

# The *Georgefischeriales*: a phylogenetic hypothesis<sup>1</sup>

Robert BAUER<sup>2</sup>, Dominik BEGEROW<sup>2</sup>, Apollonia NAGLER<sup>3</sup> and Franz OBERWINKLER<sup>2</sup>

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

<sup>3</sup> Wilhelm-Keim-Str. 6, D-82031 Grünwald, Germany.

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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 phragmobasidiolate, whereas the *Eballistraceae* and the *Georgefischeriaceae* are holobasidiolate. 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 *Phragmotaeonium* 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

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Table 1. Specimens and characters studied.

Specimens	Hosts/Substrates	Characters studied <sup>1</sup>	Sequences <sup>2</sup>	Source <sup>3</sup>
<i>Entyloma dactylidis</i>	<i>Agrostis stolonifera</i>	B, C, S, T	AF 009853 <sup>B</sup>	R.B. 915
<i>E. dactylidis</i>	<i>A. stolonifera</i>	B, C, T		R.B. 3014
<i>E. eleocharitis</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 <sup>B</sup>	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 <sup>B</sup>	HUV 15614
<i>Jamesdicksonia brunkii</i>	<i>Andropogon saccharoides</i>	B, C, S, T	AF 009875 <sup>B</sup>	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 <sup>B</sup>	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 <sup>Bo</sup>	CBS 436.72
<i>Tilletiopsis flava</i>	leaves of <i>Acer</i>	C, S	AJ 235285 <sup>Bo</sup>	CBS 401.84
<i>T. fulvescens</i>	leaves of <i>Rhus</i>	C, S	AJ 235281 <sup>Bo</sup>	N.B. 244
<i>T. fulvescens</i> 607	leaf of <i>Forsythia</i>	C, S	AJ 235282 <sup>Bo</sup>	CBS 607.83
<i>T. minor</i> 346	leaves	S	AJ 235286 <sup>Bo</sup>	CBS 346.33
<i>T. minor</i> 543	leaves	S	AJ 235287 <sup>Bo</sup>	CBS 543.50
<i>Tolyposporella chrysopogonis</i>	<i>Sorghastrum nutans</i>	T		HUV 2438

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

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

<sup>3</sup> 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<sup>®</sup> protocol (Qiagen). This dsDNA was sequenced directly using the ABI PRISM<sup>™</sup> 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

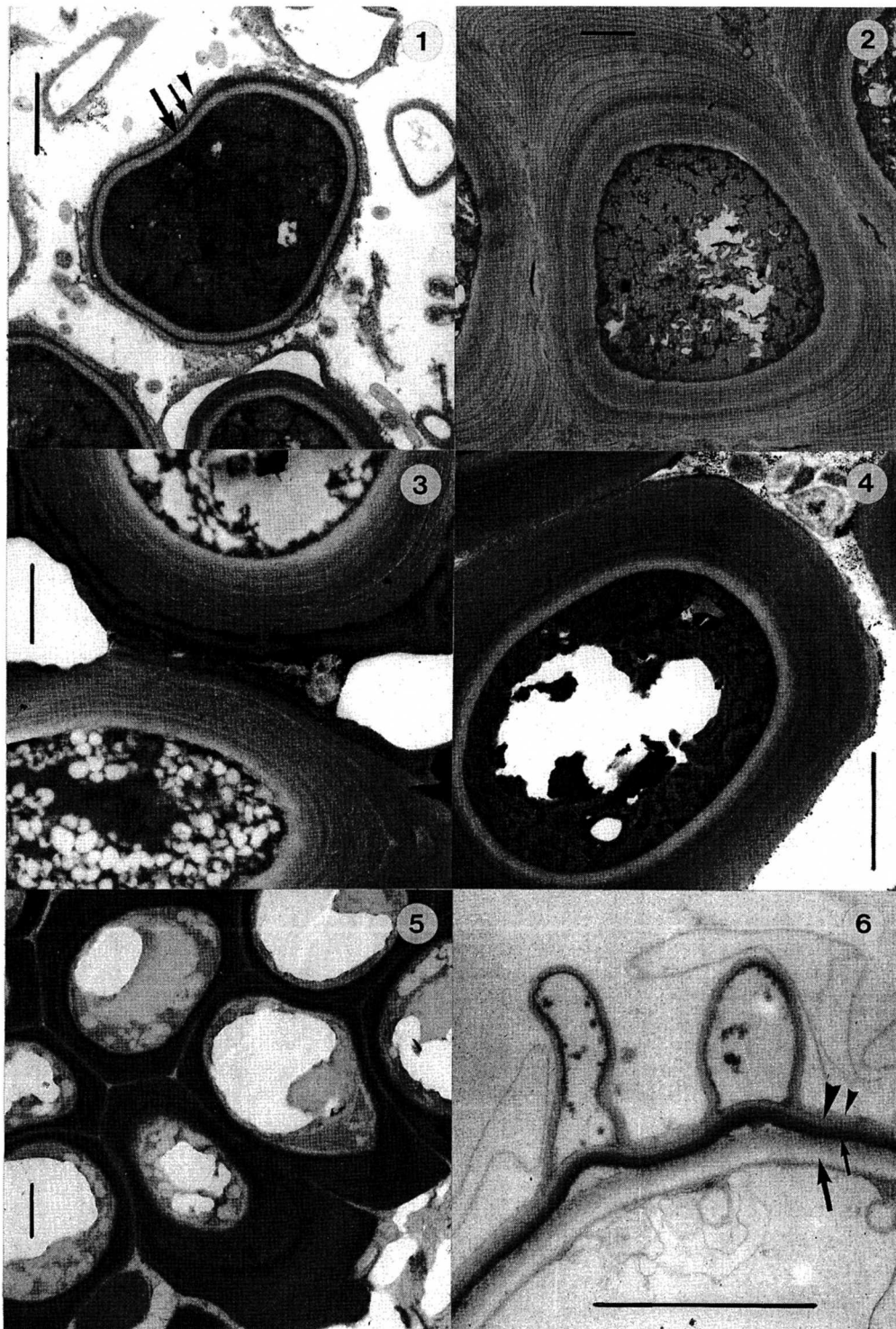
### Hyphal septation and cellular interaction

