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George K. Mugambi[1, 2, 3,*] **& Sabine M. Huhndorf**[1,†]
[1]Botany Dept., The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA
[2]Dept. of Biological Sciences, University of Illinois at Chicago, 845 W. Taylor St (MC 066), Chicago, IL 60607, USA
[3]National Museums of Kenya, Botany Dept., P.O. Box 45166, 00100, Nairobi, Kenya

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Parallel evolution of hysterothecial ascomata in ascolocularous fungi (Ascomycota, Fungi)

Abstract Ascoma morphologies have traditionally been used to classify filamentous ascomycete fungi. Phylogenies generated using DNA sequence data have since shown most of the previously identified groups not to be monophyletic. The relationships of hysterothecia-bearing ascolocularous fungi have been unsettled for many years. Recent molecular studies have shown this group as currently circumscribed not to constitute natural groupings. This study targeted wide taxon sampling to assess the validity of the current classification in the group. A data set of 40 taxa was assembled including sequence data from nuclear large subunit rDNA and translation elongation factor 1 alpha for each taxon with 68 new sequences being generated for this study and aligned with 62 sequences obtained from GenBank. Parsimony, Bayesian and Maximum Likelihood analyses were performed on the data. Mytilinidiales was recovered as a strongly supported monophyletic clade, while a monophyletic Hysteriales was also recovered but with weak support. Gloniaceae (comprising the genus *Glonium* s. str. excluding *Psiloglonium*) was shown not to be monophyletic. This study established a monophyletic hysterothecial clade nested within Pleosporales, for which we proposed a new genus, *Anteaglonium* gen. nov. including two new species, *A. globosum* sp. nov. and *A. latirostrum* sp. nov. that are described. This relationship was strongly supported and most statistical analyses support the placement. The result of this study further indicates that the hysterothecial type of ascomata may have evolved several times within the ascolocularous fungi.

Key words *Anteaglonium*, Ascomycota, evolution, Gloniaceae, Hysteriaceae, hysterothecia, LSU, Mytilinidiaceae, Pleosporales, TEF

Introduction

Traditionally, ascoma (fruit body) morphologies have been used for the taxonomic classification of major groups of filamentous ascomycetes. For example, the typical fruiting body types, cleistothecia, perithecia, apothecia and pseudothecia have in the past been used to delineate the classes, Plectomycetes, Pyrenomycetes, Discomycetes and Loculoascomycetes, respectively (Nannfeldt, 1932; Luttrell, 1951, 1955; Alexopoulos *et al.*, 1996; Lumbsch, 2000; Pöggeler *et al.*, 2006). The first molecular studies, based on restricted taxon sampling, seemed to support some of these classes such as Plectomycetes and Pyrenomycetes (e.g. Berbee & Taylor, 1992). However, subsequent molecular studies with extended taxon sampling convincingly showed that this coarse classification does not reflect phylogenetic relationships. These classical groups did not form monophyletic groups in molecular phylogenetics (Suh & Blackwell, 1999; Berbee *et al.*, 2000; Lumbsch *et al.*, 2000; Lindemuth *et al.*, 2001; Grube *et al.*, 2004; Schmitt *et al.*, 2005; Schoch *et al*. 2006) with a notable exception of a study that revealed a monophyletic loculoascomycetes (Liu *et al.*, 1999) but with no support. Hence, current classification of major clades in the Ascomycota at the class level uses correlation of several character sets, but is primarily based on phylogenies inferred by DNA sequence data (Hibbett *et al.*, 2007; Lumbsch & Huhndorf, 2007).

Several ascomycetes form rather elongate, superficial or erumpent ascomata that are often carbonaceous and open by a slit over the entire length (Fig. 1). These peculiar ascomata are called hysterothecia following Clements (1909). Hysterothecia are known from different unrelated groups of ascomycetes including Graphidaceae and Trapeliaceae in the Lecanoromycetes (Lumbsch, 1997; Staiger *et al.*, 2006; Lumbsch *et al.*,

*Corresponding author. Email: gkmugambi@gmail.com
†Email: shuhndorf@fieldmuseum.org

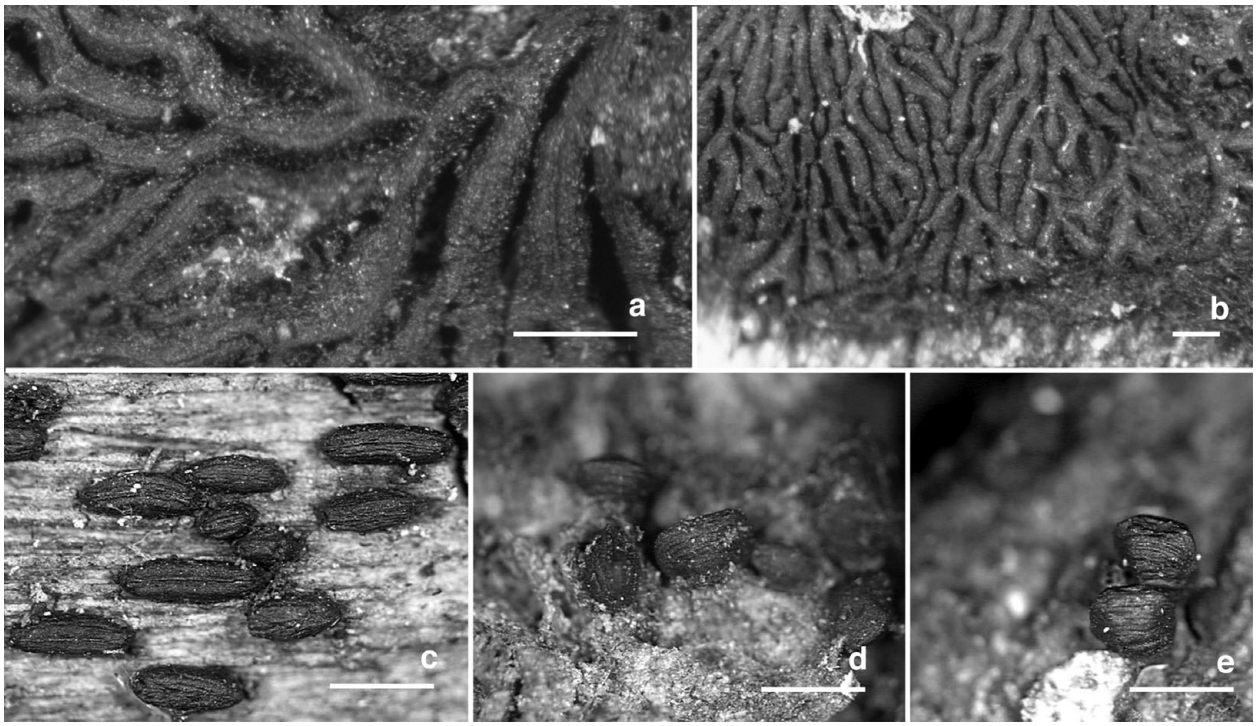

Figure 1 Ascoma types traditionally termed as hysterothecia within the ascolocularous fungi. a–c, Hysteriaceae. a, b, *Glonium stellatum*. c, *Psiloglonium clavisporum*. d, e, Mytilinidiaceae. (*Mytilinidion*). Bar lines: a–e = 1 mm.

