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Population genetic variation and species relationships of seven clams species (Family: Veneridae) from Lake Timsah (Suez Canal).

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Summary

Specimens of veneridae clams were collected from Lake Timsah (Suez Canal). These specimens were classified by morphological investigation to seven species, six genera and four subfamilies. Some specimens had shell abnormalities (about 5-7% out of the collected specimens) as response to contaminants exposure in their habitat. Random amplified polymorphic DNA (RAPD) markers were used to determine the genetic similarities between the collected species. 13 out of 21 RAPD primers generated 368 RAPD markers ranged from 130 to 2073 bp. The genetic similarity index values revealed non-significant differences in the genetic diversity between species (the value between each two species was above 0.5) The high values of similarity proved that, the collected species must be actually monophylogenetics. The similarity value between some species was higher than the others; despite these species follow different subfamilies. On the other hand, although two species follow the same genus, their similarity value was lower than that of them and the rest of the species. The unweighted pair group method using arithmetic average (UPGMA) dendrogram based on the genetic similarity index comprised two main clusters, one of which nested large cluster including six species, and the other included only one species.

Keywords: Clams; Veneridae; DNA; Genetic diversity; RAPD-PCR; UPGMA

1. Introduction

Veneridae is a cosmopolitan marine bivalve family that distributed along many Egyptian coasts, lives in sandy-site or muddy-gravel sediments. Veneridae members are filter feeders and thoroughly exposed to the local water. They extract contaminants with extracting oxygen and food and accumulate them in their bodies in a much higher concentration than in the surrounding sea water [1, 2, and 3]. The shell can be an important site of metals bioaccumulation and may act as a "toxic waste sink" to facilitate the removal of pollutants from the more metabolically active soft tissues. Metals may be either adsorbed onto the surface of the shell relatively non-specifically or integrated

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(sequestrated) into the shell matrix. Sometimes pollution cause change in shell structure and color [2]. Recent works synonymies some Veneridae subfamilies with each other due to their overall morphological similarity. The use of traditional shell-based characters alone, however, is questionable for resolving phylogenetic relationships of this family. Seilacher [4] stated that morphology is controlled not only by evolutionary heritage and necessities of adaptation to specific environments, but also by morphogenetic programs. In other words, morphology is a product of combined phylogenetic, adaptive, and constructional factors. Stanley [5] emphasized that shell cross-sectional shape, lateral profile, thickness, and surface features are related to burrowing behavior. The use of RAPD analysis in phylogenetic studies and population genetics has been observed in a wide variety of organisms [6, 7, 8 and 9]. In aquatic shellfish, this technique has successfully analyzed genetic polymorphisms at the interspecies level of bivalves, such as mussel [10], intraspecies level of scallop [11], abalone [12] and oyster [13, 14 and 15]. De Wolf et al. [16] concluded that, RAPDs are not only useful to infer genotoxic related population genetic effects by considering band intensity differences or gain/loss of RAPD bands, but they may also provide information on the overall genetic diversity and structure of populations.

Morphological characters of both Venerinae and Chioninae (Veneridae) subfamilies were evaluated by mapping them onto the resultant phylogenetic tree [17]. This study showed that, despite a high amount of homoplasy in the morphological characters, a specific subset of those characters can be used to distinguish between members of the two subfamilies. Recently, to clarify the systematic and phylogeny of Veneridae, a portion of nucleotide sequence of the gene encoding the large subunit of mitochondrial ribosomal RNA was analyzed by Ai-hui et al. [18] in 14 species representative of 10 genera belonging to 6 different subfamilies. The results indicated that the current placement of the genera into subfamilies does not always reflect a natural subdivision that based on morphological characters. In addition, the six species of Tapetinae, though confirmed to be a monophyletic clad, do not exhibit a correct attribution at the genus level. Canapa et al. [19] determined 400 base pair long portion of 16S rRNA gene sequence for many species of venerid clams and concluded that neighbor-joining and maximum parsimony trees support the rest of traditional morphological classification method at the level of the subfamilies.

Timsah Lake lies in a half-way of the Suez Canal. Although its water is salty, fresh water is coming from the Nile through Ismailia Canal, reduces its salinity and its pH value. The aim of this work was to assess the genetic diversity of venerid species collected from Lake Timsah in order to formulate hypotheses regarding the underlying mechanism that is responsible for the species genetic structure of this family.

