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## Review

# Evolutionary origin, worldwide dispersal, and population genetics of the dry rot fungus *Serpula lacrymans*

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## ABSTRACT

Recent research on the evolution, phylogeography and population genetics of the dry rot fungus *Serpula lacrymans* is reviewed. The fungus causes severe damage to construction wood in temperate regions worldwide. Multi-locus genetic analyses have shown that *S. lacrymans* includes two cryptic species currently referred to as var. *shastensis* and var. *lacrymans*. Both lineages occur naturally in high altitude regions, but var. *lacrymans* has also spread from its natural range in Asia, and established itself in indoor environments in temperate regions worldwide. Japan was apparently colonized independently from Europe, North and South America by var. *lacrymans*. The population found in Australia and New Zealand seems to represent a mixture of the Japanese and the Euro-American lineages. Little genetic variation exists in the founder populations of var. *lacrymans* worldwide. Due to the introduction of a limited number of vic (vegetative incompatibility) alleles into the founder populations, genetically different individuals often cannot recognize self from non-self through the vegetative incompatibility response. Moreover, only a low number of MAT (mating) alleles seem to be present in Europe. Genetic analyses of the European and Japanese indoor populations have shown that *S. lacrymans* mainly spreads sexually via basidiospores. Surprisingly, an excess of heterozygotes has been observed in the founder populations. This could be due to heterozygote advantage caused by a limited number of MAT alleles. Recent analyses of the *S. lacrymans* genome provide new opportunities for further research on the dry rot fungus.

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## 1. Introduction

The dry rot fungus *Serpula lacrymans* is among the economically most damaging fungi due to its ability to infest and cause aggressive brown rot decay of wooden construction materials in temperate regions worldwide (Jennings and Bravery, 1991; Schmidt, 2006). In buildings, humans have recreated its

required conditions for growth and made it a successful invasive species on a global scale. Favorable conditions for growth of mycelia seems to be a high degree of humidity, low air flow, and a temperature below 25 °C, conditions which are usually met in cellars in temperate regions. The dry rot fungus has apparently been associated with human civilization for at least several hundred years, probably more (Kausserud et al.,

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2007). Some of the first reliable reports are from wooden sailing vessels in the 17th century, where it presumably caused severe damages (Ramsbottom, 1937). These damages should not be confused with those caused by *Laetiporus sulphureus*, a fungus which might have been an even more severe destroyer of wooden ships.

Much research has been conducted during the last 200 y on the biology of *S. lacrymans*. Richard Falck initiated much of this work (e.g. Falck, 1912). It is assumed that *S. lacrymans* has a regular basidiomycete life cycle starting with a presumed short-lived monokaryotic mycelium. After dikaryotization heterokaryotic hyphae with clamps are formed making up the dominant phase of the life cycle. Both the mono- and the dikaryotic mycelia can form simple oidia for asexual dispersal when growing under unfavorable conditions, which may be an 'escape response'. The dikaryotic hyphae are able to transform into different types of hyphae, including vessel (conducting), tendril and fiber hyphae. The mycelium can form thick strands for the transport of nutrients and water, and can grow several meters across inorganic materials (Fig. 1). The general range for mycelial growth is between 3 °C and 26 °C with an optimum of 21/22 °C (Jennings and Bravery, 1991). This preference for relatively low temperatures

probably limits both the worldwide geographical distribution as well as its spatial distribution within buildings (Hegarty, 1991). In buildings, *S. lacrymans* is able to produce thick, crust-shaped fruit bodies, with rust-colored spores (Fig. 1) during the entire growing season. Fruit body size varies from a few centimeters to more than a meter in diameter and it has been calculated that  $5 \times 10^8$  spores can be produced by a 1 m<sup>2</sup> fruit body during 10 min (Falck, 1912).

During the last 10 y, numerous genetic studies of *S. lacrymans* have been conducted (Engh et al., 2010a, 2010b; Kauserud et al., 2006a, 2006b, 2007; Palfreyman et al., 2003; Skrede et al., 2011; White et al., 1997, 2001). In this review we sum up this research with a main emphasis on the evolutionary origin, phylogeography and population genetics.

## 2. Phylogeny of *S. lacrymans* and its allies

*S. lacrymans* belongs to the family Serpulaceae, apparently one of the earliest diverging lineages in Boletales (Binder and Hibbett, 2006; Binder et al., 2010). Phylogenetic analyses by Binder and co-workers indicate that saprotrophy is the plesiomorphic state in Boletales and that several bolete lineages



