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Anther smut fungi on monocots☆

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ABSTRACT

Teliospores, hyphal septa, cellular interactions, and nucleotide sequences from the ITS and LSU region of the rRNA gene of specimens of *Ustilago vaillantii s. lat.* on *Muscari* and *Scilla* species were examined and compared with findings in other *Ustilaginomycotina*. The data show that *U. vaillantii s. lat.* specimens belong to the *Urocystales* and represent the sister group of the *Urocystaceae*, standing well apart from *Vankya heufleri* and *V. ornithogali*. Within the *Urocystales*, *U. vaillantii s. lat.* is unique in sporulating in the anthers of the host plants. Accordingly, the new genus *Antherospora* is proposed for the anther smuts on *Hyacinthaceae*. In addition, our data show that there is a stringent phylogenetic correlation between the specimens of *Antherospora* and their respective hosts. Thus, the specimens on *Scilla* spp. as well as those on *Muscari* spp. form highly supported monophyla. Furthermore, on *Scilla* a phylogenetic dichotomy exists between the specimens infecting *Scilla bifolia* and those infecting *S. vindobonensis*, with the specimens of the two host species showing a difference of 17 bp in the ITS nucleotide sequences. Therefore, *A. vindobonensis* is described as a new species, and *A. scillae* and *A. vaillantii* are proposed as new combinations. Consequently, because of their sporulation in anthers and their parasitism on species of other genera of the *Hyacinthaceae*, *Ustilago albucae*, *U. peglerae*, *U. tourneuxii*, and *U. urgineae* are also ascribed to *Antherospora* as new combinations. Descriptions are given for all species.

Introduction

Anther smuts on eudicots belong to the pucciniomycotinous genus *Microbotryum* (Bauer *et al.* 1997, 2001, 2006; Begerow *et al.* 1997). Vánky (1998) discussed the history of *Microbotryum* in detail. The core *Microbotryum* spp., anther smuts parasitizing members of *Caryophyllaceae*, are important model organisms for many biological disciplines, for example genetics (e.g. Hood & Antonovics 2004), population analysis (e.g. Giraud 2004), phylogenetics (e.g. Kemler *et al.* 2006), coevolution (e.g. Begerow *et al.* 2004), and ecology (e.g. Thrall *et al.* 1993).

By contrast little is known of the ustilagomycotinous anther smuts that parasitize monocots. Thus, from the described species *Ustilago albucae*, *U. muscari-botryoidis*, *U. peglerae*, *U. scillae*, *U. tourneuxii*, *U. urgineae*, and *U. vaillantii* (see Zundel 1953, Vánky 1994, and the references therein) no ultrastructural and only scant molecular phylogenetic data are available. Merely from the ITS analysis of Roux *et al.*

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(1998) it can be concluded that *U. vaillantii* belongs to the *Ustilaginomycetes* and not to the *Microbotryales*. All these anther smut species occur on plants of the monocotyledonous family *Hyacinthaceae*. Because the new system of *Ustilaginomycetes*, now considered as the subphylum *Ustilaginomycotina* (Bauer *et al.* 2006), revealed that parallel evolution with their hosts was apparently a widespread phenomenon in the ustilaginomycotinous phylogeny (Bauer *et al.* 1997, 2001), the uniform host spectrum of the anther smuts on monocots suggests that this group is monophyletic.

Because of morphological similarities, Vánky (1994) treated *Ustilago muscari-botryoidis, U. scillae, U. tourneuxii, U. urgineae,* and *U. vaillantii* as belonging to one species, *U. vaillantii*. With the new system of *Ustilagomycotina* it becomes clear that *Ustilago* species are restricted to poacean hosts (Bauer *et al.* 1997, Begerow *et al.* 1997). Thus, based mainly on the liliaceous hosts, Ershad (2000) transferred *U. vaillantii* together with *U. heufleri* and *U. ornithogali* into the new genus *Vankya* typified by *V. ornithogali*. However, anther smuts on monocots differ in some features from foliicolous *Vankya ornithogali* and *V. heufleri*. Therefore, we carried out morphological, ultrastructural, and molecular studies in order to propose a phylogenetic hypothesis for this group.

Materials and methods

The specimens of anther smuts on members of *Hyacinthaceae* examined in this study are listed in Table 1. Additionally, DNA of the following specimens was sequenced [the first number refers to the GenBank accession number of the ITS sequence (if present) and the second number (if present) refers to the GenBank accession number of the LSU sequence]: *Melanustilospora ari* (-/EF517924), *Urocystis alopecuri* (-/EF517945), *U. beckmanniae* (-/EF517943), *U. colchici* (-/EF517933), *U. ficariae* (-/EF517938), *U. floccosa* (-/EF517937), *U. galanthi* (-/EF517922), *U. ornithogali* (-/EF517935), *U. syncocca* (-/EF517929), *Vankya heufleri* (EF667965/EF517925, EF653981), and *V. ornithogali* (EF635910/-). Nomenclatural novelties were registered in MycoBank (www.MycoBank.org, see Crous *et al.* 2004).

LM

Sori and spore characters were studied using dried herbarium material. Morphological observations and measurements of spores were made of the material mounted in lactophenol heated to the boiling point and then cooled. A Nikon Eclipse E600 was used for LM studies. In each specimen, at least 30 spores were measured.

EM

The ultrastructure of *Antherospora scillae* (syn. *Ustilago scillae*) of specimen TUB 15840 (see Table 1) was studied using a Zeiss EM 109 transmission electron microscope at 80 kV. Samples were fixed overnight with 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature. Following six transfers in 0.1 M sodium cacodylate buffer, samples were post-fixed in 1 % osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1 % aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, using 10 min changes at 25, 50, 70, 95 %, and three times in 100 % acetone. Samples were embedded in Spurr's plastic and sectioned with a diamond knife. Serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and washed with distilled water.