2. Materials and methods

2.1- Samples collection and preparation:

Samples of water, sediment and venerid specimens were collected from Lake Timsah in October (2008). Small portions of the adductor and foot muscles were excised nondestructively and flash-frozen in liquid nitrogen before stored in a -80oC freezer until further DNA analysis. In the laboratory, all specimens were morphologically identified and photographed. Whole soft tissues of the common clam were frozen till used for heavy metals determination.

2.2. Ecological investigations:

Physicochemical parameters determination:

Water temperature, hydrogen ion concentration, and dissolved oxygen were measured in situ, while salinity was measured at the laboratory by using conductivity salinometer.

Heavy metals determination:
Heavy metals including Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn were analyzed using the Atomic Absorption Spectrophotometer (AAS) (Perkin- Elmer Model 2380) in surface sea water, sediment [20], common clam whole soft tissues [21] and common clam shell [22].

Total petroleum hydrocarbons determination (TPHs):
TPHs were analyzed in surface sea water by capillary gas chromatography (GC) followed by high performance liquid chromatography (HPLC) with fluorescence detection.

2.3. Genomic DNA extraction and purification:
For each specimen, genomic DNA was isolated and purified from 30-35mg of adductor and foot muscles using Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer's instructions. Whole genomic DNA was then quantified using a spectrophotometer and diluted to a standard concentration of 20ng/ul.
A set of 21 RAPD primers comprising unique decamer nucleotides with high G+C contents ranging from 60-70% (Tm = 32 - 34 ^{o}C) were used to amplify genomic DNA for the collected samples. The sequence of the primers (5´ – 3´) is represented in Table (1). Amplification was conducted in 25 µl volumes consisting of 2.5 µl of 10x buffer (20 mM Tris- HCl, 100 mM KCL, 0.1 mM DDT, 50% Glycerol, 0.5% Tween 20 and 0.5% NP-40), 0.5 µl dNTPs mixture (25 mM each), 1.5 µl MgCl2 (25 mM), 1 µl BioReady Taq DNA polymerase (BioFlux) (2.5 U/µl), 1 µl DNA template (100 ng) , 2 µl of primer and up to 25 µl by ddH2O. Negative controls, all the components with no DNA using distilled water instead, were used to detect possible contaminations. PCR amplifications were carried out in a Gene Amplification PCR system (Perkin Elmer Model 2400) using the following conditions: Initial denaturation at 94oC (2 min.), followed by 45 cycles of 94oC (30 s), 38oC (30 s) and 72oC (60 s) with a final extension at 72oC (5 min.).

Table 1: List of arbitrary primers sequences and their G+C contents. Show only the primers sequences that gave amplification in all species

Code	Sequence 5´ to 3´	G + C%	Code	Sequence 5´ to 3´	G + C%
1	GTA GAC CCG T	60	12	TGC GCC CTT C	70
2	GGA CCC TTA C	60	13	TTC GCA CGG G	70
3	GTC GCC GTC A	70	14	GTG AGG CGT C	70
4	GGT CCC TGA C	70	15	CAA ACG TCG G	60
5	TGG ACC GGT G	70	16	CTG CTG GGA C	70
6	AGG GGT CTT G	60	17	GTG ACG TAG G	60
7	TTC CCC CGC T	70	18	CCA CAG CAG T	60
8	TTC CCC CCA G	70	19	TGA GCG GAC A	60
9	ACT TCG CCA C	60	20	GTG AGG CGT C	70
10	CAA TCG CCG T	60	21	CCA GGA GGA C	70
11	AGG GAA CGA G	60			

Amplified products were separated on 1.5% polyacrylamide gels using TBE buffer at 80V for 1.5 hours. Gels were developed in ethidium bromide baths for 15 minutes. Visualization was done with a FluorChem 8800 Imaging System (Alpha Innotech Corporation) under UV lighting and DNA bands were scored.

2.4. Statistical analysis:
Two-way ANOVA were employed to find the variances in the ecological analysis by using MSTAT-C program (MSTAT-C V.2.10). All results were significant at a probability level equal or less than 0.05 [23].Variation detected by RAPD–PCR amplification was analyzed for the presence versus absence bands. Cluster analysis of pair wise genetic similarity of different species was performed using unweighted pair group method with the arithmetic mean (UPGMA). The calculations and dendrogram were performed by using the NTSYS-pc version 2.02 software packages (Numerical Taxonomy System, Exeter Software).

