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Research Article

ZOOLOGY

Karyological and RAPD-PCR analysis of five marine fishes species (Labridae-Perciformes) From Red Sea coral reef

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Abstract

Cytogenetic studies on five Red Sea reef fish species *Larabicus quadrilineatus, Hemigymnus melapterus, Thalassoma rueppellii, Thlassoma lunare and Gomphosus caeruleus* of the family Labridae (Perciformes) were carried out; all samples were collected from Hurghada, on the Egyptian Red Sea coast. All the specimens showed a diploid chromosome number of 2n=48, they were also identical in the karyotype (all acrocentric), and in the Fundamental number FN=48. The RAPD-PCR analysis were carried out by using 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`.

The results were extremely useful to improve the importance of using RAPD-PCR in genetic analysis among the species which are identical in chromosome number and karyotype.

INTRODUCTION

Approximately 1,000 species of fishes occur in Red Sea, many reef fishes exhibit considerable variation in colour, some have a very different pattern as juveniles, others such as wrasses (Labridae) may differ markedly in colour with sexual development (Randall, 1982) Because most morphologic features of fishes have been shown to have the potential of being modified by environmental conditions a morphologically based classification should be tested by features not likely to be environmentally plastic. Chromosome structure is likely to reflect genetic divergence and have a minimum of environmental distortion (Barker, 1972).

About 13,000 marine fish species exist (Nelson, 1994) and so far fewer than 2% of these have been studied cytogenetically (Brum, 1996). 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).

Despite the variation in chromosome number observed among marine fishes, some groups such as Perciformes, with almost 7000 marine species and comprising many living marine teleosts of economic importance, show little chromosome divergence. Approximately 60% of the Perciformes species showed a karyotype characterized by 48 uniarmed (acrocentric) chromosomes (Galetti *et al*., 1999).

The use of molecular techniques has increased dramatically over the past several years, largely due to the development of the polymerase chain reaction (PCR). The random amplification of polymorphic DNA (RAPD), a methodology that employs a single short primer in PCR (Welsh and Mc Clelland 1990; Williams *et al*.,1990), has been widely used in several studies in animals including the identification of fish species (Dinesh *et al*., 1993; Borowsky *et al*.,1995; Sultmann *et al*.,1995; Partis &Wells, 1996 and Callejas &Ochando,1998)and the detection of the population genetic variability in these organisms (Johnson *et al*., 1994; Caccone *et al*., 1997; Nadig *et al*., 1998; Cagigas *et al.*, 1999 and Chong *et al*.,2000).

The purpose of the present study was to describe the chromosomal complements of five species *Larabicus quadrilineatus* (four line wrasse), *Hemigymnus melapterus* (thick lip wrasse), *Thalassoma rueppellii* (klunzinger`s wrasse), *Thlassoma lunare* (moon wrasse) *and Gomphosus caeruleus* (Red Sea bird wrasse) which are distributed in the Red Sea by using Giamsa staining and to utilize the RAPD-PCR analysis to verify the occurrence of genetic variations between the five species.

Materials and Methods

Five species of *Larabicus quadrilineatus, Hemigymnus melapterus, Thalassoma rueppellii, Thlassoma lunare and Gomphosus caeruleus* 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 Labrids 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 cyand ff and 10% glycerol. The gel was stained with ethidium bromide (1%) though 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: GSij = 2a/(2a+b+c)

Where GSij 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

A diploid number of 48 acrocentric chromosomes (FN =48) was observed by the chromosomal analysis in all studied specimens the spreads and karyotypes were illustrated in figs (1, 2, 3, 4 and5).

The G+C contents of the eight primers were 60%. 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 5 (OPA-19) and 20 (OPA-18) (12.63 bands/ primer) and had a size range from 200 bp (OPO-11) to 2500 bp (OPA-17). 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 and13).. 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 and9). 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. *T. lunare* was the species that produced the greatest number of bands (53), and *L. quadrilineatus* the lowest (42) while 52 bands in *H. melapterus*; 48 bands in *T. rueppellii*; and 48 bands in *G. caeruleus.* A total of 101 DNA bands were generated by all primers in all specimen, out of these DNA bands 62 (61.39%) were conserved among all specimens while 39 bands were polymorphic with percentage 38.61% of all the eight tested primers produced polymorphism in all specimens table10.

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

Larabicus quadrilineatus

The eight primers produced 42 bands in *L. quadrilineatus*. The number of bands amplified per primer varied from 1 band by the primer OPA-19 to 9 bands by the primer OPO-11 and had a size arranged from 250 bp by the primer OPO-11 to 2000 bp by the primer OPA-15.

Hemigymnus melapterus

The eight primers produced amplification products with *H.melapterus* of 52 bands ranging from 1 fragment by the primer OPA-19 to 10 fragments by the primer OPA-14, the size of fragments varied from 250 bp by the primer OPO-11 to 2500 bp by the primers OPA-14 and OPA-17.

Thalassoma rueppellii

All the primers amplified successfully yielded distinct RAPD patterns with *T. rueppellii,* the eight primers generated 48 fragments, the number of fragments varied from 2 by the primer OPA-19 to 9 by the primer OPA-14. The size of fragments ranged from 200 bp by the primer OPO-11 to 1900 bp by the primer OPA-17.

Thlassoma lunare

The RAPD-DNA analysis of *T.lunare* indicated that it produced 53 fragments with all eight primers, varied in number from 3 by the primer OPA-15 to 11 by the primer OPO-11, the size of these fragments ranged from 300 bp by

the primers OPA-14, OPA-19 and OPA-20 to 2000 bp by the primers OPA-18 and OPA-20.