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

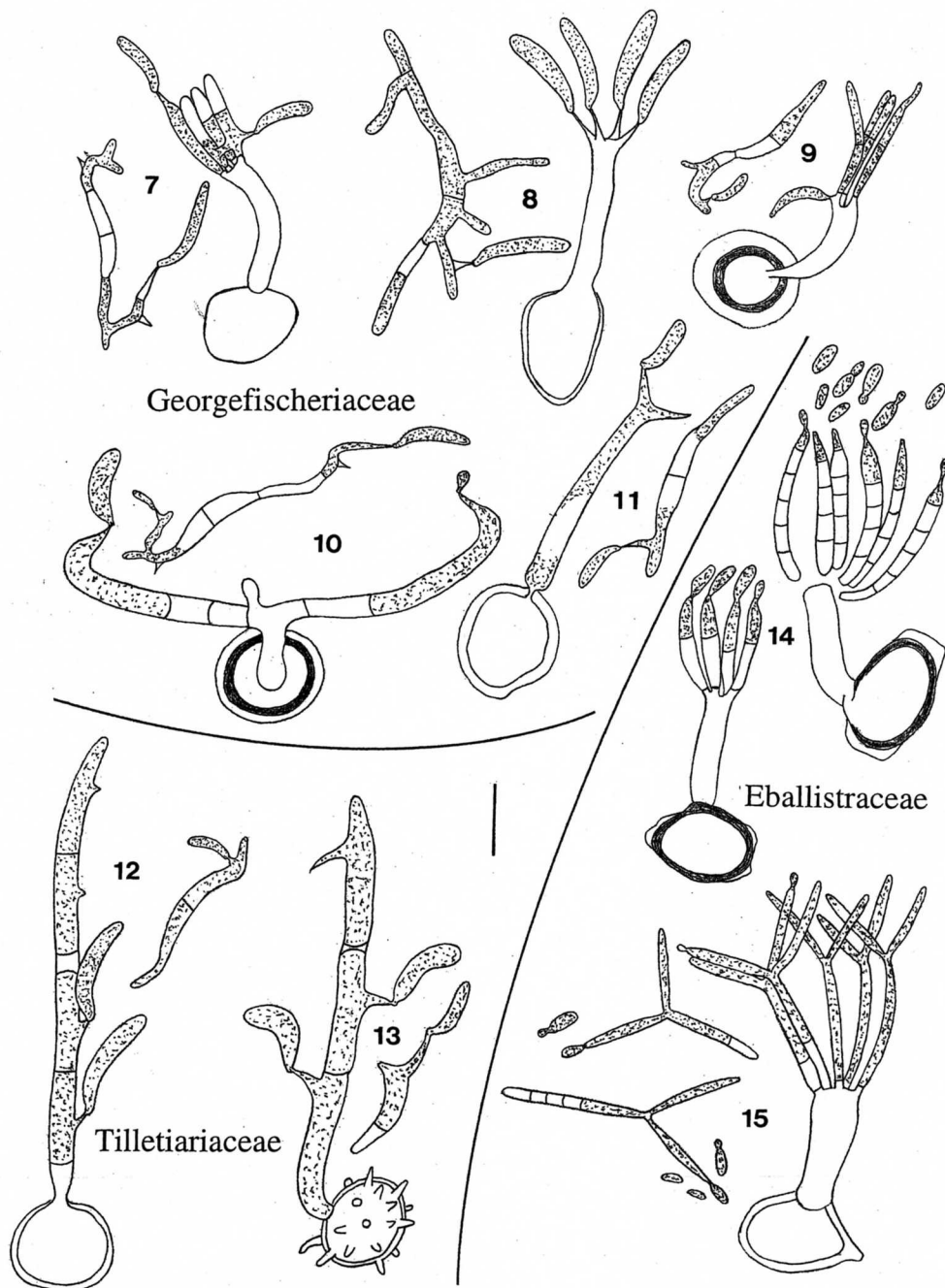
Teliospores of the phytoparasitic species listed in Table 1 developed in the intercellular spaces in the mesophyll. The mass of sporogenous hyphae was usually completely used for teliospore formation. Due to the topology of the intercellular spaces, teliospores were usually formed in more or less dense 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  $\mu$ 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



