Taxonomy and biodiversity of hysteriaceous ascomycetes in fynbos

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Received 17 March 2003, accepted in revised form 3 June 2003

Six hysteriaceous ascomycetes, Gloniopsis praelonga, Glonium chambianum, G. compactum, G. lineare, G. pusillum, and Hysterium angustatum, were commonly collected during a survey of saprobic microfungi occurring on leaf and twig litters of Proteaceae, or dead culms of Restionaceae in the Fynbos of the Cape Floral Kingdom. Several new hosts are reported for these taxa, while additional data are presented as to their morphological variation, distribution and ecology.

Introduction

The fynbos biome, which comprises 80% of the Cape Floral Kingdom in the south-western and southern Cape of South Africa, includes three vegetation types of which shrubs, the so-called ‘Fynbos’, are the most dominant. The biome is defined by moderate to high amounts of winter rain, a predominance of low to medium-height shrubs, nutrient poor soils and frequent fires (Cowling and Richardson 1995). The study on saprobic microfungi in Fynbos was initiated in 2000 and two families, monocotyledonous Restionaceae (restio) and dicotyledonous Proteaceae (protea), were chosen as host plants because of their high endemic ratios in the area, 94% and 96% respectively, accessibility, and tissue types favoured by saprobic fungi. During the course of this study several species of three genera of hysteriaceous fungi, Gloniopsis De Not., Glonium Muhl., and Hysterium Pers., were commonly encountered. The majority of these represent new records for South Africa, and are also reported on new host plants. Their morphological characteristics are described, and discussed in relation to their distribution patterns and ecology.

Hysteriaceous ascomycetes represent fungi having lirelliform ascomata, so-called hysterothecia, which are ellipsoid to elongate-shaped and open by means of longitudinal slits. The family Hysteriaceae accommodates some hysteriaceous fungi having ascomata that open by means of a longitudinal slit with two depressed flaps or lips. Gloniopsis and Glonium have hyaline dictyosporous and didymosporous ascospores respectively, whereas Hysterium has brown phragmosporous ascospores. Presently, approximately 524 names have been recorded in these three genera (http://www.speciesfungorum.org/). This large number of names, mostly generated during the 19th century, was the result of various workers trying to identify these fungi by means of macroscopic features of their ascomata, and supposed host specificity. The chaotic status that ensued was settled when Zogg (1962) monographed these genera, and this still remains the only treatment of this family. He emphasised the colour, number of septa and size of ascospores as the main criteria for separating species and genera. In so doing he recognised seven genera in the Hysteriaceae: Farlowiella, Gloniella, Gloniopsis, Glonium, Hysterium, Hysterocarina, and Hysterographium. Luttrell (1973) followed Zogg’s treatment in the subfamily Hysteroideae. Barr (1987) accepted five genera and concluded that the presence of hysterothecial ascomata was not a prerequisite of the Hysteriaceae, but rather the consistency of a three-layered peridium composed of small pseudoparenchymatous cells. In a later treatment Kirk et al. (2001) acknowledged 13 genera in the Hysteriaceae, while Eriksson (2001) recognised seven, considering a further three as doubtful. Since Zogg’s monograph, several new species have been added to this complex, resulting in five species of Gloniopsis (Amano 1983, Lorenzo and Messuti 1998), 19 species of Glonium (Amano 1983, Sivanesan and Sutton 1985, Speer 1986, Lorenzo and Messuti 1998), and 20 of Hysterium (Tilak 1963, Tilak and Rao 1965, 1966, Farr 1973, Messuti and Lorenzo 1997). Although several species of Hysteriaceae have been reported from woody substrates in South Africa by Van der Linde (1992), we were curious to know if the taxa occurring on endemic Proteaceae and Restionaceae would represent common species, or be unique to Fynbos, and whether there was any indication of host and tissue specificity among them.

