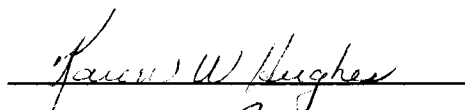
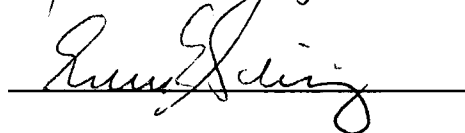


To the Graduate Council:

I am submitting herewith a thesis written by David B. G. Nicholl entitled, "Relationships with the *Pleurotus djamor* species complex." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.


Ronald H. Petersen, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Associate Vice Chancellor and Dean
of the Graduate School

RELATIONSHIPS WITHIN THE *PLEUROTUS DJAMOR* SPECIES COMPLEX

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

David B. G. Nicholl
May 1997

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ABSTRACT

Pleurotus djamor (Fr.) Boedijn is a widespread tropical to subtropical species complex. This complex has been given many names based primarily on the color of the pileus and geographic origin of the collection. Three distinct basidiome morphologies have been described: 1) off-white, tan to pallid olive; 2) pink; 3) gray fibrillose. Collections representing these forms fruited in the laboratory indicate that these forms overlap even within a single collection. Inter-collection crosses were performed to determine sexual compatibility (= dikaryotization) between collections. Results indicate universal compatibility between all morphotypes. Fruiting of these hybrid dikaryons was successful, indicating universal interfertility among these forms over diverse geographic locations. Macro- and micro- morphological characters were examined and phenetic trees of relationships within this group were created. No clear and consistent phenetic differences were discovered among collections by geography or color morph indicating a high degree of morphological overlap. Based on these criteria all color morphs should be considered to be part of a single taxon *P. djamor*.

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CHAPTER I

INTRODUCTION

Pleurotus djamor (Fr.) Boedijn is a widespread, tropical to subtropical species complex. The taxonomy of this species complex is confused with as many different schemes as authors. Relationships within the group have been primarily determined using morphological characteristics (Corner, 1981; Pegler, 1977; Singer, 1986; and Guzman *et al.*, 1992). Recent studies, however, have incorporated intercompatibility tests as additional criteria for species delimitation (Petersen, 1992; Petersen and Hughes 1993; Vilgalys and Sun, 1994; Petersen, 1995a.).

Taxonomy

Pleurotus djamor was originally described in *Herbarium Amboinense* under the name *Boletus secundus arboreus* (Rumphius, 1750). Rumphius described the species as growing from trees, with a circular-oblong to liver shape and short stalk or sometimes sessile. He also noted that caps had overlapping lobes and displayed red coloration when young, fading to white, dull-yellow, or yellowish-tan with age. The species epithet was sanctioned by Fries (1821) as *Agaricus djamor* in the tribe *Pleurotus*, subtribe *Concharia*. In 1836, Fries transferred the species epithet to the genus *Lentinus*.

Many authors have subsequently redescribed and renamed material of this species as new from different locations, but examination of this material by later authors (Pegler, 1986) has established that some of these names are superfluous. The following names have been assigned to this group by Berkeley and Broome (1887): *Agaricus flabellatus*, *A. scytocephalus*, *A. leptogramme* all described from Ceylon, the latter two species being

separated based on smaller spore size. Reexamination by Petch (1917) and Pegler (1986), however, revealed no differences. These three "species" were transferred to *Pleurotus* by Saccardo in 1887.

This species was again described as new by Petch and given the name *Cladopus fuscolamellatus*. He described the spores as oval, salmon-pink, $4.5 \times 2.3 \mu\text{m}$, and noted that the gills blackened upon drying. Later reevaluation of this fungus (Pegler, 1986) revealed these spores to be *Aspergillus* conidia, which may also account for the strange black color observed on the gills.

Other names equated with *P. djamor* by Pegler (1986) include *Crepidopus djamor* by van Overeem (1917) in India, *Lentinus goossensiae* and *Claudopus goossensiae* by Beeli in Goossens (1928). Boedijn combined the name *djamor* with *Pleurotus* in 1959 because *djamor* had priority over other later names such as *flabellatus*, as well as this being its correct generic placement.

Modern treatments of this complex leave the picture no less confused. In 1961, Singer described a new species from northern Brazil which he named *P. ostreatoroseus*. The pileus was described as whitish to sordid buff or grayish becoming pinkish when dried, with gills colored whitish to pink. The stipe was described as lateral and short to absent. Spores were described as cylindrical, smooth, thin-walled, inamyloid, measuring $7.5\text{--}10 \times 3\text{--}4 \mu\text{m}$. Basidia were listed as $15\text{--}20 \times 5\text{--}5.5 \mu\text{m}$, while cheilocystidia measured $22\text{--}40 \times 7\text{--}9 \mu\text{m}$, clavate with a mucro or subcapitate. He also noted thick-walled hyphae in the irregular hymenophore trama possibly indicating the presence of skeletal hyphae and hence a dimitic hyphal system. Another pink *Pleurotus* from the northern Far East

was also described as a new species and given the name *P. salmoneostramineus* Vas. (Vassileva, 1973). This name has been used for pink oyster mushrooms from the eastern tropics and Japan. Recent studies indicate this "species" is intercompatible with white *P. djamor* (Neda *et al.*, 1988).

Pegler (1977) described this species from East Africa under the name *P. flabellatus*, noting a pink tint in young caps, becoming white to ivory by maturity, with a finely striate margin. Caps were reniform to flabelliform with stipe absent, lateral or eccentric, white finely tomentose to glabrescent. In this description the context is mentioned as being monomitic. Spores were listed as 6-8.3 x 3-4 μm , oblong cylindric, thin walled and smooth. Cheilocystidia measured 19-35 x 5-9.5 μm , clavate to cylindric sometimes attenuated into a mucronate outgrowth at the top.

Corner (1981) reported *P. djamor* as a morphologically variable species. Color, micromorphology and habit were used to subdivide *P. djamor* into six varieties. Intermediates among these varieties were also noted. Basidia were 20-25 x 5-6 μm with four sterigmata. Cheilocystidia were clavate to subventricose, occasionally mucronate or capitate measuring 20-40 x 6-11 (-13) μm . He described the species as monomitic when young, becoming dimitic in age with numerous skeletal hyphae.

Corner's (1981) key to the varieties of *P. djamor*

1. Gills pink. Spores 7-10 x 3-4 μm 2
1. Gills white to pallid ochraceous (rarely tinged pink); sp. 5.5-8.7 x 2.5-4.5 μm 3
 2. Pileus pink var. *roseus*
 2. Pileus fuscous livid.....var. *fuscoroseus*

3. Pileus fuscous or fuliginous pruinose. Gill-edge often umber-pruinose; sp. 6-7.8 x 3.3-4.5 μm var. *fuscopruinosus*
3. Pileus white to pallid ochraceous. Gill-edge not darker.....4
4. Pileus with eccentric stem 3-4 cm x 7-8 mm, cyathiform; sp. 6.5- 7.5 x 3.8-4.5 μm var. *cyathiformis*
4. Pileus nearly or quite lateral, imbricate; stem short.....5
5. Terricolous, merismatoid; sp. 5.5-6.5 x 2.5-3 μm var. *terricolus*
5. Lignicolous, not merismatoid; sp. 6-8.7 x 3-4 μm var. *djamor*

Pegler (1986), in contrast to Pegler (1977), referred to this species as *P. djamor* and concurred with Corner (1981) that some specimens were dimitic. In addition, he included the possibility of pinkish and grayish tints in some caps. Spores were cylindric, hyaline, thin-walled, 6-9 x 3-3.7 μm . Basidia were narrowly clavate, four sterigmate, 14-20 x 5-6 μm . Cheilocystidia ranged from clavate to some with mucronate tip, and 16-23 x 6-10 μm .

Guzman *et al.* (1992) divided this species complex into separate species. In this taxonomic scheme *P. djamor* was equated to Corner's (1981) *P. djamor* var. *djamor* and var. *roseus*. In addition Corner's (1981) *P. djamor* var. *fuscoroseus* was considered equivalent to *P. ostreatoroseus* Sing. from Brazil and Mexico and with *P. salmoneostramineus* Vas. from the northern Far East. Guzman described *P. djamor* as spathulate, concave, reniform, flabelliform to subinfundibuliform with pink tones when young to whitish, yellowish, light grey to light brown with gills white to yellowish.

Spores were (6.4-) 7.2-9.6 (-10.4) x (3.2-) 4-4.8 μm , oblong-elliptic to subcylindric. Cheilocystidia were extremely variable, ranging from subcylindric, subclaviform, subventricose to subspheropendunculate sometimes with irregular constrictions and sometimes with mucro or capitate with or without mucilaginous halo. The context was monomitric when young becoming dimittic as the skeletal hyphae arise from generative hyphae in mature basidiomes. He was the only author to mention the presence of pseudocystidia. Pseudocystidia were shaped subventricose to submucronate, arising from the subhymenium and extending through the hymenium (Guzman *et al.* 1992).

Singer (1986) referred to *P. djamor* by the name *P. flabellatus* (Berk. & Br.) Sacc. These two names appear to be synonymous in most of the literature (Corner, 1981; Pegler, 1986; Guzman *et al.*, 1992). Singer (1986), however, stated that *P. flabellatus* was monomitric where Corner (1981) and Guzman *et al.* (1992) reported that *P. djamor* was dimittic. Most authors (Corner 1981, Singer 1986, and Guzman *et al.* 1992) have considered *P. opuntiae* (Dur. & Lev.) Sacc. to be a separate species based on its smaller spores and absence of cheilocystidia. Pegler (1977), however, separated *P. opuntiae* from *P. flabellatus* on the basis of the former having larger spores and lecythiform cheilocystidia.

Recently an oyster mushroom was found in New Zealand that had a gray fibrillose pileus and was identified as *P. opuntiae* because of its similarity to descriptions of that species (Reese *et al.*, 1990; Petersen, 1995a). However, it was determined to be part of the *P. djamor* species complex by intercompatibility tests (Petersen, 1995a).

Species Concepts

To unravel the taxonomy of this species complex it is necessary to understand the conceptual basis used by these authors to delimit taxa. Although rarely stated, most of these authors used a morphological species concept. This concept bases species definition and delineation on comparative morphology of critical characters (van Steenis, 1957). A critical character may be any attribute that can be qualified, quantified or assessed in some manner. Since there are a large number of possible characters it behooves the taxonomist to select characters with high information content. The selection of characters is based on current information and hypotheses about the taxa, but tradition and intuition also play a role (Kuyper, 1988). Thus the selection of characters and the importance attached to them can lead to divergent opinions on the true status of a "species". A morphological species is based on the morphological structures of a type specimen, to which the name is attached. This Linnean view of species has dominated mycological taxonomy (Smith, 1968). The 20th Century, however, has seen the proliferation of other species concepts which are more Darwinian in nature.

The first such concept espoused by Mayr (1942) is called the biological species concept (BSC). This concept separated species based on the ability of organisms to interbreed. Thus a species is a group of organisms that share a common gene pool and are separate from other gene pools. Mayr (1982) amended his theory to include an ecological aspect in which a species is "...a reproductive community of populations (reproductively isolated from others) that occupies a specific niche in nature." This theory incorporated emerging knowledge about population genetics into the notion of a

species (Sluys, 1991). Testing the biological species concept has led to the discovery of cryptic species. These are species that are morphologically inseparable, but are reproductively isolated from each other and thus have separate evolutionary fates (Stebbins, 1950; Wiley, 1981; Grant, 1981; Paris *et al.*, 1989).

The biological species concept has been critiqued on theoretical and empirical grounds by many authors (Donoghue, 1985; Wiley, 1978; Cronquist, 1988; Cracraft, 1989; Templeton, 1989). One weakness is that the concept completely ignores asexual taxa (Budd and Mishler, 1990; Wiley, 1978). In addition, there are many distinct species, such as members of *Quercus*, which often hybridize in nature. This would result in placing these separate taxa together in an unnatural species if the BSC was followed (van Valen, 1976). Self-pollinating plants also pose a dilemma for this concept, for each individual plant would have to be considered a separate species since each one is genetically isolated (Cronquist, 1988). The biological species concept has also been attacked as being non-operational. The number of crosses necessary to determine which individuals are interfertile is astronomical, and even if completed are inconclusive because natural conditions cannot be duplicated in the laboratory. It is hard to know the extent of interbreeding among populations, other than to look for morphological discontinuities, although morphological changes may not accompany reproductive isolation (Sluys, 1991; Cronquist, 1988; Nixon and Wheeler, 1990). Applying this concept in the time dimension demonstrates its non-dimensionality, because all sexual organisms are linked through ancestral populations making it difficult to determine the beginning and end of a species (Nixon and Wheeler, 1990; Sluys, 1991).

The temporal and spatial weaknesses of the BSC and the apparent subjective nature of traditional morphological species concepts led to the introduction of other species concepts. One such concept proposed by Simpson (1961) and later resurrected and defended by Wiley (1978) is called the Evolutionary Species Concept. According to this concept "a species is single lineage of ancestor-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate (Wiley, 1978)." This concept's strength is that it includes both extinct and extant taxa as well as sexual and asexual taxa. Thus under this regime a species is held together not only by gene flow but also by ecology, ontogeny, and genetics. However, there are problems when applying this concept, for instance how much diversity is allowed in a "single lineage" with a common evolutionary fate. In addition, this not an operational definition since it does not specify which traits should be used to define a species (Templeton, 1989).

The phylogenetic species concept has also been proposed as a solution to the problems of the BSC. This species concept emphasizes the idea that species are real evolutionary biological entities, not merely human constructs. Therefore "species are speciated" and must not be able to be reduced to smaller units that fulfill that same role. Under this theory species are defined as "an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a paternal pattern of ancestry and descent" (Cracraft, 1983). Implicit in this definition is the idea that each species must be monophyletic. There is debate as to whether species must be monophyletic and to whether the concept of monophyly is applicable below the species

level (Donoghue, 1985; Cracraft, 1989; Nixon and Wheeler, 1990). Sluys (1991) argued that species are not interactors in the evolutionary process but are its results. Therefore, he saw species as a resultant pattern of evolutionary process, not a "functioning unit in processes." In theory he agreed with Cracraft that species should have apomorphic characters, but empirically they are rare at the species level. This however does not make species less real merely because species (monophyletic taxa) are recognized by apomorphic characters, not defined by them (Sluys 1991).

Another concept that tries to address the weaknesses of the traditional morphological species concept and the BSC is the phenetic species concept (though not specifically given this name by its adherents). The field in which this species concept grew is called numerical taxonomy and was first proposed by Michel Adanson (1763), and later reborn by Sneath and Sokal (1963, 1973). In this concept, species are grouped together by a large number of characters (morphology, physiology, chemistry and DNA) each with an *a priori* equal weight, which are used to compute coefficients of phenetic similarity between individuals. These coefficients are then employed in an algorithm to produce a phenogram. The major advantages of this system is its repeatability and objectivity (Sneath and Sokal, 1973). This theory relies on the idea that organisms that are most closely related share the most overall similarity, although they admitted that relationships based on descent and those based on similarity may not always be congruent. They argued that current patterns are important in taxonomy, not when the resemblance originated nor how fast it has increased or decreased through time. In addition, the fossil record is so incomplete that most evolutionary scenarios are based on

phenetic relationships among extant organisms anyway. They also asserted that when fossils are available the phylogeny is to be based on phenetic similarity of the putative ancestor and descendent. To minimize the negative effects of possible non-congruence of phenetics and phylogeny they indicated that "phenetic clusters based on living organisms are more likely than not to be monophyletic sensu Henning" (Sokal and Sneath, 1973). One criticism of this concept is that the outcome often depends on what algorithm is used to construct the phenogram. In addition a large number of "meaningless" characters must be laboriously analyzed in the name of not *a priori* determining which characters should be used (i. e., giving all characters equal weight; Cronquist, 1988). There is also no objective way to determine how much phenetic divergence indicates differences at the species or any other taxonomic rank.

Objectives

Species delimitation in taxonomy is ambiguous when applying different criteria and with no *a priori* reason for assuming one is more accurate in determining true relationships within groups. There is great difficulty in finding and applying a species concept which is not only theoretically sound but practical for taxonomy. The best way to examine this ambiguity is from several perspectives. Therefore, I applied three of these concepts; morphological, phenetic, and biological, to this taxonomically difficult *P. djamor* species complex. Micro- and macromorphological comparisons, intercompatibility, and interfertility were examined for a more thorough examination of relationships between members of this species complex. Interfertility tests are rarely used for fungi because the conditions necessary to produce fruitbodies from the dikaryons of

most species are unknown (Boidin, 1986). In fact when two SSIs form a dikaryon (intercompatibility) the underlying assumption is that basidioma production, karyogamy, meiosis, spore germination, and normal growth could potentially occur given the right environment and substrate (Boidin, 1986). *Pleurotus djamor* is relatively easy to fruit in the laboratory and therefore affords an opportunity to substantiate these assumptions for collections paired in this study. Comparisons of the practicality and implications of each species concept are discussed. The specific objectives of this research were to determine the importance of coloration, geography, morphology, intercompatibility, and interfertility in dividing or unifying strains and variants as members of this species complex.

CHAPTER 2

MATERIALS AND METHODS

Mushroom & Culture Collection and Storage

Dikaryotic mycelia (dikaryons) and single spore isolates (monokaryons; SSIs) were established either from collections of mushrooms in nature or from spore prints made by international colleagues and mailed to the University of Tennessee. Mailed spore prints were made on site by placing a pileus over a piece of aluminum foil, wax paper or glass slide and allowing spores to fall on the surface. The method of establishing dikaryon cultures and SSIs depended upon the origin of the collection.

Field collected mushrooms were described in a field book, then dried, catalogued and stored in the herbarium at University of Tennessee (TENN; Holmgren *et al.*, 1981). Dikaryons were established in one of two ways from these "fresh" mushrooms: 1) A pileus or portion of a pileus was attached to the lid of a Petri plate with the lamellae facing downward. Malt extract agar (MEA) Petri plates were made with the following ingredients: 7.5 g bacto malt extract (Difco), 10 g bacto-agar (Difco), 500 ml distilled water. The plate was placed almost vertically against a support allowing spores to drop and spread across the agar surface. Spores germinated, overgrew each other, and anastomosed, creating a polyspore dikaryon. 2) A portion of a gill was removed with sterile forceps and placed on the agar surface in a Petri plate. Hyphae grew from the gill creating a dikaryotic mycelium. Monokaryotic mycelia resulted when single-spore germings were isolated with the aid of a dentist pick and dissecting microscope before

they could come into contact with each other.

Collections received as spore prints were manipulated in the following way. A sterile 1 ml pipet was used to place a small drop of sterile distilled water (sdw) on the spore print allowing spores to dislodge into the water. The drop with spores was then drawn back into the pipet. The spore suspension was placed in a test tube with 10 ml sdw. Serial dilutions were prepared, resulting in dilutions of 1.0, 0.1, 0.01 and 0.001. One ml of each dilution was transferred to Petri plates. Spores were allowed to settle to the agar surface for a short time after which excess water was decanted and the plates sealed. Dilution plates were examined daily for germination and/or contamination. Spores in the most concentrated dilutions were allowed to germinate, overgrow, and anastomose, creating a polyspore dikaryon. Germinated spores in the highest dilutions also germinated and grew for a few days. SSIs were isolated and transferred to individual plates before they overgrew each other in order to establish monokaryotic isolates for intra- and intercollection pairings.

These isolates (dikaryotic, SSI) were stored in one of two ways: 1) a plug of mycelium was made using a cork borer, and transferred to an agar (MEA) slant tube. The mycelium colonized the agar in the tube at room temperature and the tube was subsequently placed in cold storage at 5°C. 2) plugs of mycelium were made using a cork borer. These were transferred to a microvial filled with sdw and stored at room temperature.

Spawn

Dikaryon cultures were fruited under controlled laboratory conditions to reduce

environmental and host effects on morphological characters. A mixture of 200 g of rye grain, 200 ml of water and one gram of calcium sulfate were mixed in a one-quart Mason jar. The lid of the jar was punctured with an ice pick to make 15 to 20 holes for gas exchange. Lids were lined with cotton to prevent contamination. The jars were then autoclaved for one hour to sterilize the rye medium, and allowed to cool to room temperature in a laminar flow hood.

Dikaryotic mycelium was allowed to colonize an entire MEA Petri plate. The mycelium in the agar plates was cut into small squares (approx 20) and transferred to the Mason jars of rye medium. Latex gloves were used during these transfers to prevent contamination. Each jar was then shaken to evenly disperse the mycelia within the rye media for faster and homogeneous colonization. Inoculated jars were placed in a growth chamber in the dark at 75-80° F. After seven days, jars were shaken vigorously to break the mycelium and allow for recolonization ending on day 14.

Fruiting Substrate

A wheat straw substrate was created with the following ingredients: 140 g wheat straw (Agrifeed and Pet Supply), 140 g water, 12 g rice bran (EnerG Foods), 6 g of calcium sulfate, 1.5 g of calcium carbonate (modified from Stamets, 1991). These ingredients were mixed in a 12" x 24" polypropylene bag which was closed with a twist tie and autoclaved for one hour. The bags were allowed to cool to room temperature in a laminar flow hood. The mycelium-covered rye grain (spawn) was poured into the bags and the bags closed again. The bags were then shaken to disperse the spawn throughout the wheat straw substrate. These bags were placed on a lab table top. Temperature in this

room ranged from 72°-82° F, and fluorescent ceiling lights were on for approximately 8-10 hrs daily. Once fully colonized, the bags were placed under plant benches on gravel in a greenhouse. Temperature ranged from 70°-83° F, and relative humidity was maintained between 75-90%. The amount of light received was dependent upon the outside weather conditions.

Fruiting Initiation

Small circular holes (1-3 inches diam.) were cut in the bags to allow primordia formation and basidioma development. The amount of time to fruiting was highly variable with some collections fruiting within two da while others took up to six wks to begin. All collections went from primordia to mature basidioma in 3-7 da with rapid senescence after maturity.

