## Revision of the *Opegrapha* species with muriform ascospores (previously *Dictyographa*) (lichenized *Roccellaceae*)

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Abstract: Opegrapha species with muriform ascospores are revised and two species, differing only in the shape of their excipulum, are accepted: O. arabica, a widespread coastal species, and O. varians, known from mainland Yemen and Socotra. The genus Dictyographa, previously distinguished from Opegrapha by its muriform ascospores, is reduced into synonymy with Opegrapha. Dictyographa angolensis and D. sandwicensis are new synonyms of O. arabica; the type of D. cinerea was found to be a mixture of different species of the Hysteriaceae; and D. contortuplicata and D. psyllocarpa are excluded from the Arthoniales. Lectotypes are designated for D. angolensis, D. contortuplicata and D. sandwicensis.

Key words: Arthoniales, Graphidaceae, Hysteriaceae

#### Introduction

The genus *Dictyographa* Müll. Arg. was introduced by Müller (1893) to accommodate *Graphina varians* and the new species *D. arabica*, both differing from *Opegrapha* in having muriform ascospores, and from *Graphina* in having anastomosing paraphyses. Later, six other taxa were described or combined in *Dictyographa*. Meanwhile, *D. epiphylla* was excluded from the genus and combined in *Aulaxina* (Santesson 1952).

As part of a revision of the paleotropical species of *Opegrapha*, we have examined all the available material of *Dictyographa*. The aim of this paper is to present a revision of all these taxa, and to propose the synonymy of *Dictyographa* with *Opegrapha*.

#### Material and Methods

Specimens from B, BM, CBG, G, PH, TUR, UPS and the private herbaria of M. Schultz and M. R. D. Seaward have been studied. Hand-cut sections were examined in water, 5% KOH (K), or Lugol's reagent (1% I<sub>2</sub>)

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without (I) or with (K/I) KOH pre-treatment. Measurements of asci and ascospores all refer to material examined in KOH; those of ascospores do not include the perispore. Drawings were done using a drawing tube. Ascospore measurements are indicated as (minimum–)  $\tilde{X}-\sigma_X-\tilde{X}+\sigma_X$  (–maximum), followed by the number of measurements (n); the length/breadth ratio of ascospores is indicated as I/b and given in the same way. Macroscopic photos were made with an Olympus SZX12 binocular microscope, with a ColorView I digital camera and AnalySIS 5.0 software. Microscopic photos were prepared with an Olympus BX51, and the same digital camera.

#### Opegrapha Ach.

Syn. nov.: Dictyographa Müll. Arg., Bull. Herbier Boissier 1: 131 (1893); type: Dictyographa arabica Müll. Arg.; non Dictyographa Darb., Ber. Deutsch. Bot. Ges. 15: 6 (1897); type: Dictyographa gracillima (Kremp.) Darb. [=Ingaderia gracillima (Kremp.) Feige & Lumbsch]

Species of *Dictyographa* Müll. Arg. are similar to *Opegrapha* Ach. in that they both have lirelliform ascomata with a carbonized excipulum, anastomosing paraphysoids, a K/I+ blue hymenial gel, clavate asci with an apical K/I+ blue ring, I – ascospores, etc. Matzer (1996: 94) examined the type specimens of *D. arabica* and *D. varians* and concluded that the genus *Dictyographa* is related to *Opegrapha*, from which it differs only by having muriform ascospores. As this

character alone does not justify recognition of a distinct genus, we propose synonymizing *Dictyographa* with *Opegrapha*. A similar situation is known with many other recently revised genera, such as *Arthonia* (Sundin & Tehler 1998), *Acanthothecis*, *Carbacanthographis*, *Fissurina*, *Glyphis*, *Graphis* (Staiger 2002), *Haematomma* (Staiger & Kalb 1995), *Sclerophyton* (Sparrius 2004) and *Strigula* (Roux & Sérusiaux 2004), all of which in-

clude species with muriform ascospores, as well as species with only transversally septate ascospores. The species of *Dictyographa* should also be compared with the genus *Lecanographa* because of their pruinose ascomata. However, the latter genus differs from *Opegrapha* by the presence of ellipsoid to shortly lirelliform ascomata and a different ascus type (*Grumulosa*-type as defined by Egea & Torrente 1994).

