PART - A
ACKNOWLEDGEMENTS

This thesis is an attempt to the taxonomy, morphology, ecology of cestode parasites and haematology of fish, *Channa punctatus* (Bloch) which is possible only with the unselfish collaboration of the many who contributed to the instant consequence of this challenging task.

The present work has been carried out in the P.G. Department of Zoology, BIPIN BIHARI COLLEGE, Jhansi under the able supervision of Dr. A.K. Srivastav, M.Sc. D.Phil, F.A.Z., F.H.S., F.Z. S.I., F.I.A.E.S. The author express deep gratitude and indebtedness to her supervisor for his untiring and kind interest in this project. Dr. Srivastav not only suggested the problems but also guided me at all stages of this work. His constant inspiration and cordial treatment are greatly acknowledge.

The author heartily thankful to Dr. Surendra Katyal, M.D. (Path.) director of "Sant Diagnostic Centre and Blood Bank " Jhansi for providing laboratory facilities and several valuable help for the "Haematological Part" of this thesis.

The author is thankful to the authorities of Bipin Bihari College, Jhansi, particularly to Dr. U.P. Singh, Principal for being kind and considerate in allowing her for research work in the P.G. Department of Zoology.

The author express her deep gratitude to Late Dr. J. P. Tiwari, Head Department of Zoology, Bipin Bihari College, Jhansi for providing laboratory facilities during the period of present project.

The author is grateful to Late Dr. S.C. Agrawal, Late Dr. U.K. Dwivedi, Dr. A.B. Gupta, Dr. R.C. Gupta. Dr. A.S. Gurudeo, Department of Zoology, Bipin Bihari College, Jhansi for their valuable suggestions in the preparation of the present manuscript.

It gives the author for considerable delight in thanking most willingly to her ideal teachers Dr. V.I. Sharma, Dr. O.P. Yadav, and Dr. S.K. Dubey who cherished her Zoological knowledge to achieve this goal.
The author wish to record her appreciation to a good many individuals who, in a variety of ways helped herself in course of the study. The author utter her thanks to Dr. B.K. Srivastav, Dr. Nopur Mathur, Dr. Pragya Khare, Dr. Fazal Ahmed, Mrs Sharmila Roy and Rakhi Agarwal.

The author is highly thankful to Smt. Chhavikala Srivastava for her inspiration and affectionate treatment during the research period.

The author is also thankful to Mr. R. K. Chaturvedi and Mr. R.C. Srivastava, Lab assistants of Zoology Department for their co-operation during this work.

The author feels deeply grateful to her mother Smt. Radha Lohia and her father Shri Om Prakash Lohia, who very lovingly brought her up and gave her best possible education and supported at all stages of this project. The co-operation extended by her brother Anupam and both sisters Kavita and Kankna are also highly appreciable.

The author is deeply grateful to her husband Er. Mahendra K. Gupta for his deep appreciation and buoyancy and thanks are also due to her in-laws.

Thanks are also due to Mr. Surendra Gupta Director of Surendra Computers for carrying out excellent job in computerising this manuscript.

Date 17-05-2000

Shweta Lohia
INTRODUCTION

A number of fresh water fishes constitute highly nutritive food for human beings. Some of them are considered as delicacies. These edible fishes are known to harbour a number of cestode, nematode, trematode and acanthocephala parasites which cause deterioration in their health, hence their nutritive and market value is effected. The curiosity of the author to know about the helminth parasites found in such fishes lead her to undertake the present project. In the present thesis the author has restricted herself to the nature of infection of cestode parasites only. With a view to know the nature and extent of cestode infection, regular studies were under taken to record the nature of parasitism in fresh water fish *Channa punctatus* (Bloch) for two successive years. To have the idea of the state of infection in some fresh water fishes the survey was conducted in different parts of district Jhansi.

The present thesis deals with some of the interesting cestodes obtained during the survey which include the description of three new genera, six new species with a new family.

The new species *Lytocestoides nandanpurensis* n.sp. belong to the family Lytocestoidae Hunter, 1927 of the order Caryophyllidae Beneden in Carus, 1863. So far only fifteen genera have been reported from the family Lytocestoidae Hunter, 1927 from the whole world. Out of them eight genera have been reported from the oriental region having six from Indian subcontinent. The present new species *Lytocestoides nandanpurensis* n.sp. have been reported from the genus *Lytocestoides* Baylis, 1928.

