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Genetic diversity and molecular declining population of four Channid species from North India and possible strategies

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2nd international conference on Integrative Biology Summit, Chicago, USA
(August, 04-05, 2014)

Piscine Biodiversity

- Among vertebrates, fishes occupy remarkable status due their highest species diversity totalling to nearly **25,000 species** which is 3-6 times more compared to other vertebrate groups.

- India is one of the mega-biodiversity countries in the world and occupies the **9th position** in terms of freshwater mega-biodiversity (Mittermeier & Mitterrneier, 1997).

- In India, there are **2,500** species of fishes; of which **930** live in freshwater and **1570** in sea water (Kar, 2003).

- **According to Conservation Assessment & Management Plan (CAMP) workshop report (1998), 329 freshwater fishes in India are under the risk of decline or extinction.**

Decline in fish Biodiversity

- According to Conservation Assessment and Management Plan (CAMP) workshop held in 1997, 327 freshwater fishes in India are under the risk of decline or extinction.

- **Anthropogenic factors responsible for decline of fish species**
  - Pollution load into aquatic ecosystem
  - Modification/ Destruction of aquatic habitat
    - Construction of Dam
    - Diversion or reclamation of river beds for urbanization & consequent reduction of water discharge in rivers.
    - Consequent reduction in natural habitat area
  - Introduction of exotic (non-native) fish species
  - Over fishing
  - Global climatic variations

END RESULT…….

Decrease in Ichthyofaunal Diversity

Based on IUCN Red List Categories

Status of assessed freshwater fishes of India

(CAPM Workshop Report 1998)

Extinct (Ex)	***Gymnocypris biswasi***	**1**
Extinct in the Wild (EW)	***Osteobrama belaqngeri***	**1**
Critically Endangered (CR)		**47**
Endangered (EN)		**98**
Vulnerable (VU)		**82**
Lower Risk – near threatened (LR-nt)		**67**
Lower Risk – least concern (LR-lc)		**13**
Lower Risk – conservation dependent (LR-cd)		**0**
Data Deficient (DD)		**18**
Total evaluated at this workshop		**329**
Not Evaluated at this workshop (NE)		**300+**

Why catalogue and conserve biodiversity

- Biodiversity supports livelihood and sustainability development.
- To secure the IPRs related to fish biodiversity to maintain our status on our biological resvoiurs and their potential benefits.

The way forward……

Outline goals:

- Sustainable utilization of these resviours.
- Appropriate planning of biodiversity conservation & management strategies.
- Identification, listing and understanding the threat level and formulating species specific conservative plan.

Response to New Challenges & Development

- Legislation of Biological Diversity Act 2002 (BDA 2002)
- Biological Diversity Rules (2004)
- National Biodiversity Action Plan (NBAP 2008)

All aimed at launching viable Plans and Programmes & Policies towards **"Biodiversity Conservation"**

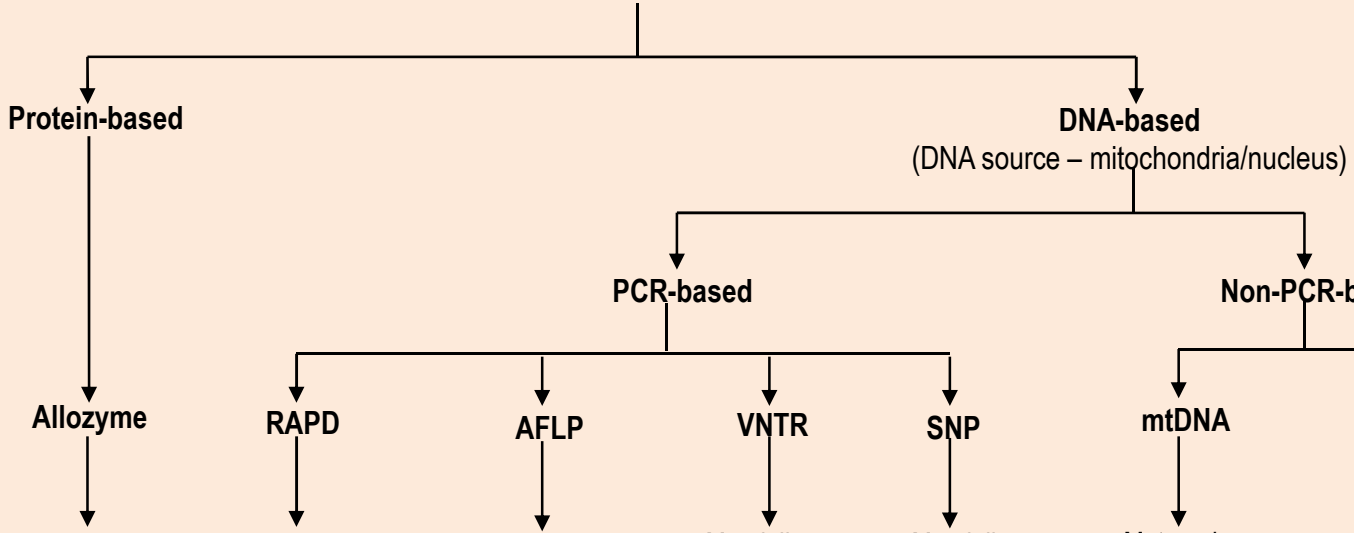
Genetic factors

- Apart from anthropogenic factors, genetic factors are also responsible for decline of fish species due to:
  - Loss in genetic variability
  - Inbreeding
  - Improper breeding programmes
- Genetic variability is important for survival of species because species having more genetic variation will be less susceptible to adverse environmental conditions.

Existing Molecular Methods for Genotyping

- Even though there are several approaches to do genotyping such as
  - **RFLP**
  - **VNTR**
  - **AFLP**
  - **SNP**
  - **RAPD**
- RAPD appears most suitable because of its
  - **Simple and Cost effectiveness**
  - **Versatility**
  - **Does not require prior sequence information**
  - **Does not need the radioactivity**

Different categories of molecular markers



	Allozyme	RAPD	AFLP	VNTR	SNP	mtDNA	RFLP
Inheritance pattern	Mendelian codominant	Mendelian dominant	Mendelian dominant	Mendelian codominant	Mendelian codominant	Maternal inheritance	Mendelian codominant
Prior sequence information required?	Yes	No	No	Yes	Yes	No*	Yes
Applications	Linkage mapping, population studies	Species and hybrid identification, fingerprinting for population studies	Population studies, linkage mapping	Paternity analysis, linkage mapping, population studies	Linkage mapping, population studies, disease diagnosis	Maternal lineage	Species identification, linkage mapping, population studies,

*Conserved PCR primer sequence information can be adopted from a related species.

Schematic division of various categories of molecular markers and their essential features.

Status of different molecular markers for study of Fish population genetics

- Globally about 100 fish species from 30 families were genetically analysed by RAPD method.

- **Indian Scenario**
  - **48** fish species studied by RAPD markers
  - **37** species by **Allozyme** marker
  - **20** species by **RFLP/ mtDNA / VNTR Assay**

Great diversity of *Channa* species

- Snakehead fishes of Channidae family, endemic to freshwater, are distributed only in **Asian** and **African** countries. They are represented by **30** species of which **27** are confined to Asian countries (Amback et al., 2006).

- In the Indian subcontinent alone, there are **12** different species of this group and **04** of them are ubiquitously distributed across the country.

- These fishes are considered highly relished food fishes, fetch good market price and are regarded as consumer's choice.

- Scientifically, they provide an interesting target for phylogenetic analysis due to the existence of several species and availability of multi species of this genus in a particular area.

- **Despite great economic importance and rich species diversity of these fishes, most of the *Channa* species are showing declining trend and hence included in the comprehensive list of different categories of declining fresh water fishes prepared at CAMP workshop (1998).**

Distribution of *Channa* species in India

C. striatus

India: **Ubiquitous**

Other Places: Southern China and Thailand

C. punctatus

India: **Ubiquitous**

Other Places: Indian Sub-continent, China

C. marulius

India: **Ubiquitous**

Other Places: Bangladesh, China, Thailand and Cambodia, Sri Lanka and Pakistan

C. gachua

India: **Ubiquitous**

Other Places: Indian Sub-continent

C. amphibius

India: **North Bengal**

Other Places: Bhutan

C. barca

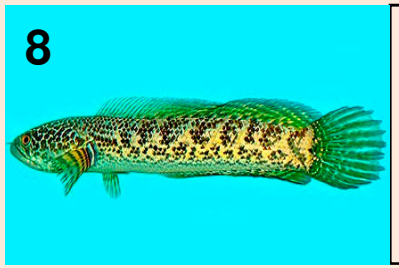
India: **West Bengal,Brahmaputra river in Assam, Ganges-**

Other Places: Bhutan

C. orientalis

India: **India**

Other Places: Afghanistan, Iran, Pakistan, Nepal, Sri Lanka, Bangladesh, Burma



C. aurantimaculata

India: **Brahmaputra River basin, northern Assam,**

Other Places: **-**

C. bleheri

India: **The Brahmaputra River basin at northern Assam,**

Other Places: **-**

C. micropeltes

India: **The Brahmaputra River basin at northern Assam, Kerala**

Other Places: Malaysia, Thailand, Vietnam

C. stewartii

India: **Eastern Himalaya Inhabits both running and standing waters**

Other Places: Nepal

C. Diplogramma

India: **Western Ghats, Kerala**

Other Places: **-**

Four different species of genus *Channa*

Heteropneustes fossilis

Clarias batrachus

Clarias gariepinus

Three catfish species

Focal Theme of the study

Out of the 7 selected species in the present study, 6 species were placed into different declining categorized according to **IUCN** criteria (International Union for Conservation of Nature) in Conservation Assessment and management Plan (**CAMP,** 1997) workshop. Hence, the focal theme of this study were:

1: ***To identify species specific DNA profiles and bands, if any, from the RAPD fingerprints.***

2: ***To evaluate comparative densitometric analysis of RAPD band profile for additional assistance in species identification.***

3: ***To establish inter-specific phylogenetic relationship among the four species of the genus Channa and intra-specific phylogenetic analysis among all seven species using the neighbor-joining method.***

❖ **Continued…….**

continued……. **‘Focal Theme of the study’**

4: ***To quantify gene diversity of the population of investigated fish species by RAPD fingerprinting.***

5: ***To assess the Genetic Similarity (SI) and Genetic Distance (GD) of the population of investigated fish species.***

6: ***To estimate polymorphic band contents (PIC) to assess its feasibility for use in population genetics.***

7: ***To identify sex-specific RAPD markers, if any.***

Primers yielding clear and reproducible DNA fingerprinting

Total 22 primers were initially screened (**Operon technologies**)

