

Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters

JEAN-MARC MONCALVO*, HUEI-FANG WANG AND RUEY-SHYANG HSEU

Applied Microbiology Laboratory, Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China

A gene phylogeny of 29 isolates of the *G. lucidum* complex collected in temperate and subtropical areas was produced by parsimony analysis from nucleotide sequence data of the internal transcribed spacer region of the ribosomal gene and from divergent domain D2 of the large ribosomal subunit gene, and serves as hypothesis of natural relationships between taxa. Results were compared with morphological, ecological, cultural and mating data. They show that extensive convergence or parallelism of morphological characters has occurred during *Ganoderma* evolution, but also that remarkable morphological difference may occur with little divergence time. Monophyletic groups correlate fairly well with geographical origin of the taxa and/or host relationships. Phylogenetically related isolates have similar culture characteristics, but they may share these characteristics with distant taxa. Therefore, culture characters are less polymorphic than morphological characters between recently diverged taxa, but are useless in recognizing monophyletic groups. Isolates belonging to the same biological species were monophyletic with one exception. A species concept based on monophyly and potential evidence of genetic isolation is proposed, and taxonomy of the *G. lucidum* complex is revised. Collections named *G. lucidum* in North America and in Asia are not conspecific with European *G. lucidum*. The sister group of European *G. lucidum* is an Argentinian taxon labelled *G. oerstedii*. North American *G. lucidum* is related to a Formosan isolate identified as *G. boninense*. *G. tsugae* is absent from Taiwan and probably also from Japan and China, although it was commonly reported there. *G. tsugae* belongs to a lineage restricted to coniferous forests in the more Northern latitudes, of which the taxonomy remains unresolved (the *G. valesiacum* complex). Correct naming and distribution of several taxa are still to be investigated. From observation of distribution of taxa within monophyletic groups it is speculated that laccate *Ganoderma* may have originated in the tropics.

A lot of taxonomic confusion has always been associated with *Ganoderma lucidum* (Curtis: Fr.) P. Karst. and allied species. They are white-rot polypore fungi having laccate basidiocarps that vary in colour from orange-red to black with several brownish gradations. Basidiocarps are stipitate or sessile. The context is uniformly coloured or duplex, ranging from nearly white to brown. The pore surface is light coloured. The outer layer of the pileus is composed of pilocystidia. Basidiospores are double-walled, slightly to strongly echinulate and truncated at the apex.

The first laccate polypores were described from Europe in the late eighteenth century. Karsten (1881) established the genus *Ganoderma* with *Boletus lucidus* Curtis as the only species, of which no authentic material remains. Steyaert (1961) designated plate no. 224 of William Curtis's *Flora Londonensis* published in 1781 as the lectotype of *Boletus lucidus*. In the original description, Curtis reported the collection from hazel. In Europe, four taxa related to *G. lucidum* are presently distinguished (Ryvarden & Gilbertson, 1993). They are *G. resinaceum* Boud., *G. pfeifferi* Bres., *G. carnosum* Pat. and *G. valesiacum* Boud. The latter two species

are restricted to conifers, and the distinction between *G. valesiacum* and *G. lucidum* remains tentative (Ryvarden & Gilbertson, 1993). *G. resinaceum* has often been confused with *G. lucidum* although morphological differences exist between the two species (Steyaert, 1972; Ryvarden & Gilbertson, 1993). *G. resinaceum* and *G. lucidum* have been reported worldwide, while *G. carnosum* seems restricted to Europe. *G. pfeifferi* was reported from Malaysia by Corner (1983).

Adaskaveg & Gilbertson (1986) reported that cultures of collections named *G. lucidum* in North America could not be differentiated from those of European *G. resinaceum* and showed that the two taxa belong to the same biological species. Steyaert (1980) had previously reported that collections named *G. lucidum* in North America might not be conspecific with European *G. lucidum*.

G. tsugae Murrill and *G. oregonense* Murrill are two species described from conifers in North America. Morphologically, they are doubtfully distinct (Gilbertson & Ryvarden, 1986) although the latter has larger basidiocarps, slightly larger spores and longer pilocystidia (Gilbertson & Ryvarden, 1986; Adaskaveg & Gilbertson, 1988). Culture characters of the two taxa are similar (Adaskaveg & Gilbertson, 1989), and they resemble those described from European *G. valesiacum* (Stalpers, 1978; Adaskaveg & Gilbertson, 1986). Stalpers

* Corresponding author, present address: Institute for Systematic Botany, University of Lausanne, CH-1015 Lausanne, Switzerland.

Table 1. Original identification, locality and source of morphological and/or cultural data of the isolates of this study

Original identification and collection No.	Geographical origin	Host	Material available*	Sources of morphological and/or cultural data
<i>G. ahmadii</i> Steyaert +FWP 14329	Pakistan	<i>Dalbergia sissoo</i>	B	Steyaert, 1972
<i>G. carnosum</i> Pat. ‡AHN 1197–121	Germany	<i>Abies</i> stump	B	This work
<i>G. lucidum</i> (Curtis: Fr.) P. Karst				
RSH RZ	Taiwan	Sawdust	C,M	Hseu, 1990
RSH 0626	Taiwan	Sawdust	C,M	Hseu, 1990
ATCC 32471	India	<i>Acrocarpus</i> sp.	M	Hseu, 1990; Wang & Hua, 1991
JMM P93-1	Philippines	Deciduous tree	B,M	This work
RYV 33217	Norway	<i>Betula</i> sp.	B	This work
CBS 270.81	France	n.a.	M	Wang & Hua, 1991
HMAS 60537	China	n.a.	M	This work
CBS 430.84	U.S.A.	<i>Quercus hypoleucoides</i>	M	Wang & Hua, 1991; Adaskaveg & Gilbertson, 1986
ATCC 46755	U.S.A.	Coniferous tree	M	Wang & Hua, 1991
RSH TEX.1	§U.S.A.?	Sawdust	M	This work
<i>G. tsugae</i> Murrill				
RSH J2	Japan	Sawdust	C,M	Hseu, 1990
RSH 1109	§Taiwan	Sawdust	C,M	Hseu, 1990
RSH BLC	§Taiwan	Sawdust	C,M	Hseu, 1990
RSH 0981	China	Coniferous tree	B,M	This work
CBS 223.48	Canada	<i>Tsuga canadensis</i>	M	Wang & Hua, 1991
CBS 428.84	U.S.A.	<i>Abies concolor</i>	M	Wang & Hua, 1991; Adaskaveg & Gilbertson, 1986
ATCC 46753	U.S.A.	n.a.	M	Wang & Hua, 1991
ATCC 46754	U.S.A.	n.a.	M	Wang & Hua, 1991
<i>G. valesiacum</i> Boud.				
CBS 282.33	U.K.	n.a.	M	Wang & Hua, 1991
<i>G. oregonense</i> Murrill				
CBS 177.30	U.S.A.	Gymnosperm wood	M	Wang & Hua, 1991
ATCC 46750	Canada	<i>Tsuga heterophylla</i>	M	Wang & Hua, 1991
<i>G. oerstedii</i> (Fr.) Torr.				
ATCC 52409	Argentina	n.a.	M	Bazzalo & Wright, 1982; Wang & Hua, 1991
ATCC 52410	Argentina	<i>Pinus</i> sp.	M	Bazzalo & Wright, 1982; Wang & Hua, 1991
<i>G. boninense</i> Pat.				
RSH RS	Taiwan	Sawdust	C,M	Hseu, 1990
<i>G. resinaceum</i> Boud.				
CBS 194.76	Netherlands	<i>Fagus sylvatica</i>	M	Wang & Hua, 1991; Adaskaveg & Gilbertson, 1986
CBS 152.27	U.K.	n.a.	M	Wang & Hua, 1991
ATCC 52411	Argentina	<i>Scutia buxifolia</i>	M	Bazzalo & Wright, 1982; Wang & Hua, 1991
<i>G. pfeifferi</i> Bres.				
CBS 747.84	Netherlands	<i>Quercus</i> sp.	M	Wang & Hua, 1991

* B, wild collected basidiocarp; C, basidiocarp produced in sawdust medium; M, mycelium in pure culture.

