

Genetic differentiation of cercariae infrapopulations of the avian schistosome *Trichobilharzia szidati* based on RAPD markers and mitochondrial *cox1* gene

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Received: 29 April 2011 / Accepted: 13 July 2011 / Published online: 28 July 2011
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Abstract Avian schistosome *Trichobilharzia szidati* is a member of the largest genus within the family Schistosomatidae (Trematoda). Population genetic structure of *Trichobilharzia* spp. schistosomes, causative agents of cercarial dermatitis in humans, has not been studied yet. The knowledge of the genetic structure of trichobilharzian populations is essential for understanding the host–parasite coevolutionary dynamics and epidemiology strategies. Here we examined genetic diversity in three geographically isolated local populations of *T. szidati* cercariae inhabiting Russia based on nuclear (randomly amplified polymorphic DNA, RAPD) and mt (*cox1*) markers. We analyzed *T. szidati* cercariae shed from seven naturally infected snails of *Lymnaea stagnalis*. Using three random primers, we demonstrated genetic variation among populations, thus posing genetic structure across geographic sites. Moreover, *T. szidati* cercariae have been genetically structured among hosts (infrapopulations). Molecular variance analysis was performed to test the significance of genetic differentiation within and between local populations. Of total parasitic diversity, 18.8% was partitioned between populations, whereas the higher contribution (48.9%) corresponds to the

differences among individual cercariae within infrapopulations. In contrast to RAPD markers, a 1,125-bp fragment of *cox1* mt gene failed to provide any significant within-species structure. The lack of geographic structuring was detected using unique haplotypes which were determined in the current work for Moscow and Western Siberian local populations as well as obtained previously for European isolates (Czech Republic and Germany). All *T. szidati*/*Trichobilharzia ocellata* haplotypes were found to be mixed across their geographical origin.

Introduction

Trichobilharzia szidati Neuhaus, 1952, visceral avian schistosome, is a member of the largest genus in the family Schistosomatidae (Trematoda) covering over 40 species. The members of *Trichobilharzia* are the most frequent causative agents of cercarial dermatitis in humans. Cercarial dermatitis is caused by cercariae when they mistake humans for waterfowl and penetrate human skin. These parasites have a two-host life cycle, with water snails and birds as the intermediate and final hosts, respectively. Usually, an egg of visceral *Trichobilharzia* is deposited in the circulation (capillaries) of the final host and needs to pass through different tissues in order to reach the lumen of the intestine and, subsequently, leave the host with feces. The eggs mature within the host and the released eggs contain fully developed miracidia that are able to hatch. Once in the water, the miracidium leaves the egg and searches for the intermediate host. Upon finding a proper molluscan host, the miracidium penetrates and develops into a mother sporocyst. The latter stage produces daughter sporocysts which migrate to the hepatopancreas where

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further development with subsequent release of cercariae continues. Once in the water, the cercariae find a proper final host, penetrate the skin, transform to schistosomula, and start to migrate to the preferred location for egg laying. Depending on the parasite species, two different routes are employed to reach these places: migration via the blood circulation or passage through the nervous tissues (peripheral nerves and the central nervous system). The first route is typical for visceral species; the second one is used by the nasal schistosome *Trichobilharzia regenti* (Horák et al. 2002; Horák and Kolářová 2011).

The European “*Trichobilharzia ocellata*” isolates from *Lymnaea stagnalis* are regarded as a synonym of *T. szidati* based on molecular analysis (Rudolfová et al. 2005). The intermediate host spectrum of *T. szidati*/*T. ocellata* is limited to *L. stagnalis* and *Stagnicola palustris* (Kock 2001). Usually *L. stagnalis* snails are infected by *T. szidati* only. However, Rudolfová et al. (2005) found a rare case of *Trichobilharzia franki* infecting *L. stagnalis* in Czech Republic. Prevalence of *T. szidati* in snails differs across localities and depends on the season ranging in Europe (Finland, Germany, France) from 0.17% to 0.82% (reviewed in Horák and Kolářová 2011). The first finding of *T. szidati* in natural definitive host—mallard (*Anas platyrhynchos*)—was reported recently by Rudolfová et al. (2007).

