RESEARCH LETTER

Phylogeographic relationships in the polypore fungus *Pycnoporus* inferred from molecular data

Laurence Lesage-Meessen[1], Mireille Haon[1], Eva Uzan[1], Anthony Levasseur[1], François Piumi[1], David Navarro[1], Sabine Taussac[1], Anne Favel[1,2] & Anne Lomascolo[1,3]

[1]INRA, UMR 1163 de Biotechnologie des Champignons Filamenteux, ESIL, Marseille, France; [2]Université de la Méditerranée, UMR 1163 de Biotechnologie des Champignons Filamenteux, ESIL, Marseille, France; and [3]Université de Provence, UMR 1163 de Biotechnologie des Champignons Filamenteux, ESIL, Marseille, France

Correspondence: Laurence Lesage-Meessen, INRA, UMR1163 de Biotechnologie des Champignons Filamenteux ESIL, 163 avenue de Luminy, CP 925, 13288 Marseille Cedex 09, France. Tel.: +33 491828608; fax: +33 491828601; e-mail: laurence.lesage-meessen@esil.univmed.fr

Received 31 January 2011; revised 17 June 2011; accepted 1 September 2011.
Final version published online 3 October 2011.

DOI: 10.1111/j.1574-6968.2011.02412.x

Editor: Nina Gunde-Cimerman

Keywords
Pycnoporus; molecular marker; phylogeny; ITS; β-tubulin; laccase.

Abstract

The genus *Pycnoporus* forms a group of four species known especially for producing high redox potential laccases suitable for white biotechnology. A sample of 36 *Pycnoporus* strains originating from different geographical areas was studied to seek informative molecular markers for the typing of new strains in laboratory culture conditions and to analyse the phylogeographic relationships in this cosmopolitan group. ITS1-5.8S-ITS2 ribosomal DNA and partial regions of β-tubulin and laccase *lac3-1* gene were sequenced. Phylogenetic trees inferred from these sequences clearly differentiated the group of *Pycnoporus cinnabarinus* strains from the group of *Pycnoporus puniceus* strains into strongly supported clades (100% bootstrap value). Molecular clustering based on *lac 3-1* sequences enabled the distribution of *Pycnoporus sanguineus* and *Pycnoporus coccineus* through four distinct, well supported clades and sub-clades. A neotropical sub-clade, grouping the *P. sanguineus* strains from French Guiana and Venezuela, corresponded to *P. sanguineus sensu stricto*. A paleotropical sub-clade, clustering the strains from Madagascar, Vietnam and New Caledonia, was defined as *Pycnoporus* cf. *sanguineus*. The Australian clade corresponded to *P. coccineus sensu stricto*. The Eastern Asian region clade, clustering the strains from China and Japan, formed a *P. coccineus*-like group. Laccase gene (*lac 3-1*) analysis within the *Pycnoporus* species can highlight enzyme functional diversity associated with biogeographical origin.

Introduction

The genus *Pycnoporus* belongs to the polyporoid white-rot fungi, the most representative group of homobasidiomycetes causing wood decay (Hibbett *et al.*, 2007). *Pycnoporus* is a genus closely related to *Trametes*, being morphologically similar in all characters except for the conspicuous bright reddish-orange colour of the basidiocarp (Ryvarden, 1991; Ryvarden & Gilbertson, 1994). Historically, four species were discerned based on their morphological features (pore size of basidiocarp and basidiospore shape) and their distribution areas (Nobles & Frew, 1962; Ryvarden & Johansen, 1980): (1) *Pycnoporus cinnabarinus*, a common species, distributed especially in the northern hemisphere, (2) *Pycnoporus puniceus*, a rare species known from Africa, India, Malaysia and New Caledonia, and characterized by a basidiocarp with large irregular pores (1–3 per mm), (3) *Pycnoporus sanguineus*, a common species distributed in tropical and subtropical regions, and (4) *Pycnoporus coccineus*, distributed in the countries bordering the Indian and Pacific Oceans. To date, the description and exploration of the *Pycnoporus* diversity has been based mainly on morphological similarity to the type specimen – referenced in international collections – although species delineation remains difficult due to highly variable macro- and micro-morphological characters. In addition, the four species of *Pycnoporus* are very similar, especially those distributed in the tropical areas and, when cultured, the high degree of similarity of their cultural characters hinders their identification.

In recent years, a more precise assessment of identification and diversity of fungi has been achieved using

molecular markers including the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, and partial sequences of genes coding for subunits of RNA polymerase, β-tubulin, cellobiohydrolase-C, topoisomerase II and manganese peroxidase (Hatsch *et al.*, 2004; Wang *et al.*, 2004; Froslev *et al.*, 2005; Tomšovský *et al.*, 2006; Hilden *et al.*, 2008).

The *Pycnoporus* genus is known to produce laccases (*p*-diphenol : oxygen oxidoreductases, EC 1.10.3.2) (Eggert *et al.*, 1998), which typically are blue copper oxidases responsible for lignin degradation and wood decay, and mmthe decomposition of humic substances in soils (Gianfreda *et al.*, 1999; Baldrian, 2006). Laccases can oxidize a wide range of compounds, including polyphenols, methoxy-substituted phenols, aromatic diamines and environmental pollutants such as industrial dyes, polycyclic aromatic hydrocarbons and pesticides (Herpoël *et al.*, 2002; Sigoillot *et al.*, 2004; Brijwani *et al.*, 2010). A recent study identified the strains *P. coccineus* MUCL 38523 (from Australia), *P. sanguineus* IMB W006-2 (from China) and *P. sanguineus* BRFM 902 (from French Guiana) as outstanding producers of high redox potential laccases, particularly suitable for white biotechnology processes such as lignin biorefinery and cosmetic applications (Uzan *et al.*, 2010, 2011). Accordingly, species of the genus *Pycnoporus* are now strong contenders for industrial applications, and so require unambiguous identification, especially for typing new strains in laboratory culture conditions.

The aim of this study was to infer phylogenetic relationships among the four species of the genus *Pycnoporus* using sequence data from the ITS region of rDNA and from partial regions of the gene encoding β-tubulin and laccase isoenzyme Lac I. This analysis leads to a discussion about geographical distribution within the *Pycnoporus* genus, with a special focus on the very closely related species *P. coccineus* and *P. sanguineus.*

Materials and methods

Fungal strains

Thirty-six strains obtained from different international collections studied: two strains of *P. puniceus*, five of *P. cinnabarinus*, 25 of *P. sanguineus* and four of *P. coccineus* (Table 1). The strains had various geographic origins: Central/South America (Cuba, Venezuela, French Guiana) (14), Europe (4), South eastern Africa (Madagascar) (1), Eastern Asia (Vietnam, China and Japan) (9), Oceania (Australia, New Caledonia and Solomon Islands) (7); one strain was of unknown origin.

