Mycol. Res. **107** (3): 291–296 (March 2003). © The British Mycological Society
DOI: 10.1017/S0953756203007408 *Printed in the United Kingdom.*

Molecular characterisation of *Armillaria* species from Zimbabwe

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Received 22 August 2002; accepted 21 January 2003.

Armillaria species are amongst the most important pathogens of trees and have a world-wide distribution. In recent years, the taxonomy of Northern Hemisphere *Armillaria* spp. has been extensively treated, but those occurring in Africa are poorly known. Previously, isolates of *Armillaria* from Zimbabwe have been grouped based on morphology and biochemical tests. In this study, six isolates representing the three previously characterized groups of *Armillaria* spp. occurring in Zimbabwe were analysed using DNA-based techniques. Three distinct clusters emerged from both PCR–RFLP and analysis of sequence data for the IGS-1 rRNA operon. The three groups corresponded to those previously identified based on morphology and biochemical tests. Differences in IGS-1 sequences strongly suggest that the Zimbabwean groups represent three distinct taxa. Isolates belonging to Group I, previously assumed to be to *A. heimii*, were similar to those identified as *A. fuscipes* from South Africa and La Reunion. Group II isolates resided in a clade apart from all other isolates and appear to represent *A. heimii*. The remaining isolates residing in Group III clustered with isolates from Zambia and Cameroon. These are different from *A. heimii* and *A. fuscipes* and apparently represent an undescribed taxon.

INTRODUCTION

The genus *Armillaria* has a world-wide distribution and includes some of the most important root pathogens of forest trees and fruit crops. The taxonomy of *Armillaria* has presented problems for many years due to the assumption that *A. mellea* is a single polymorphic species. This has lead to the grouping of many of the currently recognized *Armillaria* spp. within *A. mellea s. lat*. The re-classification of the genus, especially in Europe, where several species are known to occur, is based mainly on mating tests (Korhonen 1978). As a result, at least five biological and morphologically distinct species, previously thought to be *A. mellea*, have been described in Europe (Korhonen 1978, Pegler 2000). Application of mating tests have further led to the recognition of at least nine biological species in North America (Anderson & Ullrich 1979, Anderson 1986, Volk & Burdsall 1995) and at least ten biological species in Asia (Ota *et al*. 1998). Several species of *Armillaria* are known to occur in Australia and New Zealand and these have been reasonably well studied (Pegler *et al*. 1978, Kile & Watling 1981, 1983, 1988). However, very little is known regarding the taxonomy of species of *Armillaria* in Africa.

In one of the early studies aimed at identifying *Armillaria* spp. from Africa, Pegler (1977) recognized two species, *A. heimii* and *A. mellea*. Subsequent to this study, it was generally assumed that *A. heimii* is the major species in Africa (Mohammed *et al*. 1994). A number of studies on *Armillaria* in Africa have followed and based on morphological variation in culture, physiological, biochemical and somatic compatibility tests, four groups have been recognized. These groups are thought to represent *A. mellea* (African), *A. heimii* and two groups for which names have not been provided (Mohammed *et al*. 1994, Abomo-Ndongo & Guillaumin 1997, Chillali *et al*. 1997, Mwenje & Ride 1997). In addition to these, Coetzee *et al*. (2000) have recently shown that *A. fuscipes*, previously thought to be synonymous with *A. heimii*, is a distinct and common species in South African pine plantations.

A. heimii has been presumed to be the predominant species in Zimbabwe (Mohammed *et al*. 1994). Using pectic isozymes, especially pectic lyase patterns,

Table 1. *Armillaria* isolates from Zimbabwe and South Africa used in IGS-1 analysis.