Molecular analyses

Genomic DNA was isolated from herbarium specimens (for details see Table 1). Methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data have been described by Lutz *et al.* (2004). We determined base sequences of the 5́-end of the nuLSU rDNA, including the domains D1/D2 (LSU), and the ITS1/2 region of the rDNA, including the 5.8S rDNA (ITS). The LSU was amplified using the primer pair NL1 and NL4 (ÓDonnell 1992, 1993) or LR6 (Vilgalys & Hester 1990), respectively. The ITS was amplified using the primer pair ITS1f and ITS4 (Gardes & Bruns 1993). For amplification of both regions we adjusted the annealing temperature to 45 °C. DNA sequences prepared in the course of this study were deposited in GenBank, and their accession numbers are listed in Table 1.

To ascertain the phylogenetic positions of the analysed anther smut specimens, we followed two strategies: (1) we analysed the LSU sequences of selected anther smut specimens (compare Fig 2) together with representatives of all genera of the *Urocystales* and most of the *Ustilaginales* (GenBank accession numbers are given in Fig 2) to support their position in *Urocystales*, and (2) we analysed the concatenated ITS and LSU sequences of all anther smut specimens growing on *Muscari* and *Scilla*, respectively, within a dataset reduced to representatives of the *Urocystales* (GenBank accession numbers are given in Fig 3) to test whether distinct phylogenetic lineages within these specimens could be revealed.

Sequences were aligned for both datasets with MAFFT 5.861 (Katoh *et al.* 2002, 2005) using the L-INS-i option. Both alignments [length: 591 bp (LSU), 1311 bp (ITS + LSU); variable sites: 305 (LSU), 385 (ITS + LSU)] were used throughout their length. We avoided both manipulation of the alignment by hand and manual exclusion of any positions as recommended by Giribet & Wheeler (1999) and Gatesy *et al.* (1993), respectively.

For the LSU dataset, we used four different algorithms to analyse the phylogenetic position of the anther smuts of monocots. For NJ analysis the data were first analysed using Modeltest 3.7 (Posada & Crandall 1998) to find the most appropriate model of DNA substitution. The hierarchical likelihood ratio test and the Akaike information criterion proposed the GTR + I+ G DNA substitution model. BS values were calculated for 1 K replicates. MP was performed in two steps as described by Begerow *et al.* (2001). ML was performed using the same substitution model as for the NJ analysis, but BS analysis was only performed for 100 replicates due to time restrictions. For Bayesian analysis we used a Bayesian approach of phylogenetic inference using a MCMC technique as implemented in the computer program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Four

Table 1 – List of sequenced *Antherospora* specimens with host plants, GenBank accession numbers, spore sizes, mean spore sizes with standard deviation, and reference specimens

Species	Host	GenBank acc. no. (ITS/LSU)	Spore sizes (μm)	Mean spore sizes (μm) with S.D.	Reference specimens
Antherospora scillae	*Scilla bifolia*	EF653983/EF653965	(8–)8.5–11.5(–12.5) × (6.5–)8–10	9.928 ± 0.979 × 8.891 ± 0.613	Germany, Baden-Württemberg, Tübingen, forest S of the street Entringen-Kayh, 17 Mar 2005, *M. Kemler & M. Lutz*, TUB 15840
A. scillae	*S. bifolia*	EF653984/EF653966	7–10(–13) × 6.5–8(–8.5)	8.596 ± 1.317 × 7.461 ± 0.503	Germany, Baden-Württemberg, Heilbronn, Horkheim, garden, 17 Mar. 2003, *M. Lutz*, TUB 15842
A. scillae	*S. bifolia*	EF653985/EF653967	(7–)7.5–10(–12) × (6–)6.5–8.5(–9.5)	8.557 ± 1.003 × 7.461 ± 0.692	Germany, Baden-Württemberg, Heilbronn, SW of Horkheim, riverside of Neckar, 'Scillawäldchen', 17 Mar 2003, *M. Lutz*, TUB 15841
A. scillae	*S. bifolia*	EF653992/EF653974	7–9(–11) × (6–)7–8(–9)	7.97 ± 1.067 × 7.22 ± 0.561	Slovakia, Malé Karpaty Mts, Dubová village, oak wood near the calvary, NW from the village, 29 Mar 2004, *J. Vlčko, R. Hrivnák & J. Kochjarová*, SAV s.n., TUB 15848
A. scillae	*S. bifolia*	EF653996/EF653978	(7–)7.5–11(–12) × 6.5–9.5(–10)	9.437 ± 1.318 × 8.235 ± 0.78	Germany, Baden-Württemberg, Kirchheim/Teck, Hohenreisach, 14 Apr 2006, *N. Böhling*, TUB 15839
A. vindobonensis	*S. vindobonensis*	EF653989/EF653971	7–10(–11) × 7–9	8.68 ± 1.136 × 7.54 ± 0.783	Slovakia, Podunajská nížina, Rusovce village, in the park near the manor house, 13 Apr 2003, *K. Bacigálová*, SAV s.n. — holotype; TUB 15847, HUV 21503 — isotype
A. vindobonensis	*S. vindobonensis*	EF653990/EF653972	7–10(–12) × 7–9	8.73 ± 1.033 × 7.89 ± 0.827	Slovakia, Podunajská nížina, Chynorany village, Chynoriansky luh Nature Reserve, 26 Mar 2004, *J. Kochjarová*, SAV s.n., TUB 15846
A. vindobonensis	*S. vindobonensis*	EF653991/EF653973	7–11(–12) × (6–)7–9	8.83 ± 1.189 × 7.79 ± 0.89	Slovakia, Podunajská nížina, Jarovce/Kittsee villages, in the pleasantry, *J. Vlčko, R. Hrivnák, I. Ondrášek, M. Ujházyová & K. Ujházy*, SAV s.n., TUB 15845
A. vindobonensis	*S. vindobonensis*	EF653994/EF653976	7–9(–12) × (6–)7–8(–9)	8.09 ± 1.101 × 7.15 ± 0.435	Hungary, Dunaalmás village, 19 Mar 2003, *J. Kochjarová & J. Vlčko*, SAV s.n., TUB 15843
A. vindobonensis	*S. vindobonensis*	EF653995/EF653977	(6–)7–9(–12) × (6–)7–8(–9)	8.05 ± 1.14 × 7.17 ± 0.513	Austria, Maria–Lanzendorf village, mixed forest southern from the village, near the path to Himberg village, 22 Mar 2004, *J. Vlčko & J. Kochjarová*, SAV s.n., TUB 15837
A. vindobonensis	*S. vindobonensis subsp. borhidiana*	EF653993/EF653975	7–11(–15) × 7–10	9.07 ± 1.558 × 7.89 ± 1.109	Hungary, Mecsek Mts, Pécs, Misina hill, 10 Mar 2002, *J. Vlčko, R. Hrivnák & K. Ujházy*, SAV s.n., TUB 15844
A. vaillantii	*Muscari comosum*	EF653997/EF653979	(6–)6.5–10.5(–11.5) × 6–8(–9)	8.35 ± 1.308 × 7.105 ± 0.936	Germany, Baden-Württemberg, Kirchheim/Teck, garden Römersteinstrasse 12, 2 May 2006, *N. Böhling*, TUB 15838
A. vaillantii	*Muscari neglectum*	EF653998/EF653980	(7–)7.5–11.5(–12) × (6–)7–8.5(–10)	9.2 ± 1.361 × 7.625 ± 1.154	Slovenia, Kras, 7 km ESE from Sezana, 30 Apr 2006, *C. & K. Vánky*, HUV 21337
A. vaillantii	*Muscari neglectum*	EF653982/EF653964	(6–)7–10(–13) × (5.5–)6–8	8.44 ± 1.635 × 6.882 ± 0.696	Germany, Baden-Württemberg, Tübingen, Gabriel-Biel-Str. 5, 17 Apr 2005, *C. & K. Vánky*, HUV 21046
A. vaillantii	*Muscari tenuiflorum*	EF653986/EF653968	8–16(–16.5) × (7–)7.5–9(–10)	11.038 ± 2.521 × 8.36 ± 0.7	Germany, Sachsen-Anhalt, Saalkreis, Löbejün, Schiedsberg, 25 May 2000, *H. Jage*, GLM 47396
A. vaillantii	*Muscari tenuiflorum*	EF653987/EF653969	(6.5–)7.5–10.5(–11) × 6–9(–9.5)	9 ± 1.054 × 8.071 ± 0875	Germany, Sachsen-Anhalt, Saalkreis, Löbejün, Schiedsberg, 29 May 1999, *U. Richter*, GLM 48095
A. vaillantii	*Muscari tenuiflorum*	EF653988/EF653970	7–11.5 × (6–)6.5–8(–9)	8.2 ± 1 × 7.291 ± 0.69	Germany, Sachsen-Anhalt, Saalkreis, Löbejün, Schiedsberg, 24 May 1999, *W. Durka*, GLM 50411