There are many studies focusing on taxonomical identification of *Trichobilharzia* spp. and phylogenetic relationships assessment between different representatives of the genus. These studies involved nuclear (28S and ITS rDNA) and mt (*cox1*) markers, and many different haplotypes were discovered using them within *Trichobilharzia* spp. across geographical scale (reviewed in Jouet et al. 2010). However, population genetic structure of *Trichobilharzia* spp. has not been studied yet. Examination of parasitic genetic structure across geographic areas as well as within and among hosts on a local scale may provide information about transmission. On the other hand, this knowledge is necessary for understanding the coevolutionary dynamics and revealing host–parasite interactions involving mechanisms of local adaptations of hosts to parasites.

Molecular markers have been widely used in population genetics. Among nuclear markers, randomly amplified polymorphic DNA (RAPD) are the most popular and have been widely used for parasite identification and differentiation (e.g., Williamson et al. 1997). RAPD was applied to estimate genetic variation of mammalian schistosomes within and among hosts or within and among populations (e.g., Dabo et al. 1997; Davies et al. 1999; Sire et al. 2001). The population genetic studies involving different mt markers were also reported for *Schistosoma* parasites (Morgan et al. 2005; Attwood et al. 2008; Stothard et al. 2009).

Previously, using two random primers, we observed cercariae RAPD variability between two *Trichobilharzia* species—*T. szidati* and *T. franki* (Semenova et al. 2005). In addition, we demonstrated the utility of RAPD to study population genetic structure of *Bucephalus polymorphus* (Trematoda: Bucephalidae) using cercariae from daughter sporocysts (Korsunen et al. 2009). A comparative analysis based on the *cox1* mt gene was suitable to reveal among- and within-species variation in cercariae of *T. szidati*, *T. franki*, *T. regenti*, *T. sp. var. narochanica* originating from Russia and Belarus (Chrisanfova et al. 2009; Lopatkin et al. 2010). In another study, three groups of haplotypes were determined for cercariae of avian schistosome *Bilharziella polonica* from Belarus based on *cox1* polymorphism (Chrisanfova et al. 2011). Here we combined nuclear (RAPD) and mt (*cox1*) markers to examine genetic diversity in seven cercariae infrapopulations of *T. szidati* from three geographically isolated local groups (populations) inhabiting Russia.

Material and methods

Collection sites

T. szidati cercariae were sampled after shedding from seven naturally infected snails of *L. stagnalis* posing seven infrapopulations (all the individuals of a parasite species inhabiting a host individual at one time, Bush et al. 1997). The snails were collected in 2002 and 2005 from three localities in Russia. Among them are those collected from two Moscow freshwater ponds—Altufyevo (Lsm1, Lsm7, Lsm20) and Olympiyskaya derevnya (Lsm2, Lsm3, Lsm4) as well as Kargat River (Lsk2), basin of Chany Lake, at Novosibirsk region in Western Siberia (Table 1). We estimated *T. szidati* prevalence in snails for the season of 2005. In total snail batch, two (2.5%) out of 80 snails from Altufyevo were infected by *T. szidati* cercariae, whereas three (3.4%) out of 88 snails from Olympiyskaya derevnya were found positive. Only one (0.38%) out of 263 snails from Kargat emitted ocellate furcocercariae.

DNA extraction, amplification, and sequencing

DNA was extracted from cercariae that have been shed by each host snail using a Chelex-100 suspension containing proteinase K (0.1 mg/ml) at 50°C for 30 min (a slight modification of Walsh et al. 1991). For RAPD analysis, DNA was extracted from individual cercariae; in total, 39 cercariae were used (Table 1). For ITS2-based species identification and *cox1* amplification, DNA was extracted using ten cercariae from each infrapopulation.

Table 1 Geographical area of sampling and GenBank accession numbers of *T. szidati* nucleotide sequences used for current molecular analysis

| | Parasite infrapopulation (snail host) | Collection site | <i>n</i> | GenBank accession number | |
|---|---------------------------------------|--------------------------------|----------|--------------------------|------------------------|
| | | | | ITS2 (318 bp) | <i>cox1</i> (1,125 bp) |
| | Lsm1 | Altufyevo (Moscow) | 9 | HM016851 | JF838197 |
| | Lsm2 | Olympiyskaya derevnya (Moscow) | 4 | HM016852 | JF838198 |
| | Lsm3 | Olympiyskaya derevnya (Moscow) | 4 | HM016853 | JF838199 |
| | Lsm4 | Olympiyskaya derevnya (Moscow) | 3 | HM016854 | JF838200 |
| | Lsm7 | Altufyevo (Moscow) | 5 | HM016855 | JF838201 |
| | Lsm20 | Altufyevo (Moscow) | 4 | HM016856 | JF838202 |
| <i>n</i> number of cercariae analyzed by RAPD | Lsk2 | Kargat (Western Siberia) | 10 | HM016857 | JF838203 |

Species identification was verified based on ITS2 rDNA; the amplification reactions were performed using the primers its3Trem and its4Trem by methods according to Dvořák et al. (2002).

