To the Graduate Council:

I am submitting herewith a dissertation written by Jiankang Jin entitled "Studies *in Panellus stypticus* and the genus *Panellus* complex." I have examined the final copy of the dissertation for the form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with major in Botany.

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Associate Vice Chancellor
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STUDIES IN *PANELLUS STYPTICUS* AND THE GENUS *PANELLUS* COMPLEX

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Jiankang Jin

May 2000

DEDICATION

This dissertation is dedicated to my family:

My mother Rendi Qi

My wife Guiqin Qian

My son Juncheng Jim Jin

献给我的母亲戚顺娣，妻子钱桂琴及儿子金俊诚

Without their support and sacrifice, this research would have been impossible.

因为有了他们的支持和牺牲，本研究及本文才有可能。

道可道，非常道。名可名，非常名。
无名天地之始；有名万物之母。

—老子

The Truth that can be spoken of is not the eternal Truth;
The Name that can be named is not the eternal Name.
There was no Name for the Beginning of the Universe;
However, there was an Origin for the entire Universe.

— Lao Zi

Ancient Chinese Philosopher, ca. 2,500 years ago

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ABSTRACT

This research examined the biogeography, phylogeny and speciation processes within *Panellus stypticus*, and tried to resolve the systematics of the *Panellus* complex that includes *Panellus stypticus*, *P. mitis*, *P. serotinus* (*Sarcomyxa serotina*), *P. ringens*, *P. violaceofulvus*, *Tectella patellaris* (*P. patellaris*), *P. pusillus* (*Dictyopanus pusillus*) and *Pleurotopsis longinqua* (*P. longinquus*). Exemplars of those eight species and geographically diverse collections of *P. stypticus* were examined using isozyme analyses, morphological comparison, mating studies and molecular analyses.

In the *Panellus* complex, circumscription based on morphological characters was not well defined. Species in the complex were well separated and unique species characters made this complex morphologically diverse.

Based on morphological and molecular analyses, circumscription of the genus *Panellus* included *P. stypticus* and *D. pusillus* only, with other species in the complex assigned to separate genera. Probably additional intersterility groups exist within poroid *Dictyopanus*. Under this concept, the genus *Panellus* was defined morphologically by a panelloid stature, a lateral stipitate pileus and smooth amyloid spores. I propose that the following five genera be used to describe this complex: *Panellus* for the *Panellus stypticus – Dictyopanus pusillus* clade, *Pleurotopsis* for the *Pleurotopsis longinqua – P. ringens* clade, *Tectella* for *Tectella patellaris*, *Sarcomyxa* for *P. serotinus* and a new genus status for *P. mitis*. Thus, some species in this study should be named as follows: *Panellus pusillus* for *D. pusillus; Sarcomyxa serotina* for *P. serotinus; Pleurotopsis ringens* for *Panellus ringens*; and *Pleurotopsis longinqua* for *Panellus longinquus*. However, the designation of *Panellus violaceofulvus* to *Pleurotopsis violaceofulvum* needs further verification.

Within *Panellus stypticus*, Eastern and Pacific North American regions were clearly differentiated from each other based on the distribution of extracellular laccase (ECL1), esterase

(EST1-1) and alcohol dehydrogenase (ADH3) alleles. A connection between Pacific North America, Asia and Europe may be inferred based on the presence of the ADH3 allele in these populations. The distribution of ECL and EST alleles did not clearly separate Northern and Southern Hemisphere populations. A bimodal distribution for ECL alleles in Oceanian areas suggests local differentiation within this region. In this species, mating studies made clear that there is single intersterility group in this species though antagonistic reactions were observed between Northern Hemisphere collections and Southern Hemisphere collections, and within Oceanian collections. Spore shape and the ratio between spore length and width remained as stable and conserved characters in this species; however, many characters such as lamella height and spore size were differentiated among five geographical areas, even between neighboring areas. More diversity including basidioma general stature and lamella anastomoses was observed in Oceanian areas for those characters.

In *Panellus stypticus*, RFLP data revealed that there were seven geographical areas (populations). Sequence data for the ITS region and for the group I intron supported a separation of Northern and Southern Hemisphere populations with the New South Wales population being most closely related to Northern Hemisphere populations. Among Northern Hemisphere populations, Eastern North America was clearly unique, separated from both Eurasian and the Pacific Northwest populations.

In *Panellus stypticus*, RFLP data do not show gene flow between geographical regions. Based on ITS sequences, it is more likely that Northern Hemisphere collections were ancestral to the Southern Hemisphere collections. Tasmanian and New Zealand collections were believed to be from Australian continent. In North America, these studies suggested that allopatry produced by the Rocky Mountains may have played an important role in the early evolution and

differentiation of the species. Within the Northern Hemisphere, Eurasia or Eastern North America is the possible center of origin. The time frame for evolution and dispersal for the species is discussed.

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1. INTRODUCTION

1.1. INTRODUCTION TO THE GENUS *PANELLUS* COMPLEX

1.1.1. Nomenclatural history of the genus *Panellus* complex

A chronological history of the genus *Panellus* is given in Table 1.1. The genus was established by P. Karsten (1879) based on only two characters: "foot (= stipe) lateral; spores ellipsoid to spherical." The type species was *P. stypticus* (Bull. ex. Fr.) Karst., which was transferred from *Panus*.

Eight new species were added to the genus by Murrill (1915). *Panellus* was divided into two genera by Smith (1949), *Panellus* and *Tectella*, based on amyloid versus nonamyloid spores, the presence or absence of a stipe and presence or absence of a veil in young basidiomata. Kühner & Romagnesi (1953) included six species in *Panellus*: *P. serotinus, P. mitis, P. ringens, P. stipticus, P. patellaris* and *P. violaceofulvus*. The first three species were newly proposed species and were appended into *Panellus*.

The genus *Panellus* was delineated by Miller (1970) as "Pileus 8-45 (-100) mm broad, 5-35 mm wide, viscid to dry, glabrous to woolly-pubescent, smooth; lamellae close to subdistant, even, edges entire, white, pinkish-buff, ochraceous, olivaceous to vinaceous or brown. Stipe present or absent, if present very short (1-12 mm) and eccentric or laterally attached, dry, concolorous with the pileus. Sporocarps revive when moistened. Spores allantoid, oblong, cylindric to narrowly elliptical, smooth, thin-walled, amyloid (blue to purple in Melzer's solution). Spore print white to yellow. Cheilocystidia cylindric, fusiform, clavate to bifurcate or irregularly shaped, thin-walled, hyaline, embedded to protruding, abundant to infrequent. Pleurocystidia present or absent, when present, similar to cheilocystidia. Cuticle either a gelatinous pellicle or of interwoven thick- and thin-walled hyphea. Trama of pileus has a gelatinous upper layer and a lower layer of non-gelatinous interwoven thick- and thin-walled

Table 1.1. Chronological development of circumscription in the genus *Panellus*

Karsten, 1879	Only two characters, lateral stipe attachment and elliptical spores were used to describe the genus. The type species is *P. stypticus*. Two species, *P. stypticus* and *P. farinaceus* were originally included in the genus.
Murrill, 1915	Ten species were described, including *P. jalapesis, P. subcantharelloides, P. flabellatus, P. eugrammus, P. dealbatus, P. haematopus, P. ursinus, P. vulpinus, P. cantharelloides,* and *P. stypticus*. Among these, the first eight species were named by the author. However, most of these have been transferred to other genera.
Smith, 1949	Two genera were established in subfamily Pleurotoideae, family Tricholomataceae: *Panellus*: spores amyloid, smooth, and small; gills entire, no pleurocystidia. *Tectella*: spores not amyloid, stipe lacking; carpophores resupinate at first; spore deposit pure white; veil present on underside in young stages; hyphae of the flesh with thickened walls.
Kühner and Romagnesi, 1953	Six species were described in *Panellus*: *P. serotinus, P. mitis, P. ringens, P. stipticus, P. patellaris* and *P. violaceofulvus*. The first three species were described as new species and amended into *Panellus*.
Miller, 1970	Six species of *Panellus* described by Kühner and Romagnesi (1953) were reported from North America.
Singer, 1975	Three genera were established in tribus *Panelleae* of the family Tricholomataceae. The genus *Panellus* included subgenus *Panellus* with ten species, including *P. stypticus, P. longinquus, P. ringens* and *P. violaceofulvus*; and subgenus *serotinia* in which four species were described, including *P. serotinus*. Genus *Dictyopanus* included *D. pusillus* and two other species. Genus *Tectella* included only one species, *T. patellaris*.
Burdsall and Miller, 1975; 1978	Poroid *Dictyopanus orientalis* was transferred to *Panellus* as *P. orientalis*. Two varieties: *D. pusillus var. pseudorhipidium, D. p. var. sublamellatus* and one poroid species *D. gloeocystidiatus* were integrated into *Panellus* as *P. pusillus*. They established two subgenera *Panellus* and *Mitellus*. *Panellus* included *P. stipticus, P. pusillus* and *P. copelandii*, *Mitellus* subgen. nov. included *P. patellaris, P. serotinus, P. ringens, P. violaceofulvus*. Six species formerly in *Dictyopanus* were merged into *Panellus*.
Libonati-Barnes, 1981	*P. stipticus, P. mitis, P. serotinus P. ringens, P. violaceofulvus P. longinquus,* and *Dictyopanus pusillus* etc were included in the genus *Panellus. Tectella patellaris, Dictyopanus gloeocystidiatus, D. luminescens, D. foliicolus* were excluded from the genus.
Horak, 1983	*Pleurotopsis longinqua* was transferred from *Panellus* to *Pleurotopsis* due to its distinctly gelatinized pileocutis and distinctively southern Austral-Antarctic distribution.
Miller, 1984	*Panellus* was separated from *Pleurotus, Panus, Hohenbuehelia* and *Resuspinatus* by amyloid, smooth and elliptical spores (blue to purple in Melzer's solution).

Table 1.1. (Continued) Chronological development of circumscription in the genus *Panellus*

Libonati-Barnes, 1984	A collection from the Pacific coast of North America was classified as *Panellus longinquus*, but was slightly different from the South American holotype. The collection was given a subspecies designation: *P. longinquus ssp. Pacificus.*
Corner, 1986	The genus *Dictyopanus* treated as synonym of *Panellus*. Twenty-two new poroid and lamellate species found in Malaysia were added into *Panellus*. *P. longinquus* was tentively placed in *Panellus*. Two subgenera were established: *Megalopanellus* with smooth amyloid spores and centric stipe and *Mesopanellus* with more or less centric pileus and well developed stem (nonpleurotoid). *Mesopanellus* is marasmioid, gills decurrent or adnato-decurrent.
Petersen and McCleneghan, 1995; 1997	The establishment of a new subspecies *P. longinqua ssp. pacificus* by Libonati-Barnes & Redhead (1984) was believed to be redundant.

hyphae. Clamp connection present in all species." Subsequently Singer (1975) divided *Panellus* into three genera: *Panellus, Tetella* and *Dictyopanus*. Budsall and Miller (1975, 1978) merged *Dictyopanus* into *Panellus*, and described the genus as: "Pileus 1-25-(32) mm diameter, reniform, pubescent, white to tan. Hymenophore lamellate or poroid, lamellae close, pores 3-5/mm, edges entire, white to pale tan, some luminescent when fresh. Stipe absent or lateral, 6-12 x 3-8 mm, pubescent, concolorous with cap. Some revive when moistened. Spores (3-) 3.5-9(-11) x 1-5.5 µm, allantoid, oblong, ovoid to narrowly ovoid, smooth, hyaline, amyloid in Melzer's reagent. Cheiolocystidia 17-65 x 3.5-11 µm, simple and cylindrical to ventricose, thin walled, hyaline, smooth, abundant. Pleurocystidia present, sometimes rare, often not protruding beyond basidia. Pileus trama either a gelatinous zone or an agglutinated *textura intricata.*"

Singer (1986) had the following description about the genus: "Pileus rarely centrally (and then short-) stipitate, more frequently eccentrically or laterally stipitate or subsessile; epicutis consisting of filamentous hyphae which are more or less branched. Spore print white or whitish. Spores hyaline, cylindric, or ellipsoid to oblong, sometimes, allantoid, rarely short-ellipsoid, smooth, amyloid, acyanophilic. Cheilocystidia scattered to numerous, inconspicuous to conspicuous, rarely absent. Cystidia or pseudocystidia present or absent, sometimes matuloid. Carpophores often luminescent. Often but not always with a gelatinous upper zone. Taste mild or styptic. Often distinctly reviving when dried and remoistened. Cosmopolitan occurrence". Corner (1986) had the following description: "Pileus mesopodal, pleuropodal, or sessile, medium-sized to very small, mostly white. Gills adnate to decurrent, or with small to minute pores, edges chalky pruinose. Flesh thin, soft, tough, gelatinous. Spores white, smooth, more or less amyloid, subglobose to ellipsoid, cylindric or allantoid. Cheilocystidia usually present, thin walled. Pleurocystidia generally absent. Hyphae monomitic, usually clamped".

The genus *Dictyopanus* Pat. was established by N. Patouillard (1900) who described the genus as: "Pleuropodal or subsessile, stipe becomes small, fleshy but becomes tough in age. No

(putrid) smell. Hymenium thick layer of anastomosing, radiant pores. Cystidia none. Spores white. Lignicolous, small species, all found in warm regions. There were two species or varieties in the genus: *D. rhippidium* Bk. and *D. subpulverulentus* Bk." Singer (1975) specified the type species of this genus as *D. pusillus* (Pers. ex. Lev.) Sing. and described the genus as "carpophores pleurotoid, polyporoid; trama of pore walls gelatinous or not, hymenophore favoroid and poroid; cystidia and cheilocystidia differentiated; spores smooth, amyloid, ellipsoid to cylindric; stipe short and eccentric and lateral, trama astrigent." Although there are several morphocharacters that separate *Dictyopanus* from *Panellus* sensu Singer, spores, trama, and taste of carpophores are quite similar and were the basis for merging *Dictyopanus* into *Panellus* (Burdsall and Miller, 1975 and 1978).

The genus *Tectella* Earle was established by F. S. Earle (1909) with the type species *Tectella patellaris* (Fr.) Murr. [≡ *Panus operculatus* Berk. & Curt.]. Earle (1909) furnished the following description of the genus: "Persistent, reviving, fasciculate: pileus resupinate, poculate: lamellae concentric from a central point: spores white: veil covering the young lamellae, soon vanishing: stipe none". Singer (1986) described this genus as "pileus non-stipitate, attached laterally or at some point of the sterile surface; veil present in the form of a pellicular veil that covers the lamellae in the young specimens; spores up to 5.5 µm long, small, almost inamyloid but in accumulation amyloid; cystidia none, but cheilocystidia differentiated and typically dimorphic, entire, obtuse, often thick-walled; trama of the pileus in its upper layer strongly gelatinized with thin-filamentous hyphae. Carpophores pendulous from a pseudostipe or directly attached to the substratum. Occurred in temperate Europe, Asia and North America".

The genus *Sarcomyxa* Karst. was established by Karsten (1891), who described the genus as the following: "Pileus fleshy, young pellicle (pileocutis) with wet, viscid covering. Stipe fibrous, lateral. Veil none. Lamellae ending on stipe. Spores cylindric, curved, spore print white. Cystidia cylindric-clavate, or cylindric. The type species was *Pleurotus serotinus* (Schrad.) Fr."

Singer (1975, 1986) established the subgenus *Serotinia* (Pilat) Sing., and subgenus *Panellus* in *Panellus*. In his subgenus *Serotinia* (Pilat) Sing., *P. serotinus* (Pers. in Hofmann ex Fr.) is the type species. Another nine species were also included in this subgenus. Singer (1975) indicated the primary distinguishing character between species *P. stypticus* and *P. serotinus* was presence or absence of cystidia on the sides of lamellae.

The genus *Pleurotopsis* (P. Henn.) Earle was established by F. S. Earle in 1909. He gave the following description: " Sporocarp thin, membranous, reviving: pileus sessile, at first resupinate: lamellae radiating from a central or eccentric point: spores white or hyaline: veil none: stipe none." The type species was *P. spodoleuca* (Berk.) Earle [≡ *Marasmius spodoleucus* Berk.]. *Pleurotopsis longinqua* (Berk.) Horak (1983) was a combination transferred from *Panellus longinquus* (Berk.) Sing. by Horak (1983).

Currently *Panellus stypticus*, *P. mitis*, *P. ringens*, *P. violaceofulvus*, species in former genera *Dictyopanus* (Burdsall and Miller, 1975 and 1978) and *P. serotinus* [≡ *Sarcomyxa serotina* (Schrad.: Fr.) Karst.] are still considered species of *Panellus*. Genus *Tectella* (Earle, 1909) and species *Pleurotopsis longinqua* (Horak, 1983) are regarded as separated taxa.

1.1.2. Morphology of species in this study

Panellus stypticus (Bull. ex. Fr.) Karst. "with a luminescent and a non-luminescent race" (Singer 1986): Hesler (1960) described the species as "Cap 1/4 to 1/2 inch broad, grayish when dry, cinnamon when wet, surface breaking up to form minute, powdery or scurfy scales, odor mild, taste astringent. Gills pale brownish, close. Stipe at times apparently absent. Spores white in mass". Miller thought the genus had been thoroughly studied in Europe, the species clearly delimited and studied also in North America. He described *P. stypticus* as "Pileus 5-12-(32) x 12-15 -(25) mm, convex to plano-convex, crenelate margin, ochraceous buff, various shades of tan

or brown when dried. Lamellae close, narrow, often forked, stipe 6-12 mm long, 3-8 mm wide, usually eccentric, often lateral, minute fibrillose, dull white, spores 3.0-3.5-4.6 x 1.2-2.2 µm, elliptical to somewhat allantoid, amyloid. Basidiomata, usually in tightly packed imbricate clusters as a saprophyte, fruiting in the fall in September, October, and November in Europe and North America".

Panellus serotinus (Pers. in Hofmnn ex. Fr.) Kühner [≡ *Sarcomyxa serotina* (Schrad.: Fr.) Karst.]: "Pileus 2.5- 6.0 (-10.0) cm broad, 2.0 –3.5 cm wide, convex, fan-shaped, conchate; margin often lobed or wavy, viscid, vetiver green, olive ochre to deep colonial buff, purple over the margin, always sessile. Lamellae close to subdistant, narrow, even, clearly marginate, pale orange to honey yellow with yellowish olive to brownish olive. Spores allantoid to cylindric, with amyloid walls and yellow contents. Spore print yellow. Pleurocystidia fusiform, broadly fusiform, to clavate, thin-walled, sometimes with incrustations over the apex. Cheilocystidia nearly cylindric, fusiform to narrowly clavate, thin-walled, abundant. Cuticle with numerous tangled nearly cylindric, fusiform to long clavate, thin-walled pileocystidia. This robust species has a characteristic greenish to olivaceous color of the pileus, is viscid when fresh, and has numerous tangled pileocystidia" (Miller, 1970).

Panellus mitis (Pers. ex. Fr.) Sing.: "Pileus 5-8 mm long, 5-6 mm wide, spathulate to conchate, flat to broadly convex, a dull white ground color. Lamellae subdistant, even, narrow, white to pale pinkish cinnamon. Stipe 1-2 mm long, laterally attached. Spores allantoid to cylindric, smooth, thin-walled, amyloid. Cheilocystidia fusiform, clavate to contorted and irregular, thin-walled. The small white sporocarps containing a gelatinous upper layer in the pileus trama and at the gill edge" (Miller, 1970).

Panellus ringens (Fr.) Romagnesi: "Pileus (5-) 10-30 mm in diameter, sessile, margin even to somewhat crenate , light purple to purple drab or lilac colored with vinaceous tints fading in age to vinaceous fawn with a conspicuous pallid pubescence which is dense over the lateral

attachment of the cap to the substrate. Spores oblong to allantoid, amyloid, often hard to find. Pleurocystidia not found. Cheilocystidia clavate, cylindric, fusiform, thin-walled. This species is closely related to *P. violaceofulvus* but has smaller spores and basidia, lacks a cellular hymenopodium, and doesn't develop a venose hymenium between the lamellae" (Miller, 1970).

Panellus violaceofulvus (Batsch ex Fr.) Sing.: "Pileus 3-15 mm diameter, sessile or with a short rudimentary stipe; margin even to slightly wavy, inrolled at first, dry, brown tinted violet, or darker violet-brown with a conspicuous white, wolly pubescence. Lamellae subdistant, sometimes venose, pale violet to brownish in age. Spores cylindric to allantoid, amyloid. Cystidia absent. Subhymenium a cellular hymenopodium, particular evident near gill attachment to pileus. *P. ringens* could be misidentified as *P. violaceofulvus*" (Miller, 1970).

Dictyopanus pusillus (Pers. ex. Lev.) Sing. [≡ *P. pusillus* (Pers. ex. Lev.) Burdsall and Miller]: Burdsall and Miller (1975) had the following description for this species: "Pileus 5-20(-27) mm broad, reniform to semicircular, convex to planate, nearly white to pale tan when fresh. Reviving when moistened. Surface dry, pubescent to appressed wooly, azonate. Pores concolorous with cap both when fresh and dry, pore surface irregular. 4-5/mm across basidiocarp, elongated radially, sometimes becoming sublamellate. Luminescent when fresh. Basidiospores 4-5.5 x 2-3 μm, ovoid to broadly ovoid, adaxially flattened, hyaline, thin-walled, smooth, amyloid. Pleurocystidia lacking or present only near the pore edge, cylindrical to lanceolate, hyaline, thin-walled, smooth, protruding up to one-half of the total length. Cheilocystidia inconspicuous, cylindrical, 4-6 μm diam. Thin-walled, with refractive contents, imbedded in dense cluster of acanthophysoid end cells and some reddish-brown encrusting granules making up the pore apex. Cuticle a loose *textura intricata,* thin-walled to slightly thick-walled. Pileocystidia imbedded in cuticle, inconspicuous, cylindrical, flexuous, thin-walled, hyaline, with refractive contents. Stipe cuticle like that of pileus cuticle with caulocystidia like the pileocystidia". This species was merged into genus *Panellus* and referred to as subgenus *Panellus* by Burdsall and Miller (1975). Burdsall &

Miller (1978) determined that *P. stypticus* and *P. pusillus* (*D. pusillus*) were incompatible. Corner (1986) described this species as having a 12 mm or more fawn tan pileus, ellipsoid to allantoid small (2-3 μm) spores, acerose basidioles present, and cheilocystidia none or slightly gloeocystidial. He also mentioned that this species had a hymenial sublamellate variety, (*P. pusillus var. pseudorhipidium* Singer) in the American tropics (?= *P. pusillus var. sublamellatus* Corner).

Tectella patellaris (Fr.) Murr. [≡ *P. patellaris* (Fr.) Konr.-Maubl.]: Singer (1975) described the monotypic genus as: "Pileus non-stipitate, attached laterally, veil present that covers lamellae in the young specimens, spores long, almost inamyloid but amyloid accumulatively. Upper layer of pileus is strongly gelatinized. Based on those morphocharacters, the significant differences between (narrow-sense) *Panellus* (*sensu* Singer) and *Tectella* are that the latter has inamyloid spores but becoming accumulatively amyloid, and is veil-present in young carpophores". Miller (1970) had the following description: "Pileus 8-15 mm broad, sessile to short-stipitate, convex, viscid to glutinous at first, soon becoming fibrillose to villose and moist to dry, brown, "snuff brown." Stipe when present 1-3 mm long, 0.5-1.5 mm wide, eccentric, equal, dry, usually somewhat curved. Lamellae subdistant, narrow, "cinnamon-buff", covered at first by a thin membranous, dry "pinkish buff" veil which soon disappears leaving no traces at maturity. Spores allantoid, thin-walled, amyloid, spore print white. Cheilocystidia clavate to fusiform, thin-walled, hyaline with some yellowish contents in KOH and Melzer's solution, protruding 1/3 to ½ of total length. Pleurocystidia –none seen. Cuticle of pileus a thick gelatinous layer of interwoven hyphae, thin-walled. Trama of lamellae of interwoven hyphae, thin- and thick-walled".

Pleurotopsis longinqua (Berk.) Horak (1983) [≡ *P. longinquus* (Berk. In Hooker) Sing.]: Horak (1983) described this species as: "Distinctively southern Austral-Antarctic distribution; it is easily identified by the cylindrical to allantoid, amyloid spores and distinctly gelatinized pileocutis; basidiomata ranges from whitish, pink, copper red, grey-liliac to grey-brown. The tomentose to strigose stipe is either eccentric to lateral or occasionally absent". Horak (1983)

stated that the transfer of the species was due to its distinctly gelatinized pileocutis. Libonati-Barnes and Redhead (1984), Petersen (1992) and Petersen and McCleneghan (1995 and 1997) reported that this species is available in South America and Pacific North America.

1.1.3. Mating systems in genus *Panellus* complex

Macrae (1937; 1942) first reported *P. stypticus* was heterothallic and tetrapolar. Petersen and Gordon (1994) reported that *P. mitis, P. patellaris* and *P. ringens* all had a tetrapolar mating system. The mating system of *Dictyopanus pusillus* is also tetrapolar (Petersen et al., 1997).

1.2. INTRODUCTION TO *PANELLUS STYPTICUS*

1.2.1. Distribution of *P. stypticus*

Panellus stypticus (Bull. & Vent.: Fr.) Karsten is widely distributed in the North Hemisphere temperate zones (Miller, 1970), ranging from Alaska to Costa Rica. The species has also been collected in Tasmania and New Zealand (Petersen and Bermudes, 1992a and 1992b; and unpublished UT Herbarium data). Basidiomata form in September, October, and November in Europe and North America (Miller, 1970).

1.2.2. Mating studies within *P. stypticus*

paired Collections of *P. stypticus* from Japan and Eastern North America, and later collections from New Zealand and Russia were paired Petersen and Bermudes (1992a, 1992b). The results revealed a universal intercompatible group over these geographical regions.

1.2.3. Bioluminescence studies within *P. stypticus*

Bioluminescence is the ability of certain living things in the environment to give off light without producing heat. Bioluminescent systems in nature appear to have evolved independently for these organisms are not monophyletic (e.g., luminescent fungi, fire flies, and luminescent deep-ocean fish).

In some geographical areas, *P. stypticus* is bioluminescent. Possibly the earliest record of phosphorescence (luminescence) noted in *P. stypticus* was made by Gentry in 1885 (Macrae, 1942). Buller in 1924 described the lamellae of *P. stypticus* in North America as luminescent, while this phenomenon was not observed in European specimens (also cited by Macrae, 1942). Macrae (1942), Lingle et al. (1992), O'Kane et al. (1990a; 1990b), and Petersen and Bermudes (1992a; 1992b) all confirmed that in Eastern North America, *P. stypticus* basidiomata were luminescent. Mycelia of this species were luminescent as well (Bermudes et al., 1990).

By pairing nonluminescent monocaryons with luminescent ones, Macrae (1942) concluded that luminosity in this species was an inherited character and was governed by a single pair of Mendelian factors in which luminosity was dominant over nonluminosity. Luminosity factors were independent of intersterility factors. Lingle et al. (1992) agreed with Macrae about luminosity and stated that at least three different mutations of genes could lead to the loss of luminescence. They also reported that the maximum bioluminescence was found at 525 nm, and shifted to 528 nm in deeply pigmented basidiocarps.

After intercontinental compatibility tests, Petersen and Bermudes (1992a; 1992b) suggested that bioluminescence and compatibility were independent since luminescence seemed to be geographically restricted. This suggested that the ability or potential to interbreed must have been preserved since separation of *P. stypticus* into geographically isolated areas.

Mycelia of *P. stypticus* grown submerged were non-luminescent, but became luminescent while growing on solid substrata. Dark-grown colonies were luminescent in the center, and light-grown colonies were brightest at the periphery (Bermudes et al.,1990).

In *Panellus*, the recorded luminescent species are *P. stypticus* (North America), *P. luminescens*, *P. gloeocystidiatus*, Japanese *Dictyopanus foliicolus*, and *P. orientalis* according to Kobayashi as cited in Corner (1986).

1.3. INTRODUCTION TO MOLECULAR METHODS USED TO EXAMINE EVOLUTIONARY RELATIONSHIPS BETWEEN SPECIES AND GENERA

1.3.1. Phylogenetic analyses by ITS, SSU, LSU and mitochondrial sequences

Molecular phylogenetics leans heavily on the assumption that the phylogenies of genes within organisms reflect the phylogenies of the organisms themselves. To date, most molecular studies have concentrated on only a few genes, such as the nuclear ribosomal RNA or mitochondrial ribosomal RNA genes. Estimates of phylogenetic patterns may vary depending on whether one samples data from the mitochondrial or nuclear genome. Rates of substitution and other changes may also vary considerably between genes, and even among different regions within the same gene (Vilgalys and Hibbett, 1991).

The most commonly used sequences are the ribosomal internally transcribed spacer regions (ITS) including ITS1, ITS2 and the intervening 5.8S ribosomal RNA gene (usually used at or below species level, or for species complexes), and the ribosomal large subunit gene (LSU) usually used at or above genus level, or for genus complexes. Sequences in the intergenic spacer (IGS) region, including IGS1 and IGS2 are often used for population level comparisons.

There are a number of studies using these genes to infer phylogenies in fungi. Kretzer et al. (1996) studied 47 isolates that belonged to 38 recognized species of *Suillus* sensu lato using ITS sequences and found that *Boletinus* and *Fuscoboletinus* within *Suillus* sensu lato were not

monophyletic. One species, *Suillus granulatus* was polyphyletic and the most primitive clades within *Suillus* sensu lato were of strongly boletinoid hymenophore. Later, Kretzer and Bruns (Kretzer, 1997), using ITS sequences, proved that the secotioid genus *Gastrosuillus* was clearly derived and had evolved at least twice from *Suillus*. They proposed transferring the genus *Gastrosuillus* into *Suillus*.

O'Donnell et al. (1997) used DNA sequences of 18s and partial LSU rDNA to construct phylogenetic relationships of ascomycetous truffles, true and false morels. Results indicated several epigeous and most hypogeous taxa were misplaced taxonomically. A new phylogenetically-based classification at the family level for those fungi was thus proposed.

Phylogenetic analyses based on 18S ribosomal DNA (nuc-SSU) and the mitochondrial ribosomal small subunit (mit-SSU) sequences of homobasidiomycetes showed that *Panellus stypticus* was closely related to *P. serotinus*, all though the former contained a group I intron in the 18S ribosomal DNA (Hibbett, 1996; Hibbett et al., 1997). A single most parsimonious tree based on mit-rDNA, however, put *P. stypticus* and *P. serotinus* in different clades (Hibbett and Donoghue, 1995).

A comprehensive phylogenetic analysis of the gilled mushrooms and puffballs was performed by Hibbett et al. (1997), using nuclear and mitochondrial rDNA, Results suggested that 87% of the gilled mushrooms were in a single lineage (Euagarics), which should have arisen no later than mid-Cretaceous. Puffballs and other Gastromycetes evolved at least four times, and diverse fruiting body forms and spore dispersal mechanisms have evolved among Gastromycetes.

Phylogenetic relationships of *Lepiota* sensu lato based on LSU of rRNA were investigated. Parsimony analysis excluded tribe Cystodermeae from the family Agaricaceae and retained tribes Lepioteae and Leucocoprineae. *Agaricus* and *Coprinus* sect. *comati* were nested within the lepioid fungi (Johnson and Vilgalys, 1998).

Based on 18s rDNA sequence data, phylogenetic analyses of the order Pezizales (Ascomycota) were carried out. Separation of the order into two suborders based on suboperculum structure was disproved. Strongly supported clades (>95% bootstrap) included the Pezizaceae, the Morchellaceae, the Sarcoscyphaceae, the Helvellaceae and a clade including Sarcosomataceae and Otidiaceae (Harrington, 1999).

1.3.2. Phylogenetic analyses by RFLPs, DNA fingerprinting and DNA/DNA hybidization techniques

Restriction fragment length polymorphisms (RFLPs), and/or PCR fingerprinting etc techniques may be used for phylogenetic applications, usually at or below the rank of species. PCR fingerprinting uses the polymerase chain reaction (PCR) to amplify a region of genomic DNA, followed by digestion using restriction enzymes (Vilgalys and Hibbett, 1991).

Hibbett and Vilgalys (1991) used PCR fingerprinting to analyze the evolutionary relationship of *Lentinus*, and found that *Lentinus tigrinus* was more closely related to the Polyporaceae than to the Tricholomataceae. Other species of *Lentinus* appeared to be intermediate between Polyporaceae and Tricholomataceae. This conclusion was also supported by hyphal structure -- dimitic hyphae and hyphal pegs. These results suggested that the traditional viewpoint that Agaricales and Aphyllophorales are two separate lineages may be incorrect.

A phylogeny of the genus *Lentinula* based on RFLPs of rDNA ITS, SSU and LSU sequences suggested that the Asian-Australasian distributed *L. edodes, L. lateritia,* and *L. novazealandiae* had evolved recently and a second distinct group, the American-distributed *L. boryana* was postulated to be the earliest lineage in the genus.

Rrestriction analysis of ITS and SSU genes of rDNA was used to separate 36 isolates of four species of Gaeumannomyces (Ascomycota) into five different groups Fouly et al. (1997.

1.3.3. Isozyme techniques for systematics

Extracellular laccase (ECL) has been used in a number of studies to examine relationships within and between species. Extracellular laccase profiles in the genus *Agaricus* were dissimilar between species but largely invariant within a species (Kerrigan and Ross, 1988). Allozymes were also used to assay wild collections and domestic cultivars of *Agaricus bisporus*, and found novel genotypes existing only in wild collections, indicating *A. bisporus* was also indigenous in North America (Kerrigan, 1990). Distinct ECL patterns were observed between several species of *Pleurotus* while isolates sharing the same pattern were sexually compatible (Jin et al., 1994). In the genus *Clavicorona*, laccase and tyrosinase spot tests may be characteristic of the genus and only of very limited value in separating species, but ECL isozyme profiles were different between morphospecies, and consistent within a species. Tyrosinase profiles were variable in the genus (Wu, 1991). Extracellular laccase (ECL) patterns were confirmed of significance in systematics of *Clavicorona*, and reported that 140 collections from Europe and North America were invariant, while two collections from China were different (Wu et al., 1995).

Other enzymes have also been informative. In biological species of *Gibberella fujikuroi* and allied species, six isozymes: [fumarase, glucose phosphate isomerase (GPI), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) and triose-phosphate isomerase (TPI)] could potentially be used to distinguish different species (Huss et al., 1996). A lignin-degrading isolate could be placed in the genus *Trametes* by phenoloxidase (laccase) isoelectrofocusing electrophoresis (Mansur et al., 1997).

1.3.4. Studies with Group I introns

Group I introns are a class of genetic elements defined in part by the possession of a set of conserved sequences which enable their secondary structure to remove the intron from the RNA transcript. Some have demonstrated the ability *in vitro* to encode autocatalytic, self-splicing RNAs

(Hibbett, 1996). Group I introns were first discovered by Cech (1988) and his collaborators (Michel and Westhof, 1990).

In the ascomycetous lichen complex *Cladonia chlorophaea*, a high degree of variability in 3'-SSU rDNA size of two individuals could be accounted for by different numbers of insertions of Group I introns. In lichen-forming fungi, six different SSU positions of intron insertion were identified, some known only from lichen-forming fungi (DePriest and Been, 1992). A total of eleven insertion positions have now been reported for lichen-forming fungi and their allies. Most insertions were Group I introns (Gargas et al., 1995). DePriest (1993) reported in the same ascomycetous lichen complex, *Cladonia chlorophaea*, the variable distribution of these introns in SSU rDNA suggested that they were mobile, either by intron insertion, deletion or both, – within this species complex.

A plant host *Youngia japonica* was reported transferred the Group I intron to its parasitic fungus *Protomyces inouyei* in the course of evolution (Nishida and Sugiyama, 1995). Hibbett (1996) reported that Group I introns inserted at the same positions (position 943) in SSU rDNA of some fungi. By using intron-specific primers together with flanking rDNA primers, a PCR assay for 39 species of homobasidiomycetes revealed that introns were confined to the genera *Panellus*, *Clavicorona* and *Lentinellus*. Phylogenetic analyses of the nuclear SSU of rDNA and mitochondrial SSU of rDNA sequences suggested *Clavicorona* and *Lentinellus* were closely related, but *Panellus* was more distantly related. A phylogenetic analysis of intron sequences including mushroom introns and other introns retrieved from GenBank by BLAST search, suggested the mushroom introns were monophyletic. The distribution of host lineages and insertion sites among the introns suggested that horizontal and vertical transmission, homing, and transposition have been factors in intron evolution (Hibbett, 1996). Hibbett et al. (1997) submitted two sequences of *Panellus stypticus* and two sequences of *P. serotinus* of partial 18s ribosomal

RNA to the GenBank. One collection had 379 bp Group I insertion while the other collection and two *P. serotinus* collections did not.

Group I introns were found in rDNA of closely related plant pathogenic fungi of the family Sclerotiniaceae: genera *Botrytis*, *Dumontinia*, *Encoelia*, *Grovesinia*, *Myriosclerotinia*. Incongruent branching patterns of intron-based and rDNA ITS-based phylogenetic trees suggested that fungal host genomes and the group I introns did not share a common evolutionary history. A model of horizontal transfer of the intron via virus, viral infection, and / or transformation of new host fungus was proposed (Holst-Jensen et al., 1999).

The variation in distribution and sequence of group I introns within a short highly conserved region of rDNA presents a unique opportunity for examining the molecular evolution and mobility of group I introns within a systematics framework.

1.4. INTRODUCTION TO METHODS USED TO EXAMINE SPECIATION AND DIFFERENTIATION WITHIN A SPECIES OR SPECIES COMPLEX

1.4.1. Introduction to speciation and differentiation and their detection

Speciation is a process by which a genetically cohesive group of interbreeding individuals diverges into two or more genetically distinct groups of individuals (Petersen and Hughes, 1999). Speciation is defined as a process that is marked by formation of new species, either by splitting of a phylogenetic lineage or acquisition of reproductive isolating mechanisms to produce discontinuity between populations. Allopatric speciation has long been considered as the primary speciation mechanism, but, sympatric speciation has been reported (Gibbon, 1996). Locally adapted populations are the first stage in divergence leading to speciation (Petersen and Hughes, 1999). Gene flow, or the movement of genes between populations, determines if and how fast populations can evolve into separate species (Carlile, 1987).

To detect speciation or differentiation within a species, today's researches employ several methods. Mating tests are performed to detect if intercollection compatibility among geographical areas is reduced. Isozyme or allozyme analyses may be also performed to determine if a specific geographic area has unique enzyme profiles or bands (phenotype), indicating differentiation. Molecular analyses such as sequencing, RFLP analyses of ribosomal ITS or IGS regions, DNA hybridization, and/or DNA fingerprinting techniques in a species or species complex, may be conducted to reveal speciation or differentiation.

1.4.2. Isozyme techniques for detecting speciation and differentiation

Isozymes are multiple molecular forms of enzymes derived from the same organism (tissue and culture) that share a common catalytic activity. Different classes of enzymes may have inherently different levels of genetic variation (Acquaah, 1992). Isozymes including allozymes have been routinely used for detecting speciation and differentiation.

Extracellular laccase (ECL), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM), were used to examine collections of *Marasmius scorodonius*, and found that extracellular laccase profiles of *M. scorodonius* in North America and Europe were distinct, though all collections from two continents were sexually intercompatible. The results of these enzyme comparisons were congruent to some extent with intersterility data or morphological species or the geography in *M. scorodonius* (Gordon, 1994).

Pleurotus pulmonarius was completely intercompartible throughout the Northern Hemisphere. Among its seven geographical populations, enzyme indices of similarities ranged from 0.20 –0.63, with the majority under 0.50. Noticeably, the western North American pool shares a 0.35 similarity with the eastern N. American pool. The North China pool shared a 0.49 similarity with the Southern Chinese pool (Petersen, 1995).

McCleneghan (1996) tested eight isozymes and found five enzymes: phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), alcohol dehydrogenase (ADH), fluorescent esterase (F-EST) and hexokinase (HEX) were informative in *Pholiota alnicola.* Collections from the same geographical area tended to have the highest enzymatic similarity in the highly variable taxon *P. alnicola.* In *P. spumosa*, profiles of the five enzymes were consistent with the morphospecies.

1.4.3. Mating tests for detecting speciation and differentiation

Whether two groups are called species or not depends greatly on which species concept is adopted. In the biological species concept (BSC), the ability or inability to interbreed is basic to both allopatric and sympatric speciation. Without free gene exchange, two populations are more likely to express genetic variation (Petersen, 1995). Several studies have used mating tests to examine speciation forces in higher fungi.

Gordon and Petersen (1992) reported that *Marasmius siccus* from Asia and North America showed partial compatibility. In that species, partial compatibilities separated the Japanese population from the North Carolina- Ohio population (Petersen, 1995).

Pleurotus pulmonarius and *P. ostreatus* in North America are morphologically inseparable, but the two species do not interbreed, though they intermix throughout the entire North American temperate zone (Petersen and Hughes, 1993). Allopatry caused by continental separation played an important role in the earlier speciation process of *Pleurotus* (Vilgalys and Sun, 1994). Petersen and Ridley (1996) reported that a New Zealand *Pleurotus* isolate, NZP, was totally compatible with *P. pulmonarius* and partially compatible with three other biological species, suggesting that the NZP's mating ability has been preserved for a long time, while other species were already reproductively isolated.

Intercompatibility, partial intercompatibility, and incompatibility were shown to occur among collections of *Dictyopanus pusillus* from geographically separated locations (Petersen and McCleneghan, 1997).

In *Collybia subnuda*, two intersterility groups were found, both of which share very similar morphology and identical ITS sequences. Collection records indicated that they were sympatric throughout the Appalachian Mountains as far north as New York (Murphy, 1997). Vilgalys et al. (1986; 1987; 1991; 1994) suggested that speciation in the *Collybia dryophila* complex was gradual and involved many steps.

1.4.4. Molecular techniques for detecting differentiation and speciation

RFLP patterns and DNA sequencing have also been used to examine relationships within genera, but, the sequences selected for these studies are more variable than those used for higher level comparisons.

Populations of *Xylaria magnoliae* (*Ascomycota*) were analyzed by rDNA restriction patterns. Two populations one mile apart were found to be phenotypically different while another two collections 400 miles apart showed only small genetic differences. These findings suggested a fine-grained population structure existing for the species, and genetic exchange and spore disposal happened between widely separated localities (Gowan and Vilgalys, 1991).

At the population level, the cultivated *Agaricus bisporus* was thought to have European origin (European population). Pacific coastal populations shared much with the germplasm of cultivated mushrooms and were thought to be recent escapes from cultivated varieties. An Alberta (Canada) population was very different from the European, the Californian, and the Israeli populations and was probably native to Canada (Kerrigan et al., 1995).

Intensive sampling of the British Columbia isolates of *Armillaria* species confirmed the usefulness of a species diagnosis based on restriction site differences in the first ribosomal intergenic

spacer (IGS1). A confusion of *A. gallica* and some *A. sinapina* isolates could be resolved by analyses of the second spacer (IGS2; White et al., 1998).

1.5. GENERAL PURPOSE OF THIS STUDY

The purpose of this research was to examine biogeography, phylogeny and speciation processes within *Panellus stypticus* and to resolve the systematics of the *Panellus* generic complex.

1.5.1. In *Panellus stypticus*:

Biogeography in *Panellus stypticus*: Isozyme electrophoreses, mating tests, morphological comparisons, and molecular analyses were conducted to analyze biogeographical distribution in the species. Questions included: 1) How many intersterility groups there are in the species? 2) Is *P. stypticus* a single biological species over its entire geographical area? and 3) if collections from different geographical areas have differentiated into different populations, is there evidence that gene flow occurred between different geographical areas?

Phylogeny in *Panellus stypticus*: This research was also aimed at reconstruction of a phylogeny for populations within *P. stypticus* based on sequences of the ribosomal ITS region. Evolutionary relationships among different geographical areas (populations) and possible explanations for dispersal within the species were examined.

1.5.2. The *Panellus* complex:

This research was intended to resolve the systematics in the *Panellus* complex which includes *P. stypticus, P. mitis, P. serotinus* (*Sarcomyxa serotina*), *P. ringens, P. violaceofulvus, Tectella patellaris* (*Panellus patellaris*), *P. pusillus* (*Dictyopanus pusillus*) and *Pleurotopsis longinqua* (*Panellus longinquus*). A phylogeny based on molecular analyses was reconstructed to

circumscribe the genus *Panellus*, and evolutionary relationships of those clearly delimited species were determined.

2. MATERIALS AND METHODS

2.1. GENERIC AND SPECIFIC NAMES USED IN THIS RESERCH

Generic names in *Panellus* complex and the species within the complex were used on the basis of the following researchers' descriptions:

Genus *Panellus*: Karsten [1879 Rysslands, Finnlands och den Skandinaviska Halfons Hallsvampar. Finska Letteratur-Sallskapets Tryckeri, Helsinki 32: p248]; Burdsall and Miller [1978, Notes on the genus *Panellus*. Mycotaxon 7: p511-514].

Genus *Dictyopanus*: Patouillard [1900 Sur les families et les genres, Des Hymenomycetes. Essai Taxonomique : p137]; Synonym to *Panellus*: Corner [1986, The Agaric genus *Panellus* Karst. (including *Dictyopanus* Pat.) in Malaysia. Gard. Bull. Sing. 39: p105, 129, and 136]; Burdsall and Miller [1975, A reevaluation of *Panellus* and *Dictyopanus* (Agaricales). Beih. Nova Hedwigia. 75 (51) 79-91].

Genus *Tectella*: Earle [1909, The genera of the North American gill fungi. Bulletin of North York Botanical Garden, 5: p373]; Singer [1986, The Agaricales in Modern taxonomy. Koeltz Scientific Books, Koenigstein, p347-348].

Genus *Sarcomyxa*: Karsten (1891, Symbolae ad Mycologiam Fennicam. Societas pro Fauna et Flora Fennica 18: p62); Horak [1968, Synopsis generum Agaricalium (Die Gattungstypen der Agaricales), in Beiträge zur Kryptogamenflora der Schweiz, Band XIII. Kommissionsverlag Druckerei Buchuler + Co AG, Wabern-Bern: p555-556)].

Genus *Pleurotopsis*: Earle (1909); Horak [1968, Synopsis generum Agaricalium (Die Gattungstypen der Agaricales, in Beiträge zur Kryptogamenflora der Schweiz, Kommissionsverlag Druckerei Buchuler + Co AG, Wabern-Bern: p483-484].

Species *Panellus stypticus*: Karsten [1879 Rysslands, Finnlands och den Skandinaviska Halfons Hallsvampar. Finska Letteratur-Sallskapets Tryckeri, Helsinki 32: p248]; Burdsall and Miller [1978, Notes on the genus *Panellus*. Mycotaxon 7: p511-514].

Species *P. mitis, P. serotinus, P. ringens, P. violaceofulvus* and *Tectella patellaris* (*Panellus patellaris*): Miller [1970, The genus *Panellus* in North America. Michigan Bot. 1970: p17-30].

Species *Dictyopanus pusillus*: Corner [1986, The Agaric genus *Panellus* Karst. (including *Dictyopanus* Pat.) in Malaysia. Gard. Bull. Sing. 39: p137-137].

Species *Pleurotopsis longinqua*: Horak [1983, Mycogeography in the South Pacific region: Agaricales, Boletales. Australian Journal of Botany, Supplementary Series 10: p7].

2.2. COLLECTIONS USED IN THIS STUDY

Basidiomata collected for this study were deposited in the University of Tennessee Fungal Herbarium, Knoxville (TENN). Cultures of collections were deposited in the Department of Botany, University of Tennessee, Knoxville Culture Collection (CULTENN).

Specimens and cultures used in this study are listed in Appendix 2.1. Collections in TENN are accessed by their field number and TENN accession number. Culture collections in CULTENN, including single-spore isolates and dicaryons, are also accessed by their field number and TENN accession number.

2.3. CULTURE METHODOLOGY

2.3.1. Establishment of cultures from field-collected materials

When basidiomata were collected in the field, they were wrapped in wax paper and taken back to the lab. The collection was assigned a field number. Basidioma color, size, pileus shape, lamellae, taste, smell and other morphological data were recorded. A spore print on paper or on

agar was obtained. Basidiomata were then dried in an electric food dryer, and accessioned into TENN.

2.3.1.1. Single spore isolates

To obtain single-spore isolates, several pieces of lamellae, with or without the pileus attached, were hung over MA medium (Malt Agar medium; Appendix 2.2). When spores dropped, single-spore germlings were isolated under a dissecting microscope and inoculated onto MA medium.

2.3.1.2. Dicaryon isolates

Dicaryotic cultures were made either by isolating a plug of MA medium containing dense spores, or simply by cutting a small piece of tissue from a pileus and subculturing it onto MA medium.

2.3.1.3. Culture maintenance

Single-spore isolates were maintained on MA disks in distilled water at room temperature (ca. 23 C) for up to 5 years (Appendix 2.2). Dicaryotic cultures were inoculated on MA agar slants and incubated at room temperature (ca. 23 C) for 20- 40 days until colonized. Finally tubes were placed into a cold room at 4 C for storage for up to two years. Dicaryotic cultures were also maintained in distilled water cryovials (Appendix 2.2).

2.3.2. Growth of cultures for DNA extraction or enzyme studies

For DNA extraction or enzyme studies, a mycelial plug was incubated in PD broth (Appendix 2.2). Cultures were grown for 20 - 40 days depending on the growth rate of a collection at room temperature (ca. 23 C). Mycelium was harvested, blotted dry with a paper

towel and used directly for DNA extraction or enzyme analyses, or wrapped in aluminum foil and stored at –80 C for later use.

2.3.3. Growth of cultures for crossing studies

Single-spore isolates in distilled water cultures were drained and recultured onto MA medium in Petri dishes. Monocaryon status was confirmed by the following miniculture method: 1) a small plug of an agar culture was squashed on a glass slide with a cover slip; 2) the slide was incubated for a few days at room temperature (ca. 23 C) in a moist glass Petri dish. Slides were padded with wet tissue paper to keep them moist; 3) after growth occurred, microscopic observation was performed to check for the presence or absence of clamps.

2.4. MORPHOLOGICAL ANALYSES

2.4.1. Morphological analyses in the *Panellus* complex

2.4.1.1. Specimens examined in the *Panellus* complex

Collections examined macroscopically and microscopically in the *Panellus* complex are listed in Table 2.1. *Panellus stypticus* collections examined morphologically are listed in Appendix 2.3.

Panellus stypticus: All collections with available basidiomata were examined (Appendix 2.3). Exemplars 2675, 3342, 4319 and 6157, used for molecular analyses, were also selected for morphological comparison.

Dictyopanus pusillus: two intersterility groups, group I (collections 8265, 9408, 9522, 9803 and 9808) and group II (collection 9421) were included for morphological analyses. Collections from geographically separated areas were selected.

Panellus mitis: All three available collections, 5644, 7486 and 7494, were examined.

Table2.1. Collections examined macroscopically and microscopically in the genus *Panellus*

collection No.	TENN accession No.	species	origin
2675	48833	*Panellus stypticus*	New Zealand, Fjordland
3342	48835	*Panellus stypticus*	Russia, Premorsk
4319	50398	*Panellus stypticus*	Switzerland, Maggia
6157	52316	*Panellus stypticus*	USA, TN, Polk Co.
7699	53778	*Dictyopanus* sp.	Costa Rica, Prov. Cartago
7809	53765	*Dictyopanus* sp..	Costa Rica, Prov. San Jose
8265	54317	*Dictyopanus pusillus*	USA, FL, Ocala National Forest
9408	56382	*Dictyopanus pusillus*	Costa Rica, Prov. Alajuena
9522	56278	*Dictyopanus pusillus*	USA, FL, Quincy
9803	56342	*Dictyopanus pusillus*	Puerto Rico, El Yunque
9808	56347	*Dictyopanus pusillus*	Puerto Rico, El Yunque
9421	56395	*Dictyopanus pusillus*	Costa Rica, Prov. Cartago
5644	52389	*Panellus mitis*	USA, ID, Bonner Co.
7486	53512	*Panellus mitis*	Finland, Prov. Etela-hame
7494	53511	*Panellus mitis*	Finland, Prov. Etela-hame
6894	53450	*Panellus serotinus*	USA, NC, Macon Co
9992	58324	*Panellus serotinus*	USA, NC, Macon Co.
6711	52365	*Panellus ringens*	USA, AK, Turnagain Arm
9812	58308	*Panellus ingens*	USA, NC, Graham Co.
DAOM 198753	58323	*Panellus violaceofulvus*	Canada, Quebec, Forillon National Park
6170	52302	*Tectella patellaris*	USA, NC, Macon Co.
6486	53403	*Tectella patellaris*	USA, NY, Duchess Co.
6662	53214	*Pleurotopsis longinqua*	USA, WA, Clallam Co.
6663	53215	*Pleurotopsis longinqua*	USA, WA, Grey's Harbor Co.