GLM, Herbarium of the Staatliches Museum für Naturkunde Görlitz, Germany; HUV, Herbarium *Ustilaginales* Vánky, Tübingen, Germany; TUB, Herbarium of the Spezielle Botanik & Mykologie, Eberhard-Karls-Universität Tübingen, Germany; SAV, Herbarium of the Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia.

incrementally heated simultaneous Markov chains were run over 1 M generations using the general time reversible model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model as recommended by Huelsenbeck & Rannala (2004). Trees were sampled every 100th generation resulting in an overall sampling of 10 001 trees. From these, the first 3001 trees were discarded (burn-in = 3001). The trees sampled after the process had reached stationarity (7 K trees) were used to compute a 50 % majority rule consensus tree to obtain estimates for the PPs of groups of species. This Bayesian approach of phylogenetic analysis was repeated four times to test the independence of the results from topological priors (Huelsenbeck *et al.* 2002). Trees were rooted with members of the *Exobasidiomycetes*.

For the concatenated ITS + LSU dataset, we used both NJ analysis and a Bayesian approach for molecular phylogenetic analysis. For NJ analysis the data were first analysed using Modeltest 3.7 (Posada & Crandall 1998) to find the most appropriate model of DNA substitution. The hierarchical likelihood ratio test proposed the TrN + G DNA substitution model. BS values were calculated for 1 K replicates. The Bayesian analysis was performed as described above with the following variations: Four incrementally heated simultaneous Markov chains were run over 2 M generations, the burn-in was set to 2001. Trees were rooted with *Doassansiopsis limnocharidis* representing the *Doassansiopsidaceae*.

Pairwise relative base pair differences were calculated from the MAFFT alignment with PAUP version 4.0b10 using the PAIRDIFF command (Swofford 2001).

Results

Morphology

All specimens of the *Ustilago vaillantii s. lat.* complex studied here (see Table 1) sporulated in the anthers (rarely also on the surface of anther filaments) of their hosts. Spore sizes of the specimens are listed in Table 1. The spore sizes of the specimens on the different host species were very similar, except for specimen GLM 47396 representing *Antherospora vaillantii* (syn. *Ustilago vaillantii*). The mean spore diameter in this specimen was roughly 2 μm greater than in the other specimens (Table 1).

Septa

The septa in soral hyphae of *Antherospora scillae* (syn. *Ustilago scillae*; TUB 15840) had simple pores with more or less rounded pore lips, and they were enclosed by membrane caps (Fig 1A–B).

Cellular interaction

Antherospora scillae (syn. *Ustilago scillae;* TUB 15840) formed intracellular aseptate haustoria with an electron-opaque, vesicular matrix coating the entire haustorial cell wall (Fig 1C–D). Haustoria regularly terminated in the host cell and were morphologically distinct from the intercellular hyphae.

