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Genetic variability and transcontinental sharing of *Giardia duodenalis* infrapopulations determined by glutamate dehydrogenase gene

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ABSTRACT

Microevolutionary data of *Giardia duodenalis* sub-assemblages is a prerequisite for determining the invasion zoonotic patterns of the parasite. To infer transmission patterns that could not be differentiated by the phenotypic features, a population genetic investigation is crucial for the elucidation of the genetic structure of *G. duodenalis* among the continents. Forty *G. duodenalis* positive fecal samples were collected from different foci of Northwest Iran. The specimens were subjected to Trichrome staining and sucrose gradient flotation. DNA samples were extracted, amplified, and sequenced by targeting glutamate dehydrogenase (gdh) gene. The global *gdh* sequences of sub-assemblages AII and BIV retrieved from NCBI GenBank were analyzed to estimate diversity indices, neutrality indices, and gene migration tests. Sequencing analyses indicated various levels of genetic variability of sub-assemblages AII and BIV among the five continents. Sub-assemblage BIV had greater genetic variability (haplotype diversity: 0.975; nucleotide diversity: 0.04246) than sub-assemblage AII. The statistical *Fst* value demonstrated that the genetic structure of sub-assemblages AII and BIV are moderately differentiated between European-American populations (*Fst*: 0.05352–0.15182), whereas a significant differentiation was not seen among other geographical population pairs. We conclude that a high gene flow of *G. duodenalis* sub-assemblages AII and BIV is unequivocally sharing among the continents. The current findings strengthen our knowledge to assess the evolutionary patterns of *G. duodenalis* in endemic foci of the world and it will become the basis of public health policy to control human giardiasis.

1. Introduction

Giardia duodenalis (syn. *G. lamblia*, *G. intestinalis*; Diplomonadida) is a ubiquitous enteropathogen protozoan parasite that can cause steatorrhea diarrhea and gastroenteritis disorders in humans in both developing (incidence 15–55%) and developed (incidence 5%) countries (Adam, 1991; Feng and Xiao, 2011).

To date, at least eight assemblages (A–H) and various sub-assemblages (AI-AIII, BIII, and BIV) have been identified regarding the analysis of genetic variability (Feng and Xiao, 2011; Lasek-Nesselquist et al., 2009; Thompson et al., 2000). In order of etio-parasitological importance, the zoonotic assemblages A and B are known to be the principal causative agents of human giardiasis. However, infrequent human infections by the assemblages C, D, and E of *G. duodenalis* have been globally reported, particularly in immunocompromised individuals and children (Feng and Xiao, 2011). Domestic animals and wildlife are frequently affected by various *Giardia* subtypes including the assemblages C and D (dog), E (hoofed livestock), F (cat), G (rat), and H (marine animals) (Thompson, 2004).

In recent years, DNA-based genotyping strategies have been broadly used in the epizootiology, transmission dynamic, evolutionary patterns, and distribution of drug-resistance alleles (Criscione et al., 2005; Mahami-Oskouei et al., 2016; Mohammadzadeh et al., 2017; Spotin et al., 2017). The evolutionary potential of parasites originates from gene migration (gene flow), genetic diversity range, and the extent of diversity recombination among genomes (Andras and Ebert, 2013).

Although, an increasing effort has been undertaken to illustrate the patterns of genetic diversity and inter–intra divergence levels of *Giardia* assemblages, there are no more works on population structure and expansion patterns of *Giardia* assemblages among the continents (Choy et al., 2015; Cooper et al., 2007; Lasek-Nesselquist et al., 2010; Lasek-Nesselquist et al., 2009; Takumi et al., 2012).

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The aim of the present study was to assess the genetic variability and population structure of *G. duodenalis* from global sequence data based on glutamate dehydrogenase gene (*gdh*) to recognize how zoonotic sub-assemblages AII and BIV are shared among the continents.

2. Materials and methods

2.1. Ethical approval, sample collection, and cyst purification

All patients were fulfilled an informed consent form. The ethics consent was approved by Ethical Review Committee of Tabriz University of Medical Sciences (No: 5/4/9264). Forty *G. duodenalis* positive fecal samples were collected from patients suffering gastrointestinal complications and fatty diarrhea (steatorrhea) in Northwest Iran (East Azerbaijan and Ardabil Provinces) in period of January 2014 to December 2016. The *G. duodenalis* cysts were purified, and concentrated by sucrose gradient flotation (Galeh et al., 2016). The purified cysts were stained by the Trichrome method.

2.2. Total genomic DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA of *G. duodenalis* was extracted through the freeze-thawing method (Galeh et al., 2016). 50 μL of purified cysts were mixed with an equal volume of glass beads. After adding 25 μL of proteinase K, the suspension was incubated at 60 °C overnight. DNA extraction was performed by Stool DNA Isolation mini kit (Yekta Tajhiz Azma, Iran). A single-round PCR was developed to recognize the *Giardia* infection by targeting *gdh* gene. The PCR amplification was done by using forward primer of 5′ TCAACGTCAACCGCGGCTTCCGT3′, and reverse primer of 5′GTTGTCCTTGCACATCTCC3′ as described previously (Read et al., 2004). The PCR amplification was done in 25 μL reaction volumes including 1 μL of each forward and reverse primers (15 pmol), 0.3 μL (5u/μL) of Taq DNA polymerase (Cinacolon, Iran), 0.9 μL of MgCl2 (50 mM; Cinacolon, Iran), 0.5 μL of dNTP Mix (10 mM; Cinacolon, Iran), 2.5 μL of 10 × PCR buffer (Cinacolon, Iran), 4 μL of DNA template, 10–13 μL of deionized distilled water, and 3 μL of bovine serum albumin (0.1%; New England Biolabs). The amplicons (PCR products) were electrophoresized on 1.5% agarose gel stained with DNA safe stain (Yekta Tajhiz Azma, Iran).

2.3. Sequencing, retrieving sequence, and phylogenetic analyses

PCR products were purified and sequenced by targeting *gdh* gene (Pouya Gostar gene Company, Iran). The ambiguity sites were edited using the standard IUPAC codes. Contigs (overlapped sequences) were analyzed at consensus position using Sequencher Tmv.4.1.4 software. The population structure and genetic diversity of *G. duodenalis* were investigated in 167 (sub-assemblage AII) and 461 (sub-assemblage BIV) sequences generated at the *gdh* of the parasite retrieved from the GenBank database (Abe et al., 2005; Ankarklev et al., 2012; Babaei et al., 2008; Cacciò et al., 2008; Choy et al., 2015; Colli et al., 2015; De Liberato et al., 2015; De Lucio et al., 2016; De Lucio et al., 2015; Debenham et al., 2017; Flecha et al., 2015; Garcia-R et al., 2017; Geurden et al., 2009; Gil et al., 2017; Haramoto et al., 2012; Hatam-Nahavandi et al., 2017; Helmy et al., 2014; Hijjawi et al., 2016; Hogan et al., 2014; Hussein et al., 2009; Itagaki et al., 2005; Lasek-Nesselquist, 2010; Lasek-Nesselquist et al., 2009; Lebbad et al., 2011; Levecke et al., 2009; Martínez-Díaz et al., 2011; Oates et al., 2012; Oliveira-Arbex et al., 2016; Pelayo et al., 2008; Prystajecky et al., 2015; Robertson et al., 2007; Roellig et al., 2015; Santín et al., 2013; Souza et al., 2007; Vermeulen et al., 2015; Wang et al., 2013; Wegayehu et al., 2016). The value of gene flow for *G. duodenalis* populations was assessed using a pairwise fixation index (*F*st: F-statistics) and a number of migrants per generation (Nm) (Rozas et al., 2003). The retrieved sequences of *G. duodenalis* sub-assemblages from various hosts/sources are presented in Supplementary Table 1.

