



British Mycological
Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio



Heterobasidion occidentale sp. nov. and Heterobasidion irregulare nom. nov.: A disposition of North American Heterobasidion biological species

William J. OTROSINA^a, Matteo GARBELOTTO^{b,*}

^aUSDA Forest Service, Southern Research Station, 320 Green Street, Athens, GA 30602, USA

^bDepartment of Environmental Science, Policy and Management – Ecosystem Sciences Division, University of California at Berkeley, 137 Mulford Hall # 3114, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:

Received 13 December 2008

Received in revised form

21 July 2009

Accepted 3 September 2009

Available online 8 September 2009

Corresponding Editor:

Joey Spatafora

Keywords:

Host specificity

Mating barriers

Phylogenetic species

Species complex

Taxonomy

ABSTRACT

The genus *Heterobasidion* includes some of the most important pathogens of conifers in the world, and as such it is one of the most intensely studied genera of fungi. Because of the remarkable paucity of distinguishing morphological traits, the taxonomy of species within this genus has always been problematic. A partial resolution of the taxonomic issues regarding this genus was achieved by defining the most important and first described species within it, *Heterobasidion annosum*, as a species complex containing at least two partially intersterile biological species defined as intersterility groups (ISGs). With time, the number of ISGs has increased to include at least two distinct North American and three distinct Eurasian ISGs. Two additional, yet unnamed, taxonomic groups within *Heterobasidion* have been recently described in Japan. ISGs are distinguishable either by minor morphological differences, by partial intersterility, by ecological traits including host preference, and/or by their geographic range. Several studies employing a variety of molecular tools and analyses have confirmed the distinct genetic divergence among ISGs, identifying each of them as a monophyletic group. Using genetic markers, genotypes can always be unambiguously assigned to one ISG, and very few inter-ISG hybrids have been identified. In this paper, we summarize the available information, both genetic and ecological, that differentiates the two North American ISGs from each other and from other taxonomic units within the genus. We demonstrate that morphometric characteristics such as pore density and pore shape differentiate the two ISGs. Based on the cumulative genetic, ecological, and morphological evidence, we propose a disposition of ISGs of the North American *H. annosum* by replacing the P ISG with *Heterobasidion irregulare*, and the S ISG with *Heterobasidion occidentale*.

© 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

The genus *Heterobasidion* (Stalpers 1979) is a global complex of woody plant pathogens and saprobes whose host range comprises over 200 plant taxa, the majority of which are conifers

(Korhonen & Stenlid 1998). *Heterobasidion* has a worldwide negative impact on conifers, both ecologically and economically, by reducing site productivity and the amount of harvestable timber (Woodward et al. 1998). On the other hand, this organism is also responsible for creating forest conditions

* Corresponding author. Tel.: +1 510 643 4282; fax: +1 510 643 5436.

E-mail address: matteog@berkeley.edu

0953-7562/\$ – see front matter © 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

doi:10.1016/j.mycres.2009.09.001

conductive to regeneration, nutrient cycling, and succession (Garbelotto 2004). In either case, it is a significant ecological force directly or indirectly affecting natural processes and anthropogenic activities.

Until about 30 Y ago, *Heterobasidion* and its pathology were generally considered as monolithic, with the single species *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (s.l.) regarded as one of the major pathogens worldwide, both in terms of damages and geographic range (Otrosina & Cobb 1989; Woodward et al. 1998). Via mating studies, Korhonen (1978) delimited two intersterility groups (ISGs) of the pathogen in Europe, designating the S and P group for isolates attacking spruce (*Picea* sp.) and pine (*Pinus* sp.), respectively. Later, Capretti et al. (1990) delimited the host specific F ISG for *Heterobasidion* attacking the true fir, *Abies alba* Mill., in Italy. Subsequently, the F and S ISGs in Europe have been designated as separate taxonomic species, *Heterobasidion abietinum* and *Heterobasidion parviporum*, respectively (Niemela & Korhonen 1998). The Eurasian pine-associated species is currently referred to as *H. annosum sensu stricto* (s.s.).

Chase & Ullrich (1990a, b) conducted mating experiments using *H. annosum* s. l. isolates from North America utilizing S and P ISG testers from Korhonen (1978) and showed North American S and P groups to have a higher degree of interfertility between them than the Finnish S and P groups. However, host associations with the North American S group were highly correlated with *Abies* and *Tsuga* and the P group was correlated with *Pinus* hosts. Later, Otrosina et al. (1993) used isozyme analyses to study population structure of European and North American *Heterobasidion* ISGs. They found the North American S and P ISGs to be widely divergent from the European S, P, and F ISGs. These findings were corroborated in a series of subsequent genetic studies employing a variety of fungal isolates, genetic markers, and analyses (Asiegbu et al. 2005; Harrington et al. 1998; Johanneson & Stenlid 2003; Linzer et al. 2008; Ota et al. 2006). All studies have identified the presence of at least five Operative Taxonomic Units (OTUs) within the *H. annosum* s.l. species complex, each characterized by unique genetic and phenotypic traits. The five OTUs include the three Eurasian species and the two North American ISGs. From an evolutionary and phylogenetic perspective, the five OTUs can be separated into two distinct clades. One, which we here call the fir/spruce clade, includes the Eurasian *H. parviporum*, *H. abietinum*, the North American *H. annosum* S ISG, and possibly two recently described taxa from Japan (Tokuda et al. 2009). The other, which we call the pine clade, includes the Eurasian *H. annosum* s.s. and the North American *H. annosum* P ISG. While the taxonomic relationships among OTUs within the fir–spruce clade are not completely resolved, as highlighted by the fact that the OTUs place differently using different molecular markers (Harrington et al. 1998; Johanneson & Stenlid 2003; Linzer et al. 2008; Ota et al. 2006; Otrosina et al. 1993), the relationship between the two taxa within the pine clade seems clear. A detailed phylogeographic study of the *H. annosum* s.l. complex that employed a large worldwide representation of *Heterobasidion* genotypes of the pine clade found the Eurasian *H. annosum* s.s. and the North American P ISG to have attained reciprocal monophyly at the three loci present in both taxa (Linzer et al. 2008). The fourth locus was a mitochondrial DNA insertion absent in Eurasia. The North

American P ISG and the Eurasian *H. annosum* s.s. represent two clearly distinct sister OTUs in an overall monophyletic clade.