Molecular phylogenetic analyses

The analysis of the LSU dataset placed the anther smuts of monocots in the *Urocystales* as a sister group to the *Urocystaceae,* which comprised representatives of *Flamingomyces*, *Melanustilospora*, *Mundkurella*, *Urocystis*, *Ustacystis,* and *Vankya*. The monophyly of *Antherospora* was highly supported by all four methods, whereas the relationship between *Antherospora* and the members of the *Urocystaceae,* as well as the relationships within the *Urocystaceae* remained unresolved using LSU rDNA sequences (Fig 2).

For the combined ITS + LSU dataset, the different runs of Bayesian phylogenetic analyses that were performed and the NJ analysis yielded consistent topologies. We present the consensus tree of one run of Bayesian phylogenetic analyses to illustrate the results (Fig 3). Estimates for PPs are indicated on branches before slashes; BS values from the NJ analysis are indicated after slashes. In all analyses, specimens from *Muscari* and *Scilla* clustered together being the sister taxon of the sampled members of the *Urocystaceae*. Thus, they were separated from *V. heufleri* and *V. ornithogali*. The latter species clustered within the clade composed of representatives of the *Urocystaceae* (*Mundkurella kalopanacis, Urocystis colchici*, and *Ustacystis waldsteiniae*). Within the specimens from *Muscari* spp. and *Scilla* spp. the analyses revealed two well-supported, distinct phylogenetic lineages correlated with the two host genera. Moreover, within the specimens of each host genus the specimens split up in distinct clusters according to the host species (*Muscari tenuiflorum, M. comosum* + *M. neglectum, Scilla bifolia,* and *S. vindobonensis*).

Taxonomy

Antherospora R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **gen nov.**

MycoBank no.: MB 511407

Etym.: Referring to the sporulation in the anthers.

Fungi Urocystalium sensu Bauer *et al.* (1997) in antheris vel interdum in aliis organis floralibus interioribus Hyacinthacearum sporulantes. Sporae singulares, sine cellulis sterilibus.

Typus: *Antherospora vaillantii* (Tul. & C. Tul.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky 2008.

Members of the *Urocystales sensu* Bauer *et al.* (1997) sporulating in the anthers and occasionally also in other inner floral organs of *Hyacinthaceae*. Spores single, sterile cells lacking.

Antherospora albucae (Syd. & P. Syd.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov**.

MycoBank no.: MB 511408

Basionym: *Ustilago albucae* Syd. & P. Syd., *Wiss. Ergebn. dt. ZentAfr, Exped. 1907–1908*. **Bd. 2**: 95 (1914).

Typus: **Rwanda**: *Buganza*: S of the Lake Mohasi, on *Albuca* sp., 28 Jul 1907, collector unknown, no. 610 (type lost in B).

Sori in all swollen, deformed, globoid flowers of an inflorescence, in the anthers, ovaries and on the surface of other inner

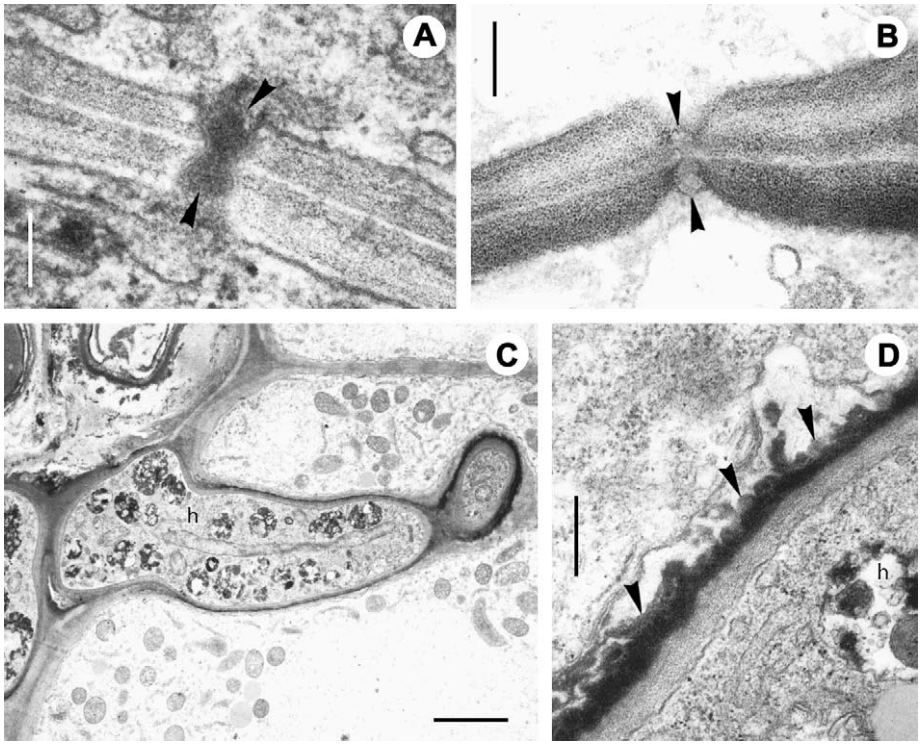

Fig 1 – Ultrastructure of *Antherospora scillae* (syn. *Ustilago scillae*). (A–B) Simple pores with two outer membrane caps (arrowheads). Bars = 0.1 μm. (C) Section through a haustorium (h). Note the electron-opaque matrix coating the haustorial wall. Bar = 2 μm. (D) Detail of a haustorium (h) showing the electron-opaque, vesicular matrix (arrowheads) coating the haustorial wall. Bar = 0.3 μm.

floral organs, producing blackish brown, powdery spore masses, at first enclosed by the outermost floral envelopes, later disclosed. *Spores* variable in shape and size, globose, ovoid, ellipsoidal, elongated, slightly irregular, sometimes curved, 9.5–22.5 × 7–14.5 μm, subglobose spores 9.5–15.5 μm in diam, yellowish brown; wall even, *ca* 0.5 μm thick, densely and minutely verruculose, spore profile smooth to finely wavy.

