

## *Graphis tetralocularis*, a new lichen with four-celled ascospores from tropical Africa

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**Abstract:** *Graphis tetralocularis* C. Bock & Hauck is described as a new species from Rwanda. It is characterized by triseptate, hyaline ascospores  $12\text{--}15\text{--}(17) \times 5\text{--}7.5 \mu\text{m}$ , a connivent, laterally carbonized exciple and by the presence of atranorin. So far, *G. tetralocularis* is only known from the type locality in central Africa, where it grows epiphytically on *Nuxia floribunda* in a dry forest.

**Key words:** *Graphidaceae*, lichenized Ascomycetes, Rwanda, taxonomy

### Introduction

More than a thousand species of the lichen family *Graphidaceae* are known, having, like many families with *Trentepohlia* photobiont, their main occurrence in the tropics (Nakano 1988; Archer 2001a; Staiger 2002). An important feature in separating species and genera of *Graphidaceae* has traditionally been ascospore characteristics, i.e., septation and colour (Müller 1880). Only a limited number of species form four-celled, colourless ascospores. This is why we were able to recognize a species of *Graphis* collected in Rwanda as a new species, though it is known so far only from the type locality. Since it clearly differs from any other known *Graphidaceae* species and since the crustose lichen flora of tropical Africa is very poorly known, we decided to describe the species in the present paper notwithstanding its unknown distribution and variability.

### Methods

Microscopic measurements were made at  $\times 400$  magnification in water. Sections were tested for colour

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reactions with Lugol's iodine. TLC was carried out according to Culberson & Ammann (1979).

### The Species

#### *Graphis tetralocularis* C. Bock & Hauck sp. nov.

Thallus crustaceus, tenuis, planus, plus minusve rimosus, albido-griseus usque ad griseo-viridis. Apothecia numerosa, nigra, simplices vel raro furcata, flexuosa, 0.5–1.5 mm lata, semi-immersa. Excipulum cum labiis convergentibus, latere fusco-fuligineum, 20–30  $\mu\text{m}$  latum, in parte basali pallide flavido-fuscum. Hypothecium pallide flavido-fuscum usque ad hyalinum. Hymenium 60–75  $\mu\text{m}$  altum, hyalinum, sed epihymenium pallide flavido-fuscum. Ascospores transverse tetraloculares, hyalinae, 12–15(–17)  $\mu\text{m}$  longae et 5–7.5  $\mu\text{m}$  latae, iodo caerulescentes. Paraphyses simplices, apicibus leviter incrassatis. Atranorinum continens.

Typus: Rwanda, Akagera National Park, 1°55'2" S, 30°42'30" E, on twig of *Nuxia floribunda*, 1369 m alt., 18 October 2003, C. Bock (GOET—holotypus).

(Figs 1–3)

*Thallus* crustose, thin, smooth,  $\pm$  cracked, whitish grey to grey-green. Soredia and isidia absent. Photobiont green, *Trentepohlia*.

*Apothecia* frequent, black, simple or rarely branched, curved, 0.5–1.5 mm long, semi-immersed. *Exciple* connivent, nearly closed, laterally carbonized, 20–30  $\mu\text{m}$  thick, basally uncarbonized, I–. *Hypothecium* 13–20  $\mu\text{m}$  thick, pale yellowish brown to hyaline, I–.

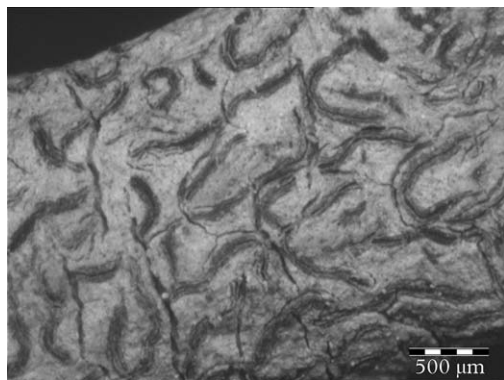


FIG. 1. *Graphis tetralocularis*, habitus (holotype in GOET).

