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Phylogenetic relationships of Australian and New Zealand *Armillaria* species

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Abstract: *Armillaria* species cause Armillaria root rot on a wide range of plant species throughout the world. Based on morphology and sexual compatibility, various species of *Armillaria* have been reported from Australia and New Zealand. These include *A. hinnulea, A. fumosa, A. pallidula, A. novae-zelandiae* and *A. luteobubalina* from Australia. In New Zealand, *A. limonea, A. novae-zelandiae, A. hinnulea* and a fourth undescribed but morphologically distinct species are recognized. To determine the phylogenetic relationships between *Armillaria* spp. from Australia and New Zealand, the ITS region (ITS 1, 5.8S rRNA gene and ITS2) of the rRNA operon was amplified and the DNA sequences determined for a collection of isolates. The ITS sequences of *A. ostoyae* (from USA) and *A. sinapina* (from USA) were included for comparison. Phylogenetic trees were generated using parsimony analysis. *Armillaria hinnulea* was found to be more closely related to *Armillaria* spp. occurring in the Northern Hemisphere than it was to the other Australian and New Zealand species. The remainder of the Australian and New Zealand *Armillaria* spp. included in this study formed a monophyletic clade and confirmed separation of species based on morphology and sexual compatibility.

Key Words: *Armillaria*, evolution, ITS, phylogeny

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INTRODUCTION

Armillaria (Fr.: Fr) Staude (Staude 1857) (Basidiomycetes, Agaricales, Tricholomataceae) species are among the most important phytopathogenic fungi. Species of *Armillaria* are necrotrophic pathogens on a large variety of plant hosts (Raabe 1962, Watling et al 1991) and mycotrophic associates of achlorophyllous plants (Watling et al 1991). Members of this genus occur worldwide and can be found in boreal, temperate and tropical regions of the world, in plantations, parks, gardens and natural forests (Hood et al 1991, Kile et al 1991).

More than 39 different morphologically and biologically defined species of *Armillaria* are known (Volk and Burdsall 1995). Morphological studies of basidiocarps identified five different *Armillaria* spp. in Australia and three species in New Zealand (Kile and Watling 1983, 1988). The five species in Australia are: *A. fumosa* Kile & Watl. (Kile and Watling 1983), *A. hinnulea* Kile & Watl. (Kile and Watling 1983), *A. novae-zelandiae* (Stev.) Herink (Stevenson 1964, Herink 1973), *A. pallidula* Kile & Watl. (Kile and Watling 1988), and *A. luteobubalina* Watl. & Kile (Podger et al 1978). *Armillaria novae-zelandiae, A. limonea* (G. Stev.) Boesewinkel (Boesewinkel 1977), *A. hinnulea*, and a fourth morphologically distinct but unnamed species are found in New Zealand (Watling et al 1991, Hood 1992, GS Ridley unpubl). Kile and Watling (1988) showed that isolates representing these morphologically distinct *Armillaria* spp. are sexually incompatible with one another and thus represent different biological species.

Northern Hemisphere *Armillaria* spp. have been well studied at the molecular level (Anderson et al 1987, 1989, Smith and Anderson 1989, Harrington and Wingfield 1995, Volk et al 1996). Several phylogenetic studies were conducted on African, North American, European and Asian *Armillaria* spp. (Anderson and Stasovski 1992, Terashima et al 1998, Chillali et al 1998, Coetzee et al 2000a, b) using internal transcribed spacer regions (ITS1 and ITS2)

and the first intergenic spacer region (IGS-1) sequence data as well as regions generated through sequencing with arbitrary primed primers (SWAPP) PCR (Piercey-Normore et al 1998). In contrast, no such studies have been conducted on the Australian and New Zealand *Armillaria* spp. Their phylogenetic relationships are thus unknown. The objective of this study was to determine the phylogenetic relationships between the Australian and New Zealand *Armillaria* species using sequence data from the ITS1, ITS2 and the 5.8S gene regions of the rRNA operon.

MATERIALS AND METHODS

Fungal isolates.—Haploid and diploid isolates of *Armillaria* spp. originating from different regions in Australia and New Zealand were obtained (TABLE I). These isolates are maintained in the culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA extraction.—Isolates were grown in liquid MY (2% malt extract and 0.3% yeast extract) at 22 C in the dark for two weeks. Mycelium was harvested by centrifugation (15 300 × g, 20 min), lyophilized and ground to a fine powder in liquid nitrogen. DNA was extracted according to the method described by Coetzee et al (2000b). RNase A (0.01 mg/μL) (Roche Diagnostics) was added to the suspension at 37 C to remove contaminating RNA.