The biological material originating from Venezuela and Vietnam was deposited in our collection, the International Centre of Microbial Resources dedicated to Filamentous Fungi (CIRM-CF, Marseille, France) through Deposit Contracts in accordance with the international convention on biological diversity. The strains from French Guiana and French New Caledonia were isolated from specimens collected between 2007 and 2010, which were assigned to *P. sanguineus* on the basis of morphological features (Ryvarden, 1991; Courtecuisse *et al.*, 1996). The other strains were obtained from International Culture Collections (Table 1). For the species *P. sanguineus*, *P. coccineus*, *P. cinnabarinus*, we designated, as, reference strain, the strain which was isolated from a specimen collected in the same geographic area as that of the original type specimen. The strain CIRM-BRFM 902 originating from French Guiana was designated as reference strain for *P. sanguineus* (L) Murrill, Surinam (Lamark, 1783), the strain MUCL 39523 originating from Australia for *P. coccineus* (Fr.) Bondartsev & Singer, Polynesia (Fries, 1851), and the strain MUCL 30555 originating from Belgium for *P. cinnabarinus* (Jacq.) P. Karst, Europe (Karsten, 1881). The strain of *Trametes suaveolens* CBS 426.61 was used as an outgroup in phylogenetic analyses.

Molecular analysis

Genomic DNA was isolated from mycelial powder (40–80 mg) as described by Lomascolo *et al.* (2002). The ITS region was amplified using the ITS1 and ITS4 primers as described by White *et al.* (1990). The degenerate primers Bsens and Brev were adapted from primers already designed to match a 133-amino-acid conserved region in β-tubulin from *Lentinula* spp. and *Pleurotus* spp. (Thon & Royse, 1999). In our study, β-tubulin gene from *Trametes versicolor*, *Polyporus lepideus*, *Schizophyllum commune*, *Coprinus cinereus*, and *Pleurotus sajor-caju* (NCBI accession numbers AY944859, AY944857, X63372, AB000116, AF132911, respectively) were aligned, and consensus primers Bsens [5′-ATCAC(A/T)CACTCI-CTIGGTGGTGG-3′] and Brev [5′-CATGAAGAA(A/G) TGIAGACGIGGG-3′] were designed. The universal genetic code was used. At degenerate positions, if three or four combinations were possible, the base was replaced by an inosine (I); otherwise, the two possible bases were kept. The two degenerate primers F2 [5′-CA(C/T)TGGCA (C/T)GG(A/G)TTCTTCC-3′] and R8 [5′-GAG(A/G) TGGAAGTC(A/G)ATGTG(G/A)C-3′] were designed to match, respectively, the copper-binding domains I and IV, highly conserved in blue copper oxidases such as laccases (Messerschmidt & Huber, 1990). The sequences of F2 and R8 were based on the alignment of the corresponding nucleotide regions of the basidiomycete laccases from *P. coccineus*, *P. sanguineus*, *Lentinula edodes*,

Table 1. Strains of *Pycnoporus cinnabarinus*, *Pycnoporus coccineus*, *Pycnoporus puniceus* and *Pycnoporus sanguineus* included in the study and newly identified at the molecular level

				NCBI accession numbers		
Original identification	Collection number*	Final identification	Geographic origin/host	ITS1-5.8S-ITS2	β-Tubulin	Lac F2-R8
P. cinnabarinus	MUCL 30555[†]	*P. cinnabarinus*	Bois de Lauzelle, Louvain-la-Neuve, Belgium/rotten wood	AF363764[‡]	FJ410367	EU683258
P. cinnabarinus	CIRM-BRFM 137[§]	*P. cinnabarinus*	Monokaryon from fruit-body of wild strain I-937	AF363757[‡]	FJ410369	AF170093
P. cinnabarinus	CIRM-BRFM 237	*P. cinnabarinus*	Russia/deadwood	FJ234205	FJ410370	EU684159
P. cinnabarinus	CIRM-BRFM 945	*P. cinnabarinus*	Castelnau-Durban, France/deadwood	FJ234206	FJ410371	EU714500
P. cinnabarinus	MUCL 38420	*P. coccineus*	Alice Springs, Australia/deadwood	AF363768[‡]	FJ410368	EU684160
P. puniceus	MUCL 47083	*P. puniceus*	Pinal del Rio, Cuba/dead fallen trunk, *Quercus cubana*	FJ234198	FJ410376	FJ425895
P. puniceus	MUCL 47087	*P. puniceus*	Pinal del Rio, Cuba/dead fallen trunk, *Quercus cubana*	FJ234199	FJ410377	FJ425896
P. sanguineus	MUCL 29375	*P.* cf *sanguineus*	Tamatave Madagascar, Indian Ocean/deadwood	AF363769[‡]	FJ410366	EU684158
P. sanguineus	CIRM-BRFM 942	*P.* cf *sanguineus*	Danang beach, Vietnam/deadwood	FJ234201	FJ410354	FJ232700
P. sanguineus	CIRM-BRFM 943	*P.* cf *sanguineus*	Mekong delta, Vietnam/wood log	FJ234202	FJ410355	FJ232701
P. sanguineus	CIRM-BRFM 979	*P.* cf *sanguineus*	Owentoro, New Caledonia/*Acacia spirobis*	FJ234184	FJ410363	EU714502
P. sanguineus	CIRM-BRFM 980	*P.* cf *sanguineus*	French New Caledonia/*Nothofagus codonandra*	FJ234203	FJ410364	EU684161
P. sanguineus	CIRM-BRFM 981	*P.* cf *sanguineus*	French New Caledonia/deadwood	FJ234204	FJ410365	EU714503
P. sanguineus	CIRM-BRFM 892	*P. sanguineus*	Kow Marsh, French Guiana/trunk of dead palm tree	FJ234185	FJ410341	EU678766
P. sanguineus	CIRM-BRFM 893	*P. sanguineus*	Kowou, French Guiana/edge of sea	FJ234186	FJ410342	EU678779
P. sanguineus	CIRM-BRFM 895	*P. sanguineus*	Paracou, French Guiana/rotten wood	FJ234187	FJ410343	EU678767
P. sanguineus	CIRM-BRFM 896	*P. sanguineus*	Paracou, French Guiana/burnt wood	FJ234188	FJ410344	EU678768
P. sanguineus	CIRM-BRFM 899	*P. sanguineus*	Laussat, French Guiana/rotten wood	FJ234190	FJ410346	EU678770
P. sanguineus	CIRM-BRFM 900	*P. sanguineus*	Laussat, French Guiana/rotten wood	FJ234191	FJ410347	EU678771
P. sanguineus	CIRM-BRFM 901	*P. sanguineus*	Sainte Elie, French Guiana/rotten wood	FJ234192	FJ410348	EU678772
P. sanguineus	CIRM-BRFM 902[†]	*P. sanguineus*	Macouria, French Guiana/burnt wood	FJ234193	FJ410349	EU678773
P. sanguineus	CIRM-BRFM 903	*P. sanguineus*	Royale Island, French Guiana/rotten wood	FJ234194	FJ410350	EU678774
P. sanguineus	CIRM-BRFM 905	*P. sanguineus*	Royale Island, French Guiana/rotten wood	FJ234195	FJ410351	EU678782
P. sanguineus	CIRM-BRFM 906	*P. sanguineus*	Royale Island, French Guiana/rotten wood	FJ234196	FJ410352	EU678776
P. sanguineus	CIRM-BRFM 881	*P. sanguineus*	Venezuela/rotten wood	FJ234197	FJ410362	EU684155
P. sanguineus	CIRM-BRFM 542	*P.* cf. *coccineus*	Unknown	FJ234200	FJ410353	EU678786
P. sanguineus	IMB W3008	*P.* cf. *coccineus*	Guangxi, Mt Daming, China/rotten wood of broad-leaves tree	AF363753[‡]	FJ410356	EU714499
P. sanguineus	IMB G53	*P.* cf. *coccineus*	Hainan, Bawangling, China/rotten wood of *Pinus* sp.	AF363763[‡]	FJ410357	EU683255
P. sanguineus	IMB H2180	*P.* cf. *coccineus*	Hainan, Qiongzhong, China/rotten hardwood	AF363770[‡]	FJ410358	EU683256
P. sanguineus	IMB G66	*P.* cf. *coccineus*	Hainan, Tunchang, China/rotten hardwood	AF363762[‡]	FJ410359	EU683257
P. sanguineus	IMB W006-2	*P.* cf. *coccineus*	Guangxi, Mt Daming, China/rotten wood of broad-leaves tree	AF363754[‡]	FJ410360	EU678784
P. sanguineus	IMB G05.10[§]	*P.* cf. *coccineus*	Monokaryon from fruit-body of wild strain IMB G05	FJ750267	FJ410361	EU678783
P. coccineus	MUCL 38527	*P.* cf. *coccineus*	Shizuoka, Izu, Japan/decaying wood *Castanea crenata*	FJ750266	FJ410372	EU683253

