Diversity of RNA viruses in declining Mediterranean forests

1 **Sergio Diez-Hermano[1*], Pedro Luis Pérez-Alonso[1], Wilson Acosta Morell[1], Jonatan Niño-**
2 **Sanchez[1], Marcos de la Peña[2], Julio Javier Diez[1*]**

3 [1] Department of Plant Production and Forest Resources, Sustainable Forest Management Research
4 Institute (iuFOR). Higher Technical School of Agricultural Engineering (ETSIIAA), University of
5 Valladolid, Palencia, Spain.

6 [2] Institute for Plant Molecular and Cellular Biology (CSIC-UPV), Valencia, Spain.

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8 ***Authors for correspondence:**

9 Julio Javier Diez Casero
10 Higher Technical School of Agricultural Engineering of Palencia (ETSIIAA), Campus La Yutera
11 Avenida de Madrid, 503, 34004 Palencia, Spain
12 Phone: +34979108420
13 E-mail: JulioJavier.Diez@uva.es
14
15 Sergio Diez-Hermano
16 Higher Technical School of Agricultural Engineering of Palencia (ETSIIAA), Campus La Yutera
17 Avenida de Madrid, 503, 34004 Palencia, Spain
18 Phone: +34979108432
19 E-mail: sergio.diez.hermano@uva.es

20 **Abstract**

21 Global change alters forestry habitats and facilitates the entry of new pathogens that don't share a co-
22 evolution history with the forest, leading them into a spiral of decline. As a result, relationships
23 between forests' organisms get disbalanced. Under this scenario, RNA viruses are of particular
24 interest, as they participate in many of such relationships thanks to their ability to infect a wide range
25 of hosts, even from different kingdoms of life. For this reason, the study of RNA viruses is essential
26 to understand how viral flow across different hosts might occur, and to prevent possible outbreaks of
27 diseases in the future. In this work the RNA virus diversity found in trees, arthropods and fungi from
28 declining Mediterranean forests is described. To this extent, three habitats (*Quercus ilex, Castanea*
29 *sativa* and *Pinus radiata*) were sampled and RNAseq was performed on tree tissues, arthropods and
30 fungi. 146 viral sequences were detected by searching for matches to conserved motifs of the RNA-
31 dependent RNA polymerase (RdRP) using Palmscan. Up to 15 viral families were identified, with
32 Botourmiaviridae (28.7%) and Partitiviridae (9.6%) being the most abundant. In terms of genome
33 type, ssRNA(+) viruses were the most represented (83.5%), followed by dsRNA (15%) and two
34 ssRNA(-) representatives. Viruses belonging to families with cross-kingdom capabilities such as
35 Hypoviridae (1), Mitoviridae (6) and Narnaviridae (5) were also found. Distribution of viruses across
36 ecosystems was: *Q.ilex* (57.5%), *P.radiata* (26.7%) and *C.sativa* (15.8%). Interestingly, two RdRP
37 sequences had no matches in available viral databases. This work constitutes a starting point to gain
38 insight into virus evolution and diversity occurring in forests affected by decline, as well as searching
39 for novel viruses that might be participating in unknown infectious pathways.

40 **Key Words:** virome, metavirome, virosphere, forest pathology, *Quercus, Castanea, Pinus*

1   Introduction

In recent decades, it has been shown that all biotic constituents of forest ecosystems can carry and be affected by viruses. In addition to plant viruses, all plant-associated organisms (bacteria, protists, fungi, vertebrate and invertebrate animals) have their own viral communities (virome), and together they form the metavirome or virosphere of the forest system. Currently, 64 virus species are known to affect 19 forest plant species in temperate and urban forests (Rumbou et al., 2021). Based on genome type (single-stranded or ss, double-stranded or ds, positive-sense, or antisense), most are (+)ssRNA viruses belonging to the *Beta-, Seco-, Bromo-, Tombus-, Virga-* and *Mayoviridae* families. In comparison, only one (-)ssRNA family has been found so far, the *Emaraviridae*, consisting of four species. Three dsRNA viruses have also been described in pine and ash, as well as three other dsDNA viruses with retrotranscriptase capacity in *Betula* sp., *Castanea sativa* and *Fraxinus americana*. Among all the viral diversity described, the genera *Emaravirus* and *Badnavirus* are considered to be tree pathogens and, therefore, causative agents of the diseases with which they are commonly associated.

