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one year or a little more. The fish can be made to breed during most of the year under laboratory conditions. About 100 to 150 eggs are laid at a time and measure 1.2 to 1.7 mm. in diameter. The embryos hatch out in 6 to 8 days if kept at 18-19°C. and take 3 to 4 weeks to reach a size of about 11 mm. when all the essential characters of the fish are developed.

The eggs were fertilized by the dry-sperm method of artificial insemination. Freshly fertilized eggs were subjected to hot or cold treatment by immersing them in water of the requisite temperature.

Observations

Production of abnormalities:

The table below classifies the abnormalities produced by hot or cold treatment of the freshly fertilized eggs.

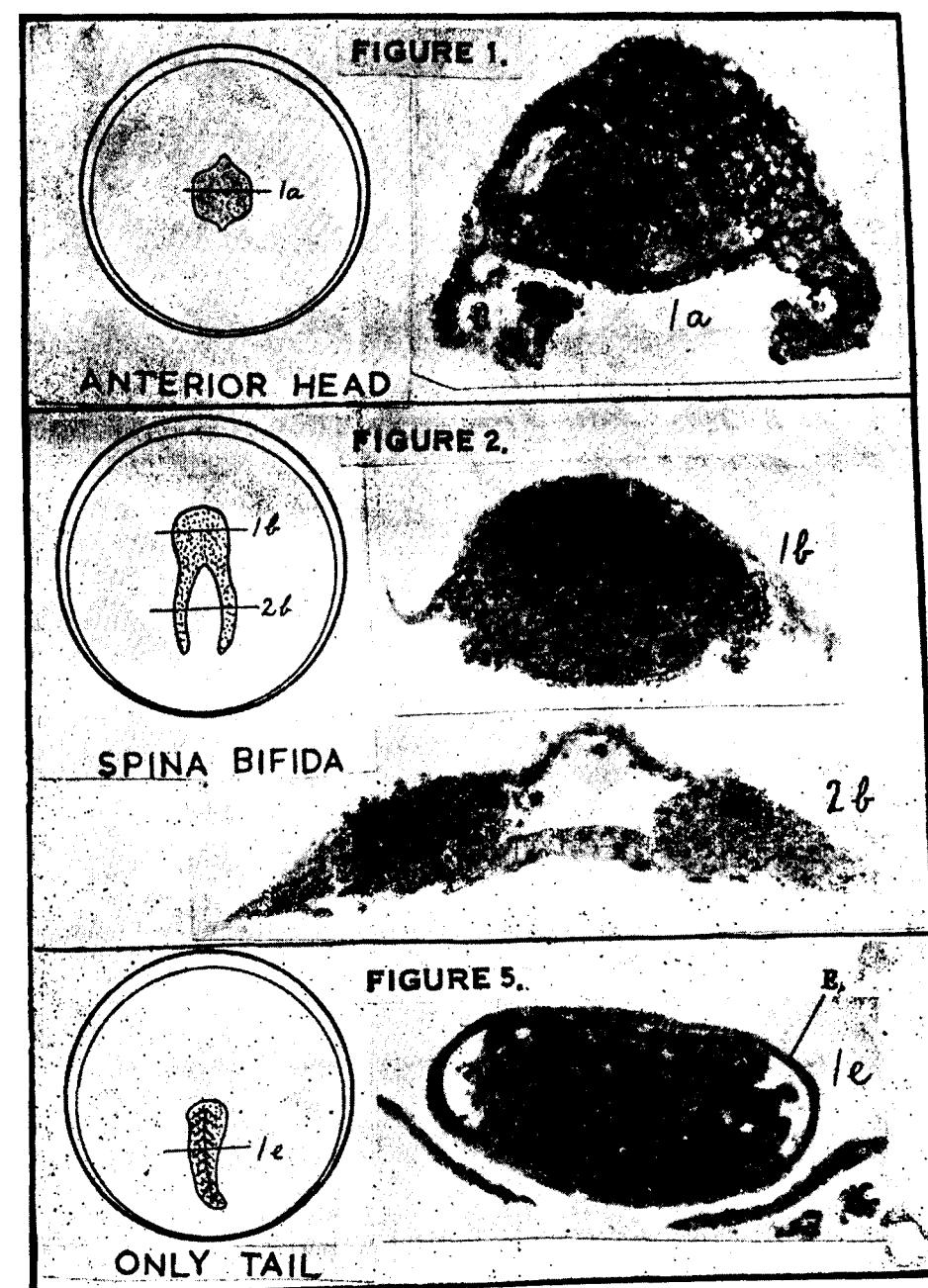
	Temperature and duration of treatment	
	32.5 to 37°C. for 5 minutes	0°C. for 1½ to 2 hours.
Total number of eggs treated	... 936	4,284
Eggs starting to gastrulate	... 437	981
Eggs with abnormal gastrulation	... 199	185
(i) Only anterior part of head present	... 60	10
(ii) Spina bifida	... 39	7
(iii) Only trunk and tail-like structure	... 0	28
(iv) Tail-like structure only	... 0	44

The proportion of abnormalities produced by hot treatment is greater than that produced by cold treatment. Also, lack of posterior parts of the embryo is much more frequent after hot treatment, while the absence of head or head and trunk structures could only be found after cold treatment.

Actual observation of the developing eggs has shown two causes for these abnormalities; firstly the incomplete formation of the germ ring which results in the blastopore failing to close and secondly the extent of the rolling in of the invaginating parts which determines the extent of differentiation. Incomplete formation of the germ ring leads to deficiencies of the posterior parts of the body and the incomplete rolling in leads to reductions of the anterior parts of the body.

Swarup

Plate 1



Only anterior part of head: (Fig. 1).

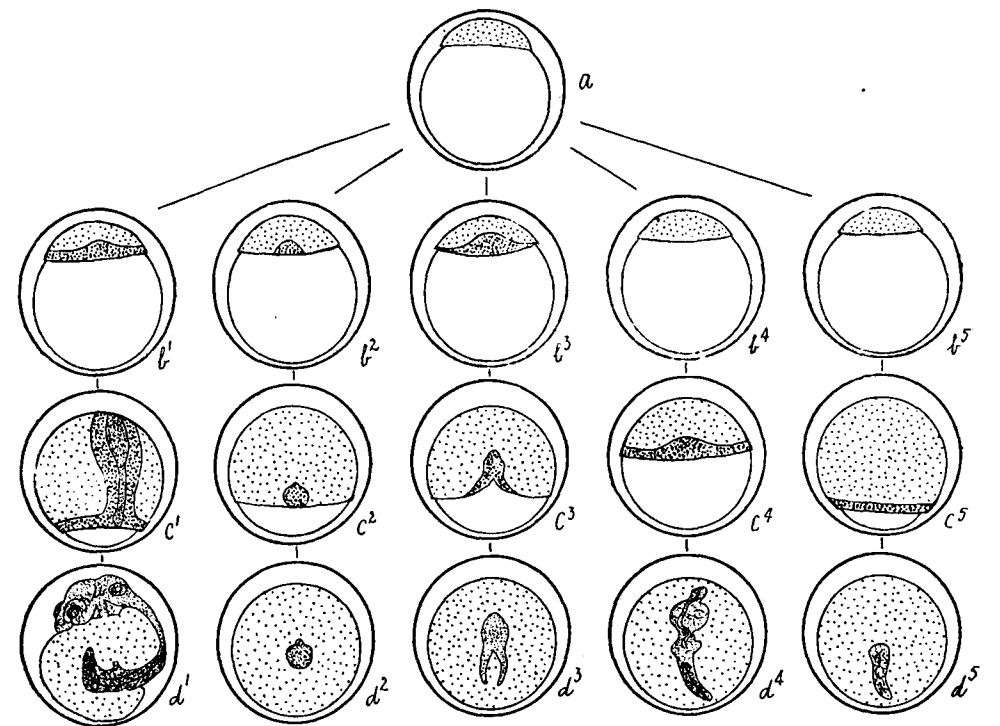
Externally it appears in the form of a small rounded raised protuberance without any sign of differentiation. A transverse section through it shows a mass of undifferentiated cells which in some cases are grouped to give the appearance of optic lobes.

Spina bifida: (Fig. 2).

Externally it appears in the form of a head-like structure attached to a bifid trunk. Both externally and in transverse sections the structure shows no differentiation.

Only trunk and tail-like structures: (Fig 3 and 4).

Externally it gives the impression that an embryo is formed which is without the head. The trunk region is sometimes quite extensive and



Text-Fig. 1. Diagram showing the formation of abnormalities.

a, Normal blastodisc at close of segmentation.

b¹, c¹ and d¹, = Normal germ ring and invagination leading to complete embryo formation.

b², c² and d², = Absence of germ ring in most part except at one point anteriorly where head like structure is formed.

b³, c³, and d³, = Germ ring formed anteriorly and laterally but absent posteriorly thus forming spina bifida.

b⁴, c⁴, and d⁴, = Germ ring formation is delayed to a later stage and in the mean time the blastodisc spreads over the yolk for some distance with the result that a structure is formed which is without the head.

b⁵, c⁵, and d⁵, = Germ ring formation is delayed to a very late stage when the expanding blastodisc reaches the animal pole and hence only tail-like structure is formed.

shows clearly a large beating heart (Fig. 3). In other cases the trunk region is small and it does not possess a heart (Fig. 4). Transverse section passing through the trunk region shows a nerve cord, two structures similar to pronephric tubules, a gut-like organ and a heart. The rest of the space is filled with undifferentiated cells. None of the sections show any trace of notochord. Sections of the tail show only undifferentiated cells which are covered over by a thin epithelium.

Tail-like structure only: (Fig. 5).

This abnormality is a tail-like structure without any trunk and head regions. A faint superficial metamerism is visible. The sections passing through the tail show no sign of differentiation. Nerve cord and notochord are absent and the entire inner mass is made up of undifferentiated cells which are covered by a thin epithelium.

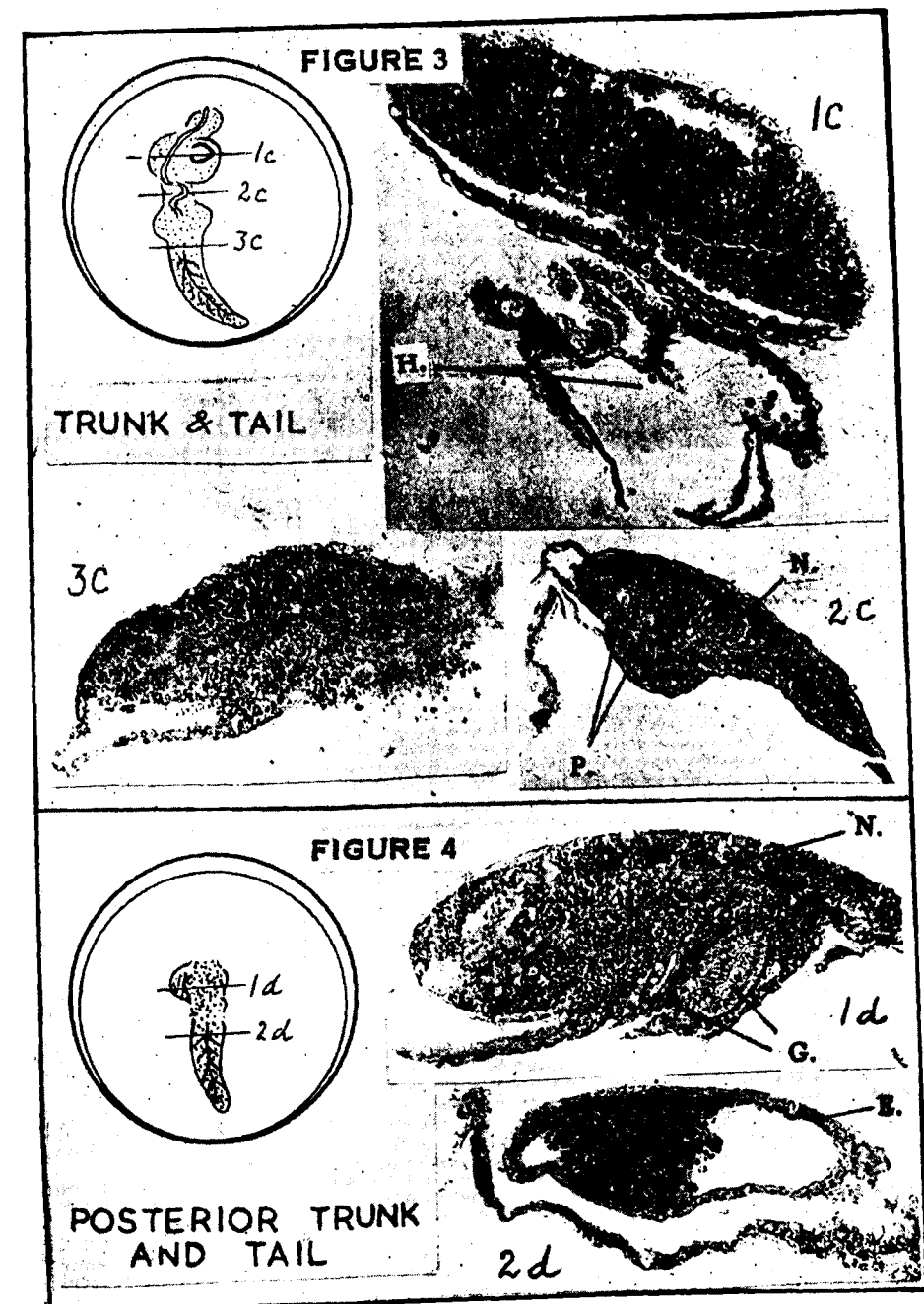
Discussion

The author has described the process of gastrulation in this fish (Swarup 1958). The blastodisc at the animal pole expands to cover the yolk and its margin thickens to form the germ ring. In-rolling of the margins of the blastodisc occurs round the circumference, but goes on most rapidly at one point where the embryo is formed. As the blastodisc covers more and more of the yolk mass, the processes of cell movements, inductions and differentiation proceed in a synchronised manner and result in the formation of a complete embryo. In other words it may be said that the two processes of epiboly and of gastrulation are interdependent in time and space in the egg's history for the normal formation of an embryo. If by any agency this relation is disturbed, malformations are created.

For the understanding of the present abnormalities a morphogenetic field radiating from the animal pole has to be postulated. Now, if the process of invagination is delayed, which actually happens in the treated eggs, the epibolic process does continue. In such an event the period of delay in invagination would determine the position of the point of invagination on the egg's surface. The point of invagination would in turn effect the extent upto which the embryonic materials have been invaginated. The decrease in invagination results in a corresponding reduction of the embryo, beginning anteriorly. It is well established that a complete embryo is formed only if the chorda-mesoderm region reaches the rostral head level. If the organiser invaginates only up to the presumptive ear level, an embryo will be formed which is normal only in regions posterior to the rhombencephalon. A still further reduction of invagination leads to a corresponding decrease in anterior structures. In the extreme case the invagination is delayed until the whole yolk except

Swarup

Plate 2



Figures 3 and 4. Diagram and sections of embryo with only trunk and tail-like structures. Mag. 162X.
E = Epithelial covering; G = Gut;
N = Nerve cord;
P = Pronephros; H = Heart.

the vegetative pole is covered by the growing blastodisc. A tail blastema only, without any trace of head or trunk regions, will develop.

In the above explanation it is considered that a complete germ ring is formed though late in relation to epibolic process. What would happen if the formation of germ ring remains incomplete? The invagination beginning at one point of the edge of the blastodisc does sometime encounter great difficulty in spreading equally around the whole egg with the result that the germ ring is incompletely formed. The incomplete formation of the germ ring however does not inhibit the overgrowth of the blastodisc over the yolk surface. The result is that the edges of the blastodisc approach each other very closely at the vegetative pole without actually forming a true blastopore. Moreover the prospective embryonic materials cease to roll in with the result that two abnormalities are formed. If only the dorsal edge of the blastodisc invaginates, a structure similar to the embryonic forebrain (prosencephalon) develops. On the other hand if the lateral edges of the disc invaginate as well as the dorsal, an undifferentiated head-like structure with a bifid trunk is formed.

Presence of undifferentiated tissue and various degrees of deficient organogenesis as is noticed in these abnormal embryos can be readily explained on the basis of weak secondary inductions as some of the primary organisers are absent in such cases.

Summary

1. Gastrulation abnormalities appear when freshly fertilized eggs of *G. aculeatus* are subjected to high or low temperatures.
2. The abnormalities noticed are the formations of (i) only anterior part of head, (ii) spina bifida, (iii) only trunk and tail-like structures, (iv) tail-like structure only.
3. It is argued that the processes of epiboly and invagination are interdependent in time and space in the eggs' history for normal formation of an embryo and if any agency disturbs this relationship abnormalities are created.

Acknowledgements

It is pleasure to thank Sir Alister Hardy, Linacre Professor of Zoology and Comparative Anatomy and Dr. M. Fischberg, Jenkinson Memorial Lecturer in Embryology for providing me facilities and guidance at the University of Oxford.

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Abnormal Development in the Temperature Treated Eggs of *Gasterosteus aculeatus* (L.); III. Twinning

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With 1 Text-Fig.

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Introduction

This article is in continuation of my previous papers (Swarup 1958, 1959) on the abnormal development of *Gasterosteus aculeatus*. Occurrence of double monstrosities in vertebrates is fairly well known in nature. Since such abnormalities throw light on the developmental processes involved, various workers have tried to produce twinning by experimental means. In the present work twins were produced when the freshly fertilized eggs were subjected to temperature treatment.

Material and Methods

G. aculeatus is a small fresh water fish; its eggs measure 1.2 to 1.7 mm. in diameter and the young ones hatch out in about a week's time at 18-19°C.

Freshly fertilized eggs were given temperature treatment by immersing them in water of the requisite temperature.

Observations

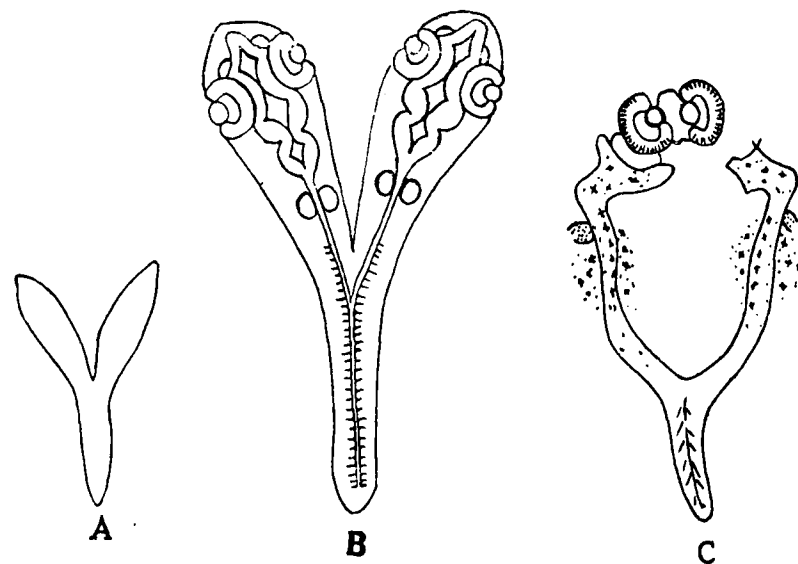
The temperature treatments which were most successful, considering the viability of the eggs, were as follows:—

Heat Treatment: 32.5 to 37.0°C. for a duration of 5 minutes.

Cold Treatment: 0°C. for a duration of $\frac{1}{2}$ to 3 hours. The heat treatment did not produce twinning though other types of abnormalities were common. Twinning was however produced in the cold treated eggs. Out of the 6063 eggs treated, 1597 started gastrulation, and 1271

formed embryos. On an examination of the embryos it was found that 3 of them showed duplication abnormalities. All the three cases were of axial duplication (twinning) and no case of lateral duplication (poly-melia or polydactyly) was observed.

The twinning observed involve only doubling of the anterior parts of the embryo and there is complete fusion in the posterior regions. Of the three twins, one pair remained undifferentiated without any sign of organogeny (fig. 1A) and died very early; one reached an advanced embryonic stage and developed normal heads with well differentiated brains.



Text-Fig. 1. Diagrams of twins; A, twins lacking morphogenesis, B, symmetrical twins, C, twins with deficient organogenesis.

and eyes (fig. 1B). The third on the other hand also reached an advanced stage but was very deficient (fig. 1C) with one tail, two deficient pigmented trunks each with a heart, almost or entirely lacking cephalic structures and with two abnormally situated pigmented eyes.

Discussion

In literature, various causes for twinning are given. Hertwig (1892) forwarded the theory that polyspermy causes twinning or polyembryony. It has since proved erroneous because in certain cases even double fertilization results in unitary organisation (Goldschmidt and Katsuki 1928; Rothenbuehler *et al* 1951). Moreover it has been proved that in amphibian eggs, such characteristics as polarity, bilaterality and other spatial relationships are determined even before fertilization. (Harrison 1945).

Spemann's (1901, 1902) well-known constriction experiments on the cleaving amphibian eggs have produced twins. It is thought that in these experiments the chordomesoderm material of the future embryo splits during gastrulation and thus causes the formation of twins. A very interesting case is reported by Schultze (1894). Twins are produced when the frog egg at the two cell stage is compressed between glass plates and turned upside down. A possible explanation is provided by suggesting that the heavy yolk, which sinks from the vegetable to the animal pole divides the archiplasm into two separate concentrations each of which is competent of embryo formation. Whatever the reason, it is clear that some internal dislocation and rearrangement of cytoplasmic constituents produce double monsters. Pasteels (1938, 1940) who has greatly extended this work interprets the facts on the basis of a double gradient concept.

That multiple centres of induction can arise and lead to the formation of twins is shown by Holtfreter (1933), Pasteels (1948) and Witschi (1952). Holtfreter's blastulae gained secondary inductive capacity when they were injured by freezing, boiling or dehydration. Pasteels, using centrifugation, induced self differentiation in ventral ectoblast which formed neural and mesodermal structures. Witschi who obtained twins in overripe eggs assigns the cause to physiologic fragmentation of the archiplasm and activation of latent inductor capacity.