† Type specimen.

‡ Authentic specimen of *G. atkinsonii* Jahn, Kotl. & Pouzar, which is a synonym of *G. carnosum* (Kotlaba & Pouzar, 1983).

§ Commercially cultivated in U.S.A. and in Taiwan, but exact origin of the strain might be Japan.

|| This isolate is labelled *G. oerstedii* in Bazzalo & Wright (1982) and in ATCC culture collection, but its name was changed to *G. resinaceum* (J. E. Wright, pers. comm.).

(1978) considered *G. valesiacum* an earlier name for *G. tsugae*, while Adaskaveg & Gilbertson (1986) stated that mating studies would be necessary prior to establishing the synonymy. *G. tsugae* was reported from Japan (Imazeki, 1939), from China (Zhao, 1989) and from Taiwan (Sawada, 1942) but not from more Southern latitudes. *G. oregonense* was not reported outside North America.

Species circumscription, phylogenetic relationships, host range and distribution of taxa of the *G. lucidum* complex are therefore unclear even among the few taxa nowadays recognized in temperate countries. In a previous work using molecular data to infer phylogenetic relationships and taxonomic segregation in the Ganodermataceae Donk, it appeared that isolates labelled *G. tsugae* in Taiwan and in

North America might not be conspecific, while an Argentinian isolate labelled *G. oerstedii* was close to North American *G. tsugae* (Moncalvo, Wang & Hseu, 1995).

In this study, we have selected 30 isolates of the *G. lucidum* complex from different geographical origins to infer their natural relationships. Preference was given to isolates having available morphological, ecological, cultural and/or mating data for comparison with molecular data. Tropical taxa were not included in this work because of the lack of adequate material in culture, and because identification of tropical collections is generally problematic (Corner, 1983). Phylogenetic characters were produced from nucleotide sequence data from the internal transcribed spacer (ITS) region of the ribosomal gene (rDNA) and from divergent domain D2 of the

large ribosomal subunit gene (LSU-D2). Gene phylogeny was constructed using parsimony analysis and serves as hypothesis of evolutionary relationships between isolates. The use of molecular data to investigate phylogenetic relationships in fungi has been reviewed by Bruns, White & Taylor (1991) and by Hibbett (1992).

MATERIALS AND METHODS

Source of materials

Organisms used in this study and their origins are given in Table 1, together with references to previous systematic studies using these collections. Sources were abbreviated as follows: FWP, Fungi of West Pakistan, in Steyaert's collection in the Jardin Botanique de Belgique (Meise, Belgium); ATCC, American Type Culture Collection (Rockville, Maryland, U.S.A.); CBS, Centraalbureau voor Schimmelcultures (Baarn, Netherlands); HMAS, Herbarium of the Microbiology Department of Academia Sinica (Beijing, China); RYV, Herbarium of Leif Ryvarden (Oslo, Norway); JAHN, ex-Jahn's Herbarium (obtained from Leif Ryvarden); ZHANG, isolates provided by Dr X. Q. Zhang (Beijing, China); RSH and JMM, author's collection. ATCC and CBS strains were purchased via the Culture Collection and Research Center (CCRC) of Hsinchu in Taiwan.

Notes about names given to the isolates

In our sample, we consider collection RYV 33217 the most similar isolate to the lectotype of *B. lucidus* for two reasons: (i) its basidiocarp morphology matches Steyaert's (1967*a*) description and picture of *G. lucidum*, of which he stated that his collection resembles Curtis's plate no. 224 in *Flora Londonensis* 1781; (ii) like Curtis's specimen, RYV 33217 was collected on hardwood in Northern Europe. Also, we consider strain *G. tsugae* CBS 223.48 correctly identified since it was collected on *Tsuga canadensis* Carr. in North America, as was the type specimen of this species (Murrill, 1902). Collection *G. ahmadii* FWP 14329 is unambiguously named because it is the type specimen of this species. Collection *G. carnosum* JAHN 1197-121 is an authentic specimen of *G. atkinsonii* Jahn, Kotl. & Pouzar, which was later considered conspecific with *G. carnosum* by Kotlaba & Pouzar (1983). Names given to the other isolates in Table 1 were often tentative: some names have been changed one or more times. For instance in CBS and in CCRC, North American isolates originally identified as *G. tsugae* were changed to *G. valesiacum*, following Stalpers (1978). Hseu (1990) renamed *G. tsugae* Japanese collections RSH 1109 and RSH J2 originally identified as *G. lucidum*. Also, the strain labelled *G. resinaceum* ATCC 52411 is named *G. oerstedii* in the ATCC catalogue, as it appeared in Bazzalo & Wright (1982), but the latter author modified his first taxonomic decision (J. E. Wright, pers. comm.).

Cultures

Cultures were kept on PDA (potato dextrose agar Difco) at 6 °C. Mycelia were grown 1–2 wk on potato dextrose (Difco)

at 25° or at 30° prior to harvesting, then lyophilized and stored at –20° until DNA isolation. Some isolates were grown on MEA (malt extract agar Difco) and/or PDA to examine their culture characteristics.

DNA isolation, PCR amplification, sequencing and phylogenetic analysis

DNAs were isolated either from lyophilized mycelia grown on potato-dextrose or from herbarium specimens. Regions targeted for PCR amplification and sequencing were the internal transcribed spacer (ITS) region and divergent domain D2 of the large ribosomal DNA gene (LSU-D2). Sequences encompassing the LSU-D2 region were recorded for all strains from corresponding positions 425 to 648 of *Saccharomyces cerevisiae* LSU-rRNA (Gutell & Fox, 1988). Oligonucleotide primers used and procedures for DNA isolation, PCR amplification, sequencing and phylogenetic analysis are described elsewhere (Moncalvo *et al.*, 1995).

RESULTS AND DISCUSSION

Nucleotide sequence variation and phylogenetic analysis

Nucleotide sequences were deposited in the European

Table 2. EMBL nucleotide sequences accession numbers of divergent domain 2 of the large ribosomal subunit gene (LSU-D2) and of the internal transcribed spacers ITS 1 and ITS 2 for the isolates listed in Table 1

Isolate no.	LSU-D2	ITS 1	ITS 2
FWP 14329	Z37018	Z37047	Z37098
RSH RZ	X78776	X78743	X78764
RSH 0626	Z37019	Z37048	Z37072
ATCC 32471	Z37020	X78744	X78765
JMM P93-1	Z37021	X78745	X78766
RYV 33217	Z37094	Z37096	Z37073
CBS 270.81	Z37022	Z37049	Z37099
HMAS 60537	Z37023	Z37050	Z37074
CBS 430.84	Z37024	Z37051	Z37075
ATCC 46755	Z37025	Z37052	Z37076
RSH TEX.1	Z37026	Z37053	Z37077
RSH J2	X78778	X78746	X78767
RSH 1109	Z37027	X78747	X78768
RSH BLC	Z37028	Z37097	Z37078
RSH 0981	Z37029	X78748	X78769
JAHN 1197-121	Z37032	Z37057	Z37082
CBS 223.48	Z37154	Z37054	Z37079
CBS 428.84	X78781	X78735	X78756
ATCC 46753	n.d.*	Z37063	Z37105
ATCC 46754	Z37030	Z37055	Z37080
CBS 282.33	Z37031	Z37056	Z37081
CBS 177.30	Z37035	Z37060	Z37100
ATCC 46750	Z37036	Z37061	Z37101
ATCC 52409	Z37033	Z37058	Z37083
ATCC 52410	X78787	X78739	X78760
RSH RS	X78777	X78749	X78770
CBS 194.76	X78785	X78737	X78758
CBS 152.27	Z37037	Z37062	Z37085
ATCC 52411	Z37034	Z37059	Z37084
CBS 747.84	X78786	X78738	X78759

* Not determined.