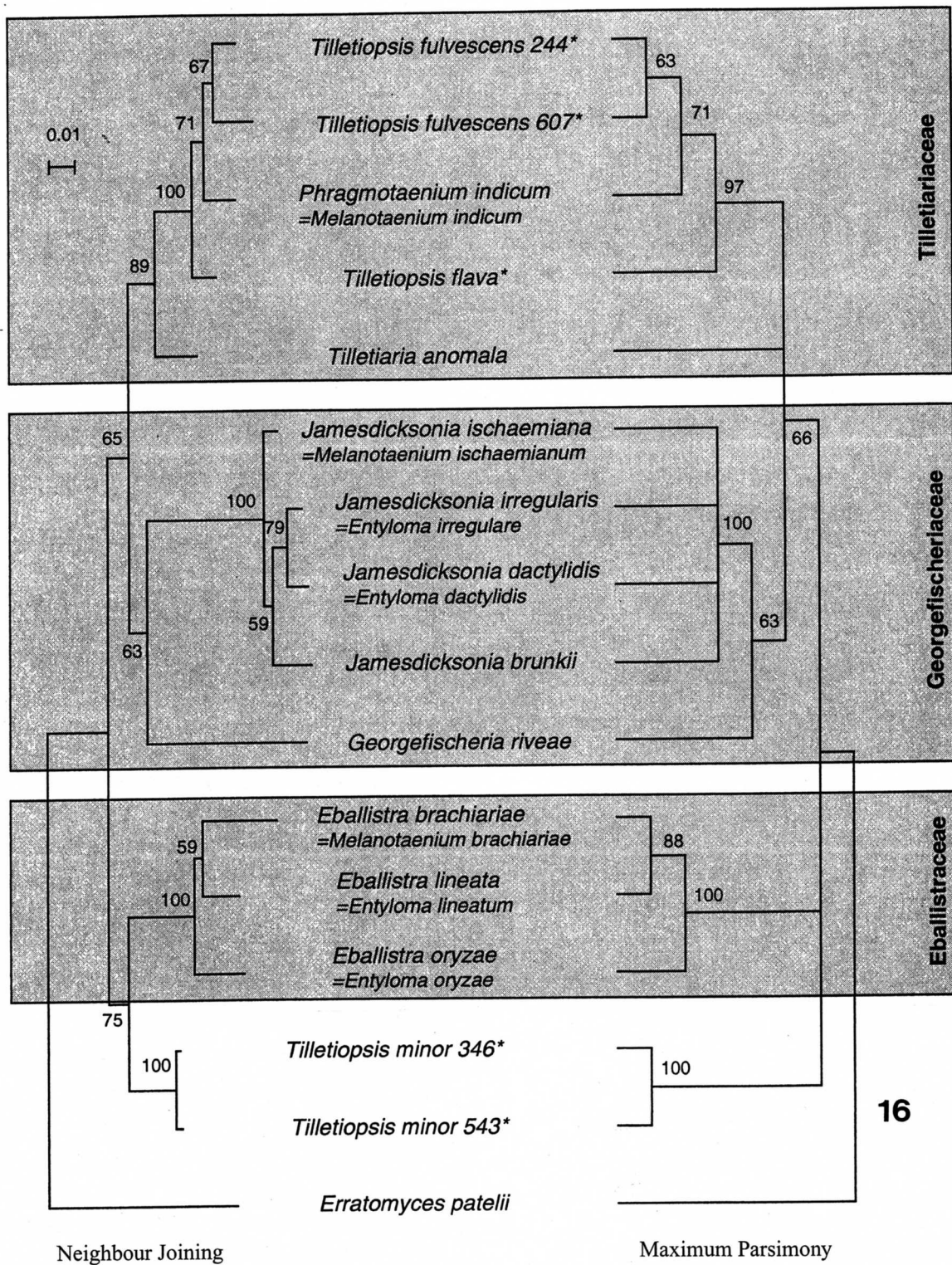
**Figs 7–15.** Typical basidia and germinating basidiospores/basidioconidia. Bar = 10  $\mu$ m. **Fig. 7.** *Entyloma irregulare* (i.e. *Jamesdicksonia irregularis*, R.B. 3015). Note the conjugation of the basidiospores. **Fig. 8.** *E. dactylidis* (i.e. *J. dactylidis*, R.B. 3014). Note the exobasidiaceous orientation of the basidiospores. **Fig. 9.** *J. brunckii*. Note the different kinds of basidiospores on the basidium. **Fig. 10.** *Georgefischeria riveae*. Note the two arms perpendicular to the long axis of the basidium. **Fig. 11.** *Melanotaenium ischaemianum* (i.e. *J. ischaemiana*). Two-sterigmate basidium. Note the exobasidiaceous orientation of the basidiospore on the basidium. **Fig. 12.** *M. indicum* (i.e. *Phragmotenium indicum*). Note the transversely septate basidium. **Fig. 13.** *Tilletiaria anomala*. Note the transversely septate basidium. **Fig. 14.** *M. brachiariae* (i.e. *Eballistra brachiariae*, HUV 15615). Two basidia in different developmental stages showing the apical budding of the basidiospores. **Fig. 15.** *Entyloma oryzae* (i.e. = *Eballistra oryzae*). Note the triradial basidiospores and their germination by budding.

