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I am submitting herewith a dissertation written by Edgar B. Lickey entitled "Systematic and Monographic Studies in *Artomyces* Jülich and *Clavicorona* Doty." I have examined the final paper copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.

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SYSTEMATIC AND MONOGRAPHIC STUDIES IN *ARTOMYCES* JÜLICH AND *CLAVICORONA* DOTY

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Edgar Ballenger Lickey
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DEDICATION

This dissertation is dedicated to my family and friends
for all their love and support.

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ABSTRACT

This study comprises a complete reevaluation of genera *Artomyces* Jülich and *Clavicorona* Doty, based primarily on analysis of nuclear ribosomal ITS 1 – 5.8S – ITS 2 DNA (ITS) sequences and complemented with morphological characterizations. *Clavicorona*, as represented by the type species *C. taxophila* was completely divergent from *Artomyces* and probably is more closely related to the *Omphalina – Gerronema – Chrysomphalina* group. A total of 16 species are recognized in *Artomyces*, including eight species described as new and one species name transferred from *Clavicorona*. Although a phylogenetic estimation based on the ITS sequences revealed little resolution in the basal nodes, five major and two monospecific clades were identified.

Broad biogeographical patterns among the species of *Artomyces* could not be identified, but intraspecific biogeographical patterns were observed within the widespread species *A. pyxidatus*. Previous studies established that populations of *A. pyxidatus* from the eastern United States, Sweden, and China were conspecific based on mating compatibility and enzyme profiles. In this study, mating compatibility was extended to include collections from Russia, Costa Rica, Mexico, and Utah. The molecular diversity within *A. pyxidatus* was examined by DNA sequence and restriction site analyses of the ITS region. A phylogenetic analysis of twelve collections based on ITS sequences revealed a broad geographical pattern in which Eurasian collections comprised a sister clade to North American collections. North American collections were further subdivided into northeastern and southwestern clades. A survey of 255 *A. pyxidatus* collections using restriction enzymes revealed variable RFLP patterns that followed similar geographical patterns.

Previous studies reported the occurrence of a Group I intron in the 18S ribosomal DNA gene in culture isolates of two of three genera of the Auriscalpiaceae that were surveyed. In this study, the presence or absence of this intron was determined for seven species of *Lentinellus*, two species of *Auriscalpium*, and 15 species of *Artomyces*. The occurrence of the intron among the species of these genera was variable. Within *Artomyces*, the intron was present in *Ar. pyxidatus* and in a newly described species from Argentina, *Ar. austropiperatus*. Within *Lentinellus*, the intron was present primarily in a clade consisting of the closely related species *L. micheneri*, *L. 'europeanus,' L. 'subaustralis'* and *L. montanus* and was variable in *L. castoreus*. Within *Auriscalpium*, the intron was present in *Au. villipes* but not in *Au. vulgare*. Heterozygosity for presence of the intron was observed. Phylogenetic estimations were generated for the intron and nuclear ribosomal internal transcribed spacer regions (ITS). Tests of congruence for the two trees indicated that the trees were significantly different.

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PART I

GENERAL INTRODUCTION

Doty (1947) proposed the genus *Clavicorona* to include what he interpreted as a group of closely related species, and he chose *Craterellus taxophilus* Thom (1904) to exemplify them. Since then it has become evident that there are observable differences between the unbranched *C. taxophila*, which fruits on leaf duff, and the pyxidately branched, lignicolous species. Jülich (1981) subsequently proposed the genus *Artomyces* to include these branched species, but his treatment was not fully accepted and the inclusive genus is still commonly called *Clavicorona.* Recent taxonomic and systematic studies within this group showed that the species exhibit relatively few distinguishing morphological characters, but that underlying biological and biochemical differences were present (Dodd 1972, Wu 1991, Wu et al. 1996). Dodd (1972), Wu (1991), and Wu et al. (1996) speculated on phylogenetic relationships among the species but with few available characters, hypotheses could not be easily tested. Since the completion of these studies, forays to New Zealand, Australia (Tasmania), Argentina, Costa Rica, and New Guinea added a number of putative new species to *Clavicorona sensu* Doty, and the availability of new molecular techniques warranted a re-examination of *Clavicorona* and *Artomyces*.

Modern taxonomy of fungi includes a combination of three primary methods including morphology, mating compatibility, and some form of molecular analysis. Morphological analysis has been a mainstay in taxonomy for obvious reasons (i.e. things that look alike are alike). However, there are relatively few morphological characters available with which to circumscribe species, and it has been shown that morphological homoplasy is not uncommon in higher fungi (Moncalvo et al. 1995, Vilgalys 1991, Bruns et al. 1991).

Mating studies have added an important biological component to circumscribing species (Boidin 1986, Petersen 1995). Recent studies have shown that significant underlying genetic variation and intersterility barriers may be hidden within a morphological species. In a classic example, *Armillaria mellea* has been shown to be composed of up to ten intersterility groups, or biological species, and these have been confirmed by phylogenetic analyses (Anderson and Ullrich 1979, Piercy-Noremore et al. 1998). Other examples include the *Collybia dryophila* complex (Vilgalys 1991) and *Xeromphalina campanella* (Johnson 1997). On the other hand, collections of *Amylostereum areolatum*, which were thought to be represented by two morphologically distinct taxa, are completely interfertile, but molecular data are lacking (Vasiliauskas et al. 1999).

Intercompatibility tests are helpful in taxonomic studies by delimiting biological species, but cannot be used in all cases. Because *Suillus* spp. are mycorrhizal, they are not amenable to *in vitro* mating studies, leaving Kretzer et al. (1996) with only morphology and DNA sequences to define and delimit species boundaries. Wu (1991) reported intercompatibility among collections of *Clavicorona colensoi*, *C. cristata*, *C. microspora*, *C. turgida*, and *C. pyxidata*. Both *C. colensoi* (*Artomyces candelabrus* in this study) and *C. cristata* showed reduced compatibility (less than 50%) among collections. More recent intercompatibility studies with collections of putative new species from Argentina were unsuccessful because monokaryon cultures grew poorly even during self-crossing attempts (Hughes, unpubl. data).

Modern DNA-based techniques have been used to elucidate relationships of some cryptic species (*Armillaria* spp., Piercy-Noremore et al. 1998; *Ganoderma lucidum*, Moncalvo et al. 1995; *Collybia dryophila*, Vilgalys 1991). By far, the workhorse of DNA studies in higher basidiomycetes has been the nuclear ribosomal gene repeat, primarily because of the relative ease of amplification and manipulation. The ITS 1 – 5.8S – ITS 2 sequences have been widely used to determine interspecific relationships and sometimes broad biogeographical patterns, both among and within species. Several taxa that have been studied using sequence comparisons and restriction fragment length polymorphisms of this region include *Flammulina* (Hughes et al. 1999, Methven et al. 2000), *Lentinula* (Nicholson et al. 1997, Hibbett et al. 1998, Mata et al. 2001), *Omphalotus* (Hughes and Petersen 1998), *Panellus* (Jin et al. 2001), *Pleurotus* (Vilgalys and Sun 1994), *Suillus* (Kretzer et al. 1996), and *Xeromphalina* (Johnson 1997).

Over the past several years there has been a growing interest in studying the genetic variability within fungal species that have broad ranges, especially those with intercontinental distributions. These taxa have been the subject of recent molecular studies to determine the amount of variation that exists among widely scattered populations (Vilgalys and Johnson 1987, Vilgalys 1991, Vilgalys and Sun 1994, McCleneghan 1996, Johnson 1997, Hughes et al. 1998, 1999, James et al. 1999, Methven et al. 2000, Jin et al. 2001). Athough biological barriers to reproduction are apparently lacking among populations of these species, geographic barriers to gene flow seem to exist as evidenced by sequence and allozymic divergence. This genetic divergence can be used to estimate the historical biogeographic patterns within species in the absence of fossil data. The pan-North Temperate species *Artomyces pyxidatus* is represented in our culture collection by more than 200 isolates originating from Europe, North America, Central America, and China, making this species another good model for such a survey.

In a study of the relationships among genera of the homobasidiomycetes, Hibbett (1996) identified a Group I intron imbedded in the nuclear ribosomal small subunit gene of *Clavicorona pyxidata*, *Lentinellus ursinus*, *L. vulpinus*, and *L. montanus*. However, he did not find it in the related species *Auriscalpium vulgare*. Hibbett (1996) speculated that the occurrence of the intron in these species was due to a single ancient event that took place before the divergence of the three genera. A preliminary survey of some *Artomyces* collections for this study revealed that not all species contain the intron and that further study was warranted.

This dissertation is composed of three parts. The first part deals with a circumscription of *Artomyces* and its relationship with *Clavicorona* sensu Jülich. This includes a complete description of previously described and proposed new species based on morphology and ITS DNA sequences. Sequences of this region were also used for phylogenetic estimations. The second part of this work concentrates on the geographic variability within the widespread *A. pyxidatus*. Enough variability was found in the ITS sequence region to construct an intraspecific phylogeny which exhibited strongly supported geographic patterns. The third section addresses the variability of the occurrence and size of the Group I intron found in the small subunit gene. All cultures of *Artomyces*, *Auriscalpium*, and *Lentinellus* were surveyed for the presence of this intron. Phylogenetic estimations based on intron and ITS sequences were compared to each other and found to be significantly incongruent. These three sections address different aspects

of the total variability found within *Artomyces,* and they extend our knowledge of the evolutionary and biogeographical history of this group.

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PART II

PHYLOGENETIC AND TAXONOMIC STUDIES IN *ARTOMYCES* AND *CLAVICORONA*

ABSTRACT

This study comprises a reevaluation of *Clavicorona* Doty and *Artomyces* Jülich, based on analysis of nuclear ribosomal ITS 1 – 5.8S – ITS 2 DNA sequences, morphological characterizations, and limited mating analyses. Sequence data and morphological characters indicated that *Artomyces* is divergent from *Clavicorona taxophila*, the type species of *Clavicorona*, and that *C. taxophila* might be closely related to the *Omphalina – Gerronema – Chrysomphalina* group. A total of 16 species are recognized in *Artomyces*, including eight species described as new. *Clavicorona microspora* was transferred to *Artomyces*, whereas *C. tuba*, which may have more in common with *Artomyces* species, was deemed *species inquirenda* until specimens can be located for examination.. A morphological description of each species is provided and an artificial key to the species of *Artomyces* is included. Mating analyses helped to identify isolates of some Costa Rican collections as either *A. pyxidatus* or *A. stephenii*. There was significant variability within the nrDNA ITS region among *Artomyces* species. Sequences ranged from 629 to 740 bp in length. Although phylogenetic estimations and distance trees based on ITS sequences revealed little resolution in the basal nodes, five major and two monospecific clades could be identified. Biogeographical patterns among the species could not be identified, but there was higher sequence divergence between collections from geographically separated populations of widely distributed species than between collections of species with restricted ranges.

INTRODUCTION

Clavicorona Doty was proposed by Doty (1947) to represent clavarioid fungi whose basidiomata are simple or pyxidately branched, possess gloeoplerous hyphae that often terminate in the hymenium as gloeocystidia, and produce small, white, hyaline, and smooth to roughened spores. *Craterellus cristatus* Kauff., *Clavaria taxophila* (Thom) Lloyd, *Clavaria pyxidata* Pers.: Fr. and *Clavaria coronata* Schw. were placed in this genus. *Craterellus taxophilus* Thom (1904) was chosen as the type species because it exemplifies the genus, there was ample material, and the type collection was well-described (Doty 1947). Corner (1950) transferred several other names into *Clavicorona*, recognizing eight species in his monograph, and increased that to eleven species in his supplement (Corner 1970). The first study to mention basidiospore amyloidity in this genus was by Leathers and Smith (1967), who divided the genus into three sections based on spore reactions to Melzer's reagent, an iodine-based solution that tests for presence of starch. Section *Clavicorona* included species with inamyloid spores; section *Coronatae* included species with dextrinoid spores; and section *Pyxidospoggus* included species with amyloid spores. Dodd (1970), after finding that all species exhibited amyloid spores, subsequently recognized only two groups, subgenera *Clavicorona* and *Ramosa*. Subgenus *Clavicorona* included those species producing unbranched, obconic basidiomata that occurred only on duff, whereas subg. *Ramosa* was characterized by usually pyxidately branched basidiomata fruiting on wood. Jülich (1981) considered the differences between the two subgenera to be more significant and proposed *Artomyces* gen. nov. for the complex that Dodd (1972) considered as subg. *Ramosa*. In his descriptions, Jülich (1981) used spore amyloidity as a primary character where *Clavicorona sensu* Jülich species exhibited inamyloid spores whereas *Artomyces* species

produced amyloid spores. Mycologists never fully accepted the name *Artomyces* and have preferred calling the entire group *Clavicorona* (e.g. Petersen 1988, Wu 1991, Wu et al. 1996, Stalpers 1996). Wu et al. (1996) completed the latest systematic treatment of *Clavicorona* based on morphological comparisons, sexual inter-incompatibility, and extracellular enzyme electrophoretic profiles. In addition to the 11 named species recognized by Dodd (1972) and the newly named *C. microspora* Wu & R.H. Petersen (1992), Wu et al. (1996) identified three putative new species from North Carolina, Puerto Rico, and New Zealand, but these were not formally described.

This study; 1) provides an estimation of phylogenetic relationships among the species of *Artomyces* and *Clavicorona* based on ITS DNA sequences and assesses the validity of Jülich's (1981) recognition of two distinct genera; 2) anaylyzes additional recent collections from Argentina, Costa Rica, Indonesia, New Guinea, New Zealand, and Tasmania; and 3) identifies and formally describes new species using morphology, ITS sequences, and mating studies.

METHODS

Cultures – Monokaryon and dikaryon cultures used in this study (TABLE 1) were established from basidiomata collected in nature. Spore prints were obtained by suspending small sections of branch material from the upper two branch ranks of fresh basidiomata over a malt extract agar plate (MEA; 15 g malt extract and 20 g agar per liter water). Once spores were deposited and began to germinate, 20 germinating spores were isolated and placed one per MEA plate, representing single-basidiospore isolates (SBIs). After growth, these isolates were used in mating compatibility studies and DNA sequencing. Remaining germinating spores were allowed to overgrow to produce "polyspore-derived" dikaryotic mycelia. Sometimes, a small section of the basidioma was placed directly onto an MEA plate, and a tissue-derived dikaryon was obtained. Monokaryons and dikaryons were then subcultured onto MEA slants for storage at 4°C and into 1.5 ml distilled water vials for storage at room temperature. These represent the culture collection (CULTENN) with the Tennessee field book (TFB) number serving as the CULTENN accession number. The rest of the fruit body was dried, assigned an accession number, and deposited in the mycology herbarium (TENN) for later morphological study and identification voucher.

Mating studies – Eight collections from Costa Rica exhibited the ability to cross *in vitro* and were surveyed for intercompatibility with four collections of *A. pyxidatus* used by Wu (1991). Intercompatibility studies were performed as described by Gordon and Petersen (1991) with the following modifications. Four SBIs from each collection were paired with four SBIs from another collection resulting in a set of four pairings between the two collections (i.e. SBIs of 9433 and 9436 were paired as 1x1, 3x5, 5x9, and 7x11, respectively). Collection 1541 (China) was represented by only one SBI, and collection 1607 (North Carolina) was represented by three SBIs. All isolates included in the mating study are given in TABLE 1. Compatible matings were determined by the presence of numerous clamp connections in and near the contact zone, viewed directly on the agar surface under 250X magnification.

TABLE 1. List of isolates and collections examined and included in the analyses.

Species	Isolate[a]	Herbarium Number[b]	Country	State or Province	Sequence[c]	RFLP	Morphology		Mating
							Complete	SEM[d]	
Artomyces adrienneae	ZT 6839	59139[e]	Argentina	Chubut		X	X		
	7384	54435	Argentina	Neuquen		X			
	7386	54404	Argentina	Neuquen		X			
	7387	54434	Argentina	Neuquen	AF454418	X	X		
	7389	54426	Argentina	Neuquen		X			
	8299	55352	Argentina	Chubut		X			
	8309	54987	Argentina	Chubut		X			
	8314	54992	Argentina	Chubut		X			
	8343	55017	Argentina	Chubut		X	X		
Ar. austropiperatus	2822	48916	New Zealand	Eastern District		X	X		
	7153	56948	New Zealand	Eastern District	AF454408	X	X		
	8335	55009	Argentina	Chubut	AF454407	X	X		
	8363	55036[e]	Argentina	Neuquen		X	X		
	ZT 6768	59138	Argentina	Neuquen		X	X		
	RV95/509	DHN 2890	Australia	Tasmania		X	X		
	RV95/511	DHN 2892	Australia	Tasmania		X	X		
Ar. candelabrus	2555	48930	New Zealand	Canterbury		X			
	2637	48917	New Zealand	Southern District	AF454419	X	X	X	
	2644	48918	New Zealand	Southern District		X			
	2667	48920	New Zealand	Southern District		X			
	2685	48919	New Zealand	Auckland		X	X		
	3922	50128	Australia	Tasmania	AF454420	X	X		
	7044	54108	New Zealand	Southern District		X			
	7067	54122	New Zealand	Southern District		X			
	7102	56874	New Zealand	Tasman		X			
	7110	56219	New Zealand	Tasman		X			
	7130	56881	New Zealand	Tasman		X			
	55157	43871	New Zealand	Tasman		X	X		

TABLE 1 cont.

Species	Isolate[a]	Herbarium Number[b]	Country	State or Province	Sequence[c]	RFLP	Morphology Complete	Morphology SEM[d]	Mating
Ar. carolinianus	1615	48890[e]	United States	North Carolina	AF454409		X		
Ar. colensoi	2679	48923	New Zealand	Auckland	AF454423	X	X	X	
	2807	48925	New Zealand	Auckland	AF454424	X	X		
	55633	44117	New Zealand	Northland		X	X		
Ar. costaricensis	7900	53848	Costa Rica	San Jose	AF454411	X	X		
	9670	56631[e]	Costa Rica	San Jose	AF454410	X	X		
	10866	59137	Costa Rica	Cartago		X	X		
Ar. cristatus	8079	55403	United States	California			X		
	DLL 8974	48928	United States	California	AF454422		X	X	
	GB 1701	#####	Sweden	Unknown	AF454421				
Ar. dichotomus	DED 7220	SF 7220	Indonesia	Java	AF454406		X		
Ar. enigmatus	3456	48929[e]	United States	Puerto Rico	AF454415		X		
	9905	56533	Costa Rica	Puntarenas	AF454414		X		
Ar. microsporus	2349	48856	Japan	Togichi	AF336138		X		
	KY 5352	#####	Japan	Unknown	AF336137				
Ar. novazealandensis	2672	48915	New Zealand	Southern District	AF454417	X	X	X	
	7023	54111[e]	New Zealand	Southern District		X	X		
	7024	#####	New Zealand	Southern District	AF454416	X			
	7025	56203	New Zealand	Southern District		X	X		
	7037	56200	New Zealand	Southern District		X	X		
	7056	56211	New Zealand	Southern District		X			
Ar. piperatus	5770	53491	Canada	British Columbia	AF454404	X	X		
	5869	51959	United States	Washington		X	X		
	6675	DAUP	Canada	British Columbia		X			
	8221	57288	United States	Alaska	AF454405	X	X	X	

TABLE 1 cont.

Species	Isolate[a]	Herbarium Number[b]	Country	State or Province	Sequence[c]	RFLP	Morphology		Mating
							Complete	SEM[d]	
Ar. pyxidatus	1190	48842	United States	New York		X			X
	1607	48852	United States	North Carolina		X	X		
	1506	48845	United States	Wisconsin		X			X
	1541	48855	China	Yunnan		X	X		X
	7263	53544	Sweden	Uppland	AF336139	X	X	X	
	9464	56438	Costa Rica	San Jose		X			X
	9465	56439	Costa Rica	San Jose		X			X
	9681	56642	Costa Rica	San Jose		X			X
	9932	56558	Costa Rica	Puntarenas		X			X
	10030	56742	Costa Rica	Heredia		X			X
	56667	48854	United States	Georgia	AF336145	X			
	GB 1279	#####	Sweden	Unknown		X			X
Ar. stephenii	7913	53798	Costa Rica	San Jose		X			
	7898	53845	Costa Rica	San Jose		X			
	7875	53762	Costa Rica	San Jose		X			
	9433	56407	Costa Rica	Cartago	AF454425	X	X		X
	9436	56410[e]	Costa Rica	Cartago		X	X		X
	9438	56412	Costa Rica	Cartago		X	X		X
	9743	58644	Costa Rica	San Jose		X			X
	11022	58630	Costa Rica	San Jose	AF454426	X			X
Ar. tasmaniensis	3905	50112	Australia	Tasmania	AF454413	X	X		
	3979	50283	Australia	Tasmania	AF454412	X	X		
	4003	50218[e]	Australia	Tasmania		X	X		
	RV95/527	#####	Australia	Tasmania		X			
Ar. turgidus	2651	48934	New Zealand	Southern District	AF454402	X			
	2682	48926	New Zealand	Auckland	AF454401	X	X	X	
	3961	50103	Australia	Tasmania		X	X		

TABLE 1 cont.

Species	Isolate[a]	Herbarium Number[b]	Country	State or Province	Sequence[c]	RFLP	Morphology Complete	Morphology SEM[d]	Mating
Ar. turgidus	7040	54099	New Zealand	Southern District		X	X		
	7109	56218	New Zealand	Tasman		X	X		
	7164	56950	New Zealand	Auckland		X	X		
	55137	44306	New Zealand	Southern District		X	X		
	RV95/875	DHN 3138	Papua New Guinea	Morobe	AF454403	X	X		
Clavicorona taxophila	5608	51926	United States	Idaho			X	X	
	9186	#####	United States	California	AF115333				
	#####	C10264	Denmark	Vestjælland			X	X	
	SAE 0890	C #####	Denmark	Nordjylland			X		
Amylostereum aureolatum	CBS334.66	#####	France	Alpes Maritimes	AF454428				
Auriscalpium villipes	4396	52353	Mexico	Veracruz	AF454429				
Au. vulgare	4242	50578	Sweden	Vastergotland	AF454430				
Lentinellus subaustralis	9159	54890	United States	Tennessee	AF454427				

[a] Isolates are often represented by a culture in the collection at the University of Tennessee (CulTenn) and DNA isolate. This number is often the field collection number. No cultures exist for *C. taxophila*. RV = Rytas Vilgalys; GB = Göteburg; ZT = Egon Horak; KY = K. Yokoyama; DED = Dennis Dejardin; SAE = S. Elborne; ##### = no isolate number.

[b] Herbarium numbers are University of Tennessee accessions (TENN) except where noted. DHN = Duke University; SF = San Francisco State University; C = Copenhagen, Museum Botanicum Hauniense; ##### = no herbarium number.

[c] GenBank Accession numbers.

[d] SEM = Scanning electron microscopy.

[e] Collections designated as type specimens. Type specimens of previously described species are cited in the text.

DNA methodology – DNA isolation protocols followed those used by Hughes et al. (1999). The nuclear ribosomal ITS 1-5.8S-ITS 2 (ITS) region was amplified using forward primer ITS 5 and reverse primer ITS 4 (White et al. 1990). The PCR protocols were: initial melting cycle of 94 C for 4 minutes, 35 repetitions of a three step amplification cycle of 94 C for 1 minute, 52 C for 1 minute, and 72 C for 1 minute, ending with an extension cycle of 72 C for 1 minute.

PCR products were directly purified using a Promega Wizard PCR Purification Kit. The purified product was sequenced using primers ITS 5 and ITS 4 on an automated ABI 373 DNA sequencer (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc.). Primers ITS 2 and ITS 3 were used in some cases to clarify ambiguous sequences resulting from insertions and/or deletions (indels) mostly resulting from poly-adenine runs (White et al. 1990). Rarely, a smaller secondary, interfering fragment of non-ITS origin would amplify. In this case, the PCR products were electrophoresed and then the ITS fragment was excised from a 1X TAE 1.5% low-melting temperature agarose gel (NuSieve GTG agarose, FMC Bioproducts) following manufacturer's directions. The nuclear ribosomal large subunit (LSU) gene was also amplified for DNA isolate 9186 of *Clavicorona taxophila* using primers LR 5 and ITS 3 and sequenced using primers ITS 2, ITS 3, ITS 4, ITS 5, LR 5, and LR 21. Primers LR 5 and LR 21 are from Vilgalys and Sun (1994). Sequences were manually corrected and aligned using the LINEUP and SEQLAB programs in the Genetics Computer Group package (GCG 2001).

Where possible, the ITS region from two isolates of each putative taxon were sequenced (TABLE 1). Proposed new species *Artomyces adrienneae*, *A. carolinensis*, and *A. dichotomus* were represented by only one sequence each. Additionally, one isolate of *Amylostereum areolatum* (Centraalbureau voor Schimmelculture isolate 334.66), *Auriscalpium villipes* (isolate 4396, TENN 52353), *Au. vulgare* (isolate 4242, TENN 50578), and *Lentinellus subaustralis* (isolate 9159, TENN 54890) were chosen to represent the outgroup because of their putative close relationship with *Artomyces* (Hibbett 1996, Ellen Larrson pers. comm.). Sequences were deposited into GenBank and their accession numbers are included in TABLE 1.

Restriction digest profiles – Variable regions within the ITS region were identified by comparing aligned sequences. Restriction sites were mapped for ITS sequences using the MAP program of GCG (GCG 2001) and restriction enzymes were selected which produced diagnostic restriction fragment length polymorphisms (RFLPs) among collections of *Artomyces* that were either morphologically similar, from the same geographical area, or both. ITS PCR products for the antipodal *Artomyces* collections and *A. piperatus* collections from the Pacific Northwest were surveyed using restriction enzymes *Acc* I, *Apa*L I, *Apo* I, *Mbo* I, *Mse* I, and *Nla* III (New England BioLabs), and *Cfo* I and *Hae* III (Promega) according to manufacturer's directions. Isolates of collections from Argentina were surveyed using *Cfo* I; isolates from Costa Rica were surveyed using *Cfo* I and *Hae* III. Restriction fragments were separated on 1X TBE 3% agarose gels stained with ethidium bromide and were visualized under ultraviolet light. Restriction sites were mapped and collections were grouped by presence or absence of variable sites.

Phylogenetic reconstruction – Phylogenetic relationships were estimated using PAUP version 4.0b8 (Swofford 1998). The total alignment consisted of 805 bp positions. Nine regions of high variability were excluded from the analysis because alignment in those regions was ambiguous and contained some large gaps (APPENDIX A). The final dataset consisted of 543 bp positions. The remaining gaps were small and variable and were treated as missing data to reduce any over exaggeration associated with "gaps-as-fifth-base." Both a heuristic search with maximum parsimony and a neighbor joining search with Kimura 2-parameter distance measure were conducted using the shortened dataset. Bootstrap analyses of 1000 replicates were generated for both.

Morphological characterizations – In almost all cases at least three collections and the type specimen of each putative taxon were critically examined both macro- and micromorphologically. Macromorphological observations included the height, width, and substrate of basidiomata as well as size and color of the stipe, the number, size, and color of branches, and color and shape of branch tips. Information was also obtained from field notes about color, odor, and taste of fresh collections. Color references in "quotes" are according to Ridgway (1912), and those in (parentheses) are from Kornerup and Wanscher (1961).

Small, 2 – 4 mm sections of branch material were taken almost exclusively from a well-developed branch one or two ranks below the tips. These were first briefly soaked with 95% EtOH, then hydrated in ddH_20 from six to twelve hours. Tissues were then sliced into thin longitudinal sections. All sections were viewed at 1500X magnification with oil immersion under bright field. Some of the sections were mounted in 3% KOH and surveyed for gloeocystidia. These same sections were then stained with Congo red, squashed, and surveyed for basidia, gloeoplerous hyphae, thick-walled hyphae, and encrusted hyphae. A second set of sections was mounted and stained with Melzer's reagent, squashed, and surveyed for basidiospores. Measurements consisted of length and width of basidia, width and projection length of gloeocystidia, width of gloeoplerous hyphae in the context, thickness of hyphal walls, and length and width of spores. Fifteen to 21 spores were measured for each collection. Averages, ranges, and standard deviation were calculated for length and width of spores and basidia. Seven specimens (TABLE 1) were chosen for scanning electron microscopy. Small bits of dried specimen were mounted onto pedicels with double-sided carbon tape, sputter-coated with gold at the EM facility at the University of Tennessee, and viewed at 10,000X magnification.

In the description section, *Clavicorona* is presented first and *Artomyces* second. Within *Artomyces,* the species are presented alphabetically. For each previously known species, a nomenclator is provided and for each new species, a proposed name and a Latin description are provided. All are followed by macro- and micromorphological descriptions of the basidiomata, a commentary, distribution data, and a list of collections used in the description. Collection data include location, date, collector, collection number, and herbarium accession number. The abbreviation TFB preceding the collection number refers to the "Tennessee Field Book" number that is also the culture number used in DNA and mating studies. Herbarium abbreviations are as follows: C = Copenhagen, Museum Botanicum Hauniense; CUP = Cornell University; DUKE = Duke

University; K = Kew; MICH = University of Michigan; SF = San Francisco State University; TENN = University of Tennessee.

RESULTS

ITS sequence characteristics – The sequence from *Clavicorona taxophila* was sufficiently divergent from sequences of *Artomyces* and the four members of the outgroup that it could not be aligned with any confidence. Approximately 900 bp of nuclear ribosomal large subunit sequence from *C. taxophila* was used for a "basic BLAST" search in GenBank. The closest match found was with a sequence of *Chrysomphalina chrysophylla* (Lutzoni 1996; GenBank Accession # U66430) used in Lutzoni's (1997) phylogenetic study of omphalinoid mushrooms. The similarity between the two sequences was about 92%.

In *Artomyces,* the amplified ITS region ranged from 629 bp in *A. stephenii* to 740 bp in *A. enigmatus.* All sequences were aligned relatively easily, and there was little difficulty aligning sequences of *Amylostereum areolatum, Auriscalpium villipes, Au. vulgare,* and *Lentinellus subaustralis* as members of the outgroup. Sequence divergence between isolates within species was low, from 0% to a maximum of 3.9% between *A. turgidus* from New Zealand and the single New Guinea isolate. Additionally, the two New Zealand isolates of *A. turgidus* originated on each of the two big islands, and they exhibited a divergence of 1.4%. Similar patterns were also seen between isolates of *A. cristatus* with 1.4% divergence between Sweden and California, *A. candelabrus* with 1.2% divergence between New Zealand and Tasmania, and *A. austropiperatus* with 0.8% divergence between Argentina and New Zealand. Endemic species such as *A. novazealandeae*, *A. tasmaniensis*, and *A. piperatus* showed 0% divergence between their respective isolates and *A. costaricensis* exhibited 0.3% divergence. However, the two isolates of *A. stephenii*, known only from Costa Rica, exhibited a 2.9% divergence. For comparison, Vasiliauskas et al. (1999) reported ITS sequence divergences at less than 2.6% among *Amylostereum laevigatum*, *A. areolatum*, and *A. chailletii*. Hughes et al. (1999) provided average ITS divergence values for the species of *Flammulina* of 0.04% and 1.6% divergence between European and North American collections, respectively. The average divergence between North American and European isolates was 1.12%, and between North American and Asian was 2.1%.

Restriction fragment analysis – A combination of eight restriction enzymes provided a good diagnostic tool (FIG 1) for assigning individual isolates into six taxa for the antipodal collections (TABLE 2). Three of these groups morphologically matched species identified as *Clavicorona turgida*, *C. candelabrum*, and *C. colensoi* by Wu (1991) and Wu et al. (1996). Morphological comparisons with type material indicated that the collections of *C. candelabrum* identified by Wu (1991) and Wu et al. (1996) were probably *C. colensoi* and vice versa. Other isolates grouped with an undescribed taxon identified by Wu et al. (1996) as "Taxon 4" (*A. tasmaniensis* in this study). Moreover, some collections identified as "Taxon 3" (Wu et al. 1996) were segregated into two distinct groups (*A. austropiperatus* and *A. novazealandensis* in this study) and both were distinct from *A. piperatus* of the American Pacific Northwest.

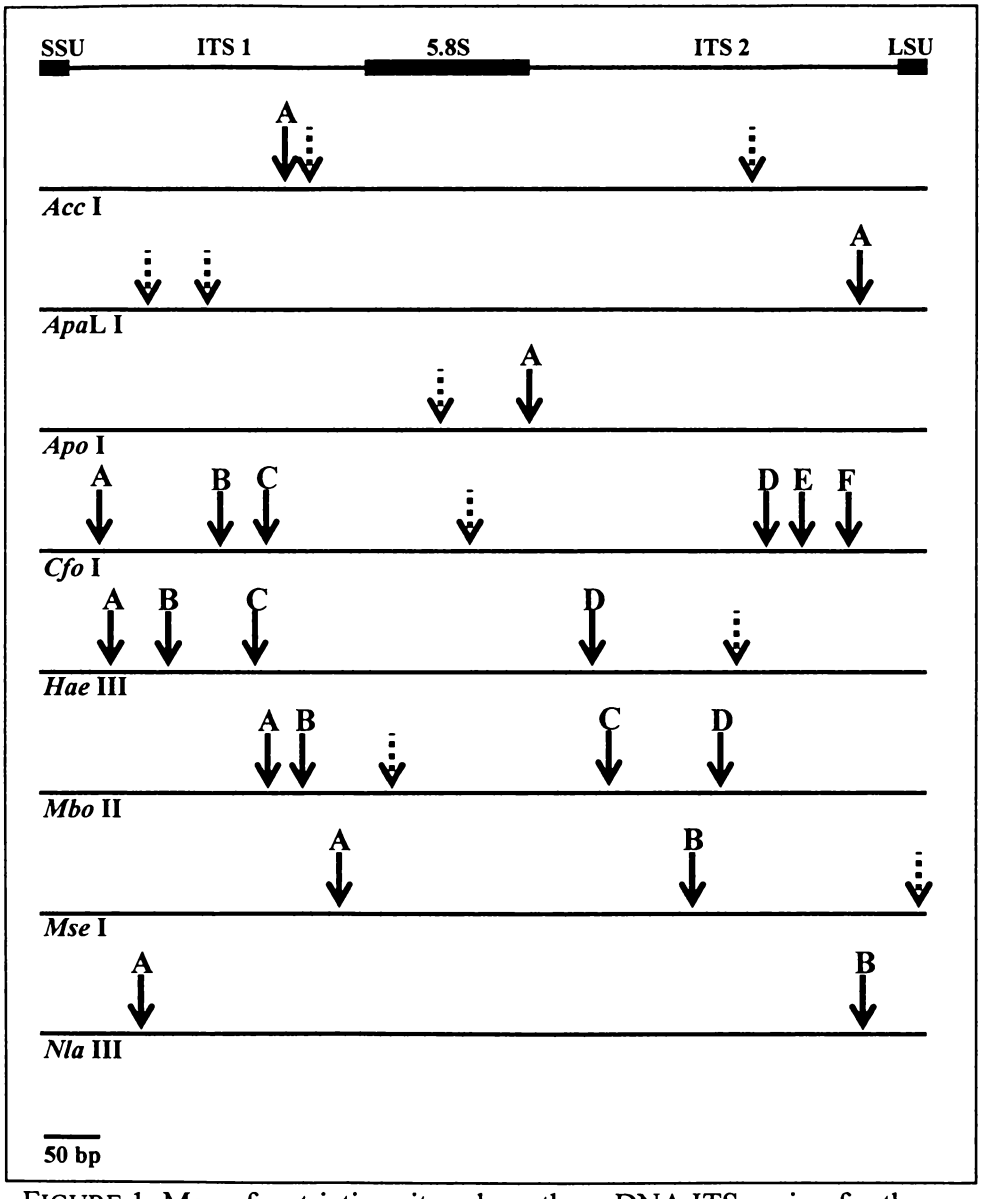


FIGURE 1. Map of restriction sites along the nrDNA ITS region for the antipodal *Artomyces* collections. Solid arrows indicate variable sites which are coded by letters. Dotted arrows indicate invariable sites.

TABLE 2. Results of the ITS RFLP survey of the antipodal *Artomyces* collections using eight restriction enzymes. *Artomyces piperatus*, Argentine *A. austropiperatus*, and *A. adrienneae* are included. Letters refer to specific restriction sites indicated in Figure 2. "0" indicates absence of variable restriction sites.

SPECIES	COUNTRY	PROVINCE	ISOLATE[a]	*Cfo* I	*Hae* III	*Acc* I	*Apa*L I	*Mbo* II	*Mse* I	*Nla* III	*Apo* I
A. adrienneae	Argentina	Chubut	8299 (55352), 8309 (54987), 8314 (54992), 8343 (55017)	A	X^b	X^b	X^b	X^b	X^b	X^b	X^b
		Neuquen	7384 (54435), 7386 (54404), 7387 (54434), 7389 (54426)	A	X^b	X^b	X^b	X^b	X^b	X^b	X^b
A. austropiperatus	New Zealand	Eastern Dist.	2822 (48916), 7153 (56948)	BD	ABCD	A	A	0	A	A	0
	Australia	Tasmania	RV95/509 (DHN 2890), RV95/511 (DHN 2892)	BD	ABCD	A	A	0	A	A	0
	Argentina	Chubut	8335 (55009)	BD	X^b	X^b	X^b	X^b	X^b	X^b	X^b
		Neuquen	8363 (55036)	BD	X^b	X^b	X^b	X^b	X^b	X^b	X^b
A. candelabrus	Australia	Tasmania	3922 (50128)	X^b	X^b	0	0	B	0	A	0
	New Zealand	Auckland	2685 (48919)	AB	AD	0	0	B	0	A	0
		Canterbury	2555 (48930)	AB	AD	0	0	B	0	A	0
		Southern Dist.	2637 (48917), 2667 (48920), 7067 (54122), 7044 (54108)	AB	AD	0	0	B	0	A	0
			2644 (48918)	AB	AD	0	0	B	0	A	0
		Tasman	7102 (56874)	AB	AD	0	0	B	0	A	0
			7110 (56219)	AB	AD	0	0	B	0	A	0
			7130 (56881), 55157 (43871)	AB	AD	0	0	B	0	A	0
A. colensoi	New Zealand	Auckland	2679 (48923), 2807 (48925)	F	A	0	0	AB	AB	AB	0
		Northland	55633 (44117)	F	A	0	0	AB	AB	AB	0
A. novazealandensis	New Zealand	Southern Dist.	2672 (48915), 7023 (54111), 7024 (#####), 7025 (56203), 7037 (56200), 7056 (56211)	A	CD	0	0	D	0	A	A
A. piperatus	Canada	British	5770 (53491), 6675 (DAUP)	B	ABCD	A	0	0	A	A	A
	United States	Alaska	8221 (57288)	B	ABCD	A	0	0	A	A	A
		Washington	5869 (51959)	B	ABCD	A	0	0	A	A	A

TABLE 2 cont.

SPECIES	COUNTRY	PROVINCE	ISOLATE	*Cfo* I	*Hae* III	*Acc* I	*Apa*L I	*Mbo* II	*Mse* I	*Nla* III	*Apo* I
A. tasmaniensis	Australia	Tasmania	3979 (50283), 4003 (50218), RV95/527 (#####)	ABD	ABC	0	0	AC	0	A	0
A. turgidus	Australia	Tasmania	3961 (50103)	ABC	AC	0	0	AB	0	0	0
	New Zealand	Auckland	2682 (48926), 7164 (56950)	ABC[c]	AC	0	0	AB	0	0	0
		Southern Dist.	2651 (48934)	ABC	A	0	0	AB	0	0	0
			7040 (54099), 55137 (44306)	ABC	AC	0	0	AB	0	0	0
		Tasman	7109 (56218)	ABC	AC	0	0	AB	0	0	0
	Papua New Guinea	Morobe	RV95/875 (DHN 3138)	ABCE	AC[c]	0	0	AB	0	0	0

[a] Isolate numbers are given with herbarium voucher numbers (in parentheses) as in Table 1. ##### = no herbarium number.

[b] X = no data.

[c] Each of these digests exhibited a single extra fragment, but additional restriction sites were not found in the sequences. This may be due to an undetected site or simply incomplete digestion.

Two Argentine isolates, 8335 and 8363, shared the same two *Cfo* I restriction sites as one of the antipodal groups found in New Zealand and Tasmania (TABLE 2). Sequences of Argentine isolate 8335 and New Zealand isolate 7153, recognized here as *A. austropiperatus*, were nearly identical. A second Argentine group of isolates, *A. adrienneae*, had *Cfo* I restriction sites in common with *A. novazealandensis* (TABLE 2), but analysis of sequences of Argentine isolate 7387 and New Zealand isolates 7024 and 2672 indicated that these species were distinct.

A combination of two restriction enzymes, *Cfo* I and *Hae* III, was used to segregate the Costa Rican collections into four distinct groups. One group was identified as *A. pyxidatus* and was verified by intercompatibility analysis. The other three groups were determined to represent the previously undescribed species *A. costaricensis*, *A. enigmatus*, and *A. stephenii*.

Phylogenetic reconstruction – With 128 parsimony-informative characters in the dataset, the heuristic search yielded twelve most parsimonious trees (MPTs) of 440 steps with a consistency index of 0.64 and retention index of 0.71 (FIG 2). Five major clades (Clades I through V) and two monospecific clades were resolved. Among the 12 MPTs, *A. tasmaniensis* was consistently placed in the basal position, but there was very low support in the arrangement of the other six clades. Clade I was composed of morphologically similar and narrowly endemic *A. dichotomus*, *A. carolinensis*, *A. piperatus*, and *A. costaricensis* as well as the more widespread *A. austropiperatus*. Clade II was a weakly supported group composed of *A. colensoi* from New Zealand, *A. cristatus* of northern coniferous forests, and *A. stephenii* of Costa Rica. Clade III was composed of *A. enigmatus*, found in Costa Rica and Puerto Rico, and Australasian *A. turgidus*. Although Dodd (1972) reported *Clavicorona turgida* specimens from the New World and India, cultures and specimens included in this study were from Australasia only. *Artomyces candelabrus* from Tasmania and New Zealand and *A. adrienneae* of Argentina comprised Clade IV. In seven of the twelve MPTs, *A. novazealandensis* weakly associated with Clades III and IV, but was placed as the third most basal in the other five MPTs. Clade V, composed of *A. pyxidatus* and *A. microsporus*, was consistently placed as the second most basal branch among the MPTs, but this was also poorly supported. Similar clades were observed when the complete dataset of 805 bp positions was used in the parsimony analysis.

No overall difference in the grouping of species was observed between parsimony and neighbor joining analyses (FIG 3). The same clades in the parsimony analysis were resolved in the neighbor joining analysis, and *A. tasmaniensis* was placed in the basal position. However, Clade I was placed in the second most basal position, as opposed to Clade V, and there was lower bootstrap support for Clade II.

Mating studies – Among the eight collections from Costa Rica surveyed in the mating analysis, two intersterility groups were identified (FIG 4). Monokaryons derived from five collections were compatible with *A. pyxidatus* exemplars. The second group was composed of monokaryons from collections 9433, 9436 and 9348 and were 100% intercompatible with each other. These were identified as belonging to *A. stephenii sp. nov.*

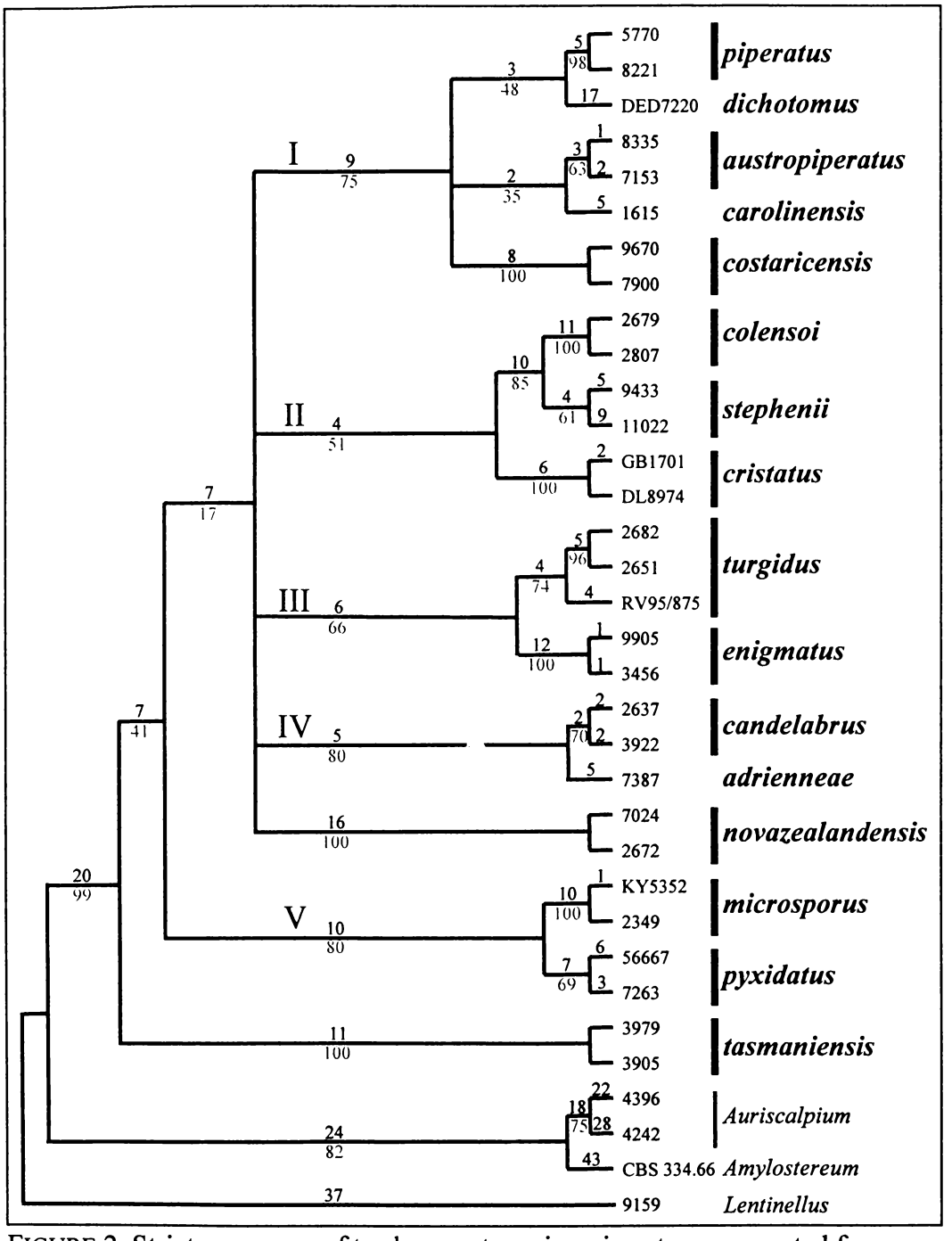


FIGURE 2. Strict consensus of twelve most parsimonious trees generated from a heuristic search of nrDNA ITS sequences of representative *Artomyces* species. Branch lengths are located above, and bootstrap values from 1000 replicates are indicated below each branch. Tree length = 440; CI = 0.64; RI = 0.71. Major clades are identified by Roman numerals.

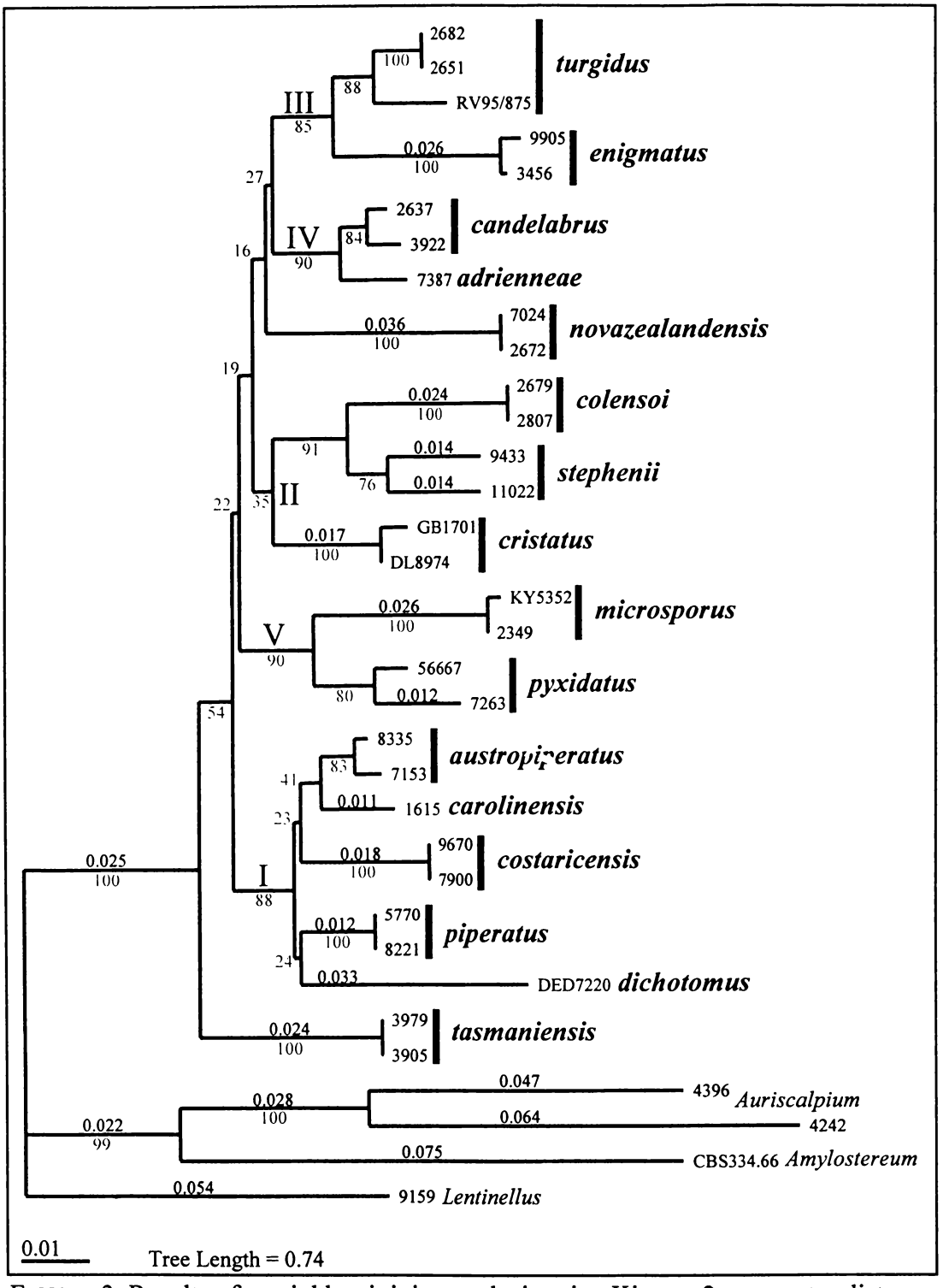


FIGURE 3. Results of a neighbor joining analysis using Kimura 2-parameter distance measure based on nrDNA ITS sequences of representative *Artomyces* species. Branch lengths longer than 0.010 substitutions/site are indicated above the branch; bootstrap values from 1000 replicates are indicated in whole numbers below or to left of each branch. Major clades are identified by Roman numerals.

	A. stephenii			*A. pyxidatus*								
				Costa Rica					WI	ME	SWE	CHI
	9433	9436	9438	9464	9465	9681	9932	10030	1506	1190	GB1279	1541
9433		4/4	4/4	0/4	0/4	0/1	0/3	0/3	0/3	0/4	0/4	0/1
9436	+		4/4	0/4	0/4	0/3	0/4	0/4	0/4	0/4	0/4	0/1
9438	+	+		0/4	0/4	0/3	0/4	0/4	0/4	0/4	0/4	0/1
9464	-	-	-		2/4	2/2	4/4	4/4	4/4	4/4	4/4	1/2
9465	-	-	-	+		3/3	4/4	4/4	4/4	4/4	4/4	1/1
9681	-	-	-	+	+		3/4	2/3	2/3	3/3	2/3	1/1
9932	-	-	-	+	+	+		4/4	4/4	4/4	4/4	1/1
10030	-	-	-	+	+	+	+		4/4	4/4	4/4	1/1
1506	-	-	-	+	+	+	+	+		4/4	4/4	1/1
1190	-	-	-	+	+	+	+	+	+		2/4	1/1
GB1279	-	-	-	+	+	+	+	+	+	+		1/1
1541	-	-	-	+	+	+	+	+	+	+	+	

FIGURE 4. Simplified results of the recognition grid of intercollection matings. Eight collections from Costa Rica and four exemplar collections representing *Artomyces pyxidatus* were included. Each cell above the diagonal indicates the number of compatible pairings out of the number attempted. Collection numbers are included in Table 1.

MORPHOLOGICAL CHARACTERIZATIONS

Clavicorona Doty. 1947. Lloydia 10: 39. *emend.* Lickey.
Type – *Craterellus taxophilus* Thom. 1904. Bot. Gaz. 37: 218.
Basidiomata obconic to coronate, unbranched, white, fruiting on duff of needles, leaves, and twigs under conifers. Clamp connections absent on generative hyphae of context. Conductive, aseptate hyphae in context sometimes terminating as pseudocystidia; contents show no reaction to sulfobenzaldehyde or Congo red. Basidiospores ellipsoid, white, hyaline, smooth, thick-walled, inamyloid.

