A reevaluation of taxa of *Clavicorona* subg. *Ramosa* based on morphology, compatibility, and laccase electrophoretic patterns

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Clavicorona subg. *Ramosa* species are not easily distinguished morphologically and the Southern Hemisphere taxa are reluctant to dikaryotize in vitro, making determination of biological species difficult. In this paper we present a comparison of basidiome and culture mat morphological data, compatibility data and laccase isozyme profiles of all taxa for which preserved basidiomata and cultures were available. Biological species were usually congruent with morphospecies. Electrophoresis of laccase allozymes revealed patterns that were largely invariant within species or within geographically isolated infraspecific subgroups and could be used to identify taxa. The conserved laccase isozyme for *Clavicorona pyxidata*, a species with a Northern Hemisphere distribution, was invariant in 140 collections from North America and Europe, but was different from two isolates from China. Results of this study confirmed that extracellular laccase patterns are of significance in the systematics of *Clavicorona*. Combined evidence from morphology, incompatibility systems and laccase electrophoretic patterns led to a revised morphological and geographical distribution analysis and a recognition of four more taxa within the subgenus.

Keywords: *Clavicorona*, systematics, laccase, mating systems, distribution.

The literature that includes treatments of *Clavicorona* is significant and includes taxonomically inclusive works (Doty, 1947; Corner, 1950, 1970; Petersen, 1988), generic floristic monographs (Dodd, 1972; Wu, 1991), and descriptions of individual species (Leathers & Smith, 1967; Wu & Petersen, 1992). The number of taxa accepted in the genus by individual authors has varied, but all authors agree that morphological characters are difficult to observe (e. g. ornamentation patterns on minute basidiospores), and are often obliterated in the preservation process (e.g. shapes and sizes of cystidia and gloeocystidia). In this genus, type specimens are of particularly limited use since they are often over a century old, and poorly preserved from the outset.

Dodd proposed two subgenera, *Clavicorona*, with unbranched fruitbodies on duff or litter, and *Ramosa*, with branched fruitbodies on wood (Dodd, 1972). Jülich segregated subg. *Ramosa* as genus *Artomyces* (Jülich, 1981). While Dodd was able to delineate the North American taxa of subg. *Ramosa*, coupling observations on fresh specimens with those on herbarium material, his coverage of extralimital taxa was primarily based on type specimens and solely on morphology. Petersen (1988) accepted only two species for New Zealand based on morphology. Wu (1991) added two additional taxa for New Zealand based on morphological analysis of additional material, sexual compatibility tests and some laccase enzyme analyses.

Production of extracellular laccase (EC 1.14.18.1 or EC 1.10.3.2) is a common property of wood-rotting basidiomycetous fungi. Fungal laccases are extracellular phenoloxidases that can oxidize monophenols, *o*- and *p*-diphenols, amino phenols and diaminoaromatic compounds (Reinhammar, 1984). They are commonly produced by white-rot fungi and may play a role in lignin degradation and/or detoxification of lignin degradation products (Ishihara, 1980; Reinhammar, 1984). Laccase electrophoretic patterns have been shown to differ only slightly within species but to differ between species and therefore have potential as a systematic tool. Blaich & Esser (1975) found only slight differences between six geographical races of *Pleurotus ostreatus*, while Kerrigan & Ross (1988) reported that isozyme patterns of extracellular laccase were largely invariant within species of *Agaricus* but dissimilar between species. Electrophoretic analysis conducted by Wu (1991) indicated that extracellular laccase banding patterns could also be of significance in the systematics of the genus *Clavicorona*.

In this study, sexual compatibility patterns and data from laccase enzyme electrophoresis were combined with morphological observations. The results expand the number of putative taxa within subg. *Ramosa* and redefine the geographical range of some taxa. We further report consistency of within-species isozyme patterns within large geographical areas and confirm that laccase may have value as a systematic tool in this subgenus.

Materials and methods

Morphology

The process of specimen documentation and preservation was summarized by Petersen (1988) and Wu (1991). In the present study, special attention was paid to basidiospore dimensions, shape, and

ornamentation, and to (gloeo-)cystidial dimensions and shape, and hymenial and/or subhymenial agglutination or gelatinization. Basidiospores were measured in 3% KOH solution. E value (length divided by width), median E value (E^m), median length (L^m) and median width (W^m) were calculated for each specimen. In the text below, color names follow Kornerup & Wanscher (1978) and those enclosed in single quotes are from Ridgway (1912). Voucher specimens were preserved in the herbarium of the University of Tennessee (TENN). Collections of *Clavicorona* used for morphological, compatibility and laccase analyses are listed in Tab. 1.

Tab. 1. – Collections of *Clavicorona* used for morphological, compatibility and laccase analyses.

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
C. candelabrum			
NEW ZEALAND			
North Island, Waitakere Mts.	2679	48923	BL
	2681	48922	BL
	2802	48924	BL
	2807	48925	BL
North Island, Waipoua	55633	44117	BL
South Island, Nelson	55721	43390	B
C. colensoi			
NEW ZEALAND			
North Island, Waitakere Mts.	2685	48919	BL
Waitakere Ranges	55218	44127	B
	PDD55203	48932	B
North Island, Titirangi	55243	44120	BCL
North Island, Urewera Nat. Pk.	518	42316	B
	562	42194	B
	571	42327	B
	574	42328	B
North Island, vic. Auckland	55895	43447	B
South Island, Craigieburn	646	42211	B
South Island, Fjiordland	2637	48917	BL
	2644	48918	BCML
	2667	48920	BML
South Island, Lewis Pass	2555	48930	BCML
South Island, Nelson Lakes	55051	43387	B
South Island, vic. Murchison	55157	43871	BL
	55031	43388	B
	55017	44094	B

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
AUSTRALIA			
New South Wales	3543	50060	L
Tasmania	3922	50128	B
	3939	50070	B
C. cristata			
UNITED STATES, CA	DDL8974	48928	BCML
FINLAND, location unknown	GB1700	none	CML
	GB1701	none	ML
C. microspora			
JAPAN, Tochigi Pref.	2349	48856	BCML
	KY5352	none	CML
C. piperata			
CANADA, British Columbia	5770	53491	BL
Vancouver Island	6675	DAVP	L
USA, WA Olympic Peninsula	5869	51959	BL
C. pyxidata			
CHINA, Jilin Prov.	1541	48855	BCML
Yunnan Prov.	1449	48279	BCML
FINLAND, Location unknown	GB1702	none	L
UNITED STATES			
AZ Coronado Forest	JPL479	none	BCL
AZ Flagstaff	ATCC22501	none	CL
GA Rabun Co.	4567	—	L
	4568	51049	L
	4573	51053	L
	5241	51893	L
	5242	51900	L
	5243	51899	L
	5484	51947	L
	5485	51948	L
	6211	51936	L
	6212	—	L
	56665	48853	BML
	56666	48887	BL
	56667	48854	BCML
	56668	48888	BL

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
IL Coler Co.	AM6658	51877	L
MD Beech Island	HHB810	none	BL
MD Prince Georges Co.	AES515	none	BL
ME Hancock Co.	4990	51411	L
MI Berrien Co.	RGT 752	RGT930701/09	
NC Haywood Co	1616	48895	BL
	1617	48900	BL
	6609	51931	L
	6610	51929	L
	6611	51932	L
	6612	51933	L
	6614	51930	L
	6615	51934	L
	6616	51935	L
	6617	51949	L
NC Jackson Co.	4806	51107	L
NC Macon Co.	1113	—	L
	1135	48914	BL
	1137	—	L
	1614	48896	BL
	1621	48898	M
	1648	none	L
	1650	none	ML
	1660	48849	BM
	1661	48850	BML
	1662	48851	BM
	2092	48933	B
	2174	48795	L
	4091	50241	L
	4598	51076	L
	4816	51117	L
	5116	51937	L
	5117	51939	L
	5168	51941	L
	5298	51876	L
	5437	—	L
	5441	—	L
	5461	—	L
	5464	51945	L
	5493	51946	L
	5519	—	L
	6307	51922	L
	6308	51921	L
	6428	51927	L
	6618	—	L
	6621	51919	L
	6622	51918	L
	6623	51915	L
	6624	51920	L
	56653	48846	BML

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
	56655	56655	BCML
	56662	48848	BML
	none	47668	L
	none	47669	L
	none	47670	L
	none	47673	L
ATCC24439	37734	CL	
NC Swain Co.	6632	51914	L
	6633	51923	L
	6634	51917	L
	56680	48891	BL
	56681	52357	L
NY St. Lawrence Co.	1190	48842	BCML
NY Tompkins Co.	1493	48911	B
	1498	48908	B
	1499	48910	B
	1704	48902	BL
	1706	48841	BM
	1707	48905	B
	1724	48906	BL
	1732	48907	B
	1733	48901	B
	1739	48903	B
	1741	48840	BM
	4938	51251	L
NY Dutchess Co.	6485	51942	L
	6488	51943	L
SC Oconee Co.	4581	51060	L
	6433	51928	L
TN Blount Co.	3710	50698	L
	4681	51944	L
	5401	51898	L
	5402	51897	L
	5404	51895	L
TN Cumberland Co.	----	36429	L
TN McMinn Co.	4563	51905	L
	4564	51906	L
TN Monroe Co.	2994	50680	L
	2995	50681	L
TN Scott Co.	5236	51901	L
	5237	51904	L
	5238	51902	L
	5299	51875	L
	5300	51880	L
	5538	51903	L
	6608	51924	L
	6619	51916	L
	6620	51913	L
TN Sevier Co.	1606	48894	BL
	1607	48852	BML
	1608	48897	BL

