

Morphological and molecular characterization of various *Artemia* strains from tropical salt pans in South East Coast of India

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Abstract: The present study documents the morphological and molecular characteristics of *Artemia* strains in four main ecological regions from South East Coast of India. Samples from tropical salt pans such as Kelambakkam (KBM), Vedaranyam (VDM), Tuticorin (TCN) and Thamaraikulam (TKM) and reference samples from GSL strain (Great Salt Lake, Utah) were analysed. Biometry of cysts, furcal morphology and molecular genetic relatedness using the technique of random amplified polymorphic DNA (RAPD-PCR) finger printing analysis were done with four *Artemia* strains. The biometry results revealed that the KBM cyst diameter was similar to GSL cyst and compared to other strains. Scanning electron micrographs (SEM) of hydrated *Artemia* cysts showed a smooth outer membrane with granular surface topography without much variation. However, the SEM structures of fractured cysts showed variation in the inner architecture patterns of egg membranes. The discrimination on furcal fin shape and its numbers of setae were more evident to distinguish *Artemia* strains studied. The TKM strain showed wide furcal groove compared to other strains. RAPD-PCR analysis showed consistent genetic differences between the VDM and TKM strains. The DNA polymorphisms were evident in all the *Artemia* strains examined and the highest percentage of polymorphic bands was found in TKM and TCN *Artemia* strains. The obtained results obviously showed that, within the collection of South Indian *Artemia* cyst samples examined, two different groups seem to exist. The morphological and molecular analysis revealed a greatest genetic difference between the *Artemia* strains which provide the genetic relatedness and the specific status of *Artemia* strains confined to South India.

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1. INTRODUCTION

The genus *Artemia* comprises a complex of bisexual species defined by the criterion of reproductive isolation of a large number of parthenogenetic and bisexual strains under the binomen *A. parthenogenetica*, composed of diploid and polyploidy strains for taxonomic convenience (Sun *et al.*, 1999). They have been recorded in over 600 coastal and inland lakes worldwide (Van Stappen, 2002). In morphological studies, the specific challenge exists in the selection of morphological character useful for description of a population or species. Several authors have used different criteria of morphological traits for strain characterization (Gilchrist, 1960 and Amat, 1980).

Biometry of cysts has been reported to be one of the important characteristics to mark the different strains of *Artemia* (Tejeda, 1987). Variations in the cyst size and thickness of outer layer would correlate the strain characterization. Geographical and physical barriers between con-specific strains must exist for reproductive isolation to evolve as a byproduct of the genetic differences (Gajardo *et al.*, 1998). Triantaphyllidis *et al.* (1997) conducted a scanning

electron microscopic study of bisexual *Artemia* populations, which revealed that populations representing the species *A. franciscana*, *A. persimilis*, *A. urmiana* and *A. sinica*. Besides recently described species from Kazakhstan have a pair of spine-like outgrowths at the basal parts of their penis, whereas populations from southern Europe and North Africa (Mediterranean populations) lack these spine-like outgrowths. Characterization of various strains of brine shrimp *Artemia* is essential to enhance its potential in aquaculture (Abatzopoulos *et al.*, 1986). However, knowledge of their morphological characteristics, biochemical variation and molecular differentiation (both genotypic and phenotypic) of a particular batch of cysts and nauplii can greatly increase the effectiveness of their usage in fish or shrimp hatchery (Browne and Bown, 1991; Pilla and Breadmore, 1994).

In recent years the use of molecular markers has gained popularity. *Artemia* inter and intra-specific biodiversity have been studied using a variety of techniques (Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) using either strains or

pooled samples for analysis. The description of the species *A. tibetiana*, the increasing availability of samples from Central and Eastern Asia have intensified the study of the phylogeny of the genus *Artemia*. Within this general framework, ample attention has been given to unravel the genetic relationships between the bisexual species from continental Asia and from the Americas (*A. franciscana* and *A. persimilis*), last but not least their link with the parthenogenetic lineage in the genus (Sun *et al.*, 1999). One of the molecular tools early employed to answer a wide range of questions regarding speciation processes has been mtDNA sequencing and RAPD-PCR analysis. Considering the pronounced tendency of the development of local adaptations, genetic and molecular surveys have been highly informative for the genus (Bossier *et al.*, 2004 and Qiu *et al.*, 2006).