Clavicorona taxophila (Thom) Doty. Lloydia 10: 39.
≡ *Craterellus taxophilus* Thom. 1904. Bot. Gaz. 37: 218.
≡ *Clavaria taxophila* (Thom) Lloyd. 1920. Myc. Notes 64: 1008.
Holotypus: USA, New York, Tompkins Co., Ithaca, Fall Creek Gorge, Thom, s.n., 19 Oct - 19 Nov 1903 (CUP-A-015445, *n.v.*).
= *Clavicorona mairei* (Battetta) Corner. 1950. Monograph, p.286.
≡ *Clavaria mairei* Battetta. 1938. Bull. Soc. Myc. 54:44.
Holotypus: unknown.
= *Clavicorona tuba* (Heim) Corner. 1950. Monograph, p.287.
≡ *Physalacria tuba* Heim. 1934. Treb. Inst. Catalana Hist. Nat. Sèr. Bot. 15:50.
Holotypus: unknown.
= *Clavaria corbierei* Bourdot & Galzin. 1927. Hym. Fr., p.112.
Holotypus: L. Corbière, s.n., Cherbourg, (P, *n.v.*).
Basidiomata (FIG 23A, B): Unbranched, truncate to coronate in stature, up to 30 mm high, up to 7mm diam. at apex, when fresh mostly white to "cartridge buff" (30A2) when young and aging to "ochraceous buff" (5A5) to "ochraceous," dried specimens "ochraceous buff" to "xanthine orange" (6C8) to "amber brown" (6D8). One specimen, C 10264, was freeze-dried and is white. Odor none; taste none based on one report.
Micromorphology: Hyphae in basal mycelia pad clamped, thick-walled (to 2µm thick), and echinate. Context hyphae thin-walled, hyaline, septate, without clamp connections. Gloeoplerous hyphae and gloeocystidia not observed. Pseudocystidia (FIG 5a) rare to common, sulfobenzaldehyde negative, not granular when stained with Congo red. Basidia (FIG 5b) narrowly clavate,16.0 – 24.0 µm long (L_m = 20.7 µm ± 2.9) X (2.8-) 3.2 – 4.4 (-4.8) µm wide (W_m = 3.8 µm ± 0.5), clamped.

Basidiospores (FIG 22A, FIG 5c) ellipsoid (E_m = 1.35 ± 0.05), (3.0-) 3.2 – 4.0 (-4.4) µm (L_m = 3.7 µm ± 0.3) X (2.2-) 2.4 – 3.2 (-3.6) µm, smooth, thick-walled (to 1 µm), usually guttulate.
Commentary: Thom (1904) described *Craterellus taxophilus* from collections he made on the Cornell University campus, explaining that the obconic, truncate stature of this organism was characteristic of *Craterellus*. In his description he reported that spores were broadly elliptical to subglobose and "3 – 4 µm," cystidia were numerous, 4 – 6 µm diam., arising from the trama and staining black in osmic acid. Lloyd (1920) transferred this species to *Clavaria*, conjecturing that the truncated apex illustrated by Thom (1904)

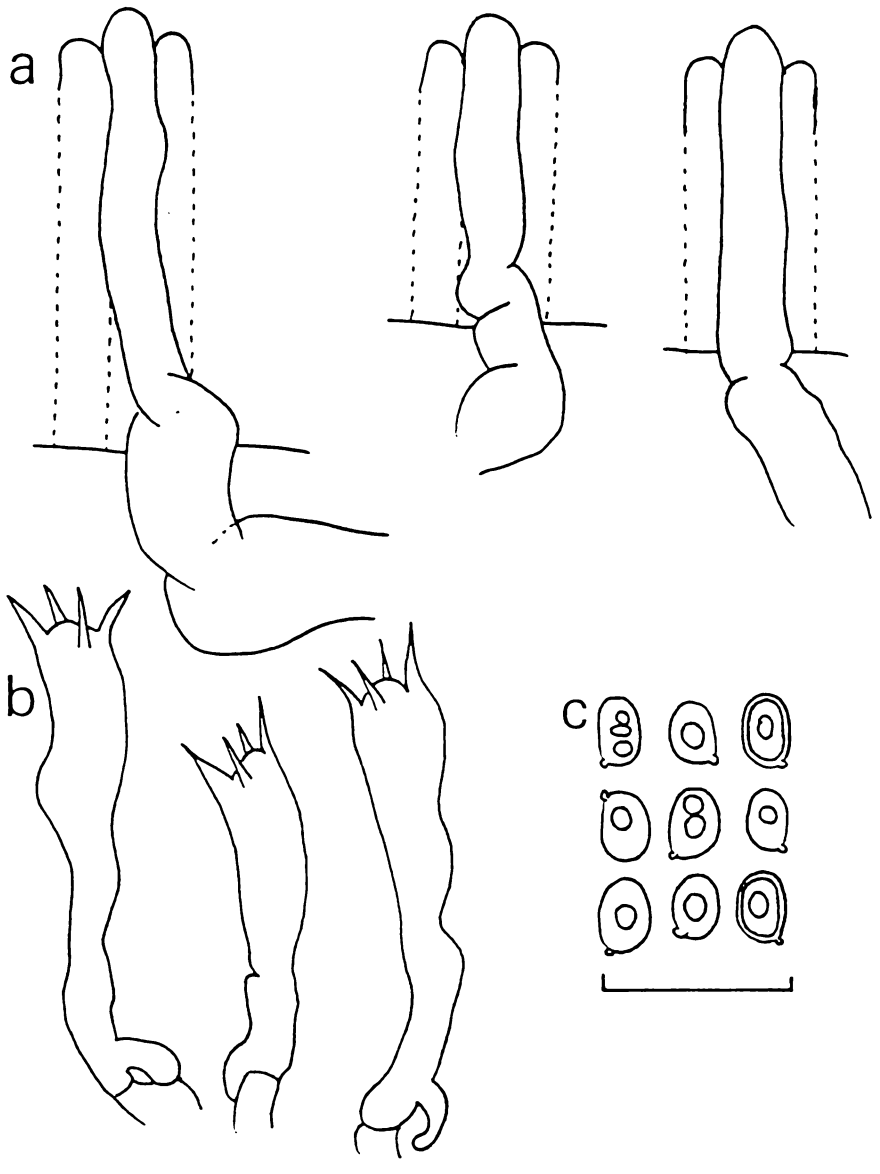


FIGURE 5. *Clavicorona taxophila*. a) pseudocystidia (TENN 51926), top of adjacent basidia and location of hymenium-subhymenium boundary are indicated; b) basidia (SAE 0890); c) basidiospores (SAE 0890), upper and lower right show wall thickness. Bar = 10 μm.

was "exaggerated" and was probably fertile. Doty (1944) reported that in the species he found fruiting under *Pseudotsuga taxifolia* the cup-shaped apex was sterile. Unfortunately, as Coker (1947) later pointed out, Doty's photograph (1944: pl. 6, Fig. B, p.79) labeled "*C. taxophila*" was incorrect. The morphological measurements Doty provided were typical of *C. taxophila*, but the picture was of some other species, perhaps *C. cristata* because it was on wood, and its apex was ringed with small branchlets. In his description, Doty (1947) reported that the gloeoplerous hyphae in the basidiome context did not have conspicuously granular contents like those of "related species," and that there were no clamp connections in the generative hyphae of the basidiome context. While it is evident that Corner (1950, 1970) did not study any specimens, he indicated he was unsure of the presence or absence of clamps. In their study of the type specimen, Leathers and Smith (1967) reported that the spores of *C. taxophila* were inamyloid, and placed it in sect. *Clavicorona*. Dodd (1972) also examined the type specimen but found that its spores were weakly amyloid, thin-walled and minutely asperulous at 3200X. He also observed that the hyphae of the context were not clamped and stated that gloeoplerous hyphae were rare. Josserand (1974) found the spores to be smooth, thick-walled and inamyloid and the context hyphae to be clampless. Likewise, Jülich (1981) reported spores as inamyloid and context hyphae without clamps, but Stalpers (1996) only mentioned the latter. Gennari and Losi (1997) found that the spores of their collection from Tuscany were inamyloid and without clamped hyphae in the context. The SEM micrographs of specimen C-10264 clearly indicate spores are smooth (FIG 22A).

Unfortunately, the type specimen of this species, which in turn happens to be the type species for *Clavicorona*, is missing from CUP (Gruff and Hodges pers. comm.). However, three ancillary specimens were borrowed from CUP where the type was located. In addition, two specimens were borrowed from C as well as two specimens from TENN to provide a range of what is considered by most to exemplify *C. taxophila*. Five of these specimens were critically examined and were nearly identical. They all exhibited small, smooth, thick-walled, inamyloid, ellipsoid basidiospores. Gloeoplerous hyphae and gloeocystidia typical of *Artomyces* were absent, although there were some analogous pseudocystidia that originated deep below the hymenium. No clamps were observed on hyphae in the context. Spore sizes found in this study agreed with measurements reported in the above citations, which were relatively constant.

DNA was isolated from three different collections of *C. taxophila*, TENN 51926, C 10264, and isolate 9186 (from D. Largent, Humboldt State University, CA). According to nuclear large subunit (LSU) and ITS ribosomal DNA sequence comparisons, *C. taxophila* is very divergent from exemplars of *Artomyces* and members of the outgroup. Their sequences could not be aligned, and *C. taxophila* sequences were left out of phylogenetic analyses. Basic BLAST searches in GenBank (www.ncbi.nlm.nih.gov) using the LSU sequences revealed a possible close association with members of the *Omphalina - Chrysomphalina - Gerronema* group.

Battetta (1938) described the apparently terricolous *C. mairei* as small, cyathiform to craterelloid with the margin of the apices of at least a couple basidiomata pleurodentate. Spores were 4.5 X 3.5 µm, smooth, inamyloid, and some form of cystidia were present. He compared this new species to *Clavaria corbierei*, but distinguished it

on the basis of its craterelloid stature, sterile apex and cystidia similar to those found in *C. pyxidata*. There was no mention of presence of clamps. Corner (1950), not having examined the type specimen, was unsure if clamps were present. He accepted this as a primitive form of *Clavicorona*, suggesting that it might be synonymous with *C. taxophila*, but he also briefly entertained the idea that this species might be a reduced *Omphalia*. However, because Battetta (1938) described the apex as "pleurodentate," that was enough to convince Corner (1950) that it was *Clavicorona*. Dodd (1972) also suggested that *C. mairei* might be a synonym of *C. taxophila*, but was unable to locate the type specimen, which is still unknown. In the absence of the type, Battetta's (1938) figures and descriptions may provide some important clues. The basidiomata illustrated in the photograph (Battetta 1938: Fig. 1, p.45) seem to indicate that the pleurodentate margin is really more lobed and eroded, but his drawings (Fig. 3-2, p.46) clearly show almost pyxidate branching. Presumably, the drawings were based on the basidiomata illustrated in the photograph, and this may or may not be the result of some exaggeration or artistic liberty. Although he did not mention so in the text, it is apparent in another drawing (Battetta 1938: Fig. 3-10, p.45) that clamps were absent from the generative hyphae in the context. Overall, the characteristics of this species are quite the same as those of *C. taxophila*. Josserand (1974) examined two specimens collected by Locquin as *C. taxophila* to determine if they could be *C. mairei*. He also was unable to locate authentic type material for *C. mairei*, but he found few differences between Battetta's (1938) description and the descriptions of *C. taxophila*, and therefore, concluded that the two names were most likely synonymous.

Heim (1934) described *Physalacria tuba* fruit bodies as resembling miniature fruit bodies of *Pistillina stilboidea*, but thought it better represented a species of *Physalacria*. Doty (1947) later synonymized *P. tuba* under *C. taxophila*, but provided no explanation. However, Corner (1950) recognized it as distinct from *C. taxophila* based on what he interpreted as textured and more elongate spores. Dodd (1972) was unable to locate Heim's authentic or type specimens and was hesitant to make any taxonomic decisions on the relationship of *P. tuba* with other members of the *Clavicorona*. However, because basidiomata are unbranched, Dodd (1972) placed this species in his subg. *Clavicorona* with *C. taxophila* and *C. mairei* and suggested all three might be synonymous.

Paechnatz (1984) reported what he identified as *C. tuba* from Germany. He described the basidiomata as pure white, unbranched, 4-18 mm tall expanding from 1 mm at base to 2.8 mm at the coronate to cup-shaped apex. Basidioma base was slightly bulbous and covered with dense, white hairs. Basidia were 2- 4-spored, 13.6 – 34.8 X 2.6 – 4.8 μm, and clamped. Hyphae of the basal mat were clamped but there was no mention of clamped hyphae in the context. Gloeocystidia were up to 8.9 μm diam. and projected up to 20 μm from hymenium. Spores were mostly long-oval to cylindric, rarely round, 2.4 – 5.2 X 1.9 – 3.3 μm, very finely warted to almost smooth, and guttulate. Paechnatz's specimen was obtained for this study from both Martin Luther University (HAL 1721) and Staatliches Museum für Naturkunde Görlitz (GLM 14223). Spore dimensions were found to be similar (3.2 – 3.8 X 2.2 – 3.0 μm) to those reported by Paechnatz (1984), but spores appeared smooth, slightly thick-walled and inamyloid. The hyphae in the basal mycelial pad were clamped and often echinate and thick-walled, but no clamps were

observed on the hyphae of the context, and gloeoplerous hyphae were not seen. Based on these observations, it is likely that Paechnatz's (1984) specimen represents *C. taxophila*.

My assessment of *Clavaria corbierei* is based on Bourdot and Galzin's (1927) description because its type specimen is unavailable at present. Basidiospores were described as smooth, hyaline, uniguttulate, subglobose to broadly ellipsoid, 3 – 4.5 (-5) X 3 – 4 µm. Corner (1967) described the spores of two collections (R.J. Bandoni B.C. 699 and J.R. Weir 16963) as smooth, inamyloid, 3 – 4 X 2.2 – 3 µm and reported that clamps are absent on the generative hyphae in the context. It was reported to fruit among moss and on needles under conifers and is known from Priest Lake, Idaho (Corner 1967), the site of several TENN collections of *Clavicorona taxophila*. Interestingly, Corner (1967, p.35) further suggested that *Clavaria corbierei* represented "a gill-less *Omphalina*, but with hyphae typical of *Clavaria*."

From data presented here, it is suggested that *Clavicorona taxophila*, *C. mairei*, *C. tuba* and *Clavaria corbierei* Bourd. & Galz. represent a single morphological species. The above nomenclator, with *Clavicorona taxophila* given priority, is tentative until authentic, if not type specimens can be found to indicate otherwise. Genetically, this group may have its closest relatives in the *Omphalina* complex. A study of the relationships with these agarics is imperative to further understand the taxonomy of this group, but it is conceivable that these coronate basidiomata represent a reduced, gill-less omphalinoid agaric species. Support of this hypothesis comes from Singer (1986) who indicated that several members of omphalinoid agarics have contextual hyphae without clamp connections.

Distribution and Habit: Collections identified as *Clavicorona taxophila* have been reported from northern California, northern Idaho, New York, British Columbia, Quebec, and Denmark, apparently associated with leaf and needle duff under *Taxus*. Gennari and Losi (1997) reported it from Tuscany, Italy under *Cupressus*, *Chamaecyparis*, and *Pinus*. *Clavicorona mairei* is known only from the type collection from Bron, France. *Clavaria corbierei* was described from Cherbourg, France and reported from British Columbia and Idaho.

Specimens: CANADA, Prov. Quebec, Portneuf Co., Duchesnay, 26 Aug. 1938, coll. Fitzpatrick, CUP-027411. DENMARK, Nordjylland, east of Himmerige, south of Ålbæk, 11 Oct. 1983, coll. S.A. Elborne, SAE 0890; Vestjælland, Sorø, "Runde mose," 16 Oct. 1976, coll. U. Søchting, (C-10264). USA, Idaho, Bonner Co., Priest Lake, 27 Sept. 1968, coll. R.H. Petersen, RHP 3958, (TENN 34137); Idaho, Shoshone Co., Coeur d'Alene National Forest, vic. Wallace, 24 Sept. 1992, coll. R.H. Petersen, TFB 5608, (TENN 51926); New York, Tompkins Co., Enfield, Enfield Gorge, 14 Oct. 1905, coll. G.F. Atkinson, (CUP-A-019487); New York, Tompkins Co., Ithaca, north side of Fall Creek by Suspension Bridge, 1 Oct. 1912, coll. Ishikawa, (CUP-A-023462a).

Artomyces Jülich. 1981. Bibliotheca Mycologica 85: 395.
Type – *Clavaria pyxidata* Pers.: Fr. 1821. Systema Mycologicum, vol.1, p.470.
Basidiomata pyxidately branched, lignicolous. Trama monomitic, but dimitic in *A. colensoi* and *A. dichotomus*. Generative hyphae inflated, thin-walled, clamped. Skeletal hyphae (when present) with walls 1.0 – 1.5 µm thick. Gloeoplerous hyphae present in trama, terminating in hymenium as gloeocystidia; contents sulfobenzaldehyde positive, granular in Congo red. Basidia 4-sterigmate, rarely 2-sterigmate, narrowly clavate to cylindric, thin-walled, clamped. Basidiospores small, white, hyaline, thin-walled, amyloid, asperulate but smooth in *A. cristatus*, often guttulate.
Commentary – Although almost all species exhibited amyloid, asperulous spores the degree of amyloidity and ornamentation seemed to vary within single collections and may be due to the stage of spore development. Spore ornamentation in several species was verified by scanning electron microscopy at 10,000X magnification (FIG 22), and some differences were observed. Spores of *A. turgidus* showed larger asperulae (FIG 22H) than those of *A. piperatus* (FIG 22F) while those of *A. cristatus* clearly exhibit smooth walls (FIG 22D). Unfortunately, the degree of difference was too small and variable to be measurable.

A few characters were inconsistent, and were not used in describing species. Leptocystidia appear to be very plastic, often varying in size and density among different sections of the same collection. Thickness of hymenium and sub-hymenium also appears to be variable depending on where along the branches a section is taken, and at what angle the sections are sliced. None of the specimens exhibited a conspicuously gelatinized hymenium. However, due to varying drying techniques, the hymenia of some specimens were poorly preserved, and this may give a gelatinized appearance.

Key to the Species of *Artomyces*

1. Basidiomata unbranched to rarely twice branched 2
1. Most basidiomata branched more than twice 3

2. Basisiomata rarely branched; spores smooth, $L_m > 5.5$ µm, cylindric, $E_m < 2.0$ ..... *A. cristatus*
2. Basidiomata unbranched; spores asperulate, $L_m < 5.5$ µm, ellipsoid ..... *A. tuba*

3. Trama with thick-walled (wall ≥ 1 µm thick) hyphae 4
3. Trama without thick-walled hyphae 5

4. Basidiomata petite; spores $L_m = 4$ µm, $E_m = 1.5$; NEW ZEALAND ..... *A. colensoi*
4. Basidiomata robust; spores $L_m = 3.6$ µm, $E_m = 1.25$; INDONESIA ..... *A. dichotomus*

5. Spores $L_m \geq 4.5$ µm 6
5. Spores $L_m < 4.5$ µm 10

6. Basidiomata petite; branches 1 mm diam. or less 7
6. Basidiomata stout; branches often up to 3 mm diam 8

7. Basidiomata white; ARGENTINA ..... *A. adrienneae*
7. Basidiomata dusky; AUSTRALIA and NEW ZEALAND ..... *A. candelabrus*

8. Basidiomata white to light colored; spores L_m = 4.5 µm;
COSTA RICA and PUERTO RICO ..... *A. enigmatus*
8. Basidiomata dusky; spores L_m = 5.0 µm 9

9. Genetic profile as in FIG 1, TABLE 2 ..... *A. austropiperatus*
9. Genetic profile as in FIG 1, TABLE 2 ..... *A. novazealandensis*

10. Spores L_m = 3.5 µm; basidiomata profusely branched; JAPAN ..... *A microsporus*
10. Spores L_m > 3.5 µm 11

11. Spores L_m = 4.1 µm, elongate, E_m > 1.4; PAN-NORTH TEMPERATE ..... *A. pyxidatus*
11. Spores broader, E_m < 1.4 12

12. Spores broadly ellipsoid, E_m < 1.3 13
12. Spores ellipsoid, E_m > 1.35 15

13. Basidiomata very stout, turning dark brown to black in drying;
branches to 3 mm diam., usually in 2-3 ranks; TASMANIA ..... *A. tasmaniensis*
13. Basidiomata stout, turning light to medium brown in drying;
branches to 2 mm diam., usually in more than 3 ranks;
NORTH AMERICA 14

14. Basidiomata often "avellaneous" (7B3); substratum conifer-wood;
AMERICAN PACIFIC NORTHWEST ..... *A. piperatus*
14. Basidiomata "buff brown" (6D4); substratum hardwood;
WESTERN NORTH CAROLINA ..... *A. carolinensis*

15. Basidiomata turning dark brown to black in drying; AUSTRALASIA ..... *A. turgidus*
15. Basidiomata turning light to medium brown in drying; COSTA RICA 16

16. Basidiomata profusely branched; branches usually in more than
5 ranks, reduced gradually upward ..... *A. stephenii*
16. Basidiomata not as well-branched; branches usually in less than
5 ranks, not noticeably reduced upward except near top ..... *A. costaricensis*

1. *Artomyces adrienneae* Lickey, *sp. nov.* [*nom. prov.*].

Basidiomata usque ad 40 mm alto et 15 mm lata, ramosus, pyxidatus, specimen exsiccatum pallidus ochraceus bubalinus ad pallidus cinnamomeus roseus. Stipes saepe praesens ad 1 (-2) cm alto. Rami gracilis dilatatus ad 1 mm; rami inferiora et rami superus alba. Apices plerumque coronatus-cristatus. Gloeocystidia communis, lavatus ad cylindraceus ad obclavatus, ad 14 µm diametro, interdum extans ultra hymenium 10 µm. Basidia circa 22.9 X 5.1 µm. Basidiosporae ellipsoideae, (3.8-) 4.2 – 5.2 (-5.6) X 2.8

– 3.6 (-3.8) µm, E_m = 1.44 ± 0.08, amyloideae, hyalinus, asperulatus. Lignatilis angiospermae praecipue Nothofagus.

Holotypus [*hic designatus*]: CHILE, Prov. Valdivia, vic. Villa d'Angostura (Argentina), 1 km on Chilean side of border, 13 April 1995, coll. R.H. Petersen, TFB 7387 (TENN 54434).

Etymology: in honor of my wife, Adrienne L.A. Lickey.

Basidiomata (FIG 23C, D): scattered to gregarious, up to 40 mm tall X 15 mm wide, petite. Branches in 3 - 5 ranks, usually 3 - 4 per node, slender, rarely more than 1 mm diam., "pure white" to white, mellowing to "fleshy buff." Stipe to 20 mm tall X 2 mm diam.; basal mycelial mat whitish. Tips mostly coronate-cristate. No odor or taste observations reported. Dried basidiomata "pale ochraceous buff" (4A2) to "pale cinnamon pink."

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 6 µm diam, aseptate. Gloeocystidia (FIG 6a) usually cylindric to clavate, sometimes obclavate, to12 µm diam., or obclavate and inflated to 14 µm diameter in subhymenium then narrowing to 6 µm in hymenium, non-emergent to protruding to 10 µm. Basidia (FIG 6b) 20.0 – 28.0 µm (L_m = 22.9 µm ± 1.6) X 4.4 – 6.0 µm (W_m = 5.1 µm ± 0.5), clamped.

Basidiospores (FIG 6c) ellipsoid (E_m = 1.44 ± 0.08), (3.8-) 4.2 – 5.2 (-5.6) µm (L_m = 4.6 µm ± 0.4) X 2.8 – 3.6 (-3.8) µm, asperulate, often guttulate.

Commentary: According to the phylogenetic estimation presented in this study, *A. adrienneae* and *A. candelabrus* comprise sister clades (FIGS 2, 3). With respect to micromorphological characters, this species is almost identical to *A. candelabrus*, with the exception of distinctly obclavate gloeocystidia in *A. adrienneae*. However, the most obvious difference is that basidiomata of *A. adrienneae* are pure white when fresh, while those of *A. candelabrus* (*q.v.*) are "light ochraceous buff" (5A4) to "tilleul buff" (7B2).

Corner (1957) probably identified these white, Argentine specimens as *Clavicorona turgida*, because his account of spores (4 – 5 X 3 – 3.5 µm) and basidia (20 – 30 X 5 – 6 µm) is identical to the above. Both his spore and basidia measurements were slightly larger than those reported for *A. turgidus* in this study. Interestingly, Corner (1957) also reported smooth spore walls, whereas those of *A. adrienneae* and *A. turgidus* are clearly asperulate. Further indication that the Argentine group is distinct from *A. turgidus* as defined here is that specimens of *A. turgidus* are usually darker colored and become dark brown when dried whereas specimens of *A. adrienneae* are always pure white and merely become cream colored when dried. Finally, the phylogenetic estimations indicated that *A. turgidus* and *A. adrienneae* are not closely related (FIGS 2, 3).

Distribution and Habit: *Artomyces adrienneae* is known from Argentina and neighboring Chile. The substrate is hardwood, most likely *Nothofagus*.

Specimens: CHILE, Prov. Valdivia, vic. Villa d'Angostura (Argentina), 1 km on Chilean side of border, 13 April 1995, coll. R.H. Petersen, TFB 7387 (HOLOTYPE - TENN 54434). ARGENTINA, Prov. Chubut, Parque Nacional Los Alerces, Lago Menendez, elev. 570 m, 28 March 1992, coll. E. Horak, ZT 6839 (TENN 59139); Prov. Chubut, Parque Nacional Lago Puela, north shore, 10 May 1996, coll. R.H. Petersen, TFB 8343 (TENN 55017).

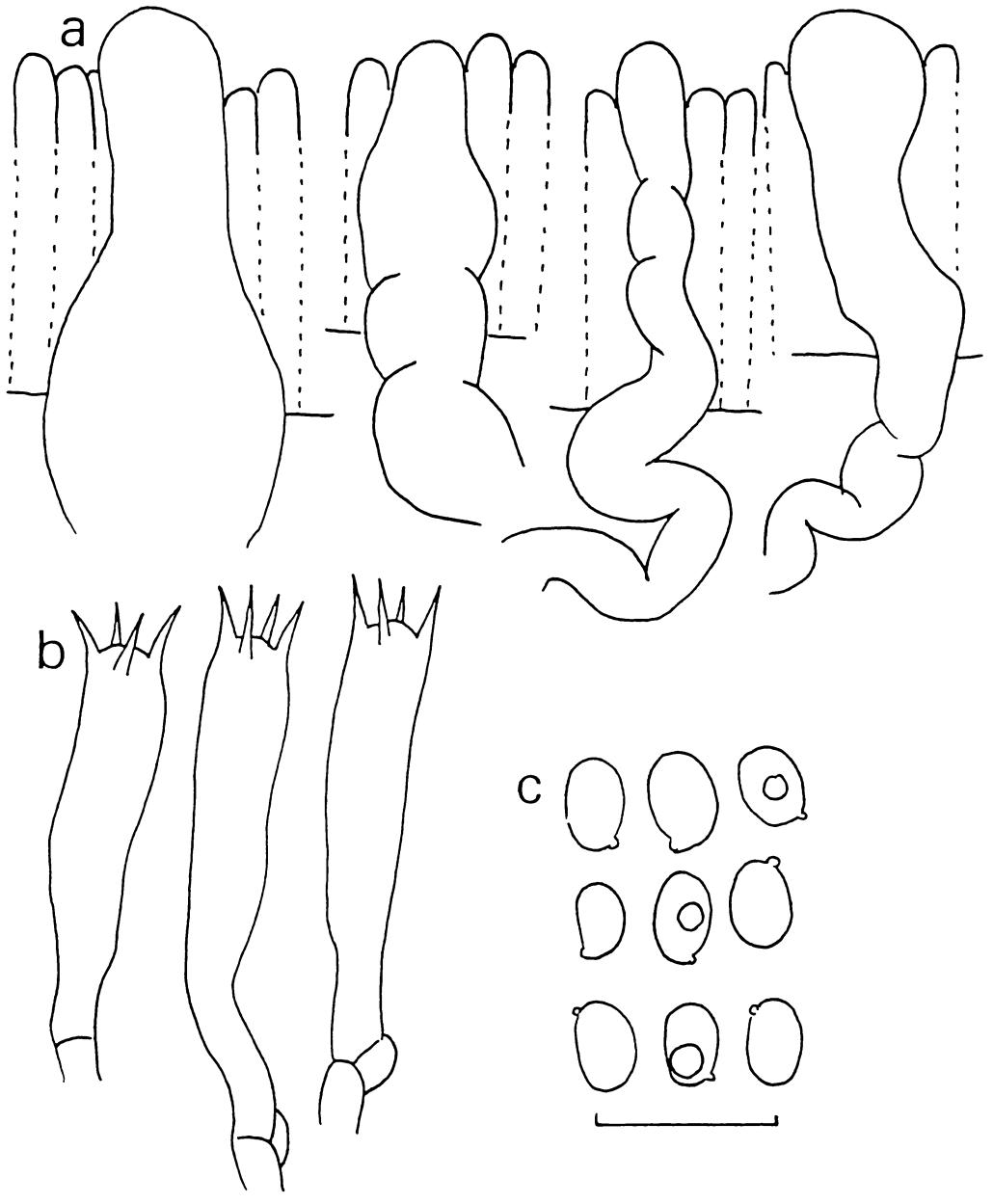


FIGURE 6. *Artomyces adrienneae* (TENN 54434). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

2. *Artomyces austropiperatus* Lickey, *sp. nov.* [*nom. prov.*].

Basidiomata usque ad 80 mm alto et 40 mm lata, ramosus, pyxidatus, specimen exsiccatum badius ad memnonius ad nigellus. Stipes praesens ad 20 mm alto. Rami crassus dilatatus ad 3 mm; rami inferiora avellaneus ad bubalinus bruneus ad cinnamomeus bubalinus; rami superus cinnamomeus bubalinus ad roseus bubalinus. Apices cuspidatus ad coronatus-cristatus. Sapor tarde vel cito acerbis. Gloeocystidia communis, cylindraceus ad late fusiformis, 2.0 – 10.0 µm diametro, saepe extans ultra hymenium ad 30 µm. Basidia circa 29.4 X 4.8 µm. Basidiosporae late ellipsoideae, (4.4-) 4.8 – 6.0 X (3.4-) 3.6 – 4.8 µm, E_m = 1.26 ± 0.02, amyloideae, hyalinus, asperulatus. Lignatilis angiospermae saepe Nothofagus. *Differt a* Artomyces novazealandensis *genetice.*

Holotypus [*hic designatus*]: ARGENTINA, Prov. Neuquen, Parque Nacional de los Arayanes, 14 May 1996, coll. R.H. Petersen, TFB 8363 (TENN 55036).

Etymology: *austro-* southern; *-piperatus* for its overall morphological resemblance and apparent close genetic relation to the Pacific Northwest species.

Basidiomata (FIG 23E, F)**:** up to 80 mm tall X 40 mm wide, usually well-branched, more or less compact, stoutish. Branches in up to six ranks, 4 – 6 per node, up to 3 mm diameter (6 mm in fresh specimen TENN 55009), "wood brown" (7C4), "vinaceous buff" (9B2), "cinnamon buff" (6B4), "pinkish buff" (6A3), to "tilleul buff" (7B2). Stipe up to 20 mm long X 4 mm diam. (5 mm in HN 2892), "olive brown" (5E5), "buff brown" (6D4), "cinnamon buff" (6B4), to "avellaneous" (7B3); basal mycelial pad creamy white to dark brown. Branch tips cuspidate to coronate-cristate. Odor described as mildly peppery, taste slowly to quickly acrid. Dried basidiomata from "Brussels brown" (6E6), "burnt umber" (7E7), "blackish brown" (7F5), "wood brown" (7C4), to "cinnamon rufous" (6D7).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae common, up to 10 µm in diam, aseptate. Gloeocystidia (FIG 7a-c) cylindric to broadly fusiform, 2.0 – 10.0 µm wide, non-emergent to protruding to 48 µm, occasional to common. Basidia (FIG 7d) 20.0 – 36.0 (-42.0) µm (L_m = 29.4 µm ± 4.8) X 4.4 – 6.4 (-7.0) µm (W_m = 5.9 µm ± 0.5), clamped.

Basidiospores (FIG 7e) broadly ellipsoid to ellipsoid (E_m = 1.26 ± 0.03), (4.4-) 4.6 – 5.6 (-6.0) µm (L_m = 5.1 µm ± 0.3) X (3.4-) 3.6 – 4.8 µm, obviously asperulate, usually guttulate.

Commentary: Specimens of *Artomyces austropiperatus* and *A. novazealandensis* are virtually identical in stature and micromorphology. Dodd (1972), Petersen (1988), and Wu (1991) considered southern collections of this morphology as representing *Clavicorona piperata* even though their spores were consistently at least 1 µm longer and 1 µm wider. Later, Wu et al. (1996) found that the southern collections had laccase electrophoretic patterns quite different from those of *C. piperata* and suggested that the southern collections represented a distinct species. The two cultures they used to represent their "Taxon 3," also included in this study, shared one common laccase band, but one culture exhibited two additional bands. According to ITS sequences, TENN 48916 (isolate 2822) was placed in the group described here as *A. austropiperatus*, whereas TENN 48915 (isolate 2672) was placed in *A. novazealandensis*.

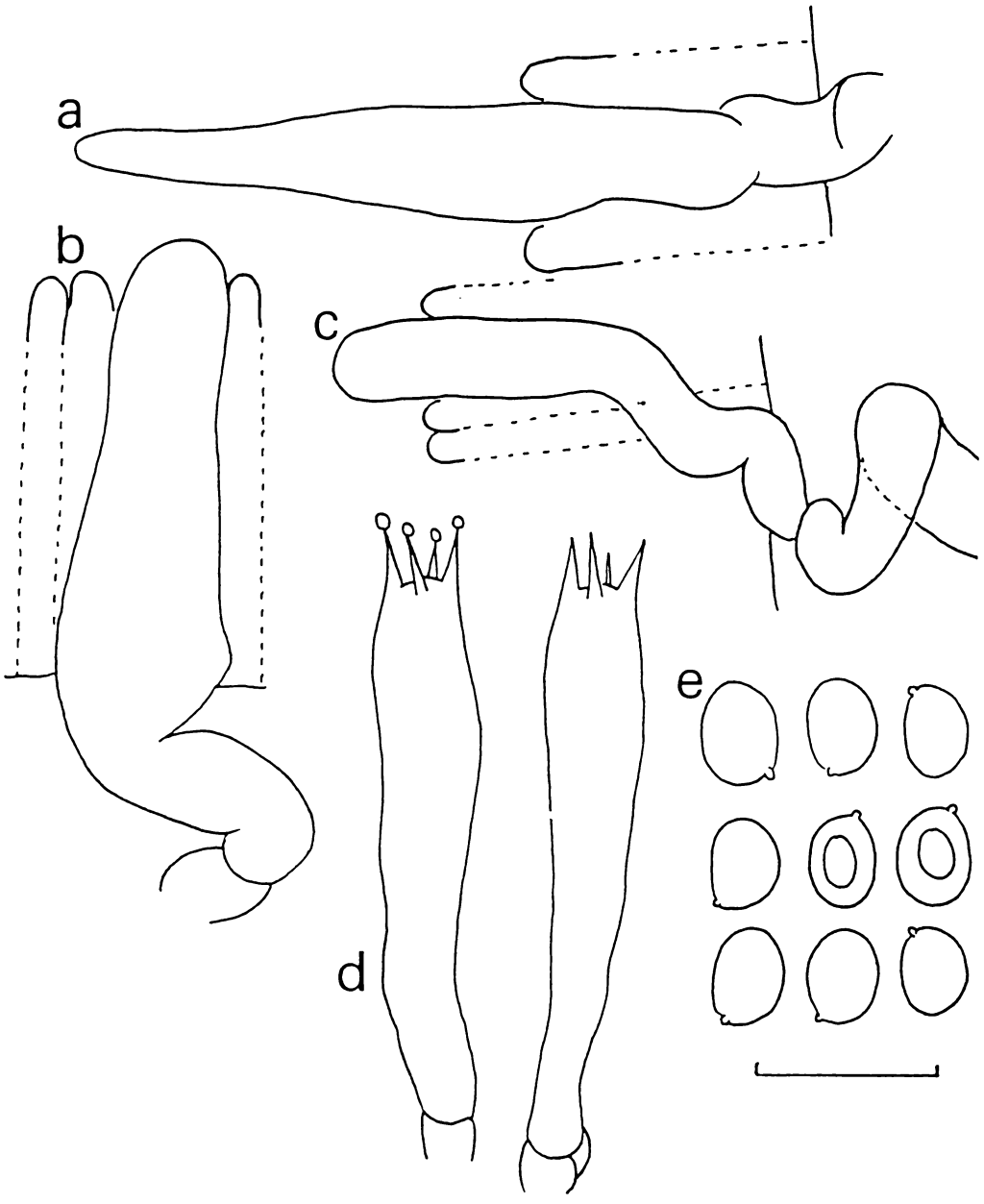


FIGURE 7. *Artomyces austropiperatus*. a) gloeocystidia (DUKE HN2890); b) gloeocystidia (TENN 48916); c) gloeocystidia (DUKE HN2892); d) basidia (DUKE HN2890); e) basidiospores (without ornamentation; DUKE HN2892). Bar = 10 μm.

These two species are significantly divergent with respect to their DNA sequences. *Artomyces austropiperatus* was nested within Clade I with *A. piperatus*, whereas *A. novazealandensis* either comprised its own unique clade or grouped with a "*candelabrus-turgidus*" clade in five of twelve MPTs. Apparently, the best way to distinguish these two cryptic species is through diagnostic restriction site data identified in this study (FIG 1, TABLE 2).

Distribution and Habit: This species is known from Argentina, Tasmania, and New Zealand (North Island). It is found primarily on *Nothofagus*.

Specimens: ARGENTINA, Prov. Chubut, Parque Nacional Lago Puela, north shore, 10 May 1996, coll. M. Rajchenberg, TFB 8335 (TENN 55009); Prov. Neuquen, Parque Nacional de la Arayanes, vic. Villa d'Angostura, 14 May 1996, coll. R.H. Petersen, TFB 8363 (HOLOTYPE - TENN 55036); Prov. Neuquen, Lago Nahuel Huapi, "Pto. Blest," 1 Apr 1992, coll. E. Horak, ZT 6768, (TENN 59138). AUSTRALIA, Tasmania, Mt. Field National Park, Russell Falls Nature Walk, 20 May 1995, coll. R. Vilgalys, RV 95-511 (DUKE HN2892); Tasmania, Mt. Field National Park, Russell Falls Nature Walk, 20 May 1995, coll. R. Vilgalys, RV 95-509 (DUKE HN2890). NEW ZEALAND, North Island, Urewera National Park, 29 May 1990, coll. R.H. Petersen and K.W. Hughes, TFB 2822 (TENN 48916); North Island, Urewera National Park, Track to Waipai Swamp, 27 May 1994, coll. R.H. Petersen, TFB 7153 (TENN 56948).

3. ***Artomyces candelabrus*** (Massee) Jülich. 1981. Bibliotheca Mycologica 85: 399.

≡ *Clavaria candelabra* Massee. 1899. Kew Bull. Misc. Info. p.172.

≡ *Clavicorona candelabrum* (Massee) Corner. 1950. Monograph, p.286.

Holotypus: MALAYSIA, Selangor, no date, coll. Ridley, Ridley 37 (K 69007 !).

Basidiomata (FIG 23G, H): scattered to gregarious, up to 35 mm tall X 35 mm wide, petite, loosely branched, consistency tough. Branches in 3 – 4 (-6) ranks, usually 2 – 3 per node, slender, rarely more than 1 mm diameter, "light ochraceous buff" (5A4), "pale ochraceous buff" (4A2), "vinaceous buff" (9B2), and "tilleul buff" (7B2). Stipe often present, to 10 mm tall X 1 mm diam., "clay color" (5C6), "avellaneous" (7B3), and "wood brown" (7C4); basal mycelial mat brown. Tips cuspidate to coronate-cristate. Odor described as "typical" (spicy), taste acrid. Dried basidiomata "cinnamon rufous" (6D7) and "amber brown" (6D8) to "auburn" (7E5) and "Vandyke brown" (7E6) to "walnut brown" (7D6) and "mars brown" (8F7).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, to 10 (-12) µm diam., aseptate. Gloeocystidia (FIG 8a-b) cylindric to often obclavate with a very inflated region (-14 µm diam.) in subhymenium narrowing to 4 – 6 (-8) µm diam. as it enters the hymenium, non-emergent to protruding to 16 µm. Basidia (FIG 8c) 18.0 – 30.0 µm (L_m = 24.9 µm ± 3.5) X 4.0 – 6.0 µm (W_m = 5.2 µm ± 0.7), clamped.

Basidiospores (FIG 8d) ellipsoid (E_m = 1.45 ± 0.08), (3.6-) 4.0 – 5.2 (-6.0) µm (L_m = 4.6 µm ± 0.4) X (2.6-) 2.8 – 3.8 (-4.0) µm, obviously asperulate, often guttulate.

Commentary: For some time there has been taxonomic confusion between *A. colensoi* and *A. candelabrus*. To distinguish *Clavicorona candelabrum* from *C. colensoi*, Corner (1950, 1970), Dodd (1972), and Wu et al. (1996) all placed emphasis on skeletal hyphae

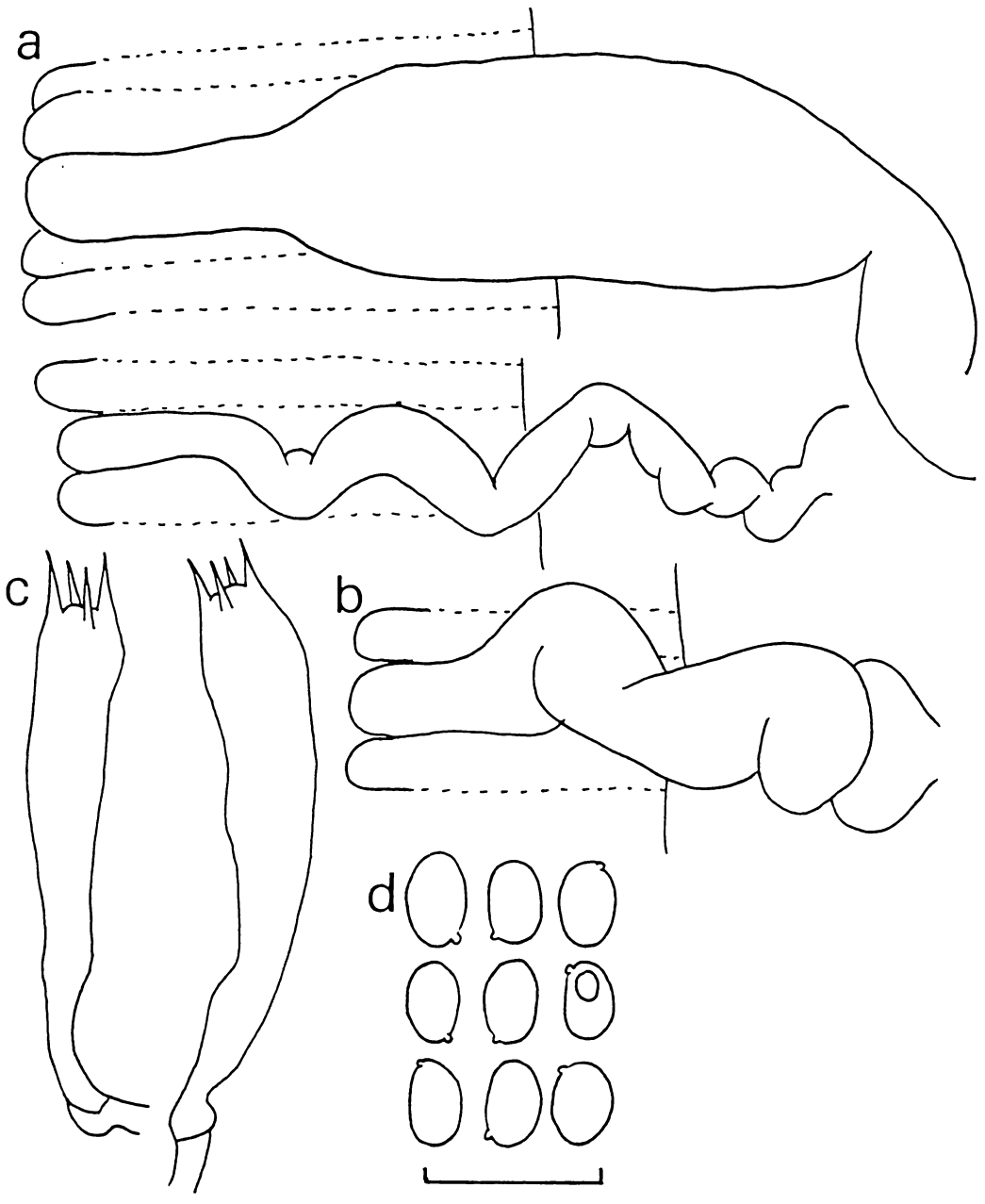


FIGURE 8. *Artomyces candelabrus*. a) gloeocystidia (TENN 48917); b) gloeocystidia (TENN 48919); c) basidia (TENN 48917); d) basidiospores (without ornamentation; TENN 48917). Bar = 10 µm.

found in the context of *A. candelabrus* basidiomata versus the monomitic construction in *C. colensoi*. Spore measurements among these studies varied and overlapped, but the general pattern was that spores of *C. colensoi* were slightly larger than those of *C. candelabrum*. Petersen (1985, 1988) treated this taxon as a synonym of *Clavicorona colensoi* because he did not find thick-walled hyphae in the *Clavaria candelabra* type specimen (Ridley 37, K 69007) and found little differences in spore sizes. In a study of enzyme banding patterns based on recent collections of putative *C. colensoi*, however, Wu et al. (1996), found differences between the two taxa, and that the group they called *C. colensoi* defined a discrete intercompatibility group. No intercompatibility results were reported for *C. candelabrum*. The segregation of the two groups was supported by DNA analysis reported in this study in which a number of the same isolates from the Wu et al. (1996) study were included. These were 2555 (TENN 48930), 2637 (TENN 48917), 2644 (TENN 48918), 2667 (TENN 48920), 2685 (48919), and 55157 (TENN 43871) for their *C. colensoi*, and 2679 (TENN 48923), 2807 (TENN 48925), and 55633 (TENN 44117) for their *C. candelabrum*. These two groups could also be distinguished by the presence or absence of thick-walled hyphae in the context and to a lesser extent spore sizes.

A review of the type specimens of *Clavaria candelabra* (Ridley 37, K 69007) and *C. colensoi* (Colenso s.n., K 69008) revealed that there might have been some sort of nomenclatural confusion. It was found that Colenso's specimen exhibited thick-walled hyphae and spores 3.6 – 4.2 µm (L_m = 3.9 µm ± 0.3) X 2.4 – 3.0 µm. Ridley's specimen lacked thick-walled hyphae and spores were (4.2-) 4.4 – 5.2 µm (L_m = 4.7 µm ± 0.3) X (3.0) 3.4 – 4.0 µm. In his study of the Colenso specimen, however, Petersen (1984) did not report the presence of thick-walled contextual hyphae.

Whether thick-walled hyphae are present or absent, sufficient molecular evidence exists that suggests there are two distinct taxa. Spore sizes, while overlapping to some degree, indicate that there may have been some taxonomic reversal. The reason for the possible mix-up could be due to differing interpretations of structures, the methods used in measuring the structures, and the interpretation of the overall data.

Another character that may be used to distinguish *A. candelabrus* from *A. colensoi* is the presence of inflated gloeocystidia that narrow in the hymenium. This character is not absolutely constant, but their presence in such an extreme form may be indicative of *A. candelabrus*. Dried specimens of *A. candelabrus* are usually lighter brown than those of *A. colensoi*, which turn almost black in drying.

Collection MEL 1053577 was examined for this study because it was identified by Wu (1996, specimen annotation) as "probably *Clavicorona pyxidata* var. *asperospora* Fawc." This collection consisted of many small fragmented, few-branched basidiomata with branches in up to three ranks, and spores 4.2 – 5.0 X 2.6 – 3.2 µm (L_m = 4.5, E_m = 1.56). Based on spore measurements and overall stature of the basidiomata, it seems likely that this collection represents *Artomyces candelabrus*. This specimen was not included in the morphological characterization above.

Distribution and Habit: The type specimen is from Malaysia, and TENN collections are from New Zealand (both major islands) and Tasmania. Substrates included with the specimens have been diffuse-porous hardwood.

Specimens: AUSTRALIA, Tasmania, Lake Pedder National Heritage Area, 26 May 1991, coll. K.W. Hughes, TFB 3922 (TENN 50128); Victoria, Wannon Dist., Lower Glenelg National Park, 14 June 1964, coll. H.M. Beauglehole, A.C. Beauglehole 6359 (MELL 1053577). MALAYSIA, Selangor, no date, coll. Ridley, Ridley 37 (HOLOTYPE - K 69007). NEW ZEALAND, North Island, Auckland, Waitakere Ranges, Farley Track, 22 May 1990, coll. R.H. Petersen, TFB 2685 (TENN 48919); South Island, Tasman Dist., 21 km SE of Murchison on road to Matakitaki, 16 April 1983, coll. R.H. Petersen, TFB 55157 (TENN 43871); South Island, Southern Dist., Fjordland, vic. Te Anau, Keplar Track, 17 May 1990, coll. R.H. Petersen, TFB 2637 (TENN 48917).

4. *Artomyces carolinensis* Lickey, *sp. nov.* [*nom. prov.*].

Basidiomata usque ad 45 mm alto et 35 mm lata, parvus ramosus, pyxidatus, specimen exsiccatum cinnamomeus-bruneus ad ochraceus-fulvus. Stipes praesens ad 1 cm alto. Rami leniter crassus dilatatus ad 2 mm; rami inferiora et superus bubalinus-bruneus. Apices cuspidatus ad coronatus-cristatus. Sapor nullus. Gloeocystidia communis, cylindraceus ad clavatus, 2.0 – 6.0 µm diametro, interdum extans ultra hymenium 4 (16) µm. Basidia circa 19.5 X 4.6 µm. Basidiosporae late ellipsoideae, 3.6 – 4.4 (-4.8) X 2.8 – 3.4 (-4.0) µm, (E_m = 1.27 ± 0.08), amyloideae, hyalinus, asperulatus. Lignatilis angiospermae.

Holotypus [*hic designatus*]: UNITED STATES, North Carolina, Haywood Co., Great Smoky Mountains National Park, Cataloochee Cove, Caldwell Creek Trail, 30 June 1989, Q. Wu, TFB 1615 (TENN 48890).

Etymology: *carolinensis* – referring to North Carolina.

Basidiomata (FIG 23I)**:** cespitose, up to 30 mm tall X 15 mm wide (field notes indicate specimen was 45 mm X 35 mm), loosely branched, stoutish. Branches in up to 4 ranks, 3 – 4 per node, to 2 mm diam, "buff brown" (6D4) throughout. Stipe up to 10 mm long X up to 2 mm diam.; basal mycelial pad tannish. Tips coronate-cristate to cuspidate. It reportedly had a peppery odor but no taste, although Wu (1991) noted taste as "very slowly acrid." Dried basidiomata "ochraceous tawny" (6C6) to "cinnamon brown" (6E8).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 8 µm diam., aseptate. Gloeocystidia (FIG 9a) cylindric to clavate, 2.0 – 6.0 µm wide, non-emergent to protruding up to 6 (-16) µm. Basidia (FIG 9b) (12.0-) 14.0 – 20.0 (-32) µm (L_m = 19.5 µm ± 5.4) X 4.0 – 4.8 (-6.0) µm (W_m = 4.6 µm ± 0.6), clamped.

Basidiospores (FIG 9c) broadly ellipsoid (E_m = 1.27 ± 0.08), 3.6 – 4.4 (-4.8) µm (L_m = 4.0 µm ± 0.3) X 2.8 – 3.4 (-4.0) µm, asperulate, often guttulate.

Commentary: Wu (1991) and Wu et al. (1996) believed this collection represented a new species, but did not name it, instead referring to it as "Taxon 1." Spore measurements taken during this study were the same as those reported by Wu et al. (1996), who suggested that this collection might represent a variant of *Clavicorona turgida*. Although "Taxon 1" exhibited a gelatinized subhymenium, they stated that it differed from *C. turgida* because it lacked a gelatinized hymenium, produced a different laccase electrophoretic pattern, and had darker basidiomata based on one collection. The

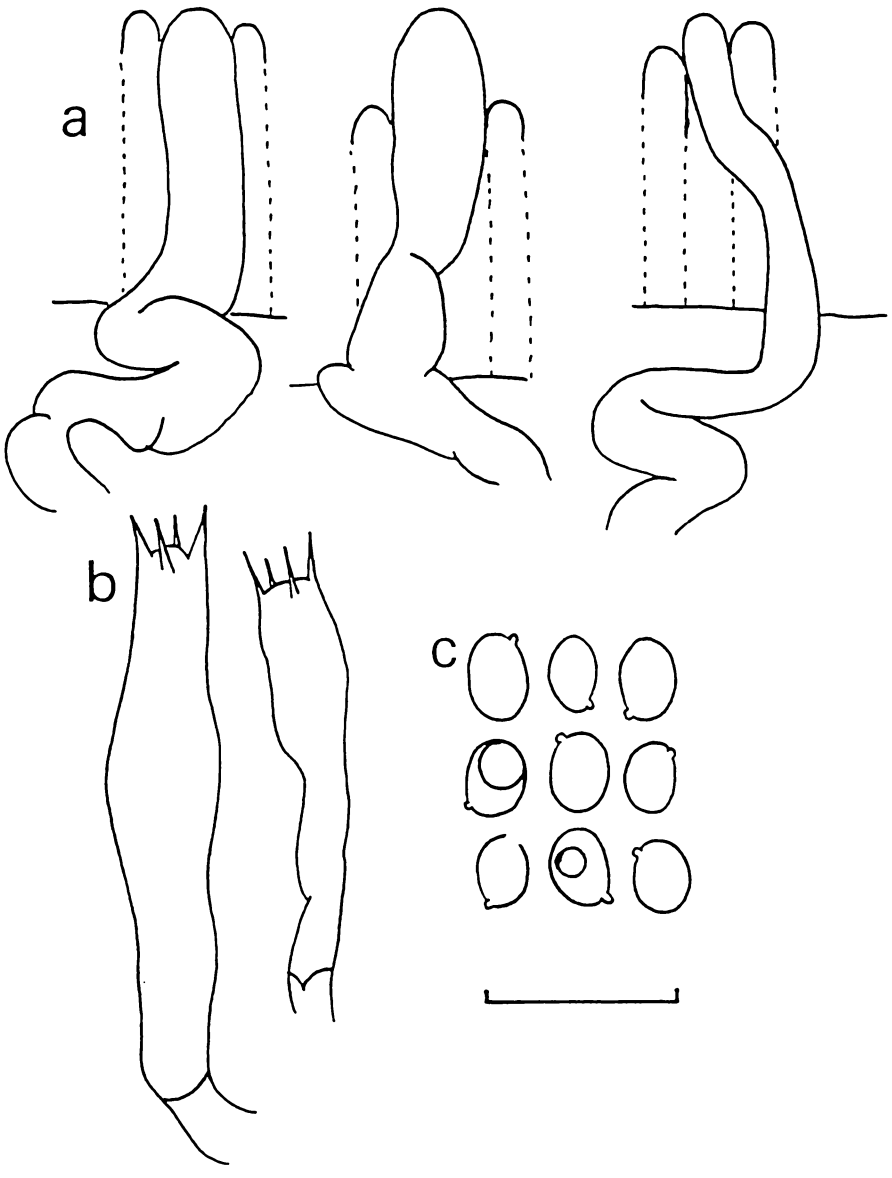


FIGURE 9. *Artomyces carolinensis* (TENN 48890). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

gelatinized subhymenium of TENN 48890 was not observed in this study. Wu et al. (1996) cited the possibility that another specimen (Atkinson, 10700 CUP) that Dodd (1972) identified as *C. turgida* could also be their "Taxon 1."