Since DNA amount extracted from individual cercaria is limited; the most intensely stained and unambiguous RAPD profiles were obtained only when the DNA sample was used for no more than three primers. Among tested random primers, those which detected the most of polymorphic markers were selected for this study. RAPD-PCR was performed with each of the selected 10-mer primers, OPA09 (5'-ACCGGACACT-3'), OPA10 (5'-AACGGGCACC-3'), and P29 (5'-CCGGCCTTAC-3'), in 25 µl volume containing 10 ng of total DNA, 75 mM Tris-HCl (pH 8.8), 20 mM (NH₄)₂SO₄, 0.01% Tween 20, 5 mM MgCl₂, 0.25 mM of each dNTPs, 1 µM of primer, and 0.6–0.7 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania). The following amplification profile was used: 2 min of denaturation at 95°C, 35 cycles of 1 min at 94°C, 1 min at 38°C, 15 s at 45°C and 2 min at 72°C, followed by the final 10 min extension at 72°C. Reaction mixture containing no template DNA was used as negative control for PCR assays.

Amplification of 1,125 bp *cox1* fragment was performed using the primer pair (Cox1_schist_5' and Cox1_schist_3') and conditions developed by Lockyer et al. (2003). The PCR products were processed using the automatic sequencing system ABI PRISM 3100-Avant (Applied Biosystems Inc, Foster City, CA, USA). The ITS2 sequences were deposited in GenBank under the accession numbers HM016851–HM016857 and GenBank accession numbers of *cox1* sequences generated from this study: JF838197–JF838203 (Table 1).

Data analysis

Reproducible, clear, scorable RAPD fragments obtained from *T. szidati* cercariae using the three primers were used for statistical analysis. For each *T. szidati* cercariae, a 1/0 (the presence/absence of the amplified bands) binary data matrix was analyzed. Intra- and interpopulation genetic

diversity was estimated based on percentage of polymorphic loci (*P*) and mean values of genotypic diversity (Shannon's index, *H*). These parameters were calculated using population genetic analysis software POPGENE ver. 1.32 (Yeh et al. 1999). Furthermore, the unweighted pair-group means of analysis (UPGMA) dendrogram based on Nei and Li's genetic distances (GD_{xy}) (Nei and Li 1979) was constructed using TREECON for Windows (Van de Peer and De Wachter 1994). Nodal support was assessed by bootstrap resampling (1,000 replicates). Analysis of molecular variance (AMOVA) was employed to quantify the amount of variation between cercariae within hosts as well as among populations and hosts using ARLEQUIN ver 3.11 (Excoffier et al. 2005).

Nucleotide sequences of 1,125 bp *cox1* mtDNA fragment were aligned and used for phylogenetic tree construction. Maximum likelihood (ML) analysis was performed in MEGA version 4.0 software (Tamura et al. 2007) using haplotypes obtained in this study and those deposited in GenBank by Lockyer et al. (2003): *T. ocellata* (AY157189, Germany), *T. szidati* (AY157191, Czech Republic), and *T. regenti* (AY157190, Czech Republic); the latter was used as the outgroup. The Hasegawa, Kishino, and Yano (HKY) model and the parameters were chosen using the hierarchical likelihood ratio test implemented in Modeltest 3.7 (Posada and Crandall 2001). Internal node support was assessed by bootstrapping (500 replicates). Analysis of nucleotide (π) and haplotype (*h*) diversities were performed using MEGA version 4.0 software.