Note: 1) In *P. stypticus*, a total 69 collections were examined (Appendix 2.3).
2) In *D. pusillus*, collections 8265, 9408, 9522, 9803,9808 belonged to intersterility group I. Collection 9421 was in intersterility group II.
3) DAOM198753 was from Canadian National Mycological Herbarium (DAOM).

Panellus serotinus: Two collections, 6894 and 9992, were examined. Collection 6881 used for molecular analyses was not included because the basidiomata were heavily contaminated. Collection 8885 designated as *P. serotinus* but with different zymograph was also included in the morphological examination.

2.4.1.2. Characters examined for every collection

Morphocharacters examined included: basidioma general stature and hymenium structure; pileus pendent, bell-shaped or not; lamellae equal length; lamella forked or not; substipitate or sessile (pseudostipitate); spore size, shape; spore surface color patches; spore reaction in Melzer's solution (Appendix 2.4); cheilocystidia length, shape; pleurocystidia length, shape; gelatinousness of pileus surface; basidioma revivability; host of basidiomata.

2.4.1.3. Measurement of morphocharacters

For microscopic characters, a basidioma was held (trapped) in pith, and dissected under a dissecting microscope at a magnification of 10 X or 20 X, depending on basidioma size. Specimens were sliced as thin as possible under the dissecting microscope. Some microscopic characters were observed at 10 X or 20 X magnification. Other micro-characters were observed with a Wild compound microscope (Switzerland) at a magnification of 187.5 X or 1875 X (oil immersion). Measurements were aided by an ocular micrometer. Drawing was free hand with emphasis on retaining the shape, superficial structure and structural ratio.

The ocular micrometer in the Wild compound microscope was calibrated with a haemocytometer (Fisher Scientific Co.) at a magnification of 1875 X. One unit of the ocular micrometer was converted to 8.2 μm (1 unit = 8.2 μm).

A second ocular micrometer in the dissection microscope (AO 41) at a magnification of 20 X was calibrated by observing a 10 mm metric ruler. One unit equaled 0.24 mm (1 unit = 0.24 mm).

2.4.1.4. Number of measurements per character

At least 10 sections were observed. Twenty to thirty lamellae were observed for cystidia at a magnification of 1875 X. If cystidia and spores were abundant, then typical cystidia and spores were selected for drawing. If cystidia or spores were scarce, more dissections were made and examined for clear images of cystidia and spores.

2.4.1.5. Number of characters examined per specimen

For microscopic characters at high magnification, at least ten spores were measured and drawn. At least five typical hymenia, cheilocystidia and pleurocystidia were measured and drawn.

2.4.1.6. UPGMA analyses

Morphological characters including macroscopic and microscopic characters in the genus *Panellus* complex were analyzed using UPGMA (Unweighted Pair Group Method using Arithmetic averages). Morphocharacters were assumed to be independent with an equal possibility of change. Characters that were not available were treated as missing data. The UPGMA tree was constructed by using Phylip (Phylogeny Inference Package, V3.5C) (Felsenstein, J. 1991).

2.4.2. Characters examined in *Panellus stypticus*

2.4.2.1. Number and selection of the specimens examined

Panellus stypticus collections examined are listed in Appendix 2.3. If a collection had enough basidiomata, then mature but intact basidiomata were selected for dissection. In some cases, there was only one or a few basidiomata available. In this case only one or the only mature fruit body was selected for dissection.

2.4.2.2. Measurement of morphocharacters in *Panellus stypticus*

Methods used in *Panellus stypticus* were the same as those used for the *Panellus* complex.

For microscopic characters observed at low magnification with the dissecting microscope, one or several moderately mature basidiomata were cut perpendicular to the stipe at a position 2/3 –3/4 of the distance from the stipe to the distal margin. Eight lamellae were measured for their height by using an ocular micrometer at a magnification of 20 X.

2.4.2.3. Number of measurements per character

The method and number of measurements per character were similar to those used in the *Panellus* complex (morphological analysis **2.4.1.4**).

2.4.2.4. Characters examined per specimen

In *Panellus stypticus*, macrocharacters examined included: basidioma general stature panelloid or not (Corner, 1986); lamellae height; lamellae forked or not; lamella anastomosis present or absent; and structure of lamella anastomosis.

Microcharacters examined included: spore size, shape; cheilocystidia length, shape; pleurocystidia length, shape; distribution of oil droplets.

2.4.2.5. Analyses of characters between collections and geographical areas

Morphological characters described above, were grouped and compared among geographical areas. For spores, a linear equation ($Y = a + b X$; in which X: spore length; Y: spore width; a: constant; b: slope of the equation) and correlation (r) between spore length and width were calculated. Average spore size and standard deviation were plotted for each geographical area.

2.5. ISOZYME ANALYSIS

2.5.1. Culture preparation

Time trials were performed to determine the best period for assaying enzyme activity (clarity of bands and appearance of weak bands) and phenotype (band mobility) over time. Cultures were sampled at 19, 23, 27, and 30 days. It was determined that when a colony reached a relative size of ca.70% of 50 ml of PD broth, (i.e. about a 26- 27 day period growth), the stage was optimum for extracting extracellular and intracellular enzymes. This time schedule was used throughout this part of the study.

2.5.2. Culture medium for extracellular laccase

Extracellular laccase (ECL) was the only extracellular enzyme examined. In *P. stypticus*, all electrophoreses, including time tests, were performed with fresh liquid cultures. In the *Panellus* complex, all electrophoreses used fresh culture medium except for a very few collections that grew rapidly. For these, approximately 1 ml liquid medium was stored at –80 C for later assays. Kerrigan and Ross (1988) reported that liquid medium for extracellular laccase (ECL) stored at –20 C would be stable for a few months.

2.5.3. Extraction of intracellular enzymes

After 27 days incubation in PD broth, mycelia were filtered through nylon mesh in a funnel. The original agar inoculum was removed and mycelium was dried with paper towels. Mycelia were used immediately or wrapped in aluminum foil and frozen for future assays.

For intracellular enzyme extraction, approximately one gram of fresh paper-dried mycelium was put into an ice-cold mortar with 500 µl of modified Correa-Victoria extraction buffer (Appendix 2.5), then ground with sterile sand. If mycelium weighed less than one gram, extraction buffer was adjusted proportionally to the weight of the mycelium. Mycelium was ground on ice and the slurry was centrifuged at 12,000 rpm for 10 min. The supernatant was used immediately or frozen at –80 C for use in the second day.

2.5.4. Techniques for making polyacrylamide gels

Electrophoreses were carried out on vertical polyacrylamide slab gels (gel size approximately 7 X 8 cm: Bio-Rad Mini protean II Multi-Casting System) using instructions by the manufacturer and a modified Laemmli buffer system described by Kerrigan and Ross (1988). Gels consisted of 4.0% stacking and 10.0% separating gels respectively. Recipes and buffers are listed in Appendix 2.5. The buffer system was a discontinous system (Kerrigan and Ross, 1988): the upper buffer was pH 8.3, lower buffer was pH 7.5 (Appendix 2.5).

2.5.5. Sample preparation

For electrophoreses, 32 µl fungal extract was combined with 1 µl 0.5% bromphenol blue tracking dye and 2 µl 50% (w/v) sucrose (Appendix 2.5). The sample was added to each gel well using small pipette tips.

2.5.6. Gel staining and recording

Gels were removed from the gel apparatus and placed in staining trays.

For extracellular laccase (ECL), gels were immersed in an ethanol solution of *o*-tolidine for 15-30 min (Appendix 2.6). After removing gels from the stain, gels were wrapped in plastic wrap for one to a few hours, a step that would darken bands significantly. Gels were photographed 1-3 times with a digital camera (Kodak, Scientific documetation and file system, model 120).

Staining for alcohol dehydrognase (ADH), superoxide dismutase (SOD), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM) and shikimate dehydrogenase (SKDH) followed Z-R.Wang's method (Wang, unpubl.). Gels for intracellular enzymes of estrase (EST), peroxidase (POD) followed A-M.Wang's method (Wang, pers. comm.). Recipes and detailed procedures for these intracellular enzymes are listed in Appendix 2.6.

2.5.7. Preliminary tests to select appropriate enzymes

Preliminary electrophoresis trials were performed using selected collections to select enzymes with clear, informative banding patterns.

2.5.8. Enzymes examined

Isozymes LAP, MDH, PGI, PGM and SOD were excluded from further analysis due to poor resolution of bands; POD was detected for all collections, but it was excluded from analyses due to ambiguous, complex patterns.

ECL was used for infraspecific analyses in *Panellus stypticus* and for separating or diagnosing species within the *Panellus* complex. ECL patterns in *Panellus serotinus*, *Panellus* sp., *Dictyopanus pusillus* and /or *Dictyopanus* sp. were evaluated. EST and ADH were also used to reveal variation within *Panellus stypticus*.

2.5.9. Controls on each gel

After preliminary studies, collection 5277 was used as the control for ECL electrophoreses. The first band below the sample well of 5277 was set as the standard. Collection 5185 was used as the control for ADH electrophoreses. The first band of 5185 was set as the standard. Collection 6880 was used as the control for EST electrophoreses. The third band of 6880 was set as the standard.

2.5.10. Data analyses

ECL banding patterns were examined to determine if there were species-specific patterns. Collections with unusual banding patterns were subjected to further morphological analysis. The first ECL band below the wells (ECL1), the fastest band (bands) of EST (EST1) and the third band of ADH (ADH3) were analyzed for infraspecific variation in *P. stypticus*.

Rf rates were calculated for bands of all the collections under analyses. Rf = (the distance of a particular band moved from the sample well) / (the distance of the front dye moved from the sample well). Rf was used for analyses in the *Panellus* complex.

Rm rates were calculated for ECL1, EST1 and ADH3 bands for all collections *in P. stypticus* collections. Relative mobility Rm = (Rf rate of a particular band) / (Rf rate of the standard band of the control).

In ECL1 and EST1, Rm rates were further sorted into six electrophoretic mobility groups based on Rm values and grouped based on the geographical origin of the collection. The number of collections in each electrophoretic mobility group in each geographical area was calculated.

2.6. MATING STUDIES

2.6.1. Self crosses to determine mating types

One to three collections were selected from each geographical area for self crosses. For each collection, normally12-14 monocaryons were paired in a semi-diallel grid. A collection which was viable and had clear-cut mating behavior was designated as the exemplar for a geographical region.

Few collections were available from the Southern Hemisphere. There were only two to three collections from New South Wales and Tasmania, with relatively low viability, so fewer than 12 monocaryons were used in self crosses. The exemplars were selected based on availability of viable monocaryons.

2.6.2. Selection of mating exemplars in *Panellus stypticus*

An exemplar was chosen from each geographical area that was viable and had typical mating behavior based on self-crosses. Seven exemplars (Table 2.2) were chosen from seven geographical areas. Self crosses for mating exemplars are listed in Appendix 2.7: Appendix 2.7.1 for exemplar 9810 (USA, TN); Appendix 2.7.2 for exemplar 9029 (USA, CA); Appendix 2.7.3 for exemplar 3180 (Russia, Premorsk); Appendix 2.7.4 for exemplar 8926 (Russia, Caucasia); Appendix 2.7.5 for exemplar 2664 (New Zealand); Appendix 2.7.6 for exemplar 3567 (Australia, New South Wales); Appendix 2.7.7 for exemplar 3586 (Australia, Tasmania).

2.6.3. Division of collections based on geographical areas in *Panellus stypticus*

A total 57 collections with moncaryons were used in the mating tests. They were divided into seven groups based on geographical areas (populations): Eastern North America, Pacific North America, Far East, Europe, New Zealand, New South Wales and Tasmania, Australia.

Table 2.2. Exemplars chosen as tester collections for mating tests

exemplars	TENN accession No.	origin	geographical areas	tester monocaryons	
9810	58306	USA, TN	Eastern North America	8: A2B2	12:A1B1
9029(A)	58325	USA, CA	Pacific North America	1:A1B1	2:A2B2
3180	48834	Russia, Premorsk	Far East	1:A1B1	2:A2B2
8926	55546	Russia, Caucasia	Europe	1:A1B1	13:A2B2
2664	48830	New Zealand	New Zealand	5:A2B2	20:A1B1
3567*	50067	Australia, New South Wales	Australia, New South Wales	1: A1B1	10:A1B2
3586	50140	Australia, Tasmania	Australia, Tasmania	4:A1B1	8:A2B2

*3567 was selected as the exemplar of New South Wales, Australia because it was the only viable collection among three collections from this area, even though its self cross showed unusual pairing behavior responses.

2.6.4. Procedures for setting up matings

Two tester monocaryons, normally two opposite mating genotypes, (i.e. A1B1 & A2B2, or A1B2 & A2B1) were chosen from each exemplar to cross to four monocaryons from each other collection in this study.

In a mating, two monocaryotic inoculum plugs were placed approximately five mm (5 mm) apart on MA medium in Petri dishes (5 cm diameter). Petri dishes were incubated at room temperature (ca. 23 C) long enough for colonies to contact and possibly to overgrow. Petri dishes were examined for mating compatibility and other mating behaviors 20-40 days after inoculation, depending on growth of the colonies.

2.6.5. Criteria for determining a successful mating

The contact zone and two opposite sides from the contact zone were observed microscopically for presence or absence of clamp connections. Abundant clamp connections were regarded as a compatible mating. No clamp connection observed in the contact zone was

regarded as an incompatible mating. On a few occasions, epifluorescence microscopy with DAPI (Appendix 2.4: Hughes, unpub.) was used to confirm the presence or absence of clamps.

2.6.6. Criteria for scoring a mating as abnormal behavior in *Panellus stypticus*

Mating behaviors were also recorded. Definitions and terms of mating behaviors are listed in Table 2.3. Abnormal mating behaviors observed in this study were antagonism, flat-like reactions and barrage-like reactions in compatible matings. Antagonism was considered as a phenomenon similar to somatic incompatibility. flat-like reactions and barrage-like reactions appearing in compatible matings were considered as atypical mating behaviors.

2.6.7. Mating studies within *Dictyopanus pusillus*

Many of the *Dictyopanus* collections were originally designated as *Dictyopanus* sp. Collection 9808 was selected as a mating exemplar due to its relatively high viability. A self-cross of 12 monocaryons of 9808 was carried out, and two monocaryons with opposite mating genotypes were designated as tester monocaryons. The two tester monocayons 9808-1 (A1B1) and 9801-3 (A2B2) were crossed with four monocaryons of all *Dictyopanus* collections with available monocaryons to determine the number of intersterility groups in *Dictyopanus* collections. The self cross of 9808 is given in Appendix 2.8.

2.6.8. Other mating studies in the genus complex

Collection 7577 (*Pleurotopsis longinqua* based on morphology) was chosen as an exemplar for this species. The self-cross of 7577 is given in Appendix 2.9. Two tester monocaryons 7577-11 (A1B1) and 7577-12 (A2B2) were used to cross to four monocaryons of every collection with monocaryons which were designated as *Panellus* sp.

Table 2.3. Definitions and terms of mating tests and behaviors

antagonism	Somatic incompatibility. Two monocaryons in a pairing produce pigment deposition in the contact line with or without a very dense mycelial build-up.
barrage reaction (B)	In a typical pairing, if two monocaryons with close affinity have common B in mating genotypes, the pairing is marked with a mycelial line built up between two parental colonies (McCleneghan, 1996).
compatible	A pairing between two monocaryons produced abundant clamp connections and a third heterozygous colony could overgrow two parental colonies in a typical pairing.
contact line, contact zone	The area or a line formed between two colonies from inoculum disks.
cross	Mating, pairing
dicaryon	An individual isolate with two nuclei but not diploid.
exemplar collection	A collection selected from a group or a geographical area to stand for the group. An exemplar is viable or has typical characteristics, such as good mating behavior for mating tests (McCleneghan, 1996).
flat reaction (F)	In a typical pairing, if two monocaryons with close affinity have common A in mating genotypes, thus the pairing is marked with a crevassse surrounded by two mycelial lines in the contact zone.
incompatible	A pairing between two monocaryons produces no clamp connections and in a typical pairing two parental colonies merge together.
mating	Cross, pairing.
monocaryon	An individual isolate with only one nucleus per cell (haploid) with one mating genotype.
pairing	Mating , cross
self cross	Monocaryotic isolates from a collection (fungal individual) paired together. Theoretically, pairings will show up a 25% compatibility for a tetrapolar fungus, and a 50% compatibility for a bipolar fungus.
tester monocaryon	Monocaryon isolates with known mating genotypes.

Seven collections originally designated as *Panellus* sp. in CulTenn had available monocaryons. They were not *Panellus stypticus, P. mitis, P. ringens, P. serotinus, Tectella patellaris*, or *Dictyopanus pusillus* based on their morphology. All seven collections crossed to exemplars 7577 (*P. longinqua*) and 9029 (*P. stypticus*).

Further mating tests using *P. stypticus* exemplar 9029 (USA, CA) with collections 3570, 7435, 7543, 7511, and 7577 (*Pleurotopsis longinqua*) were carried out.

2.7. MOLECULAR ANALYSES

2.7.1. Extraction and storage of DNA

DNA extraction followed the procedure of Carlson (Carlson et al., 1991) as modified by Hughes et al. (1998). All solutions used for DNA extraction are given in Appendix 2.10. Extraction products were verified in 0.8% agarose gel to determine DNA yield. Lamda *Hind* III was used as a standard molecular marker (Appendix 2.11). Gels were stained in 0.5 X TBE (tris-boric acid-EDTA) buffer with 0.5 µg/ml ethidium bromide.

2.7.2. PCR amplification

The ribosomal DNA repeat is illustrated in Figure 2.1. All the primers used in this research are listed in Appendix 2.12 and their positions are shown in Figure 2.1.

2.7.2.1. ITS region

The ITS1-5.8s-ITS2 region of the ribosomal DNA was amplified for sequencing and RFLP analyses using ITS4 and ITS5 as the primers (White et al, 1990). A typical PCR amplification reaction mixture is listed in Appendix 2.13. Reaction parameters for amplification of the ITS region were: 94 C for 3 min, 35 repetitions of 94 C for 1 min, 52 C for 1 min, and 72 C for 1 min. The final cycle was an extension at 72 C for 3 min. If DNA had been frozen for a long time,

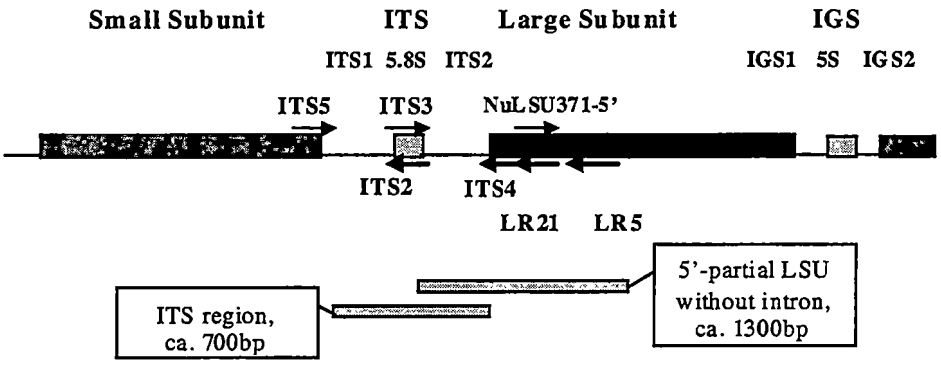


Figure 2.1. An illustration of the nuclear ribosomal DNA repeat, showing SSU, ITS, LSU and IGS, and primer sites for ITS and LSU amplification

PCR reaction mixtures were pre-heated at 94 C for 4 min before adding Taq polymerase which enhanced the ability to amplify. PCR products were verified by 0.8% agarose gel electrophoreses.

2.7.2.2. 5'-partial LSU

The 5'-end partial ribosomal LSU region was amplified for sequencing and intron detection with primers ITS3 and LR5 (White et al, 1990; Vilgalys and Sun, 1994).

Reaction parameters were: 94 C for 3 min followed by 35 repetitions of 94 C for 0.5 min, 50 C for 1 min, and 72 C for 1.5 min. The final cycle was an extension at 72 C for 3 min. Mixtures were preheated at 94 C for 4-5 min before adding Taq polymerase. PCR products were verified by 0.8% agarose gel electrophoreses.

2.7.3. Gel evaluation for size and intron presence

PCR amplification with primers ITS3 and LR5 amplified a ca. 1300 bp fragment in some collections and a ca. 1700 bp fragment in other collections. PCR amplification for all collections with available cultures in *P. stypticus*, *D. pusillus*, *P. mitis*, three *P. serotinus* collections and exemplar collections of *P. ringens*, *P. violaceofulvus* and *Pleurotopsis longinqua* were conducted. PCR products in the 5' –partial LSU were evaluated for length differences by electrophoreses on a 1.5% agarose gel. ØX174 Hae III was used as a molecular marker.

2.7.4. Purification of PCR amplification products for sequencing

For sequencing, 150-200 µl PCR product was amplified. The PCR product was electrophoresed using a one-well low-melt 1.5% TAE agarose gel (~65 C, NuSieve GTG Agarose, FMC Bioproducts) containing 0.5 µ/ml ethidium bromide. Only one product was electrophoresed at a time. The desired band was excised with a sterile scalpel. Approximately 300-450 mg of agarose with the desired PCR product was excised. Purification of the product was carried out with a Wizard PCR Kit (Promega) following manufacturer's instructions (Appendix 2.14). After the purification, the product was verified in an 0.8% or 1.5% agarose gel to determine purity, and its concentration was estimated by comparison with 200 ng of ØX174-Hae III molecular marker (Appendix 2.11).

2.7.5. Sequencing

Sequencing was carried out on an ABI 373 DNA sequencer (ABI Prism Dye Terminator Cycle, Perkin-Elmer Inc.) with primers ITS2, ITS3, ITS4, ITS5 for ITS1-5.8S-ITS2 region, and ITS3, LR5, LR21 (White et al, 1990; Vilgalys and Sun, 1994), and NuDNA5'-371 (Appendix 2.12) for the 5'- partial LSU region.

2.7.6. Sequence correction

Sequences were corrected by comparison of sequences produced by different primers. Primer ITS3 and ITS5 read from 5' to 3' direction, while primer ITS2 and ITS4 read from 3' to 5' direction. Legible sequences generated by ITS5 were compared with legible sequences of ITS2, and legible sequences generated by ITS3 were compared with legible sequences of ITS4. Sequence correction was also checked by multisequence alignment. When a different base pair or indel appeared, the base pair was checked in the original sequence electrophoregrams.

2.7.7. Sequence alignment

The GCG program (Genetic Computing Group, version 9.1, 1997) was used for sequence editing and alignment. The GCG program 'pileup', was used to generate an aligned file. Manual adjustments were made to the alignment.

2.7.8. Restriction Fragment Length Polymorphisms for comparisons within *P. stypticus*

2.7.8.1. Selection of enzymes

ITS sequences of exemplars from different geographical areas were aligned. Restriction sites were mapped using the map program in GCG. Sequence variability in regions recognized by restriction enzymes was exploited to examine geographical variability. After comparison, four restriction enzymes, *BstZ* I, *Dra* I, *Hinf* I and *Taq* I, were chosen to detect restriction site differences. Information on the four restriction enzymes is given in Appendix 2.15.

2.7.8.2. Location of restriction sites and expected fragments

The ITS sequence of each exemplar was mapped for each of the four restriction enzymes using the GCG map program. Expected fragment sizes were calculated and confirmed by 1.5% agarose gel electrophoreses.

2.7.8.3. Restriction digestion procedures

Enzyme concentration and specific buffers followed manufacturer's instructions (Appendix 2.15). Electrophoresis in 1.5% agarose was used to visualize the digestion results. ØX174, *Hae* III digestion was used as the molecular marker. Digestion with each of the four restriction enzymes were made for all of the *P. stypticus* collections. Fragment lengths of those collections were compared to exemplars with known fragment lengths.

After preliminary tests for optimal concentration of restriction enzymes, digestion conditions were as follows:

For a single digestion with *BstZ* I: 5.8 µl H_2O + 1 µl 10x buffer D (Promega) + 0.1 µl bovine serum albumin (BSA) + 0.1 µl 10u/µl *Hinf* I +3 µl PCR product. Mixture was digested at 50 C for ca.1 hr.

For a single digestion with *Dra* I: 5.6 µl H_2O + 1 µl 10x buffer B (Promega) + 0.4 µl 10u/µl *Dra* I +3µl PCR product. Mixture was digested at 37 C for ca. 1 hr 45 min.

For a single digestion with *Hinf* I, 5.8 µl H_2O + 1 µl 10x buffer B (Promega) + 0.2 µl 10u/µl *Hinf* I +3µl PCR product. Mixture was digested at 37 C for ca. 1 hr.

For a single digestion with *Taq* I, 5.6 µl H_2O + 1 µl 10x buffer E (Promega) + 0.4 µl 10u/µl *Taq* I +3µl PCR product. Mixture was digested at 65 C for ca. 1 hr 20 min.

2.7.8.4. Data analysis and documentation

Restriction fragment length polymorphism (RFLP) patterns for *P. stypticus* collections were plotted on a world map.

2.7.9. DNA sequence analysis for phylogeny within *P. stypticus* and in *Panellus* complex

2.7.9.1. Parsimony analyses

Multi-sequence alignments for the species *P. stypticus* and the *Panellus* complex were analyzed using parsimony analyses in PAUP, version 4.0. All characters were considered of equal weight and unordered. Variable characters were considered as parsimony informative. Gaps were considered as missing characters. When more than one most parsimonious trees resulted, a bootstrap 50% majority-rule consensus tree was computed.

2.7.9.2. Neighbor Joining analyses

Multi-sequence alignments for the species *P. stypticus* and the genus *Panellus* complex were also analyzed using Neighbor-Joining analyses. Neighbor-Joining analyses reflected general similarity of two individuals in a whole data set. Phenograms of the species *P. stypticus* and the genus *Panellus* complex were constructed using the Neighbor-Joining program in GCG (1997).

2.7.9.3. Bootstrap analysis

Bootstrap support for phylograms were estimated by 100 replicates of the original multisequence data set.

2.7.9.4. Trees

Nexus tree files were generated after PAUP analyses and Neighbor-Joining analyses. A text tree file was produced from the Phylip program. Nexus tree files and text tree files were imported into TreeView (Page, 1998) to display dendrograms.

2.7.9.5. Obtaining additional sequences from GenBank

During the reconstruction of a phylogeny within *P. stypticus* and within the genus *Panellus* complex, a BLAST search of ITS and LSU sequences in the GenBank (*hppt://www.ncbi.nlm.nih.gov*) produced several close matches. These sequences were downloaded and included in the phylogenetic analyses. Downloaded sequences from GenBank which were closest matches to the sequences of the genus *Panellus* complex are listed in Table 2.4.

Table 2.4. Downloaded sequences from GenBank for reconstruction of phylogeny in the genus *Panellus* complex

Species	Collec-tion or isolate	GenBank accession No.	Loca-tion in rDNA	Authors	Publication and/or source	What retrieval was based on
Panellus stypticus	DS 93-213	U59090	SSU	Hibbett, D.S.	Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi Mol. Biol. Evol. 13 (7), 903-917 (1996)	*Panellus stypticus* 4319 and *Dictyopanus pusillus* 8265, intron sequence.
Resinomycena acadiensis	DAOM 169949	AF042638	LSU	Moncalvo, J..M., Lutzoni, F., Rehner, S., Johnson, J., Vilgalys, R.	Molecular phylogeny of the Agaricales based on 25S rDNA sequences. (unpublished, in press)	*Panellus stypticus* 4319 and *Dictyopanus pusillus* 8265, LSU without Group I intron
Hygrocybe citrinopallida	930731-1	U66435	LSU	Lutzoni, F.	Phylogeny of lichen- and non lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Syst. Biol. (1997) 373-406.	*Panellus mitis* 5644; *P. serotinus* 6881; *Tectella patellaris* 6170, LSU
Pleurotopsis longinqua	RV95/47	AF042604	LSU	Moncalvo,J..M., Lutzoni,F., Rehner,S., Johnson,J., Vilgalys,R.	Molecular phylogeny of the Agaricales based on 25S rDNA sequences. (unpublished, in press)	*Pleurotopsis longinqua* 6662; *Panellus ringens* 6711; *P. violaceofulvus* CBS391.50, LSU
Mycena clavicularis	RV87/6	AF042637	LSU	Moncalvo,J..M., Lutzoni,F., Rehner,S., Johnson,J. Vilgalys,R.	Molecular phylogeny of the Agaricales based on 25S rDNA sequences. (unpublished, in press)	*Panellus stypticus* 4319; *Dictyopanus pusillus* 8265, LSU
Mycena galericulata	RV87/ 14.01	AF042636	LSU	Moncalvo, J.M., Lutzoni, F., Rehner, S., Johnson, J., Vilgalys, R.	Molecular phylogeny of the Agaricales based on 25S rDNA sequences. (Unpublished, in press)	*Panellus stypticus* 4319; *Dictyopanus pusillus* 8265, LSU
Mycena rutilanthiformis	JM96/26	AF042606	LSU	Moncalvo, J..M., Lutzoni, F., Rehner, S., Johnson, J. Vilgalys, R.	Molecular phylogeny of the Agaricales based on 25S rDNA sequences. (unpublished, in press)	*Panellus stypticus* 4319; *Dictyopanus pusillus* 8265, LSU
Saccharomyces cerevisiae	N/A	J01355	LSU	Bayev, A., Georgiev, O. I. et al.	The structure of the yeast ribosomal RNA genes. 3. precise mapping of the 18s and 25s rRNA genes and structure of the adjacent regions. Nucleic Acids Res., 9(4)789-799, 1981	N/A

3. RESULTS

3.1. RESULTS OF MORPHOLOGICAL ANALYSES

3.1.1. Morphological analyses in the genus *Panellus* complex

3.1.1.1. Macroscopic characters in the genus *Panellus* complex

Macroscopic characters of exemplars in the *Panellus* complex are listed in Table 3.1.

"Panelloid" is a descriptive term for basidioma stature as used by Corner (1986) (he didn't define the term, however). In this study, panelloid refers to a basidioma with a short conspicuous lateral stipe, which is concolorous or slightly different to the pileus, and clearly is not part of the pileus. Lamellate or poroid hymenia emerge above the stipe and the pileus is typically fan-shaped (pleurotoid). Thus "nonpanelloid" refers to basidioma statures that do not meet these characters.

The stature of *Panellus stypticus* and *Dictyopanus pusillus* could be best described as panelloid. In *Panellus stypticus,* generally all the North Hemisphere collections were considered as panelloid. Some collections in the Oceanian areas, however, were considered as nonpanelloid.

All the collections of *Dictyopanus pusillus* examined were panelloid. All other species (*P. mitis, P. serotinus, P. ringens, P. violaceofulvus T. patellaris* and *P. longinqua*) examined in the genus complex were nonpanelloid.

Basidiomata of *P. mitis* and *P. serotinus* are considered as substipitate, in which the so-called stipe is also part of the pileus, though they are also pleurotoid. *P. ringens* and *T. patellaris* are bell-shaped (pseudostipitate). The pendent pseudostipe is eccentric and is part of the pileus. They are not considered panelloid.

At least two intersterility groups (biological species) were encountered among collections originally designated as either *Dictyopanus pusillus* or *D. sp.* based on mating tests (Appendix 3.5). Among those participating in mating tests, intersterility group I of *Dictyopanus pusillus*

Table 3.1. Macroscopic morphocharacters in the genus *Panellus* complex

Collection No.	TENN Accession No.	Species	Origin	Hymenium	Basidioma stature	Stipe	Membranous veil	Pileus pubescence	Lamellae density	Lamellae equal length	Lamellae forked
2675	48833	*P. stypticus*	New Zealand, Fjordland	lamellate	panelloid	substipitate	–	–	close	–	–
3342	48835	*P. stypticus*	Russia, Premorsk	lamellate	panelloid	substipitate	–	–	close	–	–
4319	50398	*P. stypticus*	Switzerland, Maggia	lamellate	panelloid	substipitate	–	–	close	–	–
6157	52316	*P. stypticus*	USA, TN, Polk Co.	lamellate	panelloid	substipitate	–	–	close	–	–
7699	53778	*D.* sp.	Costa Rica, Prov. Cartago	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
7809	53765	*D.* sp.	Costa Rica, Prov. San Jose	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
8265	54317	*D. pusillus*	USA, FL, Ocala N. Forest	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
9408	56382	*D. pusillus*	Costa Rica, Prov. Alajuena	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
9522	56278	*D. pusillus*	USA, FL, Quincy	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
9803	56342	*D. pusillus*	Puerto Rico, El Yunque	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
9808	56347	*D. pusillus*	Puerto Rico, El Yunque	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
9421	56395	*D. pusillus*	Costa Rica, Prov. Cartago	poroid	panelloid	substipitate	–	–	N/A	N/A	N/A
5644	52389	*P. mitis*	USA, ID, Bonner Co.	lamellate	substipitate pleurotoid	substipitate	–	–	distant	–	–
7486	53512	*P. mitis*	Finland, Prov.Etela-hame	lamellate	substipitate pleurotoid	substipitate	–	–	distant	–	–
7494	53511	*P. mitis*	Finland, Prov.Etela-hame	lamellate	substipitate pleurotoid	substipitate	–	–	distant	–	–
6894	53450	*P. serotinus*	USA, NC, Macon Co	lamellate	pleurotoid	substipitate	–	–	crowded	almost +	–
9992	58324	*P. serotinus*	USA, NC, Macon Co.	lamellate	pleurotoid	substipitate	–	–	crowded	almost +	–
6711	52365	*P. ringens*	USA, AK, Turnagain Arm	lamellate	bell-shaped pendent	sessile, pseudostiptate	–	+	subdistant	–	–
9812	58308	*P. ringens*	USA, NC, Graham Co.	lamellate	bell-shaped pendent	sessile, pseudostiptate	–	+	subdistant	–	–
DAOM 198753	DAOM 198753	*P. violaceofulvus*	Canada, Quebec, Forillon National Park	lamellate	bell-shaped pendent	sessile	–	+	distant	–	–
6170	52302	*T. patellaris*	USA, NC, Macon Co.	lamellate	pendent	sessile, pseudostiptate	+	+	close	–	–
6486	53403	*T. patellaris*	USA, NY, Duchess Co.	lamellate	pendent	sessile, pseudostiptate	+	+	close	–	–
6662	53214	*P. longinqua*	USA, WA, Clallam Co.	lamellate	pleurotoid	sessile, pseudostiptate	–	–	distant	–	+
6663	53215	*P. longinqua*	USA, WA, Grey's Harbor Co.	lamellate	pleurotoid	sessile, pseudostiptate	–	–	distant	–	+

Note: 1) In *P. stypticus*, a total 69 collections were examined, see Appendix 2.6. 2) N/A: not available; + stands for yes; – stands for no.
3) In *D. pusillus*, collections 8265, 9408, 9522, 9803,9808 belonged to intersterility group I, collection 9421 was in intersterility group II.
4) Substipitate: stipe extended from pileus usually laterally; Pseudostipitate: sessile, stipe is part of pileus, usually eccentrically.

included collections 3493, 4861, 8733, 8265, 8271, 8747, 9096, 9408, 9522, 9803, 9807 and 9808. Collection 9421 belonged to intersterility group II. Macroscopically and microscopically, morphocharacters of collection 9421 were not distinct from intersterility group I, including basidioma stature, pore structure, cystidia shapes and lengths (see Table 3.2 and Table 3.3).

Panellus mitis is unique in that it grows always on coniferous hosts (collections 5644, 7486 and 7494, see Appendix 2.1), and it has a large hymenium-free area near its substipe.

Panellus serotinus is substipitate, and is the only species with crowded lamellae of almost equal length. Lamellae are white, high, smooth, and entire. Due to a thick pileus, *Panellus serotinus* was not revivable when dried specimens were rehydrated.

Panellus ringens, Panellus violaceofulvus and *Tectella patellaris* are bell-shaped, sessile or pseudostipitate species.

In *Panellus ringens*, basidiomata are violaceous or lilac, though collection 6590 is dull ochre, not violaceous. Lamella density is subdistant, dark purple or violaceous, and occasional anastomoses could be observed between lamellae. The pileus is covered with fluffy pubescence.

Panellus violaceofulvus DAOM198753 is a tiny mushroom. The diameter for DAOM198753 mature basidiomata is 2.5 –4.5 mm. Its pileus was fawn, cinnamon colored (color 7D5, Konerup, 1981, the Farben color standard), the inner side of pileus was white. All other characters resembled those of *P. ringens*, though its spores in exemplar collection DAOM198753 were a little bigger than those of *P. ringens* exemplar 6711.

In *Tectella patellaris*, one distinctive character was that it exhibited an evanescent membrane covering lamellae in young basidiomata. The pileus was also covered with pubescence.

Pleurotopsis longinqua is pleurotoid. Basidioma epicutis was quite gelatinous. Lamellae were forked, which was also unique in this genus complex.

Table 3.2. Microscopic morphocharaters in the genus *Panellus* complex (measurement in μm)

Collection	TENN Accession No.	Species	Origin	Hymenium	Pleurocystidia		Cheilocystidia		Spore	
				average length	shape	length beyond hymenium	shape	length beyond hymenium	shape	Average size	surface
2675	48833	*P. stypticus*	New Zealand, Fjordland	25.6	clavate	3.3-17.2	clavate	16.4-41.0	allentoid	3.62x2.17	smooth
3342	48835	*P. stypticus*	Russia, Premorsk	23.6	clavate	9.8-17.2	clavate	16.4-24.6	allentoid	3.61x1.62	smooth
4319	50398	*P. stypticus*	Switzerland, Maggia	15.0	clavate	16.4-20.5	clavate, ventricose	9.8-24.6	allentoid	3.54x1.89	smooth
6157	52316	*P. stypticus*	USA, TN, Polk Co.	16.4	clavate	12.5-16.4	irregular clavate	28.7-41.0	allentoid	3.80x1.86	smooth
7699	53778	*D. sp.*	Costa Rica, Prov. Cartago	6.2	clavate	9.8-16.4	cylindrical, dendritic	23.8-45.1	phaseoliform	2.66x1.60	smooth
7809	53765	*D. sp.*	Costa Rica, Prov. San Jose	5.8	clavate	4.1-8.2	clavate, dendritic,	12.3-18.0	phaseoliform	3.27x1.90	smooth
8265	54317	*D. pusillus*	USA, FL, Ocala N. Forest	6.4	clavate	16.4-23.0	clavate, dendritic	18.0	phaseoliform	4.04x2.03	smooth
9408	56382	*D. pusillus*	Costa Rica, Prov. Alajuena	6.2	clavate	12.3-16.4	clavate, dendritic	16.4-24.6	elliptical	3.46x2.33	smooth
9522	56278	*D. pusillus*	USA, FL, Quincy	6.2	clavate	9.8-13.94	clavate, dendritic	9.8-20.5	phaseoliform	3.78x1.86	smooth
9803	56342	*D. pusillus*	Puerto Rico, El Yunque	6.8	clavate	15.6-29.5	clavate, dendritic	28.7-49.2	subglobose	3.49x2.52	smooth
9808	56347	*D. pusillus*	Puerto Rico, El Yunque	5.7	clavate	14.8-23.8	clavate, dendritic	24.6-41.0	subglobose	3.44x2.54	smooth
9421	56395	*D. pusillus*	Costa Rica, Prov. Cartago	5.8	clavate	6.6-10.7	clavate, dendritic	16.4-36.9	allentoid	3.49x1.82	smooth
5644	52389	*P. mitis*	USA, ID, Bonner Co.	8.0	clavate	3.3-4.1	absent or pseudocystidia	N/A	long cylindrical	4.43x1.61	smooth, dark amyloid
7486	53512	*P. mitis*	Finland, Prov.Etela-hame	10.3	clavate	3.3-5.7	absent or pseudocystidia	N/A	N/A	N/A	N/A
7494	53511	*P. mitis*	Finland, Prov.Etela-hame	6.2	clavate	4.9-6.6	absent or pseudocystidia	N/A	long cylindrical	4.50x1.67	smooth, dark amyloid
6894	53450	*P. serotinus*	USA, NC, Macon Co	7.8	absent	N/A	clavate	6.6	long cylindrical	4.64x1.46	smooth, dark amyloid
9992	58324	*P. serotinus*	USA, NC, Macon Co.	5.5	matulloid, thick-walled	12.3	clavate	12.3	long cylindrical	4.18x1.42	dark amyloid,
6711	52365	*P. ringens*	USA, AK, Turnagain Arm	8.2	clavate, thick-walled	3.3-6.6	clavate,	4.1-5.7	allentoid	4.58x1.89	amyloid
9812	58308	*P. ringens*	USA, NC, Graham Co.	8.2	clavate, thick-walled	2.5-3.3	clavate	3.3-4.1	N/A	N/A	N/A
DAOM 198753	DAOM 198753	*P. violaceofulvus*	Canada, Quebec, Forillon National Park	25.6	clavate, thick-walled	2.5-4.1	clavate, ventricose	4.1-5.7	allentoid	5.57x2.56	amyloid
6170	52302	*T. patellaris*	USA, NC, Macon Co.	7.0	clavate, cylindrical thick-walled	13.1-13.9	clavate, thick-walled	13.9-17.2	long cylindrical	3.39x1.01	smooth, dark amyloid
6486	53403	*T. patellaris*	USA, NY, Duchess Co.	7.8	clavate, thick-walled	3.3-6.6	clavate, thick-walled	9.8-12.3	long cylindrical	3.97x1.50	smooth, dark amyloid
6662	53214	*P. longinqua*	USA, WA, Clallam Co.	23.6	clavate	2.5-4.1	clavate	2.5-4.1	phaseoliform, thick-walled	6.79x3.54	amyloid
6663	53215	*P. longinqua*	USA, WA, Grey's Harbor Co.	23.6	clavate	5.7-6.6	clavate	4.1 or absent	phaseoliform, thick-walled	5.37x3.14	amyloid

Note: 1) In *P. stypticus*, a few exemplars selected from 69 collections are listed here. 2) length of cystidia beyond hymenium: hymenium length not included.

Dried specimens of all species were rehydrated with wet paper towels for several hours to overnight. All were able to revive except *P. serotinus*. The whole basidioma of *P. mitis* became very gelatinous when rehydrated. Uniquely, when basidimata of *Pleurotopsis longinqua* were rehydrated and revived, they turned to greenish black color.

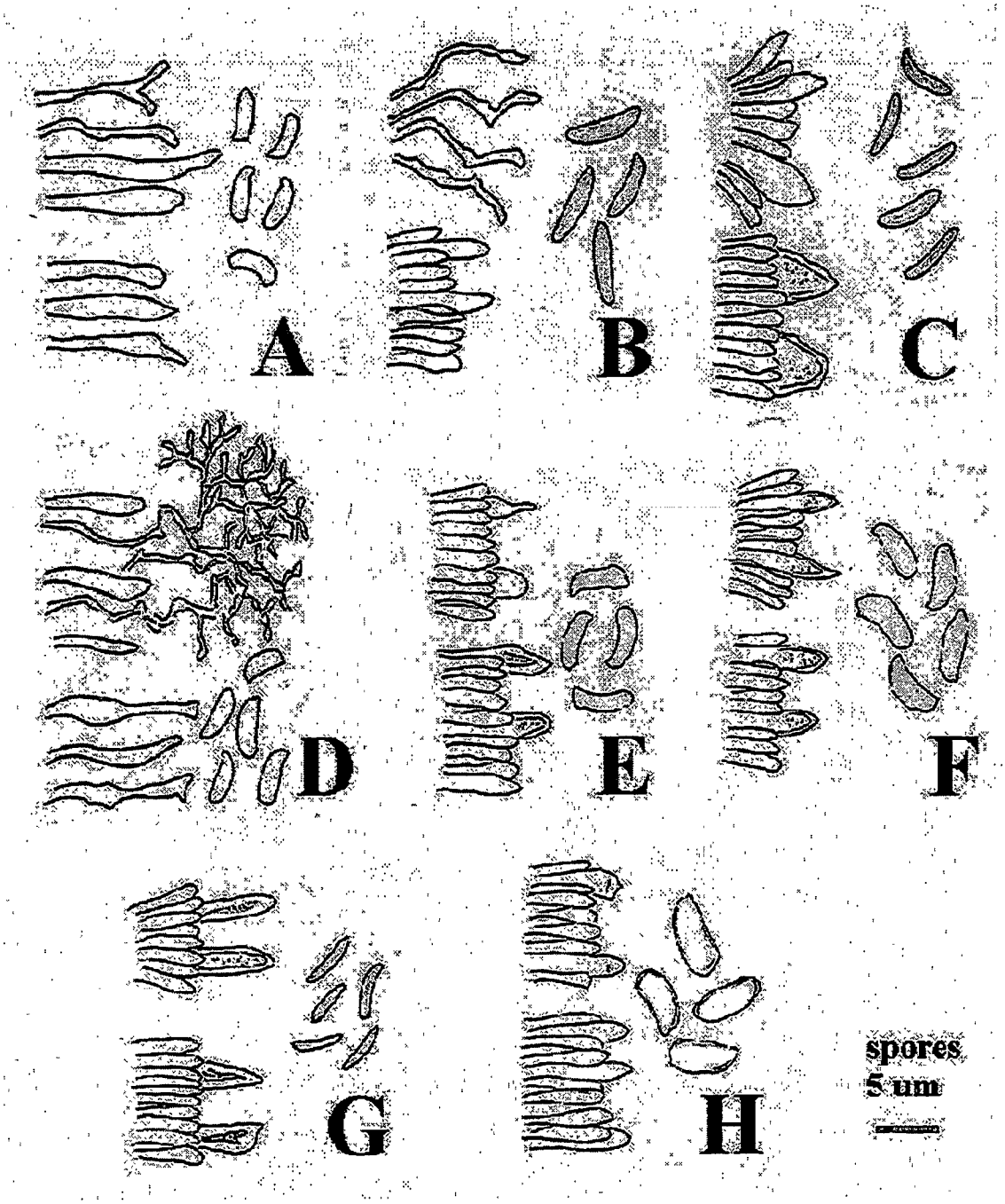
Based on macroscopic morphocharacters only, all the species in this genus complex were well-separated. Only *Dictyopanus pusillus* had poroid hymenial struture. Only *T. patellaris* had a membranous veil present in young basidiomata. Only *P. serotinus* has equal lengths for lamellae and was considered as non-revivable due to its basidioma size. Only *P. longinqua* had forked lamellae.

3.1.1.2. Microscopic characters in the genus *Panellus* complex

Microscopic characters of exemplars for the genus *Panellus* complex are listed in Table 3.2. Pleurocystidia, cheilocystidia and spore size and shape of the species for the genus *Panellus* complex are illustrated in Figure 3.1.

In *Panellus stypticus,* spores were somewhat allantoid, with a weak amyloid reaction. It was very common to find a large number of oil droplets or occasionally an oily layer surrounding lamellae. Oil droplets were usually associated with cystidia.

In *Dictyopanus pusillus,* similar oil droplets were observed and associated with cystidia. Another significant phenomenon was dendritic cheilocystidia, densely packed at lamellae margins, together with relative short embedded clavate cheilocystidia (gloeocystidia) (Corner, 1986). Microscopic observation of intersterility group I (collections 8265, 9408, 9522, 9803, 9808) revealed that spore shape and size in this group was quite diverse. Spore shape ranged from ovoid, elliptical, subglobose to phaseoliform. Spore size ranged from 2.66 X 1.60 µm to 4.04 X 2.03 µm. In intersterility group II (collection 9421), spores were allantoid with a size of 3.49x1.82 µm. Collection 9421 also had embedded clavate gloeocystidia in its lamellae tips. But



A: *P. stypticus*; B: *P. mitis*; C: *P. serotinus*; D: *D. pusillus*; E: *P. ringens*; F: *P. violaceofulvus* G: *T. patellaris*; H: *P. longinqua*

Figure 3.1. Cheilocystidia, pleurocystidia and spore size and shape of the species for the genus *Panellus* complex (Note: for cystidia of each species, above: cheilocystidia; below: pleurocystidia; the bar is only for spores)

in consideration of the fact that intersterility group I is so diverse in spore shape and size, the allantoid spore shape of collection 9421 makes it almost indistinguishable from the other intersterility group.

All the poroid collections observed above, including collection 9421, had basidioma diameter larger that 12 mm, and spores between 3-4 μm long. Morphologically *Dictyopanus pusillus* would be the proper species epithet for them according to Corner (1986).

Two collections 7699 and 7809 from Costa Rica that were not involved in mating tests and originally designated as *Dictyopanus* sp. were included in microscopic observation. Their spores were phaseoliform and relatively smaller than the above collections. Both had narrow clavate gloeocystidia at lamellae margins. Collections 7699 and 7809 cannot be separated from *Dictyopanus pusillus* by only those characters.

In *Panellus mitis*, lamella margins are characteristic in that interior hyphae become divergent and gelatinous, forming a swollen crown in which divergent hymenial hyphae formed. Cheilocystidia were absent.

In *Panellus serotinus,* one collection had thick-walled stout metulloid pleurocystidia, and the other collection lacked pleurocystidia.

In *Panellus ringens,* pleurocystidia were short, clavate and thick-walled. Cheilocystidia were inconspicuous and difficult to find. There were extended hymenial cells, sticking out of the hymenial layer, clavate in shape. In one collection, no spores were found, consistent with Miller's (1970) observation that spores in this species were difficult to find. In exemplar 6711, sometime color patches were observed on the surface of spores when they were stained in Melzer's solution.

In *P. violaceofulvus*, both pleurocystidia and cheilocystidia were short, clavate and thick-walled. Spores were allantoid, phaseoliform, sometime with color patches on the spore surface

when stained in Melzer's solution. Spores in exemplar collection DAOM198753 (5.57 X 2.56 μm) were a little bigger than those of *P. ringens* exemplar 6711 (4.58 X 1.89 μm).

In *Tectella patellaris*, both pleurocystidia and cheilocystidia were conspicuously clavate, cylindrical and thick-walled.

In *Pleurotopsis longinqua,* a conspicuous character was that its spores were very large (5.37-6.76 X 3.14-3.54 μm), and thick-walled. Sometime color patches were also observed on the surface of spores when they were stained in Melzer's solution.

Panellus stypticus and *Dictyopanus pusillus* share similar pleurocystidia and cheilocystidia shapes, often clavate and ventricose. Exemplars 4319 and 8265 share similar spore shape, though in *D. pusillus*, there was considerable diversity in spore shape. *Panellus stypticus* had allantoid spores in all the 69 world-wide collections surveyed. Spore shape in this species is quite conserved (see Morphological analyses **3.1.2.5**). In *Dictyopanus pusillus* (intersterility group I based on mating tests), spore sizes and shape varied from Florida, to Puerto Rico, and to Costa Rica. Spore shape varied from allantoid, phaseoliform, subglobose, to elliptical. Both *Panellus stypticus* and *Dictyopanus pusillus* had smooth, normal amyloid spores. *Panellus mitis P. serotinus* and *T. patellaris,* however, had darker amyloid and cylindrical spores. *Panellus ringens, P. violaceofulvus* and *P. longinqua* had relatively bigger allantoid spores, sometimes with color patches on spore surface. *Panellus serotinus, P. ringens, P. violaceofulvus* and *T. patellaris* had thick-walled pleurocystidia.