Materials and Methods

Nature reserves and undisturbed areas of the Fynbos in the Western Cape province of South Africa were visited over a
3-year period (2000–2002). Dead leaves, twigs, and old flowerheads of proteas or dead culms of restios were collected from the beds or from the standing plants. The samples were inspected immediately for fungal structures, and air-dried for later study. Air-dried samples were incubated in moisture chambers for 2–3 days before examination. Single spore colonies were established on 5% malt extract agar (MEA; Biolab, Midrand, Johannesburg), supplemented with 0.04g l–1 streptomycin sulphate. Cultural characteristics were rated for each isolate in triplicate from MEA plates after 15–30d of incubation at 25°C in the dark, and colours determined according to Rayner (1970). Microscopic observations, measurements and photography of characteristic structures were made from structures mounted in lactophenol. The 95% confidence intervals were derived from 30 observations wherever possible to determine the range of variation in size of structures, with the extremes given in parentheses. Sections of ascomata were made on a Leica CM1100 Cryostat microtome, mounted with Jung tissue freezing medium™ (Leica Instruments, Germany). Photographic images were captured with a Nikon Digital Camera DXM 1200 on a Nikon Eclipse E600 light microscope or a Nikon SMZ2800 dissecting microscope. Herbarium specimens are lodged at PREM (National Collection of Fungi, Pretoria) and reference cultures are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

**Results**

A total of six hysteriaceous species were recovered from Fynbos: *Gloniopsis praelonga*, comprising 34 collections from 12 plant genera; *Glonium*, with 21 collections representing four species (*G. cambianum*, *G. compactum*, *G. lineare*, *G. pusillum*) on 11 host genera, and *Hysterium angustatum*, comprising eight collections on six host genera. All species represent new records for South Africa, except *G. praelonga*. Furthermore, new host plants are also documented for each fungal species. The morphological variation occurring in each taxon is described below.


**Figures 1–4, 25.**

Ascomata hysterothecioid, globose to subglobose in vertical section, solitary to gregarious, erumpent to superficial with base immersed, straight to flexuous, simple to rarely branched, ellipsoid or elongated with blunt to pointed extremes, opening by a depressed longitudinal slit, 0.1–0.9mm long, 0.3–0.4mm wide, 0.1–0.2mm high (Figures 1, 25). Peridium consisting of three layers, up to 70μm in diameter, pseudoparenchymatous, outer layer dark, carboneous, inner layer hyaline, compressed, middle layer consisting of 1–2 rows of thick-walled, brown cells between outer and inner layers (Figure 25). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, numerous, 0.5–1.5μm wide. Asci bitunicate, cylindric to clavate, stipitate, octosporous, 95–113 x 15–20μm (Figure 2). Ascospores (23–)25–26(–30) x (9–)10–11(–13)μm (Mean 25.5 x 10.7μm), ellipsoid, hyaline to yellowish, with 5–7 transverse septa and 1–2 longitudinal septa, slightly constricted at the transverse septa, with a gelatinous sheath (Figure 3). The description is based on PREM57535.