In the present experiments it is possible that the temperature shock inhibited the development of a normal distribution of the presumptive areas and that due to this two centres of induction were formed on the germ ring. An indication of an abnormal distribution of the presumptive materials is given by the observation that the blastodisc in treated eggs was often less high and had a broader base than the blastodisc in control eggs. The structure of the twins formed here suggests that the two centres of induction were near each other and that the distance between them narrowed gradually during epiboly. The formation of a single tail is linked with the presence of a single blastopore.

Summary

1. Twins are produced when the freshly fertilized eggs of *G. aculeatus* are subjected to a temperature of 0°C.

2. Of the three twins produced one remained undifferentiated; the other reached an advanced stage with well developed brain and eyes while the third which also reached an advanced stage was very deficient. All the three twins died before hatching.

3. The temperature shock probably caused the fragmentation of the presumptive areas and due to this two centres of induction were formed on the germ ring.

Acknowledgment

It is a pleasure to thank Sir Alister Hardy and Dr. M. Fischberg of the University of Oxford for facilities and help during my stay at Oxford.

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Glycogen Content During the Development of *Pila virens* (Lamarck)

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(Communicated by Prof. R. V. SESHAIYA, F.Z.S.I.)

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Introduction

There have been several investigations on the glycogen content during development in vertebrates. The glycogen content of chick embryos and tissues has been studied by Sakuragi (1917), Idzumi (1924) and Murray (1925) (cited by Needham, 1931), Lee (1951), Donhoffer (1933) and by other authors, Konopacki and Konopacka (1926) (cited by Needham, 1931), Gregg and Pomerat (1942) and Tanaka (1934) have investigated the glycogen content and distribution of glycogen in the embryos. Heatley (1935) has studied the distribution of glycogen in the regions of amphibian gastrula by microchemical methods. In contrast to these several studies, the glycogen content during the development of only few invertebrates has been investigated.

The presence of glycogen in the sea urchin egg and its utilisation during development have been investigated by Ephrussi and Rapkine (1928). Lindberg (1945) investigated the metabolism of glycogen in the fertilization of the sea urchin egg. Orstrom and Lindberg (1940) have studied glycogen in *Paracentrotus lividus*. Raven (1946) in his studies on the chemical embryology of *Limnaea stagnalis*. L has investigated the glycogen distribution in the early stages of development. The present study relates to the glycogen content and its distribution in the different stages of the apple-snail *Pila virens* by different methods.

Material and Methods

Eggs were obtained from the laboratory aquaria in which adult animals had been kept alive. The embryos were removed from the eggs

and taken immediately for analysis. Hundred embryos were taken for each determination.

For determining glycogen in the early stages of development colorimetric micro-method described by Kemp *et al* (1954) was employed and the results are calculated in micrograms. For later stages Pfluger's method modified by Good *et al* (1933) was employed and the results are calculated in milligrams. The distribution of glycogen in the early stages of development was determined by adopting Bauer-Feulgen technique. The glycogen content in the sections specifically stained by Bauer-Feulgen method was determined cytophotometrically. The amount of light transmitted through the section was measured by means of Photovolt electronic colorimeter Model No. 512. The image of the section was projected at a magnification of 100 into the search unit of the photometer. A green Wratten filter was employed to accentuate absorption by the fuchsin stain. The illumination was so arranged that the photometer scale recorded 100 when the light was passing through a clear region of the slide with sections. The selectively stained region of the section was then interposed and the degree of light absorption recorded. Six readings from the different parts of the section were taken for each stage. The average value for light absorption by the sections exhibiting no glycogen detectable by the eye was calculated—and this was deducted from the average value relating to the glycogen containing portions of the sections. From the values thus obtained the mean was calculated as a measure of glycogen content.

Observations

Distribution of glycogen.

In the early oocyte there is no trace of glycogen. In the mature oocyte the glycogen granules are confined to the central region of the cytoplasm, the cortical region being entirely free of glycogen. Immediately after the egg is laid, a few scattered granules are found in the ovum. At the time of fertilization the glycogen granules are densely distributed both in the cortical and central regions of the egg. Later, during gastrulation the ectoderm, endoderm and mesoderm cells—show glycogen and in the early veliger the embryonic shell-gland which is in the form of an ectodermal invagination shows conspicuously the presence of glycogen.

Quantitative observations.

Before fertilization, the glycogen content is low. At the time of fertilization the glycogen content rises to 50 μ g. per 100 eggs as determined by colorimetric method. During segmentation stages, the glycogen content suddenly declines to 10 μ g. per 100 eggs.

Cytophotometric determinations also give the same trend of rise and decline of glycogen content. In terms of optical density the values are

8.3 before fertilization, 53.0 for the fertilized egg and 32.6 during segmentation. The endoderm and mesoderm cells in the gastrula stage have a higher content than the ectoderm cells. The values are 46.8, 52.6, and 41 for the endoderm, mesoderm, and ectoderm cells respectively. Later in the early veliger, the cytophotometric value for the shell gland is 54.1 whereas for ectoderm it is only 42. After segmentation the glycogen content per 100 embryos rises to 3970 μ g. at 134 hrs; at 192 hrs. there is a fall and thereafter there is a rapid rise, increasing to 4107 μ g. at 360 hrs. of development. The data relating to glycogen content calculated in terms of unit weight show a progressive diminution of glycogen after 192 hrs. of development, the values being 0.051 gm. at 192 hrs. and 0.019 gm. at 360 hrs. of development. The cytophotometric data relating to glycogen content in the early stages of development closely correspond to the results obtained by other methods.

Discussion

Bretschneider and Raven (1951) found an uniform distribution of glycogen throughout the egg in *Limnaea*. In *Limnaea*, Raven (1946) noticed an accumulation of glycogen at the 24-cell stage in the central protoplasm. At a 70-cell stage the cells 4a, 4b, and 4c (but not 4d) are rich in glycogen. In the gastrula stage glycogen is present at the animal pole mainly around the polar depression and the cells in the vegetal pole are rich in glycogen. The mesoderm cells and endoderm cells are poor in glycogen. In the gastrula of *Pila* the glycogen concentration is high in the endoderm and mesoderm cells—unlike what Raven found in *Limnaea*. In the egg of *Helix* May and Weinland (1953) found galactogen in considerable amount but not glycogen.

Orstrom and Lindberg (1940) demonstrated a fall of glycogen content in the egg of sea urchin *Paracentrotus lividus* immediately following fertilization. This was also confirmed in the eggs of other sea urchins, *Echinocardium cordatum* and *Psammechinus miliaris*. Histochemical studies of glycogen in the frog embryo by Konopacki and Konopacka (1926) and by Brammertz (1913) and Marcus (1906) (cited by Needham 1950) on nematode worm *Ascaris* revealed a diminution of glycogen in the eggs following fertilization. In the present study also the glycogen content of the eggs falls after fertilization from 50 μ g. to 10 μ g. as may be seen from Table I. It is probable that fertilization stimulates the oxidation of the carbohydrates as evidenced by diminution in the amount of glycogen (Brachet 1950).

That glycogen undergoes a diminution in all parts of the embryo during development is clearly established by Brachet and Needham (1935) in *Rana*, Kataoka and Tsunoo (1933) in *Cryptobranchus* and by Takamatsu (1936) in *Hynobius* (cited by Needham, 1950). There are, however, a few reports to the contrary. In the teleost *Salmo salar* investigated by Hayes and Hollett (1940) glycogen is absent at the beginning but increases steadily at the later stages. An increase in the glycogen

content during embryonic development has been reported by Nouvel (1935) in the orthonectid parasites of Ophiuroids, *Rhopalura* (cited by Needham, 1950).

Table I

Table I—Glycogen content during development of *Pila virens* (Colorimetric and chemical methods).

Stage		Glycogen per 100 embryos	Glycogen per gram wet wt.
Fertilized egg		50 μ gm.	—
Segmentation	12 hrs.	10 "	—
Blastula	20 "	35 "	—
Gastrula	24 "	50 "	—
	36 "	65 "	—
	60 "	100 "	—
	72 "	245 "	—
	84 "	295 "	—
	108 "	500 "	—
	120 "	1000 "	—
	132 "	2000 "	—
	144 "	3970 "	0.051 gms.
	192 "	1699 "	0.050 "
	240 "	2450 "	0.038 "
	288 "	4065 "	0.030 "
	360 "	4107 "	0.019 "

The glycogen content per unit weight was calculated for the embryos from 6th day till a day after hatching. For these stages the results are therefore expressed both as per 100 embryos as well as per unit weight of embryo. It is presumed that the increase in weight in the earlier stages of the embryos was not significant. A decrease in glycogen content per unit weight is noticed in *Pila virens* from sixth day onwards till a day after hatching. The results are in accordance with the findings of Brachet and Needham (1935). Kataoka and Tsunoo (1933) and Takamatsu (1936) in other embryos. The decrease in terms of unit weight, as seen from Table I is evidently due to the functioning of the different organs resulting in the utilisation of glycogen.

That glycogen and phosphatase are simultaneously present in regions which calcify has been borne out by the observations of Glock (1940), Horowitz (1942). Glock (1940) found that glycogen in primary centres of

long-bone ossification (in rats) increases from the late foetal stage to the tenth day of post-natal life and then falls off. Horowitz (1942) has stated that in foetal heads, only tissues which calcify have both alkaline phosphatase and glycogen.

The dense accumulation of glycogen granules in the early veliger stage of *Pila* implies an active centre for shell secretion. A high phosphatase activity has also been observed by the author (1959) in the shell gland region.

Summary

1. The glycogen content and distribution during the development of *Pila virens* have been determined employing cytochemical, cytophotometric and chemical methods.

2. Following fertilization of the egg there is a fall in the glycogen content.

3. The shell gland is the site of high glycogen accumulation in the early veliger stage.

4. There is an overall increase of glycogen content when calculated per 100 embryos and there is a decrease when calculated per unit weight of embryo during development.

Acknowledgments

My thanks are due to Prof. R. V. Seshaiya, Director and Professor, Marine Biological Station, Portonovo for suggesting the problem and for guidance, and to the Government of India, for the award of a senior research scholarship.

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Sex Chromosomes in Melaniid Snails. I. *Paludomus tanschaurica* (Gmelin) (Prosobranchia : Gastropoda)

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With 7 Text-Figures.

(Communicated by Prof. R. V. SESHAIYA, F.Z.S.I.)

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Introduction

Coe (1944) has remarked that "little is known regarding the physical hereditary basis of sexuality in Molluscs, for only a few doubtful examples have been reported. Perrot (1930)." Coe's remarks apply mainly to the pulmonate molluscs which are hermaphrodite. The chromosome cytology of only a few molluscs has so far been studied, for this group presents technical difficulties for proper investigation. With regard to the prosobranchs, which are dioecious, sex chromosomes have been reported in only thirteen species. Among the more important investigations relating to the sex-determining chromosomes in prosobranchs. mention may be made of the works of Kuschakewitsch (1913, 1921), Bataillon (1921), Hyman (1923), and Alexenko (1929). However, very little attention has been paid to these early works and our knowledge of the question of the hereditary basis of sexuality in dioecious molluscs is still far from complete.

The present account which is in continuation of the earlier studies of Melaniidae by the author (Jacob 1954, 1957, 1958, 1959) relates to the identification and behaviour of sex chromosomes during the maturation of the male germ cells in the prosobranch mollusc *Paludomus tanschaurica*.

Material and Methods

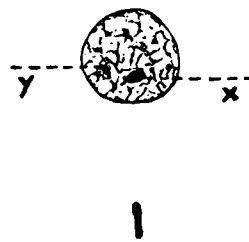
Specimens of *Paludomus tanschaurica* were collected from streams chiefly with sandy bottom and kept in the aquaria. For fixation of the

testis, Bouin's fluid, Carnoy's 1:3 acetic alcohol, San Felice's fluid Flemming's and La Cour's fixatives were used. For staining sections of material fixed in Bouin, Flemming and La Cour, Heidenhain's iron-alum Haematoxylin was employed. Gentian violet also gave good results. Sections of material fixed in San Felice were stained in Feulgen after a preliminary hydrolysis of seventeen minutes in N HCl at 58-60°C. For Carnoy fixed material the hydrolysis was for twelve minutes. Sections were cut at varying thicknesses from 8 to 14 μ .

Acetocarmine and Feulgen squashes were also prepared and proved very useful particularly for the diplotene and diakinetik stages.

Observations

The resting nuclei of the primary spermatogonia and the early primary spermatocyte are very small and do not differ markedly in size. In the resting primary spermatocyte nucleus, two strongly Feulgen-positive bodies of very unequal size may be seen (Fig. 1) amidst irregular strands of chromatin which stain very faintly with Feulgen.



Text-Fig. 1. A resting primary spermatocyte nucleus.

Leptotene. The leptotene nucleus is represented in Fig. 2 and the chromosomes do not show any polarisation or bouquet orientation. Notwithstanding the small size of the nucleus, a thick, looped and positively

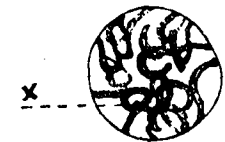


Text-Fig. 2. A leptotene nucleus.

heteropycnotic chromosome can easily be recognised. A second minute heteropycnotic element can also be made out as in the figure, but only after a careful examination of favourable preparations.

Zygotene. The pairing of homologous chromosomes is normal and complete. In the zygotene nucleus the larger of the two heteropycnotic elements is easily recognised as lying peripherally and close to the nuclear membrane. It appears as a stout curved body (Fig. 3) but the

smaller and minute heterochromosome cannot be made out with certainty amidst the mass of the zygotene bivalents which traverse the nucleus in all directions. However, it is definite that the two heterochromosomes are not associated.



Text-Fig. 3. A zygotene nucleus.

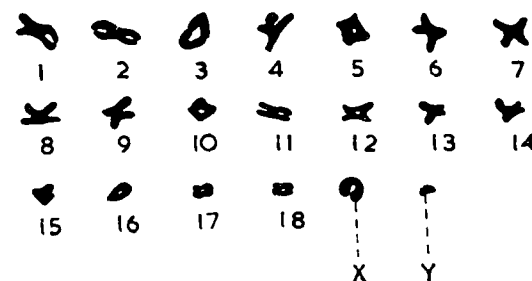
Pachytene. The closely intertwined pachytene bivalent lie irregularly in the nucleus and the bouquet orientation so characteristic of many species of animals is entirely wanting in *Paludomus tanschaurica*. Compared to what was observed in the previous stage, the larger heterochromosome which may be referred to as the X chromosome, now shows further increase in length as well as thickness, but it continues to be situated at the periphery close to the nuclear membrane (Fig. 4). The smaller heterochromosome could not be made out in this stage amidst the rather thick pachytene bivalents, only a few of which are represented in the figure.



Text-Fig. 4. A pachytene nucleus.

Diplotene and Diakinesis. These stages are best studied in acetocarmine squashes. The pachytene threads contract and split resulting in the diplotene bivalents with chiasmatal configurations. In the diakinetik stage the bivalents are characteristically distributed at the periphery of the nucleus, more or less evenly spaced and equidistant from one another. The chromosome complement shows eighteen bivalents and in addition the large and the small heterochromosomes. This corresponds to the eighteen pairs of autosomes and the two highly unequal chromosomes found in the spermatogonial metaphase of this species (Jacob 1959). In Fig. 5 is shown the entire chromosomal

complement of a diakinetid nucleus which consists of four large, eleven medium and three small bivalents besides the large X and the small Y. The latter two always remain as separate univalents. During the diakinetid stage the nucleination of the autosomal bivalents has proceeded to a great extent and they stain as deep as the heterochromosomes.

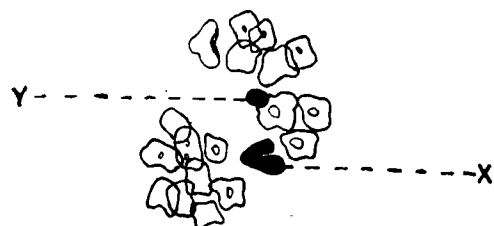


Text-Fig. 5. The diakinetid chromosomes drawn serially in order of size.

5

I Metaphase. The X and Y chromosomes, by their distinctive shape and extreme inequality in size, stand out markedly from the autosomal bivalents (Fig. 6). An interesting feature which characterises the X and Y in I metaphase is their slightly negative heteropycnosis in contrast to their positive heteropycnosis in the prophase stages. This is therefore an instance of reversible heteropycnosis. Hyman (1923) obtained in *Fasciolaria* a similar phenomenon when the X chromosome at pachytene stained less deeply than the other chromosomes, but he did not actually interpret this as a possible instance of negative heteropycnosis.

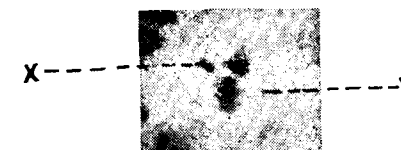
Text-Fig. 6. I metaphase plate, polar view. The univalent sex chromosomes (large X and small Y) are shown in black while the autosomal bivalents are represented in outline.



6

I Anaphase. The heterochromosomes do not have any characteristic position on the metaphase plate. But in the I anaphase they lie on either side of the equatorial plate close to the poles of the spindle. In Fig. 7 it may be noted that they have actually moved to the opposite poles far in advance of the disjoining halves of the bivalents. This instance of the precession of the heterochromosomes was first noticed by Seshaiya (1938). The marked difference in size between the two hetero-

chromosomes is clear even when they lie on the spindle. The anaphasic disjunction of the autosomal bivalents is smooth and normal.



Text-Fig. 7. I anaphase showing the precession of X and Y to the opposite poles.

7

It was shown that during the I metaphase stage the heterochromosomes are not at all paired, in fact they were never paired and showed no tendency to pair in any stages of meiosis. Their distribution in I anaphase is however quite regular, one lying on each side of the equator and close to the poles as stated earlier. Even an accidental passing of the two heterochromosomes to the same pole was never observed.

Further stages. The first maturation division is reductional for the heterochromosomes as for the other chromosomes. The identity of the heterochromosomes is lost at the I telophase and in later stages. The secondary spermatocytes do not pass into a typical resting or interphase condition but pass into the II metaphase as soon as formed.

Discussion

The characteristic staining property of the sex chromosomes, as Wilson (1911) pointed out, is helpful in identifying and tracing them from one generation to another even through the resting stages. Heteropycnosis of the sex chromosomes is a fairly wide spread phenomenon in the male meiotic phase of animals. In groups like the Heteroptera almost all species show heteropycnosis of the sex chromosomes. The behaviour and staining properties of the distinctive heterochromosomes during spermatogenesis in *Paludomus tanschaurica* leave no doubt regarding their identity as sex chromosomes. Further, a study of the spermatogonial chromosomes also showed the presence of the highly unequal pair of chromosomes besides the eighteen pairs of autosomes (Jacob 1959).

It has generally been maintained since the works of Heitz (1933), Muller and Painter (1932), White (1940, 1941) that heteropycnosis is associated with genetic inertness. In *Paludomus* the sex chromosomes must then be largely or wholly inert genetically since they contain no cytologically detectable chromatin other than heterochromatin. They are therefore probably concerned only with the genetic determination of sex and no other morphogenetic process.

The absence of pairing between X and Y and their regular segregation in spite of the lack of any association between them call for some special remarks. At the I metaphase stage they are seen as separate

bodies and their behaviour on the spindle might therefore be expected to be random, either each going to opposite poles or both going to the same pole. The association of the autosomes at I metaphase and their subsequent segregation are normally conditioned by the pairing and chiasma formation in the meiotic prophase. However, in the present instance the X and Y do not pair and yet show, not random, but regular and unfailing segregation to the opposite poles at the I division. White (1940) stated that in the multiple sex chromosomes of Arachnida the X^1 and X^2 which are homologous throughout their length are prevented from forming chiasmata by their heteropycnosis. He (White 1933) also found absence of pairing and chiasma formation between the two heteropycnotic X chromosomes in the tetraploid spermatocytes of *Schistocerca*. McNabb (1928) found that two X's are not heteropycnotic in the oocytes where they regularly pair and form chiasmata. The absence of heteropycnosis in the X chromosome of the female is regarded by White (1940) as an adaptation permitting the formation of a bivalent. Heteropycnosis in the female would be a barrier to chiasma formation. Pairing and crossing over between X and Y chromosomes are known in Mammalia, Diptera, etc. Koller (1936, 1937, 1938), Koller and Darlington (1934), Painter (1923, 1924), Perje (1948) and White (1940, 1941, 1954). But in such cases it has been pointed out that there are pairing or homologous euchromatic regions, though minute, and crossing over occurs in them. This would strengthen the assumption that heteropycnosis prevents chiasma formation. However, Pontecorvo (1943) claims that in mammals formation of chiasmata is confined to the regions that are positively heteropycnotic at pachytene. But Makino (1951) holds that the XY complex of the different species of field mice is due to "heterochromatic cohesion" and that chiasmata play no role in holding together the sex chromosomes. Smith (1952) points out that we have no evidence for regarding the physico-chemical constitution of heterochromatin as fundamentally different from that of euchromatin. If so, it would seem that there is no intrinsic barrier to crossing over within and between two segments that are respectively euchromatic and heterochromatic, provided that they pair with precision at zygotene. The behaviour of the sex chromosomes and supernumerary chromosomes (White 1933, McNabb 1928, McClintock 1933, and Ostergren 1947) seems to show that the capacity of the heterochromatic chromosomes to pair is dependent on to what degree they are diffusively distributed at zygotene and pachytene stages. In the present instance the X and Y are very condensed and appear as massive or solid structures. Their deep staining with Feulgen even from the early stages indicates that they are heavily 'charged' with nucleic acid and probably tightly spirals. The absence of pairing of X and Y might therefore be attributed to these features.

