. Sothmann]. C. Fasciculate conidiophores arising from stromata (a); conidiophores (b); conidia (c) [drawing by U. Braun].

*Filiella pastinacae* (P. Karst.) Videira & Crous, in Videira, Groenewald, Braun, Shin & Crous, Stud. Mycol. **83**: 88, 2016. Fig. 3

≡ *Cercospora pastinacae* P. Karst., Hedwigia **23**(4): 63, 1884.

≡ *Pseudocercospora pastinacae* (P. Karst.) U. Braun, Nova Hedwigia **56**(3-4): 444, 1993.

Fully synonymy, see Braun (1995).

Holotype: Finland, Mustalia, on *Pastinaca sativa*, 7 Jul. 1867, P. Karsten (H 3921).

Lesions on leaves and stems, causing yellowish to brown necrotic discolorations, variable in shape and size, finally large leaf and stem portions or entire leaves becoming necrotic. Mycelium internal. Caespituli amphigenous and on stems, punctiform, scattered or gregarious, whitish to greyish white. Stromata immersed or substomatal, 20–80 µm diam., colourless to faintly yellowish, composed of swollen hyphal cells, subcircular in outline, 2.5–8 µm diam. Conidiophores numerous, in dense fascicles, arising from stromata, erumpent, erect, straight, subcylindrical or somewhat attenuated towards the tip, unbranched, 5–25 × 2–4 µm, 0–1-septate, hyaline, thin-walled, smooth, mostly with a single terminal conidiogenous locus, apex truncated or almost so, 2.5–3.5 µm wide, unthickened, not darkened. Conidia solitary, acicular, subcylindrical, sometimes almost obclavate, straight to somewhat curved, (10–)20–48 × 2.5–4 µm, 0–3-septate, hyaline, thin-walled, smooth, apex subacute or subobtuse, base mostly truncated, occasionally short obconically truncated, 2.5–4 µm wide, hila neither thickened nor darkened.

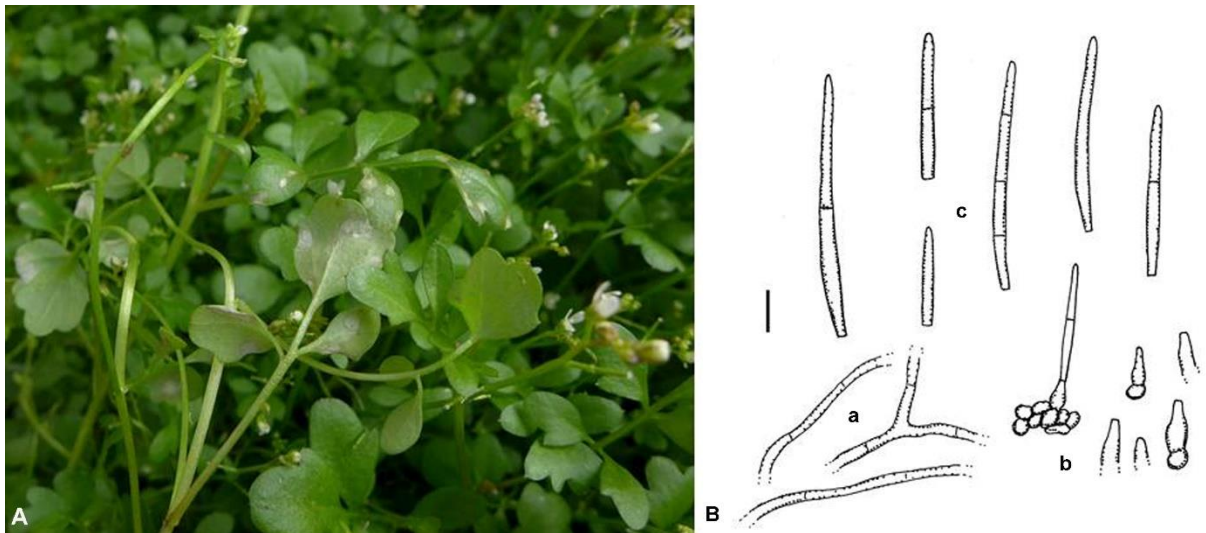
Specimen examined: Germany, Rheinland-Pfalz, Landkreis Bad Dürkheim, Leistadt, Berntal, Felsenberg, dry grasslands, on *Trinia glauca*, 13 May 2020, J. Kruse (HAL 3371 F, POLL 9789).