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
	3624	50743	L
	3650	50769	L
	5194	51938	L
	5195	51940	L
	5302	----	L
	5303	----	L
	5403	51896	L
	5412	51885	L
	5413	51884	L
	5414	51886	L
	5415	51889	L
	5416	51891	L
	5417	51883	L
	5418	51888	L
	5419	51878	L
	5420	51879	L
	6601	51901	L
	6602	51908	L
	6603	51910	L
	6604	51911	L
	6605	51912	L
	6606	51925	L
	6607	51907	L
TN Sullivan Co.	5411	51887	L
	5422	51892	L
	5423	----	BL
	5424	51894	L
	5425	51881	L
	5426	51890	L
	5427	51882	L
VA Mt Lake	HHB10654	none	L
WI Leopold Reserve	1501	48843	BL
	1502	48893	BL
	1503	48844	BCL
	1504	48879	BL
	1505	48874	B
	1506	48845	BCM
	1507	48880	BL
	1508	48892	BL
	1509	48885	BL
	1510	48883	B
	1511	48872	BL
	1512	48886	BL
WI Sauk Co.	1513	48875	BL
	1514	48872	BL
	1515	48876	BL
	1516	48878	BL
	1517	48881	BL
	1518	48884	B
	1519	48877	BL
	1520	48882	BL

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
SWEDEN	GB1137	none	L
	GB1279	none	CML
	GB1280	none	CML
SWITZERLAND	4289	50625	L
C. turgida			
NEW ZEALAND			
North Island, Waitakere Mts.	2679a	48923	L
	2682	48926	BCML
	2838	48921	B
North Island, Mt. Egmont	55137	44103	B
North Island, Urewera Nat. Pk.	55087	43389	B
South Island, Fjiordland	2651	48934	BCML
South Island, Cascade Valley	55137	44103	BCL
South Island, Nelson Lakes	55050	43389	B
AUSTRALIA, Tasmania	3961	50163	L
Taxon 1			
USA, NC Haywood Co.	1615	48890	BCL
Taxon 2			
USA, Puerto Rico	3456	48929	BL
Taxon 3			
NEW ZEALAND			
North Island, Mangamuka Sc. Res	55602	44125	B
North Island, Mt. Egmont	55189	43394	B
North Island, Urewera Nat. Pk.	2822	48916	BL
	544	42313	B
	561	42324	B
	586	42332	B
	591	42197	B
	592	42199	B
	2820	49279	B
North Island, Waipoua Kauri Res	747	42292	B
	55647	44126	B
South Island, Fox Glacier	55105	43430	B
South Island, Lewis Pass	2567	48927	B
South Island, Nelson Lakes Pk.	55058	43393	B
	55064	43433	B
	55720	43391	B

Species/Location[1]	Collection Number[2]	Herbarium Number	Use[3]
South Island, Fjiordland	2672	48915	BL
South Island, Westland	PPD55204	48931	B
Taxon 4			
AUSTRALIA, Tasmania	3905	50211	BL
	3979	50238	BL
	4003	50218	L
	4005	50197	B
	4034	50266	B

[1] Within the United States, State names are abbreviated.
[2] Collection number refers to the field notebook number and is also used to identify cultures.
[3] B = Basidiome morphology; C = Culture mat morphology; M = Mating experiment; L = Laccase electrophoretic mobility.

Cultures

Establishment of dikaryon cultures was by mass spore germination or by isolation from basidiome tissue. Monokaryon (= single-spore) isolates were made using the technique of Wu (1991). All cultures were grown and maintained on malt extract agar (MEA: 1.5% Difco malt extract; 2% Bacto-agar) or on potato dextrose agar (PDA: 3.9% Difco Bacto potato dextrose agar). Cultures were stored at 4 C in the dark.

Culture mat morphology was recorded on MEA medium using methods described previously (Wu & Petersen, 1992). Three duplicates were cultured for each collection. The diameter of colony was measured as growth rate. The total number of duplicates (n) was used to calculate mean growth rate. Terminology and codes in the text follow Nobles (1965) and Stalpers (1978). Ethanolic syringaldazine (Harkin & Obst, 1973) and aqueous p-cresol (Marr, 1979) were used to detect laccase and tyrosinase activity, respectively.

Mating experiments

Self-crosses (= pairings between sibling monokaryon isolates from a single basidiome), intercollection and interspecific matings (usually between tester strains of the various collections) were all performed on MEA at room temperature (21-23 C), using techniques summarized by Wu & Petersen (1991). For some extremely slow-growing isolates, mating experiments were conducted by placing two inoculum blocks together so that less growing time was required. Clamp connections were normally used as indication for mating compatibility. The monokaryotic and dikaryotic condition was

confirmed by mounting mycelium with DAPI (4'-6-diamidino-2-phenylindole) solution and observing under epifluorescence microscopy.

Laccase enzyme electrophoresis

For enzyme assays, a 5 mm diameter plug was removed from the culture and placed in 25 ml of potato dextrose broth (PD: 2.4% Difco potato dextrose). Preliminary experiments demonstrated that some species of *Clavicorona* excreted compounds into the medium which were self-inhibitory and ultimately killed the culture. This problem was reduced by culture in PD medium. Since preliminary trials revealed no difference in isozyme profiles in malt extract broth (ME: 1.5% Difco malt extract) or PD, culture in PD broth was selected for these studies. Cultures were sampled at intervals of one week begining 2–3 weeks after initial subculture.

Procedures for separation of laccase isozymes were modified from Kerrigan & Ross (1988). Separation was carried out on a 8 cm x 10 cm x 1.0 mm vertical polyacrylamide gel using non-denaturing conditions. The stacking gel and separating gel were 12% and 4% with 2.6% crosslinker. Buffers were: 1) upper electrode buffer: 50 mM Tris + 383 mM glycine, pH 8.3; 2) stacking and separating gel buffers: 0.375 M Tris-HCl, pH 8.8; and 3) lower electrode buffer: 63 mM Tris-HCl, pH 7.5. Twenty-seven µl of culture medium mixed with 3 µl tracking dye (41.6% bromphenol blue, 50% glycerol: modified from Sambrook & al., 1989) were loaded per well. Separation was at 200 V for 45-60 min. Laccase isozymes were visualized by immersion of gels in a 10% ethanol solution of *O*-toluidine buffered to pH 4.7 with sodium acetate (Leslie & Leonard, 1979). The staining solution was made fresh for each gel since some degradation of the solution occurred even when the solution was protected from light. Gel bands were recorded 2–3 times during staining by removing the gel from the stain and measuring visible bands. After staining for 2 hours, gels were removed from the stain, wrapped in clear plastic wrap and left overnight at room temperature. This procedure enhanced visibility of faint bands. Gels were fixed for 15 minutes in a 20% ethanol, 10% glycerol solution and dried between 2 sheets of cellophane for 48 hours then filed for later reference. In some cases, extracts were concentrated by dialysis against 1M sorbitol at 4 C for 24 hours. This resulted in a 3-4x concentration without degradation of laccase. Mobilities were determined in proportion to a standard (ATCC 24439: Dodd, 1972) which was electrophoresed on each gel. Electrophoretic mobilities were recorded as the relative mobility of a given band compared to the standard. (R_i = Mobility of band X /Mobility of the

standard). Where it was difficult to determine if two electrophoretic mobilities were identical, two extracts were combined and electrophoresed with the individual extracts on a common gel. Eight-percent gels were also used to resolve questions of identity. For purposes of this study, bands differing by $R_i < 0.02$ were considered to be the same for purposes of delineating taxa.

