**Supporting Information**

**Fig S1. Alignments of sequence records derived from two type specimens of Geometridae, one with high quality DNA (a) and one with low quality DNA (b).** The alignments show only a single representative of each distinct sequence. In many cases, there were hundreds or thousands of a particular sequence.High quality reads have high coverage across the entire 658bp barcode region and originate from a single source – indicated by a single nucleotide (color) at each position in the contig. Low quality reads do not span the entire barcode region (i.e. they have regions lacking coverage) and often originate from multiple sources – indicated by multiple nucleotides (colors) at certain positions in the contig.

***See associated image file “Figure S1.svg”Macintosh HD:Users:ageraldes:Documents:ME:temp:Figure_S1.pdf***

**S1 Table. Type specimens analyzed, including sequencing results and accession numbers.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Process ID (Sanger/NGS)** | **Age (Yrs)** | **Identification** | **Status** | **Sanger Group** | **No. NGS Reads** | **Min. Cov.** | **Max. Cov.** | **Avg. Cov.** | **Recovered bp by NGS** | **NGS Contig GenBank Acc.** | **Sequence Read Archive Acc.** |
| PNGTY381-13 / PNGTY1837-15 | 104 | *Myrioblephara mixticolor* | Syntype | HQ | 143804 | 7 | 115751 | 29924 | 658 | KR070780 | SRR1867808 |
| PNGTY404-13 /  PNGTY1827-15 | 112 | *Cassephyra plenimargo* | Holotype | HQ | 213007 | 72 | 146189 | 42992 | 658 | KR070771 | SRR1867811 |
| PNGTY417-13 /  PNGTY1843-15 | 109 | *Psilalcis auropurpurea* | Syntype | HQ | 106286 | 0 | 74012 | 20477 | 448 | KR070767 | SRR1867812 |
| PNGTY466-13 /  PNGTY1839-15 | 110 | *Paralcidia marginata* | Syntype | HQ | 221885 | 5 | 168474 | 44541 | 657 | KR070779 | SRR1867813 |
| PNGTY473-13 /  PNGTY1823-15 | 110 | *Atmoceras plumosa* | Syntype | HQ | 143340 | 30 | 76376 | 28215 | 658 | KR070768 | SRR1867814 |
| PNGTY1047-14 /  PNGTY1845-15 | 110 | *Tripteridia viridisecta* | Syntype | HQ | 188107 | 1 | 101191 | 37855 | 570 | KR070783 | SRR1867815 |
| PNGTY1070-14 / PNGTY1834-15 | 111 | *Gymnoscelis ochriplaga* | Holotype | HQ | 186897 | 1 | 103399 | 38169 | 657 | KR070772 | SRR1867816 |
| PNGTY1098-14 / PNGTY1824-15 | 110 | *Axinoptera fasciata* | Holotype | HQ | 166116 | 0 | 83389 | 31838 | 474 | KR070785 | SRR1867817 |
| PNGTY1106-14 / PNGTY1826-15 | 112 | *Calluga semirasata* | Holotype | HQ | 215946 | 1 | 154302 | 43408 | 658 | KR070769 | SRR1867818 |
| PNGTY1124-14 / PNGTY1833-15 | 123 | *Eois semirubra* | Holotype | HQ | 232024 | 106 | 143908 | 46803 | 658 | KR070773 | SRR1867819 |
| PNGTY142-12 / PNGTY1831-15 | 112 | *Collix ghosha dichobathra* | N/A | MQ | 11665 | 0 | 11082 | 2142 | 459 | KR070778 | SRR1945335 |
