

**STUDIES ON CERTAIN ASPECTS OF
TAXONOMY AND GENETICS OF SOME AIR-BREATHING FISHES
OF NORTH-EASTERN INDIA**

ABSTRACT

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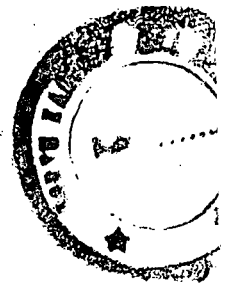
Thesis submitted in fulfilment of the requirement of the Degree of
DOCTOR OF PHILOSOPHY

To



**THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA**

NOVEMBER, 1982



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The live fishes comprise about 15% of the total marketable surplus of inland fisheries which seems to originate as captured fishery, either from wild water or from culture pond, naturally occurring with carps. Since it is an unorganised capture fishery, there is no authentic data regarding production.

However, the culture of air-breathing fishes could certainly constitute a substantial second line of production in terms of fish protein from the inland water and could help to step up the nutritional effort which is comparatively very low in India. Keeping this fact in mind, in recent years, the air-breathing fishes have caught the imagination of fishery scientists, in view of their popular demand, their nutritive value and recuperative importance and also for the need to utilize the swamps which cannot be easily made suitable for carp culture. The swamps with diverse water condition can be easily utilized by hardy fishes with air-breathing nature, thus these fishes could provide considerable scope to play an important role in the rural economy and hence it becomes imperative to organize planned effort to render these swamp production. On the other hand, while considering the development and standardization of the fishery management techniques of the air-breathing fishes, many gaps in the information were experienced in the field of biology, ecology and genetics, and therefore it is necessary to undertake the study of certain aspects of biology, systematics and genetics of these fishes. The present investigation is a part of such studies which involves a particular group of air-breathing fishes, the 'Channids' or murrels.

The murrels or the snake-headed fishes are represented by

only one genus Channa (= Ophiocephalus), comprising of six species. They have a homogenous distribution in tropical and subtropical region of the world and at least five species viz. Channa striatus, Channa barca, Channa punctata, Channa stewartii and Channa orientalis, are readily available in North-Eastern India. All the representatives are very much alike in colour and shape and therefore are often difficult to distinguish when they are of the same size. We have undertaken in our laboratory a detailed study on the biology and genetics of this group and keeping this in view, the present work has been carried out and the findings have been presented in three chapters of this thesis.

The first chapter presents the results of morphometric analysis and meristic studies of these five species while the second chapter deals with the chromosome analysis and the third chapter has been devoted to electrophoretic investigations on certain proteins.

I - Morphometric analysis and Meristic counts :

As far as the present study is concerned, a fairly successful working key for the field identification of the different species has been provided from the point of view of morphotaxonomy. All together twenty-nine morphological parameters have been studied. Though certain amount of intra-species variations are observed, measurements and counts of different body parameters show considerable amount of distinctiveness among different species. Biometric indices for fifteen body parameters have been carried out for all the five species and compared. This study, thus provides a balanced key to identify the different species, *irrespective of size, form*

and colour. Regression equations for ten body parameters have also been worked out for all the five species studied.

II - Cytogenetical Investigation :

All the five species of Channa have been subjected to chromosome analysis. The 2n numbers of different species are found to be as follows :-

<u>C. striatus</u>	2n=40 (8m + 6st + 26t), NF 54
<u>C. barca</u>	2n=38 (6m + 6sm + 4st + 22t), NF 54
<u>C. punctata</u> Var. A	2n=34 (16m + 14sm + 4t), NF 64
<u>C. punctata</u> Var. B	2n=32 (16m + 16sm), NF 64
<u>C. stewartii</u>	2n=66 (12m + 6sm + 6st + 42t), NF 90
<u>C. orientalis</u>	2n=76 (2m + 6sm + 68t), NF 84

The most interesting finding is the existence of two chromosomal races of C. punctata having 2n number as 34 and 32 respectively, which could not be detected by morphological studies. Moreover, it has been observed that all the species are markedly different from one another at their karyotypic level. Comparison made with the earlier works reveals that certain species (eg. C. orientalis) show quite a good amount of differences in their total chromosome complements. However, basing on the present finding, a hypothetical line of karyotypic speciation has also been presented. It has been suggested that species with more number of acrocentric chromosomes are nearer to the primitive teleosts while species with more number of bi-armed chromosomes are comparatively more advanced in the line of karyotypic evolution. But the karyotypes of C. stewartii and C. orientalis do not show any direct relationship with the karyotypes of C. striatus, C. barca or C. pur

III - Electrophoretic Investigations :

Characterization of different Channa species has also been tried through electrophoretic investigation of soluble tissue proteins and serum proteins along with two enzyme systems- Esterases and Lactate dehydrogenase. It has been observed that the pattern of distribution of tissue and serum proteins in all the five species are species specific as well as tissue specific, even through certain homologous bands could be detected among different species. Use of esterases results in unequivocal assignment to species because of species-specific banding pattern. To characterise the nature of esterases, inhibition experiments with different esterase activity inhibitors such as urea, CuSO_4 , Eserine sulphate, Diisopropylflurophosphate and heat, have also been performed and eight zones of esterase activity could be identified for all the five species. Lactate dehydrogenase isoenzymes show an unique distribution in all the five species studied. EDH A_4 is found to be more negatively charged than LDH B_4 and migrates furthest towards the cathode. LDH E_4 isoenzyme is present in all the species. The overall distribution and banding pattern of LDH is also medicative of the distinctiveness of the genus.

As a follow up of the present work we suggest the study of banding pattern of the karyotype to provide further insight into the karyotypic evolution within this group. We would also like to suggest further application of biochemical techniques involving a large number of enzymes and statistically significant number of individuals to find out the genetic distance between the members which we believe will have the last word in unravelling the genetic relationship of this group of fishes.

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This is to certify that the thesis entitled "STUDIES ON SOME ASPECTS OF TAXONOMY AND GENETICS OF SOME AIR-BREATHING FISHES OF NORTH-EASTERN INDIA", submitted by Mr. Nabendu Jyoti Dhar for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong (India), embodies the record of original investigations carried out under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of a Ph.D. Degree. This work has not been submitted for any Degree of any other University.

K. Chatterjee

(Dr. K. CHATTERJEE)

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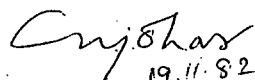
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19.11.82
(Nabendu Jyoti Dhar)

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FOREWORD

Certain freshwater fishes show unique environmental adaptations for direct use of atmospheric oxygen, in addition to their gill respiration. By virtue of their aerial respiratory habit, they can stand water deficient in oxygen, such as those of swamps and marshy areas with foul water, where the usual gill breathing fishes cannot thrive. Fishes with this particular characteristic are commonly grouped together and called as 'air breathing fishes'. There are at least eight freshwater and two marine genera of teleosts which constitute the fauna of air-breathing fishes in India, viz. Anabas, Amphipnous, Channa (Ophiocephalus), Clarias, Colisa, Heteropneustes, Notopterus, Ospromenus, Soleophthalmus and Periophthalmus.

The live fishes comprise about 15% of the total marketable surplus of inland fisheries (Jhingran, 1982) which seems to originate as captured fishery, either from wild water or from culture pond, naturally occurring with carps. Since it is an unorganised capture fishery, there is no authentic data regarding production.

However, the culture of air-breathing fishes could certainly constitute a substantial second line of production in terms of fish protein from the inland water and could help to step up the nutritional effort which is comparatively very low in India. Keeping this fact in mind, in recent years, the air-breathing fishes have caught the imagination of fishery scientists, in view of their popular demand, their nutritive value and recuperative importance and also for the need to utilize the swamps which cannot be easily made suitable for carp culture. The swamps with diverse water

condition can be easily utilized by hardy fishes with air-breathing nature, thus these fishes could provide considerable scope to play an important role in the rural economy and hence it becomes imperative to organize planned effort to render these swamp production. On the other hand, while considering the development and standardization of the fishery management techniques of the air-breathing fishes, many gaps in the information were experienced in the field of biology, ecology and genetics, and therefore it is necessary to undertake the study of certain aspects of biology, systematics and genetics of these fishes. The present investigation is a part of such studies which involves a particular group of air-breathing fishes, the 'Channids' or murrels.

The murrels or the snake-headed fishes are represented by only one genus Channa (=Ophiocephalus), comprising of six species (Menon, 1974). They have a homogenous distribution in tropical and subtropical region of the world and at least five species viz. Channa striatus, Channa barca, Channa punctata, Channa stewartii and Channa orientalis, are readily available in North-Eastern India. All the representatives are very much alike in colour and shape and therefore are often difficult to distinguish when they are of the same size. We have undertaken in our laboratory a detailed study on the biology and genetics of this group and keeping this in view, the present work has been carried out and the findings have been presented in three chapters of this thesis.

The first chapter presents the results of morphometric analysis and meristic studies of these five species while the second chapter deals with the chromosome analysis and the third chapter has

been devoted to electrophoretic investigation on certain proteins. The present study, therefore, will provide us, besides the general morphological differences, with a more comprehensive taxonomical key for identification of the different Channa species, comprising of Morpho-, Cyto- and Chemo-taxonomic parameters. On the other hand, chromosomal investigations will be helpful to unravel the phylogeny, speciation and evolution of this group. Protein electrophoresis will provide us with the information regarding protein polymorphism at intra- and interspecific levels of this group. In addition, the inhibition studies will reveal the nature and types of isoenzymes that are studied.

Jhingran, V.G. (1982) : Fish and Fisheries of India. Hindustan Publishing Company. 667 p.

Menon, A.G.K. (1974) : A check-list of fishes of Himalayan and Indo-Gangetic plains. Sp. Publ. No.1, Fishery Society of India (CIFRI, Barrackpore), 137 p.

CHAPTER-I

MORPHOMETRIC ANALYSIS AND MERISTIC COUNTS

1. INTRODUCTION

The teleostean complex comprising of more than 25,000 species, has a remarkable heterogenous composition with 32 orders and 400 families (Greenwood et al., 1966). This probably includes the list of subspecies and races, designated as separate species in different places. Therefore, to find out the exact taxonomic status of a species, it is necessary to study the morphometric and meristic features of a species, which in turn confirms the specific characters of a species, genus, family and so on. A brief survey of such investigations on Indian fishes is presented below.

The first authentic description of fishes of the Indian subcontinent can be dated back to the work of Bloch (1785) and his 'Auslandische Fische'. This is closely followed by the works of Lecepede (1798-1803), Schneider (1801) and Russell (1803). In 1822, Hamilton (Buchanan) listed the available fishes from the river Ganges. McClelland (1839) published his collection on Indian Cyprinidae with description. Sykes (1841) published his works on fishes from Dukhun. Jerdon (1849) described few species of fishes from Southern India. Bleeker (1853) described the fish fauna of Bengal. Günther (1859-1870) studied the fishes from British Museum collection and published a number of catalogues which included Indian fishes also. But the monumental works of Day (1878, 1889) on fishes from British India, Burma and Ceylon are still the most informative and useful references to Indian ichthyo-taxonomists. Since then, fishes were described from different regions by different workers such as Raj (1916), Annandalei (1918), Shaw and Shebbeare (1937) etc. Hora (1937, 1941, 1944, 1951 and 1955) has contributed much on Indian fishes along with their geographical distributions. Menon (1951, 1964 and 1974) and Mishra (1959) have

also described a large number of fishes from taxonomical point of view.

The murrels are represented by only one genus Channa. The name Channa was first used by Scopoli (1777) while describing the type Channa orientalis. Bloch (1794) gave the name Ophiocephalus for the genus while describing the type Ophiocephalus punctatus. He named them so, probably because of the similarities of structure and appearance of the head of these fishes with the snake-head (Ophidian=snake, Cephalus=head).

Regarding the classification of this group, some differences were observed between the classification of Day (1878) and the recent classification of Greenwood et al. (1966). Day grouped the murrels under the family Ophiocephalidae and brought it under the order Acanthopterygii which is characterised by spiny-rays, but he could also observe the differences in this regard of this family with other members of the order and probably that is why he grouped all the snake-headed fishes under a separate sub-division — Channiformes (the 13th group under the order Acanthopterygii). He classified this group under two genera — (i) Ophiocephalus and (ii) Channa, and described nine species under the genus Ophiocephalus and one species under the genus Channa. Regan (1929) included the murrels under the family Ophiocephalidae under the sub-order Ophiocephaloidea. The latter, along with other three sub-orders, viz. Percoidae, Gobioidae and Anabantoidae, was listed under the order Percomorpha. Greenwood et al. (1966) have grouped all the murrels under a single genus Channa and created a new order Channiformes, to accommodate only the snake-headed fishes.

Regarding the names Channa and Ophiocephalus there were certain controversy while using these in practice. Myers and Shapovalov (1931) opined that Channa Scopoli (1777) had priority over its subjective synonym Ophiocephalus Bloch 1794 (the earlier Channa Gronow 1763 has not been accepted because the 'Principle of Zoological Nomenclature' were not applied in that work). Scopoli's work (1777) has on the other hand been placed on the list of acceptable works. Smith (1945) made a plea for retention of the familiar name Ophiocephalus Bloch 1794, for the genus but the rules of nomenclature are adamant in this regard and the name Channa Scopoli 1777 should be used. Herse and Myers (1937), McAllister (1968) expressed their opinion in favour of Channa and subsequently most of the taxonomists are using this name.

Due to the presence of many overlapping characters among different members of the family (such as Ophiocephalus marulius, O. leucopunctatus and O. pseudomarulius), Day (1878) was quite confused regarding the proper identification of the species. Sometime Channa amphibious and Channa barca are considered as separate species (Shaw and Shebbeare, 1937; Reddi, 1981). Probably most controversial of all, is the species Channa orientalis or Ophiocephalus gachua. Day (1878) classified this under two separate genera according to the characteristics of the pelvic fin. Such differences are hardly observed and in fact, later on Day's Channa orientalis and Ophiocephalus gachua have been confirmed as a single species.

Altogether six Channa species are found in India (Menon, 1974). In North-Eastern region of India, all the six species are

readily available. This group of fishes are distributed throughout the world except West Europe, North America, South America and Australia. In India, they are available throughout, in ponds as well as streams and rivers, from tropical region to high mountain range.

The murrels are sometimes considered as weed fishes in carp culture ponds, because of their predatory habits on other fishes. But they may certainly provide a second line of production in terms of protein. In addition, the unutilizable swampy areas can be used with least management. Some of the murrels attain quite a large size and have commercial values. However, these fishes are not easily distinguishable because of their morphological similarities and many a time are wrongly identified. Unless there is a definite key for identification, there will always remain a problem in studying this group of fishes from different biological angles. Therefore, in the present investigation, morphotaxonomy, cytotaxonomy and chemotaxonomy are incorporated so that a proper identification formula could be evaluated. In the present chapter, the morphotaxonomical parameters for different species have been considered.

2. MATERIALS AND METHODS

2.1. The Fishes :

The materials for the present investigations comprise of five species (Fig. 1a & b) belonging to the genus Channa, viz.;

1. Channa striatus (Bloch)
2. Channa barca (Hamilton)
3. Channa punctata (Bloch)
4. Channa stewartii (Playfair)
5. Channa orientalis (Schneider)

These are included in the family Channidae, the characteristic features of which are: body elongated, the anterior portion is subcylindrical; head is depressed and has plate like scales; laterally placed eyes. The presence of five branchiostegals but no pseudobranchii; the number of gills is four; gill openings are wide and the membranes of the two sides are connected beneath the isthmus. Suprabranchial organ is not developed. On either side, an accessory hollow cavity, besides the gill cavity in the head region, acts as an accessory respiratory organ and provides an amphibious mode of respiration which helps them to thrive for a long period out of their natural habitat. Teeth are present in the jaws, vomer and palate, some of which are conical in shape. The dorsal fin is spineless, long and single. The anal fin is almost similar to dorsal except it is shorter. Pectoral as well as pelvic fins are present in the thoracic region. Scales are either large, moderate or small. Lateral line is abruptly curved and almost discontinuous. Air tube (sac) is present. Few numbers of pyloric appendages are present.

2.1.1. Synopsis of Species:

The morphological characters for different species as described by Day (1878) are as follows:-

1. Channa striatus D. 37-45, A. 23-26, L.l. 50-57, L.tr. $\frac{4\frac{1}{2}}{9}$ or $\frac{5\frac{1}{2}}{10}$. Dark gray passing in stripes into the white of the abdomen.
2. Channa barca D. 47-52, A. 34-36, L.l. 60-65, L.tr. $\frac{5}{13}$. Ventral $\frac{2}{5}$ th of length of pectoral, dark violet, spotted, as are also the fins.
3. Channa punctata D. 29-32, A. 21-23, L.l. 37-40, L.tr. $\frac{4-6}{9}$. Spotted or banded; vertical fins dark with a light edge.
4. Channa stewartii D. 39-40, A. 27, L.l. 47-50, L.tr. $\frac{4\frac{1}{2}}{9}$. Ventrals $\frac{1}{3}$ rd as long as pectoral, purplish spotted with black.
5. Channa orientalis D. 32-37, A. 21-23, L.l. 40-45, L.tr. $\frac{3}{7}$. Ventrals $\frac{2}{5}$ th as long as pectoral; pectoral fins banded; vertical fins edged with red.

2.1.2. Synonyms:

1. Channa striatus (Bloch)

(i) Ophiocephalus striatus Bloch, Naturg. Ausland. Fische., 2, p. 141, pl. 359, 1793; type locality: Malabar. Gunther, Cat. Fish. Brit. Mus., 3, p.474, 1861. Bleeker, Verh. Bat. Gen., 25, p.42, 1853. Day, Fish. India, p.366, 1876. Day, Faun. Brit. Ind. Fish., 2, p.363, 1889. Shaw and Shebbeare, J. Asiat. Soc. Beng., 3, p.124, pl.4, fig.4, 1937; North Bengal. Menon, Rec. Ind. Mus., 52, p.22, 1954, Manipur.

(ii) Ophiocephalus wrahl Lecepede's (Buffon), Hist. Nat. Poiss., 3,

p.552, 1802; type locality: Tranquebar; Hamilton, Fish. Ganges, pp.60, 367, pl.31, fig.17, 1822.

(iii) Ophiocephalus chena Hamilton, Fish. Ganges, p.62, 1822. Type locality: Goalpara, Assam.

(iv) Ophiocephalus planiceps Valenciennes (in C. & V.), Hist. Nat. Poiss., 7, p.424, 1831; type locality: Java.

(v) Ophiocephalus sowarah Bleeker, Nat. Geneesk. Arch. Ned. Indie, 2(3), p.519, 1845.

2. Channa barca (Hamilton)

(i) Ophiocephalus barca Hamilton, Fish. Ganges, pp.67, 367, pl.35, fig. 20, 1822; type locality: R. Brahmaputra, near Goalpara, Assam. Cuvier (in C. & V.) Hist. Nat. Poiss., 7, p.436, 1831. Gunther, Cat. Fish. Brit. Mus., 3, p.477, 1861. Day, Fish. India. p.365, pl. 77, fig.2, 1876. Day, Faun. Brit. India Fish., 2, p.361, fig.118, 1889.

(ii) Bora chung Russell, J. Asiat. Soc. Beng., 8, p.551, 1839.

(iii) Ophiocephalus amphibius McClelland, Calcutta. J. Nat. Hist., 5, p.275 (Vol.1, pl.11, fig.3, 1841), 1845; type locality: R. Chail (tributary of Brahmaputra), Bhutan. Shaw and Shebbeare, J. Asiat. Soc. Bengl, 3, p.119, pls. 1 and 6, 1937; North Bengal.

3. Channa punctata (Bloch)

(i) Ophiocephalus punctatus Bloch, Naturges. ausland. Fische., 7, p.139, pl.358, 1793; type locality: Coromandel coast. Bleeker, Verh. Bat. Gen., 25, p.42, 1853. Gunther, Cat. Fish. Brit. Mus., 3, p.469, 1861. Day, Fish. India, p.367, pl.78, fig.1, 1876. Day,

Faun. Brit. Ind. Fish., 2, p.364, 1889. Shaw and Shebbeare, J. Asiat. Soc. Beng., 3, p.123, pl.4, fig.1, 1937; North Bengal. Hora, Rec. Ind. Mus., 39, p.44, 1937; Nepal. Menon, Rec. Ind. Mus., 47, p.235, 1949; R. Kosi, East Nepal. Regan, Rec. Ind. Mus., 1, p.158, 1907; Nepal.

(ii) Ophiocephalus karruway Lecepede's (Buffon), Hist. Nat. Poiss., 3, p.552, 1802; type locality - Tranquebar.

(iii) Ophiocephalus lata Hamilton, Fish. Ganges, pp.63, 367, pl. 34, fig.18, 1822; type locality, R. Ganges.

(iv) Ophiocephalus indicus McClelland, Calcutta. J. Nat. Hist., 2, p.583, 1842; type locality: Loodianah and Jallalabad. Bleeker, Verh. Bat. Gen., 25, p.42, 1853.

(v) Ophiocephalus affinis Gunther, Cat. Fish. Brit. Mus., 3, p.470, 1861; type locality: Mauritius.

(vi) Channa punctata De Witt, Stanford Ichth. Bull., 7(4), p.82, 1960, Nepal.

(vii) Channa punctatus Menon, Rec. Indian Mus., 52, p.22, 1954, Manipur.

4. Channa stewartii (Playfair)

(i) Ophiocephalus stewartii Playfair, Proc. Zool. Soc. Lond., p.14, pl.3, 1867; type locality: Cachar, Assam. Day, Fish. India, p.367, pl.77, fig.3, 1876. Day, Faun. Brit. Ind. Fish., 2, p.363, 1889. Shaw and Shebbeare, J. Asiat. Soc. Beng., 3, p.123, pl.4, fig.3, 1937; North Bengal. Menon, Rec. Ind. Mus., 47, p.235, 1949; type locality: R. Kosi, East Nepal.

5. Channa orientalis (Schneider)

(i) Ophiocephalus gachua Hamilton, Fish. Ganges, pp.68, 367, pl. 21, 1822; Bengal. Bleeker, Verh. Bat. Gen., 25, p.42, 1853. Gunther, Cat. Fish. Brit. Mus., 3, p.471, 1881. Day, Fish. India, p.367, 1876. Day, Faun. Brit. Ind. Fish., 2, p.364, 1889. Shaw and Shebbeare, J. Asiat. Soc. Beng., 3, p.121, 1937; North Bengal. Menon, J. Bombay Nat. Hist. Soc., 48(3), p.540, 1949; Kumaon. Menon, Rec. Ind. Mus., 47, p.229; Hoshiarpur.

(ii) Channa orientalis Schneider, Syst. Ichth. Bloch, p.496, pl. 90, fig.2, 1801; type locality: India. Gunther, Cat. Fish. Brit. Mus., 3, p.483, 1881. Day, Fish. India, p.367, 1876. Day, Faun. Brit. Ind. Fish., 2, p.365, fig.119, 1889. De Witt, Stanford Ichth. Bull., 7(4), p.81, 1960; Nepal.

(iii) Ophiocephalus aurantiacus Hamilton, Fish. Ganges, pp.69, 368, pl.23, fig.22, 1822; type locality: Goalpara, Assam.

(iv) Ophiocephalus limbatus Cuvier (in C. & V.), Hist. Nat. Poiss., 7, p.201, 1831.

(v) Ophiocephalus coramota Cuvier (in C. & V.), Hist. Nat. Poiss., 7, p.414. (from Russel, pl.11, p.49), 1831; Vizagapatam.

(vi) Ophiocephalus fusca Cuvier (in C. & V.), Hist. Nat. Poiss., 7, 414, 1831.

(vii) Ophiocephalus marginatus Cuvier (in C. & V.), Hist. Nat. Poiss, 7, p.411, 1831; type locality: Pondicherry.

(viii) Ophiocephalus montanus McClelland, Calcutta J. Nat. Hist., 2, p.583, 1842; type locality: 'Baisoot, Jallalabad, Himalaya and

Sadon¹. Bleeker, Verh. Bat. Gen., 25, p.42, 1853.

(ix) Philypnoidees surakartensis Bleeker, Verh. Bat. Gen., 22, p. 19, 1849.

(x) Ophiocephalus kelaartii Gunther, Cat. Fish. Brit. Mus., 3, p. 472, 1861; type locality: Ceylon.

(xi) Ophiocephalus gachua malaccensis Peters, Monatsber. Akad. Wiss. Berlin, p.262, 1868.

(xii) Ophiocephalus harcourt butleri Annandale, Rec. Ind. Mus., 14, p.54, pl.2, fig.7, pl.4, fig.16, 17, 1918. Hora, Rec. Indian Mus., 22, p.208, 1921; type locality: Manipur.

(xiii) Channa burmanica Choudhury, Rec. Indian Mus., 16, p.284, pl.22, fig.4, 1919; type locality: R. Irrawady, N. Burma.

(xiv) Channa gachua Menon, Rec. Indian Mus., 52, p.22, 1954; Manipur.

2.2. Taxonomic status: (Greenwood et al. 1966)

Phylum	: Vertebrata
Sub-Phylum	: Craniata
Super-class	: Gnathostomata
Series	: Pisces
Class	: Teleostomi
Sub-class	: Actinopterygii
Super-order	: Acanthopterygii
Order	: Channiformes
Family	: Channidae
Genus	: <u>Channa</u> (Scopoli, 1777)

- Species : 1. C. striatus (Bloch)
 2. C. barca (Hamilton)
 3. C. punctata (Bloch)
 4. C. stewartii (Playfair)
 5. C. orientalis (Schneider)

2.3. Distribution and Collection Sites:

This group of fish is distributed throughout India, Burma and Ceylon. They inhabit from elevated localities and most inland districts to within the range of tides. They generally live in holes or grassy-edges of ponds, lakes, streams or rivers. They are able to change their habitat by traversing through moist piece of ground.

All these five Channa species were collected over a period of three years or so from freshwater bodies like streams, ponds, lakes etc. in and around Gauhati (Assam) and Shillong (Meghalaya). Channa orientalis was mostly collected from the streams of Shillong and adjacent areas. All other species were collected from plain areas with occasional catch of Channa stewartii and Channa punctata from Shillong.

2.4. Morphometric Measurements and Meristic Counts :

2.4.1. Morphometric Measurements:-

The different parameters employed, to study the general morphological features of the different fish species, are adopted from Ricker (1971). These are :-

- (1) Total length (TL) : This is the greatest length of the fish from its anterior-most extremity to the end of the tail fin.

- (2) Standard length (SL): It is the greatest length of a fish from its anterior-most extremity (mouth closed) to the hidden base of the median tail fin rays (where these meet the median hypural plate).
- (3) Body depth (BD): This is taken at the deepest point, exclusive of fleshy or scaly structure at fin base.
- (4) Head length (HL): This is measured with mouth closed from the tip of the snout or upper lip (whichever extends farthest forward) to the posterior edge of the opercular bone or to the extremity of the membrane margining the bone but excluding the opercular spines if these are present.
- (5) Head width (HW): This is the greatest dimension with gill covers closed in normal position.
- (6) Snout length (Sn.L): This is taken with divider from the most anterior point on the snout or upper lip (whichever extends farthest forward) to the front margin of the orbit.
- (7) Post-orbital length of Head (POL): This is the greatest distance between the hind margin of the orbit and the body opercular margin.
- (8) Suborbital depth: This is generally taken from the bony edge of the orbit to the suborbital or pre-orbital margin at its deepest point.

- (9) Inter-orbital width (IOD): This is the least bony width from orbit to orbit.
- (10) Eye diameter (ED): This is taken as length of orbit, the greatest distance between the free orbital edge and is often oblique.
- (11) Lower jaw length: It is the length of the mandible taken with one tip of the divider inserted in the posterior mandibular joint to give the maximum possible dimension.
- (12) Upper jaw length: It is taken from the anteriormost point of the premaxillary to posterior point of maxillary.
- (13) Gape width: This is the greatest transverse distance across the mouth opening, with the mouth closed.
- (14) Pre-dorsal length (PDL): Distance from the tip of the snout to the first dorsal fin ray.
- (15) Head depth (HD): This is the perpendicular distance between the end of the nape to the ventral side of the head.
- (16) Girth (G): Circumference of the body at its deepest point.
- (17) Base length of the Dorsal fin (DFB).
- (18) Base length of the Anal fin (AFB).
- (19) Height of the Pectoral fin.
- (20) Height of the Pelvic fin.

- (21) Length of the Caudal fin.
- (22) Distance between the Pelvic fin and Anal opening.
- (23) Distance between the Pelvic fin and Anal fin.

2.4.2. Meristic Counts:

- (24) Scale counts: 'Lateral line scales' represent the number of pored scales in the lateral line. 'Scale above the lateral line' are counted from the origin of the dorsal fin including the small scales and counting downwards and backward too, but including the lateral line scale. 'Scales below the lateral line' are counted similarly to those above but upward and forward from the origin of anal fin including the small scale; sometimes caudal peduncle-scales are also considered. The scales present in the head regions are also counted.

- (25) Number of rays in Dorsal fin.
- (26) Number of rays in Pectoral fin.
- (27) Number of rays in Pelvic fin.
- (28) Number of rays in Anal fin.
- (29) Number of rays in Caudal fin.

2.4.3. Regression Equation and Biometric Index:

Besides these parameters, the colouration of the fish, sex and weight are also taken into consideration. All the above mentioned

parameters are considered from one particular side of the fish. To study the morphometric characteristics, a regression method has been employed with the formula:

$$Y = a + bx$$

Where, 'x' is the variable character; 'a' is a constant value to be determined, 'b' is the regression coefficient and 'Y' is the total length. The values of 'a' and 'b' are determined by the following formula:

$$b = \frac{XY - n\bar{x}\bar{y}}{x^2 - n(\bar{x})^2} \quad a = Y - bx$$

Where n is the total number of length groups; \bar{x} is the mean of 'X' and \bar{y} is the mean of 'Y'.

Biometric index for each species has been calculated out as described by Tobor (1974), by taking the following parameters in relation to the total length and head length as mentioned below:-

Standard length, Body depth, Girth, Head length, Head width, Head depth, Pre-dorsal length, Base of Dorsal fin and Base length of Anal fin in relation to Total length, and Eye diameter, Snout length, Inter-orbital distance, Post-orbital distance, Head width and Head depth in relation to Head length, have been considered.



3.1. Morphometry and Meristic Counts of Channa striatus.

3.1.1. Morphometry :

The results obtained on morphometric study of Channa striatus have been presented in Table-1 and summarised below :

Total length: The mean value of total length (cm) is found to be 27.67 cm. with a range from 18.8 to 33.4 cm. and in percentage of standard length, it is found to be 114.34.

Standard length: The mean standard length is recorded as 24.2 cm. and the range of standard length is found to be 16.5 to 28.8 cm.

Body depth: The mean value of body depth is found to be 3.82 cm. When expressed as percentage of standard length it is found to be 15.79. The range extends from 2.65 to 4.5 cm.

Head length: The mean value of head length is recorded as 7.42 cm. with a range from 5.4 to 8.7. When expressed as percentage of standard length it is found to be 30.66.

Girth: The mean value of girth as recorded is 12.26 cm. and when expressed as percentage of standard length it is found to be 50.68. The range varies between 9.1 and 14.4 cm.

Pre-dorsal length: The mean value for pre-dorsal length is recorded as 8.14 cm and the range varies between 6.05 and 9.65 cm. When the mean value is expressed as percentage of standard length it is found to be 33.64.

Dorsal fin base length: The mean value for dorsal fin base length is recorded to be 13.83 cm. whereas the range is from 9.7

to 16.2 cm. When expressed as percentage of standard length it is found to be 57.13.

Anal fin base length: The mean value for anal fin base length is recorded to be 8.53 cm. The range varies between 5.65 and 10.2. When it is expressed as percentage of standard length, it is found to be 35.25.

Height of dorsal fin: In this case, the mean value obtained is 2.26 cm. When it is expressed as percentage of standard length, it is found to be 9.94. The minimum and maximum values are 1.35 and 3.05 cm. respectively.

Height of pectoral fins: The mean value for this parameter is 3.55 cm. with the minimum and maximum values of 2.0 and 4.6 cm. respectively. When converted as percentage of standard length it is recorded as 14.67.

Height of pelvic fins: The mean value for pelvic fin height is found to be 2.67 cm. When expressed as percentage of standard length it is found to be 11.04. The range for this parameter varies between 1.85 and 3.4 cm.

Height of Anal fin: The mean value is found to be 2.03 cm. When expressed as percentage of standard length it is recorded to be 8.54. The range varies between 1.25 and 2.45 cm.

Length of caudal fin: The mean value for this parameter is found to be 3.49 cm. The minimum and maximum values recorded are 2.3 and 4.6 cm. When the mean value is expressed as percentage of standard length it is found to be 14.45.

Distance between pelvic fin to anal opening: The mean value for this parameter is found to be 4.29 cm. When it is expressed as percentage of standard length, it is found to be 17.73. The range varies between 2.6 and 5.4 cm.

Distance between pelvic fin to anal fin: The mean value for this parameter is found to be 4.48 cm. When expressed as percentage of standard length it is found to be 18.51. The values range between 2.9 and 6.5 cm.

Head width: The mean value obtained for this parameter is 3.71 cm. with a minimum and a maximum value of 2.8 and 4.6 cm. respectively. When expressed as percentage of standard length it is observed to be 15.33.

Head depth: The mean value obtained for this parameter is 3.11 cm. When expressed as percentage of standard length it is found to be 12.85. The range varies between 1.9 and 3.95 cm.

Snout length: The mean value for snout length is calculated out to be 1.51. When this is expressed as percentage of head length it is found to be 20.35. The value ranges between 0.9 and 1.8 cm.

Eye diameter: The mean eye diameter for this fish is found to be 0.9 cm. When this is converted to percentage of head length it is found to be 12.13. The value ranges between 0.7 and 1.1 cm.

Post-orbital length: The mean value for post-orbital length is found to be 4.69 cm. with the minimum and maximum values of 3.65 and 6.1 cm. respectively. When converted the mean value as percentage of head length it is found to be 63.21.

Table-1 : Morphometric Analysis of Channa striatus

Parameters	Mean (cm)	Mean % standard length	Range (cm)
Total length	27.67	114.34	18.8 - 33.4
Standard length	24.2		16.5 - 28.8
Body depth	3.82	15.79	2.65 - 4.5
Girth	12.26	50.68	9.1 - 14.4
Head length	7.42	30.66	5.4 - 8.7
Pre-dorsal length	8.14	33.64	6.05 - 9.65
Dorsal fin base length	13.83	57.13	9.7 - 16.2
Anal fin base length	8.53	35.25	5.65 - 10.2
Height of dorsal fin	2.26	9.94	1.35 - 3.05
Height of pectoral fin	3.55	14.67	2.0 - 4.6
Height of pelvic fin	2.67	11.04	1.85 - 3.4
Height of anal fin	2.03	8.54	1.25 - 2.45
Length of caudal fin	3.49	14.45	2.3 - 4.6
Dist. betn. Pel.F. to Anal.Op.	4.29	17.73	2.6 - 5.4
Dist. betn. Pel.F. to Anal.F.	4.48	18.51	2.9 - 6.5
Head width	3.71	15.33	2.8 - 4.6
Head depth	3.11	12.85	1.9 - 3.95

Parameters	Mean (cm)	Mean % of Head length	Range
Snout length	1.51	20.35	0.9 - 1.8
Eye diameter	0.9	12.13	0.7 - 1.1
Post orbital length	4.69	63.21	3.65 - 6.1
Sub-orbital length	0.66	8.89	0.5 - 0.9
Inter orbital distance	1.79	24.12	1.25 - 2.1
Upper jaw length	3.02	40.70	2.2 - 3.4
Lower jaw length	3.23	43.51	2.3 - 3.8
Gape	1.4	18.87	1.2 - 1.7

Sub-orbital length: The mean value of this parameter is recorded to be 0.66 cm. which is 8.89% of the mean head length. The value ranges between 0.5 and 0.9 cm.

Inter-orbital distance: The mean value for this parameter is found to be 1.79 cm. When it is expressed as percentage of head length, it is found to be 24.12. The range varies between 1.25 and 2.1 cm.

Upper jaw length: The mean value for this parameter is found to be 3.02 cm. with the minimum and maximum values of 2.2 and 3.4 cm. respectively. When it is expressed as percentage of head length, it is found to be 40.70.

Lower jaw length: The mean value for this parameter is recorded to be 3.23 with the minimum and maximum values of 2.3 and 3.8 cm. respectively. When it is expressed as percentage of head length, it is found to be 43.51.

Gape width: The mean value for the gape is found to be 1.4 cm. When expressed as percentage of head length it is found to be 18.87. The minimum and maximum values recorded are 1.2 and 1.7 cm. respectively.

3.1.2. Meristic counts

The details of meristic counts are given in the Table-16 and summarised as follows:

Dorsal fin rays: The mean value is found to be 43.5. The range varies between 41 and 46.

Pectoral fin rays: The mean value is 15.86, which varies between 15-18.

Pelvic fin rays: This is found to 6 and observed to be constant.

Anal fin rays: The mean value is found to be 26.86 with the minimum and maximum values of 26 and 28 respectively.

Caudal fin rays: The mean value is found to be 15.43. This ranges between 15 and 16.

Lateral line scales: The mean number is recorded to be 60.07 which varies between 57 and 61.

Transverse scales: The mean value obtained is 7.93/11.03. This varies between $7\frac{1}{2}/10\frac{1}{2}$ to $8\frac{1}{2}/11\frac{1}{2}$.

3.1.3. Regression equations

The regression equations for various morphometric parameters studied for Channa striatus are presented in Table-2.

3.1.4. Biometric Indices

For all the characters (morphometric) studied, a mean biometric index for each 3.0 cm. length group has been analysed and presented in Table-3, Fig. 2.

3.2. Morphometry and Meristic Counts of Channa barca.

3.2.1. Morphometry

The results obtained for morphometric measurements of Channa barca have been presented in Table-4 and summarised below :

Total length: The mean value for total length is found to be 25.51 cm. When expressed as percentage of standard length it is found to be 107.37. The value ranges between 17.5 and 34.5 cm.

Table-2 : Regression Equations on Morphometric Parameters of
Channa striatus

Parameters	Regression Equations
Total length (Y) VS. Standard length (x)	$Y = 0.1754 + 1.1264 x$
Total length (Y) VS. Body depth (x)	$Y = 2.1002 + 6.6428 x$
Total length (Y) VS. Girth (x)	$Y = 2.5926 + 2.1394 x$
Total length (Y) VS. Head length (x)	$Y = 1.6740 + 3.7769 x$
Total length (Y) VS. Eye diameter (x)	$Y = 1.6014 + 31.2631 x$
Total length (Y) VS. Snouthlength (x)	$Y = 0.6004 + 18.2222 x$
Total length (Y) VS. Post-orbital length (x)	$Y = 1.9164 + 5.4745 x$
Total length (Y) VS. Inter-orbital length (x)	$Y = 0.5001 + 15.3809 x$
Total length (Y) VS. Head depth (x)	$Y = 0.5994 + 9.111 x$
Total length (Y) VS. Pre-dorsal length (x)	$Y = 0.6236 + 3.2044 x$

Table-3 : Biometric Indices in different length groups of Channa striatus

Parameters	GROUP I 18.1-21.0 cm	GROUP II 21.1-24.0 cm	GROUP III 24.1-27.0 cm	GROUP IV 27.1-30.0 cm	GROUP V 30.1-33.0 cm	GROUP VI 33.1-36.0 cm
TL/SL	1.14	1.14	1.15	1.14	1.14	1.16
TL/BD	7.12	7.65	7.18	7.08	7.37	7.42
TL/Girth	2.09	2.39	2.33	2.22	2.28	2.32
TL/HL	3.51	3.82	3.65	3.68	3.81	3.98
TL/PDL	3.15	3.72	3.39	3.39	3.42	3.67
TL/DFB	1.97	2.08	2.01	1.95	2.03	2.06
TL/AFB	3.37	3.54	3.54	3.24	3.28	3.34
TL/HW	6.69	7.41	7.45	7.75	7.52	7.26
TL/HD	9.7	9.12	9.92	8.97	8.52	9.15
HL/ED	7.9	7.75	8.29	8.33	8.38	7.64
HL/IOD	4.32	3.88	4.08	4.17	4.19	4.0
HL/Sn.L.	5.82	5.17	5.11	4.75	4.77	4.67
HL/PDL	1.50	1.48	1.43	1.47	1.42	1.46
HL/HW	1.83	1.98	2.11	2.04	1.94	1.91
HL/HD	2.77	2.38	2.45	2.44	2.24	2.3

Standard length: The mean value for standard length obtained is 23.76 cm. The range varies between 14.9 and 29.5 cm.

