

The *Georgefischeriales*: a phylogenetic hypothesis¹

Robert BAUER², Dominik BEGEROW², Apollonia NAGLER³ and Franz OBERWINKLER²

² Universität Tübingen, Botanisches Institut, Lehrstuhl Spezielle Botanik und Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.

³ Wilhelm-Keim-Str. 6, D-82031 Grünwald, Germany.

Received 17 April 2000; accepted 14 September 2000.

To obtain an understanding of the phylogenetic relationships among the *Georgefischeriales*, septation, cellular interactions, teliospores, basidia, cultures and nucleotide sequences from the 5' terminal domain of the nuclear large subunit rRNA gene were studied. Analyses of both morphological and molecular characters yield similar phylogenetic conclusions. The *Georgefischeriales* are divided into three groups, corresponding to the *Eballistraceae*, *Georgefischeriaceae*, and *Tilletiariaceae*. The basal dichotomy is between the *Eballistraceae* and the branch uniting the *Georgefischeriaceae* and *Tilletiariaceae*. The *Tilletiariaceae* are phragmobasidiate, whereas the *Eballistraceae* and the *Georgefischeriaceae* are holobasidiate. The *Eballistraceae* differ from the *Georgefischeriaceae* and *Tilletiariaceae* in the lack of the ballistospore mechanism. The systematic position of *Tilletiopsis minor* is unclear. The *Eballistraceae*, *Eballistra* and *Phragmotaenium* are proposed as new taxa. The descriptions of the *Tilletiariaceae* and *Jamesdicksonia* are emended. Except for *Entyloma majus*, *E. parvum*, *Georgefischeria*, *Jamesdicksonia brunckii*, *J. obesa*, *Tilletiaria anomala*, and *Tolyposporella chrysopognis*, the teleomorphic species of the *Georgefischeriales* are presented as new combinations.

INTRODUCTION

In the new system of *Ustilaginomycetes*, the order *Georgefischeriales* was erected for species having local interaction zones and poreless septa (Bauer, Oberwinkler & Vánky 1997). Haustoria or other intracellular fungal organs are lacking. Most *Georgefischeriales* occur on grasses and they generally sporulate in vegetative parts of their respective hosts. The teliospore masses are usually not powdery and with a few exceptions the sori are not exposed by rupture of the host tissues. Molecular analyses confirmed this group (Begerow, Bauer & Oberwinkler 1997).

Initially, based on the mode of cellular interaction and hyphal septation, *Entyloma dactylidis*, *E. irregulare*, *E. oryzae*, *Georgefischeria riveae*, *Melanotaenium brachiariae*, *M. ischaemianum*, and *Tilletiaria anomala* have been grouped in the *Georgefischeriales* (Bauer *et al.* 1997). Within the *Georgefischeriales*, these species were distributed by Bauer *et al.* (1997) between the *Georgefischeriaceae* with *E. dactylidis*, *E. irregulare*, *M. ischaemianum* and *Georgefischeria*, *Tilletiariaceae* with *T. anomala*, and the so-called *Entyloma oryzae* group with *Entyloma oryzae* and *Melanotaenium brachiariae*. By sequence analyses *Jamesdicksonia brunckii* and the conidial species *Tilletiopsis flava*, *T. fulvescens* and *T. minor* were added to the *Georgefischeriales* (Begerow *et al.* 1997, Begerow, Bauer & Boekhout 2000). These studies also revealed that the genera *Melanotaenium* and *Entyloma* are polyphyletic and that some of the species of

these two genera belong to the *Georgefischeriales*. Here, morphological and molecular characters are used in order to propose a phylogenetic hypothesis for this group.

MATERIALS AND METHODS

Specimens, the respective characters studied, and the origin of the sequences are listed in Table 1.

Basidia were obtained from teliospores spread thinly on water agar and malt-yeast-peptone agar (Bandoni 1972) in Petri dishes at room temperature. Cultures were grown on malt yeast peptone agar.

The ultrastructure of septa, cellular interactions and teliospore walls was studied with 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 postfixed 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 3 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.