In contrast to trees' virome, most viruses affecting forest fungi and oomycetes have a dsRNA genome and belong to the *Partiti-, Toti-, Curvula-* and *Reoviridae* families. Viruses with a (+)ssRNA genome are classified within the families *Hypo-, Endorna-, Botourmia-, Fusari-* and *Mitoviridae*, and some of their members have been found to be able to induce changes in the level of pathogenicity of forest pathogens of particular relevance, such as *Cryphonectria parasitica, Ophiostoma novo-ulmi, Gremmeniella abietina, Fusarium circinatum, Heterobasidion annosum* and *Hymenoscyphus fraxineus* (van Diepeningen, 2021). In addition, the first (-)ssRNA and ambivalent (combining + and - sense strands in their genome) viruses of fungal pathogens have recently been described in *Armillaria* sp. and *C. parasitica* (Forgia et al., 2021; Linnakoski et al., 2021). Finally, high viral diversity has also been found in soil pathogens such as *Rosellinia necatrix*, leading to the discovery of new *Hypoviridae* and candidates yet to be classified, such as *Fusagraviruses* and *Megatoviruses* (Arjona-López et al., 2018).

Current virus taxonomy is based on the presence of homologous genomic structures between viral families. In particular, plant viruses tend to cluster with fungal and arthropod viruses (Lefeuvre et al., 2019). This is indicative of the ability of some viruses to overcome barriers between biological kingdoms and infect a wide diversity of organisms (cross-kingdom viruses), which, in the context of forests, mainly involves trees, fungi and insects. Cross-kingdom viruses represent a considerable proportion of the fungal virome, to the extent that 50% of known fungal species carry plant viruses (Cao et al., 2022). Some members of the *Toti-, Partiti-, Endorna-* and *Chrysoviridae* families are plant viruses capable of replicating in meristematic cells and infecting fungi in vitro (Nerva et al., 2017). The first experimental evidence of a natural cross-kingdom infection was found in *Rhizoctonia solani*, which was able to acquire and transmit the Cucumber mosaic virus from its host plant (Andika et al., 2017). Fungal and plant viruses are able to act synergistically and facilitate transmission even between vegetatively incompatible fungal strains, using the plant as a transport pathway. This phenomenon has been described experimentally for the *Cryphonectria hypovirus-1* and the Tobacco mosaic virus, and active expression of the p29 gene of the former and the movement proteins (MPs) of the latter were necessary for it to take place (Bian et al., 2020). MPs are unique to plant viruses and are necessary for viral particles to move from cell to cell. However, fungal viruses, which do not usually have an extracellular phase, lack them. This display of functional cooperation gives an idea of the potential of viruses to infect unexpected organisms, regardless of the evolutionary distance that separates them.

86 Insects are also susceptible to cross-kingdom viral infections. Despite being the most numerous
87 group of vectors transmitting plant viruses, few studies have been devoted to unravelling the virome
88 of insects affecting forest species. Once inside the insect, plant viruses can develop forms of non-
89 persistent transmission (in external structures of the insect) or circular transmission (using internal
90 compartments as reservoirs) (Whitfield et al., 2015). In some cases, circular transmission may
91 involve replication of the plant virus within the cells of the insect host. In the case of European
92 forests, viruses have been found in *Lepidoptera* such as *Lymantria dispar, Thaumetopoea*
93 *pityocampa* and *Leucoma salicis*, in *Coleoptera* such as *Ips typographus* and in *Hymenoptera* such as
94 *Neodiprion sertifer* (Jakubowska et al., 2015; Paraskevopoulou et al., 2021). Fungal viruses capable
95 of infecting insects, such as some DNA viruses of the *Genomoviridae* family, have also been
96 detected (Liu et al., 2016).

