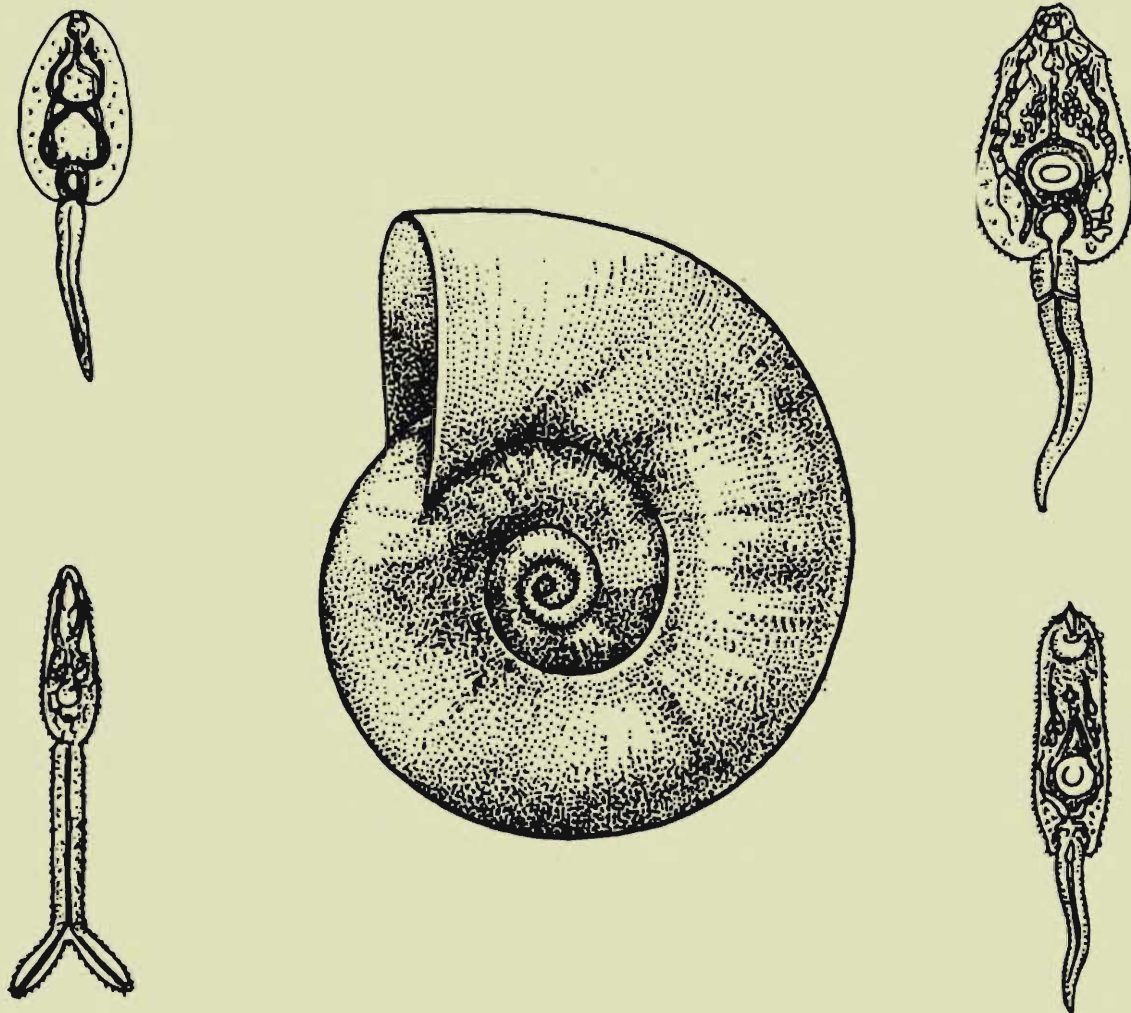


# SNAILS FLUKES AND MAN



ZOOLOGICAL SURVEY OF INDIA  
1991

# SNAILS, FLUKES AND MAN

Edited by  
Director, Zoological Survey of India



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Compiled by  
**N.V. Subba Rao, J. K. Jonathan and C.B. Srivastava**

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*Indoplanorbis exustus* in the centre with Cercariae around.

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## FOREWORD

Zoological Survey of India has been playing a key role in the identification and study of faunal resources of our country. Over the years it has built up expertise on different faunal groups and in order to disseminate that knowledge training and extension services have been devised. Hitherto the training programmes were conducted in entomology, taxidermy and ornithology. The scope of the training programmes has now been extended to other groups and the one on Snails, Flukes and Man is the first step in that direction.

Zoological Survey of India has the distinction of being the only Institute where extensive and in-depth studies are pursued on both molluscs and helminths. The training programme has been of mutual interest to malacologists and helminthologists. The response to the programme was very encouraging and scientific discussions were very rewarding. The need for knowledge and literature on molluscs was keenly felt. Helminthology is taught as a subject in a number of universities and colleges and several good text-books are available. We have therefore deliberately included more topics on molluscs, especially freshwater forms in the course contents as well as in this publication.

As there is no authoritative book dealing with Indian molluscs, it is hoped that this volume will meet the requirements of our students to some extent. It is mainly intended for helminthologists, but is expected to be used by all zoologists interested in freshwater biology.



Professor **Mohammad Shamim Jairajpuri**  
Director  
Zoological Survey of India

**Calcutta**  
**31st July, 1991**

# SNAILS, FLUKES AND MAN

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## **Snails, Flukes and Man - An Introduction**

**Mohammad Shamim Jairajpuri**

Director, Zoological Survey of India

Zoological Survey of India has been in the lead as far as basic research in malacology and helminthology is concerned. The two disciplines have grown up almost together. In fact, the thrust that was given to freshwater mollusc studies in the beginning of this century was largely due to the fact that these are intermediate hosts of helminth parasites, namely the flukes or the trematodes. During the First World War incidence of schistosomiasis was reported in the troops returning into India. The Indian troops returning from South Africa were suspected to carry the disease but there was some apprehension that a local focus might well exist. A search was initiated for an intermediate snail host in India. A survey of the freshwater molluscs of the Indian subcontinent was conducted. These and the consequent later studies culminated in the revision of freshwater molluscs of India by Annandale, Prashad, Rao and others (see Subba Rao, 1989). The monumental work on larval trematodes : *Cercariae Indica* by Sewell (1922) perhaps needs no introduction. From then on, the momentum that was built up in the study of the two groups, viz., molluscs and helminths has not only been maintained but enhanced further by the ZSI scientists like Ray, Rajagopal and Subba Rao (for details see Subba Rao, 1989); Chauhan, Soota, Hafezullah, Srivastava, etc. It is thus evident that ZSI has a long tradition of malacological and helminthological research. Soon after occupying the chair that was once held by Drs. Annandale, Sewell and other luminaries of the world of zoology, the bright past of this institute acted as a catalyst and inspired me for paving a beaming future for ZSI. Myself, being basically a parasitologist, soon after stepping into the portals of ZSI, recognised this valuable national resource, and realised that here is an opportunity and necessity for interaction between ZSI and scientific community in various universities and other research institutions. It has been an infectious inspiration which found ready recipients in my colleagues in ZSI. The result-First Training Programme on Snails, Flukes and Man.

Although Helminthology is taught as a specialisation in many of our Indian universities, Malacology does not find an important place. Zoological Survey of India is the only institute where studies are pursued in both the disciplines. Since the Survey plays a key role in the identification and study of both molluscs and helminths it has also taken up the pleasant task of organizing an interaction among malacologists and helminthologists of the country.

In general most of the helminthological studies in India tend to have bias towards either systematics or physiology of adult worms from vertebrate hosts or larval trematodes. The life cycle of a digenetic trematode cannot be completed without the assistance of an intermediate molluscan host. The distribution and epidemiology of diseases caused by these parasites in domestic and wild animals and in man depends on the presence of a susceptible and efficient intermediate host, the snail. In the control of

helminthic diseases more and more stress is given to physical perturbations rather than the use of single chemical or biological methods. In the control of schistosomiasis "environmental manipulation or alteration" is suggested as a better alternative. Moreover, now-a-days priority is accorded to environmental issues and management. Since molluscs and helminths are intergral parts of the environment, for a sound management of the same it is obvious that we should adopt a holistic approach towards its understanding. This brings us to the point that we should have an integrated approach to the problems of helminthic diseases in man and animals. Once it is accepted it becomes a corollary that a malacologist should know the essentials of helminthology and vice versa. Since both are involved in public health, their knowledge is a prerequisite for the environmentalist who advocates for a clean and healthy environment. It is also needless to say that a sound systematic knowledge of the causative organ of the disease and its spreading agent i.e., the parasite and the snail puts one on a better footing in handling the connected issues.

The first training programme on Snails, Flukes and Man is aimed at focussing our attention to some of the aspects of mutual interest to malacologist and helminthologist. Equal emphasis will be given to the study of molluscs and helminths. By snail we mean a gastropod mollusc and to know gastropods we should also know their 'kith and kin' i.e., other molluscan classes. Molluscs play an important positive role in our national economy. Frozen squid, cuttle fish and boiled clam meat along with frozen shrimp had fetched nearly Rs 600.00 crore during the year 1988-89. Frozen squid from India has become popular in Spain, France and Greece and now ranks among Indian Sea food exports. Very soon the giant African snail, *Achatina fulica* will become a source for earning valuable foreign exchange. Not only as gourmet's delight but also as objects of beauty molluscs have been appealing to man from the early ages. A number of ornamental shells and shell crafts are sold within the country and also exported providing livelihood for many people. Recent investigations have shown that some of the molluscs are sources of important bio-medical compounds (Bhakuni and Silva, 1975). The uses of molluscs are many and varied. Notwithstanding their importance, molluscs are comparatively a neglected group of animals in India. We still draw our information based on exotic text-books and species. It is time that we should get to know our Indian fauna and expose our students to the vast biological diversity that is found in our country.

As mentioned earlier helminthological investigations, hitherto have been concentrating more on adult worms or on larval trematodes. There are very few studies on the life histories and host-parasite relationships. I am positive that in the topics presented here some of the recent advances made in these two interrelated branches are highlighted and gaps in our knowledge or approach are hinted. In order to evolve a comprehensive perspective a three dimensional approach has to be applied. A combination of 'snail's eye view' 'flake's eye view' and the 'human eye view' will lead us to a better understanding and better control of the situation.

The topics presented in the book cover broadly the following aspects :

- i) magnitude of trematode infections in animals and man,
- ii) importance of molluscs in public and cattle health and in aquaculture,

- iii) importance of the life history studies of helminth parasites and the role of host ecology,
- iv) host-parasite relationships.

Since non-availability of literature is a problem that is often faced by Indian students and even experts we have compiled a few relevant topics as a sort of ready reference. Any suggestions, for its improvement or inclusion of more topics for better coverage in future, are welcome from the users/readers.

### Suggested Readings

- Annandale, N. 1920. The geographical distribution of the freshwater gastropod molluscs of the Indian empire and its direct bearing on that of human disease. *Indian J. Med. Res.*, Calcutta (Spl. congress). pp. 35-37.
- Bhakuni, D. S. & Silva, M. 1975. Biodynamic substance from marine fauna. *J. Sci. Ind. Res.*, 34 (1) : 36-53.
- Mehra, H. R. 1980. The Fauna of India and the adjacent countries. Platyhelminthes Vol I. Trematoda. Z. S.I., Calcutta. pp. 418.
- Mukherjee, R.P. 1986. The Fauna of India and the adjacent countries. The Larval Trematodes of India. Part I. Amphistome Cercariae. Z. S. I., Calcutta. pp. 87.
- Srivastva, C.B. 1982. The Fauna of India and the adjacent countries. Platyhelminthes Vol. I. Trematoda (supplement). Z.S.I., Calcutta. pp.163.
- Subba Rao, N. V. 1989. Handbook Freshwater Molluscs of India. xxiii +290 pp. Z.S.I., Calcutta.



## General Introduction to Mollusca

**N.V. Subba Rao**

Zoological Survey of India, Calcutta.

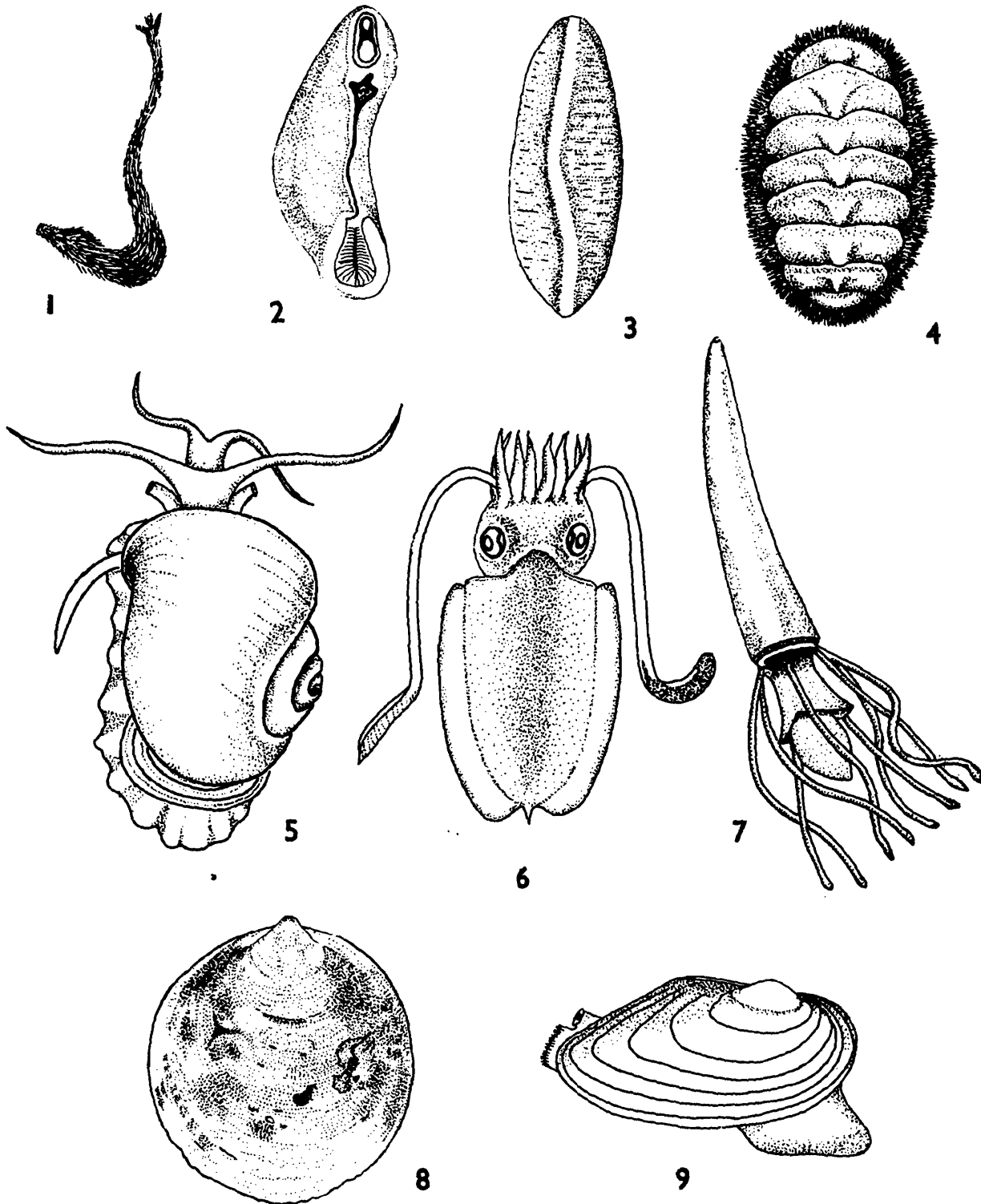
The phylum Mollusca includes a heterogeneous assemblage of organisms. A freshwater mussel looks strikingly different in structure from a squid or from a snail or a slug. The phylum cannot be easily defined or comprehended on the basis of a single character, since there is no such easily observable character that finds expression in all the molluscs (Fig. 1). However, majority of the molluscs can be recognised by the shell. A mollusc has to be recognised on the basis of a combination of traits or character states. But all the molluscs are built on a basic organizational plan.

### Phylogeny

A number of opinions have been expressed on the phylogeny of Mollusca. They have been considered as derived from Platyhelminthes (Graham, 1957; Morton and Yonge, 1963). Lemche (1961) considered Mollusca as a rather primitive group to be placed immediately above the Coelenterata. From molluscan-like ancestors Annelida and Arthropoda have evolved independently. According to Russell-Hunter (1968) Mollusca was directly derived from Turbellaria-like animal and had no connection with the stock or stocks which gave rise to the annelid-arthropod phyla.

Comparative anatomical and embryological evidences suggest phylogenetic interrelationships among flatworms, molluscs, annelids and arthropods. Fossil record draws a blank as far as their interrelationships are concerned. Soft bodied flatworms did not occur in the fossil state. Annelids were represented by a few fossils in the precambrian period, during which no fossils of other groups were represented. All the major classes of molluscs were already distinct by the cambrian period, when the first molluscan fossils were known. It is inferred that the ancestral mollusc might have occurred in precambrian period. This led some malacologists to conceptualize an artificial and ancestral molluscan model or archetype. Each of the several molluscan traits as we know them to-day are combined into this archetype. The reconstruction of molluscan evolution and phylogeny has been an intellectual exercise.

Of all the invertebrate phyla, there is striking similarity between Annelida and Mollusca. Both exhibit spiral cleavage and identical trochophore larvae. The discovery of *Neopilina*, in 1952, a living monoplacophoran, added further points in support of annelid-mollusc relationship. *Neopilina* displays an apparent metameric plan of structure in the replication of its parts. This prompted some to propose that molluscs arose from the annelids.



Representatives of Molluscan classes :

1. *Chaetoderma* sp. 2 & 3. Ventral and dorsal views of *Nemenia* sp.- class Aplacophora. Not known from India. 4. *Acanthopleura* sp. class Polyplacophora. 5. *Pila* sp. class Gastropoda 6. *Sepiella* sp. class Cephalopoda. 7. *Cadulus* sp. class Scaphopoda. 8. *Neopilina*- class Monoplacophora-Not known from India. 9. *Lamellidens* sp. class Bivalvia.

Many zoologists are of the opinion that annelids and molluscs arose from a free living flat worm-nemertine stock (Vagvolgyi, 1967, Harry, 1969; Stasek, 1972). Their ancestor, which might have looked like a small, ciliated vermiform organism had a through gut, a trochophore-like larva and pseudometamerism. The last mentioned character had got regularized in annelids, which developed more harmonious metamerism as an adaptation to the burrowing habit. The molluscs, however exhibit pseudometamerism in some primitive forms, eg. monoplacophorans and chitons but abandoned this tendency to metamerism in other classes. The two groups diverged from the common ancestor, prior to the pronouncement of either molluscan or annelidan characters. Just as in Turbellaria, molluscan foot exhibits backwardly directed waves during its locomotion. It is suggested that this rhythmic contraction of the flatworm became confined to the ventral surface of a mollusc and the dorsal parts became visceralized covered by a mantle and shell.

The theory proposed by Lang in as early as 1896, and developed recently by Stasek (1972) unfolds the molluscan frame work in four stages; ancestral form, transitional turbellariform stage, transitional molluscan stage, and advanced molluscan stage.

The ancestral form was an inhabitant of the precambrian period. It crawled about on the rocks or other hard substrata of oceans. It had a complete gut and had the ability to secrete abundant mucus, which acted as a protective cover and also as a smooth locomotory track.

In the transitional turbellariform stage, a radula and cuticle had developed. Once the skin got thickened with cuticle a ciliary respiratory mechanism had been innovated. To supply blood, a haemocoel consisting of sinuses and dorsal heart with pericardium had come into being, the last one by modification and enlargement of the gonoducts.

Transitional molluscan stage had gills contained in an incipient mantle cavity underlying the edges of the mantle. In the advanced molluscan stage, the shell in the form of a calcified cuticle has developed dorsally; gills became pedal retractors.

Thus according to Stasek (1972) secretion of a cuticle over the dorsal body surface together with an increase in size had brought in its evolution the broad distinguishing features of the phylum Mollusca.

### **Classification**

Linnaeus (1758) adopted the name, Mollusca, a term which was in fact proposed by Johnston (1650), but without developing any real concept of the phylum. Cuvier (1795) had shown better understanding of the group, and his concept approximates to modern ideas. In the beginning several other groups such as barnacles, brachiopods and other shelled forms were classified together with the molluscs.

Pelsencer (1892) classified the phylum Mollusca into five classes, namely Amphineura, Gastropoda, Scaphopoda, Lamellibranchia (Pelecypoda) and Cephalopoda. The present day classification is only a slight deviation and it had further split the class Amphineura.

Naef (1923) divided Mollusca into two subphyla namely Amphineura and Conchifera, the former including Aplacophora and Polyplacophora and the latter all the other classes. At present three subphyla namely Aculifera, Placophora and Conchifera are recognised. Although there is some consensus with regards to subphyletic division there is still a difference of opinion on the status of some classes

and their division into orders. Salvini-Plawen (1969), who contributed much to the knowledge on primitive molluscs, treats Solenogastres (= Neomeniamorpha) and Caudofoveata (= Chaetodermamorpha) as two distinct classes since the two have evolved independently. But Scheltema (1978) and Ivanov (1981) are in favour of giving these two the status of subclasses under the class Aplacophora, as they had closely linked origin and the variation seen in their body plan is not sufficient enough to elevate them to the level of classes.

Based on respiratory organs Milne Edwards (1848), divided the class Gastropoda into Prosobranchia, Opisthobranchia and Pulmonata which is acceptable even today. The one that was proposed by Spengel (1881) stresses the importance of the nervous system. He divided Gastropoda into Streptoneura and Euthyneura, which has been discontinued now. The euthyneurous condition of the nervous system has been attained in different ways in opisthobranchs and pulmonates. In the former it has been due to detorsion and in the latter it is the result of concentration of the anterior loop of the figure 8 into the head region. This single ring which innervates the visceral mass is in effect the posterior loop of the figure 8. Hence the division based on nervous system has not been favoured by many malacologists.

The two classes Scaphopoda and Cephalopoda were not subjected to many changes. Three names have been used for the other major class of Mollusca, namely, Bivalvia, Lamellibranchia and Pelecypoda. But after the publication of Treatise on Invertebrate Paleontology the term Bivalvia is accepted and has been in use at present. This term was first used by Linnaeus in the 13th edition of *Systema Naturae*, later adopted by Haas (1929) and accepted by Moore *et al* (1969). The old term Lamellibranchia has been used to a subclass.

Following Seed (1983) the classification of the phylum Mollusca is presented as follows :

Phylum Mollusca Cuvier, 1795

Subphylum Aculifera Hatschek, 1891

Class 1. Aplacophora von Jhering, 1876

Subphylum Placophora von Jhering, 1876

Class 2. Polyplacophora de Blainville, 1816

Subphylum Conchifera Gegenbaur, 1878

Class 3. Monoplacophora Wenz, 1940

Class 4. Gastropoda Cuvier, 1795

Class 5. Bivalvia Linne, 1758

Class 6. Scaphopoda Bronn, 1862

Class 7. Cephalopoda Cuvier, 1795

The relationship of classes within the phylum Mollusca is a subject of divergent opinions. Based on embryology and evolution of shell the Cephalopoda were considered by some to have evolved independently from Coelenterata. Bivalves, scaphopods and gastropods have retained tetracyclomeric symmetry as in Cephalopods. But it does not seem possible to derive bivalves from gastropods or vice versa (Lemche, 1961).

Scaphopods and bivalves have some similarity in their shells and both do not possess a distinct head (Seed, 1983). In the early gastropods there is a planospiral shell which was also found in primitive cephalopods. Both the groups have a distinct and well developed head. From the parasitological point of view (or rather from a 'flukes' eye view) Wright (1971) suggests close relationship between gastropod, scaphopod and bivalve classes and separates cephalopods from these.

According to many malacologists molluscs are divided into two subphyla Aculifera and Conchifera. the former is primitive and includes Polyplacophora and Aplacophora. They have diverged much earlier in the molluscan evolution. The Conchifera includes gastropods, bivalves, scaphopods and cephalopods and have probably arisen from the Monoplacophora (Runnegar and Pojeta, 1974; Yochelson, 1974).

Stasek (1972) proposed three separate lines of evolution within the phylum and distinguished three subphyla. One line i.e. Aculifera includes Aplacophora, which is closest to the stem group and gave rise to Solenogastres and Caudofoveata. The second line includes Placophora represented by a single class Polyplacophora. It has all the salient features of the molluscan framework. The third line is that of conchifera which includes all the major molluscan classes. Three successful stocks of the phylum, namely the Gastropoda, Bivalvia and Cephalopoda are included in this subphylum. Among other two classes only Scaphopoda shows some signs of advancement while Monoplacophora is considered to be nearer to the origin of this line.

According to Harry (1969) a prochiton stock gave rise to the Polyplacophora, which in turn were ancestors to the Aplacophora. All other molluscs arose through a separate line, the Proconchifera. Monoplacophora is the earliest derivative of this branch. Proconchifera further gave rise to a group called Mesoconchifera, which in its turn gave rise to Probivalvia and Metaconchifera. The former gave rise to Bivalvia and Scaphopoda, and from the latter arose separately Gastropoda and Cephalopoda. The last mentioned two classes only have median dorsal mantle cavity. This character is attributed to the remote promollusc by several writers. But it is thought to have arisen only at the Metaconchifera stage.

### **General Organization And Function**

Development of the parasite takes place inside the body of the mollusc. In other words host's body offers the necessary environment for the parasite to complete its life cycle and to increase its progeny. The physical and biochemical properties of various parts of the molluscan body have their impact on the invading pathogen or parasite. The molluscan body is divisible into head, foot, and a dorsal hump-like visceral region covered by a shield-like mantle. The head and foot are so combined that together it is known as head-foot region.

General form and functions of the molluscan body are given below :

1. **Molluscan skin and subdermal tissues** : The skin and subdermal tissues are the first to be contacted by an invading miracidium. The skin is composed of epithelium among which are distributed ciliated columnar cells and goblet cells. The distribution and shape of the epithelial cells vary depending on the region of the body.

Dermal secretions, which consist of mucus are important physiological phenomena

of the mollusc. The nature of these secretions which are secreted by the goblet cells, is dependent on the part of the body from where these originate. The secretions of body surface and mantle contain fluorescent substances, which are absent in the foot-sole mucus of pulmonates. Foot-sole region of gastropod is the region where the mucus secreting cells are abundant. The periphery of foot and mantle contain glandular cells whose secretions act as repellants and protect the mollusc from predators or pathogens such as miracidia.

The nature of molluscan skin secretions and their impact on attacking larvae are subjects worth pursuing.

The subdermal tissues are composed of muscle layers of varying complexity. In the foot region there is a dense tissue which prevents the entry of larvae. Even when the larvae somehow enter the tissue their further growth is prevented by the strong development of muscle layers. Where the tissues are loose larvae get an opportunity for settlement and for further growth.

**2. The Mantle and shell :** These are the two important structures which distinguish Mollusca from all other phyla. The major function of the mantle is to secrete a protective shell. The mantle encloses a cavity, known as mantle cavity, which is primarily a respiratory chamber. Within the mantle cavity are situated the ctenidium, osphradium, and the hypobranchial or mucus gland. Excreta from the anus and renal opening are discharged into it. It also houses the female genital opening and the male genital organ. The mantle cavity thus serves for respiration, excretion, defaecation and also for reproduction.

In terrestrial molluscs (pulmonates and some prosobranchs) the gills are absent and the mantle cavity with a rich supply of blood vessels serves as a lung. The mantle cavity is lined with ciliated epidermal cells which create currents by their movements. These ciliary currents assist in the oxygenation of water reaching ctenidia and in the removal of waste products. In sedentary gastropods and bivalves collection of food is done through ciliary filter feeding mechanism. In some freshwater prosobranchs, such as *Bellamya* and *Bithynia*, ciliary currents bring in additional food to these otherwise algal browsers. The ciliary currents are important as far as the trematode life cycles are concerned. The incoming currents will serve as routes of invasion for miracidia or infective eggs of the parasite. The effluent currents discharge chemical attractants or cause local turbulence which can cause stimulation to searching larvae. How far these host factors are attractants for encouraging an initial host-parasite contact is a subject of varying opinions.

The mantle edge bears three folds, namely the outer, middle and inner. The outer fold is involved in the secretion of the shell. The inner surface of the outer fold secretes the periostracum, whereas its outer surface secretes the outer calcareous layer. The inner calcareous layer is secreted by the entire mantle surface.

The molluscan shell consists of three layers, namely an outer most organic and uncalcified structure known as periostracum, a middle calcareous layer and the innermost nacreous or mother-of-pearl layer. The periostracum is composed of a number of layers, which are more in freshwater gastropods than in the marine species. The second layer is the main structure of the shell and it is usually composed of calcium

carbonate crystals. The innermost nacreous layer is found in several families of molluscs and is not found outside this group. The calcareous layer may consist of entirely aragonite or calcite crystals. These crystals are like bricks which are united by the conchiolin matrices, which correspond to concrete. The organic components of nacreous conchiolin consist of a linear network of extremely thin inter-lamellar sheets held together by transverse bridges. The nacreous conchiolin matrices vary in their structures in different groups. These structural patterns were statistically characteristic at the class level of taxonomy.

Hitherto the molluscan shell is considered to consist of three layers as mentioned above, but recently a calcareous layer (mosaicostracum) was discovered between the periostracum and outer calcareous layer. Ultra structure of the mosaicostracum is a potential tool for specific identification and for the study of evolution.

**3. Digestive system :** Digestive system converts food into a physical and chemical state suitable for proper absorption and utilization by the numerous cells of the body. It provides a dynamic environment and induces the larvae to emerge out of the eggs, when the latter are swallowed by an appropriate snail.

Biochemistry and physical properties of the alimentary system determine the compatibility or incompatibility of the molluscan host. The digestive system includes the mouth, the buccal mass-including the buccal cavity, odontophore and radula, oesophagus, stomach, digestive gland and intestine.

Externally the mouth is surrounded by horny 'lips' and 'jaw' in gastropods, a pair of fleshy palps in bivalves, and hard mandibles in cephalopods.

The buccal cavity contain two important structures, odontophore and radula. The latter is a ribbon like structure bearing a number of teeth. It scrapes the food from the substratum or cuts down solid objects into finer particles to facilitate their easy handling by the system. The radula is moved by the muscles of the buccal mass, which are supplied with haemoglobin, a red pigment. There are a pair of salivary glands opening into the dorsal surface of the buccal cavity. The secretions from the salivary glands serve as lubricants for the radula in its movement. The mucus secretions from these glands contain proteolytic enzymes and amylases. The mucus entangles the food particles which are formed into strings. These strings of food pass from the buccal cavity into a tubular oesophagus, from where they move into the stomach.

Stomach is an important and complex organ in the digestive system of molluscs. It is the site of extracellular digestion and into this open the digestive gland and intestine. The stomach consists of a style sac, which secretes a rod-like structure known as style. This crystalline style is present in some gastropods and bivalves. The style rubs against the thickened gastric shield of the stomach and breaks down in bits releasing various enzymes into the stomach. These include amylases, some times glycogenases and oxidases. In many herbivorous gastropods, especially basommatophoran snails, the anterior part of the stomach is modified as a crop or gizzard and contain sand grains to assist in the grinding of food particles.

Digestive gland forms a major part of the animal. It receives food particles from the stomach. The digestion in the gland may be either intra or extracellular or a combination of both. Extracellular digestion is highly developed in the carnivorous prosobranchs. Undigested food passes from the digestive gland back into the stomach

and thence into the intestine. In molluscs the intestine has no major absorptive function. It helps in rolling the faecal matter into pellets or chains.

It is not known exactly in which part the ingested eggs hatch. *In vitro* studies also did not give any clue. It is presumed that sequential and a series of actions by enzymes and mechanical grinding in gizzard is needed to break the opercular seal of eggs (Wright, 1971).

**4. Circulatory system :** The circulatory system provides the route as well as the environment for the initial development of the parasite. The vascular system may be open as in majority of molluscs or closed as in cephalopods. The closed system has capillaries connecting the arterial and venous systems, whereas open system has sinuses to transport blood.

Primitive gastropods have retained two auricles and the circulatory plan of the ancestral mollusc. In all other gastropods the right auricle is either vestigial or totally absent. Bivalves have two auricles enclosing a median ventricle. Gastropods also possess a single ventricle. After vertebrates, Cephalopoda is the only class to possess a fully enclosed high pressure blood system, consisting of well established arterial and venous systems. The blood is carried to all parts of the body by a single aorta, arising from the ventricle, dividing into an anterior and posterior artery. In many gastropods and bivalves there are two aortae namely posterior and anterior, the former supplying the visceral mass and the latter to head and foot.

**5. Reproductive System and Cycles :** Sexes are separate in all three minor classes and also in Scaphopoda, Cephalopoda, in majority of the bivalves and in higher prosobranch gastropods. Only in certain gastropods and bivalves one species may be dioecious while its related species may be hermaphroditic. Archaeogastropoda, among prosobranchs and opisthobranchs are protandric hermaphrodites, while pulmonates are simultaneous hermaphrodites. A few freshwater prosobranchs, i.e. thiarids are parthenogenic.

In many hermaphroditic species, mainly in opisthobranch and pulmonate gastropods and in bivalves, the change of sex is a general rule. Hermaphroditism is supposed to have arisen due to influence of ecological factors, and species which encounter difficulties for their reproductive activity, such as land molluscs have developed hermaphroditism. In hermaphrodites the male and female gametes are produced either in separate follicles or from different areas of the same follicle. There is a common hermaphroditic duct which divides into a separate sperm duct and an oviduct for discharging out the male and female gametes respectively.

In the primitive archaeogastropoda the gametes pass from the gonad through the genital duct into the right nephridium and finally into the mantle cavity. The genital duct consists of two parts, the gonoduct proper and the right nephridium. Fertilization is external and there is no need for copulatory organ.

In all the other gastropods the right nephridium has degenerated leaving only the part that function as a gonoduct. The gonoduct consists of the nephridial part of the gonoduct, the gonoduct proper and the pallial duct. The pallial part of the gonoduct is modified to store the sperm and to secrete the egg membrane. A copulatory organ had developed to transfer the sperm into the female.

Pulmonates and opisthobranchs have complex reproductive systems, which exhibit a number of variations. In these two subclasses details of reproductive system, including the structure of penis, are important in the systematics.

Two types of larvae, namely trochophore and veliger are seen in gastropod molluscs. The former is found in primitive gastropods and the latter in many marine forms. A free swimming larva is absent in some marine prosobranchs, Neogastropoda, nearly all freshwater prosobranchs (excepting *Thiara torulosa*) and in almost all pulmonates, and a tiny snail emerges out of the egg at the time of hatching.