PCR.—PCR fragments for the ITS1 and ITS 2 regions including the 5.8S gene between the small subunit (SSU) and large subunit (LSU) were obtained using the primer set ITS1 and ITS4 (White et al 1990). The IGS-1 region was amplified using the primer set P-1 (Hsiau 1996) and O-1 (Duchesne and Anderson 1990). The PCR conditions were the same as those described by Coetzee et al (2000b).

DNA sequencing.—DNA sequences were determined using an ABI PRISM® 377 DNA sequencer. The ITS region was sequenced in both directions with primers ITS1 and ITS4 and newly designed internal primers CS2B (5′ CAAGGTGCGTTCAAAGACTCG 3′) and CS3B (5′ CGAGTCTTTGAACGCACCTTG 3′). The sequence reactions were carried out using an ABI PRISM® Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin Elmer, Warrington, U.K.) according to the manufacturer's directions.

Sequence analysis.—Multiple alignments of ITS DNA sequences were done using the Clustal W version 1.6 (Thompson et al 1994) program and manually adjusted. Aligned ITS sequences for the Australian and New Zealand *Armillaria* isolates were deposited in TreeBase (accession number S569, matrix accession number M862). Phylogenetic analyses were conducted using searches based on maximum parsimony and maximum likelihood in PAUP* vers. 4 (Swofford 1998). Ambiguously aligned sequence regions were excluded from the data matrix before analysis. In the parsimony analysis, insertions and deletions (indels) of more than one base were treated in various ways to assess their influence on the topology of the trees obtained. However, with the exception of indels included without coding and gaps treated as newstate, the topology of the trees remained the same, irrespective of the indel treatment. Indels were, therefore, regarded as the result of a single evolutionary event and were coded with multistate characters (0 = deletion, >0 = insert). Phylogenetic trees were rooted to *A. ostoyae* (B481, GenBank accession number AF169645) and *A. sinapina* (B493, GenBank accession number AF169646) as the outgroup.

Most parsimonious (MP) trees were generated by heuristic searches with TBR (Tree Bisection Reconnection) branch swapping and MulTrees effective. Starting trees were obtained via stepwise addition with 100 random taxon addition sequences. MaxTrees was set to auto-increase. Zero length branches were collapsed. Parsimonious trees obtained according to the procedure described above were optimized by applying successive weighting according to the mean consistency of each parsimony informative character. This weighting scheme was applied until the number of MP trees obtained after heuristic searches had stabilized. The confidence levels of the branching points on the phylogenetic trees were determined by bootstrap (1000 replicates) (Felsenstein 1985). Heuristic searches were used in this analysis with MulTrees and TBR active. Starting trees were obtained via stepwise addition of taxa with *A. ostoyae* (B481) as the reference taxon. MaxTrees was set to auto-increase, zero length branches were set to collapse and topological constraints were not inforced. Bremer support/decay indexes (Bremer 1988, Donoghue et al 1992) were calculated for monophyletic clades using AutoDecay v. 4.0 (Eriksson 1998).

The phylogenetic relationship between *A. hinnulea* and the Northern hemisphere *Armillaria* spp. was determined in preliminary analysis. ITS sequence data for various *Armillaria* spp., with the exception of *A. fuscipes* and *A. heimii*, were obtained from GenBank. Sequences were aligned using Clustal W vers. 1.6 (Thompson et al 1994) and manually adjusted by inserting gaps. Most parsimonious trees were obtained as described for the Australian and New Zealand *Armillaria* spp. Indels were, however, included without coding in this analysis.

Phylogenetic analysis based on maximum likelihood was done to estimate nucleotide frequencies, gamma distribution and the transition/transversion (ti/tv) ratio. Search settings corresponded to the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al 1985). Starting branch lengths were obtained using the Rogers-Swofford approximation method. Molecular clock was not enforced. Starting trees were obtained via stepwise addition and the addition of sequences followed the order of taxa in the data set. Heuristic searches were conducted with TBR and MulTrees effective. MaxTrees was set to auto-increase. Branches were collapsed if branch lengths were less than, or equal to 10^{-8}.

RESULTS

PCR.—The IGS-1 region was successfully amplified using the primers P-1 and O-1. Double bands were

observed for certain isolates within the same species. IGS-1 amplicon sizes varied between 400 bp (base pairs) and greater than 1500 bp for the various *Armillaria* spp. (TABLE I). The ITS regions and 5.8S gene were successfully amplified using the primers ITS1 and ITS4. ITS amplicon sizes were the same within species but varied between 800 and 1000 bp among the different species.

Phylogenetic analysis.—Data for the ITS1 region included sequences starting 22 bp downstream from the 3′ end of the SSU while sequences for the ITS2 region stopped approximately 3 bp upstream from the 5′ end of the LSU. The total number of characters obtained after alignment by inserting gaps (without coding indels) was 867. The number of nucleotides sequenced, however, varied between 658 and 763 characters between the different isolates. The ITS1 and ITS2 regions were characterized by the presence of numerous indels. The largest indel was observed in *A. limonea* (CMW4991, CMW4992, CMW4678 and CMW4680) and was 127 bp in size. Indels were, with few exceptions, conserved within species.