Mating compatibility among the five taxa is highly variable, with the general trend of stronger mating barriers observed for non-conspecific genotypes found in sympatry (Stenlid & Karlsson 1991). Thus mating compatibility ranges from 4 % for sympatric *H. annosum* s.s. and *H. parviporum* individuals (Korhonen 1978), to almost 100 % for the allopatric *H. annosum* s.s. from Eurasia and the North American P ISG (Chase & Ullrich 1990b).

Notwithstanding the biological distinctions between *Heterobasidion* ISGs and species, globalization of economies and more efficient and rapid international transportation of goods add to the importance of clear taxonomic definitions of an organism known to be an extremely aggressive pathogen of coniferous tree species. This is of obvious importance for efforts directed toward prevention of unwanted introductions of exotic pathogens and genetic transfers worldwide. With the delimitation of the three European *Heterobasidion* species, the unresolved nomenclature of the North American S and P ISGs is source of significant confusion. Lack of official taxonomic designation is a serious problem, not only for scientists but also for those involved in formulating regulatory policies for prevention and detection of pathogen introductions. Based upon some differentiating morphological traits strongly supported by genetic and ecological evidence, we propose naming the North American *H. annosum* S and P ISGs as two new species.

Materials and methods

Field collections

During October and November 2007, we collected basidiomata of *Heterobasidion* in the Modoc National Forest in Northern California from both pine and true fir habitats, in Southern California from true fir habitats, and in the southeastern United States from pine habitat. We made an effort to collect specimens from geographically distant locations in order to include possible effects of geography on the morphological variation of basidiomata. Site characteristics and GPS coordinates are reported in Table 1.

In California habitats, basidiomata collections predicted to be the P ISG were accomplished via excavations of pine stumps. Evidence of Annosum root disease (ARD) symptoms in surrounding trees was noted. All pine isolates in California were obtained from ponderosa pine (*Pinus ponderosa* Douglas ex P. Laws. & C. Laws) stumps within the residual stands of this species. Stump diameters ranged from 50 to 100 cm. Stands from these pine collections were approximately 70–100 Y old and elevation ranged from 1536 m to 1600 m.

Collections of the S ISG from *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr. were carried out in both the Modoc National Forest in northern California and in the San Bernardino National Forest in southern California. Collections of basidiomata in northern and southern California true fir stumps were obtained via searching hollow decay columns in stump sapwood or heartwood. Stump diameters ranged

Table 1 – Sites where basidiomata of *Heterobasidion annosum sensu lato* were collected.

Site ^a	GPS coordinates	Dominant forest cover	Stump sp. disease +/-	N samples ^b
1. MNF	N41° 38.506 W120° 58.936	<i>P. ponderosa</i> 95 %	<i>P. ponderosa</i> +	2
2. MNF	N41° 58.768 W120° 52.600	<i>P. ponderosa</i> 95 %	<i>P. ponderosa</i> +	1
3. MNF	N41° 58.868 W120° 50.429	<i>P. ponderosa</i> 95 %	<i>P. ponderosa</i> +	2
4. MNF	N41° 58.873 W120° 50.506	<i>P. ponderosa</i> 95 %	<i>P. ponderosa</i> +	4
5. MNF	N41° 33.024 W120° 18.026	<i>A. concolor</i> 95 %	<i>A. concolor</i> –	3
6. MNF	N41° 30.444 W120° 17.633	<i>A. concolor</i> 95 %	<i>A. concolor</i> +	1
7. SBNF	N34° 13.733 W116° 56.045	<i>A. concolor</i> 80 % <i>P. jeffreyi</i> 20 %	<i>A. concolor</i> –	1
8. SBNF	N34° 13.506 W116° 55.372	<i>P. jeffreyi</i> 60 % <i>A. concolor</i> 40 %	<i>A. concolor</i> –	2
9. SBNF	N34° 13.454 W116° 55.096	<i>P. jeffreyi</i> 70 % <i>A. concolor</i> 30 %	<i>A. concolor</i> –	1
10. SBNF	N34° 12.961 W116° 57.547	<i>P. jeffreyi</i> 60 % <i>A. concolor</i> 40 %	<i>A. concolor</i> +	2
11. SBNF	N34° 12.810 W116° 58.366	<i>P. jeffreyi</i> 60 % <i>A. concolor</i> 40 %	<i>A. concolor</i> +	1
12. GA	N32° 15.758 W81° 43.320	<i>P. taeda</i> 100 %	<i>P. taeda</i> tree +	9

a MNF = Modoc National Forest, CA; SBNF = San Bernardino National Forest, CA; GA = Central Georgia.

b Not all samples were employed for morphological and DNA analyses (see Table 2).

from 20 cm diameter to 70 cm in northern California and 100 to 230 cm in southern California. Elevation approached or exceeded 2000 m at all true fir sites.

In the southeastern USA, one multiple basidiomata collection was made in an ARD diseased 25 Y old loblolly pine (*Pinus taeda* L.) plantation. Basidiomata were collected from the base of symptomatic trees, just beneath the current-year needle fall.

Laboratory analyses

All collections were transported to the laboratory at U.C. Berkeley, California, in an ice chest and processed. Analyses were performed on a total of 26 basidiomata from 11 sites. Tissue from the context of the basidiomata was obtained and DNA analyses were performed to determine ISG using taxon specific competitive priming PCR and direct sequencing of the ITS as described in Garbelotto *et al.* (1996). Sequences were aligned in Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA), and manually corrected before being compared to multiple ITS sequences of both the P and S ISGs of the North American *Heterobasidion*. Pore size measurements were made on specimens after photographing the hymenial layer using a camera fixed and standardized to obtain uniform distance to object (hymenial layer). Pore density was measured via randomly selecting 3–16 1.0 mm² fields on the hymenial layer of each basidiocarp, using the program Image Pro Plus[®] (Media Cybernetics, Inc., Bethesda, MD, USA). For two small samples, only two observations were possible. Shape of pores was not regular, but ranged from circular to extremely elongated (rarely quasi-daedaloid). In order to compare frequency of irregular vs. regular pores, 40 pores per basidiome were randomly selected and analyzed both quantitatively and qualitatively. For each pore, the longest (LB) and the shortest perpendicular bisects (SB) were measured, and it was noted whether: a) LB/SB > 2, and b) the pore was regular in shape,

e.g. circular or ovoid, or irregular (sinuous or contorted). This second observation is a visually intuitive trait, more restrictive than the first one, as many pores for which LB/SB > 2 have a rather regular ovoid shape and would not be classified as “irregular”. Additionally, an intermediate bisect (MB) was also measured to represent the average width of the pores and used to calculate the approximate area of the pores.