Therefore, the present paper describes the biometry of cyst, furcal morphology and molecular genetic characteristics of four *Artemia* strains confined to four main ecological regions in South East Coast of India. Although information on the distribution and biometry of *Artemia* strain from South India is limited, studies on the genetic relatedness of the *Artemia* strains are lacking. Hence, the present study investigated the cyst characteristics, furcal morphology and molecular genetic variation of South Indian *Artemia* strains.

2. MATERIALS AND METHODS

2.1 Sample collections

Sample of adult *Artemia* and cysts were collected from Kelambakkam (KBM; 10° 01' N–79° 07' E), Vedaranyam (VDM; 08° 59' N–78° 50' E), Tuticorin (TCN; 08° 50' N–78° 08' E) and Thamarakulam (TKM; 08° 04' N–77° 68' E). Both adult brine shrimp and cysts were collected and transported to the laboratory in plastic containers with habitat water. For molecular studies, the adults as well as cysts were washed with double distilled water and stored at –20°C until use.

2.2 Biometry of cysts

The cyst diameter of each strain was done using the microscope (Leica, DM2500, Germany) equipped with pre-calibrated software. The cyst diameter of each strain was measured in dried as well as hydrated cysts. As many as 100 cysts were taken randomly and used for measurements to have valid statistical inference.

2.3 Scanning Electron Microscopy

Scanning electron microscopic studies were carried out on cysts of different *Artemia* strains as described by Gilchrist (1978). For scanning electron microscopic studies, the cysts were then immersed in

10% formaldehyde for 30 minutes to remove the micro organisms and debris attached on the surface of the shell. The cysts were then fixed in 2.5% glutaraldehyde prepared in cacodylate (sodium phosphate) buffer adjusted to pH 7.4 for several hours. Then the cysts were post-fixed in 2% osmium tetroxide for 12 hours at 4°C. After post-fixation, cysts were rinsed in distilled water for few minutes and dehydrated in graded alcohol series. Then the cysts were subjected to critical point drying and glued on to standard mica squares (1cm²), mounted on metal stubs. Whole cysts and fractured cysts were gold sputter, coated (Denton sputter Coater Desk II), viewed under scanning electron microscope (Leica Cambridge, UK) and photomicrographs were taken at various magnifications.

2.4 Assessment of furcal morphology

Furcal morphology of different *Artemia* strains were analysed using a microscope (Leica, DM2500, Germany). Furca of each strain was removed carefully and mounted on microscopic the glass slide. The furcal characters such as furcal shape, number of setae on furca, furcal groove and furcal length of each strain were scored.

2.5 Molecular methods

2.5.1 Extraction of genomic DNA

The adult *Artemia* genomic DNA was extracted and used for genetic analysis as described by Bossier *et al.* (2004) with slight modifications. Frozen tissues of adult *Artemia* (100 mg) was kept at room temperature for 2 minutes to be slightly softened without thawing the tissue completely. The samples were then pulverized on ice with a sterile pestle directly into a 1.5 ml eppendorf tube. To this, 600 µl of 2X CTAB buffer (Tris-HCl 100 mM, pH 8.0, NaCl 1.4 M, EDTA 20 mM, CTAB 2 % and 0.1 mg/ml proteinase K) was added and the mixture was incubated for 30 minutes at 60°C and then centrifuged at 14000 × g for 15 minutes. The supernatants were extracted with phenol/chloroform (1:1), centrifuged at 14000 × g for 5 minutes. Extracted again with chloroform-isoamyl alcohol (24:1), and centrifuged at 14000 × g for 5 minutes and then the supernatant was collected using sterilized 200 µl micropipette tips. To this, 650 µl of water and 1300 µl of ice-cold isopropanol were added. The precipitated DNA was centrifuged at 14,000 × g for 15 minutes and resuspended in double distilled water. The pelletized DNA samples were used to remove the RNA, and then it was centrifuged at 15,000×g for 5 minutes. The collected supernatants were incubated with RNase (0.2 mg/ml) at 37°C for 15 minutes it was added with 1.5 ml of 100 % ethanol and the DNA was allowed to precipitate for two hours at –20°C. This was centrifuged again at

14000 × g for 15 minutes. The DNA pellet was washed with 70 % ethanol and left to air dry at room temperature. The dried DNA pellets were resuspended in TE buffer and stored at -80°C for further analysis.