Many freshwater snails of India breed throughout the year with a peak period just after monsoon. Host reproductive cycle can also have an effect on the development of the parasites. At least in some species it was seen that spent individuals or juveniles were more susceptible to trematode infection, while older individuals are less susceptible. It is probably due to protein reduction in blood which becomes less nutritive (Wright, 1971).

Some of the molluscs excrete hormone like substances that affect the activities of other members of their own species. Such compounds are called pheromones. Based on their activity these chemical messengers are known as triggering pheromones and primer pheromones. The former cause behavioural changes and the latter influence the reproductive system without causing immediate behavioural changes. Certain basommatophoran snails release primer pheromones which inhibit growth and fecundity. These pheromones in small quantities, are beneficial to growth and survival but in higher proportions limit the density of populations.

### **Abundance and Distribution**

Estimates of the number of molluscan species vary between 80,000 and 1,00,000. This figure is purely provisional as taxonomy of many families of molluscs is still in a confused state. It is well known that there are "lumpers" and "splitters" among taxonomists and depending on their approach the numbers of species may decrease or increase as the case may be. Some times revisionary studies of families may also bring down the number of species in the changed species concept. For example in a recent revision of the family Lymnaeidae by Hubendick the number of species have been reduced from 1000 to 40.

According to one estimate there are 50,000 (or 80,000) species of gastropods, 15,000 (or 20,000) of bivalves, 400 cephalopods 300 scaphopods and 635 species of other classes (Polyplacophora-500, Aplacophora-130 and Monoplacophora-5). Although molluscs have occupied all possible habitats except aerial, these are abundant in the marine environment, which account for more than half of the known species. Of the seven recognised classes five, namely Aplacophora, Polyplacophora, Monoplacophora, Scaphopoda and Cephalopoda are exclusively marine. The two large classes viz. Gastropoda and Bivalvia are the most successful in adapting to different marine and freshwater habitats and include 94% of the species of molluscs. The former has successfully colonised terrestrial habitats, whereas the latter could not overcome their filter feeding habit and hence had not been able to invade land.

Five major classes, namely Polyplacophora, Gastropoda, Scaphopoda, Bivalvia and Cephalopoda are represented in India. Against a total of about 400 families, it is

estimated that about 250 families occur in the Indian region. These include 5042 species in all of which 3271 are marine, 1487 are land and 284 are freshwater. The estimates of land and freshwater species are based on the actual data, since these have been systematically well worked out. But the same cannot be said about the marine molluscs of which our knowledge is far from satisfactory.

Molluscs are found in various habitats, from the deep sea (2000 fms) off Andaman and Nicobar Islands to higher elevations (about 5000 m) in the Himalaya mountains. But these are found in abundance in the rocky intertidal zone along our coast and in the coral reef ecosystem of Gulf of Mannar, Gulf of Kutch, Andaman and Nicobar Islands and Lakshadweep. In comparison, sandy coasts support less molluscan fauna which include mostly burrowing and interstitial forms.

From the malacological point of view five important zones of India can be recognised, namely Insular zones of Andaman and Nicobar Islands and Lakshadweep, North east India, Western Ghats, Plains of Peninsular India, and North-west India. In the last mentioned zone are found molluscs, which are characteristic of arid and semi-arid habitats. *Hemiplecta basileus*, the largest native imperial snail, is characteristically associated with the teak forests of south India. Land molluscs are abundant in the shady humid forest ecosystems of North east India and Western Ghats. Some of the typical land operculates are distributed in south India and Sri Lanka. A species of slug (*Anadenus altivagus*) occurs at an altitude of 4900 m in the Himalayas.

Out of the 8,765 freshwater species estimated to exist in the world, about 220 species are known from India. Majority of the species occur in streams in Western Ghats or in localised areas in North-east India and Himalaya mountains. About one third of the species are inhabitants of lentic waters and are distributed throughout the country. Some bivalves (species of *Pisidium* at about 4600 m) and even gastropods are dredged from the glacial lakes in the Himalayas. Indian gastropod fauna is remarkable in that it includes a few genera like *Cremnoconchus*, and *Neritina* whose close affinities are with marine representatives of their respective families. The subfamily Bellamyinae and the family Pilidae have Gondwanaland origin. The former is distributed in Africa, India and South east Asia and the latter in tropical South America, South Africa, India, Sri Lanka and South east Asia.

Indian freshwater bivalve fauna include two families Unionidae and Pisidiidae of great antiquity and cosmopolitan distribution and also a family of recent origin, namely Etheriidae. The last mentioned family has a discontinuous distribution occurring in South America, tropical Africa, Madagascar and India.

Marine molluscs are numerically abundant as individuals and as species. Majority of the known species are from the littoral region and search in the offshore and deep waters may reward one with new records and new species.

### Size and Diversity

World size records of shelled molluscs are available in Wagner and Abbott's Standard Catalog of shells. As far as the Indian molluscs are concerned no authentic records are maintained on the smallest or largest shell of India. However, based on the National Zoological collections in the Zoological Survey of India an idea of size range among Indian fauna is given. Excluding the microscopic forms (meiofauna) smallest specimens are from the marine genus *Cyclostrema* and the freshwater *Gyraulus*, which

measure 0.75 mm and 4.0 mm respectively. *Charonia tritonis*, collected off Nicobar Island, is the largest Indian gastropod and measures 35.0 cms. The world size record for the same species, collected from Pacific, is 48.26 cms. Among bivalves the smallest sized specimens are found in the freshwater genus *Pisidium*; *Pisidium annandalei* measures 3.00 mm. The largest bivalve in National Zoological Collection is *Tridacna maxima* measuring about 37.0 cms and occurring in the littoral zone off Andaman and Nicobar Islands. In fact (we had seen) specimens of *T maxima* measuring around 60 cms. in the Kamorta Island, (where these shells are used as containers by the nicobarese for feeding their pigs.). The largest clam shell, *Tridacna gigas* measuring 136.87 cms and weighing 507 lbs was collected off Sumatra.

Not only in size but also in shell shape, sculpture and colouration molluscs exhibit a great amount of diversity. The diversity is more pronounced in marine molluscs, which display flamboyant diversity in color and in form within and between species. In comparison, freshwater and land molluscs are less colourful.

Diversity is also evident in their feeding habits. There are herbivores, carnivores, scavengers and deposit feeders, suspension feeders, commensals and parasites. Excepting *Pila* which may show some tendency to carnivorous feeding so far no carnivorous freshwater mollusc has been reported from India. The only carnivorous land snail of India, *Gulella (Huttonella) bicolor* has been reported to feed on garden snail. All the freshwater forms are either herbivores or suspension feeders. It is the marine molluscs which occupy diverse habitats and exhibit diverse feeding habits.

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## General Introduction to Platyhelminthes

**M. Hafeezullah**

Zoological Survey of India, Calcutta.

The representatives of this phylum are commonly known as flatworms. They are acoelomate and soft-bodied, the intervening spaces between various organs and the muscles being filled with a network of connecting tissue cells and fibres called mesenchymal parenchyma. This parenchyma is commonly known as packing tissue also. The digestive tract is incomplete (with few exceptions). There are no special skeletal, circulatory and respiratory structures. The nervous system is simple. The excretory system is protonephridial i.e. consisting of flame cells, capillaries, collecting vessels, bladder and excretory pore. They are typically hermaphroditic (except schistosomes and some didymozoids).

### Classification of Platyhelminthes

Flatworms are commonly divided into three classes; *Turbellaria*, which are with rare exception free-living; *Trematoda*, all are strictly parasitic and known as "flukes", and *Cestoda*, all are strictly parasitic and known as "tapeworms", which are excluded from the scope of the present article.

### Class Turbellaria

The *Turbellaria* originated in the sea, so most of them are marine. Two of the five orders are strictly marine. The terrestrial forms have evolved from freshwater ones. They are predominantly free-living but the rhabdocoelous suborder *Temnocephalida* have members which may be commensals or ectoparasites chiefly on echinoderms, freshwater crustacea, molluscs and turtles. As an adaptation to parasitic life, they have developed tentacles anteriorly and adhesive and holdfast organs posteriorly. The suborder *Lecithophora* has a small group with doliiform (barrel-shaped) pharynx, called *Dalyellioida*. These dalyellioid turbellarians are parasites occurring within the bodies of turbellarians, molluscs and echinoderms. It is these dalyellioid rhabdocoelous turbellarians parasitic in molluscs which are undoubtedly the ancestral forms of parasitic *Trematoda*.

*Acoela* is the most primitive and *Allocoela*, *Rhabdocoela* and *Polycladida* have directly evolved from it while the *Tricladida* radiated from *Allocoela*.

### Class Trematoda

The flukes are generally divided into three subclasses, namely, *Monogenea*, *Aspidogastrea* and *Digenea*.

### *Monogenea* Van Beneden, 1858.

The *Monogenea* generally have simple and direct life cycles having a single host. The life cycles do not involve intermediate hosts and metagenesis or polyembryony is

lacking (*Gyrodactylus* is an exception). They are mostly ectoparasites of lower vertebrates (excepting *Polystoma*). The adults have a well-developed posterior sucker which is single (Monopisthocotylea Odhner, 1912) or multiple (Polyopisthocotylea Odhner, 1912). The mouth at the anterior end of the body is not surrounded by muscular oral sucker as is present in Digenea but there are paired secretory elements which secrete adhesive material. These paired secretory elements surrounding the mouth are adhesive in nature and therefore they are better known as prohaptors. The intestine may be a simple sac or more commonly it is forked with many lateral diverticula arising from the two main caeca. There is no anus. Usually they have retained eyes, whereas only free-living larval stages of some digenetic trematodes, possess ocelli.

The Monogenea are relatively very host-specific generally parasitising the same host species or related species in the same genus, so much so that sometimes host is identified if the monogenean fluke is known. Since these flukes parasitise lower vertebrates, molluscs are not involved in any way in their life-cycles, they differ sharply from the Digenea and Aspidogastrea.

The Monogenea are generally ectoparasites of fishes and amphibians. Endoparasitism is not common. They have simple life cycles involving only a single lower vertebrate host. However, *Polystomum integerrimum nearcticum*, presents atypical life cycle with an interesting combination of ecto- and endoparasitism. It occurs in the urinary bladder of frogs and produces eggs which pass to the exterior with urine or when the frogs breed in the spring. The developing larva is protected by a shell. In a few weeks the larva hatches, swims with the aid of ciliary bands, and attaches by hooklets to gills of tadpoles. If attachment is on the external gill, the larva matures sexually as an ectoparasite within a few weeks to produce viable eggs. This gill generation dies when the tadpole host metamorphoses. If the larva is attached more internally on the gill, at the time of metamorphosis of the amphibian it migrates over the body surface and through the intestine it reaches the urinary bladder where it becomes a typical adult in about three years. The ectoparasitic gill generation is smaller than the endoparasitic bladder generation. Thus in *Polystomum integerrimum nearcticum*, there occur two possible generations, a neotenic (sexually mature larva) one on young tadpoles and an endoparasitic one in older tadpoles and adult frogs.

#### Aspidogastrea Faust and Tang, 1936

This is a small group of trematodes. They are primarily the parasites of marine and freshwater snails or clams, inhabiting their mantle and pericardial and renal cavities. Incidentally they parasitise the gut of vertebrates like fishes and turtles, and the bile passage of elasmobranch fishes also. In such incidental cases the vertebrate predate on the gastropod or bivalve molluscs infected with the aspidogastrid parasites which get liberated in the gut of the vertebrate and thus a vertebrate host is also introduced in the life-history of Aspidogastrea. Actually a vertebrate appears to be necessary for maturation of most species rather than just an adjunct host helpful as a dispersing agent.

The life-history of Aspidogastrea is simple and direct as they do not show alternation of generations, but some of them have alternation of hosts. The larva or the young worm directly develops into an adult either in the same molluscan host or in

another individual of the same or different species. Sometimes when the infected mollusc (a gastropod or a bivalve) is eaten by a fish or turtle, the parasite attaches itself to the wall of the gut of the vertebrate. That is how a second host (a vertebrate) is also initiated in the life-history of *Aspidogastrea* as occurs in Digenea. In the case of *Stichocotyle nephropsis* Cunningham, 1884, the life cycle is more complicated. In this species larva gets encysted on the wall of the intestine of lobsters, *Nephrops norvegicus* and *Homarus americanus*, while adults occur in the biliary passages of elasmobranch fishes. The first stage larva may develop in a marine mollusc, thence to a lobster. This situation suggests how alternation of hosts may have evolved in *Aspidogastrea*.

*Aspidogastrid* genera are grouped into two families i.e. *Aspidogastreidae* and *Stichocotylidae*, the latter comprising a single genus *Stichocotyle* Cunningham, 1884, inhabiting bile passages or spiral valve of skates.

Although *Aspidogastrea* is a small group of flatworms, it poses a problem in determining its status *vis-a-vis* Monogenea and Digenea since some of their characteristics overlap with the latter two groups. Thus most authors consider it to occupy an independent rank intermediate in position between Monogenea and Digenea.

The *aspidogastrid* trematodes cannot belong to Monogenea since molluscs appear in their life-history, there is no oral sucker, they do not have posterior sucking disc consisting of hooks, anchors, bars and clamps, the excretory pore or pores are posterior rather than anterior in position, the intestinal tract is always rhabdocoelic in type as in the turbellarians and digenetic gasterostomes, and they are endoparasites rather than ectoparasites. On the other hand, they resemble the Digenea in most of their anatomical characters but there is no evidence of alternation of generations in *Aspidogastrea*, and the larva has no trace of ciliated epithelium. In their very large posterior sucker subdivided into compartments by septa, they differ from both Monogenea and Digenea.

### Digenea Van Beneden, 1858

Typically the digeneans are leaf-like in form but they exhibit considerable variety of forms and sizes. The life-cycle is complex and indirect i.e. metagenesis or alternation of sexual and asexual generations occurs, and two to four hosts are involved in the life-cycle. The first intermediate host is always a mollusc (with three exceptions) and in the case of man and domesticated animals it is always a snail while the definitive host is always a vertebrate for the sexual phase of the life-cycle. Interpolated in between these two hosts there may be one or two second intermediate host(s). In the case of progenesis, the vertebrate definitive host is deferred from the life-cycle and the worm sexually matures in the mollusc or crustacea. Most of the digenean adults generally occur in the intestinal track of vertebrates but they may also be found in the blood vessels, eye-sockets, liver, swimbladder, lungs and the gonads.

In the gasterostomes mouth is situated near the middle of the body and is not surrounded by a sucker. The gut is rhabdocoelous. The genital atrium is near the posterior end of the body. In the life history the gasterostomes do not use a snail as the first intermediate host. They use oysters or marine bivalves or freshwater mussels for the purpose. The sporocyst is branching type and the cercaria is "Oxhead"

Sexes are united in all the digeneans except in the family Schistosomatidae. In the family Didymozoidae sexes are united as a rule but in some genera there seems to be a gradual tendency towards sexes becoming separate while in others gonochoristicism is pronounced.

The didymozoids are aberrant forms because generally they are cyst-dwelling and some of them show pronounced gonochoristicism. They parasitise marine fishes. There seems to be a gradual dimorphism arising out of hermaphroditism in the Didymozoidae.

The life cycle of the family Didymozoidae Poche, 1907 is not completely known. Eggs are small, bean-shaped, or elliptical to oval, usually embryonated in utero. Miracidium is spined anteriorly, but not motile. The intermediate hosts, larval stages like sporocyst, redia and cercaria are not at all known. Juvenile or pre-adult stage is also not known. The larval stages encountered in invertebrates like crustacea and medusae as well as in the body cavity and tissues of fishes have been called as metacercariae or didymozoid larvae. But Yamaguti (1970, 1971, 1975) distinguished two groups i.e. Monilicaecum and Torticaecum and recognised three stages (*Premonilicaecum*, *Monilicaecum*, *Postmonilicaecum*; *Pretorticaecum*, *Torticaecum* and *Posttorticaecum*). According to him the first stage occurs in crustacea (medusae also), the second stage in fishes which serve as paratenic hosts, and *Postmonilicaecum* and *Posttorticaecum* develop to adults in the definitive hosts. As the knowledge about the complete life cycle of didymozoids is not known, it is not clear at what stage and how gonochoristicism starts and unisexuality determined in this family.

Members of the family Schistosomatidae Looss, 1899 are sexually dimorphic. Of all the dimorphic forms the best known are the schistosomes. Males and females are separate and in the blood vessels the male lives in the gynecophoric canal of the female. The life cycle does not include redial generation.

The determination and separation of sexes take place at the earliest stages of larval development. Two strains of sporocysts are produced by the miracidia so that the cercariae produced by one sporocyst will be either male producing or female producing only and not both. In the blood vessels both types of cercariae enter. Males become sexually mature much early while the females remain undeveloped for a pretty long time. The interesting situation is that in some dioecious blood flukes the females do not develop fully to maturity in the absence of the males. It has been suggested that males may be producing a pheromone essential to the females to attain sexual maturity and to maintain reproductive function, sperm or a testicular secretion is not the stimulating agent.

### Digenean Life Cycles

The life cycle of digenetic trematodes is complex. It is indirect i.e. a sexual generation alternates with a series of asexual larval generations. The first intermediate host is invariably (with three exceptions) a mollusc; the second intermediate host, if any, may be a crustacea or a fish or a mollusc; the final or definitive host is always a vertebrate. The outline of a generalised life cycle is presented here :

**Liberation of Egg:**

The life-cycle begins when the eggs of the worm escape from the body of the definitive host *via* the intestinal, genitourinary, or pulmonary tracts. In *Philophthalmus megalurus* the eggs are washed from the bird's eyes when it feeds. In *Acetodextra amiuri* (a heterophyid), the worm matures in the ovaries of catfish. The young worms invade the developing eggs of the host. Eggs are retained in the uterus (as in the above case), and the gravid worms are discharged when the host spawns. After reaching the water the worm ruptures forcibly and ejects a stream of eggs into the water.

**Hatching of Miracidium :**

When discharged in water they may be fully developed (*Opisthorchis*, *Dicrocoelium*, *Heterophyes*, *Metagonimus*) or may require some more time to complete development outside the host before hatching (*Fasciola*, *Fasciolopsis*, *Paragonimus*, *Echinostoma*). Hatching of the larva or miracidium takes place in water. In operculate egg the operculum or the lid is opened while in the nonoperculate egg the shell is thin and weak and it breaks vertically at the top and the ciliated miracidium comes out in the water.

**Miracidium :**

The ciliated miracidium actively swims in water in a characteristic rotating manner and seeks an appropriate host, which is always a mollusc. Some miracidia are attracted by their proper snail hosts as are filings to magnet. The miracidia do not feed and they die in 24 hours or less if unsuccessful in finding a proper molluscan host. They also die if they do not reach water. It then penetrates the exposed portions of the mollusc, e.g. gills (*Fasciola*), head, tentacles and foot (*Shistosoma*). In other instances (*Clonorchis* and *Dicrocoelium*) the miracidium may be eaten by the molluscan host. If the miracidium does not find the mollusc of its choice within a few hours it perishes. The cilia of the miracidium disappear or lost inside the mollusc.

**Sporocyst and Redia :**

In the body of the mollusc, the miracidium metamorphoses into a sac-like larva called sporocyst. It absorbs liquid food from the host and acts as brood pouch for the asexual development of the second generation larvae called rediae. The rediae escape through the ruptured wall of the sporocyst. These rediae then produce the third generation larvae called the cercariae which escape out through the anterior birthpore. So extensive is the multiplication of the germ cells that thousands of cercariae may develop from one miracidium. In some cases a generation of daughter sporocysts and in others a generation of daughter rediae may also develop.

**Cercaria :**

The cercariae are highly developed and are equipped with motile tails. They pass through the tissue of the mollusc into the cavity between the body and the shell, and thence into water. The liberated cercariae actively swim with their tails, the body being anterior (except in those with forked tails). After moving for some time in water, they may attach themselves to the surface film or settle to bottom.

**Metacercaria :**

In order to invade the definitive host, the cercariae become active again and penetrate the exposed surface (*Schistosoma*) and get encysted in the definitive host as metacercariae, or become encysted on aquatic plants (*Fasciola hepatica*) or in a second intermediate host (fishes as in the case of *Opisthorchis sinensis* and crustacea as in the case of *Paragonimus westermani*). When the aquatic plant or the second intermediate host is eaten by the definitive host, the metacercariae find entry into the body of the latter. After some time the encystment dissolves. The metacercariae change into adults, migrate to the locations of choice where they mature and the cycle is completed.

Variations in this life cycle may occur by the addition of an extra sporocyst or an extra redial generation. Variations may also occur in the above mentioned generalised life-history by the absence of one or two larval generations as exemplified below :

Miracidial generation absent	<i>Dicrocoelium dendriticum</i>
Sporocyst generation absent	<i>Euparyphium ilocanum</i>
Redial and encysted metacercarial generations absent.	<i>Schistosoma spp.</i>

**Significance of Life-history :**

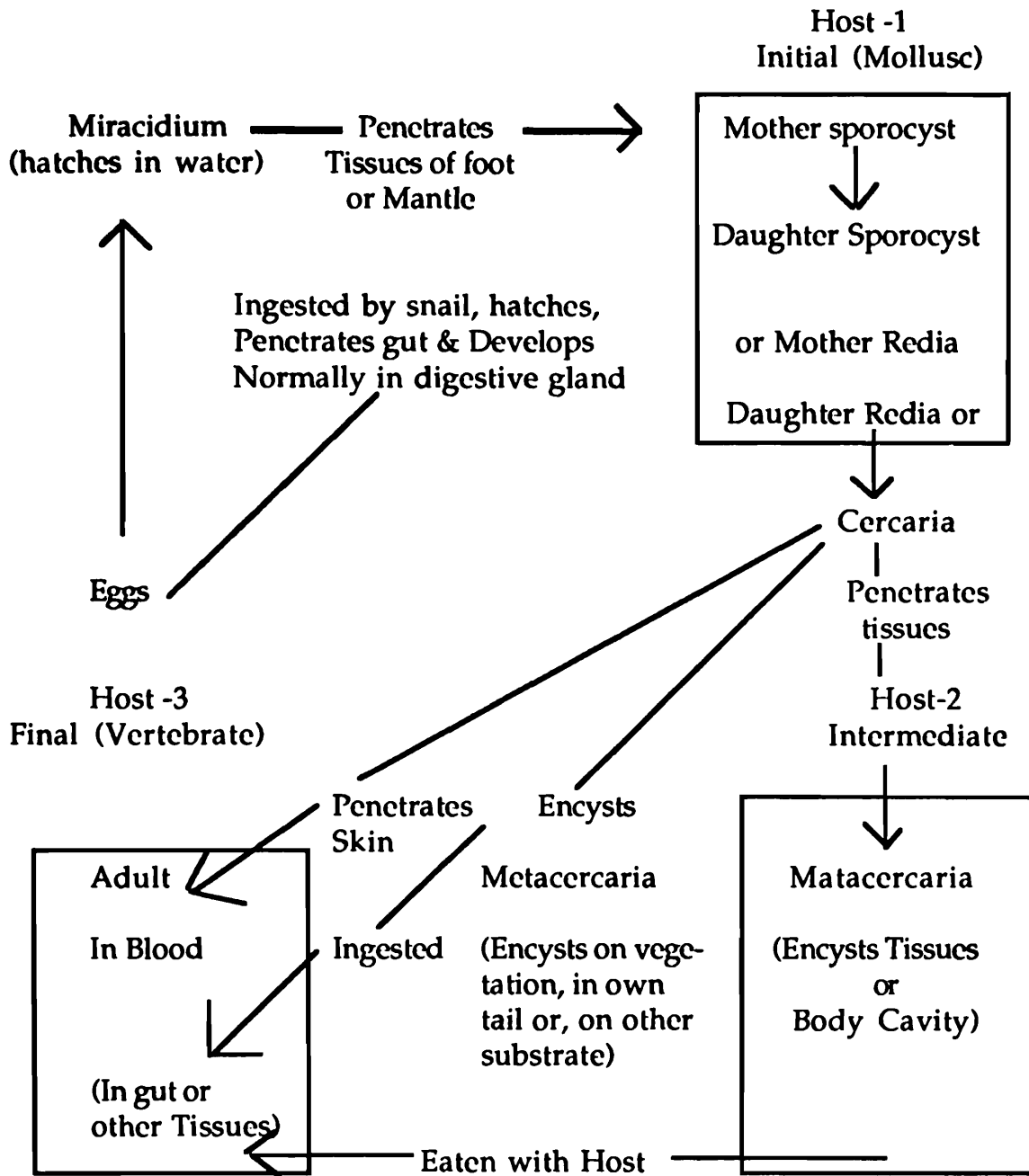
1. The knowledge of life-histories of parasites provides the only sound basis for combating parasitic infections.
2. Life history studies have demonstrated abundantly the significance of immature stages and their morphology to concepts of trematode taxa from orders to species.
3. Larval stages of flukes provide clues to proper classification. A study of cercariae has shown that some adult trematodes once thought to be closely related are not so, and others, quite dissimilar as adults, have proved to be closely related.
4. The life history studies of parasites supply phylogenetic information about the particular parasitic group.

**Progenesis**

While studying the specimens of a trematode larva recovered from a marine mollusc, *Donax vittatus* Dollfus (1913) coined the term 'metacercaria' to designate an immediate 'postcercarial stage' in the life cycle of a digenetic trematode. The metacercaria may or may not be encysted depending upon the presence or absence of cystogenous cells in the body of the cercaria. According to Stunkard (1967), the metacercaria is a 'juvenile' worm in which the development of the reproductive organs is variable in different families. Cases are encountered in literature where the animals become sexually mature before the attainment of adulthood. To designate this condition the term "progenesis" was applied by Giard (1887). Dollphus (1924) applied this designation for the gravid metacercaria of digenetic trematodes.

The appearance of the phenomenon of progenesis in the life histories of digeneans is not uncommon. The metacercaria occurring in the first intermediate host which is always a mollusc or in the second intermediate host, say, a crustacea, may become

Current Patterns Of Life Cycles Of Digenetic Trematodes



From : Hyneman, D. 1960. On the origin of complex life cycles in the digenetic flukes. In Libro Homenaje al Dr. Eduardo Caballero Y Caballero pp. 133-152

sexually mature and produce viable eggs. Thus in progenesis in a digenetic trematoda the life cycle is completed within the molluscan host or get extended up to the second intermediate host (like crustacea etc.) without the involvement of the vertebrate definitive host.

Some interesting cases of progenesis in the Digenea are given as under :

Ismail Koya and Mohandas (1982) reported *Gorgoderina* sp. from the gonad of a female green mussel, *Perna viridis* Linn. from Narakkal, Cochin. The green mussel apparently serves as the first intermediate host for the worm. The definitive host is not known, but the genus *Gorgoderina* is normally found in the urinary bladder of amphibians and teleost fishes. Although the specimens of the worm were not found encysted in the gonad of the mussel, they are egg producing ones and morphologically complete adults. It is obvious that this *Gorgoderina* sp. can exclude the definitive host from its life cycle progenetically.

*Genarchella genarchella* Trevassos, Artigas and Pereira, 1928, a hemiuroid, was described from a marine fish *Salminus maxillosus* in a lagoon near Buenos Aires, Brazil. Its intermediate snail host is *Littoridina australis* in the lagoon. There is no second intermediate host of this digenetic trematode. What happened is that some environmental changes took place and as a result the definitive marine fish host *Salminus maxillosus* became adapted to fresh water and therefore it became unnecessary for the trematode *Genarchella genarchella*. But, according to Szidat (1956), the unusual thing about the fluke was its entire life cycle could be completed within the snail only. Further, the cercarial stage also get suppressed from the life cycle. The egg produces miracidium which in turn produces rediae, and the rediae produce metacercariae. These metacercariae mature in the snail and lay numerous eggs containing the miracidia.

This is a unique case of progenesis in which the definitive host was excluded by the worm and the life cycle can be entirely completed in the snail host.

*Pseudopodocotyle bravoae* Rodriguez, 1970 was reported from the hepatopancreas of a freshwater crustacea *Pseudothelphusa (Ptychophallus) tristani* examined in Costa Rica, Central America. Apparently this freshwater crustacea serves as the second intermediate host for the worm. This is a progenetic metacercaria of which the adult, the definitive host and the first intermediate host (a mollusc) are unknown. Reproductive organs have reached sexual maturity organically and physiologically producing viable eggs.

#### Significance of Progenesis :

1. The life cycle of the digenian is shortened due to the disappearance of the vertebrate host restricting the dispersal and prolonged life of the parasite.
2. The phenomenon lends support to the thesis that mollusc was the original definitive host of the Digenea.
3. Progenesis can account for the development of the new evolutionary line sharply differentiating from ancestral types.

## Hyperparasitism

An organism which parasitises another parasite is called as a hyperparasite and the phenomenon is designated as hyperparasitism. A hyperparasite is also defined as an organism which lives parasitically in or on another parasite. The following examples explain hyperparasitism.

1. specimens of the monogenetic trematode, *Choricotyle aspinachorda*, typically attach near the bases of the legs of a symbiotic isopod, *Cymothoa excisa*, lodged in the throat cavity (frequently on gill rakers) of a pigfish. Often when a pigfish is not infested by the cymothoid isopod, it still has immature specimens of the monogenean on the gills. Primarily large adult flukes favour the isopod, and they deposit clumps of eggs on it. The combined worms and eggs on the isopod present a flowery appearance. *Choricotyle louisianensis* also has a similar relationship with a cymothoid isopod on a kingfish (a ground mullet).

2. Another species, *Udonella caligorum*, often associates with copepods (usually a species of *Caligus*) on which it attaches its eggs by filaments. Unusual because it lacks hooks or anchors, this monogenean can be commonly observed on caligids from the gills and skin of a sea catfish (hardhead catfish), seatrout, and striped mullet.

The metacercariae of *Microphallus* in the crab meat of the Blue crab are not ordinarily seen until they get hyperparasitised by a haplosporidan protozoan (*Urosporidium crescens*). The protozoan multiplies extensively until the cyst increases in size by many times and the metacercarial tissue is replaced by brown balls of spores distinguishing the metacercarial cyst. These brown balls of spores are known as "buckshot" or "pepper spots". The larval worms get severely debilitated but they do not harm the crab or the man who eats it.

The definitive hosts of the microphallid fluke in Mississippi are the raccoon and marsh rice rats. If the hyperparasitism in the metacercariae is not severe and they are healthy enough, the lifecycle is completed in the normal way.

Other encysted metacercariae may also get hyperparasitised by this or related species of the protozoans. Some also possess other kinds of protozoan hyperparasites including microsporidans, myxosporidans, flagellates and opalinids.

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## **Taxonomic Approaches to the Study of Mollusca**

**N. V. Subba Rao**

Zoological Survey of India , Calcutta.

Taxonomy is the theory and practice of classifying organisms. It involves identification of individuals or populations and placing them into previously established taxa by applying suitable nomenclature. Species is the basic unit of taxonomy and its recognition and description forms the first step in classification. A species is recognised on the basis of a single character or aggregate of characters. The concept of species is an important aspect in taxonomy.

Man had been attracted by molluscs due to their beautiful forms and colours. Amateur shell collectors have contributed much to our knowledge on molluscs by way of collection and identifying them. Initially shell and other easily accessible visual characters have formed the basis for molluscan taxonomy. But "it has been found that a classification founded on any single character, however important that may be, has always failed, for no part of the organization is invariably constant" (Darwin, 1859). Since shell shows a number of convergent characters inquiries have been directed toward other more unreversible and reliable characters. It has been pursued more seriously in freshwater molluscs than in other groups. Sometimes morphologically similar species have shown different responses to the invading parasites.

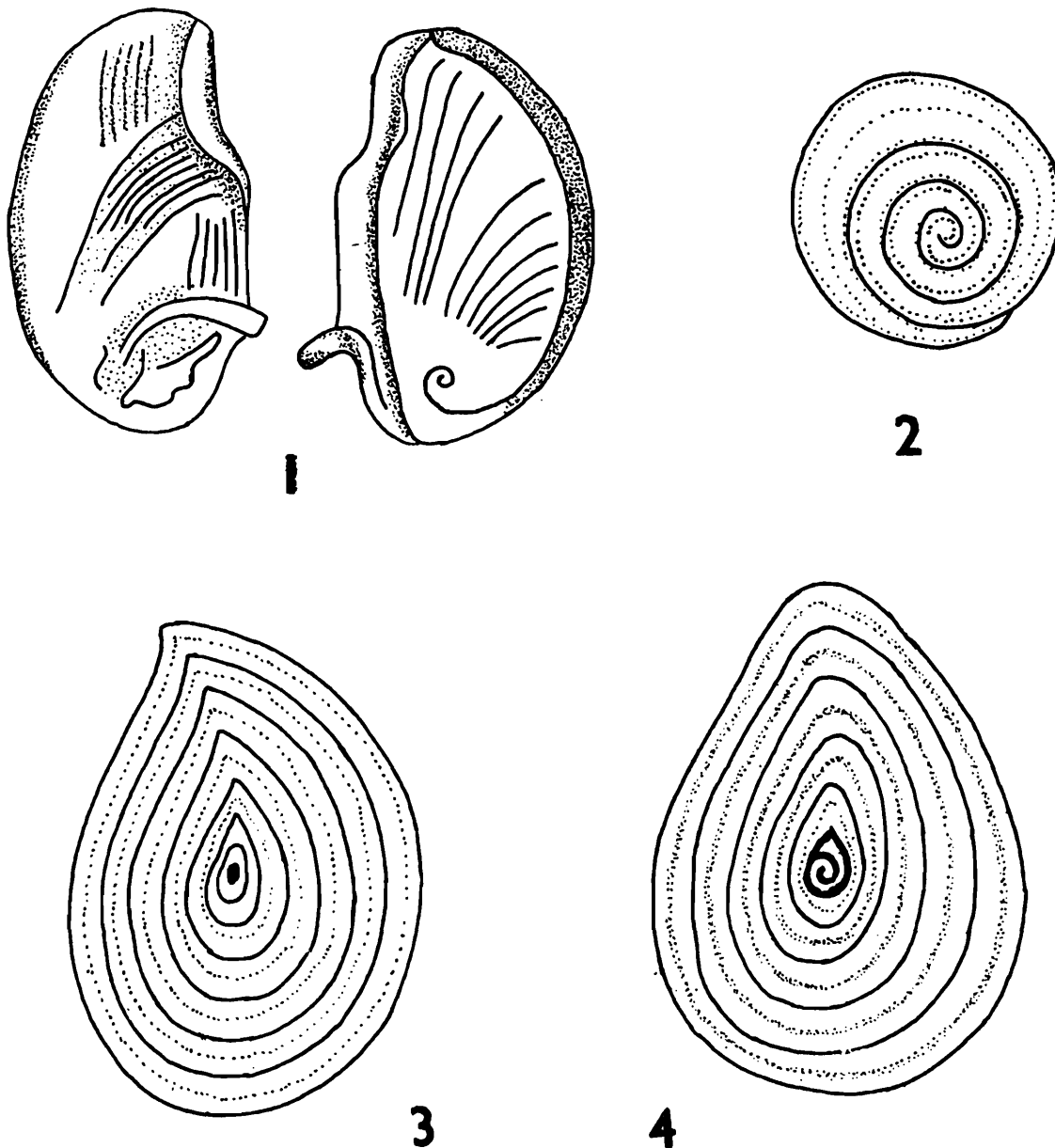
The species is a dynamic not a static unit, and dependence on one character may not reveal the correct identity of a species. When multiple characters are used in the identification and classification of a species it is imperative that "weighting" of characters should be done judiciously. Separate techniques have been developed for evaluating or assessing the value of taxonomic characters in the molluscs. Thus, in addition to classical morphological studies, a number of new techniques, namely cytological, biochemical, serological and biophysical are used in the taxonomic study of molluscs. A review of each technique and its application to different species of molluscs are given in the following pages.

