**SUPPLEMENTARY TABLES AND FIGURES**

**Table S1.** List of species providing information for the available mitochondrial genome in the suborder Troglotremata and other trematodes used in this study for sequence comparative and phylogenetic analyses

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Family/Species/Strains** | **GenBank** | **Complete**  **mtDNA as reported (bp)** | **mtDNA\***  **(*cox*3-*nad*5)** | **PCGs** | **MRGs** | **Country of collection** | **Reference**  **(if any)** |
|  | **Suborder Troglotremata** |  |  |  |  |  |  |  |
|  | **Paragonimidae** |  |  |  |  |  |  |  |
| 1 | *Paragonimus skrjabini miyazakii* | ON782295 | 17591 | 13240 | 10098 | 1714 | Japan | This study |
| 2 | *Paragonimus heterotremus* | MH059809 | 13927 | 13222 | 10101 | 1711 | China | Qian *et al*. (2018) |
| 3 | *Paragonimus heterotremus* | KY952166 | 13526 | 13230 | 10101 | 1720 | Vietnam | GenBank |
| 4 | *Paragonimus ohirai* | KX765277 | 14818 | 13222 | 10104 | 1710 | Japan | Le *et al.* (2019) |
| 5 | *Paragonimus westermani* | MN412705 | 14790 | 13215 | 10104 | 1731 | China | GenBank |
| 6 | *Paragonimus westermani* (3n) | AF540958 | 14244 | 13213 | 10101 | 1732 | South Korea | GenBank |
| 7 | *Paragonimus westermani* (2n) | AF219379 | 14965 | 13213 | 10101 | 1732 | South Korea | GenBank |
| 8 | *Paragonimus westermani* (TypeI) | KM280646 | 14965 | 13223 | 10104 | 1729 | India | GenBank |
| 9 | *Paragonimus westermani* (AP) | KX943544 | 14975 | 13216 | 10104 | 1721 | India | Biswal *et al.* (2014) |
| 10 | *Paragonimus westermani* (IND2009) | CM017921 | 20273 | 13230 | 10104 | 1729 | India | Oye *et al*. (2019*b*) |
| 11 | *Paragonimus kellicotti* | MH322000 | 13786 | 13196 | 10098 | 1711 | United States | Wang *et al.* (2018) |
|  | **Suborder Opisthorchiata** |  |  |  |  |  |  |  |
|  | **Heterophyidae** |  |  |  |  |  |  |  |
| 12 | *Haplorchis taichui* | MG972809 | 15120 | 13277 | 10164 | 1730 | Vietnam | GenBank |
| 13 | *Haplorchis taichui* | KF214770 | 15131 | 13225 | 10164 | 1730 | Laos | Lee *et al*. (2013) |
| 14 | *Metagonimus yokogawai* | KC330755 | 15258 | 13364 | 10245 | 1736 | South Korea | GenBank |
|  | **Opisthorchiidae** |  |  |  |  |  |  |  |
| 15 | *Clonorchis sinensis* | FJ381664 | 13875 | 13511 | 10209 | 1777 | Russia | Shekhovtsov *et al*. (2010) |
| 16 | *Clonorchis sinensis* | JF729303 | 13879 | 13514 | 10209 | 1778 | China | Cai *et al*. (2012) |
| 17 | *Clonorchis sinensis* | JF729304 | 13877 | 13512 | 10209 | 1777 | South Korea | Cai *et al*. (2012) |
| 18 | *Opisthorchis viverrini* | JF739555 | 13510 | 13457 | 10206 | 1767 | Laos | Cai *et al*. (2012) |
| 19 | *Opisthorchis felineus* | EU921260 | 14277 | 13499 | 10218 | 1769 | Russia | Shekhovtsov *et al*. (2010) |
| 20 | *Amphimerus* sp. | MK238506 | 15151 | 13387 | 10179 | 1754 | Ecuador | Ma *et al*. (2019) |
| 21 | *Metorchis orientalis* | KT239342 | 13834 | 13422 | 10176 | 1765 | China | Na *et al*. (2016) |
|  | **Suborder Echinostomata** |  |  |  |  |  |  |  |
