

The lichenicolous *Opegrapha* species (Roccellaceae, Ascomycota) with 3-septate ascospores on *Pertusaria* and *Ochrolechia*

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The *Opegrapha* species with 3-septate ascospores growing on *Pertusaria* and *Ochrolechia* are revised. Two species are recognized: *Opegrapha anomea* (of which *O. pertusariae*, *O. quaternella*, *O. wetmorei* and possibly *Leciographa weissii* are considered to be synonyms), and *O. blakii* Ertz & Diederich **sp. nov.** described from a sterile lichen with an *Ochrolechia*-like thallus, known from Ecuador and Venezuela. *Opegrapha anomea* and several related lichenicolous species with roundish or irregular, often multilocular ascomata are morphologically intermediate between *Opegrapha* and *Plectocarpon*, and might represent a distinct genus. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 144, 235–241.

ADDITIONAL KEYWORDS: Arthoniales – *Opegraphoidea* – *Plectocarpon* – taxonomy.

INTRODUCTION

In a recent paper, Cole & Hawksworth (2001) described the new lichenicolous fungus *Opegrapha wetmorei* M. S. Cole & D. Hawksw., growing on *Ochrolechia trochophora* in Minnesota in the USA. A careful search of the literature revealed the existence of at least five additional names referring to *Opegrapha* spp. described from *Pertusaria* or *Ochrolechia*. All have 3-septate ascospores, except *O. pertusariicola* Coppins & P. James, a relatively rare species confined to *Pertusaria leioplaca*, well characterized by 5–6-septate ascospores (Coppins & James, 1979). We herewith present a revision of all the species with 3-septate ascospores.

Some additional parasites of *Pertusaria* and *Ochrolechia* were described or combined in *Leciographa*, but appear to belong to *Dactylospora*. *Leciographa inspersa* (Tul.) Rehm and *L. homoica*

(Nyl.) Sacc. are considered to be synonyms of *Dactylospora parasitica* (Flörke) Zopf (Clauzade, Diederich & Roux, 1989). *Leciographa parellaria* (Nyl.) Sacc. is a synonym of the well-known *Dactylospora parellaria* (Nyl.) Arnold, and *Leciographa parvula* (Arnold) Sacc. (syn. *Dactylospora parvula* Arnold) is a name of uncertain application [type lost: M–]. As the epihymenium of *L. parvula* was said to be K+ red (Vouaux, 1912–14), it is not a synonym of any of the *Opegrapha* species mentioned below. The *Dactylospora* species are not discussed in the present paper and we did not revise any of their types.

MATERIAL AND METHODS

We studied specimens from BG, BR, H, M, MIN, NY and SBBG, and from the private collections of A. Aptroot, P. Diederich and K. Kalb. Hand-made sections were examined in water, 5% KOH (K), Congo Red with KOH pretreatment, or Lugol's reagent without (I) or with (K/I) KOH pretreatment. Measurements of asci and ascospores refer to material examined in KOH;

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those of ascospores do not include the perispore. Drawings were done using a drawing tube.