3.1.1.3 Phylogenetic reconstruction of the genus *Panellus* complex based on morphological characters

Macroscopic and microscopic morphocharacters from **3.1.1.1** and **3.1.1.2**, together with host substrates from which a collection was picked, were incorporated into a data matrix (Table 3.3).

Table 3.3. Data matrix of morphocharacters in the genus *Panellus* complex

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
4319 *P. stypticus*	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A
8265 *D. pusillus*	B	A	A	A	A	/	/	/	A	A	A	A	A	B	A	A	A	A	A	A
5644 *P. mitis*	A	B	A	A	A	C	A	A	A	A	A	B	B	A	/	/	B	A	B	B
9992 *P. serotinus*	A	B	A	A	A	A	B	A	B	A	B	A	A	A	A	A	B	A	B	A
6711 *P.ringens*	A	C	A	B	B	C	A	A	A	A	B	B	A	A	A	B	A	A	A	A
Daom198753 *P. violaceo-fulvus*	A	C	A	B	B	C	A	A	A	C	B	B	A	A	B	B	A	A	A	B
6170 *T. patellaris*	A	C	B	B	B	B	A	A	A	A	B	A	A	A	B	A	B	A	B	A
6662 *P. longinqua*	A	B	A	B	A	C	A	B	A	C	A	B	A	A	A	B	A	B	A	A

Note: 1) Characters used in this table are described in the text **3.1.1.3.**
2) Symbol / stands for N/A. Collection 9992 was used as one of the exemplars for morphological analyses, substituting for exemplar 6881 which was used for molecular analyses, due to the fact that basidiomata of 6881 were heavily contaminated.

In Table 3.3, characters are described as the following: 1. A. lamellate, B. poroid; 2. A. panelloid, B. pleurotoid but not panelloid; C. pendent bell-shaped; 3. A. membranous veil absent, B. membranous veil present; 4. A. stipe substipitate; B. stipe sessile, pseudostipe; 5. A. pubescence absent, B. pubescence present; 6. A. lamellae crowded, B. lamellae close, C. lamellae subdistant to distant; 7. A. lamellae not equal length, B. equal length; 8. A. lamellae not forked, B. lamellae forked; 9. A. basidiomata revivable, B. basidiomata not revivable. 10. hymenium length A. <10 μm, B. 10- 20 μm, C. > 20μm; 11. A. pleurocystidia thin-walled, B. pleurocystidia thick-walled. 12. A. length of stick-out pleurocystidia from hymenia >10 μm, B. stick-out pleurocystidia <10 μm; 13. A. cheilocystidia clavate present, B. cheilocystidia absent or pseudocystidia; 14. A. dendritic cheilocystidia absent, b. dendritic cheilocystidia present; 15. A. cheilocystidia thin-walled, B. cheilocystidia thick-walled; 16. A. length of stick-out cheilocystidia from hymenia >10 μm, B. stick out cheilocystidia <10 μm; 17. A. spores allantoid to phaseoliform, B. spores cylindrical; 18. A. spores thin-walled, B. spores thick-walled; 19. A.

spores amyloid, B. spores strongly amyloid; 20. A. host hardwood or other, but not conifer, B. host conifers (record of host of collection 9992 was not available, this datum was inferred from collection 6894, another exemplar for morphological comparison).

Morphological data (Table 3.3) were analyzed by UPGMA (Unweighted Pair Group method using Arithmatic averages) using Phylip (Phylogeny Inference Pakage, V3.5C, Felsenstein, 1991). Results are given in Figure 3.2.

The general topology of the UPGMA tree is similar to the 5'- partial LSU Neighbor-Joining tree (Molecular analyses **3.4.3.1.3**). In the UPGMA tree, *Panellus stypticus* and *Dictyopanus pusillus* formed a close clade, *P. violaceofulvus* was closer to *P. ringens* than to *P. lonqinqua*, but these three species formed a relatively tight clade. *Panellus serotinus*, *P. mitis* and *Tectella patellaris* each formed a monotyptic clade. This UPGMA tree generally agrees with parsimonious analysis and Neighbor-Joining analysis based on molecular data (Molecular analyses **3.4.3.1.2** and **3.4.3.1.3**).

3.1.2. Morphological comparison within *Panellus stypticus*

Morphological data for all 69 collections in *P. stypticus* are given in Appendix 3.1. Basidioma general stature, lamella anastomosis, lamellae forked or not, spore size, lamella height, Cheilocystidia shape and pleurocystidia shape were included.

Forked lamellae were observed only in collections 2515, 3342, 3350, 5846, 5847, 6512, 6880 and 7736. The remaining collections had unforked lamellae with rare instances of forking. This is contradictory to the report by Miller (1970).

3.1.2.1. Basidioma general stature in *P. stypticus*

Basidioma general stature (panelloid or not) was examined for 69 collections of *P. stypticus* (Appendix 3.1). All of the Northern Hemisphere collections were panelloid. In Oceanian areas,

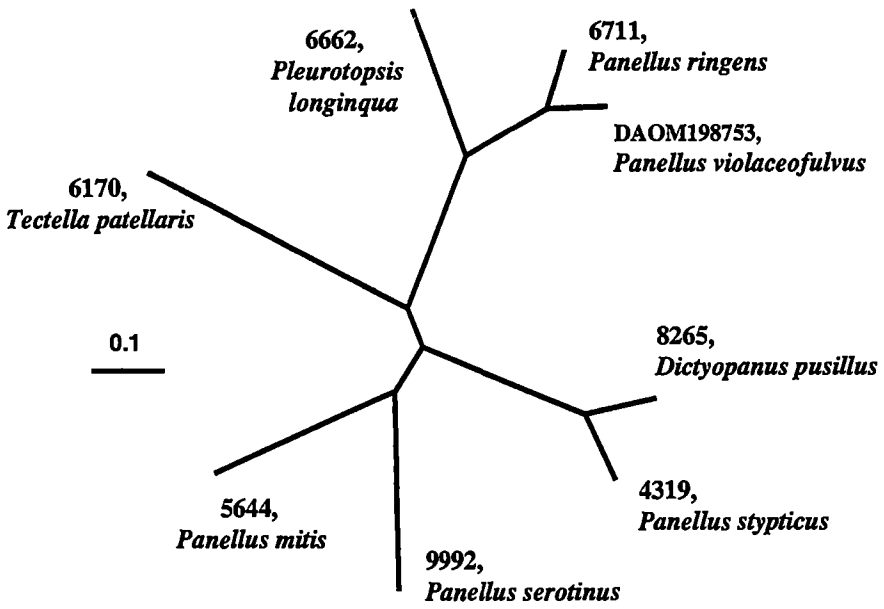


Figure 3.2. A UPGMA tree of the genus *Panellus* complex based on morphological characters.

basidioma stature was quite diverse and different from Northern Hemisphere collections. Collections from New South Wales and Tasmania of Australia were pleurotoid, but their lateral stipes were significantly shorter (collections 3544, 3567, 3586 and 3968) or absent (collection 3541). Thus they were not considered panelloid. All collections in New Zealand were panelloid except collection 2658 which showed non-panelloid stature. Noteworthy is that all of those collections were sexually compatible with Northern Hemisphere collections.

3.1.2.2. Lamella anastomoses in *P. stypticus*

Though some remnant veins were observed in New Zealand collections 2586 and 2664, and all collections from New South Wales and Tasmania (collections 3541, 3544, 3567, 3586, 3907 and 3968), collections 2646, 2658 and 2675 from New Zealand had no lamella anastomoses or remnant veins.

In the Northern Hemisphere, only one collection, 6503, was found in which part of a basidioma was devoid of anastomosis. All other collections had conspicuous or inconspicuous anastomoses. Further microscopic observation of collections 2300 and 3180, which had conspicuous anastomoses, revealed that anastomoses among lamellae were composed of a cellular matrix, not oily materials. Those anastomoses resembled shallow lamellae, on which oil droplets, clavate cystidia-like cells and basidia were observed.

3.1.2.3. Lamella height in *P. stypticus*

Lamella height was measured for all 68 collections of *P. stypticus* (Appendix 3.1).

All collections in this species were divided into seven geographical areas (populations) based on their RFLP patterns (see Molecular analyses **3.4.1**) and their geographical origin: Eastern North America, Pacific North America, Far East, Europe, New Zealand, New South Wales and Tasmania of Australia. In this analysis, due to the fact that there were three collections each in New South Wales and Tasmania of Australia, all the collections in Oceania were grouped as a geographical area. Lamella heights were calculated and grouped based on geographical areas (Table 3.4 and Figure 3.3). The Far East population had the lowest lamella height and lowest standard deviation. Oceanian collections had the highest lamella height with a relative high

Table 3.4. Mean lamellae heights (mm) of basidiomata and standard deviation in different geographical areas in *P. stypticus*

	Eastern North America	Europe	Far East	Oceania	Pacific North America
Lamellae height (mean)	0.392	0.341	0.289	0.499	0.404
Standerd deviation	0.103	0.1551	0.0468	0.1218	0.1362
No. of collections	25	11	10	12	10

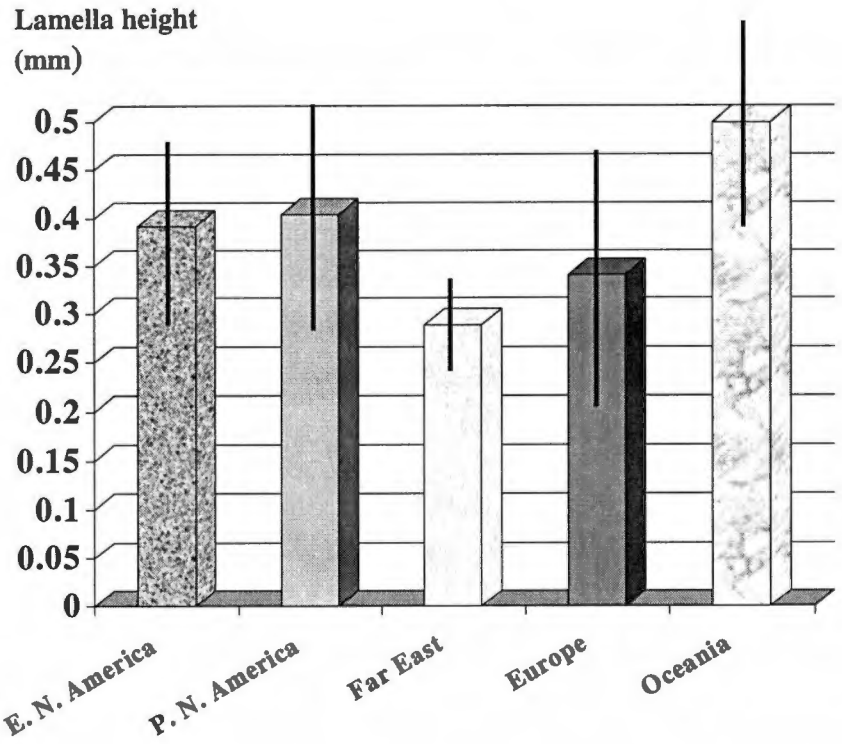


Figure 3.3. Lamella heights (column) and population standard deviation (bar) of basidiomata in different geographical areas in *P. stypticus*

standard deviation. European collections had the highest standard deviation, indicating high diversity in this area.

3.1.2.4. Cheilocystidium and pleurocystidium shape

Cheilocystidium and pleurocystidium shapes were examined in 69 collections. Cystidia were sorted into four types according to their shapes: A - clavate to ventricose; B - cystidia deformed toward dendritic; C - narrow cylindrical to filiform; D - cystidia tips deformed but not dendritic. Data are given in Table 3.5 and cheilocystidia are compared in Figure 3.4.

In this species, cheilocystidia were mainly clavate and ventricose, though dendritic cystidia constituted a significant proportion. Collections from the Far East had high rates of clavate-

Table 3.5. Distribution and occurrence of pleurocystidia and Cheilocystidia shape in different geographical areas in *P. stypticus* (%)

	Number of Collection.	Cheilocystidia shape				Pleurocystidia shape			
		A	B	C	D	A	B	C	D
Eastern North America	26	0.85	0.19	0.42	0.54	0.96	0	0.04	0.19
Europe	11	1.00	0.27	0.18	0.36	1.00	0.09	0	0.27
Far East	10	0.80	0.70	0.30	0.30	0.90	0	0.20	0.10
Oceania	12	0.83	0.25	0.58	0.25	0.75	0.08	0.42	0.25
Pacific North America	10	1.00	0.50	0.20	0.30	1.00	0	0.10	0.20

Note: 1) cystidium shape: A: clavate to ventricose. B: dendritic. C: narrowly cylindrical and filiform. D: cystidium tips deformed.
2) Occurrence was calculated as collection number with a certain cystidium category divided by total collection number examined in a geographical area.

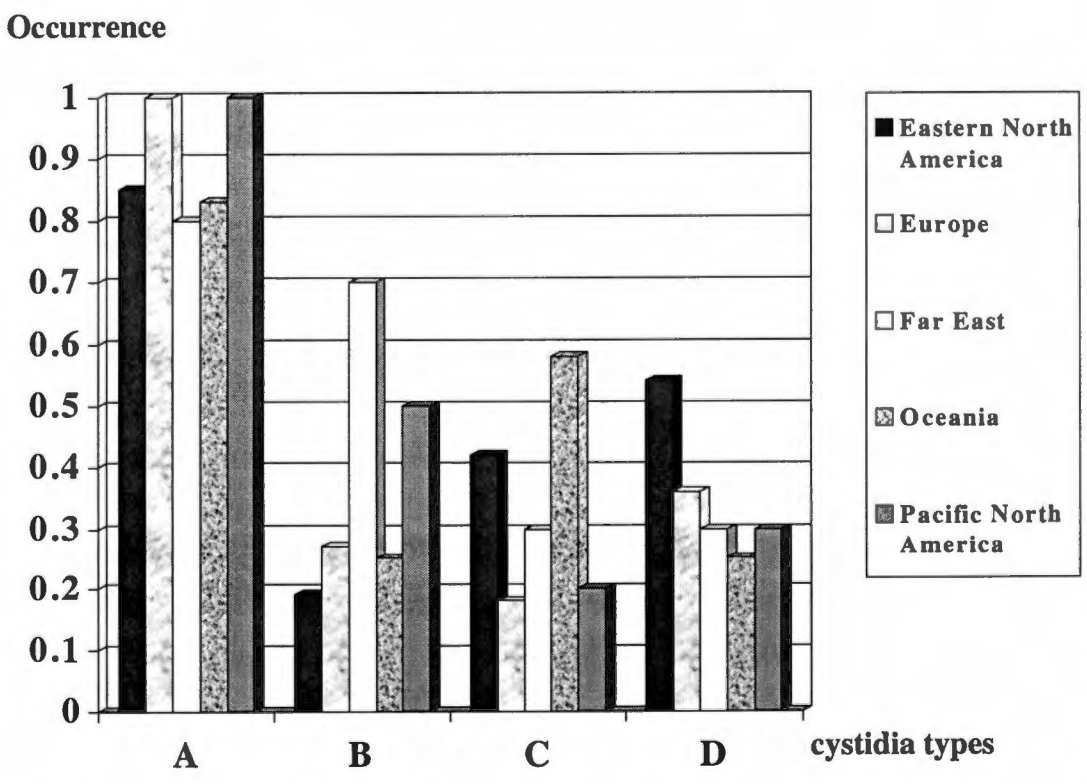


Figure 3.4. Cheilocystidia shapes and occurrence in different geographical areas in *P. stypticus* (Note: cystidia shape: A: clavate and ventricose. B: dendritic. C: narrowly cylindrical and filiform. D: cystidia tips deformed).

ventricose and dendritic cheilocystidia. In Oceanian populations, narrow cylindrical or filiform cheilocystidia comprised a significant proportion. Pleurocystidia were almost all clavate in shape except in Oceanian populations, where narrow cylindrical or filiform pleurocystidia were also the main composition, as in cheilocystidia.

3.1.2.5. Spore size

Spore sizes (length X width, in μm) of 69 collections in *Panellus stypticus* are listed in Appendix 3.1.

A linear correlation was calculated on spore sizes of those 69 collections. Taking X as the length of a spore and Y as the width of the spore, a linear equation (Figure 3.5) was obtained as: $Y = 0.0352 + 0.5001X$. The slope coefficient of the linear equation was 50.01%, and the correlation coefficient between lengths and widths of spores in 69 collections was: $r = 0.8256$, indicating that spore length and spore width were tightly correlated, and that spore shape for this species was quite conserved.

Average spore size (length X width) and the population standard deviation are listed in Table 3.6 and illustrated in Figure 3.6 for five geographical areas: Eastern North America, Pacific North America, Far East, Europe and Oceania,

The Far East area showed the smallest spore sizes and the smallest population standard deviation. Oceanian populations showed the largest spore sizes and the largest population standard deviation, indicating diversity of spore sizes in those areas.

Noteworthy is the finding that neighboring geographical areas (populations) showed conspicuous difference in spore sizes. Spore size range of Eastern North America and Pacific North America overlapped only slightly. However, Far East and Europe showed distant spore size range, indicating differentiation in spore size.

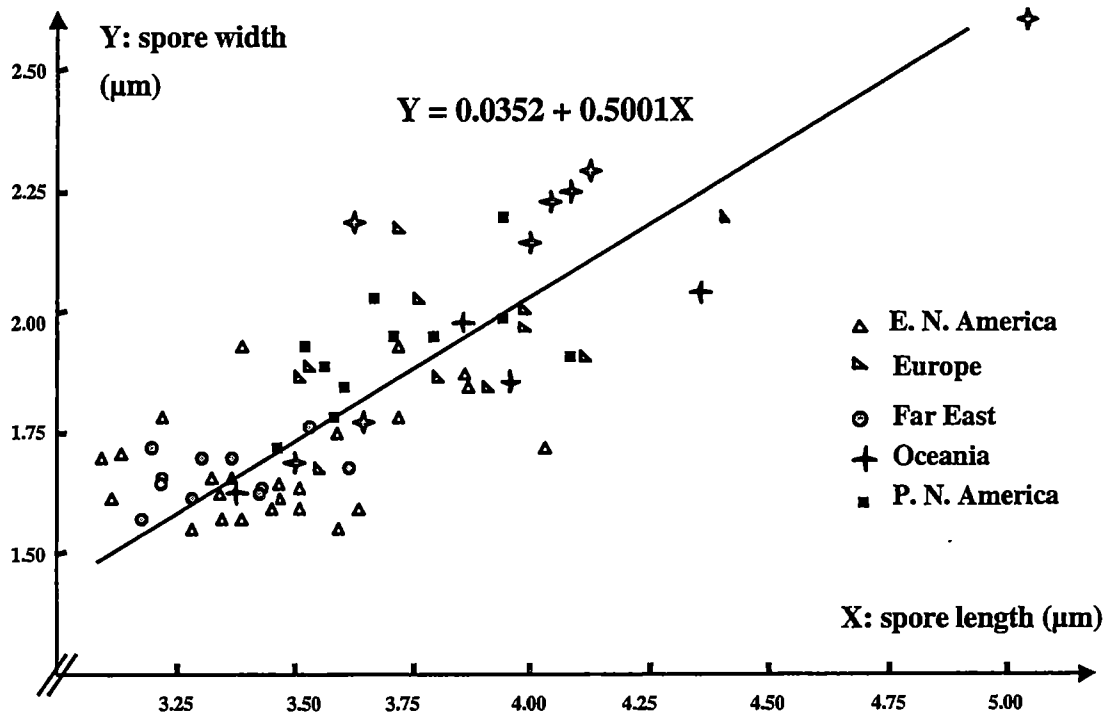


Figure 3.5. Distribution of spore sizes and their linear equation

Table 3.6. Spore size for different geographical areas in *Panellus stypticus*

	No. of collections	spore length X spore width (average ± standard deviation, in µm)
Eastern North America	26	3.453 ± 0.2305 X 1.715 ± 0.0992
Europe	11	3.827 ± 0.2751 X 1.955 ± 0.1586
Far East	10	3.305 ± 0.1344 X 1.687 ± 0.0492
Oceania	12	3.972 ± 0.4433 X 2.086 ± 0.2832
Pacific North America	10	3.704 ± 0.2132 X 1.928 ± 0.1331

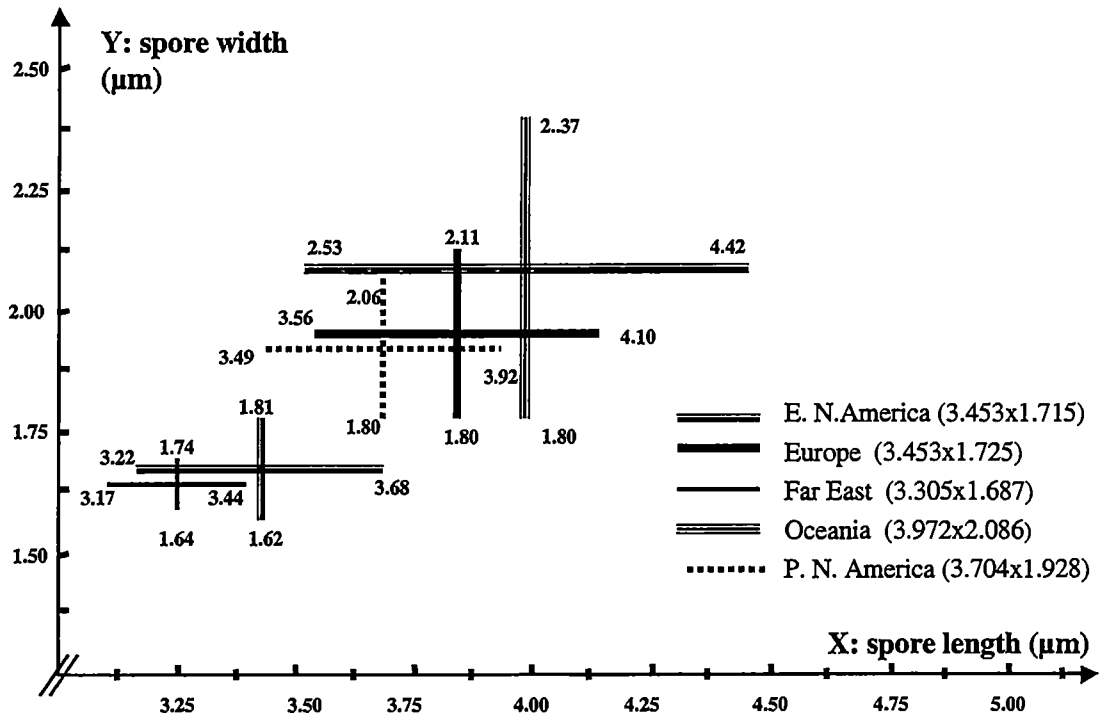


Figure 3.6. Average spore length and width (crossing point) with one standard deviation extension in five geographical areas

3.1.3. Other morphological analyses

Taxonomic placements for collections which were originally designated as species in the *Panellus* complex are given below. Taxonomic placement was based primarily on morphological characters (Table 3.7).

Collections 2646 and 3010 were typical *Panellus stypticus* morphologically. RFLP anlayses revealed that they had restriction sites consistent with *P. stypticus* and the extracellular laccase (ECL) patterns were similar to those of *Panellus stypticus* collections (Isozyme analyses **3.2.2** and **3.2.4.1**).

Collection 6590 is *Panellus ringens* based on macroscopic characters, even though the basidiomata are dull ochre, not lilac colored.

Collection 6650 had different RFLP patterns from *Panellus stypticus* collections. It is typical *Pleurotopsis longinqua* macroscopically, moreover, it turned to greenish black when rehydrated and dried, a typical character of this species. Collection 7577 had the same morphocharacters.

Collection 6881 is typically *Panellus serotinus* by macroscopic characters.

Table 3.7. Taxonomic placement for collections originally designated as species in the genus *Panellus* complex

Collection No.	TENN accession No.	Original designation	Correct placement	Basis for placement
2646	53149	*Panellus* sp.	*Panellus stypticus*	morphology, RFLP pattern, ECL profiles
3010	56674	*Panellus* sp.	*Panellus stypticus*	morphology, RFLP pattern, ECL profiles
6590	53016	*Tectella patellaris*	*Panellus ringens*	morphology
6650	53198	*Panellus stypticus*	*Pleurotopsis longinqua*	morphology, RFLP pattern
6881	53461	*Panellus stypticus*	*Panellus serotinus*	morphology, DNA data
7577	53672	*Panellus* sp.	*Pleurotopsis longinqua*	morphology

3.2. RESULTS OF ISOZYME ANALYSES

3.2.1. Selection of enzymes for analyses

Leucine aminopeptase (LAP), malate dehydrogenase (MDH), shikimate dehydrogenase (SKDH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) were excluded from analysis due to poor resolution of bands. Peroxidase (POD) was also excluded from further study because banding patterns were too complex.

Three enzymes were retained for further analyses. Extracellular laccase (ECL) bands were examined in collections of *Panellus stypticus*, as well as between species of the *Panellus* complex since there was some consistency within and between species. Esterase (EST) and alcohol

dehydrogenase (ADH) were used to reveal variation within *Panellus stypticus*. The relative mobility values for these three isozymes are listed in Appendix 3.2.1.

3.2.2. ECL profiles for different species in genus *Panellus* complex

Relative mobility (Rf) rates in the genus complex excluding *P. stypticus* are listed in Appendix 3.2.2. In collections designated as *Panellus* sp., collections 2646 and 3010 showed a pattern similar to other collections of *Panellus stypticus*. Morphological examination (see morphological analyses **3.1.3**) and RFLP analysis (see molecular analyses **3.4.1.2**) of rDNA ITS region confirmed that they were *Panellus stypticus*. Collections 3570, 7435 and 8345 each showed a non-*P. stypticus* pattern. They may be different from known species of *Panellus stypticus* and *P. serotinus* (Figure 3.7).

In *Panellus serotinus* (Figure 3.7), extracellular laccase (ECL) electrophoresis revealed that collections designated as *P. serotinus* (5624, 5699, 5729, 6167, 6846, 6894, 8121 and ASM5048), showed a similar basic pattern for the species.

3.2.3. ECL profiles for *Dictyopanus pusillus* and *Dictyopanus* sp.

Poroid collections 3493, 3513, 4460, 4536, 4861, 8265, 8271, 8733, 8747, 9096 and 9522 were designated as *Dictyopanus pusillus* or *Dictyopanus* sp., extracellular laccase (ECL) electrophoresis revealed they had a general ECL profile (Figure 3.7). Collections 3493, 4861, 8265, 8271, 8733, 8747, 9096 and 9522 were a single intersterility group. ECL profiles suggested that all of these collections shared a common ELC pattern (2-3 bands) for the intersterility group but there was considerable variation in electrophoretic mobility of individual bands suggesting a high level of genetic differentiation within this group.

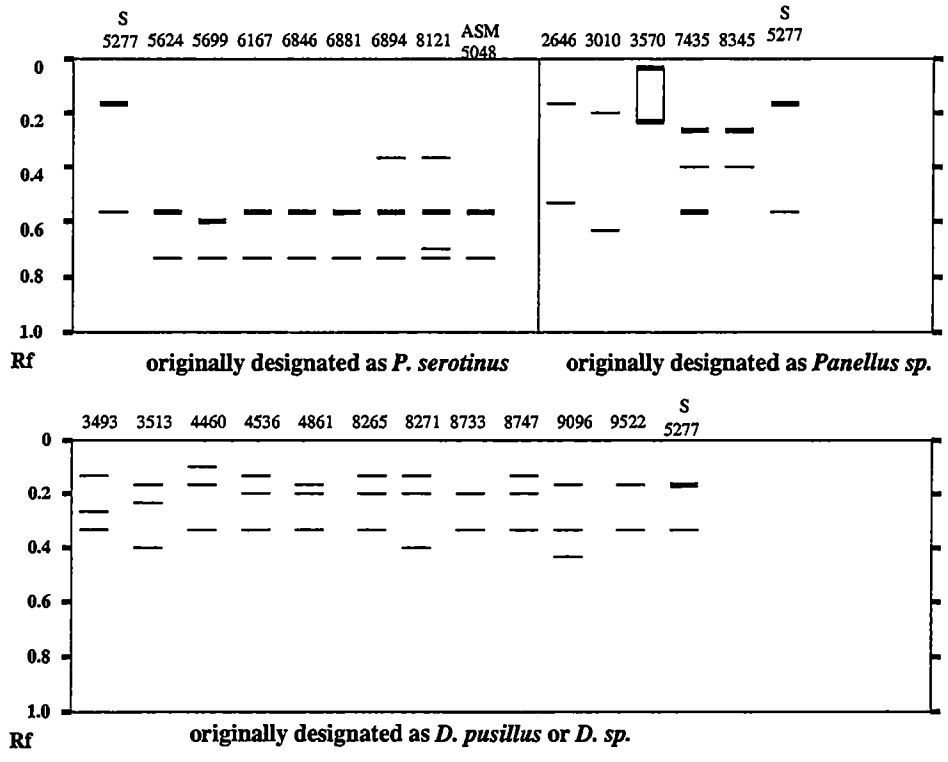


Figure 3.7. ECL zymographs of collections originally designated as *Panellus serotinus*, *Panellus* sp., and *Dictyopanus pusillus* or *Dictyopanus sp.* (S: standard)

Collection 9421, which was a separate intersterility group, was not incorporated into the electrophoresis analysis because it was obtained after isozyme analyses were finished.

3.2.4. Isozyme analyses in *Panellus stypticus*

3.2.4.1. Extracellular laccase (ECL) electrophoresis

Extracellular laccase (ECL) may be a monomer or a dimer (Acquaah, 1992). In *Panellus stypticus*, both the first band (presumed locus, ECL1) and the second band (presumed locus, ECL2) from the starting wells appeared as a single band, and ECL1 was present for every collection in the species. These data suggested that this enzyme in this species is a monomer.

ECL zymographs of *P. stypticus* collections are illustrated in Figure 3.8. The first band of extracellular laccase (ECL1) showed a single band with relatively small variation in mobility

between collections. A relative mobility (Rm) was calculated for each collection compared to the standard collection. Based on Rm rates (Appendix 3.2.1), this band was divided into six groups (slower to faster band in Rm rates): group 1= 0.76-0.85, group 2=0.86-0.95, group 3=0.96-1.05, group 4=1.06-1.15, group 5=1.16-1.25, group 6>1.26. The collections (Appendix 3.2.1) were sorted into different zones of relative mobility and then grouped based on five different geographical areas (Table 3.8). Results are given in Table 3.1 and Figure 3.9. The Oceanian area included New South Wales, Tasmania and New Zealand.

Several collections of *P. stypticus* lacked the second ECL band, so the second band was excluded from analysis.

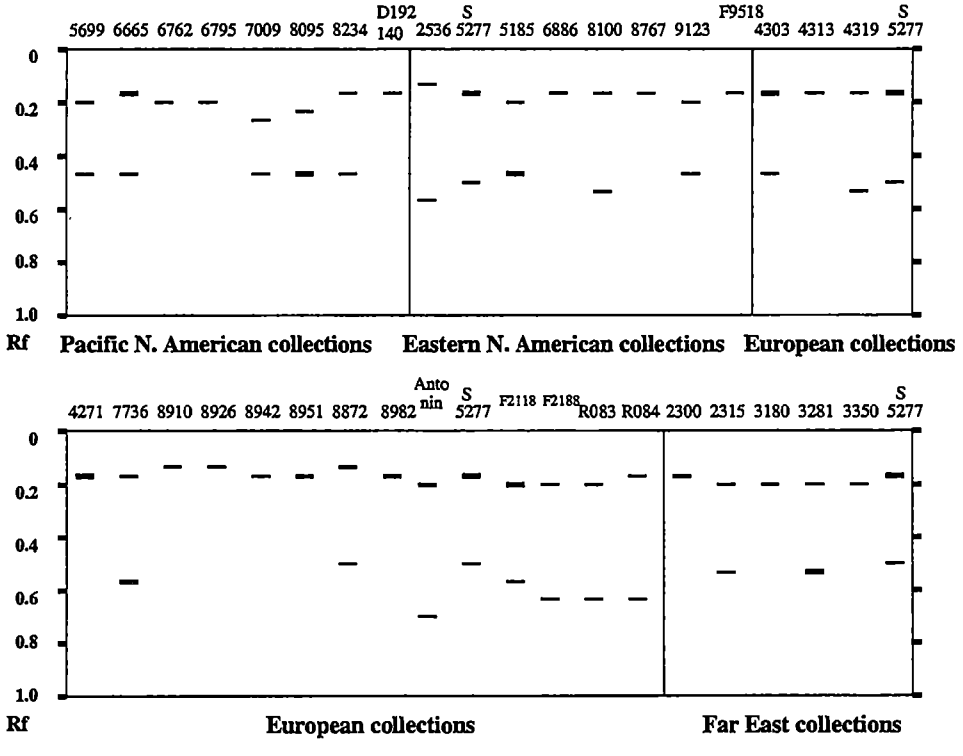


Figure 3.8. ECL zymographs of selected collections in *Panellus stypticus* (S: standard)

Based on Figure 3.9, Eastern North America and Pacific North America were most dissimilar in ECL alleles as revealed by relative mobility (Rm) rates. Eastern North America had the highest percentage of slower bands, while Pacific North America had the highest percentage of the faster bands. Eastern North America, the Far East and Europe all showed a bell-shaped curve for the distribution of Rm values. The Far East and Europe had relative mobility (Rm) rates falling in medium relative mobility (Rm) rates. Pacific North American and Oceanian areas, however, showed a bimodal distribution with high percentage in group 3 (0.96-1.05) and group 4 (1.16- 1.25) Rm rates.

Table 3.8. Pattern of ECL1 bands and Rm values in each mobility group in different geographical areas

	No. of collections	group					
		1 0.76-0.85	2 0.86-0.95	3 0.96-1.05	4 1.06-1.15	5 1.16-1.25	6 >1.26
Eastern N. America	18	5.56	38.89	44.44	11.11		
Europe	16		25.00	37.50	31.25	6.25	
Far East	9		11.11	22.22	44.44	22.22	
Oceania	11		18.18	36.36	9.09	36.36	
Pacific N. America	8			37.50	12.50	37.50	12.50

Note: Percentage (%) = (number of collections in the group) / (total collections)

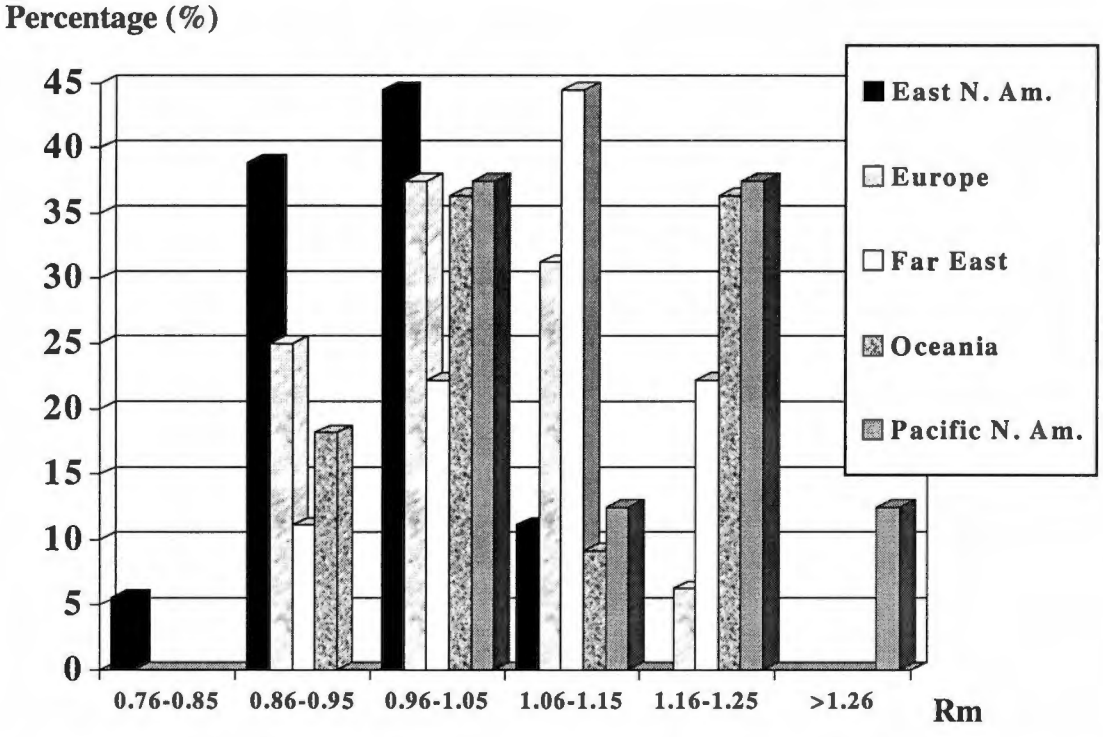


Figure 3.9. Percentage and distribution of ECL1 bands in different geographical areas

3.2.4.2. Alcohol dehydrogenase (ADH) electrophoresis

Results of ADH electrophoreses revealed that the ADH3 band (the third band from the starting point, presumed locus) was present only in Pacific North America, the Far East and Europe, while this band was absent in Eastern North America and Oceania. This band in the Far East collections was stronger than other geographical areas. Lack of heterozygosity for ADH3 bands suggested that this enzyme for this locus is probably a monomer, in contrast to reports that ADH is a dimer (Acquaah, 1992 and references therein).

ADH3 was compared for different geographical areas (Table 3.9). Electrophoreses showed that Far East collections had the highest occurrence of this band, and also had the highest band activity of this band. In Pacific North America and Europe, the percentage of collections with this

Table 3.9. ADH3 bands in different geographical areas and their percentage of occurrence

	Eastern North America	Europe	Far East	Oceania	Pacific North America
No. of collections examined	25	17	10	10	13
No. ADH3 present	0	5	8	0	5
Percentage (%)	0	29.41	80.00	0	38.46

* Percentage (%) = (number of collections in the group) / (total collections)

band decreased. This was also accompanied by decreased band activity in those two geographical areas. This band was absent in Eastern North American and Oceanian areas.

3.2.4.3. Esterase (EST) electrophoresis

Esterase (EST) displayed variable patterns for *Panellus stypticus,* but the fastest band area (furthest from starting wells) was less variable, almost present for every collection. One band (the one with slowest mobility in the furthest band area, EST1-1) was used for comparing collections in this species. These bands were divided into six groups based on their relative mobility (Rm) rates: group 1 <0.93, group 2 = 0.94-0.98, group 3 = 0.99-1.04, group 4 = 1.03-1.06, group 5 =1.07-1.12. Percentage of the occurrence of bands in five different geographical areas were calculated and illustrated in Table 3.10. and Figure 3.10.

Based on EST1-1 distribution, Eastern North America was most different in relative mobility (Rm) rates with Eastern North America having the highest rates in slower Rm rates. Eastern North America and Far East had slowest bands. Oceania and Europe showed the fastest Rm rates. Except Eastern North America, the remaining four geographical areas showed highest distribution of bands with Rm rates in Rm of 0.99 –1.04.

Table 3.10. Pattern of EST1-1 bands and percentages in each mobility group in different geographical areas

	No. of collec-tions	band No. apppeared	group				
			1 <0.93	2 0.94-0.98	3 0.99-1.04	4 1.03-1.06	5 1.07-1.12
Eastern N. America	21	18	16.67	72.22	11.11		
Europe	17	26		6.67	66.67	13.33	13.33
Far East	9	20	22.22	33.33	44.44		
Oceania	10	15			87.50		12.50
Pacific N. America	12	25		9.09	63.64	27.27	

Note: Percentage (%) = (number of collections in the group) / (total collections)

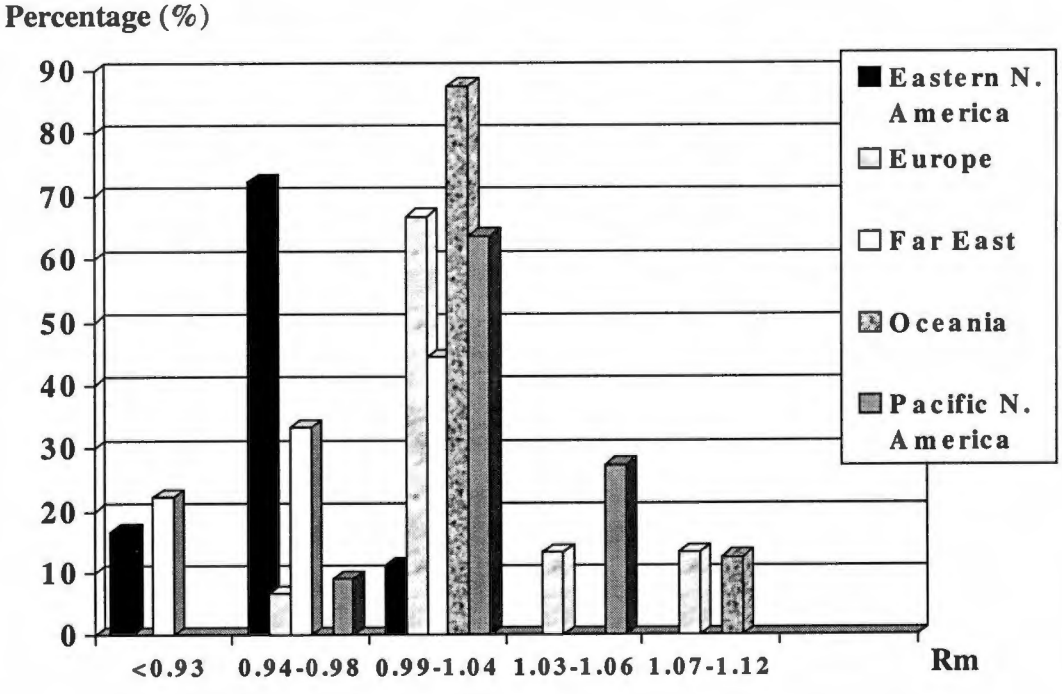


Figure 3.10. Percentage and distribution of EST1-1 band in different geographical areas

3.3. MATING STUDIES

3.3.1. Mating studies in *Panellus stypticus*

Seven mating exemplars were chosen to represent geographical regions or populations. They were: 9810 (USA, TN) for Eastern North America; 9029 (USA, CA) for Pacific North America; 3180 (Russia, Premorsk) for the Far East; 8926 (Russia, Caucasia) for Europe; 2664 New Zealand; 3567 Australian New South Wales; 3586 Australia, Tasmania.

A total of 57 collections were involved in this mating study. The mating data are listed in Appendix 3.3. Mating grids of each exemplar to collections from each of five geographical areas (Eastern North America, Pacific North America, Far East, Europe and Oceania) are listed in the following appendices:

Crosses with exemplar 9810, Appendix 3.3.1.1 –3.3.1.5.

Crosses with exemplar 9029, Appendix 3.3.2.1 –3.3.2.5.

Crosses with exemplar 3180, Appendix 3.3.3.1 –3.3.3.5.

Crosses with exemplar 8926, Appendix 3.3.4.1 –3.3.4.5.

Crosses with exemplar 2664, Appendix 3.3.5.1 –3.3.5.5.

Crosses with exemplar 3567, Appendix 3.3.6.1 –3.3.6.5.

Crosses with exemplar 3586, Appendix 3.3.7.1 –3.3.7.5.

The mating results of seven exemplars with 57 collections were summarized and listed in Appendix 3.4.

3.3.1.1. Intersterility group in *Panellus stypticus*

All 57 collections designated as *P. stypticus* and involved in this mating study belonged to a single intersterility group (the same biological species), though in some geographical areas, compatibility rates were reduced, and antagonism was observed.

3.3.1.2. Compatibility rates in *Panellus stypticus*

Exemplar 9029 from Pacific North America had the highest average compatibility rate (Appendix 3.4); and exemplar 3567 from New South Wales had the lowest average compatibility rate. Among the seven geographical areas (populations), Pacific North America had the highest average compatibility rate; New South Wales had the lowest average compatibility rate. Noteworthy is that exemplars from the Northern Hemisphere tended to have lower compatibility rates with the Southern Hemisphere populations compared to matings with the Northern Hemisphere populations, but 9029 was an exception. And second, exemplar 2664 from New Zealand showed a slightly lower compatibility rates with collections from the Northern Hemisphere. Exemplar 3586 from Tasmania showed reduced compatibility only to those from Eastern North America. Exemplar 3567 from New South Wales showed reduced compatibility to all populations, including its own population. (Table 3.11 and Figure 3.11).

Table 3.11. Mating compatibility rates of each mating exemplar to collections from each geographical area

	Pacific North America	Eastern North America	Europe	Far East	New Zealand	New South Wales	Tasmania
9810 (USA, TN)	95.74	98.18	94.68	92.59	85	79.17	91.67
9029 (A) (USA, CA)	98.84	95.76	94.62	100	100	100	100
3180 (Russia, Premorsk)	86.67	89.47	83.33	81.82	50	54.17	54.17
8926 (Russia, Caucasia)	98.86	97.37	91.86	100	55	54.17	54.17
2664 (New Zealand)	93.33	93.86	97.82	97.92	93.55	100	100
3567 (Australia, N.S.W.)	65.56	52.88	60.53	30.43	54.84	53.33	44.44
3586 (Australia, Tasmania)	98.86	80.56	93.33	91.30	100	91.67	85.71

*N.S.W: New South Wales, Australia.
*Exemplars: 9810 (USA, TN) for Eastern North America; 9029 (A) (USA, CA) for Pacific North America; 3180 (Russia, Premorsk) for the Far East; 8926 (Russia, Caucasia) for Europe; 2664 New Zealand; 3567 Australian, New South Wales; 3586 Australia, Tasmania.

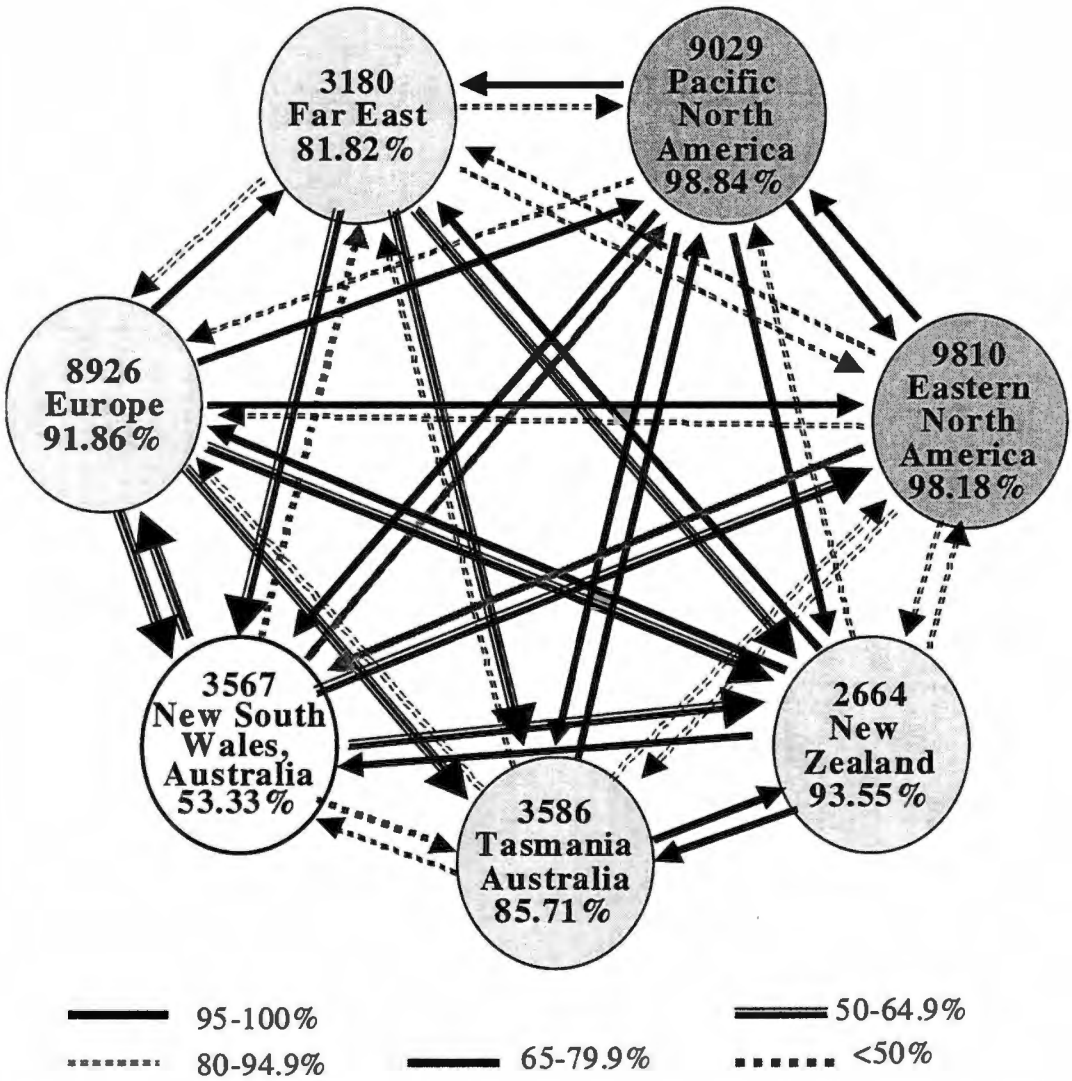


Figure 3.11. Compatibility rates of exemplars (number within circle) to other collections within the same geographical area (within circle) and to other geographical areas (lines)

Note: Circles represent geographical areas. The collection number in the circle was the exemplar in that population and the percentage inside the circle indicates the mating compatibility of the exemplar with its own geographical area (population). Shade within circles represents different levels of compatibility rates. Lines between an exemplar to other geographical areas (populations) are indicated by different styles of lines. Arrow indicates an exemplar crosses to (arrow pointing to) all the collections of another population.

3.3.1.3. Antagonistic reactions

Antagonism was a significant phenomenon in matings in this species except in crosses with exemplar 9810 from Eastern North America and exemplar 9029 from Pacific North America. Exemplar 3180 from the Far East showed increased antagonism in crosses with New South Wales and Tasmanian populations, but not with the New Zealand population. Exemplar 8926 from Europe clearly showed antagonism in crosses with Southern Hemisphere populations, but not with Northern Hemisphere populations. Exemplar 3567 of New South Wales showed very high antagonism in crosses with all other populations, including its own. Exemplar 3586 of Tasmania showed increased antagonism in crosses to all populations, except its own. A similar phenomenon was also observed in exemplar 2664 from New Zealand (Table 3.12 and Figure 3.12).

Table 3.12. Mating antagonism rates of each exemplar to seven geographical areas (populations)

	Pacific North America	Eastern North America	Far East	Europe	New Zealand	New South Wales	Tasma-nia
9810 (USA, TN)	0	0	0	0	0	0	0
9029 (A) (USA, CA)	0	0	0	0	0	0	0
3180 (Russia, Premorsk)	7.78	9.65	0	3.33	0	33.33	25
8926 (Russia, Caucasia)	0	0	0	0	22.5	33.33	25
2664 (New Zealand)	25.56	13.16	18.75	29.35	12.9	33.33	16.67
3567 (Australia, N.S.W.)	51.11	57.69	52.17	50	35.48	60	44.44
3586 (Australia, Tasmania)	29.55	21.3	28.26	16.67	28.13	45.83	14.29

Note: N.S.W.: New South Wales, Australia.