Group*/ species	Isolate no.	Country/area of origin	Host	GenBank® accession no.
Group I	CMW10165	Zimbabwe, Chimanimani	*Prunus persica*	AF489482
Group I	CMW4874	Zimbabwe, Stapleford	*Araucaria cunninghamii*	AF489481
Group II	CWM4455	Zimbabwe, Eastern Highlands Estates	*Camellia sinensis*	AF489486
Group II	CMW4456	Zimbabwe, Stapleford	*Brachystegia utilis*	AF489485
Group III	CMW10115	Zimbabwe, Harare	*Acia albida*	AF489483
Group III	CMW10116	Zimbabwe, Harare	*Newtonia buchananii*	AF489484
A. fuscipes	CMW2717	South Africa, Sabie	*P. elliottii*	
A. fuscipes	CMW2740	South Africa, Entabeni	*P. elliottii*	
A. fuscipes	CMW3167	South Africa, Sabie	*P. elliottii*	
A. heimii	CMW3173	Zambia, Dola Hill	*Tectona grandis*	
A. heimii	CWM3152	Cameroon, Western Highlands	Unknown	
A. fuscipes	CMW3164	La Reunion, Saint-Denis	*Pelargonium asperum*	
A. heimii	CMW3955	Zimbabwe, Harare	*Acacia xanthophloea*	

* Groups represent those defined by Mwenje & Ride (1996).

Mwenje & Ride (1996) showed that *Armillaria* isolates from Zimbabwe reside in three biochemically distinct groups (Groups I–III). Isozyme analysis of other enzymes such as beta-1,3 glucanases, non-specific esterases and putative suberinases further supported separation of the Zimbabwean isolates into these three groups (Mwenje & Ride 1996). In addition to these studies, Mwenje & Ride (1996) found that the isolates from Zimbabwe could be differentiated into the three groups based on their colony morphology, characteristics of their rhizomorphs and morphology of basidiocarps produced *in vitro* (Mwenje & Ride 1996). Isolates representing the three Zimbabwean *Armillaria* groups have been shown to differ in their pathogenicity on cassava (*Monihot esculenta*). Group III isolates were the most pathogenic and also have the widest distribution in the country (Mwenje, Ride & Pearce 1998). The differences in morphology, isozymes and pathogenicity among the various Zimbabwean groups suggested that they could represent distinct species.

In recent years *Armillaria* root rot has increased in importance in fruit and pine seed orchards in the eastern highlands of Zimbabwe. While death caused by *Armillaria* in Zimbabwe varies between 1 and 25% in commercial plantations, higher figures (60%) have been reported in fruit orchards, especially in *Prunus* spp. (Mwenje *et al*. 1998). Although some information is available on the distribution and pathogenicity of *Armillaria* in Zimbabwe (Mwenje *et al*. 1998), the identity of the species of *Armillaria* remains unknown.

The recent discovery of *A. fuscipes* in South Africa (Coetzee *et al*. 2000) and the availability of DNA sequence data for a collection of *Armillaria* spp. from southern Africa has provided the justification to reconsider the taxonomy of the three groups of isolates described from Zimbabwe. Since South Africa is a close neighbour of Zimbabwe, it was hypothesized that species composition in these two countries would be similar. A major limitation, however, in the classification of *Armillaria* in Zimbabwe is the rare occurrence of fruiting bodies (Swift 1968, Mwenje & Ride 1996). Although basidiocarps have been encountered in the wild (Swift 1968, Mwenje & Ride 1996), they are scarce, especially in years of low rainfall. Furthermore, the African taxa were previously shown to have a homothallic system (Abomo-Ndongo, Mohammed & Guillaumin 1997), making sexual compatibility tests with tester strains impossible. As a result of these problems, we were prompted to use a DNA-based identification procedure to identify isolates from Zimbabwe. The aim of this study was, therefore, to consider the identity of isolates residing in the three groups previously identified in Zimbabwe. This was achieved by comparing their restriction fragment length polymorphism (RFLP) and nucleotide sequences of the intergenic spacer region one (IGS-1) region of the rRNA operon with one another and with sequences published by Coetzee *et al*. (2000).