**Cultural characteristics:** Colonies sterile, 22mm in diameter, with base immersed, straight to flexuous, simple to rarely branched, ellipsoid or elongated with blunt to pointed extremes, opening by a depressed longitudinal slit, 0.1–0.9mm long, 0.3–0.4mm wide, 0.1–0.2mm high (Figures 1, 25). Peridium consisting of three layers, up to 70μm in diameter, pseudoparenchymatous, outer layer dark, carbonaceous, inner layer hyaline, compressed, middle layer consisting of 1–2 rows of thick-walled, brown cells between outer and inner layers (Figure 25). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, numerous, 0.5–1.5μm wide. Asci bitunicate, cylindric to clavate, stipitate, octosporous, 95–113 x 15–20μm (Figure 2). Ascospores (23–)25–26(–30) x (9–)10–11(–13)μm (Mean 25.5 x 10.7μm), ellipsoid, hyaline to yellowish, with 5–7 transverse septa and 1–2 longitudinal septa, slightly constricted at the transverse septa, with a gelatinous sheath (Figure 3). The description is based on PREM57535.
Figures 1–12: (1–3) *Gloniopsis praelonga* (PREM57535). (4) *Gloniopsis praelonga* (PREM57557). (5–7) *Glonium chambianum* (PREM57562). (8) *Glonium chambianum* (PREM57560). (9–12) *Glonium compactum* (PREM57570). 1, 5, 9 Hysterothecia on the host surface. 2, 6, 10 Asci. 3 Ascospore with a gelatinous sheath. 4 Ascospore without a sheath. 7, 8, 11, 12 Ascospores. Scale bars: 1, 5, 9 = 0.5mm; 2, 6, 10 = 10µm; 3, 4, 7, 8, 11, 12 = 5µm
entire margin, raised, pale olivaceous grey (21‴′′′′′′d) above, reverse smoke grey (21‴′′′′′′f), velvety. Aerial mycelium dense. Notes: Although not on Proteaceae, *Gloniopsis praelonga* has previously been recorded from South Africa (Van der Linde 1992), namely on wood of *Acacia* spp. The morphology of this species appears to be relatively uniform between different collections. Observations made from various isolates revealed, however, that the gelatinous sheaths of ascospores varied between different collections. Ascospores of some collections were clothed with a gelatinous sheath (PREM57535, PREM57536, PREM57537, PREM57528), whereas the majority of collections lacked gelatinous sheaths (Figure 4, PREM57557). Ascospore dimensions of the various collections varied from narrower (20–24 x 8–10µm in PREM57530) to wider (25–38 x 8–13µm in PREM57534), but all fell into the range (16–34 x 6–15µm) of Zogg’s description for the species (Zogg 1962). The presence of three distinct layered peridium emphasised by Barr (1987) for recognition of members of Hysteriaceae was not commonly observed. However, a middle layer of 1–2 cells thick was found between the outer and inner layers.


Figures 5–8, 27.

Ascormata hysterothecioid, subglobose to globose in vertical section, solitary to gregarious, erumpent to superficial with base immersed, straight to flexuous, simple to rarely branched, ellipsoid with blunt extremes, opening by a depressed longitudinal slit, 0.4–0.6mm long, 0.1–0.2mm wide, 0.1–0.2mm high (Figures 5, 27). Peridium consisting of two layers, up to 40µm in diameter, carbonaceous, outer layer dark, inner layer hyaline, compressed (Figure 27). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, 0.5–1.5µm wide. Asci bitunicate, cylindric to clavate, stipitate, octosporous, biseriate, 73–88 x 13–18µm cellular, hyaline, septate, flexuous, rarely branched, ellipsoidal with pointed extremes, opening by a depressed longitudinal slit, 0.4–0.6mm long, 0.1–0.2mm wide, 0.1–0.2mm high (Figures 5, 27). Peridium consisting of two layers, up to 40µm in diameter, carbonaceous, outer layer dark, inner layer hyaline, compressed (Figure 27). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, 0.5–1.5µm wide. Asci bitunicate, cylindric to clavate, stipitate, octosporous, biseriate, 73–88 x 13–18µm (Figure 6). Ascospores (18–)20–21(–23) x (4–)5–6(–7)µm (Mean 26.5 x 5.5µm), ellipsoidal with pointed extremes, opening by a depressed longitudinal slit, 0.3–0.6mm long, 0.2–0.3mm wide, 0.1–0.2mm high (Figures 9, 28). Peridium consisting of two layers, up to 50µm in diameter, pseudo-parenchymatous, carbonaceous, outer layer dark, inner layer hyaline, compressed (Figure 28). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, 0.5–1.5µm wide. Asci bitunicate, clavate, stipitate, octosporous, 65–69 x 12–15µm (Figure 10). Ascospores (24–)26–27 (–30) x (4–)5–6(–7)µm (Mean 26.5 x 5.5µm), fusiform, often curved, hyaline, with one transverse septum, slightly constricted at the septum (Figures 11, 12). The description is based on PREM57570.


