**Supplementary Table S1.** *Giardia duodenalis* reference strains used in this study.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ACCESSION****NUMBER** | **ISOLATE** | **ASSEMBLAGE/****GENOTYPE** | **HOST** | **COUNTRY** |  **CONTINENT** | **YEAR** | **REFERENCE** |
| EU769206 | Swecat171 | A | cat | Sweden | Europe | 2011 | [1] |
| EU621373 | JC002 | A | deer | Poland | Europe | 2008 | [2] |
| EU769205 | Swecat078 | A | cat | Sweden | Europe | 2008 | [1] |
| GU396696 | H3-001 | A | human | Poland | Europe | 2016 | [3] |
| HM165227 | Sweh040 | A | human | Sweden | Europe | 2016 | [4] |
| KF922979 | HC11 | A | human | Brazil a | South America | 2014 | [5] |
| KF922983 | HC29 | A | human | Brazil a  | South America | 2014 | [5] |
| KF922992 | HC44 | A | human | Brazil a | South America | 2014 | [5] |
| KF923000 | DC01 | A | dog | Brazil a | South America | 2014 | [5] |
| KM190682 | VANC/85/UBC/2 | A | human | Canada | North America | 1985 | [6] |
| KY612245 | AN1.5 | A | human | Brazil b | South America | 2014 | [7] |
| MN629930 | Z1 | A | dog | Iraq | Asia | 2020 | [8] |
| XM001705373 | WB C6 | A | culture | USA | North America | 2007 | [9] |
| GQ329671 | Sweh166 | A  | human | Sweden | Europe | 2011 | [4] |
| EU014384 | Be2 | A1 | beaver | Canada | North America | 2011 | [10] |
| HQ179591  | 46c2 | A2 | human | Australia | Oceania | 2011 | [11] |
| AY072724 | ISSGF7 | A3 | human | Italy | Europe | 2002 | [12] |
| DQ090542  | STS-U | A3 | human | Norway | Europe | 2004 | [13] |
| DQ116612 | CBHRG9 | A3 | wastewater | Mexico | North America | 2006 | [14] |
| EU014381 | AB | A3 | human | Peru | South America | 1985 | [15] |
| FJ560577 | Lim2 | A3 | human | France | Europe | 1998 | [16] |
| FJ971408 | GLT1 | A3 | human | Thailand | Asia | 2007 | [17] |
| FN386484  | I231104 | A3 | wastewater | Spain | Europe | 2004 | [18] |
| KU504738 | S42 | A3 | human | Brazil c | South America | 2011 | [19] |
| DQ090523 | BG-Ber2 | B | human | Norway | Europe | 2005 | [13] |
| AB480877  | PalH9 | B | human | Palestine | Asia | 2006 | [20] |
| AB618785  | GH-202 | B | human | Japan | Asia | 2010 | [21] |
| FJ971440 | GLT2 | B | human | Thailand | Asia | 2007 | [17] |
| FJ971461 | GL518 | B | human | Thailand | Asia | 2010 | [17] |
| FJ971482 | GL121 | B | human | Thailand | Asia | 2007 | [17] |
| KF922985 | HC32 | B | human | Brazil a | South America | 2014 | [5] |
| KF922993 | HC45 | B | human | Brazil a | South America | 2014 | [5] |
| KP687755 | VANC/90/UBC/44 | B | wastewater | Canada | North America | 1990 | [6] |
| KP687756 | "VANC/90/UBC/54 | B | beaver | Canada | North America | 1990 | [6] |
| KU504702 | S3C1 | B | human | Brazil c | South America | 2011 | [19] |
| KU504704 | S3C3 | B | human | Brazil c | South America | 2011 | [19] |
| KU504707 | S8 | B | human | Brazil c | South America | 2011 | [19] |
| KU504708 | S9 | B | human | Brazil c | South America | 2011 | [19] |
| KU504709 | S10C3 | B | human | Brazil c | South America | 2011 | [19] |
| KU504712 | S11C1 | B | human | Brazil c | South America | 2011 | [19] |
| KU504713 | S11C2 | B | human | Brazil c | South America | 2011 | [19] |
| KU504714 | S11C3 | B | human | Brazil c | South America | 2011 | [19] |
| KU504715 | S13C2 | B | human | Brazil c | South America | 2011 | [19] |
| KU504720 | S15 | B | human | Brazil c | South America | 2011 | [19] |
| KU504722 | S16C2 | B | human | Brazil c | South America | 2011 | [19] |