97 Yet, despite having more information than ever about the viral component of forest ecosystems,
98 studies on the virosphere of Mediterranean forests are still lacking, to the best of our knowledge.
99 Mediterranean forests rank among the terrestrial ecosystems threatened by globalisation and climate
100 change the most (Newbold et al., 2020). Weather predictions for the coming decades project drastic
101 reductions in precipitation patterns and rising temperatures, exposing forests in the Mediterranean
102 basin to long periods of drought and massive forest fires (Nunes et al., 2022). The high frequency of
103 climatic adversities decreases the ability of Mediterranean tree species to cope with the high number
104 of pests and diseases spread by globalisation, facilitating the invasion of new pathogens with which
105 forests have not had the opportunity to co-evolve. There is evidence that most RNA viruses, many of
106 which are known causative agents of emerging infectious diseases, are contained in forest ecosystems
107 and are often released as a result of forest disturbance and expanding human populations (Wilcox and
108 Ellis, 2006; Mackay and Arden, 2016), increasing the risk that unexpected pandemics might arise.

109 Therefore, given the capacity of RNA viruses to jump between organisms and even to alter essential
110 characteristics such as the pathogenicity level of their host, getting a better understating of the viral
111 flows that might occur in endangered ecosystems is of the essence. With this purpose, the
112 metavirome of three Mediterranean forests affected by decline is described here, by means of high-
113 throughput sequencing and for different kingdoms of life, including plants, fungi and arthropods.

114 ## 2   Materials and methods

115 ### 2.1   Sampling sites and procedure

116 Bark, wood and leaves' samples were extracted from trees of *Castanea sativa* (chestnut), *Quercus*
117 *ilex* (holm oak) and *Pinus radiata* (Monterrey pine) from Castile and Leon (Spain) forests (Table 1
118 and Figure 1). Each sampling site corresponded to a single tree species. Sampling was conducted for
119 all four sites in June and July 2021. Material from sixteen trees was sampled and pooled per plot.
120 After removing the external bark, one sample was taken per tree from the main trunk at the height of
121 50 cm over the collar, to a depth of 2-3 cm. Only xylem and the internal bark layer (phloem) were
122 considered in the analysis. Living individuals of common arthropods (ants, grasshoppers, beetles)
123 were also collected (8-10 specimens each). Samples were stored at 4ºC for one day prior to
124 processing and at -80ºC afterwards.

125 ### 2.2   Sample processing and sequencing

126 Fungi were cultured from the trees and arthropods' samples. Bark, wood and leaves were cut into
127 small pieces and disposed on Petri dishes with PDA+ampicilin (100 ug/ml) medium for 3 days at
128 25ºC. Arthropods were washed in a Ringer solution and 50 ul of the solution were poured and spread

129 also on Petri dishes with PDA+ampicilin medium for 3 days at 25ºC. Visually different fungal
130 colonies were picked, isolated and stored at -80ºC.

131 Frozen samples of bark, wood, leaves and arthropods were pulverised and pooled per sampling site
132 prior to RNA extraction. Isolated fungi were pooled likewise. Total RNA was purified from trees,
133 arthropods and fungi using Spectrum Plant kit (Sigma Aldritch, MO, USA) following the
134 manufacturer's protocol. Quality control with Qubit yielded RIN values > 7 for all extractions.
135 Samples were sent to Macrogen (South Korea) for sequencing (Illumina Miseq with TruSeq libraries
136 and ribosomal RNA depletion, 150 bp paired-end reads).