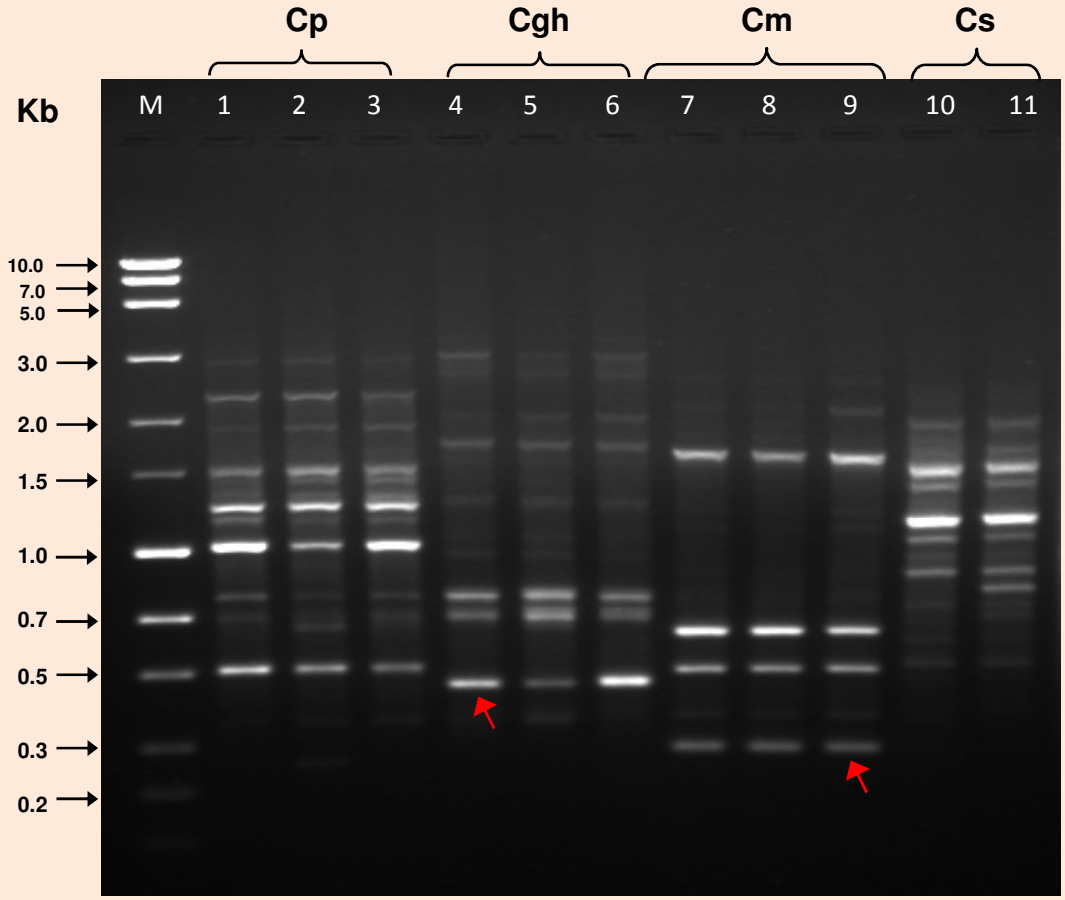
- OPA Kit (20 primers)
- OPB Kit (02 primers)

S. No.	*C. punctatus*	*C. gachua*	*C. marulius*	*C. striatus*	*H. fossilis*	*C. batrachus*	*C. gariepinus*
1	OPA1	OPA1	OPA1	OPA1	OPA1	OPA1	OPA1
2	OPA4	OPA4	OPA4	OPA4	OPA4	OPA4	OPA4
3					OPA5		
4	OPA7	OPA7	OPA7	OPA7		OPA7	OPA7
5					OPA8	OPA8	OPA8
6					OPA10		
7						OPA11	OPA11
8	OPA12	OPA12	OPA12	OPA12	OPA12	OPA12	OPA12
9	OPA18	OPA18	OPA18	OPA18	OPA18	OPA18	OPA18
10	OPA19	OPA19	OPA19	OPA19	OPA19	OPA19	OPA19
11	OPA20	OPA20	OPA20	OPA20	OPA20	OPA20	OPA20
12	OPB12	OPB12	OPB12	OPB12		OPB12	OPB12

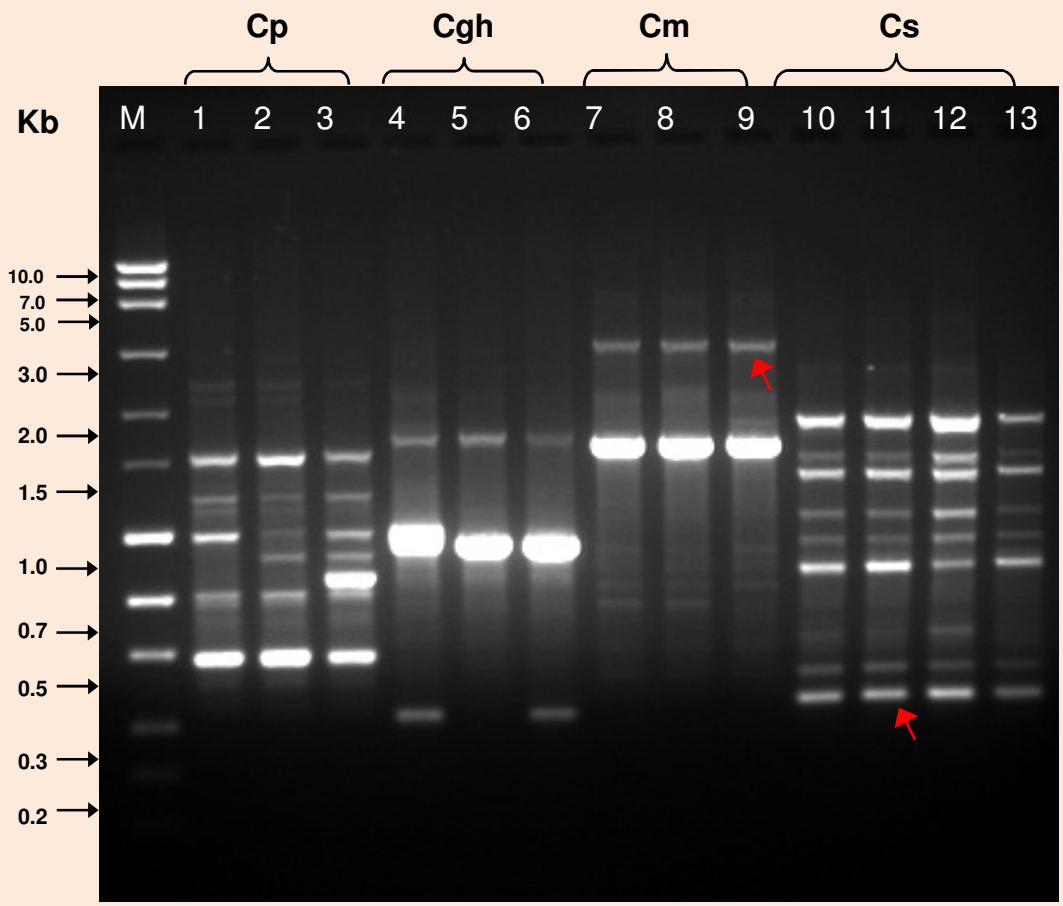
Significance of generated RAPD band profiles

There are three main applications of generated RAPD band profiles.

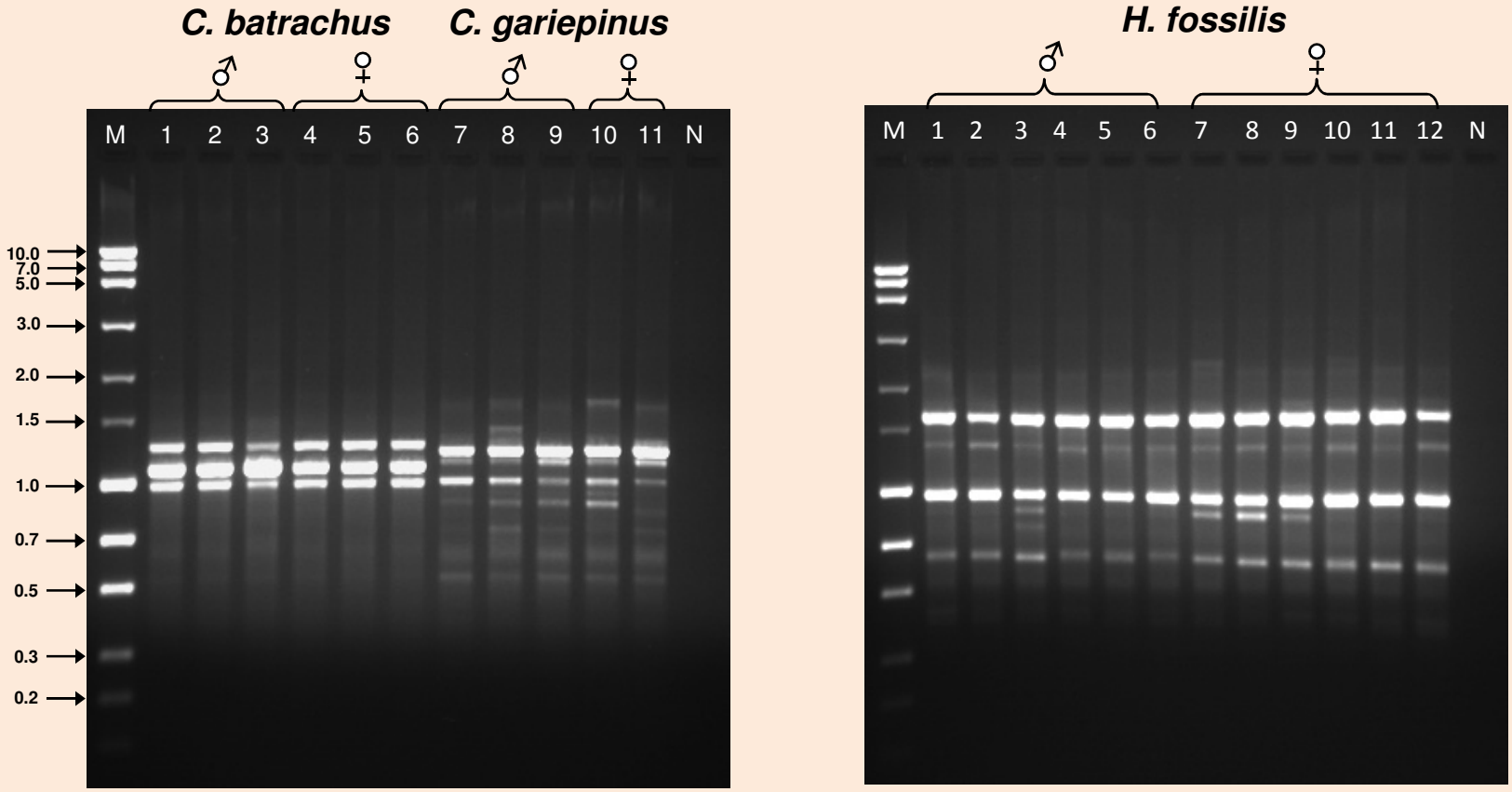
- **Species identification** at molecular level.
- **Phylogenetic relationship** among the four species of *Channa*.
- **Assessment of genetic variations** within the genome of investigated fish species.