Parsimony analysis of the ITS sequences in which indels were treated in various ways, generated MP trees that differed in length, number of trees retained, consistency index and retention index. The MP trees generated using different indel treatments were similar in topology, with some variation of branches at the tips of trees. Analysis with indels excluded and gaps treated as newstate, however, produced MP trees that differed in the placement of *A. limonea* and *A. novae-zelandiae* clades relative to other clades.

The MP tree (FIG. 1) generated with indels coded with multistate characters and gaps treated as missing, grouped the isolates of *A. hinnulea, A. luteobubalina, A. pallidula, A. fumosa, A. novae-zelandiae, A. limonea* and the unknown New Zealand species into six strongly supported monophyletic lineages. *Armillaria pallidula* isolates and *A. fumosa* isolates grouped in a strongly supported (100%) clade and could not be differentiated from each other. Isolates representing *A. novae-zelandiae* formed a sister group with the *A. pallidula–A. fumosa* group. In this analysis we were not able to clearly differentiate between *A. novae-zelandiae* isolates from New Zealand and isolates representing the same species from Australia. *Armillaria limonea* formed a basal group to the *A. pallidula–A. fumosa* and *A. novae-zelandiae* sister group. *Armillaria luteobubalina* isolates were placed basal to *A. limonea* on the most parsimonious tree. The most parsimonious tree generated from the data set placed isolates representing the unknown species basal to *A. luteobubalina. Armillaria hinnulea* was placed basal to the rest of the Australian and New Zealand species. It was intriguing that, in our preliminary study, *A. hinnulea* grouped strongly within a clade representing the Northern hemisphere *Armillaria* spp. and not in the Australian–New Zealand *Armillaria* clade. (FIG. 2).

DISCUSSION

Molecular analysis of the IGS-1 of the rRNA operon of *Armillaria* spp. from Australia and New Zealand indicated that this is a highly divergent group of fungi. In this study the IGS-1 amplicon sizes varied significantly among the species. These size differences can only be attributed to the presence of large indels. This is in contrast to the Northern Hemisphere *Armillaria* species where the IGS1–1 region was found to range between 845 bp and 920 bp among the different *Armillaria* species (Anderson and Stasovski 1992, Harrington and Wingfield 1995, Terashima et al 1998). The large size variation observed in the IGS-1 region for the Australian and New Zealand species made it unsuitable for use in a robust phylogenetic study and it was, therefore, not included in this study.

The ITS regions (ITS1, 5.8S gene and ITS2) of the rDNA operon were used as an alternative to the IGS-1 region to determine the phylogenetic relationships between the Australian and New Zealand *Armillaria* species. Anderson and Stasovski (1992) found that the ITS regions for the majority of the Northern Hemisphere *Armillaria* spp. were excessively conserved for determining the phylogenetic relationships. ITS sequence data obtained in this study indicated a higher degree of DNA sequence similarity between the various lineages but with sufficient variation to be used in phylogenetic analysis of the Australian and New Zealand *Armillaria* spp.

Cladograms generated indicated that *A. hinnulea* is more closely related to the Northern Hemisphere *Armillaria* spp. than to the other Australian and New Zealand species. In a preliminary analysis of ITS sequences for *A. hinnulea* and Northern Hemisphere and African *Armillaria* spp. (FIG. 2), *A. hinnulea* grouped within the Northern Hemisphere clade. This is in agreement with the views of Kile and Watling (1983), who indicated that *A. hinnulea* resembles the European *A. bulbosa* Velen. (synonym: *A. cepistipes*). *Armillaria hinnulea* is further distinguishable from the other Australian and New Zealand *Armillaria* spp. in general basidiocarp morphology, and is the only Australian species with clamp connections in the subhymenial layer of the basidiocarp (Kile and Watling 1983). The New Zealand population of *A. hinnulea* differs from the Australian collections by having clamps in both the subhymenium and the hymenium (GS Ridley unpubl). Sexual compatibility