The segregation of X and Y without their ever coming together may be regarded as an example of what White (1940) has described as "determinate disjunction" in Neuroptera and Heteroptera and which Darlington (1937) has termed "distance conjugation". Similar peculiar beha-

viour of the sex chromosomes is known in some other instances as well. An example of "distance conjugation" in Neuroptera has been reported by Naville and de Beaumont (1933). Cooper (1941) showed that in *Melophagus* which possesses an X and Y, these move regularly to opposite poles without previous conjunction. Payne (1910) and Troedsson (1944) have reported that the five components of compound X in *Acholla* go to one pole while the single Y goes to the opposite pole, in spite of the absence of synaptic phenomena. Such instances of the behaviour of the sex chromosomes where one chromosome (multiple or single) is supposed to determine the disjunction of another at either the first or second meiotic division without there being any connection or association between them raise doubts on the validity of the generally accepted assumption of the classical hypothesis of meiosis i.e., that reduction is dependent upon the earlier pairing and subsequent crossing over of parts of chromosomes. An understanding of the forces that govern the behaviour of these sex chromosomes may then be expected to lead to a better understanding of the fundamental nature of meiosis.

Another interesting aspect of the X and Y chromosomes in *Paludomus tanschaurica* is what appears to be a cyclical reversibility of heteropycnosis which was described earlier. Reversible heteropycnosis has been observed by White (1940) in the X chromosomes of Acrididae, by Ohmachi (1935) in Gryllidae (cited by White 1940) and quite recently by Ohno *et al* (1959) in the male rat *Rattus norvegicus*.

The extreme inequality of the X and Y chromosomes and their entirely heteropycnotic nature would seem to indicate an advanced type of sex mechanism in this snail. But in view of the paucity of investigations on the sex chromosomes of Prosobranchs it is not possible to discuss their evolution, although in the light of the present investigation and the findings of previous investigators it may be concluded that the male is the heterogametic sex in Prosobranchs.

Summary

1. The X and Y chromosomes in *Paludomus tanschaurica* have been traced from the resting primary spermatocyte to the secondary spermatocyte except in the case of Y which in zygotene and pachytene was obscured from view owing to its extreme small size and the crowded nature of the nuclei.

2. The diploid chromosome number as determined from a study of the meiotic stages is thirty eight ($36 + X + Y$) and this number corroborates that obtained from spermatogonial metaphase.

3. The first maturation division is reductional for the sex chromosomes and they precede the autosomes to the poles.

4. The sex chromosomes are entirely heteropycnotic and therefore are probably genetically inert except for the determination of sex.

5. The regular segregation of X and Y at the first meiotic division appears to be an instance of determinate disjunction.

6. The phenomenon of reversible heteropycnosis has been observed in the sex chromosomes.

7. The sex formula for the male is XY. In the light of the present study and the investigations of the earlier workers, it is concluded that the male is the heterogametic sex in Prosobranchs.

Acknowledgments

I have great pleasure in recording my grateful thanks to Professor R. V. Seshaiya, Director, Marine Biological Station, for suggesting the problem and for guidance. I am specially thankful to Professors A. D. Peacock and R. A. R. Gresson and Dr. Ann R. Sanderson for reading through. To the Government of India my thanks are due for the award of a research scholarship which enabled me to pursue this investigation.

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Explanation to Figures

Text-Figures 1 to 5 are camera lucida drawings from San Felice-Feulgen sections and magnified to X ca 2800. Text-Fig. 6 is also a camera lucida drawing but from an acetocarmine squash and magnified to X ca 1600. Text-Fig. 7 is a photomicrograph from a Bouin-Heidenhain iron-alum Haematoxylin section and magnified to X ca 1500.

Macronuclear Reorganisation in *Paramecium multimicronucleatum* (Powers & Mitchell)

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The separation of the nuclear apparatus into a micro and a macronucleus with accompanying differences in size, shape, function and mode of division is characteristic of all euciliates. The macronucleus fragments, degenerates and disappears during conjugation and it is subsequently re-formed from one of the products of the synkaryon. In the degenerating fragments the DNA content progressively diminishes and in later stages the fragments do not respond positively to any stain. Sonneborn (1947), however, was able to obtain fully viable clones of *Paramecium aurelia* in which all the macronuclear material was derived from the fragments of the old macronucleus of the exconjugants. In these cases the macronuclear anlagen was experimentally destroyed by exposing the organisms to higher temperatures. On the basis of these observations Sonneborn (1947) developed the view that the ciliate macronucleus is compound consisting of many discrete, probably diploid units or sub-nuclei.

While examining cultures of *Paramecium multimicronucleatum*, obtained locally, a number of individuals showed fragmented polymorphic macronuclei, some of them resembling stages which Diller (1936) refers to as hemixis. Stranghöner (1932) observed fragmentation of macronucleus in the present species during endomixis. Wichterman (1953) observed a similar fragmentation of the macronucleus in cultures of *Paramecia* and doubts whether these individuals are normal physiologically. This paper is an attempt to ascertain the fate of such individuals.

Re-isolation cultures of *Paramecium multimicronucleatum* grown in hay-infusion incubated at 37°C for 24 hour was satisfactory. Four lines were built from a pair of ex-conjugants, and immediately after every fission the daughter individuals were isolated and one of them was fixed and stained for cytological examination. The fission rate was high in the early days of the cultures but reached a steady state of 2.2, (averaged for 5 days) after a few days. The cultures were all dead after 230 generations extending from January 20th to March 18th, 1956, and no autogamy

or endomixis occurred in the cultures. The variations in the fission rate is in no way related to the regeneration process described below.

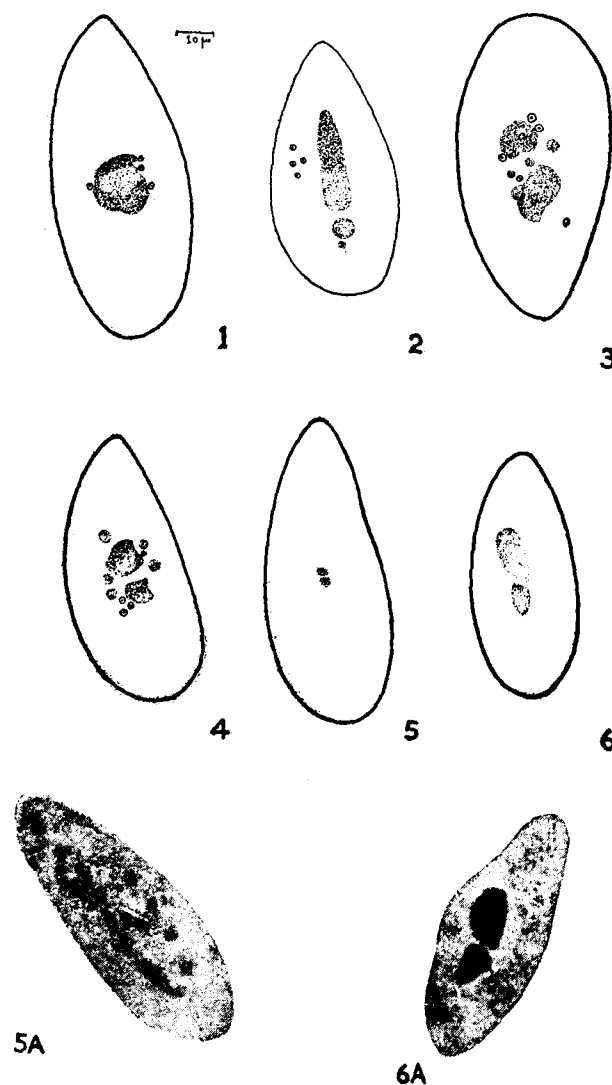
Of the four lines only the fourth one yielded interesting results and hence will be dealt with in some detail. One of the fission products of 114th fission was very slim and slowly creeping at the bottom of the culture dish, unlike its partner which was very active. Cytological preparation of the former revealed that a normal macronucleus was absent and instead there were only two small Feulgen positive bodies (Fig. 5 & 5 a). There was no trace of micronuclei.

Sub-lines derived from the fourth line were studied in detail and as many as 18 *Paramecia* were found amiconucleate containing only the two Feulgen positive bodies already referred to. By sacrificing one sub-line, it was noted that the normal sister individual does not show any macronuclear abnormalities. Five of the abnormal *Paramecia* were studied with regard to their subsequent behaviour. A macronucleus developed normally from each of two Feulgen positive bodies (Fig. 6 & 6a) and the two macronuclei segregated at the next division. There was no trace of the micronucleus. Three macronuclear regenerants underwent fission normally.

These observations lend support to the view that the macronucleus of *Paramecium multimicronucleatum* is composed of a number of sub-nuclei, but does not go so far as to demonstrate the composition of the sub-nuclei. It is possible that each fragment may contain more than one sub-nucleus. Kimball (1953) believes that the macronucleus of *Paramecium aurelia* as highly polyploid but not organised into sub-nuclei. Sonneborn (1947) on the basis of regeneration experiments with heterozygotes, coupled with genetic observations suggests a sub-nuclear organisation of the macronucleus. It is further supported by Moses' (1950) evidence that there is 40 times as much DNA in the macronucleus as in the micronucleus of *Paramecium caudatum* and by Nanny's work on mating types as given Sonneborn (1951). The probability of the sub-nucleation of the ciliate macronucleus is, however, implicit in the numerous merotomy experiments carried out by various investigators, (Weisz, 1951). Further work will probably clear the conflicting views existing in the field.

The present observations are also interesting from a different point of view. We have not seen any evidence of nuclear reorganisation taking place either by endomixis or autogamy. Examination of a number of cytological preparations has revealed extrusion of bodies from the macronucleus but these were never in significant numbers. (Figs. 2, 3 and 4). These bodies appear to be the result of irregularities in the contraction

of the macronucleus following fission. In a few instances the macronucleus was broken into two or three large bodies and in all such cases, the micronucleus was normal. Diller (1936) observed fragmentation of



Explanation of Figures

- Fig. 1. *Paramecium multimicronucleatum*, normal vegetative individual.
 Fig. 2. Fragmentation of the macronucleus during contraction following fission.
 Figs. 3 & 4. Various numbers of rounded extruded chromatin material from the macronucleus. The macronucleus is broken into two large bodies.
 Figs 5 & 5A. Amicronucleate form showing two small Feulgen positive bodies.
 Figs 6 & 6A. A later stage of the form shown in Fig. 5, showing regeneration of macronucleus from the small Feulgen positive bodies.

the macronucleus in *Paramecium aurelia* unaccompanied by micronuclear activity. He called this process *hemixis* and maintained that it is a result of the precocious division of the macronucleus during binary fission. A series of stages showing the macronucleus progressively extruding rounded chromatin bodies of variable numbers and sizes have been figured by him. These bodies are described to segregate passively during the subsequent fissions and ultimately to disintegrate. Diller (1936) calls the entire process as one of purification of the macronucleus.

It is probable that the two small Feulgen positive bodies (Fig. 5) in our preparations may represent the extruded bodies from the macronucleus, which have got segregated owing to irregularities of fission. If this is true it would imply that, the chromatin bodies extruded from the macronucleus, under certain circumstances, have the potentiality to regenerate the entire macronucleus. It is, however, difficult to explain as to why only two such bodies were present in all our preparations.

In one of our sublines in which macronuclear extrusions were noted, conjugation readily occurred when there were only eight individuals in the culture dish. It may be recalled that these are descended from a single parent and what prompted this behaviour is not clear. It may be possible, in the light of this unexpected behaviour, that the extrusions are a partial reorganization process of the macronucleus in the absence of conjugation and autogamy.

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Chromosomes of the slug *Oncidium verraculatum* Cuv. (Mollusca : Gastropoda : Pulmonata)

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(Communicated by Prof. R. V. SESHAIYA, F.Z.S.I.)

(With 3 Text-figs)

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Introduction

In a previous communication (Natarajan, 1958) the number and morphology of the chromosomes of three species of Stylommatophorous snails were reported and the present paper deals with the number, morphology and behaviour of the chromosomes of the marine slug *Oncidium verraculatum* Cuv. (Oncidiidae: Stylommatophora). The anatomy, embryology and bionomics of this slug have been described by Awati and Karandikar (1948). Studies on the gametogenesis of various species of slugs made by Gatenby (1918), Watts (1952), and Nath and Chopra (1955) relate mainly to cytoplasmic inclusions without reference to chromosomes. Perrot (1930, 1938) studied the chromosomes of about seven species of slugs belonging to the families Arionidae and Limacidae with reference to the chromosome numbers only. The present study is the first detailed one to record the morphology and behaviour of the chromosomes during spermatogenesis of a slug, especially with reference to features like chiasma frequency, terminalisation etc.

Material and Methods

Specimens of *Oncidium* are available from January to August in the mud flats in the Vellar estuary at Portonovo, South India and large numbers of them were collected at low tide.

The ovotestis, easily recognised by its brown colour, was fixed in Sanfelice's mixture and Carnoy's 1:3 acetic alcohol. These gave very good results. Iron-alum Haematoxylin, Crystal violet and Feulgen were

used for staining the sections. Aceto-carmin and aceto-orcin squashes of the ovotestis, especially after pretreatment with alpha-bromo-naphthol, were found to be of great help for the study of the various stages.

Observations

The number of chromosomes as determined from a study of a number of spermatogonial metaphase plates is $2n=36$. A polar view of one such plate is shown in Text. fig. 1. All the chromosomes are metacentrics showing two arms of equal length and hence with median centromeres. Twelve chromosomes are larger in size and lie towards the periphery of the plate, while the rest are of uniform size and lie near the centre.

During leptotene, the nucleus is traversed in all directions by faintly staining and granular threads. But these get polarised towards one pole during zygotene and thus form a typical bouquet. Subsequently, the polarisation and bouquet formation are completely lost.



Explanation to Figs. Text-figs. 1 to 3. Fig. 1. Spermatogonial metaphase—polar view—aceto-carmin squash. Fig. 2. I metaphase bivalents—aceto-orcin squash. Fig. 3. II metaphase plate.—polar view—aceto-carmin squash. All are camera lucida drawings. X ca 5000.

The calculated chiasma frequency and terminalisation coefficient values during diplotene and diakinesis are given below.

Stage	Number of nuclei	Total number of bivalents	Total number of X-ta.	X-ta per bivalent	Total number of terminal X-ta.	Terminalisation coefficient
Diplotene	10	180	212	1.18	53	0.25
Diakinesis	10	180	186	1.03	152	0.81

At diplotene, the chiasmata are seen very well and the calculated chiasma frequency at this stage is 1.18. The terminalisation coefficient value of 0.25 at this stage shows that only about one-fourth of the total number of chiasmata are terminal. During diakinesis all the bivalents appear smooth and deep-staining. The chiasma frequency is 1.03 and the terminalisation coefficient value of 0.81 indicates clearly that almost 90% of the chiasmata are terminalised by this stage. Thus, the degree of terminalisation is very high in this form between diplotene and diakinesis and almost complete by the later stage.

At the I metaphase stage (Text-fig. 2) all the eighteen bivalents become very well condensed and deep-staining. The six larger bivalents appear more or less as rings while the smaller ones are dumb-bell shaped.

A polar view of a II metaphase stage is shown in Text-fig. 3. Of the eighteen chromosomes, all of which are distinctly two-armed, six are larger while the remaining twelve are smaller in size. The larger ones lie more towards the periphery of the plate while the smaller ones are near the centre.

Discussion

The present study shows that in *Oncidium*, the karyotype consists of 36 metacentric chromosomes, of which six pairs are large and twelve are small. No other member of the family Oncidiidae has so far been studied cytologically. However, Perrot (1930, '38) studied the chromosomes of six species of slugs belonging to the family Limacidae and one species of Arionidae and found that in the former the chromosome number varies between 48 and 62 and in the latter the number is $2n=32$.

As only few species of slugs have been studied cytologically, it is difficult to make any generalisation with reference to karyological relationships. Further, the morphology of the chromosomes has not been investigated so far excepting in the present study. However, the available data indicate that the families Arionidae and Oncidiidae, with diploid chromosome numbers of 32 and 36 respectively, are more closely related to each other than to the family Limacidae, where the diploid chromosome number is much higher (varying between 48 and 62).

Summary

1. The chromosome number of the slug *Oncidium verraculatum* Cuv. is $2n=36$ and $n=18$.
2. All the mitotic chromosomes are distinctly two-armed and with median centromeres.
3. Polarisation and bouquet formation characterise the zygotene stage.
4. The terminalisation coefficient of the chiasmata has a marked increase between diplotene and diakinesis and is almost complete by the later stage.

5. With the available data it appears that the families Arionidae and Oncidiidae are more closely related to each other than to Limacidae.

Acknowledgements

I have great pleasure in expressing my sincere thanks to Prof. R. V. Seshaiya, Professor and Director, Marine Biological Station, Portonovo, for suggesting the problem and for guidance. To Dr. Joseph Jacob my thanks are due for reading through the manuscript. The award of a senior scholarship by the Ministry of Education, Government of India is also duly acknowledged.

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Alkaline phosphatase Activity in the Ovary of some Fish

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Introduction

The phosphatases are vital not only for the formation of bones but also for the metabolism of carbohydrates, nucleotides, and phospholipids (Sumner and Somers 1953). Bradfield (1951) suggested that the phosphatases may be part of the enzyme system involved in the liberation of the newly synthesised protein from a complex with the nucleic acid. The interesting correlation between the phosphatase activity and growth and differentiation was first demonstrated in chick embryogenesis by Moog (1944). The majority of the studies on the alkaline phosphatase activity in animals have been with reference to adult tissues. However, some interesting studies on the developing tissues have also been carried out. Krugelis (1947), Wicklund (1948) Gustafson and Hasselberg (1950) studied alkaline phosphatase distribution in the developmental stages of sea urchin. Fitzgerald (1949) studied the phosphatase activity in the developing grasshopper egg. Moog (1941) as stated already studied the localisation of alkaline phosphatase and acid phosphatase in the early embryogenesis of chick. Kind and Macchi (1952) investigated chemically the phosphatase activity in the tissues of *Rana pipiens*. Krugelis *et.al.* (1952) investigated the phosphatase activity and nucleic acids during the embryonic development of *Amblystoma punctatum* at different temperatures.

Lorch (1949) studied the distribution of the alkaline phosphatase in the skull of developing trout with special reference to calcification and alkaline phosphatase activity. Arvy (1955) has studied the distribution of phosphatase and lipase activity in some teleosts histochemically *viz.* *Perca*

fluviatilis, *Cyprinus carpio* L, *Tina vulgaris* Cuv, *Trutta fario* L, *Trutta iridus* Gibb, *Anguilla anguilla* Turton and *Lebistes teticulatus* Peters.

The present paper gives the results of a quantitative study of alkaline phosphatase activity in the different stages of growth of ovary of three different fishes *viz.*, *Labeo fimbriatus* (Bloch), *Mystus seenghala* (Sykes) and *Boleophthalmus boddarti* (Day).

Material and Methods

The specimens of the two species of fish, *Labeo fimbriatus* (Bloch), and *Mystus seenghala* (Sykes) were collected from the local ponds in the University campus during different seasons of the year. Specimens of *Boleophthalmus boddarti* (Day) were collected at low tide from the Velar estuary at Portonovo.

The ovaries in three clear marked stages of growth, *viz.*, Stage I (immature,) Stage II (maturing) and Stage III (mature) were taken for analysis, adopting Bower's (1954) classification. For the determination of alkaline phosphatase activity in the ovary the method adopted by Kind and Macchi (1952) with the modification of Hawk *et.al.* (1954) was followed. Fresh tissues were removed blotted quickly and weighed accurately. The tissue was homogenised with 10. c.c. of cold double distilled water, saturated with chloroform and allowed to stand in the refrigerator for 48 hours. The homogenate was centrifuged and to 1 ml of the homogenate was added 9 ml of glycerophosphate buffer (pH 9.4) and incubated for 2 hours at 37°C in an incubator. At the end of 2 hours the reaction was stopped and the proteins precipitated by adding 2 ml of 30% cold trichloroacetic acid solution. Control tubes containing buffered phosphate and 1 ml of sample were kept without incubation for the determination of initial phosphate concentration, under identical conditions.

The deproteinised samples of the experiment and the control were allowed to remain in the refrigerator for an hour and filtered. From the filtrate (both incubated and unincubated) samples of known quantity were taken for the estimation of inorganic phosphate following the procedure adopted by Fiske and Subbarao (1925) given in Hawk *et.al.* (1954). The colour developed in the reaction was read in a Lumetron colorimeter (Model 401-A) using a 650 μ filter. For the standard, a standard solution was prepared and the inorganic phosphate determined as above. The difference in the phosphate content in the incubated and unincubated gives the alkaline phosphatase activity in the ovary. The alkaline phosphatase activity is expressed in micrograms of inorganic phosphate released per gram weight of wet tissue. For each stage three determinations were made and the mean was taken for comparison.

Observations and Results

The results are shown below:

Alkaline phosphatase activity expressed in micrograms of inorganic phosphate released per gram of tissue.

(The result in each stage is the mean of three experiments. Numbers within brackets indicate range of variation)

	Stage I Immature	Stage II Maturing	Stage III Mature	Ratio
<i>Labeo fimbriatus</i> (Bloch)	348.3 (345-350)	1245 (1240-1250)	235.6 (225-247)	1 : 3.5 : 0.68
<i>Mystus seenghala</i> (Sykes)	215.6 (211-221)	511.6 (503-520)	225.0 (220-230)	1 : 2.4 : 1.04
<i>Boleophthalmus boddarti</i> (Day)	273.3 (270-275)	1500 (1500-1500)	225.0 (220-230)	1 : 5.4 : 0.8

The above data show that in general the alkaline phosphatase activity in the first and the third stages is not strikingly different, but has a markedly higher value in the second stage. In *Labeo* the alkaline phosphatase activity increases 3.5 times in the second stage and comes down in the third stage to a lower level than in the first stage. In *Mystus* the rise in the second stage is twice that in the first stage, and in the third stage is very slightly higher than in the first. In *Boleophthalmus* the rise in the second stage is 5.4 times and in the third stage falls below the level of first.

Discussion

Moog (1944) has shown that in the development of chick there are waves of formation of alkaline phosphatase at different stages in the development of at least most tissues. Moog (1944) and Krugelis (1950) have suggested that phosphatase activity is concerned with active metabolism and differentiation in tissues. In the present study it is observed that there is a marked rise and fall of alkaline phosphatase activity. The maturing stage of the ovary is evidently an active phase of synthesis as the alkaline phosphatase activity reaches a peak in this stage in all the three species. The rise and fall of alkaline phosphatase activity is analogous to that observed by Moog (1944) in the development of chick.

