Karyological investigation of Persian Gulf cuttle fish (*sepia arabica*) in the coasts of Khuzestan province

*Ashraf Jazayeri, Forough Papan, Hossein Motamedi, Soghra Mahmoudi ASL

Shahid chamran university of Ahwaz
*Corresponding Author: Jazayeriashraf@yahoo.com

Abstract: Cephalopods are a group of Molluska. Which have extensive geographical extension and variation, in the world's oceans the most important cephalopods in Persian Gulf and Oman Sea are squids and cuttle fish? Nowadays we have found that cuttle fish has an extensive application in several contexts. But there is not enough information about their biology and the amount of their storage in Iran's waters. We must considerate reservoirs more than ever. Because of its economic value and the amount cattle fish's hunting. Therefore in this research for the first time in the world, cattle fish of Persian Gulf was investigated Kariologicaly. Investigation results of metaphase plaques resultant from analyzing blood cells of cuttle fish showed that , this species has the chromosomal number of 2n=68 indeed it is found than in chromosomal extension of this species there were not identifiable sexual chromosomes. **[Ashraf Jazayeri**, Kariologicaly investigation of Persian Gulf cuttle fish (*sepia arabica*) in the coasts of Khuzestan province. Life Science Journal. 2011; 8(2):849-852] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: kariology, cattle fish, Persian Gulf, sepia Arabica

1. Introduction

Cephalopods are old animals and successful groups of Molluska. These animals live in all of the world's oceans and in various depth of the water. Generally cephalopods have 2 side symmetry are with an extended head with a crown of mobile process which surrounded the mouth. Some of them do not have a shell and some others have. Shell is covered by a cloak. Cephalopods are animals with soft body which discharge water from cloak' s hole with a funnel flush, and so in addition to repelling the wastes, make the animal to move. Their body color is so various. they can rapidly change their color and get the color of surroundings. Comparing to fish, these animals release fewer amounts of eggs in the water. Their egg's diameter is various from 0.8 to 17 mm. The total number of known species of cephalopods in the world is 1000 variety and they belong to 43 families. There are only sexes of *Sepiidae* family in Iran waters. *Sepiidae* family is known with a flat big internal shell made from carbonate at the exterior part which is contributed to animal floating control. Most important cephalopods in Persian Gulf and Oman Sea are squids and cuttle fish. Which are spreaded frequently in coastal waters and far from coast of this zone? Dominant species of cuttle fish in south waters of our country is sepia *pharaonis.*

Fig1. Sepia Arabica (cuttle fish)

***Sepia arabica* (cuttle fish) classification:**

Kingdom: Animalia
Phylum: Molluska
Class: Cephalopoda
Super order: Decapodiformes
Order: Sepiida
Family: Sepiidae
Spices: sepia arabica

sepia arabica (cuttle fish) in the world is spreaded in Indian ocean , red sea , Adan Gulf , Persian Gulf , Giboty countries , Egypt , Eritrea ,

India , Iran , Kuwait , Pakistan , Oman , Qatar , Saudi Arabia , Somalia , Srilanka , Sudan , Yemen , Emirates. Today it is found that cuttle fish have various applications in several fields. In spite of this, there is not enough information for their biology and the amount of their storage in Iran waters. Based on the last obtained information from Iran fishery, a kilogram of cuttle fish is exported with the price of about 3.4 dollars .By exporting cuttle fish in the year 2007, 3 million dollars of currency has been entered to the country. This aquatic has a valuable place among fishery products as an important export product .considering the biological and economical importance of this marine source the necessity of its biological and geographical investigation in Persian Gulf and investigation some of its characteristics especially from view point of chromosomal investigations, is very important.