Table 1. Continued

Original identification	Collection number*	Final identification	Geographic origin/host	NCBI accession numbers		
				ITS1-5.8S-ITS2	β-Tubulin	Lac F2-R8
P. coccineus	CBS 355.63	*P. coccineus*	Bougainville, Aropa Airstrip, Solomon Islands/log	AF363760‡	FJ410375	EU678785
P. coccineus	MUCL 38523†	*P. coccineus*	Victoria, Australia/ironbark pole, *Eucalyptus* sp.	FJ873395	FJ410373	EU683254
P. coccineus	MUCL 38525	*P. coccineus*	Manjimup, Western Australia/*Eucalyptus marginatus*	FJ234207	FJ410374	EU714501
Trametes suaveolens	CBS 446.61	*Trametes suaveolens*	Sonnblick, Austria	FJ750268	FJ410378	

*The strains were obtained from different collections: CBS-KNAW, Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CIRM-BRFM, Banque de Ressources Fongiques de Marseille du Centre International de Ressources Microbiennes, France; CRBIP, Institut Pasteur, Paris, France; IMB, Institute of Microbiology of Beijing, China; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium.

†Reference strain.

‡Sequences previously deposited in GenBank (Lomascolo *et al.*, 2002).

§Monokaryons obtained as described by Lomascolo *et al.* (2002) for IMB G05.10 and Herpoël *et al.* (2002) for CIRM-BRFM 137.

Coriolus hirsutus and *P. sajor-caju* (NCBI accession numbers AB072703, AY458017, AB035409, AY081775 and AJ507324, respectively).

The ITS1-5.8S rRNA gene-ITS2, laccase F2-R8 and β-tubulin Bsens-Brev fragments were amplified from 50 ng genomic DNA in 50 μL PCR reagent containing 1.5 U Expand™ High Fidelity PCR system (Roche, France) with a protocol adapted from Lomascolo *et al.* (2002). Annealing temperatures and extension times were respectively 51 °C and 1 min for ITS1/ITS4 amplification, 55 °C and 50 s for Bsens/Brev amplification and 55 °C and 2 min for F2/R8 amplification. In the case of the lacF2/R8 fragment, the PCR products were further cloned into the pGEM®-T Easy vector (Promega), following the manufacturer's protocols. The PCR products were sequenced by GATC Biotech AG (Konstanz, Germany) or Cogenics (Meylan, France). All the nucleotide sequences were deposited in GenBank under the accession numbers given in Table 1.

Intron/exon junctions, in the Bsens-Brev β-tubulin and F2-R8 laccase gene fragments, were determined on the basis of alignment with, respectively, the *Trametes versicolor* FSU 2571 and *P. cinnabarinus* BRFM 137 coding regions (NCBI accession numbers AAY40456 and AF152170; Otterbein *et al.*, 2000; Schmitt *et al.*, 2008) and by identifying the eukaryotic consensus splicing sites (5′-GT and 3′-AG nucleotides). The nucleotide sequences (only exons for β-tubulin and laccase gene fragments) were aligned using the CLUSTAL W algorithm (Higgins *et al.*, 1991). The alignments were then hand-refined. Phylogenetic analyses were performed from single genes according to the method developed for the FIGENIX platform (Gouret *et al.*, 2005) using the heuristic search for maximum likelihood trees. Bootstrap values were calculated over 1000 replicates to assess branch topology. Phylogenetic trees were rooted with *T. suaveolens* as an outgroup.

Results and discussion

The filamentous fungi, among which the genus *Pycnoporus* is considered a strong contender for white biotechnology processes, form a huge worldwide source of biological diversity that needs to be explored. In the present work, the phylogenetic relationships of a large sample of *Pycnoporus* strains of different geographical origins were analysed using three complementary DNA markers. The nuclear rDNA region, ITS1-5.8S-ITS2, was often used to infer phylogenetic relationships among wood decay basidiomycetes species within a particular genus such as *Phanerochaete* (de Koker *et al.*, 2003) or a species complex such as *Postia caesia* (Yao *et al.*, 2005) but it often fails to provide robust phylogenetic resolution among the

fungal species (Wang *et al.*, 2004). The β-tubulin gene sequences were shown to resolve phylogenetic relationships within ascomycetes genera that could not be distinguished on the basis of morphology, especially in *Aspergillus* or *Pestalotiopsis* genera (Giraud *et al.*, 2007; Hu *et al.*, 2007). The genus *Pycnoporus* is described to overproduce laccase (encoded by *lac3-1* gene) as an extracellular ligninolytic enzyme in induced culture conditions (Eggert *et al.*, 1996; Lomascolo *et al.*, 2003). To date, genes encoding laccases have not been used to gain phylogenetic information within a fungal genus.