To authenticate genetic relationships among *G. duodenalis* assemblages (A, B, C, D, and E) provided by the *gdh* gene, a phylogenetic tree was drawn using the program Splits Tree 4.0 based on the Neighbor-Net method and Median Joining character (Huson and Bryant, 2006). *Giardia ardeae* was considered as an out-group branch (Accession no: AF069060).

To reveal the genealogical relationships at intra-genetic diversity of *G. duodenalis* haplotypes, a network was constructed by PopART software using the Median Joining algorithm (Bandelt et al., 1999). The distance scale was estimated 0.01. According to the analysis of molecular variance (AMOVA), the diversity indices (Nucleotide diversity (π) and Haplotype diversity (Hd), neutrality indices (Fu's Fs statistic and Tajima's D), and *F*st index (as a scale of gene migration) were calculated using DnaSP software version 5.10 (Rozas et al., 2003). The level of genetic differentiation from metapopulations (regional population) to infrapopulations presented by *F*st index with ranging; 0–1. *F*st < 0.05 (insignificant differentiation), 0.05–0.15 (moderate differentiation), 0.15–0.25 (large differentiation) and *F*st > 0.25 (immense differentiation). The pairwise distances (percent identity and divergence) among the aligned sequences were built using the DNASTAR's MegAlign program (Table 1).

3. Result

3.1. PCR, nucleotide sequence analysis, and sequences pairwise distances

The fragment of the *gdh* gene (nearly 458-bp) was successfully amplified and sequenced for all *Giardia* isolates. Based on the multiple sequence alignment analyses, 24 (14.4%; sub-assemblage AII) and 249 (54%; sub-assemblage BIV) new haplotypes were explicitly identified in 628 geographic isolates occurred at the Asian, Australian, European, African, and American populations (Tables 2 and 3). The lowest and highest number of haplotypes of sub-assemblage BIV belong to the Americas (n = 35) and Australia (n = 73) continents (Table 2). No deletions/insertion (*I*ndel) mutations characterized in sub-assemblages AII and BIV, whereas Transition and Transversion substitutions occurred among the consensus sequences. Within the 380-bp consensus position of sub-assemblage BIV, 109 variable (polymorphic) sites were detected. Of these, 66 were parsimony-informative sites, and 43 of these were singleton variable sites. Furthermore, 17 singleton variable sites and 7 parsimony-informative sites corresponded to sub-assemblage AII.

3.2. Neutrality, diversity indices, and Fst

The haplotype numbers (Hn), the number of isolates, diversity indices, neutrality indices of *G. duodenalis* sub-assemblages BIV and AII are given in Tables 2 and 3. DNA sequencing analyses of sub-assemblages AII and BIV indicated various ranges of genetic diversity among the five continents. Current findings show that the sub-assemblage BIV has greater genetic variation (Hd: 0.975; π: 0.04246) than sub-assemblage AII (Hd: 0.378; π: 0.00326) (Tables 2 and 3). Regarding the infrapopulation geography of *G. duodenalis* sub-assemblage BIV, the highest haplotype diversity appeared to the Australia population (Hd: 0.975), while the lowest haplotype diversity occurred in the Americas populations (Hd: 0.832) (Fig. 1).

Tajima's D (-2.18344) and Fu's Fs (-50.137) indices of sub-assemblages BIV and AII demonstrated negative values in all *G. duodenalis* geographical populations, suggesting a considerable divergence from neutrality (Tables 2 and 3). The sequence pairwise distances of sub-assemblage BIV provided a divergence of 0.00–3.3% and a high percent identity of 96.5–100% among the continents (Supplementary Fig. 1). The observed and expected mismatch distribution including Raggedness r (0.0106–0.2768), R2 statistic (0.0198–0.0305), and the estimate of *Tau*: 0.000 (τ; as a moment estimator in population expansion) are shown in Fig. 2 (Supplementary information).

Table 1
Global geographical sub-assemblages of *Giardia intestinalis* used in this study based on the gdh gene.

Parasite (Assemblage)	Host/Source	Country	Accession number	Reference
G. intestinalis (A)	Homo sapiens	Canada	KM190733	Prystajecky et al. (2015)
G. intestinalis (A)	Water	Canada	KM190734	Prystajecky et al. (2015)
G. intestinalis (A)	Sewage	Canada	KM190735	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	Canada	KM190736	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	Canada	KM190737	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	Canada	KM190738	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190739	Prystajecky et al. (2015)
G. intestinalis (A)	Water	Canada	KM190740	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190741	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190742	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190743	Prystajecky et al. (2015)
G. intestinalis (A)	Dog	Canada	KM190744	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190745	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	USA	KM190746	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190747	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	Canada	KM190748	Prystajecky et al. (2015)
G. intestinalis (A)	Beaver	Canada	KM190749	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190750	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190751	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190752	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190753	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190754	Prystajecky et al. (2015)
G. intestinalis (A)	Homo sapiens	Canada	KM190755	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190725	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190726	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190727	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190728	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190729	Prystajecky et al. (2015)
G. intestinalis (A)	Dolphin	USA	GU176069	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Dolphin	USA	GU176070	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176071	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176072	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	*Larus* sp.	USA	GU176073	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Shark	USA	GU176074	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176075	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Atlantic harbor seal	USA	GU176076	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176077	Lasek-Nesselquist.et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176078	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Grey seal	USA	GU176079	Lasek-Nesselquist et al. (2010)
G. intestinalis (A)	Homo sapiens	Iran	AB434771	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434772	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434773	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434774	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434775	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434776	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434777	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434778	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434779	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434780	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434781	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	AB434782	Babaei et al. (2008)
G. intestinalis (A)	Homo sapiens	Iran	JF917086	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF917087	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF917088	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF917089	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF917090	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968195	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968196	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968200	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968201	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968202	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	JF968203	Unpublished
G. intestinalis (A)	Wastewater	Iran	KT235915	Hatam-Nahavandi et al. (2017)
G. intestinalis (A)	Wastewater	Iran	KT235916	Hatam-Nahavandi et al. (2017)
G. intestinalis (A)	Wastewater	Iran	KT235917	Hatam-Nahavandi et al. (2017)
G. intestinalis (A)	Wastewater	Iran	KT235918	Hatam-Nahavandi et al. (2017)
G. intestinalis (A)	Homo sapiens	Iran	KU565026	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	KU565027	Unpublished
G. intestinalis (A)	Homo sapiens	Iran	KU565030	Unpublished
G. intestinalis (A)	Homo sapiens	Jordan	KX228236	Hijjawi et al. (2016)
G. intestinalis (A)	Homo sapiens	Jordan	KX228237	Hijjawi et al. (2016)
G. intestinalis (A)	Homo sapiens	Jordan	KX228238	Hijjawi et al. (2016)
G. intestinalis (A)	Homo sapiens	Brazil	KT334239	Oliveira-Arbex et al. (2016)
G. intestinalis (A)	Homo sapiens	Brazil	KT334240	Oliveira-Arbex et al. 2016)

