**Supplementary Tables**

**Supplementary Table 1: Read data collected for each species.** Short read data were trimmed before any assembly process. Coverage was estimated based on our 1.07 Gb assembly size.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Technology** | **Reads** | **Bases** | **est. fold coverage** |
| *Sp. multiplicata* | Illumina | 447,040,259 | 134,112,077,700 | 125.2  |
| *Sp. multiplicata* | Pacbio | 3,582,769 | 20,842,516,014 | 19.5 |
| *Sp. multiplicata* | Oxford Nanopore | 430,065 | 952,110,011 | 0.9 |
| *Sp. bombifrons* | Illumina | 569,784,662 | 170,935,398,600 | 159.7 |
| *Sc. holbrookii* | Illumina | 254,070,350 | 41,990,135,448 | 39.4 |
| *Sc. couchii* | Illumina | 90,655,530 | 27,196,659,000 | 25.4 |

**Supplementary Table 2: Assembly characteristics at major steps.** Long reads greatly improved contiguity of the genome, producing an assembly N50 of 26,214 bp, compared to an N50 of 614 bp of the short read assembly. Duplicate contigs likely stemming from heterozygosity were removed (Methods). Given observed colinearity between the *X. tropicalis* and the *Sp. multiplicata* genomes, we performed a scaffolding of these contigs against the *X. tropicalis* genome assembly using Chromosomer1, this more than doubled genome N50 from 29,771 bp to 70,967 bp (row 4).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Assembly steps** | **Kmer size** | **Total size** | **Number of sequences** | **Longest sequence** | **N25** | **N50** | **N75** |
| Short read assembly | 91 | 1,051,349,327 | 250,0730 | 15,443 | 1,099 | 614 | 356 |
| Hybrid contig assembly | 51 | 1,169,449,223 | 111,151 | 401,788 | 63,738 | 26,214 | 9,418 |
| Duplicate removal | 51 | 1,091,640,347 | 84,984 | 401,788 | 68,199 | 29,771 | 11,387 |
| Ungapped chromosomer scaffolds | 51 | 1,073,483,482 | 49,736 | 60,197,306 | 36,398,839 | 70,967 | 19,311 |

**Supplementary Table 3. Comparison of the current state of released diploid anuran genomes.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Assembly size (Gbp)** | **Assembly N50** | **Reference** | **Database** |
| *N. parkeri* | 2.0 | 1.05 Mbp | Sun, Y.-B. *et al* | NCBI |
| *R. catesbeiana* | 5.8 | 69 Kbp | Hammond, S.A. *et al* | NCBI |
| *R. marina* | 2.5 | 168 Kbp | Edwards, R.J. *et al* | GigaDB |
| *Sp. multiplicata* | 1.1 | 70 Kbp | This study | NCBI |
| *O. pumilio* | 5.0 | 73 Kbp | Rogers, R.L. *et al* | N/A |
| *P. adspersus* | 1.6 | 157 Mbp | Denton R. D., *et al* | N/A |
| *X. tropicalis* | 1.4 | 135 Mbp | Hellsten, U. *et al* | Xenbase |

**Supplementary Table 4. Repetitive DNA in *Sp. multiplicata,* as outputted by RepeatModeler.** We also performed analysis to quantify overrepresented kmers in our short and long read set. We detected 2 elements in our kmer scans that showed evidence of enrichment: GGGGGTTATATTACTGTATACAGCGCT and CTGTCTCTTATACACATCTCCGAGCCCACG. These appear to be novel repeats, as we were unable to find a match for either of these sequences in the NCBI database. We broadly investigated the locations of repetitive elements across contigs as well as scaffolds. We found that repetitive elements were evenly distributed across the scaffolds with no obvious clustering. We found evidence that repetitive elements may have played a role in the fragmentation of the assembly as there was overrepresentation of simple AT repeats near the ends of contigs. We also found an enrichment of the second repetitive element discovered from kmer enrichment at the ends of contigs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Type** | **Subset** | **Number of elements** | **Length (bp)** | **Percentage of sequence** |
| SINEs |  | 1,474 | 81,832 | 0.01 |
|  | ALUs | 4 | 280 | 0 |
|  | MIRs | 77 | 2,833 | 0 |
| LINEs |  | 218,428 | 58,280,930 | 4.98 |
|  | LINE1 | 26,126 | 13,550,592 | 1.16 |
|  | LINE2 | 51,747 | 11,458,129 | 0.98 |
|  | L3/CR1 | 117,401 | 26,689,898 | 2.28 |
| LTR elements |  | 58,089 | 29,554,407 | 2.53 |
|  | ERVL  | 555 | 96,592 | 0.01 |