Molecular evidence indicates that *Artomyces carolinensis* is related to *A. piperatus* from the American Pacific Northwest. Morphologically, they are almost indistinguishable with nearly identical spore dimensions and overall similar basidiome stature. However, basidiomata of *A. piperatus* are often "avellaneous" (7B3) and fruit on conifer wood whereas those of *A. carolinensis* are "buff brown" (6D4) and fruit on hardwood. Although it is not a consistent character, gloeocystidia of *A. piperatus* may project farther past the hymenium than in *A. caroliniensis*. It is possible that the sole collection of *A. carolinensis* represents a disjunct variant from the Pacific Northwest, but based on DNA sequence analysis, *A. carolinensis* is divergent from *A. piperatus*. In the phylogenetic estimation, it is included in a weakly supported polytomy, with *A. dichotomus* and *A. austropiperatus*, which comprises a clade sister to *A. piperatus* (FIGS 2, 3). Although it is represented by only one collection, I decided that this species is recognizable due to its genetic distinctiveness.

Distribution and Habit: The single collection is from western North Carolina. Wu et al. (1996) reported that it fruited on conifer wood, but the substrate included with the specimen is clearly hardwood.

Specimens: UNITED STATES, North Carolina, Haywood Co., Great Smoky Mountains National Park, Cataloochee Cove, Caldwell Creek Trail, 30 June 1989, Q. Wu, TFB 1615 (HOLOTYPE - TENN 48890).

5. *Artomyces colensoi* (Berk. apud Hooker) Jülich. 1981. Bibliotheca Mycologica 85: 399.

≡ *Clavaria colensoi* Berk. apud Hooker. 1855. Flora of New Zealand 2: 186.

≡ *Clavicorona colensoi* (Berk. apud Hooker) Corner. 1950. Monograph, p.287.

Holotypus: NEW ZEALAND, no date, coll. Colenso, s.n. (K 69008 !).

Basidiomata (FIG 24A, B): often densely gregarious, up to 60 mm tall X 15 mm wide, petite, well-branched, but open, consistency tough. Branches in 3 – 4 (-6) ranks, 2 - 4 per node, slender, usually less than 1 mm diam., "avellaneous" (7B3) to "cinnamon buff" (6B4). Stipe up to 10 mm long X up to 1.5 mm diam., "fawn color" (7C5) to "army brown" (8D5); basal mycelial pad brownish. Apices coronate-cristate to cuspidate. Odor slightly peppery, taste "acrid, but also bitter." Dried basidiomata "blackish brown" (7F5) to "seal brown" (8F6) to "warm sepia" (7F6) throughout.

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, inflated, clamped, some encrusted (not observed in type specimen); 2) gloeoplerous hyphae, common, up to 10 µm in diam., aseptate; 3) thick-walled hyphae, walls 1 – 1.2 µm thick, aseptate. Gloeocystidia (FIG 10a) cylindric to ventricose, sometimes capitate, 2.8 – 10 (-14) µm wide, non-emergent to protruding up to 10 µm. Basidia (FIG 10b) (18.0-) 20.0 – 28.0 µm (L_m = 22.9 µm ± 2.0) X 3.6 – 5.2 µm (W_m = 4.4 µm ± 0.4), clamped.

Basidiospores (FIG 10c) ellipsoid (E_m = 1.48 ± 0.05), 3.6 – 4.4 (-4.8) µm (L_m = 4.0 µm ± 0.3) X 2.4 – 3.0 µm, minutely asperulous, often guttulate.

Commentary: *Artomyces colensoi* is very similar morphologically to *A. candelabrus,* and the two are often confused. In addition to the taxonomic problems presented in the

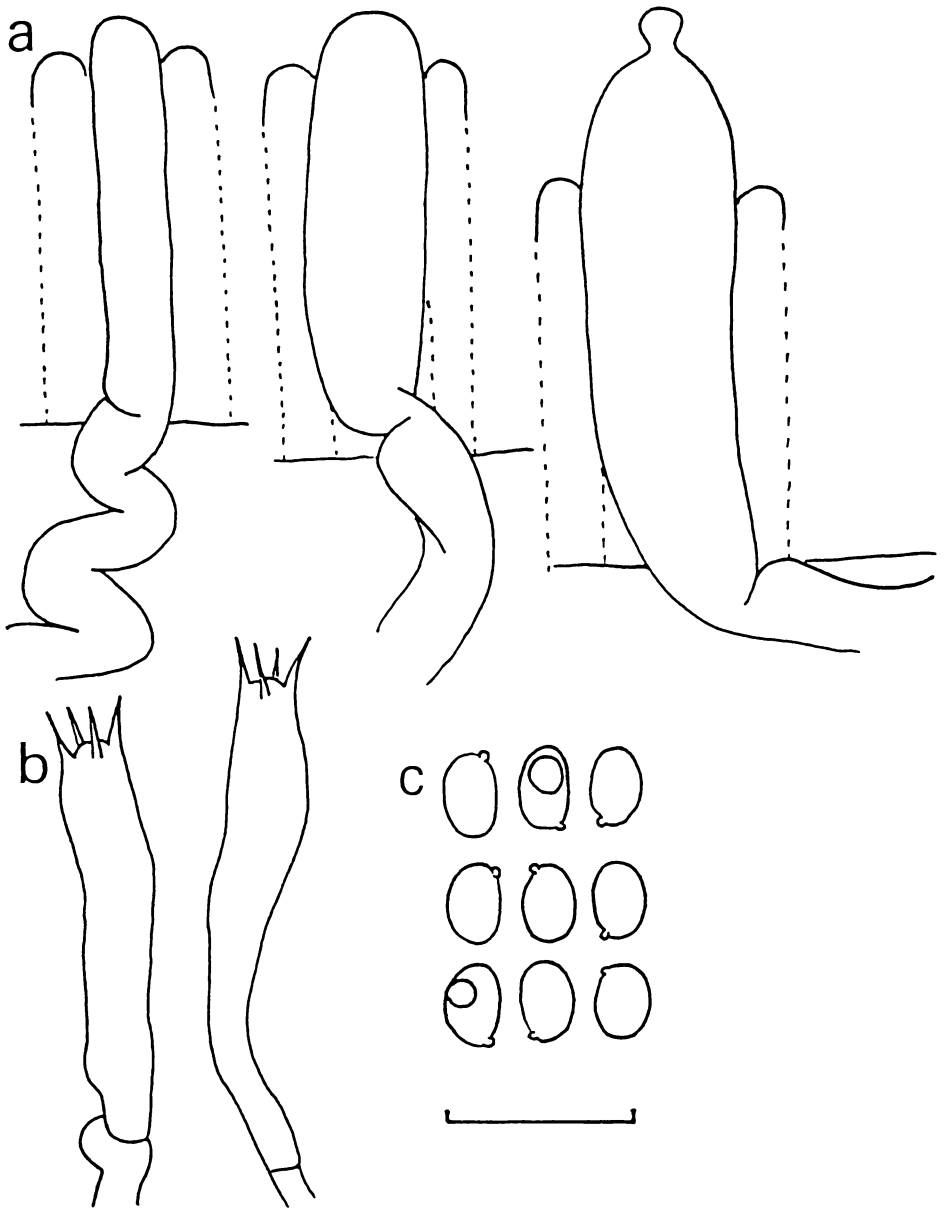


FIGURE 10. *Artomyces colensoi* (TENN 44117). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 µm.

commentary under *A. candelabrus*, Corner (1950) determined that *Clavicorona piperata* did not warrant recognition because of its similarity to *C. colensoi*. However, subsequent studies by Dodd (1972), Petersen (1988), and Wu et al. (1996) have shown that *C. piperata* (*Artomyces piperatus*) is a distinct species (see commentary under *A. piperatus*). *Artomyces colensoi* is distinct from both *A. candelabrus* and *A. piperatus* because it exhibits thick-walled hyphae in the trama, and its petite basidiomata turn almost black in drying. The only other species known to exhibit thick-walled hyphae is *A. dichotomus*, but this species exhibits stout basidiomata and has spores that are shorter (L_m = 3.6 µm ± 0.3) and more round (E_m = 1.27 ± 0.06) than *A. colensoi.*

Based on the ITS phylogenetic estimation and distance tree (FIGS 2, 3), *A. colensoi* comprises part of Clade II. In this clade, *A. colensoi* is well-supported as sister to *A. stephenii* from Costa Rica, and *A. cristatus* is weakly supported as basal in this group. Morphologically, *A. colensoi* appears quite different from *A. stephenii*. The basidiomata of *A. colensoi* are darker, both when fresh and dried, they are more petite and not profusely branched, and they exhibit thick-walled hyphae in the trama. However, the basidiospores of *A. stephenii* (3.2 – 4.0 µm X 2.4 – 3.0 µm, E_m = 1.40 ± 0.04) are only slightly shorter than *A. colensoi*. *Artomyces cristatus* is distinct in this clade because of its usually unbranched basidiomata and smooth spores which are more than twice as long as broad.

Collection MEL 1053578 was examined for this study because Wu (1996, specimen annotation) identified it as" probably *Clavicorona pyxidata* var. *asperospora* Fawc." The collection consisted of a few petite, very well-branched basidiomata with branches in up to 5 ranks, and with spores 4.0 – 4.8 X 2.6 – 3.2 µm (L_m = 4.1, E_m = 1.45).

This specimen also exhibited the slightly encrusted hyphae usually present in *A. colensoi*, but skeletal hyphae were not observed. Based on these characteristics it was tentatively identified as *A. colensoi*. If this specimen is *A. colensoi*, then the known range of this species extends outside of the North Island of New Zealand.

Distribution and Habit: Known presently from the North Island of New Zealand. Dodd (1972) examined two specimens, "Banks Peninsula, South Island, s.n. (K)" and "Black Gully, Tapanai, Otago District, South Island, 30.III.66, 28221 (PDD)," but these were not examined for verification in this study. Another specimen, MEL 1053578 from Victoria, Australia is tentatively identified as *A. colensoi*. TENN specimens fruited on diffuse-porous hardwood, probably *Nothofagus* spp.

Specimens: AUSTRALIA, Victoria, Eastern Highlands Dist., Dandenong Ranges, Sherbrooke Falls, 3 April 1933, coll. J.H. Willis, s.n. (MEL 1053578). NEW ZEALAND, no date, coll. Colenso, s.n. (HOLOTYPE - K 69008); North Island, Waipoua Kauri Reserve, Yakas Tree Track, 12 May 1983, coll. R.H. Petersen, TFB 55633 (TENN 44117); North Island, Auckland, Waitakere Ranges, Farley Track, 22 May 1990, coll. R.H. Petersen, TFB 2679 (TENN 48923); North Island, Auckland, Waitakere Ranges, Piha Valley Track, 26 May 1990, coll. R.H. Petersen and K.W. Hughes, TFB 2807 (TENN 48925).

6. *Artomyces costaricensis* Lickey, *sp. nov.* [*nom. prov.*].

Basidiomata usque ad 75 mm alto et 30 mm lata, ramosus, pyxidatus, specimen exsiccatum bubalinus bruneus ad cinnamomeus bruneus ad aurantiacus bubalinus.

Stipes praesens ad 15 mm alto. Rami crassus dilatatus ad 3 mm, parvus deminutis, rami inferiora lignum bruneus ad pallid cinnamomeus roseus ad avellaneus, superus avellaneus ad sordidus roseus bubalinus ad pallid ochraceus bubalinus. Apices cuspidatus ad coronatus-cristatus. Sapor leviter acerbis vel nullus. Gloeocystidia communis, cylindraceus, 4.0 – 7.6 µm diametro, extans ultra hymenium ad 12 µm. Basidia circa 26.4 X 4.6 µm. Basidiosporae ellipsoideae, 3.8 – 4.6 (-5.0) X (2.6-) 2.8 – 3.4 (-3.8) µm, (E_m = 1.37 ± 0.05), amyloideae, hyalinus, asperulatus. Lignatilis Quercus. *Holotypus* [*hic designatus*]: COSTA RICA, Prov. San Jose, Villa Mills (CATIE), 30 June 1998, J.L. Mata, TFB 9670 (TENN 56631).

Etymology: *costaric-*: Costa Rica; *-ensis*: emanating from.

Basidiomata (FIG 24C, D)**:** up to 75 mm tall X 30 mm wide, well-branched, more or less compact, stout. Branches in up to 5 ranks, 4 – 6 per node, long and equal, reducing little upward, up to 3 mm diam., "pale cinnamon pink" (5A2), "cream buff" (4A4), "pale ochraceous buff" (4A2), to duller than "pinkish buff" (6A3). Stipe up to 15 mm long and 2 (-5) mm diam., "wood brown" (7C4), "sayal brown" (6C5), to "avellaneous" (7B3); basal mycelial pad tan. Apices cuspidate to coronate-cristate. Odor mild, taste weakly acrid if any at all. Dried basidiomata "wood brown" (7C4), "orange buff" (5A5), "buff brown" (6D4), "cinnamon brown" (6E8) to "cinnamon rufous" (6D7).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 10 µm in diam., aseptate. Gloeocystidia (FIG 11a) occasional to common, usually cylindric, 4.0 – 7.6 µm diam., commonly protruding 4 – 8 (12) µm. Basidia (FIG 11b) 24.0 – 32.0 µm long (L_m = 24.4 µm ± 2.4) X (3.6-) 4.0 – 5.6 µm wide (W_m = 4.6 µm ± 0.6), clamped.

Basidiospores (FIG 11c) ellipsoid (E_m = 1.37 ± 0.05), 3.8 – 4.6 (-5.0) µm (L_m = 4.2 µm ± 0.3) X (2.6-) 2.8 – 3.4 (-3.8) µm, obviously asperulate, often guttulate.

Commentary: This is one of four species known from Costa Rica. *Artomyces costaricensis* is imperfectly recognizable by long, slowly reducing branches that appear equal over successive ranks. *Artomyces pyxidatus* and *A. stephenii* usually exhibit profusely, delicately branched, lighter colored basidiomata, and spores of these two species are slightly shorter and narrower than *A. costaricensis*. *Artomyces enigmatus* (*q.v.*) differs in its lighter colored basidiomata that are not as robust or well-branched as those of *A. costaricensis*. In the phylogenetic estimation, *A. costaricensis* forms a distinct branch in a larger clade composed of species allied with *A. piperatus* (FIGS 2, 3). Morphologically, this may be reflected in the overall stoutness of the basidiomata of the species in this clade.

The three specimens were collected in the same general location at the CATIE Forest Experiment Station Villa Mills in high altitude (greater than 2500 meters) oak forest. This hints of a restricted or endemic habitat type where this species may be isolated in oak forest "islands" at high elevation.

Distribution and Habit: Known only from three collections from the same location in Costa Rica, fruiting on *Quercus*.

Specimens: COSTA RICA, Prov. Cartago, Villa Mills, CATIE Forest, 23 June 2000, R.H. Petersen, TFB 10866 (TENN 59137); Prov. San Jose, Bosque Experimental de Villa Mills, 22 June 1995, K. Shanks, TFB 7900 (TENN 53848); Prov. San Jose, Villa Mills, CATIE Forest, 30 June 1998, J.L. Mata, TFB 9670 (HOLOTYPE - TENN 56631).

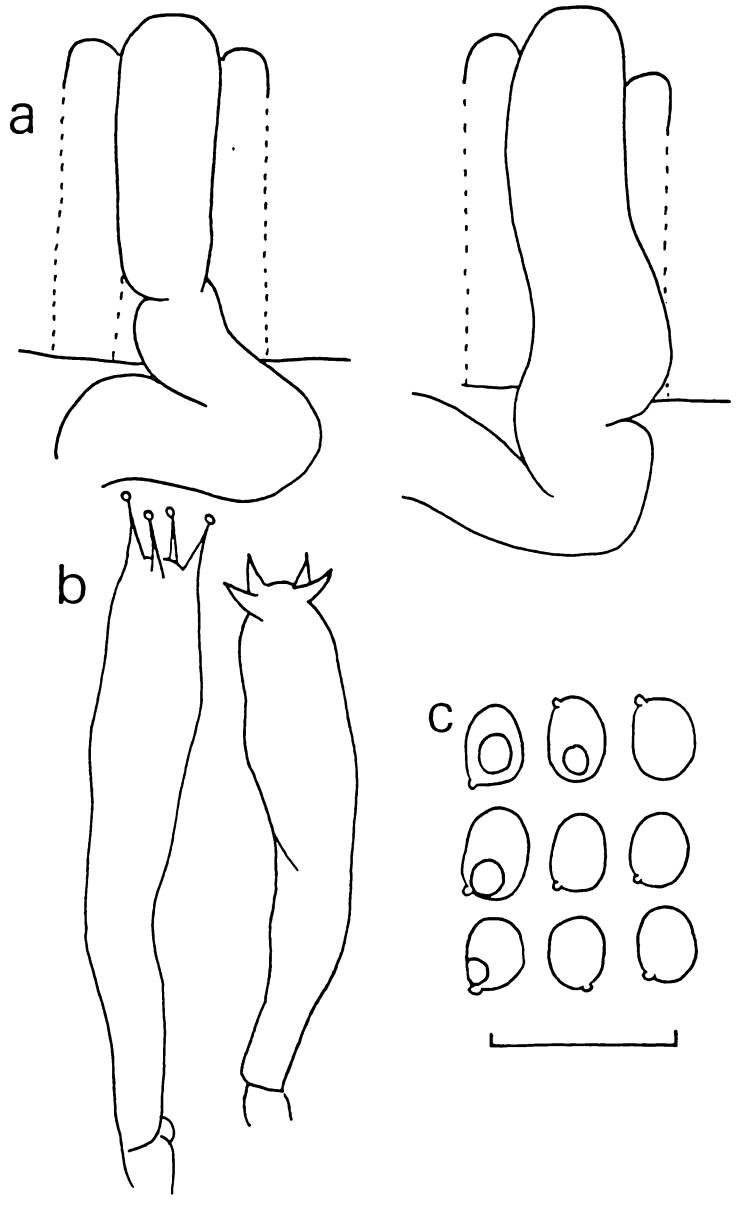


FIGURE 11. *Artomyces costaricensis* (TENN 59137). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

7. ***Artomyces cristatus*** (Kauffm.) Jülich. 1981. Bibliotheca Mycologica 85:399.
≡ *Craterellus cristatus* Kauffm. 1930. Mich. Acad. Sci. 11:172.
≡ *Clavicorona cristata* (Kauffm.) Doty. 1947. Lloydia 10:40.
Holotypus: UNITED STATES, Oregon, Takilma, 5 Dec 1925, coll. C.H. Kauffman, s.n. (MICH 10444 !).

Basidiomata (FIG 24E)**:** scattered, up to 10 mm tall X 2.5 mm diam. at expanded apex. According to Kauffman (1930), fresh specimens were 10 – 25 mm tall with expanded apex to 5 mm, "vinaceous buff" (9B2). All basidiomata observed for this study unbranched, coronate, with brown basal mycelial pad. Apices coronate-cristate. Dried basidiomata "vinaceous rufous" (7D8), "carob brown" (7F7), (brown-orange, 5D3) to (caramel brown, 6C6).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 6 µm in diam., aseptate. Gloeocystidia (FIG 12a, b) cylindric to clavate, 2.4 – 5.2 µm diam., non-emergent to protruding up to 6 µm. Basidia (FIG 12c) (20.0-) 22.0 – 28.0 µm long (L_m = 24.6 µm ± 2.2) X 4.0 – 5.6 µm wide (W_m = 4.6 µm ± 0.5), clamped.

Basidiospores (FIG 12d) cylindric (E_m = 2.38 ± 0.05), (4.8-) 5.4 – 6.0 (-7.2) µm (L_m = 5.6 µm ± 0.5) X 2.0 – 2.6 (-3.0) µm, smooth, often guttulate with large guttulae.

Commentary: Kauffman (1930) described this as a species of *Craterellus*, probably based largely on its truncate to "cornucopia-shaped" stature. He might have been influenced by Thom's (1904) *Cr. taxophilus*, but Lloyd (1920) had already transferred the latter to *Clavaria*. Doty (1944), reporting on *Clavaria* taxa from the United States northwest, treated *Craterellus cristatus* as a synonym under *Clavaria coronata* Schw. Coker (1947) recognized that *Craterellus cristatus*, *Clavaria taxophila* and *C. pyxidata* (under which *C. coronata* and *C. piperata* were included) all belonged to a unique group, but proposed no changes because Doty's (1947) work was apparently in press. Doty (1947) placed these names together under the genus name *Clavicorona*.

Spore length measurements from this study (5.6 – 6.0 µm) are longer than those reported by Kauffman (1930: 4 – 5 µm). They are slightly smaller than those reported by Wu et al. (1996: 6.0 – 7.0 µm) and Dodd (1972: 6.0 – 7.0 µm), but are more similar to those found by Overholts (1940: 5 – 6 µm) and Coker (1947: 4.6 – 6.5 µm). Basidia length measurements provided by Kauffman (1930: 65 – 70 µm), however, are significantly longer than those reported by Dodd (1972: 20 – 28 µm), Wu et al. (1996: 25 – 35 µm), and those of this study (23.0 – 28.0 µm). These inconsistencies could be due to variation among collections, but the description by Wu et al. (1992) was based in part on specimen TENN 48928.

The type specimen, represented by one basidioma, was borrowed from MICH, but micromorphological characters were not studied. Included with the type were two permanent slides prepared by W.C. Coker in 1947, but micromorphological characters were not well preserved in these slides. Because Coker (1947) and Doty (1947) provided basidiospore measurements of the type similar to that found in this study, and because the spores of *A. cristatus* are distinctive in *Artomyces*, there seemed no need to sample the type specimen.

With cylindric spores, mostly simple basidiomata, and coniferous substrate, this is a distinct morphospecies in *Artomyces*. Although it is apparently widely distributed

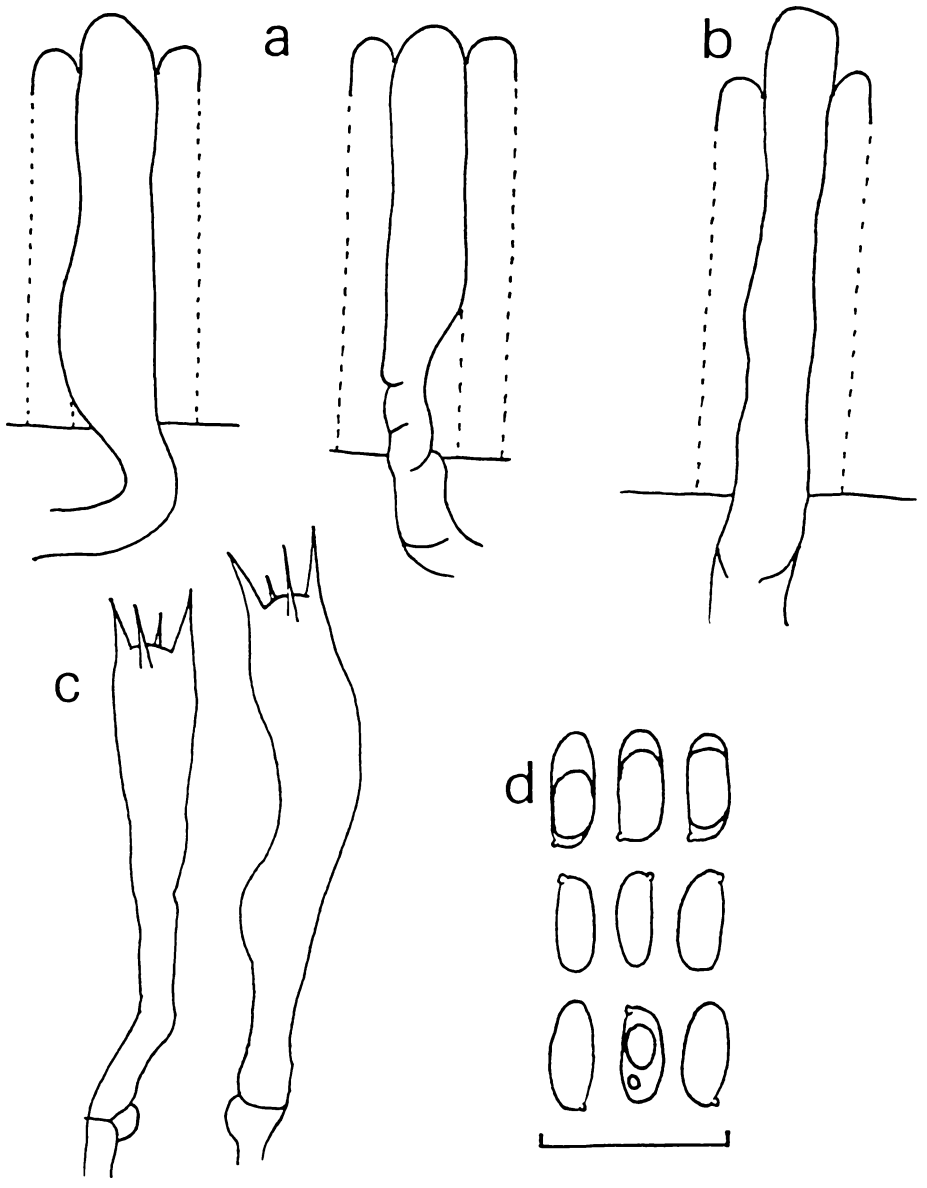


FIGURE 12. *Artomyces cristatus*. a) gloeocystidia (TENN 55403); b) gloeocystidia (TENN 48928); c) basidia (TENN 48928); d) basidiospores (without ornamentation; TENN 55403). Bar = 10 μm.

across the boreal regions of the Northern Hemisphere, populations are remarkably similar. They remain sexually intercompatible (Wu 1991) and exhibit little sequence divergence (1.0%).

Distribution and Habit: Known from American Pacific Northwest, Canada (Quebec; Overholts 1940) and Northern Europe (Daun, unpubl. data). The type specimen was reportedly collected on Douglas fir (*Pseudotsuga menziesii*). TENN 48928 was found on coniferous wood (probably *Taxus*), not *Quercus* as reported by Wu et al. (1996) and TENN 55403 also fruited on coniferous wood.

Specimens: UNITED STATES, California, Trinidad Co., Highway 299, across from Grays Fall Campground, 22 December 1989, coll. P. Largent, DLL 8974 (TENN 48928); California, Trinidad Co., Highway 299, across from Grays Fall Campground, 19 November 1996, coll. D. Largent, TFB 8079 (TENN 55403).

8. ***Artomyces dichotomus*** (Corner) Jülich. 1981. Bibliotheca Mycologica 85:399.
≡ *Clavicorona dichotoma* Corner. 1970. Nova Hedwigia 33:146.
Holotypus: INDONESIA, Java, Mt. Pangarango, 29 March 1950, coll. S.J. van Oostroom, No. 13385 (L., *n.v.*).

Basidiomata (FIG 23J)**:** up to 50 mm tall X 12 mm wide, loosely branched, stoutish, tough, pliant and leathery. Branches in up to 5 ranks, branches 2 - 4 per node, to a little more than 1 mm diam., (gray-brown, 5E3) to "blackish brown" (6F3). Three primary stems 10 mm long X 1 – 2 mm diam.; basal mycelial pad brown. Tips rounded to truncate-cuspidate to rarely coronate, with a crown of three to four short, acicular points. No odor reported, taste mildly acrid. Dried basidioma "blackish brown" (6F3) to "carob brown" (7F5-7), with a grayish-white coating.

Micromorphology: Context dense, brown-pigmented, composed of; 1) generative hyphae, thin-walled, not inflated, clamped; 2) thick-walled hyphae, walls up to 1.2 µm thick, rarely branched, aseptate; 3) gloeoplerous hyphae, common, up to 8 µm diam., aseptate. Gloeocystidia (FIG 13a) uncommon, extremely dilated to 16 µm diam. in subhymenium, quickly narrowing to approximately 3 µm diam. in hymenium. Basidia not measured.

Basidiospores (FIG 13b) broadly ellipsoid ($E_m = 1.27 \pm 0.06$), 3.2 – 4.2 µm (L_m = 3.6 µm ± 0.3) X 2.6 – 3.2 µm, minutely asperulate, guttulate.

Discussion: SF 7220 was tentatively identified as *Clavicorona candelabrum* primarily because it exhibited thick-walled hyphae in the context. However, due to some confusion the name *C. candelabrum* was misapplied to collections with thick-walled hyphae, which are correctly named *C. colensoi*. Collection SF 7220 probably does not represent *Artomyces colensoi* because it has ellipsoid spores ($E_m = 1.5$), whereas SF 7220 has broadly ellipsoid spores ($E_m = 1.3$). The phylogenetic estimation also indicates that SF 7220 (isolate DED 7220) is not associated with either *A. colensoi* or *A. candelabrus*, but is included on a unique branch in Clade I with *A. piperatus* (FIGS 2, 3).

Due to its treatment with a hazardous chemical, the type specimen, which is housed in Leiden, is unavailable for study. However, the micromorphological characters of SF 7220 are almost identical to Corner's (1970) description of his type specimen of *Clavicorona dichotoma*, even though he emphasized the dichotomous branching habit. Several nodes on SF 7220 were dichotomous, and this may be a plastic character.

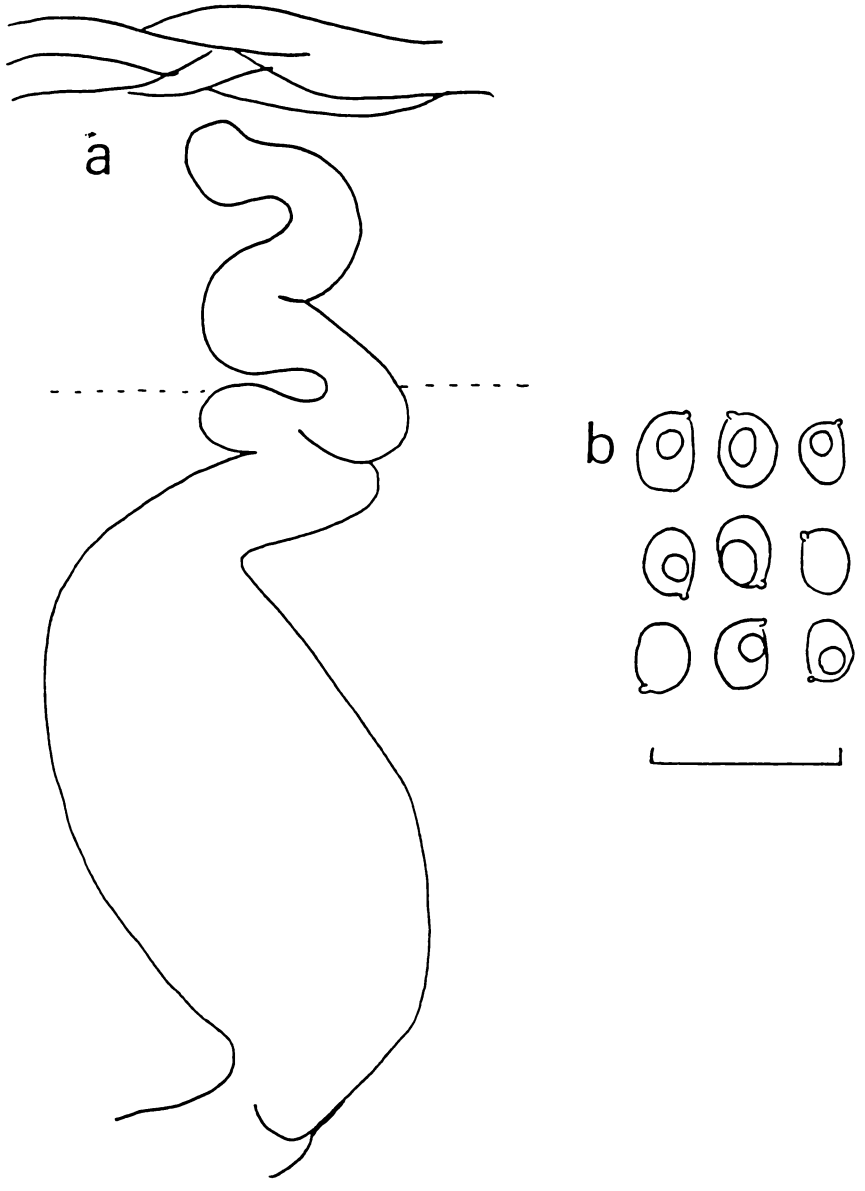


FIGURE 13. *Artomyces dichotomus* (SF 7220). a) gloeocystidium, with collapsed hymenium and ambiguous hymenium-subhymenium boundary; b) basidiospores (without ornamentation). Bar = 10 μm.

Corner's (1970) spore measurements (4 – 4.5 X 3 – 3.5 µm) are slightly larger than mine for SF 7220 (3.2 – 4.2 X 2.6 – 3.2 µm), but based on his measurements, "E" is approximately 1.3. His description of the type specimen is "grey when fresh, drying dark fuscous with a greyish hymenium" compares very well with SF 7220. The final determining factor in identifying SF 7220 as *A. dichotomus* is that it was collected less than 75 km from the type locality in Java.

Without indicating that he studied the type material, Dodd (1972) reported spore lengths (4.5 – 5 µm) slightly larger than Corner's (1970). Dodd (1972) also reported basidia measurements of 16 – 18 X 4 – 4.8 µm, where Corner (1970) did not report any.

Distribution and Habit: The only two specimens were collected in Java, less than 75 km from each other, just south of Jakarta. No substrate was included with SF 7220, but field notes state it was found "on buried rotten log in primary forest."

Specimens: INDONESIA, Java, Mt. Halimun National Park, 10 January 2001, coll. A. Wilson and D.E. Desjardin, DED 7220 (SF 7220).

9. ***Artomyces enigmatus*** Lickey, *sp. nov.* [*nom. prov.*].

Basidiomata usque ad 40 mm alto et 15 mm lata, parvus ramosus, pyxidatus, specimen exsiccatum tepidus bubalinus ad ochraceus bubalinus. Stipes nullus. Rami leniter crassus dilatatus ad 2 mm, rami inferiora avellaneus ad vinaceus bubalinus ad "tilleul" bubalinus, superus pallid roseus bubalinus ad alba. Apices cuspidatus ad coronatus-cristatus. Sapor cito intense acerbis et persistens. Gloeocystidia communis, cylindraceus ad clavatus ad anguste lecythiformis, 4.0 – 10.0 µm diametro, extans ultra hymenium ad 14 µm. Basidia circa 23.4 X 4.7 µm. Basidiosporae ellipsoideae, 3.6 – 5.2 X 2.8 – 4.0 µm, (E_m = 1.34 ± 0.07), amyloideae, hyalinus, asperulatus. Lignatilis angiospearmae.

Holotypus [*hic designatus*]: UNITED STATES, Puerto Rico, Caribbean National Forest, El Junque, 4 December 1990, coll. R.H. Petersen and S. Gordon, TFB 3456 (TENN 48929).

Etymology: *enigmatus*: from enigma because the two collections are morphologically different, but their cultures exhibited almost identical ITS sequences.

Basidiomata (FIG 24F)**:** up to 40 mm tall X 15 mm wide, loosely branched, slightly stoutish. Branches in up to 5 ranks, branches 2 - 4 per node, more or less stout, up to 2 mm diameter, "pale pinkish buff" (6A2) to pure white, bruising "tilleul buff" (7B2). Stipe inconspicuous, basidiomata branched from base, base "vinaceous buff" (9B2), "avellaneous" (7B3), "fawn color" (7C5), to "tilleul buff" (7B2), basal mycelial pad white to tan. Tips cuspidate to coronate-cristate. Odor weakly typical, taste quickly and intensely acrid, persisting. Dried basidiomata "warm buff" (5A4) to "ochraceous buff" (5A5).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 6 (-8) µm diam., aseptate. Gloeocystidia (FIG 14a, b) occasional to common, cylindric to clavate, sometimes capitate, rarely beaked, 4.0 – 10.0 µm wide, often protruding up to 14 µm. Basidia (FIG 14c) 20.0 – 32.0 µm long (L_m = 23.4 µm ± 2.6) X (4.0-) 4.4 – 5.2 µm diam. (W_m = 4.7 µm ± 0.4), clamped.

Basidiospores (FIG 14d, e) ellipsoid (E_m = 1.34 ± 0.07), 3.6 – 5.2 µm (L_m = 4.4 µm ± 0.5) X 2.8 – 4.0 µm, obviously asperulate, often guttulate.

Commentary: I decided that these two collections probably represented a single species because they exhibited almost identical ITS sequences. Conversely, they had significantly different-sized spores. The spores of TENN 48929 (Puerto Rico) were 4.4 – 5.2 X 3.2 – 4.0 µm (L_m = 4.8 µm ± 0.3), while those of TENN 56533 (Costa Rica) were 3.6 – 4.4 X 2.8 – 3.4 µm (L_m = 3.9 µm ± 0.2). Spore shapes were very similar, however, with E_m ratios of 1.39 ± 0.13 and 1.29 ± 0.07, respectively. These two collections also differ slightly in basidiomata color. The Puerto Rican specimen was described as having pure white branches [but bruising "tilleul buff" (7B2)] and a "vinaceous buff" (9B2) to "avellaneous" (7B3) to "fawn color" (7C5) base, whereas the Costa Rican specimen was described as having "pale pinkish buff" (6A2) branches and a "tilleul buff" (7B2) base. In their dried state, these two collections exhibit the same "warm brown" (5A4) to "ochraceous buff" (5A5) color.

Fresh basidiome color can be an important character, but white to atypically pale forms have been observed in other species such as *A. pyxidatus*. The New Guinea collection identified here as *A. turgidus* may be a white form of that species. Taste may add an additional character of affinity between these two collections. It was stressed in the field notes that both were quickly and intensely acrid and that this was persistent.

One possibility is that these differences observed in spore size and basidiome color are due to some degree of phenotypic plasticity. The two were collected in slightly different habitats. The Puerto Rican specimen was collected on diffuse-porous hardwood in montane rainforest while the Costa Rican specimen was collected on oak in montane oak forest.

Wu et al. (1996) suggested that TENN 48929 (isolate 3456), which they referred to as "Taxon 2," represented a distinct species. They based this on unique laccase electrophoretic patterns, incompatibility with other species in mating studies, and morphology, but they stopped short of describing it for lack of other specimens. The spore sizes they report for this collection are almost identical to those listed above, but their E_m ratio of 1.54 is slightly higher than that calculated in this study.

Distribution and Habit: Known only from two collections, one from Puerto Rico, on diffuse porous hardwood, and the other from Costa Rica on *Quercus* at high elevations.

Specimens: COSTA RICA, Prov. Puntarenas, Coto Brus, Hacienda la Amistad, 4 July 1998, coll. J. Cifuentes, TFB 9905 (TENN 56533). UNITED STATES, Puerto Rico, Caribbean National Forest, El Junque, 4 December 1990, coll. R.H. Petersen and S. Gordon, TFB 3456 (HOLOTYPE - TENN 48929).

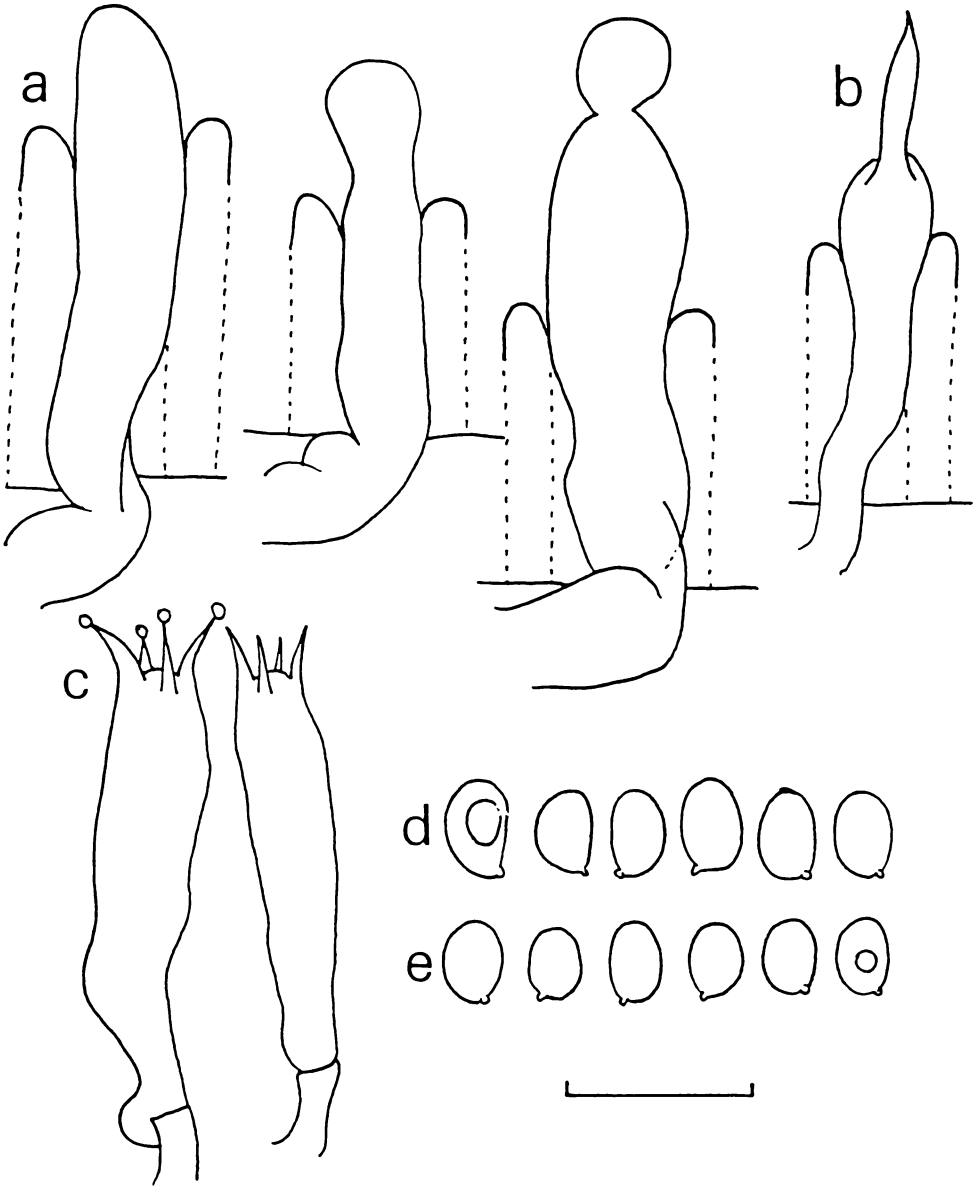


FIGURE 14. *Artomyces enigmatus*. a) gloeocystidia (TENN 56533); b) gloeocystidia (TENN 48929); c) basidia (TENN 48929); d) basidiospores (without ornamentation; TENN 48929); e) basidiospores (without ornamentation; TENN 56533). Bar = 10 μm.

10. ***Artomyces microsporus*** (Wu & R.H. Petersen) Lickey, *comb. nov.*

≡ *Clavicorona microspora* Wu & R.H. Petersen. 1992. Mycotaxon 45: 124.

Holotypus: JAPAN, Tochigi Pref., Mashiko, 29 September1989, coll. R.H. Petersen, TFB 2349 (TENN 48856 !).

Basidiomata (FIG 25D): in fragments, largest is 60 mm tall X 30 mm wide, profusely branched. Branches in up to 7 ranks, 4 – 6 per node, unequal, up to 2 mm diam., (yellowish white, 2A2), "martius yellow" (2A3), and "massicot yellow" (2A4). No conspicuous stipe present, apparently branched from base; basal mycelial pad tan. Tips coronate-cristate, "pale ochraceous buff" (4A2) and "pale pinkish cinnamon" (6A2). Taste slowly and weakly acrid. Lower branches of dried specimen "ochraceous buff" (5A5) and "capucine orange" (5A6), apices "cinnamon rufous" (6D6 – 6D7).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 6 µm diam., aseptate. Gloeocystidia (FIG 15a) cylindric, 2.8 – 4.4 µm wide, non-emergent to protruding up to 4 µm. Basidia (FIG 15b) 16.0 – 22.0 µm long (L_m = 17.5 µm ± 1.8) X 4.0 – 4.8 µm wide (W_m = 4.5 µm ± 0.3), clamped, one 2-spored observed.

Basidiospores broadly ellipsoid (E_m = 1.41 ± 0.14), 3.2 – 3.6 (4.0) µm (L_m = 3.4 µm ± 0.3) X 2.0 – 2.8 µm, minutely asperulate, often guttulate (FIG 15c).

Commentary: Wu and Petersen (1992) described basidiomata of this species as having basidia 10 – 18 X 3.0 – 4.5 µm, basidiospores 3 – 4 X 2.0 – 2.5 (-3.0) µm (E = 1.2 – 2.0, E_m = 1.5, L_m = 3.4 µm), and gloeocystidia 3.0 – 4.5 µm diam. and projecting up to 16 µm beyond basidia. With the exception of basidia size, these measurements are in agreement with those of the present study. Wu and Petersen (1992) used a combination of intercompatibility studies, laccase enzyme banding patterns, and morphological comparisons to support their designation of *Clavicorona microspora* as a distinct species and suggested its close relationship with *C. pyxidata*. This close association is strongly supported in the phylogenetic estimation in the present study where the two species are apparently sister taxa (FIGS 2, 3). Macromorphologically similar to *A. pyxidatus*, *A. microsporus* is easily distinguished based on its small, broadly ellipsoid spores.

Distribution and Habit: Known only from Japan fruiting on rotten hardwood.

Specimens: JAPAN, Tochigi Pref., Mashiko, 29 September1989, coll. R.H. Petersen, TFB 2349 (HOLOTYPE - TENN 48856).

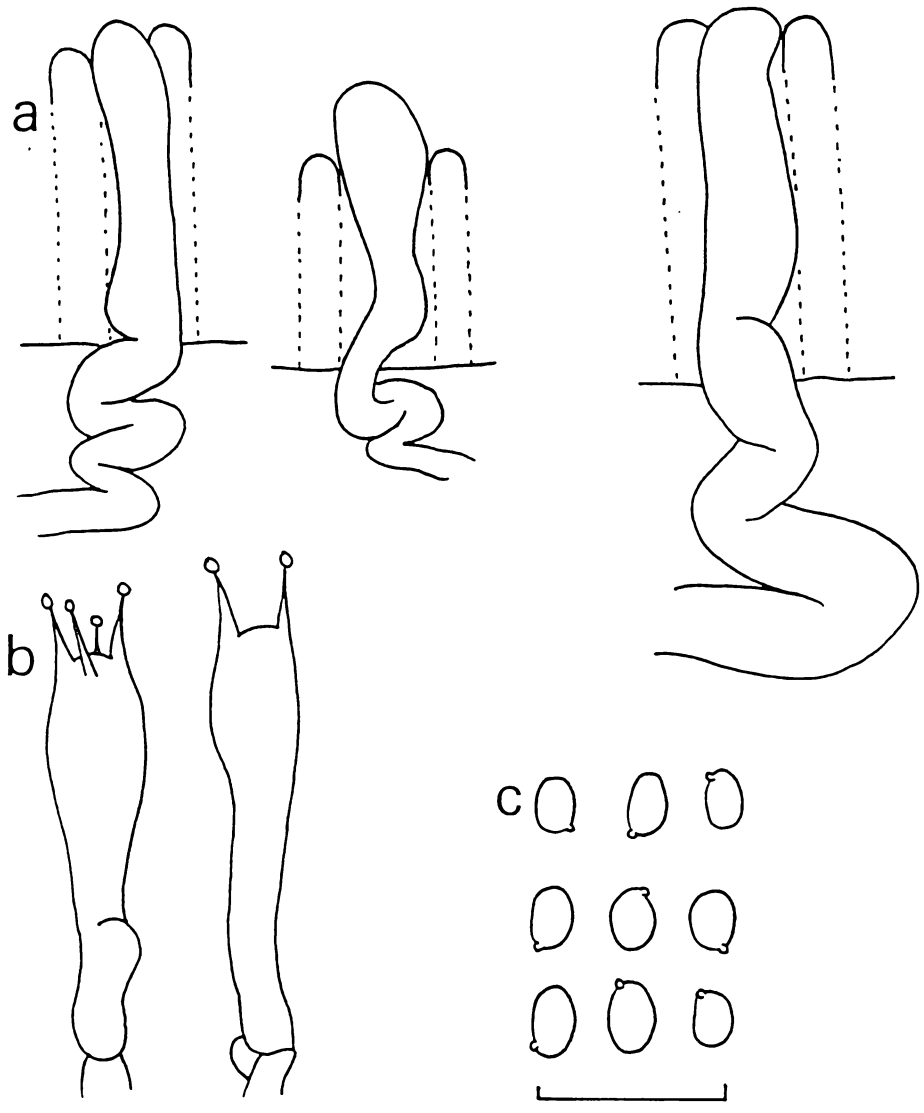


FIGURE 15. *Artomyces microsporus* (TENN 48856). a) gloeocystidia; b) basidia, 4-spored and rare 2-spored; c) basidiospores (without ornamentation). Bar = 10 μm.

11. ***Artomyces novazealandensis*** Lickey *sp. nov.* [*nom. prov.*]

Basidiomata usque ad 60 mm alto et 30 mm lata, ramosus, pyxidatus, specimen exsiccatum memnonius ad nigellus. Stipes praesens ad 15 mm alto. Rami leniter crassus dilatatus ad 2 mm, rami inferiora fuscus ad lignum bruneus, superus avellaneus ad cinnamomeus bubalinus. Apices cuspidatus ad coronatus-cristatus. Sapor intense acerbis. Gloeocystidia communis, cylindraceus ad late fusiformis, 3.0 – 12.0 µm diametro, saepe extans ultra hymenium ad 28 µm. Basidia circa 31.6 X 3.7 µm. Basidiosporae late ellipsoideae, (4.2-) 4.6 – 6.0 X 3.6 – 4.8 µm, (E_m = 1.28 ± 0.02), amyloideae, hyalinus, asperulatus. Lignatilis angiospermae saepe Nothofagus. *Differt a* Artomyces austropiperatus *genetice.*

Holotypus [*hic designatus*]: NEW ZEALAND, South Island, Fiordland, Lake Te Anau, Keplar Track, 9 May 1994, coll. R.H. Petersen, TFB 7023 (TENN 54111).

Basidiomata (FIG 24G, H)**:** up to 60 mm tall X 30 mm wide, well-branched, more or less compact, somewhat stoutish. Branches up to 6 ranks, 4 - 6 per node, up to 2 mm diam., "avellaneous" (7B3), "cinnamon buff" (6B4), to "cinnamon drab" (9C3). Stipe often conspicuous, up to 15 mm long X 4 mm diam., "benzo brown" (9D3), "fuscous" (6E4), "wood brown" (7C4), to "natal brown" (8E6); basal mycelial pad creamy white to tan. Tips cuspidate to coronate-cristate. Odor mildly to weakly peppery, taste intensely acrid and lingering. Dried basidiomata "Vandyke brown" (7E6) to "burnt umber" (7E7) to "blackish brown" (7F5).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 10 µm diam., aseptate. Gloeocystidia (FIG 16a) occasional to common, cylindric to broadly fusiform, 3.0 – 12.0 µm wide, often protruding to more than 28 (-36) µm. Basidia (FIG 16b) 24.0 – 36.0 (-44.0) µm long (L_m = 31.6 µm ± 3.7) X 4.8 – 7.6 µm wide (W_m = 6.0 µm ± 0.8), clamped.

Basidiospores (FIG 16c) broadly ellipsoid to ellipsoid (E_m = 1.28 ± 0.02), (4.2-) 4.6 – 5.6 (-6.0) µm (L_m = 5.1 µm ± 0.4) X 3.6 – 4.8 µm, asperulate, often guttulate.

Commentary: As stated before, collections of *Artomyces novazealandensis* and *A. austropiperatus* are virtually identical in stature and micromorphology. Dodd (1972), Petersen (1988), and Wu (1991) considered southern collections of this morphology as representing *Clavicorona piperata* even though their spores were consistently at least 1 µm longer and wider. Later, Wu et al. (1996) found that the southern collections had laccase electrophoretic patterns quite different from those of *C. piperata* and suggested that the southern collections represented a distinct species. The two cultures they used to represent their "Taxon 3," also included in this study, shared one common laccase band, but one culture exhibited two additional bands. According to ITS sequences, TENN 48915 (isolate 2672) is identified as belonging to the group described here as *A. novazealandensis*, while TENN 48916 (isolate 2822) is identified as *A. austropiperatus*.

