

SUMMARY

Clams are considered one of the favourite seafoods specially for the people who mainly inhabit the cities which located on marine coasts. They have high value of protein and fair amount of calcium and iron. In Egypt, many of the most commercially important are venerid clams.

Veneridae is one of the largest bivalve families, containing over 500 species of clams. The classification within this family has been controversial at least since the 1930s. The characters used for classifying this group still tend to be superficial, focusing on external features, specially those of the shell, which are adapted to their environment.

Recently the morphological characters are evaluated by mapping them onto the resultant phylogenetic tree. All the previous works encourage and through the light on the molecular genetic characters as a valuable insights for phylogenetic studies. Nucleotide sequences characteristically provide high numbers, usually hundreds or even thousands of characters of little but equal complexity. They form data sets independent from morphological data.

Venerids are filter feeders and good indicators for monitoring the pollution in the surrounding environment. They extract the contaminants with the extracting oxygen and food. They can accumulate these contaminants in their bodies much higher than in the surrounding sea water. So attention has been recently drawn to not only the phylogenetic map but also to the goals of genetic

ecotoxicology, in particular, the need to relate genotoxicity in individuals to population and community level.

The main goal of this study was collecting different venerids from different habitats and construct genetic maps to follow any genetic variations.

Seven venerid species were recorded in this study, *Callista florida*, *Dosinia radiata*, *Gafrarium pectinatum*, *Paphia undulata*, *Tapes decussatus*, *Venerupis aureus* and *Venerupis pullastra*. These species classified previously by morphological characters under four subfamilies and six genera. *Tapes decussatus* was the common species at the different stations. Some venerids which collected from Suez and Ismailia had some shell abnormalities.

The ecological studies revealed that, there are marked differences between stations (Marsa Matrouh, Alexandria, Ismailia and Suez) in the physicochemical parameters of sea water. Also, the heavy metals content in sea water, sediment, soft tissues and shell of the common clam showed marked differences between stations. Marsa Matrouh samples revealed the lowest heavy metals concentrations while Suez had the highest concentrations of Cd, Cu, Fe, Mn, Ni and Pb and Alexandria had the highest concentrations of Co and Zn. The sequence of metals concentrations was, water < shell < sediment < soft tissues for all metals except with Cd was, water < sediment < shell < soft tissues. TPHs concentration in sea water at Suez recorded higher level than that of Ismailia while at Marsa Matrouh and

Alexandria, the concentrations were under the limit of chromatography detection.

Veneridae often serve as a first intermediate host for Digenea and play a very important role in the life cycle of several of these platyhelminthes. *Tapes decussatus* samples which collected from Marsa Matrouh and Alexandria were devoided of parasites, while some samples from Ismailia and Suez were found to be infected with digenean larvae. *Tapes decussatus* from the two stations was infected with *Cercaria lata* (Digenea: Faustulidae), *Venerupis pullastra* was infected with unknown digenean species and *Venerupis aureus* was found to be infected with the two previous digenean species larvae.

The prevalence of infection by digenean larvae increased with increasing of clam size. The infected venerid species had different size, so, the mode of infection was different between them. The prevalence of infection varied from 0% in August (summer) and December (winter) to 80% in May (spring). The infection increased in female than male.

The infection level by the digenean larvae in the venerids tissues ranged from light to heavy. In the light and moderate infections, the larvae were restricted in male and female gonads, while in the heavy infection, the larvae were found around the visceral mass and inside the gonad and digestive gland. In light infection, the gonads structure did not affected while in moderate infection, the gonads lossed some parts of their structure and in heavy infection, complete castration of gonads were occurred.

The digestive gland of the normal common clam is large, tubulo-acinar, consists of primary and secondary tubules. The primary tubules are lined with simple epithelium consists of two main cell types, digestive and basophilic cells. Inside the digestive cells exhibit various degrees of vacuulations. The digestive gland of some uninfected clams which collected from Ismailia and Suez showed an increase vacuulations in the digestive cells, together with a reduction in number of basophilic cells. In heavy infection, the digenean larvae were found around the digestive gland and between the tubules. An intense autolysis was observed in all digestive cells with loss of basophilic cells and increasing the vacuolization. Despite the alterations presented, basal membranes were intact.

In the preliminary genetic diversity experiments of this study, a particular amplification program was suitable for detecting a high degree of genetic variations in the RAPD-PCR analysis of the venerid clams. The program worked with low annealing temperature at 38 °C and slightly numerous cycling at 45 cycles, and these physicochemical parameters allowed arbitrary primers to anneal several sites in a genome.

After employing 21 arbitrary primers comprising decamer nucleotides of random sequences, all of which G+C content at 60-70% with the calculated melting temperature at 32-34 °C with the seven collected species from Ismailia, 13 primers provided strongly amplified fragments. The fingerprints generated by the 13 primers revealed unique profile for each species. 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. The genetic similarity index values were calculated by pairwise comparison in the RAPD-PCR amplification profiles of any two species. There are no significant differences in the genetic diversity between the collected species; 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 genetic similarity value between *Gafrarium pectinatum* and *Tapes decussatus* was higher than that of 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 *Venerupis pullastra* follow the same genus, the genetic 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 *Venerupis pullastra*. From these genetics data, we can use the morphological characters in the classification until the families' level.

The total of 216 RAPD markers ranging from ~ 267 to ~ 1486 pb in size were produced after using 10 primers with the DNA of *Tapes decussatus* populations collected from uncontaminated and contaminated stations. Six primers gave positive results with the four

populations and produced 3-10 amplified bands with total 154 bands, while the other primers produced 6-8 bands with total 62 bands but were not reproducible with some populations.

The genetic similarity index values revealed that, there are no significant differences in the genetic diversity between Marsa Matrouh, Alexandria and Ismailia; the results between each two populations were above 0.5. The high values of similarity proved that, the three populations must be actually monophylogenetics, especially Marsa Matrouh and Alexandria, which the genetic similarity value between them was 0.795. On the other hand, the genetic similarity values between Suez and Marsa Matrouh and between Suez and Alexandria were, 0.434 and 0.422 respectively. The low values proved that, the Suez population is genetically different from the other two stations populations, while the genetic similarity value between Suez and Ismailia is high (0.723) which revealed that, the two populations are monophylogenetics. Unweighted pair group method using arithmetic average (UPGMA) dendrogram based on the genetic similarity index comprised two main clusters, one included *Tapes decussatus* populations collected from Marsa Matrouh (uncontaminated site) and Alexandria which could be genetically very related to each other. Another cluster included the populations from Ismailia and Suez (contaminated sites) which could be genetically related to each other.