Macromorphology

Photographs and morphological descriptions of each collection were made from primordia initiation through basidioma maturity. Photographs were taken with a Minolta SRT101 camera and 90MM (1:2.5) Vivitar Series 1 macro lens. Morphological descriptions and terminology were made following Kit Scates' *Easy Guide to Mushroom Descriptions*. Colors were classified according to Kernerup and Wanscher (1978). A spore print on aluminum foil was made of from basidiomata of each collection to be used for micromorphological spore measurements and description.

Micromorphology

Micromorphological terminology and sectioning techniques were applied according to Largent et. al., 1977. Dried mushrooms were reconstituted by briefly

soaking them in 95% ethanol (< 30 sec), then transferring them to water.

Micromorphology was assessed by sectioning mushrooms with a Shick double-edge razor blade and dry elderberry pith. Sections were stained with a 1% aqueous phloxine solution. A drop of this red dye was placed on a glass slide, the section of mushroom was added and allowed to soak up the dye. Excess dye was removed by blotting with a Kimwipe. A drop of 3% KOH was dropped on the section and a piece of cover glass was placed on top. The following areas of the basidioma were examined under a Nikon compound microscope with an ocular micrometer: pileipellis, context, hymenophoral trama, subhymenium, hymenium including basidia, basidiospores, cystidia, and pseudocystidia.

Phenetic Study

Macro- and micromorphological characters that varied in a quantitative or qualitative manner were used in a comparison of phenetic similarity among basidiomata from 20 selected collections. Qualitative characters were recorded as a 1 or 0 indicating presence or absence of the following characters: hygrophanous areas on the pileus, pseudocystidia (cystidioid elements), pleurocystidia, and various colors. Colors were grouped into nine classes with each collection given a 1 or 0 depending on whether or not it exhibited a color found in that class. Colors were classified as following: Class 1- white; Class 2- yellowish white, pale yellow and light yellow; Class 3- yellowish gray and grayish yellow; Class 4- brownish gray, grayish brown, yellowish brown, light brown, brown and brownish orange; Class 5- orange gray and grayish orange; Class 6- grayish red and reddish gray; Class 7- orange white and light orange; Class 8- pinkish white,

pastel red, pale red, pink; Class 9- dark brown.

The following quantitative characters were measured: basidial length, basidial width, cheilocystidial length, cheilocystidial width, spore length, and spore width. Quantitative variables were entered into a spread sheet (Excel) and a correlation matrix was created. No variables had a correlation coefficient of 0.7 or greater, indicating that all variables were independent enough to be used in the analysis. Each variable was tested for normality by creating box plots. Euclidean distances were calculated on a rectangular SYSTAT (1995) file of the following variables: basidial length, spore length, basidial width (transformed), basidial length (transformed), cheilocystidial length (transformed), and cheilocystidial width (transformed).

To include all characters in an analysis, quantitative characters were converted to character state classes in the following manner: Spore length $8.0-8.99\mu\text{m} = 0$, $9.0-9.99\mu\text{m} = 1$, $>10.0\mu\text{m} = 2$; Spore width $4.00-4.99\mu\text{m} = 0$, $>5.00\mu\text{m} = 1$; Basidia length $20.0-22.99\mu\text{m} = 0$, $23.0-24.99\mu\text{m} = 1$, $>25.0\mu\text{m} = 2$; Basidial width $5.0-5.99\mu\text{m} = 0$, $>6.0\mu\text{m} = 1$; Cheilocystidial length $18.0-19.99\mu\text{m} = 0$, $20.0-21.99 = 1$, $22.0-24.99\mu\text{m} = 2$; Cheilocystidial width $6.0-6.99 = 0$, $>7.0\mu\text{m} = 1$. These converted quantitative characters were then combined with the qualitative characters in a rectangular SYSTAT file.

All phenetic data were analyzed with a statistical program called SYSTAT (1995). Euclidean distances and Goodman Kruskal gamma coefficients (1954) were calculated and several algorithms were used to create phenetic trees displaying relationships among collections (Goodman and Kruskal, 1954). The algorithms used include average linkage (UPGMA), single linkage and complete linkage methods.

Reference Collections

Six reference collections were selected to represent the range of color morphs found in this complex as well as its diverse geographic distribution. Table 1 shows the geographic distribution of collections examined in this study. The reference collections were: 1) 6346 pink morph from Malaysia; 2) 7444 gray morph from New Zealand; 3) TMI 31760 white morph from Japan; 4) IBUG 7₁ pink morph from Mexico; 5) 7839 gray morph from Costa Rica; and 6) 3467 white morph from Puerto Rico. Twelve SSIs were paired in all combinations to perform a self-cross for each of these collections (see Fig. 1). After sufficient growth, the contact zone between donor inoculum blocks was observed under a compound microscope for the presence or absence of clamp connections and other micromorphological characteristics. The SSIs were assigned mating types based on their reactions with other SSIs. One representative of each mating type was selected as a tester strain for intercollection pairings.

Intercompatibility

Intercollection intercompatibility was determined by the ability of paired isolates to produce clamp connections (i.e. form a dikaryon). The contact zone and both sides of the plate were examined for the presence or absence of clamp connections. Obverse sides of donor colonies are checked for clamps to determine if nuclear migration was unilateral, bilateral or confined to the contact zone.

In the event of senescence or contamination of one of the original tester strains, other SSIs from the collection were chosen to complete the intercollection pairings. Tester strains from all six reference collections were paired in all possible combinations,

Table 1. Putative *Pleurotus djamor* collections examined in intercompatibility test.

Collection #	Country	Infra	Color Morph*
6346	Malaysia	Unknown	Pink
4326	Mexico	Tabasco	White
4331	Mexico	Tabasco	White
4375	Mexico	Veracruz	Gray
4381	Mexico	Veracruz	Gray
IBUG 53	Mexico	Jalapa	White
TMI 31760	Japan	Unknown	White
RDL1	Guatemala	Unknown	White
3467	USA	Puerto Rico	White
441A	USA	Florida	White
7167	New Zealand	North Island	Gray
7444	New Zealand	North Island	Gray
6241	Mexico	Tlaxcala	Gray
6270	Mexico	Veracruz	Gray
6275	Mexico	Veracruz	Gray
6278	USA	Florida	Gray
ET 3313	Mexico	Tlaxcala	Gray
PDD 88115	New Zealand	North Island	Gray
IBUG 7₁	Mexico	Jalapa	Pink
IBUG 39	Mexico	Jalapa	Pink
JAPAN	Japan	Unknown	Pink
TMI 30258	Japan	Okayama	Pink
MCADOO	Belize	Toledo	White

Table 1 continued

Collection#	Country	Infra	Color Morph*
6276	Mexico	Tlaxcala	Gray
6277	Mexico	Tlaxcala	Gray
IBUG 7 ₂	Mexico	Unknown	Pink
IBUG 3	Mexico	Unknown	White
K7018	Venezuela	Aragua	White
7839	Costa Rica	San Isidro	Gray
7840	Costa Rica	San Vito	Gray
ASM 7021	USA	Texas	Pink
VR 1786	Mexico	Unknown	Gray
MF 1-18	Mexico	Unknown	White
8013	USA	Florida	Gray
7826	Costa Rica	San Jose	White

* Color morph is assigned according to collection data or color of basidiome when first fruited.

Reference collections are in *bold italics*.

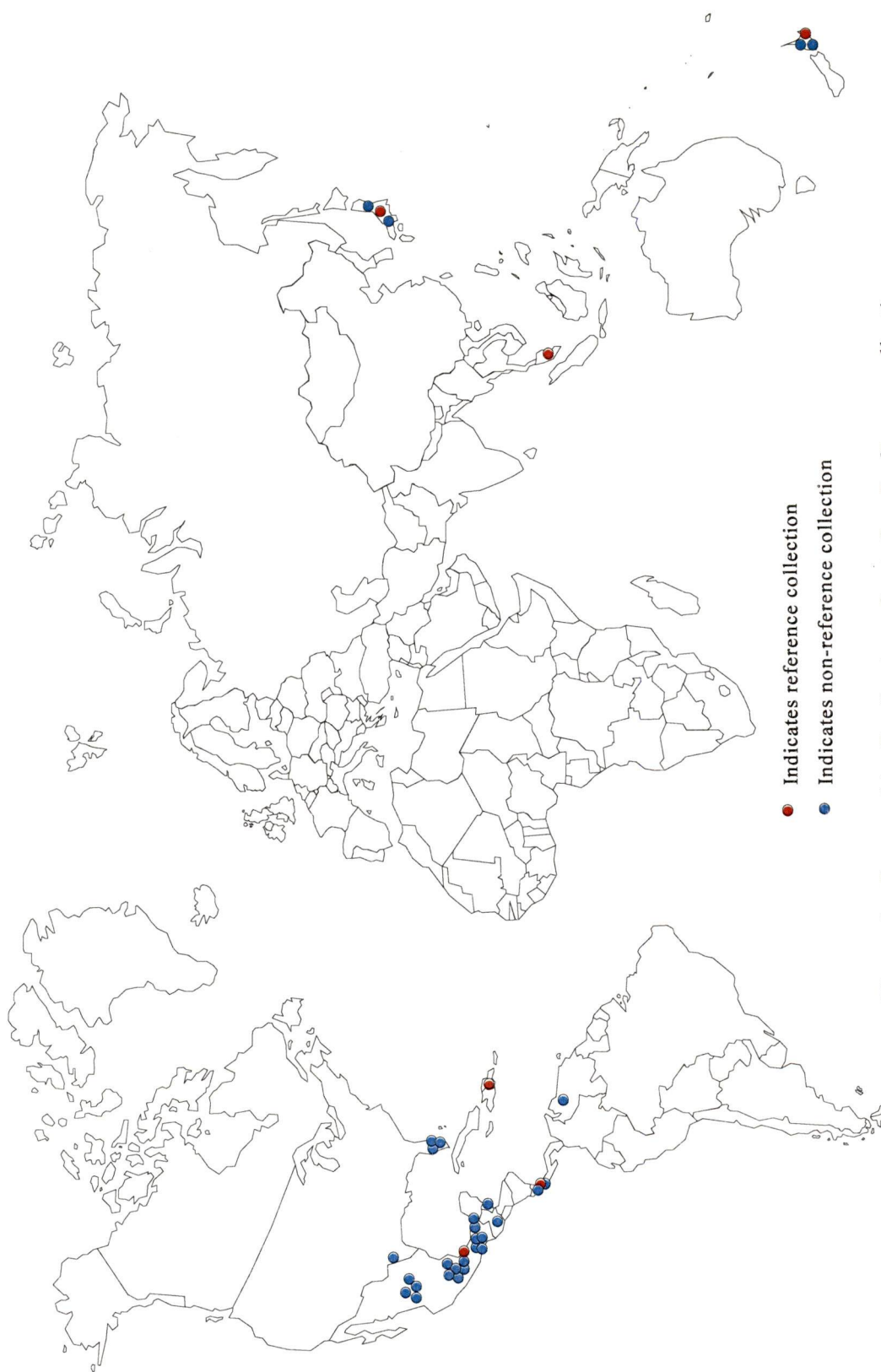


Figure 1. Geographic distribution of putative *P. djamor* collections

resulting in 16 pairings in each cross between reference collections. Twenty-nine non-reference collections were paired with one of the reference collections in a 2 x 2 cross in order to access their placement in an intercompatibility group. Table 1 lists the collections used in these intercompatibility tests and their geographic origin.

Interfertility

Four SSIs of six reference collections were crossed in all combinations. Each of these crosses resulted in a minimum of 7 dikaryons and a maximum of 16. Four "healthy" dikaryons from each cross were isolated (15 crosses with 4 "hybrid" dikaryons each for a total of 60 dikaryons). An attempt was made to fruit each of these dikaryons using the fruiting method described previously in order to access interfertility. In addition, 20 single spores germlings were isolated, allowed to colonize a MEA Petri plate and checked for clamps. The spore characteristics for each hybrid are listed in parenthesis, following its pileus color description, as follows: (# isolated/ # grew/ # monokaryotic).

Interfertility was determined by the ability of a dikaryon to produce an F_2 "hybrid" basidioma with viable spores. Four F_2 dikaryons from each reference collection pairing (16 possible dikaryons) were selected in order to evaluate the repeatability of inheritance of color morphology.

F_2 dikaryons were isolated by cutting two 2.5 cm² blocks from the contact zone. Each was transferred to a new MEA plate and allowed to colonize the plate. One of each pair of plates was used to make agar plugs as described previously for long-term water storage of the F_2 dikaryons, while the other plate was used to begin the fruiting process.

Basidioma macromorphological descriptions were made using terminology by Scates, 1982, while colors were assigned according to Komerup and Wanscher, 1983. A piece of the cap was used to isolate single spores as described above. Basidiospores were then monitored for germination over the next five days. Twenty individual single-spore germings from each "hybrid" were isolated with a dentist pick using a dissecting microscope. Each was transferred to its own Petri plate, examined for clamps (to insure monokaryotic isolates) and put into the culture collection for future research.

CHAPTER 3

RESULTS

Macromorphology

Of attempts to fruit 39 putative *P. djamor* collections, 33 were successfully fruited. All collections that did not fruit were attempted at least twice. Basidioma color was extremely variable among and even within collections. The three distinct pileus color morphs described in the *P. djamor* complex (Petersen, 1995a) [1. Off-white, tan to pallid olive (Kornerup and Wanscher 1978 terminology could include, yellowish white, light yellow, yellowish gray, grayish yellow, orange gray, grayish orange); 2. pink (pinkish-white, pastel red, pale red and pink); and 3. gray (brownish gray, grayish brown, brownish orange, and orange gray)], did not maintain their distinctiveness when fruited under standard conditions. Although some basidiomata did fit within one of these color morphs, others exhibited colors spanning two or even all three color morphs.

Fruited basidiomata were grouped according to the color ranges they displayed. Each member of a group exhibited most of the colors, but not necessarily all of the colors listed for that group. These groups overlapped and therefore were not discrete. Groups were as follows (See Table 2): 1) white, yellowish white (4A2, 4A3), yellowish gray (4B2), to grayish yellow (4B3): K-7018, TMI 31760, RDL-1, IBUG 53, and IBUG 3; 2) white, yellowish white (4A2, 4A3), light yellow (4A4), yellowish gray (4B2), grayish yellow (4B3), to orange gray (5B2), grayish orange (5B3, 5B4): 4326, 4375, 3467, MCADOO, and 6275; 3) white, yellowish white (3A2, 4A2, 4A3), yellowish gray (3B2, 4B2), grayish yellow (4B3, 4B4), yellowish brown (5D4, 5E4, 5F4), grayish brown (5D3,

Table 2. Colors exhibited by collections of *Pleurotus djamor*.

Group	Collections	Colors exhibited
1	K7018, TMI 31760, RDL1, IBUG 53, IBUG 3	white, yellowish white (4A2, 4A3), yellowish gray (4B2), grayish yellow (4B3)
2	4326, 4375, 3467, MCADOO 6275	white, yellowish white (4A2, 4A3), light yellow (4A4), yellowish gray (4B2), grayish yellow (4B3), orange gray (5B2), grayish orange (5B3, 5B4)
3	4331, 7839, 6278, 6270, 4381, 8013, 441A, 7840, VR 1786	white, yellowish white (4A2, 4A3), yellowish gray (3B2, 4B2), grayish yellow (4B3, 4B4), yellowish brown (5D4, 5E4, 5F4), grayish brown (5D3, 5E3), grayish orange (5B4, 5B3), brownish orange (5C3, 5C4), orange gray (5B2), orange white (5A2), pale orange (5A3), brownish gray(4C2)
4	PDD 88115, 7167, 7444	yellowish white (4A2), orange white (5A2), orange gray (5B2, 6B2), grayish orange (5B3, 5B4, 5B5), brownish orange (5C3, 5C4, 6C3, 6C4, 6C5), brownish gray (5C2), brown (6D4, 6D5, 6E4), grayish brown (5D3, 6E3, 7E3, 7F3) to dark brown (6F6, 6F5, 6F4, 7F4)
5	TMI 30258, JAPAN	grayish orange (6B3, 6B4), orange white (5A2, 6A2), pale orange (6A3), yellowish brown (5E4, 5F4), brownish orange (6C4, 6C5, 7C4), grayish red (7B3, 8B3, 8C3, 8C4), pinkish (7A2, 8A2, 11A2), pale red (10A3), pink (11A3)
6	IBUG 7 ₁ , IBUG 7 ₂ , IBUG 39, MF1-18, ASM 7021, 6346	white, yellowish white (4A2), yellowish gray (4B2), grayish brown (5F3, 6D3, 6E3, 7D3), light brown (6D4), brown (6E4, 7E4), brownish orange (6C3, 6C5), grayish orange (5B3, 6B4), orange gray (5B2), grayish red (7B5, 8B3), pastel red (7A4), pinkish white (7A2-11A2), pink (11A3)

5E3), grayish orange (5B4, 5B3), brownish orange (5C3, 5C4), orange gray (5B2), orange white (5A2), pale orange (5A3), and brownish gray (5C2): 4331, 7839, 6278, 6270, 4381, 8013, 441A, 7840 and VR 1786; 4) yellowish white (4A2), orange white (5A2), orange gray (5B2, 6B2), grayish orange (5B3, 5B4, 5B5), brownish orange (5C3, 5C4, 6C3, 6C4, 6C5), brownish gray (5C2), brown (6D4, 6D5, 6E4), grayish brown (5D3, 6E3, 7E3, 7F3) to dark brown (6F6, 6F5, 6F4, 7F4): PDD 88-115, 7167 and 7444; 5) grayish orange (6B3, 6B4), orange white (5A2, 6A2), pale orange (6A3), yellowish brown (5E4, 5F4), brownish orange (6C4, 6C5, 7C4), grayish red (7B3, 8B3, 8C3, 8C4), pinkish (7A2, 8A2, 11A2), pale red (10A3) and pink (11A3): TMI 30258, and JAPAN; and 6) white, yellowish white (4A2), yellowish gray (4B2), grayish brown (5F3, 6D3, 6E3, 7D3, 7E3), light brown (6D4), brown (6E4, 7E4), brownish orange (6C3, 6C5, 7C3), grayish orange (5B3, 6B4), orange gray (5B2), grayish red (7B5, 8B3), reddish gray (8B2), orange white (5A2), pastel red (7A4), pinkish white (7A2, 8A2, 9A2, 10A2, 11A2), pale red (7A3, 8A3, 9A3, 10A3), to pink (11A3): IBUG 7₁, IBUG 7₂, IBUG 39, MF 1-18, ASM 7021 and 6346. Many colors were observed that were not covered by any of the three morphotypes or that spanned across two or three “morphotypes”. Basidiomata of collection 6346, for example, were pink in the primordial stage but faded through orange-white becoming yellow-white to off-white by maturity. However, later fruiting flushes from the same bag remained pink through all stages. Basidiomata of another collection (ASM 7021) in the primordial and young stage were pink, pale red with brownish gray to grayish brown pubescence, fading to orange white, yellow-white with orange-grey pubescence. This collection could potentially be assigned to any of the three color

morphs. Basidiomata of other collections, such as members of group four, were dark brown in the primordial and young cap stage, a color not covered by any of the original morphotype groupings. Some colors exhibited by these basidiomata fell between two color morphs such as reddish gray between pink and gray morphs or orange-gray between tan and gray morphs.

The following morphological description covers all fruited basidiomata. Pileus flabelliform, spatulate, petaloid, concave, reniform to infundibulliform; surface smooth, fibrillose to tomentose over stipe area; margin opaque (PDD 88115, 7444, 7167) to hygrophanous, striate when moist, even when young becoming undulate-lobed to crenate in age. Lamellae decurrent to subdecurrent, unequal, occasionally anastomosing at base, thin, broad becoming narrower at margin, entire, white to yellowish-white (4A2, 4A3), light yellow (4A4), yellowish gray (4B2), orange white (5A2, 6A2), pale orange (6A3), grayish orange (5B6), pinkish white (7A2, 8A2, 9A2, 10A2, 11A2), pale red (7A3, 8A3, 9A3, 10A3), pastel red(8A4), or grayish red(7B3). Stipe absent or short, 5-20 mm long, eccentric or lateral, equal to tapering downward, smooth, pubescent to tomentose, usually white to yellowish white (4A2, 4A3), occasionally orange white (5A2), pinkish white (8A2, 9A2, 10A2), pale red (8A3), to grayish red (9B3). Spore prints yellowish white (4A2, 4A3), yellowish gray (3B2,4B2), grayish yellow (4B3), grayish beige (4C2), orange gray (5B2), grayish orange (5B3), pale orange(6A3), orange white (5A2,6A2), pinkish white (8A2, 9A2), pale red (8A3), reddish gray (9B3), to grayish red (8B3). Gill, stipe and spore color closely mirrored pileus color (i.e. a pink stipe, gills and spore print only occurred with a pink pileus).

Micromorphology

The following micromorphological description covers all fruited basidiomata.

Pileipellis: thin epicutis of generative hyphae; hyphae slender to somewhat inflated, 3.4-8.3 μm diam, thin- to somewhat thick-walled (wall 0.4-1.1 μm), occasionally branched, clamped, repent to suberect, often forming fascicles.

Pileus context: monomitic when young becoming dimitic with age; generative hyphae branched, septate with prominent clamp connections, 3.6-13.7 μm diam, thin to thick walled (wall 0.4-1.1 μm thick); skeletal hyphae 2.9-6.8 μm diam, thick-walled (wall 1.1-2.9 μm) creating a narrow lumen, not septate, rarely branched, often twisted, terminally attenuate.

Hymenophoral trama: monomitic at first soon becoming dimitic, irregular, predominated by skeletal hyphae (2.8-6.8 μm diam; wall 0.7-2.5 μm thick), non-septate, unbranched; generative hyphae 4.0-10.8 μm diam, septate, branched, thin-walled (wall 0.4-1.1 μm thick).