Results

Initially, species identification of cercariae was verified based on the amplification and sequence analysis of ITS2 rDNA (318 bp) through the comparison with nucleotide sequences available in GenBank. As a next step using RAPD-PCR, we analyzed a total of seven cercariae infrapopulations of *T. szidati* from three geographically isolated locations: Kargat River (Novosibirsk region) and

two freshwater ponds in Altufyevo and Olympiyskaya derevnya (Moscow). A set of three random primers (OPA09, OPA10, P29) was suitable for observing genetic diversity within populations (within infrapopulations and between them) and among them. Banding patterns obtained with the P29 primer for three cercariae from each studied infrapopulation are shown as examples in Fig. 1. RAPD profiles differed among three cercariae populations: Kargat (lanes 1–3), Altufyevo (lanes 4–12), and Olympiyskaya derevnya (lanes 13–21). Moreover, each cercariae infrapopulation within species had a unique set of RAPD profiles: Lsk2 (lanes 1–3), Lsm7 (lanes 4–6), Lsm20 (lanes 7–9), Lsm1 (lanes 10–12), Lsm2 (lanes 13–15), Lsm3 (lanes 16–18), and Lsm4 (lanes 19–21).

The three primers selected yielded a total of 57 RAPD fragments in the range of 250 to 2,200 bp. The number of reproducibly amplified fragments in each cercariae pattern varied from 13 to 20, depending on the primer used. The high level of within-snail diversity was observed. The percentage of polymorphic loci (P) within infrapopulations ranged from 17.9% (Lsm2) to 45.3% (Lsm1) (Table 2). The obtained data also demonstrate high within-population genetic diversity with population estimates being 58.4% and 70.0% for Altufyevo and Olympiyskaya derevnya respectively. The percentage of polymorphic bands within these two populations was 86.3%. Total genetic diversity within three studied populations reached 98.4%. The same tendency was found with Shannon's index (I) estimates. The mean I values within infrapopulations were 0.19, 0.12, and 0.18 for populations from Altufyevo, Olympiyskaya

derevnya, and Kargat, respectively. Estimated genetic diversity obtained with I within Altufyevo (0.31) and Olympiyskaya derevnya (0.30) populations were higher and of the same order of magnitude compared with both values obtained for within Moscow strain (0.33) or within three populations in total (0.37).

An UPGMA dendrogram was constructed based on RAPD bands obtained with primers used (Fig. 2). The dendrogram shows the division of all specimens into three distinct clusters. These clusters corresponded to populations examined in this study from geographically isolated water bodies—Altufyevo and Olympiyskaya derevnya ponds and Kargat River as well. The studied *T. szidati* populations were strongly bootstrap-supported (67, 83, 100). The topology of dendrogram also demonstrates the infrapopulation substructuring. Cercariae from Lsm1, Lsm7, and Lsm20 infrapopulations within Altufyevo population, Lsm2, Lsm3, Lsm4 within Olympiyskaya derevnya population, and Lsk2 infrapopulation formed distinct corresponding subclusters separated from each other. These subclusters had high bootstrap values (80–100). The differences between cercariae within Lsk2 infrapopulation varied in range 0.03–0.44 respectively, while the minimum and maximum values of genetic distance for the other infrapopulations were 0.21 and 0.47 (Lsm1), 0.22 and 0.50 (Lsm7), 0.18 and 0.27 (Lsm20), 0.14 and 0.23 (Lsm2), 0.21 and 0.34 (Lsm4), and 0.30 and 0.34 (Lsm3), respectively. The Lsk2 infrapopulation is the most distant relative to the others. The level of differences between populations from Altufyevo (Lsm1, Lsm7, Lsm20) and Olympiyskaya derevnya (Lsm2, Lsm3, Lsm4) was 0.74.

Genetic data were also examined using an AMOVA. Three sources of variation (differences within infrapopulations; among infrapopulations within Altufyevo and Olympiyskaya derevnya populations; between Altufyevo, Olympiyskaya derevnya, and Kargat populations) made a statistically significant contribution to the total variance observed (Table 3). The highest contribution corresponds to the differences among individual cercariae within infrapopulations (48.9%, $p < 0.0001$); 32.3% ($p < 0.0001$) of variability accounted by differences among infrapopulations within Altufyevo and Olympiyskaya derevnya populations. Of total genetic diversity, 18.8% ($p < 0.01$) were partitioned between populations.