Gomphosus caeruleus

Random amplified polymorphic DNA (RAPD) technique was used to examine the genetic variability on *G. caeruleus* produced different RAPD band patterns of number of 48 bands ranged approximately from 200 bp by the primer OPO-11 to 1900 bp by the primer OPA-17. The generated bands ranged in number from 1 by the primer OPA-19 to 12 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 similarity was observed between *L. quadrilinatus* and *G. caeruleus* (62%) and the lowest between *L. quadrilinatus* and *T. rueppellii* (42%). The UPGMA dendrogram shows two groups, group A in which *Hemigymnus melapterus* and *Thlassoma lunare* were clustered together, group B which consists of two clusters one contained *Larabicus quadrilineatus* alone while the other contained *Thalassoma rueppellii* and *Gomphosus caeruleus* 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

Karyotyping analysis:

There have been severed studies in recent times on the cytogenetic studies in the teleostei fishes, Oliveira *et al*., (2000). The karyotype with 2n = 48 acrocentric chromosomes is considered by some authors an inheritance of the first vertebrates (Ohno *et al.*, 1968; Ohno, 1970; Ohno, 1974). However, Brum (1996) and Brum and Galetti (1997) proposed that this karyotype was a synapomorphy of the groups Euteleostei and Clupeiformes conserved mainly in their marine species, which the karyotype derived from a likely ancestry of the vertebrates initially with 60 chromosomes, including the metacentric chromosomes. According to Brum (1995) and Brum *et al*. (1995), the karyotypes derived from this basal complement (48 acroncentric chromosomes) containing two armed chromosomes and resulting in a FN higher than 48 have been found in the groups of the fresh water fishes, where there are many environmental fragmentations, and also in the marine fishes with low vagility that occur in the restricted areas (Galetti *et al*., 1999). In the marine environment, the karyotypic conservation is related to the existence of physical barriers, high mobility, high size of the population and higher homogeneity of the environment conditions (Brum, 1996). The results obtained in the present study showed karyotypes with 2n = 48 chromosomes in the Labridae. The Family Labridae (Wrasses) contains approximately 500 species distributed in to 60 genera, and so far only 47 species (belonging to 21 genera) of them are cytogenetically studied (Vasil`ev,1980; Ojima & Kashiwagi, 1989; Manna,1989; Klinkhardt *et al*.,1995; Brum,1996 and Arkhipchok, 1999). The degree of karyotypic differentiation (number of changes in FN values) in the Family Labridae is inversely related to the dispersive potential provided by the extent of pelagic larval duration of each species (Molina & Galetti, 2004; Molina, 2006 and Sena & Molina 2007). 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 inversion within this group, Alvarez *et al*., 1986 (**Table 1).**

RAPD-PCR analysis:

The RAPD technique for examining genetic variability showed a number of advantages including: no prior knowledge of the DNA sequences of the investigated organism is needed (Welsh and McClelland,1990); its assay is relatively simple, rapid and independent of gene expression (Goodwin and Annis,1991); and only a small amount of DNA is required to perform the reaction (Pinochet *et al*.,1994).The results observed show that the RAPD-PCR assay is capable of revealing polymorphism in species of fishes. The eight primers produced at least two polymorphic fragments.

Some authors have pointed out that, although RAPD-PCR is a powerful technique for detecting random amplified polymorphic DNA and several reasons exist for the amplification of DNA regions, the main shortcoming of this technique is its sensitivity to changes in reaction conditions, where the use of markers often results in imperfect estimation of genetic distances between taxa of supraspecies rank (Rothuizen and Van Wolferen, 1994). In our experiments the results obtained by optimized and repeatable conditions made differences in banding patterns an improbable RAPD artifact.

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 of PCRs (Bielawski *et al*., 1995). RAPD fragments generated by OPA-14 primer produced low polymorphism (15.38%) among the studied fishes. this primer sequences may have annealed to variable sequences which might be of great utility at lower taxonomic levels, e.g. for the differentiation of very related species, however, in RAPD fragments generated by other 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.