Subhymenium: irregular globose cells (pseudoparenchyma), 3.6-16.2(-28.8) μm diam, with interwoven hyphae 5.0-8.3 μm diam, thick-walled (wall 1.1-2.9 μm), hyphae extended through the hymenium as pseudocystidia, subventricose, cylindro-clavate to cylindric, 24.12-104.4 μm long, one collection (PDD 88-115) with branched pseudocystidia.

Spores: 6.8-13.3 x 3.6-5.8 μm , hyaline, smooth, thin-walled, inamyloid, cylindric, oblong, with distinct hilar appendix.

Basidia: 16.2-32.0 x 4.7-7.9 μm , clavate, four-sterigmate, hyaline, thin-walled,

clamped.

Cheilocystidia: 14.0-35.3 x 4.7-11.9 μm , crowded on sterile lamella edge, hyaline, thin-walled, clavate, cylindric, subcylindric, ventricose, ventricose-rostrate (occasionally with long rostrum), subventricose, subventricose-capitate (occasionally bicapitate), subventricose-mucronate, subsphaeropendunculate, rarely with an apical halo drop.

Cystidioid elements (Pleurocystidia): 14.4-24.1 x 4.7-6.5 μm , abundant in notch of gill when present, subventricose-capitate to subventricose-mucronate, thin-walled, hyaline.

Phenetic Study

Spore length and spore width were examined by measuring twenty spores from each collection. Mean spore length of each collection ranged from 8.01-10.53 μm (see Fig. 2), while the means for spore width ranged from 4.36-5.15 μm (see Fig. 3). Basidial length and basidial width were examined by measuring fifteen basidia from each collection. Mean basidial length ranged from 20.69-26.64 μm (see Fig. 4) and mean basidial width ranged from 5.83-6.84 μm (see Fig. 5). Cheilocystidial length and width were examined by measuring fifteen cheilocystidia, being sure to include samples of all shape variants found in that collection. Mean cheilocystidial length ranged from 18.14-24.36 μm (see Fig. 6), while mean cheilocystidial width ranged from 6.58-8.78 μm (see Fig. 7). Visual assessment of box plots indicated that spore length and basidial length were normally distributed. Basidial width, cheilocystidial length, cheilocystidial width, and spore width showed skewed distributions indicating departure from normality. Basidial width was normalized by a hyperbolic tangent transformation. Cheilocystidial

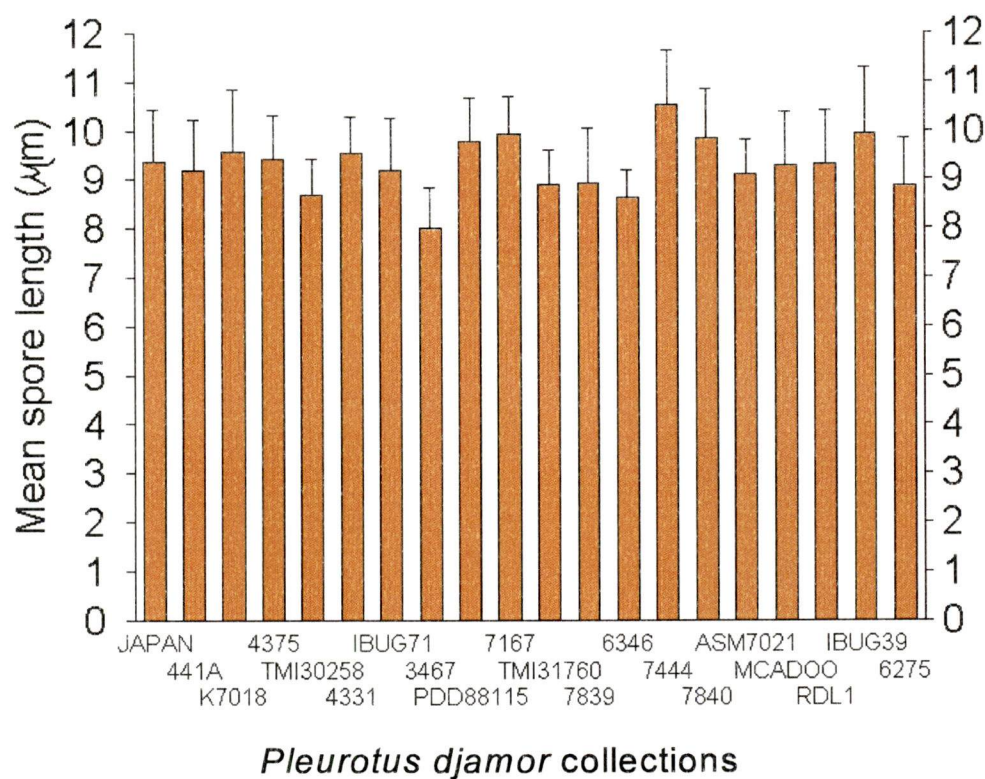


Figure 2. Mean spore length of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

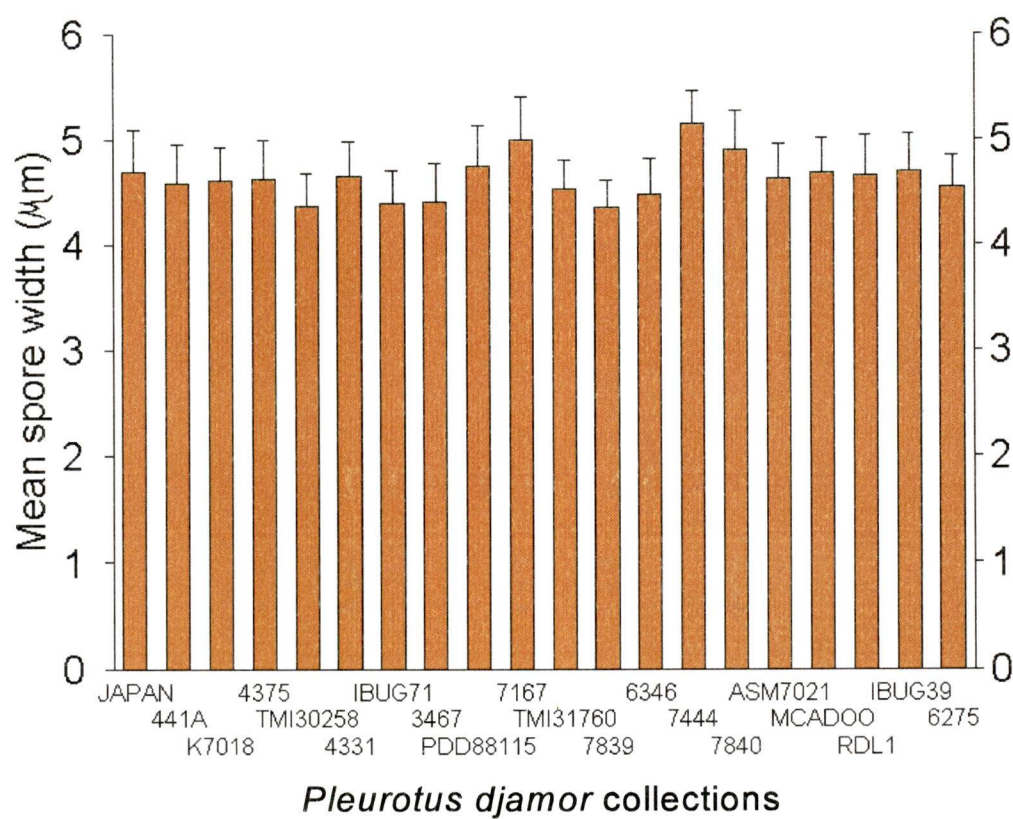


Figure 3. Mean spore width of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

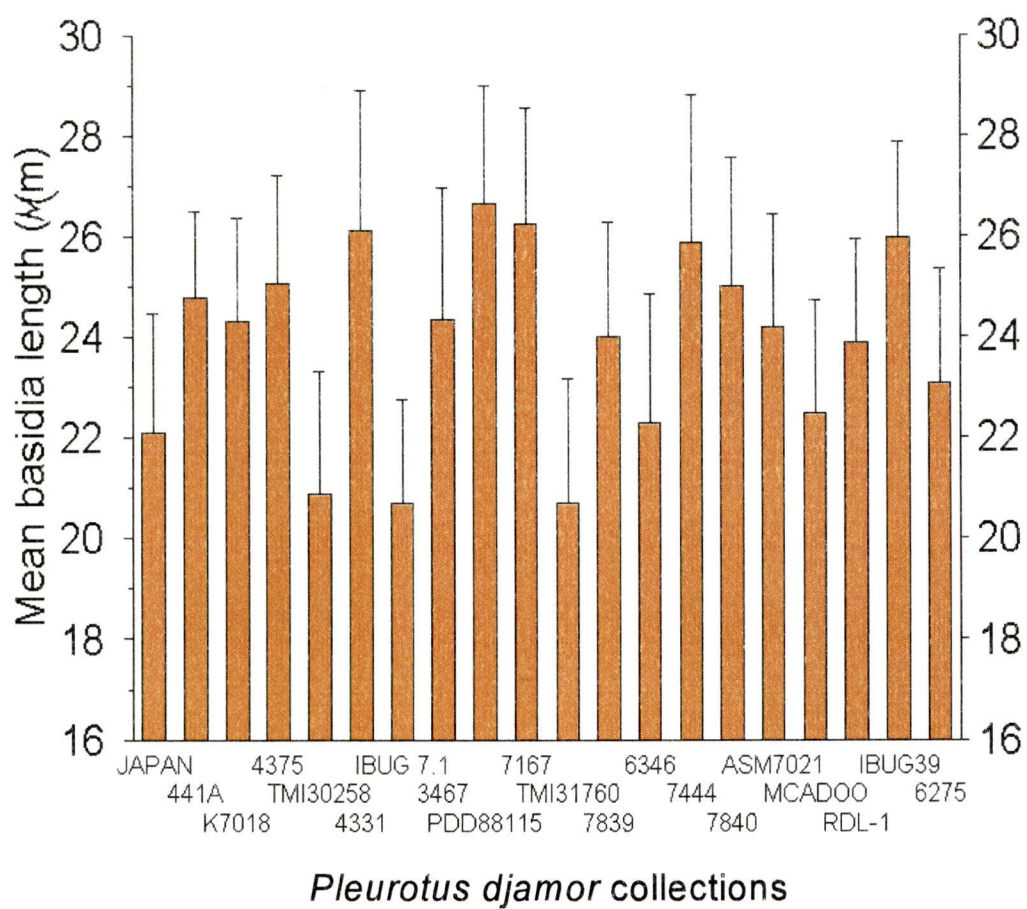


Figure 4. Mean basidia length of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

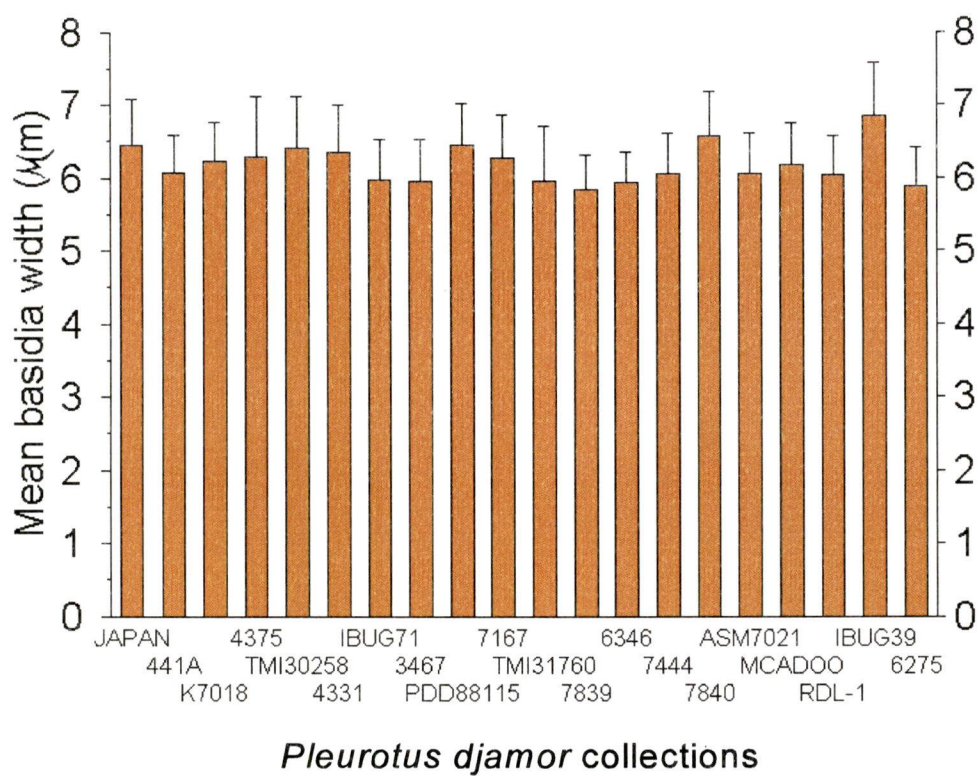


Figure 5. Mean basidial width of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

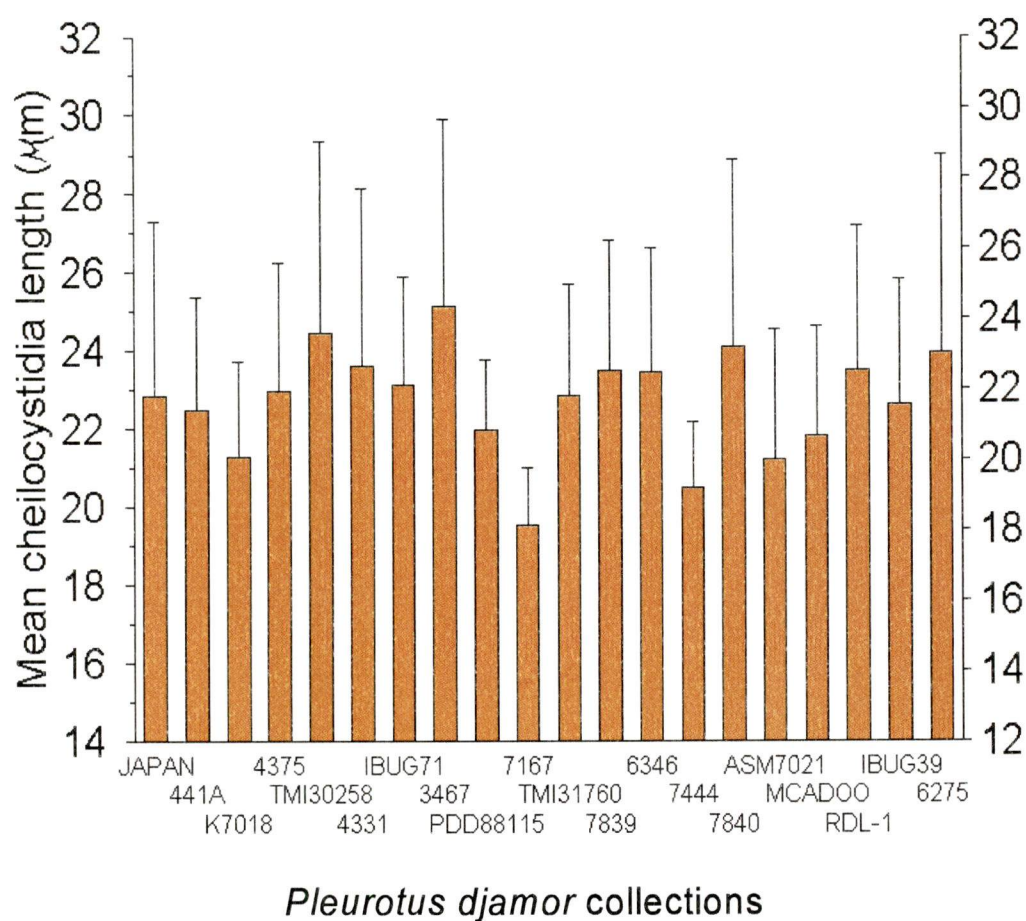


Figure 6. Mean cheilocystidial length of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

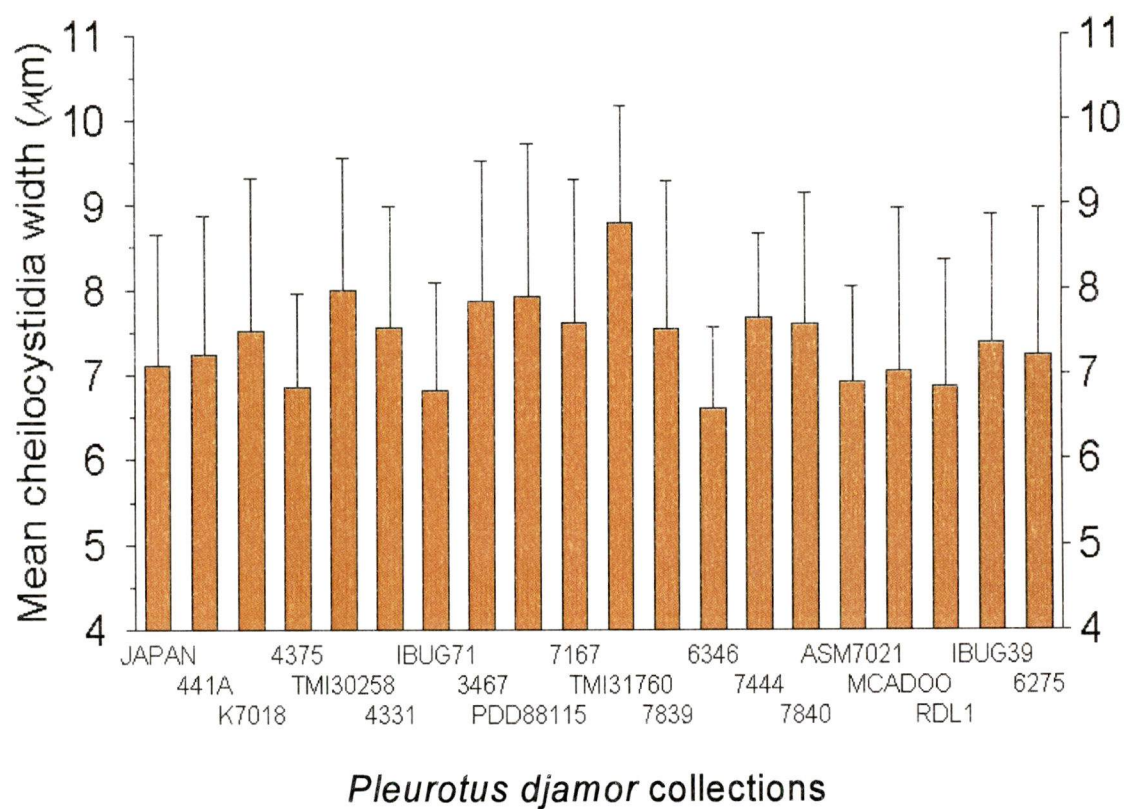


Figure 7. Mean cheilocystidial width of each collection of *Pleurotus djamor* used in phenetic analyses, measured in μm .

length was sin transformed, cheilocystidial width was arc tangent transformed, and spore width was hyperbolic tangent transformed resulting in a normal distribution for each variable. Normality was examined for transformed variables by visual assesment of box plots.

A join (agglomerative) single linkage clustering algorithm was used to create a tree of phenetic relationships (see Fig. 8). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 0.5938 between a cluster with TMI 31760 and IBUG 7₁ and a cluster containing the other 18 collections. Four major clusters were created: 1) TMI 31760, IBUG 7₁, TMI 30258; 2) 3467, 7839, RDL1, 6275, 6346, JAPAN, MCADOO; 3) K7018, ASM 7021, 441A, 4375 7840; and 4) 4331, 7167, 7444, IBUG 39, PDD 88115. A complete linkage clustering algorithm was used to create a phenogram (see Fig. 9). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 2.3645 between a cluster of seven collections (top of tree) and 13 collections (bottom of tree). These two clusters were then each divided into two major clusters creating four distinct clusters: 1) TMI 30258, IBUG 7₁, TMI 31760; 2) MCADOO, JAPAN, 6346, 6275; 3) 3467, 7839, RDL1, 4375, 441A, ASM 7021, K7018; 4) PDD 88115, IBUG 39, 7444, 7167, 4331, 7840. An average linkage clustering technique was applied to the data creating a phenogram (see Fig. 10). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 1.46 between a cluster of seven collections (top of tree) and 13 collections (bottom of tree). These two clusters were each divided into two major clusters creating 4 clusters each separating at distances around 0.45-0.66: 1) TMI 30258, IBUG 7₁, TMI 31760; 2) MCADOO, JAPAN, 6346, 6275; 3) 3467, 7839, RDL1, 4375,

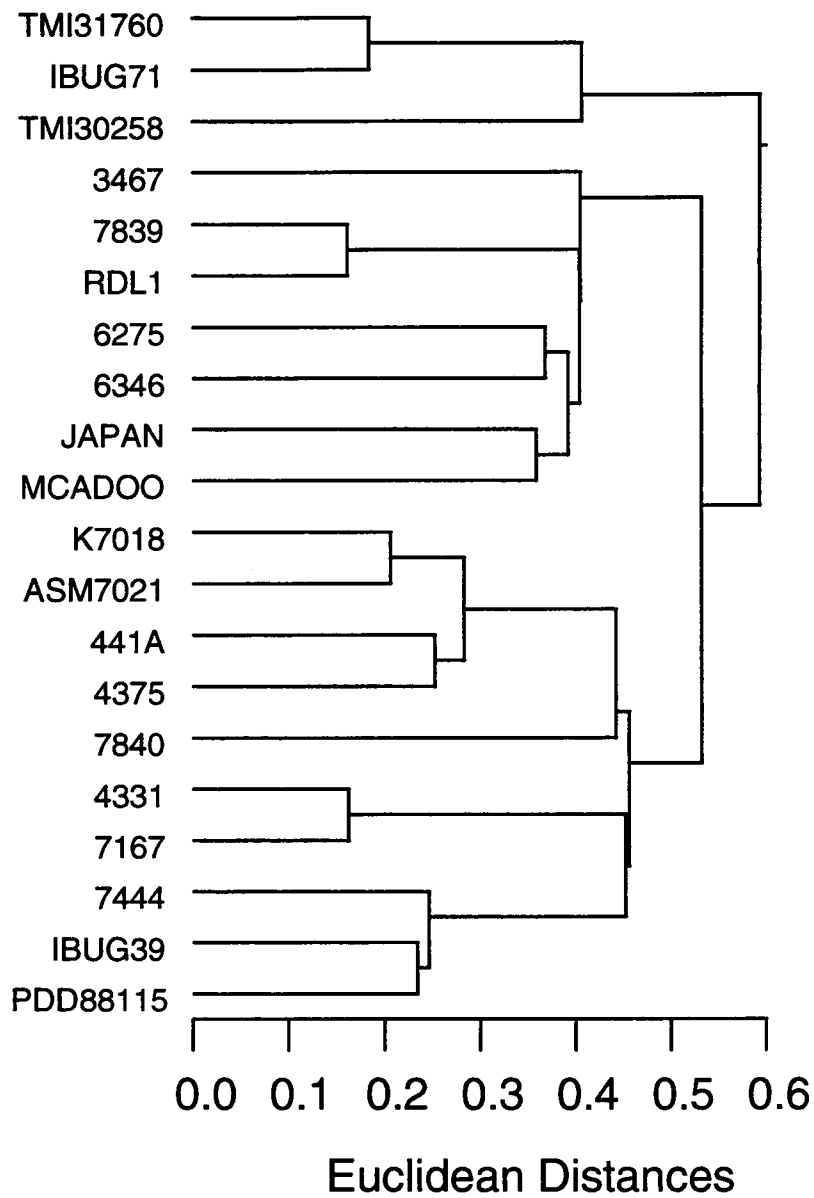


Figure 8. Phenetic tree created using a join (agglomerative) single linkage clustering algorithm of Euclidean distances to ascertain relationship among collections of *Pleurotus djamor* using only quantitative variables.

