A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales)

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Fomitiporia mediterranea is described as a new wood-decaying basidiomycete species associated with esca of grapevine in European wine-growing countries. Characters of the fruit body are essentially identical with those of the closely related species, *Fomitiporia punctata*. *Fomitiporia mediterranea* is distinct by the sequences of the ribosomal ITS1-5.8S-ITS2 region and by larger mycelial growth rates at temperatures between 15 °C and 35 °C. While *F. punctata* is confirmed as a homothallic species, *F. mediterranea* is shown to be outcrossing, exhibiting a unifactorial mating behaviour with a multiple allelism of the mating type factor, *A*; single spore isolates of *F. mediterranea* are intersterile in pairing tests with *F. punctata*. In southern Europe, *F. mediterranea* not only occurs on *Vitis vinifera*, but also on a number of other hardwood genera; it seems restricted to *Vitis vinifera* elsewhere in Europe.

Taxonomic novelty: *Fomitiporia mediterranea* M. Fischer

Esca is a widespread disease of grapevine in wine-producing countries. It occurs at different incidence in Europe, North and South America, Australia, and, possibly, New Zealand and South Africa (CHIARAPPA 2000). Esca is most prominent in the mediterranean region, where in some areas more than 50 % of the vineyards have a disease incidence ranging from 20–30 % (CORTESI, FISCHER & MILGROOM 2000). In Central Europe, observations on the epidemiology of esca have been mostly carried out in Germany, where the disease has become economically important in recent years (KASSEMEYER 1998; unpubl. data). The disease affects the wood of the trunk and the lateral branches, and symptoms can be observed on leaves and wood, more rarely also on berries and clusters.

Several genera of fungi have been reported as being associated with esca (recently reviewed by MUGNAI, GRANITI & SURICO 1999; CHIARAPPA 2000). One of the main symptoms of esca, the white rot occurring inside of the trunk and main branches, is considered to be caused by basidiomycetes, the ones mostly mentioned *Phellinus igniarius* (L.) Quél. and *Fomitiporia punctata* (P. Karst.) Murrill, formerly called *Phellinus punctatus* (P. Karst.) Pilát, and, to a lesser extent, *Stereum hirsutum* (Willd. : Fr.) Pers. (RIVES 1921, GARD 1922, CHIARAPPA 1959, 1997, MUGNAI, SURICO & ESPOSITO, 1996, LARIGNON & DUBOS 1997, MUGNAI, GRANITI & SURICO 1999, CORTESI, FISCHER & MILGROOM 2000). On the basis of mycelia isolated from infected wood of *Vitis*, *F. punctata* for a long time has been misidentified as *P. igniarius*. Only recently however, using morphological and molecular characters the basidiomycetes found associated with white rot of vines showing symptoms of esca from wine-growing areas of Italy were assigned to *F. punctata* (CORTESI, FISCHER & MILGROOM 2000). Fruit bodies, which would allow a reliable differentiation between *F. punctata* and *P. igniarius* (FISCHER 2000), only rarely occur on the infected vines and, in addition, are easily overlooked in the field.

Fomitiporia punctata has a worldwide distribution and is found on numerous hardwood genera (CUNNINGHAM 1965, GILBERTSON & RYVARDEN 1987, RYVARDEN & GILBERTSON 1994, DAI 1999). In Central Europe, *F. punctata* exists both as a parasite and a saprotroph; important host genera include *Corylus* L. and *Salix* L., whereas occurrence on *Vitis* L. is considered as rare. Typically, fresh fruit bodies occur on living trees, where they develop well above the ground on standing trunks and branches. Decay activities of the fungus are limited, and some years after infection the wood remains quite solid (JAHN 1967).

Molecular studies on European strains of *F. punctata* from several hardwood genera including *Vitis* demonstrated that after digestion of the nuclear ribosomal ITS region (ITS1, 5.8S, ITS2) the generated fragments were almost uniform and that *F. punctata* was divergent from *P. igniarius* and related taxa (CORTESI, FISCHER & MILGROOM 2000). Preliminary observations on the wood decay symptoms however point to a variation among European strains of *F. punctata*. The species is considered a distinct parasite in the mediterranean area, where distinct wood decay symptoms may be evident on *Vitis* plants only 8–10 years old (MUGNAI, GRANITI & SURICO 1999);

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besides, it is thought to cause severe damage on olive-trees (PLANK 1980). In contrast, *F. punctata* does not play an important role as a parasite in the remaining parts of Europe (JAHN 1967, RYVARDEN 1978).