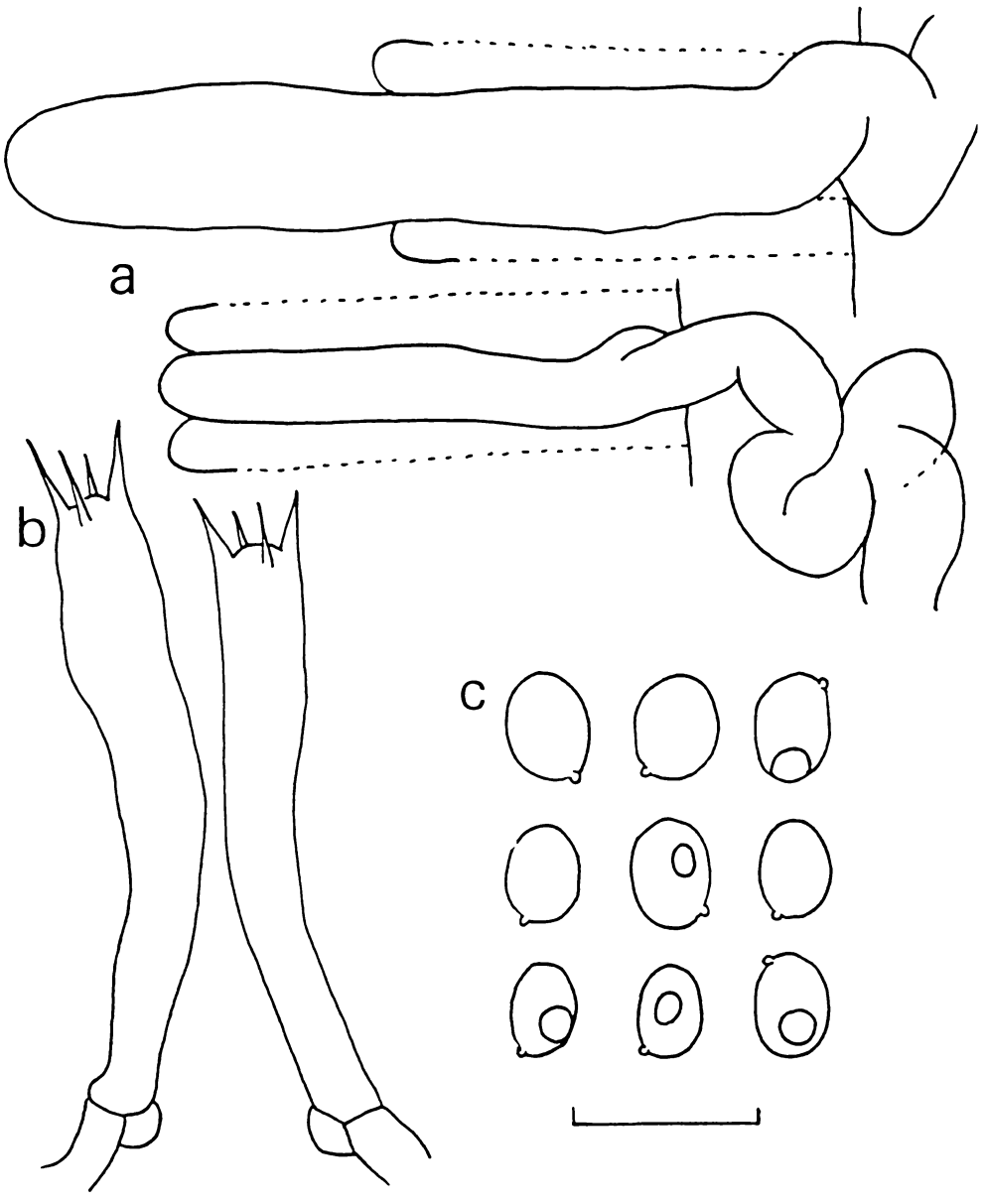


FIGURE 16. *Artomyces novazealandensis* (TENN 48915). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

Even though *A. novazealandensis* is morphologically similar to species in Clade I (FIGS 2, 3), its ITS DNA sequence is somewhat divergent from them and comprises a clade by itself. This monospecific clade is grouped with Clades III and IV in several of the most parsimonious trees, but this relationship was very weakly supported. It could be that the ITS DNA sequence has undergone rapid evolution in this species and that this species is related to the "*piperatus*" group. The branch length of *A. novazealandensis* is long compared to the others of the same rank, and its variable placement could be due to long-branch-attraction. The long branch may also represent a distinct clade with yet unknown or extinct members. Surveys of other gene sequences that are more conserved may help resolve this relationship.

Distribution and Substrate: Known only from South Island, New Zealand fruiting primarily on *Nothofagus*.

Specimens: NEW ZEALAND, South Island, Fiordland, Monowai, Borland Lodge Track, 19 May 1990, coll. R.H. Petersen, TFB 2672 (TENN 48915); South Island, Fiordland, Lake Te Anau, Keplar Track, 9 May 1994, coll. R.H. Petersen, TFB 7023 (HOLOTYPE - TENN 54111); South Island, Fiordland, Lake Te Anau, Keplar Track, 9 May 1994, coll. R.H. Petersen, TFB 7025 (TENN 56203); South Island, Fiordland, Fjordland National Park, 10 May 1994, coll. R.H. Petersen, TFB 7037 (TENN 56200).

12. *Artomyces piperatus* (Kauffm.) Jülich. 1981. Bibliotheca Mycologica 85:399.
≡ *Clavaria piperata* Kauffm.1927. Pap. Mich. Acad. Sci. 8:146.
≡ *Clavicorona piperata* (Kauffm.) Leathers & Smith. 1967. Mycologia 59:461.
Holotypus: UNITED STATES, Washington, Grays Harbor Co., Lake Quinault, 12 November 1925, coll. C.H. Kauffman, s.n. (MICH 10095 !).
= *Clavicorona avellanea* Leathers & Smith. 1967. Mycologia 59:460.
Holotypus: UNITED STATES, Idaho, Nordman, 8 October 1956, coll. A.H. Smith, Smith 54272 (MICH 5580 !).

Basidiomata (FIG 25A, B, C): up to 60 mm tall X 20 mm wide, well-branched, not compact, somewhat stoutish. Branches in up to 6 ranks, 4 - 5 per node, to 2 mm diam., "somewhat more yellow than 'tilleul buff' (7B2)." Conspicuous stipe usually present, up to 20 mm long X 3 mm diam., "avellaneous" (7B3) to "wood brown" (7C4) and "vinaceous buff" (9B2), basal mycelial pad white to brown. Tips cuspidate to coronate-cristate. Odor peppery, taste mildly acrid and lingering. Dried basidiomata "chamois" (4B4) to "Brussels brown" (6E6) to "bone brown" (7F8).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 8 µm diam., aseptate. Gloeocystidia (FIG 17a) occasional to common, cylindric, broadly fusiform, to mucronate, 2.8 – 7.0 µm diam., few protruding, to more than 24 µm. Basidia (FIG 17b) 22.0 – 30.0 (-32.0) µm long (L_m = 23.9 µm ± 2.7) X 4.0 – 5.2 (-6.0) µm wide (W_m = 4.6 µm ± 0.5), clamped.

Basidiospores (FIG 17c) broadly ellipsoid to ellipsoid (E_m = 1.31 ± 0.06), (3.2-) 3.6 – 4.4 (4.8) µm (L_m = 4.0 µm ± 0.3) X 2.6 – 3.6 µm, minutely asperulous, often guttulate.

Commentary: Doty (1944) first regarded *Clavaria piperata* as a distinct taxon, but then later synonymized it with *Clavicorona coronata* Schw. (Doty 1947). That same year,

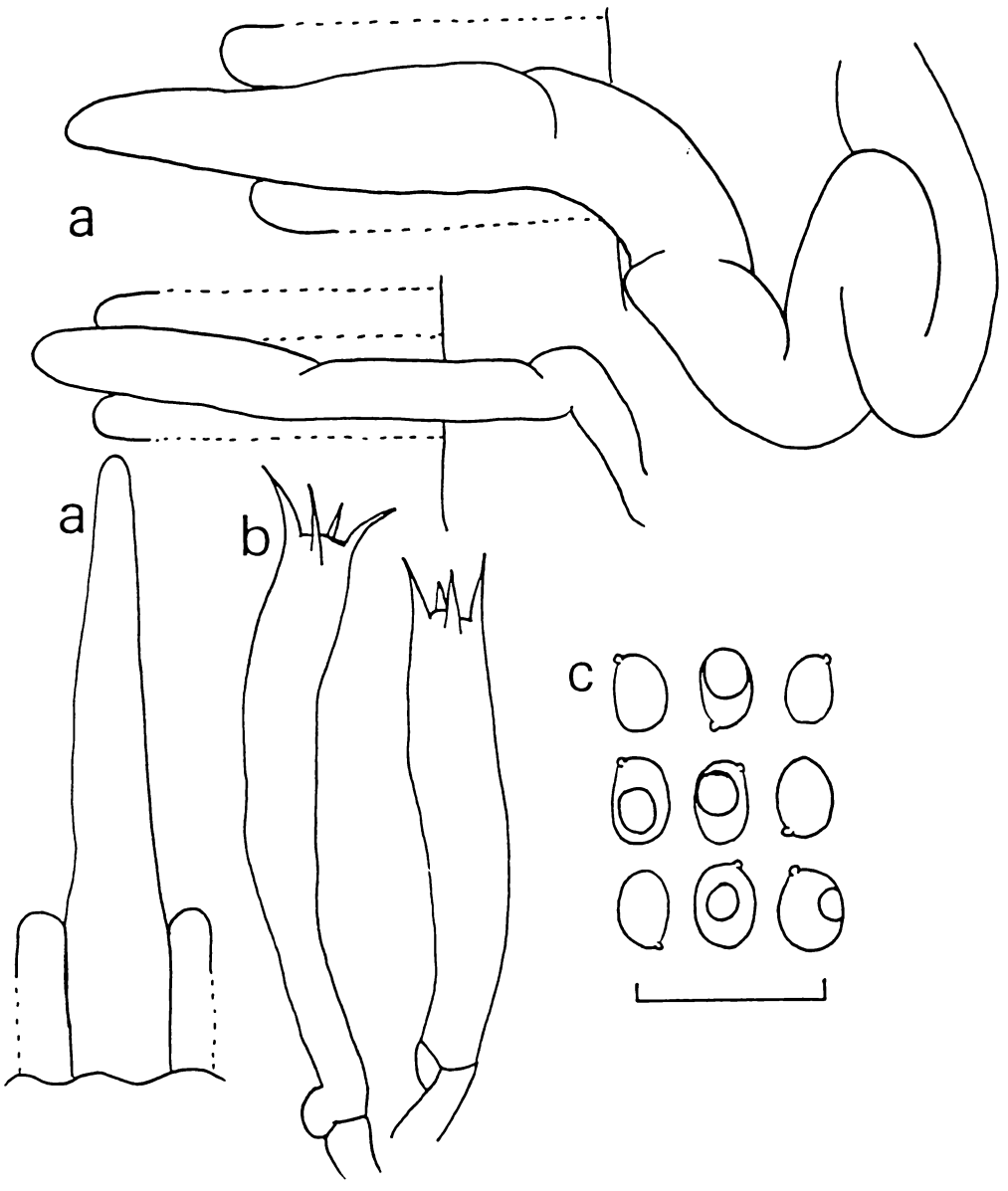


FIGURE 17. *Artomyces piperatus* (holotype of *Clavicorona avellanea*, Smith 54272, MICH 5580). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 µm.

Coker (1947) suggested that *Clavaria piperata* should be regarded only as a variety of *Clavaria pyxidata*, also due to the lack of distinguishing characters. Corner (1950), on the other hand suggested that *Clavaria piperata* might be synonymous under *Clavicorona colensoi*. Later, he apparently became unsure of its affinity and suggested that it be placed in a group with *Clavicorona pyxidata* and *C. avellanea* Leathers and Smith (Corner 1970). Leathers and Smith (1967) recognized *Clavaria piperata* and transferred the name to *Clavicorona*. At the same time they introduced their new species *Clavicorona avellanea* but did not describe its differences from *C. piperata*. Dodd (1972) synonymized *C. avellanea* under *C. piperata* concluding that the two are almost identical. Dodd (1972), however, cited collections from Australasia, as did Petersen (1988) and Wu (1991). They included Fawcett's (1939) collection of *C. pyxidata* var. *asperospora* as well as several other collections under *C. piperata*. Spore and basidia measurements reported here agree with Kauffman's (1927) description, and differ little from Leathers and Smith's (1967) description, but my measurements of spores are on the lower end of the ranges reported by Dodd (1972), Petersen (1988), and Wu (1991). Based on basidiomata and culture morphology as well as laccase electrophoretic patterns, Wu et al. (1996) suggested that the Southern Hemisphere collections probably represented a distinct species. This conclusion is supported by both the morphological and DNA sequence data presented here.

The Southern Hemisphere specimens collected under the name *Clavicorona piperata* include at least two species; *Artomyces novazealandensis* and *A. austropiperatus*. The basidiomata of these three species appear similar because they are somewhat dusky colored and robust. As previously mentioned, *A. novazealandensis* and *A. austropiperatus* are morphologically indistinguishable in that they have spores, basidia, and gloeocystidia of the same size, but these two cryptic morphospecies exhibit significantly divergent ITS DNA sequences. The spores of *A. piperatus* are similar in shape (Em = 1.31) to those of the other two species (1.28 for *A. novazealandensis* and 1.26 for *A. austropiperatus*) but are smaller. The spores of *A. piperatus* average 4.0 X 3.0 µm, while the spores of each of the other two average 5.1 X 4.0 µm. Scanning electron micrographs of the spores revealed that those of *A. novazealandensis* (FIG 22E) exhibit larger asperulae than do those of *A. piperatus* (FIG 22F). The basidia of *A. piperatus* are also smaller than those of the other two, averaging almost 5 µm shorter and 1 µm narrower.

Phylogenetically, *A. piperatus* is closest to *A. austropiperatus*, together comprising part of Clade I (FIGS 2, 3). *Artomyces novazealandensis* is somewhat divergent and comprises a clade by itself. The latter species may actually be related to the former, but there may be some influence of long-branch-attraction in the phylogenetic estimation.

Distribution and Habit: Known only from North America west of the Rocky Mountains (Alaska, British Columbia, Idaho, Washington), fruiting on rotting conifers.
Specimens: CANADA, British Columbia, Vancouver, Coquitlan Water Drainage, Pipeline Rd., 7 October 1992, coll. R.H. Petersen, TFB 5770 (TENN 53491). UNITED STATES, Alaska, Juneau, Sheep's Creek Trail, 20 September 1995, coll. R.H. Petersen, TFB 8221 (TENN 57288); Idaho, Nordman, 8 October 1956, coll. A.H. Smith, Smith 54272 (HOLOTYPE of *Clavicorona avellanea* - MICH 5580); Washington, Grays

Harbor Co., Lake Quinault, 12 November 1925, coll. C.H. Kauffman, s.n. (HOLOTYPE - MICH 10095); Washington, Olympic National Park, Soleduk Drainage, 15 October 1992, coll. S. Redhead, TFB 5869 (TENN 51959).

13. ***Artomyces pyxidatus*** (Pers.: Fr.) Jülich. 1981. Bibliotheca Mycologica 85: 399.
≡ *Clavaria pyxidata* Pers. 1794. Neues Mag. Bot. 1: 117.
≡ *Clavaria pyxidata* Pers.: Fr. 1821. Systema Mycologicum, vol.1, p.470.
≡ *Clavicorona pyxidata* (Pers.: Fr.) Doty. 1947. Lloydia 10: 43.
= *Clavaria petersii* Berkeley & Curtis. 1873. Grevillea 2: 7.
Holotypus: UNITED STATES, Alabama, no date, coll. Peters, s.n. (ANSPHILA !).
= *Clavaria coronata* Schw. 1831. Trans. of the American Philosophical Society 4: 182.
Holotypus: UNITED STATES, Pennsylvania, Northampton Co., Bethlehem, coll. Schweinitz (?), Syn.# 1041 (ANSPHILA !).

Basidiomata (FIG 25E - H)**:** gregarious to scattered, up to 100 mm tall X 40 mm wide, petite to slightly robust; branching variable, profusely branched and compact to loosely branched and open. Branches in up to 10 ranks, 5-6 per node, up to 2 mm diam.; almost white, "light buff" (3A2), "pale ochraceous buff" (4A2), to "lemon yellow" (2A7). Stipe often present, up to 25 mm tall X 2 mm diam.; basal mycelial pad pure white to rust colored. Tips cuspidate, coronate-cristate, to rarely acicular. Taste and odor mildly to strongly peppery. Dried basidiomata "pale cinnamon pink" (5A2), "cinnamon buff" (6B4), "buff brown" (6D4), "sayal brown" (6C5), "chestnut brown" (7E4), to "cinnamon rufous" (6D7).

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 8 µm diam., aseptate. Gloeocystidia (FIG 18a) occasional to abundant, cylindric to clavate, 2.4 – 10.0 µm diam., non-emergent to protruding to 12 µm, sometimes inflated to 10.0 µm in subhymenium narrowing to 6.0 µm in hymenium. Basidia (FIG 18b) (16.0-) 18.0 – 28.0 (-30.0) µm long (L_m = 21.3 µm ± 3.0) X (3.6-) 4.0 – 4.8 (-5.2) µm diam. (W_m = 4.2 µm ± 0.3), clamped.

Basidiospores (FIG 18c) elongate (E_m = 1.65 ± 0.05), 3.6 – 4.8 (5.0) µm (L_m = 4.1 µm ± 0.3) X (2.0-) 2.4 – 2.8 (-3.0) µm, minutely asperulous, often guttulate.

Commentary: Arguably, *A. pyxidatus* is the most common, widely distributed and well-known species of this genus. While variation in macroscopic characters exists, microscopic characters are relatively constant. Measurements reported here are comparable with those reported by Corner (1950), Dodd (1972), and Wu et al. (1996).

Due to the variability of this species, there was apparently some early taxonomic confusion. Schweinitz (1831) described *Clavaria coronata* as a new species, but did not include a clear diagnosis of how he distinguished this taxon from Persoon's (1794) *C. pyxidata ex* Fries' (1821), which he (Schweinitz) included in his synopsis of the fungi of North Carolina. Berkeley and Curtis (1873) described *Clavaria petersii* as a species distinct from *C. pyxidata*, also without providing diagnostic differences to distinguish the two species, and did not acknowledge Schweinitz's *C. coronata*. Burt (1922) favored keeping the three as distinct species, providing a key that emphasized slight differences in basidioma color and spore size. The next year Coker (1923) synonymized the three species under *Clavaria pyxidata*, expanding its circumscription to include wide

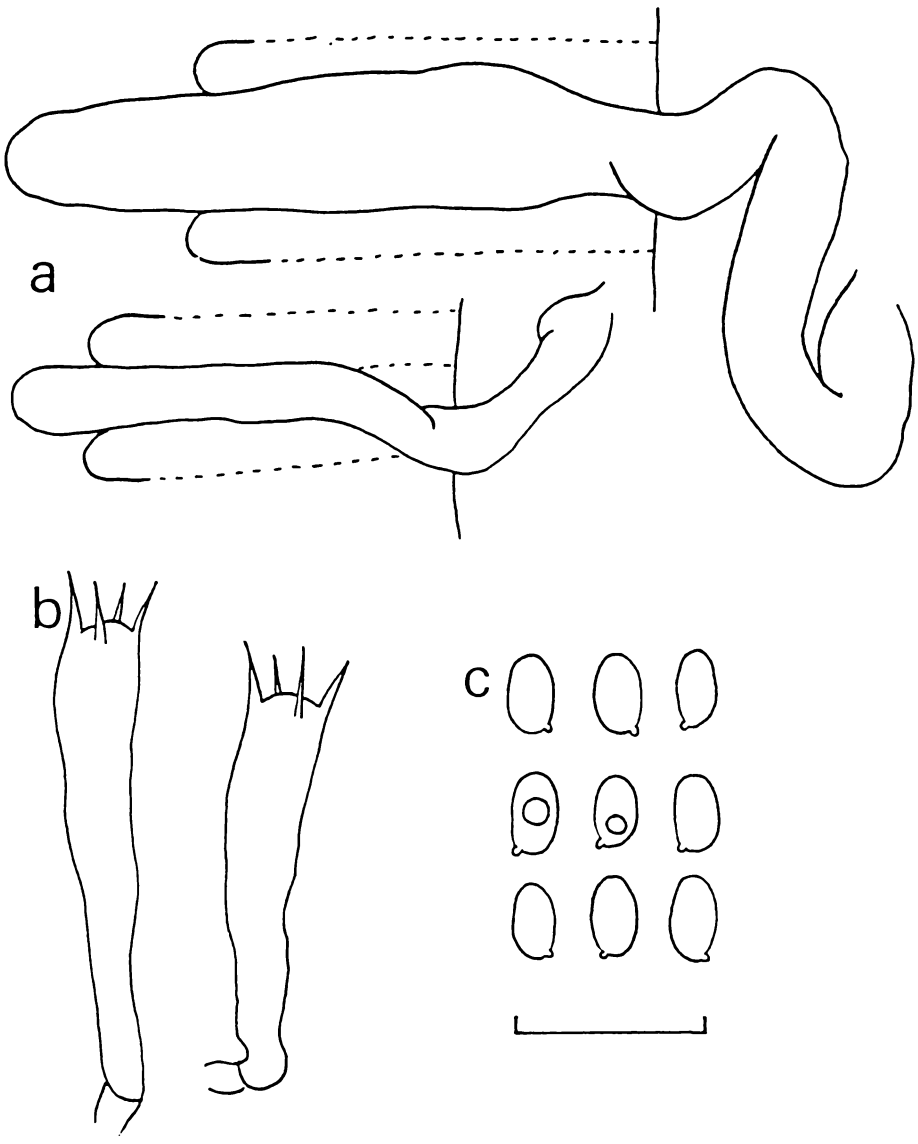


FIGURE 18. *Artomyces pyxidatus* (TENN 48852). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

morphological variability. Doty (1944), however, resurrected the name *Clavaria coronata* but misattributed it to what Kauffman (1930) had named *Craterellus cristatus*. Doty (1944) kept *C. coronata* distinct from *Clavaria taxophila* (Thom) Lloyd and *Clavaria piperata* Kauffman. Since his paper dealt with fungi of the American Pacific Northwest, *C. pyxidata* was not included, but he noted that it, too, was a distinct species "sensu Coker." Three years later Doty (1947) published his paper proposing the genus name *Clavicorona,* but apparently he changed his mind regarding *Clavicorona coronata*. This time he synonymized *Clavaria piperata* under *C. coronata* and recognized *Clavicorona cristatus*, as well as *C. pyxidata* and *C. taxophila*. Corner (1950) subsequently synonymized *Clavicorona coronata* and *Clavaria petersii* under *C. pyxidata*, recognized *C. cristata*, but suggested that *C. piperata* was a synonym under another species, *C. colensoi*. Yet again, the name *C. coronata* was resurrected, this time by Leathers and Smith (1967) who felt that it was distinct based on dextrinoid spores. Dodd (1972) reported that spores were amyloid and again synonymized *C. coronata* with *C. pyxidata*. Observations of spores from the type material of *Clavaria coronata* Schw. for this study support Dodd's (1972) assessment and taxonomic treatment.

Recent studies of the variability in basidioma morphology, culture morphology, sexual intercompatibility, phenoloxidase enzyme banding patterns and DNA sequences within *A. pyxidatus* include those by Dodd (1972), Wu et al. (1996), and Chapter III below. Although there is some variability in morphology and enzyme banding patterns, these studies have shown that wide-ranging populations remain intercompatible and exhibit little ITS DNA sequence divergence.

Distribution and Habit: *Artomyces pyxidatus* is widely distributed across much of the North Temperate Zone deciduous forest: reported from Canada (Quebec), China (Jilin and Yunnan), Costa Rica, France, Mexico, Russia, Sweden, Switzerland, and United States (eastern states, Arizona, New Mexico, Minnesota, Utah), but apparently absent from the North American Pacific coast; fruits on rotting hardwood with some unsubstantiated reports of conifer wood.

Specimens: CHINA, Prov. Yunnan, Simao Co., Mounai Mountains, 5 August 1990, Q. Wu, TFB 1541 (TENN 48855). SWEDEN, Uppland, vic. Uppsala, Gottsundabergen, 7 September 1994, coll. R.H. Petersen, TFB 7263 (TENN 53544). UNITED STATES, Alabama, no date, coll. Peters, s.n. (HOLOTYPE of *Clavaria petersii* - ANSPHILA); Alabama, no date, coll. Peters, s.n., 1860 Ravenel Fasc. 5 (ANSPHILA); Pennsylvania, Northampton Co., Bethlehem, coll. Schweinitz (?), Syn.# 1041 (HOLOTYPE of Clavaria coronata - ANSPHILA); Tennessee, Sevier Co., Great Smoky Mountains National Park, 8 June 1989, Q. Wu, TFB 1607 (TENN 48852).

13a. *Clavaria pyxidata* Pers.: Fr. **var. *asperospora*** Fawcett. 1939. Proc. Roy. Soc. Victoria 51: 15.

Holotypus: AUSTRALIA, Victoria, Sherbrooke (MEL-Fawcett 7125F *n.v.*).

Basidiomata (according to Fawcett 1939)**:** basidiomata gregarious to scattered, up to 100 mm tall, often petite, flesh tough and pliant. Branches "wood brown" (7C4) to "avellaneous" (7B3). Stipe slender, "sometimes pubescent," "cinnamon brown" (6E8); basal mycelial pad brown. Taste peppery.

Micromorphology (according to Fawcett 1939): context composed of hyphae, 3.5 µm, often inflated to 11 µm, clamped (gloeoplerous hyphae not described). Cystidia of two types; 1) "fusiform, pointed, hyaline, with sscanty cell contents;" 2) "cylindrical with rounded tips, resembling gloeocystidia of *Physalacria*." Basidia 4-spored, 3.5 – 4.6 µm diam.

Basidiospores "sub-globose 3.2 – 4.0 X 4 – 4.8 µm" (L_{median} = 4.4 µm), "hyaline, distinctly rough, pure white in mass."

Commentary: Fawcett (1939) considered this variety to be widespread in Victoria and cited its occurrence in Tasmania. Corner (1950) suggested that it might represent a "fully developed state" of *Clavicorona colensoi*, but later suggested an affinity with *C. avellanea* (Corner 1970). Although Fawcett (1939) did not identify a type specimen by number, Dodd (1972) indicated that he saw two specimens, MEL-Fawcett 7125F which he referred to as the type and MEL-Fawcett 7126F. Dodd (1972) did not provide any morphological measurements, but he stated that these collections had the same macro- and micromorphological characters as North American *C. piperata*, with which he synonymized it along with *C. avellanea*. Both Petersen (1988) and Wu (1991) agreed with this treatment, but like Dodd (1972), they did not report any morphological measurements. Wu et al. (1996) suggested that their "Taxon 4" (*Artomyces tasmaniensis* of this study) might represent *C. pyxidata* var. *asperospora*, citing similar morphological characters. Unfortunately, "type" material of var. *asperospora* was not available for either this study or that by Wu et al. (1996), but Fawcett's (1939) spore measurements might be sufficient to make some comparisons. *Artomyces tasmaniensis* and probably *A. colensoi* can be ruled out because their spore sizes are slightly smaller (L_m = 3.8 and 3.9, respectively). *Artomyces novazealandensis* and *A. austropiperatus* can be ruled out because their spores are slightly larger (L_m = 5.1 and 5.1 µm), and at least *A. novazealandensis* may be endemic to the South Island of New Zealand. By process of elimination, *Clavaria pyxidata* var. *asperospora* is probably either *A. candelabrus* or *A. turgidus*, both of which have coarsely asperulate spores.

While the type material is apparently missing, three specimens identified by Wu (1996, specimen annotation) as "probably *Clavicorona pyxidata* var. *asperospora,*" were examined for this study. However, the three seem to represent three different species; MEL 2019810 is probably *Artomyces turgidus*, MEL 1053577 is probably *Artomyces candelabrus*, and MEL 1053578 is probably *Artomyces colensoi*.

14. ***Artomyces stephenii*** Lickey *sp. nov.* [*nom. prov.*]

Basidiomata usque ad 50 mm alto et 20 mm lata, ramosissimus subtilis, pyxidatus, specimen exsiccatum cinerascens roseus bubalinus ad eburneus bubalinus. Stipes interdum praesens ad 10 mm alto. Rami gracilis dilatatus ad 1(2) mm, rami inferiora proximus lignum bruneus ad bubalinus bruneus, superus proximus avellaneus ad vinaceus bubalinus. Apices cuspidatus ad coronatus-cristatus. Sapor valde acerbis. Gloeocystidia communis, cylindraceus ad late fusiformis, 2.0 – 6.8 µm diametro, interdum extans ultra hymenium 12 µm. Basidia circa 19.7 X 4.7 µm. Basidiosporae ellipsoideae, 3.2 – 4.0 (-4.4) X (2.2-) 2.4 – 2.8 (-3.0) µm, (E_m = 1.40 ± 0.04), amyloideae, hyalinus, asperulatus. Lignatilis angiospermae.

Holotypus [*hic designatus*]: COSTA RICA, Prov. Cartago, Rio de Savegre, Cabanas Chacun, 29 June 1998, coll. R.H. Petersen, TFB 9436 (TENN 56410).
Etymology: in memory of my father, Stephen E. Lickey.
Basidiomata (FIG 26A, B, C): up to 100 mm tall X 50 mm wide, often profusely branched and compact, petite to slightly robust. Branches in up to 10 ranks, 5-6 per node, up to 2 mm diam., "vinaceous buff" (9B2) to "tilleul buff" (7B2) to "avellaneous" (7B3). Stipe up to 10 mm or branched from base, "wood brown" (7C4) to "buff brown" (6D4); basal mycelial pad whitish to dark brown. Tips cuspidate to coronate-cristate, "tilleul buff" to "pale pinkish cinnamon" (6A2) to "pale ochraceous buff" (4A2). No odor reported, taste strongly acrid. Dried basidiomata "cream buff" (4A4) to slightly more grey than "pinkish buff" (6A3).
Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 8 (-10) µm in diam., aseptate. Gloeocystidia (FIG 19a, b) occasional to abundant, cylindric to capitate, 2.0 – 6.8 µm diam., non-emergent to protruding up to12 µm. Basidia (FIG 19c) 16.0 – 26.0 µm long (L_m = 19.7 µm ± 2.2) X 3.8 – 5.6 µm diam. (W_m = 4.7 µm ± 0.5), clamped.

Basidiospores (FIG 19d) ellipsoid (E_m = 1.40 ± 0.04), 3.2 – 4.0 (-4.4) µm (L_m = 3.7 µm ± 0.2) X (2.2-) 2.4 – 3.0 µm, minutely asperulate, weakly amyloid, often guttulate.
Commentary: The general stature of basidiomata of this species, with respect to its profusely and delicately branching, is very similar to *A. pyxidatus*, which also occurs in Costa Rica. However, spores of *A. pyxidatus* are slightly longer (L_m = 4.1 µm ± 0.3) and narrower (E_m = 1.65 ± 0.05) than those of *A. stephenii*. These two species were subjected to an intercompatibility study, and it was found that each formed their own intercompatibility group (FIG 4). The SBIs representing three collections (9433, TENN 56407; 9436 TENN 56410; 9348, TENN 56412) of *A. stephenii* were 100% intercompatible with each other.

ITS DNA sequence analysis confirmed that *A. stephenii* was distinct from *A. pyxidatus* and formed a sister clade to *A. colensoi* (FIGS 2, 3). Two more species also fruit in Costa Rica, but both are morphologically and genetically distinct. *Artomyces costaricensis* has stouter, darker colored basidiomata and longer spores (L_m = 4.2 µm). *Artomyces enigmatus* also has longer spores that are slightly broader (L_m = 4.4 µm, E_m = 1.32) and its basidiomata are only slightly stouter.
Distribution and Habit: Known only from Costa Rica. Apparently fruiting only on hardwood.
Specimens: COSTA RICA, Prov. Cartago, Rio de Savegre, Cabanas Chacun, 29 June 1998, coll. R.H. Petersen, TFB 9433 (TENN 56407); Prov. Cartago, Rio de Savegre, Cabanas Chacun, 29 June 1998, coll. R.H. Petersen, TFB 9436 (HOLOTYPE - TENN 56410); Prov. Cartago, Rio de Savegre, Cabanas Chacun, 29 June 1998, coll. R.H. Petersen, TFB 9438 (TENN 56412).

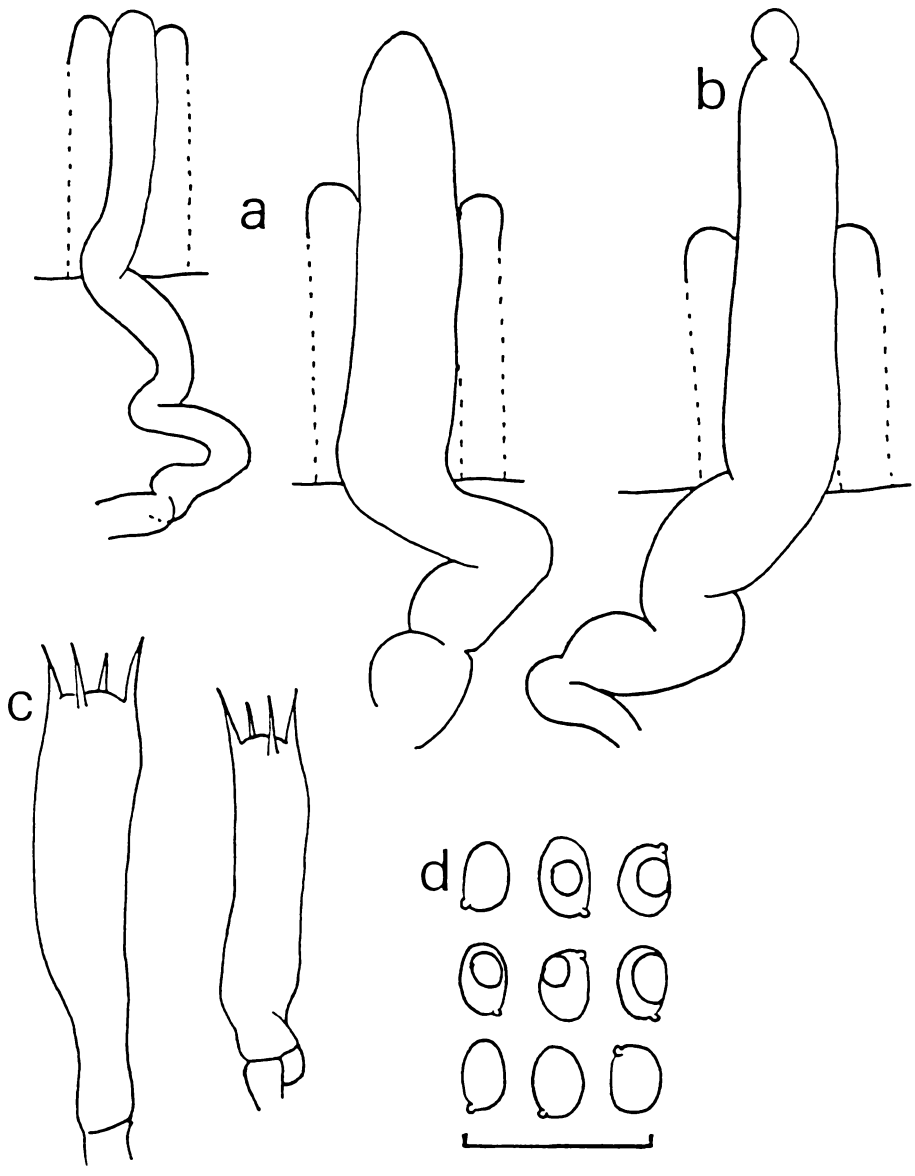


FIGURE 19. *Artomyces stephenii*. a) gloeocystidia (TENN 56410); b) gloeocystidia (TENN 56407); c) basidia (TENN 56410); d) basidiospores (without ornamentation; TENN 56410). Bar = 10 μm.

15. ***Artomyces tasmaniensis*** Lickey *sp. nov.* [*nom. prov.*]

Basidiomata usque ad 60 mm alto et 20 mm lata, parvus ramosus, pyxidatus, specimen exsiccatum badius vel nigellus. Stipes praesens ad 15 mm alto. Rami crassus dilatatus ad 3 mm, rami inferiora proximus fuscus, superus proximus cinnamomeus. Apices cuspidatus ad coronatus-cristatus. Sapor tarde acerbis. Gloeocystidia communis, cylindraceus ad digitiformis, 2.8 – 6.8 µm diametro, interdum extans ultra hymenium 17.6 µm. Basidia circa 25.2 X 4.5 µm. Basidiosporae late ellipsoideae, (3.2-) 3.6 – 4.4 X 2.6 – 3.6 µm, (E_m = 1.26 ± 0.01), amyloideae, hyalinus, asperulatus. Lignatilis angiospermae.

Holotypus [*hic designatus*]: AUSTRALIA, Tasmania, Tahune Forest Preserve, vic. Geeveston, 5 June 1991, coll. K.W. Hughes, TFB 4003 (TENN 50218).

Etymology: because this species is known only from Tasmania.

Basidiomata (FIG 26D, E, F)**:** up to 60 mm tall and 20 mm wide, loosely branched, stout. Branches in 2-3 ranks, 3 - 4 per node, stout to 3 mm diam., "deep brownish drab" (11E3), "wood brown" (7C4), "sayal brown" (6C5), and "cinnamon brown" (6E8). Stipe up to 15 mm long X 3 mm diam., "blackish brown" (7F5), "army brown" (8D5), "fuscous" (6E4), and "hair brown" (6E3); basal mycelial pad is rust colored. Tips cuspidate to coronate-cristate, "fawn color" (7C5) to "tawny olive" (5C5). Odor typical, peppery, taste slowly acrid. Dried basidiomata "chestnut" (8E7), "chocolate" (8E8), "burnt umber" (7E7), "seal brown" (8F6), to black.

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 8 µm diam., aseptate. Gloeocystidia common, cylindric to broadly fusiform, 2.8 – 6.8 µm wide, non-emergent to protruding up to17.6 µm (FIG 20a). Basidia 22.0 – 32.0 µm long (L_m = 25.2 µm ± 2.5) X 4.0 – 5.2 µm wide (W_m = 4.5 µm ± 0.4), clamped (FIG 20b).

Basidiospores broadly ellipsoid (E_m = 1.26 ± 0.01), (3.2-) 3.6 – 4.4 µm (L_m = 3.8 µm ± 0.3) X 2.6 – 3.6 µm, asperulate, often guttulate (FIG 20c).

Commentary: Basidiomata of this species are morphologically similar to those of *A. turgidus*, which also occurs in Tasmania. Basidiomata of both species are pale shades when fresh, with those of *A. tasmaniensis* being slightly darker, and basidiomata of both turn almost black when dried. However, spores of *A. tasmaniensis* are slightly shorter and wider than *A. turgidus*.

This species corresponds to "Taxon 4" of Wu et al. (1996), which they interpreted as cohesive based on intercompatibility of two isolates, a common laccase profile among three isolates, and micromorphology of five specimens (including the three in this study). This group is further held together by similarity of DNA sequences. Wu et al. (1996) suggested that this taxon might represent Fawcett's (1939) *Clavaria pyxidata* var. *asperospora*. However, the type specimen of var. *asperospora* apparently was not (and still is not) available for study, and an exact determination could not be made. Three specimens from the Melbourne (MEL) herbarium annotated *C. pyxidata* var. *asperospora* by Wu in 1996 were examined, and it was found that they were not the same as *A. tasmaniensis*, but rather that each represented a separate species as discussed earlier (see under *C. pyxidata* var. *asperospora*).

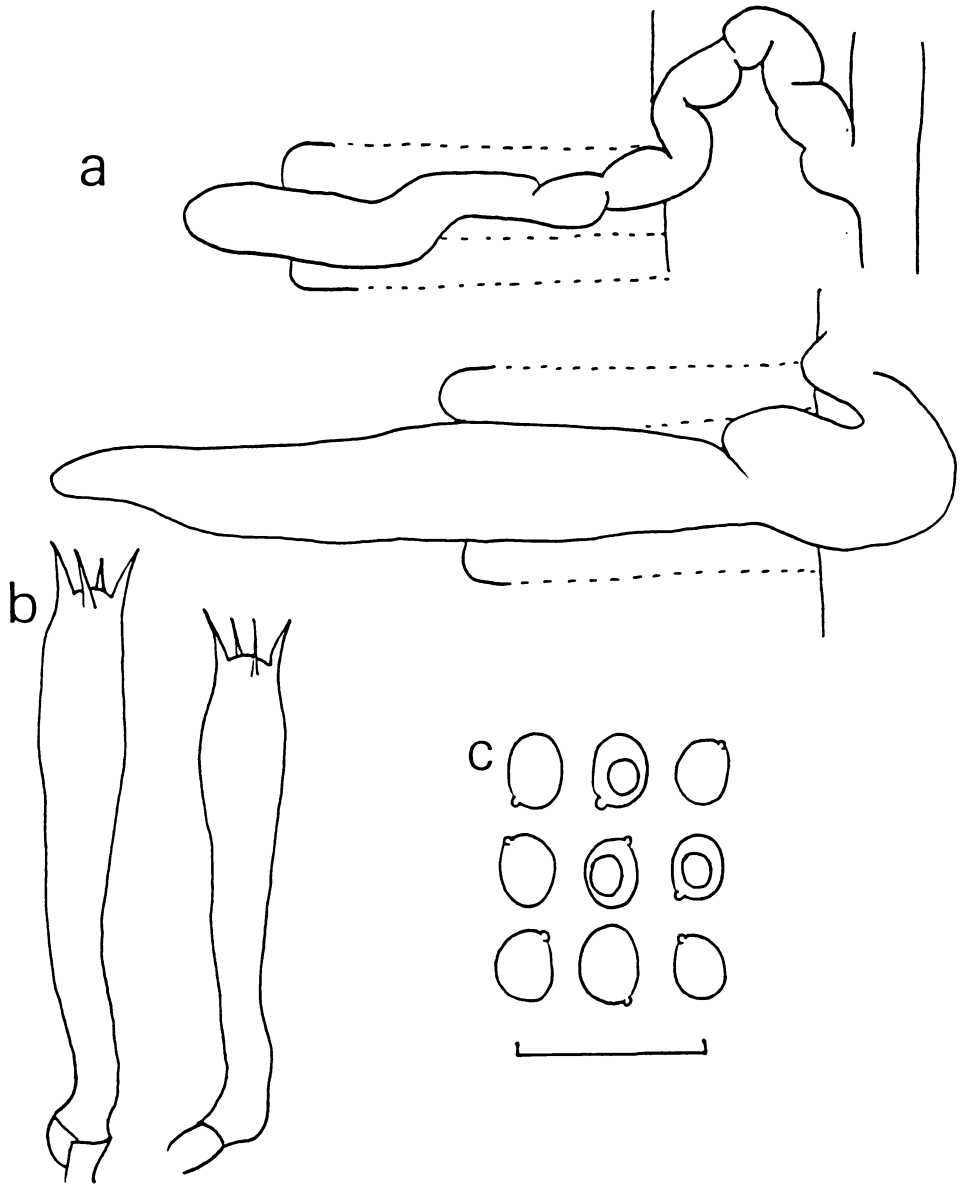


FIGURE 20. *Artomyces tasmaniensis* (TENN 50218). a) gloeocystidia; b) basidia; c) basidiospores (without ornamentation). Bar = 10 μm.

In the phylogenetic estimation and distance trees, *A. tasmaniensis* consistently comprises the basal clade in *Artomyces* (FIGS 2, 3). Although support for this relationship is not strong, it could indicate that *Artomyces* originated in the Australian region of Gondwana, and that *A. tasmaniensis* may represent characteristics of the ancestral species to *Artomyces*.

Distribution and Habit: Known only from Tasmania. Substratum included with all three specimens was identified as hardwood. Field notes indicate that TENN 50218 was fruiting on *Eucalyptus*.

Specimens: AUSTRALIA, Tasmania, Mt. Wellington, Gully at 400 m, 26 May 1991, coll. K.W. Hughes, TFB 3905 (TENN 50112); Tasmania, Mt. Field National Park, 3 June 1991, coll. R.H. Petersen and K.W. Hughes, TFB 3979 (TENN 50283); Tasmania, Tahune Forest Preserve, vic. Geeveston, 5 June 1991, coll. K.W. Hughes, TFB 4003 (HOLOTYPE - TENN 50218); Tasmania, Tahune Forest Preserve, vic. Geeveston, 5 June 1991, coll. K.W. Hughes, TFB 4005 (TENN 50197).

16. *Artomyces turgidus* (Lév.) Jülich. 1981. Bibliotheca Mycologica 85: 399.

≡ *Clavaria turgida* Léveillé. 1846. Ann. Sci. Nat. III 5: 155.

≡ *Clavicorona turgida* (Lév.) Corner. 1957. Darwiniana 11: 195.

≡ *Clavaria javanica* Sacc. & Syd. 1899. Syll. Fung., p. 238.

≡ *Clavicorona javanica* (Sacc. & Syd.) Corner. 1950. Monograph, p. 289.

≡ *Clavaria coronata* Zipp. apud Lév. 1844. Ann. Sci. Nat. III 2: 215. [*non* Schweinitz 1831].

Holotypus: INDONESIA, Java, no date, coll. Léveillé, s.n. (K 69005 !).

= *Clavaria laetissima* Berk. 1881. J. Linn. Soc. Bot. 18: 386.

Holotypus: AUSTRALIA, no date, coll. Lockyer, Lockyer 477 (K 69006 !).

Basidiomata (FIG 26G, H, I, J): up to 75 mm tall X 40 mm wide, well-branched, open to compact, petite to robust. Branches in up to 7 ranks, 2 - 4 per node, 1 - 2 mm diam., "tilleul buff" (7B2), "vinaceous buff" (9B2), and "avellaneous" (7B3). Stipe conspicuous, up to 10 mm long X 3 mm diam., "wood brown" (7C4), "benzo brown" (9D3), "light drab" (17B2), and "cinnamon buff" (6B4); basal mycelial pad cream to rust colored. Tips cuspidate to coronate-cristate, darken with age to "dusky drab" (10F3) to "dark grayish brown" (13F3). Odor typical to faintly peppery, taste bitter to intensely acrid and lingering. Dried basidiomata "xanthine orange" (6C8), "burnt umber" (7E7), "seal brown" (8F6), to black.

Micromorphology: Context composed of; 1) generative hyphae, thin-walled, often inflated, clamped; 2) gloeoplerous hyphae, common, up to 10 µm diam., aseptate. Gloeocystidia (FIG 21a, b) common, cylindric to broadly fusiform to lageniform, 2.4 – 12.0 µm diam., non-emergent to protruding up to 30 µm. Basidia (FIG 21c) 20.0 – 30.0 µm long (L_m = 23.5 µm ± 2.7) X 3.6 – 6.0 µm diam. (W_m = 5.0 µm ± 0.6), clamped.

Basidiospores (FIG 21d) ellipsoid (E_m = 1.38 ± 0.06), (3.2-) 3.6 – 4.8 µm (L_m = 4.1 µm ± 0.3) X 2.4 – 3.6 µm, obviously asperulate, often guttulate.

Commentary: Zippel (in Léveillé 1844) described a new species of *Clavaria* from Java as being similar in aspect to *C. pyxidata* Pers. but basidiomata were thinner, more yellow, and with a glabrous stipe. Apparently unaware of Schweinitz's 1831 paper,

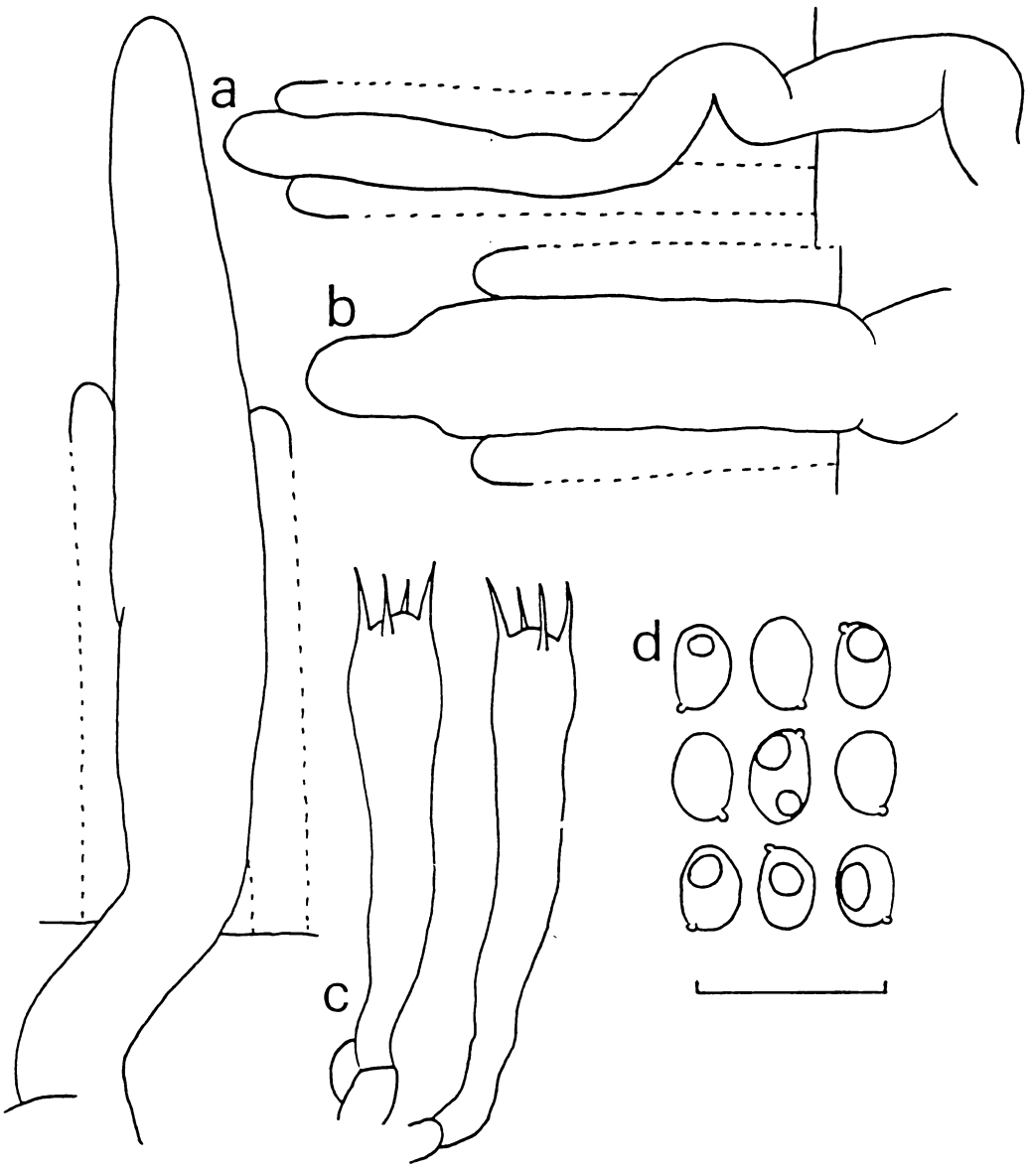


FIGURE 21. *Artomyces turgidus*. a) gloeocystidia (TENN 56950); b) gloeocystidia (TENN 54099); c) basidia (TENN 54099); d) basidiospores (without ornamentation; TENN 56950). Bar = 10 μm.

Zippel called his species *C. coronata* (see discussion under *A. pyxidatus*). Realizing the mistake, Léveillé (1846) changed the name *C. coronata* to *C. turgida*. In an unconnected attempt to remedy the homonym problem, Saccardo and Sydow (1899) overlooked Léveillé's change and proposed their own substitute name, *C. javanica*. Corner (1950) also missed Léveillé's (1846) name substitution and transferred *C. javanica* into *Clavicorona*. He later acknowledged the priority of *Clavaria turgida* and subsequently transferred that name to *Clavicorona*, and synonymized the name *Clavicorona javanica* (Corner 1957).

Berkeley (1881) described a new *Clavaria* species from Australia. Basidiomata were orange to brown and dichotomously branched, and he named it *C. laetissima*. This description was apparently from the dried specimen (Petersen 1985). Dodd (1972) synonymized this species under *Clavicorona turgida,* finding few morphological differences and observing its gelatinized hymenium, which he emphasized in his characterization of the species. Petersen (1985), however, interpreted the hymenium as collapsed, not gelatinized and found that spores were smaller (3.6 X 2.2 μm) than those Dodd (1972) reported for *C. turgida* (4.0 – 5.0 X 3.0 – 3.5 μm).

For this study, it was found that the hymenium was collapsed, but the existence of gelatinization was difficult to determine. Several specimens of *Artomyces*, not just those of *A. turgidus*, appeared to have a gelatinized hymenium due to poor drying or preservation. Examination of fresh material would be useful for this determination.

Spores of Lockyer 477 were found to be 3.2 – 4.4 (-4.6) μm (L_m = 3.9 μm ± 0.4) X 2.4 – 3.2 μm; E_m = 1.4 ± 0.1. Although somewhat at the lower end of the spore length range for *A. turgidus*, spore sizes are comparable and E_m values identical. At this point, there is no reason not to include *C. laetissima* in synonomy under *A. turgidus*.