Body depth: The mean value is recorded to be 3.51 cm. When expressed as percentage of standard length it is found to be 14.77. The range varies between 2.25 and 4.7 cm.

Girth: The mean girth obtained is 11.39 cm. When converted as percentage of standard length it is recorded to be 49.93. The value varies between 7.9 and 14.9 cm.

Head length: The mean value for this parameter is found to be 6.93 cm. with the minimum and maximum values of 5.0 and 8.95 cm. respectively. When expressed as percentage of standard length it is found to be 29.17.

Pre-dorsal length: The mean value for this parameter is found to be 7.56 cm. with the minimum and maximum values of 5.3 and 10.2 cm. respectively. When expressed as percentage of standard length it is found to be 31.82.

Dorsal fin base length: The mean value of this parameter recorded is to be 13.54 cm. The range varies between 9.35 and 18.7 cm. When converted as percentage of standard length it is found to be 56.99.

Anal fin base length: The mean value is found to be 7.71 cm. The range varies between 4.9 and 11.0 cm. When expressed as percentage of standard length it is found to be 32.45.

Height of dorsal fin: The mean value for this parameter is recorded to be 2.15 cm. The value ranges between 1.3 and 2.95 cm. When expressed as percentage of standard length, is found to be 9.63.

Table-4 : Morphometric Analysis of Channa barca

Parameters	Mean (cm)	Mean % standard length	Range (cm)
Total length	25.51	107.37	17.5 - 34.5
Standard length	23.76	-	14.9 - 29.5
Body depth	3.51	14.77	2.25 - 4.7
Girth	11.39	47.93	7.9 - 14.9
Head length	6.93	29.17	5.0 - 8.95
Pre-dorsal length	7.56	31.82	5.3 - 10.2
Dorsal fin base length	13.54	56.99	9.35 - 18.7
Anal fin base length	7.71	32.45	4.9 - 11.0
Height of dorsal fin	2.15	9.63	1.3 - 2.95
Height of pectoral fin	3.34	14.06	2.2 - 4.6
Height of pelvic fin	2.43	10.23	1.6 - 3.2
Height of anal fin	1.75	7.37	1.1 - 2.35
Length of caudal fin	3.75	15.78	2.6 - 5.0
Dist. betn. Pel.F.to A.opening	3.41	14.35	2.1 - 4.7
Dist. betn. Pel.F.to Anal fin	3.83	16.13	2.5 - 5.15
Head width	3.62	15.24	2.25 - 5.4
Head depth	3.08	12.96	1.9 - 4.3

Parameters	Mean (cm)	Mean % of Head length	Range (cm)
Snout length	1.46	21.07	1.1 - 1.8
Eye diameter	0.82	11.83	0.65 - 1.0
Post orbital length	4.82	69.55	3.15 - 6.35
Sub-orbital length	0.64	9.24	0.5 - 0.75
Inter-orbital length	1.62	23.31	1.1 - 2.2
Upper jaw length	2.74	39.49	2.1 - 3.3
Lower jaw length	3.06	44.20	2.3 - 3.85
Gape	1.16	16.69	1.05 - 1.30

Height of pectoral fin: The mean value is found to be 3.34 cm. for this parameter. The minimum and maximum values are 2.2 and 4.6 cm. respectively. When expressed as percentage of standard length it is found to be 14.06.

Height of pelvic fin: The mean value is found to be 2.43 cm. The value ranges between 1.6 and 3.2 cm. When expressed as percentage of standard length it is found to be 10.23.

Height of anal fin: The mean value for this parameter is recorded to be 1.75 cm. The minimum and maximum values are 1.1 and 2.35 cm. respectively. When this is converted as percentage of standard length it is found to be 7.37.

Length of caudal fin: The mean value for this parameter is found to be 3.75 cm. with a range between 2.6 and 5.0 cm. When expressed as percentage of standard length it is recorded to be 15.78.

Distance between pelvic fin to anal opening: The mean value for this parameter is found to be 3.41 cm. The value ranges between 2.1 and 4.7 cm. When expressed as percentage of standard length it is found to be 14.35.

Head width: The mean value for this parameter is found to be 3.62 cm. The value ranges between 2.25 and 5.4 cm. When expressed as percentage of standard length it is found to be 15.24.

Head depth: The mean value for this parameter is found to be 3.08 cm. The minimum and maximum values recorded are 1.9 and 4.3 cm. respectively. When converted to percentage of standard length it is found to be 12.96.

Snout length: The mean value for snout length is found to be 1.46 cm. The maximum and minimum values recorded are 1.8 and 1.1 cm, respectively. When expressed as percentage of head length it is found to be 21.07.

Eye diameter: The mean value for this parameter is found to be 0.82 cm. The range varies between 0.65 and 1.0 cm. When expressed as percentage of head length it is recorded to be 11.83.

Post-orbital length: The mean value is found to be 4.82 cm. The value ranges between 3.15 and 6.35 cm. When expressed as percentage of head length it is found to be 69.55.

Sub-orbital length: The mean value for this parameter is found to be 0.64 cm. The value varies between 0.5 and 0.75 cm. When expressed as percentage of head length, it is found to be 9.24.

Inter-orbital length: The mean value for this parameter is found to be 1.62 cm. The minimum and maximum values obtained are 1.1 and 2.2 cm, respectively. When expressed as percentage of head length it is found to be 23.31.

Upper jaw length: The mean value is found to be 2.74 cm. The minimum and maximum values recorded are 2.1 and 3.3 cm, respectively. When expressed as percentage of head length it is found to be 39.49.

Lower jaw length: The mean value is found to be 3.06 cm. The values ranges between 2.3 and 3.85 cm. When expressed as percentage of head length it is found to be 44.20.

Gape width: The mean value for this parameter is found to

Table-5 : Regression Equations on Morphometric Parameters of Channa barca

Parameters	Regression Equations
Total length (Y) VS. Standard length (x)	$Y = 2.9527 + 1.0694 x$
Total length (Y) VS. Body depth (x)	$Y = 1.0602 + 7.0545 x$
Total length (Y) VS. Girth (x)	$Y = 0.8219 + 2.2602 x$
Total length (Y) VS. Head length (x)	$Y = 1.5021 + 3.7079 x$
Total length (Y) VS. Eye diameter (x)	$Y = 2.1563 + 31.6250 x$
Total length (Y) VS. Snout length (x)	$Y = 1.4308 + 14.3463 x$
Total length (Y) VS. Post-orbital length (x)	$Y = 2.0632 + 5.1487 x$
Total length (Y) VS. Inter-orbital length (x)	$Y = 2.1275 + 13.6414 x$
Total length (Y) VS. Head depth (x)	$Y = 2.3761 + 7.9591 x$
Total length (Y) VS. Pre-dorsal length (x)	$Y = 1.0989 + 3.2849 x$

Table-6 : Biometric Indices in different length groups of Channa barca.

Parameters	GROUP I 15.1-18.0 cm.	GROUP II 18.1-21.0 cm.	GROUP III 21.1-24.0 cm.	GROUP IV 24.1-27.0 cm.	GROUP V 27.1-30.0 cm.	GROUP VI 30.1-33.0 cm.	GROUP VII 33.1-36.0 cm.
TL/SL	1.18	1.17	1.16	1.19	1.19	1.17	1.17
TL/BD	7.78	7.28	7.28	7.1	6.83	7.37	7.34
TL/Girth	2.22	2.2	2.23	2.25	2.19	1.28	2.32
TL/EL	3.51	3.58	3.88	3.68	3.45	3.69	3.88
TL/PDL	3.29	3.31	3.59	3.49	3.18	3.28	3.38
TL/DFB	1.88	1.9	1.97	1.899	1.73	1.89	1.84
TL/AFB	3.55	3.48	3.59	3.38	2.98	3.14	3.14
TL/HW	7.79	7.36	7.34	7.19	6.83	6.71	6.39
TL/HD	9.1	8.67	8.7	8.33	7.78	7.91	8.02
HL/ED	7.77	7.79	8.64	8.43	8.53	9.03	8.9
HL/IOD	4.39	3.76	3.78	3.82	4.05	4.06	4.05
HL/POL	1.58	1.48	1.4	1.33	1.47	1.41	1.4
HL/Sn.L.	4.39	4.43	4.55	4.78	5.06	4.92	4.94
HL/HW	2.21	2.06	1.89	1.96	1.98	1.82	1.65
HL/HD	2.59	2.42	2.57	2.26	2.25	2.14	2.07

to be 1.16 cm. The minimum and maximum values recorded are 1.05 and 1.30 cm, respectively. When expressed as percentage of head length it is found to be 16.69.

3.2.2. Meristic Counts

The details of meristic counts are presented in the Table-16. It is summarised below:

Dorsal fin rays: The mean value for this parameter is recorded to be 44.47, whereas the minimum and maximum values recorded are 43-49.

Pectoral fin rays: The mean value is found to be 16.73. The range varies between 16 and 17.

Pelvic fin rays: The number of pelvic fin rays is found to 6 and as a constant.

Anal fin rays: The mean value for this parameter is found to be 26.47. The number varies between 25 and 28.

Caudal fin rays: The mean value is recorded to be 14.27, which varies between 14 and 15.

Lateral line scales: The mean number recorded is 59.93. The range varies between 58 and 63.

Transverse scales: The mean value is found to be 8.17/11.23. It varies between $7\frac{1}{2}/10\frac{1}{2}$ and $9\frac{1}{2}/11\frac{1}{2}$.

3.2.3. Regression equations:

The regression equations for various morphometric parameters are presented in Table-5 for Channa barca.

3.2.4. Biometric indices

For all the morphometric parameters studied, a mean biometric index for each 3.0 cm. length group has been calculated out and presented in Table-6, Fig. 3.

3.3. Morphometry and Meristic Counts of Channa punctata:

3.3.1. Morphometry

The results recorded on morphometric measurements of Channa punctata have been presented in Table-7 and summarised below:

Total length: The mean value obtained for total length is found to be 14.74 cm. The range varies between 8.6 and 21.0 cm. When expressed as percentage of standard length, it is found to be 119.74.

Standard length: The mean value recorded for standard length is found to be 12.31 cm. The minimum and maximum values observed are 7.2 and 17.7 cm. respectively.

Body depth: The mean value for this parameter is recorded to be 2.53 cm. The range varies between 1.6 and 3.6 cm. When expressed as percentage of standard length it is found to be 20.55.

Girth: The mean value for girth is recorded to be 8.16 cm. with a range varying between 4.8 and 12.0 cm. When expressed as percentage of standard length it is found to be 66.29.

Head length: The mean value for this parameter is found to be 4.27 cm. with a range varying between 2.4 and 6.1 cm. When expressed as percentage of standard length, it is found to be 34.69.

Pre-dorsal length: The mean value obtained is 4.63 cm. The minimum and maximum values are 2.65 and 6.7 cm. respectively. When expressed as percentage of standard length it is found to be 37.61.

Dorsal fin base length: The mean value for dorsal fin base length is recorded to be 6.77 cm. The range varies between 3.5 and 10.3 cm. When expressed as percentage of standard length it is found to be 54.99.

Anal fin base length: The mean value for this parameter is found to be 4.58 cm. The range is from 2.5 to 7.1 cm. When expressed as percentage of standard length it is found to be 37.21.

Height of dorsal fin: The mean value is found to be 1.31 cm. with a range ranging between 0.85 and 2.2 cm. When expressed as percentage of standard length it is found to be 10.64.

Height of pectoral fin: The mean value for this parameter is recorded to be 2.14, with a range varying between 1.3 and 3.3 cm. When it is expressed as percentage of standard length it is found to be 17.38.

Height of pelvic fin: The mean value is 1.57 cm. The range is from 0.9 to 2.3 cm. When expressed as percentage of standard length it is found to be 12.75.

Height of anal fin: The mean value is found to be 1.17 cm. The range varies between 0.9 and 2.3 cm. When expressed as percentage of standard length it is found to be 9.50.

Length of caudal fin: The mean value is 2.42 cm. The range

Table-7 : Morphometric Analysis of Channa punctata.

Parameters	Mean (cm)	Mean % standard length	Range (cm)
Total length	14.74	119.74	8.6 - 21.0
Standard length	12.31	-	7.2 - 17.7
Body depth	2.53	20.55	1.6 - 3.6
Girth	8.16	66.29	4.8 - 12.0
Head length	4.27	34.69	2.4 - 6.1
Pre-dorsal length	4.63	37.61	2.65 - 6.7
Dorsal fin base length	6.77	54.99	3.5 - 10.3
Anal fin base length	4.58	37.21	2.5 - 7.1
Height of dorsal fin	1.31	10.64	0.85 - 2.2
Height of pectoral fin	2.14	17.38	1.3 - 3.3
Height of pelvic fin	1.57	12.75	0.9 - 2.3
Height of anal fin	1.17	9.50	0.9 - 2.3
Length of caudal fin	2.42	19.66	1.3 - 3.8
Dist.betn. Pel.F. to A.opening	1.59	12.92	0.9 - 2.2
Dist.betn. Pel.F. to Anal fin	2.35	19.09	1.0 - 2.5
Head width	2.65	21.53	1.5 - 4.0
Head Depth	2.34	19.01	1.3 - 3.3

Parameters	Mean (cm)	Mean % of Head length	Range (cm)
Snout length	0.74	17.33	0.5 - 1.0
Eye diameter	0.58	13.58	0.4 - 0.7
Post-orbital length	2.99	70.02	1.6 - 4.2
Sub-orbital length	0.25	5.85	0.1 - 0.4
Inter-orbital length	1.04	24.36	0.6 - 1.2
Upper jaw length	1.2	28.1	0.85 - 1.6
Lower jaw length	1.34	31.38	0.9 - 1.8
Gape	0.79	18.5	0.4 - 1.8

varies between 1.3 and 3.5 cm. When expressed as percentage of standard length it is recorded to be 19.66.

Distance between pelvic fin and Anal opening: The mean value is 1.59 cm. The minimum and maximum values observed are 0.9 and 2.2 cm. respectively. When expressed as percentage of standard length it is found to be 12.92.

Distance between pelvic fin and Anal fin: The mean value is recorded to be 2.35 cm. The range varies between 1.0 and 2.5 cm. When expressed as percentage of standard length it is found to be 19.09.

Head width: The mean value is 2.65 cm. The range varies between 1.5 and 4.0 cm. When expressed as percentage of standard length it is recorded to be 21.53.

Head depth: The mean value is found to be 2.34 cm. with a range between 1.3 and 3.3 cm. When expressed as percentage of standard length it is found to be 19.01.

Snout length: The mean length is found to be 0.74 cm. The range varies between 0.5 and 1.0 cm. When expressed as percentage of head length it is found to be 17.33.

Eye diameter: The mean value for this parameter is found to be 0.58 cm. The range varies between 0.4 and 0.7 cm. When expressed as percentage of head length it is found to be 13.58.

Post-orbital length: The mean value is recorded to be 2.99 cm. The minimum and maximum values are 1.6 and 4.2 cm. respectively. When expressed as percentage of head length it is found to be 70.02.

Sub-orbital length: The mean value for this parameter is recorded to be 0.25 cm. The range varies between 0.1 and 0.4 cm. When expressed as percentage of head length it is recorded to be 5.85.

Inter-orbital length: The mean value for this parameter is found to be 1.04 cm. The range varies between 0.6 and 1.2 cm. When expressed as percentage of head length it is found to be 24.36.

Upper jaw length: The mean value for this parameter is found to be 1.2 cm. The range varies between 0.85 and 1.6 cm. When expressed as percentage of head length it is found to be 28.10.

Lower jaw length: The mean value is 1.34 cm. The minimum and maximum ranges are 0.9 and 1.8 cm. respectively. When expressed as percentage of head length it is recorded to be 31.38.

Gape width: The mean value for gape width is recorded to be 0.79 cm. The minimum and maximum values are 0.4 and 1.8 cm. respectively. When expressed as percentage of head length it is found to be 18.50.

3.3.2. Meristic Counts

The details of the meristic counts are presented in the Table-16 and summarised below:

Dorsal fin rays: The mean value obtained is 29.32 with a range varying from 28 to 30.

Pectoral fin rays: The mean value is found to be 14.39 with a range varying from 13 to 16.

Pelvic fin rays: The mean value recorded for this parameter is found to be 5.35. The range varies between 5 and 6.

Anal fin rays: The mean value is found to be 19.9. The range varies between 19 and 22.

Caudal fin rays: The mean value recorded is found to be 13.87 with a range varying between 13 and 15 in number.

Lateral line scales: The mean value for this parameter is found to be 38.97. The range varies between 38 and 41.

Transverse scales: The mean values obtained are found to be 4.98/8.85. The range varies between $4\frac{1}{2}$ - $5\frac{1}{2}$ / $8\frac{1}{2}$ - $9\frac{1}{2}$.

3.3.3. Regression Equations:

The regression equation for various morphometric parameters are calculated out and presented in Table-8 for Channa punctata.

3.3.4. Biometric Indices:

For all the morphometric parameters studied a mean biometric indices for each 2.0 cm. length groups has been calculated out and presented in Table-9, Fig. 4.

3.4. Morphometry and Meristic Counts of Channa stewartii.

3.4.1. Morphometry

The results recorded on morphometric measurements of Channa stewartii have been presented in Table-10 and summarised below:-

Total length: The mean value for total length is found to be 12.02 cm. The minimum and maximum ranges are 8.2 and 16.1 cm.

Table-8 : Regression Equations on Morphometric Parameters of Channa punctata.

Parameters	Regression Equations
Total length (Y) VS. Standard length (x)	$Y = 1.5681 + 1.2007 x$
Total length (Y) VS. Body depth (x)	$Y = 2.5416 + 5.0016 x$
Total length (Y) VS. Girth (x)	$Y = 0.8464 + 1.6274 x$
Total length (Y) VS. Head length (x)	$Y = 0.5386 + 3.3439 x$
Total length (Y) VS. Eye diameter (x)	$Y = 4.4902 + 20.0432 x$
Total length (Y) VS. Snout length (x)	$Y = 1.1676 + 16.1112 x$
Total length (Y) VS. Post-orbital length (x)	$Y = 0.1087 + 5.1074 x$
Total length (Y) VS. Inter-orbital length (x)	$Y = 0.1091 + 14.2054 x$
Total length (Y) VS. Head depth (x)	$Y = 0.2197 + 6.2782 x$
Total length (Y) VS. Pre-dorsal length (x)	$Y = 0.6528 + 3.0941 x$

Table-9 : Biometric Indices of different length groups in Channa punctata.

Parameters	GROUP I 8.1-10.0 cm.	GROUP II 10.1-12.0 cm.	GROUP III 12.1-14.0 cm.	GROUP IV 14.1-16.0 cm.	GROUP V 16.1-18.0 cm.	GROUP VI 18.1-20.0 cm.	GROUP VII 20.1-22.0 cm.
TL/SL	1.18	1.21	1.19	1.19	1.19	1.22	1.19
TL/BD	5.41	5.83	6.01	5.56	5.96	6.18	5.83
TL/Girth	1.75	2.34	1.76	1.74	1.88	1.93	1.75
TL/HL	3.46	3.38	3.34	3.38	3.54	3.69	3.44
TL/PDL	3.23	3.23	3.15	3.13	3.20	3.29	3.13
TL/DFB	2.4	2.22	2.17	2.18	2.15	2.18	2.04
TL/AFB	3.23	3.33	3.17	3.19	3.34	3.12	2.96
TL/HW	5.58	5.59	4.67	5.44	5.67	5.81	5.25
TL/HD	6.18	6.22	6.31	6.02	6.45	6.86	6.36
HL/ED	6.25	7.29	7.07	7.47	7.51	7.69	8.71
HL/IOD	4.17	4.43	4.72	4.27	4.17	4.02	3.81
HL/POL	1.52	1.50	1.49	1.39	1.38	1.40	1.45
HL/Sn.L.	4.55	5.38	6.06	5.82	5.61	6.01	6.78
HL/HW	1.61	1.66	1.4	1.61	1.6	1.57	1.53
HL/HD	1.79	1.84	1.89	1.78	1.82	1.85	1.85

respectively. When expressed as percentage of standard length it is found to be 117.73.

Standard length: The mean value for this parameter is found to be 10.21 cm. The range varies between 6.3 and 13.4 cm.

Body depth: The mean value obtained for this parameter is 1.86 cm. with a range varying between 1.0 and 2.75 cm. When expressed as percentage of standard length it is found to be 18.22.

Girth: The mean value is found to be 6.33 cm. The range varies between 3.6 and 8.75 cm. When expressed as percentage of standard length it is found to be 61.99.

Head length: The mean value is found to be 3.02 cm. The range varies between 1.85 and 4.2 cm. When expressed as percentage of standard length it is found to be 29.58.

Pre-dorsal length: The mean value is recorded to be 3.45 cm. The range varies between 2.0 and 4.6 cm. When expressed as percentage of standard length it is found to be 33.79.

Dorsal fin base length: 5.98 cm. is found to be the mean value for this parameter. The minimum and maximum values are 3.75 and 8.25 cm. respectively. When expressed as percentage of standard length it is found to be 58.57.

Anal fin base length: 3.58 cm. is found to be the mean value. However, the range varies between 2.4 and 5.7 cm. When expressed as percentage of standard length it is recorded to be 35.06.

Table-10 : Morphometric Analysis of Channa stewartii

Parameters	Mean (cm)	Mean % Standard length	Range (cm)
Total length	12.02	117.73	8.2 - 16.1
Standard length	10.21	-	6.3 - 13.4
Body depth	1.86	18.22	1.0 - 2.75
Girth	6.33	61.99	3.6 - 8.75
Head length	3.02	29.58	1.85 - 4.2
Pre-dorsal length	3.45	33.79	2.0 - 4.6
Dorsal fin base length	5.98	58.57	3.75 - 8.25
Anal fin base length	3.58	35.06	2.4 - 5.7
Height of dorsal fin	1.08	10.58	0.8 - 1.4
Height of pectoral fin	1.74	17.04	1.1 - 2.5
Height of pelvic fin	0.77	7.54	0.6 - 1.1
Height of anal fin	0.92	9.01	0.65 - 1.2
Length of caudal fin	1.77	17.03	1.4 - 2.8
Dist. betn. Pel.F.to Anal Op.	1.23	12.05	0.8 - 1.8
Dist. betn. Pel.F.to Anal fin	1.53	14.99	1.0 - 2.1
Head width	2.06	20.18	1.2 - 3.0
Head depth	1.66	16.26	0.8 - 2.4

Parameters	Mean (cm)	Mean % Head length	Range (cm)
Snout length	0.57	18.87	0.3 - 0.8
Eye diameter	0.47	15.56	0.3 - 0.6
Post-orbital length	1.99	65.89	1.1 - 2.35
Sub-orbital length	0.24	7.95	0.15 - 0.35
Inter-orbital length	0.96	31.79	0.55 - 1.3
Upper jaw length	0.97	32.12	0.6 - 1.45
Lower jaw length	1.14	37.75	0.75 - 1.7
Gape	0.82	27.15	0.6 - 1.1

Height of dorsal fin: The mean value is found to be 1.08 cm. The range varies between 0.8 and 1.4 cm. When expressed as percentage of standard length it is found to be 10.58.

Height of pectoral fin: The mean value is found to be 1.74 cm. The range varies between 1.1 and 2.5 cm. When expressed as percentage of standard length it is found to be 17.04.

Height of pelvic fin: The mean value is found to be 0.77 cm. The range varies between 0.6 and 1.1 cm. When expressed as percentage of standard length it is found to be 7.54.

Height of anal fin: The mean value is found to be 0.92 cm. The minimum and maximum values are 0.65 and 1.2 cm. respectively. When expressed as percentage of standard length it is found to be 9.01.

Length of caudal fin: The mean value for this parameter is found to be 1.77 cm. The range varies between 1.4 and 2.8 cm. When expressed as percentage of standard length it is found to be 17.03.

Distance between pelvic fin to anal opening: The mean value is 1.23 cm. The range is from 0.8 to 1.8 cm. When converted to percentage of standard length it is found to be 12.05.

Distance between pelvic fin to anal fin: The mean value for this parameter is found to be 1.53 cm. The range is between 1.0 and 2.1 cm. When expressed as percentage of standard length it is found to be 14.99.

Head width: The mean value is found to be 2.06 cm. The range varies between 1.2 and 3.0 cm. When expressed as percentage of standard length it is found to be 20.18.

Head depth: The mean value is found to be 1.66 cm. The range varies between 0.8 and 2.4 cm. When expressed as percentage of standard length it is found to be 16.26.

Snout length: The mean value for snout length is found to be 0.57 cm. The range varies between 0.3 and 0.8 cm. When expressed as percentage of head length it is found to be 18.87.

Eye diameter: The mean value for eye diameter is found to be 0.47 cm. The range varies between 0.3 and 0.6 cm. When expressed as percentage of head length it is found to be 15.56.

Post-orbital length: The mean value for this parameter is found to be 1.99 cm. The minimum and maximum values recorded are found to be 1.1 and 2.35 cm. respectively.

Sub-orbital length: The mean value for this parameter is recorded to be 0.24 cm. The range varies between 0.15 and 0.35 cm. When expressed as percentage of head length it is found to be 7.95.

Inter-orbital length: The mean value is found to be 0.96 cm. The minimum and maximum values are 0.55 and 1.3 cm. respectively. When expressed as percentage of head length it is found to be 31.79.

Upper jaw length: The mean value obtained is 0.97 cm. The range varies between 0.6 and 1.45 cm. When expressed as percentage of head length, it is found to be 32.12.

Lower jaw length: The mean value for this parameter is found to be 1.14 cm. The range varies between 0.75 and 1.7 cm. When expressed as percentage of head length it is found to be 37.75.

Table-11 : Regression Equations on Morphometric Parameters of Channa stewartii.

Parameters	Regression Equations
Total length (Y) VS. Standard length (x)	$Y = 1.3479 + 1.0463 x$
Total length (Y) VS. Body depth (x)	$Y = 0.2734 + 6.3884 x$
Total length (Y) VS. Girth (x)	$Y = 0.2619 + 1.7057 x$
Total length (Y) VS. Head length (x)	$Y = 1.6105 + 3.4464 x$
Total length (Y) VS. Eye diameter (x)	$Y = 0.6108 + 20.0345 x$
Total length (Y) VS. Snout length (x)	$Y = 1.3002 + 19.0813 x$
Total length (Y) VS. Post-orbital length (x)	$Y = 2.7535 + 4.4012 x$
Total length (Y) VS. Inter-orbital length (x)	$Y = 1.8767 + 10.4316 x$
Total length (Y) VS. Head depth (x)	$Y = 0.4162 + 5.7359 x$
Total length (Y) VS. Pre-dorsal length (x)	$Y = 0.1997 + 3.4243 x$

Table-12 : Biometric Indices in different length groups of Channa stewartii.

Parameters	GROUP I 8.1-10.0 cm.	GROUP II 10.1-12.0 cm.	GROUP III 12.1-14.0 cm.	GROUP IV 14.1-16.0 cm.	GROUP V 16.1-18.0 cm.
TL/SL	1.21	1.18	1.18	1.16	1.21
TL/BD	7.73	6.69	5.76	5.89	8.05
TL/Girth	2.09	1.96	1.76	1.78	2.24
TL/HL	4.13	4.11	3.87	3.75	4.74
TL/PDL	3.76	3.44	3.44	3.41	3.62
TL/DFB	2.21	2.01	2.02	1.89	1.96
TL/AFB	3.98	3.25	3.16	3.40	2.82
TL/HW	6.47	5.96	5.35	5.53	7.0
TL/HD	8.89	7.12	6.64	6.80	8.05
HL/ED	6.39	5.56	6.84	7.07	6.18
HL/IOD	3.33	3.07	3.10	3.25	2.72
HL/POL	1.72	1.49	1.43	1.49	1.42
HL/Sn.L.	5.75	5.24	5.32	5.30	4.86
HL/HW	1.56	1.45	1.38	1.48	1.48
HL/HD	2.15	1.73	1.72	1.82	1.7

Gape width: The mean value for this parameter is found to be 0.82 cm. The range varies between 0.6 and 1.1 cm. When expressed as percentage of head length it is found to be 27.15.

3.4.2. Meristic Counts

The details of the meristic counts are presented in the Table-16 and summarised below:-

Dorsal fin rays: The mean value is found to be 34.23. The range varies between 31 and 38.

Pectoral fin rays: The mean value for this is found to be 13.27. The range varies between 12 and 14.

Pelvic fin rays: Six is found to be the constant number.

Anal fin rays: The mean value is found to be 22.07. The range varies between 19 and 27.

Caudal fin rays: The mean value is found to be 13.43. The range varies between 12 and 14.

Lateral line scales: The mean value is recorded to be 43.13 whereas the range varies between 39 and 49.

Transverse scales: The mean values are found to be 4.6 and 7.33. The range is recorded to be $4\frac{1}{2}$ - $5\frac{1}{2}$ / $6\frac{1}{2}$ - $8\frac{1}{2}$.

3.4.3. Regression Equations

The regression equations for various morphometric parameters studied are presented in Table-11 for this species.

3.4.4. Biometric Indices

Biometric indices for various morphometric parameters have been analysed and a mean is calculated out for each 2.0 cm. length groups and are presented in Table-12, Fig. 5.

3.5. Morphometry and Meristic Counts for Channa orientalis

3.5.1. Morphometry

The results recorded on morphometric measurements of Channa orientalis have been presented in Table-13 and summarised below :-

Total length: The mean total length is found to be 5.83 cm. The minimum and maximum values are found to be 3.6 and 8.0 cm. respectively. When expressed as percentage of standard length, it is recorded to be 120.45.

Standard length: The mean value for this parameter is found to be 4.84 cm. The range varies between 3.0 and 6.7 cm.

Body depth: The mean value for body depth is found to be 0.81 cm. The range recorded varies between 0.4 and 1.5 cm. When expressed as percentage of standard length, it is found to be 16.74.

Girth: The mean value for girth is found to be 2.83 cm. The range varies between 1.6 and 4.05 cm. When expressed as percentage of standard length it is found to be 58.47.

Head length: The mean value for this parameter is found to be 1.34 cm. The minimum and maximum values recorded are 0.9 and 2.0 cm. respectively. When expressed as percentage of standard length, it is found to be 27.69.

Pre-dorsal length: The mean value is found to be 1.73 cm. The range varies between 1.0 and 2.45 cm. When expressed as percentage of standard length it is found to be 35.74.

Dorsal fin base length: The mean value is found to be 2.68 cm. The range varies between 1.65 and 3.6 cm. When expressed as percentage of standard length it is found to be 55.37.

Anal fin base length: The mean value for this parameter is found to be 1.68 cm. The range varies between 0.9 and 2.35 cm. When expressed as percentage of standard length it is found to be 34.71.

Height of dorsal fin: The mean value for this parameter is found to be 0.48 cm. The range varies from 0.25 to 0.7 cm. When expressed as percentage of standard length it is found to be 9.92.

Height of pectoral fin: The mean value is recorded to be 1.15 cm. The range varies from 0.6 to 1.4 cm. When expressed as percentage of standard length it is found to be 23.76.

Height of pelvic fin: The mean value is found to be 0.34 cm. The range varies from 0.2 to 0.5 cm. When expressed as percentage of standard length it is found to be 7.02.

Height of anal fin: The mean value is found to be 0.39 cm. The minimum and maximum values are 0.2 and 0.6 cm. respectively. When expressed as percentage of standard length it is found to be 8.06.

Length of caudal fin: The mean value for this parameter is found to be 0.99 cm. The range varies between 0.6 and 1.3 cm. When expressed as percentage of standard length it is found to be 20.45.

Table-13 : Morphometric Analysis of Chenna orientalis (=C.gachua)

Parameters	Mean (cm)	Mean % Standard length	Range (cm)
Total length	5.83	120.45	3.6 - 8.0
Standard length	4.84	-	3.0 - 6.7
Body depth	0.81	16.74	0.4 - 1.3
Girth	2.83	58.47	1.6 - 4.05
Head length	1.34	27.69	0.9 - 2.0
Pre-dorsal length	1.73	35.74	1.0 - 2.45
Dorsal fin base length	2.68	55.37	1.65 - 3.6
Anal fin base length	1.68	34.71	0.9 - 2.35
Height of dorsal fin	0.48	9.92	0.25 - 0.7
Height of pectoral fin	1.15	23.76	0.6 - 1.4
Height of pelvic fin	0.34	7.02	0.2 - 0.5
Height of anal fin	0.39	8.06	0.2 - 0.6
Length of caudal fin	0.99	20.45	0.6 - 1.3
Dist. betn. Pel.F.to Anal op.	0.71	14.67	0.4 - 1.0
Dist. betn. Pel.F.to Anal fin	0.85	17.46	0.55 - 1.25
Head width	0.96	19.83	0.6 - 1.3
Head depth	0.68	14.05	0.45 - 0.95

Parameters	Mean (cm)	Mean % Head length	Range (cm)
Snout length	0.29	21.64	0.15 - 0.45
Eye diameter	0.24	17.91	0.15 - 0.35
Post-orbital length	0.91	67.91	0.5 - 1.3
Sub-orbital length	0.11	8.21	0.05 - 0.2
Inter-orbital length	0.44	32.84	0.3 - 0.65
Upper jaw length	0.4	29.85	0.25 - 0.6
Lower jaw length	0.46	34.33	0.3 - 0.7
Gape	0.36	26.87	0.2 - 0.55

Distance between pelvic fin to anal opening: The mean value for this parameter is recorded to be 0.71 cm. The range varies between 0.4 and 1.0 cm. When expressed as percentage of standard length it is found to be 14.67.

Distance between pelvic fin to anal fin: The mean value for this parameter is found to be 0.85 cm. The range varies from 0.55 to 1.25 cm. When expressed as percentage of standard length, it is found to be 17.46.

Head width: The mean value for this parameter is found to be 0.96 cm. The range varies between 0.6 and 1.3 cm. When expressed as percentage of standard length it is found to be 14.05.

Snout length: The mean value for this parameter is found to be 0.29 cm. The range varies between 0.15 and 0.45 cm. When expressed as percentage of head length it is found to be 21.64.

Eye diameter: The mean value is found to be 0.24 cm. The range varies between 0.15 and 0.35 cm. When expressed as percentage of head length it is found to be 17.91.

Post-orbital length: The mean value for this parameter is found to be 0.91 cm. The range varies between 0.5 and 1.3 cm. When expressed as percentage of head length, it is found to be 67.91.

Sub-orbital length: The mean value is 0.11 cm. The range varies between 0.05 and 0.2 cm. When expressed as percentage of head length it is found to be 8.21.

Inter-orbital length: The mean value for this parameter is found to be 0.44 cm. The range varies between 0.3 and 0.65 cm. When expressed as percentage of head length it is found to be 32.84.

Upper jaw length: The mean value for this parameter is found to be 0.4 cm. The range varies between 0.25 and 0.6 cm. When expressed as percentage of head length it is found to be 29.85.

Lower jaw length: The mean value for this parameter is found to be 0.46 cm. The minimum and maximum values recorded are 0.3 and 0.7 cm. respectively. When expressed as percentage of head length it is found to be 34.33.

Gape width: The mean values for this parameter is recorded to be 0.36 cm. The range varies between 0.2 and 0.55 cm. When expressed as percentage of head length it is found to be 26.87.

3.5.2. Meristic Counts

The details of the meristic counts are presented in Table-16 and summarised below:-

Dorsal fin rays: The mean value for this parameter is recorded to be 29.7, with a range varying between 28-31.

Pectoral fin rays: The mean value is found to be 12.47 with a range varying between 11 and 14.

Pelvic fin rays: The mean value for this parameter is found to be 5.87 with a range varying between 5 and 6.

Anal fin rays: The mean value for this parameter is found to be 19.97. The range varies between 17 and 21.

Table-14 : Regression Equations on Morphometric Parameters of Channa orientalis.

Parameters	Regression Equations
Total length (Y) VS. Standard length (x)	$Y = 0.8345 + 1.0553 x$
Total length (Y) VS. Body depth (x)	$Y = 1.7532 + 5.1355 x$
Total length (Y) VS. Girth (x)	$Y = 1.0135 + 1.7393 x$
Total length (Y) VS. Head length (x)	$Y = 1.2678 + 3.4707 x$
Total length (Y) VS. Eye diameter (x)	$Y = 1.7310 + 17.8680 x$
Total length (Y) VS. Snout length (x)	$Y = 1.5847 + 14.5652 x$
Total length (Y) VS. Post-orbital length (x)	$Y = 1.7101 + 4.6373 x$
Total length (Y) VS. Inter-orbital length (x)	$Y = 1.3182 + 10.6018 x$
Total length (Y) VS. Head depth (x)	$Y = 1.1367 + 6.9976 x$
Total length (Y) VS. Pre-dorsal length (x)	$Y = 5.7416 + 3.9885 x$

Table-15 : Biometric Indices in different length groups of Channa orientalis.

Parameters	GROUP I 3.1-4.0 cm.	GROUP II 4.1-5.0 cm.	GROUP III 5.1-6.0 cm.	GROUP IV 6.1-7.0 cm.	GROUP V 7.1-8.0 cm.
TL/SL	1.2	1.22	1.22	1.19	1.19
TL/BD	9.0	7.32	7.33	7.01	6.87
TL/Girth	2.25	2.05	2.08	2.04	2.06
TL/HL	4.0	4.32	4.53	4.38	4.17
TL/PDL	3.6	3.27	3.42	3.45	3.33
TL/DFB	2.18	2.15	2.26	2.12	2.17
TL/AFB	4.0	3.63	3.44	3.33	3.48
TL/HW	6.0	6.14	6.33	5.98	6.01
TL/HD	8.0	8.9	8.57	8.47	8.29
HL/ED	6.0	5.53	5.35	6.48	5.72
HL/PDL	1.8	1.59	1.32	1.51	1.51
HL/IOD	3.0	3.18	2.93	3.10	3.16
HL/Sn.L.	6.0	4.57	4.1	4.52	4.82
HL/HW	1.5	1.42	1.39	1.37	1.44
HL/HD	2.0	2.06	1.89	1.94	1.99

Table-16 : Summary of the Meristic Counts recorded in different Channa spp.

Parameters	<u>C. striatus</u>		<u>C. barca</u>		<u>C. punctata</u>		<u>C. stewartii</u>		<u>C. orientalis</u>	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
No. of rays in dorsal fin	43.5	41-46	44.47	43-49	29.32	28-30	34.23	31-38	29.7	28-31
No. of rays in pectoral fin	15.86	15-18	16.73	16-17	14.39	13-16	13.27	12-14	12.47	11-14
No. of rays in pelvic fin	6	6 Const.	6	6 Const.	5.35	5-6	6	6 Const.	5.87	5-6
No. of rays in anal fin	26.86	26-28	26.47	25-28	19.9	19-22	22.07	19-27	19.97	17-21
No. of rays in caudal fin	15.43	15-16	14.27	14-15	13.87	13-15	13.43	12-14	12.67	11-14
No. of scales along the lateral line	60.07	57-61	59.93	58-63	38.97	38-41	43.13	39-49	39.9	36-41
No. of scales above the lateral line	7.93	7½-8½	8.17	7½-9½	4.98	4½-5½	4.6	4½-5½	4.43	3½-4½
No. of scales below the lateral line	11.03	10½-11½	11.23	10½-11½	8.85	8½-9½	7.33	6½-8½	5.67	5½-6½

Caudal fin rays: The mean value is found to be 12.67. The range varies between 11 and 14.

Lateral line scales: The mean value recorded for this parameter is found to be 39.90. The range varies between 36 and 41.

Transverse scales: The mean values obtained for this parameter are found to be 4.43 and 5.67. The range varies between $3\frac{1}{2}$ - $4\frac{1}{2}$ and $5\frac{1}{2}$ - $6\frac{1}{2}$.

3.5.3. Regression Equations:

The regression equations for various morphometric parameters are calculated out and present in Table-14.

3.5.4. Biometric Indices

For various morphometric measurements recorded, a mean biometric index for each 1.0 cm. length groups has been calculated out and presented in Table-15, Fig. 6.

4. DISCUSSION

The channids, in general, are very much alike in shape and gross morphological features with slight variation in body colour and size. This is responsible for several disputable interpretations put forward by different Ichthyotaxonomists while identifying them (Day 1878, Shaw and Shebbeare 1937 and Reddy 1981). The situation has been further complicated by their wide range of distribution from the mountain range to the coastal area through the plains. Because of the plasticity of the ecological conditions of their niches, intra-species variations in the morphological features are very much expectable (Schmidt 1921, Vladykov 1934, Lindsey 1954 and Fage 1958).