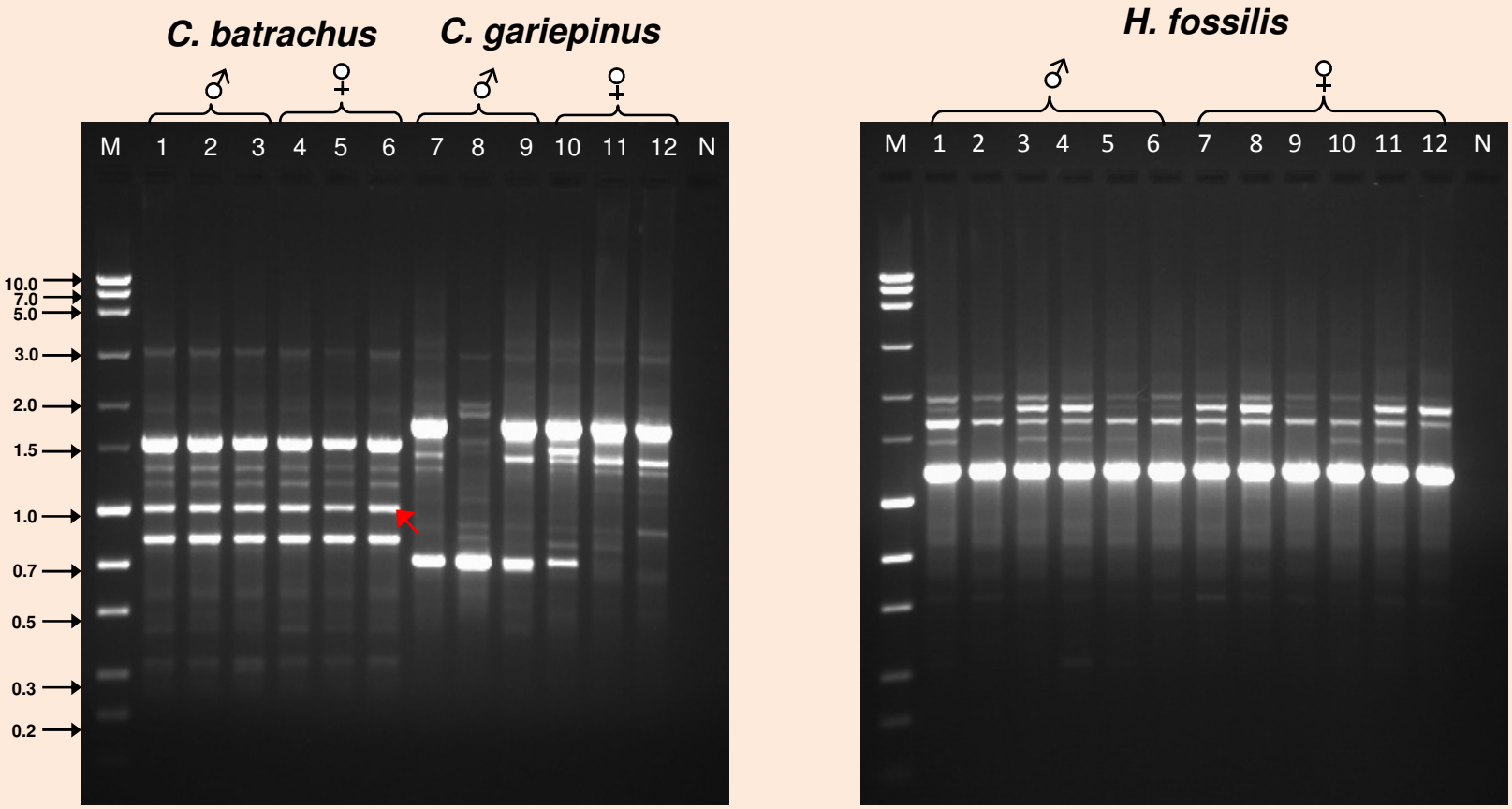
RAPD Profiling of four species of *Channa* amplified by OPA 4
(Cp= *C. punctatus*, Cgh = *C. gachua,* Cm = *C. marulius* and Cs = *C. striatus*)



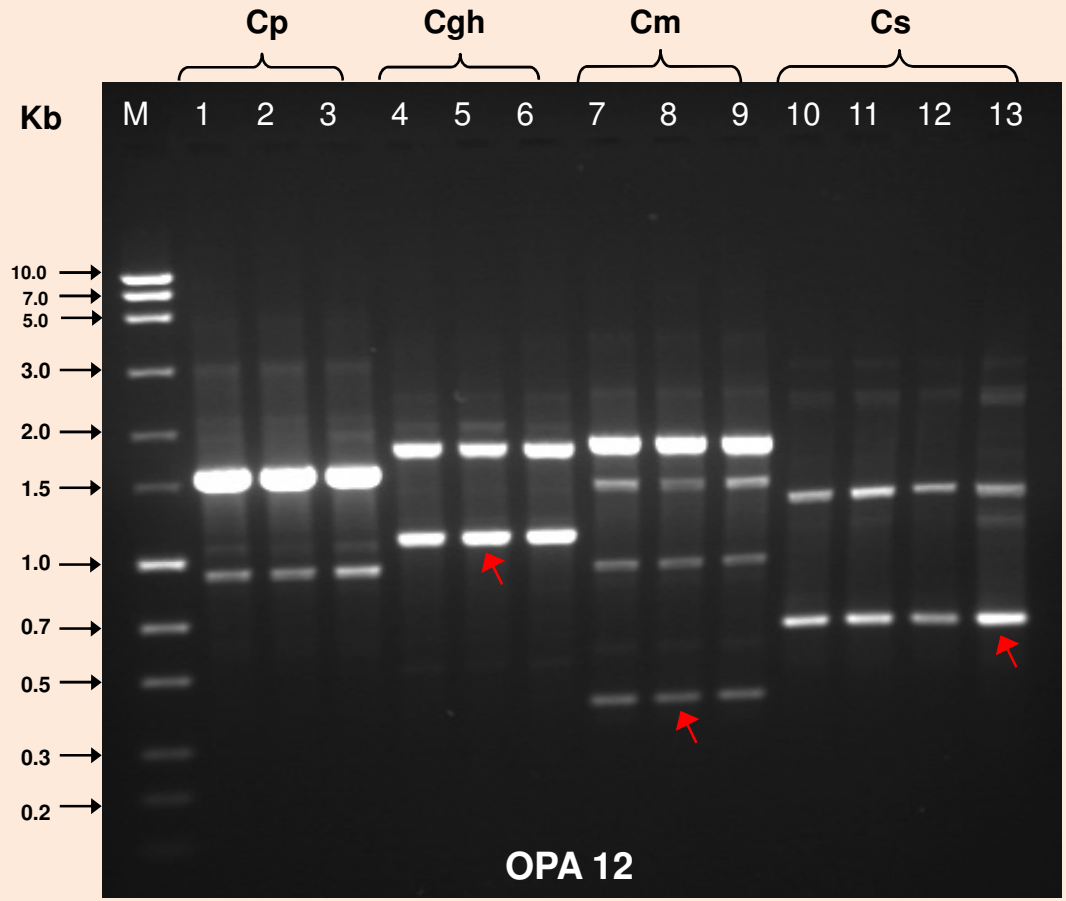
RAPD Profiling of four species of *Channa* amplified by OPA19
(Cp= *C. punctatus*, Cgh = *C. gachua,* Cm = *C. marulius* and Cs = *C. striatus*)



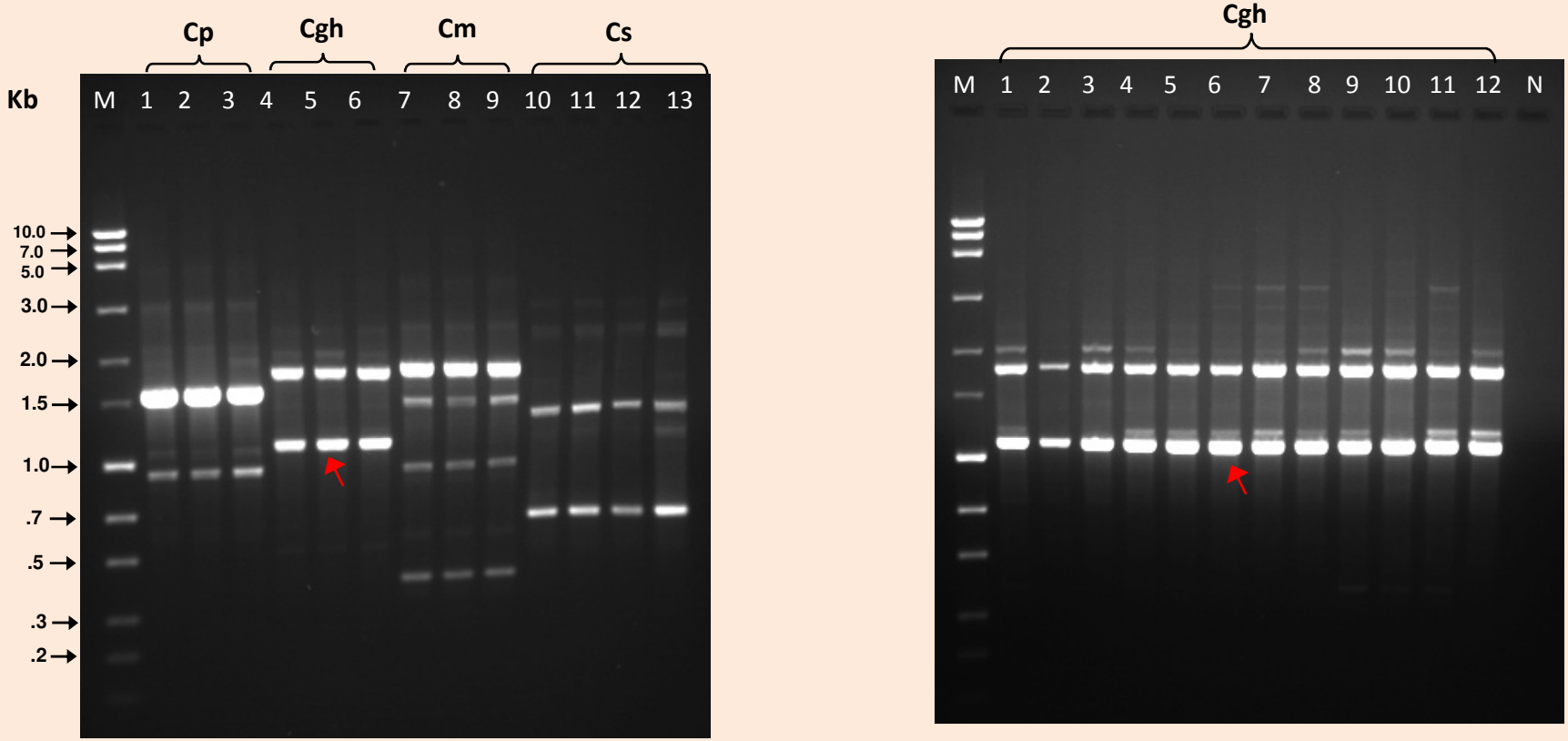
RAPD bands profile of catfish species obtained by primer OPA1