Table 3. Nucleotide sequence variation in the divergent domain 2 of the large ribosomal subunit gene* (LSU-D2) and in the internal transcribed spacer 1 (ITS 1) and 2 (ITS 2) in our sample†

Number of	rDNA region			Total
	LSU-D2	ITS 1	ITS 2	
Positions aligned	229	209	196	634
Variable positions	8	44	39	91
Phylogenetically informative sites	7	41	34	82
Phylogenetically informative sites unambiguously aligned	7	35	20	62
Gaps in the alignment	0	9	5	14
Transitions				
C <-> T	4	12	6	22
A <-> G	3	11	8	22
Transversions				
A <-> T	0	1	1	2
A <-> C	0	0	0	0
G <-> C	0	1	1	2
G <-> T	0	1	1	2
Transitions/transversions ratio	7:0	7:6:1	4:7:1	7:3:1

* Calculated from corresponding positions 425 to 648 of *Saccharomyces cerevisiae* rRNA (Gutell & Fox, 1988).
† Excluding strain ATCC 46753 (see text).

Molecular Biology Laboratory (EMBL) and given accession numbers shown in Table 2. Nucleotide sequences of the ITS region of isolate ATCC 46753 were unalignable with those of the other strains in this study. This isolate was therefore excluded from molecular analysis.

Nucleotide sequence variation in the three rDNA regions studied is reported in Table 3. In the LSU-D2 region all strains aligned unambiguously in 229 positions. Eight positions were variable. One substitution was unique to isolate ATCC 52411, while seven substitutions were phylogenetically informative. All substitutions were transitions. Several isolates share identical nucleotide sequences, resulting in the distinction of eight groups on the basis of sequence similarity. These groups are shown in Table 4.

Nucleotide sequence alignment in the ITS region produced 75 phylogenetically informative sites, of which 55 were unambiguously aligned. Short length mutations resulted in 20 positions in which different alignments were possible for some taxa. All ambiguously aligned positions were in three highly variable regions: six sites were in the central region of ITS 1, while four and 10 sites respectively were near the 5' end of the ITS 2 region. High transition biases were calculated in the two ITS regions (Table 3). The bias was almost twice as great in ITS 1 as in ITS 2.

The phylogenetic tree depicted in Fig. 1 was produced using parsimony analysis from the 82 cladistically informative characters from the combined D2 and ITS datasets. Positions with different possible alignments were recoded as previously described (Moncalvo *et al.*, 1995). Exclusion from the dataset of ambiguously aligned positions did not affect tree topology, nor did analyses using different possible alignments in the ITS region (data not shown). Different analyses only slightly affected statistical support of the branches. Parsimony analysis from the seven phylogenetically informative characters of the LSU-D2 region alone generated a 50% majority-rule consensus

tree having similar topology to the tree depicted in Fig. 1, but no branches were statistically supported by bootstrapping (data not shown).

Six monophyletic groups were resolved with a statistical confidence equal to or greater than 92%. To facilitate discussion, these groups are designated Groups 1–6 (Fig. 1). Monophyly between Groups 1 and 2 (Phylum I) is supported by 99% of bootstrap replications, and monophyly between Groups 3 and 4 (Phylum II) has a statistical support of 94%. In Fig. 1, phylogenetic relationships were evaluated using European isolates of *G. resinaceum* and *G. pfeifferi* (Group 6) as outgroups to root the tree. However, examination of the distance matrix (Table 4) shows that these isolates may not be the more divergent taxa in our sample. Thus, their use as an outgroup may misevaluate phylogenetic relationships between lineages as they are depicted in Fig. 1. Phylogenetic trees rooted with Phylum I and Group 4 respectively are shown in Figs 2 and 3. Results show that monophyly between Groups 5 and 6 (Phylum III) is supported by 92–95% of bootstrap replications. Basal relationships between Phyla I, II and III remain unresolved (Figs 1–3).

Table 4 shows that taxa sharing identical sequences in the LSU-D2 region differ by 0 to < 2% of nucleotide substitutions in the ITS region. Table 4 also indicates that more recently diverged taxa often have higher nucleotide sequence variation in the ITS 2 region than in the other regions studied, indicating that the ITS 2 region usually diverged earlier than the other regions. With longer divergence times, there was no significant difference between ITS 1 and ITS 2 variations, with one notable exception between Groups 5 and 6 (6–7% variation in ITS 1 *v.* 1.5–3% in ITS 2): that indicates that nucleotide substitution rates in the two spacers were not similar in all groups, therefore, that molecular divergence observed in the spacers is only a rough evaluation of divergence time between taxa. Calculated biases within the *G. lucidum* complex (Table 3) were higher than those reported from more divergent *Ganoderma* species (Moncalvo *et al.*, 1995), and overall results agree with observations that transition bias declines with greater divergence time, i.e. when more divergent taxa are included in the calculation, as discussed in the paper cited above.

Gene phylogeny, basidiocarp morphology and ecology

The gene phylogeny depicted in Fig. 1 shows that isolates identified as *G. lucidum* nest throughout Groups 1 to 5, that collections named *G. tsugae* classify in Groups 1, 2 and 4, and that the three isolates labelled *G. resinaceum* do not cluster together (Groups 3 and 6). The result indicates extensive parallelism or convergence in the evolution of morphological characters, resulting in phenotypically similar basidiocarps of polyphyletic origin.

In contrast, remarkable phenotypic divergence may occur with little divergence time. In Group 2, Argentinian collections of *G. oerstedii* are barely distinguished from European *G. lucidum* by sequence divergence (Fig. 1 and Table 4), but the two taxa are morphologically very distinctive (Bazzalo & Wright, 1982; Ryvarden & Gilbertson, 1993) and were even classified in different sections by Steyaert (1980). One may

Table 4. Number of nucleotide substitutions between taxa* in ITS 1 (below the diagonal) and in ITS 2 (above the diagonal). Taxa are listed in the same order as in Fig. 1. Bo in the LSU-D2 region