3. Results

3.1. Classification according to the morphometric characters:
The collected specimens were classified into seven species as follows:
1- *Callista florida* Lamarck, 1818 (Subfamily: Pitarinae Stewart, 1930)
2- *Dosinia radiata* Reeve, 1850 (Subfamily: Dosiniinae Deshayes, 1853)
3-*Gafrarium pectinatum* Linnaeus, 1758 (Subfamily: Circinae Dall, 1896)
4-*Paphia undulata* Born, 1778 (Subfamily: Tapetinae H. & A. Adams, 1857)
5-*Tapes decussatus* Linnaeus, 1758 (Tapetinae)
6-*Venerupis aureus* Gmelin, 1791 (Tapetinae)
7-*Venerupis pullastra* Montagu, 1803 (Tapetinae)
According to the morphological investigation some collected specimens had shell abnormalities (about 5-7% out of the collected specimens) as shown in Figure1.

Fig. 1: Venerid clams showing normal (on the left side) and abnormal shells (on the right side) indicated by arrows heads. Scale bar = 3 cm.

3.2- Ecological investigations:

Table 2: Physicochemical parameters values of Lake Timsah surface sea water. (Mean ± standard error of 6 determinations).

	Parameters			
	T (°C)	pH	Salinity (‰)	DO (mg/l)
M ± SE	26.200 ± 1.550	8.360 ± 0.067	40.354 ± 0.281	5.497 ± 0.184

The physicochemical parameters of surface sea water are illustrated in Table (2). ANOVA revealed highly significant differences ($P \leq 0.01$) in heavy metals values between water, sediment, soft tissues and shells. The sequence of heavy metals concentrations followed the pattern; water < shell < sediment < soft tissues for all metals except for Cd, which gave the following pattern; water < sediment < shell < soft tissues as detailed in Table (3). TPHs concentration in surface sea water was 0.31 mg/l.

Table 3: Heavy metals concentrations in surface sea water (µg/ml) and sediment, common clam (*Tapes decussatus*) whole soft tissues and shell (µg/g dry weight) of Lake Timsah. (each mean ± standard error for 6 determinations).

Metals	Parameters			
	Water	Sediment	Soft tissues	Shell
Cd	0.480 ± 0.011	4.953 ± 0.246	6.593 ± 0.460	5.309 ± 0.302
Co	1.118 ± 0.133	2.917 ± 0.296	12.543 ± 0.570	1.984 ± 0.218
Cu	4.093 ± 0.096	10.232 ± 0.824	60.887 ± 3.944	4.206 ± 0.434
Fe	11.290 ± 0.201	305.528 ± 5.923	2182.250 ± 88.172	143.849 ± 6.122
Mn	2.077 ± 0.185	33.365 ± 2.758	79.660 ± 2.034	24.710 ± 1.963
Ni	2.165 ± 0.097	24.904 ± 1.399	33.084 ± 1.950	17.305 ± 1.250
Pb	3.175 ± 0.230	45.161 ± 1.862	47.550 ± 1.724	43.810 ± 1.688
Zn	12.186 ± 0.886	47.223 ± 2.265	108.768 ± 4.280	22.639± 0.299

3.3- RAPD–PCR analysis:

A total of 368 RAPD markers ranging from 130 to 2073 bp in size were clearly identified from PCR amplification using 13 primers for each venerid species (Figure 2A and B). The greatest number of PCR bands was found with primer 12 (61 bands) while the smallest number was obtained with primer 11 (only 9 bands).

Table 4: Genetic similarity matrix index calculated by pairwise comparison of seven species of venerid clams collected from Lake Timsah. *Cf, Callista florida; Dr, Dosinia radiata; Gp, Gafrarium pectinatum; Pu, Paphia undulata; Td, Tapes decussatus; Va, Venerupis aureus* and *Vp, Venerupis pullastra.*

	Cf	***Gp***	***Td***	***Va***	***Vp***	***Pu***	***Dr***
Cf	1	0.691	0.683	0.638	0.528	0.695	0.671
Gp		1	0.789	0.679	0.545	0.703	0.679
Td			1	0.679	0.602	0.695	0.663
Va				1	0.549	0.650	0.634
Vp					1	0.630	0.549
Pu						1	0.740
Dr							1