|  | **Echinostomatidae** |  |  |  |  |  |  |  |
| 22 | *Artyfechinostomum malayanum* | OK509083 | 17175 | 13408 | 10131 | 1725 | Thailand | Pham *et al*. (2022) |
| 23 | *Artyfechinostomum sufrartyfex* | KY548763 | 14567 | 13409 | 10131 | 1728 | India | GenBank |
| 24 | *Echinostoma caproni* | AP017706 | 14150 | 13293 | 10128 | 1709 | Egypt | GenBank |
| 25 | *Echinostoma miyagawai* | MN116740 | 14468 | 13320 | 10128 | 1724 | China | Fu *et al.* (2019) |
| 26 | *Echinostoma miyagawai* | MH393928 | 14410 | 13321 | 10128 | 1763 | China | Li *et al*. (2019) |
| 27 | *Echinostoma paraensei* | KT008005 | 20298 | 13319 | 10128 | 1748 | NA | GenBank |
| 28 | *Echinostoma revolutum* | MN496162 | 17030 | 13326 | 10134 | 1733 | Thailand | Le *et al.* (2020) |
| 29 | *Hypoderaeum conoideum* | KM111525 | 14180 | 13361 | 10116 | 1730 | China | Yang *et al.* (2015) |
|  | **Fasciolidae** |  |  |  |  |  |  |  |
| 30 | *Fasciola gigantica* | KF543342 | 14478 | 13309 | 10107 | 1755 | China | Liu *et al.* (2014) |
| 31 | *Fasciola* sp. (hybrid) | KF543343 | 14453 | 13282 | 10107 | 1755 | China | Liu *et al.* (2014) |
| 32 | *Fasciola hepatica* | AF216697 | 14462 | 13305 | 10104 | 1755 | Australia | Le *et al.* (2001a) |
| 33 | *Fasciola jacksoni* | KX787886 | 14952 | 13286 | 10137 | 1743 | Sri Lanka | Rajapakse *et al*. (2020) |
| 34 | *Fascioloides magna* | KU060148 | 14047 | 13272 | 10131 | 1745 | Czech | Ma *et al.* (2016) |
| 35 | *Fasciolopsis buski* | KX169163 | 14833 | 13380 | 10122 | 1768 | China | Ma *et al.* (2017) |
|  | **Schistosomatidae (outgroup)** |  |  |  |  |  |  |  |
| 36 | *Schistosoma haematobium* | DQ157222 | 15003 | - | 10116 | - | Mali | Littlewood *et al.* (2006) |

mtDNA: the entire mitochondrial genome; mtDNA\*: coding mitochondrial genome (5’-*cox*3 to *nad*5-3’); PCGs: protein-coding genes; MRGs: mitoribosomal genes.

**Table S2.** List of trematode-universal and specific primers for long-PCR used for enrichment of the mitochondrial genome of *Paragonimus skrjabini miyazakii* for next-generation sequencing

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Primer**  **name** | **Sequence (5’ -> 3’)** | **Length/**  **Tm (oC)** | **Position** | **Binding targe (gene)** |
| 1 | PAR3F | GTGTCTKTGGAGBATGGATGG | 21/56.3 | 218–239 | *cox*3 |
| 2 | PMI2R | CACTCGCACAAAATCCCCAC | 20/53.8 | 5738–5758 | *nad*1 |
| 3 | PAR4F | GTTYTTTAYGAGGGTGCCATATTGCC | 26/56.4 | 2152–2377 | *nad*4 |
| 4 | UNI16R | TCTCGGGGTCTTTCCGTCT | 19/53.2 | 9118–9136 | *rrn*L |
| 5 | PMI3F | GGAGTGATAGGCATTCCTACGG | 22/56.7 | 7771–7792 | *cox*1 |
| 6 | GLYR | ACKAGACCHCYGACTTGGAAAGAC | 24/55.7 | 13280–13303 | *trn*G |
| 7 | \*PMI12F | AAGGGTGTTGTTTGGGGCTC | 20/53.8 | 13004–13023 | *nad*5 |
| 8 | \*PMI15R | TAGAACCCGCCTTATGGAGG | 20/53.8 | 1284–1303 | *cob* (*cyt*B) |
| 9 | \*GLYF | AGTATKYYGTCTTTCCAAGTC | 21/52.4 | 13272–13292 | *trn*G |
| 10 | \*PMI20R | ACCCTACCCCAAGAGCAATTG | 21/54.4 | 374–394 | *cox*3 |

\*Primers 7–8 and 9–10 were paired for amplifying the NCR for validation of the length and for NGS sequencing.