### **Morphological Studies**

#### **(A). Shell**

Shell is an important taxonomic character in Mollusca. Except for some opisthobranchs, land slugs, and octopus all other molluscs possess a shell. The shell may consist of a single piece (univalve in gastropods) or two valves (Bivalve) or a number of pieces (as in Polyplacophora). The univalved shells of Gastropoda can be easily differentiated into those having an operculum (operculate) and others without an operculum (non-operculate). The following shell characteristics are useful in taxonomic studies of molluscs.

**1. Presence or absence of operculum :** When molluscs are collected alive, especially the freshwater gastropods, one has to see whether the aperture is closed by an operculum or not. Molluscs belonging to the families Ancyliidae, Planorbidae and Lymnaeidae do not possess any operculum and hence these are described as non-operculate. All other freshwater molluscs are described as operculate since an operculum is present. The operculum is attached to the foot of the animal and closes the aperture when the animal is withdrawn into the shell. Based on growth lines the operculum can be described as concentric, multispiral and paucispiral. The general shape and outline of operculum are also taxonomically important ( Fig. 1).



**Fig. 1. Opercula: 1. Paucispiral with excentric nucleus; 2. Multispiral 3. Concentric (with central nucleus); 4. Concentric (with spiral nucleus).**

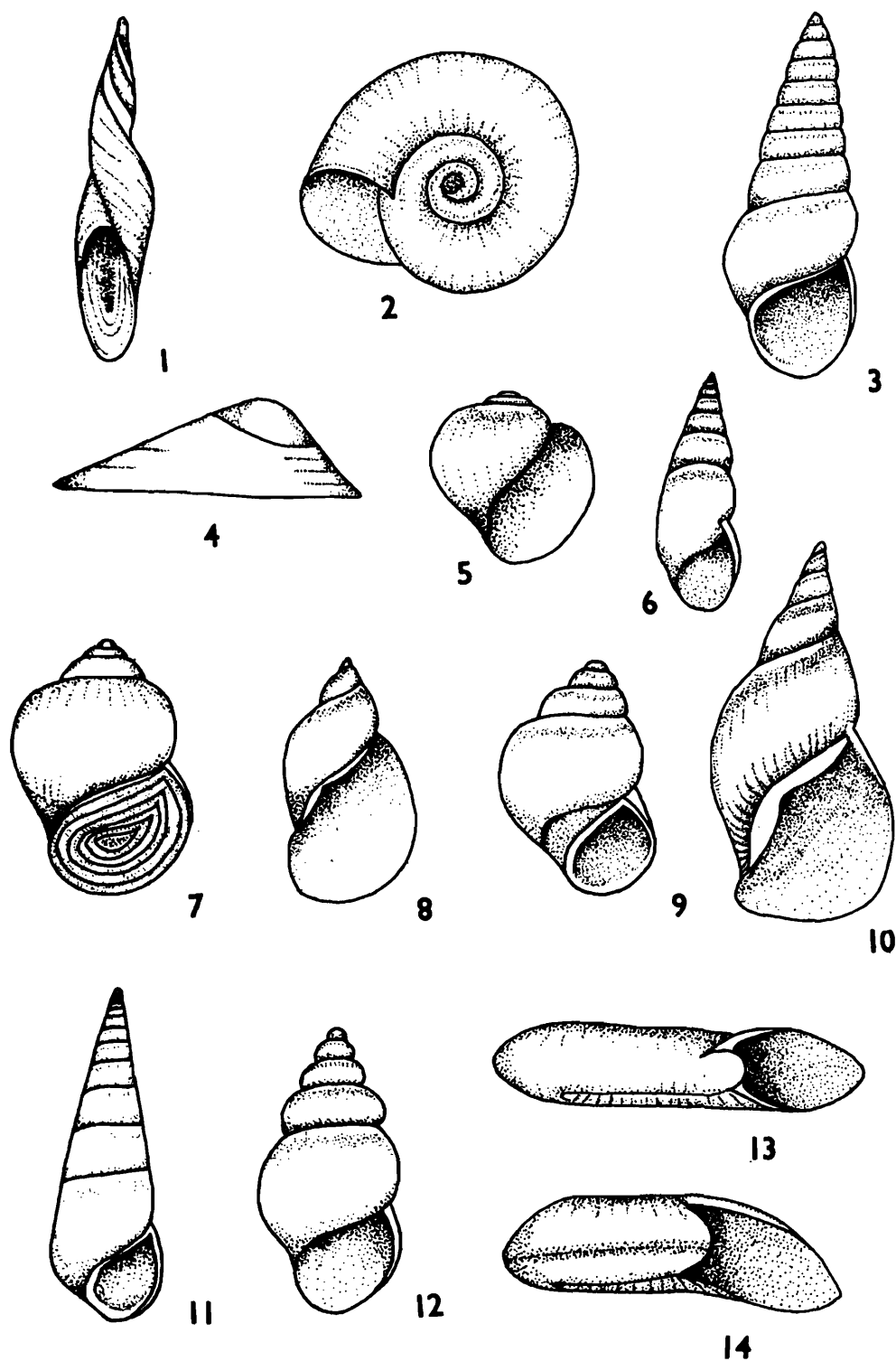


Fig. 2. Shell shapes and characteristics:

1. Sinistral; 2. Discoidal; 3. Dextral and turreted; 4. Patelliform or limpet-like; 5. Globose with rapidly increasing whorls; 6. Whorls flattened and gradually increasing; 7. Operculate and globose; 8. Non-operculate with twisted columella; 9. Elongate with callus; 10. Shell with twisted columella and elongate spire; 11. Shell with shallow sutures; 12. Shell with deep sutures and inflated whorls; 13. Discoidal with parallel aperture; 14. Discoidal with deflected aperture.

**2. Spiral coiling :** Two types of spiral coiling are seen, viz. dextral and sinistral. When a shell is held in hand with the aperture facing the observer the latter will be on the right hand side in dextral forms, while it will be on the left in sinistral forms. All freshwater molluscs of India possess dextral shells. The only exceptions are *Camptoceras*, a rare genus, and a few land species.

**3. General shape :** It is variable. It may be turreted (tower like), globose (spherical or subspherical), discoidal (round and flat like a disc) or patelliform (cap-like with a flattened cone) (Fig. 2).

**4. Whorls :** The whorls mainly consist of a body whorl, a few other spiral whorls and a few nuclear whorls (protoconch) at the apex. The number and shape of the whorls vary from family to family and even from species to species. The number of whorls has to be carefully counted. The periphery of each whorl may be rounded, angular or keeled. The whorls may increase slowly or rapidly. The suture, that is where the line of one whorl meet with the other, may be shallow or deep.

The shape and number of nuclear whorls are useful in the identification of some marine gastropods.

**5. Colour and sculpture :** Colour of the shell is an important character in the identification of marine species, but in contrast freshwater species are less colourful and drab. However, a few species have typically coloured shells. Some times there may be colour markings on the surface of the shell. But there is variation in pattern of sculpture. It may be spiral or axial and consist of lines, striae, ribs or tubercles.

**6. Aperture :** The aperture may be narrow or wide and its outline may be round, oval or lunate or a combination of two of these. It consists of an outer lip and inner lip. The aperture often contains teeth in the region of columella, inner lip, or in the outer lip. When the teeth are present in the outerlip region these are called parietal teeth and characteristic to certain genera. But majority of freshwater molluscs do not possess any teeth.

The internal axial column around which the whorls are coiled is known as columella and is seen through the aperture. It may be straight or twisted. Sometimes a plait may be present in the columella. Sometimes the columella bears a deposit known as columellar callus.

The central axis of the shell opens through a small depression near the internal lip of the aperture. It is known as umbilicus and when present the shell is described as perforate, otherwise as non-perforate.

### **(B). Internal or soft parts**

The gastropod body is divisible into a head, a foot, a pallial region and a visceral mass. The head and foot are fused and hence it is called as a head-foot region. The pallial region is enclosed in a membranous and pigmented mantle. The visceral mass is enclosed in a transparent non-pigment membrane, which is an extension of mantle.

**1. Head-foot region :** It includes three characters which are important at various levels of taxonomy. These are eyes, tentacles and radula. The first two characters are useful for distinguishing orders and families, while the last one is important at specific level.

a). **Eyes** : In freshwater snails the eyes are located at the base of a single pair of tentacles (Basommatophora), but in land molluscs they are at the tips of the posterior pair of tentacles (Stylommatophora). In neritids the eyes are situated on peduncles located on the external side of tentacles.

b). **Tentacles** : The shape and form of a tentacle is useful in distinguishing families. In the common viviparids the tentacles are long and slender and show sexual differentiation. They are long, filiform and cylindrical in Planorbidae and Bithyniidae, whereas they are flattened and triangular in Lymnaeidae. In freshwater limpets (Ancyliidae) the tentacles are short, blunt and cylindrical.

c). **Radula** : The radula is a ribbon-like structure enclosed in a sheath in the buccal mass. It consists of several transverse rows of teeth arranged in longitudinal series. Each transverse row consists of teeth distinguished into a central (or rhachidian), laterals, intermediates and marginals. The last can again be divided into internals and outers. The number, shape, size and position of the teeth and their cusps on different teeth are important characters for identification of genera and species. The central tooth may have three distinct cones named inner endocone, a median mesocone and an outer ectocone. But in many families it may be difficult to differentiate these cones very distinctly. However, the shape and number of these cones may vary from genus to genus, and even from species to species. The central tooth may be bicuspid (Planorbidae or Ancyliidae) or unicuspid (Lymnaeidae and Ancyliidae) (Fig. 3) or multicuspid (Thiaridae). The shape and structure of laterals and marginals are also important in the identification of molluscs.

2. **Pallial complex or Pallial region** : It includes three characters of importance viz. mantle edge, pseudobranch and kidney. The colour and shape of the mantle edge has to be noted. It bears digitiform processes in many members of the family Thiaridae. In general it is smooth and plain in majority of the freshwater molluscs.

a). **Secondary gill** : The pseudobranch as the name suggests is a structure which functions like a gill. It is situated on the left side near the mantle collar. It is present in the members belonging to the families Planorbidae and Ancyliidae. It may be simple, branched or folded.

b). **Kidney** : The structure of the kidney has been studied in detail in Planorbidae and Lymnaeidae. It is very much narrow and elongate in the former family, but it is large and wide in the latter. The presence or absence of a ridge on the ventral surface of the kidney differentiates certain genera and even species.

3. **The Visceral Mass** : The Genitalia- the copulatory organ and prostate gland, are the two important characters of the male genital system. The copulatory organ may be a simple verge or penis or it may consist of several structures, when it is called as a penial complex. The latter is present in Basommatophora and consists of a verge, a preputium and the penial musculature. The penis may be enclosed in a sac as in Basommatophora or naked as in viviparids and neritids. Preputium is a portion of the complex and it opens at male genital aperture. These have been used as taxonomic characters in the study of Lymnaeidae and Planorbidae. The shape and relative size of preputium and the vergic sac are of taxonomic value. The nature of the verge viz. simple or bifid; the presence or absence of a stylet on the verge; the number, shape and arrangement of penial muscles; and the presence or absence of a preputial gland and duct are of taxonomic value.

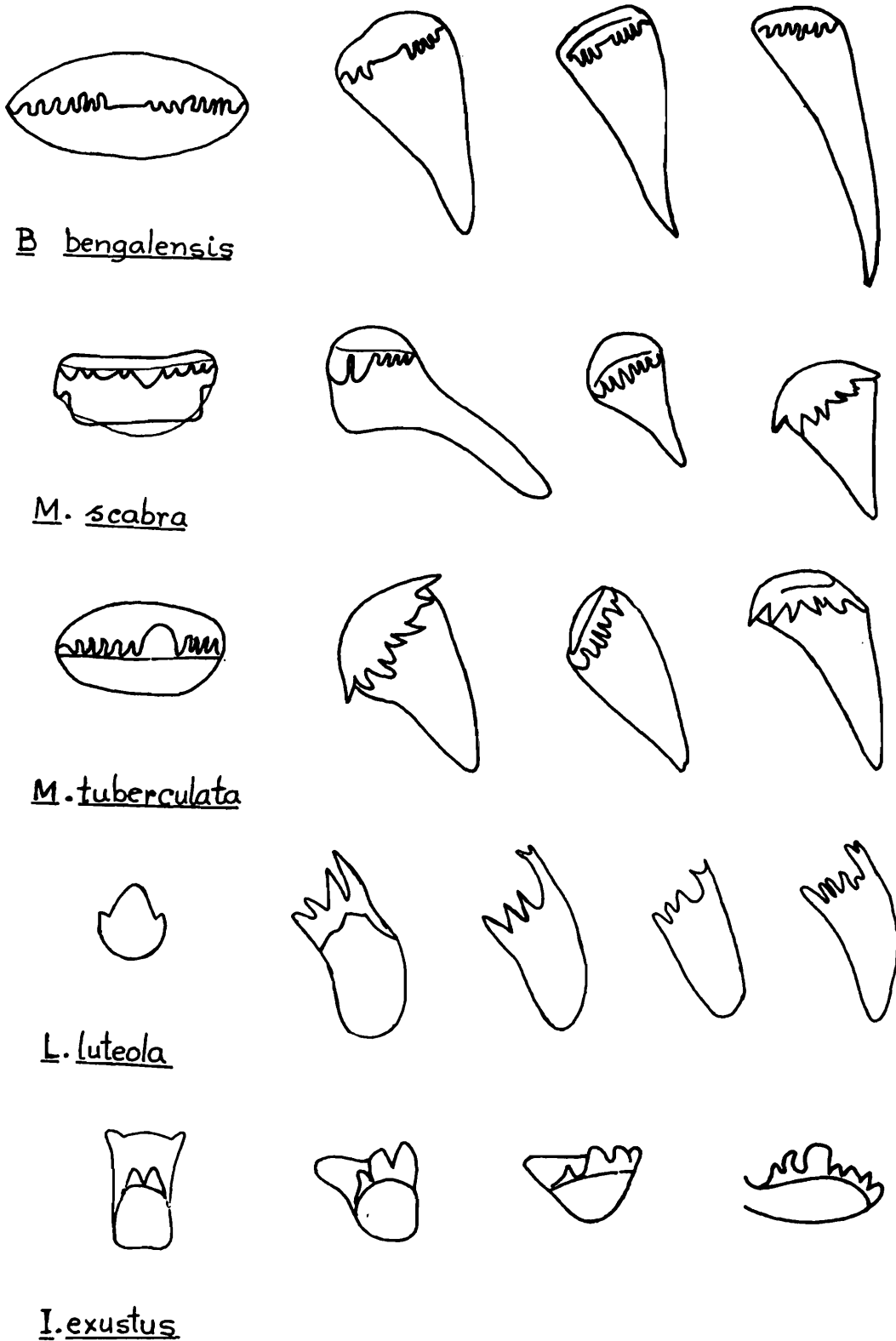


Fig. 3. Radular teeth of gastropods.

Central or rachidian, a representative of laterals, marginals and other marginals are figured for each species.

In *Segmentina* and *Hippeutis* the vergic sac bears two short flagella whereas *Gyrulus* has a conspicuous stylet at the tip of the verge.

The shape and structure of prostate gland are important characters in the study of Planorbidae and Lymnaeidae. Many of the thiarids are ovo-viviparous. Eggs are retained in a brood pouch. The position and shape of brood pouch is of considerable taxonomic significance.

**4. Egg masses :** The form and shape vary with the family. In Lymnaeidae the eggs are laid in a gelatinous, elongate and cylindrical mass. It is twisted to the left (anticlock wise). In Planorbidae egg masses are yellow and have no free 'tail' They are firm and somewhat flattened. In Lymnaeidae eggs have two membranes, viz. an internal and an external, whereas in Planorbidae and Ancyliidae the eggs are devoid of an external membrane (Fig. 4).

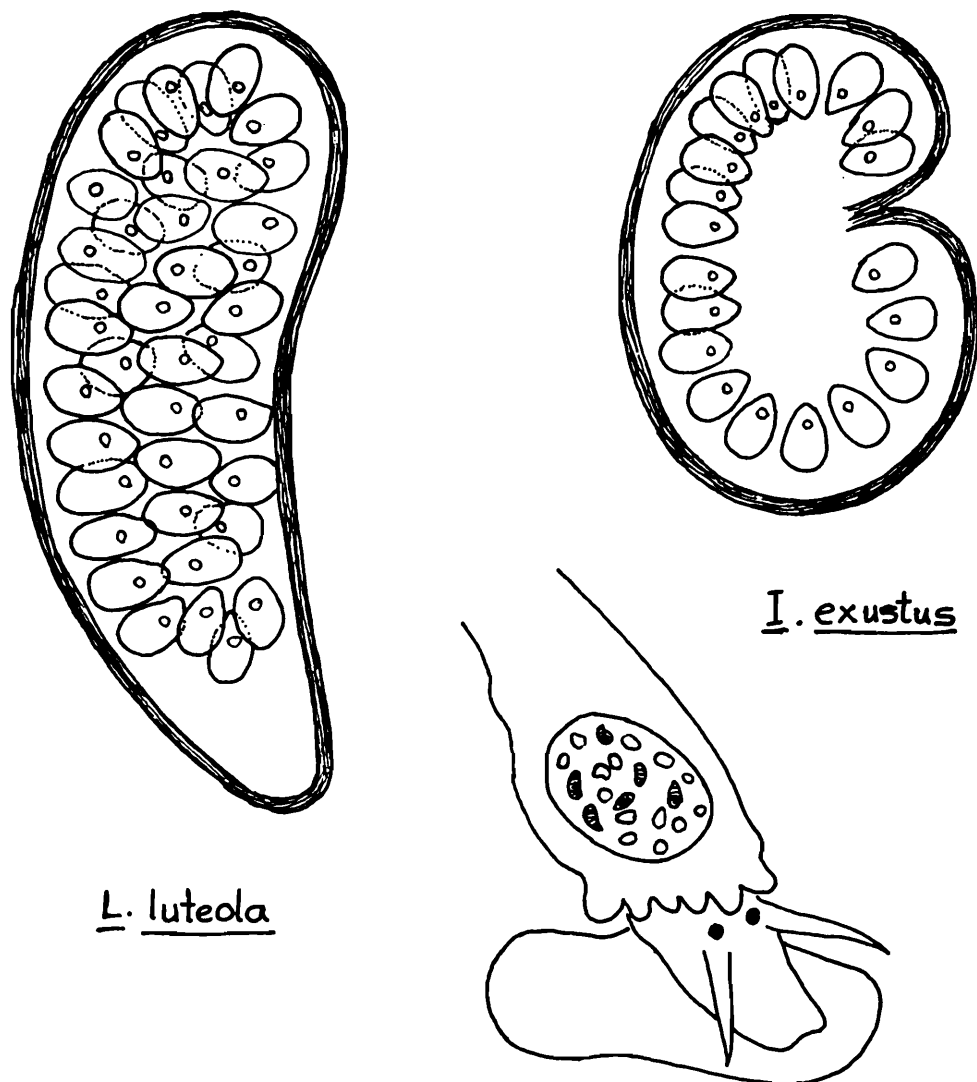


Fig. 4. Egg masses, and brood pouch of a thiarid.

### Cytological Studies

Cytotaxonomy, involving chromosome studies, have gained importance in taxonomic studies of snails during the last three decades. Chromosome morphology and behaviour, karyotype analysis and sex chromosomes were used in systematics of molluscs, especially gastropods. Reliable reports on chromosome numbers are available for classes Gastropoda, Bivalvia and Cephalopoda. Chromosome numbers are known in 567 species of gastropods and only a few species of Bivalvia and Cephalopoda. A detailed review on this subject is given by Patterson (1967, 1969). According to her, the range of haploid chromosome numbers varied from order to order. In the Archacogastropoda the chromosome number ranges from 9 to 18; in the Mesogastropoda it is 7 to 20 and in the Neogastropoda it ranges from 13 to 36. In the Neogastropoda except *Purpura lapillus*, which has 13 pairs of chromosomes, all other species have 28 to 30 or more pairs of chromosomes. The lowest chromosome number of 5 pairs is found in a species of *Catinella* (Fam. Succineidae). In aquatic pulmonate snails the chromosome number is almost the same in all the species studied (Burch, 1960). No clear cut correlation has been observed between low chromosome number and primitiveness.

Karyotype analyses are used in species discrimination among various groups (Burch, & Davis, 1967; Ramamoorthy, 1958, Patterson, 1967). Burch (1960, 1968), who attributed limited taxonomic value to chromosome number described a tissue culture technique for karyotype analyses of pulmonate snails (Burch, 1968). Polyploidy was noticed in several freshwater snails. In *Bulinus* populations it was found to cause strain differences in susceptibility to schistosome infections (Brown & Burch, 1967). Cytological studies of Indian molluscs are only a few in number (Natarajan, 1959, 1960 & 1970; Ramamoorthy, 1958, 1959 Jacob, 1959). A table showing the number of chromosomes in a few Indian species is given below:

#### Chromosome Numbers of Some Indian Species.

Species	Haploid No.	Reference
<i>Bellamyia dissimilis</i>	11	Ramamoorthy, 1958
<i>B. bengalensis</i>	11	Ramamoorthy, 1958
<i>Thiara (Melanoides) tuberculatus</i>	16	Jacob, 1959
" " (polyploid)	45-47	"
<i>T (Tarebia) lineatus</i>	35-36	"
<i>Thiara (Melanoides) torulosa</i>	18	"
<i>Thiara scabra</i>	38-39	"
<i>Paludomus tanschaurica</i>	17	"
Pleurobranchidae (3 spp)	12,13	Natarajan, 1970
Nudibranchia (16 families)	13	"
Aplysiidae (6 spp)	17	"
<i>Hydatina velum</i>	15	"
<i>Haminoea crocata</i>	17	"
<i>Smaragdinellidae</i> (3 spp)	18	"
<i>Onchidium verruculatum</i>	18	"

### Electrophoretic Studies

Electrophoretic studies have provided additional tools in taxonomy of certain medically important molluscs. However, the taxonomic value of blood protein or somatic protein is not conclusively established. Protein constitution of a planorbid blood undergoes qualitative and quantitative change during the life time of a snail. Hence, its value as a taxonomic character is ruled out (Wright & Ross, 1963). Egg proteins have been found to yield more reliable electrophoretic data. Major features of egg protein profiles in planorbid snails are found to be characteristic for certain species groups, but in general, only minor differences are noticed at species and populational level (Wright, 1971).

Enzyme systems of planorbid snails were useful in explaining phylogenetic and physiological relationships (Wright *et al*; 1960; Malek & File, 1971). Starch gel electrophoresis of certain enzyme systems especially esterases in the digestive gland are useful in evaluating the physiological heterogeneity of snail populations (Wright, 1971).

By undertaking electrophoretic study of foot muscle extract of *Semisulcospira*, Davis (1969) has shown that *Semisulcospira trachea* is a synonym of *S. libertina*. The latter species has been implicated as the first intermediate host for the human lung fluke, *Paragonimus westermani* in Japan.

The importance of disc electrophoresis in the study of molluscan taxonomy has been brought out by Davis (1967, 1969) and Davis & Lindsay (1964, 1967).

### Serological Studies

In a number of molluscs it has not been possible to define precisely a natural unit constituting a population or a subspecies or a species or a species complex. It has been mainly so in respect of intermediate snail-hosts of helminths. Till recently the medically important gastropod genus *Bulinus* has been a riddle to taxonomists. A number of species of the genus were described by using classical taxonomic approach or morphological approach. When the life history of *Schistosoma haematobium* was worked out in 1915, it was seen from literature that more than hundred species of *Bulinus* were described (Wright, 1971).

In such instances, where the classical morphological approach or biochemical approach is not satisfactory in distinguishing taxa, serological or immunological approach has proved to be useful. Immunological methods have been adopted in elucidating the systematics of freshwater prosobranch and pulmonate snails. Intra-agar double diffusion modification of the Ouchterlony gel diffusion technique has been adopted for molluscan studies by Davis (1968, 1969), Burch (1969), Burch & Lindsay (1970, 1973), Michelson (1966), Patterson (1969) Wright (1966, 1971) and Wright and Klein (1967).

Immunological reactions of antigens extracted from foot muscle proteins were used in taxonomic groupings in the family Lymnaeidae. These immuno-taxonomic studies correlated best with shell characters (Burch & Lindsay, 1973).

Gel diffusion techniques have been useful in distinguishing genera and congeneric species of planorbid snails (Michelson, 1966; Wright & Klein, 1967) of Hydrobiidae and Pleuroceridae (Burch & Lindsay, 1970; Davis, 1968, 1969).

A number of immunological tests using egg proteins were carried out in the genus *Bulinus*. Antigen of one species is pre-absorbed and the antibody of another species is allowed to diffuse through it in order to react and meet with its own antigen. From this it was possible to determine whether or not the species from which antiserum was obtained, contained the same protein furnishing the antigen. Thus by applying immunological techniques "Identity" and "Non-identity" reactions were obtained among species. Populations with the same chromosome number showed "Identity" reactions whereas those with different chromosome numbers showed "Non-identity" reactions (Burch, 1969).

Immunological techniques were used in discriminating *Bulinus* species complexes (Wright, 1971). Electrophoresis of egg proteins gave no particular character and chromatography did not reveal any fluorescent substances. Antisera were prepared in rabbits for *Bulinus obtusispira*, a natural host of *Schistosoma haematobium* on Madagascar. It was found that the species belonged to the *Bulinus africanus*-complex. But it was nonsusceptible to any strains of schistosome for which *B. africanus* is the intermediate host. It was however, susceptible to *S. haematobium*, which normally develops in *B. forskali*. As Wright (1971) comments the final laugh is with flukes and their snail hosts.

### Biophysical Methods

These include radiographic and spectroscopic methods. Autoradiographic techniques are used in the determination of uptake and deposition of radioisotopes by molluscs. A recent review has been given by Malck & Cheng (1974).

Scanning electron microscope has been used in the study of molluscan shell architecture. X-ray diffraction technique is an advancement over it. It is used in the mineralogical and crystallographic studies of molluscan shells. So far the process of biomineralization has been studied in bones and teeth but recent studies have shown that molluscan shell can be a good object to carry out such studies.

Certain shell fragments collected during archaeological and geological excavations are too small to be identified by standard zoological techniques. In such circumstances Electron microscope and X-ray diffraction techniques are providing useful information. These are also useful in studying the physical properties of pearls and nacreous conchiolin and in studying the boring pattern of wood boring molluscs. The conchiolin residue of inner nacreous layer and other ultra structures are taxonomically important. These studies can help in the identification up to family level.

The biophysical methods are also useful to elucidate the molecular structure of byssus found in some bivalves.

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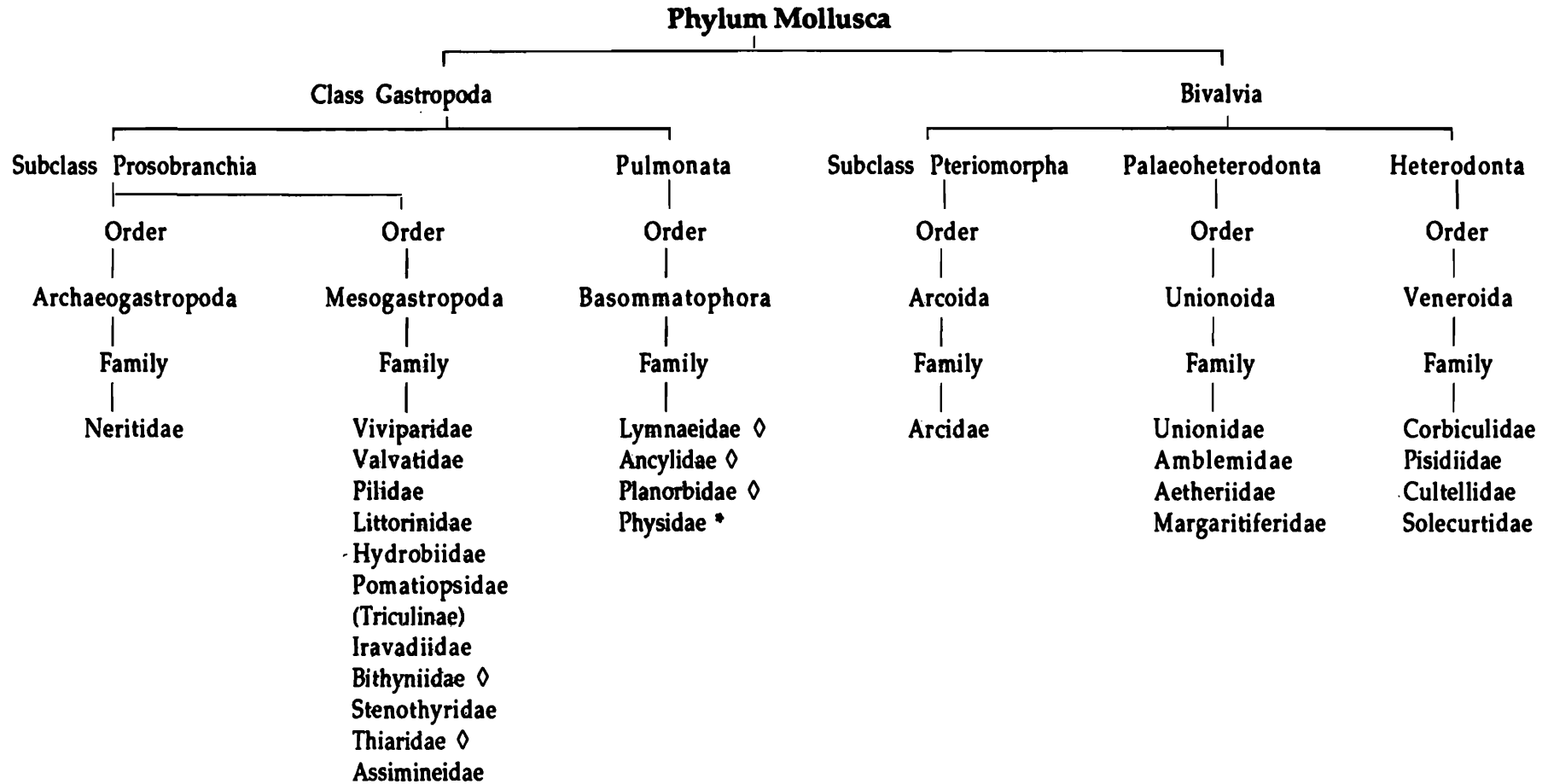
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# CLASSIFICATION OF FRESHWATER MOLLUSCA



◇ Intermediate hosts for trematodes

In India represented by fossil forms, but in Pakistan by the extant species, *Physa acuta* Draparnaud.



## Trends in Taxonomy of Trematoda

**M. Hafeezullah**

Zoological Survey of India, Calcutta.

Although attempts had been made by various authors at classifying the Trematoda since 1800, a worthwhile basis of its classification was first advanced by Burmeister (1856). Based on the nature of adhesive organs he divided the class Trematoda into three groups: (1) Pectobothrii, with hard and firm suckers; (2) Malacobothrii, with soft and flexible suckers; and (3) Aspidobothrii, with multiloculate adhesive organ, to include the then known only genus *Aspidogaster* Van Baer, 1826. On the basis of life cycle patterns of trematodes, Van Beneden (1858) divided the class Trematoda into two subclasses : (1) Monogeneses, with a single generation in the life cycle; and (2) Digeneses, with the sexual generation alternating with the asexual larval generations. Carus (1863) latinized these names to Monogenea and Digenea. But, inspite of the facts already known that *Aspidogaster conchicola* Van Baer, 1827 does not have asexual generations in the life cycle and that earlier authors included aspidogastrids with the polystomes which are monogenetic, Van Beneden (1858) did not touch Aspidobothrii of Burmeister.

Monticelli (1888) erected the family Aspidobothriidae for the genus *Aspidogaster* Van Baer, 1826, but following the rules of Zoological Nomenclature, Poche (1907) changed this family name to Aspidogastridae.

There were aberrations and anomalies in the life cycles of *Gyrodactylus* Nordmann, 1832 (*G. elegans*, *G. medius*; viviparous species showing polyembryony due to successive generations produced in the embryo), *Polystomum integerrimum* (digenetic), *Aspidogaster conchicola* (monogenetic) and the strigeids e.g. *Holostomum cornucopiae* (= *Strigea strigis*) (Metastatic i.e. development of encysted tetracotyle larva in the final vertebrate host directly from miracidium without the knowledge of intervening redial, sporocyst and cercarial stages in the life history. The encysted tetracotyle larvae developed to sexual maturity in the vertebrate hosts). Therefore, Monticelli (1892) thought that probably the nature of life cycle patterns did not form the appropriate basis for dividing the Trematoda into subdivisions. He therefore concurred with Burmeister (1856) and accepted the division of Trematoda on the basis of nature of adhesive organs. He, however, changed the names of Burmeister groups and classified the Trematoda into three groups: (1) Heterocotylea, (2) Malacocotylea, and (3) Aspidocotylea. Here, Heterocotylea is the Pectobothrii of Burmeister (1856). In 1922, it was convincingly proved that the strigeids have furcocercous cercariae in their life cycles and are strictly digenetic. Therefore they were removed from the list of anomalies. Other aberrations and anomalies listed above still exist.