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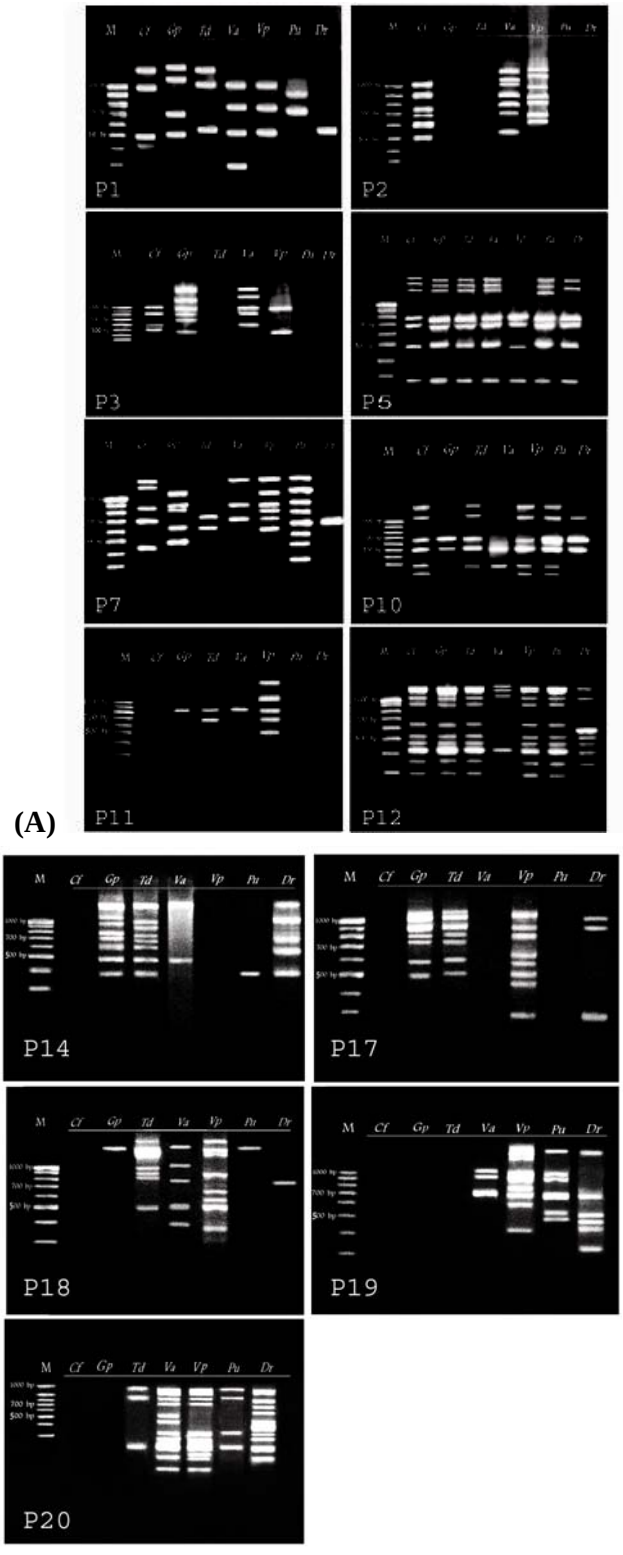

Fig. 2 (A and B): Bands generated by PCR using RAPD primers for the seven species of venerid clams collected from Lake Timsah. bp, molecular weight in base pairs; *Cf, Callista florida; Dr, Dosinia radiata; Gp, Gafrarium pectinatum;* M, DNA marker; P, primer; *Pu, Paphia undulata; Td, Tapes decussatus; Va, Venerupis aureus* and *Vp, Venerupis pullastra.*

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The genetic similarity index values were calculated by pairwise comparison in the RAPD-PCR amplification profiles of any two species (Table 4). It is noteworthy that there are no significant differences in the genetic diversity between them; the results between each two species were above 0.5. The high values of similarity proved that, the seven species must be actually monophylogenetics. The similarity value between *Gafrarium pectinatum* and *Tapes decussatus* was higher than the others, despite the two species follow different subfamilies, also the value between *Paphia undulata* and *Dosinia radiata* was high despite the two species follow different subfamilies. On the other hand, although, *Venerupis aureus* and *V. pullastra* follow the same genus, the similarity value between them was lower than that of them and most other species. The unweighted pair group method using arithmetic average (UPGMA) dendrogram based on the genetic similarity index comprised two main clusters, one of which was a nested large cluster including six species, and the other included one species (Figure 3).