Earlier workers distinguished the different Channa species on the basis of body colour and body marks. C. barca and C. striatus were differentiated from one another by the length of the vertical dark stripes which extend upto the belly in case of C. striatus, whereas do not exceed the lateral line in case of C. barca. Moreover, the belly is yellowish in colour in case of C. striatus while it is plain white in case of C. barca (Day 1878, Shaw and Shebbeare 1937). The pectoral fins of C. barca, C. striatus and C. punctata are plain, without any marking. But the pectoral fins of C. stewartii and C. orientalis are banded with yellow and black colour. It has been noticed in the present investigation that C. stewartii seems to possess three rows of alternately arranged yellow and black bands in the pectoral fins whereas in C. orientalis these bands extend upto the tip of the fins. Such differences exist within the same species, in different populations. Channa stewartii, when collected from the stream, possesses a greenish-blue tinge throughout the body, but when collected from

pond, possesses dull grey colour. Similarly, it has been observed that certain populations of C. orientalis contain one or two black spot on the extremity of the dorsal fin while others are devoid of such spots. Similar tail spot polymorphism was also observed in Xiphophorus (Borowsky 1978). Such polymorphic characters cannot be taken as diagnostic features in distinguishing different species (Mayr, 1963).

Meristic counts may or may not always be constant for a particular species, because non-genetic variations of morphological characters are common in fish (Barlow, 1961) and are always affected by environmental fluctuations during developmental stages (Lindsay 1954, Barlow 1961). The differences of the meristic counts in different species of Channa have been presented in Table-16. A comparison of the present observations on the meristic counts of different Channa species has been done with the earlier works of Day (1878) and Shaw and Shebbeare (1937) and presented in Table-17. The differences observed may be due to the differences in the environmental conditions as well as age (where mostly one year or one plus year fishes are employed) which is not mentioned in the earlier works. However, the range recorded in the present study, coincides in many cases with the previous records. Moreover, variations at population level have also been recorded by many workers (Tanning, 1944, Barlow 1961; Suzuki and Yamaguchi 1980 etc.). It has been revealed that the meristic characters exhibit plasticity under the influence of environmental factors, especially temperature during early period of life history.

Significant differences in the morphometry among different species of Channa have been observed (Tables-1, 4, 7, 10 & 13). Such

Table-17 : Comparative account of meristic counts of five species recorded by earlier workers and present study.

SPECIES	AUTHORS	MERISTIC			OBSERVATIONS		
<u>C. striatus</u>	Day, 1878	D.37-45	P.17	V.6	A.23-26	C.13	L1. 50-57
	Shaw & Shebbeare, 1937	D.37-45	-	-	A.23-26	-	L1. 50-59
	Present study	D.41-46	P.15-16	V.6	A.26-28	C.15-16	L1. 57-61
<u>C. barca</u>	Day, 1878	D.47-52	P.16	V.1/5	A.34-36	C.19	L1. 60-65
	Shaw & Shebbeare, 1937	D.51	P.17	V.1/5	A.34	C.14	L1. 78
	Present study	D.43-49	P.16-17	V.1/5	A.25-28	C.14-15	L1. 58-63
<u>C. punctata</u>	Day, 1878	D.29-32	P.17	V.6	A.21-23	C.12	L1. 37-40
	Shaw & Shebbeare, 1937	D.29-32	P.17	-	A.21-23	C.12	L1. 37-40
	Present study	D.28-30	P.13-16	V.6	A.19-22	C.12-15	L1. 38-41
<u>C. stewartii</u>	Day, 1878	D.39-40	P.17	V.6	A.27	C.14	L1. 47-50
	Shaw & Shebbeare, 1937	D.39-40	P.17	V.6	A.27	C.12	L1. 45-50
	Present study	D.31-38	P.12-14	V.6	A.19-27	C.12-14	L1. 39-49
<u>C. orientalis</u>	Day, 1878	D.34	P.14	-	A.22	C.14	L1. 41
	Shaw & Shebbeare, 1937	D.32-37	P.15	-	A.21-23	C.12	L1. 40-45
	Present study	D.28-31	P.11-14	V.6	A.17-21	C.11-14	L1. 36-41

N.B. D = Dorsal fin rays
V = Ventral fin rays
C = Caudal fin rays

P = Pectoral fin rays
A = Anal fin rays
L1 = Lateral line scales

differences are genetic or phenotypic (Kothare and Bal 1976). To determine the taxonomic uniformity, the present observations have been compared with the available standard descriptions (Day 1978, Shaw and Shebbeare 1937) and are found to be favourable, in spite of certain amount of variations.

Differences in the morphometric and meristic parameters between male and female of all the Channa species are not well defined. Sexual dimorphism could not be traced out in any species. However, it is observed that females are always larger in size than the male and numerically more in number. According to Nikolsky (1963), sex ratio varies considerably from species to species, but in most of the cases, it is close to unity. Presence of more female in nature is also recorded by Hora and Mishra (1936) in Labeo dero. Probably, this phenomenon is related to the breeding biology of the fishes.

The biometric index of different parameters are probably species specific. However, according to Gould (1966), ratio between different morphological characters of the same species does not necessarily be constant, due to variation resulting from differences in sex, race, nutrition and other environmental factors during the period of incubation and early life of larvae. Moreover, the biometric indices differ considerably in different length groups of the same species. Biometric indices for different body parameters of all the five species of Channa have been examined and it is found that in all the five species growth of standard length, dorsal fin length are more or less constant. In other words these parameters can be treated as isometric (Bayagbona 1963).

It has been observed for all the five species that the eye diameter becomes smaller in relation to head length; in other words, this parameter shows a negative allometry.

In case of Channa striatus, it is found that the rate of growth of anal fin base length, pre-dorsal length, body depth and head width in relation to total length are more or less constant. Similarly, growth of head depth, head width, post-orbital length in relation to head length are also isometric. The growth of head length and head depth in relation to total length show negative and positive allometry respectively. Similarly, growth of interorbital distance and snout length in relation to head length show negative and positive allometry respectively.

In case of Channa barca the growth of pre-dorsal length in relation to total length shows isometric growth. Similar relationship also exists in the growth of post-orbital length and inter-orbital length in relation to head length. The growth of anal fin base length, head width and depth and body depth in relation to total length show more or less positive allometry whereas growth of head length shows a negative allometry. On the other hand, head width and depth in relation to head length show a positive allometry while the snout length in relation to head length shows a negative allometry.

In case of Channa punctata, it has been observed that head width in relation to the total length and head width and depth and post-orbital length in relation to the head length show isometric growth. On the other hand, growth of head length, pre-dorsal

Table-18 : Biometric Indices in different Channa species at 7.0-10.0 cm. length group.

Parameters	<u>C. punctata</u>	<u>C. stewartii</u>	<u>C. orientalis</u>
TL/SL	1.18	1.21	1.19
TL/BD	5.41	7.73	6.87
TL/Girth	1.75	2.09	2.06
TL/HL	3.46	4.13	4.17
TL/DPL	3.28	3.76	3.33
TL/DFB	2.4	2.21	2.17
TL/AFB	3.23	3.98	3.48
TL/HW	5.58	6.47	6.01
TL/HD	6.18	8.89	8.29
HL/ED	6.25	6.39	5.72
HL/Sn.L.	4.55	5.75	4.82
HL/POL	1.52	1.72	1.51
HL/IOD	4.17	3.33	3.16
HL/HW	1.61	1.56	1.44
HL/HD	1.79	2.15	1.99

Table-19 : Biometric Indices in different Channa species at
15.0-20.0 cm. length group.

Parameters	<u>C. striatus</u>	<u>C. barca</u>	<u>C. punctata</u>	<u>C. stewartii</u>
TL/SL	1.14	1.18	1.2	1.17
TL/BD	7.11	7.51	5.85	6.24
TL/Girth	2.09	2.33	1.83	1.86
TL/HL	3.51	3.69	3.47	3.9
TL/PDL	3.16	3.45	3.17	3.44
TL/DFB	1.97	1.98	2.17	1.89
TL/AFB	3.37	3.73	3.09	3.2
TL/HW	6.69	8.17	5.88	5.75
TL/HD	9.7	9.65	6.33	6.93
HL/ED	7.9	7.77	7.64	6.98
HL/Sn.L.	5.82	4.39	5.82	5.17
HL/IDD	4.32	4.39	4.25	5.18
HL/POL	1.5	1.58	1.41	1.47
HL/HW	1.91	2.21	1.61	1.47
HL/HD	2.77	2.59	1.82	1.78

Table-20 : Biometric Indices in different Channa species at their maximum length groups.

Parameters	<u>C.striatus</u>	<u>C.barca</u>	<u>C.punctata</u>	<u>C.stewartii</u>	<u>C.orientalis</u>
TL/SL	1.14	1.18	1.18	1.21	1.2
TL/BD	7.12	7.78	5.41	7.73	9.0
TL/Girth	2.09	2.32	1.75	2.09	2.25
TL/HL	3.51	3.51	3.46	4.13	4.0
TL/PDL	5.15	3.29	3.23	3.76	3.6
TL/DFB	1.97	1.88	2.4	2.21	2.18
TL/AFB	3.37	3.55	3.23	3.98	4.0
TL/HW	6.69	7.79	5.58	6.47	6.0
TL/HD	9.7	9.1	6.18	8.89	8.0
HL/ED	7.9	7.77	8.25	6.39	6.0
HL/IOD	4.32	4.39	4.17	3.33	3.0
HL/POL	1.5	1.58	1.52	1.72	1.8
HL/Sn.L.	5.82	4.39	4.55	5.75	6.0
HL/HW	1.83	2.21	1.61	1.56	1.5
HL/HD	2.77	2.59	1.79	2.15	2.0

length, body depth and head depth in relation to total length and growth of snout length in relation to head length are negatively allometric. However, growth of inter-orbital length in relation to head length shows positive allometry.

The biometric indices of Channa stewartii reveal that the growth of pre-dorsal length in relation to total length and growth of post-orbital length and head width in relation to head length are isometric. All the remaining ratios show positive allometry.

It is also observed for Channa orientalis that the growth of head length, head width and anal fin base length in relation to total length and growth of post-orbital length, inter-orbital length, head width and depth in relation to head length are isometric in nature. However, growth of pre-dorsal length and body depth in relation to total length and growth of snout length and head depth show positive allometric relationships.

Channa barca and C. striatus are the largest among the five species studied while C. orientalis is the smallest among them. Due to the considerable range in size there is large overlapping of different species at certain size groups. A comparative study on the biometric indices has, therefore, been carried out covering all the five species to find out whether there is any species specific characters by which one species can be distinguished from the other when they are of same size. The fishes have been sorted into two different size groups. The latter are so arranged that these include more than one species. Thus, one of the size groups (7-10 cm.) covers three species (C. punctata, C. stewartii and

C. orientalis) while the other (15-20 cm.) covers four species (C. barca, C. striatus, C. punctata and C. stewartii). The findings summarised in Tables-18 & 19 along with a comparative study of biometric indices in different Channa species at the maximum size groups (Table-20), provide a reasonably balanced key to identify these species irrespective of their size, form and colour. However, it may be well to state here that all these morphological characters are not ubiquitous and morphological characteristics are not alone sufficient for species identification for this group of fishes.

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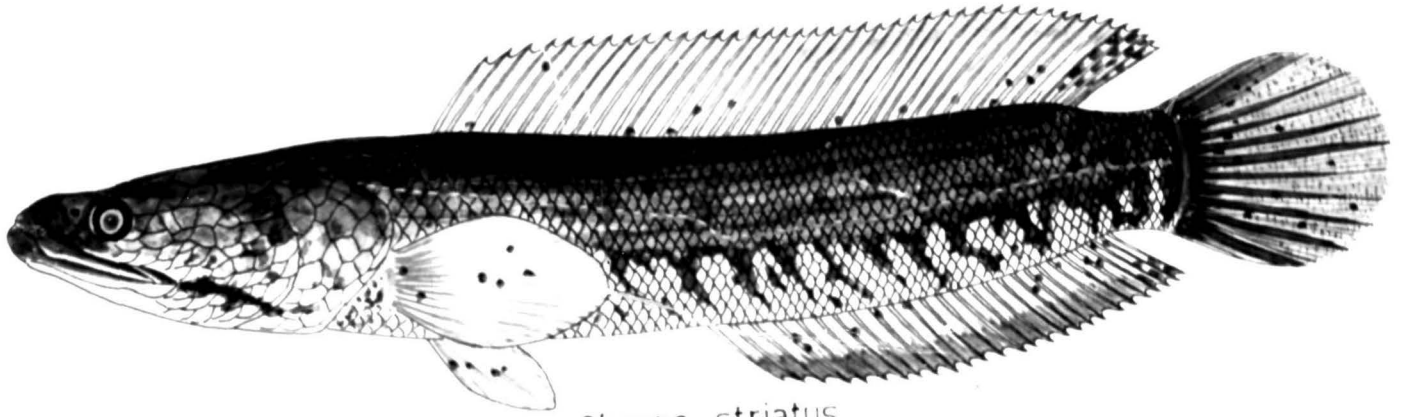
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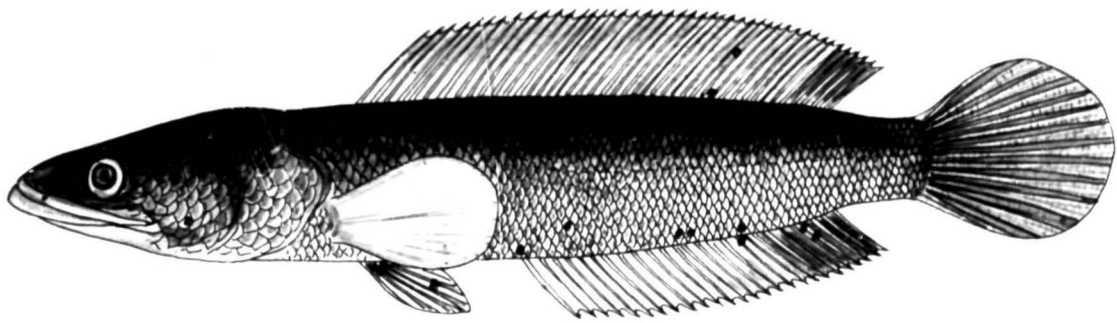
6. FIGURES AND PLATES

Figure 1a : The Fishes

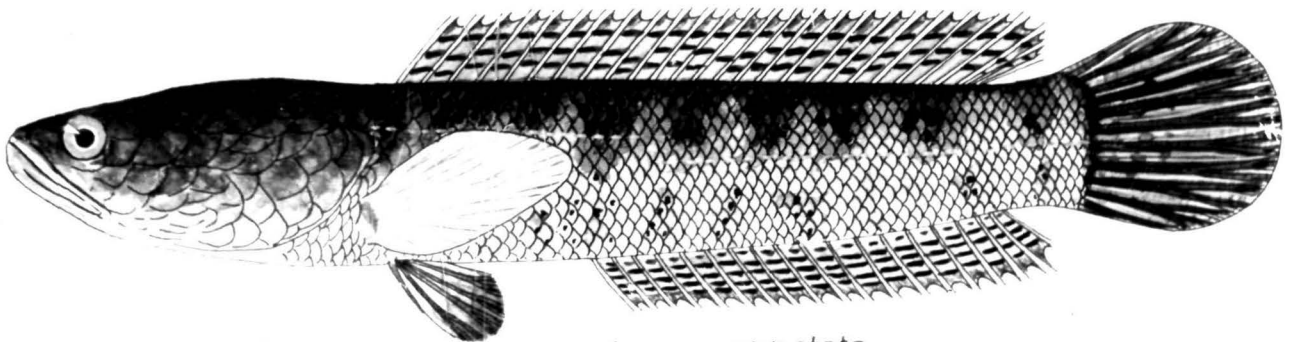
FIG. 1a



Channa striatus



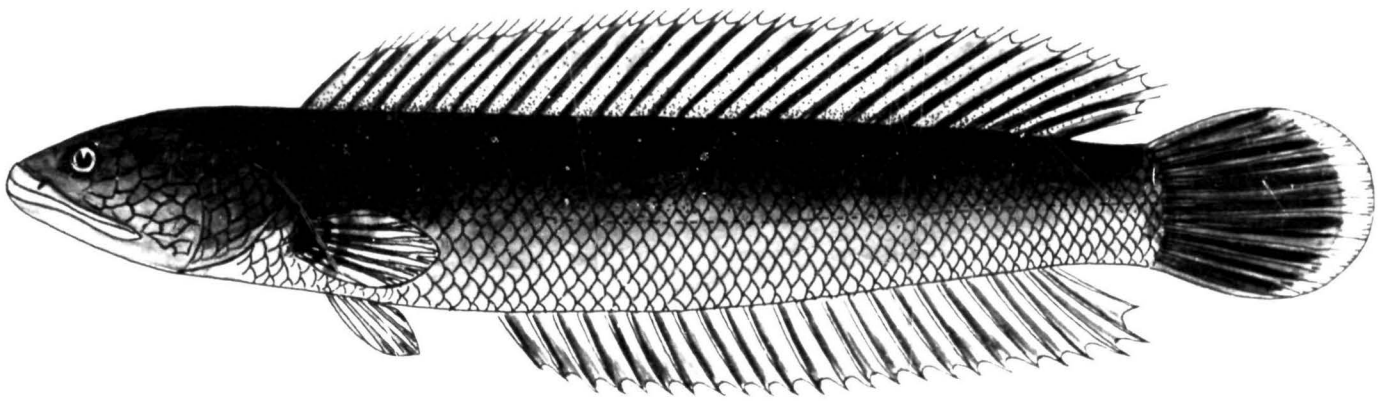
Channa barca



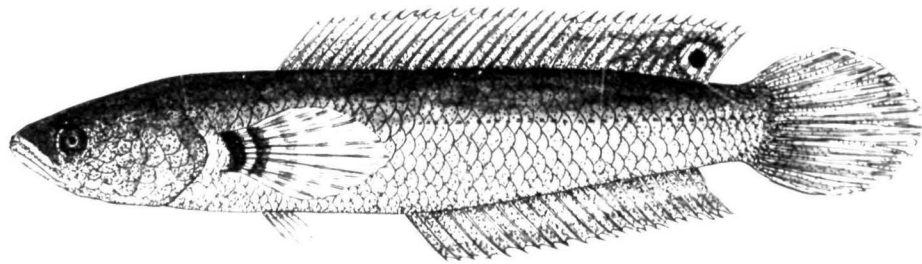
Channa punctata

Figure 1b : The fishes

FIG. 1b



Channa stewartii



Channa orientalis

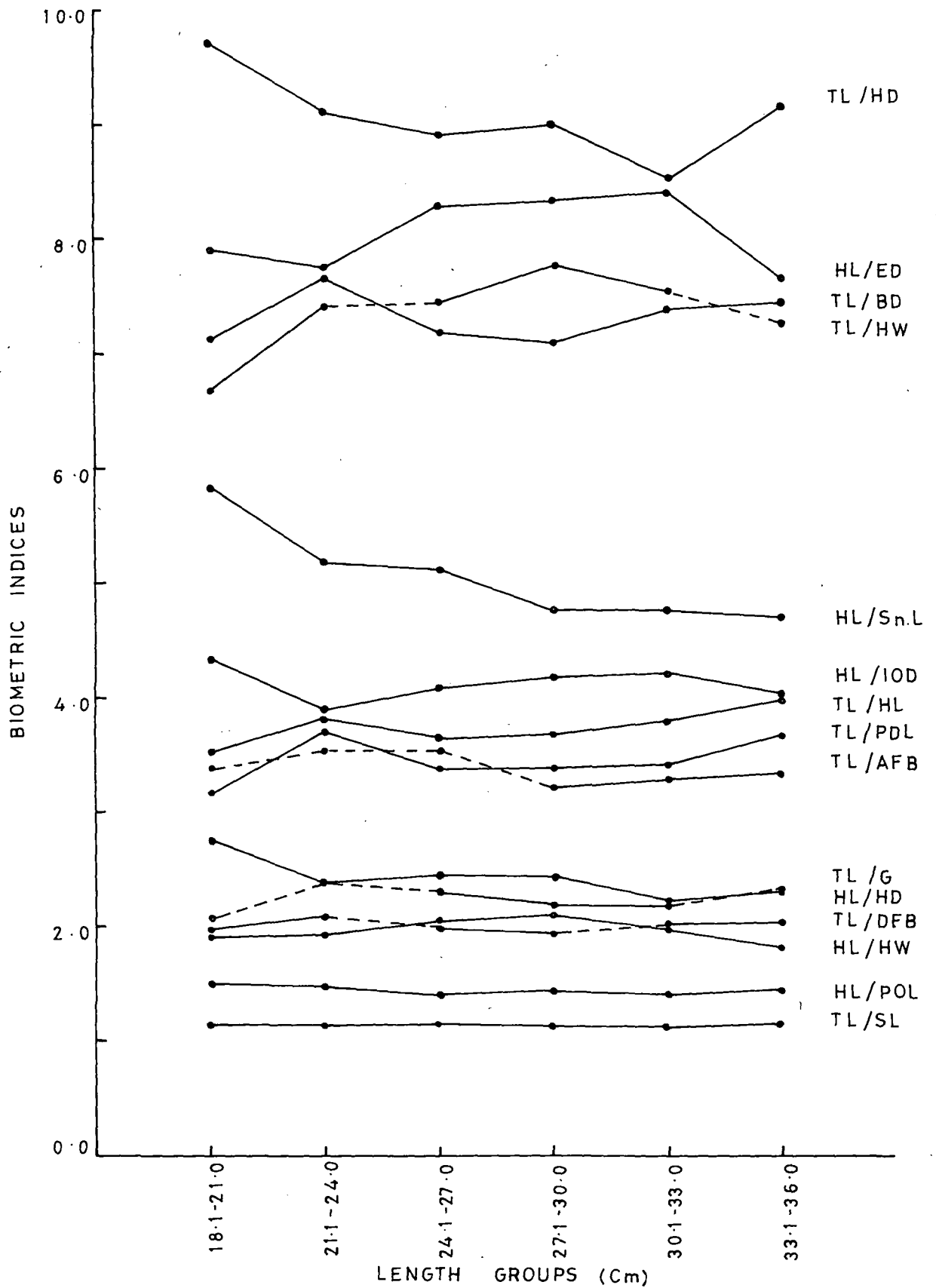


FIG. 2. BIOMETRIC INDICES OF DIFFERENT BODY PARAMETERS OF CHANNA STRIATUS

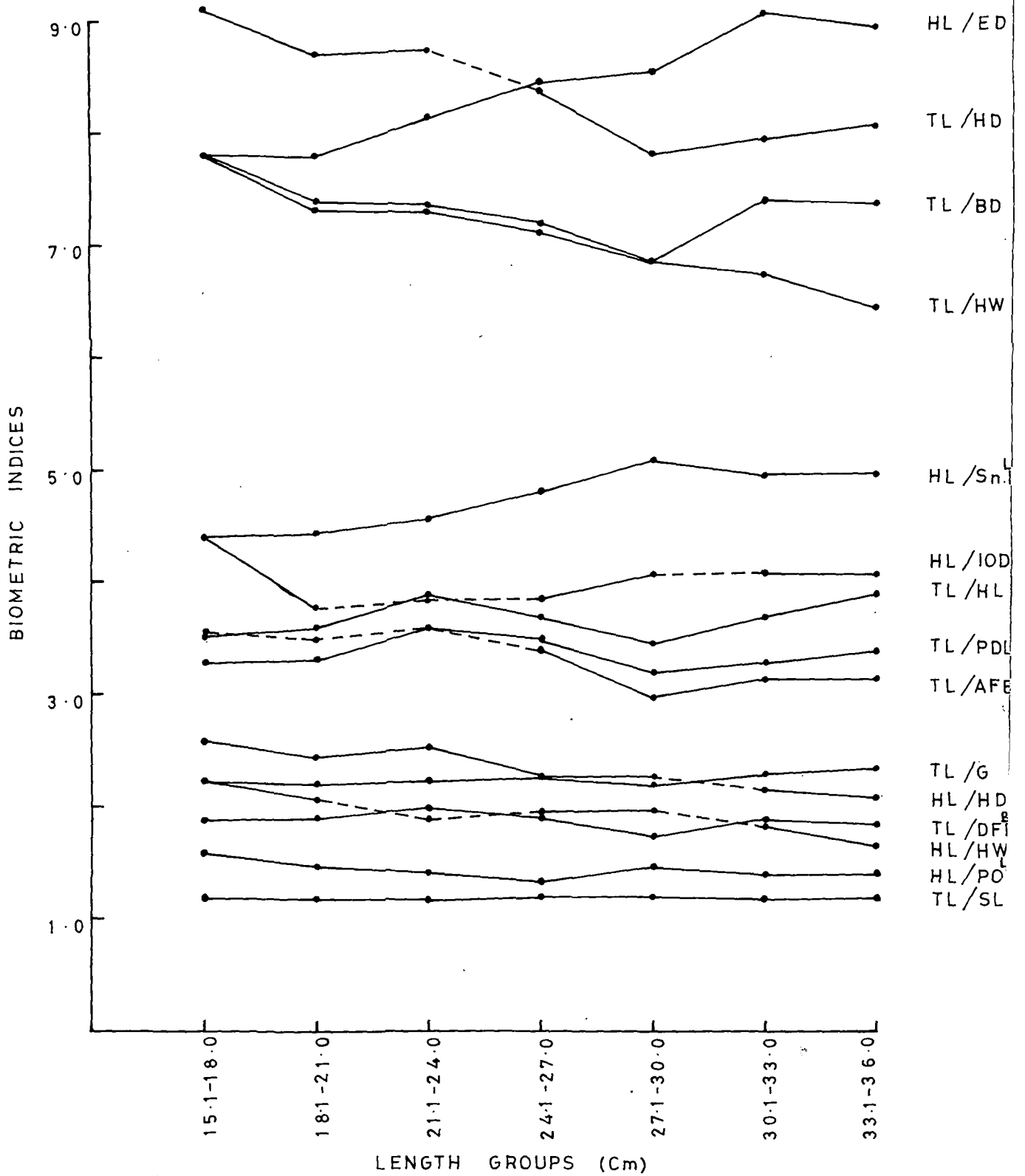


FIG. 3. BIOMETRIC INDICES OF DIFFERENT BODY PARAMETERS OF CHANNA BARCA

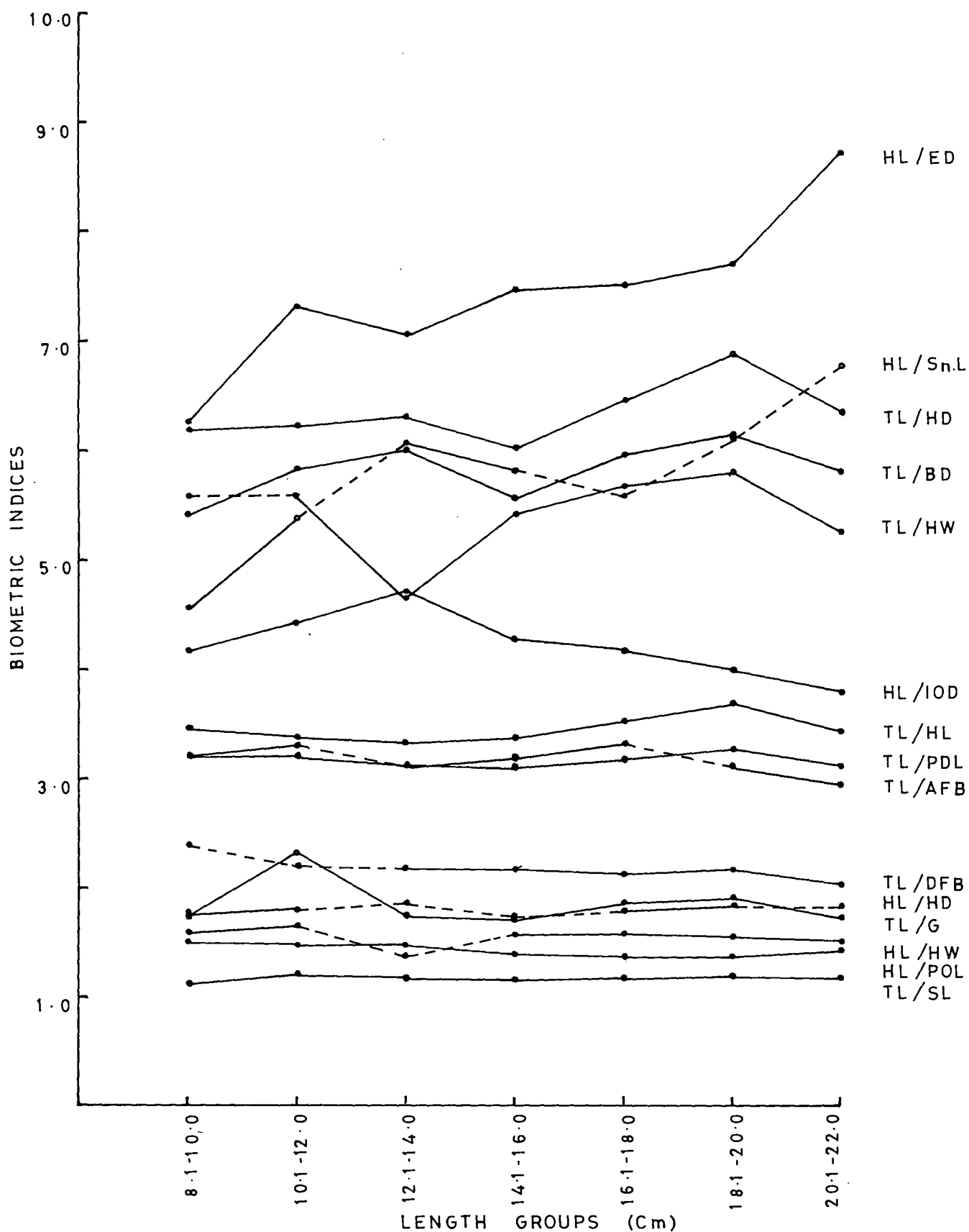


FIG. 4. BIOMETRIC INDICES OF DIFFERENT BODY PARAMETERS OF CHANNA PUNCTATA

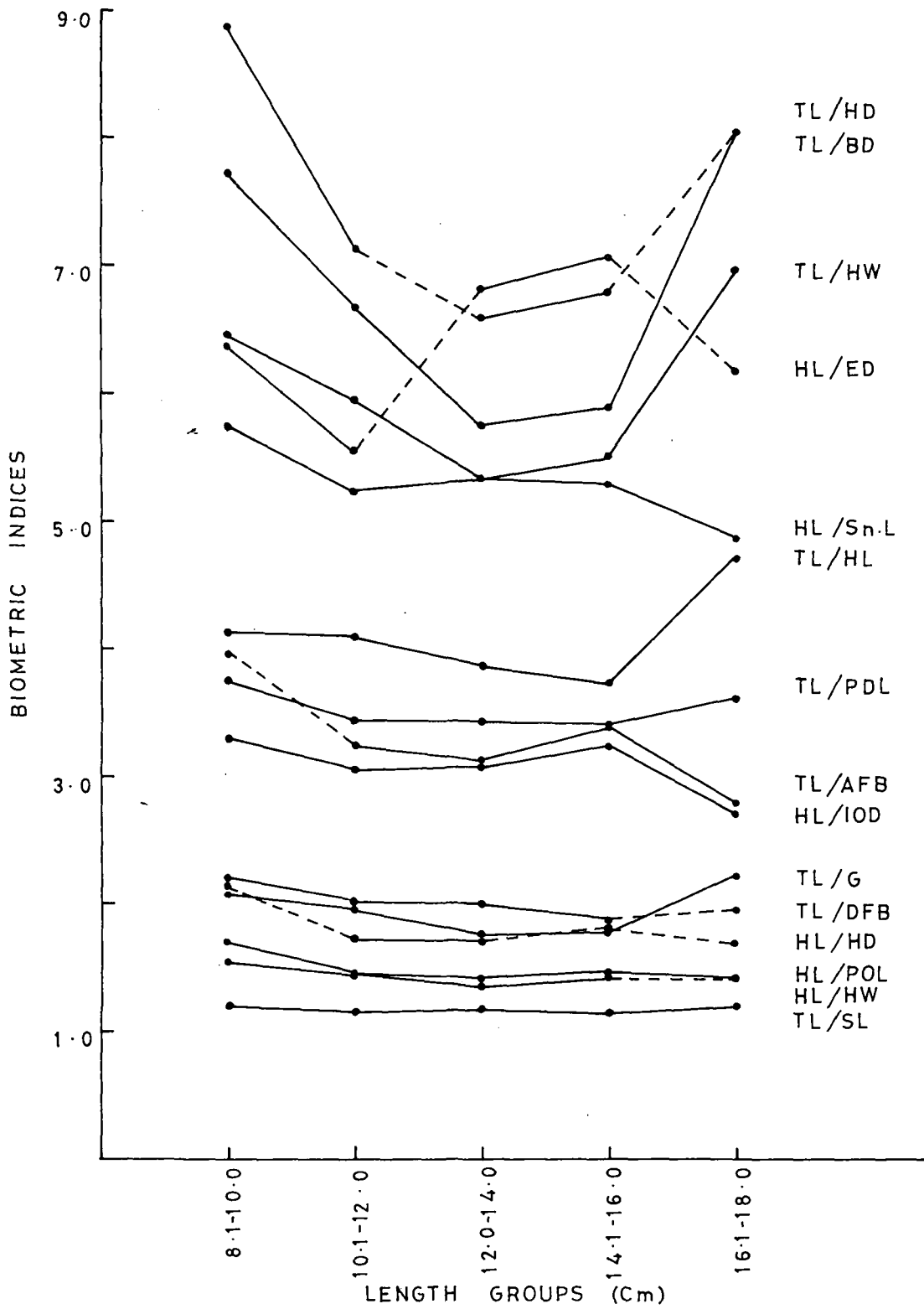


FIG. 5. BIOMETRIC INDICES OF DIFFERENT BODY PARAMETERS OF CHANNA STEWARTII

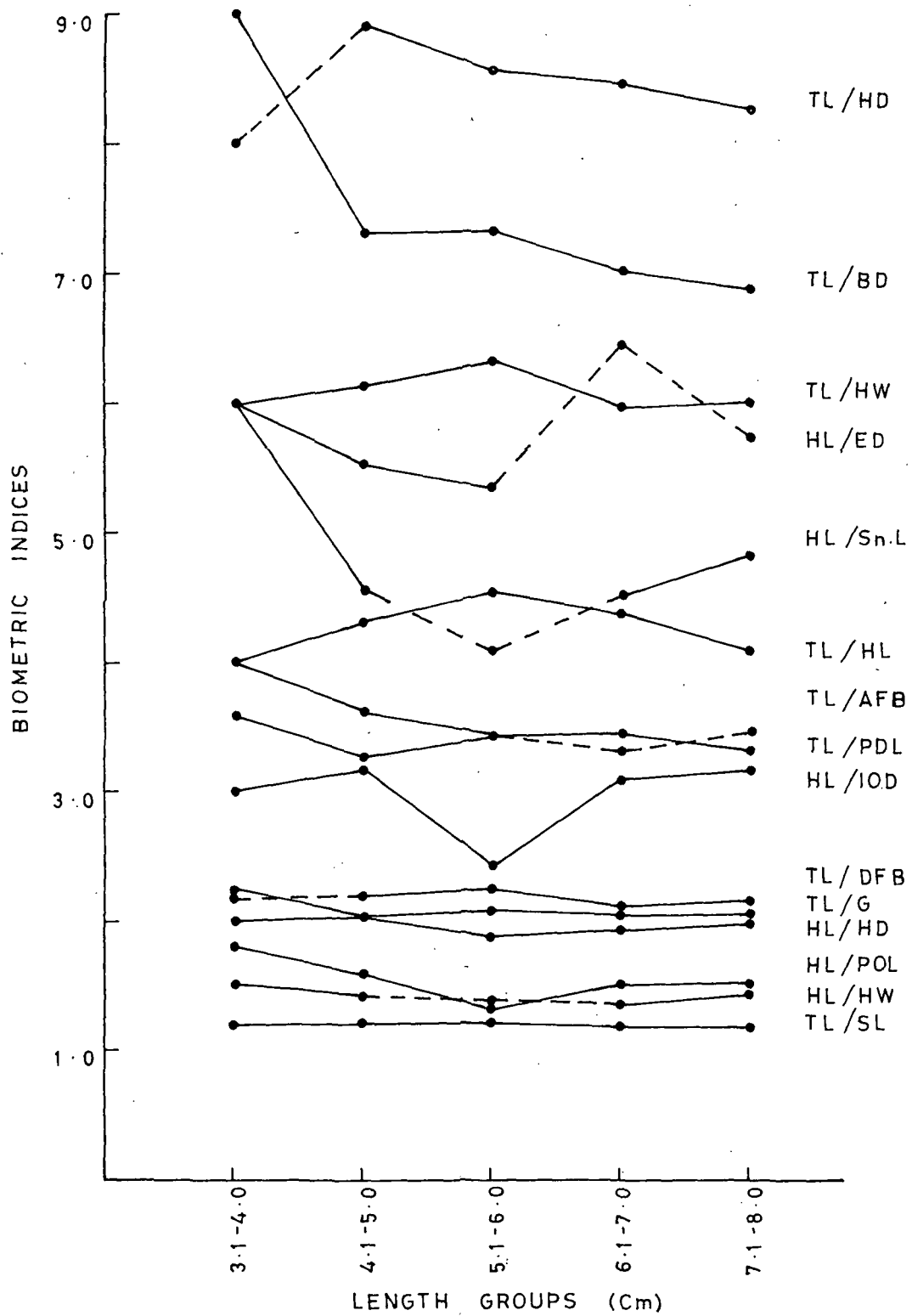


FIG. 6. BIOMETRIC INDICES OF DIFFERENT BODY PARAMETERS OF CHANNA ORIENTALIS

CHAPTER-II
CYTOGENETIC INVESTIGATION

1. INTRODUCTION

Meristic counts and morphometric analysis has been the subject of classical taxonomic investigation in the past to place different groups of animals in the system of classification and to unravel their natural relationship. It is now an accepted fact that chromosomal informations of the species are essential to the modern taxonomists since the species are considered to be the objective reality of some particular genetic community (Manna 1969). The analysis of karyotypes, diploid number, metrical data of metaphase chromosomes, behaviour and arrangement of chromosomes during division provide the taxonomists an independent set of tools to study the interrelations of different groups.

Though interest on the study of fish chromosomes can be traced back to the thirties by referring to the works of Iriki (1932a,b), Prokofieva (1934), Friedman and Gordon (1934), Makino (1934a,b, 1937, 1939), Svardson and Wickbom (1939) etc., the study of karyotypes of fishes has lagged far behind that of other vertebrates. Out of about 25,000 living species of fishes only about 900 species are chromosomally known and less than 600 species have been karyotyped. This is due to the small size and large number in most complements with the result that traditional chromosome techniques have not been useful for the study of fish chromosomes. Thus during the decade 1941-1950, only few papers on teleost chromosomes have been published (Makino 1941a,b, Svardson 1941, 1945, Svardson and Wickbom 1942 and Wickbom 1943). The following decade i.e. 1951-1960, has seen a few researchers working on fish chromosomes. Nogusa (1951, 1954, 1955a,b,c, 1957a,b,c and 1960) published a series of papers on the comparative study of chromosomes of Pisces with particular considerations on taxonomy, evolution and

sex determination. Prakken et al. (1955) studied the chromosomes of Esox lucius. Three populations of Atlantic salmon, Salmo salar from Canada were cytologically investigated by Boothroyd (1959).