Taxa	Locality†	Group 1									Group 2					Group 3					Group 4	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
1 <i>G. ahmadii</i> 14329	PK	—	2	5	2	2	5	4	4	4	5	5	5	5	5	18	20	19	19	21	18	
2 <i>G. valesiacum</i> 282.33	EC	1	—	3	0	0	3	2	2	2	3	3	3	3	5	16	18	19	17	19	16	
3 <i>G. carnosum</i> 1197–121	EC	2	1	—	3	2	4	3	3	3	4	4	4	4	6	15	17	18	16	16	15	
4 <i>G. tsugae</i> 223.48	CA	2	1	0	—	0	3	2	2	2	3	3	3	3	5	16	18	19	17	19	16	
5 <i>G. tsugae</i> 46754	US	1	0	0	0	—	1	0	0	0	1	1	1	1	3	14	16	17	15	17	14	
6 <i>G. tsugae</i> 428.84	US	3	2	1	1	1	—	1	1	1	2	2	2	2	4	15	17	18	16	18	15	
7 <i>G. lucidum</i> 46755	US	2	1	0	0	0	1	—	0	0	1	1	1	1	3	14	16	17	15	17	14	
8 <i>G. oregonense</i> 177.30	US	2	1	0	0	0	1	0	—	0	1	1	1	1	3	14	16	17	15	17	14	
9 <i>G. oregonense</i> 46750	US	2	1	0	0	0	1	0	0	—	1	1	1	1	3	14	16	17	15	17	14	
10 <i>G. lucidum</i> 33217	EC	4	3	2	2	2	3	2	2	2	—	0	0	0	2	13	15	16	14	16	13	
11 <i>G. lucidum</i> 270.81	EC	5	4	3	3	3	4	3	3	3	0	—	0	0	2	12	14	15	13	16	12	
12 <i>G. oerstedii</i> 52410	AG	4	3	2	2	2	3	2	2	2	0	1	—	0	2	13	15	16	14	16	13	
13 <i>G. oerstedii</i> 52409	AG	4	3	2	2	2	3	2	2	2	0	1	0	—	2	13	15	16	14	16	13	
14 <i>G. tsugae</i> 0981	CH	5	4	3	3	3	4	3	3	3	1	1	1	1	—	15	17	16	16	18	14	
15 <i>G. lucidum</i> RZ	TW	19	18	17	17	17	18	17	17	17	17	18	17	17	18	—	1	2	0	5	12	
16 <i>G. lucidum</i> 0626	TW	19	18	17	17	17	18	17	17	17	17	18	17	17	18	0	—	3	3	7	13	
17 <i>G. lucidum</i> 32471	IN	19	18	17	17	17	18	17	17	17	17	18	17	17	18	0	0	—	4	8	14	
18 <i>G. lucidum</i> P93-1	PH	19	18	17	17	17	18	17	17	17	17	18	17	17	18	0	0	0	—	6	13	
19 <i>G. resinaceum</i> 52411	AG	20	19	18	18	18	19	18	18	18	18	19	18	18	19	4	4	4	4	—	15	
20 <i>G. lucidum</i> 60537	CH	24	23	22	22	22	23	22	22	22	24	25	24	24	23	16	16	16	16	20	—	
21 <i>G. tsugae</i> 1109	TW	25	24	23	23	23	24	23	23	23	25	26	25	25	24	17	17	17	17	21	1	
22 <i>G. tsugae</i> J2	JP	24	23	22	22	22	23	22	22	22	24	25	24	24	23	16	16	16	16	20	0	
23 <i>G. tsugae</i> BLC	TW	24	23	22	22	22	23	22	22	22	24	25	24	24	23	16	16	16	16	20	0	
24 <i>G. lucidum</i> 430.84	US	17	18	17	17	17	18	17	17	17	19	20	19	19	18	14	14	14	14	17	20	
25 <i>G. tsp.</i> TEX.1	?	18	19	18	18	18	19	18	18	18	20	21	20	20	19	15	15	15	15	18	21	
26 <i>G. boninense</i> RS	TW	18	19	18	18	18	19	18	18	18	20	21	20	20	19	15	15	15	15	18	21	
27 <i>G. pfeifferi</i> 747.84	EC	16	17	16	16	16	17	16	16	16	18	19	18	18	17	21	21	21	21	24	23	
28 <i>G. resinaceum</i> 152.27	EC	16	17	16	16	16	17	16	16	16	18	19	18	18	17	20	20	20	20	23	23	
29 <i>G. resinaceum</i> 194.76	EC	17	18	17	17	17	18	17	17	17	19	20	19	19	18	22	22	22	22	25	24	

* As determined by pairwise distance in PAUP from nucleotide sequences alignment between taxa. One nucleotide substitution between two taxa in ITS 1 or in ITS 2 corresponds to a difference of 1.

† Abbreviated as follows: Pakistan, PK; Europe, EC; Canada, CA; U.S.A., US; Argentina, AG; China, CH; Taiwan, TW; India, IN; Japan, JP; ?, unknown.

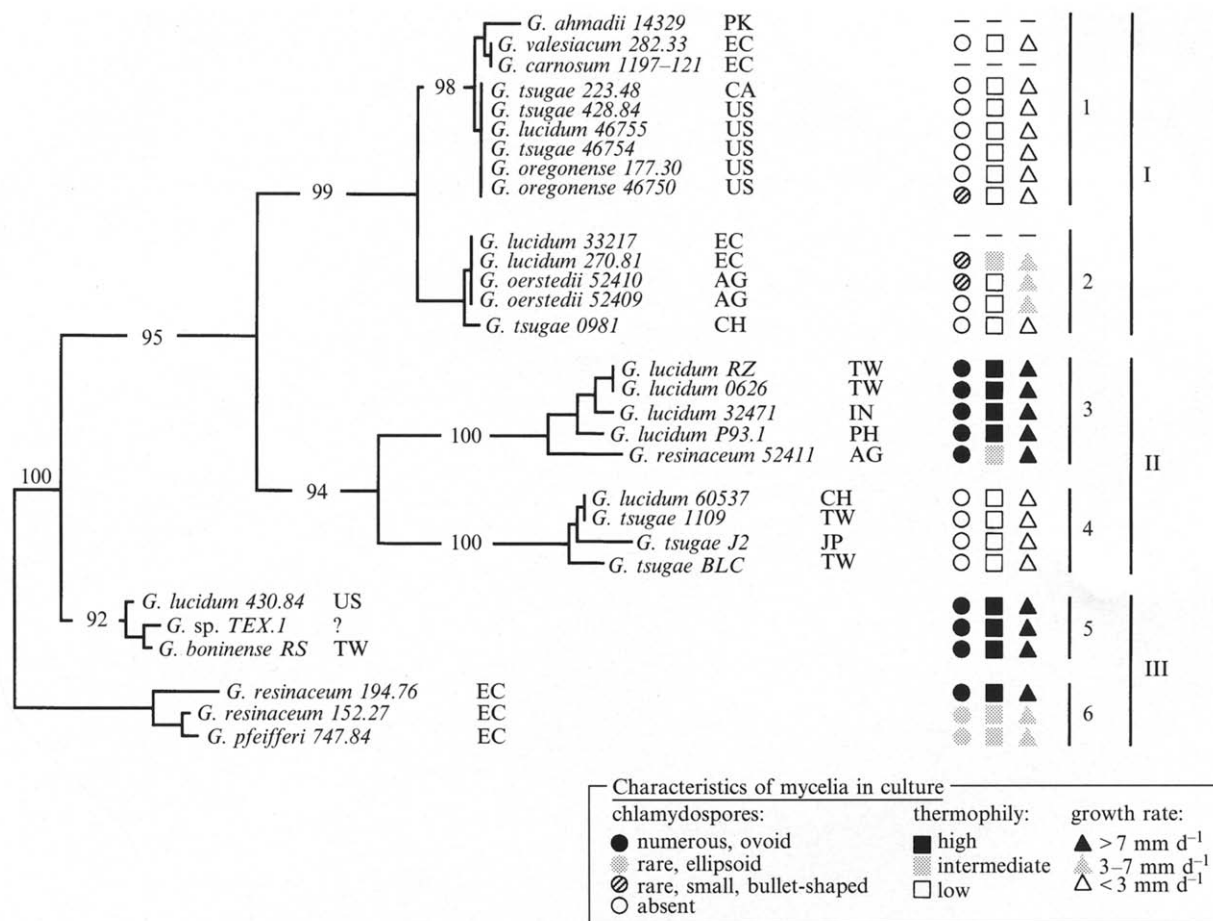
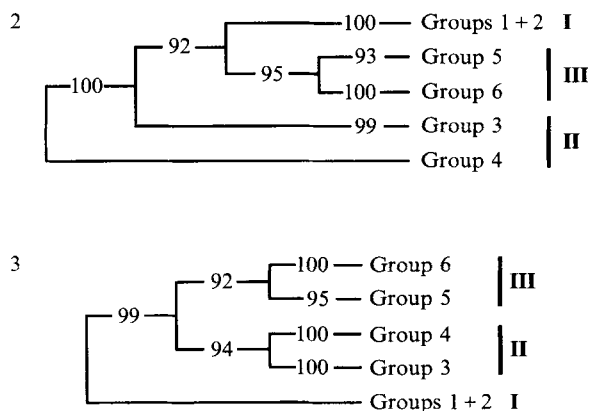