2007; Mangold *et al.*, 2008), Opegraphaceae in the Arthoniomycetes (Ertz *et al.*, 2009) and ascolocularous fungi (Luttrell, 1953). Despite their wide occurrence among ascomycetes, hysterothecial taxa in the ascolocularous fungi were generally believed to form a natural group. Consequently, they were classified under the family Hysteriaceae (Chevallier, 1826; Luttrell, 1953) and later elevated to ordinal rank, Hysteriales (Luttrell, 1955, 1973). Subsequently two families were recognised among the ascolocularous hysterothecial fungi, Hysteriaceae placed under Pleosporales (Barr, 1987, 1990), or under Hysteriales (Kirk *et al.*, 2001, 2008; Lumbsch & Huhndorf, 2007; Boehm *et al*., 2009) and family Mytilinidiaceae placed in Melanommatales (Barr, 1987, 1990), Pleosporales (Kirk *et al.*, 2008; Lumbsch & Huhndorf, 2007) or in Mytilinidiales (Boehm *et al.*, 2009).

A recent molecular study by Boehm *et al*. (2009) reviewed the phylogenetic relationships of hysteriaceous ascolocularous fungi and proposed several revisions within the group. They accepted Hysteriaceae distinct from Mytilinidiaceae and recognised its ordinal rank, Hysteriales, agreeing with the original designation by Lindau (1897). In their study many taxa in traditionally classified Hysteriales were found to be highly polyphyletic. They accepted within the order the genera *Gloniopsis* De Not., *Hysterium* (Pers.) Fr., *Hysterographium* Corda, *Psiloglonium* Höhn and *Rhytidhysteron rufulum* (Spreng.) Speg. that was previously placed in Patellariaceae. The genus *Ostreichnion* Duby, which previously was treated in Mytilinidiaceae, was also found to belong within Hysteriales. The genera *Hysterium*, *Hysterographium*, *Gloniopsis* were found to be highly polyphyletic with *Hysterographium fraxini* (Pers.) De Not. and *Hysterium hyalinum* Cooke & Peck grouping together outside the order (Boehm *et al*., 2009). At the same time the genus *Farlowiella* Sacc. that traditionally has been treated in Hysteriaceae was found to belong outside the group. A new family Gloniaceae was erected to accommodate a distinct lineage within the subclass Pleosporomycetidae restricted to subiculate *Glonium* Muhl. taxa, which included the type species of the genus, *Glonium stellatum* Muhl. (Boehm *et al*., 2009). Their non-subiculate counterparts were found to nest within the Hysteriales and the genus *Psiloglonium* was revived to accommodate the group (Boehm *et al*., 2009). The distinction of subiculate and non-subiculate groups in the traditional genus *Glonium* had previously been proposed by von Höhnel (1918), and later by Petrak (1923a, 1923b) and Barr (1987). Boehm *et al*. (2009) also proposed order Mytilinidiales within subclass Pleosporomycetidae to accommodate taxa currently classified in Mytilinidiaceae.

Previous molecular studies aimed at studying the evolution of fruiting body types included only a few hysterothecial fungi (Liu *et al.*, 1999; Lumbsch *et al.*, 2000; Lindemuth *et al.*, 2001) and until recently the phylogeny of these fungi was poorly understood. Lindemuth *et al.* (2001) showed that *Glyphium* Nitschke *ex* F. Lehm. with hysterothecial ascomata does not belong in Dothideomycetes (the core group of ascolocularous fungi) but in the Chaetothyriomycetidae. The comprehensive study by Boehm *et al.* (2009) demonstrated that ascolocularous hysterothecial fungi as currently circumscribed are not monophyletic but fall into at least four distinct lineages. The discovery of these unrelated hysterothecia-bearing lineages stimulated us to further investigate the phylogenetic relationships of these fungi, by including in the analyses some key