Duke HN 3138 (isolate RV95-875) from Papua New Guinea was described as being pure white, 4 cm tall, and with slender branches (2 – 3 mm). Sequence analysis indicated little divergence between this and the two *A. turgidus* collections, and it is included here in this description because of the overall morphological similarity of basidiomata. Corner (1950, p.289) designated a type for *Clavicorona javanica*, describing it as "wholly waxy white" in contrast to Zippel's (1844) note of yellowish basidiomata. Although this specimen from Java must be disregarded as type because Zippel's had priority (Petersen 1984), Corner (1950) did refer to white basidiomata. Later he ascribed the name *Clavicorona turgida* to several "pure white" collections from Argentina (Corner 1957) and Chile (Corner 1970). These specimens were not obtained for examination for this study, but the reported spore measurements correspond very well with *Artomyces adrienneae*, described in this study as new. Phylogenetic estimations indicate that *A. adrienneae* is allied with *A. candelabrus* (FIGS 2, 3). Dodd (1972) examined several collections which he identified as *Clavicorona turgida* from North Carolina, Costa Rica, Cuba, and Brazil, but he explained that they were slightly more robust that Australasian collections. These specimens were not obtained for this study, and these collections remain unverified. Collection MEL 2019810 was examined for this study because it was identified by Wu (1996, specimen annotation) as "probably *Clavicorona pyxidata* var. *asperospora* Fawc." This collection consisted of one robust, well-branched basidoma with six ranks of branches, and had spores 4.0 – 4.8 X 2.8 – 3.4 μm (L_m = 4.5, E_m = 1.5). It is here identified as *Artomyces turgidus*.

In a recent study, Wu et al. (1996) found that two isolates (2651 and 2682) were sexually intercompatible. They also reported a unique electrophoretic banding pattern in *Clavicorona turgida* for extracellular laccase.

Distribution and Habit: Known in this study from Indonesia, New Guinea, Australia, and New Zealand. If verified, specimens cited by Dodd (1972) might increase this range to include the New World. Substratum included in several collections was identified as hardwood.

Specimens: AUSTRALIA, no date, coll. Lockyer, Lockyer 477 (HOLOTYPE of *Clavaria laetissima* - K 69006); Tasmania, Rosebery, slopes of Mt. Murchison, 30 May 1991, coll. R.H. Petersen and K.W. Hughes, TFB 3961 (TENN 50103). INDONESIA, Java, no date, coll. Léveillé, s.n. (HOLOTYPE of *Clavaria coronata* - K 69005). NEW ZEALAND, North Island, Auckland, Waitakere Ranges, Farley Track, 22 May 1990, coll. R.H. Petersen, TFB 2682 (TENN 48926); North Island, Auckland, Waitakere Ranges, vic. Huia, 31 May 1994, coll. R.H. Petersen, TFB 7164 (TENN 56950); South Island, Cascade Valley, 11 April 1983, coll. R.H. Petersen, TFB 55137 (TENN 44103); South Island, Fjordland, Fjordland National Park, Hollyford Rd., 10 May 1994, coll. G. Ridley, TFB 7040 (TENN 54099); South Island, Nelson Co., vic. St. Arnaud, Nelson Lakes National Park, Lake Rotoroa, 18 May 1994, coll. P. Austwick, TFB 7109 (TENN 56218). PAPUA NEW GUINEA, Morobe Prov., Border Track, Kuper Range Field Station, 11 July 1995, coll. R. Vilgalys, RV-95-875 (DUKE HN 3138).

Excluded Species

Artomyces divaricatus (Leathers & Smith) Jülich. 1981. Bibliotheca Mycologica 85:399.
≡ *Clavicorona divaricata* Leathers & Smith. 1967. Mycologia 59:456-462.
Holotypus: UNITED STATES. Idaho, Priest Lake, 18 October 1956, coll. A.H. Smith, Smith 54971 (MICH 5581 !).

Commentary: Leathers and Smith (1967) described this collection as a new species of *Clavicorona*, citing its branched, coralloid, lignicolous habit, pseudocystidia containing "refractive granular content," and amyloid spores. They indicated that young (smaller) spores were strongly amyloid, but did not mention older (larger) spores. In his examination of the specimen, Dodd (1970) found that the spores were smooth and weakly amyloid. Furthermore, he stated that gloeoplerous hyphae and gloeocystidia were present but rare. Consequently, Dodd (1970) included this as a valid species of *Clavicorona*.

Upon examination of this specimen for this study, it was apparent that the majority of the spores and those associated with basidia were inamyloid, smooth, and thin-walled. Spores were cylindric ($E_m = 2.1 \pm 0.4$), 5.0 – 7.6 µm X 2.4 – 3.2 µm. A second set of smaller spores were broadly ellipsoid ($E_m = 1.2 \pm 0.1$), (3.0 – 4.0 µm X 2.2 – 3.4 µm), strongly amyloid, asperulate, and thick-walled (up to 1.0 µm), but these were much less common and did not appear to be associated with basidia. These were considered to be contaminants. Furthermore, no pseudocystidia (gloeocystidia) or gloeoplerous hyphae typical of *Artomyces* were observed in the specimen with KOH, sulfobenzaldehyde, or Congo red reagents. A search through Corner's (1970) key revealed that this specimen most likely represents *Lentaria albovinacea* Pilát. In his description of *L. albovinacea*, Corner (1970) suggested the synonymy of *C. divaricata*,

but he apparently did not examine Smith's specimen for confirmation, relying instead on Leathers and Smith's (1967) description.

DISCUSSION

The morphological and molecular data presented here support Jülich's (1981) recognition of *Artomyces* as a genus distinct from *Clavicorona*. Furthermore, it is apparent that these two genera are not closely related, and that *Clavicorona taxophila* has affinities with members of the *Omphalina – Gerronema – Chrysomphalina* group. Because its basidiomata are simple, unbranched, and coronate, *C. taxophila* has been assumed to be a primitive form of the branched species (Corner 1950). This concentration on the macroscopic character of *C. taxophila* basidiomata seems to have overshadowed the distinctly different underlying microscopic characters such as smooth, thick-walled, inamyloid spores, the clampless generative hyphae, and the lack of a typical gloeoplerous system.

The amount of DNA sequence divergence between pairs of isolates of widely distributed *Artomyces* species, such as *A. austropiperatus, A. candelabrus, A. cristatus, A. pyxidatus,* and *A. turgidus*, may be due to range disjunctions and geographic isolation. In the pan-Temperate *A. pyxidatus*, sequence data suggest that there is no gene flow occurring between the European and North American populations, and these populations have probably been isolated for a long period of time (Chapter III). Intraspecific disjunction patterns such as this could be explained by either long-distance spore dispersal and/or vicariance events among once widespread populations. However, in the absence of fossil data, the times of isolation are speculative. Using geologic history, a fairly well-supported intraspecific phylogeny, and limited fossil data, Hibbett (2001) hypothesized that divergent populations of *Lentinula* in the Old World were due mostly to vicariance events and were rarely due to long-distance spore dispersal events. On the other hand, little or no sequence divergence was observed between isolates of more restricted species, such as *A. costaricensis, A. novazealandensis*, *A. piperatus*, and *A. tasmaniensis*, indicating that there are few, if any, geographic isolation mechanisms.

The lack of fossil record and the poor resolution in the basal nodes makes identifying the geographic and evolutionary origin of *Artomyces* very difficult. Cain (1944) stated that geographic regions of high species diversity within genera have been used to determine centers of geographic origin, but he cautioned that a reliance on this criterion is problematic because secondary points of radiation are possible. The region of highest *Artomyces* species diversity and density is the Australasian region where seven out of the 17 species occur, six of which occur in New Zealand and Tasmania alone, and four of which are endemic or narrowly restricted to that area. An eighth species occurs in Argentina. Conversely, the Northern Hemisphere has nine species, with four occurring in Costa Rica alone, and seven with narrowly restricted ranges. The higher density of species in New Zealand and Tasmania and the putative basal phylogenetic position of *A. tasmaniensis* suggest a Gondwanan center of origin. However, there are still large geographic areas such as India and Africa that remain under-studied. If a Southern Hemisphere origin of *Artomyces* is assumed, then the next task is identifying a more specific site. The Southern Hemisphere species seem particularly closely associated with *Nothofagus*, with only a few accounts of *Eucalyptus* and *Leptospermum* as substrate. If a

Nothofagus association is assumed, and this association was historical, then a history of *Artomyces* may be hypothesized based on the biogeography of *Nothofagus* for which there is a very extensive fossil record. *Nothofagus* has been hypothesized to have originated in Australasia as well, but recent molecular work in combination with fossil evidence has shown stronger support for a South American center of origin (Swenson and Hill 2001). They further suggest that New Zealand was a point of secondary radiation.

From this putative Southern Hemisphere origin, it is still unclear what routes of radiation the species of *Artomyces* have taken to reach to its present-day distribution. No discernable overall geographic pattern among *Artomyces* species was observed in the phylogenetic estimation (FIG 2) or the neighbor joining tree (FIG 3). Undersampling, the extinction of intercalary taxa, higher than expected long-distance dispersal, and/or variable rates of gene evolution within the ITS gene could account for the lack of patterns among species. This apparent lack of geographic patterns is inconsistent with those found in *Lentinula* whose species fall into discrete clades that match their geographic distribution based on both large subunit and ITS regions (Hibbett et al. 1998). Hibbett (2001) estimated a molecular clock based on vicariance events calibrated with geologic events. However, within a few clades in *Artomyces*, it was apparent that there were intercontinental sibling species pairs. The species pair *dichotomus-piperatus* was not strongly supported, but species pairs *colensoi-stephenii*, *turgidus-enigmatus*, *candelabrus-adrienneae*, and *microsporus-pyxidatus* were strongly supported. Disjunct species pairs could result from repeated dispersal events, vicariance, or the independent radiation of ancestral species that were separated when the continents of Gondwana separated. Because there was low branch support, analysis of other genes, particularly ones that could resolve the basal nodes, would be needed to verify this relationship.

The observed distribution of some characteristics on the estimated phylogeny (FIGS 2, 3) is interesting and noteworthy. Species with robust or stout basidiomata are found in two clades, including all members of Clade I (the "*piperatus*" group) and *A. novazealandensis*, which forms a clade by itself, but does not associate with Clade I (FIGS 2, 3). Because of the stoutness of its basidiomata, *A. novazealandensis*, which is virtually indistinguishable morphologically from *A. austropiperatus*, was originally considered to be *A. piperatus* (Dodd 1972, Petersen 1988, and Wu 1991). However, based on ITS sequences, *A. novazealandensis* is evidently in a different clade. Likewise, the three profusely branched species also occur on two distinct clades, with *A. microsporus* and *A. pyxidatus* on Clade V and *A. stephenii* on Clade II. "Pure white" basidiomata have apparently been derived at least three separate times. All collections of *A. adrienneae* (Clade IV) are white, whereas only one collection each of *A. turgidus* and *A. enigmatus,* both in Clade III, exhibit white basidiomata. In the latter two species, the white form may be just an occasional occurrence, but in the Argentinean species, apigmented basidiomata seem to be consistent. A "conifericolous" habit has occurred at least two separate times. Both *A. piperatus* (Clade I) and *A. cristatus* (Clade II) seem to prefer rotten conifer wood, whereas all other species seem to prefer hardwood. There are some reports of *A. pyxidatus* occurring on softwood, but in all specimens studied for this project, the wood has been hardwood, with an apparent preference for diffuse porous woods such as *Acer*, *Liriodendron*, and *Populus*.

With no fossil record it is difficult to determine the stature of the basidiomata of the *Artomyces* ancestor. *Artomyces tasmaniensis* was consistently placed in the basal phylogenetic position in the genus, and although bootstrap support for the basal branch was not strong (FIGS 2, 3), it could indicate that *A. tasmaniensis* represents characteristics of the ancestral species. However, there was no resolution in the ITS phylogeny to determine the relationship between *Artomyces* and members of the outgroup. Based on their micromorphological similarity, Mass Geesteranus (1976) grouped *Clavicorona* (branched coralloid with a smooth hymenium), *Lentinellus* (pileate with a lamellate hymenium), and *Auriscalpium* (pileate with a toothed hymenium) into Auriscalpiaceae. Recent work by Ellen Larsson (pers. comm.) further indicated the close molecular (large subunit nrDNA) and morphological association of *Amylostereum* (pileate with a smooth hymenium) with the members of this family. In the present study ITS sequence data supported these hypotheses and showed that *Amylostereum,* represented by *Am. areolatum* isolate CBS 334.66, and *Auriscalpium,* represented by *Au. villipes* isolate 4396 and *Au. vulgare* isolate 4242, are sister taxa. With such diversity in basidiomata stature among these putatively closely related genera, it is difficult to determine which stature is most primitive. Branched basidiomata of *Lentinellus cochleatus* have been observed in both culture (Miller and Stewart 1971) and nature (Maas Geesteranus 1975), as well as in cultures of *Auriscalpium vulgare* (pers. obser.) indicating that the ability to branch is present in at least those two genera. Deeper nodes, however, need to be resolved with an analysis of more conserved DNA sequences to evaluate the relationships among the four genera. Current research by Ellen Larsson and Nils Hallenberg at Gothenborg University, Sweden on the relationships of several members of the Hericiales may reveal these relationships.

GENERAL CONCLUSIONS

Artomyces Jülich is a distinct genus separate from *Clavicorona* Doty *sensu* Jülich, which may have more affinity with the omphalinoid agarics. Based on a combination of molecular analyses of the nuclear ribosomal ITS region and morphological comparisons, there is strong support for at least eight new species in addition to the nine previously described. The eight new species were identified and formally described. *Artomyces divaricatus* was excluded because it was identified as belonging to *Lentaria. Clavicorona microspora* was transferred into *Artomyces*, while *C. tuba*, which may have more in common with *Artomyces* species, was deemed *species inquirenda* until specimens can be located for examination. Broad geographic patterns in the context of the ITS phylogenetic estimation are indiscernible, but there appear to be a few cases of intercontinental sibling species. Intraspecific geographic patterns were also observed.

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PART I APPENDIX

FIGURE 22. Scanning electron micrographs of basidiospores. Bar = 1.0 μm.

A) *Clavicorona taxophila* (C 10264).
B) *Artomyces candelabrus* (TFB 2637, TENN 48917).
C) *A. colensoi* (TFB 2679, TENN 48923).
D) *A. cristatus* (DLL8974, TENN 48928).
E) *A. novazealandensis* (TFB 2672, TENN 48915).
F) *A. piperatus* (TFB 8221, TENN 57288).
G) *A. pyxidatus* (TFB 1607, TENN 48852).
H) *A. turgidus* (TFB 2682, TENN 48926).

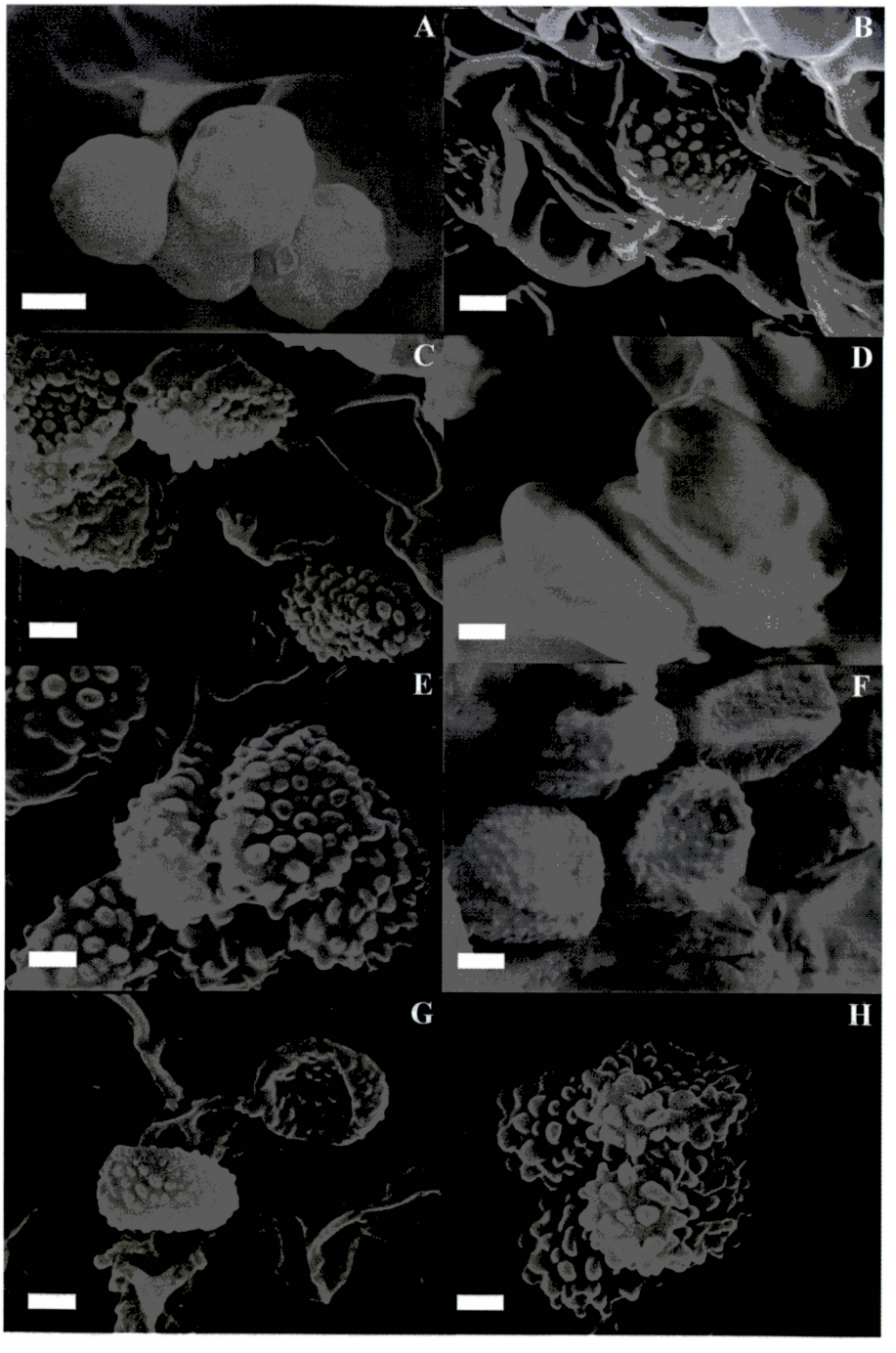

A
B
C
D
E
F
G
H

FIGURE 23. Photographs of typical fresh and dried basidiomata. Large scale blocks = 1 cm; numbers on collection labels in A 4 mm tall; numbers in C, E, F, and H 4.5 mm tall.

A) *Clavicorona taxophila*, fresh collection TFB 5608 (TENN 51926).
B) *C. taxophila*, dried specimens (l-r) C 10264, TENN 51926, and SAE 0890.
C) *Artomyces adrienneae*, fresh collection TFB 7349 (TENN 54434).
D) *A. adrienneae*, dried specimen TENN 59139 (ZT 6839).
E) *A. austropiperatus*, fresh collection TFB 8363 (holotype TENN 55036).
F) *A. austropiperatus*, fresh collection TFB 8335 (TENN 55009).
G) Holotype of *Clavaria candelabra* K 69007 (Ridley 37).
H) *A. candelabrus*, fresh collection TFB 2637 (TENN 48917).
I) *A. carolinensis*, holotype TENN 48890 (TFB 1615).
J) *A. dichotomus*, dried specimen SF 7220 (DED 7220), photo by D.E. Desjardin.

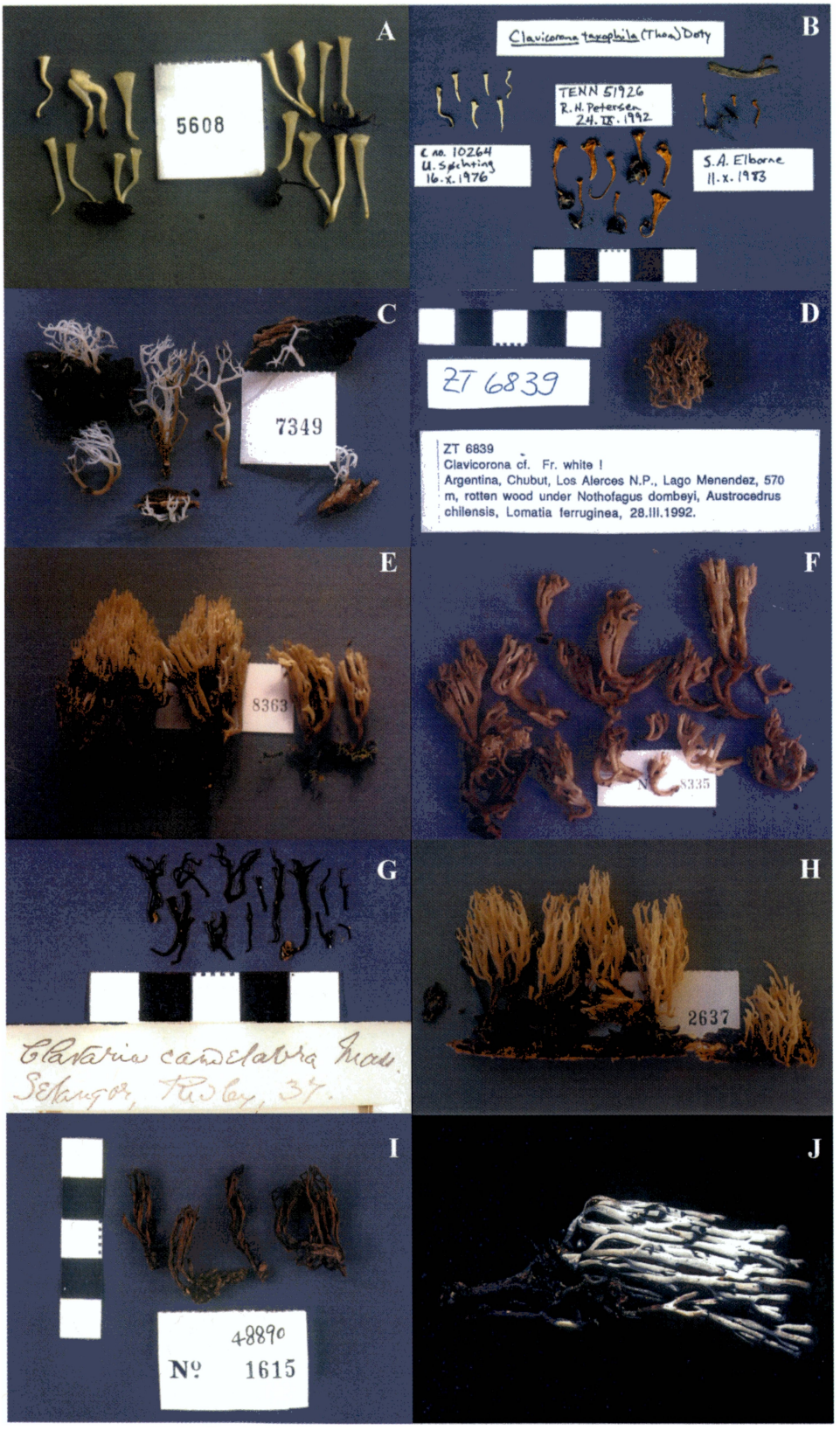

A
5608
B
Clavicoma taxophila (Thaxt.) Doty
TENN 51926
R. H. Petersen
24. IX. 1992
C no. 10264
U. Spielvinyl
16. X. 1976
S.A. Elborne
11. X. 1983
C
7349
D
ZT 6839
ZT 6839
Clavicorona cf. Fr. white !
Argentina, Chubut, Los Alerces N.P., Lago Menendez, 570 m, rotten wood under Nothofagus dombeyi, Austrocedrus chilensis, Lomatia ferruginea, 28.III.1992.
E
8363
F
8335
G
Clavaria candelabra Linn.
Selangor, Ridley, 37.
H
2637
I
48890
Nº 1615
J

FIGURE 24. Photographs of typical fresh and dried basidiomata. Large scale blocks = 1 cm; numbers in B 4.5 mm tall; numbers in C and D 4 mm tall.

A) Holotype of *Clavaria colensoi* K 69006 (Colenso s.n.).
B) *A. colensoi*, fresh collection TFB 2679 (TENN 48923).
C) *A. costaricensis*, fresh collection TFB 9670 (holotype TENN 56631).
D) *A. costaricensis*, fresh collection TFB 10866 (TENN 59137).
E) Holotype of *Craterellus cristatus* MICH 10444 (Kauffman 5Dec1925).
F) *A. enigmatus*, dried specimens TENN 56533 (TFB 9905) and holotype TENN 48928 (TFB 3456).
G) *A. novazealandensis*, dried specimen TENN 48915 (TFB 2672).
H) *A. novazealandensis*, holotype TENN 54111 (TFB 7023).

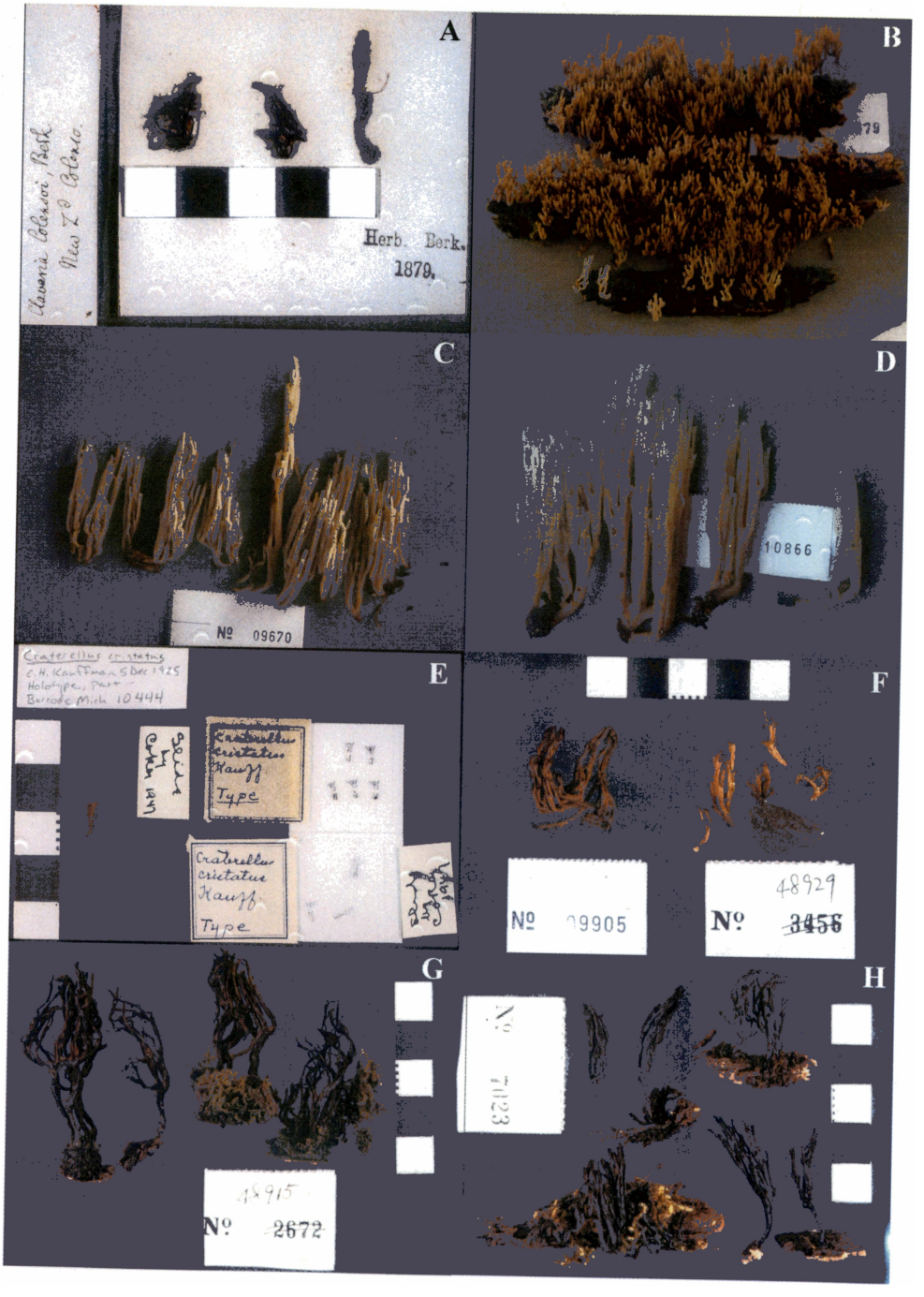

A
Herb. Berk.
1879.
B
C
№ 09670
D
10866
E
Craterellus cristatus
C.H. Kauffman 5 Dec 1925
Holotype, Part
Bernice Mich 10444
Craterellus cristatus Kauff Type
Craterellus cristatus Kauff Type
F
№ 09905
48929
№ 3456
G
48915
№ 2672
H

FIGURE 25. Photographs of typical fresh and dried basidiomata. Large scale blocks = 1 cm; numbers in C and H 4 mm tall.

A) Holotype of *Clavaria piperata* MICH 10095 (Kauffman 12Nov1925).
B) Holotype of *Clavicorona avellanea* MICH 5580 (Smith 54272).
C) *Artomyces piperatus*, fresh collection TFB 5770 (TENN 53491).
D) Holotype of *Clavicorona microspora* TENN 48856 (TFB 2349).
E) Holotype of *Clavaria coronata* (Schweinitz) ANSPHILA (Syn # 1041).
F) Holotype of *Clavaria petersii* ANSPHILA (Peters s.n.).
G) Ancillary specimen of *C. petersii* ANSPHILA (Peters s.n.).
H) *A. pyxidatus*, fresh collection TFB 9235 (TENN 55966).

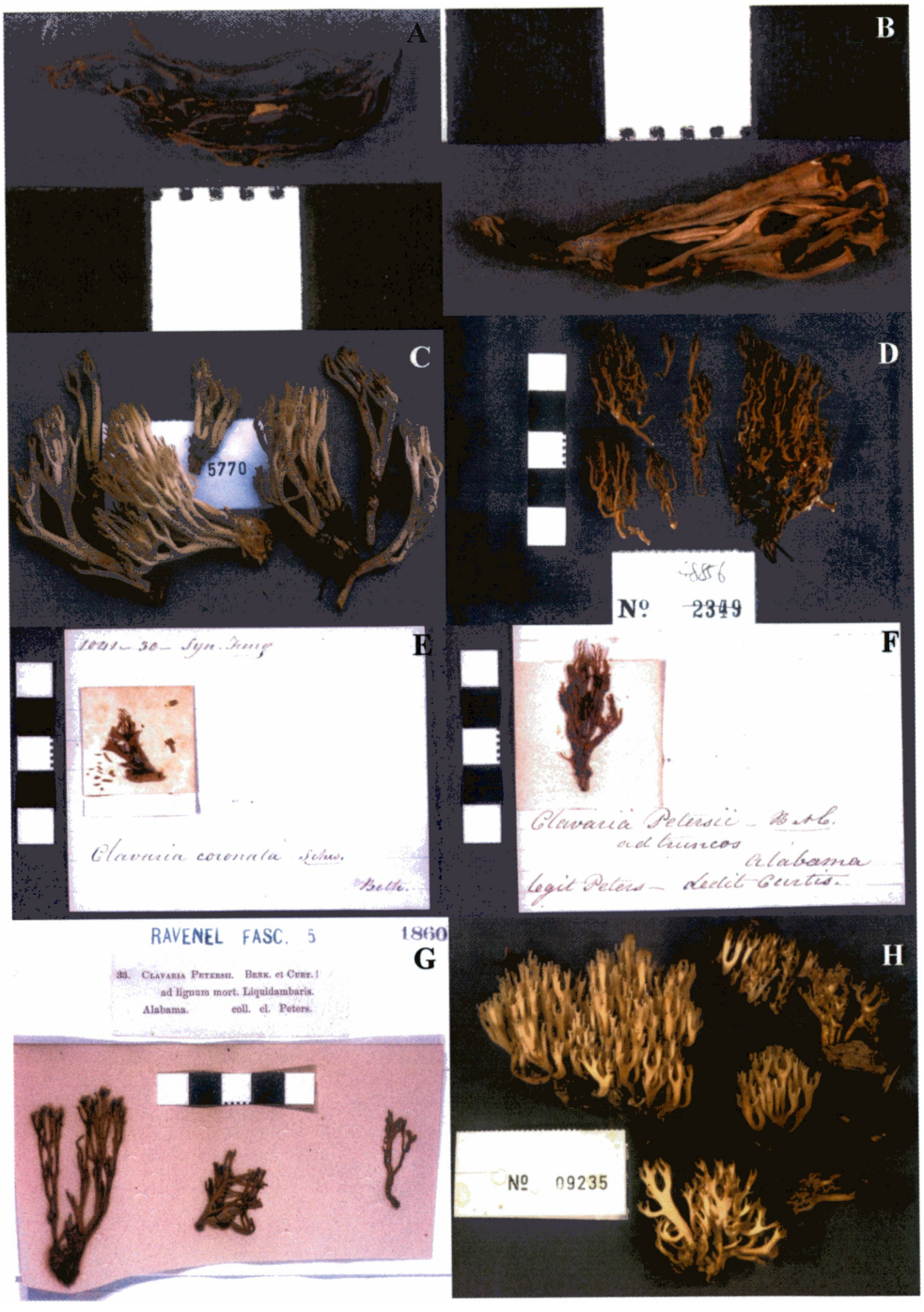

A
B
C
5770
D
Nº 2349
E
Clavaria coronata Schw.
F
Clavaria Petersii
ad truncos
Alabama
RAVENEL FASC. 5
1860
G
33. Clavaria Petersii. Berk. et Curt.
ad lignum mort. Liquidambaris.
Alabama. coll. cl. Peters.
H
Nº 09235

FIGURE 26. Photographs of typical fresh and dried basidiomata. Large scale blocks = 1 cm; numbers in C, E, F, I, and J 4.5 mm tall.

A) *Artomyces stephenii*, dried specimens TENN 56412 (TFB 9438) and holotype TENN 56410 (TFB 9436).
B) *A. stephenii*, dried specimen TENN 58644 (TFB 9743).
C) *A. stephenii*, fresh collection TFB 7898 (TENN 53845).
D) *A. tasmaniensis*, holotype TENN 50218 (TFB 4003).
E) *A. tasmaniensis*, fresh collection TFB 3979 (TENN 50283).
F) *A. tasmaniensis*, fresh collection TFB 3905 (TENN 50112).
G) Holotype of *Clavaria coronata* (Zippel) K 69005 (Léveillé s.n.).
H) Holotype of *Clavaria laetissima* K 69006 (Lockyer s.n.).
I) *A. turgidus*, fresh collection TFB 3961 (TENN 50103).
J) *A. turgidus*, fresh collection TFB 2820 (TENN 49279).


A
№ 09438
№ 09436
B
№ 09743
C
7898
D
E
F
№ 3905
G
Clavaria contorta. Typ. Leo.
H
Clavaria laetissima
LocKyer 477
I
№ 3961
J
2820

PART III

BIOGEOGRAPHICAL PATTERNS IN *ARTOMYCES PYXIDATUS*

This chapter is a virtually unaltered version of a paper by the same name that was accepted for publication in the journal Mycologia in 2002.

Lickey, E.B., K.W. Hughes, and R.H. Petersen. 2002. Biogeographical patterns in *Artomyces pyxidatus.* Mycologia 94. In Press.

ABSTRACT

Artomyces pyxidatus (Auriscalpiaceae) is a lignicolous, coralloid basidiomycete found throughout temperate regions of the Northern Hemisphere. Previous studies established that populations from the eastern United States, Sweden, and China were conspecific based on mating compatibility and enzyme profiles. In this study, mating compatibility was extended to include collections from Russia, Costa Rica, Mexico, and Utah. The molecular diversity of *A. pyxidatus* was examined by DNA sequence and restriction site analyses of the nuclear ribosomal internally transcribed spacer region (ITS1-5.8S-ITS2). A phylogenetic analysis of twelve isolates based on ITS sequences revealed a broad geographical pattern in which Eurasian isolates comprise a sister clade to North American isolates. North American isolates appear to be further subdivided into northeastern and southwestern clades. A survey of 255 *A. pyxidatus* isolates using restriction enzymes revealed variable RFLP patterns that follow similar geographical patterns.

INTRODUCTION

Artomyces pyxidatus (Pers:Fr) Jülich, commonly known as *Clavicorona pyxidata* (Pers:Fr) Doty, is a branched, coralloid, lignicolous basidiomycete which occurs in North Temperate deciduous forests. In his study of genus *Clavicorona*, Dodd (1972) erected subgenus *Ramosa* to include the branched, lignicolous, amyloid-spored species. Jülich (1981) elevated this group to genus rank indicating that morphological differences between subgenera *Ramosa* and *Clavicorona* were significant enough to warrant genus level status. He established the name *Artomyces*, and chose *A. pyxidatus* as the type species. Recent sequence data from large subunit (27S) and internal transcribed spacer (ITS) regions of nuclear ribosomal DNA support this genus rank treatment (Chapter II). Morphological and cultural characterizations of *A. pyxidatus* were elucidated by Dodd (1972), James and McLaughlin (1988), Wu (1991), and Wu et al. (1995). Additionally, Wu (1991) and Wu et al. (1995) determined that isolates of *A. pyxidatus* from China, Sweden, and the eastern United States were all intercompatible and suggested that they represented a single biological species. A different laccase enzyme banding pattern, however, was observed for isolates from China (Wu 1991, Wu et al. 1995).

There are many examples of Homobasidiomycete species that have very broad geographic distributions and whose populations remain sexually intercompatible *in vitro*, even though they may be continents apart. These include *Collybia dryophila* (Vilgalys and Johnson 1987, Vilgalys 1991), *Flammulina velutipes* (Petersen et al. 1999), *Panellus stypticus* (Petersen and Bermudes 1992a, 1992b, Jin 2000), *Pholiota spumosa* (McCleneghan 1996), *Pleurotopsis longinqua* (Petersen 1992, Petersen and McCleneghan 1995, 1997), *Pleurotus ostreatus* and *P. pulmonarius* (Petersen and

Hughes 1993, Petersen 1995a, 1995b), *Schizophyllum commune* (Raper et al. 1958), and *Xeromphalina campanella* (Johnson 1997). These taxa have been the subject of recent genetic studies to determine the amount of variation that exists among widely disparate populations (Vilgalys and Johnson 1987, Vilgalys 1991, Vilgalys and Sun 1994, McCleneghan 1996, Johnson 1997, Hughes et al. 1998, 1999, James et al. 1999, Methven et al. 2000, Jin et al. 2001). While biological barriers to reproduction are apparently lacking among populations of these taxa, geographic barriers to gene flow seem to exist as evidenced by sequence and allozymic divergence.

The purpose of the present study was to elucidate biogeographic patterns among collections of *Artomyces pyxidatus*. We report mating compatibility patterns of collections from Costa Rica, Mexico, Russia, and Utah with previous collections and examine biogeographical patterns using DNA sequence and restriction fragment length polymorphism (RFLP) frequencies of the nuclear ribosomal ITS area.

METHODS

Cultures.–Monokaryon and dikaryon cultures used in this study (TABLE 1) were established from basidiomata collected in nature and are maintained in the culture collection at the University of Tennessee as described by Hughes et al. (1999).

Mating studies.–In addition to previously available cultures, single basidiospore isolates (SBIs) were established from spore prints of recent collections from Russia, Mexico, Costa Rica, and Utah. Intercompatibility studies were performed as described by Gordon and Petersen (1991) with the following modifications. Four monokaryons from each collection were paired with four monokaryons from another collection resulting in a set of four pairings between the two collections (i.e. monokaryons of 9932 and 8396 were paired as 1x3, 7x5, 9x7, and 11x9, respectively). Five collections from Costa Rica, eight from Mexico, one from Russia, and one from Utah were included in this test of intercompatibility. Where possible, *A. pyxidatus* monokaryons from collections used by Wu (1991) were included in this study. Two collections (8896 and 8950 from Russia), were paired only with 8723 (Mexico) and 8927 (Russia). Collection 1541 (China) was represented by only one monokaryon, and collections 1607 (North Carolina) and 8927 (Russia) were represented by only three monokaryons. All isolates included in the mating study are indicated in TABLE 1. Compatible matings were determined by the presence of well-formed clamp connections in and near the contact zone, viewed directly on the agar surface under 250X magnification.

DNA methodology.–DNA isolation protocols followed Hughes et al. (1999). The nuclear ribosomal ITS 1-5.8S-ITS 2 (ITS) region was amplified using forward primer ITS 5 and reverse primer ITS 4 (White et al. 1990). The PCR protocols were: initial melting cycle of 94 C for 4 minutes, 35 repetitions of a three step amplification cycle of 94 C for 1 minute, 52 C for 1 minute, and 72 C for 1 minute, ending with an extension cycle of 72 C for 1 minute.

Twelve isolates of *A. pyxidatus* were chosen for sequencing in order to represent populations from a wide geographic range (TABLE 1). Additionally, two isolates *A. microspora* were chosen for outgroup comparison because of its close relationship with

TABLE 1. *Artomyces pyxidatus* isolates included in this study.

Country	Province	Isolate Number[b] and TENN Herbarium Number	Restriction Enzymes[a]						
			***Bsa*JI**	***Bsr*I**	***Cfo*Ia**	***Cfo*Ib**	***Ear*I**	***Tse*I**	***Xho*I**
China	Jilin	1449 (48279)	0	0	1	1	0	0	0
	Yunnan	1541[c,d] (48855)	0	0	1	1	0	0	0
Russia	Caucasia	8897[d] (55517), 8903[c] (55523), 8927[c,d] (55547), 8950[d] (55610)	0	0	1	1	0	0	0
	Leningrad	10605 (58265), 10611 (58271), 10629 (58288), 10630 (58289), 10631 (58290), 10640 (58247)	0	0	1	1	0	0	0
Sweden	unknown	GB 1137, GB 1279[d], GB 1280, GB 1702	0	0	1	1	0	0	0
	Uppland	7246 (53620), 7255 (53509), 7263[c] (53544), 7268 (53634), 7269 (#####), 7298 (53578)	0	0	1	1	0	0	0
Switzerland	Maggia Valley	4289 (50625)	0	0	1	1	0	0	0
Costa Rica	Heredia	10030[d] (56742)	1	1	0	0	1	1	1
	Puntarenas	9932[c,d] (56558)	1	1	0	0	1	1	1
	San Jose	9464[d] (56438), 9465[d] (56439), 9681[d] (56642)	1	1	0	0	1	1	1
		10873 (58737)	0	1	0	0	1	1	1
Mexico	Mexico	8396[d] (55067), 8409[d] (55236), 8437[d] (55266), 8439[d] (55268), 8440[d] (55269)	1	1	0	0	1	1	1
		8408[d] (55235)	1	1	0	0	0	1	1

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
Mexico	Nayarit	8723[c,d] (55152), 8726 (55155), ET 3817[d]	1	1	0	0	1	1	1
		8725 (55154), 8751 (55180)	0,1	1	0	0,1	1	1	1
Canada	Quebec	9255 (55986)	0	1	0	0,1	1	1	1
		9256 (55987)	0	1	0	1	1	1	1
United States	Arizona	ATCC 22501	0,1	1	0	0,1	1	1	0,1
		H 7524[c], JPL 479	1	1	0	0	1	1	1
	Georgia	4567 (51048), 4568 (51049), 5485 (51948), 6211 (#####), 6507 (51958),56665 (48853), 56667[c] (48854), 56668 (48888)	0	1	0	1	1	1	1
		4573 (51053), 5241 (51893), 5242 (51900), 5243 (51899), 6216 (51957), 56666 (48887)	0	1	0	1	1	1	0,1
		5484[c] (51947)	1	1	0	0	1	1	1
	Illinois	ASM 6658	0	1	0	1	1	1	0,1
	Maine	4990 (51411)	0	0,1	0	1	1	1	0,1
	Maryland	AES 515	1	1	0	0	1	1	1
		HHB 810	0	1	0	1	1	1	0,1
	Michigan	7945 (#####)	0	1	0	1	1	1	1
	Minnesota	6932 (#####)	0,1	0,1	0	0,1	1	1	1
		6941 (#####)	0	1	0	1	1	1	0,1

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
United States	Mississippi	9201 (#####)	0	1	0	1	1	1	0
	New York	1190[d] (48842)	0,1	1	0	0,1	1	1	0,1
		1704 (48902), 4938[c] (51251)	0	1	0	1	1	1	0,1
		1724 (48906)	0	1	0	1	1	1	0
		6485 (51942), 6488 (51943)	0	1	0	1	1	1	1
	North Carolina	1135 (48914)	0	1	0	0,1	1	0,1	1
		1607[d] (48852), 4091 (50241), 4598 (51076), 5116 (51973), 5441 (51759),5555 (52488), 6226 (51953), 6611 (51932), 6624 (51920), 6633 (51923),9238 (55969), 9239 (55970), 9241 (55972), 9243 (55974), 9245 (55976),9271 (56459), 9281 (56359), 56653 (48846), 56662 (48848), ##### (47669), ASU 1231, DODD 38	0	1	0	1	1	1	0,1
		1608 (48897), 1614 (48896), 1618 (49873), 1648 (#####), 1650 (#####), 2174 (48795), 4816 (51117), 5048 (51451), 5117 (51939), 5168 (51941), 5298 (51876), 5437 (51755), 5461 (51777), 5464 (51945), 5493 (51946), 6308 (51921), 6513 (51950), 6522 (51952), 6610 (51929), 6615 (51934), 6616 (51935), 6617 (51949), 6621 (51919), 6622 (51918), 6623 (51915), 9240 (55971), 9246 (55977), 9270 (56458), 9277 (56355), 9726 (57111), 56655 (48847), ASU 1238	0	1	0	1	1	1	1

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
United States	North Carolina	1617 (48900), 1661 (48850), 5072 (51471), 6219 (51956), 6428 (51927), 6609 (51931), 6614 (51930), 6618 (#####), 9259 (55990), 9272 (56351), 9279 (56357)	0	1	0	1	1	1	0
		4806 (51107)	1	1	0	0	1	1	1
		6307 (51922)	1	1	0	1	1	1	1
		6612 (51933), 9244 (55975)	0,1	1	0	0,1	1	1	1
		6632 (51914)	0,1	1	0	1	1	1	0,1
		9242 (55973)	1	1	0	1	1	1	1
		9269 (56457)	0	0	0	1	0	1	1
		9278 (56356)	1	0,1	0	0,1	1	1	1
		9300 (57086)	0	0,1	0	1	1	1	1
		9724 (57109)	0	1	0	0	1	1	1
		56680 (48891)	0	1	0	1	0	1	0,1
		56681 (52357), ##### (47673)	0	1	0	0,1	1	1	1
		##### (47668), ##### (47670)	0,1	1	0	1	1	1	1
	South Carolina	4580 (51059), 4581 (51060)	0	1	0	1	1	1	1
		5519 (51799)	0	1	0	1	1	1	0
		6433 (51928)	0	1	0	1	1	1	0,1

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
United States	Tennessee	1606 (48894), 4563 (51905), 5303 (51618), 6601 (51909), 6602 (51908),6605 (51912), 6607 (51907), 9225 (55956), 9229 (55960), 9230 (55961),9232 (55963), 9234 (55965), 9235 (55966), ##### (36429)	0	1	0	1	1	1	0,1
		2994 (50680), 2995 (50681), 3624 (50743), 3650 (50769), 3710 (50698),4564 (51906), 4681 (51944), 5194 (51938), 5195 (#####), 5236 (51901),5300 (51880), 5302 (51617), 5401 (51898), 5403 (51896), 5411 (51887),5416 (51891), 5417 (51883), 5418 (51888), 5420 (51879), 5422 (51892), 5423 (#####), 5425 (51881), 5426 (51890), 5427 (51882), 6603 (51910),6604 (51911), 6606 (51925), 6608 (51924), 6620 (51913), 9220 (55951),9221 (55952), 9226 (55957), 9227 (55958), 9228 (55959), 9233 (55964), 9250 (55981), 9253 (55984), ATCC 24439	0	1	0	1	1	1	1
		5237 (51904), 5238 (51902), 5404 (51895), 5413 (51884), 6619 (51916)	0	1	0	1	1	1	0
		5299 (51875)	1	1	0	0	1	1	1
		5402 (51897)	0	0,1	0	1	1	1	1
		5412 (51885)	0	0,1	0	0,1	1	1	0,1
		5414 (51886), 5419 (51878	0,1	1	0	1	1	1	1
		5415 (51889)	0,1	0,1	0	1	1	1	0,1

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
United States	Tennessee	5538 (51903)	0,1	1	0	0,1	1	1	1
		9222 (55953), 9223 (55954), 9231 (55962)	0,1	1	0	0,1	1	1	0,1
		9224 (55955)	0,1	1	0	1	1	1	0,1
	Texas	9828 (58807)	0	1	0	1	1	1	1
	Unknown	RGT 752	0	1	0	1	1	1	1
	Utah	9705 (57091)	0	1	0	1	1	1	0,1
		9706 (57092)	1	1	0	0	1	1	1
		9707[c,d] (57093), 9711 (57097)	0,1	1	0	0,1	1	1	0,1
		9708 (57094)	0	1	0	1	1	1	1
		9709 (57095)	0,1	1	0	0	1	1	1
		9710 (57096)	0,1	1	0	0,1	1	0,1	1
	Virginia	HHB 10654	0,1	1	0	1	1	1	1
	Wisconsin	1501 (48843)	0,1	0,1	0	0,1	1	1	1
		1502 (48893), 1508 (48892), 1513[c] (48875), 1515 (48876), 1517 (48881),1520 (48882)	0	1	0	1	1	1	0,1
		1503 (48844), 1504 (48879), 1514 (48872), 1519 (48877)	0	1	0	1	1	1	1
		1506[d] (48845), 1507 (48880), 1516 (48878)	0	1	0	1	1	1	0

TABLE 1. cont.

Country	Province	Isolate Number[b] and TENN Herbarium Number	*Bsa*JI	*Bsr*I	*Cfo*Ia	*Cfo*Ib	*Ear*I	*Tse*I	*Xho*I
United States	Wisconsin	1509 (48885), 1511 (48872)	0,1	1	0	0,1	1	1	0,1
		1512 (48886)	0,1	0,1	0	0,1	1	1	0,1

[a] Presence (1) or absence (0) of restriction sites for the enzymes surveyed. Heterozygotes are identified by 0,1.

[b] All isolates originated from University of Tennessee collections except those with preceding initials. These isolates came from; AES and JPL = Forest Products Lab Madison, ASM = Andrew Methven, ASU = Coleman McCleneghan, ATCC = American Type Culture Collection, DODD = Jim Dodd, ET = Estrada Torres, GB = Gothenberg Culture Collection, H = Roy Halling, HHB = Hal Burdsall, RGT = Greg Thorn.

[c] Isolates which were sequenced for the nrDNA ITS region.

[d] Isolates included in the inter-compatibility study.

A. pyxidatus (Chapter II). PCR products were directly purified using a Promega Wizard PCR Purification Kit. The purified product was sequenced using primers ITS 5 and ITS 4 on an automated ABI 373 DNA sequencer (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc.). Primers ITS 2 and ITS 3 were used in some cases to clarify ambiguous sequences resulting from insertions and/or deletions (indels). Rarely, a smaller secondary, interfering fragment of non-ITS origin would amplify. In this case, the PCR products were first electrophoresed and then the ITS fragment was excised from a 1X TAE 1.5% low-melting temperature agarose gel (NuSieve GTG agarose, FMC Bioproducts) following manufacturer's directions. Sequences were manually corrected and aligned using the LINEUP and SEQLAB programs in the Genetics Computer Group package (GCG 2001), and deposited in GenBank. Accession numbers for *A. pyxidatus* isolates are: 1541 = AF336140, 7263 = AF336139, 8927 = AF336141, 8903 = AF336142, 8723 = AF336143, 5484 = AF336146, h7524 = AF336148, 9709 = AF336149, 9932 = AF336150, 4938 = AF336147, 1513 = AF336144, and 56667 = AF336145. Accession numbers for *A. microspora* isolates are: ky5352 = AF336137 and 2349 = AF336138. The sequence alignment is included in APPENDIX B.

Variable regions within the ITS1-5.8S-ITS2 region were identified by comparing aligned sequences. Restriction sites were mapped for ITS sequences using the MAP program of GCG (GCG 2001) and restriction enzymes were selected which produced restriction fragment length polymorphisms (RFLPs) among *A. pyxidatus* collections. ITS PCR products of all collections in the study were surveyed using restriction enzymes *Bsa*J I, *Bsr* I, *Ear* I, *Tse* I, *Xho* I (New England BioLabs), and *Cfo* I (Promega) according to manufacturer's directions. Restriction fragments were separated on 1X TBE 3% agarose gels stained with ethidium bromide and were visualized under ultraviolet light. Fragment sizes were estimated and compared with predicted fragment sizes based on the mapped ITS sequences. Isolates were then scored for presence or absence of restriction sites.

Phylogenetic reconstruction.–Phylogenetic relationships were estimated using PAUP 4.0 (Swofford 2000). Two apomorphic insertions were detected among *A. pyxidatus* sequences and were excluded from the analysis. Gaps in *A. pyxidatus* sequences that resulted from the alignment with *A. microspora* (the designated outgroup) were treated as missing data. A branch-and-bound search was used to find the most parsimonious tree. Two bootstrap analyses of 100 replicates were conducted with both a branch-and-bound search using maximum parsimony and a neighbor-joining search (Saitou and Nei 1987) using Jukes-Cantor distance measures (Jukes 1969). Sequence alignments and trees were deposited in TreeBASE (Study Accession # S661, Matrix Accession # M1037).