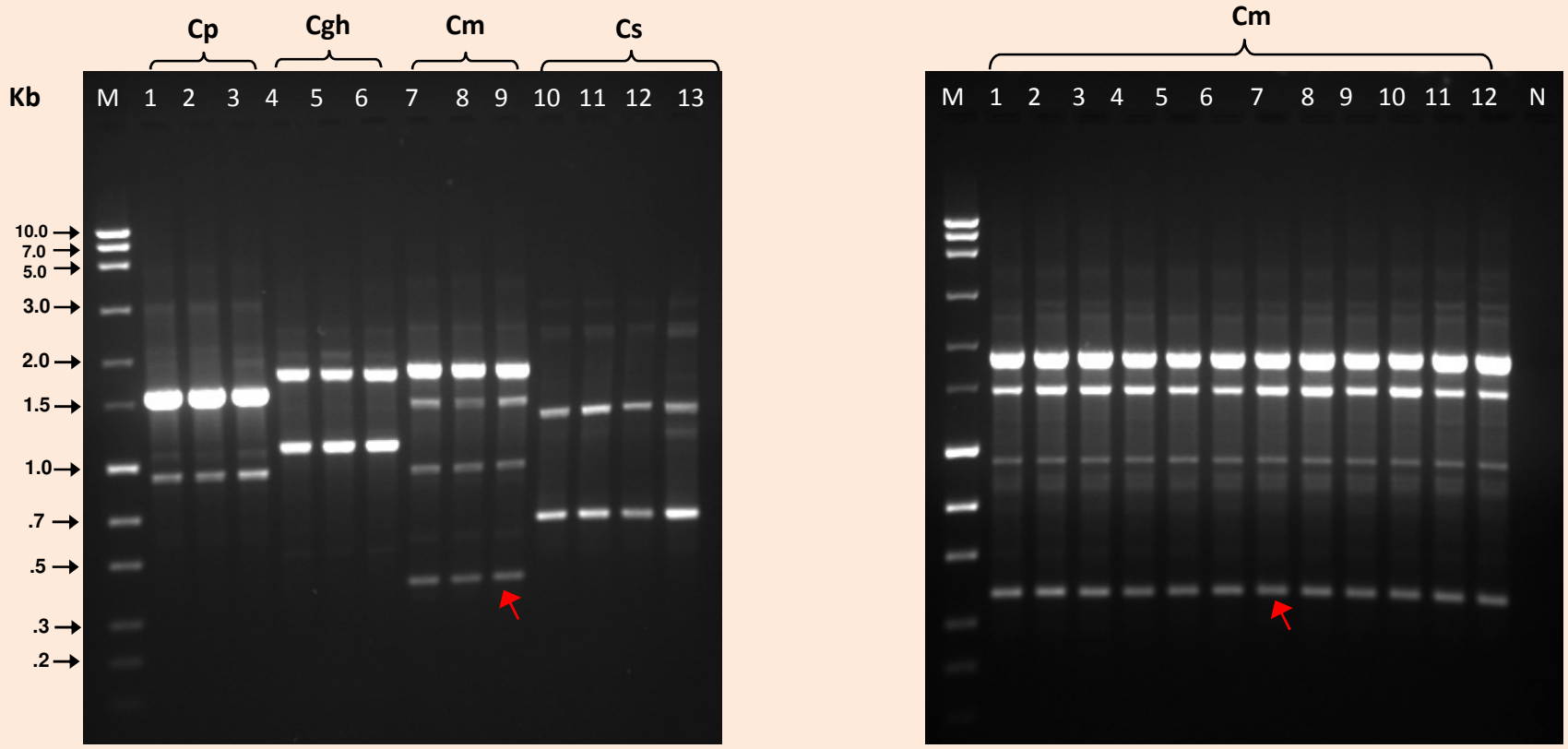
RAPD bands profile of catfish species obtained by primer OPA19



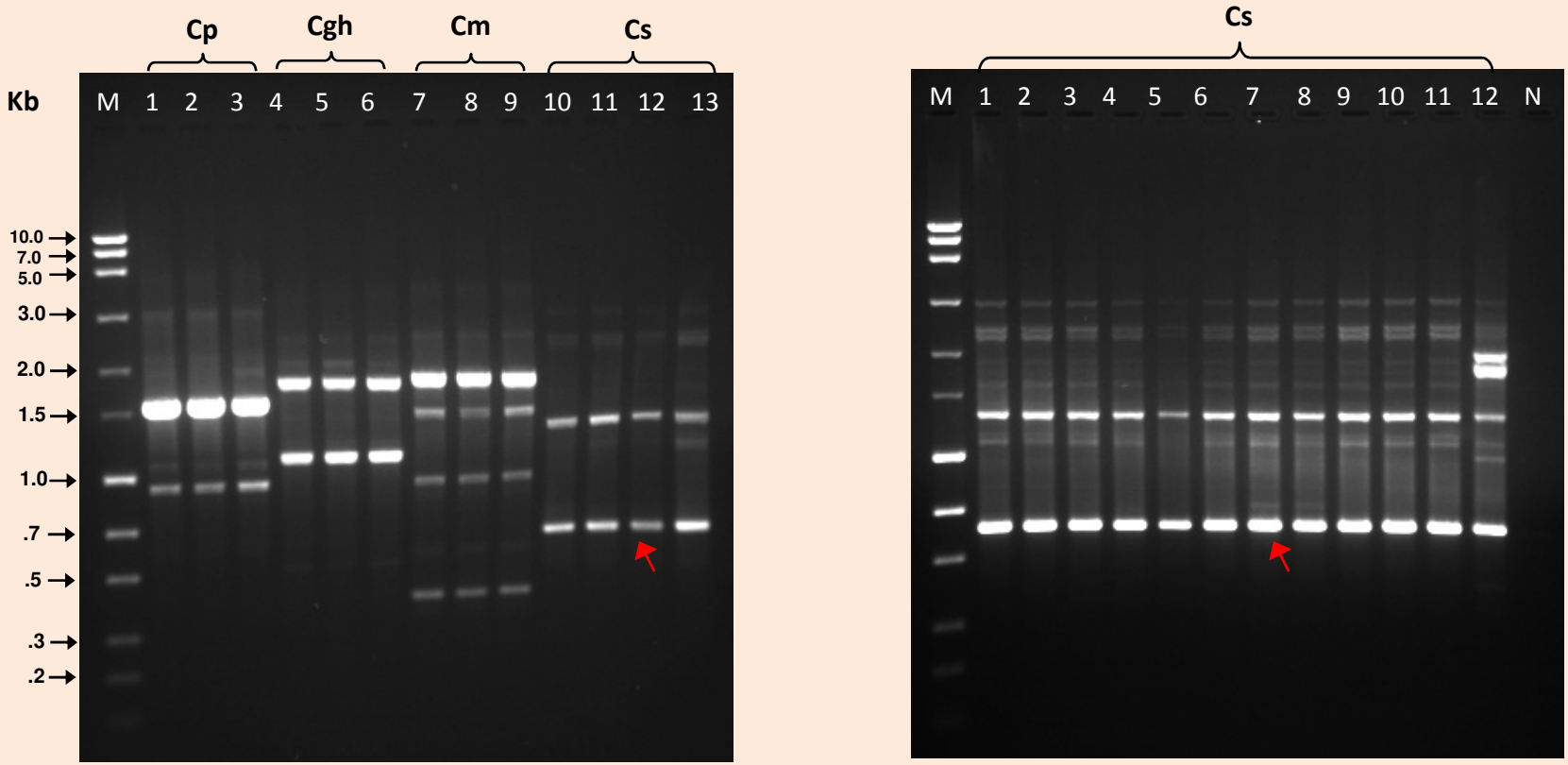
RAPD Profiling of four species of *Channa* amplified by OPA12
(Cp= *C. punctatus*, Cgh = *C. gachua,* Cm = *C. marulius* and Cs = *C. striatus*)



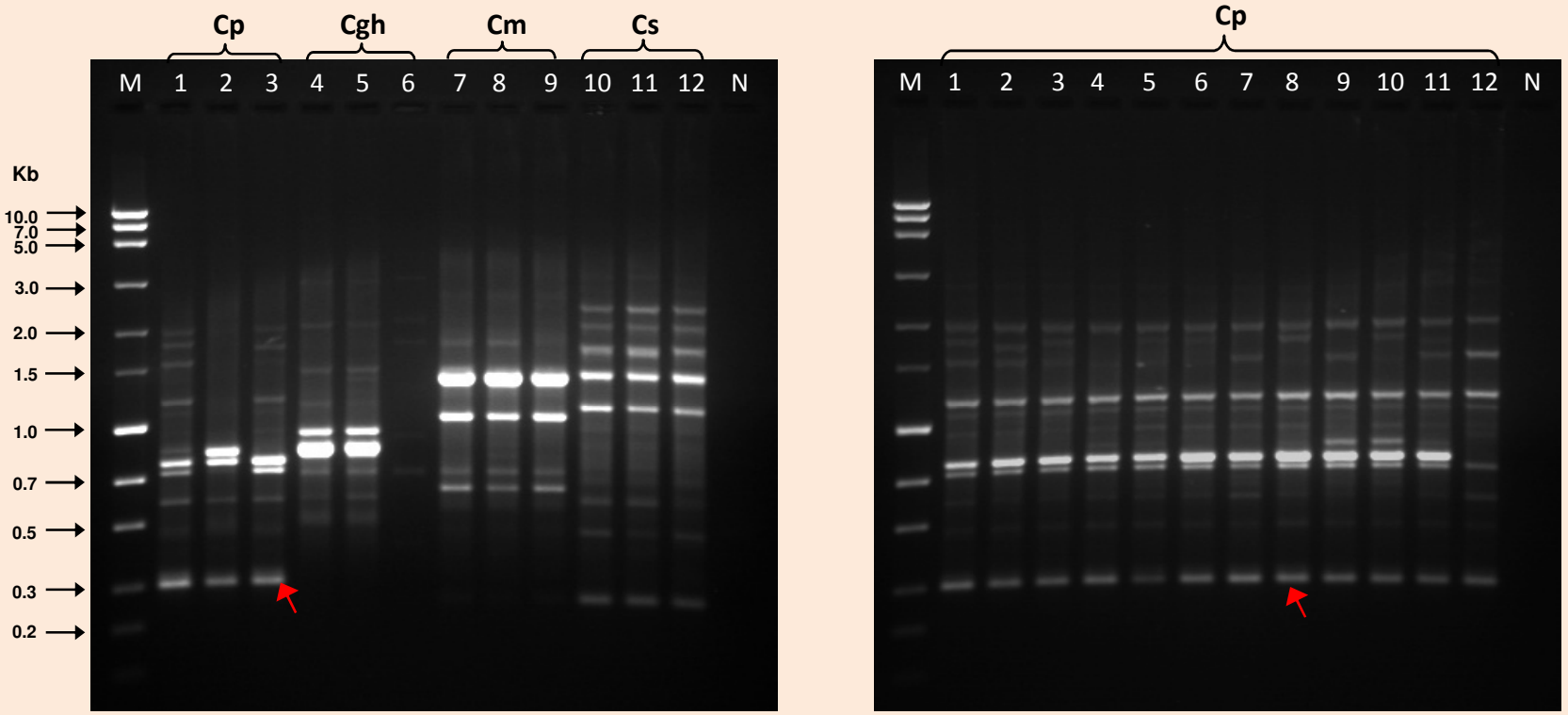
RAPD bands profile of *Channa gachua* obtained by primer OPA12