The study of amino acids in the three stages of the ovary made by the author has also shown that the second stage of the ovary has a maximum concentration of amino acids, and the high activity of the alkaline phosphatase in this stage of the ovary in the three fishes investigated may be correlated to an active protein synthesis.

Summary

1. The alkaline phosphatase activity has been determined in the three different stages of growth of the ovary of *Labeo fimbriatus* (Bloch), *Mystus seenghala* (Sykes) and *Boleophthalmus boddarti* (Day).

2. The phosphatase activity rises to a peak in the maturing stage (Stage II) and declines in the mature stage (Stage III) to about the same level as or slightly lower level than in the first stage.

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Triactinomyxon sp. (Actinomyxid Sporozoan: Protozoa) Parasitic in a Fresh-water Oligochaete

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(With 1 Text Fig.)

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Introduction

The Actinomyxidia, an order of parasitic Protozoa of the subclass Cnidosporidia, were discovered and named by Stolc. No attention was focussed on this order of interesting group of parasites in India and in the Asiatic countries. The order has seventeen known species belonging to seven genera of the two families, Tetractinomyxidae and Triactinomyxidae Kudo, (1946). Of these, eight species belong to the genus *Triactinomyxon* stolc, 1889 (7), to which the present species also belongs. Of these eight, six are recorded from the intestinal wall of three tubificid worms (1, 2, 3, 4, 5, 8), one from the intestinal wall of a lumbricid worm and one from the intestinal wall of a maidid worm (6).

In the course of a study of the taxonomy and biology of the fresh-water Oligochaeta of Cuddapah, India, one worm belonging to the family Naididae, *Pristina longiseta* Ehrenberg, was found infected with several spore-cysts of an actinomyxid parasite. Six cysts in various stages of development were found in the gut-wall of the middle segments and were absent from the anterior 10 segments and posterior 5 segments. They were found protruding into the coelom of the segments as spherical bodies. The presence of the cysts in the absorptive part of the intestine and their absence in the anterior and posterior segments, indicate that they derive the nourishment from the intestinal wall and not from the coelomic fluid bathing them.

The cysts contain the spores in different stages of development. The spores are the free-living stage in the life-history of the parasite and the media for the spread of the parasite into new hosts. As a ripe spore cyst was not available, nearly ripe spores were liberated from a spore-cyst for studying their structure.

Description of *Triactinomyxon* SP.

The spore approaching maturity (Fig. 1a) is colourless, transparent and rocket-shaped. It consists of a vertical barrel-shaped central axis, having apically three polar capsules and radiating basally three similar transparent flap-like vertical valves at equal angles to one another form-

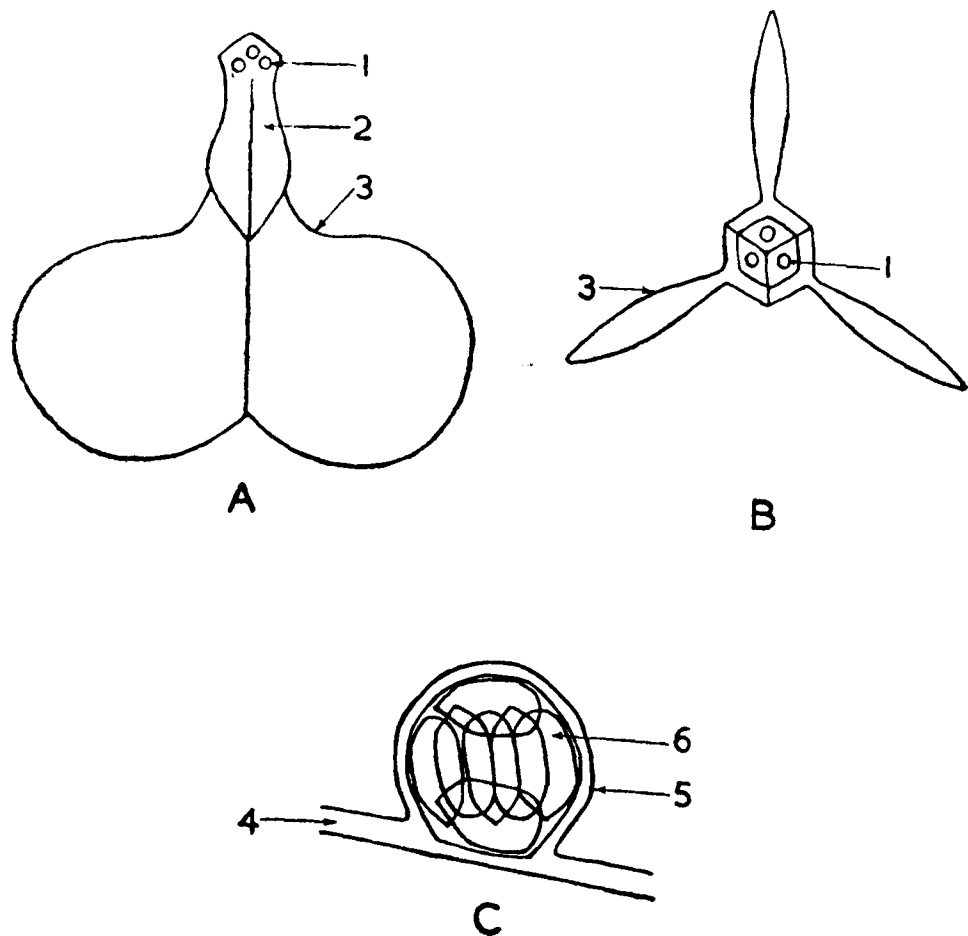


Fig. 1 *Triactinomyxon* sp.: A. lateral view of the spore, /2000; B. apical view of the spore, /2000; C. spore-cyst attached to the host's intestinal wall, /1250. 1, polar capsule, 2, barrel-shaped central axis, 3, ray of the tri-radiate base, 4, intestinal wall of the host, 5, spore-cyst, 6, immature spore.

ing a tri-radiate base (Fig. 1b), with which the spore stands. The polar capsules are spherical as their filaments are not discharged. Ventral to the capsules the axis contains a compact mass of greenish sporoplasm undifferentiated into uninucleate sporoplasms. In the unripe spore the polar filaments are not discharged and the uninucleate sporoplasms are not differentiated.

The cyst (Fig. 1c) contains eight bodies, the developing spores. They are all similar in size and shape in a cyst. The sizes and shapes are different in different cysts. The immature spores are smaller and nearly spherical in the smaller cysts and larger and ovoid in the larger cysts. These two conditions of the spores represent younger and older stages respectively in the development of spores as in *T. legeri* and *T. naidanum*. Early developmental stages of the cysts in the life-history are not seen.

The eight bodies in the cyst are the central axes of the developing spores, with their basal valves crumpled and packed behind them as parachutes behind parachuters. In a liberated spore, the valves uncrumple and spread out to form a tri-radiate base to stand on it with the axis of the spore projected vertically upwards.

Of the several hundreds of worms of other species of fresh-water oligochaetes examined during the study, no other oligochaete was found infected with this actinomyxid parasite, although another parasite, *T. naidanum* Naidu was found infecting and specific to another fresh-water oligochaete, *Nais communis punjabensis* Stephenson. This parasite also appears to be specific to *Pristina longiseta* Ehrenberg.

Dimensions of the unripe spore:—height 60μ diameter at the base 65μ diameter at the mid-axis 15μ Locality, Cuddapah, Andhra Pradesh, India. Collected by K. Vanamala Naidu.

Discussion

Eight species have so far been recorded for the genus *Triactinomyxon* Stolc, each with a specific shape of the spore and valves, and definite number of uninucleate sporoplasms. They are: *T. naidanum* Naidu, 1956(6), with 12 sporoplasms found in *Nais communis punjabensis* belonging to the family Naididae; *T. ignotum* Stolc, (8), with 8 sporoplasms, *T. legeri* Mackinnon and Adam, (5), with 24 sporoplasms, *T. dubium* Granata, (3), with 32 sporoplasms, actually a new name for an unnamed species of *Triactinomyxon* described by Leger(4), and *T. mrazeki* Mackinnon and Adam, (5), with 50—100 sporoplasms, all the above four species are recorded from *Tubifex tubifex* belonging to the family Tubificidae; *T. ohridensis* Georgevitch (1), with 8 sporoplasms, found in a tubificid worm, *Tubifex ohridensis*; *T. magnum* Granata, (2),

with 16 sporoplasms found in *Limnodrilus udekemianus* of the Tubificidae: and *T. petri* Georgevitch, (1), with 8 sporoplasms from an unidentified worm belonging to the family Lumbricidae.

The present species with a rocket-shaped spore, with short and barrel-shaped central axis and short and wide valves, differs from all the eight known species. Before Georgevitch, (1), discovered two new species, *T. ohridensis* and *T. petri*, both with 8 sporoplasms in their spore there were only five species known, each with a particular number of sporoplasms. Now there are three species with 8 sporoplasms. As such the determination of the species on the basis of sporoplasm number no longer seems to hold good. The present species, in which the sporoplasm number could not be seen due to unripe condition of the spore, is evidently a new species as it has a distinctive rocket-shaped spore. This spore-shape is not found in the other eight species. However, pending the examination of the ripe spore, the final determination of the present species is deferred.

Remarks

This parasite is rare as of the numerous worms of *Pristina longiseta* examined only one contained the spore-cysts.

Summary

An actinomyxid, *Triactinomyxon* sp., distinguished from other members of the genus by the rocket-shaped spore with flap-like vertical radiating valves is described from the intestinal wall of an Indian fresh-water oligochaete, *Pristina longiseta*. This is the second record of an actinomyxid in Asia and also in a member of the oligochaete family Naididae.

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Observations on and Diagnosis of the Flagellate Genus *Pseudotrichonympha* Grassi and Foa 1911

BY M. VITTAL,

(Communicated by Dr. M. A. MOGHE, F.Z.S.I.)

(with 1 Text Fig. and 1 table)

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Introduction

This paper is a record of observations on the flagellate genus *Pseudotrichonympha* Grassi and Foa 1911, *Coptotermes heimi* Wasm. and *Heterotermes malabaricus* Snyder, collected from Dharwar (Bombay).

Material and Technique

The termites are commonly found infesting wood, books, cardboards, files and paper during the rainy season.

Smears of the termite intestinal contents were made in a weak saline solution of 0.4% strength on slides without using any albumen adhesive. Of the various fixatives and stains used, Schaudinn's fluid followed by Delafield's haematoxylin gave the best results.

Distribution

The termites of the genera *Coptotermes*, *Heterotermes*, *Schedorhinotermes* and *Rhinotermes* (F. Rhinotermitidae) are known to harbour the several species of the genus *Pseudotrichonympha* (Table I).

TABLE 1. Distribution and morphological features of the species of *Pseudotriconympha* recorded so far.

Species	Host	Length	Breadth	Flagella	Nucleus	Remarks
<i>P. hertwigi</i> (Hartmann) Grassi and Foa 1911.	<i>Coptotermes hartmanni</i>	330-750 mic.	40-60 mic.	—	Anterior with large karyosome	Big species.
<i>P. hertwigi</i> var. major Grassi 1917.	<i>Coptotermes lacteus</i>	—	—	Absent in posterior extremity.	Round and anterior.	Form long and slender, with surface striations reaching upto the base of the rostral tube.
<i>P. hertwigi</i> var. minor Grassi 1917.	<i>Coptotermes jostedti</i>	—	—	—	—	Surface striations extend upto the zone of the cap, the only structure free from it.
<i>P. magnipapillosa</i> Grassi 1917.	<i>Schedorhinotermes putorius</i>	—	—	—	—	Cap and rostral tube very big.
<i>P. parvipapillosa</i> Grassi 1917.	<i>Schedorhinotermes putorius</i>	—	—	—	—	Cap and rostral tube very short.
<i>P. grassii</i> Koidzumi 1921.	<i>Coptotermes formosanus</i>	200-300 mic, rarely 500	50-120 mic.	Free portions of flagella in anterior region 6-8 mic. and 25-30 mic. resp. Body flagella 16-20 mic.	Round and anterior. 16-20 mic. diameter.	Body fusiform. Posterior end glabrous. Division forms frequent. Bell (campanula) 20 mic. in height and 40-50 mic. across its maximum width.

Species	Host	Length	Breadth	Flagella	Nucleus	Remarks
* <i>P. pristina</i> (Imms) Culter 1921,	<i>Archotermopsis wroughtoni</i>	167-280 mic.(Imms) 134-259 (Cutler)	57-144 mic. (Imms) 100-226 (Cutler)	Of two kinds. Longer ones 14-16 mic. and rest 12 mic.	19 mic. in diameter.	No campanula
<i>P. sphaerophora</i> Dunckerley 1923.	<i>Rhinotermes nasutus</i>	230 mic.	65 mic.	—	—	Oval form. Constant presence of one or more brownish, non-staining spherical bodies as large as the nucleus (25 mic. diameter) in the endoplasm.
<i>P. belari</i> DeMello 1927.	<i>Heterotermes indicola</i>	180-561 mic.	60-221 mic.	Of three kinds. 1st series 20-22 mic. 2nd " 44-50 mic. 3rd " 8-22 mic.	17-30 mic. diameter	Shape markedly variable. Campanula superimposed (?) with a free posterior margin.
<i>P. pseudotriconympha</i> spp. Kirby 1932.	<i>Heterotermes aureus</i>	245 mic.	70 mic.	Present in posterior extremity.	Round and anterior 16 mic. diameter.	Shape pisiform. Surface striations laeotropic. Parabasal apparatus present.
<i>P. ramani</i> DeMello 1935.	Unidentified	100-210 mic.	80-132 mic.	—	Lies freely	Campanula, "reinforcing bottle shaped bodies" (in rostral region) and "radial flagella" present.

* The validity of the inclusion of this species in the genus *Pseudotriconympha* is doubtful.

Species	Host	Length	Breadth	Flagella	Nucleus	Remarks
<i>P. samani semi-nuda</i> DeMello 1935.	<i>Heterotermes</i> spp.	—	—	—	—	No campanula. 2nd series of flagella extraordinarily big, their turned bunch reaching the level of the mushroom-shaped cap.
<i>P. hertwigi</i> var. <i>simpler</i> DeMello 1935.	<i>Coptotermes</i> spp.	100-410 mic.	13-94 mic.	—	Lies freely.	Posterior extremity not glabrous. Lack of well developed fan-shaped "myonemes".
<i>P. cardiformis</i> Karandikar and Vittal 1954.	<i>Heterotermes malabaricus</i> & <i>Coptotermes heimi</i>	100-225 mic.	50-150 mic.	Of three types. 1st type 6-8 mic. 2nd type 18-20 mic. 3rd type 12-14 mic.	14-27 mic. diameter.	Generally heart-shaped. Campanula anterior and medially placed. Endoplasmic inclusions and sphaerita-like micro-organisms present in endoplasm.
<i>P. pisciformis</i> K. & V. 1954.	do.	100-230 mic.	35-75 mic.	1st type 6-8 mic. 2nd type 14-16 mic. 3rd type 8-14 mic.	10-18 mic. diameter.	Resembles the form of a cylindrical fish. Campanula disposed obliquely on one side of the median axis. Posterior end hyaline and finger-like.
<i>P. subapicalis</i> K. & V. 1954.	do.	200-300 mic.	100-210 mic.	—	15-25 mic. diameter.	Campanula distinctly subapical.

Taxonomic Features

Shape and Size. The *shape* in species of this genus varies from a heart-shaped appearance to a strikingly pisciform shape, and the *size* generally ranges from 100-300 microns in length and 50-150 microns in width.

Campanula. The body of *Pseudotrichonympha* is very clearly demarcated by a narrow, circlet-like line into two unequal regions: a very small, anterior, bell-like portion called the campanula and posterior to it a very extensive area forming the major portion of the body (Karandikar and Vittal, 1954). Grassi (1917) and Koidzumi (1921) while describing this structure failed to notice the narrow, circlet-like line demarcating it from the rest of the body which they called the "bell". DeMello (1927) described the campanula in *P. belari* as a super-imposed structure possessing a free margin.

Grassi, Koidzumi, DeMello and Karandikar and Vittal noted two types of granular layers, a darker inner and a lighter outer layer in the ectoplasm of the campanular portion of *Pseudotrichonympha*.

Rostral tube. The presence of the rostral tube in *Pseudotrichonympha* has been clearly indicated in all accounts concerning the said genus though different workers have used different names for its designation (Kirby, 1932). It is a tiny, thick-walled, deeply stainable, dumbbell-shaped tubule occupying a central, vertical position in the campanula of *Pseudotrichonympha*. It is closed posteriorly, so much so the body endoplasm does not continue into it and is surrounded by a single, dark layer of ectoplasm which is closely applied to the already thickened wall of the rostral tube (Karandikar and Vittal, 1954). With the exception of Grassi who distinguished two layers, all other authors, have noticed only one layer of ectoplasm surrounding the rostral tube.

Blepharoplast. This is the name used by Franca and later adopted by Kirby (1932) to the hemispherical granule situated at the anterior end of the rostral tube in the base of the cap in the genus *Trichonympha*. In *Pseudotrichonympha* also such a body is present in an identical position to which the name blepharoplast is used in this account because it suggests a blepharoplast in position and method of division (Kirby, 1932). This structure has been observed by Grassi, Koidzumi and Karandikar and Vittal as an enlargement at the anterior end of the rostral tube and not sharply demarcated from the rostral tube proper. DeMello (1927) observed the blepharoplast in *P. belari* as a hollow, hemispherical body rising above a superior transversal meniscus at the anterior end of the rostral tube.

The author has carefully examined this structure in *P. subapicalis*. The blepharoplast in this species is a hemispherical granule lying on and separated from the flat surface of the anterior end of the rostral tube.

Rostral fissure. Grassi (1917) while enumerating the characters of the *Pseudotriconympha* species expresses the belief that the "citartrosi" (rostral fissure) does exist in *Pseudotriconympha* but that it is only very slightly developed. Koidzumi (1921) does not make any reference to this structure in his description of the genus *Pseudotriconympha*. DeMello and Monteiro (1942) while suggesting the entire characterisation of the order Hypermastigina have reiterated the absence of rostral fissure in species belonging to the genus *Pseudotriconympha*. Karandikar and Vittal (1954) in their study of the *Pseudotriconympha* species stated that the rostral fissure is not visible.

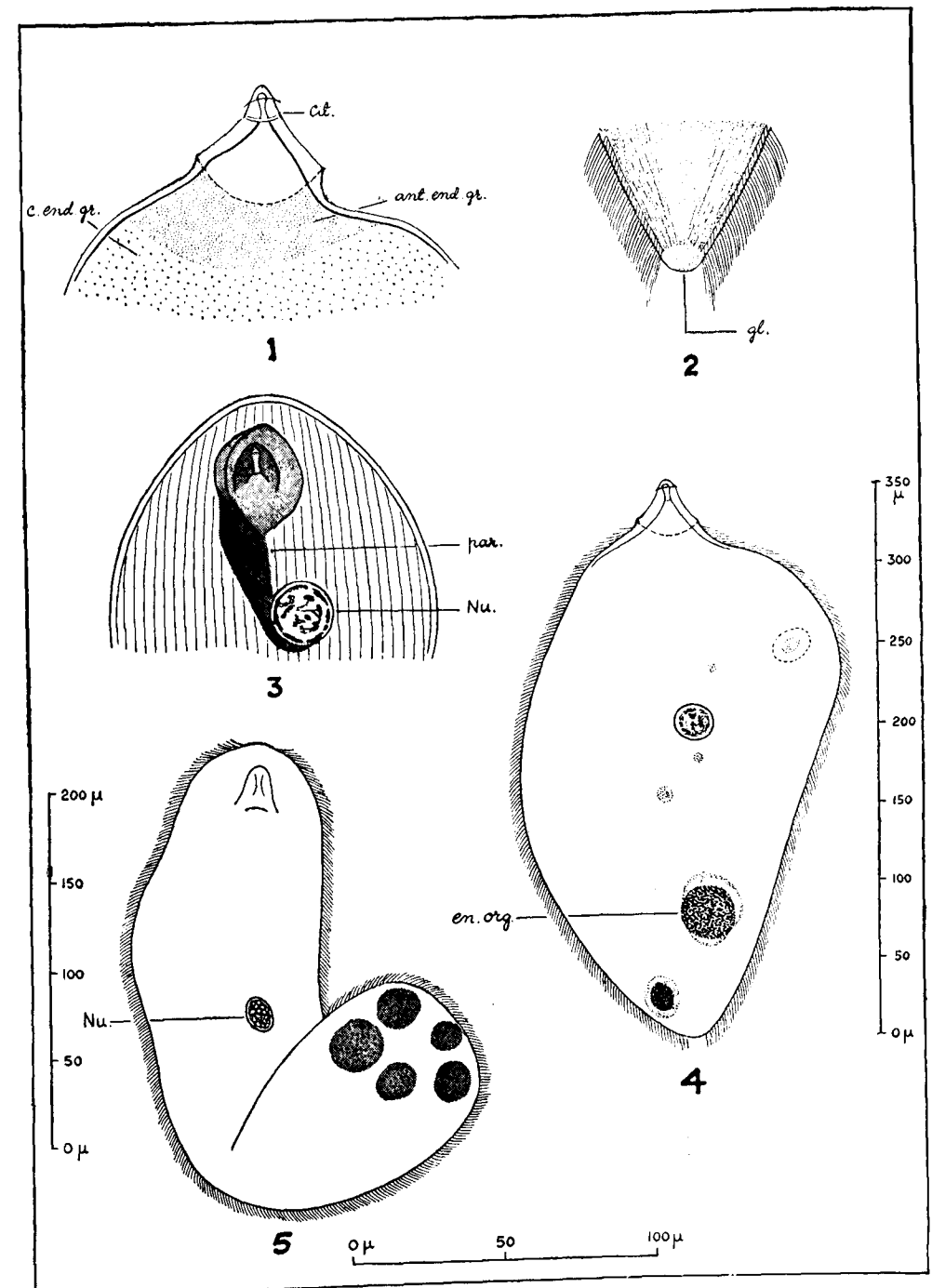
The author has re-examined this structure in *P. subapicalis* from *Coptotermes heimi* Wasm. The rostral fissure is visible and is only slightly developed as observed by Grassi. (Text Fig. 1).