The understanding of the epidemiology of esca is limited by the fact that only little is known about the spread of the disease in nature. Different modes of disease spread should result in different genetic structures of populations. While MUGNAI, GRANITI & SURICO (1999) and POLLASTRO et al. (2000) found some evidence for a spatial aggregation of infected plants in Italy, BALDACCI, BELLI & FOGLIANI (1962) as well as CORTESI, FISCHER & MILGROOM (2000) found esca symptoms to be more spatially random. The pronounced genetic diversity of populations of *F. punctata* within vineyards, demonstrated by RAPD markers (PEROS, JAMAUX-DESPREAUX & BERGER 2000) and somatic incompatibility types (CORTESI, FISCHER & MILGROOM 2000), indicates that population structures are essentially shaped by sexual recombination. In previous studies however taxa belonging to *Fomitiporia* have been shown to exhibit an asexual, i.e. homothallic, mode of reproduction (FISCHER 1996). Basically, investigations on mating behaviour in species of *Fomitiporia* are impeded by the fact that, for unknown reasons, germination rates of basidiospores are very low at best under laboratory conditions (FISCHER 1987).

The present study includes 23 strains identified as *F. punctata*, mostly isolated from *Vitis*, but also from other host plants. Strains were collected in Italy, Germany, Estonia, and Finland. In particular, the following problems should be addressed: 1) On the basis of a sequence analysis of the nuclear ribosomal ITS region (ITS1, 5.8S, ITS2) an accurate resolution should be obtained between the isolates of *F. punctata*, originating from vine and non-vine substrates. In order to assess the extent of molecular variation within *F. punctata*, several strains each of the closely related taxa, *Phellinus igniarius*, and *F. robusta* (P. Karst.) Fiasson & Niemelä, were included in the phylogenetic analysis. 2) A reliable and simple protocol should be developed for the germination of basidiospores of *F. punctata*. Eventually, single spore mycelia should be used for pairing tests, both within strains (pattern of sexuality) and between strains (multiple allelism, species delimitation). 3) The obtained data should be compared with more traditional characters such as morphology of fruit bodies and cultural behaviour.

Material and methods

Fungal material and culturing

The strains used are listed in Tab. 1. Specimens are deposited at the herbarium of the University of Regensburg (REG). Mycelial cultures were grown on malt extract medium (ME; 2 % agar, 2 % malt extract, 0.05 % yeast extract) under permanent dark conditions.

Comparative microscopy

Sections of fruit bodies were placed on a slide in a drop of Melzer´s reagent or lactophenol-cotton blue (MEIXNER 1975); examinations were at 500x or 1250x under phase contrast optics. Twenty observations were recorded for measurements of basidiospores.

Mycelial growth

For determination of temperature requirements selected strains of *F. punctata* were incubated on ME at 15 °C, 21 °C, 30 °C, and 35 °C. These strains were CA3, VD44, Asti2, LM2, TW7.98a, TW7.98b, 45/23, 85-74, 87-511, Dai2727, and 99-105. Mycelial growth was measured by calculating the mean of two perpendicular colony diameters. Two repeats were performed for each isolate.

Isolation of single spore isolates

A section of the hymenium was attached to the inside of a Petri plate lid. Discharged spores were dispersed with Ringer´s solution (NaCl, 0.225%; KCl, 0.01%; $CaCl_2$, 0.0045%, $NaHCO_3$, 0.005% in distilled water) or with sterile cultural filtrate (ME) of the corresponding strain. Plates with dispersed spores were incubated at 15 C, 21 C, and 30 C under daylight conditions.

Pairing tests

Intrastrain pairings were made using 8 single spore mycelia, which were paired in all possible combinations. Paired mycelia were incubated for 4–5 wk before examination. For interstrain pairings two different isolates per mating type were selected from each strain as testers; these were paired in all possible combinations.

DNA isolation and PCR amplification

Whole cell DNA was isolated from fresh mycelium; isolation was essentially as described by LEE & TAYLOR (1990). Quantity and quality of the DNA were examined on 1% agarose gels. Isolated DNA was diluted 1 : 100 in distilled water. The polymerase chain reaction (PCR) was used to amplify a portion of the nuclear encoded ribosomal DNA unit defined by the primer combination prITS5 and prITS4 (for primer sequences, see WHITE et al. 1990). The fragment spans the entire ITS1 region, the 5.8S rRNA gene, and the ITS2 region.

The PCR reactions were set up in 100 μl volumes and were overlayed with two drops of mineral oil. Hot start PCR was applied throughout (D'AQUILA et al. 1991). Forty cycles were performed on a TRIO-Thermoblock (Biometra, Germany), using the following parameters: 95 °C denaturation step (1 min), 50 °C annealing step (1 min), 72 °C primer extension (1 min). A final incubation step at 72 °C (7 min) was added after the final cycle. 5 μl of each PCR reaction were electrophoresed on 1 % agarose gels. DNA molecular weight marker VI (Roche Diagnostics, Germany) was used as standard. The amplified products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) following the ma-