MATERIALS AND METHODS

Origin of isolates

The identity and geographical origin of the isolates used in this study are given in Table 1. The six Zimbabwean isolates were chosen specifically to represent equal numbers of the three groups previously defined based on their morphological and biochemical characteristics (Mwenje & Ride 1996). The Zimbabwean isolates are maintained in the *Armillaria* culture collection at the National University of Science and Technology in Bulawayo, Zimbabwe as well as the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Two *Armillaria* isolates from South Africa representing *A. fuscipes* were from the culture collection (CMW) of the FABI. The *Armillaria* isolates from South Africa were previously identified based on their basidiocarp morphology as well as their IGS-1 sequences and RFLP profiles. Isolates were maintained on 2% malt extract agar (MEA) slants and stored at 4 °C.

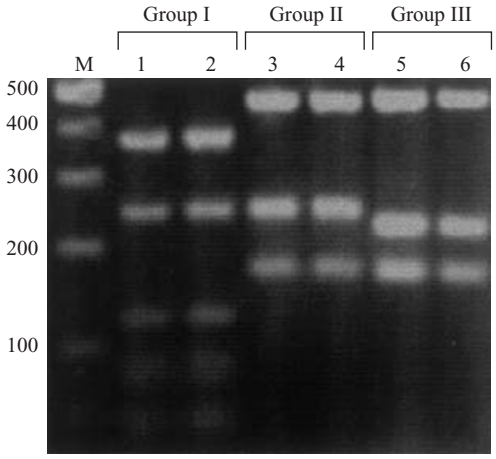

Fig. 1. A 3% agarose gel showing *Alu*I restriction fragments for *Armillaria* isolates used in this study. Lanes 1–6 show profiles for isolates CMW10165 (1), CMW04874 (2), CMW4455 (3), CMW4456 (4), CMW10115 (5) and CMW10116 (6). Lanes labelled with M indicates a 100 bp molecular marker with band sizes in base pairs.

Mycelium production and DNA preparation

Cultures were grown in the dark under stationary conditions at 26 ° in 250 ml conical flasks containing 50 ml liquid MY medium (3% malt extract and 0.3% yeast extract). Mycelium was harvested after 3 wk by filtration using Whatman no. 1 filter paper. The mycelium was freeze-dried and ground to a fine powder. DNA extractions from the powdered mycelium were achieved using the protocols of O'Donnell, Cigelnik & Nirenberg (1998). The DNA pellets were rehydrated in 50 µl of ddH_2O and stored at −20 ° until further use.

Polymerase Chain Reaction (PCR) and RFLP analyses

PCR

The first intergenic spacer region (IGS-1) between the 3′ end of the large subunit (LSU) ribosomal RNA (rRNA) gene and the 5′ end of the 5S gene of *Armillaria* isolates was amplified using PCR. Amplicons were obtained with primer pair P-1 (Hsiau 1996) and 5S-2B (5′-CAC CGC ATC CCG TCT GAT CTG CG-3′) as used by Coetzee *et al*. (2000) for amplification of *Armillaria* isolates from South Africa. The PCR reaction mixture included *Taq* polymerase (1.75 U) (Roche Diagnostics, Randburg), PCR buffer with $MgCl_2$ supplied by the manufacturer, additional $MgCl_2$ (0.25 mM), dNTPs (1 mM), primers (0.01 µM of each), and approximately 80 ng of template DNA. PCR conditions were an initial denaturation at 94 ° for 3 min followed by 35 cycles of denaturation (30 s, 94 °), primer annealing (30 s, 58 °), and elongation (1 min, 72 °). A final step of 7 min at 72 °C was allowed for complete elongation of the amplicons. PCR products were electrophoresed on an ethidium bromide stained agarose gel (1%) and visualized under UV illumination.

RFLP

IGS-1 amplicons obtained for the isolates from Zimbabwe were digested without prior purification as described by Harrington & Wingfield (1995). Restriction profiles were obtained by digesting 18 µl of the PCR product with the restriction endonuclease *Alu* I (20 U) for 5–7 h at 37 °. The resulting PCR–RFLP fragments were separated on a 3% agarose gel stained with ethidium bromide and visualized under UV illumination. Fragments smaller than 100 bp (base pairs) were not considered due to their low visibility. RFLP fragment sizes were determined and compared with those previously published for African *Armillaria* species (Coetzee *et al*. 2000).