Faust and Tang (1936) erected the subclass Aspidogastrea for the family Aspidogastridae independent and equal in rank to the two subclasses Monogenea Van

Beneden and Digenea Van Beneden, considering the position of Aspidogastrea as intermediate between Monogenea and Digenea. Thus, it is seen that Burmeister (1856), Monticelli (1892) and Faust and Tang (1936) recognised an independent status of subclass to the aspidogastrids. Stunkard (1963), however, opposed the idea that they are "intermediate in position between the Monogenea and Digenea" He argues that "a species either does or does not have an alternation of generations in the life cycle" and emphasises that the aspidogastrids form an aberrant group and occupy an anomalous position." "It appears that the aspidogastrids and digenetic forms have descended from a common turbellarian-like ancestor which initially became parasitic in molluscs, and that the aspidogastrids never acquired asexual methods of reproduction and became mature in their molluscan hosts or in vertebrates that feed on these hosts, whereas members of the digenea developed polyembryonic asexual reproduction in the molluscs and, with the acquisition of vertebrate hosts, sexual maturity was more and more deferred to worms in the definitive hosts. The frequent appearance of progenesis in digenetic species and the demonstration by Stunkard (1959) that the life cycle of *Asymphylogora aminicola* can be completed in the snail without the intermediation of the usual fish host lends support to the thesis that, originally the digenea were parasites of molluscs and that, secondarily, they acquired an asexual method of reproduction and vertebrate hosts. The acquisition of the longer-lived, wider ranging vertebrate hosts facilitated dispersal and prolonged the life of the parasites, thus increasing reproductive capacity and survival value for the species."

Baer and Euzet (1961) proposed that the Monogenea should be separated from the Trematoda so as to recognise it as a separate and independent class at par with the class Trematoda itself-a scheme which is supported by Mehra (1980), Lamoth-Argumendo and Cruz (1982) and others. The argument advanced in support of this theory is, that the monogenean order Chimaericoloidea (genera *Chimaericola* and *Callorhynchicola*) has close affinity with the cestodarian group Gyrocotyloidea. There are morphological similarities, both in their larvae and nature of their development. The Gyrocotyloidea appear to develop like Monogenea without the interpolation of an intermediate host in the life cycle. Further, Gyrocotyloidea and Chimaericoloid both parasitise primitive and archaic selachian fish genera, *Chimaera* and *Challorhynchus*. Most probably Gyrocotyloidea radiated from Chimaericoloidea. Stunkard (1962, 1963) opposes this scheme. He agrees that there is morphological similarity between the above mentioned monogenean and cestodarian groups and both of them were undoubtedly derived from free-living turbellarian-like ancestors, but the separation of Monogenea from the class Trematoda on the basis of differences in life cycle is not consistent because certain of the accepted Monogenea (gyrodactylids and polystomes) are not monogenetic. He explains that the gyrodactylids are viviparous, with successive generations and the polystomes parasitising amphibia are digentic. Consequently, for these groups the designation Monogenea is inappropriate. Stunkard advocates that once the nature of the adhesive organs is accepted as the basis of classification of Trematoda, the problems of aberration and anomaly immediately disappear. He therefore divides the class Trematoda into two subclasses :

- 1) Pectobothridia Burmeister, 1856,
- 2) Malacobothridia Burmeister, 1856.

He further divides Malacothridea into two orders :

- 1) *Aspidobothrea* Burmeister, 1856,
- 2) *Digena* Van Beneden, 1858.

Mehra (1980) did not agree to this scheme of classification of Trematoda and asserts that "The emphasis on the life history as the basis of classification should be maintained, as it gives an indication of the evolutionary history of the classes Monogenea and Trematoda", accepting the former as independent class at par with the latter.

Bacr and Joyeux (1961) proposed to restrict the Trematoda to three independent subclasses-Aspidogastrea, Digena and Didymozoida, but Stunkard (1963) opposed the idea of separating the didymozoids (represented by the only family Didymozoidae) from Digena.

**Conclusion :** Generally the nature of adhesive organs is not accepted as the basis of classification of the class Trematoda although it solves the problems of aberration and anomaly posed by gyrodactylids, polystomes and aspidogastriids. The nature of life cycle pattern is universally accepted as the basis of classification, and thus the class Trematoda is divided into three subclasses : 1) Monogenea, 2) Aspidogastrea, and 3) Digena. Monogenea as a full-fledged class independent and at par with the class Trematoda is followed only by few authors including Mehra (1980).

#### **Subclass Digena :**

In Digenean parasites there are two types of characters, one which are primitive providing trustworthy indications of phylogenetic relationships while others are adaptive which develop in the life history to lead parasitic mode of life. Such characters are not indicative of genetic relationship. The old classifications of the digenean flukes were of necessity and convenience and were made utilising adaptive characters, such as suckers, digestive and reproductive organs and therefore they were not reflecting natural relationship. That is why earlier classifications of Digena proved unsatisfactory. Recently, more reliance has been placed on the excretory system which is supposed to be the most conservative system and is least altered in adaptation to parasitism. It maintains a constant pattern throughout larval stages and adult life, and furnish an additional basis for evolving modern and natural classification. La Rue (1938) pointed out that a taxonomic system which really indicates relationship must be based on comparative anatomy of all the stages of life cycle, specially of the miracidia and cercariae. Systematists all over the world, specially H. W. Stunkard, R.M. Cable, and numerous others have contributed a lot to the study of digenean life cycles. Today there is a treasure of determined life cycles in the literature of Zoology, and a digenean classification based on true relationship is now emerging, although allocation of some groups is still speculative.

Generally the method of development of the excretory system, the characters of the larval stages, mainly miracidia and cercariae, and the life cycles as a whole give clues to determine true relationships and thereby help to trace ancestries on one hand and phylogenetic tendencies on the other. Thus, natural groups of higher taxa from family level above are formed and allocated along evolutionary lines. Sometimes adult morphology is also helpful in this exercise, but generally a group of adult characters

are carefully evaluated in determination of genera and species. To achieve this a species must be carefully, correctly and completely described and compared with the allied ones. Intraspecific variations in the population must be noted. All this is subject to live study of the flukes in the field, correct method of collection and preservation, proper staining and differentiation, and dexterity at the use of microscope. Availability of correct and complete information on species is prerequisite for the investigators working on the systematics of higher taxa.

Genera and even species of flukes are assessed on the basis of adult morphology and anatomy. Adult characters like presence or absence of spines on the tegument or on the oral sucker around the mouth, number and position of suckers, location of genital pore, nature and position of testes and ovary, extent of uterus, nature and distribution of vitellaria, presence or absence of cirrus and cirrus pouch, presence or absence of external seminal vesicle, pattern of digestive system, details of the excretory system, etc. are employed to designate genera or species. A trend has developed among workers in trematodology to use statistics or morphometric characters to describe new species. Well, this should be avoided as far as possible as this creates confusion. Of course, sometimes only one or two morphological characters are strong enough to designate a new species or erect a new genus.

Recently, La Rue (1957) proposed a new system of classification of Digenea. He formed apparently natural groups of flukes on the basis of the nature and method of development of excretory system as well as morphological characters of larval stages, mainly miracidia and cercariae. Thus, he divided the subclass Digenea into two superorders, namely, Anepitheliocystida and Epitheliocystida. Anepitheliocystida contains 3 orders, Strigeatoidea, Echinostomida and Rencolida; while Epitheliocystida has 2 orders, Opisthorchiida and Plagiorchiida. Obviously, each order has been further divided into suborders, superfamilies and families.

Even this system of classification, although much improved, has its own limitations. The same excretory pattern (arrangement of flame cells and empirical formulae) may apply to more than one family and may not hold for all members of the same family. Mehra (1980) pointed out that "the development of the epithelial excretory bladder in the cercariae is a secondary character having no phylogenetic significance and not showing closer relationship of the groups in Epitheliocystida" He therefore suggested suppression of La Rue's superorders, and gave his own scheme of classification of Digenea employing miracidial, cercarial and adult characters. He divided the subclass Digenea into 5 orders, namely, Fasciolatoidea Szidat, 1936, Strigeatoidea La Rue, 1926, Azigeatoidea Mehra, 1957, Hemiuritoidea Mehra, 1957, and Gorgoderida Mehra, 1958. He further divided each order into suborders, superfamilies and families.

### Origin of Digenean Flukes

It is purely a speculative and hypothetical exercise to trace the origin of a particular animal group but such speculations are based on some strong and basic biological facts which appeal to the mind. In the process there may be some unclear spots needing more and more clarity. New information such as discovery of new species of animals or phenomena or facts are added to decrease the speculative elements.

Structural similarities both larval and adult, embryology, functions of organs, comparative parasitism, etc. are usual tools which provide evidence of ancestry.

Several theories of the origin of parasitic flatworms have been postulated but the planuloid-acoeloid one seems to be most convincing. It is supported by the study of comparative parasitism and the fact that evolution of the parasite shows a correlation with the evolution of the host. There is no difficulty in visualising an evaluation from planuloid ancestors to an acoeloid turbellarians. A planula is the ciliated larva of coelenterates. Probably the planuloid ancestor had a ciliated epidermis, had no mouth and no digestive tract, and ingesting and digesting like protozoans. It is further presumed that this planuloid ancestor gave rise to two divergent groups-coelenterates and acoelous turbellarians. The ciliated and free-living aquatic order Acoela (class TURBELLARIA) form the most primitive group which gave rise to the order Rhabdoceles. The rhabdoceles have a mouth and a rhabdoceles (sac-like or unbranched) gut, protonephridia, oviducts, and nervous system. This turbellarian order has two suborders, (1) Temnocephalida, and (2) Lecithophora. The temnocephalids do not have cilia, have developed tentacles anteriorly and one or two adhesive organs posteriorly. They are ectocommensals on freshwater animals like crustaceans, snails and turtles. They are not parasites, and hence do not depend on their hosts for food. They eat insect larvae, rotifers, small crustaceans and other aquatic organisms, capturing them from the surrounding water. The suborder Lecithophora has the real parasitic rhabdoceles. It has a small group called Dalyellioida having anteriorly directed mouth, doliiform (barrel-shaped) pharynx, rhabdoceles gut, and a single gonopore. These dalyellioids parasitise molluscs, echinoderms and other turbellarians. Thus, it is here in the dalyellioids where the molluscs first got associated as hosts with rhabdoceles turbellarians, and the echinoderms are situated in the main line of the evolution of vertebrates. Probably the ancestral adult digenean primarily parasitised molluscs. With the appearance of vertebrates in the evolutionary history, the digeneans became structurally more and more specialised developing elaborate adhesive organs already present in the rhabdoceles turbellarians and modified reproductive system to produce greater number of eggs, and became adapted to vertebrate hosts but retained their dependence upon molluscan hosts. It is generally accepted that molluscs are the original definitive hosts of digenean flukes and, as evolution continued, vertebrates were secondarily acquired in their life cycles in which they attained sexual maturity for undergoing sexual reproduction and wide dispersal of species. Evidence of this hypothesis is to be found in frequent cases of progenesis encountered in digenetic trematodes, in which the vertebrate host is deferred and the fluke sexually matures in the molluscan host completing the life cycle therein.

There is also a mesozoan theory of the origin of digenean flukes. The Mesozoa has two orders, Dicyemida and Orthonectida, the two groups differing in their life cycle. As a matter of fact, the Mesozoa as a whole group of minute organism parasitic in cephalopods and other marine invertebrates is controversial so far as its phylogenetic origin is concerned. So much so that it is even possible that the two orders are diphyletic, i.e. they are not related with each other. There is difference of opinion among authors whether the simple structure of mesozoans is primitive and degenerate in nature or the simplicity is the result of parasitism and therefore progressively specialised. Due to this division of opinion some give them the status of a phylum

while others consider them as a class of parasitic flatworms. As the relationship of the mesozoans themselves is obscure, a discussion of the origin of digean flukes through them is not considered necessary here.

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# Cytotaxonomical Techniques for the Study of Molluscs and Helminths

**Ashok K. Singh**

Zoological Survey of India, Calcutta.

Cytotaxonomy is the study of the natural relationships of organisms by a combination of cytology and taxonomy.

The foundation of cytotaxonomy was laid when the principle of chromosome individuality was accepted and the chromosome theory of heredity became established.

It was noticed that the karyotype characterized by the chromosome number, size and morphology, was a definite and constant character of each species. Zoologists and Botanists promptly realized that it was also a conservative set of morphological feature, and structural changes in them could be a basis for the study of evolutionary relationships within and between species. We may admit that karyotype has a lower adaptive value as compared to other morphological features (e.g. colouration, size, appendage morphology, cutaneous production etc.). For this reason karyotype variations reflected general phylogenetic lines.

Chromosomal studies may be carried out as a technique of taxonomy, where one is using the karyotype in the same way as the shape of genitalia, sculpturing on the pronotum, wing venation etc. We should never forget that essentially, evolution is a cytogenetic process.

In karyotype analysis chromosome banding techniques have demonstrated some structural differences between karyotypes that were formerly indistinguishable. They help us to interpret chromosomal rearrangements, such as inversions and translocations, and to understand and to identify differences between the karyotypes of related species.

## **Karyotypic Analysis**

White (1978) recognized six levels of karyotypic analysis currently found in literature viz. :

**Alfa Karyology** : Only chromosome numbers and approximate sizes have been determined. At present this is the only practicable level of analysis in yeast and most protozoa, fungi and algae.

**Beta karyology** : Chromosome numbers and lengths of chromosome arms are known, i.e. centromeric positions have been accurately located. Sex chromosomes, if present, have been identified. Most karyotypic data are of this type.

**Gamma karyology** : Giemsa and fluorescent banding techniques have been carried out and maps showing locations of the main C,G- and Q- bands are available.

**Delta karyology** : Locations of satellite DNAs, nucleolar organizers, and 5-sr RNA loci have been determined.

**Epsilon karyology** : On the basis of lampbrush chromosome analysis, the main distinctive loops and other land marks have been mapped.

**Zeta karyology** : On the basis of polytene chromosome analysis, thousands of bands and other land marks (puffs, nucleolar organizers, etc.) have been mapped.

Each type brings very different amounts and types of information to problems of evolution and speciation, based on different materials and techniques.

### Techniques

For the chromosomal preparations we always prefer actively dividing cells, of which gonads are the best source of mitotic and meiotic stages. In molluscs the sexes are usually separate, although certain groups are hermaphrodites. The gametes in hermaphrodites may be produced in separate male and female gonads (testes and ovaries) or in the same gonads (ovotestes). Several techniques for the molluscan chromosomes have been devised and improved (Burch 1968, Patterson 1971, Stern 1975, Prasad & Das 1978). The following two procedures have been worked out.

A. Squash preparation, and B. Air dry preparation.

These procedures may form the basis of other methods to be improvised later by molluscan cytotaxonomists.

Colchicine is injected prior to dissection, to arrest metaphase. Its effect varies in different animal groups. Its method of application is preferably by injection into animals, and the range of concentration is also wide. This drug is exceptionally suitable for the study of chromosome structure, and metaphase arrest, provided a strict control is maintained over the concentration and period of treatment. This has to be standardized for the molluscan material. Make 1% colchicine solution (10 mg in 10 ml of distilled water)- inject 0.03 ml of this solution into the animal weighing roughly 200 gms., adjust concentration and volume of colchicine according to the need.

### Techniques to be followed for Molluscs

**A. Squash preparation** : The squash method has a great advantage in that the entire process is rapid and also much more suitable for critical observation. In properly prepared squashes, one can carry out observation on separated single cells; moreover the cell being released of its compactness, undergoes much enlargement in volume affording wider space for the chromosomes to become scattered. Owing to these advantages, it has become the universal routine method in chromosome work.

- i. Inject the animal with colchicine solution (in case of land snail through the apex of the shell).
- ii. After 3-4 hours, dissect out desired tissue (testes, ovotestes) in distilled water at room temperature. Clean and mince the tissue and transfer it to (a) 0.56% KCl for 5-15 minutes or (b) 0.9% sodium citrate solution for 30-60 minutes.

(This hypotonic treatment is used for almost all animal

preparations. It is based on the principle of swelling the cells for dispersion of chromosomes. The solution to be effective, must be of low osmotic pressure. The timings and concentrations are to be worked out for the molluscan material. This treatment is very critical).

- iii. Transfer the tissue in freshly prepared fixative (1 part glacial acetic acid + 3 parts methanol). Allow fixing up to a minimum of 30 minutes. Material can be stored in this fixative even for 2-3 months in a refrigerator.

(The purpose of the fixation is to kill the tissue without causing any distortion of the components to be studied, as far as is practicable. Fixation as is now realized must be critical. It should not only increase visibility of chromosome structure but should also clarify the details of chromosome morphology, such as chromatic and heterochromatic regions, primary and secondary constrictions).

- iv. Transfer the fixed material to 50% acetic acid till it becomes soft.
- v. Squash the material with a drop of 50% acetic acid between well cleaned slides and coverslips. Store the slides in vapours of 50% acetic acid in refrigerator for overnight.

(Special care has to be taken while squashing. The coverslip should not move and the slides are to be protected from dust as far as possible).

- vi. Next morning take out the slides, bring them to room temperature and immerse in 1:3 acetic acid methanol mixture for an hour.
- vii. Remove the coverslips with the sharp edge of a blade while immersed. Dry the slides and coverslips at room temperature in a dust free chamber.
- viii. Stain in buffer Giemsa (pH 6.8-6.9). Rinse quickly in two coupling jars of distilled water. Check its staining under microscope. Dry under lamp, scan and finally mount in DPX.

**B. Air dry preparation :** This technique is applied principally to tissues of any organism which can be converted easily into cell suspensions.

- i. After 3-4 hours of colchicine injection, tissue is dissected out, cleaned, minced and transferred into a centrifuge tube. One third (1/3rd) of the tube is filled with 0.9% sodium citrate solution.
- ii. Agitate the tube with a rubber agitator and then add 0.9% sodium citrate solution to make the volume 10 ml. If any particulate piece of tissue remains inside, remove it from the cell suspension by decanting into another tube.
- iii. Keep it for 30-40 minutes for the hypotonic treatment.

- iv. Centrifuge it at 1000-1200 r.p.m. for 5 minutes. Pour off supernatant slowly without disturbing sedimented cells. Agitate the tube for some time.
- v. Add two drops of freshly prepared fixative and agitate. Add 4-5 drops more and agitate. Fill the tube to 10 ml. mark with fixative by squirting the fluid from pipette with force, so that it becomes homogeneous. Keep it for 30 minutes for fixation. This can be stored in the refrigerator (at 4 °C) for over-night fixation. (The duration of fixation may be prolonged further).
- vi. Centrifuge it at 1000-1200 r.p.m. for 5 minutes, pour out all fixative and keep the tube inverted on a piece of blotting paper to drain off fixative as much as possible. Take care that cells remain at the top of the tube. Repeat steps v & vi.
- vii. Finally resuspend the pellet in 0.5 ml. to 1 ml. fixative depending on the amount of the pellet.
- viii. Take clean slide and put a drop of the cell suspension from a pasteur pipette held at a height of one meter from the slide. Dry the slides either in air or exposed to flame, mark them with diamond pencil and keep in a clean slide box. One can check the quality of the preparation after staining (as in step viii squash preparation). The chromosomes should be elongated and well spread and the sister chromatids should lie parallel to each other.

#### Techniques to be followed for Trematodes

Most trematodes are hermaphroditic, with well developed and complex male and female reproductive organs. The squash technique is much more suitable for this animal. The different schedules of squashing can be divided into two categories, namely, softening, performed prior to staining and softening, clearing and staining accomplished in the same fluid.

#### A. Acetic-carmines/ Acetic-orcein squash technique :

- i. In order to get good spreading of the chromosomes, the parasites are pretreated for 2 hours at room temperature in 10 ml. 0.9% NaCl (normal mammalian saline) with 2 ml. of 0.05% colchicine.
- ii. Fix the parasite in 1 : 3 aceto alcohol for 24 to 48 hours kept in refrigerator.
- iii. Dissect out the testes.
- iv. Stain the testes in acetic-carmines. The solution serves the double purpose of fixation and staining as acetic acid is a good fixative of chromatin and is a rapidly penetrating fluid.

(Belling suggested the addition of ferric hydroxide in acetic carmine during its preparation for the intensification of colour. The addition of a few drops of ferric chloride or ferric acetate

solution also serves the intensity of colour. Acetic-orcein can also be used in the same way and has the added advantage that no iron mordanting is necessary).

- v. Tease the testes into small bits on a slide, put a drop of stain and squash between slide and coverslip, under folds of blotting paper.
- vi. Slides free from air bubble are sealed with paraffin/aceto gelatin solution to protect from drying. Scann immediately and photomicrograph.

(Material, which provide difficulty in staining due to possible inadequate fixation can be fixed in Carnoy's fluid prior to acetic-carmin staining).

- B. For the Giemsa staining of trematode chromosomes, squash method of mollusc can be applied fully after colchicine treatment and proper fixing of testes.

Following technical points should always be remembered :

- i. "When fixation of tissue has been inadequate, various artefacts may be produced in metaphase chromosomes. Following acetic fixation chromosomes may have a swollen, bloated and vacuolated appearance. If penetration of the fixative into the moribund tissue is delayed, one may get 'clumping' of the chromosomes, with fusion of some of them into aggregates. Frequently associated with clumping is the appearance of stainable connecting strands between the chromosomes. It should be unnecessary to point out that all these types of appearances are essentially in constant and variable from cell to cell, and that they are not seen in life or in material that has been prepared by the best techniques" (White, 1973).
- ii. "It is a common observation that size differences exist among mitotic metaphase chromosomes from different tissues. This may be caused by differential penetration of fixing and hydrolysing chemicals so that outer cells may have more prolonged treatment. Comparison of chromosome size among individuals must be made from the same tissues under uniform fixation, hydrolysis and staining. The kind of stain employed, depending upon its solvents, may swell or shrink the chromosomes. Comparisons between tissues of two individuals is difficult unless one uses sectioned material or careful squashing so that the original positions of the cells are maintained. Differences in pretreatment may effect relative arm lengths compared to untreated material. For example, Smith (1965) found that untreated spermatogonial metaphase chromosomes of *Chilocorus* appear metacentric. However, after short periods arms become strikingly asymmetrical causing greater contraction than in heterochromatic arms" (Jackson, 1971).

- iii. Genotypic control of chromosome size is evident. Chromosomal genes influence the quantity and quality of DNA protein coils and folds. Such genotypic control may vary among species, and within the same organism there is tissue specific control.

### Karyotyping and Construction of Idiograms

The cells with good chromosome spreads are photomicrographed. The diploid number (2n) is determined by basic or most predominant number observed in the individual. Cytologists cut out the individual chromosomes from the photomicrographs of the metaphase chromosomes to construct karyotype. The term karyotype describes a display of chromosomes such that they are lined up starting with the longest and continuing in order of diminished size. The agreement dictates that the shorter chromosome arm points to the top of the plate. The cut out chromosomes which appear similar in morphology and staining intensity are paired. The morphology of an individual chromosome depends upon its total length and the position of the centromere. The position of the centromere is indicated either by the arm ratio i.e.,

$$\frac{\text{length of the long arm } q}{\text{length of short arm } p} \text{ or the centromeric index (C),}$$

i.e.,  $\frac{\text{length of short arm } p}{\text{length of whole chromosome } p+q}$

In some animal groups centromeres cannot be located, so usually chromosomal lengths alone provide workable methods.

An idiogram is a diagrammatic karyotype based on chromosome measurements of many cells. Even the most accurate actual photograph does not alone present the idiogram. The procedure is to measure the length of each arm or the entire chromosome with the help of their photomicrographs. This is usually done with the dial caliper. Their relative percentage lengths in relation to the total length of the complement is calculated. The measurements are used in drawing comparative idiograms, an analysis of which forms the basis of cytotaxonomical conclusions.

It is essential to stress the need for care and attention to detail in cytotaxonomic work, if one is to get the most out of it. It is almost always desirable to work on individually numbered specimens which are finally preserved with the number as well as the locality of collection, and deposited in some major national museum, where they can be checked, if necessary, by later workers. In groups where a large percentage of the species are still undescribed, the cytotaxonomist may have to be satisfied with referring to his species by letters or numbers, rather than the latin names, pending full monographic treatment by a competent taxonomist. Where a cytotaxonomist publishes work on a named species, it is desirable for him to make it clear by whom the material was identified, since there is always a possibility of misidentification.

References on Mollusc and Trematode cytogenetics have been appended, and it is for the workers in the groups to judge, plan and execute their further research programme.

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## **Systematics and Ecology of Freshwater Gastropods of Parasitological Importance**

***N.V. Subba Rao and S. C. Mitra***

Zoological Survey of India, Calcutta.

From the parasitological point of view four classes of Mollusca namely, Gastropoda, Scaphopoda, Bivalvia and Cephalopoda are important since all of the known molluscan hosts of flukes belong to these classes. Cephalopods are the only known hosts for the dicyemid mesozoa. About 30 species of cephalopods are reported to have been infected with larval and adult trematodes (Overstreet & Hocbert, 1975). No parasites have so far been recorded from the members of the classes Aplousobranchia, Polyplacophora and Monoplacophora.

Among the freshwater molluscs gastropods are more important as intermediate hosts and hence deserve special treatment as presented below.

In all, over 100 species of freshwater gastropods are reported to serve as intermediate hosts for schistosomes, liver flukes, intestinal and lung flukes. Fortunately, in India the number of species is much less being only 14, out of which 10 species (Pls I-III) are considered important since adult trematodes are recorded from them. (Table 1)

Nematodes are encountered less frequently than trematodes in gastropods. The gut, lumen, the lung or various tissues offer sites for settlement of these parasites.

Freshwater gastropods are grouped into two subclasses, Prosobranchia and Pulmonata. The latter is more important than any other group since, majority of the species implicated as intermediate hosts of helminth parasites belong to it. In fact out of 100 species recorded as intermediate hosts only 18 are prosobranchs and the remaining are pulmonates. Of the 18 families of freshwater molluscs occurring in India, two, namely Lymnaeidae and Planorbidae, both pulmonates, deserve special mention as most of the snails in which helminth parasites develop belong to these two families. However, a mention is also made of the other prosobranch families of some parasitological significance.

### **Systematics: Family Planorbidae**

It is one of the most important family from medical and veterinary point of view. The shell is discoidal or globose or physoid. The animal is sinistral with pulmonary and genital apertures on the left side. The tentacles are long, filiform, cylindrical and bear eyes on their inner bases. The radula includes a bicuspid central tooth, large bi or tricuspid lateral tooth and long, narrow and multicuspoid marginal teeth. A pseudobranch, on the left side, preputium and vergic sac, haemolymph reddish, since it contains haemoglobin, are some other important characters.

Table - I  
FRESHWATER SNAIL HOSTS

Family	No. of Indian Genera	No. of Indian species	Distribution of the family	Species reported as intermediate host	Remarks
Pilidae	2	6	Asia, Africa, N. & S America	<i>Pila globosa</i> (Swainson) <i>Pila scutata</i> (Mousson)	Nematode parasite
Viviparidae	4	8	World wide	<i>Bellamya bengalensis</i> (Lamarck)	<i>Angiostrongylus cantonensis</i> was recorded in Thailand.
Bithyniidae	5	10	Europe, Asia, Africa Australia & N. America	<i>Gabbia orcula</i> (Frauenfeld)	
Thiaridae	5	29	Asia, Africa, Central & S. America, Pacific & Caribbean islands	<i>Thiara tuberculata</i> (Mueller) <i>Thiara granifera</i> (Lamarck)	
Lymnaeidae	1 (5 subgenera)	13 (12 sub-specific forms)	World wide	<i>Lymnaea acuminata</i> (Lamarck) <i>L. luteola</i> (Lamarck) <i>L. auricularia</i> (Linnaeus)	
Planorbidae	6	18	World wide	<i>Gyraulus convexiusculus</i> (Hutton) <i>G. euphraticus</i> (Mousson) <i>Indoplanorbis exustus</i> (Deshayes) <i>Segmentina trochoidea</i> (Benson) <i>Hippeutis umbilicalis umbilicalis</i> (Benson)	
Ancylidae	1	5	World wide	<i>Ferrissia tenuis</i> (Bourguignat)	

Five subfamilies are recognised (Malek & Cheng, 1974) : Bulininae, Planorbinae, Segmentininae, Helisomatinae and Planorbulinae. Only the first three families are distributed in India.

### Subfamily Planorbinae

There is no preputial gland and vergic sac is without flagella. The family includes three genera viz. *Gyraulus*, *Planorbis* and *Biomphalaria*. The last mentioned is an important genus from the standpoint of public health but fortunately for us it is not represented in India. Several species of the genus *Biomphalaria* serve as intermediate hosts for *Schistosoma mansoni*. The genus *Gyraulus* is widely distributed in India and is represented by several species, whereas *Planorbis* has been recorded from Kashmir and Tibet only. Although both have discoidal shells they can be distinguished from the nature and number of their whorls. But the most important structure is verge, which bears a stylet in *Gyraulus* and without it in *Planorbis*.

#### Genus *Planorbis* Geoffroy, 1767

Occurs throughout Europe, Northern Asia, Asia Minor and Northern Africa.

The genus is represented by two species in India, *Planorbis planorbis tangitarensis* Germain and *P. rotundatus* Poiret, both occurring in Kashmir. No parasites are recorded from any of the species.

#### Genus *Gyraulus* Charpentier, 1837

Shell small, disc-like, thin, transparent or translucent, whorls 3-4, rapidly increasing, with or without peripheral keel, body whorl slightly deflected at aperture which is oblique and simple.

World -wide. North and south America, West Indies, Europe, Asia, Africa, Malaysia, Fiji, New Caledonia, Philippines and Pacific Islands.

Commonly occurs in ponds, ditches, drains etc. Usually found attached to leaves and roots of aquatic vegetations. Eggs are deposited in numbers in a gelatinous mass, attached to weeds or other structures submerged in water.

Though earlier reports included a large number of species as occurring in Indian region including Bangladesh, Burma, Pakistan and Sri Lanka, recent studies by Subba Rao (1989) has brought down the number to nine including some imperfectly known species. Only two among them are implicated as intermediate hosts.

#### *Gyraulus convexiusculus* (Hutton)

**Distribution :** India : Widely distributed. Iran to Philippines.

**Remarks :** Shell small, greatly flattened, whorls 4-5 , periphery subangulate, sutures impressed, finely obliquely striate, umbilicus wide.

**Parasites :** Larval forms of *Giganticotyla crumenifer*, *G. explanatum*, *Fasciola elongatus* and also *Cercaria chugathi*, *C. gyraulusi*, *C. rithorensis*, *C. furgolensis* were recorded.

#### *Gyraulus euphraticus* (Mousson)

**Distribution :** India : Bihar, Punjab, West Bengal. Baluchistan, Afghanistan.

**Remarks :** Shell strongly resembles the preceding species but much more sharply

keeled around the periphery and a little more coarsely sculptured, body whorl slightly deviating from the spiral axis.

**Parasites** : Larval forms of *Echinostoma ilocanum*, a parasite of rats and human beings and *Diplodiscus amphicrus* were recorded from the snail. Sewell (1922) recorded a few cercariae.

#### Subfamily **Bulininae**

Shell ovoid or elongate and acuminate as in *Camptoceras* or disc-shaped as in *Indoplanorbis*; sinistral.

Includes three genera, *Bulinus* Mueller, *Indoplanorbis* Annandale and Prashad and *Camptoceras* Benson. The species of *Bulinus* transmit human blood fluke, *Schistosoma haematobium*, *S. interculatum* and amphistome *Paramphistomum microbothrium* (cattle, sheep and goat) and *Gastrodiscus aegypticus* (horse); they are distributed in Africa, Middle East and Southern Europe. The two genera *Indoplanorbis* and *Camptoceras* are distributed in India.

#### Genus *Indoplanorbis* Annandale & Prashad, 1921

Shell large and thick, discoidal, spire sunken, sutures impressed, aperture ear-shaped.

Animal sinistral, foot broad and short, posteriorly pointed, branchial process lobed.

Ponds, ditches and canals with or without vegetations.

It is a monotypic genus and is represented by a single widely distributed species, *I. exustus* (Deshayes).

**Distribution** : India : Widely distributed. Persia, Pakistan, Burma, Malaya, Vietnam, Thailand, Sumatra, Java and Celebes.

**Parasites** : Trematodes of horse, goat, sheep, camel, dog, buffalo and other cattle develop to cercarial stage in this snail. It serves as the intermediate host for the largest number of cercariae. The important parasites recorded are : *Schistosoma indica*, *S. nasalis*, *S. spindalis*, *Fasciola gigantica*, *Paramphistomum mehri*, *P. explanatus*, *Gastrodiscus secundus*, *Petagifer srivastavi*, *Plasmiorchis orientalis*, *Pseudodiscus collinsii*, *Gastrothylas crumenifer*, *Enterohaemotrema plaeorticum*, *Cotylopholrum cotylopholrum*, *Cotylophora indica*, *C. bhaleraoi*, *C. mathurapurensis*. Besides these parasites, about 57 types of cercariae were recorded from this snail.

#### Genus *Camptoceras* Benson, 1842

*Camptoceras* is represented by three species in India. *C. (C.) terebra* Benson, *C. (Culmenella) hirasei* Clench and *C. (Culmenella) subspinosa* Annandale & Prashad, all occurring in northern India including Kashmir in the west and Manipur in the east. No parasites are recorded.

#### Subfamily **Segmentininae**

Shell small, low, flattened, smooth, glossy; aperture heartshaped.

Following Subba Rao (1989) only two genera *Segmentina* and *Hippeutis* are recognised.

#### Genus *Segmentina* Fleming, 1817

Shell small, glossy, thin, convex above and flattened below, body whorl large, carinate or angulate; internally divided by white transverse lamellae; aperture sub-triangular.

North Africa, Europe and Asia

Represented by one subgenus *Polypylis* Pilsbry, 1906 and three species in India. Only one species is treated here.

*Segmentina (Polypylis) trochoidea* (Benson)

**Distribution** : India : Karnataka, Tamilnadu, West Bengal, Assam. Burma, Sri Lanka.

**Remarks** : Shell very small, trochoid, whorls 3 1/2 periphery acutely angulate, umbilicus closed or narrow, apertural lamellae weak.

**Parasites** : *Fasciolopsis buski* was found to develop in this snail in Assam.

Genus *Hippeutis* Charpentier, 1837

Shell small, discoidal, similar to that of *Segmentina* but without the internal lamellae.

Europe, South east Asia, China, Taiwan, the Philippines and Japan.

A single species with its subspecies is considered here.

*Hippeutis (Helicorbis) umbilicalis umbilicalis* (Benson)

**Distribution** : India : Uttar Pradesh – Kumaon Lakes, Bihar, West Bengal, Assam, Manipur. South east Asia, Southern China, Taiwan, the Philippines.

**Remarks** : Periphery bluntly angulate, whorls roundly convex above.

**Parasites** : It is an intermediate host of *Fasciolopsis buski*.

The snail was experimentally infected with *Gastrodiscoides hominis*, an intestinal parasite of man and several other species of mammals including the domestic pig. Human infections of this parasite were reported from Assam, Bengal, Bihar and Orissa. Experimental infection of this snail with *Fasciolopsis buski* was also reported.