RESULTS

Intercompatibility.–Almost all collections were intercompatible with at least one of four monokaryon pairings producing clamp connections (FIG 1). One exception was the pairing between collection 8723 (Mexico) and 10030 (Costa Rica) in which none of the eight pairings produced clamp connections. Two collections showed reduced intercompatibility, collection ET3817 from Mexico and collection 9681 from Costa Rica. However, this reduction in dikaryotization ability was not shared by other collections from the same geographical regions and may reflect individual genotypes. Other crosses

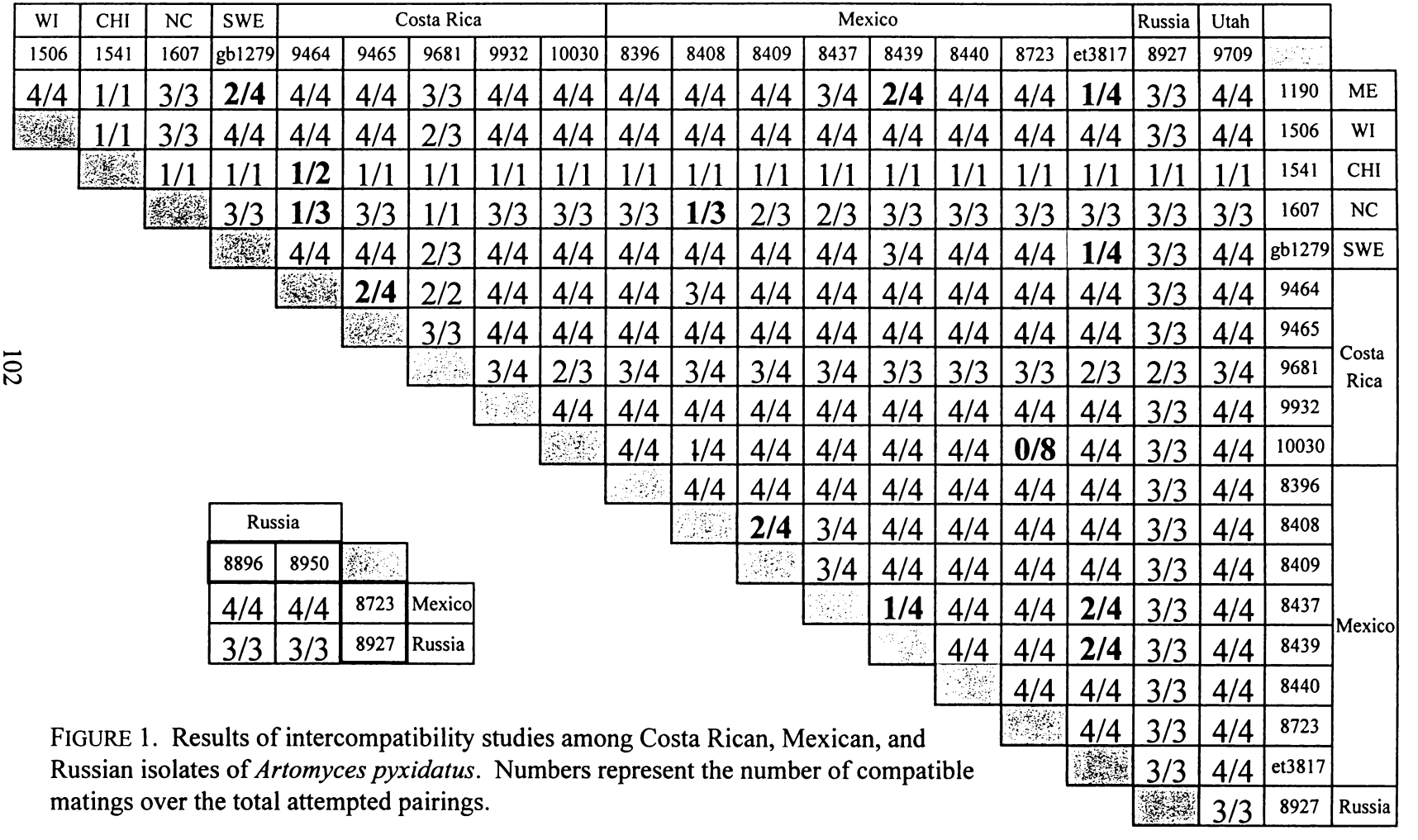


FIGURE 1. Results of intercompatibility studies among Costa Rican, Mexican, and Russian isolates of *Artomyces pyxidatus*. Numbers represent the number of compatible matings over the total attempted pairings.

also showed reduced intercompatibility. These included 9464 (Costa Rica) x 1607 (North Carolina), 8408 (Mexico) x 1607, 8437 (Mexico) x 8439 (Mexico).

ITS sequence characteristics.–The length of the ITS region varied from 633 - 650 bp. Variation in sequence length was partially due to four indels. Two indels were apomorphic; a 17-bp tandem duplication present near the 3'-end of ITS 1 in collection 5484 (Georgia), and a two-bp tandem duplication near the 3'-end of ITS 2 in collection 8927 (Caucasia). Collections 7263 (Sweden), 1541(China), 8927 (Caucasia) and 8903 (Caucasia) all shared a variable T repeat in the ITS 1 region that was absent from remaining collections, and collections 8273, 5484, h7524, 9709 and 9932 had a one-base deletion in the middle of the ITS 1 region. There were at least 17 bp differences (approximately 2.5% sequence divergence) in the ITS region between New World and Eurasian isolates.

Phylogenetic reconstruction.–A parsimony analysis of the ITS sequences using a branch-and-bound search yielded two most parsimonious trees of 65 steps (FIG 2; TreeBase accession # S661, matrix # M1037). Bootstrap analyses showed strong support for three major branches with little resolution at the tips. The neighbor-joining tree had the same overall topology as the tree produced by the branch-and-bound searches. Two major branches were resolved within *A. pyxidatus*; a Eurasian clade and a New World clade. Within the New World clade, there appears to be a well-supported separation between eastern isolates and southern isolates with overlap in Georgia. A similar but less well-supported separation occurs in the Eurasian clade where there was an apparent separation between European isolates and the single Asian isolate.

Restriction fragment analysis.–Seven variable restriction sites were identified and mapped for six enzymes (FIG 3). Four of these were diagnostic for segregating Eurasian and New World isolates. *Bsr* I, *Ear* I, and *Tse* I restriction sites were present in New World isolates and absent from Eurasian isolates. The fourth site, *Cfo* I site "a", was present in Eurasian isolates and absent from New World isolates. Three exceptions were found for the *Ear* I site [isolates 8408 (Mexico), 9269 (North Carolina), and 56680 (North Carolina)], and one exception was found for the *Bsr* I site (isolate 9269, North Carolina). There were several isolates that are apparently heterozygous for the presence of the *Bsr* I and *Tse* I sites. This was not confirmed by sequence analysis, but 24-hour digestions with high enzyme concentrations yielded the same result.
A geographic pattern among isolates was observed in the distribution of restriction sites *Bsa*J I and Cfo I site "b", and apparent heterozygous isolates exist for both enzymes. *Cfo* I site "b" was present in all Eurasian isolates, and the presence of this restriction site was maintained at higher allele frequencies in eastern North American populations than in those sampled in Utah, Arizona, Mexico, and Costa Rica (FIG 4). The reverse is the case for enzyme *Bsa*J I. Restriction sites for this enzyme were found to be at higher frequencies in populations sampled in Utah, Arizona, Mexico, and Costa Rica, than in those from eastern North America, and were absent from Eurasian isolates (FIG 4). Restriction sites for enzyme *Xho* I were also absent from Eurasian isolates and variably

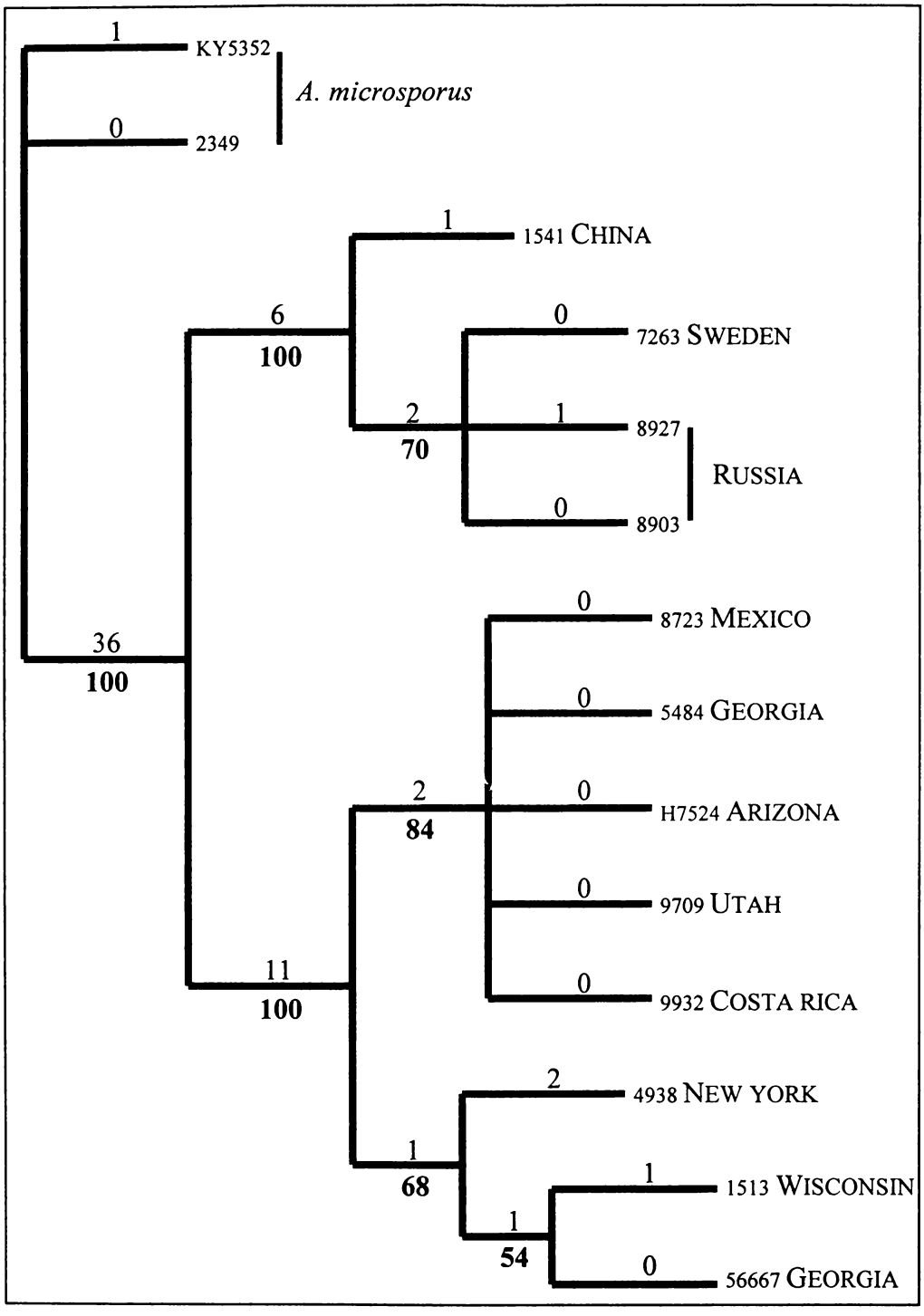


FIGURE 2. One of two most parsimonious trees for *Artomyces pyxidatus* collections. The two trees differed only in the arrangement of Swedish and Caucasian (Russian) collections. Tree length = 65. Bootstrap values (bold) are for the node to the right of the value. Distances are given above each line. Consistency index = 0.97. Retention index = 0.98.

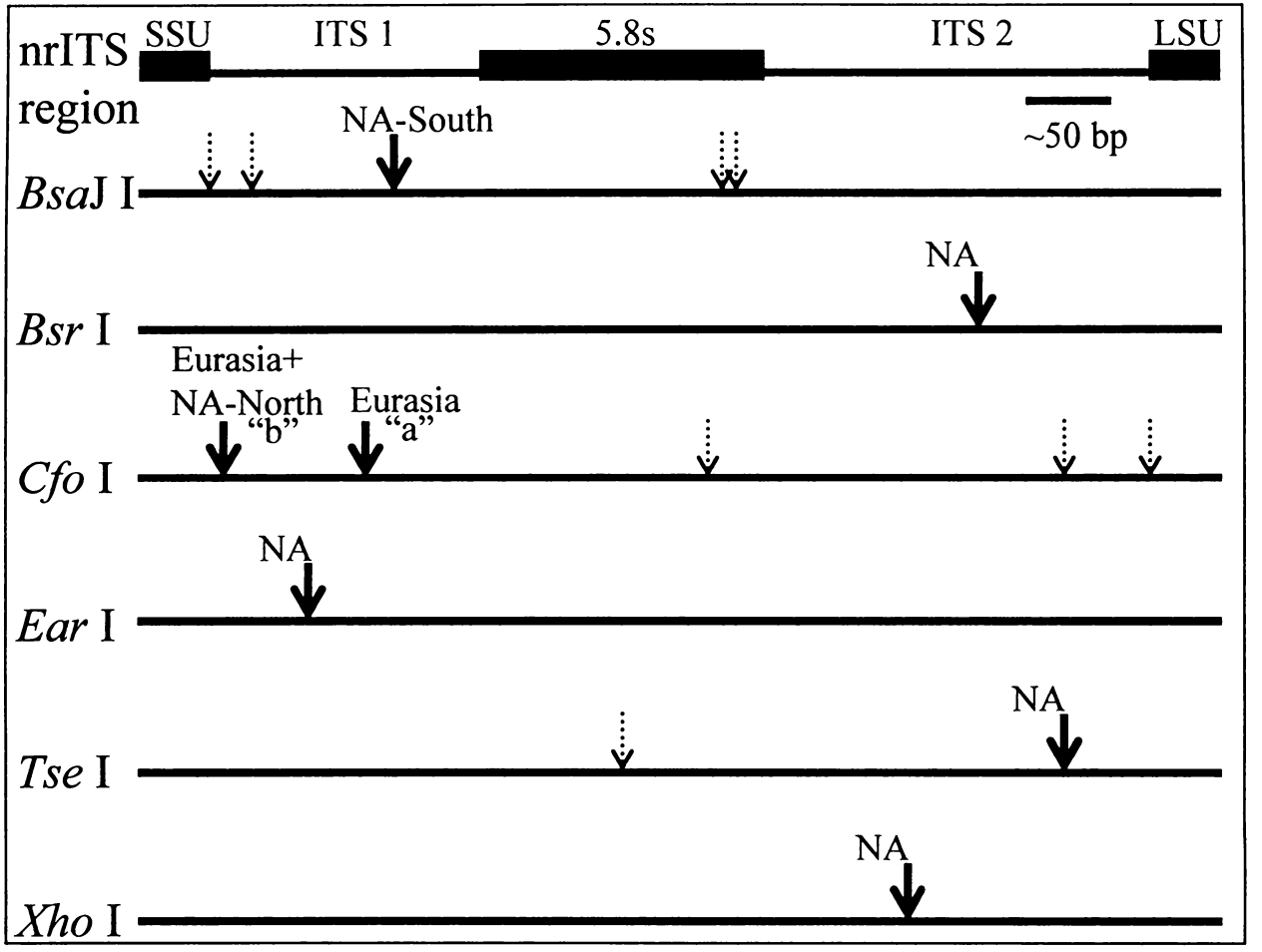
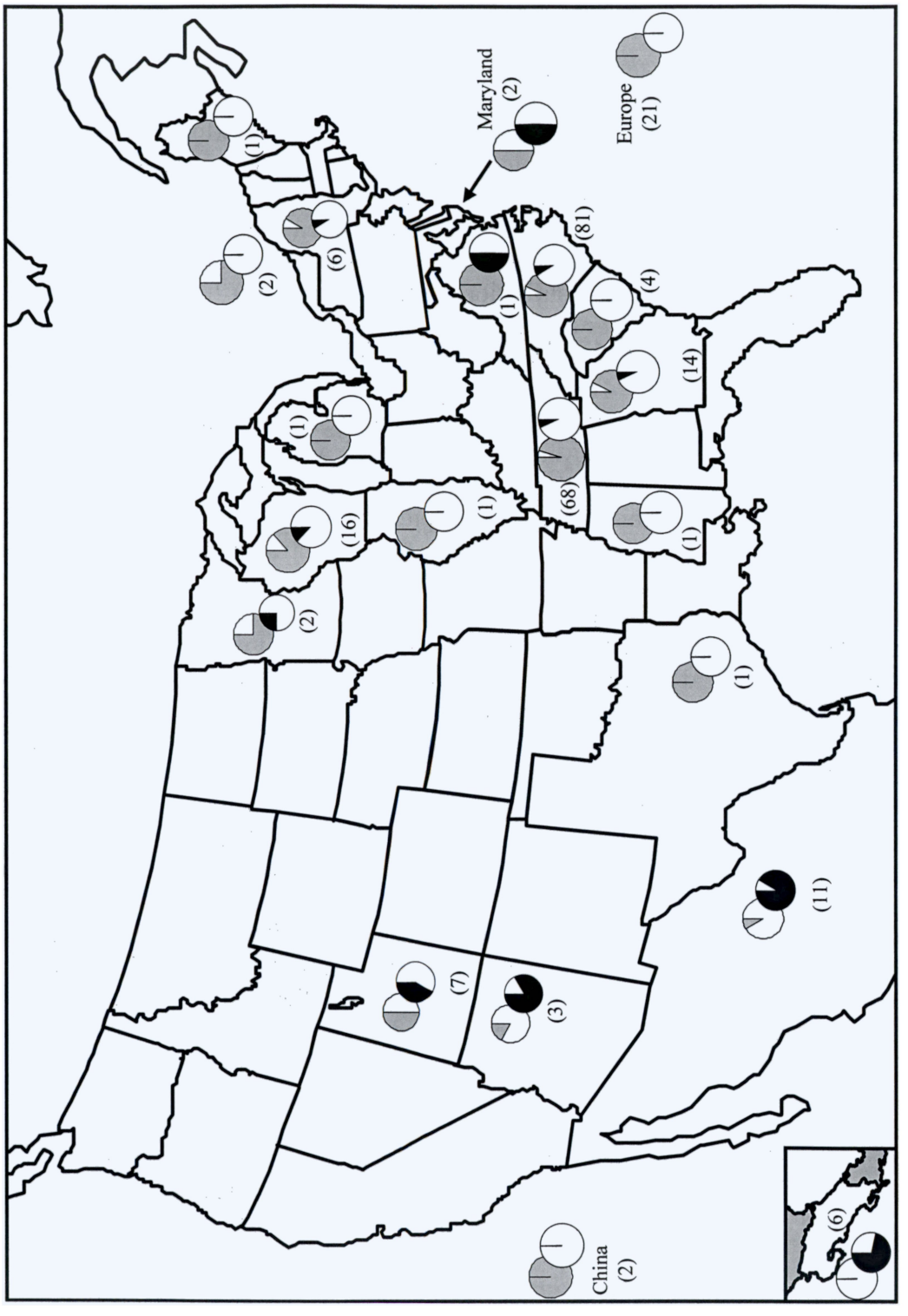


FIGURE 3. Restriction site map for the enzymes used in this study and their relative location in the nuclear rDNA ITS region. Variable sites are identified by solid arrows. Invariable sites are identified by dotted arrows. The geographical area in which each restriction site is found is indicated (NA = North America).

FIGURE 4. Geographic distribution of RFLP allele frequencies in *Artomyces pyxidatus*. Presence of restriction site is indicated by the shaded portion of the graphs; grey for *Cfo* I and black for *Bsa*J I. Pie graphs indicate allele frequencies for all isolates collected in each locale (state, country, or continent) and do not indicate exact location. Numbers in parentheses indicate sample size.


Maryland
(2)
Europe
(21)
(1)
(2)
(6)
(1)
(81)
(4)
(14)
(1)
(68)
(16)
(1)
(1)
(2)
(1)
(11)
(7)
(3)
China
(2)
(6)

present at high frequencies in New World isolates, but resulted in no discernable geographic pattern.

DISCUSSION

Separation of Europe and North American collections.–Artomyces pyxidatus is widely distributed throughout the Northern Temperate Zone with an extension into the high altitude areas of the North and Central American sub-tropical zone (Mexico and Costa Rica). Isolates from all populations sampled were intercompatible *in vitro*, but collections from different continents were genetically divergent. For ITS sequences, there was approximately a 2.5% sequence divergence between New World and Eurasian isolates. This divergence is greater than that observed for other mating-compatible basidiomycete species with similar distributions. European and North American collections of *Flammulina velutipes* differed by 1.2% (Hughes et al. 1999), *Pleurotus pulmonarius* European and North American collections showed a 0.5% sequence difference (Vilgalys and Sun 1994) and *Panellus stypticus* collections differed by 0.7% (unpub. data). Continent-specific RFLP patterns based on ITS sequence differences confirmed that European and North American populations were genetically distinct.

Additional evidence for reproductive separation between continents is found in analysis of an 18S Group I intron in *A. pyxidatus* previously discovered by Hibbett (1996). All collections of *A. pyxidatus* contained a Group I intron in the 18S ribosomal RNA gene (Chapter IV). However, New World isolates, including the three with geographically inconsistent RFLP patterns, contained an unknown insertional element within the 18S Group I intron. This unknown insertional element was absent from all Eurasian isolates examined (Chapter IV). Presence of the "unknown" insertional element in collections that were exceptions to biogeographical RFLP patterns suggests that these anomalies may be due to mutations resulting in loss of a restriction site rather than to European introductions into North America.

Presence of widespread *A. pyxidatus* sequence and RFLP patterns unique to either North America or Europe, suggests that populations on these two landmasses are effectively geographically isolated. ITS sequence differences would further suggest that these geographical domains have been separated for a long time. In contrast, an allozyme study of *Schizophyllum commune,* which has an almost global distribution, indicated that this species maintains high levels of genetic variation and no fixed allelic differences were observed among populations (James et al. 1999). It was hypothesized that some low levels of gene flow may have occurred through long-distance spore dispersal, and/or that effective population sizes were large enough to overcome the effects of genetic drift.

Biogeographical history.–The apparent reproductive isolation of Europe and North America raises questions as to when these populations were last connected. Several researchers have concluded that effective reproductive isolation may have occurred millions of years ago [Vilgalys and Sun 1994 (*Pleurotus*), Wu and Mueller 1997, Redhead 1988, Hughes et al. 1999 (*Flammulina*), Methven et al. 2000 (*Flammulina*), Coetzee et al. 2000 (*Armillaria mellea*) and Jin et al. 2001(*Panellus stypticus*)]. All of these have been hypothesized to be very old species with Laurasian distributions. Their

populations may have been separated with the disappearance of the last North Atlantic land bridge in the Eocene (~40 Mya), or more recently with the disappearance of any of the Bering land bridges in the Pleistocene (~1.6 Mya). During periods when land bridges existed, forests, and probably their fungal counterparts, were much more widely distributed across the connected continents of the Northern Hemisphere (Graham 1999).

Our data do not address either the temporal or geographic origin of species *A. pyxidatus*, however, the European and North American populations must have shared a gene pool at some point in the past. Both the Bering land bridge and the North Atlantic land bridge seem to be equally feasible possibilities for places of last contact. The Bering land bridge was an important corridor for the migration of fauna and flora through the Middle Pliocene (~3.5 Mya) and through its intermittent existence during the Quaternary Period (~1.6 Mya) (Graham 1999). Evidence from biogeographical studies with *Flammulina velutipes* and *Panellus stypticus* suggest that populations of these species migrated between Asia and western North America (Methven et al. 2000, Jin et al. 2001). It is possible that *A. pyxidatus* followed that route. However, *A. pyxidatus* is not known from west of the Rocky Mountains, and the forests that presently occupy that area are largely coniferous. *Artomyces pyxidatus* seems to occur only on decaying hardwood logs in deciduous forests, and in North America it appears to have a preference for diffuse-porous wood such as *Acer*, *Liriodendron*, and *Populus* (our personal observation). Up to the Late Tertiary (~3.4 Mya), climates were warmer and western North America may have been more suitable for deciduous forests favored by *A. pyxidatus*. During the later Quaternary period, however, the western US and Canada maintained mostly boreal to tundra vegetation types (Delcourt and Delcourt 1993). If a Bering land bridge was a migration route for *A. pyxidatus*, it may have been extirpated from the western US and Canada during the Quaternary period.

The North Atlantic land bridge was much more ancient, and the separation between North America and Europe is estimated to have been complete by the Eocene (~40 Mya) (Graham 1999). It was also an important corridor for the exchange of flora and fauna throughout much of the Tertiary, where deciduous forests presumably extended uninterrupted across North America, Europe and Asia (Graham 1999). If this was the point of last contact, and the time of separation was approximately 40 Mya, then *A. pyxidatus* and other fungi with similar distributions are extremely old. Alternatively, after the disappearance of the land bridge, islands such as Greenland and Iceland could have provided "stepping-stones" during warmer periods before and during the Quaternary. Long-distance spore dispersal has not been studied in *Clavicorona*, but basidiospores of *Heterobasidium annosum* have been estimated to travel at least 200 miles (322.6 km) (Rishbeth 1959). Presently, populations of *A. pyxidatus* are known from as far north as Maine and Sweden, but *A. pyxidatus* has not been collected from Greenland (Rostrup 1888, Kobayasi et al. 1971, Watling 1977,1983, Knudsen et al. 1993).

A North Atlantic connection is suggested by RFLP frequencies (FIG 4). Even though there is measurable ITS sequence divergence between Eurasian and New World isolates, eastern North American isolates share alleles for *BsaJ* I and *Cfo* I "b" with Eurasian isolates. This is consistent with a North Atlantic land bridge connection but might also be explained by rare long-distance spore dispersal. With the prevailing winds

moving in an easterly direction, it is conceivable that spores could have been dispersed long distances from eastern North America to Europe. A founder effect may have caused the resulting populations in Europe to be fixed for the shared alleles *Bsa*J I and *Cfo* I "b".

Within the New World, isolates can be subdivided into a northeastern and a southwestern group based on ITS sequence data (FIG 2). This pattern may reflect the influence of glaciation on populations of *A. pyxidatus* and its host trees. Throughout the Quaternary, the ranges of forest species across the Northern Hemisphere contracted and expanded with the advance and retreat of glaciers (Delcourt and Delcourt 1993). Genetic divergence is hypothesized to have occurred when populations were forced southward into isolated refugia during glacial maxima. No fixed restriction site differences were observed in the ITS region among North American isolates, but there are clearly differences in allele frequencies in the two restriction sites *Cfo* I site "b" and *Bsa*J I (FIG 4). *A. pyxidatus* was collected in Mississippi (9201), Louisiana (not cultured), and eastern Texas (9828), and there is a potential for gene flow between northeastern populations and southwestern populations through this Gulf Coast corridor. The ITS phylogeny (FIG 2) places collections from Georgia in both the northeastern clade (collection 56667) and the southwestern clade (collection 5484). Possibly, the southeastern US represents a hybrid zone where these two populations may introgress.

Geographically consistent crossing barriers *in vitro* were not observed in this study or in earlier studies (Wu 1991, Wu et al. 1995) in spite of sequence and RFLP differences. This suggests that mating ability is conserved across diverging allopatric populations and that reproductive isolation is not congruent with sequence divergence.

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PART IV

VARIABILITY AND PHYLOGENETIC INCONGRUENCE OF AN 18S nrDNA GROUP I INTRON IN *ARTOMYCES*, *AURISCALPIUM*, AND *LENTINELLUS* (AURISCALPIACEAE: HOMOBASIDIOMYCETES)

ABSTRACT

Artomyces, *Auriscalpium* and *Lentinellus* are three genera within the Auriscalpiaceae (Homobasidiomycetes). Their macromorphology is highly divergent: *Artomyces* produces basidia and spores on coralloid branches, *Auriscalpium* on teeth hanging from a pileus, and *Lentinellus* is a lamellate fungus. The three genera shared a number of micromorphological characters and previous studies also suggested that they shared a similar Group I intron in the 18S ribosomal gene, possibly of common origin. To examine this further, the presence or absence of the Group I intron in the 18S ribosomal DNA gene was determined for seven species of *Lentinellus*, two species of *Auriscalpium*, and 15 species of *Artomyces*. Occurrence of the intron among the species of these genera is variable. Within *Artomyces*, the intron was present in *Ar. pyxidatus* and in a newly described species from Argentina, *Ar. austropiperatus*. Within *Lentinellus*, the intron was present primarily in a clade consisting of the closely related species *L. micheneri*, *L. flabelliformis*, *L. "subaustralis"* (*nom. prov.*) and *L. montanus* and was variable in *L. castoreus*. Within *Auriscalpium*, the intron was present in *Au. villipes* but not in *Au. vulgare*. Heterozygosity for presence of the intron was observed.

Independent phylogenetic estimations were generated for the intron and nuclear ribosomal internal transcribed spacer regions (ITS). Tests of congruence for the two trees indicated that the trees were significantly different. Some of the discontinuity between the two phylogenies is due to placement of the *Ar. austropiperatus* intron within the *Lentinellus* intron clade. Variability in length of the intron was observed in populations of the pan-Northern Temperate species *Ar. pyxidatus* and was due to the presence of an additional unknown insertional element found only within North American collections of *Ar. pyxidatus* and absent from European and Asian collections.

INTRODUCTION

Group I introns are mobile, intervening sequences, that can catalyze their own excision through characteristic secondary and tertiary structure (Cech 1988, 1990; Dujon 1989; Shub et al. 1994; Westhof and Michel 1996). They have several conserved regions that allow secondary structure to form through base pairing, and these regions have been used to categorize these introns into classes (Cech 1988, 1990; Westhof and Michel 1996). Group I introns have been found in nuclear and organelle genes of eukaryotes, as well as in eubacteria and viruses (Dujon 1989, Shub et al. 1994). Although introns with apparent sequence homology have been found in related host taxa, consistent with a presumed common evolutionary origin, their occurrence may also be variable among closely related host taxa suggesting multiple insertional events (Woodson 1996). Some shared occurrences appear to be ancient in origin, such as the intron occurring in the leucine tRNA gene that occurs in both cyanobacteria and chloroplasts, while others appear to be much more recent in origin (Turmel et al. 1995). More than 200 different Group I introns have been identified occurring in a wide variety of genes in different hosts (Damberger and Gutell 1994). Particular attention has been paid to the introns found in nuclear, organelle, and bacterial ribosomal RNA genes (Woodson 1996).

Group I introns seem to be especially prevalent in the nuclear small subunit ribosomal RNA genes of fungi. These include the Ascomycete *Cryptendoxyla hypophloia* (Suh et al. 1999), mycorrhizal *Cenococcum geophilum* (Shinohara et al.

1996), lichen forming *Cladonia chlorophaea*, *Lecanora dispersa*, *Calicium tricolor*, *Porpidia crustulata*, as well as a non-lichen forming ally *Mycocalicium albonigrum* (DePriest and Been 1992, DePreist 1993, Gargas et al. 1995). They have also been found in plant parasitic fungi *Protomyces*, *Phialophora gregata*, *Botrytis, Dumontinia, Encoelia, Grovesinia, Myriosclerotinia,* and *Sclerotinia* (Nishida and Sugiyama 1995, Chen et al. 1998, Holst-Jensen et al. 1999), and the entomothphagus *Cordyceps* (Nikoh and Fukatsu 2001).

In a phylogenetic study of homobasidiomycetes using the nuclear small subunit ribosomal RNA gene, Hibbett (1996) found that *Panellus stypticus* and confamilial *Artomyces pyxidatus* (≡ *Clavicorona pyxidata*; see Chapter II), *Lentinellus montanus, L. ursinus* and *L. omphalodes* all contained a Group I intron in the same position corresponding to base 943 of the small subunit ribosomal gene of *Escherichia coli* (see Gargas et al. 1995). The intron was apparently absent in another member of the Auriscalpiaceae, *Auriscalpium vulgare.* Because the intron occurred in two separate evolutionary lineages, *Panellus stypticus* and *Artomyces-Lentinellus*, he concluded that the intron was probably acquired in two separate events, adding that the introns in *Artomyces* and *Lentinellus* were more similar to each other than either was to the intron in *Panellus stypticus*. Once acquired in the Auriscalpiaceae lineage, Hibbett suggested that the intron was probably lost in *Auriscalpium* (Hibbett 1996). A possible heterozygous condition for presence/absence of the intron in one isolate of *L. montanus* and an isolate of *L. ursinus* was also noted (Hibbett 1996). For his survey, Hibbett (1996) sampled only one specimen each of *Ar. pyxidatus, Au. vulgare, L. omphalodes* (*L. micheneri* in this study) and *L. ursinus* (probably *L. omphalodes* in this study) and two specimens of *L. montanus*.

In this study, the range and extent of the occurrence of the Group I intron in the 18S nrDNA gene was examined among and within species of *Artomyces*, *Auriscalpium*, and *Lentinellus*. Phylogenetic analyses were undertaken to elucidate the evolutionary history of the intron and to determine if these estimations were congruent with those based on ribosomal ITS 1 – 5.8S – ITS 2 sequences.

METHODS

Cultures and DNA Extraction – Monokaryon and dikaryon cultures used in this study (TABLE 1) were established from basidiomata collected in nature and are maintained in the culture collection at the University of Tennessee as described by Hughes et al. (1999). These include 84 isolates of seven species of *Lentinellus*, 15 isolates of 2 species of *Auriscalpium*, and 325 isolates of 15 species of *Artomyces*. Each collection is represented by a voucher specimen and, for most cases, dikaryon and monokaryon cultures. The DNA extraction protocol followed that used by Hughes et al. (1999).

PCR Amplification – The region of the nuclear ribosomal 18S gene in which the intron was located was amplified using the forward primer SR1c and reverse primer NS6 (Hibbett 1996; TABLE 2). Cycle parameters were 94°C for 4 minutes followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 2 minutes with a final extension at 72°C for 1 minute (modified from Hibbett 1996). Direct visualization of PCR products was accomplished by gel electrophoresis in 1.5% 1X TBE agarose gels

TABLE 1. Collections examined for presence or absence of a Group I intron.

Taxon	Country	Province	Isolate[a]	Intron
Artomyces adrienneae	Argentina	Chubut	8299 (55352), 8309 (54987), 8314 (54992), 8343 (55017)	no
		Neuquen	7384 (54435), 7386 (54404), 7387 (54434), 7389 (54426)	no
Ar. austropiperatus	Argentina	Chubut	***8335***[b] (55009), 8363 (55036)	yes
	Australia	Tasmania	RV95-509 (DHN 2890), RV95-511 (DHN 2892)	no
	New Zealand	North Island	2822 (48916), 7153 (56948)	no
Ar. candelabrus	Australia	Tasmania	3922 (50128)	no
	New Zealand	Auckland	2685 (48919)	no
		Canturbury	2555 (48930)	no
		Fiordland	2637 (48917), 2644 (48918), 2667 (48920)	no
		Matakitaki	55157 (43871)	no
		South Island	7067 (54122), 7102 (56874), 7110 (56219), 7130 (56881)	no
Ar. carolinensis	United States	North Carolina	1615 (48890)	no
Ar. costaricensis	Costa Rica	Cartago	10866 (58728)	no
		San Jose	7900 (53848), 9670 (56631)	no
Ar. colensoi	New Zealand	Auckland	2679 (48923)	no
		North Island	28)7 (48925), 55633 (44117)	no
Ar. cristatus	Sweden	unknown	GB1701 (#####)	no
	United States	California	8079 (55403), DL8974 (#####)	no
Ar. enigmatus	Costa Rica	Puntarenas	9905 (56533)	no
	United States	Puerto Rico	3456 (48929)	no
Ar. microsporus	Japan	Tochigi	2349 (48856)	no
		Yokoyama	KY5352 (#####)	no
Ar. novazealandensis	Australia	New South Wales	3543 (50060)	no
	New Zealand	Fiordland	2672 (48915), 7023 (54111), 7024 (#####), 7025 (56203), 7037 (56200), 7044 (54108)	no
		South Island	7056 (56211)	no

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
Ar. piperatus	Canada	British Columbia	5770 (53491), 6675 (#####)	no
	United States	Alaska	8221 (57288)	no
		Washington	5869 (51959)	no
Ar. pyxidatus	Canada	Quebec	9255 (55986), 9256 (55987)	yes[c]
	China	Jilin	1449 (48279)	yes
		Yunnan	***1541***[b] (48855)	yes
	Costa Rica	Heredia	10030 (56742)	yes[c]
		Puntarenas	9932 (56558)	yes[c]
		San Jose	9464 (56438), 9465 (56439), 9681 (56642), 10873 (58737)	yes[c]
	Mexico	Mexico	8396 (55067), 8408 (55235), 8409 (55236), 8437 (55266), 8439 (55268), 8440 (55269)	yes[c]
		Nayarit	8723 (55152), 8725 (55154), 8726 (55155), 8751 (55180) ET3817 (#####)	yes[c]
	Russia	Caucasia	8897 (55517), 8903 (55523), 8927 (55547), 8950 (55610)	yes
		Leningrad	10605 (58265), 10611 (58271), 10629 (58288), 10630 (58289), 10631 (58290), 10640 (58247)	yes
	Sweden	unknown	GB1137 (#####), GB1279 (#####), GB1280 (#####), ***GB1702***[b] (#####)	yes
		Uppland	7246 (53620), 7255 (53509), 7263 (53544), 7268 (53634), 7269 (#####), 7298 (53578)	yes
	Switzerland	Maggia	4289 (50625)	yes
	United States	Arizona	ATCC 22501(#####), ***H7524***[b] (#####), JPL479 (#####)	yes[c]
		Georgia	4567 (51048), 4568 (51049), 4573 (51053), 5241 (51893), 5242 (51900), 5243 (51899), 5484 (51947), 5485 (51948), 6211 (#####), 6216 (51957), 6507 (51958), 56665 (48853), 56666 (48887), ***56667***[b] (48854), 56668 (48888)	
		Illinois	ASM6658 (#####)	yes[c]
		Maine	4990 (51411)	yes[c]

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
Ar. pyxidatus	United States	Maryland	AES515 (#####), HHB810 (#####)	yes[c]
		Michigan	7945 (#####),	yes[c]
		Minnesota	6932 (#####), 6941 (#####)	yes[c]
		Mississippi	9201 (#####)	yes[c]
		New York	1190 (48842), 1704 (48902), 1724 (48906), 4938 (51251), 6485 (51942), 6488 (51943)	yes[c]
		North Carolina	1135 (48914), 1607 (48852), 1608 (48897), 1614 (48896), 1617 (48900), 1618 (49873), 1648 (#####), 1650 (#####), 1661 (48850), 2174 (48795), 4091 (50241), 4598 (51076), 4806 (51107), 4816 (51117), 5048 (51451), 5072 (51471), 5116 (51973), 5117 (51939), 5168 (51941), 5298 (51876), 5437 (51755), 5441 (51759), 5461 (51777), 5464 (51945), 5493 (51946), 5555 (52488), 6219 (51956), 6226 (51953), 6307 (51922), 6308 (51921), 6428 (51927), 6513 (51950), 6522 (51952), 6609 (51931), 6610 (51929), 6611 (51932), 6612 (51933), 6614 (51930), 6615 (51934), 6616 (51935), 6617 (51949), 6618 (#####), 6621 (51919), 6622 (51918), 6623 (51915), 6624 (51920), 6632 (51914), 6633 (51923), 9238 (55969), 9239 (55970), 9240 (55971), 9241 (55972), 9242 (55973), 9243 (55974), 9244 (55975), 9245 (55976), 9246 (55977), 9259 (55990), 9269 (56457), 9270 (56458), 9271 (56459), 9272 (56351), 9277 (56355), 9278 (56356), 9279 (56357), 9281 (56359), 9300 (57086), 9724 (57109), 9726 (57111), 56653 (48846), 56655 (48847), 56662 (48848), 56680 (48891), 56681 (52357), ##### (47668), ##### (47669), ##### (47670), ##### (47673), ASU 1231 (#####), ASU 1238 (#####), Dodd 38 (#####)	yes[c]
		South Carolina	4580 (51059), 4581 (51060), 5519 (51799), 6433 (51928)	yes[c]

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
Ar. pyxidatus	United States	Tennessee	1606 (48894), 2994 (50680), 2995 (50681), 3624 (50743), 3650 (50769), 3710 (50698), 4563 (51905), 4564 (51906), 4681 (51944), 5194 (51938), 5195 (#####), 5236 (51901), 5237 (51904), 5238 (51902), 5299 (51875), 5300 (51880), 5302 (51617), 5303 (51618), 5401 (51898), 5402 (51897), 5403 (51896), 5404 (51895), 5411 (51887), 5412 (51885), 5413 (51884), 5414 (51886), 5415 (51889), 5416 (51891), 5417 (51883), 5418 (51888), 5419 (51878), 5420 (51879), 5422 (51892), 5423 (#####), 5425 (51881), 5426 (51890), 5427 (51882), 5538 (51903), 6601 (51909), 6602 (51908), 6603 (51910), 6604 (51911), 6605 (51912), 6606 (51925), 6607 (51907), 6608 (51924), 6619 (51916), 6620 (51913), 9220 (55951), 9221 (55952), 9222 (55953), 9223 (55954), 9224 (55955), 9225 (55956), 9226 (55957), 9227 (55958), 9228 (55959), 9229 (55960), 9230 (55961), 9231 (55962), 9232 (55963), 9233 (55964), 9234 (55965), 9235 (55966), 9250 (55981), 9253 (55984), ATCC 24439 (#####), ##### (36429)	yes[c]
		Texas	9828 (58807)	yes[c]
		Unknown	RGT752 (#####)	yes[c]
		Utah	9705 (57091), 9706 (57092), 9707 (57093), 9708 (57094), 9709 (57095), 9710 (57096), 9711 (57097)	yes[c]
		Virginia	HHB10654 (#####)	yes[c]
		Wisconsin	1501 (48843), 1502 (48893), 1503 (48844), 1504 (48879), 1506 (48845), 1507 (48880), 1508 (48892), 1509 (48885), 1511 (48872), 1512 (48886), ***1513***[b] (48875), 1514 (48872), 1515 (48876), 1516 (48878), 1517 (48881), 1519 (48877), 1520 (48882)	yes[c]

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
Ar. stephenii	Costa Rica	Cartago	9433 (56407), 9436 (56410), 9438 (53412)	no
		San Jose	7875 (53762), 7898 (53845), 7913 (53798), 9743 (58644), 11022 (58630)	no
Ar. tasmaniensis	Australia	Tasmania	3905 (50112), 3979 (50283), 4003 (50218), RV95-527 (#####)	no
Ar. turgidus	Australia	Tasmania	3961 (50103)	no
	New Guinea	Morobe	RV95-875 (DHN 3138)	no
	New Zealand	Auckland	2682 (48926)	no
		Fiordland	2651 (48934)	no
		North Island	7164 (56950)	no
		South Island	7040 (54099), 7109 (56218), 55137 (44306)	no
Auriscalpium vulgare	Finland	Etala-Hame	7458 (53581)	no
	Guatemala	Sacatepequez	4850 (51148)	no
	Mexico	Tlaxcala	6234 (52891), ET3318 (#####), ME1145 (#####)	no
	Sweden	Uppland	7295 (53594)	no
		Vastergotland	4202 (50538), 4242 (50578)	no
	Switzerland	Maggia	4291 (50627)	no
	United States	Idaho	5580 (52356), 5634 (52355)	no
		Minnesota	6920 (56826), 6946 (56823)	no
Au. villipes	Mexico	Veracruz	***4396***[b] (52353), 6271 (52354)	het[d]
Lentinellus castoreus	Austria	Burgenland	ANM 511 (#####)	no
	Australia	New South Wales	3538 (50166)	no
	Costa Rica	Alajuena	9405 (56380)	no
		San Jose	7808 (53758), 7876 (53748), 7880 (53831)	no
	Mexico	Nayarit	8759 (55187)	het[d]
	Russia	Caucasia	8946 (55606)	no
	Switzerland	Maggia	4321 (50400)	yes
	United States	California	9547 (56260), DDL 9369 (#####)	no
		Illinois	ANM 495 (#####)	yes

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
L. castoreus	United States	Florida	8270 (54331)	no
			9149 (54880)	yes
		Louisiana	***8685***[b] (56292), 9208 (55942)	yes
		Missouri	ANM 492 (#####)	yes
		North Carolina	***4101***[b] (50687), 8768 (55196)	yes
		Tennessee	2036 (48636), 4065 (50260), 9254 (55985)	yes
L. cochleatus	Austria	Unknown	***9985***[b] (#####)	yes
L. crawfordii	New Zealand	South Island	7104 (56878)	no
L. cystidiosus	Mexico	Mexico	8452 (55281)	het[d]
L. flabelliformis	Austria	Unknown	9980 (WU945214), ***9981***[b] (58504)	yes
	Sweden	Uppland	7292 (53622),	yes
L. micheneri	Finland	Etela-Hame	7468 (53587), 7491 (53646)	yes
	Greenland	West Greenland	9776 (58674), 9777 (58675), 9785 (58683), 9786 (58684), 9795 (58693)	yes
	Siberia	Lake Baikal	9978 (#####)	yes
	Sweden	Vastergotland	4243(50579)	yes
	United States	Alaska	***6701***[b] (53028), 8199 (53880)	yes
		Washington	PBM 1723 (#####)	yes
L. montanus	United States	Montana	OKM6414 (#####), ***OKM27329***[b] (#####)	yes
L. "pseudoflabelliformis"	United States	Washington	5702 (52535)	no
L. pulvinulus	Argentina	Santa Catalina	10740 (#####)	no
L. subargillaceus	United States	California	10233 (57143)	no
L. "subaustralis"	United States	North Carolina	4433 (50931), 6293 (52942), 9177 (#####)	yes
		Tennessee	3402 (48996), 6303 (53125), ***9159***[b] (54890), 9712 (57098)	yes
L. tridentinus	Russia	Caucasia	8967 (55627)	no

TABLE 1. cont.

Taxon	Country	Province	Isolate[a]	Intron
L. ursinus	Mexico	Mexico	8456 (55285)	no
		Tlaxcala	6259 (52917), 8711 (55141)	no
	New Zealand	South Island	7104 (56878)	no
	United States	Missouri	ANM 493 (#####)	no
		North Carolina	2209 (48123), 2210 (48125), 6631 (53195), 8862 (55482)	no
		South Carolina	9986 (57001)	no
		Tennessee	3404 (49190), 5324 (51639)	no
		Wisconsin	ANM 473 (#####)	no
L. vulpinus	Sweden	Uppland	7267 (53539)	no
Lentinellus sp.	Russia	Primorsk	3211 (49529)	no

[a] All isolates originated from University of Tennessee collections except those with preceding initials. These isolates came from; AES and JPL = Forest Products Lab Madison, Wisconsin; ANM = Andrew Miller; ASM = Andrew Methven; ASU = Coleman McCleneghan; ATCC = American Type Culture Collection; DDL = David Largent; DODD = Jim Dodd; ET = Arturo Estrada-Torres; GB = Gothenberg (Sweden) Culture Collection; H = Roy Halling; HHB = Harold Burdsall; OKM = Orson Miller; RGT = Greg Thorn; RV = Rytas Vilgalys. TENN herbarium accession numbers in parentheses. Where noted; ##### = no accession number, DHN = Duke, WU = Wien University.

[b] Ribosomal ITS and Group I sequences were obtained from these isolates. These are highlighted with bold italic type.

[c] Group I intron contains an additional insertional element.

[d] "Het" indicates putative heterozygous state for presence and absence of Group I intron.

TABLE 2. List of primers used for PCR assay and sequencing of 18S nuclear ribosomal DNA and the associated Group I intron.

Primers	Sequence 5' --> 3'	Position in yeast[a]
SR1c[b]	AGC AGC CGC GGT AAT	567-581
NS51[c]	GGG GGA GTA TGG TCG CAA GGC	1095-1116
NS41[c]	CCC GTG TTG AGT CAA ATT A	1169-1187
NS6[d]	GCA TCA CAG ACC TGT TAT TGC CTC	1404-1427
18SII[e]	GAC GCG CAT ATA GTC AGC	
18SISF[e]	GAC AGG TTC AGA GAC TAG AT	

[a]Rubstov et al. (1980).
[b]Hibbett (1996).
[c]Kerry O'Donnell cited in Hibbett (1996).
[d]White et al. (1990).
[e]Primers derived for use in this study. Primer 18SISF is situated across conserved region "R" of the intron. Primer 18SII is a reverse primer located in the secondary insert approximately 300 bp from the 5' end of the conserved region "S".

with ethidium bromide stain. A few representative isolates were electrophoresed in a 1.5% agarose gel with a 1X alkaline buffer according to FMC Bioproducts (1997).

The nuclear ribosomal ITS 1-5.8S-ITS 2 (ITS) region was amplified using forward primer ITS 5 and reverse primer ITS 4 (White et al. 1990). Cycle parameters were an initial melting cycle of 94 C for 4 minutes, 35 repetitions of a three step amplification cycle of 94 C for 1 minute, 52 C for 1 minute, and 72 C for 1 minute, ending with an extension cycle of 72 C for 1 minute.

Southern Blots - Southern Blots were performed according to Amersham (1987) for a few representative samples. DNA was fixed to Amersham Hybond N+ nylon membranes by exposure to ultraviolet light. A probe was obtained from an isolate of *Ar. adrienneae*, a species that does not contain the intron. The PCR product was digested with *Hae* III, and a 148 bp fragment was excised from a 1X TAE 1.5% low-melting temperature agarose gel (NuSieve GTG agarose, FMC Bioproducts). This fragment corresponds to bases 1265 to 1403 in the small subunit gene of *Saccharomyces cerevisiae* (Rubstov et al. 1980). The fragment was purified using a Promega Wizard PCR Purification Kit following manufacturer's directions. The purified product was then labeled with P_{32} and used to probe the blots (Sambrook et al. 1989).

DNA Sequencing, alignment, and comparison. - Five isolates of *Ar. pyxidatus*, two isolates of *L. castoreus*, and one isolate each of *Ar. austropiperatus*, *Au. villipes*, *L. cochleatus*, *L. montanus*, *L. micheneri*, *L. flabelliformis*, and *L. "subaustralis"* (*nom. prov.*) were chosen for sequencing of both the 18S Group I intron and the ITS region (TABLE 1). PCR products were electrophoresed and then excised from a 1X TAE 1.5% low-melting temperature agarose gel and purified as described above. Purified products were sequenced with an automated ABI 373 DNA sequencer (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc) with primers NS41 and NS51 (TABLE 2) for the intron and primers ITS 5 and ITS 4 for ITS (White et al. 1990). Two additional primers (TABLE 2) were needed to accommodate the longer intron length of some *Ar. pyxidatus* isolates. Sequences of each gene were manually corrected and aligned using the LINEUP and SEQLAB programs in the Genetics Computer Group package (GCG 2001). Sequences of the small subunit gene of *Saccharomyces cerevisiae* (GenBank J01353; Rubstov et al. 1980) and sequences for *Ar. pyxidatus* and *L. omphalodes* (GenBank U59066 and U59078, respectively; Hibbett 1996) were compared to the intron alignment for reference purposes.

Phylogenetic reconstruction -- Phylogenetic relationships of the intron and the ITS 1 – 5.8S – ITS 2 region were estimated separately using PAUP 4.0 (Swofford 1998). Ambiguous regions where alignment was tenuous were excluded from the analyses. This removed many large gaps where indels were too variable to confidently "gap-code." Remaining gaps were small and were treated as missing data to avoid any character duplication associated with "gaps-as-fifth-character" where indels of two or more positions are counted as more than one event. The final alignments used in the analyses are included in APPENDIX C. A branch-and-bound search was used to find the most

parsimonious trees, and bootstrap analyses of 100 replicates were conducted with a branch-and-bound search using maximum parsimony.

Phylogenetic comparisons – Several comparisons were made between the phylogenetic estimations based on intron and ITS sequences with a direct focus on levels of character incongruence. Incongruence metrics of Mickevich and Farris (1981) and Miyamoto (in Kluge 1989 and Swofford 1991) (I_{MF} and I_M, respectively), the homogeneity test (HT_F) of Farris et al. (1994), and Templeton's (1983) significantly least parsimonious tests (SLP_T) were calculated using PAUP and have been thoroughly described by Johnson and Soltis (1998). The parameters for HT_F were 1000 replicates using a branch-and-bound search.

RESULTS

PCR Assay for Group I intron -- The expected length of the 18S sequence without the intron was approximately 860 bp, based on *Saccharomyces cerevisiae* sequence (Rubstov et al. 1980). All isolates of *Auriscalpium vulgare* exhibited a PCR product of this size. Both isolates of *Au. villipes*, however, exhibited two bands, one approximately 860 bp, the other approximately 1300 bp. This larger fragment was not observed in any of the *Au. vulgare* isolates and was shown by sequencing to contain the Group I intron.

The taxonomy and nomenclature of *Lentinellus* is presently in a state of flux and reorganization, but taxonomic groups have tentatively been delimited. The following taxonomic groupings and names are sensu RHP (work in progress). Seven species of *Lentinellus* exhibited a larger fragment. The larger fragment was found in *L. cochleatus, L. montanus, L. micheneri, L. flabelliformis, L. "subaustralis"*, and was variable in *L. castoreus*. A heterozygous condition was observed in *L. cystidiosus*. The larger fragment is apparently absent in isolates of *L. vulpinus, L. ursinus, L. subargillaceus, L. "pseudoflabellifomis," L. crawfordii, L. tridentinus*, and *L. pulvinulus*.

Increased fragment sizes were observed in only two of the 15 species of *Artomyces* surveyed. All isolates of *Ar. pyxidatus* and only Argentinean isolates of *Ar. austropiperatus* exhibited this large fragment. The large fragment in all Eurasian *Ar. pyxidatus* isolates and in the *Ar. austropiperatus* isolate was approximately 1300 bp indicating presence of the intron. Considerable variation, however, was found in fragment sizes among North American *Ar. pyxidatus* isolates. A comparison of fragment lengths of several North American *Ar. pyxidatus* isolates and isolate 7387 (*Ar. adrienneae*) which lacks the intron is shown in FIG 1. The size of these PCR fragments ranged from approximately 1300 bp to a little more than 2100 bp. As with *Au. villipes*, a fragment of 860 bp was sometimes observed in *Ar. pyxidatus* isolates, but was always in a heterozygous condition.

Oftentimes multiple primary and secondary bands were present in North American collections of *Ar. pyxidatus*. The high annealing temperature (60C) in the PCR cycle should have been sufficiently stringent to avoid non-target amplification. Southern blots probed with a 148 bp 18S fragment showed that the variable primary and the multiple secondary bands were likely of small subunit origin and not the result of non-target priming and amplification. Alkaline gels, which should prevent secondary structure formation, indicated that the multiple bands were probably not the result of heteroduplexing or other secondary structure.