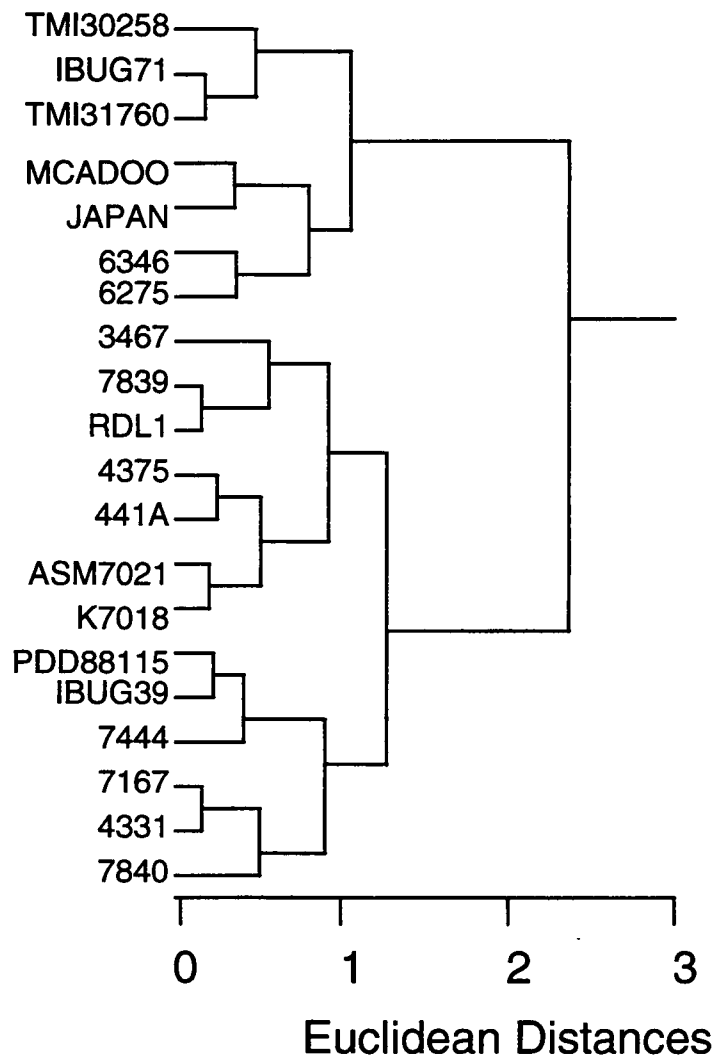


Figure 9. Phenetic tree created using a complete linkage clustering algorithm of Euclidean distances to ascertain relationship among collections of *Pleurotus djamor* using only quantitative variables.

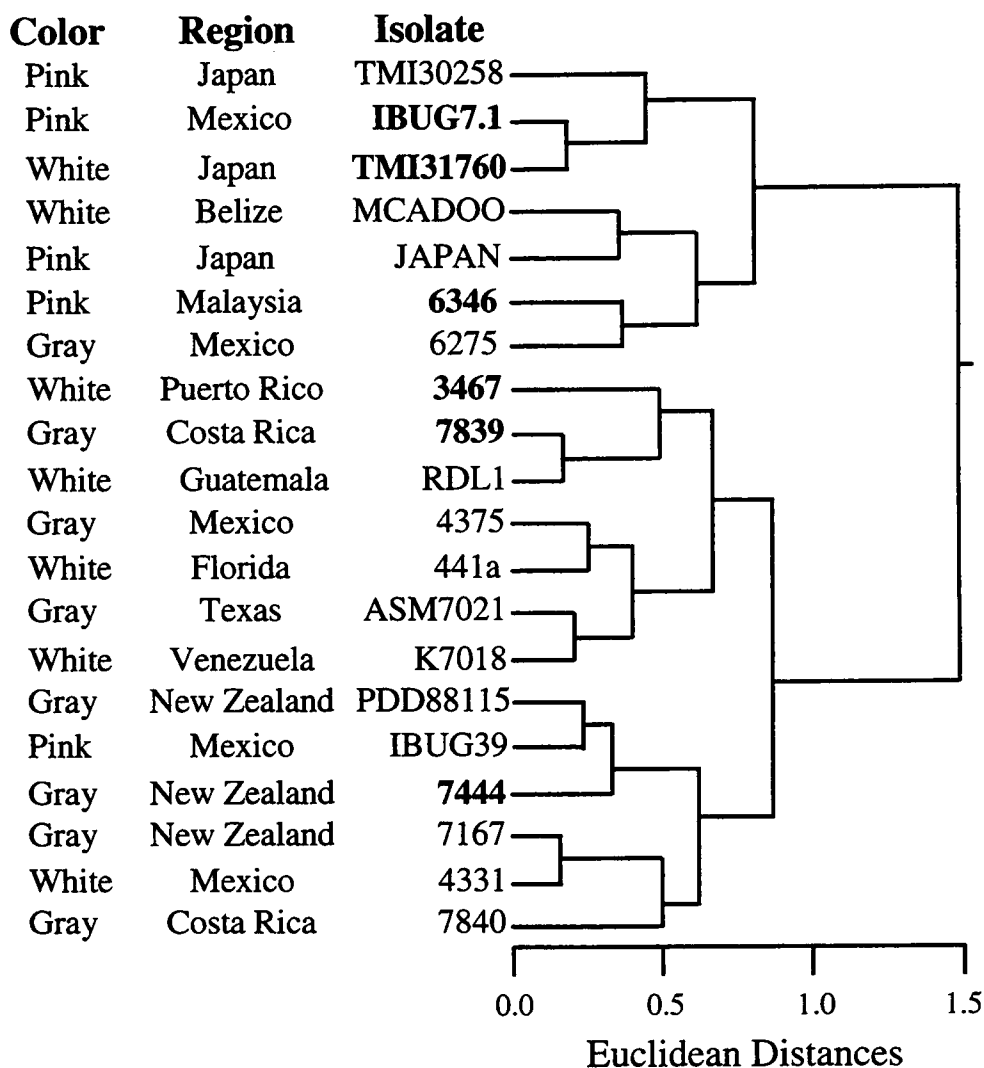


Figure 10. Phenetic tree created using an average linkage clustering algorithm of Euclidean distances to ascertain relationship among collections of *Pleurotus djamor* using only quantitative variables. Bold face = "Reference" Collections.

441A, ASM 7021, K7018; 4) PDD 88115, IBUG 39, 7444, 7167, 4331, 7840.

Phenotypic distances were calculated using Gamma coefficients on qualitative characters. A phenogram was created using the average linkage method (see Fig. 11). Distances ranged from 0.0 to 1.28. The collections were grouped into three major clusters with little to no separation of collections within these clusters: 1) 6346, K7018, RDL1, TMI 31760, IBUG 7₁, IBUG 39, 4375, 3467, MCADOO, 6275, 4331, 441A, 7840, and 7839; 2) TMI 30258, JAPAN; and 3) ASM 7021, 7167, 7444, PDD 88115. This phenogram showed little separation between collections indicating low resolution.

Gamma coefficients were calculated and a join cluster algorithm using the average linkage method was used to create a phenogram (see Fig. 12). Distances ranged from 0 to 1.06 with five major clusters separating from each other at a distance of 0.5 or greater. Cluster one contained only ASM 7021. Cluster two included PDD 88115, 7167, and 7444. Cluster three contained 4331, 7840, 7839, 6275, 3467, 441A, TMI 31760, MCADOO, RDL1, K7018, IBUG 39, 4375. Cluster four contained IBUG 7₁ and 6346. Cluster five included TMI 30258 and JAPAN. A single linkage algorithm was applied to the data to create a phenogram (see Fig. 13). Distances ranged from 0 to 0.46 indicating little resolution. Three major clusters could be discerned: 1) JAPAN, TMI30258; 2) 6346, IBUG 7₁, MCADOO, TMI 31760, 441A, 3467, 4331, 7840, 7839, 4375, K7018, RDL1, IBUG 39, 6275, PDD 88115, 7167, 7444; 3) only ASM 7021. A complete linkage algorithm was also used to create a phenogram (see Fig. 14). Distances ranged from 0 to 1.97. Two major clusters were separated at a distance of 1.97: 1) 7444, 7167, PDD 88115, ASM 7021; and 2) the sixteen remaining isolates. This second cluster was

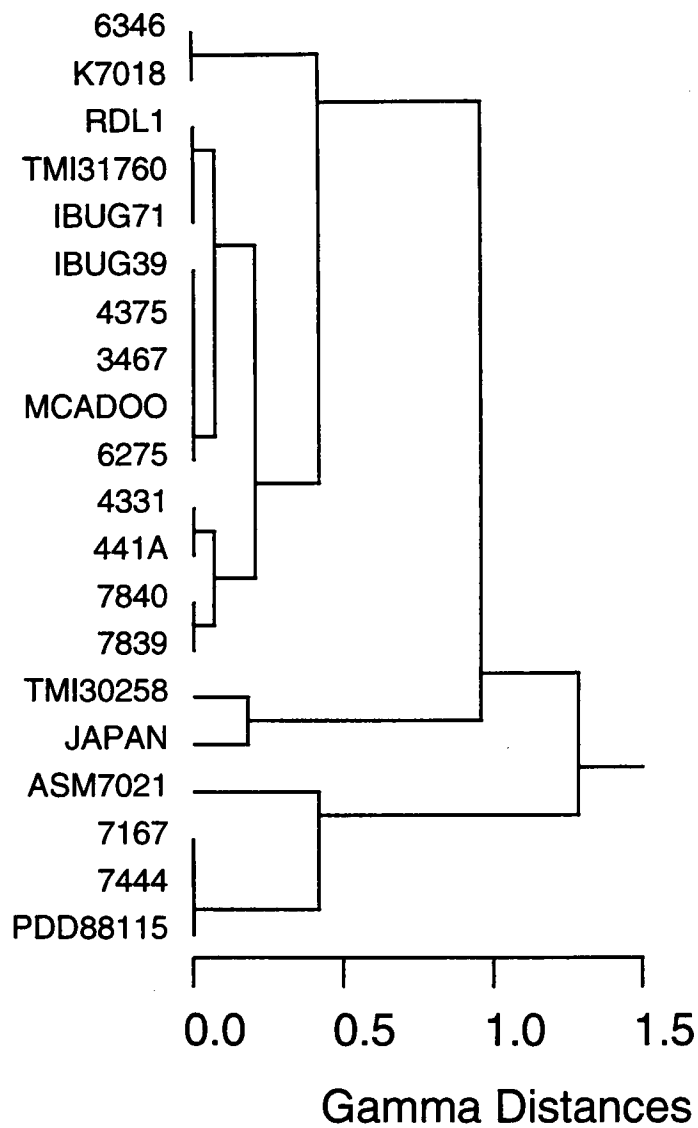


Figure 11. Phenetic tree created using an average linkage clustering algorithm of Gamma distances to ascertain relationship among collections of *Pleurotus djamor* using only qualitative variables.

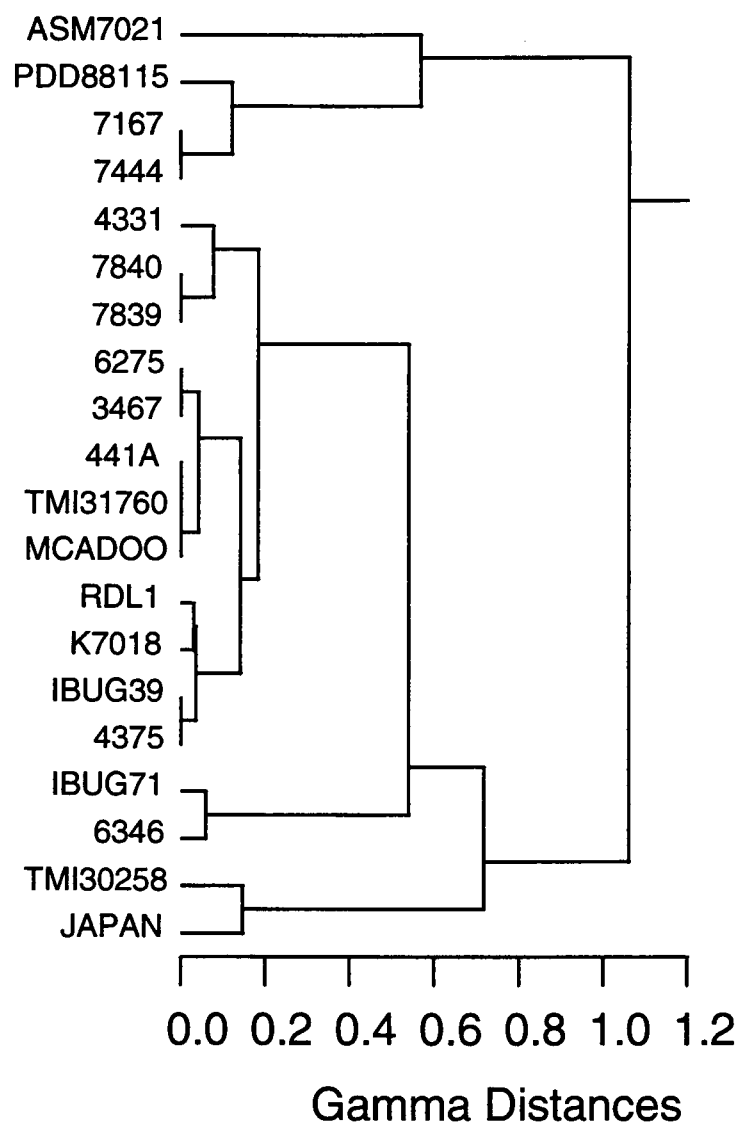


Figure 12. Phenetic tree created using an average linkage clustering algorithm of Gamma distances to ascertain relationship among collections of *Pleurotus djamor* using both converted quantitative and qualitative variables.

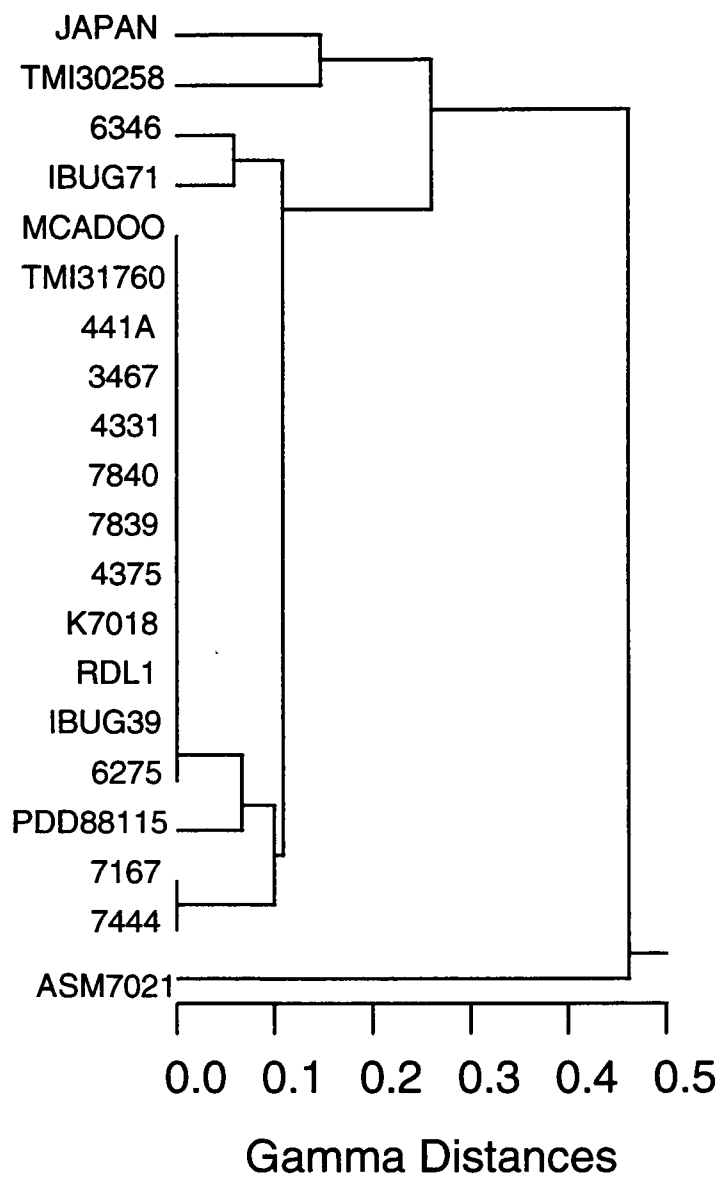


Figure 13. Phenetic tree created using a single linkage clustering algorithm of Gamma distances to ascertain relationship among collections of *Pleurotus djamor* using both converted quantitative and qualitative variables.

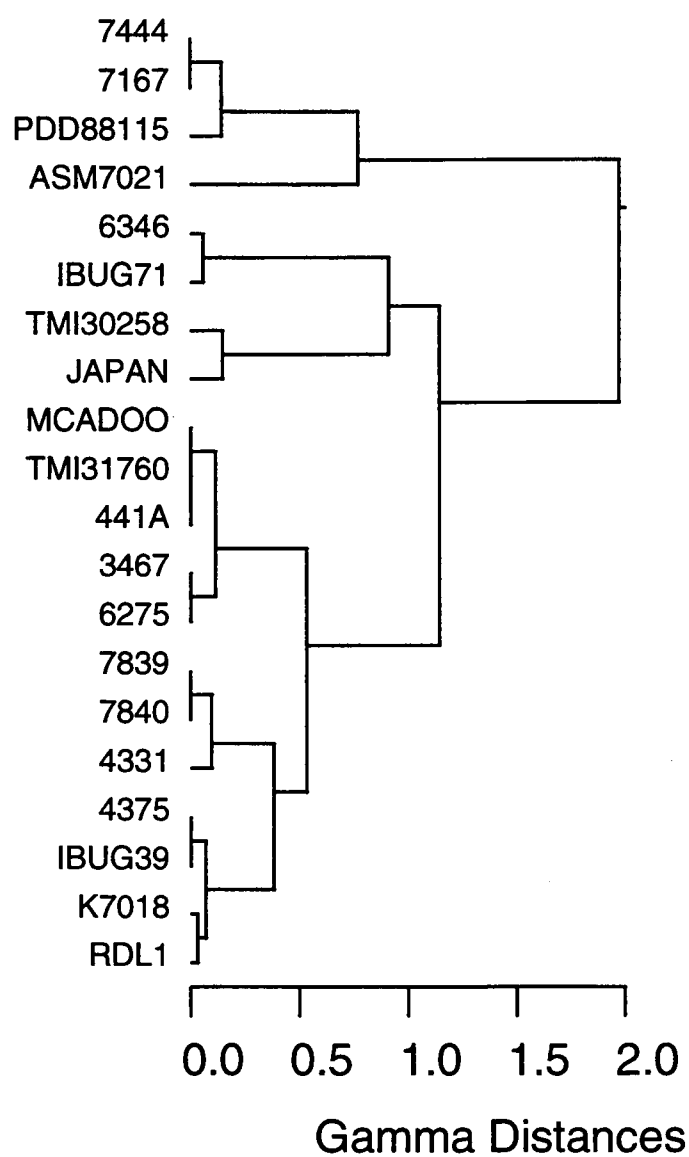


Figure 14. Phenetic tree created using a complete linkage clustering algorithm of Gamma distances to ascertain relationship among collections of *Pleurotus djamor* using both converted quantitative and qualitative variables.

subdivided into two clusters separating at a distance of 1.14: 1) 6346, IBUG 7₁, TMI 30258, JAPAN; and 2) MCADOO, TMI 31760, 441A, 3467, 6275, 7839, 7840, 4331, 4375, IBUG 39, K7018, RDL1.

Self Crosses

Five reference collections were self-crossed in order to determine their mating alleles for use as tester strains. Tester strains are denoted by asterisks. Collection 7444 had already been self-crossed prior to this study. Isolate 14* = A_1B_1 ; 15* = A_2B_2 ; 19* = A_2B_1 .

