**Table S1.** List of primers used for direct virus RT-PCR detection

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus** | **Direction** | **Sequences (5‘-3‘)** | **Tm (°C)** | **Product size (nt)** |
| Armillaria ambi-like virus 1 | Forward | CCCCTCATGGTCACTATGGATATG | 61 | 800 |
| Reverse | CTCGCTGAGCCTCTCTACATCTT |
| Armillaria ostoyae ambi-like virus 2 | Forward | CGAGATGTTAGCCCTATACCCATC | 60 | 487 |
| Reverse | GGTGTAGGAACTGTCTGTACTCTG |
| Armillaria ostoyae ambi-like virus 3 | Forward | CCGACTATAGGATTGTCCCTTCAG | 60 | 477 |
| Reverse | CATCTGTCTTATCAGCTCTCTGGG |
| Armillaria ostoyae ambi-like virus 4 | Forward | CTATTCCCTTCCGACTTCTTTCCC | 61 | 588 |
| Reverse | CTCTATCGAGACCAGATCCCAGATC |
| Armillaria ostoyae tymovirus 1 | Forward | GAGCTCTAGGTGAAGTGGAGTATG | 60 | 731 |
| Reverse | CTCTCTTATAGGAACTCTAGGCGC |

**Table S2. A,** Pairwise Sequence Comparison (PASC) percentages (%) based on nucleotide sequence of ambi-like viruses hosted by *Armillaria cepistipes* and *ostoyae*. **B**, PASC % based on RdRP amino acid (aa) sequence of ambi-like viruses hosted by *Armillaria cepistipes* and *A. ostoyae*.

**A** **B**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **AoALV1** | **AoALV2** | **AoALV3** |
| **AoALV2** | 19.53 |  |  |
| **AoALV3** | 77.46 | 18.65 |  |
| **AoALV4** | 90.93 | 19.20 | 77.52 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **AoALV1** | **AoALV2** | **AoALV3** |
| **AoALV2** | 27.66 |  |  |
| **AoALV3** | 31.40 | 31.90 |  |
| **AoALV4** | 85.73 | 29.82 | 30.09 |

**Table S3.** Types and genomic position of the ribozymes detected in *Armillaria* ambiviruses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Virus name** | **Contig name** | **Ribozyme (+)** | **e-value** | **Ribozyme (-)** | **e-value** |
| **AoALV1** | ambi\_spadesMETA\_NODE\_2\_length\_6862 | HHRz (4510-4564) | 1.4e-07 | HHRz (330-254) | 4.6e-11 |
| **AoALV2** | ambi\_spadesMETA\_NODE\_4\_length\_6697 | HPRz (4424-4531) | 5,00E-08 | HPRz (4987-4884) | 5.7e-10 |
| **AoALV3** | ambi\_spadesMETA\_NODE\_8\_length\_4562 | HHRz (1727-1803) | 7.7e-09 | HHRz (2068-2014) | 1.1e-08 |
| **AoALV4** | ambi\_spadesMETA\_NODE\_9\_length\_4549 | HHRz (1-41) | 0.021 | HHRz (333-256) | 2.5e-09 |

\*nt positions of the region spanning the ribozyme in the original virus contig. The (+) polarity is defined as the RNA strand coding for the polymerase (ORFA).