



Cytogenetic characterization and Molecular genetic variations of five marine species of Perciform fishes

KEYWORDS

Genetics - Cytogenetic – Chromosomes – DNA - RAPD-PCR – Fishes – Labridae - Perciformes

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ABSTRACT

The chromosomal analysis of five species of order Perciformes, species *Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Cheilinus mentalis*, *Cheilinus digrammus* and *Cheilio inermis* were carried out; all samples were collected from Hurgada, on the Egyptian Red Sea coast. The diploid chromosome numbers and fundamental numbers for five species under study were $2n=40$ and $FN=54$, $2n=40$ and $FN=54$, $2n=44$ and $FN=60$, $2n=40$ and $FN=52$ and $2n=48$ and $FN=66$ respectively, they were different in their karyotypes. The RAPD-PCR analysis was carried out on species under studies by using eight primers. All eight primers amplified successfully on the genomic DNA extracted from all studied fish species. Using RAPD-PCR is very useful in determination genetic molecular variations and relationship degree between the species which belong to same family.

Introduction

The Red Sea is home of unique fauna due to its almost isolated position near the Indian ocean, over ten percent of its fish species are endemic found exclusively between the Suez canal in the north and the Gate of Lamentations (Bab-el-Mandab) in the south Debelius, (2007).

The genetic structure represents one of a very important elements for the taxonomy used by the modern cytotaxonomists, so the chromosomal analysis and karyotype studies have become an invaluable tool contributing to the solution of many systematic and evolution problems Rab et al. (1991). Cytogenetic is a branch of genetics that is concerned with study of the structure and function of the cell, especially the chromosomes Baltimore and Williams, (2006).

Karyological studies have provided basic information on the number, size and morphology of chromosomes that is important to undertake chromosome manipulations in fish Khan et al. (2000). Furthermore, the karyotype might characterize a species through the determination of the details (number, size and structure) of its chromosomes Alcocer et al. (1999). Besides karyotype analysis helps to predict, with considerable certainty, the fertility or sterility of hybrids by comparing the number and morphology of the chromosomes of parental species Serebryakova, (1972).

The diploid chromosome number varies from $2n=22-26$, in some species of Nototheniidae, an Antarctic fish group, to $2n=240-260$ in some anadromous Acipenseridae, which show several micro-chromosomes Ozouf-Costaz et al. (1997).

The family Labridae (Wrasses) contains approximately 500 species distributed into 60 genera, and so far only 47 species (belonging to 21 genera) of them are cytogenetically studied (Arkhipchuk, (1999); Brum, (1996); Klinkhardt et al. 1995; Manna, 1989; Ojima and kashiwagi, (1989) and Vasil'ev, (1980)).

In the Labridae, although most species have been reported with $2n=48$, the number of chromosome arms (FN) is often higher ($FN=48-90$), indicating a predominant occurrence of pericentric inversions within this group Alvarez et al. (1986).

Random amplified polymorphic DNA (RAPD) has been widely used in the last decade in species identification program, and in assessing genetic variations among different taxa at DNA level because of its cost effectiveness and simple operation without requiring prior knowledge of species DNA sequences (Schnell et al. (1995) and Frankel et al. (1997).

Atienzar et al. (2002) and Brahmane et al. (2006) and (2008), reported that, the main advantages in the use of the RAPD method lie in its rapidity, applicability to any organism without prior information on the nucleotide sequence, cell cycle, karyotype and potential detection of a variety of DNA damage and mutations at the whole genome level. Random amplified polymorphic DNA (RAPD) is one of the molecular techniques that has benefited from the advent of the PCR.

According to Atienzar and Jha (2006) and Fouz et al. (2007), the RAPD method has been initially used to detect polymorphism in genetic mapping, taxonomy and phylogenetic studies and later in genotoxicity and carcinogenesis studies. In addition, the fact that the RAPD assay allows the visualization of a wide range of PCR products may explain why this assay is a preferred choice. Also the random amplified polymorphic DNA (RAPD) is a useful assay for the detection of genotoxin-induced damage and mutations.

In spite of the fact there have been considerable development of cytotaxonomic studies in the last twenty years, very little information are available on the cytogenetic characteristics of marine fishes in Egypt especially in the Red Sea. So the aim of the present study is fill this gap and also to indicate the taxonomic and phylogenetic relationship of five species of order Perciformes, *Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Cheilinus mentalis*, *Cheilinus digrammus*, *Cheilio inermis*.

Materials and Methods

Five species of *Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Cheilinus mentalis*, *Cheilinus digrammus* and *Cheilio inermis*, were collected from Red Sea (Egypt), transported to the laboratory of "National Institute of Oceanography and Fisheries (Red Sea Branch)" and kept alive until processed.

Chromosome preparation and staining:

Metaphases were obtained from cephalic kidney, spleen and gills after injection of 0.05% colchicines for approximately 2 hr, following the standard air-drying procedure Nirchio and Cequea (1998). Chromosomal morphology was analysed through high quality spread photographs according to Leven et al. (1964).

Polymerase Chain Reaction (PCR) protocol:

The genomic DNA was extracted using ALPHA DNA kits following the manufacturer's protocol. eight primers OPA12, 5'-TCGGCGATAG -3', OPA14, 5'-TCTGTGCTGG -3', OPA15, 5'-TTCCGAACCC -3', OPA17, 5'-GACCGCTTGT -3', OPA18 5'-AGGTGACCGT -3', OPA19 5'-CAAACGTCGG -3', OPA20, 5'-GTTGCGATCC -3' and OPO11 5'-GACAGGAGGT -3', were designed and worked consistently among Labrida species, yielding a product of 200 – 2700 base pairs. Thermal cycling in PCR reaction consisted of an initial step of 95 C for 1 min followed by 55 cycles of 20 s at 94 C, 30 s at 37 C and a final extension of 2 min at 72 C as described by Nadig et al. (1998).