Note: During the preparation of Braun's (1995) monographic treatment of *Cercospora* and allied genera, type material of *Cercospora pastinacae* had not been traced at herbarium H. Therefore, Braun (1995) designated a neotype. The holotype of this species has recently been found, so that the previous neotypification is now obsolete.

#### (4) *Neopseudocercospora capsellae* on *Cardamine occulta*

*Neopseudocercospora capsellae* (Ellis & Everh.) Videira & Crous, in Videira, Groenewald, Braun, Shin & Crous, Stud. Mycol. **83**: 86, 2016.

Fully synonymy, see Braun (1995).



**Fig. 3:** *Neopseudocercospora capsellae*. A. Symptoms on leaves. B. Immersed hyphae (a); conidiophores (b); conidia (c) [drawing by U. Braun].

Leaf spots amphigenous, subcircular to somewhat irregular in outline, 1–6 mm diam., dull greenish, margin indefinite. Caespituli hypophyllous, whitish, greyish white or with a faintly ochraceous tinge, punctiform to effuse. Mycelium internal; hyphae branched, hyaline, septate, thin-walled, smooth, 2–3 µm wide. Conidiophores solitary to loosely or dense aggregated, arising from immersed swollen hyphal cells, 2–4 µm diam., or aggregations of such cells, erumpent, erect, straight, subcylindrical to conical, lageniform or slightly geniculate-sinuous, unbranched, short, 5–20 × 2–4 µm, aseptate, i.e.,

conidiophores reduced to conidiogenous cells, hyaline, thin-walled, smooth, mostly with a single terminal conidiogenous locus, truncated, neither thickened nor darkened. Conidia solitary, cylindrical, subacicular or somewhat attenuated towards both ends, straight to curved, 18–65 × (1.5–)2–3 µm, 0–3-septate, hyaline, thin-walled, smooth, apex subobtuse to subacute, base truncated to very short obconically truncated, 1–2 µm wide, hila neither thickened nor darkened.

Specimen examined: Germany, Rheinland-Pfalz, Bad Dürkheim, Bürgermeister-Gropp-Straße, 49°27'32.88"N, 8°8'59.25"E, c. 140 m alt, in a flower pot, on *Cardamine occulta*, 30 Jan. 2020, J. Kruse (HAL 3376 F, POLL 9792).

Notes: Braun (1995) described and illustrated *Pseudocercospora capsellae* and discussed the taxonomy of this species. Various species names previously described from different brassicaceous genera were reduced to synonymy with *P. capsellae*. Videira et al. (2016) clarified the phylogeny of this species, based on material from its type host, *Capsella bursa-pastoris*, and reallocated it to the new genus *Neopseudocercospora*. The question whether *N. capsellae* constitutes a single species with wider host range within the *Brassicaceae* or if it rather represents a complex of cryptic, morphologically barely distinguishable but genetically distinct species on different host genera can only be answered on the basis of results of further sequence analyses. At present, the collection on *Cardamine occulta* can only be assigned to *N. capsellae* as described in Braun (1995, under *P. capsellae*). *Cardamine occulta* is, in any case a new host for this species. *Cardamine occulta*, previously often referred to as *C. flexuosa*, is an Asian species, introduced and synanthropic in Europe. The nomenclature, taxonomy and European distribution of this species have recently been discussed by Marhold et al. (2016).

## Acknowledgements

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