The First Report for the invasion of Artemia franciscana Kellogg 1906in Tashk and Bakhtegan Lakes, Iran

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Abstract: Artemia, a small crustacean, with high commercial value is a valuable model organism for researchers. This creature by tolerating extreme range of different environmental conditions was dispersed to more than 600 and 18 sites over the world and Iran, respectively. Tashk and Bakhtegan Lakesare one of the natural parthenogenetic Artemia habitats in Iran. Due to occurrence of an unknown bisexual Artemia in Tashk Lake, the species of this un-endemic Artemia was inferred. In this regard, four different molecular markers as Na/K ATP-ase, 12S-16Sby PCR-RFLP technique and COI and HSP26 by sequencing and subsequent Genbank data were studied. The conducted analyses with emphasizing to ability of molecular techniques for identifying unknown species characterized the new population as A. franciscanain these two lakes. These analyses also revealed a molecular diversity between the sequenced genes with the data found in the Genbank.
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1. Introduction

Brine shrimp Artemiais one the important zoo-planktons in aquaculture. Having special physiological adaptabilities such as the capability of producing resistant cysts, a very effective osmotic pressure regulation system, Artemia has been adapted to the life in salty and very salty water. In fact, among Metazoan, Artemia is the only creature that can endure high degrees (up to 300 gr per liter) of saltiness (Browne, 1992). In addition to its great nutritional value (fatty acids and necessary proteins), Artemia is an appropriate research model in molecular and evolutionary experiments. This genus harbors two bisexual (comprising of six species) and parthenogenetic strains (Van Stappen, 2008). Each of these populations has been adapted to different climates and habitats based on their molecular characteristics and physiologic. In 1995, the first official paper has referred to only 80 areas as Artemia habitats (Abonyi, 1915), while around 600 geographical regions has been introduced as Artemia habitats in the latest list of the year 2002 (Van Stappen et al, 2002). The fast dispersion of Artemia around the earth and also the discovery of new regions through developments in specific researches is the reason for the change in dispersion list of Artemia. Likewise, the extinction of Artemia in some regions (such as Lamington region in England and Shurabil Lake in Iran) can also make changes in the habitat and dispersion list of Artemia (Van Stappen, 2008).

So far, 18 different sites of Artemia have been reported in Iran (Abatzopoulos et al, 2006; Asem et al, 2009). All of these sites, except Urmia Lake, have the endemicparthenogenetic Artemia. The geographical position of Bakhtegan Lake, the lake in Fars province of Iran, is 53º/ 50`N and 29º/ 40`E and it is located at a distance of 80 Km from the east of Shiraz. Tashk Lake, the other lake in Fars province,in geographical position of 53º/ 50`N and 29º/ 60`Eis located at a distance of 50-160 Km from the east of Shiraz (Agh, 2007).Tashk lake was previously introduced as Brackish Lake that had connection to Bakhtegan Lake via a connective bridge. Therefore, the saltiness of water has been increased which has made this region a biologically suitable place for parthenogenetic Artemia (Agh, 2007). The temperature of these lakes which has adaptability to thermo iso-plates of the region is between less than 5 degrees and more than 40 degrees and even reaches to 45ºC in sloughs (Alamdari, 1987). There is no idea about the first report ofparthenogeneticArtemiain these lakes, but several reports has been recorded about the existence of parthenogeneticArtemia in these lakes in 1980, 1984, 2002, and 2007 (Geddes, 1980; Browne et al, 1984; Van Stappen et, 2002; Agh, 2007).

A. franciscana, as one of the very piotatic bisexual species in the world (with great frequency74

sites) is the dominant Artemiaspecies in Great Salt Lake, U.S. (Van Stappen, 2002). In fact, the high and fast adaptability capacity of this species in new ecosystems has caused it to be selected for commercial Pond production industry in most of the countries (Amat et al, 2007). The molecular and physiological capacity of A. franciscana to a wide domain of ecological conditions and also the high growth velocity and reproduction potential of this Artemia has caused it to be easily dispersed around the world.