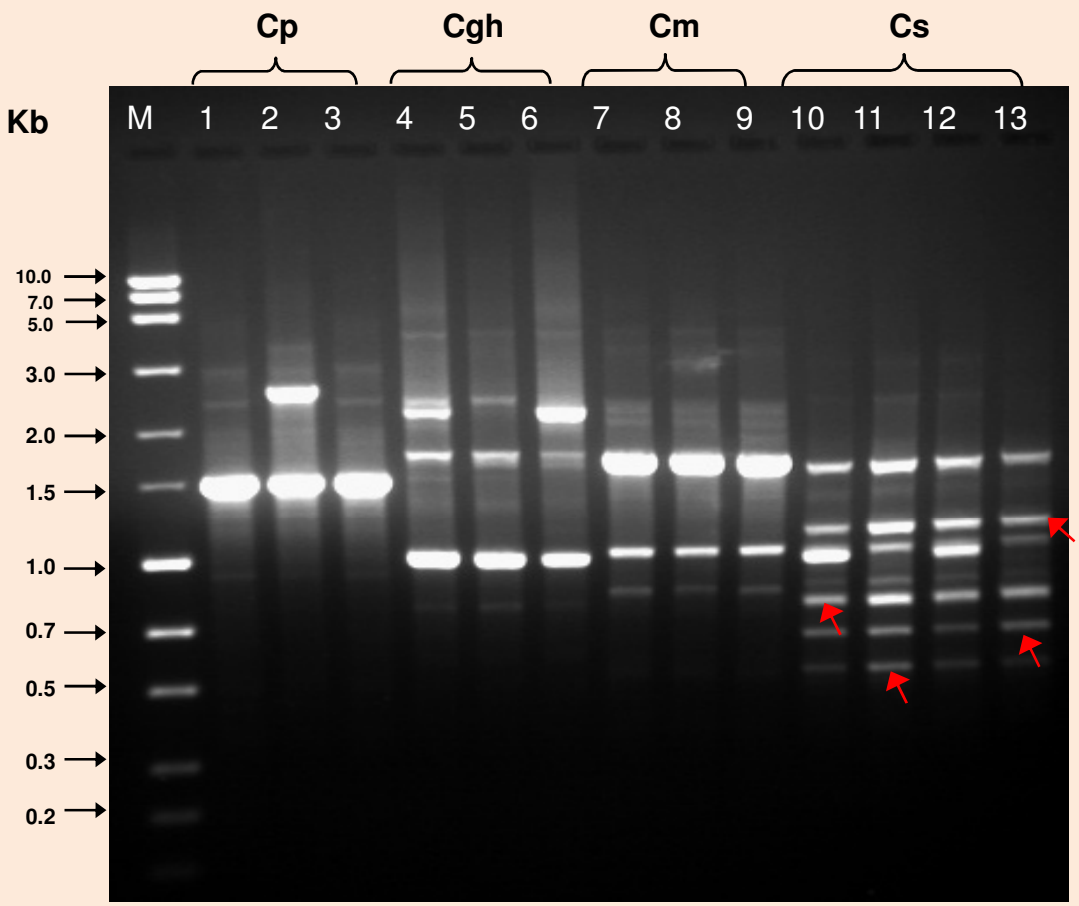
RAPD bands profile of *Channa marulius* obtained by primer OPA12



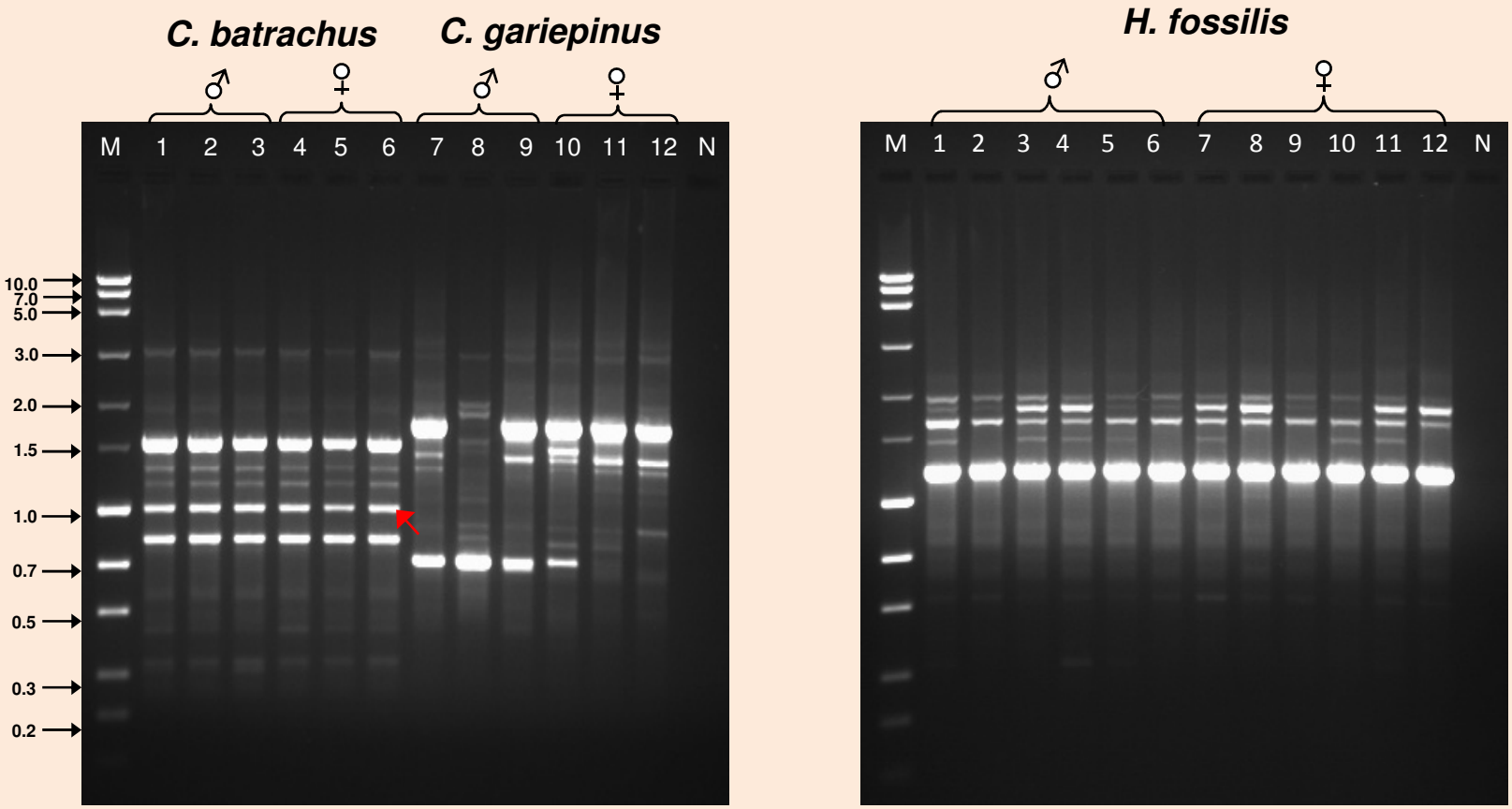
RAPD bands profile of *Channa striatus* obtained by primer OPA12



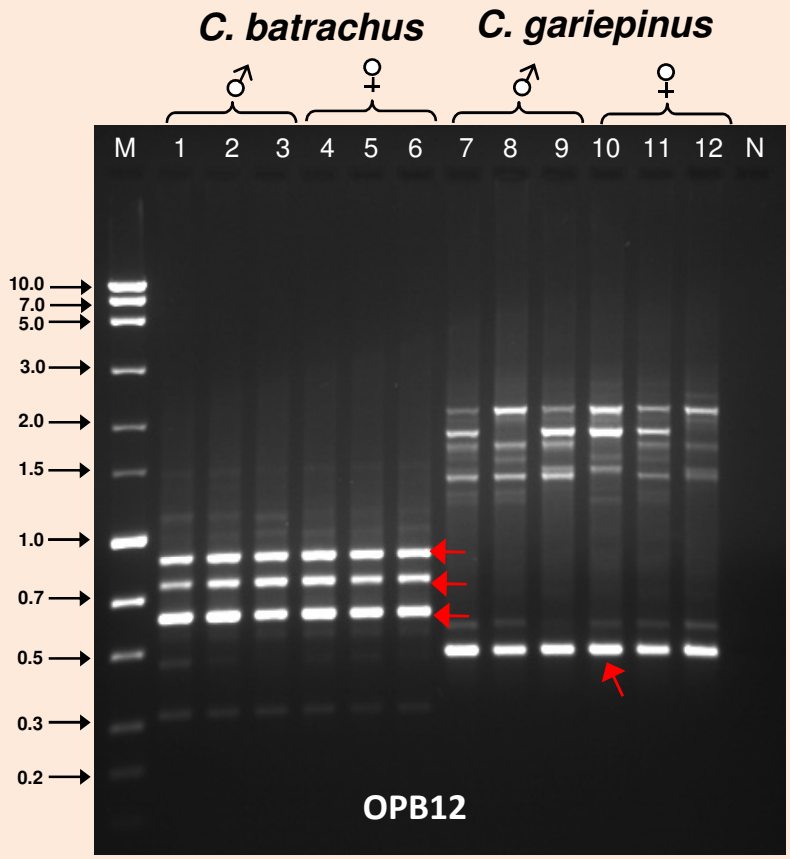
RAPD bands profile of *Channa punctatus* obtained by primer OPA7