2.3 Bioinformatic analysis

138 Raw reads were cleaned and trimmed using Cutadapt v.3.5 (-q 20) (Martin, 2011). Contigs were
139 assembled with SPAdes v.3.15.5 (rnaviralSPAdes.py) (Prjibelski et al., 2020) and scanned for the
140 presence of RNA-dependent RNA polymerase (RdRp) sequences using Palmscan v1.0 (Babaian &
141 Edgar, 2022), a software that searches sequencing data for matches with the conserved motifs A, B
142 and C (the "palmprint") from the primary protein sequence of RdRp. RdRp sequences that matched
143 any non-viral organism in nr NCBI database were discarded. Viral identities were assigned to the
144 remaining RdRps by aligning the found sequences against PALMdb (Edgar et al., 2022) and RdRp-
145 scan (Charon et al., 2022) databases using DIAMOND v2.1.8 (Buchfink et al., 2021). Taxonomy
146 resolution was stablished according to different percent identity thresholds (species at >90%, genus at
147 >70% and family at >30%). Multiple sequence alignment was performed with MAFFT (E-INS
148 iterative refinement method) (Katoh et al., 2019) and used as input for phylogenetic tree building on
149 IQTREE (default parameters) (Nguyen et al., 2015) with ultrafast bootstrap (Hoang et al., 2018) and
150 automatic model selection (Kalyaanamoorthy, 2017).

151 Viral diversity analyses were performed and visualised in R environment 4.2.1 (R Core Team, 2022)
152 using the packages *ggtree* (Yu, 2020), *ggmsa* (Zhou et al., 2022) and *seqinr* (Charif et al., 2023).
153 Sequence logos were plotted with Skylign (Wheeler et al., 2014)

3 Results

3.1 Taxonomy and distribution of RdRps

156 A total of 146 RdRps were found among all analysed samples. Of these, 122 (83%) were assigned to
157 existing viral groups with different levels of resolution, 22 (15%) had matches in current databases
158 but lacked taxonomy assignment and 2 were absent from any database (Table 2 and Table 3). Most of
159 the RdRps were found in the habitat of *Q. ilex* (57.5%), followed by *P. radiata* (26.7%) and *C. sativa*
160 (15.8%) (Figure 2 and Table 4). Regarding sample type, half of the RdRps were found in tree
161 samples (46.5%), followed by arthropods (21.2%), fungi from arthropods (16.4%) and fungi from
162 trees (15.7%).

163 The most numerous viral families were Botourmiaviridae (28.7%), Partitiviridae (9.6%), Mitoviridae
164 (4.1%) and Narnaviridae (4.1%) (Figure 3). In terms of genome type, ssRNA(+) viruses were the
165 most represented (83.5%), followed by dsRNA (15%) and two ssRNA(-) representatives, Chuviridae
166 and Phenuiviridae, found in arthropods from pine and chestnut habitats, respectively.

167 The distribution of RdRps revealed that sample types have different viral profiles (Figure 3). Some of
168 the families were exclusive to fungi (Totiviridae, Fusariviridae, Hypoviridae and Polymycoviridae)
169 or arthropods (only in chestnut habitat: Iflaviridae, Nodaviridae, Phenuiviridae; and only in pinus

170   habitat: Sinhaliviridae, Chuviridae and Dicistroviridae). One family was found to be exclusive to
171   holm oak's habitat (Tombusviridae), regardless of sample type. Endornaviridae family was found
172   only in pines whereas Botybirnavirus was exclusive to holm oaks.

3.2   Phylogeny of RdRps

174   MSA of the RdRps resulted in the dendrogram shown in Figure 4. RdRps from trees and arthropods'
175   samples clustered in two distinct groups, while RdRps from isolated fungi were interspersed, forming
176   heterogeneous subgroups. No grouping by habitat was observed except for most RdRps from holm
177   oak samples clustering together within the Lenarviricota supernode. All of the RdRps identified up to
178   species levels belonged to arthropods' samples: Lake sinai virus 1 and 2, Deformed wing virus,
179   Black queen cell virus and Hubei picornalike virus 15, in the Kitrino- and Pisuviricota nodes. In
180   nodes where two RdRp from trees' fungi and arthropods' fungi were paired, as is the case for some
181   Betapartitivirus and Narnaviridae (bottom part of the tree), their sequences shared 100% identity. In
182   all other cases the homology in the conserved RdRp motifs A, B and C was at least greater than 50%.