Each sample was analyzed in agarose gel prepared in 10 mM tris-HCL (ph 7.6), 10 mM EDTA, 0.005% bromophenol blue, 0.005% xylene cyanide and 10% glycerol. The gel was stained with ethidium bromide (1%) through adding 2 ul of this stain/100ml agarose gel and photographed under ultraviolet light. The marker is composed of fourteen chromatography – purified individual DNA fragments (n base pairs): 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, it contains two reference bands (1000 and 500 bp) for easy orientation.

Data analysis

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the 5 sample fish accessions. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical.

The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient Sneath and Sokal, (1973).

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where GS_{ij} is the measure of genetic similarity between individuals i and j , a is the number of bands shared by i and j , b is the number of bands present in i and absent in j , and c is the number of bands present in j and absent in i .

The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) Sneath and Sokal, (1973).

Results

Karyotype analysis:

The chromosomal analysis of five species of order Perciformes, family Labridae: *Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Cheilinus mentalis*, *Cheilinus digrammus*, and *Cheilio inermis* including chromosome number, fundamental number and karyotypes were investigated as follows:

1-Cheilinus lunulatus

The chromosome number of *Cheilinus lunulatus* was scored, the diploid set consists of $2n=40$ and fundamental number

(FN)=54 as shown in (Fig 1). The karyotype consists of three groups; group A contains five metacentric pairs of chromosomes, group B which composed of two submetacentric and group C which consists of thirteen acrocentric pairs of chromosomes.

2-Cheilinus abudjubbe

The photographs of cell spread and karyotypes of this species was found to have a diploid chromosome number of $2n=40$ and fundamental number FN=54, as shown in (fig. 2). This number of chromosomes are allocated into four groups; group A consists of four metacentric pairs of chromosomes; group B is composed of one submetacentric pair; group C has two subtelocentric pairs of chromosomes and group D which composed of thirteen pairs of acrocentric chromosomes.

3- Cheilinus mentalis

The diploid chromosome number of *Cheilinus mentalis* is $2n=44$ and FN=60. For karyotyping, the chromosomes of 10 well-spread metaphase plates were cut out from photographs and paired on the bases of centromeric position and size as shown in fig. (3), and four different groups formed: group A has three metacentric pairs of chromosomes; group B is composed of three submetacentric pairs of chromosomes; group C which consists of two subtelocentric pairs of chromosomes and group D which consists of fourteen pairs of acrocentric pairs of chromosomes.

4-Cheilinus digrammus

The chromosomal analysis of this fish indicated that the diploid chromosome number $2N=40$ and the fundamental number is FN=52 (fig. 4), the chromosomes are arranged in four groups: group A composed of three metacentric chromosome; group B consists of two submetacentric chromosomes; group C has only one subtelocentric chromosome and group D consists of fourteen pairs of acrocentric chromosomes.

5- Cheilio inermis

Chromosomal analysis of *Cheilio inermis* showed the diploid chromosome number of $2n=48$ and FN=66. The mitotic metaphase spread and karyotype are shown in fig. (5). The chromosomes are allocated into four groups; group A consists of four metacentric pairs of chromosomes; group B is composed of one submetacentric chromosome group C composed of four subtelocentric chromosomes and group D contains fifteen acrocentric pairs of chromosomes.

RAPD-PCR analysis:

Eight single primers (OPA-12, OPA-14, OPA-15, OPA-17, OPA-18, OPA-19, OPA-20 and OPO-11) were used in the present investigation to determine the genetic differences among five species of family Labridae (*Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Cheilinus mentalis*, *Cheilinus digrammus*, and *Cheilio inermis*). All eight primers amplified successfully on the genomic DNA extracted from all studied fish species. The eight primers yielded amplification products in the five species of the family Labridae. The number of fragments amplified per primer varied between 4 (OPA-19) and 18 (OPA-18) (11.38 bands/ primer) and had a size range from 190 bp (OPO-18) to 2700 bp (OPA-12). The DNA fragments generated by the eight primers from the genomic DNA of the five species were separated using Agarose gel electrophoresis and illustrated in figs (6, 7, 8, 9, 10, 11, 12 and 13). The banding patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized in tables (2, 3, 4, 5, 6, 7, 8 and 9). The number and positions of the bands depended on species and primer as shown in these tables.

The number of bands was variable in each species. *Cheilinus lunulatus* was the species that produced 46 bands, and in *Cheilinus abudjubbe* 44, while in *Cheilinus mentalis* 43 bands; 54 bands in *Cheilinus digrammus*; and 50 bands in *Cheilio inermis*. A total of 91 DNA bands were gener-

ated by all primers in all specimen, out of these DNA bands 65 (71.43%) were conserved among all specimens while 26 bands were polymorphic with percentage 28.57% of all the eight tested primers produced polymorphism in all specimens table 10.

Following are the amplification results of the five species obtained by the examined primers:

Cheilinus lunulatus

All primers amplified yielded distinct RAPD pattern with *Cheilinus undulatus*, the eight primers generated 46 fragments, ranging from 1 by the primer OPA-19 to 11 by the primer OPO-11 and the size of these fragments arranged from 250 bp by the primers OPA-14 and OPA-18 to 1900 bp by the primer OPA-17.

Cheilinus abudjubbe

The RAPD DNA analysis indicated that all amplified primers produced fragments with this fish, 44 are all the fragments varied from 1 by the primers OPA-15 and OPA-19 to 10 by the primer OPO-11, the size of these fragments varies from 250 bp by the primers OPA-14 and OPA-18 to 2500 bp by the primers OPA-14 and OPA-17.