DNA sequencing

IGS-1 sequences for six *Armillaria* isolates representing the three *Armillaria* groups from Zimbabwe (Table 1) were determined using an ABI PRISM™ 377 automated DNA sequencer. Sequence reactions were carried out using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase FS (Applied Biosystems, Johannesburg) according to the specification of the manufacturers. The IGS-1 region was sequenced in both directions using primers P-1, 5S-2B, MCP-2, MCP-2R, MCP-3, MCP-3R, 5S-3MC, 5S-3MCR, MCP-2A, MCP-2AR, 5S-5MC, and 5S-5MCR (Coetzee *et al*. 2000).

Sequence analyses

Sequence data obtained for the six *Armillaria* isolates from Zimbabwe were aligned with alignments of Coetzee *et al*. (2000) available in TreeBase (matrix number: M859, study number: S566). Sequence alignments in poorly aligned regions, resulting from large indels, were manually optimized. Indels of two or more bases were treated as a single evolutionary event by a multi state coding system. The relationships between the isolates were determined by distance analysis in PAUP* version 4 (Swofford 1998) using mean character differences to accommodate coded indels. Trees were constructed using the Neighbour-Joining tree building algorithm (Saitou & Nei 1987). Outgroups were not available for the data set, and trees were therefore rooted to midpoint. Support for the differentiation between Group II and Group III isolates was obtained by using Group I as the outgroup in bootstrap analysis. The sequence matrix and neighbour-joining tree generated in this study has been deposited in TreeBASE (study accession no. S840, matrix accession no. M1357).

RESULTS

Amplification of IGS-1 and PCR–RFLPs

PCR amplication of the IGS-1 region of *Armillaria* isolates resulted in amplicons of different sizes. Isolates

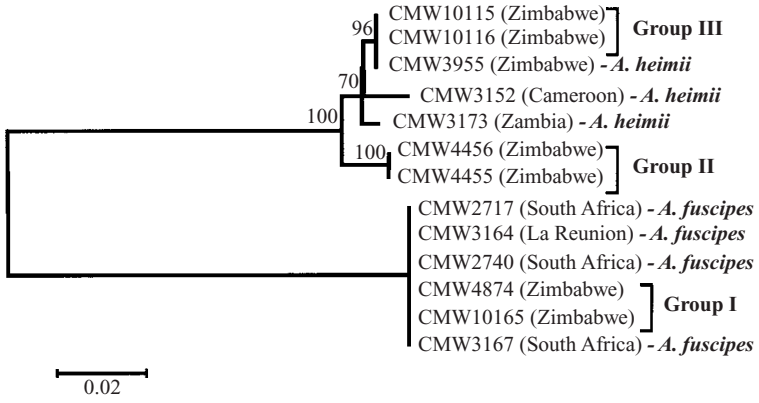

Fig. 2. Phylogram generated with the neigbour-joining tree building algorithm in PAUP*. Bar = total nucleotide differences between taxa. Bootstrap values (%) are indicated above the branches.

representing Zimbabwean Group I (CMW10165 and CMW04874) gave a PCR fragment approximately 1200 bp in size. Group II (CMW4455 and CMW4456) and Group III (CMW10115 and CMW10116) isolates resulted in an amplified fragment size of approximately 900 bp.

RFLP profiles obtained after digestion of amplicons with the restriction enzyme *Alu*1 differed for all three of the previously defined Zimbabwean groups of isolates (Fig. 1). The two isolates chosen to represent Group I (CMW10165 and CMW4874) had similar RFLP patterns with fragments of approximately 380, 255 and 130 bp. Isolates representing Group II exhibited a distinct pattern with fragment sizes of approximately 480, 255 and 175 bp. Group III isolates had an RFLP pattern with fragment sizes of approximately 480, 230 and 175 bp. No differences were observed in RFLP profiles for isolates within the same group and groups could thus be easily separated using this technique.

