The Georgefischeriales: a phylogenetic hypothesis

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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, Georgefischeriacae, and Tilletiaraceae. The basal dichotomy is between the Eballistraceae and the branch uniting the Georgefischeriacae and Tilletiaraceae. The Tilletiaraceae are phragmobasidiate, whereas the Eballistraceae and the Georgefischeriacae are holobasidiate. The Eballistraceae differ from the Georgefischeriacae and Tilletiaraceae in the lack of the ballistospore mechanism. The systematic position of Tilletiopsis minor is unclear. The Eballistraceae, Eballista and Phragnotoanum are proposed as new taxa. The descriptions of the Tilletiaraceae and Jamesdicksonia are emended. Except for Entyloma majus, E. parvum, Georgefischeria, Jamesdicksonia brunckii, J. ochsa, Tilletiaria anomalum, and Tolyposorella chrysopogonis, 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 sorus 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 dacytidis, E. irregularare, E. oryzae, Georgefischeria riceae, Melanotanum brachiares, M. ischaemiamum, 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 Georgefischeriacae with E. dacytidis, E. irregularare, M. ischaemiamum and Georgeficheria, Tilletiariaaceae with T. anomalum, and the so-called Entyloma oryzae group with Entyloma oryzae and Melanotanum brachiares. By sequence analyses Jamesdicksonia brunckii and the conidial species Tilletiopsis flavus, T. fulosescens and T. minor were to the Georgefischeriales (Begerow et al. 1997, Begerow, Bauer & Boekhout 2000). These studies also revealed that the genera Melanotanum 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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1 Part 185 in the series Studies in Heterobasidionycestes from the Botanical Institute, University of Tübingen.
Table 1. Specimens and characters studied.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Hosts/Substrates</th>
<th>Characters studied1</th>
<th>Sequences3</th>
<th>Source4</th>
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<tr>
<td>Entyloma dactylidis</td>
<td>Agrostis stolonifera</td>
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<td>R.B. 915</td>
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<td>(as E. eleocharitidis)</td>
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<td></td>
<td>Isotype</td>
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<td>Zizania aquatica</td>
<td>S, T</td>
<td>AF 229351*</td>
<td>HUV 15050</td>
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<td>E. majus</td>
<td>Sporobolus spicata</td>
<td>T</td>
<td>BPI 175837</td>
<td>Type</td>
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<tr>
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<tr>
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<td>P. trivialis</td>
<td>B, C, T</td>
<td>R.B. 3015</td>
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<tr>
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<td>M.P. 1965</td>
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<td>E. oryzae</td>
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<td>B, C, T</td>
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<td>Eleocharis acicularis</td>
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<td>Erratomyces patellii</td>
<td>Phaseolus vulgaris</td>
<td>S, used as outgroup</td>
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<td>C, S</td>
<td>AJ 235281(c)</td>
<td>N.B. 244</td>
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<td>CBS 607.83</td>
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<td>AJ 235286(c)</td>
<td>CBS 346.33</td>
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<td>Sorghastrum nutans</td>
<td>T</td>
<td>HUV 2438</td>
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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 Ustilaginies Vánky, Tübingen, Germany; M.P., Herbarium M. Piepenbrinck, 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 eleocharitidis, E. lineatum, E. majus, E. purpurum, E. scirpula, Jamesicksonia brunkii, J. obsa, Melaenaenium indicum, and Tolyposporella chrysopogonis also have the typical characters of the Georgofischeriales: 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 Piepenbrinck, 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 Jamesicksonia obsa (Fig. 3), J. brunkii (Fig. 4), Tolyposporella chrysopogonis (Fig. 5), and especially Georgofischeria rivosiae (Fig. 2), but not in the other species
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 *Tolypospora chrysopogonis*, the teliospores were arranged in distinct balls (Fig. 5), whereas in
Figs 7–15. Typical basidia and germinating basidiospores/basidioconidia. Bar = 10 μm. Fig. 7. *Entyloma irregularare* (i.e. *Jamesdicksonia irregularis*, R.B. 3013). Note the conjugation of the basidiospores. Fig. 8. *E. dactylidis* (i.e. *J. dactylidis*, R.B. 3014). Note the exobasidioleaceous orientation of the basidiospores. Fig. 9. *J. brunii*. Note the different kinds of basidiospores on the basidium. Fig. 10. *Georgefischeria ripeae*. Note the two arms perpendicular to the long axis of the basidium. Fig. 11. *Melanotaenium ischaemianum* (i.e. *J. ischaemianum*). Two-sterigate basidium. Note the exobasidioleaceous orientation of the basidiospore on the basidium. Fig. 12. *M. indicum* (i.e. *Phragmotaenium 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. *Eballistra oryzae* (i.e. = *Eballistra oryzae*). Note the triradiate 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 irregularare* (Fig. 7), *E. dactylidis* (Fig. 8), *Jamesdicksonia brunii* (Fig. 9), *Georgefischeria ripeae* (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 irregularare* (Fig. 7), *Melanotaenium*
Neighbour Joining

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 patelli. 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 dactylicsis (Fig. 8), Georgelschiera 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 irregularae*. The conjugated basidiospores germinated apically or laterally while still connected to the basidium, producing basidioconidia (Fig. 7). Basidia of *Entyloma dactylidis* (Fig. 8) and *Melanotaenium ischaemianum* (Fig. 11) possessed a specific orientation of the basidioconidia: the hilar appendices of the ballistosporic 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-sterigate (Figs 8 and 11).

