Table S1. PCR assays.Cycling conditions for all PCRs used in this publication. Reaction mix consisted of TopBio 2× MasterMix (TopBio, Vestec, Czechia), 0.8 μM of each primer, and 2.0 μl of extracted DNA in a total volume of 25 μl. In the 2nd round of the nested PCR assay, 1 μl of PCR product from the 1st round was used as a template.

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| --- | --- | --- | --- | --- | --- | --- |
| Assay | PCR round | Primer ID | Primer sequence (5´-3´) | Amplicon (bp) | Cycling conditions | Reference |
| diagnostic PCR for *Anoplocephala* (ITS2) | 1st | S18 | TAA CAG GTC TGT GAT GCC | ~1 100 | initial denaturation 3´ at 96°C, 35× 15´´ at 95°C, 15´´ at 55°C, 30´´ at 72°C and final extension 12´at 72°C. | Jousson et al. 1999 |
| L3T | CAA CTT TCC CTA CGG TAC TTG |  | Jousson et al. 1999 |
| 2nd | AP-ITS2\_3F | AAT TGT GGG GGC TTC TCT TA | ~240 | initial denaturation 3´ at 96°C, 35× 15´´ at 95°C, 15´´ at 55°C, 9´´ at 72°C and final extension 5´at 72°C. | Drogemueller, 2004 |
| AP-ITS2\_2R | ATA AAG AAA GGC ACG AGG T |  | Drogemueller, 2004 |
| ITS1 - *Bertiella* specific |  | 201\_F | TAT TGC CTA CCT TCG GTG G | ~300 | initial denaturation 3´ at 96°C, 35× 15´´ at 95°C, 15´´ at 60°C, 7´´ at 72°C and final extension 2´at 72°C. | this study |
| 520\_R | TGT AAT AGA ACT CGA CGC ATA G |  |  |
| 18S SSU (Anoplocephalids) |  | BF | GGA CAC TAT GAG GAT TGA CAG A | ~600 | initial denaturation 3´ at 96°C, 35× 15´´ at 95°C, 15´´ at 55°C (optionally 52°C), 10´´ at 72°C and final extension 5´at 72°C. | Doležalová et al. 2015 |
| 18S\_2445R | TTG GTC GTC TTC TCA GCA |  | this study |
| ITS1 (Anoplocephalids) |  | BertITS1\_F | CTG CGG AAG GAT CAT TAC AC | ~600 | initial denaturation 3´ at 96°C, 35× 15´´ at 95°C, 15´´ at 55°C (optionally 52°C), 10´´ at 72°C and final extension 5´at 72°C. | McLennan 2017 |
| BertITS1\_R2 | GCA GTC TGC GAT TCA CAT TA |  | McLennan 2017 |

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