**Table S3.** Amplicons of the complete mitochondrial genome of *Paragonimus skrjabini miyazakii* by LPCR using trematode-universal and specific primers and used for next-generation sequencing

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Primer pair** | **Length of amplicons** | **Position in the genome** | **Spanning**  **region** |
| 1 | PAR3F-PMI2R | 5.5 kb | 218–5758 | *cox*3–*nad*1 |
| 2 | PAR4F-UNI16R | 7 kb | 2152–9136 | *nad*4–*rrn*L |
| 3 | PMI3F- GLYR | 5.5 kb | 7771–13303 | *nad*1–*trnG* |
| 5 | PMI12F-PMI15R | 5.9 kb | 13004–1303 | *nad*5–*cob* |
| 6 | GLYF-PMI15R | 4.9 kb | 13272–394 | *trn*G–*cox*3 |

**Table S4.** Statistics of codon usage in the mitochondrial genomes of 11 *Paragonimus* strains/species of the family Paragonimidae (suborder Troglotremata) (Platyhelminthes: Trematoda)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| aa | Codon | *P. s. miyazakii*  (Pmiy-OkuST1-JP)  (Japan)  ON782295 | | *P. heterotremus*  (Phet-LC-VN) (Vietnam)  KY952166 | | *P. heterotremus*  (Phet –GX-CN)  (China)  MH059809 | | *P. ohirai*  (Pohi-Kino-JP)  (Japan)  KX765277 | | *P. westermani*  (Pwes(2n)-Haenam-KR)  (Korea) AF540958 | | *P. westermani*  (Pwes(3n)-Bogil-KR)  (Korea)  AF219379 | | *P. westermani*  (Pwes-(dog1)-CN)  (China)  MN412705 | | *P. westermani*  (Pwes-TypeI-IN)  (India)  KM280646 | | *P. westermani*  (Pwes-AP-IN)  (India)  KX943544 | | *P. westermani*  (Pwes-IND2009-IN)  (India) CM017921 | | *P. kellicotti*  (Pkel-Ozark-US)  (United States)  MH322000 | |
| No | % | No | % | No | No | % | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % |
| **Ala** | GCG | 36 | 1.07 | 37 | 1.10 | 38 | 1.13 | 28 | 0.83 | 51 | 1.51 | 50 | 1.49 | 52 | 1.54 | 48 | 1.43 | 49 | 1.45 | 49 | 1.46 | 44 | 1.31 |
|  | GCA | 10 | 0.30 | 19 | 0.56 | 18 | 0.53 | 23 | 0.68 | 13 | 0.39 | 12 | 0.36 | 12 | 0.36 | 15 | 0.45 | 12 | 0.36 | 14 | 0.42 | 13 | 0.39 |
|  | GCT | 75 | 2.23 | 68 | 2.02 | 64 | 1.90 | 66 | 1.96 | 59 | 1.75 | 61 | 1.81 | 57 | 1.69 | 61 | 1.81 | 60 | 1.78 | 59 | 1.75 | 67 | 1.99 |
|  | GCC | 22 | 0.65 | 20 | 0.59 | 22 | 0.65 | 18 | 0.53 | 29 | 0.86 | 29 | 0.86 | 30 | 0.89 | 26 | 0.77 | 27 | 0.80 | 23 | 0.68 | 18 | 0.54 |
