

***Discostroma ficicola* sp. nov. (Amphisphaeriaceae)
and a key to species of *Discostroma***

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Paulus B.C., Gadek P.A. & Hyde K.D. (2006) *Discostroma ficicola* sp. nov. (Amphisphaeriaceae) and a key to species of *Discostroma*. *Sydowia* 58 (1): 76 – 90.

An investigation of microfungal diversity in a tropical rainforest of North Queensland, Australia, yielded a new species of *Discostroma* on decaying leaves of *Ficus pleurocarpa*. *Discostroma ficicola* is described and illustrated and compared with similar species. A synopsis of the genus *Discostroma* and a key to the genus are provided.

Key words: Ascomycetes, *Discostromopsis*, leaf litter fungi.

A study to estimate microfungal diversity in decaying leaves of four rainforest tree species in a tropical rainforest in North Queensland, Australia (Paulus *et al.* 2003 a, 2003 b) resulted in the discovery of a new species of *Discostroma* Clem. on decaying leaves of *Ficus pleurocarpa* F. Muell. We provide descriptions and illustrations of *Discostroma ficicola* sp. nov., comparisons with similar species and a synopsis of the genus in this paper.

Clements (1909) introduced *Discostroma* with a new combination, *D. rehmi* (Schnabl) Clem. Von Arx (1974) considered *D. rehmi* synonymous with *Metasphaeria massarina* Sacc., and introduced a new combination, *D. massarinum* (Sacc.) Arx (as *D. massarina*; Arx 1974). Currently 28 species are included in this genus (Müller & Loeffler 1957, Eriksson 1974, Brockmann 1976, Barr 1983, Sivanesan 1983, Eriksson 1992, Huhndorf 1992, Barr 1993 1994, Yuan & Barr 1994, Okane *et al.* 1996, Hatakeyama & Harada 2004). Of these, four species were described by Swart (1979) in *Discostromopsis* H.J. Swart, but his genus was considered synonymous with *Discostroma* by Sivanesan (1983) and the four species were redispersed to the later genus (Sivanesan & Shivas 2002b). Keys to species of *Discostroma* were provided by Brockmann (1976) and Barr (1994). Fungi included in *Discostroma* are mainly biotrophic (Brooks & El Alaily 1939; Kang *et al.* 1998, Samuels & Blackwell

2001), but fruiting bodies of *Discostroma* have also been reported from decaying plant material such as twigs (Brockmann 1976), leaves (Barr 1993, this study) and cones of *Pinus* (Müller & Loeffler 1957). The placement of *Discostroma* in the Amphisphaeriaceae *sensu stricto*, as suggested by its morphology and its mode of life (Samuels *et al.* 1987), was supported by molecular phylogenetic analyses (Kang *et al.* 1998, Jeewon *et al.* 2002, Smith & Hyde 2003). Using a molecular approach, Jeewon *et al.* (2002) also confirmed the anamorph-teleomorph connections between *Discostroma* and *Seimatosporium* Corda, which had been previously known from cultural studies alone (Müller & Shoemaker 1965, Brockmann 1976, Okane *et al.* 1996).

Materials and Methods

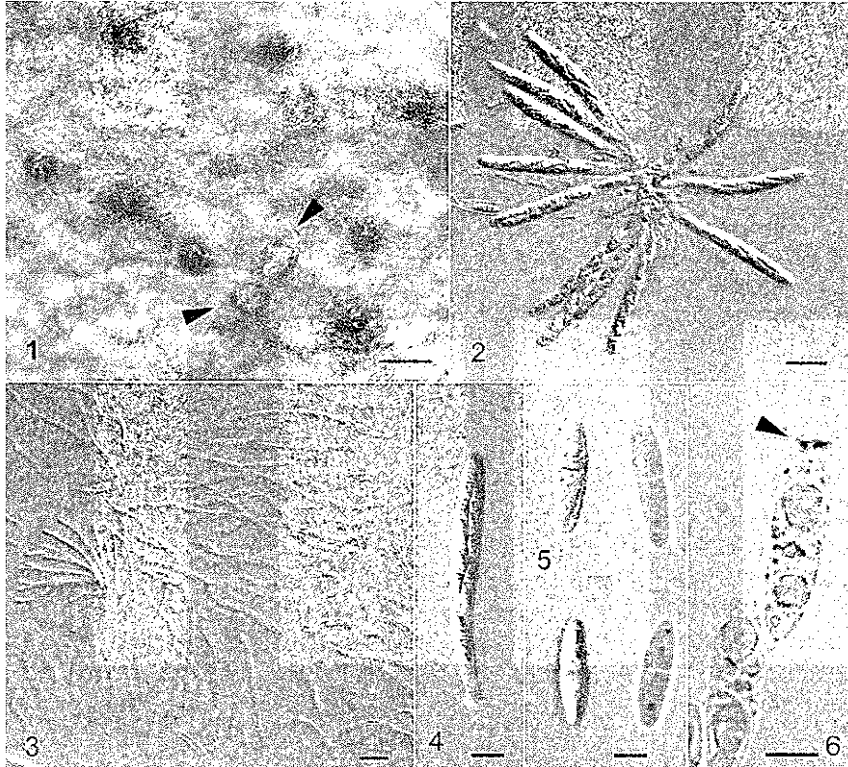
The study site comprises of upland rainforest near Topaz, Queensland, Australia (700 m a. s. l.). The forest has been classified as complex mesophyll rainforest (Type 1b rainforest; Tracey 1982) and has a high diversity of tree species. Decaying leaves were collected under six individual trees of *Ficus pleurocarpa*.