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

Species	Locality	2N	FN	Karyotypic Formula	Reference
Bodianus axilaris		48	86	8m+30 sm+10st/a	Ojima and Kashiwagi, 1980
B. loxozonus		48	82	8m+26sm+14st/a	Ojima and Kashiwagi, 1980
B. mesothorax		48	74	8m+18sm+22st/a	Ojima and Kashiwagi, 1980
B.rufus		48	80	8m+14 sm+10st+16a	Sena,2003
B.insularis		48	78	6m+14 sm+10st+18a	Sena,2003
Cheilinus bimaculatus	Mar Tirreno, Nettynol (Roma)	32	38	4m+2sm+26st/a	Ojima and Kashiwagi, 1980
C.fasciatus		48	60	12sm+34st/a	Ojima, 1983
Cirrhilabrus cyanopleura		34	46	10m+2sm+22st/a	Ojima and Kashiwagi, 1980
C. temminckii		34	46	10m+2sm+22st/a	Ojima and Kashiwagi, 1980
Crenilabrus melops		46	56	10 m and 36 st	Cataudella *et al*.,1973
C. griseus		48	76	2m+26sm+20st/a	Klinkhardt *et al*., 1995
C. ocellatus		38	84	36m/sm/st+12a	Klinkhardt *et al*., 1995
C. quinquemaculatus		38	74	14m+22sm+2st	Klinkhardt *et al*., 1995
C. tinca		48	82	34m/sm/st+14a	Klinkhardt *et al*., 1995
Epibulus insidiator		48	60	4m+8sm+36st/a	Klinkhardt *et al*., 1995
Hemipteronotus dea		44	44	44st/a	Ojima and Kashiwagi, 1980
H. taeniurus		48	52	4sm+44st/a	Ojima and Kashiwagi, 1980
Symphodus mediterraneus		46	52	6m/sm+40st/a	Cano *et al*., 1982
S.melops		46	92	46m/sm	Lopez *et al*., 1989
S.roissali		38	76	10m+28m/sm/st	Lopez *et al*., 1989
S. scina		48	86	2m+36sm+10st/a	Klinkhardt *et al*., 1995
Xyrichthys pavo		48	56	8sm+40a	Klinkhardt *et al*., 1995
X. dea	Nara, Japan	44	44	44a	Vitturi *et al*., 1989
X. twistii		44	44	44a	Ueno and Takai,2000
X.novacula		22	40	18m/sm+4a	Ueno and Takai,2000
Cheilio inermis	Mar Tirreno, Civitavecchia	48	72	12m+12sm+24st/a	Ojima and Kashiwagi, 1980
Coris aygula		48	60	6m+6sm+36st/a	Ojima, 1983
C. gaimardi		48	60	2m+10sm+36st/a	Ojima and Kashiwagi, 1980
C. julis		48	58	10m/sm+38st/a	Duchac *et al*.,1982
C. multicolor		48	62	6m+8sm+34st/a	Ojima and Kashiwagi, 1980
Gomphosus varius	Brazilian coastline	48	48	48st/a	Ojima and Kashiwagi, 1980
Halichoeres radiates		48	48	48a	Sena & Molina, 2007
H. brasiliensis		48	48	48a	Sena & Molina, 2007
H. poeyi		48	52	4m+44st/a	Sena & Molina, 2007
H.centriquadus		48	48	48st/a	Ojima and Kashiwagi, 1980
H. kallochroma		48	48	48st/a	Ojima and Kashiwagi, 1980
H. melanochir		48	50	2m+46st/a	Ojima and Kashiwagi, 1980
H.poecilopterus		48	54	4m+2sm+42st/a	Ojima and Kashiwagi, 1980
H. prosopeion		48	50	2m+46st/a	Ojima and Kashiwagi, 1980
H. tenuispinnis		48	50	2m+46st/a	Ojima and Kashiwagi, 1980
H. trimaculatus		48	48	48st/a	Ojima and Kashiwagi, 1980
Hemigymus fasciatus		48	60	6m+6sm+36st/a	Ojima, 1983
Hologymnosus semidiscus		48	86	8m+30sm+10st/a	Ojima and Kashiwagi, 1980
Labroides dimidiatus		48	48	48st/a	Ojima and Kashiwagi, 1980
Pseudolabrus japonicas		48	52	2m+2sm+44st/a	Ojima and Kashiwagi, 1980
Stethojulis banddanesis		48	52	4m+44st/a	Ojima and Kashiwagi, 1980
S. interrupta		48	50	2sm+46st/a	Ojima and Kashiwagi, 1980
S. strigiventer		48	50	2m+46st/a	Ojima and Kashiwagi, 1980
Thalassoma cupid		48	48	48st/a	Ojima and Kashiwagi, 1980
T. amblycephalum		48	48	48st/a	Ojima and Kashiwagi, 1980
T. lunare		48	48	48st/a	Ojima and Kashiwagi, 1980
T. lutenscens		48	48	48st/a	Ojima and Kashiwagi, 1980
T. pavo		48	48	48a	Cano *et al*., 1982
T. bifasciatum	South-West Puerto Rico	48	48	48a	Ramon, 2002
uinquevittatum		48	48	48st/a	Ojima and Kashiwagi, 1980

Thalassoma lunare *Zanclus cornutus* *Arius subrostratus*	West coast of India	48 48 54	48 48 96	48 a. 48 a. 22 m+16 sm+10 st+10a.	Kushwaha *et al*., 2011
Larabicus quadrilineatus *Hemigymnus melapterus,* *Thalassoma rupellei,* *Thlassoma lunare and* *Gomphosus caeruleus*	Hurghada, Red Sea, Egypt.	48 48 48 48 48	48 48 48 48 48	48 a. 48 a. 48 a. 48 a. 48 a.	Present study

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

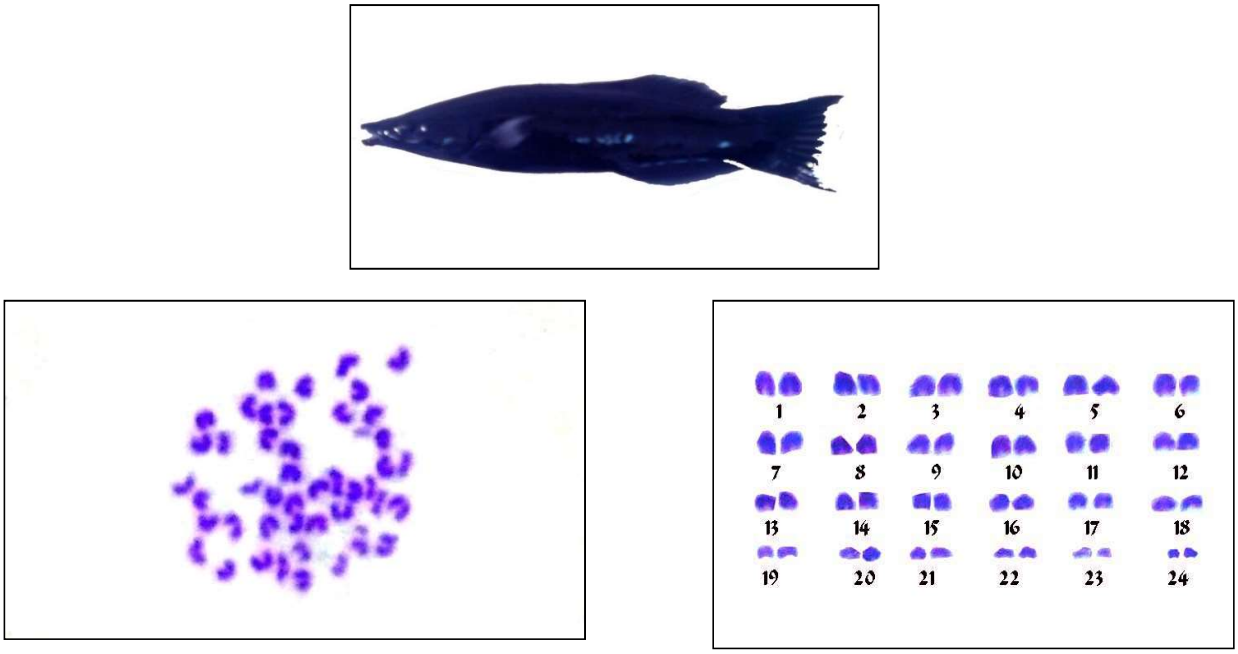


Fig (1): A coloured photograph, Chromosomes spread and karyotype of ***Larabicus quadrilineatus***

