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Molecular identification and phylogenetic analysis of free-living amoeba in the water resources of Arak, Iran

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ABSTRACT

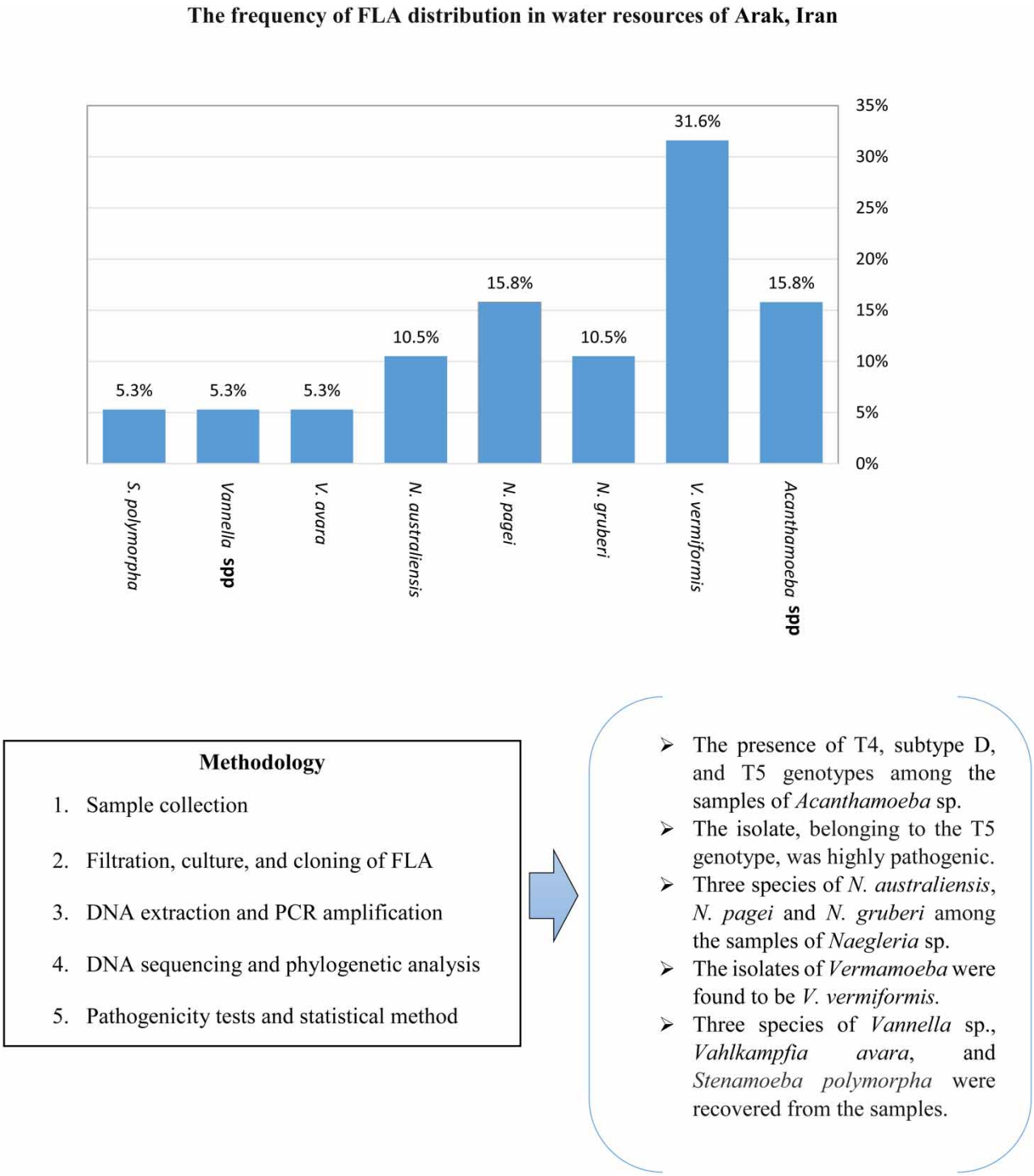
The aim of the present study was to detect free-living amoeba (FLA) in the water resources of Arak, Iran using molecular tools. A total of 154 samples were collected from different water supplies. Molecular analyses, sequencing, and phylogenetic study were conducted to confirm the species and genotypes of FLA. Fisher exact test was used to determine the significance. Of 154 water samples, 19 (12.3%) samples were tested positive for FLA. Three genotypes of *Acanthamoeba* including T4, subtype D, and T5 were identified among the isolates. The pathogenicity assay showed that the isolate of *Acanthamoeba* in drinking water was highly pathogenic. Three species of *Naegleria*, including *N. australiensis*, *N. pagei*, and *N. gruberi* were found among the samples. Six isolates of *Vermamoeba* were identified as *V. vermiformis*. Meanwhile, three other species including *Vannella* sp., *Vahlkampfia avara,* and *Stenamoeba polymorpha* were also recovered from the water samples. Statistical analysis showed a significant difference between the various water resources contaminated with FLA. This is the first study to reveal the presence of *S. polymorpha* in water sources in Iran. According to the findings of the present study, health officials should be beware of potential public health impacts of FLA in water resources.

Key words: Arak, FLA, genotype, Iran, phylogenetic study, *Stenamoeba*

HIGHLIGHTS

- Different species of *Acanthamoeba*, as well as *Vermamoeba vermiformis*, *Naegleria australiensis*, *N. pagei*, and *N. gruberi* were found in water resources.
- Three species of *Vannella*, as well as *Vahlkampfia avara* and *Stenamoeba polymorpha* were recovered from the water samples.
- This study is the first of its kind revealing the presence of *S. polymorpha* in Iran.