The new genus *Pseudobilobulata* n.g. represnet the family Capingentidae Hunter, 1930 of the order Caryophyllidea Beneden in Carus, 1863. So far only nine genera have been reported from the family Capingentidae from the whole world. Out of them five genera have been reported from the oriental region and Indian subcontinent. The present new genus is the sixth from Indian subcontinent.
The new species *Cephalochlamys orchhaensis* n. sp. belong to the family Cephalochlamydidae Yamaguti, 1959 of the order Pseudophyllidea Carus, 1863. So far only one species have been reported from the genus *Cephalochlamys* Blanchard, 1908 from the whole world. Present species *Cephalochlamys orchhaensis* n. sp. first report from India.

The new species *Philobythos prasadi* n. sp. belong to the family Philobythiidae, Campbell 1977 of the order Pseudophyllidea Carus, 1868. So far only one species have been reported from the genus *Philobythos* Campbell, 1977 from the whole world. Present species *Philobythos prasadi* n. sp. first report from India and second in world.

The new genus *Sukhobythos capoori* n.g., n.sp. Campbell, 1977 of the order Pseudophyllidea Carus, 1868. So for only two genera have been reported from the family Philobythiidae Campbell, 1977 from the whole world. Both genera have been reported from the marine teleosts from north and northwest Atlantic region. The present new genus is the third from Indian subcontinent and first from fresh water fishes:

The new family Jalpiidae n.f. comes closer to philobythiidae Campbell, 1977 order Caryophyllidea Beneden in carus, 1868. *Jalpos* n.g. first report of n.f. Jalpiidae.

With a view to discover the cestode host relationships examination of the fresh water fish, *Channa punctatus* (Bloch) has been performed for two successive years. The prevalence, mean intensity and relative density of cestode infection has been worked out in relation to the body weight, sex of the host and cloacal temperature of the host.

Life evolved first in the water. The primitive unicellular animals took its oxygen and nutrition from water and excreted its waste products into it. But with the development of the multicellular forms, this simple arrangement of drainage and supply could no longer hold. The cells in the deeper parts of the body could not come into free contact with surrounding water and would therefore suffer. To overcome this difficulty a system of inter-communicating channels developed pervading the whole animal body and opening upon the exterior. Through these channels the water could freely flow in and out and in this way the deeper cells could
satisfy their needs. This channel system represents the primitive circulatory system.

In the course of evolution that enclosed water has undergone profound modifications and has been transformed into what we call now blood. Although blood has departed a long way form its primitive ancestor yet in its inorganic composition it still maintains a close resemblance with water.

Blood may be described as a specialised connective tissue in which liquid portion named as plasma. The corpuscles (red blood cells and white blood cell) and the platelets are suspended in the plasma. These cells are continually being distroyed either because as a result of their functional activities and replaced by newly formed cells.

The study of fish blood have their own importance as Hickey (1976) has given more and more stress on regular haematological observations of fishes for their proper maintenance and production, since these form a very valuable source of high energy and protein rich food particularly when more than 50% of humun population all over the globe is suffering severly from mal-nutrition. Hence fish health should be a matter of great importance to all human being specially to the fish biologiate.

During the course of study some fishes harbouring cestode parasites were found. This provide an excellent oppertunity to study physiopathological changes.
Several workers have contributed to the knowledge of cestode taxonomy from the Indian subcontinent.

Southwell's contribution has been classical. Apart from his classical volume of "Fauna of British India" his pioneering contributions include the descriptions of many new species. In 1913 Southwell reviewed the cestode material then existing in the Indian museum collection. They included *Gangesia bengalensis* (1913), *Gigantoline magna* (1915) from fresh water fishes.


The important contribution of Dollfus comprise *Senga besnardi* (1934) *Senga ophiocephalina* (1934) and *Senga pycnomera* (1934).


Shinde G.B. described a number of known and unknown cestodes. His important contributions are *Circumoncobothrium ophiocephali* (1968), *Lytocestoides aurangbadensis* and *Circumoncobothrium raoii* (1976 with Jadhav), *Uncibilicularis southweli* (1976 with


Besides the major contributions of the aforesaid workers a number of stray papers have been published by Mathur (1992) and Mathur and Srivastav, A.K. (1996).

Very scanty work has been done on the ecology on the cestode parasites, Mathur (1992) has tried to make relation of cestode parasites with the *Heteropneustes fossilis* (Bloch.) of Bundelkhand region of Uttar Pradesh.

The effect of length and weight on the Total Erythrocyte Count (TEC) have been noted by Dombrowsky (1953), Preston (1960), Ostrumova (1960 a, b) and Joshi and Tandon (1977 a).