Cheilinus mentalis

The RAPD analysis of *C. mentalis* illustrated that all primers except OPA-12 gave 43 fragments, ranging from 2 bands by the primer OPA-19 and 10 bands by the primers OPA-18 and OPO-11. The size of fragments ranged from 250 bp by the primer OPA-14 to 2100 bp by the primer OPA-17.

Cheilinus digrammus

Random amplified polymorphic DNA (RAPD) technique was used to examine the genetic variability on *C. digrammus* produced different RAPD band patterns of number of 54 bands ranged approximately from 190 bp by the primer OPA-18 to 2700 bp by the primer OPA-12. The generated bands ranged in number from 2 by the primer OPA-19 to 10 by the primer OPA-18.

Cheilio inermis

Random amplified polymorphic DNA (RAPD) technique was used to examine the genetic variability on *Cheilio inermis* produced different RAPD band patterns of number of 50 bands ranged approximately from 250 bp by the primer OPA-18 to 2100 bp by the primer OPA-17. The generated bands ranged in number from 2 by the primer OPA-19 to 11 by the primer OPA-18.

The Dice coefficient illustrated the genetic similarity between the five species, and the UPGMA clustering pattern are shown in Table (11) and Figure (14). The highest genetic distance was observed between *Cheilinus lunulatus* and *Cheilinus abudjubbe* (80%) and the lowest between *Cheilinus lunulatus* and *Cheilinus mentalis* (53%). The UPGMA dendrogram shows two groups, group A in which *Cheilinus lunulatus* and *Cheilinus abudjubbe* were clustered together, group B which consists of two clusters one contained *Cheilio inermis* alone while the other contained *Cheilinus mentalis* and *Cheilinus digrammus* indicating the close genetic relationship among species.

To the best of author knowledge these results are reported for the first time in Egypt, this investigation confirmed that the polymerase chain reaction is very important nowadays to the taxonomists specially in sibling species in addition to the morphological and anatomical characters.

Discussion

Cytogenetic analysis:

The present work is contribution to the karyological data on marine fish species, a group that has been relatively less studied than freshwater species. Considering the order Perci-

formes, karyotypes of approximately 600 species have been analyzed so far, which correspond to 7% of the number of described species for this order Affonso, (2000).

The present cytogenetic studies; chromosome number, karyotype and fundamental number of five species of order Perciformes belonging to family Labridae are agree with that reported by several authors (Cano et al. (1981); LeGrand and Fitzsimons, (1988); Caputo et al. (1996b); Aguilar and Galetti, (1997); Galetti et al. (1999); Affonso et al. (2002); Nagpure et al. (2006); Rocha and Molina, (2008); Kushwaha et al. (2011) and Calado et al. (2012)) who reported that the order Perciformes show little chromosome divergence, approximately 60% of the Perciformes species showed a karyotype characterized by 48 uniarmed (acrocentric) chromosomes.

The chromosome number $2n=40$ and $2n=48$ of the present study of ten species belonging to family Labridae is agree with that reported by (Ojima and Kashiwagi (1980), Cano et al. (1982), Ojima, (1983), Klinkhardt et al. (1995), Ramon, (2002), Sena, (2003) and Kushwahe et al. (2011)) who concluded that the diploid chromosome number of family Labridae ranged from $2n=22$ to $2n=54$.

In the family Labridae, although most species have been reported with $2n=48$ the number of chromosome arms (FN) is often higher (48 - 90), indicating a predominant occurrence of pericentric inversions within this group Alvarez, et al. (1986).

RAPD-PCR analysis:

This study reports on the use of RAPD markers for studying genetic similarity among five species (family, Labridae) of Red Sea coral reef fishes in Egypt for the first time. The RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms (Chalmers et al. (1992); Bardakci and Skibinski, (1994); Greef and Triest, (1999); Barman et al. (2003); Soliman et al. (2003) and Ali, (2003))

RAPD bands in this study were always variant (i.e. strong, faint, fuzzy and sharp bands) generated with each primer because one or more copies of DNA may exist per genome or may be attributed to the varying of the annealing process between the primer and the DNA, this problem of mixed bands shows the well known sensitivity PCR's Bielawski et al. (1995).

RAPD fragments generated by the all eight primers, there were high degree of polymorphism; their sequences may be considered as more conserved sequences which are most useful in higher taxonomic levels and evolutionary relationships.

The description of this similarity coefficient is not simple, especially when more than one character is involved in the same cluster. Thus *Cheilinus lunulatus* and *Cheilinus mentalis* and *Cheilinus digrammus* are found to have a similarity coefficient of 80%, while between *Cheilinus lunulatus* and *Cheilinus mentalis* is 53%, between *Cheilinus mentalis* and *Cheilinus digrammus* is 63%, and between *Cheilinus lunulatus* and *Cheilio inermis* 63%. (The UPGMA dendrogram among the species under study is presented in fig (14).

According to Atienzar and Jha (2006) and Fouz et al. (2007), the RAPD method has been initially used to detect polymorphism in genetic mapping, taxonomy and phylogenetic studies and later in genotoxicity and carcinogenesis studies. In addition, the fact that the RAPD assay allows the visualization of a wide range of PCR products may explain why this assay is a preferred choice. Also the random amplified polymorphic DNA (RAPD) is a useful assay for the detection of genotoxin-induced damage and mutations.

It can be concluded also that, RAPD-PCR could prove to be a useful tool for estimating the genetic variability and degree of similarity among fish species.