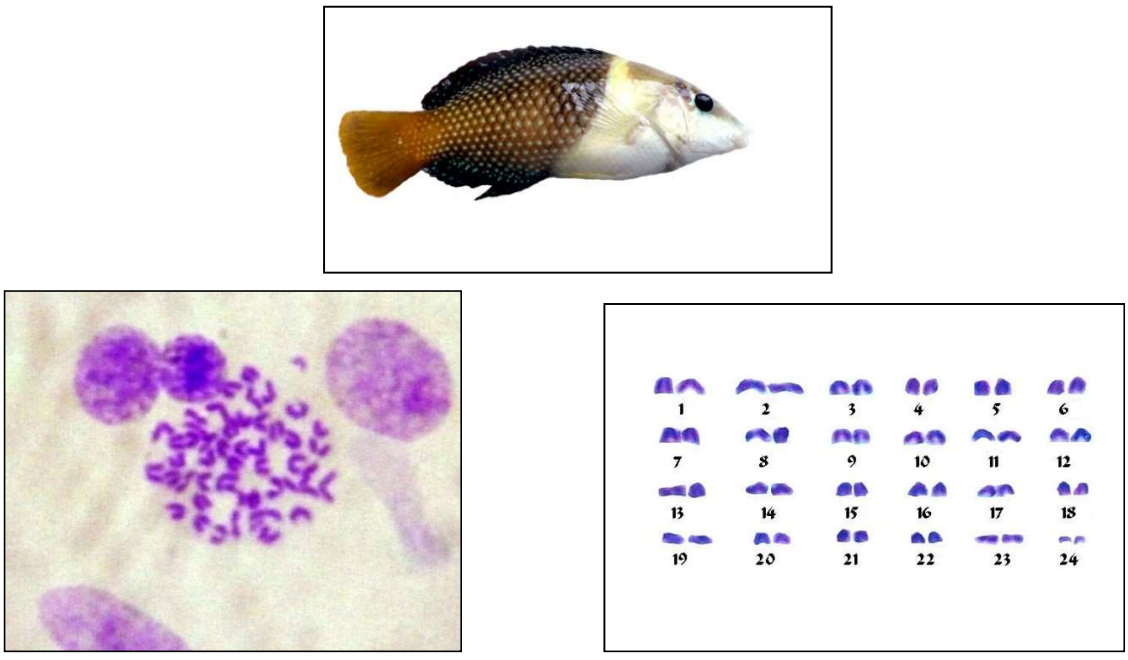
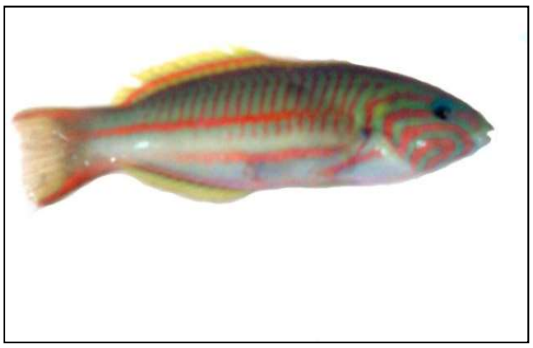
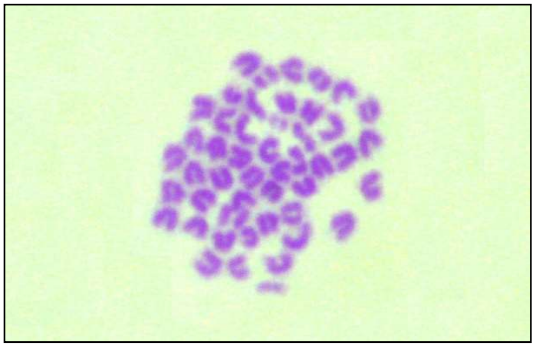


Fig (2) A coloured photograph, Chromosomes spread and karyotype of ***Hemigymnus melapterus***





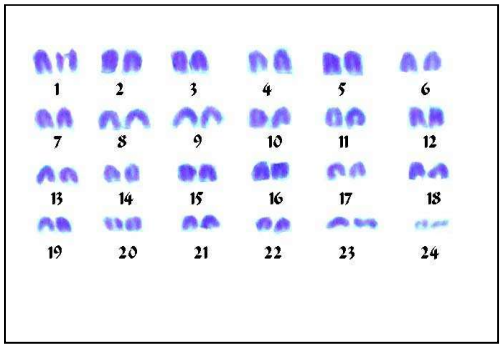
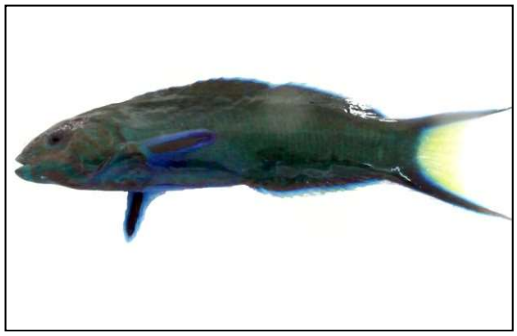
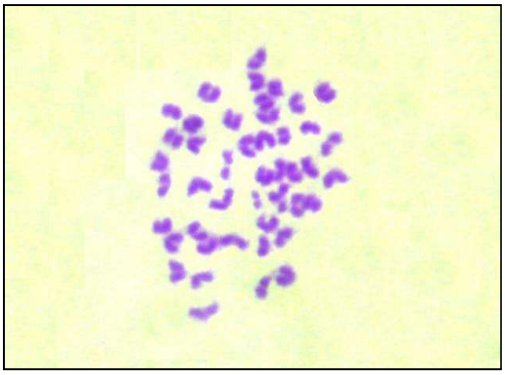