Ten single-spore isolates of collection 7839 were paired in all combinations and a tetrapolar mating system was revealed (see Fig. 15). Isolates 2*, 3, 7 = A_1B_1 ; 6*, 8 = A_2B_2 ; 12*, 13 = A_1B_2 ; 4, 5*, 11 = A_2B_1 . Isolate 1 was a contaminant.

Eleven single-spore isolates of collection 6346 were paired in all combinations and a tetrapolar mating system was revealed (see Fig. 16). Isolates 1, 5*, 7, 9, 12, 14, 17 = A_1B_1 ; 4, 13* = A_2B_2 ; 11* = A_1B_2 ; 8* = A_2B_1 .

Twelve single spore isolates of collection IBUG 7₁ were paired in all combinations and a tetrapolar mating system was revealed (see Fig. 17). Isolates 2*, 4, 14, 16 = A_1B_1 ; 10, 11*, 18 = A_2B_2 ; 1, 7*, 8, 12, 20* = A_1B_2 . The fourth mating type (A_2B_1) was not present in this sample.

Twelve single-spore isolates of collection TMI 31760 were paired in all possible combinations and a tetrapolar mating system was revealed (see Fig. 18). Isolates 1, 6*, 8, 10 = A_1B_1 ; 4*, 12 = A_2B_2 ; 5, 7* = A_1B_2 ; 2, 3, 9*, 11 = A_2B_1 . In all the above pairings no clear barrage or flat reactions could be discerned therefore mating types are assigned

	A_1B_1			A_2B_2		A_1B_2		A_2B_1		
	2	3	7	6	8	12	13	4	5	11
2		-	-	+	+	-	-	-	-	-
3	-		-	+	+	-	-	-	-	-
7	-	-		+	+	-	-	-	-	-
6	+	+	+		-	-	-	-	-	-
8	+	+	+	-		-	-	-	-	-
12	-	-	-	-	-		-	+	+	+
13	-	-	-	-	-	-		+	+	+
4	-	-	-	-	-	+	+		-	-
5	-	-	-	-	-	+	+	-		-
11	-	-	-	-	-	+	+	-	-	

Figure 15. Self cross grid of collection 7839. Pluses indicate compatibility (i.e., formation of clamp connections), while minuses indicate incompatibility.

	A_1B_1							A_2B_2		A_1B_2	A_2B_1
	1	5	7	9	12	14	17	4	13	11	8
1		-	-	-	-	-	-	+	+	-	-
5	-		-	-	-	-	-	+	+	-	-
7	-	-		-	-	-	-	+	+	-	-
9	-	-	-		-	-	-	+	+	-	-
12	-	-	-	-		-	-	+	+	-	-
14	-	-	-	-	-		-	+	+	-	-
17	-	-	-	-	-	-		+	+	-	-
4	+	+	+	+	+	+	+		-	-	-
13	+	+	+	+	+	+	+	-		-	-
11	-	-	-	-	-	-	-	-	-		+
8	-	-	-	-	-	-	-	-	-	+	

Figure 16. Self cross grid of collection 6346. Pluses indicate compatibility (i.e., formation of clamp connections), while minuses indicate incompatibility.

	A_1B_1				A_2B_2			A_1B_2				
	2	4	14	16	10	11	18	1	7	8	12	20
2		-	-	-	+	+	+	-	-	-	-	-
4	-		-	-	+	+	+	-	-	-	-	-
14	-	-		-	+	+	+	-	-	-	-	-
16	-	-	-		+	+	+	-	-	-	-	-
10	+	+	+	+		-	-	-	-	-	-	-
11	+	+	+	+	-		-	-	-	-	-	-
18	+	+	+	+	-	-		-	-	-	-	-
1	-	-	-	-	-	-	-		-	-	-	-
7	-	-	-	-	-	-	-	-		-	-	-
8	-	-	-	-	-	-	-	-	-		-	-
12	-	-	-	-	-	-	-	-	-	-		-
20	-	-	-	-	-	-	-	-	-	-	-	

Figure 17. Self cross grid of collection IBUG 7₁. Pluses indicate compatibility (i.e., formation of clamp connections), while minuses indicate incompatibility.

	A_1B_1				A_2B_2		A_1B_2		A_2B_1			
	1	6	8	10	4	12	5	7	2	3	9	11
1		-	-	-	+	+	-	-	-	-	-	-
6	-		-	-	+	+	-	-	-	-	-	-
8	-	-		-	+	+	-	-	-	-	-	-
10	-	-	-		+	+	-	-	-	-	-	-
4	+	+	+	+		-	-	-	-	-	-	-
12	+	+	+	+	-		-	-	-	-	-	-
5	-	-	-	-	-	-		-	+	+	+	+
7	-	-	-	-	-	-	-		+	+	+	+
2	-	-	-	-	-	-	+	+		-	-	-
3	-	-	-	-	-	-	+	+	-		-	-
9	-	-	-	-	-	-	+	+	-	-		-
11	-	-	-	-	-	-	+	+	-	-	-	

Figure 18. Self cross grid of collection TMI 31760. Pluses indicate compatibility (i.e., formation of clamp connections), while minuses indicate incompatibility.

arbitrarily.

Twelve single-spore isolates of collection 3467 were paired in all combinations and a tetrapolar mating system was revealed (see Figure 19). Isolate 1, 2*, 4, 5, 10, 11*, 12 = A_1B_1 ; 8* = A_2B_2 ; 3, 7*, 9 = A_1B_2 ; 6* = A_2B_1 .

Primary mating alleles (A_1B_1 , A_2B_2) were assigned arbitrarily. Subordinate mating alleles (A_1B_2 , A_2B_1) were assigned based upon a flat reaction seen as a thin clear line along the contact zone with associated hyphal lysis which is indicative of a common A-allele.

Intercompatibility

Four single-spore isolates (SSIs) from each of six reference collections were paired with four single spores isolates of the other five reference collections.

Collection **7839** was compatible with 3467, 7444, IBUG 7₁, 6346 and TMI 31760 (see Fig. 20). The only exception was SSI 6346:4 which was incompatible with all four SSIs of 7839. Nuclear migration was reciprocal. However, SSI 6346:11 allowed nuclear migration from the contact zone in only one of the four pairings.

Collection **6346** was fully compatible with TMI 31760 (see Fig. 21). 6346 showed some reduced compatibility with 3467, IBUG 7₁, 7444 and 7839. 6346 x TMI 31760: all uncontaminated pairings were positive. 6346 x 3467: 11 of 16 were compatible. 6346 x IBUG 7₁: 12 of 16 were compatible. 6346 x 7444: 7 of 16 were compatible (6 were contaminated). 6346 x 7839 see above. In several of these crosses there was no nuclear migration into one or both of the collections, but there was no consistent pattern.

Collection **IBUG 7₁** was compatible with 7444, TMI 31760, 3467, 6346 and 7839

	A_1B_1							A_2B_2	A_1B_2			A_2B_1
	1	2	4	5	10	11	12	8	3	7	9	6
1		-	-	-	-	-	-	+	-	-	-	-
2	-		-	-	-	-	-	+	-	-	-	-
4	-	-		-	-	-	-	+	-	-	-	-
5	-	-	-		-	-	-	+	-	-	-	-
10	-	-	-	-		-	-	+	-	-	-	-
11	-	-	-	-	-		-	+	-	-	-	-
12	-	-	-	-	-	-		+	-	-	-	-
8	+	+	+	+	+	+	+		-	-	-	-
3	-	-	-	-	-	-	-	-		-	-	+
7	-	-	-	-	-	-	-	-	-		-	+
9	-	-	-	-	-	-	-	-	-	-		+
6	-	-	-	-	-	-	-	-	+	+	+	

Figure 19. Self cross grid of collection 3467. Pluses indicate compatibility (i.e., formation of clamp connections), while minuses indicate incompatibility.

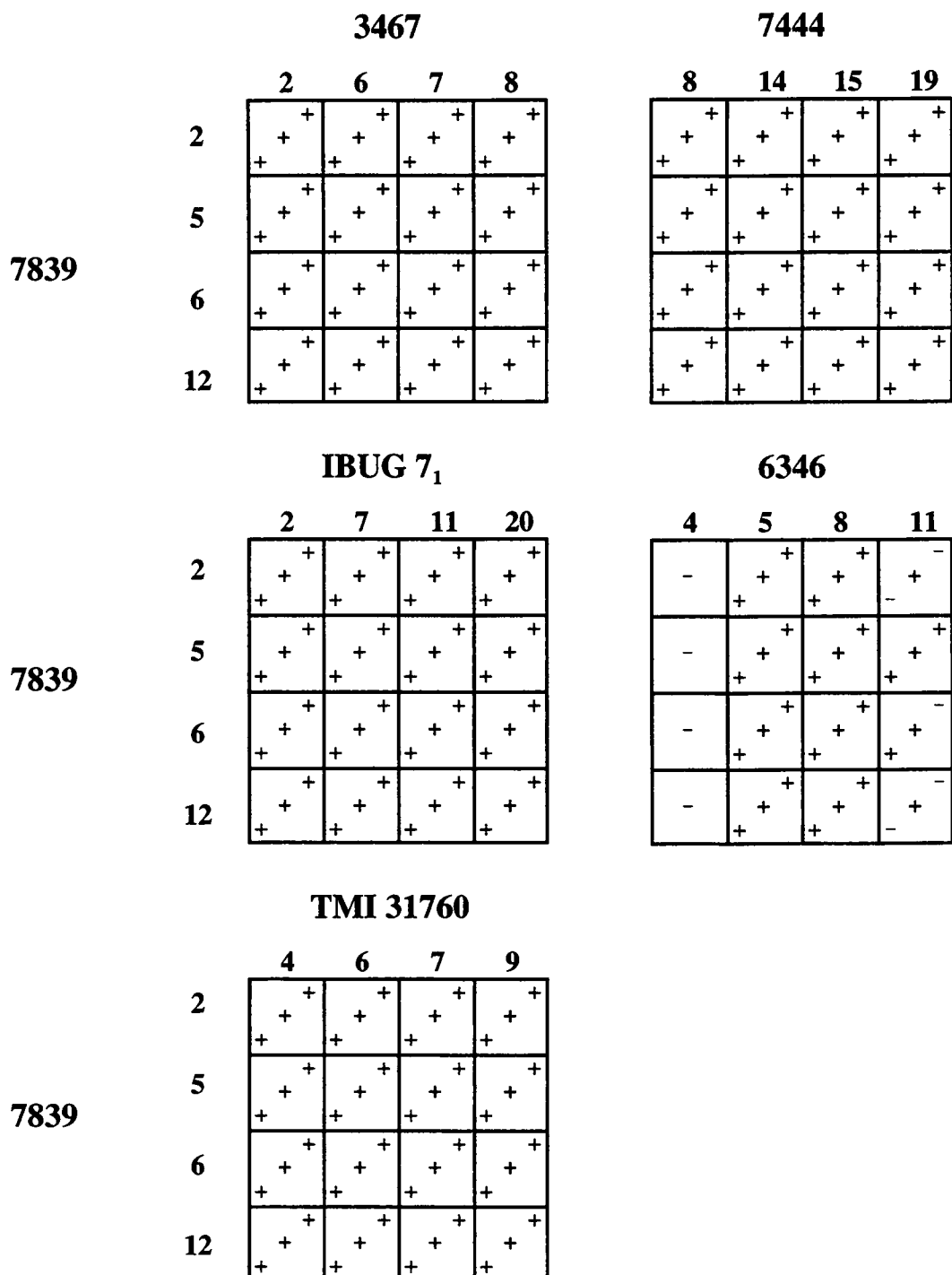


Figure 20. Inter-collection compatibility pairings using haplont (single-spore) isolates of Collection 7839 with 3467, 7444, IBUG 7₁, 6346, and TMI 31760

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

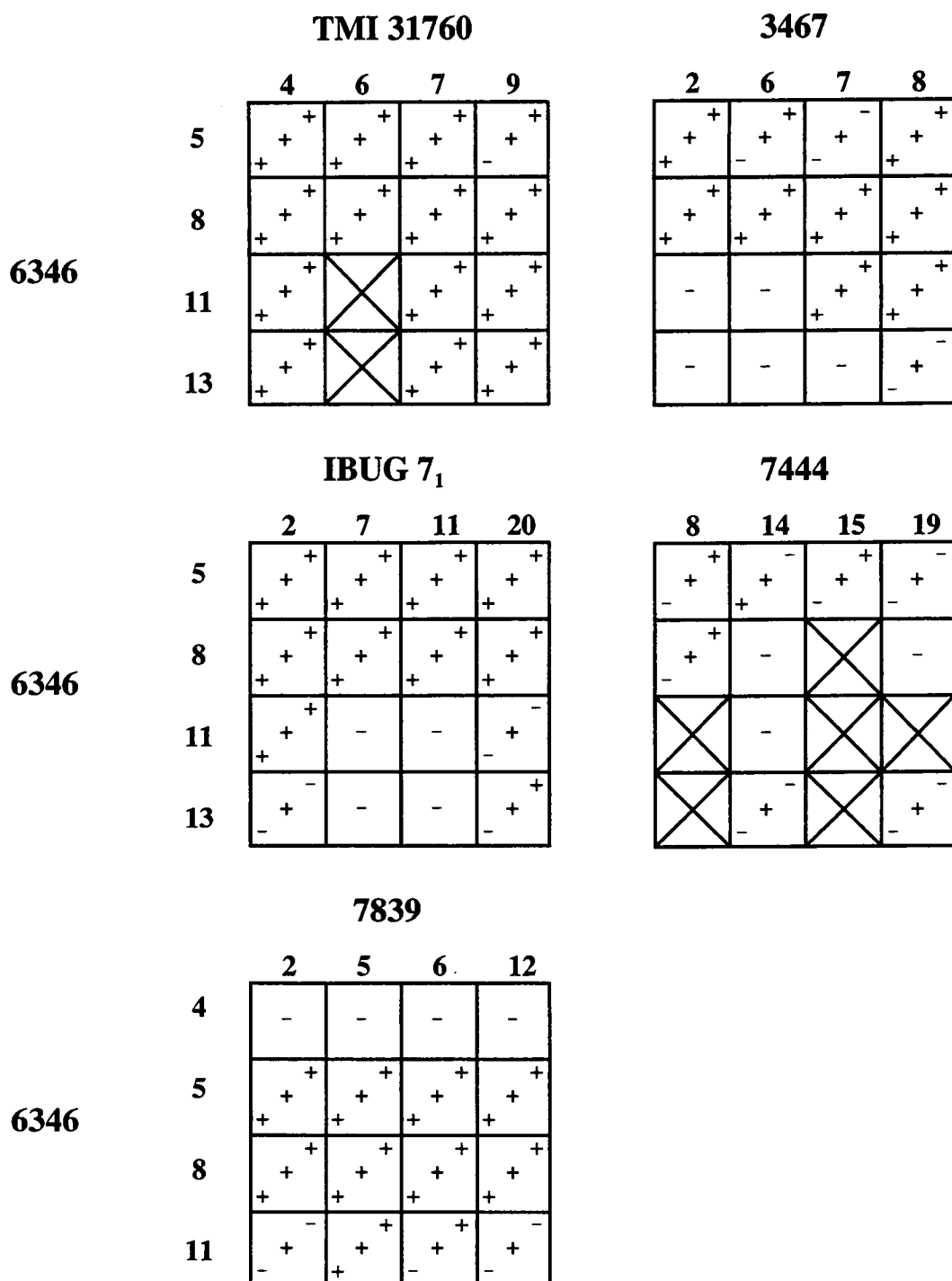


Figure 21. Inter-collection compatibility pairings using haplont (single-spore) isolates of Collection 6346 with TMI 31760, 3467, IBUG 7₁, 7444, and 7839

PAIRINGS Center (+/-) **MIGRATIONS** Corner (+/-)

(+) Compatible Pairings Upper right (+/-) Nuclear migration into column collection

(-) Incompatible Pairings Lower left (+/-) Nuclear migration into row collection

(x) Plate not examined because of contamination.

(see Fig. 22). IBUG 7₁ x 7444: 14 of 16 pairings were compatible (one was contaminated). IBUG 7₁ x TMI31760: all pairings compatible. IBUG 7₁ x 3467: 12 of 16 pairings were compatible. IBUG7₁ x 6346, 7839 see above. In several pairings of IBUG 7₁ with 7444 and TMI 31760 there was no nuclear migration into one or both of the collections, but with no consistent pattern. In pairings of IBUG 7₁ and 3467 there was bilateral nuclear migration in all compatible pairings.

Collection **3467** was compatible with 7444, TMI 31760, IBUG 7₁, 6346 and 7839 (see Fig. 23). 3467 x 7444: all pairings compatible. In two of these pairings 3467:8 x 7444:11, 19 there was only nuclear migration into collection 3467. In the remaining 14 pairings there was bilateral nuclear migration. 3467 x TMI 31760: all pairings were compatible. In five of these pairings there was no nuclear migration into one of the collections but with no consistent pattern. In the other 11 pairings nuclear migration was bilateral. 3467 x IBUG 7₁, 6346 and 7839 see above.

Collection **7444** was compatible with TMI 31760, 3467, IBUG 7₁, 6346 and 7839 (see Figure 24). 7444 x TMI 31760: 15 of 16 pairings were compatible. In five pairings there was no nuclear migration into one or both of the collections. In the other 10 pairings nuclear migration was bilateral. 7444 x 3467, IBUG 7₁, 6346 and 7839 see above.

Collection **TMI 31760** was compatible with 7444, 3467, IBUG 7₁, 6346 and 7839 (see Fig. 25). For details of pairings see above.

Other (non-reference) putative *P. djamor* collections were then paired in a 2 x 2 cross with one of the six reference collections to access its membership in this intercompatibility group. Reference collection 7839 was paired with the following

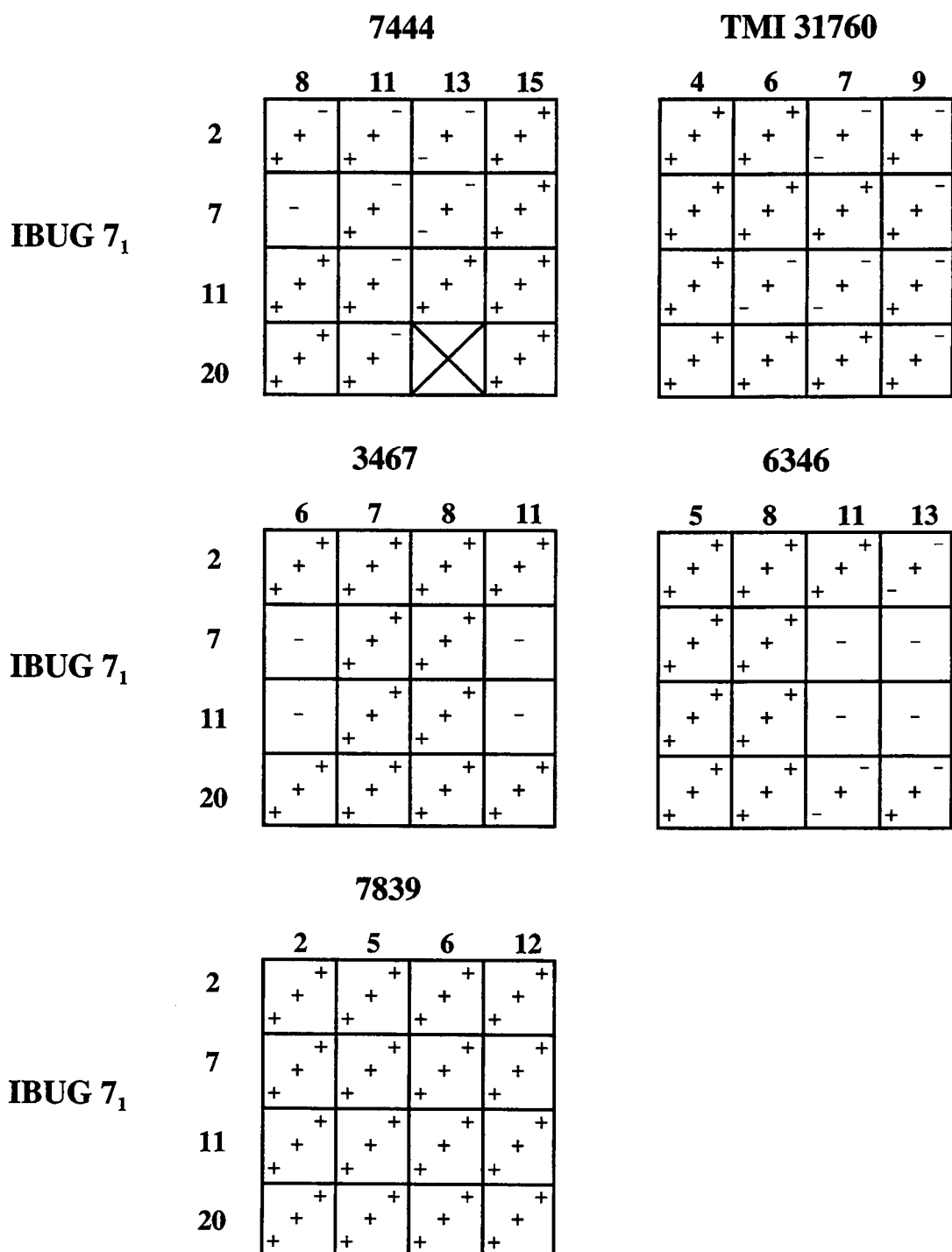


Figure 22. Intercollecion compatibility pairings using haplont (single-spore) isolates of Collection IBUG 7₁ with 7444, TMI 31760, 3467, 6346, and 7839

PAIRINGS Center (+/-) MIGRATIONS Corner (+/-)

(+) Compatible Pairings Upper right (+/-) Nuclear migration into column collection