Slides were prepared from ascomata removed from decaying leaves following incubation in humid chambers, which contained tissue paper moistened with sterile distilled water. Some slides were stained with Melzer's reagent to test amyloidity of the ascal apical apparatus. The range of measurements is derived from examining a minimum of 25 ascospores 20 asci, 10 ascomata per specimen mounted in sterile distilled water. The arithmetic mean and standard deviation of ascospore, ascal and ascomatal dimensions were calculated in Microsort Excel (Microsoft Corporation, Redmond). Spores, asci and ascomata were measured at their widest point. Slides were rendered semipermanent by the addition of 90% lactic acid. Photographs were taken on an Olympus BX50 microscope using an Olympus CA 35 AD-4 camera. Photos were scanned and plates were assembled using Adobe Photoshop 5.0 (Adobe Systems Inc., San Jose). The type specimen is deposited at the Plant Pathology Herbarium (BRIP), Queensland, department of Primary Industries.

Taxonomy

Discostroma ficicola Paulus, P. Gadek & Hyde K. D. **sp. nov.** Figs. 1–6.

Ascomata numerosa, epiphylla, nigra, globosa vel subglobosa, immersa vel semi-immersa, clypeo circulari et nigro, 112.5 – 175 × 112.5 – 150 µm. Asci cylindrici vel anguste ellipsoidei, unitunicati, 8 spori uniseriati vel biseriati, annulo amyloideo, 2.5 × 1.5 µm, pulvillo dicto atramento scriptorio coerulescente praediti, (80–) 90 – 110 × 10 – 12 µm. Ascosporae ellipsoideae, uniseptatae, hyalinae, guttulateae, 17 – 20 × 5 – 6.5 µm. Anamorphosis ignota.



Figs. 1 – 6. *Discostroma ficicola* (BRIP 29181). 1. Ascomata on leaf surface. 2. Asci. 3. Paraphyses. 4. Ascus. 5. Ascospores. 6. Amyloid ascal apparatus (arrowed) and ascospores (note slight constriction at septa). Scale bars: 1 = 100 μm ; 2 = 25 μm ; 3, 5 – 6 = 5 μm ; 4 = 10 μm .

Holotypus. AUSTRALIA, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, in decaying leaves of *Ficus pleurocarpa*, 8 Feb 2002, leg. B. Paulus and I. Steer, BRIP 29181.

Etymology. *ficicola* (Latin – *Ficus* = dweller), referring to the strangler fig *Ficus pleurocarpa* from which this fungus was identified.

Habitat. in decaying leaves of *Ficus pleurocarpa*.

Ascomata numerous, mostly epiphyllous, black, globose or subglobose with circular black clypeus visible on leaf surface, submerged when young, semisubmerged with central papilla emerging when mature, macroscopically appearing as pustules on leaf surface, 112.5 – 175 \times 112.5 – 150 μm (mean \pm SD = 138.4 \pm 17.6 \times 133.7 \pm 10.8 μm , n = 10), peridium composed of several layers of dark brown, irregular cells. Paraphyses abundant, persistent, hyaline, smooth, septate, sometimes inflated at base with long, tapering apical