We also used the 1,125-bp fragment of *cox1* mt gene to examine intraspecific genetic variance of *T. szidati* cercariae. The high haplotype diversity was observed ($h = 100\%$), i.e., for each cercariae infrapopulation, a unique haplotype was detected, while the nucleotide diversity in the total sample was low ($\pi = 0.9\%$). ML dendrogram was constructed based on the *cox1* nucleotide sequences obtained for seven studied cercariae infrapopulations and two 1,125-bp *cox1* sequences available in GenBank (GenBank accession numbers

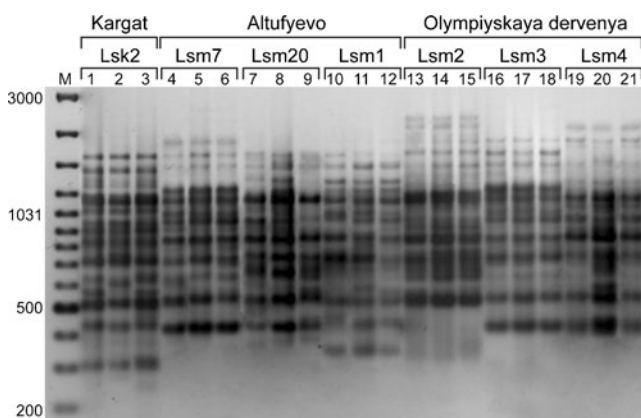


Fig. 1 RAPD profiles of *T. szidati* cercariae from three local populations (Kargat, Altufyevo, and Olympiyskaya derevnya) obtained with the P29 primer. Lane M molecular size marker (100-bp ladder), lanes 1–3 cercariae from Lsk2 infrapopulation, lanes 4–6 cercariae from Lsm7 infrapopulation, lanes 7–9 cercariae from Lsm20 infrapopulation, lanes 10–12 cercariae from Lsm1 infrapopulation, lanes 13–15 cercariae from Lsm2 infrapopulation, lanes 16–18 cercariae from Lsm3 infrapopulation, lanes 19–21 cercariae from Lsm4 infrapopulation

Table 2 RAPD variability among and within seven *T. szidati* cercariae infrapopulations originating from three geographically isolated local populations

| Population | Infrapopulation | <i>n</i> | <i>P</i> (%) | <i>I</i> ($\pm \sigma$) |
|--|---------------------|----------|--------------|---------------------------|
| Altufyevo (Moscow) | Lsm1 | 9 | 45.3 | 0.21 (0.26) |
| | Lsm7 | 5 | 43.2 | 0.26 (0.27) |
| | Lsm20 | 4 | 21.6 | 0.12 (0.24) |
| | Σ | 18 | 36.7 | 0.18 (0.26) |
| | Lsm1 + Lsm7 + Lsm20 | 18 | 70.0 | 0.31 (0.26) |
| | Lsm2 | 4 | 17.9 | 0.10 (0.21) |
| Olympiyskaya derevnya (Moscow) | Lsm3 | 4 | 26.8 | 0.14 (0.24) |
| | Lsm4 | 3 | 19.5 | 0.11 (0.22) |
| | Σ | 11 | 21.4 | 0.12 (0.23) |
| | Lsm2 + Lsm3 + Lsm4 | 11 | 58.4 | 0.30 (0.29) |
| Kargat (Novosibirsk region) | Lsk2 | 10 | 38.4 | 0.18 (0.26) |
| Altufyevo + Olympiyskaya derevnya | | 29 | 86.3 | 0.33 (0.21) |
| Kargat + Olympiyskaya derevnya + Altufyevo | | 39 | 98.4 | 0.37 (0.17) |

n number of cercariae analyzed, *P* percentage of polymorphic loci, *I* mean values of genotypic diversity (Shannon’s index), σ standard deviation

AY157189, AY157191) (Fig. 3). The topology of tree did not reveal subdivision into haplotype lineages. All *T. szidati*/*T. ocellata* haplotypes were found to be mixed across their geographical origin. The Moscow haplotypes from Altufyevo (Lsm1, Lsm7, Lsm20) and Olympiyskaya derevnya (Lsm2, Lsm3, Lsm4) were not segregated according to their geographical origin. The Western Siberian haplotype (Lsk2) was placed among the Moscow (Lsm20), Czech Republic (GenBank accession number AY157191), and Germany (GenBank accession number AY157189) haplotypes. The European haplotypes also did not form distinct lineage. Thus, compared with results coming from RAPD analysis, *cox1* domain failed to differentiate neither the Russian

populations nor the Russian and European sets of haplotypes between each other.