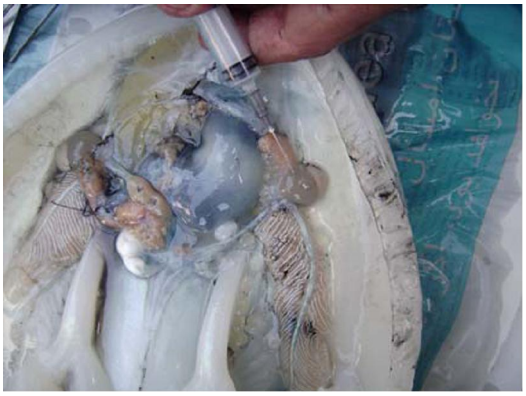
2. Material and Methods

In this investigation a research ship with motor power of 280 horse power, equipped with trawler net .sampling was performed monthly during a year from Mar.2007 until Feb.2008, in hunting place (Bahrekan). Samples were transferred to laboratory alive in a plate which had enough air. For preparing desired chromosomal extensions, two methods were used which include blood cell' s analysis and preparing a slide from metaphasic gill tissue (after injecting kolchesin). In the first method environmental blood analysis was like this: immediately after hunting, animal' s cloak was cut and some blood is extracted from main heart and animal's gill hearts with an injector mixed with sodium heparin for carrying blood samples. A special refrigerator for carrying laboratory samples was used. After arriving laboratory, samples were frizzed in 4^{0} c. for analyzing blood cells a RPM1 1640 tissue culture was used. About 10 drops of serum and 0.1 cc of PHA was added to analysis environment (as a mitogen) and then the amount of 1cc of heparins blood was added to each analysis vial (all of the above stages were perfumed in sterilized environment) .

Then analysis environments were kept in incubator with 25 ^{0}c for 4 days, and they were shaking slowly every 24 hours.

After 72 hours, cells extraction from analysis environment was perfumed. In such a way that tissue culture contents were transferred to centrifuge pipe and were centrifuge for 6 minutes by 1000 g, and surface solution was removed. Then 3 ml of hypotonic solution was added slowly to cell mass by shaking (used hypotonic solution was potassium chloride 0.75 molar). Pipes were kept in laboratory

Fig2. Sampling the blood from heart of cuttle fish



temperature for 20 minutes and were centrifuged for 6 minutes with 1000 g and again surface layer was removed. Then by adding fresh and cold fixative, the last step of centrifuge was performed and again surface layer was removed. Some drops of fixative added to final residual, and were combined perfectly this residual was used for producing microscopically slide. For preparing extension no the slide, the dropping droplet method was used on clean and cold glasses (From 50 cm away). Then prepared slides were staining with Gimsa 1% in dry weather of laboratory. In microscopic studies, after observing adequate metaphasic plaques, desired chromosomal expiations were photographed by a microscope equipped with a camera.

In second method, after transferring a live sample to the laboratory, the sample is weighted rapidly and 0.02 ml kolchesin solution was injected to muscle peritoneal area by an insulin injector per a gram of body weight. Then the sample was kept in aquarium for 5 hours, sample was analyzed , and gills and kidney tissue was separated .after pressing the tissues , they were poured to vials and 0.36% of potassium chloride hypotonic solution was added to it (as same volume as applied tissue) and was kept in room temperature for 45 minutes . In the next stage, fresh and cold fixative was added it in the amount of one third of solution volume (ratio 3:1 Ethanol to acetic acid). Then first stage of centrifuge was performed f0r 10 minutes with 1000 g. After supernatant layer was removed and fixative was added to bottom residual, again, it was incubated for 1 hour in room temperature. And washing stages were performed two times. Finally 1 ml of fixative was added to the residual and getting lam and staining was perfumed like previous stage.

3. Results

Results of Karyological investigations on this species showed that, the chromosomal number of sepia Arabica species is 2n= 68 which includes 3 pairs of met centric, 14 pairs of sub Meta centric and 17 pairs of Telo centric. Indeed it is not observed any identifiable sexual chromosomes.

