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Monograph on *Nichollsia Khasiensis* Chopra & Tiwari 1950 (Crustacea Isopoda Phreatoicoidea ; Nichollsidae)

L. P. Gupta

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MONOGRAPH ON *NICHOLLSIA KASHIENSIS* CHOPRA & TIWARI 1950 (CRUSTACEA, ISOPODA, PHREATOICOIDEA, NICHOLLSIDAE)

By

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Introduction

The discovery of a phreatoicid isopod in wells at Varanasi (Chopra, 1947) was hailed as an incident of great zoogeographical importance because, barring a couple of species of phreatoicids from the Cape Province of South Africa, the living species of the suborder phreatoicoidea of isopods are now confined to the Australian continent, Tasmania and New zealand. Described by Chopra and Tiwari (1950) as a new species under a new genus, *Nichollsia kashiense* was found to be common in deep wells at Varanasi, and some specimen were also available from a well at Lohagara near Naini in Allahabad District of Uttar Pradesh. A few year later, Tiwari (1955) described another species, *Nichollsia menoni*, from an abandoned well in Monghyr in Bihar State, extending the distribution of the genus eastward in the Gangetic Plain. Subsequent surveys have revealed that *Nichollsia kashiensis* occurs in several other localities in U. P. and Bihar, i. e., Ramnagar (opposite Varanasi on the right bank of Ganga), Chapra and Patna (Gupta, 1980). In a personal communication to Dr. K. K. Tiwari, Prof. P. J. Sanjeeva Raj of Madras Christian College, Tambaram (Madras), informs that he has material of phreatoicid isopods from Andhra Pradesh (locality not revealed) in South India obtained during deep drilling operations for boring tube wells.

The phreatoicoidea is a primitive suborder of isopods which seems to have evolved during the palaeozoic in the old landmass known as Gondwanaland. Apart from Australia, Newzealand and Tasmania where several living species belonging to many genera still survive in surface as well as subterranean waters, recent representatives of this group have a discontinuous distribution in India, in the Gangetic Plain and Andhra Pradesh (Peninsular India), and in the Cape Province of South Africa. Outside the Australian continent, the only fossil representatives of this group have been reported from Siberia by Birstein (1962) who recorded *Paleophreatoicus soyanensis* from Permian beds. Judging from the present and past distribution, it appears that phreatoicoidea must have evolved during palaeozoic and had wide distribution in the old landmass of Gondwanaland, and has now disappeared from most of the Afro-Euro-Asian landmass, leaving living relicts in South Africa and India, and sizeable population in Australasia.

Inspite of its zoogeographical interest and biological antiquity, practically no work has been done on the anatomy, physiology, histology etc. of the phreatoicids, though the group seems to have received considerable attention from taxonomists. Nicholls (1943) in a monograph (in two parts) has thoroughly revised the group, giving detailed description of all the known species till then. Except for a brief account of the anatomy of *Mesamphisopus capensis* by Barnard (1927) and *Colubotelson thomsoni* by Engemann (1964), we have practically no information about the internal morphology and other aspects of phreatoicoidea. The group shows considerable ecological diversity inhabiting several aquatic and semi-aquatic niches on surface and in subterranean habitats and its morphological characters show an interesting assemblage of primitive and specialised features. It was, therefore, considered desirable to have detailed and in depth investigation on at least one species of phreatoicid isopod to findout the similarities that the group shares with other isopods and differences that separate them from each other. Because of its easy availability and hardiness under captivity (individuals can survive for several months in water from ponds or well at room temperature and with very little care), *Nichollsia kashiensis* was selected for the purpose.

The work is divided into a number of chapters, each dealing with one topic. The account begins with description of the external morphology, ecology and observations on feeding habits, locomotion, respiration, etc. of specimens in captivity. In subsequent chapters each organ system is dealt with separately, and the material includes morphology, gross structure and histology of each system. Where possible, experiments on live specimens to elucidate the process of ingestion of food, digestion, defecation etc., and of circulation of blood, palpitation of heart etc., were done. The result is a comprehensive account of the Indian species of phreatoicoidea, *Nichollsia kashiensis*. As such, it is the first work of its kind done on a representative of Isopoda, phreatoicoidea.

Separated as they are by time span and geographic space from their relatives in Australisia and South Africa, the Indian representatives of suborder are naturally, widely, divergent from the main evolutionary branch of the group extant in Australia, New Zealand, etc. Comparison with the Australian and S. African kins brings out certain interesting morphological and anatomical similarities and differences, which show the common affinities and evolutionary divergence of the different

stems spatially separated from each other by discontinuous stretches of land and oceans. The details of similarities and divergences are discussed separately in each.

While it is not possible to generalise on the basis of single species, it is hoped that similar studies will be undertaken on selected species of Phreatoicoid isopods from Australia and South Africa so as to provide comparative material that could throw light on the evolutionary history of the group and its relationship with other crustaceans.

DISTRIBUTION

So far, species of the genus *Nichollsia* have been recorded from a number of isolated localities in Uttar Pradesh and Bihar in the Gangetic Plains (Fig. 1). From Uttar Pradesh. *N. kashiensis* has been recorded from deep wells at Lohagara (near Naini in Allahabad District), Varanasi and Ramnagar (oppostive Varanasi on the right bank of Ganga). In Bihar, two species of this genus occur. *N. kashiensis* has been collected by the author from Chapra and Patna. Another species, *Nichollsia menoni*, has been deseribed from Monghyr.

It appears that diligent surveys in the Gangetic Plains are likely to reveal that this genus may be more widespread in the subterranean waters.

Though no published report is now available, it appear that subterranean Phreatoicoids are also available in Andhra Pradesh in South India (pers. commun., P. J. Sanjeeva Raj).

MATERIAL AND METHODS

Animals were collected from the wells with the help of plankton and ordinary nets which were dropped and left overnight and then taken out. For collection of bottom detritus an iron bucket full of water was dropped from the surface and left at the botton for 40-48 hours. While falling on the ground, the bucket strikes at the bottom and the silt mud and loose detritus is stirred and floats in the water. A part of this floating detritus and silt settles in the bucket. By this method more than 1 kg. of bottom sample was collected in one attempt.

For collecting water samples and recording temperature at the bottom and other depths, a simple device described below and improvised by the author was successfully used.

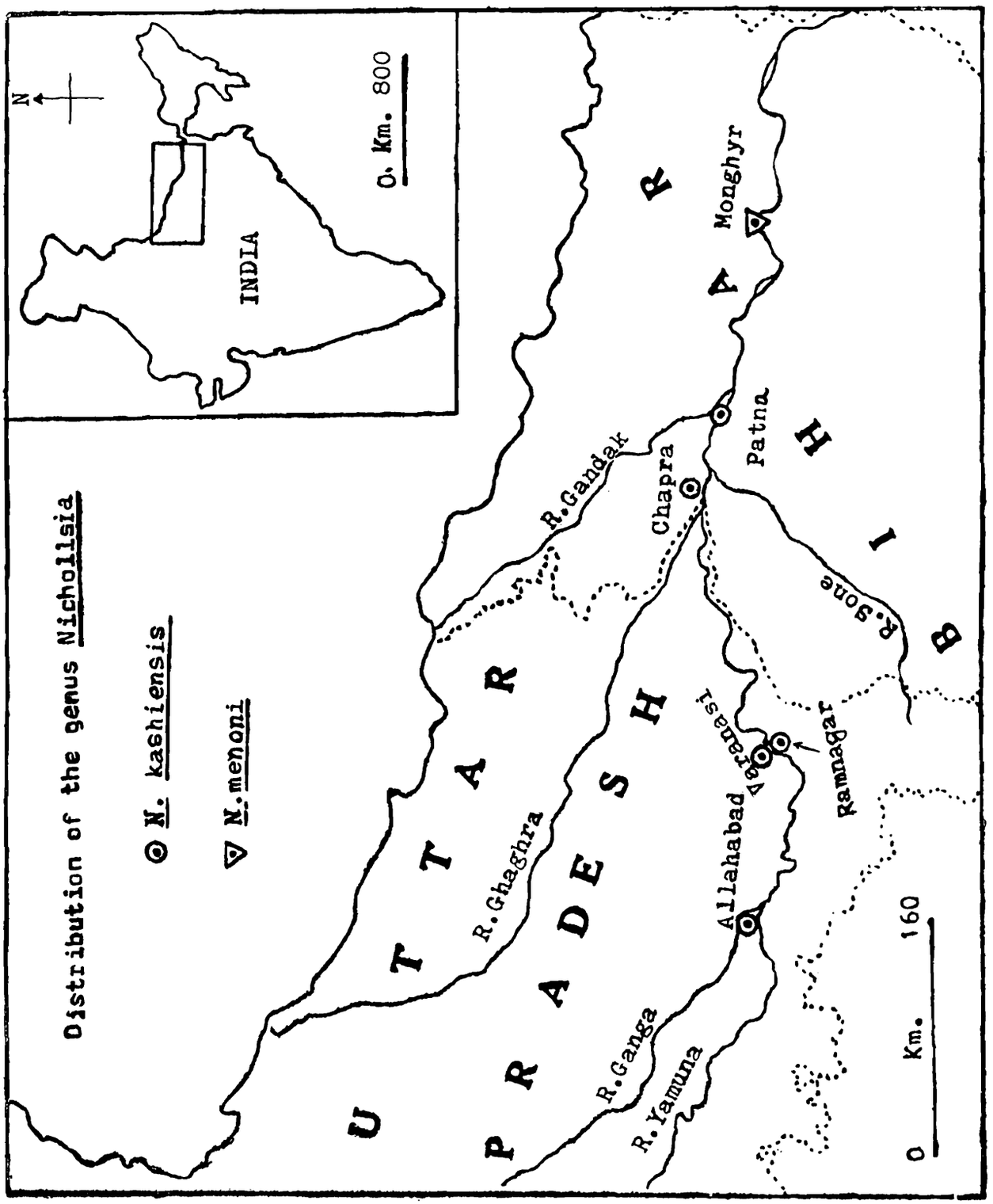


Fig. 1. Distribution of the genus *Nichollsia* in India.

Description (Fig. 2) :

It consists of a vacuum flask (f) held in position by means of two square metal plates (p.1 p.2). The plates have four small holes each on the four corners enabling them to be coupled together by means of metal rods (r) around the flask. The mouth of the flask is closed by a rubber cork reinforced with two brass rings (rg). The upper ring provides attachment to a triangular metal suspender (s). Four

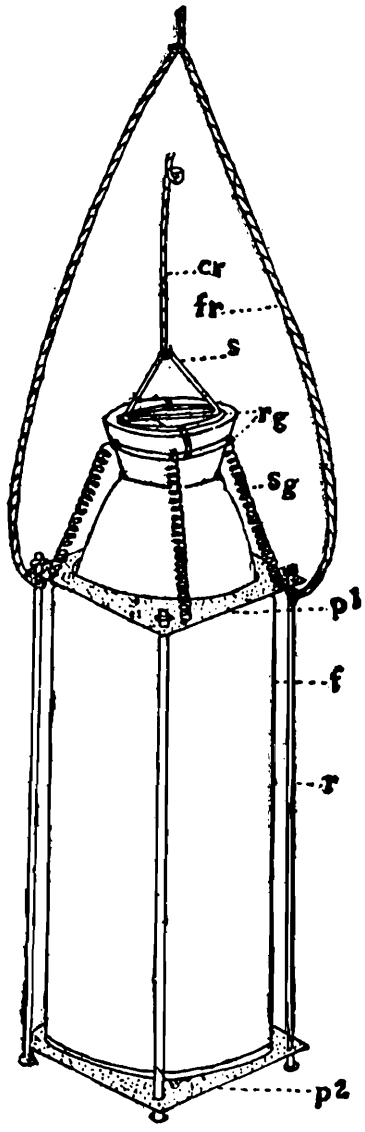


Fig. 2. Sketch of the water sampler.

springs (sg) connecting the upper plate with the lower ring hold the cork firmly, pressed against the mouth of the flask. Pieces of thick silk thread of different length tied along with the springs, limit the upward movement of the cork while opening the immersed flask.

The suspender and the upper plates are tied with long ropes (cr & fr) of different sizes or colour.

Operation :

A weight of suitable size is attached to the underside of the lower plate (A full sized brick has been successfully tried). The apparatus is then lowered into the well taking care not to let the cork rope sag or come too close to the flask rope to get entangled with it. When the apparatus reaches the bottom or any predetermined depth, the cork rope is pulled leaving the flask rope loose. This would result in lifting the cork and letting water gently into the flask accompanied by liberation of air bubbles. When bubbles cease to appear on the surface it can be presumed that the flask is full with water. The flask should be left open for a minute or two more for safety. After a few jerks to expel any air bubbles that might possibly stick to the inside of the flask, the cork rope is loosened closing the mouth firmly. Now the flask is pulled up without causing tension on the cork rope. The temperature of the water sample could be noted with a thermometer after removing the cork. Now samples of water could be drawn by an aspirator for cnemical analysis.

The following precautions, before the operation, would ensure better results : (i) Clean the inside of the flask thoroughly to prevent the air bubbles from sticking to it. (ii) Water proof the vacuum flask by plasticine to prevent entry of water between the flask proper and its metal cover.

Structure of different organs of digestive system was determined by dissections, whole mounts, and longitudinal, transverse and horizontal sections. For fixation, different fixatives were used : alcoholic bouin's Heidenhain's Susa, Zenker's solution, Helly's fixative, Carnoy's and Kolmer's fluid. Thionin (0.3%) in 10% formalin was also used as fixative and stain. Helly's fixative gave good result with digestive gland while Alcoholic bouin's and Susa gave better result as a general fixative. Thionin did not give cytological details. Stains used were Ehrlich's haematoxylene and Delafield's haematoxylene counterstained with Eosin. Mallory's triple and Hubschman's modification (1962) of Azan stain were found illustrative.

Movement of digestive fluid to the intestine was observed in isolated alimentary canal material kept in crustaceans normal saline

(Van Harrevald's 1936). Thick sections of whole animal or part e.g., head, thorax and abdomen were cut with the help of blade from the wax blocks to find out the disposition and reiative position of the internal organs and their constituents inside the body cavity.

Movement of the mouthparts during feeding was observed in the aquairium directly. In this pH of different parts of digestive system was recorded with the help of narrow range pH paper (B. D. H.).

Muscles of the digestive system were early traceable in specimens cleared in cedarwood oil after proper dehydration.

Apart from the routine histological techniques, a vital dye was injected to locate different glands in the body. For this purpose, trypan blue (1%) was injected in to the pericardium was picked up by the segmental excretory glands. This stain was made permanent in whole mounts by the following procedure :

1% Trypan blue in normal saline 3-4 hours.
↓
10% formalin in normal saline, 10 mins.
↓
Aquous Bouin's fluid, 10 mins.
↓
Distilled water, 10 mins.
↓
70% Alcohol, 10 mins.
↓
90% Alcohol, 10 mins.
↓
Absolute Alcohol, 30 mins.
↓
Absolute Alcohol, 15 mins.
↓
Xylol, 5 mins.
↓
Mount in D. P. X.

Live adults were injected with vital dyes for tracing out the course of blood. For this purpose one percent trypan blue was found very useful. A mixture of carbon particles with the above dye was also tried successfully. The fluid was injected bit by bit with the help of fine metal injection needle into pericardial cavity without damaging the heart. As the freshly moulted animals are transparent, the movement of dye could be observed under stereoscopic binocular against transmitted light. The needle was inserted between the 4th and 5th abdominal segments laterally to its mid dorsal line.

Helly's fixative proved to be better for circulatory system. Sections were stained with Mallory's triple, Azan (modified) and Haematoxylene and Eosin. For whole mount of the heart, the animals were fixed in Kolmer's fluid for 24 hours then dissected and stained. Heart beat was noted with the help of stop watch.

Routine fixatives and stains were used for morphology and histology of the pleopods. For experiments on respiration thoroughly cleaned (10 or 15) animals were starved for 48 hours and were weighted. After drying with the help of blotting paper the animals were weighted and kept in reagent bottles as described by Job (1960) with known volume of well water. After 2nd and 3 hours the water was analysed by Winkler's (Welch, 1948) method. In controlled the water was analysed before and after the experiments.

The gross arrangement of the ganglia and the nervous system is best studied by dissections of starved animals. Before dissection the animals were fixed in Bouin's fluid or first fixed in formalin (10%) and then stained in picric acid. This treatment gave yellow colour and rigidity to the nerves. For histological studies different fixatives were used, these are Bouin's fluid, Heidenhain's Susa, Carnoy and Thionin (0.3%). Before fixation the tips of appendages were encised for better penetration of the fixatives, sections were cut as usual from 6μ to 8μ and stained in Eosin/haematoxylene, Mallory's triple and Heidenhains Azan (modified by Hubschman, 1962) stain.

For chromosomal studies the animals were dissected in normal saline and testicular lobes were transferred to distilled water and following process was adopted for squash preparation.

Distilled water, 5-10 mins.
↓
Fix in Aceto-alcohol (1 : 3), 30-40 mins.
↓
Stain in 2% in Lacto-Aceto-orcein, 30-40 mins.
↓
Transfer to the clean slide in 45% Acetic acid and spread well and put cover slip.
↓
Press gently then observe and spread under microscope.
↓
Remove the coverslip in 1 : 3 Aceto-Alcohol.
↓
n-Butyl Alcohol, 10 mins. (two changes).
↓
Mount in Euparol.

Similar technique was employed for chromosomes of embryos.

GENERAL ECOLOGY

Nichollsia inhabits freshwater wells which are fed by subterranean channels. Each well represents an isolated system where the prevailing conditions drastically influence the biomass. There is less competition, the physical, chemical and biotic factors are limited and more or less uniform. Fecundity of the community as a whole is low and populations are stable. During the survey of the well fauna in Eastern U. P. and Bihar, it was observed that none of the two wells is exactly similar faunistically. However, wells harbouring *Nichollsia kashiensis* show close faunistic similarity.

Most of the wells in Bihar are accessible to superficial or surface waters either by seepage or overflow ; therefore in many wells the fauna is a complex mixture of surface and subterranean forms. Very few wells are inhabited by a purely subterranean community.

Total depth of wells surveyed, varied from 30 ft. to 55 ft. in Bihar and 50 ft. to 150 ft. or above in Eastern U. P. The water column was between 25 ft. to 100 ft. with a diameter ranging from 6 ft. to 10 ft. These wells are bound by brick walls. The bottom is made of sandy loam and silt.

Extreme narrowness of the wells acts as a barrier to light penetration to a higher depth. For most part of the year the light rays do not fall vertically above the water surface, therefore, their penetration to the bottom is doubtful. The day length at depth is very ahort and almost nil at the bottom of deeper wells. This renders the growth of rich vegetation at higher depths, and these are confined to the growth of microflora only. The microflora of a well from where *Nichollsia kashiensis* was collected consists of *Ocillatoria* sp., *Ulothrix sp.*, *Microcystis* sp., *Calothrix* sp., *Phyllobium* sp., *Microspora* sp., and *Moss* sp.

Temperature in the wells does not vary much. It remains between 25°C to 27°C and there is a difference of 0.5°C in the temperature of surface and bottom water. It rises from end of June and remains within 27°C up to October, going down 25°C maintaining constancy otherwise. From this point of view the well is a homogeneous ecosystem. The temperature is also maintained by upwelling currents from the bottom bringing this uniformity. *Nichollsia* is found in those wells where the water is being regularly drawn out. As mentioned the water level is maintained constantly by seepage from the ground source.

The water level variation is up to 15 ft. during rainy season. High water column creats a change in the pressure at the bottom. At every 60 ft. of water depth there is a rise of approximatly 1 atmosphere pressure and pressure influences, to some extent, the physiology of the animal without any visual effect.

Rainy season brings lot of changes in the concentration of water solutes from the surrounding channels promoting vegetative growth in the wells. The well water remains alkaline throughout the year between pH 7 and 8.2.

Biological community. Animals associated with *Nichollsia kashiensis* are very few and are listed below :

1. *Protozoa*—very few species are present.

2. *Insecta*—very few chironomous larvae, confined to upper zone of the well.

3. *Crustacea*

Copepoda—Cyclops sp.

Ostracoda—Physocypria furfuracea.

Amphipoda—Gammarus sp., *Indoniphargus* sp. (with *N. menoni*).

Breeding in all crustacea inhabiting the wells is a synchronized event, consequently influx in their population occurs at the same time of the year, resulting in increase of faecal discharge, exuviae and the dead animals in the habitat. As mentioned elsewhere the moulting *Nichollsia* are attacked by amphipods, ostracods and their own colleagues. Cannibalism may not be so frequent in natural habitat as it is observed in the aquarium, because the space in aquarium is limited and is comparatively overcrowded,

All the copepods, ostracods and amphipods associated with *Nichollsia* at Varanasi show subterranean adaptations like loss of eyes, loss of colour pigment etc.

Vertical Migration :

In all most all the wells it was found that individuals of *Nichollsia* migrate upward along the wall and while taking water one very often comes across few specimens in his bucket. This is very common during the winter season. This phenomenon was noted from November to January. It is well known that they are not good swimmers, therefore

the possible and only reason for migration above the bottom seems to be the dearth of food. They come up to the surface for grazing on vegetation growth on the bricks. Influx of population due to release of broods may be the other possible reason for such migrations.

Parasites. In its natural environment *Nichollsia kashiensis* not confronted by many parasites. There appear to be very few external or internal parasites.

External parasites :

In deeper wells at Varanasi, *Nichollsia kashiensis* is not found with external parasites. But specimens collected at Patna, where the wells are not deep, were found infected with protozoans and fungi. The fungi cause black spots on the body wall of the animal. Their infection intensity is reduced by using Sodium Chloride in the aquarium.

In aquarium *N. kashiensis* is attacked by snail *Indoplanorbis* sp., amphipods and the ostracod *Stenocypris malcolmsoni.*

Internal parasites :

Many secimens of male, female and youngs have been examined for histological purposes, but only one male specimen collected from Ramnagar (Opposite Varanasi, U. P.) was found infected with gregarine parasites. Eggs and cysts of parasite were found in the body cavity particularly around the vas deferens in the region of the penis.

Observations in the Aquarium :

When live *Nichollsia kashiensis* were transferred to the aquarium, their mortality for about a month, was very high later they adjusted and mortality rate went down. Many aspects of behaviour related to feeding, respiration, moulting and reproduction have been given in the respective chapters in detail.

Before transferring the animals, the aquarium water was treated with Methylene blue, Sodium thiosulphate and Sodium Chloride at different times for dechlorination and check of fungal growth etc. Similar treatment was given to aquatic plants, added to the aquarium for experiments on food and feeding behaviour of the animal.

Different types of Substratum like, mud, pebbles and stone chips were tried, but red sand of middle order was found very suitable for

successful rearing of these animals in the aquarium. Few stone chips or cement blocks offered them shelter and rough surfaces for ecdysis.

Pond, river or tap waters (not underground) did not induce breeding for longer time. The underground water supplied through tap at Patna could induce breeding in the laboratory. It needed frequent change of water for maintaining temperature between 25°C to 28°C.

Animals were maintained and could survive well between the range of 19°C to 34°C temperature variation in the aquarium. Sudden change from 25°C to 35°C caused death of many individuals if the higher temperature was maintained for more than 3 hours. Moulting, feeding and pleopod beating were very much effected by fluctuation in temperature.

Locomotion : *Nichollsia kashiensis* is not a swimmer and its mode of locomotion is by walking and running. As usual it has developed a half hanging stance, highly adapted for clinging.

For interpretation and understanding of the gaits of *Nichollsia* we have to look at the arrangement and orientation of the legs. The posterior 3 pairs of legs are directed backwardly and are generally passive during walking and remain above the ground. They help the animal in walking along the wall of the aquarium or in hanging the animal from a substrate in upright position.

The anterior 4 pairs of legs usually participate during walking. Amongst these 4 pairs only 2nd, 3rd and 4th peraeopods always participate in sequence of 4, 2, 3 or 3, 4, 2 whatever way we interprete it. The gnathopods move either together (left and right) or alternate with one another. Sometimes the animal keeps its gnathopods raised with its dactylus folded over the propodus.

During walking, the anterior legs touch the ground or substratum by their mero-corpal joint or tips of the dactylus or both at a time. *Nichollsia* can walk backward but not very effectively. They can climb on the walls.

When disturbed or obstructed *Nichollsia* often turns back by rolling on its head and back or moved forward by vigorous blows of abdomen which drives tne animal forward.

Sometimes the animal stands on its uropods and swims up with the longitudinal axis of the body in a vertical position by wriggling movement. In this type of upswimming the animal sometimes rotates

anticlockwise around its longitudinal axis. While falling down in the water the animal fans out its pleopods at right angle to the longitudinal axis.

Burrowing : *Nichollsia* has been found burrowing in the soft mud or sand of the aquarium. This was carried out with the help of strong gnathopods.

Response to light : *Nichollsia* in aquarium has been found to avoid light. They aggregate towards the darker end of the aquarium lying down in crevices or under vegetative or algal masses.

Aggregation : Very often they are found crowding in one or the other corner of the aquarium. Aggregation in wood louse has been atributed by Sutton (1972) as a result of thigmokinetic behaviour. He is not certain about the biological significance of this behaviour in oniscoids, similar position is maintained in *Nichollsia kashiensis*.

Many workers (Banta, 1910 a, b,) ; Fage, 1955 ; Ginet, 1960 ; Vandel, 1965) have studied the effect of light on subterranean forms and have found that they are negatively phototropic, Ginet (*op. cit.*) found that strong light is harmful to *Niphargus* and a light intensity of 20000 lux causes the death of *N. virei* in two to three days while *Gammarus pulex* withstands 40,000 lux for 25 days. However *Nichollsia kashlensis* was not experimented from this point of view but aquarium was exposed to sunlight during day time for many days without any apparent harmful effect.

CLASSIFICATION

Chopra & Tiwari (1950) described the genus and species *Nichollsia kashiensis* from India. They placed it in the sub-family Nichollsinae of family Amphisopidae belonging to the suborder Phreatoicoidea. Later on Tiwari (1955b) erected a new family Nichollsidae to accommodate this genus. In the same year Tiwari (1955a) described another species *Nichollsia menoni* from India (Bihar). So far only these two species are known. The following key is adopted from Nicholls (1943) Tiwari (1955) :

I. Key to the suborder of Isopoda :

A. Uropods lateral—

C. Body sub-cylindrical, fusiform ; pleon appearing compressed. Uropods ambulatory ; pleopods natatory and respiratory ... Phreatoicoidea

D. Body depressed (Semi-cylindrical) :

E. Uropods forming, with tailpiece, a caudal fan ; pleopods natatory and respiratory ... Cymothoidea

F. Uropods valve like, inflexed, meeting beneath pleopods which are largely branchial ... Idoteoidea

B. Uropods terminal, body depressed—

G. Pleopods exclusively branchial

J. Third pair generally modified into a thin opercular plate ... Aselloidea

K. Pleopods never covered by opercular plate, parasitic forms ... Bopyroidea

H. Pleopods adapted for air breathing ... Oniscoidea

II. Key to the families of suborder Phreatoicoidea :

A. Lacinia mobilis on both mandibes.

C. Endopodite of pleopods mesially cleft ; Outer ramus of uropod longer than the inner. (Monotypic : India) ... Nichollsidae

D. Endopodite of pleopods entire ; outer ramus of uropod shorter than the inner ... Amphisopidae

(S. Africa, Australia, Tasmania).

B. Lacinia mobilis only on the left mandible... Phreatoicidae

(Bassian region of Australia, New Zealand).

III. Key to the species of *Nichollsia* :

1. The postero-lateral edge of telson with two widely separated notches and undulating in its upper half ... *N. kashiensis*

2. The postero-lateral edge of telson is smooth in its upper half ... *N. menoni*

Both the species have been reported from India only.