GRAPHICAL ABSTRACT



INTRODUCTION

Free-living amoebae (FLAs) are amphizoic protozoans causing severe diseases such as fatal encephalitis and cutaneous ulcers. The most pathogenic of the amoeba include *Acanthamoeba* spp., *Balamuthia mandrillaris,* and *Naegleria fowleri* (Visvesvara *et al.* 2007; Hajialilo *et al.* 2015). The Vahlkampfiidae family of FLA is composed of important genera, distributed

within a wide range of aquatic and soil habitats, worldwide. Two genera of *Naegleria* and *Vahlkampfia* are the most frequently identified FLA in the environment (Ithoi *et al.* 2011; El-Badry *et al.* 2020; Paknejad *et al.* 2020).

In general, the FLA cysts, especially those of *Acanthamoeba*, are resistant to treatment with chlorine and monochloramine (Dupuy *et al.* 2014). Therefore, the water resources are regarded as suitable niches for FLA, through which the most common amoeba are transmitted to humans via contaminated water (Lorenzo-Morales *et al.* 2015; Abdul Majid *et al.* 2017). Water activities such as swimming and bathing as well as using contaminated water to wash contact lenses are considered as major risk factors for amoebic keratitis, worldwide (Khan 2006). In Iran, the most described cases are related to *Acanthamoeba* keratitis (AK) in the country, which frequently occurs among contact-lens users (Niyyati & Rezaeian 2015). T4 genotype is introduced as a common and more pathogenic *Acanthamoeba* genotype compared to other genotypes (Schuster & Visvesvara 2004; Khan 2006; Nuprasert *et al.* 2010; Hajialilo *et al.* 2016). Several genotypes of *Acanthamoeba* are isolated from the water resources of Iran (Behnia *et al.* 2017; Paknejad *et al.* 2020; Rafsanjani *et al.* 2020; Mahmoudi *et al.* 2021). Also, some species of other FLAs, such as *Naegleria* spp., *Vermamoeba vermiformis*, and *Vannella* sp. are isolated from the water niches of the country (Javanmard *et al.* 2017; Niyyati & Latifi 2017; Paknejad *et al.* 2020).

A pilot study conducted on FLA within the study region (2014), revealed the presence of *Acanthamoeba* cysts based on morphological features in the rural water sources of Markazi province, Iran (Mosayebi *et al.* 2014). Another study isolated *Acanthamoeba* by culture and morphological features from a pool in Arak (Sarmadian *et al.* 2020). No molecular investigations were conducted on water resources in the study area. The aim of the present study was the detection and molecular characterization of FLA isolated from the water resources of Markazi province in Iran.

METHODS

Isolates

During April to September 2019, a total of 154 samples were collected from the water samples of a swimming pool (the samples were collected from the water surface of the main pool and hot Jacuzzi tub) (n=45), drinking water (n=30), park water supplies (n=22), mosques (ponds used for ablution) (n=41), and several other locations in the northern, eastern, southern, and western parts of the raceway (the origin of aqueduct water and groundwater) (n=16) in Arak, the capital of Markazi province of Iran (34 °00′ N 49 °40′ E). The city is located on the crossroad of northern, eastern, southern, and western provinces of Iran (Figure 1). Arak has a relatively cold and dry climate. The samples collected from the swimming pool water were obtained from four sides of the main pool and the hot Jacuzzi tubs. The water samples were collected into

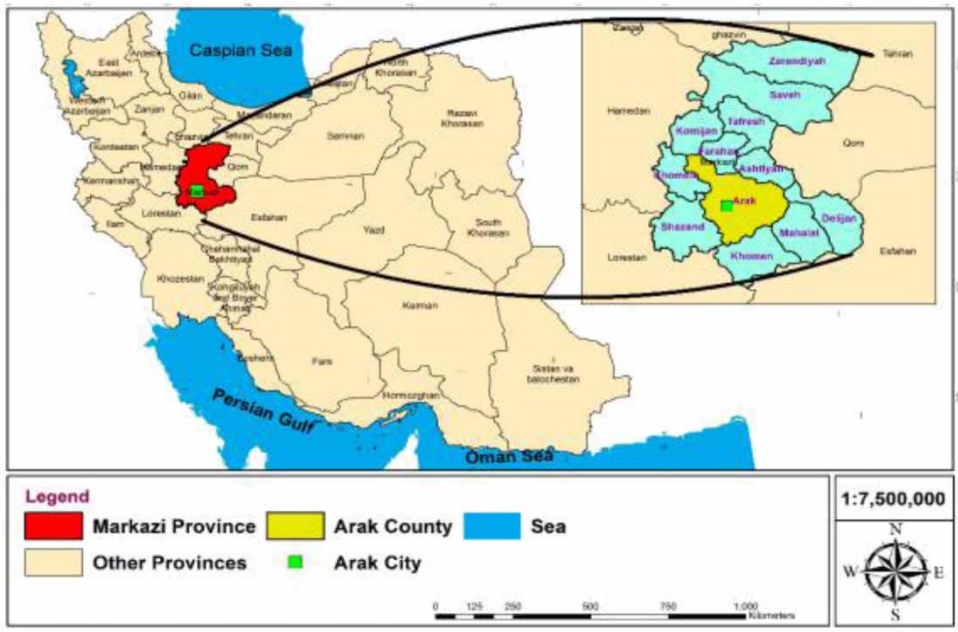


Figure 1 | Map of the Markazi province, located on the crossroad of northern, eastern, southern, and western provinces of Iran.