Family Lymnaeidae

From parasitological point of view Lymnaeidae is a very important family of common freshwater pulmonate snails. The shell is dextral, ovate oblong, thin and light, spire pointed and variable in height, body whorl inflated and proportionately large, columella typically twisted; aperture rather large, lip thin and simple.

Foot broad, tentacles bluntly triangular.

World-wide. Inhabits all types of freshwater bodies, common in stagnant water ponds, tanks, lakes etc. with or without vegetation. Eggs laid on weeds etc. in clusters as gelatinous mass.

Represented in India by a single genus *Lymnaea* Lamarck.

The systematics of the genus *Lymnaea* is still in a confused state. On the basis of its extremely variable shell characters, a large number of species and subspecific forms were described by various authors. Hubendick (1951), made a revisionary study of the family based on anatomical characters and as a result of it, the number of species and forms were drastically brought down. But more recent studies by Burch and Lindsay (1973) proved that classical taxonomic divisions within the family based on shell characters are more reliable. In this context we are inclined to follow the arrangement

of Annandale & Rao (1925) for the Indian forms.

**Genus *Lymnaea* Lamarck, 1799**

Shell thin, body whorl large, spire exerted, columella spirally twisted.

Subba Rao (1989) recognises 17 species as occurring in India and adjacent countries. Six subgenera *Pseudosuccinea*, *Galba*, *Radix*, *Lymnaea* and *Stagnicola* are recognised. Only species having parasitological significance are dealt with here.

***Lymnaea (Pseudosuccinea) acuminata* Lamarck**

**Distribution :** India : Common throughout.

**Remarks :** Shell thin, ovate, spire short, apex acute. Aperture wide. Shell in this species shows considerable variations based on which a number of forms have been created. The forms which are fairly constant and usually recognised are, *L. acuminata* f. *rufescens* Gray (spire fairly produced, aperture uniformly less expanded), *L. acuminata* f. *gracilior* Martens (elongate and narrow with a pinkish tinge), *L. acuminata* f. *chlamys* Benson (spire short and globose with a golden yellow colour).

This species usually occurs in permanent water bodies with abundant vegetation.

**Parasites :** Larval forms of some important parasites of sheep, cattle and man are recorded from this snail. It was recorded as the intermediate host of different flukes viz. *Fasciola gigantica*, *F. hepatica*, *Schistosoma indica*, *S. nasalis*, *S. spindalis* and *Clinostomum giganticum*, *Echinostoma* sp. About 15 cercariae are recorded from this snail.

*Echinostoma revolutum*, *E. malayanum*, f. *hepatica* and *Orientobilharzia turkestanicum* develop in *L. acuminata* f. *rufescens*.

***Lymnaea (Pseudosuccinea) luteola* Lamarck**

**Distribution :** India : Common throughout.

**Remarks :** Shell thin, glossy, body whorl less inflated and laterally compressed a little; spire comparatively longer and gradually tapering. Aperture angulately narrowed above. As in the preceding species a number of forms have been described under this species also. The following forms are fairly constant and are commonly recognised; *L. luteola* f. *australis* Annandale & Prasad (smaller with a comparatively longer spire), *L. luteola* f. *ovalis* Gray (spire short and abruptly pointed, body whorl globose and inflated), *L. luteola* f. *succinea* Deshayes (close to f. *impura*, broader with a less acute spire).

**Parasites :** It is one of the important snails serving as an intermediate host of some parasites of cattle, pig, dog etc. Parasites recorded are : *Schistosoma nasalis*, *S. spindalis*, *S. sios*, *S. incognitum*, *Fasciola gigantica*, *F. hepatica*, *Clinostomum giganticum*, *Orientobilharzia deltae*, *Echinoparphium bugulai*.

Besides the parasite *Fischoederius elongatus*, about 10 cercariae were recorded from the form *succinea* Deshayes.

***Lymnaea (Radix) auricularia* (Linnaeus)**

Subgenus *Radix* Montfort includes four species among which only *L. (R) auricularia* (Linnaeus) is of significance in the present context.

**Distribution** : India : Kashmir. It is a palaeartic species which has its distribution extended to Kashmir.

**Remarks** : Shell large, inflated, columella strongly twisted, the fold obliterating the umbilicus, spire deeply sunken into the body whorl.

**Parasites** : Only *Orientobilharzia turkestanicum* was known to develop in this snail in Srinagar area (Dutt & Srivastava, 1964).

#### Family Ancyliidae

Shell small, cap-like or patelliform, aperture large, animal completely covered by shell, foot broad, tentacles short, blunt and cylindrical.

World-wide in distribution.

Represented by a single genus *Ferrissia* Walker.

Genus *Ferrissia* Walker, 1903.

Shell with radiately striate apex.

Australia, New Zealand, S. E. Asia, and S. E. Africa.

Five species are recognised from India, among which only *F. tenuis* (Bourguignat) is implicated doubtfully as an intermediate host.

#### *Ferrissia tenuis* (Bourguignat)

**Distribution** : Peninsular India.

**Remarks** : Shell minute, fragile, apex obtuse, aperture oblong.

Attached to stones, pebbles or floating objects in rivers or streams with moderate flow. Eggs are laid singly on decaying leaves etc., in a gelatinous mass.

**Parasites** : The species was reported to be an intermediate host of human blood fluke, *Schistosoma haematobium* in Gimvi village in the Ratnagiri district, Maharashtra. But a survey by the senior author in the area around Gimvi village did not show any conclusive evidence to support that the snail is an intermediate host of schistosome.

#### Order Mesogastropoda

##### Family Piliidae

Shell operculate, large, perforate or imperforate with an inflated body whorl, spire short. Operculum large, usually calcareous. Animal with a pair of filiform tentacles.

#### Genus *Pila* Roeding, 1798

Shell very large, aperture large but not expanded, outerlip slightly thickened; operculum calcareous, thick, with a lateral nucleus (nearer to columella).

Asia and Africa.

Subba Rao (1989) recognised five Indian species. Among these, from only *Pila globosa* (Swainson) were recorded a few larval trematodes.

#### *Pila globosa* (Swainson)

**Distribution** : It is the commonest species occurring widely in the greater part of India, except Punjab and Himachal Pradesh in the north and also in the Peninsular India, where it is replaced by *Pila virens* (Lamarck).

Two varieties, *incrassatula* and *minor*, both described by Nevill are recorded from West Bengal.

**Parasites :** The following larval trematodes were recorded : *Artyfechinostomum sufratyfes*, *Diplodiscus* sp., *Echinostoma cercaria*, *C. andhraensi*, *C. pigmentata* and *Xiphidocercaria* sp.

#### Family Viviparidae

Shell moderately large, whorls rounded with or without bands. Aperture sub-circular, operculum horny, concentric.

World -wide.

Represented in India by one subfamily Bellamyinae.

Among five Indian species only *Bellamyia bengalensis*, the most common species is dealt here.

#### *Bellamyia bengalensis* (Lamarck)

**Distribution :** India : Common throughout, and also Burma.

**Remarks :** Shell thin, smooth with a few dark bands, embryonic shell acutely keeled.

Based on shell characters, principally on the form of spire, a good number of infraspecific forms were described. The following forms are fairly constant and are usually recognised, *B. bengalensis* f. *typica* (Lamarck), the most widely distributed form, with evenly convex whorls, *B. bengalensis* f. *annandalei* (Kobelt) a thinner and conical form with nearly straight sides of whorls, *B. bengalensis* f. *doliaris* (Gould), a form with a biangulate body whorl.

**Parasites :** Though a few larval trematodes were recorded viz., *Cercaria asiatica* Sinha & Prasad, *Thapariella anastomusa* Srivastava, *Cercaria thaparai* Rai and *C. kumaonensis* Singh & Malaki, it is not considered to be a significant intermediate host.

#### Family Bithyniidae

Shell small, ovate or elongate-turreted, narrowly perforate or imperforate with convex whorls; aperture round or ovate; peristome often thickened, operculum thick, calcareous with a subcentral nucleus.

World-wide.

Two subfamilies, Bithyniinae and Mysorellinae and 5 Indian genera are recognised.

#### Subfamily Bithyniinae

It includes 10 Indian species among which only *Gabbia orcula* is important.

#### *Gabbia orcula* (Frauenfeld)

**Distribution :** India: Common throughout. Occurs in slow moving freshwater streams, canals, stagnant water pools, wet paddy fields; usually found among weeds.

**Remarks :** Shell globose, imperforate, smooth, body whorl well rounded, columellar margin a little reflected. Includes a variety, *producta* (Nevill), a more elongate form.

**Parasites :** Though Sewell (1922) recorded a few cercariae from it there are no subsequent records.

## Family Thiaridae

It is the largest family of Indian freshwater prosobranchs. Shell ovately conical to elongate turreted, smooth or variously sculptured with ridges, nodules, spines etc. Aperture ovate or pear shaped, sometimes rather canaliculate.

World-wide. Prefers streams or rivers, but also found in stagnant ponds or lakes etc.

The taxonomy of this large family is in a confused state. Subba Rao (1989) recognised four subfamilies : Thiarinae, Melanopsinae, Melanatrinae and Paludominac.

### Subfamily Thiarinae

Shell imperforate, ovately conical to elongate, smooth or with sculpture. Aperture elongate, angulate above. Operculum with a basal nucleus.

Asia, Africa, Central and South America, Caribbean and Pacific islands.

#### *Thiara (Melanoides) tuberculata* (Mueller)

**Distribution :** India : Very widely distributed throughout the whole of South-east Asia. It occurs in practically all possible bodies of stagnant and slow moving freshwaters; ponds, streams, ditches, drains, water mains, extending to slight brackish water.

**Remarks :** shell elongate with reddish brown dots and flames, sculptured conspicuously with vertical ribs and spiral striae, whorls 10-12, fairly rounded. Shows considerable variation in sculpture, colouration and length-breadth ratio. One subspecies *T. (M.) tuberculata crebra* (Lea) is recorded from Andaman and Nicobar Islands.

**Parasites :** A number of cercariae were recorded from the snail. Sewell (1922) recorded *Cercaria indica*, in addition *C. aciculate*, *C. bristiae*, *C. bristpennata*, *C. claudiglandula*, *C. cordata*, *C. cruciata*, *C. beschiensi*, *C. dissimilis*, *C. fulvier*, *C. fulvaculata*, *C. goratiensis*, *C. nepprenocephalus*, *C. indicus*, *C. kukrailensis*, *C. bhimtalensis*, *C. naukuchiensis*, *C. pinjorensis* and also avian parasites like *Paragonimus westermani*, *Stellantchasmus formosanus*, *Transversospoma patialensis* are recorded (Subba Rao, 1989).

*Thiara (Tarebia) granifera* (Lamarck), is also recorded elsewhere as a host of *Paragonimus westermani*.

Subfamily Melanatriinae includes two genera *Sulcospira* Troschel, 1958 and *Brotia* H. Adams, 1866, each represented by a single species. Of these two, *Brotia costula* (Rafinesque) is a highly variable and widely distributed edible species from which *Cercaria indica*, was recorded by Sewell (1922).

### Ecology

The freshwater molluscs inhabit all types of aquatic bodies, ponds, ditches, lakes, streams, rivers, with or without vegetation, with or without stones, boulders etc.

Broadly the freshwater habitats can be grouped into two categories such as, ponds, ditches, paddy fields etc., with more or less stagnant water (lentic type) and streams, rivers and canals with flowing water (lotic type).

Pilids, viviparids, lymnaeids and planorbids are usually found in stagnant waters whereas thiarids, ancylicids are usually the inhabitants of flowing waters. In this

context it may be mentioned that in North Bengal (Jalpaiguri district) at certain places the course of the rivers (e.g. Torsa, Raidak) changed due to some reasons or other. As a result the original course at such points became rather dead and turned into something like a patch of stagnant water with consequent growth of aquatic weeds etc. This led to the occurrence of stagnant water forms like planorbids, lymnaeids and viviparids. But these were absent in the regular course of the river, where usual riverine forms, such as *Brotia* sp., *Paludomus* sp., *Ferrissia* sp. were found.

Intermediate snail hosts, namely *Gyraulus convexiusculus*, *G. euphraticus*, *Segmentina trochoidea*, *Hippeutis unibilicalis umbilicalis*, *Lymnaea acuminata*, *Gabbia orcula* occur among weeds. Usually they remain attached to leaves and roots of vegetations. *Gabbia orcula* is sometimes found on muddy bottom also. *Lymnaea acuminata* attaches itself to floating leaves, sticks etc. and mostly remains close to the edge of water, but often crawling out of water. Naturally vegetation is a prerequisite for the occurrence of such species.

On the other hand, two of the most important species viz., *Indoplanorbis exustus* and *Lymnaea luteola* although prefer weeds are equally at ease on muddy substratum. These were reported to be abundant in ponds where no vegetation of any kind was noticed in any season of the year and where during the summer the water level goes down drastically turning the pond into a puddle. Planorbidae and Lymnaeidae have successfully colonised transient freshwater habitat since they have broad physiological and ecological requirements. But at the same time they are restricted by their respiratory activity, which involves filling up their lungs with atmospheric oxygen. As a result most of these snails are found not immersed in water. They cannot tolerate the turbulence of large rivers.

Two other species, *Pila globosa* and *Thiara tuberculata* show remarkable adaptations. *Pila globosa* normally inhabits stagnant water with vegetations, occurring mostly in the littoral zones of ponds but often extending to wet or semi dry paddy fields. To a large extent it is amphibious in nature. During its aquatic phase it excretes ammonium compounds and during its terrestrial phase, it excretes uric acid. The latter is an adaptation to conserve water and found in land gastropods. At Sagar island, while on tour, we have seen this snail to occur freely in fields flooded with the saline water from the adjacent Muriganga river along with such primary estuarine species as *Littorina melanostoma*, *Stenothyra deltae*, *Assimineia brevicula* etc., in the month of September when the salinity was a bit low. *Thiara tuberculata* normally occurs in all water bodies, ponds, canals, rivers, streams and also water mains, extending to brackish water. During the course of our series of surveys of ponds around Calcutta, it was seen to occur in abundance in muddy drains throughout the year including summer, with very little flow of water. Its population density in some of these drains was exceeding 1000/m<sup>2</sup>.

Though the young ones of the common pond snail, *Bellamya bengalensis* remain attached to floating sticks or weeds, adults are usually found on the muddy bottom of ponds, ditches etc. Some of the infra-specific forms of this widely distributed polytypic species are known to occur in very foul water. During the recent survey of Uttar Pradesh, at Saharanpur these snails were seen to occur abundantly in a canal of foul water, a sewerage for the whole township.

*Ferrissia tenuis*, the freshwater limpet occurs in flowing streams and rivers and remains attached to floating sticks, leaves or submerged stones etc.

Many of these freshwater habitats dry up during the summer. So the inhabiting snail fauna has to face an annual spell of dry and unfavourable period. This they overcome by burying themselves under dead weeds or mud. At least the apertural portions are buried under mud or sand. While a high mortality rate is conceded it is nonetheless an established fact that many of the snails do successfully overcome such intervening drought spell. (Laboratory experiments have proved that *Bulinus truncatus* a planorbid intermediate host species can breed with double the normal intensity, after desiccation).

An important phenomenon which plays a definite role in the life of these snails, is that a majority of the freshwater bodies are discontinuous and temporary. This results in two things, firstly the discontinuous habitats create isolated local populations of snail fauna giving rise to divergent infra-specific forms. Secondly at the same time owing to the geologically temporary nature of these habitats these divergent forms do not get time to evolve to a higher level. Perhaps the high degree of infra-specific variations displayed by several of these species like, *Lymnaea acuminata*, *L. luteola*, *Bellamya bengalensis*, *Thiara tuberculata* and also *Indoplanorbis exustus*, can be linked to this factor. Habitat diversity, in this context, seems more important since all the species which exhibit such divergent forms are remarkable for their adaptations to a varied range of habitats.

### Snail Susceptibility and Host Specificity

While analysing the compatibility/incompatibility of the larval trematodes vis-a-vis their molluscan hosts, certain factors need to be kept in view. A high degree of specificity occurs on the part of the parasites. Parasites are usually adapted either to a single species or a few allied species in a single genus or at least in a same family. A particular trematode may penetrate several species but its subsequent fate is governed by a few yet uncertain physiological and biochemical phenomena. If it is successful it goes on producing cercariae or is encapsulated by some cellular reactions. In susceptible snails usually such reactions do not occur, though there are reports of it happening in case of some dying or moribund cercariae.

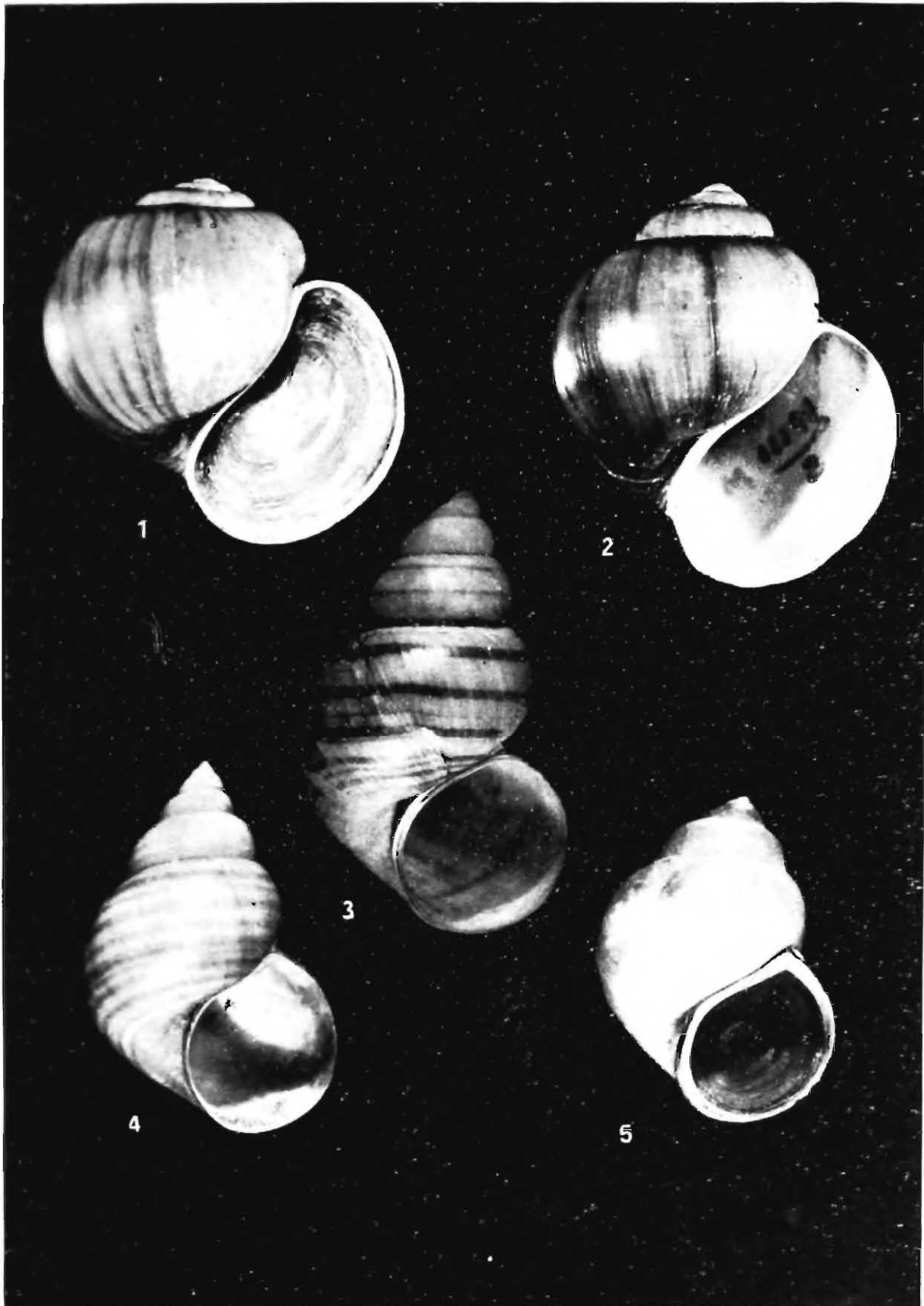
Host specificity is best demonstrated by human-infecting schistosomes. Species of schistosomes are reported to develop in closely allied species of hosts. In Africa a similarity has been noticed in the broad ecological requirements of both man and planorbid snails, which provide an ideal scenario for the schistosomes to survive. Sometimes human activities may create conditions leading to large concentrations of snails. Similarly, *Fasciolopsis buski* develops only in *Segmentina* and *Hippeutis*, two allied genera under the same subfamily. Again, *Fasciola hepatica* and *F. gigantica* develop only in *Lymnaea* and the African genus *Bulinus* is susceptible to the restrictive local strains of schistosomes. Since particular snail hosts have particular habitat requirements trematodes also follow the same characteristic habitat pattern. Exceptions such as absence of a particular trematode infection inspite of the presence of the susceptible snail may be due to some ecological (topographical) or physiological factors. Actually the whole process of host-parasite relationship involves a number of

stages like, first contact, invasion, establishment within the host and finally the escape process. Obviously all these factors equally contribute in regulating the nature of compatibility/incompatibility of the snail (Malek & Cheng, 1974).

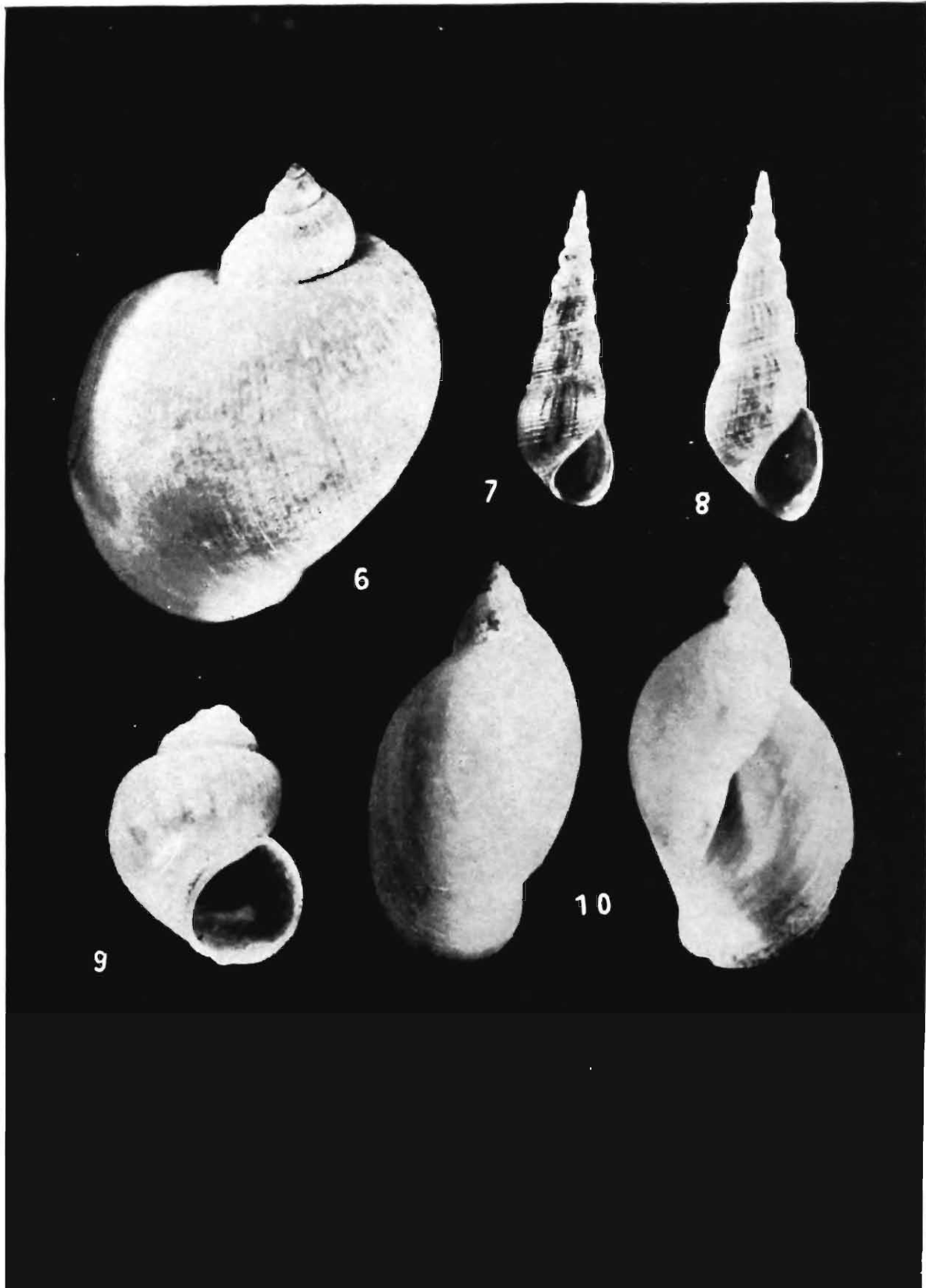
Each organism shows diverse responses to the environment in which it inhabits. The snail has to respond to the environmental changes brought out in a normal way and also by the parasites. The host provides the environment for the parasites. Both the host and parasites are the integral parts of the ecosystem. The underlying causes of the integration of the species into an ecosystem is best understood through biochemical ecology. Each organism has its own chemical language. Through this organisms establish 'contacts' with other organisms and the biotope. Biochemical ecology deciphers these codes. The concept of biochemical ecology is relatively new and takes the study of ecology to the molecular level (Gilles, 1972).

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1. *Pila globosa*;      2. *Pila scutata*;      3. *Bellamya bengalensis* f. *typica*;  
4. *B. bengalensis* f. *annandalei*;      5. *B. bengalensis* f. *doliaris*.



6. *Lymnaea auricularia*; 7. *Thiara (Melanoides) tuberculata*; 8. *T (M.) pyramis*  
[ *T (M.) tuberculata*]; 9. *Gabbia oracula*; 10. *Lymnaea acuminata* f. *typica*.



11 *L. luteola* f. *typica*; 12. *Indoplanorbis exustus*

## **Biology of Gastropod Molluscs in relation to Trematode Infections**

**B. Das Gupta**

Ananda Mohan College, Calcutta.

The snail is an essential first intermediate host, or only intermediate host of different digenetic trematodes. The presence of susceptible snail host is the primary requirement for the establishment of trematode infection. The appearance and survival of the parasites are influenced by general biotic factors associated with aquatic environments as well as by the more intimate physiological and immunological interchanges present in any host-parasite relationships. The parasites are affected variously by changes in the host activity, abundance, feeding patterns, migratory and swarming behaviour and also by changes in the temperature, light, pH, O<sub>2</sub>, surface tension of the water body etc. which interact in a complex manner. The fecundity of the parasite also provides a considerable advantage for the completion of its life cycle.

Level of infection in snail host is dependent on the appearance of new generation, immunity to infection in some part of their life and death of the old snails. The habitat preference of the snail also plays an important role in larval infection. Abiotic factors like precipitation, temperature, light and pH. etc. are considered to be the factors which have obvious and important impact on the vegetational distribution of the freshwater habitats on which, in turn, the distribution of the gastropod snails depends.

### **Trematode Parasites of Snails in India**

In India a number of workers have reported larval trematodes from snail host of a particular locality or from a new snail host. Rao (1932-33) made a comparative study of cercarial fauna in Madras and recorded 11 species—6 from *Indoplanorbis exustus* and 5 from *Lymnaea luteola* and later by Peter (1958), Rao & Ayyar (1932) in Assam; Bhalerao (1943) from Hyderabad; Malaki & Singh (1962) from Kumaon hills, Sahai (1969-70) from U.P.; Mohandas (1976-1977) from Kerala; Muralcedharan *et. al.* (1977) from Karnataka. Survey of larval trematodes and their snail hosts in West Bengal was restricted mostly to the area in and around the city of Calcutta (Sewell, 1922). Das Gupta (1973) reported 41.1% and 18% infection in *L. acuminata* and *I. exustus* respectively in the suburbs of Calcutta. Das Mahapatra (1983, 1984) and Majumdar (1983) made investigations on the larval trematodes of fresh-water snails and their host-parasite relationships from 11 districts of West Bengal.

Wide range of variation in the percentage of infection in total snail population is available from different countries. It has been observed that the population of snail species and the frequency of infection varies from place to place and also with the

seasons. The studies by Das Mahapatra (1983) and Majumdar (1983) revealed that the incidence of infection in snails with larval trematodes is higher in ponds than in any other habitats. Sewell (1922), Rao (1973), Mohandas (1974), Muraleedharan *et al.* (1977) and others reported similar observations from India.

Considerable variations in percentage of infection with a single species are also on record. A snail may serve as an intermediate host for more than one species of trematode parasites but usually harbouring one at a time. *Gabbia travancorica*, *I. exustus* and *Thiara (Melanoides) tuberculata* serve as intermediate hosts for 12, 15, and 17 types of cercariae respectively (Sewell, 1922). Rao (1932-33) described 6 and 5 species of cercariae in *Indoplanorbis exustus* and *L. luteola* respectively. Singh (1959) reported 10 types of cercariae in *I. exustus*. Das Gupta (1973) recorded 3 species of cercariae each in *L. acuminata* and *I. exustus*. Mohandas (1974) reported 13 species of cercariae in *I. exustus*, 11 species from *L. luteola*, 4 species from *Digoniostoma pulchella*, 3 species in *M. tuberculatus*. Das Mahapatra *et al* (1984) reported 20 species of cercariae from 10 species of snails in West Bengal.

Sewell (1922), Rao (1932-33), Bhalerao (1943), Peter (1954), Singh (1959), Mukherjee (1966), Sahai (1969-70), Jain (1970), Mohandas (1974), Muraleedharan *et al* (1977), Das Mahapatra (1984) have recorded double or multiple infection in different snail hosts in India.

#### **Seasonal Variation of Snail Population and their Infection with Trematode Parasites**

It is generally believed that the density of snail population in a locality varies in different seasons of the year. We are unanimous that the population has one peak in a year, but the peak has been reached in post-monsoon in different snails.

Trematode larvae are found throughout the year but a seasonal fluctuation in their number is of common occurrence (Kemp and Gravely, 1919). Sewell (1922) considered the possibility of a relationship that existed between seasonal variation in *T. (M.) tuberculata* population and larval trematode infection. Seasonal variation in infection of snails with trematode larvae have been reported by many workers in India: Das Gupta (1973), Madhavan (1973), Jain (1976), Pandey (1978), Majumder (1983) and Das Mahapatra (1983, 1984).

Several workers reported two peaks of cercarial infection. Das Mahapatra (1984) recorded that there is only one peak in most of the snails in West Bengal and this is confined to monsoon through post-monsoon. Breeding behavior, habitat preference of snail host, immunity of infection in some stages of life and mortality of old snails also had their impact on the seasonal variations of infection.

#### **Age Sensitivity of the Host Snail**

The rate of trematode infection varies with the size and age of the different host species. Some snails are immune to infection at their older age, but found 100% infection at young stage (Amcel, 1934), while some other snails are more resistant in young stage (Sewell, 1922). Das Gupta (1973) observed that the susceptible stages of *L. accuminata* and *I. exustus* are 12-18 mm and 5-8 mm size respectively.

#### **Histopathology**

In course of development and multiplication, trematode larvae obtain their

nourishment from vital organs of snail hosts, where they are lodged and cause mechanical, morphological and physiological damage to the organs (Ward, 1907; Brumpt, 1922). Authors agree on the nature and extent of damage but they differ on the factors which cause the damage.

The most commonly encountered histopathological changes in molluscs, especially gastropods, are those associated with the presence of larval trematodes. These range from extremely severe to minor alterations. This topic has been comprehensively reviewed by Cheng and Snyder (1962) and only a few additional studies have been reported since that review.

**Damage to haemolymph vessels :** Pan (1965) has demonstrated that the walls of the aorta and the arteries of *Biomphalaria glabrata* infected with *Schistosoma mansoni* become thickened after the sixth week of infection. The cells lining these vessels are hypertrophic and hyperplastic and some undergo division. The muscular tunic of the arteries and aorta is thickened and is intensely eosinophilic. In addition, the cells comprising the vessels, connective tissue tunics are usually hypertrophic and contain lightly basophilic, abundant cytoplasm and oval chromatin-rich nuclei. These changes are most conspicuous in small arteries.

Digestive gland is the chief site of infection. The damage ranges from slight, involving only dislocation of the surrounding digestive lobules (Pratt and Lindquist, 1943) to rather severe, involving mechanical destruction of cells coupled with autolysis and parasite induced lysis (Wright, 1966; Boral & Das Gupta, 1983).

The rediae probably reach the digestive gland via blood stream (Rees, 1934). While lodged between the digestive gland tubules, they grow rapidly both in size and number and may cause obliteration of the lumina of the tubules and blocking of interlobular spaces (Rees, 1934). In extreme cases the membranous mantle covering the digestive gland becomes overstretched and ruptures. Blocking of lumina of the tubules and interlobular spaces as reported by others could not be detected even in extreme cases though rupture of the mantle was recorded in a number of specimens.

Mechanical damage of the digestive gland tissue due to parasitization has been reported by Faust (1920), Agersborg (1924), Hurst (1927) and Rees (1934), and our findings in *Lymnaea acuminata* are in complete agreement with their observations.

Interestingly, due to loss of pigment, the blackish brown colour of the digestive gland turns pale yellow. Similar change was noted in *Physa* (Hurst, 1927), *Patella* (Rees, 1934) and *Melanoides* (Lal and Premvati, 1958).

Appearance of a transverse partition near the base of some of the absorptive cells of the digestive gland enclosing the nucleus, and thus giving a squamous appearance to the cells, rather than columnar has been reported from *Patella* (Rees, 1934) and *Melanoides* (Lal and Premvati, 1955). No such change, however, could be detected in *L. acuminata*. In summary, histopathological features associated with the digestive glands of molluscs parasitized by larval trematodes include the following (Malek and Cheng, 1974) :

1. Rupturing of the tunica propria.
2. Disarrangement of the acinar tubules.
3. Mechanical compression and rupturing of cells.

4. Lysing of cells by enzymes and excreta from parasites.
5. Starvation autolysis of cells.
6. Presence of large numbers of ferment globules.
7. Increase in number of calcium spherites.
8. Increase in lipid content in cells.
9. Reduction in amount of stored glycogen in cells.
10. Alteration from columnar to cuboidal or squamous epithelium.
11. Increase in number of cytoplasmic vacuoles.
12. Possible occurrence of abnormal mitotic figures.

