

A new species of *Volvocisporium* from Namibia, *V. grewiae* sp. nov. (Microstromatales, Ustilaginomycota)

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Abstract A new species of *Volvocisporium* (Ustilaginomycota, Microstromatales) has been found on *Grewia* cf. *flavescens* (Tiliaceae) in Namibia. *V. grewiae* is the second representative of this genus worldwide and the first one from the African continent based on morphological and molecular data.

Introduction

Although plant parasitic micromycetes play an important role within the global ecosystem, large deficiencies exist in the knowledge of their number and diversity, especially in tropical and subtropical regions of Africa, Asia and South America. Our work within the BIOTA (Biodiversity Monitoring Transect Analysis) Southern Africa project contributes to filling such gaps by investigating the rust fungus flora (Uredinales) of Southern Africa. Within the frame of this project several field trips to Namibia were carried out to collect rust fungi in several regions of the country. On one of these field trips a hitherto unknown species of Ustilaginomy-

cotina was found on *Grewia* cf. *flavescens* in the Caprivi Strip, the north-easternmost region of Namibia. As *Volvocisporium grewiae* is only the second representative of this genus hitherto known, our findings supply valuable information on the host range, geographical distribution and morphological variability of this poorly known genus.

Materials and methods

Morphology

One dried herbarium specimen collected on a field trip to Namibia in April/May 2004 was examined by standard light microscopy (oil immersion) and by scanning electron microscopy (SEM). For light microscopical investigations with a Zeiss Axioskop, the spores were scraped from the surface of leaves with a razor blade, mounted and stained in lactophenol containing 0.05% cotton blue. About 20 basidia and basidiospores were measured to ascertain the morphological characters of the species. The results were documented by free-hand line drawings and by photographic pictures made with the integrated camera MC 80. For SEM investigations, samples from dried herbarium material were hand-cut with a razor blade, mounted flatly on a SEM stub and coated with gold in a sputter-coater. The samples were examined with a Cambridge Stereoscan 250 Mk 2. For examination of the sorus structure, cryosections were made. Small pieces of the dried herbarium material were soaked for 24 h in Tissue-Tek O.C.T. Compound 4583 (Sakura Fine-tek) and sectioned in a Leitz Kryostat 1720 at -20°C . Sections of 15 μm thickness were collected on a microscope slide and embedded and stained in lactophenol with cotton blue.

Taxonomical novelties *Volvocisporium grewiae* Ritschel & Oberw.

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The sections were observed with a Zeiss Axioskop and documented by line drawings as well.

Molecular phylogeny

For the phylogenetic analyses, DNA was isolated from herbarium specimen and partial LSU rDNA was amplified as described earlier (Begerow et al. 2001). The alignment was built with MAFFT 3.85 (Kato et al. 2002) using the accurate and interactive refinement method. After trimming off ends, the LSU alignment consisted of 554 bp. Phylogenetic analyses were carried out using PAUP* 4.0b10 (Swofford 2001). Modeltest 3.0 (Posada and Crandall 1998) was carried out to determine a model of DNA substitution that fits the data set. GTR+I+G was selected from the Akaike information criterion for the LSU alignment (base frequencies: $\pi_A=0.2445$, $\pi_C=0.1986$, $\pi_G=0.2989$, $\pi_T=0.2580$; substitution rates: A/C=0.7221, A/G=2.6571, A/T=0.7745, C/G=0.3248, C/T=5.8762, G/T=1.0000; gamma shape parameter=1.1100; percentage of invariant sites=0.4496). Neighbor-joining analysis was done using genetic distances according to the specified substitution model. Parsimony analysis was conducted in two steps where the first with 10,000 random additions without swapping resulted in several islands. Subsequent TBR swapping resulted in two most parsimonious trees for the LSU alignment with 685 steps (CI=0.498; RI=0.642; RC=0.320), 1,000 replicates were used for bootstrap analyses. For Bayesian analysis, four incrementally heated simultaneous MCMC Markov chains were run over 1,000,000 generations using the general time reversible model (six rate classes) including a proportion of invariant sites and gamma distributed substitution rates of the remaining sites (GTR+I+G). Trees were sampled every 100th generation, resulting in an overall sampling of 10,000 trees. From these, the first 3,000 trees were discarded (as burnin). MrBayes (Huelsenbeck and Ronquist 2001) was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the *a posteriori* probabilities. The genbank accession numbers are given in Fig. 3.