500 mL sterile flasks, and then transferred to the Department of Medical Parasitology and Mycology at the School of Medicine, Arak University of Medical Sciences, Iran.

Filtration, culture, and cloning of FLA

All water samples were filtered through nitrocellulose membrane filters (0.45 μm pore size) using a vacuum pump. The membranes were cultured on 1.5% non-nutrient agar (NNA) plates, seeded with heat-inactivated suspension of *Escherichia coli*. The plates were incubated at 30 °C for up to 30 days to grow the FLA and in the meantime, the plates were examined under light microscope on a daily basis. Cloning and purification were performed to obtain clean plates with minimal bacterial and fungal contamination (Paknejad *et al.* 2020; Rafsanjani *et al.* 2020).

DNA extraction and PCR amplification

The FLAs were collected in sterile phosphate buffered saline (PBS) from the positive plates. DNA extraction was carried out by High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's recommended protocol with glass beads treatment. PCR optimization was performed using specific primers of *Acanthamoeba* spp. (JDP1 5′-GGCCCAGATCGTTTACCGTGAA-3′and JDP2 5′-TCTCACAAGCTGCTAGGGAGTCA-3′) *Naegleria* spp. (NA1 5′-AACCTGCGTAGGGATCAT-3′ and NA2 5′-TTTTCTTTTCCTCCCCTTAT-3′) *Vermamoeba* spp. (5′-GCT CCA ATA GCG TAT ATT AA-3′ and 5′-AGA AAG AGC TAT CAATCT GT-3′) and *B. mandrillaris* (5′-Balspec16S: 5′-CGCATGTATGAA-GAAGAC CA-3′ and 3′-Balspec16S: 5′-TTACCTATATAATT GTCGATACCA-3′) (Hajialilo *et al.* 2015; Niyyati *et al.* 2016; Paknejad *et al.* 2020). Standard PCR was performed in a 30-μL volume containing a ready-made mixture of Amplicon (Taq DNA Polymerase Master Mix RED, Denmark), template DNA, 0.1 μM of each primer, and distilled water. Thermal cycling conditions were conducted according to the previous studies at annealing temperatures of 64 °C (45 s), 57 °C (20 s), 50 °C (45 s), and 56 °C (1 min) for *Acanthamoeba* spp., *Naegleria* spp., *Vermamoeba* spp., and *B. mandrillaris*, respectively (Hajialilo *et al.* 2015; Niyyati *et al.* 2016; Paknejad *et al.* 2020; Rafsanjani *et al.* 2020). Finally, the PCR products were electrophoresed on agarose gel (2% w/v), followed by ultraviolet visualization.

DNA sequencing and phylogenetic analysis

DNA sequencing of PCR products was achieved using an automatic ABI3130 sequencer machine (Applied Biosystems, USA). The obtained sequences were edited manually by chromas (Version 1.0.0.1) software, and were compared by aligning the query sequence against the eukaryotic sequences using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) on the NCBI nucleotide sequence database. The sequences were deposited in the NCBI GenBank database. A phylogenetic analysis of the sequences obtained for the FLA isolates was conducted using the neighbor-joining method. Some additional isolates from Iran that overlap the sequences in the current study were also included to provide additional context. MEGAX was used to identify the best model for analysis of this dataset (Kumar *et al.* 2018). Consequently, the K2+G+I or HKY+G was used for maximum likelihood analysis. The bootstrap number of 1000 replicates was considered (Figures 2–4).

Pathogenicity tests and statistical method

Osmo-tolerance and thermo-tolerance methods were performed for pathogenicity assays. The osmo-tolerance assay was evaluated based on the growth ability of *Acanthamoeba* isolates in two concentrations of 0.5 and 1 M mannitol. In thermo-tolerance assays, the growth ability of *Acanthamoeba* isolates was surveyed at 37 and 40 °C. The samples received daily follow-up for a duration of 1 week (Khan 2001; Lasjerdi *et al.* 2015; Hajialilo *et al.* 2016). The Fisher exact test was used to determine the significance. A P value of <0.05 was considered statistically significant for the differences observed. SPSS software version 16.00 (SPSS Inc., Chicago, IL, USA) was applied for data analysis.

RESULTS

Of 154 water samples from different water resources of the city of Arak, 33 (21. 4%) samples were tested positive for FLA using culture along with direct microscopy. Nineteen samples (57. 6%) were tested positive for FLA with PCR amplification, of which three isolates (15. 8%) showed specific bands for *Acanthamoeba*. In addition, PCRs were also positive by the primers used for *Naegleria* and *Vermamoeba* in eight isolates (42%) for each FLA. The PCR results were negative for *B. mandrillaris* among the samples. The frequency distribution of FLA was calculated for drinking water (15. 8%), swimming pool water (10.5%), raceway water (47%), park water supplies (15. 8%), and mosques (10.5%).