Fig (3) A coloured photograph, Chromosomes spread and karyotype of ***Thalassoma rupellei***





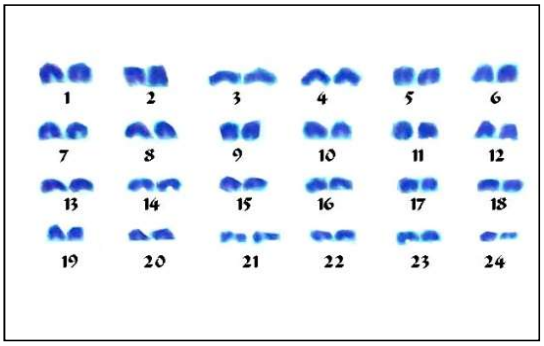


Fig (4) A coloured photograph, Chromosomes spread and karyotype of ***Thalassoma lunare***

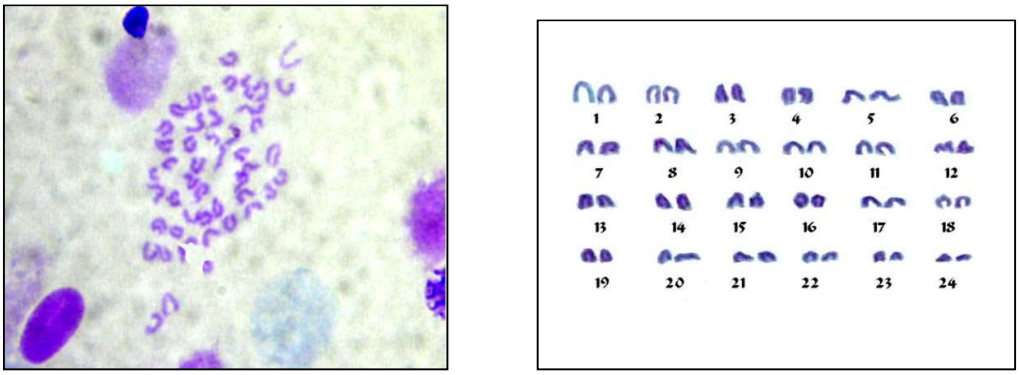


Fig (5) A coloured photograph, Chromosomes spread and karyotype of ***Gomphosus caeruleus***

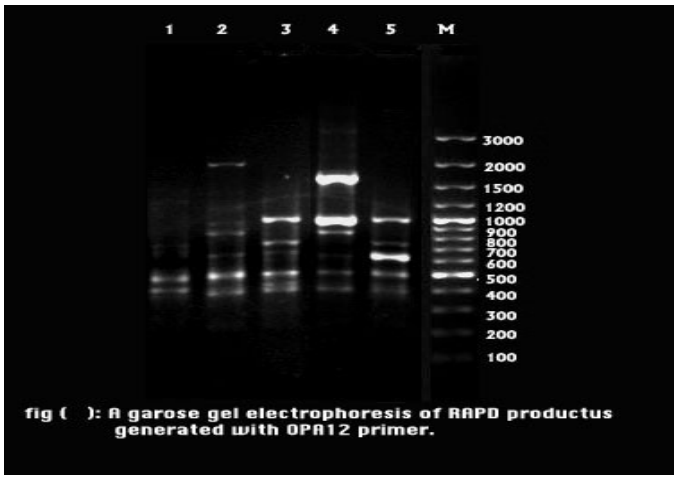


Fig (6): Agarose gel electrophoresis of RAPD products generated with OPA-12.
Where *1- Larabicus quadrilineatus, 2-Hemigymnus melapterus 3-Thalassoma rueppellii, 4-Thlassoma lunare, 5-Gomphosus caeruleus.*

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**2000**	**0**	**1**	**0**	**0**	**0**
2	**1600**	**0**	**0**	**0**	**1**	**0**
3	**1050**	**0**	**1**	**1**	**1**	**1**
4	**880**	**0**	**1**	**0**	**1**	**0**
5	**750**	**1**	**0**	**1**	**0**	**1**
6	**600**	**1**	**1**	**1**	**1**	**1**
7	**530**	**0**	**0**	**1**	**0**	**0**
8	**400**	**1**	**1**	**1**	**1**	**1**
9	**350**	**1**	**0**	**1**	**0**	**1**
10	**310**	**0**	**1**	**1**	**1**	**1**

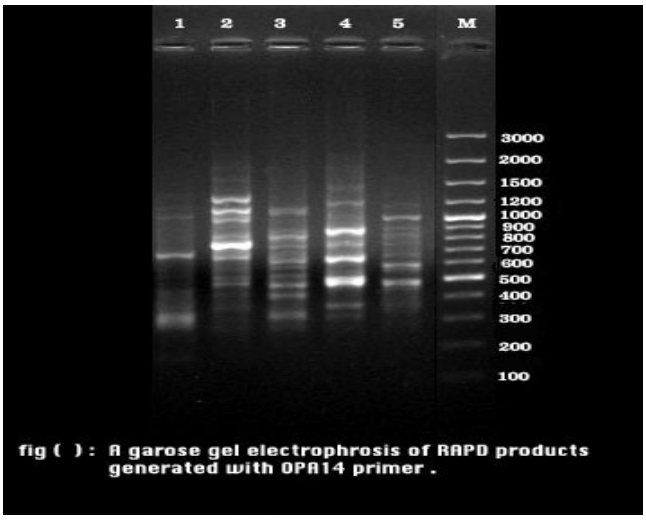


Fig (7): Agarose gel electrophoresis of RAPD products generated with OPA-14.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**2500**	**0**	**1**	**0**	**0**	**0**
2	**1350**	**0**	**1**	**0**	**0**	**0**
3	**1150**	**1**	**1**	**1**	**1**	**1**
4	**1050**	**1**	**1**	**0**	**1**	**1**
5	**970**	**1**	**1**	**1**	**1**	**0**
6	**860**	**0**	**1**	**1**	**1**	**1**
7	**780**	**0**	**1**	**1**	**1**	**1**