**Fig. 1 – Growth habits of *Serpula lacrymans*. (A) Fruit bodies and mycelia of var. *lacrymans* in a building. (B) Rhizomorphs/hyphal cords of var. *lacrymans*. (C) Fruiting of var. *lacrymans* in nature in the Czech Republic. (D) Fruiting of var. *shastensis* in nature. (E) Typical substrate of var. *shastensis* at Mount Lassen, California.**

have independently adapted to ectomycorrhizal (ECM) symbiosis. Traditionally, Serpulaceae was considered a strictly saprotrophic family, but molecular phylogenetic analyses have shown that the ECM-forming genera *Austropaxillus* and *Gymnopaxillus* are nested within *Serpula* (Binder and Hibbett 2006; Jarosh 2001; Skrede et al., 2011). *Austropaxillus* and *Gymnopaxillus* form ECM with roots of *Nothofagus* and *Eucalyptus* trees and are known only from the temperate Southern Hemisphere. In Fig. 2, a schematic phylogenetic tree of Serpulaceae is shown based on the multi-locus phylogenies published in Skrede et al. (2011). According to Binder and Hibbett (2006) and Skrede et al. (2011) *Austropaxillus*/*Gymnopaxillus* forms a monophyletic sister to the *S. lacrymans*/*Serpula himantoides* group, making *Serpula* paraphyletic as currently circumscribed.

*S. himantoides* and *S. lacrymans* are sister taxa and form a monophyletic group (Fig. 2). The taxonomic circumscriptions of the saprotrophic *S. himantoides* and *S. lacrymans* have varied through time: they have been lumped as one based on morphology (Cooke, 1957) or recognized as two separate species based on mating experiments (Harmsen et al., 1958). *S. lacrymans* was further subdivided into two lineages representing the var. *lacrymans* and var. *shastensis* (Harmsen, 1960). The morphotaxon *S. himantoides* includes probably five cryptic species, most of them with a primary affinity to southern South America and North America (Carlsen et al., 2011).

A clade comprising the saprotrophic species *Serpula incrassata*, *Serpula pulverulenta* and *Serpula similis* (Fig. 2) has been considered a sister to all other Serpulaceae (Skrede et al., 2011). *S. incrassata* seems to have a strictly North American distribution (Cooke, 1957). The less studied *S. pulverulenta*, which also appears in houses, has at least a European and probably also a North American distribution (Harmsen, 1954; Christiansen, 1960). The branch leading to *S. similis* is very long (Skrede et al., 2011), indicating an increased rate of molecular evolution in this lineage. *S. similis* is known from tropical regions in Africa and Southeast Asia, where it is found on bamboo and hardwoods (Carlier et al., 2004; Ginns, 1971).

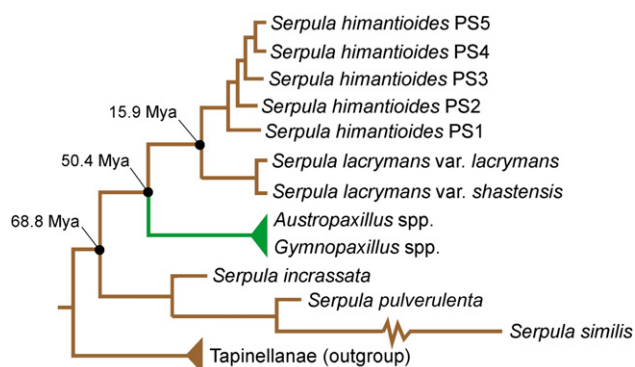


**Fig. 2 – A schematic phylogenetic tree of Serpulaceae based on results from Skrede et al. (2011) and Carlsen et al. (2011). Brown branches reflect saprotrophism while the green branch reflects an ectomycorrhizal nutritional mode. Approximate divergence times of three nodes are superimposed (Skrede et al., 2011).**

### 3. Historical biogeography of *S. lacrymans*

According to estimated divergence times in Skrede et al. (2011), Serpulaceae diverged from the Tapinellanae during the Late Cretaceous (Fig. 2). The mean crown-group age of Serpulaceae was estimated to be between 80 and 58 MyBP (Skrede et al., 2011). This is congruent with the estimated origin of the Gymnosperm genus *Pinus* (evolved between 155 and 87 MyBP; Won and Renner, 2006), which seems to be the main substrate of Serpulaceae in the Northern Hemisphere. However, more than 20 My years differ between the estimates from two studies that have dated the split between the ECM group *Austropaxillus*/*Gymnopaxillus* and the brown rot species *S. lacrymans*/*S. himantoides* (35 MyBP, Eastwood et al., 2011; 57 MyBP, Skrede et al., 2011). Hence, including the 95 % highest node density values for both studies, we can only state that the clades diverged some time between 66 and 15 MyBP (Eastwood et al., 2011; Skrede et al., 2011). Fossil evidence of an ECM root-tip supports ECM fungi having evolved at least 50 MyBP (LePage et al., 1997). The divergence between *S. lacrymans* and *S. himantoides* is estimated to have occurred in the Miocene; the mean age estimate being 16 MyBP.

North America was suggested as the most likely ancestral area for Serpulaceae. This was followed by the expansion of the *Austropaxillus*/*Gymnopaxillus* clade to South America and further to Australia/New Zealand. The *S. lacrymans*/*S. himantoides* clade probably occurred in North America during the Early Eocene to Mid Miocene and then spread across the Beringian land bridge to Asia and Europe. *S. himantoides* is also present in South America, and long distance dispersal is needed to explain the expansion of *S. himantoides* and the *Austropaxillus*/*Gymnopaxillus* clade to South America during this time period (Skrede et al., 2011).