Comments: The holotype specimen of *Ustilago albucae* has been destroyed in 1943 during the Berlin fire along with the greater part of the Berlin herbarium. Thus, the present description is based on non-type specimens PREM 15450 and 23630, on *Albuca altissima* Dryand. from South Africa.

Antherospora peglerae (Bubák, Syd. & P. Syd.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov**.
MycoBank no.: MB 511409
Basionym: *Ustilago peglerae* Bubák, Syd. & P. Syd., *Ann. Mycol.* **12**: 264 (1914).
Typus: **South Africa**: *Cape Prov.*, Kentani, on *Ornithogalum lacteum*, 12 Nov 1913, *A. Pegler* (PREM 7101 — holotype, HUV 18240! — isotype).

Sori in all deformed, globoid flowers of an inflorescence, in the anthers, often on the filaments, more rarely also on the surface of gynoeceum, producing dark brown, powdery spore masses, enclosed for a long period by the outermost floral envelopes. *Spores* vary considerably in shape and size, usually ellipsoidal, elongated, pyriform, often curved, kidney-shaped, sometimes also asymmetrically, more rarely subglobose, 9–24(–27) × 7–12 μm, yellowish or pale olivaceous–brown; wall even, *ca* 0.5 μm thick, finely and densely verruculose, spore profile finely rough.

Comments: Antherospora peglerae differs from the other species by larger, usually elongated, often curved spores.

Antherospora scillae (Cif.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov**.
MycoBank no.: MB 511410
Basionym: *Ustilago scillae* Cif., *Ann. Mycol.* **29**: 24 (1931).
Typus: **Germany**, Baden, Rastatt, Ottersdorfer Wald, on *Scilla bifolia*, Apr and May 1875, *J. Schröter* (HUV 4934 — lectotype designated by Vánky 1985: 249, isolectotypes in Rabenh., *Fungi eur.* no. 2098, among others in WRSL s.n.).

Sori in the anthers and on the surface of anther filaments producing dark olive–brown, powdery mass of spores, initially enclosed by the floral petals, later disclosed. Infection systemic, all anthers and flowers of an inflorescence are infected. *Spores* quite regular in shape, globose, subglobose, ovoid, or very rarely ellipsoidal, tear-shaped or elongated, 7–11.5(–13) × (6–)6.5–9.5(–10), olive–brown, often lighter coloured on one side; wall *ca* 0.5 μm thick, thinner on the lighter side, finely and densely verruculose, spore profile finely wavy.

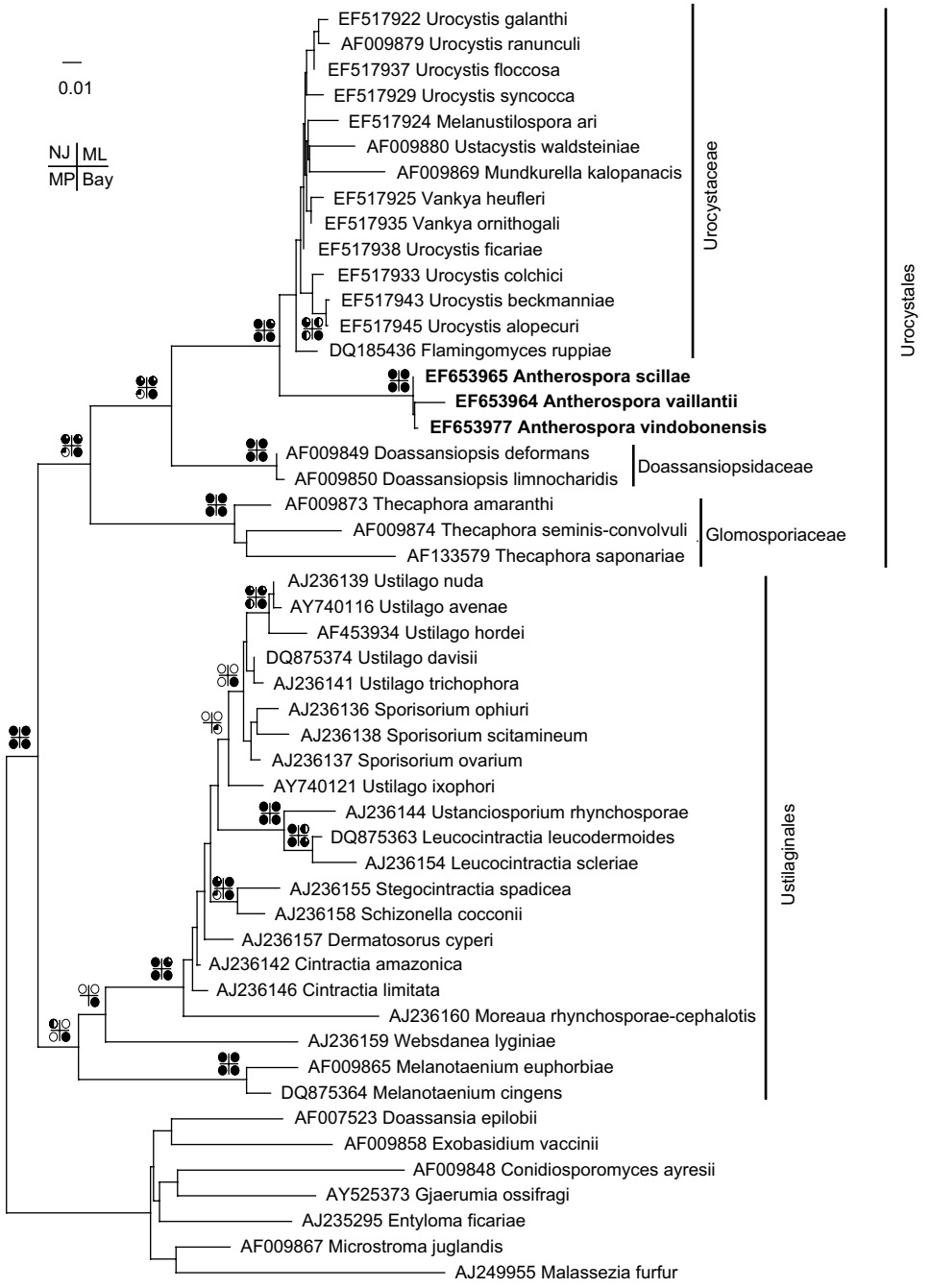

Fig 2 – Phylogenetic hypothesis based on NJ analysis using the GTR + I + G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites. Support values are given as indicated for NJ, MP, ML, and bayesian inference (Bay). For NJ, MP, and ML the classes represented by open and partially or fully closed circles are 50–75, 76–85, 86–90, 91–95, 96–100. For bayesian inference the classes were adjusted to 70–80, 81–90, 91–95, 96–98, 99–100.