In this study, amplification of the ITS1-5.8S-ITS2 region yielded fragments 550–650 bp in length. After clean-up, the 36 sequences of *Pycnoporus* strains were aligned in 467 nucleotide positions (see Supporting Information, File S1). The sequencing analysis showed that the ITS1 and ITS2 regions were different in the strains studied, due to nt-insertions/deletions or substitutions, whereas the 5.8S rRNA gene sequences (157 bp long) were conserved for all the taxa. Within the ITS1 sequences, 44 of the 131 aligned positions (33.6%) varied among the strains of *Pycnoporus*. Within the ITS2 sequences, 36 of the 177 aligned positions (20.3%) varied among the strains of *Pycnoporus.* The most informative nucleotide sites (32 in all) were extracted from the alignment of the ITS1-5.8S rRNA gene-ITS2 sequences and are depicted in Table 2. Characterized by multiple nt-insertion events, up to 21 (see File S1), the sequences of the *P. puniceus* strains are not reported on this table. This sequence specificity was further confirmed by clustering ITS sequences available on GenBank (accession numbers FJ372685 and FJ372686) from Thai strains of *P. puniceus*. C and T insertions (at positions 48 and 452, respectively), and C at position 126 (instead of T) were shown to be specific to the *P. cinnabarinus* species. All the strains of *P. sanguineus* from Madagascar, Vietnam, French Guiana, New Caledonia and Venezuela exhibited identical ITS1 and ITS2 sequences. A common T/G and A/C substitution (at positions 43 and 113) was observed for the Chinese strains of *P. sanguineus*, including CIRM-BRFM 542 of unknown origin, and for all strains of *P. coccineus*. T/C and C/T substitutions (at positions 323 and 333) were shown to be specific to the East Asian strains of *P. sanguineus* and *P. coccineus*. Likewise, the ITS1 and ITS2 sequences of the strain MUCL 38420 (from Australia) classified as *P. cinnabarinus* were identical to those of both *P. coccineus* strains from Australia (MUCL 38523 and MUCL 38525), strongly suggesting taxonomic misidentification of the specimen. The strain MUCL 38420 was collected in Australia at the beginning of the 20th century; at that time, *P. coccineus* had not yet been described (Ryvarden & Johansen, 1980). In addition, the species *P. cinnabarinus* is known to be especially distributed in the temperate northern regions (Nobles & Frew, 1962).

Amplification of β-tubulin encoding gene fragments yielded 400-bp products on average. Comparison between gene and predicted cDNA fragment sequences showed that the corresponding coding region was interrupted by one intron. Interestingly, the intron length was 53, 54, 55 and 59 bp respectively for the species *P. puniceus*, *P. cinnabarinus*, *P. sanguineus* and *P. coccineus*, except for the Chinese *P. sanguineus* strains (including CIRM-BRFM 542), for which intron length was similar to that of *P. coccineus* species (59 bp instead of 55 bp). Identity between the partial predicted cDNAs was 78% on average. However, the amino acid sequences of the deduced partial proteins were 100% similar for all the strains.

β-Tubulin-encoding gene fragments, sequenced for the first time in *Pycnoporus* strains, were aligned in 263 nucleotide positions, and 55 of them (21%) varied among the strains of *Pycnoporus* (see File S2). The partial alignment depicted in Table 3 shows the most informative nucleotide sites, 26 in all. Compared with all the *P. coccineus* and *P. sanguineus* strains, specific variations occurred in six positions for the strains of *P. puniceus* and nine positions for the strains of *P. cinnabarinus*. Among the *P. sanguineus* and *P. coccineus* strains, sequence identities were observed for the strains of *P. sanguineus* from Vietnam, New Caledonia and Madagascar (except at position 260), and for those from French Guiana and Venezuela. C at position 98 and T at position 253 were common characters in all the strains of *P. coccineus* (including MUCL 38420) and in the Chinese strains of *P. sanguineus* (including CIRM-BRFM 542). C/G substitution at positions 152 and 206 was specific to the East Asian strains of *Pycnoporus*, and T/C substitution (at position 56) was specific to the Australian strains of *Pycnoporus.*

The phylogenetic trees inferred from ITS1-5.8S-ITS2 and β-tubulin gene sequences (Figs 1 and 2) clearly differentiated the group of *P. cinnabarinus* strains from the group of *P. puniceus* strains (100% bootstrap support). The group of the *P. coccineus* strains from Australia (including strain MUCL 38420), the *P. sanguineus* strains from China (including CIRM-BRFM 542 of unknown origin) with the Japanese strain of *P. coccineus*, and the strain of *P. coccineus* from the Solomon Islands (positioned alone), formed a well supported clade (84% bootstrap value with ITS). Due to the high similarity of their ITS sequences, the strains of *P. sanguineus* from Madagascar, Vietnam, New Caledonia, French Guiana and Venezuela could not be distinguished phylogenetically. β-Tubulin molecular data might be of slightly more help than ITS data to disclose genetic polymorphism within these *P. sanguineus* strains with two groups, although weakly supported (Fig. 2).

Table 2. Informative nucleotide sites from the alignment of the ITS1-5.8S rRNA gene-ITS2 regions for *Pycnoporus cinnabarinus*, *Pycnoporus sanguineus* and *Pycnoporus coccineus*

		Position in the sequence alignment*																																				
		Internal transcribed spacer1 (ITS1)														[5.8 S rRNA gene]						Internal transcribed spacer2 (ITS2)																
		1	18	20	25	28	34	43	48	64	74	75	83	96	100	113	117	118	126		293	309	311	323	335	356	370	373	378	445	452	533	543	545	565	
Pcin30555†	Belgium	A	G	G	C	G	C	G	C	G	A	C	T	A	A	C	T	C	C	[…]	A	T	C	C	T	C	A	T	T	T	T	T	T	C	
Pcin137	France (MK)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[…]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pcin237	Russia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[…]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pcin945	France	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[…]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pcin38420	Australia	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang892	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang893	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang895	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang896	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang899	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang900	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang901	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang902†	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang903	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang905	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang906	French Guiana	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang881	Venezuela	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang29375	Madagascar	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang942	Vietnam	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang943	Vietnam	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang979	New Caledonia	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang980	New Caledonia	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang981	New Caledonia	.	.	.	.	.	.	.	-	.	T	.	.	.	.	.	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Psang542	Unknown	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangW3008	China	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangG53	China	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangH2180	China	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangG66	China	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangW006-2	China	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
PsangG05.10	China (MK)	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
Pcoc38527	Japan	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	T	C	.	G	.	.	.	-	.	.	.	
Pcoc355.63	Solomon Islands	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Pcoc38523†	Australia	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	
Pcoc38525	Australia	.	.	.	.	.	.	T	-	.	T	.	.	.	.	A	.	.	T	[…]	.	.	.	.	.	.	G	.	.	.	-	.	.	.	

Psang, *Pycnoporus sanguineus*; Pcin, *Pycnoporus cinnabarinus;* Pcoc, *Pycnoporus coccineus*; MK for Monokaryon.

*Vertical numbering.

†Reference strains of *Pycnoporus* species; dot for nucleotide identical to the first reference sequence (from *Pycnoporus cinnabarinus* MUCL 30555); hyphen for gap.

Table 3. Informative nucleotide sites from the alignment of the partial regions of the β-tubulin gene for all the strains of *Pycnoporus* examined in this study

