**Additional file 1: Table S1**. Description of primers used in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer name** | **Primer sequence (5′ to 3′)** | **Length (nt)** | **Position in SSU rRNA†** | **Application** |
| Microspor\_270F‡ | GCCTGAGATGGCTMYACG | 21 | 297 | Universal primers for Primary PCR |
| Microspor\_840R‡ | GTTGAGTYAAATTAAGCMGCACA | 23 | 884 |
| Microspor\_446F‡ | GRATGCTGCAGTTAAARDGTC | 21 | 477 | Universal primers for Secondary PCR |
| Microspor\_776R‡ | TCACYCTTGCGAGCRTACTAT | 21 | 819 |
| Microsp\_univ01F | CACCAGGTTGATTCTGCCTGAC | 21 | 1 | Amplification of long SSU rRNA |
| Microsp\_univ02R | GYTRCCTTGTTACGACTT | 18 | 1337 |
| Microsp\_Salam\_S1F | GTACGATGTGCAGGTAAAGTA | 21 | 385 | Specific primers for detection; Primary PCR |
| Microsp\_Salam\_S1R | ACGTCCTTTATCATCGGAATC | 21 | 683 |
| Microsp\_Salam\_S2F | ACGATGTGCAGGTAAAGTATG | 21 | 387 | Specific primers for detection; Secondary PCR |
| Microsp\_Salam\_S2R | CGAGTCATCAAATTTCACCTC | 21 | 625 |

† Position refers to the position of the primer’s 5′ end in the SSU rRNA gene sequence of the *Vavraia*-like parasite determined by this study (GenBank: MH918740).

‡ Adapted from [7]. The number within the primer name indicates the position of the primer’s 5′ end in the reference sequence XR\_002670150 (SSU rRNA gene from *Encephalitozoon intestinalis* ATCC 50506).

**Additional file 1: Table S2.** Comparison between clinical descriptions and PCR detection of microsporidia of the six salamanders from the 2013 outbreak

|  |  |  |  |
| --- | --- | --- | --- |
| Salamander specimens | Clinical implication and histopathology | PCR detection of microsporidia | |
| Universal primers | Specific primers |
| S1 | Mild rhabdomyositis | + | + |
| S2 | Subacute rhabdomyositis | + | + |
| S3 | Intestinal cestodiasis, ovarian folliculitis, multifocal rhabdomyositis and steatitis | \_ | + |
| S4 | Cestodiasis | \_ | + |
| S5 | Moderate lymphocytic rhabdomyositis | \_ | + |
| S6 | Rhabomyositis, intralesional *microsporidia* | + | + |

**Additional file 1:** **Table S3.** The detection of microsporida in salamander food sources and inveterbrates in the environment using primers developed in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Original location on the SMARC** | | **PCR Detection** |
| Snails *(Helisoma anceps )* | QW-5: A flow-through tank system for salamanders | | + |
| Snails *(Elimia comalensis)* | Circular snail tank | | + |
| Amphipoda *(Hyalella azteca)* | Water raceways 1 and 2 | | - |
| Black worms *(Lumibriculus variegatus)* | EN4-4/QW-7 : A flow-through tank system for salamanders | | - |
| Brine shrimp *(Artemia salina)* | BSB Brine Shrimp Tank | | - |
| Fish food flakes | Freezers | | - |
| Water fleas (Cladocera) | Ponds D8/B6: | Two separate ponds to raise invertebrates to feed salamanders | +/- |
| Cyclopods (Cyclopoida) | Ponds D8/B6 | -/+ |
| Seed shrimps (Ostracod) | Ponds D8/B6 | -/- |
| Copepods (Calanoida) | Ponds D8/B6 | -/- |
| Back swimmer (Notonectidae) | Ponds D8/B6 | -/- |