Comments: The present description is based on the specimens included in molecular phylogenetic analyses. Our data suggest that there is high host specialization amongst species of *Antherospora* infecting *Scilla*, and we cannot rule out that *A. scillae* is restricted to *Scilla bifolia*.

In the literature there are numerous reports of various species of *Scilla* infected by anthericolous smuts. Until molecular data are available for these specimens, we suggest that these smuts are referred to as *Antherospora scillae s. lat.* Simultaneously, we tentatively prefer to keep the name *Antherospora scillae s. str.* to specimens on *Scilla bifolia*.

Antherospora tourneuxii (A. A. Fisch. Waldh.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov**.

MycoBank no.: MB 511411

Basionym: *Ustilago vaillantii* var. *tourneuxii* A. A. Fisch. Waldh., *Verh. Bot. Ver. Prov. Brandenb.* **22**: 65 (1880).

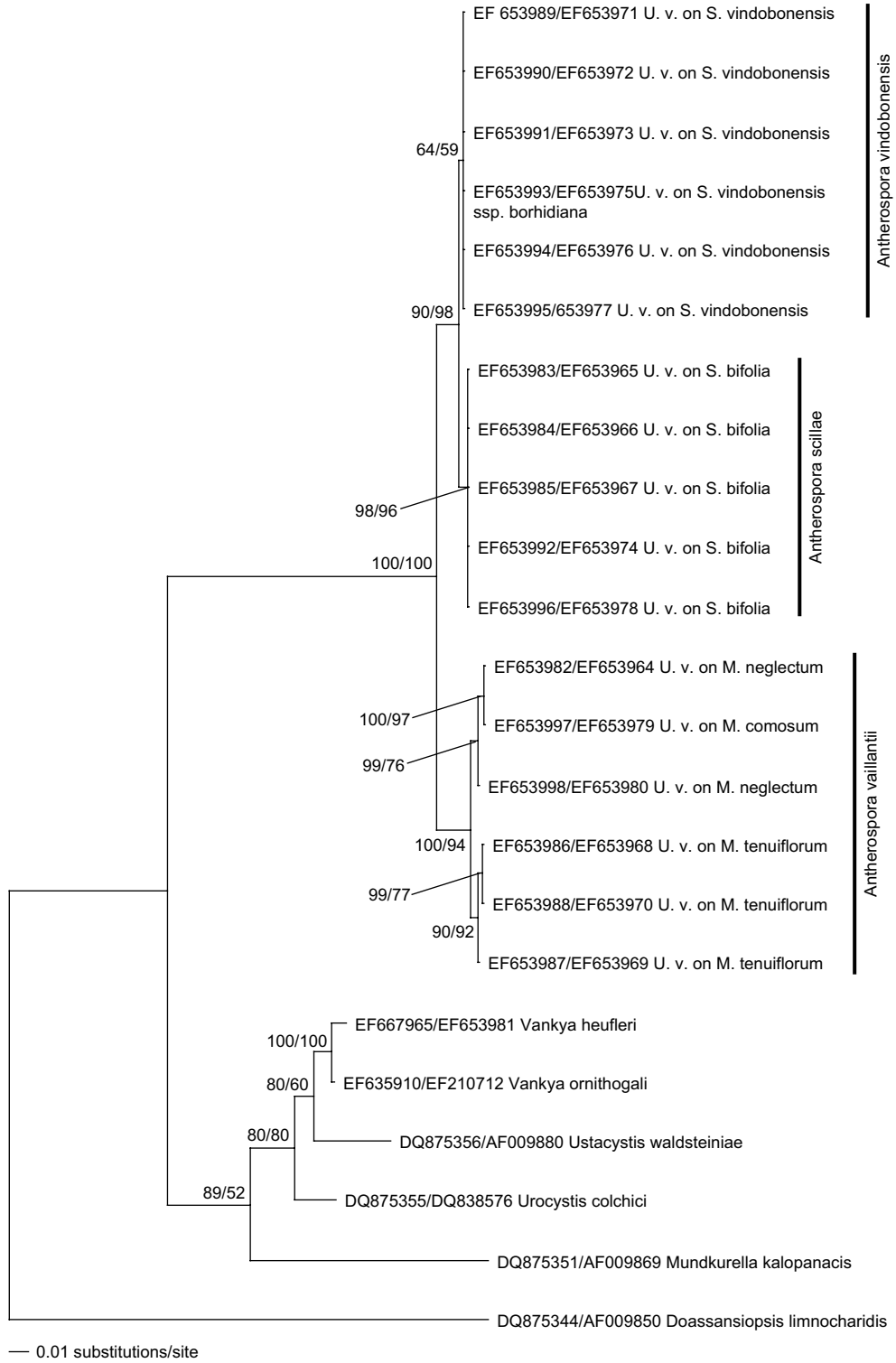

Fig 3 – Bayesian inference of phylogenetic relationships within the sampled *Urocystales*: MCMC analysis of an alignment of concatenated ITS and LSU base sequences using the GTR + I + G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 50 % majority-rule consensus tree computed from 18 K trees that were sampled after the process had reached stationarity is shown. The topology was rooted with *Doassansiopsis limnocharidis*. Numbers on branches before slashes are estimates for PPs, numbers on branches after slashes are percentage BS values of 1 K replicates. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. Our suggestions for species boundaries (right column) are indicated. M., *Muscari*; S., *Scilla*; U., *Ustilago*; v., *vaillantii*.