DNA was isolated from cultures or herbarium specimens using the SDS method as described previously (Begerow *et al.* 1997). The 5' region of the nuclear large subunit of the rRNA

¹ Part 185 in the series *Studies in Heterobasidiomycetes* from the Botanical Institute, University of Tübingen.

Table 1. Specimens and characters studied.

Specimens	Hosts/Substrates	Characters studied ¹	Sequences ²	Source ³
<i>Entyloma dactylidis</i>	<i>Agrostis stolonifera</i>	B, C, S, T	AF 009853 ^B	R.B. 915
<i>E. dactylidis</i>	<i>A. stolonifera</i>	B, C, T		R.B. 3014
<i>E. eleocharitidis</i>	<i>Eleocharis dulcis</i>	T		HUV 12056
(as <i>E. eleocharidis</i>)				Isotype
<i>E. lineatum</i>	<i>Zizania aquatica</i>	S, T	AF 229351*	HUV 15050
<i>E. majus</i>	<i>Sporobolus spicata</i>	T		BPI 175837
				Type
<i>E. irregulare</i>	<i>Poa trivialis</i>	B, C, S, T	AF 229352*	R.B. 919
<i>E. irregulare</i>	<i>P. trivialis</i>	B, C, T		R.B. 3015
<i>E. oryzae</i>	<i>Oryza sativa</i>	S, T	AF 229353*	M.P. 1965
<i>E. oryzae</i>	<i>O. sativa</i>	B, C, T		HUV 16399
<i>E. parvum</i>	<i>Eleocharis acicularis</i>	T		BPI 176157
				Syntype
<i>Erratomyces patelii</i>	<i>Phaseolus vulgaris</i>	S, used as outgroup	AF 009855 ^B	M.P. 1991
<i>Entyloma scirpicola</i>	<i>Scirpus articulatus</i>	T		HUV 5754
				Isotype
<i>Georgefischeria riveae</i>	<i>Rivea hypocrateriformis</i>	B, C, S, T	AF 009861 ^B	HUV 15614
<i>Jamesdicksonia brunkii</i>	<i>Andropogon saccharoides</i>	B, C, S, T	AF 009875 ^B	HUV 17816
<i>J. obesa</i>	<i>Dichanthium annulatum</i>	T		HUV 5371
<i>Melanotaenium brachiariae</i>	<i>Brachiaria distachya</i>	B, C, T		HUV 15615
<i>M. brachiariae</i>	<i>B. distachya</i>	B, C, S, T	AF 009864 ^B	HUV 17510
<i>M. ischaemianum</i>	<i>Ischaemum semisagittatum</i>	B, C, S, T	AF 229354*	HUV 17524
				Topotype
<i>M. indicum</i>	<i>I. indicum</i>	B, C, S, T	AF 229355*	HUV 18022
				Paratype
<i>Tilletiaria anomala</i>	decaying wood	B, C, S, T	AJ 235284 ^{Bo}	CBS 436.72
<i>Tilletiopsis flava</i>	leaves of <i>Acer</i>	C, S	AJ 235285 ^{Bo}	CBS 401.84
<i>T. fulvescens</i>	leaves of <i>Rhus</i>	C, S	AJ 235281 ^{Bo}	N.B. 244
<i>T. fulvescens</i> 607	leaf of <i>Forsythia</i>	C, S	AJ 235282 ^{Bo}	CBS 607.83
<i>T. minor</i> 346	leaves	S	AJ 235286 ^{Bo}	CBS 346.33
<i>T. minor</i> 543	leaves	S	AJ 235287 ^{Bo}	CBS 543.50
<i>Tolyposporella chrysopogonis</i>	<i>Sorghastrum nutans</i>	T		HUV 2438

¹ B, Basidia; C, Culture; S, Sequence; T, Teliospores, hyphae and cellular host-parasite interaction

² Origin of sequences: B, Begerow *et al.* (1977); Bo, Boekhout, Fell & O'Donnell (1995); *, new sequences