Interest in the chromosome study of fishes greatly increased during the next decade (1961-1970) and as a result about 250 species were chromosomally worked out (Gyldenholm and Scheel, 1971). The diversified implications of chromosome studies could be well understood by reviewing the following literatures. Sick et al. (1962) have given a comparative account of haemoglobin patterns and chromosome numbers of American, European and Japanese eels (Anquilla). Lieder (1963) has investigated on the presumptive sex chromosomes in Perca, Acerina and Anquilla. Simon and Dollar (1963) have discussed the cytological aspects of speciation in two North American teleosts, Salmo gairdneri and S. clarki lewisi. Ojima et al. (1963) have described the chromosomes of the members of Salmonidae from the point of Cytotaxonomy. There was a great controversy regarding the nomenclature of chromosome types, which was, however, overcome later on as Levan et al. (1964) proposed that the chromosomes should be categorised according to their centromeric position, which was universally adopted in 1966 at the Paris Conference. Roberts (1964) has investigated the chromosome complements of twenty species of the family Centrarchidae. Post (1965) has worked out the chromosome numbers in some teleostean fishes. Ohno and Atkin (1966) have investigated the DNA values and chromosome number of eight species of fishes. Becak et al. (1966) have investigated the chromosomal polymorphism in different individuals of the green sunfish Lepomis cyanellus as an evidence of somatic segregation. Chen and Ebeling (1966, 1968) and Chen

(1968, 1969) have given comparative account of chromosome morphology with special reference to heterogamety in certain fish species. Yamada (1967) has studied the chromosomes in the embryonic cell of a goby Chaenogobius brotaenia. Ohno et al. (1967, 1968, 1969) have made significant contribution on the chromosome morphology, ploidy level and evolution from fish to higher groups. Nygren et al. (1968a & b) have made cytological studies on perch, pike and salmon. Muramoto et al. (1968, 1969) have studied the chromosomes of the fish order Ostariophysi. Booke (1968, 1970) has determined the DNA values and karyotypes of different coregonine fishes of the Great Lakes, U.S.A. and enumerated the findings from the point of Cytotaxonomy. Wolf et al. (1969) have analysed the DNA content and chromosome sets of various cyprinid species and discussed the mechanism of polyploidization in this group. Mayers and Roberts (1969) have studied five populations of Alosa pseudoharengus cytologically and found chromosomal homogeneity in all the populations. Prehn & Rasch (1969) and Rasch et al. (1970) have studied the chromosomes of poeciliid species and their hybrids from Mexico. Hitotsumachi et al. (1969) have given a comparative account of karyotypes in several species of Japanese loaches. Ojima and his co-workers (1969, 1970) have also studied the chromosomes of Japanese loaches and cyprinids. Chen and Ruddle (1970), Chen and Reisman (1970) have given a detailed account of chromosomes of several species of Fundulus and sticklebacks respectively. Ebeling and Chen (1970) have reviewed the chromosomal heterogamety in teleostean fishes. Kobayasi et al. (1970) have compared the chromosomes of the members of the genus Carassius. The application of cytogenetical and biochemical studies in solving phylogenetic

problems in the family Salmonidae has been discussed by Behnke (1970). Roberts (1970) has studied the chromosome morphology and speciation in the Atlantic salmon Salmo salar. Fukuoka and Niiyama (1970) have reported the somatic chromosomes of ten species of pleuronectid fishes.

During the last decade, a renewed and vigorous interest in studying the fish chromosomes has been observed. This has been largely due to technological advances, by modifying the air drying technique for chromosome preparation which provides comparatively better resolution of individual chromosomes within a complement. The realisation that karyotypic comparisons are crucial in assessing phylogenetic relationships within various taxa, has been equally significant.

A comparative study on chromosomes of twenty species of kill-fish from the genus Fundulus has been done by Chen (1971). Kang and Park (1971, 1973a,b, 1974) have worked out the cytogenetics of Korean fresh-water fishes. Mayers (1971) has analysed the chromosome complements of the genus Salvelinus. Nishikawa et al. (1971) have reported the chromosome numbers in two eel species (Anquilla). Beamish et al. (1971) have investigated the karyotypes and DNA values of the members of the suborder Esocoidae. Beamish and Tsuyuki (1971) have studied the biochemical and cytological differences found in the longnose sucker (Catostomus catostomus) and large and dwarf forms of the white sucker (C. commersoni). Ebeling et al. (1971) have investigated the increase in genome size in some deep sea teleostean species. Uyeno and Miller (1971) have reported the presence of multiple sex chromosomes in Mexican cyprinodontid fishes. Nygren and his collaborators (1971a,b,c,d,e,

1972, 1974, 1975a,b) have made extensive study on the chromosome characterization of a large number of fishes and their hybrids. Campos and Hubbs (1971, 1973) have stressed the taxonomic implications of karyotypes of gambusiine fish. The karyotypes and DNA values of 15 Japanese cyprinid fishes have been investigated by Ojima et al. (1972). The karyotypes and electrophoretic variations for 14 species of the genus Corydoras have been reported by Scheel et al. (1972). Derberovic and Sofradzija (1972) have reviewed the karyological data of freshwater fishes from Yugoslavia. Karyotypes of the teleost family Esocidae have been investigated by Davisson (1972). Fukuoka (1972) has studied the chromosomes of rainbow trout Salmo gairdneri irideus and reported the variation found in chromosome number of this fish. Passakas and Klekowski (1972) have analysed the chromosomes of European eel (Anguilla anguilla) with a note on sex determination. Cimino (1972) has examined egg production, polyploidization in a diploid, all female fish of the genus Poeciliopsis. Chernenko (1972) has studied the evolution and cytotaxonomy of the family Salmonidae. Scheel (1972) has described rivuline karyotypes and their evolution. Ross (1973) has investigated the chromosome complements of five etheostomine fishes of the family Percidae. Kirpichnikov (1973) has investigated the karyotype and evolution in Cyclostomata and fishes. Denton and Howell (1973) have worked out the chromosomes of African polypterid fishes. Capanna et al. (1973) have investigated the karyotypes of some hybrid species from the order Salmoniformes. Nikol'skij and Vasil'ev (1973) have recorded some regularities in the distribution of chromosome number in fishes. Cataudella and his co-workers (1973a,b, 1974, 1977a,b) have reported the chromosome morphology

of several Mediterranean mullets, cyprinids and other fishes. Uyeno, individually and with his co-workers (1973) has provided a comparative account of chromosomal morphology of a large number of fish species. Ojima et al. (1973) have worked out the karyotypes of acchelognathine fishes of Japan with a discussion on phylogenetic relationship among them. Banding techniques with particular reference to fish chromosomes are very few. Howell and Bloom (1973) have used differential fluorescence banding to study the chromosomes and spermatozoa of mud-minnows. Abe and Muramoto (1974) have investigated the chromosome morphology of two salmonid species, Salvelinus leucomaenis and S. malma by using differential staining. Chen and Ebeling (1974, 1975) have made a cytotaxonomical studies of Californian myctophoid fishes and also made karyological studies on some Xiphophorine fish hybrids. The chromosome studies on Japanese gobioid fish have been extensively done by Arai and Sawada (1974, 1975) and Arai et al. (1974). Fontana and Colombo (1974) have reported the chromosomes of Italian sturgeons. Karyotypes of nine species of fishes from the family Salmonidae have been studied by Muramoto et al. (1974). Kajdanova (1974) has investigated the karyosystematics and chromosome polymorphism in the family Salmonidae. A comparative study on chromosomes of twelve gobioid fishes from Japan has been done by Nishikawa et al. (1974). Ueno (1974) has investigated the chromosomal polymorphism and isoenzyme variation in different populations of Pseudobagrus aurantiacus. Itahashi and Kawase (1975) have worked out the karyotypes of five species of Cyprinodontidae. LeGrande (1975) has studied the karyotypes of six species of Louisiana flatfishes. Zenzes (1975) has studied the C-banding patterns in Salmo trutta. Thorgaard (1976) has studied

the Robertsonian polymorphism and constitutive heterochromatin distribution in chromosomes of Salmo gairdneri. Howell and Villa (1976) have studied the chromosomal homogeneity in two sympatric cyprinid fishes of the genus Rhinichthys. Park and Kang (1976) have discussed the karyotype conservation and DNA variations in anguilloid fishes. Takahasi and Oka (1976) have studied the karyotypes and electrophoretic variations in loaches of the genus Cobitis. A cytotaxonomical study of some freshwater cottoid fishes has been done by Abe (1976). Burtzev et al. (1976) have studied the karyology of the Acipenseridae in relation to hybridization and taxonomical problems. Passakas (1976) has confirmed the chromosome set of Anguilla anguilla. Kobayasi (1976) has compared the chromosome numbers of different races of spinous loaches, Cobitis biwa. Dingarkus and Howell (1976) have analysed the karyotypes and found out an evidence of tetraploidy in the North-American paddle fish Polyodon spatula. Ueno and Ojima (1976, 1977) have investigated the diploid-tetraploid complexes in the genus Cobitis and described the chromosome complements of two species of the genus Coreoperca with reference to karyotypic differentiation and evolution.

Vasil'ev (1977) has studied the polyploidization and evolution of karyotypes in the family Salmonidae. The chromosome complements of different subspecies and species of Xiphophorus have been investigated by Förster and Anders (1977). Avise and Gold (1977a,b) and Gold and Avise (1977) have studied the cytogenetics of a large number of species of North American fishes. Michele et al. (1977) have studied the karyotypes of some species of the family Loricariidae. Nishikawa and his co-

workers (1977, 1978) have compared the karyotypes of different species of Japanese fishes. Taki and his collaborators (1977a,b) have given a comparative account of chromosome number of Puntius from different zoogeographical regions. Kobayasi et al. (1977) have studied the chromosomes of the hybrids between different Carassius species and subspecies. Thorgaard (1977) has reported the heteromorphic sex chromosomes in male rainbow trout. Beamish and Miller (1977) have investigated the chromosomes of gila trout, Salmo gilae from cytotaxonomical point. Sofradzija (1977) has investigated the karyology and cytotaxonomy of the Leuciscus species from the waters of Bosnia and Herzegovina. Black and Howell (1978) have described a distinct chromosomal race of the cyprinodontid fish Fundulus notatus from the upper Tombigbee river system of Alabama and Mississippi. Ojima and Ueda (1978) have shown the presence of a new C-banded marker chromosome in carp-tuna hybrid. Passakas (1978) has studied the C-banding pattern in the chromosomes of European eel Anguilla anguilla. Viktorovsky (1978) has investigated karyotypic evolution in charrs of the genus Salvelinus. Gold et al. (1978, 1979a,b) have reported the gross karyotypic change and evolution in the North American fishes. Vasil'ev et al. (1978) have investigated the chromosomes of cyprinid fishes along with their hybrids. LeGrande (1978, 1980, 1981) and LeGrande and Cavender (1980) have studied the karyotypes of several species of North American catfishes. Bunch and Nadler (1980) have studied the Giemsa-banding pattern of chromosomes of the tahr and discussed the chromosomal evolution in the tribe Caprini. Sola (1980) and Sola et al. (1981) have studied the chromosomes of eel in relation to cytotaxonomy and

sex determination and reviewed the cytotaxonomical works on fishes. Bertollo et al. (1981) have analysed the chromosomes of Hoplias lacerdae from taxonomical point of view. Johnson et al. (1981) have described the karyotype of seven species of Galaxias (galaxiidae) from Tasmania and interpreted the evolutionary relationships.

It appears from the foregoing review that since 1960 great interest has been shown in the area of fish cytogenetics. This has resulted in improved methodology for obtaining and studying fish chromosomes. Most of the methods are offshoots from mammalian chromosome techniques and has been extensively reviewed by Denton (1973) in his book "Fish Chromosome Methodology". Since the publication of this book, a number of papers on fish chromosome techniques have been published. Through leucocyte culture, Grammeltvedt (1975) has obtained the chromosomes of Salmon. Toledo and Ferrari (1976) has modified the squash technique for chromosome studies in fishes. Badr and El-Dib (1977) have described the process of fixation and staining of Tilapia tissues for chromosome examination. Kligerman and Bloom (1977) have described a method for rapid chromosome preparations from solid tissues of fishes. Ida et al. (1978) have described a technique to prepare fish chromosomes by in vitro colchicine treatment. Park and Park (1979) have given a culture technique using marine fish kidney to obtain their chromosomes. Smith and Lemoine (1979) have found out that colchicine treatment results to polyploidy in brook trout.

Studies on fish chromosome in India have started probably with the work of Sharma et al. (1960) when they determined the diploid chromosome number in three species of fishes. Srivastava

and Kaur (1962, 1964) have carried out the chromosome studies in certain freshwater fishes. Nayyar (1964, 1965, 1966, 1967) has worked out the chromosome numbers in about twentythree species of fishes. Srivastava and Das (1968, 1969) have investigated the chromosomes in Clarias batrachus and certain other teleostean fishes. Natarajan and Subrahmanyam (1968) have observed the chromosome number in Tilapia mossambica. Effect of calcium treatment on fish chromosome has been investigated by Subrahmanyam (1969). Prasad and Manna (1970) have investigated the somatic chromosomes of a major carp Cirrhina mrigala. Subrahmanyam and Natarajan (1970) have investigated the somatic chromosomes of Therapon cuvier. Manna and Prasad (1971) have put forward a hypothesis on the mechanism of fish chromosome evolution. Prasad (1971) and Prasad and Manna (1971) have observed the somatic and germinal chromosomes of about sixteen freshwater fishes. Subrahmanyam and Ramamoorthi (1971) have investigated the chromosomes of the estuarine worm eel Moringua linearis. Chatterjee and Majhi (1973) have investigated the chromosomes of Mugil parsia. Khuda-Bukhs and Manna (1973, 1974) have studied the karyotypes of nine species of fishes including two major Indian carps Catla catla and Labeo bata. Nanda (1973) has investigated the chromosomes of Mystus vittatus and Ompok pabda. Prasad and Manna (1973) have shown with cytological evidences that there exist two forms of Mystus vittatus with 2n number 54 and 56 as a result of pericentric inversion and not because of Robertsonian fusion. Manna and Prasad (1973) have investigated the chromosomes of three species of fishes from the genus Channa. Vasudevan et al. (1973) and Prasad and Manna (1974) have investigated the chromosome complements of

Heteropneustes fossilis. Manna and Khuda-Bukhsh (1974) and Manna and Prasad (1974a) have investigated the chromosomes of two hybrid fishes and fishes from the family Gobiidae respectively. Natarajan and Subrahmanyam (1974) have analysed the chromosomes of some teleosts from the Perdonovo waters. Khuda-Bukhsh (1975) has investigated the somatic chromosomes of an exotic fish, Puntius japonicus. Rishi (1976) has studied the chromosomes of four species of fishes. Khuda-Bukhsh and Manna (1976) have studied the karyotypes of two species of mullets. Rishi and Gaur (1976) have investigated the chromosomes of jet-black molly and reported the presence of female heterogamety in it. Das and Kar (1977) have studied the somatic chromosomes of a siluroid fish Rita chrysea. Khuda-Bukhsh and Manna (1977) have studied the somatic and germinal chromosomes of an aquarium fish Mollienisia latipinna. Manna and Khuda-Bukhsh (1977) have studied the karyomorphology and cytological evaluation of the cyprinid fishes and provided a check list of chromosome numbers in cyprinid fishes. Khuda-Bukhsh (1978) has studied the somatic chromosomes of an estuarine fish Trypanchen vagina (Gobiidae). Manna and Khuda-Bukhsh (1978) have studied the karyotypes of three species of fishes. The chromosomal homogeneity of two Indian catfishes has been reported by Rishi (1978a). The Giemsa-banding pattern in the chromosome of Channa punctatus has been reported by Rishi (1978b). Choudhury et al. (1979) have investigated the chromosomes of six species of marine fishes. Khuda-Bukhsh (1979a) has investigated three species of fishes, showing the presence of heteromorphic, homomorphic and rod-like chromosomes. The chromosomes of two hill stream fishes, Barilius bendelisis and Rasbora daniconius have been studied by Khuda-Bukhsh (1979b). Patro and

Prasad (1979) have investigated the chromosomes of six Indian marine fishes. Khuda-Bukhsh (1980) and Khuda-Bukhsh et al. (1980) have further investigated three species of hill stream fishes with a report on high chromosome number in Tor putitora. Khuda-Bukhsh (1981) has again investigated two species of hill stream fishes, Labeo diplostomus and Garra gotyla gotyla cytologically. Rishi (1981), Rishi and Haobom (1981) and Rishi and Singh (1981) have investigated the chromosome complements in six species of fishes. Sharma and Tripathi (1981) have investigated the chromosomes of a siluroid fish Wallago attu from Jammu.

The hitherto available cytological information about Channidae is inadequate and controversial. Nayyar (1966) studied the germinal chromosomes of C. marulius, C. striatus, C. punctata (= C. punctatus). His result (C. punctata, $2n=34$) has been contradicted by Manna and Prasad (1973) and Rishi (1973) according to whom the diploid number is 32. However, the karyotype study of this fish by Manna and Prasad (1973) and Rishi (1973) is not in full agreement to each other. Similarly, the diploid number for C. striatus as determined by Nayyar (1966) and Manna and Prasad (1973) is 40, but the detailed karyotype analysis is full of contradiction. The present investigation therefore, was undertaken to determine the diploid number and chromosome morphology of five Channa species to study the extent of intra- and inter-specific variations and to analyse the phylogenetic relationship among the various members of the family Channidae.

2. MATERIALS AND METHODS

2.1. The Fishes

Materials for the present study comprised of five species of *Channa* viz., *C. striatus*, *C. barca*, *C. punctata*, *C. stewartii* and *C. orientalis* (as described in Chapter 1) and were collected from Gauhati (Assam) and Shillong (Meghalaya).

2.2. Chromosome preparation:

Adult individuals received an intramuscular or intraperitoneal injection of 0.05% Colchicine solution at a rate of 1 ml/100 gm. body weight. The injected fishes were then released in a well aerated aquarium and sacrificed after 2-4 hours depending on the condition of the fish.

Cytological preparation for chromosomal slides were made from kidney and gonads following the technique of Baker (1970) with necessary modifications as described below. The tissues were taken out, cut into fine pieces with the help of a scissor in hypotonic solution (tri-sodium citrate solution 0.09%), flushed vigorously to make a homogenous cell suspension and allowed to stand for about 45 minutes. The homogenous suspension thus prepared was centrifuged at 1500-2000 rpm. for about 12-15 minutes. The supernatant was removed and the residue was fixed in about 4 ml. of freshly prepared modified Carnoy's fixative (Acetic acid: Absolute alcohol, 1:3 v/v) and resuspended by vigorous flushing, till a homogenous solution was obtained. This solution was allowed to fix for about 30 minutes at room temperature. The suspension was then again centrifuged at 1500-2000 rpm. for at least 10-15 minutes and the supernatant was again discarded. The cell mass at the bottom of the tube was resuspended in about 1 ml. of fixative. Two or three drops of the suspension were then placed on a clean

slide (chilled in absolute alcohol) for overnight for better cleaning and also to minimize the heat) from a distance and ignited by passing the slide over a flame. Then the slide was kept for proper drying. This preparation was either directly used for staining or kept in a dust-free box for further use.

The dried slide were stained with Giemsa stain solution. The working stain solution was prepared as follows :

(i) Preparation of buffer solution: A buffer solution of pH 6.8 or 7.2 was prepared by mixing two stock solutions of $m/15$ di-sodium hydrogen orthophosphate and $m/15$ Potassium dihydrogen orthophosphate in equal volume.

(ii) 10 ml of Giemsa stock solution was mixed thoroughly with 90ml. of the above buffer solution.

(iii) The dried slides were then stained with the above solution for about 10-15 minutes.

(iv) The stained slides were then washed in distilled water and allowed to dry completely. After drying, the slides were mounted in D.P.X. mountant after a dip in Xylene for 5-7 minutes. Whenever necessary, photomicrographs were taken of the chromosomal spreads and karyotypes and idiograms were prepared.

2.3. Chromosome analysis:

The following parameters were considered while studying the chromosome morphology:

- (i) Length of the long arm of the chromosome.
- (ii) Length of the short arm of the chromosome.

$$(iii) \text{ Arm-ratio or 'r' } = \frac{\text{Length of the long arm}}{\text{Length of the short arm}}$$

$$(iv) \text{ Centromeric Indices } = \frac{\text{Length of the short arm}}{\text{Length of the long arm}} \times 100$$

(v) Total length of the chromosome.

(vi) Relative length of the chromosome:

$$RL = \frac{\text{Total length of a chromosome}}{\text{Total length of the whole chromosome set}}$$

(vii) Number of arms or NF value: total number of arms in a complete chromosome set.

(viii) Centromeric position and chromosome type.

The position of the centromere was determined according to Levan *et al.* (1964) by dividing the long arm by the short arm to provide an arm ratio (r). If 'r' is 1.0 to 1.7, the centromere has been considered as median and the chromosome has been designated as 'm' type (metacentric); a 'r' of 1.7 to 3.0 reflects a submedian centromere and the chromosome has been referred as 'sm' type (sub-metacentric). A 'r' between 3.0 to 7.0 indicates a subterminal centromere and the chromosome has been designated as 'st' type (subtelocentric). A 'r' of 7.0 to infinity indicates a terminal centromere and the chromosome has been referred as 't' type (terminal).

Basing on the centromeric position and chromosome type, karyotypes and idiograms were prepared.

3. RESULTS

The diploid number for each species has been determined by studying a large number of well spread metaphase complements from both the sexes. Chromosomes have been categorised following the suggestion of Levan et al. (1964) and grouped in the karyotype according to the types and arranged serially according to the decreasing order of length. Only the mean values of all the measurements, taken from different chromosome sets, were used in the tables for karyotypic studies.

3.1. Chromosome of C. striatus:

To establish the diploid chromosome number of this species, 147 well spread metaphase complements have been studied. As the chromosome number is found to vary between 36 and 42, the frequency of occurrence has been found out for the exact 2n number, as follows :

No. of chromosomes (2n)	36	38	40	42
No. of nuclei scored	2	12	127	6

Thus the chromosome counting shows a definite peak at 40 (86.39%) and this has been considered as the 2n number for C. striatus (Plate I).

The karyotype of C. striatus indicates the presence of 4 pairs of metacentric chromosomes with median centromere, 3 pairs of sub-telocentric chromosomes with sub-terminal centromere and 13 pairs of telocentric (or acrocentric) chromosomes with terminal centromere (Fig. 1, Table-1). Thus it is seen that the karyotype consists of 7 pairs of bi-armed and 13 pairs of single-armed chromosomes. No heteromorphic chromosome pair could be detected

Table-1 : Karyomorphological studies of the chromosome complement of Channa striatus.

Sl. No.	Length of short arm (μ)	Length of long arm (μ)	Total length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome Type
1.	2.68	2.82	5.50	10.52	1.05	48.73	m
2.	2.22	2.25	4.47	8.55	1.01	49.66	m
3.	1.91	1.94	3.85	7.36	1.02	49.61	m
4.	1.53	1.67	3.20	6.12	1.09	47.80	m
5.	0.85	2.75	3.60	6.89	3.24	23.61	st
6.	0.71	2.72	3.43	6.56	3.83	20.69	st
7.	0.56	2.54	3.10	5.93	4.54	10.06	st
8.	-	-	2.78	5.32	∞	-	t
9.	-	-	2.51	4.80	∞	-	t
10.	-	-	2.36	4.51	∞	-	t
11.	-	-	2.22	4.25	∞	-	t
12.	-	-	2.08	3.98	∞	-	t
13.	-	-	1.97	3.77	∞	-	t
14.	-	-	1.94	3.71	∞	-	t
15.	-	-	1.81	3.46	∞	-	t
16.	-	-	1.69	3.23	∞	-	t
17.	-	-	1.67	3.19	∞	-	t
18.	-	-	1.53	2.93	∞	-	t
19.	-	-	1.46	2.79	∞	-	t
20.	-	-	1.11	2.12	∞	-	t

in either sexes. The 'NF' value (no. of arms) for this species is found to be 54. The mean length of chromosome ranges from 5.50 micra to 1.11 micra.

3.2. Chromosomes of C. barca:

160 well spread metaphase complements have been employed for chromosome counting to establish the diploid number. It has been observed that chromosome number, sometimes varies between 36 and 42 and therefore, to get the exact diploid number, the frequency of occurrence has been found out as shown below :

<u>No. of chromosomes (2n)</u>	<u>36</u>	<u>38</u>	<u>40</u>	<u>42</u>
No. of nuclei scored	6	132	20	4

Thus the chromosome counting shows a definite peak at $2n = 38$ (82.5%) and this has been considered as the $2n$ number for C. barca (Plate II).

From the karyomorphological analysis, it has been found that the karyotype of C. barca consists of 8 pairs of bi-armed and 11 pairs of single-armed chromosomes, which can be further resolved into 3 pairs of metacentric chromosomes with median centromere, 3 pairs of submetacentric chromosomes with sub-median centromere, 2 pairs of subtelocentric chromosomes with sub-terminal centromere and 11 pairs of telocentric (or acrocentric) chromosomes with terminal centromere (Fig. 1, Table-2). However, no sex chromosomes or heteromorphic pair could be detected in either sexes. The 'NF' value for this species is found to be 54. The mean chromosome length varies between 3.38 micra to 0.78 micra.

Table-2 : Karyomorphological studies of the Chromosome complement of Channa barca

S1. No.	Length of short arm (μ)	Length of long arm (μ)	Total length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome type
1.	1.67	1.71	2.38	10.37	1.02	49.41	m
2.	1.56	1.62	3.18	9.76	1.04	49.06	m
3.	0.91	0.94	1.85	5.68	1.03	49.19	m
4.	0.89	1.78	2.67	8.19	2.0	33.33	sm
5.	0.88	1.73	2.61	8.01	1.97	33.72	sm
6.	0.67	1.38	2.05	6.29	2.06	32.68	sm
7.	0.45	1.56	2.01	6.17	3.47	22.39	st
8.	0.43	1.33	1.76	5.40	3.09	24.43	st
9.	-	-	1.56	4.79	α	-	t
10.	-	-	1.46	4.48	α	-	t
11.	-	-	1.45	4.45	α	-	t
12.	-	-	1.34	4.11	α	-	t
13.	-	-	1.21	3.71	α	-	t
14.	-	-	1.17	3.59	α	-	t
15.	-	-	1.14	3.49	α	-	t
16.	-	-	1.06	3.25	α	-	t
17.	-	-	1.01	3.10	α	-	t
18.	-	-	0.89	2.73	α	-	t
19.	-	-	0.78	2.39	α	-	t

3.3. Chromosomes of C. punctata:

297 well spread metaphase complements have been used for chromosome counting to determine the $2n$ number for this species, the result of which has convincingly demonstrated the existence of two forms of C. punctata viz: individuals with $2n = 34$ grouped together as C. punctata var. A and individuals with $2n = 32$, grouped as C. punctata var. B.

3.3.1. C. punctata var. A

Karyotypic analysis of C. punctata var. A reveals the presence of 15 pairs of bi-armed and 2 pairs of single armed chromosomes in the total complement (Plate III). On further investigation, it has been observed that the karyotype consists of 8 pairs of metacentric chromosomes with median centromere, 7 pairs of submetacentric chromosomes with submedian centromere and 2 pairs of telocentric chromosomes with terminal centromere (Fig. 1, Table-3). Here also no heteromorphic pair or marker chromosomes could be demarcated. The 'NF' value for this variety is found to be 64. The mean chromosome length ranges between 2.04 micra and 0.88 micra.

3.3.2. C. punctata var. B

The karyotype consists of 16 pairs of bi-armed chromosomes (Plate-IV), characterized by the presence of 8 pairs of metacentric chromosomes with median centromere and 8 pairs of submetacentric chromosomes with sub=median centromere (Fig. 1, Table=4). No heteromorphic pair or marker pair could be detected. The 'NF' value for this variety is also found to be 64, as in the case of C. punctata var. A. The mean chromosome length varies between 3.84 micra and 1.79 micra.

Table-3 : Karyomorphological studies of the chromosome complement of Channa punctata (Variety-A)

Sl. No.	Length of short arm (μ)	Length of long arm (μ)	Total length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome Type
1.	2.51	2.53	5.04	10.71	1.01	49.80	m
2.	2.23	2.25	4.48	9.52	1.01	49.78	m
3.	2.08	2.11	4.19	8.90	1.01	49.64	m
4.	2.01	2.09	4.10	8.71	1.04	49.02	m
5.	1.45	1.51	2.96	6.29	1.04	48.99	m
6.	1.4	1.5	2.90	6.16	1.07	48.28	m
7.	1.36	1.38	2.74	5.82	1.01	49.64	m
8.	1.12	1.13	2.25	4.78	1.01	49.78	m
9.	1.25	2.25	3.50	7.44	1.18	35.71	sm
10.	1.07	1.87	2.94	6.25	1.75	36.39	sm
11.	0.83	1.51	2.38	5.06	1.82	34.87	sm
12.	0.75	1.49	2.24	4.76	1.99	33.48	sm
13.	0.63	1.24	1.87	3.97	1.97	33.69	sm
14.	0.48	1.22	1.70	3.61	2.54	28.24	sm
15.	0.44	1.21	1.65	3.51	2.75	26.67	sm
16.	-	-	1.25	2.66	∞	-	t
17.	-	-	0.88	1.87	∞	-	t

pla-4 : Karyomorphological studies of the chromosome complement of Channa punctata (Variety-B).

Sl. No.	Length of short arm (μ)	Length of long arm (μ)	Total Length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome Type
1.	1.91	1.93	3.84	9.61	1.01	49.74	m
2.	1.40	1.49	2.97	7.44	1.01	49.83	m
3.	1.42	1.44	2.86	7.16	1.01	49.65	m
4.	1.42	1.43	2.85	7.13	1.01	49.82	m
5.	1.28	1.31	2.59	6.48	1.02	49.42	m
6.	1.19	1.21	2.40	6.01	1.01	49.58	m
7.	1.17	1.18	2.35	5.88	1.01	49.79	m
8.	1.12	1.13	2.25	5.63	1.01	49.78	m
9.	1.06	1.91	2.97	7.34	1.80	35.69	sm
10.	0.87	1.84	2.71	6.79	2.11	32.10	sm
11.	0.75	1.81	2.56	6.41	2.41	29.29	sm
12.	0.68	1.39	2.07	5.18	2.04	32.85	sm
13.	0.64	1.38	2.02	5.06	2.15	31.68	sm
14.	0.63	1.28	1.71	4.78	2.03	32.98	sm
15.	0.62	1.18	1.80	4.51	1.90	34.44	sm
16.	0.62	1.17	1.79	4.48	1.89	34.64	sm

3.4. Chromosomes of C. stewartii:

188 well spread metaphase complements have been employed to determine the diploid number for this species. The variation in chromosome number is found to be 62 and 68. So, to establish the 2n number, the frequency of occurrence has been found out as follows:

<u>No. of chromosomes (2n)</u>	62	64	66	68
<u>No. of nuclei scored</u>	6	16	161	5

Thus it has been observed that the chromosome counting shows a definite peak at 66 (85.64%) and therefore this number is considered as the diploid number for C. stewartii (Plate V).

The karyotype consists of 12 pairs of bi-armed and 21 pairs of single armed chromosomes which could be characterized by the presence of 6 pairs of metacentric chromosomes with median centromere, 3 pairs of submetacentric chromosomes with submedian centromere, 3 pairs of subtelocentric chromosomes with sub-terminal centromere and 21 pairs of telocentric chromosomes with terminal centromere (Fig. 2, Table-5). The 'NF' value is found to be 90. The mean chromosome length ranges from 4.42 micra to 0.43 micra. Heteromorphic pair or sex chromosomes could not be differentiated.

3.5. Chromosomes of C. orientalis:

208 well spread metaphase complements have been used for chromosome counting to determine the diploid number for this species. In the studied chromosome complements, the number is found to be varying between 74 and 80. However, to determine

Table-5 : Karyomorphological studies of the chromosome complement of Channa stewartii.

Sl. No.	Length of short arm (μ)	Length of long arm (μ)	Total length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome Type
1.	2.13	2.29	4.42	7.67	1.08	48.19	m
2.	1.64	1.73	3.37	5.85	1.05	48.66	m
3.	1.43	1.52	2.95	5.12	1.06	48.47	m
4.	1.29	1.36	2.65	4.59	1.05	48.68	m
5.	1.26	1.27	2.53	4.39	1.01	49.80	m
6.	0.86	0.89	1.75	3.04	1.03	49.14	m
7.	1.12	2.08	3.20	5.55	1.85	35.00	sm
8.	1.14	1.97	3.11	5.39	1.73	36.66	sm
9.	0.58	1.86	2.44	4.23	3.21	23.77	st
10.	0.57	1.71	2.28	3.96	3.0	25.00	st
11.	0.57	1.57	2.14	3.71	2.75	26.64	sm
12.	0.42	1.29	1.71	2.97	3.07	24.56	st
13.	-	-	2.15	3.73	α	-	t
14.	-	-	2.03	3.52	α	-	t
15.	-	-	1.73	3.00	α	-	t
16.	-	-	1.57	2.72	α	-	t
17.	-	-	1.56	2.71	α	-	t
18.	-	-	1.37	2.38	α	-	t
19.	-	-	1.36	2.36	α	-	t
20.	-	-	1.36	2.36	α	-	t
21.	-	-	1.29	2.24	α	-	t
22.	-	-	1.28	2.22	α	-	t
23.	-	-	1.21	2.09	α	-	t
24.	-	-	1.14	1.98	α	-	t
25.	-	-	1.02	1.77	α	-	t
26.	-	-	1.07	1.86	α	-	t
27.	-	-	0.93	1.61	α	-	t
28.	-	-	0.86	1.49	α	-	t
29.	-	-	0.85	1.48	α	-	t
30.	-	-	0.71	1.23	α	-	t
31.	-	-	0.58	1.01	α	-	t
32.	-	-	0.57	0.99	α	-	t
33.	-	-	0.43	0.75	α	-	t

Table-6 : Karyomorphological studies of the chromosome complement of Channa orientalis

Sl. No.	Length of short arm (μ)	Length of long arm (μ)	Total Length (μ)	% Relative length	Arm Ratio (r)	Centromeric Indices	Chromosome Type
1.	1.14	1.15	2.29	8.07	1.01	49.78	m
2.	0.86	1.72	2.58	7.18	2.0	33.33	sm
3.	0.71	1.71	2.42	6.74	2.41	29.34	sm
4.	0.58	1.29	1.87	5.20	2.22	31.02	sm
5.	-	-	1.79	4.98	α	-	t
6.	-	-	1.71	4.76	α	-	t
7.	-	-	1.57	4.37	α	-	t
8.	-	-	1.43	3.98	α	-	t
9.	-	-	1.36	3.79	α	-	t
10.	-	-	1.29	3.59	α	-	t
11.	-	-	1.14	3.17	α	-	t
12.	-	-	1.07	2.98	α	-	t
13.	-	-	0.93	2.59	α	-	t
14.	-	-	0.91	2.53	α	-	t
15.	-	-	0.86	2.39	α	-	t
16.	-	-	0.84	2.34	α	-	t
17.	-	-	0.83	2.31	α	-	t
18.	-	-	0.79	2.19	α	-	t
19.	-	-	0.77	2.14	α	-	t
20.	-	-	0.71	1.98	α	-	t
21.	-	-	0.71	1.98	α	-	t
22.	-	-	0.66	1.87	α	-	t
23.	-	-	0.64	1.78	α	-	t
24.	-	-	0.58	1.61	α	-	t
25.	-	-	0.57	1.59	α	-	t
26.	-	-	0.57	1.59	α	-	t
27.	-	-	0.56	1.56	α	-	t
28.	-	-	0.52	1.45	α	-	t
29.	-	-	0.51	1.42	α	-	t
30.	-	-	0.50	1.39	α	-	t
31.	-	-	0.43	1.19	α	-	t
32.	-	-	0.42	1.17	α	-	t
33.	-	-	0.41	1.14	α	-	t
34.	-	-	0.41	1.14	α	-	t
35.	-	-	0.36	1.00	α	-	t
36.	-	-	0.35	0.97	α	-	t
37.	-	-	0.29	0.81	α	-	t
38.	-	-	0.28	0.78	α	-	t

the exact $2n$ number, the frequency of occurrence for such variants are found out as shown below:

<u>No. of chromosomes ($2n$)</u>	<u>74</u>	<u>76</u>	<u>78</u>	<u>80</u>
No. of nuclei scored	2	174	28	7

Thus the chromosome counting shows the peak when $2n = 76$ (82.71%) and this has been considered as the diploid number of this species (Plate VI).

It has been found that the karyotype of C. orientalis consists of 4 pairs of bi-armed and 34 pairs of single armed chromosomes. The karyotype is characterized by the presence of 1 pair of metacentric chromosomes with median centromere, 3 pairs of submetacentric chromosomes with sub-median centromere and 34 pairs of telocentric chromosomes with terminal centromere (Fig. 2, Table-6). The 'NF' value is found to be 84. The mean length of chromosomes varies between 2.29 micra and 0.28 micra. No heteromorphic pair or sex chromosomes could be detected in the karyotype.



4. DISCUSSION

Chromosome studies in fishes have been found to be extremely difficult because of their small size and high number and probably, therefore, out of the 25,000 existing fish species, only 1000 or so are cytologically worked out (Sola et al. 1981). However, with the advances in the methodology, our knowledge on fish chromosomes is being enriched everyday.

Review of literature reveals that there exists at present a considerable uncertainty and confusion among fish cytogeneticists in interpreting the nature of the 'st' type of chromosomes with reference to their number of arms. While a large number of workers has considered the 'st' type as single armed, an equally large number has considered the 'st' type as bi-armed and there are instances that researchers who earlier considered the 'st' as single armed later changed their stand in favour of the bi-armed status (Manna and Prasad, 1973 and Manna and Khuda-Bukhsh 1977). We are of the opinion that the 'st' type of chromosomes should be considered as bi-armed while determining the NF value, because the very basis of categorising the chromosome depends upon the arm ratio and an arm ratio less than 7.0 recognises the presence of two arms in the chromosomes concerned (Table-7).

The diploid chromosome number of C. striatus was first reported by Nayyar (1966) according to whom, the karyotypes consisted of 10 metacentric and 30 rods which amount to NF 50. Manna and Prasad (1973) while reporting the same diploid number ($2n=40$) for C. striatus gave a chromosome formula: $2n = 8m + 2sm + 16st + 14t$ which according to the present concept accepts the NF as 66. The present investigation confirms the diploid number as 40.

However, the chromosome formula $2n = 8m + 6st + 26t$, determined by us does not fully agree with the findings of Manna and Prasad (1973). While the number of metacentrics is same i.e. 8 in both the findings, the gross differences can be boiled down to the single submetacentric pair they have recorded in the karyotype as according to their observation: "The karyotype consisted of 4 pairs of metacentrics, 1 pair of submetacentrics and 15 pairs of rod-shaped ones". A pericentric inversion in a rod shaped chromosome may convert it to a submetacentric one which may well account for the difference in the two karyotypes. It may be well to state here that different karyotypes with a constancy in the diploid number has been reported in Anguilla anguilla (Chiarelli et al. 1969, Passakas 1978, Sola et al. 1980), Anguilla australis (Nishikawa et al. 1971, Sola et al. 1980), Anguilla rostrata (Ohno et al. 1973, Sola et al. 1980), Oncorhynchus kisutch (Simon 1963, Uyeno 1972), Salmo gairdneri (Cuellar and Uyeno 1972, Muramoto et al. 1974, Thorgeard 1976, 1977), Salvelinus fontinalis (Davisson et al. 1972, Cataudella et al. 1973b, Muramoto et al. 1974), Acheilognathus lanceolatus (Ojima et al. 1973) and a large number of other fish species.

The chromosomes of Channa barca is being reported for the first time. The diploid number is 38 and the chromosome formula $2n = 6m + 6sm + 4st + 22t$ and the NF is calculated as 54.

The karyotype study of Channa punctata shows the existence of two forms. Nayyar (1966) reported a diploid number of 34 (16 metacentrics and 18 acrocentrics) from populations collected from the river Yamuna and its adjoining tributaries near Delhi. Rishi (1973) observed a diploid number of 32 (5 pairs of metacentric, 9

Table-7 : Gross karyotypic analysis of different Channa spp. studied.

Species	2n	m	sm	st	t	NF
1. <u>Channa striatus</u>	40	8	-	6	26	54
2. <u>Channa barca</u>	38	6	6	4	22	54
3. <u>Channa punctata</u> (Variety-A)	34	16	14	-	4	64
4. <u>Channa punctata</u> (Variety-B)	32	16	16	-	-	64
5. <u>Channa stewartii</u>	66	12	6	6	42	90
6. <u>Channa orientalis</u>	76	2	6	-	68	84

pairs of submetacentric and 2 pairs of acrocentric chromosomes) from populations collected from ponds near Kurukshetra. Manna and Prasad (1973) also obtained a diploid number of 32 (origin presumably Kalyani, West Bengal) and observed "all the chromosomes had a second arm of variable length and none was devoid of it". Our observations from specimens originating from ponds and the Brahmaputra systems around Gauhati, Assam have revealed the presence of two forms with fundamental differences in chromosome number and morphology. The form A has a diploid number of 34 ($16m + 14sm + 4t$) and form B possesses a diploid number of 32 ($16m + 16sm$). Though the $2n$ numbers of the two forms apparently correspond with the previous works (Manna and Prasad 1973, Rishi 1973; $2n=32$ and Nayar 1966; $2n=34$), the karyotype compositions and NF values differ considerably. The existence of more than one chromosomal forms (with different diploid numbers) has been reported in several fish species. Manna and Prasad (1974b) have reported the occurrence of two forms of Mystus vittatus (Bagridae) having 54 and 58 as diploid numbers. They have claimed that pericentric inversion or centromeric shift and not simple Robertsonian principle is the underlying cause. Recently LeGrande and Cavender (1980) have discovered two chromosomal races of Noturus flavus (Siluriformes: Ictaluridae) with diploid numbers of 48 and 50 which appear to be easily explainable as a Robertsonian rearrangement. Robertsonian rearrangements refer to the fusion of two non-homologous single armed chromosomes to give rise to a bi-armed one and conversely the formation of two single armed chromosomes by the fission and/or dissociation of a bi-armed chromosome. Such events have frequently been credited as a major operative procedure in the evolution

of fish karyotype (Gold 1979). It has been claimed that centric fusions are more common than centric dissociations (Denton 1973, LaGrande 1978) thus suggesting that decrease in diploid number is the more favoured pathway in karyotypic evolution in fish. Considering this concept we would like to suggest the two pairs of 't' chromosomes of form A have given rise to one pair 'sm' chromosome of form B through fusion. The difference in metrical analysis of the two forms may be easily explained by taking into consideration that some amount of chromatin may be deleted at various times during the transformations. It may be well to re-collect here that morphometric analysis and meristic counts (Ref. Chapter I) failed to differentiate the two forms. A detail investigation involving breeding experiments would perhaps be the only way to dissect the taxonomic status of the two forms. Nevertheless, the present finding reaffirms the importance of chromosomes as a valuable tool for the taxonomists.