DNA sequence analysis

The IGS-1 data set included a total of 1191 characters after alignment with inclusion of gaps. Forty-eight indels were replaced with multi-state characters yielding a total of 715 characters that were used in a distance analysis. The absolute lengths of the IGS-1 sequences determined for the isolates from Zimbabwe were compared with the aligned sequences from Coetzee *et al.* (2000) after exclusion of 50 characters from the 3′ end of the IGS-1 sequence matrix published by Coetzee *et al.* (2000). In this study the IGS-1 region sequenced for isolates of Group I (CMW10165 and CMW4874) was the same as that for isolates from South Africa (CMW2717 and CMW2740 and CMW3167) and La Reunion (CMW3164) and ranged from 1041 to 1044 bp. Isolates representing Zimbabwean Group II (CMW4456 and CMW4455), Group III (CMW10115 and CMW10116) and a previously sequenced Zimbabwean isolate CMW3955 (Coetzee *et al.* 2000) had an absolute length of 832 bp. Isolates from Cameroon and Zambia had absolute lengths of 816 and 803 bp, respectively.

DNA sequence analysis using the neighbour-joining analysis, grouped isolates into three major clusters that were consistent with those previously defined based on biochemical and morphological characteristics (Fig. 2). *Armillaria* isolates belonging to Group I grouped with *A. fuscipes* from South Africa (CMW2717 and CMW2740 and CMW3167) and La Reunion (CMW3164). Group II isolates (CMW4455 and CMW4456) grouped together and were separate from those representing Group III and these were not close to any other previously named species of *Armillaria*. Isolates from Zambia (CMW3173), Cameroon (CMW3152) and Zimbabwe (CMW3955) for which sequence data were available grouped with isolates of Group III (CMW10115 and CMW10116) (Fig. 2). Of these isolates, the previously sequenced Zambabwean isolate (Coetzee *et al.* 2000) was more closely related to the Group III isolates than to the isolate from Cameroon, previously thought to represent *A. heimii*.

DISCUSSION

This study confirms previous investigations (Swift 1968, 1972, Mwenje & Ride 1996) in which *Armillaria* isolates from Zimbabwe were shown to represent more than one taxon. In addition, we have shown that the three groups, defined based on isozyme differences by Mwenje & Ride (1996), can also be clearly separated on the basis of RFLP and DNA sequence differences. With these additional data, we believe that there is sufficient evidence to accept that the three groups represent distinct species of *Armillaria*. Of these, one group appears to represent *A. fuscipes*, one is close to what is thought to be *A. heimii* and one represents an undescribed species.

Group I isolates from Zimbabwe were identified as *A. fuscipes* based on their IGS-1 DNA sequence similarity with this species. The fact that this species is reflected in one of the three groups of *Armillaria* defined by Mwenje & Ride (1996) is not surprising, since this species was recently described from South African forest plantations (Coetzee *et al.* 2000). *A. fuscipes* was

first described by Petch (1909) from Sri Lanka and was thought to be introduced from West Africa (Pegler 1986). Pegler (1986), as well as Kile & Watling (1988), considered *A. fuscipes* and *A. heimii* to be conspecific and the name *A. heimii* has been used by various authors for the African taxon. Coetzee *et al.* (2000), however, showed that there is a clear difference at the molecular and morphological level between *A. fuscipes* and *A. heimii* and that they should be treated as separate taxa. The fact that isolates previously thought to represent *A. heimii* (Mwenje & Ride 1997) are most likely *A. fuscipes*, suggests that the latter species is the more widely distributed.

Armillaria isolates representing Group I and now recognized as *A. fuscipes*, have been found in forest plantations and fruit tree orchards as well as in indigenous woodlands in Zimbabwe (Mwenje & Ride 1996, Mwenje *et al.* 1998). Group III isolates have the widest distribution and this fungus is thought to be the most pathogenic (Mwenje *et al.* 1998). This suggests that *A. fuscipes* is not the most important pathogen amongst *Armillaria* spp. in Zimbabwe.