Tab. 1: List of fungal taxa and strains

Species (strain number, date, location, collector)	Substrate	GenBank accession number
Fomitiporia robusta (P. Karst.) Fiasson & Niemelä:		
89-828[1], 28.8.1989, Estonia, AB[3]	Quercus L.	AF515565
96-515[1], 15.5.1996, Germany (Bavaria), LK	Fraxinus excelsior L.	AF515560
Phellinus igniarius (L.) Quél.:		
85-625[1], 25.6.1985, Germany (Bavaria), MF	Salix caprea L.	AF515573
TN5758[1], 25.5.1994, Finland, TN	Salix L.	AF515574
Strains identified as Fomitiporia punctata (Karst.) Murrill:		
CA3*[2], VIII-1997, Italy (Tuscany), PC	Vitis vinifera (DC.) Beg.	AF515575
VD24*[2], VIII-1997, Italy (Tuscany), PC	Vitis vinifera	AF515571
VD44*[2], VIII-1997, Italy (Tuscany), PC	Vitis vinifera	AF515572
Asti2*[2], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515577
Asti3*[2], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515576
Asti4*[2], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515578
Asti5*[2], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515579
Asti6*[2], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515580
LM1*[1], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515567
LM2*[1], X-1998, Italy (Piedmont), LM	Vitis vinifera	AF515566
K9/9*[2], II-2000, Germany (Baden-Württemberg), IM	Vitis vinifera	AF515568
3/22*[1], VIII-2001, Germany (Baden-Württemberg), MF	Vitis vinifera	AF515583
45/23*[1], VIII-2001, Germany (Baden-Württemberg), MF	Vitis vinifera	AF515585
85-74[1], 4.7.1985, Germany (Bavaria), MF	Salix caprea L.	AF515563
87-511[1], 11.5.1987, Germany (Bavaria), WP	Rhamnus cathartica L.	AF515564
89-826b[1], 26.8.1989, Estonia, AB	Sorbus aucuparia L.	AF515562
940417.3*[1], IX-1994, Italy (Liguria), OH	Olea europaea L.	AF515582
Dai2727[1], 5.10.1997, Finland, YCD	Sorbus aucuparia	AF515561
TW7.98a*[1], VII-1998, Italy (Lombardy), TW	Acer negundo L.	AF515569
TW7.98b*[1], VII-1998, Italy (Lombardy), TW	Lagerstroemia indica L.	AF515570
CSF1.96*[2], II-1999, Italy (Emilia Romagna), SdM	Actinidia chinensis Planch.	AF515581
99-105*[1], 5.10.1999, Italy (Lazio), MF	Corylus avellana L.	AF515586
01-1010[1], X-2001, Germany (Baden-Württemberg), MF	Syringa vulgaris L.	AF515584

* Strains actually belonging to F. mediterranea; [1] mycelium isolated from fruit bodies; [2] mycelium isolated from infected wood; [3] AB Andreas Bresinsky, LK Lothar Krieglsteiner, MF Michael Fischer, TN Tuomo Niemelä, PC Paolo Cortesi, LM Laura Mugnai, IM Ingo Morgenstern, WP Wolfgang Paulus, OH Ottmar Holdenrieder, YCD Yu-Cheng Dai, TW Tobias Wagner, SdM Stefano diMarco

nufacturer's instructions. DNA was suspended in 50 µl Tris-HCl buffer (10 mM, pH 8.0).

Sequencing

All strains listed in Tab. 1 were included in the sequencing experiments. Instead of mycelium derived from fruit bodies, single spore isolates were used for strains 3/22, 45/23, 01-1010, and TN5758, designated 3/22.7, 45/23.3, 01-1010.3, and TN5758.1, respectively. Fragments were sequenced with the AmpliTaq DNA Polymerase FS Dye Terminator Cycle Sequencing kit (Perkin Elmer, USA), using 2 µl of premix, 1 µl of the primers (8 pmol of prITS1 and prITS4, respectively), and 3.5 µl of the PCR products. The reactions were set up in 11 µl volumes, and were overlayed with one drop of mineral oil.

Sequences were generated in two directions and twenty-five amplification cycles were carried out, using the following parameters: 96 °C denaturation step (30 s), 59 °C annealing step (15 s) for prITS1, 53 °C annealing step (15 s) for prITS4, 60 °C primer extension (4 min). DNA was precipitated by addition of 2 µl of NaAc (3 M, pH 4.8) and 55 µl of EtOH 100 %, and was then washed with 150 µl of EtOH 70 %. The DNA pellet was resuspended in 1 : 4 EDTA (50 mM, pH 8.0) : formamide.

The electrophoresis was done with an ABI 373A Automatic Sequencer (Perkin Elmer). After processing the raw data with SeqEd (version 3.0), the sequences were aligned using the ClustalX (version 1.64b) program (THOMPSON et al. 1997). A final alignment was performed by eye. Alignment gaps were treated as missing data. All positions were included in

the final alignment. The sequences obtained have been deposited in GenBank (for numbers, see Tab. 1), sequence alignments have been deposited in TreeBASE as submission no. SN 1139.