Flagella. There are three types of flagella which cover the body in *Pseudotriconympha*. The flagella of the first type are relatively short taking their origin from the thick layer of basal granules situated next to the rostral tube. The flagella of the second type are the longest. They arise in a thick circular band just below the first type of flagella, their basal granular layer being arranged all round the rostral tube (Karandikar and Vittal, 1954). Posterior to the circular band of flagella the flagella of the third type arise in laetotropic rows from the basal granules situated in the "ribbon-like" bands covering the body in a likewise direction. These are a little longer than the flagella of the first type which extending posteriorly right upto the whole length of the body leave however, a small glabrous area at the posterior extremity (Text Fig. 2).

Endoplasm. The endoplasm in *Pseudotriconympha* is distinguishable into two zones: the broad, "breast-like" anterior fifth portion of the body being formed of clear, finely dotted endoplasm comparable to the anterior endoplasmic granules in *Trichonympha* and the remaining four-fifths of the body being full of coarse and deeply stainable granules. Grassi, Koidzumi and Karandikar and Vittal have described these granular layers.

Nucleus. The position of the nucleus is variable in *Pseudotriconympha*. It is usually anterior; sometimes it is found in the middle third of the body and very rarely much distally.

Parabasal apparatus. Concerning this structure Grassi (1917) has remarked that he has not found in *Pseudotriconympha* any trace of those cords which form the characteristic apparatus for the suspension reference to the parabasal cords while giving the diagnostic characters of *Pseudotriconympha*. DeMello and Monteiro (1942) have stated that the parabasal apparatus is absent in *Pseudotriconympha*. Karandikar and



Test-figs. 1 to 5 *Pseudotriconympha subapicalis* Karandikar and Vittal 1954 from the termite host *Coptotermes heimi* Wasmann. Fig. 1, Anterior region $\times 440$, Fig. 2, Posterior extremity $\times 440$, Fig. 3, Individual showing the parabasal apparatus $\times 440$, Fig. 4, Individual showing entozoic organisms $\times 220$ and Fig. 5, Individual showing sphaerita-like microorganisms $\times 200$.

ante. end. gr. anterior endoplasmic granules; c. end. gr. coarse endoplasmic granules; cit. citartrosi (rostral fissure); en. org. entozoic organisms; gl. glabrous extremity; nu. nucleus; par. parabasal apparatus.

Vittal (1954) did not find any trace of the parabasal cords in *Pseudotriconympha*. But what is interesting is that* Kirby (1932) observed in *Pseudotriconympha* from *Heterotermes aureus* parabasal cords like those in *Triconympha*.

The author has observed cords somewhat similar to those observed by Kirby in the vicinity of the nucleus in *P. subapicalis* from *C. heimi* Wasm. (Text fig. figure 3), which presumably are of the nature of parabasal cords.

Entozoic organisms. Grassi, Koidumi, DeMello and Kirby have not found any entozoic organisms in the *Pseudotriconympha* species they studied. But Karandikar and Vittal (1954) found sphaerita-like microorganisms in abundance in the endoplasm of the *Pseudotriconympha* species from *H. malabaricus* Snyder and in relatively less numbers in those from *C. heimi* Wasm. (Text figs. figures 3 and 4).

Endoplasmic inclusions. The endoplasmic inclusions commonly found in *Pseudotriconympha* are the undigested remains of the fibres of wood which form the diet of their termite hosts. The debris of wood in *Pseudotriconympha* is not limited to the portion behind the nucleus but is found even a little anteriorly leaving free only a limited anterior zone which takes the form of a "breast". Apart from the fibres of wood, certain other endoplasmic inclusions have been observed in the body of *Pseudotriconympha* (Dunkerley, 1923. Diagnosis Karandikar and Vittal, 1954).

The diagnosis of the genus *Pseudotriconympha* in the light of the above is given below.

Relatively large hypermastigote flagellates presenting a variety of forms from the perfect heart-shaped appearance to a pisciform shape; body divisible into two unequal regions comprising of a very small, anterior, bell-like portion called the campanula and posterior to it a very extensive area forming the major portion of the body; rostral tube in the campanular region closed posteriorly, so much so, the body endoplasm does not continue into it; blepharoplast a hemispherical granule rising above a transversal superior miniscus at the anterior end of the rostral tube in the base of the cap; rostral fissure when visible slightly developed; flagella covering the body of three types which differ from each other in their length and location; posterior extremity usually glabrous; endoplasm distinguishable into two zones; position of nucleus variable; parabasal cords in the vicinity of the nucleus known; entozoic organisms and

* Kirby in a letter (dated October 25, 1951) says: "The figure of *Pseudotriconympha* from *Heterotermes aureus* which I published in 1932 does not show the parabasal apparatus correctly. It was difficult to see anything with the technique used and the cords shown are not present in silver impregnated material I have of other species. Though I have not had silver preparations of the species from *H. aureus*, it is probable that they are not there either. What seems to be the parabasal bodies in this other material are numerous comparable to those of *Holomastigotoides* instead of *Triconympha*."

endoplasmic inclusions present; no sexual forms known; xylophagous; described species entozoic in termites.

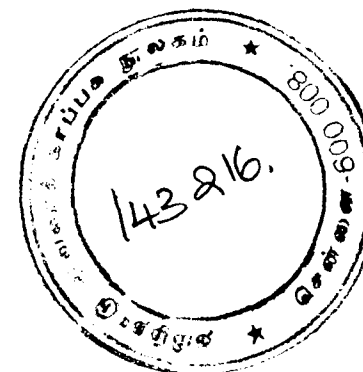
Generitype. *P. hertwigi* (Hartmann) symbiont of *Coptotermes hartmanni* Holmgren, Brazil.

Acknowledgements

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Seasonal Histological changes in the Testis of the Catfish *Mystus seenghala* (sykes)

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(With 2 Plates)

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Introduction

The exhaustive studies of the gonadal cycle of fishes which have been published so far are of foreign species. Some of the outstanding contributions on the testicular cycle are by Turner (1919) and Craig-Bennett (1931). Van Oordt (1925), Jones (1940), Weisel (1943), Matthews (1938) and James (1946) have studied in detail the histological changes in the testes of *Xiphophorus helleri*, *Salmo salar*, *Oncorhynchus nerka*, *Fundulus heteroclitus*, *Lepomis macrochirus* and *Huro salmoides* respectively. Observations on the sexual cycle in relation to environment have been made in *Fundulus heteroclitus* (Matthews, 1939), *Phoxinus* (Bullough, 1939, 1940) and *Galeichthys felis* (Frederick, 1941). There have also been noteworthy contributions on the histology of the germ cells in *Cottus bairdii* (Hann, 1927), on the origin of germ cells and spermatogenesis in *Betta splendens* (Bennington, 1936) and the regeneration of gonads in *Plecoglossus ativelis* after spawning (Kazuo Suzuki, 1939).

Sufficient data on the bionomics and breeding habits of Indian fishes of economic importance are available (Jones, 1946, 1950, 1951). But investigations on the histology of the gonadal cycle of Indian species are meagre. Gosh and Kar (1952) and Sathyanesan (1958) have worked on the seasonal histological changes occurring in the gonads of *Heteropneustes fossilis* and *Cirrhina reba* respectively. The present study is on the histological variations of the testis during the sexual cycle. Besides, such a study is of importance for the accurate determination of the time and the duration of spawning of the species.

Material and Methods

Specimens of *Mystus seenghala* were collected from the river Ganges at Banaras, every month for a complete year. The standard length of the specimens was recorded. The anterior, middle and posterior parts of the testis were separately fixed in Bouin's picro-formol acetic and Zenker's fixatives. The total weight of the testis was determined before transferring to 70% alcohol. Sections were cut at 4 and 6 μ thickness and stained in Erlich's haematoxylin, Heidenhain's iron haematoxylin and Delafield's haematoxylin. Eosin and orange-G were used as counter-stains.

The mean weekly temperature of the water was regularly recorded. The condition factor of the testis for all the twelve months was calculated by using the formula—

$$C = \frac{\text{weight of the gonad}}{L^3 \times 10^{-8}} \quad (\text{Allison, 1951})$$

Where C is the condition factor and L is the standard length of the fish.

General Morphology of the Testis

The testes of *M. seenghala* are paired elongated, flattened structures with lobulated margins, and situated ventro-lateral to the air bladder to which they are attached by the mesorchia. Posteriorly the testis on either side lie close together with their sperm ducts running adjacently in the median line (Pl. 3, Fig. 2) which unite further behind to open into the common urinogenital papilla along with the urinary bladder. In the female the genital opening is separate, the papilla is less-prominent, and the ureter alone opens into it. These features distinguish the two sexes in the adult state.

The testis is covered with a vascular tunica of connective tissue. The seminiferous tubules anastomose and form a net-work. The term lobule is also used by workers (Turner, 1919) as synonymous with tubule. The testis during its seasonal cycle exhibits a period of activation, growth, maturity, depletion and rest.

The classification of Bennett, cited by James (1946), with little modification is advantageously used in the present study to describe the gross morphological changes of the testis.

Stage A: (Resting condition)—The testis is slender and translucent. This condition prevails from August to December.

Stage B. (Slightly enlarged condition)—During this stage the testis shows a little enlargement, and becomes opaque. This stage is noticeable in January and in the early part of February.

Stage C. (Greatly enlarged condition)—The testis is much more enlarged than before, and tinted dull-white. The duration of this stage is

very short as it quickly passes into stage D. This condition is found in February.

Stages D and E. (Spawning and partly spent conditions)—Fishes belonging to these stages are available from March to May. Due to their quick succession and overlapping, these two stages are treated together.

Stage F. (Completely depleted condition)—The testis is flesh coloured and much reduced in size. Specimens with spent testis are found in June and July.

Seasonal Histological Changes

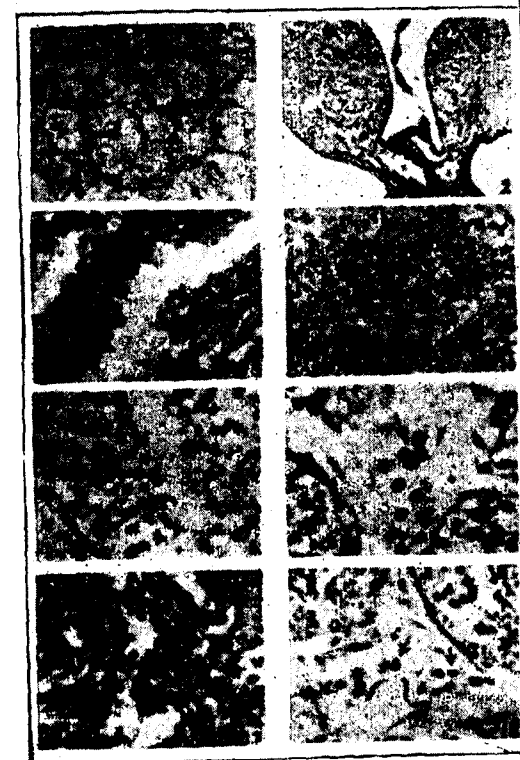
Stage A. The testis consists of seminiferous tubules surrounded by connective tissue. In *M. seenghala* there is no radial septation of the testis as in the salmon (Weisel, 1943) and the core of connective tissue reported in perch (Turner, 1919) is wanting. There are no distinct cortical and medullary zones as have been described in *Fundulus* (Matthews, 1938). The spermatogonia are the only germ cells present and are arranged along the tubular wall. Often these germ cells totally displace the tubular lumen and the individual tubule looks like a cord of cells (Pl. 3, Fig. 1). The spermatogonia measure 7.5 to 15 μ in diameter. The interstitial cells are seen inbetween contiguous tubules, at such places where the intertubular space is wide. Their cytoplasm is meagre and the nucleus is well defined. These cells may be easily distinguished from the variously shaped and scattered nuclei of the connective tissue elements. The structure of the last or posterior part of the testis is different from that of the rest. In this region well-defined cavities are present. But the spermatogonia which are characteristic of the anterior testis are totally absent. Such cavities are often seen interconnected and an excessive growth of connective tissue is evident between them. These cavities are lined with small cells with scanty cytoplasm (Pl. 3, Fig. 3).

Stage B. During this stage spermatogenesis is in progress. The dividing germ cells are seen in the form of 'nests' or clusters (Pl. 3, Fig. 4 & 5) and those of an individual nest are, more or less, in the same stage of maturation. As pointed out by Matthews (1938), Van Oordt (1925) and James (1946), it is difficult to interpret the different germ cells; the primary and secondary spermatocytes in particular. The secondary spermatocytes appear to be smaller than the primary (Pl. 3, Fig. 5). Their nuclei are usually spherical but the chromatin gives a mottled appearance due to its irregular distribution. The diameter of the spermatocytes ranges from 5 to 8 μ . The spermatids show more affinity to stain and are much smaller, having an average diameter of 2 to 2.5 μ . In few tubules sperms are present. The sperm head is stained deeply but the tail lacks affinity for stain (Pl. 3, Fig. 5). The sperms do not exhibit any regular arrangement as reported in perch (Turner, 1919) and *Xiphophorus* (Van Oordt, 1925). The interstitial cells do not present any variation during preceding stage Pl. 3, Fig. 7). The posterior testis is similar to that of the

Satyaneesan

Plate 3

PLATE I.



- Fig. 1—Photograph showing the spermatogonia completely replacing the lumina of the tubules. Stage A ... X 480
- Fig. 2—Photograph showing portions of the posterior testis with their sperm ducts (marked X). Stage A ... X 25
- Fig. 3—Photograph showing the cavity of the posterior testis with the lining cells. Absence of spermatogonia may be noted. Stage A ... X 750
- Fig. 4—Photograph showing the testis in an actively dividing phase and the germ cells are in different stages of maturation. Stage B ... X 480
- Fig. 5—Photograph showing spermatogonia, spermatocytes and sperm. Stage B ... X 720
- Fig. 6—Photograph showing the interstitial cells in between tubules (marked by arrow). Stage B ... X 760
- Fig. 7—Photograph showing the cavity of the sterile posterior testis with the lining cells. Absence of dividing germ cells and the presence of deeply staining connective tissue elements may be noted. Stage B ... X 560
- Fig. 8—Photograph showing shrunken interstitial cells in between contiguous tubules (marked by arrow). Stage D ... X 640

Stage C. There is a noticeable reduction in the number of spermatogonia and a relative increase in the number of 'nests' of actively dividing germ cells and sperm masses. As the sperms are formed, the thin wall of the 'nests' gives way and the sperms are liberated. When several such 'nests' belonging to the same tubule liberate their sperm contents, the sperm-filled lumen of the tubule is discernible. An increase in the number of interstitial cells as compared with the foregoing stage is obvious. Some isolated groups of these cells are outstandingly large (Pl. 4, Fig. 1) in comparison to others (Pl. 4, Fig. 2). Matthews (1938) has recorded in *Fundulus* "clusters of peculiar cells", and he presumed them to be interstitial cells. He did not locate these cells in the testis fixed either immediately before or during the spawning season. The posterior testis remains the same without any appreciable change.

Stages D and E. The lumina of the tubules are almost filled with sperms, and the 'nests' of dividing germ cells are much less (Pl. 4, Fig. 5). The spermatogonia are fewer (Pl. 4, Fig. 4) and they are referred to in literature as reserve germ cells (Kazuo Suzuki, 1939), resting germ cells (Weisel, 1943; Jones, 1940) and dormant germ cells (Hann, 1927). This is reported to be the parent cell for the new generation of germ cells (Hann, 1927; Weisel, 1943; Matthews, 1938). The tubular wall is thin, due to distension, and in some cases communication is established between the sperm-filled lumina of the neighbouring tubules. There is an apparent increase in number of the interstitial cells during stages C and D. Few groups are prominent by their large size; some possess cytoplasmic vacuoles (Pl. 4, Fig. 3) and some others are shrunken and pycnotic (Pl. 3, Fig. 8). The cavities of the posterior testis are distended with sperm (Pl. 4, Fig. 6) and the connective tissue element in between is comparatively thin. Many of these cavities are interconnected and finally they open into the sperm duct which is situated at the inner margin. The partly spent testes exhibit varying quantities of sperm, suggesting that in this species, the extrusion of milt takes place in instalments. The expulsion of sperm is followed by the division of the resting germ cells.

Stage F. The tubules become more compact but the sperm masses are always present. The resting germ cells are in active stages of division. The persistence of sperm and the actively dividing germ cells stated above during this phase reminds one of the condition obtained in stage-B testis. But in stage-F the divisions end with the formation of spermatogonia which remain dormant throughout the accompanying stage. These germ cells occupy the wall of the tubule all around, thus progressively narrowing down the tubular lumen (Pl. 4, Fig. 7). The groups of large interstitial cells which are seen during the preceding stages could not be detected. Matthews (1938) has reported the frequent occurrence of large vacuoles in these cells in the spent testis of *Fundulus*. In the posterior testis the cavities are almost devoid of sperms and show varying degrees of contraction (Pl. 4, Fig. 8). In some, along with the clumps

of relict sperm, a number of their lining cells are also seen in the lumen.

The condition factor readings appear to have a correlation with the testicular cycle. This value exhibits gradual increase in stage-B as the testis is activated.

The maximum is reached during stages C to E, followed by a decline during the fall as shown below:—

Months	Condition factor	Stages	Average water temperature in °C
January	0.25	B	18.6
February	0.49	C	20.5
March	0.53	D & E	25.0
April	0.45		27.3
May	0.40		30.0
June	0.27	F	29.4
July	0.17		28.8
August	0.08	A	28.7
September	0.06		28.2
October	0.05		27.4
November	0.06		23.1
December	0.07		19.0

Discussion

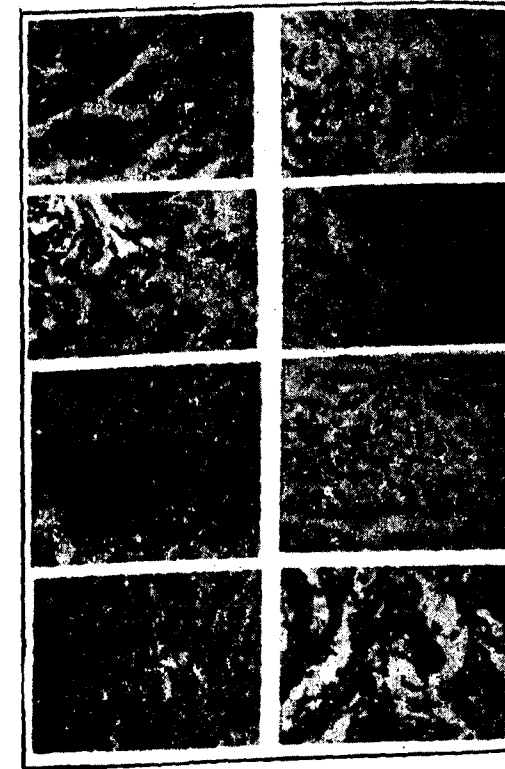
The sexes can be distinguished in the adult *M. seenghala*. The urinogenital papilla of the male is more prominent than the urinary papilla of the female. A distinct separate genital opening is situated close to the urinary papilla in the female. Mookerjee *et al* (1940) have recorded the presence of papilla only in the male of *Macrones gulio*.

The anterior part of the testis of *M. seenghala* is functional and the posterior part is sterile. In the 'transitional' portion between these two regions the germ cells are seen towards the periphery in varying quantities which progressively decrease in number further backwards and finally disappear in the posterior region. The posterior testis is made up of net-work of cavities lined by small cells and is continuous with the anterior testis. It is likely that these cavities represent the lumina of the tubules and the lining cells may be the degenerate germ cells. It appears that this part of testis having abandoned its germinal potentiality has assumed the secondary function of serving as conduits for the passage of milt. In *Polypterus*, Kerr (1919) has recorded the posterior testis to be sterile and made up of net-work of cavities. Jungerson (cited by Kerr, 1919) reported homologous condition in many young teleosts. The pre-

Satyanesan

Plate 4

PLATE II



- Fig. 1—Photograph showing a group of interstitial cells which are prominent by their large size. Stage C ... X 580.
- Fig. 2—Photograph showing the group of interstitial cells (marked by arrow). Stage C ... X 720.
- Fig. 3—Photograph showing vacuolated interstitial cells (marked by arrow). Stage L ... X 720.
- Fig. 4—Photograph showing few resting spermatogonia along the wall of the sperm filled tubule. Stage D ... X 680.
- Fig. 5—Photograph showing seminiferous tubules whose lumina are filled with sperm. Stage D ... X 140.
- Fig. 6—Photograph showing a cavity of the sterile posterior testis distended with sperm. The lining cells are seen all around. Stage D ... X 750.
- Fig. 7—Photograph showing the relict sperm in the lumina of the tubule whose wall lodges germ cells. Stage F ... X 590.
- Fig. 8—Photograph showing the collapsed cavity of the sterile posterior testis which is denuded of their sperm contents. Stage F. ... X 400.

sence of sterile posterior testis in the adult *M. seenghala* may be the persistence of the young teleostean feature.

Craig-Bennett (1931) has correlated seasonal cycle in the interstitial cells of the testis with the secondary sexual character in *Gasterosteus aculeatus*. This view gains further support from the findings of Courrier (vide Craig-Bennett, 1931). Champy (1923) and Essenberg (1923) showed that there is no correlation between the secondary sex characters and development of the interstitial cells (vide Craig-Bennett, 1931). Van Oordt (1925) observed that in *Xiphophorus* 'sex hormones are not secreted by the interstitial cells at least exclusively'. Matthews (1938) could not find evidences to establish the secretory nature of the interstitial cells. In *M. seenghala*, the interstitial cells are identifiable throughout the year. They exhibit a numerical increase during stages C and D. During these phases isolated groups of these cells are prominent by their large size. Some possess cytoplasmic vacuoles and some others are shrunk and pycnotic. In spite of these apparent quantitative and qualitative variations it is difficult to assign any functional correlation with certainty.

Turner (1919) has recorded a cord of germ cells outside the body of the testis from which the testis is periodically replenished with germ cells. But as reported by Hann (1927) such a migration of germ cells from outside is not evident in *M. seenghala*. Division of the resting spermatogonia takes place in this species immediately after spawning as in the case of *Cottus bairdii* (Hann, 1927) and perch (Turner, 1919). And these new germ cells remain dormant throughout the resting phase.