#### Key to the species of Opegrapha with muriform ascospores

#### Opegrapha arabica (Müll. Arg.) Vain.

Catal. Welwitsch Afric. Plants 2: 442 (1901).—Dictyographa arabica Müll. Arg., Bull. Herbier Boissier 1: 131 (1893); type: Yemen, mit Roccella montagnei am Schemsan über Aden, 1892, Schweinfurth [L. Arab. No. 14] (G!—holotype).

Syn. nov.: Opegrapha angolensis Vain., Catal. Welwitsch. Afric. Plants 2: 441 (1901).—Dictyographa angolensis (Vain.) A. Zahlbr., Cat. Lich. Univ. 2: 285 (1924); type: Angola, Loanda, Boa Vista, ad ramulos Balsamodendri, Febr. 1854, C. Welwitsch 361 (TUR-Vainio 28154!—lectotype, here designated; BM!—isolectotype).

Syn. nov.: Dictyographa sandwicensis A. Zahlbr., in Magnusson & Zahlbruckner, Arkiv för Botanik 31A(1): 27 (1944); type: Hawaiian Islands, Kauai, Hanalei, corticolous, Jan. 1910, U. Faurie (UPS!—lectotype, here designated).

(Figs 1, 2, 6B & 7)

Thallus white or pale grey, inconspicuous or thin, c. 0.1 mm thick, continuous or with few to numerous cracks; surface smooth and  $\pm$  shiny, sometimes  $\pm$  white pruinose.

Ascomata numerous,  $\pm$  dense, scattered, rarely in groups of 2–5, lirelliform, slightly to more rarely strongly flexuous, simple or with one or two short to long branches, first immersed in the thallus, becoming  $\pm$  half immersed, more rarely entirely sessile over an inconspicuous or thin thallus, 0.3-2.5 (-3) × 0.2-0.3 mm; excipulum black, usually epruinose; hymenial disc slit-like or narrowly to  $\pm$  widely exposed, white pruinose. *Excipulum* dark brown, K+ slightly oliva-

ceous, broadly discontinuous under the hypothecium, 25–60 µm wide laterally. Hypothecium hyaline to pale brown, 7–20 μm tall, I+ red. Hymenium hyaline, not inspersed with oil droplets, 75-110 µm tall; hymenial gel I+ directly red, K/I+ blue. Epihymenium hyaline to dark brown, K+ slightly olivaceous, I+ red or bluish. Paraphysoids richly branched and anastomosing, 1 µm wide, apically slightly enlarged, up to 2 μm wide. Asci clavate, 4–8-spored, 65–  $100 \times 19-26 \,\mu\text{m}$ , with a small ocular chamber and a distinct apical K/I+ blue ring. Ascospores hyaline, submuriform, oblong, upper half slightly wider, sometimes slightly constricted in the middle and appearing  $\pm$  biclavate, with 7–9 transverse septa, 1–6 cells divided by one or two longitudinal septa, septation starting with one median  $(-37) \times (5.5-)6.5-8.8(-12) \mu m$ , 1/b 2.7-3.8(n=132); perispore hyaline, 2–3 µm thick; brown pigmentation not observed.

*Pycnidia* rare, usually found near the margin of the thallus, entirely immersed in the thallus, visible as pale to dark brown spots of c.  $60-100 \, \mu m$  diam.; wall broadly discontinuous below, dark brown; conidia hyaline, aseptate, sickle-shaped,  $13.5-19.0 \times 0.5-1.0 \, \mu m$ .

Chemistry. Thallus K-, C-, KC-, Pd-; UV+ white; TLC: no acids detected.

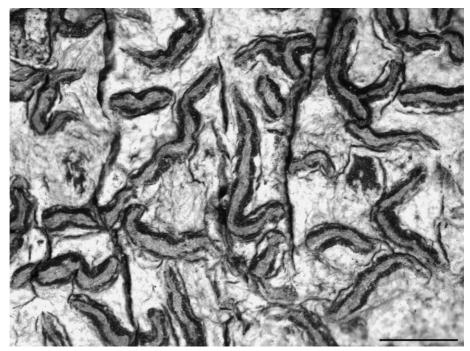


Fig. 1. Opegrapha arabica, thallus with lirellae (Streimann 42471). Scale bar=1 mm.

