

## Two new species of *Multiclavula* (lichenized basidiomycetes) from savanna soils in Rwanda (East Africa)

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Two new species of *Multiclavula* are described from Rwanda: *M. akagerae*, with a thallus consisting of turgescens glomerules, and *M. rugaramae*, with a thallus composed of flattened, rounded, marginate, and dispersed squamules. They grow on soil in frequently burned savannas or on lateritic soils in eastern Rwanda. The genus *Lepidostroma* should probably be reduced into synonymy with *Multiclavula*. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 155, 457–465.

ADDITIONAL KEYWORDS: Akagera – Butare – *Lepidostroma* – Rugarama – taxonomy.

### INTRODUCTION

Recent fieldwork in the eastern parts of Rwanda (East Africa) led to the discovery of two species of basidiolichens growing on soil in frequently burned savannas or on lateritic soils; both produce a typical persistent thallus and ephemeral clavarioid fruiting bodies of the *Multiclavula* type. They are described as new to science in the genus *Multiclavula* R. H. Petersen. The two taxa represent the first records of this genus from Africa.

### MATERIAL AND METHODS

This study is based on field observations, on detailed examination of the collections made by the authors, and on herbarium material of related species (B and GZU). No colour charts have been used. The material was examined in distribution water or in lactophenol cotton-blue (LCB). The measurements always refer to

water mounts. Air-dried herbarium material for study by scanning electron microscopy (SEM) was mounted on polished aluminium stubs using a transparent two-component epoxy glue, gold coated in a Balzers Union SCD 040 sputter, and examined with a Cambridge Stereoscan S 200 scanning electron microscope.

### GENERIC CONCEPT

Both species described in this paper are typical clavarioid basidiomycetes with simple, orange or reddish, tubular basidiocarps, that are rather tough when fresh and collapse and become very brittle when dry; hymenium tissue covers the whole upper part of the basidiocarps and comprises only subclavate or clavate basidia; spores are ovoid, with a distinct eccentric apiculus, colourless, thin-walled, and guttulate. Both species are lichenized and have a permanent thallus with typical habit and anatomy.

Species with such characteristics, formerly assigned to *Clavulinopsis* Overeem subgenus *Eu-Clavulinopsis* and/or *Lentaria* Corner subgenus *Lentariopsis*, have

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been transferred to the genus *Multiclavula* by Petersen (1967); 13 species were recognized and several were said to be associated with algae or with bryophyte protonemata. The species associated with algae are considered as truly lichenized, and are now included in lichens checklists; for example, three species are included in the checklists for Austria and Fennoscandia [*M. corynoides* (Peck) R. H. Petersen, *M. mucida* (Pers.) R. H. Petersen, and *M. vernalis* (Schwein.) R. H. Petersen; Hafellner & Türk, 2001; Santesson *et al.*, 2004].

A detailed anatomical study of these species, especially their means of association with algae (the 'lichen thallus'), is provided by Oberwinkler (1970, 1984). In *Multiclavula corynoides* and *M. mucida*, the thallus is a gelatinous film with flattened to globular structures, either distinct or aggregated, containing the coccomyxoid algae. Within these structures, the algal cells are surrounded by hyphae, forming a pseudoparenchymatous layer; typical dolipores are present at the septa. In the terricolous *M. corynoides* and *M. vernalis*, these tiny globules can be massively produced and become easily detached from the main thallus, and thus act as diaspores; they are named bulbils by Poelt & Obermayer (1990). Our own observations on European material of these three species (collections from B and GZU!) confirm these features.

One of the species found in Rwanda, here described as *M. akagerae*, has a thallus composed of much larger glomerules, which lose their turgescence when dry, and its photobiont is not a coccomyxoid but a chlorococcoid alga. We believe that such discrepancies in photobiont and thallus structure do not warrant any generic distinction.

Petersen published the original description of *Multiclavula* in January 1967 (*vide* Oberwinkler, 1970). In August of the same year, Mägdefrau & Winkler (1967) published the new genus *Lepidostroma* with the single, newly described species *L. terricolens* found on soil in the Santa Marta range in Columbia. It has since been found in Costa Rica within the framework of the Ticolichen project ([http://www.fieldmuseum.org/research\\_collections/botany/botany\\_sites/ticolichen/images.html](http://www.fieldmuseum.org/research_collections/botany/botany_sites/ticolichen/images.html)). Oberwinkler (1984: 758, 773) studied the type collection and demonstrated that it is identical to *Multiclavula calocera* (Martin) R. H. Petersen, a species also known only from the Santa Marta mountain range. If the genus *Lepidostroma* is accepted, the correct name for the species is thus *L. calocerum* (Martin) Oberw.

The thallus of this species is completely different and composed of flattened, reniform to lobate (rarely rounded), tiny squamules, with an upper and lower cortex (each made of a single layer of cubic to parallelepipedic cells), and a loose medulla containing the photobiont cells which belong to a chlorococcoid algal

genus. Such a thallus is almost identical to that of the second species found in Rwanda, and it thus could be described in the genus *Lepidostroma*.

