

## The relationships of *Virgariella verrucosa* sp.nov. on *Aloe* leaves from Turkey

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*Virgariella verrucosa* sp.nov., a distinctive species with reticulately ornamented conidia, is described from dead leaves of *Aloe* sp. collected in Turkey. All other known species of *Virgariella* have smooth conidia. A key to accepted species is given. The new species is compared with some taxa in *Virgaria*, *Virgariella*, *Cordana*, and *Pleurophragmium*, elements of which are heterogeneous, and some other less closely related genera.

Keywords: taxonomy, *Aloe*, hyphomycetes, generic concepts.

The genus *Virgariella* was introduced by Hughes (1953) and, although the name implies it is related to *Virgaria* Nees, there was no discussion of its affinities. The two genera were distinguished in Ellis (1971) by the denticulate conidiogenous loci in *Virgaria* and non-protuberant conidiogenous loci in *Virgariella*. At that time, both were considered to differ from *Cordana* Preuss and *Pleurophragmium* Costantin (genera with protuberant or denticulate conidiogenous loci) in having aseptate rather than septate conidia. However, a later treatment of *Pleurophragmium* by Ellis (1976) included species with both septate and aseptate conidia, and a discussion of *Cordana* by Vasant Rao & de Hoog (1986) also included the description of an aseptate species. These two studies tended to obscure the generic limits not only of *Cordana* and *Pleurophragmium* but they also called into question how *Virgariella* should be circumscribed. During the same period, there were also revisions of both *Virgariella* by Kirk (1981) and *Cordana* by de Hoog (1973a), in which several species were reassessed and excluded from both genera. To some degree, these studies have reduced what was a developing heterogeneity in the concepts of the genera concerned. Subsequently, *Pleurophragmium* was reduced to synonymy with *Dactylaria* Sacc. by de Hoog (1985), and, although the type species of *Pleurophragmium* was inexplicably omitted from the treatment of *Dactylaria*, this work similarly reduced the heterogeneity of the genus as conceived by Ellis (1976).

These various approaches have clarified generic concepts to a limited extent in the complex by reducing or rationalizing the numbers of acceptable taxa in individual genera. However, there is still no clear assessment of the value of available criteria in terms of what is of primary or secondary importance in defining the genera. It has become clear in comparable groups of fungi, such as the *Cercospora* complex (Pons & al., 1985), that although conidium development and conidiogenous cell growth may be identical in a range of generic taxa that appear to be superficially close, there are subtle differences in the nature of the conidiogenous loci and the secession scars left on conidia and conidiogenous cells. These are of more fundamental importance in grouping or separating genera than aspects of conidial and conidiophore morphology. Such differences concern the relationships of the loci to proliferation of the conidiogenous cells before, during and after conidia have been formed, the flush or protuberant (denticulate) nature of the scars on the conidiogenous cells, and the presence or absence and degree of wall thickening of the scars on the conidiogenous cells and conidia. Of secondary value are some features of conidial, conidiogenous cell, and conidiophore morphology because these show degrees of variation within species and within genera that make their worth at the generic level somewhat less important.

### Material and methods

Collections examined are maintained in herb. IMI and designated by IMI accession numbers where appropriate.

For scanning electron microscopy a piece of the freshly collected material on *Aloe* was excized, mounted on a stub and gold coated in a Polaron ES100 machine under vacuum for 4 min. Electron micrographs were taken on an Hitachi S570 scanning electron microscope at 15 KV.

### Taxonomy

#### *Virgaria*

(Fig. 1B)

In the type species of *Virgaria*, *V. nigra* (Link) Nees (IMI 98707), pale brown conidiophores are conspicuously and repeatedly branched; conidiogenous loci are numerous, scattered irregularly over the length of the conidiogenous cell, minute and slightly protuberant with distinctly thickened scars; conidia are aseptate, brown, smooth, reniform, the base barely protuberant to flush but with a thickened scar; secession is schizolytic.