Fig. 1. Gene phylogeny of 29 isolates of the *G. lucidum* complex produced from rDNA sequences of the ITS and LSU-D2 regions compared with characteristics developed by mycelia in culture. The tree depicted is one of 18 equiparsimonious trees from a heuristic search in PAUP, using TBR branch swapping and 20 random addition sequences (Swofford, 1990). The other equiparsimonious trees differ only in the arrangement of terminal branches. Tree length = 113 steps; consistency index = 0.788; retention index = 0.917. Isolates in Group 6 were chosen as outgroups to root the tree. Values above branches are confidence levels after 100 bootstrap replications. Geographic origin of the isolates is abbreviated as follows: Pakistan, PK; Europe, EC; Canada, CA; U.S.A., US; Argentina, AG; China, CH; Taiwan, TW; India, IN; Philippines, PH; Japan, JP; unknown, ? Thermophily in culture was categorized as follows: high thermophily, optimum growth at 28–32°, still growing at 38°; low thermophily, optimum growth at 22–26°, not growing at 38°. Growth rates were determined at optimum growth temperature.



Figs 2–3. Schematic representation of phylograms produced from 100 bootstrap replications using TBR branch swapping and simple addition sequences in PAUP (Swofford, 1990), when isolates of Group 4 (**Fig. 2**) and Groups 1 and 2 (Phylum I) (**Fig. 3**) are chosen as outgroups to root the tree. Values above branches are bootstrap confidence levels.

argue that allopatry played a major role in the phenotypic divergence between Argentinian *G. oerstedii* and European *G. lucidum*. However, high phenotypic divergence associated with low divergence time is also observed among sympatric taxa: in Europe, *G. valesiacum* and *G. carnosum* are clearly distinguished in both macroscopic and microscopic characters (Ryvarden & Gilbertson, 1993), but the two taxa diverged recently, as indicated in Fig. 1 and Table 4.

All in all, rDNA gene phylogeny seems independent of the evolution of morphological characters. Group 6 is the only group in which members were found to share a morphological synapomorphy. This group is composed of European collections of *G. resinaceum* and *G. pfeifferi* which are characterized by a resinous layer on the upper crust (Ryvarden & Gilbertson, 1993).

Host relationships and geographical origin provide better support for the gene phylogeny in Fig. 1. All European and North American taxa restricted to coniferous trees (*G. valesiacum*, *G. carnosum*, *G. tsugae* and *G. oregonense*) cluster in

Group 1. The other isolates collected on conifers are in Group 2, together with European *G. lucidum* which was reported on both hardwood and softwood (Ryvarden & Gilbertson, 1993). Also, all strains identified as *G. tsugae* or *G. lucidum* from Far East Asia are in Phylum II (Groups 3 and 4) while Phyla I and II are essentially composed of isolates from Europe and temperate America (Fig. 1).

Gene phylogeny and culture characteristics

The characters most useful in distinguishing between *Ganoderma* cultures are chlamydospore production, growth rate and thermophily (Nobles, 1948; Adaskaveg & Gilbertson, 1986, 1989; Hseu, 1990; Wang & Hua, 1991). Figure 1 shows that there is a remarkable correlation between these characters in our sample: fast-growing cultures are thermophilic and produce numerous ovoid chlamydospores, while slow-growing cultures are not thermophilic and do not produce chlamydospores. In the *G. lucidum* complex chlamydospores are: (i) ovoid, abundantly produced (in Groups 3, 5 and 6); (ii) ellipsoid, rarely formed (in Group 6); (iii) small, bullet-shaped, rarely produced, not formed in every culture medium (Groups 1 and 2); (iv) absent (Groups 1, 2 and 4) (Fig. 1).

The taxonomic value of small, bullet-shaped chlamydospores, which are scarcely formed by some isolates, needs further scrutiny. Wang & Hua (1991) reported that kind of chlamydospore being produced by European *G. lucidum* CBS 270.81 and by *G. oerstedii* ATCC 52410, while *G. oerstedii* ATCC 52409 does not form chlamydospores. We confirm these observations, although Bazzalo & Wright (1982) did not report chlamydospores in cultures of *G. oerstedii* ATCC 52410. Although different in chlamydospore production, there is no molecular divergence between the two isolates of *G. oerstedii* (Table 4). Also, the two isolates of *G. oregonense* (Group 1) share identical nucleotide sequences (Table 4) but only one of them (ATCC 46750) produces a few small bullet-shaped chlamydospores. Adaskaveg & Gilbertson (1989) did not report chlamydospores in collections of *G. oregonense*, nor from other coniferous-growing taxa in North America.

Nobles (1948) emphasized chlamydospore production in distinguishing between cultures of *G. tsugae* and *G. lucidum* in North America. She stated that *G. lucidum* isolates produce numerous chlamydospores while *G. tsugae* isolates do not produce chlamydospores. Because Nobles's work was taken as reference by other authors (Adaskaveg & Gilbertson, 1986; Hseu, 1990), several isolates of the complex were identified either as *G. lucidum* or *G. tsugae* on that basis. However, Nobles (1948) did not examine European *G. lucidum*, which does not produce numerous ovoid chlamydospores in culture (Wang & Hua, 1991). Thus, cultural features and phylogenetic relationships (Fig. 1) support the distinction between European and North American *G. lucidum*.

Culture characteristics distinguish between Phyla I and III, and between Groups 3 and 4 within Phylum II (Fig. 1). Also, although they do not distinguish between all groups, they support fairly well the more terminal clades (Fig. 1). This result indicates that characters developed by mycelia in culture are less polymorphic than morphological characters between recently diverged taxa. Therefore, culture characters are useful

in distinguishing between taxa, but since distant taxa may share similar culture characters, these are useless in identifying monophyletic lineages and assessing natural relationships in *Ganoderma*.

Intraspecific sequence divergence in *Ganoderma*: biological species concept v. phylogenetic species concept

The biological species concept (Mayr, 1965; Dobzhansky, 1970) defines species by the interfertility criterion. In higher fungi, interfertility studies are generally restricted to mating tests between monokaryons (Boidin, 1980). Hseu (1990) showed that Asian collections labelled *G. tsugae* (Group 4) and *G. lucidum* (Group 3) represent two biological species. Figure 1 shows that these two biological species are monophyletic, and that they recently diverged. Sequence variation within the two species is similar: there was 0 substitution in the D2 region, and respectively 0–0.5 and 0–2% in the ITS 1 and ITS 2 regions (average ITS variation 0–1.25%) (Table 4). That matches previous studies that reported typically 0–2% of intraspecific nucleotide sequence variation in the ITS region among fungal species, with few exceptions, as reviewed by Moncalvo (1995).