Distribution and ecology. Opegrapha arabica is a widespread coastal species known from Angola, Kenya, Tanzania, Seychelles (Mahé and Aldabra), Yemen, Papua New Guinea, Australia and Hawaii. It has been collected on Adansonia, Avicennia marina, Balsamodendron, Plumiera, Rhizophora and Xylocarpus granatum in Africa, on Clerodendrum, Cocos nucifera, Ficus reflexa, Lumnitzera, Pandanus tectorius, Pemphis, Sideroxylon inerme, Thespesia populneoides in the Seychelles, and on Casuarina equisetifolia in Australia, always in coastal vegetation, especially mangroves.

Notes. The description of Dictyographa sandwicensis was published by A. H. Magnusson, based on a manuscript of A. Zahlbruckner, who died in 1938 (Magnusson & Zahlbruckner 1944). No specimens under that name are present in W, WU and PAD, but one has been found in UPS and we have therefore designated it as the lectotype. The specimen Nannfeldt 22037a (UPS) helps us to understand the

great variability of the external characters of O. arabica. The lirellae are first immersed in the thallus (as in the holotype), later become  $\pm$  half immersed (as in the type specimen of D. sandwicensis), with a slit-like hymenial disc. The ascomata can sometimes be entirely sessile with an inconspicuous or thin thallus (as in the type of O. angolensis) and the hymenial disc can become  $\pm$  widely exposed and white pruinose (as in Streimann 42471).

Additional specimens examined. Angola: Loanda, prope Praia de Zamba, ad ramulos Adansoniae, C. Welwitsch 369 (TUR-Vainio 28155!, BM!—syntypes of Opegrapha angolensis); Loanda, in insula Loanda, ad truncos Rhizophorarum, C. Welwitsch 351 (TUR-Vainio 28153!, BM!—syntypes of Opegrapha angolensis).— Kenya: Coastal prov.: Kilifi distr., Mida Creek, Mida, <10 m, 3°19'S, 39°58'E, on old fallen branches of Avicennia marina in the mangrove zone, 1970, Lundqvist 6374-b (UPS); ibid., on A. marina, Santesson 20924a, 20925a (UPS); ibid., on bark of Avicennia, 1972, Nannfeldt 22039 (UPS); Kwale distr., Moonlight Bay c. 20 km S of Mombasa, on the beach, on Plumiera, 1972, Nannfeldt 22037a (UPS).—Tanzania: Tanga, 2.5 km NE of Pangani, 5°25′S, 38°59′E, on branches of Xylocarpus granatum (mangrove), 1971, Moberg 1502

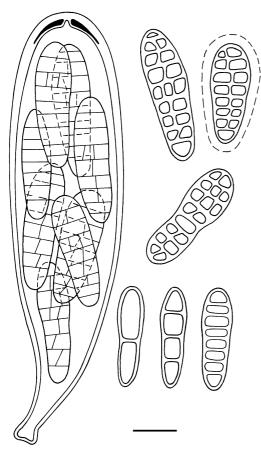


Fig. 2. Opegrapha arabica (Streimann 42471), ascus with apical K/I+ blue ring, and ascospores (three mature above, three immature below). Scale bar= $10 \ \mu m$ .

(UPS); ibid., Santesson 23495 (UPS; Moberg Lich. Sel. Exs. Upsal. 213).—Seychelles: Mahé: North Point, on Cocos, 1994, Feuerer 60588 (hb. M.R.D. Seaward). Aldabra: Cinque Cases, on Sideroxylon inerme, 1973, Hnatiuk 730191 (BM); ibid., on Pandanus tectorius, Hnatiuk 730148, 730149 (BM); ibid., on Thespesia populneoides, Hnatiuk 730156 (BM); ibid., on Lumnitzera, Hnatiuk 730135 (BM); ibid., on Ficus reflexa, Hnatiuk 730120 (BM); ibid., Ile Polymnie, on Clerodendrum, Hnatiuk 730981 (BM); ibid., on Cocos nucifera, Hnatiuk 731280, 731839 (BM); ibid., La Gigi, on twigs of Pemphis, Hnatiuk 730107, 730108 (BM).-Papua New Guinea: Madang prov.: c. 10 km W of Bogia, Laing Island in Hansa Bay, 4°10.4'S, 144°52.5'E, 1 m, coastal primary forest, E-side, 1995, Sipman 38751 (B).—Australia: Northern Territory: Melville Island, Condor Point, 11°44'S, 131°17'E, 2 m, strand vegetation dominated by Acacia auriculaeformis, Gyrocarpus, Thespesia and Diospyros maritima, on Casuarina equisetifolia, 1989, Streimann 42471 (CBG, B).