The divergence of *S. lacrymans* var. *lacrymans* and *S. lacrymans* var. *shastensis* occurred between 13 and 4 MyBP (9 MyBP, Skrede et al., 2011). The distribution of var. *shastensis* mainly in North America and of var. *lacrymans* in eastern Eurasia supports a trans-Beringian distribution with subsequent vicariance. The continuous boreal forest across Beringia from about 14 to 3.5 MyBP suggested by Sanmartín et al. 2001 supports the vicariance hypothesis.

### 4. The geographic range of *S. lacrymans* in nature and man-made habitats

*S. lacrymans* is found in buildings in temperate regions in Eurasia, North and South America, as well as in Oceania (Australia/New Zealand). In obvious contrast to the frequent indoor occurrence of *S. lacrymans*, its absence in nature has remained an enigma for many years. Bridge Cooke (1943) reported *S. lacrymans* var. *himantoides* from Mt. Shasta in California based on material redescribed by Harmsen (1960) as *S. lacrymans* var. *shastensis*. Bridge Cooke (1943) described it as common in the Mt. Shasta region, and this has been confirmed by Kauserud and collaborators, who found it frequently at the forest limit on Mt. Shasta and Mt. Lassen. Bagchee (1954) revisited an area in Himachal Pradesh in the Himalayas from where *S. lacrymans* had been reported from

1929 to 1946. He found it in three localities in the south-western Himalayas and further records from the area were added by Rattan (1977) and Bech-Andersen (1993). Ahmad (1972) added a few records from north-eastern Pakistan. Some of these findings have been confirmed later to belong to var. *lacrymans* (Kauserud et al., 2007). Knudsen's experience from the Russian expeditions indicates that it is a rare species in these regions, found only once in an area with many suitable trunks where it was intensively searched for. Bech-Andersen and collaborators tried unsuccessfully three times

to find it in the Pakistani (1983), Indian (1987) and Chinese (1990) Himalayas, but finally succeeded in India in 1992 (Bech-Andersen, 1993). Based on the recent number of additional, unpublished records by Knudsen et al. from Siberia, Parmasto from Caucasus and Xiao-Lan from China, a clearer picture of its ecology and distribution in nature is emerging (Table 1).

*S. lacrymans* seems to be a Holarctic species complex. In Eurasia it is distributed from Far East Russia (134°E) to the Caucasus (41°E) and from central Asia (53°N) to southern