part, 3–9 µm wide at base, tapering to 2 µm wide at apex, which is obtuse or slightly inflated. Asci unitunicate, cylindrical or narrowly clavate (when ascospores have obliquely or overlapping uniseriate arrangement) or narrowly ellipsoid (when ascospores are biseriate), 8-spored, apex rounded with a J+ subapical annulus, approximately 2.5 µm wide and 1.5 µm high, pulvillus staining slightly blue with ink, pedicel short with inflated blunt base, (80–) 90–110 µm × 10–12 µm (mean ± SD = 97.9 ± 10.3 × 10.6 ± 0.8 µm, n = 20). Ascospores oblong to ellipsoid, sometimes inequilateral, with one median septum, slightly constricted or not constricted at the septum, hyaline, smooth-walled, usually guttulate, 17–20 × 5–6.5 µm (mean ± SD = 18.7 ± 1.2 × 5.7 ± 0.7 µm, n = 25). Anamorph not observed.

Notes. For the generic placement of our taxon, we considered genera developing under clypei, with hyaline ascospores, and asci with an amyloid apical ring. *Hyponectria* Sacc., *Physalospora* Niessl and *Rachidicola* Hyde K.D. & J. Fröhl. were excluded on the basis of ascospore septation, and *Charonectria* Sacc. and *Arwidssonia* B. Erikss. on ascomatal differences (Niessl 1876, Saccardo 1878, 1880, Hyde & Fröhlich 1995, Sivanesan & Shivas 2002a, Wang & Hyde 1999). Within the Amphisphaeriaceae *sensu stricto*, *Ellurema* Nag Raj & W.B. Kendr. also has hyaline ascospores and an amyloid annulus but differs from our specimens in ascospore septation, ascus shape and abundance of paraphyses (Nag Raj & Kendrick 1985, Kang *et al.* 1999). With the exception of *Discostroma*, other genera within the Amphisphaeriaceae differ in ascospore pigmentation and/or septation or amyloidity of the ascus ring (Kang *et al.* 1998). Several species of *Discostroma* (e.g. *D. cupulum*, *D. empetri*, *D. hyperboreum*, *D. ledi*, *D. rhododendri*, *D. succineum* and *D. tostum*) resemble *D. ficicola* in having ellipsoid, hyaline ascospores with strictly one single median septum and an amyloid ascus ring (Table 1). Two species, *D. pachystimae* and *D. caninum*, also form hyaline ascospores with predominantly one septum, but they may form additional septa (Table 1). *Discostroma ficicola* differs clearly from all species with similar ascospores in their lengths (key, Table 1).

Table 1 provides a summary of characters of 28 species of *Discostroma*. A key to the genus is included below.

Key to *Discostroma*

1. Ascospores always muriform 2
1. Ascospores predominantly with transverse septa only 4
2. Ascospores dark brown, mostly with 3 transverse and one longitudinal septum, 18–20 (–34) × 8–10 (–12.5) µm
..... *Discostroma muricatum*
2. Ascospores hyaline 3

3. Ascospores with 3 transverse septa and 3–4 longitudinal septa
19–22 (–24) × 10–13 μm *Discostroma propendulum*
3. Ascospores with 3–7 (mostly 5) transverse septa and up to
3 longitudinal septa, pinkish in mass, 18–25 × 8.5–11 μm
. *Discostroma massarinum*
4. Ascospores always with one transverse septum 5
4. Ascospore septation variable 11
5. Ascospores 13–16 × 5–6 μm, asci 65–80 × 7.5–11.5 μm
. *Discostroma succineum*
5. Asci larger 6
6. Ascospore length ≥ 14 μm 7
6. Ascospore length predominantly ≤ 14 μm 8
7. Ascospores 17–20 × 5–6.5 μm, asci (80)90–110 × 10–12 μm . . .
. *Discostroma ficicola*
7. Ascospores 14–17 × 7–8 μm, asci 90–100 × 11–12 μm
. *Discostroma hyperboreum*
8. Ascospores becoming yellowish to dull brown at maturity,
10.5–13 (–16.5) × 5–6.5 μm, finely verruculose as longitudinal
striae *Discostroma cupulum*
8. Ascospores remain hyaline at maturity, smooth 9
9. Ascospores 12–13.5(–15) × 5.5–7 μm, asci 80–93 × 9–10.5 μm
. *Discostroma empetri*
9. Ascospore width ≤ 5.5 μm 10
10. On dead stems of *Epilobium*, ascospores 8–13.5 × 3–4.5 μm,
ellipsoid to slightly curved, asci 62–85 × 5–7 μm
. *Discostroma tostum*
10. On leaves of *Ledum groenlandicum*, ascospores 10.5–12 × 4–
4.5 μm, ellipsoid, asci 63–75 × 6–7 μm *Discostroma ledi*
10. On leaves of *Rhododendron californicum*, ascospores 10–13 ×
4.5–5.5 μm, oblong ellipsoid, asci 60–88 × 6.5–9 μm
. *Discostroma rhododendri*
11. Ascospores predominantly 1-septate but additional septa may
develop 12
11. Ascospores predominantly with more than one septum or with
approximately equal proportions of 1, 2 and 3 septa 15
12. Ascospore length mostly ≥ 12 μm 13
12. Ascospore length mostly ≤ 12 μm 14
13. Ascospores mostly 1-septate, rarely 3-septate, ellipsoid to fusoid,
hyaline, 12–14 × 4.5–5 μm, ascomata erumpent
. *Discostroma pachystimae*
13. Ascospores mostly 1-septate but also 2- or 3-septate, ellipsoid,
hyaline to yellowish, 12–15 (–17) × 5.3–6.5 μm, ascomata visible
as round, raised dark brown shiny areas *Discostroma caninum*
14. Ascospores 10–12(–13) × 4–5(–5.3) μm, 1-septate, sometimes
2-3-septate, asci 75–100 × 5.3–7.4 μm *Discostroma rosae*