EXTERNAL MORPHOLOGY

Chopra and Tiwari (1950) have given full description of the external morphology of *Nichollsia kashiensis* in so far as it was relevant to the taxonomy of the species. During the course of my studies, I have been able to discover extra features overlooked by Chopra and Tiwari. The present narative, though drawing largely from the earlier account also furnishes additional morphological information about the species.

Body (Fig. 3A, Pl. I.). The body is long, slender and vermiform. Width is uniform throughout. Length of body measures 14 to 16 times its width in adult specimens. Except for a few short and stiff hairs, the body is smooth and devoid of any sculpturing.

Head (Fig. 3B). Head is rather short. It is somewhat shallow, being longer than deep. Width and depth are equal. Its dorsal surface is faintly convex and shape is roughly rectangular in dorsal view. The antero-dorsal margin of the head is strongly convex, the front sloping steeply forwards. Fronto-lateral corner (fr. p.) is produced into a large angular projection on each side. The subocular incisure is deep. Antero-ventral corner of the head is bluntly angular, and behind this the sub-orbital notch (sub. orb.) is conspicuous. A shallow genal groove (gen. gr.) begins from the hinder angle of the sub-ocular incisure and ends in the sub-orbital notch. Behind the sub-orbital notch, the ventral edge of the head forms an oblique, sloping line with the mandible. Posterior process is wanting and post-mandibular portion of the ventral margin of head is horizontal, and equal to mandibular region. Postero-dorsal margin of head is convex, the sides descending obliquely forwards up to the middle, from where they descend vertically to meet the ventral border. This vertical lower half of the postero-lateral edge is slightly overlapped by antero-ventral expansion of the first peraeon segment. Cervical groove (c. gr.) is conspicuous only on sides. In younger examples and in some adult also, (Fig. 8A) a faint groove, parallel to the ventral edge of head is present, and it meets the ascending arm of the cervical groove. Eyes are absent. The ocular area (which is very prominent in fresh specimens) is seen as a shallow triangular depressions preserved in spirit for a long time.

Peraeon (Fig. 3A). Peraeon is long with uniform width and depth. It is wider than deep. The first segment (per. 1) is very short in mid-dorsal line, measuring from one-third to one-fifth of the dorsal

length of head. The antero-dorsal margin is strongly concave, the corresponding posterior margin being straight, The antero-ventral border of this segment is expanded and overlaps the lower half of the head. Segments two to four show a progressive increase in length, the fourth being the longest ; posterior to fourth the remaining segments

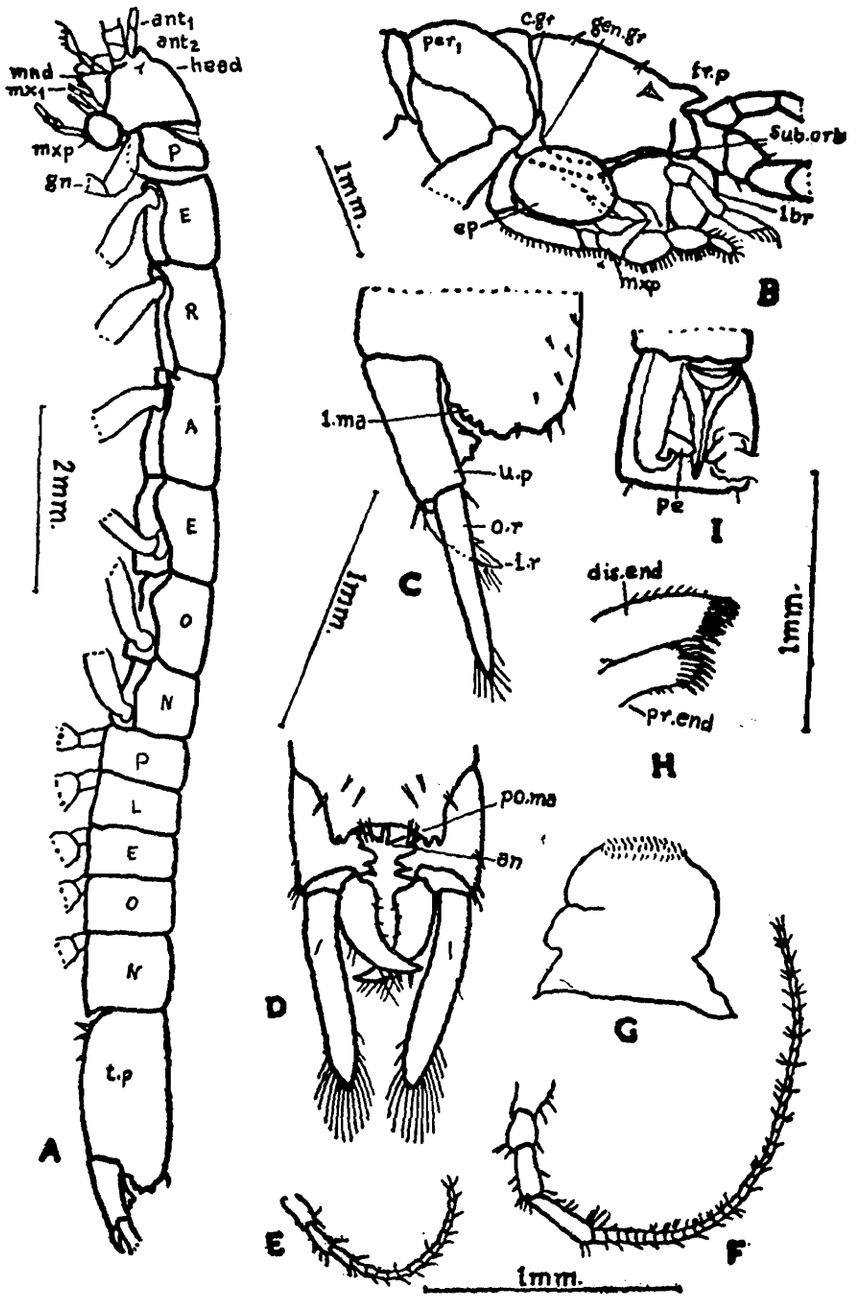


Fig. 3. (From Chopra & Tiwari 1950).

A. Lateral view of the animal. B. Lateral view of head. C. Posterior end of tailpiece and uropod. (♀). D. Dorsal view of the same. E. Antennule. F. Antenna. G. Labrum. H. Maxillule. I. Ventral view of seventh peraeon. ♂.

progressively decrease in length. Each segment is longer than wide and wider than deep. The anteroventral corners of second to fourth segments are produced in front of their respective coxal attachments. Postero-ventral corners are rounded. In fifth to seventh segments both antero and postero-ventral corners are rounded and ventral edges are slightly emarginate in the region where the legs are attached. The sterna of all segments are compressed, and produced posteriorly, this compression being more conspicuous in the posterior segments. They are keeled in the middle.

Pleon (Fig. 3A, C, D.). Pleon, when compared with that of other subterranean genera of the family Amphisopidae, is rather long. The great increase in length of the pleon is partly due to elongation of tail piece (t. p.), which is more than half as long as the rest of pleon segments taken together. Pleon is as wide as peraeon, but deeper than it owing to a slight downward extension of the pleura, which, however, do not cover the sympodites of pleopods. First four pleon segments are short, sub-equal and wider than long, the fifth is longer than the rest. Depth of the pleon segments is distinctly greater than their length or width. Free pleural margins of each segment are straight, posterior corners angular, and anterior one's blunt. The tail piece (sixth pleon segment and telson) is one and a half time as long as deep, and twice as long as wide. It's dorsal surface is rounded and ventral surface flat. The antero-lateral margin of tail piece obliquely ascends upwards, attachment with the fifth pelon segment being in the upper half. Antero-ventral angle is rounded. Ventral margin is straight, and anteriorly it bears three backwardly directed spines. Telsonic apex (Po. ma. and la. m.) is strongly emarginate. Dorsally the telson (po. ma.) is of the shape of a shallow horse-shoe. Below the Postero-dorsal corner, the free lateral edge (l. ma.) is turned downwards and backwards in an undulating curve, the upper half containing two rather widely separated notches and the lower half being more compactly crenulated. As it reaches the region of uropodal peduncle (u. p.), this descending edge turns anteriorwards, running parallel to, and in contact with the peduncle, behind which it steeply turns down vertically to meet the ventral edge of tail-piece at right angles. The ventral surface of tail piece is flat with parallel sides, the anterior margin straight, and posterior margin produced mesially into an angle between uropodal peduncles. Anus (an) is a vertical slit on the posterior face, above the insertion of peduncle of uropods. The

anal opening is guarded by the two semicircular anal plates (Fig. 17C, an. pl.) which are cuticular.

Appendages :

Antennule (Fig. 3E). Antennule is large and filiform, consisting of 13-16 joints in adult. It exceeds the peduncle of antenna by four to six of its terminal joints. First three or four joints are longish, the distal joints become progressively short and narrow. There are usually five types of setae present mostly near the distal end of each segment. The most important of these are the olfactory setae or Aesthetascs (Fig. 8B aes.). They are as supposed, chemosensory in function. Simple spines around the aesthetascs are considered by Rath (1891) as protecting setae to the olfactory setae. Number of aesthetascs varies in the developmental stages of the animal. There is a single aesthetasc at the terminal end of the antennule in the newly hatched young. In a large adult male of 3 cm these setae are present on 10 distal segments of the left antennule and 9 on right on its inner side. There is variation in the number of segments bearing aesthetascs on left and right antennule of the same animal. Number of segments bearing aesthetascs in female is less than the number in male of the same size. Generally there is a pair in each segments but some times there are 3 in some segments. The terminal aesthetasc is always single. Each aesthetasc has a stalk and the main body. The main body is a cylindrical elongated tube with wide lumen and possesses a large apical pore. Annulation is absent. In an adult individual the main body of aesthetasc is 0.05 mm in length while the stalk is 0.03 mm while the maximum diameter of the main body is 7μ. Other main types of setae present on antennule are plumose and rod or simple setae (Fig. 8B pl. se, s. se.). Simple setae are mostly confined to the distal segments while plumose setae are confined to the basal segments. Plumose setae are considered to be auditory in function. Few microtrichs arranged in crescent forms are present on the first peduncle segment.

The antennule of *N. kashiensis* is longer than that in other subterranean genera. Nicholls (1943, p. 6) believes this type of antennule to be more primitive than the short, club shaped antennule found in a large number of Phreatoicids. A long filiform antennule is characteristic of the Amphisopinae and Phreatomerinae among Amphisopids.

Antenna (Fig. 3F). Antenna is moderately long, being somewhat less than half the length of body. The five jointed peduncle is almost

half as long as the flagellum. First three joints of peduncle are short and subequal, fourth is longer and fifth is twice the length of the fourth. Flagellum consists of thirty to forty five joints. The basal joints are longer, and length of the joints progressively diminishes towards the distal extremity. A few stiff hairs are present. These are plumose, rod and acuminate setae. The plumose setae are confined to proximal segments while the acuminate and rod setae are mostly on the distal segments.

Labrum (Fig. 3G). It is large and asymmetrical and resembles that of *Mesamphisopus capensis* (Barnard, 1914, p. 233, and Nicholls, 1943, p. 31). There are very fine setae arranged along the median ridge on the ventral (Fig. 10B) side of the labrum. The open surface is also provided with fine setae near its anterior margin. All the ventral setae are directed inward towards mouth.

Mandible (Fig. 4A) : Both mandible possess lacinia mobilis. They form an oblique sloping line with the ventral edge of head, behind the suborbital notch in which fits the fulcral process of mandible. The left mandible is larger than the right. The incisor (i. pr.) of the left mandible bears four strongly chitinised teeth. Its lacinia mobilis (la. m) is tridentate and chitinised. Spine row (sp.) is borne on a ridge sub-parallel to the lacinia mobilis and carries about half a dozen spines, pectinate on one side. This is followed by a row of plumed setae, about eight or nine in number, borne on a ridge between the base of spine row and molar. Molar process (m. pr.) is well developed ; its anterior and lower margins are fringed with cilia, the lower most five or six of which are long and setose. These lower setose cilia actually lie dorsal to the molar and they reach to the opening of the oesophagus and help in directing food directly into the mouth. The shallow convex shaft also appears to be ciliated. Ridge pattern on the left molar surface is concentric but on the right (Fig. 9B) it is transverse. Significance of this asymmetry in ridge pattern on the two molar surfaces is not very clear. It seems that it provides more efficient mechanism for crushing and grinding detritus and vegetable matter. So far as I know, in almost all other members of the suborder Phreatoicoidea the ridge pattern on both mandibles is transverse and this may be considered as a family character. A fringe of cilia also borders the bend between the incisor and molar. Acetabular process (ace.) is well developed. Fulcral process (f. pr.) is coniconical and prominent. The palp (mnd. p.) is three jointed, first joint

is short, second nearly double the length of first, and third sub-equal to second and compressed. Inner margin of the second joint is ciliated. The third joint is broadly triangular, its distal half on the inner side being concave. The apex of the third joint bears eight or nine long

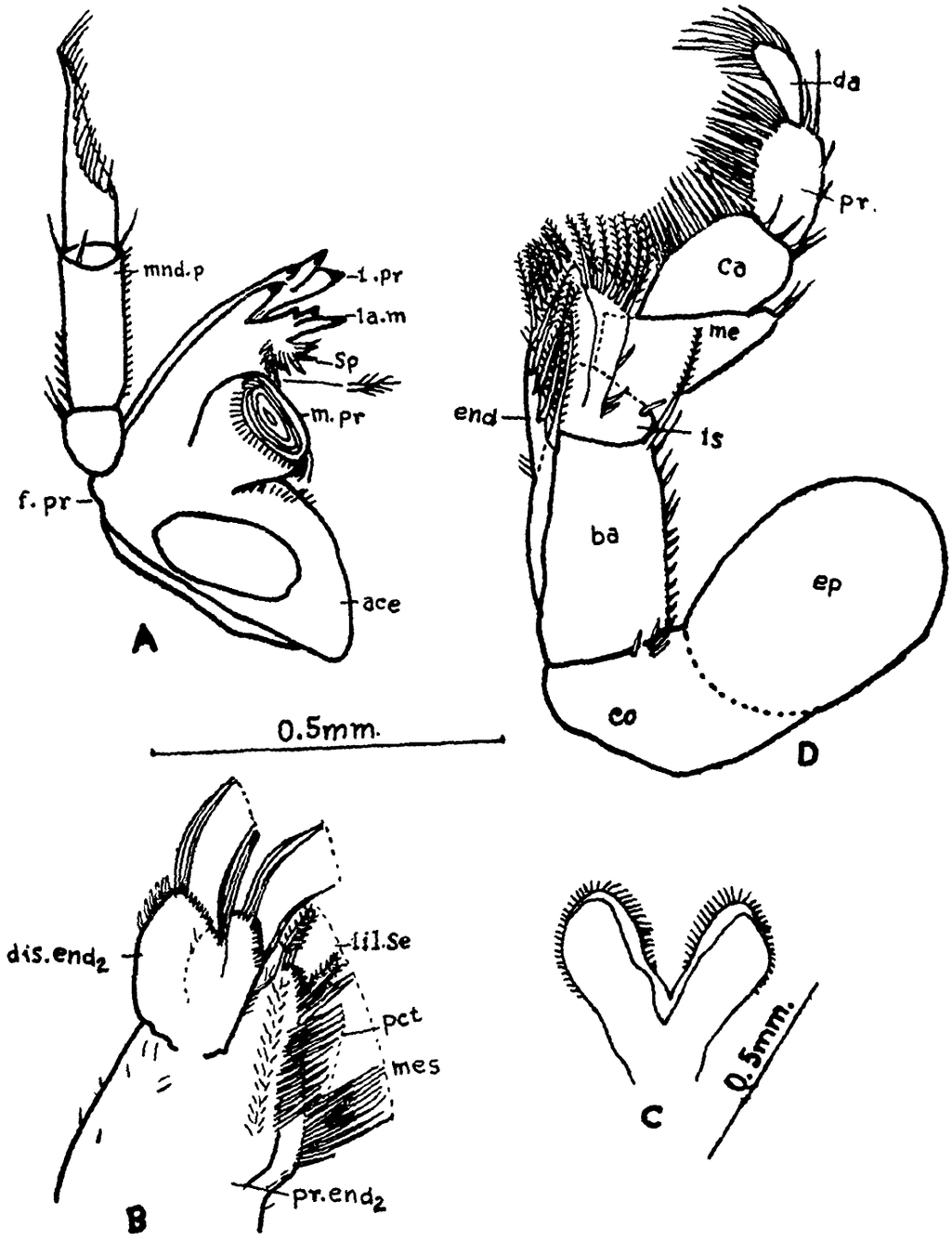


Fig. 4. (From Chopra & Tiwari 1950).

A. Left mandible anterior view. B. Right maxilla posterior view. C. Labium. D. Right maxilliped.

finely denate setae ; the inner concave margin carries a fringe of about two dozen shorter dentate setae. Other joints are beset with scattered stiff setae. The dentate setae on the palp help in cleaning the antenna and antinnules. There is, on the outer surface of the palp, a well developed system of fine hairs (microtrichs) of a much smaller magnitude (approximately 2-8μ). Typically they are arranged in short crescentic rows. The number of these crescentic rows are less on the first and third but much more on the second segment. The hairs are much smaller on the first segment than others. In the right mandible, incisor is weekly chitinised, lacinia is non chitinised and its teeth are finely serrate, and the fringe of cilia on the margin of molar shaft is longer. The spine row carries a smaller number of pectinate and plumed spines.

Maxillula (Fig. 3H). Proximal endite (Pr. end.) is short, its apex broad and truncated. The apex carries a row of nine hairy spines of which the first and last are short. The distal endite (dist. end.) is longer, and somewhat broader than the proximal. The apex bears about fifteen pectinate spines, disposed in transverse rows of three each in the proximal series and five or six in the distalmost. These spines are slightly chitinsed. The distal half of the posterior edge of the proximal endite and that of the anterior edge of distal endite are fringed with cilia.

Maxilla (Fig. 4B). Maxilla shows typical amphisopid condition. The proximal endite (Pr. end 2) is triangular with a broad base and narrow, truncated apex. The mesial edge (mes.) is almost straight, with no sharp demarcation between the basal and apical regions. The row of filtratory setae (fil. se.), about 36 in number, is well developed, but the setae do not appear to be ciliated. Submarginally, posterior surface bears a row of about fifteen pectinate spines (pct.) which continue up to the apex, and behind this row of spines there is a row of dense cilia. Basally the posterior surface is fringed with small cilia. On the anterior side there are four or five setae in the submarginal concavity at the base. Apex of this endite bears about fifteen setose spines and its distal free margin is ciliated. The middle and outer lobes of the distal endite (dis. end$_2$) are broader and more elevated than the proximal endite. The apex is oblique anteriorly and bear dentate spines, which are about a dozen in number on the middle lobe and about a dozen and a half on the outer. Free margins of these lobes are ciliated. The outer lobe, behind the apical dentate spines,

caries a few short and simple spines. Maxillae are symmetrical in both sexes. The basal portion bears crescents of microtrichs towaads inner margin.

Libium (Fig. 4C). Is large and bilobed. Apex of each lobe is densely ciliated with 4 conate setae on the inner side ; towards the base, the cilia are shorter and sparse.

Maxilliped (Fig. 4D ; 7A, B) : Maxilliped does not show any marked departure from the usual Phreatoicine pattern. Coxa (co.) is short and its epipodite (ep.) is large, broadly elliptical and its dorsal margin is free from cilia and hairs. Fine elongated hairs are present along its lower and anterior margin. The inner surface has a group of hairs along the mid longitudinal line and another group of longer hairs

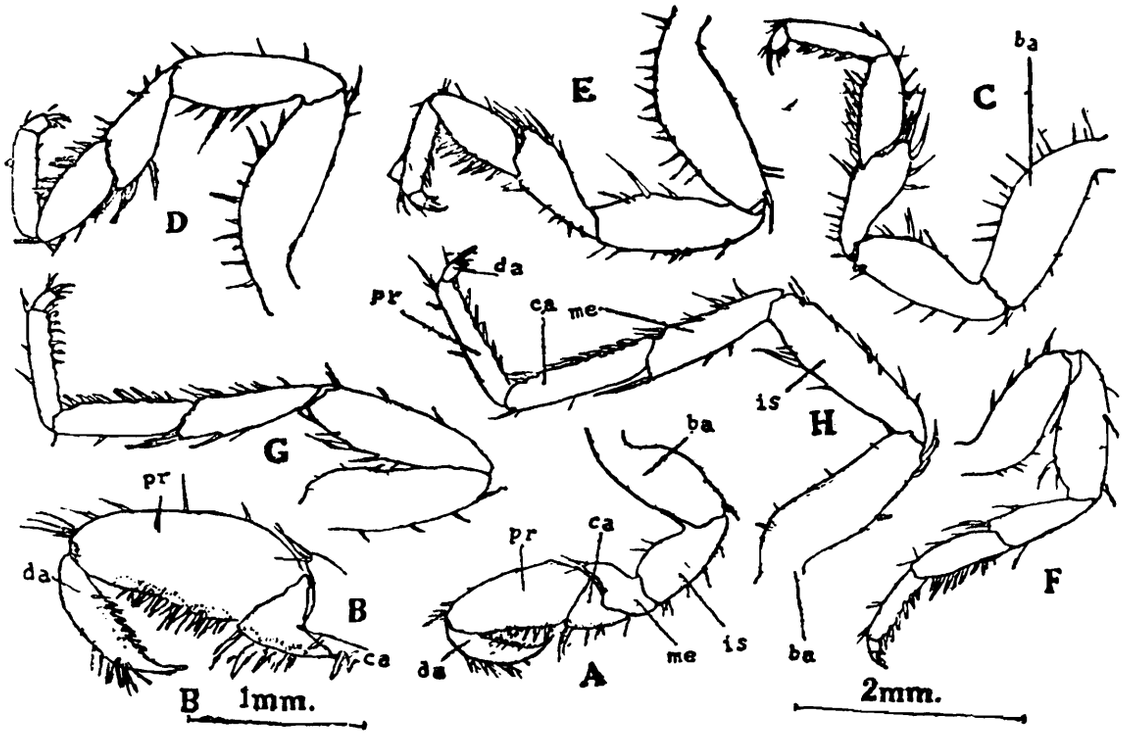


Fig. 5. (From Chopra & Tiwari 1950).

A & B. Gnathopod (male). C. Second peraeopod (male). D. Third peraeopod (male). E. Fourth per aeopod (male). F. Fifth peraeopod (male). G. Sixth peraeopod (male). H. Seventh peraeopod (male).

along the line subparallel to the ventral margin. Basis (ba.) is twice as long as broad, fringed with a row of simple, short setae on its outer edge, and a single large plumed spine on the posterodistal corner. Endopodite (end.) arises in the middle of the basis. It is broad distally and extends upto the end of triangular merus. Its antero-mesial and

distal edges are fringed with long, plumed setae. A single large coupling hook (co. h.) is present in the usual position. The coupling hook is curved a little in its distal half and possesses two rows of teeth, seven in each row at its termination. These teeth help in interlocking the two hooks. Posterior edge is bare except for a few short simple simple setae. Ischium (is.) is short. Merus (me.) is subtriangular, with a short postero-distal projection bearing two simple setae. Carpus (ca.) and propodus (pr.) are elongated oval, fringed with a row of long simple setae on the anterior, and only a few setae on the posterior edge. Dactylus (da.) is short, narrow and elongate, with apex fringed with long setae. The microtrich system (Fig. 7A, 9F) is well developed on the maxilliped. The microtrichs are present in the form of crescentic rows and straight rows on coxa, basis and endopodite.

Peraeopods (Fig. 5A-H). Peraeopods resemble those of *Hyperoedesipus plnmosus* (Nioholls and Milner, 1923 pp. 23-33, pls. II-V and Nicholls, 1943, pp. 49-57, figs. 12, 13) except that, gnathopod and fourth peraeopod are not sexually modified in males. Coxae of all peraeopods are fused with the epimera of their respective segments ; the first four pairs are attached on the anterior and last three on the posterior end of the segments.

Gnathopod (Fig. 5A, B). It is similar in both sexes, resembling that of the female of *Hyperoedesipus*. It is short and stout. Coxa is broad. Basis (ba.) is long and moderately expanded. Ischium (is.) is shorter than basis. Merus (me.) is sub-quadrangular, and its antero-distal angle is some what produced. Carpus (ca.) is short and triangular. Propodus (Pr.) is about as long as the basis and moderately expanded. It is narrow basally ; its anterior (dorsal) edge is convex and posterior ventral straight. Its posterior margin, bears five or six spines, and a sub-marginal row of a few short hairs. The dactylus (da.) is shorter than the ventral margin of propodus and is usually flexed below the latter. Its inner edge, facing the propodus is straight, the outer edge being somewhat convex. Distally it tapers into a long, narrow, inwardly bent claw. The posterior edge (which faces the propodus when in flexed condition) is dentate, carrying about eight short forwardly directed teeth and sub-marginal row of six setae. Free edges of all joints of the gnathopod are beset with scattered spines, which are more numerous on the anterior margin of the dactylus and antero-distal angle of palm. The carpus bears four short spines and about half a

dozen setae on its posterior edge. Number of teeth and spines on the posterior margin of different segments of the gnathopod on left and right side are usually not the same in the same or different individuals.

Of the succeeding pairs of peraeopods, those of the anterior series of three are some what stouter than those of the posterior series. In the second, third and fourth peraeopods bases are expanded a little and individual joints are some what compressed. Merus (me.) is slightly produced antero-distally. Posterior three pairs, fifth, sixth and seventh are slender and their individual joints are elongated, the antero-distal projection of merus being more obsolete. Dactylus in all peraeopods is very short and ends in a stout, curved claw which bears a short unguis at its base. Second peraeopod in some large adult males bears two unguis or short teeth at its base. The posterior border of propodus bears a row of spines four or five in the anterior series, and seven or eight in the posterior. Edges of all legs are fringed with setae and spines, which are more numerous on the posterior edge of carpus, and anterior distal angle of merus. The setae and spines on the ventral margin of these peraeopods varies, usually one less or one more than the other individual.

Coxae of seventh peraeopods bear a pair of long penes (Fig. 31 pe.).

Pleopods (Fig. 6 ; 7 C-E). Pleopods are foliaceous, respiratory and natatory in function. Epipodites are absent, and the sympodites are not covered by the downward extenaion of pleura of pleon segments. Coupling hooks are absent, but each sympodite bears two entangling setae on the inner side, carried on a slight ridge, the upper setae being long and the lower one very short. Exopodites and endopoditee are smooth and exopodites are provided with few simple small spines scattered along the margines and surface while endopodites are are free from any setae. Exopodite Lobe is very laterally displaced. Endopodites are very reduced and cleft in the middle. On exopodite below the junction of lobe along the inner margin there are a number of crescents of microtrichs (m. tr.) arranged linearly. Their number and arrangement however differs from those of *Asellus aquaticus* (Needham, 1942). They are present on the anterior and posterior face of the exopod and their number varies in different pleopods from 20 on first pleopod, 14 on 2nd, 3rd, and 4th and 9 on 5th pleopod.

Unlike other phreatoicids, the posterior pleopods are not much shorter than the anterior ones. First three pairs are sub-equal, fourth slightly shorter and broader than the first and the fifth is a little shorter than the fourth.