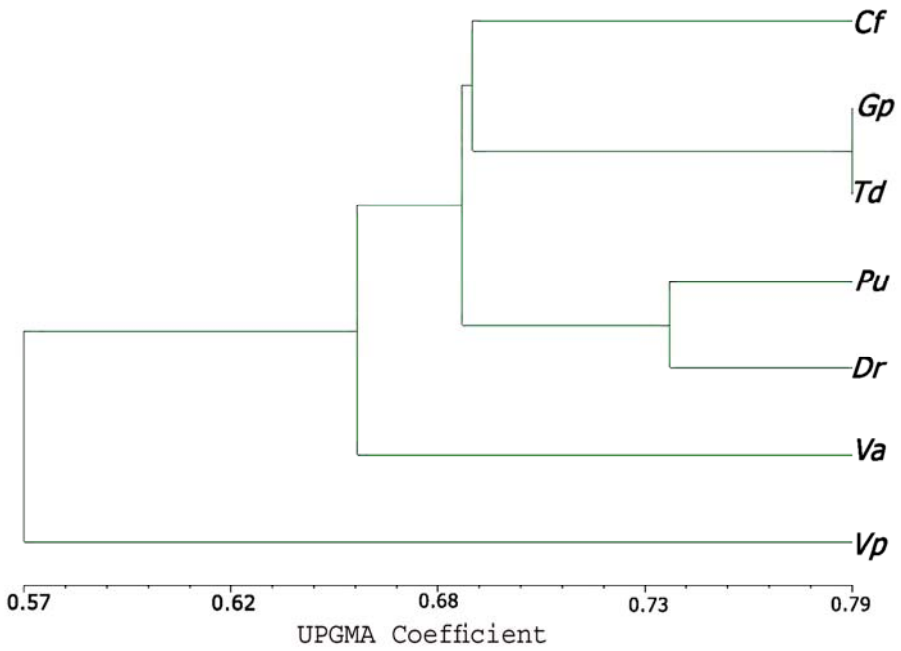

Fig. 3: Unweighted pair group method using arithmetic average (UPGMA) dendrogram based on genetic similarity index values calculated from data of 13 arbitrary primers for seven species of venerid clams collected from Lake Timsah. *Cf, Callista florida; Dr, Dosinia radiata; Gp, Gafrarium pectinatum; Pu, Paphia undulata; Td, Tapes decussatus; Va, Venerupis aureus and Vp, Venerupis pullastra.*

4. Discussion

The higher concentration of heavy metals and TPHs in Lake Timsah is due to the passage of ships through Suez Canal which increases the pollution of water [24]. By comparing the measured heavy metals concentrations in water, sediment, soft tissues and shell, it was found that, the highest metal concentrations were in soft parts, followed by shell, sediment and finally in the water. This pattern of concentration gradient was proved for all investigated metals except for Cd, which accumulated in the shell in higher values than in sediment. These results agree with other reports [1, 2, 3 and 25] and attributed to the fact that clams are biomonitoring, accumulate contaminants in their bodies in levels much higher than in the surrounding environment.

The metals concentration was much lower in the shell than in the soft tissues similar to those observed in other molluscs [26, 27, 28, 29, 30, 31, 32 and 33]. These phenomena is in accordance with the fact that metal variability in the shells is less associated with weight, size, season, physiological condition, etc. than in the soft tissues [34]. In other molluscs, higher iron concentrations were found in the shells rather than in the soft tissues as observed by Ireland & Wootton [35] and Szefer & Szefer [36 and 37]. These differences may be due to the cleaning process applied prior to analysis in the shells was inadequate as stated by Cravo et al. [25].