The plant names follow the International Plant Names Index (2006) and the herbarium acronyms are according to the Index Herbariorum (Holmgren and Homlgren 1998, onwards) which is available online.

Results and discussion

Volvocisporium grewiae Ritschel & Oberw. sp. nov.
on *Grewia* cf. *flavescens* (Tiliaceae) (Figs. 1, 2)

Morbus fungosus maculas dilute cinnamomeas ad castaneas pagina abaxiali foliorum evocat quae primum circulares sunt

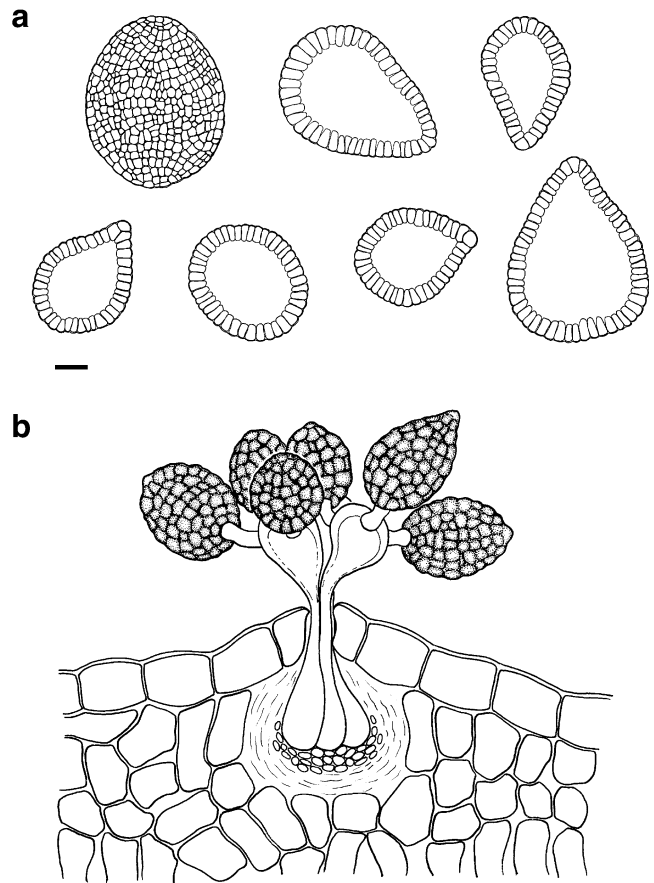
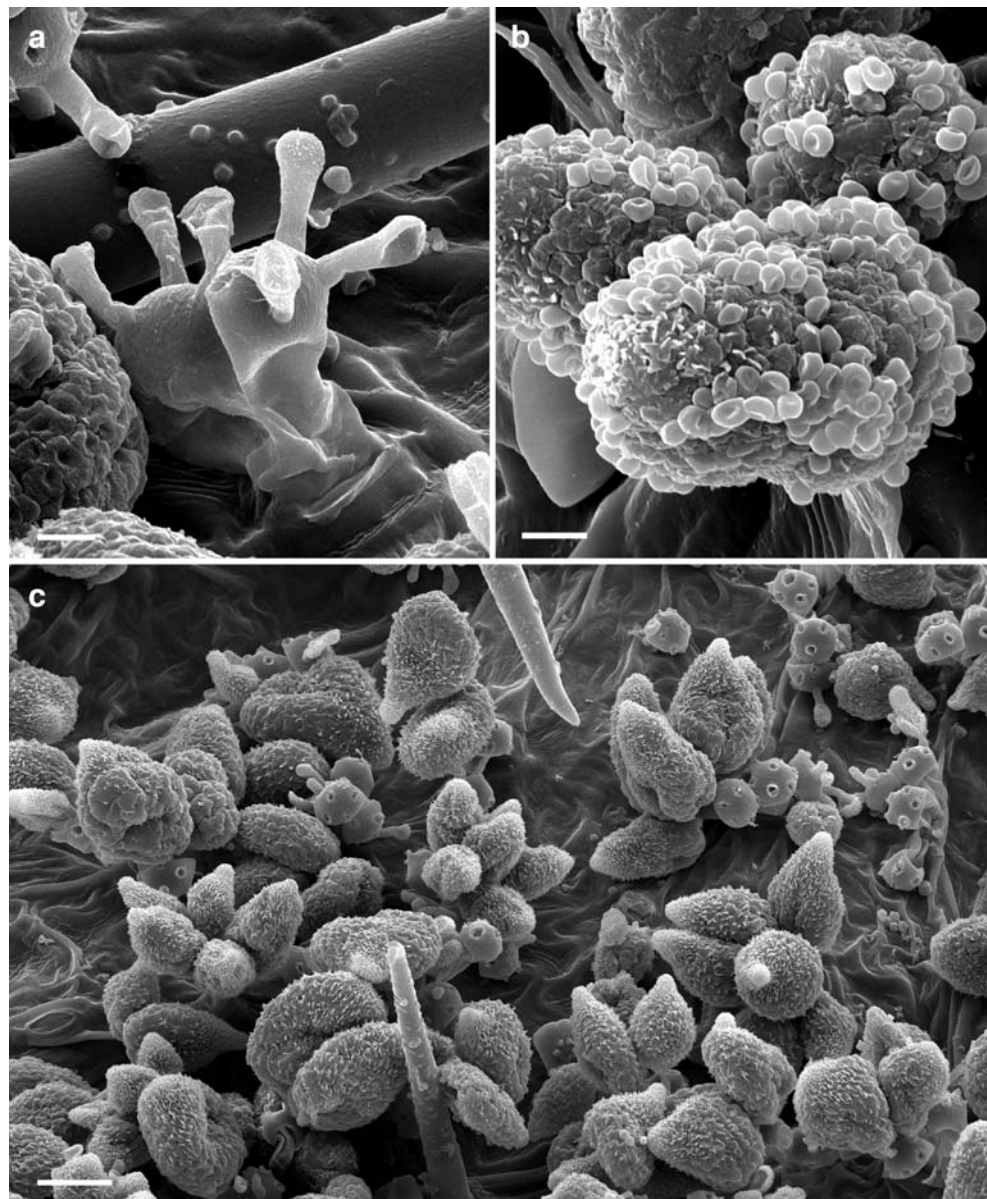