	Species	Collection (Locality and Source)	nuLSU	TEF
Gloniaceae	*Glonium stellatum* I	USA, Smoky Mts (A. Miller 32), F	GQ221887	GQ221926
Hysteriales	*Glonium chambianum* I	USA, Smoky Mts. (A. Miller 1484), F	GQ221883	–
	Gloniopsis praelonga I	Kenya, Taita hills (G. Mugambi 426N), EA	GQ221901	GQ221913
	Gloniopsis praelonga II	New Zealand (S. Huhndorf 5211.1), F	GQ221905	GQ221923
	Gloniopsis praelonga III	New Zealand (S. Huhndorf 5280), F	GQ221912	GQ221914
	Hysterium angustatum I	New Zealand (S. Huhndorf 5211), F	GQ221906	GQ221921
	Hysterium angustatum II	New Zealand (S. Huhndorf 5216), F	GQ221908	GQ221933
	Hysterium angustatum III	Kenya, Malindi (G. Mugambi 243A), EA	GQ221899	GQ221928
	Hysterium angustatum IV	Kenya, Taita hills (G. Mugambi 123N), EA	GQ221900	GQ221927
	Hysterium pulicare I	USA, Smoky Mts (A. Miller 85), F	GQ221898	GQ221934
	Hysterium pulicare II	USA, Smoky Mts (A. Miller 1455), F	GQ221904	GQ221932
	Hysterium pulicare III	Kenya, Mt. Kenya (G. Mugambi 1234), EA	GQ221897	GQ221929
	Hysterium sp1	USA, Smoky Mts (A. Miller 1442), F	GQ221884	GQ221930
	Hysterium sp2	USA, Smoky Mts (A. Miller 1495), F	GQ221885	GQ221931
	Hysterium sp3	USA, Smoky Mts (A. Miller 1443), F	GQ221882	–
	Hysterographium flexuosum I	Kenya, Kakamega forest (G. Mugambi 1262c), EA	GQ221886	GQ221935
	Hysterographium mori I	USA, Indiana Dunes (S. Huhndorf 5273), F	GQ221910	GQ221936
	Hysterographium mori II	Kenya, Mt. Kenya (G. Mugambi 1214), EA	GQ221895	–
	Hysterographium mori III	Cuba, Sacti Spiritus (S. Huhndorf 557), F	GQ221896	–
	Hysterographium mori IV	Kenya, Malindi (G. Mugambi 1190), EA	GQ221892	–
	Hysterographium subrugosum I	Kenya, Malindi (G. Mugambi 1010), EA	GQ221891	–
	Psiloglonium clavisporum I	Kenya, Malindi (G. Mugambi 344A), EA	GQ221889	–
	Psiloglonium lineare I	USA, Smoky Mts (A. Miller 1557), F	GQ221873	GQ221920
	Rhytidhysteron rufulum I	Kenya, Malindi (G. Mugambi 361A), EA	GQ221893	–
Pleosporales	*Anteaglonium abbreviatum* I	USA, Smoky Mts. (A. Miller 925.1), F	GQ221877	GQ221924
	Anteaglonium abbreviatum II	Kenya, Malindi (G. Mugambi 1029), EA	GQ221878	GQ221915
	Anteaglonium globosum I	USA, Indiana Dunes (S. Huhndorf 5283)	GQ221911	GQ221919
	Anteaglonium globosum II	USA, Smoky Mts. (A. Miller 925.2)	GQ221879	GQ221925
	Anteaglonium latirostrum I	Kenya, Taita hills (G. Mugambi L100N.2), EA	GQ221876	GQ221938
	Anteaglonium latirostrum II	Kenya, Taita hills (G. Mugambi 1119), EA	GQ221874	GQ221937
	Anteaglonium parvulum I	New Zealand (S. Huhndorf 5223), F	GQ221909	GQ221918
	Anteaglonium parvulum II	Kenya, Taita hills (G. Mugambi 1218), EA	GQ221880	GQ221922
	Anteaglonium parvulum III	Kenya, Taita hills (G. Mugambi 219N), EA	GQ221881	GQ221916
	Anteaglonium parvulum IV	New Zealand (S. Huhndorf 5210), F	GQ221907	GQ221917

Table 1 Species and specimens from which new sequences were obtained for this study with locality and voucher and GenBank accession numbers.

taxa that were not treated previously. The aim of this study was to elucidate the phylogenetic relationships of hysterothecia-bearing fungi in the class Dothideomycetes, as well as shed light on the validity of hysterothecium as a taxonomic character as currently applied. Here, we present phylogeny resulting from combined analyses of two nuclear DNA markers, the ribosomal large subunit (LSU) and translation elongation factor 1 alpha (TEF).

Materials and methods

Taxon sampling and morphological analyses

The taxa sequenced for this study are listed in Table 1 together with their associated locality information and GenBank accession numbers. For microscopy and image capture, the ascomata were squash-mounted in water and images of micromorphological structures captured with a Dage DC-330 video system mounted on a Zeiss Axioskop microscope. Representative species of genera within Hysteriaceae and Mytilinidiaceae were included in the study. A total of 40 taxa were newly sequenced for this study (Table 1) while the rest of the sequences were obtained from the GenBank (Table 2). Voucher specimens of the taxa used for this study are deposited in F and the Kenyan material in EA.

DNA extraction, PCR amplification, sequencing and sequence alignment

Genomic DNA was extracted from dried ascomata using the Dneasy Plant Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Nuclear LSU was amplified using LROR, LR6 and LR3 primers (Vilgalys & Hester,

	Taxa	nuLSU	TEF
Botryosphaeriales	*Botryosphaeria dothidea*	DQ678051	DQ767637
	Macrophomina phaseolina	DQ678088	DQ677929
Capnodiales	*Capnodium coffeae*	DQ247800	DQ471089
	Cladosporium cladosporioides	DQ678057	DQ677898
	Mycosphaerella punctiformis	DQ470968	DQ471092
Dothideales	*Delphinella strobiligena*	DQ470977	DQ471100
	Dothidea sambuci	AY544681	DQ497606
Dothideomycetes	*Farlowiella carmichaeliana*	AY541492	DQ677931
	Helicomyces roseus	DQ678083	DQ677928
	Hysteropatella clavispora	AY541493	DQ677901
Eurotiomycetes	*Capronia pilosella*	DQ823099	DQ840565
	Dermatocarpon miniatum	EF469160	DQ782893
	Eupenicillium limosum	EF411064	EF411070
	Glyphium elatum	AF346420	FJ161099
Geoglossaceae	*Geoglossum nigritum*	AY544650	DQ471044
	Trichoglossum hirsutum	AY544653	DQ471049
Gloniaceae	*Glonium circumserpens*	FJ161200	FJ161108
	Glonium stellatum	FJ161179	FJ161095
Hysteriales	*Gloniopsis praelonga* (A)	FJ161173	FJ161090
	Gloniopsis praelonga (B)	FJ161195	FJ161103
	Gloniopsis smilacis	FJ161174	FJ161091
	Hysterium angustatum (A)	FJ161207	FJ161111
	Hysterium angustatum (B)	FJ161180	FJ161096
	Hysterium insidens	FJ161182	FJ161097
	Hysterium pulicare	FJ161201	FJ161109
	Hysterographium fraxini	FJ161171	FJ161088
	Hysterographium mori (A)	FJ161196	FJ161104
	Hysterographium mori (B)	FJ161198	FJ161106
	Hysterographium subrugosum	FJ161210	
	Ostreichnion curtisii	FJ161176	FJ161093
	Psiloglonium clavisporum	FJ167526	FJ161105
	Psiloglonium compactum	FJ161172	FJ161089
	Psiloglonium simulans	FJ161178	FJ161094
Lecanoromycetes	*Lecanora hybocarpa*	DQ782910	DQ782901
	Lobaria scrobiculata	AY584655	DQ883768
	Peltigera degenii	AY584657	DQ782897
Leotiomycetes	*Botryotinia fuckeliana*	AY544651	DQ471045
	Coccomyces strobi	DQ470975	DQ471099
	Dermea acerina	DQ247801	DQ471091
Magnaporthaceae	*Magnaporthe grisea*	AB026819	Genome
Myriangiales	*Myriangium duriaei*	DQ678059	DQ677900
Mytilinidiales	*Lophium mytilinum* (A)	FJ161203	FJ161110
	Lophium mytilinum (B)	DQ678081	DQ677926
	Mytilinidion mytilinellum	FJ161184	FJ161100
	Mytilinidion resinicola	FJ161185	FJ161101
	Mytilinidion rhenanum	FJ161175	FJ161092
	Mytilinidion scolecosporum	FJ161186	FJ161102
Pleosporales	*Alternaria alternata*	DQ678082	DQ677927
	Bimuria novae-zelandiae	AY016356	DQ471087
	Cochliobolus heterostrophus	AY544645	DQ497603
	Delitschia winteri	DQ678077	DQ677922
	Dendryphiella arenaria	DQ470971	DQ677890
	Leptosphaeria maculans	DQ470946	DQ471062
	Lewia eureka	DQ678044	DQ677883

Table 2 Species and specimens used in this study from which sequences were obtained from GenBank.