In *M. seenghala* temperature might be a factor affecting the growth and maturity of the gonads as in *Gasterosteus* (Craig-Bennett, 1931), *Fundulus* (Matthews, 1939) and *Galeichthys* (Frederick, 1941). Bullough (1939) observed that in minnows, long daily hours of artificial light in winter and a temperature of 17°C induced early maturation of the gonads. Further work by him (1940) showed that "the internal rhythm is capable of acting in the absence of these external seasonal changes which may normally reinforce it and render it more precise in its time of action". Marshall (1956) states that apart from external influences there is an inherent tendency towards a reproductive rhythm in fishes which discharge their generative products in bulk. A large percentage of the population of *M. seenghala* breeds during March to May. However, late spawners are encountered in June, July and in early August. Karamchandani and Motwani (1955) reported breeding of *Rita rita* in river Ganges from March to August. Khan (1924) found that *Rita rita*, *Mystus seenghala* and *Wallago attu* breed from June to August in the Punjab. He observed that the breeding of *Pseudeutropius garua* commences earlier in March.

The existence of the internal reproductive rhythm is well established (Marshall 1956 and others). The external environmental factors do have

some influence on the activation of spawning in many species. It is possible depending on the early or delayed onset of favourable environmental conditions the breeding period may be shifted a little one way or other.

Summary

In the adult *M. seenghala* the sexes can be distinguished with ease.

The posterior one-fourth of the testis is sterile and is formed of network of cavities, homologous to the structure described in the young teleosts (Jungersoa, vide Kerr, 1919) which bear semblance to the condition obtaining in *Polypterus* (Kerr, 1919).

The functional part of the testis during its seasonal cycle exhibits a period of activation, growth, maturity, depletion and rest.

The interstitial cells exhibit some apparent variations during the pre-spawning and spawning periods, for which it is difficult to draw any functional correlations.

This species breeds in the river Ganges from March to May. The late spawners encountered are not taken into account. Temperature appears to be an environmental factor influencing spawning.

A correlation between the variations in the condition factor value and the stages in the seasonal cycle of the testis is attempted.

Acknowledgements

My thanks are due to Dr. A. B. Misra, Professor of Zoology, Banaras Hindu University, for his guidance and help throughout this work. I am also grateful to the Government of India for the award of a Scholarship which made this work possible.

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Two New Species of Cestodes belonging to the Family Dilepididae Fuhrmann, 1907.*

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(Department of Zoology, Lucknow University, Lucknow)

(With 6 Text-figures)

(Communicated by Dr. M. B. LAL, F.Z.S.I.)

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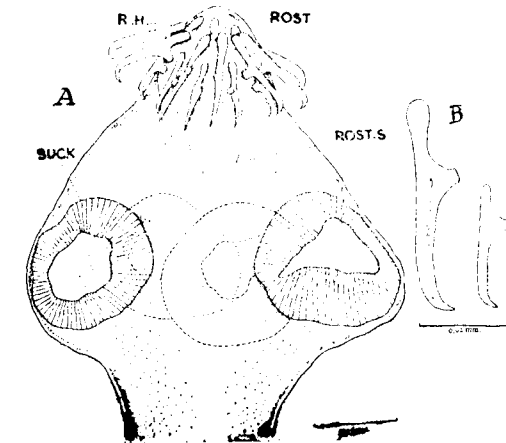
Introduction

The material which forms the basis of the present paper was collected from birds in two different parts of Uttar Pradesh. A large number of specimens were recovered from the intestine of *Ardea purpurea* Linn. shot in the environs of Lucknow and three mature and one immature specimens were collected from the intestine of a red wattled Lapwing, *Lobivaellus indicus* Boddaert, shot down in the environs of Pilibhit. Subsequent examination of six *A. purpurea* from several places failed to produce any further specimens. On a detailed study it was concluded that the specimens are new to science; those from *Ardea purpurea* belonging to the genus *Parvitaenia* Burt, 1940 and those of *Lobivanellus indicus* belonging to the genus *Panuwa* Burt, 1940.

Parvitaenia purpurea n. sp.

Length of the strobila 4.5-6.6 mm. with 40-60 segments. Scolex more or less triangular; basal portion broader 0.22 mm. $\times$ 0.19-0.20 mm. Average diameter at the top (apex) 0.09 mm. Rostellum powerful and highly muscular 0.08-0.095 mm. $\times$ 0.05 mm. Rostellum bears a double crown of 20 hooks, the large hooks alternating with the smaller hooks. Large hooks 0.047-0.052 mm. in length. Smaller hooks 0.027-0.030 mm. in length. Suckers globular and muscular 0.08-0.10 mm. in diameter. Neck short

0.10 mm. $\times$ 0.07 mm. Anterior immature segments longer than broad 0.15 mm. $\times$ 0.075 mm. Mature and gravid segments broader than long, the former 0.16 mm. $\times$ 0.21 mm. and the latter 0.20 mm. $\times$ 0.33 mm. Genital pores alternate irregularly, situated in a notch-like depression



Text-Fig. 1. A. Scolex of *Parvitaenia purpurea*, B. Rostellar hooks (enlarged)

near about the anterior third of the lateral margin of the segments. Dorsal and ventral longitudinal excretory vessels measure 0.0036 mm. and 0.0062 mm. in diameter respectively with a transverse vessel in the posterior part of the segment. Cirrus sac distinguishable into two parts; proximal globular portion and the distal narrower portion. It measures 0.15-0.175 mm. in length and 0.06-0.075 mm. in width extending upto the middle of the segment. Cirrus and ductus ejaculatorius coiled within the cirrus sac. Cirrus sac, a little prior to its entrance into the genital cloaca, is provided with a ring like muscular band (m.b.). Tests 6-9 in



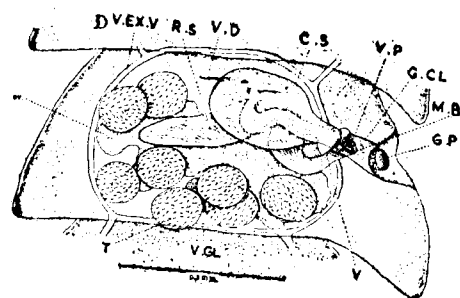
Text-Fig. 2. Rostellar hooks of known species of *Parvitaenia*. 1. *P. ardeolae* Burt 1940. 2. *P. cochlearii* Coll, 1955. 3. *P. purpurea* n. sp.

number (diameter 0.07-0.08 mm. $\times$ 0.05-0.06 mm.) situated dorsally to the ovary and almost surrounding it except on the poral side and always limited within the ventral longitudinal excretory vessels. Ovary (0.28 mm. across the segment) extending up to the inner borders of both the poral and the aporal ventral longitudinal excretory vessels at the sides. Vagina located posterior to the cirrus sac. Receptaculum seminis large and elongated and shows two prominent twists. Vitelline gland compact, posterior to ovary, 0.06 mm. in diameter. Uterus an irregularly lobed sac partially divided by several incomplete septa. Eggs and oncospheres not fully developed.

*Part of thesis approved by the University of Lucknow for the award of the Ph.D. degree

Type locality: Lucknow.

Type: in the helminthological collection, University of Lucknow.



Text-Fig. 3. Mature segment of *Parvitaenia purpurea*.

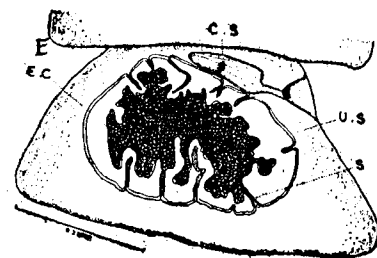
Type host: *Ardea purpurea* Linn.

Habitat: Intestine.

Discussion

There are only two species of *Parvitaenia* Burt, 1940 reported so far, *P. ardeolae* Burt, 1940 and *P. cochlearii* Coil, 1955. The present form can be easily separated from both species by its relative and absolute size of the cirrus sac, larger size of strobila, larger testes and the

Text-Fig. 4. Gravid segment of *Parvitaenia purpurea*.



spiny cirrus (in the case of *P. cochlearii* the spines are present at the base of the cirrus, otherwise the cirrus is unarmed). In addition, the rostellar hooks show marked difference in the curvature of the handle and the form of the guard.

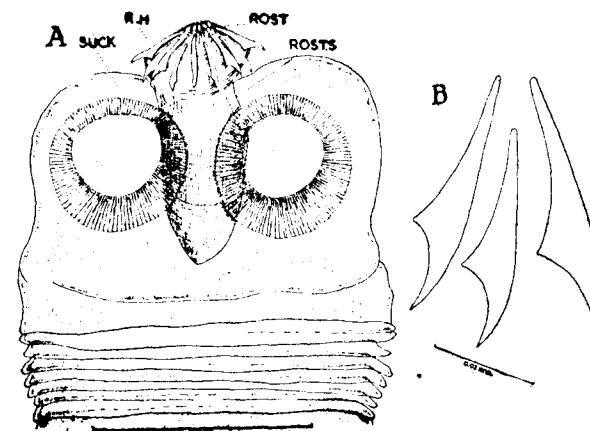
Panuwa valelli n. sp.

Length of the mature strobila 19-24 mm. with 75-80 segments. Scolex markedly developed, wider than long 0.17-0.21 mm. $\times$ 0.27-0.31 mm. Rostellum well developed 0.16-0.18 mm. $\times$ 0.07-0.09 mm. Rostellum armed with a double row of 16 hooks, the handle of the hook being twice as long as the blade with a small guard. Large hooks 0.048-0.057 mm. alternate with smaller hooks 0.035-0.039 mm. Rostellar sac 0.215 mm. long extending much beyond the lower level of the suckers. Suckers

well developed, rounded or oval 0.10-0.126 mm. in diameter. Neck indistinct, segmentation starting almost behind the scolex. Fully mature segments 0.38-0.46 mm. $\times$ 0.85-0.94 mm. Gravid segments 1.15-1.20 mm. $\times$ 0.83-1.20 mm. Strobila margin serrated. Genital pores alternate irregularly, located slightly anterior to the middle of the lateral margin

Text-Fig. 5A. Scolex of *Panuwa valelli* n. sp.

B. Rostellar hooks (enlarged) of *P. valelli*.



of the segments opening into a well developed genital cloaca. Cirrus sac slightly anteromedian in position 0.15-0.17 mm. $\times$ 0.029-0.035 mm. Cirrus sac extends a little beyond the ventral poral longitudinal excretory vessel and dorsal to it. Vas deferens greatly convoluted. Testes 16-20 in number (diameter 0.09-0.08 mm.) rounded or oval mainly posterior to the ovary. Ovary bilobed (one of the lobes being subdivided into small lobulations) located posterior to the coils of the vas deferens and slightly poral in position. Ovary 0.30 mm. across. Vagina short posterior to cirrus sac (but never ventral to it) and dorsal to poral longitudinal excretory vessels. Receptaculum seminis large and fusiform 0.055-0.087 mm. $\times$ 0.027-0.038 mm. Vitelline gland posterior to ovary and slightly poral in position measuring 0.025-0.035 mm. $\times$ 0.05-0.06 mm. Uterus breaking up into 250-300 egg capsules in each segment, each with a single egg. Eggs and onchospheres 0.06-0.07 mm. and 0.048-0.050 mm. in diameter respectively. Embryonic hooks 0.015-0.020 mm. in length.

Type host: *Lobivanellus indicus* Boddaert.

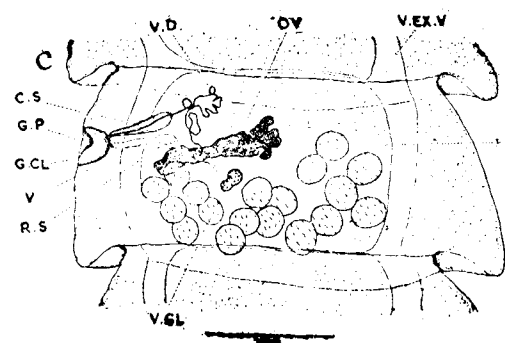
Habitat: Intestine.

Type locality: Pilibhit. U.P.

Type: In the helminthological collection, University of Lucknow.

Discussion

The genus *Panuwa* was established by Burt (1940) with *P. lobivanelli* as its genotype which is the only species known so far. The present form shows contrasting differences in the size and shape of the rostellar hooks, size of the cirrus sac, the number of egg capsules per segment and the size of the eggs.



Text Fig. 6. Mature segment of *P. valelli*.

Abbreviations used

c.s., cirrus sac; cc., egg capsules; g.cl., genital cloaca; g.p., genital pore; m.b., muscular band; ov., ovary; r.h., rostellar hook; rost., rostellum; rost. s., rostellar sac; r.s., receptaculum seminis; s., septum; suck., sucker; t., testis; u.s., uterine sac; v., vagina; v.d., vas deferens; v.ex.v., ventral longitudinal excretory vessel; v.gl., vitelline gland; v.p., vaginal pore.

Summary

Parvitaenia purpurea n.sp. and *Panuwa valelli* n.sp. are described from two different birds in U.P. *P. purpurea* is distinguished from the other two known species of the genus by the size of the cirrus sac, strobila, testes and the shape of the rostellar hooks. *P. valelli* is separated from the only one known species by a number of features.

Acknowledgments

I wish to express my grateful thanks to Professor M. B. Lal for his kind interest in guiding the present work.

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Development of *Calappa lophos* (Herbst) and *Matuta lunaris* Forskal (Crustacea: Brachyura)

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(With 32 Text-figures)

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Introduction

In the family *Calappidae*, there have been some observations on the development of the genus *Calappa*, but there is so far no account of the early development of the genus *Matuta*. Smith (1880b) described the megalopa of *Calappa mormorata* without a figure. Labour (1944) described and figured the megalopa of *Calappa flammea* from the shallow waters of Reach and according to her the megalopa described by Smith was that of *C. flammea*. She (L. 1944) described and figured the megalopa of *Cycloes bairdri* which changed to a young crab. By rearing a berried female in the laboratory, Aikawa (1937) obtained and described with figures the first zoea of *C. lophos* but did not record where and at what stage the eggs hatch. The observations recorded below on *C. lophos* supplement those by Aikawa (1937).

Calappa lophos having carapace length 93.5 mm. and breadth 50 mm. in berry was caught on the 2nd July 1953 in the off-shore waters of the Lawson's Bay, Visakhapatnam along with fishes in a fishing net. The eggs were in late stages of development, dirty orange shade, spherical and of diameter 320-330 μ .

Matuta lunaris in berry was caught in a net while fishing at the Lawson's Bay, Visakhapatnam on the 11th January 1951 and subsequently several ovigerous crabs were collected and reared in the laboratory. The carapace lengths and widths of the berried crabs vary from 31-35 mm. in length and 33-38 mm. in breadth respectively. The late eggs were dark gray, spherical and of diameter 400-410 μ whereas the early eggs were

orange, spherical and diameter 310-310 μ . The definition of the larval stage is the same as used in an earlier account (Raja Bai, 1959).

Observations

Early development of *Calappa lophos*: The ovigerous crab (P. I, Figs. 1-18) was not aggressive. It was always seen with its chelae interlocked in front. When the crab was placed in a basin of sea water it was observed that a large quantity of water was taken in and discharged in a stream at the anterior part of the buccal cavern just over the interlocked chelae. The crab was then transferred to an aquarium in which it was submerged and it was noticed that there was now only a small vertical water current.

On the next day (3-7-53) the heart-beats of the larvae inside the egg membranes were noticed. A day later (4-7-53) the eggs hatched and were liberated as prezoa. The prezoa performs vigorous movements of the abdomen by which the larva swims. The pre-zoea sheds the abdomen by which it swims. The pre-zoea sheds also its thin pre-zoeal membrane and within 1-3 hours changes over to the early first zoea. The early zoea extends its exopod setae of the maxillipedes within 10 minutes and reaches the late first zoea stage. Many died on the second day and the last batch of about 50 died three days after hatching.

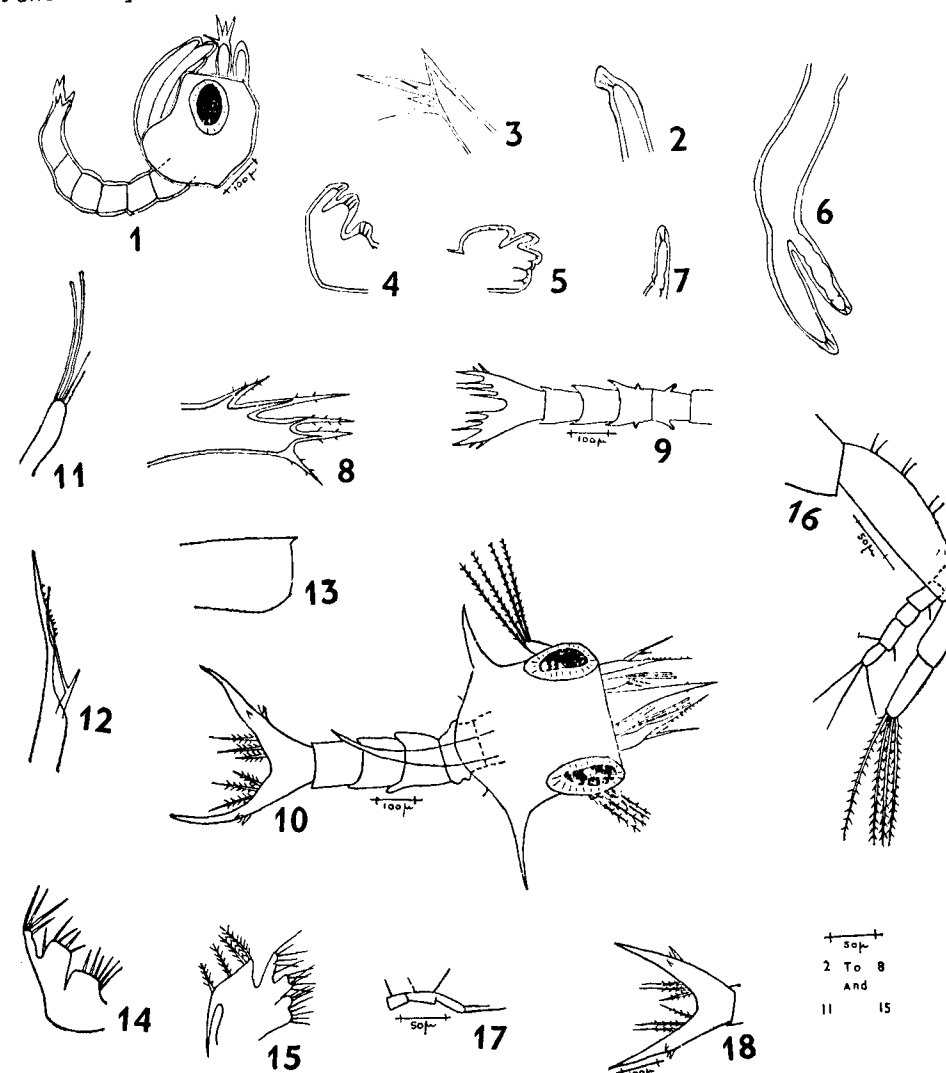
2. Pre-zoea: It is enclosed by a very thin pre-zoeal membrane and the maxillipedes are free and covered over by separate pre-zoeal membranes (Fig. 1).

The abdomen consists of five segments excluding the telson. The telson forks are rudimentary and enclosed by flat setose spines (Fig. 8).

The eye is sessile. The first antenna is a conical process bearing a rudimentary aesthete and a spine (Fig. 2). The second antenna bears two rudimentary spine-like processes, one of which bears a spinule (Fig. 3). The mandible is not visible. The coxa, the basis and the endopod of the first maxilla bear 3, 3 and 2 setae respectively (Fig. 4). The coxa and the basis of the second maxilla are bilobed and bear 1-2 and 2-2 setae respectively. The unsegmented endopod bears a seta whereas the exopod bears 3 rudimentary setae (Fig. 5).

The coxa and the basis of the first maxillipede are devoid of setae. The endopod is indistinctly five-segmented and tipped with 2 setae. The exopod bears 3 apical setae (Fig. 6). The second maxillipede is as rudimentary as the first maxillipede. The endopod is indistinctly 3 segmented and tipped with 2 setae (Fig. 7).

3. Early first zoea. It has all the three kinds of carapace spines well developed and the exopod setae not extended. The abdomen is advanced over that of the previous stage in that the second and the



Legends:—Text-figs. 1-18—*Calappa lophos* (Herbst)

1. Pre Zoea from the egg.
2. First antenna of the pre-zoea.
3. Second antenna of the pre-zoea.
4. First maxilla of the pre-zoea.
5. Second maxilla of the pre-zoea.
6. First maxillipede of the pre-zoea.
7. The endopod of the second maxillipede of the pre-zoea.
8. Telson of the pre-zoea.
9. Abdomen of the early first zoea.
10. Late first zoea.
11. First antenna of the late first zoea.
12. Second antenna of the late first zoea.
13. Mandible of the late first zoea.
14. First maxilla of the late first zoea.
15. Second maxilla of the late first zoea.
16. First maxillipede of the late first zoea.
17. Endopod of the second maxillipede of the late first zoea.
18. Telson of the late first zoea.

third abdominal segments have a pair of knobs of which those of the second segment are much larger than those of the third segment. The third, the fourth and the fifth abdominal segments have lateral spines, which are somewhat obtuse at the terminal end. The lateral spines of the fifth abdominal segment are very much smaller than those of the third and the fourth segments. The telson has still the rudimentary forks but the pair of lateral spines on each side of the telson are discernible (Fig. 9).

4. *Late first zoea* has all the 3 kinds of carapace spines. The rostral and the dorsal spines are at an acute angle to the body. The rostral spine has 2 denticles on it (Fig. 10).

The second and the third abdominal segments bear each a pair of knobs. The third, the fourth and the fifth abdominal segments bear lateral spines. The telson forks bear a hair in addition to a pair of lateral spines already present in the early first zoea on each fork; between the forks there are 6 plumose setae (Fig. 18).