| PNGTY158-12 / PNGTY1838-15 | 111 | *Papuarisme brunneata* | Holotype | MQ | 6479 | 0 | 5747 | 1165 | 569 | KR070787 | SRR1945382 |
| PNGTY189-12 / PNGTY1835-15 | 109 | *Hyposidra apicifulva* | N/A | MQ | 62208 | 0 | 45441 | 12301 | 570 | KR070781 | SRR1945383 |
| PNGTY801-13 / PNGTY1836-15 | 101 | *Milionia knowlei* | Syntype | MQ | 44190 | 1 | 43301 | 9153 | 658 | KR070777 | SRR1945384 |
| PNGTY587-13 / PNGTY1846-15 | 118 | *Ctimene basistraga obsoleta* | Syntype | MQ | 546 | 0 | 105 | 31 | 323 | KR070784 | SRR1946575 |
| PNGTY639-13 / PNGTY1842-15 | 102 | *Pseudeusemia bursadoides dignitosa* | Syntype | MQ | 134542 | 37 | 82031 | 27382 | 658 | KR070774 | SRR1945385 |
| PNGTY917-13 / PNGTY1840-15 | 108 | *Pingasa nobilis furvifrons* | Holotype | MQ | 46793 | 6 | 24276 | 9516 | 658 | KR070782 | SRR1945386 |
| PNGTY923-13 / PNGTY1821-15 | 121 | *Aeolochroma caesia* | Holotype | MQ | 68837 | 3 | 43442 | 14002 | 658 | KR070764 | SRR1945387 |
| PNGTY957-13 / PNGTY1844-15 | 106 | *Sarcinodes subvirgata* | Holotype | MQ | 99655 | 86 | 42405 | 20379 | 658 | KR070762 | SRR1945388 |
| PNGTY971-13 / PNGTY1828-15 | 105 | *Celerena lerne amplimargo* | Holotype | MQ | 113363 | 1 | 42333 | 21657 | 569 | KR070763 | SRR1945389 |
| PNGTY475-13 / PNGTY1832-15 | 104 | *Dyscheralcis retroflexa* | Syntype | LQ | 2681 | 0 | 1278 | 424 | 514 | KR070766 | SRR1867935 |
| PNGTY1146-14 / PNGTY1822-15 | 110 | *Alcis irrufata* | Holotype | LQ | 49944 | 2 | 37401 | 8881 | 657 | KR070770 | SRR1867936 |
| PNGTY1155-14 / PNGTY1830-15 | 120 | *Cleora repetita* ab. *suffusa* | Holotype | LQ | 7632 | 1 | 5708 | 731 | 454 | KR070765 | SRR1867937 |
| PNGTY008-12**\*** / N/A | 109 | *Spectrobasis differens* | Syntype | LQ | 1468 | 0 | 1157 | 280 | 237 | N/A | SRR1867938 |
| PNGTY073-12**\*** / N/A | 104 | *Desmoclystia unipuncta* | Syntype | LQ | 320 | 0 | 116 | 40 | 357 | N/A | SRR1867939 |
| PNGTY102-12**\*** / N/A | 120 | *Sterrhochaeta minuta* | Syntype | LQ | 3081 | 0 | 215 | 91 | 324 | N/A | SRR1867940 |
| PNGTY120-12**\*** / N/A | 118 | *Propithex alternata* | Holotype | LQ | 14863 | 0 | 1554 | 163 | 323 | N/A | SRR1867941 |
| PNGTY756-13 / PNGTY1825-15 | 105 | *Bursadopsis plenifascia* | Syntype | LQ | 133263 | 4 | 71444 | 20742 | 634 | KR070786 | SRR1867942 |
| PNGTY1080-14 / PNGTY1829-15 | 117 | *Chloroclystis rufofasciata* | Syntype | LQ | 4411 | 0 | 1685 | 647 | 419 | KR070775 | SRR1867943 |
| PNGTY1128-14 / PNGTY1841-15 | 112 | *Polyacme straminea* ab. *brunneata* | Holotype | LQ | 141402 | 23 | 71197 | 28117 | 658 | KR070776 | SRR1867944 |

The four Process ID’s marked with an asterisk (\*) represent specimens where NGS analysis generated sequence reads from multiple species. HQ – high quality; MQ – medium quality; LQ – low quality; N/A – not applicable.