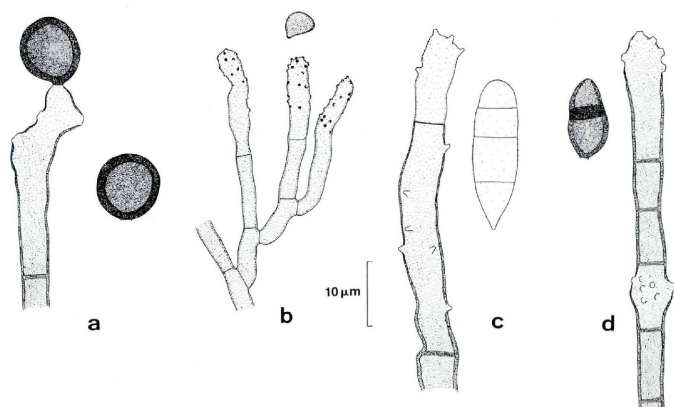


Fig.1.— a. *Virgariella globigera* (IMI 248562), conidium and part of a conidiophore.— b. *Virgaria nigra* (IMI 98707), conidium and branched conidiophores.— c. *Pleurophragmium parvisporum* (IMI 168725), conidium and part of a conidiophore.— d. *Cordana pauciseptata* (IMI 69977), conidium and part of a conidiophore.— Bar: 10 µm.

### *Virgariella*

(Fig. 1A)

In the type species of *Virgariella*, *V. atra* Hughes (IMI 248562), brown conidiophores are unbranched; conidiogenous loci are few, restricted to the apices of the conidiogenous cell, very rarely intercalary, and clearly formed in association with holoblastic sympodial proliferative growth of the conidiogenous cell, initially protuberant, cylindrical and truncate with unthickened scars, later usually collapsing to appear flush; conidia are aseptate, brown, smooth, globose, the base barely protuberant to flush, unthickened but with a minute marginal frill; secession is schizolytic.

### *Pleurophragmium*

(Fig. 1C)

In the type species of *Pleurophragmium*, *P. parvisporum* (Preuss) Holubová-Jechová [syn. *P. simplex* (Berk. & Broome) Hughes] (IMI 168725), brown conidiophores are unbranched; conidiogenous loci are few, restricted to the apices or the upper regions of the conidiogenous cells and clearly formed in association with holoblastic sympodial to irregular proliferative growth of the conidiogenous cell, protuberant and tapered with unthickened scars; conidia are (1–4) 3-septate, pale brown, verruculose to smooth, fusiform to subclavate, the base truncate, not protuberant, unthickened with no marginal frill; secession is schizolytic.

**Cordana**

(Fig. 1D)

In the type species of *Cordana*, *C. pauciseptata* Preuss (IMI 69977), brown conidiophores are unbranched; conidiogenous loci are few, formed at the apices of the conidiogenous cells which then proliferate holoblastically once or more successively along the main axis to form distinct conidiogenous nodes at higher levels, protuberant and truncate with unthickened scars; conidia are 1-septate, brown, smooth, ellipsoid to fusiform, the base flush to protuberant, unthickened and with no marginal frill; secession is schizolytic.

**Collection on *Aloe***

An abundant collection of a species belonging to this complex was made in Turkey on dead leaves of an *Aloe* species, but its generic placement is equivocal. The brown conidiophores are unbranched and the few to many conidiogenous loci are either restricted to one conidiogenous region at the apex or occur over the upper part of the conidiogenous cell (Figs 2–4). Conidia are dark brown, aseptate, and conspicuously reticulately ornamented, with a distinct protuberant basal marginal frill which could indicate rhexolytic secession (Figs 2, 5–8). The conidiogenous loci are unthickened, cylindrical, truncate and protuberant and remain so after conidial secession (Figs 2–3).

That loci are not thickened is of primary taxonomic importance and clearly excludes the species from *Virgaria*. The unthickened conidiogenous loci could place the collection in *Virgariella*, *Pleurophragmium* or *Cordana* but the relationships here are more problematic and depend on a combination of secondary characters. The presence of intercalary conidiogenous nodes is typical of *Cordana*, and even in *C. solitaria* Vasant Rao & de Hoog, the only species with aseptate conidia in the genus, they are present. In not developing such intercalary conidiogenous nodes, the species can be excluded from *Cordana*. Conidia in species of *Pleurophragmium* [or *Dactylaria* sect. *Pleurophragmium* (Cost.) de Hoog] are not only 1–6 septate but also hyaline or occasionally pale brown rather than very dark brown, smooth or verruculose, and thin-walled. There is considerable variation in the nature of the conidiogenous loci and scars in the genus; in the type species, scars are tapered, a feature that is quite different from the unthickened cylindrical appearance in *Virgariella* and *Cordana*. This variability is insufficiently wide to include the *Aloe* fungus in *Pleurophragmium*. Not one of the 41 species accepted in *Dactylaria* (syn. *Pleurophragmium*) by de Hoog (1985) has either aseptate or very dark brown reticulately ornamented conidia.