2.5.2 RAPD-PCR analysis

The polymerase chain reaction was performed in a Thermal Cycler (ASTEC™, PC-818 Programmable Thermal Controller) to produce multiple copies of DNA. The genomic DNA extracted for the different strains such as KBM, VDM, TCN, TKM and GSL reference strain were subjected to RAPD-PCR analysis. A total of twenty random primers (RAPD kit: OPK-01 to OPK-20) were tested for RAPD-PCR analysis. The sequences of the primers (Sigma, Bangalore) used are given in Table 1.

Table 1. RAPD primer sequences

Primers	Sequence 5' to 3'	Sequence 5' to 3'
OPK-01	CATTCGAGCC	AATGCCCCAG
OPK-02	GTCTCCGCAA	TGGCCCTCAC
OPK-03	CCAGCTTAGG	GGTTGTACCC
OPK-04	CCGCCCAAAC	CCCGCTACAC
OPK-05	TCTGTTCGAGG	CTCCTGCCAA
OPK-06	CACCTTTCCC	GAGCGTCGAA
OPK-07	AGCGAGCAAG	CCCAGCTGTG
OPK-08	GAACACTGGG	CCTAGTCGAG
OPK-09	CCCTACCGAC	CACAGGCGGA
OPK-10	GTGCAACGTG	GTGTCGCGAG

The RAPD-PCR reaction was performed with twenty primers (OPK-01 to OPK-20). The RAPD-PCR was carried out with each primer in a total reaction volume of 50 µl containing 5.0 µl of PCR buffer (10 mM Tris-HCl at pH.8.3, 50 mM KCl, 2.5 mM MgCl₂), 0.5 µl of each dATP, dGTP, dTTP and dNTP, 0.5 pmol of primers and 2.0 U of *Taq*-DNA polymerase. All the above components were made up to 50 µl in a 100 µl PCR tubes. The thermal cycling amplification conditions were as follows: the initial denaturation at 94°C for 5 minutes, final denaturation at 94°C 1 minute, annealing at 35°C for 1 minute and primer extension at 72°C for 2 minutes and final extension at 72°C for 7 minutes. Amplified products were resolved by 2 % agarose gel electrophoresis performed in 1X TAE buffer and stained with ethidium bromide. The agarose gel profiles were photographed using the gel documentation system (Gel Doc™, BIO-RAD).

2.6 Data analysis

Results obtained in cysts biometry data were analyzed using SPSS (Version 17.0) for analysis of variance. The phylogenetic analysis was conducted using in PHYLIP 3.6. Phylogenetic relationships

were estimated using neighbor-joining (NJ) analysis. NJ analysis was performed using Jukes-Cantor and pair-wise deletion options. Trees based on genetic distances were constructed by the NJ method and cluster analyses on DNA banding patterns between the strains were calculated.

3. RESULTS

3.1 Biometry of *Artemia* cysts

Dry cyst of KBM strain measured around 228.43±9.82 µm in diameter comparable to reference strain of GSL (225.93±2.78 µm) (Table 2).

Table 2. Biometry of *Artemia* cysts

Strains	Cyst diameter (µm)		F-ratio	Sig.
	Dry cysts	Hydrated cysts		
KBM	228.43±9.82	256.30±4.32	10.994	(P<0.005)
VDM	247.73±5.77	267.16±3.69	12.847	(P<0.003)
TCN	245.02±6.67	276.48±3.99	14.936	(P<0.003)
TKM	256.20±5.35	285.80±3.28	14.590	(P<0.003)
GSL	225.93±2.78	243.28±4.39	14.220	(P<0.003)

The dried cysts of VDM, TCN and TKM strains measured to 247.73±5.77 µm, 245.02±6.67 µm and 256.20±5.35 µm in diameter respectively. After hydration, the cyst diameter of GSL increased to 243.28±4.39 µm, whereas the KBM, VDM, TCN and TKM cysts measured to 256.30±4.32 µm, 267.16±3.69 µm, 276.48±3.99 µm and 285.80±3.28 µm respectively. Interestingly, the biometry results revealed that the KBM cyst diameter was similar to GSL reference cyst. The diameter of different *Artemia* cysts varied significantly among the *Artemia* strains studied (P<0.005).

