

**INDOOR FUNGAL DESTROYERS OF WOODEN
MATERIALS – THEIR IDENTIFICATION IN PRESENT
REVIEW**

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ABSTRACT

The wood-destroying fungi traditionally were separated from one another primarily on a basis of their sporocarp and/or strain morphology. Their diversity and simple macro- and

micromorphology of fungal structures have been major obstacles for more rapid progress in this regard. However, over the past two decades, there has been substantial progress in our understanding of genetic variability within traditionally recognized morphospecies. In this study we have overviewed genetic variation and phylogeography of macrofungi, which are important destroyers of wooden materials indoor of buildings. Several morphologically defined species of these fungal destroyers (*Coniophora puteana*, *C. olivacea*, *C. arida*, *Serpula himantioides*) have been shown to actually encompass several genetically isolated lineages (cryptic species). The protective efficacy against cryptic species within traditionally recognized morphospecies through laboratory tests (EN 113) and field trials (EN 252) might be sufficient to better prognosis of decay development in wooden materials for hazard assessment and for proper conservation and management plans.

KEYWORDS: Wood protection, wood-destroying fungi, cryptic species.

INTRODUCTION

Various physical and mechanical properties of wood are changed, obviously impaired, due to its damaging by biological and environmental factors (Reinprecht 2011, 2013). Its damage can be caused by wood-destroying fungi, insects, fire, wind, rain, and air pollution. Wood-destroying fungi are the most frequent biodegradation agents of logs, timber and wood products. Rotten wood has a lower density, worse mechanical properties and its physical properties are changed individually according to the kind of rot (Reinprecht et al. 2007). Therefore, detailed knowledge of decomposition processes does not only aid prognosis of decay development in wooden materials for hazard assessment but also allows the identification of wood decay fungi that can be used for biotechnology processes in the wood industry (Schwarze 2007, Reinprecht 2016).

However, the wood-destroying fungi traditionally were separated from one another primarily on a basis of morphologic characters. Their vast diversity and simple macro- and micromorphology of fungal structures (e.g. hyphae, mycelium, sporocarps) have been major obstacles for more rapid progress in this regard. Moreover, since a sporocarp is seldom present and colonized wood shows frequently no or only meagre surface mycelium, diagnostic features other than those important ones usually provided by the sporocarp and/or mycelium must be sought. However, over the past two decades, there has been substantial progress in our understanding of genetic variability within traditionally recognized wood-destroying fungi. These morphospecies are known to occur as complexes of cryptic species and irrespective of the distance in terms of their phylogenetic relationships, many of them have been identified using molecular markers in recent decades (Gáper et al. 2013). Species complexes are composed of genetically isolated lineages (cryptic species) that are not distinguishable on the basis of purely morphological criteria (Le Gac et al. 2007). Which markers that are most suitable to reveal cryptic fungal species vary among fungal groups. Some analyses indicate that three sequenced loci might be sufficient to separate between cryptic species within indoor wood-destroying macrofungi (Skrede et al. 2012). Balasundaram et al. (2015) provide overview of the ten different DNA markers, two representing mitochondrial DNA (COX and mtSSU) and eight representing nuclear DNA (*gpd*, *hsp*, ITS, LSU, SSU, *rpb2*, *tef* and *tub*). Their study indicates that *rpb2*, *tef*, *tub* and *hsp* may give a good indication about the existence of cryptic species in fungal destroyers of wooden materials. Using recent results in literature data, we aimed to answer the following questions: (1) Are there genetically isolated lineages (cryptic species) within the most important indoor wood-destroying

macrofungi that have not been distinguished based on morphological criteria? (2) Is there a geographical segregation of the different cryptic species?

MATERIAL AND METHODS

All reliable information on the important fungal destroyers of wooden materials was collected and corroborated through the evaluation of literature in libraries and searches in online databases using Google Scholar, SciFinder and Web of Knowledge.

RESULTS AND DISCUSSION

Some important fungal destroyers of wooden materials are genetically heterogeneous fungi

The important fungal destroyers that attack indoor wooden materials are: *Serpula lacrymans* (Wulfen) J. Schröt., *Coniophora puteana* (Schumach.) P. Karst., *Fibroporia vaillantii* (DC.) Parmasto = *Antrodia vaillantii* (DC.) Ryvarden and rarely also *Serpula himantioides* (Fr.) P. Karst., *Serpula pulverulenta* (Sowerby) Bondartsev = *Leucogyrophana pulverulenta* (Sowerby) Ginns, *Leucogyrophana mollusca* (Fr.) Pouzar, *Hydnomerulius pinastri* (Fr.) Jarosch & Besl = *Leucogyrophana pinastri* (Fr.) Ginns & Weresub, *Meruliporia incrassata* (Berk. & M.A. Curtis) Murrill, *Coniophora marmorata* Desm., *Coniophora arida* (Fr.) P. Karst., *Amyloporia sinuosa* (Fr.) Rajchenb., Gorjón & Pildain = *Antrodia sinuosa* (Fr.) P. Karst., *Antrodia xantha* (Fr.) Ryvarden and *Antrodia serialis* (Fr.) Donk (Reinprecht 2008).