|  | ERVL-MaLRs | 25 | 1,430 | 0 |
|  | ERV\_classI | 5,256 | 2,224,606 | 0.19 |
|  | ERV\_classII | 1,125 | 60,728 | 0.01 |
| DNA elements |  | 220,710 | 45,278,690 | 3.87 |
|  | hAT-Charlie | 7,350 | 606,067 | 0.05 |
|  | TcMar-Tigger | 564 | 77,182 | 0.01 |
| Unclassified |  | 1,196,208 | 215,660,787 | 18.44 |
| Small RNA |  | 6,395 | 534,323 | 0.05 |
| Satellites |  | 4,496 | 576,825 | 0.05 |
| Simple repeats |  | 364,651 | 28,449,523 | 0.05 |
| Low complexity |  | 36,062 | 1,923,725 | 0.16 |

**Supplementary Table 5. Genomic level comparison of diploid anuran genomes.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Assembly size (Gbp)** | **Repetitive content** | **Genes** | **Transcript length (bp)** | **Gene length (bp)** | **Reference** | **Database** |
| *N. parkeri* | 2.0 | 48% | 23,408 | 1,382 | 42,081 | Sun, Y.-B. *et al* | NCBI |
| *R. catesbeiana* | 5.8 | 62% | 22,204 | 720 | 7,144 | Hammond, S.A. *et al* | NCBI |
| *R. marina* | 2.5 | 64% | 25,846 | 1,204 | 18,800 | Edwards, R.J. *et al* | GigaDB |
| *Sp. multiplicata* | 1.1 | 32% | 19,639 | 1,370 | 9,399 | This study | NCBI |
| *X. tropicalis* | 1.4 | 35% | 21,067 | 1,300 | 1,6500 | Hellsten, U. *et al* | Xenbase |

**Supplementary Table 6. Description of three genes with increased copy number in *Sp. multiplicata* relative to the other published anuran genomes.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Uniprot match** | ***N. parkeri*** | ***R. catesbeiana*** | ***R. marina*** | ***Sp. multiplicata*** | ***X. tropicalis*** | **Function** |
| HYAS3 | 2 | 1 | 2 | 7 | 1 | Hyaluronic acid production, critical for cell surface  |
| NOD2A | 2 | 1 | 2 | 12 | 5 | TGF-β signal factor controlling developmental patterning |
| ZP3 | 3 | 4 | 3 | 9 | 2 | Zona pellucida glycoprotein, involved in egg sperm recognition |

**Supplementary Table 7. Expansion of nodal in anurans.** Including the other members of the nodal gene family as well as reducing target coverage requirements to ≥50% reveals evidence of 24 copies of nodal in *Spea multiplicata.* 22 of these are most closely related to *xnr5* and *xnr6*. The remaining 2 were most closely related to *xnr2*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Gene****name** | **SWISSprot match** | **Gene length** | **Match length** | **Match coverage** | **Percent identity** |
| *N. parkeri* | nodal homolog | NOD4A | 405 | 402 | 100 | 51.4 |
| *N. parkeri* | nodal homolog | NODAL | 394 | 406 | 97 | 56.5 |
| *N. parkeri* | nodal 2-A-like | NOD2A | 386 | 405 | 95 | 51.6 |
| *N. parkeri* | nodal 2-A-like | NOD2A | 458 | 405 | 99 | 48.1 |
| *N. parkeri* | nodal 2-A-like | NOD2B | 382 | 384 | 93 | 46 |
| *N. parkeri* | nodal 2-A-like | NODAL | 378 | 406 | 92 | 36.5 |
| *R. catesbeiana* | PIO13389.1 | NOD2A | 223 | 405 | 55 | 49.6 |
| *R. catesbeiana* | PIO32278.1 | NOD2A | 516 | 405 | 94 | 53.9 |
| *R. catesbeiana* | PIO32279.1 | NODAL | 331 | 406 | 81 | 57.3 |
| *R. marina* | RMA\_00017829 | NODAL | 704 | 403 | 96 | 52.2 |
| *R. marina* | RMA\_00020594 | NOD2A | 471 | 405 | 76 | 52.2 |
| *R. marina* | RMA\_00020595 | NOD2A | 414 | 405 | 94 | 52 |
| *R. marina* | RMA\_00027748 | NOD2A | 1502 | 405 | 94 | 48.5 |
| *R. marina* | RMA\_00029607 | NOD2A | 319 | 405 | 76 | 50.1 |
| *R. marina* | RMA\_00040870 | NOD4A | 404 | 402 | 99 | 54.1 |
| *S. multiplicata* | g11187 | NOD2A | 365 | 405 | 90 | 46.8 |
| *S. multiplicata* | g11188 | NOD2A | 365 | 405 | 90 | 47 |
| *S. multiplicata* | g11568 | NOD2A | 365 | 405 | 90 | 47.3 |
| *S. multiplicata* | g11569 | NOD2A | 320 | 405 | 79 | 49.3 |
| *S. multiplicata* | g11677 | NOD2A | 365 | 405 | 90 | 46.8 |
| *S. multiplicata* | g16257 | NOD2A | 208 | 405 | 51 | 47.5 |
| *S. multiplicata* | g17861 | NOD2A | 234 | 405 | 58 | 41.8 |
| *S. multiplicata* | g20382 | NOD2A | 365 | 405 | 90 | 46.8 |
| *S. multiplicata* | g20383 | NOD2A | 365 | 405 | 90 | 47.3 |
| *S. multiplicata* | g22585 | NOD2A | 329 | 405 | 81 | 45.4 |
| *S. multiplicata* | g25137 | NODAL | 451 | 406 | 97 | 50.8 |