Fig. 1 *Volvocisporium grewiae* Ritschel & Oberw. sp. nov. **a** Multi-cellular, hollow-bodied basidiospores. **b** Diagrammatic median view of a group of basidia with immature basidiospores. Scale bars = 10 μ m

*deinde confluent et decolorationem vel mortem foliorum hospiti efficiunt; probasidia substomatalia, c. 20–30 μ m longa, basaliter bulbosa et usque ad 5–8 μ m lata, singula ad octona in contexto denso hypharum; metabasidia subglobosa ad clavata, maturitate ex stomatibus extrudentia, (11)12–16(20) \times 10–12(13) μ m, pariete c. 1 μ m crasso lateraliter, incrassato usque ad 3 μ m apicaliter, sterigmatibus 3–8, 5–6(8) μ m longis, 2–3 μ m latis; basidiosporae gastroideae, primum lachrymiformes et ex cellulis paucis compositae, deinde plusminusve ovoideae et moruloides, (35) 39–54(60) \times (20)25–43(50) μ m (medium 46 \times 34.5 μ m), ex cellulis numerosissimis, 2–4 μ m diam., irregulariter subpolyhedricae, pallide luteolae vel cinnamomeae compositae. In foliis *Grewiae* cf. *flavescens* Juss.*

Infection spots on the abaxial surface of leaves, pale cinnamon to chestnut brown, at first circular in shape, 1–10 mm diam., later confluent and irregular, covering large areas of the whole leaf, often accompanied by pale yellowish to pale brown discolored or necrotic spots on the adaxial leaf side; **probasidia** substomal, each of them c. 20–30 μ m long and basally bulb-like swollen up to

Fig. 2 *Volvocisporium grewiae* Ritschel & Oberw. sp. nov. SEM. **a** Basidia, growing through a stoma. **b** basidiospores forming subglobose yeast cells. **c** Abaxial surface of a leaf of *Grewia* cf. *flavescens* covered with basidia and basidiospores of *V. grewiae*. Scale bars: **a** = 10 μ m, **b** = 5 μ m, **c** = 20 μ m