(-) Incompatible Pairings Lower left (+/-) Nuclear migration into row collection

(x) Plate not examined because of contamination

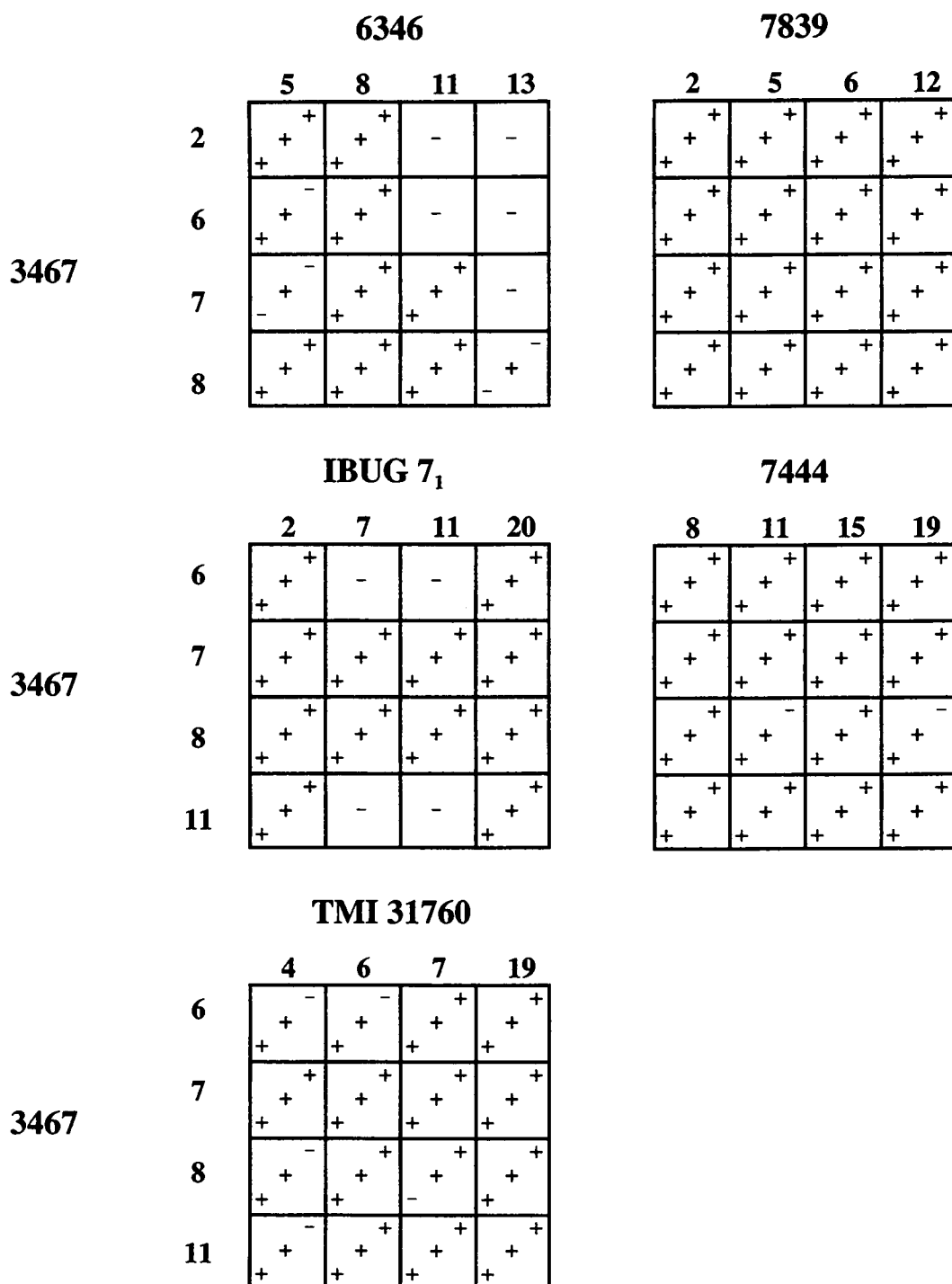


Figure 23. Inter-collection compatibility pairings using haplont (single-spore) isolates of Collection 3467 with 6346, 7839, IBUG 7₁, 7444, and TMI 31760

PAIRINGS Center (+/-) (+) Compatible Pairings (-) Incompatible Pairings (x) Plate not examined because of contamination	MIGRATIONS Corner (+/-) Upper right (+/-) Nuclear migration into column collection Lower left (+/-) Nuclear migration into row collection	
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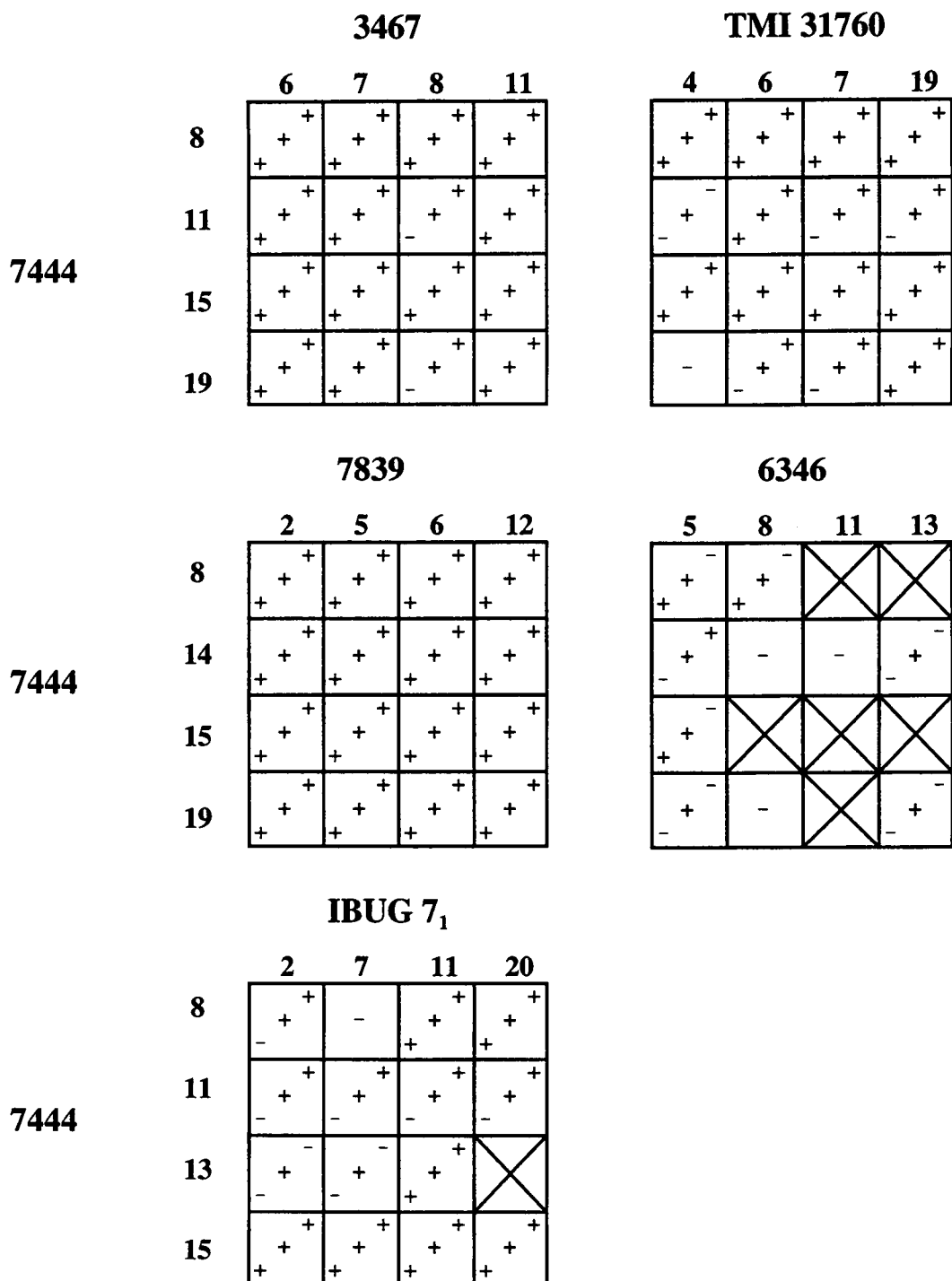


Figure 24. Inter-collection compatibility pairings using haplont (single-spore) isolates of Collection 7444 with 3467, TMI 31760, 7839, 6346, and IBUG 7₁

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

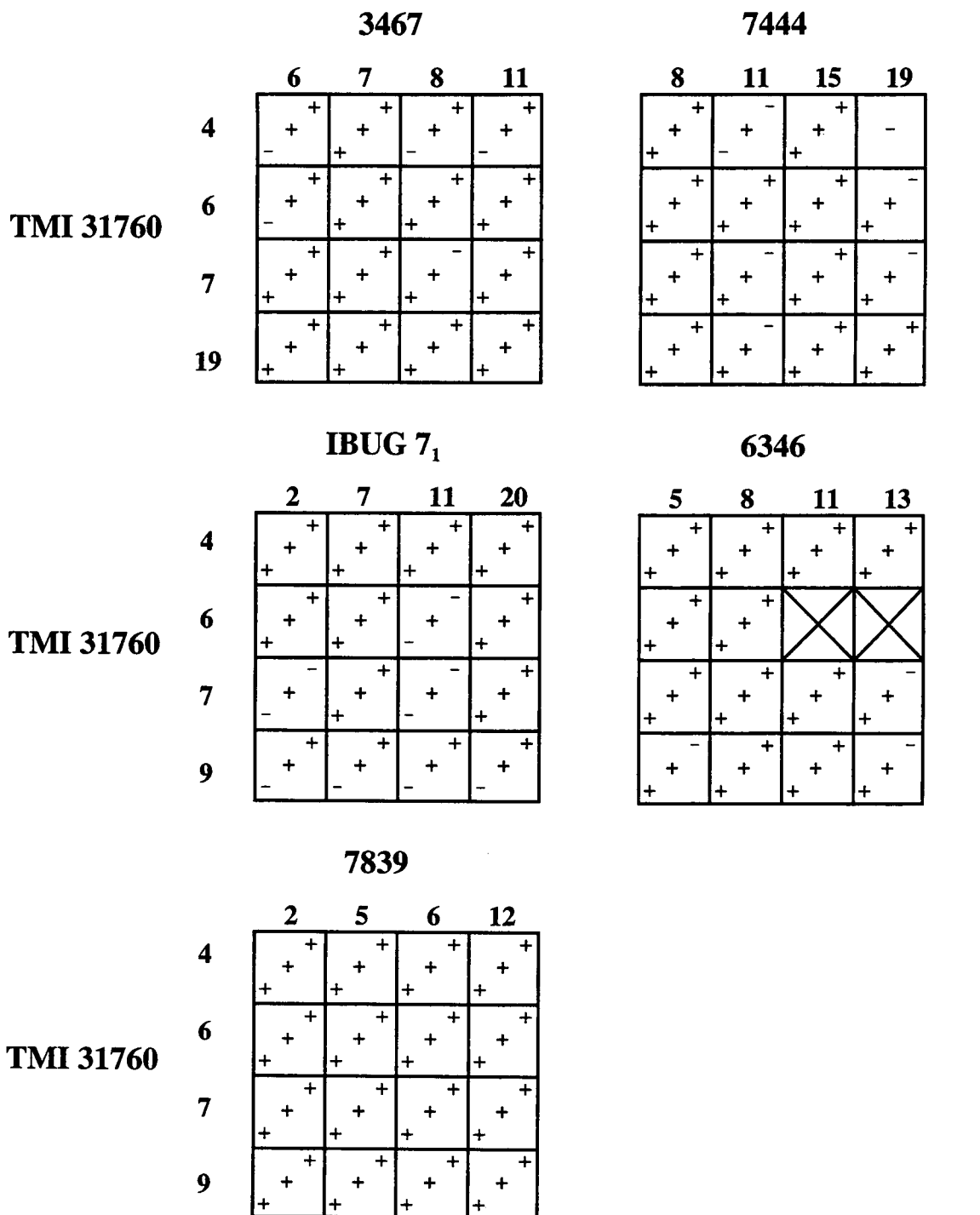


Figure 25. Intercollection compatibility pairings using haplont (single-spore) isolates of Collection TMI 31760 with 3467, 7444, IBUG 7₁, 6346, and 7839

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

collections: 6276, RDL1, 6275, 6270, 8013, K7018, and IBUG 3. All pairings were compatible except 6276 which was incompatible in all four pairings (see Fig. 26). Reference collection 3467 was paired with the following collections: MCADOO, 7840, 4326, IBUG 53, 4375, 6277, 4331, and 6241. All pairings were compatible with bilateral nuclear migration except 6277 and 6241 which were each incompatible in all four pairings (see Fig. 27). Reference collection IBUG 7₁ was paired with the following collections: IBUG 7₂, ASM 7021, ET-3313, and IBUG 39. ASM 7021 was compatible with IBUG 7₁ while IBUG 7₂, ET 3313, and IBUG 39 were all incompatible (see Fig. 28). Reference collection 7444 was paired with the following collections: 7167, PDD 88115 and Villegas Rios 1786. All of these pairings were compatible with bilateral nuclear migration except 7444:14 x 7167:11 which only produced clamp connections in the contact zone (see Fig. 28). Reference collection 6346 was paired with the following collections: JAPAN, TMI 30258, MF 1-18, and IBUG 7₂. All pairings were compatible (see Fig. 29). Reference collection TMI 31760 was paired with 441A and 6278. Both pairings were compatible with bilateral nuclear migration (see Fig. 29).

Collection 6241 differed from "*P. djamor*" in several macromorphological features; a strongly inrolled margin, distinctly infundibuliform pileus with floccose gray patches, stipe long and tapering, and nutty (asine) smell. This collection was paired with the following collections: 7826, 6276, ET 3313 and 6277 (see Fig. 30). Pairings with 6276 and ET 3313 were compatible with bilateral nuclear migration. Two of four pairings with 6277 were compatible and two were incompatible. Collection 7826 was incompatible in all four pairings. Collection 7826 was also paired with 3467, 7444, TMI

		6276		RDL1		6275		6270	
		8	9	2	14	2	13	2	12
7839	2	-	-	+	-	+	+	+	+
	12	-	-	+	+	+	+	+	+
		8013		K7018		IBUG 3			
		3	5	10	14	11	12		
7839	2	-	+	+	+	+	+		
	7	-	+	+	+	+	+		

Figure 26. Inter-collection compatibility pairings using haplont (single-spore) isolates of Collection 7839 with 6276, RDL1, 6275, 6270, 8013, K7018, IBUG3

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

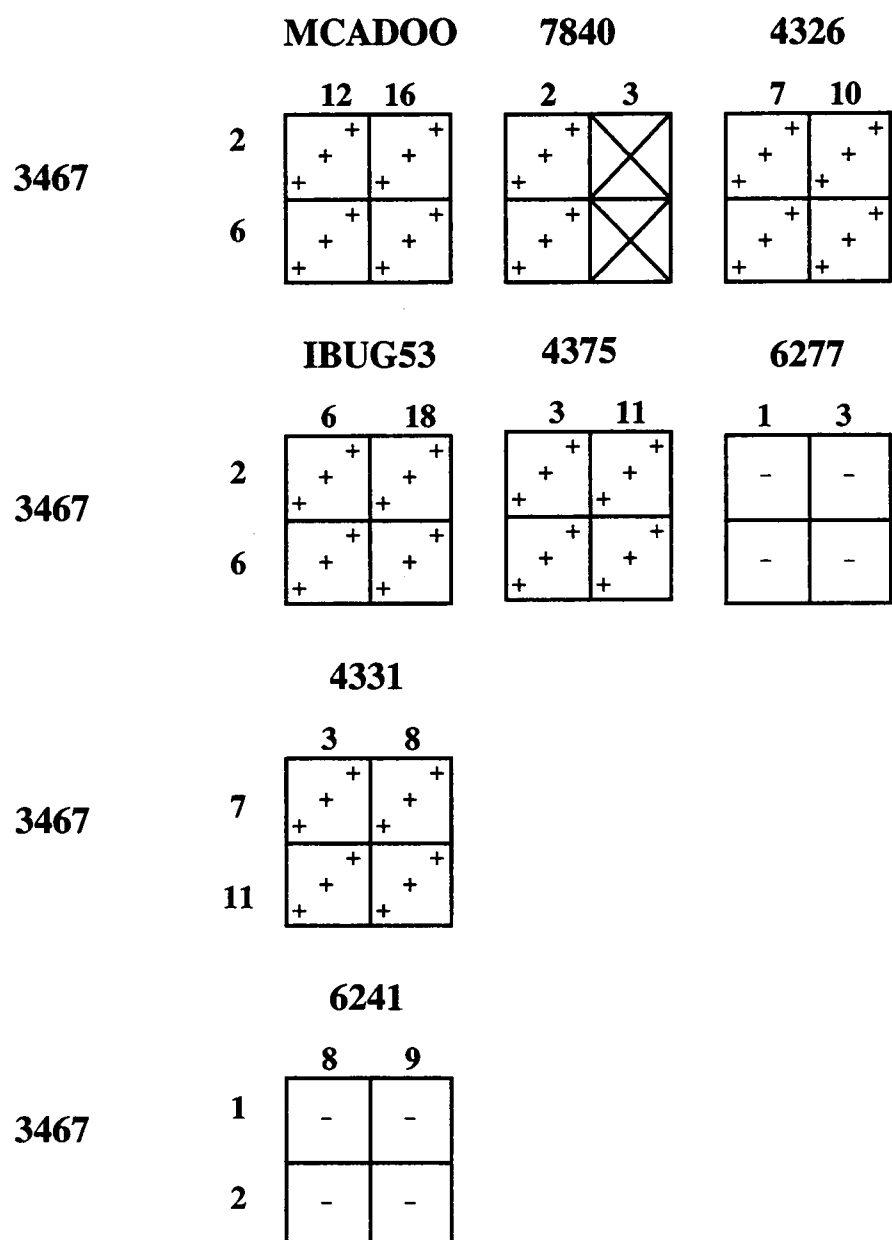


Figure 27. Intercollecion compatibility pairings using haplont (single-spore) isolates of Collection 3467 with MCADOO, 7840, 4326, IBUG53, 4375, 6277, 4331 and 6241

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

		IBUG 7 ₂		ASM 7021		ET 3313		IBUG 39	
		1	10	1	4	5	11	N-5	N-11
IBUG 7 ₁	1	-	-	+	+	-	-	-	-
	10	-	-	+	+	-	-	-	-
		7167		PDD 88115		VILLEGAS-RIOS 1786			
		1	11	3	7	6	13		
7444	11	+	+	X	+	+	+		
	14	+	+	+	+	+	+		

Figure 28. Inter-collection compatibility pairings using SSIs of collection IBUG 7₁ with IBUG 7₂, ASM 7021, ET 3313, and IBUG 39 as well as collection 7444 with 7167, PDD 88115, and VILLEGAS-RIOS 1786.

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

		JAPAN	TMI 30258	MF1-18	IBUG 7₂																
		1 20	9 12	2 14	3 6																
6346	5	<table><tr><td></td><td>+</td></tr><tr><td>+</td><td>+</td></tr></table>		+	+	+	<table><tr><td></td><td>+</td></tr><tr><td>+</td><td>+</td></tr></table>		+	+	+	<table><tr><td></td><td>+</td></tr><tr><td>+</td><td>+</td></tr></table>		+	+	+	<table><tr><td></td><td>+</td></tr><tr><td>+</td><td>+</td></tr></table>		+	+	+
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Figure 29. Inter-collection compatibility pairings using SSIs of collection 6346 with JAPAN, TMI 30258, MF1-18, and IBUG 7₂. Collection TMI 31760 with 441A and 6278.

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

		7826		6276		ET 3313		6277	
		6	10	8	9	5	11	1	3
6241	1	-	-	+	+	+	+	+	-
	12	-	-	+	+	+	+	+	-
		3467		7444		TMI 30258		6241	
		1	2	1	12	14	13	8	9
7826	4	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-

Figure 30. Inter-collection compatibility pairings using SSIs of collection 6241 with 7826, 6276, ET 3313, and 6277 as well as collection 7826 with 3467, 7444, TMI 30258, and 6241.

PAIRINGS	Center (+/-)	MIGRATIONS	Corner (+/-)
(+)	Compatible Pairings	Upper right (+/-)	Nuclear migration into column collection
(-)	Incompatible Pairings	Lower left (+/-)	Nuclear migration into row collection
(x)	Plate not examined because of contamination		

30258 and 6241. All of these pairings were incompatible (see Fig. 30). For a summary of all intercompatibility tests see Table 3.

Interfertility

All reference collections were intercompatible and interfertile (see Fig. 31). Each reference collection pairing produced from one to four hybrid fruitings with viable spores. Reference collection (F_1) colors are listed in Table 4. Collection **7839** (see Fig. 32) displayed yellowish white, yellowish gray, orange gray, grayish orange, yellowish brown, and grayish brown. Collection **6346** (see Fig. 33) exhibited white, yellowish white, yellowish gray, grayish brown, reddish gray, orange white, pinkish white, pale red, and pink. Collection **IBUG 7₁** (see Fig. 34) demonstrated white, yellowish white, yellowish gray, orange white, pinkish white, pale red, and pink. Collection **3467** (see Fig. 35) ranged from white, yellowish white, yellowish gray, grayish yellow, to orange gray. Collection **7444** (see Fig. 36) displayed yellowish white, light brown, brown, grayish brown, brownish orange, grayish orange, and dark brown. Collection **TMI 31760** (see Fig. 37) showed the least variation and ranged from white, yellowish white, to yellowish gray. All reference collections had a hygrophanous margin by maturity except 7444.

F_2 Hybrid **6346:8 x 7839:12** ranged from pastel red, pale red, pinkish white, fading to orange white, pale orange, yellowish white, white, (20/17/16). F_2 basidiomata 6346:8 x 7839:2 varied from pinkish white to orange white, grayish orange, yellowish white to white, (20/20/19). F_2 basidiomata 6346:11 x 7839:5 ranged from pastel red, pale red, pinkish white, orange white, grayish red, brownish orange, grayish orange to yellowish white, (20/16/12). F_2 basidiomata 6346:5 x 7839:2 exhibited white, yellowish

Table 3. Summary of intercompatibility tests.