The presence of contaminants may affect the integrity of some collected shells. Black et al. [38] revealed that shell can be an important site of metal bioaccumulation and may act as a toxic waste sink to facilitate removal of pollutants from the soft tissues, which are adsorbed on the surface of the shells and may be integrated into the shell matrix. Once metals are trapped in the shell matrix, little metal release is possible unless breakdown of the shell matrix occurs. The detected abnormalities may affect the complete closing of valves while clams can tolerate long periods of unsuitable conditions by closing their valves as stated by Eversol [39] In the current study, RAPD-PCR technique was used to evaluate the classical identification and genetic variations among the examined venerid species [40, 41 and 9]. RAPDs are commonly used as markers to discriminate particular taxa by the presence/absence of certain diagnostic bands as mentioned by Barman et al. [42]. Aranishi & Okimoto [15] revealed that, polymorphisms derived from priming sites, which are randomly distributed throughout a genome, are inherited in a Mendelian fashion and result in differing amplification products useful as genetic markers. Out of the examined 21 arbitrary primers comprising unique decamer nucleotides, only 5 different primers gave positive results with the seven clams' species and produced 1-10 amplified bands with total 187 bands. Other 8 primers produced 1-11 amplified bands with total 181 bands, but were not reproducible with some species. An optimized RAPD-PCR amplification program with the thirteen primers allowed us to identify a total of 368 RAPD markers for each species. The differences of resulting DNA profiles among species as mentioned by Fritsch & Rieseberg [43] depending on the (1) presence/absence of priming sites, (2) priming complementary completeness/ incompleteness or (3) distance between priming sites. The genetic similarity index values were calculated by pairwise comparison in the RAPD-PCR amplification profiles of any two of seven venerid species revealed that, the seven species must be actually monophylogenetics. These data support the morphological studies which grouped these species in one family (Veneridae). The genetic similarity value between *Gafrarium pectinatum* and *Tapes decussatus* was higher than the others despite the two species follow different subfamilies; also the value between *Paphia undulata* and *Dosinia radiata* was high despite the two species follow different subfamilies. On the other hand, although, *Venerupis aureus* and *V. pullastra* follow the same genus, the similarity value between them was lower than that of them and most other species. From these genetics data, the morphological characters could be used in the classification until the families' level. Also the present results showed that, RAPD is useful technique for phylogenetic analysis among closely related individuals than morphological investigations. This agrees with Andre et al. [44], Futoshi & Takane [45], Baozhong et al. [7] and

Ibrahim et al. [46]. Seilacher [4] stated that morphology is controlled not only by evolutionary heritage and necessities of adaptation to specific environments, but also by morphogenetic programs. In other words, morphology is a product of combined phylogenetic, adaptive, and constructional factors.

5. References

1. Farag, E. - Dekinesh, S. - El- Odessy, H.: Trace elements accumulation in soft parts of six edible bivalve molluscs inhabiting Alexandria coast, Egypt. Egy. Ger. Soc. Zool., 31 (D): 27-36 (2000).

2. El-Gamal, M. - Sharshar, Kh. Heavy metals partitioning between soft tissues and shells of some freshwater and marine mollusks as accumulation indicators for their monitoring in the environment. Egy. Ger. Soc. Zool., 45 (D): 141-161 (2004).

3. Hamed, M. - Emara, A.: Marine molluscs as biomonitors for heavy metal level in the Gulf of Suez, Red Sea. Mar. Syst., 60: 220-234 (2006).

4. Seilacher, A.: Arbeitskonzept zur konstruktion morphologie. Lethaia, 3: 393-396 (1970).

5. Stanley, S.: Relation of shell form to life habits in the Bivalvia (Mollusca). Geol. Soc. America, Inc., Mem. 125: 296 pp. (1970).

6. Williams J. - Kubelik, A. - Livak, K. - Rafalski, J. - Tingey S.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6513-6535 (1990).

7. Baozhong, L. - Feng, Y. - Bo, D. - Jianhai, X.: The comparison between allozyme and RAPD markers for the population genetic structure analysis of scallop *Chlamys farreri.* Chinese J. Oceano. & Limnol. 24 (3): 295-299 (2006).

8. Marin, S. - Haye, P. - Marchant, S. - Winkler, F.: Molecular markers used to analyze species-specific status in abalones with ambiguous morphology. J. Shellfish Res. 26(3): 833-837 (2007).

9. Sleem, S. - Ali, T.: Application of RAPD-PCR in taxonomy of certain freshwater bivalves of genus *Caelatura.* Global J. Molecu. Scie. 3 (1): 27-31(2008).

10. Rego, I. - Martinez, A. - Gonzalez-Tizon, A. - Vieites, J. - Leira, F. - Mendez, J.: PCR technique for identification in the Pacific cod *Gadus macrocephalus* around Japan revealed by mtDNA and RAPD markers. Fish Sci., 64: 673-679 (2002).