First pleopod (Fig. 7C) has an elongate oval, smooth exopodite

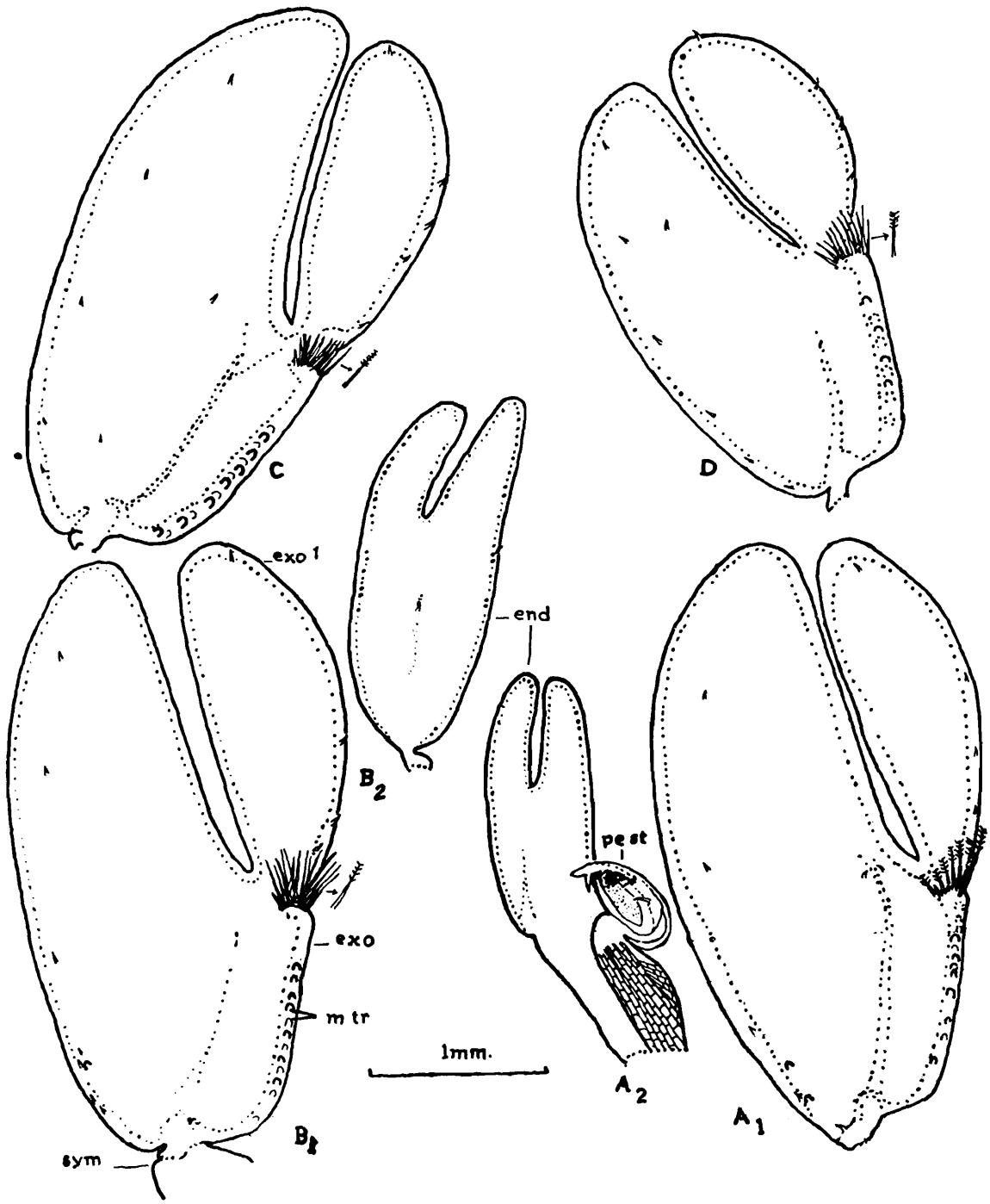


Fig. 6. A_1 A_2 Second pleopod (exopod & endopod). B_1 B_2. Third pleopod (Exopod & endopod). C. Fourth pleopod (exopod). D. Fifth pleopod (exopod).

(exo.) whose apex is entire and somewhat narrow. The endopodite (end.) is very narrow and about half as long as the exopodite. A median longitudinal cleft extends from the distal extremity, up to one third the length of the entire endopod, giving it an appearance of a 'tuning fork'.

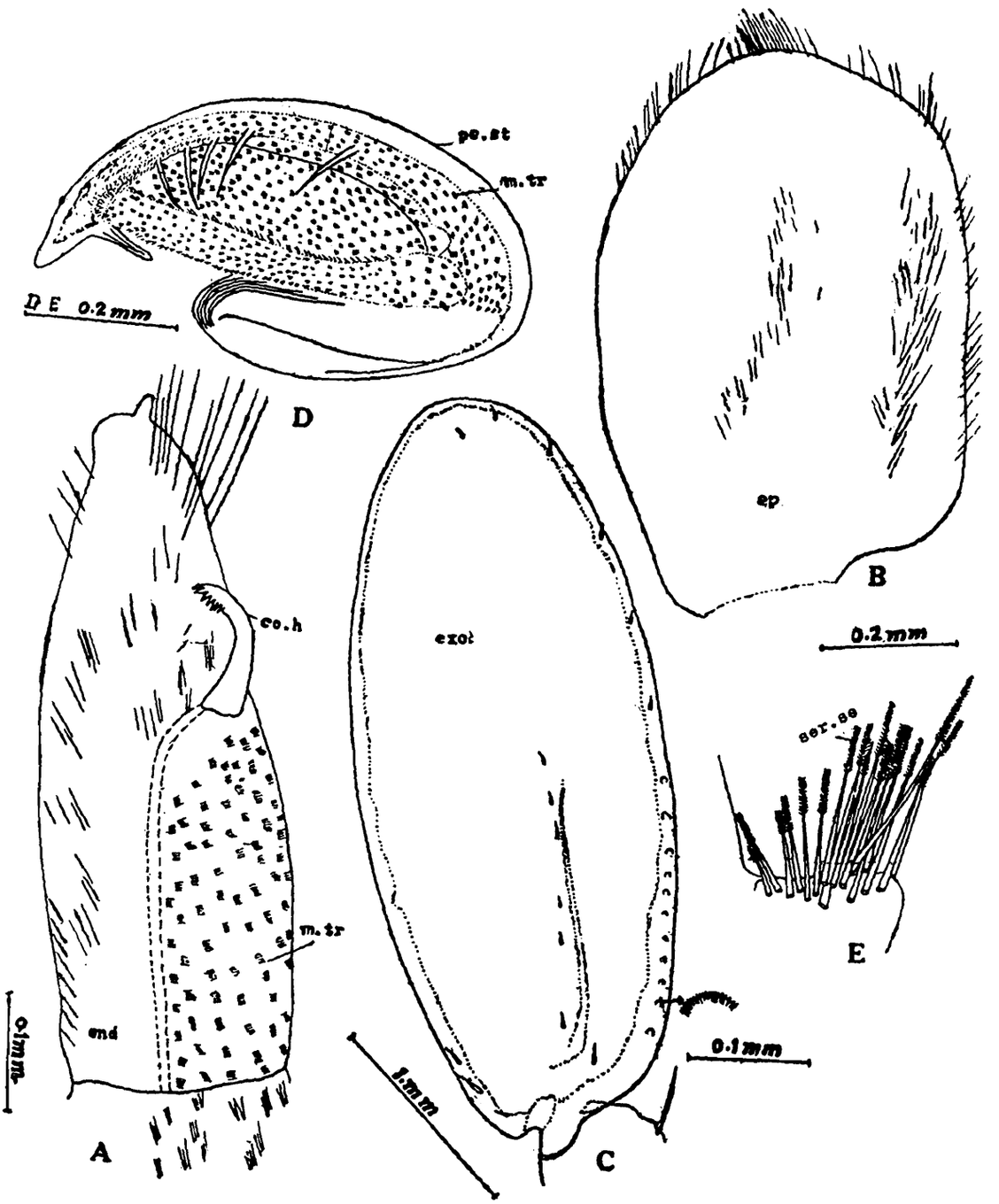


Fig. 7. A. Endopodite of maxilliped B. Epipodite of maxilliped. C. Exopod. of first pleopod. D. Penial stylet. E. Serrate setae on the junction of exopod. lobe.

Exopodites of second to fifth pleopods are bilobed. In second pleopod (Fig. 6A1, A2) the exopodite, which is slightly longer than that of the first, is broadly, triangular with a narrow rounded apex. The inner margin is oblique up to two-thirds of its length, where it meets the shaft that bears the lateral lobe (exo.l.). The lateral lobe is long and narrow and springs from a triangular shaft, which projects from the inner margin in the basal third of exopod. The lobe is narrow basally and broadens out apically. External to the insertion of the lobe, the shaft bears numerous serrated spines. Presence of serrated spines and microtrichs along the inner margin and above the afferent canal indicates that they are probably sensory and have some relation with the flow of the blood. The endopodite in female is similer to that of the first pleopod. In males, however, endopodite of second pleopod bears a complex penial stylet (Fig. $6A_2$; 7D, pe. st.). In this pleopod, the endopodite is long and consists of a muscular peduncular region and a distal forked portion, with a feeble constriction at the junction of the two regions. The penial stylet springs from the apex of the peduncular region, and except at the point of attachement, is completely free from the endopedite. The muscular base of the stylet lies apposed to the endopod for some distance, beyond which it takes a sharp downwards bend. The body of the stylet consists of a thick muscular rim, enclosing a membranous area. The inner margin (which is away from the endopodite) is strongly convex and thick, and projects beyond the straight outer margin (facing the endopod) in the form of a beak. The margin facing the endopodite is thin and straight and there is a stout spine at the angle where the outer and inner margins meet. The inner margin bears five, less stout, narrow spines. The outer and inner margins of the penial stylet enclose between them a deep groove on the anterior face, lined by a membranous surface, looking very much like an elongated sauce pan. The whole cavity and anterior floor of penial stylet is covered by fine microtrichs or cilia arranged in groups of two on the outer rim and three and four on the floor of the depression ot cavity. Just behind the subterminal spine the microtrichs are single. The five spines mentioned above on the inner margin, overhang above the cavity of the stylet.

Third pleopod (Fig. 6B1, B2) appears to be somewhat longer than the preceding two. It is similar in structure to the second pleopod of female. Fourth (Fig. 6C) and fifth (Fig. 6D) pleopods are slightly

shorter, fifth being the shorter of the two. They are similar to the third but both the exopod and endopod are broader, more rounded and less elongate.

Uropod (Fig. 3C, D). Peduncle (u. p.) is short and stout and extends beyond the telsonic edge. Its inner mesial edge is ridged and fringed with a few short, stiff setae, and the disto-mesial angle is produced into three large tubercles. Outer ramus (o. r.) of uropod is longer than the inner. It is lamellar and narrow towards the apex, which bears a tuft of long spines. Inner ramus (i. r.) is short, stout and sickle shaped. It is broad basally and tapers towards the apex. It bears on the outer margin sub-apically, a few short setae. In adult males (Pl. I, fig. 1) the outer ramus is very long, and may be one and a half times as long as the tail piece. In females the outer ramus (Pl. I, fig. 2) is much shorter than the tail piece, although still longer than the inner ramus.

Fcmales : Females are smaller in size than males. They do not differ much from the males in general structure, except in the outer ramus of the uropod, which is shorter than the tail piece. There are four pairs of oostegites on peraeopods one to four. Each oostegite is as long as the basis, and broadly quadrangular in shape. There is a pair of small oostegites termed as coxal lobes present on the posterior side of the maxillipeds.

Head and Endophragmal system (Fig. 17A) : The ventral surface of the head consists of chitinous membrane in which a system of calcified bars has developed to support the oral appendages and their muscles. This system is similar to that of *Ligia oceanica* in general features (Tiwari, 1952). The important structures in maxillo-sternal framework, briefly mentioned are the median bar (med. b.), the maxillular sclerite (mx_1scl.), the maxillary sclerite (mx_2scl.), and the alar bar.

The endophragmal system (Tiwari, 1952) consists of tergal and sternal alae. The tergal alae are vestigeal. The sternal alae (st. al.) are a pair of horizontal semitubular triangular capsules enclosing maxillary glands (Pl. III st. al) fused along their inner edge. It also consists of Pharyngeal process (ph. pr.), maxillo-pterygoid process (pty.), and maxillary sternite (st.).

Characters of newly hatched young, size 4-4.5mm. (Figs. 8-12).

1. Frontal lamina and lateral process are not distinct but small notches indicate their limit.

2. Occipital (cervical) groove is absent. It is also absent in *Phreatoicoides terricola.*

3. Suborbital notch is present.

4. *Antennule* (Fig. 8B). It has only 6 segments, the 4th segment is subequal to the first which is the longest. Third segment is the smallest. There is only one aesthetasc at the tip of the antennule. Other setae are plumose, simple and serrate.

5. *Antenna* (Fig. 8C). There are 5 peduncular and 13 flagellar segments. The flagellar segments gradually become longer towards the distal end. The terminal segment bears 7 long simple (rod) setae.

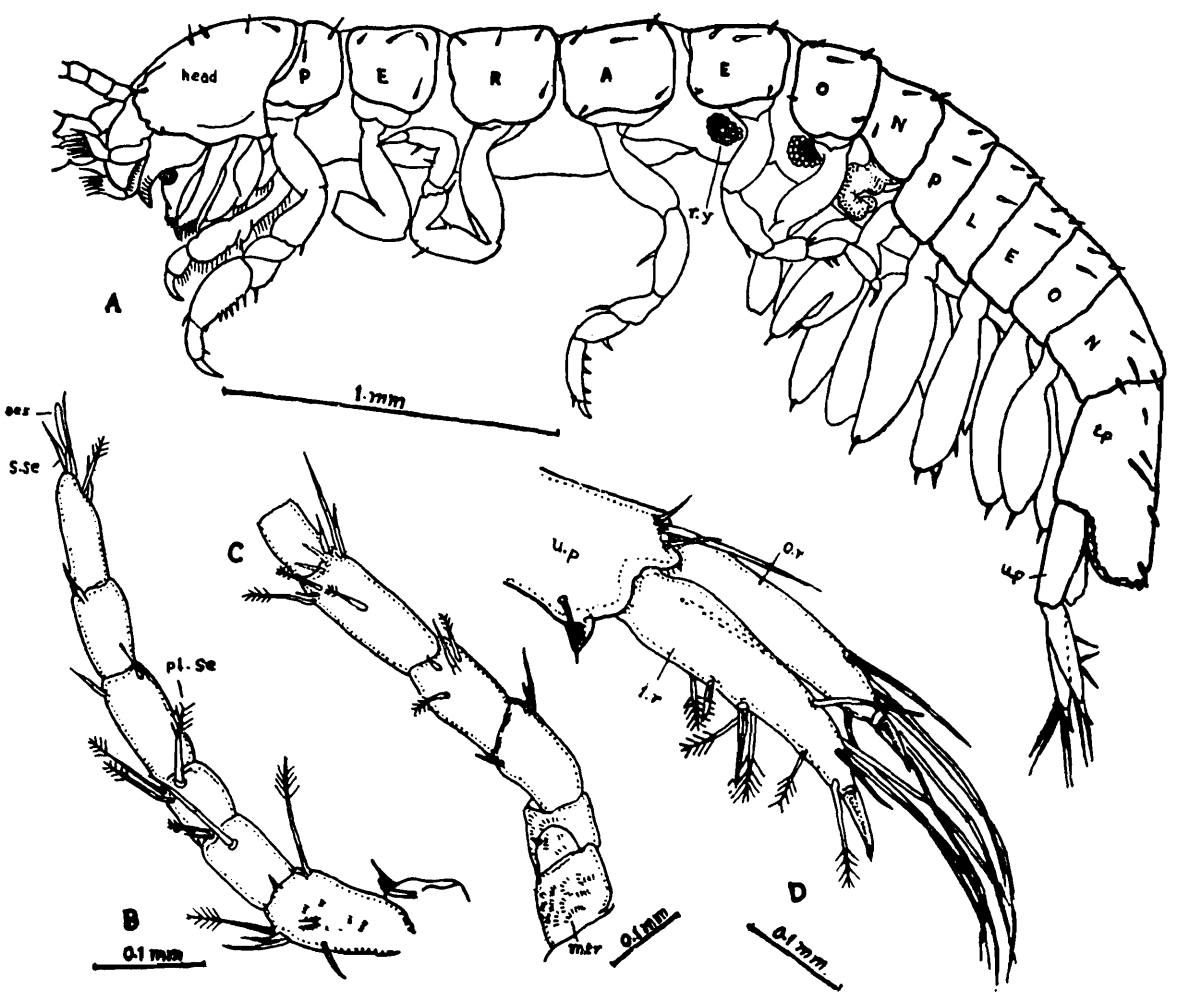


Fig. 8. A. Lateral view of young. B. Antennule of young. C. Antenna of young. D. Right uropod of young.

The first peduncular segment bears a lobe like outgrowth at its termination above the second segment. The first two peduncular segments have microtrichs arranged in crescents. The tubercle or lobe may have some similarity with the antennary cones of *Gammarus pulex* (Cussans, 1904) or other amphipods without any functional significance.

6. *Labrum* (Fig. 10A, B). The distal margin of the labrum has a conical middle lobe with long hairs along the margin and crescents of microtrichs on dorsal surface. Ventral surface has median rows of fine cones and spines directed inwards.

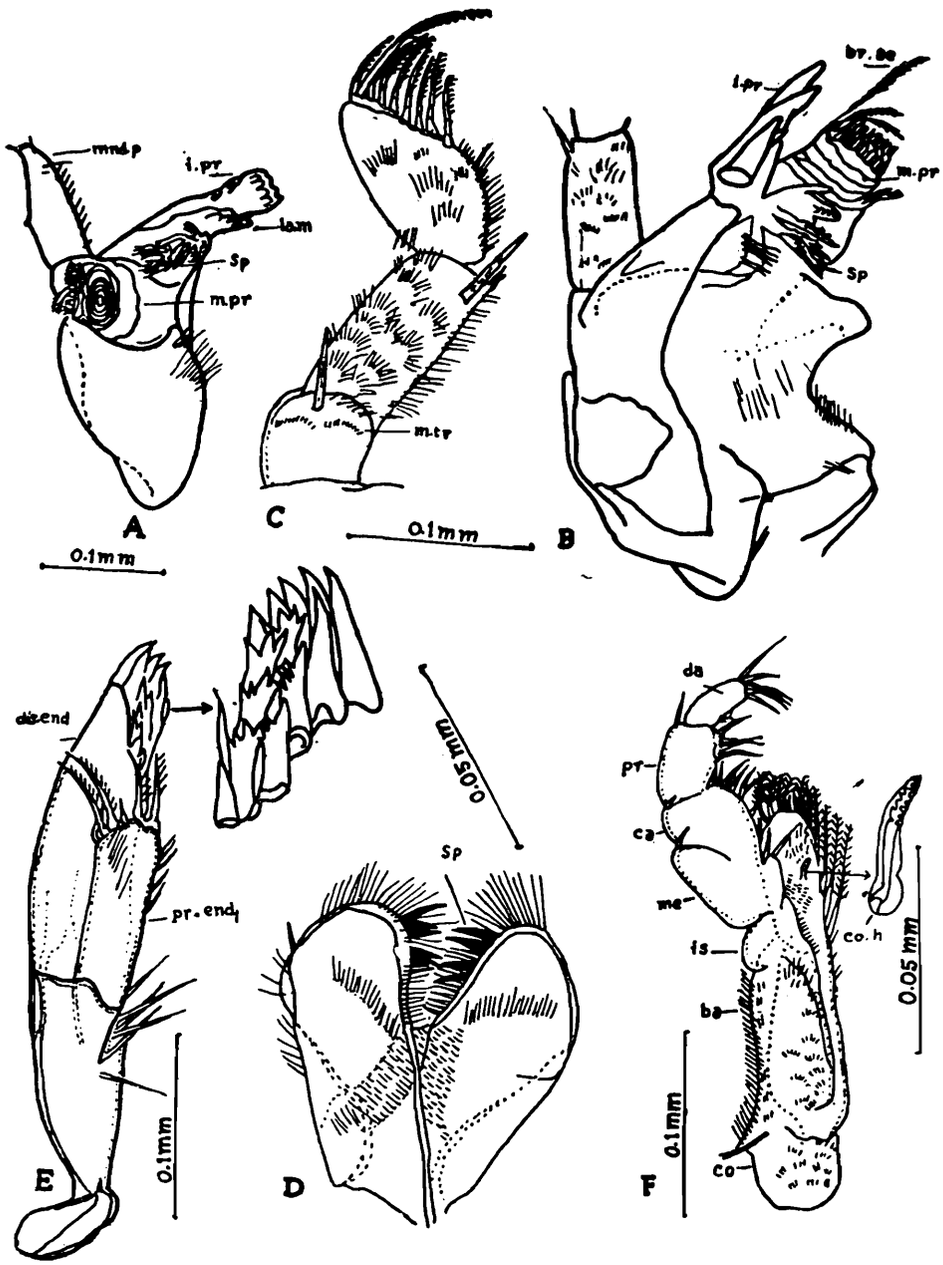


Fig. 9. A Left mandible of young. B. Right mandible of young. C. Mandibular palp of young D. Labium of young. E. Maxillule of young. F. Maxilliped of young.

7. *Mandible* (Fig. 9A, B, C.). The four teeth of the incisor in left mandible are not as prominent as on the right. Whip like brush setae are present on both mandibles. This type of setae has not been noticed in adults. As in adult the molar ridges are concentric on left and transverse on right. The palp has nine plumose or serrate spines

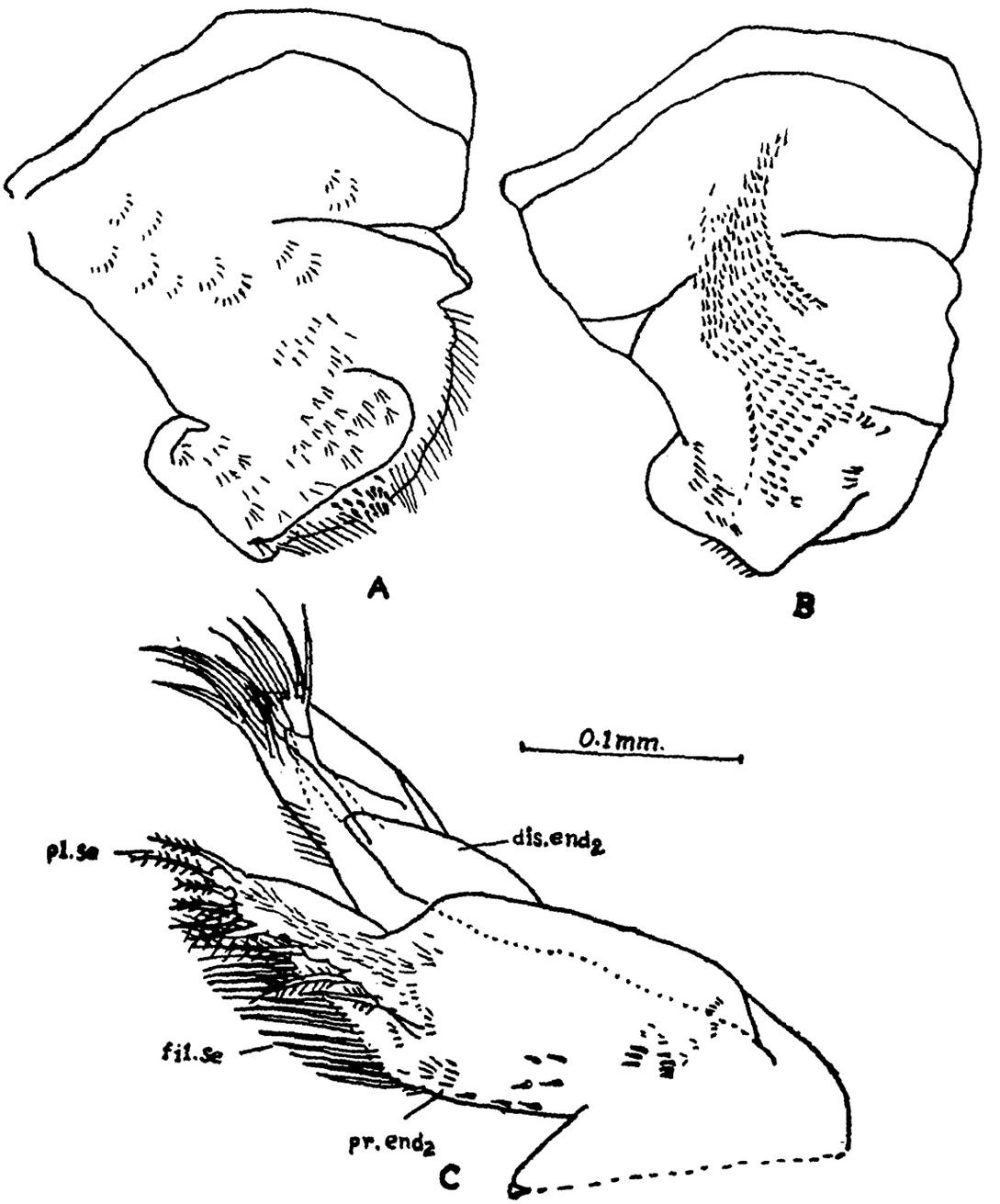


Fig. 10. A. Labrum of young (dorsal view). B. Labrum of young (Ventral view). C. Maxilla of young.

at the termination. Inner margin of 2nd and 3rd is ciliated. Microtrichs on all segments are present in crescent rows.

8. *Paragnaths* (Fig. 9D). There is significant variation from adult.

9. *Maxillula* (Fig. 9E). There are 7 hairy spines at the apex of

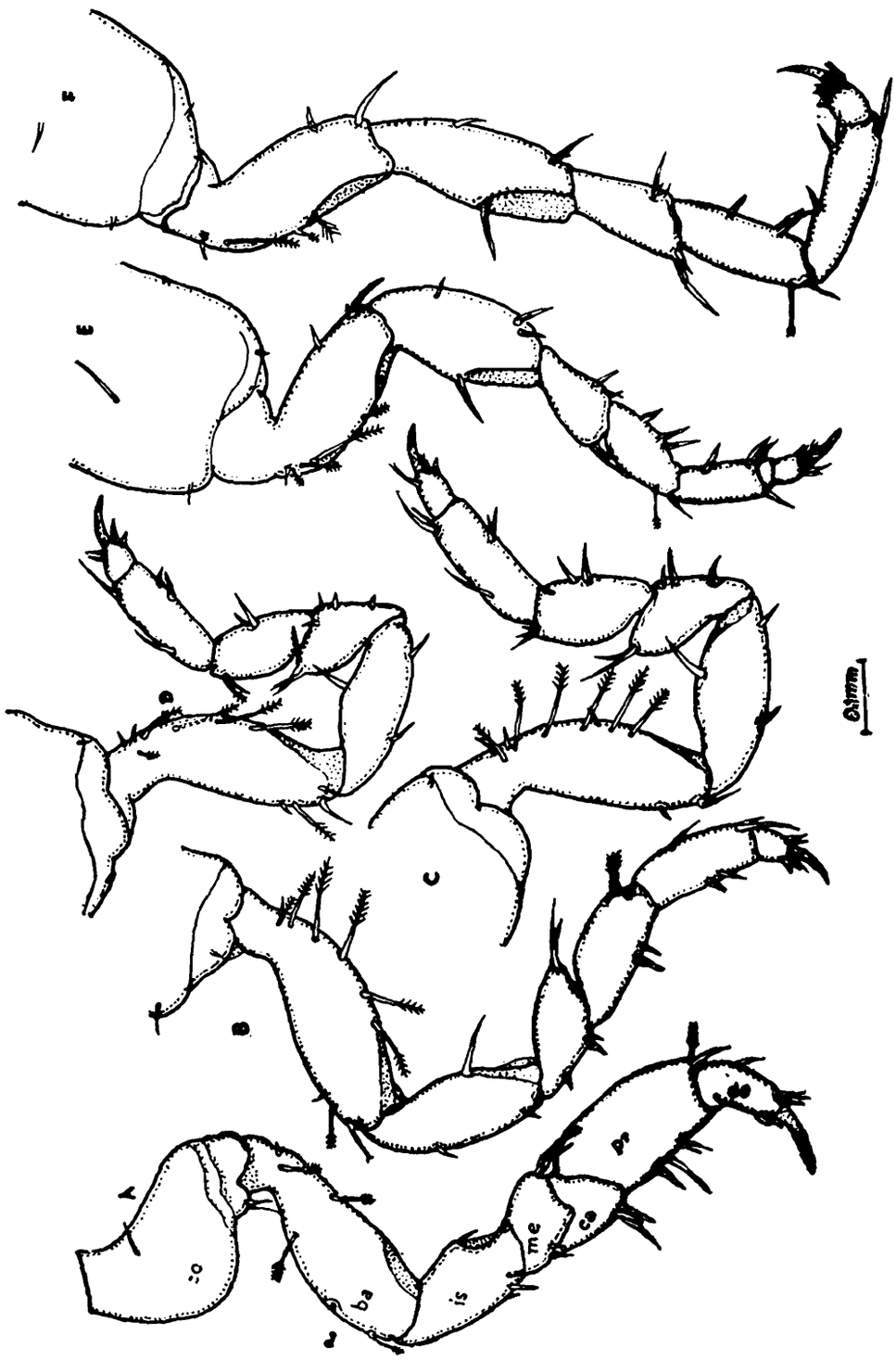


Fig. 11. A-F. Peraeopods 1-6 of young.

proximal endite of which the first and last are simple spines. The distal endite has 6 cuspidate spines in two distal rows and 6 pectinate in 3 rows, 2 in each along with one cuspidate spine on the sides of the sides of the proximal two rows. There are no cilia.