TABLE I. *Armillaria* isolates used in phylogenetic analysis

Species	IGS amplicon size (ca)	Culture No.	Alternative number	Host	Origin	Collector	Genbank accession No.
Armillaria hinnulea	660 bp	CMW4990	3512/13	Basidiocarp on *Nothofagus* sp.	South Island, New Zealand	GS Ridley	AF329905
		CMW4988	3511/15	Basidiocarp on *Nothofagus* sp.	South Island, New Zealand	GS Ridley	AF329906
		CMW4987	3511/10	Basidiocarp on *Nothofagus* sp.	South Island, New Zealand	GS Ridley	AF329907
		CMW4983	Lot2(11)	Basidiocarp on *Nothofagus* sp.	Australia	—	AF329908
A. luteobubalina	610 bp	CMW4978	MtCole1(18)	Unknown	Victoria, Australia	—	AF329909
		CMW4979	MtCole1(1)	Unknown	Victoria, Australia	—	AF329910
		CMW3942	659.85	*Eucalyptus regnans*	Australia	GA Kile	AF329911
		CMW4977	SA(6)	Unknown	South Australia	—	AF329912
		CMW5704	WA31(5)	Unknown	Western Australia	—	AF329913
A. pallidula	400 bp	CMW4972	Qld5761	Unknown	Queensland, Australia	—	AF329914
		CMW4968	3626, ATCC 66124	*Pinus caribaea* var. *hondurensis*	Australia	P Gordon	AF329915
A. fumosa	400 bp	CMW4960	Qld.Coll.9(4)	Unknown	Queensland, Australia	GA Kile	AF329916
		CMW4957	123	Basidiocarp on *Eucalyptus* sp.	Tasmania, Australia	GA Kile	AF329917
		CMW4955	123.1	Basidiocarp on *Eucalyptus* sp.	Tasmania, Australia	GA Kile	AF329918
		CMW4956	123.2	Basidiocarp on *Eucalyptus* sp.	Tasmania, Australia	GA Kile	AF329919
		CMW4959	Qld.Coll.8(1)	*P. radiata*	Queensland, Australia	GA Kile	AF329920
		CMW4967	Qld.Coll.9(3)	*P. radiata*	Queensland, Australia	GA Kile	AF329921
A. novae-zelandiae	830 bp	CMW4963	121, ATCC 66127, DAR41512	Basidiocarp on *Antherosperma moschatum*	Tasmania, Australia	GA Kile	AF329922
		CMW4966	Lot4(4)	Unknown	Australia	—	AF329923
		CMW4964	Qld.Coll.10(3)	Basidiocarps on *P. radiata*	Queensland, Australia	GA Kile	AF329924

TABLE I. Continued

Species	IGS amplicon size (ca)	Culture No.	Alternative number	Host	Origin	Collector	Genbank accession No.
		CMW4986	3505/15	Basidiocarps from *Nothofagus fusca* and *N. solandri* forest	South Island, New Zealand	GS Ridley	AF329925
		CMW4722	G3.0.34.4	Rhizomorphs from *Beilschmiedia tawa* forest	North Island, New Zealand	IA Hood	AF329926
A. limonea	580 bp	CMW4991	3522/2	*P. radiata*	North Island, New Zealand	GS Ridley	AF329927
		CMW4992	3522/13	*P. radiata*	North Island, New Zealand	GS Ridley	AF329928
		CMW4678	A3.4.26.3	Rhizomorphs from *Beilschmiedia tawa* forest	North Island, New Zealand	IA Hood	AF329929
		CMW4680	C3.28.0.1	Rhizomorphs from *Beilschmiedia tawa* forest	North Island, New Zealand	IA Hood	AF329930
Armillaria sp.	>1500 bp	CMW5597	A35.4	*Nothofagus fusca*	North Island, New Zealand	IA Hood	AF329931
		CMW4994	4698/10	*Nothofagus* sp.	North Island, New Zealand	GS Ridley & JF Gardener	AF329932
		CMW4993	4698/9	*Nothofagus* sp.	North Island, New Zealand	GS Ridley & JF Gardener	AF329933