It has been noted that the Total Erythrocyte Count (TEC) values also fluctuate during different seasons. Syrov (1970), Joshi and Tandon (1977 b) and Khan (1977) have reported higher Total Erythrocyte Count (TEC) values during summer months. A general fall in Total Erythrocyte Count (TEC) value is reported.

Total Leucocytes Count (TLC) have been made on a large variety of fish fauna as described by Malassez (1872), Hall et al (1926), Reznikoff and Reznikoff (1934), Albritton (1952), Mott (1957), Lyask (1959), Preston (1960), Hawe and Goodnight (1962), Puchkov (1964), Andrew (1965), Mcknight (1966), Srivastava (1968 b), Blaxhall (1972), Hickey (1976), Tandon and Joshi (1976), Joshi and Tandon (1977 b) and Pandey and Pandey (1977).

Besides various other factors influence of Total Leucocytes Count (TLC) values in fishes, such as varying length and weight (Joshi and Tandon 1977 a), Temperature (Slicher, 1961; Einszpron Orecka, 1970; Farghaly et al 1973 and Pandey, 1977) Season (Plessis,
1958; Murachi 1959 and Khan 1977) and Parasites (Tandon and Joshi, 1973; Pandey and Pandey, 1974 and Ikeda et al, 1976) have been reported.

Haemoglobin (Hb) contents of the fish blood under normal and different ecophysiological conditions have been pooled out by many workers Hall, et al, (1926); Hall and Gray, (1929); Reznikoff and Reznikoff, (1934); Fieid et al, (1943); Higginbotham and Meyer, (1948); Kisch, (1949 a); Scholander and Vandam, (1957); Ostrumova (1960 a, b); Preston (1960); Pradhan (1961); Hawe and Goodnight, (1967); Mulcahy, (1970); Dey and Upadhyaya, (1972); Rao and Behura, (1973); Bagchi and Ibrahim, (1974); Pandey and Pandey, (1977); and Prasad et al, (1977).

Concentration of haemoglobin in most of the teleosts is said to be higher than the elasmobranch fishes (Hall and Gray, 1929). Active fishes with higher rate of body metabolism are reported to contain more haemoglobin in their blood than the sluggish forms Hall and Gray, (1929), Root, (1931), Shubnikov, (1959), Hawe and Goodnight, (1962), Kiawe et al, (1963) and Cameron, (1970). Influence of age, length, weight, sex and temperature on the haemoglobin concentration of fish blood have also been reported by several workers Lysaya, (1951), Ostrumova, (1960 a, b), Molner et al, (1967), Pradhan, (1961), Hocachaka, (1961), Banerjee, (1966), Kuzmian, (1966), Radzinskaya, (1967), De wilde and Houston, (1967), Mulcahy, (1976), Joshi and Tandon, (1977) and Pandey, (1977). Seasonal changes have also been found in Haemoglobin (Hb) contents, Schayer, (1925), Preston, (1960), Radzinskaya, (1966), Griggs, (1968), Smisnova, (1970), Syrov, (1970), Foda, (1974), and Joshi and Tandon, (1977) have worked on the seasonal variation in Haemoglobin contents of fishes.

Observation on the Erthrocyte Sedimentation Rate (ESR) have been made by Schumacher et al (1956), Murachi (1959), Preston (1960), Sano (1960), Sinderman and Meirs (1961), Barnheart (1969), Blaxhall (1972) and Pandey and Pandey (1977). Thus comparatively less attention has been paid to this part of fish haematology.

The studies regarding the absolute values of the blood are most same, Mean Corpuscular Haemoglobin (MCH) value of fishes has been reported mainly by Weinreb et al (1972), Ikeda et al (1970) and Prasad et al (1977).

The studies made on the Mean Corpuscular Haemoglobin Concentration (MCHC) are mainly those reported by De Wilde and Houston (1967), Weinreb et al (1972), Fange and Sjöbeck (1975), Boivio et al (1974 b and 1975) and Prasad et al (1977).
MATERIAL AND METHODS

The alimentary canal of the host was removed and cut open in normal saline water in troughs or petridishes. It was lightly shaken and the contents decanted several times. The intestine and its contents containing parasites were examined thoroughly under a binocular microscope to ensure that none of the parasite is left behind. In some cases as the scolices were deeply embedded it was found necessary to take them out by scraping the mucosa of the intestine with a sharp scalpel or by releasing the scolices with a pair of needles. Later portion of the mucosa attached to the cestode in the normal saline water. The worms were stretched in luke warm water and in case of larger worms by lifting them by the help of needles or forceps against the edges of petridishes repeatedly for several times and later on fixed in 5% formalin or alcoholic Bouin's fluid. Fixed and washed worms were stored in 5% formalin till needed for study.