10. *Maxilla* (Fig. 10C). There is not much variation except the lesser number of setae and spines.

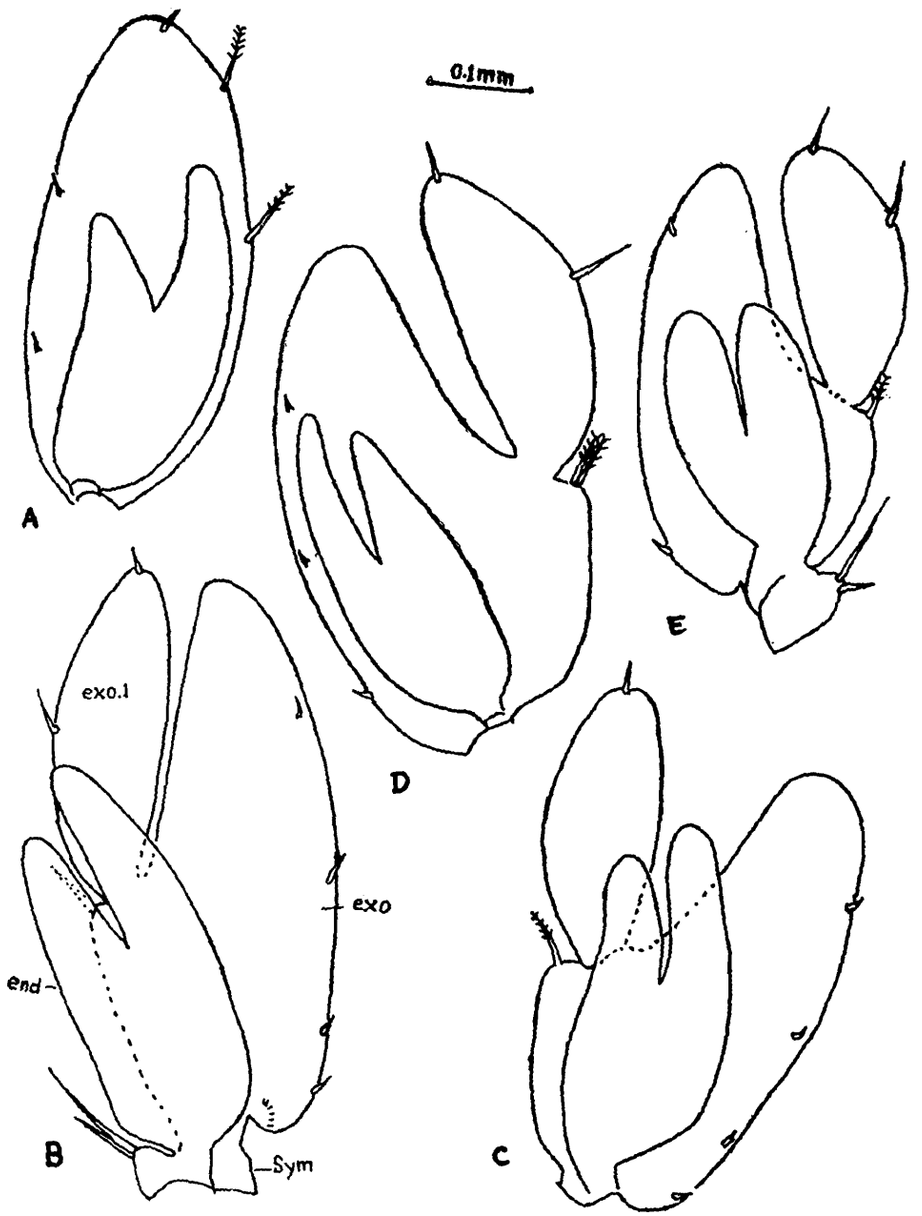


Fig. 12. A-E-Pleopod 1-5 of young.

11. *Maxilliped* (Fig. 9F). Epipodite has hairs on its dorsal margin. The shape is not very much elliptical but little elongated and

slightly narrow distally. The coupling hook is very much similar to *Limnoria* (Menzies, 1956). Other parts of the maxilliped are similar to the adult except lesser number of setae and spines.

12. *Peraeopods* (Fig. 11 A-F). Gnathopod has elongated, robust dactylopodite and propodite than other peraeopods. Number of denticles are only two behind the claw in gnathopod. Other setae and spines are much less than adult form. Seventh peraeopod is absent and is seen in the segment as a curved bud in transparent young.

13. *Pleopods* (Fig. 12-E). 1st pleopod has two plumose setae in the distal half along the inner margin while in adult they are absent. Other 3 spines are present. Microtrichs are absent.

Other pleopods : There are one or two plumose setae near junction of lobe on the exopodite. Microtrichs are absent except a crescent at the base of the outer margin of exopod in second pleopod. All the endopods are free from setae and microtrichs. Penial stylet is absent.

14. *Telson* (Fig. 8A) is high near the junction of uropod.

15. *Uropod* (Fig. 8D). The peduncle has only one tubercle at its distomesial angle. The outer and inner ramii are exactly similar except that the inner margin of inner ramus bears 5 plumose setae and 2 simple spines. Both ramii are straight and have terminations in claws just like peraeopods. The inner ramus is equal or little longer than the outer ramus and is totally different from the inner ramus of the adult in which it is sickle shaped.

Body Wall And Moulting

The body wall of *N. kashiensis* can be divided into three parts namely, the integument, the hypodermis, and the connective tissue.

Integument :

The integument or the so called cuticle can be divided into an outer thin layer of "epicuticle" and an inner thick layer of "endocuticle".

Epicuticle :

The epicuticle is very thin and more or less a homogeneous (Pl. II, fig. 1, ep. c.) layer. It is largely responsible for the restricted permeability of the cuticle. The epicuticle differs from the chitin-containing endocuticle in not being composed of closely packed laminae.

It is readily stained with Acid fuchsin and as mentioned above does not contain chitin (Yonge, 1932). In Mallory's triple stain, Stevenson (1968) recognised two parts of epicuticle which take red stain of different consistency at different stages of moulting in *Orconectes sanborni* (Faxon). These are epicuticle and mesocuticle. In some of the histological sections of *N. kashiensis* the two distinct regions were noticed where the epicuticle is deep and mesocuticle is light red. These two layers may be marked as outer and inner epicuticle. Such distinction in *Nichollsia* is possible only at certain stages of moulting. Epicuticle is thickest in the region of molar surface of the mandible. In this region thickness of the epicuticle is 4 to 5 times to that of normal thickness on the dorsal part of the body.

Endocuticle :

The endocuticle in crustacea in general is further divided in to 3 main regions these are, Pigmented layer, Calcified layer and uncalcified or membranous layer. These divisions are mainly based on observations of Decapod cuticle (Vitzou, 1881 ; Dennel, 1960 ; Travis, 1955 ; Lockwood, 1968).

In *Nichollsia* however such divisions are not distinct in early stages but in the advanced stages of moulting the endocuticle looks stratified with varying number of lamellae in different regions of the body. The number of lamellae varies from 4 to 8 and goes even up to 14 at some places. These lamellae are horizontal and are cross striated. Such cross striations are more prominent in sections fixed and stained in 0.3% Thionin. In some sections the inner part of the endocuticle has shown an unstratified narrow layer stained deeper (blue) which may represent the uncalcified or membranous layer. The pigment layer is practically absent. The whole of the endocuticle takes blue colour in Mallory's triple stain.

The lamellae are separable. Drach (1953) made observations on the lamellae in *Homarus gammarus* with the aid of electron microscope and inferred that the dark lamellar zones consist of fibrils which extend horizontally and the intervening lighter zones are due to fibrils which pursue a curved path from one lamella to the next. Similar disposition of fibres were reported by light microscope (Dennel, 1926, 1960) in *Astacus*. In *Nichollsia* the lamellae become thinner towards the hypodermis.

Pore canals : Pore canals are absent, perhaps due to subterranean made of life of the animal.

Tegumental glands :

These are confined to a pair of glands in each segment in the coxal base and other segments of the appendages. These are not distributed in the general body wall. In this regard *Nichhollsia* agrees with *Maja* (Decapod) for the epicuticle is a premoult secretion and is formed before the endocuticle has appeared (Drach, 1939). Whether the coxal glands are tegumental glands or modified segmental glands is controversial.

Hypodermis :

The hypodermal layer is composed of a single layer of cuboidal cells. The cells contain granular protoplasm (Pl. II hyp., lip. gr.) during the advanced stages of moulting. These lipoid granules are stained red with eosin. The nuclei are large and more or less spherical.

Beneath the hypodermis, their is a layer of connective tissus which in not often visible in sections.

Moulting :

Like every crustacean, *Nichollsia kashiensis* also periodically secretes a new exoskeleton and shed the old. It moults several times during its life time. Ecdysis like other isopods is completed in two stages.

A few days before moulting, *Nichollsia* stops feeding and becomes totally inactive. When the posterior half of the body is ready to moult the skin splits between the 4th and 5th thoracic segments and the animal drags itself free with its front limbs. A few days later the performance is repeated in reverse when the head end is shed. Usually the laps of time between the posterior and anterior moults is 24 hours but it may extend upto 70 hours under the influence of physical factors. On both occasions the cast skin is often eaten by. In laboratory cultures cannibalism of moulting *Nichollsia* by their more mobile neighbours is of common occurrence. Some of the indivuduals die during casting of anterior skin.

During ecdysis lateral fissures develop on the two sides of the body between the tergum and sternum. Posterior exuviae include

the intima of hind gut while the anterior includes that of the foregut. At the time of moulting the animal finds a hide out. The animal makes the old skin loose by rubbing its body against rough surfaces and by forward and backward movements in the crevices. The anterior skin under ecdysis is pulled out with the help of inner ramii of the telson. Mouth parts also help in removal of the posterior skin.

The intermoult period varies from 15 days in the youngest to 60 days in the oldest individuals. In normal adults this period is 30 days. Old males attaining length of 30 mm are above, often succumbed during ecdysis.

Visible signs of moulting are the appearance of white bands along the dorsal surface of the body in anterior segments, where the cuticle becomes very hard and brittle. Generally such signs appear about 70-80 hours before the posterior skin is shed. Few hours before anterior moult new claw formation is visible inside the gnathopods and mandibles.

After posterior moult the animal can be seen moving (Pl. I, fig. 2.) here and there but after the anterior moult it regains movement of appendages only after the laps of 12-15 hours and resumes feeding after 20-40 hours depending on the temperature of the environment.

Frequency of moulting increases with the frequent change of water and rise of temperature, provided other conditions are favourable.

A moulting *Nichollsia* under ecdysis is most helpless creature and is exposed to cannibalism and predation.

Following specific histological changes are seen during the moulting period of *Nichollsia kashiensis*.

Hypodermis :

The cytoplasm of cells become granular (Pl. II, fig. 2. lip. gr.). The lipoid granules are stained red with eosin. Throughout the body wherever the new epicuticle under the old cuticle has been formed such granules have disappeared, It acts as a storage organ for reserved lipoid materials during moulting.

Coxal glands :

These glands become prominent with lipoid dorplets (granules) around their nuclei (Fig. 19A-I),

Athrocytes or Branchio-pericardial organs :

Athrocytes (Pl. VI, fig. 1, 2, athr.) become very active during moulting and pick up tripan blue stain prominently when injected in the animais. Histologically these athrocytes show heavy accumulation of droplets around their nuclei which disappear after the moulting is over.

Hepatopancreas :

Like other glands the digestive glands also show heavy accumulation of dark granules (Pl. IV) around their nuclei. Activity of these glands is increased and are seen even in the animals starved during moulting.

Setae :

There are two main type of setae grouped according to their function and morphology.

(a) Mechanical setae

(b) Sensory setae

Mechanical setae have no core of living tissue and are varied in shape according to their function. They occur in the stomach. They are composed of cuticle with an outer coating of epicuticle.

Sensory setae (Fig. 27D, E) :

They contain living tissue which usually extends some distance into the setae from the base. The seta is supplied with nerve elements. These setae can be organ of touch, balance, chemoreception and pressure. Chemosensory setae are called as "Aesthetascs" confined to the anterior segments of the antennules. Plumose setae also called as acoustic or, auditory setae are distributed to the antennae, and other appendages of the body.

The Muscular System

Muscles moving the segments of the body and the appendages can be seen with their points of insertion, through the transparent exoskeleton. In addition to those of the gut and the heart, the muscles may be divided into three groups namely (1) muscles moving the segments of the body, (2) muscles moving the appendages (3) muscles in connection with the gastric mill. The third group has been

dealt with in the digestive system. This grouping is being followed from Hewitt (1907).

Muscles moving the segments of the body (Fig. 14, 15).

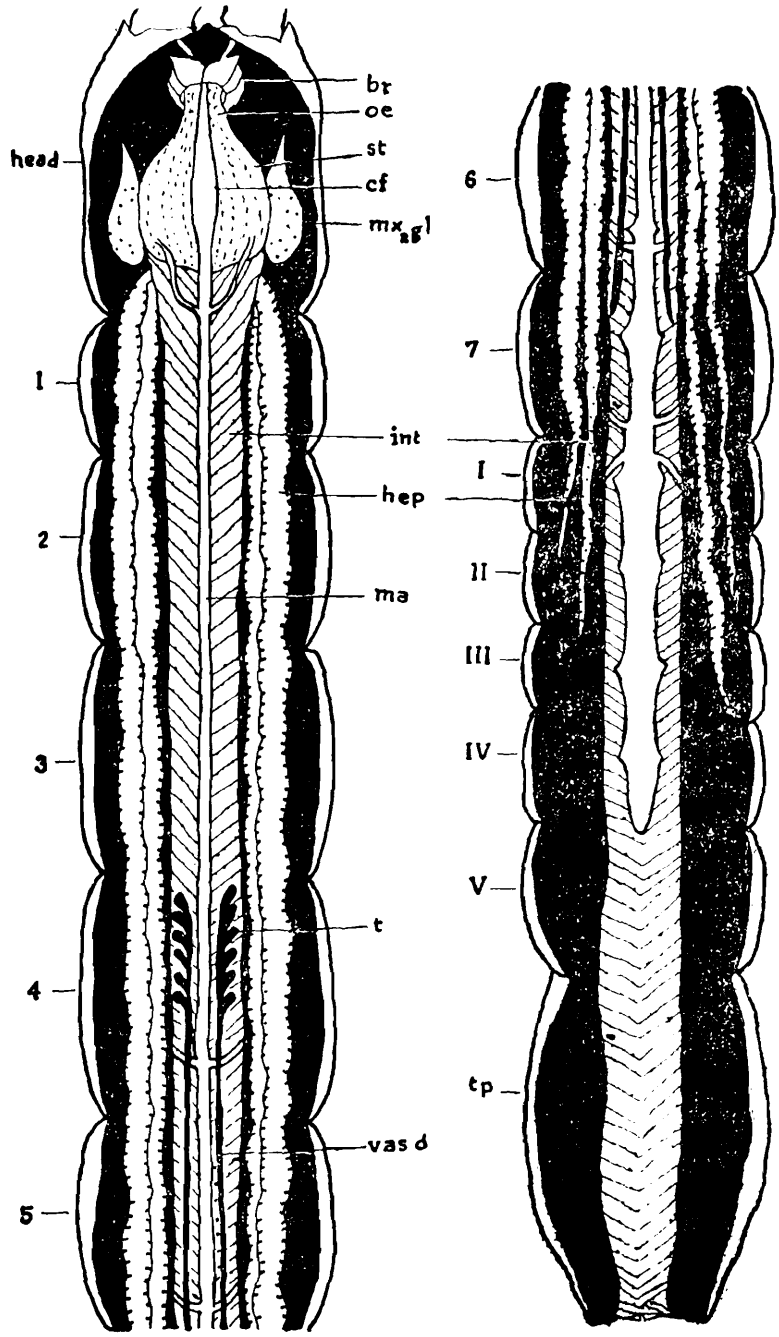


Fig. 13. Visceral anatomy of *N. kashiensis*.

When *Nichollsia* is observed from dorsal side the dorsal longitudinal muscles are seen lying parallel, on the two sides of the heart and dorsal aorta. These are dorsal longitudinal (d. l. m.) muscles. There are three bands of muscles of varying thickness on each side in a

segment in thoracic region and four bands in each abdominal segment. In fifth abdominal segment the number of bands is much more and virtually cover the whole of the dorsal half of the body. This perhaps due to the long and heavy telson which is so strongly flexed or moved in different actions of the animal. All the muscle bands on one side are an exact mirror image of those, on the opposite side. Apart from these longitudinal segmental bands there are a pair of intersegmental bands on each side running below the dorsal longitudinal muscles between the two segments. They are actually dorsal oblique muscles (d. o. m.) originating from the postero-dorsal cuticular invagination of the segment behind. It proceeds forward and extend obliquily into the segment infront.

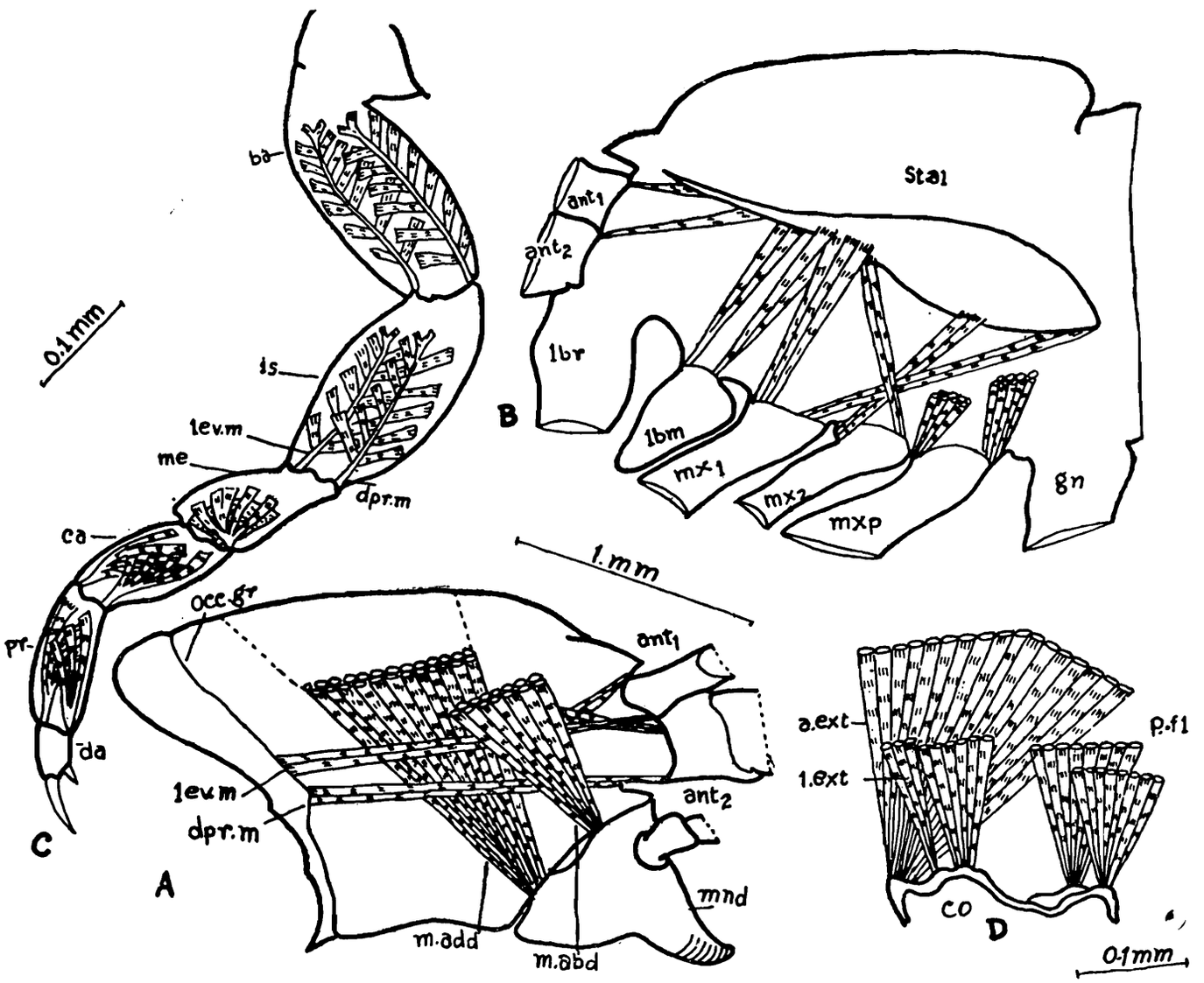


Fig. 14. A. Muscles of mandible, antennule and antenna. B. Muscles of other mouthparts. C. Muscles of thoracicleg. D. Muscles of coxa in thorax.

The point of origin of this muscle is dorsal while the point of attachment is lateral on the anterior end of the segment. Thus this muscle covers two segments completely. All the dorsal muscles originate from the anterior end of the segment, run posteriorly traversing the segment and are inserted on the anterior margin of the posterior segment behind the intersegmental membrane. This helps in the telescopic movement of the body segments.

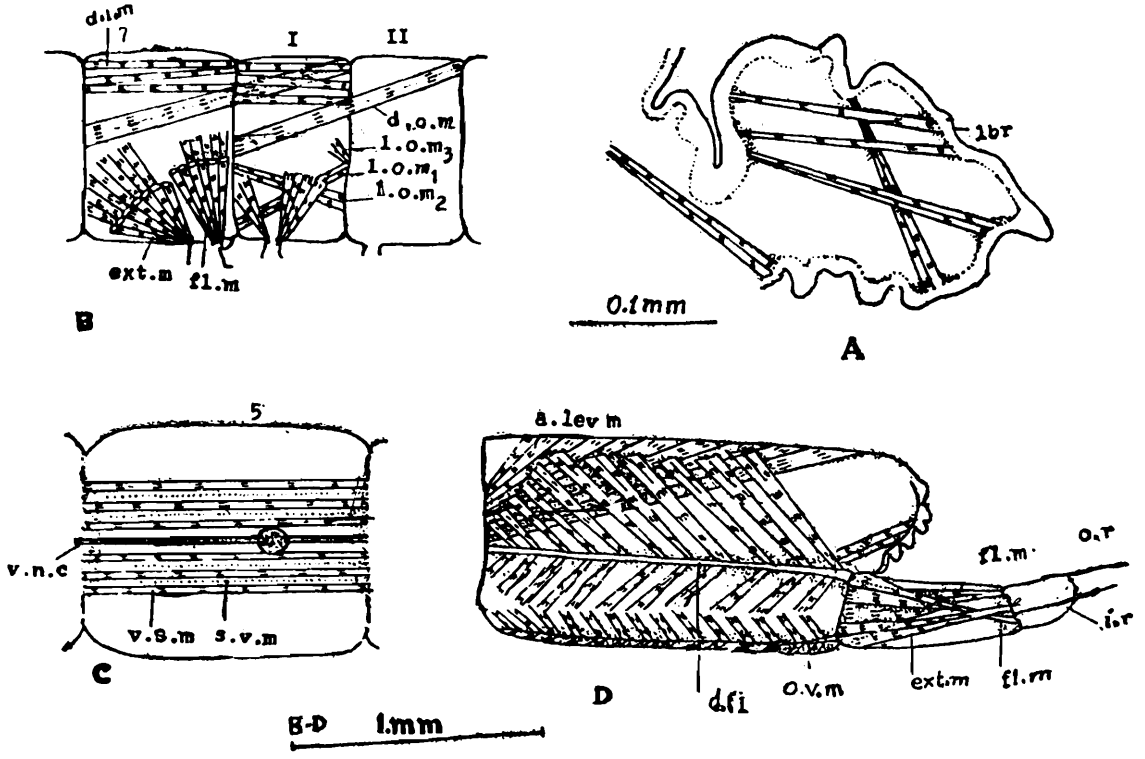


Fig. 15. A. Muscles of labrum. B. Thoracic and abdominal muscles. C. Ventral muscles of third peraeon. D. Muscles of Telson and uropod.

There are three sets of oblique muscles on the lateral sides in each segment of thorax and abdomen. Abdomen here includes only up to 5th abdominal segment. Out of these three lateral oblique muscles two cross each other, one (lom. 1) originating from the mid-lateral part of posterior margin and running down obliquely to the ventral margin at the anterior end of the same segment. The other (lom. 2) follows the same pattern but in opposite direction. Another, the third muscle originates from the same point of mid lateral line at the posterior end of the segment and is attached to the hypodermis infronr (lom. 3). It is a small muscle and does not cover the length of the segment.

Muscles of the telson are very strongly developed. There is a thick band of fan shaped muscle, anterior levator muscle (a. lev. m.) which originates from the anterior lateral margin of the telson and fans out posteriorly and covers a large area of the telson from anterior to posterior of its side. Other strongly developed muscles of telson are concerned with the uropod, and are dealt with in the following para.

Ventral Muscles : (Fig. 15C).

In each segment of thorax and abdomen on the two sides of the nerve cord tbere are 3 sets of muscles on each side running parallel to the nerve cord. These are ventral segmental (v. s. m.) muscles. There are a pair of superficial ventral muscles (s. v. m.) on each side lying between the ventral muscles.

Muscles moving the appendages :

Muscles of the head appendages occupy greater part of the cephalic cavity (Fig. 14A, B). The most prominent of these are a symmetrical pair of bundles called "mandibular adductor" (m. add.) formed of numerous bands and connected to the mandible. On each side, the bands originate from the inner surface of the cranium and converge towards the base of the mandible, which is hollow and box like, the muscle bands enter its cavity and are attached to the inner surface of the mesial margin by a broad tendon.

The mandibles are pulled apart by a comparatively short and slender band called the "mandibular abductor" (m. abd.) which originates from lateral edge of the cranium and is attached laterally but but dorsal to the adductor muscle on the outer side of the mandible.