183   RdRps existing in current databases but lacking a taxonomy (NA in Figure 4) were classified as
184   Kitrino-, Duplorna- or Pisuviricota. As for the unknown RdRps (with bona-fide A, B and C motifs
185   but absent from databases), one shared high sequence similarity with the Hypoviridae representative
186   and was found in the same sample type (fungi from holm oaks), whereas the other was located in the
187   vicinity of ssRNA(-) viruses (Chuviridae and Phenuiviridae) but shared no common origin with
188   them.

4   Discussion

190   The study of environmental viromes has grown in popularity thanks to recent major advances in
191   massive sequencing technologies. The scale of these analyses is highly variable, with some studies
192   focusing on a particular species (Faizah et al., 2020; Wu et al., 2015), complete ecosystems (Hurwitz
193   & Sullivan, 2013) or different environments (Daugrois et al., 2021; Ma et al., 2021). However, most
194   of the advances in plant virology focus on crops or fruit trees of economic interest, which contrasts
195   the scarcity of such studies in forestry (Rumbou et al., 2021). Reducing the scope even more to
196   Mediterranean forests, with the Mediterranean basin being considered a biodiversity hotspot (Médail
197   et al., 2019), hardly any information is found. In the present work, focused on 3 Mediterranean forest
198   habitats in north-western Spain, 146 RdRps of viral origin have been found, of which 22 have no
199   clear taxonomy yet and 2 are completely unknown. This shows the great lack of knowledge about
200   viral diversity that exists in our own forests.

201   Most of the reported RdRps have been identified as ssRNA(+) viruses, which are accepted to be the
202   evolutionary origin of the other two identified groups, ssRNA(-) and dsRNA (Koonin et al., 2015,
203   2021). Holm oak's habitat showed the highest viral richness (57.5% of all the RdRps). Holm oak
204   samples were taken in a "dehesa", a unique ecosystem in the Iberian Peninsula that has been
205   modified by man to simultaneously obtain livestock and non-timber forest resources. This makes
206   dehesas an ecosystem with particularly high biodiversity (Moreno et al., 2016; Rodríguez-Rojo et al.,
207   2022), so it is not surprising that there is also a high viral diversity that has never been investigated.

208   Some of the viral families have been found to be ubiquitous across sample types and habitats, which
209   could be interpreted as a first indication of their cross-kingdom potential. This is the case for
210   Botourmiaviridae, Partitiviridae, and Narnaviridae. Many genera in Botorumiaviridae family are
211   known to infect fungi, and some of them have been identified on grapevine leaves affected by the
212   oomycete *Plasmopara viticola* (Chiapello et al., 2020). Mycoviruses of this family can persist in their

213 fungal host without the need for a capsid and are thought to require only the RdRp to replicate (Wang
214 et al., 2020). Only the genus Ourmiavirus infects plants exclusively (Rastgou et al., 2009). The RdRp
215 of this genus is most similar to that of the genera Mitovirus and Narnavirus (family Narnaviridae)
216 and its movement protein (MP) is similar to that of the family Tombusviridae. The envelope protein
217 bears some similarity to that of some plant or animal viruses. These viruses are very easily
218 transmitted mechanically and no vector has been identified, so it is thought that there could be a
219 horizontal transmission (Ayllón et al., 2020).

220 The Narnaviridae family has the simplest genome of all RNA viruses, encoding only one polypeptide
221 in which RdRp is found. Within this family, the genus Narnavirus replicates in the cytoplasm, while
222 Mitoviruses replicate in mitochondria and can cause hypovirulence in pathogenic fungi (de Rezende
223 et al., 2021; Hillman & Cai, 2013). Phylogenetically, Narnaviruses are much more closely related to
224 Ourmiaviruses (family Botourmiaviridae) than to Mitoviruses. Although no Mitoviruses have been
225 found outside fungi, we've found one RdRp identified as a Mitovirus in a pine tree sample.
226 Nonetheless, Mitovirus sequences have been found before in plant mitochondrial sequences (Hong et
227 al., 1998; Marienfeld et al., 1999). As for the Partitiviridae family, many of its genera have
228 characteristic hosts, which can be plants (Deltapartitivirus), fungi (Gammapartitivirus) and protozoa
229 (Cryspovirus). Many sequences of this family have also been identified in arthropods (Cross et al.,
230 2023). Here we've identified one genus, Betapartitivirus, whose hosts can be plants and fungi.