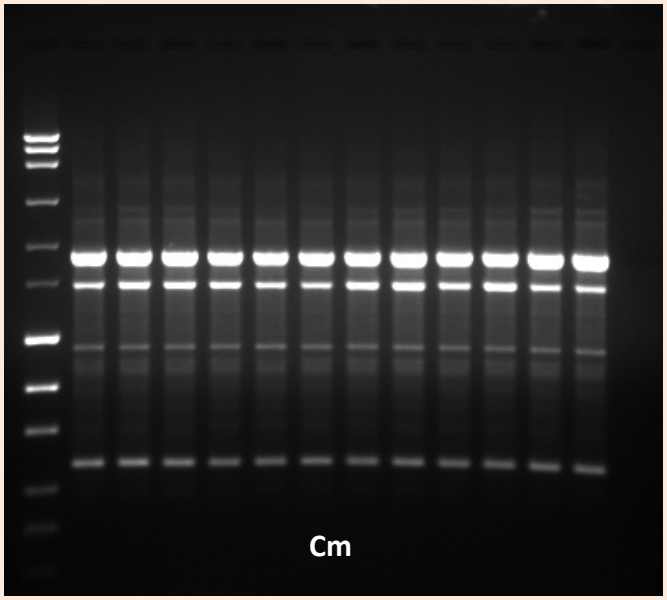
RAPD Profiling of four species of *Channa* amplified by OPA 20
(Cp= *C. punctatus*, Cgh = *C. gachua,* Cm = *C. marulius* and Cs = *C. striatus*)

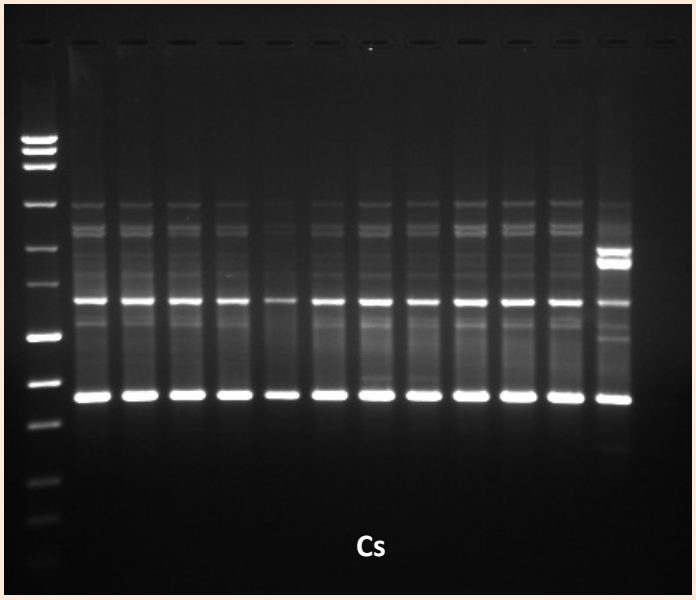


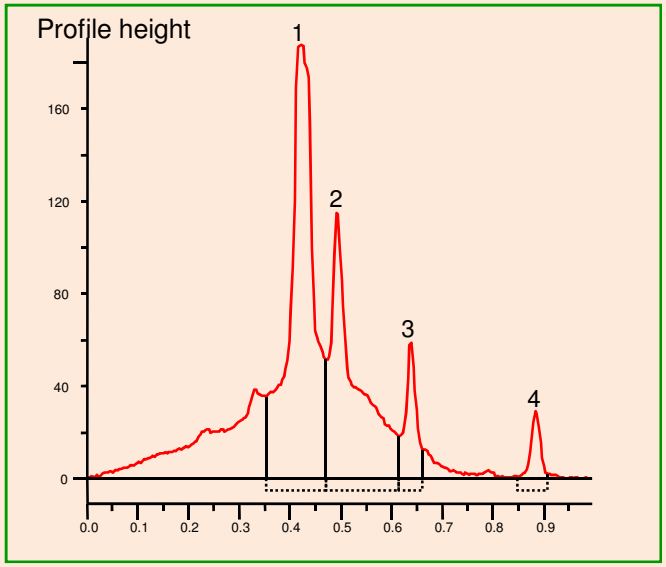
RAPD bands profile of catfish species obtained by primer OPA19