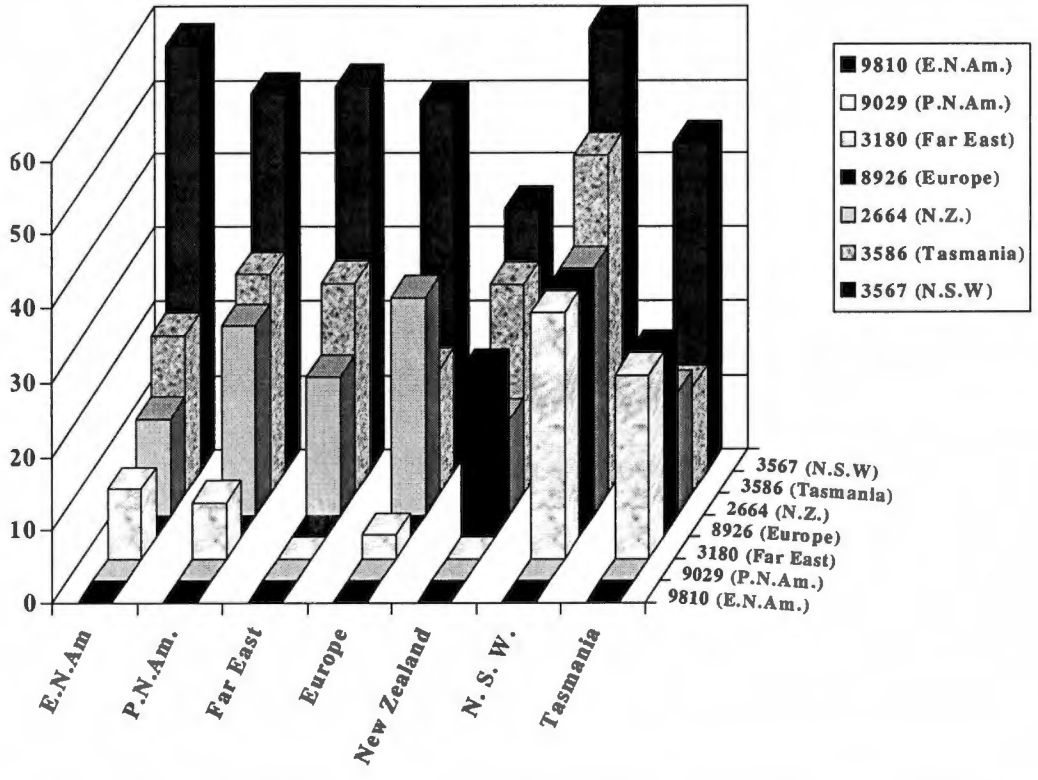


Figure 3.12. Antagonism rates of seven exemplars to collections from seven geographical areas

3.3.1.4. Other abnormal mating behavior

Other atypical mating behaviors, such as F/+ (Flat-like reaction with presence of clamps) and B / + (Barrage-like reaction with presence of clamps) reactions were observed. Among the seven exemplars, exemplar 9029 from CA, USA showed no such abnormal behaviors. Exemplar 3180 from Premorsk, Russia displayed the highest frequency of such behavior, followed by exemplar 2664 from New Zealand, exemplar 3567 from New South Wales and exemplar 3586 from Tasmania of Australia (Table 3.13).

Another significant phenomenon observed in mating tests was that Southern Hemisphere collections tended to show more strangulate hyphae (hyphae club-shaped toward one direction in

Table 3.13. Abnormal mating behavior (F/ + and B / +) of each exemplar to seven populations

	Eastern North America	Pacific North America	Far East	Europe	New Zealand	New South Wales	Tasmania
9810 (USA, TN)	4	0	0	0	0	2	1
9029 (A) (USA, CA)	0	0	0	0	0	0	0
3180 (Russia, Premorsk)	13	4	5	25	8	3	2
8926 (Russia, Caucasia)	3	10	0	7	12	4	13
2664 (New Zealand)	5	16	16	15	0	5	1
3567 (Australia, N.S.W.)	5	1	2	9	0	0	0
3586 (Australia, Tasmania)	18	1	10	16	4	0	4

*N.S.W.: New South Wales of Australia.

most cases, sometimes ventricose, sometimes swollen in many parts) than Northern Hemisphere collections. Exemplars 2646 (New Zealand), 2675 (New Zealand), 3541 (New south Wales, Australia), 3544 (New south Wales), 3567 (New south Wales), 3968 (Tasmania) were observed to have extensive strangulate hyphae in intercollection matings and self-crosses. On a few occasions, this kind of hyphae was also observed in crosses with Northern Hemisphere collections 9810 (USA, TN), and 9525 (Canada, Montreal). In a self cross of *Dictyopanus pusillus* 9808, strangulate hyphae were also observed.

3.3.2. Results of matings within *Dictyopanus pusillus* and *Dictyopanus sp.*

In TENN, 14 collections with moncaryons were originally designated as *D. pusillus* or *Dictyopanus sp.* Among them, collections 3878, 8265, 8271, 8747, 9408, 9421 were originally designated as *D. pusillus*. Collections 3493, 4861, 7114, 8412, 8733, 9096, 9522, 9803, 9807 and 9808 were designated as *Dictyopanus sp.*

Collection 9808 was chosen as the exemplar for mating tests. Its self cross is given in Appendix 2.8. Single-spore isolates 9808-1 (A1B1) and 9808-3 (A2B2) were used as tester

monocaryons. Intercollection matings of exemplar 9808 in crosses with the above collections are listed in Appendix 3.5. Mating results revealed that collections 3493, 3878, 4861, 8265, 8733, 8747, 9096, 9408, 9522, 9803, 9807, 9808 were the same intersterility group, i.e. a biological species. Moreover, only a few minor abnormal B-like but compatible matings were observed in these matings. Collection 9421, however, was completely incompatible to exemplar 9808. Thus, there are two intersterility groups (biological species) represented by the *Dictyopanus* collections. One intersterility group was 9421 from Costa Rica. The other intersterility group included collections 3493, 3878, 4861, 8265, 8733, 8747, 9096, 9408, 9522, 9803, 9807 and 9808.

3.3.3. Results of other mating tests

In TENN, seven collections originally designated as *Panellus* sp. had monocaryons and were put into mating tests. Collection 7577 (*Pleurotopsis longinqua* based on morphology) was chosen as an exemplar. The self cross of 7577 is listed in Appendix 2.9. Two tester monocaryons, 7577-11 (A1B1) and 7577-12 (A2B2), were used to cross to four monocaryons of every collection with monocaryons and designated as *Panellus* sp. Result of the intercollection matings revealed that all other collections designated as *Panellus* sp. were completely incompatible to 7577 with stunted growth or antagonism (Appendix 3.6.1).

A further cross of *P. stypticus* exemplar 9029 (A) (USA, CA) to collections designated as *Panellus* sp. (3570, 7435, 7543, 7511), and *Pleurotopsis longinqua* (7577) determined that all matings were incompatible with more or less antagonism (Appendix 3.6.2).

3.4. RESULTS OF MOLECULAR ANALYSES

3.4.1. RFLP studies within *P. stypticus*

3.4.1.1. Sequence comparison of the ITS1-5.8s-ITS2 ribosomal gene

Ten exemplars from different geographical areas were chosen for molecular analyses: Eastern North American exemplars 6157 (TN, USA) and 9525 (Canada, Montreal); Pacific North American exemplars 9029A (CA, USA) and 8234 (AK, USA); Far East exemplar 3342 (Premorsk, Russia); European exemplars 4319 (Maggia, Switzerland) and 4271 (Vasgotland, Sweden); Australian New South Wales exemplar 3541; Australian Tasmanian exemplar 3586; and New Zealand exemplar 2675.

A multisequence alignment of ten exemplars in *P. stypticus* with the outgroup 8265 *D. pusillus* and together with restriction sites marked is given in Appendix 3.7. The PCR product between primers ITS4 and ITS5 gave out a fragment of ca 700 bp for the Northern Hemisphere collections of *P. stypticus*. In the ITS region, the Eastern North American exemplar 6157 (USA, TN) was 588 bp long, 9525 (Canada, Montreal) was 589 bp. The Pacific North American exemplars 9029A (USA, CA), 8234 (USA, Alaska), the Far East exemplar 3342 (Russia, Premorsk), the European exemplars 4319 (Switzerland, Maggia) and 4271 (Sweden, Vasgotland) were 587 bp long in the ITS region. Compared to the length of exemplar 6157, New Zealand exemplar 2675 accumulated a total of 91 bp deletion in its ITS region, giving out 497 bp; while the Australian New South Wales exemplar 3541 had a total of 18 bp deletion, leaving 570 bp; the Australian Tasmanian exemplar 3586 had a total deletion of 24 bp, resulting in 564 bp. Electrophoreses of the PCR products in the ITS region showed that PCR products from these Oceanian areas were the same in length to the sequenced exemplars.

3.4.1.2. Mapping, restriction sites in the ITS region and expected fragments

ITS sequences of the ten exemplars were aligned in Appendix 3.7. Restriction enzymes were selected that differentiated between polymorphisms. Four restriction enzymes *BstZ* I, *Dra* I, *Hinf* I and *Taq* I, were chosen to the detect restriction site difference between collections. The positions and expected fragment lengths by those four restriction enzymes were calculated and are given in Table 3.14.

3.4.1.3. Distribution of the restriction sites by enzymes *BstZ* I, *Dra* I, *Hinf* I and *Taq* I

Four enzymes, *BstZ* I, *Dra* I, *Hinf* I and *Taq* I, were used to reveal the presence or absence of restriction sites for all collections in this study. Digestion results for those four enzymes are listed in Appendix 3.8, and are shown in Figure 3.13. *BstZ* I separated Eastern North American from other Northern Hemisphere collections. The Far East collection (3281, Premorsk, Russia) lost a *BstZ* I restriction site. Tasmanian and New Zealand Collections all lacked a *BstZ* I site due to a fragment deletion. *Dra* I separated European collections and New Zealand collections from other collections. European collections and New Zealand collections differed in one restriction site. A European collection 8910 (Caucasia, Russia) lost a *Dra* I restriction site. *Hinf* I merely separated New Zealand collections from others due to a long deletion. *Taq* I indicated that exemplar 3342 (Premorsk, Russia) was a mutant, which gained an aditional *Taq* I restriction site. Digestion by *BstZ* I indicated that collections from Eastern North America were different from the Pacific North American collections including Alaskan collection, 8234. Noteworthy is that Far East and New South Wales collections have the same RFLP patterns as Pacific North American collections for these enzymes.

Collections 2646 and 3010 were originally designated as *Panellus sp*, and had the same *Dra* I pattern as other *P. stypticus* collections. The origin of collections RC/F95.083 and RC/F95.084 was unknown, but the *BstZ* I pattern implicated a European origin.

Table 3.14. Restriction sites and fragment lengths of 10 exemplars in the ITS region

Geographical region	Eastern North America		Pacific North America		Far East		Europe		New South Wales		Tasmania		New Zealand	
exemplar	6157, (9525)		9029A, 8234		3342		4319, 4271		3541		3586		2675	
Electrophoregram length	675		674		674		674		651		651		584	
ITS region length	588, (589)		587		587		587		570		564		497	
enzyme	P	L	P	L	P	L	P	L	P	L	P	L	P	L
BstZ I			200	200	200	200	200	200	201	201				
	588	**588**	587	387	587	387	587	387	570	369	564	**564**	497	**497**
Dra I							150	**150**					64	**64**
	473	473	473	473	473	473	473	**323**	467	467	459	459	405	**341**
	588	115	587	114	587	114	587	114	570	103	564	105	497	92
Hinf I	130	130	130	130	130	130	130	130	130	130	134	134		
	314	184	314	184	314	184	314	184	315	185	307	173	252	**252**
	322	8	322	8	322	8	322	8	323	8	315	8	260	8
	588	266	587	265	587	265	587	265	570	247	564	249	497	237
Taq I	34	34	34	34	34	34	34	34	34	34	35	35	35	35
					95	**61**								
	261	227	261	227	261	**166**	261	227	262	228	254	219	199	164
	320	59	320	59	320	59	320	59	321	59	313	59	258	59
	588	268	587	267	587	267	587	267	570	249	564	251	497	239

Note: 1) Origin of exemplars: 6157: TN, USA; 9029A: CA, USA; 8234: AK, USA; 3342: Premorsk, Russia; 4319: Maggia, Switzerland; 4271: Vasgotland, Sweden; 3541: New south Wales, Australia; 3586: Tasmania, Australia; 2675: New Zealand.

2) P stands for the position of a restriction site in the ITS region (Appendix 3.7); L stands for a fragment length cut by a restriction enzyme. Bold numbers are those distinct fragment lengths for the exemplars.

3) The positions and fragment lengths indicated above were based on Appendix 3.7 (ITS begins at ATTGAATAC, and LSU begins at ATTTGACCT). PCR products by using primers ITS4 and ITS5 include partial SSU and partial LSU. Electrophoregram length referred to the sequenced length between primers ITS4 and ITS5, but didn't include the sequences of the two primers.

4) Exemplar 9525 (Montreal, Canada) was one base pair longer than exemplar 6157 (TN, USA), which inserted in position 164 in Appendix 3.7.

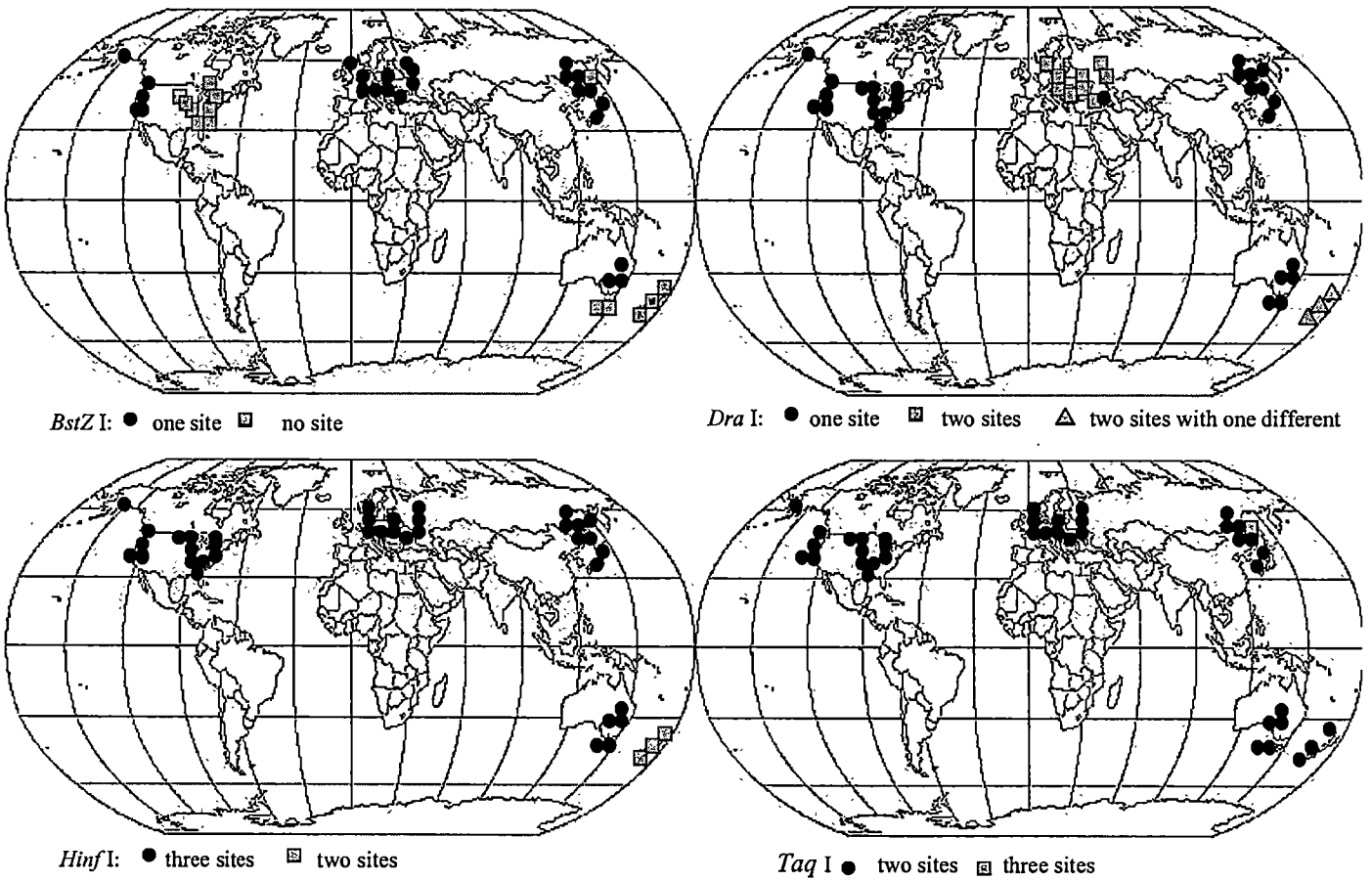


Figure 3.13. Distribution of RFLP patterns for restriction enzymes *BstZ* I, *Dra* I, *Hinf* I and *Taq* I in *Panellus stypticus* (Note: New Zealand and Tasmanian collections had no *BstZ* I site due to a fragment deletion; New Zealand collections had another deletion that led to the loss of a *Hinf* I restriction site)

3.4.2. Group I introns in 5'- partial LSU in genus *Panellus* complex

3.4.2.1. Intron presence or absence in genus *Panellus* complex

Alignment of 5' – partial LSUSequences of exemplars of *P. stypticus* and *D. pusillus*, *Panellus ringens, P. violaceofulvus* and *Pleurotopsis longqinqua, T. patellaris, P. mitis*, and *P. serotinus* indicated that the 5'-partial LSU in *P. stypticus* exemplar 4319 and *D. pusillus* exemplar 8265 harbored an intron. The insertion was ca. 400 bp inserted at yeast ribosomal DNA LSU position 931 (Bayev et al., 1981). The alignment of exemplars of the above species is given in Appendix 3.9, showing the insertion of the intron in *P. stypticus* and *D. pusillus*.

A BLAST search of GenBank using the intron sequences of *P. stypticus* exemplar 4319 (Maggia, Switzerland) and *Dictyopanus pusillus* exemplar 8265 (FL, USA), produced a match with a Group I intron (Hibbett, 1996, Genbank No. PSU59090), from position 943 of the SSU of ribosomal DNA in *P. stypticus* (Appendix 3.12).

Sequencing revealed that exemplars *Panellus ringens* 6711, *P. violaceofulvus* CBS391.50, *Pleurotopsis longqinqua* 6662, *T. patellaris* 6170, *P. mitis* 5644, and *P. serotinus* 6180 all lacked the intron. Further, PCR products of the 5' – partial LSU of the three *P. serotinus* collections and three *P. mitis* collections lacked an intron (Appendix 3.10).

3.4.2.2. Intron distribution in *P. stypticus*

The 5'- partial LSU fragment length was used as an indicator of Group I intron presence.

In *P. stypticus*, all 76 collections with cultures were examined for the presence of the intron. All 65 collections from Northern Hemisphere, representing Eastern North America, Pacific North America, Far East and Europe were ca. 1700 bp, suggesting intron presence, however one collection (5277, NC, USA), was heterozygous with two bands. One band was ca. 1700 bp and the other was ca. 1300 bp in length. In the Southern Hemisphere, six collections

from New Zealand and two collections from Tasmania were ca. 1300 bp, while three collections from New South Wales of Australia were ca. 1700 bp (see Appendix 3.10).

To make sure that the insert was a Group I intron for Southern Hemisphere collections, collection 3541 from New South Wales, Australia, was chosen for sequencing. The sequencing result revealed that 3541 harbored a Group I intron (Appendix 3.12).

3.4.2.3. Intron distribution in *Dictyopanus* sp.

In poroid *D. pusillus* and *D.* sp., all 15 collections were ca. 1700 bp in length, however one collection, 8733 from Estado Nayarit, Mexico was heterozygous, with two bands: a ca. 1300 bp band and a ca.1700 bp band (see Appendix 3.10).

3.4.2.4. Results of intron sequence comparisons

The best match of a BLAST search in the GenBank by using the intron sequence of *P. stypticus* collection 4319 (Maggia, Switzerland) was a *P. stypticus* SSU Group I intron (Hibbett, 1996) which was 67.95% homologous to the intron of *P. stypticus* exemplar 4319. In contrast, inserts in the 5'- partial LSU of *P. stypticus* exemplar 4319 and *D. pusillus* (collection 8265) were 83.8% homologous, and the intron of collection 4319 (Maggia, Switzerland) was 97.0% homologous to that of *P. stypticus* exemplar 3541 (New South Wales, Australia). The intron of *Dictyopanus pusillus* exemplar 8265 (FL, USA) was 83.8% homologous to the intron of *Panellus stypticus* collection 4319 (Maggia, Switzerland), and was 84.2% homologous to *Panellus stypticus* exemplar 3541 from New South Wales, Australia (Appendix 3.12).

3.4.3. Phylogenetic reconstruction

3.4.3.1. Phylogenetic reconstruction in the genus *Panellus* complex

3.4.3.1.1. Parsimony analyses in the genus *Panellus* complex based on 5'- partial LSU sequences

Panellus stypticus, P. mitis, P. ringens, P. violaceofulvus, P. serotinus, Dictyopanus pusillus, Tectella patellaris and *Pleurotopsis longinqua* were grouped for phylogenetic analyses. A multisequence alignment of this genus complex together with sequences obtained from GenBank is given in Appendix 3.11. The closest sequence homology for *D. pusillus* and *P. stypticus* was AF042638 (*Resinomycena acadiensis*).

Based on the multisequence alignment in Appendix 3.11, including all species in the *Panellus* genus complex and the closest outgroup, *Resinomycena acadiensis*, but excluding other related species, a single most parsimonious tree was produced (below Figure 3.14). The tree length had 143 steps. The length for each clade is indicated above the branch and bootstrap support was indicated in rectangles below the branch. There were five clades, each approximately equidistant from each other. *Panellus stypticus* and *D. pusillus* formed a close clade. The outgroup *Resinomycena acadiensis* was most closely related to the clade consisting of *P. stypticus* and *D. pusillus. Panellus ringens, P. violaceofulvus* and *Pleurotopsis longinqua* formed another very close clade. The remaining three species, *T. patellaris*, *P. mitis*, and *P. serotinus*, formed three independent monotypic clades. The position of *T. patellaris* was poorly supported. Positions of the remaining clades were well supported (Figure 3.14). *Panellus violaceofulvus* (CBS391.50) is remarkably similar to *Pleurotopsis longinqua* (1 bp difference); however, *P.violaceofulvus* collection CBS391.50 was received as a culture from CBS and is not anchored by a herbarium specimen. Therefore, it's accurate identification cannot be confirmed. In the Figure 3.14, species with bold names are considered to be within the genus *Panellus* sensu

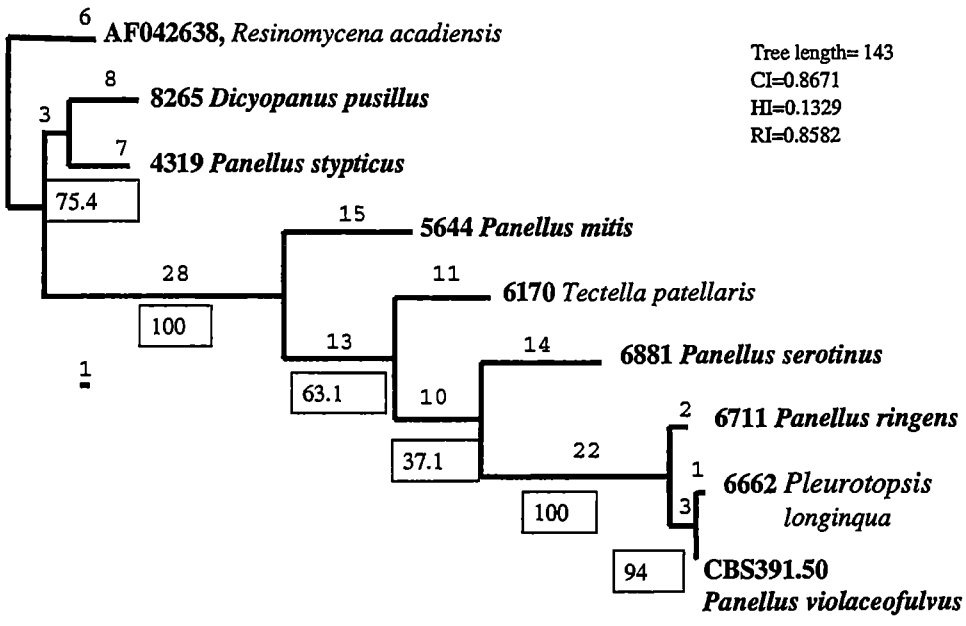


Figure 3.14. A single most parsimonious tree of the genus *Panellus* complex based on 5'- partial LSU
[Note: 1) upper number: clade length; lower number: bootstrap support. 2) species names with bold face are currently retained in genus *Panellus*.]

Burdsall and Miller (1975 and 1978), *Pleurotopsis longinqua* and *Tectella patellaris* are nested within *Panellus* in this concept.

3.4.3.1.2. Phylogenetic reconstruction of the genus *Panellus* complex together with other closely matched sequences

Six sequences and the yeast sequence listed in Table 2.4 were downloaded from GenBank by a BLAST search based on 5' - partial LSU sequences of exemplars for the genus *Panellus* complex. Those sequences except the yeast sequences were included in a phylogenetic analysis (sequence alignment in Appendix 3.11). Two most parsimoneous trees were produced with tree length of 215 steps (see below Figure 3.15 and Figure 3.16).

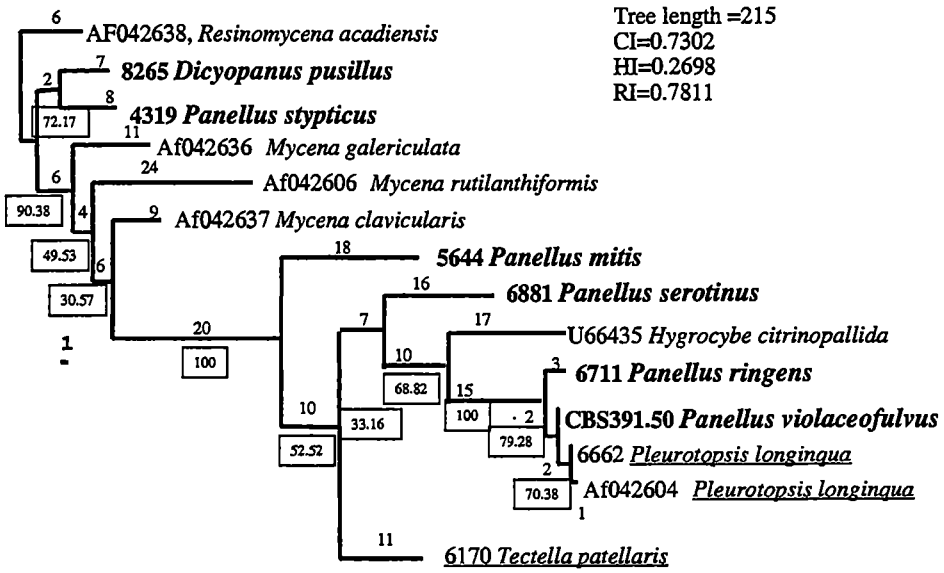


Figure 3.15. One of the two most parsimonious trees of the genus *Panellus* complex and close taxa based on 5'- partial LSU of ribosomal DNA

[Note: 1) upper number: clade length, lower number: bootstrap support. 2) Bold face indicates those in genus *Panellus*, underlined indicates those still in *Panellus* complex.]

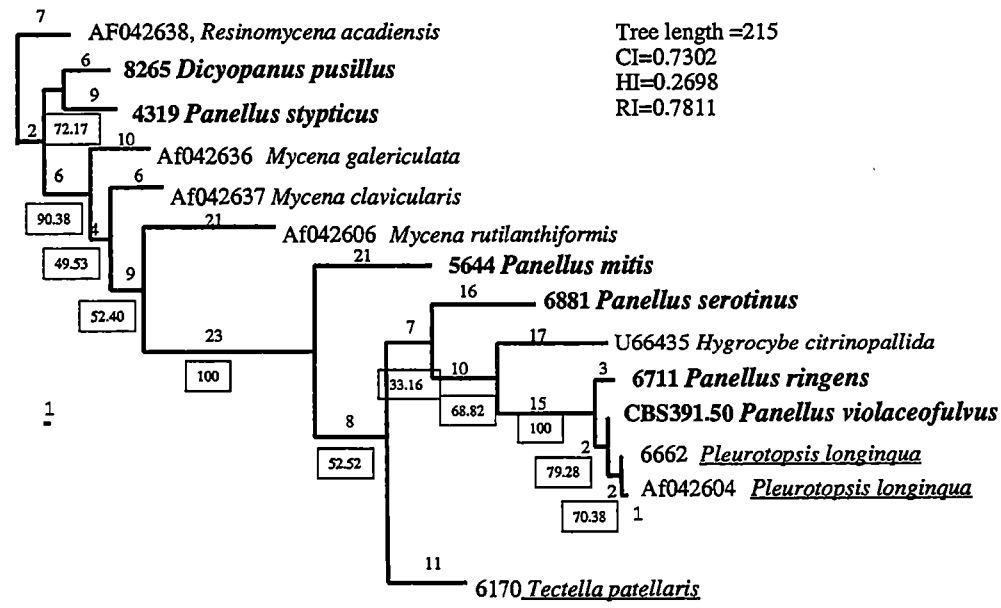


Figure 3.16. The other of the two most parsimonious tree of the genus *Panellus* complex and close taxa based on 5'- partial LSU of ribosomal DNA

[Note: 1) upper number: clade length, lower number: bootstrap support. 2) Bold face indicates those in genus *Panellus*, underlined indicates those still in *Panellus* complex.]

The topology of the genus *Panellus* complex in those two trees was not changed. The positions of AF042606 *Mycena rutilanthiformis* and AF042637 *Mycena clavicularis* were poorly supported, but the three *Mycena* species together with *Resinomycena acadiensis, P. stypticus* and *D. pusillus* formed a well supported clade. *Panellus ringens, P. violaceofulvus* and *Pleurotopsis longqinqua* formed another well supported clade. These results are consistent with the analyses above. Two independent monotypic clades *T. patellaris* and *P. serotinus* with long branches, were still poorly supported. U66435 *Hygrocybe citrinopallida* was nested between a clade consisting of *T. patellaris* and *P. serotinus* and a clade consisting of *Panellus ringens, P. violaceofulvus* and *Pleurotopsis longinqua.* It is obvious that the current genus *Panellus*, with or without *Tectella patellaris* and *Pleurotopsis longinqua*, is polyphyletic.

3.4.3.1.3. Neighbor-Joining analyses in the genus *Panellus* complex based on 5'- partial LSU sequences

Phylogenies based on parsimony analysis (**3.4.3.1.1**) and Neighbor-Joining analyses (Figure 3.17) were identical. There were five clades, each approximately equidistant from each other. *Resinomycena acadiensis* was most closely related to a clade consisting of *P. stypticus* and *D. pusillus. Panellus ringens, P. violaceofulvus* and *Pleurotopsis longqinqua* formed another very close clade. The remaining three species, *T. patellaris*, *P. mitis*, and *P. serotinus*, formed three independent monotypic clades. *Panellus violaceofulvus* (CBS391.50) was very close to *Pleurotopsis longinqua.*

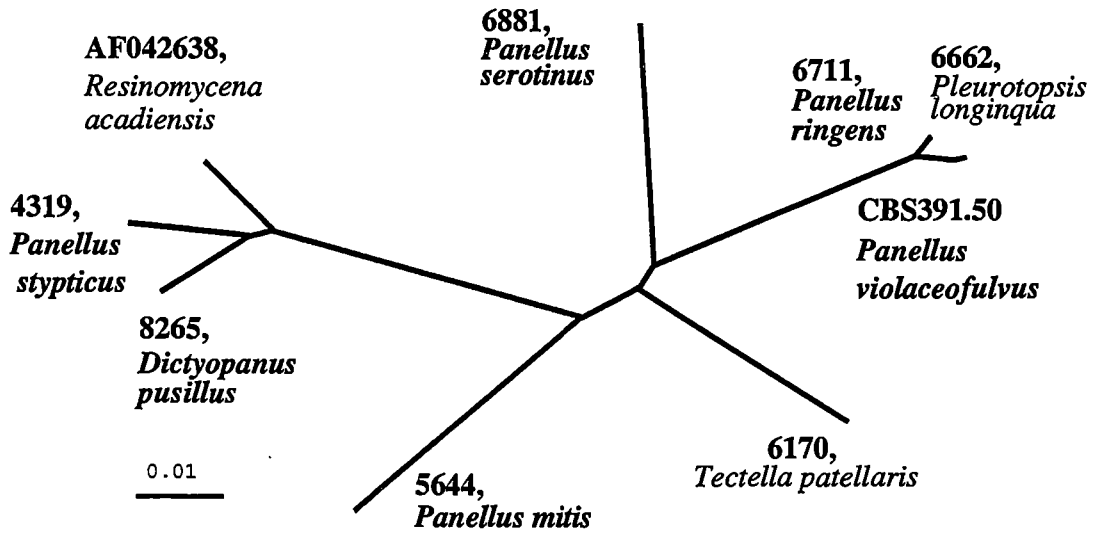


Figure 3.17. A Neighbor-Joining Tree of Genus *Panellus* Complex Based on Partial LSU

(Note: Bold face indicates those retained in genus *Panellus*.]

3.4.3.2. Phylogenetic reconstruction in *Panellus stypticus*

3.4.3.2.1. Parsimony analyses based on the ITS sequences

Parsimony analysis was used to examine the relationships between 10 geographically diverse exemplars in *P. stypticus*. Based on LSU sequences of exemplar 4319, the closest outgroup was *Dictyopanus pusillus*. Collection 8265 (*Dictyopanus pusillus*) served as the outgroup. Two most parsimonious trees were produced. They were identical except for the relative positions of the three Oceanian exemplars. One cladogram placed collection 3541 as basal to the Southern Hemisphere collections (Figure 3.18), and the other placed 3586 as basal (Figure 3.19). In Figure 3.18, the clade containing 3586 and 2675 was poorly supported with a bootstrap value of 56.17%, but the whole Oceanian clade is well supported with 97.67% bootstrap support. In Figure 3.18, *P. stypticus*

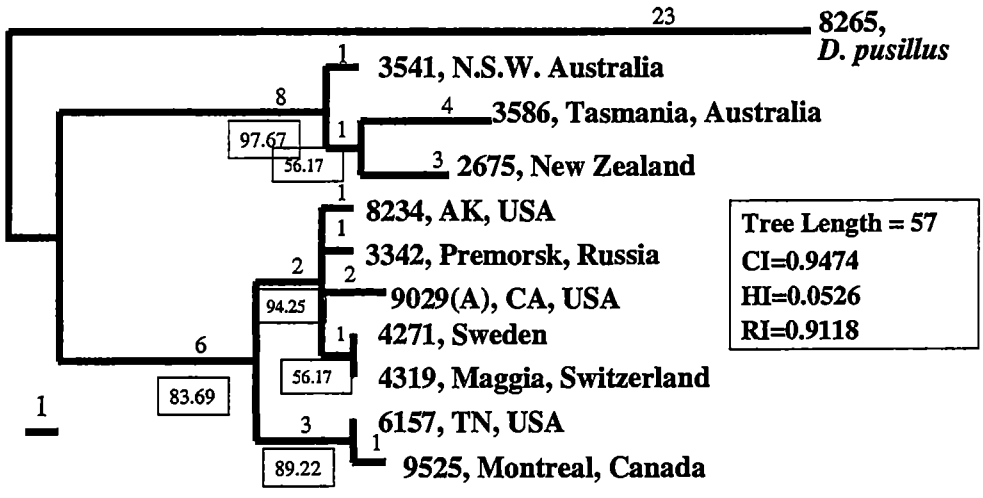


Figure 3.18. One of the two most parsimonious trees of 10 exemplars in *Panellus stypticus* based on ITS sequences

(Note: Upper number: clade length; Lower number: bootstrap support)

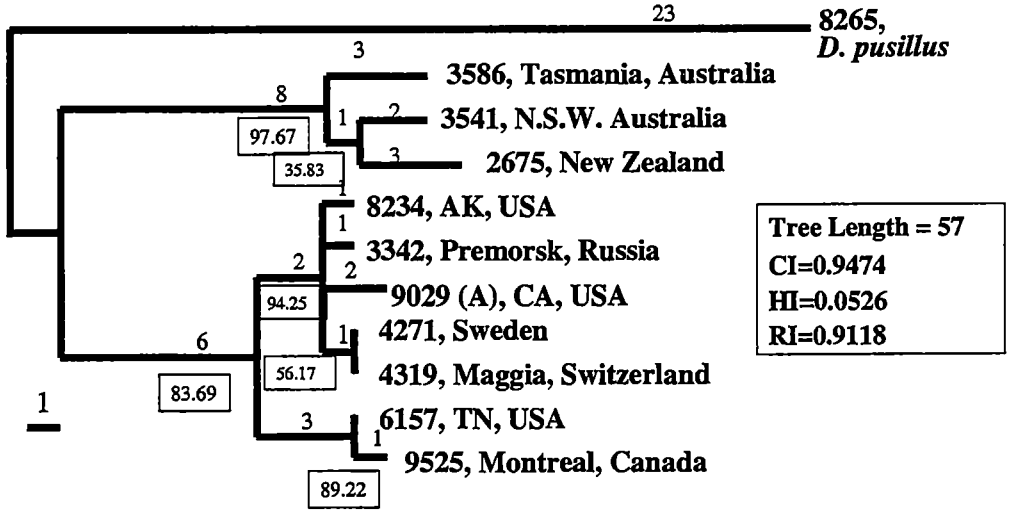


Figure 3.19. The other of the two most parsimonious trees of 10 exemplars in *Panellus stypticus* based on ITS sequences

(Note: Upper number: clade length; Lower number: bootstrap support)

collections were divided into two major well supported clades: a Northern Hemisphere clade and the Oceanian clade. In the Northern Hemisphere clade. Two collections from Eastern North America formed a clade. Collections from Pacific North America, the Far East, and Europe formed another well supported clade.

3.4.3.2.2. Neighbor-Joining analyses in *Panellus stypticus*

The Neighbor-Joining program was also used to reconstruct phylograms for the 10 exemplars in the species (Figure 3.20). The result of Neighbor-Joining analysis was almost identical to the two cladograms produced by parsimony analyses. There were two major clades, a Northern Hemisphere clade and a Southern Hemisphere clade. The Northern Hemisphere clade was further divided into an Eastern North America clade and a clade consisting of Pacific North America, the Far East and Europe. In the Southern Hemisphere clade, collection 3586 of Tasmania, Australia, was basal.

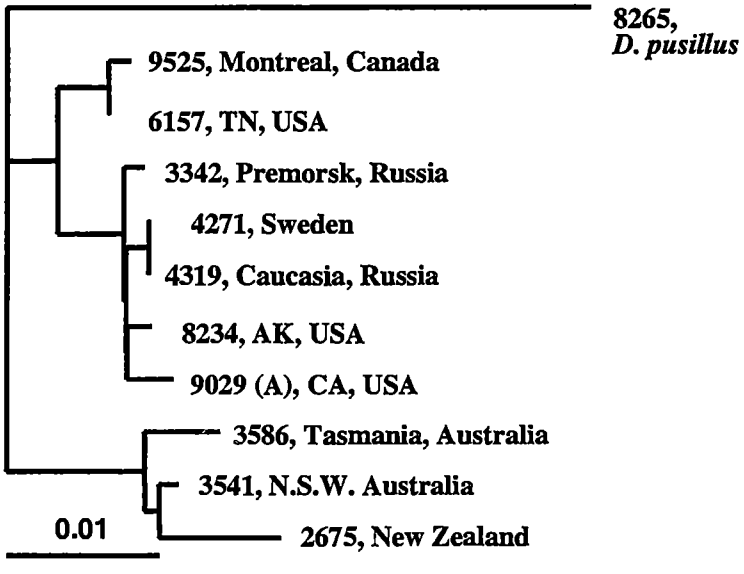


Figure 3.20. A Neighbor-Joining tree of 10 exemplars in *Panellus stypticus* based on ITS Sequences

4. DISCUSSIONS AND CONCLUSIONS

4.1. MORPHOLOGICAL ANALYSES

4.1.1. Morphologic analyses in the genus *Panellus* complex

4.1.1.1. Macroscopic and microscopic characters

Macroscopic and microscopic morphocharacters clearly separated all of the species in this genus complex, which is consistent with Miller's (1970) report.

All collections in *Dictyopanus pusillus* examined were panelloid. Northern Hemisphere *Panellus stypticus* collections and some collections from the Oceanian areas were also panelloid. A few collections from Oceanian areas, however, were nonpanelloid. All other species, *P. mitis, P. serotinus, P. ringens, P. violaceofulvus, T. patellaris* and *P. longinqua*, were nonpanelloid. Burdsall and Miller (1975 and 1978) merged *Dictyopanus* into *Panellus*, based in part on similar basidiome stature. Even with a few non-panelloid collections from the Southern Hemisphere, panelloid stature is a significant macroscopic character to circumscribe the clade consisting of *P. stypticus* and *D. pusillus*. This cohesive grouping was supported by phylogenies based on molecular data and morphological data.

If the observed weak amyloid reaction in *P. stypticus* and *D. pusillus* can be regarded as a normal amyloid reaction in the genus, then the amyloid reaction of two collections of *T. patellaris* in this study was strongly amyloid. Thus the amyloid reaction of spores in *T. patellaris* in this research was contradictory to the previous reports (Smith, 1949; Kühner and Romagnesi, 1953). The finding that forked lamellae were rare in *P. stypticus* was also contradictory to previous reports (Miller, 1970).

4.1.1.2. Phylogeny based on morphocharacters

The UPGMA tree based on morphocharacters had similar topology to phylograms obtained from parsimony analysis and Neighbor-Joining analysis of the 5'–partial LSU gene. *Panellus stypticus* and *D. pusillus* formed a clade, and *P. ringens*, *P. violaceofulvus* and *Pleurotopsis longinqua* formed another clade.

The grouping of two species, *P. stypticus* and *D. pusillus*, which included a lamellate and a poroid species, raised a question about the evolution of lamellate and poroid hymenial structure (see below). In the clade composed of *P. ringens, P. violaceofulvus* and *P. longinqua*, pleurotoid *P. longinqua* is macroscopically quite different from the sessile pendent *P. ringens* and *P. violaceofulvus*.

4.1.1.3. Circumscription of the genus *Panellus*

The genus *Panellus* has been circumscribed in a number of different ways by previous researchers. The genus was established by P. Karsten (1879) with only two characters, lateral stipe attachment and elliptical spores with the type species, *P. stypticus* (Bull. ex. Fr.) Karst. Karsten (1891) established a separate genus, *Sarcomyxa*, with *S. serotina* as the type species. Earle (1909) established the monotypic genus *Tectella* with the type species *Tectella patellaris* (Fr.) Murr. Smith (1949) erected two genera, *Panellus* and *Tectella*, within the subfamily Pleurotoideae (Tricholomataceae). *Tectella* was separated based on presence of nonamyloid spores, lack of stipe, and presence of a veil in the early stages of fruitbody development. Kühner and Romagnesi (1953), however, included *P. serotinus* (i.e., *Sarcomyxa serotina*), *P. mitis*, *P. ringens*, *P. patellaris* (i.e., *Tectella patellaris*), *P. violaceofulvus*, and *P. stypticus* within *Panellus*. They also mentioned that an amyloid reaction in *P. patellaris* (*T. patellaris*) was difficult to observe (but see above). Miller (1970) reported in North America that there were six species in the genus based on their possession of white, allantoid to narrowly elliptical amyloid

spores, a trama of thick-walled hyphae, even gill edges, a sessile or short stipitate basidiocarp, cheilocystidia and lignicolous habitat. Burdsall and Miller (1975) merged poroid *Dictyopanus* into *Panellus*, based on similarities of ovoid spores, a well developed stipe, and the ability to revive when rehydrated. They established two subgenera under *Panellus*: subgenus *Panellus* for *P. stypticus, P. pusillus* and *P. coplandii*, and subgenus *Mitellus* for *P. mitis, P. patellaris, P. serotinus, P. ringens,* and *P. violaceofulvus*. Later, Miller (1984) noted that the genus was easily separated from other pleurotoid genera such as *Pleurotus, Panus, Cheimonophyllum, Pleurocybella, Hohenbuehelia* and *Resupinatus* in that the spores of the genus were amyloid in addition to elliptical and smooth. Singer (1975) proposed a tribus *Panelleae* under Tricholomataceae, with three genera, *Panellus*, *Dictyopanus* and *Tectella*. *Panellus* contained two subgenera *Panellus* and *Serotinia*. Horak (1983) transferred *P. longinquus* to the genus *Pleurotopsis* as *P. longinqua* due to its gelatinous pileocutis. Corner (1986), in agreement with Burdsall and Miller (1978), treated *Dictyopanus* as a synonym of *Panellus* in his wide circumscription of the genus but tentatively included *Panellus longinquus* in *Panellus*.

According to Karsten's circumscription, the genus *Panellus* had a lateral stipe attachment and elliptical spores. However, in this study, the spores of the type species *P. stypticus* were primarily allantoid based on 69 world-wide collections. In only a very few collections were elliptical spores observed.

In this study, all species had amyloid spores although the degree of staining varied. Based on spore shape and the extent of spore staining in Melzer's reagent, species could be further divided into three groups: 1) *Panellus stypticus* and *Dictyopanus pusillus* with allantoid spores; 2) *P. mitis, P. serotinus* and *Tectella patellaris* with cylindrical spores; and 3) *P. ringens, P. violaceofulvus* and *Pleurotopsis longinqua* with allantoid spores showing color patches on the surface of the spores. Burdsall and Miller (1970), in agreement with findings above, described spores of *Panellus* (including *Dictyopanus, P. mitis, P. patellaris, P. serotinus, P. ringens,* and

P. violaceofulvus) as "allantoid, oblong, ovoid to narrowly ovoid, smooth, hyaline, amyloid in Melzer's reagent," but did not subdivide the genus based on micromorphology even though significant morphological differences exist within this group. *Panellus ringens* and *P. violaceofulvus* are sessile, pendent, eccentrically attached to substrate, and are nonpanelloid, as is *Tectella patellaris*. Clearly, those three species do not possess the character of lateral stipe attachment, which was established for the genus by Karsten (1879). Further, in *Tectella patellaris*, a membranous veil exists in young basidiomata. This is unique in this group and was the basis for describing *Tectella* as a separate monotypic genus (Earle, 1909). *Pleurotopsis longinqua* has a gelatinous pileocutis, unique to this group.

Vilgalys et al. (1991) stated that a genus should be based on monophyly and emphasize evolutionary history. A phylogeny based on morphocharacters indicated that the *Panellus* genus complex was quite diverse and probably not monophyletic. Only the *P. stypticus* and *D. pusillus* clade and the *P. ringens*, *P. violaceofulvus* and *Pleurotopsis longinqua* clade formed a well-supported monophyletic group. *Panellus mitis, P. patellaris, P. serotinus,* each formed a monotypic clade.

Based on the above, circumscription of the genus *Panellus* could include *P. stypticus* and *D. pusillus* only with the other *Panellus* species assigned to separate genera. Probably, additional intersterility groups exist within poroid *Dictyopanus* (see Corner, 1986) and, pending further studies, these may also belong to this clade. Under this concept, the genus *Panellus* could be defined morphologically as having panelloid stature, a lateral stipitate pileus, and smooth amyloid spores.

4.1.2. Morphological analyses within *Panellus stypticus*

Overall, morphological variability was greater in the Southern Hemisphere. There was a high proportion of collections that were nonpanelloid and devoid of lamella anastomosis in

Oceanian areas but not in the Northern Hemisphere. Spore length and width was also more variable (Figure 3.6) with larger spores overall. Spore length and width for the Eastern US were distinct from those of Pacific North America.

4.1.3. Conclusions of morphological analyses

In the *Panellus* complex, circumscription based on morphological characters was not well defined. Although species in the complex were well separated, unique species characters made this complex morphologically diverse. An examination of the chronological development in the circumscription of this genus showed that there was no single character or group of characters that could be used for defining *Panellus*.

Circumscription of the genus *Panellus* could include *P. stypticus* and *D. pusillus* only in this *Panellus* complex studied. Probably, additional intersterility groups exist within poroid *Dictyopanus*. Genus *Panellus* could be defined morphologically as having panelloid stature, a lateral stipitate basidioma, and smooth amyloid spores.

In *Panellus stypticus*, spore shape and the ratio between spore length and width remained as stable and conserved characters. In this species, however, many characters such as lamella height and spore size were differentiated among five geographical areas, even between neighboring areas. Collections from Australia and New Zealand were considerably more diverse for morphological characters including spore size, basidioma stature and lamella anastomoses.

4.2. ISOZYME ANALYSES

4.2.1. Extracellular laccase (ECL) patterns for the genus complex and *P. stypticus*

Two conclusions could be made based on ECL analyses: 1) There are characteristic patterns for *P. stypticus, P. serotinus,* and *D. pusillus* (Figure 3.7 and Figure 3.8) that can help identify these species; 2) Within *P. stypticus*, there is considerable variation in electrophoretic

mobility within and between geographical areas. Changes in electrophoretic mobilities are due to the gradual accumulation of mutations that result in a change in the surface charge of a protein (Acquaah, 1992). The observed electrophoretic variability, therefore, represents underlying genetic variability. This is in contrast to studies by Wu et al. (1995) who found little variability in *Clavicorona pyxidata* with the exception of two collections from China. Gordon and Petersen (1997, 1998), however, found differences in *Marasmius* ECL patterns between Europe and Eastern North America.

In *Panellus stypticcus*, five geographical areas differed in the distribution of ECL1 alleles. Interestingly, the most divergent areas from each other were Eastern North America and Pacific North America. Europe and the Far East were more similar. Pacific North America and Oceanian areas showed a bimodal distribution, suggesting further differentiation within these geographical areas.

4.2.2. Alcohol dehydrogenase (ADH) electrophoreses in *P. stypticus*

Electrophoreses suggested that band ADH3 was unique and present only in Pacific North America, the Far East and Europe. This result suggested an affinity between collections from these regions.

4.2.3. Esterase (EST) electrophoreses in *P. stypticus*

As with ECL, Eastern North America showed higher percentage in slower bands in EST1-1. Oceanian areas were quite divergent in EST1-1 distribution.

4.2.4. Conclusions in isozyme analyses

Eastern and Pacific North American regions are clearly differentiated from each other based on the distribution of extracellular laccase (ECL1) and Esterase (EST1-1) alleles. This

separation is also consistent with the finding of an unique allele, ADH3, in Pacific North America that is absent in Eastern North America.

A connection between Pacific North America, Asia and Europe may be inferred based on the presence of the ADH3 allele in these populations. The distribution of ECL1 and EST1-1 alleles, however, did not clearly separate Northern and Southern Hemisphere populations. A bimodal distribution for ECL and EST1-1 alleles in Oceanian areas suggests local differentiation within this region.

4.3. MATING STUDIES

4.3.1. Mating studies in *Panellus stypticus*

This study confirmed that there is a single intersterility group (biological species) of *P. stypticus* existing over Eurasia, North America, New Zealand, Australian New South Wales and Tasmania. This is consistent with previous studies (Petersen and Bermudes, 1992a and 1992b). In *P. stypticus*, however, overall mating compatibility rates were reduced between the Northern and Southern Hemisphere collections and mating reactions classified as "antagonistic" were commonly observed. In contrast, ability to intercross remained high within the Northern Hemisphere collections and antagonistic reactions were not observed. Collections within the Southern Hemisphere showed reduced self compatibility and antagonistic reactions occurred between and within the three geographical areas in the Southern Hemisphere.

4.3.2. Mating studies in *Dictyopanus pusillus*

Result of mating tests in collections originally designated as *Dictyopanus pusillus* and *Dictyopanus sp.* revealed that at least two intersterility groups (biological species) were harbored in these morphologically indistinguishable collections. Thus, there may be sibling species within *D. pusillus*. Microscopic examination revealed extensive variation in spore size and shape in this

species. More collections from a wider geographical area, and further analyses may be needed for a better understanding of species circumscription, species structure, variation and phylogeny for this species. Spore shape in *D. pusilus* was highly variable in contrast to *P. stypticus*. In P. stypticus, spore shape and the ratio between spore length and width remained conserved and stable over wide geographical areas.

4.3.3. Conclusion of mating studies

Reduced intercompatibility between the Northern and Southern Hemisphere populations of *P. stypticus* was observed. This, coupled with reduced intercompatibility within and between populations from the Southern Hemisphere, is suggestive of founder effects in which drift and inbreeding in small isolated populations reduce available genetic diversity and result in intrapopulation and interpopulation barriers to gene exchange (Hartl, 1988).