It is fascinating that the clavarioid lichenized basidiomycetes are able to produce two different types of thallus. They are analogous to those formed by omphalinoïd lichenized species (*Omphalina* Qué. *s.l.*). Indeed, the *Botrydina*-type thallus of omphalinoïd species is also composed of spherical (or almost so) glomerules enclosing coccomyxoid algae with a pseudoparenchymatous outer layer, whereas the *Coriscium*-type thallus is composed of flattened, tiny and fragile, corticate squamules, with coccomyxoid algae, whose cells are tightly surrounded by hyphae.

Detailed phylogenetic analysis of the genus *Omphalina* as a whole (Lutzoni & Vilgalys, 1995; Lutzoni, 1997), including DNA sequences of the nuclear ribosomal repeat unit, has shown that the lichenized species are monophyletic. Species of *Omphalina* with *Botrydina*- and *Coriscium*-type thalli are thus demonstrated to form a well-supported clade. They are now assigned to the separate genus *Lichenomphalia* Redhead *et al.* (Redhead *et al.*, 2002).

Unfortunately, our attempts to extract DNA from the clavarioid species from Rwanda failed, and we were unable to demonstrate that they are congeneric. Nevertheless, we suggest that they are, and that the species with a thallus composed of glomerules and the species with a *Lepidostroma*-type thallus belong to a monophyletic group and thus to the same genus. If demonstrated by further DNA analyses, this hypothesis implies that *Lepidostroma* should be reduced into synonymy with *Multiclavula*. Redhead & Kuyper (1987) have published a key for the species of *Lichenomphalia* entirely based on thallus characters, including those provided by the cortex and subtending hyphae. Such a tool is also very much needed for the clavarioid lichens.