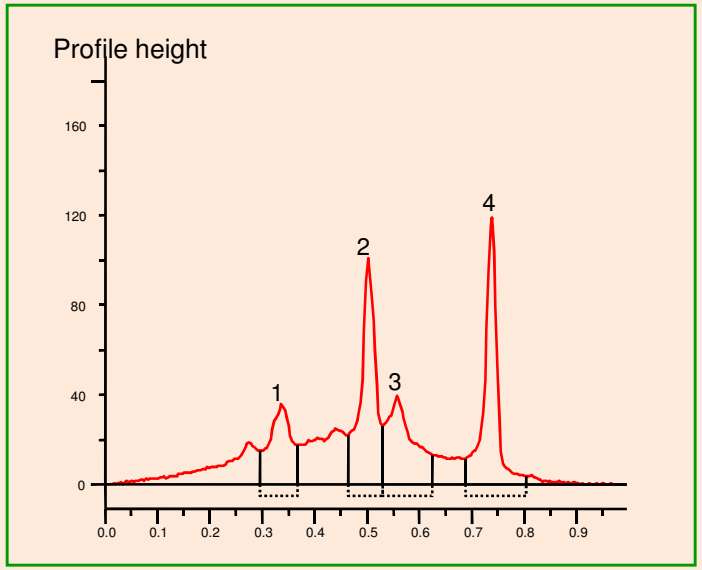


RAPD bands profile of catfish species obtained by primer OPB12

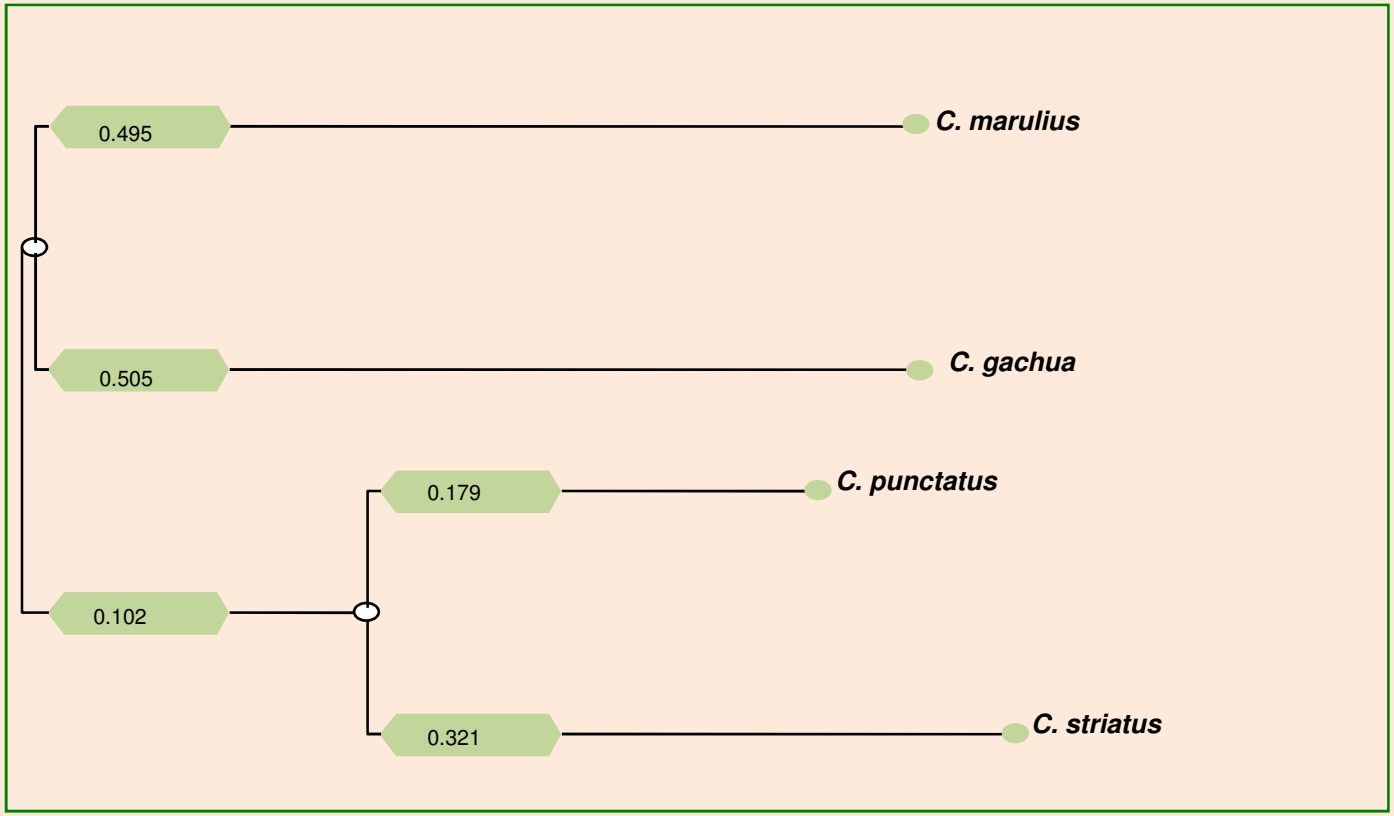




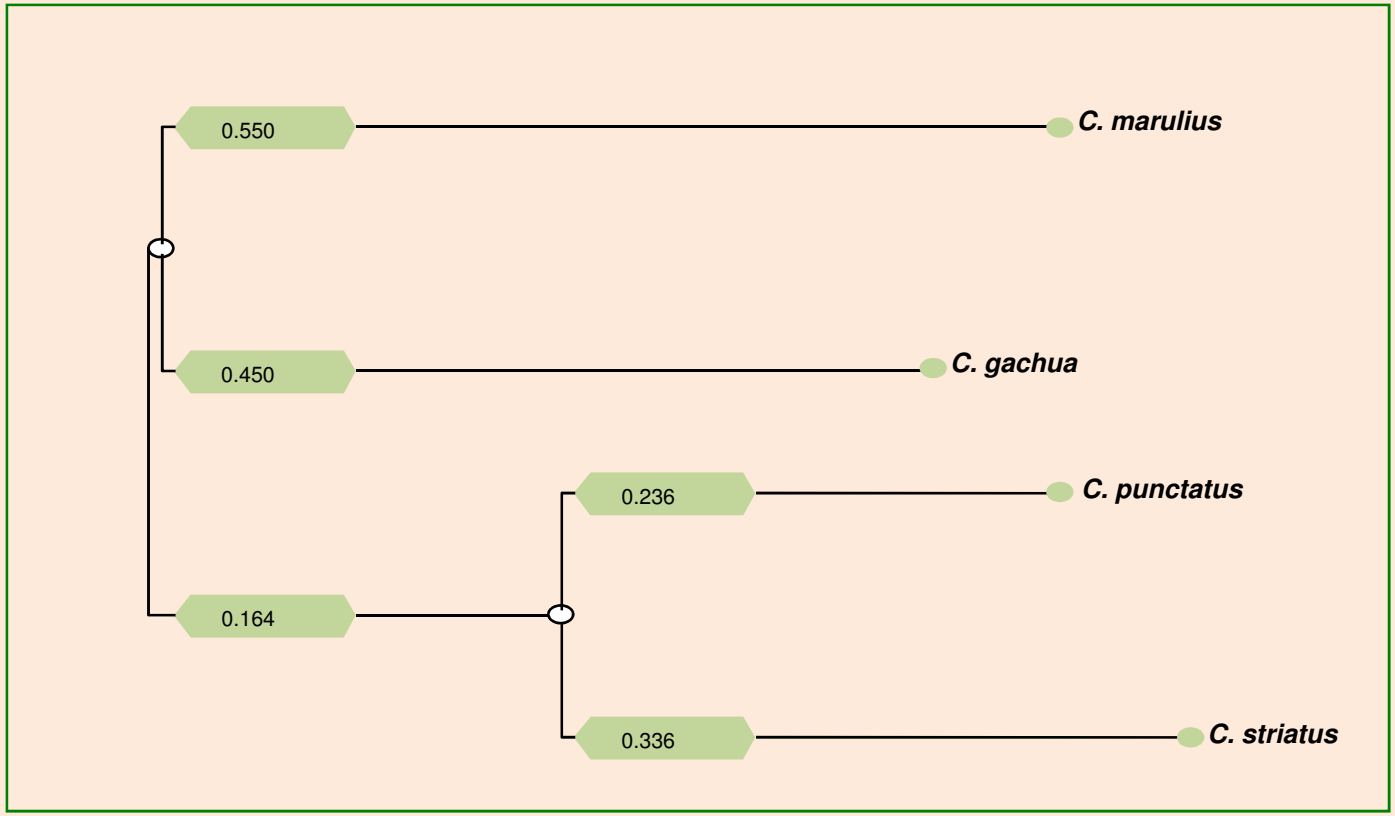




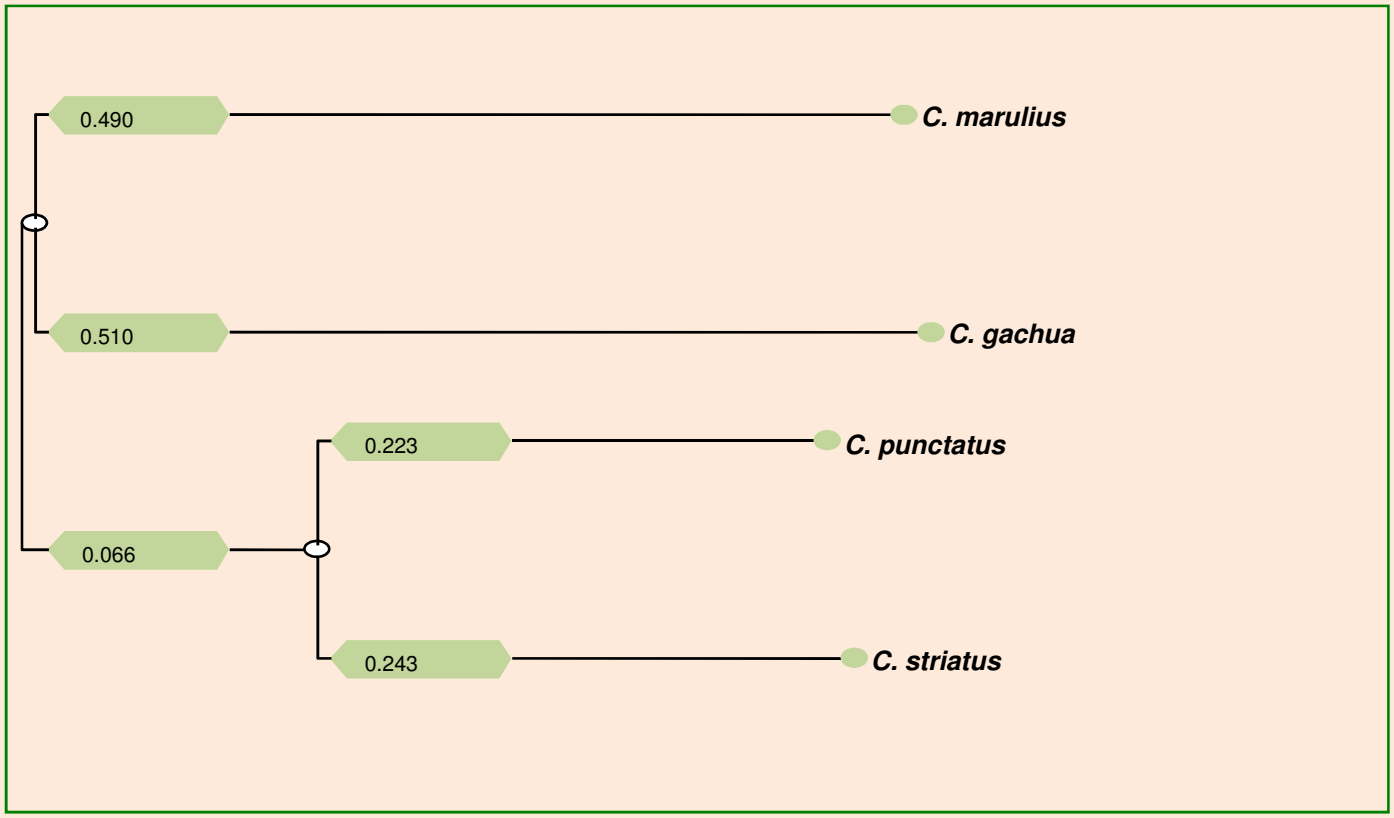
Peaks of RAPD band profiles of *C. marulius* & *C. striatus* showing relative quantitative PCR products with OPA12



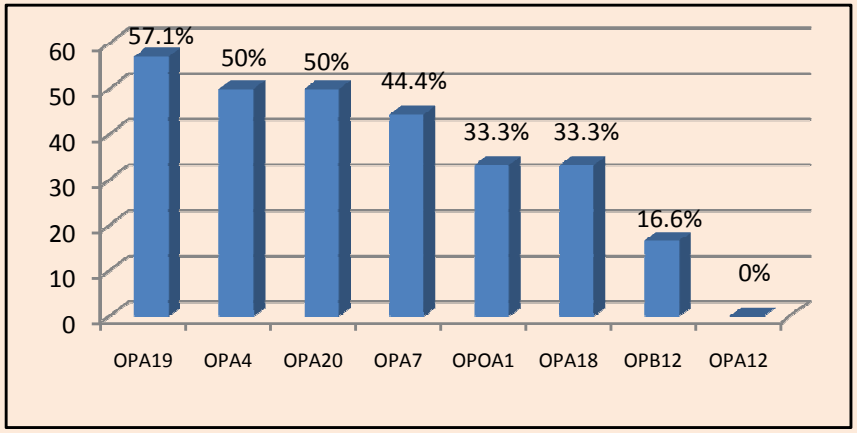
Cluster Analysis showing genetic relatedness of four different *Channa* species with OPA4



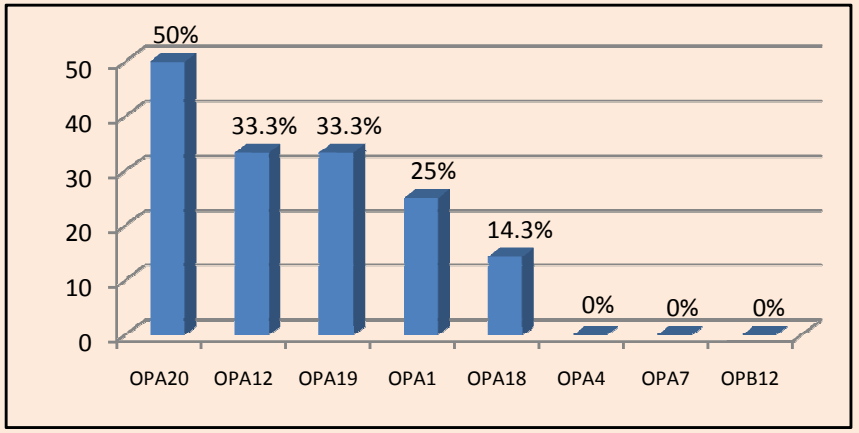
Cluster Analysis showing genetic relatedness of four different *Channa* species with OPA7



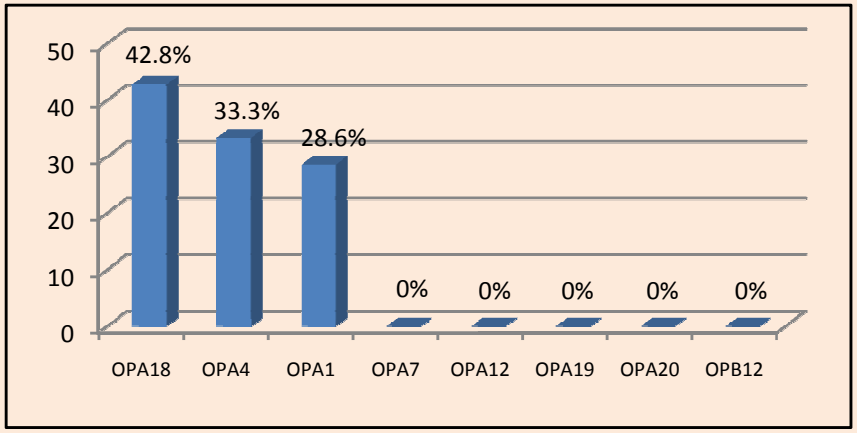
Cluster Analysis showing genetic relatedness of four different *Channa* species with OPA19



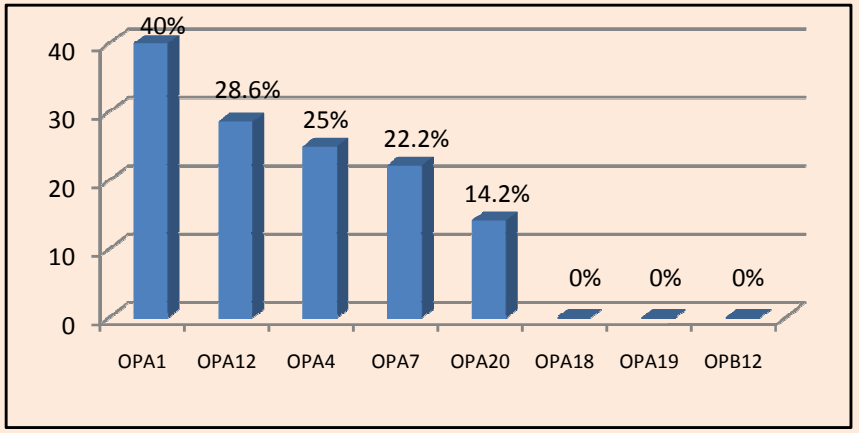
C. punctatus



C. gachua



C. marulius



C. striatus

Polymorphic band content of *Channa* species obtained with different primers

Genetic analyses values of four *Channa* species.