Table (1): Review of the cytogenetic data in the Family Labridae

| Species | Locality | 2N | FN | Karyotypic Formula | Reference | |
|---------------------------------|---------------------------------------|---------------------------------------|--------|---------------------------|---------------------------|---------------------------|
| <i>Cheilinus bimaculatus</i> | Mar Tirreno, Nettynol (Roma) | 32 | 38 | 4m+2sm+26st/a | Ojima and Kashiwagi, 1980 | |
| <i>C.fasciatus</i> | | 48 | 60 | 12sm+34st/a | Ojima, 1983 | |
| <i>Cirrhilabrus cyanopleura</i> | | 34 | 46 | 10m+2sm+22st/a | Ojima and Kashiwagi, 1980 | |
| <i>C. temminckii</i> | | 34 | 46 | 10m+2sm+22st/a | Ojima and Kashiwagi, 1980 | |
| <i>Crenilabrus melops</i> | | 46 | 56 | 10 m and 36 st | Cataudella et al.1973 | |
| <i>C. griseus</i> | | 48 | 76 | 2m+26sm+20st/a | Klinkhardt et al. 1995 | |
| <i>C. ocellatus</i> | | 38 | 84 | 36m/sm/st+12a | Klinkhardt et al., 1995 | |
| <i>C. quinque maculatus</i> | | 38 | 74 | 14m+22sm+2st | Klinkhardt et al. 1995 | |
| <i>C. tinca</i> | | 48 | 82 | 34m/sm/st+14a | Klinkhardt et al.,1995 | |
| <i>Epibulus insidiator</i> | | 48 | 60 | 4m+8sm+36st/a | Klinkhardt et al. 1995 | |
| <i>Hemipteronotus dea</i> | | 44 | 44 | 44st/a | Ojima and Kashiwagi, 1980 | |
| <i>H. taeniurus</i> | | 48 | 52 | 4sm+44st/a | Ojima and Kashiwagi, 1980 | |
| <i>Symphodus mediterraneus</i> | | 46 | 52 | 6m/sm+40st/a | Cano et al. 1982 | |
| <i>S.melops</i> | | 46 | 92 | 46m/sm | Lopez et al. 1989 | |
| <i>S.roissali</i> | | 38 | 76 | 10m+28m/sm/st | Lopez et al. 1989 | |
| <i>S. scina</i> | | 48 | 86 | 2m+36sm+10st/a | Klinkhardt et al. 1995 | |
| <i>Xyrichtys pavo</i> | | 48 | 56 | 8sm+40a | Klinkhardt et al. 1995 | |
| <i>X. dea</i> | | 44 | 44 | 44a | Vitturi et al. 1989 | |
| <i>X. twistii</i> | | 44 | 44 | 44a | Ueno and Takai,2000 | |
| <i>X.novacula</i> | | 22 | 40 | 18m/sm+4a | Ueno and Takai,2000 | |
| <i>B.rufus</i> | | Nara, Japan | 48 | 80 | 8m+14 sm+10st+16a | Sena,2003 |
| <i>B.insulari</i> | | 48 | 78 | 6m+14 sm+10st+18a | Sena,2003 | |
| <i>Cheilio inermis</i> | | Mar Tirreno, Civi- tavecchia | 48 | 72 | 12m+12sm+24st/a | Ojima and Kashiwagi, 1980 |
| <i>Coris aygula</i> | 48 | | 60 | 6m+6sm+36st/a | Ojima, 1983 | |
| <i>C. gaimardi</i> | 48 | | 60 | 2m+10sm+36st/a | Ojima and Kashiwagi, 1980 | |
| <i>C. julis</i> | 48 | | 58 | 10m/sm+38st/a | Duchac et al. 1982 | |
| <i>C. multicolor</i> | 48 | | 62 | 6m+8sm+34st/a | Ojima and Kashiwagi, 1980 | |
| <i>Gomphosus varius</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>Halichoeres radiates</i> | 48 | | 48 | 48a | Sena & Molina, 2007 | |
| <i>H. brasiliensis</i> | 48 | | 48 | 48a | Sena & Molina, 2007 | |
| <i>H. poeyi</i> | 48 | | 52 | 4m+44st/a | Sena & Molina, 2007 | |
| <i>H.centriquadus</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>H. kallochroma</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>H. melanochir</i> | 48 | | 50 | 2m+46st/a | Ojima and Kashiwagi, 1980 | |
| <i>Hemigymus fasciatus</i> | 48 | | 60 | 6m+6sm+36st/a | Ojima, 1983 | |
| <i>Hologymnosus semidiscus</i> | 48 | | 86 | 8m+30sm+10st/a | Ojima and Kashiwagi, 1980 | |
| <i>Labroides dimidiatus</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>Pseudolabrus japonicas</i> | 48 | | 52 | 2m+2sm+44st/a | Ojima and Kashiwagi, 1980 | |
| <i>Stethojulis banddanesis</i> | 48 | | 52 | 4m+44st/a | Ojima and Kashiwagi, 1980 | |
| <i>S. interrupta</i> | Brazilian coastline | | 48 | 50 | 2sm+46st/a | Ojima and Kashiwagi, 1980 |
| <i>S. strigiventer</i> | 48 | | 50 | 2m+46st/a | Ojima and Kashiwagi, 1980 | |
| <i>Thalassoma cupid</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>T. amblycephalum</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>T. lunare</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>T. lutenscens</i> | 48 | | 48 | 48st/a | Ojima and Kashiwagi, 1980 | |
| <i>T. pavo</i> | 48 | 48 | 48a | Cano et al. 1982 | | |
| <i>T. bifasciatum</i> | 48 | 48 | 48a | Ramon, 2002 | | |
| <i>T. quinquevittatum</i> | 48 | 48 | 48st/a | Ojima and Kashiwagi, 1980 | | |
| <i>Thalassoma lunare</i> | West coast of India | 48 | 48 | 48 a. | Kushwaha et al. 2011 | |
| <i>Zanclus cornutus</i> | | 48 | 48 | 48 a. | | |
| <i>Arius subrostratus</i> | | 54 | 96 | 22 m+16 sm+10 st+10a. | | |

(2N) diploid number, (FN) fundamental number, (a) acrocentric, (st) subtelocentric, (sm) sub-metacentric and (m) metacentric.