the other species they were dispersed singly or in irregular groups.

### Basidia

Two different main types of basidia were found in the *Georgefischeriales*: teliospore germination resulted in holo-

basidia with terminal basidiospores in *Entyloma irregulare* (Fig. 7), *E. dactylidis* (Fig. 8), *Jamesdicksonia brunckii* (Fig. 9), *Georgefischeria riveae* (Fig. 10), *Melanotaenium ischaemianum* (Fig. 11), *M. brachiariae* (Fig. 14), and *Entyloma oryzae* (Fig. 15), whereas *Melanotaenium indicum* and *Tilletiaria anomala* produced phragmobasidia with lateral basidiospores (Figs 12–13). In addition, *Entyloma irregulare* (Fig. 7), *Melanotaenium*



**Fig. 16.** Phylogenetic hypotheses derived from analyses of a 506 bp alignment of the 5' end of the nuclear large subunit rRNA gene, rooted with *Erratomyces patelii*. Bootstrap values (1000 replicates) under 50% are not shown. Anamorphic species are marked by asterisks. In maximum parsimony the strict consensus tree of 7 most parsimonious trees (297 steps) found by heuristic analysis is illustrated.

*brachiariae* (Fig. 14) and *Entyloma oryzae* (Fig. 15) had more or less symmetrical basidiospores that were passively released, whereas asymmetrical, ballistic basidiospores were produced on the basidia of *Entyloma dactylidis* (Fig. 8), *Georgefischeria riveae* (Fig. 10), *Melanotaenium ischaemianum* (Fig. 11), *M. indicum* (Fig. 12) and *Tilletiaria anomala* (Fig. 13). In *Jamesdicksonia brunkii*, only a few basidia were observed.

Connected to these, two kinds of basidiospores were found: symmetrical fusiform basidiospores of the gastroid type, and ballistic basidiospores on short sterigmata (Fig. 9). The fusiform basidiospores, but not the ballistic basidiospores, usually became septate while still connected to the basidia. Most of the segments of these basidiospores germinated at both poles, producing ballistoconidia.

Basidiospores conjugated on the basidium only in *Entyloma irregulare*. The conjugated basidiospores germinated apically or laterally while still connected to the basidium, producing ballistoconidia (Fig. 7). Basidia of *Entyloma dactylidis* (Fig. 8) and *Melanotaenium ischaemianum* (Fig. 11) possessed a specific orientation of the basidiospores: the hilar appendices of the ballistospore basidiospores were abaxially orientated. The basidia of *Entyloma dactylidis* differed from those of *Melanotaenium ischaemianum* in the number of basidiospores. *E. dactylidis* had basidia with an apical whorl of (3-)4(-6) basidiospores, whereas the basidia of *M. ischaemianum* were generally two-sterigmate (Figs 8 and 11).

On the one hand, *Georgefischeria riveae* was unusual in having two-spored basidia usually with two long apical arms more or less perpendicular to the long axis of the basidium (Fig. 10), whereas on the other, *Entyloma oryzae* was unusual in producing triradiate basidiospores (Fig. 15).