Maximum parsimony (MP) analysis was performed with PAUP 4.0b2 (SWOFFORD 1999) using heuristic search, with tree-bisection-reconnection (TBR) branch-swapping algorithm, MulTrees option in effect, and zero length branches collapsed. All characters were of unordered type and had equal weight. A two-step analysis was performed: step one used 100 heuristic searches, keeping up to ten trees per replicate; step two used TBR branch swapping on the shortest trees found in step one. Maxtrees option was set to 1000. To estimate the branching order, a bootstrap analysis (FELSENSTEIN 1985) was run with 1000 heuristic replicates. For neighbour-joining analysis, a distance matrix was generated using DNA DIST, a program from the PHYLIP 3.5c package (FELSENSTEIN 1995) integrated in ClustalX. The calculation was performed using the Kimura 2 model and a transition:transversion ratio 2:1. Bootstrap values for internal nodes were calculated by 1000 replications.

Results

Molecular sequence data

The alignment included 23 strains identified as *Fomitiporia punctata*, and two strains each of *F. robusta* and *P. igniarius*. The final alignment resulted in 772 nucleotides with 190 variable characters, 146 of which were parsimony-informative. The most parsimonious analysis with PAUP produced many most parsimonious trees with 228 steps each, consistency index (CI) 0.921, and retention index (RI) 0.945. The aligned region contains a small portion of the flanking 18S and 28 S genes, the complete ITS1 region, the complete 5.8S gene, and the complete ITS2 region.

The size of the sequenced region was variable within and between taxa. For *P. igniarius*, it was 643 or 647 nucleotides; for *F. robusta*, it was 725 or 726 nucleotides; for *F. punctata*, the strains from *Vitis* and/or the mediterranean area were 740-744 bp, whereas the strains from Central and Northern Europe originating from non-*Vitis* hosts were 719 or 720 nucleotides.

Sequence variability was most prominent for the ITS1 region. For the taxa of *Fomitiporia*, one major insertion was found between nucleotides 50 and 57 (*F. robusta*), whereas major deletions were located between nucleotides 199 and 204 (*F. punctata* from Central and Northern Europe), and between nucleotides 248 and 253 (*F. robusta*). Within the ITS2 region two major deletions were noted, located between nucleotides 536 and 539, and between 722 and 730 (both *F. robusta* and *F. punctata* from Central and Northern Europe). Compared with the three taxa of *Fomitiporia*, the ITS regions were distinctly smaller in the strains of *P. igniarius*. The sequences of the 5.8S gene were almost identical for the isolates under study, without exception the length was 169 nucleotides.

The variability of the examined DNA region is reflected in the generated phylogenetic trees, where the strains are subdivided into four separated groups (Figs. 1, 2). Both in the neighbour-joining tree (Fig. 1; bootstrap value 100 %) and the strict consensus tree (Fig. 2; 100 %), strains from *Vitis* and/or Italy, containing *Acer* (strain TW7.98a), *Actinidia* (CSF1.96), *Corylus* (99-105), *Lagerstroemia* (TW7.98b), and *Olea* (940417.3) as host plants, were grouped together in a single clade, sharply delimited from the remaining strains of *F. punctata*, which appeared as sister group to *F. robusta* (bootstrap values 89 % and 97 %, respectively). *Phellinus igniarius* was distinctly divergent from the taxa of *Fomitiporia*.

Characters of fruit bodies and mycelia

Although of different geographic origin and host, collections of *F. punctata* were not distinguishable in morphology and anatomy of fruit bodies. Typically, fruit bodies occurring on *Vitis* were found in the uppermost part of the trunk, next to the pruning wounds (Fig. 3; strain 3/22). Two classes of basidiospore size were noted in several collections from Italy and in Dai 2727 from Finland. Most basidiospores measured 6–7 x 5–6 μm, whereas others were smaller, with dimensions of only 5–5.5 x 4–5 μm. Probably the smaller sizes are due to immature spores detached from the basidium. With one exception no setae were found in the specimens examined; one single seta, located in the hymenium and 15 x 4.5 μm in size, was observed in VD 44 (from *Vitis vinifera*, Italy).

In agreement with previous reports (STALPERS 1978, FISCHER 1987, 2000), the appearance of cultured mycelium was variable for isolates of *Fomitiporia punctata* and two mycelial types were distinguished: In type B („bleaching-type"; FISCHER 1987), comprising the majority of isolates, mycelial cultures were cottony to woolly, with aerial hyphae yellowish to brownish; in such cultures pigmentation of the medium was weak or lacking; under 21 °C conditions, growth rate was fast, approximately 3–4.5 cm in 2 wk. Type S („staining type"; FISCHER 1987) was characterized by a sparse development of aerial hyphae, a modest to strong pigmentation of the medium and a slow growth, 1.5–2.5 cm in 2 wk. Type B was more common with the temperatures applied in this study, especially so in the strains from Italy and/or *Vitis*, type S was more often observed in the strains from Central and Northern Europe. Basically, the mycelial type could vary in subsequent inoculations.