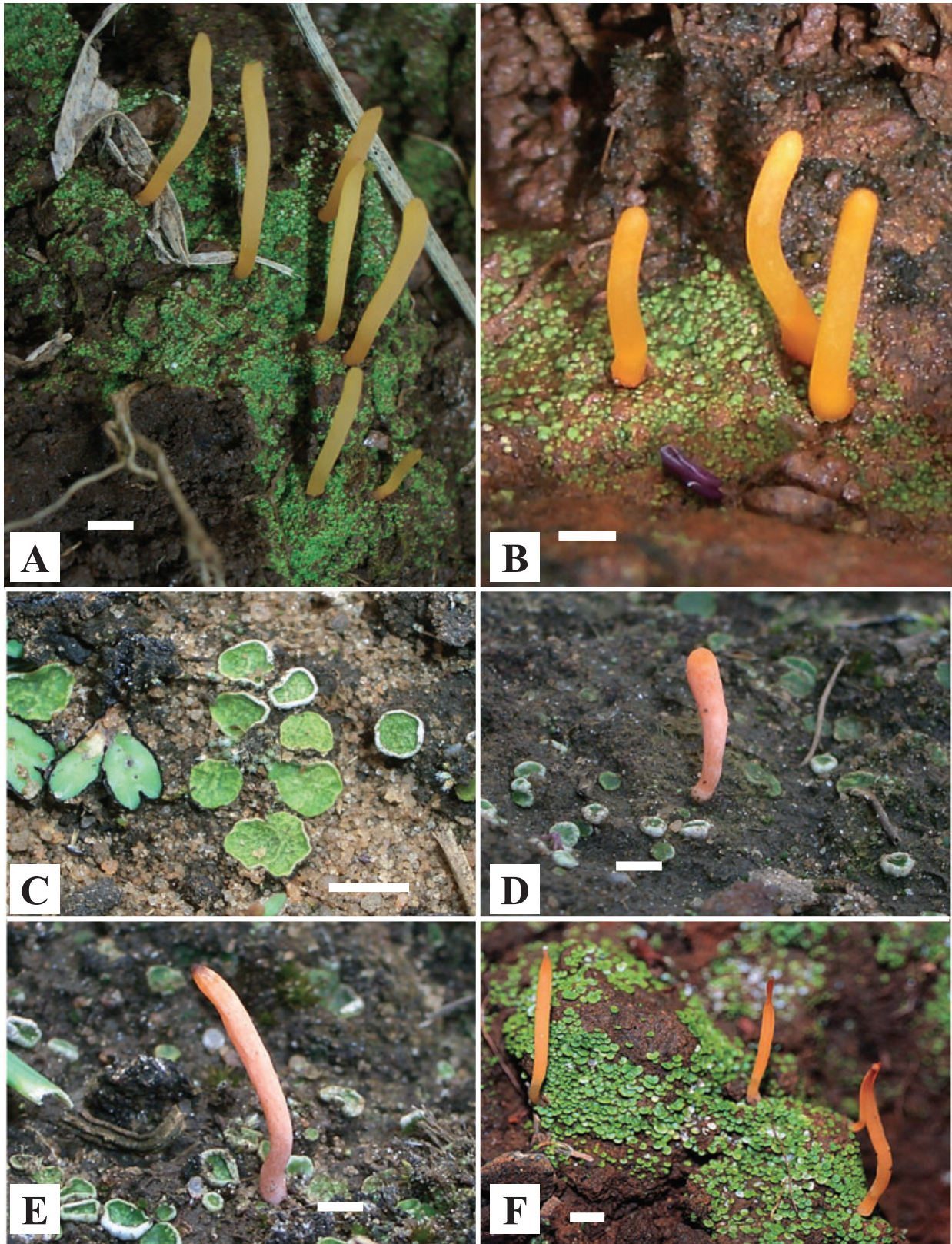
## THE SPECIES

***MULTICLAVULA AKAGERAE* EB. FISCH., ERTZ,  
KILLMANN & SÉRUS., SP. NOV.  
(FIGS 1A, B, 2A, 3A, B)**

*Type:* RWANDA: prov. Kibungo, Akagera National Park, foot of Mt. Mutumba, open savannas and wooded gallery thickets along a small intermittent river, on open lateritic soil in burnt savanna, 01°38'51.6"S, 30°39'53.7"E, 1450 m, iv.2005, D. Ertz, E. Fischer, D. Killmann & E. Sérusiaux (*Holotype:* BR; *Isotypes:* KOBL, LG).

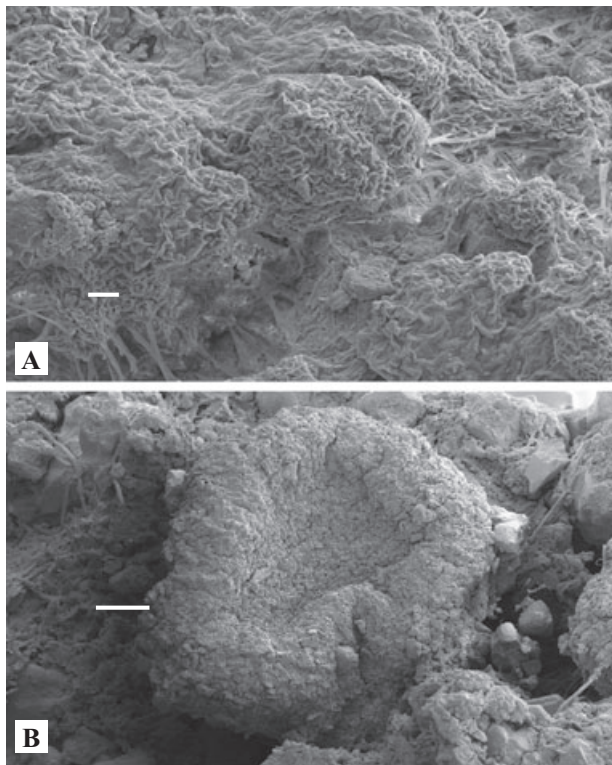
*Diagnosis:* A *Multiclavula vernalis* differt photobionte chlorococcoideo, glomerulis thalli profunde sinuatis et basidiocarpis pallidioribus. A *M. clara* basidiis valde longioribus et sporibus majoribus distincta est.





**Figure 1.** A, *Multiclavula akagerae*, Butare; B, *M. akagerae* (type); C–E, *Multiclavula rugaramae* (type), C showing two left thalli of *Riccia okahandjana*; F, *Multiclavula calocera*, Costa Rica (photography by R. Lücking). Scale bar, 2 mm.





**Figure 2.** A, thallus of *Multiclavula akagerae*. B, thallus of *M. rugaramae*. Scale bar: A, 30  $\mu\text{m}$ ; B, 100  $\mu\text{m}$ .

**Description:** THALLUS very distinct, yellowish green to deep green and composed of discrete, turgescer, and slightly convex glomerules, usually contiguous, rounded or irregularly, slightly to distinctly lobulate, 0.2–0.5 mm in diameter when wet; glomerules collapsing when dry and thus forming a thin, green, irregular crust of dispersed, flattened, and tiny squamae. Photobiont: chlorococcoid colonies with spherical to ellipsoid green cells (8–)10–12  $\mu\text{m}$  in diameter, with a (sub)central pyrenoid. Cortex well developed, c. 10  $\mu\text{m}$  thick, composed of a single layer of cells which appear to have a deeply lobate shape in front view and thus look like jigsaw pieces. Subtending hyphae thin-walled, 2–2.5  $\mu\text{m}$  thick, with clamps.

**BASIDIOCARPS** filiform, simple, very rarely branched, slightly inflated at the base and attenuated at the apex, 0.5–1.5 cm long and 0.3–0.4 mm thick when dry, rather tough and slightly elastic when wet and very brittle when dry, pale orange to orange, with base more reddish and apex much paler, surface slightly but distinctly (best seen under the dissecting microscope) velvety, cylindrical and turgescer when wet and with a few distinct and irregular longitudinal furrows when dry. Central hyphae bright orange in section, densely agglutinated and rounded to distinctly polygonal in cross-section, very long (up to

50  $\mu\text{m}$  long), thin-walled, simple or rarely branched, with conspicuous clamps throughout the basidiocarp. Sterile hyphal elements apparently present in the hymenium, slightly inflated at their apices but not protruding out of the hymenium (confusion with young basidia?). Basidia subclavate, 35–40  $\times$  c. 10  $\mu\text{m}$ , basally clamped, with four sterigmata. Basidiospores ovoid, hyaline and thin-walled, with a small, usually eccentric apiculus, and one to three guttules (easily seen in living material), 8–10  $\times$  4–5  $\mu\text{m}$ .