All statistical analyses were done with the program JMP, Version 7 (SAS Institute Inc., Cary, NC, USA). Pore density was compared among basidiomata using nested ANOVA in the “test model” function in JMP, selecting for the LSD model. This analysis allows the nesting of multiple pore density measurements within each basidiomata sample, thus avoiding pseudo-replication, and determines effect significance both for ISG and isolates. Analysis on the shape of pores was performed as follows: for each ISG number of pores with LB/SB > 2 and number of irregular pores were independently tabulated, and Fisher’s exact tests were run on each dataset to determine significant differences in frequencies of elongated and irregular pores between the two ISGs. Analysis on size of pores was done using an estimate of the area obtained by multiplying $LB/2 \times MD/2 \times \pi$, and performing a nested ANOVA as described for pore density analyses.

After morphometric and DNA analyses, basidiomata of representative holotypical and epitypical specimens for each ISG were freeze-dried and deposited in the herbarium at the University of California, Berkeley. Vouchers’s identification numbers are UC1935442 and UC1935443.

Results

Habitat and macroscopic characteristics of basidiomata

P ISG

In three drier sites of the Modoc National Forest (CA), characterized by the presence of pine and juniper, all nine

basidiomata recovered from pine stumps were typed as P ISG through DNA analyses. Not all nine were employed for the morphological study, as at least two were small “popcorn” basidiomata (see below). In western North America, ecological habitat itself cannot be relied upon to classify the P ISG, because pine stumps are routinely infected by S ISG as well (Otrošina *et al.* 1992), depending upon the density and proximity of *Abies* sp. On the other hand, where symptomatic pine and western juniper (*Juniperus occidentalis* Hook) occur near pine-thinning stumps, our experience and observations over the years indicate a high probability that recovered basidiomata will be of the P ISG (Otrošina *et al.* 1993). In the southeastern, southern and midwestern states of the USA, only the P ISG has ever been reported. As expected, all nine collections from that region belonged to that ISG.

Our observations indicate the ecological situation governs where basidiomata are produced. In the western United States, the P ISG is generally found inside pine stumps, often close to the interior, where sufficient moisture is likely to be present during the long, hot, and dry periods in some of the interior western states and on the semiarid east sides of the Sierra Nevada, Cascades and Transversal mountain ranges from eastern Washington and Oregon through southern California. Notably, the P ISG in western North America produces both large (up to 30 cm in length) shelf-shaped basidiomata that may be alive for several years and small (0.5–1 cm in diameter) incipient basidiomata referred to as “popcorns” because of their appearance (Fig 1). These smaller annual basidiomata are often found under the bark of decaying stumps or under the bark of roots in stumps or dead trees. East of the Rocky Mountains, P ISG basidiomata are mostly found fruiting near ground level just beneath the fresh, undecomposed duff on the bases of symptomatic, diseased trees and infected stumps (Fig 2). The climate there is moist and humid, particularly during late fall through early spring when fruiting is abundant. Generally, the P ISG basidiomata for both western and eastern United States are smaller in size than those of the S ISG, but exceptions do occur. Resupinate forms are possible for both ISGs.



Fig 1 – These small *Heterobasidion irregulare* basidiomata can be found under the bark at the base or in the primary roots of dead trees and stumps. They are referred to as “popcorns” because of their appearance.



Fig 2 – Fruiting by *Heterobasidion irregulare* is greatly influenced by environmental conditions. While it mostly fruits inside stumps in the dry western part of North America, it fruits in the duff at the base of dead trees and stumps in the more mesic Eastern states, as shown in this picture.

North American S ISG

Generally, the habitat for basidiomata of the S ISG is within *Abies* sp., particularly *Abies concolor*, stumps. While it is very rare to find P ISG infecting stumps of true fir (*Abies* sp.), S ISG basidiomata are frequently found in pine stumps (Garbelotto *et al.* 1999; Otrošina *et al.* 1992, 1993). All ten basidiomata collected for this study in true fir stumps were typed as S ISG through DNA analyses. True firs typically occur at higher elevations where there is more moisture and the temperatures are cooler. Fruiting is frequently found within decay columns, often referred to as chimneys, within the sapwood of cut stumps (Fig 3). These decay columns indicate infection by the fungus prior to tree-felling, and therefore active pathogenesis (Otrošina & Cobb 1989). The basidiomata are long-lived (Fig 4), often larger than those of the P ISG and can frequently



Fig 3 – Most basidiomata of *Heterobasidion occidentale* are found in stumps, either under an intact top layer, if infection by the pathogen occurred on the stump surface, or within decay columns as the one shown here, if infection occurred on standing trees.



Fig 4 – Both North American *Heterobasidion* species can form multilayered basidiocarps that will be productive for several years. Here, a section of a *Heterobasidion occidentale* basidiocarp, showing the stratification of annual hymenial layers.

attain sizes of 0.4 m × 0.3 m in length × width (Fig 5). In true fir and pine stumps infected post tree-felling, i.e. through the stump surface, basidiomata are often resupinate or semi-resupinate and produced beneath the intact stump surface.