Isolates were able to grow at all temperatures tested, i.e. between 15 C and 35 C. The strains included in the tests all showed optimum growth at 30 C and throughout were of the B-type. For all temperatures, strains originating from *Vitis* and/or Italy obtained distinctly larger growth rates than those from Central and Northern Europe collected on non-*Vitis* hosts. Within 8 d, the former group had growth rates of 1.6 cm, 2.4 cm, 3.2 cm, and 2.5 cm at 15 °C, 21 °C, 30 °C, and 35 °C, respectively, while the latter group had growth rates of 1.3 cm, 1.9 cm, 2.5 cm, and 1.6 cm.

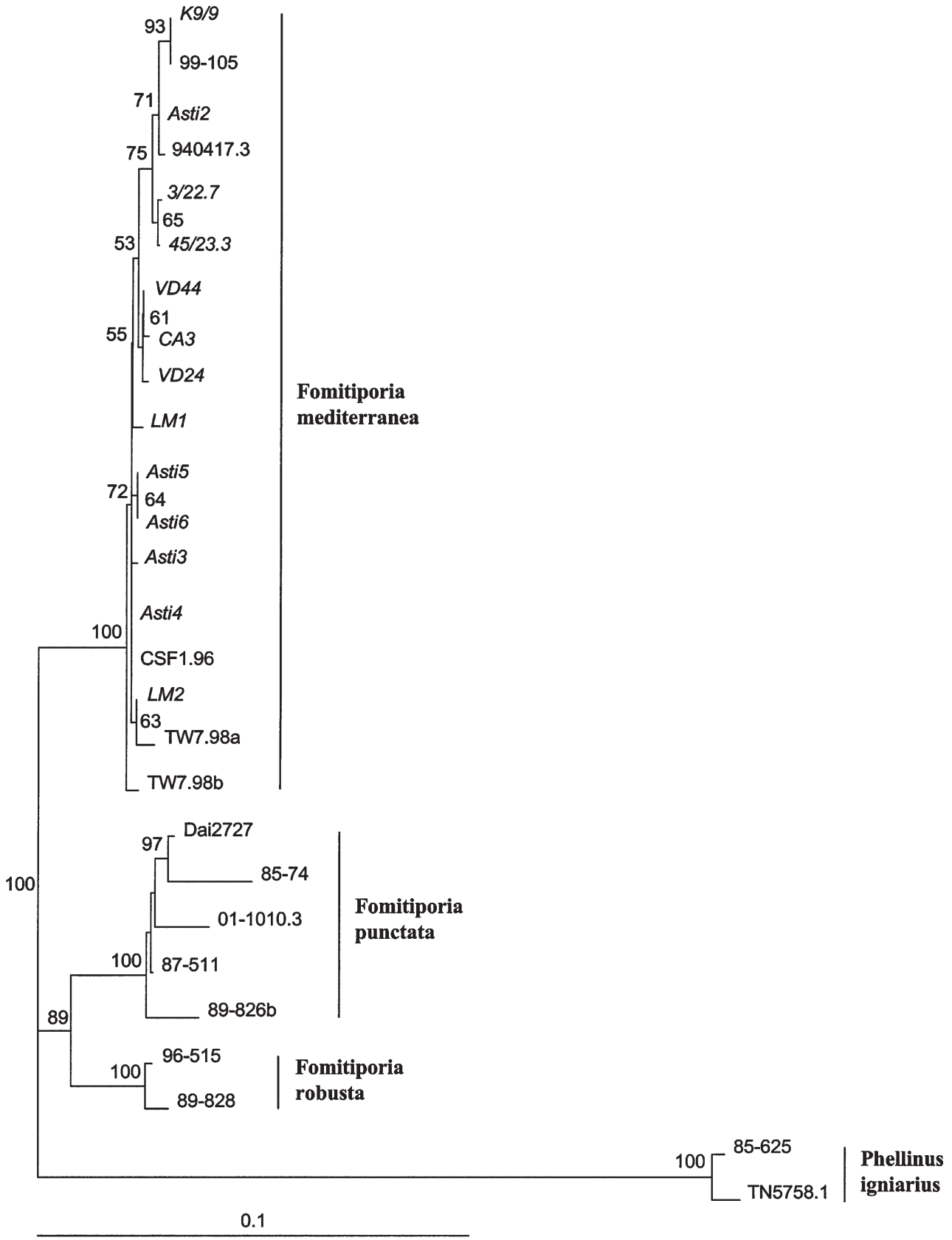

Fig. 1: Phylogenetic relationships of taxa of *Fomitiporia* and *Phellinus igniarius* inferred from the nuclear ITS1-5.8S-ITS2 region using the neighbour-joining method. The tree was rooted with the isolates belonging to *P. igniarius*. Bootstrap values of 50% or greater from 1000 bootstrap replications are indicated for the corresponding nodes. Branch lengths are proportional to distance. The proposed specific designation is explained in the text. Strains originating from *Vitis* are indicated in italics