### Cultural characteristics

Two different cultural growth forms were found in the *Georgefischeriales*. In *Entyloma irregulare* (Fig. 7), *E. dactylidis* (Fig. 8), *Jamesdicksonia brunckii* (Fig. 9), *Georgefischeria riveae* (Fig. 10), *Melanotaenium ischaemianum* (Fig. 11), *M. indicum* (Fig. 12), and *Tilletiaria anomala* (Fig. 13), discharged ballistic basidiospores and/or the resulting ballistoconidia germinated essentially identically. After discharge, the ballistic propagules became two-celled by the formation of a transverse septum and began to germinate at both poles. Subsequently, a *Tilletiopsis*-like culture generating pseudomycelia with retraction septa and ballistoconidia developed.

In contrast, in *Melanotaenium brachiariae* (Fig. 14) and *Entyloma oryzae* (Fig. 15), another cultural growth form developed from the basidiospores. The basidiospores in these two species budded apically in a yeast-like manner producing subspherical to ellipsoidal yeast cells. While still germinating, basal vacuolation in the basidiospores pushed the cytoplasm into the buds, and the emptied regions were periodically walled off by retraction septa (Figs 14–15). The resulting yeast cells budded in the same manner. Thus, a yeast phase without the formation of pseudohyphae and ballistoconidia was formed.

### Molecular analyses

We did not obtain PCR products from the herbarium specimens of *Entyloma eleocharitis*, *E. majus*, *E. parvum*, *E. scirpicola*, *Jamesdicksonia obesa* and *Tolyposporella chrysopogonis*. The sequences of the other species listed in Table 1 were analyzed with two methods, and the resulting trees are shown in Fig. 16. Using *Erratomyces patelii* as root, in the neighbour joining analysis the species of the *Georgefischeriales* were distributed into three groups, which were congruent to the *Georgefischeriaceae*, *Tilletiariaceae* and the so-called *Entyloma oryzae* group (sensu Bauer *et al.* 1997). Maximum parsimony resulted in a similar topology without significant differences. The differences between the two methods concerned the phylogenetic placements of *Tilletiaria anomala* and *Tilletiopsis minor*, and the internal arrangement of the species representing

the *Georgefischeriaceae* (Fig. 16). In neighbour joining, *T. anomala* was located at the base of the group representing the *Tilletiariaceae* with bootstrap support of 89%, whereas the maximum parsimony consensus topology showed a polytomy at this level. Likewise, in contrast with neighbour joining, in maximum parsimony most species of the group representing the *Georgefischeriaceae* appeared in a polytomy. In addition, the two strains of *Tilletiopsis minor* tested appeared in neighbour joining in a dichotomy with the group representing the *Eballistraceae* with medium statistical support, while the maximum parsimony analysis showed a polytomy at this level.

## DISCUSSION

### The system

Analyses of both the morphological and molecular characters yielded essentially identical phylogenetic conclusions: the subgroups of the *Georgefischeriales* reported by Bauer *et al.* (1997) and Begerow *et al.* (1997, 2000) are also evident in this study. These are the *Tilletiariaceae*, *Georgefischeriaceae*, and the so-called *Entyloma oryzae* group (the *Eballistraceae*, as proposed below). 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. In addition to the studies of Bauer *et al.* (1997) and Begerow *et al.* (1997, 2000), in the present study *Entyloma eleocharitis*, *E. lineatum*, *E. majus*, *E. parvum*, *E. scirpicola*, *Jamesdicksonia obesa*, *Melanotaenium ischaemianum*, *Melanotaenium indicum*, and *Tolyposporella chrysopogonis*, were identified as members of the *Georgefischeriales*.

### Georgefischeriaceae

Among the *Georgefischeriales*, the formation of holobasidia and ballistospore propagules characterizes the *Georgefischeriaceae*. The *Georgefischeriaceae* share the formation of holobasidia with the *Eballistraceae* and the formation of ballistic propagules with the *Tilletiariaceae*. Although the combination of holobasidia and the presence of the ballistospore mechanism clearly separates the *Georgefischeriaceae* from the other members of the order, no apomorphy is obvious. The basidia observed in *Entyloma dactylidis* and *Melanotaenium ischaemianum* with basidiospores having a characteristic abaxial orientation of the hilar appendices are typical for the *Exobasidiaceae*, but occur also in species of the *Tilletiales* and *Dossansiales* (Oberwinkler 1977, 1982, Ingold 1995, Vánky & Bauer 1996, Bauer *et al.* 1997, 1999, Begerow *et al.* 2000). Therefore, we consider the exobasidaceous basidium as apomorphic for the *Exobasidiomycetidae* and, therefore, plesiomorphic for the *Georgefischeriales*. Accordingly, the presence of ballistic propagules in the *Ustilaginomycetidae* and *Exobasidiomycetidae* (for the subclasses see Bauer *et al.* 1997) indicates that the ballistospore mechanism was already established before the *Georgefischeriales* diverged from the other groups of the *Exobasidiomycetidae*.