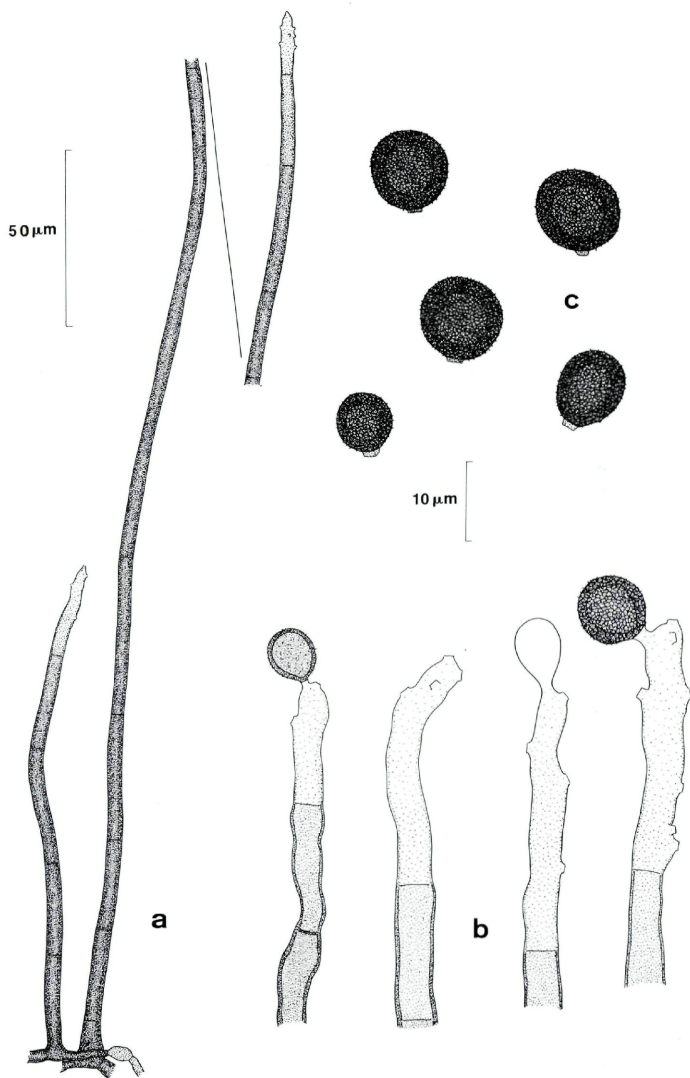


Fig.2.- *Virgariella verrucosa* (IMI 349855).- a. Conidiophores.- b. Apical parts of conidiophores with conidiogenous cells and developing conidia.- c. Mature conidia.- Bar: 10 μm.

The fact that in the *Aloe* fungus the denticles remain intact rather than collapsing might mitigate against inclusion of the species in *Virgariella*. However, of the three genera available in this complex it is probably the most suitable, although the apparently rhexolytic rather than schizolytic type of conidial secession is not really characteristic of *Virgariella*. SEM studies (Figs 3,7,8) show the scar faces on both conidia and conidiogenous cells to be ragged, not smooth, suggesting that a separating cell is perhaps involved in secession rather than the splitting of a multilayered septum. Walker & Minter (1981), faced with such problems in the '*Nematogonium*, *Gonatobotrys*, *Gonatobotryum* complex', postulated that the distinction between the two types of secession is not clear-cut. No ultrastructural evidence has been produced to confirm or refute this.