Because nucleotide sequence divergence varies between different lineages (Bruns *et al.*, 1992; Moncalvo *et al.*, 1995), it is clear that sequence divergence alone is useless to separate species until variation in a given genomic region for a given species is established. For instance, if intraspecific nucleotide divergence determined above is similar throughout our sample, then all strains in Group 1 would be conspecific, as would be those in Group 2, and these two lineages would be barely separated at the species rank (Table 4). Also, *G. resinaceum* and *G. pfeifferi* would be conspecific (Group 6, Table 4). Taken together, these results indicate that the more recently diverged species of the complex may not readily be differentiated from nucleotide sequence divergence in the ITS region, as in *Armillaria* (Fr.) Staude (Anderson & Stasovski, 1992).

Adaskaveg & Gilbertson (1986) reported that North American *G. lucidum* and European *G. resinaceum* 194.76 are intercompatible, i.e. belong to the same biological species. However, in our gene phylogeny the two taxa do not cluster together: North American *G. lucidum* is in Group 5 while *G. resinaceum* 194.76 is in Group 6 (Fig. 1). The phylogenetic species concept (Mishler & Brandon, 1987) proposes that a species should be monophyletic. Application of the monophyly criterion to combine these two intercompatible taxa into a single species would therefore drag *G. boninense* and *G. pfeifferi* into the synonymy of *G. resinaceum*. That is hardly acceptable from morphological and cultural differences. In addition, Table 4 shows that molecular divergence between Groups 5 and 6 has occurred in both the LSU-D2 and ITS regions (4–5% of overall ITS variation), indicating that taxonomic divergence has occurred. There is a dissimilarity in sequence variation between ITS 1 and ITS 2 between isolates of these groups, respectively 6–7 v. 1.5–3%. That dissimilarity is remarkable in our sample as in general substitutions in the two spacers are roughly comparable (Table 4), but remains unexplained.

Thus, there is apparently a conflict between the biological species concept and the phylogenetic species concepts in interpreting relationships between North American *G. lucidum* and European *G. resinaceum*. However, demonstration of intercompatibility *in vitro* does not prove that the two taxa really are interfertile, i.e. that viable basidiospores will be produced, nor that genetic exchange is effective *in vivo*.

Therefore, we propose a species model in *Ganoderma* that uses both the monophyly criterion and the interfertility criterion: a species would be circumscribed from both evidence of monophyly and potential evidence of reproductive isolation from the closest lineage. In absence of mating data, a monophyletic taxon would be distinguished at the species rank when it includes collections that share at least one synapomorphy that enables its distinction from the closest lineage, when this synapomorphy may be indicative of absence of genetic exchange between the two lineages, hitherto of taxonomic divergence. This synapomorphy may be a morphological, ecological, physiological or molecular trait. That is, for the time being we refrain from considering Phylum III a single species in absence of evidence of genetic exchange throughout the lineage, and we do not accept conspecificity between North American *G. lucidum* and *G. resinaceum*.

Taxonomic conclusions

Results above show that there is little, if any, correlation between rDNA gene phylogeny and morphology in the *G. lucidum* complex. Therefore, it may be questionable whether gene phylogenies reflect organismal phylogeny. However, high phenotypic plasticity in *Ganoderma* was evidenced by several authors (Steyaert, 1967*a, b*, 1972; Bazzalo & Wright, 1982; Ryvarde, 1991; Chen, 1993), and since our dataset resolves clades that are supported by characteristics developed by mycelia in culture, geographic origin and/or host relationships, and also by mating data in Phylum II, we do assume that the rDNA gene phylogeny depicted in Fig. 1 provides information about natural relationships between taxa of this study. We propose the following taxonomy:

Group 1. *G. ahmadii* and the *G. valesiacum* complex (*G. valesiacum*, *G. tsugae*, *G. oregonense* and *G. carnosum*)

G. ahmadii is the only taxon of the group collected on hardwood (Table 1) and it differs by having smaller basidiospores and a darker context. Although its phylogenetic distinction from the other taxa clustered in this group is not supported by bootstrapping (Fig. 1), we consider *G. ahmadii* a distinct species. It diverged from the other isolates of the group by 0.75–2% of nucleotide substitutions in the ITS region, while intertaxa variation within coniferous-growing collections ranges between 0 and 1% (Table 4). The species was reported only from Pakistan, India and South China (Steyaert, 1972; Zhao, 1989).

North American isolate ATCC 46755 was identified as *G. lucidum* probably on the basis of basidiocarp morphology, since host relationships, culture characters and molecular data

all clearly indicate that it belongs either to *G. tsugae* or to *G. oregonense* (Table 1 and Fig. 1). From morphological, ecological and cultural similarities, these two species may be conspecific with *G. valesiacum* (Stalpers, 1978; Adaskaveg & Gilbertson, 1986; Gilbertson & Ryvarde, 1986). Also, the low nucleotide sequence divergence observed between the three taxa (Table 4) is indicative of little divergence time. However, these taxa are not separate from *G. carnosum* from our dataset (Fig. 1 and Table 4); therefore, if we accept conspecificity between *G. valesiacum*, *G. tsugae* and *G. oregonense*, then *G. carnosum* should also be placed into the synonymy of *G. valesiacum*. In Europe, *G. carnosum* is clearly separated from *G. valesiacum* by having a more robust basidiocarp, a blackish pilear surface and wider basidiospores that appear distinctly rough under the microscope, and by host relationships (*Abies* or *Picea v. Larix*) (Ryvarde & Gilbertson, 1993). Nucleotide sequence data from a more variable genomic region are necessary to infer a gene phylogeny in the group, and interfertility studies are needed to know if this lineage is composed of a single biological species, in which case one may distinguish geographical or ecological variants, or if taxa of the complex are reproductively isolated. Further scrutiny will necessitate population studies in a biogeographic context with exact reference to host relationships.

For the time being, we consider the *G. valesiacum* complex (*G. valesiacum*, *G. tsugae*, *G. oregonense* and *G. carnosum*) a taxon that diverged recently. The group is apparently restricted to coniferous forests in the Northern Hemisphere but may be absent from China and is certainly absent from Japan and Taiwan, as indicated by the phylogenetic positions of Asian isolates identified as *G. tsugae* (Groups 2 and 4).

Group 2. *G. lucidum*, Argentinian collections labelled *G. oerstedii* and a coniferous-growing collection from China

The two isolates of *G. lucidum* share identical nucleotide sequence and differ from Argentinian collections labelled *G. oerstedii* by only one nucleotide substitution (0.25%) (Table 4). There are some variations in culture characteristics between these isolates (Fig. 1). Voucher collections of *G. oerstedii* isolates were described in Bazzalo & Wright (1982), who stated that they coincide with all features of the holotype of the species described from Costa Rica, except that the spores are somewhat more polymorphic. *G. oerstedii* and *G. lucidum* differ in pilocystidia and basidia shape, context colour and spore size (Bazzalo & Wright, 1982; Ryvarde & Gilbertson, 1993). For the time being, and in the absence of mating data between these isolates, these morphological differences taken with geographical distribution (South America and Europe), make us keep the two taxa as distinct species, with *G. oerstedii* as a tentative name for the Argentinian taxon (J. E. Wright, pers. comm.).

Results indicate that *G. lucidum sensu stricto* is present from Northern to Southern Europe, and is probably absent from North America, Taiwan and Japan. Its distribution area may extend southward to Central Africa from where Ryvarde & Johansen (1980) reported specimens that match the European description. The lineage (Group 2) would then encompass South America where it produces distinctive basidiocarps,

labelled *G. oerstedii* in Argentina. The Eastern distribution of *G. lucidum sensu stricto* is not known, but its lineage extends to North West China, as indicated by the placement of Chinese collection ZHANG 0981 in Fig. 1.