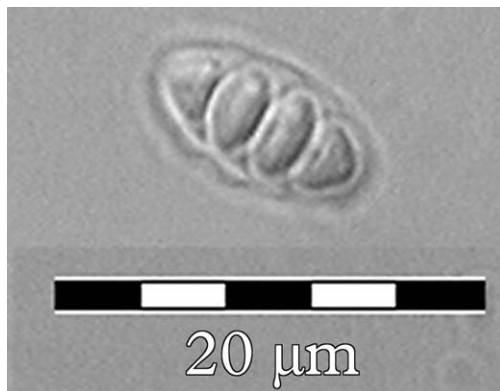


FIG. 3. *Graphis tetralocularis*, ascospore (holotype in GOET).

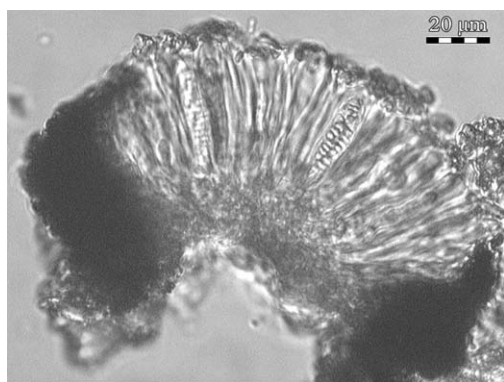


FIG. 2. *Graphis tetralocularis*, section of apothecium (holotype in GOET).

*Parathecium* hyaline, I<sup>-</sup>. *Hymenium* 60–75 μm tall, hyaline, not interspersed, I<sup>-</sup>. *Epihymenium* pale yellowish brown, I<sup>-</sup>. *Asci* 45–50 × 7–15 μm, with eight spores. *Ascospores* colourless, transversely triseptate, 12–15(–17) × 5–7.5 μm, ellipsoid, occasionally with pointed ends, I<sup>+</sup> blue. *Paraphyses* simple, 0.5–1 μm wide, apices ± thickened.

*Pycnidia* not observed.

*Chemistry.* Atranorin (traces) detected by TLC.

*Distribution and habitat.* *Graphis tetralocularis* is known only from the type locality, a dry forest with *Nuxia floribunda* Benth., *Haplocoelum gallense* (Engl.) Radlk. and *Strychnos usambarensis* Gilg. in Rwanda.

### Discussion

*Graphis tetralocularis* differs from other species of *Graphidaceae* by its four-celled ascospores, apothecia with only laterally carbonized exciples and by its chemistry. Atranorin was detected by TLC, but its content was not sufficient to cause a clear reaction in the spot test with K or P. Atranorin has not been so far reported from the *Graphidaceae*, which are generally poor in lichen substances (Staiger 2002). The partly carbonized exciple, the hyaline, I<sup>+</sup> blue ascospores and eight-spored asci characterize *G. tetralocularis* as a member of *Graphis* s. str. (Staiger 2002). *Glyphis*, which is closely related to *Graphis* in terms of exciple and spore characteristics, differs by branched, gelatinous paraphyses with brown granular tips.

The characters above separate *G. tetralocularis* from all *Graphidaceae* previously known from Africa. Dodge (1964) reported several *Graphis* species with triseptate ascospores. The apothecia of *G. afzelii* Ach. and *G. timidula* Nyl. differ by their white-pruinose exciple. *Graphis timidula* has, moreover, larger ascospores (27–36 × 11–13 μm) than *G. tetralocularis* (Dodge 1964). *Graphis afzelii* contains crystals of lecanoric acid on the surface of short, hyaline hyphae, which form the white pruina on the exciple (Brodo *et al.* 2001; Staiger 2002). The presence of lecanoric acid is unique to *G. afzelii* and the