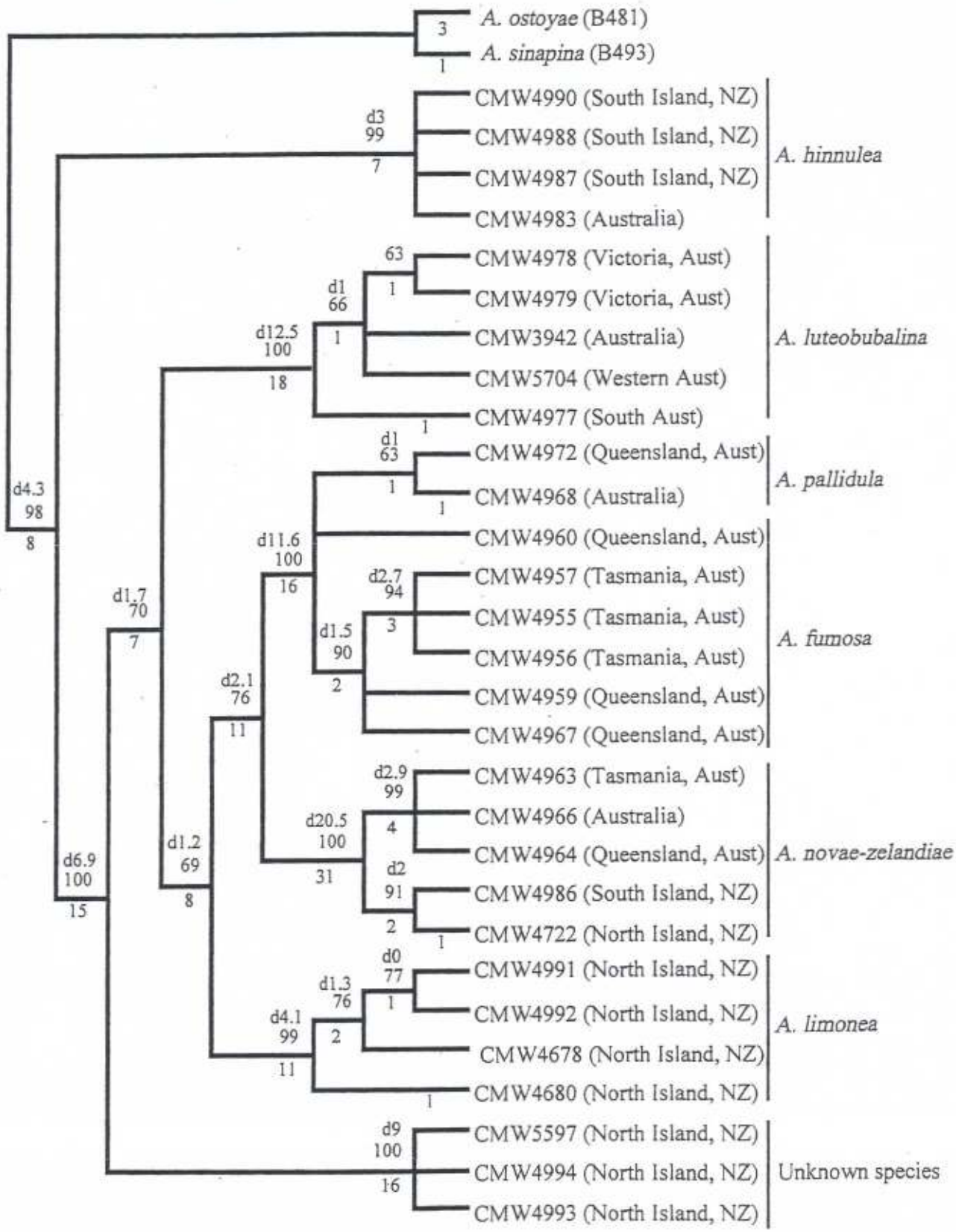

FIG. 1. One of the most parsimonious trees generated after a heuristic search from the ITS sequence data with indels coded and gaps treated as missing. Bootstrap (1000 replicates) values and Bremer support indexes for the branching nodes are indicated above the tree branches. Values below the branches are the branch lengths. Number of parsimony informative characters = 113, length of tree = 202, CI = 0.880 and RI = 0.967.