**Table 1 – Information about localities where *Serpula lacrymans* has been found in nature**

| Country  | Locality                                    | Coordinate        | Altitude m | Date       | Host                                              | Collector                    |
|----------|---------------------------------------------|-------------------|------------|------------|---------------------------------------------------|------------------------------|
| USA      | California, Mt. Shasta                      | 41°18'N, 122°18'W | 1400–2700  | 1917       |                                                   |                              |
| USA      | California, Mt. Shasta                      | 41°18'N, 122°18'W | 2500       | 19.07.1956 | <i>Abies magnifica</i> var. <i>shastensis</i>     | Cooke 30312                  |
| USA      | California, Lassen Volcanic Nat. Pk         | 40°29'N, 121°30'W | 2300       | 04.07.1965 | <i>Abies magnifica</i> var. <i>shastensis</i>     | Cooke 35010                  |
| USA      | California, Mt. Shasta                      | 41°18'N, 122°18'W | 2600       | 18.07.1966 | <i>Abies magnifica</i> var. <i>shastensis</i>     | Cooke 37279                  |
| USA      | California, Lassen Volcanic Nat. Pk         | 40°29'N, 121°30'W | 2300       | 19.07.1956 | <i>Abies magnifica</i> var. <i>shastensis</i>     | Cooke 30401                  |
| USA      | California, Mt. Shasta                      | 41°18'N, 122°18'W | 1900       | 19.07.1966 | <i>Abies concolor</i>                             | Cooke 37306                  |
| India    | Himachal Pradesh, Kullu, Parbati Rg., Pulga | 31°59'N, 77°26'E  | 2500–3500  | Oct. 1929  | <i>Abies pindrow</i>                              | Unknown                      |
| India    | Shimla Hills, Narkanda, Khanoti forest      | 31°06'N, 77°10'E  | 3000       | 15.06.1946 | <i>Abies pindrow</i>                              | Bagchee                      |
| India    | Uttar Pradesh, Mundali, Lakho               | 30°45'N, 77°48'E  | 2500–3500  | 13.10.1943 | <i>Picea</i> or <i>Abies</i>                      | Bagchee                      |
| India    | Himachal Pradesh, Manali                    | 32°16'N, 77°10'E  | 2500–3500  | 25.09.1940 | <i>Abies pindrow</i>                              | Bagchee, KB-102              |
| India    | Himachal Pradesh, Kullu, Dhara, Pulga       | 31°59'N, 77°26'E  | 2500–3500  | 21.10.1940 | <i>Picea smithiana</i> , <i>Pinus wallichiana</i> | Bagchee                      |
| India    | Himachal Pradesh, Bashahar, Narkanda        | 31°27'N, 77°45'E  | 3000       | 06.06.1946 | <i>Abies pindrow</i>                              | Bagchee                      |
| India    | Himachal Pradesh, Bashahar, Narkanda        | 31°27'N, 77°45'E  | 3000       | 13.06.1946 | <i>Abies</i> sp., <i>Picea</i> sp.                | Bagchee                      |
| India    | Himachal Pradesh, Kullu, Parbati Rg., Pulga | 31°59'N, 77°26'E  | 2500–3500  | 13.10.1952 | <i>Picea smithiana</i>                            | Bagchee                      |
| India    | Himachal Pradesh, Bashahar, Iranda          | 31°27'N, 77°38'E  | 2500–3500  | 17.10.1941 | <i>Picea smithiana</i>                            | Bagchee                      |
| India    | Himachal Pradesh, Kullu, Parbati Rg.        | 31°57'N, 77°06'E  | 2500–3500  | 20.10.1952 | <i>Pinus wallichiana</i>                          | Bagchee KB-87, 90            |
| India    | Jammu & Kashmir, Gulmarg, Ningel Nullah     | 34°05'N, 74°38'E  | 2500–3000  | 26.08.1972 | <i>Abies pindrow</i>                              | Rattan RSD 6667              |
| India    | Himachal Pradesh, Bashahar, Narkanda        | 31°27'N, 77°45'E  | 3000       | 21.08.1992 | Old conifer stump                                 | Unknown                      |
| Pakistan | Khyber-Pakhtunkhwa, Swat, Kalam             | 34°47'N, 72°22'E* | 2000       | 23.08.1952 | <i>Cedrus deodara</i>                             | Ahmad                        |
| Pakistan | Khyber-Pakhtunkhwa, Mansehra                | 34°20'N, 73°12'E* | 650–2000   | 25.08.1959 | <i>Cedrus deodara</i>                             | Ahmad                        |
| China    | Xinjiang, Tian Shan                         | 41°17'N, 80°13'E  | up to 7400 | 22.06.1977 | Unknown                                           | Mao Xiao-Lan & Wen Hua-An 20 |
| Russia   | Altay Rep., Teletskoye Lake, Kyga River     | 51°21'N, 87°51'E  | 440        | 13.08.2001 | <i>Abies sibirica</i> , <i>Pinus sibirica</i>     | Knudsen                      |
| Russia   | Far East, Alin Sichote Mts., Lazovsky Res.  | 43°16'N, 134°03'E | 360        | 06.08.2003 | <i>Pinus koraiensis</i>                           | Mukhin                       |
| Russia   | Western Sayan Mts., 10 km S. of Tanzybey    | 53°03'N, 92°54'E  | 450        | Aug. 04    | <i>Pinus sibirica</i>                             | Knudsen                      |
| Russia   | Caucasus, Karachaevo-Cherkesskaya AO        |                   | 1900–2000  | 26.09.1968 | <i>Abies nordmanniana</i>                         | Parmasto 53341               |
| Russia   | Caucasus, Karachaevo-Cherkesskaya AO        | 43°27'N, 41°45'E  | Unknown    | 26.09.1968 | <i>Abies nordmanniana</i>                         | Parmasto                     |

Himalaya (30°N). In North America *S. lacrymans* var. *shastensis* is only known from the Cascade Mountains in south-western USA (40–41°N, 121–122°W). It is absent from tropical, subtropical, arctic and subarctic areas. Further, it is also absent from boreal areas, although both *Picea abies* and *Pinus sylvestris* could potentially act as hosts. The natural habitats are generally at high elevations in mountains, mostly >2000 m, except for the northernmost records from Russia, which vary from 4 to 600 m. No records are known from lowlands. The localities are often shaded northern- or western-facing slopes with a deep snow-cover in wintertime and remaining cool during summertime due to the high elevations, with maximum temperatures not averaging much more than 20 °C. In wintertime the temperatures may be very low, but this is compensated by the thick snow-cover acting as a protecting buffer against strong, desiccating, cold winds.

*S. lacrymans* occurs only on conifers. Known hosts include *Abies concolor*, *Abies magnifica*, *Abies nordmanniana*, *Abies pin-drow*, *Abies sibirica*, *Cedrus deodara*, *Picea smithiana*, *Pinus koraiensis*, *Pinus sibirica* and *Pinus wallichiana*. Species of *Abies* seem to be especially important hosts. In some areas *Abies* is the only host, in other areas it is mixed with other hosts, but no records are known outside the natural distribution of *Abies*. Hence, a possible distribution can be postulated based on the coherent distribution of *Abies*-species between 53° and 31°N. Even the few colonizations into European forests (Czech Republic) from indoor populations occur within the natural distribution of *Abies alba*, although only one record is from this host here (Czech Republic), the majority being from *Picea abies* (Kotlaba, 1992). *S. lacrymans* has mainly been found on large trunks in nature, often >1 m in diameter at the base. The fruit bodies appear most often on the underside, often near the base of the trunk or among the roots. The fruit bodies in the trunks are often connected to the soil with rhizomorphs or mycelia, possibly transporting water and nutrients to the active mycelia.