| KU504723 | S16C4 | B | human | Brazil c | South America | 2011 | [19] |
| KU504731 | S24C2 | B | human | Brazil c | South America | 2011 | [19] |
| KU504732 | S24C3 | B | human | Brazil c | South America | 2011 | [19] |
| KY612242 | S8C5 | B | human | Brazil c | South America | 2011 | [19] |
| LC508615 | K6 clone1 | B | human | Kenya | Africa | 2013 | [22] |
| MT542771 | 5 | B | human | Brazil c | South America | 2021 | [23] |
| AY072725 | Nij5 | B1 | human | The Netherlands | Europe | 2002 | [24] |
| AY072726 | LD18 | B2 | human | Belgium | Europe | 2002 | [24] |
| AY072727 | BAH8 | B3 | human | Australia | Oceania | 1999 | [12] |
| JF422719 | BRAdogD15 | C | dog | Brazil a | South America | 2009 | [25] |
| KF923019 | VET01 | D | dog | Brazil a  | South America | 2014 | [5] |
| AY072729 | P15 | E | pig | Czech Republic | Europe | 1996 | [12] |
| AY647264 | A101 | F | cat | Italy | Europe | 2004 | [26] |

a Atlantic Forest biome; b Cerrado biome; c Amazon biome.

**Reference:**

1. Lebbad M, Mattsson JG, Christensson B, Ljungström B, Backhans A, Andersson JO, et al. From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. Vet Parasitol. 2010;168(3-4):231-9. doi: 10.1016/j.vetpar.2009.11.003. PMID:19969422.
2. Solarczyk P, Majewska AC, Moskwa B, Cabaj W, Dabert M, Nowosad P. Multilocus genotyping of *Giardia duodenalis* isolates from red deer (Cervus elaphus) and roe deer (Capreolus capreolus) from Poland. Folia Parasitol (Praha). 2012;59(3):237-40. doi: 10.14411/fp.2012.032 PMID: 23136805.
3. Solarczyk P, Werner A, Majewska AC. Genotypowanie izolatów *Giardia duodenalis* uzyskanych od ludzi w zachodnio-centralnej Polsce [Genotype analysis of *Giardia duodenalis* isolates obtained from humans in west-central Poland]. Wiad Parazytol. 2010;56(2):171-7. PMID: 20707303.
4. Lebbad M, Petersson I, Karlsson L, Botero-Kleiven S, Andersson JO, Svenungsson B, et al. Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. PLoS Negl Trop Dis. 2011;5(8):e1262. doi: 10.1371/journal.pntd.0001262 . PMID: 21829745.
5. Durigan M, Abreu AG, Zucchi MI, Franco RMB, de Souza AP. Genetic Diversity of *Giardia duodenalis*: Multilocus Genotyping Reveals Zoonotic Potential between Clinical and Environmental Sources in a Metropolitan Region of Brazil. PLoS ONE. 2014 9(12): e115489. doi:10.1371/ journal.pone.0115489.
6. Prystajecky N, Tsui CK, Hsiao WW, Uyaguari-Diaz MI, Ho J, Tang P, et al. *Giardia* spp. Are Commonly Found in Mixed Assemblages in Surface Water, as Revealed by Molecular and Whole-Genome Characterization. Appl Environ Microbiol. 2015;81(14):4827-34. doi: 10.1128/AEM.00524-15 PMID: 25956776.
7. Nunes BC, Calegar DA, Pavan MG, Jaeger LH, Monteiro KJL, Dos Reis ERC, et al. Genetic diversity of *Giardia duodenalis* circulating in three Brazilian biomes. Infect Genet Evol. 2018;59:107-112. doi: 10.1016/j.meegid.2018.02.001 PMID: 29410226.
8. Hassan,Z.I. Unpublished. Avaible in {HYPERLINK “<https://www.ncbi.nlm.nih.gov/nuccore/MN629930>”}.
9. Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, et al. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. Science. 2007;317(5846):1921-6. doi: 10.1126/science.114383 PMID: 17901334.
10. Teodorovic S, Braverman JM, Elmendorf HG. Unusually low levels of genetic variation among Giardia lamblia isolates. Eukaryot Cell. 2007;6(8):1421-30. doi: 10.1128/EC.00138-07. PMID: 17557879; PMCID: PMC1951139.