An alternative placement for this species is in a genus with brown holoblastic conidia that clearly secede rhexolytically, such as *Geniculisporium* Chesters & Greenhalgh, *Dematophora* Hartig, *Hansfordia* Hughes, *Nodulisporium* Preuss, or possibly in similar genera seceding schizolytically such as *Conoplea* Persoon or *Ramichloridium* Stahel ex de Hoog (Tab. 1). The remains of the separating cell in each of these rhexolytic genera is not only evident on the conidium but also at the conidiogenous locus. This does not happen

Tab. 1.— *Virgariella* and genera with which it is related.

	Conidiomata	Conidiophores	Setae	Conidiogenous cells		Conidiogenous loci			
						frequency	proliferation	protrusion	shape
<i>Conoplea</i>	hyphal	branched	absent	integrated	apical	few	holoblastic symphydial	flush	N/A
<i>Cordana</i>	hyphal	unbranched	absent	integrated	apical/intercalary	few	holoblastic nodal	protuberant	truncate
<i>Dematophora</i>	synnematal	branched	absent	integrated	apical	few	holoblastic symphydial	flush	N/A
<i>Geniculisporium</i>	hyphal	branched	absent	integrated	apical	few	holoblastic symphydial	flush	N/A
<i>Hansfordia</i>	hyphal	branched	present	integrated	apical	few	holoblastic symphydial/irregular	protuberant	truncate
<i>Nodulisporium</i>	synnematal	branched	absent	integrated	apical/intercalary	few	holoblastic symphydial	flush/slightly protuberant	N/A
<i>Plesiothremium</i>	hyphal	unbranched	absent	integrated	apical/intercalary	few	holoblastic symphydial/irregular	protuberant	tapered
<i>Ramichloridium</i>	hyphal	branched/unbranched	absent	integrated	apical/intercalary	many/few	holoblastic symphydial/irregular	slightly protuberant	N/A
<i>Virgaria</i>	hyphal	branched	absent	integrated	apical/intercalary	many	holoblastic irregular	protuberant	N/A
<i>Virgariella</i>	hyphal	unbranched	absent	integrated	apical/intercalary	few	holoblastic symphydial	protuberant then collapsed	cylindrical/truncate

in the *Aloe* fungus and is the primary reason for not placing the species in this group. In addition, in these genera the conidiophores are conspicuously and irregularly branched. In *Conoplea* they are also typically torsive, in *Dematophora* they are always synnematos, in *Nodulisporium* they may be mononematous or synnematos, and in *Hansfordia* they have long apical sterile elements. Quite apart from these features, there are other differences. In *Conoplea* the thick-walled ornamented conidia have either germination slits or pores. In the other three genera, the conidia are thin-walled and very pale in colour, but sometimes with conspicuous ornamentation [as in *Hansfordia pulvinata* (Berk. & M.A.Curtis) Hughes]. *Geniculosporium* and *Dematophora* are the anamorphs of Xylariaceae, but no correlations are known for *Hansfordia* or *Conoplea* species.

These differences justify the description of the fungus on *Aloe* as a new species.

*Virgariella verrucosa* Sutton, sp. nov. (Figs 2–8)

Coloniae atro brunneae, conspicuae, fasciculatae et separatae vel extensive confluentes. Mycelium partim superficiale, partim in substrato immersum, ex hyphis medio brunneis, laevibus, ramosis, septatis, 2.5–3.5  $\mu\text{m}$  compositum.

N/A: not applicable.

thickening	Conidial ascension	Conidia						Conidial scars		
		septa	solitary/catenate	pigmentation	ornamentation	shape	wall thickness	protrusion	marginal frill	thickening
none	schizolytic	0	solitary	mid-dark brown	verruculose/smooth	ellipsoid/obovoid/pyriform	thick	flush	none	none
none	schizolytic	(0-1)	solitary	mid-dark brown	smooth	ellipsoid/fusiform	thick	flush/protuberant	minute	none
slight	rhexolytic	0	solitary	hyaline	smooth	obovoid	thin	flush	distinct	slight
slight	rhexolytic	0	solitary	pale brown	smooth	obovoid	thin	flush	distinct	slight
none	rhexolytic	0	solitary	hyaline/pale brown	smooth/verruculose	ellipsoid/globose	thin	flush	distinct	none
none	rhexolytic	0	solitary	pale brown	smooth/verruculose	ellipsoid	thin	flush	minute	none
none	schizolytic	(0-1-3 (-3-4))	solitary	pale brown	smooth/verruculose	fusiform/subclavate	thin	flush/protuberant	none	none
none	schizolytic	0-1 (-3)	solitary, catenate	hyaline/pale brown	smooth/verruculose	ellipsoid/fusiform/globose	thin	flush/protuberant	none	none
distinct	schizolytic	0	solitary	brown	smooth	reniform	thin	flush	none	distinct
none	schizolytic/rhexolytic	0	solitary	pale-dark brown	smooth/verruculose	globose/ovoid/obovoid	thin/thick	flush/protuberant	minute	none