The detailed chromosome analysis of Channa stewartii has been carried out for the first time ($2n=66$; $NF=90$; $12m + 6sm + 6st + 42t$).

The karyotype of Channa orientalis (= C. gachua; Menon 1974) as described here, differs markedly from that of C. gachua as presented by Manna and Prasad (1973). In contrast to our findings of 76 chromosomes, of which 2 belong to 'm' type, 6 to 'sm' type and the rest (68) to 't' type, they have reported a diploid number of 78 consisting of 12m, 12sm, 4st, 14t and 36T chromosomes. It may be stated that earlier C. orientalis and C. gachua (O. gachua) were regarded as separate species (Day 1878).

The females of both the forms of C. punctata have slightly larger chromosomes than that of the males. However, in the other Channa species the variations observed were not significant. The difference may be attributed to some unknown physiological factors essentially under genic control (Manna and Prasad 1973). In a further analysis it has been revealed that the total length of chromosomes in different species varies from 32.58 micra to 57.82 micra and two groups can be tentatively made on this basis viz.

(i) C. barca (32.58 micra), C. orientalis (35.94 micra) and C. punctata var. B (39.94 micra) and (ii) C. punctata var. A (47.07 micra), C. striatus (52.28 micra) and C. stewartii (57.82 micra).

It is interesting to note that the NF values of C. punctata var. A and C. punctata var. B (NF=64) and that of C. barca and C. striatus (NF=54) are same, but they differ in the total length of their chromosomes. It has also been noticed that the maximum and minimum lengths of the bi-armed chromosomes for all the species are within the same range except in C. striatus which is characterized with comparatively larger chromosomes. In case of single armed chromosomes, except C. striatus, all the species show a decreasing trend in their mean length with the increasing number.

The teleosts in general, display relatively high diploid numbers (around 50), whereas in the comparatively advanced group (Neoteleosts) the diploid chromosome number rarely exceeds 24 (Hinegardner and Rosen 1972, Stingo 1979). Karyotype made up of bi-armed chromosomes merely differing in size are considered as symmetrical, while the karyotypes exhibiting marked differences in the chromosome shape and size are defined as asymmetrical. The latter is further termed as unimodal when they lack microchromosomes

(Stebbins 1971, Morescalchi 1975). It appears, therefore, there exists considerable degree of chromosomal heterogeneity among the Channids. While C. punctata var. β qualifies to be categorised under the type symmetrical, the rest can be labelled as asymmetrical: Unimodal type.

According to Gold (1979), three primary mechanisms viz. polyploidy, aneuploidy and Robertsonian rearrangement can be held responsible for changes in chromosome number to be established during the course of evolution. The difference in the karyotype and the pathway of speciation has been convincingly explained through Robertsonian alterations in a large number of fish family (Simon 1963, Denton 1973, Park 1974, LeGrande 1980). However, it is extremely difficult to explain the great diversity in the karyotype of the different Channa species solely through Robertsonian principles. The basic inference from Robertsonian principles is change in diploid number without accompanying change in NF (Booke 1974, LeGrande 1981). However, according to the present investigation, not only there is a wide range in the diploid number (32-76) among the different members, the NF values also vary significantly (64-90). In absence of data on comparative nuclear DNA values of these five species, no comment on diploid-tetraploid relationship seems to be justified. Moreover, the analysis of total chromosomal length and studies on meiotic behaviour (Manna and Prasad 1973, Dhar unpublished), is not indicative of polyploidy among the different species. The importance of pericentric inversion and/or centromeric shift along with Robertsonian events in fish evolution have been stressed by various workers (Manna and Prasad 1971, LeGrande 1981). The present knowledge of the karyology of the

Channidae does not reveal a clear cut pathway of karyotypic evolution. However, considering all the information available, we are of the opinion that Robertsonian alterations superimposed with pericentric inversion and/or centromeric shift have been the major mechanism behind karyotypic evolution in Channidae. The presence of asymmetrical karyotype in all the Channa species except C. punctata var. B tends to support the concept that all of them are comparatively primitive than the latter. It is now widely accepted that the most fundamental karyotype of fishes consists of approximately 48 acrocentric chromosomes (Nogusa 1960, Post 1965) and there is a tendency for evolving groups of fishes to have fewer chromosomes through bi-arm formation (Danton 1973, LeGrande 1978). Recently Manna and Khuda-Buksh (1977) have suggested that instead of all being of rod-shaped, the primitive karyotypes consisted of 48 chromosomes with different morphology as it would involve less structural rearrangements in the evolution of different karyotypes than if they were to be derived from all rods. Both C. striatus and C. barca with $NF=54$ and diploid number of 40 and 38 respectively seem to be close to the primitive karyotype. The karyotype of the former might have given rise to that of the latter by the fusion of 4 't' chromosomes and slight modification in the position of the centromere of some bi-armed chromosomes through centromeric shift. Robertsonian alteration through fusion of 8 't' and pericentric inversion in 10 't' will result in the karyotype of C. punctata var. A. It has already been suggested that centric fusion involving 4 't's of C. punctata var. A will give rise to the karyotype of C. punctata var. B. While the inter-relationship between the karyotypes of C. orientalis and

C. stewartii is apparent (20 't' change to 10 bi-armed through fusion and 6 't' become bi-armed by pericentric inversion), it is extremely difficult to find out the relationship of these two species to the 'so-called' main line in the Channid speciation. C. orientalis has a diploid number exactly double of that of C. barca. May be mainly polyploidy coupled with Robertsonian alterations or pericentric inversion had a role to play. It may also be argued that by a complex series of pericentric inversions, centric dissociations, C. orientalis evolved from C. barca. In the karyotypic evolution of Chiroptera, the direction of Robertsonian alterations (fission/fusion) seems to move indifferently in both directions (Capanna and Civitelli 1970). But it should also be remembered that the usual trend as observed in fish karyotype evolution is indicative towards reduction of chromosome number. Recently LeGrande (1981) has suggested the possible occurrence of centromeric terminalization prior to fusion as an operative factor in the evolution of Ictalurid karyotypes. This may also be applicable to the complexity of relationship between these two karyotypes. Considering all the available facts and the concept that in the absence of the primitive number in several families because of marked differences in the karyotypes, the karyotype evolution in fish has been multidirectional (Denton 1973, Park 1974), we have suggested the possible ways of speciation in this family (Fig.3). Perfection of the banding techniques for fish, is hoped to unravel the problem in future.

It is well accepted that correlations between morphologic and karyologic evolution are hard to understand (Benazzi 1973). Though the morphotaxonomists have placed the snake-heads under a distinct

order with a single family, it is often difficult to identify them by morphometric and meristic criteria. Karyologically, however, each species is remarkably distinct. Again on the question whether these fishes as a group should be treated as primitive or advanced, karyologic data leads us to utter confusion. If we consider their air-breathing nature, the symmetrical karyotype of C. punctata var. B, should be regarded as an advanced or specialized group. The asymmetrical karyotype of the rest of the species, on the other hand, is indicative of the reverse. In addition, sufficient fossil records are not available to throw any light.

Though there are few reports on the presence of sex chromosomes or heterogametic individuals in fishes (Lieder 1963, Chen 1969, Ebeling and Chen 1970, Uyeno and Muller 1971, Rishi and Gaur 1976, Förster and Anders 1977, Cataudella and Sola 1977, Thorgaard 1977, Filho et al. 1980), no heteromorphic pair or sex chromosomes could be detected in the presently studied species which is in accordance with the current concept that the sex chromosomes in fish are in very low grade of differentiation and hence not morphologically distinguishable.

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6. FIGURES AND PLATES

Plate-I : Metaphase spread from kidney and
karyotype of Channa striatus
(a) Male (b) Female (c) Karyotype
(bar represents 10 μ)

PLATE - I

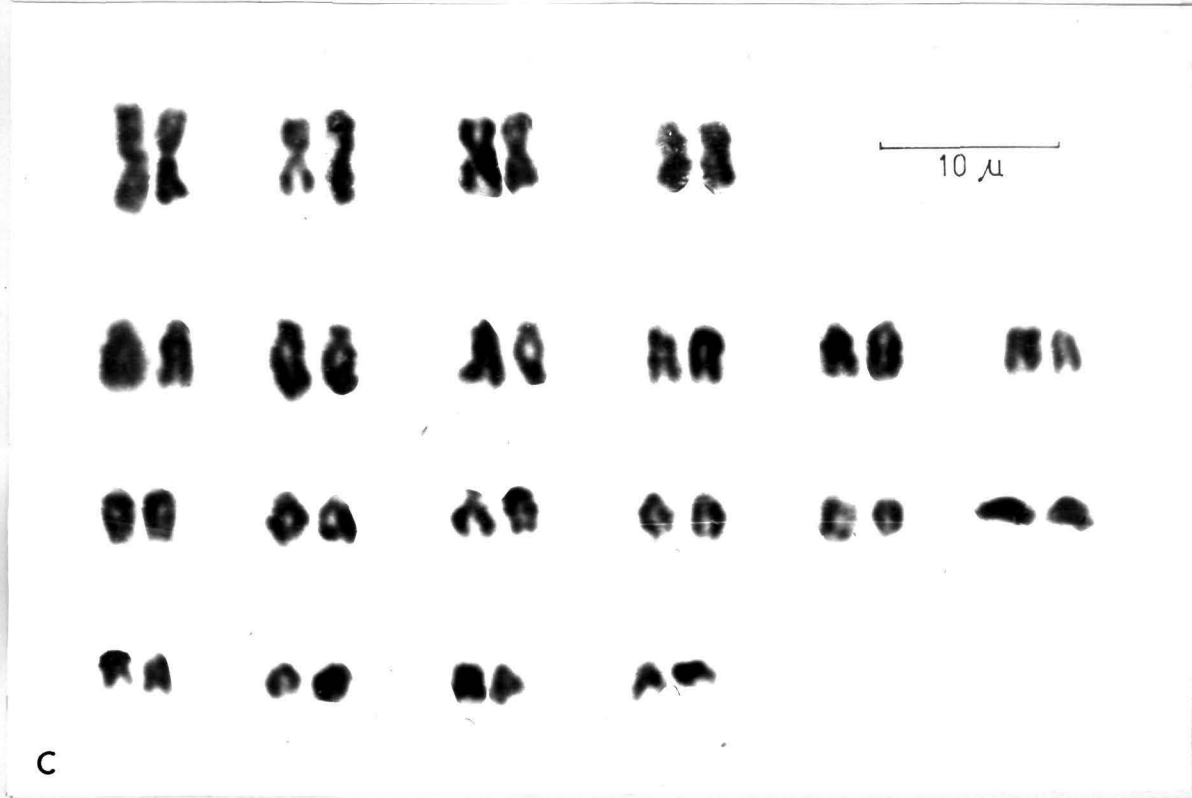
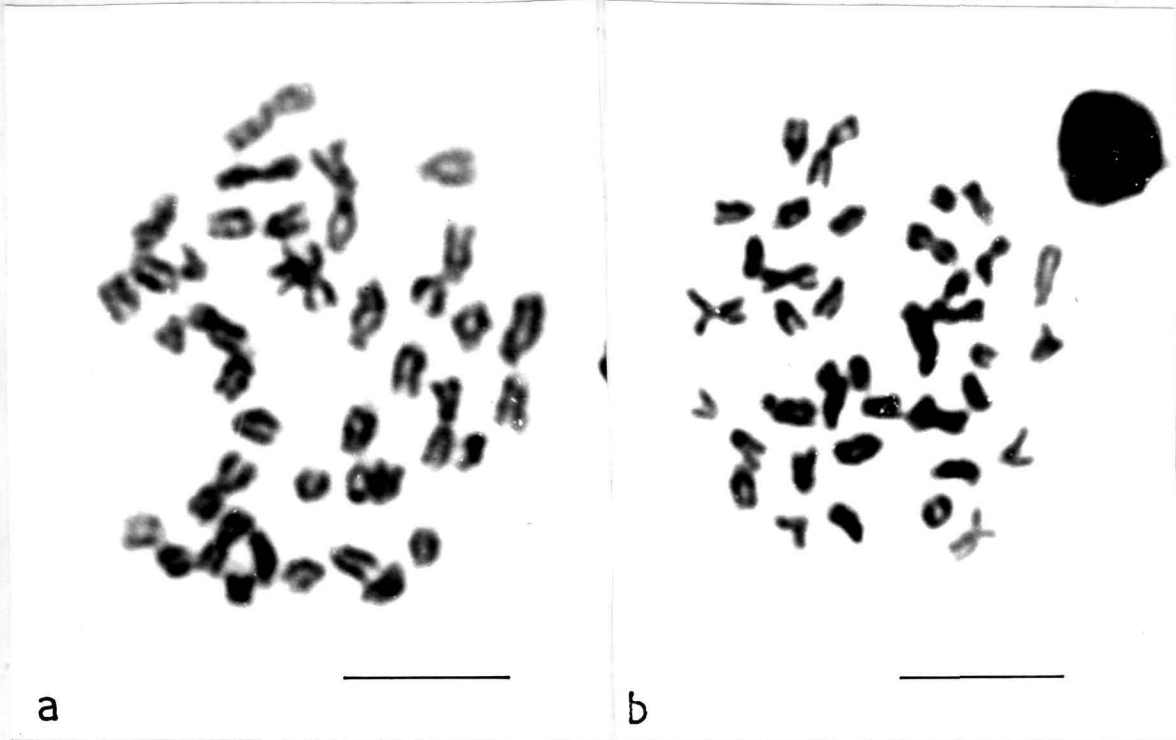


Plate-II : Metaphase spread from kidney
and karyotype of Channa barca
(a) Male (b) Female (c) Karyotype
(bar represents 10 μ)

PLATE - II

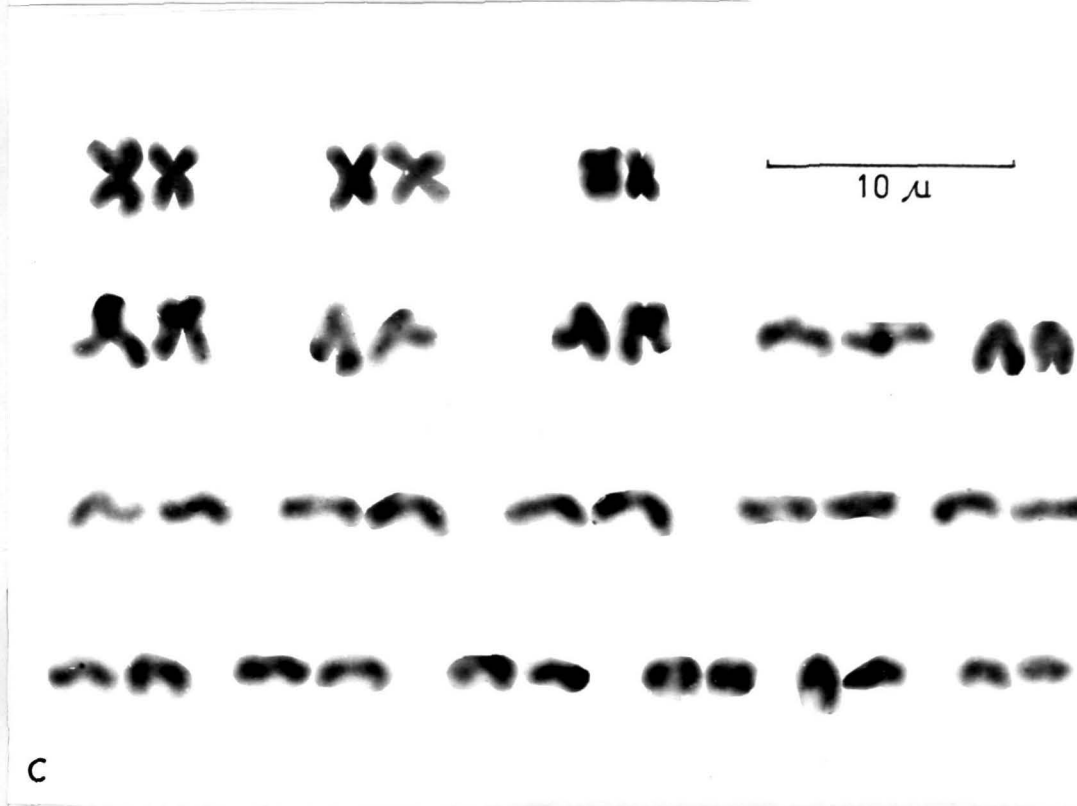
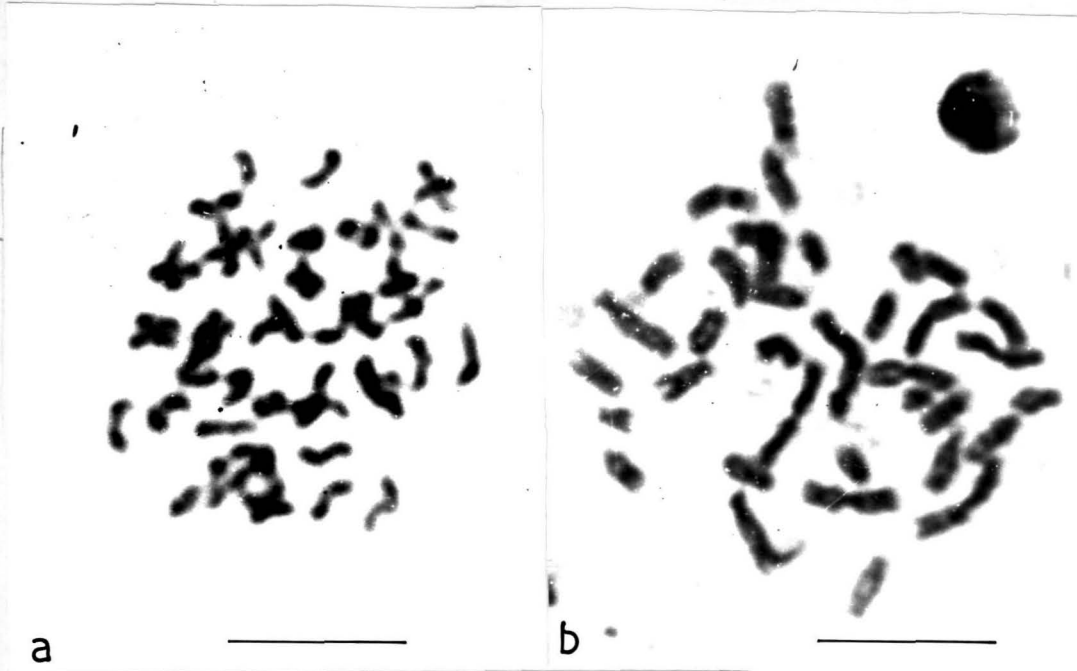
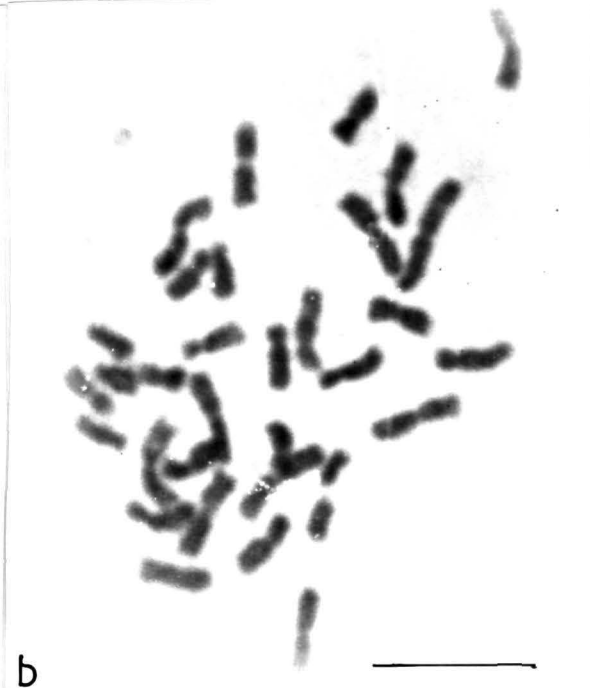


Plate-III : Metaphase spread from kidney
and karyotype of Channa punctata Var. A.
(a) Male (b) Female (c) Karyotype
(bar represents 10 μ)

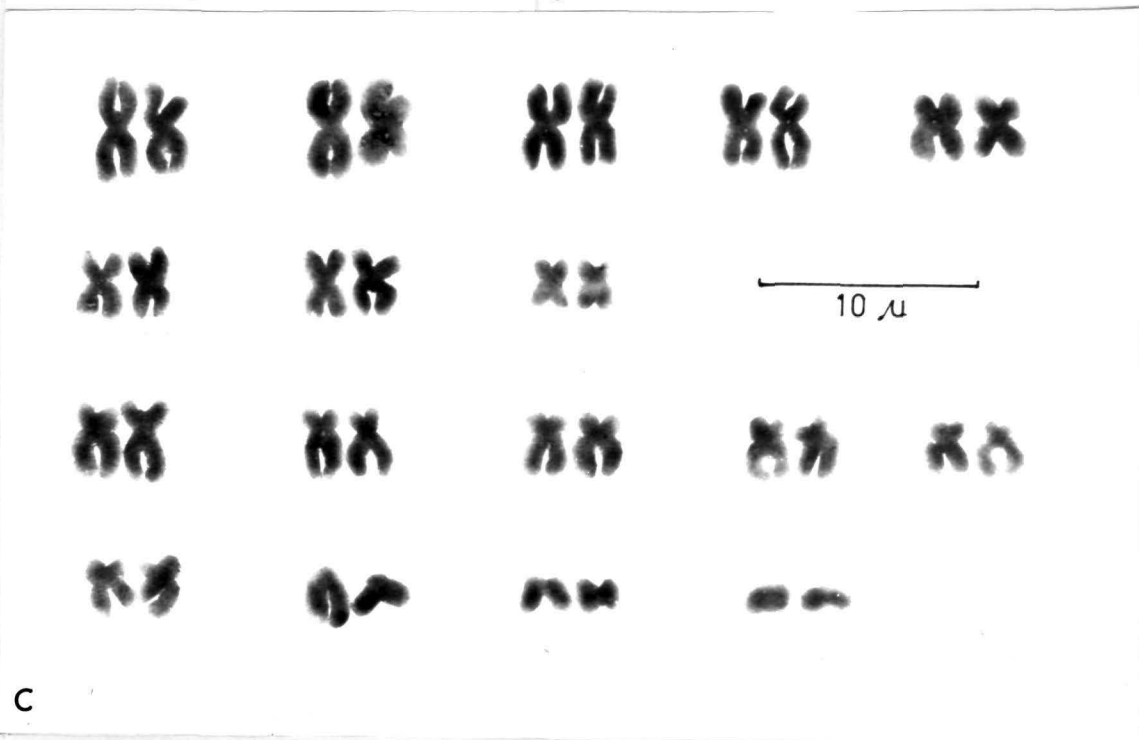
PLATE-III



a



b



c

Plate-IV : Metaphase spread from kidney and
karyotype of Channa punctata Var. B.
(a) Male (b) Female (c) Karyotype
(bar represents 10 μ).

PLATE-IV

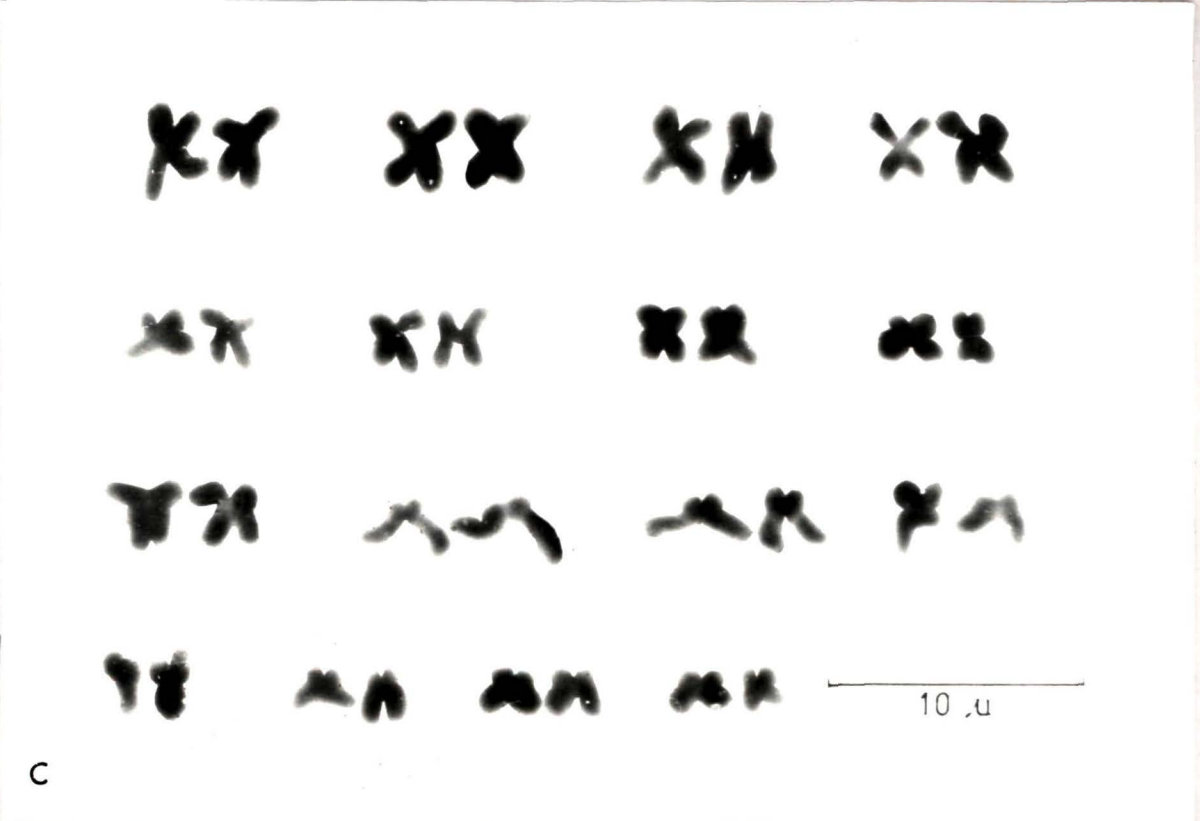
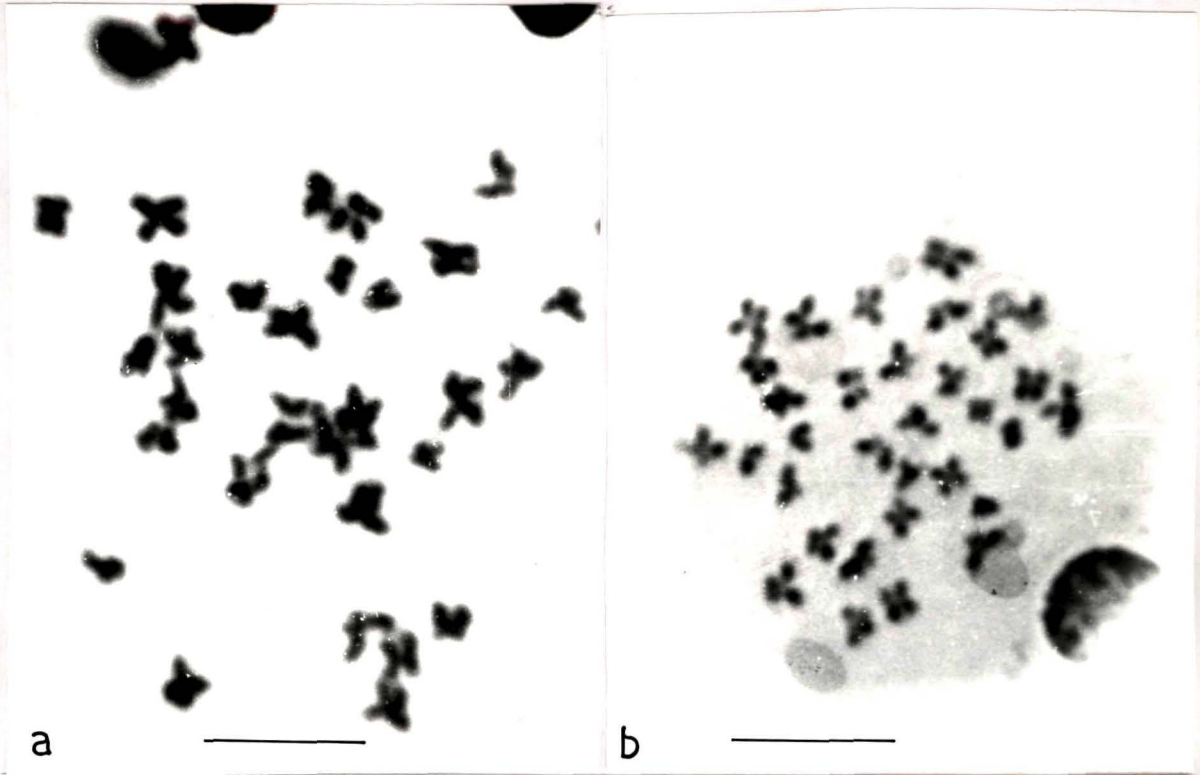
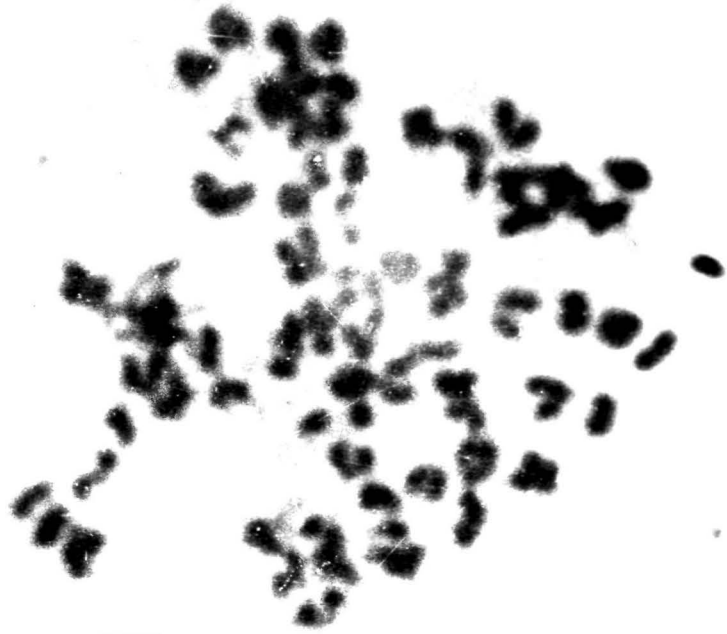


Plate-V : Metaphase spread from kidney and
karyotype of Channa stewartii

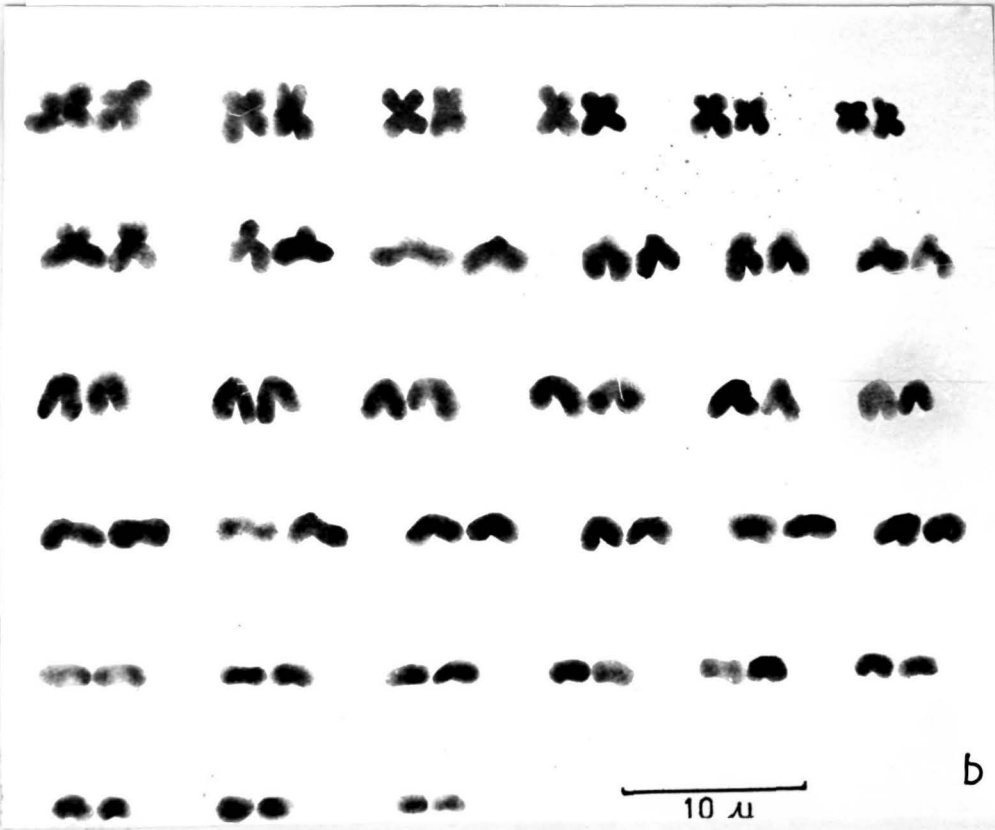
(a) Male (b) Karyotype

(bar represents 10 μ)

PLATE - V



a



b

Plate VI : Metaphase spread from kidney and
karyotype of Channa orientalis

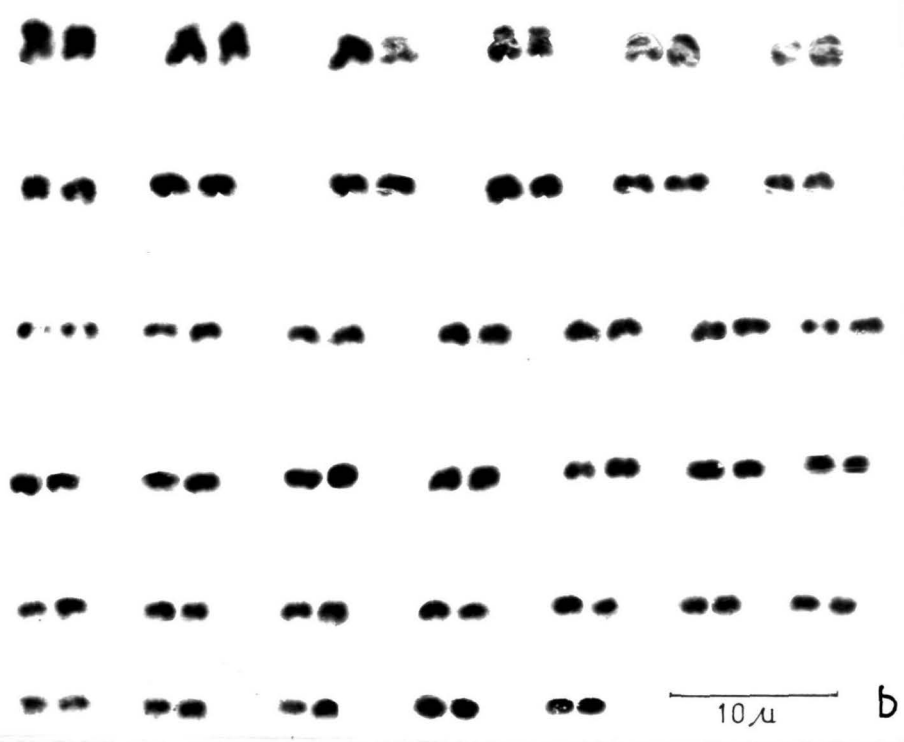
(a) Male (b) Karyotype

(bar represents 10 μ)