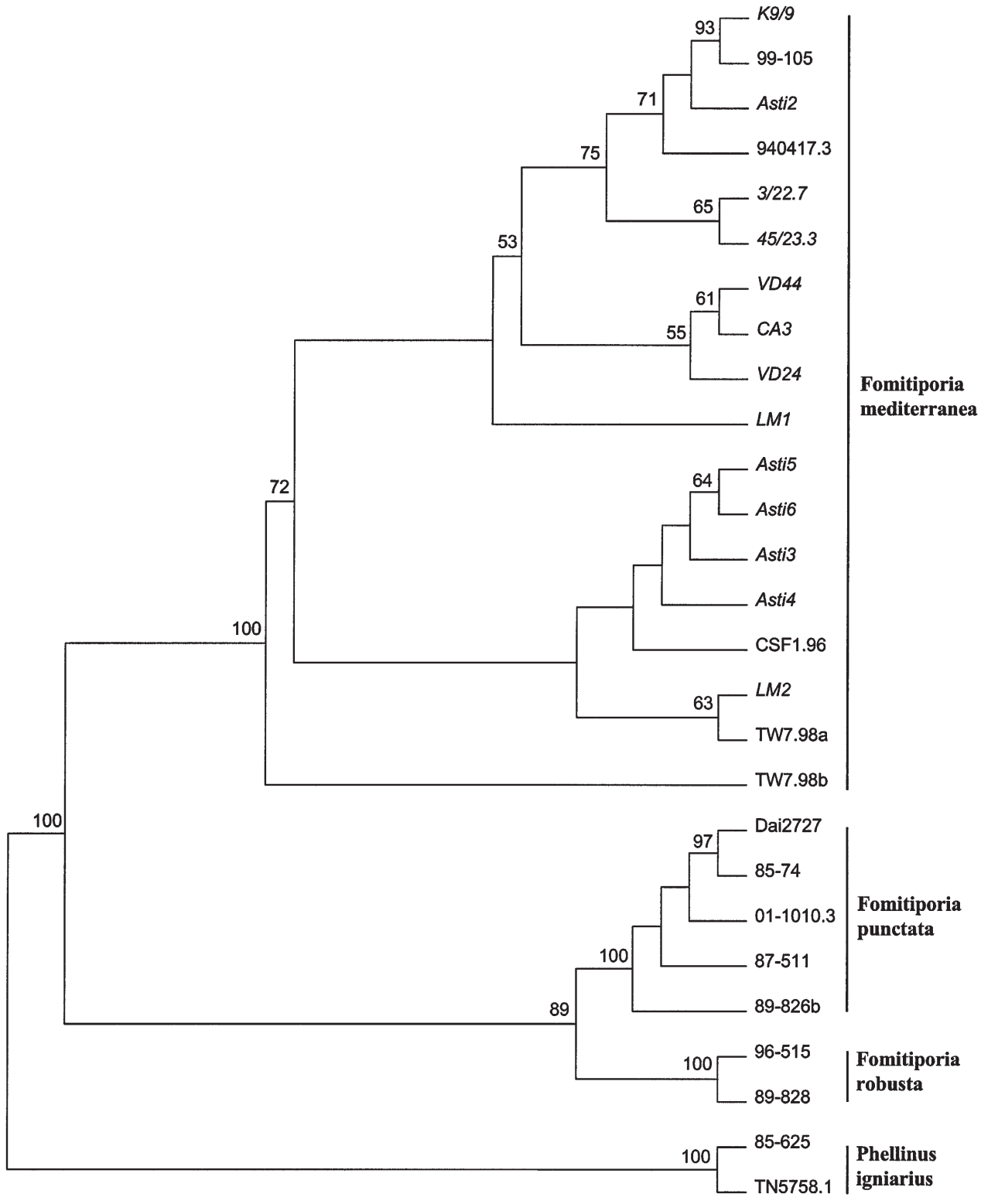

Fig. 2: Strict consensus tree (228 steps) of many most parsimonious trees inferred from the nuclear ITS1-5.8S-ITS2 region. The tree was rooted with the isolates belonging to *P. igniarius*. Bootstrap values of 50% or greater from 1000 bootstrap replications are included for the corresponding nodes. The proposed specific designation is explained in the text. Strains originating from *Vitis* are indicated in italics

Spore germination and pairing tests

Spore germination tests were performed with three strains, namely 3/22 and 45/23, from *Vitis*, and 01-1010, from *Syringa*. Several parameters were found to have a positive effect on spore germination: instead of incubating plates with spore prints at room temperature, they were kept at higher temperatures, preferably 30 °C; after first germination events spores were favourably dispersed with a sterile-filtrated cultural filtrate of the corresponding strain. Application of Ringer´s solution was nearly equally as effective. Germination seemed to be further enhanced when spores were densely packed on the medium, a phenomenon also noted in previous experiments on germination behaviour of xerophilic fungi (BOIDIN 1958). All in all, germination rates still were below 1 % in this study, and it took between 8 d and approximately 4 wk to get first germination.