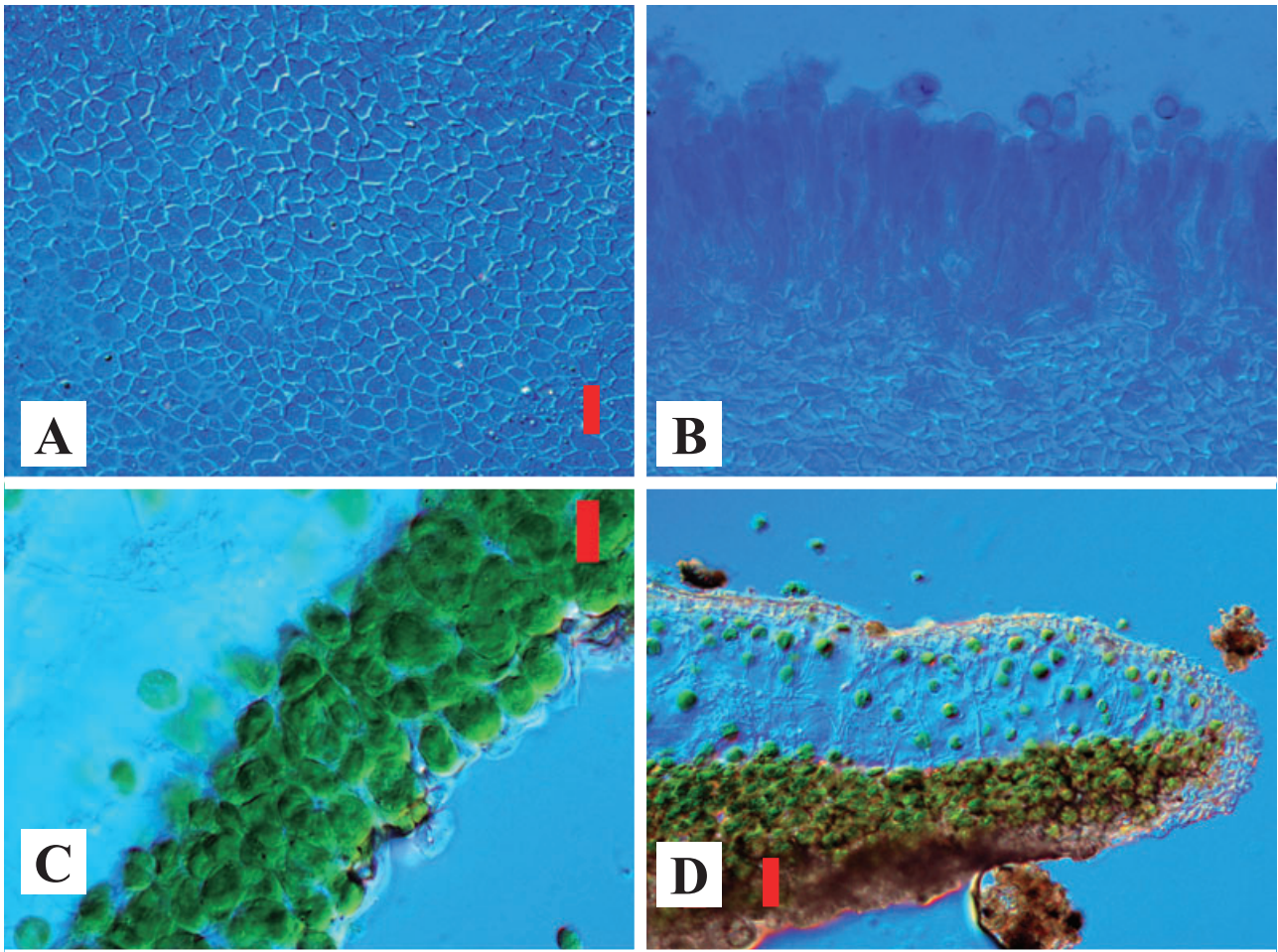
**Etymology:** This new species is named after the Akagera National Park in eastern Rwanda where its type locality is located.

**Other specimens examined:** RWANDA: prov. Butare, Butare, IRST park with isolated trees on regularly cut meadows and plantations, on earth embankment, 02°37'0.20"S, 29°44'0.45"E, c. 1690 m, x.2003, E. Fischer & D. Killmann (KOB). *Ibidem*, iii.2005, D. Ertz, E. Fischer, D. Killmann & E. Sérusiaux (BR, KOB, LG).