The whole mounts were stained in either Borax carmine or Mayer's Haemalum. The Mayer's Haemalum proved to be the best stain for cestodes. Whole mounts were either cleared in xylol or clove oil. For sectioning the material was cleared in xylol embedded in histowax and cut at 0.006 - 0.008 mm stained with Delafield's Haematoxyline and Eosin and mounted in canada balsam. The worms have also been studied in living condition.

Only camera lucida drawings were made. All the measurements have been given in millimeters unless other wise stated. Averages taken on the basis of the study of three to ten worms except in cases where still fewer worms were obtained.

During the course of study the total number of hosts thus examined was 168. The hosts examined belong to 8 species of fishes.

For the study of cestodes host relationship *Channa punctatus* (Bloch) was selected. The live fishes were obtained through local fish catchers. A thorough study of five fishes were made in every month. This was continued for two successive years. Following process was used in the study of cestode host relationship.
(a) Live fishes were weighted individually.

(b) Sexes were identified.

(c) The alimentary canal of the fish was cut open in the normal saline solution in a petridish.

(d) The four kinds of parasites viz. cestodes, nematodes, trematodes and acanthocephala were collected and counted separately in each infection.

(e) The morphological studies of the cestodes, thus obtained were performed and their diagnosis completed on the basis of the study of permanent stained slides.

A total number of 110 *Channa punctatus* (Bloch) were examined and 62 of them were found infected for helminth infection. The total number of 209 helminth parasites were obtained which included 17 cestodes, 26 trematodes and 116 acanthocephala.

During the ecological studies prevalence, mean intensity and relative density were calculated. The definitions given by Morgolis et al 1982 were followed.

1. **Prevalence** - Number of individuals of a host species infected with a particular parasite species divided by number of hosts examined.

   \[
   \text{Prevalence} = \frac{\text{Number of host infected}}{\text{Number of host examined}}
   \]

2. **Mean Intensity** - Total number of host individuals of a particular parasite species in a sample of a host species divided by number of infected individuals of the host species in the sample.

   \[
   \text{Mean Intensity} = \frac{\text{Total number of cestodes obtained}}{\text{Total number of hosts infected.}}
   \]

3. **Relative Density** - Total number of individuals of a particular parasite species in a sample of host divided by total number of individuals of the host species.
Relative Density = \frac{\text{Total number of cestodes obtained}}{\text{Total number of hosts examined}}

Prevalence, Mean intensity and Relative density of cestode parasites were calculated, annual, season wise and month wise in relation to the following parameters:-

(a) Body weight of the host.

(b) Sex of the host.

(c) Cloacal Temperature of the host.

The fish *Channa punctatus* was selected to make normal and experimental studies on various haematological parameters. The fishes were caught with the help of local fishermen and animal catcher of department. These fishes were kept in the aquarium. For taking blood each fish was taken out of aquarium with the help of small butterfly net and immediately made unconscious either by stunning it with a sharp blow on the head or decapitation by severing the spinal cord. The caudal part of the fish is cut down and the blood is taken out from the vein and stored in clean vial with the help of Double oxalate as anticoagulent.

A thin blood film was made by spreading a drop of blood evenly across a clean grease free slide using a smooth edged spreader. Blood slides were stained in Leishman's stain or Wright's stain and were mounted in D.P.X.

The Red Blood Corpuscles (RBC) and White Blood Corpuscles (WBC) counts were recorded with the help of haemocytometer which includes two graduated pipette in which dilution is done. One pipette with a red bead is used for counting RBC while the other with a white bead for counting WBC. Besides those pipette the haemocytometer includes a glass slide ie Neubauer's Counting Chamber. It bears two counting chambers with a coverslip. Take the pipette meant for RBC which is already rinsed with alcohol or spirit or other and thoroughly dried. Take care that air bubbles would not be included. The pipette should now be transfered to the container of hayem's solution which is carefully sucked upto 101 mark
and blood is throughly mixed with hayem's fluid. Before starting to count RBC in this
diluted blood, place the coverslip on the counting chambers. The coverslip is supported
upon the slide plateform but remains separated from the central plateforms by a distance
of 0.1 mm First reject 3 or 4 drops of the mixture from the pipette. Now apply the tip of
the pipette between the coverslip and the platform and allow few drops of blood mixture
to flow in the narrow space between the coverslip and the counting chambers. If necessary
both chambers may be filled in this manners. Blood mixture remains filled up between
the coverslip and counting chambers because of capillary action. Air should not be taken
into and also pouring excess blood mixture so that the H-shaped groove remains free
from it. When the counting chambers are properly flooded the slide may kept aside for a
few minutes so that the RBC settle down on the bottom floor of the two counting
chambers. Now transfer the slide gently and carefully under the microscope without
disturbing the setteleed RBC and start counting them.