Synonyms.: *Ustilago tourneuxii* (A. A. Fisch. Waldh.) Maire, *Recueil Trav. Cryptog. déd. Louis Mangin*: 358 (1931).
Yenia tourneuxii (A. A. Fisch. Waldh.) Liou, *Contrib. Inst. Bot. Natl. Acad. Peiping* **6**: 45 (1949).
Typus: **Egypt**: *Mariut, nr Alexandria*, on *Bellevalia trifoliate,* 20 Feb 1880, *P. Ascherson* (type where?).

Sori in the anthers, producing dark olive–brown, powdery mass of spores. *Spores* subglobose, ovoid, ellipsoidal, elongated, usually obtuse, occasionally curved and subangular, 7–16 × 6–10 μm; wall conspicuously verrucose.

Comments: As no material of this species could be obtained for examination, the present description is taken from Maire (1931: 358) who studied the smut of *Bellevalia mauritanica*, collected in Algeria, which may also represent a species different from that of *B. trifoliata*, on which *Ustilago tourneuxxi* was originally described. In the protologue of Fischer von Waldheim (1880: 65), the spore measurements were 10–18 × *ca* 10 μm.

Antherospora urgineae (Maire) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov**.
MycoBank no.: MB 511412
Basionym: *Ustilago urgineae* Maire, *Recueil Trav. Cryptog. déd. Louis Mangin* 359 (1931).
Typus: **Morocco**: Larache, on *Urginea maritima*, 10 Dec 1929, *R. Maire* (MPU, Herb. Maire 9956! – lectotype designated by Vánky 1991: 166).

Sori in all deformed, globoid flowers of an inflorescence, in the anthers and on the surface of inner floral organs producing blackish brown, powdery spore masses, enclosed for a long period by the outermost floral envelopes. *Spores* subglobose, ovoid, ellipsoidal to slightly irregular, rarely tear-shaped, varying in size, 9.5–15(–17.5) × 7–12 μm, yellowish brown; wall even, 0.5–0.8 μm thick, finely, densely verruculose, spore profile very finely wavy.

Antherospora vaillantii (Tul. & C. Tul.) R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **comb. nov.**
MycoBank no.: MB 511413
Basionym: *Ustilago vaillantii* Tul. & C. Tul., *Ann. Sci. Nat., Bot., sér.* 3 **7**: 90 (1847).
Synonyms: *Yenia vaillantii* (Tul. & C. Tul.) Liou, *Contrib. Inst. Bot. Natl. Acad. Peiping* **6**: 45 (1949).
Vankya vaillantii (Tul. & C. Tul.) Ershad, *Rostaniha* **1**: 69 (2000).
Typus: **France**: on *Muscari comosum, S. Vaillant* (type where?).
Ustilago muscari-botryoidis Cif., *Ann. Mycol.* **26**: 14 (1928).
Typus: **Italy**: *Piemonte*, Cuneo prov., Alba distr., Moretta, verso Santa Rosalia, on *Muscari botryoides*, 1921, *R. Ciferri* (type where?).

Sori in the anthers and on the surface of inner floral organs, producing dark olive–brown, powdery mass of spores, enclosed by the floral petals. Infection systemic, all fertile, deformed, globoid or ellipsoid flowers of an inflorescence are infected. *Spores* very variable in shape and size, globose, subglobose, ovoid, curved, pyriform, tear-shaped, or irregularly elongated, (6–)6.5–10.5(–11.5) × 6–8(–9) μm, olive–brown; wall even, *ca* 0.5 μm thick, finely and densely verruculose, spore profile finely wavy.

Comments: Phylogenetically, the specimens of *Antherospora* on *Muscari* cluster together, but form several well-supported separate lineages with considerable genetic distances, which probably refer to different cryptic species. The present description is based on the specimen TUB 15838 on *Muscari comosum*, which is the type host of *Antherospora vaillantii*. Spore sizes do not differentiate the different lineages. The notable exception is specimen GLM 47396 on *M. tenuiflorum*, which deviates from remaining specimens on this host and on *Muscari* in general. It has much larger spores (see Table 1), but identical ITS and LSU sequences compared with the specimen GLM 50411 on *M. tenuiflorum*. This large-spored specimen may be atypical for what is commonly observed in this group. We refrain from formally naming the lineage on *M. tenuiflorum* because of the still unknown phylogenetic relation of *Ustilago muscari-botryoidis* on *Muscari botryoides* to this group. This latter name is tentatively accepted as a synonym of *A. vaillantii*.

Antherospora vindobonensis R. Bauer, M. Lutz, Begerow, Piątek & Vánky, **sp. nov.**
MycoBank no.: MB 511414
Etym.: Scilla vindobonensis

Sori in antheris, interdum superficiales in filamentis, massam sporarum pulverulentam fuscoolivaceam, primo a petalis obtectam, demum patentem generantes. Infectio systemica, vulgo omnibus antheribus plantae infectis, nonnumquam antheris vel floribus inflorescentiae singularibus non infectis. Sporae globosae, subglobosae, ovoideae, ellipsoideae, irregulariter elongatae vel curvatae, (6–)7–11(–15) × (6–)7–9(–10) μm, olivaceae, interdum in uno latere dilutae, dense verruculosae, a latere visae subtiliter sinuatae. Sequentiae acidi nucleici ITS et partium D1/D2 submonadis majoris ribosomalis holotypi in collectione sequentiarum acidi nucleici NCBI (www.ncbi.nlm.nih.gov) numeris EF653989 et EF653971 depositae sunt.