14. Ascospores $7 - 12 \times 3 - 4 \mu\text{m}$, usually 1-septate, sometimes with further transverse septa, asci $55 - 70 \times 5 - 7 \mu\text{m}$ *Discostroma strobiligenum*
15. Ascospores mostly 2-septate, becoming 3-septate when mature, $16 - 20 \times 14 - 22 \mu\text{m}$, hyaline when young, brown when mature *Discostroma tricellulare*
15. Ascospores mostly 3-septate or approximately equal proportions of 1, 2 or 3 septa 16
16. Ascospores 1, 2, or 3 septa often in the same ascus, $11 - 14 \times 4.5 - 5 \mu\text{m}$ *Discostroma polymorphum*
16. Ascospores predominantly 3-septate 17
17. Ascus ring distinct and J+ 18
17. Ascus ring indistinct and J- 23
18. Ascospores hyaline to yellowish 19
18. Ascospores pigmented 22
19. Ascospores with predominantly 3 transverse septa and, rarely, one longitudinal septum, $16.5 - 20(-25) \times 7.5 - 9(-10) \mu\text{m}$, asci $105 - 130 \times 8.5 - 13.3 \mu\text{m}$ *Discostroma sanguineae*
19. Ascospores with only transverse septa 20
20. Ascospores $12 - 18 \times 4 - 6 \mu\text{m}$, asci $70 - 111 \times 6 - 10 \mu\text{m}$ *Discostroma botan*
20. Ascospore width $\geq 6 \mu\text{m}$ 21
21. Ascospores $18 - 26 \times 6 - 8 \mu\text{m}$, asci $90 - 130 \times 9 - 11(-15)$, ascomata $200 - 250(-350) \mu\text{m}$, on leaves of *Pentaphylloides fruticosus* *Discostroma fruticosum*
21. Ascospores $15.5 - 24 \times 6.5 - 9 \mu\text{m}$, asci $100 - 160 \times 9 - 13 \mu\text{m}$, ascomata $300 - 450 \times 250 - 495 \times 250 - 495 \mu\text{m}$, on leaves of *Rubus* spp. *Discostroma rubicola*
22. Ascospores olivaceous, $15 - 17 \times 9 \mu\text{m}$, asci $100 \times 9 - 10 \mu\text{m}$, ascomata $270 - 450 \times 200 - 300 \mu\text{m}$ *Discostroma fuscillum*
22. Ascospores light brown, $15 - 18 \times 6.5 - 9 \mu\text{m}$, asci $117 - 150 \times 8 - 10 \mu\text{m}$, ascomata $400 - 600 \times 210 - 400 \mu\text{m}$ *Discostroma saccardoanum*
23. Ascospores brown, echinulate, $11.5 - 13.8 \times 4 - 4.5 \mu\text{m}$ *Discostroma osyridis*
23. Ascospore hyaline 24
24. Ascospores $14 - 18 \times 4 - 5 \mu\text{m}$, anamorph *Seimatosporium kriegianum* *Discostroma callistemonis*
24. Ascospore length $\geq 17 \mu\text{m}$ 25
25. On dead leaves of *Leptospermum juniperinum*, ascospores $17 - 23 \times 4.5 - 6 \mu\text{m}$, anamorph *Seimatosporium leptospermi* *Discostroma leptospermi*
25. Ascomata on dead leaves of *Melaleuca* 26
26. Anamorph *Seimatosporium elegans*, on leaves of *Melaleuca ericifolia*, ascospores $17 - 23 \times 4.5 - 6 \mu\text{m}$.. *Discostroma elegans*