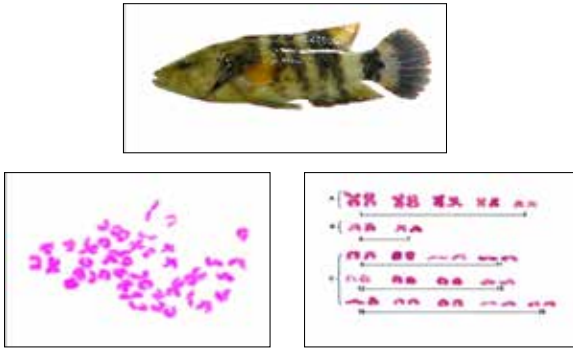


Fig (1): A coloured photograph, Chromosomes spread and karyotype of *Cheilinus lunulatus*.

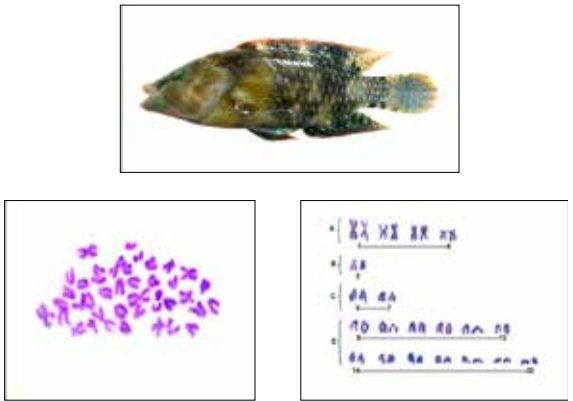


Fig (2) A coloured photograph, Chromosomes spread and karyotype of *Cheilinus abudjubbe*.

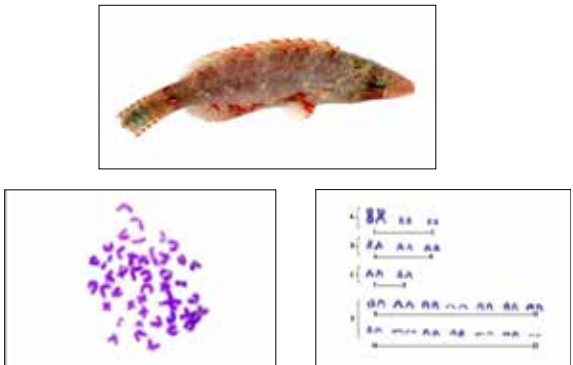


Fig (3) A coloured photograph, Chromosomes spread and karyotype of *Cheilinus mentalis*.

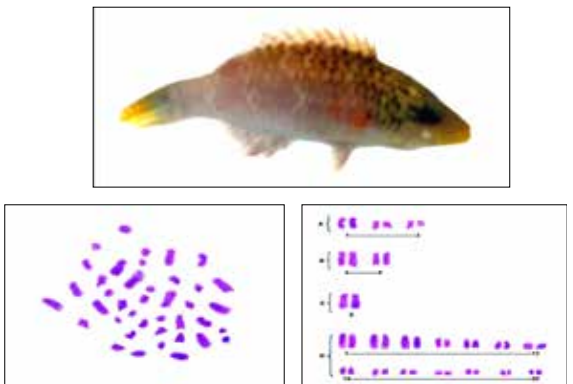


Fig (4) A coloured photograph, Chromosomes spread and karyotype of *Cheilinus digrammus*.

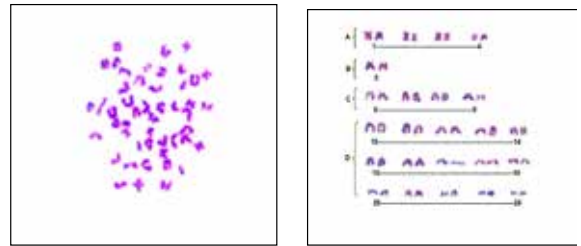
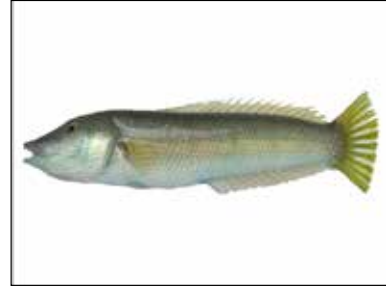


Fig (5) A coloured photograph, Chromosomes spread and karyotype of *Cheilio inermis*.

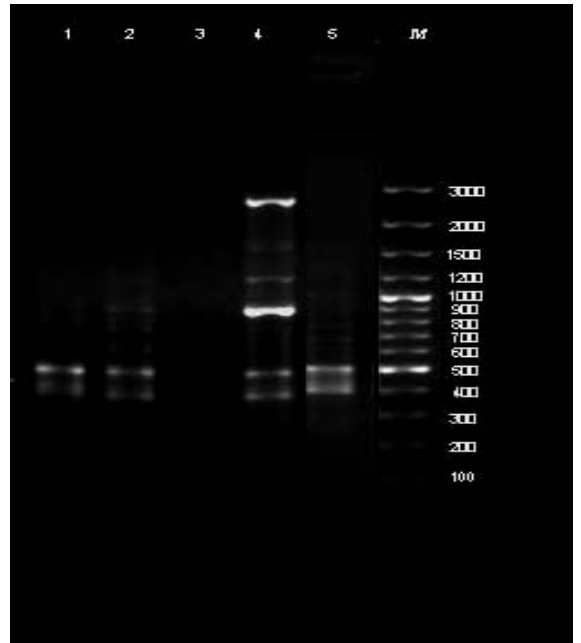


Fig (6): Agarose gel electrophoresis of RAPD products generated with OPA-12.