As mentioned above, certain traditionally recognized wood-destroying morphospecies or morphological species are known as complexes of cryptic biological species with similar phenotypes. For example, *Laetiporus sulphureus* s.l. comprises some closely related taxa (Vasaitis et al. 2009). Similarly, *Fomes fomentarius* (L.) Fr. has been found to have some closely related ITS lineages/sublineages in the world (Gáper et al. 2016). Some strains of *Fomitopsis officinalis* (Vill.) Bondartsev & Singer have also been sequenced and 16 rDNA ITS sequences of the same show a significant level of sequence divergence between some of them, indicating the existence of cryptic taxa. Even in the case of *Piptoporus betulinus* (Bull.) P. Karst., in which species identification is comparatively easy, the rDNA ITS sequence divergence indicates the presence of distinct clusters within this species (Dresch et al. 2015). These problems also apply to some above-mentioned indoor destroyers of wooden materials, namely *Serpula himantioides*, *Coniophora puteana* and *C. arida*. The dry rot fungus *Serpula lacrymans* is genetically almost homogeneous morphospecies.

Serpula lacrymans

The dry rot fungus *Serpula lacrymans* had been considered a heterogeneous species until recently when the existence of two varieties was showed (Kausserud et al. 2007c, Schmidt and Huckfeldt 2011, Balasundaram et al. 2015). One nonaggressive of them was *Serpula lacrymans* var. *shastensis* (Harmsen) Ginns & M.N.L. Lefebvre, residing naturally in North America. The variety *shastensis* was first proposed by North American mycologists Ginns and Lefebvre (1993). The type of variety was selected by Danish biologist Harsmen as *Merulius lacrymans* var. *shastensis* Harsmen: "Fruitbody thin, resupinate, tough and flexible when dry; pores very coarse; mycelium in pure culture thinner and looser than in the main species. Type specimen: Mt. Shasta, California, USA, July, 19, 1956, leg. W. Bridge Cooke, no. 30312." (Harsmen 1960). The other

aggressive variety was *Serpula lacrymans* var. *lacrymans*, which is known from all continents, both from buildings and a few natural habitats (Kausrud et al. 2007c, Schmidt and Huckfeldt 2011). Only low genetic diversity and geographic structuring has been detected in this aggressive variety (Skrede et al. 2013, Maurice et al. 2014). These two varieties were also shown as two main lineages (cryptic species) by AFLPs analysis (amplified fragment length polymorphisms), DNA sequences and microsatellites (Kausrud et al. 2007c). Nevertheless, nowadays, the dry rot fungus *Serpula lacrymans* is considered a homogeneous taxon (CABI 2016).

Serpula himantioides

Despite the *Serpula lacrymans* homogeneity, in the sister species *Serpula himantioides*, four genetic markers (*tub*, *hsp*, LSU and ITS) and AFLPs analysis gave high support for the delimitation of five different lineages/cryptic species (Kausrud et al. 2006, Carlsen et al. 2011, Balasundaram et al. 2015) named SH1-SH5. Lineage SH1 appears to include South American strains. SH2 and SH3 include North American strains. SH4 include North American and Norway strains. The widespread lineage SH5 has been detected on all continents except for South America and Antarctica (Fig. 1). Moreover, high genetic diversity has been detected in this lineage among the strains from East Asia and North America (Carlsen et al. 2011).



Fig. 1: A worldwide geographical distribution of the *Serpula himantioides* lineages/cryptic species named SH1-SH5, *Coniophora puteana* lineages/cryptic species named CP1-CP3, *C. arida* lineages/cryptic species named CA1-CA5, and *C. olivacea* lineages/cryptic species named CO1-CO6. This distribution map was constructed from a base map of the world from the DIVA GIS Development Team (2011).

Coniophora spp.