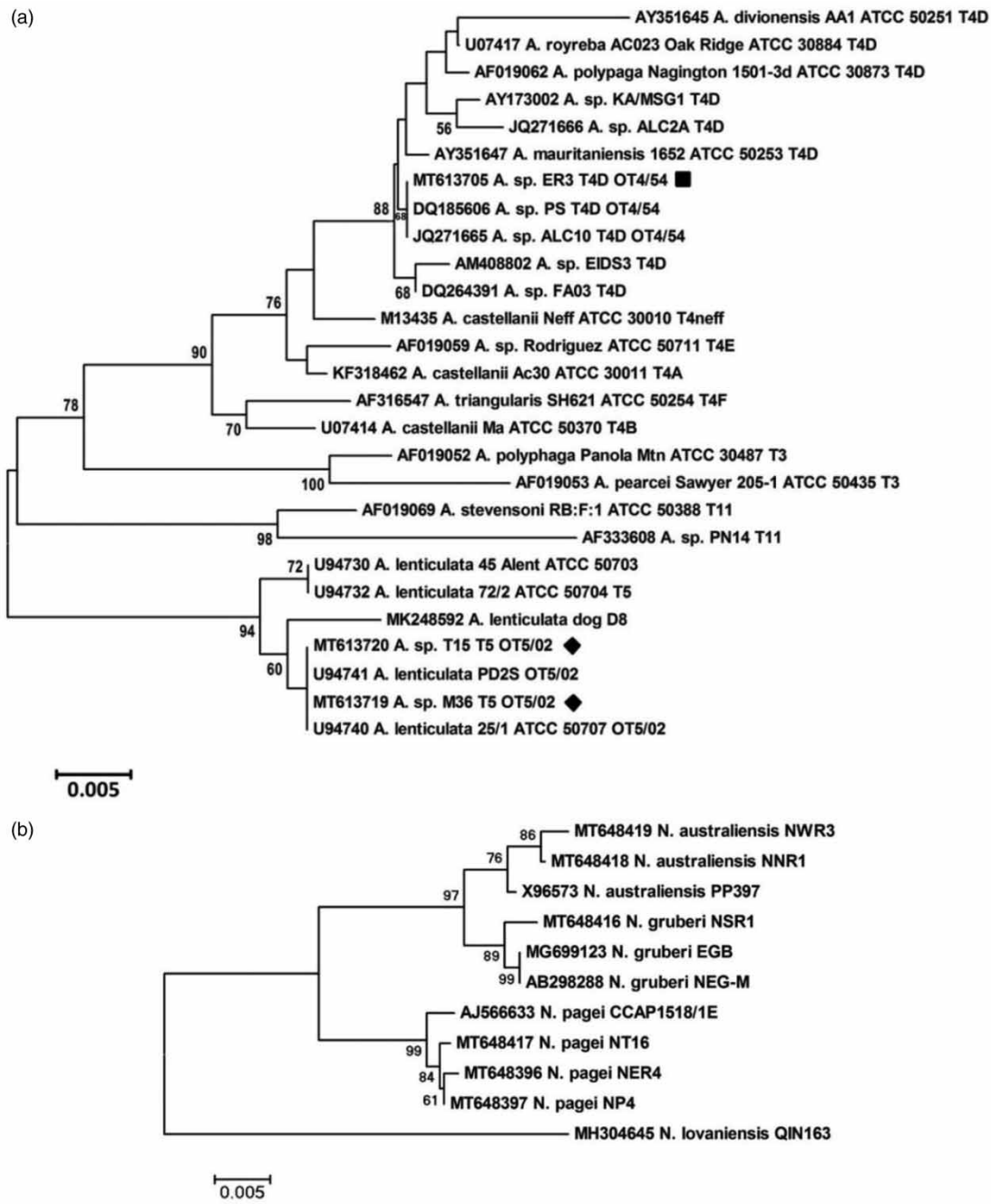


Figure 2 | (a) Neighbor-joining tree for *Acanthamoeba* isolates. ■=T4 Isolate ER3; shown with other representative *Acanthamoeba* samples of sequence type subtype T4D including two isolates carrying allele OT4/54; ◆=T5 isolates M36 and T15; shown together with other sequence type T5 isolates carrying allele OT5/02. (b) Phylogenetic relationships for the ITS1-5.8SrRNA-ITS2 region between the isolates of *Naegleria* identified in this study and the standard isolates from *N. australensis, N. pagei*, and *N. gruberi*. The constructed tree was obtained by using the neighbor-joining method and the maximum composite likelihood setting. The sequence of the region from *N. lovaniensis* was used to root the tree.

Sequencing and phylogenetic analyses revealed the presence of T4, subtype D, and T5 genotypes among the *Acanthamoeba* isolates (Figure 2(a)). The pathogenicity assay showed that the T15 isolate, belonging to the T5 genotype, was highly pathogenic, whereas two isolates including ER3 and M36 genotypes were found to be non-pathogenic. Three species of *Naegleria* including *N. australiensis* (10. 5%), *N. pagei* (15. 8%), and *N. gruberi* (10. 5%) were present among the samples. The phylogenetic relationships between the isolates obtained in this study are shown in Figure 2(b). Six isolates