³ BPI, US National Fungus Collections, Beltsville, USA; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; HUV, Herbarium Ustilaginales Vánky, Tübingen, Germany; M.P., Herbarium M. Piepenbring, Tübingen, Germany; N.B., Culture collection of T. Nakase, Saitama, Japan; R.B., Herbarium R. Bauer, Tübingen, Germany.

gene was amplified using the polymerase chain reaction and the primers NL1 and NL4 (O'Donnell 1993). The PCR product was purified using the QIAquick[®] protocol (Qiagen). This dsDNA was sequenced directly using the ABI PRISM[™] Dye-Termination Cycle Sequencing Kit (Applied Biosystems) on an automated sequencer (ABI 373A, Applied Biosystems). An alignment of 506 basepairs was created using MEGALIGN of the Lasergene-package (DNASTAR). The PHYLIP package, version 3.572 (Felsenstein 1995), was used to perform the following analyses: neighbour joining of a distance matrix (Kimura 2-parameter model, transition to transversion rate: 2.0) with 1000 bootstrap replicates and maximum parsimony (heuristic, the jumble option turned on 10 replicates) with 1000 bootstrap replicates. Sequences are deposited in Genbank (see Table 1).

RESULTS

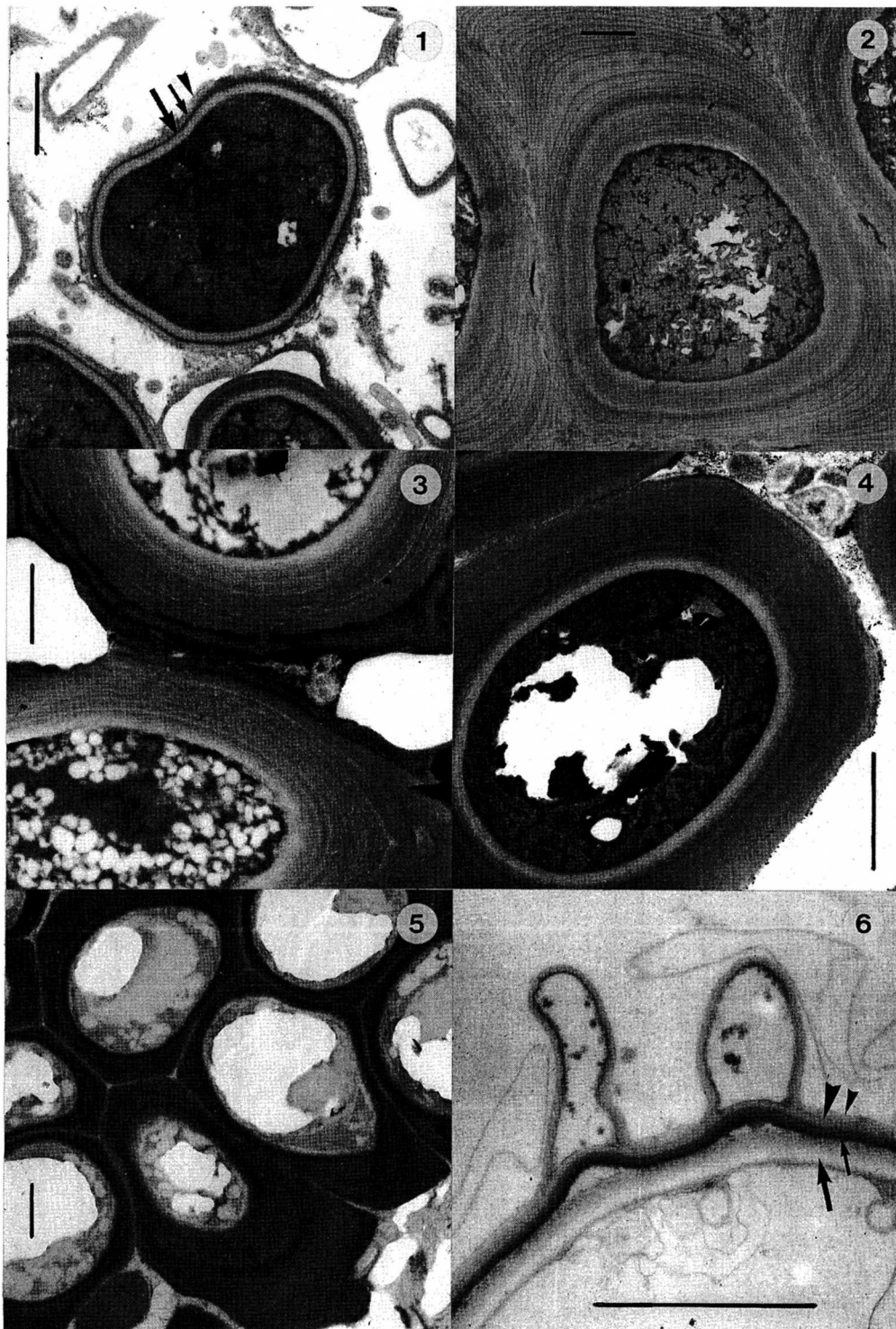
Hyphal septation and cellular interaction