| *S. multiplicata* | g25860 | NOD2A | 365 | 405 | 90 | 46.8 |
| *S. multiplicata* | g2598 | NOD2A | 365 | 405 | 90 | 47 |
| *S. multiplicata* | g3034 | NOD2A | 284 | 405 | 70 | 47 |
| *S. multiplicata* | g30506 | NOD2A | 315 | 405 | 78 | 45.1 |
| *S. multiplicata* | g30507 | NOD2A | 365 | 405 | 90 | 46.6 |
| *S. multiplicata* | g34467 | NOD2A | 365 | 405 | 90 | 47.3 |
| *S. multiplicata* | g34468 | NOD2A | 365 | 405 | 90 | 45.6 |
| *S. multiplicata* | g34476 | NODAL | 367 | 403 | 91 | 53.4 |
| *S. multiplicata* | g3511 | NOD2A | 208 | 405 | 51 | 47.5 |
| *S. multiplicata* | g3513 | NOD2A | 316 | 405 | 78 | 43.9 |
| *S. multiplicata* | g38079 | NOD2A | 362 | 405 | 89 | 46.1 |
| *S. multiplicata* | g5200 | NOD2A | 365 | 405 | 90 | 45.7 |
| *S. multiplicata* | g9681 | NOD2A | 329 | 405 | 81 | 45.4 |
| *X. tropicalis* | LOC100488935 | NOD3A | 397 | 401 | 99 | 95.5 |
| *X. tropicalis* | LOC100491713 | NOD3C | 401 | 401 | 100 | 95 |
| *X. tropicalis* | LOC101732995 | NOD3A | 401 | 401 | 100 | 99 |
| *X. tropicalis* | LOC101734157 | NOD2A | 386 | 405 | 95 | 52.8 |
| *X. tropicalis* | LOC101734231 | NOD2A | 386 | 405 | 95 | 53.3 |
| *X. tropicalis* | nodal | NOD3B | 401 | 401 | 100 | 99.8 |
| *X. tropicalis* | nodal1 | NODAL | 403 | 403 | 100 | 99.5 |
| *X. tropicalis* | nodal2 | NOD2A | 405 | 405 | 100 | 89.9 |
| *X. tropicalis* | nodal5 | NOD2A | 386 | 405 | 95 | 53.6 |
| *X. tropicalis* | nodal6 | NOD2A | 382 | 405 | 93 | 58.6 |

**Supplementary Table 8. Genes showing different patterns of molecular evolution in *Scaphiopus* and *Spea*.** Significantly different selection pressures were determined if the two group (i.e., two genus) model was statistically superior to a single factor model according to a likelihood ratio test. ‘ω’ is the dN/dS value of a given gene under the specified category (i.e., single model or genus-specific model) and ‘-lnL refers to the loglikelihood of a given model design.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **gene** | ***X. tropicalis* match** | **Swissprot match** | **Model 1** **-lnL** | **Model 1 ω** | **Model 2** **-lnL** | **Model 2 ω *Spea*** | **Model 2 ω *Scaphiopus*** | **p-value** | **q-value** |
| g10134 | LOC100493382 | UBP13\_DANRE | -602.8 | 0.1726 | -590.4 | 2.8667 | 0.0095 | 6.6E-07 | 5.5E-05 |
| g1015 | tsc22d3 | TPD52\_HUMAN | -515.9 | 0.1153 | -511.6 | 0.8994 | 0.0001 | 3.3E-03 | 4.0E-02 |
| g1017 | tsc22d3 | TPD52\_HUMAN | -512.8 | 0.1171 | -508.0 | 0.8043 | 0.0001 | 1.9E-03 | 2.8E-02 |
| g10677 | tox4 | TOX4\_XENTR | -570.9 | 0.0872 | -564.8 | 1.1463 | 0.0001 | 5.1E-04 | 1.1E-02 |
| g1071 | snx12 | SNX12\_HUMAN | -756.7 | 0.0093 | -752.3 | 0.0001 | 0.1440 | 3.0E-03 | 3.8E-02 |
| g10887 | cs | CISY\_XENTR | -1455.4 | 0.0296 | -1449.9 | 0.2403 | 0.0040 | 9.9E-04 | 1.7E-02 |
| g11371 | fam167a | F167A\_HUMAN | -923.4 | 0.2872 | -916.5 | 4.0825 | 0.0371 | 2.0E-04 | 5.7E-03 |
| g117 | ccdc115 | NH2L1\_XENTR | -1292.4 | 0.2590 | -1282.6 | 1.6489 | 0.0623 | 9.8E-06 | 5.5E-04 |
| g11731 | LOC100498221 | RDH16\_HUMAN | -1695.5 | 0.2383 | -1687.1 | 0.6609 | 0.1609 | 4.3E-05 | 1.8E-03 |
| g11732 | taok2 | TAOK2\_XENLA | -1106.9 | 0.0152 | -1100.5 | 0.0263 | 0.0007 | 3.2E-04 | 7.5E-03 |
| g11875 | LOC100145107 | HBB2\_XENTR | -826.0 | 0.5821 | -804.9 | 10.0000 | 0.0371 | 8.1E-11 | 2.7E-08 |
| g12141 | snai3 | SNAI2\_HUMAN | -1371.5 | 0.3387 | -1366.6 | 0.0564 | 1.3937 | 1.7E-03 | 2.6E-02 |
| g12484 | b4galnt4 | B4GN4\_HUMAN | -1247.4 | 0.0787 | -1240.1 | 0.6056 | 0.0060 | 1.3E-04 | 4.4E-03 |
| g12815 | polr1d.2 | RPAC2\_XENLA | -547.7 | 0.0842 | -541.2 | 0.8065 | 0.0001 | 2.9E-04 | 7.3E-03 |
| g12861 | flnc | FLNA\_HUMAN | -3610.0 | 0.0683 | -3603.9 | 0.2654 | 0.0386 | 4.8E-04 | 1.0E-02 |
| g12863 | fam91a1 | F91A1\_HUMAN | -1229.6 | 0.1270 | -1222.8 | 0.9405 | 0.0081 | 2.3E-04 | 6.4E-03 |
| g12892 | fam131b | F131B\_HUMAN | -1505.9 | 0.0598 | -1496.5 | 1.1962 | 0.0001 | 1.6E-05 | 7.9E-04 |
| g12893 | fam131b | F131B\_HUMAN | -1334.4 | 0.0663 | -1320.6 | 0.1187 | 0.0599 | 1.4E-07 | 1.5E-05 |