## 5. Does *S. lacrymans* include two species?

Specimens of *S. lacrymans* from California were ascribed to var. *shastensis* while all other collections, mainly from buildings in Europe, were ascribed to var. *lacrymans* (Harmsen, 1960). One collection (Parmasto) from high altitudes in the Caucasus also showed affinity to var. *shastensis* based on genetic analyses (Kausserud et al., 2007). The single occurrence of var. *shastensis* in the Caucasus is puzzling, but indicates that it may have an unrecognized wide and coherent or somewhat patchy natural distribution in mountainous regions in the Northern Hemisphere. However, this finding should be confirmed as this sequence was generated using a nested-PCR approach which involves higher risks for contamination. The *shastensis* variety has, in contrast to var. *lacrymans*, never been detected in buildings. Morphologically, the fruit bodies produced by var. *shastensis* are slightly thinner than those of var. *lacrymans*. Moreover, thicker mycelial strands are normally produced by var. *lacrymans* (Harmsen, 1960). Whether these morphological differences are genetically based or just reflect phenotypic plasticity is, however, unknown.

Employing various genetic markers, Kausserud et al. (2007) demonstrated that the two varieties are highly differentiated genetically; the independent genetic markers consistently split them into two sister groups. Mating compatibility in terms of prezygotic clamp connections between primary (monokaryotic) mycelia has been observed between the two lineages (Harmsen, 1960; Kausserud et al., 2007). However, no germinating spores were obtained from the fruit bodies that were formed *in vitro* (Kausserud et al., 2007). Moreover, in connection with the latter study, dikaryotic mycelia of the two varieties as well as the 'hybrid dikaryon' were grown *in vitro* in microcosms (Fig. 3). Interestingly, the hybrid dikaryon grew significantly worse than the var. *lacrymans* and var. *shastensis* dikaryons (Fig. 3). The large genetic difference between the two varieties, the significant morphological

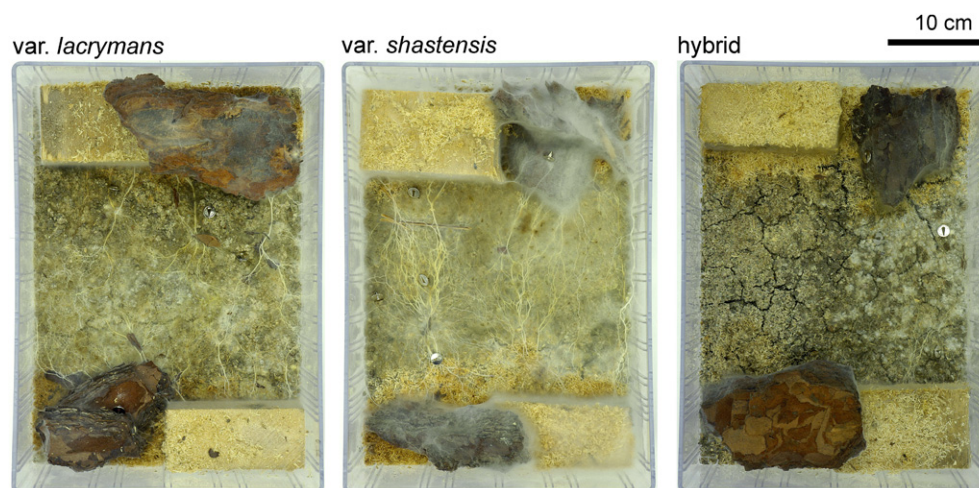


**Fig. 3** – Microcosms studies of growth of dikaryotic strains of *Serpula lacrymans* var. *lacrymans*, var. *shastensis* and their hybrid. The strains were inoculated on the wooden cubes and bark of *Pinus sylvestris* using agar plugs with growing mycelia. The boxes were kept at room temperature (about 25 °C) for 4 weeks. The two parental strains showed a far better growth than the hybrid, possibly reflecting prezygotic mating barriers.

differentiation between them, the lack of hybrids or introgressed genotypes in field material, and the poor *in vitro* growth ability of the hybrid dikaryon suggest that *var. shastensis* and *var. lacrymans* are reproductively isolated species. Hence, some unrecognized pre- and postzygotic barriers may exist between the two forms.

## 6. Phylogeography of *var. lacrymans*

For a long time the location from which *S. lacrymans* originated, spread out and colonized the human domain was enigmatic. Findings in the Himalayas led to the suspicion that the source population for the European population resides there. White *et al.* (1997, 2001) were able to obtain a culture of *S. lacrymans* from the Himalayas and genetic analyses demonstrated that it belonged to *var. lacrymans* (see also Palfreyman *et al.*, 2003). Kauserud *et al.* (2007) analyzed the genetic variation among collections of *var. lacrymans* from buildings worldwide, as well as from various natural habitats using multiple DNA sequence loci, AFLPs and microsatellites (Högberg *et al.*, 2006). A tentative phylogeographic migration scenario for *var. lacrymans*, based on the findings in Kauserud *et al.* (2007) is depicted in Fig. 4.