Isolate ZHANG 0981 recently diverged from the taxa above (Fig. 1 and Table 4). Its basidiocarp morphology and culture characteristics match those of *G. tsugae*, and like this species, it was collected on conifer wood. Ryvar den & Gilbertson (1993) reported that *G. lucidum* grows on both hardwood and coniferous wood in Northern Europe, as does the Argentinian taxon of this group (Bazzalo & Wright, 1982). Further scrutiny in Group 2 (the *G. lucidum* complex *sensu stricto*) is obviously needed.

Group 3. An Asian species mistaken as *G. lucidum* and an Argentinian isolate mistaken as *G. resinaceum*

The Asian species matches both the intercompatibility criterion (Hseu, 1990) and the monophyly criterion (Fig. 1) and seems widespread throughout tropical and subtropical Asia, but has yet to be correctly named. Since several laccate *Ganoderma* species with unclear taxonomic circumscription and status were described from tropical Asia, we refrain from describing this taxon as new for the time being.

Argentinian strain ATCC 52411 is monophyletic with the Asian taxon above, with statistical support of 100% (Fig. 1). However, there were two nucleotide differences in the D2 region and 9–12 (about 2.25–3%) in the ITS region (Table 4), indicating divergence. Although basidiocarp morphology and culture characters of strain ATCC 52411 resemble those of the Asian taxon, differences are found in pilocystidia shape and thermophily of mycelia in malt-agar (Bazzalo & Wright, 1982; Hseu, 1990; Fig. 1). Thus, for the time being, we do not consider the Argentinian collection 52411 conspecific with the Asian species. Its correct name has yet to be determined. This isolate was first identified as *G. oerstedii* (Bazzalo & Wright, 1982), then as *G. resinaceum* from culture similarities (J. E. Wright, pers. comm.). Figure 1 shows that it certainly does not belong to *G. resinaceum* (Group 6) which was described from Europe, while *G. oerstedii* remains a possible name if Argentine isolates in Group 1 were misnamed.

Group 3 extends from India to Argentina and is split into two taxa between Asia and South America. This suggests its occurrence throughout the Pacific Islands. It is certainly absent from Europe and North America.

Group 4. An Asian species mistaken as *G. tsugae*

Taiwanese and Japanese isolates of this species match both the intercompatibility criterion (Hseu, 1990) and the monophyly criterion (Fig. 1). From culture and molecular evidence (Fig. 1) we assume that the Chinese strain HMAS 60537, although identified as *G. lucidum*, is conspecific with the other isolates of this group. The correct name of this species has yet to be determined.

In the Northern Hemisphere, this species is certainly restricted to East Asia. There is no indication of its presence or absence southward of Taiwan.

Group 5. North American *G. lucidum* and Formosan *G. boninense*

North American *G. lucidum*, Formosan *G. boninense* and an unidentified strain from unknown origin are monophyletic with a statistical support of 92% of bootstrap replications (Fig. 1) and differ only by about 0.5% of nucleotide substitutions in the ITS region (Table 4), indicative of little divergence. The three isolates are not distinguished in culture (Fig. 1). The voucher collection of the unidentified species was not available during the course of this study. This isolate is commercially cultivated in Texas and may originate from Japan (P. S. Lewis, pers. comm.). The Formosan isolate has been identified as *G. boninense* (Hseu, 1990) since it matches Steyaert's description of this species (Steyaert, 1967b), which is characterized by very slightly echinulate basidiospores that are somewhat elongated. That character distinguishes the Formosan collection from North American *G. lucidum*. Mating studies would show whether or not the two taxa are conspecific. If so, and if the Formosan collection has been correctly identified, then *G. boninense* may be the correct name for North American collections mistaken as *G. lucidum*.

G. boninense was described from Bonin Island. It has been reported throughout the Pacific Islands to Sri Lanka, and from Australia, Taiwan, Japan and China (Steyaert, 1967b; Zhao, 1989). This distribution suggests an outward radiation from South East Asia, and makes the presence of *G. boninense* in North America likely. The distribution map of '*G. lucidum*' in North America (Gilbertson & Ryvar den, 1986) indicates that the taxon is widespread in the Southern region but is apparently absent from the Pacific North West and Central regions. That would agree with the hypothesis of a tropical origin for the North American taxon.

Group 6. *G. resinaceum* and *G. pfeifferi*

Isolate *G. resinaceum* CBS 152.27 clusters with *G. pfeifferi* and *G. resinaceum* CBS 194.76 stands apart (Fig. 1). The former two isolates differ only by two nucleotide substitutions (0.5%), both located in the ITS 1 region. *G. resinaceum* CBS 194.76 differs from them by one nucleotide substitution in the D2 region and by 5–7 (1.25–1.75%) in the ITS region (Table 4). The result strongly suggests that the two isolates named *G. resinaceum* may not be conspecific, and indicates that strain CBS 152.27 probably belongs to *G. pfeifferi*. Culture characteristics support this observation (Wang & Hua, 1991 and Fig. 1). Ryvar den and Gilbertson (1993) stated that basidiocarps of the two species may be confused but the basidiospores are different. Unfortunately, the voucher collections were not available to us.

G. pfeifferi seems restricted to Eurasia with scarce records in the more Eastern regions (Corner, 1983). *G. resinaceum* is cosmopolitan in its present circumscription, since Steyaert (1980) and Ryvar den (1985) considered numerous names as synonyms. However, the Argentinian isolate tentatively identified as such (Group 3, Fig. 1) belongs to a different species. Intercompatibility with phylogenetically divergent taxa (North American *G. lucidum* in Group 5), low molecular divergence with a morphologically distinct taxon (*G. pfeifferi*)

and numerous synonyms suggest that *G. resinaceum* is a species complex.

Strain ATCC 46753; not a *Ganoderma*

A note in the ATCC catalogue indicates that North American isolate ATCC 46753 was first named *G. tsugae* by Mounce but was later considered *G. lucidum* by Smith. Overall culture characteristics of this strain (Wang & Hua, 1991) match none of those reported for North American *Ganoderma* species by Adaskaveg & Gilbertson (1989), nor do they match those of other *Ganoderma* taxa that were described in Wang & Hua (1991). This isolate produced nucleotide sequences in the ITS regions which were unalignable with those of other *Ganoderma* species. In contrast, ITS nucleotide sequences of *Ganoderma* taxa aligned fairly well with those of isolates of related genera *Amauroderma* and *Fomitopsis* (Moncalvo *et al.*, 1995). From these observations, we conclude that strain ATCC 46753 is not a *Ganoderma* species. Without the voucher collection in hand, and in the absence of a nucleotide sequence database for polypores, we were unable to identify this strain.

Systematics perspectives and nomenclature

Ryvarden (1991) stated that the high phenotypic plasticity observed in *Ganoderma* indicates that the group is young and that strong speciation has not yet been achieved. Ryvarden's (1991) hypothesis was supported from molecular evidence (Moncalvo *et al.*, 1995). This study provides novel evidence of the recent origin of laccate *Ganoderma* species. Besides showing low divergence time between species of the *G. lucidum* aggregate, results indicate that temperate taxa believed to be widespread (*G. lucidum* and *G. tsugae*) are in fact geographically restricted, and therefore might be too young to have spread worldwide.

Distribution of Groups 2 and 3 encompasses the tropics and suggests a radiation from equatorial regions: so does the *G. boninense* aggregate (Group 5). A large number of laccate *Ganoderma* species have been described from the tropics. It has been speculated that the tropics act as a pump to generate taxa because of optimal climatic conditions enhancing biological diversity there, therefore that higher taxa may have a tropical origin (Jablonski, 1993). Since laccate *Ganoderma* species are of recent origin, the group may be appropriate to test this hypothesis. A research perspective is to infer evolutionary relationships between tropical and temperate taxa. That would provide novel insights into the origin and evolution of laccate *Ganoderma*.