11. Patwary, M. - Kenchington, E. - Bird, C.- Zouros, E.: The use of random amplified polymorphic DNA markers in genetic studies of the sea scallop, *Placopecten magellanicus* (Gmelin, 1971). J. Shell Fish Res., 13: 547–553 (1994).

12. Huang, B. - Peakall, R. - Hanna, P.: Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar. Biol., 136: 207–216 (2000).

13. Hirschfeld, B. - Dhar, A. - Rask, K. - Alcivar-Warren, A.: Genetic diversity in the eastern oyster (*Crassostrea virginica*) from Massachusetts using the RAPD technique. J. Shell fish Res., 18: 121-125(1999).

14. Klinbunga, S. - Ampayup P.- Tassanakajon, A. - Jarayabhand, P. - Yoosukh, W.: Genetic diversity and molecular markers of cupped oysters (genera Crassostrea, Caccostrea and Striostrea) in Thailand revealed by randomly amplified polymorphic DNA analysis. Mar Biotech. 3: 133–144 (2001).

15. Aranishi, F. - Okimoto, T.: Genetic relationship between cultured populations of Pacific oyster revealed by RAPD analysis. J. Appl. Genet., 45 (4): 435-443 (2004).

16. 16- De Wolf, H. - Blust, R. - Backelijau, T.: The population genetic structure of *Littorina littorea* (Mollusca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. Science of the total Environ. 325 (1-3): 59–69 (2004).

17. Kappner, I. – Bieler, A.: Phylogeny of venus clams (Bivalvia: Venerinae) as inferred from nuclear and mitochondrial gene sequences. Mol. Phylo. & Evol., 40(2): 317-331(2006).

18. Ai-hui - Zhao-xia, L. - Gong-neng, F.: Phylogenetic relationship of the genus *Meretrix* (Mollusca: Veneridae) based on mitochondrial COI. Gene Sequences, 30 (3): 233-239 (2009).

19. Canapa, A.- Marota, I.- Rollo, F. - Olmo, E.: Phylogenetic analysis of Veneridae (Bivalvia): Comparison of molecular and palaeontological data. J. Mol. Evol. 43: 517-522 (1996).

20. 20- Coughlan, B.- Hartl, M.- O'Reilly, S.- Sheehan, D.- Morthersill, C- van Pelt, F.- O'Halloran, J. - O'Brien, N.: Detecting genotoxicity using the Comet assay following chronic exposure of Manila clam *Tapes semidecussatus* to polluted estuarine sediments. Mar. Pollu. Bulle. 44: 1359-1365 (2002).

21. Dybern, B.: Field sampling and preparation of subsamples of aquatic organisms for analysis of metals and organochlorines, FAO, Fisher Tech. Paper, 212: 1-13 (1983).

22. Cravo, A. - Foster, P. - Bebianno, M.J.: Minor and trace elements in the shell of *Patella aspera* (Roding 1998). Environ. Interna. 28, 295-302 (2002).

23. Bailey, N.: Statistical Methods in Biology. 2nd Ed. (Biological Science Texts): 216 pp. (1981).

24. Hamed, M.A.: Determination of some micro – elements in aquatic ecosystem and their relation to the efficiency of aquatic life. Ph. D. Thesis, Faculty of Science, Mansoura University. p 134 (1996).

25. Cravo, A.- Bebianno, M. - Foster, P.: Partitioning of trace metals between soft tissues and shells of *Patella aspera.* Env. Int., 30(1): 87- 98 (2004).

26. Segar, D.- Collins, J.- Riley, J.: The distribution of the major and some minor elements in marine animals. Mar. Biol. Ass. U.K., 51: 131–136 (1971).

27. Carriker, M. - Palmer, R.- Sick, L.- Jonhson, C.: Interaction of mineral elements in sea water and shell of the oyster (*Crassostrea virginica* "Gmelin") cultured in controlled and natural systems. J. Exp. Mar. Biol. Ecol., 46: 279 – 296 (1980).

28. Walsh, K. - Dunstan, R. - Murdoch, R.: Differential bioaccumulation of heavy metals and organopollutants in the soft tissue and shell of the marine gastropod, *Austrococklea constricta.* Arch. Environ. Contam. Toxicol. 28(1): 35-39 (1995).