Other muscle bands which control the movement of the head appendages are those of first and second antennae (lev. m., dpr. m), first and second maxillae, hypopharynx and maxilliped. The muscles of antennae are attached to the sides and dorsal to stomach in the head and cuticular invagination of occipital groove, so that these appendages can be moved in any direction. Imms (1940) classified the antennae of arthropods into two groups, 'segmented' and 'annulated' types. In malacostraca including Isopoda the antennae are annulated, where the peduncle is provided with the muscles while the flagellum is annulated and does not contain any muscle. Therefore, the flagellum always moves as an entire organ with the help of peduncular muscles.

Muscles of labrum are not much different from those of *Diastylis rathkei* (Haffer, 1965). Most of these muscles are protractors as described by Haffer, and are having origin and insertions between the dorsal and ventral wall (Fig. 15A) of the labrum. There are muscles arising from base of the labrum and are attached to the dorsolateral edge of the head behind the base of antenna II.

Muscles of hypopharynx, maxillae and maxilliped, however, are not so prominent as those controlling movement of the mandibles and also have a different origin. These muscles originate from the inner surface of the endophragmal skeleton, particularly the sternal alae, maxillary sclerite and the cuticular invagination of the occipital groove.

Unlike the muscles of cephalic appendages, the muscles of thoracic legs are six prominent bands originating from different points, on the lateral sides of the tergum (Fig. 14D) at different levels. All these muscles converge to the proximal border of the basal segment of the appendage. From their positions in relation to the tergum these muscle bands of each side can be grouped into three sets, two anterior, two lateral, and two posterior bands. The two anterior bands are large and broad of which one is outer and the other is inner. The posterior (p. fl.) bands are flexors and are less prominent than anterior bands but more stronger than the outer laterals. The anterior pairs (a. ext.) and outer lateral (1. ext.) muscles are extensor muscles. Disposition of these muscles facilitates the forward and also the outward movement of thoracic appendages.

The orientation of anterior, outer lateral, and posterior bands of muscles in 5th, 6th and 7th thoracic segments is just reverse due to posterior shifting of the appendages.

Apart from the above mentioned muscles each appendicular segment (other than dactylopodite) bears two sets of muscles one is depressor and the other is levator (lev. m., dpr. m.). These muscles help in walking of the animal.

The five pair of abdominal appendages possess separately operating muscles. In each segment on each side there are only two sets of muscles. These are anterior and posterior sets and can be called as extensor and flexor muscles (ext. m., fl. m.). In comparison to thoracic legs the muscles of abdominal appendages are weaker.

There are three strong muscles (Fig. 15D) operating with each uropod. These are dorsal flexor muscles (d. fl.) originating from the lateral wall of the telson and are attached with the dorsal edge of uropod at its base. The outer (o.v.m.) and inner ventrals are extensors originated from the uropodal base. The outer rami (o.r.) has flexor (fl.m.) and an extensor muscle (ext.m.) while the inner rami has only flexor (fl.m.) muscle which is prominent.

In comparison to *Ligia oceanica* (Hewitt, loc. cit.) the longitudinal muscles on sternum in *Nichollsia* are not very prominently developed. Further the telson in *Nichollsia* is strongly developed and consequently the muscles are also well developed. The telson and inner rami work as defensive organ in this animal.

As in other Arthropods, all muscle fibres in *Nichollsia* are striated, the striation often showing with extreme clearness. The muscles are attached to the cuticle, intercepting the hypodermis, with the help of tonofibrils.

Food Feeding and Digestive System

Little is known about the internal anatomy of Phreatoicids. Exeept a few accounts giving morphology of the foregut of *Mesamphisopus* (=*Phreatoicus*) *capensis* (Barnard) and *Notamphisopus* (=*Phreatoicus*) *kirki* (Chilton), Barnard, (*1914*, *1927*) ; *Colubotelson thomsoni*, (Engemann, 1964) and *Nichollsia kashiensis*, (Tiwari & Ram, 1972) nothing is known about their digestive anatomy. Since *Nichollsia* Chopra and Tiwari is a subterranean form, its anatomy is of considerable importance. Their omnivorus habit and lower metabolism has effected the digestive system to a considerable extent.

There are many accounts giving morphology and physiology of the digestive system in various isopods (other than Phreatoicoidea) and amphipods (Cussans, 1904 ; Geldard, 1907 ; Hewitt, 1907 ; Ide, 1892 ; Yonge 1924 ; Nicholls, 1931, Stevenson, 1961 and John, 1968).

There is further paucity of literature on the subterranean forms. The subterranean forms shows a remarkable degeneration or absence of tegumental glands in stomodeal region of the foregut and also in simplification of intestine in its gross morphology.

Application of different terminology in foregut has been discussed by Nicholls (*loc. cit.*), Haffer (1965) and Tiwari and Ram (*loc. cit.*).

There is difference of opinion on the functional aspects of proventriculus and its internal appendages. Similarly the functional aspect of intestine and hepatopancreas has been controversial. Present studies on *Nichollsia kashiensis* has added to our knowledge on these aspects and might help in solving some of the controversies.

The whole alimentary canal including digestive glands occupies one third of body cavity of the animal of which the digestive glands alone occupy three times more speace than foregut and hindgut together.

Food and feeding habit :

Nichollsia is an intermittent feeder. Its feeding is totally stopped during the period of ecdysis and transfer of the eggs to the brood pouch. It is omnivorus in its feeding habit.

N. kashiensis is primarily a detritus feeder. The subterranean habitat offers a monotonus condition. This condition is in sharp contrast to that of *Ligia*, (Carefoot, 1973a) and other carnivorous isopods whose energy requirement is more than *Nichollsia* and they are more active animals.

Examination of the gut contents reveals that the animal feeds on the detritus which includes everything in decomposition along with the mud, sand and protozoans and decaying matter at bottom of the well.

Analysis of bottom sample of a pucca well at Varanasi.

This well is inside a house campus and has a Neem (*Azadirachta indica*) tree by its side.

Weight of the sample ... ... 1Kg.

I. *Plant material in different state of decomposition.*

1. Leaves, twigs and flowers of the Neem tree (*Azadirachla indica*) about 50 gm.
2. Algae and mosses.
3. Sunhemp fibres of the rope used for drawing water.

II. *Decaying dead animals.*

(a) *Crustaceans*

4. Faeces, shells etc. and dead ostracods ;

(a) *Crustaceans*

5. Faeces, exuviae etc. and dead copepods ;
6. „ „ „ „ „ Amphipods
7. „ „ „ „ „ *Nicholllsia*

(b) *Insects*

8. Chironomus larvae, faeces and exuviae
9. Dead ants.

(c) *Protozoans.*

III. *Other organic materials*

10. Pieces of cloth, about 300 gms.

IV. *Size group, percentage of the clay particles.*

1.2μ	—	10μ	...	60% Approx imate
11μ	—	30μ	...	20% „
30μ	—	90μ	...	10% „
90μ	—	100μ	...	10% „

Some common microflora thriving in the well :

1. *Moss* sp.
2. *Ocillatoria* sp.
3. *Ulothrix* sp.
4. *Microcystis* sp.
5. *Calothrix* sp.
6. *Phyllobium* sp.
7. *Microspora* sp.

Above data reveals the organic richness of the habitat which provides sufficient nutrition to the animals.

Observations in Aquarium : Coprophagus habit.

Nichollsia has been found feeding on their own faeces along with other food material. Coprophagus habit is common amongst isopods. Sutton (1972) attributed the coprophagus habit, as a mechanism for maintaining the copper content in the body as well as blood of crustaceans. Wieser (1966) has noted higher concentration of copper in the

digestive gland of crustaceans. Bacterial activity may well make available a lot of other things in the faeces, which may partly explain why wood lice can live for so long in cultures without fresh food. *Nichollsia* has also been found to survive for months without fresh food.

Cannibalism :

Normally they do not attack live individuals of their own but during moulting when an animal is inactive, particularly during ecdysis, they are frequently attacked. If any individual is punctured and its internal organs are exposed, it is attacked by other individuals. Gravid females were found eating their embryos and youngs from the brood pouch. These embryos were being attacked by other individuals also. They eat their own skin casts after moulting.

Interesting observations were made when live pieces of earthworms were added in the aquarium. These live pieces whether small or large were vigorously attacked by *Nichollsia*, either singly or collectively. Snatching of pieces amongst them was common. A small piece of 5 cm was fully consumed in 5 hours by a group of four individuals feeding at a time. When live ostracods and other crustacea were crushed and added to the aquarium they were immediately consumed.

In normal conditions *Nichollsia* feed on aquarium plants like *Hydrila* etc. When starved they feed on bread pieces, boiled egg, black paper and carmine. Readymade fish food for aquarium fishes was successfully attempted. In aquarium the author maintained them on fine sand substratum where the chances of infection was found minimum. When *Stenocypris* (an ostracod) population was high in the sand, they very often attacked moulting *Nichollsia*, endevouring them completely. This might be one of the reasons why *Nichollsia* did not thrive in open waters. Apart from the sand and mud they feed on algal and fungal growth and the crust formations on the glass wall and cement blocks provided for shelter in the aquarium.

Food preference :

To determine their food preference *Nichollsia* were kept in the aquarium and offered algae and sand mixture and also with dead spiders, odonate nymphs and other dead insects. Insects and spiders were not disturbed even after fifteen days while the algae and sand with detritus was consumed in a very short time.

It is reasonable at this level to think that *Nichollsia* is primarily a detritus feeder. Although its attractions to the live pieces of earthworm and crushed ostracods is noncomplementary behaviour.

While moving here and there the food at the bottom is recognised only when it is touched by the first antennae or other mouth parts. It was observed that this animal very frequently touched the ground with the tips of its first antennae. Live flesh of an animal is recognised from a distance because it emits its strong odours which are percieved by the Chemosensory aesthetascs of the first antennae.

It is possible that setae present on mouth parts are also chemosensory and assist in selection and preference of food material from the ground or detritus.

The digestive system consists of mouth parts, foregut and the hind gut. The midgut is represented by digestive glands. These organs are being dealt with separately.

Mouth parts :

All the paired appendages of the mouth parts originate behind the oral cavity. They project forwards beneath the head with their tips coming to lie below or just behind the oral cavity.

The oral cavity (Fig. 17B o. cav.) is surrounded by the oral appendages, these include the single labrum and other paired appendages like mandibles, paragnaths, maxillules, maxillae and maxillipeds. Inward movement of this so called upper lip helps in scraping and pushing the food to the incisor and the molar processes.

The mandibles lie on either side of the oral aparture and are well developed masticating organs. The molar process helps in grinding the food material while the cutting edges help in tearing, rupturing and transferring food from the incisor to the molar part.

The paragnaths form the posterior boundary and ventral floor of the oral cavity. The long setae on the inner margins of the paragnaths enclose the gap between the two lobes and thus the floor is completed. The paragnath lobes lie close behind the mandibles, the cleft between them allowing the food being pushed from below between the mandibles by the maxillules. The distal endite of maxillules reaches little beyond the incisor process of the mandible.

Maxilliped takes active part in feeding. Inner margin of the maxilliped is armed with large number of elongated setae of which the proximals are plumed or brush setae, while distals are long simple setae. The brush setae of endites close the gap between the two maxillipeds. Further the coupling hooks keep the two sides intact. The epipodites are placed vertically on both sides of the head and form the outer boundary of the feeding chamber prohibiting the outward flow of food particles coming with the water current and passing posteriorward.

The gnathopods aid in feeding by holding the food mass and by combing the mouth parts and antennae. Unlike other legs, the dactylopodite is palmate and bears a number of toothed, comblike spines and setae on its inner margin. It helps, in digging the mud or sand and in search of food.

Feeding mechanism

(1) *Movement of mouth parts.*

Labrum moves inward while the mandible's movement is transvers to the body axis and alternate with the labrum. The left incisor overlaps the right, slides upward tearing the food particles and directing it to the molar processes via lacinia mobilis. The right molar process which is smaller in dimension and transversely ridged, strikes on the concentrically ridged large molar surface of the left. The setae on the margins of the molars help in retaining the organic food on the molar surface for proper crushing and mastication. The molars are so close to the oral opening that it directly transfers the food to the oesophagus which is further assisted and sucked by the oesophageal dilators. These dilators help in dilating the oesophagus and drawing in and moving the food posterior from the molars. The mandibular palp has not been observed taking part in feeding. The paragnaths move as usual in forward and backward directions. The two lobes from the ventral floor of the oral cavity, from this floor the food collected, is easily transferred to the molar processes.

Movement of maxillules on both sides is simultaneous as well as alternate. They move alternately to the mandibles. Their movement is forward, backward and also inward. The outer lobes with chitinised setae help in bringing the food to the incisors and also in puncturing them. It is the most active mouth part. Both the maxillules meet in

middle near the incisor of the mandibles. Maxillules transfer the food particles brushed forward by the maxillae and also the maxillipeds. Movement of the maxillae is also forward-backward. The setae on the inner margin of the maxillae help in filtering the food-carrying water and retaining the food particles which are transported ahead through the maxillules to the incisor and molars and then to the oesophagus. Maxillae do not act as tearing or piercing organs. Maxilliped has its endite well armed with feathered setae. The distal four segments are provided with elongated simple setae on their inner margins. The feathered or the so called pectinate setae of the endites of the two sides meet and form a filter floor. There are two types of movement in the maxilliped, the up and the down movement. It helps in clearing the accumulated, unconsumed food or sand particles which fall down from the mouthparts. In the second type the propodus and dactylus move laterally downward and inward. When the animal is feeding on detritus the water with food particles is directed towards the mouth by the lateral inward beating of the dactylus, the current passes back through various parts of the oral appendages and is filtered.

Different modes of feeding.

(1) *Biting and scraping* :

From the functional point of view all the mouth parts but the maxillae are built on a similar plan. Ventrally placed on each appendage are heavily chitinized structures for biting or scraping the food material. Such structures including conate setae on the inner surface of the labrum help in scraping the food from the surfaces. During feeding, the maxillules come closer as the mandibles separate, and in doing so they abrade the edge of the weed and facilitate the work of the mandible when these next bite. The maxilla does seem to take part in abrading and piercing the food with the help of its stiff setae. The gnathopods help scraping the leaf surfaces.

(2) *Pushing* :

Above the biting and scrapping parts of the appendages are structures concerned with the removal of food particles from the biting parts and with its transfer to the molar processes for chewing. Serving to transfer food upwards along the mandibles are the rows of toothed spines and the lacinia mobilis of each side. By their very arrangement

this should push food upward everytime the mandibles come together. Cannon & Manton (1927a) in describing thc asymmetrical arrangement of the mandibles in *Hemimysis* imphasized the function of these parts in transferring the food from incisor to the molar processes. The molar process of right mandible alongwith its crushing action also slides inward so that the food is pushed up. In addition to those on the mandibles, there are toothed spines on the maxillules. They aid in pushing the food upward while the labrum goes on pushing the food in the oral cavity.

At the time when the food is bitten by the mandibles, is likely to fall between them as they retract, the maxillules are coming together and in doing so they will push inwards any food which is likely to fall, and the toothed nature of the spines should make them more efficient in this respect.

Pushing was effective when filamentous tissue like algal threads and sunhemp fibres were fed to the animal. When the fibres reach the stomach, pushing is further activated by lateral ampullae and their bifid and multifid hooks. It was so effective that the two ends of the fibres were visible through the two ends of the alimentary canal and the entire length of fibre could be taken out.

(3) *Brushing* :

Escaping particles of food are probably dealt with by the plumose or brush setae, on the maxillules, maxillae and maxilliped. It is difficult to observe these setae in action, but from general movement of the appendages, some idea of their function may be inferred.

The plumose setae move mainly forward and backward, aince they are on the inner parts of the appendages, and the setae of each member of the appendages meet in the mid-vetral line. The three pairs of appendages normally move out of phase with each other and particles of food are probably brushed forwards from one to the other untill they are combed off the setae of the maxillules by the spines on the paragnath. From there the food particles will be pushed between the mandibles by toothed spines on the outer endite of the maxillules and by the brush setae on the inner endite of that appendage. Once between the mandibles, food passes up to the molar process by virtue of lateral movements of the mandibles themselves.

(4) *Combining* :

It is necessary in such a method of feeding where small particles of food will tend to cling to various appendages, for there to be combing mechanism to remove such debris. Comb spines on gnathopods and the long smooth spines on the inner border of the distal segments of maxilliped aid in keeping the mouth parts free of debris. In addition, short smooth spines on the paragnath, comb the food particles from the brush setae of the inner endites of the last three pairs of mouth-parts. Other structures which probably aid in combing the mouthparts are the spines on the distal endite.

(5) *Trituration* :

All movements of the mouthparts other than labrum or mandibles and outer lobes of maxillules, serve to push the food forwards and upwards to the molar processes of the mandibles. The asymetrical ridges on the two molars slide across each other to crush the food, from there the triturated food passes to the foregut.

As already mentioned, in continuous feeding the trituration is not complete because the food quickly passes behind, to the foregut. This process is continued by hooks of lateral ampullae in the foregut. When fed on algal filaments, in faecal matter many cells of the algae remain entire with their chlorophyll entact showing that unless punctured they are not acted upon by the digestive enzymes.

Since *Nichollsia* is detritus feeder, during feeding it digs out or scoops the soft bottom with the help of its gnathopods aided by second thoracic legs, the floating particles of clay, silt, sand and other organic matters are swiftly directed to the mouthparts by the distal segments of the maxilliped. The excess and unwanted sand particles are allowed to fall down from the oral chamber passing through and in between the appendages.

Alimentary canal (Fig. 13).

The alimentary canal is divided into three regions, fore, mid and hind-gut, and extends from mouth to the anus. The mouth is subterminal and leads directly to the oesophagus, which soon dilates to form the stomach. Oesophagus and stomach constitute the foregut and its inner chitinous linning is continuous with the integument, a condition which is also found in the hindgut, while the midgut, which lies

between these two regions including digestive glands, lacks this linning. In *Nichollsia* the midgut is so much reduced that it is virtually composed of opening of the digestive gland (hepatopancreas), since only the area immediately around the opening of the gland lacks a chitinous linning. Such kind of short midgut is common in Isopods and has also been reported in Decapods (Pike, 1947 ; Pillai, 1960).

The hindgut or the, so called, intestine is a straight tube from stomach to anus. The lumen of the intestine is more or less uniform in diameter underlined by a flexible cuticle or intima. In the rectal region, rectal pads are present. The anal slit is vertical and guarded by semicircular valves called anal plate (Fig. 17C, an. pl.) controlled by muscle. The hindgut in telson region ascends to the dorsal half and consequently the anal opening is also placed in the dorsal half on posterior face of the telson.

Foregut :

It has already been described by Tiwari & Ram (1972) in detail and only a passing reference to its constituent parts will be given here.

It has two parts, viz., the oesophagus and stomach (proventriculus).

Oesophagus ;

The mouth opening leads into a short and narrow oesophagus which runs obliquely backwards and upwards. It is almost rectangular in cross section with tetraradiate lumen due to presence of four longitudinal ridges. These ridges in *Sphaeroma terebrans* have been termed by John (loc. cit.) as labral, laterals and metastomal ridges. All the four ridges project into the lumen. As in *Sphaeroma terebrans* and Decapods (John, *loc. cit.*) the labral ridge is the continuation of the labrum into the wall of the oesophagus. It continues posteriorly into the stomach and forms the dorsal ampulla. The lateral ridges are broader than the labral. The metastomal ridge on the floor of the oesophagus is prominent only in the middle and posterior region. The oesophagus is little dilated in middle of its length.

The oesophageal intima is comparatively thicker and wrinkled. In cross section the epicuticle is not distinct, but the endocuticle picks up the blue colour of Azan (modified). Beneath the cuticle there is a layer of columnar epithelial cells which are comparatively tall in the longitudinal ridges. They contain clear protoplasm and elongated nuclei

placed near the lumen. There are no vacuoles. The epithelial layer is followed by a thin layer of basement membrane and thin circular muscle and on its outer surface there are scattered strands of longitudinal muscles. Like *Spelaeomysis longipes*, (Nath & Pillai, 1972) the salivary glands and tegumental glands in oesophagus are absent in *Nichollsia kashiensis*. The deficiency of longitudinal muscles is compensated by the greater development of gastric muscles. In *Nichollsia* the anterior, posterior and lateral oesophageal dilators (Fig. 16) are better developed than in other isopods. These muscles pass through the epithelium and the fibres are attached to the inner cuticle. These muscles help in the suctorial action of oesophagus.

Stomach : (Figs. 13, 16, 17C ; Pl. III, figs. 1, 2, St.).

The oesophagus opens into the wide pearshaped stomach. The oesophagus and stomach are lying in cephalic and first free thoracic segments supported from below by sternal alae of the endophragmal skeleton (Pl. III, fig. 2, st. al.). Entrance to the stomach from the oesophagus is guarded by one dorsal, two lateral and one ventral ampulla. Other appendages of stomach are anterior dorsal lamella (a. d. l.), posterior dorsal lamella (p. d. l.) posterior ventral (p. v. l.) lamella, bristle (b. p.) plates, central (c. v.) valve and ventral (v. v.) valve. Two filter channels are formed on floor of the stomach. Filter I is formed by bristle plates and ventral ampulla, while filter II is formed by anterior ventral lamellae, median wedge, central valve and ventral valve. Filter II has (Pl. III fig. 1) an outer (o. f. c.) and inner filter (i. f. c.) channels. The outer filter channel ends blindly at its posterior end but is connected with the inner filter channel through the sieve of very fine setae. The inner filter channels open posteriorly into the common chamber where the intestine and hepatopancreas of two sides meet together. Opening of inner filter channels are guarded by the ventral valve.

Histology :

As stated above, the foregut is formed by the invagination of body wall with its inner linning made of cuticle which is thicker in the stomach. This thick cuticle is produced into many inward projecting folds armed with large number of setae, bristles and hooks. The hooks pick up deep red stain showing their epicuticular nature and mechanical function. Beneath the cuticle, there is a layer of epithelial cells which

is not uniform throughout. Separation of the cuticle from the underlying epithelium occurs, particularly where the cuticle is thick. This feature has been noted by a number of authors in various Crustacea (Geldard, 1907, Rehorst, 1914 ; Nicholls, 1931b, and Martin, 1964). While this may be in part an artefact due to fixation, it may also be due to the separation of the old cuticle from the underlying epithelium in moulting stages of the animal when the new cuticle has been formed. Martin (*loc. cit.*) considers that it occurs in the early stages of premoult, but the author has found that it occurs only when the new cuticle is already formed. However, the spaces between the cuticle and epithelium are formed due to differential contraction of the two components during fixation. The foregut is joined with the hindgut in its dorsal region by a thin strand (Pl. VI, fig. 3). The inner surface of the anterior ventral lamellae forming the outer wall of the outer filter chamber of filter II the median wedge and the central valve have porous chitinous linning. Similar cuticle have been reported in *Ligia* (Nicholls, loc. cit.), *Asellus* (Rehorst, loc. cit), *Marinogammarus* (Martin, loc. cit.), and *Sphaeroma* (John, loc. cit.). As in *Sphaeroma*, in *Nichollsia* also beneath the porous linning (Pl. III, fig. 1, ept.) there is a layer of tall columnar cells with granular protoplasm and basal nuclei. According to John (loc. cit.) the cells are secretory and secretions are digestive in nature. The outer part of the stomach wall is formed of a thick layer of connective tissue.

Musculature of the foregut (Fig. 16).

In *N. kashiensis* the gastic musculature consists of the oesophageal dilators, cardiac dilators, pyloric muscles and the gastrics. These muscles are better developed in this species than most of other isopods and obviously compensate the poor development of circular and longitudinal muscles in the wall of foregut.

The foregut musculature can be divided into two groups :

(i) Extrinsic and (ii) Intrinsic.

Extrinsic muscles are those which originate from the wall of the foregut and are attached to the body wall. Intrinsic muscles are those which originate and are attached on the gut wall.

Extrinsic muscles :

There are 5 pairs of extrinsic muscles at the anterior end of oesophagus. These are two pairs of dorsal, two pairs of ventral and a

pair of lateral dilators. They originate from the oesophagus and run anteriorly and posteriorly attaching themselves on the dorsal, ventral and lateral surfaces of the cuticle around the mouth and behind. The two lateral oesophageal dilators originate anterior to the origin of dorsal (dod II) and ventral dilators (vod II).

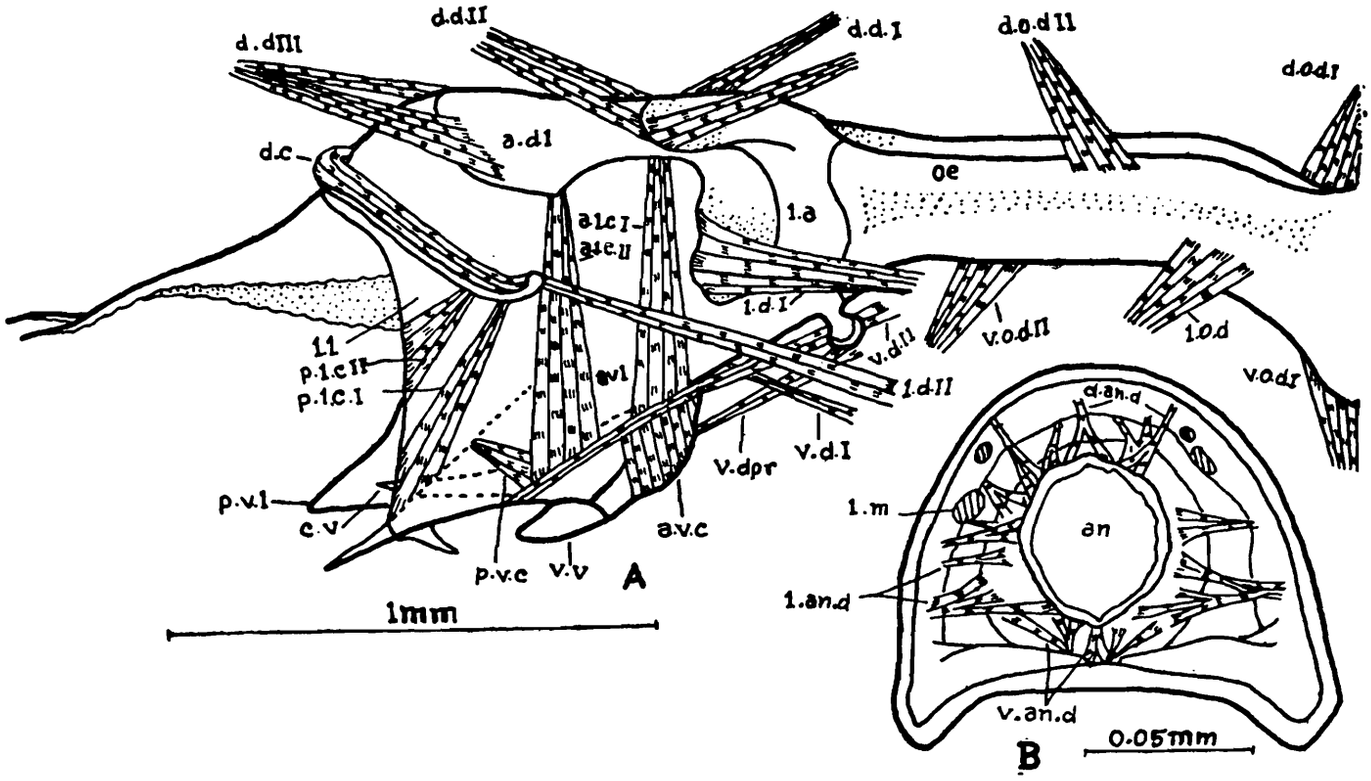


Fig. 16. A. Musculature of the foregut. B. Muscles of the anal region.