The studies have indicated that the molecular adaptability power of the Artemia (speciallyA. franciscana) is the main reason for this wide dispersion. It is inferred that some genes of heat shock proteins (HSPs) or mitochondrial genes can show these molecular changes quickly in a way that they have been considered as the successful adaptability criterion to new conditions. The researches have indicated that these chaperonproteins has a vital role in increasing physiological adaptability of the living creature facing with unpleasant biological conditions (Clegg et al, 2000). These proteins change a lot in Artemia which have experienced a successful period in a new and different habitat (Bossier et al, 2009). It has been proven that other proteins of this family also have significant role in stress tolerance and creating molecular adaptability (Federand and Hofmann, 1999; Prohaszka and Fust, 2004).

In order to investigate the species diversity of Artemia, different methods have been introduced including different types of morphometric and molecular techniques. Mitochondrial genome performance has been confirmed as an appropriate option for identification of indefinite taxon, analysis of species creation and even identification of Artemia population (Avise, 2000; Bossier et al, 2004). Likewise, a molecular method has been presented recently in order to recognize the level of parthenogenetic from bisexuality level which can be used for the separation of these two levels (Manaffar et al, 2011).

Since A. urmiana is the only endemic bisexual Artemiapopulation observing a huge population of bisexual Artemia in Tashk and Bakhtegan Lakes, the identification of level and species of the observed Artemia was considered as the main goal of the present study.

2. Material and Methods

Artemia cysts were collected from four different parts of Tashk and Bakhtegan Lakes which located in Fars provincein July 2011(Figure 1). The cysts were hatched after rinsing and purification in standard laboratory conditions in Urmia Lake water which was diluted to the saltiness of 35 gr per liter, a temperature of 27º, pH=8, and equipped with aeration systems and enough light (LavensandSorgeloos, 1996). Instar I were naupliitransferred into one liter bottles containing 80 gr per liter saline water in 4 repetitions and reached for 20 days with mixture of enriched yeast with fatty acid, and unicellular Alga Dunaliellasalina (Coutteau et al, 1992).

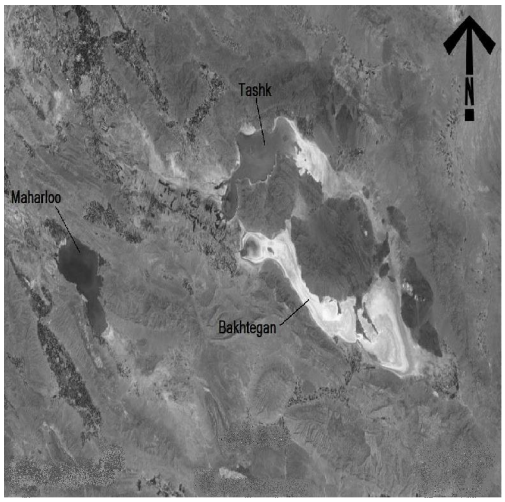

Figure1- Location of Tashk and Bakhtegan Lakes which are near the Maharloo Lake.

2.1. Molecular analysis

The DNAwere extracted from cyst individuals of cyst using Chelex method (Estoup et al, 1996). In order to extract DNAs of the mature Artemia samples, the CTAB method was used (Doyle and Doyle, 1990).

In order to investigate the species diversity of unendemic ofArtemiapopulation in Tashk and Bakhtegan Lakes, 20 samples (cyst and mature Artemia) were used. To this end, genetic parts of Na/K ATPase (a part of core genome for identification of bisexually or parthenogenetic) (Manaffaret al, 2011) and 12s-16s (a part of mitochondrial genome for identification of Artemiaspecies(Bossier et al, 2004) were used.

PCR program and also used primers have been summarized in Table 1. The PCR product were analyzed in all experiments using the electrophoresis of 2% Agarose gel and photographed by Gene Flash gel. Documentation system after approving the quality of PCR product in RFLP technique, in order to characterize strain and species of Artemia the exon-7 fragment of Na- K ATPase gene and the 12s-16s gene fragment were digested by Tru1I and Hpa II restriction digestion enzymes andthey were analyzed on 2% Agarose gel. Fragments of HSP26 and COI genes were also sequenced by Sina Gene Company. (Folmeret al, 2006).