Cultural characteristics: Colonies sterile, 14.2mm in diameter on MEA after 30d at 25°C in the dark, circular with an entire margin, raised, olivaceous grey (21‴′′′′′′k), velvety. Aerial mycelium dense. Notes: The first record of the species from Africa was on *Strychnos aculeata* Solereder. (Loganiaceae, a dicotyledon), a host on which it was rarely encountered (Zogg 1962). This species differs from most *Glonium* species, which have erumpent to almost superficial ascomata with immersed bases, by having ascomata that are deeply imbedded in the host substratum (Figure 28). The present species was the most common *Glonium* species encountered during the survey of Fynbos, and was collected from monocotyledinous restios only. *Restio* species have larger ascospore dimensions than those of the *Strychnos* species (24–28 x 5–6µm), but smaller ascii than those of the *Strychnos* species (90–130 x 8–13µm).


Figures 13–16, 26.

Ascormata hysterothecioid, solitary to gregarious, almost rectangular in vertical section, erumpent to superficial, straight to flexuous, ellipsoidal with blunt to pointed
extremes, opening by a depressed longitudinal slit, 0.2–0.4mm long, 0.1–0.2mm wide, up to 0.1mm high (Figures 13, 26). Peridium consisting of three layers, up to 12.5µm in diameter, pseudoparenchymatous, carbonaceous, outer layer dark, present only in sides and top, inner layer hyaline, compressed, middle layer consisting of 1–2 rows of thick-walled, brown cells between outer and inner layers at sides and 3–4 rows at the bottom (Figure 26). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, 1.5–2.5µm wide. Asci bitunicate, cylindrical to clavate, stipitate, octosporous, 43–55 x 9–13µm (Figure 14). Ascospores (12–)13–14(–15) x (4–)5µm (Mean 13.5 x 4.7µm), ellipsoid with upper cell often broader, hyaline, with 1 transverse septum (Figures 15, 16).


Notes: This species is known worldwide from various dicotyledonous plants (Zogg 1962). Ascomata were so small that the typical hysterothecioid character was not easily recognised, while this was not the case for the other hystericaceous fungi reported on in this study (Figure 26). The carbonaceous, dark outer layer was absent at the bottom of the ascomatal peridium. This resulted in the middle layer expanding, consisting of up to 3–4 thick-walled cells. G. lin-eare represents one of a few species collected from dead leaves of proteas.


Figures 17–20, 29.

Ascomata hysterothecioid, subglobose to globose in vertical section, solitary to gregarious, erumpent to superficial with base immersed, straight to flexuous, ellipsoidal with pointed extremes, opening by a depressed longitudinal slit, 0.2–0.3mm long, 0.1–0.2mm wide, up to 0.1mm high (Figures 17, 29). Peridium consisting of three layers, up to 17.5µm in diameter, psudoparenchymatous, carbonaceous, outer layer dark, inner layer hyaline, compressed, middle layer consisting of 1–2 rows of thick-walled, brown cells (Figure 29). Pseudoparaphyses cellular, hyaline, septate, flexuous, branched, 1.5–2µm wide. Asci bitunicate, cylindrical to claviform, stipitate, octosporous, 33–48 x 8–11µm (Figure 18). Ascospores (9–)11–12(–13) x 4–5µm (Mean 11.3 x 4.6µm), ellipsoid, with upper cell often larger, hyaline, with 1 transverse septum (Figures 19, 20). The description is based on PREM57574.