IGS-1 DNA sequence analyses of isolates representing Zimbabwean groups II and III revealed a high number of differences in the presence of indels between the two groups. Mwenje & Ride (1996) indicated that groups II and III can be separated based on their cultural and biological characterstics as well the as morphology of *in vitro* produced basidiocarps. Results obtained from RFLP and IGS-1 DNA sequence data analyses, in the present study, support the separation of Group II and III isolates and therefore, support those of Mwenje & Ride (1996). The differences found between the groups II and III from Zimbabwe, by Mwenje & Ride (1996) and in this study, suggest that they could represent two distinct species.

In this study, isolates of *Armillaria* representing Group III formed a monophyletic group with isolates from Cameroon, Zambia and Zimbabwe, thought to represent *A. heimii* (Coetzee *et al.* 2000). Basidiomes of *Armillaria* spp. are rare in Zimbabwe and only those linked to Group III were previously found and described in detail from this country (Mwenje & Ride 1996). The morphology of these fruit bodies, however, differed from *A. heimii* in all characteristics (Mwenje & Ride 1996) and also from those of *A. fuscipes* described in South Africa (Coetzee *et al.* 2000). A possible explanation for this contradiction is that cultures that have been treated as *A. heimii*, do not reflect this species. Resolution of this question will be difficult as it will require collections of isolates from fruit bodies in the area where *A. heimii* was first described.

Isolates representing Group II are significantly different to *A. heimii* based on IGS-1 sequence data. Identification of isolates residing in this group is currently not possible. Since the onset of studies on *Armillaria* in Zimbabwe in 1989, over 60 isolates of this fungus have been collected in native forests and in plantations (unpublished data). Of particular interest is the fact that only two of these belong to Group II. These collections were primarily from diseased trees and support our view that the fungus is relatively unimportant as a pathogen. It is hoped that in the future, basidiocarps representing Group II isolates will be found so that this fungus can be provided with a name.

ACKNOWLEDGEMENTS

We thank the members of the Tree Pathology Co-operative Programme (TPCP), the Mellon Foundation, the National Research Foundation (NRF) and the THRIP initiative of the Department of Trade and Industry (DTI) South Africa as well as UNESCO-ANSTI and the Southern African Regional Co-operation in Biochemistry, Molecular Biology and Biotechnology (SARBIO) for financial support. We also acknowledge efforts of Julia A. Hasler (SARBIO Project Coordinator) to promote research collaboration in Southern Africa and without which the senior author would not have been able to undertake this study.

REFERENCES

Abomo-Ndongo, S. & Guilaumin, J.-J. (1997) Somatic compatibility among African *Armillaria* isolates. *European Journal of Forest Pathology* **27**: 201–206.

Abomo-Ndongo, S., Mohammed, C. & Guillaumin, J.-J. (1997) Sexual behaviour of *Armillaria heimii* and *A. mellea* isolates from Africa. *European Journal of Forest Pathology* **27**: 207–224.

Anderson, J. B. (1986) Biological species of *Armillaria* in North America: redesignation of groups IV and VIII and enumeration of voucher strains for other groups. *Mycologia* **78**: 837–839.

Anderson, J. B. & Ullrich, R. C. (1979) Biological species of *Armillaria* in North America. *Mycologia* **71**: 402–414.

Chillali, M., Idder-Ighili, H., Guillaumin, J.-J., Mohammed, C. & Botton, B. (1997) Species delimitation in the African *Armillaria* complex by analysis of the ribosomal DNA spacers. *Journal of General and Applied Microbiology* **43**: 23–29.

Coetzee, M. P. A., Wingfield, B. D., Coutinho, T. A. & Wingfield, M. J. (2000) Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa. *Mycologia* **92**: 777–785.

