|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **ITS1** | **ITS2** | **18S** | ***Cytb*** | ***cox1*** |
| **PCR Mix** | | | | | |
| PCR Buffer (5X) | 10 µl | 10 µl | 5 µl | 10 µl | 10 µl |
| dNTPs (10mM) | 2 µl | 1 µl | 1 µl | 1 µl | 1 µl |
| MgCl2 (25 mM) | 6 µl | 6 µl | 4 µl | 4 µl | 4 µl |
| Forward Primer (10 μM) | 5 µl | 5 µl | 5 µl | 5 µl | 5 µl |
| Reverse Primer (10 μM) | 5 µl | 5 µl | 5 µl | 5 µl | 5 µl |
| Template DNA | 5 µl | 5 µl | 5 µl | 5 µl | 5 µl |
| *goTaq* DNA polymerase | 0,5 µl | 0,5 µl | 0,5 µl | 0,5 µl | 0,5 µl |
| Autoclaved distilled water to | 100 µl | 50 µl | 50 µl | 50 µl | 50 µl |
| **PCR Primers** | | | | | |
| Forward Primer | NC5 (Gasser et al., 1996) | senITS2 (Vobis et al., 2004) | 18SF (Kaewmongkol et al., 2011) | CytbF (Dittmar &Whiting, 2003) | LCO1490 (Folmer et al., 1994) |
| Reverse Primer | ITS1rev (Marrugal et al.., 2013) | ITS2R (Luchetti et al., 2007) | 18SR (Kaewmongkol et al., 2011) | A5F (Dittmar &Whiting, 2003) | HCO2198 (Folmer et al., 1994) |
| **PCR Conditions** | | | | | |
| Initial Denaturing | 94 ºC for 5´ | 94 ºC for 5´ | 96 ºC for 2´ | 95 ºC for 12´ | 96 ºC for 2´ |
| Number of cycles | 35 | 35 | 45 | 30 | 40 |
| Denaturing | 94 ºC for 30´´ | 94 ºC for 60´´ | 94 ºC for 50´´ | 95 ºC for 30´´ | 94 ºC for 30´´ |
| Annealing | 58 ºC for 30´´ | 55 ºC for 60´´ | 58 ºC for 60´´ | 40 ºC for 30´´ | 50 ºC for 30´´ |
| Primer extension | 72 ºC for 90´´ | 72 ºC for 60´´ | 72 ºC for 90´´ | 68 ºC for 2´ | 72 ºC for 60´´ |
| Final extension | 72 ºC for 5´ | 72 ºC for 10´ | 72 ºC for10´ | 68 ºC for 7´ | 72 ºC for 7´ |

Table S1**.** PCR mix, primers and conditions used for each molecular marker sequenced in this study.

**Folmer, O., Black, M., Hoeh,W., Lutz, R.& Vrijenhoek, R.** (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular *Marine Biology and Biotechnology* **3**, 294–299.

**Gasser, R.B., Nansen, P. & Guldberg, P.** (1996) Fingerprinting sequence variation in ribosomal DNA of parasites by DGGE. *Molecular and Cellular Probes* **10**, 99–105.