### Pathophysiology

**Effect on fecundity :** It has been stated that the presence of certain helminth parasites, especially larval trematodes, is known to eliminate or reduce the production of eggs by molluscs. If the larval trematodes are rediae, the hosts gonadal tissues are totally or partially destroyed as the result of direct ingestion. On the other hand, if the larval trematodes are sporocysts, parasitic castration apparently is due to physiological stress.

**Changes associated with parasitic castration :** In addition to reduction or cessation of egg laying, several other interesting manifestations of parasitic castration have been reported. Wesenberg Lund (1934) appears to be the first to have reported that mollusks parasitized by larval trematodes become abnormally large. Specifically, he found that parasitized *Lymnaea* have become larger.

Sex reversal is another phenomenon that has been associated with parasitic castration. Pelsencer (1906, 1928) was the first to point out that the penis of snail harbouring larval trematodes is reduced. This, in his opinion, is due to partial or complete castration of the host. It was Wesenberg Lund (1931), however, who first proposed the theory that changes of sex in Mollusca might be directly the result of parasitization by larval trematodes.

**Effect on Growth :** The most detailed study to date on possible enhanced growth of parasitized snails is that by Pan (1965). He has shown that adolescents of *Biomphalaria glabrata*, 7.3 mm in shell diameter, mass infected with *Schistosoma mansoni*, have greater shell diameters than uninfected snails between the second and sixth week after infection.

**Effect on Shell Morphology :** In addition to differences in the degree of calcification and the dimensions of the shells of parasitized and non parasitized molluscs, another type of abnormality is known. Specifically, Sturrock and Sturrock (1971) have reported that the shell of *Biomphalaria glabrata* is commonly distorted when infected with *Schistosoma mansoni*.

**Effect on Longevity :** Despite extremely severe histopathological alteration in parasitized molluscs, especially in instances of trematode parasitization, helminthic diseases of molluscs may be considered chronic rather than virulent and or acute. In other words, the molluscan host is not killed in a short period of time.

**Effect on Heart Rate :** Lee and Cheng (1970) were the first to report that the heart rate of *Biomphalaria glabrata* has significantly increased as the result of parasitization by *Schistosoma mansoni*. A more detailed analysis (Lee and Cheng, 1971) has revealed that when the heart rates of snails (NH albino strain, 10 mm in shell diameter) exposed to the miracidia are compared to those of uninfected controls over a period of 8 weeks postinfection, there is a significant decrease from the fourth week in case of the former.

**Effect on Respiration :** Since Von Brand *et al.* (1950) have demonstrated that there is a correlation between the heart rate of *Biomphalaria glabrata* and oxygen consumption, Lee and Cheng (1971) have conducted studies to ascertain the oxygen consumption of snails uninfected and infected with *Schistosoma mansoni*.

**Effect on Thermal tolerance :** Vernberg and Vernberg (1961) have established that molluscs infected with larval trematodes have a lower maximum thermal tolerance limit. They investigated *Nassarius obsoletus* parasitized by either *Lepocreadium ovalis* sporocysts or *Zoogonus rubellus* sporocysts. Similarly, Etges and Gresso (1965) have noted that specimens of *Biomphalaria glabrata* infected with *Schistosoma mansoni* died at higher temperatures while uninfected ones survived.

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## **Snail Borne Diseases and their Role in Veterinary Public Health**

**Gita Biswas**

IVRI Regional Station, Belgachia, Calcutta.

Livestock of a country contributes towards augmentative protein production, subsidiary occupation supplementary income, vital draught power and supply of organic manure. Hence for agricultural country like India livestock is a pivot. Even with present poor mode of livestock keeping practices, the contribution of Animal Husbandry Sector towards Nation's economy is upto Rs 16,500 crores (Anon, 1987).

According to FAO Statistics on livestock population (1988) India possesses cattle 201,401; buffalo 75,605; pig 10,519; sheep 56,980; goat 108,493; chicken 206,000 and duck 29,000. This vast animal populations are continuously overburdened with heavy load of communicable infections of bacterial, viral and parasitic origin. And under this iceberg of communicable diseases a considerable number is of zoonotic importance. Their successful propagation in developing country like India is effected by various factors viz. :

- a) Weak epidemiological surveillance.
- b) Poor knowledge in ecology.
- c) Less attention by public health workers.
- d) Insufficient dialogue between medical personnel, veterinarian & scientists of allied field.

The exact magnitude of the losses caused by animal diseases in human health and losses to agricultural economy still remain undetermined. This is mainly because of lack of or less prominent clinical manifestation in many of the infections. Particularly parasites invade host in crippling fashion and produce corrosive and hidden pathology making animal moribund. Poor farmer who spends money for feeding and rearing the animal gets no return.

Water resources development, such as dam construction and irrigation schemes are taking place on a large scale in India. It gives opportunity to the snail fauna to increase and in turn to enhanced exposure to trematode infection.

Trematodes with reservoirs of infection in domestic, pet and wildlife impose a particular serious burden of ill health on a vast number of people, who live in rural area and earn their livelihood through animal keeping and other associated forms of agriculture. In India this high risk group comprise upto 90% of total population (WHO, 1979). Close association of people with their animals occurs throughout large area and

often under unsatisfactory sanitary condition. Under suitable circumstances man becomes susceptible to a varying degree with a very wide variety of trematode infection which have their natural reservoirs in animals. Either circumstances have not existed for man to encounter many of these flukes in past or the more or less rare encounters have not been recognised or recorded.

The trematodes which parasitize domestic animals and man belong to the sub class Digenea and order Prosostomata. Life cycle involves not only an alternation of generation but also an alternation of hosts; the definitive host being a vertebrate and first intermediate host being always a snail. All important parasitic flukes of domestic animals and man belong to the following families :

- |                      |                       |
|----------------------|-----------------------|
| 1) Schistosomatidae, | 2) Fasciolidae,       |
| 3) Dicrocoelidae,    | 4) Paramphistomidae,  |
| 5) Notocotylidae,    | 6) Opisthorchidae,    |
| 7) Heterophyidae,    | 8) Echinostomatidae,  |
| 9) Plagiorchiidae,   | 10) Troglotrematidae. |

### **Veterinary Public Health**

It is a component of public health activities devoted to the application of professional veterinary skills, knowledge and resources to the protection and improvement of human health. Both physician and veterinarian undertake very similar types of training and develop similar skills and knowledge in their complementary branches of medicine. Hence public health veterinarians can extend their cooperation in various contexts of public health. The following few aspects can be considered as the main roles of veterinarian in public health activities.

1. **Veterinary context** : Veterinarians can save human beings from malnutrition. By eliminating major animal diseases, veterinarian makes animals more productive, both quantitatively and qualitatively.

2. **Public health context** : Veterinarian helps in diagnosis, surveillance and control of zoonosis, diseases and infections naturally transmitted between man and vertebrate animal (WHO, 1959).

3. **Generalist context** : Food protection, environment protection, laboratory services, biomedical research etc.

In snail borne diseases public health activities of veterinarian are reflected by his ability to control zoonosis, control animal diseases as well as to undertake research on trematodes and their snail intermediate host.

### **Snail Borne Diseases/ Infection**

**A. Schistosomiasis (bilharziasis)** : It is one of the most wide spread trematode infections. Among parasitic diseases position of schistosomiasis is next to malaria from public health point of view. Schistosome infections can be categorised into two groups : (i) Human schistosomiasis and (ii) Animal schistosomiasis.

#### **(i) Human Schistosomiasis**

There are three species, which are dealt below :

i) *Schistosomia mansoni* : Adult trematode occurs in portal, hepatic and mesenteric veins of man. Symptomatology is related to the location of the parasite in the affected host. Diagnosis depends on the demonstration of egg in the stool and by immunological tests namely Intradermal test, CFT, IFAT, ELISA, Miracidial immobilisation test.

**Occurrence** : Africa, Arabian peninsula, north eastern south America and the Caribbean area. Not recorded from India.

**Animal Reservoir** : Gerbil and Nile rat in Egypt, rodents in south Africa, rodent and wild mammals as well as cattle in Brazil, baboons and dogs in East Africa.

**Intermediate Host** : Snails of the genus *Biomphalaria*.

**Period of communicability** : Usually 1-2 years but may be for longer period.

**Method of control/ Preventive measures are as follows :**

1. Proper disposal of stool.
2. Treatment of snail breeding place with molluscicides.
3. Use of cercaria-repellent.
4. Education of public.

**Control measures of patients are as follows :**

- 1) In endemic area reporting of health authority.
- 2) Examination of contact person.

ii) *S. japonicum* : As in *S. mansoni*, adult trematode occurs in portal, hepatic and mesenteric vein hence diagnosis etc. are same as in the case of *S. mansoni*.

**Occurrence** : China, Japan, Philippines, Sulawesi, Laos and Thailand. Not reported from India.

**Animal Reservoir** : Dog, cat, pig, cattle, water buffalo, horse, field mice and wild rat. In Philippines 25% of the total transmission is attributed to animal reservoir.

**Intermediate host** : Snails of the genus *Oncomelania*.

**Period of communicability and Method of control** : As in *S. mansoni*.

iii) *S. haematobium* occurs primarily in the vein in urinary bladder. Hence ova comes out with urine. Diagnosis etc. as in *S. mansoni*.

**Occurrence** : Parts of Africa, in Madagascar and also sporadic in Middle East, Cyprus, Southern Portugal, Mauritius. An endemic focus has also been reported from India in Ratnagiri district of Maharashtra (Gadgil & Shah, 1952). Upto 25% of the human population was found to be infected. Later, infections were also reported from Madurai and other parts of Maharashtra.

**Animal Reservoir** : Baboon and monkey in East Africa, rodent in Kenya and South Africa, pig in Nigeria and chimpanzees in West Africa. In India convincing records are yet to be obtained.

**Intermediate host** : *Bulinus truncatus* in Africa, *Planorbium metidgenensis* in Morocco and Portugal. In India *Ferrissia tenuis*.

Other schistosomes : namely *S. intercalatum*, *S. mattei* need adequate study.

### (ii) Animal Schistosomiasis

Schistosomiasis in domestic animals is widely distributed in India and following species of blood flukes of veterinary importance have been recorded.

1. *Schistosoma indicum* Montgomery (1906),
2. *S. incognitum* Chandler (1926),
3. *S. nasale* Rao & Ayyar, 1933,
4. *Orientobilharzia bomfordi* Dutt & Srivastava 1955,
5. *O. datti* Dutt & Srivastava, 1955,
6. *Bivitellobilharzia nairi* Dutt & Srivastava 1955,
7. *S. bovis* Blanchard 1895- needs confirmation.

*S. incognitum* : Adult trematode is recorded in portal, hepatic and mesenteric vein of pig, dog and some times sheep. Diagnosis by demonstration of ova in stool and immunological tests.

**Occurrence** : India.

**Zoonotic potentiality** : Chandler (1926) while examining presumably human stool in Bengal first recorded this schistosome ova. Dutt (1967) and Ahluwalia (1968) studied in detail the host-parasite relationship in monkeys and expressed that this parasite is of considerable importance from the zoonotic point of view.

**B. Cercarial dermatitis** : A world wide problem. Exposure to the cercariae results in sensitization which on renewed contact with cercariae and their penetration of the dermis produces according to the degree of sensitization, at first a burning sensation followed by itching and severe dermatitis.

**Occurrence** : World wide.

**Etiology** : Bird schistosomes : *Ornithobilharzia* sp., *Austroilharzia* sp., *Trichobilharzia* sp. and also mammalian schistosomes.

**C. Fascioliasis** : It is an infection caused by liver flukes viz., *Fasciola hepatica* and *F. gigantica*, which occur in the bile ducts of cattle, buffalo, sheep, goat, pig, rabbit, elephant and rarely in man.

In India, *F. hepatica* occurs at high altitudes like Darjeeling, Kashmir and intermediate host being *Lymnaea acuminata*. *F. gigantica* occurs in plains. Most of the reports of human infection refer to single cases or to very small number. Only two human endemic foci one in Malawi (2.4%) and the other in Peru (4.5-34%) were reported so far.

**D. Amphistomiasis** : The infection is caused by several species of the family Paramphistomidae, viz., *Gygentocotyle explanatum*, *Cotylophoran catylophoran*, *Gastrothylax crumenifer* etc. In India, *Indoplanorbis exustus* mainly acts as intermediate host. Animals affected are cattle, buffalo, sheep, goat etc. Immature fluke causes acute to peracute infection leading to death.

Amphistome species *Gastrodiscoides hominis* has zoonotic potentialities. The trematode occurs in the intestine of pig and man. Endemic focus has been found in Assam.

**E. Dicrocoeliasis :** The causative fluke, *Dicrocoelium dendriticum* occurs in the bile duct of sheep, goat, cattle, pig, donkey, rabbit and rarely man. Many a time human patient shows false positivity after consumption of infected liver as the eggs are not affected by digestive juice. So for confirmation patient must stop eating meat at least for three days. In India, *Macrochlamys candida*, a land snail, acts as first intermediate host and *Formica sp.* serves as second intermediate host.

**F. Notocotylidae infection :** Several species under this family parasitize fowl, duck and other birds. Poultry and duckery have been important cottage industries in our country and hence research to gain knowledge regarding this fluke is essential.

**G. Opisthorchiasis (clonorchiasis) :** This liver-fluke disease caused by *Opisthorchis sp.* is wide spread in Asia and Europe. Dog, cat, pig and man act as definitive hosts. In India, this fluke is very common. Restudy of the life cycle is essential.

**H. Heterophyes infection :** Very small (1.0-1.5 mm x 0.5-0.7mm) intestinal fluke. Commonly affects dog, cat, and man. In India, it is very common. First, intermediate host is snail and second intermediate host is fish. Study of life cycle of the fluke is essential.

**I. Echinostome infection :** *E. recurvatum*, *E. paraulum*, *E. revolutum* are the common intestinal parasites of birds. Infection often causes severe enteritis and affects production. There is strong demand to conduct study on life cycle in India.

The trematode, *Paryphostomum sufratyfex* belongs to family Echinostomidae. Commonly occurs in pig and pathogenic to man. Author and her associates have detected an endemic focus near Barrackpore. It points out the need for further research.

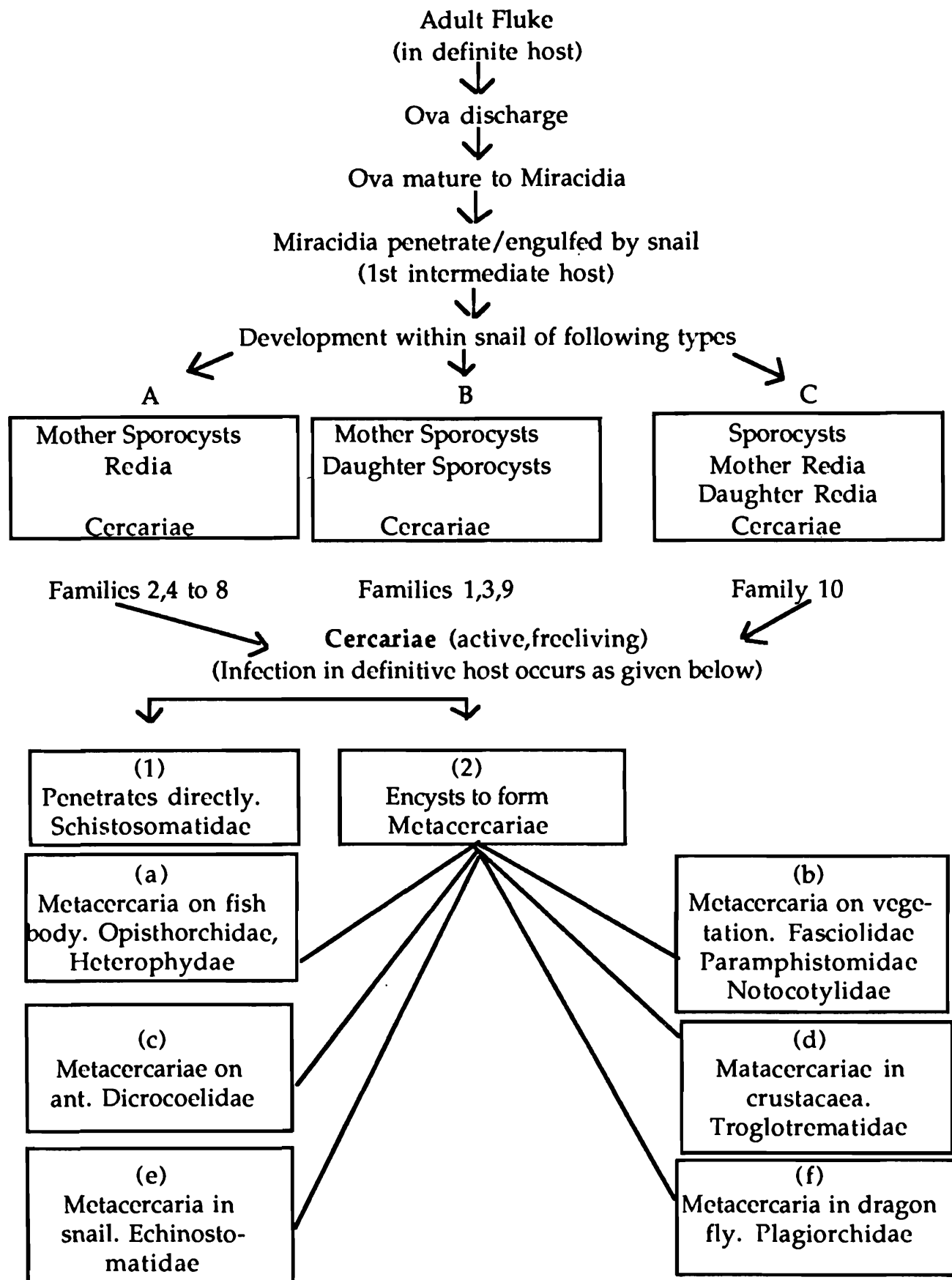
**J. Prosthogonimus infection :** *Prosthogonus sp.* belongs to family Plagiorchidae. The trematode occurs in the intestine of bird. Indian poultry suffers from infections by *P. indicus*. Detailed research on this parasite is essential.

**K. Troglotrema infection :** Flukes of this family are commonly called 'Lung flukes. Type species *Paragonimus westermani* occurs in branchi, lung and occasionally in liver and spleen of man, dog, cat, tiger, wolf, mangoose, pig etc. In India, it has been recorded in animals.

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Scheme Summarizing The General Life Cycle Pattern

## Laboratory Rearing of Medically and Economically Important Molluscs

**S. K. Raut**

University of Calcutta.

Molluscs are important both from medical and agricultural view points. Reports on the successful rearing technique of medically and economically important molluscs are still wanting. Successful snail-host rearing method in laboratory is required in such basic studies as : snail-host-parasite relationships, population dynamics, effects of molluscicides, snail control method, among others. A detailed account on the life-history parameters of snails and slugs of agri-horticultural importance, especially as food, is very much needed with a view to encourage or discourage the population build-up of these species, as the case may be.

In India, the members of the families Lymnaeidae and Planorbidae are registered intermediate hosts of a number of helminth parasites which cause diseases in man and domestic animals. Of the terrestrial molluscs, the giant African land snail, *Achatina fulica* and the slug, *Laevicaulis alte* are known to cause serious damage to agri-horticultural plants. Besides, these land snails have also been registered as the intermediate hosts of the nematode parasite, *Angiostrongylus cantonensis*, causative agent of the disease, eosinophilic meningoencephalitis, both in man and monkey.

As these diseases effect our socio-economy, attempts are being made to eradicate the same. On the other hand, to save the great loss to our agri-horticulture due to the attack of snails and slugs malacologists are looking for an effective control measure. Moreover, the recent export possibility of *A. fulica* as a source of food demands an urgent need of developing a successful mass rearing technique.

Of the many reported intermediate snail hosts of trematode worms, *Lymnaea luteola*, *L. acuminata*, *Indoplanorbis exustus*, *Gyraulus covexiusculus*, and the land forms, the snail *Achatina fulica* and the slug *Laevicaulis alte* are considered for the mass rearing technique under laboratory conditions.

### Methods of Culture

Freshwater snails could be cultured very easily in glass and/ or plastic containers. If pond water is used in the culture, the water has to be changed regularly, but after two or three days if the snails are given aquatic algae, weeds or other vegetations as their food. If land vegetations are used as food, the water has to be changed within a period of 24-hours. Otherwise, the snails may die due to fouling of water following decomposition of the vegetation. The snails, though deposit their egg capsules on the wall of the container, deposition of egg capsules on the vegetation is a common

phenomenon. Since the left-over vegetations are to be removed to avoid water fouling these egg capsules could safely be removed from the vegetation with the help of a fine scalpel. These egg capsules can easily be maintained in a separate container. It is to be noted that the whole culture stock would be spoiled if dead snails are allowed to decompose in the rearing tank. The water fouling capacity of decomposed snail tissues is many times higher than those of the decomposed plant tissues. It would be wise to place small pebbles in the culture containers to provide resting sites for the snails. Of course, in all cases the upper tip of the pebble would be kept exposed to the air so that the snails may rest at the edge of water, as per habit of some species. To have the maximum production, the factors like DO, pH, salinity, hardness, etc. of the water should be maintained at par. The oxygen could be maintained by the use of an air-bubbler; the hardness could be maintained by the use of  $\text{CaCO}_3$  and the salinity could be maintained by adding NaCl at regular intervals.

The terrestrial molluscs are to be kept in terrarium. The terrarium must be filled with loose, moist soil up to 8 cm of its height. The soil moisture should be kept at 40-45% by spraying water on the soil at regular intervals depending upon the season. Since land snails are cryptic, the terrarium should be placed preferably in a dark place.

Be it freshwater or land mollusc culture, special attention has to be paid to maintain strict hygienic conditions by removing faecal pellets and other undesirable wastes from the tray or terrarium regularly.

#### Factors Influencing the Rearing of Snails and Slugs

1. **Influence of food on the growth of the snails and slugs** : Growth rate in snails and slugs varied with the type of food. It is recorded that the freshwater snail, *L. luteola* added, on an average, 1.9 mm and 60.6 mg per week to its shell length and body weight respectively while fed with lettuce. Likewise, weekly addition in shell length and body weight was 1.9 mm and 57.7 mg, 0.62 mm and 2.0 mg and 0.77 mm and 4.3 mg while fed with mustard leaves, algae and *Lemna* respectively. Under similar conditions, *I. exustus* added 1.2 mm, 0.4 mm and 0.35 mm to the shell diameter and 28.1, 1.1. and 0.7 mg to body weight when reared with mustard, algae and *Lemna* respectively. *G. convexiusculus* added weekly 0.59, 0.18 and 0.26 mm to shell diameter and 1.8, 0.2 and 0.35 mg to body weight while cultured with mustard, algae and *Lemna* respectively. The giant African land snail, *A. fulica* while fed on lettuce added 1.5 mm and 0.46 mg respectively to the shell length and body weight per week. The slug *L. alte* added 0.42 mm to the body length and 0.05 mg to the body weight per week when fed with lettuce but added 0.31 mm and 0.03 mg per week to the respective parameters when fed on amaranth.

2. **Influence of temperature on the growth of snails and slugs** : Like food, temperature also played significant role in regulating the growth rate in snails and slugs. The snail, *L. luteola* while reared under constant temperatures with the supply of lettuce as food, weekly added 0.22, 0.46, 0.71 and 2.17 mm to the shell length while maintained at constant 10, 15, 20, 25 and 30°C temperatures, but at room temperatures (16-36°C) the weekly addition was 1.91 mm. Similar trend of growth in body weight has also been exhibited by this snail species at these temperatures. Weekly addition to shell length and to body weight exhibited by the snail, *L. acuminata* under similar conditions was little bit higher but the trend of growth was same. The land snail, *A. fulica* and the

slug, *L. alte* failed to survive at 10 and 15°C constant temperatures. At 20, 25, 30°C constant temperatures and also at room temperatures *A. fulica* weekly added 0.29, 1.16, 1.14 and 1.16 mm respectively to the shell length and 0.01, 0.14, 0.35 and 0.44 mg respectively to the body weight. *L. alte* though failed to survive at 10 and 15°C added 0.198, 0.498, 0.414 and 0.52 mm to the body length and 0.01, 0.06, 0.05 and 0.35 mg to the body weight respectively at 20, 25, 30°C and room temperatures.

**3. Impact of salinity on the growth of snails :** Of the chemical factors of pond water, salinity is important one. Rearing of *L. luteola*, *I. exustus* and *G. convexiusculus* at different concentrations of NaCl revealed that these snails are unable to thrive at 3200 mg concentration of NaCl/ lit. Moreover, *I. exustus* also failed to survive at concentration 2400 mg/ lit. *L. luteola* weekly added 2.13, 1.7 and 1.41 mm to the shell length and 73.6, 40.8 and 6.23 mg to the body weight respectively while cultured at 100 mg, 1600 mg and 2400 mg concentrations of NaCl/lit. In these concentrations of NaCl weekly addition to the shell diameter and to the body weight for *G. conexusculus* was recorded 0.47, 0.5 and 0.43 mm, and 1.07, 1.01 and 0.59 mg respectively. *I. exustus* added weekly 1.02 and 0.92 mm to the shell diameter and, 18.72 and 13.74 mg to the body weight when maintained at 100 and 1600 mg concentration of NaCl/lit respectively.

**4. Influence of temperature on the age of attainment of sexual maturity in snails :** With all other conditions unchanged, the snail *L. luteola* attained sexual maturity at the age of 221, 83, 44, 25 and 41 days while reared at 15, 20, 25, 30°C constant temperatures and at room temperatures (16-36°C) respectively. Under same temperature grades *L. acuminata* attained sexual maturity at the age of 266, 119, 54 and 51 days respectively. *I. exustus* attained sexual maturity after 59 and 49 days when reared at 25°C and room temperatures (19-33°C) respectively. At constant 15 and 20°C, *I. exustus* attained sexual maturity after a considerable length of time (days) while at 35°C it died prior to the attainment of sexual maturity, at the age of 76 days.

**5. Influence of temperature on mortality of snails and slugs :** The snails *L. luteola* exposed to 10, 15, 20, 25, 30°C and room temperatures experienced 50% mortality after 7, 27, 112, 47, 33 and 54 days respectively. And, under similar conditions 100% snails died within 308, 354, 130, 74, 62 and 79 days respectively. In, *L. acuminata* 50% individuals died within 11, 49, 197, 73, 50 and 68 days, and 100% individuals died within 326, 342, 224, 139, 75 and 87 days respectively at 10, 15, 20, 25, 30°C and room temperatures. At 10°C fifty per cent of *L. alte* died within 6 days and 100% died within 11 days. At 15, 20, 25, 30°C and room temperatures 50% *L. alte* died within 12, 282, 355, 84 and 296 days respectively while 100% mortality was recorded by 19, 380, 478, 241 and 528 days respectively at these temperatures.

**6. Influence of temperature on the longevity of snails and slugs :** The snails *I. exustus* failed to survive at 10°C while *L. luteola* and *L. acuminata* failed to survive at 35°C. At 15°C *L. luteola* survived for 354 days and *L. acuminata* survived for 342 days. The life span of the snail was gradually shortened with the rise of temperature. At 30°C *L. luteola* and *L. acuminata* survived for 62 and 75 days respectively. Contrast to this *I. exustus* survived only for 51 days while reared at 15°C. Interestingly, at 35°C it thrived for a period of 76 days. At room temperatures (19-33°C) *L. luteola*, *L. acuminata*, *G. convexiusculus* and *I. exustus* survived for 79, 87, 158 and 264 days in that order. Though

no study has been made on the influence of temperature on the survival rate of *A. fulica* it survived for 1562 days under simulating field conditions in Calcutta. *L. alte* did not survive beyond 11 days at 10°C but thrived successfully for 528 days in laboratory.

**7. Effect of crowding on the longevity of snails :** The snails *L. luteola* survived for a period of 82, 74 and 54 days and *I. exustus* survived for a period of 92, 91 and 88 days when cultured with 1/8 and 25 individuals per tray in that order.

**8. Effect of crowding on the fecundity of snails :** The snails produced less number of eggs with the increase of density of egg laying individuals per tray. *L. luteola* deposited 32, 13 and 4 egg capsules, *I. exustus* deposited 91, 60 and 31 and *G. convexiusculus* deposited 32, 28 and 26 egg capsules respectively when 1, 10 and 20 individuals were reared per tray.

**9. Influence of temperature on development and hatching of eggs :** The freshly laid eggs of *L. luteola*, *L. acuminata*, *I. exustus* and *G. convexiusculus* when left in trays with pond water (DO : 14.08mg/lit; CO<sub>2</sub> : 7.3mg/lit; Hardness : 451 ppm; Salinity : 0.86 ppm) kept at 5, 10, 15, 20, 25, 30, 35, 40°C constant temperatures and at room temperatures (18–35°C) exhibited different results. The eggs, irrespective of snail species did not hatch at 5 and 40°C while eggs of *I. exustus* and *G. convexiusculus* did not hatch at 10°C. It is noted that the eggs of *L. luteola* required 56, 34, 15, 12, 7, 11 and 10 days for development for hatching when maintained at 10, 15, 20, 25, 30, 35°C and room temperatures in that order. Similarly, for other species, the developmental period was shortest at 35°C and a gradually longer developmental period has been noted with the lowering of temperature from 35°C to 10°C in *L. acuminata* and to 15°C in *I. exustus* and *G. convexiusculus*. In *L. luteola*, *L. acuminata* and *I. exustus* hatching percentages were gradually less with the lowering of temperatures from 30°C to 10°C; at 35°C the percentage was slightly less than that recorded at 30°C. However, in all snail species, the percentage of hatching was maximum (98–100%) at room temperatures. Interestingly, 100% hatching has been noted in case of *G. convexiusculus* irrespective of temperatures from 15 to 35°C.

In land forms, the snail *A. fulica* required 20, 17, 14, 16 and 12 days to complete the development of eggs for hatching while exposed to 20, 25, 30, 35°C and room temperatures respectively. The eggs of the slug *L. alte* completed development within 21, 17, 13 and 16 days while maintained at 20, 25, 30°C and room temperatures in that order. At other temperatures, the eggs did not hatch. In both the species hatching percentage was highest at room temperatures. This was followed by 25, 30, 20 and 35°C in that order in *A. fulica* and 30, 25 and 20°C in that order in *L. alte*.

**10. Influence of pH on hatching of eggs :** The eggs of *L. luteola* maintained at pH 6, 8, 9 and 9.5 required 10, 9, 12 and 15 days respectively for development and hatching. The eggs did not hatch at pH 5 and 10. In pond water (pH : 7.1-7.8) development of eggs was completed within 9 days. The hatching percentages were 100, 96.07, 98.38, 83.87 and 35.71 at pH 7.1-7.8, 6, 8, 9 and 9.5 in that order.

**11. Impact of salinity on the development and hatching of eggs :** The eggs exposed to different concentrations of NaCl (from 100 mg/lit to 6400 mg/lit) exhibited a varying range of developmental period and the percentage of hatching. In *L. luteola* hatching

took place at all the concentrations up to 4800 mg/lit. The incubation period at 100 mg/lit was 8 days for *L. luteola* and 7 days for *I. exustus*. The hatchability percentage was also gradually less with the increase of concentration of NaCl in both the species. In *L. acuminata*, the incubation period was 14 days at 100 mg/lit concentration of NaCl while 400 mg/lit concentration the same was 29 days. Above this concentration the eggs did not hatch. The percentage of hatching was 96.55 at 100 mg/lit concentration. This was followed by a gradual less percentage of hatching of eggs with the increase of concentration of NaCl. At 400 mg/lit the hatching percentage was lowest (12%).

In summary the following suggestions may be taken into account :

- i) Lettuce may be recommended as an ideal food.
- ii) Room temperature may be considered as the best thermal condition for culture. But , sometimes maintenance of constant temperature, at 25 or 35<sup>0</sup>C may be ideal for culture of some species.
- iii) Pond water (approximately, DO : 14.08 mg/lit; CO<sub>2</sub> <: 7.3 mg/lit; Hardness : 451 ppm; Salinity: 0.08 ppm) may be used for achieving best growth, production and population build-up of lymnacid and planorbid snails.
- iv) Normal temperature with 85-95% RH and 40-45% soil moisture may be considered ideal for the culture of terrestrial molluscs, especially for *A. fulica* and *L. alte*.
- v) As per need the culture may be designed with less number of individuals in a container to have a maximum production.
- vi) Tap water may be used in the culture following filtration through activated charcoal and aeration for one week.

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## Molluscs in Aquaculture

*N. V. Subba Rao and A. Dey*

Zoological Survey of India, Calcutta.

There has been an unprecedented increase in man's use of natural living resources during the last few decades due to the rise in human population. In order to meet the world protein demands and to improve the dietary standard of human beings, aquaculture has been conceived and developed. "Kyoto declaration on aquaculture", in the year 1975, had been a significant step for the promotion of aquaculture. Sophisticated aquacultural practices have substantially increased the global production figures. According to one estimate, aquaculture production constitutes 66% fish, 17.5% sea weed, 16.2% molluscs and 0.3% crustaceans. Aquaculture techniques are useful not only in increasing food production but also in conservation of certain important species. Although fishes constitute the main component in the aquaculture, molluscs are also a good source to increase production. Food value of several molluscs, such as oysters, mussels, clams, cockles, cuttle fishes and squids is well known. The global demand in molluscs is so much that in the year 1980 the nominal catches totalled 4,950,718 tonnes which include 972,885 tonnes of oysters, 613,965 tonnes of mussels, 364,173 tonnes of scallops, 1,176,771 tonnes of clams, and 7,572,098 tonnes of cephalopods (FAO, 1981). Molluscs are also cultured for the production of luxury items, such as pearls. Aquaculture may be the answer for starved masses and for the retrieval of over-exploited natural shell resources. An increase in shell fish production can augment our protein-rich food supplies and brighten up our economy. Cultivation of molluscs is at present carried out mostly through coastal aquaculture. But from the data collected during our surveys we have seen that there is a scope to take up farming of molluscs in freshwater aquaculture (Subba Rao, 1989). Snail farming may soon develop into an important technological innovation in India.

### Molluscs in Coastal Aquaculture

Marine molluscs play a significant role in the coastal aquaculture. The commercially important molluscs are edible oysters, pearl oysters, mussels, clams and cockles, chank, cuttle fish and squid. These molluscs are fished mostly from natural stocks and it has not been possible to meet the growing demand from this source alone. Central Marine Fisheries Research Institute has taken up the culture of molluscs and achieved success in developing suitable techniques for certain species. Shell fisheries can be broadly categorised under the following head : i) Edible shell fisheries, ii) Pearl fisheries, iii) Chank fisheries and iv) Lime shell fisheries. Some of the important molluscs which are drawing attention in coastal aquaculture are given below :

**Oyster culture :** The world oyster demand has been on the rise to-day and may increase to 2 millions tonnes by 2000 AD. The world's major producers of oyster are USA

(41%), Japan (19.1%), followed by the Republic of Korea, France and Mexico. A small amount is also produced in Taiwan, China, Canada, New Zealand, Great Britain and FRG. In India, the culture of oyster was initiated but the production is not very significant.