There are three pairs of dorsal, (d.d.I. to d.d.III.), two pairs of lateral (ld I, II) and two pairs of ventral dilators (vd I, II.) on the stomach. Out of these seven pairs, three pairs are attached to the four ampullae *viz.*, dorsal ampulla, lateral ampulla and ventral ampulla. All the muscles are paired. The dorsal dilator II (d.d.II) originates from the base of the anterior dorsal lamella while the dorsal dilator III (d.d.III.) originates from the posterior margin of the said lamella. The lateral dilator muscle I (l.d.I) originates from the inner wall of the lateral ampulla, while the lateral dilator II (l.d.II.) originates from the tip of dorsal cuticular sclerite on the lateral sides. The ventral dilator (ve.I) is attached to the ventral ampulla and ventral dilator II (vd.II) is attached to the upper margin of the filter apparatus. All these extrinsic muscles, except dorsal dilator II, III, which are attached to the posterior dorsal margin of the head, are attached to the bases of antennae and around.

Intrinsic muscles :

These muscles are absent from the oesophagus. Stomach possesses seven pairs of such muscles. There is one dorsal constrictor (dc.) muscle which makes half circle along the anterior margin of posterior dorsal lamella. Laterally there are four pairs of intrinsic muscles these are, anterior lateral constrictors I and II (ale. I, alc. II) and posterior lateral constrictors (plc. I and plc. II). Anterior lateral constrictors

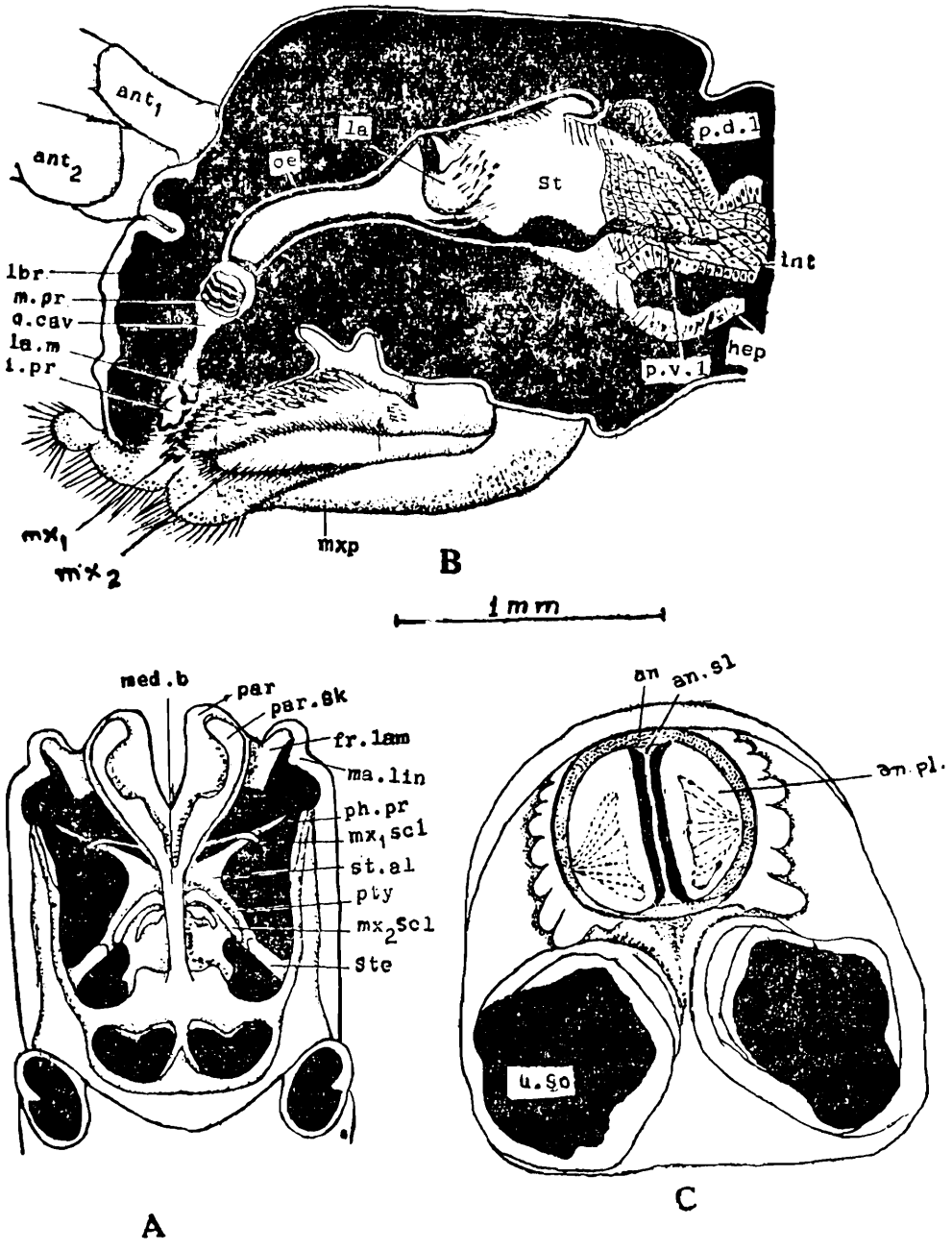


Fig. 17. A. Head showing endophragmal system. B. Foregut and mouth parts in sagital section of head. C. Posterior face of telson showing anal plates.

are attached between the lateral margin of anterior dorsal lamella and dorsal margin of filter apparatus. The posterior lateral constirctors are attached between the cuticular frame of posterior dorsal lamella and posterior margin of lateral lamella. There are two sets of ventral constrictor. Anterior ventral constrictor (a.v.c.) envelops the anterior half of the filter apparatus and stretches between the two cuticular frames on the sides of anterior ventral lamella. The other is posterior ventral constrictor (pvc.) which is paired and is attached between the lateral margin of posterior ventral lamella and the posterior end of outer filter channel. There is a small muscle which originates from the anterior end of the median wedge and attached on the cuticular horns of the stomach. This is ventral depressor (v.dpr.).

Functions of Provcntriculus (Stomach).

Observations on a semitransparent living specimen reveals that the proventriculus contracts, whether or not it contains food. Movement of food in the stomach is obscured due to the thick muscles enveloping it in the head region, but from the structure of constituent parts and attachment of muscles their function can easily be deduced.

The stomach primarily triturates the food material as an auxiliary to the mouthparts and secondarily it acts as a sieve or filter for further retention in the cavity, of such food that have not been sufficiently divided. Digestive juice from the gland also acts in the region of filter chamber. Presence of bifid and multifid hooks on the lateral ampullae is an indication that food is triturated in the stomach. In continuous feeding the food is not retained for longer and trituration is therefore incomplete, because it passes out of the stomach before it is completely triturated.

In *Nichollsia kashiensis* after mastication and trituration, the food passes to the oesophagus and enters the stomach between the bristle plates and ventral ampulla below and the lateral and dorsal ampullae above. Here the food is held up and liquid portion of the food is squeezed through filter I carrying with it only very fine particles. The same motion which compresses the food will tend to force it back into the larger space of the foregut, by virtue of the backwardly projecting hairs with which it will be in contact all around. Contraction of the oesophageal dilators is alternate with one another from anterior to

posterior and also with circular muscle of its wall. This affects the movement of food inside the oesophagus. It is also being substantiated by pushing from mandibles. Actions of the four ampullae are affected by dorsal dilator muscle I (d. d. I), lateral dilators (Id. I) and ventral dilator (v.d. I). When food moves further behind, the dorsal constrictor (d.c.) and posterior lateral constrictor I and II (p.l.c. I & II) bring the posterior dorsal lamellae and posterior ventral lamellae close so that stomach is closed from behind. This helps in filling of the stomach and allowing action of the ampullae in trituration of the food. During this process the triturated food is compressed and squeezed between the dorsal wall and ventral floor of the stomach. The liquid and fine particles of food pass down to filter II in its outer filter channel. Simultaneous contraction of muscle sheath (a. v. c.) across the ventral convex surface of the foregut draws apart the anterior ventral lamellae and thus pushes up the filter apparatus. On return of the parts to their normal position, the ventral depressor (v. d. r.) brings down the floor of filter II and thus the liquid and fine particles of food coming down from filter I with other food is caught between the outer filter channels and median wedge is pressed through the inner filter channel to the common duct of hepatopancreas. The coarse food particles and other food mass passes back to the intestine. When the food is in process in stomach it is possible that digestive juice from hepatopancreas enters the stomach through the intestine. The digestive juice never enters the stomach through inner filter channel because it is always one way traffic due to the presence of ventral valve which allows the food to pass out from filter channel to the hepatopancreas. Movement of fluid from the hepatopancreas to the intestine was observed in the isolated alimentary canal kept in normal saline in watch glass under the stereoscopic binocular microscope. It was found that the fluid moved to intestine and behind. However, its flow to stomach was not confirmed. Many authors (Kanneworff and Nicolaisen, 1969 etc.) have suggested that this fluid goes to stomach and digestion in part may occur there. The process of flow of digestive fluid to the intestine is further aided by rhythmical contractions of hepatopancreatic tubules in *Nichollsia*. Such contractions were observed in the living animal through the transparent ventral surface of a recently moulted specimen, to consist of waves passing forward along the tubules expelling their contents. On relaxation of the muscles of the tubules, liquid from the stomach was observed to inter into the lumen. The more solid portions

of the food with the digestive enzymes, passes into the intestine, where further digestion and absorption occurs.

Opening of stomach and backward movement of food is affected by dorsal dilator II and dorsal dilator III and lateral dilator (1. d. II) and ampullar muscles.

Digestive gland or hepatopancreas (Figs. 18, 21B & Pl. IV).

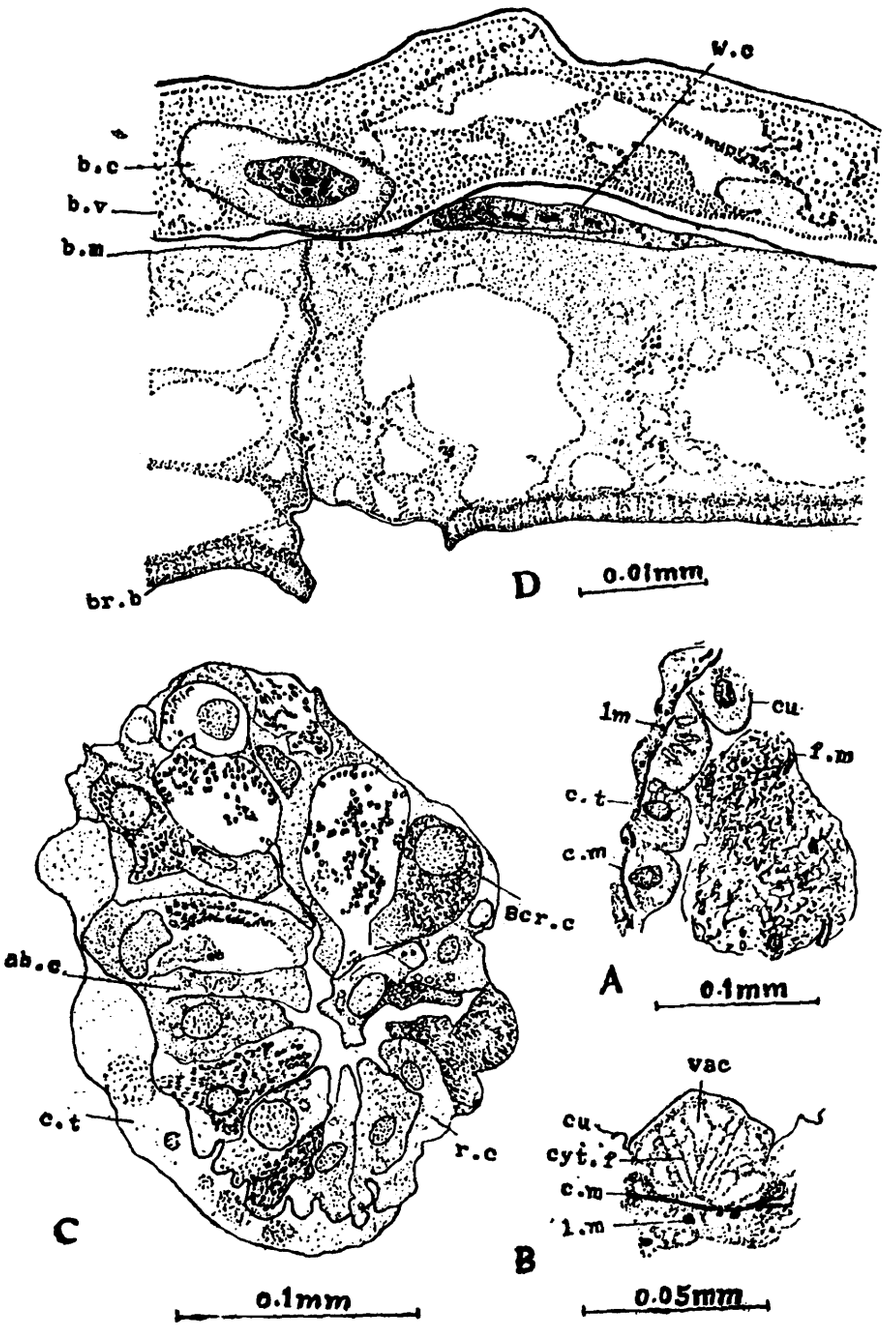


Fig. 18. A. T. S. intestine showing diffused cuticle. B. T. S. intestine showing vacuoles. C. T.S. hepatopancreas. D. Magnified view of hepatopancreas epithelium.

Morphology ;

The digestive gland consists of two pairs of blind tubules opening into the alimentary canal at the junction of the foregut and intestine, on ventral side in the maxillipedal region. These tubules extend posteriorly on either side of the intestine as far as the fourth abdominal segment in adult animals and only upto 6th thoracic segment in youngs. At their blind ends they taper off and get inserted on the neighbouring

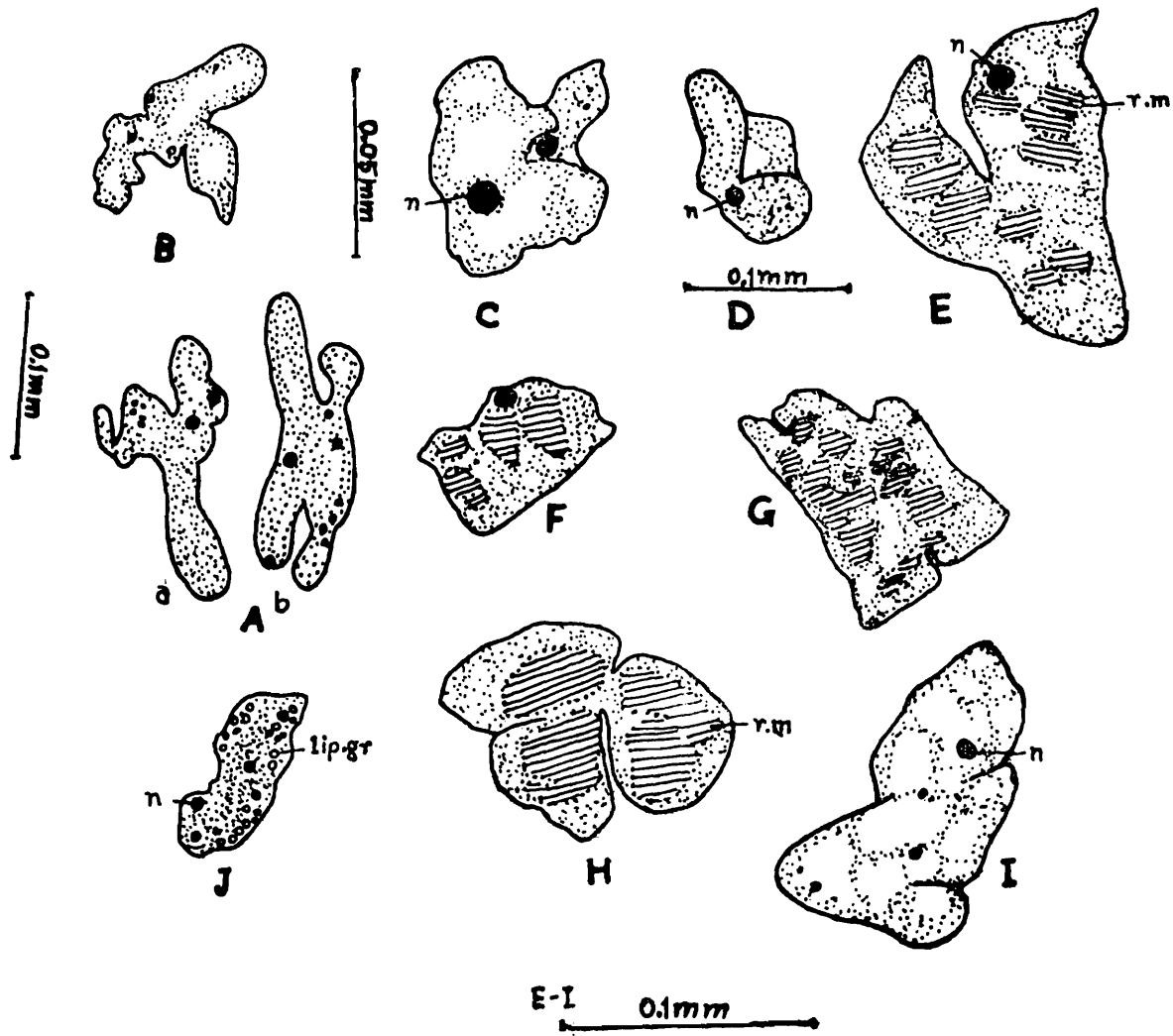


Fig. 19. A. L. S. antennal gland B. L. S. mandibular gland. C. L. S. maxillipedal gland D. L. S. coxal gland of first thoracic segment. E-I. Coxal glands in thoracic segment II-VI. J. Section of an athrocyte.

structures by connective tissue coat on their outer sides. The components of each pair are situated one below the other (Pl. IV, fig. 3 ; hep.) and each tubule is constricted at interval so that sometimes it has a beaded appearence. This contriction is caused by longitudinal muscle arranged spirally on the gland tubule. Each pair of a side has a common

duct proximally which run transversely and join medially with its counterpart below the stomach in a common chamber where the two inner filter channels of filter II open and are guarded by the ventral valve lying in the chamber. The communication between the chamber and intestine is guarded by the central valve.

Hepatopancreas look yellow with dark brownish granules through the semitransparent cuticle of the animal.

Each tube has been divided in three regions, an anterior and à posterior one which are more or less shorter regions and are less active in comparison to longer and physiologically very active middle portion.

Some authors (Alikhan, 1969 ; Martin, 1964) have considered these zones as nonsecretory, secretory and germinative zones. Guieysse-Pellissier (1913) has considered only two zones, as zone of germination and zone of growth, which has been termed as embryonic zone and transitional zone by Nath and Pillai (1972).

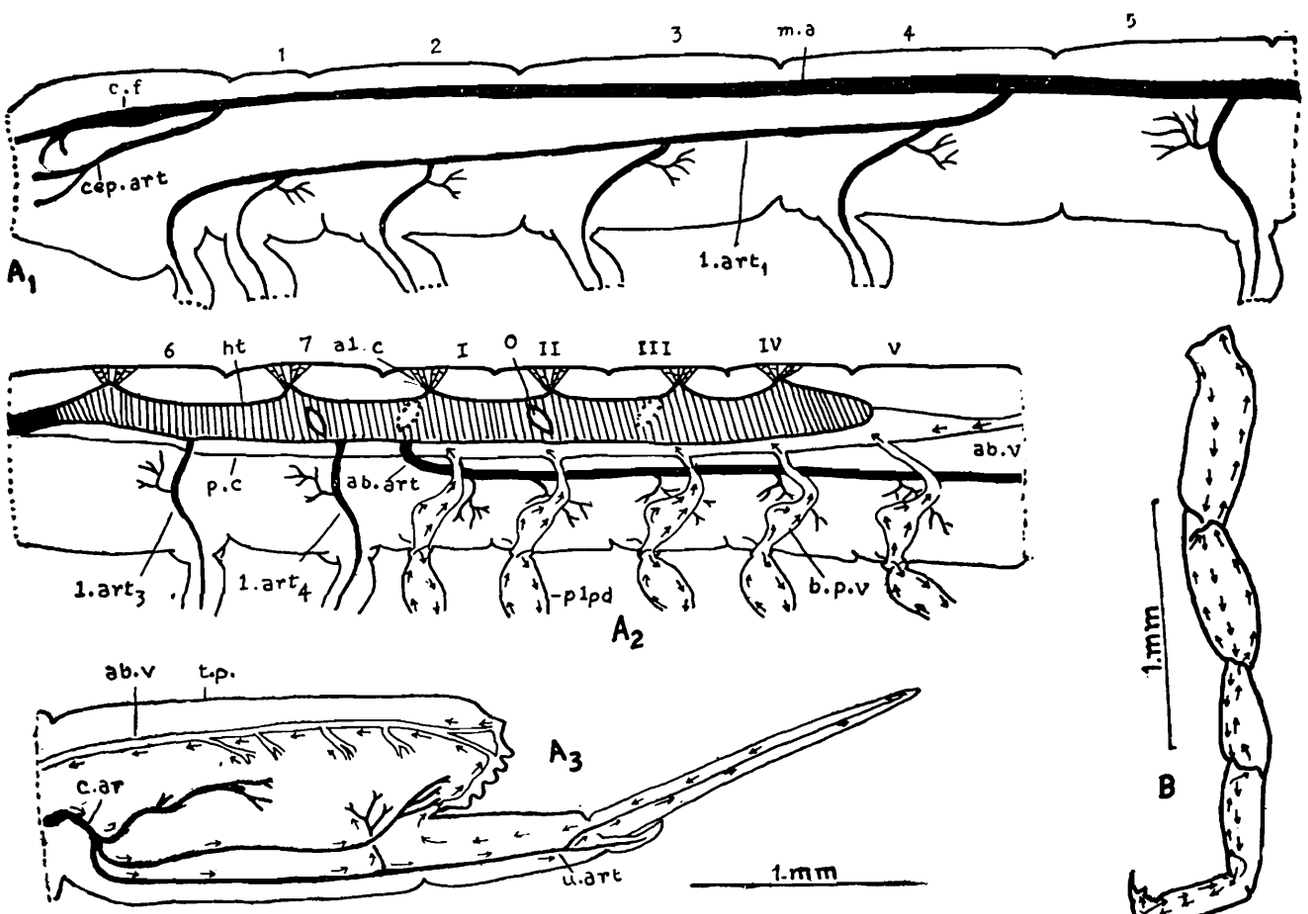


Fig. 20. $A_1A_2A_3$. Circulatory system in the body of *N. kashiensis*. B. Circulation in thoracic leg.

Histology of the digestive gland :

In transverse section each tubule is composed externally of connective tissue in which a network of fibres and isolated blood vessels are embedded (Fig. 18C, D). Internally an epithelium nearly fills the whole of lumen of the tubule and consists of two distinct cell types

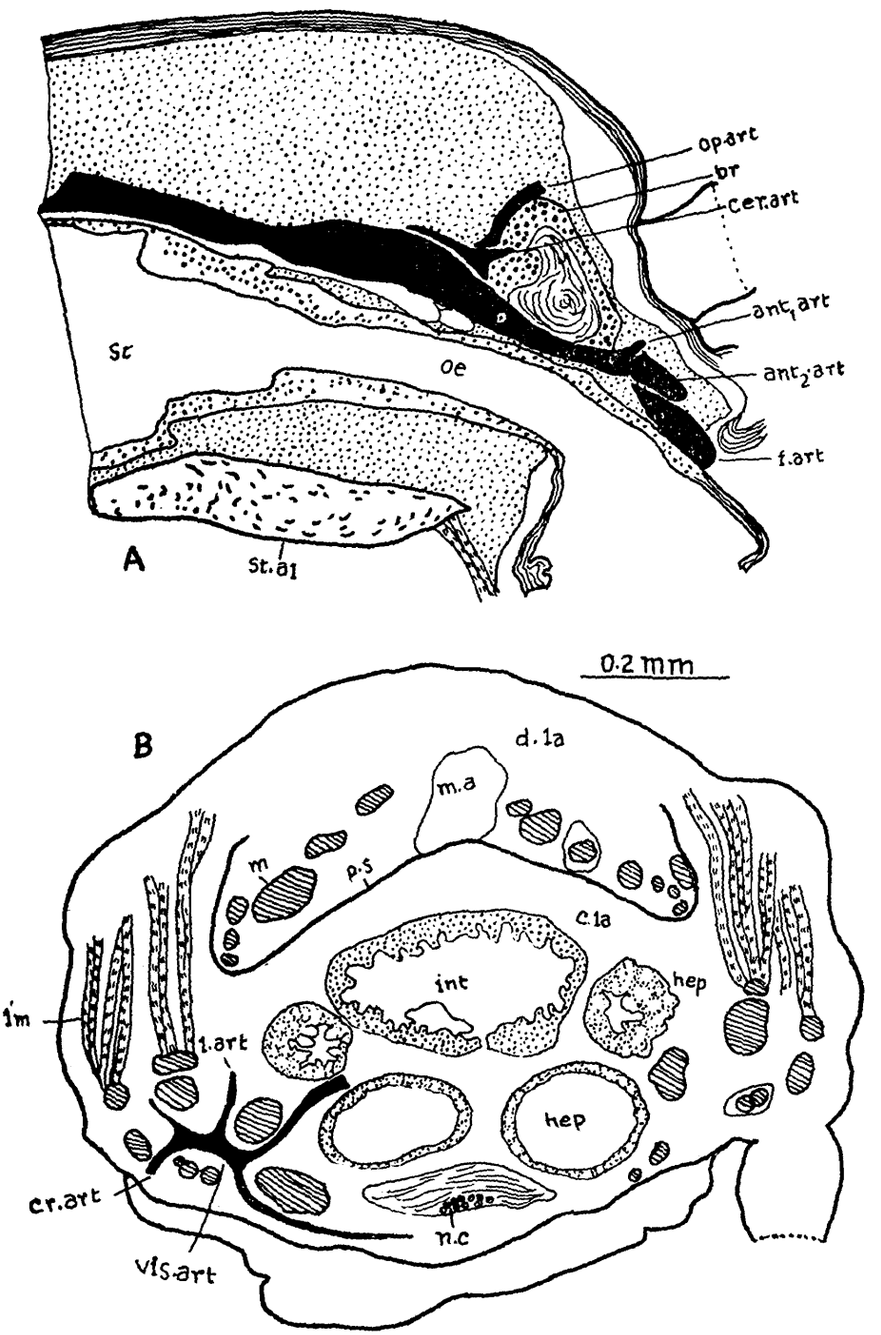


Fig. 21. A. Sagital section of head showing vascular system. B. T. S. Thorax showing ramification of lateral artery.

with a third type of replacement cells which are not very distinct. These are, absorptive cells, secretory cells and replacement cells.

These cells are evident in the middle region of tubule in the whole mounts (Pl. IV, fig. 1, 2.). The distal or terminal zone or tip of the tubule does not show this differentiation fig. 2.) but in the proximal region cells in active absorption and secretion may be observed. Black granules with discharged fluid into the lumen could be seen in the distal region of the tubules (scr.). In this region cells are smaller with small nuclei measuring between 6-10μ while further anterior the cells are somewhat larger with nuclei 20μ in diameter. In matured zone the cells are much larger with nuclei between 10-20μ in diameter. Each tubule is composed of 12-15 rows of cells in cross section. Lloyd (1908) noted 10 rows in *Asellus* and more than 12 in *Porcellio*. Each absorption cell at its basal region is broad, flat and hexagonal in shape. In some cells the nucleus shows two nucleoli a common feature in Crustacea (Launoy, 1903 ; Shyamasundary and Verghese, 1973).

Absorption cells. (Fig. 18 & Pl. IV ab. c.).