The three morphospecies, *Coniophora puteana*, *C. arida* and *C. olivacea* comprise fourteen cryptic species (Balasundaram et al. 2015). They were detected using multi-locus DNA sequencing of the three DNA loci (*tub*, *tef* and ITS), and a good correspondence was observed between these three genetic markers and the ALFP data (Kausrud et al. 2007a,b, Skrede et al. 2012). These analyses revealed the occurrence of three cryptic species within the morphospecies *C. puteana* named CP1-CP3 (Kausrud et al. 2007b, Skrede et al. 2012) in the world, six cryptic species in *C. olivacea* in North America and five in *C. arida* in North America (Kausrud et al. 2007a, Skrede et al. 2012). The *C. puteana* lineage CP3 occurs only in North America, whereas the other two lineages have a wider distribution: the lineage CP1 occurs in North America, Europe and NE Asia and the lineage CP2 in North America, Europe, India and Oceania

(Fig. 1). Moreover, Kausserud with co-workers suggested hybridization among cryptic species in the two morphospecies, *C. puteana* and *C. arida* (Kausserud et al. 2007a, b).

***Amyloporia* and *Antrodia* spp.**

Using multi-locus DNA sequencing of the two DNA loci (ITS and LSU), Ortiz-Santana and her co-workers suggest that *Amyloporia sinuosa*, *Antrodia xantha* and *Antrodia serialis* may represent genetically heterogeneous morphospecies. However, to define these complexes more strains and sequences of additional gene regions are needed in that these morphospecies are poorly represented in their study (Ortiz-Santana et al. 2013).

Strain differences in substrate utilisation and host range have been described for a variety of fungal species. These strain-specific differences have been demonstrated for different strains of some medicinal mushrooms [e. g. *Fomitopsis pinicola* (Sw.) P. Karst. (Basidiomycota), *Ophiocordyceps sinensis* (Berk.) G. H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Ascomycota)], some plant pathogens [e. g. *Piptoporus betulinus* (Bull.) P. Karst. (Basidiomycota), *Fusarium avenaceum* (anamorph of *Giberella avenacea* R.J. Cook, Ascomycota)] and some other fungi (Meng et al. 2013, Sørensen and Giese 2013, Dresch et al. 2015). Moreover, Sørensen and Sondergaard reported the need to use the same yeast extracts brand in secondary metabolite analyses when repeating experiments (Sørensen and Sondergaard 2014). For example, the polysaccharopeptides isolated from different sources of *Trametes versicolor* (L.) Lloyd (wild sporocarp, mycelial biomass of submerged culture, or cultivated sporocarp) differ in composition and physiological activity (Cui and Christi 2003). Finally, Gersonde already in 1958 states that there was strain variation with regard to the sensitivity of *Coniophora puteana* to wood preservatives (Gersonde 1958). Therefore, we assume that the establishment of culture collections of fungal destroyers of wooden materials with different geographical origin is of great importance to study their biological and biochemical characteristics, as well as to estimate the resistance of the treated wood against wood-destroying fungi. In the wood industry, identification of different lineages (cryptic species) can be essential for a better prognosis of decay development in wooden materials for hazard assessment and for proper conservation and management plans.

Methods for identification of genetically isolated lineages (cryptic species) within fungal destroyers of wooden materials

Sporocarp morphology, strain characteristics, phospholipid analysis and immunological assays have been the classical means to identify wood-decaying fungi obtained from indoor wooden materials, but wood decay is also caused by both closely related morphospecies and cryptic species that are very difficult to identify using these means (Reinprecht 2008, Kirker 2014). However, over the last two decades there have been significant advances made for diagnostic not only closely related morphospecies but also to identify the cryptic species within traditional morphological taxa: DNA-based molecular genetics methods and protein-based MALDI-TOF (Matrix-assisted laser desorption/ionization - time of flight) mass spectrometry.

Molecular methods are based mainly on polymerase chain reaction (PCR) amplification and subsequent sequence or fragmentation analysis of selected DNA regions (markers). For resolution at and below the generic level the nuclear ribosomal ITS region is a first candidate. For some groups of fungi, other genes or markers give better resolution at the species level (Balasundaram et al. 2015). The application of these methods for analysis of genetic variability of wood-destroying fungi is documented in the first part of our article. Two other PCR based DNA fingerprinting techniques, capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) and denaturing high-performance liquid chromatography (D-HPLC), were used to

identify *Serpula lacrymans* and to profile wood-rot basidiomycetous fungi in the built environment (Maurice et al. 2011). Råberg et al. (2005) shown that intra-specific sequence variation in the internal transcribed spacer of the rDNA in *Coniophora puteana* allows identification of this species by terminal restriction fragment length polymorphism analysis (T-RFLP). The application of DNA-based molecular tools has overcome limits of traditional methods and classical protein-based methods, allowing for rapid analysis of genetic variability of wood-destroying fungi directly from wood without the need of a time-consuming isolation step.