RESULTS

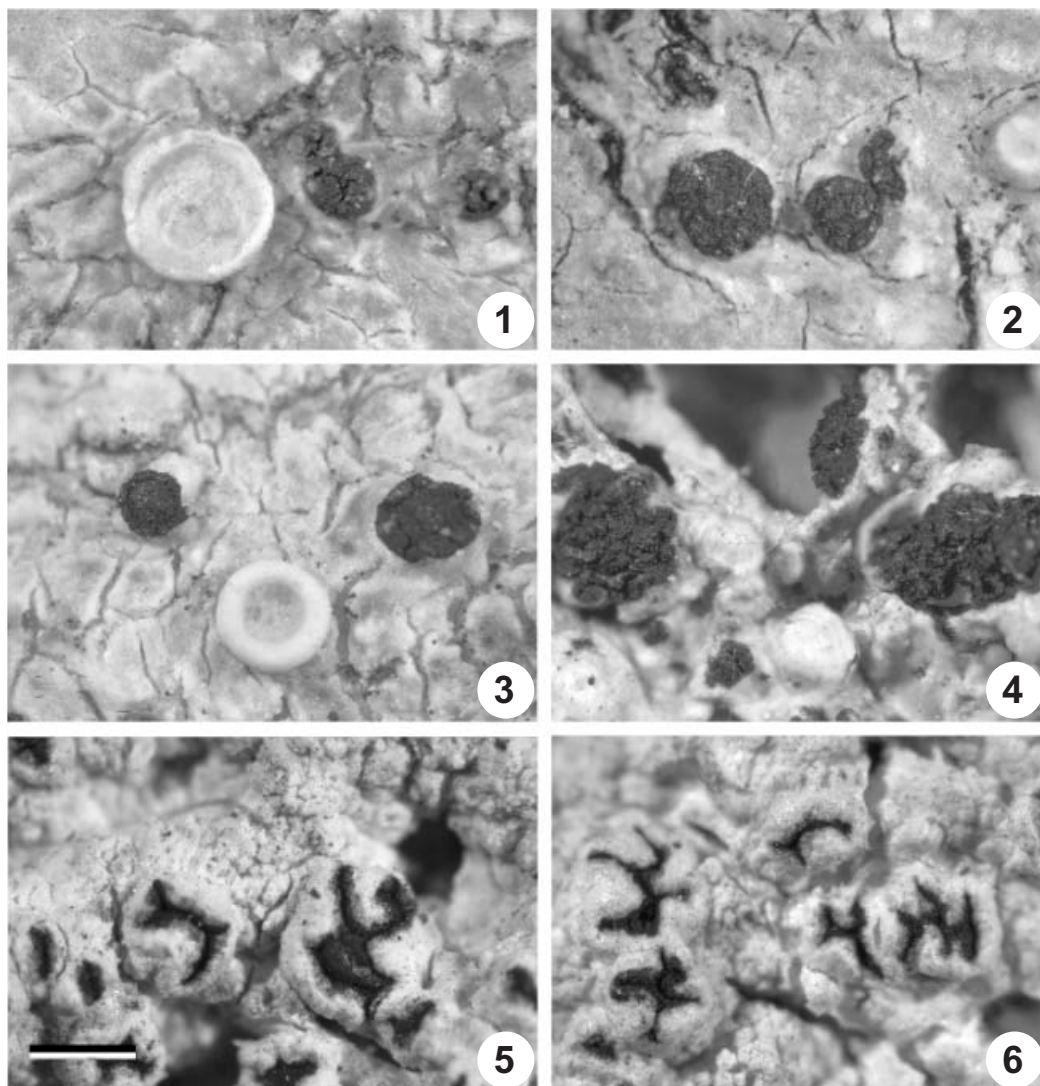
OPEGRAPHA ANOMEA NYL. (FIGS 1–4, 8)

Actes Soc. linn. Bordeaux 3 (1): 399 (1857); *Leciographa anomea* (Nyl.) Deichm.-Br. & Rostr., *Bot. Tidskr., I. R.* 3: 253 (1869); *Dactylospora anomea* (Nyl.) Arnold, *Flora* 57: 109 (1874); *Mycobilimbia anomea* (Nyl.) Sacc. & D. Sacc., in Saccardo, *Sylloge fung.* 18: 185 (1906). *Type*. France, Puy-de-Dôme, 'ad abietes prope Mont Dore Arvernica', on corticolous *Pertusaria*

amara, W. Nylander (H-NYL 6581 ! – lectotype, here designated; H-NYL 6580 ! – paratype).