8	**700**	**1**	**0**	**1**	**1**	**1**
9	**600**	**0**	**0**	**1**	**1**	**1**
10	**500**	**0**	**1**	**1**	**1**	**1**
11	**430**	**0**	**1**	**1**	**0**	**0**
12	**360**	**0**	**1**	**0**	**1**	**0**
13	**300**	**1**	**0**	**1**	**1**	**0**

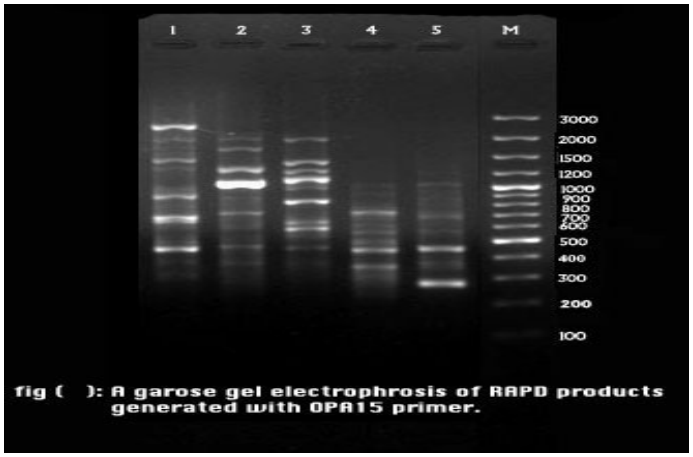


Fig (8): Agarose gel electrophoresis of RAPD products generated with OPA-15.

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

Band No.	***RAPD marker base pair***	***L. quadrilineatus***	***H. melapterus***	***T. rupellei***	***T. lunare***	***G. caeruleus***
1	**2000**	**1**	**0**	**0**	**0**	**0**
2	**1700**	**1**	**1**	**1**	**0**	**0**
3	**1400**	**0**	**1**	**0**	**0**	**0**
4	**1300**	**1**	**0**	**1**	**0**	**0**
5	**1000**	**0**	**1**	**1**	**0**	**0**
6	**900**	**1**	**0**	**0**	**0**	**0**
7	**700**	**0**	**1**	**0**	**0**	**0**
8	**650**	**1**	**1**	**1**	**0**	**0**
9	**600**	**0**	**0**	**1**	**0**	**0**
10	**550**	**0**	**0**	**0**	**1**	**0**
11	**500**	**1**	**1**	**1**	**1**	**1**
12	**350**	**1**	**1**	**0**	**1**	**1**
13	**280**	**0**	**0**	**0**	**0**	**1**

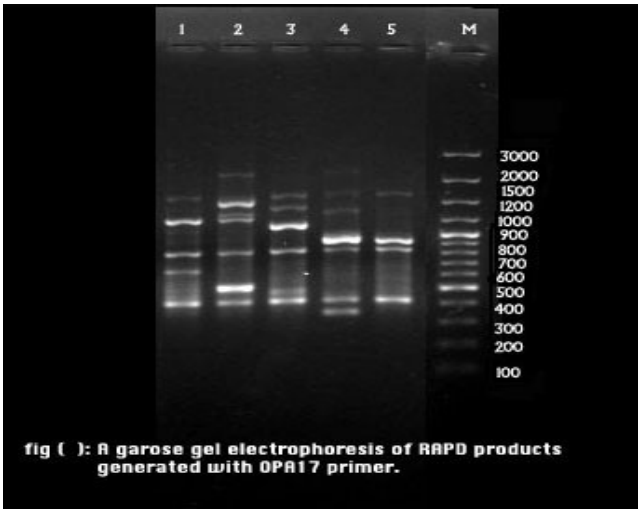


Fig (9): Agarose gel electrophoresis of RAPD products generated with OPA-17.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**2500**	**0**	**1**	**0**	**0**	**0**
2	**1900**	**1**	**1**	**1**	**1**	**1**
3	**1500**	**0**	**1**	**1**	**1**	**0**
4	**1200**	**1**	**1**	**0**	**0**	**0**
5	**1000**	**0**	**0**	**1**	**0**	**0**
6	**760**	**0**	**0**	**0**	**1**	**1**
7	**700**	**1**	**1**	**1**	**1**	**1**
8	**500**	**1**	**0**	**0**	**0**	**0**
9	**450**	**0**	**1**	**0**	**0**	**0**
10	**400**	**0**	**0**	**1**	**0**	**0**
11	**350**	**1**	**1**	**1**	**1**	**1**
12	**300**	**0**	**0**	**0**	**1**	**0**