Table 1. primers and PCR program

	Forward and reverse primers	programPCR
Na/K ATPase	-cca-aac-gta-tgg-ctt-c-3' 5'-cag -agc-acg-act-gca-aga-3' 5'-gaa-ttc	94°C 2 Min 35 cycle (94°C 2 Min, 56°C 25 Sec, 72°C 1 Min) 72°C 3 Min
COI	-atc-ata-aag-ata-tgt-g-3' 5'-ggt-aca -tga-cca-aaa-aat-ca -3' 5'-taa-act-tca-ggg	95°C 3 Min 33 cycle (95°C 1Min, 50°C 1 Min, 72°C 1.20 Min) 72°C 10 Min
12S-16S	-cca-aac-gta-tgg-ctt-c-3' 5'-cag -agc-acg-act-gca-aag-3' 5'-gaa-ttc	95°C 2 Min 34 cycle (94°C 1.15 Min, , 52°C 1 Min, 72°C 2 Min) 72°C 4 Min
HSP 26	-gga-gaa-gaa-tga-gaa-g-3' 5' -tgg-acg-tgt-cca-tat-tc-3' 5'-tct-ctt	94°C 2 Min 35 cycle (94°C 15 Sec, 54°C 25 Sec, 72°C 30 sec) 72°C 4 Min

3. Resalts

The analysis of the implied a 700 bpwhich can be related to cytochrome oxidase gene and 217 bp fragment which can be related toheat shock gene of HSP 26 indicated that the sequence of the PCR product also checked ingenbank data base by Blast Run. Enzymatic cutting of piece number 280 bp of core genome which is produced by Tru1I showed that the created cutting pattern in Artemiasamples of Tashk and BakhteganLakes, are exactly similar to the pattern of Bisexual Artemia (Figure 2).

In order to analyze the species of Artemia, the enzymatic cutting of piece number 1500 bp of 12s-16s mitochondrial genome was used. Enzyme cutting by the limitative HpaII enzyme created the profile figure of A. franciscana (Figure 3). Analysis of the sequence results using Genbank internet search by Blast software emphasized that the above bisexual Artemia belongs to the bisexual American Artemia in molecular structure of this gene in comparison to genes recorded in gene bank.

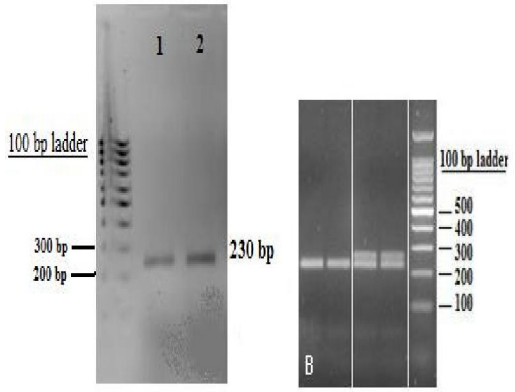

Figure 2- Agarose gelofrestriction enzymefragment of280bpregion ImageA):230bpbandwas produced from Brine Shrimpsamples (1: Tashk Lake , 2: Bakhtegan Lake)

Image B): taken fromthe referencegel. Manaffaret al, 2011 pattern,cuttwobandsofparthenogeneticArtemia and Single-band patternisindicative ofbisexualArtemia.

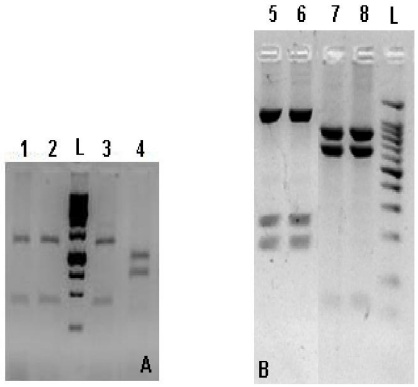

Figure 3. Agarose gelelectrophoresisof12S-16Sfragment of1500bpregionof theenzyme restriction by the enzymeHpaII.images were : Image A) Restriction enzymefragmentabovetheexotic Artemia in Tashk (sample 1) Bakhtegan (sample 2) control samples (3.A. franciscanaand 4.A. sinica)with 1 Kb marker. Referencegelfrom (Bossier et al, 2004) samples 5 and 6 related to A. franciscana , samples 7 and 8 related to A.sinica with 100 bp ladder.