closely related *G. oryzoides* Leight. within the *Graphidaceae*; consequently Kalb & Staiger (2001) separated these two species from *Graphis* in the re-erected genus *Dyplolabia* A. Massal. *Graphis hyalinella* Müll. Arg. differs from *G. tetralocularis* by immersed apothecia and by the absence of a parathecium (Dodge 1964). Apothecia of *G. gomphospora* Müll. Arg. are wider (1–2 mm) than those of *G. tetralocularis* and have sulcate lips (Dodge 1964). *Graphis nigeriensis* C. W. Dodge has the hyaline parathecium in common with *G. tetralocularis*, and both species have ascospores of similar size (16 × 8 µm). However, these species differ by the darker thallus of *G. nigeriensis* and by the apothecial lips, which are persistent in *G. tetralocularis*, but break away and expose the disc in older apothecia of *G. nigeriensis* (Dodge 1953, 1964). *Graphis subolivacea* Zahlbr. is distinguished from *G. tetralocularis* by larger ascospores (18–22 × 8.5–9 µm) and by its olive, K+ red thallus (Zahlbruckner 1926; Dodge 1964). *Graphis myriocarpoides* Vain. differs from *G. tetralocularis* by its K+ red thallus, longer ascospores (17–18 × 5–7 µm) and apothecia with sulcate lips (Vainio 1929; Dodge 1964). Ascospores of *G. triticella* Vain. have a similar size (14–17 × 6–8 µm) to those of *G. tetralocularis*, but *G. triticella* has, like *G. myriocarpoides* and *G. subolivacea*, a K+ red thallus (Vainio 1929; Dodge 1964). *Graphis thoroldi* C. W. Dodge is characterized by very small apothecia, which are only 0.5–0.6 mm long (Dodge 1953). *Graphis palmensis* Vain. differs from *G. tetralocularis* by larger ascospores (18–20 × 7–8 µm) and by conspicuous plasmodesmata that connect the protoplasts of the individual cells of the ascospores (Dodge 1964).

Numerous *Graphidaceae* species in and outside Africa with hyaline, four-celled ascospores are easily distinguished from *G. tetralocularis* by the completely uncarbonized exciple. Because of the uncarbonized exciple these species do not belong to *Graphis* s. str. and were assigned by Staiger (2002) to *Acanthothecis* Clem., *Fissurina* Fée, *Gymnographa* Müll. Arg. or *Platythecium* Staiger. The type species of *Gymnographa*, *G. medu-*

*ulina* Müll. Arg., however, is a synonym of *Phaeographis eludens* (Stirt.) Shirley (Archer 2001b). A further species with an uncarbonized exciple in the *Graphidaceae* was recently described as *Anomomorpha sordida* Staiger (Staiger 2002). The correct generic affiliation of *G. platycarpella* Müll. Arg. and *G. schizogramma* Vain. (Wirth & Hale 1963) is unclear, as specimens of these species were not examined by Staiger (2002). Further characters separating species of the family with an uncarbonized exciple from *G. tetralocularis* can be found in Wirth & Hale (1963), Galloway (1985), Awasthi (1991), Coppins *et al.* (1992), Archer (1999, 2001a), Staiger & Kalb (1999) and Staiger (2002).

The South American *Platythecium acutisporum* Staiger is carbonized at the base (Staiger 2002). *Graphis karstenii* Zahlbr. from India has a divergent exciple and a widely open disk. *Graphis furfuracea* Leight. from Sri Lanka differs from *G. tetralocularis* by its larger ascospores (22–36 × 10–12 µm) and a dimidiate exciple, i.e., by the absence of the basal part of the exciple (Awasthi 1991). Apothecia of *G. implexula* Stirt. from India have pale yellow lips and a yellowish thallus. The eastern Asian, saxicolous *G. cervina* Müll. Arg. has 4–7-locular ascospores. Specimens of this species with triseptate spores can be separated from *G. tetralocularis* by the thick, yellowish thallus, the basally carbonized exciple and the presence of norstictic acid (Staiger 2002, Nakanishi *et al.* 2003). The Australian *G. albonitens* Müll. Arg. and *G. elixii* Archer have laterally carbonized exciples like *G. tetralocularis*, but *G. albonitens* has completely immersed apothecia with a slit-like appearance and a hymenium that is 100–125 µm tall (Archer 1999). *Graphis elixii* contains 2-methoxypsoromic acid, has a fawn thallus and larger ascospores ([16–]18–20 × 8–10 µm) than *G. tetralocularis* (Archer 1998, 2001c). The saxicolous *G. howeana* Archer, which was described from Australia (Archer 2001a), has four-celled ascospores of similar size (14–16 × 5–7 µm) like *G. tetralocularis*, but lacks a proper exciple, has a taller hymenium and contains no lichen

substances. *Graphis nigririmis* (Nyl.) Müll. Arg. differs from *G. tetralocularis* by the apically carbonized exciple, the taller hymenium and larger ascospores of  $18\text{--}22 \times 10\text{--}14 \mu\text{m}$  (Archer 2001a).

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