Taxa	nuLSU	TEF
Ophiosphaerella herpotricha	DQ767656	DQ767639
Phaeodothis winteri	DQ678073	DQ677917
Phaeosphaeria eustoma	DQ678063	DQ677906
Phaeosphaeria nodorum	EU754175	Genome
Phoma herbarum	DQ678066	DQ677909
Pleomassaria siparia	DQ678078	DQ677923
Pleospora herbarum (A)	DQ678049	DQ677888
Pleospora herbarum (B)	DQ247804	DQ471090

Table 2 Continued.

1990), while TEF was amplified using EF1–526F, EF1–983F, EF1–1567R, Ef-df and EF-gr primers obtained from the Fungal Tree of Life database (http://ocid.nacse.org/research/aftol/primers.php). The choice of gene marker was dictated by the availability of supplementary sequences from GenBank. Translation elongation factor 1 alpha (TEF) is much more variable than the LSU at least for this group of fungi and therefore important in resolving terminal nodes. Polymerase chain reaction (PCR) was carried out using the following protocol: the final volume of the PCR reaction was 25 μl and contained 2.5 μl buffer, 2.5 μl dNTP mix, 1 μl of each primer (10 μm), 5 μl of BSA, 0.25 μl taq polymerase, 2 μl genomic DNA extract and 10.75 μl deionised water. The reaction was allowed to run for 34 cycles. The annealing temperature was set at 58 °C reducing by 1 °C each cycle for total of eight cycles and then at 50 °C for the remaining cycles. The fragments were sequenced using the Big Dye Terminator reaction kit (ABI PRISM, Applied Biosystems, Forster City, USA). Sequencing was performed using the same set of primers as PCR. Sequences were aligned using multiple sequence alignment program, MUSCLE v3.6 (Edgar, 2004) and manually edited. Ambiguously aligned regions were excluded from the analyses. A total of 115 ambiguously aligned characters were excluded, 31 of these from LSU and 84 from TEF. The TEF sequences were analysed using nucleotide alignments.

Phylogenetic analyses

Phylogenetic analyses included Maximum Parsimony (MP), Maximum Likelihood (ML) and a Bayesian approach for the single-gene and combined data sets. Maximum parsimony (MP) analyses were carried out using PAUP*version 4.0b10 (Swofford, 2001). Heuristic searches were carried out employing TBR branch swapping, unordered characters, addition sequence set at random and MulTree option in effect. To estimate branch support, bootstrapping (Felsenstein, 1985) was performed employing 1000 replicates using 10 random sequence addition replicates with the above settings. MODELTEST v3.7 (Posada & Crandall, 1998) was used to determine the best-fit model of evolution before the data sets were subjected to Bayesian and Maximum Likelihood analyses. Maximum likelihood (ML) analyses were carried out using GARLI v0.951 (Zwickl, 2006) employing GTR+I+G model of evolution with fixed parameters, base frequencies and among site variation. The other settings in the program were left in their default mode. Bayesian analyses employing a Markov chain Monte Carlo (MCMC) were carried out using MrBayes v3.1 (Huelsenbeck & Ronquist, 2001). Four MCMC chains were run simultaneously for 2–5 million generations for single locus and combined loci, utilising GTR+I+G the best-fit model of evolution for the data, trees were sampled every 100th generation and printed every 500 generations. The temperature of the cold chain was left in the program's default setting. AWTY program by Nylander *et al.*, (2007) was used to check the stationarity of Bayesian tree sampling procedure produced by MrBayes program by plotting the split frequencies of run 1 versus run 2 as well as a plot of cumulative split frequency. The trees obtained before the MCMC chains achieved stationarity were discarded and posterior probabilities determined using the sumt command in MrBayes.

Hypotheses tests

Since the results of our phylogenetic analyses, which suggested a new lineage within the Pleosporales are incongruent with the current concept of the Hysterialean fungi, we tested whether our data are sufficient to reject the following four alternative hypotheses: (1) monophyly of *Anteaglonium* Mugambi & Huhndorf gen. nov. + Hysteriaceae *s. str.*, (2) monophyly of *Anteaglonium* + Mytilinidiaceae, (3) monophyly of *Anteaglonium* + Gloniaceae *sensu* Boehm *et al.* (2009), and (4) monophyly of *Anteaglonium* + Hysteriaceae *s. str.* + Mytilinidiaceae + Gloniaceae. Such topologies might be present in suboptimal trees not present in the 50% majority-rule consensus tree of the MCMC sampling, which may not be significantly worse than the obtained topology. Hypotheses tests were performed using Tree-PUZZLE 5.2 (Schmidt *et al.*, 2002) with the combined data set employing the GTR+I+G nucleotide substitution model. Two different tests were carried out, the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) and the expected likelihood weight (ELW) test following Strimmer and Rambaut (2002).

Results

Phylogenetic analyses

We generated a total of 68 new sequences (nuclear LSU and TEF) for this study (Table 1). The sequences were aligned with those of 62 taxa obtained from GenBank (Table 2). The final

combined gene alignment comprised 96 taxa with 2182 characters for which both data sets were available. Since the topology of the phylogenies obtained by the single-gene and combined gene analyses were similar (target clades had support of equal or greater than 70% Bootstrap and 95% Bayesian posterior probabilities), we only present results of combined gene analyses (Fig. 2). Branch support is indicated by Maximum Parsimony Bootstrap (BS) values equal or above 70%, while Bayesian Posterior Probabilities (PP) equal or above 95% are indicated by bold branches. MrBayes was run for 5 million generations and the first 10 000 trees were discarded as burnin, which was well after the sampling had reached stationarity.