#### Opegrapha varians (Müll. Arg.) Vain.

Catal. Welwitsch Afric. Plants 2: 442 (1901).—Graphina varians Müll. Arg., Proceed. Roy. Soc. Edinburgh 11: 468 (1882).—Graphis varians (Müll. Arg.) Stizenbg., Bericht über die Tätigk. St. Gallisch. Naturw. Gesellsch. 1889–90: 187 (1891).—Dictyographa varians (Müll. Arg.) Müll. Arg., Bull. Herbier Boissier 1: 131 (1893); type: Socotra, in ramis Dracaenae, 700 m, 1881, Schweinfurth (G!—holotype).

(Figs 3, 4, 5, 6A & 7)

Thallus pale grey to olivaceous, c. 0.2 mm, rimose to areolate; surface smooth, matt to slightly shiny,  $\pm$  white pruinose.

Ascomata numerous,  $\pm$  dense, scattered, rarely in groups of 2-5, lirelliform, rarely almost punctiform, straight, curved, often slightly flexuous, simple, more rarely with one to three branches,  $\pm$  half immersed the thallus to sessile,  $0.5-2(-3) \times$ 0.2-0.3 mm; excipulum black, usually epruinose; hymenial disc soon narrowly to  $\pm$  widely exposed, densely white pruinose. Excipulum dark brown, K+ olivaceous, continuous under the hypothecium (sometimes thinner than laterally, but never discontinuous),  $40-85 \,\mu\text{m}$  wide laterally,  $30-75 \,\mu\text{m}$ wide basally. Hypothecium pale brown, 30-45 μm tall, I+ red. Hymenium hyaline, not inspersed with oil droplets, 100–110 µm tall; hymenial gel I+ directly red, K/I+ blue. Epihymenium pale to dark brown, K+ olivaceous, I+ red. Paraphysoids branched, anastomosing, 1 µm wide, apically slightly enlarged, up to 2 µm wide. Asci clavate, (6-)8-spored,  $90-105 \times 20-22 \mu m$ , with a small ocular chamber and a distinct apical K/I+ blue ring. Ascospores hyaline, submuriform, oblong, upper half slightly wider, slightly constricted in the middle, with 8-10(-11) transverse septa, often 1-7cells divided by one or two longitudinal septa, septation starting with one median transverse septum, I -, (22.5-)24.6-31.9 $(-36) \times (6.5-)7.2-8.6(-9) \mu m$ , 1/b 3.3-3.9 (n=46); perispore hyaline, 2–2·5 µm thick; brown pigmentation not observed.



Fig. 3. Opegrapha varians, thallus with lirellae (Hein 7992b2). Scale bar=1 mm.

*Pycnidia* rare, near the margin of the thallus, entirely immersed in the thallus, visible as pale to dark brown spots of c. 50–80  $\mu$ m diam.; wall dark brown near the ostiole, pale brown to almost hyaline below; conidia hyaline, aseptate, sickle-shaped,  $13-20 \times 0.5-1 \mu m$ .

Chemistry. Thallus K-, C-, KC-, Pd-; UV+ white; TLC: no acids detected.

Distribution and ecology. Opegrapha varians is known from two specimens from mainland Yemen and Socotra (Sipman 2002), collected from twigs of *Boscia arabica* and *Dracaena* sp.

Notes. Opegrapha varians is microscopically similar to O. arabica. The species differ in the structure of the excipulum: continuous under the hymenium in O. varians and discontinuous in O. arabica. In dead lirellae, this character is visible under the binocular microscope, as the entire

hymenium quickly disappears, whereas the more resistant excipulum persists for a longer time. In *O. varians*, the excipulum then looks like a blackish canoe. This is never the case in *O. arabica* where the degraded lirellae are composed of the two lateral parts of the excipulum between which the substratum (bark) is exposed.

Additional specimen examined. Yemen: Gov. Al Mahra, 15 km W of Al Fatk, southern flank of Jabal Faydami, E of highest peak, seasonally humid (luff) side, 350–450 m, 52°35′E, 16°30′N, steep rocky slopes and cliffs with ledges, clefts and fissures (affected by monsoonal mist), sandstone, on twigs of Boscia arabica tree (Hein 7992a), with Diploicia canescens, 2000, Hein 7992b2 (B).