Syn. nov. *Opegrapha quaternella* Nyl., *Flora* 68: 449 (1885); *Mycobilimbia quaternella* (Nyl.) Vouaux, *Bull. trim. Soc. mycol. France* 29: 440 (1913); *Opegraphoidea quaternella* (Nyl.) Fink, *The Lichen Flora of the United States*, University of Michigan Press, p. 102 (1935). *Type*: USA, Massachusetts, New Bedford, on *Pertusaria velata*, 1877, H. Willey (H-NYL 6341 ! – lectotype, here designated).

Syn. nov. *Leciographa pertusariae* Vouaux, in Pitard & Harmand, *Bull. Soc. Bot. France* 58, Mém. 22: 70



Figures 1–6. Figs 1–4. Ascomata of *Opegrapha anomea* on thallus of *Ochrolechia*. Fig. 1. Young, subspherical ascomata opening following irregular cracks (Tønsberg 25166); Fig. 2. Larger ascomata with a completely cracked surface (Tønsberg 15298); Fig. 3. Older ascomata, in which the upper sterile layer has mostly disappeared, except along the margin (Tønsberg 25166); Fig. 4. Large, mature ascomata of an irregular shape (holotype of *Opegrapha wetmorei*). Figs 5, 6. Ascomata of *Opegrapha blakii* sp. nov. (holotype). Scale bar = 500 µm.

(1911); *Opegrapha pertusariae* (Vouaux) Hafellner, *Herzogia* 10: 17 (1994). Type: Tenerife, Añavigo, 1000 m, on *Persea*, on *Pertusaria exalbescens*, February 1906, C. J. Pitard (PC – lectotype, here designated).

Syn. nov. *Opegrapha wetmorei* M. S. Cole & D. Hawksw., *Mycotaxon* 77: 322 (2001). Type: USA, Minnesota, Lake County, Superior National Forest, east of Pine Lake, north of Toimi, 19 miles south-east of Hoyt Lakes, 47°27'51"N, 91°45'26"W, on *Ochrolechia trochophora*, on *Thuja*, 21.vi.1999, C. M. Wetmore 82244 (MIN ! – holotype).

?*Leciographa weissii* Körb., *Verhandl. zool.-botan. Ges. Wien* 17: 707 (1867). Type: Jugoslavia, 'hab. lichenem in thallo sterili *Pertusariae* et *Ochrolechia* parasitice crescentem ad *Quercuum* cortices c. Podi prope Megline nec non c. Comolaz in valle Ombla Dalmatiae', E. Weiss (WRS� –, L –; type lost?).