4.4. MOLECULAR ANALYSES

4.4.1. Group I intron

Panellus stypticus and *Dictyopanus pusillus* shared a homologous Group I intron. The remaining species of this genus *Panellus* complex: *Panellus mitis, P. serotinus, P. ringens, P. violaceofulvus, Tectella patellaris,* and *Pleurotopsis longinqua,* lacked this intron. The presence of the intron supported the close relationship between *Panellus stypticus* and *Dictyopanus pusillus*, and separated the two species from the rest of the genus complex, which confirmed the proposed relationship between these two groups (Burdsall and Miller, 1975 and 19778) and suggested that the intron was present in the ancestral species prior to the separation of *Dictyopanus pusillus* and *Panellus stypticus.*

Within *P. stypticus,* all Northern Hemisphere and Australian New South Wales collections had this intron, while this intron was absent in Tasmanian and New Zealand collections. A

97.0% identity of the intron in New South Wales exemplar 3541 and the European exemplar 4319 confirmed their homology. This finding supports a hypothesis that there is a closer relationship between New South Wales and the Northern Hemisphere collections than between other Southern Hemisphere collections and the Northern Hemisphere collections.

4.4.2. LSU sequence studies in the genus *Panellus* complex

In this study, results of parsimony analysis and Neighbor-Joining analysis based on 5' – partial LSU analysis produced a phylogram with *Panellus stypticus* and *Dictyopanus pusillus* in a clade. *Panellus ringens, P. violaceofulvus* and *Pleurotopsis longinqua* formed a second monophyletic clades, while *P. mitis, P. serotinus* and *Tectella patellaris* each formed a monotypic clade between the above two clades. A UPGMA tree based on morphocharacters in the genus complex generated a similar topology. The above facts suggested there were five genera in this *Panellus* complex. Parsimony analyses of this genus complex together with downloaded sequences from GenBank showed that species in this genus complex were polyphyletic, and that those clades were well supported.

In this genus complex, the unexpected grouping of *P. ringens, P. violaceofulvus* and *P. longinqua* raised some interesting questions, since the culture used for LSU sequencing representing *P. violaceofulvus* was not anchored with a herbarium specimen. If the culture truly is *P. violaceofulvus*, then there is a remarkable morphological divergence between *Pleurotopsis longinqua* and *P. violaceofulvus* but little change in the LSU sequence. Alternately, the culture could represent *Pleurotopsis longinqua*; however, *P. longinqua* is known from Australia, New Zealand and New Guinea and the Pacific North West (Horak, 1983; Libonati-Barnes and Redhead, 1984), and also from South America (Petersen and McCleneghan, 1995 and 1997). It is not known from Europe and this would be an unexpected range extension. It is clear that this area needs further examination with new material.

4.4.3. RFLP patterns in *Panellus stypticus*

Results of the RFLP analyses revealed that there were five RFLP patterns produced by four restriction enzymes. These five patterns represent only two or three base pair (bp) differences in the ITS region between two neighboring areas, (i.e. there are only two bp differences between the Far East and Europe, three bp difference between the Far East and Pacific North America, and two bp differences between the Far East and Alaska). RFLP patterns, however, were consistent within geographical regions proposed on the basis of potential geographical barriers to gene exchange (Eastern North America, Pacific North America, Far East, Europe, Australian New South Wales, Australian Tasmania, and New Zealand). This suggests that geographical barriers such as mountains, deserts and oceans are real barriers for these fungi.

4.4.4. ITS sequence studies in *Panellus stypticus*

All the exemplars in the Northern Hemisphere had similar ITS lengths but the three Oceanian exemplars had a number of deletions in both the ITS1 and ITS2 regions. Compared to the Northern Hemisphere exemplars, New Zealand exemplar 2675 had the most fragment deletions; the New South Wales exemplar had the fewest fragment deletions. The Tasmanian exemplar was in between.

4.4.5. Conclusions in molecular analyses

In *P. stypticus*, RFLP data were consistent with the hypothesis that there were seven geographical areas (populations). Sequence data for the ITS region and for the group I intron supported a separation of Northern and Southern Hemisphere populations with the New South Wales population being most closely related to Northern Hemisphere populations. Among

Northern Hemisphere populations, Eastern North America was clearly unique, separated from both Europe and the Pacific Northwest populations.

4.5. THE GENUS *PANELLUS* - SUMMARY AND RECOMMENDATIONS

Basidiomycetes or basidiomycota are basically divided as Agaricales versus Aphyllophorales (sensu Fries), or Polyporaceae versus Agaricaceae and Bolitaceae sensu Smith (Smith, 1949). Lamellate versus poroid hymenphores are typical characters that would determine an order or family level.

Pegler (1983) suggested that the pleurotoid genera *Lentinus, Pleurotus* and *Panus* were derived from polyporoid ancestry, i.e., pleurotoid fungi and Agaricales were convergent in the evolution of hymenium structure (cited by Hibbett, 1991). Conversely, Corner (1981) considered that monomitic hyphal structure was primitive and thus proposed an alternate phylogeny in which *Panus, Pleurotus* and *Lentinus* were a basal, paraphyletic group from which the Agaricales and Polyporaceae evolved.

In this genus complex, a phylogeny based on the 5'-partial LSU gene revealed that *P. stypticus* and *D. pusillus* were nested in *Mycena* and *Resinomycena* clades. *Hygrocybe* was nested between a clade consisting of *P. mitis*, *P. serotinus*, *Tectella patellaris* and *P. ringens* and a clade consisting of *P. violaceofulvus* and *Pleurotopsis longinqua*. Thus, lamellate and poroid species, pleurotoid and nonpleurotoid genera were nested together. While the question of whether pleurotoid genera were evolutionarily basal for agarics cannot be resolved by this study, this study did provide some information about whether poroid or lamellate hymenium were key criteria for orders. Since poroid *D. pusillus* was nested within lamellate *Mycena* and *Resinomycena* clades, and within *Panellus* in a strict sense, the poroid hymenium might be a derived character.

Sequence and morphological data supported Burdsall and Miller's (1975, 1978) merger of *Dictyopanus* into *Panellus*. These two species clearly were in the same clade, supported by parsimony analysis, Neighbor-Joining analysis based on LSU sequences, and the presence of a Group I intron in the nuclear LSU. The transfer of *Pleurotopsis longinqua* from *Panellus* was warranted, based on the genetic distance from the *P. stypticus-D. pusillus* clade but would require an additional transfer of *P. violaceofulvus* and *P. ringens* to maintain the coherence of this clade. More evidence (morphological and molecular) is needed for the transfer of *P. violaceofulvus*. *Tectella patellaris* has already been assigned a separate generic designation (Earle, 1909). *P. serotinus* was well separated from either of the terminal clades and formed a monotypic clade. I suggest adopting Karsten's (1891) genus *Sarcomyxa* for the species. *Panellus mitis* was also well separated from either of the terminal clades and probably requires separate generic assignment. Burdsall and Miller (1975) established a subgenus *Mitellus* under genus *Panellus,* which included *P. mitis, P. serotinus, P. ringens, P. violaceofulvus* and *P. patellaris* (*T. patellaris*), and *P. mitis* was specified as the type species for subgenus *Mitellus*. We suggest that if *P. mitis* is erected to a separate genus, *Mitellus* would be an appropriate name for the new genus.

I suggest that five genera be used to describe this complex: *Panellus* for the *Panellus stypticus – Dictyopanus pusillus* clade, *Pleurotopsis* for *Pleurotopsis longinqua – P. ringens –P. violaceofulvus* clade, *Tectella* for *Tectella patellaris* , *Sarcomyxa* for *P. serotinus* and a new genus status for *P. mitis*. Thus species in this study should be named as follows: *Panellus pusillus* for *D. pusillus; Sarcomyxa serotina* for *P. serotinus; Pleurotopsis ringens* for *Panellus ringens* and *Pleurotopsis longinqua* for *Panellus longinquus*. However the placement of *Pleurotopsis violaceofulvum* for *Panellus violaceofulvus* needs more evidence.

4.6. POPULATIONS AND GENE FLOW WITHIN *PANELLUS STYPTICUS*

Several lines of evidence supported the biogeographical separation of the Pacific Coast of North America from Eastern North America. Parsimony and Neighbor-Joining analyses based on ribosomal ITS sequences supported two clades in the Northern Hemisphere, an Eastern North American clade and a Pacific North American - Eurasian clade. RFLP analyses in the ribosomal ITS region revealed that the Eastern North American clade had an unique RFLP pattern. The presence of bioluminescence in Eastern North America, not observed in Pacific North American collections, is further evidence for separation of these two geographical domains. Mating barriers, however, were not observed between these two areas suggesting that mating systems are evolutionarily conserved and must have been preserved since separation of *P. stypticus* into geographically isolated populations. The reason(s) for separation of Eastern North American populations from Western North American populations must remain speculative; however, an obvious geographical barrier is the Rocky Mountains.

Several lines of evidence support a connection between Pacific North America and Asia or Eurasia. As noted above, ITS sequence data place collections from these regions into a single clade. European and Asian collections, however, differed in RFLP patterns suggesting geographical separation of these regions, perhaps caused by the Tibetan Plateau, Gobi desert, and/or Ural mountains. An evolutionary relationship between the Pacific North American collections and Asian collections was suggested by a shared unique allele for ADH3 and by the similarity of ECL alleles.

4.7. EVOLUTION WITHIN *PANELLUS STYPTICUS* AND ORIGIN OF *P. STYPTICUS*

Data from this study did not determine the center of origin for *Panellus* (i.e., *P. stypticus* and *Dictyopanus pusillus*). ITS sequence data and presence or absence of a Group I intron did, however, provide some information about putative patterns of evolution and dispersal in *P.*

stypticus. With a clear separation of *P. stypticus* into Northern and Southern Hemisphere clades, the first question is "which clade is basal?"

Alignment of ITS region for both *Panellus stypticus* and *Dictyopanus pusillus* sequences (Appendix 3.7) made clear that at least four gaps present in Oceanian deletions were occupied by sequences that were similar in Northern Hemisphere *Panellus stypticus* and *Dictyopanus pusillus* collections. One explanation for this finding is that the longer Northern Hemisphere sequence is plesiomorphic and deletions found in the Southern Hemisphere collections were derived. In this scenario, the Northern Hemisphere collections would be ancestral to the Southern Hemisphere collections. An alternate possibility, however, is that Southern Hemisphere *P. stypticus* is ancient and has given rise to both Northern Hemisphere *P. stypticus* and *D. pusillus*. Similar patterns of evolution have been observed in *Pleurotus* in which *Pleurotus populinus* is buried within the *P. ostreatus* clade (Vilgalys and Sun, 1994) and in *Flammulina* in which *F. mexicana* is buried within the *F. populicola* clade (Hughes et al., in press). Both hypotheses are consistent with the presence of a Group I intron in Northern Hemisphere *P. stypticus* and *D. pusillus* collections but not in collections from New Zealand and Tasmania. The *D. pusillus* intron is divergent, however, from both *P. stypicus* introns, a finding that would support separation of *P. stypticus* from *D. pusillus* prior to the separation of Southern Hemisphere *P. stypticus* from Northern Hemisphere *P. stypticus*. Further, ECL profiles were more similar within *P. stypticus* than between P. stypticus and *D. pusilus*. Together, these data suggest that Northern Hemisphere *P. stypticus* was ancestral to the Southern Hemisphere collections.

Dispersal from the Northern Hemisphere to the Southern Hemisphere may have been from Asia. Dispersal via Australia is supported by the presence of a Group I intron in the New South Wales collections but not in New Zealand or Tasmania, and by shared RFLP patterns between New South Wales and Far East. Of the three populations sampled, the New Zealand population has the most divergent ITS sequence. The populations from New South Wales and Tasmania are

more similar. The hypothesis of dispersal from Asia is also supported by evidence from isozyme analyses which showed that enzyme mobility patterns were most divergent between Eastern North American and Oceanian collections. Decreased ability to intercross observed within Southern Hemisphere populations and a series of ITS deletions not found in the Northern Hemisphere are consistent with evolution in the Southern Hemisphere as a series of founder events, as are differences in lamella height and spore size. The genetic divergence in Tasmania and New Zealand could be explained by founder events resulting in inbreeding and genetic drift (Hartle, 1988).

New Zealand was separated from Australian continent 60-70 mya (Smith and Briden, 1977). Tasmania, however, separated from the Australian continent much later, and was reconnected by a land bridge to the Australian continent 18,000 years ago during the glacial maxima (Brown and Lomolino, 1998). It is possible, therefore, that the establishment of Tasmanian population was a relatively recent event. A question remains as to when the New Zealand populations were established. It is likely that this may have been more recent than 60-70 mya (separation of New Zealand from Gondwana) because there is insufficient genetic divergence to support isolation for that time frame.

It was not possible to suggest a center of origin within the Northern Hemisphere. Data presented above are consistent with a hypothesis of an earlier isolation of the Eastern North American populations from the Pacific North American and Eurasian populations, and a later isolation of Pacific North American populations from Asian populations and Asian populations from European populations. A land connection between Eastern North America and Eurasia was present beginning 70 – 80 mya (million years ago) (Smith and Briden, 1977) and an epicontinental sea (about 65-70 mya) separated Pacific North America from Eastern North America (Graham, 1993) During the Eocene (50 mya), the climate warmed and plant populations pushed northward. At this time, exchange was possible between Europe and North America via

the North Atlantic land bridge or through limited water gaps until the late Eocene (30mya) (Wolfe 1975, Tiffney 1985, Graham 1993). At this time, the Rocky Mountains, which began forming in the Cretaceous, were continuing to rise and may have prevented or reduced gene exchange between North American East and West coast populations. In contrast, connections between Pacific North America and Asia were present sporadically in the Pliocene (3.5 mya; Hopkins, 1967) via the Bering Land Bridge and may have been available for migration as recently as 10,000 years ago (Hopkins, 1967). Cold northern climates and glaciation during these more recent times, however, may have prohibited plant and fungal migration. Separation of Europe and Asia may have occurred with the rise of the Tibetan Plateau and the subsequent emergence of the Gobi desert beginning ca. 20 mya (million years ago) with the collision between the Indian and Eurasian plates (Smith et al., 1981).

The above scenario assumes that the species *Panellus stypticus* existed prior to the loss of the North Atlantic Land Bridge, ca. 30 million years ago. There is virtually no fossil record for fungi but the few data points that are available suggest that this is possible. Based on ribosomal 18S sequences, the basidiomycetes may have originated 300-400 mya with mushrooms (macrobasidiomycete) arising 65 mya. (Berbee and Taylor, 1993). Hibbett et al., (1997) identified a putative *Mycena* in Dominican amber deposits dated at 15-20 mya and an ancient *Marasmius*-like fruitbody dated to the mid-Cretaceous, 90-94 mya. Biological data showing continent-specific distributions in the Northern Hemisphere also suggest that current fungal species may have existed prior to modern continental separations. ITS sequences of European and North American collections of *Flammulina velutipes* differed by a single restriction site and the European form was not found in North America (Hughes et al, 1999). *Pleurotus pulmonarius* and *P. ostreatus* collections showed intercontinental differences (Vilgalys and Sun, 1994; Petersen, 1995) as did collections of *Agaricus bisporus* (Kerrigan et al., 1995) and *Marasmius androsaceus* (Gordon and Petersen, 1997).

Regardless of how populations of this species became established in different geographical regions, gene exchange between populations became restricted and local differentiation occurred as shown by morphological, enzymatic and RFLP differences between geographical areas.

REFERENCES

Acquaah, G. 1992. *Practical Protein Electrophoresis for Genetic Research.* Dioscorides Press, Portland.

Bayev, A., O. I. Georgiev, A. A. Hadjiolov, N. Nikolaev, K. G. Skryabin, and Z. V. M. 1981. The structure of the yeast ribosomal RNA genes. 3. Precise mapping of the 18s and 25s rRNA genes and structure of the adjacent regions. *Nucleic Acids Research* 9: 789-799.

Berbee, M. L., and J. W. Taylor. 1993. Dating the evolutionary radiations of the true fungi. *Canadian Journal of Botany* 71: 1114-1127.

Bermudes, D., V. L. Gerlach, and K. H. Nealson. 1990. Effects of culture conditions on mycelial growth and luminescence in *Panellus stypticus*. *Mycologia* 82: 295-305.

Brown, J. H., and M. V. Lomolino. 1998. *Biogeography.* Sinauer Associates, Inc., Sunderland, Massachusettes.

Burdsall, B. H., and O. K. Miller. 1975. A reevaluation of *Panellus* and *Dictyopanus* (Agaricales). *Beih. Nova Hedwigia* 51: 79-91.

Burdsall, B. H., and O. K. Miller. 1978. Notes on the genus *Panellus*. *Mycotaxon* 7: 511-514.

Carlile, M. J. 1987. Genetic exchange and gene flow: their promotion and prevention. Pages 203-214 in A. D. M. Rayner, C. M. Brasier, and D. Moore, eds. *Evolutionary Biology of the Fungi.* Cambridge University Press, Cambridge.

Carlson, J. E., L. K. Tulsieram, J. C. Glaubitz, V. M. K. Luk, C. Kauffeldt, and R. Rutledge 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. *Theor. and Appl. Genet.* 83: 194-200.

Cech, T. R. 1988. Conserved sequences and structures of group-I introns: building an active site for RNA catalysis--a review. *Gene* 73: 259-271.

Corner, E. J. H. 1981. The agaric genera *Lentinus*, *Panus*, and *Pleurotus* with particular reference to Malaysian species. *Beih. Nova Hedwigia* 69: 1-169.

Corner, E. J. H. 1986. The Agaric genus *Panellus* Karst. (including *Dictyopanus* Pat.) in Malaysia. *Gard. Bull. Sing.* 39: 103-147.

DePriest, P. T. 1993. Molecular innovations in lichen systematics: the use of ribosomal and intron nucleotide sequences in the *Cladonia chlorophaea* complex. *Bryologist* 96: 314-325.

DePriest, P. T., and M. D. Been. 1992. Numerous Group I introns with variable distributions in the ribosomal DNA of a lichen fungus. *J. Mol. Biol.* 228: 315-321.

Dujon, B. 1989. Group-I introns as mobile genetic units: facts and mechanistic speculations--a review. *Gene* 82: 91-114.

Earle, F. S. 1909. The genera of the North American gill fungi. *Bulletin of New York Botanical Garden,* 5: 373.

Felsenstein, J. 1991. *PHYLIP (Phylogeny Inference Package), version 3.5c*. Department of Genetics, University of Washington, Seattle, WA.

Fouly, H. M., H. T. Wilkinson, and W. Chen. 1997. Restriction analysis of internal transcribed spacers and the small subunit gene of ribosomal DNA among four *Gaeumannomyces* species. *Mycologia* 89: 590-597.

Gargas, A., P. T. DePriest, and J. W. Taylor. 1995. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Mol. Biol. Evol.* 12: 208-218.

GCG. 1997. Genetics Computer Group. Oxford Molecular Group, Inc.

Gibbon, A. 1996. On the many origins of species. *Science* 273: 1946-1949.

Gordon, S. A. 1994. Infraspecific variation within three species of *Marasmius* (Tricholomataceae, Agaricales, Basidiomycotina). Dissertation, The University of Tennessee, Knoxville. P249.

Gordon, S. A., and R. H. Petersen. 1992. Interbreeding populations of some *Marasmius* species. *Mycologia* 84: 204-208.

Gowan, S., and Vilgalys R. 1991. Ribosomal DNA length polymorphisms within populations of *Xylaria magnoliae* (*Ascomycotina*). *American Journal of Botany* 78: 1603-1607.

Graham, A. 1993. History of the vegetation: Cretaceous (Maastrichtian) - Tertiary. Pages 57-70 in Flora of North America Editorial Committee, ed. *Flora of North America.* Oxford University Press, New York.

Harrington, F. A., D. H. Pfister, D. Potter and M. J. Donoghue. 1999. Phylogentic studies within the *Pezizales*. I. 18s rRNA sequence data and classification. *Mycologia* 91: 41-50.

Hartl, D. L. 1988. *A Primer of Population Genetics*. Sinauer Associates, Inc., Sunderland, MA.

Hesler, L. R. 1960. *Mushrooms of the Great Smokies*. The University of Tennessee Press, Knoxville.

Hibbett, D. S. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. *Mol. Biol. Evol.* 13: 903-917.

Hibbett, D. S., D. Grimaldi, and M. J. Donoghue. 1997. Fossil mushrooms from miocene and cretaceous ambers and the evolution of the homobasidiomycetes. *Am. J. Bot.* 84: 981-991.

Hibbett, D. S., Pine, E.M., Langer, E., Langer, G., and Donoghue, M.J. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proc. Nat. Acad. Sci. USA* 94: 12002-12006.

Hibbett, D. S., and M. J. Donoghue. 1995. Progress toward a phylogenetic classification of the Polyporaceae through parsimony analyses of mitochondrial ribosomal DNA sequences. *Can. J. Bot.* 73: S853-S861.

Hibbett, D. S., and R. Vilgalys. 1991. Evolutionary relationships of *Lentinus* to the Polyporaceae: evidence from restriction analysis of enzymatically amplified ribosomal DNA. *Mycologia* 83: 425-439.

Hillis, D. M., C. Moritz, and B. K. Mable, eds. 1996. *Molecular Systematics*. Sinauer Associates, Inc., Sunderland, MA.

Holst-Jensen, A., M. Vaage, T. Schumacher and S. Johanson. 1999. Structural characteristics and possible horizontal transfer of Group I introns between closely related plant pathogenic fungi. *Mol. Biol. Evol.* 16: 114-126.

Hopkins, D. M. 1967. The Cenozoic history of Beringia - A synthesis. Pages 451-485 in D. M. Hopkins, ed. *Neogene and early Quaternary vegetation of north-western North America and northeastern Asia.* Stanford University Press, Stanford.

Horak, E. 1968. Synopsis generum Agaricalium (Die Gattungstypen der Agaricales), in Beiträge zur Kryptogamenflora der Schweiz, Band XIII, Kommissionsverlag Druckerei Buchuler + Co AG, Wabern-Bern: p741.

Horak, E. 1983. Mycogeography in the South Pacific region: Agaricales, Boletales. *Austral. J. Bot. Suppl. Ser.* 10: 1-41.

Hughes, K. W., L. L. McGhee, A. S. Methven, J. Johnson, and R. H. Petersen. 1999. Patterns of geographic speciation in the genus *Flammulina* based on sequences of the ribosomal ITS1-5.8S-ITS2 area. *In review* .

Huss, M. J., C. L. Campbell, D. B. Jennings, and J. F. Leslie. 1996. Isozyme variation among biological species in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* 62: 3750-3756.

Jin, J.-K., and C.-L. Li. 1994. Studies on extracellular laccase (ECL) isozymes and moncaryotic pairing compatibility in relation to taxonomic identification of *Pleurotus* species. *Jiangsu Journal of Agriculture Science* 10: 11-17.

Johnson, J., and R. Vilgalys. 1998. Phylogenetic systematics of *Lepiota sensu lato* based on nuclear large subunit rDNA evidence. *Mycologia* 90: 971-979.

Karsten, P. A. 1879. Rysslands, Finnlands och den Skandinaviska Halfons Hallsvampar. *Finska Letteratur-Sallskapets Tryckeri, Helsinki* 32: 248 p.

Karsten, P. A. 1891. Symbolae ad Mycologiam Fennicam. *Societas pro Fauna et Flora Fennica* 18: 62.

Kerrigan, R. W. 1990. Evidence of genetic divergence in two populations of *Agaricus bisporus*. *Mycol. Res.* 94: 721-733.

Kerrigan, R. W., D. B. Carvalho, P. A. Horgen, and J. B. Anderson. 1995. Indigenous and introduced populations of *Agaricus bisporus*, the cultivated button mushroom, in eastern and western Canada: Implications for population biology, resource management, and conservation of genetic diversity. *Can. J. Bot.* 73: 1925-1938.

Kerrigan, R. W., and I. K. Ross. 1988. Extracellular laccases: Biochemical markers for *Agaricus* systematics. *Mycologia* 80: 689-695.

Kornerup, A., and J. H. Wanscher. 1981. *Farben (Color standard).* Taschenlexikon der Farbe, Sadolin & Holmblad, Kopenhagen.

Kretzer, A., Y. Li, T. Szaro, and T. D. Bruns. 1996. Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications. *Mycologia* 88: 776-785.

Kretzer, A., and B. T.D. 1997. Molecular revisitation of the genus *Gastrosuillus*. *Mycologia* 89: 586-589.

Kühner, R., and H. Romagnesi. 1953. *Flore analytique des champignons superieurs.* Masson et Cie, Paris.

Largent, D., D. Johnson, and R. Watling. 1977. *How to identify mushrooms to genus III. Microscopic features.* Mad River Press, Inc., Eureka.

Libonati-Barnes, S. D. 1981. Systematics of Tectella, Panellus, Hohenbuehelia and Resupnatus (Tricholomataceae) in the Pacific Northwest. Dissertation, The University of Washington, Seattle, WA. P366.

Libonati-Barnes, S. D., and S. A. Redhead. 1984. *Panellus longinquus subsp. pacificus*, a new west coast North American agaric associated with red alder. *Mycotaxon* 20: 205-212.

Lingle, M. L., D. Porter, and D. J. O'Kane. 1992. Preliminary analysis of genetic complementation of bioluminescence in *Panellus stypticus* isolated from pine and hardwood. *Mycologia* 84: 94-104.

Lutzoni, F. M. 1997. Phylogeny of lichen- and non lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Syst. Biol.* 1997: 373-406.

Macrae, R. 1937. Interfertility phenomena of the American and European form of *Panellus stypticus* (Bull.) Fries. *Nature* 139: 674.

Macrae, R. 1942. Interfertility studies and inheritance of luminescence in *Panus stypticus*. *Can. J. Res.* C20: 411-434.

Mansur, M., and T. Suarez. 1997. Identification of a laccase gene family in the new lignin-degrading basidiomycete CECT 20197. *Applied and Environmental Microbiology* 63: 2637-2646.

Michael, F., and E. Westhof. 1990. Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. *J. Mol. Biol.* : 585-610.

Miller, O. K. 1970. The genus *Panellus* in North America. *Michigan Bot.* 1970: 17-30.

Miller, O. K. 1984. New concepts in our understanding of *Pleurotus*, *Hohenbuehelia* and their allies. *Korean J. Mycol.* 84: 189.

Murphy, J. F. 1997. Intersterility groups in *Collybia subnuda*. *Mycologia* 89: 566-577.

Murrill, W. A. 1915. *North American Flora* 9: p246.

Nicholson, M. S., B. A. Bunyard, and D. J. Royse. 1997. Phylogeny of the genus *Lentinula* based on ribosomal DNA restriction fragment length polymorphism analysis. *Mycologia* 89: 400-407.

Nishida, H., and J. Sugiyama. 1995. A common Group I intron between a plant parasitic fungus and its host. *Mol. Biol. Evol.* 12: 883-886.

O'Donnell, K., E. Cigelnik, N. S. Weber, and J. M. Trappe. 1997. Phylogenetic relationship among ascomycetous truffles and the true and false morels inferred from 18s and 28s ribosomal DNA sequences analysis. *Mycologia* 89: 48-65.

O'Kane, D. J. 1990. Spectral analysis of bioluminescence of *Panellus stypticus*. *Mycologia* 82: 595-606.

O'Kane, D. J., W. A. Lingle, D. Porter, and J. E. Wampler. 1990. Localization of bioluminescent tissue during basidiocarp development in *Panellus stypticus*. *Mycologia* 82: 595-606.

Page, R. D. M. 1998. TreeView. Version 1.52. Division of Environmental and Evolutionary Biology, IBLS, The University of Glasgow., Glasgow. http://taxonomy.zoology.gla.ac.uk/rod/rod.html.

Patouillard, N. 1900. Sur les families et les genres, Des Hymenomycetes. *Essai Taxonomique* : 137.

Pegler, D. 1983. The genus *Lentinus*, a world monograph. *Kew Bull.* 10: 1-281.

Petersen, R. H. 1992. Mating systems in three New Zealand agarics. *New Zealand Journal of Botany* 30: 189-197.

Petersen, R. H. 1995. There's more to a mushroom than meets the eye: Mating studies in the Agaricales. *Mycologia* 87: 1-17.

Petersen, R. H., and D. Bermudes. 1992a. Intercontinental compatibility in *Panellus stypticus* with a note on bioluminescence. *Persoonia* 14: 457-463.

Petersen, R. H., and D. Bermudes. 1992b. *Panellus stypticus*: geographically separated interbreeding populations. *Mycologia* 84: 209-213.

Petersen, R. H., and K. W. Hughes. 1993. Intercontinental interbreeding collections of *Pleurotus pulmonarius* with notes of *P. ostreatus* and other species. *Sydowia* : 139-152.

Petersen, R. H., and K. W. Hughes. 1999. Species and speciation in mushrooms. *Bioscience* 49: 440-452.

Petersen, R. H., and S. C. McCleneghan. 1995. Mating systems of antipodal agarics: an unreported taxon and range extensions. *New Zealand J. Bot.*33: 93-98.

Petersen, R. H., and S. C. McCleneghan. 1997. Reports on long-distance sexual compatibility in Agaricales. *Nordic J. Bot.* 17: 419-432.

Petersen, R. H., and G. S. Ridley. 1996. A New Zealand *Pleurotus* with multiple-species sexual compatibility. *Mycologia* 88: 198-207.

Singer, R. 1975. *The Agaricales in Modern Taxonomy*. Edition III. J. Cramer, Chicago.

Singer, R. 1986. *The Agaricales in Modern taxonomy*. Edition IV. Koeltz Scientific Books, Koenigstein.

Smith, A. 1949. *Mushrooms in their Natural Habitats*. Sawyer's Inc., Portland.

Tiffney, B. H. 1985. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *J. Arnold. Arbor.* 62: 487-516.

Vilgalys, R. 1986. Phenetic and cladistic relationships in *Collybia* sec. *levipedes* (fungi: Basidiomycetes). *Taxon* 35: 225-233.

Vilgalys, R. 1991. Speciation and species concepts in the *Collybia dryophila* complex. *Mycologia* 83: 758-773.

Vilgalys, R., and D. S. Hibbett. 1991. Phylogenetic implications of generic concepts in fungal taxonomy: the impact of molecular systematic studies. Pages 73-91. *A Herbette Symposium in Lausanne*, Lausanne, France.

Vilgalys, R., and J. L. Johnson. 1987. Extensive genetic divergence associated with speciation in filamentous fungi. *Proc. Natl. Acad. Sci. USA* 84: 2355-2358.

Vilgalys, R., and O. K. Miller. 1987. Mating relationships within the *Collybia dryophila* group in Europe. *Transactions of the British Mycological Society* 89: 295-300.

Vilgalys, R., and B. L. Sun. 1994a. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. *Proc. Natl. Acad. Sci. USA* 91: 4599-4603.

Vilgalys, R., and B. L. Sun. 1994b. Assessment of species distributions in *Pleurotus* based on trapping of airborne basidiospores. *Mycologia* 86: 270-274.

White, E. E., C. P. Dubetz, M. G. Cruickshank, and D. J. Morrison. 1998. DNA dianostic for *Armillaria* species in British Columbia: within and between species variation in the IGS-1 and IGS-2 regions. *Mycologia* 90: 125-131.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. *PCR Protocols, A Guide to Methods and Applications.* Academic Press, San Diego.

Wolfe, J. A. 1975. Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62: 264-279.

Wu, Q.-X. 1991. *Systematics in the genus Clavicorona: morphology, intercompatibility, and phenyloxidase isozyme.* Dissertation, The University of Tennessee, Knoxville. p203.

Wu, Q.-X., K. W. Hughes, and R. H. Petersen. 1995. A reevaluation of taxa of *Clavicorona* subg. *Ramosa* based on morphology, compatibility and laccase electrophoretic patterns. *Sydowia* 47: 89-124.

Appendices

Appendix 2.1. Collections used for morphological, isozyme, mating and molecular studies

Collection No.	TENN Accession No.	Species	Country	Locale	Host	Collector	Date collected	Note and original designation
2300	49841	*Panellus stypticus*	Japan	Gunma, Pref.	hardwood stump	RHP	9.26.89	
2315	48229	*P. stypticus*	Japan	Tochigi Pref.	*Chaemaecypanus*	RHP	9.27.89	
2416	N/A	*P. stypticus*	USA	IL, Jackson Co.	N/A	NAMA	10.20.89	no dikaryon available
2536	48764	*P. stypticus*	USA	TN, Sevier Co.	hardwood log	RHP	1.19.90	
2586	48831	*P. stypticus*	New Zealand	Cantabury	*Nothofagus* log	RHP	5.12.90	*P. stypticus*
2646	53149	*P. stypticus*	New Zealand	Fjordland	*Nothofagus* log	RHP	5.17.90	*P. stypticus*, originally as *P. sp*
2658	48837	*P. stypticus*	New Zealand	Fjordland	rotten *Nothofagus*	RHP	5.18.90	
2664	48830	*P. stypticus*	New Zealand	Fjordland	*Nothofagus*	RHP	5.19.90	
2675	48833	*P. stypticus*	New Zealand	Fjordland	*Nothofagus*	RHP	5.19.90	
2810	48775	*P. stypticus*	New Zealand	Urewera	*Nothofagus*	RHP & KWH	5.29.90	
3010	56674	*P. stypticus*	USA	NC, Macon Co.	N/A	RHP	7.16.90	*P. stypticus*, originally as *P. sp*
3180	48834	*P. stypticus*	Russia	Premorsk	*Betula* log	RHP	9.9.90	
3281	48832	*P. stypticus*	Russia	Premorsk	hardwood log	RHP	9.14.90	
3342	48835	*P. stypticus*	Russia	Premorsk	rotten *Quercus*	RHP	9.22.90	
3350	48836	*P. stypticus*	Russia	Premorsk	rotten *Quercus*	RHP	9.22.90	
3541	50039	*P. stypticus*	Australia	New South Wales	*Eucalyptus* log	RHP	5.16.91	
3544	50059	*P. stypticus*	Australia	New South Wales	*Eucalyptus* bough	RHP	5.16.91	
3567	50067	*P. stypticus*	Australia	New South Wales	N/A	A. Wood	5.21.91	
3586	50140	*P. stypticus*	Australia	Tasmania	hardwood log	KWH	5.21.91	
3907	50110	*P. stypticus*	Australia	Tasmania	rotting Sassafras	RHP	5.26.91	no dikaryon available
3968	50099	*P. stypticus*	Australia	Tasmania	dead *Eucalyptus*	RHP& KWH	5.30.91	
4271	50607	*P. stypticus*	Sweden	Vastergotland	*Betula*	RHP	9.24.91	
4303	50384	*P. stypticus*	Switzerland	Maggia	*Betula / Alnus*	RHP	10.1.91	
4313	50393	*P. stypticus*	Switzerland	Maggia	*Fagus* stump	RHP	10.2.91	
4319	50398	*P. stypticus*	Switzerland	Maggia	rotting *Alnus*	RHP	10.3.91	
5185	52701	*P. stypticus*	USA	NC, Macon Co.	N/A	SCM	7.26.92	
5259	51577	*P. stypticus*	USA	NC, Macon Co.	*Betula* branch	RHP	7.27.92	
5277	51595	*P. stypticus*	USA	NC, Macon Co.	*Betula* branch	RHP	7.30.92	
5450	51768	*P. stypticus*	USA	SC, Oconee Co.	hardwood	RHP	8.18.92	
5471	52632	*P. stypticus*	USA	NC, Macon Co.	hardwood	RHP	8.19.92	
5699	52421	*P. stypticus*	USA	WA, Lewis Co.	hardwood log	KWH	10.2.92	
5703	52533	*P. stypticus*	USA	WA, MRNP	hardwood log	KWH	10.2.92	
5846	52233	*P. stypticus*	USA	WA, Jefferson Co.	Alder	SCM	10.14.92	

Appendix 2.1. (Continued) Collections selected for isozyme, morphological, mating and molecular studies

Collection No.	TENN Accession No.	Species	Country	Locale	Host	Collector	Date collected	Note and original designation
5847	52276	*P. stypticus*	USA	WA, Jefferson Co.	N/A	KWH	10.14.92	
6157	52316	*P. stypticus*	USA	TN, Polk Co, Cherokee	N/A	SCM	11.7.92	
6494	53409	*P. stypticus*	USA	NY, Duchess Co.	N/A	SCM	9.26.93	
6501	52960	*P. stypticus*	USA	NC, Macon Co.	*Quercus / Betula*	RHP	8.7.93	
6503	52951	*P. stypticus*	USA	NC, Macon Co.	*Betula / Prunus*	RHP	8.7.93	
6504	52961	*P. stypticus*	USA	GA, Rabun Co.	hardwood	RHP	8.8.93	
6506	52962	*P. stypticus*	USA	GA, Rabun Co.	hardwood	RHP	8.8.93	
6512	52966	*P. stypticus*	USA	NC, Jackson Co.	hardwood	RHP	8.9.93	
6548	52991	*P. stypticus*	Russia	Premorsk	Hardwood	RHP	9.8.93	
6576	53006	*P. stypticus*	Russia	Premorsk	*Quercus?*	RHP	9.9.93	
6586	53013	*P. stypticus*	Russia	Premorsk	hardwood	RHP	9.12.93	
6592	53018	*P. stypticus*	Russia	Premorsk	hardwood	RHP	9.13.93	
6665	53217	*P. stypticus*	USA	WA, Snohomish Co.	Alder	KWH	10.24.93	
6734	53048	*P. stypticus*	USA	WA, Clallam Co.	Hardwood	KWH	10.15.93	
6762	53076	*P. stypticus*	USA	WA, Clallam Co.	*Alnus*	RHP	10.19.93	
6795	53109	*P. stypticus*	USA	WA, Clallam Co.	Alder	RHP	10.24.93	
6801	53420	*P. stypticus*	USA	NY, Albany Co.	N/A	SCM	9.28.93	
6880	53460	*P. stypticus*	USA	NY, Schuyler Co	N/A	SCM	10.10.93	
6886	53453	*P. stypticus*	USA	VA, Grayson Co.	N/A	SCM	10.11.93	
7009	N/A	*P. stypticus*	USA	WA, Whatcom Co.	hardwood	RHP	10.27.93	
7736	54676	*P. stypticus*	Russia	Caucasia	*Tilia/ Quercus/Fagus*	J. Johnson	9.9.96	
7749	54709	*P. stypticus*	Russia	Caucasia	N/A	J. Johnson	9.11.96	
7954	54356	*P. stypticus*	USA	MN, Clearwater Co.	*Betula?*	RHP	8.24.95	
7961	54381	*P. stypticus*	USA	MN, Beltrami Co.	*Betula*	RHP	8.25.95	
8095	55419	*P. stypticus*	USA	CA, Trinity Co.	N/A	KWH	9.15.95	
8100	56337	*P. stypticus*	USA	TN, Knoxville	N/A	KWH	11.10.97	
8234	55360	*P. stypticus*	USA	AK, Vic. Juneau	hardwood bough	KWH	9.21.95	
8767	55195	*P. stypticus*	USA	NC, Macon Co.	*Acer* twig?	RHP	7.4.96	
8872	55492	*P. stypticus*	Russia	Caucasia	hardwood	RHP	9.8.96	
8910	55530	*P. stypticus*	Russia	Caucasia	hardwood	RHP	9.12.96	
8926	55546	*P. stypticus*	Russia	Caucasia	hardwood	RHP	9.15.96	
8942	55602	*P. stypticus*	Russia	Caucasia	*Quercus* log	RHP	9.19.96	
8951	55611	*P. stypticus*	Russia	Caucasia	*Alnus*	RHP	9.20.96	originally as *P. stypticus*
8982	55642	*P. stypticus*	Turkey	Trabzon	*Picea*	RHP	9.26.96	

Appendix 2.1. (Continued) Collections used for morphological, isozyme, mating and molecular studies

Collection No.	TENN Accession No.	Species	Country	Locale	Host	Collector	Date collected	Note and original designation
9029A	58325	*P. stypticus*	USA	CA, Trinity Co.	N/A	RHP	N/A	
9123	55750	*P. stypticus*	USA	LA, E. Batton Rouge Parish	hardwood	RHP	5.25.97	
9525	56339	*P. stypticus*	Canada	Montreal, Ursule Falls	N/A	J. Jin	8.3.97	
9801	56340	*P. stypticus*	USA	NC, Cataloochee	rotten hardwood	J. Jin	5.29.98	
9811	58307	*P. stypticus*	USA	TN, Washington Co.	N/A	E. Lickey	11.15.98	
9814	58310	*P. stypticus*	USA	TN, Blout Co.	Rotten wood	D. Kruegar	11.29.98	
Daom 192140	Daom 192140	*P. stypticus*	Canada	British Columbia	N/A	N/A	N/A	
Antonin	N/A	*P. stypticus*	Czech	N/A	N/A	N/A	N/A	
F2118	N/A	*P. stypticus*	Germany	N/A	N/A	N/A	N/A	
F2188	N/A	*P. stypticus*	Austria	N/A	N/A	N/A	N/A	
F9518	N/A	*P. stypticus*	Canada	Ontario	N/A	N/A	N/A	
RC/M 95.021	N/A	*P. stypticus*	N/A	N/A	N/A	N/A	N/A	
RC/F 95.083	N/A	*P. stypticus*	France?	N/A	N/A	N/A	N/A	
RC/F95. 084	N/A	*P. stypticus*	France?	N/A	N/A	N/A	N/A	
3493	49311	*D. pusillus*	USA	Puerto Rico	hardwood log	RHP	12.8.90	originally as *D. sp.*
3878	55852	*D. pusillus*	Mexico	Est. Chiapas	hardwood	T. Ahtti	10.18.91	
4861	51160	*D. pusillus*	Guatemala	Dpto. Escuintla	hardwood	Y. Sonerhamp	7.1.92	originally as *D. sp.*
8265	54317	*D. pusillus*	USA	FL, Ocala Nat Forest	hardwood	RHP & KWH	11.16.95	
8271	54318	*D. pusillus*	USA	FL, Ocala Nat Forest	hardwood	RHP & KWH	11.17.95	
8733	55162	*D. pusillus*	Mexico	Est. Nayarit	hardwood	RHP	7.18.96	originally as *D. sp.*
8747	55176	*D. pusillus*	Mexico	Est. Nayarit	hardwood	J. C. Fuentes	8.20.96	
9096	N/A	*D. pusillus*	USA	LA, St. Martin's Parish	rotten wood	KWH	5.23.97	
9408	56278	*D. pusillus*	Costa Rica	Prov. Alajuena	hardwood bough	RHP	6.27.98	
9421	56395	*D. pusillus*	Costa Rica	Prov. Cartago	hardwood	R. E. Halling	6.28.98	
9522	56278	*D. pusillus*	USA	FL, Hwy 267, Quincy	oak or cypress	SCM	5.29.97	originally as *D. sp.*
9803	56342	*D. pusillus*	Puerto Rico	El Yunque, Biol Station	deciduous trunk	J.-K. Jin	6.9.98	originally as *D. sp.*
9807	56346	*D. pusillus*	Puerto Rico	El Yunque, Carribean National Forest	rotten log	J.-K. Jin	6.11.98	originally as *D. sp.*

Appendix 2.1. (Continued) Collections used for morphological, isozyme, mating and molecular studies

Collection No.	TENN Accession No.	Species	Country	Locale	Host	Collector	Date collected	Note and original designation
9808	56347	*D. pusillus*	Puerto Rico	El Yunque, Carribean National Forest	rotten log	J.-K. Jin	6.11.98	originally as *D. sp.*
3513	50185	*D. sp.*	Australia	New South Wales	N/A	RHP	5.14.90	
3518	50160	*D. sp.*	Australia	New South Wales	N/A	RHP	5.14.90	
3526	50184	*D. sp.*	Australia	New South Wales	hardwood bark	RHP	5.15.91	
4460	50952	*D. sp.*	Puerto Rico	Toro Negro	N/A	RHP	5.27.92	
4535	51015	*D. sp.*	Puerto Rico	El Verde Station	broadleafed wood	RHP	5.31.92	
7699	53778	*D. sp.*	Costa Rica	Prov. Cartago	N/A	R. E. Halling	6.13.95	
7809	53765	*D. sp.*	Costa Rica	Prov. San Jose	*Quercus* log	RHP	6.15.95	
6650	53198	*P. longinqua*	USA	WA, Jefferson Co.	alder	KWH	10.19.93	originally as *P. stypticus*
6662	53214	*P. longinqua*	USA	WA, Callam Co.	alder	KWH	10.20.93	
6663	53215	*P. longinqua*	USA	WA, Grey's Harbor Co.	unknown	KWH	10.21.93	
5644	52389	*P. mitis*	USA	ID, Bonner Co.	conifer twig	RHP	9.26.92	
7486	53512	*P. mitis*	Finland	Etela-hame Prov.	*Pinus* bark	RHP	9.14.94	
7494	53511	*P. mitis*	Finland	Etela-hame Prov.	*Picea* bark	RHP	9.15.94	
6705	53031	*P. ringens*	USA	AK, Anchorage	*Alnus*	RHP	9.15.99	originally as *T. patellaris*
6711	52365	*P. ringens*	USA	AK, Turnagain Arm	hardwood	RHP	9.17.93	
9812	58308	*P. ringens*	USA	NC, Graham Co.	unknown bark	E. Lickey	11.19.98	
CBS 391.50	CBS 391.50	*P. violaceofulvus*	N/A	N/A	N/A	R. Kühner	N/A	CBS Catalogue, 1990
DAOM 198753	58323	*P. violaceofulvus*	Canada	Quebec, Forillon N. Park	fir branch	S.A. Redhead	10.2.88	Canadian DAOM collection
5624	52387	*P. serotinus*	USA	ID, Kootenai Co.	hardwood	SCM	9.24.92	
5659	52448	*P. serotinus*	USA	ID, Boundary Co.	*Populus*	RHP	9.27.92	
5729	52554	*P. serotinus*	USA	WA, Pierce Co.	N/A	RHP	10.3.92	
6167	52312	*P. serotinus*	USA	NC, Macon Co,	hardwood?	SCM	11.11.92	
6715	53036	*P. serotinus*	USA	AK, Turnagain Arm	hardwood	RHP	9.17.93	no dikaryon
6846	53433	*P. serotinus*	USA	NY, Cortland Co	maple	SCM	10.4.93	
6881	53461	*P. serotinus*	USA	NY, Schuyler Co.	N/A	SCM	9.9.93	originally as *P. stypticus*
6893	53462	*P. serotinus*	USA	GA, Rabun Co.	N/A	SCM	11.10.93	no dikaryon
8121	53885	*P. serotinus*	USA	AK, Vic.Anchorage	*Alnus*	RHP	9.11.95	
A5048	N/A	*P. serotinus*	USA	N/A	N/A	N/A	N/A	
6894	53450	*P. serotinus*	USA	NC, Macon Co	yellow birch	SCM	11.15.93	originally as *P. serotinus*?
9992	58324	*P. serotinus*	USA	NC, Macon Co.	N/A	RHP	6.14.98	

Appendix 2.1. (Continued) Collections used for morphological, isozyme, mating and molecular studies

Collection No.	TENN Accession No.	Species	Country	Locale	Host	Collector	Date collected	Note and orginal designation
3570	50065	*P. sp.*	Australia	New South Wales	N/A	A. Wood	5.21.91	may not be *Panellus*, originally as *P. sp.*
7435	54086	*P. sp*	New Zealand	North Island	fern *Rachis*	A. Methven	7.4.94	originally as *P. sp.*
7511	53673	*P. sp*	New Zealand	South Island	N/A	A. Methven	5.18.91	originally as *P. sp*
7577	53672	*P. sp*	New Zealand	North Island	N/A	A. Methven	5.27.94	originally as *P. sp.*
8345	55019	*P. sp.*	Argentina	Prov. Chubut	*Hometia*	RHP	5.10.96	
8458	55287	*P. sp.*	Mexico	Est. Mexico	*Abies / Pinus* log	RHP	7.7.96	
4306	N/A	*T. patellaris*	Switzerland	Maggia	standing *Alnus*	RHP	10.1.91	
5558	52491	*T. patellaris*	USA	NC, Haywood Co. GSMNP	*Betula /Prunus*	RHP	9.5.92	
6170	52302	*T. patellaris*	USA	NC, Macon Co. Chattooga	hardwood	SCM	11.12.92	
6202	56667	*T. patellaris*	USA	NC, Macon Co.	hardwood	RHP and KWH	1.9.93	
6486	53403	*T. patellaris*	USA	NY, Duchess Co.	hardwood	SCM	9.26.93	
6490	53405	*T. patellaris*	USA	NY, Duchess Co.	N/A	SCM	9.26.93	
6590	53016	*T. patellaris*	Russia	Premorski Reg.	hardwood	RHP	9.13.93	
6703	53030	*T. patellaris*	USA	AK, Anchorage	*Alnus*	RHP	9.15.93	
6716	53037	*T. patellaris*	USA	AK, Turnagain Arm	*Alnus*	RHP	9.15.93	
6933	N/A	*T. patellaris*	USA	MN, Vic. Bemidji	birch bark	Nama	8.25.95	
7972	54364	*T. patellaris*	USA	MN, Beltrami Co.	*Alnus*	T. Volk	8.25.9	
8274	N/A	*T. patellaris*	USA	AK, Girdwood	*Alder*	KWH	9.10.95	

Note: collecor: KWH: Karen W. Hughes; RHP: Ronald H. Petersen; SCM: S. Coleman McCleneghan.

Appendix 2.2. Growth and storage media

1. Malt Agar medium (MA): Difco malt extract 15g, Difco agar 20g, water 1000ml.
2. PD broth (PD): Difco potato dextrose 20g, water 1000ml.
3. Distilled water storage cryovials: 2 ml sterile cryovials, were filled with 1.5 –1.8 ml sterile distilled water, three to five plugs (0.7 cm diameter) of inoculum were immersed in serile distilled water. Cryovials were maintained at room temperature.