Discussion

In the present study, combined analysis based on RAPD markers and *cox1* mt gene have been applied to investigate the intraspecific genetic structure of *T. szidati* cercariae sampled from three geographically isolated populations. *Cox1* failed to provide any significant within-species structure. The lack of geographic structuring was detected using unique haplotypes which were determined in the

Fig. 2 UPGMA dendrogram constructed based on Nei and Li’s genetic distances for 39 *T. szidati* cercariae from seven hosts originated from three local populations (Altufyevo, Olympiyskaya derevnya, and Kargat)

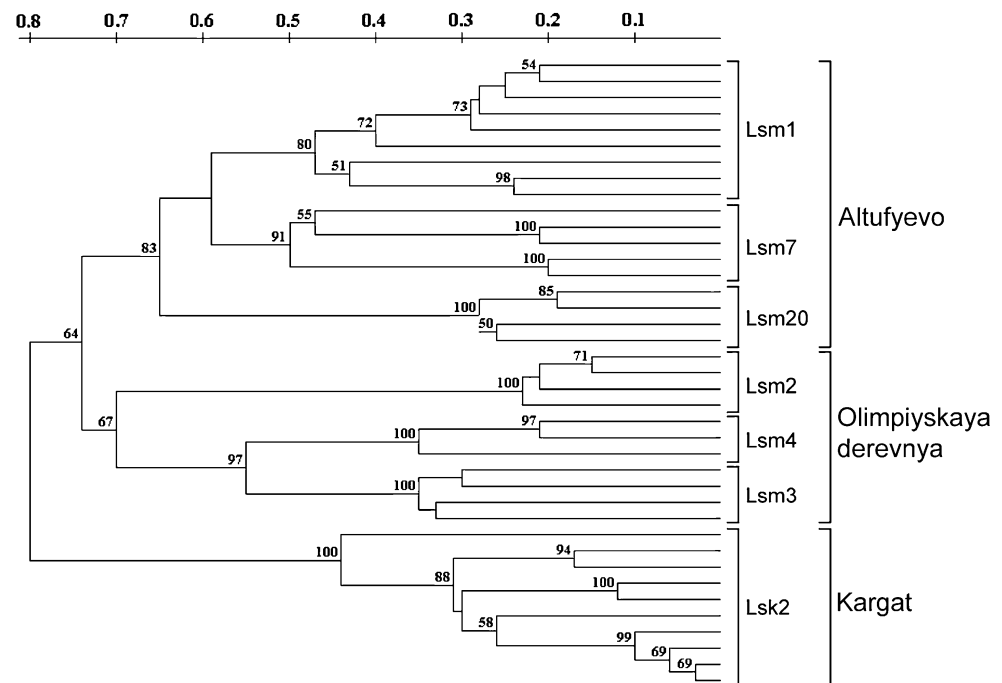


Table 3 Results of molecular variance (AMOVA) analysis for three populations of *T. szidati* cercariae

| Source of variation | d.f. | Sum of squares | Variance component | Percent | <i>p</i> |
|---|------|----------------|--------------------|---------|----------|
| Among Altufyevo, Olympiyskaya derevnya and Kargat populations | 2 | 344.6 | 6.394 | 18.8 | <0.01 |
| Among infrapopulations (hosts) within Altufyevo and Olympiyskaya derevnya populations | 4 | 267.0 | 10.995 | 32.3 | <0.0001 |
| Within infrapopulations (hosts) | 32 | 532.9 | 16.654 | 48.9 | <0.0001 |
| Total | 38 | 1147.5 | 34.043 | | |

The degrees of freedom, sum of squares, variance components, fraction of total variance contributed by each component, and the probability of obtaining a more component estimate by chance alone (*p* value)

current work for Moscow and Western Siberian local populations as well as obtained previously by Lockyer et al. (2003) for European isolates of *T. szidati*/*T. ocellata* (Czech Republic and Germany). Likewise, the high haplotype diversity ($h=85.6\%$) and no geographical substructuring was determined based on the 1,125-bp-long *cox1* fragment for another avian schistosome (*B. polonica*) originating from Belarusian lakes (Chrisanfova et al. 2011). In total, 11 haplotypes forming three lineages were detected with one of the lineages being intermediate between two others. However, these haplotype lineages did not demonstrate any geographical specificity. The high values of *cox1* haplotype diversity ($h=80\text{--}100\%$) were previously determined for *T. szidati*, *T. franki*, and *T. sp. var. narochanica* (proposed new species) cercariae originating from Belarus (Chrisanfova et al. 2009) as well as for *T. szidati*, *T. franki*, and *T. regenti* cercariae infrapopulations from Russia (Moscow and Moscow region) (Lopatkin et al. 2010). The unique *cox1* haplotypes obtained through these studies appeared to indicate the high *T. szidati* haplotype diversity across the species geography in general. Additionally, for *cox1* haplotypes obtained herein, genealogical network was constructed