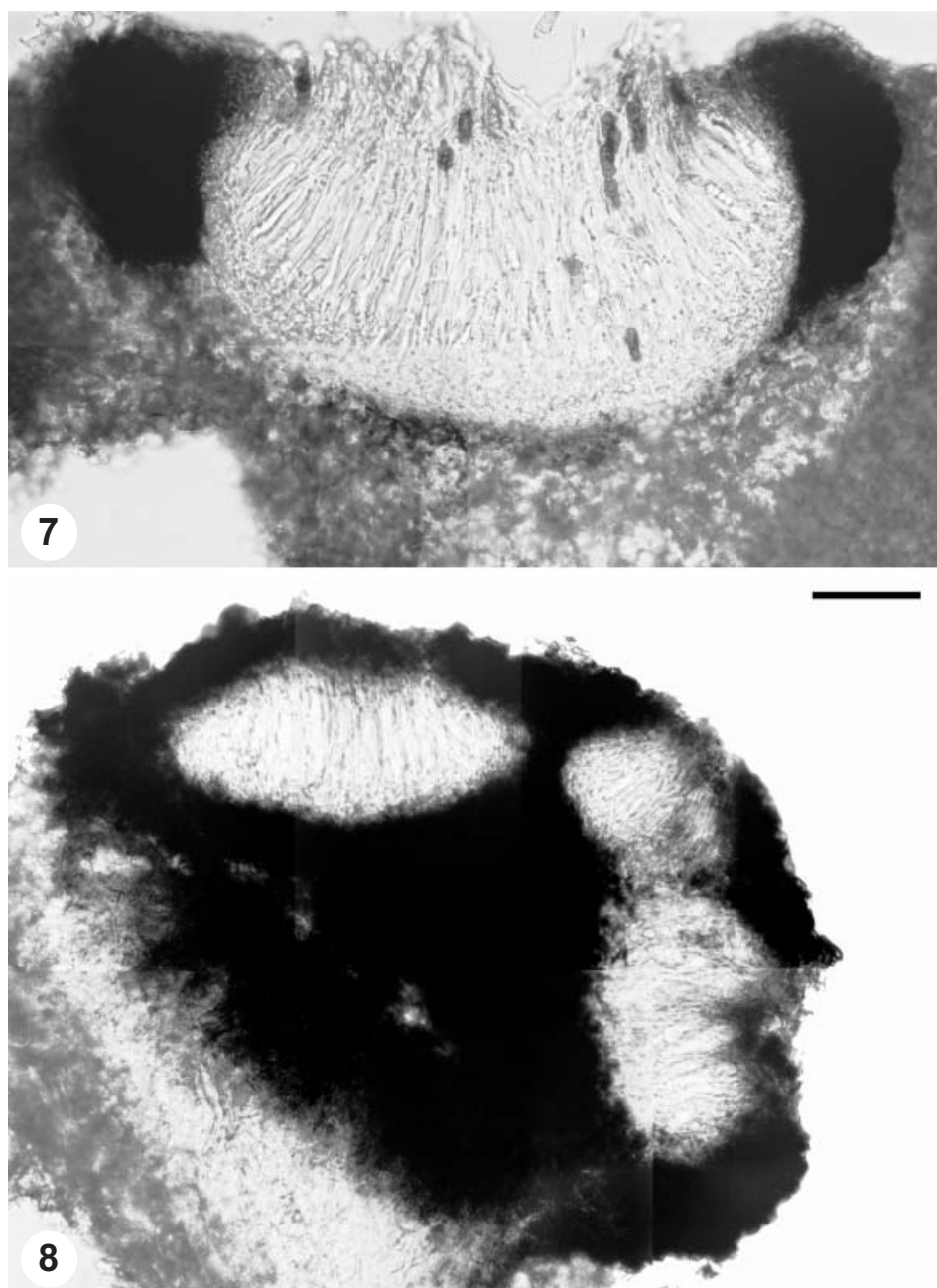
Description. Ascomata lichenicolous, black, epruinose; when young, roundish, subspherical, partly immersed in the host thallus and breaking through the host cortex; upper sterile stromatic layer soon breaking along irregular slits giving the ascoma a verruculose appearance, with remnants often remaining on margin and/or upper surface of the ascoma; mature ascomata regularly appearing aggregated in groups reaching 4 mm in diameter, as a result of concentric growth of one single ascoma in a later stage of development; in section, ascomata unilocular to multilocular. Exciple laterally and basally very variable in thickness (Fig. 8), dark brown, K-. Hypothecium hyaline, reduced, indistinguishable from the subhymenium. Hymenium hyaline, K-, I+ blue, then red, K/I+ blue, 75–85 µm thick; epihymenium brownish, K-; subhymenium hyaline, 7–10 µm. Paraphyses branched and anastomosed, 2–2.5 µm thick, apically indistinctly swollen. Asci subclavate, 4(–8)-spored, 35–40 × 8–13 µm, wall I-, K/I-, except for an apical K/I+ blue ring. Ascospores hyaline, becoming brown and coarsely verrucose at maturity, elongate ellipsoid, 3-septate, slightly constricted at the medium septum, 17–26 × 6.5–9 µm, with a distinct, hyaline perispore 0.5 µm thick; brown pigment located on the ascospore wall. Pycnidia unknown.