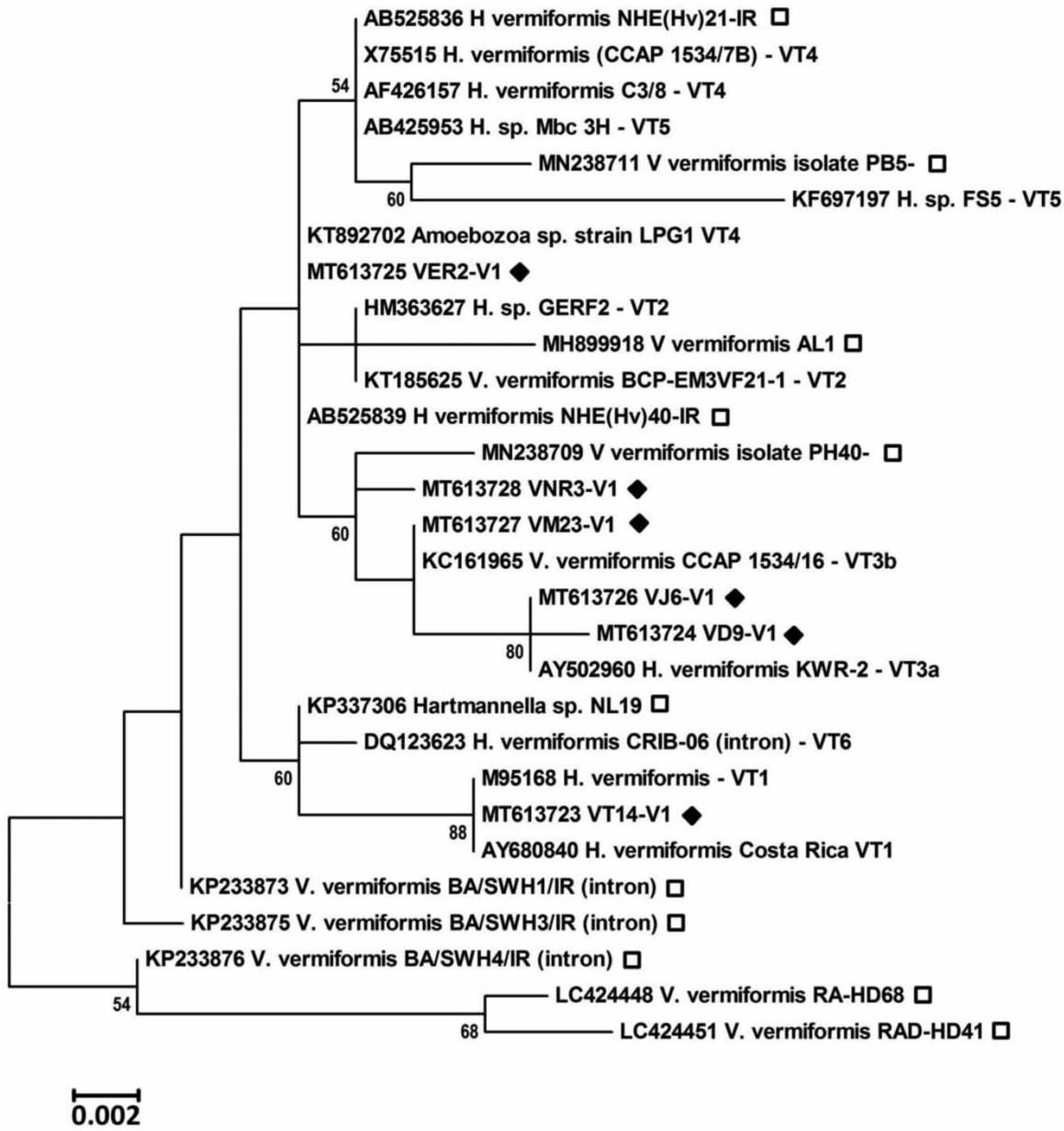


Figure 3 | Phylogenetic relationships between the isolates of *Vermamoeba*. Standard sequences labeled with VT# (*Vermamoeba* Type) to indicate which clade a sequence was assigned to in analysis of 39 isolates with almost complete 18S rRNA gene sequences. ◆=isolates from this study. □=Iranian isolates from other studies.

of *Vermamoeba* (31. 6%) were found to be *V. vermiformis* based on the 18S rRNA gene (Figure 3). Interestingly, three species of *Vannella* sp., *Vahlkampfia avara*, and *Stenamoeba polymorpha* were recovered from the water samples of the region (Table 1) (Figure 4). The VJ6 isolate of *V. vermiformis* was only found in the hot Jacuzzi tub water sample. The frequency of FLA in the water resources is demonstrated in Table 2 and Figure 5. Overall, statistical analysis showed that there were significant differences between the various water supplies contaminated by FLA ($P<0.001$).

DISCUSSION

The findings of the present study provided an expanded knowledge about the molecular distribution of FLA in various water resources in the region. The distribution of FLA was determined for drinking water (15. 8%), swimming pool water (10.5%),