Appendix 2.3. *Panellus stypticus* collections used for morphological analyses

Collec -tion No.	TENN Acces- sion No.	Species	Country	Locale	Geographical designation
2300	49841	*Panellus stypticus*	Japan	Gunma, Pref.	Far East
2315	48229	*Panellus stypticus*	Japan	Tochigi Pref.	Far East
2536	48764	*Panellus stypticus*	USA	TN, Sevier Co.	Eastern N. America
2586	48831	*Panellus stypticus*	New Zealand	Cantabury	Oceania
2646	53149	*Panellus stypticus*	New Zealand	Fjordland	Oceania
2658	48837	*Panellus stypticus*	New Zealand	Fjordland	Oceania
2664	48830	*Panellus stypticus*	New Zealand	Fjordland	Oceania
2675	48833	*Panellus stypticus*	New Zealand	Fjordland	Oceania
2810	48775	*Panellus stypticus*	New Zealand	Urewera	Oceania
3010	56674	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
3180	48834	*Panellus stypticus*	Russia	Premorsk	Far East
3281	48832	*Panellus stypticus*	Russia	Premorsk	Far East
3342	48835	*Panellus stypticus*	Russia	Premorsk	Far East
3350	48836	*Panellus stypticus*	Russia	Premorsk	Far East
3541	50039	*Panellus stypticus*	Australia	New South Wales	Oceania ,
3544	50059	*Panellus stypticus*	Australia	New South Wales	Oceania
3567	50067	*Panellus stypticus*	Australia	New South Wales	Oceania
3586	50140	*Panellus stypticus*	Australia	Tasmania	Oceania
3907	50110	*Panellus stypticus*	Australia	Tasmania	Oceania
3968	50099	*Panellus stypticus*	Australia	Tasmania	Oceania
4271	50607	*Panellus stypticus*	Sweden	Vastergotland	Europe
4303	50384	*Panellus stypticus*	Switzerland	Maggia	Europe
4313	50393	*Panellus stypticus*	Switzerland	Maggia	Europe
4319	50398	*Panellus stypticus*	Switzerland	Maggia	Europe
5185	52701	*Panellus stypticus*	USA	NC, Macon Co,	Eastern N. America
5259	51577	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
5277	51595	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
5450	51768	*Panellus stypticus*	USA	SC, Oconee Co.	Eastern N. America
5471	52632	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
5699	52421	*Panellus stypticus*	USA	WA, Lewis Co.	Pacific N. America
5703	52533	*Panellus stypticus*	USA	WA, MRNP	Pacific N. America
5846	52233	*Panellus stypticus*	USA	WA, Jefferson Co.	Pacific N. America
5847	52276	*Panellus stypticus*	USA	WA, Jefferson Co.	Pacific N. America
6157	52316	*Panellus stypticus*	USA	TN, Polk Co, Cherokee	Eastern N. America
6229	52883	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
6494	53409	*Panellus stypticus*	USA	NY, Duchess Co.	Eastern N. America
6501	52960	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
6503	52951	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
6504	52961	*Panellus stypticus*	USA	GA, Rabun Co.	Eastern N. America
6506	52962	*Panellus stypticus*	USA	GA, Rabun Co.	Eastern N. America
6512	52966	*Panellus stypticus*	USA	NC, Jackson Co.	Eastern N. America
6548	52991	*Panellus stypticus*	Russia	Premorsk	Far East
6576	53006	*Panellus stypticus*	Russia	Premorsk	Far East
6586	53013	*Panellus stypticus*	Russia	Premorsk	Far East
6592	53018	*Panellus stypticus*	Russia	Premorsk	Far East
6665	53217	*Panellus stypticus*	USA	WA, Snohomish Co.	Pacific N. America
6734	53048	*Panellus stypticus*	USA	WA, Clallam Co.	Pacific N. America

Appendix 2.3. (Continued) *Panellus stypticus* collections used for morphological analyses

Collec-tion	CulTenn Acces-sion No.	Species	Country	Locale	Geographical designation
6762	53076	*Panellus stypticus*	USA	WA, Clallam Co.	Pacific N. America
6795	53109	*Panellus stypticus*	USA	WA, Clallam Co.	Pacific N. America
6801	53420	*Panellus stypticus*	USA	NY, Albany Co.	Eastern N. America
6880	53460	*Panellus stypticus*	USA	NY, Schuyler Co	Eastern N. America
6886	53453	*Panellus stypticus*	USA	VA, Greyson Co.	Eastern N. America
7736	54676	*Panellus stypticus*	Russia	Caucasia	Europe
7749	54709	*Panellus stypticus*	Russia	Caucasia	Europe
7954	54356	*Panellus stypticus*	USA	MN, Clearwater Co.	Eastern N. America
8095	55419	*Panellus stypticus*	USA	CA, Trinity Co.	Pacific N. America
8100	56337	*Panellus stypticus*	USA	TN, Knoxville	Eastern N. America
8234	55360	*Panellus stypticus*	USA	AK, Vic. Juneau	Pacific N. America
8767	55195	*Panellus stypticus*	USA	NC, Macon Co.	Eastern N. America
8910	55530	*Panellus stypticus*	Russia	Caucasia	Europe
8926	55546	*Panellus stypticus*	Russia	Caucasia	Europe
8942	55602	*Panellus stypticus*	Russia	Caucasia	Europe
8951	55611	*Panellus stypticus*	Russia	Caucasia	Europe
8982	55642	*Panellus stypticus*	Turkey	Trabzon	Europe
9123	55750	*Panellus stypticus*	USA	LA, E. Batton Rouge Parish	Eastern N. America
9525	56339	*Panellus stypticus*	Canada	Montreal, Ursule Falls	Eastern N. America
9801	56340	*Panellus stypticus*	USA	NC, Cataloochee	Eastern N. America
9811	58307	*Panellus stypticus*	USA	TN, Washington Co.	Eastern N. America
9814	58310	*Panellus stypticus*	USA	TN, Blount Co.	Eastern N. America

Note: 1) Geographical designation was based on RFLP analyses and biogeography (Molecular analyses **3.4.1**)

2) Collection 8982 belonged to the European population based on RFLP pattern, and was geographically closer to Europe than to Far East.

Appendix 2.4. Melzer's solution for microscopy and DAPI for microscopical epiflurescence

1. Melzer's solution (Largent, Johnson and Walting, 1977):

Add 1.5 g Iodine, 5.0g Potassium Iodine and 100 mg chloral hydrate to 100 ml H_2O. Heat the mixture but don't boil.

2. DAPI: It should be kept in refrigerator (4 C) and can be kept for several years. When in use, obeserve immediately. Epifluorescence will quench over time (Hughes, unpublished).

a. 0.5 M Sodium Phosphate stock solution: dibasic, 71.01 g dry Na_2HPO_4 dissolved and diluted in a liter of dd H_2O.

b. 0.5 M Citric Acid stock solution: 105.06 g dry C6H6O7.H_2O dissolved and diluted in a liter of dd H_2O.

c. McIlvaine's buffer: pH 4.4, mix 35.28 ml of 0.5 M Sodium phosphate stock with 22.36 ml of 0.5 M Citric Acid stock, bring to 200 ml with dd H_2O.

d. DAPI: 0.5 µg/ ml, 4'-6-diamidino-2-phenylindole, add 50 µg DAPI to 100 ml McIlvaine's buffer.

Appendix 2.5. Solutions used for isozyme electrophoreses

1. Correa- Victoria extraction buffer (modified) (McCleneghan, 1996):

   Tris 0.6g, sucrose 17.0g, ascorbic acid 0.1g, cysteine 0.1g, in about 500 – 700 ml H_2O, adjust to pH7.2 with 0.03 M citric acid, finally add H_2O to 1000 ml.

2. Sample buffer:

   a. Bromophenol tracking dye: 0.5% bromophenol blue dissolved in 1x running electrode buffer (pH8.3).

   b. 50% (w/v) sucrose solution: 5g sucrose in 10 ml 1x running buffer (pH8.3).

   c. Sample added to each well: 1µl 0.5% bromophenol tracking dye, 2 µl 50% sucrose solution (w/v) and 32 µl enzyme extract.

3. Solutions for 4.0% stacking and 10% separating gels:

   a. 4x stacking gel buffer: 0.5 M Tris-HCl: 6.0g Tris, pH 6.8 adjusted with HCl, to 100 ml H_2O.

   b. 4x separating gel buffer: 1.5 M Tris-HCl: 18.2g Tris, pH 8.8 adjusted with HCl, to 100 ml H_2O.

   c. 30% acrylamide-Bis stock: 15.0g (acrylamide 14.6g and Bis 0.4g), at first add 60-80 ml H_2O to dissolve acrylamide-Bis by stirring, finally adjust to 100 ml.

   d. 10% $(NH_4)_2S_2O_3$: 0.1g ammonium persulfate in 1 ml H_2O. Prepare fresh before use.

   e. TEMED: original solution.

4. Preparation of the 4.0% stacking and 10% separating gels:

	volume for 4% stacking gel		volume for 4% separating gel	
solution	2 gels	4 gels	2 gels	4 gels
4x stacking/separating buffer	0.76 ml	1.52 ml	2.52 ml	5.04 ml
dd H_2O	1.88 ml	3.76 ml	4.08 ml	8.16 ml
30% acrylamide-Bis stock	0.42 ml	0.84 ml	3.34 ml	6.68 ml
10% $(NH_4)_2S_2O_3$	23.4 µl	46.8 µl	70.0 µl	140.0 µl
TEMED	3.88 µl	7.76 µl	3.52 µl	7.04 µl

5. Buffer system (discontinuous system) (Kerrigan and Ross, 1988)

a. 1 x running buffer (also upper chamber buffer): dilute 1/5 from a 5x stock running buffer. 5 x stock running buffer: Tris 15.0g, glycine 72.0g, add H_2O to 1000 ml, pH 8.3.

b. 1x lower electrode buffer (Kerrigan and Ross, 1988): dilute 1/5 from a 5x stock lower buffer. 5x stock lower electrode buffer: Tris 18.9g, adjusted with HCl to pH 7.5, add H_2O to 500 ml.

Appendix 2.6. Stains for extracellular laccase (ECL) and intracellular enzymes (ADH, SOD, LAP, MDH, PGM, PGI, SKDH)

1) Extracellular laccase (ECL) stain:

   a. Staining buffer: NaAc (sodium acetate)16.4g + HAc (acetic acid) 11.5 ml. Add H_2O to 900 ml.

   b. When staining a gel, add 0.1g *o*-tolidine, 10 ml 95% ethanol and 90 ml staining buffer, prepare fresh.

   c. Gels were immersed in an ethanol solution of *o*-tolidine for 15-30 min, then they were rinsed and wrapped in plastic film for weak bands to appear.

2) Intracellular enzymes of ADH, SOD, LAP, MDH, PGI, PGM and SKDH (Z.-R.Wang's method, unpublished):

   a. Solutions for staining of intracellular enzymes:

      1) 0.1 M Tris-HCl pH 8.0: Dissolve 12.11 g Tris in 800 ml H_2O. Stir and adjust to pH 8.0. Add H_2O to final volume of 1000 ml.

      2) 20 units/ ml Glucose-6-phosphate dehydrogenase: 500 units of G-6-P dehydrogenase dissolved in 25 ml H_2O. Prepare just before use due to its unstability.

      3) 2.5% frutose-6-phosphate: 0.5 g frutose-6-phosphate dissolved with 20 ml H_2O.

      4) 1% NAD: 1 g NAD (β-nicotinamide adenine dinucleotide) dissolved in 100 ml H_2O.

      5) 0.5% NADP: 0.5 g NADP (β-nicotinamide adenine dinucleotide phosphate, sodium salt) dissolved in 100 ml H_2O.

      6) 0.5% MTT: 0.5 g MTT (3-[4, 5 –dimethylthiazol—2yl]-2, 5diphenyltetrazolium bromide; thiazolyl blue) dissolved in 100 ml H_2O.

      7) 1% PMS: 0.2 g PMS (phnazine methosulfate) dissolved in 20 ml H_2O.

      8) 5% glucose-1-phosphate disodium: 1 g glucose-1-phosphate disodium dissolved in 20 ml H_2O.

9) Potasium –phosphate buffer (pH 6.6): 27.25 g KH_2PO_4, with 11.40 K_2HPO_4 in 250 ml H_2O.

10) 1% Leucine-ß-naphthylamide: 0.5 g Leucine-ß-naphthylamide dissolved in 50 ml H_2O.

11) 2 M malic acid, pH8.0: 13.41 g DL-malic acid dissolved in 50 ml H_2O, bring to pH 8.0 with NaOH.

b. Recipes for staining of intracellular enzymes:

	PGI	PGM	LAP	6PGDH	SKDH	MDH	ADH
Tris-HCl, pH8.0	3 ml	3 ml		3 ml	3 ml	3 ml	3 ml
10%, $MgCl_2$,	1 ml	1 ml		1 ml			
Glucose-6-phosphate dehydrogenase	5 drops	5 drops					
Frutose-6-phosphate	5 drops						
1% NAD							
0.5% NADP	5 drops	5 drops		5 drops	5 drops	5 drops	5 drops
0.5% MTT	5 drops	5 drops		5 drops	5 drops	5 drops	5 drops
1% PMS	2 drops	2 drops		2 drops	2 drops	2 drops	2 drops
1%Agarose	6-7 ml	6-7 ml		6-7 ml	6-7 ml	6-7 ml	6-7 ml
5% glucose-1-phosphate disodium		8 drops					
Potasium – phosphate buffer, pH 6.6			5 ml				
1% leucine-ß-naphthylamide			ca. 20 drops				
Fast black K salt			0.5g /20ml H_2O				
0.002g Shikimic acid					0.002g		
2 M malic acid, pH8.0						1 ml	
6-gluconic acid				0.01g			
95% ethanol							10 drops
dd H_2O			ca. 50 ml				

Note: alcohol dehydrognase (ADH), superoxide dismutase (SOD), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH)

c. Procedures for intracellular enzymes ADH, SOD, LAP, MDH, PGI, PGM, and SKDH (Z.-R. Wang's method, unpublished):

1) After electrophoresis, dissemble a gel from the electrophoresis apparatus, rinse the gel in dd H_2O and put the gel into a tray.

2) Dissolve 1 g agarose in 100 ml dd H_2O (1%) in a microwave oven completely. When solidified, store it in a refrigerator.

3) Before staining re-dissolve the 1% agarose in a microwave oven.

4) Pipette 6-7 ml dissolved 1% agarose in a small beaker, add other solutions as indicated as above for a particular enzyme, swirl gently and quickly mix solutions well.

5) Pour the liquid mixture onto a gel quickly and thoroughly cover the gel surface, let it cool and solidify.

6) Wrap the gel and the covering agarose layer with plastic film. Wait for bands to appear, and peel off the covering agarose layer. Record the bands in the gel.

3. Recipes for staining intracellular enzymes of estrase (EST), peroxidase (POD) (A.-M. Wang's method, unpublished, personal communication)

a. Estrase (EST): 30 mg alpha-naphthyl acetate was dissolved in 3 ml acetone, then 30 mg $NaPO_3$ (0.2 M, pH 6.4) was added. A 30 mg Fast Blue RR salt was ground and mixed in immediately before staining.

b. Peroxidase (POD): Benzidine stock solution 1.0 ml; dd H_2O 18.6 ml; 3% H_2O_2 0.4 ml. Benzidine stock solution: dissolve 2 g Benzidine in 18 ml acetic acid. Bring to 90 ml with H_2O.

Appendix 2.7. Self crosses for mating exemplars

Appendix 2.7.1 for 9810 (USA, TN);
Appendix 2.7.2 for 9029 (USA, CA)
Appendix 2.7.3 for 3180 (Russia, Premorsk);
Appendix 2.7.4 for 8926 (Russia, Caucasia);
Appendix 2.7.5 for 2664 (New Zealand);
Appendix 2.7.6 for 3567 (Australia, New South Wales);
Appendix 2.7.7 for 3586 (Australia, Tasmania).

Appendix 2.7.1. Self cross of Eastern North American exemplar 9810 (USA, TN)

1		-	-	-	-	-	-	-	$-_B$	$-_B$	-	$-_F$
2	-		-	-	-	-	-	$-_B$	$-_B$	$-_B$	$-_F$	$-_F$
5	-	-		-	-	-	-	-	$-_B$	$-_B$	$-_F$	$-_F$
9	-	-	-		-	-	$-_B$	$-_B$	$-_B$	$-_B$	-	-
10	-	-	-	-		-	$-_B$	$-_B$	$-_B$	$-_B$	-	-
11	-	-	-	-	-		$-_B$	$-_B$	$-_B$	$-_B$	$-_F$	$-_F$
3	-	-	-	$-_B$	$-_B$	$-_B$		-	-	-	+	+
6	-	$-_B$	-	$-_B$	$-_B$	$-_B$	-		-	-	+	+
7	$-_B$	$-_B$	$-_B$	$-_B$	$-_B$	$-_B$	-	-		-	+	+
12*	$-_B$	$-_B$	$-_B$	$-_B$	$-_B$	$-_B$	-	-	-		+	+
4	-	$-_F$	$-_F$	-	-	$-_F$	+	+	+	+		-
8*	$-_F$	$-_F$	$-_F$	-	-	$-_F$	+	+	+	+	-	
	1	2	5	9	10	11	3	6	7	12*	4	8*
	A2B1	A2B1	A2B1	A2B1	A2B1	A2B1	A1B1	A1B1	A1B1	A1B1	A2B2	A2B2

Note: 1) * 12 (A1B1) and 8 (A2B2) were chosen as two tester monocaryons.
2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.7.2. Self cross of Pacific North America exemplar 9029 (USA, CA)

1*		+	+	$-_B$	$-_B$	-	-	$-_F$	$-_F$	$-_F$
2*	+		-	$-_F$	$-_F$	-	-	$-_B$	$-_B$	$-_B$
14	+	-		$-_F$	$-_F$	-	$-_B$	$-_B$	$-_B$	$-_B$
4	$-_B$	$-_F$	$-_F$			+	+	+	+	+
5	$-_B$	$-_F$	$-_F$			+	+	+	+	+
3	-	-	-	+	+		-	-	-	-
7	-	-	$-_B$	+	+	-		-	-	-
8	$-_F$	$-_B$	$-_B$	+	+	-	-		-	-
9	$-_F$	$-_B$	$-_B$	+	+	-	-	-		-
16	$-_F$	$-_B$	$-_B$	+	+	-	-	-	-	
	1*	2*	14	4	5	3	7	8	9	16
	A1B1	A2B2	A2B2	A2B1	A2B1	A1B2	A1B2	A1B2	A1B2	A1B2

Note: 1) *1 (A1B1) and 2 (A2B2) were chosen as tester monocaryons.
2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.7.3. Self cross of Far East exemplar 3180 (Russia, Premorsk)

1*		-	+	$-_F$	$-_F$	-
17	-		+	$-_F$	$-_F$	$-_B$
2*	+	+		-	-	-
6	$-_F$	$-_F$	-		$-_B$	+
18	$-_F$	$-_F$	-	$-_B$		+
13	-	$-_B$	-	+	+	
	1*	17	2*	6	18	13
	A1B1	A1B1	A2B2	A1B2	A1B2	A2B1

Note: 1) * 1 (A1B1) and 2 (A2B2) were tester monocaryons.
2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.7.4. Self cross of European exemplar 8926 (Russia, Caucasia)

1*		+	+	+	+	+	−	−	$-_F$	−
4	+		−	−	−	−	$-_F$	−	−	−
5	+	−		−	−	−	−	−	$-_B$	$-_B$
9	+	−	−		−	−	$-_F$	−	−	−
12	+	−	−	−		−	$-_F$	$-_B$	−	−
13*	+	−	−	−	−		$-_F$	$-_B$	−	−
6	−	$-_F$	−	$-_F$	$-_F$	$-_F$		+	+	+
7	−	−	−	−	$-_B$	$-_B$	+		−	−
8	$-_F$	−	$-_B$	−	−	−	+	−		−
11	−	−	$-_B$	−	−	−	+	−	−	
	1*	4	5	9	12	13*	6	7	8	11
	A1B1	A2B2	A2B2	A2B2	A2B2	A2B2	A2B1	A1B2	A1B2	A1B2

Note: 1) *1 (A1B1) and 13 (A2B2) were tester monocaryons.
2) + stands for compatible reaction; − stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.7.5. Self cross of New Zealand exemplar 2664 (New Zealand)

12		+!	+!	−	−!
3	+!		N/A	−!	$-_B$!
7	+!	N/A		N/A	$-_B$!
5*	−	−!	N/A		+!
20*	−!	$-_B$!	$-_B$!	+!	
	12	3	7	5*	20*
	A1B2	A2B1	A2B1	A2B2	A1B1

Note: 1) * 20 (A1B1) and 5 (A2B2) were tester monocaryons.
2) + stands for compatible reaction; − stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction; ! means epiflurescence was used to check clamps. N/A meant not observed due to low viability.

Appendix 2.7.6. Self cross of New South Wales exemplar 3567 (Australia, New South Wales)

1*		-	-	-	-	-	-	-
5	-		-	-	+!	-	-	-
6	-	-		+!	+!	-	-	+!
7	-	-	+!		-	-	-	-
10*	-	+!	+!	-		-	-	-
11	-	-	-	-	-		-	-
15	-	-	-	-	-	-		-
17	-	-	+!	-	-	-	-	
	1*	5	6	7	10*	11	15	17

Note: 1) * Mating behaviors in this collection were very bizarre, in that hyphae were strangulated, clamps became difficult to observe. 1 and 10 were chosen as tester monocaryons due to their high viability.

2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction; symbol ! means epiflurescence was used to check clamps.

Appendix 2.7.7. Self cross of exemplar 3586 (Australia, Tasmania)

4*		+!	+!	+!	+!	+!	-!
6	+!		-	-	-	-	$-_B$
7	+!	-		-	-	-	$-_B$
8*	+!	-	-		-	-	-
10	+!	-	-	-		-	$-_B$
15	+!	-	-	-	-		$-_B$
17	-!	$-_B$	$-_B$	-	$-_B$	$-_B$	
	4*	6	7	8*	10	15	17
	A1B2	A2B2	A2B2	A2B2	A2B2	A2B2	A1B2

Note: 1) *4 (A1B1) and 8 (A2B2) were tester moncaryons.

2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction; symbol ! means epiflurescence was used to check clamps.

Appendix 2.8. Self cross of *Dictyopanus pusillus* exemplar 9808 (USA, Puerto Rico)

1*		-	+	+	+	+	-	$-_B$	$-_B$	$-_B$	-	$-_F$
5	-		+	+	+	+	-	-	-	$-_B$	$-_B$	$-_B$
2	+	+		$-_B$	$-_B$	$-_B$	-	$-_F$	$-_F$	$-_F$	$-_F$	$-_B$
3*	+	+	$-_B$		-	-	$-_F$	$-_F$	$-_F$	$-_F$	$-_F$	$-_B$
8	+	+	$-_B$	-		$-_B$	$-_F$	-	$-_F$	$-_F$	-	-
9	+	+	$-_B$	-	$-_B$		$-_F$	$-_F$	$-_F$	$-_F$	$-_F$	-
4	-	-	-	$-_F$	$-_F$	$-_F$		-	-	-	-	+
6	$-_B$	-	$-_F$	$-_F$	-	$-_F$	-		-	-	-	+
7	$-_B$	-	$-_F$	$-_F$	$-_F$	$-_F$	-	-		-	-	+
10	$-_B$	$-_B$	$-_F$	$-_F$	$-_F$	$-_F$	-	-	-		-	+
11	-	$-_B$	$-_F$	$-_F$	-	$-_F$	-	-	-	-		+
12	$-_F$	$-_B$	$-_B$	$-_B$	-	-	+	+	+	+	+	
	1*	5	2	3*	8	9	4	6	7	10	11	12
	A1B1	A1B1	A2B2	A2B2	A2B2	A2B2	A2B1	A2B1	A2B1	A2B1	A2B1	A1B2

Note: 1) * 1 (A1B1) and 3 (A2B2) were chosen as tester monocaryons.
2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.9. Self cross of 7577 which was originally designated as *Panellus sp*, but it is *Pleurotopsis longinqua*

1		-	-	-	+	+	+	$-_B$	-	-	$-_B$	-
6	-		-	-	+	+	+	-	-	$-_B$	-	-
8	-	-		-	+	+	+	-	$-_B$	$-_B$	$-_B$	-
11*	-	-	-		+	+	+	$-_F$	-	-	-	$-_B$
2	+	+	+	+		-	-	$-_B$	-	-	-	-
10	+	+	+	+	-		-	$-_B$	$-_B$	$-_B$	-	-
12*	+	+	+	+	-	-		$-_B$	-	-	$-_F$	-
3	-	-	-	$-_F$	$-_B$	$-_B$	$-_B$		-	-	+	+
4	-	-	-	-	-	$-_B$	-	-		-	+	+
9	-	-	-	-	-	$-_B$	-	-	-		+	+
7	$-_B$	-	$-_B$	-	-	-	$-_F$	+	+	+		$-_B$
14	-	-	-	$-_B$	-	-	-	+	+	+	$-_B$	
	1	6	8	11*	2	10	12*	3	4	9	7	14
	A1B1	A1B1	A1B1	A1B1	A2B2	A2B2	A2B2	A1B2	A1B2	A1B2	A2B1	A2B1

Note: 1) * 11 (A1B1) and 12 (A2B2) were chosen as tester monocaryons.
2) + stands for compatible reaction; - stands for incompatible reaction; $-_F$ stands for flat reaction; $-_B$ stands for barrage reaction.

Appendix 2.10. Solutions used for DNA extraction

1. Solutions for DNA extraction

   a. Carlson lysis buffer (Carlson et al.; 1991):

      100 mM Tris, pH 9.5 (1.21g Tris, add HCl, adjust to pH 9.5);

      20 mM EDTA (0.76 g/ 100ml H_2O);

      1.4 M NaCl (8.18g/ 100ml H_2O);

      2% CTAB (2g /100ml H_2O);

      1% PEG (polyethylene glysorol) 8000 (1g/100ml H_2O);

      Note: add 2 µl ß-mercaptoethanol per 1 ml of buffer before use.

   b. Chloroform: isoamyl alcohol = 24:1 (v/v), with glass pipettes in hood.

   c. 100% Ethanol, ice cold.

   d. Isopropanol (isopropyl alcohol).

   e. TE (Tris-EDTA) buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0.

   f. RNAse plus, DNAse free.

2. DNA extraction protocol

   a. Prepare fungal tissue: for CTAB preserved tissues, blot off CTAB. For frozen materials and fresh materials, weigh ca. 300 mg tissue.

   b. Prepare Carlson lysis buffer: In hood pipette 750 µl Carlson lysis buffer in a 1.5 ml tube. Add 1.5µl ß-mercaptoethanol. Heat tubes at 74 C.

   c. Warm pestle and morter in heating block at 74 C.

   d. Label tubes. Three tubes for each specimen are needed.

   e. Grind 300 mg tissue thoroughly in morter with sterile sands. Add 750 µl preheated Carlson lysis buffer. Scrape slurry back to the tube. Incubate at 74 C for digestion for 30-60 min.

   f. Reverse sample every 10 min.

g. Centrifuge sample at 10,000 rpm for 10 min at room temperature. Remove supernatant to a new tube.

h. Cool sample to room temperature. In hood, add 1 volume of chloroform: isoamyl alcohol (24:1 mix well by inversion.

i. Centrifuge at 10,000 x g for 10 min.

j. Transfer upper phase to a clean tube. Add 1 volume isopropanol. Invert several times to mix and incubate at room temperature for 30 min to precipitate DNA.

k. Centrifuge at 5000 x g, 4 C for 20 min to sediment DNA.

l. Wash pellet with 250 µl ethanol. Pour off ethanol and dry pellet. Spin at 5000 x g at 4 C for 10 min.

m. Add 50 µl TE buffer, resuspend DNA.

n. Add 2 µl RNAse (10x), incubate at 37 C for 30 min.

Appendix 2.11. Molecular markers

1. Lamda *Hind* III molecular marker

band	fragment size (bp)	Percentage (%)	quantity (ng) in 100 ng
1	23,130	47.69	47.7
2	9,416	19.41	19.4
3	6,557	13.52	13.5
4	4,361	8.99	9.0
5	2,322	4.79	4.8
6	2,027	4.18	4.2
7	567	1.16	1.2
8	125	0.26	0.3
total	48,502		

2. ØX174-Hae III molecular marker

band	fragment size (bp)	percentage (%)	quantity (ng) in 10µl (200 ng)
1	1,353	25.12	50.2
2	1,078	20.01	40.0
3	872	16.19	32.4
4	603	11.20	22.4
5	310	5.76	11.5
6a	281	5.22	10.4
6b	271	5.03	10.1
7	234	4.34	8.6
8	194	3.6	7.2
9	118	2.19	4.4
10	72	1.34	2.6
total	5,368		

Appendix 2.12. Primers used for amplification of ITS and 5'-LSU regions

primer	purpose	direction	sequence
ITS2	ITS	3'	GCTGCGTTCTTCATCGATGC
ITS3	ITS, LSU	5'	GCATCGATGAAGAACGCAGC
ITS4	ITS	3'	TCCTCCGCTTATTGATATGC
ITS5	ITS	5'	GGAAGTAAAAGTCGTAACAAGG
LR5	LSU	3'	TCCTGAGGGAAACTTCG
LR21	LSU	3'	ACTTCAAGCGTTTCCCTTT
NuLSU371'-5	LSU	5'	AAAAGAACTTTGGAAAGAGAGT

Reference: ITS2, ITS3, ITS4 and ITS5 from White et al 1990; LR5 and LR21 from Vilgalys and Sun, 1994; NuLSU371'-5 was synthesized by Genosys.

Appendix 2.13. Solutions for PCR amplification

1. Reaction mixture for PCR amplification (50 µl)

   27 µl H_2O;

   5 µl 10x buffer without $MgCl_2$ (Promega);

   6 µl $MgCl_2$ (25mM) (Promega);

   8 µl dNTP (10 mM for each nucleotide);

   1 µl primer A (10µM), 1 primer B (10µM);

   1 µl DNA solution of the collection to be amplified;

   1 µl Taq polymerase (1.25 unit /µl) (Promega).

2. dNTP (10 mM) preparation: to 360 µl H_2O, add 10 µl dATP (100 mM), 10 µl dTTP (100 mM), 10 µl dCTP (100 mM) and 10 µl dGTP (100 mM). Mix well.

3. Taq polymerase (1.25 unit/ µl) preparation: 10.4 µl H_2O, 1.6 10x buffer 4 µl Taq polymerase (5 unit/ µl) stock (Promega), mix well, store at –20 C or –80 C.

Appendix 2.14. Low-melt agarose electrophoresis and Promega Wizard Microcolumn PCR product purification procedure

1. Prepare 1.5% low-melt agarose (0.375 g NuSieve GTG agarose in 25 ml 1x TAE, 5µl 10 mg/ml ethidium bromide per 100 ml of 1x TAE buffer), melt agarose completely and pour gel with one well. When cool, place the gel in the electrophoresis apparatus.
2. Mix 1x Tris-acetic acid-EDTA (TAE) buffer. Add 5µl 10 mg/ml ethidium bromide per 100 ml of 1x TAE buffer to the buffer and mix well. Bring up to top edge of gel.
3. Add 1.5 – 2 µl 10x tracking dye to 150-200µl PCR product and mix well. Add PCR product into the well of the gel.
4. Run the gel with at 100 v to separate bands to a desirable distance.
5. Cut 300 mg to 450 mg low-melt agarose containing the desired band viewed with a UV transluminator and transfer to a 1.5 ml centrifuge tube.
6. Turn on heating block and keep temperature at 70 C. Heat the centrifuge tube in and let agarose melt.
7. When the gel is melted, add 1 ml resin (Promega) in and mix well, but don't vortex. Transfer the mixture into a syringe. And push the mixture through an attached Wizard microcolumn. Wash the Wizard microcolumn (Promega) with 2 ml 80% isopropanol through the attached syringe. Centrifuge the microcolumn at 10,000 for 2 min to dry resin.
8. In a new centrifuge tube, add 50 ml sterile ddH_2O into the microcolumn and wait a few minutes. Centrifuge the tube with the microcolumn on it at 10,000 rpm for 20 sec to get purified DNA.
9. Verify the purified DNA and estimate its concentration in a regular 0.8% agarose gel by adding 10 µl ØX174-Hae III as a standard molecular marker.

Appendix 2.15. Restriction enzymes *BstZ* I, *Dra* I, *Hinf* I and *Taq* I

	recognition sequence	buffer	digestion temperature	digestion time
BstZ I	C^GGCCG	D	50 C	1 hr to 1 hr 10 min
Dra I	TTT^AAA	B	37 C	1 hr 45 min to 2 hrs
Hinf I	G^ANTC	B	37 C	1 hr to 1 hr 20 min.
Taq I	T^CGA	E	65 C	1 hr 20 min to 1 hr 25 min

Note: All enzymes were from Promega. Enzyme concentration and specific buffers followed manufacturer's instruction. N stands for A, C, G or T.

Appendix 3.1. *Panellus stypticus* collections and their morphological characters

Collection No.	TENN Accession No.	Country	Locale	Panelloid	Anastomosis	Lamellae forked	Spore size (µm) (average)	Lamella height (mm)	Cheilocystidia	Pleurocystidia
2300	49841	Japan	Gunma, Pref.	+	+	-	3.34x1.64	0.26	AB	AC
2315	48229	Japan	Tochigi Pref.	+	+	-/+	3.21x1.66	0.29	A	AC
2536	48764	USA	TN, Sevier Co.	+	+	-	4.05x1.72	0.55	--CD	A--D
2586	48831	New Zealand	Cantabury	+/-	+/-	-	5.12x2.68	0.81	A	no
2646	53149	New Zealand	Fjordland	-	-	-	3.84x1.98	0.34	A-CD	A
2658	48837	New Zealand	Fjordland	+	-	-	4.38x2.30	0.39	A	A
2664	48830	New Zealand	Fjordland	+	+/-	-	3.98x1.85	0.42	A	AB
2675	48833	New Zealand	Fjordland	+	-	-	3.62x2.17	0.63	A	A--D
2810	48775	New Zealand	Urewera	+	+/-	-	4.02x2.12	0.53	AB	AB
3010	56674	USA	NC, Macon Co.	+	+	-	3.71x1.88	0.48	A-CD	A--D
3180	48834	Russia	Premorsk	+	+	-	3.49x1.78	0.25	AB	A
3281	48832	Russia	Premorsk	+	+	-	3.20x1.62	0.26	-B	A
3342	48835	Russia	Premorsk	+	+	-/+	3.61x1.75	0.36	---D	A
3350	48836	Russia	Premorsk	+	+	-/+	3.25x1.64	0.32	AB-D	A
3541	50039	Australia	New South Wales	+/-	+/-	-	4.03x2.21	0.46	ABC	A--D
3544	50059	Australia	New South Wales	+/-	+/-	-	3.42x1.73	0.54	--C	A-C
3567	50067	Australia	New South Wales	+/-	+/-	-	4.08x2.32	0.50	ACD	A-C
3586	50140	Australia	Tasmania	+/-	+/-	-	3.72x1.76	0.60	ABC	--CD
3907	50110	Australia	Tasmania	+/-	+/-	-	3.39x1.68	0.56	--CD	--C
3968	50099	Australia	Tasmania	+/-	+/-	-	4.06x2.23	0.48	AC	A-C
4271	50607	Sweden	Vastergotland	+	+	-	3.71x2.16	0.11	AC	A
4303	50384	Switzerland	Maggia	+	+	-	4.00x1.99	0.44	A--D	A--D
4313	50393	Switzerland	Maggia	+	+	-/+	4.00x1.95	0.46	A	A
4319	50398	Switzerland	Maggia	+	+	-	3.54x1.89	0.29	AB-D	A--D
5185	52701	USA	NC, Macon Co,	+	+	-	3.30x1.68	0.35	--CD	A
5259	51577	USA	NC, Macon Co.	+	+	-	3.26x1.62	0.46	A-CD	A
5277	51595	USA	NC, Macon Co.	+	+	-	3.64x1.85	0.38	--C	A
5450	51768	USA	SC, Oconee Co.	+/-	+	-	3.56x1.53	0.26	--CD	A
5471	52632	USA	NC, Macon Co.	+	+	-	3.59x1.59	0.29	AB-D	A
5699	52421	USA	WA, Lewis Co.	+	+	-	3.95x2.22	0.64	A	A--D
5703	52533	USA	WA, MRNP	+	+	-	3.39x1.72	0.22	ABC	A
5846	52233	USA	WA, Jefferson Co.	+	+	-/+	3.53x1.79	0.30	A-CD	A
5847	52276	USA	WA, Jefferson Co.	+	+	-/+	3.73x1.90	0.32	AB-D	A
6157	52316	USA	TN, Polk Co, Cherokee	+	+	-	3.80x1.86	not measured	A-CD	A
6229	52883	USA	NC, Macon Co.	+	+	-	3.39x1.67	0.30	A-CD	A
6494	53409	USA	NY, Duchess Co.	+	+	-	3.29x1.62	0.64	A	A
6501	52960	USA	NC, Macon Co.	+	+	-	3.40x1.68	0.23	A	A--D
6503	52951	USA	NC, Macon Co.	+	+/-	-	3.58x1.82	0.32	A--D	A-C
6504	52961	USA	GA, Rabun Co.	+	+	-	3.23x1.61	0.40	A--D	A
6506	52962	USA	GA, Rabun Co.	+	+	-	3.46x1.68	0.33	ABCD	A
6512	52966	USA	NC, Jackson Co.	+	+	-/+	3.30x1.94	0.33	AB	no
6548	52991	Russia	Premorsk	+	+	-	3.21x1.66	0.28	ABC	A
6576	53006	Russia	Premorsk	+	+	-	3.24x1.70	0.38	AB	A
6586	53013	Russia	Premorsk	+	+	-	3.18x1.71	0.26	ABCD	A
6592	53018	Russia	Premorsk	+	+	-	3.32x1.71	0.23	A-C	A--D
6665	53217	USA	WA, Snohomish Co.	+	+	-	3.54x1.94	0.28	AB	A
6734	53048	USA	WA, Clallam Co.	+	+	-	3.94x1.98	0.40	AB	A
6762	53076	USA	WA, Clallam Co.	+	+	-	3.59x1.88	0.56	A	A
6795	53109	USA	WA, Clallam Co.	+	+	-	3.60x1.85	0.46	AB	A
6801	53420	USA	NY, Albany Co.	+	+	-	3.39x1.64	0.61	A--D	A--D
6880	53460	USA	NY, Schuyler Co	+	+	-/+	3.80x1.85	0.37	A--D	A
6886	53453	USA	VA, Greyson Co.	+	+	-	3.44x1.66	0.48	ABC	A
7736	54676	Russia	Caucasia	+	+	-/+	3.88x1.85	0.22	AB	A
7749	54709	Russia	Caucasia	+	+	-	3.48x1.72	0.19	A-CD	A
7954	54356	USA	MN, Clearwater Co.	+	+	-	3.10x1.70	0.34	A	A
8095	55419	USA	CA, Trinity Co.	+	+	-	4.10x1.94	0.30	A	A-CD
8100	56337	USA	TN, Knoxville	+	+	-	3.14x1.64	0.42	A	A
8234	55360	USA	AK, Vic. Juneau	+	+	-	3.67x2.06	0.56	A--D	A
8767	55195	USA	NC, Macon Co.	+	+	-	3.22x1.79	0.33	A-CD	A

Appendix 3.1. (Continued) *Panellus stypticus* collections and their morphological characters

Collection	CulTenn Accession No.	Country	Locale	Panelloid	Anastomosis	Lamellae forked	Spore size (µm) (average)	Lamella height (mm)	Cheilocystidia	Pleurocystidia
8910	55530	Russia	Caucasia	+	+	-	3.74x1.84	0.49	A--D	A
8926	55546	Russia	Caucasia	+	+	-	3.51x1.87	0.36	AB	AB
8942	55602	Russia	Caucasia	+	+	-	3.71x1.98	0.34	A	A
8951	55611	Russia	Caucasia	+	+	-	4.42x2.33	0.19	A	A--D
8982	55642	Turkey	Trabzon	+	+	-	4.11x1.92	0.66	A	A
9123	55750	USA	LA, E. Batton Rouge Parish	+	+	-	3.67x1.75	0.28	AB	A
9525	56339	Canada	Montreal, Ursule Falls	+	+	-	3.39x1.66	0.39	A	A--D
9801	56340	USA	NC, Cataloochee	+	+	-	3.32x1.70	0.45	A	A
9811	58307	USA	TN, Washington Co.	+	+	-	3.14x1.71	0.32	A	A
9814	58310	USA	TN, Blount Co.	+	+	-	3.60x1.73	0.49	A	A

Note: 1) Panelloid stature: +: panelloid; -: not panelloid; +/-: ambiguous.

2) Lamellae anastomosis: +: anastomosed; +/-: ambiguous, remnant anastomosis; -: no anastomosis.

3) Lamellae forked: -/+: a few forked lamellae observed; -: no forked lamellae observed.

4) Four types of cystidia: A- clavate and ventricose. B- cystidia deformed toward dentritic. C- narrow cylindric and filiform. D - cystidia tips deformed but not dendritic..

Appendix 3.2. Collections used for isozyme analyses and their relative mobility rates (Rm) (in *P. stypticus*) and mobility rates (Rf) (in *Panellus* complex)

Appendix 3.2.1. Collections of *P. stypticus* used for isozyme analyses and the relative mobility rates (Rm) for ECL1, ADH3 and EST1

Collection No.	TENN Accession No.	Species	Country	Locale	ECL 1 (upper band) Rm	ADH 3 (third band) Rm	EST 1 (front bands) Rm
2300	49841	*P. stypticus*	Japan	Gunma, Pref.	1.12	1.30	0.93, 0.98
2315	48229	*P. stypticus*	Japan	Tochigi Pref.	1.12	N/A	0.93, 0.98
2536	48764	*P. stypticus*	USA	TN, Sevier Co.	0.90	N/A	0.97, 1.03
2586	48831	*P. stypticus*	New Zealand	Cantabury	0.99	N/A	1.02
2646	53149	*P. stypticus*	New Zealand	Fjordland	0.93		
2658	48837	*P. stypticus*	New Zealand	Fjordland	1.14	N/A	N/A
2664	48830	*P. stypticus*	New Zealand	Fjordland	0.95	N/A	1.03, 1.08
2675	48833	*P. stypticus*	New Zealand	Fjordland	1.04	N/A	N/A
2810	48775	*P. stypticus*	New Zealand	Urewera	1.04	N/A	1.03, 1.07
3010	56674	*P. stypticus*	USA	NC, Macon Co.	0.88		
3180	48834	*P. stypticus*	Russia	Premorsk	1.14	1.32	
3281	48832	*P. stypticus*	Russia	Premorsk	1.20	1.44	1.00, 1.06
3342	48835	*P. stypticus*	Russia	Premorsk		N/A	0.94, 0.99
3350	48836	*P. stypticus*	Russia	Premorsk	1.12	1.33	0.96, 0.99, 1.04
3541	50039	*P. stypticus*	Australia	New South Wales	1.16	N/A	1.03, 1.08
3544	50059	*P. stypticus*	Australia	New South Wales	1.28	N/A	1.08, 1.13
3567	50067	*P. stypticus*	Australia	New South Wales	1.02	N/A	1.02, 1.08
3586	50140	*P. stypticus*	Australia	Tasmania	1.22	N/A	1.03, 1.08
3968	50099	*P. stypticus*	Australia	Tasmania	1.22	N/A	1.03, 1.08
4271	50607	*P. stypticus*	Sweden	Vastergotland	1.07	N/A	1.08, 1.12
4303	50384	*P. stypticus*	Switzerland	Maggia	1.00	1.34	1.08, 1.12
4313	50393	*P. stypticus*	Switzerland	Maggia	0.96	N/A	1.03
4319	50398	*P. stypticus*	Switzerland	Maggia	1.00	N/A	1.04
5185	52701	*P. stypticus*	USA	NC, Macon Co.	1.06	1*	
5259	51577	*P. stypticus*	USA	NC, Macon Co.	0.92	N/A	0.97, 1.04
5277	51595	*P. stypticus*	USA	NC, Macon Co.	1.00	N/A	0.94, 0.98
5450	51768	*P. stypticus*	USA	SC, Oconee Co.		N/A	0.96, 1.00
5471	52632	*P. stypticus*	USA	NC, Macon Co.	0.96	N/A	0.95, 1.00
5699	52421	*P. stypticus*	USA	WA, Lewis Co.	1.23	N/A	1.05, 1.10
5703	52533	*P. stypticus*	USA	WA, MRNP		N/A	0.99, 1.04
5846	52233	*P. stypticus*	USA	WA, Jefferson Co.		N/A	0.98, 1.03
5847	52276	*P. stypticus*	USA	WA, Jefferson Co.		N/A	1.01, 1.08
6157	52316	*P. stypticus*	USA	TN, Polk Co, Cherokee	0.96	N/A	0.95, 1.00, 1.05
6229	52883	*P. stypticus*	USA	NC, Macon Co.	0.89	N/A	
6494	53409	*P. stypticus*	USA	NY, Duchess Co.		N/A	0.90, 0.95, 1.00
6501	52960	*P. stypticus*	USA	NC, Macon Co.	0.95	N/A	1.02
6503	52951	*P. stypticus*	USA	NC, Macon Co.	0.97	N/A	
6504	52961	*P. stypticus*	USA	GA, Rabun Co.	0.85	N/A	0.98, 1.03
6506	52962	*P. stypticus*	USA	GA, Rabun Co.	0.94	N/A	0.97, 1.03
6512	52966	*P. stypticus*	USA	NC, Jackson Co.	0.94	N/A	0.95, 0.99
6548	52991	*P. stypticus*	Russia	Premorsk	1.18	1.34	1.00, 1.04
6576	53006	*P. stypticus*	Russia	Premorsk	0.92	1.44	1.00, 1.04
6586	53013	*P. stypticus*	Russia	Premorsk	1.04	1.33	0.97, 1.00, 1.05

Appendix 3.2.1. (Continued) Collections of *P. stypticus* used for isozyme analyses and the relative mobility rates (Rm) for ECL1, ADH3 and EST1

Collection	CulTenn Acces-sion No.	Species	Country	Locale	ECL 1 (upper band) Rm	ADH 3 (third band) Rm	EST 1 (front bands) Rm
6592	53018	*P. stypticus*	Russia	Premorsk	1.02	1.44	1.00, 1.05
6665	53217	*P. stypticus*	USA	WA, Snohomish Co.	1.05	1.34	1.00, 1.05
6734	53048	*P. stypticus*	USA	WA, Clallam Co.		N/A	1.00, 1.05
6762	53076	*P. stypticus*	USA	WA, Clallam Co.	1.17	1.34	1.05, 1.10
6795	53109	*P. stypticus*	USA	WA, Clallam Co.	1.10	1.34	1.00, 1.05
6801	53420	*P. stypticus*	USA	NY, Albany Co.		N/A	0.96, 1.00
6880	53460	*P. stypticus*	USA	NY, Schuyler Co		N/A	1.00, 1.06
6886	53453	*P. stypticus*	USA	VA, Greyson Co.	0.96	N/A	0.93, 1.00
8234	55360	*P. stypticus*	USA	AK, Vic. Juneau	1.00	1.33	1.03, 1.07
8767	55195	*P. stypticus*	USA	NC, Macon Co.	1.00	N/A	0.94, 0.98
8872	55492	*P. stypticus*	Russia	Caucasia	0.89	N/A	1.00
8910	55530	*P. stypticus*	Russia	Caucasia	0.91	1.35	1.00
8926	55546	*P. stypticus*	Russia	Caucasia	0.89	1.35	1.00, 1.05
8942	55602	*P. stypticus*	Russia	Caucasia	1.06	N/A	1.00, 1.06
8951	55611	*P. stypticus*	Russia	Caucasia	0.96	N/A	1.00, 1.05
8982	55642	*P. stypticus*	Turkey	Trabzon	0.89	1.34	0.97, 1.01
9029A	58325	*P. stypticus*	USA	CA, Trinity Co.		N/A	0.99, 1.04
9123	55750	*P. stypticus*	USA	LA, E. Batton Rouge Parish	1.12	N/A	0.98, 1.00
9525	56339	*P. stypticus*	Canada	Montreal, Ursule Falls		N/A	0.93, 0.98
9801	56340	*P. stypticus*	USA	NC, Cataloochee		N/A	0.92, 1.00
Daom 192140	Daom 192140	*P. stypticus*	Canada	British Columbia	1.03	1.35	1.05, 1.11
Antonin	N/A	*P. stypticus*	Czech	N/A	1.09	1.31	1.01, 1.05
F2118	N/A	*P. stypticus*	Germany	N/A	1.15	N/A	1.06
F2188	N/A	*P. stypticus*	Austria	N/A	1.16	N/A	1.01, 1.06
F9518	N/A	*P. stypticus*	Canada	Ontario	1.00	N/A	0.97, 1.04
RC/F 95.083	N/A	*P. stypticus*	France?	N/A	1.09		1.02, 1.07
RC/F95. 084	N/A	*P. stypticus*	France?	N/A	1.04		1.02, 1.07

Note: 1) Bands ECL1, ADH3 and EST1 each were presumed to be controlled by a single locus.
2) The standard collection for ECL1 bands was the 5277 ECL1 band. The standard collection for ADH3 bands was 5185. The standard collection for ECL1 bands was 6880 ECL1 band.
3) N/A: not available.

Appendix 3.2.2. Collections used for isozyme analyses in the genus *Panellus* complex and their ECL mobility (Rf) rates

Collection	CulTenn Accession No.	Species	Country	Locale	ECL Rf values (all bands)
2646	53149	*P. stypticus*	New Zealand	Fjordland	0.21, 0.50
3010	56674	*P. stypticus*	USA	NC, Macon Co.	0.21, 0.64
5277*	51595	*P. stypticus*	USA	NC, Macon Co.	0.18, 056 /(0.13, 0.33)
3493	49311	*D. pusillus*	USA	Puerto Rico	0.14, 0.28, 0.34
4861	51160	*D. pusillus*	Guatemala	Dpto. Escuintla	0.14, 0.19, 0.33
8265	54317	*D. pusillus*	USA	FL, Ocala Nat Forest	0.12, 0.19, 0.33
8271	54318	*D. pusillus*	USA	FL, Ocala Nat Forest	0.11, 0.19, 0.39
8733	55162	*D. pusillus*	Mexico	Est. Nayarit	0.20, 0.33
8747	55176	*D. pusillus*	Mexico	Est. Nayarit	0.11, 0.17, 0.34
9096	N/A	*D. pusillus*	USA	LA, St. Martin's Parish	0.15, 0.36
9522	56278	*D. pusillus*	USA	FL, Hwy 267, Quincy	0.13, 0.35
3513	50185	*D. sp.*	Australia	New South Wales	0.18, 0.23, 0.39
4460	50952	*D. sp.*	Puerto Rico	Toro Negro	0.11, 0.17, 0.33
4536	N/A	*D. sp.*	N/A	N/A	0.11, 0.19, 0.33
5624	52387	*P. serotinus*	USA	ID, Kootenai Co.	0.57, 0.76
5659	52448	*P. serotinus*	USA	ID, Boundary Co.	0.60, 0.76
6167	52312	*P. serotinus*	USA	NC, Macon Co,	0.29, 0.38, 0.55, 0.76
6846	53433	*P. serotinus*	USA	NY, Cortland Co	0.56, 0.76
6881	53461	*P. serotinus*	USA	NY, Schuyler Co.	0.56, 0.76
6894	53450	*P. serotinus*	USA	NC, Macon Co	0.38, 0.56, 0.76
8121	53885	*P. serotinus*	USA	AK, Vic.Anchorage	0.38, 0.55, 0.73, 0.76
ASM5048	N/A	*P. serotinus*	USA	N/A	0.55, 0.75
3570	50065	*P. sp.*	Australia	New South Wales	0.04, 0.26
7435	54086	*P. sp.*	New Zealand	North Island	0.33, 0.46, 0.60
8345	55019	*P. sp.*	Argentina	Prov. Chubut	0.33, 0.43

Note: 1) * Collection 5277 (*P. stypticus*) was the standard collection for ECL electrophoreses, Rf rates for its two bands changed when the buffer system was reprepared (see diagrams in Figure 3.7).
2) Rf = (distance that a band moved)/ (distance that the dye moved).