Phylogenetic analyses recovered strongly supported monophyletic Mytilinidiales and a monophyletic Hysteriales that lacked significant support (Fig. 2), which generally agreed with the results of Boehm *et al*. (2009). Gloniaceae, which was erected for subiculate taxa in *Glonium* by Boehm *et al*. (2009), was found to be polyphyletic. A new strongly supported lineage, comprising of taxa previously placed in *Glonium*, was recovered nested within Pleosporales distant from Gloniaceae (Fig. 2). A new genus, *Anteaglonium* is erected and described below to accommodate the group. The clade comprises taxa previously recognised as *Glonium abbreviatum* (Schwein.) M. L. Lohman, *G. parvulum* (W. R. Gerard) Sacc., and two new species that are described below (Fig. 2). The genera currently accepted in the family Hysteriaceae, *Gloniopsis*, *Hysterium* and *Hysterographium* are not monophyletic (Fig. 2), confirming recent results by Boehm *et al.* (2009). Mytilinidiaceae forms supported a sister relationship with Gloniaceae (Fig. 2).

Hypotheses tests

Since the placement of *Anteaglonium* within Pleosporales was unexpected, assessment of alternative phylogenetic hypotheses was conducted (Table 3). The tests significantly reject the monophyly of (1) *Anteaglonium*+ Hysteriaceae *s. str.*, (2) *Anteaglonium* + Mytilinidiaceae and (3) *Anteaglonium* + Gloniaceae. However, the monophyly of the three families and genus *Anteaglonium* (*Anteaglonium* + Mytilinidiaceae + Gloniaceae + Hysteriaceae *s. str.*) could not be rejected by the Shimodaira–Hasegawa test but was significantly rejected by the ELW test (Table 3).

Discussion

Our analyses recovered a new hysterothecial group nested within Pleosporales and statistical tests of alternative hypotheses are significantly rejected (Table 3). The clade occurs in a strongly supported relationship within Pleosporales and taxa in the group are placed in a new genus, *Anteaglonium*. The phylogenetic analyses indicate that ascolocularous fungi bearing hysterothecial ascomata are not monophyletic (Fig. 2). Therefore, our study rejects the traditional classification of ascolocularous hysterothecial taxa, which agrees with findings by Boehm *et al.* (2009). The results presented here and the studies by Boehm *et al.* (2009) demonstrate that the hysterothecial ascoma morphology has evolved independently several times within the ascolocularous fungi thus underscoring the unreliability of its use in classification.

Little is known about the developmental origin of hysterothecial morphology in most groups of ascolocularous fungi. The only detailed developmental study on the group is that carried out by Luttrell (1953) on *Glonium stellatum*, which was assumed to represent the entire hysterothecial group. In light of the new phylogenetic data, more studies on the developmental origin of the hysterothecial morphology in the independent lineages of ascolocularous fungi are required. Such detailed ontogenetic studies are necessary to better understand the evolution of the character and especially its independent origins. For example, it has been demonstrated that repeated evolution of closed fruiting bodies is linked to ascoma development in the largest lichenised fungal group, Lecanoromycetes (Schmitt *et al*., 2009).

The recovery of a strongly supported hysterothecial clade nested within Pleosporales in our analyses necessitated the establishment of a new genus *Anteaglonium* (described below) to accommodate the taxa in this monophyletic clade. We feel justified in placing the new taxa in a single genus based on some shared key morphological characters and the strong support the

Null hypothesis	Log L	Difference	Shimodaira–Hasegawa test	Expected likelihood weight test
Monophyletic *Anteaglonium* + Hysteriaceae	−14944.16	79.01	0.0060[a]	0.0000[a]
Monophyletic *Anteaglonium* + Mytilinidaceae	−14942.66	77.51	0.0100[a]	0.0000[a]
Monophyletic *Anteaglonium* + Gloniaceae	−14937.32	72.22	0.0020[a]	0.0010[a]
Monophyletic *Anteaglonium* + Hysteriaceae + Mytilinidaceae + Gloniaceae	−14905.66	40.50	0.0880[b]	0.0344[a]
Unconstrained	−14865.15	0.0000	1.0000[b]	0.9990[b]

Table 3 Probabilities of the four phylogenetic hypotheses being significantly worse than the observed.