PLATE-VI



a



10 μ

b

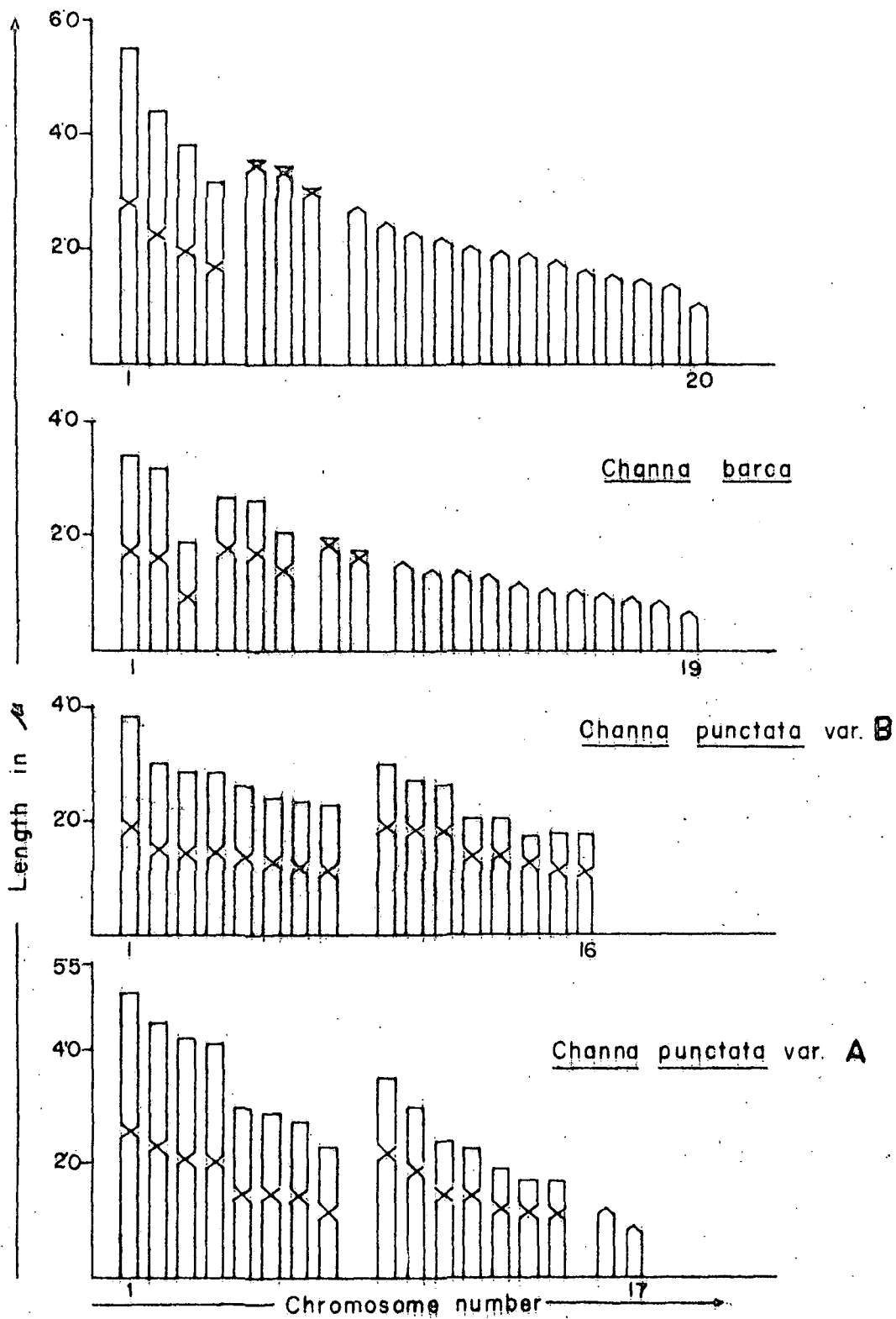


Fig. 1. IDIOGRAMS OF DIFFERENT CHANNA SPECIES

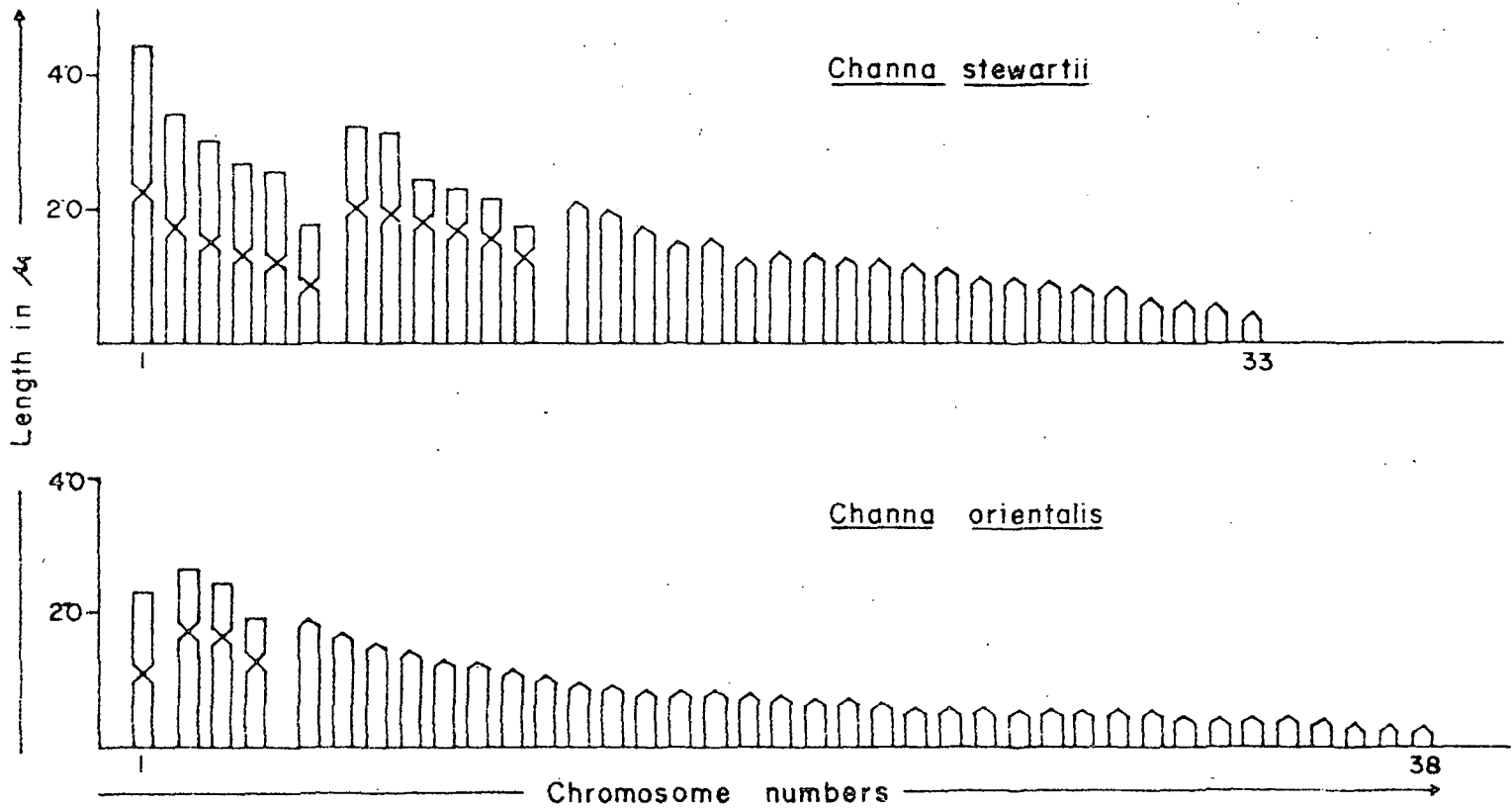


Fig. 2. IDIOGRAMS OF DIFFERENT CHANNA SPECIES

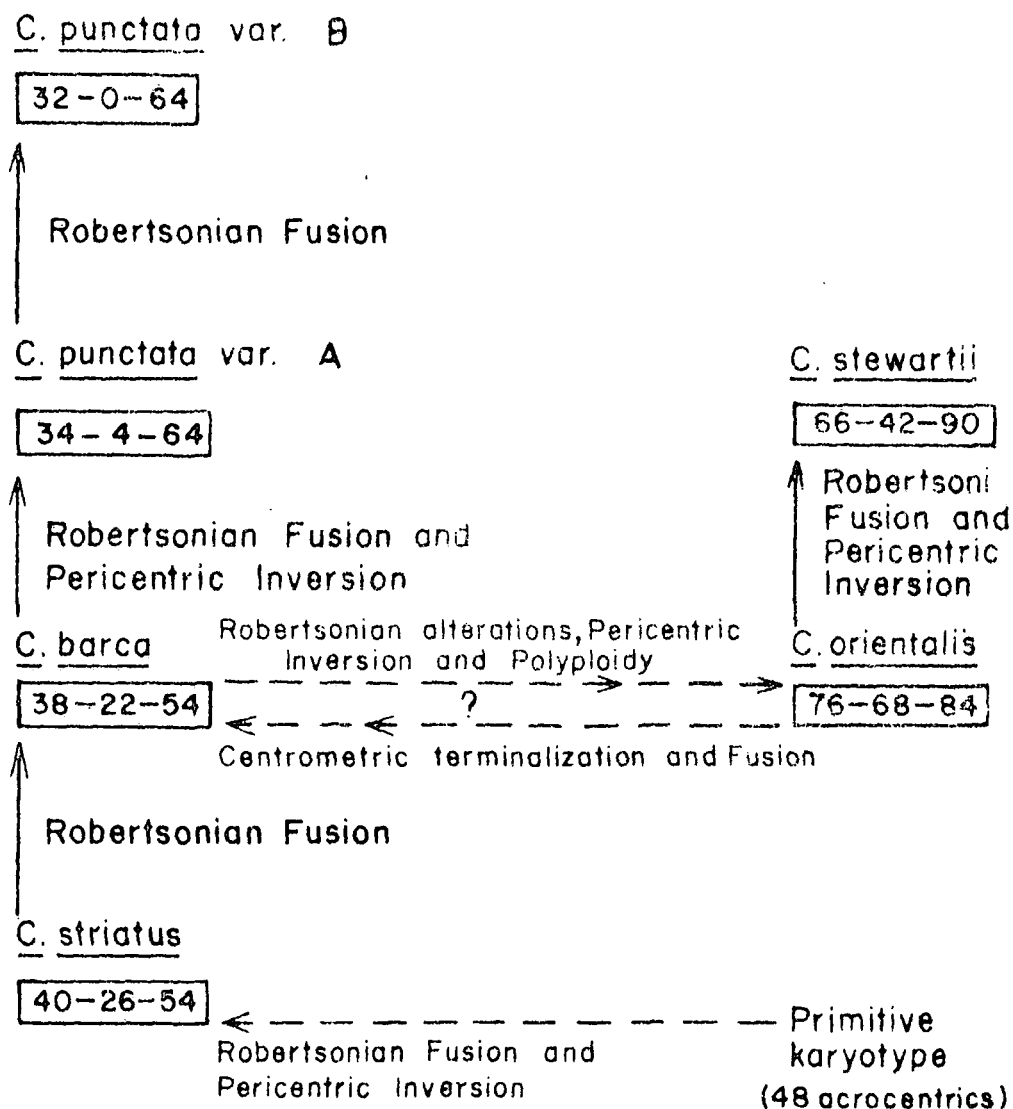


Fig. 3 Hypothetical karyotype evolution among different *Channa* species. The first number from the left, inside the box, represents the diploid chromosome number, the middle number represents the number of acrocentric and the last number represents the NF value.

CHAPTER-III
ELECTROPHORETIC INVESTIGATION

1. INTRODUCTION

Due to the existence of morphological plasticity among individuals, conventional morphological characters are often found to be insufficient to give the exact data to resolve species problem. On such cases, biochemical and physiological approaches are found to be potentially more useful adjustment to the conventional taxonomic criteria.

The exploitation of biochemical events in terms of taxonomy mainly depends upon the presence of enormous specificity of macromolecules and metabolic processes at every taxonomic level which are basically same in the lowest and highest organisms. Moreover, numerous fundamental questions concerning the species-structure and process of speciation seem capable of being answered by studies concentrated at the molecular level.

Differences in the morphological features between and within a species are greatly influenced by different environmental factors (Barlow 1961). It is therefore, presumed that such phenotypic differences may be reflected in their physiological and biochemical processes. The sequential events of biochemical changes have been found to be controlled by differential activity of appropriate genes. Through the process of genetic transcription and translation, new proteins with specific genetic information from DNA molecule are synthesized. These new proteins are either structural proteins or functional proteins. The functional proteins are nothing but enzymes. In other words, it can be said that enzymes being proteins, are the products of genes. So alteration in the enzyme activity indicates the alteration of the activity of corresponding gene (Markert and Ursprung 1974).

It is well established that the metabolic pathways are multistep processes, each step is catalysed by a particular functional protein or enzyme. It has been further observed that each cell type and tissue, contributing to a specific organ, contains a specific set of enzymes. The presence of a specific enzyme (i.e. functional protein) in a particular organ of an organism enables the organ to perform a specific function. Thus, it is expected that the morphological adaptations to environmental fluctuations are closely related to the biochemical adaptations through changes in the functional proteins. It has been observed that the relationships between certain enzyme and morphological differentiation is so close that often the appearance or disappearance of a specific enzyme is considered as a 'biochemical marker' (Scholl and Anders 1973, Brody et al. 1976, Reinitz 1977).

The variation in the primary structure of proteins, leading to the surface change can be detected by electrophoresis and this technique has been found to be useful in studying problems involving different taxonomic ranks from relationships between orders to relationship between species. In this aspects, 'Polyacrylamide gel electrophoresis (PAGE)' is found to be more advantageous than other existing electrophoretic techniques as it gives a better resolution of separated proteins and ^{is the} most economic as well as easy to carry out. The study of electrophoretic pattern of enzymes and other proteins reveal the overall genic variation and can be used to examine the genetic relationship between two or more forms. Moreover, electrophoresis is capable of revealing a large portion of allelic variation at a specific locus, making it possible to calculate the frequency of specific alleles in different populations (Case et al. 1975).

Studies on proteins with reference to fish groups are so enormous that any attempt to review all the literatures will always remain inadequate. However, it may be noted that a negligible percentage of these works has been devoted to taxonomic problems (O'Rourke 1974). A very comprehensive review is presented here which could be taken as a representative one, mostly dealing with investigations on tissue and serum proteins, esterases and lactate dehydrogenase (LDH) with particular reference to the genetics and systematics of the fish concerned. Though the first attempt on such investigation can be dated back to Nuttal's (1904) work on comparative serological study on certain fish species, the next fifty years almost drew a blank until Connell (1953) reported the pattern of the skeletal muscle proteins of codling by electrophoretic technique. Lillevik and Schelomer (1961) have studied the species differentiation in fish by electrophoretic analysis of skeletal muscle proteins. Booke (1964) has reviewed the variations found in fish serum proteins in relation to species specificity, phylogeny and environmental conditions. Sanderz (1964) has electrophoretically investigated the serum proteins in three trout species and their hybrids within the family Salmonidae. Markert and Faulhaber (1965) have investigated the lactate dehydrogenase isoenzyme patterns in 30 fish species and observed that all these species have one major isoenzyme system with two minor ones (in eye and gonad) and on the basis of this they grouped these fishes into four categories. Nyman (1965, 1966) has investigated the species specific proteins in fishes along with intra- and inter-specific variations found in them and suggested their usefulness in biochemical systematics. Shaw (1965) has reviewed the variations

found in different enzyme systems through electrophoretic technique and commented on their significance in biological sciences and research. Nyman (1967) has reviewed the protein variation found in salmonidae. Tsuyuki et al. (1968) have studied the contribution of protein electrophoresis to rockfish systematics.

Chen and Tsuyuki (1970) has investigated the protein electropherograms of Tilapia mossambica and T. melanoptera. Clayton and Franzin (1970) have studied the muscle lactate dehydrogenase isoenzymes of lake whitefish (Coregonus clupeaformis) which provided an additional evidence for the probable tetraploid nature of salmonid fishes. Polymorphisms of lactate dehydrogenase and glycerol 3-phosphate dehydrogenase isoenzymes in mergim populations from English channel and approaches, have been studied by Dando (1970). Holmes and Whitt (1970) have investigated the esterase isoenzymes of Fundulus heteroclitus during developmental stages. Lindsay et al. (1970) have investigated the presence of protein variations in different sympatric populations of Coregonus clupeaformis and described the possibility of speciation under selective pressure. Interspecific differentiations of serum proteins in the blood of black sea fish (mullet) have been investigated by Senkevich and Kulikova (1970). The presence of LDH E₄ isoenzyme in addition to the usual A₄ and B₄ isoenzymes in Xiphophorus helleri eye have been studied by Whitt and Booth (1970) and it has been suggested that probably it plays an important role in the biochemistry of vision.

Dando (1971) has studied the lactate dehydrogenase polymorphism in flatfish (Heterostomata) where species-specific isoenzymes were detected in heart and muscle extracts, with individual

variations in certain species. Adaptive lactate dehydrogenase variations in the geographic populations of the crested Blenny Anoplarchus have been reported by Johnson (1971).

Vrijenhoek (1972) has revealed genetic relationship of hybrids to their progenitors using LDH isoenzymes as gene markers in Pociliopsis. The presence of mono and polymorphic protein loci in the population of tetraploid salmon species Oncorhynchus keta has been reported by Altukhov et al. (1972). The occurrence of centric fusion and trisomy for the LDH-B locus in brown trout Salvelinus fontinalis have been studied by Davisson et al. (1972). Thirtyone species from the family Scorpaenidae have been electrophoretically investigated for six enzyme systems by Johnson et al. (1972) which could be used as chemotaxonomic parameters. The biochemical genetics of the atlantic salmon Salmo salar have been discussed from the point of significance in population identifications.

Baron (1973) has investigated the serum proteins in Sardinella aurita. Davisson et al. (1973) have analysed the pseudolinkage of LDH loci in the teleost genus Salvelinus by doing repeated hybridization at inter specific level. Genetic polymorphisms of LDH isoenzymes in the carp Cyprinus carpio have been studied by Engel et al. (1973). Hamoir et al. (1973) have discussed the muscle protein patterns of coelocanth Latimeria chalumnae. Johnson et al. (1973) have investigated the genetic polymorphisms and heterozygosity in three species of rock fish genus Sebastes. Kirpichnikov (1973) has investigated the biochemical polymorphism and microevolution in fishes. Scholl and Anders (1973) have investigated the electrophoretic variations of enzyme proteins in

platyfish and sword-tails (Poeciliidae). Scholl (1973) has compared a randomly selected sample of loci (including those for LDH) in Xiphophorine species to comment on the extent of biochemical evolution in this fish genus. Truveller et al. (1973) have studied the proteins in herring Clupea harengus by electrophoretic methods. Whitt et al. (1973) have studied the developmental and biochemical genetics of lactate dehydrogenase isoenzymes in fish.

O'Rourke (1974) has extensively reviewed the biochemical and immunological investigations done on fish groups and discussed their implications as taxonomical tools. Truveller et al. (1974) have investigated the distribution of esterases in Cyprinus carpio by using polyacrylamide gel electrophoresis. Tsai and Yang (1974) have studied the serum and muscle proteins of Blennioid fishes from Taiwan. Yoneda and Ishihaka (1974) have studied the blood serum proteins from Chum salmon Oncorhynchus keta and cherry salmon O. masou by using disc electrophoresis.

Basasibwaki (1975) has compared the electrophoretic patterns of lactate dehydrogenase and malate dehydrogenase isoenzymes in five lake victoria cichlid species. Bastos et al. (1975) have investigated the electrophoretic patterns of muscle proteins of the fish genus Lutjanus. Noire Mester and Tesio (1975) have studied certain Blennidae electrophoretically with special consideration to systematics.

Allendorf et al. (1976) have studied the genetic variation in Scandinavian brown trout Salmo trutta and found distinct sympatric populations among them. Brassington and Ferguson (1976) have studied the LDH and esterase enzyme systems in roach and rudd and

in their natural hybrids and investigated their utility in stock identification. Brody et al. (1976) have described the genetic control of three protein markers in carp populations and their implication in breeding have been discussed. Champion and Whitt (1976) have investigated the different gene expression in multi-locus isoenzymes of the developing green sunfish. Dzhabarov (1976) has performed an electrophoretic study of the blood serum proteins of Chalcalburnes chalcoides and Lucioperca lucioperca. Moav et al. (1976) have discussed the advantages and methods for application of electrophoretic genetic markers to fish breeding. Reshetnikov (1976) has discussed the electrophoretic method and its applications to systematize the fish group Salmonidae. Yardley and Hubbs (1976) have electrophoretically investigated two species of Mosquito fish with notes on genetic subdivision.

Ahuja et al. (1977) have investigated the tissue specific esterases in the Xiphophorin fish Platypoecilus maculatus, Xiphophorus helleri and their hybrids. Alexander (1977) has studied the protein concentration in the serum of the Atlantic salmon from Northwest England and Northern Ireland. Allendorf et al. (1977) have detected the isoenzyme loci in brown trout and discussed the findings from population levels. Child (1977) has studied the biochemical polymorphism in char. Franzin and Clayton (1977) have studied different enzyme systems in various geographic populations of Uncerhynchus clupeaformis and discussed the evolutionary patterns of speciation. Association of alloenzymes and temperature in crested Blenny Anoplarchus purpurescens has been investigated by Johnson (1977). Leslie and Vrijenhoek (1977) have analyzed the natural populations of Poeciliopsis monacha with reference to

alloenzyme inheritance and patterns of mating. Markert (1977) has reviewed the applications of isoenzyme studies in biological and medical research. Menzel (1977) has worked out on morphological and electrophoretic identifications of a hybrid cyprinid fish (Notropis atherinoides X N.c. cornutus) with a discussion on implications of the study on evolution of Notropis albeolus. Reinitz (1977a) has investigated the inheritance of muscle and liver types of supernatant NADP dependent isocitrate dehydrogenase in rainbow trout Salmo gairdneri along with a test for association of transferring and lactate dehydrogenase phenotypes with weight gain. He (1977b,c) has also studied thirteen protein systems in rainbow trout and cutthroat trout and their hybrids and found that only phosphoglucose isomerase could be used for the distinction of the two species studied. Valenta et al. (1977) have investigated the genetic polymorphisms and isoenzyme patterns of lactate dehydrogenase in tench (Tinca tinca), crucian carp (Carassius carassius) and common carp (Cyprinus carpio).

Braune (1978) has studied the immunological properties of lactate dehydrogenase isoenzyme in trout. Buth and Burr (1978) have investigated the isoenzyme variability in the cyprinid genus Compostoma. Cross (1978) has investigated four isoenzymes in tissues of interspecific hybrids of the fish family Cyprinidae. Herzberg (1978) has studied esterase patterns of the surface mucus of Tilapia species and suggested their implications in identification. Kimura (1978a) has investigated the protein polymorphism and genetic variation in the populations of the loach Cobitis delicata. Electrophoretic variations of enzymatic and nonenzymatic proteins were also investigated in eight populations of Misgurnus

angiollecaudatus by him (1978b) from the point of protein polymorphism and geographic variations. Nefedov et al. (1978) has investigated the muscle esterase polymorphism in horse mackerel of the Northeast Atlantic. Paulov and Hensel (1978) has described the protein spectra in the skin, swim-bladder and serum of few members of cyprinid fishes. The genetic basis of protein (LDH, MDH, GPI and PGM enzyme systems) polymorphisms in Fundulus heteroclitus have been investigated by Place and Power (1978). Ribeiro and Toledo (1978) have studied the lactate dehydrogenase isoenzyme in neotropical fishes. Genetic variability of proteins in six species belonging to diploid and tetraploid forms from the order Salmoniformes has been investigated for different populations by Salmenkova and Omelchenko (1978). Shami and Bearbmore (1978) have studied the enzyme variation found in the guppy Poecilia reticulata. A biochemical method basing on enzyme variability has been suggested by Sidell et al. (1978) to distinguish striped bass and white perch larvae. Taniguchi and Tashima (1978) have studied the genetic variation of liver esterases in red-sea bream. Valenta (1978) has investigated the polymorphism of A, B and C loci of lactate dehydrogenase isoenzyme in European fish species belonging to twentyone genera of Cyprinidae. Wiseman et al. (1978) has given an electrophoretic evidence for subspecific differentiation and intergradation in Etheostoma spectabile.

Diebig et al. (1979) have investigated the biochemical polymorphism in muscle and liver extracts and in the serum of the rainbow trout Salmo gairdneri. Brody et al. (1979) have compared the Chinese and European races of common carp by studying thirty-three isoenzyme loci. Buth (1979a,b,c) has investigated the isoenzyme

variability in a number of Cypriniformes fishes with reference to the genetics and biochemical systematics. Fujio and Kato (1979) have carried out starch gel electrophoresis for fifteen enzymes in 41 species and discussed the genetic variations found in different populations. Hadfield et al. (1979) have studied the clinal variation in electrophoretic and morphological characters between two nominal species of the genus Pseudomugil. Kijima and Fujio (1979) have investigated the geographical distribution of IDH and LDH isoenzymes in chum salmon populations. Klar and Statnaker (1979) have investigated the variation found in muscle lactate dehydrogenase in snake valley cutthroat trout Salmo clarki subspecies. Kornfield et al. (1979) have studied the biochemical and cytological differences found in cichlid fishes of the sea of Galilee. Fish species identification by using thin layer isoelectric focussing has been enumerated by Lundstrom (1979). Morizot and Siciliano (1979) have studied the polymorphisms, linkage and mapping of four enzyme loci in the fish genus Xiphophorus. Nagai and Sadaaki (1979) have distinguished the Japanese, American and European eel by studying the isoenzyme patterns in them. Philipp et al. (1979) have investigated 33 loci of enzymes and isoenzymes for two closely related species of fish Micropterus salmoides salmoides and M. dolomieu and compared the evolutionary pattern of differential gene expression. Lopes et al. (1979) have given a phylogenetic interpretation of chromosomal and electrophoretic data obtained from Columbiformes. Saseaman and Yoshiyama (1979) investigated the geographic variation of lactate dehydrogenase isoenzymes in Anoplurachus purpureus. Starmach (1979) has done electrophoretic separation of blood serum lactate dehydrogenase, transferrin and

esterases on polyacrylamide gel in seven carp breed lines. Enzyme polymorphisms in carp Cyprinus carpio of Rupsha breed have been discussed from genetic point of view by Tiit (1979). Polymorphic proteins in carps (C. carpio) and in tinch (Tinca tinca) have been investigated by Valenta et al. (1979).

Busack et al. (1980) have investigated the Eagle lake trout morphologically, cytologically and biochemically and hypothesized that Eagle lake trout has originated from rainbow trout, either by immigration or introduction of the latter to that particular locality. Tetraploid catostomid genus Hypentelium has been subjected to electrophoretic analysis of about 40 presumptive isoenzyme loci by Buth (1980) who discussed the findings from the point of evolution and provided a biochemical key for identification. Buth et al. (1980) have investigated 23 presumptive enzyme loci in ten populations of the percid sub-genus Microperca and discussed their relationships and differences among all the members of the sub-genus. Comparini and Robino (1980) have reported the existences of two species of Anguilla leptocephali as confirmed by electrophoretic studies. Studies on isoenzyme patterns in armoured catfishes by Dunham et al. (1980) have shown the levels of duplicate gene expression in them. Ferris and Whitt (1980) have studied the genetic variability in species with extensive gene duplication in tetraploid catostomid fishes. Fischer et al. (1980) have enumerated the evolution of five multiple isoenzyme systems in chordates. Grant et al. (1980) have used biochemical genetic variants for identification of sockeye salmon Oncorhynchus nerka stock in Cook Inlet, Alaska. Grant and Utter (1980) have studied the tissue enzyme variations in Theragra chalcogramma from the point of population structure and

stock identification. Turner et al. (1980a) have investigated differential gene duplication in a Mexican fish (genus Skiffia). Sakaizumi et al. (1980) have described the alloenzyme variation in wild populations of the fish Oryzias latipes. Evolutionary genetics of a gynogenetic fish Poecilia formosa have been studied electrophoretically by Turner et al. (1980b). Winans (1980) has studied the geographic variation in the milk-fish Chanos chanos with biochemical evidences.

Review of literature reveals that works on biochemical genetics of Indian fishes are very scanty in comparisons to the works done in the rest of the world. However, Chandrasekhar (1959) has studied the blood proteins of five Indian carps belonging to the family Cyprinidae. Das (1961) has investigated the blood biochemistry for three Indian carps. A comparative study on the tissue proteins of some catfishes has been carried out by Hussain and Siddique (1974). Menezes (1975) has investigated the eye lens and serum proteins of Sardinella sardinella longiceps by using electrophoretic techniques. Krishnaja and Rege (1977, 1979) have made electrophoretic studies on the genetics of two species of Indian carp and their fertile hybrids. Basu et al. (1981) have studied the egg proteins in Notopterus notopterus and Mystus vittatus. Dhar and Chatterjee (1982) have made electrophoretic investigations on the protein variations in two Channa species.

It is amply clear from the foregoing review that although the fish fauna is very rich in India, not a very large number of species/genus/family has been subjected to biochemical investigations on taxonomic problems. The main purpose of this study is to

determine the amount of variation within and between the different Channa species so as to unravel their relationship. Subsequently, we have used electrophoretic estimates of soluble proteins, esterases and LDH enzymes as an independent tool for determining the genetic characteristics of the various members under this wide-ranging genus.

2. MATERIALS AND METHODS

2.1. The Fishes:

In the present investigation, five species of Channa, viz. C. striatus, C. barca, C. punctata, C. stewartii and C. orientalis have been employed. The fishes were collected from and around Gauhati (Assam) and Shillong (Meghalaya). To avoid ontogenic problems, fishes were collected during the same season and from almost the same environment. Only the adult fishes were used for the study. Fishes were brought to the laboratory in live condition and acclimatized in a well aerated aquarium for atleast seven days, and then subjected to investigations.

2.2. Polyacrylamide-Gel Electrophoresis:

Polyacrylamide gel electrophoresis of serum, soluble tissue proteins and two enzyme systems viz. Esterases and LDH of the different fish species was performed according to Davis (1964) with suitable modifications whenever necessary.

2.2.1. Preparation of tissue homogenates:

The different tissues employed were brain, heart, liver, kidney, muscle and eye besides the blood serum. Tissue homogenates were prepared as follows: as soon as the fishes were sacrificed, the particular tissues were immediately removed from the animal and placed in a cold isotonic solution, (NaCl, 0.15M). After a couple of minutes, the tissue was blotted and weighed accurately and placed in a glass homogenizer, containing measured volume of cold homogenization medium (0.25M Sucrose solution). The tissue were homogenized carefully in the medium keeping the tubes in cold ice-mass so that no denaturation occurs. Generally 10%, 5% or 2.5% homogenates were prepared depending on the maximum weight of the tissue taken.

2.2.2. The Extractions:

The structural elements were removed from the homogenates by differential centrifugation. Tissue extracts were obtained by centrifuging the homogenates at 15,000 x g for 15 minutes at 4°C. The resulting clear supernatant contained the soluble protein. The residue was discarded. The clear supernatant was subjected for further analysis.

For separation of serum, blood samples were collected in clean glass centrifuge tubes after cutting the caudal region of the fish and allowed to stand for one hour at around 37°C for clotting. The samples were then centrifuged at 2,500 rpm. for 15 minutes. The clear sera, thus obtained were used for subsequent analysis (Chandrasekhar 1959).

2.2.3. Electrophoresis:

When particles of effective charge (Q) is forced to migrate in a viscous medium (liquid or gel) by action of an electric field (potential gradient, E.), the phenomenon is generally called as Electrophoresis (Maurer 1971). The driving force which acts upon the particle migrating with constant velocity is equal to the frictional resistance (F) which the particle must overcome in the medium, i.e.

$$QE = F$$

The electrophoretic mobility of a particle is defined as

$$m = \frac{d}{tE} = \frac{V}{E} = \frac{Q}{F} = \left(\frac{Cm^2}{\text{Volt. X Sec.}} \right)$$

where 'd' is the migration distance of the particle in time 't'. V is the velocity and 'F' is the frictional resistance.

In disc electrophoresis, a discontinuous separating system is used with regards to pH value, buffer composition and gel pore-size in which Polyacrylamide gel serves as the matrix.

Disc electrophoresis is carried out with small columns of polyacrylamide gel consisting of three layers, in suitable container like cylindrical tubes. The three layers are- (i) a large pore spacer or staking gel, (ii) a small pore separation or running gel in which the sample constituents are separated and (iii) a large pore sample gel containing the sample solution. Electrophoresis is performed with a vertical column of gels attached to two different reservoirs- sample gel uppermost, attached to an upper reservoir and the lower end submerged in the buffer solution of the lower reservoir. Electrodes are placed in each reservoir and polarity is set so that the sample ions migrate towards the small pore gel. A voltage is applied for a specific time. The gel is then removed from the container and placed for a period of time in a solution of protein fixative and stained. Unbound dye is removed from the gel slowly by washing in 7% acetic acid and then the gel is preserved in a suitable solution.

2.2.3.1. Reagents:

Stock solutions : These solutions ^{are} stored in brown bottles in a refrigerator and their shelf life are upto several months.

Stock A (pH 8.9) 1N.HCL : 48 ml	: The volume is made upto 100 ml. with distilled water.
Tris (hydroxymethyl) methylamine : 36.6 gm	
N, N, N, N - Tetramethyl-Ethylenediamine : 0.23 ml	
(TEMED)	

Stock B (pH 6.7) 1N.HCL : 48 ml | The volume is made
 TRIS : 5.98 gm | upto 100 ml with
 TEMED : 0.46 ml . | distilled water.

Stock C Acrylamide : 30 gm | The volume is made
 NN-Methylenebis- | upto 100 ml with
 acrylamide (BIS) : 0.8 gm | distilled water.

Stock D Acrylamide : 10 gm | The volume is made
 BIS : 2.5 gm | upto 100 ml with
 | distilled water.

Stock E Riboflavin : 4.0 mg
 Water (dist.) : 100 ml

Stock F Sucrose : 40 gm
 Water (dist.) : Volume is made upto 100 ml
 with distilled water.

Stock G Ammonium Per-
 sulphate : 0.14 gm
 Water (dist.) : Volume is made upto 100 ml
 with distilled water.

1M. Stock Buffer Solution (pH 8.3) TRIS : 6 gm
 Glycine : 28.8 gm

For reservoirs, a 10% strength is used, out of this stock solution. Water : Volume is made upto 1000 ml with distilled water.

Washing solution for destaining and storing. Acetic Acid : 70 ml
 Water : Volume is made upto 1000 ml with distilled water (7% Sol.).

Fixative Stain Solution (for protein) : 1% Coomassie Brilliant blue of 0.5% Amino Black is prepared in 7% Acetic Acid Solution.

Sometimes 12% TCA was used as protein fixative prior to the staining and 1% Bromophenol blue was also used as indicator solution.

2.2.3.2. Equipments used:

The size of the cylindrical gel tubes used were about 10 cm. in length with an inner diameter of 5 mm. These tubes were fixed vertically in the electrophoresis running chamber, consisting primarily of upper and lower chamber and with platinum electrodes in each chamber. The upper and lower chambers were the buffer reservoirs. Arrangements were there to hold the gel tubes vertically in the upper reservoir and to make a link with the lower reservoir. The additional equipments comprise of electrodes, cables and a power supply (Systronic 604), gel tube racks for loading, rubber caps for closing one end of the gel tube and polymerizing lamps, needle and syringe, test-tubes etc.

2.2.3.3. Gel Systems and their composition:

1. Separation Gel System : It was prepared always just before use by mixing the stock solution in the following proportions (mixing ratio V/V) :

Stock A	- 1 part
Stock C	- 2 parts
Water (dist.)	- 1 part
Stock G	- 4 parts

2. Spacer Gel System : It was prepared by mixing the stock solutions in the following proportions just before use as follows:

Stock B	⊖ 1 part
Stock D	- 2 parts
Stock E	- 1 part
Stock F	- 4 parts

2.2.3.4. Procedure:

About 1 ml. to 1.5 ml. (upto 7 cm. in length of the gel tubes) of the separation gel system was poured into the gel tubes which were capped at the bottom. Care was taken not to form any air bubble in the gel column. A few drops of distilled water were carefully layered above the separation gel system to provide a plain gel surface. The gel tubes were then left undisturbed for about 2-3 hours at room temperature for polymerization of the separation gel system. After polymerization, the water from the top of the gel column were removed carefully and about 0.2 ml. of the spacer gel system was poured carefully into the gel tubes (about 1 cm. length of the gel tube was covered). A few drops of water were again layered above this gel system and the gel tubes were left under fluorescence light for 30-45 minutes to polymerize.

The gel tubes were then fixed vertically as described by Davis (1964) in the disc electrophoresis chamber. The upper and lower chambers of the apparatus were filled with 1M Tris-Glycine buffer (pH 8.3). About 0.1 ml. of the test solution was directly poured over the spacer gel system after removing the water layer. The remaining vacant part of the gel-tube was filled with the buffer, used for the chambers. Then both the upper and lower chambers were filled with buffer completely. One drop of 1% Bromophenol blue indicator solution was mixed in the upper buffer chamber. After all these preparations, the apparatus was placed at 4-5°C (inside a refrigerator) and the two electrodes were connected with the electrophoresis power supply. A current strength of 1.5 mA/gel-tube was passed through for a period of 10 minutes. Then the current strength was raised upto 3 mA/gel-tube. When the bromophenol blue

indicator ring had reached the bottom of the gel-tube, the current supply was stopped and the gel-tubes were removed from the upper chamber. With the help of a long needle and water force, the gels from the gel-tubes were removed carefully and immediately subjected to different treatments to obtain different banding patterns.

2.2.4. Visualization of serum and soluble tissue proteins:

For protein bands, the gels were immediately transferred to 12% TCA solution and kept there for about half an hour for protein fixation. Then these were removed to staining solutions (Amido Black or Coomassie brilliant blue) and allowed to stand for about 15-20 minutes, depending upon the concentration of the staining solutions. After removing from the stain, gels were washed in distilled water and stored in 7% Acetic acid solution for destaining. By changing the acetic acid solution for several times (until the bands became clear), destaining was performed and then they were stored in 7% acetic acid solution permanently.

The visible bands were counted and categorised as dark, medium and light bands depending upon the intensity of the staining. Electrophoretic mobility or RF values were determined for each bands. Photographs were taken and banding patterns and gels were drawn for diagrammatic representation. Numbering of the bands for protein is done from cathode to anode (No. 1 being the least migrated band). But for esterases, it is done just in reverse way, the farthest migrated band (towards anode) is designated as band number one. In case of LDH, it is same as esterases.

2.2.5. Separation of Isoenzymes:

Since isoenzymes of an enzyme system are protein of the same

configuration but with slight differences in the molecular weight and electrophoretic mobility, the above mentioned separation technique can be used for their detection. In this case, the gels were treated with specific stain mixtures for specific enzyme system with incubation period. However, in certain steps, certain modifications were made to get proper bands. Fixation was done with 7% Acetic Acid Solution.

A. Lactate dehydrogenase (LDH)

For lactate dehydrogenase isoenzyme study, the above mentioned technique was used for separation. Only instead of indicator solution, the running time (about 2 hours) was considered. The staining solution was prepared (for 6 gel-tubes) as follows :-

1M Tris-HCL (pH 8.0)	- 2.5 ml.
1N Lithium lactate Std. Solution	- 0.5 ml.
NAD (β-Nicotinamide adenine dinucleotide)	- 80 mg.
PMS (Phenazine methosulphate)	- 1.2 mg.
NBT (p-Nitroblue tetrazolium chloride)	- 8.0 mg.

Then the whole mixture was diluted with distilled water and the volume was made upto 50 ml. After the running, the gels were directly placed in the above solution mixture and then incubated at 37°C for 15 minutes. LDH bands were violet in colour.

B. Esterases

In the case of esterases also the same technique was used, only the gel running time was about 90 minutes. Esterases were studied by using α-naphthylacetate as the substrate. The staining solution was prepared (for 6 gel-tubes) as follows :-

Substrate = 0.5 gm. of α -naphthylacetate
 25 ml. of Acetone
 25 ml. of Water (distilled)

Solutions = 50 mg. Fast blue R R salt
 2.5 ml. of 1M Tris-HCL Solution (pH 7.0)
 1.5 ml. of Substrate

In the above mixture, distilled water was added and the volume was raised to 50 ml. After placing the gels in this staining solution, they were incubated at 37°C for about 15 minutes. The esterases bands appeared as brickish colour rings.

The number of bands for both the cases were determined and the electrophoretic mobility for each band was calculated out and studied properly. Photographs were taken and diagrams were also made.

2.2.6. Inhibition studies of Isoenzymes:

Both Lactate dehydrogenase isoenzymes and Esterases were subjected to different inhibition studies, using a number of inhibitor. The different inhibitors used were as follows :-

For LDH = (i) Heating upto 55°-65°C

For Esterases (i) Eserine sulphate - $10^{-3}M$ to $10^{-5}M$ Soln.
 (ii) Diisopropylfluorophosphate - 10^{-3} to $10^{-5}M$
 (iii) Cupric Sulphate - $10^{-3}M$ Solution
 (iv) Urea - 10M Solution

(c)

Procedure: (1) During inhibition studies, the gels were allowed to stand in the usual staining buffer which also contained the inhibitor with the required strength at room temperature for about 30 minutes. In case of LDH, gels were directly incubated at 37°C for 15 minutes.

For esterases, after the first step, the gels were stained by the usual procedure, only the stain contained the substrate which was not applied in the first step. Incubation time was 15 minutes at 37°C.

For heat inactivation, the extracts were placed in test tubes and subjected to heating as mentioned for an hour or so. Then they were cooled, centrifuged and subjected to electrophoresis and finally staining was done as usual.

These gels were subjected to densitometric analysis with a spectrophotometer (Beckman) for exact correlation of the bands.

2.3. Quantitative Estimation of Proteins:

Quantitative estimation of protein is necessary so that a known amount of protein can be loaded from the sample of different tissues for electrophoresis. Protein was determined according to the procedure of Lowry et al. (1951).

Principle: Proteins when react with Folin Ciocalteu (Phenol) reagent, form a blue colour, the intensity of which at 750 nm. is proportional to the amount of proteins present in a sample. The final colour reactions are the bi-uret reaction of phosphomolybdic phosphotungstic reagent by tyrosine and tryptophan present in the treated protein.

Reagents : Stock A : 1% CuSO_4 solution
 Stock B : 2% K-Na-tartrate
 Stock C : 2% Na_2CO_3 in 0.1N. NaOH.

0.5 ml of the stock A was mixed with 0.5 ml of Stock B and

the volume was made upto 50 ml. with stock C, just before use and this was treated as alkaline solution. Folin-Ciocalteau reagent was prepared by diluting this with equal amount of distilled water just before use (this made it 1N in acid).

Procedure: 5 ml of alkaline solution was added to 1 ml. of test solution. They were mixed thoroughly and allowed to stand at room temperature for 10 minutes. 0.5 ml. of distilled F-C-reagent was added rapidly with immediate mixing. After 30 minutes, read the extinction against appropriate blank at 750 nm. in a spectrophotometer (Beckman). Protein concentration was determined after preparing a standard curve, using Bovine serum albumen.

For convenience, during experiment at least three amounts of sample with different dilutions were used. These are as follows :-

0.2 ml. of sample diluted with 0.3 ml. of dist. water.

0.1 ml. of sample diluted with 0.4 ml. of dist. water.

0.01ml. of sample diluted with 0.49 ml. of dist. water.

3. RESULTS

3.1. Cytoplasmic soluble proteins:

3.1.1. Channa striatus :

The total number of bands in brain, heart, liver, kidney, muscle, eye and serum extracts have been found to be 7, 11, 9, 10, 10, 14 and 13 respectively (Fig.1; Plate I). In brain, band numbers 2 and 6 have been found to be dark, band number 4 is medium and the rest are found to be light in staining intensity: in heart, band numbers 1, 2, 3 and 6 are found to be as dark bands while the rest could be categorised under light bands. In liver also, only two categories of bands have been observed, band numbers 5 and 6 as dark bands, while the rest are light bands. Similarly in kidney, band numbers 3 and 6 have been found to be comparatively darker than the rest of the bands. In muscle, most of the bands were categorised as dark bands except band numbers 2, 4, 7 and 9. In eye, band numbers 3, 7 and 10 are categorised as dark bands and the rest are of light category. In serum, except band numbers 5, 6, 11 and 13, all the visible bands are categorised as dark bands. It has been observed that though certain tissues exhibited equal numbers of bands, the distribution and patterns have been found to be distinct from each other. On the other hand, certain bands in different tissues have shown similar mobilities. Such bands are considered as homologous bands (Fig. 1).