(data not shown) which demonstrated a “star-tree” structure. A starburst phylogeographic pattern is an expected signature for an abundant species that has expanded its range rather recently from small numbers of founders (Avice 2000). In contrast to the avian schistosomes, geography-based differentiation was reported for mammalian schistosomes. Attwood et al. (2008) found that three of four studied *Schistosoma mekongi* populations from Cambodia and Southern Laos were distinguishable at *cox1* and 12S mt loci. Furthermore, the unique haplotype patterns representing two evolutionary lineages with strong spatial specificity were detected for Ugandan *Schistosoma mansoni* from Lake Albert and Lake Victoria using DNA barcoding, sequence analysis of two partially overlapping *cox1* regions—ASMIT (396 bp) and MORGAN (617 bp) (Stothard et al. 2009). Morgan et al. (2005) used partial fragments of *cox1*, *rrnL-rrnS*, *cytb-nad4L-nad4*, and *nad1* covering more than 2,500 bp of mtDNA to describe within-species diversity of *S. mansoni* originating from Africa and the New World. Considerable geographical structure, at global and regional scales, was found based on 85 unique mt haplotypes separated into five distinct lineages.

Using RAPD-PCR, we demonstrate cercariae genetic variation among populations, thus posing genetic structure across geographic sites. Cercariae from Altufyevo were found to be more similar to those from Olympiyskaya derevnya than compared with Kargat samples. The geographical distance between two Moscow populations is about 25 km. Kargat population located in Western Siberia is much more geographically isolated from two others (about 2,600 km). Moreover, using random primers, it was shown that *T. szidati* cercariae have been genetically structured not only between local populations but among hosts within geographic sites as well. Clustering of cercariae into seven groups corresponding to their snail hosts as visualized on UPGMA dendrogram is seemed to indicate the occurrence of parasite–host interactions. In our previous work, substructuring at the both infrapopulation and population levels was detected based on RAPD markers for *B. polymorphus* cercariae from daughter sporocysts (Korsunen et al. 2009). In general, the

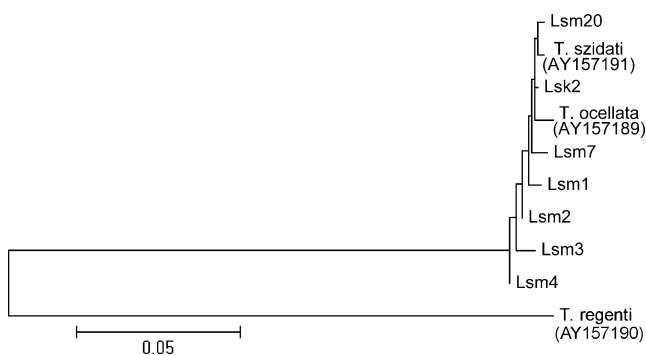


Fig. 3 Phylogenetic tree based on the 1,125-bp *cox1* of *T. szidati*/*T. ocellata* cercariae constructed using maximum likelihood method (HKY model of substitution). The scale shows the number of nucleotide substitutions per site between DNA sequences. Sequence of *T. regenti* (GenBank accession number AY157190) was used as outgroup

parasite substructuring among hosts as well as their high heterogeneity within hosts both detected by RAPDs may reflect some molecular adaptations occurring between intermediate host and parasite. Lively (1989) showed strong local adaptation by parasite in reciprocal cross-infection experiment using *Potamopyrgus antipodarum* as the host and *Microphallus* sp. (Trematoda: Microphallidae) as the parasite. Snails from several New Zealand lake populations were experimentally exposed to parasites from the same lakes. The results showed that snails are more susceptible to parasites from the same locality, i.e., the parasites are adapted to infecting snails from their local host population.