The basidia found in *Jamesdicksonia brunckii* suggest that the ballistic basidiospore occurring on the basidia of *Entyloma*

*dactylidis* and *Melanotaenium ischaemianum* is homologous to the passively released gasteroid type occurring on the basidia of *Entyloma irregulare*. On the basidia of *J. brunckii* both kinds of basidiospores were observed. It is known that aerial basidia of the exobasidiaceous type tend to form symmetrical, passively released basidiospores if the basidia come in contact with the agar during development (Bauer *et al.* 1999). As in *J. brunckii*, these basidiospores become septate while still connected to the basidia. Thus, external environmental conditions may be responsible for the different kinds of basidiospores observed in *J. brunckii*. In addition, in contrast with the description, the basidium of *J. brunckii* illustrated in Durán (1972: fig. 17) resembles that of the ballistic, exobasidiaceous type. This is also true for *J. obesa*, the type of *Jamesdicksonia*. The basidia illustrated in Thirumalachar, Pavgi & Payak (1960) resemble those of the ballistic type, whereas the basidia illustrated in Raghunath (1969) resemble those of *Entyloma irregulare*.

We observed fusion of compatible basidiospores on the holobasidia only in *Entyloma irregulare*, but this also occurs in *E. eleocharitis* (Pavgi & Singh 1969, 1970) and *E. scirpicola* (Thirumalachar & Dickson 1949). Although not explicitly described, the illustrations in Pavgi & Singh (1969, 1970) and Thirumalachar & Dickson (1949) suggest that the 'secondary sporidia' are ballistic.

Multilamellate teliospore walls which become gelatinous and swell in water are considered as an important generic characteristic for *Jamesdicksonia* (Walker & Shivas 1998). However, *Jamesdicksonia* shares this feature with *Georgefischeria* and *Tolyposporella chrysopogonis* (belonging to the *Tilletiariaceae*, see below). Therefore, this feature appears not to be indicative of a natural relationship and cannot be used as a diagnostic character of *Jamesdicksonia*. In fact, our molecular analyses reflect this situation. In interpreting the molecular trees, *Jamesdicksonia brunckii* is more closely related to *Entyloma dactylidis*, *E. irregulare*, and *Melanotaenium ischaemianum* than to *Georgefischeria riveae* or, *vice versa*, *G. riveae* stands in an isolated position within the clade representing the *Georgefischeriaceae*. This isolated position of *Georgefischeria* is also well reflected by morphological, ecological and coevolutionary characteristics. Thus, the two species of *Georgefischeria* form lightly coloured teliospores and sori, at least the basidia in *G. riveae* possess long arms perpendicular to the long axis of the basidium, they grow intracellularly in the xylem and intercellularly in the phloem, they cause hypertrophy and parasitize *Convolvulaceae* (Narasimhan *et al.* 1963). In contrast, *Entyloma dactylidis*, *E. eleocharitis*, *E. scirpicola*, *Jamesdicksonia brunckii*, *J. obesa* and *Melanotaenium ischaemianum* are united in having darkly pigmented teliospores and sori, they grow only intercellularly, they do not cause hypertrophy, they parasitize grasses, and their basidia are of the normal type. To accommodate these species in the *Georgefischeriaceae*, the description of *Jamesdicksonia* is emended as follows:

**Jamesdicksonia** Thirum., Pavgi & Payak, *Mycologia* **52**: 478 (1960).

Members of the *Georgefischeriales* sensu Bauer *et al.* (1997) having holobasidia, ballistic propagules and darkly pigmented

teliospores and sori. Known species are parasitic on *Poaceae* and *Cyperaceae*.

Type: *Jamesdicksonia obesa* (Syd. & P. Syd.) Thirum., Pavgi & Payak 1960.

#### Other species

**Jamesdicksonia brunckii** (Ellis & Galloway) J. Walker & R. G. Shivas, *Mycol. Res.* **102**: 1212 (1998).

**Jamesdicksonia dactylidis** (Pass.) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Thecaphora dactylidis* Pass., *Ann. Sci. Nat., Bot., ser. 6*, **4**: 231 (1876).

**Jamesdicksonia eleocharitis** (Sawada) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Ustilago eleocharitis* Sawada, *Taiwan Agric. Res. Inst. Rept.* **85**: 39 (1943); as '*eleocharidis*'.

**Jamesdicksonia irregularis** (Johanson) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Entyloma irregulare* Johanson, *Öfvers. Förh. Kongl. Svenska Vetensk.-Akad.* **41**: 159 (1884).

**Jamesdicksonia ischaemianum** (Thirum. & Pavgi) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Melanotaenium ischaemianum* Thirum. & Pavgi, *Sydowia* **20**: 25 (1967).