3.1.2. Channa barca :

The total number of soluble protein bands in brain, heart, liver, kidney, eye, muscle and serum extracts have been represented by 12, 13, 7, 15, 12, 12 and 11 bands respectively (Fig.2; Plate II). In brain, band numbers 5, 9, 10 and 11 could be designated as dark bands while in heart extract, band numbers 3, 4, 6 and 10 are dark

enough. But in liver extract, only band number 5 has been found to be of dark category. In kidney extract, band numbers 2, 6, 7 and 9 could be considered as dark bands while in eye extract, band numbers 2, 5 and 7 are dark in intensity. In muscle extract, band numbers 1, 4, 6, 8 and 11 are categorised as dark bands while in serum 8 bands (i.e. number 1, 2, 4, 5, 7, 8 and 10) have been found to be dark in intensity. The rest of the bands in all the tissues are either light or medium. Similar to the previous observation in C. striatus, here also it has been observed that some of the tissues possess same number of bands but their distribution and patterns are totally tissue-specific. However, few homologous bands have also been observed in different tissues (Fig. 2).

3.1.3. Channa punctata :

Channa punctata shows the presence of the maximum number of bands of soluble proteins in all the tissues. Their total number in brain, heart, liver, kidney, eye, muscle and serum have been found to be 10, 17, 13, 14, 12, 7 and 15 respectively (Fig.3; Plate III). In brain, band numbers 4, 7 and 10, in heart band numbers 1, 4, 6 and 9, in liver band numbers 1, 4, 5 and 8, in kidney band numbers 4, 7 and 10, in eye band numbers 1, 3, 5, 6, 9 and 11 and in serum band numbers 1, 3, 7-12 and 15 have been categorised as dark bands. In muscle, except band number 6, all other bands are found to be dark in intensity. The distribution and patterns of bands in different tissues are found to be distinct from one another though certain homologous bands are also observed in different tissues.

3.1.4. Channa stewartii :

The total number of protein bands in brain, heart, liver,

kidney, muscle and eye extracts have been found to be 13, 10, 11, 9, 8 and 11 respectively (Fig.4; Plate IV). In brain, band numbers 11 and 13 are dark, band numbers 3, 4, 8 and 10 are of medium category and the rest are light in intensity. In heart, band number 3 is dark in intensity, band numbers 7 and 8 are medium bands and the rest are light bands. In liver, no dark bands could be detected. However, band numbers 5, 6, 7 and 9 are of medium type. In kidney, band number 5 is a dark band, while band numbers 4, 6, and 7 are medium bands and the rest are light bands. In muscle, band numbers 5 and 4 are considerably dark while band number 7 is of medium type and the rest are light bands. In eye, band numbers 1, 4, 8 and 11 are of medium category and the rest are light in intensity. Here also some homologous bands have been found among different tissue proteins. In certain tissues (heart and eye) the total number of bands are found to be the same, but the distribution and patterns are quite distinct from each other (Fig. 4).

3.1.5. Channa orientalis :

The total number of protein bands found in brain, heart, liver, kidney, muscle and eye are 10, 13, 12, 14, 11 and 12 respectively (Fig.5; Plate V). In brain extract, three dark bands (1, 8 and 10) and two medium bands (3 and 5) have been observed. In heart, band number 7 is comparatively darker than the rest while band numbers 2, 4, 9 and 11 could be categorised as medium bands. In liver, band number 6 is found to be dark while band numbers 4 and 9 are of medium category. In kidney extract, band numbers 6 and 11 are dark type while band numbers 5 and 14 are of medium type. In muscle extract, band numbers 2, 4, 7, 9 and 11 are of

dark type while in eye extract, band number 4 is of dark type and band numbers 1, 2, 6 and 7 are of medium category. The rest of the bands in different tissues can be categorised under light type. The distribution and patterns of bands in different tissues are found to be tissue-specific though certain bands have shown similar mobilities in different tissues.

3.2. Esterases :

In the present investigation, the maximum number of esterase activity zone is found to be eight which can be resolved to the maximum number of 10 bands. The esterase zones are numbered Est.1 to Est.8 according to their relative mobilities, the most anodal zone being referred to as Est.1 and the most cathodal zone as Est.8. An identical pattern of bands have been found in both the sexes within a species. All the esterases are probably polymeric since more than one allelic variants are observed in each zone. The esterases are classified according to Holmes and Masters (1967). The details of the distribution and pattern of esterases in different tissues of different species of Channa have been summarized below.

3.2.1. Channa striatus :

The esterase activities in brain, heart, liver, kidney, muscle, eye and serum are found to be represented by 6, 6, 7, 8, 4, 5 and 9 bands respectively (Fig.6; Plate I). It has been observed that except serum esterases all other tissue esterases are mostly confined between the zones Est.4 and Est.6. However, one minor bands of liver esterases and one band of kidney esterases are also found in Est.8 and Est.7 zones respectively. The serum esterases are distributed in the zones Est.5 - Est.7.

3.2.2. Channa barca :

The total number of bands of esterase activities in brain, heart, liver, kidney, muscle, eye and serum have been found to be 4, 6, 2, 4, 4, 7 and 9 respectively (Fig.7; Plate II). It has been observed that most of the esterase activities are concentrated in Est.5 and Est.6 zones, except in kidney where it is extended upto Est.4 zones. Few minor bands of esterase activity are also observed in Est.7 and Est.8 zones.

3.2.3. Channa punctata :

The esterase activities in C. punctata are found to be unique of its kind, distributed from Est.1 zone (Fig.8; Plate III). However, most of the esterase bands are found in Est.2, Est.3 and Est.4 zones. The total number of esterase bands in brain, heart, liver, kidney, muscle and eye are found to be 9, 7, 10, 7, 7 and 6 respectively. Serum esterases of this species has not been subjected to examination.

3.2.4. Channa stewartii :

The total number of bands of esterase activity in brain, heart, liver, kidney, muscle and eye have been found to be 5, 4, 7, 4, 4 and 5 respectively. (Fig.9; Plate IV). Most of the bands are concentrated in Est.4, Est.5 and Est.6 zones, though a few bands are also found in Est.8 zone. Brain, heart and muscle tissues possess only minor bands while liver exhibits the maximum number of major bands.

3.2.5. Channa orientalis :

The total number of bands of esterase activities in brain,

heart, liver, kidney, muscle and eye are found to be 3, 3, 6, 3, 2 and 4 respectively. It is further observed that except Est.1 and Est.2 zones esterase activities are wide-spread from Est.3 to Est.8 (Fig. 10; Plate V). Moreover, each zone of esterase activity contains mostly one esterase band,

3.2.6. Classification of Esterase zones with specific inhibitors:

Different tissue esterases from different Channa species have been subjected to specific inhibitors such as Urea (10 M), CuSO_4 (10^{-3}M), Eserine sulphate (10^{-3}M) and Diisopropylfluorophosphate (DFP, 10^{-3}M) to find out the nature or type of esterases present in the different activity zones (Plate VI). It has been observed that most of the esterase activities are localized in Est.4 to Est.6 zones in most of the tissues as well as species. Only few minor bands are distributed in other esterase zones. The effects of specific inhibitors on different esterase zones are summarised below :-

Table-1 : Classification of Esterases in the Channids on the basis of sensitivity to specific inhibitors.

Specific inhibitors	E S T E R A S E				Z O N E S			
	1	2	3	4	5	6	7	8
Urea (10 M)	++	+	+	++	+++	+	-	-
CuSO_4 (10^{-3}M)	-	-	-	-	+	-	-	-
*Es (10^{-3}M)	+++	+++	+++	+++		+++	-	+
**DFP (10^{-3}M)	+++	+	+	+++		+++	-	-

Inhibitory effect = + slight; ++ Moderate and +++ Complete
 *Es = Eserine sulphate; **DFP = Diisopropylfluorophosphate

From the above inhibition experiments, it can be presumed that Est.1, Est.4 and Est.6 zones are mostly composed of Choline or Acetylcholine esterases while Est.7 zone is probably reflecting Acetyl-esterases. On the other hand, Est.5 zone may be composed of Arylesterases. The nature of esterases in Est.2, Est.3 and Est.8 are not very clear in this investigation. However, traces of carboxylesterases are probably present in these zones.

3.3. Lactate dehydrogenase (LDH) isoenzymes :

LDH is a tetrameric molecule, encoded by two separate subunits, designated as 'A' and 'B' in most of the vertebrate tissues including fish. When LDH isoenzymes of 'A' and 'B' subunits are composed of one type of molecule, they are termed as LDH A_4 (or LDH 5) and LDH B_4 (or LDH 1) isoenzymes respectively. The combinations of these two homopolymers, which are termed as LDH A_3B_1 (LDH 4), A_2B_2 (LDH 3) and A_1B_3 (LDH 2), always show their activities within the LDH A_4 and LDH B_4 zones. Another LDH isoenzyme, found in eye tissue and encoded by a different subunit 'E', has higher relative electrophoretic mobility than the other two (A_4 and B_4) and is termed as LDH E_4 isoenzyme. In normal cases, LDH A_4 shows comparatively more activity in the skeletal muscle than LDH B_4 whereas LDH B_4 is more active in heart muscle than LDH A_4 . In most of the cases, it is found that LDH B_4 is composed of more net negative charges than LDH A_4 and migrates towards the anode while A_4 remains relatively confined towards the cathodal end. But in certain cases, the 'A' subunit bears more net negative charges than the 'B' subunit and as a result LDH A_4 migrates farthest towards anode while LDH B_4 remains confined towards the cathodal end. In the present investigation, a similar observation is made for all

the Channa species which is further confirmed by urea and heat inhibition studies. LDH E_4 is always found near the anodal end.

3.3.1. C. striatus :

It has been observed that most of the tissues exhibit two to three LDH activity zones (Fig.11; Plate I). LDH A_4 is found to be present in brain, liver, muscle and eye tissues while LDH B_4 is predominant in heart tissue though it is also observed in muscle tissue with lesser intensity. LDH A_2B_2 is found in heart, kidney and serum. LDH A_3B_1 is found in all the tissues except kidney and muscle. LDH A_1B_3 is not observed in any of the tissues.

3.3.2. C. barca :

In this fish, most of the tissues exhibit one to a maximum three LDH activity zone (Fig.12; Plate II). LDH A_4 is found to be predominant in brain and muscle tissues whereas LDH B_4 is found in heart, liver and eye tissues. Except muscle and serum, all the tissues possess LDH A_2B_2 . In heart and eye tissues, in addition to LDH A_2B_2 , one more LDH activity zone viz. A_3B_1 could be traced. No LDH A_1B_3 is found to be present in any of the tissues.

3.3.3. C. punctata :

Except muscle and serum, all other tissues exhibit all the five major LDH isoenzymes (Fig. 13; Plate III). In addition to this, one or two minor LDH activity zones could be traced in certain tissues. Muscle and serum show only two LDH activity zones i.e. A_4 and B_4 isoenzymes.

3.3.4. C. stewartii :

It has been observed for this species that except kidney,

all other tissues exhibit LDH A_4 isoenzyme, while LDH B_4 is found to be present in heart, liver, kidney and eye tissues (Fig. 14; Plate IV). LDH A_2B_2 isoenzyme is exhibited by all the tissues alongwith LDH A_3B_1 isoenzyme though the latter is absent in liver and muscle tissues.

3.3.5. C. orientalis :

In this fish, LDH A_4 isoenzyme is found to be present in all the tissues. LDH B_4 isoenzyme is prominent in brain tissue (Fig. 15; Plate V). On the other hand, LDH A_2B_2 isoenzyme is present in brain, heart and kidney tissues. The other two forms (A_3B_1 and A_1B_3) are not so distinct.

3.3.6. Eye-specific LDH E_4 isoenzyme :

It has been observed that the eye extract of all the five Channa species, in addition to the usual A_4 and B_4 LDH isoenzymes, exhibits a third type of LDH isoenzyme with faster relative electrophoretic mobility towards anode. This is termed as LDH E_4 isoenzyme (Fig. 11-15; Plate VI). Moreover, sometimes certain LDH bands appear beyond the LDH A_4 isoenzyme in eye tissue as well as in other tissues like brain and heart. These are probably heteropolymers containing 'E' and 'A' or 'B' subunits.

The heat stability of LDH E_4 isoenzymes in relation to the other two LDH isoenzymes (A_4 and B_4) has also been examined (Plate-VI). It has been found that heating at 65°C for 15 minutes or below, has little effect on any of the LDH isoenzymes. But above 15 minutes duration or so, heat shows adverse effect on LDH A_4 isoenzyme which is found to be less stable than LDH B_4 isoenzyme though

E_4 LDH isoenzymes is not at all affected even upto 30 minutes duration. Above 30 minutes all the LDH activities are removed completely.

Liver specific LDH isoenzyme :

In certain cases, it is found that when the eye specific LDH isoenzyme is not present, a liver specific LDH isoenzyme is there but with a reverse mobility. To confirm this, reverse electrophoresis of liver tissue extract has been performed, and no LDH activity zone could be detected in any of the species.

4. D I S C U S S I O N

The proteins along with the nucleic acids are the most important components which regulate all biological processes since genetic information transferred by the nucleic acids is ought to reveal many interesting aspects of evolution and heredity. It will also help to rationalize the chemotaxonomic approach to systematize the animal kingdom.

In general, sex, spawning, food, age, hibernation, disease, osmotic pressure, temperature, light, oxygen depletion and other seasonal factors have some role on the total protein species of a fish (Booke 1964). To minimize the influences of such factors, adult fishes were obtained during the same season and almost from the same environmental conditions taking sex and age as constant. It may therefore, be presumed that the patterns and distribution of the electrophoretic bands in different tissues and serum could not be affected by factors other than genetic, so that species specificity could be understood only from the genetic level.

It has been observed that the patterns and distributions of soluble protein bands in all the species is species-specific. It may be mentioned that a number of homologous bands in the corresponding tissues of different species could be detected due to similar mobilities. Similarly, corresponding tissues of different species, sometimes possess the same number of bands but differing from each other in mobility and type. For example, the number of protein bands in the eye tissue of C. barca, C. punctata and C. orientalis are 12 but differ from each other in mobility and staining intensity. The liver tissue in all the species has shown the minimum number of bands. Most of the protein bands in the

brain tissue exhibit phenotypes with moderate to lighter staining intensity. This may probably be due to lesser protein content in brain than in the other tissues. It may be interesting to note that most of the muscle protein bands in all the species show the maximum absorption of dye.

The blood tissue is perhaps more dependent on the physiological state of an organism than any other tissue. Though it is believed that serum proteins show species-specific pattern (Booke 1964), due to the limited number of population studied, no general conclusion could be drawn in this regard. However, in the present investigation, serum protein has shown considerable differences in the species studied. Though it has been earlier reported that water soluble liver and muscle proteins may not show species specificity (Nyman 1965a,b,c), species-specific patterns and interspecies variations of tissue and serum proteins have been found in other fishes also (Chandrasekhar 1959, Tsuyuki et al. 1966, Johnson et al. 1972, Tsai and Yang 1974 and many others) and such variations are expected to have great taxonomic value. The distinct electrophoretic differences, observed among the members of the Channids may also be used for identification, to find out their relationships and differentiation as have been employed for other fishes (Reinitz 1977a,b, Buth 1977, Menzel 1977, Buth et al. 1980). In the present investigation it has been observed that though the distribution and pattern of protein bands exhibit considerable similarity in different species (as has been expressed through the presence of homologous protein bands in muscle and serum proteins) demonstrating their close relationships, they differ considerably in their total protein patterns thus reflecting the differences in the genetic make up.

Esterases are a few groups of enzymes differing in their functions. As their very name indicates, the principal features of the various esterases concern hydrolysis of esters like acetate and butyrates. They are inherited codominantly i.e. without forming the hybrid rings. They are monomers or dimers. Holmes and Masters (1967) have distinguished six different groups in guinea-pig viz. slow Carboxyl-esterases, fast Carboxyl-esterases, Choline esterase, Acetylcholine esterase, Acetyl-esterases and Aryl-esterases.

The total number of esterase bands in fish ranges from 5 in Ribulus marmoratus (Massaro et al. 1975) to 15 in Fundulus heteroclitus (Holmes and Whitt 1970). In most other teleosts (See Ahuja et al. 1977 for detail) there are 7 to 9 esterase bands. The channids exhibit a maximum number of 10 bands in the liver of C. punctata. However, the different esterase bands of the channids can be conveniently grouped into 8 zones numbered as Est.1 to Est. 8. Of all the tissues examined the liver usually displays nearly the maximum number of bands representative of a species, the only exception being C. barca where it displays the minimum number of bands. In Fundulus out of a total of 15 bands, the liver displays 11 bands (Holmes and Whitt 1970). In most other teleosts the liver shows the maximum number of bands (i.e. 7 to 9) representative of the species (see Ahuja et al. 1977). It may be stated that skeletal muscle tissue displays 14 out of the 15 bands present in Fundulus while most of the other teleosts exhibit 2 to 6 bands in muscle. The channids usually display 2 to 4 bands in the skeletal muscle tissue except C. punctata which exhibits 7 bands in the muscle.

The inhibitor sensitivity studies have been performed to dissect the nature of coding loci for esterases. However, all the bands are not interpretable genetically. It appears at least three zones code for Cholin or Acetylcholin esterases, one each for aryl- and acetyl- esterases. Carboxyl-esterases are probably coded by the rest of the zones.

It is quite clear from the nature and pattern of distribution of the esterase bands, aided with the inhibition experiments that the identification and differentiation of the five species is very much possible, thus providing once again the usefulness of esterases from the point of biochemical taxonomy.

The lactate dehydrogenase enzyme (LDH E.C. 1.1.1.27) is one of the most extensively studied enzyme systems. LDH converts pyruvate to lactate with a concomitant production of NAD^+ by oxidation of NADH . This allows the continuation of glycolysis which again permits the continued production of ATP for energy. LDH is reported to be present in almost all groups of vertebrates and invertebrates (Markert and Ursprung 1974). In most vertebrates, LDH is a tetrameric molecule and usually two separate loci have been identified which code for the two subunits viz. A and B. In many tissues these two subunits indiscriminately associate with each other to form five tetrameric isoenzymes, viz. A_4 , A_3B_1 , A_2B_2 , A_1B_3 and B_4 . All these isoenzymes are electrophoretically separable because the two types of subunits differ in their net charge (Apella and Markert 1961, Markert 1962). In many species of fish fewer than these five isoenzymes are found due to restrictions on subunit association or tetrameric instability (Markert and Faulhaber 1965, Whitt 1970a,b). In most of the teleosts, there occurs a

retinal-specific LDH, the E locus which possesses a remarkable anodal mobility (Markert and Faulhaber 1965, Massaro and Markert 1968). Furthermore, a liver specific LDH isoenzyme, viz. the F locus with cathodal mobility has been detected in cyprinids (Klose et al. 1969, Kapes and Whitt 1972), gadoids (Odense et al. 1969, Lush 1970, Sensabaugh and Kaplan 1972). It is assumed that the two LDH genes A and B had been present early in teleost evolution. By duplication of the B gene, a third LDH gene had originated. In the group of fish leading to cypriniformis etc. this locus evolved in its regulation and other properties to an isoenzyme (F_4) synthesized predominantly in liver while in the other line leading to most orders of teleosts it became restricted to retinal (E_4) function (Whitt et al. 1973). Thus, in the advanced teleosts the two loci became mutually exclusive in their function resulting in the adaptive radiation of the teleosts (Fujio and Kaneko 1980, Fisher et al. 1980).

All the channid species under present investigation are provided with the eye specific LDH isoenzyme indicating their advanced nature. In majority of the fishes the LDH B subunits bears more net negative charge than the A subunit as a result of which the letter remains confined towards the cathodal end. The comparative distribution and pattern in heart and skeletal muscle tissue and the inhibition studies have shown a reversed relative electrophoretic mobility of these two subunits in the five Channa species as compared to most other vertebrates. The Channids, therefore, stand out as a group from most of the teleosts in this context. At the species level, it has been observed that certain species exhibit all the five LDH isoenzymes of A and B subunits, while in some other only a few of them are reflected indicating

perhaps the loss of capacity of the A and B subunits to recognize each other. Even the pattern and distribution of LDH isoenzymes in different tissues of the same species show variation which may be due to tissue specificity.

It has been observed in the present investigation that all the tissues of Channa striatus and C. barca possess the usual isoenzyme pattern as found in other fishes (Markert and Faulhaber 1965). Most of the tissues contain two or three LDH isoenzymes. However, muscle tissue in both the species contain only one isoenzyme, LDH 1 or A_4 . Other tissues contain LDH 1 (A_4) and LDH 5 (B_4) and sometimes an intermediate LDH 3 (A_2B_2) which is heteropolymer of the other two. Heart tissue of C. striatus contains four LDH activity zones of which two zones may represent two allelic forms of LDH 1 (A_4). C. punctata on the other hand, shows LDH isoenzyme pattern (of A and B subunits) similar to that of mammals. Except muscle, all other tissues contain five or more LDH activity zones. In brain and liver, in addition to the probable five zones, two and one minor zones are also detected respectively. Muscle has only two zones of activity, probably LDH 1 (A_4) and LDH 5 (B_4). Presence of five LDH isoenzymes are also reported in Whitting (Markert and Faulhaber 1965). The LDH isoenzyme pattern in C. stewartii and C. orientalis are quite distinct from other species of Channa, because these two species show all the bimodal distribution of LDH isoenzymes having two to five or more zones of activity. In both the species, muscle tissue possesses only two isoenzymes while brain and kidney have four zones of LDH activity. However, the heart LDH of C. orientalis reflects only two LDH isoenzymes, while in C. stewartii the same tissue shows four bands. The liver tissue

of C. stewartii possesses five zones of activity, while C. orientalis only three. Though it is reported that liver contains only LDH B₄ in fish (Markert and Faulhaber 1965), exception to this finding is also observed in other fishes (Whitt et al. 1973).

Since the advent of electrophoretic survey of protein variation, a large body of data has accumulated, clearly indicating that most natural populations of animals and plants maintain considerable level of protein polymorphism (Leslie and Vrijenhoek 1977). The level of polymorphism increases with the increase in population size. In the present study, not a large number of polymorphic protein loci could be detected in any of the species. Moreover, protein heterogeneity is determined by differences in the species ecology (Kirpichnikov and Muske 1980) which was eliminated in the present investigation. The relationships between protein loci and environment are very complicated. Organisms respond differently to alterations of various environmental factors (Kirpichnikov and Muske 1980). But adaptive divergence, may bear little electrophoretic divergence (Avisé et al. 1975, Larson and Highton 1978). Probably, that is why, though two chromosomal races of C. punctata were detected (see Chapter II), they could not be identified morphologically as well as electrophoretically. Again, the protein polymorphisms are mostly the results of geographical variation accomplished with genetic variation (Lindsay et al. 1970, Franzin and Clayton 1977, Tabachnick 1977, Kimura 1978a,b, Brody et al. 1979, Diebig et al. 1979, Winans 1980). In the present investigation all the species were collected from almost same

geographic locations with little differences in the environmental conditions and probably, because of this, no polymorphic populations could be detected. Whatever differences have been observed among individuals, cannot be considered under the phenomenon of polymorphism because they were not statistically significant as the number of populations studied has been very small. Perhaps by increasing the population number and size, some detectable biochemical polymorphism in terms of protein variation could be traced which, however, is beyond the scope of the present investigation.

5. REFERENCES

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6. FIGURES AND PLATES

Figure 1 : Diagrammatic illustration of the soluble
protein phenotypes of Channa striatus.

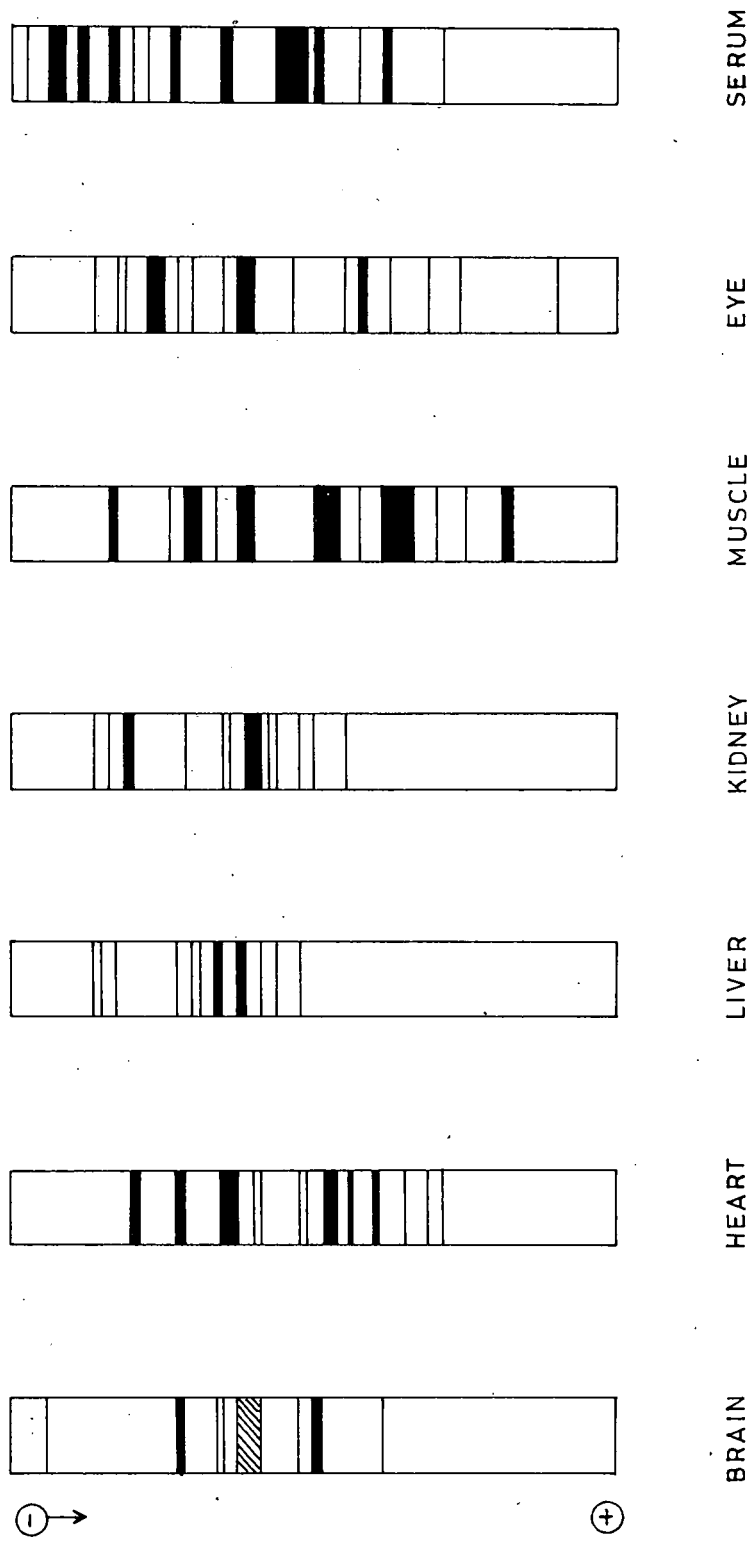


FIG. 1

Figure 2 : Diagrammatic illustration of the soluble protein phenotypes of Channa barca.

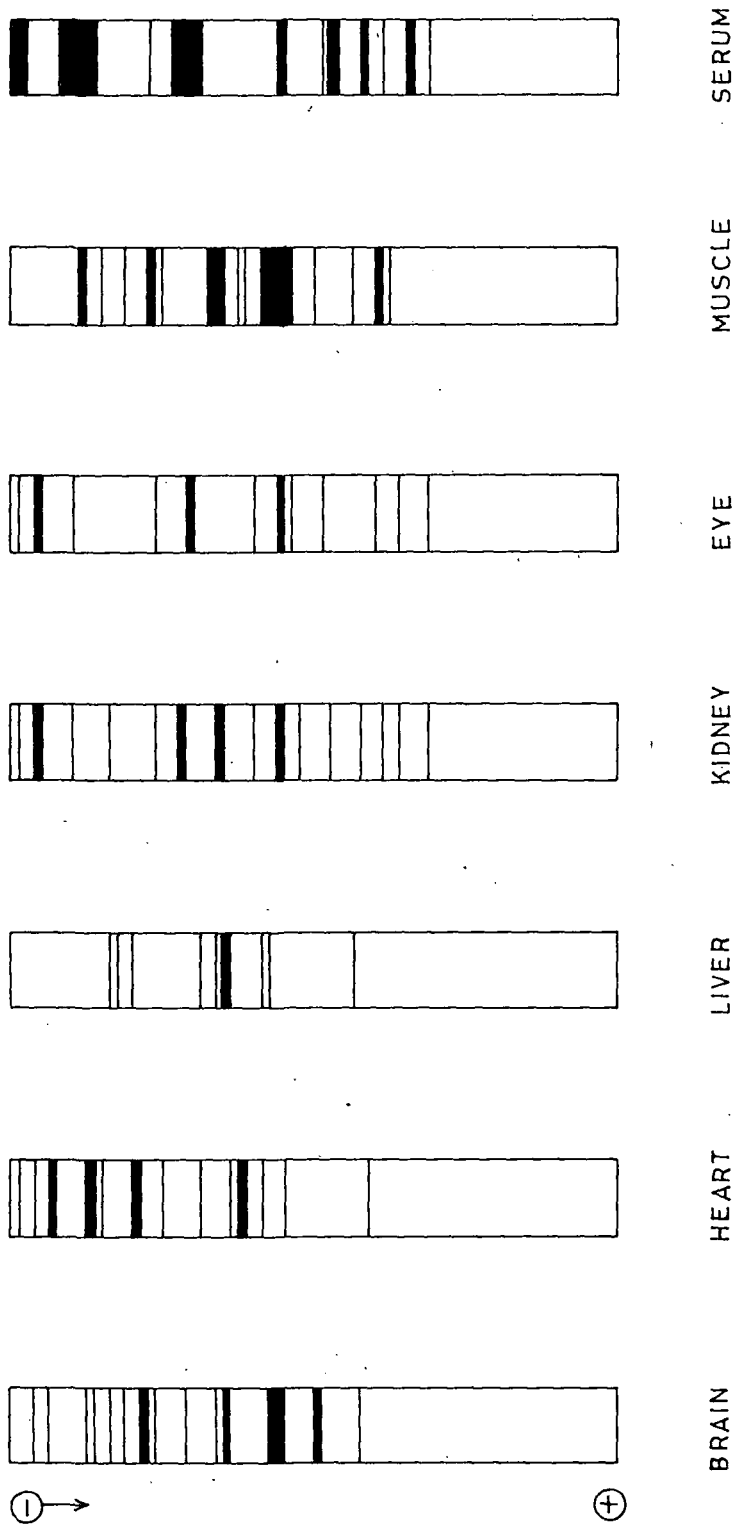


FIG. 2

Figure 3 : Diagrammatic illustration of the soluble
protein phenotypes of Channa punctata .

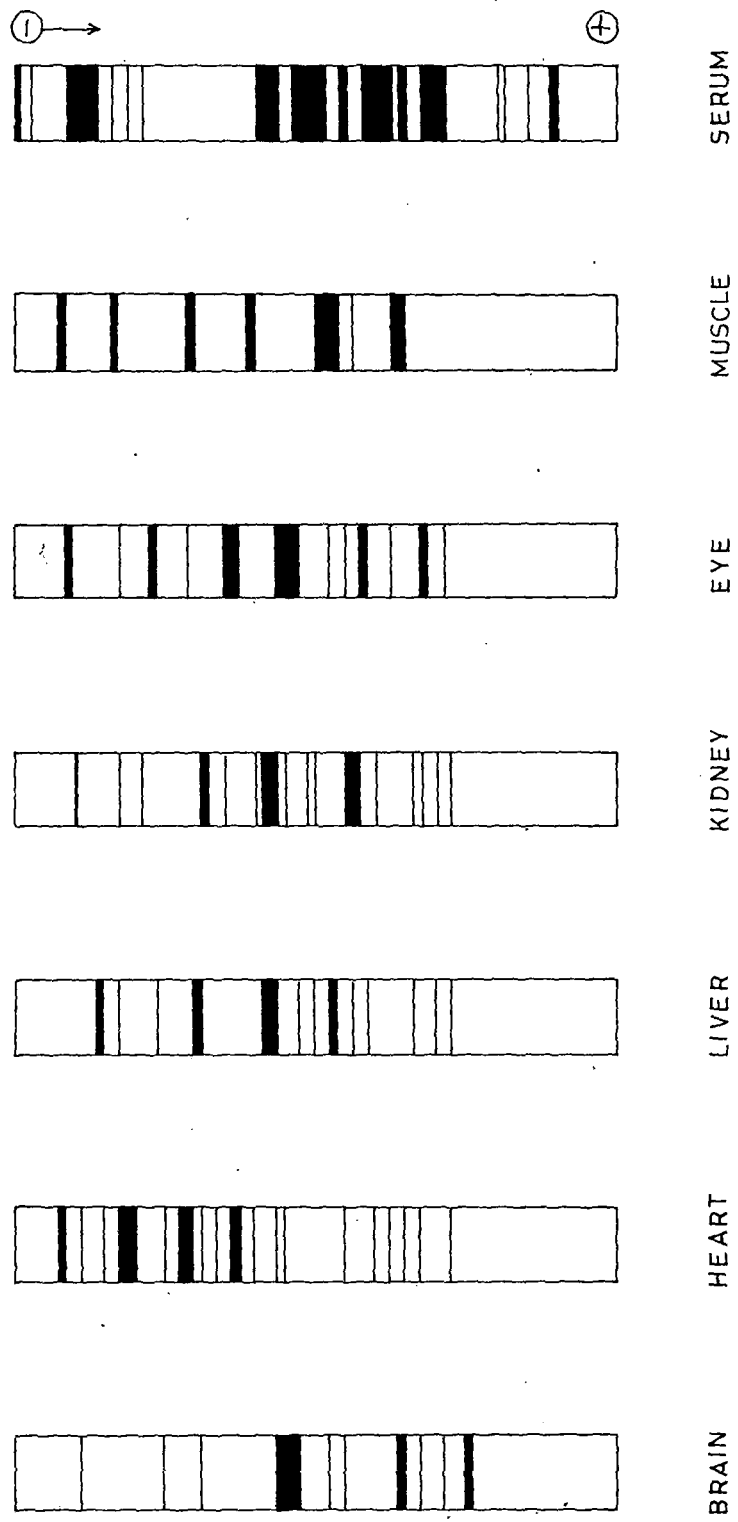


FIG. 3

Figure 4 : Diagrammatic illustration of the soluble protein phenotypes of Channa stewartii.

Figure 5 : Diagrammatic illustration of the soluble protein phenotypes of Channa orientalis.

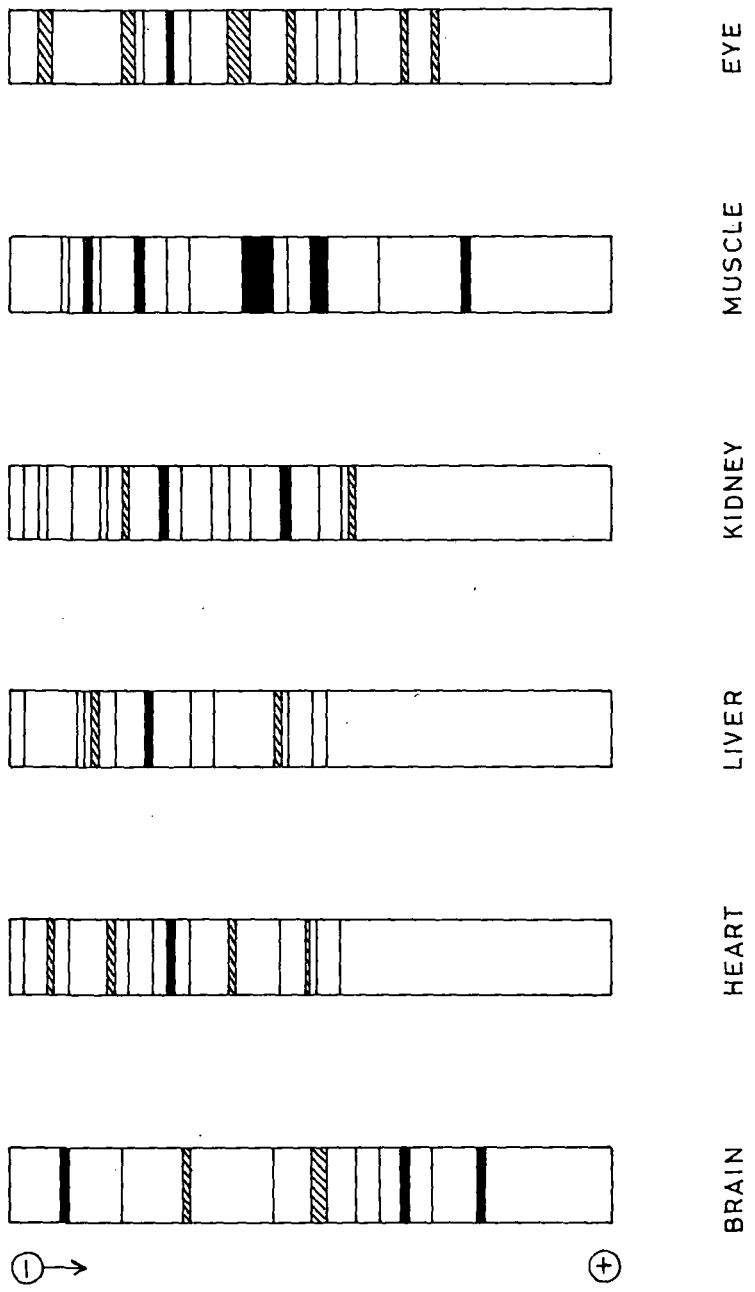


FIG. 5

Figure 6. : Diagrammatic illustration of Esterase phenotypes of Channa striatus.

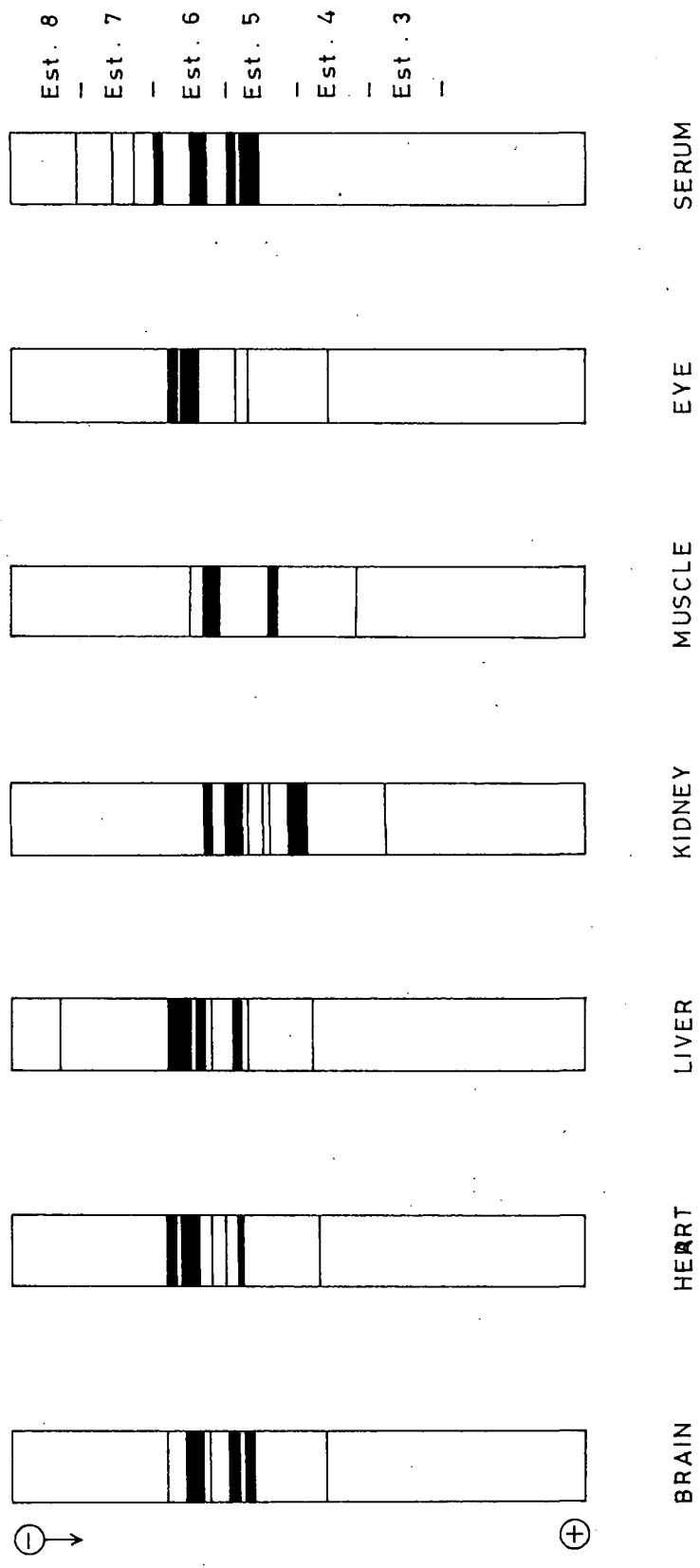


FIG. 6

Figure 7 : Diagrammatic illustration of Esterase phenotypes of Channa barca.