| g13150 | LOC100492966 | O52A1\_HUMAN | -1370.0 | 0.2126 | -1361.5 | 3.3201 | 0.0291 | 3.6E-05 | 1.6E-03 |
| g13767 | atxn3 | ATX3\_HUMAN | -843.8 | 0.0753 | -839.7 | 0.0057 | 0.7380 | 4.0E-03 | 4.6E-02 |
| g13992 | not | NOTO\_XENLA | -1099.8 | 0.1216 | -1095.3 | 0.0056 | 0.6973 | 2.7E-03 | 3.6E-02 |
| g13993 | not | NOTO\_XENLA | -1095.7 | 0.1097 | -1087.2 | 0.0001 | 0.7263 | 3.5E-05 | 1.6E-03 |
| g14393 | grin2b | NMDE2\_XENLA | -617.9 | 0.0855 | -613.1 | 0.3895 | 0.0624 | 2.1E-03 | 2.9E-02 |
| g14927 | plaa | PLAP\_XENLA | -916.4 | 0.1002 | -911.7 | 0.0942 | 0.1686 | 2.0E-03 | 2.9E-02 |
| g15077 | gmpr2 | GMPR2\_HUMAN | -1852.2 | 0.0484 | -1845.0 | 0.0020 | 0.2632 | 1.4E-04 | 4.5E-03 |
| g15087 | tbc1d9b | TBC9B\_HUMAN | -1434.7 | 0.0429 | -1430.4 | 0.1970 | 0.0001 | 3.4E-03 | 4.2E-02 |
| g15248 | myo18b | MY18A\_HUMAN | -435.4 | 0.4798 | -428.7 | 0.0001 | 1.6539 | 2.6E-04 | 6.8E-03 |
| g15278 | ppcdc | COAC\_HUMAN | -377.0 | 0.3873 | -370.1 | 5.2004 | 0.0390 | 2.1E-04 | 5.9E-03 |
| g15420 | rnf34 | RNF34\_HUMAN | -2171.4 | 0.0869 | -2167.3 | 0.0274 | 0.3982 | 4.1E-03 | 4.8E-02 |
| g15892 | synj2 | SYNJ2\_HUMAN | -489.0 | 0.1620 | -483.8 | 2.8634 | 0.0485 | 1.3E-03 | 2.1E-02 |
| g16767 | zg16 | ZG16\_HUMAN | -501.4 | 0.1211 | -496.5 | 2.7921 | 0.0537 | 1.7E-03 | 2.5E-02 |
| g16939 | rpusd1 | RUSD1\_DANRE | -464.0 | 0.1169 | -453.5 | 1.2218 | 0.0001 | 4.9E-06 | 3.0E-04 |
| g17467 | hoxc4 | HXD4A\_DANRE | -747.5 | 0.1579 | -741.9 | 1.6533 | 0.0118 | 7.6E-04 | 1.4E-02 |
| g18054 | pgap3 | PGAP3\_XENLA | -1307.3 | 0.1687 | -1302.2 | 0.2369 | 0.0001 | 1.4E-03 | 2.2E-02 |
| g18311 | mafg | MAFG\_HUMAN | -1201.1 | 0.0355 | -1192.8 | 0.2080 | 0.0271 | 4.8E-05 | 1.9E-03 |
| g1861 | fermt2 | FERM2\_HUMAN | -1610.0 | 0.0941 | -1605.9 | 0.0001 | 0.1074 | 3.9E-03 | 4.6E-02 |
| g18637 | chmp2a | CHM2A\_XENLA | -1010.4 | 0.0960 | -986.6 | 1.7430 | 0.0205 | 5.1E-12 | 3.3E-09 |
| g19099 | spata6 | SPAT6\_HUMAN | -764.9 | 0.2960 | -757.5 | 1.7840 | 0.0098 | 1.3E-04 | 4.4E-03 |
| g19401 | cyb5r4 | NB5R4\_XENTR | -1604.7 | 0.1527 | -1597.9 | 0.0109 | 0.9682 | 2.3E-04 | 6.4E-03 |
| g19524 | pak3 | PAK3\_XENLA | -785.3 | 0.0396 | -772.2 | 1.5501 | 0.0026 | 3.2E-07 | 2.9E-05 |
| g19551 | atp1a1 | AT1A1\_XENLA | -5412.5 | 0.0236 | -5407.2 | 0.0555 | 0.0176 | 1.1E-03 | 1.9E-02 |
| g19630 | mfge8 | EDIL3\_HUMAN | -996.0 | 0.1363 | -989.5 | 0.6095 | 0.0076 | 3.2E-04 | 7.5E-03 |
| g19914 | ubr1 | UBR1\_HUMAN | -2171.2 | 0.1929 | -2166.8 | 0.5445 | 0.1231 | 3.3E-03 | 4.0E-02 |
| g20142 | fermt2 | FERM2\_HUMAN | -3241.7 | 0.0835 | -3235.6 | 0.0001 | 0.0930 | 4.7E-04 | 1.0E-02 |
| g20308 | fcn2 | FCN1A\_XENLA | -1348.6 | 0.1600 | -1343.9 | 0.3778 | 0.0906 | 2.1E-03 | 2.9E-02 |
| g20505 | nrxn3 | NRX3A\_HUMAN | -714.7 | 0.0183 | -701.5 | 0.3023 | 0.0043 | 2.9E-07 | 2.7E-05 |
| g21205 | nqo1 | NQO1\_HUMAN | -1588.9 | 0.2178 | -1581.4 | 0.0682 | 0.9483 | 1.1E-04 | 4.0E-03 |
| g21206 | nqo1 | NQO1\_HUMAN | -1713.0 | 0.2201 | -1707.0 | 1.0144 | 0.1221 | 5.2E-04 | 1.1E-02 |
| g21659 | dph5 | DPH5\_HUMAN | -1497.8 | 0.0246 | -1493.4 | 0.2441 | 0.0037 | 3.0E-03 | 3.8E-02 |
| g21857 | gnat1 | GNAT1\_HUMAN | -591.7 | 0.1129 | -585.8 | 1.5532 | 0.0198 | 6.0E-04 | 1.2E-02 |
| g2204 | dpf3 | REQUB\_XENLA | -503.0 | 0.0522 | -498.8 | 0.0001 | 0.3885 | 4.0E-03 | 4.6E-02 |
| g2209 | ttc9 | TTC9A\_HUMAN | -896.1 | 0.1457 | -891.7 | 0.4326 | 0.1279 | 3.2E-03 | 4.0E-02 |
| g22100 | pdzrn3 | PZR3B\_DANRE | -1994.1 | 0.3186 | -1985.0 | 3.3467 | 0.0402 | 2.0E-05 | 9.3E-04 |
| g22198 | pdgfa | PDGFA\_XENLA | -416.5 | 0.7708 | -411.3 | 0.0471 | 10.0000 | 1.3E-03 | 2.1E-02 |
| g22638 | znf516 | REST\_DANRE | -458.8 | 0.1188 | -453.8 | 0.0001 | 0.5813 | 1.6E-03 | 2.5E-02 |
| g22710 | phka1 | KPB1\_HUMAN | -2636.1 | 0.0950 | -2631.5 | 0.0167 | 0.1217 | 2.4E-03 | 3.3E-02 |
| g23338 | rab31 | RAB31\_HUMAN | -338.5 | 0.2719 | -334.2 | 1.3395 | 0.0001 | 3.7E-03 | 4.4E-02 |