For the two strains originating from *Vitis* intrastrain pairing of single spore mycelia resulted in three distinct types of reaction: 1) Intermingling of mycelia occurred in selfing reactions, 2) a pigmented line of demarcation was formed in pairings of incompatible reactions ($A=$), and 3) a secondary mycelium developed in pairings of compatible isolates ($A\neq$). Secondary mycelia were formed in the contact zone, overgrowing the paired isolates. In general, formation of secondary mycelia was reduced in intrastrain pairings when compared to compatible interstrain pairings (see below). Single spore mycelia of a single strain segregated into two groups, designated A_1 and A_2. The obtained results correspond well with a unifactorial, bipolar pattern of sexuality.

In strain 01-1010, collected on *Syringa*, no such pattern could be observed. All pairings resulted in slightly antagonistic reactions, and no secondary mycelia were developed. This is in agreement with former observations on the pairing behaviour of *F. punctata* (FISCHER 1996).

In interstrain pairings the three collections fell into two groups, demonstrating complete intersterility. The first group is composed of the two stocks from *Vitis vinifera*; compatibility between these stocks was complete and the pairings resulted in the distinct formation of a secondary mycelium in the contact zone. The two strains from *Vitis* were fully intersterile in pairings with strain 01-1010 (from *Syringa*). In all these pairings, a pigmented line of demarcation (DL) was formed.

In an additional experiment, single spore mycelia of strain 85-74 (from *Salix*), isolated previously, were paired with the testers of the strains above. As demonstrated before (FISCHER 1996), 85-74 exhibits a homothallic mode of reproduction. In the present study, testers of 85-74 formed a DL in pairings with 3/22 and 45/23, indicating a negative reaction. In pairings with 01-1010 the reactions were similar to those desribed for the intrastrain pairings of 01-1010.

These results cleary show that two intersterility groups exist among the strains considered as *F. punctata*.

Fig. 3: *Fomitiporia mediterranea* (strain 3/22, Germany): fruit body formed on 25 year old *Vitis vinifera* cv. Müller-Thurgau

Taxonomic conclusions

The strains enclosed in this study fall into four groups, three of which representing well known species, i.e. *F. punctata*, *F. robusta*, and *P. igniarius*. A fourth group of strains, originally filed under *F. punctata*, is well separated by molecular data, growth behaviour of cultured mycelium, and results of pairing tests. Therefore, a specific taxonomic status is proposed for the isolates collected on *Vitis* and/or originating from Italy:

***Fomitiporia mediterranea* M. Fischer, sp. nov.**

Basidiomata perennia, resupinata; superficies pororum luteobrunnea ad brunnea, pori circulares, 5–8 in quoque millimetro; systema hypharum dimiticum, omnia septa fibulis egentia; hyphae skeletales luteobrunneae, 2.5–4.5 μm latae, hyphae generativae hyalinae, septatae, 2.5–3.5 μm latae; sporae ellipsoideae ad subglobosae, hyalinae, crassitunicatae, cyanophilicae et amyloideae, 6–7 x 5–6 μm.

Holotypus 45/23 in REG, collectus a M. Fischer, in *Vitis vinifera* in Germania, 2001.

Characters of the fruit bodies (Fig. 3)

Fruit bodies resupinate, inseparable, woody hard; up to 15 mm thick; margin narrow, yellowish brown. Pore surface yellowish brown to pale brown; pores circular, 5–8 per mm, dissepiments

thick, entire. Context golden brown, up to 2 mm thick; tubes concolorous with pore surface, up to 13 mm long. Hyphal system dimitic; septa without clamp connections; tissue darkening with KOH. Skeletal hyphae golden brown, rarely branched, aseptate, 2.5–4.5 µm wide; generative hyphae hyaline, thin-walled, frequently branched, simple septate, 2.5–3.5 µm wide; setae essentially absent, very rarely in the hymenium of one specimen (VD 44), slightly ventricose, 15 x 4.5 µm; spores broadly ellipsoidal to subglobose, hyaline, thick-walled, smooth, dextrinoid and cyanophilous, (5.5)6–7(7.5) x (4.5)5–6 (6.5) µm.

Discussion

The sequences of the nuclear encoded ITS region, growth characteristics of cultured mycelium, and pairing tests of single spore isolates showed that a hitherto unknown basidiomycete taxon associated with esca occurs on *Vitis vinifera*. Besides *Vitis*, this taxon, described here as *F. mediterranea*, can be found on several cultivated plant species in Italy and probably elsewhere in the mediterranean area, but with the data at hand seems restricted to *Vitis vinifera* in the other European wine-growing countries. *Vitis vinifera* has been cited as one of the hosts for *F. punctata* (RYVARDEN & GILBERTSON 1994), but such findings probably are assignable to *F. mediterranea*. The species apparently is well adapted to areas with higher temperatures, which is supported by the tests on mycelial growth rates. At all temperatures tested, *F. mediterranea* showed faster growth than *F. punctata*, and this was most evident under 30 °C and, especially, 35 °C conditions.