3.2 Morphology of cysts

Scanning electron micrographs of hydrated *Artemia* cysts showed smooth outer membrane with granular surface. There is no specialized area interpreted as a micropylar region seen on the outer surface of the cyst. Before hydration, the cyst resembles a hardened structure. After hydration in seawater (35 g L⁻¹), the spherical cysts assume a much larger size. This transformation is the result of rapid water absorption of the cyst. Scanning electron micrographs of cysts of four different *Artemia* strains clearly indicated that the surface topography is smooth and without any ornamentation. An in-depth study on the egg membranes clearly indicated the subtle variation in architecture of alveolar layer and thickness of the egg membranes of the four strains studied. The cross section of the cyst shows the presence of outer tertiary layer and an innermost cuticular layer. The tertiary layer consists of an outer cortex and an inner alveolar layer (Figure 1).

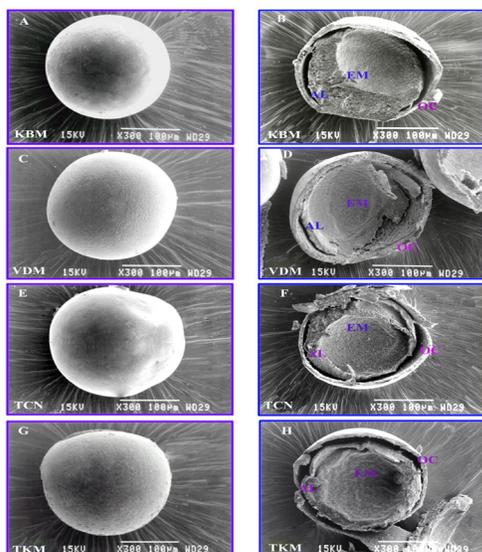


Figure 1. Scanning electron micrographs of cysts

All the cysts showed an outer thick cortex membrane and an inner alveolar layer which both constitute the tertiary layers. The embryonic mass is seen covered by membrane within the embryonic cuticle. The innermost membrane is the embryonic cuticle which encircles the embryonic mass.

3.3 Furcal morphology *Artemia* strains

The furca of each strain showed that natatory setae of 6 to 11 on each side of the distal exite and its setules were very lengthy in nature. Furca of KBM strain showed as many as 8 to 11 setae on its distal exite and it was interrelated to TCN strain (Figure 2).

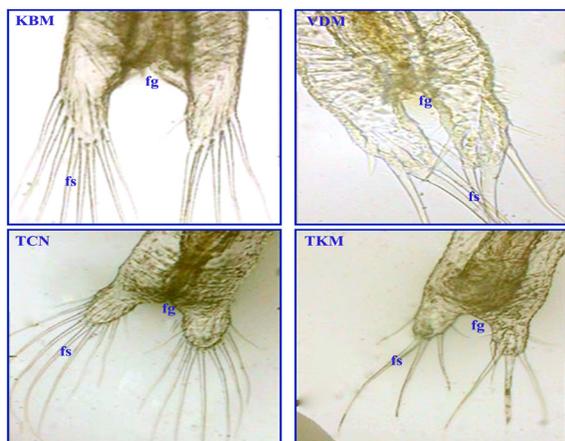


Figure 2. Furcal morphology of *Artemia* strains

In comparison, the TCN strain had the much wider and deeper furcal groove. However, the TKM strain showed wide furcal groove when compared to KBM strain. At the same time, the end part of the telson segment showed considerable variation between KBM and VDM strains.