		Position in the sequence alignment*																										
		5	14	29	32	41	50	56	71	98	134	152	167	173	176	179	182	197	206	213	221	224	239	243	246	253	260
Pcin30555†	Belgium	C	A	T	T	T	C	C	T	T	T	G	C	C	T	C	G	C	G	T	T	C	G	T	C	T	C
Pcin137	France (MK)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pcin237	Russia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pcin945	France	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pcin38420	Australia	G	G	C	C	C	T	T	.	C	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	.	.
Ppun47083	Cuba	G	G	C	C	C	G	.	G	C	.	.	T	T	C	T	A	T	.	C	C	T	A	C	.	.	.
Ppun47087	Cuba	G	G	C	C	C	G	.	G	C	.	.	T	T	C	T	A	T	.	C	C	T	A	C	.	.	.
Psang892	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang893	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang895	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang896	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang899	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang900	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang901	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang902†	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang903	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang905	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang906	French Guiana	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang881	Venezuela	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	C	.
Psang29375	Madagascar	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	.
Psang942	Vietnam	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	G
Psang943	Vietnam	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	G
Psang979	New Caledonia	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	G
Psang980	New Caledonia	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	G
Psang981	New Caledonia	G	G	C	C	C	G	.	.	.	C	.	.	.	C	.	.	T	.	C	C	T	.	C	A	C	G
Psang542	Unknown	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangW3008	China	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangG53	China	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangH2180	China	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangG66	China	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangW006.2	China	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
PsangG05.10	China (MK)	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
Pcoc38527	Japan	G	G	C	C	C	G	.	.	C	C	C	.	.	C	.	.	.	C	.	C	T	.	C	A	.	.
Pcoc355.63	Solomon Islands	G	G	C	C	C	G	.	.	C	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	.	.
Pcoc38523†	Australia	G	G	C	C	C	T	T	.	C	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	.	.
Pcoc38525	Australia	G	G	C	C	C	T	T	.	C	C	.	.	.	C	.	.	.	.	.	C	T	.	C	A	.	.

Psang, *Pycnoporus sanguineus*; Ppun, *Pycnoporus puniceus*; Pcin, *Pycnoporus cinnabarinus*; Pcoc, *Pycnoporus coccineus*; MK, Monokaryon (see Table 1).

*Vertical numbering; dot for nucleotide identical to the first reference sequence (from *Pycnoporus cinnabarinus* MUCL 30555); hyphen for gap.

†Reference strains of *Pycnoporus* species.

In this study, the functional *lac3-1* gene, which protein products showed high variability in enzymatic activity between the species of *Pycnoporus* (Uzan *et al.*, 2010), was targeted to infer the phylogenetic relationships within the genus *Pycnoporus*, and especially within the *P. sanguineus* and *P. coccineus* species. PCR amplification resulted in laccase F2-R8 products of about 1640 bp. Comparison between gene and predicted cDNA fragment sequences showed that the corresponding partial coding regions were interrupted by eight introns. A positional homology among these introns could be observed. It is noteworthy that the eight intron lengths were strictly similar for the East Asian strains of *Pycnoporus* on the one hand, and for the Australian strains on the other (data not shown). The nine exons corresponded to sequences of 1182 nucleotides. The 36 deduced partial proteins (corresponding to about 75–80% of the full length protein) displayed sequence similarity ranging from 87.6% to

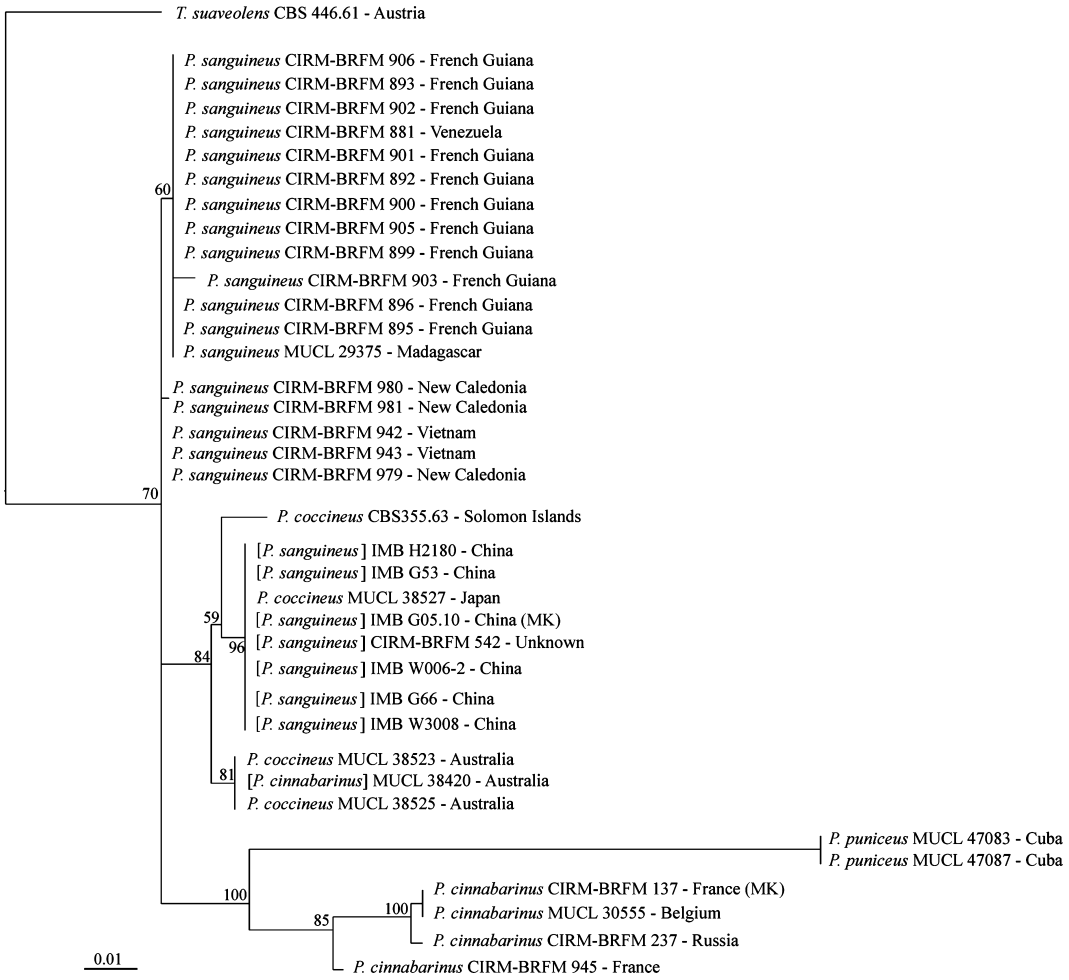

Fig. 1. Maximum likelihood phylogenetic tree based on ITS1-5.8S-ITS2 sequences of the 36 *Pycnoporus puniceus*, *Pycnoporus cinnabarinus*, *Pycnoporus sanguineus* and *Pycnoporus coccineus* strains studied. Model of nucleotide substitution HKY was chosen; transition weighted four times over transversion and log likelihood = −1254.30132. Estimated base frequencies were: *f*(A) = 0.21956, *f*(C) = 0.23793, *f*(G) = 0.25956 and *f*(T) = 0.28295. Numbers are percentages from 1000 bootstrap replicates. The scale bar represents one nucleotide substitution per 100 nucleotides. Clearly misidentified strains in brackets (see Table 1).

99.7%. The 36 laccase sequences from *Pycnoporus* strains were aligned in 1185 nucleotide positions after hand-refining (see File S3). These regions of the laccase gene had 33% variable positions among the strains of *Pycnoporus* studied. Informative nucleotide site variations were localized in the conserved copper-binding domains, especially domains II and III with T/C substitution specific to the East Asian strains of *Pycnoporus*.