29. El-Nady, F.: Heavy metals exchange among the aquatic environment of the Mediterranean coast of Egypt. Indian J. Mar. Sci., 25(3): 225-233 (1996).

30. Giusti, L.- Williamson, A.- Mistry, A.: Biologically available trace metals in *Mytilus edulis* from the coast of northeast England. Environ. Int., 25(8): 968-981(1999).

31. De Wolf, H- Ulomi, S.- Backeljau, T.- Pratap, H.- Blust, R.: Heavy metal levels in the sediments of four Dartes Salaam mangroves. Accumulation and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda). Environ. Int., 26 (4): 243-249 (2001).

32. Szefer, P. - Frelek, K.- Szefer, K.- Lee, Ch-B- Kim, B-S- Warzocha, J.- *et al.*: Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis trossulus* from the southern Baltic. Environ. Pollut. 120: 423- 444 (2002).

33. Ravera, O. - Cenct, R.- Beone, G.- Dantas, M. - Lodigiani, P.: Trace element concentrations in freshwater mussels as related to those in their environment. J. Limnol., 62 (1): 61 –70 (2003).

34. Lingard, S. - Evans, R.- Bourgoin, B.: Methods for the estimation of organic-bound and crystal – bound metal concentrations in bivalves shells. Bull. Environ. Contam. Toxicol. 48: 179–184 (1992).

35. Ireland, M. - Wootton, R.: Distribution of lead, zinc, copper and manganese in marine gastropods, Thais lapillus and littorina littorea, around the coast of Wales. Environ. Pollut. 12: 27-41(1977).

36. Szefer, P. - Szefer, K.: Occurrence of ten metals in *Mytilus edulis* L. and *Cardium glaucum* L., from Gdansk Bay. Mar. Pollut. Bull., 16 (11): 446- 450 (1985).

37. Szefer, P. - Szefer, K.: Metals in molluscs and associated bottom sediments of the southern Baltic. Helgol Meeresunters, 44: 424- 441(1990).

38. Black, M. - Ferrell, J.- Horning, R.- Martin, L.: DNA strand breakage in freshwater mussels *Anodonta grandis* exposed to lead in the laboratory and field. Environ. Toxicol. Chem. 15 (5): 802-808 (1996).

39. Eversole, A.: Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic) hard clam. U.S. Fish Wild. Serv. Biol. Rep. 82 (11.75). U.S. Army Crops of Engineers, TR El.82.4. 33 pp. (1987).

40. Paraense, W.- Pointier, J.- Dealy, B.- Pernot, A.- Incani, R.- Balzan, C.- Chrosciechowski, P.: *Piomphalaria pronsa* (Gastropoda: Planorbidae): A morphological and biochemical study. Mem. Inst. Oswaldo. Cruz. 87: 171-179 (1992).

41. Spatz, L.- Vidigal, T.- Silva, M.- Cappa, S.- Carvalho, O.: Characterization of *Biomphalaria orbignyi, Biomphalaria peregrine* and *Biomphalaria oligosa* by polymerase chain reaction and restriction enzyme digestion of the internal transcribed spacer region of the RNA ribosomal gene. Mem. Inst. Oswaldo. Cruz. 95(6): 807-814 (2000).

42. Barman, H.- Barat, A.- Yadav, B.- Banerjee, S.- Mehar, P.- Reddy, P.- Jana, R.: Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. Aquaculture. 217, 115-123 (2003).

43. Fritsch, P. - Rieseberg, L.: The use of random amplified polymorphic DNA (RAPD) in conservation genetics, in: T.B.Smither, R.K. Wayne (Eds.), Molecular Genetic Approaches in Conservation. Oxford University Press, 55-73 (1996).

44. Andre, C.- Lindegarth, M.- Jonsson, P.- Sundberg, P.: Species identification of bivalve larvae using random polymorphic DNA (RAPD). J. Mar. Biol. Assoc. UK, 79(3): 563-565 (1999).

45. Futoshi, A. - Takane, O.: Genetic relationship between cultured populations of Pacific oyster revealed by RAPD analysis. J. Appl. Genet., 54(4): 435-445 (2004).

46. Ibrahim, A.- Aly, R.- Kenchington, E. - Ali, T.: Genetic polymorphism among five populations of Pinctada radiate from the Mediterranean coast in Egypt indicated by RAPD-PCR technique. Egypt J. Zool., 50: 467-477 (2008).