| g23556 | coro2b | COR2B\_XENLA | -2820.7 | 0.1865 | -2811.5 | 0.0617 | 0.7571 | 1.8E-05 | 8.6E-04 |
| g23737 | polr2d | AMERL\_HUMAN | -1188.9 | 0.1834 | -1183.3 | 0.1807 | 0.1818 | 8.0E-04 | 1.5E-02 |
| g24002 | LOC100492966 | CO3\_HUMAN | -3668.6 | 0.2279 | -3653.9 | 0.0621 | 0.8954 | 5.7E-08 | 7.4E-06 |
| g24008 | LOC101735171 | HBB2\_XENTR | -1593.2 | 0.3394 | -1583.8 | 1.7070 | 0.0554 | 1.4E-05 | 7.3E-04 |
| g24150 | LOC100488377 | UNC4\_HUMAN | -1316.2 | 0.2965 | -1300.9 | 0.3072 | 0.3009 | 3.2E-08 | 4.5E-06 |
| g24419 | LOC105948455 | SVBP\_DANRE | -1263.6 | 0.4560 | -1258.2 | 0.3622 | 0.9036 | 9.8E-04 | 1.7E-02 |
| g24561 | krt5.2 | K2C6A\_HUMAN | -1716.2 | 0.2213 | -1711.2 | 0.7942 | 0.1626 | 1.6E-03 | 2.5E-02 |
| g24936 | LOC100497729 | CNFN\_XENTR | -912.4 | 0.1444 | -905.9 | 1.3415 | 0.0328 | 3.2E-04 | 7.5E-03 |
| g25011 | LOC100495065 | MYH3\_HUMAN | -816.8 | 0.0469 | -811.6 | 0.0055 | 0.4771 | 1.2E-03 | 2.1E-02 |
| g25175 | dync2h1 | DYHC2\_HUMAN | -1112.5 | 0.0247 | -1104.8 | 0.0079 | 0.2567 | 8.9E-05 | 3.4E-03 |
| g25600 | krt35 | KRT36\_HUMAN | -2037.3 | 0.2710 | -2027.6 | 0.8682 | 0.1554 | 1.0E-05 | 5.6E-04 |
| g25634 | LOC100493251 | UN93A\_XENLA | -745.1 | 0.3669 | -738.9 | 2.2970 | 0.0471 | 4.0E-04 | 8.8E-03 |
| g2565 | celf3 | CELF4\_XENTR | -460.1 | 0.1317 | -455.9 | 0.4971 | 0.0001 | 3.7E-03 | 4.5E-02 |
| g25695 | atp6v1a | VATA\_HUMAN | -1996.7 | 0.0180 | -1992.4 | 0.1728 | 0.0022 | 3.4E-03 | 4.1E-02 |
| g25820 | LOC100492966 | CO3\_HUMAN | -5268.3 | 0.2141 | -5244.8 | 0.0528 | 0.9982 | 7.3E-12 | 3.6E-09 |
| g25939 | LOC100488078 | CP4AB\_HUMAN | -3145.2 | 0.1038 | -3139.0 | 0.2699 | 0.0724 | 4.6E-04 | 9.9E-03 |
| g25963 | ankrd13b | AN13B\_HUMAN | -2808.1 | 0.0971 | -2797.7 | 0.8957 | 0.0001 | 4.9E-06 | 3.0E-04 |
| g26118 | syne1 | SYNE1\_HUMAN | -3875.2 | 0.6134 | -3839.4 | 7.8515 | 0.0654 | 2.6E-17 | 5.2E-14 |
| g2651 | cacna1e | CAC1E\_HUMAN | -988.9 | 0.0839 | -983.1 | 0.0001 | 1.2265 | 6.4E-04 | 1.2E-02 |
| g26543 | abca5 | ABCA5\_HUMAN | -509.1 | 0.0718 | -501.9 | 2.2252 | 0.0001 | 1.6E-04 | 5.0E-03 |
| g27008 | LOC101733681 | CAC1D\_HUMAN | -3348.6 | 0.0335 | -3341.3 | 0.1374 | 0.0216 | 1.2E-04 | 4.4E-03 |
| g27017 | glra3 | GLRA3\_HUMAN | -2205.2 | 0.0517 | -2189.5 | 0.8960 | 0.0159 | 2.1E-08 | 3.4E-06 |
| g27186 | gatad2a | P66A\_HUMAN | -2213.7 | 0.0713 | -2200.4 | 0.6299 | 0.0001 | 2.6E-07 | 2.6E-05 |
| g27464 | s100a11 | S10AB\_HUMAN | -1238.0 | 0.1187 | -1233.9 | 0.0001 | 0.1371 | 4.1E-03 | 4.8E-02 |
| g27489 | LOC105945936 | CP2S1\_HUMAN | -1098.5 | 0.2816 | -1087.3 | 0.0524 | 1.3001 | 2.2E-06 | 1.6E-04 |
| g27758 | spdef | SPDEF\_HUMAN | -437.3 | 0.0001 | -425.7 | 0.0001 | 0.0001 | 1.4E-06 | 1.1E-04 |
| g28362 | atad1 | ATAD1\_XENTR | -540.5 | 0.0867 | -533.9 | 0.0062 | 0.3063 | 2.9E-04 | 7.3E-03 |
| g28462 | wnt11r | WNT11\_XENLA | -440.2 | 0.0323 | -431.3 | 0.0001 | 1.3225 | 2.4E-05 | 1.1E-03 |
| g28493 | pak3 | PAK3\_XENLA | -788.9 | 0.0384 | -776.6 | 1.1660 | 0.0026 | 6.7E-07 | 5.5E-05 |
| g28859 | atad1 | ATAD1\_XENTR | -2714.7 | 0.3692 | -2703.8 | 1.8518 | 0.0651 | 3.3E-06 | 2.2E-04 |
| g2917 | ctdspl | CTDSL\_HUMAN | -272.7 | 0.0114 | -264.3 | 0.0001 | 0.4948 | 4.1E-05 | 1.7E-03 |
| g29382 | tmem17 | TM17A\_XENTR | -817.2 | 0.0347 | -812.4 | 0.2612 | 0.0001 | 2.0E-03 | 2.9E-02 |
| g30097 | gtf2h1 | TF2H1\_HUMAN | -2796.1 | 0.1039 | -2789.7 | 0.0001 | 0.1185 | 3.3E-04 | 7.7E-03 |
| g30620 | hpn | HEPS\_HUMAN | -1608.6 | 0.0744 | -1601.5 | 0.5601 | 0.0069 | 1.8E-04 | 5.3E-03 |
| g30676 | ctbp1 | CTBP1\_HUMAN | -492.8 | 0.0436 | -488.1 | 0.4517 | 0.0001 | 2.2E-03 | 3.0E-02 |
| g31455 | frmd8 | FRMD8\_XENTR | -733.8 | 0.1820 | -720.0 | 0.0001 | 0.1900 | 1.6E-07 | 1.6E-05 |
| g31508 | cacna1f | CAC1D\_HUMAN | -1319.3 | 0.0367 | -1312.6 | 0.2436 | 0.0115 | 2.7E-04 | 7.1E-03 |
| g31526 | nin | IN80D\_XENLA | -442.6 | 0.1982 | -437.6 | 0.0001 | 0.2072 | 1.7E-03 | 2.5E-02 |
| g32315 | LOC100493382 | NDUV3\_HUMAN | -1821.8 | 0.2238 | -1806.4 | 2.9167 | 0.0252 | 2.9E-08 | 4.3E-06 |