Results

Key to taxa of *Clavicorona* subg. *Ramosa*

1. Gloeoplerous hyphae absent; basidiomata dichotomously branched 2
1. Gloeoplerous hyphae present; basidiomata usually pyxidately branched 3

2. Skeletal hyphae abundant; basidiospores subglobose ..... (*C. dichotoma*)
2. Skeletal hyphae absent; basidiospores elliptical to allantoid ..... (*C. divaricata*)

3. Distributed in the Northern Hemisphere 4
3. Distributed in the South Pacific regions (New Zealand, Australia) 9

4. Basidiomata minute (< 1 cm high), rarely branched; basidiospores elongate-elliptical (E=2.3–3.5; E^m=2.8) ..... *C. cristata*
4. Basidiomata medium to large size (> 2 cm high), densely branched; basidiospores elliptical to subglobose (E=1.2–2.0; E^m<2.0) 5

5. Basidiomata white; subtropical distribution (Puerto Rico) ..... Taxon 2
5. Basidiomata colored; temperate distribution 6

6. Western North American distribution; consistent laccase band at R_i=0.72 ..... *C. piperata*
6. Eastern North American, European or Asian distribution; consistent laccase band not at Ri=0.72 7

7. Subhymenium gelatinized; basidiomata camel-brown; culture mat growing slowly on MEA (< 2 cm/4 weeks) ..... Taxon 1

7. Subhymenium not gelatinized; basidiomata lighter colored; culture mat growing fast on MEA (> 5 cm/4 weeks) 8

8. Basidiomata with pale yellowish apices; basidiospores longer than 4.0 µm [4.0–5.0(–6.0) µm] ..... *C. pyxidata*
8. Basidiomata with light orange colored apices; basidiospores shorter than 4.0 µm (3.0–4.0 µm); incompatible with *C. pyxidata* ..... *C. microspora*

9. Thick-walled tramal hyphae abundant; basidiomata densely gregarious ..... *C. candelabrum*
9. Thick-walled tramal hyphae absent; basidiomata not densely gregarious 10

10. Hymenium and subhymenium gelatinized; basidiomata pale in color ..... *C. turgida*
10. Hymenium and subhymenium not gelatinized; basidiomata brownish 11

11. Basidiomata slender (stipes 1 mm wide); consistent laccase band at R_f=1.18 ..... *C. colensoi*
11. Basidiomata stout (stipes > 1 mm wide); consistent laccase band at R_f=0.78 or R_f=0.71 12

12. Basidiospores 5.0–6.0 x 4.0–5.0 µm; consistent laccase band at R_f=0.78 ..... Taxon 3
12. Basidiospores 4.0–4.4 x 2.8–4.0 µm; consistent laccase band at R_f=0.71 ..... Taxon 4

Clavicorona candelabrum (Mass.) Corner, Ann. Bot., Mem. 1: 286, 1950; Dodd (1972), Fig. 1h.

Basidiomata up to 3 x 2.5 cm, slender, pyxidately branched, densely gregarious, arising from a brown mycelial mat; stipe short, up to 3 mm diam; stipe and lower branches brown (7C4); ('wood brown,' 'fawn color,' 'fuscous,' 'army brown,' 'benzo brown'); upper branches Sahara (6C5) to chocolate brown (6E8) upward ('avellanous,' 'cinnamon buff,' 'light cinnamon drab'); branching up to 3 ranks; apices bluntly coronate, concolorous with upper branches or paler; consistency tough; drying brownish black or black; taste quickly acrid, sometimes also bitter; odor typical of the genus. – Tramal hyphae of three types: 1) inflated generative hyphae, 4–15 µm diam, clamped, hyaline to yellowish, brown to reddish brown when aggregated, densely packed, parallel, hard to separate, thin- to

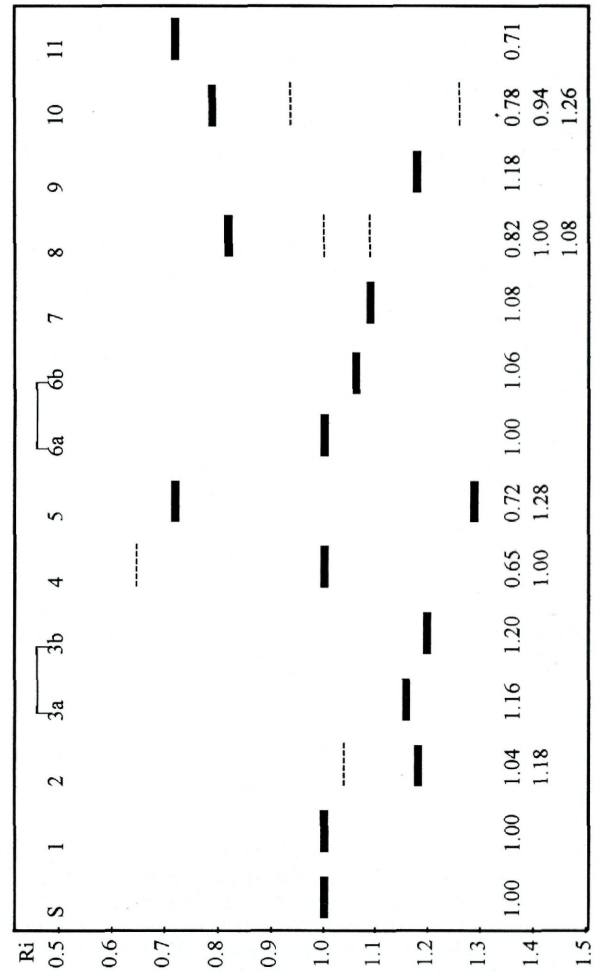

Fig. 1. – Laccase electrophoretic mobilities. – S = Standard (*C. pyxidata* isolate ATCC24439); 1 = *C. candelabrum*; 2 = *C. colensoi*; 3 = *C. cristata* (3a from California, 3b from Finland); 4 = *C. microspora*; 5 = *C. piperata*; 6 = *C. pyxidata* (6a from North America/Europe (154 collections), 6b from China); 7 = *C. turgida*; 8 = Taxon 1; 9 = Taxon 2; 10 = Taxon 3; 11 = Taxon 4. Variable bands are shown by dotted lines.

slightly thick-walled, (wall up to 1.0 µm thick); 2) hyphae with torulose wall thickenings, coupled with occurrence of rudimentary dichophyses, 3–8(–15) µm diam (wall up to 2 µm thick); and 3) gloeoplerous hyphae abundant, 4–15 µm diam; contents yellowish, dense, refractive, stained black in sulfo-benzaldehyde solution. Subhymenium approx. 20 µm thick, not obviously gelatinous. – Hymenium more than 20 µm thick; basidia 15–20 x 4–5 µm, clavate, hyaline, clamped at base, 4–sterigmate; sterigmata up to 4 µm long; gloeocystidia ventricose, projecting up to 30 µm beyond hymenium. – Basidiospores 4.0–5.0 x 2.0–3.2 µm (L^m=4.3 µm; W^m = 2.7 µm; E=1.33–2.0; E^m=1.61), broadly elliptical, weakly amyloid, minutely roughened, uniguttulate; apiculus typical of the genus.

Habitat and distribution. – On rotten *Leptospermum* wood. *Clavicorona candelabrum* has been reported from Malaya, North Borneo, Singapore and the Solomon Islands (Dodd, 1970), Philippines (Dogma, 1966), and from New Zealand (Wu, 1991 and this study).

Culture mat morphology. – The culture mat of *C. candelabrum* was characterized by slow growing, dull yellowish pigmentation, presence of gloeoplerous hyphae and spherical swollen hyphae.

Mating experiments. – A self-cross among single-spore isolates of collection 2979 (New Zealand) revealed a bifactorial mating system. Intercollection matings could not be performed due to the slow growth of monokaryon cultures and a general reluctance by donor mycelia to intermingle. Paired intercollection monokaryon cultures incubated for several months did not successfully dikaryotize.

Laccase electrophoretic pattern. – Five collections from New Zealand (North Island) presented a consistent single band with R_i = 1.00 (Fig. 1).

Clavicorona candelabrum exhibits the following separating taxonomic characters: 1) Basidiomata slender, densely gregarious in habit; 2) basidiomata light brown to chocolate brown (6C5, 6E8), drying brownish black or black; 3) tramal hyphae often with wall thickening and rudimentary dichophyses; and 4) basidiospores 4.0–5.0 x 2.0–3.2 µm, finely but distinctly roughened.

Examination of the type specimen of *C. candelabrum* (K) confirmed the presence of the unique thick-walled tramal hyphae. In all specimens used in this study, such thick-walled hyphae were observed to give rise to blunt, short-armed dichophysis-like side

branches. Such structures may support a putatively close relationship between *Clavicorona* and *Vararia* (Steglich & Sterner, 1988; Gluchoff-Fiasson & al., 1983). According to Corner (1970), the only other *Clavicorona* species having thick-walled hyphae is *C. dichotoma* Corner described from Java. However the type specimen of *C. dichotoma* (S. J. van Ootstroom 13385, L) showed distinctive dichotomous branching pattern, abundant typical skeletal hyphae and no gloeoplerous hyphae.

The two species that produce slender basidiomata, *C. candelabrum* and *C. colensoi*, are separated as follows: 1) thick-walled hyphae present in the former; 2) densely gregarious habit of the former (scattered basidiomata in the latter); 3) the culture mat of *C. candelabrum* produced darker pigments; and 4) different laccase patterns (R_i = 1.00 in the former; R_i = 1.18 in the latter).

Clavicorona colensoi (Berk. apud Hooker) Corner, Ann. Bot. Mem. 1: 287, 1950; Dodd (1972), Fig. 1c.