The eye is sessile. The first antenna has at the apex 2 unequal aesthetes and 2 unequal spines (Fig. 11). The second antenna has a long spiny process. The pointed exopod is slightly less than $1/3$ length of the spiny process and has a long stiff hair at $1/3$ its length (Fig. 12). Aikawa has described the pointed exopod provided with a spinule for the first zoea of *Calappa lophos*. The mandible is rudimentary with one tooth (Fig. 13) whereas Aikawa (1937) mentions teeth on the chewing surface of the mandible. The coxa, the basis and the endopod of the first maxilla bear 6, 5 and 4 setae (Fig. 14). The coxa, the basis and the endopod of the second maxilla are bilobed and are armed with 5-3, 4-2 and 2-4 setae of which some are plumose. The exopod bears 4 plumose setae (Fig. 15).

The coxa of the first maxillipede is short and devoid of setae. The basis is armed with 2-2-2-2 setae on its inner border. The endopod is 5-segmented bearing 0-1-0-0-2-4 setae from the proximal to the distal segment. The exopod is two segmented and is tipped with 4 plumose setae (Fig. 16). The coxa and the exopod of the second maxillipede are similar to those of the first maxillipede; the basis bears 1-1-1 setae. The endopod is three segmented bearing 1-2-2 setae from the proximal to the distal segment (Fig. 17).

Measurements of the late first zoea.	Body length	...	1.1 mm.
Length from the rostral spine to the dorsal spine	...	1.066 mm.	
Length of the rostral spine	...	300 μ	
Length of the dorsal spine	...	466 μ	
Length of the lateral spine	...	180 μ	
Length of the abdomen to the tip of the telson fork	...	830 μ	
Length of the telson to the tip of the fork	...	300 μ	

Colouration: The eyes are jet black. The mandibles, the first and the second abdominal segments are of brilliant orange colour. The lateral carapace is slightly orange. Except for this, the larva is colourless.

Early Development of *Matuta lunaris*: (Text Figs. 19-32).

1. The eggs of *M. lunaris* hatch among the pleopods in the pre-zoea stage and are liberated in the same stage. The larvae shed their skin in about 2 hours. The early first zoea which emerges from the pre-zoea reaches the late first zoea stage in about half an hour. The larvae swim only in the late first zoea unlike in *Calappa lophos* where the larvae swim in the pre-zoea stage.

2. *Pre-zoea* is enclosed by the pre-zoeal membrane (Fig. 19). The abdomen is not segmented.

The eye is sessile. The tips of the first and the second antennae are seen (Fig. 20). The mandible, the first maxilla and the second maxilla are not visible.

The protopodite of the first maxillipede is not differentiated. The endopod is not segmented. The exopod is without setae (Fig. 21). The second maxillipede is similar to the first maxillipede excepting the exopod which bears two rudimentary setae.

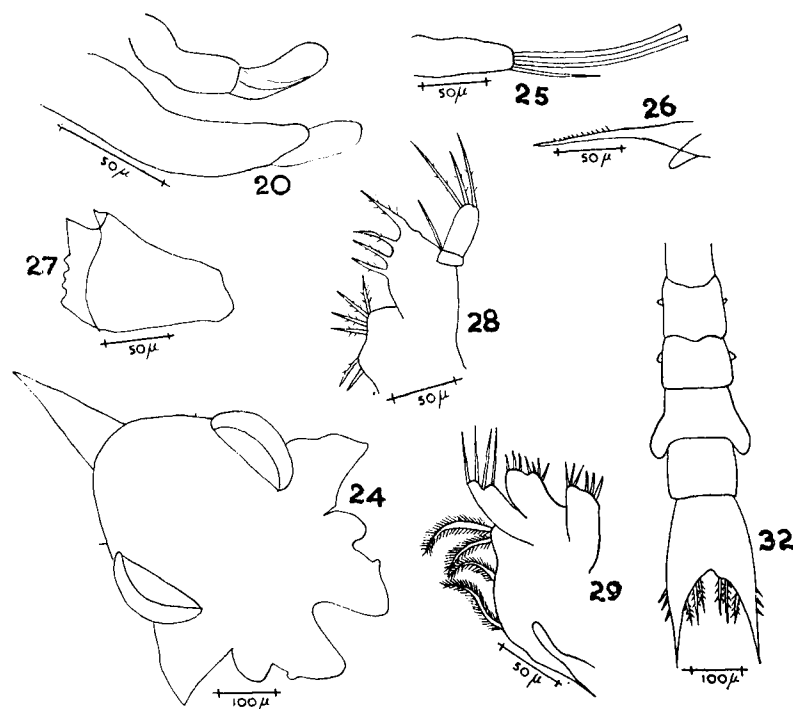
3. *Early first zoea*. It is almost similar to the late first zoea except that in the former the setae of the exopod of the first and the second maxillipedes are rudimentary and are not extended as in the late first zoea.

Late first zoea has only the dorsal and the rostral carapace spines. The dorsal carapace spine is normal to the body whereas the rostral spine which is slightly longer than the dorsal is at an acute angle to the body. A hair is present on either side of the dorsal spine (Figs. 23 and 24).

The abdomen consists of five segments excluding the telson. The second and the third segments each bears a pair of lateral knobs. The fourth segment is broad and the postero-lateral corners are lobed and clasp the anterior border of the fifth segment. The telson bears 3 lateral spines on each side; between the forks there are 6 plumose setae.

The eye is sessile. The first antenna is a conical process with two long equal aesthetes at the apex and a spine (Fig. 25). The exopod of the second antenna is less than $1/9$ th the spiny process (Fig. 26). The cutting edge of the mandible bears few teeth (Fig. 27). The coxa, and the basis of the first maxilla bear 2-4 and 4 setae respectively.

The endopod is two segmented, the distal segment is long and is tipped with 3 setae whereas the proximal segment is short and bears a single seta. Most of the setae are plumose (Fig. 28). The coxa, the basis and the endopod of the second maxilla are armed with 3-3, 4-3 and 2-2 setae respectively. The exopod is bordered with 4 plumose setae (Fig. 29).



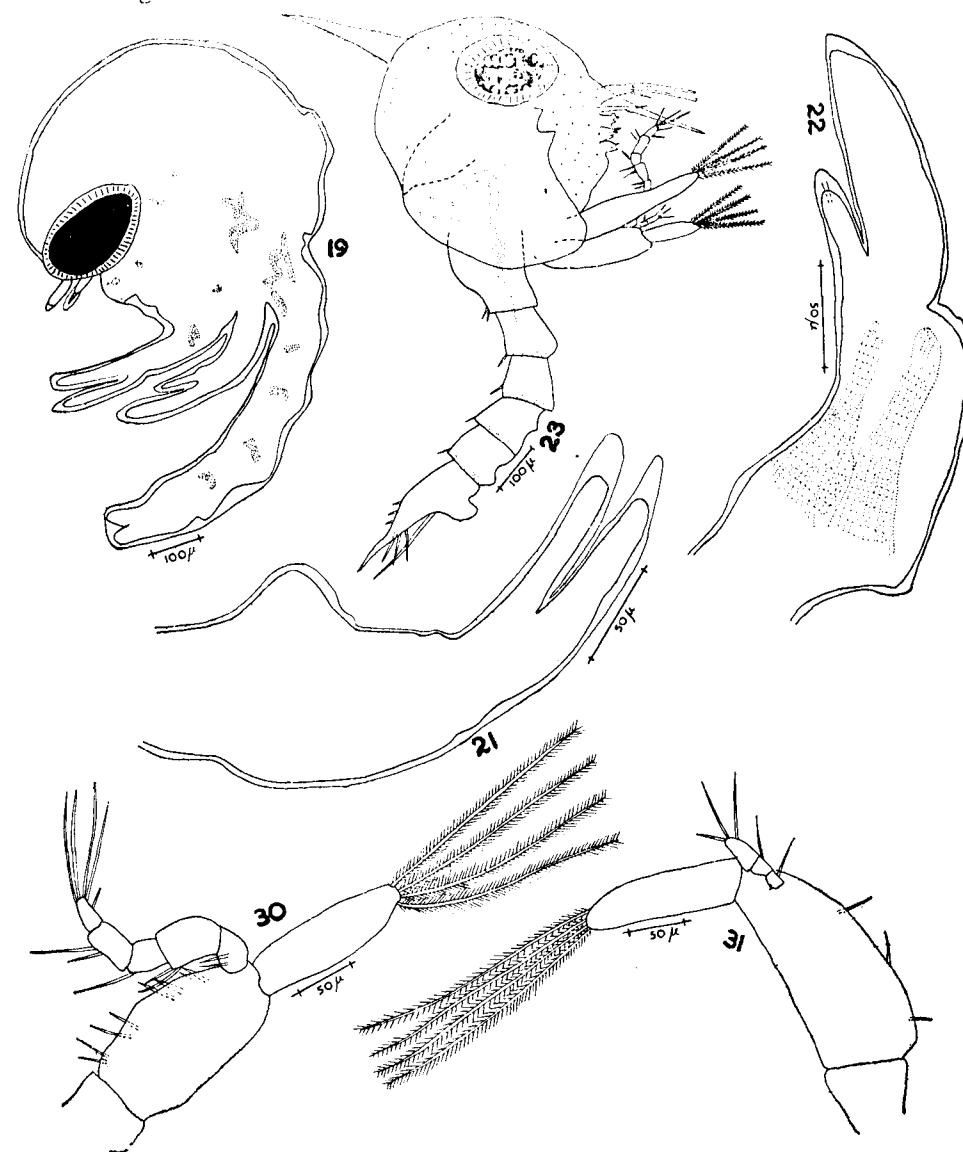
Legends:—Plaet, Figs. 19-32—*Matuta lunaris* (Forsk.)

- 20. First and second antenna of the pre-zoea.
- 24. Cephalothorax—front view of the late first zoea.
- 25. First antenna of the late first zoea.
- 26. Second antenna of the late first zoea.
- 27. Mandible of the late first zoea.
- 28. First maxilla of the late first zoea.
- 29. Second maxilla of the late first zoea.
- 32. Abdomen of the late first zoea.

The coxa of the first maxillipede is short and devoid of setae whereas the long basis bears on the inner side 2-2-2-2 setae. The endopod is 5 segmented and is armed with 2-2-1-2-5 setae from the proximal to the distal segment. The exopod is tipped with 4 natatory setae (Fig. 30). The coxa of the second maxillipede is similar to that of the first maxillipede. The basis bears 1-1-1 setae. The endopod is three segmented bearing 1-1-3 setae from the proximal to the distal segment. The exopod is similar to that of the first maxillipede (Fig. 31).

Measurements of the late first zoea:

Body length	... 1.216 mm.
Length from the rostral to the dorsal spine	... 880 μ
Length of the rostral spine	... 266 μ



- 19. Pre-zoea.
- 21. First maxillipede of the pre-zoea.
- 22. Second maxillipede of the pre-zoea.
- 23. Late first zoea.
- 30. First maxillipede of the late first zoea.
- 31. Second maxillipede of the late first zoea.

Length of the dorsal spine	...	250 μ
Length of the spiny process of the second antenna	...	150 μ
Length of the exopod of the second antenna	...	16 μ
Length of the abdomen to the tip of the telson fork	...	750 μ

Colouration. The eyes are green. The cephalothorax and the protopodites are yellowish. A black chromatophore is present in the mouth region. A large black chromatophore runs from the neighbourhood of the post cardiac region to the first abdominal segment. Black chromatophores with yellow are present in the second to the fifth segment. The telson is devoid of chromatophores.

General considerations. The early development of *Matuta lunaris* is described here for the first time. Supplementary notes such as the stage at which the larvae hatch, the pre-zoea and the early first zoea are described for *Calappa lophos*.

The main characters of the late first zoea of *Calappa lophos* and *Matuta lunaris* are as follows:—The zoea of *C. lophos* has all the three kinds of carapace spines, namely the dorsal, the rostral and the lateral spines, whereas the zoea of *M. lunaris* has only the dorsal and the rostral carapace spines. The exopod of the second antenna of *C. lophos* is pointed and bears a stiff hair at about 1/3 its length; it is slightly less than 1/3 the spiny process. In *M. lunaris* the exopod of the second antenna is knob-like and slightly less than 1/9th the spiny process. The third the fourth and the fifth abdominal segments of the zoea of *C. lophos* bear lateral spines; the telson has a pair of spines and a hair on each of its forks. In *M. lunaris* the fourth abdominal segment is broad and the postero-lateral corners are lobed and clasp the anterior border of the fifth segment; telson has 3 lateral spines on each side.

The local Calappids namely *Calappa lophos* and *Matuta lunaris* strengthen Aikawa's view that the calappid zoea has attained typical brachyuran form differing from other zoeae of Oxystomata.

Acknowledgment

I wish to thank Dr. Kandula Pampapathi Rao, Head of the Department of Zoology, Sri Venkateswara University, Tirupati, for offering valuable suggestions.

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A Record of the pearl Oyster *Pteria lotorium* (Lamarck) off Tuticorin

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(With 2 Text Figs.)

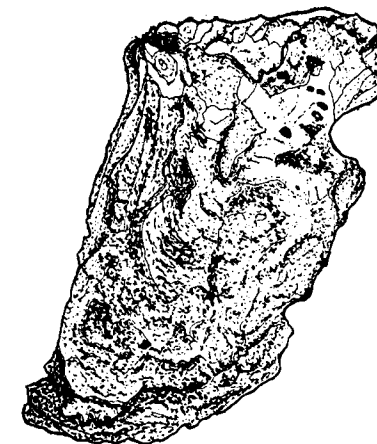
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Introduction

During the pearl-fishing operations conducted at Tuticorin in March 1958, a French diving expert, Mr. Dumas, brought up a few pearl oyster shells of more than average size while diving at 14 fathoms, east off the coast of Tuticorin. Mr. S. Mahadevan, Research Assistant, Tuticorin Fisheries Biological Station, was kind enough to send me a single large right valve of one of these shells for my examination and comment.

Description of the Shell

The shell is large, much larger than an average shell of the ordinary pearl oyster, *Pinctada vulgaris* and is obliquely elongated dorso-ventrally,



Text-Fig. 1. Right valve of *Pteria lotorium* (Lamarck), from Tuticorin (outer view) X 2/3.

the shell being much higher than long. The hinge margin is straight with a well developed anterior auricle, but the posterior auricle is very

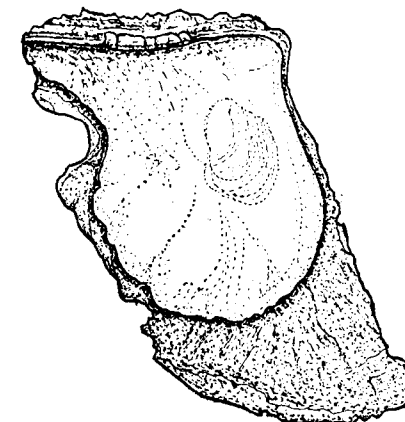
slightly developed. The front margin of the shell bears a deep byssal notch below the region of the anterior auricle. The nacreous layer is well developed. The longest dorsoventral axis of the shell is inclined at an angle of about 45° to the hinge margin. The nacreous layer on the inner surface of the shell is bordered by a broad, black, glossy marginal area which is very extensive towards the ventral margin of the shell, covering an area of almost half that of the pearly layer. The adductor impression is large and well impressed. The outer surface is rough, blackish brown and is composed of a number of overlapping scaly layers. The measurements of the shell are as follows:—Longest dorso-ventral axis (i.e., the oblique length from anterior end of hinge margin to the middle of the ventral margin): 205 mm.; Hinge margin; 105 mm.; Perpendicular dorso-ventral axis (from centre of the hinge margin to the ventral margin): 129 mm.; Length of shell (from front to hind margin) at its middle: 110 mm.

Taxonomic diagnosis

After careful examination of the specimen and a study of the available literature on the subject, I have been able to refer it to the genus *Pteria* Scopoli, 1777. But according to Jameson, (*Proc. Zool. Soc. London*, 1901, I, p. 372), the genus *Pteria* itself is split up into three sections or sub-genera, namely, *Margaritifera* (*Pinctada*), *Pteria* s. str. and *Electroma*. In the *Margaritifera* group, the longest dorso-ventral axis of the valves is approximately at right angles to the hinge line. In *Pteria* s.str. and *Electroma*, on the other hand, the shell is oblique (i.e., the long axis of the valves is directed obliquely backwards, as in the present specimen). In *Electroma*, however, the hinge is short and the posterior auricle is absent. Since the present shell is oblique and at the same time has a fairly long hinge and a well marked posterior auricle, it clearly belongs to the *Pteria* s. str. section of the genus.

As regards its specific identification, I have carefully compared the shell with the figures and descriptions in Reeve's *Conchologia Iconica* and find that it corresponds almost exactly in shape and size and other characters with the species named *Pteria lotorium* Lamarck (named as *Avicula lotorium* Reeve, *Avicula* being a synonym for *Pteria*). It is figured next to *P. macroptera* in which the anterior auricle is markedly elongated in a beak-like projection, and some authors even regard *P. lotorium* as a variety of *P. macroptera*, but in *P. lotorium* the anterior auricle is blunt and not produced, exactly as in the present specimen, and *P. lotorium* should therefore be regarded as a distinct species. Reeve also agrees with this view and has separated the two as distinct species. I have therefore identified the present specimen as *P. lotorium* (Lamarck), and it appears to be the first record of this species from Indian waters. The border of the pearly layer has a slight coppery red tinge as stated in Reeve's description.

However, it is well to remember, when identifying pearl oysters, that the species of *Margaritifera*, *Pteria*, etc., are difficult to separate from one another by hard and fast rules owing to the absence of well marked diagnostic characters and to the extraordinary amount of individual



Text-Fig. 2. Right valve of *Pteria lotorium* (Lamarck), from Tuticorin (inner view), X 2/3.

variation. It is probable that there might be extensive beds of this species off the coast of Tuticorin. It is therefore worth exploring this region intensively for further records of this species.

The species of *Pinctada* so far recorded from the Indian waters are *P. vulgaris*, *P. margaritifera* and *P. anomioides* and those of *Pteria* most commonly met with in the Bay of Bengal and Gulf of Manaar are *P. chinensis*, *P. chemnitzii* and *P. castanea*.

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Bholanath :

Plate 5

Animal Remains of the Mid-First Century B.C. to Second century A.D. from Taxilla (Sirkap), Western Pakistan

BY BHOLA NATH

(Vertebrate Zoologist, Department of Anthropology (Government of India), Indian Museum, Calcutta).

With 1 Text-figure and 2 Plates.

(Communicated by Dr. M. L. ROONWAL).

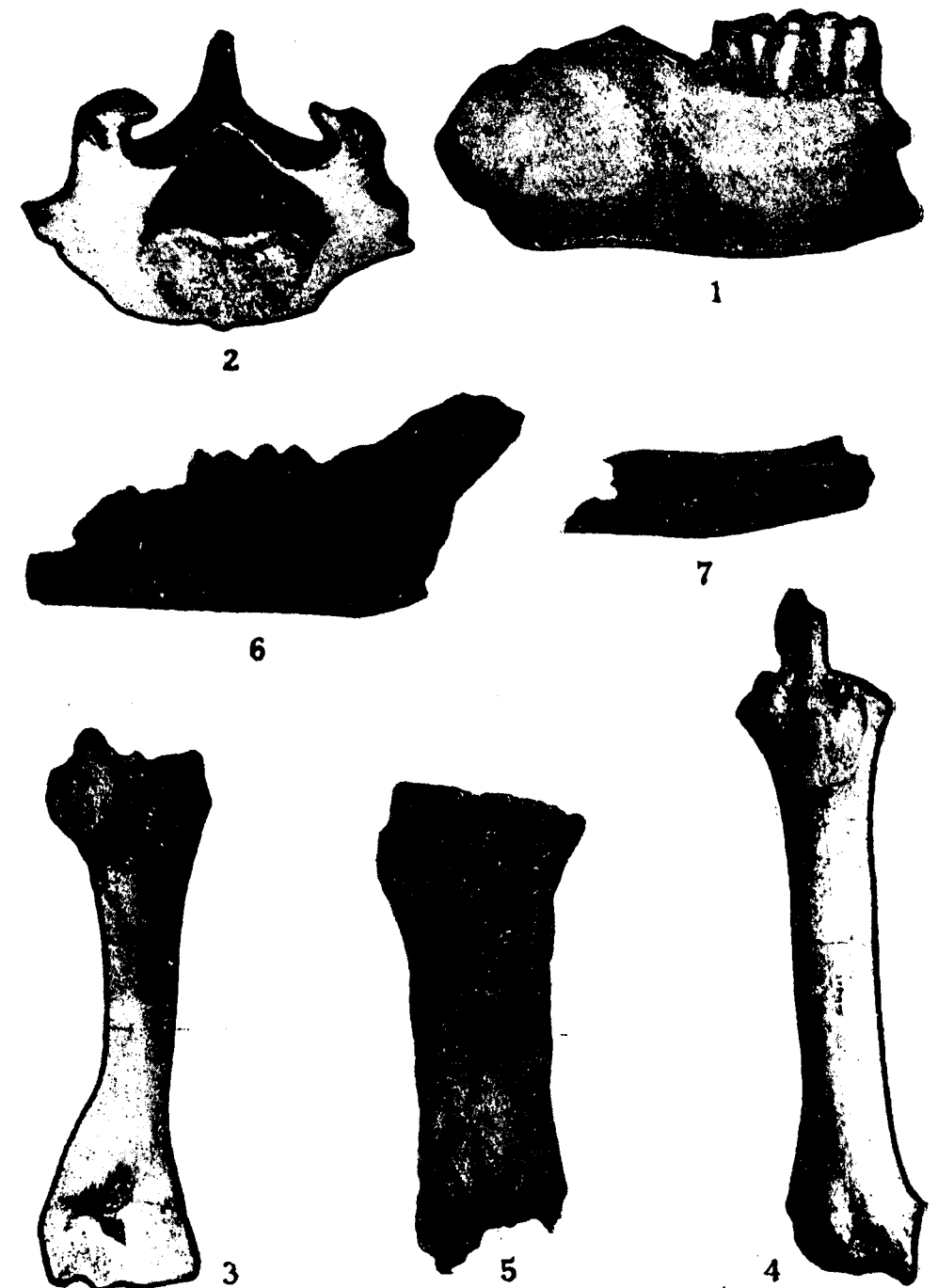
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(a) <i>Equus asinus</i> Linn.	...	
(b) <i>Equus? caballus</i> Linn.	...	
(c) <i>Bos indicus</i> Linn.	...	
(d) <i>Bos (Bubalus) bubalis</i> Linn.	...	
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Introduction

The excavation of the prehistoric sites of Taxilla, which is 22 miles north of Rawalpindi (West Punjab), now in Western Pakistan, has brought to light a wealth of historical or semi-historical associations of an exceptionally circumstantial nature. The city of Taxilla for nearly a thousand years ranked high amongst the cities of Asia, and its civilization of that time provided a remarkable intermingling of Indian and Western elements.