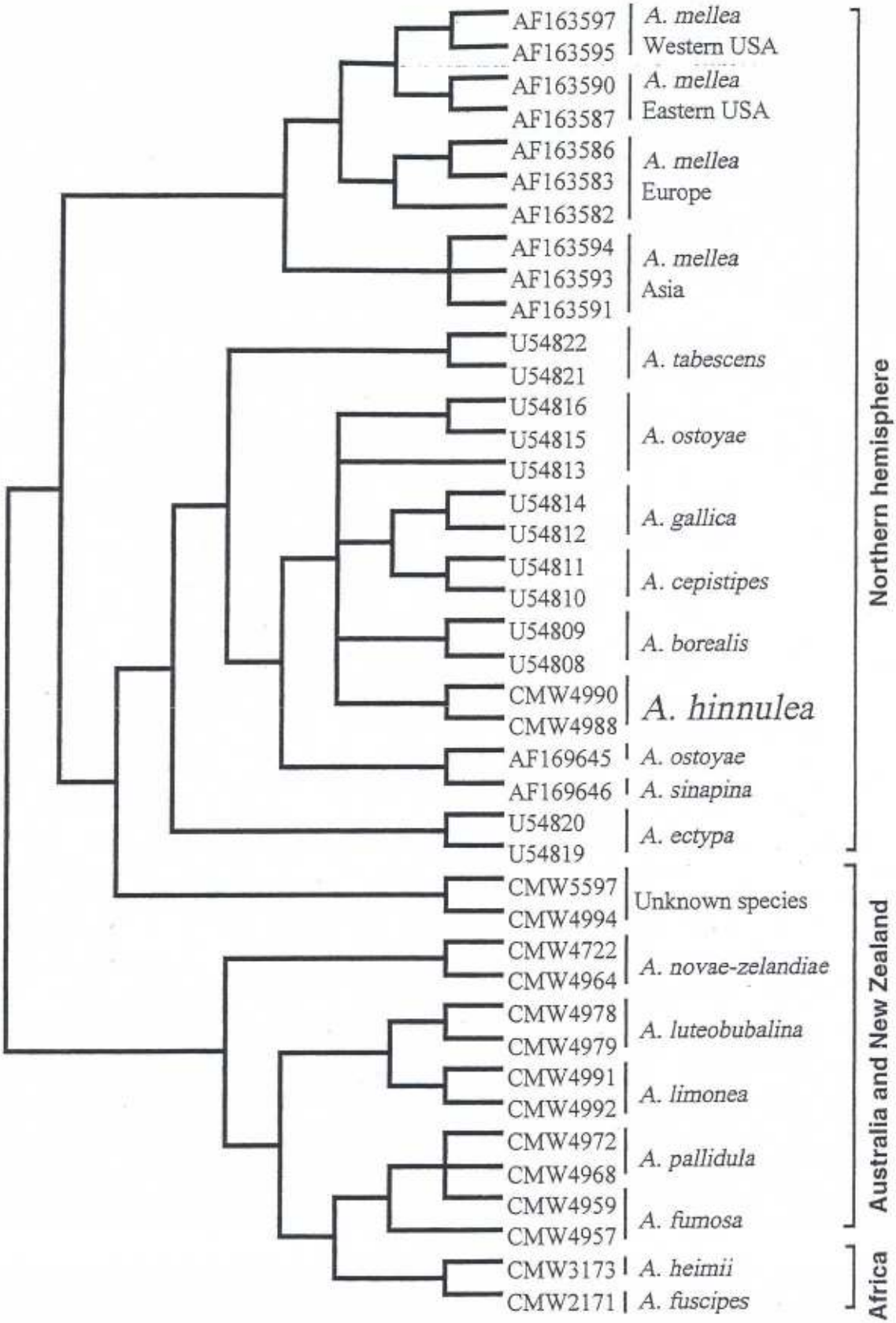

FIG. 2. Strict consensus tree from 210 MP trees for *Armillaria* spp. from the Northern and the Southern hemisphere. Number of parsimony informative characters = 339, length of tree = 485, CI = 0.786, RI =0.894. AF and U numbers refer to GenBank accession numbers.

studies (Kile and Watling 1988) confirmed the separation of *A. hinnulea* from the other *Armillaria* spp. based on morphology and indicated that this is a distinct species. Our grouping of the *A. hinnulea* isolates in a strongly supported monophyletic clade distant to the other Australian and New Zealand *Armillaria* spp. is thus in congruence with the differentiation of this species based on morphology and sexual compatibility tests.

Using interfertility tests, *A. hinnulea* isolates from Australia and putative *A. hinnulea* isolates from the central North Island of New Zealand were shown not to be conspecific by Kile and Watling (1988). Cladograms generated in the current study support this observation where the isolate of *A. hinnulea* (CMW4983) from Australia and the putative *A. hinnulea* isolates from the New Zealand North Island (CMW5597, CMW4994 and CMW4993) segregated in different clades. However, isolates derived from basidiomes collected in the South Island of New Zealand and identified as *A. hinnulea* based on micro-morphology were grouped into the same clade as the Australian isolate of *A. hinnulea*. This indicates that *A. hinnulea* is present in the South Island of New Zealand and is the same species as that occurring in Australia. It also indicates the presence of a new undescribed species in the central North Island of New Zealand.

Armillaria fumosa and *A. pallidula* could not be separated based on their ITS sequence differences resulting in one strongly supported monophyletic group. *Armillaria pallidula*, while sharing some morphological features with *A. fumosa*, was shown to be a distinct biological species (Kile and Watling 1988). *Armillaria pallidula* was described from one location in Queensland but possibly overlaps *A. fumosa* in geographic distribution (Kile and Watling 1988). Data presented in this study indicate that the two species are closely related and are probably sibling species. Differences in morphology and mating type were not congruent with the differentiation at ITS level.

Analysis of the ITS sequence data showed that the *A. novae-zelandiae* isolates from New Zealand and Australia belong to a single monophyletic clade and basal to the *A. pallidula–A. fumosa* group. *Armillaria novae-zelandiae* occurs in the temperate rainforests of southeastern Australia and New Zealand (Hood 1989). Macro- and micro-morphology of the type material from New Zealand (Stevenson 1964) was similar to that morphology of basidiocarps found in Australia (Kile and Watling 1983). Kile and Watling (1983) also found that the vegetative morphology of the Australian and New Zealand isolates of *A. novae-zelandiae* is very similar. At the biological species level, it was shown that *A. novae-zelandiae* isolates from New Zealand, mainland Australia and Tasmania are sexually compatible (Kile and Watling 1983). Morphological descriptions, sexual compatibility tests and ITS sequence analyses presented here indicated that *A. novae-zelandiae* from Australia and New Zealand are very closely related.