11. Wielinga C, Ryan U, Andrew Thompson RC, Monis P. Multi-locus analysis of *Giardia duodenalis* intra-Assemblage B substitution patterns in cloned culture isolates suggests sub-Assemblage B analyses will require multi-locus genotyping with conserved and variable genes. Int J Parasitol. 2011;41(5):495-503. doi: 10.1016/j.ijpara.2010.11.007 PMID: 21176781.
12. Cacciò SM, De Giacomo M, Pozio E. Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int J Parasitol. 2002;32(8):1023-30. doi: 10.1016/s0020-7519(02)00068-1 PMID: 12076631.
13. Robertson LJ, Hermansen L, Gjerde BK, Strand E, Alvsvåg JO, Langeland N. Application of genotyping during an extensive outbreak of waterborne giardiasis in Bergen, Norway, during autumn and winter 2004. Appl Environ Microbiol. 2006;72(3):2212-7. doi: 10.1128/AEM.72.3.2212-2217.2006 PMID: 16517674.
14. Di Giovanni GD, Betancourt WQ, Hernandez J, Assadian NW, Flores Margez JP, Lopez EJ. Investigation of potential zooanthroponotic transmission of cryptosporidiosis and giardiasis through agricultural use of reclaimed wastewater. Int J Environ Health Res. 2006;16(6):405-18. doi: 10.1080/09603120601095100 PMID: 17164167.
15. Teodorovic S, Braverman JM, Elmendorf HG. Unusually low levels of genetic variation among *Giardia lamblia* isolates. Eukaryot Cell. 2007;6(8):1421-30. doi: 10.1128/EC.00138-07 PMID: 17557879.
16. Bonhomme J, Le Goff L, Lemée V, Gargala G, Ballet JJ, Favennec L. Limitations of tpi and bg genes sub-genotyping for characterization of human *Giardia duodenalis* isolates. Parasitol Int. 2011;60(3):327-30. doi: 10.1016/j.parint.2011.05.004 PMID: 21627998.
17. Kosuwin R, Putaporntip C, Pattanawong U, Jongwutiwes S. Clonal diversity in *Giardia duodenalis* isolates from Thailand: evidences for intragenic recombination and purifying selection at the beta giardin locus. Gene. 2010;449(1-2):1-8. doi: 10.1016/j.gene.2009.09.010 PMID: 19796671.
18. Alonso JL, Amorós I, Cuesta G. LNA probes in a real-time TaqMan PCR assay for genotyping of *Giardia duodenalis* in wastewaters. J Appl Microbiol. 2010;108(5):1594-601. doi: 10.1111/j.1365-2672.2009.04559.x PMID: 19840182.
19. Coronato Nunes B, Pavan MG, Jaeger LH, Monteiro KJ, Xavier SC, Monteiro FA, et al. Spatial and Molecular Epidemiology of *Giardia intestinalis* Deep in the Amazon, Brazil. PLoS One. 2016;11(7):e0158805. doi: 10.1371/journal.pone.0158805 PMID: 27392098.
20. Hussein AI, Tokoro M. Unpublished. Avaible in {HYPERLINK “<https://www.ncbi.nlm.nih.gov/nuccore/AB480877>”}.
21. Abe N, Teramoto I. Molecular evidence for person-to-person transmission of a novel subtype in *Giardia duodenalis* assemblage B at the rehabilitation institution for developmentally disabled people. Parasitol Res. 2012;110(2):1025-8. doi: 10.1007/s00436-011-2564-4 PMID: 21786066.
22. Tokoro M, Mizuno T, Hendarto J, Matey EJ, Songok EM, Ichimura H. Unpublished. Avaible in {HYPERLINK “<https://www.ncbi.nlm.nih.gov/nuccore/LC508615>”}.
23. Köster PC, Malheiros AF, Shaw JJ, Balasegaram S, Prendergast A, Lucaccioni H, et al. Multilocus Genotyping of *Giardia duodenalis* in Mostly Asymptomatic Indigenous People from the Tapirapé Tribe, Brazilian Amazon. *Pathogens*. 2021;10(2):206. https://doi.org/10.3390/pathogens10020206.