Intercompatibility group	Collections
1. <i>Pleurotus djamor</i>	7839, 6346, 7444, IBUG 7 ₁ , 3467, TMI 31760, RDL1, 6275, 6270, 8013, K7018, IBUG3, MCADOO, 7840, 4326, IBUG 53, 4375, 4331, IBUG 7 ₂ , 7167, PDD 88115, VR 1786, JAPAN, TMI 30258, MF 1-18, 441A, 6278, ASM 7021
2. <i>Pleurotus opuntiae</i>	6241, 6276, 6277, ET 3313
3. <i>Pleurotus</i> sp.	7826

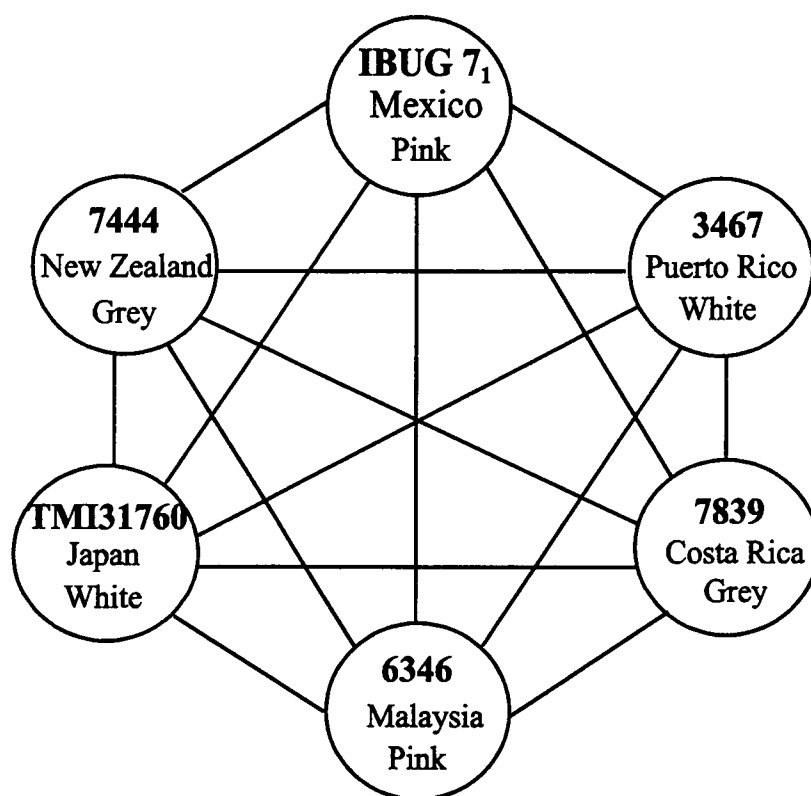


Figure 31. Lines connected between reference collections indicate positive intercompatibility and interfertility tests.

Table 4. Colors exhibited by reference collections of *Pleurotus djamor*.

Reference Collection	Colors from nature	Colors of fruited collection
7839	“ivory yellow”, “deep olive buff”, “pale olive buff”, “light grayish olive”	yellowish white (4A2), yellowish gray (4B2), orange gray (5B2), grayish orange (5E3), yellowish brown (5E4)
6346	pink	pink (11A3), pale red (10A3), pinkish white (11A2-7A2), orange white (5A2), reddish gray (7B5), yellowish gray (4B2), yellowish white (4A2), white
3467	“tilleal buff” to “pale pinkish cinnamon”	white, yellowish white (4A2), yellowish gray (4B2), grayish yellow (4B3), orange gray (5B2)
IBUG 7 ₁	pink	pink (11A3), pale red (10A3), pinkish white (11A2-8A2), orange white (5A2), yellowish gray (4B2), yellowish white (4A2), white
7444	“fuscous”, “tawny olive”, “chamois”, off-white to white	dark brown (6F4), grayish orange (5B3, 5B5), grayish brown (5D3), brownish orange (5C4), brown (6D4, 6E4), yellowish white (4A2)
TMI 31760	white	white, yellowish white (4A2), yellowish gray (4B2)

Colors in “” are from Ridgeway (1912).

Colors in () are from Kornerup and Wanscher (1978).



Figure 32. Photograph of reference collection 7839 basidiomata



Figure 33. Photograph of reference collection 6346 basidiomata



Figure 34. Photograph of reference collection IBUG 7₁ basidiomata



Figure 35. Photograph of reference collection 3467 basidiomata



Figure 36. Photograph of reference collection 7444 basidiomata



Figure 37. Photograph of reference collection TMI 31760 basidiomata

white, pale yellow, yellowish gray, grayish yellow, orange gray, and grayish orange, (20/20/18).

F₂ Hybrid **7444:14 x 7839:12** ranged from brownish orange, grayish orange, grayish brown, yellowish white, to white, (20/5/4). No hygrophanous areas were seen. F₂ basidiomata 7444:15 x 7839:6 varied from light brown, brownish orange, grayish orange, orange gray, to yellowish white, white, (20/10/8). Pileus margin became hygrophanous in age. F₂ basidiomata 7444:15 x 7839:2 ranged from brownish orange, grayish orange, orange gray, to yellowish white, (20/17/14). Pileus margin became hygrophanous in age.

F₂ Hybrid **3467:7 x 7839:5** ranged from white, yellowish white, grayish yellow, grayish orange, to grayish brown, (20/18/17). F₂ basidiomata 3467:2 x 7839:12 displayed colors of orange gray, brownish orange, yellowish white, and yellowish gray, (20/19/19). F₂ basidiomata 3467:8 x 7839:2 varied from white, yellowish white, yellowish gray, to grayish yellow, (20/19/18).

F₂ Hybrid **TMI 31760:9 x 7839:2** varied from white, yellowish white, orange gray, to grayish orange, (20/20/17). F₂ basidiomata TMI 31760:7 x 7839:2 displayed colors of white, yellowish white, yellowish gray, grayish orange, orange gray, brownish orange, and grayish brown, (20/20/20). F₂ basidiomata TMI 31760:4 x 7839:12 ranged from orange gray, grayish orange, yellowish gray, yellowish white to white, (20/20/20). F₂ basidiomata TMI 31760:4 x 7839:5 displayed colors from brown, grayish brown, brownish orange, greyish orange, yellowish gray to yellowish white, (20/20/20).

F₂ Hybrid **IBUG 7₁:20 x 7839:2** ranged from orange gray, grayish orange, brownish orange, yellowish gray, yellowish white, pale yellow to white, (20/20/14). F₂

basidiomata IBUG 7₁:7 x 7839:6 varied from white, orange gray, grayish orange, to yellowish white, (20/20/16).

F₂ Hybrid **3467:7 x 6346:11** displayed colors from pinkish white, brownish orange, grayish brown, orange white, grayish orange, to yellowish white, (20/20/20). F₂ basidiomata 3467:2 x 6346:8 ranged from pale red, pinkish white, orange white, yellowish white to white, (20/20/20). F₂ basidiomata 3467:8 x 6346:8 varied from white, pinkish white, pale red, pastel red, reddish gray, orange white, pale orange, orange gray to yellowish white, (20/20/19).

F₂ Hybrid **IBUG 7₁:20 x 6346:8** ranged from pinkish white, pale red, pale orange, orange white, grayish red, brownish orange to white, (20/20/19). F₂ basidiomata IBUG 7₁:11 x 6346:8 displayed colors from pale red, pinkish white, orange white, yellowish white, to white, (20/20/20). F₂ basidiomata IBUG 7₁:2 x 6346:8 varied from pastel red, pale red, pinkish white, grayish red, brownish orange, to grayish orange, (20/20/18). F₂ basidiomata IBUG 7₁:7 x 6346:5 ranged from white, orange gray, pale yellow, yellowish white, yellowish gray to grayish yellow, (20/20/18).

F₂ Hybrid **TMI 31760:4 x 6346:8** varied from pinkish white, pale red, orange gray, grayish orange, brownish orange, pale orange, orange white to white, (20/20/20). F₂ basidiomata TMI 31760:4 x 6346:5 ranged from orange gray, grayish orange, orange white, yellowish white to white (20/20/20). F₂ basidiomata TMI 31760:6 x 6346:8 exhibited colors from pinkish white, orange white, yellowish gray, yellowish white to white, (20/13/13).

F₂ Hybrid **7444:8 x 6346:5** ranged from dark brown, grayish brown, brownish

orange, grayish orange, orange gray, orange white to yellowish white, (20/13/11). Pileus became hygrophanous streaked in age. F₂ basidiomata 7444:14 x 6346:5 varied from brown, light brown, grayish brown, brownish orange, orange gray, grayish orange, grayish yellow, yellowish white, to white, (20/18/17). No hygrophanous areas were seen on the pileus. F₂ basidiomata 7444:15 x 6346:5 ranged from dark brown, brown, grayish brown, yellowish brown, brownish orange, grayish orange, yellowish gray, yellowish white to white, (20/16/15). Pileus margin became hygrophanous at maturity.

F₂ Hybrid **TMI 31760:4 x IBUG 7₁:7** exhibited colors from brownish gray, grayish brown, orange gray, grayish orange, yellowish white, yellowish gray to white, (20/17/15). F₂ basidiomata TMI 31760:4 x IBUG 7₁:11 ranged from orange gray, grayish orange, yellowish gray, yellowish white to white, (20/20/18). F₂ basidiomata TMI 31760:7 x IBUG 7₁:20 varied from white, yellowish white, to grayish orange, (20/20/17).

F₂ Hybrid **3467:8 x IBUG 7₁:11** ranged from orange gray, grayish orange, grayish brown, yellowish white, pale yellow, yellowish gray, grayish yellow to white, (20/?/?). F₂ basidiomata 3467:8 x IBUG 7₁:20 varied from orange gray, grayish orange, brownish gray, yellowish gray, yellowish white to white, (20/19/17). F₂ basidiomata 3467:7 x IBUG 7₁:7 exhibited colors from grayish orange, grayish brown, orange gray, yellowish gray, yellowish white to white, (20/?/?). F₂ basidiomata 3467:6 x 7444:20 ranged from brownish orange, brownish gray, grayish brown, orange white, yellowish gray, to yellowish white, (20/20/17).

F₂ Hybrid **7444:13 x IBUG 7₁:11** varied from brown, grayish brown, brownish orange, grayish orange, orange gray, yellowish white to white, (20/17/17). Pileus margin

became hygrophanous in age.

F₂ Hybrid **7444:8 x 3467:11** varied from brown, grayish brown, brownish orange, grayish orange, orange gray, orange white, pale orange, yellowish white to white, (20/15/14). F₂ basidiomata 7444:15 x 3467:7 ranged from brown, grayish brown, light brown, brownish orange, grayish orange, orange gray, light orange, yellowish gray to yellowish white, (20/9/9). F₂ basidiomata 7444:8 x 3467:6 varied from grayish brown, brownish orange, orange gray, pale orange, orange white, yellowish gray, yellowish white to white, (20/14/14). Pileus margin became hygrophanous in age for all of these hybrids.

F₂ Hybrid **TMI 31760:7 x 3467:11** ranged from grayish brown, brownish orange, grayish orange, orange gray, yellowish white to white, (20/18/18). F₂ basidiomata TMI 31760:19 x 3467:11 varied from white, yellowish gray, to yellowish white, (20/19/18). F₂ basidiomata TMI 31760:4 x 3467:7 displayed colors from grayish brown, brownish orange, orange gray, grayish orange, yellowish gray, yellowish white to white, (20/19/19). F₂ basidiomata TMI 31760:19 x 3467:7 ranged from grayish brown, brownish gray, orange gray, yellowish white to white, (20/20/19).

F₂ Hybrid **TMI 31760:4 x 7444:15** varied from dark brown, yellowish brown, grayish brown, brownish orange, grayish orange, orange gray, grayish yellow to yellowish white, (20/17/16). F₂ basidiomata TMI 31760:7 x 7444:8 ranged from dark brown, grayish brown, brownish orange, grayish orange, orange gray, yellowish gray, yellowish white to white, (20/15/13). F₂ basidiomata TMI 31760:19 x 7444:15 exhibited colors from dark brown, brown, light brown, yellowish brown, grayish brown, grayish orange, orange gray, to yellowish-white, (20/17/16). F₂ basidiomata TMI 31760:19 x 7444:8

ranged from dark brown, brown, grayish brown, grayish orange, brownish orange, orange gray, orange white, yellowish white to grayish orange, (20/14/14). In all of these hybrids pileus margin became hygrophanous by maturity.

CHAPTER 4

DISCUSSION

Cultures of mushrooms collected under the names *P. djamor*, *P. ostreatoroseus*, *P. salmoneostamineus*, *P. opuntiae*, and *P. flabellatus* were fruited under controlled laboratory conditions to standardize environmental and host effects on morphological characters. These fruitings produced pileus colors that were extremely variable between collections and often within a collection (See Table 2). Some collections fell within one of the major color morphs. TMI 31760, K7018, RDL1 and IBUG 53 ranged from white, yellowish white, yellowish gray to grayish yellow, and fell within the white-tan *P. djamor*. Other collections such as 6346 and ASM 7021 spanned two or three color morphs. Collection 6346 began as pinkish white, pastel red, reddish gray, fading to orange white, and becoming yellowish gray to yellowish white by maturity. A second flush of 6346 fruit bodies from the same "bag" remained pastel red to pinkish white through maturity. Collection ASM 7021 was pink to pastel red with grayish brown to brownish gray pubescence in the primordia and young basidiomata, fading to orange white, yellow white with orange gray pubescence by maturity. Later flushes from the same bag never exhibited pink colors. Still other collections such as 7839, 7840, 6270, 441A and 8013 ranged from yellowish gray, grayish yellow, grayish brown, brownish gray, orange gray, brownish orange, orange white to white. These collections blur the line between white-tan and gray morphs and exhibited an orange-white color which is not covered by any of the major color morphs. Collections PDD 88115, 7167, and 7444 were all dark brown in the primordial stage another color not described for any of the color

morphs. These collections also exhibited colors found in other members of the complex from yellowish white, orange-white, orange-gray, brownish orange, brownish gray to grayish brown. In addition, these three collections were all united by a lack of hygrophanous areas on the pileus, and geographic origin (New Zealand). Color morphology therefore is extremely variable even under controlled fruiting conditions, drawing into question the validity of species epithetes in this complex based on basidioma color.

These results align closely with Corner's (1981) study of the *P. djamor* complex from Malaysia in which he subdivided the species into six varieties based on color, micromorphology and habit as follows: 1) pileus and gills white to pallid ochraceous; pileus laterally attached for *P. djamor* var. *djamor*, pileus eccentric for *P. djamor* var. *cyathiformis*, and pileus merismatoid from terricolous habit for *P. djamor* var. *terricolus*. *P. djamor* fruited in the current study ranged from flabelliform with a lateral stipe to infundibulliform (cyathiform) with an eccentric stipe even within a single collection, dictated by whether fruitbodies were emerging from the sides or top of a bag. In addition, since no species in the genus *Pleurotus* are known to grow from the soil, *P. djamor* var. *terricolus* is likely just a typical white *P. djamor* growing from buried wood. 2) Corner (1981) also described two varieties with pink gills. *P. djamor* var. *roseus* had a pink pileus while *P. djamor* var. *fuscroseus* had a fuscous livid pileus. In controlled fruitings, collections such as TMI 30258 or 6346 displayed pileus colors that span both of these varieties. In fact, Corner (1981) mentioned the occurrence of intermediates among all these varieties. Only one variant, *P. djamor* var. *fuscopruinosus*, named because of its

fuscous pileus and umber-pruinose gill edge, was not observed in any collections used in my experiment.

Other recorders of this complex, Pegler and Heinemann (1972) from Central Africa, and Pegler (1977) from East Africa (both under the name *P. flabellatus*), mentioned that the young caps have a pink tint becoming white to ivory by maturity. Pegler (1986), describing the fungus from Sri Lanka, correctly referred to this species as *P. djamor*, and described the pileus with initially pinkish tints becoming white to cream colored or ochraceous brown with grayish tints. Although Guzman *et al.* (1992) separated pink *P. ostreatoroseus* from *P. djamor*, they noted the presence of pink tones in young caps in the pileus description for *P. djamor*. In 1995, Guzman *et al.*, changed their view of this complex. They named two pink varieties; *P. djamor* var. *roseus* with pink pileus and white spore print, and *P. djamor* var. *salmoneostramineus* with a pink pileus and salmon to pinkish brown spore print. These names are based on a sample of two collections from the field subsequently fruited in the laboratory. In addition they admit that under poor light conditions var. *roseus* fades to whitish and var. *salmoneostramineus* becomes pallid brownish beige. Cedano *et al.* (1993), however, stated that the pink color was lost in *P. ostreatoroseus*, fading to grayish or cream when exposed to light too long. These descriptions from widespread geographic locations indicate the plethora of colors that this species complex potentially exhibits.

Murakami and Takemaru (1990) found that a wild collection of *P. salmoneostramineus* occasionally formed albino basidiocarps when fruited in the laboratory. The albino form was isolated and crossed with the typical pink morph.

Offspring ratios indicated that the albino form was formed from a simple recessive mutation. They called into question the separation of *P. salmoneostramineus* from *P. djamor*.

Micromorphology described for members of the *P. djamor* complex has also been variable. The context has been described as both monomitic (Pegler and Heinemann, 1972; Pegler, 1977; Singer, 1986) and dimitic (Singer, 1961; Corner 1981; Pegler 1986; Guzman *et al.*, 1992; Cedano *et al.*, 1993; Guzman *et al.*, 1995). A monomitic hyphal system has only generative hyphae, which are characterized by thin walls, cross walls and clamp connections. A dimitic hyphal system has both generative hyphae and skeletal hyphae, the later are characterized by thick walls and no septa. Skeletal hyphae arise from generative hyphae (Corner, 1953).

Singer (1961) did not describe hyphal construction of the pileus context. He did mention hyphae with thick walls in the hymenophoral trama, indicating a dimitic system. He also mentioned that all hyphae bore clamps, not a characteristic of skeletal hyphae (Corner, 1981; Pegler, 1986; Guzman *et al.*, 1992; and current study) drawing into question what type of hyphae Singer observed. Corner (1981), Pegler (1986), and Guzman *et al.* (1992) all described the context as monomitic when young, generative hyphae with clamp connections, becoming dimitic in age with long, twisted, terminally attenuate, thick-walled skeletal hyphae. Corner (1981) described the skeletal hyphae as rarely branched, while Guzman *et al.* (1992), Cedano *et al.* (1993) described the skeletal hyphae as unbranched. Pegler (1986) did not specifically mention the branching state of the skeletal hyphae. In the current study, skeletal hyphae were common, however,

branched skeletal hyphae were rarely observed. The basic description of the pileus context given by Corner (1981), Pegler (1986) and Guzman *et al.* (1992) agreed with the morphology exhibited by collections of *P. djamor* examined in this study, including the monomitic context of young specimens

The pileipellis has been described as a thin layer (cutis or epicutis) of repent, radially parallel, hyaline generative hyphae (Pegler and Heinemann, 1972; Pegler, 1977; Corner, 1981; Pegler, 1986; Guzman *et al.*, 1992; Cedano *et al.*, 1993; Guzman *et al.*, 1995). Pegler (1986) described the hyphae as rarely branched, often forming fascicles. In his earlier work on this species, Pegler and Heinemann (1972) and Pegler (1977), made no mention of any fascicles in the epicutis. Corner (1981) described the hyphae as mostly unbranched and often fasciculate, with no special cystidia. Guzman *et al.* (1992), however, referred to these fascicles as ventricose-mucronate to subclaviform dermatocystidia up to 36 μm . In a later work on the “pink varieties” of *P. djamor* Guzman *et al.* (1995) referred to these fascicles as subcylindric to subclavate pileocystidia 14.4–44 μm long. Cedano *et al.* (1993) described the fascicles as pileocystidia 18.4–48.8 μm long, cylindrical, subclavate, sometimes mucronate or with an ovoid to globose apex,. Singer (1961) did not describe the micromorphology of the pileipellis. The differences reported may merely reflect differences in terminology, with Guzman *et al.* (1992, 1995) and Cedano *et al.* (1993) considering these hyphae as differentiated cystidia while Pegler (1986) and Corner (1981) did not view them as specialized hyphae worthy of cystidial terminology. In this study, the pileipellis was seen as an epicutis composed of filamentous to somewhat inflated clamped generative hyphae

that were repent to suberect, often forming fascicles. The hyphae were not viewed as specialized enough to warrant cystidial terminology.

The hymenophoral trama was dimitic, irregular (interwoven), with hyaline, clamped, generative hyphae and unbranched skeletal hyphae (Singer, 1961; Pegler, 1986; Guzman *et al.*, 1992; Cedano *et al.*, 1993; Corner, 1981; Guzman *et al.*, 1995). Pegler and Heinemann (1972) and Pegler (1977) also described the hymenophoral trama as irregular but with only clamped generative hyphae. The current study found the hymenophoral trama to be dimitic and irregular except in very young specimens in which it was monomitic.

The subhymenium was described by Corner (1981) and Pegler (1986) as well-developed with interwoven hyphae, while Singer (1961) described it tersely as well-developed. Pegler and Heinemann (1972) and Pegler (1977) found the subhymenium to be well-developed and composed of pseudoparenchymatous cells. Guzman *et al.* (1992) reported the subhymenium as comprising irregular globose elements and intermixed hyphae with thick walls, projected through the hymenium as pseudocystidia. This is the only report of pseudocystidia found in any descriptions of this species. Descriptions of this species by Cedano *et al.* (1993) and Guzman *et al.* (1995) did not mention the subhymenium. The current study found the subhymenium to be pseudoparenchymatous often with pseudocystidia. Pseudocystidia were found in collections from all geographic areas. One specimen (PDD 88115) produced branched pseudocystidia.

Basidia were described by all authors as hyaline, narrowly clavate with four sterigmata (Singer, 1961; Pegler and Heinemann, 1972; Pegler, 1977; Corner, 1981;

Pegler, 1986; Guzman *et al.*, 1992,1995; Cedano *et al.*, 1993). The reported size of basidia has varied greatly (See Table 5). Basidia measured in this study were variable spanning the ranges described above $16.2\text{-}32.04 \times 4.68\text{-}7.92\mu\text{m}$.