On the one hand, *Georgiescheria rietana* 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 *Georgiescheriales*. In *Entyloma irregularae* (Fig. 7), *E. dactylidis* (Fig. 8), *Jammedicksonia brunckii* (Fig. 9), *Georgiescheria rietana* (Fig. 10), *Melanotaenium ischaemianum* (Fig. 11), *M. indicum* (Fig. 12), and *Tilletiaria anomala* (Fig. 13), discharged ballistospores and/or the resulting ballistocoidia 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 pseudomycelium with retraction septa and ballistocoidia developed.

In contrast, in *Melanotaenium brachiaires* (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 ballistocoidia was formed.

**Molecular analyses**

We did not obtain PCR products from the herbarium specimens of *Entyloma eleochariitis*, *E. majus*, *E. parvum*, *E. scirpicola*, *Jammedicksonia obesa* and *Tolyposorella 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 *Errotomyces patellii* as root, in the neighbour joining analysis the species of the *Georgiescheriales* were distributed into three groups, which were congruent to the *Georgiescheriales*, *Tilletiariae* 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 *Georgiescheriaeae* (Fig. 16). In neighbour joining, *T. anomala* was located at the base of the group representing the *Tilletiariaeae* 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 *Georgiescheriaeae* 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 *Georgiescheriales* reported by Bauer et al. (1997) and Begerow et al. (1997, 2000) are also evident in this study. These are the *Tilletiariaeae*, *Georgiescheriaeae*, and the so-called *Entyloma oryzae* group (the *Eballistraceae*, as proposed below). The *Tilletiariaeae* are phragmobasidiates, whereas the *Eballistraceae* and the *Georgiescheriaeae* are holobasidiates. The *Eballistraceae* differ from the *Georgiescheriaeae* and *Tilletiariaeae* 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 eleochariitis*, *E. lineatum*, *E. majus*, *E. parvum*, *E. scirpicola*, *Jammedicksonia obesa*, *Melanotaenium ischaemianum*, *Melanotaenium indicum*, and *Tolyposorella chrysopogonis*, were identified as members of the *Georgiescheriales*.

**Georgiescheriaeae**

Among the *Georgiescheriales*, the formation of holobasidia and ballistosporic propagules characterizes the *Georgiescheriaeae*. The *Georgiescheriaeae* share the formation of holobasidia with the *Eballistraceae* and the formation of ballistic propagules with the *Tilletiariaeae*. Although the combination of holobasidia and the presence of the ballistospore mechanism clearly separates the *Georgiescheriaeae* 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 *Exobasidiales*, but occur also in species of the *Tilletiales* and *Dossianales* (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 *Exobasidomyctidae* and, therefore, plesiomorphic for the *Georgiescheriaeae*. Accordingly, the presence of ballistic propagules in the *Leukogynomyxidae* and *Exobasidomyctidae* (for the subclasses see Bauer et al. 1997) indicates that the ballistospore mechanism was already established before the *Georgiescheriales* diverged from the other groups of the *Exobasidomyctidae*.

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

*ductilis* and *Melanotaenium ischaemianum* is homologous to the passively released gastroid type occurring on the basidia of *Entyloma irregularis*. On the basidia of *J. bruncki* 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. bruncki*, 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. bruncki*. In addition, in contrast with the description, the basidium of *J. bruncki* 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 Thirmulalchar, Pavg & Payak (1960) resemble those of the ballistic type, whereas the basidia illustrated in Raghu Nath (1969) resemble those of *Entyloma irregularis*.

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

Multilamellate teliospores walls which become gelatinous and swell in water are considered as an important generic characteristic for *Jamesdicksonia* (Walker & Shivis 1998). However, *Jamesdicksonia* shares this feature with *Geofischeria* and *Tolypospora chrysopogonias* (belonging to the *Tillettiaceae*, 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 bruncki* is more closely related to *Entyloma ductilis*, *E. irregularis*, and *Melanotaenium ischaemianum* than to *Geofischeria riviæ* or, vice versa, *G. riviæ* stands in an isolated position within the clade representing the *Geofischeriaceae*. This isolated position of *Geofischeria* is also well reflected by morphological, ecological and coevolutionary characteristics. Thus, the two species of *Geofischeria* form brightly coloured teliospores and sorus, at least the basidia in *G. riviæ* 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 *Convolutulaceae* (Narasimhan et al. 1963). In contrast, *Entyloma ductilis*, *E. eleocharitis*, *E. scirpicola*, *Jamesdicksonia bruncki*, *J. obesa* and *Melanotaenium ischaemianum* are united in having darkly pigmented teliospores and sorus, 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 *Geofischeriaceae*, the description of *Jamesdicksonia* is emended as follows:


Members of the *Geofischeriales sensu* Bauer et al. (1997) having holobasidia, ballistic propagules and darkly pigmented teliospores and sorii. Known species are parasitic on *Poaceae* and *Cyperaceae*.