Typus: **Slovakia**: *Podunajská nížina*, Rusovce village, in the park near the manor house, on *Scilla vindobonensis,* 13 Apr 2003, *K. Bacigálová* (SAV s.n. — holotypus; HUV 21503, TUB 15847 — isotypi).

Sori in the anthers and sometimes also on the surface of anther filaments, producing dark olive–brown, powdery mass of spores, at first enclosed by the floral petals, later disclosed. Infection systemic, usually all anthers of a plant being infected, but sometimes some anthers or flowers of an inflorescence may escape the infection. Spores globose, subglobose, ovoid, ellipsoidal, irregularly elongated or curved, (6–)7–11(–15) × (6–)7–9(–10) μm, olive–brown, often lighter coloured on one side; wall *ca* 0.5 μm thick, thinner on the lighter side, finely, densely verruculose, spore profile finely wavy. The ITS and LSU sequences from DNA isolation from the holotype (SAV s.n.) are deposited in GenBank as EF653989 and EF653971, respectively.

Other specimens examined: On *Scilla vindobonensis*: **Austria**: Maria–Lanzendorf village, mixed forest south of the village, near the way

to Himberg village, 22 Mar 2004, *J. Vlčko & J. Kochjarová*, SAV s.n.—**Hungary**, Dunaalmás village, 19 Mar 2003, *J. Kochjarová & J. Vlčko*, SAV s.n.—**Slovakia**: *Podunajská nížina:* Chynorany village, Chynoriansky luh Nature Reserve, 26 Mar 2004, *J. Kochjarová*, SAV s.n.; Jarovce/Kittsee villages, *J. Vlčko, R. Hrivnák, I. Ondrášek, M. Ujházyová & K. Ujházy*, SAV s.n.

On *Scilla vindobonensis* subsp. *borhidiana*: **Hungary**, *Mecsek Mts*, Pécs, Misina hill, 10 Mar 2002, *J. Vlčko, R. Hrivnák & K. Ujházy*, SAV s.n.

Comments: In our molecular phylogenetic analyses the specimens on *Scilla vindobonensis* from Austria, Hungary and Slovakia form a sister taxon to the specimens of *Antherospora scillae* on *S. bifolia* from Germany and Slovakia. There is a difference of 17 bp in the ITS nucleotide sequences between the two lineages. Therefore, we describe *Antherospora* on *S. vindobonensis* as a separate, host-specific species morphologically similar to *A. scillae*, but different phylogenetically and ecologically. Thus, *A. vindobonensis* is a classical example of a cryptic species.

Discussion

Phylogenetic position of Antherospora

Our phylogenetic analyses with a broad sampling of *Ustilaginomycotina* demonstrates that the anther smuts on monocots form a highly supported monophyletic group, sister taxon to the *Urocystaceae* within the *Urocystales* (Figs 2–3). In addition, in the LSU and ITS/LSU analyses the specimens stand well apart from *Vankya heufleri* and *V. ornithogali* (Figs 2–3). This phylogenetic hypothesis agrees well with the morphological and ultrastructural data. As in the *Doassansiopsidaceae* and *Urocystaceae* (Bauer *et al.* 1997, 2001; Begerow *et al.* 1997), the studied specimen TUB 15840 interacts with its host by the formation of haustoria with an electron-opaque, vesicular matrix coating the fungal cell wall, and the simple pores are enclosed by two membrane caps. Furthermore, as in the other *Urocystaceae*, the teliospores in the anther smuts are pigmented (Vánky 1994). The specimens differ from the other members of the *Urocystaceae* and *Urocystales* for all in the sporulation in the anthers of their respective hosts. This difference clearly separates the specimens from *Vankya ornithogali*, *V. heufleri* (see Ershad 2000) and from all other members of the *Urocystales* (Bauer *et al.* 1997, 2001; Begerow *et al.* 2006). For these reasons the genus *Antherospora* is proposed for the anther smuts on *Hyacinthaceae*.

Intrageneric situation

Morphologically, the anther smuts on *Hyacinthaceae* are very similar. Thus, Vánky (1994) treated *Ustilago muscari-botryoidis*, *U. scillae, U. tourneuxii, U. urgineae* and *U. vaillantii* as belonging to one species, *U. vaillantii*. However, our ITS/LSU analyses reveal that the host phylogeny plays a dominant role in the evolution of these anther smuts. There is a correlation between the parasites and their respective hosts. Thus, the specimens on *Muscari,* as well as those on *Scilla,* form well-supported phylogenetic lineages. In addition, on *Scilla* there is a phylogenetic dichotomy between the specimens infecting *S. bifolia* and those infecting *S. vindobonensis* with a difference of 17 bp in the ITS nucleotide sequences between the specimens from the two host species. Obviously, the specimens on *S. bifolia* and those on *S. vindobonensis* represent two different species. Accordingly, *Antherospora vindobonensis* is described as a new species and *A. scillae* is presented as a new combination.

In *Muscari* the correlation between the parasites and the host species is less stringent. Certainly, the monophyly of the specimens on *Muscari* is highly supported, but the basal bifurcation shows a well-supported clade of the specimens on *M. tenuiflorum* on the one hand, and on *M. neglectum* and *M. comosum* on the other. In addition, the branch uniting the specimens HUV 21046 on *M. neglectum* and TUB 15838 on *M. comosum* is highly supported. We refrain from formally naming the lineage on *M. tenuiflorum* because of the still unknown phylogenetic relation of *U. muscari-botryoidis* on *M. botryoides* to this group, and present only *A. vaillantii* as new combination.

In general, our data reveal a stringent correlation of these parasites with their respective host genera. Therefore, we accept the already described species of anther smuts on host species belonging to other hyacinthaceous genera and present them as new combinations. These are *A. albucae* on *Albuca, A. peglerae* on *Ornithogalum*, *A. tourneuxii* on *Bellevalia,* and *A. urgineae* on *Urginea* species (Zundel 1953, Vánky 1994).

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