**Taxonomic notes:** Following the identification key provided by Petersen (1967: 207–208), this species is close to *M. vernalis* (Schwein.) Petersen, *M. clara* (Berk. & Curtis) Petersen, and *M. calocera* (Martin) Petersen (key entry: 11) because of the clamped hyphae of its basidiocarps, the colour and the branching pattern of its basidiocarps, and the strictly four-sterigmata basidia. *Multiclavula vernalis* is easily distinguished by its coccomyxoid photobiont, much smaller thallus glomerules (50–80  $\mu\text{m}$  in diameter vs. 200–500  $\mu\text{m}$  when wet in *M. akagerae*), which clearly act as diaspores (unlikely in *M. akagerae*), different pattern of the cortex in thallus glomerules (rounded to polygonal cells vs. jigsaw-like in *M. akagerae*), and more flexible basidiocarps. *Multiclavula clara* is so far only known from Cuba and, although its thallus has not yet been described, it is distinguished from *M. akagerae* by its much shorter basidia (15–24  $\mu\text{m}$  vs. 35–40  $\mu\text{m}$  in *M. akagerae*) and smaller spores (6.5–8  $\times$  3.5–4.5  $\mu\text{m}$  vs. 8–10  $\times$  4–5  $\mu\text{m}$  in *M. akagerae*). *Multiclavula calocera* has a *Lepidotroma*-type thallus with a completely different anatomy, and is much closer to *M. rugaramae* (see below).

The thallus glomerules of *M. akagerae* are very diagnostic: they have a noncoccomyxoid photobiont, a cortex with jigsaw-like cells, and they collapse when dry. They are unlikely to act as propagules.

**Distribution and habitat:** The species was first found in 2003, and revisited in 2005, on a single earth embankment inside a park in Butare (altitude, 1690 m), where it is plentiful. Other lichen species growing on the earth embankment in the same local-



**Figure 3.** A, *Multiclavula akagerae*, transverse section of central axis of basidiomata, detail; B, *M. akagerae*, transverse section of hymenium; C, *M. calocera*, photobiont layer; D, *M. calocera*, squamule, transverse section. Scale bar: A–C, 10  $\mu\text{m}$ ; D, 30  $\mu\text{m}$ .

ity include *Agonimia opuntiella*, *Cladonia subpityrea*, *Diploschistes muscorum* ssp. *bartlettii*, *Endocarpon pallidum*, *Gyalidea* cf. *japonica*, three different species of *Lepraria*, and *Trapeliopsis gelatinosa*.

A second locality was eventually found in (sub) natural conditions, in the Akagera National Park at Mt. Mutumba (Fig. 5A), where an open regularly burnt savanna with *Boscia angustifolia*, *Ozoroa reticulata*, *Bothriochloa insculpta*, *Loudetia kagerensis*, *Striga asiatica*, *Striga linearifolia*, and *Cynium tubulosum* ssp. *montanum* occurs. The species was abundant on muddy lateritic soil by a small channel of intermittent running water. The substrate is thus quite unstable; few other lichen species can colonize it and mostly grow on small stones: *Acarospora* sp., *Agonimia opuntiella*, *Arthonia* sp. (overgrowing mats of terricolous green algae), *Buellia* sp., two species of *Endocarpon*, *Lecidella* sp., *Lepraria* sp., *Rinodina* sp., and *Verrucaria* sp. We are convinced that this locality