Basidiomata up to 2.5 cm high, 1.5 cm wide, slender, delicate, divaricately and pyxidately branched, scattered, arising from an off-white mycelial mat; stipes short, up to 2.5 mm long, 1 mm wide, or absent, red-haired (6C4), ('avellaneous'), to light brown (6D5) ('wood brown'); branches in 2–4 ranks, greyish orange (6B4) to pale orange (5A3) ('pale ochraceous buff' to 'tilleul buff'); internodes diminishing gradually; apices paler, orange white (6A2) to white, coronate and cuspidate; texture pliable; basidiome drying dark brown; taste quickly acrid; odor typical. – Tramal hyphae 4–15 µm wide, thin- to slightly thick-walled (wall up to 0.5 µm thick), inflated, clamped, densely interwoven, not gelatinous, hyaline, yellowish brown when aggregated. Subhymenium approx. 20 µm thick; hyphae 2–6 µm wide, clamped, tightly interwoven; gloeoplerous hyphae abundant, 4–15 µm wide, with refractive yellowish contents, elongated, stained black in sulfo–benzaldehyde solution, clamped at base, usually ending in gloeocystidia in hymenium. – Hymenium up to 25 µm thick; basidia 15–20 x 3.5–5.0 µm, clavate, thin-walled, hyaline, 4–sterigmate; sterigmata up to 4 µm long, slightly curved; gloeocystidia 4–13 wide, inflated, with yellowish refractive contents, projecting up to 30 µm beyond basidia, ventricose; leptocystidia not observed. – Basidiospores 4.5– 6.0 x 3.0–4.5 µm (L^m = 5.2 µm; W^m = 3.5 µm; E = 1.25–1.83; E^m = 1.48), elliptical to broadly ovoid, amyloid, white in mass, thin-walled, minutely roughened, uniguttulate in contents; apiculus typical of the genus.

Habitat and distribution. – On rotten wood of *Nothofagus* and *Leptospermum*. New Zealand, Australia (including Tasmania).

Culture mat morphology. – Isolates examined: 55243, 2555, 2644. – Mean growth rate: (n = 3 x 3) 1 wk, 0.5 cm; 2 wks, 1.8 cm; 3 wks, 3.3 cm; 4 wks, 3.8 cm; 5 wks, 4.8 cm; 6 wks, 5.5 cm. – Macromorphology: Advancing zone appressed, margin even; aerial mycelium poorly developed, silky, yellow, or with a few white radiating strands; reverse unchanged; odor like lemon or not distinctive. – Micromorphology: Advancing zone hyphae 1.5–3 µm diam, hyaline, infrequently branched, non-amyloid, thin-walled, clamped; aerial hyphae rare, similar to advancing zone hyphae; submerged hyphae 1.5–3 µm diam, hyaline, dextrinoid, *textura intricata*, frequently branched, clamped; gloeoplerous hyphae rare, with granular, dense, yellowish content; crystals parallelogram-shaped, few, 5–10 µm long. – Phenoloxidase reactions: Week II: Laccase (+), Tyrosinase (-). Week VI: Laccase (+), Tyrosinase (-). – Nobles code: 2a. 3. 11. 14. 15. 26. 37. 38. 47. 50. 55. 60. – Stalpers code: 1. 9. 13. 14. 15. 20. 35. 37a. 39. 45. 52. 73. 83. 89. 94. 95. 97. 99.

Mating experiments. – Self-crosses of collection 2555 revealed a bifactorial mating system. However, self-crosses of collections 2644 and 2667 were unsuccessful and that of 2685 produced very few compatible pairings. Single spore isolates of collections 2667 and 2644 were randomly selected to pair with each of the four mating types of 2555. The intercollection matings within the species showed low percentage partial intercompatibility (Wu, 1991).

Laccase electrophoretic pattern. – Seven collections from New Zealand showed a consistent single laccase band at R_i = 1.18. Collection 3543 from Australia produced a single laccase band with electrophoretic mobility = 1.17. While slight, the difference was reproducible. A secondary band at R_i = 1.04 was occasionally observed (Fig. 1).

Clavicorona colensoi can be separated from others as follows: 1) slender, delicately branched, scattered basidiomata; 2) basidiomata moderately colored ('vinaceous buff,' 'wood brown'), significantly paler where protected from direct light; 3) abundant gloeoplerous hyphae, often ending as broad, clavate to stoutly fusiform, hardly emergent gloeocystidia; 4) basidiospores 4.5–6.0 x 3.0–4.5 µm; and 5) bright yellow pigmentation of culture mat on MEA.

Dodd (1970) examined the type specimen from Kew, drawing attention to the similarity of microscopic characters of *C. colensoi* and

C. piperata (western North America). He commented correctly, however, that basidiome macroscopic appearance was dissimilar, with *C. piperata* forming larger, stouter basidiomata than *C. colensoi*. In addition, the basidiospore measurements of the type specimen of *C. colensoi* have a wider range than that given by Dodd (type: 4.5–6.0 x 3.0–4.5 µm; Dodd: 4.0–5.0 x 3.0–3.5 µm). The morphological differences between *C. colensoi* and *C. candelabrum* are reviewed above under *C. candelabrum*.

Because the species is diagnosed by a number of unique microscopic characters, the morphospecies would appear to be homogeneous. Supporting this conclusion, intercollection mating data showed at least partial intercollection intercompatibility. The lack of mating types might contribute to the partial intercompatility among collections. The laccase enzyme mobility of *C. colensoi* collections exhibited only minor variation.

Clavicorona cristata (Kauffm.) Doty. Lloydia 10: 40, 1947; Dodd (1972), Fig. 1e.

Basidiomata up to 8 mm high, 1 mm wide, narrowly coniform, with broad coronate apices, rarely branched, gregarious, arising from a small, brown mycelial patch, light yellowish brown above, light greyish yellowish brown downwards; texture tough, pliable; taste unknown. – Tramal hyphae 4–10 µm wide, thin-walled, hyaline, clamped, inflated, not gelatinous. Subhymenial hyphae 2–5 µm wide, clamped, tightly interwoven; gloeoplerous hyphae abundant, 3–6 µm diam, elongated, with yellowish, refractive contents, stained black in sulfo-benzaldehyde solution, extending into hymenium. – Hymenium more than 35 µm thick; basidia 25–35 x 4–5 µm, clavate, thin-walled, clamped at base, 4–sterigmate; sterigmata up to 4 µm long, slightly curved; gloeocystidia up to 5 µm wide, mucronate or clavate, rarely projecting up to 5 µm beyond basidia; leptocystidia rare, unbranched, filamentous, 2 µm wide, projecting up to 10 µm beyond basidia. – Basidiospores 5.6-7.0 x 2.0-2.8 µm (L^m=6.1 µm; W^m=2.2 µm; E=2.3-3.5; E^m=2.8), elongate-elliptical to allantoid, thin-walled, hyaline, uniguttulate, white in mass, amyloid, minutely roughened; apiculus typical.

Habitat and distribution. – On rottern log of Douglas fir (type) and oak (DLL 8974). North America (California, Oregon, Washington and Quebec) and Finland.

Culture mat morphology. – Isolates examined: DLL8974, GB1700. – Mean growth rate: (n = 2 x 3) 1 wk, 0.1 cm; 2

wks, 0.2 cm; 3 wks, 0.5 cm; 4 wks, 0.7 cm; 5 wks, 1.2 cm; 6 wks, 1.7 cm. – Macromorphology: Advancing zone submerged; margin slightly fringed; hyphae silky; aerial mycelium cottony, cream colored; reverse unchanged; odor not distinctive. – Micromorphology: Advancing zone hyphae 1.5–3 µm diam, hyaline, infrequently branched, loosely interwoven, non-amyloid, thin-walled, clamped; aerial hyphae 2–5 µm diam, thin-walled, infrequently branched; sprouting-clamps present. Submerged hyphae 1.5–3 µm diam, hyaline, non-dextrinoid, loosely interwoven, frequently branched, more or less diverticulate, clamped. Gloeoplerous hyphae present, with granular, dense content. Bipyramidal crystals abundant, 5–10 µm long. – Phenoloxidase reactions: Week II: not tested. Week VI: Laccase (+), Tyrosinase (-). – Nobles code: 2a. 3. 5. 11a. 15. 26. 36. 38. 47. 54. 60. – Stalpers code: 1. 10. 13. 21. 31. 37a. 39. 45. 52. 73. 80. 83. 90. 94. 95. 97. 99.

Mating experiments. – A self-cross among 13 single-spore isolates of DLL 8974 (northern California) revealed a bifactorial mating system. Self-crosses among single-spore isolates of GB 1700 and GB 1701 (Finland) also showed bifactorial mating systems (R. Daun, pers. comm.). Intercollection matings of tester strains of DLL 8974 and single-spore isolates of GB 1700 and GB 1701 showed that the two Finnish collections were fully intercompatible with each other and that the Californian collection was partially intercompatible with the Finnish collections.

Laccase electrophoretic pattern. – A single laccase band of $R_f = 1.16$ was produced by DLL 8974 (California), while the Finnish material (GB 1700, GB 1701) produced a single band of $R_f = 1.20$ (Fig. 1).

Based on study of fresh material and the holotype, *Clavicorona cristata* is distinguished by the following characters: 1) diminutive size, rarely over one centimeter high, and sparse (sometimes absent) branching; 2) unique long narrow basidiospores (5.6–7.0 x 2.0–2.8 µm; $E^m = 2.8$); 3) northern distribution pattern, including North America and Fennoscandia; 4) Cultures of *C. cristata* on MEA were characterized by extremely slow growth and occurrence of verticillate and sprouting clamp connections.