In USA 40% of total oyster is derived from bottom culture using the seed from domestic sources or imported from Japan. Raft culture and artificial rearing in controlled environment are feasible but uneconomical. In Japan 'hanging method' i.e. raft, and long line or rack culture are used. The stick culture is of less importance. In Korea the raft culture method produced nearly 100,000 tonnes. The important cultured species are *Ostrea edulis*, *Crassostrea angulata*, *C. gigas* and *C. commercialis*.

In India the culture of oyster, *Crassostrea madrasensis* (= *C. cuttackensis*) has been taken up at Tuticorin and Mandapam Camp. It includes the collection of spat and growing the same to adult. For collection of spat, different methods have been tried; like lime-coated tiles, oyster shells strung on galvanised iron wire, empty coconut shells, rubberised coir mat etc. The time of spat collection varies with species, locality, fluctuation in temperature, salinity and tide. For culturing them up to adult stage the rack culture, long line culture, pole culture, and tray culture are adopted. Induced breeding of oyster was attempted by thermal stimulation, lowering the salinity and stripping. But development was hampered by ciliate infection.

Some experiments were carried out at Pulicat Lake on the east coast. Dead oyster shells and asbestos sheets were used for catch material and mid water culture in wooden trays was found advantageous. October-November and February-March were found to be intensive and moderate spat fall periods, respectively.

The following are some of the aspects that need special attention for the improvement of the oyster culture :

- i) availability of suitable space and environmental conditions (water quality, natural food supply).
- ii) suitable seed supply
- iii) selection of suitable species and genetic improvement of stocks.
- iv) provision of feeds for artificial or intensive culture systems.
- v) control of diseases, predators and fouling organisms.
- vi) type of harvesting method.

**Mussel culture :** The total world production of mussel is nearly 5000,000 tonnes, with Spain and the Netherlands being the leading producers. The other countries are France, Italy and Philippines. The methods of culture include bottom culture in the Netherlands, bouchet culture in France, raft culture in Spain, rack culture in Italy and submergd culture in Philippines. Mytilids are very suitable for culture as they convert marine phytoplankton into nutritious and palatable food.

In India two species, namely *Perna viridis* (Linnaeus), commonly known as green mussel and *Perna indica* Kuraikose and Nair, commonly known as brown mussel are used for culture. The former is distributed all along the Indian coast whereas the latter is restricted from Quilon on the west coast to Tirunelveli on the east. In the experiments conducted at Calicut centre of CMFRI on green mussel (*P. viridis*), 428 tonnes/ hectare were produced in the open sea in a period of six months by rope culture. At Vizhinjam the brown mussels are cultured by suspended or raft culture. The seeds are collected from

the natural beds and transplanted to the ropes. An annual growth rate of 34.68 grms was recorded and production was about 22.97 kg/ meter length of rope. In 1975, culture of green mussel was taken up in open sea at Kozhikode by raft culture technique. About 53.3 mm length of wire within five months showed increase in weight from 1.48 grm to 28.7 grm and the production was about 235 tonnes/ hectare. These results have shown the prospectus of culturing mussel in our inshore waters. Raft culture of mussel to suit open sea conditions are under way. Development of mussel culture as an industry needs suitable farming technology which is being attempted by CMFRI.

**Clam & Cockles culture :** The clam and cockles are of considerable food value. They are the bay clam, *Meretrix meretrix* (Linnaeus) ; backwater clam, *M. casta* (Gmelin); inflated clam, *Marcia pinguis* (Schroeter); black clam, *Villorita cyprinoides* (Gray), cockle clam, *Gafrarium tumidum* Roeding; false clam, *Paphia malabarica* Dillwyn; *P. marmorata* (Reeve) and wedge clam, *Donax cuneatus* Linnaeus and a few other venerid species.

Culture of the clam is still not up to the mark not only in India but also in the other parts of the globe. Culture of *Meretrix casta* (Gmelin) has been started at Porto Novo and that of *Anandara granosa* (Linnaeus) at Kakinada.

**Pearl culture :** The pearl oyster is a well known mollusc, which has been cultured from a long time and the technique for which has been perfected in Japan. Several species of pearl oyster, namely *Pictada fucata* (Gould), *P. maxima* (Roeding), *P. margaritifera* (Linnaeus) and *Pteria penguin* (Roeding) are used in the farming. In Japan maximum annual production of 35 tonnes of pearls is obtained from one species i.e. *P. fucata*.

In India the pearl culture project was started in 1972 at Tuticorin with a field laboratory and open sea oyster farm at Veppalodai. The raft culture technique was adapted in *P. fucata*. The technique involves grafting a piece of mantle of the donor oyster in the gonadial or hepatopancreatic region of the recipient oyster, followed by the implantation of spherical shell bead nucleus. In 3 to 18 months the pearl attains maturity and grows to 3 or 8 mm size depending on the conditions. There has been 60 to 70% success in these culture operations. The technology involved in pearl culture has been explained by Alagaraswami (1985).

Besides the above mentioned molluscs, the chank (*Turbinella pyrum* Lamarck), cuttle fish and squid are very important from the fisheries view point but commercially viable culture techniques are yet to be developed for these molluscs.

## **Molluscs in Freshwater Aquaculture**

### **Background information**

The freshwater aquaculture is also important in the development of additional food resource for the increasing human population of our country. Inland aquaculture is primarily limited to raising of fish and to some extent finfish (crustaceans).

During the course of faunistic surveys it has been observed by us that there is a regular sale of shell fish in several markets of Bihar, West Bengal, Mizoram and Arunachal Pradesh. Shell fish are harvested from the natural resources by different methods and brought to the market. There are no regular markets in some places but people use the shell fish as food very frequently.

**Bihar :** Although there are about 22 species of freshwater molluscs occurring in Bihar, only four species are favoured as food, namely *Pila globosa* (Swainson), *Bellamya bengalensis* (Lamarck), *Lamellidens marginalis* (Lamarck) and *L. corrianus* (Lea) in the south Bihar. There is a regular sale of shell fish in the markets of districts of East and West Singbhum, Gumla, Lohardanga, Ranchi, Palamau, Hazaribagh and Giridih. Most of these markets are weekly or biweekly and the rate of shell-fish varies from Rs. 1/- to 3/- per kg. *B. bengalensis* is favoured as food by all sections of people while the other three species are eaten by the tribals.

**West Bengal :** Freshwater molluscs are sold in markets in the districts of Purulia, Bankura (part), Midnapore (part), Darjeeling and Jalpaiguri. All the four species which are favoured as food in Bihar are consumed by the people in Lower Bengal. In the districts of Darjeeling and Jalpaiguri of North Bengal *Brotia costula* (Rafinesque) is eaten by the workers of the tea gardens.

There is a regular market in Jhargram where the pond snail, *Bellamya bengalensis*, *Pila globosa*, *Lamellidens marginalis* and *L. corrianus* are sold. More than six bags of *Bellamya* sp. (approximately 200 kg) are sold at the rate of Rs 3/- to 4/- per kg., and *Pila* at the rate of Rs. 2/- per kg. *Lamellidens* are sold in lots and each lot consisting of 8 to 10 full grown specimens costs Rs 2/-. Sometimes the soft parts are removed from the shell and sold in lot, each lot costing Rs 1/- to 2/-. *Brotia* was sold in the 'hat' (weekly/biweekly) market in teagarden areas at the rate of Rs. 1/- per lot which contain 18 to 20 nos.

**Mizoram :** Molluscs form a staple food for the people of Mizoram (Subba Rao and Dey, 1986). Local people (Mizo & Riang) are very fond of shell fish. Flesh of seven species is consumed by the people who either collect themselves the snails from the streams or purchase from the market. The snails are collected from the local streams and are regularly sold in Aizawl market. The shell fish are brought in bamboo basket and sold to the customers by measuring in a tin, the contents of which may weigh 250 grms (approximately); 15 to 20 average sized specimens at the rate of Rs. 1/- in the case of *Brotia* and 40 to 50 average sized *Paludomus* at the rate of Rs. 2/-. Shell-fish are also sold in a packet made of fresh banana leaves, each of which contain 20 to 25 specimens of *Paludomus* or 10 to 12 specimens of *Brotia* was priced at Rs. 1/- per packet.

**Arunachal Pradesh :** Shell-fish are also consumed in Arunachal Pradesh, although no regular marketing was observed during the visit. Shell-fish, mainly *Bellamya bengalensis* (Lamarck), *Parreysia sikkimensis* (Lea) and *P. caerulea* are collected from streams in the Lohit and Tirap districts.

Shell-fish are eaten throughout the year, except during the moonsoon months, but maximum being during the summer (March to May). It depends mainly on the availability of shell-fish and the level of consumers which varies from state to state. It is a very much favoured item for low income groups, but in Chhotanagpur area of Bihar *Bellamya* is held as a prestigious item of food, by people of all classes.

Shell-fish are collected from the natural sources by hand picking, fishing nets or by leaf-trapping, especially in some parts of Chhotanagpur (Bihar). In the leaf trapping the palm leaves are placed in the littoral region of a pond or a ditch in the evening. A number of *Bellamya* get attached to the leaf and next day these are taken out of the water and the snails are hand picked.

## List of edible freshwater molluscs

Sl. No.	Name of the species	Local name	State in which used as food
1.	<i>Pila globosa</i> (Swainson)	Ghonga (Kurmali and Mundari) Genda (Ho) Marang rookai (Santhali) Genri (non tribal of Bihar) Samuk (Bengali) Genda (Oriya)	Bihar, Orissa, West Bengal
2.	<i>Bellamya bengalensis</i> (Lamarck)	Ghongi/Googli (Kurmali and Mundari, Chota Genda (Ho) Rookai (Santhali) Samuk (non tribal of Bihar and Bengali) Genda Samuk (Oriya)	Arunachal Pradesh (Tirap), Bihar, Orissa and W. Bengal
3.	<i>Brotia costula</i> (Rafinesque)	Chenkai (Mizo) Chikangbook (Riang) Samuk (W. Bengal)	W. Bengal (North Bengal), Arunachal Pradesh, Mizoram
4.	<i>Paludomus conica</i> (Gray) & <i>P. blanfordiana</i> Nevill	Chenkai (Mizo) Chikangbook (Riang)	Arunachal Pradesh Mizoram
5.	<i>Lamellidens marginalis</i> (Lamarck) & <i>L. corrianus</i> (Lea)	Chachni (Kurmali and Mundari) Marang Rakai (Santhali) Jhinuk (non tribal of Bihar) Jhinuk/Katli (W. Bengal)	Bihar, Orissa and W. Bengal
6.	<i>Solenaia soleniformis</i> (Benson)	Tuikop (Mizo) Clampy (Beta)- Riang	Mizoram
7.	<i>Trapezoideus exolecens exolecens</i> (Gould)	Tuikop (Mizo) Clampy (Beti)- Riang	Mizoram

A list of edible molluscs is given on page 89.

**Shell Fisheries in North Bihar :** Freshwater shell fish plays an important role in cottage industries in Mehshi town of East Champaran (Bihar). Freshwater bivalve shells are used in manufacturing of shell buttons, ornaments and also preparation of slaked lime. Shell grit, dust and residual pieces are used in the preparation of poultry feed and mosaic tiles respectively (Banerjee and Satish, 1988; Datta Munshi and Chaudhury, 1988). The important bivalve species are *Parreysia (Radiatula) caerulea* (Lea), *P. (Parreysia) favidens* (Benson) and *Lamellidens corrianus* (Lea). According to Banerjee and Satish (1988), the annual requirement of raw shells by Shell Button Industries, Mehshi is around 700 tonnes. Such huge amount of raw material demand had resulted in the over exploitation of local resources leading ultimately to their depletion (Subba Rao, 1989).

From the foregoing account it is clear that freshwater shell fishes are important as food, as producers of pearls and as raw material to shell industry. It points out the need for sustained utilisation of the species involved and for renewal of shell fish resources by aquaculture.

#### **Freshwater Pearls—A Proposal for Culture**

Pearl culture in freshwater mussels has been in practice in China and Japan, with freshwater mussels viz. *Cristataria plicata* and *Hyriopsis schlegelai* respectively (Mizumoto, 1976). The importance of water quality has been stressed in the pearl farming (Mizumoto, 1976). It was found that conditions of water, which favour good pearl growth need not always produce good quality pearls. From our observations it has been seen that aquatic bodies in West Bengal offer natural conditions for the formation of pearls in the common freshwater mussel. So far, the natural occurrence of pearls in *L. marginalis* has been recorded in West Bengal only. There is a scope to initiate freshwater pearl culture in the state (Anonymous, 1990). A survey, with reference to freshwater mussels, has to be undertaken in the other states of our country.

The farming procedure summed up by Mizumoto (1976) with reference to marine pearl oyster may be applied in the case of Indian freshwater mussel also. The procedure involves the following steps :

- i) Preparation of host shell
- ii) Nucleus insertion
- iii) Convalescence
- iv) Pearl formation
- v) Nurturing procedure

**i) Preparation of host shell :** In nature, pearls may be formed in different regions of the molluscan body. In *P. fucata*, the best site for pearl production is considered to be the gonad. In pearl farming a nucleus is inserted in the gonadial region. From experience it is inferred that spent individuals, i.e. individuals whose gonads are devoid of gametes, which is usually after spawning, serve as good hosts. In the case of *Lamellidens marginalis* the suitable site and time are yet to be investigated.

**Nucleus insertion :** It involves selection of a suitable donor shell for obtaining nuclear material, and graft tissue from the freshwater mussel. The shell of *Parreysia* spp.

which are thicker than many other species, can be used for the preparation of nuclei. Shell of sacred chank, *Turbinella pyrum* can also provide the required nuclei. After cutting out bangles and other ornaments from the shell, the residuals can be finished into nuclei of different grades.

Graft tissue has to be obtained from the pallial zone of the living freshwater mussel. The operation on the living mussel has to be conducted very carefully without causing much injury to the mussel. In the case of *P. fucata*, graft tissue is made into 2-3 square mm pieces. The graft tissue has to cover at least one third of the nucleus. The size of the nucleus determines the size of the piece of graft tissue.

After obtaining the nucleus and graft tissue, the next step involves operation of the freshwater mussel and insertion of these into the gonadial region. The time and number of nuclei to be inserted have to be determined.

iii) **Convalescence** : After operation, the mussels should be allowed to recover from the disturbance caused during operation. In the case of pearl oysters culture cages consisting of heavy wire frames (68x60x30 cm) covered with fine mesh wire netting are used. Since *P. fucata* is epifaunal it is easy to operate these cages. But in the case of *L. marginalis* which is a benthic form, the cages by suspension may not be very suitable. But the same can be used with some alterations as it had been done in Bangladesh.

iv) **Pearl formation** : The graft tissue on the nucleus grows into a 'pearl sac.' The epithelial cells of the pearl sac deposit nacreous layer around the nucleus, ultimately leading to the formation of a pearl. In the pearl oyster it takes usually three to four years for pearls of commercial value to develop.

v) **Nurturing Method** : In marine farming rafts of different kinds are used but nurturing techniques have to be developed in the case of freshwater mussel.

On the basis of above mentioned data it is clear that there is scope in our country to introduce and encourage culture of some more species of molluscs which are hitherto exploited from nature.

- i) Molluscs play an important role not only in coastal aquaculture but with proper techniques, can also become important components in freshwater aquaculture.
- ii) Culture of a few species of freshwater snails and mussels can be initiated. Culture of the following can be taken up: *Pila globosa* (Swainson) and *Pila virens*. (Lamarck) for their edible value and *Lamellidens marginalis* (Lamarck) for its pearls.
- iii) A stable and steady supply of seedlings have to be maintained for which suitable breeding technique should be developed.
- iv) The demand in domestic market is less for snails and mussels, but these can be developed into valuable foreign exchange earners.
- v) Better methods for preservation and marketing are to be devised.

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## **Histochemistry and Pathology of Snails and Flukes**

***K. Hanumantha Rao***

**Ex. Professor, Andhra University, Visakhapatnam.**

Molluscs, especially gastropods and lamellibranchs, are well known as intermediate hosts of trematode parasites. Some cestodes and nematodes also use gastropods and bivalves as hosts. Although lodged in calcareous shells, the foot, mantle edges, the head and tentacles are usually exposed during movement and foraging activities in gastropods. Constant current of water enters and leaves the siphons of bivalves. There is thus tremendous scope for a variety of organisms to parasitise or be associated as commensals etc. Except for a few cases, digenetic trematodes use molluscs, chiefly gastropods for the proliferation of asexual stages. What then are the host-parasite relations/adaptations to ensure a balance so that the host and parasite coexist with minimum of discomfort on each side? This is the essence of parasitism now coming under the more broader discipline called symbiosis.

The digestive gland of the snail is the preferred location. But the wandering spirit of some of the parasites is phenomenal. The emission of cercariae is in a circadian fashion. Mostly the cercariae emerge out at dawn but there are cases of emission starting at dusk. Genetic determination of this chronobiological event has been hinted in some cases.

The distribution of trematode stages and the damage they cause to the snail's tissues vary with the species. The digestive tubules are surrounded by connective tissue, which is subject to damage. There are variations from species to species.

Work on vulnerability to parameters of the environment (biotic and abiotic) in infected versus uninfected snails has been conducted. As far as the asexual stages are concerned there is histocompatibility. Rediae or sporocysts go on proliferating and cercariae wriggle out, course through the tissues and emerge out. However, transplantation of larval stages to fresh hosts directly, either intraspecifically or interspecifically (Host-wise), is difficult to sustain. Destruction of the stages occur. Host's haemocytes and mechanism for encapsulation are brought into action. Among the molluscan hosts of medical importance parasite's (schistosome) strain differences vis-a-vis resistance to infection are known. Literature on the above aspects is voluminous and several aspects of damage of host's tissue and reactions of host's tissues are documented. Gigantism in the infected snails may occur. Neuroendocrine mechanisms are perhaps involved here. There are biochemical studies to determine the level of proteins, carbohydrates and specific enzymes like aminotransferase. Differences between healthy and parasitised snails are brought out in this respect.

Trematode metacercariae also find an abode in many gastropods and lamellibranchs. A variety of situations occurs. Host-reactions are more perceptible in this case. Encapsulation is the usual defence.

The extent of pathology depends on the number of parasites and site of encystment. For instance a nerve may be disrupted or only a muscle may be torn. The adaptation of parasites in the choice of location are most interesting. In the case of *Leucochloridium panadoxum*, which is usually a bird parasite, the colourful sporocysts lie pulsating in the tentacle of the snail. Bird's eye view is anticipated by these parasites. The birds nick the pulsating, motile tentacles and swallow the sporocysts loaded with tail-less cercariae. In so far as metacercariae or the cestoda infective stages are concerned cellular reactions and encapsulation process may differ from site to site.

The greatest difficulty in platyhelminth research has been the lack of ability to know the snail by its correct name. Specialists have to be consulted. A second important aspect to remember is the use of molluscs, especially snails, in physiological research with respect to parasitology. Take the case of the genus *Pila* : the South Indian apple-snail is *Pila virens* while *Pila globosa* has a more northern distribution. Yet in South India investigators have constantly used the name *globosa*. Again these snails have the capacity to aestivate. In work on biochemistry of such snails investigators have not made it clear whether the specimens they used for particular biochemical study were free of infection or not. All biochemical and physiological studies on gastropods are prone to be wrong unless it is made clear that the pool of specimens used contained all healthy uninfected animals.

Work on various aspects of histochemistry of tissues of gastropods, both infected and uninfected, aestivated and normal, has revealed abilities of these hosts to withstand severity of infection. During aestivation of host the trematode stages also undergo a state of diapause or even dedifferentiation. Regeneration of tissue is possible. One interesting aspect is that concerning the amoebocyte organ. In infected snails the amoebocytes producing organ undergoes hypertrophy. Well developed granulomas consist of a central core of parasitic material surrounded by hypertrophic, phagocytic amoebocytes which in turn are surrounded by fibroblasts. But the parasites, at least sporocysts and redia have in the course of evolution undergone adjustment. More or less symbiosis is the answer for this situation. The host has to seal off an unaccustomed guest and a parasite of great familiarity does ward off all danger signals. In fact in some cases a state of molecular mimicry has been postulated i.e., the parasite mimics the hosts' antigens. These are eclipsed antigens constantly baffling the host's defence mechanisms involving immunology.

Considerable host specificity may be displayed. Miracidia of *Schistosoma mansoni* are attracted by planorbids but not by bulinids. Whereas *S. haematobium* is attracted by bulinid snails. Strain differences within the species are also significant. It has been suggested that mucous patterns of snail's foot and mantle differ from species to species. The differences in the components may be important in determining the attraction of miracidia to specific hosts. Exclusively terrestrial life cycles may occur as in the case of *Paradistomoides orientalis*, a trematode in the gall bladder of *Calotes versicolor*.

Activities of infected molluscs may alter. Uninfected snails are negatively phototropic, infected snails seek the light. In *Bulinus truncatus* infected with

*Schistosoma haematobium*, a three fold reduction in egg production per clutch is reported. Susceptibility to temperature and reduction in fecundity increases with thermal stress. In cases of double infections, the rediae of the species may devour the rediae of the other species (Echinostome rediae devour other species). Histochemical changes relate to reduction of glycogen content and aberrations in enzymes. Inhibition of gametogenesis, castration and sex reversal are known to occur. But regeneration may occur. Zoonotic importance of snails should not be lost sight of, especially of snails like *Pila virens*, *P. globosa*, which are of large size and are esteemed as good food in the rural areas. There have been several cases of infection with echinostomes in snail tissues and the apple-snails come in very handy both for cercariae to encyst and human to consume.

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## **Amphistomes of Domesticated Animals of India and their Life Histories**

**R.P. Mukherjee**

Zoological Survey of India, Calcutta.

Amphistomes constitute an important and interesting group of trematode parasites infecting both cold and warm blooded animals including man and the domesticated animals. The disease caused by these trematodes to ruminants is known as amphistomiasis. About 62 species of amphistomes have been listed by Yamaguti (1971) that are reported from the domesticated ruminants of the world. Mukherjee and Chauhan (1965) and Dutt (1980) have listed 23 and 20 species of amphistomes respectively from the domesticated ruminants of India.

### **Importance of Amphistomes**

The amphistomiasis in live stock is now regarded as a disease of great economic importance. The first amphistome of ruminants, *Paramphistomum cervi*, was reported by Zeder as early as 1790 but their association with clinical symptoms had been diagnosed by Baldrey in 1906 when he correlated the sickness in sheep with immature amphistomes that had been recovered from the small intestine. Since then the disease has been reported from cattle, goat, sheep and buffalo in many countries of the world. In the past, these trematodes were regarded as harmless. However, in recent years the magnitude of amphistomiasis has been realized through a number of reports in various states of India. Initially the effect of infection by these parasites may not be quite distinct but ultimately it leads to the loss of health and decreases the capacity of resistance to various diseases. The effected animals were often seen lying down and if raised would stagger and fall. They also developed anaemia, debility, persistent diarrhoea, unthriftiness and oedema of the submaxillary space. The dung was found to be blood-stained and usually contained large number of immature amphistomes and mucous. The infected animals show rise in body temperature and their milk yield decreases.

**Species of ruminants affected :** All the species of domesticated, young and adult, ruminants are parasitised by amphistomes. However, the number of outbreaks of disease in cattle and buffaloes are lesser than in sheep and goat. This could be due to general resistance of adult bovines to the disease.

**Seasonal occurrence :** Outbreaks of amphistomiasis are seasonal in occurrence, generally between September and April. The animals pick up the infection while grazing on marshy areas or on pastures inundated during the rainy season. Stall-fed animals may pick up the infection from the fodder or imperfectly dried hay collected from the infected fields or pastures. Infection may also occur when the animals graze on the green vegetations surrounding the tanks, ponds, pools and on banks of rivers.

**Morbidity and mortality :** In domestic ruminants the morbidity and mortality rates are very high. In sheep, morbidity and mortality rates are reported to be about 15% to 86% and 35% to 90% respectively. In goats the corresponding figures are 44% to 69% and 45% to 88% respectively. Death rates in cattle and buffaloes are less and it is reported to be 21% to 39% in cattle and 10% to 20% in buffaloes.

**General account :** Amphistomes have thick and cylindrical or oval body which may be curved ventrally. It may be flat and divided into anterior and posterior portions. The cuticle is strong, elastic and may be thick. It bears longitudinal or transverse ridges or papillae. Some have prominent ventral pouch. The ventral acetabulum may be terminal or subterminal with strongly developed musculature. The oral sucker may be terminal or subterminal and may be provided with oral pouches. The cuticular lining of the cavity of the oral sucker may be provided with papillae. The oesophagus may be short or long, straight or 'J' shaped and may be provided with a posterior bulb. Intestinal caeca may be long or short, straight or wavy. The caeca are generally long, reaching the pre or mid acetabular zone but sometimes may be exceptionally short, terminating near the middle of body. They may be more or less straight or wavy or have a number of distinct bends. The terminal parts may be directed ventrally or dorsally or in a few cases may be turned medially lying one behind the other. The lumen is lined by a stratum of enteric columnar epithelium.

The excretory vesicle is elongated, antero-dorsal to the acetabulum and opens by a pore on the dorsal side of body. It may cross the Laurer's canal in which case the excretory pore is located anterior to the opening of the Laurer's canal and where there is no crossing the excretory pore lies posterior to Laurer's canal.

The two testes are generally pre-ovarian and may be in a tandem, diagonally tandem or side by side position. They may be entire, lobed or have cauliflower-like ramifications. The seminal vesicle is thin walled and may be short and narrow having only a few convolutions, or long and narrow having many loosely set coils, or long and wide having numerous tightly coiled loops occupying much space. The pars musculosa is thick walled with well developed circular and longitudinal muscular layers. It may be short or moderately long. The pars prostatica may be short and barrel shaped to pyriform, or long and conical or very long and nearly cylindrical. Cirrus pouch is lacking in most of the families, excepting a few such as Cladorchiidae.

The ovary is rounded, median, mostly post-testicular and rarely inter-testicular. The ootype is surrounded by well developed Mehli's glands. The uterus has generally only ascending loop and runs medially. In some genera (e.g. *Gastrothylax*) it crosses from one side to the other in the middle of the body and lies almost parallel to the male duct but on the opposite side. The vitellaria are follicular and are scattered in the ventral and lateral regions of the body between oesophagus and the acetabulum.

The genital opening or pore is surrounded by a thick portion of body which is known as genital bulb. It comprises the terminal parts of the male and female ducts (Pars prostatica and metraterm), genital papilla, genital atrium, genital folds, ventral atrium and ventral folds. The genital papilla, genital folds and ventral folds are usually provided with sphincters papillae, genital sphincters and ventral sphincters respectively. In some species the genital bulb is very much retracted to form a deep genital pit (*Calicophoron* sp.). In others it is generally protruded forming a genital pillar.

**Causative amphistomes :** The precise information is lacking on the pathogenic species of amphistomes. The immature forms are supposed to cause the disease and in many cases the identity of the species is based on the examination of these immature forms. Pathogenic amphistomes which cause disease in the domesticated ruminants belong to three genera, viz., *Paramphistomum*, *Cotylophoron* and *Gastrothylax*. The only species of amphistome that is found in man and pig in India is the *Gastrodiscoides hominis* and it proves to be highly pathogenic in man.

**Snail vectors :** The fresh water snails, which act as vectors to different amphistomes in domesticated ruminants are *Indoplanorbis exustus*, *Cyraulus convexiusculus*, *Digoniostoma pulchella*, *Lymnaea acuminata* and *Lymnaea luteola*.

### Life History

The studies on the life cycle of amphistomes furnish information regarding pathogenesis, epidemiology and control of the parasites. The data on the incubation period of the eggs, prepatent period in the snail host, morphology of the cercariae, infective and longevity of the metacercariae and the development in the mammalian host are particularly important. The life cycles of only a few amphistomes of domesticated animals of India have been elucidated so far. The different stages of the life cycles are as follows :

**Egg :** The eggs are oval in shape and fairly large in size. They are whitish in colour and are transparent. The shell has a distinct operculum at one end and a knob-like thickening at the other end. The egg is packed with yolk cells and the ovum is centrally located. Freshly laid eggs contain the developing embryo. The incubation period of the egg depends upon the temperature and varies from species to species.

**Miracidium :** They possess 20 ciliated epidermal plates arranged in 4 tiers of 6, 8, 4 and 2 cells respectively. The larva contains a central spherical nerve mass or brain, an apical gland, one or two pairs of penetration glands, and excretory system containing a pair of flame cells opening by separate excretory pores, germinal balls and longitudinal and circular muscle cells. Miracidium enters the snail host and further development takes place inside the intermediate host.

**Sporocyst :** The sporocysts are found in the mantle tissue, digestive gland or head-foot region of the molluscan host. They may also occur in the body space surrounding the digestive tract either free or loosely attached to the outer wall of the intestine by a mucoid substance. Young sporocysts are usually irregular, pyriform or oval but on maturity they become saccular. The sporocyst contains the germinal balls and cells and a pair of flame cells with ducts. The maturity of the sporocyst depends upon the season. The mature sporocyst contains fully grown rediae, developing rediae and germ balls. The redia gets liberated from the sporocyst by the rupture of the anterior wall or through a terminal opening of the sporocyst. The sporocyst continues to produce rediae till all the germinal cells are exhausted.

**Redia :** The rediae emerge out of the sprocyst while they are still immature and attain maturity in the tissues of the snail hosts. Generally they occur in the digestive gland and the reproductive organs of the hosts. Some are also found in the body space surrounding the digestive tract and also in the mantle tissue. Under optimum conditions they attain maturity in about 10-15 days after liberation from the sporocyst. Mature rediae are sausage shaped. The digestive system consists of a mouth, muscular pharynx,



**Cercaria** : The prepatent period in the snail host varies from species to species and also depends on the environmental temperature. The cercariae have pigmented body with numerous cystogenous cells and an unpigmented simple tail, which helps it to swim. As in the adults, the acetabulum is located terminally or subterminally on the posterior end of the body. The two pigmented eye spots are conical or irregular in shape. The main excretory canals are provided with the highly refractile excretory granules. The excretory system is useful in the identification and classification of cercariae. The arrangement of the genital rudiments is same as in that of adults. The oral sucker, which may be with or without oral pouches, is situated anteriorly. The digestive system is same as that of the adult forms.

The immature cercariae are liberated from the rediae and attain maturity in the host tissue.

**Metacercaria** : The cercariae swim for a short period in the water and at the end of this period they encyst to form metacercariae. They encyst on leaves, stems, aquatic plants, grass blades, on the walls of the container or on the surface of water. Generally the cercariae prefer to form cysts on the smooth green surface of the grass blades. The cyst wall consists of three layers – an outer unpigmented layer of mucopolysaccharide, an inner opaque and brownish layer – the cyst wall proper which is lipid in nature and a third inner most layer formed from a colourless fluid secreted by the cercariae.

The encysted metacercariae mature in about 24 hours and become infective. Under laboratory condition the metacercariae remain viable for a considerable period.

**Development in the final host** : On ingestion by the definitive host, the metacercariae excyst in its small intestine. Excystment is effected by the ruminal fluid (pepsin and hydrochloric acid in the abomasum and trypsin and bile salts in the small intestine). The excysted metacercariae attach to the mucosa of the small intestine and then gradually migrate anteriorly. The time taken to migrate by the immature flukes to reticulum and rumen and the maturation of the flukes into the adult parasites vary from species to species.

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**TABLE**  
**AMPHISTOMES OF DOMESTICATED ANIMALS AND MAN**

Family/Subfamily/Genus	Species	Host	Snail Host	Distribution
Fam. Paramphistomidae				
Subfam. Paramphistominae				
1. Paramphistomum	1. <i>P. cervi</i> .	Sheep, Goat, Cattle, Buffalo	Indoplanorbis exustus	Widely distributed
	2. <i>P. gotoi</i> .	Buffalo		U.P.
2. Calicophoron	* 1. <i>C. calicophorum</i>	Cattle, Buffalo, Sheep, Goat		Widely distributed
	* 2. <i>C. cauliorchis</i>	Cattle, Buffalo		U.P., M.P., Punjab.
	3. <i>C. orientalis</i>	Goat		U.P.
	4. <i>C. papillosum</i>	Cattle, Buffalo		Punjab, U.P., T.N.
3. Orthocoelium	* 1. <i>O. scoliocoelium</i>	Cattle, Buffalo, Sheep, Goat	Digoniostoma pulchella	U.P., M.P., W.B.
	2. <i>O. dawesi</i>	Cattle		T.N.
	3. <i>O. nasmarki</i>	Sheep		U.P.
	4. <i>O. spinicephalus</i>	Buffalo		U.P.
	5. <i>O. orthocoelium</i>	Cattle, Buffalo		U.P., M.P.
	6. <i>O. dicranocoelium</i>	Cattle, Buffalo		U.P., M.P.
4. Cotylophoron	1. <i>C. cotylophorum</i>	Cattle, Buffalo, Sheep, Goat	Indoplanorbis exustus	Widely distributed
	* 2. <i>C. indicum</i>	Cattle, Buffalo, Sheep, Goat	-do-	-do-
	3. <i>C. madrasensis</i>	Sheep		T.N.
	4. <i>C. bareilliensis</i>	Goat		U.P.
5. Gigantocotyle	* 1. <i>G. explanatum</i>	Cattle, Buffalo, rarely in Sheep, Goat	Gyraulus convexiusculus	Widely distributed
Subfam. Cladorchinae.				
6. Pfenderius	1. <i>P. papillatus</i>	Elephant		Andamans
	2. <i>P. birmanicus</i>	Elephant		Burma
	3. <i>P. heterocaeca</i>	Elephant		Andamans, Burma
Subfam. Olveriinae				
7. Olveria	* 1. <i>O. indica</i>	Cattle, Buffalo, Sheep, Goat	Gyraulus convexiusculus	U.P., M.P.
	2. <i>O. bosii</i>	Buffalo, Sheep, Goat		U.P.
Subfam. Gastrodiscinae				
8. Gastrodiscus	1. <i>G. aegyptiacus</i>	Horse		Assam, W.B.
	* 2. <i>G. secundus</i>	Horse, Mules, Ass, Elephants	Indoplanorbis exustus	Assam, T.N. AP.
9. Gastrodiscoides	* 1. <i>G. hominis</i>	Pig, Man	Helicorbis coenosus	Assam, W.B., Bihar, Orissa, U.P.
10. Homalogaster	1. <i>H. paloniae</i>	Sheep, Cattle		Assam, U.P.
Subfam. Gastrothylacinae				
11. Gastrothylax	* 1. <i>G. crumenifer</i>	Cattle, Buffalo, Sheep, Goat	Gyraulus convexiusculus	Widely distributed
12. Carmyerius	1. <i>C. spatiosus</i>	Cattle		U.P., M.P.
13. Fiscoederius	* 1. <i>F. elongatus</i>	Cattle, Buffalo, Sheep, Goat	Lymnaea luteola	Widely distributed
	2. <i>F. cobboldii</i>	Cattle, Buffalo		U.P., M.P., W.B. T.N., Punjab
Subfam. Johnsonitrematinae				
14. Johnsonitrema	1. <i>J. magnum</i>	Cattle		
Subfam. Pseudodiscinae				
15. Pseudodiscus	* 1. <i>P. collinsi</i>	Horse, Ass, Elephant	Indoplanorbis exustus	Widely distributed
	2. <i>P. hawkesi</i>	Elephant		Assam, Bihar, Punjab, T.N.