Microscopic analyses

Of the 26 basidiomata analyzed, 13 from 7 sites were assigned to the S ISG, and 13 from 4 sites were assigned to the P ISG. Average pore density was 7.3 ± 0.12 and 8.6 ± 0.07 pores mm^{-2} (mean $\pm$ SE) for the P and S ISGs, respectively. Average pore density was significantly lower for PISG than for S ISG basidiomata, with ISG and basidioma both having a significant ($P < 0.0001$) effect on the overall difference (Table 2). If only California populations were analyzed, average pore density for P basidiomata was 5.8 ± 0.2 pores mm^{-2} (mean $\pm$ SE). The more marked difference in average pore density between sympatric S and P ISG individuals makes this trait more useful



Fig 5 – The pore surface of a sporophore of *Heterobasidion occidentale*.

for the differentiation between the two species in areas where they co-exist. Lower pore density may be determined either by larger pores or by larger spaces among pores. A nested ANOVA using approximate area values obtained by multiplying $\text{LB}/2 \times \text{MB}/2 \times \text{II}$ indicated there were significant differences among basidiomata but not between ISGs in pore area. However, Fisher exact tests indicated that P ISG basidiomata were characterized by a significantly larger percentage of: a) irregular pores as visually determined, and, b) elongated pores determined as number of pores for which $\text{LB}/\text{SB} > 2$ (Table 2).

Differences in pore density, size and shape cannot be discerned by looking at a single specimen or even at multiple specimens with the naked eye. However, we found it possible to notice these differences by comparing images of the pore surface taken under the dissecting scope at 10× magnification, side by side (Fig 6).

Taxonomy

Heterobasidion irregulare nom. nov. Garbelotto & Otrosina

MB 515278

Synonym *Polyporus irregularis* Underwood, Torrey Bot Club. Bull. 24: 85, 1987.

Replacing the reference to the North American *Heterobasidion annosum* P ISG.

Lectotype: New York Botanical Garden, Voucher ID: NY 730756. Collected under a pine log in 1896 in Auburn, Alabama, USA.

Epitype selected here: U.C. Herbarium, University of California, Berkeley, Voucher ID: UC1935442. Collected in this study from a *Pinus ponderosa* stump in the Modoc National Forest (CA, USA), site 3 (Table 1).

Basidiomata ranging in size from 0.5 to 30 cm in length. Morphologically variable, ranging from resupinate to applanate, depending upon age and habitat. Pore density 7.3 ± 0.12 pores mm^{-2} (mean $\pm$ SE). Approximately 11 % of pores are distinctively not circular or elliptical, but rather elongated, rarely quasi-daedaloid. The fresh pore surface is cream-colored becoming yellow-brown with age. Found with some effort within interior of pine stumps >40 cm in diameter in the western United States and fruiting outside symptomatic pine and pine stumps >25 cm in the southeastern United States. Hymenial layer usually one to few strata in cross section. Broad host range including conifers and angiosperms, but notably found on trees of the genera *Pinus*, *Juniperus*, and *Libocedrus*. It causes a deadly root rot of conifers by attacking the cambium of roots and root collars; excellent saprobe, it causes a white stringy dry decay of wood. Genetically distinguishable from the Eurasian *H. annosum* s.s. for the diagnostic presence of a large intron of variable size, but over 1500 bp, in the 5' (ML5-ML6) region of the mitochondrial large subunit rDNA.

Heterobasidion occidentale sp. nov. Otrosina & Garbelotto MB 515277

Etym: Referring to its occurrence limited to western North America.

Replacing the reference to the North American *Heterobasidion annosum* S ISG.

Table 2 – Morphometric measurements and comparative statistics of pore layers of S and P ISG basidiomata of North American *Heterobasidion annosum*.

Sample	Site ^a	ISG	Pore density ^b Mean (SE)	Elongated pores ^b (%)	Irregular pores ^b (%)
1	7	S	6.1 (0.1)	34	19
2	8	S	6.8 (0.2)	64	11
3	8	S	7.4 (0.4)	65	11
4	9	S	9.0 (0.3)	80	2
5	9	S	9.3 (0.2)	81	0
6	9	S	11.1 (0.3)	23	0
7	10	S	8.7 (0.2)	48	6
8	10	S	8.3 (0.2)	28	3
9	11	S	8.6 (0.4)	27	0
17	5	S	10.3 (0.3)	88	9
18	5	S	7.3 (0.2)	na	na
19	5	S	10.6 (0.3)	na	na
20	6	S	9.6 (0.4)	66	0
All S basidiomata			8.6 (0.07)	53	6
11	1	P	5.7 (0.2)	74	0
12	3	P	4.6 (0.2)	na	na
13	4	P	7.5 (0.3)	82	0
15	4	P	6.1 (0.6)	80	7
16	4	P	5.2 (0.1)	85	9
22	12	P	6.7 (0.2)	50	3
23	12	P	6.9 (0.5)	68	30
24	12	P	10 (0.5)	44	12
25	12	P	9.4 (0.3)	71	13
27	12	P	8.9 (0.2)	65	19
28	12	P	9.7 (0.6)	na	na
29	12	P	8.4 (0.3)	25	0
31	12	P	9.0 (0.5)	51	15
All P basidiomata			7.2 (0.1)	64	9.7
Nested ANOVA	DF	F	P	Fisher's exact test	Fisher's exact test
Effect of sample [ISG]	17	24.6	<0.0001	P = 0.001	P = 0.03
ISG	1	190.3	<0.0001	(P ISG > S)	(P ISG > S)

a See Table 1 for description of study sites.

b See Materials and methods.

Holotype: U.C. University of California, Berkeley. Voucher ID: UC1935443. Collected in an *Abies concolor* stump in the San Bernardino National Forest (CA, USA), site 7 (Table 1).

Latin Description: *Sicut Heterobasidion irregulare*, sed **dissimile per maiorem numerum pororum et per minorem numerum pororum irregularium**. [Similar to *H. irregulare*, distinguishable by its higher average pore density, and by the lower frequency of irregular pores.]

Basidiomata generally ≥ 5.0 cm often up to $0.4 \text{ m} \times 0.3 \text{ m}$ (length $\times$ width) within sapwood decay columns of larger (>30.0 cm diameter) true fir stumps. When fresh, the pore surface is slightly off-white, somewhat brighter than *H. irregulare*. Pore density is 8.6 ± 0.07 pores mm^{-2} . Approximately 6 % of pores are distinctively not circular or elliptical, but rather elongated and rarely quasi-daedaloid. Basidiomata perennial having several strata visible in cross section, most commonly applanate, but also resupinate, in particular if found under the intact top layer of a stump. Broad host range, but limited to conifers, notably found on trees belonging to the genera *Abies*, *Sequoiadendron*, *Tsuga*, *Pseudotsuga*, and *Picea*. It causes a sap

rot disease in infected trees that reduces tree vigor, and a heartrot in larger trees that weakens the stability of infected trees. An excellent saprobe, it causes a white stringy dry decay of wood. Genetically distinguishable from congeneric species by a variety of methods including isozyme, RAPDs and DNA sequence analysis of part of the glyceraldehyde 3-phosphate dehydrogenase gene.