4. Discussion

The present study is considered as the first report about the observation of A. franciscanain Tashk and Bakhtegan Lakes, the lakesof Fars province. Some scientific analysis had already confirmed the existence of A. franciscana in Iran Plateau (natural habitat of Artemia) (Manaffar et al, 2008). In present study, it was attempted to use new technique which forecast genetic distinction patterns and extend diversity in several creatures with little error (Chow et al, 2006). The merits of the used molecular methods for strain and species identification of Artemia was already approved. But, setting sequences of cytochrome oxidase and also small heat shock protein and the analysis of its sequence with samples of gene bank indicates a genetic difference of more than 30%between tested Artemia and existed samples from the above-mentioned website.Of course, the effect of other phenomenon such as Founder effect and genetic drift should not be neglected. Regardless it should be noted that this new population have managed to adapt themselves to Tashk and Bakhtegan Lakes successfully.

However, with regard to the probable method of transferring this species of Artemia to Tashk and Bakhtegan Lakes, it should be mentioned that although

the Artemia egg is generally transferred by wind and aquatic birds (Persoone and Sorgeloos, 1980), but from 1970s until now, humans have been responsible for dispersion of Artemia, speciallyA. franciscana. Regarding to existence of A. franciscana in Maharloo Lake and also close distance ofthese three lakes, it is suggested that A. franciscana have been transferred from Maharloo Lake to these Lakes by birds.

Artemia has shown the highest level of phonotypical and genetic flexibility and with a very high reproduction speed, quick adaption to difficult conditions and molecular adaptability with environment it has been dispersed successfully in Asia, Europe, and America and has often caused the elimination of local Artemia (Browne et al., 1988; Kappas et al, 2004; Pogge, 2004). With the transfer of this species in 1970s to the island of Pacific Ocean and Brazil, it was announced that the above species will probably substitute other species including A. Salina (Van Stappen et al, 2002). However, the first report about the offensive power of A.franciscana is related to Camara in 2001 who has reported that this species is located in Rio Grand do Norte in northern Brazil. Other similar report have been recorded in Portugal (Amat et al, 2005), France (Thiery, 1992), Egypt (Triantaphyllidiset al, 1998), Italy (Mura et al, 2004), Spain and Morocco (Amat et al, 2007). Research done by Kappas in 2004 on non-local A. franciscana in Vietnam indicated that there are significant differences between local American A.franciscana and commercial Vietnamese Artemia which had been transferred to Vietnam 10 years ago.

It should be noted that the permanent settlement of non-local Artemia population and the development in dispersion of A.franciscana around the world have been one of the note worthy issues in recent years (Abatzopoulos et al, 2006; Amat et al, 2005, green et al, 2005; Mura et al, 2006). At present, A. franciscana is considered as the dominant population in the west of Mediterranean Sea, Slat mines in Portugal, Mediterranean beach of France, and the Cadiz Gulf in Spain (Amat et al, 2005). This study has proved that A. franciscana has been able to eliminate local population within a few years (Amat et al, 2005; Green et al, 2005; Amat et al, 2007). Researches in Iran have also indicated that A. franciscana has managed to become a dominant population in Nogh Pool of Rafsanjan (which was a natural habitat for parthenogenetic Artemia) in a contest with parthenogeticArtemia (Abatzopouloset al, 2006).

According to the results of the present study the found genetic diversity in A. franciscana and also its potential capability, it is expected that this bisexual species may be able to completely eliminate local parthenogeneticArtemia population in the future.