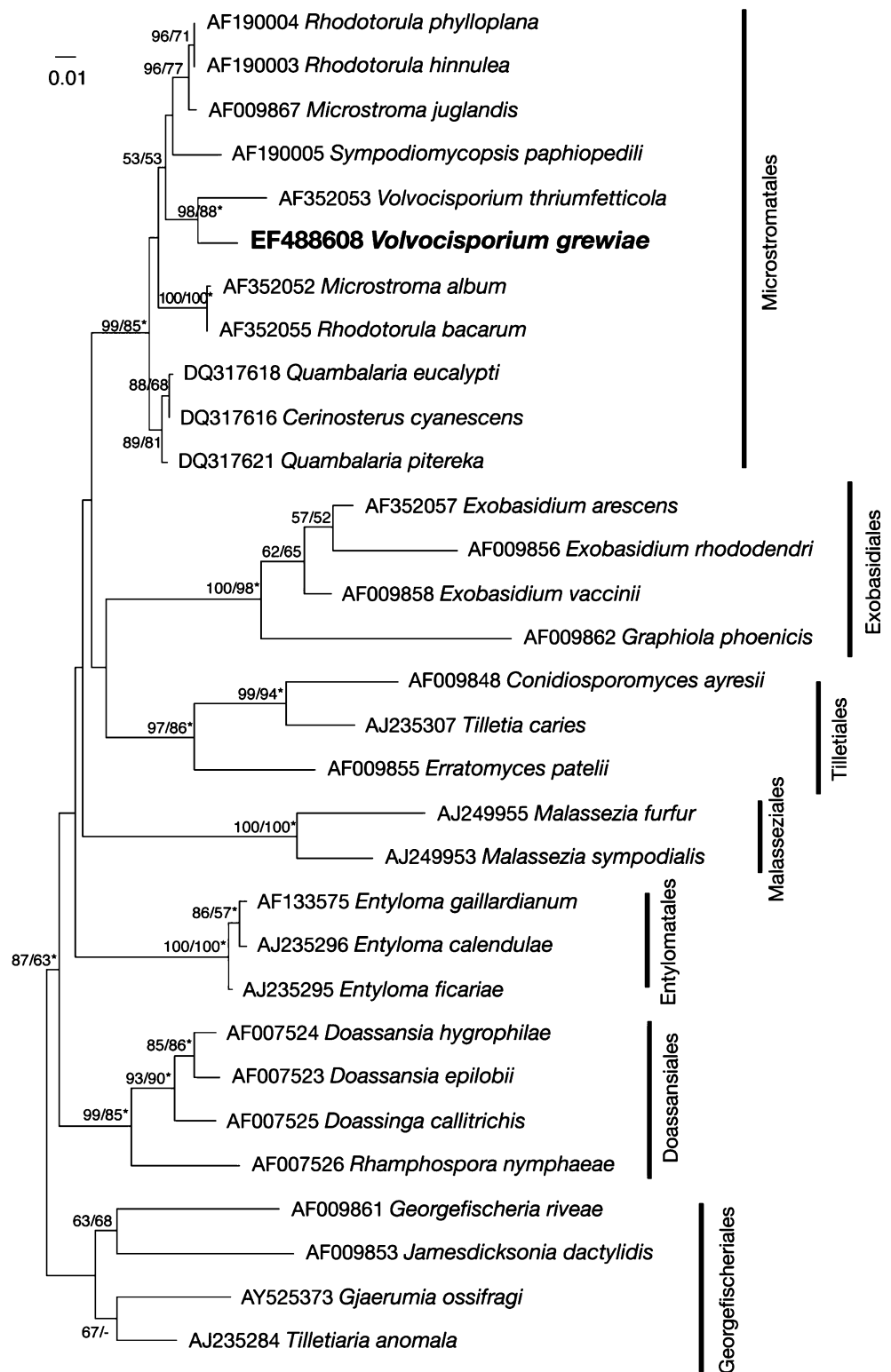
5–8 μ m, one to eight of them embedded in a matrix consisting of densely packed hyphae; **metabasidia** subglobose to clavate, breaking through the stomata at maturity, (11)12–16(20) \times 10–12(13) μ m, wall laterally c. 1 μ m thick, apically thickened up to 3 μ m, sterigmata 3–8, each of them 5–6(8) μ m long and 2–3 μ m wide; **basidiospores** gastroid, at first consisting of only a few cells and lacrimiform, later ovoidal with a small conical umbo or slightly irregular in shape, forming a hollow body of (35)39–54(60) \times (20)25–43(50) μ m (mean 46 \times 34.5 μ m), composed of hundreds of cells, each of them 2–4 μ m diam. and irregularly angular in shape, pale yellowish to cinnamon brown.