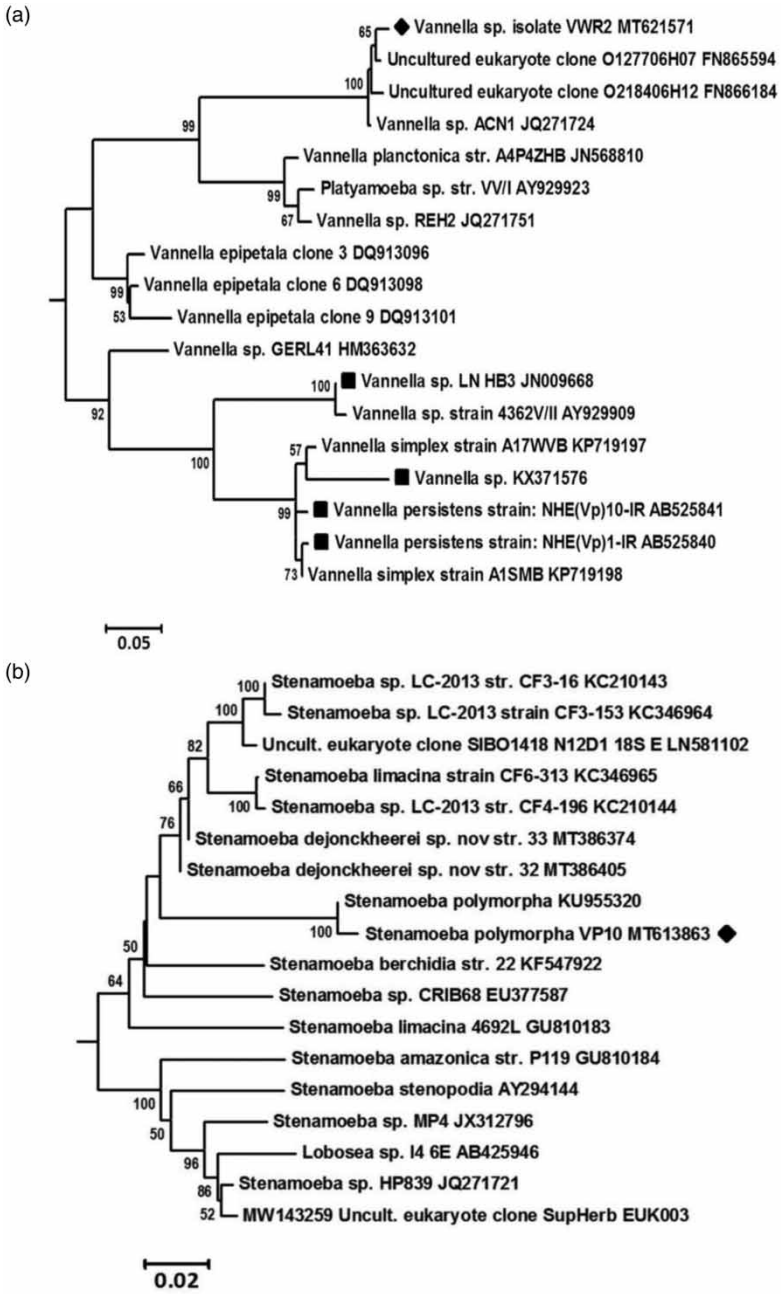


Figure 4 | (a) Phylogenetic relationships, based on the 18S rRNS gene sequences, between the isolate VWR2 (◆) and other closely related members of the genus *Vannella,* and also with other isolates classified within the *Vannella* sp. but cultured from Iranian environmental sources (■). (b) Phylogenetic relationships between the isolates of *Stenamoeba* taxa. ◆=isolate VP10.

raceway water (47%), park water supplies (15. 8%), and mosques (10.5%). All types of water sources are treated with chlorine except the raceway water. The sources of raceway water are aqueduct water and groundwater; so, raceway water is not treated (chlorinated). The prevalence of FLA in the raceway water samples was high. This may be attributed to the diverse origins of water resources supplying this area.

The present study clarified the existence of three isolates of *Acanthamoeba* including one isolate of type T4, subtype D, and two isolates of type T5. Among the Iranian isolates, the T4 sequence type encompasses 689 (78%) of the isolates, slightly larger than the proportion of such isolates in the total DNA databases (Fuerst 2014) (https://u.osu.edu/acathamoeba). The

Table 1 | Species and genotype of the FLAs isolated from the water resources of Arak, Iran

Number	Code	Species	Source	Genotype	Accession no.
1	ER3	*Acanthamoeba* sp.	Raceway	T4	MT613705
2	M36	*Acanthamoeba* sp.	Mosque	T5	MT613719
3	T15	*Acanthamoeba* sp.	Drinking water	T5	MT613720
4	VT14	*V. vermiformis*	Drinking water	–	MT613723
5	VD9	*V. vermiformis*	Swimming pool	–	MT613724
6	VER2	*V. vermiformis*	Raceway	–	MT613725
7	VJ6	*V. vermiformis*	Hot Jacuzzi tub	–	MT613726
8	VM23	*V. vermiformis*	Mosque	–	MT613727
9	VNR3	*V. vermiformis*	Raceway	–	MT613728
10	VSR4	*N. gruberi*	Raceway	–	MT613729
11	NSR1	*N. gruberi*	Raceway	–	MT648416
12	NER4	*N. pagei*	Raceway	–	MT648396
13	NP4	*N. pagei*	Park	–	MT648397
14	NT16	*N. pagei*	Drinking water	–	MT648417
15	NNR1	*N. australiensis*	Raceway	–	MT648418
16	NWR3	*N. australiensis*	Raceway	–	MT648419
17	NP10	*V. avara*	Park	–	MT648420
18	VWR2	*Vannella* sp	Raceway	–	MT621571
19	VP10	*S. polymorpha*	Park	–	MT613863

Table 2 | Frequency distribution of water contamination status in the water resources of Arak, Iran

	Status		
	Negative	Positive	Total
Park	19 (86.4%)	3 (13.6%)	22
Drinking water	27 (90.0%)	3 (10.0%)	30
Swimming pool	43 (95.6%)	2 (4.4%)	45
Mosque	39 (95.1%)	2 (4.9%)	41
Raceway	7 (43.8%)	9 (56.3%)	16
Total	135 (87.7%)	19 (12.3%)	154

$P<0.001$.

T4 isolate ER3 carries the allele OT4/54 (Fuerst & Booton 2020). This allele has been observed almost 40 times among more than 5700 *Acanthamoeba* isolates that have been analyzed globally and for which the genotype has been deposited in the international DNA databases. Among the isolates carrying allele OT4/54, 16 cases have been obtained within Iran, all but three from water samples. In addition to the isolates from the Markazi province, the isolates carrying this allele have also been obtained during the studies in Ilam, Isfahan, Mazandaran, Tehran, and Zanjan provinces (Nazar *et al.* 2011; Saberi *et al.* 2019).