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Table 1 (*continued*)

Parasite (Assemblage)	Host/Source	Country	Accession number	Reference
G. intestinalis (A)	Homo sapiens	Brazil	KT334254	Oliveira-Arbex et al. (2016)
G. intestinalis (A)	Homo sapiens	Brazil	KT334241	Oliveira-Arbex et al. (2016)
G. intestinalis (A)	Homo sapiens	Brazil	KJ741313–KJ741315	Colli et al. (2015)
G. intestinalis (A)	Homo sapiens	Brazil	EF507647	Souza et al. (2007)
G. intestinalis (A)	Homo sapiens	Malaysia	JX266827	Unpublished
G. intestinalis (A)	Homo sapiens	Malaysia	JX266828	Unpublished
G. intestinalis (A)	Homo sapiens	Malaysia	JX266829	Unpublished
G. intestinalis (A)	Homo sapiens	Malaysia	JX266830	Unpublished
G. intestinalis (A)	Homo sapiens	Malaysia	JX266831	Unpublished
G. intestinalis (A)	Wastewater	Japan	AB638272–AB638273	Haramoto et al. (2012)
G. intestinalis (A)	Homo sapiens	Spain	KT310354	De Lucio et al. (2015)
G. intestinalis (A)	Homo sapiens	Spain	KT310353	De Lucio et al. (2015)
G. intestinalis (A)	Homo sapiens	Spain	KT310352	De Lucio et al. (2015)
G. intestinalis (A)	Dog	Spain	KX757733–KX757739	Gil et al. (2017)
G. intestinalis (A)	Homo sapiens	Sweden	GQ329674–GQ329676	Lebbad et al. (2011)
G. intestinalis (A)	Wastewater	Japan	AB638277–AB638281	Haramoto et al. (2012)
G. intestinalis (A)	Homo sapiens	Japan	AB195222–AB195224	Abe et al. (2005)
G. intestinalis (A)	Dog	Japan	AB199735	Itagaki et al. (2005)
G. intestinalis (A)	Homo sapiens	Egypt	KJ124981	Helmy et al. (2014)
G. intestinalis (A)	*Rupicapra rupicapra rupicapra*	Italy	KT270858	De Liberato et al. (2015)
G. intestinalis (B)	Homo sapiens	Brazil	KT334250–KT334253	Oliveira-Arbex et al. (2016)
G. intestinalis (B)	Homo sapiens	Egypt	KJ124969–KJ124980 and KJ124982	Helmy et al. (2014)
G. intestinalis (B)	Homo sapiens	Ethiopia	KP899852–KP899871	De Lucio et al. (2016)
			KP899883–KP899885	Wegayehu et al. (2016)
			KT948092–KT9480100	Flecha et al. (2015)
			KP026306–KP026309	
G. intestinalis (B)	Homo sapiens	Uganda	JQ303248	Ankarklev et al. (2012)
G. intestinalis (B)	Homo sapiens	China	JX994231–JX994236	Wang et al. (2013) Unpublished
	Wastewater		KR902358–KR902359	
	Dog/Cat		KU156634–KU156636	
G. intestinalis (B)	Homo sapiens	India	JF918436–JF918459	Debenham et al. (2017)
			JN616253–JN616256	
G. intestinalis (B)	Homo sapiens	Jordan	KX228243–KX228245	Hijjawi et al. (2016)
G. intestinalis (B)	Homo sapiens	Malaysia	KT124833–KT124850	Choy et al. (2015) Unpublished
			KC313926–KC313937	
			JX266835–JX266836	
G. intestinalis (B)	Homo sapiens	Palestine	AB295649–AB295654	Hussein et al. (2009)
G. intestinalis (B)	Marsupial	Australia	KP756608–KP756613	Vermeulen et al. (2015)
G. intestinalis (B)	Ape	Australia	AY178752–AY178756	Unpublished
	Homo sapiens		AY178738–AY178739	
	ND		JN204450–JN204453	
G. intestinalis (B)	Homo sapiens/Cattle	New Zealand	KY124012–KY124100	Garcia-R et al. (2017)
G. intestinalis (B)	*Colobus guereza*	Belgium	FJ890942	Levecke et al. (2009)
G. intestinalis (B)	Hamadryas baboon	Belgium	FJ890943	Levecke et al. (2009)
G. intestinalis (B)	lack-headed spider monkey	Belgium	FJ890944	Levecke et al. (2009)
G. intestinalis (B)	Chimpanzee	Belgium	FJ890945	Levecke et al. (2009)
G. intestinalis (B)	Chimpanzee	Belgium	FJ890946	Levecke et al. (2009)
G. intestinalis (B)	Lemur catta	Belgium	FJ890947	Levecke et al. (2009)
G. intestinalis (B)	Lemur catta	Belgium	FJ890948	Levecke et al. (2009)
G. intestinalis (B)	*Varecia variegata*	Belgium	FJ890949	Levecke et al. (2009)
G. intestinalis (B)	Chimpanzee	Belgium	FJ890950	Levecke et al. (2009)
G. intestinalis (B)	Homo sapiens	Belgium	EU847734	Geurden et al. (2009)
G. intestinalis (B)	Dog	Australia	AY178749–AY178751	Unpublished
G. intestinalis (B)	Barbary macaque	Italy	EU637583–EU637588	Caccio et al. (2008)
G. intestinalis (B)	Homo sapiens	Norway	DQ923581–DQ923589	Robertson et al. (2007)
			DQ090532–DQ090540	
G. intestinalis (B)	Homo sapiens	Sweden	HM136880–HM136891	Lebbad et al. (2011)
G. intestinalis (B)	Homo sapiens	Spain	KT310360–KT310374	De Lucio et al. (2015)
G. intestinalis (B)	Dog	Spain	KX757741–KX757747	Gil et al. (2017)
G. intestinalis (B)	Lemur catta	Spain	HQ616621–HQ616624	Martínez-Díaz et al. (2011)
G. intestinalis (B)	Lactuca sativa	Brazil	KJ741292	Colli et al. (2015)
G. intestinalis (B)	Homo sapiens	Brazil	KJ741300	Colli et al. (2015)
G. intestinalis (B)	Dog	Brazil	KJ741296	Colli et al. (2015)
G. intestinalis (B)	Lactuca sativa	Brazil	KJ741293	Colli et al. (2015)
G. intestinalis (B)	Homo sapiens	Brazil	KJ741299	Colli et al. (2015)
G. intestinalis (B)	Beaver	Canada	KM190702	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190703	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190704	Prystajecky et al. (2015)
G. intestinalis (B)	Beaver	Canada	KM190705	Prystajecky et al. (2015)
G. intestinalis (B)	Beaver	Canada	KM190706	Prystajecky et al. (2015)
G. intestinalis (B)	Beaver	Canada	KM190707	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190708	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190709	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190710	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190711	Prystajecky et al. (2015)

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Table 1 (*continued*)