# Excluded species Dictyographa cinerea (C. Knight & Mitt.) Müll. Arg.

Bull. Herbier Boissier 2, appendix I: 78 (1894).— Opegrapha cinerea C. Knight & Mitt., Trans. Linn. Soc. London 23: 101 (1860); type: New Zealand, Knight (BM!—lectotype designated by Hayward 1977).

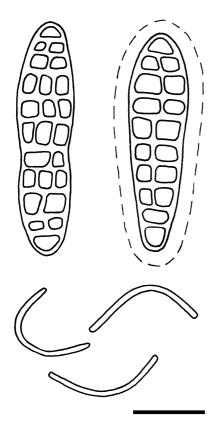


FIG. 4. Opegrapha varians, two ascospores and three conidia (Hein 7992b2). Scale bar=10  $\mu m$ .

Several type specimens are present in BM. We examined them all and concluded that none of them belongs to the Roccellaceae, because of the negative iodine reactions of the hymenium (I - and K/I -) and the missing K/I+ blue apical ring of the asci. They are probably all members of the Hysteriaceae. The lectotype specimen designated by Hayward (1977) (BM000764659) consists of three pieces of bark (two of which are grouped and one is separate) with nonpruinose, black, shiny, slit-like lirellae and 3-septate ascospores. They obviously belong to two distinct species of non-lichenized fungi of the Hysteriaceae. The separate piece has hyaline to pale brown, 3-septate ascospores constricted at the septa, measuring  $26 \times 9 \,\mu\text{m}$ . Following the identification key of Zogg (1962), it might belong to

Gloniella. The two grouped pieces have dark brown, 3-septate ascospores,  $18-21 \times 7-$ 8 µm. This specimen clearly belongs to the genus Hysterium and might be H. pulicare Pers.:Fr., according to the identification key of Zogg (1962). One of the isolectotypes from BM (BM000764658) is identical to the specimen of *Hysterium*. Another isolectotype (BM000764446) belongs abbreviatum (Schw.) Lohman. It fits perfectly the description of that species in Zogg (1962): ascomata elongate with a welldeveloped black basal and marginal tissue; hymenium hyaline, not inspersed with numerous oil droplets, K/I -; paraphysoids branched, 1–1·5 μm diam.; asci cylindrical,  $40-45 \times 5-6 \,\mu\text{m}$ , 8-spored, with a small ocular chamber, entirely K/I -; ascospores hyaline, 1-septate, more or less strongly constricted at the septa,  $6.5-7.5 \times 2.8-3.2 \,\mu\text{m}$ .

In the protologue of O. cinerea, the ascospores are described as "brown, 3-septate, sometimes 1-septate, and then contracted in the middle, frequently mural, measuring from 0.00025 to 0.00075 of an inch". Figure 18c on Plate 11 accompanying the protologue shows "spores, in which the different degrees of septation are seen". This figure shows three asci, one containing 1-septate ascospores (perhaps observed from the isolectotype that we identified as abbreviatum), one 3-septate ascospores (probably Hysterium cf. pulicare) and a third one containing muriform ascospores. We did not observe muriform ascospores in any of the type specimens from BM.

Graphina hartmanniana Müll. Arg. was reduced into synonymy with *D. cinerea* by Archer (2003). The holotype (Australia, Queensland, Toowoomba, *Hartmann*, G!—holotype) does not belong to the *Roccellaceae*, because of the negative iodine reactions of the hymenium (I – and K/I –) and lacking the K/I+ blue apical ring of the asci. The fungus is obviously non-lichenized and has the following characters: ascomata lirelliform with a slit-like opening; excipulum dark brown to black, continuous under the hymenium; hypothecium hyaline, *c.* 45–65 μm thick; hymenium hyaline *c.* 100 μm



Fig. 5. Opegrapha varians, ascospores (Hein 7992b2). Scale bar=10 μm.