3.4 RAPD-PCR profiles

The extracted genomic DNA from different *Artemia* strains was screened using random primers by polymerase chain reaction. A total of twenty RAPD primers (OPK-01 to OPK-20) were tested for their ability to provide scorable DNA banding patterns. Of the various primers used, OPK-07, OPK-08 and OPK-20 yielded a clear and consistent DNA banding pattern. Among the twenty RAPD primers screened, three primers showed clear and distinct DNA banding pattern. Of which, the primer OPK-20 generated an enhanced degree of DNA polymorphism over the other than two primers. The RAPD-PCR results of all genomic DNA isolated from different *Artemia* strains were compared with each other strains and the presence of common bands were also scored.

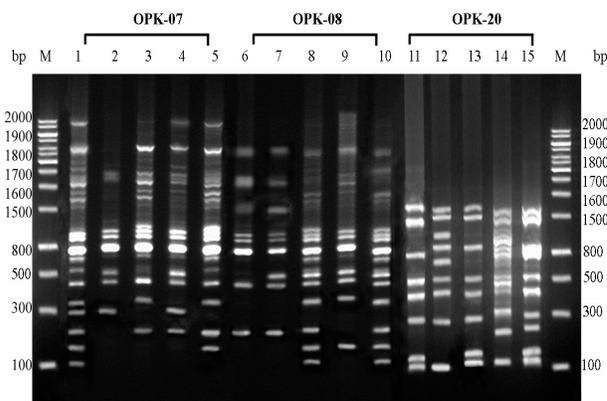


Figure 3. RAPD-PCR amplification products using primers OPK-07 (1–5), OPK-08 (6–10) and OPK-20 (11–15) for the five *Artemia* strains, Great Salt Lake (GSL), Kelambakkam (KBM), Vedaranyam (VDM), Tuticorin (TCN) and Thamaraiikulam (TKM).

As many as 13 bands were scored for GSL reference strain. In KBM strain, out of 5 bands scored (RAPD-PCR profile generated by using primers OPK-07 and OPK-08), 3 bands were found to be monomorphic and 2 bands as polymorphic (Figure 3). In VDM strain, among the 11 bands scored, 7 bands were monomorphic and remaining 4 bands were polymorphic. Out of 9 bands generated in TCN strain, 6 bands were monomorphic and the 3 bands were polymorphic. The TKM strain generated 16 bands, of which 10 bands were monomorphic and the remaining 6 bands were polymorphic. The phylograms derived from the RAPD-PCR profiles of different *Artemia* strains (primer OPK-07) generated maximum of four clusters than the other primer OPK-20. In the KBM strain of 12 bands (RAPD-PCR profile generated by primer OPK-20), 6 bands were monomorphic and the 6 bands were also polymorphic. In the VDM strain, of 8 bands scored, 4

bands were monomorphic and remaining 4 bands were polymorphic. The TCN strain scored 9 bands and of which 6 bands were monomorphic and remaining 3 bands were polymorphic. The TKM strain generated 7 bands, out of which 6 bands were monomorphic and remaining 1 band was polymorphic.

Furthermore, the similarity index based on the RAPD-PCR profiles was calculated. The VDM *Artemia* strain indicated the similarity index value of 0.83 to 0.97. Similarly, the RAPD-PCR profile of the *Artemia* strains such as GSL, KBM, VDM, TCN and TKM were subjected to phylogram analysis. The primer OPK-07 generated a maximum number of clusters compared to other primers. The DNA banding pattern obtained from the OPK-07 and OPK-08 primers confirmed the clustering patterns.

3.5 DISCUSSION

In India, the bisexual *Artemia* strains are found in coastal saltworks along the Southeast coast of India. Although studies have been made on the distribution and biology of *Artemia* not much information is available on genetic characterization of the *Artemia* strains confined to Southeast coast of India. Preliminary characterization of various *Artemia* strains has been attempted by choosing a cyst diameter as one of the parameters (Tejeda, 1987). For cyst biometrics four *Artemia* strains were analyzed, and the results obtained display wide divergence. Interestingly, the biometry cysts revealed that the KBM cyst size is greatly related to GSL reference cyst compared to other cysts. These relatively large cyst sizes are comparable to values reported for parthenogenetic populations elsewhere in the world, which tend to produce larger cysts than bisexual populations (Lavens and Sorgeloos, 1996).