| g32328 | vps13a | VP13A\_HUMAN | -1470.5 | 0.1483 | -1466.0 | 0.0001 | 0.1785 | 2.7E-03 | 3.6E-02 |
| g3316 | chfr | CHFR\_XENTR | -1905.9 | 0.1482 | -1898.8 | 1.3083 | 0.0318 | 1.8E-04 | 5.3E-03 |
| g33967 | odf3 | ODF3A\_XENTR | -1634.6 | 0.2078 | -1629.4 | 0.0318 | 0.2667 | 1.3E-03 | 2.1E-02 |
| g33970 | odf3 | ODF3A\_XENTR | -1973.5 | 0.2035 | -1966.1 | 0.0149 | 0.2531 | 1.2E-04 | 4.4E-03 |
| g34021 | senp8 | SENP8\_HUMAN | -1180.0 | 0.0943 | -1174.1 | 0.8176 | 0.0085 | 6.2E-04 | 1.2E-02 |
| g34491 | LOC101731432 | ELL2\_HUMAN | -791.8 | 0.0373 | -775.8 | 0.0021 | 0.6603 | 1.5E-08 | 2.7E-06 |
| g34891 | LOC100497845 | AGRB2\_HUMAN | -919.1 | 0.2297 | -914.4 | 1.4074 | 0.0923 | 2.2E-03 | 3.1E-02 |
| g35249 | sec23a | SC23A\_XENTR | -1338.5 | 0.0418 | -1333.9 | 0.2198 | 0.0189 | 2.3E-03 | 3.1E-02 |
| g35360 | cacna1c | CAC1C\_HUMAN | -643.5 | 0.0737 | -635.7 | 0.0001 | 0.6559 | 8.1E-05 | 3.2E-03 |
| g35409 | pcid2 | PCID2\_XENLA | -938.0 | 0.0546 | -932.9 | 0.3950 | 0.0001 | 1.4E-03 | 2.2E-02 |
| g3558 | maats1 | CFA91\_HUMAN | -1449.6 | 0.1321 | -1442.4 | 1.4890 | 0.0081 | 1.5E-04 | 4.8E-03 |
| g35613 | tgfbr1 | TGFR1\_HUMAN | -2147.7 | 0.0947 | -2143.0 | 0.0001 | 0.1068 | 2.0E-03 | 2.9E-02 |
| g35713 | tmod4 | TMOD4\_HUMAN | -555.4 | 0.0345 | -549.1 | 0.1915 | 0.0001 | 3.7E-04 | 8.3E-03 |
| g36089 | LOC100498621 | ZNT9\_HUMAN | -789.0 | 0.2576 | -784.8 | 6.3764 | 0.0431 | 3.8E-03 | 4.5E-02 |
| g36266 | vash2 | VASH2\_HUMAN | -1060.9 | 0.0366 | -1056.2 | 0.0001 | 0.3981 | 2.2E-03 | 3.1E-02 |
| g3637 | atp6v1a | VATA\_HUMAN | -1602.4 | 0.0132 | -1597.0 | 0.1961 | 0.0001 | 9.6E-04 | 1.7E-02 |
| g36977 | faxc | FAXC\_XENTR | -618.8 | 0.2051 | -614.1 | 0.6438 | 0.0001 | 2.1E-03 | 2.9E-02 |
| g37293 | kif1a | KIF1A\_HUMAN | -2722.5 | 0.0382 | -2718.1 | 0.0001 | 0.0529 | 3.0E-03 | 3.8E-02 |
| g37339 | pid1 | PCLI1\_HUMAN | -974.3 | 0.1429 | -962.1 | 0.6888 | 0.0001 | 8.3E-07 | 6.5E-05 |
| g38247 | cog5 | COG5\_HUMAN | -360.9 | 0.1943 | -355.9 | 0.4231 | 0.1739 | 1.4E-03 | 2.3E-02 |
| g38345 | myo1e.1 | MYO1E\_HUMAN | -569.4 | 0.0226 | -562.3 | 0.0023 | 0.1306 | 1.6E-04 | 5.1E-03 |
| g38924 | cct7 | TCPH\_HUMAN | -2188.0 | 0.0512 | -2181.8 | 0.0218 | 0.0558 | 4.5E-04 | 9.8E-03 |
| g38986 | rpsa | RSSA\_XENTR | -1379.9 | 0.0224 | -1373.4 | 0.0001 | 0.2063 | 3.0E-04 | 7.5E-03 |
| g39077 | adam28 | ID2B\_HUMAN | -3608.3 | 0.4204 | -3601.4 | 1.1302 | 0.3204 | 2.1E-04 | 5.9E-03 |
| g3909 | cfap45 | CFA45\_HUMAN | -1222.1 | 0.1520 | -1216.3 | 0.0001 | 0.2057 | 6.8E-04 | 1.3E-02 |
| g39132 | leprotl1 | LERL1\_HUMAN | -803.3 | 0.1015 | -794.9 | 0.0096 | 0.8451 | 4.1E-05 | 1.7E-03 |
| g39373 | hbg2 | HBB2\_XENLA | -1003.5 | 0.5434 | -984.0 | 9.8853 | 0.0421 | 4.3E-10 | 1.1E-07 |
| g39390 | LOC100489851 | CRG1\_RANTE | -1035.7 | 0.1042 | -1028.9 | 2.6719 | 0.0568 | 2.4E-04 | 6.4E-03 |
| g39581 | spop | SPOPA\_XENLA | -969.4 | 0.0182 | -951.6 | 0.2276 | 0.0001 | 2.5E-09 | 4.9E-07 |
| g39924 | LOC100485772 | FBCDB\_XENLA | -1087.6 | 0.2417 | -1082.6 | 0.3316 | 0.2226 | 1.6E-03 | 2.5E-02 |
| g40152 | med20 | MED20\_XENLA | -831.8 | 0.1053 | -827.5 | 1.1428 | 0.0001 | 3.2E-03 | 4.0E-02 |
| g40367 | csmd2 | CSMD2\_HUMAN | -707.6 | 1.3612 | -702.0 | 10.0000 | 0.3277 | 8.4E-04 | 1.5E-02 |
| g40593 | cyfip1 | CYFP1\_HUMAN | -952.0 | 0.0531 | -947.0 | 0.0001 | 0.2856 | 1.5E-03 | 2.3E-02 |
| g40599 | tubgcp5 | GCP5\_HUMAN | -980.9 | 0.1005 | -975.8 | 1.5739 | 0.0082 | 1.3E-03 | 2.2E-02 |
| g41140 | ints4 | INT4\_XENLA | -1599.7 | 0.0819 | -1581.2 | 0.0001 | 0.0893 | 1.1E-09 | 2.5E-07 |
| g41141 | kctd14 | KCD14\_HUMAN | -1175.6 | 0.0452 | -1168.6 | 0.0024 | 0.6452 | 1.9E-04 | 5.5E-03 |
| g41145 | kctd14 | KCD14\_HUMAN | -1392.0 | 0.0396 | -1382.8 | 0.0019 | 0.5242 | 1.8E-05 | 8.6E-04 |
| g41225 | MGC107841 | HBB2\_XENTR | -3924.2 | 0.2331 | -3918.4 | 0.6642 | 0.1844 | 6.4E-04 | 1.2E-02 |
| g41571 | LOC100489684 | CRG1\_RANTE | -1396.1 | 0.1045 | -1391.0 | 0.2228 | 0.0650 | 1.3E-03 | 2.2E-02 |
| g41647 | LOC100497503 | CP2C8\_HUMAN | -2302.1 | 0.3328 | -2291.7 | 1.5118 | 0.1381 | 5.5E-06 | 3.3E-04 |