Sixty *Acanthamoeba* isolates, belonging to the sequence type T5, have been obtained during different studies in Iran (representing 6.5% of the total, close to the 6.8% seen in the international DNA databases). The two sequence type T5 isolates in the current study, M36 and T15, carried the allele T5/02 (Fuerst & Booton 2020), which is the most frequently encountered T5 allele in the DNA databases. Twenty of the *Acanthamoeba* T5 isolates from Iran carried the T5/02 allele. In addition to our studies, other such isolates have also been recovered from different parts of the country including East Azerbaijan, Guilan,

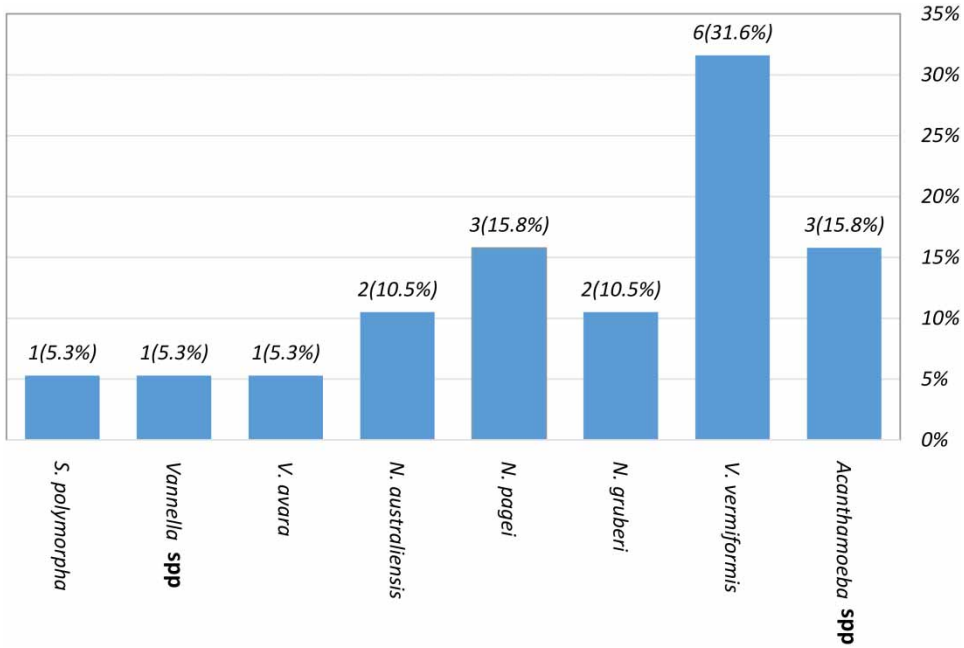


Figure 5 | The frequency of FLA distribution in water resources of Arak, Iran.

Isfahan, Shiraz, Sistan and Baluchestan, and Tehran provinces (Nazar *et al.* 2011; Behniafar *et al.* 2015; Memari *et al.* 2015; Aghajani *et al.* 2016; Armand *et al.* 2016).

The two studies on water resources of villages and swimming pools in Markazi province both showed the presence of *Acanthamoeba* contamination by culture and morphological features (Mosayebi *et al.* 2014; Sarmadian *et al.* 2020). Moreover, findings of the present study indicate that the T5 genotype isolated from the drinking water samples was a pathogenic organism, implying *Acanthamoeba* resistance to common disinfectants. Therefore, health officials should take more strict measures for the quality of water resources, especially the drinking water.

Isolates of amoebae that can be assigned to the genus *Naegleria* have been observed in a number of different studies across Iran. So far, a large number of isolates have been identified using molecular genetic methods. These isolates represent at least 12 species of *Naegleria* (*N. americana*, *N. australiensis*, *N. canariensis*, *N. carteri*, *N. clarki*, *N. dobsoni*, *N. fultoni*, *N. gruberi*, *N. lovaniensis*, *N. pagei*, *N. philippinensis*, *N. polaris*). The majority of these isolates have been assigned to the species *N. australiensis*. The next most frequently encountered species has been the isolates of *N. pagei* (Niyyati *et al.* 2012, 2015; Lasjerdi *et al.* 2015; Javanmard *et al.* 2017; Latifi *et al.* 2017, 2020; Feiz Haddad *et al.* 2019, 2020; Solhjoo *et al.* 2020; Mahmoudi *et al.* 2021). In this study, we identified seven isolates assigned to the genus *Naegleria*. Six of these were identified using the ITS1-5.8SrrNS-ITS2 region which occurs between the 18S rRNS and 28S rRNA genes of the ribosomal RNA transcriptional unit. The remaining isolate was identified based on the sequence of a portion of the 18S rRNS gene. Of the seven isolates in this study, three species were represented, with three isolates identified by genotyping as *N. pagei* (NT16, NP4, NER4), two as *N. australiensis* (NWR3, NNR1), and two as *N. gruberi* (NSR1, VSR4). All isolates of *Naegleria* in this study were identified to species level with high confidence (>99.5%). In other studies of *Naegleria* in Iran, more than 100 isolates, distributed among various species of *Naegleria*, have been obtained from 14 provinces of Iran (Ahvaz, Ardebil, Fars, Golestan, Guilan, Kerman, Khuzestan, Mazandaran, North Khorasan, Qazvin, Arak, Razavi Khorasan, Semnan, and Tehran) (Niyyati *et al.* 2012, 2015; Javanmard *et al.* 2017; Latifi *et al.* 2017, 2020; Feiz Haddad *et al.* 2020; Solhjoo *et al.* 2020; Mahmoudi *et al.* 2021).