\*Common in occurrence.

## Schistosomes and Schistosomiasis with particular Reference to India

**C.B. Srivastava**

Zoological Survey of India, Calcutta.

Blood flukes, infesting warm blooded animals, are well known among trematodes because of two reasons; they are the only trematodes showing sexual dimorphism and they cause the dreaded disease 'schistosomiasis'. These are included in the family Schistosomatidae Looss, 1899, while the blood flukes of chelonians and fishes are placed in the families Spirorchidae and Sangunicolidae respectively. Mammalian schistosomes have received by far the greatest attention of research workers world over because of the importance of their host. These blood flukes inhabit the hepatic portal system and pelvic veins of birds and mammals, including man. Present account deals with the worms of the subfamily Schistosomatinae Looss, 1899 which infect mammals. (Table I)

### General Morphology

**Males** : are generally broader and larger than females. Ventral surface is incurved to form the gynaecophoric canal in which the female remains *in situ*. Spines are present often on the body surface or may be restricted to inner surface of suckers. Both suckers are present close together. Acetabulum is better developed in males than in females. Pharynx is absent. A long oesophagus leads to the intestinal crura, which are fused posteriorly to form a single stem. There are several testes, arranged in one or two rows before the beginning of the gynaecophoric canal. Cirrus pouch may or may not be present. Seminal vesicle is present in pretesticular position. Genital pore is postacetabular, anterior to the beginning of gynaecophoric canal.

**Females** : are slender and thread-like. Cuticle is normally aspinose except in suckers and posterior end of body. Both the suckers are in the anterior region. Pharynx is absent. Oesophagus is long and divides into two caeca anterior to acetabulum. Caeca join to form a common caecum in posterior half of the body, and terminate at proximal end. Ovary is oval or elongate, usually caudad rarely cephalad of the equatorial plane. Laurer's canal absent. Vitelline follicles situated on either side of common caecum, extend from caecal union to almost posterior end of body. Uterus is long, straight, containing few eggs. Eggs are oval or fusiform, non-operculate, with terminal or lateral spine or with a rudimentary lateral spine, containing ciliated miracidium. Excretory vesicle is tubular, very short, and with terminal pore.

For oviposition the female leaves the gynaecophoric canal of the male and pushes herself in the smallest possible vessel where it lays eggs. The total number of eggs laid

in a day may vary from 150 to 500 depending on the species. The eggs work their way into the intestine or the urinary bladder, as in *Schistosoma haematobium*, and reach the exterior. By this time they contain fully developed miracidium. The disease produced by those species whose eggs are evacuated through the tissue of gut is known as *intestinal schistosomiasis* while that produced by *S. haematobium* is commonly spoken as *urinary* or *vesical schistosomiasis*.

**Life history :** The life history of Schistosome follows the general pattern of development of any digenetic trematode with some modifications. The details of the life cycle and the factors influencing it are discussed in the following pages.

### History of Human Schistosomiasis

Although ancient Egyptian physicians were acquainted with haematuria and had several prescriptions for its treatment, Bilharz (1852) was the first to discover a human blood fluke in portal vein of an Egyptian peasant at Kasr-El-Aini and designated it as *Distoma haematobium*. Cobbold (1859) suggested a new genus *Bilharzia* for the parasite, in honour of its discoverer, but three months earlier Weinland (1858) had named it *Schistosoma* and thus the parasite became *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858. The presence of schistosome ova in mummies which fell 3,000 to 4,000 years ago was reported by Sir Armund Ruffer (1910) who also demonstrated microscopically the presence of calcified blood-fluke ova in the nuclei of urinary calculi and in the cortex of kidneys of mummies (1200–1090 B.C.). Cobbold (1857) had also shown that this parasite was not confined to human species or to Egypt, by describing a worm clearly of the same genus from an ape dying in the London Zoological Society Garden. First attention for the wider distribution of schistosomes was drawn by a London physician, John Harley, who was practicing in Cape of Good Hope and called the species *Distomum capensis*. While the information on life history and other aspects of the blood fluke was being accumulated it appeared that *S. haematobium* is not the only species of that genus to infect man. In April 1904, Professor Kasturada of the Pathological Institute, Okayama, found schistosome egg in the faeces of a typhoid patient and named the parasite as *S. japonicum*, as the eggs had no terminal spine.

In India, human schistosomiasis was reported as early as 1878 in an English patient (Hatch, 1878). Later reports included urinary schistosomiasis from Bombay Presidency and other areas (Povell, 1903; Sewell, E.P. 1904); presence of eggs of *S. haematobium* and *S. bovis* was detected from a native of Madras (Christopher and Stephens, 1905). Sporadic reports of urinary schistosomiasis came from different parts of the country viz. Rajkot, Madras, Goa, Calcutta, Rawalpindi (Wardrop, 1906; Hooten, 1914; Harkness, 1922; Chandler, 1926; De Mellow, 1936; Andreason and Suri, 1945).

After the World War I (1914–18) it was feared that Indian Troops returning from Egypt, East Africa, Mesopotamia and other endemic areas may carry the infection to India as also the African Troops stationed in different parts of the country might have spread the infection. The matter was seriously taken up by the Indian Government. Soon a search started for the suitable intermediate host which might aid in the establishment of schistosomiasis in India. The main onus fell on the Zoological Survey of India and snails from the areas where Troops were stationed (Secunderabad, Waltair, Calcutta) were examined both for natural and experimental infections (Kemp

and Gravely, 1919; Sewell, R.B.S. 1919, 1920, 1922; Annandale and Sewell, 1920). None of the 13 species of snails examined were positive either for natural or experimental infection. The cercariae recovered from all these studies resulted in the publication of a monograph, "*Cercariae Indicae*" by Sewell (1922). It was argued that in the absence of a suitable intermediate host human schistosomiasis can not establish in India. Opinions, however, differed as many thought that the parasite may adopt another host (Milton, 1919; Soparker, 1919; Bhalerao, 1938; Chauhan, 1962; Baugh, 1978). A case of haematuria due to *S. haematobium* infection was reported from Gimvi Village, Guhagar Taluka in Ratnagiri district (DeSa and Monterio, 1949). This gave the clue for a thorough investigation of the area which resulted in the establishment of an endemic 'focus' of the disease in 'Gimvi' village (Gadgil and Shah, 1952). Efforts were made to find the vector snail. First *Paludomus obesa* was incriminated but later *Ferrisia tenuis* was declared as vector snail (Gadgil and Shah, 1955, 1965; Shah and Gadgil, 1955, 1955a; Gadgil, 1962). The identity of the 'Gimvi' schistosome was doubted (Varma, 1955, Anon, 1956) and it still remains an open question. Other endemic foci were located in village 'Tirupparankumdaram' in Madurai district (Sundarajulu, 1967) and Lohager in Raipur district (Srivastava and Arora, 1969). These cases need confirmation. The snail *Bellamya dissimilis* from a pond of the former village did not discharge any furcocercous cercariae though 3 specimens of *Lymnaea luteola* from the latter village did discharge schistosome cercaria suggestive of *S. haematobium*. Thus the question of existence of human schistosomiasis in India remains unanswered.

### History of Animal Schistosomiasis

The earliest record of occurrence of animal schistosomes in India dates back to Cobbold (1828). Bomford (1886, 1887) reported the presence of *S. haematobium* eggs in the large intestine of two bullocks of Calcutta. Montgomery (1906) described *S. bomfordi* and *S. spindalis* from the portal veins of cattle; *S. indicum* from that of horse and *S. bovis* from sheep and opined that eggs reported by Bomford might be that of *S. indicum*. The occurrence of *S. bovis* was doubted by subsequent workers as this parasite does not occur in India. Malkani (1932) reported a form of nasal schistosomiasis in which cauliflower-like growth developed on the nasal septum of cattle. Rao (1933, 1933a) and Malkani (1933) published detailed account of this disease and Rao (1934) named this parasite as *S. nasalis*. Occurrence and etiology of *S. indicum* was discussed by Datta (1933), Malkani (l.c), Leese (1911) and others from various parts of the country. Bhalerao (1934) described male of *S. japonicum* from pig on the basis of material sent to him by M.A. Maplestone which later proved to be *S. incognitum* described by Chandler (1926) on the basis of eggs. Rao and Ayyar (1933) reported *S. suis* from the portal vein of pig in Madras which was later proved to be identical with *S. incognitum*. Swaminathan (1934) reported the occurrence of this parasite in dogs from Jabalpur. Mudaliar and Ramanujachari (1945) described *S. nairi* from the liver of an elephant at Coimbatore, which was later transferred to the genus *Bivitellobilharzia* by Dutt and Srivastava (1955). They (1952) had also described *Ornithobilharzia dattai* from sheep and goats, completed its life history and published (1961, 1962) detailed account and made a comparative study of miracidia. Srivastava and Trisul recorded *S. turkestanicum* in cattle from Kashmir and completed (1964) the life history of this fluke. Dutt and Srivastava (1955, 1962) erected the genus *Orientobilharzia* to accommodate their species *O. dattai* and also transferred *S. turkestanicum* to it. Dutt and

Srivastava (1968) made further studies on the life cycle and pathogenicity of *S. nasale* from cattle and buffalo. This parasite was recorded from a goat in West Bengal by Sengupta and Sinha (1966). Srivastava and Dutt (1961, 1962) published detailed account on the morphology and life history of *S. indicum*. Dutt and Srivastava (1965) showed that *Macaca mulatta* is very susceptible to *S. incognitum* infection. It can thus be seen that there are seven species of schistosomes infecting animals and one species suspected to infect man. The details are summarised in Table I. Chauhan, Srivastava and Chauhan (1973) and Baugh (1978) gave comprehensive accounts of schistosome study in India. Little work has been done on bird schistosomes in India except that of Mehra (1940), Lal (1937) and Baugh (1963).

### Ecology and Behaviour of Parasite and Host

The relation between the parasite and its environment has always attracted the attention of workers in Parasitology. It will continue to be a basic biological problem and may, some day, elucidate the phenomenon of animal parasitism, its essence and origin. The more we learn about it the more complex the problem becomes.

The characteristic feature of parasitism is the fact that, in each analysis beginning from the cellular level, we must take into account for a parasite two environments. First its immediate environment or the host body; and second the external environment proper as known for free living organisms.

These two types of environments have been reviewed in respect of schistosomes.

**Eggs :** The schistosome eggs after being laid in the capillaries reach the intestinal lumen or urinary bladder in case of *S. haematobium*. In 6-7 days after oviposition the miracidium is fully developed. If the eggs fail to reach the above locations they are phagocytosed or more rarely, calcified in various tissue sites in which they occur. Sinha and Srivastava (1965) studied the effect of certain physico-chemical factors on the viability of the eggs of *S. incognitum*. At low temperature (i.e. 15-30.5<sup>0</sup>c) the eggs remain viable for 7-10 days, while in summer months (at 21-45<sup>0</sup>c) this period is reduced to 5-6 days. Similarly low humidity desiccates the eggs and in high humidity of about 79% the eggs remain viable for 4-5 days. If water is sprinkled on faeces containing eggs the viability of eggs varies with the degree of fermentation. The sex is determined in the egg stage itself and one egg produces either male or female miracidia. But the actual process is not known.

**Hatching of eggs :** The factors affecting the hatching of schistosome eggs have been studied by Standen (1949, 1951), Sinha and Srivastava (1965) and Rawan (1956, 1957). Osmotic pressure, light and water play major role in its hatching. On dilution of the faeces, or urine in case of *S. haematobium*, in the presence of light the hatching rapidly occurs. The hatching mechanism is dual. Firstly, in liquids of low osmotic pressure water is presumably taken in but the egg is unable to burst until exposed to light. This may be due to the action of a light released substance, possibly an enzyme. The mechanical pressure exerted by the active miracidium inside the egg-shell also helps it. The osmotic pressure has even more marked effect, within quite narrow range, and plays a major role in prevention of premature hatching. Thus hatching of eggs of *S. mansoni* is almost completely inhibited by 0.6% NaCl sol. and extensive hatching does

not occur until the dilution of 0.1% is reached. Hence the eggs in blood, gut contents or urine will only hatch on reaching water. The mechanism of this inhibition is not known. The optimum temperature required for hatching these eggs varies from 26-28°C and the process is almost completely inhibited at 4°C and 37°C. It can be restored when eggs are again put back to optimum temperature. The inhibitory action of these factors is clearly to the advantage of parasite that embryonated eggs should not hatch prematurely while still within the host.

**Miracidium** : The Miracidium is positively phototropic and negatively geotropic. It has sufficient food reserve to enable it to swim for 24-36 hours, after which it dies if a favourable molluscan host is not available. In all cases where the miracidium hatches in the surrounding environment, it is definitely attracted to certain species of snails and not to others, as can be deduced both from the distribution of cercariae within their host and from the experimental investigations conducted in the laboratory. The reason for this attraction is as yet unknown and may be perhaps related to the nature of skin secretions of the snail (probably sulphur contents), parts of which might possibly be soluble in water and thus create an aura of specific attraction around each snail. Such a mechanism must be extremely delicate. Vogel (1934) showed that the miracidium of *Opisthorchis felineus* is attracted to the prosobranch snail, *Bithynia leachi*, but not to the closely related species, *B. tentaculata*, which also occurs and usually more abundantly in the same biotope. The miracidium of *S. japonicum* is attracted to oncomelanid snails whereas that of *S. mansoni* penetrates only into planorbids. Consequently the miracidia of two different species of the same genus manifest totally different tropism which is all the more marked as the snail hosts themselves belong to two distinct suborders. It should, however, be borne in mind that penetration of miracidium into a snail host does not necessarily enable it to continue its normal development. It must also be able to overcome successfully the natural defenses that snail puts up to any parasite. Ecological segregation of parasites influences the distribution of cercariae and consequently also of metacercariae, if present in the life cycle.

After penetration in the snail host the miracidium does not lose the cilia immediately but uses it for passage to deeper tissue of the host. The miracidium then transforms into a single smooth-walled sporocyst. This in turn produces daughter sporocysts which make their way into lymph spaces and so to the lymph sinuses of the digestive gland. Here they grow and when mature produce typical furcocercariae, about 2,00,000 in *S. mansoni*, which escape from the snail. The development of the intra-molluscan phase also requires an optimum range of temperature.

**Cercariae** : The emergence of cercariae is periodic, and in nature tends to occur in direct sunlight between 9 a.m. and 2 p.m. but this process is inhibited or partly inhibited by temperatures lower than 21°C. Sinha and Srivastava (1965) studied various factors involved in the emergence of cercariae of *S. incognitum* and found that fresh water stimulated the emergence and the time of emergence was from 8 a.m. to 12 noon. It is possible that emergence of cercariae may be correlated, in some way, with the habit of definitive host. The question has never been fully investigated. Bueding and Most (1953) found that cercariae of *S. mansoni* are very sensitive to lack of oxygen and die within an hour under completely anaerobic condition *in vitro*. In many cases

cercariae die in the skin after penetration or even due to exhaustion during penetration causing dermatitis. The cercariae after penetration reach venous circulation either directly or through lymph vessels. The early stages of development inside the definitive host body are imperfectly known, but it is believed that the schistosomulae (as the young ones are commonly known) pass through the lungs after 4-14 days. They then pass to the intrahepatic portal vessels in about 8 days, where early stages of maturation occur. Standen (1953) found that in mixed infections with male and female cercariae paired worms may be found in the mesenteric and portal veins after about 26 days, but majority leave on about 30th day. In animals infected by male cercariae only the males develop normally but there is a tendency for worms to delay their migration from liver to portal system. The addition of female to an already established male infection greatly increases the proportion of worms migrating into portal system. Vogel (1941) and Smyth (1961) mentioned that in mice infected with female cercariae of *S. mansoni* or *S. japonicum* only the growth and maturation of adult females is greatly stunted, and virtually no migration into mesenteric veins occurs. The addition of males to an all-female-infection has a striking effect. Pairing commences as soon as the males are mature, the females develop normally and the migration of the paired worms takes place as in normal infection. It is likely that the failure of females to migrate into portal and mesenteric veins is due to physical weakness of the females and their inability to migrate against the blood stream unless carried by the larger and more powerful male. The failure of genitalia to mature in the absence of male suggests the existence of possible male stimulation factor although this has never been proved. Price (1931), El-Gindy (1950, 1951) and Kagan *et. al.* (1954) conducted experiments with *Schistosomatium douthitti* and found that in this species both males and females develop normally in unisexual infection in mice. The sexual maturity is also reached in 10-15 days. This rapid maturation contrasts strangely with that of *S. mansoni*, where sexual maturity requires more than twice as long i.e. 28 days. The marked discrepancy has, at least partly, a nutritional basis. In *S. mansoni* the passage through the lungs is slow (4-14 days) and majority of schistosomulae do not reach the liver before about 8th day after infection, which means that they do not come in contact with veins of the liver carrying their rich supply of amino-acids and carbohydrates until that time.

Vogel (1941, 1943) in a mixed species infection of schistosomes showed that the male of one species and the female of another will pair and produce eggs of the female type; the eggs, however, are produced parthenogenetically. He also found that in unusual hosts, an infection with males produces a proportion of imperfect hermaphrodites. It seems highly probable that bisexualism, in blood flukes of warm blooded animals, is a secondary phenomenon. Cameron (1964), however, believes that these forms are physiologically hermaphroditic even if physically dioecious.

In mammals, the peripheral blood stream as an environment is relatively poor in carbohydrates and protein break down products of low molecular weight. The portal system, on the other hand, carries intestinal break down products from the duodenum and is rich in glucose and amino-acids, in addition to the protein available in the plasma and blood cells. It would represent an environment of a level sufficient to satisfy the metabolic demand of an egg producing trematode. This provides such an environment to the parasite is evidenced by the efficiency with which a number of species of schistosomes grow and reproduce there. The chemical composition and

quality of soluble food material in the blood stream will vary with the feeding habit of the host. As a source of nutrient, its value must be considered in relation to the morphology and physiology of the organism utilising it. To the blood flukes, e.g. *S. mansoni*, which possesses both a gut and a well developed digestive enzyme system (Smyth, 1961) the quantity of blood protein in the plasma and blood cells represents a diet of potentially high nutritional level. Faust and Meleney (1924) and Hsu (1938) studied the food of *S. japonicum* and found that it can digest red blood cells and other components of the blood but not the mononuclear, polymorphonuclear and eosinophilic leucocytes. Hsu (1938) also found that the physiology of digestion in the two sexes of this worm is also different to a certain degree. Gonnert (1955) made electron microscope study of schistosome cuticle and found it to be considerably vacuolated suggesting that cuticular as well as intestinal absorption is possible.

In mammalian blood, apart from protein and the usual inorganic constituents, the substances likely to be of physiological importance to a parasite are fats, in the form of neutral fats, (or triglycerides), lecithin and cholesterol; aminoacids and carbohydrates. Blood parasites have at their disposal complex protein molecules, both in blood cells (e.g. haemoglobin) and in the serum. With modern techniques, serum proteins are separated electrophoretically into free components i.e. albumins, two alpha globulins, one betaglobulin and gama globulin. Globulin, particularly gama globulins, are concerned in immunity reactions. Immunity or resistance as defined by Smyth (1961), is a physiological response by the host to a previous or present contact with the parasite, the nature of the response being such that it is directed against the establishment and survival of parasite. The immunity or resistance may be natural or acquired. In acquired immunity again there are two types viz. naturally acquired or artificially induced. This second type is generally concerned in the study of immunology. The details of immunological studies in diagnosis of Schistosomiasis has been discussed by Kagan and Pellegrino (1961) and Kagan (1968).

From the foregoing account it is evident that much remains to be done in the context of both human and animal schistomomiasis. It can be summarised as below :

1. The areas from where human schistosomiasis have been reported should be thoroughly surveyed both from the snail and schistosome points of view, to settle the question whether human schistosomiasis has taken a foothold in the country.
2. Possibility of zoonosis of animal schistosomes should be explored.
3. The search for reservoir host should be made.
4. Possibility of antagonism in the schistosome cercariae and other cercariae should be studied as Joe (1964) has shown that echinostome cercariae are antagonistic to schistosome cercariae in Malaya.

If these investigations are completed they will go a long way in formulating measures for the control and eradication of schistosomiasis from the country.

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**TABLE I** Schistosomes with their hosts and distribution.

Species	Host	Distribution	Intermediate host	Authors
1. <i>Schistosoma haematobium</i> (Bilharz, 1852)	Man, Monkey expt. Host. rat, mice	Africa, Australia, Asia (Arabia, Cyprus) India (Gimvi vill., Ratnagiri) Mesopotamia, Palestine and Persia and Europe (Greece and Portugal.)	<i>Bulinus contortus</i> , <i>B. dybowski</i> , <i>B. innersi</i> (Egypt); <i>Physopsis</i> <i>africana</i> (Belgian Congo, Natal, Transval); <i>P. globosa</i> (Nyasaland and Sierra Leone); <i>P. nasuta</i> (Tanganyika Territory); <i>Lymnea</i> <i>natalensis</i> (South Africa); <i>Planor-</i> <i>bis dufourli</i> (Portugal) and <i>Ferrisia</i> <i>tenuis?</i> (India).	Bilharz (1852), Diesing (1858) Weinland (1858), Leiper (1915) Gadgil & Shah (1954) Shah and Gadgil (1955)
2. <i>Schistosoma indicum</i> Montgomery, 1906	Horse, donkey, camel, sheep, goat cattle, buffalo, zebra, antelope. Expt. Sheep, goat, donkey, buffalo- calf, rabbit, guineapig, white mouse.	India (Practically from all the states)	<i>Indoplanorbis exustus</i>	Montgomery (1906), Srivastava & Dutt (1951) Dutt & Srivastava (1962)
3. <i>Schistosoma incognitum</i> Chandler, 1926	Pig, dog, expt. Pig, dog, cat, sheep, goat, cattle rabbit, giunea pig, rat.	India (U.P., Madras Bihar, West Bengal)	<i>Lymnea luteola f australis</i> <i>succinea</i> , <i>ovalis</i> .	Chandler (1926), Bhalerao (1934), Rao (1935), Khaw (1947), Sinha et Srivastava (1954, 1956, 1960).
4. <i>Schistosoma spindale</i> Montgomery 1906	Buffalo, bandicoot. Expt. Cattle, buffalo, goat, guinea-pig	India, Sumatra, South Africa	<i>Indoplanorbis exustus</i> . <i>Lymnea acuminata</i>  <i>Lymnea luteola</i>	Montgomery (1906), Liston & Soparker (1918), Soparker(1921)  Fairley & Mackie (1925, 1930); Farley & Jasudasan (1927, 1930 a, b), Malkani (1932)
5. <i>Schistosoma nasale</i> Rao, 1933	Cattle, buffalo, goat Expt. calf, cattle, sheep, goat, guineapig	India, Burma	<i>Lymnea luteola</i> ( <i>cercariae Indicae xxx</i> ) <i>Indoplanorbis exustus</i>	Rao (1933 a, b), Malkani (1933), Dutt & Srivastava (1968) Sengupta & Sinha (1966)
6. <i>Orientobilharzia bomfordi</i> (Montgomery, 1906)	Cattle, buffalo	India (Mukteswar)	Not known	Montgomery (1906), Dutt & Srivastava (1956)
7. <i>Orientobilharzia dattai</i> (Dutt & Srivastava, 1955)	Buffalo. Expt. Buffalo-calf, goat, calf, rabbit, guineapig, mouse	India (Bareilly)	<i>Lymnea luteola</i>	Dutt & Srivastava (1952, 1955, 1961b, 1962a,b,c)
8. <i>Orientobilharzia turkes-</i> <i>stanicum</i> (Skrjabin, 1913)	Cattle, buffalo, sheep, goat, water buffalo, horse, donkey, mule, camel. Expt. Hill bull, rabbit, gunieapig, albinorat, albino mice, sheep	Russian Turkestan, Kazakas- tan, France, Bagdad, Mangolia, Iran, Pakistan, India (Kashmir), China, Korea, Azrebaidzan	<i>Lymnea tenera euphratica</i> <i>Lymnea auricularia</i> <i>L. acuminata rufescens</i>	Skrjabin (1913, 1951), Dutt & Srivastava (1955, 1964), Srivas- tava and Trisul (1957), Machttee (1936) Abdusalam & Sanwar (1954)
9. <i>Bivitellobilharzia nairi</i> (Mudalier et Ramanujachari, 1945)	Elephant	India (Coimbatore)	Not known	Mudalier et Ramanujachari (1945), Bhalerao (1947), Dutt & Srivastava (1955)



## Review of Paragonimiasis in India

**R. K. Ghosh**

Zoological Survey of India, Calcutta.

The lung fluke, *Paragonimus*, is a harmful parasite causing paragonimiasis with suggestive symptoms of tuberculosis in man and animals. This is specially a lung parasite but has also been recorded from brain, spinal cord and other organs in man, cat, dog, fox, pig, goat, cattle etc. It is reported that this fluke is associated with Jacksonian epilepsy, eosinophilic meningitis and some other nervous diseases. These parasites are normally found in encysted condition in pairs in the lung, though single or several specimens have also been recorded in a cyst.

### Distribution

Geographical distribution of *Paragonimus*, extends over the parts of Asia, Africa and America. The disease is more prevalent in China, Japan, Philippines and Korea where crabs which act as second intermediate host of *Paragonimus*, are often consumed raw with salt or drunk in wine or vinegar. In some of these places as much as 40% of human population has been infected. As reports of human paragonimiasis from neighbouring countries of India have been gradually accumulating in literature it is feared that paragonimiasis might also become a problem in India. Fortunately, there are not many authentic reports of paragonimiasis in man from India. Leiper (1913) recorded a doubtful case of paragonimiasis in a tribe of India as the sputum was suspected to contain egg. Surveyer (1919) reported a case of lung fluke in a Chinese in India, which was however believed to be not of Indian origin. Lane and Low (1923) included whole of India in the range of distribution of the disease, Baylis (1929) however, excluded that possibility. Faust (1930) included Bengal and Assam while Chandler and Read (1961) indicated parts of India as endemic focii of human paragonimiasis. Although there had been no report of larval or adult *Paragonimus* in animals or man from Nepal, Iwamura (1965) reported the occurrence of *Paragonimus* eggs in the sputum of patients suffering from tuberculosis.

India is the first Asian country from where *Paragonimus compactus* was recorded by Cobbold (1859) in a mongoose, *Herpestes edwardsi*. Of course the oldest record of the lung fluke is from Brazil from where Diesing (1850) described *P. rude* in an otter, *Lutra braziliense*. The most important species of lung fluke, *P. westermani*, which occasionally infects man, was described by Kerbert (1878) from the lungs of Bengal tiger which died in the zoo-garden in Amsterdam. After three years he obtained some more specimens from another tiger from the zoo-garden in Hamburg and described its anatomy in detail. Veevers (1923) studied lung flukes from the Indian mongoose and cats which died in London zoo, and identified them as *P. compactus* and *P. westermani* respectively. Gulati (1926) described *P. edwardsi* obtained from lungs of palm civet,

*Paradoxurus grayi* in Kumaon hills. Rao (1935) recorded *P. westermani* in dogs from Malabar and Coimbatore and also in a panther shot at Coorg. Srivastava (1935) reported the same from cats of Uttar Pradesh. Miyazaki (1970) reported that he studied some specimens of *P. westermani* from tiger from Pantnagar sent to him by Dr. Suresh Singh from Indian Veterinary Research Institute. Dutt & Gupta (1978), recorded paragonimiasis in a bear cat from 'Zoo-park', Chandigarh. Ravikumar *et. al.* (1979) also recorded *P. westermani* from mongoose, *Herpestes edwardsi* from Visakhapatnam.

### Taxonomy of Indian Species

Ward and Hirsch (1915) made a detailed study on the speciation of the genus *Paragonimus*. They recognised structure and cuticular spines in mature specimens as reliable characters for species differentiation. They held the following species as valid, *P. westermani* from tiger in India; *P. ringari* from man in Orient and *P. kellicoti* from dog, cat and pig in America. Kabayashi (1917, 1918) made detailed study on the taxonomy of the genus *Paragonimus*, based on a large number of specimens from natural and experimental hosts. He opined that spine character is not at all reliable and the genus *Paragonimus* is monotypic, represented by a single species, *P. westermani*. Ameel (1934) also doubted the validity of spine character but tentatively recognised four species. Wu (1939) and Caballero (1946) maintained that this genus is represented by a single species.

Miyazaki (1939) described *P. ohirai* from Japan and within a span of fifty years or so as many as 29 species have been recognised from different parts of Asia, Africa and America by some authors in a wide variety of natural and experimental hosts with contradictory opinions on the synonymy and range of distribution of different species.

As far as records from India are concerned most of the workers believe that *P. edwardsi* Gulati, 1926 should be regarded as synonym of *P. westermani*. (Chen, 1940, Yakogawa *et. al.*, 1960, and Miyazaki, 1970). But opinion differs with regard to *P. compactus*. Veevers (1923), Ameel (1934) and Miyazaki (1970) appear to have recognised the species as valid while Yokogawa *et. al.*, (1960), and Yamaguti (1971) considered this species as synonym or of doubtful identity. Miyazaki (1970) observed that authentic identification of the species of this genus is not possible through studies on adult specimens only but metacercarial stage must be taken into account. However, till date no metacercarial stage of this genus has been reported from any crustacean host from India. The normal molluscan host recorded for *P. westermani* in other Asian countries is also unknown from India. Sporadic reports from animals have of course been made from both north and south India, confirming wider distribution of this lung fluke in India.

### Causes of Infection

Apparently the human infection with this lung fluke is predominant only in those countries where raw or imperfectly cooked crabs or crayfish are eaten. Food habit in most parts of India prevents the wide spread infection. Crabs are eaten by a large population in India but they are cooked in such a manner that infective stage of metacercaria of lung fluke can not survive. Yokogawa (1952), Komiya (1952) and some other workers while investigating the lung fluke disease in Japan found that human infection is possible not only by eating infected crabs, but also while handling or during transportation or when chopping and crushing of infected crabs at the time of cooking.

Moreover the symptoms of this disease are quite similar to that of tuberculosis, such as intermittent cough, blood stained sputum, mild anaemia, slight fever, weariness etc. As such it is quite probable that lung fluke infection is being overlooked as a case of tuberculosis. Proper investigation should be conducted for metacercarial stage in crabs as well as presence of eggs in the sputum of suspected tuberculosis patients not responding to specific treatments.

#### Molluscan hosts

- |  |                                     |
|--|-------------------------------------|
| 1. <i>Assiminea lutea</i>                    | 10. <i>Thiara granifera</i>         |
| 2. <i>A. parasitologica</i>                  | 11. <i>T. toucheana</i>             |
| 3. <i>Bithynellaniipponica akiyoshiensis</i> | 12. <i>Tricula gregoriana chiui</i> |
| 4. <i>Brotia asperata</i>                    | 13. <i>T. minima</i>                |
| 5. <i>Oncomelania nosophora</i>              |                                     |
| 6. <i>Paludinella japonica</i>               |                                     |
| 7. <i>P. debilis</i>                         |                                     |
| 8. <i>Pomatiopsis lapidaria</i>              |                                     |
| 9. <i>Semisulcospira libertina</i>           |                                     |

Although there are several names listed in the old literature as mentioned above, recent authors (Pace, 1973; Malek and Cheng, 1974) have cited only a few species, namely, *Semisulcospira libertina*, *Thiara granifera*, *T. toucheana*, *T. tuberculata*, *Tricula minima* and *T. gregoriana chiui*.

#### Crustacean host

- |   |                                      |
|---|--------------------------------------|
| 1. <i>Potamon miyazakii</i>                             | 10. <i>P. dugasti</i>                |
| 2. <i>P. dehaani</i>                                    | (= <i>Somanniathelphusa sinensis</i> |
| 3. <i>P. sinensis</i>                                   | <i>dugasti</i> )                     |
| 4. <i>P. yaenansis</i>                                  | 11. <i>Sesarma intermedia</i>        |
| 5. <i>P. rathbuni</i>                                   | 12. <i>S. dehaani</i>                |
| 6. <i>P. denticulatus</i>                               | 13. <i>S. hamatocheir</i>            |
| 7. <i>Parathelphusa maculata</i>                        | 15. <i>Chasmagnathus conexus</i>     |
| (= <i>P.(P.) tridentata</i> )                           | 16. <i>Eriocheir japonicus</i>       |
| 8. <i>P. grapsoides</i>                                 | 17. <i>Procambarus clarkii</i>       |
| 9. <i>P. germaini</i> 14. <i>Helice tridens tridens</i> | 18. <i>Sudanautes africanus</i>      |
| (= <i>Somanniathelphusa germaini</i> )                  |                                      |

#### Conclusion

Paragonimiasis is a major public health problem in Japan, China, Korea, Formosa, Manchuria, the Phillipine Islands and to some extent in other surrounding South-east Asian countries including some Pacific Islands. Fortunately Paragonimiasis has not been a major health problem in India. No cercaria, metacercaria or adult *Paragonimus* spp. have been recovered from snails, crabs or man so far. A few records of detection of *Paragonimus* eggs in sputum of persons suffering from lung infection are of-course available in the literature. On the other hand records of *Paragonimus* spp. from tiger, dog, cat, mongoose etc., from various parts of India clearly point out the possibility of its occurring in human being also. The snail host of *Paragonimus* spp. mainly belong to

the family Thiaridae and in some cases the amphibious snails of the Hydrobiidae. The family Thiaridae and the species *T. granifera* and *T. tuberculata* are having their range of distribution in India. Similarly the crab hosts belonging to the genera *Potamon*, *Geothelphusa*, *Parageothelphusa* are also available in India. As such systematic studies are very much needed to find out the larval hosts of *Paragonimus*. It can be concluded by endorsing the view of Yokogawa et. al., (1960) that, 'more studies are badly needed on the adult and larval stages of the species of *Paragonimus* in India'.

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