231 Regarding the family Tombusviridae, it has been described in animals (Yin et al., 2022), plants
232 (Lappe et al., 2022) and fungi (Botella et al., 2022), matching our finding of this family in all sample
233 types although restricted to holm oak habitat. Tombusviridae infections are usually limited to the root
234 system, but they can infect the whole plant thanks to their MPs (Canuti et al., 2023). Tombusviridae
235 are transmitted mechanically, by contact, through seeds or through a vector such as a fungus or an
236 insect, depending on the genus. The Solemoviridae family infects plants, but it is known that one of
237 its modes of transmission is also the use of insects as vectors (Sõmera et al., 2021), which may be
238 one of the reasons why we have found this family only in arthropods.

239 For some families, only one RdRp has been detected in the samples analysed, but evidence of its
240 ability to infect several kingdoms has been described. This is the case for the Totiviridae, Chuviridae,
241 Phenuiviridae, Chrysoviridae, Polymicoviridae, Endornaviridae and two riboviruses with incomplete
242 classification: the genus Botybirnavirus and the species Hubei picorna-like virus 15 (unclassified
243 ribovirus). The family Totiviridae is associated with latent infections in fungi and protozoa. The
244 families Chuviridae and Phenuiviridae, exclusive to insects, are the only ones that possess a ssRNA(-
245) genome. Many endogenous viral elements inserted within the host genome have been related to
246 Chuviridae infections (Dezordi et al., 2020). The Chrysoviridae family infects fungi, plants and
247 insects (Kotta-Loizou et al., 2020). The Polymycoviridae family affects fungi in which it causes
248 hypovirulence or hypersensitivity to antifungals or bacteria, and is even capable of modulating
249 carbon, nitrogen and iron metabolism (Kotta-Loizou & Coutts, 2022). Endornaviridae are ssRNA(+)
250 viruses that do not have a true capsid as the genome is encapsulated together with a viral replicase
251 and are able to infect plants and fungi. The genus Botybirnavirus (Orthornavirae) has fungi as natural
252 hosts (Hough et al., 2023), however, we've found this RdRp in a holm oak tree sample, which could
253 be indicative of some cross-kingdom capability.

254 Finally, some limitations of the present study should be noted. First, many sequences in viral
255 databases have none or incomplete taxonomic assignment, and the high genetic variation of viruses
256 makes it difficult to obtain an accurate assignment at genus or species level. Recurrent updating of
257 the RdRps databases is necessary to facilitate the assignment of new ones. Secondly, although some

258 of the virus families described may be "cross-kingdom" because they contain members that infect
259 several kingdoms, it is more common for individual viruses to specialise in hosts of a single
260 kingdom, as is the case, for example, with the Totiviridae family, where some genera infect fungi and
261 others protozoa. Similarly, the *in silico* analysis performed does not allow us to verify whether
262 finding RdRps in organisms from different kingdoms than expected implies that the host is
263 susceptible to the virus, or that it only acts as a vector or asymptomatic carrier.

5   Conclusion

265 In this work we found great variation in the diversity of RNA viruses of declining Mediterranean
266 forests, with holm oak forest having the highest richness and chestnut the lowest. Up to 15 viral
267 families were identified overall, with Botourmiaviridae and Partitiviridae being the most abundant. In
268 terms of genome type, ssRNA(+) viruses were the most represented, followed by dsRNA and two
269 ssRNA(-) representatives. Viruses belonging to families with cross-kingdom capabilities such as
270 Hypoviridae, Mitoviridae and Narnaviridae were also found. Lastly, two RdRP sequences had no
271 matches in available viral databases and should be further investigated.

6   Data availability statement

273 Data and scripts used in this study are available in the following GitHub repository:
274 https://github.com/serbiodh/2023_Virome_MedForests. Raw sequencing data are available at NCBI
275 SRA under BioProject PRJNA1032577.