Appendix 3.3. Seven exemplars in crosses with collections from different geographical areas

Appendix 3.3.1. Crosses of exemplar 9810 with collections from different geographical areas

Appendix 3.3.1.1. Crosses of exemplar 9810 with Eastern North American population

9810-8	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	3	4	7	20	1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

9810-8	+	+!	+	+	+	-!	-!	+	+	+	+	+	+	+	+B	+B
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+B	+B
	1	2	3	7	1	3	4	6	1	2	4	5	1	2	3	4
	6506				6512				6880				6886			

9810-8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	77954				8100				8767				9123			

9810-8	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+
	1	3	4	7	1	2	3	5
	9525				9801			

Note: 1) 9810-8: A2B2; 9810-12: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.1.2. Crosses of exemplar 9810 with Pacific North American population

9810-8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	-F	+	+	+	+
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

9810-8	+	+	+	+	+	+	+	+	-F	+	+	-B	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

9810-8	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	3	4	5	6	4	7	13	14	2	3	5	6	1	2	8	13
	7009				8095				8234				9029			

Note: 1) 9810-8: A2B2; 9810-12: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.1.3. Crosses of exemplar 9810 with Far East population

9810-8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-F	-F
	4	5	6	8	3	4	5	6	1	2	17	18	1	2	4	5
	2300				2315				3810				6548			

9810-8	+	-	+	+	+	+	+	+	+	+	+	
9810-12	+	+	+	+	+	+	+	+	+	+	+	
	2	3	4	5	1	3	4	5	1	2	3	
	6576				6586				6592			

Note: 1) 9810-8: A2B2; 9810-12: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.1.4. Crosses of exemplar 9810 with European population

9810-8	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	3	13	15	17	4	5	6		1	3	6	9	1	3	5	8
	4271				4313				4319				8872			

9810-8	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	2	7	8	1	3	4	5	1	2	3	4	3	4	5	7
	8910				8926				8942				8951			

9810-8	+	+	+	+	+	+	+		+				+	+	+	+
9810-12	+	+	+	+	+	+	+		+				+	+	+	+
	1	2	3	4	1	2	3		1				1	2	3	4
	8982				RC/F95.083				RC/F95.084				RC/M95.021			

Note: 1) 9810-8: A2B2; 9810-12: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.1.5. Crosses of exemplar 9810 with Oceanic populations

9810-8	+	+	+!	+	+	+	+	+	+	+	+	+	+	+	+	+
9810-12	+	+	+	+	+	+	+	+	+	+	+	+	-!	+	-!	-!
	1	6	7	8	1	11	12	14	3	5	12	20	1	8	16	20
	2586 NZ				2658 NZ				2664 NZ				2675 NZ			

9810-8	+	+	-!	+	-F	+	+	+	-F	+	+F	+	+	+	+	+!
9810-12	+	-!	+!	+	-F	+	+	+	+	+	-F	-B	+	+	+	+!
	6	7	12	13	1	2	3	5	4	8	10	20	1	10	11	14
	2810 NZ				3541 NSW				3544 NSW				3567 NSW			

9810-8	+	+	+	-	+	+F	+	+	+	+	+!	+
9810-12	+	+!	+	-	+	+	+	+	+	+	+	+
	4	6	7	15	3	11	12	18	12	13	16	20
	3586 Tasmania				3907 Tasmania				3968 Tasmania			

Note: 1) 9810-8: A2B2; 9810-12: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.2. Crosses of exemplar 9029 (A) with collections from different geographical areas

Appendix 3.3.2.1. Crosses of exemplar 9029 (A) with Eastern North American population

9029-1	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	-		+	+	+	+	+	+	+	+
	3	4	7	20	1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	2	3	7	1	3	4	6	1	2	4	5	1	2	3	4
	6506				6512				6880				6886			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	-	+	+	+	+	+	+	+	+	+	+	+
	1	3	4	7	1	2	3	5	1	3	8	12
	9525				9801				9810			

Note: 1) 9029-1: A1B1; 9029-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.2.2. Crosses of exemplar 9029 (A) with Pacific North American population

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
	3	4	7	10	4	5	6	12	2	4	6	9	1	4	6	7
	5699				5703				5846				6665			

9029-1	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	3	4	6		2	5	7	8	2	3	7	8	3	4	5	6
	6734				6762				6795				7009			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	/
	4	7	13	14	2	3	5	6	2	3	4	8
	8095				8234				5847			

Note: 1) 9029-1: A1B1; 9029-2: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.2.3. Crosses of exemplar 9029 (A) with Far East population

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	5	6	8	3	4	5	6	1	2	17	18	1	2	4	5
	2300				2315				3810				6548			

9029-1	+	+	+	+	+	+	+	+	+	+	+	
9029-2	+	+	+	+	+	+	+	+	+	+	+	
	2	3	4	5	1	3	4	5	1	2	3	
	6576				6586				6592			

Note: 1) 9029-1: A1B1; 9029-2: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.2.4. Crosses of exemplar 9029 (A) with European population

9029-1	+	+	+	+	+	-	+		+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	3	13	15	17	4	5	6		1	3	6	9	1	3	5	8
	4271				4313				4319				8872			

9029-1	+	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	2	7	8	1	3	4	5	1	2	3	4	3	4	5	7
	8910				8926				8942				8951			

9029-1	+	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	2	3	4	1	3	5	8	1	2	3	1	1	2	3	4
	8982				Antonin				RC/F95.083			084	RC/M95.021			

Note: 1) 9029-1: A1B1; 9029-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.2.5. Crosses of exemplar 9029 (A) with Oceanic population

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	6	7	8	1	11	12	14	3	5	12	20	1	8	16	20
	2586 NZ				2658 NZ				2664 NZ				2675 NZ			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	7	12	13	1	2	3	5	4	8	10	20	1	10	11	14
	2810 NZ				3541 NSW				3544 NSW				3567 NSW			

9029-1	+	+	+	+	+	+	+	+	+	+	+	+
9029-2	+	+	+	+	+	+	+	+	+	+	+	+
	4	6	7	15	3	11	12	18	12	13	16	20
	3586 Tasmania				3907 Tasmania				3968 Tasmania			

Note: 1) 9029-1: A1B1; 9029-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.3. Crosses of exemplar 3180 with collections from different geographical areas

Appendix 3.3.3.1. Crosses of exemplar 3180 with Eastern North American population

3180-1	+	+	+	+	+	-	-	+	+	+	+	+	+	A+	-	+
3180-2	-	+	-	-	+F	+	+	+	+F	+	+	+	+	+	+	+
	3	4	7	20	1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

3180-1	-	+	A+	A+	-	A+	+	+	+	+	+	+	+	+	+	+
3180-2	+	+	+	+F	+F	+	+	+	+	+	+	+	+	+F	-	+
	1	2	3	7	1	3	4	6	1	2	4	5	1	2	3	4
	6506				6512				6880				6886			

3180-1	+	+	+	+	+	+	+	+	+F	+	-	+	A+	A+	A+	+
3180-2	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

3180-1	A+	+	+	+	+	+F	+F	+F	A+	A+F		
3180-2	+	+	+	A+	+	+F	+F	+F	+	-		
	1	3	4	7	1	2	3	5	8	12		
	9525				9801				9810			

Note: 1) 3180-1: A1B1; 3810-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.3.2. Crosses of exemplar 3180 with Pacific North American population

3180-1	+	-	+	+	A+	+	+	+F	+	/	+	+	A+	+	A+	+
3180-2	+	+	+	+	+	+	+	+F	A+	/	+	A-	+	-	+	A-
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

3180-1	+	+	+	+	+	+	+	+	+	-	-	+	A+	+	+	+
3180-2	+F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

3180-1	+	-	-	-	+	+	+	-	+	+	-	-	+	+		
3180-2	+	+	+	+	+	+	+	+F	+	+	+	-	+	+		
	5	6	7	8	4	7	13	14	2	3	5	6	1	2		
	7009				8095				8234				9029			

Note: 1) 3180-1: A1B1; 3810-2: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.3.3. Crosses of exemplar 3180 with Far East population

3180-1	+	+	+	+	+	+	+	+	+	+	+	+	+F	-	+	+F
3180-2	+	+	+	+	+	+	+	-	+	+	-	-	+F	-	+	+
	4	5	6	8	3	4	5	6	1	2	4	5	2	3	4	5
	2300				2315				6548				6576			

3180-1	+	+	+	+	+	+F	+	
3180-2	+	+	+	+	+	-	+F	
	1	3	4	5	1	2	3	
	6586				6592			

Note: 1) 3180-1: A1B1; 3810-2: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.3.4. Crosses of exemplar 3180 with European population

3180-1	+F	+F	+	+	+	+	+F		+	-	+F	+	+	-	+F	-
3180-2	+F	+	-	+	+	+	+F		+F	+F	+F	+	+	-	+	+F
	3	13	15	17	4	5	6		1	3	6	9	1	2	3	4
	4271				4313				4319				8982			

3180-1	+	+	+	+F	+	-	-	+	A+	-	+	+	+	-	-	+F
3180-2	+	+	+	+	+	-	-	+	A+	+	+	+	+F	+	+F	+F
	1	2	7	8	3	4	5	7	1	2	3	4	1	3	5	8
	8910				8951				8982				Antonin			

3180-1	+	+F	+	-	+F	+	+F	+F	+	+	+	+	-	+		
3180-2	+F	+B	A+	-	+	+	+F	+F	+	+	+F	+	+	+		
	1	2	3	4	1	2	3	1	1	3	5	8	1	13		
	RC/M95.021				RC/F95.083			084	8872				8926			

Note: 1) 3180-1: A1B1; 3810-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.3.5. Crosses of exemplar 3180 with Oceanic populations

3180-1	+	A+	-	-	-	+	-	+	-	A+	+F	A+	A+	-	-	+B
3180-2	+	A+	A+	-	+	-	-	-	A+	+B	+F	-	A+	A-	+B	+F
	1	6	7	8	1	11	12	14	3	5	12	20	1	8	16	20
	2586 NZ				2658 NZ				2664 NZ				2675 NZ			

3180-1	-	-	-	+F	-	A+	-	A+	-	+	A+	+B	A-	-	-	-
3180-2	-	-	+F	-	-	-	-	A+	+F	+	A+	+F	A-	+	+	A+
	6	7	12	13	1	2	3	5	4	8	10	20	1	10	11	14
	2810 NZ				3541 NSW				3544 NSW				3567 NSW			

3180-1	-	-	A+	+F	-	+	+F	+	-	-	+	-
3180-2	+	A+	-	-	A+	A+	+	-	-	A+	A+	-
	4	6	7	15	3	11	12	18	12	13	16	20
	3586 Tasmania				3907 Tasmania				3968 Tasmania			

Note: 1) 3180-1: A1B1; 3810-2: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.4. Crosses of exemplar 8926 with collections from different geographical areas

Appendix 3.3.4.1. Crosses of exemplar 8926 with Eastern North American population

8926-1	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
8926-13	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	3	4	7	20	1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

8926-1	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+
8926-13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	2	3	7	1	3	4	6	1	2	4	5	1	2	3	4
	6506				6512				6880				6886			

8926-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8926-13	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

8926-1	+	+	+	+	+	+	+	+	+	+		
8926-13	+	+	+	+	+	+	+	+	+	+		
	1	3	4	7	1	2	3	5	8	12		
	9525				9801				9810			

Note: 1) 8926-1: A1B1; 8926-13: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.4.2. Crosses of exemplar 8926 with Pacific North American population

8926-1	+F	+	+F	+	+	+	+	+	+	+	+F	+	+	+	+	+
8926-13	+	+	+	+	+	+	+	+B	+	+	+	+	+	+	+	+
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

8926-1	+B	+	+	+	+	+	+		+	+	+	+	+	+	+	+
8926-13	+B	+	+	+	+F	+	+		+	+	+	+	+	+	+	+
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

8926-1	+	+	+	-	+	+	+	+B	+	+	+F	+F	+	+		
8926-13	+	+	+	+	+	+	+	+	+	+	+	+	/	/		
	3	4	5	6	4	7	13	14	2	3	5	6	1	2		
	7009				8095				8234				9029			

Note: 1) 8926-1: A1B1; 8926-13: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.4.3. Crosses of exemplar 8926 with Far East population

8926-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8926-13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	5	6	8	3	4	5	6	1	2	4	5	2	3	4	5
	2300				2315				6594				6576			

8926-1	+	+	/	+	+	+	+	
8926-13	+	+	/	+	+	+	+	
	1	3	4	5	1	2	3	
	6586				6592			

Note: 1) 8926-1: A1B1; 8926-13: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.4.4. Crosses of exemplar 8926 with European population

8926-1	+	+	+B	+	+	+	+		+	+	+	+	+	+B	+	+B
8926-13	+	+	+B	+	+	+	+		-	+	+	-	+	+	+	+B
	3	13	15	17	4	5	6		1	3	6	9	1	2	3	4
	4271				4313				4319				8942			

8926-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8926-13	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
	1	2	7	8	3	4	5	7	1	2	3	4	1	3	5	8
	8910				8951				8982				Antonin			

8926-1	-	-	+	-	+	+	+	+F	+	+	+	+
8926-13	+	+	+	+	+	+	+	+F	+	+	+	+
	1	2	3	4	1	2	3	1	1	3	5	8
	RC/M95.021				RC/F95.083			084	8872			

Note: 1) 8926-1: A1B1; 8926-13: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.4.5. Crosses of exemplar 8926 with Oceanic populations

8926-1	+F	+	+	+	+	+F	+	+	+	+	+	+B	+F	+F	+	+
8926-13	+	+	+F	+F	+	+	+	+	+	+	+	+	+F	+F	+	+
	1	6	7	8	1	11	12	14	3	5	12	20	1	8	16	20
	2586 NZ				2658 NZ				2664 NZ				2675 NZ			

8926-1	+F	+	+	+	+	+	+	+	+	+B	-	+	-	-	-	A+B
8926-13	+	+	+	+	+	+	+	+	+	+	+	+	-	+F	+	A+B
	6	7	12	13	1	2	3	5	4	8	10	20	1	10	11	14
	2810 NZ				3541 NSW				3544 NSW				3567 NSW			

8926-1	+F	+	+F	+F	+B	+	+	+	+F	+	+F	+
8926-13	+F	+	+F	+F	+B	+	+	+	+F	+	+F	+
	4	6	7	15	3	11	12	18	12	13	16	20
	3586 Tasmania				3907 Tasmania				3968 Tasmania			

Note: 1) 8926-1: A1B1; 8926-13: A2B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.5. Crosses of exemplar 2664 with collections from different geographical areas

Appendix 3.3.5.1. Crosses of exemplar 2664 with Eastern North American population

2664-5	A-	+	+		A+	+	+		+F	+	+	+	A+	+	+	+
2664-20	+	+	+		+F	+	+		+F	+	+	+	A+	+	+	+
	3	4	20		1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

2664-5	+	+	+	A+	+	+	+	A+	A+	A+	A+		+	+	+	A+
2664-20	+	+	-	+	+	+	+	A+	A+	+	+		+	+	+	A+
	1	2	3	7	1	3	4	6	1	2	4		1	2	3	4
	6506				6512				6880				6886			

2664-5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
2664-20	+	+	+	+	-	A-	+	+	+	+	+	+	+	+	+	-
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

2664-5	+	+	+	+	+	+	+	+	+	+	+	+
2664-20	+	+	+	-	+	+	+	+	+	+	+	+
	1	3	4	7	1	2	3	5	1	3	8	12
	9525				9801				9810			

Note: 1) 2664-5: A2B2; 2664-20: A1B1.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.5.2. Crosses of exemplar 2664 with Pacific North American population

2664-5	+	+B	A+	+	+F	+F	A+	+B	A+	+F	A+	+	+	+B	+	+F
2664-20	A+	+B	A+	+	+F	+F	+	+F	+F	+	+	+F	+	A+	+	+
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

2664-5	A-	+	+F	-	+	+	A+		+	+	+	+	A+	+	A+	+
2664-20	A+	+	A-	-	+	+	A+		+	A+	+	+	+	+	A+	+
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

2664-5	-	+	-	+	+	+	A+	+	+	+F	A+	+	+	+		
2664-20	A+	+	A+	+	+	+	A+	A+	+	+B	A+	+	+	+		
	5	6	7	8	4	7	13	14	2	3	5	6	1	2		
	7009				8095				8234				9029			

Note: 1) 2664-5: A2B2; 2664-20: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.5.3. Crosses of exemplar 2664 with Far East population

2664-5	+	+F	A+	+	+	+	+	+	A+	+	+F	+	A+	+B	+B	+F
2664-20	A+	+	A+	+	+	+	+	+F	+F	+B	+F	+	A+	A+	+B	+
	4	5	6	8	3	4	5	6	1	2	4	5	2	3	4	5
	2300				2315				6548				6576			

2664-5	+	+	+		+F	+	+F		A+	+B		
2664-20	+	+	+		+B	+B	+B		A+	-		
	1	3	5		1	2	3		1	2		
	6586				6592				3180			

Note: 1) 2664-5: A2B2; 2664-20: A1B1.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.5.4. Crosses of exemplar 2664 with European population

2664-5	A+	+	A+	+	+F	+	+B		-	A+	+F	+	+	A+	+	A+
2664-20	A+	A+	A+	+	+F	+	+B		+F	A+	+F	+F	+F	A+	+	A+
	3	13	15	17	4	5	6		1	3	6	9	1	2	3	4
	4271				4313				4319				8942			

2664-5	+	+	A+	+	+	+	+	+	A-	A+	+	A+	+	+	+	+
2664-20	+	+F	+F	A+	+F	+	+	+	A+	+	+B	+	+	+	+	+
	1	2	7	8	3	4	5	7	3	4	1	13	1	3	5	8
	8910				8951				8926				8872			

2664-5	+	+	+	+	+	+	+	+	A+	A+	+	A+	A+	+	A+	+
2664-20	+	+	+	+	+	+	+F	+	A+	A+	+F	A+	A+	+	A+	+
	1	2	3	4	1	3	5	8	1	2	3	4	1	2	3	1
	8982				· Antonin				RC/M95.021				RC/F95.083			084

Note: 1) 2664-5: A2B2; 2664-20: A1B1.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.5.5. Crosses of exemplar 2664 with Oceanic populations

2664-5	+	+	/	+	A+	+	+	+	+	-	-	+	A+	+	+	+
2664-20	+	+	+	+	A+	+	+	+	+	A+	+	+	+	+	+	+
	1	6	7	8	1	11	12	14	1	8	16	20	6	7	12	13
	2586 NZ				2658 NZ				2675 NZ				2810 NZ			

2664-5	+	+	+	+	A+B	A+B	+F	+	A+	+	+	A+	+	+	+	+
2664-20	+	+	+	+	+	A+B	+F	+	A+	+	A+	A+	+	+	+	+
	1	2	3	5	4	8	10	20	1	10	11	14	4	6	7	15
	3541 NSW				3544 NSW				3567 NSW				3586 Tasmania			

2664-5	A+	+	+		+F	+	+	+
2664-20	A+	+	+		A+	+	A+	+
	11	12	18		12	13	16	20
	3907 Tasmania				3968 Tasmania			

Note: 1) 2664-5: A2B2; 2664-20: A1B1.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.6. Crosses of exemplar 3567 with collections from different geographical areas

Appendix 3.3.6.1. Crosses with exemplar 3567 with Eastern North American population

3567-1	A-	A-	A-		A-	-			+	A+	A+	A+	A-	A-	A-	A+
3567-10	/	A-	A-		A-	A-			+B	/	+	A+	-	A-	+B	A+
	3	4	20		1	2			1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

3567-1	A-	-	+	A-	A+	A+	A-	A-	A-	A-			A-	A-	A-	A-
3567-10	+	-	+	+F	A-	A+	A+	+F	A+	A+			-	A-	-	A-
	1	2	3	7	1	3	4	6	2	4			1	2	3	4
	6506				6512				6880				6886			

3567-1	-	A-	A+	A+	A-	A-	A-	-	-	A+	-	-	A-	A+	A+	A+
3567-10	A+	A+	+	+	+	+	A+	+	-	A+	A-	+	A-	A+	A+	A+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

3567-1	-	+	-		A+	A-	A-	/	-	+	+	+
3567-10	+	A+	+		+B	/	+	-	-	+	+	+
	1	3	7		1	3	5	8	1	3	8	12
	9525				9801				9810			

Note: 1) 3567-1: A1B1; 3567-10: A1B2.

2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.6.2. Crosses with exemplar 3567 with Pacific North American population

3567-1	A+	A-	A-	A+	A-	+	+	A+	+	+	A+	A+	A-	-	A+	A-
3567-10	+	A-	A+	+	+	+	+	+	+	+	-	A-	+	A+	+	A-
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

3567-1	A-	+	A+	A+	+	-	-		A+	A+	A+	A+	-	A+	-	A-
3567-10	A+	+	+	+	A-	+	-		A+	+	A+	+	A+	+	A+	A-
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

3567-1	A+	A-	-	A-	A+	A+	-	-	-	A-	-	A+	+	+		
3567-10	A+	A-	A-	+B	+	+	A+	A+	+	A-	A+	-	+	+		
	5	6	7	8	4	7	13	14	2	3	5	6	1	2		
	7009				8095				8234				9029			

Note: 1) 3567-1: A1B1; 3567-10: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.6.3. Crosses with exemplar 3567 with Far East population

3567-1	-	A+	A-	A-	+	-	-	A-	-	/	A-	A-	A-	-	A-	+
3567-10	A-	A-	A-	-	A-	-	-	A-	/	A+	+	-	A-	A-	+F	+
	4	5	6	8	3	4	5	6	1	2	4	5	2	3	4	5
	2300				2315				6548				6576			

3567-1	A-	+	+		A-	A-	A-		A-	A-		
3567-10	+F	+	+		-	A-	+		-	+		
	1	3	5		1	2	3		1	2		
	6586				6592				3810			

Note: 1) 3567-1: A1B1; 3567-10: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.6.4. Crosses of exemplar 3567 with European population

3567-1	+	A+	-	+	A+	-			-	-	-	+	/	/	A+	A-
3567-10	+	+	A-	+	+F	+			A+	A+	+F	A+	+	A+	+	A+
	3	13	15	17	4	5			1	3	6	9	1	2	3	4
	4271				4313				4319				8942			

3567-1	A+	A+	-	+F	A-	-	A-	A+	-	-	-		A-	/	A-	A+
3567-10	+	+F	A+	+F	+	A+	A+	+	A-	-	+F		+	+	A+	+
	1	2	7	8	3	4	5	7	3	1	13		1	3	5	8
	8910				8951				8926				8872			

3567-1	A+	-	+F	A+	A-	A-	A-	-	+	A+	A+	+	A-	-	-	-
3567-10	+F	+F	-	A-	A-	+	A+	+	+	+	A+	+F	A+	A+	A+	+
	1	2	3	4	1	3	5	8	1	2	3	4	1	2	3	1
	8982				Antonin				RC/M95.021				RC/F95.083			084

Note: 1) 3567-1: A1B1; 3567-10: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.6.5. Crosses of exemplar 3567 with Oceanic populations

3567-1	A-	A+	/	/	/	A+			-	-	-	-	A+	+	A-	+
3567-10	+	-	+	+	+	/			+	+	+	+	+	A-	A-	+
	1	6	7	8	3	12			1	11	12	14	1	8	16	20
	2586 NZ				2664 NZ				2658 NZ				2675 NZ			

3567-1	A-	-	-	+	A-	-	A+	A+	-	-	A+	A+	+	+		
3567-10	+	A-	A-	A+	A+	A-	+	+	/	-	+	A+	+	+		
	6	7	12	13	1	2	3	5	4	8	10	20	7	15		
	2810 NZ				3541 NSW				3544 NSW				3586 Tasmania			

3567-1	A-	-	A+		-	A-	-	-
3567-10	A-	+	A+		A-	A-	A-	+
	11	12	18		12	13	16	20
	3907 Tasmania				3968 Tasmania			

Note: 1) 3567-1: A1B1; 3567-10: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.7. Crosses of exemplar 3586 with collections from different geographical areas

Appendix 3.3.7.1. Crosses with exemplar 3586 with Eastern North American population

3586-4	-	-	+F		+F	-	-		+F	-	+	+	A+	-	+F	+
3586-8	A-	-	-		A+	A+	A+		-	A-	+	A-	-	+	-	-
	3	4	20		1	2	7		1	2	4	6	2	11	12	18
	2416				2536				6494				6501			

3586-4	+B	+F	+F	+	+F	+	+	A+	-	A+	A-		+	+F	-	A-
3586-8	+	A+	A+	A+	+	+	+F	A+	+	-	A-		+	+	A+	A+
	1	2	3	7	1	3	4	6	1	2	4		1	2	3	4
	6506				6512				6880				6886			

3586-4	A+	+	+	+	+	A+	+	+	+	+	+F	+F	+	+	+	+
3586-8	+	+	+	+	+	+	A+	+	+	+B	+B	+	+	+	+	+
	2	5	11	13	1	2	3	4	3	5	7	16	1	3	4	5
	7954				8100				8767				9123			

3586-4	+	+	+	-	+B	+	+F	-	+	+		
3586-8	+	+	+B	-	A+	A+	+F	-	/	/		
	1	3	7		1	2	3	8	8	12		
	9525				9801				9810			

Note: 1) 3586-4: A1B1; 3586-8: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.7.2. Crosses with exemplar 3586 with Pacific North American population

3586-4	+	A+	A+	+	+	+	+	+	+	+	+	+	+	+	+	+
3586-8	A+	+	A+	+	+	+	A+	+	+	A+	+	+	+	+	+	A+
	3	4	7	10	4	5	6	12	2	4	6	9	2	3	4	8
	5699				5703				5846				5847			

3586-4	A+	+	+	+	A+	+	+	A+	+	+	+	+	+	+	A+	A+
3586-8	A+	+	A+	+	+	+	+	A+	+	A+	+	+	+	+	A+	A+
	1	4	6	7	3	4	6		2	5	7	8	2	3	7	8
	6665				6734				6762				6795			

3586-4	+	+	+	+	+	+	+	A+	+	A+	A+	+F	+	+		
3586-8	+		A+	+	+	+	+	A+	+	A+	A+	A-	/	/		
	5	6	7	8	4	7	13	14	2	3	5	6	1	2		
	7009				8095				8234				9029			

Note: 1) 3586-4: A1B1; 3586-8: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.7.3. Crosses of exemplar 3586 with Far East population

3586-4	A+	A-	A-	+	+	+	A+	A+	+F	+F	A+	+	A+	-	+F	+F
3586-8	A+	+	A+	+	+	+	A+	A+	A+	+F	+	+	+B	+	+	+F
	4	5	6	8	3	4	5	6	1	2	4	5	2	3	4	5
	2300				2315				6548				6576			

3586-4	+	+	+		+F	+F	+F		-	+		
3586-8	+	+	+		+	A+	+		/	/		
	1	3	5		1	2	3		1	2		
	6586				6592				3810			

Note: 1) 3586-4: A1B1; 3586-8: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.7.4. Crosses of exemplar 3586 with European population

3586-4	+	+F	+	+	+	+F	+		+	-	+	+	+	A+	+	+
3586-8	A+	+	A+	+F	+	+F	+		+	+	+	+	+	A+	+	A+
	3	13	15	17	4	5	6		1	3	6	9	1	2	3	4
	4271				4313				4319				8942			

3586-4	+	A+	-	+	+	+	+F	+	-	+	+F	+F	+	+	+	+
3586-8	+	+	+	+	+	+	A+	+	A+	+	/	/	+	+	+F	A+
	1	2	7	8	3	4	5	7	3	4	1	13	1	3	5	8
	8910				8951				8926				8872			

3586-4	+	+	+	+	+F	-	+F	+	+F	A+	+	A+	-	+F		+
3586-8	+	-	+	+	+F	+B	+F	+F	+	A+	+	A+	A+	A+		+
	1	2	3	4	1	3	5	8	1	2	3	4	1	3		1
	8982				Antonin				RC/M95.021				RC/F95.083			084

Note: 1) 3586-4: A1B1; 3586-8: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.3.7.5. Crosses of exemplar 3586 with Oceanic populations

3586-4	+	+	+	A+	/	+			+F	+	+F	+	A+	+	+	+
3586-8	A+	+	+	A+	/	+			A+	A+	A+	A+	+	A+	+	+
	1	6	7	8	3	12			1	11	12	14	1	8	16	20
	2586 NZ				2664 NZ				2658 NZ				2675 NZ			

3586-4	+	+	+F		+	+	-	+	A+	A+	A+	+	-	+	+	A+
3586-8	+	+	+B		+	+	A+	+	A+	A+	A+	+	A+	+	A+	A+
	6	7	12		1	2	3	5	4	8	10	20	1	10	11	14
	2810 NZ				3541 NSW				3544 NSW				3567 NSW			

3586-4	A-	+	-		+F	+	A+	+
3586-8	+B	+	A+		+F	+	+B	A+
	11	12	18		12	13	16	20
	3907 Tasmania				3968 Tasmania			

Note: 1) 3586-4: A1B1; 3586-8: A1B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.4. Mating results of seven exemplars with 57 collections

		Eastern N. Am.	Pacific N. Am.	Far East	Europe	New Zealand	N. S. W.	Tasmania
9810	Total matings	110	94	54	94	40	24	24
Eastern N.	Collections	14	12	7	13	5	3	3
America	Monocaryons	55	47	27	47	20	12	12
	Compatibles	108	90	50	89	34	19	22
8: A2B2	Incompatibles	2	4	4	3	6	5	2
12:A1B1	A reactions(all)	0	0	0	0	0	0	0
	F.B / +	4	0	0	0	0	2	1
9029 (A)	Total matings	118	86	54	93	40	24	24
Pacific N.	Collections	15	11	7	13	5	3	3
America	Monocaryons	59	43	27	47	20	12	12
	Compatibles	113	85	54	88	40	24	24
1:A1B1	Incompatibles	5	1	0	5	0	0	0
2:A2B2	A reactions(all)	0	0	0	0	0	0	0
	F.B / +	0	0	0	0	0	0	0
3180	Total matings	114	90	44	90	40	24	24
Far East	Collections	15	12	6	13	5	3	3
	Monocaryons	57	45	22	45	20	12	12
	Compatibles	102	78	36	75	20	13	13
1: A1B1	Incompatibles	12	12	8	15	0	11	11
2:A2B2	A reactions(all)	11	7	0	3	0	8	6
	F.B / +	13	4	5	25	8	3	2
8926	Total matings	114	88	44	86	40	24	24
Europe	Collections	15	12	6	12	5	3	3
	Monocaryons	57	45	22	43	20	12	12
	Compatibles	111	87	44	79	22	13	13
1:A1B1	Incompatibles	3	1	0	7	18	11	11
13:A2B2	A reactions(all)	0	0	0	0	9	8	6
	F.B / +	3	10	0	7	12	4	13
2664	Total matings	114	90	48	92	31	24	24
New	Collections	16	12	6	14	4	3	3
Zealand	Monocaryons	57	45	24	46	16	12	11
	Compatibles	107	84	47	90	29	24	24
5:A2B2	Incompatibles	7	6	1	2	2	0	0
20:A1B1	A reactions(all)	15	23	9	27	4	8	4
	F.B / +	5	16	16	15	0	5	1
3567	Total matings	104	90	46	76	31	15	18
Autralian	Collections	16	12	7	14	5	2	3
New South	Monocaryons	54	45	24	45	18	8	9
Wales	Compatibles	55	59	14	46	17	8	8
1: A1B1	Incompatibles	49	31	32	30	16	7	10
10: A1B2	A reactions(all)	60	46	24	38	11	9	8
	F.B / +	5	1	2	9	0	0	0
3586	Total matings	108	88	46	90	32	24	14
Australian	Collections	15	12	7	14	5	3	2
Tasmania	Monocaryons	55	45	24	46	16	12	7
	Compatibles	87	87	42	84	32	22	12
4:A1B1	Incompatibles	21	1	4	6	0	2	2
8:A2B2	A reactions(all)	23	26	13	15	9	11	4
	F.B / +	18	1	10	16	4	0	4

Note: 1) Exemplars: 9810 for Eastern N. America; 9029 (A) for Pacific N. America; 3180 for Far East; 8926 for Europe; 2664 for New Zealand; 3567 for Australian New South Wales; 3586 for Australian Tasmania.

2) A reactions (all): Antagonistic reactions in compatible or incompatible matings; F.B / +: Flat-like and Barrage-like reactions appeared in compatible pairings.

Appendix 3.5. Crosses of exemplar 9808 with collections designated as *D. pusillus* or *D. sp.*

9808-1	+	+	+		+	+	+	+	+	+	+		+	+	+	+
9808-3	+	+	+		+	+	+	+	+	+	+		+	+	+	+
	8	16	17		1	3	4	5	4	16	19		2	3	4	5
	3493				3878				4861				8265			

9808-1	+	+	+	+	+B	+	+	+	+	+	+	+	+	+	+	+
9808-3	+	+	+	+	+B	+	+	+	+	+	+	+	+	+	+	+
	1	2	3	6	3	7	8	10	4	6	7	9	2	5	8	9
	8733				8747				9096				9408			

9808-1	A-	-	A-	A-	+	+	+	+	+	+	+	+	+	+		
9808-3	A-	-	A-	A-	+	+	+	+	+	+	+	+	+	+		
	1	3	4	6	1	2	3	4	1	3	4	5	1	2		
	9421				9522				9803				9807			

9808-1	A-	A-		
9808-3	A-	A-		
	1	2		
	9029 *P.stypticus*			

Note: 1) 9808-1: A1B1; 9808-3: A2B2; see Appendix 2.8 for self cross of collection 9808.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.
3) 9029 is an exemplar of *P. stypticus* from CA, USA.

Appendix 3.6. Intercollection mating tests for collections originally designated as *Panellus sp.*

Appendix 3.6.1. Crosses of exemplar 7577 (*P. longinqua*) with collections originally designated as *Panellus sp.*

7577-11	-	-	-	-	-	-	-	-	-	-			-	A-	A-	A-
7577-12	-	-	-	-	-	-	-	-	A-	-			A-	A-		A-
	1	2	10	12	1	4	7	9	4	7			1	5	8	9
	3570				7435				7543				7511			

7577-11	-	A-	A-	-	A-	A-	A-	A-
7577-12	A-	A-	A-	-	A-	A-	A-	A-
	1	2	3	6	1	2	3	4
	8486				8498			

Note: 1) 7577-11: A1B1; 7577-12: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.6.2. Crosses of *P. stypticus* exemplar 9029 (A) with collections originally designated as *Panellus sp.*

9029-1	A-	A-	A-	A-	A-		A-	/	A-	A-
9029-2	-	-	A-	A-	A-		A-	A-	A-	A-
	10	12	4	7	4		11	12	11	12
	3570		7435		7543		7511		7577	

Note: 1) 9029-1: A1B1; 9029-2: A2B2.
2) symbol +: compatible; -: incompatible; +B: Barrage-like reaction in compatible matings; +F: Flat-like reaction in compatible matings; !: epifluorescence used to check clamps; A: antagonism; /: not available due to low viability or contamination.

Appendix 3.7. A multisequence alignment of the ITS region in *P. stypticus* with restriction sites

9525-1: monocaryon, exemplar from Montreal, Canada.
6157: exemplar from TN, USA.
4271-3: monocaryon, exemplar from Vasgotland, Sweden.
4319: exemplar from Maggia, Switzerland.
8234: exemplar from AK, USA.
3342: exemplar from Premorsk, Russia.
9029A: exemplar from CA, USA.
3541: exemplar from Australian New South Wales.
3586: exemplar from Australian Tasmania.
2675: exemplar from New Zealand.
8265: exemplar from *Dictyopanus pusillus*, served as the closest outgroup.

```
                        SSU <--1 --> ITS
        1                          1                                  50
9525-1  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  6157  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
4271-3  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  4319  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  8234  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  3342  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
 9029A  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  3541  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GTTGACGCTG
  3586  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTTTGGGT GTTGACGGTG
  2675  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTTTGGGT GTTGACGCTG
  8265  TGAACCTGCG GAAGGATCAT TATTGAATAC GCTTT.GGGT GCTGATGCTG

        51    TaqI                                DraI,2675      100
9525-1  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  6157  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
4271-3  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  4319  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  8234  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  3342  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
 9029A  GCCTTTCGAG GCATGT.GCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  3541  GCCTTTCGAG GCATGT.GCT CGCGTTCAAA .CTGTTTAA. TCTTCACTTG
  3586  GCCTTTCGAG GCATGTTGCT CGCATTCAAA ACTGTTTAA. TCTTCACTTG
  2675  GCCTTTCGAG GCATGT.GCT CGCATTCAAA .CTGTTTAAA TCTTCACTTG
  8265  GCCTTTCGGG GCATGT.GCT CGCATTCAAA .CTATTTAA. TCTTCACTTG

        101                 TaqI,3342                              150
9525-1  TGCACCTTTT .GTAGTCTTT TGAAGTCATC TCCGCTGCAA ATGCGGG.TT
  6157  TGCACCTTTT .GTAGTCTTT TGAAGTCATC TCCGCTGCAA ATGCGGG.TT
4271-3  TGCACCTTTT .GTAGTCTTT TGAAGTCATC TCCGCTGCAA ATGCGGG.TT
  4319  TGCACCTTTT .GTAGTCTTT TGAAGTCATC TCCGCTGCAA ATGCGGG.TT
  8234  TGCACCTTTT .GTAGTCTTT TGAAGTCATC TCCGCTGCAA ATGCGGG.TT
  3342  TGCACCTTTT .GTAGTCTTT CGAAGTCATC TCCGCTGCAA ATGCGGG.TT
 9029A  TGCACCTTTT .GTAGTCTTT TGAAGTCATT TCCGCTGCAA ATGCGGG.TT
  3541  TGCACCTTTT .GTAGTCTTT TGAAGTCGTC TCCGCTGCAA ATGCGGGTTT
  3586  TGCACCTTTT TGTAGTCTTT TGAAGTCGTC TCCGCTGCAA ATGCGGGTTT
  2675  TGCACCTTTT .GTAGTC... .......... .......... ..........
  8265  TGCACCTTTT .GTAGTCTTT .GAGGTTGTC TCCGCTGCAA ATGCGGGTTT
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Appendix 3.7. (Continued) A multisequence alignment of the ITS region in *P. stypticus* with restriction sites

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          151   HinfI                          DraI,4319                200
9525-1    TTTGGG..AG TCTTG.TCTC CCTGGGCTTC AGAGACTATG TTTTCATATA
  6157    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AGAGACTATG TTTTCATATA
4271-3    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AAAGACTATG TTTTCATATA
  4319    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AAAGACTATG TTTTCATATA
  8234    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AAAGACTATG TTTTCATATA
  3342    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AAAGACTATG TTTTCATATA
 9029A    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AAAGACTATG TTTTCATATA
  3541    TTTGGG..AG TCTTG.TCTC CCTGGGCTTC AGAGACTATG TTTTCATATA
  3586    TTTGGG..AG TCT.G.TCTC CCTGGGCTTC AGAGACTATG TTTTCATATA
  2675    .TTGGG..AG .......... .......... AGAGACTATG TTTTCATATA
  8265    TCAGGGTTGA .CTTGCTCTC CCTGGGCTTC AAAGACTATG TTTTCATATA

          201                                BstZI                    250
9525-1    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT TGGCCGCGAG GCCTTTATAA
  6157    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT TGGCCGCGAG GCCTTTATAA
4271-3    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
  4319    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
  8234    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
  3342    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
 9029A    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
  3541    CA.TTGAAAA GTTACAGAAT GTCTTCTTAT CGGCCGCGAG GCCTTTATAA
  3586    CG.TTGAAAA GTTACAGAAT GTCTT.T... .......GAG GCCTTTATAA
  2675    CA.TTCAAAA GTTACAGAAT GTCTT..... .......GAG GCCTTTATAA
  8265    CATTTGAAAA GTTATTGAAT GTCTTCTTAT CGGCCGCAAG GCCTT..TAA

          251     1-> 5.8s,157bp,                                TaqI 300
9525-1    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  6157    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
4271-3    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  4319    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  8234    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAA
  3342    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
 9029A    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  3541    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  3586    AGTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  2675    AGTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG
  8265    ACTTAATACA ACTTTCAACA ACGGATCTCT TGGCTCTCCC ATCGATGAAG

          301                                                HinfI 350
9525-1    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  6157    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
4271-3    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  4319    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  8234    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  3342    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
 9029A    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  3541    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  3586    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  2675    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
  8265    AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
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Appendix 3.7. (Continued) A multisequence alignment of the ITS region in *P. stypticus* with restriction sites

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   351 TaqI HinfI                                                  400
9525-1 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  6157 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
4271-3 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  4319 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  8234 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  3342 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
 9029A TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  3541 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  3586 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  2675 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC
  8265 TCGAATCTTT GAACGCACCT TGCGCCCTTT GGTATTCCGA AGGGCATGCC

       401      5.8s<---1                                          450
9525-1 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
  6157 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
4271-3 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
  4319 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
  8234 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
  3342 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
 9029A TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT GTGGTCTTCT
  3541 TGTTTGAGTG TCATTAAATT ATCAACCTTG GAGGTCTTTC .........CT
  3586 TGTTTGAGTG TCATTAAATT ATCAACCTTG GAGG.CTTTT C.......CT
  2675 TGTTTGAGTG TCATTAAATT ATCAACCTTG GAGG.CTTTC .........CT
  8265 TGTTTGAGTG TCATTAAATT ATCAACCTTG AAGG.CTTTT ...GTCTTCT

       451                                                     500
9525-1 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  6157 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
4271-3 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  4319 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  8234 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  3342 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
 9029A AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  3541 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  3586 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC
  2675 AGGCTTGGAT GTGAGGGCTT TTGCTGGCTT CCTTCAGTGG ATTGGTCTGC
  8265 AGGCTTGGAT GTGAGGGCTT ..GCTGGCTT CCTTCAGTGG ATTGGTCTGC

       501  DraI                                               550
9525-1 TCCCTTTAAA TGCATTAGTA GGGTCCTCTT GTGGAACCGT CGCTCGGTGT
  6157 TCCCTTTAAA TGCATTAGTA GGGTCCTCTT GTGGAACCGT CGCTCGGTGT
4271-3 TCCCTTTAAA TGCATTAGTA GGATCCTCTT GTGGAACTGT CGCTCGGTGT
  4319 TCCCTTTAAA TGCATTAGTA GGATCCTCTT GTGGAACTGT CGCTCGGTGT
  8234 TCCCTTTAAA TGCATTAGTA GGATCCTCTT GTGGAACTGT CGCTCGGTGT
  3342 TCCCTTTAAA TGCATTAGTA GGATCCTCTT GTGGAACTGT CGCTCGGTGT
 9029A TCCCTTTAAA TGCATTAGTA GGATCCTCTT GTGGAACTGT CGCTCGGTGT
  3541 TCCCTTTAAA TGCATTAGTG GGTTCC.... ..ACAA..GT CGCTCGGTGT
  3586 TCCCTTTAAA TGCATTAGTG GGTTCC.... ..ACAA..GT CGCTCGGTGT
  2675 TCCCTTTAAA TGCATTAGTG GGTTCC.... ..ACAA..GT CGCTCGGTGT
  8265 TCCCTTCAAA TGCATTAGTG GGAT.CTCTT GCAGAACCGT CACTTGGTGT
```

Appendix 3.7. (Continued) A multisequence alignment of the ITS region in *P. stypticus* with restriction sites

```
         551                                                    600
9525-1   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  6157   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
4271-3   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  4319   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  8234   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  3342   GATAA.TTAT CTACGCCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
 9029A   GATAA.TTAT CTACGCTGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  3541   GATAA.TTAT CTACACCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  3586   GATAATTTAT CTACACCGTA TTGACTTGCA AT.GAGAG.C CTGCTTATAA
  2675   GATAAATTAT CTACACCGTA TTGACTTGCA .........C CTGCTTATAA
  8265   GATAA.TTAT CTACGCC.TC CTGACTTGCA ATTGAGAGTC TTGCTCATAA

                                ITS <--1 --> LSU
         601                          1                    650
9525-1   CCGTGTTCGG ACAAGCTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
  6157   CCGTCTTCGG ACAAGCTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
4271-3   CTGTCTTCGG ACAA.CTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
  4319   CTGTCTTCGG ACAA.CTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
  8234   CTGTCTTCGG ACAA.CTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
  3342   CTGTCTTCGG ACAA.CTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
 9029A   CTGTCTTCGG ACAA.CTTC. TTTAATATTT GACCTCAAAT CAGGTAGGAC
  3541   CCGTCTTCGG ACAAGC.... TTTGATATTT GACCTCAAAT CAGGTAGGAC
  3586   CCGTCTTCGG ACAAGC.... TTTGATATTT GACCTCAAAT CAGGTAGGAC
  2675   CC........ ..AACCGTAG TTTGACATTT GACCTCAAAT CAGGTAGGAC
  8265   CCGTCTTCGG AC.AGCTT.. TTCAACATTT GACCTCAAAT CAGGTAGGAG

         651         664
9525-1   TACCCGCTGA ACTT
  6157   TACCCGCTGA ACTT
4271-3   TACCCGCTGA ACTT
  4319   TACCCGCTGA ACTT
  8234   TACCCGCTGA ACTT
  3342   TACCCGCTGA ACTT
 9029A   TACCCGCTGA ACTT
  3541   TACCCGCTGA ACTT
  3586   TACCCGCTGA ACTT
  2675   TACCCGCTGA ACTT
  8265   TACCCGCTGA ACTT
```

Note: Shaded areas are restriction sites for the restriction enzymes indicated above. Bases in bold indicate their difference from other sequences at the same position.

Appendix 3.8 Collections in *Panellus stypticus* used for RFLP analyses and their digestion results

Collection No.	UTK Herbarium accession No.	Country	Locale	No. of sites in ITS region cut by restriction enzymes			
				BstZ I	*Dra* I	*Hinf* I	*Taq* I
2300	49841	Japan	Gunma Prefecture	1	1	3	2
2315	48229	Japan	Tochigi Prefecture	1	1	3	2
2536	48764	USA	TN, Sevier Co.	0	1	3	2
2586	48831	New Zealand	Cantabury	0	2!	2	2
2658	48837	New Zealand	Fjordland	0	2!	2	2
2646	53149	New Zealand	Fjordland		2!		
2664	48830	New Zealand	Fjordland	0	2!	2	2
2675	48833	New Zealand	Fjordland	0	2!	2	2
2810	48775	New Zealand	Urenera	0	2!	2	2
3010	56674	USA	NC, Macon co.		1		
3180	48834	Russia	Premorsk	1	1	3	2
3281	48832	Russia	Premorsk	0	1	3	2
3342	48835	Russia	Premorsk	1	1	3	3
3350	48836	Russia	Premorsk	1	1	3	2
3541	50039	Australia	N.S.W.	1	1	3	2
3544	50059	Australia	N.S.W.	1	1	3	2
3567	50067	Australia	N.S.W.	1	1	3	2
3586	50140	Australia	Tasmania	1	1	3	2
3968	50099	Australia	Tasmania	1	1	3	2
4271	50607	Sweden	Vastergotland	1	2	3	2
4303	50384	Switzerland	Maggia	1	2	3	2
4313	50393	Switzerland	Maggia	1	2	3	2
4319	50398	Switzerland	Maggia	1	2	3	2
5185	52701	USA	NC, Macon Co,	0	1	3	2
5259	51577	USA	NC, Macon Co.	0	1	3	2
5277	51595	USA	NC, Macon Co.	0	1	3	2
5450	51768	USA	SC, Oconee Co.	0	1	3	2
5471	52632	USA	NC, Macon Co.	0	1	3	2
5699	52421	USA	WA, Lewis Co.	1	1	3	2
5703	52533	USA	WA, MRNP	1	1	3	2
5846	52233	USA	WA, Jefferson Co.	1	1	3	2
5847	52276	USA	WA, Jefferson Co.	1	1	3	2
6157	52316	USA	TN, Polk Co,	0	1	3	2
6229	52883	USA	NC, Macon Co.	0	1	3	2
6494	53409	USA	NY, Duchess Co.	0	1	3	2
6501	52960	USA	NC, Macon Co.	0	1	3	2
6503	52951	USA	NC, Macon Co.	0	1	3	2
6504	52961	USA	GA, Rabun Co.	0	1	3	2
6506	52962	USA	GA, Rabun Co.	0	1	3	2
6512	52966	USA	NC, Jackson Co.	0	1	3	2
6548	52991	Russia	Premorsk	1	1	3	2
6576	53006	Russia	Premorsk	1	1	3	2
6586	53013	Russia	Premorsk	1	1	3	2
6592	53018	Russia	Premorsk	1	1	3	2
6665	53217	USA	WA, Snohomish Co.	1	1	3	2
6734	53048	USA	WA, Clallam Co.	1	1	3	2
6762	53076	USA	WA, Clallam Co.	1	1	3	2
6795	53109	USA	WA, Clallam Co.	1	1	3	2
6801	53420	USA	NY, Albany Co.	0	1	3	2
6880	53460	USA	NY, Schuyler Co	0	1	3	2
6886	53453	USA	VA, Greyson Co.	0	1	3	2
7009	N/A	USA	WA, Whatcom Co.	1	1	3	2

Appendix 3.8 (Continued) Collections in *Panellus stypticus* used for RFLP analyses and their digestion results

Collection No.	UTK Herbarium accession No.	Country	Locale	No. of sites in ITS region cut by restriction enzymes			
				BstZ I	*Dra* I	*Hinf* I	*Taq* I
7736	54676	Russia	Caucasia	1	2	3	2
7749	54709	Russia	Caucasia	1	2	3	2
7954	54356	USA	MN, Clearwater Co.	1	1	3	2
7961	54381	USA	MN, Beltrami Co.	1	1	3	2
8095	55419	USA	CA, Trinity Co.	1	1	3	2
8234	55360	USA	AK, Victoria Juneau	1	1	3	2
8767	55195	USA	NC, Macon Co.	0	1	3	2
8872	55462	Russia	Caucasia	1	2	3	2
8910	55530	Russia	Caucasia	1	1	3	2
8926	55546	Russia	Caucasia	1	2	3	2
8942	55602	Russia	Caucasia	1	2	3	2
8951	55611	Russia	Caucasia	1	2	3	2
8982	55642	Turkey	Trabzon	1	2	3	2
9029A	58325	USA	CA, Trinity Co.	1	1	3	2
9123	55750	USA	LA, Batton Rouge	0	1	3	2
9525	56339	Canda	Montreal, Ursule Falls	0	1	3	2
Antonin	N/A	Czech	N/A	1	2	3	2
Rc/f083	N/A	France?	N/A		2		
Rc/f084	N/A	France?	N/A		2		

Note: 2!: in restriction enzyme *Dra* I digestion, New Zealand collections showed a different site from the site European collections had. +: intron present; -: intron absent; +/-: heterozygous. Collections 2646 and 3010 were originally designated as *Panellus sp.* RC/f083 and RC/f084 were unknown of origin, though *Dra* I confirmed of their European origin.