Notes: This species has a worldwide distribution, and is known from numerous host plants (Zogg 1962). The chief generic feature of Hysterium species is their pigmented phragmospores. Approximately 20 species have thus far been described. Specific differentiation of Hysterium is mainly based on ascospore morphology, size and the number of septa. Since sepal numbers vary within a species, averages and extremes are taken into consideration. Approximately half of the Hysterium species produce 4-celled ascospores. All of them have concolorous ascospores, except for H. pulicare, which has versicolorous ascospores with darker median cells and pale end cells. Observations of the present collections, however, revealed a mixture of concolorous and
versicolorous ascospores. The majority of ascospores were concolorous, with less than 5% being versicolorous in a single ascoma (Figure 24).

Discussion

Until recently, the Hysteriaceae were only known to be represented in South Africa by 12 species from five different genera (Van der Linde 1992). Most of these collections were obtained from dead woody substrates, and are represented in the National Collection of Fungi (PREM) in Pretoria as Gloniella bambusae, Gloniopsis praelonga, Glonium abbreviatum, Hysterographium acaciae, H. flexuosum, H. fraxini, H. mori, H. spinicolum, H. subrugosum, Hysterium insidens, H. sinense and H. velloziae.

A total of 63 hysteriaceous fungi was collected during the present survey, which is approximately 12.5% of the total number of ascomycetes collected. The Hysteriaceae was the most commonly collected family next to the Lophiostomataceae. Previous data generated from this survey revealed the presence of unique and novel fungal species occurring only in Fynbos (Lee and Crous 2003a, 2003b, 2003c, Lee et al. 2003). Although no new hysteriaceous ascomycetes were found on endemic Proteaceae and Restionaceae, the majority of the taxa collected proved to be new records for South Africa when compared to data by Van der Linde (1992).

Of all the members of the Hysteriaceae collected, Gloniopsis praelonga was the species most commonly encountered. This taxon occurred on twigs of Proteaceae and culms of Restionaceae in most areas sampled. The genus Glonium was the second most frequently collected, and showed the highest specific diversity (Figure 31). Three collections of Glonium chambianum, a species recorded as rare in northern Africa (Zogg 1962), were made from three localities set far apart from one another. Host-exclusivity, coined by Zhou and Hyde (2001), namely the unique occurrence of a saprobic fungus on a restricted range of related host plants, was observed for Glonium compactum from the collections made during the survey. Of the two host types investigated, this species was only ever found on restios, and then only in areas where some degree of disturbance was observed. Glonium lineare, considered to be a cosmopolitan species on various substrates (Zogg 1962), was found only once on dead leaves of Protea, whereas G. pusillum, considered rather a rare species by Zogg (1962), was more frequently collected from both proteas and restios.

Hysteriaceous fungi with darkly pigmented ascospores such as Hysterium and Hysterographium were found less commonly in Fynbos. Hysterographium, of which six species were reported from the eastern and south-eastern region of South Africa (Van der Linde 1992), was not found during this investigation, while Hysterium, represented by H. angustatum, was collected on nine separate occasions. The present

study was conducted on limited host plants in a restricted area, namely Fynbos, located in the southern and southwestern Cape of South Africa. Taking into account that South Africa has seven plant biomes of different vegetation and climate, ranging from Fynbos to grassland, desert and forest, a more comprehensive collection on other host plants could mean the extinction of the other. However, this is not applicable to the majority of saprobic fungi, as they are predominantly assumed to be non-host specific. A study of the succession of saprobic fungi in burned areas is needed to bring more understanding of post-fire ecology of the Fynbos mycota. In areas in Fynbos where fires have recently occurred (Jonkershoek Nature Reserve), *Gloniopsis praelonga* was infrequently found on burnt twig litter of proteas. This was in contrast, however, to members of *Glonium* and *Hysterium*, which were found to be absent.

Acknowledgements — The authors are thankful to the Western Cape Nature Conservation Board for permission to collect indigenous plants. The officers at Kogelberg Nature Reserve, Jonkershoek Nature Reserve, Cape Point National Park, Kirstenbosch National Botanical Garden, and University of Stellenbosch Botanical Garden are also thanked for help during field trips.

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