| **Cys** | TGT | 93 | 2.76 | 87 | 2.58 | 87 | 2.58 | 94 | 2.79 | 70 | 2.08 | 72 | 2.14 | 69 | 2.05 | 79 | 2.35 | 84 | 2.49 | 85 | 2.52 | 80 | 2.38 |
|  | TGC | 16 | 0.48 | 20 | 0.59 | 18 | 0.53 | 12 | 0.36 | 39 | 1.16 | 38 | 1.13 | 40 | 1.19 | 29 | 0.86 | 24 | 0.71 | 25 | 0.74 | 22 | 0.65 |
| **Asp** | GAT | 55 | 1.63 | 60 | 1.78 | 59 | 1.75 | 56 | 1.66 | 60 | 1.78 | 60 | 1.78 | 59 | 1.75 | 55 | 1.63 | 54 | 1.60 | 54 | 1.60 | 56 | 1.66 |
|  | GAC | 15 | 0.45 | 7 | 0.21 | 9 | 0.27 | 10 | 0.30 | 6 | 0.18 | 6 | 0.18 | 7 | 0.21 | 12 | 0.36 | 14 | 0.41 | 12 | 0.36 | 9 | 0.27 |
| **Glu** | GAG | 62 | 1.84 | 63 | 1.87 | 64 | 1.90 | 65 | 1.93 | 68 | 2.02 | 68 | 2.02 | 66 | 1.96 | 61 | 1.81 | 65 | 1.92 | 66 | 1.96 | 68 | 2.02 |
|  | GAA | 17 | 0.51 | 18 | 0.54 | 15 | 0.45 | 12 | 0.36 | 13 | 0.39 | 13 | 0.39 | 15 | 0.45 | 18 | 0.53 | 18 | 0.53 | 17 | 0.51 | 10 | 0.30 |
| **Phe** | **TTT** | **341** | **10.13** | **303** | **9.00** | **301** | **8.94** | **325** | **9.65** | **208** | **6.19** | **208** | **6.18** | **214** | **6.35** | **255** | **7.57** | **253** | **7.49** | **263** | **7.81** | **325** | **9.66** |
|  | TTC | 28 | 0.83 | 44 | 1.31 | 45 | 1.34 | 36 | 1.07 | 130 | 3.90 | 131 | 3.90 | 124 | 3.68 | 93 | 2.76 | 82 | 2.43 | 85 | 2.52 | 30 | 0.89 |
| **Gly** | GGG | 116 | 3.45 | 100 | 2.97 | 99 | 2.94 | 88 | 2.61 | 107 | 3.18 | 105 | 3.12 | 106 | 3.15 | 103 | 3.06 | 110 | 3.25 | 109 | 3.24 | 111 | 3.30 |
|  | GGA | 48 | 1.43 | 35 | 1.04 | 37 | 1.10 | 68 | 2.02 | 43 | 1.28 | 43 | 1.28 | 43 | 1.28 | 58 | 1.72 | 48 | 1.42 | 54 | 1.60 | 38 | 1.13 |
|  | GGT | 121 | 3.60 | 150 | 4.46 | 146 | 4.34 | 132 | 3.92 | 128 | 3.80 | 130 | 3.86 | 129 | 3.83 | 109 | 3.24 | 116 | 3.42 | 110 | 3.27 | 137 | 4.07 |
|  | GGC | 35 | 1.04 | 32 | 0.95 | 36 | 1.07 | 19 | 0.56 | 43 | 1.28 | 42 | 1.25 | 43 | 1.28 | 39 | 1.16 | 36 | 1.07 | 33 | 0.98 | 23 | 0.68 |
| **His** | CAT | 50 | 1.49 | 48 | 1.43 | 49 | 1.46 | 55 | 1.63 | 32 | 0.95 | 33 | 0.98 | 34 | 1.01 | 36 | 1.07 | 37 | 1.10 | 39 | 1.16 | 51 | 1.52 |
|  | CAC | 8 | 0.24 | 11 | 0.33 | 10 | 0.30 | 3 | 0.09 | 28 | 0.83 | 27 | 0.80 | 26 | 0.77 | 28 | 0.83 | 26 | 0.77 | 24 | 0.71 | 10 | 0.30 |