Parasite (Assemblage)	Host/Source	Country	Accession number	Reference
G. intestinalis (B)	Water	Canada	KM190712	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190713	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190714	Prystajecky et al. (2015)
G. intestinalis (B)	Beaver	Canada	KM190715	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190716	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190717	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190718	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190719	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190720	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KM190721	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190722	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190723	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190724	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190725	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190726	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190727	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190728	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KM190729	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KP687769	Prystajecky et al. (2015)
G. intestinalis (B)	Beaver	Canada	KP687770	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687771	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KP687772	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687773	Prystajecky et al. (2015)
G. intestinalis (B)	Homo sapiens	Canada	KP687774	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687775	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687776	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687777	Prystajecky et al. (2015)
G. intestinalis (B)	Water	Canada	KP687778	Prystajecky et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780947	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780945	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780943	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780936	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780938	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780929	Roellig et al. (2015)
G. intestinalis (B)	*Cynomys ludovicianus*	USA	KP780933	Roellig et al. (2015)
G. intestinalis (B)	Marine animals; *Larus argentatus* (Herring gull), Dolphin, Seal	Canada	EU362930–EU362954	Lasek-Nesselquist et al. (2009)
G. intestinalis (B)	Felis silvestris catus	USA	JX448639–JX448643	Oates et al. (2012)
G. intestinalis (B)	Gorilla beringei beringei	Rwanda	JX839873 and JX839875–JX839877	Hogan et al. (2014)
G. intestinalis (B)	Homo sapiens	Canada	EF685679–EF685685	Lasek-Nesselquist et al. (2009)
G. intestinalis (B)	Homo sapiens	Brazil	KJ741301–KJ741312 KJ741316–KJ741328	Colli et al. (2015)
G. intestinalis (B)	Homo sapiens	Brazil	KT334242–KT334253	Oliveira-Arbex et al. (2016)
G. intestinalis (B)	Homo sapiens	Brazil	EF507646	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	USA	EF685679	Lasek-Nesselquist et al. (2010)
G. intestinalis (B)	Homo sapiens	Brazil	EF507654	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	Brazil	EF507664	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	Brazil	EF507664	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	Brazil	EF507672	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	Brazil	EF507682	Souza et al. (2007)
G. intestinalis (B)	Homo sapiens	Brazil	EF507668	Souza et al. (2007)
G. intestinalis (B)	Horse	Colombia	JX972185–JX972186	Santín et al. (2013)
G. intestinalis (B)	Homo sapiens	Cuba	EU594663–EU594667	Pelayo et al. (2008)

ND: Not determined.

The *F*st index of *G. duodenalis* sub-assemblage BIV ranged from low (0.00857) to moderate values (0.07391) (Table 4). The statistical *F*st indicates that the genetic structure of *G. duodenalis* sub-assemblage BIV is moderately differentiated between European-American (*F*st: 0.05352, Nm: 4.42), and African-American populations (*F*st: 0.07391; Nm: 3.13) (Table 4), whereas a significant differentiation (*F*st: 0.00857–0.04242; Nm: 4.42–28.92) was not seen among other geographical population pairs (Asian-European, Asian-African, Asian-Australian, Asian-American, European-African, and European-Australian).

The highest genetic differences in support of *G. duodenalis* sub-assemblage AII identified in Asian-American (*F*st: 0.13474), and European-American (*F*st: 0.15182) population pairs, however no genetic difference identified in the Asian-European populations (*F*st: −0.00366; Nm: −68.57) (Table 5) (Fig. 3).

3.3. Phylogenetic tree and haplotype network

To demonstrate the topology of identical haplotypes, a phylogenetic network was constructed based on geographical sequences of *duodenalis* assemblages A, B, C, D, and E (Supplementary Fig. 3). The statistical parsimony network was built to discriminate a genealogical correlation among the identified haplotypes of *G. duodenalis* sub-assemblages BIV and AII (Figs. 4 and 5). The constructed haplotype network demonstrated star-like characteristics in large infrapopulations including G.d1 (Asia, Americas, and Europe: 29%) and G.d12 (Asia, Americas, and Europe: 57.5%) as the most common haplogroups of *G. duodenalis* sub-assemblage AII (Fig. 4).

The haplotypes G.d9 (Brazil; human isolate) and G.d109 (Japan; dog isolate) were shared between haplogroups G.d1 and G.d21 (Fig. 4). Three principal common haplogroups G.d17 (Asia, the Americas, and Europe: 57.5%), G.d39 (the Americas, Australia, Africa, and Europe: 17.9%), and G.d125 (Australia and Europe: 14%) corresponded to *G.*

Table 2
Diversity and neutrality indices of *Giardia duodenalis* sub-assemblage BIV isolates based on nucleotide sequences of gdh gene. N: number of isolates; Hn: number of haplotypes; Hd: haplotype diversity; Nd: nucleotide diversity.

Continent		Diversity indices					Neutrality indices	
	Country (Hn)	N	Hn	Hd ± SD	Number of segregating sites	Nd (π) ± SD	Tajima's D*	Fu's Fs statistic**
Asia	China (12) Jordan (3) Malaysia (26) Palestine (6) India (22)	76	49	0.950 ± 0.011	35	0.00959 ± 0.01872	−1.49695	−50.137
Europe	Belgium (7) Czech Republic (4) Italy (6) Norway (18) Spain (18) Sweden (18)	78	49	0.950 ± 0.014	76	0.01927 ± 0.01872	−1.72043	−30.827
Africa	Egypt (13) Ethiopia (34) Uganda (1)	61	43	0.940 ± 0.022	69	0.01346 ± 0.00048	−2.18344	−36.442
Australia	Australia (13) New Zealand (61)	112	73	0.975 ± 0.007	102	0.04246 ± 0.05851	−0.89596	−34.036
Americas	Canada (7) Colombia (2) Cuba (4) USA (24) Brazil (10)	134	35	0.832 ± 0.025	38	0.00840 ± 0.01183	−0.86875	−12.729
Total		461	249 (54%)					

* $P < 0.01$.
** $P < 0.02$

duodenalis sub-assemblage BIV (Fig. 5). These findings reflect that the sharing of the identical population of *Giardia* isolates occurred among the various geographical regions. The haplotype network analysis indicated the spread of the haplotype G.d29 from Ethiopia (Host: Homo sapiens; Accession no: KP899858) to American, Asian, and European populations (Fig. 5).

4. Discussion

In the current investigation, we evaluate the genetic variability, transmission patterns, and population structure of *G. duodenalis* sub-assemblages AII/BIV from global sequence data based on *gdh* gene. So far, there is an insufficient population genetic study on this protozoon (Choy et al., 2015); most of investigations have focused on recombination or sexual reproduction in *G. duodenalis* (Cooper et al., 2007; Lasek-Nesselquist et al., 2009; Takumi et al., 2012).

The degree of genetic variability was greater among the sub-assemblage BIV sequences (Hd: 0.975) compared to sub-assemblage AII (Hd; 0.378), which could be due to the difference in the mode of transmission between affected hosts.

Andersson (2012) suggested that the ameiotic crossing that has recently occurred either between nuclei or within the nucleus prior to reaching homogenization is accountable for the occurrence of the high diversity level of sub-assemblage BIV. It has also proposed that high genetic variability can regularly occur following high gene flow in pathogens because high gene migration enhances the effective infra-population size in a variety of geographical areas where the heterogeneity traits are potentially dominant (Lasek-Nesselquist et al., 2009).

Moreover, the low level of allelic sequence heterozygosity (ASH) among assemblage A was considered to be due to asexual reproduction that occurred to lose the allelic variation (Choy et al., 2015). Earlier studies have demonstrated an extensive ASH in the BIV sub-assemblage (isolate GS: 0.53%), a value of up to 50-fold higher than those identified in AI (isolate WB < 0.01) and AII (isolate DG: 0.037%) sub-assemblages (Adam et al., 2013; Franzen et al., 2009; Morrison et al., 2007).