These cells are called columnar cells (Nath and Pillai, 1972). These cells are about 20μ long and 8μ broad and freely distributed all along the organ. Their nuclei are (4-5μ) oval and mostly situated in the centre and have one or two nucleoli. The chromatin granules are diffused. The cytoplasm appears frothy due to the presence of small vacuoles which may be centres of stored food. These cells are in different stages of vacuole formations. They are more numerous posteriorly with their free ends, which have a brush border, projecting freely into the lumen of the tubule and have broad bases with the infoldings of basal membrane.

Secretory cells :

These cells have also been called as gland cells. They are 35-50μ in length and 15-20μ in width. Large vacuoles inside restrict the cytoplasm to thin peripheral linings. Such cells are also called extrusion cells (Pl. IV, fig. 3, 4, ext. c.), since they extrude the contents of vacuoles into the lumen of hepatopancreas. Vacuole is filled with granular material staining dark blue with Mallory's triple or haematoxylene. The nuclei are large (8-10μ) and spherical taking amoeboid shape in some cases. The brush border is closely visible (Fig.

18D, 2. br. b.). Small spherical bodies or granules are generally aggregated around the nucleus in mass and later occupy the whole cell. Similar granules have been noticed by Frenzel (1884) and Chandy (1938). Such granules have been termed as Zymogen granules (Murlin, 1902, Chandy, loc. cit.). In starved animals granules disappear. Number of secretory cells increases anteriorly.

While in secretion, secretory cells alternate with the absorptive cells (Pl. IV, fig. 1). In active secretion the tubules show cells in different stages of gradual transformation from absorptive to secretory phase and it is probable that the cell in beginning is absorptive and later becomes glandular, an idea supported by many workers (Nusbaum & Hilarowicz, 1920 ; Agrawal, 1962 ; Davis & Burnett, 1964).

All the tubules do not show same stage of secretion. The dorsal tubules (Fig. 21B) take the first course in action and their cells get very much shrunken owing to discharge of their secretory fluid into lumen and the cavity becomes wide. This is followed by the ventral pair which becomes active, where the cells become very large, being full of copious secretion and large vacuoles, ready to discharge and the bulged out cells occupy practically whole of the lumen. This effect is more marked anteriorly than posteriorly. Thus the active phase alternates in dorsal and ventral pair of tubules.

A basement membrane is seen at the proximal side of the epithelium (Fig. 18D b. m.) with wavy appearance. It is very thin and a wandering cell (w. c.) as has been named (Ogasawara, 1968) is also seen on the outer side of it. Actually this cell seems to give rise to the basement membrane and has a thin flat elongated nucleus. Such cells are not frequented in the sections. The basement membrane in Isopods is very thin in comparison to Decapods (Ogasawara, op. cit.).

The striated or the brush border is 2μ thick with a dense (br. b.) outer and an inner terminal region, facing the lumen, while in between them is the middle portion of thin consistency showing straight striations. In optical microscope it is not possible to give any interpretation for this zonation of the brush border acurately. However, it probable that these dense regions correspond to the dense regions found in the midgut diverticulum of the copepod *Calanus helgolandicus* (Ong & Lake, 1969). Patane and Cicero (1965) have found the presence of microvilli in brush border of the cells of *Idotea*.

Fasting results in the disappearance of granules and vacuoles in the larger cells and widening of the lumen with brush border becoming more prominent,

When animals were starved for 24-48 hrs. and again fed on alcoholic carmine and sections were cut it was found that carmine particles have not entered the hepatopancreas but the cells are seen active being enlarged and filling up the lumen of the tubules. Thus it is probale that only selected fine food particles could through the filter apparatus. This does not effect the entrance of soluble forms to the tubules. Entrance of food in the alimentary canal initiates secretion by the glands.

Release of secretion :

Activity of the secretory cells is judged from the nature of full grown secretory cells transformed into a pear shaped cell and providing clear evidence of extrusion. It is being further strengthened by the presence of spherical vacuolated secretory cells with nuclei (Pl. IV, fig. 4. ext. c.), in the lumen. Thus it is a holocrine secretion followed by rupture of cells and intraluminar digestion of food. It may be mentioned that released secretory cells have been found in transverse sections of the tubules. Release of secretory product by rupture or burst of the cell (merocrine secretion) can also be observed and it is possible that both processes are involved in release of secretory products. Similar observations have been made by Shyamasundari and Verghese (1973) in Amphipods. In crustaceans there are variable reports on the mode of secretions. In decapod *Carcinus maenas*, merocrine activity has been reported by Stainer *et al* (1968), while Hirsch and Jacobs (1929, 1930) have reported holocrine secretions in *Astacus leptodactylus*. Martin (1964), found merocrine activity in *Marinogammarus obtusatus*.

Intestine :

In *Nichollsia kashiensis* the intestine is an elongated straight tubular structure lying in middle of the body cavity. In telson the tube ascends to the dorsal half of the body cavity, opening posteriorly with a vertical slit. The whole tube is underlined by chitinous layer. It is flexible and capable of enormous expansion, serving to store large quantity of of rapidly bolted food, so that the lumen expands to 6 times to its normal diameter. When empty the lumen obliterates and intestine becomes flat. Typhlosole is absent.

At the anterior junction, the intestinal epithelium gets thinned and altogether takes the thickness of a strand. At its posterior extrimity intestine is constricted, demarcating the rectum. It is short and possesses dilator (Pl. III, fig. 3 d. m.) muscles.

Histology of Intestine :

Outer surface of the intestinal wall is formed of an investment of connective tissue (Fig. 18A, B. ct.). Beneath this there are longitudinal muscles inside to which are circular muscles (1m., cm.). Inner to the muscle bands, there is a thin, deeply staining layer of basement membrane· The epithelium lies below the basement membrane. It is formed of a continuous layer of the protoplasm without cell boundaries but the foldings of cuticular invaginations give impression of individual cells which contain nuclei and numerous rhin cytoplasmic fibrils. At the anterior end the epithelial cells are long and columnar with elongated nuclei in the middle.

The syncitial nature of epithelium can be made out when the intestine is filled with the food material and lumen is fully expanded. The epithelium becomes very narrow and individuality of cells is obliterated. Epithelium is thinner in anterior region where cytoplasm fibres are more or less absent.

In telson region the intestine has 18 rows of cells in cross section. These can be divided into 4 groups of small and large cells. Two groups of small cells in groups of 4 and 5, face diagonally. Similarly other groups of 4 and 5 large cells, face each other. The number of rows increases anteriorly in the abdominal region making zonation and distinction of small and large cells difficult. The small cells are taller than broad while the large cells are cuboidal with broad bases. Large cells in hindgut possess large spherical nuclei (20 μ in diameter) while small cells possess comparatively smaller nucleus which is basal in both types. Large cells are more vacuolated having larger vacuoles around the nucleus. In some cells the nucleus is supported by muscle fibres invading the cell cytoplasm.

Cells in contact with food mass are with many vacuoles and show thin and diffused chitinous intima, where as it is ihick and dense in other regions (Fig. 18 A, B). In starved animals the rectal epithelium is thrown into folds.

Peritrophic membrane is not distinguishable in sections, but it is clear that food is bound in a thick mass in the posterior part of the hindgut.

Rectum :

At the posterior end of the intestine there is a short region with thick and folded epithelium (Pl. III. fig. 3), called rectal pad r. pd.) well supplied with muscles. This portion is demarcated from intestine by a sphinctor. Epithelium is a syncytium and continuous with that of the intestine. The anal slit is guarded by two semicircular (Fig. 17C, an. pl.) chitinous plates acting as valves. There is a small amount of non-vacuolated connective tissue outside the muscles of the rectum. Presence of vacuolated cells in intestine and rectum evidences more absorption in this region.

Musculature :

Apart from the regular outer longitudinal and inner circular muscles, there are few dorsolateral muscles originating from intestine and are attached on the body wall. Such muscles are visibly present in fifth abdominal segment. In telson large number of muscles are attached ventrally. In the rectum between the two ends of constrictions run longitudinal muscles, action of which closes the narrow lumen of the rectum. This way the food is retained here for longer time and passes slowly. There are also oblique transverse muscles around the rectum. Beyond and behind the rectal pad there are rows of dorsal, lateral and ventral extrinsic muscles (Fig. 16B), these are all dilators. Anal valves are provided with closing muscles one on each side.

Function :

Food moved behind with the peristaltic movemnt of the intestine. When it reaches to 5th pleon a small piece from food mass is broken away and ascends into the caudal part gradually moving to the rectum, where it is retained between folds of the rectal pad, heavy absorption of liquid from the food mass takes place. Afterwards the faecal pellet is thrown out by the action of muscles. In *Nichollsia* it has been observed that just after faecal discharge the opened and closed 5-6 times which indicates that there is anal uptake of water to act as an enema. Such anal uptake of water has been observed in many other

crustaceans, e. g., in *Caridina* (Pillai, 1960), and *Bathyporeia sarsi* (Kanneworff and Nicolaisen, loc. cit.) and other crustaceans (Fox, 1952).

Chitinous intima in the intestine of *Nichollsia* does not show pores or meshes, but it acts as a permeable membrane which thins out and allows food absorption in soluble forms. In this respect *Nichollsia* is much closer to *Ligia* and differs from *Sphaeroma terebrans*. However, presence of black granules like those of hepatopancreas, in the basal region of epithelial cells of intestine may be the case of particle absorption through the chitinous intima.

Movement of food in alimentary canal :

For this study animals were starved for 72 hours and since they are coprophagus, their faeces were periodically removed to avoid feeding on it. After 72 hours it was found that the alimentary canal does not contain any food material, Starved animals were fed on black paper. An average sized (2.4 cm.) adult took 2 hrs. to fill the whole alimentary canal. Such individuals were removed to separate jars without any food to study the rate of their faecal discharge and movement of food. The faecal pellets were removed very often to avoid feeding on them.

In these experiments it was found that the animal required approximately 20 hours to empty its gut of black paper at 29-30°C and 25 hours at 24-25°C.

Moore (1975) found that *Asellus* required approximately 26 hours to empty its gut of algae and plant at 15°C and 75 hours at 5°C while the corresponding value for *Gammarus* were 18 hours and 40 hours.

Defecation :

In a normal feeding individual kept in aquarium at 31-33°C, faecal pellets are discharged every 5-40 minutes. The faecal matter is retained for 15 to 40 minutes between the rectal pads and then expelled out with sudden jerk.

In a carnivorous *Eurydice pulchra*, faecal matter is discharged in a loose unbound condition (Jones, 1968), not in the form of pellets. This is probably due to change in the food habit.

Peristaltic movement :

Peristaltic wave starts from posterior end of intestine and takes 3 seconds to reach the anterior end and vice-versa and interval between two waves varries from 7 to 15 seconds in a normal adult individual.

pH of the gut :

From table II it will be seen that pH of the gut varies very slightly from one end to the other, whether the animals are starved or well fed. As a result of starvation, pH of the intestine was found to be slightly lower. Hepatopancreas has significantly low pH than intestine and is unaffected by starvation. This Foregut has more or less same pH as other parts of the intestine. This shows that digestive juice reaches to the foregut also.

Although no chemical tests were employed, on the basis of observations some conclusions on the presence or absence of two particular enzymes, can be inferred. Animals were fed on algal filaments collected from wells and the faecal mater was teased and examined under binocular microscope. It was found that green filaments were intact without any damage to their cellular wall, thus the cytoplasm and consequently the chlorophyll remained unaffected by the digestive

TABLE II

(pH of the gut)

Animals under normal feeding

Sl. *No.*	*Stomach*	*Intestiue*	*Excreta*	*Hepatopancrens*	*Haemolymph*
1.	6.5	6.6	6.7	5.4	6.8
2.	6.7	6.7	6.8	5.8	6.9
3.	6.7	6.7	6.7	6.00	6.8
4.	6.4	6.8	6.7	6.00	6.7
5.	6.7	6.7	6.6	5.8	6.8
Mean	6.6	6.7	6.7	5.8	6.8

pH after 48 hrs. of starvaton.

Sl. *No.*	*Stomach*	*Intestine*	*Excreta*	*Hepatopancreas*	*Haemolymph*
1.	6.4	6.5	—	5.5	6.8
2.	6.5	6.4	—	5.8	6.9
3.	6.6	6.1	—	5.8	6.8
4.	6.7	6.5	—	6.00	6.7
5.	6.5	6.5	—	5.5	6.8
Mean	6.5	6.4	—	5.7	6.8

enzymes. This also indicates that the particular enzyme 'cellulase' which could effect the cell wall is absent. while those cells, punctured by the mouth parts or hooks and spines of ampullae have been acted by digestive enzymes, completely loosing their green colour. Nicholls (1931) found that cellulase is absent in *Ligia* which is probably due to the omnivourous feeding habit.

Similary chitinous parts of insects as well as exuviae of its own remain unaffected by digestive enzymes and were found intact in their faecal pellets. This observation shows that chitinase is absent in *Nichollsia.*

EXCRETORY SYSTEM

All the crustacea possess a pair of functional "segmental excretory organs" (Cannon, 1931 ; p. 478). Situated in the segment of either the second antenna or the second maxilla ; occassionally both pairs are developed. In forms with free larvae one pair functions in the larva and the other in the adult, in entomostraca it is the antennal and maxillary respectively and in Decapoda the reverse.

Occurrence of one or the other pair in adult crustacea does not follow taxonomic classification at all closely and it seems probable that an explanation of their distribution is to be sought in functional factors such as the direction of water currents in the neighbourhood of the mouth. Thus in Decapods with an anteriorly directed respiratory current the antennal organ is functional whereas in isopods, where water appears to pass out behind the maxillipeds in the process of feeding, it is the maxillary organ.

The organs are probably the remaining representatives of an originally complete segmental series and may therefore be homologous with the nephridia or nephromixia a of annelids or with the coxal glands (Goodrich, 1895). They consist of an "end sac", closed proximally and leading distally into a duct which inturn opens to the exterior, either directly or via an "exit tube" (Manton, 1928a ; p.424) of small ectoderm cells. There are, as stated by Needham (1942) two forms of the organ (Burian and Muth, 1924). One has a duct of a few (3 or 4) large cells with intracellular lumen, and no exit tube while the other has a multicellular duct with multicellular lumen and a well developed exit tube. These have been referred as 'paucicellular' and 'multicellular' types respectivelly by Needham (loc. cit.). There has been some recent additions to the studies on the excretory organs of Isopoda (Needham, 1942a ; Gorvett, 1946 ; Patane, 1962).

Description : Excretion in *Nichollsia kashiensis* is carried out by three different systems.

1. The segmental excretory organs.
2. The Hepatopancreas.
3. The tegumental glands or rosette glands.

Of these three systems the first and third will be dealt in this section. The other system is discussed in the section dealing with the hepatopancreatic gland in digestive system.

In *Nichollsia* the segmental excretory organs can be described in the following heads :

1. Segmental glands of the head.
2. Segmental glands of the thorax.
3. Athrocytes or the branchio-pericardial organs of the abdomen.

Segmental glands of the head (Fig. A, B, C & Pl. V).

In *Nichollsia kashiensis* apart from a pair of well developed maxillary glands, there are a pair of small vestigial glands in each of antennal, mandibular and maxillipedal segments. The antennal gland is situated behind the base of the antenna and infront of the brain. It is a paucicellular gland having a small group of cells enclosing a small intracellular, ill defined lumen but having (Fig. 10A) no duct of any kind. These cells develop a very large intracellular vacuole with fine granular contents and prominent large spherical nuclei. In thionin stain nuclei take a very deep blue stain. In animals injected with 1% trypan blue the glands take up the blue stain and can be very easily seen through the transparent cuticle of the animal and their external morphology can be traced out. While studying the histology with Azan's (modified) stain (Hubshman *loc. cit.*) it was noticed that the granules pick up the deep red while other portions encluding nucleus are stained orange colour. This perhaps indicates the incretory nature of these glands and support the view expressed by Needham (1942) that antennal glands or the so called Nemec's gland may well be an organ of internal secretion in the adult. Each gland in its outline is more or less 'y' shaped and become more prominent during moulting.

There is a mandibular gland (Fig. 19B) lying below the antennal gland. It does not show much variation from the latter in its histomorphology. In shape it is different but overall 'y' shape is maintained

in this gland. In Heidenhain's Azan (modified) the red drops are seen around the nuclei while in thionin stain the lumen is clearly seen. In transverse section of the animal these glands are seen lying on two sides of the suboesophageal ganglia towards the periphery. They contain black granules similar to those of hepatopancreas accumulated in their lumen and it is so nearer to the sternal and tergal junction that its opening outside seems a probability. However the author has not been able to trace out any opening either morphologically or histologically but the absorption and aggregation of black granules in the gland indicates that these are functional and are enclosed in haemocoelic space. Nerve supply to these glands were not traced. This gland is also incretory and its position coincides with that of Ter-Poghossian's organ, but histologically it is much different from the latter.

There is present a small coxal gland above the maxilliped. The lay out of the maxillipedal gland (Fig. 19C) is similar to those of other coxal glands of thorax.

The maxillary gland (Pl. V, fig. 1-4).

A pair of glands lies on the two sides of the stomach and are enclosed in the capsule formed by the invagination of the sternal alae. Only the end sac and the coils of the duct are enclosed in the capsule while the bladder and the exit duct lie outside, below the capsule. The end sac is placed ventral to the duct coils and towards the mesial edge of the capsule. The coil of the duct encloses the end sac from dorsal, outer lateral and anterior sides. Posteriorly the end sac communicates with the atrium (atr.) a swollen proximal part of the duct. Details of coiling are not easy to follow in the adult owing to the deposition of reserve material in the surrounding tissues and even in the duct cells. The duct starting from the posterior end of the sac (e. s.) proceeds dorsal and anterior to it and forms convoluted coils. The end sac, contrary to the conditions described in *Asellus aquaticus* (Needham, 1942) is elongated and lying parallel to the long axis of the body.

The maxillary gland in *Nichollsia kashiensis* has the following components :

1. The end sac.
2. The atrium.

3. The coiled duct.
4. The bladder.
5. Exit duct.

The end sac leads to the atrium by a narrow opening. This opening is guarded by a sphinctor valve (sph. v.) which lies at the posterior end of the sac. There are four sphinctor cells whose free ends protrude into the atrium of the duct. In *Asellus* there are contractile fibrils (Needham, 1942), but in *Nichollsia* such fibrils are not distinct to be mentioned here. It is possible that communication between the two chambers (*i. e.* end sac and atrium is operated by the increased fluid pressure in the end sac or the volume of sphinctor cells. The above operation of valve is postulated in the absence of intracellular fibrils which I have been unable to render in histological preparations of *Nichollsia kashiensis*.

Histologically the organ shows essential similarity with that in other crustacea (Needham, 1942). Cells of the end sac are relatively small and narrow with regular nuclei and clear cytoplasm and are set rather loosely, as a single layered epithelium on the basement membrane which binds them externally. A characteristic 'brush border' is seen as a dense layer on the surface of the duct facing the lumen. The duct wall is syncytial and the lumen is intracellular with the nuclei and cytoplasm pushed aside, wherever the nuclei are present. The lumen of the duct also gets progressively narrower distally. The bladder in *Nichollsia* (bl) is a part of the exit tube, like that of *Asellus* (Needham, 1942), Stomatopoda (Woodland, 1913), Amphipoda (Vejdovsky, 1901) etc.

The whole organ is bathed by the fluid of the body cavity (haemocoel) and blood cells appear to be very abundant dorsolaterally near the proximal coil of the duct. The blood cells in the vicinity of end sac and duct coils, are loaded with large number of droplets of varying sizes taking deep red stain in the modified Azan. These cells probably carry the excretory matter which is excreted out through the gland. From the histological preparations it appears (e. s.) that secretion in the end sac and gland duct is holocrine in nature. Contrary to the condition in *Asellus aquaticus* the gland in *Nichollsia* is enclosed in the capsule formed by the sternal alae or sternal plate of the endophragmal system on the two sides of the mid-longitudinal plane. The spaces among the coils tend to become much reduced by accumulation

of reserve material in both, duct and connective tissues. The duct abruptly becomes wide and form the bladder.

The bladder is a thin walled dilated structure. It is broad dorsally and narrows down progressively in the form of a triangle. The exit duct is small and has cellular sphinctor (Pl. V, fig. 4) at the bend of the first endite. There seems to be a small valve at the junction of the duct and bladder projecting into the latter but this observation is not varified further in other sections.

Segmental glands or coxal glands of the thorax (Fig. 19 D-I).

A pair of the coxal glands are present in each segment infront of the coxal region of the legs situated between the flexor and extensor muscles of the body. The second thoracic gland is the largest with more numbcr of cells involved in its formation. As already mentioned these glands are more or less, Y shaped though there may be little variation in this shape. These glands are a group of (5-7) cells having intracellular lumen without any clear demarcation of its inner wall. They become more prominent during the moulting period and possibly store the reserve material at the onset of moulting, which is released and reconsumed during the formation of new cuticle at the end of moulting. There is no outside communication in any of the glands. They are lying in the efferent sinuses of the pereopods in the coxal region and blood passes around them while returning to the heart.

Histologically they are similar to those of antennal and mandibular glands and may be, like other glands, of incretory nature. The nuclei are larger and spherical unlike those of non secretory tissues.

Histology of the Maxillary gland :

The wall of the duct is syncytial, exit duct is thin walled and possess clongated nuclei giving the impression of its ectodermal origin. The wall of the excretory duct does not have a uniform thickness which is mostly due to displacement of nucleus and greater quantity of cytoplasm towards the periphery.

The end sac has a uniform and thin wall. The ampulla or atrium has thicker wall than the end sac. The cytoplasm is in almost all cases granular varying in density in different parts. The inner wall of the duct is thicker than the outer wall. The cytoplasm of the duct possesses large number of vacuoles by the side of its nuclei. Some of

the duct nuclei show double nucleoli and many chromatin masses. In cross section the brush border of the duct is seen in three different phases. In first phase there are few scattered granules seen along the border. In the second phase the density of granules is increased while in third phase it is uniformly thick and without granules. In response to these changes, corresponding changes have been noticed in the cytoplasm.

Histology of other glands of head and thorax :

The antennary gland has many deeply stained yellow to red granules or globules of different sizes around the yellow or orange nucleus in Azan's stain, this trend is maintained in all other glands. Cytology of these glands is similar to trophocytes described by Balashov (1963) in moulting tick *Hyalomma asiaticum*. Those trophocytes are large and contain glycoprotein inclusions. However in *Nichollsia* the nature of inclusions was not determined but it is quite certain that such inclusions, taking deep red colour in Azan, are invariably present at the time of moulting.

Athrocytes or Branchio-pericardial organs :

Athrocytes are present in abdominal segments from first to fifth on both sides of the body in the branchio-pericardial veins (sinuses) (Pl. VI, fig. 1, 2). Each sinus is the exapansion of efferent artery leading from pleopod to the pericardium. They are so wide that they enclose a wide triangular space with the posterior arm elongated and leading to the pericardial chamber. Because of this arrangement the blood spreads out in the sinus and retained till the whole sinus is filled up and then passes to the pericardial chamber.

The floor of the vein (sinus) possess groups of cells enclosed in vesicles (Athr.) called 'Athrocytes'. Number of cells in vesicles varies from one to five or even more. Some times 3 to 4 vesicles are seen proliferating from one point. As mentioned above vesicles are formed by the outward buldging or swelling of the floor. During moulting period the athrocytes become large and contain large thick masses of yellowish brown reserves around their nuclei. When trypan blue is injected in the circulatory system they pick up blue stain and have very distinct boundary (fig. 2). Athrocytes are larger towards the postero-dorsal corner of the sinus. It was Patane (1962) who named

the vesicles as 'Athrocytes' in *Idotea baltica basteri* Aud. Each athrocyte, in active phase histologically contains a number (Fig. 19J) of nuclei, small spherules taking red eosin stain (lip. gr.) and dirty green mass of reserve material in the form of fine but dense granules. When teased the athrocytes, the lipoid droplets were seen floating on the water surface. These droplets show yellow colouration in seetions stained with Mallory's triple.

Tegumental glands.

In adult *Nichollsia* there are two sets of rosette glands one on each side in the base of second maxilla in the basipodite and the other set in the labrum. In maxilla each half contains two lobes. In labrum a group of 5 to 6 cells are arranged in the form of a berry around a central cell which is probably the duct cell. They are very similar to the tegumental glands of *Porcellio* (Gorvett, 1946). The number of cells participating in gland formation in maxilla is more than labrum.

There is a small gland like structure at the base of the antenna on on its ventral side. Apart from the above described structures few scattered nephrocytes pick up injected trypan blue, such structures have been noted mainly at the joints of appendages.

CIRCULATORY SYSTEM

In *Nichollsia* the vascular system consists of the pericardial cavity, the heart arteries and a series of blood spaces or sinuses representing the venous system.

The pericardium (Fig. 20 p.c.) is a dorso-ventrally flattened sac, situated on the dorsal side of the body enclosing the heart between the anterior border of the sixth thoracic segment and the posterior border of the fourth abdominal segment. It is bound dorsally by the connective tissue of the body wall, laterally by the dorsal segmental muscles and the dorso-ventral muscle bands and ventrally by the alimentary canal.

Floor of the pericardium is formed of a horizontal septum which separates the heart from the alimentary canal. The horizontal septum (Fig. 22A, p. s.) alone is clear in *Nichollsia kashiensis*, but at some places the lateral septa are also evident bordering the muscles. Infront

it gradually tapers and at the hind end the pericardial chamber continues and extends as a narrow channel as abdominal vein (ab. v.) which collects blood from the telson and delivers to the pericardium. On each lateral side there are five apertures in linear succession through which the branchio-pericardial (b. p. v.) veins open. Thus it will be seen that the pericardial cavity communicates with branchio-pericardial veins laterally and abdominal vein on the posterior side, apart from the small sinuses on the dorsal side. The horizontal septum continues below the dorsal aorta upto first thoracic segment. Alary muscles are segmental (al. c.) and weakly developed in *Nichollsia kashiensis*.

Heart

In *Nichollsia kashiensis* the heart is a single chambered elongated tubuler sac of spirally (Fig. 20, 22, 23, s. m.) arranged striated muscles pierced by afferent and efferent apertures. It extends from the fourth abdominal segment to the sixth thoracic segment (Fig. 20) anteriorly. Posteriorly it ends blindly in the fourth pleon while anteriorly continues into median aorta. It rests completely on the floor of the pericardial cavity and the dorsal wall of the heart is attached to the integument in the middle of each segment by a bundle of connective tissue fibres (al. c.) ; these are the alae cordis, between which the heart hangs freely. Continuation of these connective fibres is spread over the heart longitudinally. Apart from the dorsal alae cordis there are two pairs of lateral and a pair on ventrolateral sides connecting the heart with the lateral and horizontal pericardial walls. At places the heart is connected with pericardium ventrally by a group of small and fine connective threads numbering about 10-12 on each side of the midventral line (Fig. 23B, ct. f.). The heart in cross section is circular in anterior, egg shaped in the middle and elliptical in the posterior region. Length of the heart is one third of the body length which is unusual. An elongated hearted in semiterrestrial Isopods has been considered (Silen, 1954) as more evolved condition. In *Nichollsia* it seems to be an adaptive feature for semicylindrical and elongated body form which is an adaptation (Chilton, 1894 ; Fage, 1955) to the subterranean habitat.

