**Supplementary material, Tables S1.** Polymerase chain reaction recipes and thermal profiles used for *CO*1 and *ITS*2 gene amplification

For fresh material (*i.e*., preserved in 95% ethanol, stored in freezer)

Per sample (for *CO*1; see Folmer *et al.* (1994) for description of primers):

|  |  |
| --- | --- |
| DNA water  | 9.05 μL |
| Q5 buffer (5×)(https://www.neb.ca/q5) | 3 μL |
| dNTPs (10 mM) | 0.3 μL |
| *LCO*1490 (10 μM) | 0.75 μL |
| *HCO*2198 (10 μM) | 0.75 μL |
| Q5 polymerase (2 U/μL) | 0.15 μL |
| Template DNA  | 1 μL |
| Total reaction volume | 15 μL |

\*Use “Step up / Touch down” thermal profile outlined in Astrin *et al.* (2012)

Per sample (for *ITS*2; see Andreev *et al.* (1998) for description of primers):

|  |  |
| --- | --- |
| DNA water  | 9.05 μl |
| Q5 buffer (5×) | 3 μl |
| dNTPs (10 mM) | 0.3 μl |
| *ITS*2F (10 μM) | 0.75 μl |
| *ITS*2R (10 μM) | 0.75 μl |
| Q5 polymerase (2 U/μL) | 0.15 μl |
| Template DNA  | 1 μl |
| Total reaction volume | 15 μl |

Thermal profile (for *ITS*2)

|  |  |  |
| --- | --- | --- |
| Initial | 98 °C | 10 seconds |
| Denaturing | 98 °C | 10 seconds |
| Annealing | 56 °C | 30 seconds |
| Extension | 72 °C | 30 seconds |
|  |  | # cycles: 34 |
| Final | 72 °C | 5 minutes |

For older, pinned material (used for both *CO*1 and *ITS*2):

|  |  |
| --- | --- |
| DNA water  | 32.5 μL |
| Q5 buffer (5×) | 10 μL |
| dNTPs (10 mM) | 1 μL |
| Fw. primer (10 μM) | 2.5 μL |
| Rev. primer (10 μM) | 2.5 μL |
| Q5 polymerase (2 U/μL) | 0.5 μL |
| Template DNA  | 1 μL |
| Total reaction volume | 50 μL |

\*Note that thermal profiles remain the same when using this higher polymerase chain reaction volume