Most genetic variation appeared among specimens collected from the natural environment in northeast Asia, and it was concluded that the species has its natural distribution there, from where it has spread out in connection with human activities (Kauserud *et al.*, 2007). The indoor Japanese population has a unique genetic composition and probably

represents an independent dispersal event (Kauserud *et al.*, 2007). Indoor collections from Europe, northeastern parts of North America, South America (one collection from Peru) and southern Australia/New Zealand (referred to as the 'Cosmopolitan group' in Kauserud *et al.*, 2007) were genetically highly homogeneous, suggesting a common source. Additional collections from South America (Chile) which have recently been analyzed genetically confirmed their belonging to the 'Cosmopolitan group' (unpublished). One tentative scenario is that the 'Cosmopolitan lineage' first spread to Europe, from where it was transported to other continents on infected wood. One possible way of dispersal was probably wooden sailing vessels, where *S. lacrymans* was reported to be a problem (Ramsbottom, 1937). Interestingly, some Asian/Japanese alleles were also recorded among the specimens from Australia/New Zealand, indicating two independent migrations followed by admixture (Kauserud *et al.*, 2007). Specimens of *var. lacrymans* collected in nature in the Czech Republic (Fig. 1c) were also analyzed in Kauserud *et al.* (2007) and turned out to be genetically highly similar to the indoor population. One may speculate that if a naturally established European population exists, a higher genetic variation should be expected. It was therefore hypothesized that the Czech collections represent re-naturalized specimens that had spread from neighboring buildings (Kauserud *et al.*, 2007). Specimens collected in nature in the Himalayas were genetically the most similar to the 'Cosmopolitan group', supporting the earlier speculations about the Himalayas as a source for the European population (Kauserud *et al.*, 2007).

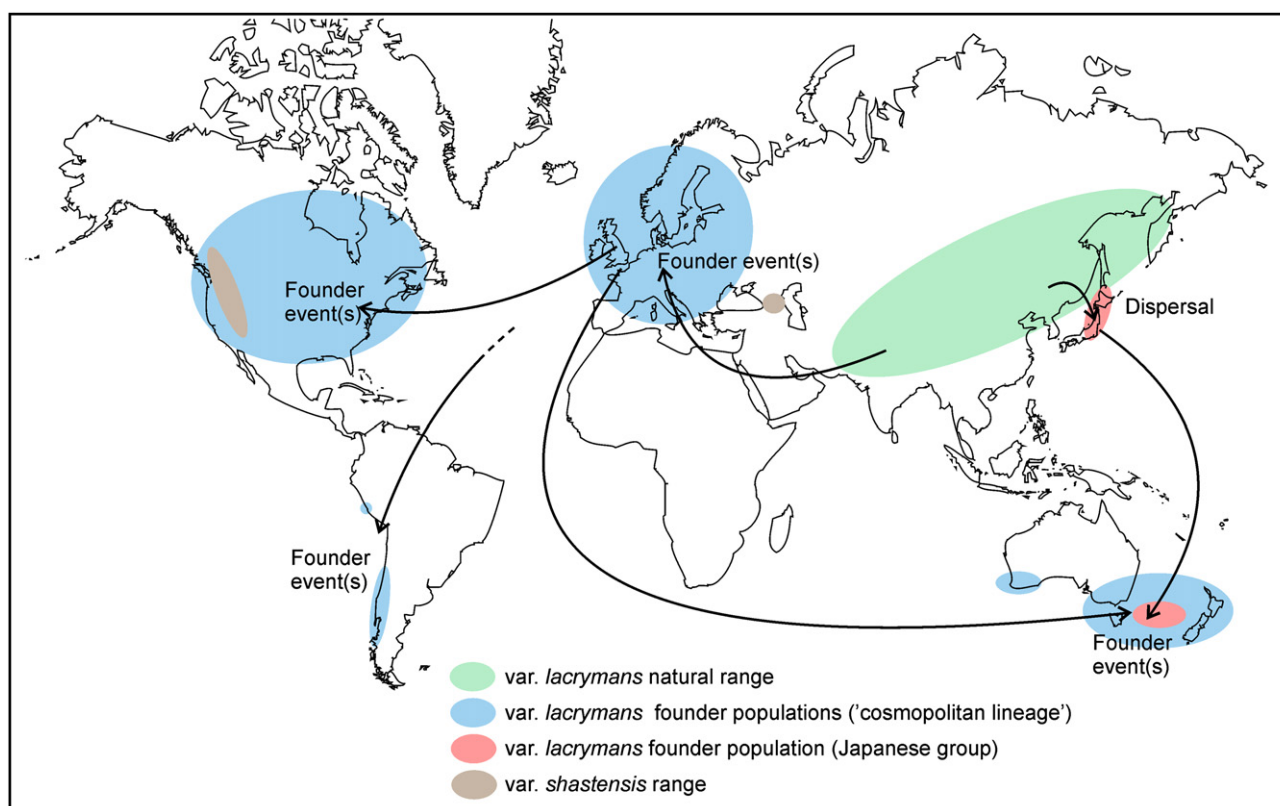


**Fig. 4** – Approximate distribution of *Serpula lacrymans* and hypotheses for worldwide spread-out based on Kauserud *et al.* (2007). The natural range of *var. shastensis* might be far wider than hitherto observed.