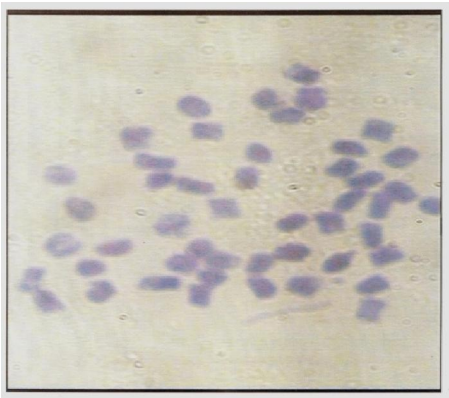


Figure.3. Metaphasic chromosomal expantion of sepia arabica

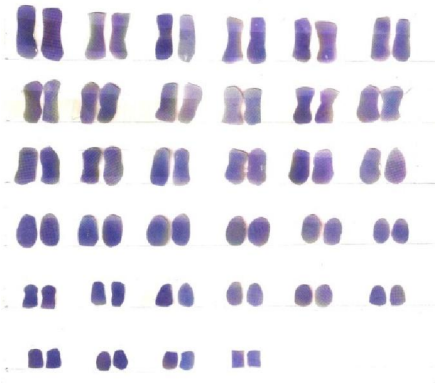


Fig4. Karyotype of sepia arabica

4. Discussion

Philogenetic studies on the basis of recognizing mitochondria and core genome help a lot to classify organisms such as cephalopods. In this study which is perfumed for the first time on sepia Arabica species, number of chromosomes were investigated and counted and identified. chromosomal number of this species is 2n= 68 ,which is different comparing to the number of the chromosomes of other species of Persian gulf cuttle fish *sepia pharaonis* (2n=48) .So far chromosomal number was reported for those groups of Molluska which their numbers in cephalopods are more than the other cephalopods .Also in cephalopods, chromosomal number was reported to be between 52-112 .Which is different from chromosomal number in the other Molluska .Notilus species shows the least chromosomal number between cephalopods ,Maybe this is the singe of an inheritance attribute for cephalopods . In the other research 3 groups of cephalopods were investigated considering the number of chromosomes. (Inaba 1985), Results showed that there was a diploid situation in some of them, for example chromosomal numbers 52 and 56. Also in the other research (Inaba 2007) on octopus was showed (octopus *vulgaris*). Besides obtaining chromosomal number of 2n= 56 for this species. It is stated that there were not any sexual chromosomes for males.

However variation in aquatics, chromosomal numbers can have several reasons such as external fecundation and occurring the polyploidy phenomenon. It seems that it is necessary to have more studies specially on the DNA molecule level to enable us have judgment about genomic change of various species of aquatics from invertebrates to vertebrates .There for , Molluska can be very great examples for extensive genetic researches because they have some unique characteristics.

Today by performing exact researches and using new technique in molecular genetic studies it is tried to review the animals, classic classifications based on cladestic measures and correct if necessary. So it is evident that it needs basic studies about genomic identifying of aquatic species.

References:

1. Aoyama, T. and Nguyan, T. Stock assessment of cuttlefish off the coast of the people's Democratic Republic of Yemen. Shimonoseki University of Fisheries, 1989; 37(203):61-112.
2. Bondmaid, L., Saiahi, A. and Boucher-Rodoni, R. Are 28s r DNA and 18s r DNA information for cephalopod phylogeny, Bulletin of Marine Science, 2003; 71:197-208.
3. Dewitt, j.m.j. Origins of polyploidy. Biological Relevance, W.H. Lewise, ed. Plenum press, New York. 1980; 3-15.
4. Graham, J.P., Mastic, L.C. and Boyle, P.R. Morphometric variation in Loligo forebsi and L. vulgaris: regional, seasonal, sex, maturity and worker differences. Fisheries Research, , 1994; 21: 127-148.
5. Inaba, A. Notes on the chromosomes of two species of octopods (Cephalopoda, Mollusca), Japans Journal of Genetic. 2007; 34: 137-139.
6. Khromov, D.N. Distribution patterns of Sepiidae. Smithsonian Institution Contribution to Zoology, 1998; 586: 191-206.
7. Meriem, S.B., Mathews, C.P., Al- Mamry, J. and Al- Rosadi, I. Stock assessment of the cuttlefish stock

Sepia pharaonis in the Gulf of Oman. International Conference On Fisheries, Aquaculture and Environment in the NW Indian Ocean, Sultan Qaboos University, Mascot, Sultanate of Oman, 2001; 7: 91-97.
Nakamura, H.K. A review of Mollusca cytogenetic for Mollusca chromosome. Bivalvia, Polyplacophora and Cephalopoda, Venus Japens Journal Malacology. 1985; 44: 193-226.
8. Nishinguchi, M.K., Mapes, R. Cephalopoda. In: Mollusca evolution. Journal of Mollusca Studies, 2007; 1464-3766.
9. Roper, C.F., Sweeney, M.J. and Nauen, C.E. Cephalopods of the world. An Annotated and Illustrated Catalogue of Species Know to date. Volume I. Chambered Nautilus and Sepioids. 2005: 106-108.
10. Silas, E.G., Sarvesan, R., Nair, K.P., Sastri, Y.A., Sreenivasan, P.V., Meiyappan, M.M., Vidyasagar, K., Rao, K.S. and Rao, B.N. Some aspects of the biology of cuttlefishes. Cephalopod bionomics fisheries and resources of the exclusive economic zone on India. Central marine Fisheries Research Inst. Cochin, India. 1985; 37:49-70.
11. Strugnell, J. and Nishinguchi, M.K. Molecular phylogeny of coleoid cephalopods (Mollusca: Cephalopoda) inferred from three Mitochondrial and Nuclear loci: A comparison of alignment, implied alignment and analysis methods. 2007: 205- 220.
12. Verbeke, W. Agriculture and the food industry in the information age. Journal of Agricultural and Economics. Oxford University Press, 2005; 32: 347-368.
13. Yuen main. G. and Yutaka, N.;
Karyological studies on seven cephalopods. The Japanese Journal of Malacology. 2005; 49(2): 126-145.

6/3/2011