The taxonomic etymology and discussion of other species of *Heterobasidion* are reviewed by Niemela & Korhonen (1998). There are currently 10 named species of *Heterobasidion*, including the two new species we propose.

Discussion

There is considerable morphological plasticity among *Heterobasidion irregulare* and *Heterobasidion occidentale* basidiomata. The morphological feature that seems to be most consistent relative to distinguishing between the two is pore density. Our pore density measurements are consistent with those of

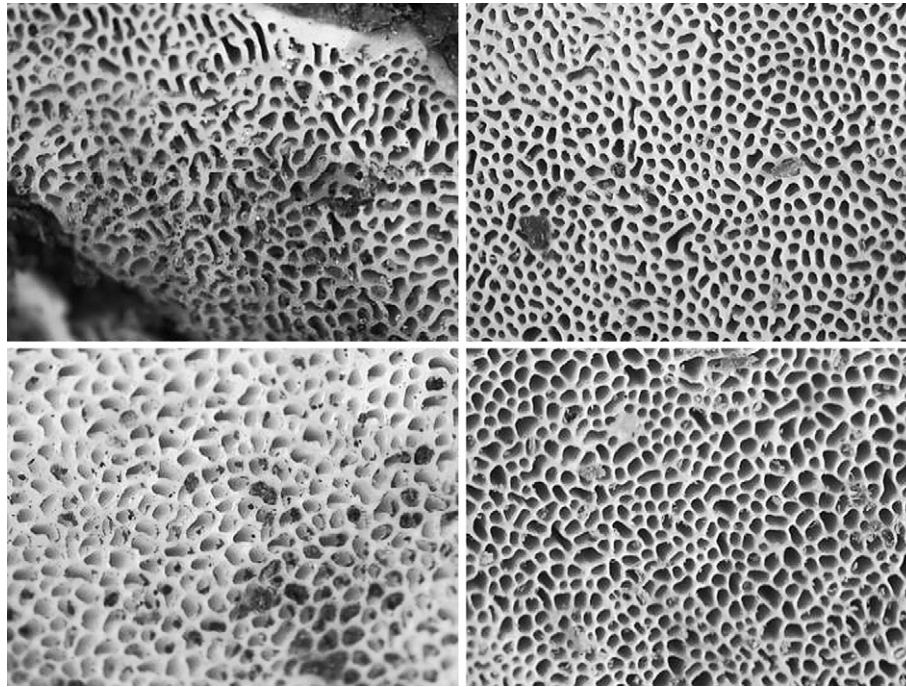


Fig 6 – These images show side by side the pore surface of two *Heterobasidion irregulare* (left column) and two *Heterobasidion occidentale* (right column) basidiomata at 10× magnification.

Heterobasidion basidiomata collected from *Tsuga heterophylla* ((Raf.) Sarg.) in British Columbia, which all evidence indicates are *H. occidentale* (Hood 1985). Pore shape and size may be less dependable, as both are greatly affected by the angle at which the hymenium is growing. Even with experience, it is difficult to distinguish between the species via morphology alone, even when found on hosts they are known to be specialized on. This is especially true when finding basidiomata in pine stumps in western North America, as both *H. irregulare* and *H. occidentale* may occur in this situation (Filip & Morrison 1998; Harrington et al. 1989; Otrosina et al. 1992, 1993; Garbelotto et al. 1996). On the other hand, these studies also showed that when the fungus was found in association with symptomatic intact pine trees (*Pinus jeffreyi* E. Murray, *Pinus ponderosa* Douglas ex P. Laws. & C. Laws, *Pinus coulteri* D. Don, *Pinus monophylla* Torr. & Frem.), true fir (*Abies concolor* (Gordon & Glend.) Lindl. Ex Hildebr., *Abies magnifica* Andr. Murray,) giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buckholz), as well as other conifer species, they correspond to either *H. irregulare* (pines, junipers, incense cedars) or *Heterobasidion occidentale* (true firs, giant sequoias, hemlocks). This strong specificity between *Heterobasidion irregulare* and *H. occidentale* and their respective host taxa may be one driver of their genetic differentiation. Our *H. irregulare* collections were associated with symptomatic ponderosa pine, western juniper (*Juniperus occidentalis* Hook.), and loblolly pine (*Pinus taeda* L.). The *H. occidentale* collections were associated with symptomatic *A. concolor* trees or decadent stands.

As stated in the descriptions, the location of basidiomata on the tree or stump is apparently governed by ecological circumstances as much as any inherent host-pathogen influence. In the southeastern USA, basidiomata of *H. irregulare*

are usually found external to symptomatic pine trees, fruiting in the duff at the base of the tree or stump. In contrast, it is rare for basidiomata of *H. irregulare* to be found fruiting external to stumps or hosts in western North America. To our experience and knowledge, *H. irregulare* rarely occurs as a saprotrophic, secondary colonizer of true fir stumps (Otrosina et al. 1992), and has never been reported as a primary pathogen of true firs (Garbelotto et al. 1996, 1997, 1999). The known natural range of *H. irregulare* includes Canada (Quebec and Ontario), most of the USA and Mexico where pine hosts are present, Cuba, and the Dominican Republic (Anonymous 1980).

H. occidentale does not occur in eastern North America, but in western North America it can colonize and fruit within pine stumps where it virtually resembles *H. irregulare* in gross morphology and habit. This is generally thought to be a product of the drier habitat where stumps of pine species can remain intact for over 50 Y after felling and support fruiting deep within (Filip & Morrison 1998; Otrosina & Cobb 1989). In true fir stands at higher elevation, where the climate is cool and moist relative to ponderosa and Jeffrey pine habitat, *H. occidentale* basidiomata can attain considerable size within decay columns of larger stumps. The known natural range of *H. occidentale* is limited to western North America from Alaska to Southern Mexico, spreading East as far as the Rocky Mountains (Anonymous 1980).