Appendix 3.9. A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
8265-lsu        Dictyopanus pusillus        Length:  1384 bp
4319-lsu        Panellus stypticus          Length:  1384 bp
6662-lsu        Pleurotopsis longinqua      Length:   987 bp
cbs39150        Panellus violaceofulvus     Length:   987 bp
6711-lsu        Panellus ringens            Length:   987 bp
6881-lsu        Panellus serotinus          Length:   987 bp
6170-lsu        Tectella patellaris         Length:   987 bp
5644-lsu        Panellus mitis              Length:   987 bp
af042638        Resinomycena acadiensis     Length:   987 bp
J01355(yeast)   Saccharomyces cerevisiae    Length:   988 bp


      1   1→ beginning LSU                                      50
 8265-lsu  ATTTGACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 4319-lsu  ATTTGACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 6662-lsu  ATTTGACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 cbs39150  ATTTGAACTC AAATCAGGTA GGACTAACCG CTGAACTTAA GCATATCAAT
 6711-lsu  TTTGAACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 6881-lsu  TTTGAACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 6170-lsu  TTTTGACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 5644-lsu  TTTTGACCTC AAATCAGGTA GGACTACCCG CTGAACTTAA GCATATCAAT
 af042638  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu  GTTTGACCTC AAATCAGGTA GGAGTACCCG CTGAACTTAA GCATATCAAT

          51   1→ sequences used for phylogeny reconstruction   100
 8265-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 4319-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 6662-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 cbs39150  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 6711-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 6881-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 6170-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 5644-lsu  AAGCGGAGGA AAAGAAACTA ACAAGGATTC CCCTAGTAAC TGCGAGTGAA
 af042638  ~~~~~GAGGA AAAGAAACTA ACCAGGATTC CCCTAGTAAC TGCGAGTGAA
yeast-lsu  AAGCGGAGGA AAAGAAACCA AC.CGGATTG CCTTAGTAAC GGCGAGTGAA

          101                                                  150
 8265-lsu  GCGGGAAAAG CTCAAATTTA AAATCTGGCG GTTTT....G CCGTCCGAGT
 4319-lsu  GCGGGAAAAG CTCAAATTTA AAATCTGGCG GTTCT....G CCGTCCGAGT
 6662-lsu  GAGGGAAAAG CTCAAATTTA AAATCTGGCG GTTCTTGCAG CCGTCCGAGT
 cbs39150  GAGGGAAAAG CTCAAATTTA AAATCTGGCG GTTCTTGCAG CCGTCCGAGT
 6711-lsu  GAGGGAAAAG CTCAAATTTA AAATCTGGCG GTTCTTGCAG CCGTCCGAGT
 6881-lsu  GCGGGAAAAG CTCAAATTTA AAATCTGGCG GTCCTTGCGG CCGTCCGAGT
 6170-lsu  GCGGGAAAAG CTCAAATTTA AAATCTGGTG GTCCTTGTGG CTATCCGAGT
 5644-lsu  GCGGGAAAAG CTCAAATTTA AAATCTGGCG GCCCTCGCGG TCGTCCGAGT
 af042638  GCGGGAAAAG CTCAAATTTA AAATCTGGCG GTTCT....G CCGTCCGAGT
yeast-lsu  GCGGCAAAAG CTCAAATTTG AAATCTGGTA CCT....TCG GTGCCCGAGT
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
            151                                                       200
 8265-lsu   TGTAATTTAG AGA.AGTGTT ATCCGTGCTG GACCGTGTAC AAGTCTCCTG
 4319-lsu   TGTAATTTAG AGA.AGCGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 6662-lsu   TGTAATTTAG AGA.AGTGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 cbs39150   TGTAATTTAG AGA.AGTGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 6711-lsu   TGTAATTTAG AGA.AGTGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 6881-lsu   TGTAATCTAG AGA.AGTGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 6170-lsu   TGTAATCTAG AGA.AGTGCT ATCCGCGCTG GACCGTGTAC AAGTCTCTTG
 5644-lsu   TGTAATCTAG AGA.AGTGCT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
 af042638   TGTAATTTAG AGA.AGTGTT ATCCGCGCTG GACCGTGTAC AAGTCTCCTG
yeast-lsu   TGTAATTTGG AGAGGGCAAC TTTGGGGCCG TTCCTTGTCT ATGTTCCTTG

            201                                                       250
 8265-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 4319-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 6662-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTACCA
 cbs39150   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 6711-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 6881-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 6170-lsu   GAATAGAGCA TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
 5644-lsu   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGCCA CGGACTGCCA
 af042638   GAATGGAGCG TCATAGAGGG TGAGAATCCC GTCTTTGACA CGGACTGCCA
yeast-lsu   GAACAGGACG TCATAGAGGG TGAGCATCCC G....TGTGG CGAGGAGTGC

            251                                                       300
 8265-lsu   GGACAT.TGT GATGCGCTCT CAAAGAGTCG AGTTGTTTGG GAATGCAGCT
 4319-lsu   GGGCAT.TGT GATGCGCTCT CAAAGAGTCG AGTTGTTTGG GAATGCAGCT
 6662-lsu   GGGCTT.TGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 cbs39150   GGGCTT.TGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 6711-lsu   GGGCTT.TGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 6881-lsu   GGGCTT.TGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 6170-lsu   GGGCTTATGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 5644-lsu   GTGCTTATGT GATGCGCTCT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT
 af042638   GGGCAT.TGT GATGCGCTCT CAAAGAGTCG AGTTGTTTGG GAATGCAGCT
yeast-lsu   GGTTCTTTGT AAAGTGCCTT CGAAGAGTCG AGTTGTTTGG GAATGCAGCT

            301                                                       350
 8265-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGG GAGAGACCGA
 4319-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGG GAGAGACCGA
 6662-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 cbs39150   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 6711-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 6881-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 6170-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 5644-lsu   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
 af042638   CAAAATGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGG GAGAGACCGA
yeast-lsu   CTAAGTGGGT GGTAAATTCC ATCTAAAGCT AAATATTGGC GAGAGACCGA
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
           351                                                 400
 8265-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 4319-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 6662-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 cbs39150  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 6711-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 6881-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 6170-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 5644-lsu  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
 af042638  TAGCGAACAA GTACCGTGAG GGAAAGATGA AAAGAACTTT GGAAAGAGAG
yeast-lsu  TAGCGAACAA GTACAGTGAT GGAAAGATGA AAAGAACTTT GAAAAGAGAG

           401                                                 450
 8265-lsu  TTAAACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 4319-lsu  TTAAACAAGT ACGTGAATTG TAGAAAGGGA AACGCTTAGA AGTCAGTCGC
 6662-lsu  TTAAACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 cbs39150  TTAAACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 6711-lsu  .T.AACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 6881-lsu  .TAAACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 6170-lsu  .T.AACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
 5644-lsu  .TAAACAGTA CGTGAAATTG TTAAAAGGGA AACGCTT.GA AGTCAGTCGC
 af042638  TTAAACAGTA CGTGAAATTG TTGAAAGGGA AACGCTT.GA AGTCAGTCGC
yeast-lsu  TGAAAAAGTA CGTGAAATTG TTGAAAGGGA AGGGCATTTG A.TCAGACAT

           451                                                 500
 8265-lsu  ACTGTTCAGA ACTCAGCCTT GC.....TTT GCTTGGTGTA CTTTCTGTTC
 4319-lsu  GCCGCTCAAA ACTCAACCTG GC.....TCT GCTTGGTGTA CTTTCTGTTC
 6662-lsu  GTTGGCTGGG GATCAACCTT GCT.T..TTT GCTTGGCGTA CTTCTCAGTT
 cbs39150  GTTGGCTGGG GATCAACCTT GCTCT..TTT GCTTGGCGTA CTTCTCAGTT
 6711-lsu  GTTGGCTGGG GATCAACCTT GCTCT..TTT GCTTGGCGTA CTTCCCAGTT
 6881-lsu  GTCGTCAGGG GATTAACCTT GCATT..TTT GCTGGGCGTA CTTCCCTGTT
 6170-lsu  GTTGGCCGGG GATCAACCTT GCATTT.TTT GCTGGGTGTA CTTCCTGGTT
 5644-lsu  GTCGTTCAGG GATCAACCTT GCATTTATTT GCTGGGCGTA CTTCCTGTTG
 af042638  GCCGTTCAGA ACTCAGCCTT GC.....TTT GCTTGGTGTA CTTTCTGTCT
yeast-lsu  GGTGTTTTGT GCCC..TCTG CTCCTTGTGG GTAGGGGAAT CTCGCATTTC

           501                                                 550
 8265-lsu  GGTGGGTCAG CATCAGTTTT GGTCGGTGGA TAAAGGCTCA GAGAATGTGG
 4319-lsu  GGTGGGTCAG CATCAATTTT GGTCGGTGGA TAAAGGCTCA .AAAATGTGG
 6662-lsu  GATGGGTCAG CATCAGTTTT GACCGCTGGA TAAAGATTGA GGGAATGTGG
 cbs39150  GATGGGTCAG CATCAGTTTT GACCGCTGGA TAAAGATTGA GGGAATGTGG
 6711-lsu  GATGGGTCAG CATCAGTTTT GACCGCTGGA TAAAGGTTGA GGGAATGTGG
 6881-lsu  CGACGGTCAG CATCAATTTT GACCGTCGGA TAAAGGCTTG GGGAATGTGG
 6170-lsu  GACGGGTCAG CATCAATTTT GACTGTTGGA TAAAGGCTTG GGGAATGTGG
 5644-lsu  GACGGGTCAG CATCAGTTTT GATCGGTGGA TAAAGGCTAG GGGAATGTGG
 af042638  GGCGGGTCAG CATCAATTTT GGTCGGTGGA TAAAGGCTCA GAGAATGTGG
yeast-lsu  ACTGGGCCAG CATCAGTTTT GGTGGCAGGA TAAATCCATA GGAATGTAGC
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
           551                                                        600
 8265-lsu  CATC.TTCGG GTGTGTTATA GCTCTGGGTC GCATGCATCG GCTGGGATTG
 4319-lsu  CATC.TTCGG GTGTGTTATA GCTCTGGGTC GCATGCATCG GCTGGGATTG
 6662-lsu  CATC.CTCGG ATGTGTTATA GCCCTTAGTT GTATACAGCG GTTGGGACTG
 cbs39150  CATC.CTCGG ATGTGTTATA GCCCTTAGTT GTATACAGCG GTTGGGACTG
 6711-lsu  CATC.TTCGG ATGTGTTATA GCCCTCAGCT GTATACAGCG GTTGGGACTG
 6881-lsu  CATCTTTCGG ATGTGTTATA GCCCTGGGTC GCATACGACG GTTGGGATTG
 6170-lsu  CATC.TTCGG ATGTGTTATA GCCCTTGGTC GCATACAACG GTTGGGATTG
 5644-lsu  CACC.TTCGG GTGTGTTATA GCCCTTGGTC GCATACATCG GTTGGGATTG
 af042638  CA..CTTCGG GTGTGTTATA GCTTTGGGTC GCATACATCG GCTGGGATTG
yeast-lsu  TTGCCTCGGT AAGTATTATA GCCTGTGGGA ATA.CTGCCA GCTGGGACTG

           601                                                        650
 8265-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTAC TTG.TACCAC GTACGTGCTT
 4319-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTAC TTG.TACCAC GTACGTGCTT
 6662-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTCT TTG..ACCAC GTACGTGCTT
 cbs39150  AGGAACTCAG CACGCCGCAA GGCCGGGTCT TTG..ACCAC GTACGTGCTT
 6711-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTCT TTG..ACCAC GTACGTGCTT
 6881-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTGT TCG.CACCAC GTACGTGCTT
 6170-lsu  AGGAACTCAG CACGCCGCAA GGCCGGGTAC TTGTTACCAC GTACGTGCTT
 5644-lsu  AGGAACTCAG CACGCCGAAA GGCCGGGTA. GCAATACCAC GTACGTGCTT
 af042638  AGGAACTCAG CACGCCGCAA GGCCGGGTAC TTG.TACCAC GTACGTGCTT
yeast-lsu  AGGACTGCGA CGTAAGTCA. .......... .......... ..........

           651                                                        700
 8265-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 4319-lsu  AGGATGCTGG CATAATGGCT TTAATCAACC CGTCTTGAAA CACGGACCAA
 6662-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 cbs39150  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 6711-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 6881-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 6170-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 5644-lsu  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
 af042638  AGGATGCTGG CATAATGGCT TTAATCGACC CGTCTTGAAA CACGGACCAA
yeast-lsu  AGGATGCTGG CATAATGGTT ATATGCCGCC CGTCTTGAAA CACGGACCAA


           701                                                        750
 8265-lsu  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 4319-lsu  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 6662-lsu  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 cbs39150  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 6711-lsu  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 6881-lsu  GGAGTCTAAC ATGCCCGCGA GTGTTTGGGT GGTAAACCCG AGCGCGTAAT
 6170-lsu  GGAGTCTAAC ATGCCCGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
 5644-lsu  GGAGTCTAAC ATGCCCGCGA GTGTTTGGGT GGTAAACCCG AGCGCGCAAT
 af042638  GGAGTCTAAC ATGCCTGCGA GTGTTTGGGT GGAAAACCCG AGCGCGTAAT
yeast-lsu  GGAGTCTAAC GTCTATGCGA GTGTTTGGGT GTAAAACCCA TACGCGTAAT
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
           751                                                        800
 8265-lsu  GAAAGTTGAA AGTTG.AGAT CCCTGTCGTG GGGAGCATCG ACGCCCGGAG
 4319-lsu  GAAAG.TGAA AGTTG.AGAT CCCTGTCGTG GGGAGCATCG ACGCCCGGAG
 6662-lsu  GAAAG.TGAA AGTTG.AGAT CTCTGTCGTG GAGAGCATTG ACGCCCAGAC
 cbs39150  GAAAG.TGAA AGTTG.AGAT CTCTGTCGTG GAGAGCATTG ACGCCCAGAC
 6711-lsu  GAAAG.TGAA AGTTG.AGAT CTCTGTCGTG GAGAGCATTG ACGCCCAGAC
 6881-lsu  GAAAG.TGAA AGTTG.AGAT CTCTGTCGTG GAGAGCATCG ACGCCCGGAC
 6170-lsu  GAAAG.TGAA AGTTG.AGAC CTCTGTCGTG GAGGGCATCG ACGCCCGGAC
 5644-lsu  GAAAG.TGAA AGTTG.AGAT CTCTGTCATG GAGAGCATCG ACGCCCGGAC
 af042638  GAAAG.TGAA AGTTG.AGAT CCCTGTCGTG GGGAGCATCG ACGCCCGGAG
yeast-lsu  GAAAGTGAAC GTAGGTTGGG GCCTCGCAAG AGGTGCACAA TCGACCGATC

           801                                                        850
 8265-lsu  CAGACCTTCT GTGACGCATC CGCGGTAGAG CATGTATGTT GGGACCCGAA
 4319-lsu  CAGACCTTTT GTGACGCATC CGCGGTAGAG CATGTATGTT GGGACCCGAA
 6662-lsu  CAGACCTTTT GTGACGGACC TGCGGTAGAG CATGTATGTT GGGACCCGAA
 cbs39150  CAGACCTTTT GTGACGGACC TGCGGTAGAG CATGTATGTT GGGACCCGAA
 6711-lsu  CAGATCTTTT GTGACGGACC TGCGGTAGAG CATGTATGTT GGGACCCGAA
 6881-lsu  CAGAACTTTT GTGACGGATC TGCGGTAGAG CGTGTATGTT GGGACCCGAA
 6170-lsu  CAGACCTTTT GTGACGGATC CGCGGTAGAG CGTGTATGTT GGGACCCGAA
 5644-lsu  CAGACCTTTT GTGACGGATC CGCGGTAGAG CGTGTATGTT GGGACCCGAA
 af042638  CTGATCTTTT GTGA..NATC CGCGGTAGAG CATGTATGTT GGGACCCGAA
yeast-lsu  CTGATGTCTT CGGATGGATT TGAGTAAGAG CATAGCTGTT GGGACCCGAA

           851                                                        900
 8265-lsu  AGATGGTGAA CTATGCCTGA ATAGGGCGAA GCCAGAGGAA ACTCTGGTGG
 4319-lsu  AGATGGTGAA CTATGCCTGA ATAGGGCGAA GCCAGAGGAA ACTCTGGTGG
 6662-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 cbs39150  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 6711-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 6881-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 6170-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 5644-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG
 af042638  AGATGGTGAA CTATGCCTGA ATA.GGCGAA GCCAGAGGAA ACTCTGGTGG
yeast-lsu  AGATGGTGAA CTATGCCTGA ATAGGGTGAA GCCAGAGGAA ACTCTGGTGG


           901                                                        950
 8265-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 4319-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 6662-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 cbs39150  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 6711-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 6881-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 6170-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 5644-lsu  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
 af042638  AGGCTCGTAG CGATTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
yeast-lsu  AGGCTCGTAG CGGTTCTGAC GTGCAAATCG ATCGTCGAAT TTGGGTATAG
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
                sequences used for
           phylogeny reconstruction ← 1                    1→ Group I intron
           951                                             1          1000
 8265-lsu  GGGCGAAAGA CTAATCGAAC CATTTGTGTT GCTACTTGGT GGTTGAGAGG
 4319-lsu  GGGCGAAAGA CTAATCGAAC CATTGGTG.T GCTACTTGGT GGTTAATAGG
 6662-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTCC~~~~ ~~~~~~~~~~
 cbs39150  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTTC~~~~ ~~~~~~~~~~
 6711-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTTC~~~~ ~~~~~~~~~~
 6881-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTTCC~~~ ~~~~~~~~~~
 6170-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTTCT~~~ ~~~~~~~~~~
 5644-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGTTC~~~~ ~~~~~~~~~~
 af042638  GGGCGAAAGA CTAATCG~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu  GGGCGAAAGA CTAATCGAAC CATCTAGTAG CTGGTTCC~~ ~~~~~~~~~~

           1001                                                    1050
 8265-lsu  .CAGTTGAAA GACTTTGCCT AGTGA.GAGT TGAGGATTGC GACACCGTCA
 4319-lsu  CCGGTTGAAA GACCTAGCCT AGTGATTTGT TGAGGATTGC GACACTGTCA
 6662-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

           1051                                                    1100
 8265-lsu  AATTGCGGGG ACGTCCTTAG AGCC.TTTGC TACCGCCTGT GCACTGAAAA
 4319-lsu  AATTGCGGGG ACGTCCTTAG AGCCTTTTGC TACCGCCTCT ATGCTGAAAA
 6662-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
            1101                                                    1150
 8265-lsu   GTGT.GCAGG CACCAGGGTA ATGACCTCGG GTATGGTAAT AATGCAAAGG
 4319-lsu   GTGTAGCTGG CACCAGGGTA ATGACCTCGG GTATGGTAAT AACGCAAAGG
 6662-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

            1151                                                    1200
 8265-lsu   ATTGGATAAT CCGCAGCCAA GTCCTAAGGG TAGTCAACGA CCTATGGATG
 4319-lsu   ATTGGATAAT CCGCAGCCAA GTCCTAACGG TCGTAGCTGA CTTATGGATG
 6662-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

            1201                                                    1250
 8265-lsu   CAGTTCACAG ACTAAATGGC GGTGGGTGTT G..TTGAAAA CAATGCTTAA
 4319-lsu   CAGTTCACAG ACTAAATGGC AGTGGGTGTT GCTTCAATAG CAATGCTTAA
 6662-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

            1251                                                    1300
 8265-lsu   GATATAGTCG GTCCCGTCGC GAGAGCGAAC TGGCAGATGA AGGCTTGCTG
 4319-lsu   GATATAGTCG GTCCCGTCGT GAAAGCGAAC TGGCAGATGA AGGCTTTGTT
 6662-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

Appendix 3.9. (Continued) A LSU multisequence alignment of exemplars of the genus *Panellus* complex

```
           1301                                                      1350
 8265-lsu  TCTCCTCAAC TTTTAAGGAC GCTTGCCTGG AGCTGCTGGG AGGTCATTTA
 4319-lsu  GTCACCTCAA CTATAATGAC ACTGGTCTGG AGCTGCT.GG AAGTCTGTTA
 6662-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 cbs39150  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6711-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6881-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 6170-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 5644-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 af042638  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
yeast-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~


           1351                                 1384
 8265-lsu  AAAAGGCCTT GGGGTAGAAT GCTATAGTGT TCC~
 4319-lsu  AGTCAGACTT GGGGTAAAAT GCTAGTAGCT GTTC
 6662-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 cbs39150  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 6711-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 6881-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 6170-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 5644-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
 af042638  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
yeast-lsu  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~
```

Appendix 3.10. Collections surveyed for presence or absence of Group I intron

Collection No.	TENN Accession No.	Species	country	locale	Intron present in LSU
2300	49841	*Panellus stypticus*	Japan	Gunma, Pref.	+
2315	48229	*Panellus stypticus*	Japan	Tochigi Pref.	+
2536	48764	*Panellus stypticus*	USA	TN, Sevier Co.	+
2586	48831	*Panellus stypticus*	New Zealand	Canterbury	-
2646	53149	*Panellus stypticus*	New Zealand	Fjordland	-
2658	48837	*Panellus stypticus*	New Zealand	Fjordland	-
2664	48830	*Panellus stypticus*	New Zealand	Fjordland	-
2675	48833	*Panellus stypticus*	New Zealand	Fjordland	-
2810	48775	*Panellus stypticus*	New Zealand	Urewera	-
3010	56674	*Panellus stypticus*	USA	NC, Macon Co.	+
3180	48834	*Panellus stypticus*	Russia	Premorsk	+
3281	48832	*Panellus stypticus*	Russia	Premorsk	+
3342	48835	*Panellus stypticus*	Russia	Premorsk	+
3350	48836	*Panellus stypticus*	Russia	Premorsk	+
3541	50039	*Panellus stypticus*	Australia	N.S.W.	+
3544	50059	*Panellus stypticus*	Australia	N.S.W.	+
3567	50067	*Panellus stypticus*	Australia	N.S.W.	+
3586	50140	*Panellus stypticus*	Australia	Tasmania	-
3968	50099	*Panellus stypticus*	Australia	Tasmania	-
4271	50607	*Panellus stypticus*	Sweden	Vastergotland	+
4303	50384	*Panellus stypticus*	Switzerland	Maggia	+
4313	50393	*Panellus stypticus*	Switzerland	Maggia	+
4319	50398	*Panellus stypticus*	Switzerland	Maggia	+
5185	52701	*Panellus stypticus*	USA	NC, Macon Co,	+
5259	51577	*Panellus stypticus*	USA	NC, Macon Co.	+
5277	51595	*Panellus stypticus*	USA	NC, Macon Co.	+/-
5450	51768	*Panellus stypticus*	USA	SC, Oconee Co.	+
5471	52632	*Panellus stypticus*	USA	NC, Macon Co.	+
5699	52421	*Panellus stypticus*	USA	WA, Lewis Co.	+
5703	52533	*Panellus stypticus*	USA	WA, MRNP	+
5846	52233	*Panellus stypticus*	USA	WA, Jefferson Co.	+
5847	52276	*Panellus stypticus*	USA	WA, Jefferson Co.	+
6157	52316	*Panellus stypticus*	USA	TN, Polk Co,	+
6229	52883	*Panellus stypticus*	USA	NC, Macon Co.	+
6494	53409	*Panellus stypticus*	USA	NY, Duchess Co.	+
6501	52960	*Panellus stypticus*	USA	NC, Macon Co.	+
6503	52951	*Panellus stypticus*	USA	NC, Macon Co.	+
6504	52961	*Panellus stypticus*	USA	GA, Rabun Co.	+
6506	52962	*Panellus stypticus*	USA	GA, Rabun Co.	+
6512	52966	*Panellus stypticus*	USA	NC, Jackson Co.	+
6548	52991	*Panellus stypticus*	Russia	Premorsk	+
6576	53006	*Panellus stypticus*	Russia	Premorsk	+
6586	53013	*Panellus stypticus*	Russia	Premorsk	+
6592	53018	*Panellus stypticus*	Russia	Premorsk	+
6665	53217	*Panellus stypticus*	USA	WA, Snohomish Co.	+
6734	53048	*Panellus stypticus*	USA	WA, Clallam Co.	+
6762	53076	*Panellus stypticus*	USA	WA, Clallam Co.	+

Appendix 3.10. (Continued) Collections surveyed for presence or absence of Group I intron

Collection No.	TENN Accession No.	Species	country	locale	Intron present in LSU
6795	53109	*Panellus stypticus*	USA	WA, Clallam Co.	+
6801	53420	*Panellus stypticus*	USA	NY, Albany Co.	+
6880	53460	*Panellus stypticus*	USA	NY, Schuyler Co	+
6881	53461	*Panellus stypticus*	USA	NY, Schuyler Co.	+
6886	53453	*Panellus stypticus*	USA	VA, Greyson Co.	+
7009	N/A	*Panellus stypticus*	USA	WA, Whatcom Co.	+
7736	54676	*Panellus stypticus*	Russia	Caucasia	+
7749	54709	*Panellus stypticus*	Russia	Caucasia	+
7954	54356	*Panellus stypticus*	USA	MN, Clearwater Co.	+
7961	54381	*Panellus stypticus*	USA	MN, Beltrami Co.	+
8095	55419	*Panellus stypticus*	USA	CA, Trinity Co.	+
8234	55360	*Panellus stypticus*	USA	AK, Victoria Juneau	+
8767	55195	*Panellus stypticus*	USA	NC, Macon Co.	+
8872	55462	*Panellus stypticus*	Russia	Caucasia	+
8910	55530	*Panellus stypticus*	Russia	Caucasia	+
8926	55546	*Panellus stypticus*	Russia	Caucasia	+
8942	55602	*Panellus stypticus*	Russia	Caucasia	+
8951	55611	*Panellus stypticus*	Russia	Caucasia	+
8982	55642	*Panellus stypticus*	Turkey	Trabzon	+
9029	55674	*Panellus stypticus*	USA	CA, Trinity Co.	+
9123	55750	*Panellus stypticus*	USA	LA, Batton Rouge	+
9525	56339	*Panellus stypticus*	Canda	Montreal, Ursule Falls	+
DAOM 192140	DAOM 192140	*Panellus stypticus*	Canada	British Columbia	+
Antonin	N/A	*Panellus stypticus*	Czech	N/A	+
5644	52389	*Panellus mitis*	USA	ID, Bonner Co.	-
7486	53512	*Panellus mitis*	Finland	Etela-hame Prov.	-
7494	53511	*Panellus mitis*	Finland	Etela-hame Prov.	-
5729	52554	*Panellus serotinus*	USA	WA, Pierce Co.	-
6846	53433	*Panellus serotinus*	USA	NY, Cortland Co	-
6881	53461	*Panellus serotinus*	USA	NY, Schuyler Co.	-
6711	52365	*Panellus ringens*	USA	AK, Anchorage	-
CBS391.50	CBS391.50	*Panellus violaceofulvus*	N/A	N/A	-
6662	53214	*Pleurotopsis longinqua*	USA	WA, CAllam Co.	-
6170	52302	*Tectella patellaris*	USA	NC, Macon Co.	-
3493	49311	*Dictyopanus pusillus*	USA	Puerto Rico	+
4861	51160	*Dictyopanus pusillus*	Guatemala	Dpto Escuintla	+
8265	54317	*Dictyopanus pusillus*	USA	FL, Ocala Nat Forest	+
8271	54318	*Dictyopanus pusillus*	USA	FL, Ocala Nat Forest	+
8733	55162	*Dictyopanus pusillus*	Mexico	Est. Nayarit	+/-
8747	55176	*Dictyopanus pusillus*	Mexico	Est. Nayarit	+
9096	N/A	*Dictyopanus pusillus*	USA	LA, St. Martin's Parish	+
9522	56278	*Dictyopanus pusillus*	USA	FL, Hwy 267, Quincy	+

Appendix 3.10. (Continued) Collections surveyed for presence or absence of Group I intron

Collec-tion No.	TENN Accession No.	Species	country	locale	Intron present in LSU
3518	50160	*Dictyopanus sp*	Australia	N.S.W.	+
3526	50184	*Dictyopanus sp*	Australia	N.S.W.	+
4460	50952	*Dictyopanus sp*	Puerto Rico	Toro Negro	+
4535	51015	*Dictyopanus sp*	Puerto Rico	El Verde Station	+
7699	53778	*Dictyopanus sp*	Costa Rica	Prov. Cartago	+
7809	53765	*Dictyopanus sp*	Costa Rica	Prov. San Jose	+

Note: Symbol + meant presence of the intron; - meant absence of the intron

Appendix 3.11. A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences for phylogeny reconstruction

```
6881-lsu    Panellus serotinus          Length:   840 bp
5644-lsu    Panellus mitis              Length:   840 bp
cbs39150    Panellus violaceofulvus     Length:   840 bp
6711-lsu    Panellus ringens            Length:   840 bp
4319-lsu-m  Panellus stypticus          Length:   840 bp
8265-lsu-m  Dictyopanus pusillus        Length:   840 bp
6170-lsu    Tectella patellaris         Length:   840 bp
6662-lsu    Pleurotopsis longinqua      Length:   840 bp
af042604    Pleurotopsis longinqua      Length:   840 bp
u66435      Hygrocybe citrinopallida    Length:   840 bp
af042638    Resinomycena acadiensis     Length:   840 bp
af042636    Mycena galericulata         Length:   840 bp
af042637    Mycena clavicularis         Length:   840 bp
af042606    Mycena rutilanthiformis     Length:   840 bp

            1                                                      50
  6170-lsu  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  6881-lsu  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  5644-lsu  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  6662-lsu  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGAGGG
  af042604  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGAGGG
  cbs39150  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGAGGG
  6711-lsu  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGAGGG
    u66435  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
8265-lsu-m  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
4319-lsu-m  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  af042638  GAGGAAAAGA AACTAACCAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  af042636  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  af042637  GAGGAAAAGA AACTAACCAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG
  af042606  GAGGAAAAGA AACTAACAAG GATTCCCCTA GTAACTGCGA GTGAAGCGGG

            51                                                    100
  6170-lsu  AAAAGCTCAA ATTTAAAATC TGGTGGTCCT TGTGGCTATC CGAGTT.GTA
  6881-lsu  AAAAGCTCAA ATTTAAAATC TGGCGGTCCT TGCGGCCGTC CGAGTT.GTA
  5644-lsu  AAAAGCTCAA ATTTAAAATC TGGCGGCCCT CGCGGTCGTC CGAGTT.GTA
  6662-lsu  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT TGCAGCCGTC CGAGTT.GTA
  af042604  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT TGCAGCCGTC CGAGTT.GTA
  cbs39150  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT TGCAGCCGTC CGAGTT.GTA
  6711-lsu  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT TGCAGCCGTC CGAGTT.GTA
    u66435  AAAAGCTCAA ATTTAAAATC TGGCGGTCCT TGCGGCTGTC CGAGTT.GTA
8265-lsu-m  AAAAGCTCAA ATTTAAAATC TGGCGGTTTT ....GCCGTC CGAGTT.GTA
4319-lsu-m  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT ....GCCGTC CGAGTT.GTA
  af042638  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT ....GCCGTC CGAGTT.GTA
  af042636  AAAAGCGGAA ATTTAAAATC TGGCGGTCCT TGCGGCCGTC CGAGTTAGTA
  af042637  AAAAGCTCAA ATTTAAAATC TGGCGGTTCT TGTAGCCGTC CGAGTT.GTA
  af042606  AAAAGCTCAA ATTTAAAATC TGGCGGTTCC ....ACCGTC CGAGTT.GTA
```

Appendix 3.11. (Continued) A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences

```
                101                                                   150
  6170-lsu  ATCTAGAGAA GTGCTATCCG CGCTGGACCG TGTACAAGTC TCTTGGAATA
  6881-lsu  ATCTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  5644-lsu  ATCTAGAGAA GTGCTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  6662-lsu  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  af042604  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  cbs39150  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  6711-lsu  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
    u66435  ATCTAGAGAA GTGTTTTCCG TGCTGGACCG TGTACAAGTC TCTTGGAATA
8265-lsu-m  ATTTAGAGAA GTGTTATCCG TGCTGGACCG TGTACAAGTC TCCTGGAATG
4319-lsu-m  ATTTAGAGAA GCGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  af042638  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  af042636  ATTTAGAGAA GTGTTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG
  af042637  ATTTAGAGAA GTGTTATCCG CGTTGGACCG TGTACAAGTC TCCTGGAATG
  af042606  ATTTAGAGAA GTGCTATCCG CGCTGGACCG TGTACAAGTC TCCTGGAATG

                151                                                   200
  6170-lsu  GAGCATCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCT
  6881-lsu  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCT
  5644-lsu  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGCCACGGAC TGCCAGTGCT
  6662-lsu  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TACCAGGGCT
  af042604  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TACCAGGGCT
  cbs39150  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCT
  6711-lsu  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCT
    u66435  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TACCAGTGCT
8265-lsu-m  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGACA
4319-lsu-m  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCA
  af042638  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCA
  af042636  GAGCGTCATA CAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCA
  af042637  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TNNCAAGGCT
  af042606  GAGCGTCATA GAGGGTGAGA ATCCCGTCTT TGACACGGAC TGCCAGGGCA

                201                                                   250
  6170-lsu  TATGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  6881-lsu  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  5644-lsu  TATGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  6662-lsu  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  af042604  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  cbs39150  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  6711-lsu  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
    u66435  T.TGTGATGC GCTCTCAAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
8265-lsu-m  T.TGTGATGC GCTCTCAAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
4319-lsu-m  T.TGTGATGC GCTCTCAAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  af042638  T.TGTGATGC GCTCTCAAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  af042636  T.TGTGATGC GCTCTCGAAG A.TCGAGTTG TTTGGGAATG CAGCTCAAAA
  af042637  T.TGTGATGC GCTCTCGAAG AGTCGAGTTG TTTGGGAATG CAGCTCAAAA
  af042606  T.TGTGATGC GCTCTCAAAG AGTCGAGTTG TTTGGGATTG CAGCTCAAAA
```

Appendix 3.11. (Continued) A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences

```
            251                                                       300
  6170-lsu  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  6881-lsu  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  5644-lsu  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  6662-lsu  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  af042604  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  cbs39150  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
  6711-lsu  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
    u66435  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGCGAGAG ACCGATAGCG
8265-lsu-m  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG
4319-lsu-m  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG
  af042638  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG
  af042636  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG
  af042637  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG
  af042606  TGGGTGGTAA ATTCCATCTA AAGCTAAATA TTGGGGAGAG ACCGATAGCG

            301                                                       350
  6170-lsu  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  6881-lsu  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  5644-lsu  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  6662-lsu  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  af042604  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  cbs39150  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  6711-lsu  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
    u66435  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
8265-lsu-m  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
4319-lsu-m  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  af042638  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  af042636  AACAAGTACC GTGAGGNAAA GATGAAAAGA GAGTT..AAA GAGAGTTAAA
  af042637  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA
  af042606  AACAAGTACC GTGAGGGAAA GATGAAAAGA ACTTTGGAAA GAGAGTTAAA

            351                                                       400
  6170-lsu  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
  6881-lsu  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTCGTC
  5644-lsu  CAGTACGTGA AATTGTTAAA AGGGAAACGC TTGAAGTCAG TCGCGTCGTT
  6662-lsu  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
  af042604  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
  cbs39150  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
  6711-lsu  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
    u66435  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGGC
8265-lsu-m  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCACTGTT
4319-lsu-m  CAGTACGTGA AATTGTAGAA AGGGAAACGC TTGAAGTCAG TCGCGCCGCT
  af042638  CAGTACGTGA AATTGTTGAA AGGGAAACGC TTGAAGTCAG TCGCGCCGTT
  af042636  CATGACGTGA AATTGCTGAA AGGGAAACGC TTGAAGTCAG TCGCGTCGTT
  af042637  CAGTACGTGA AATTGCTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGTT
  af042606  CAGTACGTGA AATTGCTGAA AGGGAAACGC TTGAAGTCAG TCGCGTTGTT
```

Appendix 3.11. (Continued) A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences

```
            401                                                  450
  6170-lsu  CGGGGATCAA CCTTGCA.TT TTTTGCTGGG TGTACTTCCT GGTTGACGGG
  6881-lsu  AGGGGATTAA CCTTGCA..T TTTTGCTGGG CGTACTTCCC TGTTCGACGG
  5644-lsu  CAGGGATCAA CCTTGCATTT ATTTGCTGGG CGTACTTCCT GTTGGACGGG
  6662-lsu  TGGGGATCAA CCTTGC...T TTTTGCTTGG CGTACTTCTC AGTTGATGGG
  af042604  TGGGGATCAA CCTTGC..TT TTTTGCTTGG CGTACTTCTC AGTTGATGGG
  cbs39150  TGGGGATCAA CCTTGC..TC TTTTGCTTGG CGTACTTCTC AGTTGATGGG
  6711-lsu  TGGGGATCAA CCTTGC..TC TTTTGCTTGG CGTACTTCCC AGTTGATGGG
    u66435  CGAGGATCAA CCTTGCA... ATTTGCTGGG CGTACTTCTC GGTTGATGGG
8265-lsu-m  CAGAACTCAG CCTTGC.... .TTTGCTTGG TGTACTTTCT GTTCGGTGGG
4319-lsu-m  CAAAACTCAA CCTGGC.... .TCTGCTTGG TGTACTTTCT GTTCGGTGGG
  af042638  CAGAACTCAG CCTTGC.... .TTTGCTTGG TGTACTTTCT GTCTGGCGGG
  af042636  CAGGACTCAG CCTTGC.... .TTTGCTTGG TGTACTTTCT GTTCGACGGG
  af042637  CAGGACTCAG CCTTGC.... TTTTGCTTGG TGTACTTCTT GTTCGACGGG
  af042606  CGGGATTCAA C.TT..CAGC TTCTGCTAGG TGGATTTCCT GTTCGACGGG

            451                                                  500
  6170-lsu  TCAGCATCAA TTTTGACTGT TGGATAAAGG CTTGGGGAAT GTGGCATCTT
  6881-lsu  TCAGCATCAA TTTTGACCGT CGGATAAAGG CTTGGGGAAT GTGGCATCTT
  5644-lsu  TCAGCATCAG TTTTGATCGG TGGATAAAGG CTAGGGGAAT GTGGCACCTT
  6662-lsu  TCAGCATCAG TTTTGACCGC TGGATAAAGA TTGAGGGAAT GTGGCATCCT
  af042604  TCAGCATCAG TTTTGACCGC TGGATAAAGA TTGAGGGAAT GTGGCATCCT
  cbs39150  TCAGCATCAG TTTTGACCGC TGGATAAAGA TTGAGGGAAT GTGGCATCCT
  6711-lsu  TCAGCATCAG TTTTGACCGC TGGATAAAGG TTGAGGGAAT GTGGCATCTT
    u66435  TCAGCATCAA TTTTGACCGT TGGATAAAGG TTGGGGGAAT GTGGCATCTT
8265-lsu-m  TCAGCATCAG TTTTGGTCGG TGGATAAAGG CTCAGAGAAT GTGGCATCTT
4319-lsu-m  TCAGCATCAA TTTTGGTCGG TGGATAAAGG CTCAGAAAAT GTGGCATCTT
  af042638  TCAGCATCAA TTTTGGTCGG TGGATAAAGG CTCAGAGAAT GTGGCA.CTT
  af042636  TCAGCATCAA TTTTGGTCGG TGGATAAAGG CTCAGAGAAT GTGGCATCTT
  af042637  TCAGCATCAA TTTTGGTCGG TGGATAAAGG CTTAGAGAAT GTGGCATCTT
  af042606  TCAGCATCAG TTTTGGCCGG TGGATAAAGG TTCAGAGAAT GTGGCATCCT

            501                                                  550
  6170-lsu  CGGATGTGTT ATAGCCCTTG GTCGCATACA ACGGTTGGGA TTGAGGAACT
  6881-lsu  CGGATGTGTT ATAGCCCTGG GTCGCATACG ACGGTTGGGA TTGAGGAACT
  5644-lsu  CGGGTGTGTT ATAGCCCTTG GTCGCATACA TCGGTTGGGA TTGAGGAACT
  6662-lsu  CGGATGTGTT ATAGCCCTTA GTTGTATACA GCGGTTGGGA CTGAGGAACT
  af042604  CGGATGTGTT ATAGCCCTTG GTTGTATACA GCGGTTGGGA CTGAGGAACT
  cbs39150  CGGATGTGTT ATAGCCCTTA GTTGTATACA GCGGTTGGGA CTGAGGAACT
  6711-lsu  CGGATGTGTT ATAGCCCTCA GCTGTATACA GCGGTTGGGA CTGAGGAACT
    u66435  CGGATGTGTT ATAGCCCTTA GTTGTATACA ACGGTTGGGA TTGAGGAACT
8265-lsu-m  CGGGTGTGTT ATAGCTCTGG GTCGCATGCA TCGGCTGGGA TTGAGGAACT
4319-lsu-m  CGGGTGTGTT ATAGCTCTGG GTCGCATGCA TCGGCTGGGA TTGAGGAACT
  af042638  CGGGTGTGTT ATAGCTTTGG GTCGCATACA TCGGCTGGGA TTGAGGAACT
  af042636  CGGGTGTGTT ATAGCTCTGG GTCGCATACA TCGGCTGGGA TTGAGGAACT
  af042637  CGGGTGTGTT ATAGCTCTGG GTCGCATACA TCGGCTGGGA TTGAGGAACT
  af042606  CGGATGTGTT ATAGCTCTGG GTCGCATACA TCGGCTGGGA CTGAGGAACT
```

Appendix 3.11. (Continued) A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences

```
            551                                                    600
  6170-lsu  CAGCACGCCG CAAGGCCGGG TACTTGTTAC CACGTACGTG CTTAGGATGC
  6881-lsu  CAGCACGCCG CAAGGCCGGG TGTTCG.CAC CACGTACGTG CTTAGGATGC
  5644-lsu  CAGCACGCCG AAAGGCCGGG TA.GCAATAC CACGTACGTG CTTAGGATGC
  6662-lsu  CAGCACGCCG CAAGGCCGGG TCTTTG..AC CACGTACGTG CTTAGGATGC
  af042604  CAGCACGCCG CAAGGCCGGG TCTTTG..AC CACGTACGTG CTTAGGATGC
  cbs39150  CAGCACGCCG CAAGGCCGGG TCTTTG..AC CACGTACGTG CTTAGGATGC
  6711-lsu  CAGCACGCCG CAAGGCCGGG TCTTTG..AC CACGTACGTG CTTAGGATGC
    u66435  CAGCACGCCG CAAGGCCGGG G.TTT..ACC CACGTTCGTG CTTAGGATGC
8265-lsu-m  CAGCACGCCG CAAGGCCGGG TACTTG.TAC CACGTACGTG CTTAGGATGC
4319-lsu-m  CAGCACGCCG CAAGGCCGGG TACTTG.TAC CACGTACGTG CTTAGGATGC
  af042638  CAGCACGCCG CAAGGCCGGG TACTTG.TAC CACGTACGTG CTTAGGATGC
  af042636  CAGCACGCCG CAAGGCCGGG TACTTG.TAC CACGTACGTG CTTAGGATGC
  af042637  CAGCACGCCG CAAGGCCGGG TACTTG.TAC CACGTACGTG CTTAGGATGC
  af042606  CAGCACGCCG CAAGGCCAGG ...TTTCGAC CATGTTCGTG CTTAGGATGC

            601                                                    650
  6170-lsu  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  6881-lsu  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  5644-lsu  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  6662-lsu  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  af042604  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  cbs39150  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  6711-lsu  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
    u66435  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
8265-lsu-m  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
4319-lsu-m  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  af042638  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  af042636  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  af042637  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT
  af042606  TGGCATAATG GCTTTAATCG ACCCGTCTTG AAACACGGAC CAAGGAGTCT

            651                                                    700
  6170-lsu  AACATGCCCG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  6881-lsu  AACATGCCCG CGAGTGTTTG GGTGGTAAAC CCGAGCGCGT AATGAAAGTG
  5644-lsu  AACATGCCCG CGAGTGTTTG GGTGGTAAAC CCGAGCGCGC AATGAAAGTG
  6662-lsu  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  af042604  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  cbs39150  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  6711-lsu  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
    u66435  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
8265-lsu-m  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
4319-lsu-m  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  af042638  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  af042636  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  af042637  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
  af042606  AACATGCCTG CGAGTGTTTG GGTGGAAAAC CCGAGCGCGT AATGAAAGTG
```

Appendix 3.11. (Continued) A LSU multisequence alignment of the genus *Panellus* complex with downloaded sequences

```
            701                                                  750
  6170-lsu  AAAGTTGAGA CCTCTGTCGT GGAGGGCATC GACGCCCGGA CCAGACCTTT
  6881-lsu  AAAGTTGAGA TCTCTGTCGT GGAGAGCATC GACGCCCGGA CCAGAACTTT
  5644-lsu  AAAGTTGAGA TCTCTGTCAT GGAGAGCATC GACGCCCGGA CCAGACCTTT
  6662-lsu  AAAGTTGAGA TCTCTGTCGT GGAGAGCATT GACGCCCAGA CCAGACCTTT
  af042604  AAAGTTGAGA TCTCTGTCGT GGAGAGCATT GACGCCCAGA CCAGACCTTT
  cbs39150  AAAGTTGAGA TCTCTGTCGT GGAGAGCATT GACGCCCAGA CCAGACCTTT
  6711-lsu  AAAGTTGAGA TCTCTGTCGT GGAGAGCATT GACGCCCAGA CCAGACCTTT
    u66435  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA CCAGACCTTT
8265-lsu-m  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCAGACCTTC
4319-lsu-m  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCAGACCTTT
  af042638  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCTGATCTTT
  af042636  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCTGACCTTC
  af042637  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCAGACCTTC
  af042606  AAAGTTGAGA TCCCTGTCGT GGGGAGCATC GACGCCCGGA GCAGACCTTT

            751                                                  800
  6170-lsu  TGTGACGGAT CCGCGGTAGA GCGTGTATGT TGGGACCCGA AAGATGGTGA
  6881-lsu  TGTGACGGAT CTGCGGTAGA GCGTGTATGT TGGGACCCGA AAGATGGTGA
  5644-lsu  TGTGACGGAT CCGCGGTAGA GCGTGTATGT TGGGACCCGA AAGATGGTGA
  6662-lsu  TGTGACGGAC CTGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  af042604  TGTGACGGAC CTGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  cbs39150  TGTGACGGAC CTGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  6711-lsu  TGTGACGGAC CTGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
    u66435  TGTGACGGAT CCGCGGTTGA GCATGTATGT TGGGACCCGA AAGATGGTGA
8265-lsu-m  TGTGACGCAT CCGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
4319-lsu-m  TGTGACGCAT CCGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  af042638  TGTGA..NAT CCGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  af042636  TGTGACGCAT CCGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  af042637  TGTGA..NAT CCGCGGTAGA GCATGTATGT TGGGACCCGA AAGATGGTGA
  af042606  TGTGACGCAT CCGCGGTAGA ACATGTATGT TGGGACCCGA AAGATGGTGA

            801                                          840
  6170-lsu  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  6881-lsu  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  5644-lsu  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  6662-lsu  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  af042604  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  cbs39150  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  6711-lsu  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
    u66435  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
8265-lsu-m  ACTATGCCTG AATAGGGCGA AGCCAGAGGA AACTCTGGTG
4319-lsu-m  ACTATGCCTG AATAGGGCGA AGCCAGAGGA AACTCTGGTG
  af042638  ACTATGCCTG AATA.GGCGA AGCCAGAGGA AACTCTGGTG
  af042636  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  af042637  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
  af042606  ACTATGCCTG AATAGGGTGA AGCCAGAGGA AACTCTGGTG
```

Note: Sequences were aligned based on homology. Intons of collections 4319 (*P. stypticus*) and 8265 (*D. pusillus*) were cut off.

Appendix 3.12. A multisequence alignment of the Group I intron

3541-inton:	*Panellus stypticus*	LSU	New South Wales, Australia	Length: 475
4319-lsu-i:	*Panellus stypticus*	LSU	Caucasia, Russia	Length: 475
8265-lsu-i:	*Dictyopanus pusillus*	LSU	Florida, USA	Length: 475
psu59090-i:	*Panellus stypticus*	SSU	(Hibbett, 1996)	Length: 475

```
            1                                                        50
3541-intron ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
4319-lsu-i  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
8265-lsu-i  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
psu59090-i  AAACTTGTTA GCCACAGTTA ACTCTGCACT AAAAAGCAGC CCTAACGGGT

            51                                                      100
3541-intron ~~~~~~~~TC GAACCATTAC TTGGTGGTTA ATAGG.CGGT TGAAAGACCT
4319-lsu-i  ~~~~~~~~~T GGTGTGCTAC TTGGTGGTTA ATAGGCCGGT TGAAAGACCT
8265-lsu-i  ~~~~~~~~TT GTGTTGCTAC TTGGTGGTTG AGAGG.CAGT TGAAAGACTT
psu59090-i  GAGGTGGTTC AGCCCTAGAG TGCGCAATGA CTCTTAAATC TAAATGCTAG

            101                                                     150
3541-intron GGCCTAGTGA TTTGTTGAGG A.TTGCGACA CTGTCAAATT GCGGGGACGT
4319-lsu-i  AGCCTAGTGA TTTGTTGAGG A.TTGCGACA CTGTCAAATT GCGGGGACGT
8265-lsu-i  TGCCTAGTGA .GAGTTGAGG A.TTGCGACA CCGTCAAATT GCGGGGACGT
psu59090-i  TCTGTGCATC CTTGTGTTGC ACGGGCGAGA CCTTCAAACT GCGGGAAACT

            151                                                     200
3541-intron CCTTAGAGCC TTTTGCTACC GCCTC.TAT. GCTGAAAAGT ATAGCTGGCA
4319-lsu-i  CCTTAGAGCC TTTTGCTACC GCCTC.TAT. GCTGAAAAGT GTAGCTGGCA
8265-lsu-i  CCTTAGAGCC .TTTGCTACC GCCTG.TGC. ACTGAAAAGT GT.GCAGGCA
psu59090-i  CCTTAGAGC. TTTCACTACC GC.TGCTATC ACTGAAAAGT GTAGTCTGCA

            201                                                     250
3541-intron CCAGGGTAAT GACCTCGGGT ATGGTAATAA CGCAAAGGAT TGGATAATCC
4319-lsu-i  CCAGGGTAAT GACCTCGGGT ATGGTAATAA CGCAAAGGAT TGGATAATCC
8265-lsu-i  CCAGGGTAAT GACCTCGGGT ATGGTAATAA TGCAAAGGAT TGGATAATCC
psu59090-i  CCAGGGTAAT GACCTCGGGT ATGGTAATAA CGTGAAAGAT TGGACAATCC

            251                                                     300
3541-intron GCAGCCAAGT .CCTAACGGT CGTAGCTGAC TTATGGATGC AGTTCACAGA
4319-lsu-i  GCAGCCAAGT .CCTAACGGT CGTAGCTGAC TTATGGATGC AGTTCACAGA
8265-lsu-i  GCAGCCAAGT .CCTAAGGGT AGTCAACGAC CTATGGATGC AGTTCACAGA
psu59090-i  GCAGCCAAGT CCCTAAAGGT AGTTATTGAC CTACGGGAAA GGTTCAGAGA

            301                                                     350
3541-intron CTAAATGGCA GTGGGTGTTG CTT..... AATGCTTAAG ATATAGTCGG
4319-lsu-i  CTAAATGGCA GTGGGTGTTG CTTCAATAGC AATGCTTAAG ATATAGTCGG
8265-lsu-i  CTAAATGGCG GTGGGTGTTG .TTGAA.AAC AATGCTTAAG ATATAGTCGG
psu59090-i  CTAGATGGAG GTTGGT.... ..CAACTAAG TTGGCTTAAG GTATAGTCCG
```

Appendix 3.12. (Continued) A multisequence alignment of the Group I intron

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            351                                                    400
3541-intron TCCCGTCGCG AGAGCGAACT GGCAGATGAA GGCTTTGTTG TCACCTCAAC
4319-lsu-i  TCCCGTCGTG AAAGCGAACT GGCAGATGAA GGCTTTGTTG TCACCTCAAC
8265-lsu-i  TCCCGTCGCG AGAGCGAACT GGCAGATGAA GGC.TTGCTG TCTCCTCAAC
psu59090-i  TCTCTGCATG AAAGTGCAAG AGGTTTGAAA TG~~~~~~~~ ~~~~~~~~~~

            401                                                    450
3541-intron TA.TAATGAC ACTGGTCTGG AGCTGCTGGC AAGTCTGTTA AGTCAGACTT
4319-lsu-i  TA.TAATGAC ACTGGTCTGG AGCTGCTGG. AAGTCTGTTA AGTCAGACTT
8265-lsu-i  TTTTAAGGAC GCTTGCCTGG AGCTGCTGGG AGGTCATTTA AAAAGGCCTT
psu59090-i  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

            451                    475
3541-intron GGGGTAAAAT GCTA.TAGCT GTTCC
4319-lsu-i  GGGGTAAAAT GCTAGTAGCT GTTC~
8265-lsu-i  GGGGTAGAAT GCTA.TAG.T GTTCC
psu59090-i  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~
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VITA

Jiankang Jin was born on December 12, 1964 on Xishan Island, Wu County, Suzhou City, Jiangsu Province, China. He received his elementary and middle school education in four schools on the island. In September 1982, he entered Nanjing Agricultural University with major in horticulture for higher education. In July 1986, he graduated from the university with Bachelor's degree. In the same year, he was admitted into an intercollegiate program between Jiangsu Provincial Academy of Agricultural Sciences and Nanjing Agricultural University with a research specialty in edible mushrooms under the guidance of professor Helin Xu. He graduated in July 1989 with a Master of Agronomy from Nanjing Agricultural University.

Concurrently, he worked in the provincial Academy as a research assistant until in 1992 with the title assistant research fellow.

In 1991, he was Project Leader for a 3- year biotechnology project, "Application of cell engineering techniques in edible mushrooms" which was granted by the Biotechnology Committee of the Academy.

In May 1993, he went to Chile as a technical advisor to explore the feasibility of "Mycelial growth and mushroom cultivation," an international project between the Chilean Foreign Ministry and the China National Science and Technology Commission. He returned to China in December 1993.

In January 1994, he was appointed as the chief of the research group of edible fungi in the Institute of Vegetable Crops of the Academy.

In 1995, he won the award " Cross-century talented young researcher" of the Jiangsu Provincial Academy of Agricultural Sciences System.

During his ten years' research in the Academy, he published more than 20 academic and technical papers in edible fungi research, participated in writing of the book "Shiitake mushrooms in China," and won 4 research awards for research participation.

In August 1996, he was admitted into Ph.D program in The University of Tennessee, doing research in fungal systematics under the guidance of Dr. Karen W. Hughes and Dr. Ronald H. Petersen. His dissertation was entitled "Studies in *Panellus stypticus* and the genus *Panellus* complex". During his graduate study, he won the Sarif Fellowship, awarded by the University of Tennessee on the basis of research excellence.