24. Paz e Silva FM, Monobe MM, Lopes RS, Araujo JP Jr. Molecular characterization of *Giardia duodenalis* in dogs from Brazil. Parasitol Res. 2012;110(1):325-34. doi: 10.1007/s00436-011-2492-3 PMID: 21695567.
25. Cacciò SM, De Giacomo M, Pozio E. Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype Giardia duodenalis cysts from human faecal samples. Int J Parasitol. 2002;32(8):1023-30. doi: 10.1016/s0020-7519(02)00068-1 PMID: 12076631.
26. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Cacciò SM. Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol. 2005;35(2):207-13. doi: 10.1016/j.ijpara.2004.10.022 PMID: 15710441.

**Supplementary Table 2.** Molecular diversity indexes of *Giardia duodenalis* based on β-giardin locus (592 bp, n=106).

|  |  |  |
| --- | --- | --- |
| **Assemblage** | **Region (N)** | **Statistics** |
| **H ± SD** | **Nº of haplotypes** | **Nº of** **polymorphic sites** | **Nº of substitutions** | **Nº of transitions** | **Nº of transversions** |
|  | South America (31) | 0.879 ± 0.037 | 14 | 38 | 39 | 21 | 18 |
| **Assemblage A** | North America (4) | 0.666 ±0.204 | 2 | 4 | 4 | 4 | 0 |
| Europe (10) | 0.822 ± 0.096 | 5 | 15 | 15 | 15 | 0 |
| Asia (2) | 1.000 ± 0.500 | 2 | 4 | 4 | 4 | 0 |
| All continents (49) | 0.854 ± 0.029 | 16 | 50 | 50 | 33 | 18 |
| All Amazon biome (5) | 0.800 ± 0.164 | 3 | 8 | 8 | 6 | 2 |
| **Amazon biome\* (4)** | 0.833 ± 0.222 | 3 | 8 | 8 | 6 | 2 |
| All Cerrado biome (8) | 0.964 ± 0.077 | 7 | 30 | 31 | 15 | 16 |
| **Cerrado biome\* (7)** | 1.000 ± 0.076 | 7 | 30 | 31 | 15 | 16 |
| **Caatinga biome\* (5)** | 0.700 ± 0.218 | 3 | 2 | 2 | 2 | 0 |
| All Atlantic Forest biome (13) | 0.859 ± 0.063 | 6 | 6 | 6 | 6 | 0 |
| **Atlantic Forest biome\* (9)** | 0.750 ± 0.112 | 4 | 4 | 4 | 4 | 0 |
| Atlantic Forest biome (4) | 0.833 ± 0.222 | 3 | 2 | 2 | 2 | 0 |
|  | All Brazil (31) | 0.879 ± 0.037 | 14 | 38 | 39 | 21 | 18 |
| **Assemblage B** | South America (41) | 0.918 ± 0.033 | 23 | 36 | 36 | 31 | 6 |
| North America (2) | 1.000 ± 0.500 | 2 | 3 | 3 | 3 | 0 |
| Europe (3) | 1.000 ± 0.272 | 3 | 7 | 7 | 7 | 0 |
| Asia (5) | 1.000 ± 0.126 | 5 | 5 | 5 | 5 | 0 |
| All continents (51) | 0.921 ± 0.028 | 28 | 37 | 37 | 32 | 6 |
| All Amazon biome (37) | 0.899 ± 0.039 | 19 | 24 | 24 | 23 | 1 |
| **Amazon biome\* (21)** | 0.757 ± 0.086 | 7 | 8 | 8 | 7 | 1 |
| Amazon biome (16) | 0.991 ± 0.025 | 15 | 21 | 21 | 21 | 0 |
| All Atlantic Forest biome (3) | 1.000 ± 0.272 | 3 | 10 | 10 | 7 | 3 |
| Atlantic Forest (2) | 1.000 ± 0.500 | 2 | 5 | 5 | 4 | 1 |
| All Brazil (41) | 0.918 ± 0.033 | 23 | 36 | 36 | 31 | 6 |
| **ALL (106) \*\*** |  | 0.951 ± 0.009 | 50 | 125 | 147 | 105 | 42 |

H ± SD: gene diversity ± standard deviation. (bp): base pair. \* Sequences obtained in this study \*\*ALL: included assemblages A, B, C, D, E and F. Further details of reference strains can be found in Supplementary Table S1. Only groups with more than 1 sequence are shown.