The procedure of counting WBC is the same as that of the RBC. The WBC were
counted in the four corners of one square mm in the central ruled area on both the slides
of the counting chambers of the haemocytometer. The WBC were recognised by the
refractile appearence and by the slight colour given to them by the stain contained in the
diluting fluid. The cells touching the boundary lines were not counted.

The method to estimate the flood based on the principle of making an acid haematin
solution of blood under experimentation in the graduated tube and comparing it with the
sealed comparison tube containing the standered acid haematin.

The graduated tube cleaned with distilled water and then with methylated spirit or 90% alcohal
was thoroughly dried before being used. Now with the help of dropper the N/10 HCl
solution was filled in the graduatetted tube and filled by sucking fresh blood of *Channa punctatus*
up to the mark of 20 cm. The blood of micropipette was added to the N/10HCl solution in
the graduated tube. The pipette should be introduced carefully into the tube and its lower
mouth should pass right upto the bottom into HCl solution. When blood had been expelled
the pipette was rinsed twice or thrice by distilled water. Every time the contents of micropipette
should be expelled into the graduated tube. The Acid haematin solution was thoroughly stirred with the help of a glass rod and then allowed to stand at least for 10 minutes. Acid haematin solution was gradually diluted by adding distilled water drop by drop with the addition of each drop of distilled water. The solution should be stirred and its colour match with that of the standered sealed tubes. This should be continued till colour of the acid haematin solution just fades away as compared to that of the standered comparison tubes. The reading before the colour just fades away as compared to that of the standered comparison tube. The reading before the colour just fade was taken as the correct and final reading. In addition in conjuuction with accurate estimation of Haemoglobin (Hb) and Red Blood Corpuscles (RBC) count. Packed cell volume(PCV) was observed in centrifuge at 3,000 rpm using Wintrobe tubes. Erythrocyte Sedimentation Rate (ESR) was observed by Westegren tube method. Knowledge of the PCV enables the calculation of "absolute" values.

The Mean corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) have been reffered to as "absolute" values. These values calculated from the results of the red cell count, haemoglobin estimation and packed cell volume.

1. **The Mean Corpuscular Volume (MCV)** which represent the average volume of the red cells. It is calculated from the red cell count and packed cell volume (PCV). The packed cell volume as a percentage is divided by the red cell count in millions per cubic milimeter and multipliedby 10. the answer is expressed in cubic micro (μμ).

\[
MCV = \frac{PCV \times 10}{TEC}
\]

2. **The Mean Corpuscular Haemoglobin (MCH)** represents the average weight of haemoglobin contained in each cell. The influenced by the size of the cell and the concentration of the haemoglobin in the cell. It is calculated from the haemoglobin and red cell count. The haemoglobin in grams per 100 ml is divided by the red cell count in millions per cubic milimeter and multiplied by 10. The answer is expressed in micro micro grams (μμg).
MCH = \frac{Hb \times 10}{TEC}

3. The Mean Corpuscular Haemoglobin Concentration (MCHC) represents the average concentration of haemoglobin in the red cells. It is calculated from the haemoglobin and packed cell volume. The haemoglobin in grams per 100 ml being divided by the packed cell volume, the result being multiplied by 100 and expressed as percentage.

MCHC = \frac{Hb \times 100}{PCV}
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<thead>
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<th>Number examined</th>
<th>Number Infected with cestode</th>
<th>Cestodes obtained</th>
</tr>
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<tr>
<td>Channa marulius</td>
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<td></td>
<td></td>
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<td>3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Labeo rohita</td>
<td>10</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Mastacembelus armatus</td>
<td>12</td>
<td>7</td>
<td>Jalpos pahujenesis n.g., n.sp.</td>
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<td></td>
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<td>Sukhobhythos capoori n.g., n.sp.</td>
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