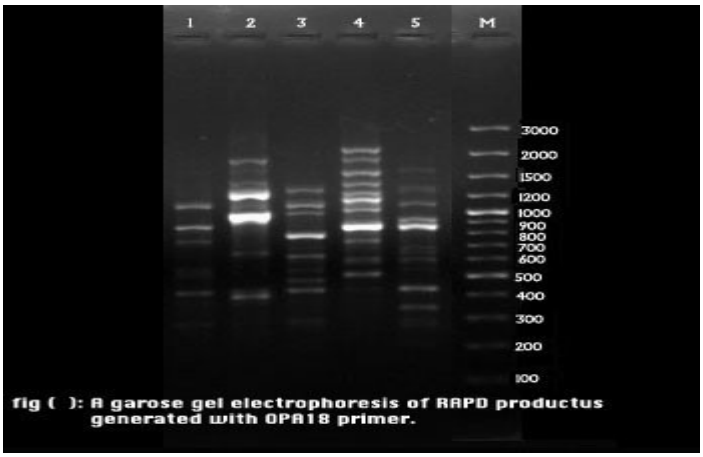


Fig (10): Agarose gel electrophoresis of RAPD products generated with OPA-18.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**2000**	**0**	**0**	**0**	**1**	**0**
2	**1900**	**0**	**0**	**0**	**1**	**0**
3	**1750**	**0**	**1**	**0**	**1**	**0**
4	**1500**	**0**	**1**	**0**	**1**	**1**
5	**1400**	**0**	**1**	**0**	**1**	**0**
6	**1250**	**0**	**1**	**0**	**1**	**1**
7	**1150**	**1**	**0**	**1**	**0**	**1**
8	**1050**	**1**	**1**	**1**	**0**	**1**
9	**950**	**0**	**1**	**0**	**0**	**1**
10	**870**	**0**	**1**	**0**	**0**	**1**
11	**800**	**1**	**0**	**0**	**1**	**1**
12	**760**	**0**	**0**	**1**	**0**	**0**
13	**700**	**0**	**0**	**0**	**1**	**0**
14	**620**	**1**	**0**	**0**	**0**	**1**
15	**550**	**0**	**0**	**0**	**0**	**1**
16	**500**	**1**	**0**	**1**	**0**	**1**
17	**450**	**0**	**0**	**1**	**1**	**0**
18	**370**	**0**	**1**	**0**	**0**	**0**
19	**320**	**0**	**0**	**0**	**0**	**1**
20	**250**	**1**	**0**	**1**	**0**	**1**

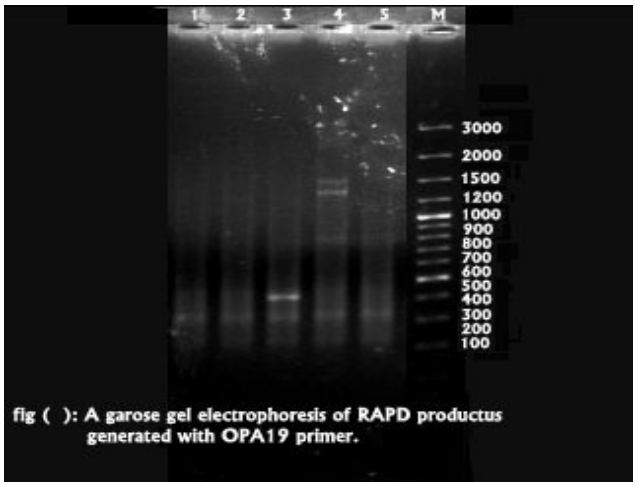


Fig (11): Agarose gel electrophoresis of RAPD products generated with OPA-19.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**1600**	**0**	**0**	**0**	**1**	**0**
2	**1400**	**0**	**0**	**0**	**1**	**0**
3	**900**	**0**	**0**	**0**	**1**	**0**
4	**400**	**0**	**0**	**1**	**0**	**0**
5	**300**	**1**	**1**	**1**	**1**	**1**

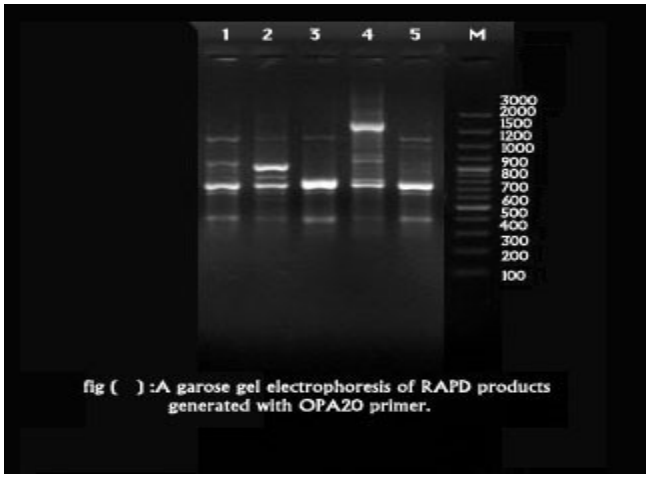


Fig (12): Agarose gel electrophoresis of RAPD products generated with OPA-20.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**2000**	**0**	**0**	**0**	**1**	**0**
2	**1500**	**1**	**1**	**1**	**0**	**1**
3	**1300**	**0**	**0**	**0**	**0**	**1**
4	**900**	**1**	**1**	**0**	**0**	**0**
5	**700**	**0**	**1**	**0**	**0**	**0**
6	**600**	**0**	**0**	**0**	**1**	**0**
7	**550**	**1**	**1**	**1**	**1**	**1**
8	**500**	**1**	**1**	**1**	**0**	**1**
9	**300**	**1**	**1**	**1**	**1**	**1**

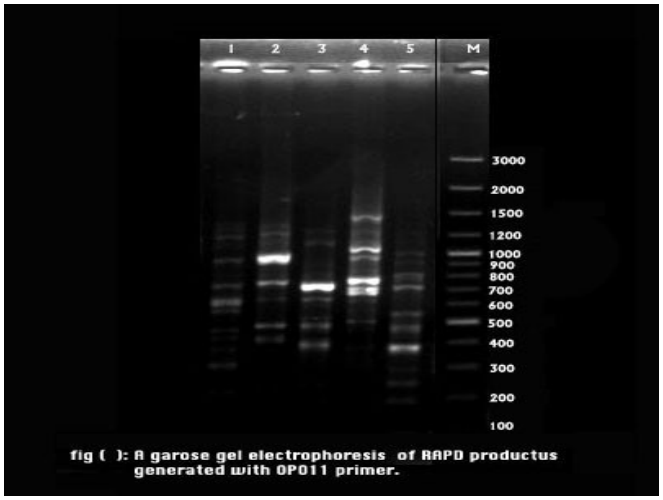


Fig (13): Agarose gel electrophoresis of RAPD products generated with OPO-11.