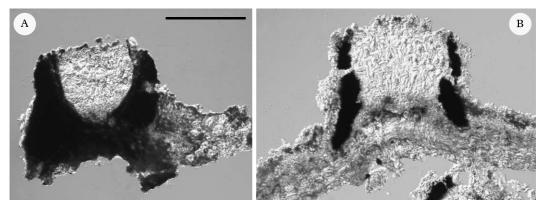


Fig. 6. Opegrapha species, cross sections of lirellae. A, O. varians (Hein 7992b2); B, O. arabica (Nannfeldt 22037a). Scale: A & B=0.2 mm.

tall; paraphysoids branched and anastomosing, 1  $\mu$ m thick; asci cylindrical-clavate, 8-spored; ascospores submuriform, 19–23 × 7–8  $\mu$ m, with 5–7(–8) transverse septa and 1–2 longitudinal septa; perispore hyaline, 2–2·5  $\mu$ m thick. It is a member of the *Hysteriaceae* and might belong to the genus

Gloniopsis De Not. because of the hyaline, muriform ascospores.

### Dictyographa contortuplicata Müll. Arg.

Bull. Herbier Boissier 2: 91 (1894); type: Central Paraguay [erroneously indicated as Bolivia on the



Fig. 7. Known distribution of Opegrapha arabica (■) and O. varians (★).

lectotype specimen and in the protologue?], *T. Morong* 1440, ex herb. *Eckfeldt* (G!—lectotype, here designated; PH!—isolectotype).

We did not find any ascospores, even after examining several ascomata in both type specimens. The entire hymenium, including immature asci (containing no ascospores), is I- and K/I-. Moreover, the paraphyses are simple. The species does not belong to the *Roccellaceae*, but seems to be a member of the *Graphidaceae*. In the protologue, ascospores are said to be 8 per ascus, hyaline,  $35 \times 8 \,\mu m$  and submuriform ("8–10-loculares, loculi 2-locellati").

The two putative type specimens contain contradictory information, as the specimen from G is said to come from Bolivia, and the one from PH is noted as being from Paraguay. However, an examination of both specimens convinced us that they are from the same collection. As the specimen from G is accompanied by original drawings and notes of J. Müller, it is clear that that specimen was used for the protologue, and therefore we are designating the specimen from G as lectotype.

From the original description and the notes on the lectotype, it appears that the specimen was collected in Bolivia by J. W. Eckfeldt. The notes accompanying the PH specimen inform us that T. Morong collected it in Central Paraguay and gave it to J. W. Eckfeldt (PH specimen), who sent a duplicate to J. Müller (G specimen). As T. Morong collected much in Paraguay and apparently did not collect in Bolivia, we consider that the indication 'Bolivia' is erroneous.

#### Dictyographa epiphylla A. Zahlbr.

Ann. Crypt. Exot. 1: 123 (1928).—Aulaxina epiphylla (A. Zahlbr.) R. Sant., Symb. Bot. Upsal. 12(1): 305 (1952); type: Java, Schiffner 3474 (W—holotype, non vidi).

The current name is *Aulaxina epiphylla* (A. Zahlbr.) R. Sant., a species of *Gomphillaceae* (Santesson 1952).

#### Dictyographa psyllocarpa (Leight.) Redinger

Arkiv för Botanik 29A: 49 (1940).—Opegrapha psyllocarpa Leight., Transact. Linn. Soc. London 25: 455

(1866); type: Brazil, Santarem, R. Spruce 306 (BM!—holotype).

A complete description and an illustration of the ascospores can be found in Redinger (1940) who combined the species in Dictyographa because of the muriform ascospores and the branched paraphyses. Examination of the type specimen revealed non-pruinose slit-like lirellae, an I – and K/I – hamathecium, K/I - asci (devoid of an apical K/I+ blue ring), and brown, muriform ascospores,  $20.5-27.5 \times 9.5-12 \,\mu\text{m}$ . The margins of the lirellae have 2-3 tiny longitudinal corrugations visible on the surface and look like those of some Hysteriaceae. Although the hamathecium of the type specimen is in a poor condition, the species surely does not belong to the Roccellaceae, because of the negative iodine reactions of the hymenium, the missing K/I+ blue apical ring of the asci, the dark brown, muriform ascospores and the general appearance of the ascomata. Since no hyaline ascospores were observed, it is not clear if the ascospores were hyaline when young, or if they were brown from the beginning. The species seems to be non-lichenized and might belong to the Hysteriaceae. Within this family, the species possibly belongs to the genus Hysterographium that has brown, muriform ascospores (Zogg 1962).

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