AMOVA analysis confirmed that the percentage of RAPD variance attributable to differences between cercariae within infrapopulations (snails) is significant and higher than the percentage of variation due to differences among infrapopulations within Altufyevo and Olympiyskaya der-evnya populations as well as differences between the three populations. Unfortunately, it was not possible to estimate the contribution of differences among infrapopulations within population from Kargat River since only one infrapopulation was available for the study. Previously, the prevalence of within-hosts/within-populations component rather than among-hosts/among-populations component in total parasitic diversity and multiple snail infections were determined based on RAPD markers in mammalian schistosome *Schistosoma haematobium* (Dabo et al. 1997; Davies et al. 1999).

In our study, the genetic heterogeneity of *T. szidati* cercariae within infrapopulations was demonstrated based on UPGMA analysis and AMOVA results. Snail infections with multiple parasite genotypes could be responsible for parasite genetic diversity within hosts. In natural populations, over 50% of snails which are infected by *S. mansoni* and *S. haematobium* contained multiple genotypes, with the maximum genotypically unique miracidia per snail being nine (reviewed in Yin et al. 2008). On the other hands, cercariae heterogeneity could be generated during the clonal reproduction in intermediate host. The variable occurrence of W1 and W2 elements originating from genomic instability of these repetitive elements was detected within and among daughter sporocyst generations of *S. mansoni* cultured in vitro (Grevelding 1999; Bayne and Grevelding 2003). Because an unexpected heterogeneity was found even among clonal cercariae originating from monomiracidial snail infections, the authors suggested that mitotic recombination events could occur during the asexual life stage of schistosomes. Previously, based on multi-locus microsatellite analysis, near-identical multi-locus genotypes (niMLGs), i.e., genotypes that differed at one or a very few loci from a frequently observed MLGs (Yin et al. 2008), were identified for *S. mansoni* (Gower et al. 2007) and *Schistosoma japonicum* (Yin et al. 2008).

Yin et al. (2008) revealed the presence of at least nine niMLG among worms arising from monomiracidial snail infection and regarded somatic mutations as the most likely explanation of this phenomenon. Recently, based on RAPD markers, we revealed significant genetic heterogeneity between cercariae within parthenites (sporocysts) for six trematode species from Schistosomatidae, Strigeidae, Gorgoderidae, Bucephalidae, Diplostomatidae, and Plagiorchiidae families (Semyenova et al. 2007). RAPD variability within daughter sporocysts was also determined in population genetic studies of some trematodes: *Microphallus pygmaeus* and *Microphallus pseudopygmaeus* (Khalturin et al. 2000) and *B. polymorphus* (Korsunen et al. 2009). Using *cox1* fragment, we did not reveal more than one haplotype in each of the cercariae infrapopulations that appeared to indicate the monomiracidial infection of the studied snails. However, such finding may be the consequence of snail infection caused by several closely related miracidia with *cox1* identical haplotypes. The second explanation for *T. szidati* cercariae heterogeneity was detected in this study—somatic mutation events during parthenogenesis rounds within snails. Furthermore, we also suggest that both explanations may be the case.

Migration of definitive host is known to be the major factor which forms the population structure of parasite (Jarne and Theron 2001). In the case of our study migration of *T. szidati*, definitive host (anatid birds) in large distances and in microspatial scale (between localities within Moscow region) appeared to promote gene flow between parasitic populations influencing the distribution of parasite genetic variability. Such factors as genetic drift and mutation, founder effect, etc. may also be responsible for the distribution of genetic diversity within and among *T. szidati* populations. Population genetic structure of *T. szidati* intermediate and definitive hosts should be studied to analyze the forces acting upon the parasite population structure. The overall lack of genetic structure in mallards (the main definitive host of trichobilharzians) from Western Russia and North Asia was demonstrated using 5'-end of the mtDNA control region (Kulikova et al. 2005). It was suggested that mallards exhibit considerable population connectedness and relatively high gene flow. To assess the *T. szidati* population genetic structure in details, additional studies that involve the use of different nuclear and mt markers and more cercariae isolates covered the larger geographical area should be carried out. The genetic structure inferring of other *Trichobilharzia* species is of the most interest as well.

Acknowledgments We would like to thank Dr. Natalya Yurlova and Dr. Elena Serbina for their help in collecting of samples. This work received financial support from the Russian Foundation for Basic Research (09-04-01611, 08-04-12204), RFC (02.740.11.0088), and President RF Program of Leading Scientific Schools (S. S. -2107.2008.4).

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