Scanning electron microscopic studies on cysts of each strain showed a smooth surface topography of the cyst without any specific ornamentation. Hydrated cyst clearly showed an absence of any specialized micropylar region. The cross section of the cyst shows the presence of outer tertiary layer and an innermost cuticular layer. The tertiary layer consists of an outer cortex and an inner alveolar layer. The alveolar layer is characterized by alveolar mesh, followed by cuticular layer, which encircles the embryo (Gilchrist, 1978). Of all the egg membranes, the alveolar layer showed subtle variations in the alveolar mesh of the strains studied.

The furcal morphological characteristics significantly contribute to the discrimination among the four *Artemia* strains. Further, variation of furcal fin shape and its numbers of setae are more evident to differentiate the strain characterization. The presence of definite and elaborate furcal morphology of the

KBM strain is characterized and compared with other strains. In comparison, the TCN strain had the much wider and deeper furcal groove. However, the TKM strain showed wide furcal groove when compared to KBM strain. At the same time, the end part of the telson segment showed substantial variation between KBM and VDM strains.

A variety of DNA fingerprinting techniques have been used for describing the diversity within the genus *Artemia*, namely RAPD (Random Amplified Polymorphic DNA) (Badaracco *et al.*, 1995; Sun *et al.*, 1999), Amplified fragment length polymorphism (AFLP) (Triantaphyllidis *et al.*, 1997 and Sun *et al.*, 1999). Molecular characterization studies, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) showed variations among the *Artemia* strains. The results further supported by biometric and RAPD data of the different *Artemia* strains. Doubtless considerable genetic differentiation exists between the KBM and VDM *Artemia* strains. The primer OPK-07 produced a maximum number of clusters than the other primers. The similarity index values recorded a maximum of 0.97 and the minimum of 0.83 based on the RAPD profiles generated for the reference GSL strain.

Morphological data can be substantially reinforced by genetic evidence (Abreu-Grobois and Beardmore, 1991; Triantaphyllidis *et al.*, 1997; Sun *et al.*, 1999; Bossier *et al.*, 2004 and Qiu *et al.*, 2006). A genetic study of *Artemia* strains in Southeast Asia using genetic marker shows relatively high genetic diversity within both local and wild strains, but wild strains tended to have higher (within strain) genetic diversity than local strains. Using these techniques, the level of mitochondrial DNA variation documented within the strains of cultured and wild strains. Genetic differences can be studied by a variety of techniques. Random amplified polymorphic DNA (RAPD-PCR) is one of the widely used molecular methods for strain genetic diversity studies. Some of the tools have also served for the characterization of anostracans and the genus *Artemia* using the cysts. The mitochondrial DNA analysis of bisexual and parthenogenetic brine shrimp (Perez *et al.*, 1994) by using different molecular approaches has been used to study speciation in *Artemia*. The highly repetitive DNA sequence in parthenogenetic has been investigated by Badaracco *et al.* (1991). Interestingly, recent researches based on molecular markers were also carried out on the phylogeny, speciation and evolution of *Artemia*. Similarly, in the present study biometry, morphological characteristics and DNA pattern in KBM bisexual *Artemia* strain seemed to be similar to GSL strain. Amat *et al.*, (2005) reported that *A. franciscana* is an exotic invasive species and there is

being extended that distribution to Portugal, Spain, France, South Asian countries as well as north of Morocco. Neither the intra and inter-strain genetic variability at a given time is known for all tested samples or its temporal stability. Given the fact that mitochondrial rDNA polymorphism is normally found at the genus or species level and due to allopatric distribution of *Artemia*, it was anticipated that this marker would be able to differentiate samples at the species or sub-species level (Palumbi, 1996).