[a] Significant values
[b] Non-significant values

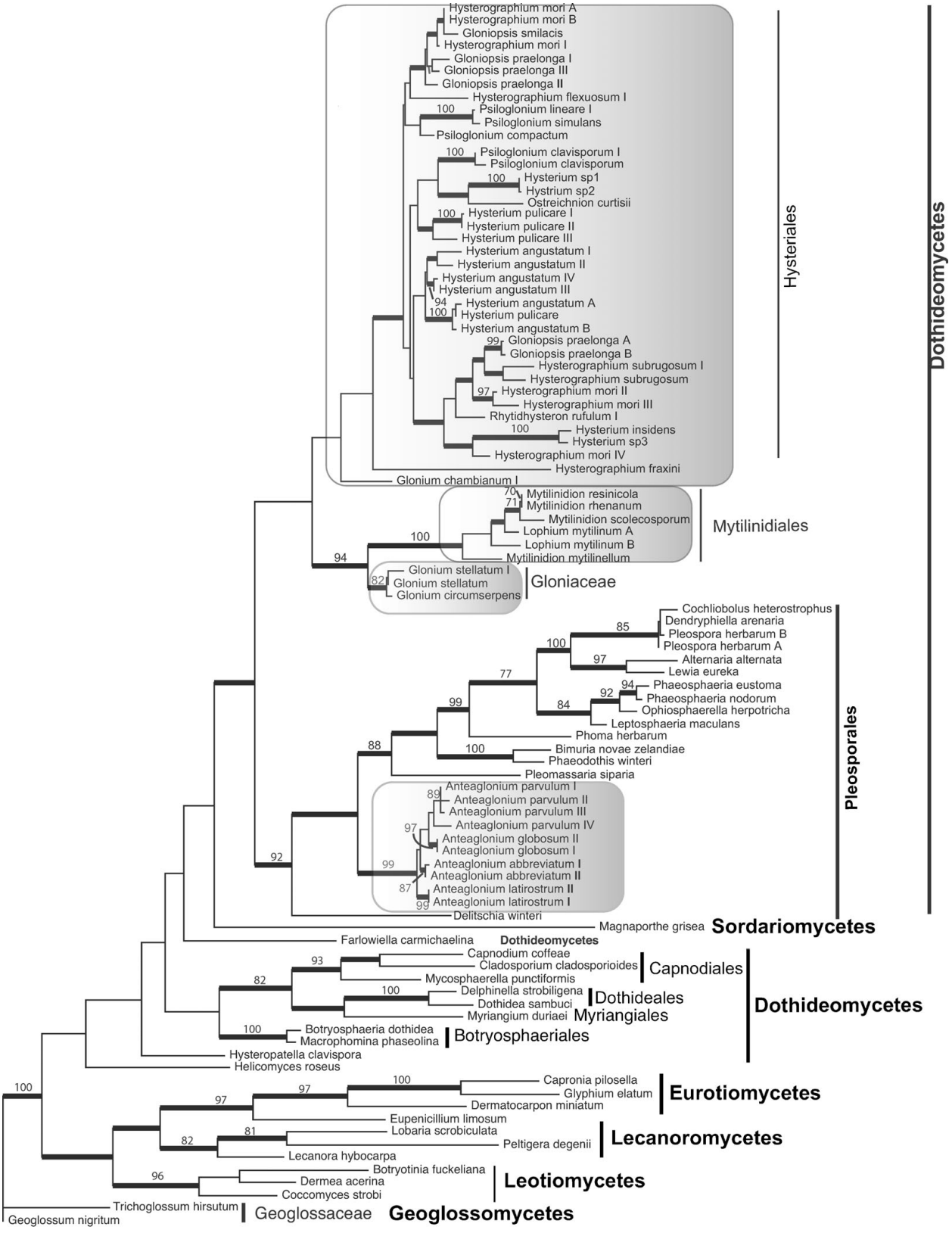

Figure 2 Phylogenetic tree obtained from Maximum Likelihood analysis based on combined nuclear ribosomal large subunit DNA and Translation elongation factor 1 alpha showing relationships between hysteriaceous fungi within the ascolocularous fungi. *Anteaglonium* is recovered as a new lineage within Pleosporales. Bootstrap support values ≥ 70% are shown above branches and Bayesian support ≥ 95% is indicated by thickened branches.

group receives in our molecular analyses (Fig. 2). The species present in this clade possess ascomata that are carbonaceous, either elongate or subglobose to globose and open by a longitudinal slit, while the asci are cylindric and ascospores fusiform to oblong. The phylogenetic position of *Anteaglonium* within Pleosporales is currently not resolved. In an additional analysis focusing on Pleosporales and using nuclear LSU sequence data (data not shown) it clustered with a group including species of *Ostropella* (Sacc.) Höhn. and *Xenolophium* Syd. However, this relationship was not supported.

Lohman (1937) synonymised *Anteaglonium parvulum* (= *Glonium parvulum*) with *A. abbreviatum* (= *G. abbreviatum*) in his study of *Glonium* in the southeastern USA. *Anteaglonium parvulum* shares a similar ascus and ascospore morphology with *A. abbreviatum*, but the two species differ in ascomatal characters (as discussed below) and molecular analyses based on taxa from wide geographic location (Table 1) are consistent with the distinction of these two taxa (Fig. 2). *Anteaglonium parvulum* differs from *A. abbreviatum* by having ascomata sitting on substratum lacking a dark crust, ascomata with acuminate ends and thus appearing fusiform with rounded ends. Ascomata apices are also largely acuminate in *A. parvulum* as opposed to almost flattened in *A. abbreviatum*. *Anteaglonium globosum* differs from *A. abbreviatum* and *A. parvulum* by having globose to subglobose ascomata, very rarely fusing to appear elongate and having roughened walls. Ascomata open by a slit that is often indistinct and sits on a dark crust that bears sparse, short, septate subiculum. The ascoma wall surface bears a sparse, brown, septate tomentum. It produces profuse greenish compounds in KOH, as opposed to *A. abbreviatum,* which produces some KOH pigment that can easily be missed, while *A. parvulum* lacks the KOH extractable compounds entirely. *Anteaglonium latirostrum* differs from all other species in the genus by possessing ascomata apices that are raised and laterally compressed. The ascospores in this species are also larger, 1–4 septate, turning pale brown with age and possessing a mucilaginous sheath.

Taxonomy

The placement of *Glonium abbreviatum* and its segregates outside Gloniaceae and nested within Pleosporales makes the description of a new genus necessary. The genus *Anteaglonium* Mugambi & Huhndorf gen. nov. includes *Anteaglonium abbreviatum* (Schwein.) Mugambi & Huhndorf comb. nov., *Anteaglonium parvulum* (W.R. Gerard) Mugambi & Huhndorf comb. nov., and two new species, one from Kenya and another from North America that are described below.

Anteaglonium Mugambi & Huhndorf, gen. nov.

Ascomata erumpentia ad superficialia, solitaria vel parva ad dense aggregata, globosa, subglobosa vel elongata, fusiformis ad oblonga, atrobrunnea ad anthracina in denigratus crusta interdum cum subiculo sparsus hyphis brunneis vel omnis absens. Aperiens longitudinalis rima, peridium laevis vel fretus, KOH extractus pigmentum praesens vel absens. Asci cylindrici, octospori, 1–2 seriatim. Ascosporae fusiformes ad oblongae, septatae, hyalinae vel pigmentae.

Ascomata erumpent to superficial, solitary or in small to large clusters, sitting on thin darkened crust with or without sparse dark brown septate subiculum or both lacking, globose to subglobose or elongate, fusiform to oblong, brown to shiny black, opening by pronounced or indistinct longitudinal slit running entire length of fruit body or apex raised and laterally compressed, wall smooth or finely longitudinally striate or roughened, tomentum present or lacking, KOH extractable pigments present or absent, fruiting body contents pigmented or not. Asci cylindrical, short pedicillate, 8-spored uniseriate or biseriate. Ascospores fusiform to oblong, septate, constricted at the primary septum, hyaline or pigmented.

Typus generis: *Anteaglonium abbreviatum* (Schwein.) Mugambi & Huhndorf.

Etymology: *Antea* (*lat.*) = formerly, meaning formerly belonging to *Glonium.*

The genus *Anteaglonium* can be distinguished from *Glonium* by its possession of smaller ascomata that are either elongate or globose and its small ascospores usually less that 8 μm long except in *A. latirostrum*. However, in the latter species the ascomata are globose often with raised laterally compressed apices, characters not present in *Glonium*.

Anteaglonium abbreviatum (Schwein.) Mugambi & Huhndorf, comb. nov.

≡ *Hysterium abbreviatum* Schwein., Trans. Am. phil. Soc., New Series 4(2): no. 2094. 1832. Basionym
≡ *Glonium abbreviatum* (Schwein.) M.L. Lohman, Bull. Torrey bot. Club 64: 64. 1937.