We saw no basidiomata of the Finnish collections, but intercompatibility of isolates from these collections with isolates from the Californian specimen established that all three belonged to a single biological species. Partial intercompatibility between Finnish and Californian collections was observed and is consistent with observations on widely separated collections of other biological

species (Gordon & Petersen, 1991; Hilber, 1982; Petersen & Halling, 1993). Laccase mobility data differed, supporting the mating experiment results and suggesting that gene flow between western North American populations and those from Fennoscandia is probably absent, allowing the gradual accumulation of genetic differences. Dodd (1972) reported collections of *C. cristata* from Quebec, and mating studies and laccase experiments on populations from that area would take on added importance in light of the data furnished here.

Corner (1950, 1970) suggested that *C. cristata* might be a juvenile state of *C. pyxidata* or *C. colensoi*. Dodd (1972) accepted *C. cristata* as a distinct morphospecies. The culture, mating and laccase data obtained in this study fully supported Dodd's conclusion that *C. cristata* is a well supported species.

Clavicorona microspora Wu & Petersen, Mycotaxon 45: 124, Fig. 1, 1992.

Basidiomata and culture mat morphology, habitat, and mating experiments were previously reported (Wu & Petersen, 1992).

Laccase electrophoretic pattern. – Isolates of two collections from Japan produced a laccase band of R_i = 1.00, sometimes with a faint band of R_i = 0.65 (Fig. 1).

Clavicorona piperata (Kauffm.) Leathers & Smith, Mycologia 59: 461, 1967; Dodd (1972), Fig. 1b.

Basidiomata stout, over 4 cm high, gregarious, branching in 2–4 ranks, 'avellaneous' to 'wood brown' at base, upper branches 'tilleul buff' or somewhat yellower; apices coronate. – Tramal hyphae 3–10 µm diam, inflated, clamped, hyaline, thin-walled; gloeoplerous hyphae abundent, 3–8 µm diam, extending to hymenium. Subhymenial hyphae 2-3 µm diam, clamped. – Hymenium up to 30 µm thick; basidia 25–30 x 4–5 µm, clavate, 4–sterigmate; gloeocystidia 4–6 µm, projecting beyond basidia, clavate or ventricose. – Basidiospores 3.5–4.5 x 2–3.5 µm (L^m = 3.9 µm; W^m = 2.8 µm; E = 1.2-1.7; E^m = 1.4), ovoid to subglobose, thin-walled, white, minutely roughened, amyloid.

Habitat and distribution. – On rotten conifer wood; western North America from northern California to British Columbia, east to the western Rocky Mountains.

Mating experiments. – Mating system unknown; no intercollection matings performed.

Laccase electrophoretic pattern. – Three Pacific Northwest collections consistently produced a laccase band at $R_f = 0.72$. A second band at 1.28 was seen in two of the three isolates (Fig. 1).

The following characters separate *C. piperata* from other similar taxa: 1) broad basidiospores (E^m = 1.4, comparing *C. pyxidata*: E^m = 1.8); 2) robust basidiomata usually branched in 2–4 ranks (those of *C. cristata* branch in 1–2 ranks maximum only); 3) habit on conifer wood (*C. pyxidata* is usually found on hardwood). Dodd (1972) extended the distributional range to Australia based on examination of the type specimen of *C. pyxidata* var. *asperospora* Fawc. that he cited as a synonym of *C. piperata*. We now believe that *C. pyxidata* var. *asperospora* represents a different taxon (see Taxon 4).

Both Petersen (1988) and Wu (1991) reported *C. piperata* from New Zealand, but new morphological and culture data indicate that the New Zealand collections should be separated into Taxon 3 (see below). Laccase mobility data also support such a hypothesis.

Clavicorona pyxidata (Pers : Fr.) Doty, Lloydia 10: 43, 1947; Dodd (1972), Fig. 1a.

Morphology. – See Wu & Petersen, 1991.

Habitat and distribution. – On rotten deciduous wood, rarely on conifer wood. North Temperate forests of Europe, Fennoscandia, North America (Rocky Mountains and East), and temperate Asia.

Culture mat morphology. – Isolates examined: 24439, 1190, 1503, 1506, JPL479, 56655, 24439, 56667, 1449, 1541, GB1279, GB1280. – Mean growth rate: (n = 12 x 3) 1 wk, 1.0 cm; 2 wks, 2.8 cm; 3 wks, 4.5 cm; 4 wks, 6.2 cm; 5 wks, plate covered. – Macromorphology: Advancing zone appressed; margin even; silky; aerial mycelium poorly developed, silky, hyaline or with a few white radiating strands; reverse unchanged; odor like lemon or not distinctive. – Micromorphology: Advancing zone hyphae 1.5–4 µm diam, hyaline, infrequently branched, non-amyloid, thin-walled, clamped; aerial hyphae rare, similar to advancing zone hyphae. Submerged hyphae 1.5–3 µm diam, hyaline, dextrinoid, clamped, loosely interwoven, frequently branched, clamped. Gloeoplerous hyphae rare, with granular, dense contents. – Phenoloxidase

reactions: Week II: Laccase (+), Tyrosinase (-). Week VI: Laccase (+; 1503 & 1506: -), Tyrosinase (-). – Nobles code: 2a. 3c. 11i. 15. 26. 36. 38. 45. 50. 54. 55. 60. – Stalpers code: 1. 8. 13. 14. 15. 20. 30. 36. 37a. 39. 45. 52. 73. 89. 90. 94. 95. 97. 99.

Mating experiments. – Our work confirms reports by Dodd (1970) and James (1983) of a bifactorial mating system in the species (Wu, 1991). Collections which were tested for intercompatibility were 56662, 56663, 56655 and 1650 from North Carolina, 56667 and 56665 from Georgia, 1607 from Tennessee,1190, 1706 and 1741 from New York, 1506 from Wisconsin, 1449 and 1541 from China and GB1279 and GB1280 from Sweden. All were intercompatible. Tester strains of 11 widely scattered collections of *C. pyxidata* were paired with tester strains of two collections of *C. microspora*. All pairings were incompatible (Wu & Petersen, 1991).

Laccase electrophoretic pattern. – Cultures of all collections from North America and Europe (ca. 154 collections) produced a consistent prominent laccase band of $R_j = 1.00$, sometimes with faint ancillary bands of $R_j = 0.46$- 0.80. Two collections from China showed a consistent single laccase band of $R_j = 1.06$ (Fig. 1).

Clavicorona pyxidata is the most commonly collected species in the genus within its distributional range. Basidiomata of *C. pyxidata* are quite variable in color (from 'vinaceous buff' to 'wood brown' shades, to bright lemon yellow), stature (branched in 2-5 ranks; with discrete stipe or nearly astipitate; strigose at base to almost smooth; with extensive basal mat or nearly without). Basidiomata of one collection from China (Yunnan) were large, copiously and openly branched, and somewhat pallid in color, while basidiomata of the other Chinese collection closely resembled North American and European material.

Infraspecific macroscopic variation has lead to a proliferation of proposed names (See synonymy furnished by Dodd, 1970). Until this time, the species concept has been held together by: 1) narrow basidiospores [4.0–5.0(–6.0) x 2.0–2.5(–3.0) μm, with average length/width ratio of 1.8–2.0, Wu & Petersen, 1991]; 2) geographic distribution; 3) relatively rapid growth on MEA; and 4) habit on rotten hardwood stumps and logs.

Although differing in laccase enzyme electrophoretic mobility, mating experiments by Wu (1991) and this study have shown that the Chinese collections belong to the same biological species as those from North America and Europe. The discrepancy in laccase mobility may reflect the imposition of a long-term geographical reproductive

barrier between populations of the biological species, although not yet reflected in ability to interbreed *in vitro*.

Clavicorona turgida (Lev.) Corner, Darwiniana 11: 195, 1957; Dodd (1972), Fig. 1g.

Basidiomata up to 5.5 x 3 cm, somewhat stout, pyxidately branched, gregarious, drying dark brown to black; stipe short, brownish orange (6C4) to cinnamon (6D5) ('light drab,' 'clay color'); branches in 3–4 ranks, light orange (5A4) to brownish orange (6C5; 'light ochraceous buff,' 'vinaceous buff'); internodes diminishing gradually; apices cream colored (4A3) or brownish orange (7C4; 'cream buff,' or 'wood brown'), sometimes minutely bruised to light brown (7D4; 'natal brown'), coronate; consistency fleshy, not tough; taste acrid; odor typical. – Tramal hyphae 4–12 µm diam, inflated, clamped, thin- to slightly thick-walled (wall <1 µm thick), hyaline, yellowish when aggregated, more or less gelatinous; gloeoplerous hyphae abundant, 3–12 µm diam, with dense refractive contents, yellowish, staining black in sulfo-benzaldehyde solution. Subhymenium 20 µm thick, gelatinized. – Hymenium about 20 µm thick, gelatinized; lower 3/4 part of individual basidium not distinct; basidia up to 20 x 5–6 µm, 4-sterigmate; sterigmata 4–5 µm long; gloeocystidia not observed; leptocystidia up to 5 µm diam, filamentous, projecting up to 8 µm from hymenium. – Basidiospores 4.0–5.5 x 2.5–4.0 µm (L^m=4.9 µm; W^m=3.2 µm; E=1.25-1.8; E^m=1.53), broadly elliptical, thin-walled, obviously asperulous, uniguttulate, amyloid; apiculus typical.