Apart from purely fundamental research goals such as phylogeny and species description, molecular tools have been important and used in applied research fields like the authentication of commercial cyst samples. Besides, it is essential to trace back their geographic background and to assess their purity in terms of strain composition. The technique proved sufficient to identify most *Artemia* species down to the species level, but discrimination between parthenogenetic strains, *A. urmiana* and *A. tibetiana* based on the use of this molecular marker remained problematic altogether, given the current resolution of the technique and the current status of reference samples being analysed. The dendrogram suggests that the strains in these two clusters are not identical and that some genetic dissimilarity between them might exist. The South Indian *Artemia* strains, although these samples were collected from different prevailing environmental conditions. This suggests high genetic dissimilarities between these samples.

According to the present investigation, considerable morphological differences were observed in bisexual strains of South India. On the divergent, RAPD results indicated a close relationship between VDM and TKM *Artemia* strains, which has been confirmed by many other studies using different approaches (Beardmore and Abreu-Grobois, 1983; Abreu-Grobois and Beardmore 1991; Browne *et al.*, 1991; Abatzopoulos *et al.*, 1997; Triantaphyllidis *et al.*, 1997; Sun *et al.*, 1999; Bossier *et al.*, 2004 and Qiu *et al.*, 2006). The present study documents that despite these small genetic differences between these *Artemia* strains, detected with the RAPD markers, sufficient isolation may have happened in other genes allowing the establishment of morphological differences. Accordingly, it would be interesting to carry out more specialized genetic examinations in order to exhibit the basis for the morphological deviations with among the bisexual *Artemia* strains. Genetic diversity data will be useful for management strategies that maintain genetic diversity, such as the wild collection as well as cultured *Artemia* strain.

CONCLUSIONS

A detailed survey of the tropical solar saltpan locations of Kelambakkam (KBM), Vedaranyam (VDM), Tuticorin (TCN) and Thamaraiikulam (TKM) was made to document and catalogue the occurrence of *Artemia* strains in four main ecological regions along the South East Coast of India. From the observations, the variations have been noted with respect to the furcal shape, fin and cyst biometric characters of different strains of *Artemia*. In conclusion, the biometry results revealed that KBM cyst diameter was similar to GSL reference cyst. The TKM strain showed wide furcal groove compared to other strains. Therefore, the genetic relatedness of *Artemia* strains were analysed using RAPD-PCR fingerprinting technique. Three random primers were produced a clear and distinct banding pattern. The primer OPK-20 was generated an enhanced degree of polymorphism compared to other primers. The RAPD-PCR profiles clearly indicated genetic dissimilarity between the KBM and TKM strains. The RAPD-PCR finger printing analysis revealed that the greatest genetic differences among the *Artemia* strains in order to provide the genetic evidence and specific status of *Artemia* strains confined to South India.

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REFERENCES

- 1 Abatzopoulos, T.J., Kastritsis, C.D., and Triantaphyllidis, C.D. 1986. A study of karyotypes and heterochromatic associations in *Artemia* with special reference to two N. Greek populations. *Genetica*. 71: 3-10.
- 2 Abatzopoulos, T.J., Triantaphyllidis, G., Beardmore, J., and Sorgeloos, P. 1997. Cyst membrane protein composition as a discriminant character in the genus *Artemia*. *Journal of Marine Biological Association*. 77: 265-268.
- 3 Abreu-Grobois, F.A., and Beardmore, J.A. 1991. Genetic characterization and intra-generic relationships of *Artemia monica* Verrill and *A. urmiana* Gunther. *Hydrobiologia*. 212: 151-168.

