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Taxonomy, biogeography and importance of *Heterobasidion* viruses

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ABSTRACT

The genus *Heterobasidion* consists of several species of necrotrophic and saprotrophic fungi, and includes some of the most detrimental organisms in boreal conifer forests. These fungi host a widespread and diverse mycovirus community composed of more than 16 species of *Partitiviridae*, a species of *Narnaviridae* and one taxonomically unassigned virus related to the Curvularia thermal tolerance virus. These viruses are able to cross species borders, co-infect single host strains and cause phenotypic changes in their hosts. The abundance of viruses increases over time in *Heterobasidion* infection centers, and they are targeted by fungal RNA interference. Long-term field studies are essential for obtaining a comprehensive view of virus effects in the nature.

1. *Heterobasidion* biology and influence on practical forestry

The genus *Heterobasidion* includes some of the most disastrous forest pathogens in boreal forests worldwide. The annual economic losses caused by these fungi exceed 50 and 800 million euros only in Finland and Europe, respectively, and more damage occurs in other parts of the Northern hemisphere. The *Heterobasidion annosum sensu lato* species complex includes five species of white rot fungi. In Europe, three species cause forest damage: *H. annosum sensu stricto* is able to infect several tree species including pines and spruces, *H. parviporum* causes root and stem decay mainly on Norway spruce (*Picea abies*), and in southern Europe *Heterobasidion abietinum* attacks *Abies* species (Niemelä and Korhonen, 1998). Two species of the *H. annosum s. lat.* complex occur in North America: *H. irregulare* and *H. occidentale*. The former of these was introduced to Italy during the second world war (Gonthier et al., 2007), and hybridized with the European *H. annosum s. str.* (D'Amico et al., 2007 Gonthier and Garbelotto, 2011). The genome of *H. irregulare* has been sequenced and annotated (Olson et al., 2012).

The center of evolution of *Heterobasidion*spp. is most probably in East Asia, although speciation has also occurred in Europe (Chen et al., 2015; Dai et al., 2003). This view is supported by the occurrence of several endemic species in eastern and southern Asia and Oceania: *Heterobasidion araucariae* and species forming the *Heterobasidion insulare sensu lato* species complex (*Heterobasidion insulare*, *Heterobasidion ecrustosum*, *Heterobasidion australe*, *Heterobasidion orientale*, *Heterobasidion linzhiense, Heterobasidion amyloideum and Heterobasidion tibeticum*) (Buchanan, 1988; Chen et al., 2014; Dai et al., 2007; Dai and Korhonen, 2009; Tokuda et al., 2009).

Strains of *Heterobasidion* spp. are efficient in infecting healthy trees: spore-mediated infections occur frequently on fresh stump surfaces, from which the mycelium may grow via root contacts to neighboring trees (Stenlid and Redfern, 1998). Thus, once the stand is contaminated, the fungus continues its growth from tree to tree. In practical forestry, *Heterobasidion* species are controlled by tree species selection (i.e., rotation), chemical or biological stump treatments against spore infection, and stump removal. However, none of these methods gives full protection against the pathogen. Change of the tree species is not always possible due to soil characteristics, herbivores or economic reasons. Stump treatment is not effective in already contaminated stands, and even when used on healthy sites its efficiency is less than 100%. Stump removal leaves infected roots in the site, and therefore removes only a fraction of the pathogen population (Piri and Hamberg, 2015). It may also interfere with the nutrient balance of the soil. Therefore, new solutions to minimize *Heterobasidion* damages are needed, and the possibility of using mycoviruses as biocontrol agents against the host fungus deserves investigation.

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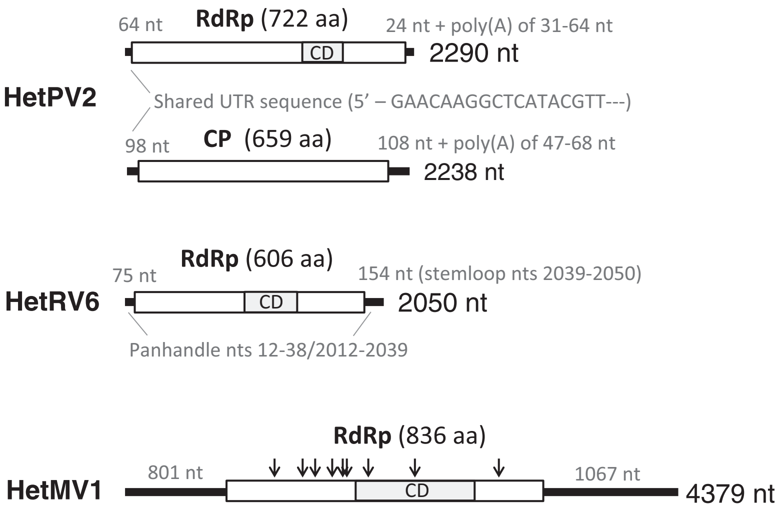

Fig. 1. Genome organization of HetPV2 (*Partitiviridae*, *Betapartitivirus*), HetRV6 (unassigned, CThTV-related) and HetMV1 (*Narnaviridae*). The untranslated regions are shown in black. The conserved core RdRP regions (CD) are shown in grey. The arrows in the HetMV1 genome indicate the positions of UGA codons within the RdRP encoding region, which encode tryptophan using the mitochondrial translation table (UGA is a stop codon when translated by standard codon usage).