Otrosina et al. (1993) presented evidence for considerable genetic divergence between the North American S and P ISGs. Laboratory pairings have shown that interfertility between them is approximately only 18 % (Chase & Ullrich 1990a; Harrington et al. 1989). In all published studies (Asiegbu et al. 2005; Harrington et al. 1998; Johanneson & Stenlid 2003;

Linzer et al. 2008; Otrosina et al. 1993), the two North America ISGs belong to two very distinct clades within the species complex. As a result, the taxa currently designated *Heterobasidion annosum* s.l. represent a paraphyletic group. Despite comprehensive surveys in California, where both ISGs are sympatric, a natural hybrid has been described only once (Garbelotto et al. 1996). Thus, taking into consideration the ecology, type of disease, host preference, low interfertility, and abundant genetic information, there is ample support for the designation of the two North American ISGs as distinct species. Based on our morphological observations, a specimen preserved at the New York botanical garden under the name of *Polyporus irregularis* (Underwood 1897; Overholts 1953) fits the description of the North American *H. annosum* P ISG. We attempted the DNA sequencing of several nuclear and mitochondrial loci from this specimen, including the multicopy ITS of the nuclear ribosomal operon, without success. Nonetheless, the specimen was collected from pine in Alabama (USA), where only the P ISG has ever been reported. Thus, we accept the priority of *P. irregularis* as the first name ever applied to this taxon in North America and designated the binomial *H. irregulare* as the new species name for the former *H. annosum* P ISG.

Not only are the two North American ISGs clearly distinct from one another, but there is sufficient evidence to deem them distinct from the already described and related Eurasian species. The North American *H. occidentale* is considered to be closely related to the Eurasian *Heterobasidion parviporum* and *Heterobasidion abietinum*, but it is clearly genetically distinct from both (Garbelotto et al. 1998; Harrington

et al. 1998; Johanneson & Stenlid 2003; Ota et al. 2006). The North American *H. irregulare* is closely related to the Eurasian *H. annosum* s. s. and is almost 100 % interfertile with it (Chase & Ullrich 1990a). Likewise, *H. irregulare* and *H. annosum* s.s. both have a broad host range and an affinity for *Pinus* spp, on which they both cause a cambium disease of roots and root collars. However, two studies of representative world-wide samples of isolates clearly indicated these to be closely related sister taxa which have attained reciprocal monophyly and diverged considerably, presumably because of allopatric separation (Linzer et al. 2008; Otrosina et al. 1993). In particular, Linzer et al. (2008) provide a convincing framework to explain the evolution of the two taxa into distinct species, and show that all studied genotypes clearly fall into one of the two species without ambiguity. Because the two taxa have been long confined to separate continents, direct selection pressure to reduce fertility between them is unlikely. Genetic isolation often precedes both morphological differentiation and full reproductive isolation in fungi and other microbes (Taylor et al. 2006). We emphasize that the three Eurasian and the two North American taxa are morphologically similar to one another, and when in sympatry they can be differentiated by their ecology (see Table 3 for a summary of field diagnostic features) or more certainly by means of the genetic analyses mentioned throughout this manuscript.

The reported introduction of *H. irregulare* into Italy during World War II (Gonthier et al. 2004, 2007), where the native *H. annosum* s.s is present, provides an excellent opportunity to highlight more significant differences between the two

Table 3 – Some diagnostic differences among the 5 taxa formerly included in the *Heterobasidion annosum* species complex that may be of use in the field where basidiocarps are found.

	<i>H. annosum</i>	<i>H. irregulare</i>	<i>H. parviporum</i>	<i>H. abietinum</i>	<i>H. occidentale</i>
Natural range	Eurasia	North America	Europe	Central, Southern, & Eastern Europe	Western North America
Exotic range	na	Central Italy	na	na	na
Major hosts	<i>Pinus</i> spp.	<i>Pinus</i> spp, <i>Libocedrus</i> , <i>Juniperus</i> spp	<i>Picea</i> spp. or <i>Abies</i> in Eastern Russia	<i>Abies</i> spp.	<i>Abies</i> spp., <i>Tsuga</i> spp., <i>Pseudotsuga</i> spp. & <i>Sequoiadendron</i>
Secondary significant hosts	Stumps of <i>Picea</i>	na	Saplings of <i>Pinus</i> spp.	na	Stumps of <i>Juniperus</i> and <i>Pinus</i> spp.
Location of sporocarps	Root collar and primary roots of dead trees	Root collar (Eastern North America, Italy), underbark of buttress and under intact stump surface (Western USA)	Root collar and decay pockets within boles	Root collar, along roots, and in decay pockets	Decay pockets in stumps and fallen trees, under intact surface of pine stumps
Length of sporocarps	Up to 30 cm	Up to 30 cm in North America. In Central Italy sporocarps can be 30–40 cm, and larger than <i>H. annosum</i>	Up to 30 cm	In colder areas (Central-Eastern Europe) up to 30 cm, in mesic warm areas up to 40–45 cm	Up to 40 cm
Mean pore density in mm ² (SD) and % of irregular pores	8 (0.3) (no data available)	7.3 (0.12) 11 %	13.4 (0.4) (no data available)	12.5 (0.3) (no data available)	8.6 (0.07) 6 %
na = not applicable.					

taxa. In the zone of invasion, pine mortality is more elevated in association with the exotic, than with the native species (D'Amico *et al.* 2007; Gonthier *et al.* 2007). A recent study has shown that in areas of sympatry, the exotic *H. irregulare* is characterized by constant and high basidiospore production, while the native *H. annosum* s.s. is characterized by fluctuations between high basidiospore production in the winter and low or absent release of basidiospores in the summer (Garbelotto *et al.* 2008). Thus, there are significant ecological differences between the two taxa that further support their designation as two distinct species. Delimiting the two North American species of *Heterobasidion* is thus critical for policy development for those charged with regulating and limiting introductions of plant pathogens, as underscored by the recent discovery of the *H. irregulare* introduction into Italy (Gonthier *et al.* 2004). With increasing globalization of economies and increasing transport of raw wood and plant products, clarifying taxonomic positions of taxa such as the *Heterobasidion* complex is an important step in regulatory processes.