Sequence comparisons – The aligned sequences of the 18S Group I intron were compared to those by Hibbett (1996) and Rubstov et al. (1980; *Saccharomycyes cerevisiae* 18S rRNA, GenBank J01353). The alignment verified that the increased PCR fragment size in isolates of *Lentinellus*, *Artomyces*, and *Auriscalpium* were due to what Hibbett (1996) identified as a Group I intron in a position corresponding to base 943 of *Escherichia coli* (Gargas et al. 1995), and base 1156 of the *S. cerevisiae* sequence.

The sequence comparisons also verified that the even larger and highly variable fragments that were amplified from North American *Ar. pyxidatus* isolates were due to an additional insertion located between conserved regions R and S of the Group I intron (FIG 2). These secondary insertions ranged in size from about 100 to over 1000 bp with differences in length apparently caused by indels of variable size. All secondary insertions were alignable on the 3' and 5' ends of the longest insertion sequenced (56667 from Georgia) with significant sections missing from the middle (FIG 2). A basic Blast search in GenBank revealed no matches for these insertions, but a search for potential secondary structure using RNAdraw (Matzura and Wennborg 1996) indicated that secondary structure was strongly probable. A search of the unknown sequences using the GCG program PEPDATA indicated that several putative open reading frames (ORFs) were present.

Phylogenetic Reconstruction – A branch-and-bound search of the Group I intron sequences for the representative isolates (TABLE 1) yielded a single most parsimonious tree of 117 steps (FIG 3). Bootstrap analysis indicated strong support for an *Ar. pyxidatus* clade and a clade composed of *L. montanus, L. "subaustralis," L. micheneri, L. flabelliformis*, and both isolates of *L. castoreus* ("core" *Lentinellus* group). However, *Ar. austropiperatus* and *L. cochleatus* were paired on a well-supported branch sister to the *Lentinellus* clade. The single *Au. villipes* isolate composed a branch between *Ar. pyxidatus* and the *Ar. austropiperatus – Lentinellus* clade.

A second branch-and-bound search using ITS sequences of these isolates yielded three most parsimonious trees of 150 steps (FIG 3). Bootstrap analysis again indicated strong support for an *Ar. pyxidatus* clade and the "core" *Lentinellus* group. Unlike the intron phylogeny, however, *Ar. austropiperatus* grouped strongly with *Ar. pyxidatus* while the *Au. villipes* isolate formed a long, unsupported branch with *L. cochleatus* and the "core" *Lentinellus* group.

Phylogenetic comparisons – The intron and ITS phylogenies appeared topologically different (FIG 3). High incongruence metrics indicated that there was heterogeneity between the datasets with $I_{MF} = 0.17$ and $I_M = 0.65$. An analysis of the datasets using the partition homogeneity test described by Farris et al. (1994) revealed that the heterogeneity was sub-structured among taxa (TABLE 3). When all groups were analyzed together there was significant heterogeneity between the datasets of intron and ITS sequences. However, when subsets *Artomyces* and *Ar. pyxidatus* were analyzed separately, the datasets were not significantly heterogeneous. When subsets *Lentinellus*, *Lentinellus* with *A. villipes*, and *Lentinellus* without *L. cochleatus* were analyzed separately, the datasets were marginally significant ($P = 0.085$, 0.038, and 0.057, respectively).

FIGURE 1. Variability in PCR product length among *Artomyces pyxidatus* isolates. Lanes are: A, Phi X *Hae* III size marker; B, GB 1702 without 2^{o} insert (Sweden); C, 9255 (Canada); D, 9256 (Canada) ; E, 8396 (Mexico); F, *Ar. adrienneae* 7388 (without intron); G, 8896 without 2^{o} insert (Russia); H, 4567 (Georgia); I, 4568 (Georgia); J, 4573 (Georgia); K, Phi X *Hae* III; L, Lambda *Hin*d III size marker; M, 56667 (Georgia); N, 1508 (Wisconsin); O, 1513 (Wisconsin); P, H7524 (Arizona); Q, 4990 (Maine); R, HBB810 (Maryland); S. 9229 (Tennessee); T, 9230 (Tennessee); U, 9232 (Tennessee); and V = Phi X *Hae* III size marker.

Templeton's (1983) "significantly least parsimonious" (SLP_T) test also show that the intron and ITS datasets were not homologous with each other's topologies ($P < 0.001$, TABLE 4). The intron dataset was not congruent with any of the three most parsimonious ITS phylogenies, and the ITS dataset was not congruent with the most parsimonious intron phylogeny.

DISCUSSION

Data from PCR assay and 18S DNA sequences confirmed Hibbett's (1996) observation of the presence of a Group I intron in *Lentinellus* and *Artomyces* for the species that he surveyed. However, the Group I intron was not found in all species of these two genera, and it was found in at least one *Auriscalpium* species. In the isolate he identifies as *L. ursinus,* Hibbett (1996) was not able to detect the intron by PCR amplification with primers NS6 and SR1c but was able to determine its presence by using a combination of intron-specific primers. The apparent absence of the intron was attributed to a PCR bias where intron-less regions in a heterozygote amplified at a higher frequency than regions containing the intron (Hibbett 1996). In light of recent taxonomic studies (Petersen, unpubl.), the identity of this isolate may be one of three species; *L. castoreus*, *L. ursinus*, or *L. vulpinus*. In the present study, the intron was not found in either *L. ursinus* or *L. vulpinus*, but its presence was found to be variable in *L. castoreus* and heterozygous in isolate 8759 (Mexico). This indicates that the "*L. ursinus*" isolate in Hibbett (1996) was probably another heterozygous *L. castoreus*. The putative heterozygous pattern was observed in isolates of some other species as well where at least two distinct fragments appeared in the primary PCR products. Sometimes one or more fragments would appear less concentrated, lending support to the idea of PCR bias.

The results of this study indicate that there is an additional secondary insertion within the Group I intron between conserved regions R and S. In many cases, there seemed to be considerable length heterogeneities within the ribosomal repeat. Based on sequence data, it appeared that the plethora of length differences is due to significant and variable degradation in the middle of the secondary insert (FIG 2). These secondary insertions have only been found in the North American populations of *Ar. pyxidatus*, and there appeared to be no geographical pattern to these insertions. These large insertions may either be another Group I intron based on putative secondary RNA-like structure and open reading frames (ORFs), or some other type of transposable element. They appeared to have no sequence similarity with anything in the GenBank database and may represent a new element. Literature searches have not yielded any reports of similar situations. DePriest and Been (1992) and DePriest (1993) noted significant size variations of the small subunit nuclear ribosomal DNA gene in the lichen forming fungus *Cladonia chlorophaea*. Sequence and restriction site analysis revealed that the cause of the variation was due to several different Group I introns inserted at five different sites (DePriest and Been 1992, DePriest 1993), but little variation in the size of the intron was observed.

Phylogenetic estimations based on intron sequences indicated that strong phylogenetic signal exists (FIG 3) and the strong similarity of intron sequences within the Auriscalpiaceae suggested an ancient infection. The presence of the intron in species that are closely related suggests a common insertional event, at least for *Lentinellus*. When

FIGURE 2. Relative locations of the Group I intron and secondary insert. The arrow indicates the position homologous to 943 of *Escherichia coli* (Gargas et al. 1995) and 1156 of *Saccharomyces cerevisiae* (Rubstov et al. 1980). Also illustrated are the regions of homology in secondary insertion sequences of four North American *Ar. pyxidatus* isolates. European isolate (GB 1702) does not contain the insertion. Solid lines represent completely aligned areas while dotted lines represent "missing" sections.

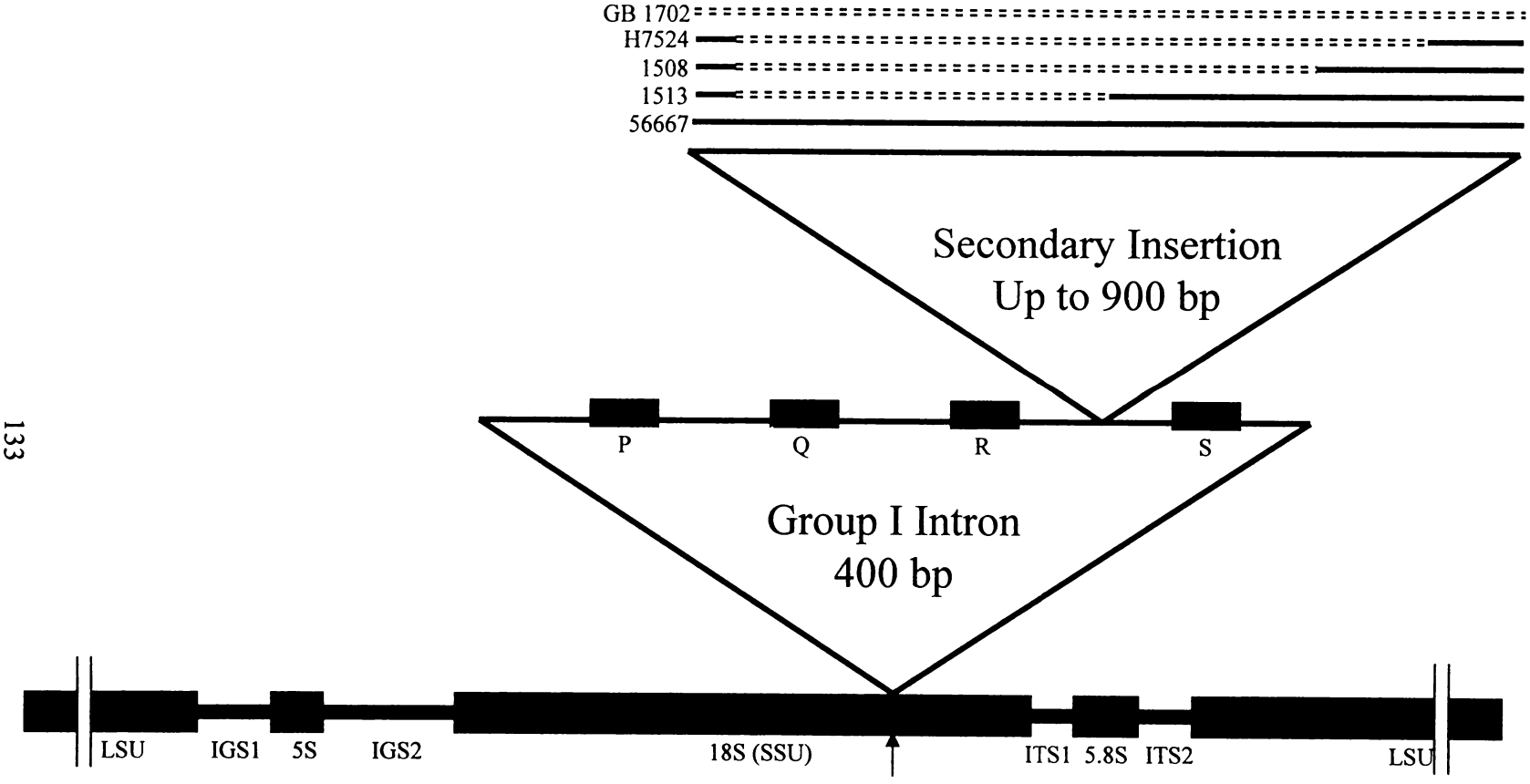

GB 1702
H7524
1508
1513
56667
Secondary Insertion
Up to 900 bp
P
Q
R
S
Group I Intron
400 bp
LSU
IGS1
5S
IGS2
18S (SSU)
ITS1
5.8S
ITS2
LSU

FIGURE 3. Comparison of phylogenetic estimations for Group I intron and ITS sequences of isolates of *Artomyces*, *Auriscalpiumn* and *Lentinellus*. Both trees are the result of branch and bound searches with maximum parsimony. Numbers above branches represent branch length, while numbers below branches represent bootstrap values from 100 replicates. The intron tree is the single most parsimonious tree 117 characters long, CI = 0.78, and RI = 0.86. The ITS tree is one of three most parsimonious trees 150 characters long, CI = 0.81, and RI = 0.87.

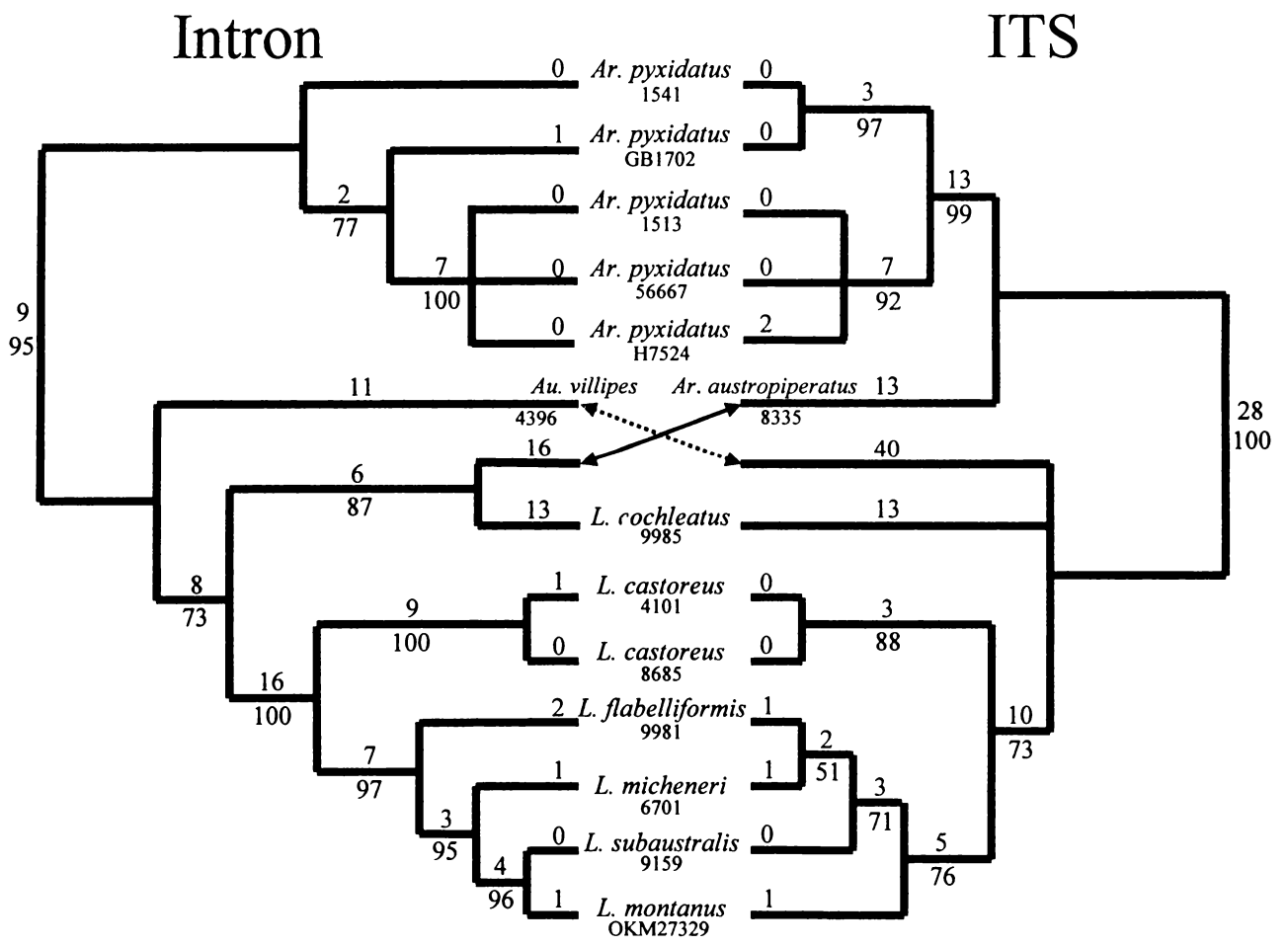

Intron
ITS
Ar. pyxidatus 1541
Ar. pyxidatus GB1702
Ar. pyxidatus 1513
Ar. pyxidatus 56667
Ar. pyxidatus H7524
Au. villipes 4396
Ar. austropiperatus 8335
L. cochleatus 9985
L. castoreus 4101
L. castoreus 8685
L. flabelliformis 9981
L. micheneri 6701
L. subaustralis 9159
L. montanus OKM27329

TABLE 3. Results of the Homogeneity Test (HT_F) of Farris et al. (1994).

Taxonomic Combinations	P[a]
All taxa	0.002
Artomyces pyxidatus and *Lentinellus*	0.041
Ar. pyxidatus without *Ar. austropiperatus*	1.000
Ar. pyxidatus with *Ar. austropiperatus*	1.000
Lentinellus	0.085
Lentinellus without *L. cochleatus*	0.038
Lentinellus with *Auriscalpium*	0.057

[a] P values above 0.05 indicate the probability that the datasets (intron vs. ITS sequences) are homogeneous.

TABLE 4. Results of the Significantly Least Parsimonious test (SLP_T) of Templeton (1983).

		Difference			
Dataset	Alternate topology	Positive	Negative	Net	P[a]
ITS	Intron	28	5	23	0.0001
Intron	ITS 1	14	1	13	0.001
	ITS 2	14	1	13	0.001
	ITS 3	15	1	14	0.0005

[a]The datasets were compared to the alternate topologies to determine if they resulted in as parsimonious a tree or more compared to the most parsimonious topology. Positive values quantify the number of steps added to the dataset to fit the alternate topology. Negative values quantify the reduction in the number of steps needed. P values below 0.05 indicate a rejection of the null hypothesis that the datasets result in similar parsimonious outcomes.

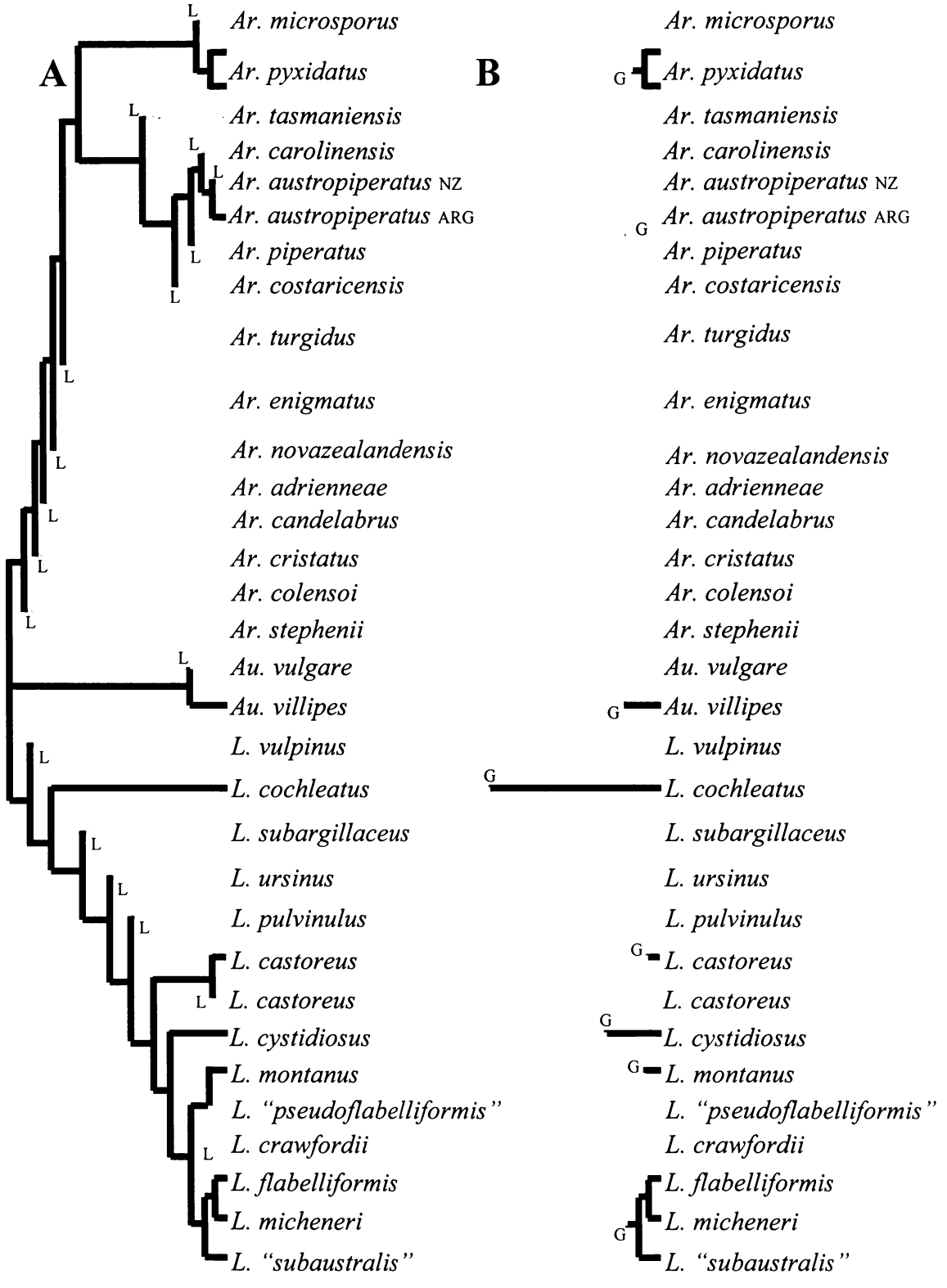
the intron phylogeny was compared to phylogenies based on ITS sequences, however, significant heterogeneity existed between the two datasets (TABLES 3, 4). It was evident that some of the heterogeneity between datasets was due in part to the discordance within the *Lentinellus* group (TABLE 3). Within *Lentinellus*, the intron was present in four closely related species with little ITS sequence divergence. Incongruence between datasets may be due to minor ITS sequence differences affecting phylogenetic placement or may be the result of ribosomal gene recombination or conversion before speciation was complete. There was also conflict in the placement of the *Ar. austropiperatus* intron within the *Lentinellus* clade (FIG 3).

Alternatively, horizontal transmission could be responsible for the conflicting phylogenies, and a "gain-only" model may seem most parsimonious (FIG 4A). Group I introns are by definition self-excising elements, often exhibiting protein coding sequences which are thought to be mobile and perhaps transmissible (Cech 1988, Turmel et al.1995). Nishida and Sugiyama (1995), Hibbett (1996), Holst-Jensen et al. (1999) have presented potential examples of horizontal transmission by Group I introns, but the mechanism of transmission from one host to another or possible vectors remain unknown. It might be assumed that for transmission to occur, an organism infected with the intron must be in close proximity to an uninfected one. With limited fossil records, ancient distributions of fungi remain largely unknown. However, present day distributions of the taxa with the Group I intron are very wide-ranging; *Artomyces pyxidatus* (1541, China; GB1702, Sweden; 1513, Wisconsin; 56667, Georgia; H7524, Arizona), *Ar. austropiperatus* (8335, Argentina), *Lentinellus castoreus* (8685, Louisiana and 4101, North Carolina), *L. "subaustralis"* (9159, Tennessee), *L. micheneri* (6701, Alaska), *L. montanus* (OKM, Montana), *L. flabelliformis* (9981, Austria), *L. cochleatus* (9985, Austria), and *Auriscalpium villipes* (4396, Mexico). Because of the long geographic distances separating these taxa, the lack of recombination, and presence of strong phylogenetic signal, it appears unlikely that there was recent horizontal transmission among these taxa.

Based on his higher taxonomic level phylogenetic estimation using 18S ribosomal DNA sequences, Hibbett (1996) hypothesized that the Auriscalpiaceae clade may have gained the intron in a single event via horizontal transmission. He also suggested that the intron was vertically inherited within the group, and that the intron was lost on the branch leading to *Auriscalpium*. The expanded dataset in the present study shows that many species in Auriscalpiaceae do not contain this intron in this position. If the intron presence in the Auriscalpiaceae is due to a single transmission event, it is likely that there were repeated losses of the intron along several evolutionary branches (FIG 4B).

Several mechanisms have been proposed which could result in the loss of an intron, and in some cases, losing an intron may be easier than gaining one, especially if there is a mutation in the homing recognition sequence (Dujon 1989, Lambowitz and Belfort 1993). An alternative hypothesis to many losses is that the introns are never really lost and are present, lying latent somewhere in the genome. Potential harboring places would include the mitochondria where it may be transposed into the nuclear genome (Turmel et al. 1995). However, Hibbett (1996) did not find the intron in other genomic DNA when he used intron specific primers.

FIGURE 4. Representation of an estimated phylogeny of *Artomyces*, *Auriscalpium* and *Lentinellus*. Relationships are based on Hibbett's (1996) 18S study and ITS data presented in Chapter II. The two trees illustrate; A) a numerous-loss (L) model; B) a gain-only (G) model. Shading indicates presence (black) or absence (gray) of the Group I intron.


A
B
Ar. microsporus
Ar. pyxidatus
Ar. tasmaniensis
Ar. carolinensis
Ar. austropiperatus NZ
Ar. austropiperatus ARG
Ar. piperatus
Ar. costaricensis
Ar. turgidus
Ar. enigmatus
Ar. novazealandensis
Ar. adrienneae
Ar. candelabrus
Ar. cristatus
Ar. colensoi
Ar. stephenii
Au. vulgare
Au. villipes
L. vulpinus
L. cochleatus
L. subargillaceus
L. ursinus
L. pulvinulus
L. castoreus
L. castoreus
L. cystidiosus
L. montanus
L. "pseudoflabelliformis"
L. crawfordii
L. flabelliformis
L. micheneri
L. "subaustralis"

Two species, *Artomyces austropiperatus* and *Lentinellus castoreus* exhibit variable presence for the intron (i.e. only some isolates contain the intron). The intron is apparently only found in Argentinean isolates of *Ar. austropiperatus*, and absent in isolates from Tasmania and New Zealand. A similar pattern was observed among isolates of *L. castoreus*, but there is little geographic separation. With few exceptions, isolates from Europe and eastern North America contain the intron, but it is apparently absent in isolates from western North America and Central America. A heterozygous condition was also observed in an isolate from Mexico. These patterns may be due to independent infection of populations in one region or the loss of the intron in the others. The presence of the intron over broad geographical areas suggests that its presence is stable over time, and in the case of the Mexican isolate of *L. castoreus*, subject to gene flow in "hybrid zones."

Group I introns have been identified in the ribosomal genes of a number of fungi but their origin is unknown. The existence of genetically similar intron sequences in several members of the Auriscalpiaceae may provide evidence that their origin is due to a single infection event that occurred before the divergence of current genera and species with consequent losses of the intron along several lineages. However, intron similarity could also be due to repeated horizontal events from a common source or even a combination of horizontal and vertical transmission. The presence of a second insertional element in North and Central American *Ar. pyxidatus* isolates suggests that these elements were present before the radiation of *Ar. pyxidatus* in the New World and lends credence to a hypothesis of a single infection followed by radiation.

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PART V

GENERAL CONCLUSSIONS

Genetic diversity within the traditional genus *Clavicorona* was evaluated using morphology, mating studies and nuclear ribosomal ITS sequences. This study demonstrated that *Artomyces* Jülich, formerly *Clavicorona* subg. *Ramosa* (Dodd 1972), is a distinct genus separate from *Clavicorona* Doty *sensu* Jülich. It was also found that *Clavicorona taxophila* may be allied with the omphalinoid agarics. Within *Artomyces*, there is support for eight new species in addition to the eight previously described.

Within *Artomyces*, biogeographical origins and migrations were not discernable in phylogenies based on ITS sequences. However, two geographic regions of high species diversity were identified. Out of the 17 species recognized in this study, six occur in New Zealand and Tasmania, four of which are endemic or narrowly restricted to that region. Four species occur in Costa Rica, two of which are apparently endemic to that region. These observations lend support to national efforts to conserve biodiversity in both the Antipodes and in Costa Rica. A correlation of sequence divergence and the size of a species' geographic range was observed. As might be expected, there is a higher level of ITS DNA sequence divergence between isolates of species with broad ranges (e.g. *A. pyxidatus* and *A. turgidus*). There also appear to be a few cases of intercontinental sibling species (e.g. *A. colensoi-A. stephenii* and *A. candelabrus-A. adrienneae*).

Within *A. pyxidatus*, intraspecific geographic patterns were observed. An ITS sequence divergence of 2.5 % was found between North American and European isolates, and diagnostic restriction sites indicated that there is no evidence of gene flow between these two regions. Furthermore, differences in allele frequencies of restriction sites were found between northeastern and southwestern North American isolates. Northeastern isolates exhibited higher frequencies of alleles at two restriction sites that are characteristic of European isolates. This indicates that the geographic location of last contact was probably through a North Atlantic connection. Geographically consistent crossing barriers *in vitro* were not observed in this study in spite of sequence and RFLP differences. This implies that mating ability is conserved across diverging allopatric populations and that reproductive isolation is not congruent with sequence divergence.

The existence of genetically similar Group I intron sequences in three genera of the Auriscalpiaceae may suggest that the intron origin is due to a single infection event that occurred before the divergence of current genera and species, with subsequent losses of the intron along several lineages. However, intron similarity could also be due to repeated horizontal events from a common source or even a combination of horizontal and vertical transmission. Incongruence between intron and ITS phylogenies in some of the internal nodes may signify horizontal transmission, whereas incongruence within the "core" *Lentinellus* group may be due to lineage sorting. No significant incongruence was found between the phylogenies within *Ar. pyxidatus*, but sequence divergence between North American and European isolates was observed. The presence of a secondary insertional element only in North and Central American isolates suggests that these elements were present before the radiation of *Ar. pyxidatus* in the New World. This lends credence to a hypothesis of a single infection followed by radiation.

APPENDICES

APPENDIX A

SEQUENCE ALIGNMENT OF THE nrDNA ITS REGION FOR *ARTOMYCES* PHYLOGENY

Appendix A – Sequence alignment of nrDNA ITS region for *Artomyces* phylogeny.[a]

```
          1                                    SSU | ITS1                50
2682      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAGA ......AAGG
2651      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAgA ......AAGG
RV95/875  TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAG A.....AAGG
KY5352    TTCCGTAGGT GAACcTGcGG AAGGATCATT AAAGCAATG. ....CCAAGG
2349      TTCCGTAGGT GAACcTGCGG AAGGATCATT AAAGTAATG. ....CCAAGG
5770      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAG .....CAAGG
8221      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAG .....CAAGG
DED7220   TTCCGTAGGT GAACcTGCGG AAGGATCATT ACAGTAGAAA A....CAAGG
8335      TTCCGTAGGT GAACcTGCGG AAGGATCATT ACAGTAAAAA G....CAAGG
7153      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA G....CAAGG
1615      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
9670      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA AAA..CAAGG
7900      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA AAAAACAAGG
3979      TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGTAAAAA AA...CAAGG
3905      TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGTAAAAA AA...CAAGG
56667     TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAA. ....CCAAGG
7263      TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAA. ....CCAAGG
9905      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA GA....AAGG
3456      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA GA....AAGG
7024      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA AA...CCAGG
2672      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA AA...CCAGG
7387      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
2637      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAACG .....CAAGG
3922      TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACA .....CAAGG
GB1701    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
DL8974    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
2679      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
2807      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
9433      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
11022     TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAA .....CAAGG
9159      TTCCGTAGGT GAACCTGCGG AAGGATCATT ACTGTAAA.. ....CAAAGG
CBS334.66 TTCCGTAGGT cAACCTGCGG AAGGATCATT ATCGAATTCA G.....AAGG
4396      TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGAAGCTC G....AAGGG
4242      ttccgTAGGT GAAC.TGCGG AAGGATCATT ATCGAAAAAG ..........
```

[a]The small subunit (18S), ITS 1, 5.8S, ITS 2, and large subunit regions are identified by lines. Shaded regions indicate areas excluded from the phylogenetic analysis because they could not be confidently aligned.

```
          51                                                        100
2682      TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC AAGTGCACGC
2651      TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC AAgTGCACGC
RV9585    TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC AAGTGCACGC
KY5352    TCGCGGGCA. GTCGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
2349      TCGCGGGCA. GTCGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
5770      TCGCG.ACA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
8221      TCGCG.ACA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
DED7220   TCGCG.GCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
8335      TCGTG.GCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
7153      TCGTG.GCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGT
1615      TCGTG.ACA. GTGGCTGGCC CTCC..... .AGG....GC ATGTGCACGC
9670      TCGCG.GCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGT
7900      TCGCG.GCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGT
3979      TCGTGCGCC. GTCGCTGGCC CCTC..... .GGG....GC ATGTGCACGC
3905      TCGTGCGCC. GTCGCTGGCC CCTC..... .GGG....GC ATGTGCACGC
56667     TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
7263      TCGCGCGCA. GTCGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
9905      TCGCGCGCA. GTTGCTGGCC CCCC..... .GGG....GC AAGTGCACGC
3456      TCGCGCGCA. GTTGCTGGCC CCCC..... .GGG....GC AAGTGCACGC
7024      TCGCGCGCA. GTCGCTGGTC CTCC..... .GGG....GC ATGTGCACGC
2672      TCGCGCGCA. GTCGCTGGTC CTCC..... .GGG....GC ATGTGCACGC
7387      ACGTGCGCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
2637      TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
3922      TCGCGCGCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
GB1701    TCGCGTGCA. GTTGCTGGCC CTCT..... .GGG....GC ATGTGCACGC
DL8974    TCGCGTGCA. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
2679      TCGTGTGCT. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
2807      TCGTGTGCT. GTTGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
9433      ACGCGTGCC. GTCGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
11022     ACGTGCGCC. GTCGCTGGCC CTCC..... .GGG....GC ATGTGCACGC
9159      CCGAACGGTT GTCGCTGGTC CTCC..... .GGG....AC ATGTGCACAC
CBS334.66 CTT..GGGTT GTAGCTGGTC TTTC..... .GGGACAAG. ...TGCTCGC
4396      CCCT.AGGTT GTCGCTGGCT TATCCCCTTG CGGGACAAGC ATGTGCACGC
4242      CTTT.CGGTT GTTGCTGGCT CCCTC..TCG CAGGG.GGGC ATGTGCACAC
```

```
          101                                                        150
2682      CGT.GTCCG. .TATCCTTCT T........A CA..CCCC.T GTGCACCTTT
2651      CGT.GTCCG. .TATCCTTCT T........A CA..CCCC.T GTGCACCTTT
RV9585    CGC.GTCCG. .CATCCTTCT T........A CA..CCCC.T GTGCACCTTT
KY5352    CGC.TTCCG. .CATCCCCCC C..TCTTCCA CACCCCCC.T GTGCACCTTT
2349      CGC.TTCCG. .CATCCCCCC CCCTCTTCCA CACCCCCC.T GTGCACCTTT
5770      CGT.GGCCG. .CATCCTTCT TT.......A CA..CCCC.T GTGCACCTTT
8221      CGT.GGCCG. .CATCCTTCT TT.......A CA..CCCC.T GTGCACCTTT
DED7220   CCT.GGCTG. .CATCCTTCT TT.......A CA..CCCC.T GTGCACCTTT
8335      CGC.GGCCG. .CATCCTTCT T........A CA..CCCC.A GTGCACCTTT
7153      CGC.GGCCG. .CATCCTTCT T........A CA..CCCC.T GTGCACCTTT
1615      CAT.GGCCG. .CATCCTTCT T........A CA.CCCCC.T GTGCACCTTT
9670      CGC.GGCTG. .CATCCTTCT T......ACA CA.CCCCC.T GTGCACCTTT
7900      CGC.GGCTG. .CATCCTTCT T......ACA CA.CCCCC.T GTGCACCTTT
3979      CGC.GGCCG. .CATCCTTCT T........A CACCCCCC.T GTGCACCTTC
3905      CGC.GGCCG. .CATCCTTCT T........A CACCCCCC.T GTGCACCTTC
56667     CGC.TTCCG. .CATCCTCTT CT.......A ....CCCC.T GTGCACCTTT
7263      CGC.TTCCG. .CATCCTCTC TC.......A ....CCCC.T GTGCACCTTT
9905      CGC.GTCCG. .CATCCTTCT ........CA CA..CCCC.T GTGCACCTTT
3456      CGC.GTCCG. .CATCCTTCT CT......CA CA..CCCC.T GTGCACCTTT
7024      CGC.GTCCG. .CATCCTTCT C........A CA..CCCC.T GTGCACCTTT
2672      CGC.GTCCG. .CATCCTTCT C........A CA..CCCC.T GTGCACCTTT
7387      CGC.GTCCG. .CATCCTTCT C........A CA..CCCC.T GTGCACCTTT
2637      CGC.GTCCG. .CATCCTTCT CT........ CA..CCCC.T GTGCACCTTT
3922      CGC.GTCCG. .CATCCTTCT CT........ CA..CCCC.T GTGCACCTTT
GB1701    CGC.GTCCG. .CATCCTTCT C........A CA..CCCC.T GTGCACCTTT
DL8974    CGC.GTCCG. .CATCCTTCT C..... ..A CA..CCCC.T GTGCACCTTT
2679      CGC.GTCCG. .CATCCTTCT T........A CA..CCCC.T GTGCACCTTT
2807      CGC.GTCCG. .CATCCTTCT T........A CA..CCCC.T GTGCACCTTT
9433      CGT.GTCCG. .CATCCTTCT T........A CA..CCCC.T GTGCACCTTT
11022     CGC.GTCCG. .CATCCTTCT c........A CA..CCCC.T GTGCACCTTT
9159      CTTCGGTCG. ACATCCTTC. .........A CA..CCCC.T GTGCACCTCT
CBS334.66 CCTTGC.... ...TCCTTCC G........A CACACCC..T GTGCACTCCC
4396      CTCTGGTTCT CAATCCTTCA .........A CACACCCCTT GTGCACCTTC
4242      C...GATT.T CA.TCCTTC. .........A CACACCCCAT GTGCACCTTC
```

```
          151                                                    200
2682      GCGTGGGGC. .GCCGCT.GG CCTCTCT... .....GGCTT TCGGTGCGCC
2651      GCGTGGGGC. .GCCGCT.gG CCTCTCT... .....GGCTT TCGGTGCGCC
RV9585    GCGTGGGGC. .GCCGCT.GG CCTCTCT... .....GGCTT TCGGTGCGCC
KY5352    GCGTGGGGT. .GCCGTTGGG CTTCGTC... .....GCCTT TCGGTGCTCT
2349      GCGTGGGGT. .GCCGTTGGG CTTCGTC... .....GCCTT TCGGTGCTCT
5770      GCGTGGGGC. .GCCGTC.GG CCACG..... .....GCCTT T.GGTGCTCC
8221      GCGTGGGGC. .GCCGTC.GG CCACG..... .....GCCTT T.GGTGCTCC
DED7220   GCGTGGGGC. .GCCGAT.GG CTGCC..... .....GCCTT T.GGTGCTCC
8335      GCGTGGGGC. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTCC
7153      GCGTGGGGC. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTTC
1615      GCGTGGGGC. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTCC
9670      GCGTGGGGT. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTTC
7900      GCGTGGGGT. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTTC
3979      GCGTGGGGC. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTCC
3905      GCGTGGGGC. .GCCGTC.GG CCGCC..... .....GCCTT .CGGTGCTCC
56667     GCGTGGGGT. .GCCGCC.GG CTTTTCAA.. .....GCCTC .CGGTGCTCC
7263      GCGTGGGGC. .GCCGCT.GG CTTTTCTTTT TAAA.GCCTC .TGGTGCTCC
9905      GCGTGGGACC CGCCGCCT.G CTCGTAGCCT TTACTTGCTT TCGGTGTTCC
3456      GCGTGGGACC .GCCGCC.GG CTCGTAGCCT TTACTTGCTT TCGGTGT.CC
7024      GCGTGGGGT. .GCCGCT.GG CCCTTT.... .....GGCCT CCGGTGCTCT
2672      GCGTGGGGT. .GCCGCT.GG CCCTTT.... .....GGCCT CCGGTGCTCT
7387      GCGTGGGGT. .GCCGTTTGG CTTTT..... .....GCCCT TCGGTGCTCT
2637      GCGTGGGGC. .GCCGTTTGG CTTTTTTT.. .....GCCCT TCGGTGCTTC
3922      GCGTGGGGC. .GCCGTTTGG CTTTTTT... .....GCCCT TTGGTGCTCC
GB1701    GCGTGGGGT. .GCCGTC.GG CTTTGTT... .....GCCTT .CGGTGCTCT
DL8974    GCGTGGGGT. .GCCGTC.GG CTTTGTT... .....GCCTT .CGGTGCTCT
2679      GCGTGGGGC. .ACTGTT.GG CT..GTT... .....GCCTT .CGGTGCTCT
2807      GCGTGGGGC. .ACTGTT.GG CT..GTT... .....GCCTT .CGGTGCTCT
9433      GCGTGGGGT. .GCCGTC.GG CC..GCC... .....GCCTT .CGGTGCTCT
11022     GCGTAGGGT. .GCCGTC.GG CC..GCC... .....GCCTT .tGGTGCTCT
9159      GCGTGTGGTT CCCCTCCT.C GCTTCGGCT. .......TGT GGGGGGCGCC
CBS334.66 GCGTGGGCTG .......CGG TGA..... .......CTT CGGTTGCCGT
4396      GCGTGGCGGG GA......GG CCTTTCAA.. .....GCCTT TTCGTC....
4242      GCGTGGTTTG TACCTCTTTT ACC..... ........AG G.GGGAACAC
```

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          201                                                  250
2682      .GCGTCTTCT T.....ACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
2651      .GCGTCTTCT T.....ACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
RV9585    .GCGTCTTCt T.....ACAC ACGCC.AC.G TATGTCATCA GAATGTCT.A
KY5352    .GCGTCTCTT ACCGCGACAC ACGCC.AC.G CATGTCTTCA GAATGTCT.A
2349      .GCGTCTCTT ACCGCGACAC ACGCC.AC.G CATGTCTTCA GAATGTCT.A
5770      .GCGTTTTCT CT....ACAC ACACC.AC.G TATGTCTACA GAATGTCT.A
8221      .GCGTTTTCT CT....ACAC ACACC.AC.G TATGTCTACA GAATGTCT.A
DED7220   .GCGTTTTCT AC....ACAA ACGCC.AT.G TATGTCTACA GAATGTcT.A
8335      .GCGTTTTCT ATCT..ACAC ACACC.AC.G TATGTCTACA GAATGTCT.A
7153      .GCGTTTTCT ATCT..ACAC ACACC.AC.G TATGTCTACA GAATGTCT.A
1615      .GCGTTTTCT CT....ACAC ACACC.AC.G TATGTCTACA GAATGTCT.A
9670      .GCGTTTTCT CT....ACAC ACACC.AC.G TATGTCTACA GAATGTCTTA
7900      .GCGTTTTCT CT....ACAC ACACC.AC.G TATGTCTACA GAATGTCTTA
3979      .GCGTCTTCT TCACACACCC CCCCC.TC.G TATGTTTTCA GAATGTCT.A
3905      .GCGTCTTCT TCACACACCC CCCCC.TC.G TATGTTTTCA GAATGTCT.A
56667     .GCGTCT.CT T...ACACAC ATACC.AC.G CATGTCTTCA GAATGTCT.A
7263      .GCGTCTCTT ....ATACAC ATACC.AC.G CATGTCTTCA GAATGTCT.A
9905      CGCGTCTCTT T.....ACAC ACGCC.AC.G TATGTCTTCA GAATGTTT.A
3456      CGCGTCTCTT T.....ACAC ACGCC.AC.G TATGTCTTCA GAATGTTT.A
7024      .GCGTCTT.. ACCCACACAC ACACC.AC.G TATGTTTTCA GAATGTCT.A
2672      .GCGTCTT.. ACCCACACAC ACACC.AC.G TATGTTTTCA GAATGTCT.A
7387      .GCGTCTT.. ......ACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
2637      .GCGTTCTT. ......ACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
3922      .GCGTCTT.. ......ACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
GB1701    .GCGTCCTCT T.....ACAC ACACC.AC.G TATGTCTTCA GAATGTTT.A
DL8974    .GCGTCCTCT T.....ACAC ACACC.AC.G TATGTCTTCA GAATGTTT.A
2679      .GCGTCTTCT T...ATACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
2807      .GCGTCTTCT T...ATACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
9433      .GCGTCTTCT T...ACACAC ACACC.AC.G TATGTCTTCA GAATGTCT.A
11022     .GCGTCTTCT T...ACACAC ACGCC.AC.G TATGTCTTCA GAATGTCT.A
9159      CGCGTCTTCT T...ATAAAC A.CCCTT..G TATGTCTTAC GAATGTCT.A
CBS334.66 GCCCGCGATT TT..ATACA. ..CTCTTT.G TATGTCT.CA GAATGTCT..
4396      .GCGCTTTCT ....ACACAA A.CTCTAC.G CATGTCTACA GAATGTCT.A
4242      CGCGTTTTCT ....ACACA. ..CTCTTTTG TATGTCTTCA GAATGTCT.A
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	251			ITS1 | 5.8S		300
2682	C..TTGCGAT	AAAAAGCAAA	.TGTAATACA	ACTTTCAACA	ACGGATCTCT	
2651	C..TTGCGAT	AAAAAGCAAA	.TGTAATACA	ACTTTCAACA	ACGGATCTCT	
RV9585	C..TTGCGAT	AAAAAGCAAA	.TGTAATACA	ACTTTCAACA	ACGGATCTCT	
KY5352	...TTGCGAT	AAAAAGCAAA	.CAGAATACA	ACTTTCAACA	ACGGATCTCT	
2349	...TTGCGAT	AAAAAGCAAA	.CAGAATACA	ACTTTCAACA	ACGGATCTCT	
5770	C..TTGCGAT	CAAAAGCAAA	..TTAATACA	ACTTTCAACA	ACGGATCTCT	
8221	C..TTGCGAT	CAAAAGCAAA	..TTAATACA	ACTTTCAACA	ACGGATCTCT	
DED7220	C..TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
8335	C..TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
7153	C..TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
1615	C..TTGCGAT	GAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
9670	C..TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
7900	C..TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
3979	C..TTGCGAT	AAAAAGCAAA	.TCGAATACA	ACTTTCAACA	ACGGATCTCT	
3905	C..TTGCGAT	AAAAAGCAAA	.TCGAATACA	ACTTTCAACA	ACGGATCTCT	
56667	...TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
7263	...TTGCGAT	AAAAAGCAAA	.CTTAATACA	ACTTTCAACA	ACGGATCTCT	
9905	C..TTGCGAT	AAAAAGCAAA	ATCGAATACA	ACTTTCAACA	ACGGATCTCT	
3456	C..TTGCGAT	AAAAAGCAAA	ATCGAATACA	ACTTTCAACA	ACGGATCTCT	
7024	C..TTGCGAT	AAAAAGCAAA	.TCTAATACA	ACTTTCAACA	ACGGATCTCT	
2672	C..TTGCGAT	AAAAAGCAAA	.TCTAATACA	ACTTTCAACA	ACGGATCTCT	
7387	C..TTGCGAT	AAAAAGCAAA	.TTTAATACA	ACTTTCAACA	ACGGATCTCT	
2637	C..TTGCGAT	AAAAAGCAAA	.TTGAATACA	ACTTTCAACA	ACGGATCTCT	
3922	C..TTGCGAT	AAAAAGCAAA	.TTGAATACA	ACTTTCAACA	ACGGATCTCT	
GB1701	C..TTGCGAT	AAAAAGCAAC	.TATAATACA	ACTTTCAACA	ACGGATCTCT	
DL8974	C..TTGCGAT	AAAAAGCAAC	.TATAATACA	ACTTTCAACA	ACGGATCTCT	
2679	C..TTGCGAT	AAAAAGCAAA	.TTAAATACA	ACTTTCAACA	ACGGATCTCT	
2807	C..TTGCGAT	AAAAAGCAAA	.TTAAATACA	ACTTTCAACA	ACGGATCTCT	
9433	C..TTGCGAT	AAAAAGCAAA	.TTAAATACA	ACTTTCAACA	ACGGATCTCT	
11022	C..TTGCGAT	AAAAAGCAAA	.TTAAATACA	ACTTTCAACA	ACGGATCTCT	
9159	CTA.TGCGAT	AAAAAGCA..	.TCTAATACA	ACTTTCAACA	ACGGATCTCT	
CBS334.66	...TTGCGTG	TTGC.GCA..	.TCTAATACA	ACTTTCAACA	ACGGATCTCT	
4396	TTGTTGCGAT	GTAACGCA..	.TCTAATACA	ACTTTCAACA	ACGGATCTCT	
4242	TTGTTGCGAT	ACAACGCA..	.TCCAATACA	ACTTTCAACA	ACGGATCTCT	