| **Ile** | ATA | 41 | 1.22 | 27 | 0.80 | 26 | 0.77 | 37 | 1.10 | 22 | 0.65 | 21 | 0.63 | 22 | 0.65 | 22 | 0.65 | 19 | 0.56 | 22 | 0.65 | 36 | 1.07 |
|  | ATT | 118 | 3.51 | 101 | 3.00 | 101 | 3.00 | 137 | 4.07 | 57 | 1.69 | 55 | 1.63 | 58 | 1.72 | 73 | 2.17 | 71 | 2.10 | 73 | 2.17 | 108 | 3.21 |
|  | ATC | 19 | 0.56 | 25 | 0.74 | 25 | 0.74 | 11 | 0.33 | 47 | 1.40 | 48 | 1.43 | 47 | 1.40 | 34 | 1.01 | 38 | 1.12 | 38 | 1.13 | 17 | 0.51 |
| **Lys** | AAG | 49 | 1.46 | 50 | 1.49 | 51 | 1.51 | 53 | 1.57 | 51 | 1.52 | 50 | 1.49 | 49 | 1.46 | 47 | 1.40 | 49 | 1.45 | 49 | 1.46 | 47 | 1.40 |
| **Leu** | **TTG** | **270** | **8.02** | **262** | **7.78** | **264** | **7.84** | **263** | **7.81** | **232** | **6.89** | **233** | **6.92** | **234** | **6.95** | **245** | **7.27** | **252** | **7.46** | **251** | **7.45** | **273** | **8.11** |
|  | TTA | 137 | 4.07 | 92 | 2.73 | 99 | 2.94 | 162 | 4.81 | 35 | 1.04 | 34 | 1.01 | 34 | 1.01 | 65 | 1.93 | 60 | 1.78 | 70 | 2.08 | 111 | 3.30 |
|  | CTG | 27 | 0.80 | 62 | 1.84 | 59 | 1.75 | 14 | 0.42 | 148 | 4.40 | 148 | 4.40 | 145 | 4.31 | 95 | 2.82 | 92 | 2.72 | 88 | 2.61 | 42 | 1.25 |
|  | CTA | 20 | 0.59 | 20 | 0.59 | 18 | 0.53 | 18 | 0.53 | 13 | 0.39 | 12 | 0.36 | 13 | 0.39 | 17 | 0.51 | 18 | 0.53 | 18 | 0.53 | 23 | 0.68 |
|  | CTT | 105 | 3.12 | 120 | 3.56 | 122 | 3.62 | 118 | 3.50 | 80 | 2.38 | 80 | 2.38 | 83 | 2.46 | 106 | 3.15 | 105 | 3.11 | 104 | 3.09 | 111 | 3.30 |
|  | CTC | 15 | 0.45 | 29 | 0.86 | 26 | 0.77 | 7 | 0.21 | 71 | 2.11 | 72 | 2.14 | 70 | 2.08 | 42 | 1.25 | 45 | 1.33 | 45 | 1.34 | 21 | 0.62 |
| **Met** | ATG | 89 | 2.64 | 93 | 2.76 | 93 | 2.76 | 92 | 2.73 | 85 | 2.53 | 87 | 2.59 | 86 | 2.55 | 87 | 2.58 | 86 | 2.54 | 87 | 2.58 | 91 | 2.70 |
| **Asn** | AAA | 18 | 0.54 | 21 | 0.62 | 22 | 0.65 | 18 | 0.53 | 26 | 0.77 | 26 | 0.77 | 26 | 0.77 | 27 | 0.80 | 27 | 0.80 | 26 | 0.77 | 24 | 0.71 |
|  | AAT | 40 | 1.19 | 43 | 1.28 | 41 | 1.22 | 51 | 1.51 | 26 | 0.77 | 24 | 0.71 | 24 | 0.71 | 32 | 0.95 | 35 | 1.04 | 33 | 0.98 | 38 | 1.13 |
|  | AAC | 12 | 0.36 | 8 | 0.24 | 11 | 0.33 | 6 | 0.18 | 20 | 0.59 | 20 | 0.60 | 22 | 0.65 | 19 | 0.56 | 20 | 0.59 | 17 | 0.51 | 9 | 0.27 |