In addition to the species investigated ultrastructurally by Bauer *et al.* (1997), *Entyloma eleocharitis*, *E. lineatum*, *E. majus*, *E. parvum*, *E. scirpicola*, *Jamesdicksonia brunkii*, *J. obesa*, *Melanotaenium indicum*, and *Tolyposporella chrysopogonis* also

have the typical characters of the *Georgefischeriales*: the mature septa in soral hyphae were poreless and small local interaction sites without interaction apparatus were present at the host-parasite interface. These characters were discussed and illustrated in detail by Bauer *et al.* (1997) and are therefore only briefly summarized here.

Teliospores

Teliospores of the phytoparasitic species listed in Table 1 developed in the intercellular spaces in the mesophyll. The mass of sporogenous hyphae was usually completely used for teliospore formation. Due to the topology of the intercellular spaces, teliospores were usually formed in more or less dense packets. The teliospore wall consisted of an electron-opaque exosporium (for the terminology see Piepenbring, Bauer & Oberwinkler 1998a), occasionally covered by remnants of the sheath and the wall of the sporogenous hypha, and an electron-transparent endosporium (Figs 1–6; layers labelled in Figs 1 and 6). In young teliospores the endosporium may be lacking (Figs 2 and 5). In *Jamesdicksonia obesa* (Fig. 3), *J. brunkii* (Fig. 4), *Tolyposporella chrysopogonis* (Fig. 5), and especially *Georgefischeria riveae* (Fig. 2), but not in the other species



Figs 1–6. Teliospores seen by TEM. Bars = 2 μ m. **Fig. 1.** *Entyloma dactylidis* (i.e. *Jamesdicksonia dactylidis*, R.B. 915). Teliospore wall with sheath (arrowhead), exosporium (small arrow) and endosporium (large arrow). **Fig. 2.** *Georgefischeria riseae*. Note the multilamellate nature of the teliospore wall. **Fig. 3.** *J. obesa*. The exosporium shows a multilamellate substructure. **Fig. 4.** *J. brunckii*. Note the laminations in the exosporium. **Fig. 5.** *Tolyposporella chrysopogonis*. Part of a teliosporeball. Note the laminated exosporium of the teliospores. **Fig. 6.** *Tilletiaria anomala*. Teliospore wall with the original wall of the sporogenous hypha (small arrowhead), sheath (large arrowhead), exosporium (small arrow) and endosporium (large arrow). Note that the wall of the ornaments is continuous with the sheath.

studied (Figs 1 and 6), the exosporium was thick and had a multilamellate substructure. In addition, teliospores were echinulate in *Tilletiaria anomala* (Fig. 6) and smooth in the other teleomorphic species listed in Table 1 (Figs 1–5). Unique

for the *Ustilaginomycetes*, the ornamentation in *Tilletiaria anomala* was continuous with the sheath and not with the exosporium (Fig. 6). In *Tolyposporella chrysopogonis*, the teliospores were arranged in distinct balls (Fig. 5), whereas in