Habitat and distribution. – On rotting wood, including *Leptospermum* and *Nothofagus*. *Clavicorona turgida* has been reported from Southeast Asia, Australia, New Zealand, South America, Central America and Cuba.

Culture mat morphology. – Isolates examined: 2651, 2682, 44103. – Mean growth rate: (n = 3 x 3) 1 wk, 0.5 cm; 2 wks, 1.7 cm; 3 wks, 2.5 cm; 4 wks, 3.8 cm; 5 wks, 5.5 cm; 6 wks, plate covered. – Macromorphology: Advancing zone appressed; margin more or less fringed; silky; aerial mycelium white, pellicular or subfelty in dark, prostrate in light, zonate; reverse unchanged. odor like lemon or not distinctive. – Micromorphology: Advancing zone hyphae 1.5–3 µm diam, hyaline, infrequently branched, non-amyloid, thin-walled, clamped; knots of hyphae mostly intercalary; aerial hyphae 1.5–3 µm diam, similar to advancing zone hyphae; gloeoplerous hyphae 1.5–3 µm diam; prostrate hyphae like submerged

hyphae, dextrinoid, stained purple in Melzer's reagent. Submerged hyphae 2–6 μm diam, hyaline, dextrinoid, textura intricata, frequently branched, clamped. Gloeoplerous hyphae rare, 2–6 μm diam. Crystals parallelogram-shaped, few, 4–8 μm long. – Phenoloxidase reactions: Week II: Laccase (+), Tyrosinase (-). Week VI: Laccase (+), Tyrosinase (-). – Nobles code: 2a. 3. 11i. 15. 22. 36. 38. 46. 50. 55. 60. – Stalpers code: 1. 9. 13. 24. 29. 30. 37a. 39. 45. 53. 61. 65. 73. 83. 89. 94. 95. 97. 99.

Mating experiments. – Self-crosses among single-spore isolates of collections 2651 and 2682 from New Zealand revealed a bifactorial mating system. Intercollection matings between testers strains of these two collections were invariably intercompatible. Tester strains of *C. turgida* (collection no. 2682) were paired with those of *C. pyxidata* (5 collections, from North America, China and Sweden); all pairings were incompatible. Likewise, pairings of tester strains of *C. turgida* (collections 2651 and 2682) with those of *C. candelabrum* (collection 2679) were incompatible.

Laccase electrophoretic pattern. – Two collections from New Zealand had a single laccase band at R_i = 1.08. Collection 55137 (New Zealand), originally identified as *C. piperata*, also had an electrophoretic mobility of 1.08. Reexamination of the specimen allowed placement in *C. turgida* on both morphological and electrophoretic grounds. Collection 2679A showed the electrophoretic pattern of *C. turgida* (R_i = 1.08), but the morphology of *C. candelabrum* (smaller, more delicate basidiomata with slender branches). Basidiomata of 2679A and 2679 (*C. candelabrum*) were intermingled on the same log, but basidiomata of 2679A were lighter in color. Assignment of 2679A to *C. turgida* is tentative and based on electrophoretic data alone. This collection may represent an immature form of *C. turgida* or a hybrid. Collection 3961 from Tasmania was assigned to *C. turgida* by morphology, but the laccase mobility differed slightly (R_i = 1.09) (Fig. 1).

Clavicorona turgida bears the following separating taxonomic characters: 1) relatively stout basidiomata usually branched in several ranks, often with short or absent stipe; 2) fresh basidiomata relatively pale in color ('vinaceous buff,' 'cream buff'), but drying dark brown or brown-black; 3) basidiomata solitary to gregarious, but not crowded; 4) gelatinous subhymenium and hymenium; 5) basidiospores 4.0–5.5 x 2.5–4.0 μm.

Dodd (1970) examined the type specimen of *C. turgida* and drew attention to gelatinization of the hymenium and the subhymenium as a diagnostic character. Our examination of the type specimen

confirms Dodd's observation. Using this character, we bring together several collections under this name, and mating data combined with laccase enzyme mobility data seem to support the taxon as homogeneous.

Cultures used by Wu (1991) were not those used in the present study, but consistency of name used in the present study was attained through microscopic examination of a common collection (2682), and electrophoretic data from two collections in common to both studies (2651, 2682). The two collections belong to a single biological species.

Unnamed taxa

Taxon 1 (North Carolina)

Taxon 1 is represented by a single collection (no. 1615) from North Carolina where *Clavicorona pyxidata* is commonly distributed. Morphological characters which separate this collection are as follows: 1) subglobose to broadly ovoid basidiospores (3.8–4.5 x 3–4 µm; E^m = 1.23; *C. pyxidata* basidiospores are ellipsoid); 2) robust, copiously branched basidiomata on conifer wood (*C. pyxidata* usually fruits on hardwood); 3) gelatinized subhymenium (that of *C. pyxidata* is not so); 4) basidiomata camel-brown ; and 5) fawn to olive-brown coloration change of the dikaryon culture mat on media PDA (Wu, 1991; no other species shares this behavior).

Habitat and distribution. – On rotten conifer wood. North America (North Carolina).

Culture mat morphology. – Isolates examined: 1615. – Mean growth rate: (n = 2) 1 wk, 0.2 cm; 2 wks, 0.5 cm; 3 wks, 1.0 cm; 4 wks, 1.5 cm; 5 wks, 2.0 cm; 6 wks, 2.5 cm. – Macromorphology: Advancing zone submerged; margin fringed; aerial mycelium absent or poorly developed, silky, hyaline; reverse unchanged; odor not distinctive. – Micromorphology: Advancing zone hyphae 1.5–3 µm diam, hyaline, infrequently branched, non-amyloid, thin-walled, clamped, non-diverticulate. Aerial hyphae rare, similar to advancing zone hyphae. Submerged hyphae 1.5–3 µm diam, hyaline, dextrinoid, *textura intricata*, frequently branched, clamped. Gloeoplerous hyphae not observed. – Phenoloxidase reactions: Week II: not tested. Week VI: Laccase (+), Tyrosinase (-). – Nobles code: 2a. 3. 7. 11i. 36. 38. 54. – Stalpers code: 1. 10. 13. 20. 30. 37a. 39. 45. 52. 90.

Mating studies. – Mating system unknown: basidiospores did not germinate and crosses have not been performed.

Laccase electrophoretic pattern. – There is a major laccase band at R_i = 0.82 and light secondary bands at R_i = 1.00 and R_i = 1.08 (Fig. 1).

Taxon 1 is similar to *C. turgida* in gelatinous subhymenium and basidiospore sizes, but they differ in the color of basidiomata, culture mat morphology and laccase electrophoretic patterns. A collection by Atkinson (10700, CUP) from North Carolina, cited under *C. turgida* by Dodd (1972) may be taxon 1.

Taxon 2 (Puerto Rico)

Basidiomata of this single collection (no. 3456) are easily separated from those of all other taxa by the following characters: 1) pure white basidiomata; 2) subtropical distribution; 3) subhymenium and hymenium are not gelatinizing; 4) basidiospores 4–5.5 x 3–4 μm (E^m = 1.54).

Habitat and distribution. – On rotten hardwood stick. Puerto Rico.

Culture mat morphology. – Culture mat of Taxon 2 resembles that of *C. pyxidata*, but with a much slower growth rate on MEA.

Mating experiments. – Self-cross of 12 single spore isolates revealed a typical bifactorial mating system. Tester strains of Taxon 2 were paired with that of *C. microspora* (2349), *C. pyxidata* (56667 and 1541) and *C. turgida* (2651); all pairings were incompatible.

Laccase electrophoretic pattern. – Wu (1991) reported two intensive laccase bands at R_i = 1.19 and 1.27 (10% gels). The present study found a single band at R_i = 1.18, probably corresponding to the band at R_i = 1.19. The R_i = 1.27 band may be a variable band (Fig. 1).

The white basidiomata of Taxon 2 resembles *C. turgida*, but Taxon 2 exhibits smaller basidiomata and no sign of gelatinous texture. Basidiospore dimensions of collection 3456 are close to those of *C. colensoi* and *C. piperata*, but its basidiomata color is different.

Clavicorona pyxidata varies in color during development, but it is never pure white as is this collection, besides *C. pyxidata* produces narrower basidiospores.

Taxon 2 (collection 3456) may represent a distinct species. This conclusion is supported by morphology, incompatibility, and laccase electrophoretic data, but we wish to obtain more collections before proposing a name.