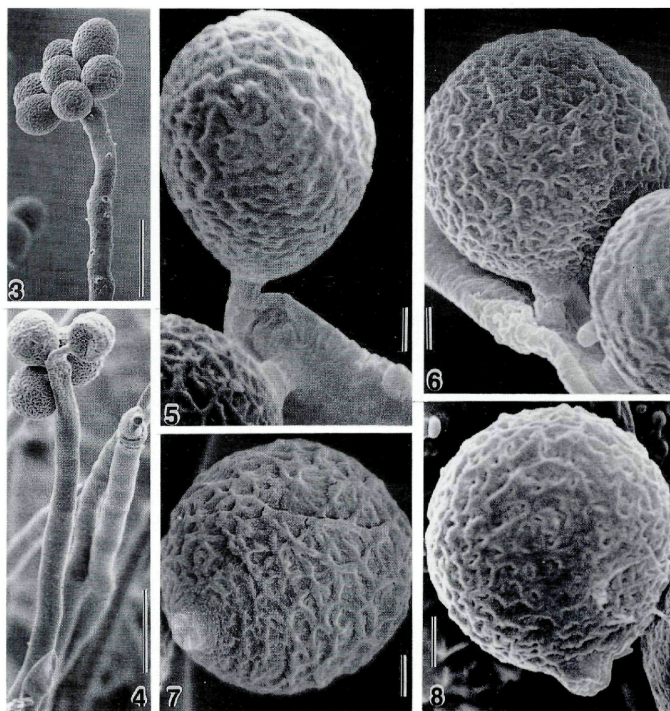
*Conidiophora macronematosa*, mononematosa, typice lateraliter ex hypha horizontali oriunda, erecta, saepe recta sed raro leniter flexuosa, 8–14 septata, laevia, non-ramosa, atro brunnea, ad apicem pallidiora, 140–405 x 3–4.5  $\mu\text{m}$ . Cellulae conidiogenae in conidiophoris incorporate, apicales, laeves, pallide brunneae, 8–36 x 3–4  $\mu\text{m}$ , proliferationibus irregulariter sympodialibus, cum 1–20 locis conidiogenis apicalis vel in cellula apicalia dispersis; loci hyalini, non incrassati, leniter vel raro valde protuberantes, truncati, usque ad 1  $\mu\text{m}$  diam. Conidia holoblastica, valde atro brunnea, crassitunicata, sphaerica, aseptata, verrucosa (ornamento reticulato), 7–10.5  $\mu\text{m}$  diam., cum segmento margine distincto; ? rhexolytica secedentia.

Colonies dark brown, conspicuous, fasciculate and separate or extensively confluent.— Mycelium partly superficial, partly +immersed in the substratum, composed of medium brown, smooth, branched, septate hyphae 2.5–3.5  $\mu\text{m}$  wide.— Conidiophores macronematous, mononematous, each typically arising laterally from an horizontal hypha, erect, mostly straight but occasionally slightly flexuous, 8–14 septate, smooth, unbranched, dark brown, becoming paler only near the apices, 140–405 x 3–4.5  $\mu\text{m}$ .— Conidiogenous cells integrated, apical, smooth, pale brown, 8–36 x 3–4  $\mu\text{m}$ , proliferating sympodially and irregularly, with 1–20 conidiogenous loci restricted to the apices or scattered over the apical cell; loci hyaline, unthickened, slightly or more rarely markedly protuberant, truncate, up to 1  $\mu\text{m}$  diam.— Conidia holoblastic, very dark brown, thick-walled, spherical, aseptate, verrucose (ornamentation reticulate), 7–10.5  $\mu\text{m}$  diam., with a distinct basal frill; secession possibly rhexolytic.