**Other species**


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


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


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


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


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


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

Phragmobasidia with lateral ballistic basidiospores represent an apomorphy for the *Tillettiaceae*, as defined above. We found this basidial type in *Melanotaenium indicum* and *Tillettiaria anomala*. The formation of ballistic spores in *Melanotaenium* was already described by Bandoni & Johri (1972). In addition, *Tolypospora chrysopogonias* may form the same basidial type. Although not explicitly described, the illustrations in Thirmulalchar, Whitehead & O'Brien (1967) suggest that the basidiospores are ballistic. As in *T. chrysopogonias*, hyphal germination of the basidial segments was also occasionally observed in *M. indicum*. In neighbour joining and, except for *Tillettiaria 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 *Tillettiaria anomala* and the other members of the *Tillettiaceae* 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 Tilletiari a anomal a, Tilletiopsis fulvescens, and T. flav a have been discovered only as cultures and their life strategies are therefore unknown. Interestingly, however, M. indicum, like Tilletiaria anomal a, occasionally forms teliospores and basidium in culture. Therefore, we speculate that Tilletiaria anomal a, Tilletiopsis fulvescens and T. flav a are phytoparasites, probably on Poaceae.

The three teleomorphic species identified in this group differ in sporation. Melanotaenium indicum and Tilletiaria anomal a produce single teliospores (Vánký 1997, Bandoni & Johri 1972), whereas the teliospores in Tolyposporella chrysopogonis are dispersed in distinct balls (Clinton 1902, Thirmalachar a t al. 1967). Multilateralate teliospore walls occur only in T. chrysopogonis. The teliospores of T. anomal a, but not of the two other species, are echinulate. Unique for the Ustilaginomycetes, the ornamentations in T. anomal a 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.

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

*Eym.: Phragmites* (from phragmobasidium, *taenium from Melanotaenium* (taenia = band).

Fungi Georgescheriales sensu Bauer et al. (1997) basidiis transverse septatis teliosporique singularibus levibus.


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

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


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

Fungi Georgescheriales sensu Bauer et al. (1997) holobasidiis basidiosporis conidiosque non conidiabantis.

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

Members of the Georgescheriales sensu Bauer et al. (1997) having holobasidia and lacking ballistical basidiospores and ballistoconidia.

The loss of the ballistospore mechanism represents an apomorphy for the Eballistrateae (as proposed here). *Melanotaenium brachiatiae* and *Entyloma oryzae* form holobasidia with passively released basidiospores in both species bud in a yeast-like manner (see also Singh & Pavi 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 Eballistrateae. This placement is statistically well supported by bootstrap values of 100% in both analyses. Therefore, we ascribe this parasite to this group.

Melanotaenium brachiatiae differs from Entyloma oryzae in basidiospore morphology. Basidiospores are more or less cylindrical in form in Melanotaenium brachiatiae (Singh & Pavi 1973) and triadrate in Entyloma oryzae. The triadrate basidiospores resemble radiate conidia of aquatic hyphomyctes (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 Georgescheriales, the new family, and also a new genus are proposed.

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

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

Descriptio analoga familiae Eballistrateae.

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

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


**Eballista brachiatiae** (Viégas) R. Bauer, Begerow, A. Nagler & Oberw., *comb. nov.*


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


**Tilletiopsis minor**

The anamorphic Tilletiopsis minor shares the Tilletiopsis-like growth in culture with the Georgescheriales and Tilletiariaceae, but not with the Eballistrateae. 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 Eballistrateae 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 brachiatiae, a member of the Eballistrateae (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 Georgescheriales.
The Georgefischeriales

Key to the families and genera of Georgefischeriales

1 Phragmobasidia present (Tilletiariaceae)
   Holobasidia present
   2(1) Teliospores echinulate
   - Teliospores smooth
   3(2) Single teliospores present
   - Sporeballs present
   4(1) Ballistosporic propagules present (Georgefischeriaceae)
   - Ballistosporic propagules absent (Ballistraceae)
   5(4) Teliospores lightly coloured
   - Teliospores darkly pigmented

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 Melanotrium with black sori on grasses (e.g. cf. the synonyms of E. dactyliidis 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. inicum 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. inicum 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 sorus 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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