| g41764 | l3mbtl3 | LMBL4\_HUMAN | -3767.1 | 0.1580 | -3756.4 | 0.0376 | 0.5088 | 3.8E-06 | 2.5E-04 |
| g42163 | LOC101731432 | RRP44\_HUMAN | -1101.0 | 0.1819 | -1080.9 | 1.1150 | 0.0574 | 2.4E-10 | 6.8E-08 |
| g42175 | adam28 | ATS10\_HUMAN | -1720.8 | 0.2210 | -1714.9 | 0.7435 | 0.1456 | 5.9E-04 | 1.2E-02 |
| g42416 | MGC89248 | RDH16\_HUMAN | -615.4 | 0.0844 | -609.6 | 4.6101 | 0.0165 | 6.9E-04 | 1.3E-02 |
| g42511 | LOC100497729 | CNFN\_XENTR | -912.4 | 0.1444 | -905.9 | 1.3415 | 0.0328 | 3.2E-04 | 7.5E-03 |
| g4290 | r3hdm2 | SYVN1\_DANRE | -364.7 | 0.0975 | -360.5 | 0.0203 | 1.5389 | 3.5E-03 | 4.3E-02 |
| g4514 | trim9 | TRIM9\_HUMAN | -1624.4 | 0.1203 | -1619.5 | 0.0001 | 0.1554 | 1.7E-03 | 2.6E-02 |
| g4578 | uba6 | UBA6\_HUMAN | -1085.5 | 0.1232 | -1080.8 | 0.0089 | 0.5184 | 2.1E-03 | 2.9E-02 |
| g4863 | kif5b | KINH\_HUMAN | -1933.3 | 0.0361 | -1929.0 | 0.1135 | 0.0234 | 3.4E-03 | 4.2E-02 |
| g4890 | pgc | PEPC\_HUMAN | -786.4 | 0.1618 | -781.9 | 10.0000 | 0.1274 | 2.7E-03 | 3.6E-02 |
| g5141 | LOC100485974 | RHG39\_HUMAN | -554.3 | 0.0230 | -549.8 | 0.0001 | 0.1066 | 2.8E-03 | 3.6E-02 |
| g520 | cdip1 | CDIP1\_XENLA | -1007.4 | 0.2020 | -999.9 | 0.0438 | 0.2331 | 1.1E-04 | 4.0E-03 |
| g5259 | dock9 | DOCK9\_HUMAN | -379.5 | 0.0491 | -374.3 | 0.0001 | 10.0000 | 1.3E-03 | 2.2E-02 |
| g5286 | sspo | ATL5\_HUMAN | -1814.3 | 0.2044 | -1800.3 | 0.0372 | 1.5788 | 1.2E-07 | 1.4E-05 |
| g538 | sec31b | SC31B\_HUMAN | -889.5 | 0.1219 | -878.4 | 0.6886 | 0.0001 | 2.4E-06 | 1.7E-04 |
| g620 | LOC100497460 | C2G1P\_HUMAN | -2906.4 | 0.2300 | -2901.9 | 0.5790 | 0.1963 | 2.7E-03 | 3.6E-02 |
| g6283 | chmp2a | CHM2A\_XENLA | -1014.3 | 0.0895 | -991.2 | 1.3879 | 0.0203 | 1.1E-11 | 4.4E-09 |
| g6323 | LOC100145082 | ST1C4\_HUMAN | -2047.1 | 0.1320 | -2037.6 | 0.0616 | 0.6063 | 1.3E-05 | 7.1E-04 |
| g6358 | brinp2 | BRNP3\_HUMAN | -932.6 | 0.2829 | -922.7 | 10.0000 | 0.0373 | 8.5E-06 | 4.9E-04 |
| g6678 | taf2 | TAF2\_DANRE | -999.2 | 0.1600 | -994.8 | 0.0145 | 1.5835 | 3.0E-03 | 3.8E-02 |
| g6875 | nr6a1 | NR6A1\_XENLA | -594.7 | 0.2180 | -588.3 | 0.0117 | 1.2940 | 3.5E-04 | 7.9E-03 |
| g7180 | tmem55b | PP4P1\_XENLA | -525.1 | 0.1423 | -518.1 | 0.0126 | 2.4106 | 1.8E-04 | 5.3E-03 |
| g7391 | dscaml1 | DSCL1\_HUMAN | -558.9 | 0.1046 | -554.1 | 0.0337 | 0.5132 | 2.0E-03 | 2.9E-02 |
| g7555 | cog1 | COG1\_HUMAN | -660.2 | 0.5069 | -653.6 | 0.0137 | 2.7297 | 2.9E-04 | 7.3E-03 |
| g7561 | LOC100489851 | CRG1\_RANTE | -1963.5 | 0.1078 | -1959.4 | 0.5086 | 0.0902 | 4.4E-03 | 5.0E-02 |
| g7620 | hbg2 | HBB2\_XENLA | -1006.6 | 0.5573 | -981.2 | 10.0000 | 0.0417 | 9.8E-13 | 9.7E-10 |
| g7688 | smim18 | SIM18\_HUMAN | -764.2 | 0.1566 | -758.2 | 0.9261 | 0.0267 | 5.3E-04 | 1.1E-02 |
| g7766 | acox1 | ACOX1\_HUMAN | -735.1 | 0.0930 | -727.8 | 1.1980 | 0.0001 | 1.3E-04 | 4.4E-03 |
| g7772 | unc13d | UN13D\_HUMAN | -824.6 | 0.1862 | -819.4 | 0.0241 | 0.7904 | 1.2E-03 | 2.1E-02 |
| g80 | myh3 | MYH2\_HUMAN | -10165.9 | 0.0626 | -10159.8 | 0.1270 | 0.0555 | 4.9E-04 | 1.0E-02 |
| g800 | hsd17b10 | HCD2\_HUMAN | -2313.3 | 0.0267 | -2307.4 | 0.0001 | 0.2910 | 5.9E-04 | 1.2E-02 |
| g8010 | LOC105947758 | CAD23\_HUMAN | -531.5 | 0.0928 | -524.9 | 2.8005 | 0.0117 | 2.9E-04 | 7.3E-03 |
| g8401 | slc25a40 | S2540\_XENTR | -1113.5 | 0.1337 | -1107.8 | 0.9791 | 0.0301 | 7.4E-04 | 1.4E-02 |
| g9435 | rab11a | RB11A\_HUMAN | -1137.6 | 0.0139 | -1130.4 | 0.0942 | 0.0065 | 1.5E-04 | 4.8E-03 |
| g9487 | pabpn1l | EPA2A\_XENLA | -1163.2 | 0.1299 | -1156.8 | 0.6734 | 0.0240 | 3.7E-04 | 8.3E-03 |
| g9818 | LOC100493382 | C4BPA\_HUMAN | -676.4 | 0.1924 | -662.1 | 3.5302 | 0.0086 | 8.4E-08 | 1.0E-05 |

**Supplementary Table 9. Functions of genes under positive selection in *Sp. multiplicata*.** Functions were determined by exploring the gene description on Xenbase or, if no description was available, by exploring the description on the Uniprot database. ‘NA’ indicates that no meaningful description could be found.