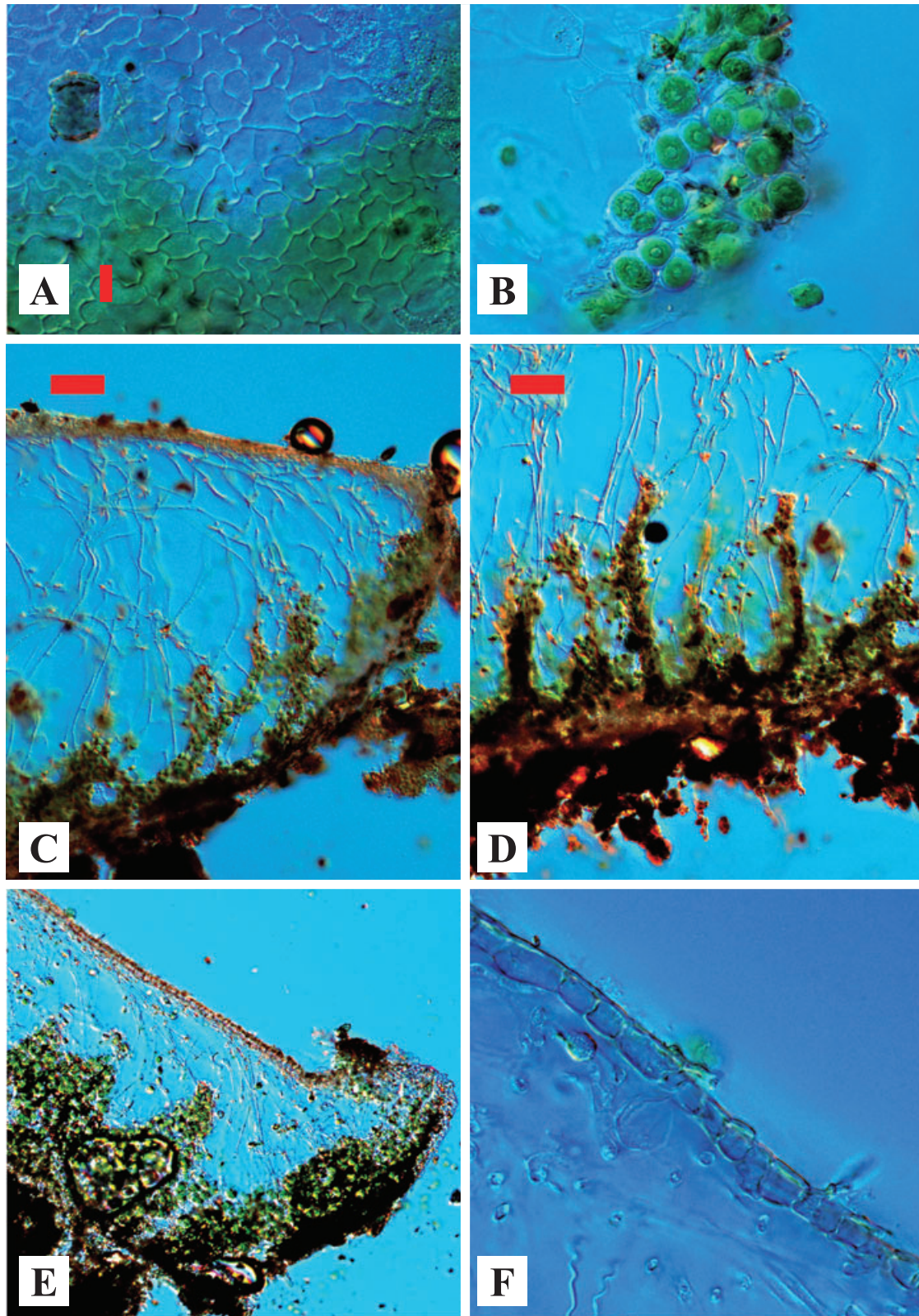
is the primary natural habitat of *M. akagerae*, and the material collected here is therefore chosen as the type; the species was eventually able to colonize an earth bank in anthropogenic conditions (parkland) within the city of Butare.

***MULTICLAVULA RUGARAMAE*** EB. FISCH., ERTZ, KILLMANN & SÉRUS., SP. NOV. (FIGS 1C–E, 2B, 4)

*Type:* RWANDA: prov. Kibungo, quartzitic outcrops at Rugarama with sparse vegetation, on soil, 02°08'05.0"S, 30°40'51.3"E, 1600–1690 m, iv.2005, E. Fischer & D. Killmann (*Holotype:* LG; *Isotype:* KOBL).

*Diagnosis:* *Multiclavula calocera* affinis sed differt squamulis rotundatis margine albido inflato, cellulis algarum cum pyrenoidibus, in parte inferiore





**Figure 4.** *Multiclavula rugaramae*: A, cortex of squamule; B, photobiont layer; C–E, squamule, transverse section; F, cortex of squamule, transverse section. Scale bar: A, B, F, 10  $\mu\text{m}$ ; C, D, 30  $\mu\text{m}$ .

medullae in columnis pyramidalibus, filiformibus vel irregularibus projectantibus et basidiocarpis roseis vel rubescentibus.

*Description:* THALLUS very distinct, green to dark green, composed of dispersed (rarely contiguous and never confluent), rounded, or almost so, patches, up to 1.5–2.0 mm in diameter and *c.* 0.2–0.4 mm thick, slightly swollen when wet, not collapsing but becoming very fragile when dry. When young, squamules concave, with a conspicuous, whitish or greyish, raised and somewhat swollen margin, when mature almost flat and with a less conspicuous margin; upper surface pale to deep green with conspicuous pale green to almost translucent maculae. Rhizinomorphs usually abundant under the lower surface and forming a hyphal network of *c.* 1–5 mm deep under the squamules, but sometimes almost missing; hyphae forming these rhizinomorphs (subtending hyphae?) thin-walled, 3.5–4.0  $\mu\text{m}$  thick, without clamps. Upper cortex pseudoparenchymatous, *c.* 8–10  $\mu\text{m}$  thick, composed of a single cell layer, cubic or parallelepipedic in cross-section and jigsaw-like (rarely polygonal) in surface view; same structure for the lower cortex; medulla loose, *c.* 200–350  $\mu\text{m}$  thick, with anastomosed and sparsely branched hyphae, 1.5–3.0  $\mu\text{m}$  thick, with clamps; algal cell layer dense and rather compact in the lower part of the medulla and projecting upwards in pyramidal, filiform, tulip-shaped or irregular columns, up to half or three-quarters of the medulla height (this feature is assumed to be responsible for the pale maculae on the upper surface). Photobiont: chlorococcoid colonies with spherical to ellipsoid green cells, 8–12  $\mu\text{m}$  in diameter, with a conspicuous (sub)central pyrenoid.