The current study used sequences from the 18S rRNA gene to identify six isolates from the genus *Vermamoeba*. At present, all amoebae assigned to this genus are classified as either *V. vermiformis* or unidentified *Vermamoeba* sp. The genetic variability between the isolates assigned to *Vermamoeba* is much less than that found between the isolates of *Acanthamoeba* or species of *Naegleria*. Nevertheless, it does appear that the phylogenetic structure within *Vermamoeba* genus is in association

with several small variable regions of the 18S rRNA gene. Comparisons of 39 sequences from the DNA databases for the isolates that include almost complete 18S rRNA gene suggest that there may be at least six clusters within *V. vermiformis* (Figure 3).

Globally, the 18S rRNA sequences of more than 900 *Vermamoeba* isolates have been deposited in the nucleotide databases. Most of these are partial 18S rRNA sequences, and only less than 40 sequences cover most of the gene. A full length sequence of the gene would be approximately 1830 nucleotides. Because the variable regions are short in length and spread over the total length of the gene, there is no consensus on the 18S rRNA gene region that could be used to characterize the *Vermamoeba* isolates. Consequently, comparing sequences from different isolates in the DNA databases may be difficult to perform, since different deposited sequences may have only a minor or no overlap, representing different parts of the gene.

From the various studies in Iran, many sequences have been obtained from different provinces, such as Arak, Ardebil, Fars, Guilan, Kerman, Markazi, Semnan, Shiraz, and Tehran (Nazar *et al.* 2012; Armand *et al.* 2016; Atyabi *et al.* 2019; Feiz Haddad *et al.* 2019). With respect to the sample source, the sequences were obtained from soil or dust samples, as well as water resources.

In this study, the 18S rRNA gene of a single isolate, VWR2, was identified as associated with the genus *Vannella*. Previously, the 18S rRNA gene of four other isolates identified as belonging to the taxa of *Vannella* was obtained in Iran.

The sequence of VWR2 was compared to other sequences from *Vannella*, and a tree was constructed that included the closest sequences within GenBank, as well as other sequences of *Vannella* isolated from across the country. The phylogenetic tree obtained suggests that the VWR2 isolate is part of a taxon within the genus *Vannella* that has not been associated with any previously classified species (Figure 4(a)). This is the first such isolate in Iran, identified by molecular methods. The VWR2 isolate forms a clade with a second unclassified isolate of *Vannella* (JQ271724), previously obtained from the gills of a fish sampled in Spain (Dyková & Kostka 2013). In addition, two uncultured eukaryotic clones, obtained from the waters of the Rio Tinto, Spain, belong to this clade. Other Iranian isolates belonging to the genus *Vannella* have been identified as *V. persistens* (Nazar *et al.* 2012). By analyzing the isolate VWR2, molecular information has been gathered on four isolates cultured from Iranian sources and identified as representing the taxa in the genus *Vannella*. None of these previous isolates belong to the genetic clade to which VWR2 has been assigned (Figure 4(a)). All of *Vannella* isolates identified in this study are presented in Table 3 (Lasjerdi *et al.* 2011b; Nazar *et al.* 2012; Niyyati & Latifi 2017).

The sequence for the isolate NP10, involving the ITS1-5.8S rRNA-ITS2 region of the ribosomal rRNA transcript, is identified as representing the species *V. avara*. It has a 100% sequence match over a partial 318 bp fragment within the sequence length from the type strain of *V. avara*, CCAP CCAP1588/1AT, originally analyzed by De Jonckheere & Brown (2005). Ten other sequences from the isolates identified with *V. avara*, exist in the DNA databases, all of which are essentially identical. These include three other isolates obtained from Iran (Lasjerdi *et al.* 2011a, 2015; Rahdar *et al.* 2016). Six other isolates of more divergent forms of *Vahlkampfia* have been obtained in Iran, but the sequence of the ITS regions from the various taxa of *Vahlkampfia* show substantial differentiation, and a valid taxonomic tree including the non-*V.* avara forms is not provided because of difficulty in identifying homologous sites, even within the 5.8S rRNA sequence. In the current study, the FLA *S. polymorpha* was isolated in the country. It is noteworthy that there is no previous report of such amoeba in Iran. Up to now, at least 13 species of the amoeba have been identified from fishes, thermal spring, soil samples, and diarrheic stool of horse in different countries (Page 1969; Smirnov *et al.* 2007; Dyková *et al.* 2010; Geisen *et al.* 2014; Peglar *et al.* 2016; Borquez-Román *et al.* 2020), therefore, more accurate in-depth studies should be conducted to identify other FLAs in the country.

Table 3 | Molecular identification of *Vannella* isolates cultured from Iranian sources of reference strains used in the study

Accession no.	Taxon	Isolate name	Source	Reference
KX371576	*Vannella* sp.	Abe Ask	Hot spring	Niyyati & Latifi (2017)
AB525840	*Vannella persistens*	NHE(Vp)1-IR	Water – park fountain	Nazar *et al.* (2012)
AB525841	*Vannella persistens*	NHE(Vp)10-IR	Water – pond	Nazar *et al.* (2012)
JN009668	*Vannella* sp.	LN_HB3	Biofilm	Lasjerdi *et al.* (2011b)
MT621571	*Vannella* sp.	VWR2	Water	This study

CONCLUSION

The present study demonstrated the existence of different *Acanthamoeba* genotypes including type T4, subtype D, and type T5. In addition, three species of *Naegleria*, i.e. *N. australiensis*, *N. pagei*, and *N. gruberi* as well as *V. avara*, *V. vermiformis*, *S. polymorpha* and *Vannella* sp. were identified among the samples. The findings of the present study suggest the abundance of different FLAs in water resources of the province. This implies the need for application of appropriate *in vivo* and *in vitro* pathogenicity tests to clarify the risk of amoeba transmission to humans.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict.

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