Distribution and hosts. We have examined specimens from France (Puy-de-Dôme, Pyrénées-Atlantiques, Vosges), Canada (British Columbia, Nova Scotia) and the USA (Alaska, California, Massachusetts, Minnesota, Mississippi, Washington), on *Ochrolechia* species, including *O. androgyna* and *O. trochophora* (thallus and margin of apothecia), and on *Pertusaria*, including *P. amara*, *P. epixantha* and *P. velata* (thallus). The species is furthermore known from the

Canary Islands (Tenerife) on *Pertusaria exalbescens* (type of *L. pertusariae*) and from Norway on *P. amara* (Holien, 2001).

Discussion. Examination of the type material of *Opegrapha anomea*, *O. quaternella* and *O. wetmorei* showed that they all represent the same species, for which *O. anomea* is the oldest name available. We were not able to trace any type material of *Leciographa weissii*, but the original description, although relatively short, and the hosts suggest the same species. The type of *O. pertusariae*, located in PC, was examined by Hafellner (1994), but, unfortunately, was not available to us for study. Hafellner (1994) stated that *O. pertusariae* is distinguished from *O. anomea* by ascospores becoming brownish at maturity, whilst they remain hyaline in *O. anomea*. During the study of our rich material of *Opegrapha* on *Pertusaria*, we were able to demonstrate that this character is highly variable and reflects a degree of maturity. Moreover, we observed that mature ascospores in the type material of *O. anomea* are brown with a verrucose pigmentation. We conclude that *O. pertusariae* does represent an additional synonym of *O. anomea*.

Cole & Hawksworth (2001) described the ascomata as being 'shortly lirelliform to rounded, with a single slit exposing the hymenium or with several slits extending to the margin, black, shiny, with convex raised margins'. The study of abundant material, mainly from North America, allowed us to recognize a very great variability within the species, and improve our understanding of the development of the ascomata. When young, ascomata are blackish, roundish, subspherical, sometimes little elongate, partly immersed in the host thallus and breaking through the host cortex. The upper sterile stromatic layer of ascomata breaks along irregular slits giving them a verruculose appearance. When the ascoma grows, remnants of the initial upper layer often remain present on the margin and on the upper surface of the ascoma. In some specimens, these remnants entirely disappear over the whole ascomatal surface, except at the margin, where they appear as an elevated, raised, cracked exciple around a smooth, flat, or slightly concave or convex ascomatal 'disc'. Such ascomata have the appearance of lirellae of *Opegrapha* species with roundish to ellipsoid ascomata. Other ascomata, in which the entire surface remains convex and partly covered by sterile remnants of the original stromatic cover, are reminiscent of species of *Plectocarpon*. We were occasionally able to observe *Plectocarpon*-like ascomata growing beside *Opegrapha*-like ascomata. In some specimens (e.g. the types of *O. quaternella* and *O. wetmorei*), the ascomata regularly appear aggregated in groups reaching 4 mm in diameter; they



Figures 7–8. Fig. 7. Section through an ascoma of *Opegrapha blakii* sp. nov. (holotype), showing the dark lateral exciple that is reduced below the hymenium. Fig. 8. Section through a multilocular ascoma of *Opegrapha anomea* (Tønsberg 29854), showing the massive lateral and basal exciple, and the stromatic tissue partly covering the hymenium. Scale bar = 50 µm.

obviously represent ascomata at an older stage of development, that became larger by concentric growth.

In section, distinctly multilocular to almost continuous unilocular hymenia were seen. However, in this latter case the hymenium is often intermixed with sterile stromatic tissue, dividing it indistinctly into several fertile loculi. Furthermore, sterile

stromatic fragments are often present over the hymenium.