BASIDIOCARPS filiform, simple, cylindrical, and  $\pm$ abruptly attenuated in a pointed apex, or sometimes distinctly clavate, especially when young, 0.3–1.3 cm long and 0.2–0.4 mm thick when dry, rather tough and slightly elastic when wet and very brittle when dry, pale pinkish to bright red, with base more reddish and apex much paler, surface distinctly (best seen under the dissecting microscope) velvety, cylindrical, and turgescens when wet and usually without distinct longitudinal furrows when dry. Central hyphae bright red or with a red ring encircling a paler centrum, densely agglutinated and rounded to distinctly polygonal in cross-section, very long (up to 50  $\mu\text{m}$  long), thin-walled, simple or rarely branched, with conspicuous clamps throughout the basidiocarp. Sterile elements absent in the hymenium. Basidia subclavate or clavate, 35–40(–44)  $\times$  8–10  $\mu\text{m}$ , basally clamped, with four sterigma. Basidiospores ovoid, hyaline and thin-walled, with a small, usually eccentric apiculus, and one to three guttules (easily seen in living material), 9–11  $\times$  4–6  $\mu\text{m}$ .

*Etymology:* This new species is named after its type locality at Rugarama in eastern Rwanda, where impressive quartzitic outcrops offer a splendid view of the lakes, swamps, and dry forests of the Akagera National Park.

*Other specimens examined:* RWANDA: prov. Kibungu, quartzitic outcrops at Nyarubuye with scattered trees and vegetation, on soil, 02°08'54.0"S, 30°44'44.1"E, 1800 m, iv.2005, D. Ertz, E. Fischer, D. Killmann & E. Sérusiaux (LG). *Ibidem*, Akagera National Park, Sports Fishing Camp at Lac Ihema, on lateritic crusts, 01°52'25.1"S, 30°44'36.8"E, 1290 m, iv.2005, D. Ertz, E. Fischer, D. Killmann & E. Sérusiaux (BR, KOBL, LG).

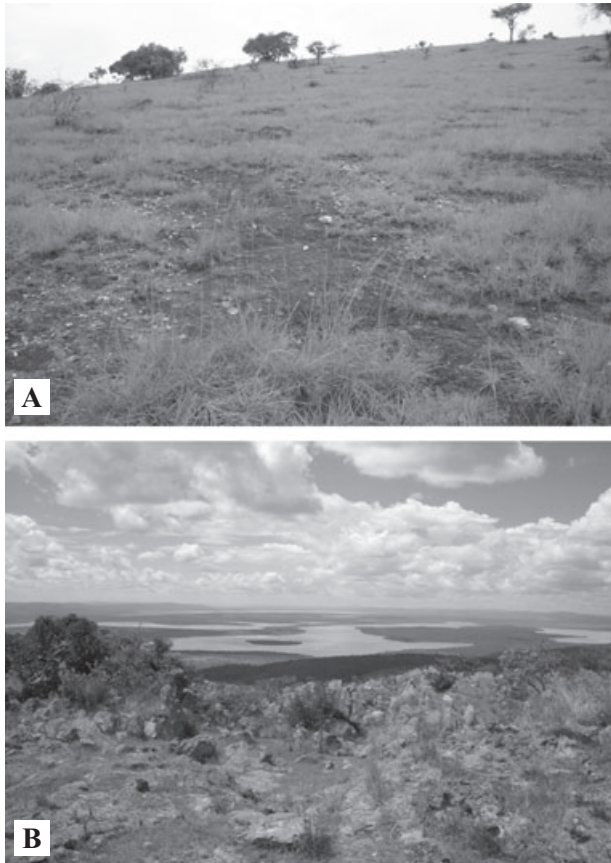
*Taxonomic notes:* As clear from the original description and illustrations, this species is close to *Lepidostroma terricolens* Mägdefrau & Winkler (1967), which was eventually shown to be identical with *Multiclavula calocera* (Oberwinkler, 1984): both species have a thallus composed of tiny greenish squamules with a pseudoparenchymatous upper and lower layer, a unique feature amongst the clavarioid lichenized basidiomycetes (Figs 1F, 3C, D). This thallus type is rather similar to the *Coriscium* type in *Lichenomphalia*, the omphaloid lichenized genus (Redhead *et al.*, 2002).

Both species also share the main characters of the basidiocarps of *Multiclavula*, and there is no doubt that they belong to the same group. As indicated above under the generic discussion, we believe that a single clade is involved, and that both thallus types (*Botrydina* type, as in *M. akagerae*, and *Coriscium* type, as in *M. calocera* and *M. rugaramae*) are represented within a single genus.