Primer	GS	GD	I	H	PC %
OPA1	0.92	0.08	0.205	0.142	33.3
OPA4	0.83	0.17	0.344	0.247	50.0
OPA7	0.89	0.11	0.233	0.161	44.4
OPA12	1.00	0.00	0.000	0.000	0.00
OPA18	0.94	0.06	0.225	0.154	33.3
OPA19	0.87	0.13	0.309	0.213	57.1
OPA20	0.94	0.06	0.088	0.041	50.0
OPB12	0.96	0.04	0.115	0.083	16.6

C. punctatus

Primer	GS	GD	I	H	PC %
OPA1	0.94	0.06	0.157	0.100	25.0
OPA4	1.00	0.00	0.000	0.000	0.00
OPA7	1.00	0.00	0.000	0.000	0.00
OPA12	0.88	0.12	0.217	0.142	33.3
OPA18	0.99	0.01	0.086	0.059	14.3
OPA19	0.97	0.03	0.200	0.137	33.3
OPA20	0.90	0.10	0.220	0.153	50.0
OPB12	1.00	0.00	0.000	0.000	0.00

C. gachua

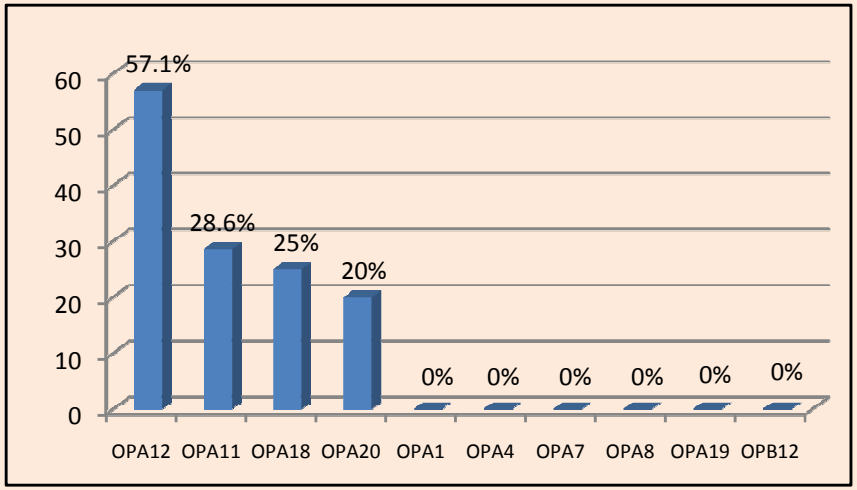
Primer	GS	GD	I	H	PC %
OPA1	0.94	0.06	0.146	0.100	28.6
OPA4	0.91	0.09	0.317	0.222	33.3
OPA7	1.00	0.00	0.000	0.000	00.0
OPA12	1.00	0.00	0.000	0.000	00.0
OPA18	0.94	0.06	0.270	0.189	42.8
OPA19	1.00	0.00	0.000	0.000	00.0
OPA20	1.00	0.00	0.000	0.000	00.0
OPB12	1.00	0.00	0.000	0.000	00.0

C. marulius

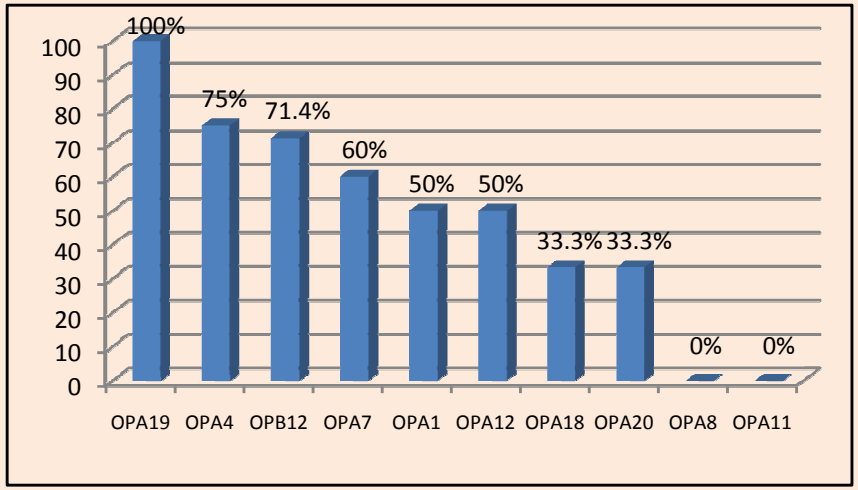
Primer	GS	GD	I	H	PC %
OPA1	0.89	0.11	0.188	0.122	40.0
OPA4	0.92	0.08	0.123	0.081	25.0
OPA7	0.94	0.06	0.110	0.072	22.2
OPA12	0.95	0.05	0.189	0.123	28.6
OPA18	1.00	0.00	0.000	0.000	00.0
OPA19	1.00	0.00	0.000	0.000	00.0
OPA20	0.97	0.03	0.060	0.036	14.2
OPB12	1.00	0.00	0.000	0.000	00.0

C. striatus

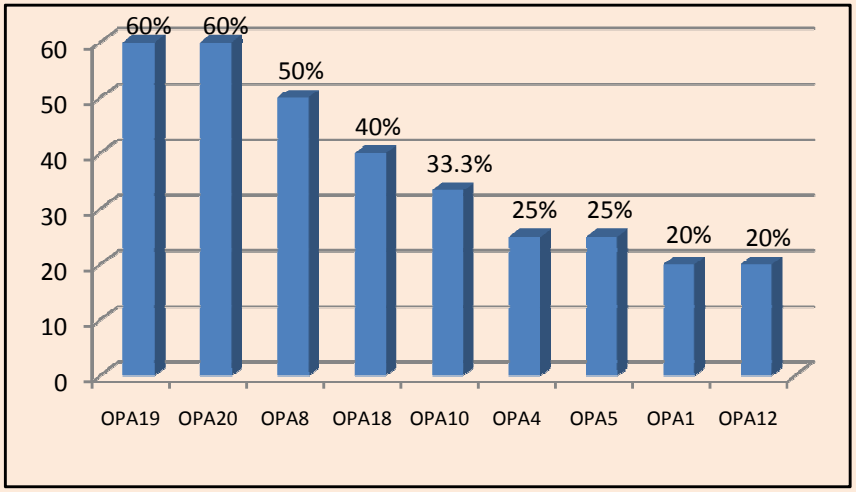
GS = Genetic similarity; **GD** = Genetic distance; **I** = Shannon's information index; **H** = Gene diversity; **PC%** = Polymorphic band content



C. batrachus



C. gariepinus



H. fossilis

Polymorphic band content of catfish species obtained with different primers

Genetic analyses values of three catfish species.

Primer	GS	GD	I	H	PC %
OPA1	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**
OPA4	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**
OPA7	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**
OPA8	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**
OPA11	0.93	0.07	0.165	0.112	28.6
OPA12	0.84	0.16	0.359	0.251	**57.**1
OPA18	0.94	0.06	0.144	0.098	25.0
OPA19	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**
OPA20	0.87	0.13	0.136	0.098	20.0
OPB12	**1.00**	**0.00**	**0.000**	**0.000**	**00.0**

C. batrachus

Primer	GS	GD	I	H	PC %
OPA1	0.81	0.19	0.227	0.161	**50.0**
OPA4	0.65	**0.35**	**0.409**	**0.268**	**75.0**
OPA7	0.79	0.21	**0.331**	**0.227**	**60.0**
OPA8	**1.00**	0.00	0.000	0.000	00.0
OPA11	**1.00**	0.00	0.000	0.000	00.0
OPA12	0.89	0.11	0.338	0.242	50.0
OPA18	0.89	0.11	0.159	0.100	33.3
OPA19	0.49	**0.51**	**0.467**	**0.310**	**100**
OPA20	0.93	0.07	**0.225**	**0.145**	33.3
OPB12	0.78	0.22	**0.445**	**0.303**	**71.4**

C. gariepinus

Primer	GS	GD	I	H	PC %
OPA1	**0.95**	**0.05**	0.095	0.060	20.0
OPA4	0.92	0.08	0.154	0.106	25.0
OPA5	**0.96**	**0.04**	0.169	0.121	25.0
OPA8	0.94	0.06	0.088	0.041	50.0
OPA10	0.93	0.07	0.165	0.110	33.3
OPA12	**0.98**	**0.02**	0.120	0.082	20.0
OPA18	0.92	0.08	0.229	0.148	**40.0**
OPA19	0.84	0.16	0.380	0.265	**60.0**
OPA20	0.80	0.20	0.240	0.154	**60.0**

H. fossilis

GS = Genetic similarity; **GD** = Genetic distance; **I** = Shannon's information index; **H** = Gene diversity; **PC%** = Polymorphic band content

CONCLUSIONS

1. The present study has clearly established the molecular basis of the identification of all seven investigated fish species.

2. Among all the primers OPA12 & OPA 7 is nearly regarded most suitable primers for *Channa* species, primer OPA8 for all catfish species and OPA11 and OPB12 for two *Clarias* species.

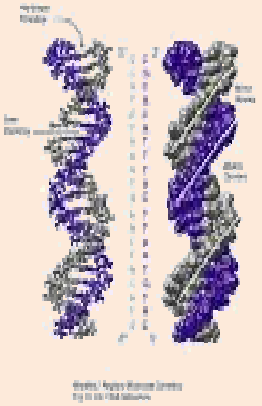
3. *C. gariepinus* shows the highest polymorphism and hence regarded as very hardy whereas *C. batrachus* shows great degree of vulnerability.

4. Among the Channid group. *C. punctatus* appears hardy and *C. marulius* shows highest degree of susceptibility. The other two species i.e., *C. gachua* and *C. striatus* fall in between the two.

5. Based on molecular phylogeny *C. punctatus* and *C. striatus* are in one cluster hence close to each other compared to *C. marulius* and *C. gachua* which shows relatedness between themselves hence put in another cluster.

6. This study has generated highly significant baseline data on the economically important food fishes of India and can be utilized in the effective management of successful propagation of these species by fishery biologists.

Thank You



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