The grouping of *A. limonea* isolates in a monophyletic clade basal to the *A. novae-zelandiae* clade supports the differentiation of these two species based on vegetative and basidiocarp morphology. The vegetative morphologies of *A. limonea* and *A. novae-zelandiae* are distinctly different and can be used to differentiate between isolates representing these species (Shaw et al 1981). *Armillaria novae-zelandiae* and *A. limonea* can be separated on micro-morphology, particularly on the structure of the pileipellis (GS Ridley unpubl). Comparisons between the descriptions of the macro-morphology of *A. limonea* (Stevenson 1964) and *A. novae-zelandiae* (Stevenson 1964, Kile and Watling 1983) indicated that they are distinct species. The grouping of *A. limonea* from New Zealand basal to *A. novae-zelandiae* and not to the Australian *A. pallidula–A. fumosa* clade is supported by their biogeography. It is apparent that *A. limonea* and *A. novae-zelandiae*, although divergent in morphology, are very similar in ITS sequences and, therefore, phylogenetically related.

Armillaria luteobubalina grouped basal to *A. limonea* on the cladogram generated in this study. *Armillaria luteobubalina* is the most prevalent *Armillaria* sp. in Australia and is widely distributed in New South Wales, Victoria, South Australia, Western Australia, Tasmania and parts of southeast Queensland (Kile and Watling 1981, 1983, Pearce et al 1986, Shearer and Tippett 1988, Shearer 1994). The grouping of *A. luteobubalina* close to the New Zealand *A. limonea* and not the other *Armillaria* species from Australia is interesting since there are limited similarities in their basidiocarp morphology (Stevenson 1964, Podger et al 1978). These two species, however, are similar in their yellow pigmentation of the pileus. Based on sexual compatibility tests, Kile and Watling (1988) showed that *A. limonea* and *A. luteobubalina* are distinct biological species.

Our results indicate that the unknown *Armillaria* species included in this study is different from the other Australian and New Zealand *Armillaria* spp. This species is only known in the central North Island of New Zealand (Hood 1992). Haploid cultures of this fungus were crossed with haploid tester strains of *A. novae-zelandiae* but failed to form dikaryons (Hood and Sandberg 1987). It was thus suggested that the unknown North Island of New Zealand fungus probably represented *A. hinnulea* (Kile and Watling 1983). Haploid isolates were, however, incom-

patible with *A. hinnulea* tester strains from Australia (Kile and Watling 1988) and the micromorphology of the basidiocarps of the two species also differed (Hood 1989). Based on our phylogenetic analysis and evidence from the reported sexual compatibility tests and morphology of the basdiocarps, we believe that this is a distinct taxon that needs to be formally described.

Large indels were present in both ITS1 and ITS2 regions obtained in this study. To reduce the effect of the indels we applied a multistate coding system, by which blocks of indels were replaced by numeric characters. This coding system resulted in an increase in resolution at the branch tips of the trees obtained after heuristic searches. Phylogenetic analysis based on ITS data in this study showed that the *Armillaria* spp. from Australia and New Zealand, with the exception of *A. hinnulea*, formed a strongly supported monophyletic group and that they are separated from one another.

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LITERATURE CITED

Anderson JB, Bailey SS, Pukkila PJ. 1989. Variation in ribosomal DNA among biological species of *Armillaria*, a genus of root-infecting fungi. Evolution 43:1652–1662.

———, Petsche DM, Smith ML. 1987. Restriction fragment polymorphisms in biological species of *Armillaria mellea*. Mycologia 79:69–76.

———, Stasovski E. 1992. Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycologia 84:505–516.

Boesewinkel HJ. 1977. New plant disease records in New Zealand: records in the period 1969–1976. N Z J Agric Res 20:583–589.

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

Chillali M, Wipf D, Guillaumin JJ, Mohammed C, Botton B. 1998. Delineation of the European *Armillaria* species based on the sequences of the internal transcribed spacer (ITS) of ribosomal DNA. New Phytol 138:553–561.

Coetzee MPA, Wingfield BD, Coutinho TA, Wingfield MJ. 2000a. Identification of the causal agent of Armillaria root rot in pine plantations in South Africa. Mycologia 92:777–785.

———, ———, Harrrington TC, Dalevi D, Coutinho TA, Wingfield MJ. 2000b. Geographical diversity of *Armillaria mellea* s. s. based on phylogenetic analysis. Mycologia 92:105–113.

Donoghue MJ, Olmstead RG, Smith JF, Palmer JD. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. Ann Mo Bot Gard 79:333–345.

Duchesne LC, Anderson JB. 1990. Location and direction of transcription of the 5S rRNA gene in *Armillaria*. Mycol Res 94:266–269.