**Jamesdicksonia scirpicola** (Thirum. & J. D. Dicks.) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Entyloma scirpicola* Thirum. & J. D. Dicks., *Am. J. Bot.* **36**: 404 (1949).

**Tilletiariaceae** R. T. Moore, *Bot. Marina* **23**: 364 (1980).

Members of the *Georgefischeriales* sensu Bauer *et al.* (1997) having phragmobasidia.

Phragmobasidia with lateral ballistic basidiospores represent an apomorphy for the *Tilletiariaceae*, as defined above. We found this basidial type in *Melanotaenium indicum* and *Tilletiaria anomala*. The formation of ballistosporic phragmobasidia in *T. anomala* was already described by Bandoni & Johri (1972). In addition, *Tolyposporella chrysopogonis* may form the same basidial type. Although not explicitly described, the illustrations in Thirumalachar, Whitehead & O'Brien (1967) suggest that the basidiospores are ballistic. As in *T. chrysopogonis*, hyphal germination of the basidial segments was also occasionally observed in *M. indicum*. In neighbour joining and, except for *Tilletiaria anomala*, in maximum parsimony, this group is well supported by bootstrap resampling. Interestingly, both maximum parsimony and neighbour joining analyses illustrated in Begerow *et al.* (2000) as well as the neighbour joining analysis of this study place *Tilletiaria anomala* and the other members of the *Tilletiariaceae* tested on a common branch with bootstrap support of 89–100%, while the maximum parsimony analysis of this study shows a polytomy at this level.

*Melanotaenium indicum* and *Tolyposporella chrysopogonis* are parasitic on *Poaceae*, while *Tilletiaria anomala*, *Tilletiopsis fulvescens*, and *T. flava* have been discovered only as cultures and their life strategies are therefore unknown. Interestingly, however, *M. indicum*, like *Tilletiaria anomala*, occasionally forms teliospores and basidia in culture. Therefore, we speculate that *Tilletiaria anomala*, *Tilletiopsis fulvescens* and *T. flava* are phytoparasites, probably on *Poaceae*.

The three teleomorphic species identified in this group differ in sporulation. *Melanotaenium indicum* and *Tilletiaria anomala* produce single teliospores (Vánky 1997, Bandoni & Johri 1972), whereas the teliospores in *Tolyposporella chrysopogonis* are dispersed in distinct balls (Clinton 1902, Thirumalachar *et al.* 1967). Multilamellate teliospore walls occur only in *T. chrysopogonis*. The teliospores of *T. anomala*, but not of the two other species, are echinulate. Unique for the *Ustilaginomycetes*, the ornamentations in *T. anomala* are formed by the sheath. In the other *Ustilaginomycetes* studied, the ornamentation develops either in a separate developmental phase after the formation of the sheath and prior to the formation of the exosporium, is part of the exosporium (Piepenbring, Bauer & Oberwinkler 1998a, b).

To accommodate these species in the *Tilletiariaceae*, in addition to the description of the *Tilletiariaceae* presented above, a new genus is proposed for *Melanotaenium indicum*.

**Phragmotenium** R. Bauer, Begerow, A. Nagler & Oberw., **gen. nov.**

*Etym.*: *Phragmo* (from phragmobasidium), *taenium* from *Melanotaenium* (taenia = band).

Fungi *Georgefischeriales* sensu Bauer *et al.* (1997) basidiis transverse septatis teliosporisque singularibus levibus.

*Typus*: *Phragmotenium indicum* (K. Vánky, M. S. Patil & N. D. Sharma) R. Bauer, Begerow, A. Nagler & Oberw.

Members of the *Georgefischeriales* sensu Bauer *et al.* (1997) having transversely septate basidia and single, smooth teliospores.

**Phragmotenium indicum** (K. Vánky, M. S. Patil & N. D. Sharma) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Melanotaenium indicum* K. Vánky, M. S. Patil & N. D. Sharma, *Mycotaxon* **65**: 149 (1997).

**Eballistraceae** R. Bauer, Begerow, A. Nagler & Oberw., **fam. nov.**

Fungi *Georgefischeriales* sensu Bauer *et al.* (1997) holobasidiis basidiosporis conidiisque non coniectabilibus.

*Typus*: *Eballistra* R. Bauer, Begerow, A. Nagler & Oberw.

Members of the *Georgefischeriales* sensu Bauer *et al.* (1997) having holobasidia and lacking ballistic basidiospores and ballistoconidia.

The loss of the ballistospore mechanism represents an apomorphy for the *Eballistraceae* (as proposed here). *Melanotaenium brachiariae* and *Entyloma oryzae* form holobasidia with passively released basidiospores in both species bud in a yeast-like manner (see also Singh & Pavgi 1973). Subsequently, a budding yeast phase without ballistoconidia and hyphae

develops. Basidia and cultural characteristics are unknown from *Entyloma lineatum*. However, *E. lineatum* appears in neighbour joining as well as in maximum parsimony within the clade representing the *Eballistraceae*. This placement is statistically well supported by bootstrap values of 100% in both analyses. Therefore, we ascribe this parasite to this group.