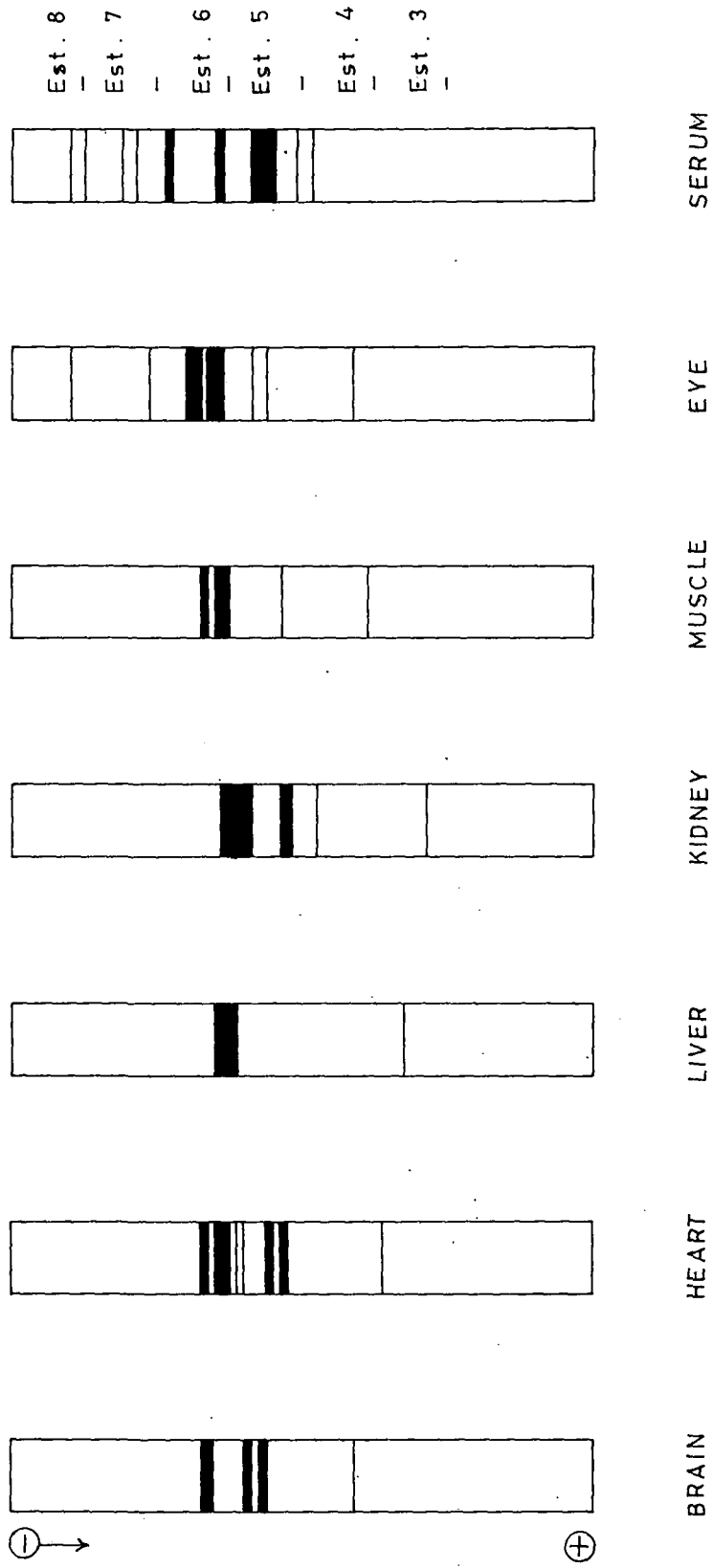


FIG. 7

Figure 8 : Diagrammatic illustration of Esterase phenotypes of Channa punctata.

Figure 9 : Diagrammatic illustration of Esterase phenotypes of Channa stewartii.

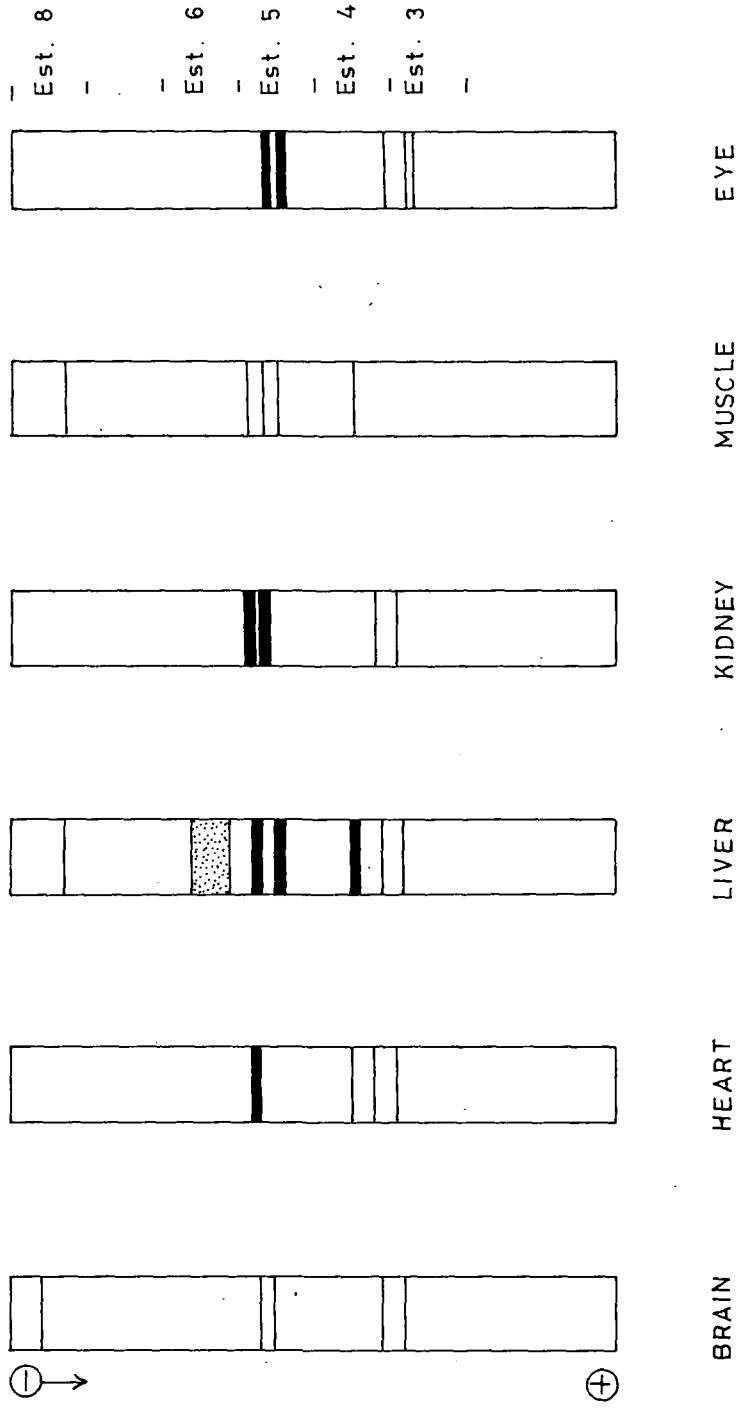


FIG. 9

Figure 10 : Diagrammatic illustration of Esterase phenotypes of Chenna orientalis.

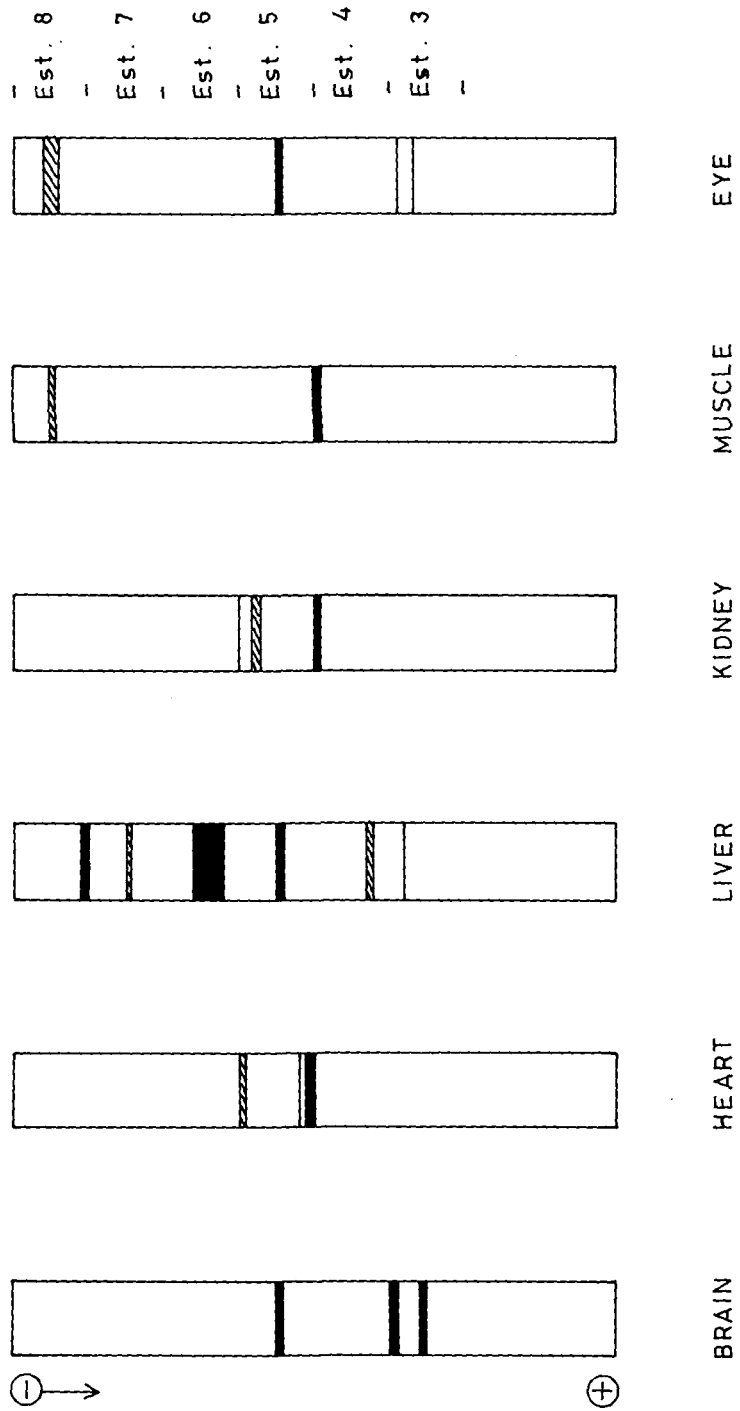


FIG. 10

Figure 11 : Diagrammatic illustration of LDH phenotypes of Channa striatus.

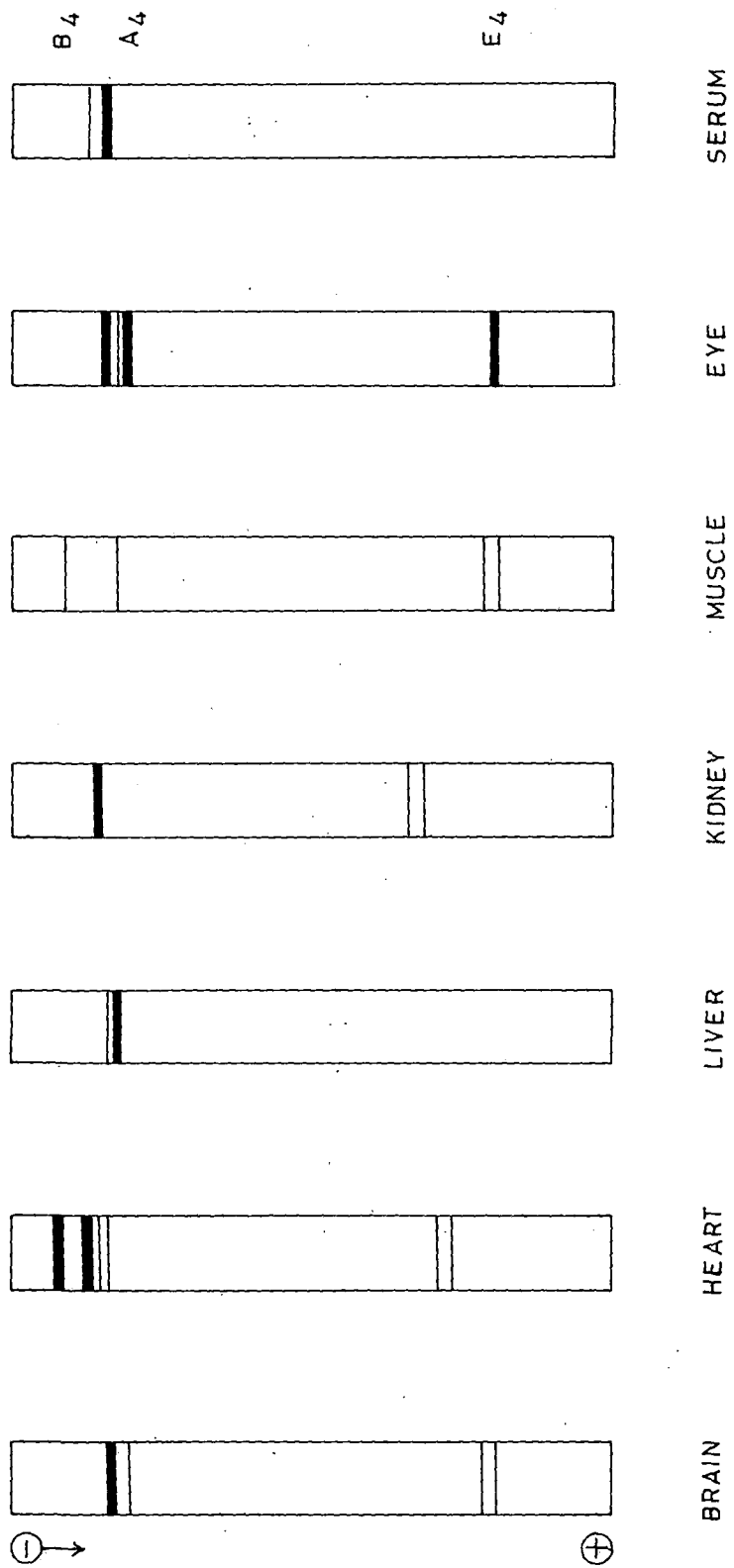


FIG. 11

Figure 12 : Diagrammatic illustration of LDH phenotypes of Channa barca.

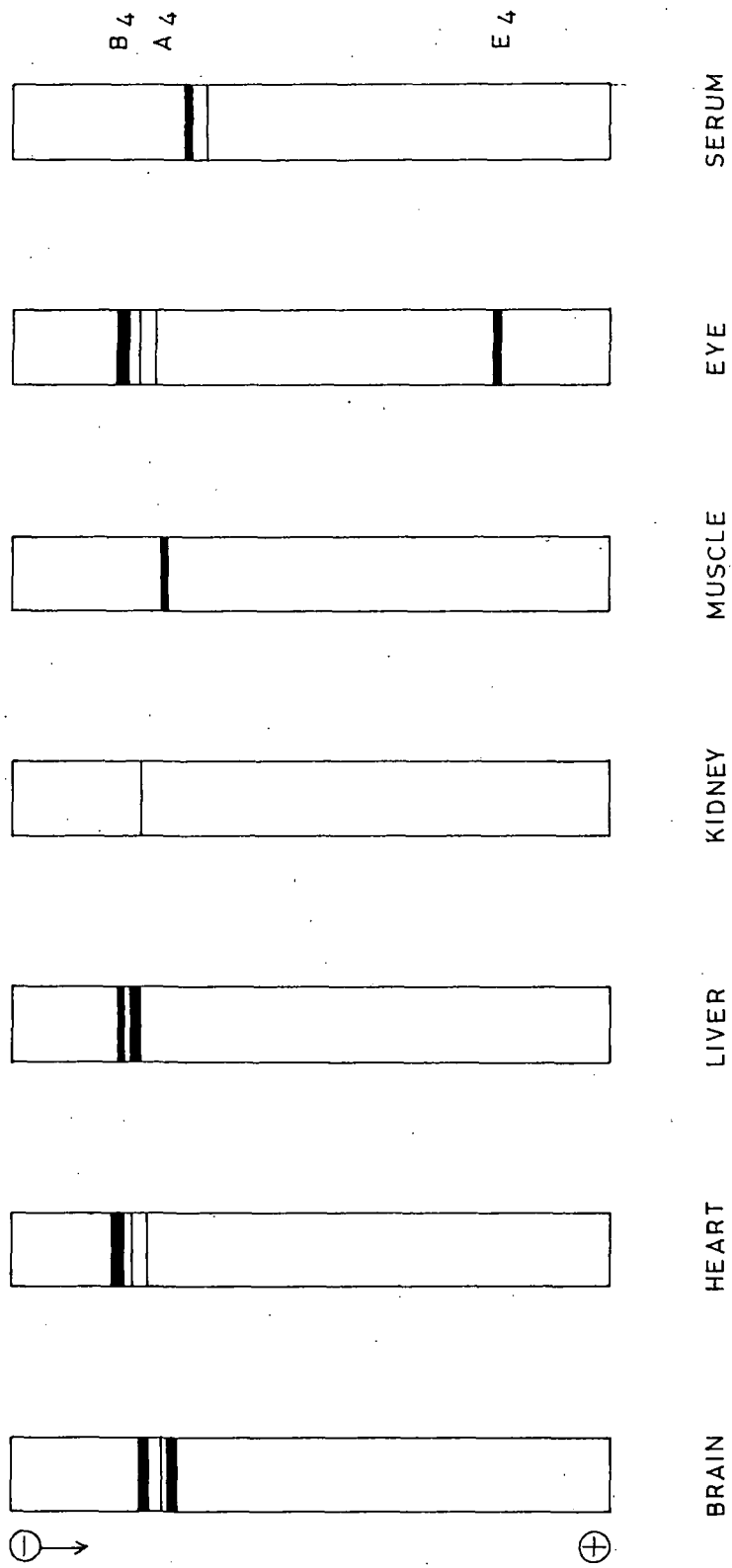


FIG. 12

Figure 13 : Diagrammatic illustration of LDH phenotypes of Channa punctata.

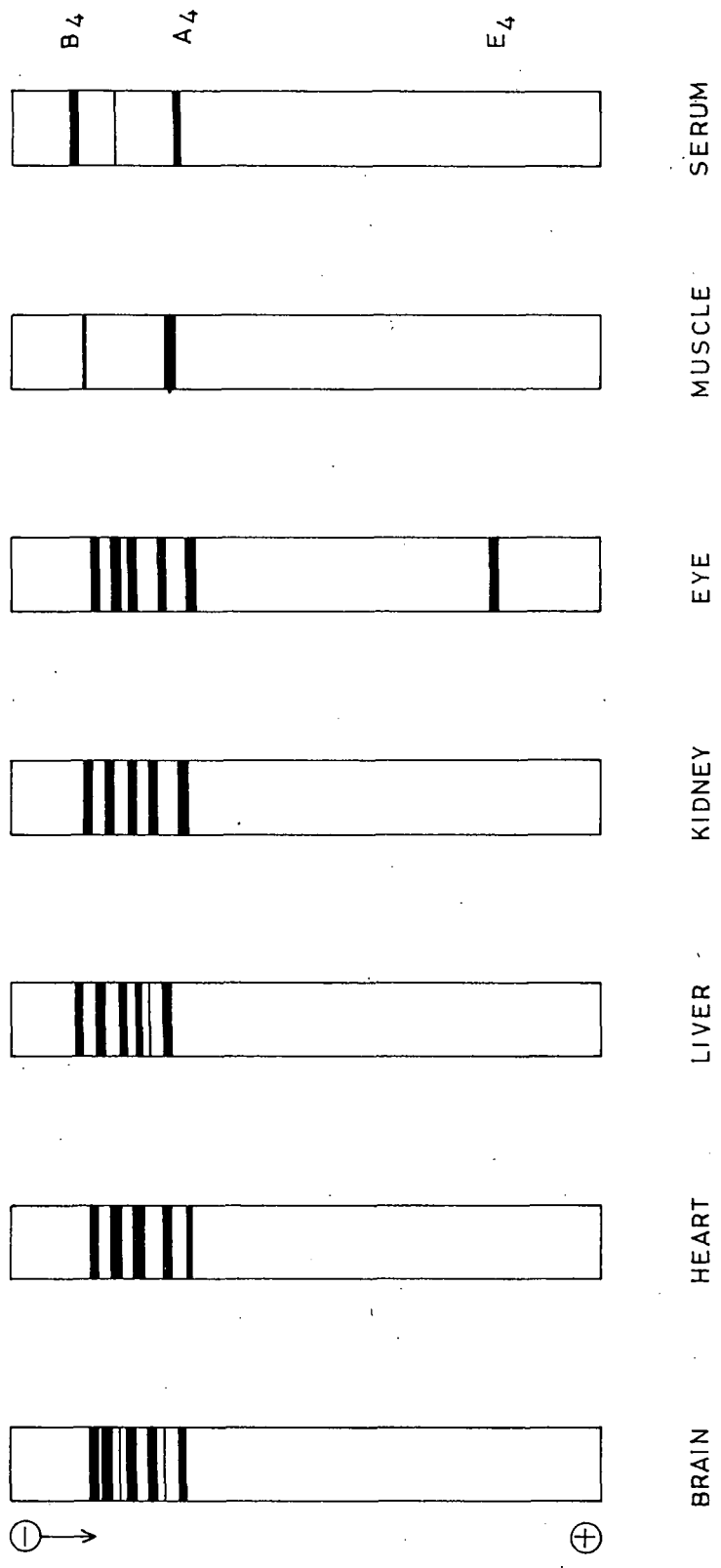


FIG. 13

Figure 14 : Diagrammatic illustration of LDH phenotypes of Channa stewartii.

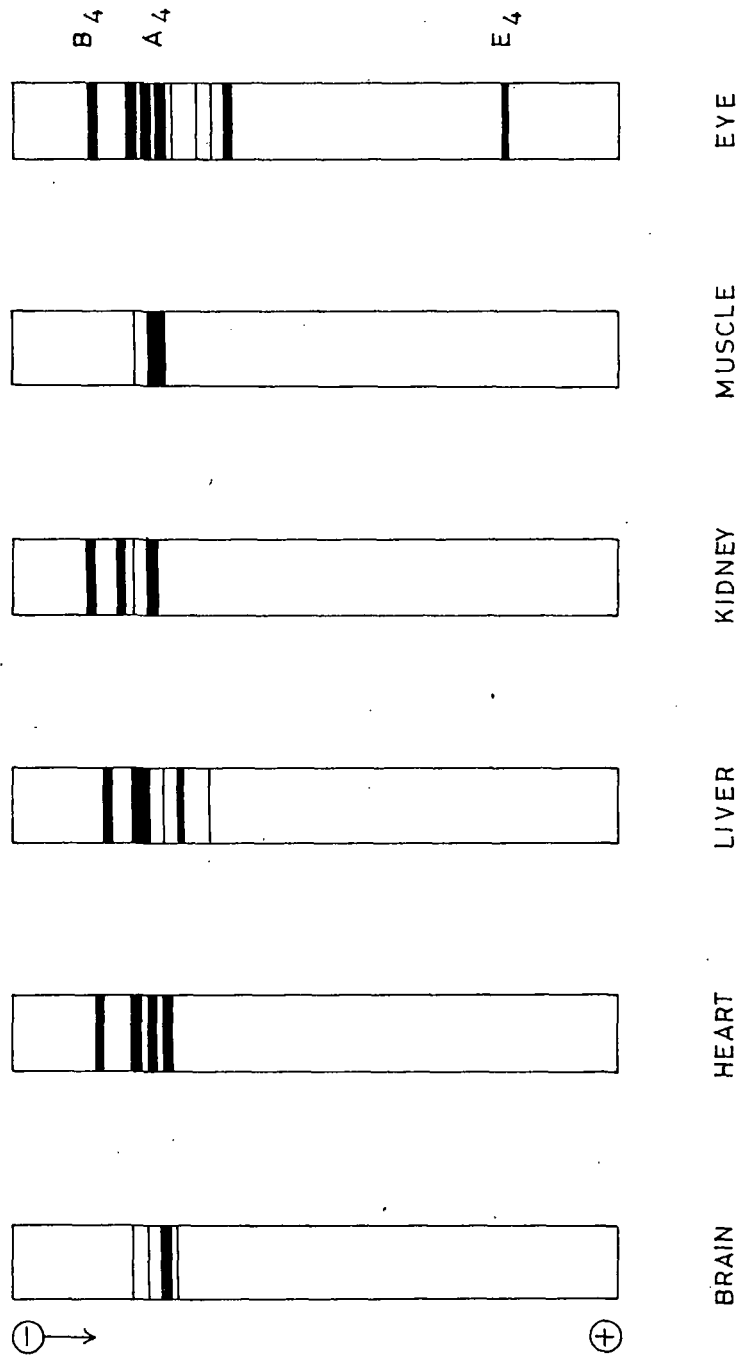


FIG. 14

Figure 15 : Diagrammatic illustration of LDH phenotypes of Channa orientalis.

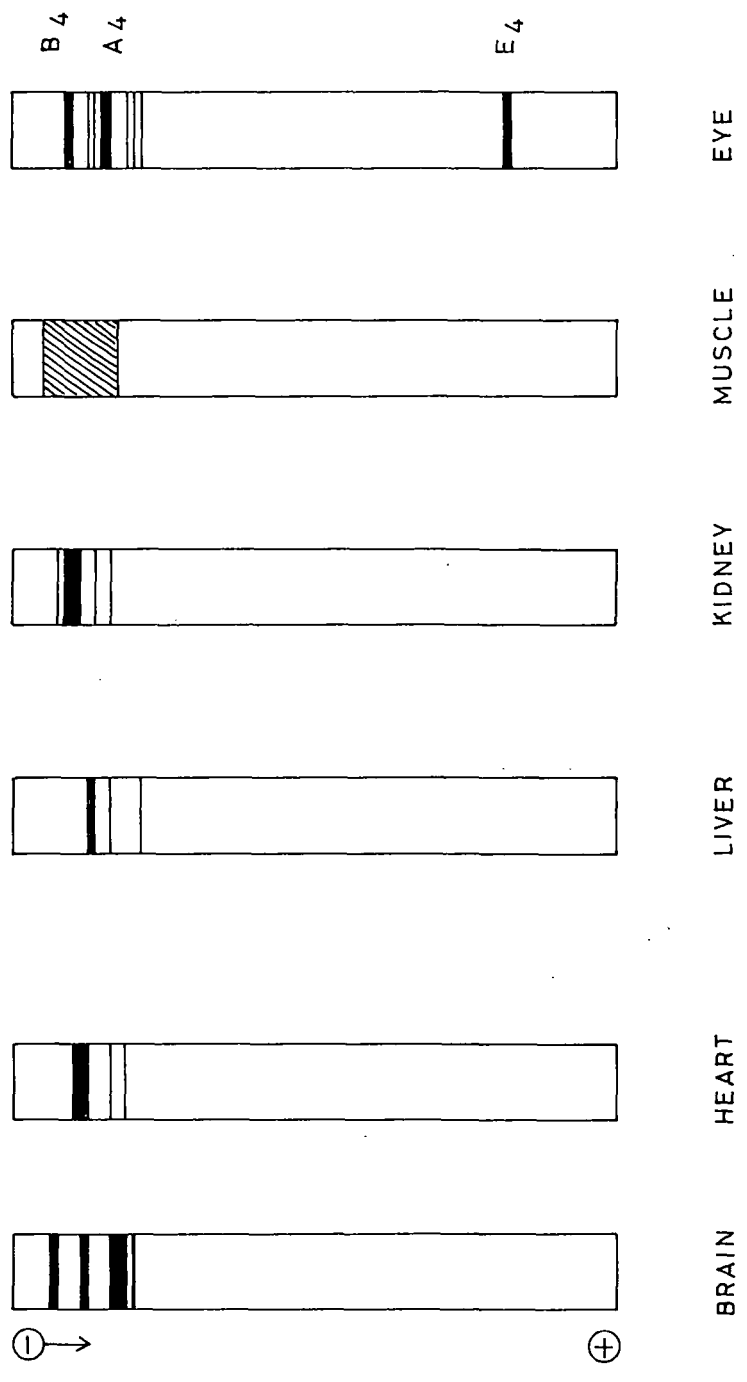
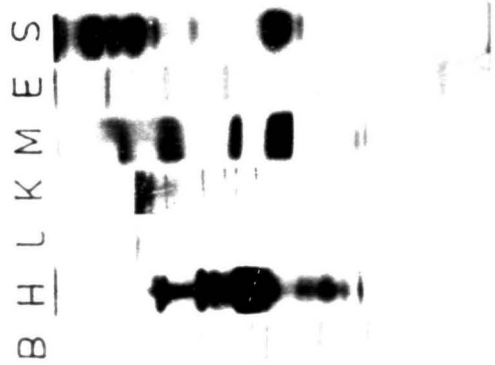


FIG. 15

Plate-I : Tissue-specific soluble protein,
esterases and LDH (A and B subunits)
patterns in Channa striatus.

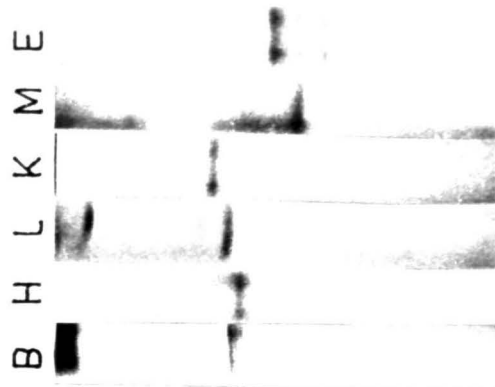
PLATE-I



PROTEIN



ESTERASES



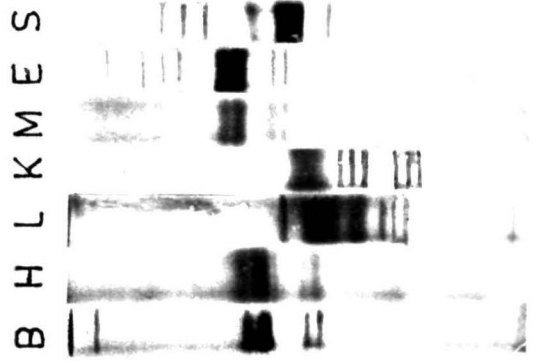
LDH

Plate II : Tissue-specific soluble protein,
esterase and LDH (A and B subunits)
patterns in Channa barca.

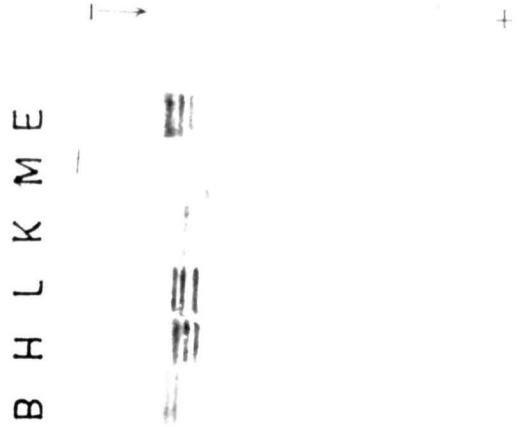
PLATE - II



PROTEIN



ESTERASES

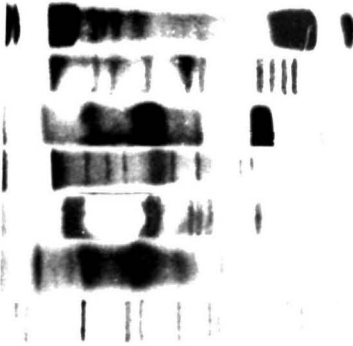


LDH

Plate-III : Tissue-specific soluble protein,
esterase and LDH (A and B subunits)
patterns in Channa punctata.

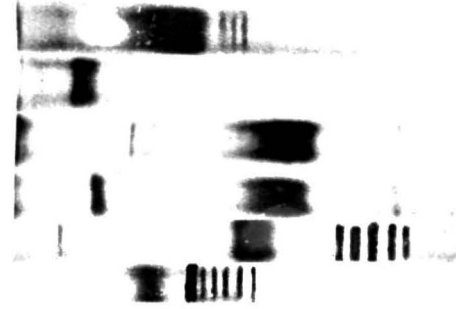
PLATE - III

B H L K M E S



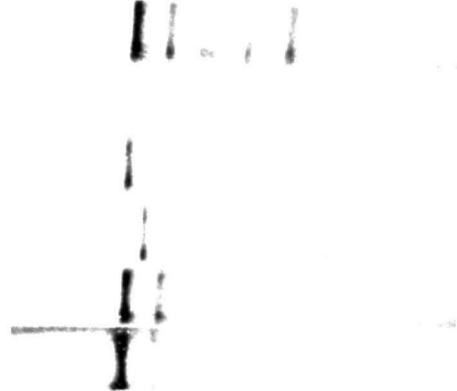
PROTEIN

B H L K M E



ESTERASES

B H L K M E

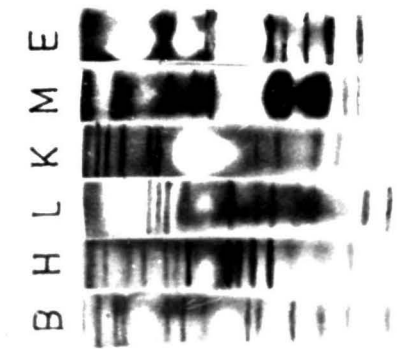
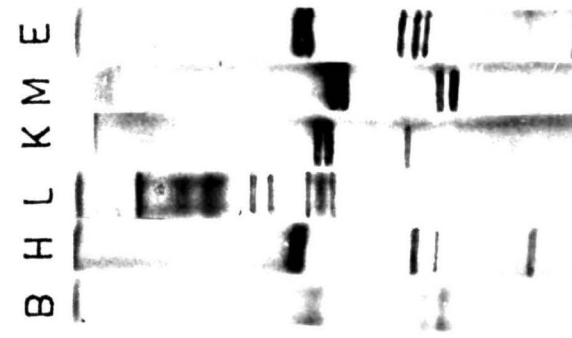
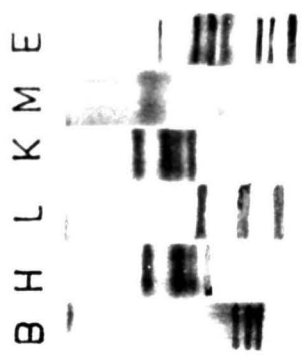


LDH

T

Plate-IV : Tissue-specific soluble protein,
esterase and LDH (A and B subunits)
patterns in Channa stewartii

PLATE -IV



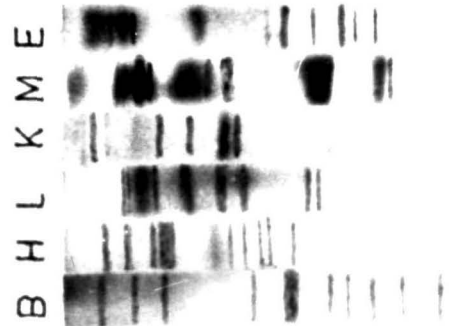
LDH

ESTERASES

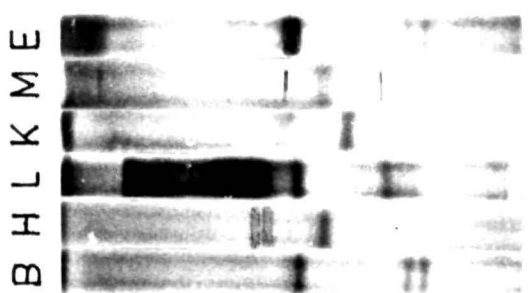
PROTEIN

Plate-V : Tissue-specific soluble proteins,
esterase and LDH (A and B subunits)
patterns in Channa orientalis.

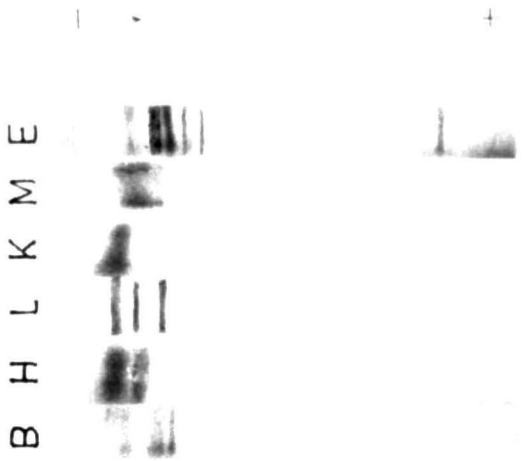
PLATE - V



PROTEIN



ESTERASES



LDH

Plate=VI

(a) Sensitivity of liver esterases of Channa striatus to various inhibitors.

(b) Sensitivity of liver esterases of Channa punctata to various inhibitors.

C = Control

U = Urea 10M

Cu = $\text{CuSO}_4 \cdot 10^{-3}\text{M}$

Es = Eserine sulphate, 10^{-3}M

DFP = Diisopropylflurophosphate 10^{-3}M

(c) LDH isoenzyme patterns showing theElocus in Channa punctata along with the heat stability experiment.

(d) LDH isoenzyme patterns showing theElocus in Channa striatus along with the heat stability experiment.

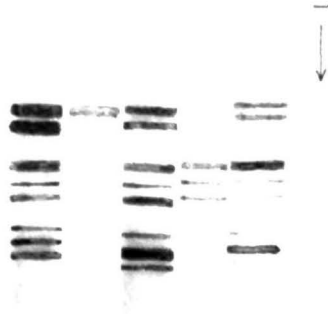
E = Control;

E' = Heat treatment at $55^{\circ}\text{-}65^{\circ}\text{C}$
for 15 minutes

E'' = Heat treatment at $55^{\circ}\text{-}65^{\circ}\text{C}$ for 20 minutes.

PLATE - VI

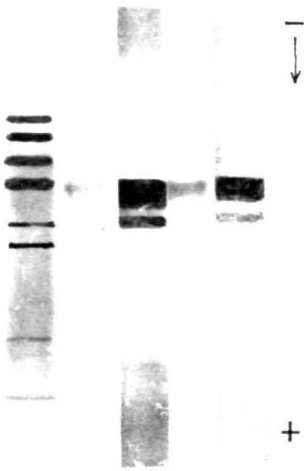
C U Cu Es DFP



ESTERASES

a +

C U Cu Es DFP



ESTERASES

b +

E E' E'' E E' E''



LDH

c d +

CONCLUDING REMARKS

The systematics of air-breathing fishes belonging to the genus Channa presents serious difficulties leading to disagreements among taxonomists on the identity of some of the species. The study of fish cytogenetics and genetics is very promising in terms of the solution of these problems. The objective of this research was to present a comprehensive morphometric and meristic, karyotypic and electrophoretic description of the five Channa species widely found in North-Eastern India, and to supplement the earlier studies and provide further insight into the question of interrelationship among these species.

As far as the present study is concerned, we have been fairly successful in providing a working key for the field identification of the different species from the point of view of morphotaxonomy. The cytogenetic data demonstrates that all the species are markedly different at the karyotypic level. In addition, the existence of two (chromosomal) races of the species C. punctata has also been established. We have also been able to characterize electrophoretically the different Channa species. Use of either soluble muscle protein or esterases results in unequivocal assignment to species because of species-specific banding patterns. The overall distribution and banding pattern of LDH is also indicative of the distinctiveness of the genus.

As a follow up of the present work we suggest the study of banding pattern of the karyotype to provide further insight into the karyotypic evolutions within this group. We would also

like to suggest further application of biochemical techniques involving a large number of enzymes and statistically significant number of individuals to find out the genetic distance between the members which we believe will have the last word in unraveling the phylogenetic relationship of this group of fishes.

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