Teodorovic et al. (2007) clarified a low level of inter-genetic diversity between *G. duodenalis* sub-groups AI and AII populations by targeting six coding and four non-coding regions including Ferredoxin, Ferredoxin intron, Beta giardin, Actin, RPL, CPN60, Intergenic 1 and 2, RPL intron, and TPI.

On the other hand, Franzen et al. (2009) illuminated that the genomic differences between assemblages A and B may explain some of the clinical and observed biological discrepancies, and proposed that *G. duodenalis* assemblages A and B could be two distinct species.

Table 3
Diversity and neutrality indices of *G. duodenalis* sub-assemblage AII isolates based on nucleotide sequences of gdh gene. N: number of isolates; Hn: number of haplotypes; Hd: haplotype diversity; Nd: nucleotide diversity.

Continent		Diversity indices					Neutrality indices	
Country		N	Hn	Hd ± SD	Number of segregating sites	Nd (π) ± SD	Tajima's D*	Fu's Fs statistic**
Asia	China - Jordan - Malaysia - Iran -Japan	56	10	0.378 ± 0.078	16	0.00326 ± 0.00385	−2.09299	−4.765
Europe	Italy - Spain - Sweden	24	7	0.447 ± 0.447	8	0.00200 ± 0.00227	−3.485	−3.485
Americas	Canada - USA - Brazil	87	7	0.548 ± 0.00120	7	0.00280 ± 0.00158	−1.073	−1.073
	Total	167	24 (14.4%)					

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			
1		99.7	99.7	98.6	99.4	98.3	98.8	99.4	98.3	98.3	98.0	98.8	97.7	98.6	98.3	99.1	98.3	97.7	98.0	99.7	99.1	99.1	99.1	99.1	99.1	1	KP899872.	
2	0.3		99.4	98.3	99.1	98.6	98.6	99.1	98.6	98.0	98.3	99.1	98.0	98.8	98.6	98.8	98.6	98.0	98.3	99.4	98.8	98.8	98.8	98.8	98.8	2	KP899873.	
3	0.3	0.6		98.3	99.1	98.6	99.1	99.1	98.6	98.6	98.3	98.6	97.4	98.8	98.6	98.8	98.0	97.4	97.7	99.4	99.4	99.4	99.4	99.4	99.4	3	KP899874.	Africa
4	1.5	1.8	1.8		99.1	98.0	98.0	98.0	97.4	97.4	96.5	98.0	96.8	97.7	97.4	98.8	98.0	97.4	97.7	98.3	98.3	98.3	98.3	98.3	98.3	4	KP899876.	
5	0.6	0.9	0.9	0.9		98.8	98.3	98.8	97.7	97.7	97.4	98.8	97.7	98.6	98.3	99.7	98.8	98.3	98.6	99.1	98.6	98.6	98.6	98.6	98.6	5	KP899877.	
6	1.8	1.5	1.5	2.1	1.2		98.3	97.7	98.8	97.7	98.0	98.8	97.7	99.1	98.8	99.1	98.3	98.3	98.0	98.6	98.6	98.6	98.6	98.6	98.6	6	EU594667.	
7	1.2	1.5	0.9	2.1	1.8	1.8		98.8	99.4	99.4	98.0	97.7	97.1	98.6	98.8	98.6	97.7	97.7	97.4	99.1	99.7	99.7	99.7	99.7	99.7	7	KU156636.	Asia
8	0.6	0.9	0.9	2.1	1.2	2.4	1.2		98.3	98.3	98.0	98.8	97.7	98.6	98.8	98.6	98.8	98.3	98.6	99.1	98.6	98.6	98.6	98.6	98.6	8	KU156634.	
9	1.8	1.5	1.5	2.7	2.4	1.2	0.6	1.8		98.8	98.0	98.3	97.1	98.6	98.8	98.0	97.7	97.7	97.4	98.6	99.1	99.1	99.1	99.1	99.1	9	KR902358.	
10	1.8	2.1	1.5	2.7	2.4	2.4	0.6	1.8	1.2		97.4	97.1	97.1	98.0	98.3	98.0	97.1	97.1	96.8	98.6	99.1	99.1	99.1	99.1	99.1	10	KR902359.	
11	2.1	1.8	1.8	3.6	2.7	2.1	2.1	2.1	2.1	2.7		98.0	97.4	98.8	98.6	97.7	97.4	97.4	97.1	98.3	98.3	98.3	98.3	98.3	98.3	11	KY124081.	
12	1.2	0.9	1.5	2.1	1.2	1.2	2.4	1.2	1.8	3.0	2.1		98.3	98.6	98.8	98.6	98.8	98.3	98.6	98.6	98.0	98.0	98.0	98.0	98.0	12	KY124082.	
13	2.4	2.1	2.7	3.3	2.4	2.4	3.0	2.4	3.0	3.0	2.7	1.8		98.0	98.3	98.0	97.7	97.7	97.4	98.0	97.4	97.4	97.4	97.4	97.4	13	KY124083.	Australia
14	1.5	1.2	1.2	2.4	1.5	0.9	1.5	1.5	1.5	2.1	1.2	1.5	2.1		99.1	98.8	98.6	98.6	98.3	98.8	98.8	98.8	98.8	98.8	98.8	14	KY124084.	
15	1.8	1.5	1.5	2.7	1.8	1.2	1.2	1.2	1.2	1.8	1.5	1.2	1.8	0.9		98.6	98.8	98.8	98.6	98.6	98.6	98.6	98.6	98.6	98.6	15	KY124086.	
16	0.9	1.2	1.2	1.2	0.3	0.9	1.5	1.5	2.1	2.1	2.4	1.5	2.1	1.2	1.5		98.6	98.6	98.3	99.4	98.8	98.8	98.8	98.8	98.8	16	DQ090533	
17	1.8	1.5	2.1	2.1	1.2	1.8	2.4	1.2	2.4	3.0	2.7	1.2	2.4	1.5	1.2	1.5		99.4	99.7	98.0	97.4	97.4	97.4	97.4	97.4	17	DQ090536	Europe
18	2.4	2.1	2.7	2.7	1.8	1.8	2.4	1.8	2.4	3.0	2.7	1.8	2.4	1.5	1.2	1.5	0.6		99.7	98.0	97.4	97.4	97.4	97.4	97.4	18	DQ090537	
19	2.1	1.8	2.4	2.4	1.5	2.1	2.7	1.5	2.7	3.3	3.0	1.5	2.7	1.8	1.5	1.8	0.3	0.3		97.7	97.1	97.1	97.1	97.1	97.1	19	DQ090538	
20	0.3	0.6	0.6	1.8	0.9	1.5	0.9	0.9	1.5	1.5	1.8	1.5	2.1	1.2	1.5	0.6	2.1	2.1	2.4		99.4	99.4	99.4	99.4	99.4	20	DQ090539	
21	0.9	1.2	0.6	1.8	1.5	1.5	0.3	1.5	0.9	0.9	1.8	2.1	2.7	1.2	1.5	1.2	2.7	2.7	3.0	0.6		100.0	100.0	100.0	100.0	21	KT334245.	
22	0.9	1.2	0.6	1.8	1.5	1.5	0.3	1.5	0.9	0.9	1.8	2.1	2.7	1.2	1.5	1.2	2.7	2.7	3.0	0.6	0.0		100.0	100.0	100.0	22	KT334246.	
23	0.9	1.2	0.6	1.8	1.5	1.5	0.3	1.5	0.9	0.9	1.8	2.1	2.7	1.2	1.5	1.2	2.7	2.7	3.0	0.6	0.0	0.0		100.0	100.0	23	KT334247.	Americas
24	0.9	1.2	0.6	1.8	1.5	1.5	0.3	1.5	0.9	0.9	1.8	2.1	2.7	1.2	1.5	1.2	2.7	2.7	3.0	0.6	0.0	0.0	0.0		100.0	24	KT334248.	
25	0.9	1.2	0.6	1.8	1.5	1.5	0.3	1.5	0.9	0.9	1.8	2.1	2.7	1.2	1.5	1.2	2.7	2.7	3.0	0.6	0.0	0.0	0.0	0.0		25	KT334249.	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			