Taxon 3 (New Zealand)

Taxon 3 can be separated by the following characters: 1) basidiospores large and broad (5.0-6.0 x 4.0-5.0 μm; E^m = 1.19), prominently roughened; 2) tramal hyphae inflated, up to 16 μm broad; 3) gloeocystidia clavate to sublanceolate, conspicuous (lanceolate and inconspicuous in *C. piperata*); 4) nodulose to chlamydospore-like swellings on dikaryon mycelium; 5) conspicuous white basidiome basal mat when dry; and 6) distribution in New Zealand.

Habitat and distribution. – On rotten wood, including *Nothofagus*. New Zealand.

Culture mat morphology. – The culture mat of Taxon 3 on MEA is characterized by dull yellowish coloration, extremely slow growth rate, and formation of chlamydospore-like swellings.

Mating experiments. – Mating system unknown: numerous pairings of single-spore isolates of two collections on a variety of solid and liquid media failed to produce clamped hyphae.

Laccase electrophoretic mobility. – Cultures from two collections (2672 and 2822) from New Zealand produced one laccase band at R_i = 0.78; one of these cultures produced two additional bands at R_i = 0.94 and 1.26 (Fig. 1).

Based on comparison of herbarium specimens of these two collections to others in our herbarium (TENN, cited by Petersen, 1988), taxon 3 appears to be one of the most commonly collected species of *Clavicorona* in the southern Pacific. One of the morphological differences between Taxon 3 and *C. piperata* is basidiospore size (*C. piperata*: 3.5–4.5 x 2–3.5 μm; Taxon 3: 5.0–6.0 x 4.5–5.0 μm). Moreover, the basidiomata of Taxon 3 are deeper brown-colored than those of *C. piperata*. In the absence of an exhaustive study of type specimens, we do not feel ready to propose a new name for this taxon because there are numerous names available.

Wu (1991) reported restricted growth and production of chlamydospore-like structures on cultures of a New Zealand

collection (2822). An additional collection/culture (2672) with identical structures has been added. The two isolates which we retain in culture, both from New Zealand, respond differently to culture on PDA, with isolate 2822 turning the medium bright lemon yellow and 2672 not.

Taxon 4 (Tasmania)

Basidiomata stout, rooting (collections 4003, 4005, 4034) or slender, gregarious but not densely so (collections 3905, 3979); base 'fuscous' to 'hair brown' (collections 4003, 4005), 'sayal brown' (collections 4034, 4039), 'blackish brown' (collections 3905, 3979), upper branches 'benzo brown', 'drab', 'cinnamon brown', 'wood brown', 'sayal brown' (4003, 4005), 'cinnamon buff' (4034, 4039), 'deep brownish drab' or 'army brown' (3905, 3979). Apices 'tawny olive' (4003, 4005), 'cream buff' (4034, 4039). – Basidiospores faintly rough (3905, 3979) to echinulate (4003, 4003, 4034), globose to subglobose (4.0–4.4 x (3.2–)3.6–4.0 um; E^m = 1.12; 4003, 4005, 4034) and 4–4.4 x 2.8–3.8 um (E^m = 1.26; collections 3905, 3979). – Gloeocystidia about 5 µm diam, extended beyond basidia, fusiform or clavate (4003, 4005, 4034), lanceolate to fusiform (3905, 3979).

Culture mat morphology. – Data not available.

Mating experiments. – Mating system unknown; crosses between collections with two differing morphologies (3979 x 4003) were compatible.

Laccase electrophoretic mobility. – A single laccase band at R_i = 0.71 was observed for collections 3905, 3979 and 4003 (Fig. 1).

The taxon is represented by five collections (3905, 3979, 4003, 4005 and 4034) from Tasmania, only three of which were in culture. Based on morphological characters these collections appear to be *Clavaria pyxidata* var. *asperospora* Fawc. which was described from Australia (Fawcett, 1939). This taxon differs from typical *Clavicorona pyxidata* in several major characteristics including basidiomata color, basidiospores, habitat and distribution. Dodd (1972) considered *C. pyxidata* var. *asperospora* a synonym of *C. piperata* based on examination of herbarium specimens. Despite some similarities in morphology, the two taxa differ in habitat (*Eucalyptus* and *Nothofagus* vs. conifer), geographic distribution (Australia vs. North America), and laccase electrophoretic patterns. We now consider these collections to belong to a separate species from *C. piperata*.

There is considerable macromorphological variation in this taxon but a common laccase electrophoretic profile, micromorphology and intercompatibility of different phenotypes suggests that this is a single polymorphic taxon. Because the type specimen of *C. pyxidata* var. *asperospora* is not available to us at this time, we are not ready to make taxonomic treatment.

Discussion

Morphological characters used in delineating taxa

Subgenus *Ramosa* is unique among clavarioid fungi because of the pyxidately branched and cup-shape tipped branches on the fruit body. All taxa included in this study show this type of branching pattern, with the exception of *C. cristata* (rarely branched, but with cup-shaped apices). The unique branching and tip pattern is missing in two other taxa in the subgenus, i. e. *C. divaricata* Leathers & Smith (unbranched to dichotomously branched) and *C. dichotoma* Corner (typically dichotomously branched). Neither of the two taxa is included in this study because cultures were not available. However our examination of their type specimens revealed that gloeoplerous hyphae were absent in *C. divaricata* or they were replaced by skeletal hyphae in *C. dichotoma*. The skeletal hyphae of *C. dichotoma* are fundamentally different from the thick-walled hyphae of *C. candelabrum*. Thus, the placement of *C. dichotoma* and *C. divaricata* in the genus of *Clavicorona* is questionable.

The color of basidiomata is used as a valuable characteristics for classification of the genus *Clavicorona*, as for many other higher basidiomycetes. Dodd (1972), however, reported that basidiomata colors were variable in *C. pyxidata* with age and weather conditions during fructification. In this study we found that collections of *C. pyxidata* from various geographic areas were variable in coloration, from cream to very bright yellow, to tan, without particular pattern. Petersen (1988) also noted color changes in *C. colensoi* during basidiome development. This suggests to be cautious of intermediate states of basidiome development when using color of basidiomata to delimit taxa in *Clavicorona*. Use of fruitbody sizes should be combined with other characters in identifying species. Basidiomata of *C. pyxidata* range from 3 to 20 cm high and the actual size may be related to the micro-habitat of individuals and collection time. On the other hand, *C. colensoi* can often be distinguished from *C. piperata* and *C. turgida* by their smaller fruitbodies. There is no adequate substitute for freshly collected basidiomata as a basis for

morphological analysis. Immediate examination (before drying) may reveal additional macro- and micromorphological characters not observable even on 'adequately' dried specimens.

Certain micromorphological characters seem more useful to the taxonomy of the genus. The presence of thick-walled hyphae in *C. candelabrum* has been used to separate it from any other species of the genus. Such separation is supported by laccase data within a restricted geographic locality, i. e. New Zealand. A gelatinous subhymenium and hymenium has been used to characterize *C. turgida*, and the species was reported from a wide geographic range including Southeast Asia, Australia, New Zealand, South and Central America, and North Carolina. Our study confirmed the occurrence of *C. turgida* in Australia and New Zealand. Establishment of cultures from a collection from North America (North Carolina) enabled us to perform culture mat morphology and enzyme electrophoresis. By comparing specimens with these additional data with herbarium specimens cited by others, we identified that the North Carolinian collections actually represent a distinct taxon, i. e. Taxon 1. The procedure of using a single character or even several morphological characters to group allopatric populations into a taxon may cover up genetic divergence as illustrated by *C. pyxidata*. The characteristically narrow basidiospore seem to hold together the northern hemisphere distributed *C. pyxidata* populations, but laccase data indicated that allopatric populations are genetically divergent. The data obtained here support the findings by many others that many 'widely distributed' species that were grouped by either a single or a set of morphological characters are most likely genetically divergent at least to some extent, e. g. *Pleurotus ostreatus* (Prillinger & Molitoris, 1979), *Heterobasidion annosum* (Otrosina & al., 1988), *Agaricus bisporus* (Kerrigan, 1990), *Laccaria laccata* (Mueller, 1992).

Taxonomic value of culture mat morphology

The culture study was intended to document mycelium behavior on medium for as many *Clavicorona* species as possible and hopefully to discover useful morphological characters. Nobles (1965) and Stalpers (1978) developed a series of key code designations with which to identify wood-inhabiting Aphyllophorales. Both used malt extract agar (MEA) as a standard medium to analyze many species from a wide range of genera, and often obtained diagnostic characters for identifying genus and species. For that reason, MEA was used in this study to describe culture mat morphology. This medium (i.e. MEA), however, may not be sufficient for identification at the specific level. Because congeneric species may share some common physiological

features, they cannot be expected to be differentiated on the medium which is useful for delimitation of species of different genera or families. Utilization of different kinds of media may be valuable for intrageneric taxonomy. Desjardin (1990) reported in *Marasmius* that very little culture morphology variation between species was observed among isolates grown on MEA, whereas distinct morphological characters were observed among isolates grown on PDA. Previous observations (Wu, 1991) on culture mat morphology of *Clavicorona* were in agreement with the results obtained in *Marasmius*.