References

1. Abatzopoulos, T.J., Agh, N. Van Stappen, G., Razavi Rouhani, S.M., Sorgeloos, P., 2006. Artemia sites in Iran. Journal of the Marine Biological Association of the United Kingdom 86, 299-307.
2. Abonyi, A., 1915. Experiment elle Datenzum Erkennen der Artemia- Gattung. Zeitschrift fur WissenschaflicheZoologie 114, 95-168.
3. Agh, N., 2007. Characterization of Artemia population from Iran. PhD thesis. Ghent University, Belgium, P 8-16.
4. Alamdari, A., 1987. Limnology and preserving of ecologic dynamic of Bakhtegan wetland.
5. Amat, F., Hontoria, F., Ruiz, O., Green, A.J., Sanchez, M.I., Figuerola, J., Hortas, F., 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean, Biological Invasions 7, 37-47.
6. Amat, F., Hontoria, F., Navarro, J.C., Vieira, N., Mura, G., 2007. Biodiversity loss in the genus Artemia in the Western Mediterranean Region, Limnetica 26, 387-404.
7. Asaadi, R., Sardashti, M., Karami, M., Golzar, Y., 2011. The visit report of Tashk, Maharlo and Bakhtegan Wetlands. Under supervision of Ministry of Power, 4 pp.
8. Asem, A., Atashbar, B., Rastegar-Pouyani, N., Agh, N., 2009. Zoology in the middle east 47, 1-4.
9. Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA, 447 pp.
10. Bossier, P., Xiaomei, W., Catania, F., Dooms, F., Van Stappen, G., Naessens, E., Sorgeloos, P., 2004. An RFLP database for authentication of commercial cyst samples of the brine shrimp Artemia spp. (International Study on Artemia LXX). Aquaculture 231, 93-112.
11. Bossier. P., Gajardo, G., Beristain, P., 2009. Species-specific RFLP pattern in the Heat Shock Protein 26 gene a single–locus tool for species identification and experimental testing of habitat-induced isolation in the New world Artemia species. Molecular Ecology Resources. Vol.10, pp. 229-231.
12. Browne, R.A., Sallee, S.E., Grosch, D.S., Sergreti, W.O. Purser, S.M., 1984. Partitioning genetic and environmental components of reproduction and life span in Artemia. Ecology 65, 949-960.
13. Browne, R.A., Davis, L.E., Sallee, S.E., 1988. Temperature effects on life history traits and relative fitness of sexual and asexual Artemia. Journal of Experimental Marine Biology and Ecology 124, 1-20.
14. Browne, R., 1992. Population genetics and ecology of Artemia: Insights into parthenogenetic reproduction. Elsevier Science Publishers Ltd. Trends in Ecology and Evolution 7, 232-237.
15. Camara, M.R., 2001. Dispersal of Artemia franciscana Kellogg (Crustacea; Anostraca) populations in the coastal saltworks of Rio Grande do Norte, northeastern Brazil, Hydrobiologia 466, 145-148.
16. Chow, S., Suzuki, N., Imai, H., Yoshimura, T., 2006. Molecular species identification of spiny lobster phyllosoma larvae of the genus Panulirus from the Northwestern Pacific Marine Biotechology 8, 260-267.