Where 1-*Cheilinus lunulatus*, 2-*Cheilinus abudjubbe*, 3-*Cheilinus mentalis*,

4-*Cheilinus digrammus*, 5-*Cheilio inermis*

| Band No. | RAPD marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 2700 | 0 | 0 | 0 | 1 | 0 |
| 2 | 1200 | 0 | 0 | 0 | 1 | 0 |
| 3 | 880 | 1 | 1 | 0 | 1 | 0 |
| 4 | 750 | 1 | 1 | 0 | 1 | 1 |
| 5 | 600 | 1 | 1 | 0 | 1 | 0 |
| 6 | 530 | 0 | 1 | 0 | 1 | 0 |
| 7 | 400 | 1 | 1 | 0 | 1 | 1 |
| 8 | 350 | 1 | 1 | 0 | 1 | 0 |
| 9 | 310 | 1 | 1 | 0 | 1 | 1 |

Table (2): Survey of RAPD markers using primer OPA-12 of five labrids specimens.

Where 1-Cheilinus lunulatus, 2-Cheilinus abudjubbe, 3-Cheilinus mentalis,

4-Cheilinus digrammus, 5-Cheilio inermis.

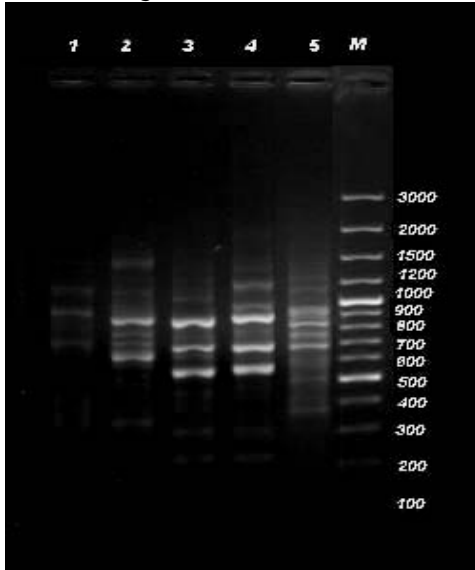


Fig (7): Agarose gel electrophoresis of RAPD products generated with OPA-14.

| Band No. | RAPD marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 2500 | 0 | 1 | 0 | 0 | 0 |
| 2 | 1350 | 0 | 0 | 0 | 1 | 0 |
| 3 | 1150 | 1 | 1 | 0 | 0 | 1 |
| 4 | 1050 | 1 | 1 | 1 | 0 | 0 |
| 5 | 970 | 0 | 0 | 0 | 1 | 0 |
| 6 | 860 | 1 | 1 | 1 | 1 | 1 |
| 7 | 780 | 1 | 1 | 1 | 1 | 1 |
| 8 | 700 | 1 | 1 | 1 | 1 | 0 |
| 9 | 600 | 0 | 0 | 0 | 0 | 1 |
| 10 | 500 | 0 | 0 | 1 | 0 | 1 |
| 11 | 360 | 0 | 1 | 0 | 0 | 1 |
| 12 | 300 | 0 | 0 | 1 | 1 | 0 |
| 13 | 250 | 1 | 1 | 1 | 1 | 0 |

Table (3): Survey of RAPD markers using primer OPA-14 of five labrids specimens.

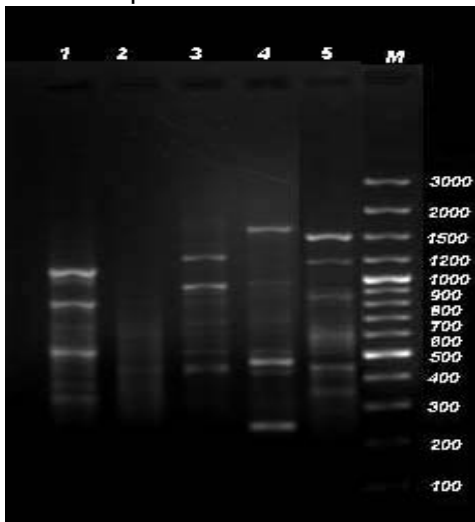


Fig (8): Agarose gel electrophoresis of RAPD products generated with OPA-15.

| Band No. | RAPD marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 1400 | 0 | 0 | 0 | 1 | 1 |
| 2 | 1000 | 1 | 0 | 0 | 0 | 0 |
| 3 | 900 | 0 | 0 | 1 | 1 | 1 |
| 4 | 700 | 0 | 0 | 0 | 0 | 1 |
| 5 | 650 | 1 | 0 | 0 | 0 | 1 |
| 6 | 600 | 1 | 0 | 1 | 1 | 1 |
| 7 | 550 | 1 | 0 | 0 | 1 | 0 |
| 8 | 500 | 1 | 1 | 1 | 1 | 1 |
| 9 | 400 | 1 | 0 | 0 | 0 | 0 |
| 10 | 350 | 1 | 0 | 0 | 0 | 1 |
| 11 | 280 | 0 | 0 | 0 | 1 | 0 |

Table (4): Survey of RAPD markers using primer OPA-15 of five labrids specimens.