|  |  |  |  |
| --- | --- | --- | --- |
| *Spea* gene | Generic function | *X. tropicalis* match | Swissprot best match |
| g8010 | cell adhesion | LOC105947758 | CAD23\_HUMAN |
| g16767 | digestive system related | zg16 | ZG16\_HUMAN |
| g32315 | electron transport chain | LOC100493382 | NDUV3\_HUMAN |
| g39390 | eye formation | LOC100489851 | CRG1\_RANTE |
| g15278 | glycolysis | ppcdc | COAC\_HUMAN |
| g40367 | immune response | csmd2 | CSMD2\_HUMAN |
| g9818 | immune response | LOC100493382 | C4BPA\_HUMAN |
| g36089 | intracellular homeostasis | LOC100498621 | ZNT9\_HUMAN |
| g26118 | intracellular organization | syne1 | SYNE1\_HUMAN |
| g26543 | lipid metabolism | abca5 | ABCA5\_HUMAN |
| g42416 | metabolism | MGC89248 | RDH16\_HUMAN |
| g15892 | nervous system development | synj2 | SYNJ2\_HUMAN |
| g6358 | neural development | brinp2 | BRNP3\_HUMAN |
| g4890 | protein metabolism | pgc | PEPC\_HUMAN |
| g10134 | protein modification | LOC100493382 | UBP13\_DANRE |
| g22100 | protein modification | pdzrn3 | PZR3B\_DANRE |
| g39373 | oxygen transport | hbg2 | HBB2\_XENLA |
| g7620 | oxygen transport | hbg2 | HBB2\_XENLA |
| g11875 | oxygen transport | LOC100145107 | HBB2\_XENTR |
| g13150 | smell | LOC100492966 | O52A1\_HUMAN |
| g11371 | NA | fam167a | F167A\_HUMAN |
| g25634 | NA | LOC100493251 | UN93A\_XENLA |

**Supplementary Table 10. Results from gene ontology (GO) analysis of genes under positive selection in *Spea*.** ‘Functional grouping’ was determined by exploring the term’s page on QuickGO (see Methods for more details).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Term** | **χ2** | **p** | **q** | **Term description** | **Functional grouping** |
| GO:0009108 | 88.454 | 0.012 | 0.065 | coenzyme biosynthetic process | enzyme synthesis |
| GO:0015937 | 88.454 | 0.013 | 0.065 | coenzyme A biosynthetic process | enzyme synthesis |
| GO:0045494 | 88.454 | 0.014 | 0.065 | photoreceptor cell maintenance | eye function |
| GO:0045959 | 88.454 | 0.013 | 0.065 | negative regulation of complement activation, classical pathway  | immune function |
| GO:1903027 | 88.454 | 0.014 | 0.065 | regulation of opsonization | immune function |
| GO:0006997 | 88.454 | 0.011 | 0.065 | nucleus organization | intracellular organization |
| GO:0090286 | 88.454 | 0.013 | 0.065 | cytoskeletal anchoring at nuclear membrane | intracellular organization |
| GO:0090292 | 88.454 | 0.014 | 0.065 | nuclear matrix anchoring at nuclear membrane | intracellular organization |
| GO:0034375 | 88.454 | 0.010 | 0.065 | high-density lipoprotein particle remodeling  | lipid and protein metabolism |
| GO:0010745 | 88.454 | 0.011 | 0.065 | negative regulation of macrophage derived foam cell differentiation  | lipid metabolism |
| GO:0043691 | 88.454 | 0.011 | 0.065 | reverse cholesterol transport  | lipid metabolism |
| GO:0033344 | 88.454 | 0.013 | 0.065 | cholesterol efflux  | lipid metabolism |
| GO:0006829 | 88.454 | 0.013 | 0.065 | zinc ion transport  | zinc transport |

**Supplementary Table 11. Assembly statistics of short read libraries for *Sp. bombifrons, Sc. couchii,* and *Sc. holbrookii*.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Kmer size** | **Total size** | **Number of sequences** | **Longest sequence** | **N25** | **N50** | **N75** |
| Sp. bombifrons | 101 | 956,925,739 | 3,134,405 | 7,759 | 734 | 444 | 217 |
| Sc. couchii | 51 | 1,111,494,020 | 869,0179 | 2,463 | 346 | 187 | 85 |
| Sc. holbrookii | 81 | 1,015,691,853 | 4,300,914 | 4,686 | 664 | 385 | 160 |

**References**

1 Tamazian, G. *et al.* Chromosomer: a reference-based genome arrangement tool for producing draft chromosome sequences. *Gigascience* **5**, 38, doi:10.1186/s13742-016-0141-6 (2016).