The delimitation between *Opegrapha* and *Plectocarpus* is not at all clear to us; several species included in *Opegrapha*, *Opegraphoidea* or *Leciographa* present, like *O. anomea*, broad, roundish, uni- to multilocular ascomata that are often irregular and very variable in form and development. These include species

such as *Opegrapha physciaria* (Nyl.) D. Hawksw. & Coppins (Coppins, James & Hawksworth, 1992), *O. plectocarpoidea* Diederich (Aptroot *et al.*, 1997), *O. pulvinata* Rehm (Coppins, 1987), *O. sphaerophorica* Isbrand & Alstrup (Isbrand & Alstrup, 1992), *O. zwackhii* (A. Massal. ex Zwackh) Källsten ex Hafellner (Hafellner, 1994) and *Opegraphoidea stau-rothelica* Fink ex Hedrick (Hedrick, 1933). Whether these species belong to *Opegrapha* s.s., or whether they represent a distinct genus cannot be decided with our current knowledge based on morphological data only. Molecular phylogenetic studies are required to improve our knowledge of the relationships among typical and 'intermediate' taxa.

In the original description of *O. wetmorei*, the spores were said to be subhyaline, becoming pale brown, with walls coarsely ornamented with refractive warts c. 0.5 µm diameter. We were able to demonstrate that the spore wall is covered by granules of brown pigment giving them the appearance of ornamentation. As in most *Opegrapha* species with brownish ascospores, the granules are formed in the perispore that later collapses, depositing them on the outside of the spore wall. In *O. pulvinata*, Coppins (1987) observed that the brownish pigment is located in the spore wall, not in the perispore. The location of this pigment in the ascospores of many *Plectocarpon* and *Opegrapha* species has never been studied in the past, but might prove to be an important taxonomic character in this group.

Additional specimens examined (unless otherwise mentioned, all on *Ochrolechia*). CANADA: BRITISH COLUMBIA. N of Whistler, N of Pemberton Valley, 9–10 km from Lillooet River bridge along the road to Bralorne, 50°32'N, 123°03'W, 400 m, 1989, *Tønsberg* 12784 (BG L-69105). NOVA SCOTIA. Shelburne County, along Hwy 302 just S of Upper Ohio, at Philip Lake, 43°58'N, 62°25'W, on *O. androgyna*, 1999, *Harris* 43047 (NY). USA: ALASKA. Cordova, Copper River delta, along Copper River Hwy, SE of the airport, 60°29.0'N, 145°25.9'W, 30 m, 2001, *Tønsberg* 29854 (BG L-71738). Kodiak Island Borough, Kodiak Island E, NW of Kodiak, Anton Larsen Bay SW, 1.6 mi along the road from the head of the bay, 57°52'N, 152°38'W, 10 m, 1991, *Tønsberg* 15298 (BG L-69074). CALIFORNIA. Santa Barbara County, Santa Cruz Island, Ridge Road at Saucos Canyon Road Gate, on *P. amara*, 1997, *Tucker* 35850 (BR, SBBG, hb Diederich). Santa Cruz Island, pine forest along ridge road at Sances Gate, 34°00'30"N, 119°48'30"W, 400 m, on *P. amara*, 1994, *Nash* 32275 (ASU). Santa Cruz Island, 4.5 km E of radar station, ridge crest down N slope, 34°00'15"N, 119°37'30"W, oak-pine woodland, on *P. amara*, 1994, *Nash* 32454 (ASU). Santa Barbara County, Santa Rosa Island, pass along the main road just SE of Black