**Table S2. Primers used in the first (PCR1) and second (PCR2) reactions to allow the analysis of 10 specimens in an Ion Torrent PGM run.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PCR** | **Code** | **Primer Name** | **Sequence (5'-3')** | **MID** | **Adapter** |
| PCR1 | F1 | **ARTH-NGS-F1.1-ion1** | **CTAAGGTAACATTCAACCAATCATAAAGATATTGG** | None | None |
| F2 | **ARTH-NGS-F2.1-ion1** | **CTAAGGTAACATTRRWRATGATCAARTWTATAAT** | None | None |
| F3 | **ARTH-NGS-F3.1-ion1** | **CTAAGGTAACTTATAATTGGDGGRTTTGGWAATTG** | None | None |
| F4 | **ARTH-NGS-F4.1-ion1** | **CTAAGGTAACAGWAGWATWRTWRAWAVWGG** | None | None |
| F5 | **ARTH-NGS-F5.1-ion1** | **CTAAGGTAACATTTTTWSWCTWCATWTDGCWGG** | None | None |
| F6 | **ARTH-NGS-F6.1-ion1** | **CTAAGGTAACTATTTGTWTGAKCWRTWKKWATTAC** | None | None |
| PCR1 | R1 | **ARTH-NGS-R1.1-ion1** | **CTAAGGTAACWGGTATWACTATRAARAAAATTAT** | None | None |
| R2 | **ARTH-NGS-R2.1-ion2** | **TAAGGAGAACTCARAAWCTWATRTTRTTTADWCG** | None | None |
| R3 | **ARTH-NGS-R3.1-ion3** | **AAGAGGATTCARDGGDGGRTAWACWGTTCAWCC** | None | None |
| R4 | **ARTH-NGS-R4.1-ion4** | **TACCAAGATCGTWGWAATRAARTTDATWGCWCC** | None | None |
| R5 | **ARTH-NGS-R5.1-ion5** | **CAGAAGGAACGTTARWARTATDGTRATDGCWCC** | None | None |
| R6 | **ARTH-NGS-R6.1-ion6** | **CTGCAAGTTCTAAACTTCTGGATGTCCAAAAAATCA** | None | None |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACATTCAACCAATCATAAAGATATTGG** | IonXpress1 | A |
| F2 | **ARTH-NGS-F2.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACATTRRWRATGATCAARTWTATAAT** | IonXpress1 | A |
| F3 | **ARTH-NGS-F3.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress1 | A |
| F4 | **ARTH-NGS-F4.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACAGWAGWATWRTWRAWAVWGG** | IonXpress1 | A |
| F5 | **ARTH-NGS-F5.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress1 | A |
| F6 | **ARTH-NGS-F6.2-ion1** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress1 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACATTCAACCAATCATAAAGATATTGG** | IonXpress2 | A |
| F2 | **ARTH-NGS-F2.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACATTRRWRATGATCAARTWTATAAT** | IonXpress2 | A |
| F3 | **ARTH-NGS-F3.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress2 | A |
| F4 | **ARTH-NGS-F4.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACAGWAGWATWRTWRAWAVWGG** | IonXpress2 | A |
| F5 | **ARTH-NGS-F5.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress2 | A |
| F6 | **ARTH-NGS-F6.2-ion2** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress2 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCATTCAACCAATCATAAAGATATTGG** | IonXpress3 | A |
| F2 | **ARTH-NGS-F2.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCATTRRWRATGATCAARTWTATAAT** | IonXpress3 | A |
| F3 | **ARTH-NGS-F3.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCTTATAATTGGDGGRTTTGGWAATTG** | IonXpress3 | A |
| F4 | **ARTH-NGS-F4.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCAGWAGWATWRTWRAWAVWGG** | IonXpress3 | A |
| F5 | **ARTH-NGS-F5.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCATTTTTWSWCTWCATWTDGCWGG** | IonXpress3 | A |
| F6 | **ARTH-NGS-F6.2-ion3** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATTCTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress3 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCATTCAACCAATCATAAAGATATTGG** | IonXpress4 | A |
| F2 | **ARTH-NGS-F2.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCATTRRWRATGATCAARTWTATAAT** | IonXpress4 | A |
| F3 | **ARTH-NGS-F3.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCTTATAATTGGDGGRTTTGGWAATTG** | IonXpress4 | A |