26. Anamorph *Seimatosporium dilophosporum*, on dead leaves of *Melaleuca squarrosa*, ascospores $19 - 21 \times 4 - 6 \mu\text{m}$
 *Discostroma stoneae*

Discussion

Discostroma is defined by a relatively broad generic concept. It is characterized by the presence of stroma and/or clypeus, ostiolate perithecium, unitunicate asci with an amyloid or inamyloid apical annulus, pulvillus or plug-like structure in the ascus apex which stains with ink (not consistently noted by authors of new species descriptions) and ellipsoid, hyaline or pigmented ascospores, which may be transversely septate or muriform, and are usually arranged uniseriately within the ascus. For many species, a *Seimatosporium* anamorph (synonym: *Sporocadus* Corda *sensu* Brockmann 1976) with holoblastic conidial ontogeny and percurrent enteroblastic proliferation of the conidiogenous cell has been reported. Molecular data will show whether the genus is monophyletic as currently circumscribed.

Ascomatal morphology deserves further examination in the genus *Discostroma*, particularly as stromata and clypei development may vary depending on substratum texture and developmental stage (Swart 1979, Lu & Hyde 2000, this paper). Rudimentary information is currently available only for some *Discostroma* species (Table 1). They seem to have been described mainly on the basis of their natural substrata (e.g., *D. ledi*, *D. tostum* and *D. rhododendri*; Table 1, Key). Fungal plant pathogens and endophytes commonly display a degree of host-specificity (Zhou & Hyde 2001, Photita *et al.* 2004). Because some *Discostroma* species have been reported only from particular plant genera, the inclusion of substratum as an important taxonomic character may be justified. However, ascomata of *Discostroma* have also been reported from decaying plant material and do not seem to be host-specific (Müller & Loeffler 1957, Brockmann 1976, Swart 1979, Barr 1993, this study). In addition, we observed that *D. ficicola* was able to colonise sterilised leaves of *Ficus pleurocarpa* at different stages of decay (data not shown). Further studies are required to confirm whether some species of *Discostroma* are saprobic for the major part of their life cycle and to confirm or refuse substratum or host as a valid taxonomic character.

Acknowledgements

Funding for this project was provided by: the Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity,

University of Hong Kong; the Cooperative Research Centre for Rainforest Ecology and Management and the School of Tropical Biology, James Cook University. B.P. thanks N. Tucker for providing access to one of the collection sites, S. McKenna for identification of plant species and C. Pearce and I. Steer for their company on collection trips. B. Bussaban and C. McCulloch are gratefully acknowledged for assistance with photography, and B. Bussaban and H. Leung for assistance in obtaining literature. E. McKenzie, S. Pennycook and two anonymous reviewers are thanked for critical comments on the manuscript.

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(Manuscript accepted 20 Dec. 2005; Corresponding Editor: U. Peintner)

Table 1. Summary of morphological characters of species in *Discostroma* (in alphabetical order)