H. annosum s.l. has become a model system for the study of fungi and plant-pathogen interactions. The genetics of intersterility between species (Chase & Ullrich 1990a, b), somatic compatibility among individuals of the same species (Hansen *et al.* 1993a, b), interspecific hybridization (Garbelotto *et al.* 1996, 2004, 2007; Olson & Stenlid 2001), di-mon mating (Garbelotto *et al.* 1999; Johanneson & Stenlid 2004), nuclear ratios (James *et al.* 2008; Ramsdale & Rayner 1994, 1996), and plant response to pathogen infection (Abu *et al.* 2004; Fossdal *et al.* 2006; Karlsson *et al.* 2003) are just some of the topics that have been studied in depth using this organism. Because of its importance, the entire genome of a *Heterobasidion* homokaryon is currently being sequenced (Stenlid *et al.* 2008). That homokaryon belongs to the North American *H. annosum* P ISG, a designation that is obscure to all scientists except for a handful of specialists. A resolution of the nomenclature within the *Heterobasidion* complex and the designation of all OTUs within it as species will be a significant advancement clarifying the scope of research focused on *Heterobasidion* spp., strengthening all communication efforts related to such research.

Acknowledgements

This work is dedicated to our mentor, Professor Emeritus Fields W. Cobb Jr.

We are thankful to Ellen Crocker for her essential role played in the laboratory portion of this study, to Rachel Linzer for critically reviewing the manuscript, and to Todd Osmundson and Lydia Baker for performing the genetic analyses. We also thank Dr. John T. Kliejunas (Retired), USDA Forest Service, Forest Health Protection, Region 5, and Ms. Lynn Burgess, USDA Forest Service, Southern Research Station, for their invaluable assistance in obtaining our field collections. We also acknowledge the participants of the 12th IUFRO conference on roots and butt rots of forest trees that, in August 2008, unanimously voted in favor of a new classification of the North American ISGs of *Heterobasidion annosum* as distinct species.

REFERENCES

- Anonymous, 1980. *Heterobasidion annosum*. [Distribution map]. Distribution maps of plant diseases, map 271. CAB International, Wallingford, UK.
- Abu SM, Li GS, Asiegbu FO, 2004. Identification of *Heterobasidion annosum* (S-type) genes expressed during initial stages of conidiospore germination and under varying culture conditions. *FEMS Microbiology Letters* 233: 205–213.
- Asiegbu FO, Adomas A, Stenlid J, 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. s.l. *Molecular Plant Pathology* 6: 395–409.
- Capretti P, Korhonen K, Mugnai L, Romagnoli C, 1990. An intersterility group of *H. annosum* specialized to *Abies alba*. *European Journal of Forest Pathology* 20: 231–240.
- Chase TE, Ullrich RC, 1990a. Five genes determining intersterility in *Heterobasidion annosum*. *Mycologia* 82: 73–91.
- Chase TE, Ullrich RC, 1990b. Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. *Mycologia* 82: 67–72.
- D'Amico L, Motta E, Annesi T, Scire' M, Luchi N, Hantula J, Korhonen K, Capretti P, 2007. The North American P group of *Heterobasidion annosum* s.l. is widely distributed in *Pinus pinea* forests of the western coast of central Italy. *Forest Pathology* 37: 303–320.
- Filip GM, Morrison DJ, 1998. North America. In: Woodward S, Stenlid J, Karjalainen R, Huttermann A (eds), *Heterobasidion annosum: Biology, Ecology, Impact and Control*. CAB International, Wallingford, UK, p. 589.
- Fossdal CG, Hietala M, Kvaalen H, Solheim H, 2006. Changes in host chitinase isoforms in relation to wounding and colonization by *Heterobasidion annosum*: earlier and higher level of defense response in 33-year-old resistant Norway spruce clone. *Tree Physiology* 26: 169–177.
- Garbelotto M, Ratcliff A, Bruns TD, Cobb FW, Otrosina WJ, 1996. Use of taxon-specific competitive priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum*. *Phytopathology* 86: 543–551.
- Garbelotto M, Slaughter G, Popenuck T, Cobb Jr FW, Bruns TD, 1997. Secondary spread of *Heterobasidion annosum* in white fir root-disease centers. *Canadian Journal of Forest Research* 27: 766–773.
- Garbelotto M, Otrosina WJ, Cobb FW, Bruns TD, 1998. The European S and F intersterility groups of *Heterobasidion annosum* may represent sympatric protospecies. *Canadian Journal of Botany* 76: 397–409.
- Garbelotto M, Cobb Jr FW, Bruns TD, Otrosina WJ, Popenuck T, Slaughter G, 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. *Phytopathology* 89: 546–554.
- Garbelotto M, 2004. Root and butt rot diseases. In: Burley J, Evans J, Youngquist JA (eds), *The Encyclopedia of Forest Sciences*, vol. 2. Elsevier, Oxford, pp. 750–758.
- Garbelotto M, Gonthier P, Linzer R, Nicolotti G, Otrosina W, 2004. A shift in nuclear state as the result of natural interspecific hybridization between two North American taxa of the basidiomycete complex *Heterobasidion*. *Fungal Genetics and Biology* 41: 1046–1051.
- Garbelotto M, Gonthier P, Nicolotti G, 2007. Ecological constraints limit the fitness of fungal hybrids in the *Heterobasidion annosum* species complex. *Applied and Environmental Microbiology* 73: 6106–6111.
- Garbelotto M, Linzer R, Nicolotti G, Gonthier P, 2008. Comparative analyses of phenotypic and ecological traits of North American and European isolates of *Heterobasidion annosum*. In: Garbelotto M, Gonthier P (eds), *Proceedings of the 12th*