Phylogenetic construction of our worldwide sample of *Pycnoporus lac3-1* sequences led to distinct groups that were correlated with the geographic origin of the strains (Fig. 3). Based on the evolutionary concept for plant diversity (Li, 2008), the distribution of *Pycnoporus* strains was characterized in terms of floristic regions, corresponding to geographic area with a relatively uniform composition of plant species (Takhtajan, 1986). Consistent with ITS and β-tubulin phylogenies, molecular clustering based on *lac3-1* sequence analysis grouped the *P. cinnabarinus* and *P. puniceus* strains into two highly supported specific lineages. The *P. sanguineus* and *P. coccineus* strains were distributed through four distinct, well supported clades and sub-clades. A neotropical sub-clade grouped the *P. sanguineus* strains from French Guiana and Venezuela – and the reference strain CIRM-BRFM 902 – corresponding to *P. sanguineus sensu stricto*. A paleotropical sub-clade clustered the strains from Madagascar, Vietnam and New Caledonia, and could be defined as *Pycnoporus* cf. *sanguineus*. The Australian clade of *P. coccineus*, including the reference strain MUCL 39523, corresponded to *P. coccineus sensu stricto*. This clade also included the Malesian strain from the Solomon Islands, positioned separately, consistent with the high

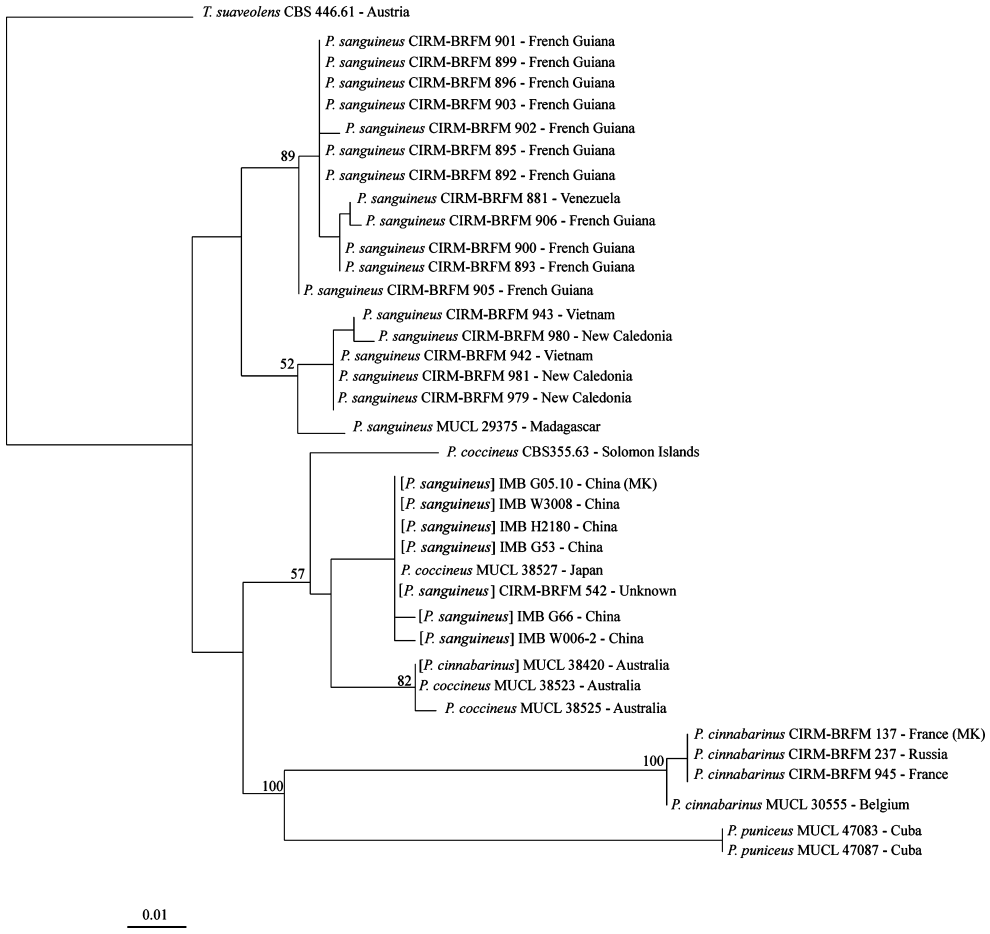

Fig. 2. Maximum likelihood phylogenetic tree based on partial region of the β-tubulin gene sequences of the 36 *Pycnoporus cinnabarinus*, *Pycnoporus coccineus*, *Pycnoporus puniceus* and *Pycnoporus sanguineus* strains studied. Model of nucleotide substitution HKY was chosen; transition weighted four times over transversion and log likelihood = −909.56923. Estimated base frequencies were: *f*(A) = 0.18825, *f*(C) = 0.34650, *f*(G) = 0.22688 and *f*(T) = 0.23838. Numbers above branches indicate that given branches were supported more than 50% from 1000 bootstrap replicates. The scale bar represents one nucleotide substitution per 100 nucleotides. Clearly misidentified strains in brackets (see Table 1).

level of endemic species in that country (Udvardy, 1975). The fourth group was the Eastern Asian region clade, clustering the strains from China, including CIRM-BRFM 542 of unknown origin and the strain MUCL 38527 from Japan. The strains of this last clade shared polymorphism in ITS and β-tubulin sequences with *P. coccineus sensu stricto* strains, as well as intron length in β-tubulin gene sequences, known to be characteristic of a lineage in basidiomycetes (Begerow *et al.*, 2004). This suggests a misidentification of Chinese specimens, very recently confirmed by macroscopic observation of basidiocarps. The high degree of similarity of the morphological characters between *P. sanguineus* and *P. coccineus* and the high variability of specimens across the season and the geographical area could explain this field misidentification (Nobles & Frew, 1962). Accordingly, the Eastern Asian region strains of *Pycnoporus* (from China and Japan), together with the related strain CIRM-BRFM 542 (suspected to be of East Asian descent), formed a *P. coccineus*-like group defined as *Pycnoporus* cf. *coccineus* (Fig. 3).

Biogeographic phylogenetic structure was related in polyporoid fungi such as *Grifola frondosa*, separating Eastern North American strains from Asian strains, and no morphological distinction was detected between them (Shen *et al.*, 2002). In the *Ganoderma applanatum/australe* species complex, eight distinct clades were strongly correlated with the geographic origin of the strains, and corresponded to mating groups (Moncalvo & Buchanan, 2008). Interestingly, the East Asian clade in our study corresponded to the functional group of *Pycnoporus* strains previously reported for their high level of laccase