Anteaglonium globosum Mugambi & Huhndorf, sp. nov. (Fig. 3)

Ascomata superficialia, globosa ad subglobsa, raro elongata, 182–360 μm diam., 96–130 μm alta, anthracina, parva ad dense aggregata in denigrata crusta cum subiculo sparsus hyphis brunneis, aperiens longitudinalis rima. Peridium carbonarium cum sparsus atrobrunneum sepatum tomentum, 2–4 μm diam., KOH extractus pigmentum praesens. Pseudoparaphyses numerosae, 2–3 μm diam., septatae. Asci cylindracei, 48–56 × 5–6 μm, octospori. Ascosporae fusiformes, 1-septatae, constrictae, hyalinae, 6–7 × 2–3 μm.

Ascomata superficial, globose to subglobose, only rarely fused appearing elongate, 182–360 wide and 96–130 μm high, black to almost shiny black, occurring in small to large clusters always on black thin crust of carbonaceous material that possess a sparse, short, dark brown and septate subiculum. Opening usually by an inconspicuous slit that runs along the entire length of ascoma. Ascomatal wall carbonaceous, surface roughened, often with sparse short brown septate tomentum, 2–4 μm wide, inner walls composed of small pseudoparenchymatous cells, wall tissues produce profuse greenish KOH extractable pigment. Ascoma centrum hyaline, pseudoparaphyses thin, 2–3 μm wide, septate, numerous, branching and fusing profusely above the asci. Asci cylindrical, short pedicillate, 48–56 × 5–6 μm, 8-spored obliquely to irregularly uniseriate. Ascospores fusiform with

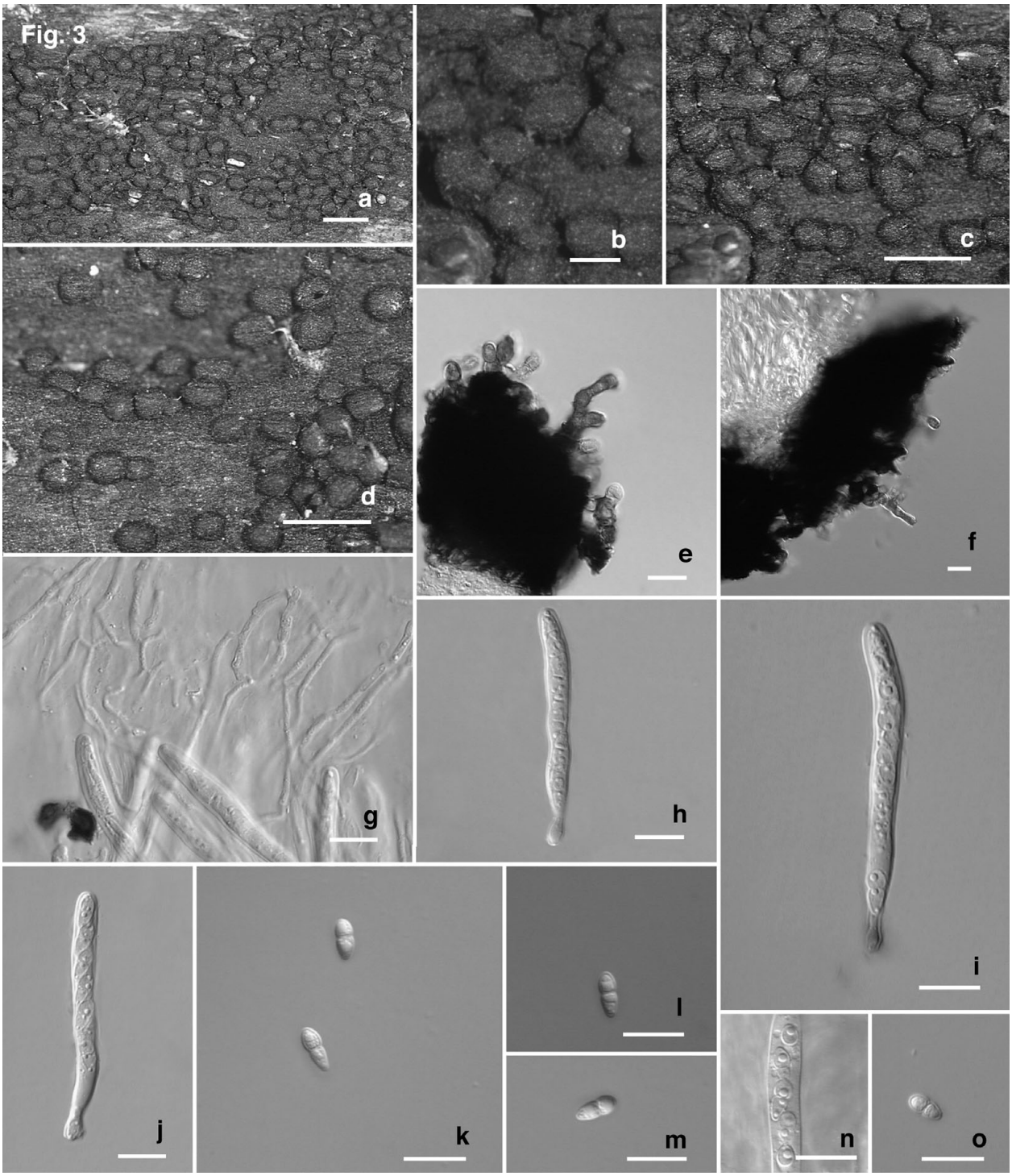

Figure 3 *Anteaglonium globosum*. a–d, Ascomata on the substrate. e, f, Ascomal wall and setae. g, Pseudoparaphyses. h–j, Ascus. k–o, Ascospores. Bar lines: a, c, d = 500 μm; b = 300 μm; f = 20 μm; g–o = 10 μm. Figs from A. Miller 925.2 (F holotype).

rounded ends, 1-septate, constricted at septum, lower cell usually narrower, hyaline, mucilaginous sheath lacking, 6–7 × 2–3 μm. Anamorph not known.

Etymology: *Globosus* (*lat.*) = rounded, referring to the shape of the ascomata.

Typus: USA: Tennessee, Sevier Co., Great Smoky Mountains National Park, 22 May 2006, A. Miller 925.2 (F-holotype).

Habitat: On decorticated woody branches on the ground, in forested areas.

Known distribution: So far only known from eastern and midwestern North America.

Additional specimen examined: USA: Indiana, Porter Co., Indiana Dunes State Park, 23 August 2008, S. Huhndorf 5283 (F).