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

Band No.	*RAPD marker base pair*	*L. quadrilineatus*	*H. melapterus*	*T. rupellei*	*T. lunare*	*G. caeruleus*
1	**1500**	**0**	**0**	**0**	**1**	**0**
2	**1300**	**0**	**1**	**0**	**0**	**0**
3	**1200**	**1**	**1**	**1**	**1**	**0**
4	**1100**	**0**	**0**	**1**	**1**	**0**
5	**1000**	**0**	**0**	**0**	**1**	**1**
6	**930**	**1**	**1**	**1**	**1**	**1**
7	**800**	**0**	**0**	**1**	**1**	**1**
8	**750**	**1**	**1**	**1**	**1**	**1**
9	**620**	**0**	**0**	**0**	**1**	**0**
10	**550**	**1**	**0**	**0**	**0**	**1**
11	**500**	**0**	**0**	**1**	**1**	**0**
12	**460**	**0**	**1**	**1**	**1**	**1**

13	420	1	0	0	0	0
14	370	1	1	0	1	1
15	350	1	0	0	0	0
16	300	1	0	0	0	1
17	280	0	0	0	0	1
18	250	1	1	0	0	0
19	200	0	0	1	0	1

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 polymer-phic bands	Polymorphism %
		(1) *L. quadrilineatus*	(2) *H. melapterus*	(3) *T. rupellei*	(4) *T. lunare*	(5) *G. caeruleus*			
1	OPA-12	4	6	7	6	6	10	3	30.00
2	OPA-14	5	10	9	10	7	13	2	15.38
3	OPA-15	7	7	6	3	3	13	7	53.85
4	OPA-17	5	7	6	6	4	12	6	50
5	OPA-18	6	8	6	9	12	20	7	35
6	OPA-19	1	1	2	4	1	5	4	80
7	OPA-20	5	6	4	4	5	9	4	44.44
8	OPO-11	9	7	8	11	10	19	6	31.58
total		42	52	48	53	48	101	39	38.61

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

	1	2	3	4	5
1	100				
2	53	100			
3	60	56	100		
4	42	57	55	100	
5	62	56	62	57	100

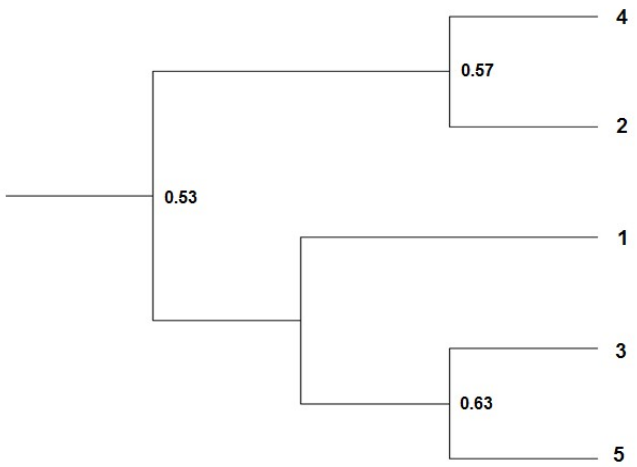


Fig (14): The evolutionary tree of the five marine fish species

الملخص العربى

الأنماط الوراثية وتحليل بلمرة الدنا العشوائي لخمس أنواع أسماك بحرية (عائلة: الملاص) من الشعب المرجانية في البحر الاحمر.

عبد الباسط مسعود عبيد1 – علي حسين أبو المعاطي2 – محمد ابو الرجال3 – زينب عبد الخالق مرعي4

4,1 معمل الوراثة الخلوية - قسم علم الحيوان – كلية العلوم بقنا – جامعة جنوب الوادي.

2 قسم علم الحيوان – كلية العلوم ببورسعيد – جامعة بورسعيد. 3 قسم علوم البحار – كلية العلوم ببورسعيد – جامعة بورسعيد.

تناول هذا البحث دراسة الفروق الوراثية بين خمس انواع من اسماك البحر الاحمر تنتمي الى عائلة الملّاص، من خلال اجراء الفحوص و المعاملات المختبرية الوراثية الدقيقة و الاسس العلمية الحديثة لتحديد اوجه التشابه و الاختلاف بين هذه الانواع من خلال تحديد العدد الكروموسومى الزوجى و الخرائط الصبغية للنواع محل الدراسة و تحديد بعض مقاطع الحمض النووى الدى اوكسى ريبوز (د.ن.أ) باستخدام تفاعل سلسلة البلمرة العشوائى لهذه الانواع ودراسة الفروق الجزيئية بين هذه الانواع من خلال استخدام ثمانية بادئات بطول عشر نيكليوتيدات و توضيح ايضا العلاقة التقاربية بين هذه الانواع داخل هذه العائلة.

أوضحت النتائج المتحصل عليها للانواع محل الدراسة أنه بالرغم من أن غالبية الانواع تحتوى على نفس عدد الصبغيات الوراثية وأيضا نفس الكاريوتيب و نفس عدد الفتائل الصبغية الفعالة، كما فى أنواع ملاص رباعى الخطوط و ملاص عريض الشفه و ملاص أبو ربيع و ملاص قمرى و ملاص البحر الاحمر الطائر 2ن=48، FN =48 و الكاريوتيب عبارة عن مجموعة واحدة من الكروموسومات الطرفية الا ان استخدام تفاعل سلسلة البلمرة العشوائى لهذه الانواع أوضح وجود درجات متفاوتة من التقارب و الاختلاف بين هذه الانواع، فمثلا درجة التقارب بين ملاص رباعى الخطوط و الانواع التالية وهى ملاص عريض الشفه و ملاص أبو ربيع و ملاص قمرى و ملاص البحر الاحمر الطائر هى 53%، 60%، 42% و62% .

وتعتبر هذه الدراسة مدخلا جديدا لاسس علم التصنيف الذى بنى على الوسائل الحديثة و هى استخدام التقنية الوراثية الحديثة فى تدعيم التصنيف التقليدى الذى يعتمد على الصفات المورفولوجية و التشريحية.