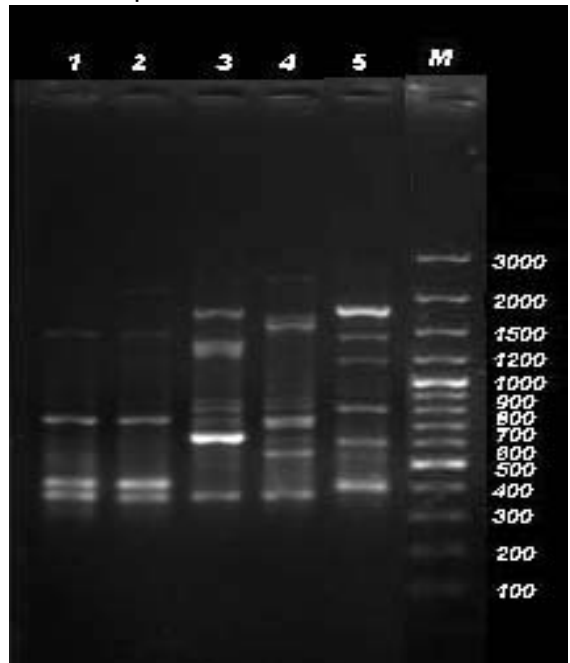


Fig (9): Agarose gel electrophoresis of RAPD products generated with OPA-17.

| Band No. | RAPD Marker bp | 1 | 2 | 3 | 4 | 10 |
|----------|----------------|---|---|---|---|----|
| 1 | 2500 | 0 | 1 | 0 | 0 | 0 |
| 2 | 2100 | 0 | 0 | 1 | 1 | 1 |
| 3 | 1900 | 1 | 1 | 0 | 1 | 1 |
| 4 | 1500 | 0 | 0 | 1 | 0 | 1 |
| 5 | 1200 | 0 | 0 | 0 | 0 | 1 |
| 6 | 760 | 0 | 0 | 1 | 1 | 0 |
| 7 | 700 | 1 | 1 | 1 | 1 | 1 |
| 8 | 550 | 0 | 0 | 1 | 1 | 0 |
| 9 | 500 | 0 | 0 | 0 | 1 | 1 |
| 10 | 400 | 1 | 1 | 0 | 0 | 0 |
| 11 | 350 | 1 | 1 | 1 | 1 | 1 |

Table (5): Survey of RAPD markers using primer OPA-17 of five labrids specimens.

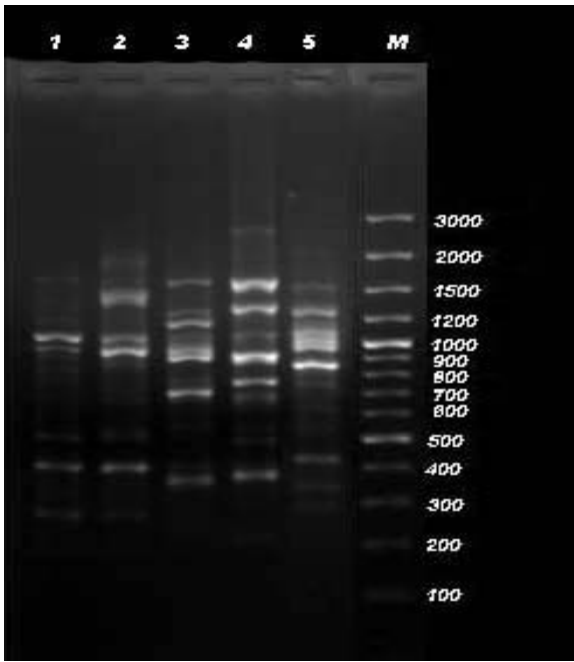


Fig (10): Agarose gel electrophoresis of RAPD products generated with OPA-18.

| Band No. | RAPD Marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 1900 | 0 | 1 | 0 | 0 | 0 |
| 2 | 1500 | 1 | 0 | 1 | 1 | 1 |
| 3 | 1400 | 0 | 0 | 1 | 0 | 0 |
| 4 | 1250 | 0 | 0 | 1 | 0 | 1 |
| 5 | 1150 | 1 | 1 | 0 | 1 | 1 |
| 6 | 1050 | 1 | 1 | 1 | 1 | 1 |
| 7 | 950 | 1 | 1 | 1 | 1 | 0 |
| 8 | 870 | 1 | 1 | 1 | 1 | 0 |
| 9 | 800 | 0 | 0 | 0 | 0 | 1 |
| 10 | 760 | 0 | 0 | 0 | 0 | 1 |
| 11 | 700 | 0 | 0 | 1 | 1 | 1 |
| 12 | 620 | 0 | 0 | 1 | 0 | 0 |
| 13 | 550 | 0 | 0 | 1 | 1 | 1 |
| 14 | 500 | 1 | 1 | 0 | 1 | 1 |
| 15 | 370 | 0 | 0 | 1 | 1 | 0 |
| 16 | 320 | 0 | 0 | 0 | 0 | 1 |
| 17 | 250 | 1 | 1 | 0 | 0 | 1 |
| 18 | 190 | 0 | 0 | 0 | 1 | 0 |

Table (6): Survey of RAPD markers using primer OPA-18 of five labrids specimens.

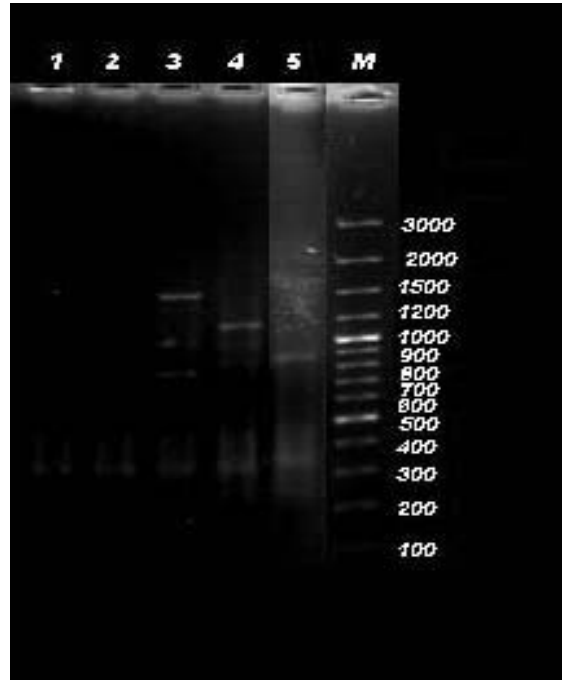


Fig (11): Agarose gel electrophoresis of RAPD products generated with OPA-19.