| F4 | **ARTH-NGS-F4.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCAGWAGWATWRTWRAWAVWGG** | IonXpress4 | A |
| F5 | **ARTH-NGS-F5.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCATTTTTWSWCTWCATWTDGCWGG** | IonXpress4 | A |
| F6 | **ARTH-NGS-F6.2-ion4** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGATCTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress4 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACATTCAACCAATCATAAAGATATTGG** | IonXpress5 | A |
| F2 | **ARTH-NGS-F2.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACATTRRWRATGATCAARTWTATAAT** | IonXpress5 | A |
| F3 | **ARTH-NGS-F3.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress5 | A |
| F4 | **ARTH-NGS-F4.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACAGWAGWATWRTWRAWAVWGG** | IonXpress5 | A |
| F5 | **ARTH-NGS-F5.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress5 | A |
| F6 | **ARTH-NGS-F6.2-ion5** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress5 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCATTCAACCAATCATAAAGATATTGG** | IonXpress6 | A |
| F2 | **ARTH-NGS-F2.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCATTRRWRATGATCAARTWTATAAT** | IonXpress6 | A |
| F3 | **ARTH-NGS-F3.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCTTATAATTGGDGGRTTTGGWAATTG** | IonXpress6 | A |
| F4 | **ARTH-NGS-F4.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCAGWAGWATWRTWRAWAVWGG** | IonXpress6 | A |
| F5 | **ARTH-NGS-F5.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCATTTTTWSWCTWCATWTDGCWGG** | IonXpress6 | A |
| F6 | **ARTH-NGS-F6.2-ion6** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTTCTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress6 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCATTCAACCAATCATAAAGATATTGG** | IonXpress7 | A |
| F2 | **ARTH-NGS-F2.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCATTRRWRATGATCAARTWTATAAT** | IonXpress7 | A |
| F3 | **ARTH-NGS-F3.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCTTATAATTGGDGGRTTTGGWAATTG** | IonXpress7 | A |
| F4 | **ARTH-NGS-F4.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCAGWAGWATWRTWRAWAVWGG** | IonXpress7 | A |
| F5 | **ARTH-NGS-F5.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCATTTTTWSWCTWCATWTDGCWGG** | IonXpress7 | A |
| F6 | **ARTH-NGS-F6.2-ion7** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress7 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACATTCAACCAATCATAAAGATATTGG** | IonXpress8 | A |
| F2 | **ARTH-NGS-F2.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACATTRRWRATGATCAARTWTATAAT** | IonXpress8 | A |
| F3 | **ARTH-NGS-F3.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress8 | A |
| F4 | **ARTH-NGS-F4.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACAGWAGWATWRTWRAWAVWGG** | IonXpress8 | A |
| F5 | **ARTH-NGS-F5.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress8 | A |
| F6 | **ARTH-NGS-F6.2-ion8** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress8 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACATTCAACCAATCATAAAGATATTGG** | IonXpress9 | A |
| F2 | **ARTH-NGS-F2.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACATTRRWRATGATCAARTWTATAAT** | IonXpress9 | A |
| F3 | **ARTH-NGS-F3.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress9 | A |
| F4 | **ARTH-NGS-F4.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACAGWAGWATWRTWRAWAVWGG** | IonXpress9 | A |
| F5 | **ARTH-NGS-F5.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress9 | A |
| F6 | **ARTH-NGS-F6.2-ion9** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress9 | A |