In general, the growth rate of *Clavicorona* isolates on MEA is distinct for different species and consistent among isolates of the same species. Color, texture and odor of culture mats also are valuable macromorphological characters in identifying species. Presence of clamped, inflated generative hyphae, spherical swollen hyphae, gloeoplerous hyphae, and crystal formation are common to all species. An individual species, however, may be characterized by certain unique features. The culture mat of *C. colensoi*, for example, was characterized by bright yellow coloration; *C. microspora* by rapid fruiting on agar media; *C. cristata* by the extremely low growth rate on all tested media; and Taxon 1 by a fawn coloration on PDA. Dikaryotic hyphae of *C. cristata* exhibited normally paired nuclei separated by septa with typical clamp connections. However a few verticillate and sprouting clamp connections were observed. The significance of formation of the verticillate clamp connections is difficult to understand.

Biological species and genetic divergence

Sexual compatibility has been used as a leading criterion of species concepts as proposed by Mayr (1942) and others. We have attempted to utilize this concept, and insofar as possible (monokaryon isolates of some taxa did not anastomose, some basidiospores did not germinate, etc.), the criteria appeared valid. Intercollection matings among putatively conspecific collections generally proved partially (i.e. *C. cristata*) to completely (i.e. *C. pyxidata*) intercompatible. Multiple allelism phenomena of mating factors were observed in *Clavicorona* species with which intercollection mating experiments were conducted. Although performed less frequently, 'interspecific' matings were never compatible and often exhibited strong antagonism phenomena. Thus, use of the biological species concept for appropriate fungi seems worthwhile, at the very least to indicate genetic differences.

The biological species concept depends on strong conservation of multigenic sexual recognition, hyphal anastomosis, dikaryotization,

and clamp connection formation mechanisms in the face of long distance and, probably, long-term separation. Such conservation has been demonstrated repeatedly (Anderson, 1986; Gordon & Petersen, 1991; Petersen & Halling, 1993). In the case of *C. pyxidata*, sexual compatibility indicated close genetic relatedness while laccase electrophoretic patterns indicated lack of recent gene exchange, especially across significant geographical barriers. All collections of *C. pyxidata* used in this study were within the accepted limits of a single morphospecies. At the same time, their total intercompatibility fulfills the definition of a single biological species (see Mayr, 1942). Extracellular laccase electromorphs, however, suggest that collections (= populations) from China are genetically different from populations of all other locations from which cultures were available to us. A similar situation was found in the *Collybia dryophila* complex where genetic divergence was associated with allopatry but not incompatibility (Vilgalys, 1991).

In compatible pairings between Californian and Finnish collections of *C. cristata*, both false and true clamp connections were observed. The true clamp connections are a sign of a compatible mating, whereas the false clamp connections indicate that nuclear migration is blocked due to genetic or biochemical factors other than mating type alleles. Inhibition of nuclear migration may be an indication of reproductive isolation. Difference in laccase mobility of the two *C. cristata* populations support the view that allopatric speciation may be in progress.

Accurate interpretation of mating reactions is the basis for understanding compatibility. In all matings, dikaryotization followed by formation of clamp connections was used as the only reliable evidence of compatibility. In species such as *C. microspora*, *C. pyxidata* and Taxon 2, clamp connections were abundant and usually could be observed all over in compatible matings, whereas in other species such as *C. cristata, C. colensoi* and *C. candelabrum*, clamp connections were scarce in compatible matings and could be found only in contact zones. In most matings of *Clavicorona*, barrage reactions were identified by raised mycelia in the contact zone and were used as an indication of common-B pairing. Sometimes the occurrence of barrage, however, was variable and unpredictable in different pairings of different collections. Some common-B pairings did not form typical barrage morphology and barrages were observed occasionally in compatible matings or common-A matings. This is in agreement with James' (1983) observation in *C. pyxidata* and many other studies in basidiomycetes (e. g. Raper, 1966; Esser & Hoffman, 1976). False clamp connections usually occurred on heterokaryotic hyphae resulting from common-B matings. False clamp connections are incomplete clamp connections, with septa preventing nuclear

migration. They can be easily identified under epifluorence microscopy. James' observation (1983) that false clamp connections were produced sporadically and in small numbers in common-B matings of *C. pyxidata* was confirmed by this study.

In collections of a few *Clavicorona* species (*i. e. C. candelabrum* and *C. colensoi*), self-crosses were not productive (i.e. pairings were dominated by incompatible matings), even on media of different concentration and after extended periods of time. There were several possible explanations for the failure to produce compatible matings, including: 1) the medium used for mating tests might not be suitable for the physiological requirements of the species, and matings, therefore, were inhibited; 2) mating ability might be lost or impaired; and 3) there may be separate genes controlling 'fertility' (in the sense of Chase & Ullrich, 1990).

Validity of extracellular laccase mobility as a systematic tool

Our data support the conclusions of Kerrigan & Ross (1988) that extracellular laccase mobility patterns may be of value in basidiomycete systematics. Most *Clavicorona* species produced relatively simple and reproducible laccase patterns. This property makes the comparison of laccase electrophoretic banding patterns a valuable taxonomic tool. The same property was found in some other species, for example, *Polyporus brumalis*, *Leptoporus litschaueri* (Blaich & Esser, 1975), and *Agaricus* (Kerrigan & Ross, 1988).

Laccase electrophoretic mobility within *C. pyxidata* was invariant over a wide geographical area (Rocky Mountains to Europe). Laccase electrophoretic mobilities for two widely separated Chinese isolates (Jilin Province in the northeast and Yunnan Province in southwest China) differed from that of North American and European isolates but were identical to each other. The two Chinese collections were intercompatible with North American and European strains. Laccase was invariant within *C. colensoi* (New Zealand and Australia) and only minor variants were observed within *C. turgida* (Tasmania and New Zealand) and *C. candelabrum* (New Zealand). In general, all tested species produced uniquely characteristic laccase banding patterns consistent within species and different from other species. Where minor differences were observed, they were between geographically separated isolates. Collections that were partially intercompatible were also separated by laccase banding pattern, as in *C. cristata*.

Extracellular laccase electrophoretic mobility patterns were useful in the systematics of this genus in two ways: 1) In delineating

species: laccase patterns share an almost invariable relationship to individual morphotaxa. Our analysis of laccase electrophoretic patterns caused a reappraisal of morphological characters. 2) In revealing significant reproductive barriers as in *C. pyxidata* and *C. cristata*. This, in turn, revealed differences between taxa at a more fastidious level than previously considered. In this way, our study is similar to work on *Armillaria*, in which intercompatibility studies caused a reappraisal of morphological and ecological characters, and delineation of several taxa previously treated as synonyms or previously undescribed (Anderson, 1986; Anderson & al., 1980). All such studies point towards acceptance of a pluralistic species concept (Mishler & Donoghue, 1982) in which several criteria delineate species (For example, see studies by Sieber-Canavesi & al., 1991; Sieber & al., 1991; Chang & Mills, 1992). These studies also support literature on the usefulness of laccase electrophoresis in fungal systematics, once proper biogeographical considerations are made.

Geographic isolation and distribution of the subgenus *Ramosa*

In the case of *C. pyxidata*, it is evident that genetic differentiation is due to long distance geographic separation. The two 'enzymatic species' are sheltered under the name *C. pyxidata*: these entities, while retaining the potential to interbreed *in vitro* with other worldwide populations, apparently are functionally allopatric, and have been separated from others for long enough to have genetic changes reflected in laccase enzyme differences. While the Pacific Ocean may serve as an obvious reproductive barrier to the east, separation from Europe is more difficult to explain. The temperate forests of China are separated from those of Europe by northern coniferous forest across Russia, temperate grasslands and desert to the north and west and by the Himalaya Mountains to the south and east. These regions may offer poor habitat for *C. pyxidata* with its requirement for hardwood substrate. Thus, Chinese and European populations may be effectively separated by geographical barriers and inhospitable ecosystems. Such data complement similar findings by Petersen & Halling (1993) in *Oudemansiella mucida*. Data gathered for species of *Marasmius* (S. A. Gordon, unpubl.), have demonstrated that the Atlantic Ocean is an effective reproductive barrier to at least some agarics, allowing isozyme differences to accumulate. Whether or not the Atlantic Ocean has acted as such a barrier within *C. pyxidata* cannot be ascertained from the data reported in this study and further studies are required. Likewise, *C. cristata* was collected from the northern coniferous forests of California and Finland. Here, laccase

isozyme patterns were distinctly different within this biological species, indicating effective geographical isolation.

The Northern Hemisphere harbors at least five species (*C. pyxidata*, *C. piperata*, *C. cristata*, *C. microspora*, and 'Taxon 1'). The south Pacific Ocean land masses (New Zealand, Tasmania) appear to support several taxa, some described (i.e. *C. colensoi*, *C. turgida*, and *C. candelabrum*), others not (Taxon 3 and 4). Thus, while traditional literature might indicate that *C. pyxidata* dominates the North Temperate Zone and occurs in a cosmopolitan distribution, several other taxa with more restricted geographic distribution are recognizable. The presence of at least four taxa in New Zealand, a relatively small geographical area, may reflect habitat diversity in New Zealand or suggest that the South Pacific area be a center of diversity for the subgenus.

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