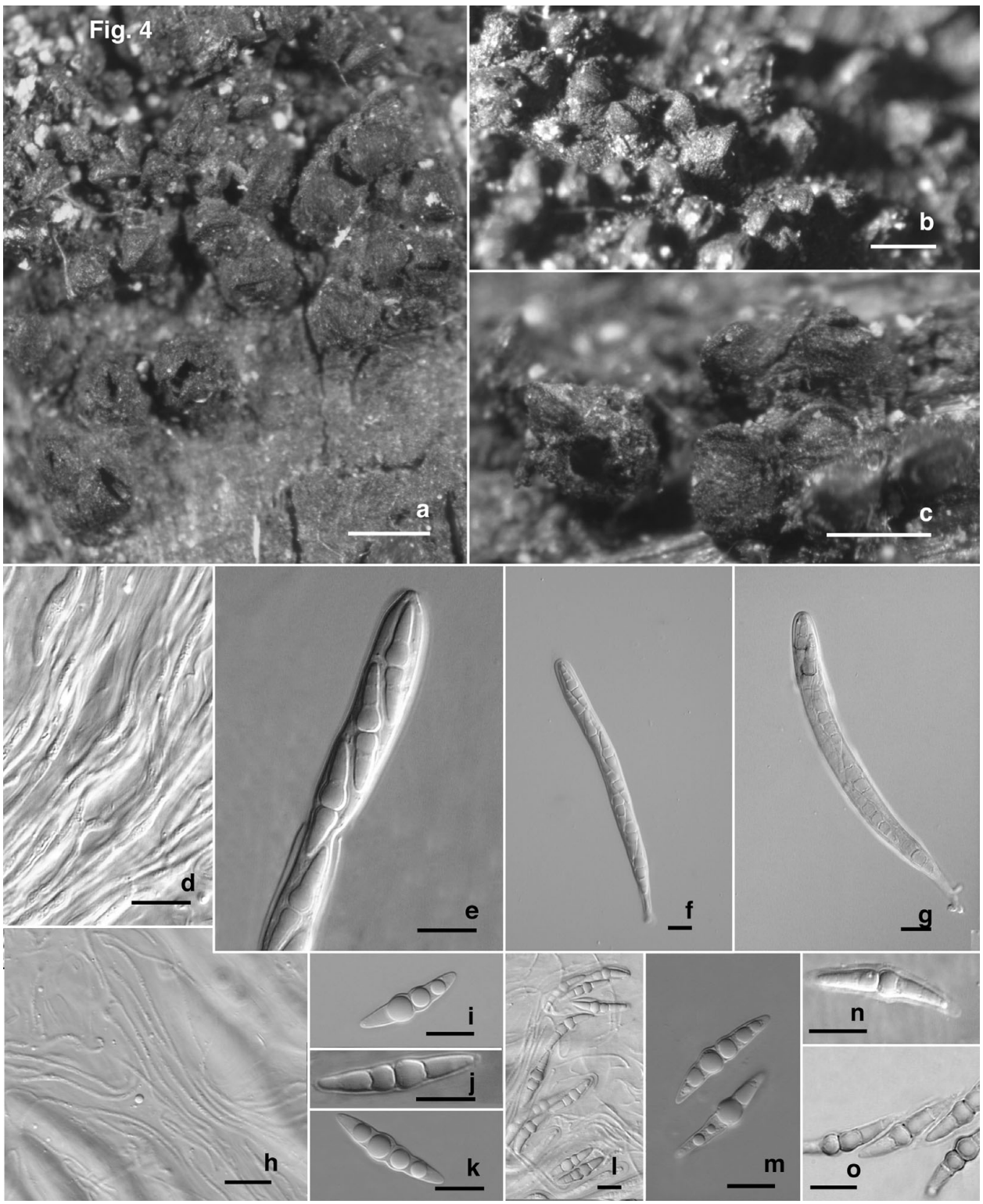

Figure 4 *Anteaglonium latirostrum*. a–c, Ascomata on the substrate. d, h, Pseudoparaphyses. e–g, Ascus. i–o, Ascospores. Bar lines: a–c = 400 μm; d–o = 10 μm. Figs from G. Mugambi 1119 (EA holotype).

Anteaglonium parvulum (W.R. Gerard) Mugambi & Huhndorf, comb. nov.

≡ Hysterium parvulum W.R. Gerard, *Bull. Torrey bot. Club* 5: 40. 1874. Basionym.
≡ *Glonium parvulum* (W.R. Gerard) Sacc., Syll., 2: 735. 1883.

Anteaglonium latirostrum Mugambi & Huhndorf, sp. nov. (Fig. 4)

Ascomata erumpentia ad superficialia, atrobrunnea, globosa ad subglobosa, solitares vel aggregata in denigrata crusta vel absens, 314–412 μm diam., 414–461 μm alta, aperiens

longitudinalis rima, interdum apicem lateralis compressus. Peridium carbonarium, pseudoparaphyses numerosae, ramosae, septatae. Asci cylindrici, octospori, partim duo seriatim, 115–124 × 9–10 μm. Ascosporae fusiformes, hyalinae, interdum atrobrunneae, 1–4 septatae, constrictae, habens mucosae vaginae, 22–28 × 4–6 μm.

Ascomata erumpent to superficial, brown to dark brown almost appearing black, globose to subglobose, aggregated or solitary, growing on thin darkened crust or crust lacking, 414–461 μm high and 314–412 μm wide, opening by a slit that run over the entire length of fruiting body or apex slightly to significantly raised and laterally compressed, ascomatal wall smooth and carbonaceous, ascoma centrum sometimes appearing pinkish in colour, with branched, septate, numerous thin pseudoparaphyses that are longer than asci. Asci cylindrical, short pedicillate, 8-spored partially biseriate, 115–124 × 9–10 μm. Ascospores fusiform, hyaline often becoming pale brown at maturity, 1–4 septate, constricted at the primary septum, usually with up to four large guttules, with mucilaginous sheath which often disappears at maturity, 22–28 × 4–6 μm. Anamorph not known.

Etymology: *Late* (*lat.*) = broadly and *rostrum* (*lat.*) = beak, describing the flattened apices of the ascomata.

Typus: Kenya: Rift Valley province, Kajiado district, Ngong hills, 16 June 2005, G. Mugambi 1119 (EA-holotype, Isotype-F).

Habitat: On decorticated woody substrate on the ground in tropical moist and dry highland forests.

Known distribution: So far only known from two forests in Kenya.

Additional specimen examined: Kenya: Coast Province, Taita Taveta district, Taita hills, Ngangao forest, 12 April 2005, G. Mugambi L101N (EA); Taita Taveta district, Taita hills, Ngangao forest, 12 April 2005, G. Mugambi L100N.2 (EA).

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