## 7. Population genetics of indoor var. *lacrymans* populations

Large indoor populations of *S. lacrymans* have established in buildings in Europe and Japan (Horisawa et al., 2004). The population genetic structures of these two populations have been investigated using various genetic markers, including microsatellites (Högberg et al., 2006; Engh et al., 2010a). Higher levels of genetic variation were found in Japanese populations compared to Europe (Engh et al., 2010a), indicating a higher gene flow and rate of dispersal from the Asian mainland population to Japan compared to Europe. Moreover, a clearer geographic sub-structure was also observed in Japan compared to Europe. This could indicate that the Japanese population was established earlier, enabling more differentiation to take place between Hokkaido and Honshu. Although low levels of genetic variation exist, the genotype distributions in both populations were largely in accordance with Hardy–Weinberg expectations and linkage equilibrium across loci was mainly observed (Engh et al., 2010a). These results indicate that sexual recombination and spread by basidiospores dominates. Hence, clonal or vegetative dispersal has seemingly little impact on shaping the population genetic structure in these two large indoor populations. Noteworthy, excess of heterozygotes were observed in several microsatellite loci, which is normally not expected. One reason for this could be the presence of a low number of MAT alleles (see below) enabling only mating between a certain proportion of genetically divergent homokaryons. In natural populations of basidiomycetes a high number of MAT alleles are normally present (Heitman et al., 2007) meaning that most monokaryons (from different fruit bodies) will be able to form dikaryons. Other reasons could be the presence of more than two different nuclei in the mycelia, or alternatively, close linkage between the analyzed microsatellite loci and loci governed by frequency dependent selection such as the MAT and vic loci (see below).

## 8. The distribution of VC types in var. *lacrymans*

In fungi, the vegetative incompatibility response is used to recognize self from non-self mycelia (Worrall, 1997). The response might be important for resource capture and hindering other mycelia from invading own territory. Moreover, it might also be important to limit the transmission of pathogens, e.g. mycoviruses (Cortesi et al., 2001). Theoretically, the vic loci are governed by frequency dependent selection due to rare allele advantage, because the possession of rare vic alleles to a higher extent enables recognition of non-self. In basidiomycetes, the established secondary mycelia must possess identical alleles at all vegetative incompatibility loci to be vegetatively compatible (Malik and Vilgalys, 1999). Hyphal anastomosis between individuals, which have alternative allelic specificities at any vic locus, usually results in compartmentation and death of the hyphal fusion cell.

The appearance of widespread VC types in *S. lacrymans* was first demonstrated by Kauserud (2004). Further, among 75

analyzed dikaryons, the vegetative incompatibility responses could largely be explained by a vegetative incompatibility system governed by two biallelic vic loci (Kauserud et al., 2006b). A basidiomycete population with two polymorphic biallelic vic loci allows nine different allelic combinations and hence, nine VC types. This is sufficient to explain the diversity of VC types in the 75 field isolates of var. *lacrymans* investigated in Kauserud et al. (2006a), where eight VC types were observed. In theory, the introduction of one single *S. lacrymans* dikaryon into Europe, being heterozygous in both vic loci, could account for the entire vic allele diversity.

A higher VC type diversity was observed in Japan (Engh et al., 2010a), where most confrontations among dikaryons resulted in the formation of incompatibility zones. This corresponds well with the independent observations of a higher genetic variation (as observed with DNA sequences, AFLPs and microsatellites) in Japan versus Europe (Kauserud et al., 2007; Engh et al., 2010a). The low diversity of vic alleles in Europe confirms that this population was established through a founder event. The introduction of new vic or MAT alleles into Europe may increase the fitness of the European *S. lacrymans* population. In the Dutch elm disease fungus *Ophiostoma novo-ulmi*, selective acquisition of MAT and vic alleles have occurred through interspecific crosses with the sister species *Ophiostoma ulmi*, probably increasing the fitness of the European *O. novo-ulmi* population (Paoletti et al., 2006).

## 9. Diversity of mating alleles in var. *lacrymans*

In general, basidiomycetes have either a bipolar or a tetrapolar mating system (Fraser et al., 2007). *S. lacrymans* possess a tetrapolar mating system (Kauserud et al., 2006b; Schmidt and Moreth-Kebernik, 1991). Hence, for two primary mycelia of *S. lacrymans* to mate they need to have different MAT alleles (mating types) at both loci. The two different loci are often termed MAT A and MAT B. MAT A locus encodes homeodomain transcription factors important for clamp connection formation. The MAT B locus encodes pheromone and pheromone reception genes that are important for recognition of appropriate mate. Together they control the nucleus pairing and movement in the cells during mating (see Brown and Casselton, 2001; Heitman et al., 2007). As for the vic loci, the MAT loci are thought to be influenced by frequency dependent selection (rare allele advantage) since primary mycelia possessing rare alleles have a greater probability of mating than mycelia possessing common alleles (May et al., 1999; Richman, 2000). This rare allele advantage is expected to maintain a high allelic richness of MAT alleles.