On leaves of *Grewia* cf. *flavescens* Juss.

Type material Africa, Namibia, Caprivi region, campsite at the main road B8 near Popa Falls, 18°06'46.9"S, 21°34'52.9" E, on *Grewia* cf. *flavescens* Juss., leg. A. Ritschel & E. Uhlmann, 11 May 2004. Holotype (PREM), Isotype (Z+ZT).

The type species of *Volvocisporium* was originally described from India under the name *Muribasidiospora triumfeticola* M.S. Patil (Patil 1977). Its classification in *Muribasidiospora* was mainly based on the formation of multicellular basidiospores, which was at that time regarded as the most important criterion for a membership of the latter genus (Rajendren 1968). Begerow et al. (2001) studied all known species of *Muribasidiospora* comparing their morphology, ultrastructure and molecular characters and found differences between *M. triumfeticola* and the other repre-

Fig. 3 Molecular phylogeny of Exobasidiomycetes based on neighbor-joining analysis of LSU rDNA sequences. The topology was rooted with members of the Geogefischeriales. The numbers refer to percentage bootstrap values of 1,000 replicates of neighbor-joining and maximum parsimony analysis respectively, values smaller than 50% are not shown; the asterisks highlight groups, which are supported with a posterior probability of 1.0 in the bayesian analysis. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site



representatives of *Muribasidiospora*, *M. celtidis* (T.S. Ramakr. & K. Ramakr.) Kamat & Rajendren, *M. hesperidium* (Maire) Kamat & Rajendren, and *M. indica* Kamat & Rajendren. *M. triumfeticola* differs from the latter not only in ultrastructural features such as the shape of the septal pores and the

interaction sites at the host–parasite interfaces, but also in the formation of apically thick-walled metabasidia and in the unique type of multi-cellular, hollow-bodied basidiospores. A further discrepancy was observed in the shape of the yeast cells, which are filiform in the latter three species versus

subglobose to shortly ellipsoidal in *M. triumfetticola*. Interestingly, morphological structures similar to those observed in *M. triumfetticola* were also found in another group of Ustilaginomycotina, in members of the order Microstromatales which seem to differ from *M. triumfetticola* mainly in the development of basidia and basidiospores. As these findings were supported by the results of the molecular study the new genus *Volvocisporium* and the new family Volvocisporiaceae were implemented and their single representative *V. triumfetticola* rearranged from the order Exobasidiales into the Microstromatales (Begerow et al. 2001). The specimen from Namibia shares with this species morphological features like the special type of multicellular, balloon-shaped basidiospores (Figs. 1a, 2c), basally swollen probasidia and metabasidia whose apical wall is distinctly thickened (Figs. 1b, 2a), subglobose yeast cells (Fig. 2b) and thus belongs undoubtedly to *Volvocisporium*. Differences exist, however, in the dimension of the metabasidia as well as in the diameter and appearance of the basidiospores. Whereas the metabasidia of the Namibian sample are only 11(12)–16(20) μm long, those of *V. triumfetticola* reach a length of 58–85 μm and are therefore distinctly larger. Being 28–37 \times 22–28 μm , the basidiospores of *V. triumfetticola* are much smaller and also differ in the oval (Patil 1977) to globose shape (Begerow et al. 2001).

The phylogenetic analysis of LSU rDNA sequences results in a well supported monophyletic group for *Volvocisporium grewiae* together with *V. triumfetticola* (Fig. 3). The fungus from Namibia is therefore proposed as the new species *Volvocisporium grewiae*. The topology of the Microstromatales is very similar to the data presented by De Beer et al. (2006) and the relationship between the families remain unclear. However, the molecular distances and the separation of the two *Volvocisporium* species seem to justify the family rank for the monophyletic group with

the unique combination of characters. The new information on the second species highlight the importance of new collections of plant parasites.

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