| PCR2 | F1 | **ARTH-NGS-F1.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACATTCAACCAATCATAAAGATATTGG** | IonXpress10 | A |
| F2 | **ARTH-NGS-F2.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACATTRRWRATGATCAARTWTATAAT** | IonXpress10 | A |
| F3 | **ARTH-NGS-F3.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACTTATAATTGGDGGRTTTGGWAATTG** | IonXpress10 | A |
| F4 | **ARTH-NGS-F4.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACAGWAGWATWRTWRAWAVWGG** | IonXpress10 | A |
| F5 | **ARTH-NGS-F5.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACATTTTTWSWCTWCATWTDGCWGG** | IonXpress10 | A |
| F6 | **ARTH-NGS-F6.2-ion10** | **CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACTATTTGTWTGAKCWRTWKKWATTAC** | IonXpress10 | A |
| PCR2 | R1 | **ARTH-NGS-R1.2-ion1-trP1** | **CCTCTCTATGGGCAGTCGGTGATCTAAGGTAACWGGTATWACTATRAARAAAATTAT** | IonXpress1 | trP1 |
| R2 | **ARTH-NGS-R2.2-ion2-trP1** | **CCTCTCTATGGGCAGTCGGTGATTAAGGAGAACTCARAAWCTWATRTTRTTTADWCG** | IonXpress2 | trP1 |
| R3 | **ARTH-NGS-R3.2-ion3-trP1** | **CCTCTCTATGGGCAGTCGGTGATAAGAGGATTCARDGGDGGRTAWACWGTTCAWCC** | IonXpress3 | trP1 |
| R4 | **ARTH-NGS-R4.2-ion4-trP1** | **CCTCTCTATGGGCAGTCGGTGATTACCAAGATCGTWGWAATRAARTTDATWGCWCC** | IonXpress4 | trP1 |
| R5 | **ARTH-NGS-R5.2-ion5-trP1** | **CCTCTCTATGGGCAGTCGGTGATCAGAAGGAACGTTARWARTATDGTRATDGCWCC** | IonXpress5 | trP1 |
| R6 | **ARTH-NGS-R6.2-ion6-trP1** | **CCTCTCTATGGGCAGTCGGTGATCTGCAAGTTCTAAACTTCTGGATGTCCAAAAAATCA** | IonXpress6 | trP1 |

The “Code” column refers to primer labels in Fig. 1. The COI binding region within each primer sequence is shown in black, while the 10bp tail (PCR1) or MID tag (PCR2) is shown in blue. The “key sequence” (required for Ion Torrent sequencing) is shown in green and the sequencing adapters are shown in red. The 10bp tails on the PCR1 primers are technically IonXpress MID tags, but they serve only to block short amplicons from acting as primers during PCR1. They were chosen over random decamer tails to maximize primer-template matching in PCR2. The same forward and reverse PCR1 primers are used for all ten samples in the first round of PCR. In the second round of PCR, samples are discriminated by using ten different sets of MID-tagged forward PCR2 primers (the same set of PCR2 reverse primers is used for all ten samples).

**Table S3**. **Components of PCR reactions in the NGS protocol**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **PCR 1.1** | **PCR 1.2** | **PCR 2.1, 2.2, 2.3, 2.4** | **PCR 2.5** | **PCR 2.6** |
| **10% Trehalose** | 5.125 µL | 5.25 µL | 5.75 µL | 5.875 µL | 6.0 µL |
| **H2O** | 0.13 µL | 0.13 µL | 0.13 µL | 0.13 µL | 0.13 µL |
| **5X Buffer** | 2.5 µL | 2.5 µL | 2.5 µL | 2.5 µL | 2.5 µL |
| **25 mM MgCl2** | 1.25 µL | 1.25 µL | 1.25 µL | 1.25 µL | 1.25 µL |
| **10 µM primers** | 0.125 µL each | 0.125 µL each | 0.125 µL each | 0.125 µL each | 0.125 µL each |
| **10 µM dNTP** | 0.0625 µL | 0.0625 µL | 0.0625 µL | 0.0625 µL | 0.0625 µL |
| **Taq (5U/ µL)** | 0.06 µL | 0.06 µL | 0.06 µL | 0.06 µL | 0.06 µL |
| **Template** | 2 µL | 2 µL | 2 µL | 2 µL | 2 µL |
| **TOTAL** | 12.5 µL | 12.5 µL | 12.5 µL | 12.5 µL | 12.5 µL |

Reactions differ only in the number of primers and the amount of trehalose. Trehalose sourced from Fluka Analytical; Hyclone ultra-pure water from Thermo Fisher Scientific; Buffer (- MgCl2), MgCl2, and Taq polymerase from KAPA Biosystems (while standard CCDB protocols utilize Platinum Taq, KAPA Taq was found to be less prone to co-amplifying trace amounts of residual DNA - derived from the Taq manufacturing process - and is therefore more amenable to high cycle PCR); primers from Integrated DNA Technologies.