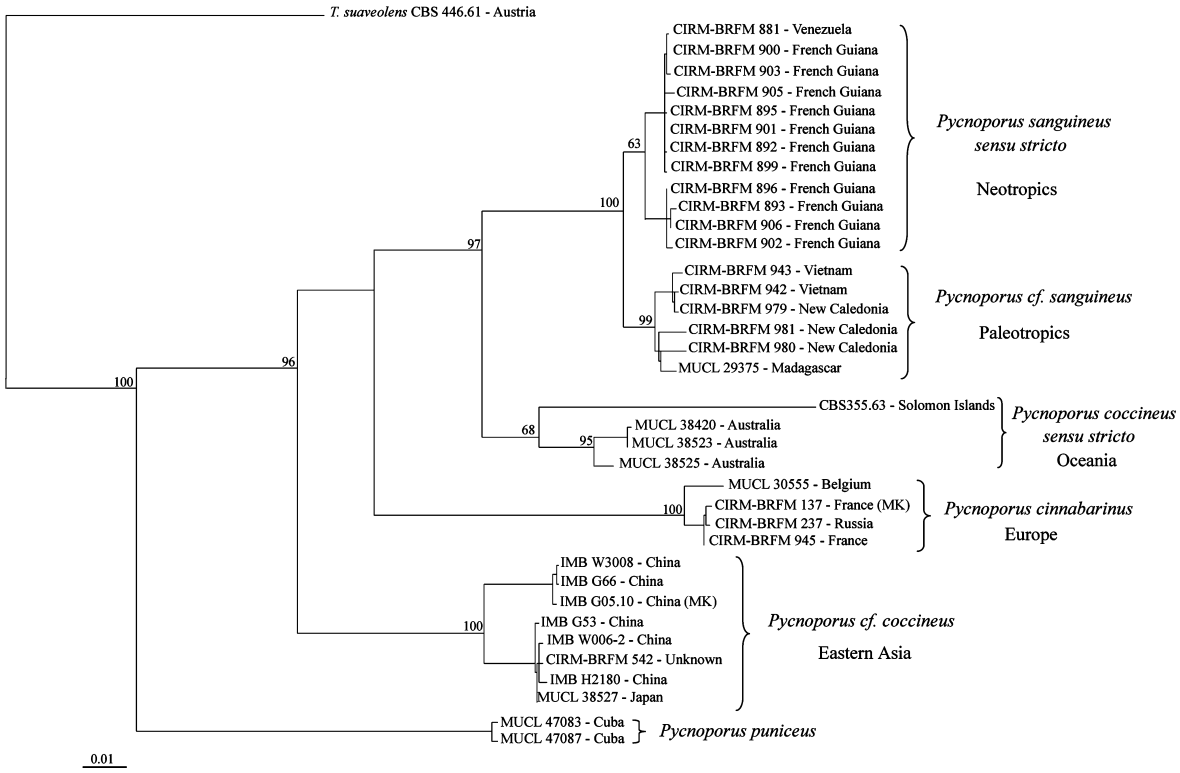

Fig. 3. Maximum likelihood phylogenetic tree based on F2-R8 partial laccase gene sequences of the 36 *Pycnoporus cinnabarinus*, *Pycnoporus coccineus*, *Pycnoporus puniceus* and *Pycnoporus sanguineus* strains studied. Model of nucleotide substitution HKY was chosen; transition weighted four times over transversion and log likelihood = −6703.69432. Estimated base frequencies were: *f*(A) = 0.19881, *f*(C) = 0.34192, *f*(G) = 0.23740 and *f*(T) = 0.22187. Numbers above branches indicate that given branches were supported more than 50% from 1000 bootstrap replicates. The scale bar represents one nucleotide substitution per 100 nucleotides. Clearly misidentified strains in brackets (see Table 1).

production (Lomascolo *et al.*, 2002). Correlation between phylogenetic proximity of fungal strains and enzymatic property similarity has been described previously, e.g. a monophyletic group of *Phlebia* strains was characterized by its similar ability to degrade recalcitrant organopollutants (Kamei *et al.*, 2005). In the same way, molecular clustering of isolates of *Aspergillus niger* aggregate group could be related to their ability to produce various types of feruloyl esterases, enzymes involved in the biodegradation process of the cell-wall polymers (Giraud *et al.*, 2007).

In conclusion, the analysis of the three genomic fragments, corresponding to rRNA, β-tubulin and *lac3-1* gene regions, with respect to *Pycnoporus* species, could provide effective, essential molecular tools for the routine identification and comparison of strains in laboratory culture conditions. For the first time, the laccase gene *lac3-1* was used to infer the phylogeny of *Pycnoporus* species and could highlight enzyme functional diversity associated with biogeographical origin. Special attention was given to the closely related species *P. sanguineus* and *P. coccineus*, which display very similar characters but are geographically discontinuous populations, indicating that biogeography has played a strong role in determining evolutionary units in the genus *Pycnoporus*. The current defining of species in basidiomycetes is still frequently delicate and should combine molecular tools with classic morphological data and mating-type experiments.

Acknowledgements

The authors thank Prof K.A. To from the University of Hanoi and Dr M. Coussot of the Centre International de Recherche Agronomique pour le Développement (CIRAD, France) for specimens of *P. sanguineus* from Vietnam and French New Caledonia, respectively. The authors also are grateful to Prof. Regis Courtecuisse (Université de Lille II, France) who provided the expertise in identification of *Pycnoporus* species collected on the French territories. The authors also sincerely thank Dr Stéphane Welti for valuable suggestions and Dr Jean-Guy Berrin for practical assistance. This work was supported financially by the Commission of the European Communities, specifically

the BIORENEW project (NMP2-CT-2006-026456 'White biotechnology for added value products from renewable plant polymers: design of tailor-made biocatalysts and new industrial bioprocesses').

References

Baldrian P (2006) Fungal laccases – occurrence and properties. *FEMS Microbiol Rev* **30**: 215–242.

Begerow D, Beate J & Oberwinkler F (2004) Evolutionary relationships among β-tubulin gene sequences of basidiomycetous fungi. *Mycol Res* **108**: 1257–1263.

Brijwani K, Rigdon A & Vadlani PV (2010) Fungal laccases: production, function, and applications in food processing. *Enzyme Res* **2010**: 149748.

Courtecuisse R, Samuels GJ, Hoff M, Rossman AY, Cremers G, Huhndorf SM & Stephenson SL (1996) Check-list of fungi from French Guiana: Studies in the flora of the Guianas, No. 80. *Mycotaxon* **LVII**: 1–85.

Eggert C, Temp U & Eriksson KEL (1996) The lignolytic system of the white-rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. *Appl Environ Microbiol* **62**: 1151–1158.

Eggert C, Lafayette P, Temp U, Eriksson KEL & Dean JFD (1998) Molecular analysis of a laccase gene from the white-rot fungus *Pycnoporus cinnabarinus*. *Appl Environ Microbiol* **64**: 1766–1772.

Fries EM (1851) Novae symbolae mycologicae. *Nova Acta Regiae Societatis Scientiarum Upsaliensis, Ser* **31**: 17–136.

Froslev TG, Matheny PB & Hibbett DS (2005) Lower level relationships in the mushroom genus *Cortinarius* (Basidiomycota, Agaricales): a comparison of RPB1, RPB2, and ITS phylogenies. *Mol Phylogenet Evol* **37**: 602–618.

Gianfreda L, Xu F & Bollag JM (1999) Laccases: a useful group of oxidoreductive enzymes. *Bioremediat J* **3**: 1–25.

Giraud F, Dupont J, Haon M, Bouzid O, Alibeu O, Navarro D, Sage L, Seigle-Murandi F, Asther M & Lesage-Meessen L (2007) Phylogenetic analysis of the *Aspergillus niger* aggregate in relation to feruloyl esterase activity. *Res Microbiol* **158**: 413–419.

Gouret P, Vitiello V, Balandraud N, Gilles A, Pontarotti P & Danchin EGJ (2005) FIGENIX: intelligent automation of genomic annotation: expertise integration in a new software platform. *BMC Bioinformatics* **6**: 198–219.