Finally, several isolates in our sample have to be renamed. Nomenclature relies on basidiocarps designated as type specimens, of which, generally, no culture was derived. Since morphology in *Ganoderma* was found to be too variable to identify collections and to distinguish between species, one way to fix the nomenclature would be to study type specimens at the molecular level. That has been undertaken in this work since we have produced sequences from the *G. ahmadii* holotype. Using a nucleotide sequence database of type specimens, virtually any *Ganoderma* collection could be

correctly named and synonymies could be established with better confidence. ITS sequences may serve this purpose, but inter and intraspecies nucleotide variation in the ITS region has yet to be investigated between and within a much larger number of intersterility groups than has been done here.

This research was supported by a post-doctoral grant from the Swiss National Science Foundation to the first author (J.M.M.), and in part by a grant (no. 8209004) from the Microbiological Research Foundation of the Republic of China to the last author (R.S.H.). We thank Jeff Leonard for proofreading the manuscript.

REFERENCES

- Adaskaveg, J. E. & Gilbertson, R. L. (1986). Cultural studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. *Mycologia* **78**, 694–705.
- Adaskaveg, J. E. & Gilbertson, R. L. (1988). Basidiospores, pilocystidia, and other basidiocarp characters in several species of the *Ganoderma lucidum* complex. *Mycologia* **80**, 493–507.
- Adaskaveg, J. E. & Gilbertson, R. L. (1989). Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G. tsugae*. *Mycological Research* **92**, 182–191.
- Anderson, J. B. & Stasovski, E. (1992). Molecular phylogeny of northern hemisphere species of *Armillaria*. *Mycologia* **84**, 505–516.
- Bazzalo, M. E. & Wright, J. E. (1982). Survey of the Argentine species of the *Ganoderma lucidum* complex. *Mycotaxon* **16**, 293–325.
- Boidin, J. (1980). La notion d'espèce. III. Le critère d'interfertilité ou d'intercompatibilité: résultats et problèmes. *Bulletin de la Société Mycologique de France* **96**, 43–57.
- Bruns, T. D., Vilgalys, R., Barns, S. M., Gonzales, D., Hibbett, D. S., Lane, D. J., Simon, L., Stickel, S., Szaro, T. M., Weisburg, W. G. & Sogin, M. L. (1992). Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. *Molecular Phylogeny and Evolution* **1**, 231–241.
- Bruns, T. D., White, T. J. & Taylor, J. W. (1991). Fungal molecular systematics. *Annual Review of Ecology and Systematics* **22**, 525–564.
- Chen, C. S. (1993). Methods for inducing various morphological fruiting body of *Ganoderma tsugae*. *Transactions of the Mycological Society of Republic of China* **8**, 9–16.
- Corner, E. J. H. (1983). *Ad Polyporaceas. I. Amauroderma and Ganoderma*. J. Cramer: Vaduz, Liechtenstein, 182 pp.
- Dobzhansky, T. (1970). *Genetics of the Evolutionary Process*. Columbia University Press: New York, 505 pp.
- Gilbertson, R. L. & Ryvarden, L. (1986). *North American Polypores*. Fungiflora: Oslo, Norway, 433 pp.
- Guteli, R. R. & Fox, G. (1988). A compilation of large subunit RNA sequences presented in a structural format. *Nucleic Acids Research* **16**, r175–r269.
- Hibbett, D. S. (1992). Ribosomal RNA and fungal systematics. *Transactions of the Mycological Society of Japan* **33**, 533–556.
- Hseu, R. S. (1990). An identification system for cultures of *Ganoderma* species. Ph.D. Dissertation, National Taiwan University, Taipei, Taiwan, 169 pp.
- Imazeki, R. (1939). Studies in *Ganoderma* of Nippon. *Bulletin of the Tokyo Science Museum* **1**, 29–52.
- Jablonski, D. (1993). The tropics as a source of evolutionary novelty through geological time. *Nature* **364**, 142–144.
- Karsten, P. A. (1881). *Enumeratio Boletinearum et Polyporearum Fennicarum, Systemate Novo Dispositarum*. *Revue de Mycologie* **3**, 16–19.
- Kotlaba, F. & Pouzar, Z. (1983). Taxonomic and nomenclatural notes on *Trametes cervina* and *Ganoderma atkinsonii*. *Česká Mykologie* **37**, 49–51.
- Mayr, E. (1965). *Animal Species and Evolution*. Belknap Press: Cambridge, MA.
- Mishler, B. D. & Brandon, R. N. (1987). Individuality, pluralism, and the phylogenetic species concept. *Biology and Philosophy* **2**, 397–414.
- Moncalvo, J. M. (1995). Evaluation of fungal biological diversity in the

- tropics: systematics perspectives. In *Tropical Mycology* (ed. K. K. Janardhanan & C. Rajendran). India.
- Moncalvo, J. M., Wang, H. H. & Hseu, R. S. (1995). Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* **87**, 223–238.
- Murrill, W. A. (1902). The polyporaceae of North America. I. The genus *Ganoderma*. *Bulletin of the Torrey Botanical Club* **29**, 599–608.
- Nobles, M. K. (1948). Studies in forest pathology. VI. Identification of culture of wood-rotting fungi. *Canadian Journal of Research* **26**, 281–431.
- Ryvarden, L. (1985). Type studies in the Polyporaceae. 17. Species described by W. A. Murrill. *Mycotaxon* **23**, 169–198.
- Ryvarden, L. (1991). *Genera of Polypores. Nomenclature and Taxonomy*. Fungiflora: Oslo, Norway, 363 pp.
- Ryvarden, L. & Gilbertson, R. L. (1993). *European Polypores*. Fungiflora: Oslo, Norway, 387 pp.
- Ryvarden, L. & Johansen, I. (1980). *A Preliminary Polypore Flora of East Africa*. Fungiflora: Oslo, Norway, 636 pp.
- Sawada, K. (1942). Descriptive catalogue of the Formosan fungi. Formosa Government Research Institute, Department of Agriculture, Report no. 83, 101–110 [in Japanese].
- Stalpers, J. A. (1978). Identification of wood-inhabiting fungi in pure culture. *Studies in Mycology* **16**, 1–248.
- Steyaert, R. L. (1961). Note on the nomenclature of fungi and incidently of *Ganoderma lucidum*. *Taxon* **10**, 251–252.
- Steyaert, R. L. (1967a). Considérations générales sur le genre *Ganoderma* et plus spécialement sur les espèces européennes. *Bulletin de la Société Royale Botanique de Belgique* **100**, 189–211.
- Steyaert, R. L. (1967b). Les *Ganoderma* palmicoles. *Bulletin du Jardin Botanique National de Belgique* **37**, 465–492.
- Steyaert, R. L. (1972). Species of *Ganoderma* and related genera mainly of the Bogor and Leiden herbaria. *Persoonia* **7**, 55–118.
- Steyaert, R. L. (1980). Study of some *Ganoderma* species. *Bulletin du Jardin Botanique National de Belgique* **50**, 135–186.
- Swofford, D. (1990). *PAUP 3.0L: Phylogenetic Analysis Using Parsimony*. Illinois Natural History Survey: Champaign, U.S.A.
- Wang, B. C. & Hua, J. (1991). *A Cultural Atlas of Some Ganoderma Cultures*. Publication of the Food Industry Research and Development Institute (FIRDI): Hsinchu, Taiwan, 131 pp.
- Zhao, J. D. (1989). *The Ganodermataceae in China*. J. Cramer: Berlin–Stuttgart, Germany, 176 pp.

(Accepted 27 April 1995)