| **Pro** | CCG | 20 | 0.59 | 27 | 0.80 | 23 | 0.68 | 20 | 0.59 | 26 | 0.77 | 27 | 0.80 | 27 | 0.80 | 27 | 0.80 | 26 | 0.77 | 26 | 0.77 | 23 | 0.68 |
|  | **CCA** | 11 | 0.33 | 10 | 0.30 | 13 | 0.39 | 11 | 0.33 | 6 | 0.18 | 5 | 0.15 | 5 | 0.15 | **5** | **0.15** | **5** | **0.15** | **5** | **0.15** | 6 | 0.18 |
|  | CCT | 44 | 1.31 | 44 | 1.31 | 48 | 1.43 | 51 | 0.51 | 44 | 1.31 | 41 | 1.22 | 43 | 1.28 | 39 | 1.16 | 40 | 1.18 | 39 | 1.16 | 46 | 1.37 |
|  | CCC | 14 | 0.42 | 11 | 0.33 | 8 | 0.24 | 10 | 0.30 | 14 | 0.42 | 18 | 0.54 | 15 | 0.45 | 18 | 0.53 | 19 | 0.56 | 20 | 0.59 | 18 | 0.54 |
| **Gln** | CAG | 24 | 0.71 | 24 | 0.71 | 25 | 0.74 | 25 | 0.74 | 25 | 0.74 | 25 | 0.74 | 25 | 0.74 | 24 | 0.71 | 24 | 0.71 | 24 | 0.71 | 24 | 0.71 |
|  | **CAA** | 6 | 0.18 | 6 | 0.18 | 5 | 0.15 | 6 | 0.18 | **3** | **0.09** | **3** | **0.09** | **3** | **0.09** | 6 | 0.18 | 4 | 0.12 | 5 | 0.15 | 5 | 0.15 |
| **Arg** | CGG | 18 | 0.54 | 16 | 0.48 | 16 | 0.48 | 13 | 0.39 | 28 | 0.83 | 28 | 0.83 | 27 | 0.8 | 23 | 0.68 | 23 | 0.68 | 22 | 0.65 | 11 | 0.33 |
|  | **CGA** | 7 | 0.21 | **3** | **0.09** | **3** | **0.09** | **7** | 0.21 | 4 | 0.12 | 5 | 0.15 | 4 | 0.12 | 9 | 0.27 | 10 | 0.30 | 9 | 0.27 | 9 | 0.27 |
|  | CGT | 34 | 1.01 | 38 | 1.13 | 38 | 1.13 | 42 | 1.25 | 27 | 0.80 | 27 | 0.80 | 28 | 0.83 | 29 | 0.86 | 30 | 0.89 | 29 | 0.86 | 39 | 1.16 |
|  | **CGC** | **5** | **0.15** | 6 | 0.18 | 7 | 0.21 | **1** | **0.03** | 11 | 0.33 | 11 | 0.33 | 10 | 0.3 | 6 | 0.18 | 7 | 0.21 | 6 | 0.18 | **5** | **0.15** |
| **Ser** | AGG | 77 | 2.29 | 75 | 2.23 | 76 | 2.26 | 70 | 2.08 | 91 | 2.70 | 88 | 2.61 | 89 | 2.64 | 78 | 2.32 | 82 | 2.43 | 74 | 2.20 | 70 | 2.08 |
|  | AGA | 27 | 0.80 | 36 | 1.07 | 33 | 0.98 | 35 | 1.04 | 16 | 0.48 | 18 | 0.54 | 17 | 0.51 | 39 | 1.16 | 37 | 1.10 | 38 | 1.13 | 31 | 0.92 |
|  | AGT | 58 | 1.72 | 54 | 1.60 | 50 | 1.49 | 64 | 1.90 | 42 | 1.25 | 44 | 1.31 | 45 | 1.34 | 55 | 1.63 | 55 | 1.63 | 58 | 1.72 | 63 | 1.87 |
|  | AGC | 16 | 0.48 | 17 | 0.51 | 19 | 0.56 | 10 | 0.33 | 26 | 0.77 | 25 | 0.74 | 24 | 0.71 | 17 | 0.51 | 19 | 0.56 | 13 | 0.39 | 20 | 0.59 |