17. Clegg, J.S., Jackson, S.A., Van Hoa, N., Sorgeloos, P., 2000. Thermal resistance, developmental rate and heat shock proteins in Artemia franciscana from San Francisco Bay and southern Vietnam. Journal of Experimental Marine Biology and Ecology 252, 85-96.
18. Coutteau, P., Brendonck, L., Lavens, P., Sorgeloos, P., 1992. The use of manipulated baker'syeast as an algal substitute for laboratory culture of Anostraca, Hydrobiologia 234, 25-32.
19. Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13-15.
20. Estoup, A., Largiader, C.R., Perrot, E., Chourrout, D., 1996. Rapid one-tube DNA extraction for reliable PCR detectionof fish polymorphic markers and transgenes. Molecular marine biology and biotechnology 5, 295-298.
21. Feder, M.E., Hofmann, G.E., 1999. Heat Shock Proteins. Molecular Chaperones, and the Stress Response. Annual Review of Physiology 61, 243-282.
22. Folmer, O.M., Black, W., Hoeh, R., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Mulecular Marine Biology and Biotechnology 3, 294-299.
23. Geddes, M.C., 1980. The brine shrimp Artemia and Parartemia in Australia. In: Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds.), The Brine Shrimp Artemia, Vol. 3, Universa Press, Wetteren, Belgium, pp. 57-65.
24. Green, A.J., Sanchez, M.I., Amat, F., Figuerola, J., Hontoria, F., Ruiz, O., Hortas, F., 2005. Dispersal of invasive and native brine shrimps Artemia(Anostraca) via waterbirds. Limnology and Oceanography 50, 737-742.
25. Kappas, I., Abatzopoulos, T.J., Van Hoa, N., Sorgeloos, P., Beardmore, A.J., 2004. Genetic and reproductive differentiattion of Artemia franciscanain a new environment. Journal of Marine biology 146, 103-117.
26. Kellogg, V.A., 1906. A new Artemia and its life condition. Science 24, 594-596.
27. Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of live food for Aquaculture. Laboratory of Aquaculture and Artemia Reference Center, University of Gent, Belgium. Published by: Food and Agriculture Organization of the unitednations (FAO Fisheries Technical paper). P 1-295.
28. Manaffar, R., Falahati, A., Moshtagiyan, A., Mosavi, S.M., Atashbar, B., Asem, A., 2008. First report for existence of Artemia franciscana coexistence with endemic parthenogenetic Artemia population in inside of the Lake Maharlu, Iran. Conference of world aquaculture, Bussan, Korea.
29. Manaffar, R., Zare, S., Agh, N., Abdolahzadeh, N., Soltanian, S., Sorgeloos, P., Bossier, P., Van Stappen, G., 2011. SNP detection in Na/K ATP-ase gene a1 subunit of bisexual and parthenogenetic Artemia strains by RFLP screening. Molecular Ecology Resources 11, 211-214.
30. Mura, G., Amat, F., Abatzopoulos, T.J., Moscatello, S., 2004. First record of Artemia franciscana in an Italian saltwork, Fifth International Large Branchiopod Symposium, Book of abstracts Toodyay, Western Australia, pp. 35-36.
31. Mura, G., Kappas, I., Baxevanis, A.D., Moscatello, S., D'Amico, Q., Lopez, G.M., Hontoria, F., Amat, F., Abatzopoulos, T.J., 2006. Morphological and molecular data reveal the presence of the invasive Artemiafranciscanain Margherita di Savoiasalterns (Italy). International Review. Hydrobiology 91, 539-554.
32. Persoone, G., Sorgeloos, P., 1980. General aspects of the ecology and biogeography of Artemia. In: The brine shrimp Artemia. Vol.3. Ecology, Culture, Use in Aquaculture, Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds.), Universa Press, Wettern, Belgium, pp. 3–24.
33. Pogge, C., 2006. Bio 25, Sanfrancisco bay ecology, brine shrimp, P.1-8.
34. Prohaszka, z., Fust, G., 2004. Immunological aspects of heat- shock proteins- The optimum stress of life. Molecular immunology 41, 29-44.
35. Qui, Z., Bossier, P., Wang, X., 2006. Diversity structure and expression of the gene for p26 a small heat shock protein from Artemia. Genomics 88, 230-240.
36. Triantaphyllidis, G. V., Abatzopoulos, T.J., Sorgeloos, P., 1998. Review of the biogeography of the genus Artemia (Crustacea, Anostraca), Journal of Biogeography 25, 213-226.
37. Thiéry, R., Robert, F., 1992. Bisexual populations of the brine shrimp Artemia in Sète-Villeroi and Villeneuve saltworks (Languedoc, France), International Journal of Salt Lake Research 1, 47-63.
38. Van Stappen, G., 2002. Zoogeography. In: Abatzopoulos, Th.J., Beardmore, J.A., Clegg, J.S.,Sorgeloos, P. (Eds.). Artemia: basic and applied biology. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 171-224.
39. Van Stappen, G., 2008. Artemia biodiversity in Central and Eastern Asia. Ph.D Thesis. University of Ghent, Ghent, Belgium, P 1-179.

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