Eriksson T. 1998. AutoDecay ver. 4.0. Program distributed by the author. Bergius Foundation, Royal Swedish Academy of Science, Stockholm.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Harrington TC, Wingfield BD. 1995. A PCR-based identification method for species of *Armillaria*. Mycologia 87: 280–28.

Hasegawa M, Kishino H, Yano T-A. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174.

Herink J. 1973. Taxonomie václavky obecné *Armillaria mellea* (Vahl ex Fr.) Kummer. In: Hasek J, ed. Sympozium o vaclavky obecne. September 1972. Lesnicka fakulta VSZ Brno. p 21–50.

Hood IA. 1989. Armillaria root disease in New Zealand forests. N Z J For Sci 19:180–197.

Hood IA. 1992. An illustrated guide to the fungi on wood in New Zealand. Auckland: Auckland University Press. 424 p.

———, Redfern DB, Kile GA. 1991. *Armillaria* in planted hosts. In: Shaw CG, Kile GA, eds. Armillaria root disease. USDA Agricultural Handbook No. 691. Washington, DC: USDA. p 122–149.

———, Sandberg CJ. 1987. Occurrence of *Armillaria* rhizomorph populations in the soil beneath indigenous forests in the Bay of Plenty, New Zealand. N Z J For Sci 17:83–99.

Hsiau PT-W. 1996. The taxonomy and phylogeny of the mycangial fungi from *Dendroctonus brevicomis* and *D. frontalis* (Coleoptera: Scolytidae) [Ph.D. Thesis]. Ames, Iowa: Iowa State Univ. 92 p.

Kile GA, McDonald GI, Byler JW. 1991. Ecology and disease in natural forests. In: Shaw CG, Kile GA, eds. Armillaria root disease. USDA Agricultural Handbook No. 691. Washington, DC: USDA. p 102–121.

———, Watling R. 1981. An expanded concept of *Armillaria luteobubalina*. Trans Br Mycol Soc 77:75–83.

———, Watling R. 1983. *Armillaria* species from south-eastern Australia. Trans Br Mycol Soc 81:129–140.

———, 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*. Trans Br Mycol Soc 91:305–315.

Pearce MH, Malajczuk N, Kile GA. 1986. The occurrence and impact of *Armillaria luteobubalina* in the Karri (*Eu-*

calyptus diversicolor F. Muell.) forest of western Australia. Aust Forest Res 16:243–259.

Piercey-Normore MD, Egger KN, Bérubé JA. 1998. Molecular phylogeny and evolutionary divergence of North American biological species of *Armillaria*. Mol Phylogenet Evol 10:49–66.

Podger FD, Kile GA, Watling R, Fryer J. 1978. Spread and effects of *Armillaria luteobubalina* sp. nov. in an Australian *Eucalyptus regnans* plantation. Trans Br Mycol Soc 71:77–87.

Raabe RD. 1962. Host list of the root rot fungus, *Armillaria mellea*. Hilgardia 33:25–88.

Shaw CG, MacKenzie M, Toes EHA, Hood IA. 1981. Cultural characteristics and pathogenicity to *Pinus radiata* of *Armillaria novae-zelandiae* and *A. limonea*. N Z J For Sci 11:65–70.

Shearer BL. 1994. The major plant pathogens occurring in native ecosystems of south-western Australia. J R Soc West Aust 77:113–122.

———, Tippett JT. 1988. Distribution and impact of *Armillaria luteobubalina* in the *Eucalyptus marginata* forest of south-western Australia. Aust J Bot 36:433–445.

Smith ML, Anderson JB. 1989. Restriction fragment length polymorphisms in mitochondrial DNAs of *Armillaria*: identification of North American biological species. Mycol Res 93:247–256.

Staude F. 1857. Die schwämme Mitteldeutschlands insbesonderes des Herzogthums. Coburg. 148 p.

Stevenson G. 1964. The Agaricales of New Zealand: V. Tricholomataceae. Kew Bull 19:1–59.

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

Terashima K, Cha JY, Yajima T, Igarashi T, Miura K. 1998. Phylogenetic analysis of Japanese *Armillaria* based on the intergenic spacer (IGS) sequences of their ribosomal DNA. Eur J For Pathol 28:11–19.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

Volk TJ, Burdsall HH. 1995. A nomenclatural study of *Armillaria* and *Armillariella* species (Basidiomycotina, Tricholomataceae). Oslo: Eko-trykk A/S. 121 p.

———, ———, Banik MT. 1996. *Armillaria nabsnona*, a new species from western North America. Mycologia 88:484–491.

Watling R, Kile GA, Burdsall HH. 1991. Nomenclature, taxonomy, and identification. In: Shaw CG, Kile GA, eds. Armillaria root disease. USDA Agricultural Handbook No. 691. Washington, DC: USDA. p 1–9.

White TJ, Bruns T, Lee S, Tailor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.