|  | TCG | 29 | 0.86 | 34 | 1.01 | 35 | 1.04 | 17 | 0.51 | 50 | 1.49 | 49 | 1.46 | 50 | 1.49 | 34 | 1.01 | 37 | 1.10 | 32 | 0.95 | 26 | 0.77 |
|  | **TCA** | 11 | 0.33 | 4 | 0.12 | **3** | **0.09** | 19 | 0.56 | 7 | 0.21 | 6 | 0.18 | 6 | 0.18 | 16 | 0.48 | 14 | 0.41 | 18 | 0.53 | 11 | 0.33 |
|  | TCT | 116 | 3.45 | 122 | 3.62 | 121 | 3.59 | 137 | 4.07 | 79 | 2.35 | 80 | 2.38 | 79 | 2.35 | 99 | 2.94 | 105 | 3.11 | 98 | 2.91 | 128 | 3.80 |
|  | TCC | 24 | 0.71 | 23 | 0.68 | 22 | 0.65 | 14 | 0.42 | 52 | 1.54 | 51 | 1.52 | 53 | 1.57 | 34 | 1.01 | 41 | 1.21 | 36 | 1.07 | 24 | 0.71 |
| **Thr** | ACG | 19 | 0.56 | 25 | 0.74 | 26 | 0.77 | 22 | 0.65 | 37 | 1.10 | 36 | 1.07 | 36 | 1.07 | 36 | 1.07 | 37 | 1.10 | 38 | 1.13 | 22 | 0.65 |
|  | **ACA** | 11 | 0.33 | 6 | 0.18 | 5 | 0.15 | 14 | 0.42 | 4 | 0.12 | **3** | **0.09** | 4 | 0.12 | 6 | 0.18 | 4 | 0.12 | 6 | 0.18 | 6 | 0.18 |
|  | ACT | 37 | 1.10 | 42 | 1.25 | 42 | 1.25 | 42 | 1.25 | 25 | 0.74 | 26 | 0.77 | 26 | 0.77 | 37 | 1.10 | 33 | 0.98 | 35 | 1.04 | 47 | 1.40 |
|  | ACC | 10 | 0.30 | 11 | 0.33 | 10 | 0.30 | 4 | 0.12 | 18 | 0.54 | 20 | 0.59 | 19 | 0.56 | 11 | 0.33 | 9 | 0.27 | 10 | 0.30 | 14 | 0.42 |
| **Val** | GTG | 116 | 3.45 | 116 | 3.45 | 121 | 3.59 | 92 | 2.73 | 166 | 4.93 | 169 | 5.20 | 168 | 4.99 | 158 | 4.69 | 159 | 4.70 | 155 | 4.60 | 110 | 3.27 |
|  | GTA | 35 | 1.04 | 21 | 0.62 | 20 | 0.59 | 44 | 1.31 | 15 | 0.45 | 17 | 0.51 | 17 | 0.51 | 26 | 0.77 | 25 | 0.74 | 28 | 0.83 | 32 | 0.95 |
|  | **GTT** | **191** | **5.67** | **208** | **6.18** | **211** | **6.27** | **205** | **6.09** | **153** | **4.54** | **152** | **4.51** | **152** | **4.51** | **183** | **5.43** | **180** | **5.33** | **188** | **5.58** | **219** | **6.51** |
|  | GTC | 22 | 0.65 | 40 | 1.19 | 37 | 1.10 | 23 | 0.68 | 85 | 2.53 | 83 | 2.47 | 82 | 2.44 | 43 | 1.28 | 45 | 1.33 | 42 | 1.25 | 18 | 0.54 |
| **Trp** | TGG | 76 | 2.26 | 74 | 2.20 | 75 | 2.23 | 56 | 1.66 | 91 | 2.70 | 91 | 2.70 | 92 | 2.73 | 86 | 2.55 | 83 | 2.46 | 81 | 2.41 | 89 | 2.64 |
|  | TGA | 37 | 1.10 | 40 | 1.19 | 39 | 1.16 | 55 | 1.63 | 20 | 0.59 | 20 | 0.59 | 21 | 0.62 | 31 | 0.92 | 34 | 1.01 | 31 | 0.92 | 22 | 0.65 |