7   Author contributions

277 SDH, JNS and WAM collected the data. JNS and WAM carried out the laboratory procedures. SDH,
278 PLPA and MP analysed the data. SDH, PLPA and JJD wrote the manuscript. SDH, JNS and JJD
279 designed the sampling scheme. All authors contributed to the article and approved the submitted
280 version.

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10   Conflict of interest

288 The authors declare that the research was conducted in the absence of any commercial or financial
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477 **12   Figure legends**

478 **Figure 1. Map of sampling sites. A** Map of Spain. Castile and Leon region is highlighted with a
479 black and thick contour. Orange and purple areas correspond to Salamanca and Leon provinces,
480 respectively. **B** Municipalities from where samples were taken (top: Leon, bottom: Salamanca). **C**
481 Sampling areas (white polygons) overlayed on top of physical maps. Coloured lines in La Alamedilla
482 correspond to four sampling transects.

483 **Figure 2. RdRp counts by habitat and sample type.**

484 **Figure 3. Distribution of viral families per habitat, sample type and genome type.** Colour's
485 gradient from white to purple indicates abundance.

486 **Figure 4. Phylogenetic tree of RdRps.** Left: consensus tree with bootstrap confidence values. Each
487 branch is labelled with the taxonomic level followed by the identity assigned to that particular RdRp.
488 Vertical rectangles with black outline indicate viral phyla (-viricota). Right: multiple sequence
489 alignment with A, B and C motifs and their corresponding logos. Blue and red regions indicate well-
490 conserved residues.

491 **13   Tables**

492 **Table 1.** Description of sampling sites.

Location	Province	Characteristics	Sampled species
La Alamedilla	Salamanca	Elevation: 756 m Rainfall: 600-854 mm	*Quercus ilex*
Las Médulas	León	Elevation: 511 m Rainfall: 400-500 mm	*Castanea sativa*
Villafranca del Bierzo	León	Elevation: 505 m Rainfall: 400-500 mm	*Pinus radiata*

493 **Table 2.** Taxonomic distribution of RdRps.

Phylum	Class	Order	Family	Genus	Species	Not assigned[1]	Unknown[2]
4	14	12	67	20	5	22	2

494 [1]RdRps found in databases that had no taxonomy assigned yet
495 [2]RdRps that could not be found in any database

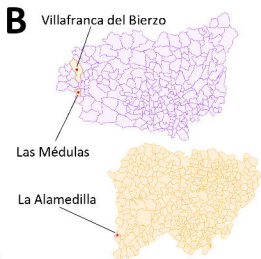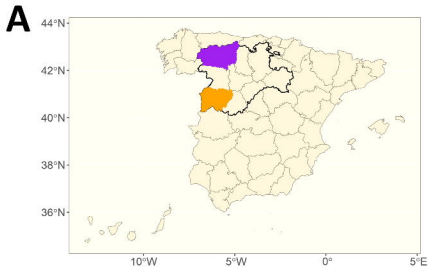496 **Table 3.** Taxonomic assignment of RdRps per genome type.

Genome

	ssRNA(+)	ssRNA(-)	dsRNA
Family (-viridae)	Botourmia- Dicistro- Endorna- Fusari- Hypo- Ifla- Mito- Narna- Sinhali- Solemo- Tombus-	Chu- Phenui-	Chryso- Partiti- Polymyco- Toti-
Genus (-virus)	Alphanoda- Alphanecro- Mito- Narna- Ourmia-		Alphachryso- Betapartiti- Botybirna- Victori-
Species	Black queen cell virus Deformed wing virus Lake Sinai Virus Lake Sinai Virus 2 Hubei picorna-like virus 15		

497   **Table 4. Count of RdRps per habitat and sample type.**

	Tree	**Fungi from tree**	**Arthropod**	**Fungi from arthropod**
Chestnut	1	2	11	9
Pine	16	7	9	7
Holm oak	51	14	11	8

498

A

B Villafranca del Bierzo

Las Médulas

La Alamedilla

C

La Alamedilla	Las Médulas	Villafranca del Bierzo
(*Quercus ilex*)	(*Castanea sativa*)	(*Pinus radiata*)