In recent years a new protein-based method for the analysis of fungal variability emerged - MALDI-TOF mass spectrometry. MALDI-TOF MS analysis is an analytical method based on the fast and precise assessment of the mass of molecules in a variable range of 100 Da to 100 KDa (Cobo 2013). The general principle of MALDI TOF MS revolves around the rapid photo-volatilization of a sample (usually cell protein extracts or whole cells) embedded in a UV-absorbing matrix followed by time-of-flight spectrometry. The biomolecules (mainly proteins and peptides) are then both desorbed and ionized by charge transfer matrix molecules by absorbing the energy of a short laser pulse. The desorbed and ionized molecules are first accelerated in an electrical field and then separated according to their mass, producing a mass spectrum that is characterized by both the mass and the intensity of the ions. The result is a spectral fingerprint that is then searched for in the appropriate database for the identification of the microorganism, comparing with reference spectra (van Belkum et al. 2012). When strains are used, the method is fast, because no complicated sample preparation is required (Ahmad et al. 2012). Schmidt and Kallow (2005) used MALDI-TOF MS for differentiation of mycelia within pairs of the closely related indoor wood decay fungi *Serpula lacrymans*, *S. himantioides*, *Coniophora puteana*, *C. marmorata*, *Fibroporia vaillantii* and *Amyloporia sinuosa* and found the method suitable to identify unknown samples by spectra comparison. In our study we used the MALDI-TOF MS for the analysis of 5 *Fomes fomentarius* strains (Pristaš et al. 2017).

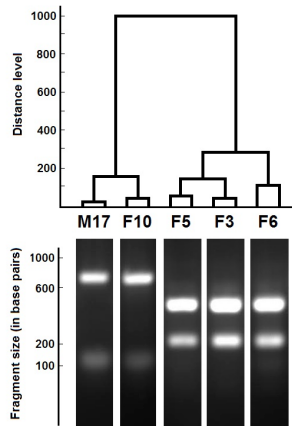


Fig. 2: Top - Dendrogram showing the relatedness of *Fomes fomentarius* sublineage A2 (strains M17, F10) and *F. fomentarius* lineage B (strains F5, F3, F6) based on comparison of mass spectra obtained by MALDI-TOF MS analysis.

Bottom - species-specific profiles of digestion of amplified ITS sequences of the same strains obtained by ITS-PCR-RFLP analysis.

Within the northern hemisphere in North America, North Africa, Asia, and all over Europe, *F. fomentarius* belongs to the common fungal destroyers in broadleaved logs in the forest and at a sawmill (Júdová et al. 2012, Reinprecht 2008). The existence of two cryptic species, namely the sublineage A2 and the lineage B, among its strains has been clearly established in Europe (Júdová et al. 2012, Pristaš et al. 2013, Gáper et al. 2016). The patterns observed by species-specific profiles of digestion of ITS sequences of each strain obtained by ITS-PCR-RFLP analysis are compatible with MALDI-TOF mass spectrometry (Fig. 2).

CONCLUSIONS

The dry rot fungus *Serpula lacrymans* is almost genetically homogeneous species. Only low genetic diversity and geographic structuring has been detected in the morphospecies. These morphological species have been shown to actually encompass several genetically isolated lineages (cryptic species): *Serpula himantioides*, *Coniophora puteana*, *C. arida* and *C. olivacea*. Recently, some authors suggest that *Amyloporia sinuosa*, *Antrodia xantha* and *A. serialis* may also represent genetically heterogeneous morphotaxa.

There is a geographical segregation of the different *Serpula himantioides* lineages named SH1 - SH5. Lineage SH1 includes strains from South America, SH2 and SH3 include North American strains, SH4 includes strains from North America and Europe, and SH5 includes strains from all continents except for South America and Antarctica. Similarly, the *Coniophora puteana* lineage CP3 occurs only in North America, whereas the other two lineages have a wider distribution: the lineage CP1 occurs in North America, Europe and NE Asia and the lineage CP2 in North America, Europe, India and Oceania.

Over the last two decades there have been significant advances made for identification of cryptic species: molecular genetics methods and MALDI-TOF mass spectrometry.

We assume that the identification of different lineages (cryptic species) can be essential for wood protection and use of wooden materials.

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