Fig 1. The sequence pairwise distances (divergence and percent identity) of *G. duodenalis* sub-assemblage BIV among the sequences circulating from GenBank database determined by the *gdh* gene.

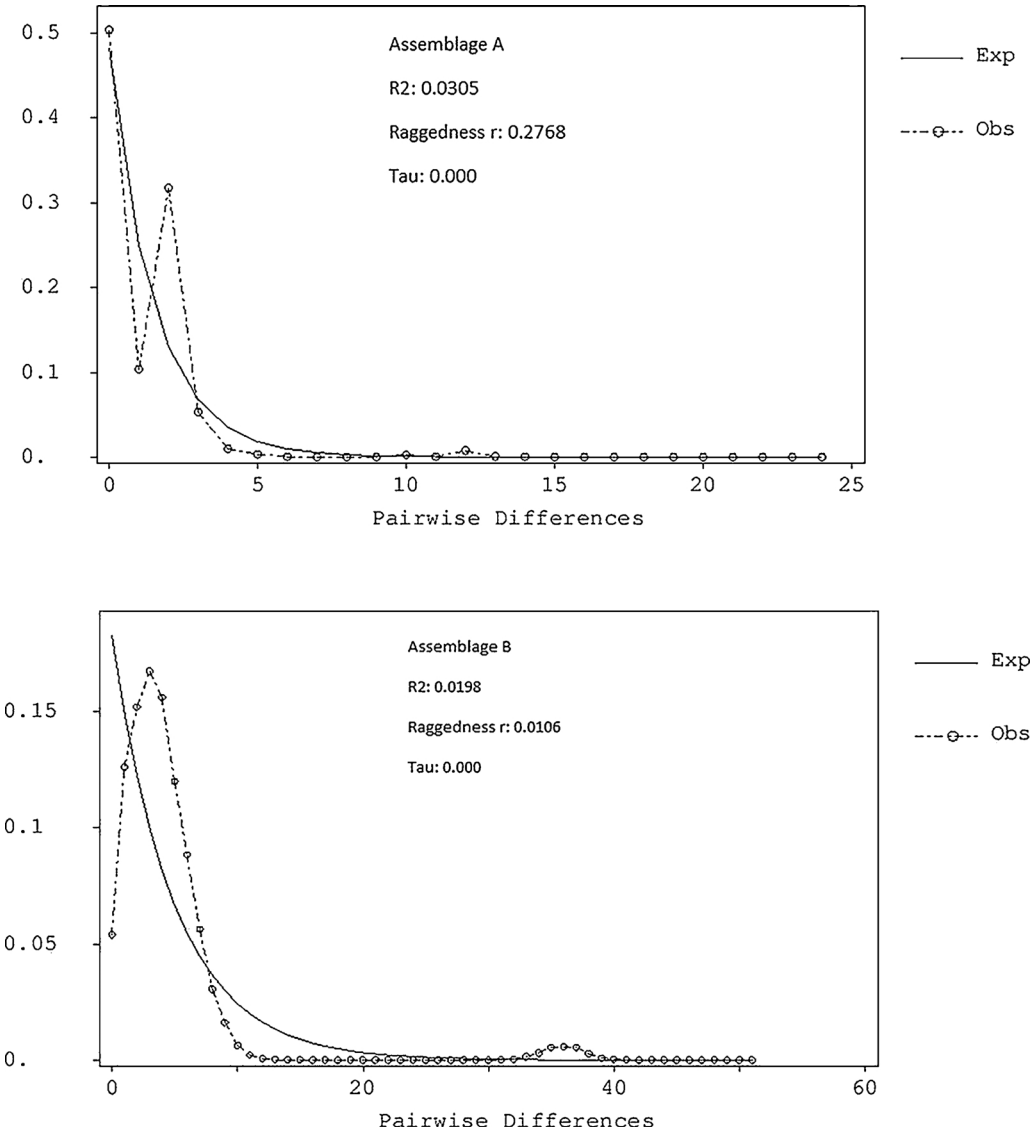

Fig. 2. Expected and observed mismatch distribution for *G. duodenalis* sub-assemblages BIV and AII inferred by *gdh* gene.

Table 4
Pairwise *F*st values (bottom left) and estimated number of migrants (Nm) per *G. duodenalis* sub-assemblage BIV (top right) of worldwide population.

Continent		Populations				
	Country	Asia	Europe	Africa	Australia	Americas
Asia	China - Jordan - Malaysia - Palestine -India	–	9.52	15.54	28.92	5.64
Europe	Belgium – Czech Republic - Italy - Norway - Spain - Sweden	0.02559	–	12.51	19.52	4.42
Africa	Egypt- Ethiopia- Uganda	0.01583	0.01959	–	11.57	3.13
Australia	Australia - New Zealand	0.00857	0.01265	0.02115	–	5.55
Americas	Canada - Colombia - Cuba - USA - Brazil	0.04242	0.05352	0.07391	0.04310	–

Table 5
Pairwise *F*st values (bottom left) and estimated number of migrants (Nm) per *G. duodenalis* sub-assemblage AII (top right) of worldwide population.

Continent		Populations		
	Country	Asia	Europe	Americas
Asia	China - Jordan - Malaysia - Iran - Japan	–	−68.57	1.61
Europe	Italy - Spain - Sweden	−0.00366	–	1.40
Americas	Canada - U.S.A - Brazil	0.13474	0.15182	–

In this study, a moderate genetic variability (Hd: 0.378–0.548; Hn: 24) of *G. duodenalis* sub-assemblage AII was identified among the Asian (Malaysia, Iran, Japan, China, and Jordan), European (Sweden, Italy, and Spain) and American (USA, Brazil, and Canada) populations. Interestingly, the Median Joining Network of sub-assemblage AII showed that haplotypes G.d9 (human isolate from Brazil) and G.d109 (dog isolate from Japan) were shared between two principal common haplogroups G.d1 (Asia, Europe, and the Americas) and G.d21 (Asia, Europe, and Americas). However, we cannot judge explicitly the potential transmission of sub-assemblage AII from pet animals to humans, and this can only being conclusively demonstrated in longitudinal studies assessing the assemblage/sub-assemblage of all *Giardia*-positive samples from humans and animals sharing the same household (Ballweber et al., 2010).