| Band No. | RAPD marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 1600 | 0 | 0 | 1 | 0 | 0 |
| 2 | 1100 | 0 | 0 | 0 | 1 | 0 |
| 3 | 900 | 0 | 0 | 0 | 0 | 1 |
| 4 | 300 | 1 | 1 | 1 | 1 | 1 |

Table (7): Survey of RAPD markers using primer OPA-19 of five labrids specimens.



Fig (12): Agarose gel electrophoresis of RAPD products generated with OPA-20.

| Band No. | RAPD marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 1500 | 1 | 1 | 1 | 1 | 1 |
| 2 | 1300 | 0 | 0 | 0 | 1 | 0 |
| 3 | 1100 | 0 | 0 | 1 | 0 | 0 |
| 4 | 800 | 0 | 0 | 0 | 1 | 1 |
| 5 | 700 | 0 | 1 | 0 | 0 | 0 |
| 6 | 550 | 1 | 1 | 1 | 1 | 1 |
| 7 | 500 | 1 | 1 | 1 | 0 | 1 |
| 8 | 300 | 1 | 1 | 1 | 1 | 1 |

Table (8): Survey of RAPD markers using primer OPA-20 of five labrids specimens.



Fig (13): Agarose gel electrophoresis of RAPD products generated with OPO-11.

| Band No. | RAPD Marker bp | 1 | 2 | 3 | 4 | 5 |
|----------|----------------|---|---|---|---|---|
| 1 | 1300 | 1 | 1 | 0 | 0 | 0 |
| 2 | 1200 | 1 | 1 | 1 | 1 | 0 |
| 3 | 1100 | 0 | 0 | 1 | 1 | 0 |
| 4 | 1000 | 0 | 0 | 1 | 1 | 1 |
| 5 | 930 | 1 | 1 | 1 | 1 | 0 |
| 6 | 870 | 1 | 0 | 0 | 0 | 1 |
| 7 | 800 | 0 | 0 | 1 | 1 | 0 |
| 8 | 750 | 1 | 0 | 0 | 0 | 1 |
| 9 | 620 | 1 | 1 | 1 | 0 | 1 |
| 10 | 550 | 1 | 1 | 0 | 0 | 1 |
| 11 | 500 | 1 | 1 | 1 | 0 | 0 |
| 12 | 460 | 1 | 1 | 1 | 0 | 1 |
| 13 | 420 | 0 | 0 | 0 | 1 | 0 |
| 14 | 370 | 1 | 1 | 1 | 1 | 1 |
| 15 | 350 | 1 | 0 | 0 | 0 | 1 |
| 16 | 300 | 0 | 1 | 0 | 0 | 1 |
| 17 | 280 | 0 | 1 | 1 | 1 | 0 |

Table (9): Survey of RAPD markers using primer OPO-11 of five labrids specimens.

Table (10): Number of amplified and polymorphic DNA-fragments in the five specimens.

| No. | Primer code | No. of amplified bands | | | | | Total amplified bands | No. of polymorphic bands | Polymorphism % |
|-------|-------------|------------------------|---------------------------|-------------------------|-------------------------|---------------------|-----------------------|--------------------------|----------------|
| | | (1) Cheilinus latus | (2) Cheilinus abud-jubbe, | (3) Cheilinus mentalis, | (4) Cheilinus digrammus | (5) Cheilio inermis | | | |
| 1 | OPA-12 | 6 | 7 | 0 | 9 | 3 | 9 | 2 | 22.22 |
| 2 | OPA-14 | 6 | 8 | 7 | 7 | 6 | 13 | 4 | 30.76 |
| 3 | OPA-15 | 7 | 1 | 3 | 6 | 7 | 11 | 4 | 36.36 |
| 4 | OPA-17 | 4 | 5 | 6 | 7 | 7 | 11 | 2 | 18.18 |
| 5 | OPA-18 | 7 | 7 | 10 | 10 | 11 | 18 | 7 | 38.88 |
| 6 | OPA-19 | 1 | 1 | 2 | 2 | 2 | 4 | 3 | 75.00 |
| 7 | OPA-20 | 4 | 5 | 5 | 5 | 5 | 8 | 3 | 37.50 |
| 8 | OPO-11 | 11 | 10 | 10 | 8 | 9 | 17 | 1 | 5.88 |
| total | | 46 | 44 | 43 | 54 | 50 | 91 | 26 | 28.57 |

Table (11): Similarity Matrix UPGMA Jaccard's Coefficient.

| | 1 | 2 | 3 | 4 | 5 |
|---|-----|-----|-----|-----|-----|
| 1 | 100 | | | | |
| 2 | 80 | 100 | | | |
| 3 | 53 | 52 | 100 | | |
| 4 | 58 | 57 | 63 | 100 | |
| 5 | 63 | 53 | 53 | 52 | 100 |

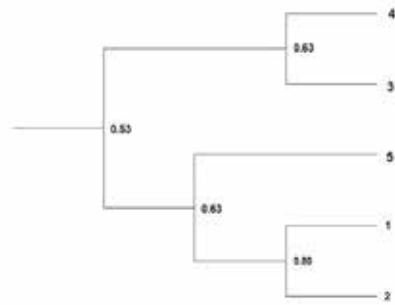


Fig (14): The evolutionary tree of the five marine fish species

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