*Melanotaenium brachiariae* differs from *Entyloma oryzae* in basidiospore morphology. Basidiospores are more or less cylindrical in form in *Melanotaenium brachiariae* (Singh & Pavgi 1973) and triradiate in *Entyloma oryzae*. The triradiate basidiospores resemble radiate conidia of aquatic hyphomycetes (Ingold 1979). It is therefore probable that the radiation of the basidiospores in *E. oryzae* has evolved in adaptation to water dispersal. Thus, the aquatic nature of rice, the host of *E. oryzae*, might be reflected by the morphology of its parasite.

To accommodate these three fungi in the *Georgefischeriales*, the new family, and also a new genus are proposed.

**Eballistra** R. Bauer, Begerow, A. Nagler & Oberw., **gen. nov.**

*Etym.* *e-* (Lat.), without; *ballistra* (Gr.), catapult; referring to the character that no ballistic propagules are formed.

Descriptio analoga familiae *Eballistracearum*.

*Typus*: *Eballistra oryzae* (Syd. & P. Syd.) R. Bauer, Begerow, A. Nagler & Oberw.

**Eballistra oryzae** (Syd. & P. Syd.) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Entyloma oryzae* Syd. & P. Syd., *Annls Mycol.* **12**: 197 (1914).

**Eballistra brachiariae** (Viégas) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Melanotaenium brachiariae* Viégas, *Bragantia* **4**: 748 (1944).

**Eballistra lineata** (Cooke) R. Bauer, Begerow, A. Nagler & Oberw., **comb. nov.**

Basionym: *Ustilago lineata* Cooke, *Fungi. Amer.*, Exs. no. 789 (1882).

**Tilletiopsis minor**

The anamorphic *Tilletiopsis minor* shares the *Tilletiopsis*-like growth in culture with the *Georgefischeriaceae* and *Tilletiariaceae*, but not with the *Eballistraceae*. It appears, however, in the neighbour joining analysis of this study, but not in the maximum parsimony analysis, as a sister taxon of species representing the *Eballistraceae* with a bootstrap support of 75%. Additionally, in both neighbour joining and maximum parsimony analysis illustrated in Begerow *et al.* (2000), *T. minor* represents the sister species of *Melanotaenium brachiariae*, a member of the *Eballistraceae* (bootstrap values 62 and 87%, respectively). Interpreting the morphological and molecular data, it is conceivable that *T. minor* is a representative of a hitherto unknown fourth group of the *Georgefischeriales*.



**Key to the families and genera of *Georgefischeriales***

1	Phragmobasidia present ( <b>Tilletiaceae</b> )	2
	Holobasidia present	4
2(1)	Teliospores echinulate	<b>Tilletiaria</b>
	Teliospores smooth	3
3(2)	Single teliospores present	<b>Phragmotaeonium</b>
	Sporeballs present	<b>Tolyposporella</b>
4(1)	Ballistosporic propagules present ( <b>Georgefischeriaceae</b> )	5
	Ballistosporic propagules absent ( <b>Eballistraceae</b> )	<b>Eballistra</b>
5(4)	Teliospores lightly coloured	<b>Georgefischeria</b>
	Teliospores darkly pigmented	<b>Jamesdicksonia</b>

**The dilemma of *Georgefischeriales* systematics**

The currently identified species of the *Georgefischeriales* may reflect only the 'tip of the iceberg' of this group. There are numerous described species of *Entyloma* and *Melanotaenium* with black sori on grasses (e.g. *cfr* the synonyms of *E. dactylidis* in Vánky 1994). Our study suggests that all these species are members of the *Georgefischeriales*. *E. majus* and *E. parvum* on the one hand, and *M. indicum* and *M. ischaemianum* on the other, may reveal the systematic dilemma of these species. Modes of hyphal septation and cellular interaction indicate that *E. majus* and *E. parvum* belong to the *Georgefischeriales* (see above), but without data concerning basidial morphology, culture characteristics and/or DNA sequences, it is not possible to ascribe these two fungi to any of the genera and families of the *Georgefischeriales*. For example, teliospores and sori of *M. indicum* are very similar to those of *M. ischaemianum*. Furthermore, both fungi parasitize species of the grass genus *Ischaemum* and they were collected in the same geographical area in India (Vánky 1997). However, these two species have different basidia and they belong to different genera (see above). In general, the dilemma of *Georgefischeriales* systematics can be summarized as follows: the morphology of sori and teliospores alone is insufficient to ascribe the numerous existing candidates for the *Georgefischeriales* to any of the taxa of this group.

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