- 4 Amat, F. 1980. Diferenciación Y distribución de las poblaciones de *Artemia* (Crustaceo, Branquiopodo) de España. I. Análisis Morfológico. Estudios alométricos referidos al crecimiento ya la forma. *Investigación Pesquera*. 44: 217-240.
- 5 Amat, F., Hontoria, F., Ruiz, O., Green, A.J., Sanchez, M.I., Figueroa, J., and Hortas, F. 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean. *Biological Invasions*. 7: 37-47.
- 6 Badaracco, G., Bellorini, M., and Landsberger, N. 1995. Phylogenetic study of bisexual *Artemia* using random amplified polymorphic DNA. *Journal Molecular Evolution*. 41: 150-154.
- 7 Badaracco, G., Tubiello, G., Benfante, R., Cotelli, F., Maiorano, D., and Landsberger, N. 1991. Highly repetitive DNA sequence in parthenogenetic *Artemia*. *Journal Molecular Evolution*. 32: 31-36.
- 8 Beardmore, J.A., and Abreu-Grobois, F.A. 1983. Taxonomy and evolution in the brine shrimp *Artemia*. In: *Protein Polymorphism: Adaptive and Taxonomic Significance*, eds. G.S. Oxford and D. Rollinson, pp 153-164. The Systematics Association Special, Academic Press, London and New York.
- 9 Bossier, P., Xiaomei, W., Catania, F., Doms, S., Van Stappen, G., Naessens, E., and 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.
- 10 Browne, R.A., and Bowen, S.T. 1991. Taxonomy and population genetics of *Artemia*. In: *Artemia Biology*, eds. R.A. Browne, P. Sorgeloos and C.N.A. Trotman, pp 221-235. CRC Press, Boca Raton, Florida, USA.
- 11 Browne, R.A., Sorgeloos, P., and Trotman, C.N.A. 1991. *Artemia Biology*, pp 1-374. Boca Raton: CRC Press.
- 12 Gajardo, G., Colihueque, N., Parraguez, M., and Sorgeloos, P. 1998. International study on *Artemia* LVIII. Morphological differentiation and reproductive isolation of *Artemia* populations from South America. *International Journal of Salt Lake Research*. 7: 133-151.
- 13 Gilchrist, B.M. 1960. Growth and form of the brine shrimp *Artemia salina* (L.). *London, Proceedings Zoological Society*. 134: 221-235.
- 14 Gilchrist, B.M. 1978. Scanning electron microscope studies of the egg shell in some Anostraca (Crustacea, Branchiopoda). *Cell Tissue Research*. 193: 337-351.
- 15 Lavens, P., and Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture. *FAO Fisheries Technical Paper No. 361*, Rome. 295 p.
- 16 Palumbi, S.R. 1996. Nucleic Acids II: the polymerase chain reaction. In: *Molecular Systematics*. Sinauer Associates, eds. D.M. Hillis, C. Moritz and B.K. Mable, pp 205-247. Sunderland, USA.
- 17 Perez, M.L., Valverde, J.R., Batuecas, B., Amat, F., Domenech, A., Marco, R., and Garesse, R. 1994. Speciation in the *Artemia* genus: mitochondrial DNA analyses of bisexual and parthenogenetic brine shrimp. *Journal of Molecular Evolution*. 38: 156-168.
- 18 Pilla, E.J.S., and Beardmore, J.A. 1994. Genetic and morphometric differentiation in Old World bisexual species of *Artemia*. *Heredity*. 73: 47-56.
- 19 Qiu, Z., Bossier, P., Wang, X., Bojikova-Fournier, S., and MacRae, T.H. 2006. Diversity, structure and expression of the gene for p26, a small heat shock protein from *Artemia*. *Genomics*. 88: 230-240.
- 20 Sun, Y., Zhong, Y., Song, W.Q., Zhang, R.S., Chen, R.Y., and Sorgeloos, P. 1999a. Detection of genetic relationships among four *Artemia* species using randomly amplified polymorphic DNA (RAPD). *International Journal of Salt Lake Research*. 8: 139-147.
- 21 Tejada, N.I.D. 1987. Preliminary characterization of four populations of *Artemia* from the Dominican Republic. In: *Artemia Research and its application*, eds. P. Sorgeloos, D.A. Bengston, W. Decler and E. Jaspers, p 380. Morphology, Genetics, Strain Characterization, Toxicology, Universa Press, Wetteren; Belgium.
- 22 Triantaphyllidis, G., Criel, G.R., Abatzopoulos, T.J., and Sorgeloos, P. 1997. International study on *Artemia*. LIII. Morphological study of *Artemia* with emphasis to old world strains bisexual population. *Hydrobiologia*. 357: 139-153.
- 23 Van Stappen, G. 2002. Zoogeography. In: *Artemia Basic and Applied Biology*, eds. T.J. Abatzopoulos, J.A. Beardmore, J.S. Clegg and P. Sorgeloos, pp 171-215. Kluwer Academic Publishing, Dordrecht, The Netherlands.

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