Spores have been described as thin-walled, inamyloid, hyaline, oblong-elliptic to subcylindric with well-defined hilar appendix (Singer, 1961; Pegler and Heinemann, 1972; Pegler, 1977; Corner, 1981; Pegler, 1986; Guzman *et al.*, 1992; Cedano *et al.*, 1993; Guzman *et al.*, 1995). While the overall description of the spores was the same, their reported sizes varied (See Table 5). Cedano *et al.* (1993) reported the largest variance in spore size ranging from $(5.6\text{-}) 7.2\text{-}10.4(-11.2) \times 3.2\text{-}4 (-4.8)\mu\text{m}$. This larger variance was also observed in the current study with spores measuring $6.84\text{-}13.32 \times 3.6\text{-}5.76\mu\text{m}$. Spore sizes varied widely within collections from all regions.

Cheilocystidia size has been described by several authors (See Table 5). Singer (1961) reported cheilocystidia as clavate, hyaline rarely with a mucro or subcapitate, then he goes on to say they are variable in size and shape, with some swollen venticose bodies. Pegler and Heinemann (1972) and Pegler (1977) also reported variable cheilocystidia clavate to cylindric, sometimes constricted with a obtuse apex, at times narrowed into elongate mucronate up to $14\mu\text{m}$ long. Pegler (1986) described a more limited range of cheilocystidia shapes as clavate, sometimes with mucronate apex. According to Corner (1981) cheilocystidia are clavate, subventricose, some mucronate or capitate. Guzman *et al.* (1992) described extremely variable cheilocystidia as subclaviform, subcylindric, subspheropendunculate, subventricose, sometimes with irregular constrictions, frequently with a mucro or capitate, occasionally with an appendix up to $13\mu\text{m}$ long, or apical

Table 5. Summary of reported basidia, cheilocystidia, and spore sizes for the *Pleurotus djamor* species complex.

Authors	Cheilocystidia	Basidia	Spores
Singer (1961)	22-40 x 7-9 μm	15-20 x 5-5.5 μm	7.5-10.3 x 3-4 μm
Pegler and Heinemann (1972), Pegler (1977)	19-35 x 5-9.5 μm	19-21 x 3.5-4.5 μm	6-8.3 x 3-4 μm
Corner (1981)	20-40 x 6-11 (-13) μm	20-25 x 5-6 μm	5.5-10 x 2.5-4.5 μm
Pegler (1986)	16-23 x 6-10 μm	14-20 x 5-6 μm	6-9 x 3-3.7 μm
Guzman <i>et al.</i> (1992)	(12.8-) 16-32 x (4-) 5-9 (-11) μm	(16.8-) 20-28 (-40) x (3.5-) 4-5.6 (-6.4) μm	6.4-10.4 x 3.2-4.8 μm
Cedano <i>et al.</i> (1993)	(14.4-) 16-33.6 (-44.4) x 4.8-11.2 (-13.2) μm	18.4-27.2 x 4.8-8 μm	(5.6-) 7.2-10.4 (-11.2) x 3.2-4 (-4.8) μm
Guzman <i>et al.</i> (1995)	(16-) 18.4-28 x 6.4-9.6 (-12) μm	(20-) 24-28 x 4.5-5.6 (-6.4) μm	6.4-10.4 x 3.2-4.8 μm
Current study	14.0-35.3 x 4.7-11.9 μm	16.2-32.0 x 4.7-7.9 μm	6.8-13.3 x 3.6-5.8 μm

mucilaginous halo or drop. Guzman *et al.* (1995) simply defined cheilocystidia as widely claviform for the two pink “varieties” of *P. djamor*. Cedano *et al.* (1993) reported variable cheilocystidia, clavate, subcylindrical, subventicose, spheropendunculated, with or without a mucron, or apex, rarely with two apices, sometimes with apex scantily inflated.

In the current study, cheilocystidia were observed that were variously shaped, ranging across the spectrum of all the shapes listed above. These cheilocystidia were also variable in size which is in general agreement with Guzman *et al.* (1992) and Cedano *et al.* (1993) (See Table 5). Other authors, measurements also fell within this range (Singer 1961; Pegler and Heinemann, 1972; Pegler, 1977; Corner, 1981; Pegler, 1986; Guzman *et al.* 1995) although some were much more limited in range (Pegler, 1986; Guzman *et al.* 1995). Cheilocystidia with a long appendix were mentioned by Pegler and Heinemann (1972) and Pegler (1977) for Central Africa and East Africa respectively. Guzman *et al.* (1992) described this long appendix for material from Central America, Cuba, Kenya, Ethiopia, and Australia. However, no authors explicitly stated in which collections it was observed. This shape was observed in the current study in only two collections from Japan (TMI 30258, JAPAN). Another rare shape are cheilocystidia with two globose heads (bicapitate) reported only by Cedano *et al.* (1993) for Mexican collections, while in the current study it was observed in three collections, two Mexican collections (IBUG 7₁, 4331) and one Japanese collection (TMI 31760).

Pleurocystidia were stated not to exist or were not described, indicating they were not present, according to early micromorphological descriptions of *P. djamor* (Singer,

1961; Pegler and Heinemann, 1972; Pegler, 1977; Corner, 1981; Pegler, 1986; Guzman, 1992). Cedano *et al.* (1993) stated that pleurocystidia were present in only one specimen, and were subventricose, clavate, mucronate or with an appendix $4-8.8 \times .8-2\mu\text{m}$. Guzman *et al.* (1995) described pleurocystidia as absent, but described cystidioid elements as $14.4-20 (-26.4) \times (4-) 4.8-6.4$, ventricose, mucronate or lecythiform, frequently with an apical mucilaginous drop, common on sides of gills. The location of these cystidioid elements begs the question why these were not considered pleurocystidia. Pleurocystidia were found in this study in many collections from diverse geographic areas including Central America (7839, MCADOO, 7840, 4331, RDL1, IBUG 39, IBUG7₁, IBUG3, 4375 6275), North America (441A), Japan (TMI 30258, TMI 31760, JAPAN) and New Zealand (PDD 88115). They varied from clavate to ventricose, ventricose-rostrate, mucronate or often capitate as indicated by Cedano *et al.* (1993) and Guzman *et al.* (1995). The lack of pleurocystidia mentioned by some authors may have resulted because pleurocystidia were not thought to exist in this species and therefore these authors did not carefully search for them.

There appear to be no particular micromorphological features which subdivide this complex by any broad geographic area or by any color morph. Although no definitive character exists to separate these collections, a phenetic study of overall similarity using (macro- and micromorphological) characters was undertaken. This was done in order to ascertain whether some more subtle but consistent differences occurred that could be detected between collections by processing phenetic data.

When just quantitative micromorphological characters were used to delineate

phenetic relationships, there was some clustering by location (see Fig. 10) but the groupings were not clearcut. All the Asian collections (6346, TMI 30258, TMI 31760 and JAPAN) were in the same major branch starting at distance at 0.80, but three Central American collections (IBUG 7₁, MCADOO, 6275) were also intermixed. New Zealand collections (PDD 88115, 7444, 7167) in the same branch beginning at a distance of .61 but again several Central American collections (IBUG 39, 4331, 7840) were interspersed. The last remaining cluster began at a distance of 0.66 and contained collections from South America, Central America and North America but with no geographic pattern evident. Three different clustering algorithms were used for single, average, and complete linkage. All three trees were almost identical (see Figs 8, 9, 10), varying mostly in the distances at which different clusters diverged. It appears that there is high similarity, based on micromorphological characters, among collections from diverse geographic locations. These collections may not be taxonomically separable by geographic location due to large variation in characters between collections within an area and large amount of character variation overlap between collections from distant origins.

Qualitative characters were also used to create a tree of phenetic relationships (see Fig. 11). There were three major clusters created, one containing collections from Asia and the New World, a second cluster comprised two collections from Japan, while the third cluster included collections from New Zealand and North America. The large number of collections that were not separated by any distance indicates that this tree has little resolution. In addition there is no tight grouping by location other than the three New Zealand collections that form a branch. These New Zealand collections are likely

held together by a lack of hygrophanous areas on the pileus and their unique dark brown coloration when young.

Both data sets were combined in an analysis of all characters using single, average and complete linkage algorithms. The average linkage method created a tree with five major branches. Branch one contained only a North American collection, the next major branch to this one contained only New Zealand collections. The third major branch contained collections from the New World tropics and one collection from Japan. The fourth cluster contained two collections one from Mexico and one from Malaysia. The last branch contained two collections from Japan (see Fig. 12). Although there appears to be some grouping by geographic origin (New Zealand and Japan) again it is not consistent with a new world (IBUG 7₁) and Asian (6346) collection also being most phenetically similar. Using a single linkage algorithm, collections shifted, with many New World collections now becoming part of the New Zealand branch (see Fig. 13). ASM 7021 diverged distantly from all other collections, while two clusters remained the same as above (JAPAN and TMI 30258; 6346 and IBUG 7₁). The complete linkage algorithm phenogram was almost identical to the average linkage tree, differing mainly in distances at which clusters diverged (see Fig. 14). The large differences between the single and average linkage trees indicate sizeable noise in the data, with collections displaying much overall phenetic similarity. The large number of color characters may overwhelm the analysis, creating trees heavily but not entirely determined by pileus coloration. Also it must be noted that some information is lost by converting quantitative characters into qualitative ones. For example, a collection with mean spore length of 8.9

μm will be grouped with collections with mean spore length of $8.0\ \mu\text{m}$ while it is technically more similar to a collection with a mean spore length of $9.1\ \mu\text{m}$.

Overall there appears to be a high amount of morphological phenetic similarity and overlap between collections in all of these analyses (quantitative, qualitative, and combined). The New Zealand collections clustered together in all analyses although not always by themselves. This indicates that these collections are very similar to each other and may represent a separate group. In addition they share the exclusive characteristics of the absence of hygrophanous areas on the cap and dark brown coloration when young. However, there is certainly much phenetic similarity with other collections of *P. djamor* and because only three collections were available it would be unwise to make definitive statements about their specific status until more collections are examined. Other groupings by major geographic area (see Fig. 10) also indicate that geographic barriers (e.g., oceans) may prohibit these collections from interbreeding with other populations thus preventing one large panmitic taxon. If this is true then character variation is high and mostly overlapping, making morphological differentiation difficult to discern. If these populations have been isolated since continents separated then morphological characters have not diverged under the influence of genetic drift, selection, mutation or other evolutionary factors. The other possibility is that these populations are part of one large panmitic taxon that has the ability for long-distance spore dispersal or are interbreeding by human intervention (i.e., cultivation). Because this species is fruited for consumption over much of the world, fruitbodies may result from strains that originated in another part of the world, potentially releasing spores that became part of the local

gene pool.

Reference collections were selected to represent the range of major color morphs and geographic origins present in this species complex. All six reference collections were intercompatible with a minimum of 7 and a maximum of 16 of 16 pairings compatible for all crosses. Some absence of compatible pairings in intercollection grids was a result of contamination of the Petri plate [i.e., several pairings of 6346 x 7444 (see Fig. 21)]. In addition there were some strain-specific responses (i.e., 6346:4 was incompatible in all its pairings with 7839 yet all other pairings between these collections were compatible.)

SSIs of *P. djamor* are not vigorous for prolonged periods of time. Continued subculturing of SSIs, necessary in order to produce sufficient mycelia for pairings, often caused their growth to slow down and sometimes effectively stop. This not only increased their potential for contamination, but made pairings between the same tester strains difficult to accomplish across all collections, because new SSIs frequently had to be established from the culture collection. Pairings between slow-growing SSIs often were incompatible where vigorously growing SSIs from the same collections were compatible. For example, the growth of 6346:13 was extremely slow and formed a dikaryon in only one pairing with 3467 (see Fig. 23). 6346:8 had a healthy growth rate and was compatible in all pairings with 3467. Other incompatible pairings may be a result of shared mating alleles between collections. Collection IBUG 7₁ SSIs 7 and 11 were incompatible with 3467 SSIs 6 and 11. IBUG7₁:7 and 11 share the same B mating allele, as do 3467:6 and 11, therefore it is possible that that both collections carry the same B allele. This incompatibility was also observed for 6346:11 and 13 x IBUG 7₁:7

and 11. Collection 6346:11 and 13 also share a common B allele, indicating 6346 and IBUG 7₁ may also share a B allele. More pairings would be required to validate the presence of shared mating alleles. Overall it appears that these reference collections are universally intercompatible with some strain-specific responses (physiological) present.

Other putative *P. djamor* collections were paired with one of these reference collections to assess their potential inclusion in this intercompatibility group. The following collections exhibited full compatibility with one of the reference collections: RDL1, 6275, 6270, K7018, IBUG 3, MCADOO, 7840, 4326, IBUG53, 4375, 4331, ASM 7021, 7167, PDD 88115, Villegas-Rios 1786, JAPAN, TMI 30258, MF1-18, 441A and 6278 (see Table 4). This compatibility combined with overall macro- and micromorphology (of collections examined), indicate that these collections are members of the *P. djamor* complex. Collection 8013 SSI pairings were 50% compatible 50% incompatible with Collection 7839. This may be a strain-specific response or these collections may share mating alleles as described previously.

Collections 6276, 6277, 6241, ET 3313, IBUG 39, 7826 were incompatible with reference collections in all crosses. IBUG 39 was successfully fruited and was macro- and micromorphologically congruent with other collections from the *P. djamor* intercompatibility group. These monokaryons were made using a stress-creating technique called neohaploidization (Petersen, 1992). These neohaplonts were extremely slow growing with much hyphal lysis, indicating that they may not behave “normally” when paired with other SSIs. Unfortunately time did not permit new SSIs from fruited material to be crossed with a reference collection.

Collections 6276, 6277, and ET 3313 never fruited so basidiome morphology of these was not assessed. Collection 6241, however, did fruit and displayed morphological characteristics distinct from *P. djamor*, including a strongly inrolled margin, distinctly infundibulliform pileus with floccose gray patches, long tapering stipe and a nutty smell. This collection was tentatively given the name "*P. agaves*" (Petersen, 1995a) and has now been identified as *P. opuntiae* (Petersen, pers. comm.). SSIs of other collections, originally collected as "*P. opuntiae*" (6276, 6277, and ET 3313), were then paired with SSIs of 6241. Almost all pairings were compatible (see Fig. 30) indicating these collections all belong to the same intercompatibility group, separate from *P. djamor*. The only incompatible pairings were 6277:3 x 6241:1,12. This could be either strain-specific response or the presence of shared mating alleles. IBUG 7₂ was unique because it was incompatible in all pairings with IBUG 7₁ and compatible in all pairings with 6346. The most likely explanation is since both collections (IBUG 7₁ and IBUG 7₂) are from Mexico they may have the same mating alleles. Collection 7826 was incompatible with all other collections indicating it is part of a separate intercompatibility group (see Fig. 30).

Interfertility tests were performed between all reference collections (see Fig. 31). F₂ dikaryons were successfully fruited with viable monokaryotic spores which grew normally, indicating that not only are these collections compatible (which may be determined by a small number of genes), but were similar enough for haploid genomes to line up and go through karyogamy and meiosis.

F₂ hybrid color morphology was as variable as the parental reference collections that produced them. Basidiomata of collection 6346 ranged from pink, pale red, pinkish

white, reddish gray, yellowish white to white while basidiomata collection 7839 were yellowish white, yellowish gray, orange-gray to grayish brown. Four hybrids were produced, one varied from pastel red, pinkish white, to orange-white, yellowish white, two others varied from pastel red, pinkish white, but also displayed some grayish orange and brownish orange colors, to yellowish white. While the fourth hybrid never exhibited any pinkish colors, basidiomata ranged from white, yellowish white, yellowish gray, orange-gray, to grayish orange. Collection IBUG 7₁ which ranged from pink, pale red, pinkish white, orange white, yellowish gray, yellowish white was crossed with 7839. No hybrid basidiomata produced any pink colors, and varied from white, yellowish white, yellowish gray, grayish orange, to orange gray. Hybrids of 6346 and IBUG 7₁ all exhibited pinkish white to pink colors, but some displayed colors not specifically described from the parents, such as brownish orange or grayish orange. Collection 7444 had unique characteristics, including no hygrophanous areas and dark brown coloration, not present in other members of this complex. F₂ hybrid from 7444:15 x 6346:5 seemed to display characteristics of 7444 when young with dark brown, brown, grayish brown, or yellowish brown pileus becoming more like 6346 in age, fading to yellowish gray, yellowish white to white, with a hygrophanous margin. However, F₂ hybrid 7444:14 x 6346:5 never exhibited any hygrophanous areas on the pileus. In addition, crosses between "pink" reference collections (IBUG 7₁, 6346) and 7444 produced no basidiomata with evident pink coloration. Collection 3467 was white, yellowish white, yellowish gray, grayish yellow to orange-gray, while collection TMI 31760 ranged from white, yellowish white, to yellowish gray. F₂ basidiomata from these crosses displayed colors as

dark as grayish brown, to brownish gray, and brownish orange which were not exhibited by the parents. Based on all these results, color morphology in this species is complex, possibly variably inherited and certainly variably expressed.

Spore survival after germination appeared to be lower in some hybrids between collection 7444 and other reference collections. 7444:14 x 7839:12: only four of 20 SSIs survived; 7444:15 x 7839:6, 8 SSIs survived, 7444:15 x 7839:2, 14 SSIs survived. Other reference collection crosses often showed greater SSI survivorship of isolated spores. In the four crosses of TMI 31760 x 7839, 17, 20, 20 and 20 SSIs survived out of 20 spores isolated for each. However, other reference collections such as IBUG 7₁:20 x 7839:2, IBUG 7₁:7 x 7839:6 had lower survivorships, 14 and 16 SSIs survived respectively out of 20 isolated for each. This potential reduced survivor rate between 7444 and other collections can only be stated tentatively because of the low sample size. It is difficult to discern whether this truly indicates reduced vigor or fitness between collections, or is simply variation resulting from the harvesting method itself.

Several authors have examined intercompatibility in this species complex. Neda *et al.* (1988) found *P. salmoneostramineus* to be compatible with *P. djamor*. Petersen (1992) and Petersen and Hughes (1993) reported *P. ostreatoroseus* (Mexico) was compatible with *P. salmoneostramineus* (Japan). However, they also found two collections of the white to tan morphs of *P. djamor*, 3467 from Puerto Rico and 4326 from Mexico, to be incompatible with *P. ostreatoroseus* and *P. salmoneostramineus* (Petersen and Hughes, 1993). This most likely is a result of some strain-specific response, given the small number of crosses performed. The current study, using

collection 3467 found it to be compatible with different "*P. djamor*" morphotypes from widespread geographic areas.

Petersen (1995) found compatibility among pink, gray and white to tan morphological variants of this complex. Guzman *et al.* (1995) described two pink varieties of *P. djamor*, var. *roseus* and var. *salmonostramineus*, as compatible with typical white *P. djamor*. In addition they mentioned that there were no micromorphological characters that distinguished these varieties. Studies by Cedano *et al.* (1993) and Guzman *et al.* (1995) also stated that these "pink" varieties or "species" can lose their color, making them indistinguishable from white-tan *P. djamor*. Interestingly, Cedano *et al.* (1993) reported that under intense light conditions the pink color is lost while Guzman *et al.* (1995) stated that the pink color is lost under low light conditions. The current study found that loss or retention of pink coloration was not simply dependent on light intensity. The variable coloration within collections indicated that the pink color is probably affected by a variety of environmental conditions combined with the genetic makeup of the organism.

The widespread intercompatibility and interfertility reported for members of the *P. djamor* complex place many diverse collections into one "biological" species. It should be noted that several collections (6277, 6276, ET313, 6241) in the intercompatibility group appear to be a distinct species *P. opunitae*. However, several collections that were collected as *P. opunitae* were found to be *P. djamor* as determined by morphology and intercompatibility (441A, 7839, 6278, 6270). Following the phenetic species concept there is no morphological dissimilarity that clearly subdivides this

complex. There is a suggestion that the New Zealand collections examined are more similar to each other than other collections of *P. djamor* but with much phenetic overlap. Following a more traditional morphological species concept one could propose that the New Zealand collections are a subspecies of *P. djamor*, based on their unique characters of dark brown coloration and lack of hygrophanous areas on the pileus, but certainly more collections need to be examined to substantiate the consistency of these differences. The three species concepts utilized (biological, phenetic and morphological) provide similar results with none furnishing any consistent differences on which to base segregation of any collections of *P. djamor* examined in this study. Several lines of evidence suggest there may be some differentiation between members of this complex. There was clustering of collections from the same broad geographic area together in phenograms, albeit with collections from other areas interspersed. The morphological differences for New Zealand collections and the possible reduction in spore viability of hybrids from the New Zealand reference collection 7444 also suggest some differentiation by geography. In addition, Petersen and Ridley (1996) found a New Zealand *Pleurotus* with multiple-species sexual compatibility, highlighting the need for caution in extrapolating species status based solely on intercompatibility between allopatric populations.

The *P. djamor* species complex has been examined phylogenetically using DNA sequences and isozymes (Vilgalys and Sun, 1994; Zervakis and Labarere, 1992; Zervakis *et al.*, 1994), and found to segregate as a distinct clade compared to other *Pleurotus* taxa. Phylogenetic relationships within this species complex have not been examined. A study of *Pleurotus ostreatus* (Matsumoto and Fukumasa-Nakai, 1995) using restriction

fragment length polymorphisms revealed that geographic distances between natural populations, from Japan, Germany, Czechoslovakia, Hungary, France, Korea and U.S.A., were correlated with genetic divergence. Based on their results for *P. ostreatus* and the paucity of information on long-distance spore dispersal in mushrooms, genetic analysis such as DNA sequencing, RFLP's or protein electrophoresis appears to be the next major step in understanding gene flow between individuals and populations in this widespread *P. djamor* complex.

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