Specimen examined.—TURKEY: Myra, Anatolia, in foliis emortuis *Aloe* sp., 2 Sept.1991, B.C.Sutton, IMI 349855, HOLOTYPE.

Three species were originally included in *Virgariella* by Hughes (1953), and although *V. fuscopurpurea* (Berk. & M.A.Curtis) Hughes [syn. *Virgariella fusca* (Cooke) Hughes] and *V. globigera* (Sacc. & Ellis) Hughes have similar conidial sizes to *V. verrucosa*, they differ in having smooth conidia. Kirk (1981) described *V. ovoidea*, a species with conidia of a similar size to *V. verrucosa*, but they are ovoid rather than spherical, smooth and pale brown with only the base slightly darker. The other original species, *Virgariella atra* Hughes, and *V. caribaensis* Holubová-Jechová apud Holubová-Jechová & Mercado Sierra (1986) have larger conidia than *V. verrucosa* and they are also smooth. There have been other additions to the genus by de Hoog (1973b), de Hoog in de Hoog & Hermanides-Nijhof (1977) and Matsushima (1975) but Kirk (1981) has clearly shown that these species should be excluded from the genus for reasons other than questionable modes of secession. There is also some superficial similarity with *Ramichloridium indicum* (Subram.) de Hoog in de Hoog





Figs 3–8.— *Virgariella verrucosa* (IMI 349855).— 3. Cluster of conidia at the apex of a conidiophore and scars evident lower down at conidiogenous loci.— 4. Conidium attached at protuberant denticle.— 5. Two denticles with attached conidia.— 6. Secession of conidium by irregular circumscissile rupture of the denticle.— 7. Conidium showing reticulate surface ornamentation.— 8. Conidium showing reticulate surface ornamentation and protuberant basal scar.— Scales: figs 3,4 = 10  $\mu$ m; figs 5–8 = 1  $\mu$ m.

& al. (1977) which has conidia of similar size and shape to *V. verrucosa*. However the conidiophores are only up to 130  $\mu$ m long and the conidia are thin-walled, pale brown and occasionally 1-septate.

#### Key to accepted species of *Virgariella*

- 1. Conidia smooth ..... 2
- 1\*. Conidia verruculose, thick-walled, 7.0–10.5  $\mu$ m diam. ....  
     ..... *V. verrucosa*

2. *Conidia globose* ..... 3  
2\*. *Conidia not globose* ..... 4  
3. *Conidiophores up to 150 µm long x 4 µm wide; conidia 6.5–9.5 µm diam.* ..... *V. globigera*  
3\*. *Conidiophores 90–200 µm long x 4.5–6.0 µm wide; conidia 11.5–16.5 µm diam.* ..... *V. caribaensis*  
4. *Conidiophores short, 25–60 µm long; conidia ovoid, 8.0–9.5 x 5.0–6.0 µm* ..... *V. ovoidea*  
4\*. *Conidiophores exceeding 60 µm in length* ..... 5  
5. *Conidia 11–15 µm long x 8–13 µm wide, evenly pigmented* ...  
..... *V. atra*  
5\*. *Conidia 5.0–7.5 µm long x 4.0–5.0 µm wide, base darker brown*  
..... *V. fuscopurpurea*

### Excluded species

*V. fusca* (Cooke) Hughes, Can. J. Bot. 31: 654. 1953.

This is a synonym of *V. fuscopurpurea* (Berk. & Curt.) Hughes, *vide* Hughes (1958).

*V. hippotrichoides* (Corda) de Hoog, Stud. Mycol. 15: 205. 1977.

This name was transferred to *Pleurophragmium* by Ellis (1976), but this is not correct and there is no suitable genus available for the species, *vide* Kirk (1981).

*V. oblonga* Matsush., Icon. Microfung. Matsush. lect.: 163. 1975.

This is incorrect in *Virgariella*, *vide* Kirk (1981).

*V. psilonoides* (Preuss) de Hoog, Verh. K. Ned. Akad. Wet. ser. 2, 61: 66. 1973.

This is not congeneric with *Virgariella*, *vide* Kirk (1981).

*V. sphaerica* Matsush., Icon. Microfung. Matsush. lect.: 163. 1975.

This is incorrect in *Virgariella*, *vide* Kirk (1981).

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