Schmidt and Moreth-Kebernik (1991) crossed monokaryons from 10 dikaryotic strains from Europe and Australia and found four different A factors and five different B factors. However, whether A and B factors corresponded to MAT A or MAT B genes was unknown. The presence of four and five different mating type factors were confirmed by Kauserud et al. (2006a) when monokaryons obtained from six different European dikaryons were crossed.

Steps toward a molecular characterization of the diversity of MAT alleles in *S. lacrymans* have been taken (Engh et al., 2010b). However, finding conserved regions for primers within

the MAT regions have proven difficult. Thus, to target closely linked genetic regions to the MAT genes and use these as proxies for studying the allelic diversity of MAT genes has been successful (James et al., 2004; Kües et al., 2001). James et al. (2004) characterized primers in the *mip* (mitochondrial intermediate peptidase) gene, which is within 1000 bp from one of the MAT A homeodomain genes for all basidiomycetes investigated (James, 2007). Amplification of the *mip* gene in a large population of *Serpula* identified three main clades of European *mip* alleles. These clades were largely congruent with the three main MAT A alleles (Engh et al., 2010b). Further work is under way in our group to characterize the allelic diversity of mating type genes in *S. lacrymans*.

## 10. Genomics of var. *lacrymans*

Because of the aggressive type of brown rot and the capacity to break down cellulose, *S. lacrymans* was genome sequenced by the U. S. Government Department of Energy Joint Genome Institute. Two monokaryotic genomes were sequenced, s7.9 (Sanger sequencing) and s7.3 (454 pyro sequencing technology). The *S. lacrymans* genome was assembled to 42.7 Mb in size, over 36 main scaffolds, with 12,789 gene models (Eastwood et al., 2011).

*S. lacrymans* depolymerizes the lignin component of the wood to access the cellulose. The cellulose is then efficiently decomposed and utilized. The brown rot residues (lignin residues) contribute about 30 % of the carbon of the soil in boreal forests and functions as a carbon sink in temperate regions (Eriksson et al., 1990). The brown rot process in *S. lacrymans* involves an attack of the plant cell wall by generating hydroxyl radicals extracellularly via the Fenton reaction, and possibly less by cellulose decomposing enzymes. However, some other carbohydrate active plant cell wall degrading enzymes are active, e.g. pectinases and hemicellulases. The involvement of a Fenton based reaction is further supported by *S. lacrymans* producing iron-reducing phenolates (e.g. variegatic acid) in contact with wood (Eastwood et al., 2011). These phenolates can regenerate the divalent iron needed to produce the hydroxyl radicals in the Fenton reaction. When the *S. lacrymans* genomes, transcriptome and proteome were compared to other sequenced white rot, brown rot and ectomycorrhizal fungi it became clear that certain protein families have experienced reductions and losses in the evolutionary pathway from a white rot fungus. These protein families include enzymes important for decomposing plant cell walls, such as hydrolytic CAZy genes. Oxidoreductase genes have experienced duplications in white rot fungi, but not in brown rot fungi. These results indicate that brown rot species have lost the expensive enzymatic plant cell wall decomposing system, and rather depend on alternatives (like Fenton reaction).

## 11. Conclusions and further perspectives

*S. lacrymans* is naturally distributed in holarctic high altitude regions where it is found on coniferous logs of large dimensions. Here it seems to be a geophilous fungus able to transport water from the ground and into the decaying logs through the rhizomorphs and performs highly effective

brown rot decay. Suitable niches have been recreated by humans in buildings (mainly cellars), where a combination of mineral and wooden material is often present. The ability to produce thick strands that can stretch several meters across inorganic material is highly suitable for the in-house life-style. From its natural range in northeast Asia *S. lacrymans* has spread out to become a successful invasive species in temperate regions worldwide. As a continuation of the genome analysis of *S. lacrymans* (Eastwood et al. 2011), an interesting task will be to analyze the molecular basis for the success of var. *lacrymans* as a global invasive species. The Serpulaceae also offers an interesting system to study the evolution of ectomycorrhizal symbiosis, as the ectomycorrhizal genera *Austropaxillus* and *Gymnopaxillus* are nested within Serpulaceae (Binder and Hibbett, 2006; Skrede et al., 2011).

*S. lacrymans* may also be a suitable model for studying the genetic basis of strand formation. Furthermore, the achievements within *Serpula* population biology and genomics, in combination with the low cost of large scale DNA-sequencing, provide a unique opportunity to address evolutionary questions within the framework of comparative and population genomics.

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