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          301                                                        350
2682      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2651      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
RV9585    TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
KY5352    TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2349      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
5770      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
8221      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
DED7220   TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
8335      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
7153      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
1615      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
9670      TGGCTCTcgc atcgatgaag aacgcagcGA AATGCGATAA GTAATGTGAA
7900      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
3979      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
3905      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
56667     TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
7263      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
9905      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
3456      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
7024      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2672      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
7387      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2637      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
3922      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
GB1701    TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
DL8974    TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2679      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
2807      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
9433      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
11022     TGGCTCTcgc atcgatgaag aacgcagcga aatgcgataa gtAATGTGAA
9159      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
CBS334.66 TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
4396      TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
4242      TGGTTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA
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          351                                                        400
2682      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2651      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
RV9585    TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
KY5352    TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2349      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
5770      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
8221      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
DED7220   TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
8335      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
7153      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
1615      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
9670      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
7900      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
3979      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
3905      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
56667     TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
7263      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
9905      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
3456      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
7024      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2672      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
7387      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2637      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
3922      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
GB1701    TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
DL8974    TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2679      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
2807      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
9433      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
11022     TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
9159      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCACCCCTT
CBS334.66 TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
4396      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCCCCTT
4242      TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCTCCTT
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          401                                                5.8S | ITS2        450
2682      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTC.
2651      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTC.
RV9585    GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTC.
KY5352    GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TTC
2349      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TTC
5770      GGTATTCcGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCC
8221      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCC
DED7220   GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCT
8335      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTCT
7153      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTCT
1615      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCT
9670      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCC
7900      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCACC.TCC
3979      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TTC
3905      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TTC
56667     GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TCT
7263      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACC.TCT
9905      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTCC
3456      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCTCC
7024      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGATAAATT CTCACCCCC.
2672      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGATAAATT CTCACCCCC.
7387      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCCG
2637      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCCG
3922      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCCG
GB1701    GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCC.
DL8974    GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCC.
2679      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCC.
2807      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCC.
9433      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCC.
11022     GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAACTT CTCACCCCCC
9159      GGTATTCCGA GGGGTACGCC TGTTTGAGTG TCGTGAAATT CTCAACCCC.
CBS334.66 GGTATTCCGA GGGGCACACC TGTTTGAGTG TCGTGAATTT CTCAACTCC.
4396      GGTATTCCGA GGGGCACGCC TGTTTGAGTG TCGTGAAATT CTCAACCCT.
4242      GGTATTCCGA GGAGCACGCC TGTTTGAGTG TCGTGAAATT CTCAACCCTT
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          451                                                    500
2682      ACCTCCCTTT TC...GAAGG GTTGGGCT.. ...cGAGGCG TGGATG.TGG
2651      ACCTCCCTTT TC...GAAGG GTTGGGCT.. ...CGAGGCG TGGATG.TGG
RV9585    ACCTCCCTTT CC...GAAGG GTTGGGCT.. ...CGAGGTG TGGATG.TGG
KY5352    ACCCCCCTTT TC...GGAGG AGGGGTT... ...GG.GGCG TGGACGTTGG
2349      ACCCCCCTTT TC...GGAGG AGGGGTT... ...GG.GGCG TGGACGTTGG
5770      ATCCCTCTTT TTT.GGAAGG GT.GGGTT.. .......GCG TGGACG.TGG
8221      ATCCCTCTTT TTT.GGAAGG GT.GGGTT.. .......GCG TGGACG.TGG
DED7220   GCCttTCTTT TTT..GATGG GT.GGCT... .......GCG TGGATgTTGG
8335      ACCTTTTTTT TTTT.GAAGG GT.GGTT... .......GCG TGGACG.TGG
7153      ACCTTTCTTT TTT..GAAGG GT.GGTT... .......GCG TGGACG.TGG
1615      ACCTCTCTTT TT...GA.GG GT.GGCT... .......GCG TGGACG.TGG
9670      ACCTCTCTTT TT...GAAGG GT.GGTT... .......GGG TGGATG.TGG
7900      ACCTCTCTTT TT...GAAGG GT.GGTT... .......GGG TGGATG.TGG
3979      ACCCCTCTTT T....GAAGG GT.GGT.... .......GCG TGGACG.TGG
3905      ACCCCTCTTT T....GAAGG GT.GGT.... .......GCG TGGACG.TGG
56667     ATCCCC.TTT TC...GGAGG GC.GGT.... ...GG.GGCG TGGATG.TGG
7263      ATCCCC.TTT TC...GGAGG GC.GGT.... ...GG.GGCG TGGATG.TGG
9905      CCCTCCCTTT TC...GAAGG GGTGGGCTT. ...GGAGGTG TGGAC.TTGG
3456      CCCTCCCTTT TC...GAAGG GGTGGGCTT. ...GGAGGTG TGGAC.TTGG
7024      .ACCCCCTTT ATTTTGAAGG GTCGGTAT.. ...GG.GGCG TGGACGT.GG
2672      .ACCCCCTTT ATTTTGAAGG GTCGGTAT.. ...GG.GGCG TGGACGT.GG
7387      CACCTTTCTT TT...GAAGG GTCGATTT.. ...GG.GGCG TGGATGT.GG
2637      CACCTTTCTT TT...GAAGG GTCGATTC.. ...GG.GGCG TGGACGT.GG
3922      CACCTTTCTT TTT..GAAGG GTCGATTC.. ...GG.GGCG TGGATGT.GG
GB1701    .ATCCCCCTT TTC..GGAGG GCCGGCTTT. ...GG.GGTG TGGACGT.GG
DL8974    .ATCTC.CTT TTC..GGAGG GG....TT.. ...GG.GGTG TGGACGT.GG
2679      .ACCTC.CTT TTC..GAAGG TTAGGCTT.. ...GG.GGTG TGGACTTTGG
2807      .ACCTC.CTT TTC..GAAGG TTAGGCTT.. ...GG.GGTG TGGACTTTGG
9433      .GTTTC.CTT TTC..GAAGG GAT.....   ...GG.GGTG TGGACG.TGG
11022     .GTTTC.CTT TTC..GAAGG GAC.....   ...GG.GGCG TGGACG.TGG
9159      .GCCCCCTTT TGC..GAGGG GT......TG T.CGGTGGCT TGGAC.TTGG
CBS334.66 .GCCTCCTTT .GC..GGGAG .......... CGCGG.GGCT TGGAG.TTGG
4396      .GCCCCCTTT TGT..AAGAA A...G.GGTG TGCACGGGCT TGGAC.TTGG
4242      ..CCCCCTTT TAC..GAGGC GGGCTT.... ....GTGGAT TGGAC.TTGG
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          501                                                        550
2682      AGGCTTT..G CCGG.AA... ........TC ..GCCCCC.. .....tAGGT
2651      AGGCTTT..G CCGG.AA... ........TC ..GCCCCC.. .....TAGGT
RV9585    AGGCTTT..G CCGG.AA... ........TC ..GCCCCC.. .....GAGGC
KY5352    AGGTCTT..G CCGGAAA... ........CC C.ACCCCCTT ..........
2349      AGGTCTT..G CCGGAAA... ........CC C.ACCCCCTT ..........
5770      AGGCCTT..G CCGGGAA... ........AC C.AACCGCTT ..GC.GGGAA
8221      AGGCCTT..G CCGGGAA... ........AC C.AACCGCTT ..GC.GGGAA
DED7220   AGGGCTTT.G CCGGGCA... ........TC CTGACCGCTT ..Gc.GGgAG
8335      AGGCCTT..G CCGG.AA... ........AC C.AACCGCTT ..GC.GGGAG
7153      AGGCCTT..G CCGG.AA... ........AC C.AACTGCTT ..GC.GGGAG
1615      AGGCCTT..G CCGGGAG... ........AC C.GACCGCTT ..GC.GGGAG
9670      AGGCCTT..G CCGGGGA... ........AC CCGACCGCTT ..GC.GGGAG
7900      AGGCCTT..G CCGGGGA... ........AC CCGACCGCTT ..GC.GGGAG
3979      AGGGCTT..G CCGGAAGAAA T......CCA CC..TCGCTT .CGC.GTGGT
3905      AGGGCTT..G CCGGAAGAAA T......CCA CC..TCGCTT .CGC.GTGGT
56667     AGGTCTT..G CCGGAAA... ........CGC CT......CT .C...GAGGT
7263      AGGTCTT..G CCGGAAA... ........CGC CC......TT .C...GAGGC
9905      AGGCTTT..G CCGGGATTTC ACCCCCACCC CCGATCCCTT CCGC.AAGGA
3456      AGGCTTT..G CCGGGATTTC ACCCCCACCC CCGATCCCTT CCGC.AAGGA
7024      AGGCCTT..G TCGG.AATCT ........CCC CC......TT GTGT.ACGAG
2672      AGGCCTT..G TCGG.AATCT ........CCC CC......TT GTGT.ACGAG
7387      AGGCCTT..G CCGG.AATCT CT.....GCC GC......TT GT.C.GGGAG
2637      AGGCCTT..G CCGG.AATCT CT.....GCC GC......TT GT.C.GGGAG
3922      AGGCCTT..G CCGG.AATCT CT.....GCC GC......TT GT.C.GGGAG
GB1701    AGGCCTT..G CCGG.AACAA ACCC...... .....TCCTT ACCC.GAGGG
DL8974    AGGCCTT..G CCGG.AACAA AACCCC ... .....TCCTT ACCC.GAGGG
2679      AGGCTTTT.G CCGG.ATA.. .......... ....TCCCTT ACTTTGAGGG
2807      AGGCTTTT.G CCGG.ATA.. .......... ....TCCCTT ACTTTGAGGG
9433      AGGCCCTT.G CCGGGAGACT TACAATCCCC C......... ..........
11022     AGGCC.TT.G CCGGGAGACT TA.AATCCCC CCC....... ..........
9159      AGG.CTTTTG CCGGG.AAAG GGTTTCGA.C CCGCTC.... ..........
CBS334.66 AGG.CTT..G C.GGGCGT.. .......... AAG....... ..........
4396      AGGTCTTTTG CCGGCTTT.. .......... ........CG CTCTCGAGCA
4242      AGGTCTTTTG CCGGCGTTTT .ACT...... AAG....... ..........
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          551                                                        600
2682      GGt..... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
2651      GGC..... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
RV9585    GGT..... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
KY5352    .......... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
2349      .......... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
5770      GGGAA..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
8221      GGGAA..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
DED7220   GGAAA..... ..........  ......TCGG CTCCTCTGAA ATGCATCAGT
8335      GGAA...... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
7153      GGAA...... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
1615      GAAA...... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
9670      GGGAAA.... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
7900      GGGAAA.... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
3979      GGTAG..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
3905      GGTAG..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
56667     GAAG...... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
7263      AAAG...... ..........  ......TCGG CTCCTCTCAA ATGCATTAGT
9905      AAGGGCGAGG GTGTGCGCGC GTGTGTTCGG CTCTTCTCAA ATGCATTAGT
3456      AAGGGCGAGG GTGTGCGCGC GCGTGTTCGG CTCCTCTCAA ATGCATTAGT
7024      GG....AGAG GT........  .....TCCGG CTCCTCTGAA ATGCATTAGT
2672      GG....AGAG GT........  .....TCCGG CTCCTCTGAA ATGCATTAGT
7387      GGGA...... ..........  ......TCGG CTCCTCTGAA ATACATTAGT
2637      GGGGA..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
3922      GGGGA..... ..........  ......TCGG CTCCTCTGAA ATGCATTAGT
GB1701    GGGACG.... ..........  ......TCGG CTCCTCTGAA ACGCATTAGT
DL8974    G..ACG.... ..........  . ....TCGG CTCCTCTGAA ACGCATTAGT
2679      AAA....... ..........  ......TCGG CTCCTCTTAA ATGCATTAGC
2807      AAA....... ..........  ......TCGG CTCCTCTTAA ATGCATTAGC
9433      .......... ..........  ........GG CTCCTCTCAA ATGCATCAGT
11022     .......... ..........  ........GG CTCCTCTCAA ATGCATTAGC
9159      .......... ..........  ......TCGG CTCCTCTCGA AGGCATTAGT
CBS334.66 .......... ..........  ......TCCG CTCCTCTCAA ATGCATTAGT
4396      AAGG...... ..........  ......TCGG CTCCTCTCAA AGGTATTAGC
4242      .......... ..........  ......TCGG CTCCTCTTAA ATGTATTAGT
```

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          601                                                    650
2682      GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
2651      GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
RV9585    GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
KY5352    GGCGACCTCC CCAGCGTTG. ....GCCTTC GGTGTGATAA TCGTCTACGC
2349      GGCGACCTCC CCAGCGTTG. ....GCCTTC GGTGTGATAA TCGTCTACGC
5770      GGAGA..CCC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTcTACGC
8221      GGAGA..CCC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
DED7220   GGGACA..CC CCAGTGTTG. ....ACCTTC GGTGTGATAA TTGTCTATGC
8335      GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
7153      GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
1615      GGAGA..CCC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
9670      GGAGA..CCC CCAGCGTTG. ....GCCTTT GGTGTGATAA TTGTCTACGC
7900      GGAGA..CCC CCAGCGTTG. ....GCCTTT GGTGTGATAA TTGTCTACGC
3979      GGAGA...CC CCAGCGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
3905      GGAGA...CC CCAGCGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
56667     GGAGA...CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
7263      GGAGA...CC CCAGCGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
9905      GGAAGGACCC CCAGCGTCG. ....GCCTTC GGTGTGATAA TTGTCTACAC
3456      GGAAGGACCC CCAGCGTCG. ....GCCTTT GGTGTGATAA TTGTCTACAC
7024      GGAAGACCCC CCAGCGTCG. ....GCCTTC GGTGTGATAA TTGTCTACGC
2672      GGAAGACCCC CCAGCGTCG. ....GCCTTC GGTGTGATAA TTGTCTACGC
7387      GGA.GA..CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
2637      GGA.GA..CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
3922      GGA.GA..CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
GB1701    GGA.GA..CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
DL8974    GGA.GA..CC CCAGTGTTG. ....GCCTTC GGTGTGATAA TTGTCTACGC
2679      AGA.GACCCC CCAGTGTTG. ....GCCTTT GGTGTGATAA TTGTCTACGC
2807      AGA.GACCCC CCAGTGTTG. ....GCCTTT GGTGTGATAA TTGTCTACGC
9433      GGA.GA..CC CCAGTGTTG. ....GCCTTT GGTGTGATAG TTGTCTACGC
11022     GGA.GA..CC CCAGTGCTG. ....GCCTTT GGTGTGATAA TTGTCTACGC
9159      AGGA....CC CTT.TGC.G. ....GCCT.C GGTGTGATAA TTGTCTACGC
CBS334.66 GAAG.....C CCAG...TG. ....GCCT.C GGCGTGATAA TTGTCTACGT
4396      AGGGGAAACC CC....TTC. ....GCCATC GGTGTGATAA TTATCTACGC
4242      AG..GA.CCT TCAG..TTGA AGAAGCC.TC GGTGTGATAA TTATCTACGC
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          651                                                        700
2682      CGCTGGG..C CGCACGT..C GTGG.GA..C CTGCTT.CTA ACCT....GC
2651      CGCTGGG..C CGCACGT..C GTGG.GA..C CTGCTT.CTA ACCT....GC
RV9585    CGCTGGG..A CACGCGT..C GCGG.GA.CC C.GCTT.CTA ACCT....GC
KY5352    CGTCGGGGCT TACGCG..CA GTGGAGA..C CTGCTT.CAA AC.TC...GC
2349      CGTCGGGGCT TACGCG..CA GTGGAGA..C CTGCTT.CAA AC.TC...GC
5770      CGTGGGG.TT CACGCGGAGT GGGG.GA..C CTGCTT.CCA ACCTTT..GC
8221      CGTGGGG.TT CACGCGGAGT GGGG.GA..C CTGCTT.CCA ACCTTT..GC
DED7220   CGTGGGGgGT CAtAGAGAGC Ga...GA..c ctgccT.Cta accTTTT.GC
8335      CGTGGGG..T TGCGCAAA.. GTGG.GA..C CTGCTT.CTA ACCTTT..GC
7153      CGTGGGG..T TGCGCAAA.. GTGG.GA..C CTGCTT.CTA ACCTTT..GC
1615      CGTGGGG..C TGCGCAAA.. GTGG.GA..C CTGCTT.CTA ACCTC...GT
9670      CGTGGGG..T TGCGCGAAGC GGGG.GA.CC CTGCTT.CTA ACCTT...GT
7900      CGTGGGG..T TGCGCGAAGC GGGG.GA.CC CTGCTT.CTA ACCTT...GT
3979      CGACGGG..T TGCGCG..TC GTGG.GA.CC CTGCTT.CCA ACCTC...GC
3905      CGACGGG..T TGCGCG..TC GTGG.GA.CC CTGCTT.CCA ACCTC...GC
56667     CGTTGGG..C TGCGCG..TC GCGG.GA.CC C.GCTT.CGA AC.TT...GC
7263      CGTCGGG..T TGCGCG..TC GCGG.GA..C CTGCTT.CGA AC.TT...GC
9905      CGTCGGGG.C CACGCTTTGA GTGGGGAACC C.GCTTTCTA ACC.C...GC
3456      CGTCGGGG.C CACGCTTTGA GTGGGGAACC C.GCTTTCTA ACC.C...GC
7024      CGTTGGG..T CACGCGT..C GTGG.GACAC C.GCTT.CCA ACCCTTT.GT
2672      CGTTGGG..T CACGCGT..C GTGG.GACAC C.GCTT.CCA ACCCTTT.GT
7387      TGTTGTG..C CACACGT..C GTGG.GA..C CTGCTT.CTA ACCC....GT
2637      TGTTGTG..C CGCACGT..C GTGG.GA..C CTGCTT.CTA ACCC....GT
3922      TGTTGTG..C CGCACGT..C GTGG.GA..C CTGCTT.CTA ACCC....GT
GB1701    CGTTTGGG.C TACGCGT..C GCGG.GA.CC C.GCTT.CCA A.CCT...GT
DL8974    CGTTGGG..C TACGCGT..C GCGG.GA..C CTGCTT.CCA A.CCT...GT
2679      CGTTGAG..C TGCACGTT.. GTGG.GA..C CTGCTT..CA AACCT...GT
2807      CGTTGAG..C TGCACGTT.. GTGG.GA..C CTGCTT..CA AACCT...GT
9433      CATTGGGT.C TGCGCGTT.. GTGG.GA..C CTGCTT.CCA A.CCT...GT
11022     CATTGGGT.C CGCGCATC.. GTGG.GA..C CTGCTT.CCA A.CCTT..G.
9159      CGT.GGGTTT AGCATGTCAT G.GG.GA.CC C.GCTT.CCA A.CC......
CBS334.66 CCAAGG..TT .GTCTGTA.. GCGG....TT TCGCTT.CTA A.CC......
4396      CGTCGGCCCT CGGTGGTGAA CG.ACCCG.. ..GCTT.CGA A.CC......
4242      C...GGC..T CGTGTGCTAT ATTCACTGTA .TG.TT..GA A.CCTGCTTC
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           701                                                        750
2682       CGTTGTGCCT CCC...CTTC CTTgggggGg T.gGggGC.. ....GTGTAC
2651       CGTTGTGCCC CCC..TCCTC CTCGCGAGGT T.GGCGGC.. ....GTGTAC
RV9585     CGTCGCGCCG CGCCCTCCTC CTCGCGAGGT T.CGTGC... GTGTGTGTAC
KY5352     CGCCACACGT GTTTTCCTTC T.....GGGA ...TGC.GT. GCATGTGGG.
2349       CGCCACACGT GTTTTCCTTC T.....GGGA ...TGCAGT. GCATGTGGG.
5770       C.......GC CT.TTCG... ......AGGT ..GTGCAA.. ..........
8221       C.......GC CT.TTCG... ......AGGT ..GTGCAA.. ..........
DED7220    T.......GC cTCTTCG... ......AGGT ..CTGCAA.. ..........
8335       C.......AC CT.TTCG... ......AGGT ..GTGCA... ..........
7153       C.......AC CT.TTCG... ......AGGT ..GTGCA... ..........
1615       TGTGCT..GC CT.TTCG... ......AGGT ..GTGCAA.. ..........
9670       CGTGCTT.GC CT.TTCG... ......AGGC ..TTGCCC.. ..........
7900       CGTGCTT.GC CT.TTCG... ......AGGC ..TTGCCC.. ..........
3979       C.TGC...GC CT.TTCG... .TT...AGGC ..GTGCGG.. ..........
3905       C.TGC...GC CT.TTCG... .TT...AGGC ..GTGCGG.. ..........
56667      CGTCGT..GT CC.TTC.... .TT...GGAc ..GCGCGCA. ..........
7263       CGTCGC..GT CC.TTC.... .TC...GGAC ..GCGCGCA. ..........
9905       CGTC.GCCGT GAGCCACCCT CTTGCGAGGG TCGTGCGTTG CGCGCCGTAC
3456       CGTC.GCCGT GAGCCACCCT CTTGCGAGGG TCGTGCGTTG CGCGCCGTAC
7024       CGTTCGTC.. ..GCCTTCA. ......AGG. T.GTGCGGAC GGCAT.....
2672       CGTTCGTC.. ..GCCTTCA. ......AGG. T.GTGCGGAC GGCAT.....
7387       CGTT.GTC.. ..GCCTTTTA .CT...AGG. ...TG.GCAC .GCA......
2637       CGTC.GTCGC T.GCCTTTTG .TT...AGG. ...TG.GCAC .GCA......
3922       CGTC.GCC.. ..GCCTTTTG .TT...AGG. ...TG.GCAC .GCA......
GB1701     TGTC.GTCGT C.TTC..... ......GGAA ......GGC. .GCA......
DL8974     TGTC.GTCAT C.TTC..... ......CGA. ......GGGC .GCG......
2679       .GTC.GCCAT T.GCC..... ...GAGAGGC ATATACATGT .GCGC.....
2807       .GTC.GCCAT T.GCC..... ...GAGAGGC ATATACATGT .GCGC.....
9433       .GTC.GTGCT T......... ......... .........A .GCGC.....
11022      CGTC.GTGCT T......... ......... .........A TGTGC.....
9159       .....GTCTC GC........ ...AAGGGAC A......... ..........
CBS334.66  .....GTCCT TC........ .....GGGA. ..........  ..........
4396       .....GTCTC GC........ ...AAGGGAC AAA.......  ..........
4242       TAACCGTCTC GC........ ...AAGGGAC AAA.......  ..........
```

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          751          ITS2 | LSU                                      800
2682      GTGCTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
2651      GTGCTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
RV9585    GTGCATTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
KY5352    ...CTTTG.A TCAAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
2349      ...CTTTG.A TCAAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
5770      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
8221      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
DED7220   ...CTTTT.A TTGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
8335      ...CTTTC.A TTGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
7153      ...CTTTC.A TTGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
1615      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
9670      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGTGGGACT ACCCGCTGAA
7900      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGTGGGACT ACCCGCTGAA
3979      ...CTTCT.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
3905      ...CTTCT.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
56667     ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
7263      ...CCTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
9905      GTGCTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
3456      GTGCTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
7024      ...CATTC.A TCGAAAGTGG GCCTCAAATC AGGCGGGACT ACCCGCTGAA
2672      ...CATTC.A TCGAAAGTGG GCCTCAAATC AGGCGGGACT ACCCGCTGAA
7387      ...cTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
2637      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
3922      ...CTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
GB1701    ACACTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
DL8974    ACACTTTC.A TCGAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
2679      ..ACGTTT.A TCAAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
2807      ..ACGTTT.A TCAAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
9433      GCACGTTT.A TCAAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
11022     GCGCGTTT.A TCaAAACTTG ACCTCAAATC AGGCGGGACT ACCCGCTGAA
9159      ...CTTTCAA TCGAAACTTG ACCTCAGATC AGGCGGGACT ACCCGCTGAA
CBS334.66 ...CATTC.A TCGAAACTTG ACCTCAGATC AGGTGGGACT ACCCGCTGAA
4396      ..TTTTTAAA TCGAACCTTG ACCTCAGATC AGGCGGGACT ACCCGCTGAA
4242      .TTAAATT.A TCGAACCTTG ACCTCAGATC AGGCGGGACT ACCCGCTGAA
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```
            801
2682        CTTAA
2651        CTTAA
RV9585      CTTAA
KY5352      CTTAA
2349        CTTAA
5770        CTTAA
8221        CTTAA
DED7220     CTTAA
8335        CTTAA
7153        CTTAA
1615        CTTAA
9670        CTTAA
7900        CTTAA
3979        CTTAA
3905        CTTAA
56667       CTTAA
7263        CTTAA
9905        CTTAA
3456        CTTAA
7024        CTTAA
2672        CTTAA
7387        CTTAA
2637        CTTAA
3922        CTTAA
GB1701      CTTAA
DL8974      CTTAA
2679        CTTAA
2807        CTTAA
9433        CTTAA
11022       CTTAA
9159        CTTAA
CBS334.66   CTTAA
4396        CTTAA
4242        CTTAA
```

APPENDIX B

SEQUENCE ALIGNMENT OF THE nrDNA ITS REGION FOR *ARTOMYCES PYXIDATUS* PHYLOGENY

Appendix B – Alignment of the nrDNA ITS region for *Artomyces pyxidatus* phylogeny.[a]

```
          1                                    SSU | ITS1                50
ky5352    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGCAATGC CAAGGTCGCG
  2349    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAATGC CAAGGTCGCG
  7263    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  1541    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  8927    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  8903    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  8723    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  1513    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
 56667    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  5484    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  4938    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
 h7524    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  9709    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG
  9932    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAAAC CAAGGTCGCG

          51                                                    100
ky5352    GGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCCCCCC
  2349    GGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCCCCCC
  7263    CGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  1541    CGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  8927    CGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  8903    CGCAGTCGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  8723    TGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  1513    CGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
 56667    CGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  5484    TGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  4938    CGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
 h7524    TGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  9709    TGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
  9932    TGCAGTTGCT GGCCCTCCGG GGCATGTGCA CGCCGCTTCC GCATCC....
```

[a]The small subunit (18S), ITS 1, 5.8S, ITS 2, and large subunit regions are identified by lines. For analysis, all gaps were condensed to on base position.

```
        101                                                        150
ky5352  C..TCTTCCA CACCCCCCTG TGCACCTTTG CGTGGGGTGC CGTTGGGCTT
  2349  CCCTCTTCCA CACCCCCCTG TGCACCTTTG CGTGGGGTGC CGTTGGGCTT
  7263  ...TCTCTC. .ACCCC..TG TGCACCTTTG CGTGGGGCGC CGCTGG.CTT
  1541  ...TCTCTC. .ACCCC..TG TGCACCTTTG CGTGGGGCGC CGCTGG.CTT
  8927  ...TCTCTC. .ACCCC..TG TGCACCTTTG CGTGGGGCGC CGCTGG.CTT
  8903  ...TCTCTC. .ACCCC..TG TGCACCTTTG CGTGGGGCGC CGCTGG.CTT
  8723  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCTGG.CTT
  1513  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCCGG.CTT
 56667  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCCGG.CTT
  5484  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCTGG.CTT
  4938  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCCGG.CTT
 h7524  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCTGG.CTT
  9709  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCTGG.CTT
  9932  ...TCTTCT. .ACCCC..TG TGCACCTTTG CGTGGGGTGC CGCTGG.CTT

        151                                                        200
ky5352  ..C.....GT CGCCTTTCGG TGCTCTGCGT CTCTTACCGC GACACACGCC
  2349  ..C.....GT CGCCTTTCGG TGCTCTGCGT CTCTTACCGC GACACACGCC
  7263  TTCTTTTTAA AGCCTCT.GG TGCTCCGCGT CTCTTAT... .ACACATACC
  1541  TTTTTTTTAA AGCCTCT.GG TGCTCCGCGT CTCTTAT... .ACACATACC
  8927  TTTTTTT.AA AGCCTCT.GG TGCTCCGCGT CTCTTAT... .ACACATACC
  8903  TTCTTTTTAA AGCCTCT.GG TGCTCCGCGT CTCTTAT... .ACACATACC
  8723  TTC......A AGCCTC..GG TGCTCCGCGT CTCTTAC... .ACACATACC
  1513  TTC......A AGCCTCC.GG TGCTCCGCGT CTCTTAC... .ACACgTACC
 56667  TTC......A AGCCTCC.GG TGCTCCGCGT CTCTTAC... .ACACATACC
  5484  TTC......A AGCCTC..GG TGCTCCGCGT CTCTTAC... .ACACATACC
  4938  TTC......A AGCCTCC.GG TGCTCCGCGT CTCTTAC... .ACACATACC
 h7524  TTC......A AGCCTC..GG TGCTCCGCGT CTCTTAC... .ACACATACC
  9709  TTC......A AGCCTC..GG TGCTCCGCGT CTCTTAC... .ACACATACC
  9932  TTC......A AGCCTC..GG TGCTCCGCGT CTCTTAC... .ACACATACC

        201                                                        250
ky5352  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  2349  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  7263  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  1541  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  8927  ACGCATGTCT TCAGAATGTC TATTGCGAt. ......... ......AAAA
  8903  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  8723  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  1513  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
 56667  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  5484  ACGCATGTCT TCAGAATGTC TATTGCGATA GAATGTCTAT TGCGATAAAA
  4938  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
 h7524  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  9709  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
  9932  ACGCATGTCT TCAGAATGTC TATTGCGAT. ......... ......AAAA
```

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          251      ITS1 | 5.8s                                        300
ky5352    AGCAAACAGA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  2349    AGCAAACAGA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  7263    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  1541    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  8927    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  8903    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  8723    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  1513    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
 56667    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  5484    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  4938    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
 h7524    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  9709    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA
  9932    AGCAAACTTA ATACAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA

          301                                                  350
ky5352    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  2349    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  7263    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  1541    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  8927    TGAAGAACGC AGCGAAATGC GATAAGTaAT GTGAATTGCA GAATTCAGTG
  8903    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  8723    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  1513    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
 56667    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  5484    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  4938    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
 h7524    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  9709    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
  9932    TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG

          351                                                  400
ky5352    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  2349    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  7263    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  1541    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  8927    AATCaTCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  8903    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  8723    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  1513    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
 56667    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  5484    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  4938    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
 h7524    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  9709    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
  9932    AATCATCGAA TCTTTGAACG CACCTTGCGC CCCTTGGTAT TCCGAGGGGC
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          401                5.8s | ITS2                          450
ky5352    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTTCACCCCC CTTTTCGGAG
  2349    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTTCACCCCC CTTTTCGGAG
  7263    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
  1541    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
  8927    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
  8903    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
  8723    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTTGGAG
  1513    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
 56667    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
  5484    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTTGGAG
  4938    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTCGGAG
 h7524    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTTGGAG
  9709    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTTGGAG
  9932    ACGCCTGTTT GAGTGTCGTG AACTTCTCAC CTCTATCCCC .TTTTTGGAG

          451                                                  500
ky5352    GAGGGGTTGG GGCGTGGACG TTGGAGGTCT TGCCGGAAAC CCACCCCCTT
  2349    GAGGGGTTGG GGCGTGGACG TTGGAGGTCT TGCCGGAAAC CCACCCCCTT
  7263    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCCTT
  1541    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCCTT
  8927    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCCTT
  8903    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCCTT
  8723    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
  1513    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
 56667    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
  5484    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
  4938    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .TG..CCTCT
 h7524    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
  9709    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT
  9932    GGCGG..TGG GGCGTGGATG T.GGAGGTCT TGCCGGAAA. .CG..CCTCT

          501                                                  550
ky5352    .......... TCGGCTCCTC TCAAATGCAT TAGTGGCGAC CTCCCCAGCG
  2349    .......... TCGGCTCCTC TCAAATGCAT TAGTGGCGAC CTCCCCAGCG
  7263    CGAGGCAAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGCG
  1541    CGAGGCGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGCG
  8927    CGAGGCAAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGCG
  8903    CGAGGCAAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGCG
  8723    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
  1513    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
 56667    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
  5484    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
  4938    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
 h7524    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
  9709    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
  9932    CGAGGTGAAG TCGGCTCCTC TCAAATGCAT TAGTGGAGA. ..CCCCAGTG
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          551                                                        600
ky5352    TTGGCCTTCG GTGTGATAAT CGTCTACGCC GTCGGGGCTT ACGCGCAGTG
  2349    TTGGCCTTCG GTGTGATAAT CGTCTACGCC GTCGGGGCTT ACGCGCAGTG
  7263    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTCGGG..TT GCGCGTCGCG
  1541    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTCGGG..TT GCGCGTCGCG
  8927    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTCGGG..TT GCGCGTCGCG
  8903    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTCGGG..TT GCGCGTCGCG
  8723    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
  1513    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
 56667    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
  5484    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
  4938    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGcG
 h7524    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
  9709    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG
  9932    TTGGCCTTCG GTGTGATAAT TGTCTACGCC GTTGGG..CT GCGCGTCGCG

          601                                                        650
ky5352    GAGACCTGCT TCAAACTCGC CGCCACACGT GTTTTCCTTC TGGGATGC.G
  2349    GAGACCTGCT TCAAACTCGC CGCCACACGT GTTTTCCTTC TGGGATGCAG
  7263    G.GACCTGCT TCGAACTTGC CGTCGCGT.. .....CCTTC TCGGACGC.G
  1541    G.GACCTGCT TCGAACTTGC CGTCGCGT.. .....CCTTC TTGGACGC.G
  8927    G.GACCTGCT TCGAACTTGC CGTCGCGT.. .....CCTTC TCGGACGC.G
  8903    G.GACCTGCT TCGAACTTGC CGTCGCGT.. .....CCTTC TCGGACGC.G
  8723    G.GACCCGCT TCAAACTTGC CGTCGTGT.. .....CCTTC TTGGACGC.G
  1513    G.GACCCGCT TCGAACTTGC CGTCGTGT.. .....CCTTC TTGGACGC.G
 56667    G.GACCCGCT TCGAACTTGC CGTCGTGT.. .....CCTTC TTGGAcGC.G
  5484    G.GACCCGCT TCAAACTTGC CGTCGTGT . .....CCTTC TTGGACGC.G
  4938    G.GACCCGCT TCaAACTTGC CGTCGTGT.. .....CCTTC TTGGAGGC.G
 h7524    G.GACCCGCT TCAAACTTGC CGTCGTGT.. .....CCTTC TTGGACGC.G
  9709    G.GACCCGCT TCAAACTTGC CGTCGTGT.. .....CCTTC TTGGACGC.G
  9932    G.GACCCGCT TCAAACTTGC CGTCGTGT.. .....CCTTC TTGGACGC.G

          651                    ITS2 | LSU                          700
ky5352    TGC..ATGTG GGCTTTGATC AAAACTTGAC CTCAAATCAG GCGGGACTAC
  2349    TGC..ATGTG GGCTTTGATC AAAACTTGAC CTCAAATCAG GCGGGACTAC
  7263    CGC..A.... ..CCTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  1541    CGC..A.... ..CCTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  8927    CGCGCA.... ..CCTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  8903    CGC..A.... ..CCTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  8723    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  1513    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
 56667    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  5484    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  4938    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
 h7524    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  9709    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
  9932    CGC..A.... ..CTTTCATC GAAACTTGAC CTCAAATCAG GCGGGACTAC
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          701
ky5352    CCGC
  2349    CCGC
  7263    CCGC
  1541    CCGC
  8927    CCGC
  8903    CCGC
  8723    CCGC
  1513    CCGC
 56667    CCGC
  5484    CCGC
  4938    CCGC
 h7524    CCGC
  9709    CCGC
  9932    CCGC
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APPENDIX C

SEQUENCE ALIGNMENTS OF THE 18S GROUP I INTRON AND nrDNA ITS

Appendix C, Part 1. 18S Group I intron[a].

```
          1                              SSU | intron                    50
1513      ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CGC..AAC A..GCTT.AG
56667     ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CGC..AAC A..GCTT.AG
H7524     ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CGC..AAC A..GCTT.AG
GB1702    ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CGC..AAC A..GCTT.AG
1541      ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CGC..AAC A..GCTT.AG
8335      ATTGACGGAA GGGCACCACC AGGAGTAAA. ..CG.TTCAC A..GCT..AG
4101      ATTGACGGAA GGGCACCACC AGGAGTAAAC AACGCT.AAT A..GCCTAAG
8685      ATTGACGGAA GGGCACCACC AGGAGTAAAC AACGCT.AAT A..GCCTAAG
9159      ATTGACGGAA GGGCACCACC AGGAGTAAAC .ACGCT.AAC AA.GCCT.AG
OKM7      ATTGACGGAA GGGCACCACC AGGAGTAAAC .ACGCT.AAC AA.GCCT.AG
6701      ATTGACGGAA GGGCACCACC AGGAGTAAAC .ACGCT.AAC AA.GCCT.AG
9981      ATTGACGGAA GGGCACCACC AGGAGTAAAC .ACGCT.AAC AA.GCCT.AG
9985      ATTGACGGAA GGGCACCACC AGGAGTAAAC ..CGC..AAC A..GCTTAAG
4396      ATTGACGGAA GGGCACCACC AGGAGTAAAC CAAGCTTCAC AACGCTTTAG

          51                                                      100
1513      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTTA
56667     CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTTA
H7524     CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTTA
GB1702    CCGCAGTGGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTT.A
1541      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTT.A
8335      CCGCAGTAGC TCTGCTCCGA ATAGCAGCCC GAAAGGGTGA GGTGGTTT..
4101      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTC.
8685      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTC.
9159      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCT GAAAGGGTGA GGTGGTTTC.
OKM7      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCT GAAAGGGTGA GGTGGTTTC.
6701      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCT GAAAGGGTGA GGTGGTTTC.
9981      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGTTTC.
9985      CCGCAGTCGC TCTGCTCCGA ACAGCAGCCT GAAAGGGTGA GGTGGT....
4396      CCGCAGTAGC TCTGCTCCGA ACAGCAGCCC GAAAGGGTGA GGTGGGTTC.
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[a]Intron is inserted at base 1165 of yeast (Rubstov et al. 1980). Regions P, Q, R, and S are indicated by shading, and the position of the secondary insert is identified.

```
          101                                                        150
1513      AGCCTTAAAG CG.AAATGCT AGTCTCCCTC GTGGG..GGG CGACACCTTC
56667     AGCCTTAAAG CG.AAATGCT AGTCTCCCTC GTGGG..GGG CGACACCTTC
H7524     AGCCTTAAAG CG.AAATGCT AGTCTCCCTC GTGGG..GGG CGACACCTTC
GB1702    AGCCTTAAAG CG.AAATGCT AGTCTCCCTC GTGGG..GGG CGACACCTTC
1541      AGCCTTAAAG CG.AAATGCT AGTCCCCCTC G.GGG..GGG CGACACCTTC
8335      .GCCTTAAAT GT.AAATGCT AGTCCTTTCC TCGCGGGGGG CGACACCTTC
4101      AGCCCCAAAT CT.AAATGCT AGTCTGCCTT C.GGGT.GGG CGACACCTTC
8685      AGCCCCAAAT CT.AAATGCT AGTCTGCCTT C.GGGT.GGG CGACACCTTC
9159      AGCCCCAAAT CT.AAATGCT AGTCCGCCTC C.GGGT.GGG CGACACCTTC
OKM7      AGCCCCAAAT CT.AAATGCT AGTCCGCCTC C.GGGT.GGG CGACACCTTC
6701      AGCCCCAAAT CT.AAATGCT AGTCTGCCTT C.GGGT.GGG CGACACCTTC
9981      AGCCCTGAAT CT.AAATGCT AGTCTGCCTT C.GGGT.GGG CGACACCTTC
9985      GGCCTTGAAC GTTAAATGCT AGTCCCTCTT C.GGAG.GGG CGACACCTTC
4396      .GCCT.AAAT .T.AAATGCT AGTCC...TT GTGAGA.GGG CGACACCTTC

          151    P                                                   200
1513      AAATTGACGG GGAACTCCTA AAGCTCAGAG CACCAAGCTT CGT..TCCGA
56667     AAATTGACGG GGAACTCCTA AAGCTCAGAG CACCAAGCTT CGT..TCCGA
H7524     AAATTGACGG GGAACTCCTA AAGCTCAGAG CACCAAGCTT CGT..TCCGA
GB1702    AAATTGACGG GGAACTCCTA AAGCTCAGAG CACCAAGCTT CGT..TCCGA
1541      AAATTGACGG GGAACTCCTA AAGCTCAGAG CACCAAGCTT CGT..TCCGA
8335      AAATTGTCGG GGAACTCCTA AAGCTCAGAA CACCAAGCTC CGT..TCCGA
4101      AAATTGACGG GGAACTCCTA AAGCTCAGTA CACCAAGCCT CATA.TCCGA
8685      AAATTGACGG GGAACTCCTA AAGCTCAGTA CACCAAGCCT CATA.TCCGA
9159      AAATTGACGG GGAACTCCTA AAGCTCGGTG CACCAAGCCC CATAATCCGA
OKM7      AAATTGACGG GGAACTCCTA AAGCTCGGTG CACCAAGCCC CATAATCCGA
6701      AAATTGACGG GGAACTCCTA AAGCTCGGTG CACCAAGCCC CATGATCCGA
9981      AAATTGACGG GGAACTCCTA AAGCTCAGTG CACCAAGCCC CAT.ATCCGA
9985      AAATTGACGG GGAACTCCTA AAGCTCAGGA CACCAAGCTC CGT..TCCGA
4396      AAATTGTCGG GGAACTCCTA AAGCTCAGAA CACCAAGCTT CGT..TCCGA

          201                                                        250
1513      AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACTCTCG
56667     AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACTCTCG
H7524     AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACTCTCG
GB1702    AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACTCTCG
1541      AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACTCTTG
8335      AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTATGGTAA AAG.CTTCTG
4101      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTATGGTAA AAGAGA.CTG
8685      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTATGGTAA AAGAGA.CTG
9159      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGCCA.CTG
OKM7      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGCCA.CTG
6701      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACA.CTG
9981      AAGGGTGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAGACA.CTG
9985      AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTATGGTAA AAGAGT.CTG
4396      AAGGGCGGGG TGGCCAGGTT AATAGCCTCG GGTACGGTAA AAG.CTTCTG
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          251                      Q                                300
1513      AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGGC TTATGGGACA
56667     AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGGC TTATGGGACA
H7524     AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGGC TTATGGGACA
GB1702    AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGGC TTACGGGACA
1541      AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGGC TTACGGGACA
8335      AGATGTTACA ATGGACAATC CGCAGCCAAG GCCCTAAGCC G.ATGGGACA
4101      AGATGTCACA ATGGACGATC CGCAGCCAAG GCCCTACGTT GTATGGGACA
8685      AGATGTAACA ATGGACGATC CGCAGCCAAG GCCCTACGTT GTATGGGACA
9159      AGATGTTACA ATGGACAATC CGCAGCCAAG GCCCTACGTC GTACGGGACA
OKM7      AGATGTTACA ATGGACAATC CGCAGCCAAG GCCCTACGTC GTACGGGACA
6701      AGATGTTACA ATGGACAATC CGCAGCCAAG GCCCTACGTC GTACGGGACA
9981      AGATGTTACA ATGGACGATC CGCAGCCAAG GCCCTACGTC GTACGGGACA
9985      AGATGTTACA ATGGATGATC CGCAGCCAAG GCCCTAAGTC TTACGGGACA
4396      AGATGTAACA ATGGACGATC CGCAGCCAAG GCCCTACGTC GTACGGGACA

                                                  2° insert
          301    R                                              S  350
1513      GGTTCAGAGA CTAGATGGAG GTGGGTCATT GTATGGCTTA AGGTATAGTC
56667     GGTTCAGAGA CTAGATGGAG GTGGGTCATT GTaTGGCTTA AGGTATAGTC
H7524     GGTTCAGAGA CTAGATGGAG GTGGGTCATT GTATGGCTTA AGGTATAGTC
GB1702    GGTTCAGAGA CTAGATGGAG GTGGGTCGTC GCGCGGCTTA AGGTATAGTC
1541      GGTTCAGAGA CTAGATGGAG GTGGGTCGTC GCGCGGCTTA AGGTATAGTC
8335      GGTTCAGAGA CTAGATGGAG GTGGGTCTTT GTCAGGCTTA AGGTATAGTC
4101      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
8685      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
9159      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
OKM7      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
6701      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
9981      GGTTCAGAGA CTAGATGGAG GTGGGTCGTT GCACGGCTTA AGGTATAGTC
9985      GGTTCAGAGA CTAGATGGAG GTGGGTTGCT GCCTGGCTTA AGGTATAGTC
4396      GGTTCAGAGA CTAGATGGAG GTGGGTCG.C GCGCGGCTTA AGGTATAGTC

          351                        intron | SSU   387
1513      CGTTCGCGAG CGAAAGCTCG ATGAGGCGGG AGCCTGC
56667     CGTTCGCGAG CGAAAGCTCG ATGAGGCGGG AGCCTGC
H7524     CGTTCGCGAG CGAAAGCTCG ATGAGGCGGG AGCCTGC
GB1702    CGTTCGCGAG CGAAAGCTCG ATGAGGCGGG AGCCTGC
1541      CGTTCGCGAG CGAAAGCTCG ATGAGGCGGG AGCCTGC
8335      CGTCCACGAG CGAAAGCTCG ACGGG.CGGG AGCCTGC
4101      CGTCTACATG TGAAAACTTG .TGGGGCGGG AGCCTGC
8685      CGTCTACATG TGAAAACTTG .TGGGGCGGG AGCCTGC
9159      CGGCTGCACA CGAAAATGTG .C.AGGCGGG AGCCTGC
OKM7      CGTCTGCACA CGAAAATGTG .C.AGGCGGG AGCCTGC
6701      CGGCTGCACA CGAAAATGTG .T.AGGCGGG AGCCTGC
9981      CGGCTGCACA CGAAAATGTG .T.AGGCGGG AGCCTGC
9985      CGTCCGCGAG CGAAAGCTCG AT.GGGTGGG AGCCTGC
4396      CGTCCTCGGG CGAGAGCTCG A..AGGCGGG AGCCTGC
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Appendix C, Part 2. ITS Region[a].

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                                                SSU | ITS1
        1                                           |                  50
1513    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACC AAAGTTGCTG
56667   TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACC AAAGTTGCTG
H7524   TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACC AAAGTTGCTG
GB1702  TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACC AAAGTCGCTG
1541    TTCCGTAGGT GAACCTGCGG AAGGATCATT AAAGTAAACC AAAGTCGCTG
8335    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACAGTAAAAC AAAGTTGCTG
4101    TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGTAAA.C AATGTCGCTG
8685    TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGTAAA.C AATGTCGCTG
9159    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACTGTAAA.C AATGTCGCTG
OKM     TTCCGTAGGT GAACCTGCGG AAGGAtCATT ACTGTAAG.C AATGTCGCTG
6701    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACTGTAAA.C AATGTCGCTG
9981    TTCCGTAGGT GAACCTGCGG AAGGATCATT ACTGTAAA.C AATGTTGCTG
9985    TTCCGTAGGT GAACCTGCGG AAGGATCATT ATCGTAAA.C AATGTTGCTG
4396    TTCCGTAGGT GAAcCTGCGG AAGGATCATT ATCG.AAG.C TCTGTCGCTG

        51                                                  100
1513    GCC.CTCC.G GGG....CAT GTGCACGCC. GCTTCCG... CATCCTCTTC
56667   GCC.CTCC.G GGG....CAT GTGCACGCC. GCTTCCG... CATCCTCTTC
H7524   GCC.CTCC.G GGG....CAT GTGCACGCC. GCTTCCG... CATCCTCTTC
GB1702  GCC.CTCC.G GGG....CAT GTGCACGCC. GCTTCCG... CATCCTCTCT
1541    GCC.CTCC.G GGG....CAT GTGCACGCC. GCTTCCG... CATCCTCTCT
8335    GCC.CTCC.G GGG....CAT GTGCACGCC. GCGGCCG... CATCCTTCTT
4101    GTC.CTCC.G GGA....CAT GTGCACGCCC TCGGTCGTTA CATCCTTC..
8685    GTC.CTCC.G GGA....CAT GTGCACGCCC TCGGTCGTTA CATCCTTC..
9159    GTC.CTCC.G GGA....CAT GTGCACACCT TCGGTCG..A CATCCTTC..
OKM     GTC.CTCC.G GGA....CAT GTGCACGCCC TCGGTCG..A CATCCTTC..
6701    GTC.CTCC.G GGA....CAT GTGCACACCT TCGGTCG..A CATCCTTC..
9981    GTC.CTCC.G GGA....CAT GTGCACACCT TCGGTCG..A CATCCTTC..
9985    GCC.CCCCTG GGA..GGCAT GTGCACGCCC ATGGTCG... CATCCTTC..
4396    GCTTCCCCTG GGACAAGCAT GTGCACGCCT CTGGTTCTCA .ATCCTTCAA

        101                                                 150
1513    T..ACCCC.T GTGCACCTTT GCGTGG.GGT GCATGT.CTT CAGAATGTCT
56667   T..ACCCC.T GTGCACCTTT GCGTGG.GGT GCATGT.CTT CAGAATGTCT
H7524   T..ACCCC.T GTGCACCTTT GCGTGG.GGT GCATGT.CTT CAGAATGTCT
GB1702  C..ACCCC.T GTGCACCTTT GCGTGG.GGC GCATGT.CTT CAGAATGTCT
1541    C..ACCCC.T GTGCACCTTT GCGTGG.GGC GCATGT.CTT CAGAATGTCT
8335    .ACACCCC.A GTGCACCTTT GCGTGG.GGC GTATGT.CTA CAGAATGTCT
4101    .ATACCCC.T GTGCACCTCT GCGTG.TGGT GTATGTTCTT ATGAATGTCT
8685    .ATACCCC.T GTGCACCTCT GCGTG.TGGT GTATGTTCTT ATGAATGTCT
9159    .ACACCCC.T GTGCACCTCT GCGTG.TGGT GTATGT.CTT ACGAATGTCT
OKM     .ACACCCC.T GTGCACCTCT GCGTG.TGGT GTATGT.CTT ACGAATGTCT
6701    .ACACCCC.T GTGCACCTCT GCGTG.TGGC GTATGT.CTT ACGAATGTCT
9981    .ACACCCC.T GTGCACCTCT GCGTG.TGGC GTATGT.CTT ACGAATGTCT
9985    .ACACCCC.T GTGCACCTCT GCGTG..GGT GTATGT.CTT CAGAATGTCA
4396    CACACCCCTT GTGCACCTTC GCGTGGCGGG GCATGT.CTA CAGAATGTCT
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[a]The small subunit, ITS 1, 5.8S, ITS 2, and large subunit regions are identified.

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        151                          ITS1 | 5.8S                    200
1513    A.T..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
56667   A.T..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
H7524   A.T..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
GB1702  A.T..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
1541    A.T..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
8335    ACT..TGCGA TAAAAAGCAA CTTAATACAA CTTTCAACAA CGGATCTCTT
4101    ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
8685    ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
9159    ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
OKM     ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
6701    ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
9981    ACTA.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
9985    AC.A.TGCGA TAAAAAGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT
4396    ATTGTTGCGA TGTAACGCA. TCTAATACAA CTTTCAACAA CGGATCTCTT

        201                                                    250
1513    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
56667   GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
H7524   GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
GB1702  GGCTCTCgca tcgatgaaga acgcagcGAA ATGCGATAAG TAATGTGAAT
1541    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
8335    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
4101    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
8685    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
9159    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
OKM     GGCTCTCGCA TCGATGAAGA ACGCAGCCAA ATGCGATAAG TAATGTGAAT
6701    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
9981    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
9985    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
4396    GGCTCTCGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT

        251                                                    300
1513    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
56667   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
H7524   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
GB1702  TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
1541    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
8335    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
4101    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
8685    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
9159    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
OKM     TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
6701    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
9981    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
9985    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCACCCCTTG
4396    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACCTT GCGCCCCTTG
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          301                                   5.8S | ITS2          350
1513      GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCA.CCTCTA
56667     GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCA.CCTCTA
H7524     GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCA.CCTCTA
GB1702    GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCA.CCTCTA
1541      GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCA.CCTCTA
8335      GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAACTTC TCACCCTCTA
4101      GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.A
8685      GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.A
9159      GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.G
OKM       GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.A
6701      GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.G
9981      GTATTCCGAG GGGTACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCC.G
9985      GTATTCCGAG GGGTACGCCT GTCTGAGTGT CGTGAAATTC TCAACCCC.A
4396      GTATTCCGAG GGGCACGCCT GTTTGAGTGT CGTGAAATTC TCAACCCT.G

          351                                                   400
1513      TCCCCTTTT. .CGGAGGGCG GT.GG..GGC GTGGATGTGG AGGTCTT..G
56667     TCCCCTTTT. .CGGAGGGCG GT.GG..GGC GTGGATGTGG AGGTCTT..G
H7524     TCCCCTTTT. .TGGAGGGCG GT.GG..GGC GTGGATGTGG AGGTCTT..G
GB1702    TCCCCTTTT. .CGGAGGGCG GT.GG..GGC GTGGATGTGG AGGTCTT..G
1541      TCCCCTTTT. .CGGAGGGCG GT.GG..GGC GTGGATGTGG AGGTCTT..G
8335      CCTTTTTTTT TTGAAGGGTG GTT.....GC GTGGACGTGG AGGCCTT..G
4101      CCCCCTTTT. GCGAGGGGC. GTCGGT.GGC TTGGACTTGG AGGCCTTT.G
8685      CCCCCTTTT. GCGAGGGGC. GTCGGT.GGC TTGGACTTGG AGGCCTTT.G
9159      CCCCCTTTT. GCGAGGGGTT GTCGGT.GGC TTGGACTTGG AGG.CTTTTG
OKM       CCCCCTTTT. GCGAGGGGT. GTCGGT.GGC TTGGACTTGG AGG.CTTT.G
6701      CCCCCTTTT. GCGAGGGGC. GTCGGT.GGC TTGGACTTGG AGG.CTTTTG
9981      CCCCCTTTT. GCGAGGGGC. GTCGGT.GGC TTGGACTTGG AGG.CTTT.G
9985      CCCCCTTTT. GCGAGGGGC. ATTGG..GGA TTGGACTTGG AGG.CTTT.G
4396      CCCCCTTTT. GTAAGAAAGG GTGTGCCGGC TTGGACTTGG AGGTCTTTTG

          401                                                   450
1513      CCGTCGGCTC CTCTCAAATG CATTAGTAGT GTTGGCCTTC GGTGTGATAA
56667     CCGTCGGCTC CTCTCAAATG CATTAGTAGT GTTGGCCTTC GGTGTGATAA
H7524     CCGTCGGCTC CTCTCAAATG CATTAGTAGT GTTGGCCTTC GGTGTGATAA
GB1702    CCGTCGGCTC CTCTCAAATG CATTAGTAGC GTTGGCCTTC GGTGTGATAA
1541      CCGTCGGCTC CTCTCAAATG CATTAGTAGC GTTGGCCTTC GGTGTGATAA
8335      CCGTCGGCTC CTCTGAAATG CATTAGTAGT GTTGGCCTTC GGTGTGATAA
4101      CCGTCGGCTC CTCTCGAATG CATTAGC.TT GC.GGCCT.C GGTGTGATAA
8685      CCGTCGGCTC CTCTCGAATG CATTAGC.TT GC.GGCCT.C GGTGTGATAA
9159      CCGTCGGCTC CTCTCGAAGG CATTAGTTTT GC.GGCCT.C GGTGTGATAA
OKM       CCGTCGGCTC CTCTCGAAGG CATTAGTTTT GC.GGCCT.C GGTGTGATAA
6701      CCGTCGGCTC CTCTCGAAGG TATTAGTTTT GC.GGCCT.C GGTGTGATAA
9981      CCGTCGGCTC CTCTCGAAGG CATTAGTTTT GC.GGCCT.C GGTGTGATAA
9985      CTGTCGGCTC CTCTCAAAGG CATTAGCTTT GC.GGCCT.C GGTGTGATAA
4396      CCGTCGGCTC CTCTCAAAGG TATTAGCTTC ....GCCATC GGTGTGATAA
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          451                                                      500
1513      TT.GTCTACG CCGTTGGGCT GCGCGGGACC CG.CTTCGAA C.TTTC..AT
56667     TT.GTCTACG CCGTTGGGCT GCGCGGGACC CG.CTTCGAA C.TTTC..AT
H7524     TT.GTCTACG CCGTTGGGCT GCGCGGGACC CG.CTTCAAA C.TTTC..AT
GB1702    TT.GTCTACG CCGTCGGGTT GCGCGGGACC TG.CTTCGAA C.CTTC..AT
1541      TT.GTCTACG CCGTCGGGTT GCGCGGGACC TG.CTTCGAA C.CTTC..AT
8335      TT.GTCTACG CCGTGGGGTT GCGCGGGACC TG.CTTCTAA CCTTTC..AT
4101      TT.GTCTACG CCGTGGGCTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
8685      TT.GTCTACG CCGTGGGCTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
9159      TT.GTCTACG CCGTGGGTTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
OKM       TT.GTCTACG CCGTGGGTTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
6701      TT.GTCTACG CCGTGGGTTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
9981      TT.GTCTACG CCGTGGGTTT A.GCGGGACC CG.CTTCCAA CCTTTC.AAT
9985      ATCATCTACG CCATGGGTTT A.GCGGGACT TG.CTTCCAA CCTTTT..AT
4396      TT.ATCTACG CCGTCGGCCC TCGG..GACC CGGCTTCGAA CCTTTTAAAT

          501                                                544
1513      CGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
56667     CGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
H7524     CGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
GB1702    CGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
1541      CGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
8335      TGAAACTTGA CCTCAAATCA GGCGGGACTA CCCGCTGAAC TTAA
4101      CGAAACTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
8685      CGAAACTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
9159      CGAAACTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
OKM       CGAAACTTGA CCTCAGATCA GGCGGgACTa cccgctgaac ttaa
6701      CGAAACTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
9981      CGAAACTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
9985      CGAAACTTGA CCTCAGATCA GGTGGGACTA CCCGCTGAAC TTAA
4396      CGAACCTTGA CCTCAGATCA GGCGGGACTA CCCGCTGAAC TTAA
         ITS2 | LSU
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VITA

Edgar Ballenger Lickey was born in Athens, Georgia on May 19, 1970 to Stephen and Nancy Lickey. He grew up in Richmond, Virginia, attended public schools there and graduated from Hermitage High School in June, 1988. That fall, he attended St. Andrews Presbyterian College in Laurinburg, North Carolina where he earned a Bachelor of Science degree in Biology in May, 1992. In August, 1992, he was awarded a graduate assistantship at Appalachian State University in Boone, North Carolina and earned a Master of Science in Biology with a concentration in ecology in August, 1996. That fall he was accepted into the Botany Department at the University of Tennessee, Knoxville, where he completed the Doctor of Philosophy degree in botany. While at UTK, he took a leave of absence spring semester 1998 to teach the Introduction to Botany at Appalachian State as visiting adjunct instructor. From January 2000 to December 2001 he worked in the Division of Biology at UTK as genetics lab preparatory, and is currently a post-doctoral research associate in the Botany Department at UTK.

He is currently a member of the Southern Appalachian Botanical Society, the Association of Southeastern Biologists, the Botanical Society of America, the American Society of Plant Taxonomists, and the American Mycological Society.

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