Ballweber et al. (2010) reported that the functional role of dogs stays unresolved in a transmission of human giardiasis. Taken together, the limited evidence from most previous studies implies that zoonotic transmission of assemblages A and B is very uncommon (Cooper et al., 2010; De Lucio et al., 2017; Inpankaew et al., 2014; Traub et al., 2004; Volotão et al., 2007).

However, a number of reports propose that dogs might perform a transitional role, since they can sporadically carry assemblage A (Ballweber et al., 2010). Although obtained data from a small-scale investigation provides slight evidence for zoonotic transmission of

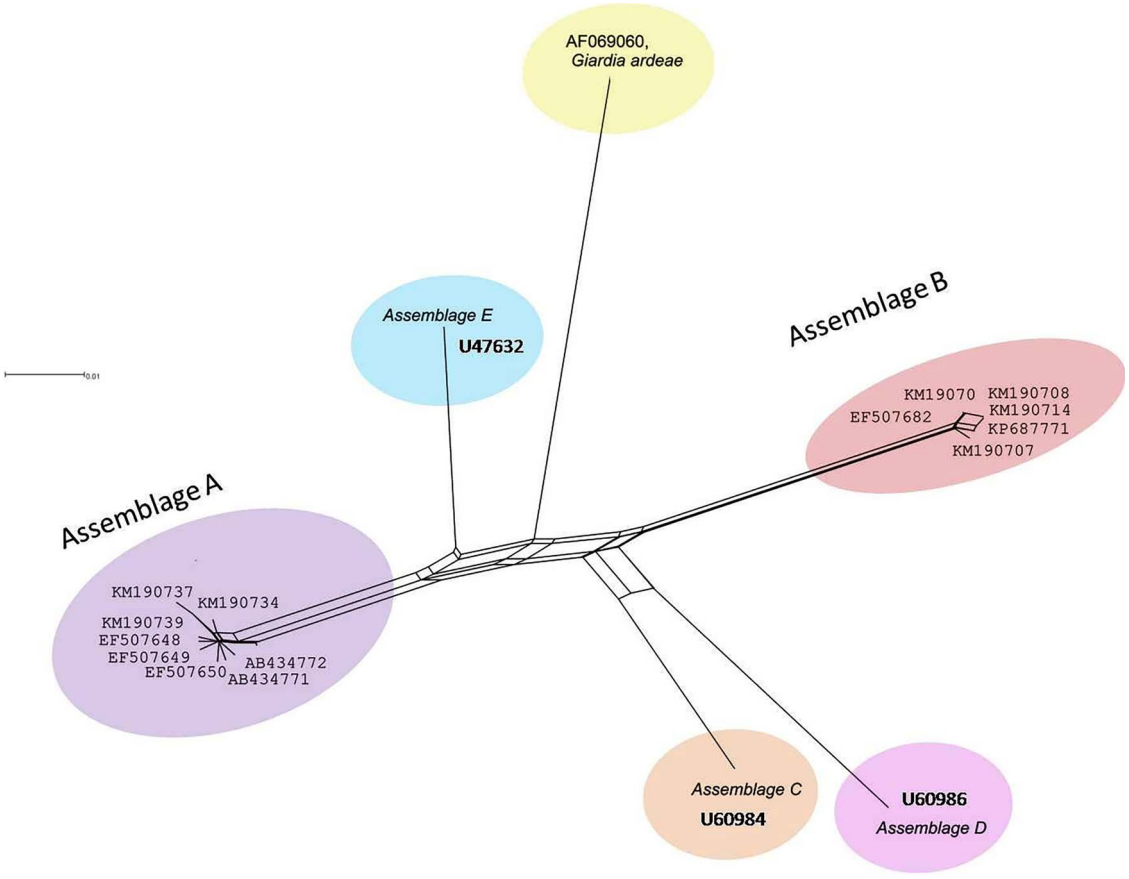

Fig. 3. Neighbor-Net graph drawn by different assemblages of *G. duodenalis* by using the Splits Tree 4.0 program.

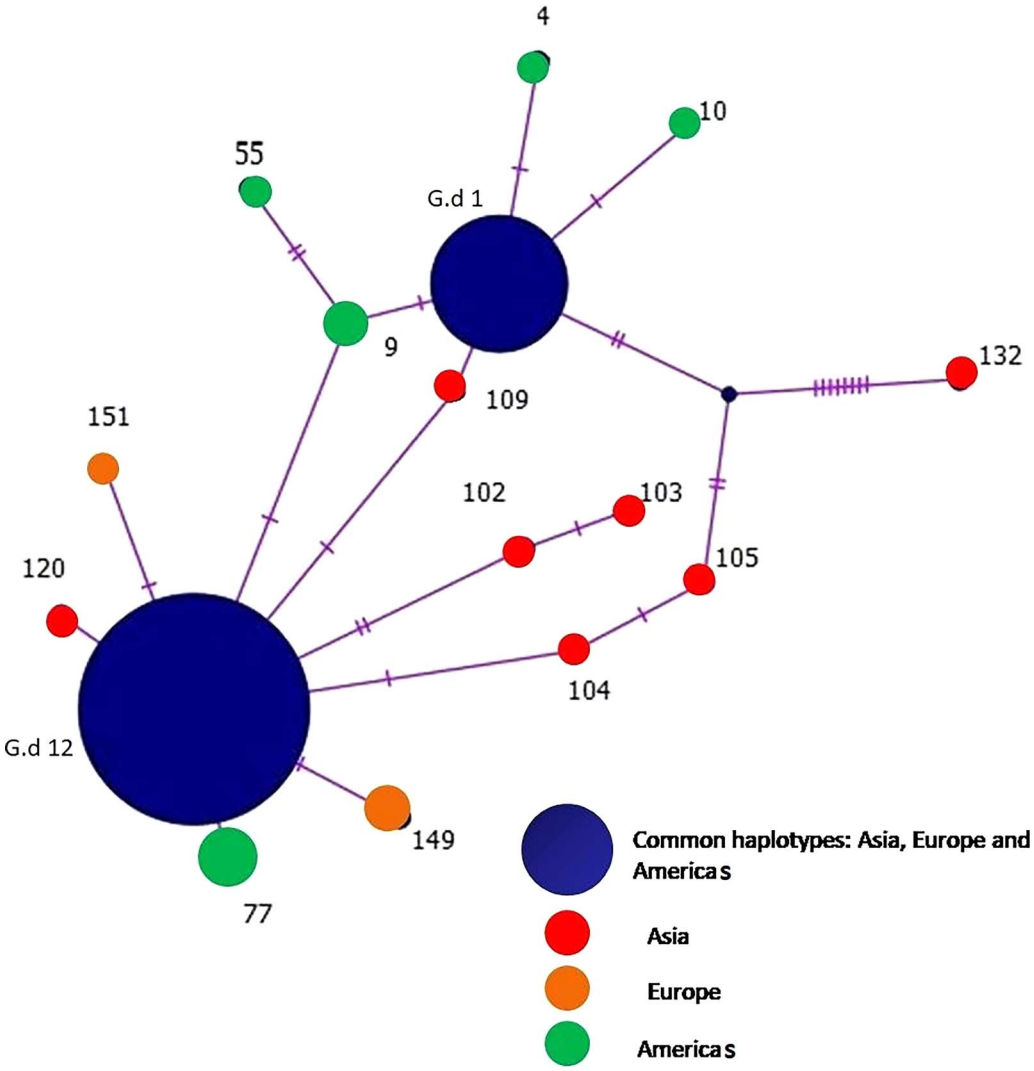

Fig. 4. Median Joining Network of *G. duodenalis* sub-assemblage AII based on *gdh* sequences from various geographical regions of the world.

assemblage A, the potential role of dogs should not be neglected.