A recent collection of *M. calocera* from Costa Rica has been examined. Its thallus differs from that of *M. rugaramae* by the following characters: squamules reniform to deeply lobate, rarely (and most usually only when young) rounded, up to 2.0–2.5 mm in their longest dimension, upper surface dark green, without any paler spots or maculae, without any swollen, whitish margin; upper part of the medulla thinner (*c.* 60–70  $\mu\text{m}$ ) with a rather dense network of 2–3  $\mu\text{m}$  thick hyphae with numerous clamps and with photobiont cells regularly arranged throughout its height; lower part of the medulla with densely packed photobiont cells which never form pyramidal columns projecting upwards; photobiont cells without pyrenoids. Otherwise, the basidiocarps of *M. calocera* are very similar, except that they are orange, and not pinkish or reddish as in *M. rugaramae*.

*Distribution and habitat:* The type locality is a nice, although regularly burnt, quartzitic and sandstone outcrop at *c.* 1700 m dominating the Akagera depres-





**Figure 5.** A, type locality of *Multiclavula akagerae* at Mt. Mutumba, Akagera National Park. B, habitat of *M. rugaramae* at Nyarubuye.

sion (Fig. 5B); tree and bush species include *Cassia mimosoides*, *Indigofera asparagoides*, and *Parinari curatellifolia*, with the following herbaceous plants and ferns: *Aeollanthus repens*, *Albuca abyssinica*, *Aloe bukobana*, *Asplenium stuhlmannii*, *Boophone disticha*, *Crassula alba*, *Craterostigma lanceolatum*, *Loudetia simplex*, *Microchloa kunthii*, *Pellaea calomelanos*, *Polystachya dendrobiiflora*, *Selaginella niamniamensis*, *Sopubia conferta*, *Sopubia karaguensis*, *Sporobolus festivus*, *Streptocarpus bindseili*, and *Vernonia perotteti*. Lichen species on nearby outcrops include *Diploschistes actinostomus*, *Caloplaca cinnabarina*, *Xanthoparmelia congensis*, several other crustose species (a yellow *Acarospora* sp., several *Buellia* sp., and *Protoparmelia* sp.), and a few cyanolichens. The only other terricolous lichen species found here is a *Gyalideopsis* sp., unfortunately with immature asci.

In the second locality (Nyarubuye), the tree and shrub flora around the quartzitic outcrops include *Albizia petersiana*, *Hymenodictyon floribundum*, *Parinari curatellifolia*, *Protea madiensis*, and *Psorospermum febrifugum*, and the lichen flora is much richer.

The lichen species mentioned for the type locality also occur, with the following additions: *Bulbothrix decurtata*, *Canoparmelia rodriguesiana*, *Canoparmelia zimbabwensis*, *Lecidella enteroleucella*, *Parmotrema pseudograyanum*, and *Peltula clavata*, all growing on rocks, and *Toninia nigropallida* growing on lateritic soil.

The third locality near Lac Ihema in the Akagera National Park is a lateritic crust (ferricrete) with very shallow soil and the following vascular plants: *Craterostigma plantagineum*, *Craterostigma lanceolatum*, *Craterostigma hirsutum*, *Lindernia philcoxii*, *Loudetia kagerensis*, and *Microchloa kunthii*. Here, a rich and highly specialized bryophyte flora with *Riccia atropurpurea*, *R. congoana*, *R. lanceolata*, *R. moenkemeyeri*, *R. okahandjana*, *R. crinita*, and *Bryum arachnoideum* occurs (Fischer, 1995). Lichen species found in the same niche as *M. rugaramae* include *Acarospora* sp. (large, pruinose, and mostly dispersed squamules with a raised margin, a single apothecium per squamule, ascospores < 100 per ascus, globose, 5–9 µm in diameter), *Endocarpon pusillum*, *Heppia lutosa*, *Heteroplacidium* sp., *Lichinella stipatula*, *Peltula obscurans* var. *hassei*, *Psora* sp., *Pyrenopsis* sp., *Psorula rufonigra*, and *Spilonema revertens*.

The anatomy of the squamules of both *M. rugaramae* and *M. calocera* is remarkable as it resembles that of the 'window lichens' described from southern Africa (Vogel, 1955; Büdel & Schultz, 2003). It shows an inverse internal thallus morphology, in which the photobiont layer is situated in the lower medulla and exposed to the substrate. In a species of *Buellia* (Vogel, 1955), in which the upper surface was almost impenetrable to light from above, the light had to pass through the translucent quartz pebbles on which the lichen was growing. In the case of *Peltula inversa* (Büdel & Schultz, 2003), the lichen was hypolithic and growing underneath translucent quartz pebbles. In *M. rugaramae*, the squamules are growing on earth, not allowing the passage of light from underneath. The inverse thallus anatomy can, however, be considered as an adaptation to hot and dry conditions during the dry seasons. *Multiclavula calocera* grows in a montane rainforest area where this explanation may not be appropriate.

*Material of Multiclavula calocera* (= *Lepidostroma terricolens*) examined: COSTA RICA: prov. Puntaneras, San Vito de Coto Bruyz, Estación Biológica Las Cruces, humid road bank in cultivated area, 8°47.1'N, 82°57.6'W, 1200 m, x.2004, H. Sipman 53371 (B).

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