Hatsch D, Phalip V & Jeltsch JM (2004) Use of genes encoding cellobiohydrolase-C and topoisomerase II as targets for phylogenetic analysis and identification of *Fusarium*. *Res Microbiol* **155**: 290–296.

Herpoël I, Jeller H, Fang G, Petit-Conil M, Bourbonnais R, Robert JL, Asther M & Sigoillot JC (2002) Efficient enzymatic delignification of wheat straw pulp by a sequential xylanase-laccase treatment. *J Pulp Paper Sci* **28**: 67–71.

Hibbett DS, Binder M, Bischoff J *et al.* (2007) A higher-level phylogenetic classification of the fungi. *Mycol Res* **111**: 509–547.

Higgins DG, Bleasby AJ & Fuchs R (1991) CLUSTAL W: improved software for multiple alignment. *Comput Appl Biosci* **8**: 189–191.

Hilden KS, Bortfeldt R, Hofrichter M, Hatakka A & Lundell TK (2008) Molecular characterization of the basidiomycete isolate *Nematoloma frowardii* b19 and its manganese peroxidase places the fungus in the corticoid genus *Phlebia*. *Microbiology* **154**: 2371–2379.

Hu H, Jeewon R, Zhou D, Zhou T & Hyde KD (2007) Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: evidence from rDNA and beta-tubulin gene phylogenies. *Fungal Divers* **24**: 1–22.

Kamei I, Suhara H & Kondo R (2005) Phylogenetical approach to isolation of white-rot fungi capable of degrading polychlorinated dibenzo-*p*-dioxin. *Appl Microbiol Biotechnol* **69**: 358–366.

Karsten PA (1881) Enumeratio Boletinearum et Polyporearum Fennicarum, systemate novo dispositarum. *Rev Mycol*, **3**: 16–19.

de Koker TH, Nakasone KK, Haarhof J & Burdsall HH (2003) Phylogenetic relationships of the genus *Phanerochaete* inferred from the internal transcribed spacer region. *Mycol Res* **107**: 1032–1040.

Lamark J-B (1783) *Agaricus ruber*. *Encycl Méth Bot (Paris)* **1**: 50.

Li DZ (2008) Floristics and plant biogeography in China. *J Integr Plant Biol* **50**: 771–777.

Lomascolo A, Cayol JL, Roche M, Guo L, Robert JL, Record E, Lesage-Meessen L, Ollivier B, Sigoillot JC & Asther M (2002) Molecular clustering of *Pycnoporus* strains from various geographic origins and isolation of monokaryotic strains for laccase hyperproduction. *Mycol Res* **106**: 1193–1203.

Lomascolo A, Record E, Herpoël-Gimbert I, Delattre M, Robert JL, Georis J, Dauvrin T, Sigoillot JC & Asther M (2003) Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. *J Appl Microbiol* **94**: 618–624.

Messerschmidt A & Huber R (1990) The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin: modelling and structural relationships. *Eur J Biochem* **187**: 341–352.

Moncalvo J-M & Buchanan PK (2008) Molecular evidence for long distance dispersal across the southern hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycol Res* **112**: 425–436.

Nobles MK & Frew BP (1962) Studies in wood-inhabiting hymenomycetes. V. The genus *Pycnoporus* Karst. *Can J Bot* **40**: 987–1016.

Otterbein L, Record E, Longhi S, Asther M & Moukha S (2000) Molecular cloning of the cDNA encoding laccase from *Pycnoporus cinnabarinus* I-937 and expression in *Pichia pastoris*. *Eur J Biochem* **267**: 1619–1625.

Ryvarden L (1991) Genera of Polypores, nomenclature and taxonomy. *Synopsis Fungorum 5*. Fungiflora, Oslo.

Ryvarden L & Gilbertson RL (1994) *Meripilus – Tyromyces. European Polypores*, Part 2. Fungiflora, Oslo.

Ryvarden L & Johansen I (1980) A preliminary polypore flora of East Africa. *Synopsis Fungorum 5*. Fungiflora, Oslo.

Schmitt I, Partida-Martinez LP, Winkler R, Voigt K, Einax E, Dolz F, Telle S, Wostemeyer J & Hertweck C (2008) Evolution of host resistance in a toxin-producing bacterial-fungal alliance. *ISME J* **2**: 632–641.

Shen Q, Geiser DM & Royse DJ (2002) Molecular phylogenetic analysis of *Grifola frondosa* (maitake) reveals a species partition separating eastern North American and Asian strains. *Mycologia* **94**: 472–482.

Sigoillot C, Record E, Belle V, Robert JL, Levasseur A, Punt PJ, van den Hondel CAMJJ, Fournel A, Sigoillot JC & Asther M (2004) Natural and recombinant fungal laccases for paper pulp bleaching. *Appl Microbiol Biotechnol* **64**: 346–352.

Takhtajan A (1986) *Floristic Regions of the World*. University of California Press, Berkeley.

Thon MR & Royse DJ (1999) Partial β-tubulin gene sequences for evolutionary studies in the Basidiomycotina. *Mycologia* **91**: 468–474.

Tomšovský M, Kolaří M, Pažoutová S & Homolka L (2006) Molecular phylogeny of European *Trametes* (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences. *Nova Hedwigia* **82**: 269–280.

Udvardy MDF (1975) *A Classification of the Biogeographical Provinces of the World*. IUCN occasional paper No. 1. International Union for Conservation of Nature and Natural Resources, Morges.

Uzan E, Nousiainen P, Balland V, Sipila J, Piumi F, Navarro D, Asther M, Record E & Lomascolo A (2010) High-redox potential laccases from the ligninolytic fungi *Pycnoporus coccineus* and *P. sanguineus* suitable for white biotechnology: from gene cloning to enzyme characterization and applications. *J Appl Microbiol* **108**: 2199–2213.

Uzan E, Portet B, Lubrano C, Milesi S, Favel A, Lesage-Meessen L & Lomascolo A (2011) *Pycnoporus* laccase-mediated bioconversion of rutin to oligomers suitable for biotechnology applications. *Appl Microbiol Biotechnol* **90**: 97–105.

Wang Z, Binder M, Dai YC & Hibbett DS (2004) Phylogenetic relationships of *Sparassis* inferred from nuclear and mitochondrial DNA and RNA polymerase sequences. *Mycologia* **96**: 1015–1029.

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* (Innis MA, Gelfand DH, Sninsky JJ & White TJ, eds), pp. 315–322. Academic Press, San Diego.

Yao YJ, Pegler DN & Chase MW (2005) Molecular variation in the *Postia caesia* complex. *FEMS Microbiol Lett* **242**: 109–116.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Alignment of the ITS1-5.8S rRNA gene-ITS2 regions (467 nt positions) for the 36 *P. cinnabarinus*, *P. sanguineus* and *P. coccineus* strains studied.

File S2. Alignment of partial regions (263 nt positions) of the gene coding for β-tubulin, for the 36 *P. cinnabarinus*, *P. sanguineus* and *P. coccineus* strains studied.

File S3. Alignment of partial regions (1185 nt positions) of the gene coding for the laccase isoenzyme Lac I, for the 36 *P. cinnabarinus*, *P. sanguineus* and *P. coccineus* strains studied.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.