The sharing of the haplotypes G.d9 and G.d109 between haplogroups G.d1 and G.d12 may probably support that these haplotypes appeared in various countries and extend into the mentioned populations. Secondly, G.d9 and G.d109 have emerged in each continent, deriving from their common haplotypes. The Median Joining Network of sub-assemblage BIV indicates that the identical haplogroups G.d17, G.d39, and G.d125 are unequivocally sharing among the Australian, European, Asian, American, and African populations.

The highest gene flow was observed (*F*st: 0.00857–0.04242; Nm: 4.42–28.92) in sub-assemblage BIV between Asian-European, Asian-African, Asian-Australian, Asian-American, European-African, and European-Australian population pairs. In view of the rapid globalization in various countries, the ecological alterations, host mobility, trading of livestock, and occurrence of a bottleneck event and founder effect can be plausibly addressed as principal causative agents of *Giardia* gene migration. Moreover, it is documented that a dynamic sexual cycle exists between *Giardia* isolates since recombination can lead to homogeneity in the *Giardia* genome (Teodorovic et al., 2007).

Current results show that the highest genetic differences of *G. duodenalis* assemblage AII were observed between Asian-American (*F*st: 0.13474) and European-American (*F*st: 0.15182) population pairs. In a similar study, Choy et al. (2015) evaluated the genetic differentiation and gene flow of *G. duodenalis* assemblage A based on triosephosphate isomerase gene across the continents. They also showed that *G. duodenalis* assemblage A is genetically well differentiated between Asian-Australian, (*F*st: 0.35464) and Asian-American populations (*F*st: 0.38293), while the *F*st value conveyed moderate differentiation (0.05–0.15) between Australian-European, Australian-African, and American-African population pairs. However, no significant genetic differentiation was appeared (*F*st < 0.05) between Australian-American, Asian-European, Asian-African, and European-African population pairs (Choy et al., 2015).

The occurrence of significant neutrality indices (Tajima's D and Fu's Fs: −2.00813 to −2.194) in support of all *Giardia* populations implies an excess of low incidence of mutants compared to the expectations under neutral developments such as population size equilibrium, presence of purifying selection, model of neutral mutation, genetic drift, and population extension after the bottleneck event (Choy et al., 2015; Teodorovic et al., 2007).

Moreover, based on the unimodal mismatch distribution test, the *Tau* value (as a divergence time for unequal population sizes and a moment estimator in population dispersal) revealed that the *G. duodenalis* sub-assemblages BIV and AII have recently experienced a population expansion among the continents.

We conclude that a high gene flow of *G. duodenalis* sub-assemblages BIV and AII is explicitly sharing among the continents. Current findings strengthen our knowledge of transmission dynamics, dispersion of drug-resistant alleles, and evolutionary patterns of giardiasis in different geographical regions of the world, also it will become the basis of public health policy to control human giardiasis. To complete the theoretical evolutionary scenario, further exploration is necessitated to

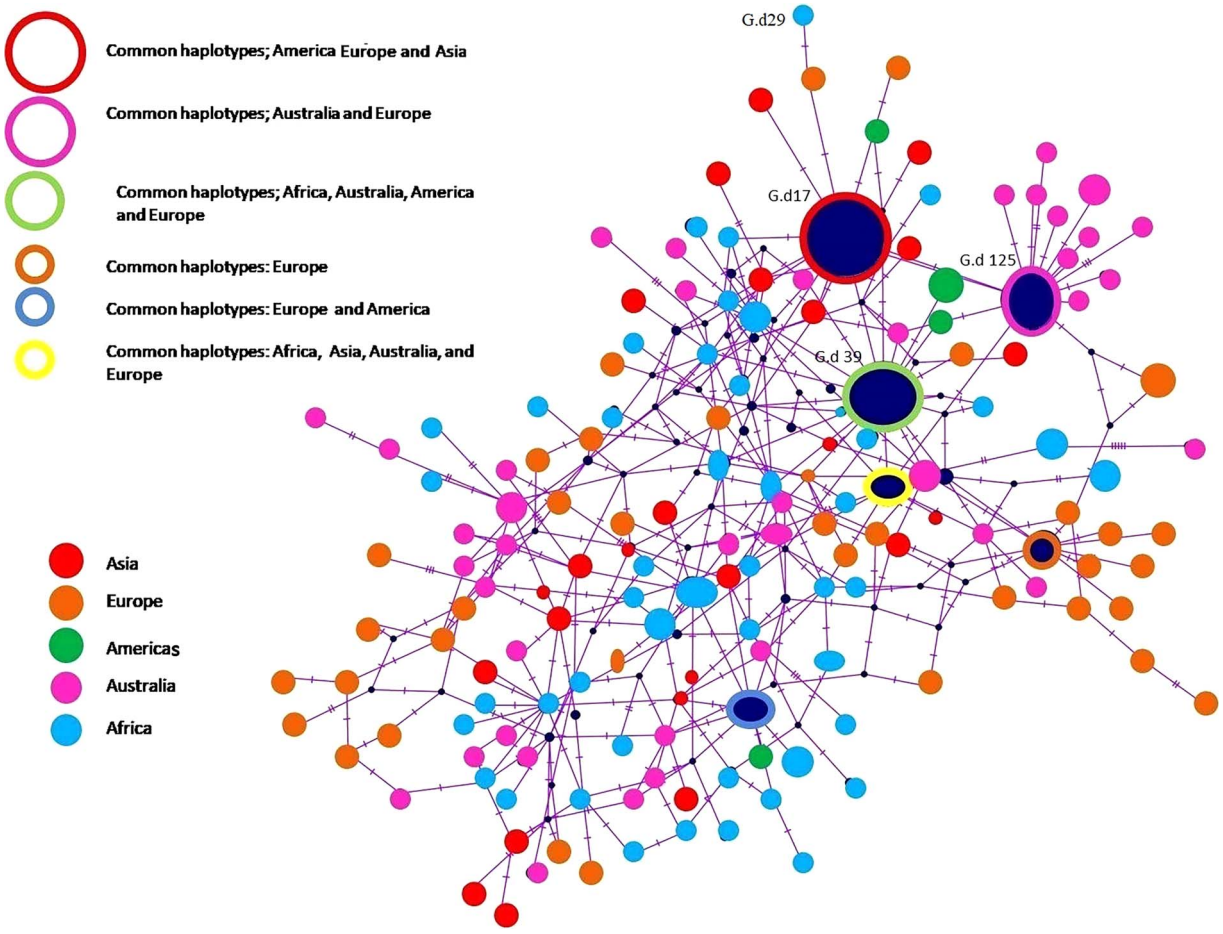

Fig. 5. Median Joining Network of *G. duodenalis* sub-assemblage BIV based on *gdh* sequences from various geographical regions of the world.

develop next generation sequencing and/or multilocus microsatellite typing of the *G. duodenalis* sub-assemblages inferred by non-adaptive genomes.

Disclosure of conflict of interest

Authors announce that there is no conflict of interest to declare.

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