2. The incidence and taxonomy of viruses in *Heterobasidion* spp.

H. annosum and *H. parviporum* have been shown to host dsRNA viruses in a frequency of approximately 15% in Europe and western Asia (Ihrmark, 2001). Most of the virus species observed so far belong to the family *Partitiviridae* (Ihrmark, 2001; Kashif et al., 2015; Vainio et al., 2010, 2011a,b, 2013a; 2015b). Partitiviruses infecting *Heterobasidion* spp. have bisegmented dsRNA genomes encoding a coat protein (CP) and an RNA-dependent RNA polymerase (RdRP) (Fig. 1), which are packed in separate virions.

To date, thirteen different species of partitiviruses have been described in *Heterobasidion* spp. (Table 1), and all can be assigned to genus *Alphapartitivirus* or *Betapartitivirus* (Nibert et al., 2014). However, according to recent species delimitation criteria (www.ictvonline.org), virus strains classified as HetPV1 may be assigned to at least two additional species, that we propose to be renamed as HetPV10-oc1 and HetPV11-au1 (these strains were formerly named as HetRV1-oc1 and HetRV1-au1, respectively; Vainio et al., 2011a). Taken together, *Heterobasidion* partitiviruses are extremely polymorphic (Fig. 2): in the genus *Alphapartitivirus* they form at least five different clusters composed of 9–11 different species and in *Betapartitivirus* two separate clusters with four species (Kashif et al., 2015). Interestingly, *Heterobasidion* alphapartitiviruses are relatively closely related to many plant-infecting partitiviruses, for example the white clover cryptic virus 1 (Fig. 2).

Heterobasidion partitiviruses are found throughout the world (Europe, Asia and North America) and in both pathogenic and saprotrophic *Heterobasidion* species (all species of *H. annosum sensu lato* as well as two species of the *H. insulare* species complex). However, all species of *Partitiviridae* are relatively rare and constitute less than 30% of dsRNA infections hosted by *Heterobasidion* spp. (Vainio et al., 2011a).

The most common mycovirus species in *Heterobasidion* spp. is a taxonomically unassigned virus species Heterobasidion RNA virus 6 (HetRV6), which constitutes more than 70% of all the dsRNA viral infections (Vainio et al., 2012). This virus has spread to Europe and North-America (Table 1), but has not been found to infect species of *H. insulare s. lat.* in East Asia. In contrast to partitiviruses, HetRV6 seems to be a single virus species with relatively low overall sequence polymorphism. HetRV6 occurs in many different *Heterobasidion* species originating from distant locations (e.g., Europe, Siberia, USA), and most of the observed differentiation seems to be host or geography-related (Vainio et al., 2012). Nevertheless, a high number of different HetRV6 strains can be found even in single forest plots (Vainio et al., 2015b).

HetRV6 is distantly related to the Curvularia thermal tolerance virus (CThTV; Márquez et al., 2007), and both viruses seem to be members of a new virus family with nine members described so far (Botella et al., 2015), most of them cryptic (Fig. 3). The genome structure of this family appears to be variable as four species (Fusarium graminearum dsRNA mycovirus-4, CThTV, Rhizoctonia solani dsRNA virus 1 and Cryphonectria parasitica bipartite mycovirus) have (at least) two genome segments, whereas only one genome segment (encoding a polymerase gene) has been found in five species (HetRV6, Alternaria longipes dsRNA virus 1, Beauveria bassiana non-segmented virus 1, Ustilago virens dsRNA virus 4 and Gremmeniella abietina RNA virus 6; Fig. 1). Based on RdRP sequence similarity, this new putative mycovirus family is distantly related to partitiviruses, and also plant-infecting species of *Potyviridae*.

Finally, a mitovirus designated as Heterobasidion mitovirus 1 (HetMV1-an1; family *Narnaviridae*) was very recently observed in *H. annosum* with small RNA deep sequencing (Vainio et al., 2015a). As typical for mitoviruses, HetMV1-an1 encodes a single protein, the RdRP (Fig. 1), but the genome size of HetMV1-an1 is somewhat larger than typical for mitoviruses (Hillman and Cai, 2013), and it shares only about 33% protein level sequence identity with its closest relative, Clitocybe odora mitovirus (Heinze, 2012). The *H. annosum* isolate hosting HetMV1 has a 200-fold quantity of HetMV1 transcripts compared to a co-infecting partitivirus (HetPV13-an1) (Vainio et al., 2015a). Despite this, the virus was not detected by traditional virus screening assays based on CF11 chromatography, and whether or not mitoviruses occur frequently in other strains of *Heterobasidion* remains to be investigated. In general, mitoviruses are common among fungi (Hillman and Cai, 2013), but only four mitovirus species have so far been described in basidiomycetes (Fig. 4).

3. Transmissibility of *Heterobasidion* viruses (laboratory transmission experiments and phylogenetic evidence)

Several studies have shown that viruses are transmitted both within and between *Heterobasidion* species on artificial growth medium (Hyder et al., 2013; Ihrmark et al., 2002; Jurvansuu et al., 2014; Vainio et al., 2010, 2011a, 2011b, 2012; 2013b, 2015b). This can be considered rather unusual, as vegetative (somatic) incom-