The excavation of this prehistoric site has brought to light the remains of three big cities—the Bhir Mound, Sirkap, and Sirsukh, besides a large number of isolated monuments, mainly Buddhist stupas and monasteries, scattered over the face of the surrounding country, the oldest and most conspicuous of them being the imposing Dharmarajika Stupa.

The present report deals with a small collection of animal remains, excavated from the Sirkap site of Taxilla, made during the field season of 1945, and Sri A. Ghosh (1948) published a report of the sites, particularly



those of Bhir Mound and Sirkap. The date assigned to the sites is from mid-first century B.C. to second century A.D. The excavation of this site was previously carried out by Cunningham (1871), and later on John Marshal carried on the excavation in 1912, continued it for twenty years, and gave a full account of various sites and the structural remains in his Taxilla report (1951).

The animal remains are mostly fragmentary and fragile. However, along with the animal remains, some of the long bones such as the humerus, radius and ulna as well as some of the tarsal bones such as calcaneum and astragalus, are almost intact. The organic material of the bones has disappeared to a great extent and they are impregnated with the inorganic material. Most of the bones consist of fragments only, and hence are unsuitable for measurements, etc.

The animal remains from Taxilla resemble in general in their species those of Harappa described by Prashad (1936), those of the Harappa 1946 collections recently worked by Nath (unpublished report) and also those of Mohenjodaro (Sewell and Guha, 1931), Hastinapura (Nath, 1955), Maski (Nath, 1957) and Rupar (Nath, unpublished report). The find of a single remains of a long-horned humped cattle from Taxilla is noteworthy since it has not been recorded from any other prehistoric sites of India so far, except at Harappa (Prashad, 1936).

As in the case of Anau (Duerst, 1904), Mohenjodaro, Harappa, Hastinapura, Maski and Rupar, there is a large number of bones which belong to young animals, indicating thereby that the people practised a full-fledged domestication of these animals.

The following five species are represented in the present collections from Taxilla (Sirkap):—*Equus asinus* Linn.; *Equus? caballus* Linn.; *Bos indicus* Linn.; *Bos (Bubalus) bubalis* Linn.; *Sus scrofa cristatus* Wagner.

As compared with specimens from Harappa, Mohenjodaro and Hastinapura this number is very small which is evidently due to the limited extent of excavation. In this report each individual bone is marked with SKR which denotes Sirkap site of Taxilla from which the bone was excavated, and which in some cases is followed by a Registered Number depicted on the bone, as given in the Excavation Register of the Archaeological Survey of India along with the particulars of "find-spot," i.e., the locus which denotes the fixed landmarks of the excavated surface; the stratum denotes the layer of soil from which the find was obtained at a particular depth from the surface level.

Acknowledgements

I wish to express my thanks to Dr. M. L. Roonwal, Director, Zoological Survey of India, for kindly going through the paper and offering valuable suggestions.

Systematic Account of the Animal Remains.

Class (MAMMALIA).

Order Perissodactyla.

FAMILY EQUIDAE.

(a) *Equus asinus* Linn. (Pl. 6, Figs. 1-3).
(The Domestic Ass).

SKR-394. Facial part of a skull (Tables 1 & 2) consisting of the following:—

Premaxilla with the body (corpus mandibulae) devoid of incisor teeth; maxilla with its palatine process, and having the maxillary tuberosity; with the 2nd, 3rd & 4th premolars and 1st, 2nd & 3rd molars of both the sides; and with a single canine tooth of the left side.

It resembles closely the facial part of the skull of an ass, *Equus asinus* Linn., obtained from Harappa (Prashad, 1936, p. 28), as well as a modern specimen of the skull of an ass present in the collection of the Zoological Survey of India. All the cheek teeth are of "pli-caballin" (a minute fold near the base of the spur or protocone) which is very characteristic of the cheek teeth of the horse.

Table 1—Measurements (in mm.) of the facial part of the skull in *Equus asinus*.

Maximum length of the facial part of skull	...	261 mm.
Maximum length of the body of Premaxilla	...	52 mm.
Width of the skull opposite 4th premolar	...	97 mm.
Height of the skull in front of molar 1	...	108 mm.
Length of diastema	...	47 mm.
Maximum length of the right tooth-row	...	135 mm.

Table 2—Measurements (in mm.) of the upper jaw teeth of *Equus asinus*.

	Taxilla SKR-394	954B/N Harappa After Prasad.	Sub- recent Harappa After Prasad	Indian Museum skull
<i>Premolar 2</i>				
Length of crown	...	31	—	—
Width of crown	...	21	—	—
Length of protocone	...	6	—	—
Length from posterior margin of crown to antecrochet.	...	11	—	—
Length from posterior margin of crown to ant. lobe of protocone.	...	15	—	—

Table 2—(Contd.)

Measurements (in mm.) of the upper jaw teeth of *Equus asinus*.

	Taxilla SKR-394	954 B/N Harappa After Prasad	Sub- recent Harappa After Prasad	Indian Museum skull
<i>Premolar 3</i>				
Length of crown.	...	23	—	—
Width of crown.	...	24	—	—
Length of protocone.	...	10	—	—
Length from posterior margin of crown to antecrochet.	...	15	—	—
Length from posterior margin of crown to ant. lobe of protocone.	...	18	—	—
<i>Premolar 4</i>				
Length of crown	...	22	23	18
Width of crown	...	24	22	21
Length of protocone	...	16	11	9
Length from posterior margin to ant. lobe of protocone.	...	16	20	13
<i>Molar 1</i>				
Length of crown.	...	18	20	13
Width of crown.	...	21	23	20
Length of protocone.	...	8.5	9	7
Length from post. margin of crown to antecrochet.	...	15	12	9
Length from post. margin of crown to ant. lobe of protocone.	...	16	17	12
<i>Molar 2</i>				
Length of crown.	...	20	21	16
Length of protocone.	...	8	10	7
Width of crown.	...	21	21	20
Length from post. margin of crown to antecrochet.	...	13	12	9
Length from post. margin of crown to ant. lobe of protocone.	...	16	16	13
<i>Molar 3</i>				
Length of crown.	...	24	19	22
Width of crown.	...	20	17	18
Length of protocone.	...	11	7	10
Length from post. margin of crown to antecrochet.	...	16	12	18

(b) *Equus? caballus* Linn. (Pl. 1, Figs. 4 & 5).
(The Horse).

THE following is the description of PLATE I.

The bone, which the scientist now refers to the *Stratum* of the site, was excavated, which in these cases is followed by the number of the stratum given in the Excavation Register of the Department of Archaeology of the Government of India. The other particulars, i.e. XVI—XXVII; stratum (a) denotes the stratum, i.e. the locus or the first landmarks of the excavated surface; and the stratum denotes the layer of soil from which the bone was obtained, and which is then followed by the particulars of depth at which the bone was situated from the surface level.

Fig. 1. New to the facial part of a skull of *Peromyscus* excavated at T.

Fig. 2. Lateral view of the rostral part of skull.

Fig. 4. Left astragalus. SKR—XXVI—XXVII, stratum I, excavated at Taxilla.

5. Left astragalus of a modern specimen of horse in the

Fig. 6. Fragment of the left humerus of long-horned race. SKF
L. II. (9). Excavated at Taxilla.

Fig. 7. Left humerus of a long-horned race from Mound F/VI Harappa. (After Prasad, 1936.)

SKR-XXVII-XXVIII; stratum (7). Distal fragment of 3rd and 4th metatarsal.
SKR-II'-III'; stratum (8). Fragment of a thoracic vertebra with neural arch only.

- SKR-I-III; stratum (9). Lumber vertebra with body, neural arch, and with anterior and posterior articular processes.
- SKR-335. Left horizontal ramus of mandible without body and with 2nd and 3rd molar teeth.
- SKR-337; stratum (12); Level II 6'-0" B.D.; square LIV-1-1" x 4'-3" x 6'-0". One incisor tooth.
- SKR-387. One incisor tooth.
- SKR-445. Complete right radius and ulna.
- SKR-471/2. Shaft of right femur without proximal and distal extremities and with supra-condyloid fossa.
- SKR-471/1. Shaft of right tibia without proximal and distal extremities.
- SKR-471-3. Fragment of the shaft of humerus.
- SKR-437. One complete right humerus.
- SKR-504. Square XLV-2'7" x 4'5" x 5'9". Right upper 1st molar tooth.
- SKR-68. Square XXIII 1'2" x 2'7" x 4'; stratum (4). 3rd incisor tooth.
- SKR-I'-II'; stratum (9). Distal fragment of left humerus of long-horned type.

These remains of the humped cattle are mostly fragmentary. However, some of the long bones such as the humerus, radius and ulna as well as a few tarsal bones such as the calcaneum and astragalus, are rather well preserved and almost intact. Unfortunately, not a single complete skull is available and it is difficult, therefore, to take measurements for comparison with those of other forms. The few teeth available do not show any special peculiarities and generally resemble those of the recent domestic humped cattle of India.

The lower jaw (Pl. 5, Fig. 1) is incomplete but resembles in all respects that of a recent specimen in the Department; its maximum length is 175 mm., while the combined length of its 2nd and 3rd molar teeth is 59 mm.

The photograph of a better preserved and conspicuous lumber vertebra is reproduced on Pl. 5, Fig. 2; its maximum length of the body is 41 mm., while its maximum breadth anteriorly is 40 mm. and posteriorly 54 mm. The maximum distance between two anterior articular processes is 72 mm., while that between two posterior articular processes is 72 mm.

Of the limb-bones the measurements of the well preserved and intact specimens of humerus and radius are given in Table 4 and photographs in Pl. 5, Figs. 3 and 4.

Table 4—Measurements in millimeters of the long bones of *Bos indicus*.

	Length	Proximal diameter	Median diameter	Distal diameter
Radius-Ulna-SKR-445 Taxilia	309	82	37	72
Humerus-SKR-437 Taxilla	297	94	45	81

The measurements of the long bones resemble closely the smaller, short-horned variety of Harappa (Prashad, 1936, pp. 38-39), which is also the form now met with in India. The other remains also resemble that of the smaller short-horned type of the present day. However, amongst them there is a single solitary find of the humerus of a massive and large-horned race of the humped cattle. Below are given (Table 5) for comparison the measurements of this specimen along with those of the large-horned race of the humped cattle from Harappa present in the Department.

Table 5—Measurements of the distal fragment of humerus of the large-horned race of *Bos indicus* Linn.

	Distal epiphyseal breadth	Breadth of the trochlea
Humerus—		
Large-horned race from Site SKR-I-II; Str. (9); Taxilla	102	88
Large-horned race from Cemetery site H/C, Harappa	103	92
Large-horned race from Site F/VI, Harappa	124	126

It is evident that in dimensions the single distal fragment of the humerus, referred to above, resembles closely those of the large-horned race of the humped cattle from Harappa (Prashad, 1936, pp. 38-39), particularly the specimen of a large-horned race from Cemetery Site, H/C Harappa. The photographs of the specimen excavated at Taxilla are reproduced, along with the above-mentioned specimen of a large-horned humped cattle from Harappa, on Pl. 5, Figs. 6 and 7.

Thus, the presence of two distinct forms of humped cattle from the prehistoric site of Taxilla is quite evident, as at Harappa (Prashad, 1936, p. 37), viz., (i) the large, massive form probably of the type of long-horned, humped cattle; and (i) a smaller form with short horns, as at present in India.

The remains from Taxilla also resemble very closely those of Mohenjodaro (Sewell and Guha, 1931, p. 654), Harappa (Prashad, 1936, pp. 32-37), Hastinapura (Nath, 1955, pp. 110-114), Maski (Nath, 1957, pp. 123-125), and Rupar (Nath, unpublished report).

- (d) *Bos (Bubalus) bubalis* Linnaeus (Pl. 5, Fig. 5).
(The Indian Domestic Buffalo).

SKR-I-II; stratum (9). Proximal fragment of the right scapula with glenoid cavity, neck and without tuber scapulae and blade; fragment of a rib.

SKR-II-III; stratum (9). Proximal fragment of the left tibia without proximal epiphysis of a young one.

SKR-II-III; stratum (8). One fragment of a lumbar vertebra with a portion of spinous process and mammillary process; one fragment of a lumbar vertebra with a portion of the neural arch without transverse and spinous processes.

These remains of the domestic buffalo are all fragmentary and are of no use for exact measurements. The photograph of a fragment of scapula is, however, reproduced on Pl. 5, Fig. 5; its maximum length is 157 mm., and the maximum width at the proximal end 69 mm.

The find of a limited number of fragments of bones of *Bos (Bubalus) bubalis* Linn. indicates that the inhabitants probably did not maintain large herds of this form (cf. *Bos indicus* Linn. above).

The remains of the buffaloes from the prehistoric site of Taxilla show very close structural resemblance with the skeleton of modern domestic buffalo in the Department. They are also similar to those of Mohenjodaro (Sewell and Guha, 1931, p. 659), Harappa (Prashad, 1936, pp. 43-44), Hastinapura (Nath, 1955, p. 115), Maski (Nath, 1957, p. 126) and Rupar (Nath, unpublished report).

FAMILY SUIDAE.

- (e) *Sus scrofa cristatus* Wagner (Pl. 5, Figs. 6 and 7).
(The Indian Domestic Pig).

SKR-II-III; stratum (5). Fragment of a thoracic rib.

SKR-I-II; stratum (7). Fragment of the superior border of the right horizontal ramus of the right horizontal ramus of the mandible without teeth.

SKR-II-III; stratum (8). Fragment of the left horizontal ramus of the mandible with 1st and 2nd molar teeth of a young individual.

These remains of the domestic pig are all fragmentary and belong to young individuals. It is, therefore, difficult to take measurements for comparison with other forms, but a careful examination of the bones and teeth refer them without any hesitation to the domestic race of the Indian pig, *Sus scrofa cristatus* Wagner.

The photographs of the fragment of a left horizontal ramus of the mandible, along with one of a right ramus of mandible, are reproduced on Pl. 5, Figs. 6 and 7 respectively. The maximum length of the fragment of the left horizontal ramus of the mandible is 115 mm., while the maximum length of the fragment of superior border of right ramus of mandible is 84 mm.

The remains of the pig from Taxilla are also similar to those of Mohenjodaro (Sewell and Guha, 1931, p. 660), Harappa (Prashad, 1936, p. 54), Hastinapura (Nath, 1955, p. 118), and Rupar (Nath, unpublished report).

Summary

This paper deals with a small collection of animal remains of the mid-first century B.C. to the second century A.D., obtained from the prehistoric site of Taxilla, about 22 miles north of Rawalpindi, in Western Pakistan. The number of species represented is 5, as follows:—*Equus asinus* Linn.; *Equus? caballus* Linn.; *Bos indicus* Linn.; *Bos (Bubalus) bubalis* Linn.; *Sus scrofa cristatus* Wagner. These remains are those of the domesticated animals maintained by the inhabitants of Taxilla.

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The Tata Medal Lecture

Some Aspects of the Embryology of Scorpions

Lecture delivered in Delhi on the 21st January, 1959, before the Annual General Meeting of the Zoological Society of India, on the occasion of receiving the Sir Dorabji Tata Gold Medal (Third Award) of the Society.

BY A. P. MATHEW,

(Retired Professor of Zoology, University College, Trivandrum)

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Introduction

I am very happy to be here today to receive the Sir Dorabji Tata Medal which the Zoological Society of India has so graciously awarded to me. I am very thankful to you all and am greatly honoured by this distinction. I am very grateful for the kind words of appreciation expressed by the President and for the warm words of encouragement in the Citation. May I also express my gratitude to the Vice-Chancellor of the University of Kerala who made it possible for me to be present here today as a delegate from that University.

The work in consideration of which this award is made (Mathew, 1956a, b; 1957) was done by me something like a hobby—outside my regular work—which is teaching. I, therefore, consider this as a recognition for the amateur researcher and as an encouragement to the many others like me, who, having some other task which is the "bread-earner", devote their spare time for research because of the love and the joy of doing it. Only yesterday Dr. A. Lakshmanaswamy Mudaliar in his Presidential Address at the Inaugural Session of the Indian Science Congress

gave us a beautiful account of the attitude of a good number of scientific workers as "seeking science for its own sake". To "see" and "know" are the highest rewards that the true scientific worker seeks and he can find satisfaction in nothing else.

Friends, thanking you all for the kind sentiments expressed and for your distinguished presence, I would have liked to stop. But the President and the Secretary-General have reminded us that in accordance with the time-honoured custom, the recipient of this award is to make a brief address on some salient features in the contribution meriting the award, and I shall say a few words about some aspects of the embryology of scorpions, as illustrated by the Indian species *Heterometrus scaber* Thorell.

Embryology of Scorpions

(a) Viviparity in scorpions

Viviparity of scorpions has been known from very early times. Generally they have heavily yolked eggs. Viviparity in them is merely retention of the eggs within the maternal body where development takes place. This is the condition in Buthidae. In them, the course of development is typical, with a germ band. In the Scorpioninae, however, the eggs are minute and have little yolk. The yolkless egg, developing inside the maternal body into a fully-formed scorpion, naturally shows many differences from the typical course of development as seen in the other types. Some of these differences, as made clear in my study of the development of *Heterometrus scaber* (1948, 1956a), may be mentioned.

(b) Persisting polar cells in *Heterometrus scaber*

The polar bodies persist and develop into a group of cells by which the embryo is attached to the follicle. Since the polar cells are at the anterior end of the developing embryo, fixation by them ensures proper orientation of the embryo. As the embryo is to be fed orally from the distal end of the follicle which forms the feeding mechanism, correct orientation of the embryo is of vital importance. Later, these cells give rise to a membrane surrounding the embryo. This membrane is comparable to the trophamnion of certain viviparous insects; probably they are developed for the same purpose.

(c) "Simultaneous gastrulation"

Gastrulation is peculiar. Sections prepared by me have shown that there is a hollow blastula in which, however, there is already an inward

proliferation of cells. Numerous sections were examined but I have missed the hollow sphere—so I have ventured to suggest that this precocious tendency to gastrulation may be called "*simultaneous gastrulation*". The proliferation is rapid and most sections of this stage show an already well-developed solid gastrula.

(d) Time-factor in development (heterochrony; adaptation.)

Another interesting feature is the precocity in the development of certain structures and delay in the development of others. Taking the development of *Euscorpius* as typical, we find here marked alterations in the time and order of appearance of certain structures. For instance, the very first structure that arises in the gastrula is an enormous stomodaeum and this is soon followed by the appearance of the rudiments of the chelicerae. Strangely enough, these are the very structures to be formed very late in the development of *Euscorpius*. Soon after these, the midgut also is established; in *Euscorpius* the midgut develops only very late. On the other hand, the limb rudiments appear in *Euscorpius* early, with the exception of the chelicerae which arise late. Here the chelicerae appear very early and the other limbs appear much later.

This displacement (heterochrony) in time of the appearance of certain structures in ontogeny, however, is not without significance. Following the development, one can easily see that these displacements in time are in adaptation to the particular conditions and needs of the developing embryo. The embryo, as it has no yolk provision, has to be nourished from the outside. This is done by the development of an elaborate mechanism producing nutrient matter which is fed to the embryo through the mouth. Naturally, the stomodaeum and midgut have to be developed early and since the feeding mechanism is held in position by the rudiments of the chelicerae, these too are formed very early.

(e) The feeding mechanism

Perhaps the most interesting feature I have described is the feeding mechanism by which the embryo is nourished to its full growth with material secreted from the maternal tissues. The mechanism is a simple one. When maturation of the egg takes place, a group of follicular cells lying at the distal end of the diverticulum proliferates onwards, forming a finger-shaped process called the appendix. Soon this differentiates into three regions—a terminal glandular region, a canaliferous middle region

and a converging proximal region. A little later the proximal part gets chitinised and is held between the two chelicerae, its terminal opening being directed to the mouth of the embryo. Examining early living embryos one notices pulsating movements of the midgut as the food is sucked in. Secreted food material is sucked into the midgut which is seen to be filled with a yellow nutrient matter very similar to yolk.

Heterometrus, however, is not the only instance of a scorpion with this type of feeding mechanism. Sometime ago, O. Pflugfelder (1930) described something similar in *Hormurus*, and recently M. Vachon (1950) has demonstrated another in *Ischnurus*. I have suggested that this curious type of feeding mechanism may be regarded as a natural "feeding bottle" with an automatic filling device.

(f) *Other interesting aspects*

There are many other interesting aspects in the study of scorpion embryology, such as the changes undergone by the diverticulum in shape and structure accompanying the growth of the embryo, the presence of the precheliceral and pregenital segments, the origin of the midgut epithelium, and the regeneration of the uterine mucosa after it has broken down completely during one reproductive cycle. Some of these have been dealt with in the memoir above cited (Mathew, 1956a.) and others are still being studied.

Once again, ladies and gentlemen, may I thank you all very much as I close these few words of mine.

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The Sir Dorab Tata Gold Medal has been awarded to Dr. A. P. Mathew, Retired Professor of Zoology at the University College, Trivandrum, for his important contributions to zoology published during the three-year period 1955-57.

Dr. Mathew has during this period published outstanding work on the embryology and biology of scorpions. In particular, his work on the embryology of the Indian scorpion, *Heterometrus scaber*, Thorell is an exceptionally complete and thorough piece of work and constitutes an original contribution of a high order both to descriptive as well as theoretical embryology of the invertebrates. His further studies on that species, especially those on breeding biology, the structure of the female reproductive organs and on maturation and fertilization of the ovum, are also of great interest.

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