Species	Ascomata	Asci ^a	Ascospores ^b	Habitat	Anamorph
<i>Discostroma botan</i> Sat. Hatakeyama & Y. Harada Mycoscience 45: 106-111 (2004)	200-250 × 300-360 µm, perithecia slightly papillate, immersed.	(62-)70-111 × 6-10 µm, J ⁺ , uniseriate.	12-18 × 4-6 µm, hyaline, 3 transverse septa.	Stems of <i>Paeonia</i> <i>suffruticosa</i>	<i>Seimatosporium</i> <i>botan</i> Sat. Hatakeyama & Y. Harada
<i>Discostroma</i> <i>callistemonis</i> (H.J. Swart) Sivan, Trans. Br. mycol. Soc. 73: 217 (1979)	170-190 × 80-100 µm, perithecia under distinct dark clypeus.	65-75 × 9-10 µm, J ⁺ , partly biseriolate.	14-18 × 4-5 (-4.5) µm, hyaline, 3 transverse septa.	Leaves of <i>Callistemon</i> <i>paludosus</i>	<i>Seimatosporium</i> <i>kriegerianum</i> (Bres.) Morgan-Jones & E. Sutton
<i>Discostroma curinum</i> Brockmann Sydowia 28: 328 (1976)	280-360 × 150- 200 (300) µm, stroma of intra-epidermal, thick- walled hyphae.	85-120 × 6-9 µm, J ⁺ .	12-15 (-17) × 5.3- 6.5 µm, hyaline to yellowish, mostly 1-septate, but also 2- or 3-septate.	Dead twigs of <i>Rosa</i> spp.	<i>Sporocactus caninus</i> (Brun.) Brockmann
<i>Discostroma cupulum</i> (Ellis) M.E. Barr Mycotaxon 46: 55 (1993)	200-280 × 100-180 µm, clypeus present.	55-75 × 6-10 µm, J ⁺ .	10.5-13 (-16.5) × 5-6.5 µm, hyaline becoming light yellowish brown to dull brown, 1-septate.	Dead, overwintered, attached leaves of <i>Quercus coccinea</i>	Not reported
<i>Discostroma elegans</i> (H.J. Swart) Sivan, Trans. Br. mycol. Soc. 73: 217 (1979)	120-130 × 30-90 µm, perithecia frequently flattened by resistance of host tissue.	70-80 × 10 µm, J ⁺ , partly biseriolate.	18-22 × 4-5.5 µm, hyaline, 3 transverse septa.	Dead leaves of <i>Metaleuca ericifolia</i>	<i>Seimatosporium</i> <i>elegans</i> H.J. Swart
<i>Discostroma empetri</i> M.E. Barr Mycotaxon 51: 200 (1994)	200-290 µm in diameter, surrounded by circular, shiny clypeus.	80-93 × 9-10.5 µm, J ⁺ .	12-13.5 (-15) × 5.5-7 (-8) µm, hyaline, 1-septate.	Dead leaves of <i>Empetrum nigrum</i>	Not reported

Species	Ascomata	Asci ^a	Ascospores ^b	Habitat	Anamorph
<i>Discostroma ficicola</i> Paulus, Gadek & K.D. Hyde	112.5 - 175 × 112.5 - 150 μm, black, circular clypeus visible, at maturity erumpent and appearing as pustules.	(80 -) 90 - 110 × 10 - 12 μm, J*; uniseriate or biseriate	17 - 20 × 5 - 6.5 μm, hyaline, 1-septate.	Decaying leaves of <i>Ficus pleurocarpa</i>	Not observed
<i>Discostroma</i> <i>fruticosum</i> Z. Q. Yuan & M. E. Barr	200 - 250(-350) μm, erumpent to superficial.	90 - 130 (- 150) μm, J*.	18 - 26 × 6 - 8 μm, hyaline, (1 -) 3-(- 5) septate.	Twigs of <i>Pentaphylloides</i> <i>fruticosa</i>	Not reported
Sydowia 46: 330 (1994)					
<i>Discostroma fuscelum</i> (Beuk. & Broome) Huhndorf	300 - 500 μm diameter, 150 - 250 μm high, beneath a blackened clypeus.	(101) 115 - 132 (- 137) × 7 - 12 (- 14) μm, J*.	15 - 21 × 5 - 9.5 μm, hyaline, 3 (seldom - 4 or more) transverse septa, sometimes with 1 longitudinal septum.	<i>Cornus</i> , <i>Prunus</i> , <i>Ribes</i> , <i>Ros</i> , <i>Rubus</i> , <i>Viburnum</i> and <i>Vaccinium</i> spp.	<i>Seimatosporium</i> <i>lichenicola</i> (Corda) Shoemaker & <i>E. Müll.</i>
Ill. Nat. Hist. Surv. Bull. 34: 529 (1987)					
<i>Discostroma</i> <i>hypophloeum</i> (P. Karst.) O. E. Erikss.	250 μm in diameter, hypophyllous.	90 - 100 × 11 - 12 μm, J*.	14 - 17 × 7 - 8 μm, hyaline, 1-septate.	Leaves of <i>Andromeda</i> <i>tetragona</i>	Not reported
Non-lichenized Pyrenomyces of Sweden, p. 7 (1992)					
<i>Discostroma ledi</i> M. E. Barr	200 - 245 × 170 - 245 μm, beneath a shallow clypeus.	63 - 75 × 6 - 7 μm, J*.	10.5 - 12 × 4.5 - 5 μm, hyaline, 1-septate.	Leaves of <i>Ledum</i> <i>groenlandicum</i>	Probably <i>Sporocadus</i> sp.
Mycotaxon 51: 201 (1994)					