Mountain, 33°58'45"N, 120°04'30"W, 300 m, mixture of oaks, grassland and chaparral, on *Pertusaria*, 1994, *Nash* 32588 (ASU). MISSISSIPPI. Jasper County, Bienville National Forest, Forest Serv. Road 506–3 at E edge of national forest by small cemetery, 32°12'N, 89°13'W, 105 m, on *P. epixantha*, 1992, *Harris* 28865 p.p. (NY). WASHINGTON. Clallam County, Olympic Nat. Park, Lake Crescent, the shore SE of Pyramid Mtn summit, NNW of Barnes Point, 48°04'N, 123°48'W, 180 m, 1997, *Tønsberg* 25041 (BG L-71775). Kittitas County, 23 km (direct) SSE of Snoqualmie Pass, S of Hwy 90, SW of Kachess Lake, between Yakima River and road FR 4283, 47°16'N, 121°17'E, 680 m, 1997, *Tønsberg* 25166 (BG L-35363). San Juan County, Shaw Island, the small peninsula between Squaw Bay and Indian Cove, just E of Squaw Bay, 48°33.6'N, 122°56.6'W, 10 m, on *P. amara*, 2000, *Tønsberg* 28758 (BG L-71947). FRANCE: PYRÉNÉES-ATLANTIQUES. SW de Larrau, près de la route vers l'Espagne, on *P. amara*, 1990, *Diederich* 9179 (hb. Diederich). VOSGES. 1 km WNW of Kastelberg, Faigue d'Artimont, on *P. amara*, 1975, *van der Knaap* & *van Dobben* (hb. Aptroot, hb. Diederich).

OPEGRAPHA BLAKII ERTZ & DIEDERICH SP. NOV.

(FIGS 5–7, 9)

Opegrapha lichenicola insignis ascomatibus atris, epruinosis, lirellatis, angustis ad ellipsoideis, saepe ramosis, in gallis albidis 0.5–2 × 0.3–1.2 mm, excipulo laterale atrobrunneo, K-, 40–65 µm, excipulo basale pallide brunneo 18–26 µm, hypothecio indistincto, hymenio 85–115 µm, ascis subclavatis, 58–63 × 14–17 µm, 4-sporis, ascosporis 3-septatis, hyalinis ad brunneis, verrucosis, 17–21 × 6.5–7.5 µm, perisporo hyalino 0.5 µm.

Type. VENEZUELA, Tachira, distr. Jauregui, bei El Hato, zwischen Bailadores und Pregonero, 8°05'N, 71°55'W, 2750 m, 13.viii.1989, K. & A. *Kalb* 29389 (hb. *Kalb* – holotype; hb. Diederich – isotype); *ibid.*, K. & A. *Kalb* 29390 (hb. *Kalb*; BR – topotypes).

Description. Ascomata lichenicolous, dispersed, not agglomerate, lirellate, with a narrow to broad, slit-like to rarely ellipsoid, often branched opening and a distinctly visible, black, epruinose disc, surrounded by a thick, whitish, elevated margin of the host, sometimes appearing as white galls, often covering and hiding the black exciple of the parasite; galls 0.5–2 × 0.3–1.2 mm, disc 0.1–1.5 × 0.05–0.4 mm; thalline margin 0.1–0.2(–0.3) mm. Exciple laterally thick, dark brown, K-, 40–65 µm, with only the inner part continuing basally into a thin, pale brown, layer, 18–26 µm thick. Hypothecium hyaline, reduced, indistinguishable from the subhymenium.