In both phylogenetic trees (Figs. 1, 2), *F. mediterranea* forms a well supported extra clade (100 %), whereas *F. punctata*, comprising strains from Central and Northern Europe, is revealed as next related to *F. robusta* (bootstrap values 89 % and 97 %).

The ITS region was largest for the strains of *F. mediterranea,* with 740-744 nucleotides; the fragment was smaller for *F. robusta*, with 725-726 nucleotides, and *F. punctata*, with 719-720 nucleotides. Within *Phellinus s. lat.* Quél. (including *Fomitiporia*), the enlargement of the ITS region has been shown to be combined with more derived characters of the fruit body or the nuclear behaviour (FISCHER 1996, unpublished results). In this way *F. mediterranea* may represent a phylogenetically young taxon. The size increase of the ITS region of *F. mediterranea* is mostly due to several small insertions located in the ITS1 and ITS2 regions. Two insertions, one each for ITS1 and ITS2, were found to be diagnostic, the first one positioned between position 199 and 204, the second one between position 536 and 539.

By morphological and anatomical means, no differentiation was possible between fruit bodies of *F. mediterranea* and *F. punctata*. The latter is generally considered a setae-less species (JAHN 1967, RYVARDEN & GILBERTSON 1994), and essentially the same is true for *F. mediterranea*. Another taxon very closely related to *F. punctata* is *F. pseudopunctata* (A. David et al.) Fiasson, reported from *Cytisus* L., *Erica* L., and *Quercus* L. in a locality of the French mediterranean coast (DAVID, DEQUATRE & FIASSON 1982). However, *F. pseudopunctata* is separated from *F. punctata* and *F. mediterranea* by its numerous setae found in the dissepiments. As a common feature, fruit bodies are always resupinate in all these species, whereas they are usually pileate in the other European members of *Fomitiporia*.

The occurrence of *F. mediterranea* on *Vitis vinifera* as a main substrate is a striking phenomenon, but this might be connected with the comparatively large number of studies dealing with *Vitis*. At least for the mediterranean area there is good evidence that *F. mediterranea* is common on other hosts than *Vitis* as well. More than 100 years ago occurrence of a „*Polyporus*-like" fungus on olive trees was reported by HARTIG (1893) and it was shown afterwards that most likely this statement pointed to *Phellinus punctatus* (PLANK 1980). Wood decay in *Olea europaea* is widely distributed all over southern Europe and, based on the data presented in this study, is probably caused by *F. mediterranea*.

Usually, lignicolous fungi are easy to cultivate and basidiospores germinate readily under appropriate conditions. Single spore mycelia derived from germinated spores can be used as testers in intraspecific and interspecific pairing tests and in this way may help to resolve the delimitations between uncertain taxa (BOIDIN 1986, FISCHER 1987, PETERSEN 1994). As an exception, basidiospores of *Fomitiporia* hardly germinated up to now (FISCHER 1987, 1996). In the present study several hints are presented to obtain improved germination rates for *F. mediterranea* and, partly, *F. punctata*. Intraspecific pairing tests demonstrated the former taxon to be outcrossing, while the latter was confirmed as homothallic. Only recently, the mode of reproduction was described as an important genus-specific character within *Phellinus s. lat.*, and species of *Fomitiporia* were all cited as homothallic (WAGNER & FISCHER 2000). This statement has been weakened by the data obtained in the present study.

In *F. mediterranea*, development and growth potential of secondary mycelium was stronger in interstrain pairings than in intrastrain pairings. In this way mycelia formed via interstrain pairings may prevail under natural conditions, resulting in an even more distinct rate of outcrossing.

With few exceptions, dispersal of lignicolous basidiomycetes is by airborne basidiospores only. Studies on the population structure of *F. mediterranea* (then designated as *F. punctata*) indicate that basidiospores are the likely way of dispersal for this fungus (CORTESI, FISCHER & MILGROOM 2000; for a somewhat differring concept, see MUGNAI, GRANITI & SURICO 1999). Subject to the putative dispersal by airborne spores each individual of *F. mediterranea*, within and outside of vineyards, may act as a potential source of inoculum.

While there is good evidence that *F. mediterranea* is widely distributed in wine-growing countries of southern Europe, its exact geographic distribution and host preferences remain

essentially unsolved. In the meantime the author has accumulated a considerable number of fungal cultures isolated from a wide variety of vine cultivars from all around the world, including South America, Australia, and Europe. It is not known yet to what extent this collection is assignable to *F. mediterranea*, but investigations on a broader scale are presently under way.

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