Harrington, T. C. & Wingfield, B. D. (1995) A PCR based identification method for species of *Armillaria*. *Mycologia* **87**: 280–288.

Hsiau, P. T. W. (1996) *The taxonomy and phylogeny of the mycangial fungi from* Dendroctonus brevicomis *and* D. frontalis (*Coleoptera: Scolytidae*). PhD Thesis. Iowa State University. 92 p.

Kile, G. A. & Watling, R. (1981) An expanded concept of *Armillaria luteobubalina*. *Transactions of the British Mycological Society* **77**: 75–83.

Kile, G. A. & Watling, R. (1983) *Armillaria* species for South-Eastern Australia. *Transactions of the British Mycological Society* **81**: 129–140.

Kile, G. A. & Watling, R. (1988) Identification and occurrence of Australian *Armillaria* species, including *A. pallidula* sp. nov. and comparative studies between them and non-Australian tropical and Indian *Armillaria*. *Transactions of the British Mycological Society* **91**: 305–315.

Korhonen, K. (1978) Interfertility and clonal size in the *Armillaria mellea* complex. *Karstenia* **18**: 31–42.

Mohammed, C., Guillaumin, J.-J., Botton, B. & Intini, M. (1994) Species of *Armillaria* in tropical Africa. In *Proceedings of the 8th International Conference on Root and Butt Rots IUFRO Working Party S750.07* (M. Johansson & J. Stenlid, eds): 402–410. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Mwenje, E. & Ride, J. P. (1996) Morphological and biochemical characterisation of *Armillaria* isolates from Zimbabwe. *Plant Pathology* **45**: 1031–1051.

Mwenje, E. & Ride, J. P. (1997) The use of pectic enzymes in the characterisation of *Armillaria* isolates in Africa. *Plant Pathology* **46**: 341–354.

Mwenje, E., Ride, J. P. & Pearce, R. B. (1998) Distribution of Zimbabwean *Armillaria* groups and their pathogenicity on cassava. *Plant Pathology* **47**: 623–634.

O'Donnell, K., Cigelnik, E. & Nirenberg, H. I. (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* **90**: 465–493.

Ota, Y., Matsushita, N., Nagasawa, E., Terashita, T., Fukuda, K. & Suzuki, K. (1998) Biological species of *Armillaria* in Japan. *Plant Disease* **82**: 537–543.

Pegler, D. N. (1977) *A Preliminary Agaric Flora of East Africa*. [Kew Bulletin Additional Series No. 6.] HMSO, London.

Pegler, D. N. (1986) *Agaric Flora of Sri Lanka* (*Armillaria*). [Kew Bulletin Additional Series No. 12.] HMSO, London.

Pegler, D. N. (2000) Taxonomy, nomenclature and description of *Armillaria*. In *Armillaria Root Rot: biology and control of honey fungus* (R. T. V. Fox, ed.): 81–93. Intercept, Andover.

Petch, T. (1909) New Ceylon fungi. *Annuals of the Royal Botanical Garden Peradeniya* **4**: 299–307.

Podger, F. D., Kile, G. A., Watling, R. & Fryer, J. (1978) Spread and effect of *Armillaria luteobubalina* sp. nov. in an Australian *Eucalyptus regnans* plantation. *Transactions of the British Mycological Society* **71**: 77–87.

Saitou, N. & Nei, M. (1987) The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.

Swift, M. J. (1968) Inhibition of rhizomorph development by *Armillaria mellea* in Rhodesian forest soils. *Transactions of the British Mycological Society* **51**: 241–247.

Swift, M. J. (1972) The ecology of *Armillaria mellea* (Vahl ex Fries) in the indigenous and exotic woodlands of Rhodesia. *Forestry* **45**: 67–86.

Swofford, D. L. (1998) *PAUP*: Phylogenetic Analysis Using Parsimony* (**and Other Methods*). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Volk, T. J. & Burdsall, H. H. (1995) *A nomenclatural study of Armillaria and Armillariella species*. [Synopsis Fungorum No. 8.] Fungiflora, Oslo.

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