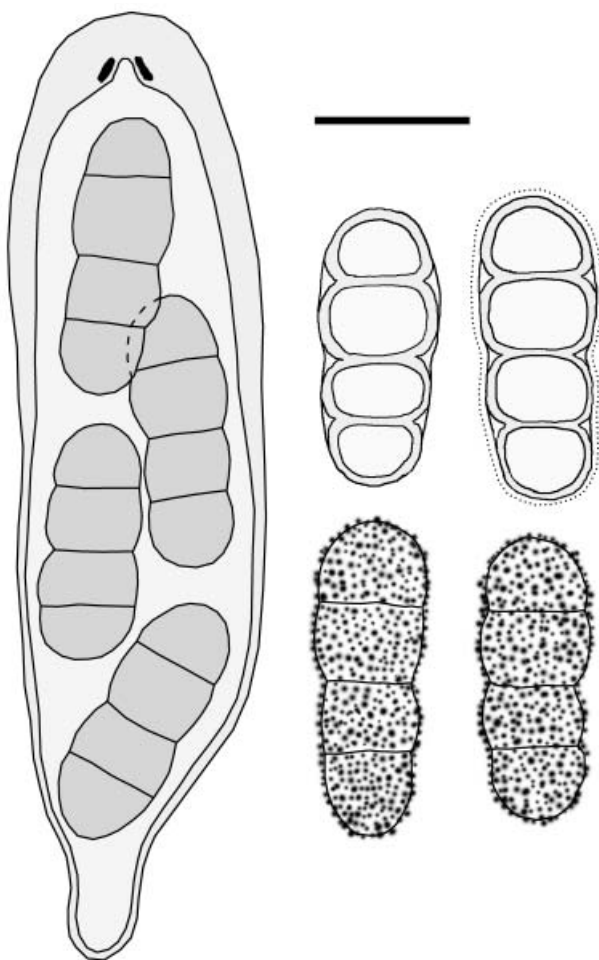


Figure 9. *Opegrapha blakii* sp. nov. (holotype). Ascus with four ascospores observed in K/I; two hyaline ascospores in section (perispore represented in one ascospore); two mature, brown, verrucose ascospores in surface view. Scale bar = 10 μ m.

Hymenium hyaline, K-, I+ blue, then red, K/I+ blue, 85–115 μ m thick; epihymenium brownish, K-; subhymenium hyaline, 7–10 μ m. Paraphyses branched, anastomosed, 2–2.5 μ m thick, apically indistinctly swollen, up to 3.5 μ m. Asci subclavate, 4-spored, 58–63 \times 14–17 μ m, wall I-, K/I-, except for an apical K/I+ blue ring. Ascospores hyaline, becoming brown and coarsely verrucose at maturity, elongate ellipsoid, 3-septate, slightly constricted at the septa, 17–21 \times 6.5–7.5 μ m; perispore of hyaline ascospores 0.5 μ m thick, indistinct in brown ascospores. Pycnidia unknown.

Etymology. Named after Klaus Kalb (*blak* in reverse order), collector of the new species.

Distribution and host. Known from Ecuador and Venezuela. It grows on an unidentified, sterile, corti-

colous, crustose lichen, with a greyish white thallus, and indistinct to irregular, sometimes pustulate or confluent soralia; thallus K-, C + red, KC + red, UV- (gyrophoric acid). The host appears to be the same for all known specimens and looks very similar to *Ochrolechia androgyna*. We did not study it further, as the *O. androgyna* group is in an urgent need of critical revision in Europe, making definitive identification of the South American specimens premature at this time. The host thallus is not much affected by the presence of the parasite, except that it is distinctly elevated around each ascoma.

Discussion. Macroscopically, this species looks very distinct with its lirellate, branched ascomata usually surrounded by a prominent whitish margin formed by the host thallus. Although the host is not identified with certainty, we consider it appropriate to study this species together with the other *Opegrapha* species growing on *Ochrolechia* and *Pertusaria*. Microscopically, we were surprised to find almost no difference between *O. blakii* and *O. anomea*, and for some time we wondered if the former could be an extreme form of the latter, possibly representing a reaction to a specific host thallus. However, careful examination of microscopical sections of many ascomata of both species convinced us that they are in fact distinct. Apart from the macroscopic differences, one additional difference is always present. In *O. blakii*, the exciple is laterally thick, prominent and always dark brown, but basally distinctly thinner, and always much paler, often almost appearing as discontinuous with the lateral exciple. In *O. anomea*, the lateral and basal exciple are both dark brown and continuous, and the basal exciple is usually much thicker than that of *O. blakii*. There are additional differences: the asci in *O. anomea* are much shorter than those in *O. blakii*, the hymenium is lower and the ascospores are slightly broader.

Additional specimen examined. ECUADOR: AZUAY. c. 35 km südlich von Cuenca, 3200 m, Waldreste in einem Grasparamo, 1987, Kalb 18420 (hb. Kalb).

ACKNOWLEDGEMENTS

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