- International Conference on Root and Butt Rots of Forest Trees, Berkeley, California – Medford, Oregon, 12th–19th August 2007. The University of California, Berkeley, USA, pp. 129–132.
- Gonthier P, Warner R, Nicolotti G, Mazzaglia A, Garbelotto MM, 2004. Pathogen introduction as a collateral effect of military activity. *Mycological Research* **108**: 468–470.
- Gonthier P, Nicolotti G, Linzer R, Guglielmo F, Garbelotto M, 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology* **16**: 1389–1400.
- Hansen EM, Stenlid J, Johansson M, 1993a. Genetic control of somatic incompatibility in the root-rotting basidiomycete *Heterobasidion annosum*. *Mycological Research* **97**: 1229–1233.
- Hansen EM, Stenlid J, Johansson M, 1993b. Somatic incompatibility and nuclear reassortment in *Heterobasidion annosum*. *Mycological Research* **97**: 1223–1228.
- Harrington TC, Worrall JJ, Rizzo DM, 1989. Compatibility among host-specialized isolates of *Heterobasidion annosum* from western North America. *Phytopathology* **79**: 290–296.
- Harrington TC, Stenlid J, Korhonen K, 1998. Evolution in the genus *Heterobasidion*. In: Delatour C, Guillaumin JJ, Lung-Escarment B, Marcais B (eds), *Root and Butt Rots of Forest Trees: Proceedings of the 9th International Conference on Root and Butt Rot*. INRA, France, pp. 63–74.
- Hood IA, 1985. Pore width in *Heterobasidion annosum* (Fries) Brefeld. *New Zealand Journal of Botany* **23**: 495–498.
- James TY, Stenlid J, Olson A, Johannesson H, 2008. Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. *Evolution* **62**: 2279–2296.
- Johannesson H, Stenlid J, 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood decay fungus *Heterobasidion annosum*. *Molecular Phylogenetics and Evolution* **29**: 9–101.
- Johannesson H, Stenlid J, 2004. Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. *Fungal Genetics and Biology* **41**: 563–570.
- Karlsson M, Olson Å, Stenlid J, 2003. Expressed sequences from the basidiomycetous tree pathogen *Heterobasidion annosum* during early infection of Scots pine. *Fungal Genetics and Biology* **39**: 31–59.
- Korhonen K, 1978. Intersterility groups of *Heterobasidion annosum*. *Communicationes Instituti Forestalis Fenniae* **94**: 1–25.
- Korhonen K, Stenlid J, 1998. Biology of *Heterobasidion annosum*. In: Woodward S, Stenlid J, Karjalainen R, Huttermann A (eds), *Heterobasidion annosum: Biology, Ecology, Impact and Control*. CAB International, Wallingford, UK, pp. 43–70.
- Linzer RE, Otrosina WJ, Gonthier P, Bruhn J, Laflamme G, Bussi  res G, Garbelotto M, 2008. Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and of human-mediated, long range dispersal. *Molecular Phylogenetics and Evolution* **46**: 844–862.
- Niemela T, Korhonen K, 1998. Taxonomy of the genus *Heterobasidion*. In: Woodward S, Stenlid J, Karjalainen R, Huttermann A (eds), *Heterobasidion annosum: Biology, Ecology, Impact and Control*. CAB International, Wallingford, UK, pp. 27–33.
- Olson A, Stenlid J, 2001. Mitochondrial control of fungal hybrid virulence. *Nature* **411**: 438.
- Ota Y, Tokuda S, Buchanan PK, Hattori T, 2006. Phylogenetic relationships of Japanese species of *Heterobasidion* – *H. annosum sensu lato* and an undetermined *Heterobasidion* sp. *Mycologia* **98**: 717–725.
- Otrosina WJ, Chase TE, Cobb Jr FW, Korhonen K, 1993. Population structure of *Heterobasidion annosum* from North America and Europe. *Canadian Journal of Botany* **71**: 1064–1071.
- Otrosina WJ, Chase TE, Cobb Jr FW, 1992. Allozyme differentiation of intersterility groups of *Heterobasidion annosum* isolated from conifers in the western United States. *Phytopathology* **82**: 540–545.
- Otrosina WJ, Cobb Jr. FW, 1989. Biology, ecology, and epidemiology of *Heterobasidion annosum*. In: Otrosina WJ, Scharpf RF, (tech. coords.), *Symposium on Research and Management of Annosus Root Disease (Heterobasidion annosum) in Western North America*. USDA Forest Service Gen. Tech. Rept. PSW 116.
- Overholts LO, 1953. *The Polyporaceae of the United States, Alaska and Canada*. University of Michigan Press, Ann Arbor, (2nd printing, 1967). 466 pp.
- Ramsdale M, Rayner ADM, 1994. Distribution patterns of number of nuclei in conidia from heterokaryons of *Heterobasidion annosum* (Fr) Bref and their interpretation in terms of genomic conflict. *New Phytologist* **128**: 123–134.
- Ramsdale M, Rayner ADM, 1996. Imbalanced nuclear ratios, postgermination mortality and phenotype-genotype relationships in allopatrically-derived heterokaryons of *Heterobasidion annosum*. *New Phytologist* **133**: 303–319.
- Stalpers JA, 1979. *Heterobasidion (Fomes) annosum* and the Bon-darzewiaceae. *Taxon* **28**: 414–417.
- Stenlid J, Karlsson JO, 1991. Partial intersterility in *Heterobasidion annosum*. *Mycological Research* **95**: 1153–1159.
- Stenlid J, Garbelotto M, K  es U, Anderson JB, Martin F, Solheim H, 2008. Sequencing the genome of the forest pathogen *Heterobasidion annosum*. In: Garbelotto M, Gonthier P (eds), *Proceedings of the 12th International Conference on Root and Butt Rots of Forest Trees, Berkeley, California – Medford, Oregon, 12th–19th August 2007*. The University of California, Berkeley, USA p. 69.
- Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobsom D, 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philosophical Transactions of the Royal Society B* **361**: 1947–1963.
- Tokuda S, Hattori T, Dai YC, Ota Y, Buchanan PK, 2009. Three species of *Heterobasidion* (Basidiomycota, Hericiales), *H. parviporum*, *H. orientale* sp. nov and *H. ecrustosum* sp. nov from East Asia. *Mycoscience* **50**: 190–202.
- Underwood LM, 1897. Some new fungi, chiefly from Alabama. *Bulletin of the Torrey Botanical Club* **24**: 81–86.
- Woodward S, Stenlid J, Karjalainen R, H  ttermann A, 1998. Preface. In: Woodward S, Stenlid J, Karjalainen R, H  ttermann A (eds), *Heterobasidion annosum: Biology, Ecology, Impact and Control*. University of Michigan Press, Ann Arbor Wallingford, UK, pp. xi–xii.