| **Tyr** | TAT | 127 | 3.77 | 114 | 3.39 | 116 | 3.45 | 137 | 4.07 | 48 | 1.43 | 47 | 1.40 | 46 | 1.37 | 77 | 2.29 | 79 | 2.34 | 79 | 2.35 | 130 | 3.86 |
|  | TAC | 24 | 0.71 | 33 | 0.98 | 33 | 0.98 | 13 | 0.39 | 102 | 3.03 | 102 | 3.03 | 104 | 3.09 | 68 | 2.02 | 70 | 2.07 | 69 | 2.05 | 23 | 0.68 |
| **stop** | TAG | 9 | 0.27 | 10 | 0.3 | 11 | 0.33 | 11 | 0.33 | 11 | 0.33 | 11 | 0.33 | 11 | 0.33 | 10 | 0.30 | 9 | 0.27 | 8 | 0.24 | 11 | 0.33 |
|  | TAA | 3 | 0.09 | 2 | 0.06 | 1 | 0.03 | 1 | 0.03 | 1 | 0.03 | 1 | 0.03 | 1 | 0.03 | 2 | 0.06 | 3 | 0.09 | 4 | 0.12 | 1 | 0.03 |

**Note:** aa: amino acid abbreviated by three letters (DDBJ: <http://www.ddbj.nig.ac.jp/sub/ref2-e.html>). Species: Pmiy: *Paragonimus* *skrjabini* *miyazakii* (OkuST1 strain); Phete: *P. heterotremus* (LC and GX strains); Pohi: *P. ohirai* (Kino strain); Pwes: *P. westermani* (2n; 3n) (Haenam; Bogil; (dog1); Type I; AP; and ID2009 strains); Pkel: *P. kellicotti* (Ozark strain). The most frequently used codons (Phe-TTT; Leu-TTG) and the least frequently used codons (varying numbers) in mitogenomes of 11 *Paragonimus* strains/species are highlighted.

**Supplementary Figure legends**

**Fig. S1.** Detailed display of the mitogenome of *Paragonimus skrjabini miyazakii* with the annotated genes, intergenic, and non-coding regions with long and short tandem repeats (17,591 bp, Pmiy-OkuST1-JP). The names of genes (PCGs, MRGs, tRNAs) and repeats (LRUs, SRUs) and their lengths (in brackets) are marked at their sites; sequences of tRNAs are highlighted, and the intergenic sequence (int. seq.) connecting the latest SRU to *cox*3 is underlined.

**Fig. S2.** Drawings of predicted structures of 22 transfer RNAs in the mitogenome of *Paragonimus skrjabini miyazakii*, arranged in the order they are present in the mitogenome. Each tRNA (here abbreviated as trn) is named according to the one-letter amino-acid abbreviation, with the exception of those specifying Serine, S1 and S2; and Leucine, L1 and L2 (L1, CUN; L2, UUR; S1, AGN; and S2, UCN); DHU arms are missing in tRNASer1(AGN) and in tRNASer2(UCN). Names of the structural components of a tRNA gene are indicated in the tRNAGlu (*trn*E) structure.