Number of ostia (O.) is two pairs arranged segementally from 7th thoracic to 3rd abdominal. The ostia are arranged on the left (7th thoracic and 3nd pleon) and right side (1st and 3rd pleon) of the

heart alternately. The left ostia are situated nearer to the ventral line while the right ostia are nearer to the dorsal line. They are oriented obliquely and parallel to the cardiac spiral muscles. The ostial flaps (o. f.) show clear muscle fibres (Fig. 23A, m. f.) expanded in the middle

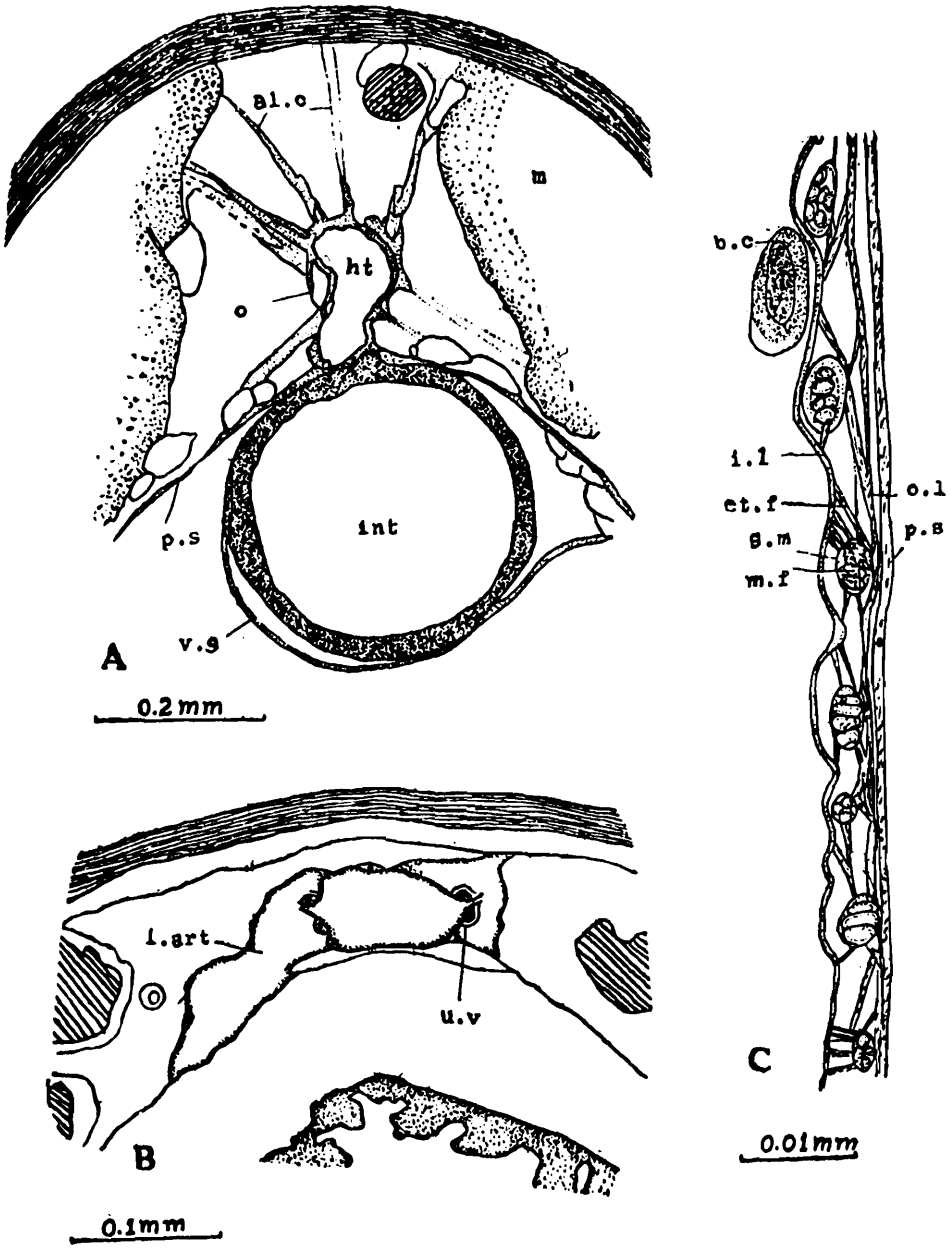


Fig. 22. A. T. S. pleon through heart. B. T. S. through median aorta branching. C. Magnified view of Heart wall.

and converging to the dorsal and ventral corners of the ostium. Muscles of each flap form a separate band which continues with the other muscle bands (s. m.) of the heart wall. Opening of the ostium

is affected by contraction of muscle fibres spread on the flaps and closing is affected by inner pressure of the fluid of the heart. As Maynard (1960) has pointed out, the ostial flaps are primarily muscular and apparently lateral extensions of the ostial muscles.

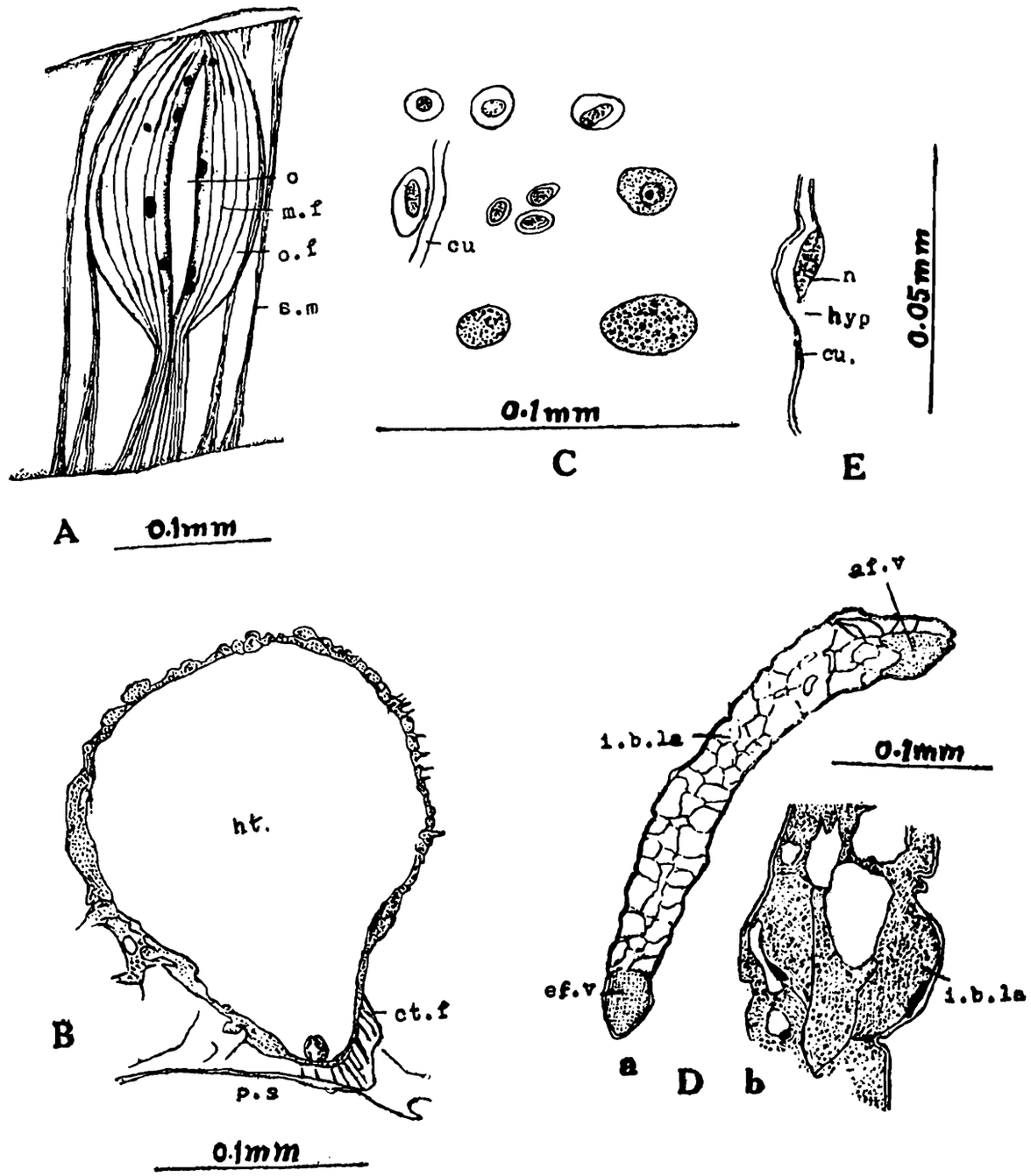


Fig. 23. A. Magnified view of ostium. B. T. S. heart. C. Different types of blood cells, D. a. T. S. Exopod (pleopod), D. b. Magnified view of exopod T. S., E. cuticle of the pleopods.

Histology of the Heart :

The heart is formed of spiral (s. m.) muscles enclosed in connective tissue layers (Fig. 22C). On the outer surface the outer layer is thicker than the inner layer. The inner layer is much thinner with few nuclei placed at distances. At higher magnifications each muscle is a bundle of 4 to 5 myofibrils (m. f.) enclosed in a membrane in its cross section. Each muscle is connected with outer and inner layers as well as its neighbouring muscles by strands of connective (cf. f.) tissues.

The Arterial System :

The median aorta (Pl. VI (3), Fig. 20, 21) which is also termed as superior aorta (Delage loc. cit.) emerges from the terminal end of the heart in the 6th thoracic segment. It runs forward close to the dorsal body wall attached to it by a series of connective tissue strands. On reaching the first thoracic segment it gives rise to a pair of lateral branches denoted here as 'cephalic artery' (cep. art.) extending in opposite direction along the dorsal body wall. They form a number of branches extending and opening among the dorso-ventral muscles of the head. The median aorta on reaching above the anterior dorsal lamella and dorsal ampulla widens and forms the anterior heart (Fig. 20, c. f.) or the so called 'cor-frontale'. Cor-frontale (c. f.) is more or less a thick walled portion of median aorta which contracts and expands independent of the heart. Anterior to the stomach the aorta gives rise to a pair of arteries on each side, one going to the anterior lateral muscles and the other going down along the anterior margin of the stomach. The median aorta now runs ahead above the oesophagus in its dorsal groove and before entering the brain it gives rise to a pair of arteries (ophthalmic artery) one on each side (op. art.) running along the outer grooves between the proto-and deuto-cerebral lobes ; in between these two arteries there arises a single dorsal branch which enters the brain (Fig. 21A, cer. art.) dorsally and anteriorly between the deutocerebral lobes. This particular artery is called as 'cerebral artery' which ramify in the brain. The median aorta continues below the brain and comes out anterior to it where it bifurcates, each branch running along and in front of the circumoesophageal commissure of its side thus surrounding the oesophagus. Each branch (called as 'facial' artery) gives off branches to labrum, antenna I and antenna II. The

facial artery (f. art.) then descends down, as has been stated above, along the oesophageal commissure and join below the suboesophageal ganglion. The appendicular arteries generally accompany and follow the course of appendicular nerves.

The lateral branches from the median aorta possess semilunar valves (Fig. 22B u. v.) which are cellular flaps with distinct nuclei on the sides of the flaps. It gives unidirectional flow of blood *i. e.*, from aorta to arteries. Presence of cellular flaps in the valves is a significant feature in *Nichollsia*.

Thoracic arteries :

Median aorta in the fourth thoracic segment gives rise to a pair of lateral arteries one on each side. This lateral artery which can be called as first lateral artery (second of Silen, 1954) runs outward and forward subparallel to the median aorta upto the head where it descends down and terminates in the maxilliped. Before it gives rise to a branch in each of the thoracic segments 1 to 4. In 5-7th thoracic segments independent thoracic arteries arise directly from the median aorta (5th) or, the heart (6th & 7th) and these are termed as 2nd, 3rd and 4th lateral (1 art $_1$.-1. art$_4$) arteries.

Each thoracic artery outwardly and posteriorly in 1-4 and anteriorly in the remaining thoracic segments, gives off a small dorsal branch arborising into fine branches. Then the artery runs antero-ventrally in the first four segments and posteroventrally in last three thoracic segments entering the appendages. It is called 'crural artery'. Before entering the appendage or the thoracic leg the lateral artery gives off an outward branch entering the muscles and an inward visceral branch dividing and upper one going to intestine (Fig. 21B vis. art.), and hepatopancreas, and the other lower one going below the nerve cord. This inner branch is called as visceral artery. Shifting in the position of arterial branches in the last three thoracic segments is in correlation with the posterior shifting of the appendages. Supply of an artery to the maxilliped from the first lateral branch is also notable in the absence of the so called first artery which is also present in *Ligia* and other terrestrial isopods, (Silen loc. cit.). In this respect *Nichollsia* is much nearer to *Asellus aquaticus* where the first lateral artery (Silen loc. cit.) is absent.

Abdominal arteries

The abdominal artery (Fig. 20 a b. art.) originates from the side of the heart in the first abdominal segment. It runs at right angle to the long axis of the body for a short distance and curves backward as a straight vessel parallel to the heart upto the posterior end of the fifth segment giving off five small lateral branches one in each segment of the five abdominal segments. Each of these branches terminates in the dorso-lateral body wall after giving off numerous smaller vessles to the dorso-ventral muscle bands attached to the base of the pleopod. Near the antero-lateral border of the telson the abdominal artery enters the telson called 'caudal artery' and runs down vertically and backwardly (c. art.) dividing into three main branches. The first branch, *i. e.*, the dorsal branch divides and redivides and supplies to the anterior muscle of the telson and intestine. The second or the inner branch runs to the posterior end of the telson and supplies to the ventral part of the rectum and posterior muscles. The third or the ventral branch, the 'uropodal artery' (u. art.) continues behind and enters the uropod. Before entering the uropod it gives off a small branch going up vertically and ramifying above. At the termination of the peduncle of uropod the artery bifurcates and branches enter the outer and inner ramii.

The Venous system :

Blood from the interstitial lacunae is reassembled in the general body cavity which is divided longitudinally into three main lacunes. The dorsal lacuna (Fig. 21B d. la) (or the pericardial chamber) is separated by the pericardial septum lying above the digestive system enclosing heart, dorsal aorta and dorsal muscles. The median or central lacuna (c. la.) is the largest and is bordered between the pericardial septum dorsally, and ventral septum ventrally. The ventral septum is not as prominent and continuous as the pericardial septum, but it is evident in the thoracic and anterior abdominal segments. The ventral septum lies above the ventral nerve. The organs lying in the central lacuna are intestine, hepatopancreas and gonads. The ventral lacuna lies below the ventral septum enclosing the nervous system and ventral muscles. The three main lacunae are interconnected at places and thus the venous blood passes from one to the other.

The central lacuna receives blood from the cephalic region and digestive organs while the ventral lacuna receives blood from thoracic appendages, lateral body wall and the sternum. Apart from these the blood from the appendages is collected in the lateral lacunes and passes backward on the ventral side of the head and thorax. The central lacuna continues behind up to anus surrounding the hind gut.

The venous blood from the thoracic lateral lacunes is received in the median sternal sinus in the abdominal region. The median sternal sinus in the abdominal region is divided by septa (Pl. VI, fig. 4) into many narrow lacunes, and may be connected with one another. Although the septate lacuna has been considered by Silen (loc. cit.) as a more evolved character represented in terrestrial Isopods, presence of these septa might be an adaptive feature for regulating the flow and maintaining the pressure of the blood. *Nichollsia* is primarily a bottom dweller where it has to maintain its internal pressure for adjusting the outside pressure of the water column.

From the sides of the median sternal sinuses are given off five pairs of symmetrically arranged afferent branchial vessels close to each other. Each afferent branchial vessel bifurcates after entering the peduncle of the pleopod of the corresponding segment and one of these branches enter the exopod and the other to the endopod. In both the exopod and endopod the afferent branchial continues the course along the inner border.

There are five branchiae or pleopods on each side, each with an exopod and an endopod. Except for the difference in size all the pleopods are same. Both the exopods and endopod are formed of two juxtaposed membranes, between them are enclosed two marginal vessles, the one along the inner border is the afferent branchial vessel and the other along the outer border is efferent branchial. The space between the two membranes and in between the two marginal vessels is occupied by a number of flattened interconnected vesicles of lacunar spaces called as intra-branchial lacunes (John, 1968), the number and extent of (i. b. la.) which vary in different branchiae and also the different regions of the same branchiae (Fig. 23D, i. b. la.).

The venous blood from the afferent branchial vessel traverses through the intra-branchial lacunes, where oxygenation takes place, to the efferent branchial vessel on the opposite side. The efferent vessel from the exopod and endopod of each pleopod join together forming

the common branchio-pericardial vein in the peduncle. This arrangement is repeated in all the five pairs of branchiae so that the exopod and endopod of all the pelopods perform respiratory function.

The blood cells enter the afferent vessel swiftly, but their movement in the intra-branchial lacunes is comparatively very slow. This movement facilitates the retention of blood and exchange of gases from water to cell and vice versa through the thin wall of the pleopods which is hardly 0·7μ in thickness.

Branchio-pericardial vein (Fig. 20 & Pl VI).

Branchio-pericardial vein of each segment opens independently into the pericardium. Thus there are five pairs of such veins in *Nichollsia*. Each vessel (b.p.v.) is the extension of efferant vessel coming out of the peduncle of the pleopod. After its emergence from the pleopod it widens gradually above between the two lateral muscles of the pleopod of its respective segement and on reaching the mid-lateral side of the segment, forms a more or less triangular space. This wide space communicates through its postero-dorsal corner to the pericardial chamber. This vein is bound anteriorly and posteriorly by walls. Thus each vein or sinus is enclosed by septa or wall from three sides *i.e.*, ventral or inner and two lateral sides in its cross section.

The floor of the veins is full of large vesicles enclosing 2 or more cells under a membrane. These cells have been named differently by different authors viz , Nephrocytes (Silen, 1954) or athrocytes (Patane, 1962).

The blood :

The blood is colourless and contains nucleated corpuscles which vary in size and shape. As in most arthropods, it is very coagulable. In circulation some large granular cells are observed with refrectile spherules. Number of these refractile cells, which are large in size and variable in shape, increases just after moulting. There is much discrepancy, contradiction and uncertainty in the morphology and terminology of various types of cells in blood of crustacea.

It is probable that many so called cell types are merely stages in the development of a singl type. Large number of cells with refractile granules or spherules in different stages of concentration have been noted in the pleopods of *Nichollsia* after injection of trypan blue.

However, some of the cell types observed in *Nichollsia kashiensis* have been illustrated (Fig. 23C) from sections of the animal. Various types of blood cells in different species of cruastacea have been illustrated by George and Nicholls (1948). Pouchet (1882) considers ovoid blood cells as a permanent form and as they grow older they present a number of granulations with their nucleus often small or altogether lost. Proleucocytes seem to be the basic types in crustacea.

Flow of blood :

The blood moves away from the heart through the arteries to the lacunes in various parts of the body. These in turn communicate with sinuses from where the blood is taken to the ventral lacune lying below the gut. From the ventral lacune it is passed into the pleopods and thence through the branchio-pericardial veins to the pericardium and then enter the heart through the ostia completing the cycle. Course of the blood in the posterior abdominal region has been some what different *i.e.* blood from the central lacune and that from the abdominal arteries reaches to the posterodorsal region of the telson (Fig. 20) where it is collected by the abdominal vein (ad.v.) and carried to the pericardium as shown in the illustrations.

Thus in *Nichollsia kashiensis* all the venous blood returning to the heart do not pass through the pleopods. Blood from the heart goes to the telson and uropods through the abdominal arteries and collected through the abdominal veins in the pericardial chamber. The venous blood from the uropods comes out and moves out off along the lateral margin of the anus and join the abdominal vein. The abdominal vein receives blood from other parts of the telson through many veins and also from the central lacuna opening along the posterior margin of the hind gut.

Thus it is clear that this blood do not pass through the pleopods and may remain unoxygenated or it may be partly oxygenated in the uropods and the thin cuticle along the outer margin of the anus and telson. A large number of blood cells are found accumulated at the posterior end of the heart, significance of which is not clear, *i.e.*, whether they are cells under senescence or newly formed cells.

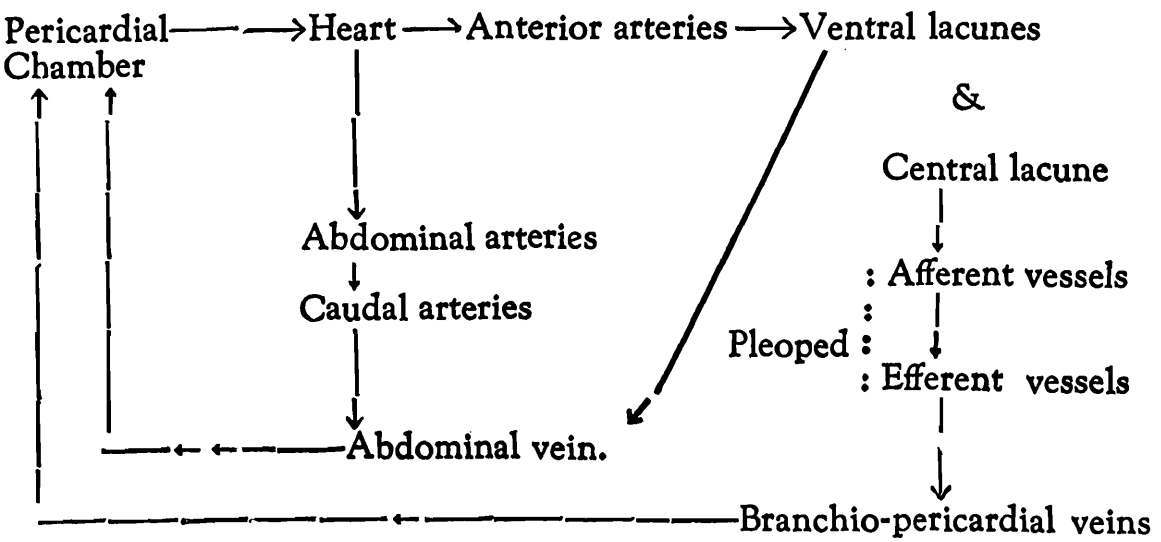
It has been noticed that the blood flow increases after moulting with increase in the number of refractile cells.

In thoracopods the arterial blood passes with high speed along the inner margin, while returning, the flow is comparatively very slow because it runs through the interstitial spaces and covers wider areas. The two courses, *i. e.*, arterial and venous flow are separated by speta in the distal four segments of the thoracopods while in the basis and ischium these are separated by muscles. The arteries seem to have definite vessels.

Heart beat :

Normal heart beat is co-ordinated but not simultaneous action. Contraction of the heart starts from the posterior termination and quickly proceeds anteriorwards. During contraction the ostia are closed and blood passes to the anterior aorta. During expansion a vacuum is created in the heart drawing the blood in from the pericardium through the ostia. The pressure in pericardial chamber falls, consequently it receives blood from the branchiopericardial veins as well as abdominal veins. Thus a continuous flow of the blood from heart to arteries, lacunes and veins is maintained.

In crustacea in general the rate of heart beat is influenced by many factors including body size, activity, temperature, respiratory stress, light and blood composition. In present case only two factors (body size and temperature) were taken for the study purpose.



Circulation in Nichollsia kashiensis

Effect of body size (Fig. 24).

Animals of different lengths were acclimatized to a particular temperature for observations on the heart beat. They were all in intermoult

period. Heart beat was counted with the help of a stop watch. Since the animals are semitransparent, movement of the heart could be easily observed by hand lens or under low magnification of stereoscopic binocular microscope aganist transmitted light.

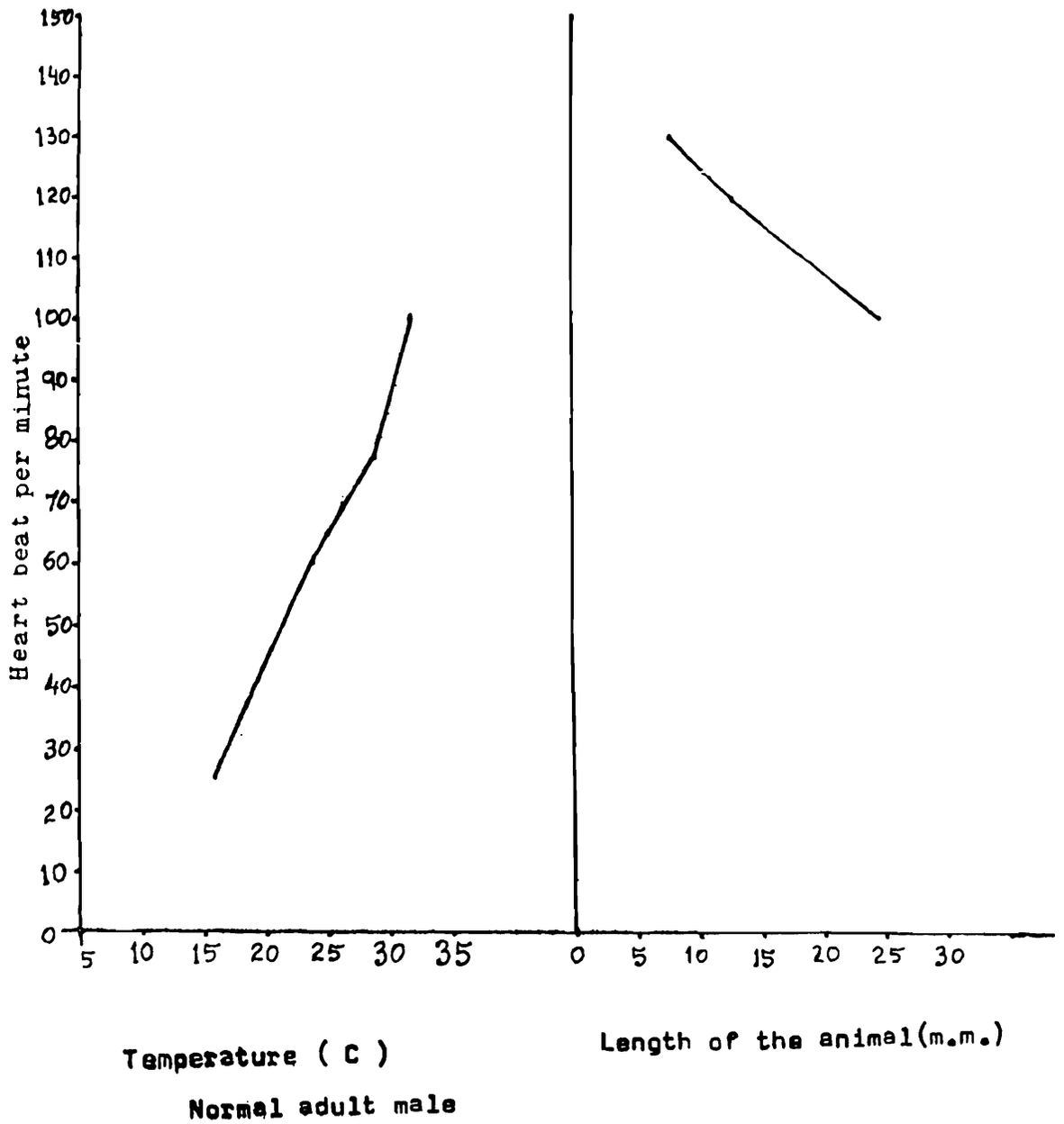


Fig. 24. Effect of temperature and body length on the heart beat.

The result was in conformity with the general rule that heart rate that heart rate varies inversly with the body size. Individuals of 7-8 mm. size group had an average of 130 beats per minute at 32°C while 10-12 mm. group had 120 beats. Adult males of 22-25 mm. length were found with an average of 100 beats per minute. However, a

normal female at 32°C was found to have only 90 beats per minute. Thus it is in agreement with the general observations on other malacostraca (Schwartzkopff, 1955 Dauscher & Flindt, 1969) like *Gammarus pulex* and *Asellus aquaticus*.

Effect of temperature (Fig. 24).

The heart rate is directly proportional to the temperature within the normal environmental range. Animals acclimatised for different temperatures were taken for the purpose. Animals were all adult males without much variations in their body length. The heart beat was noted within the normal survival range (16°-32°C) of temperatures. At 32°C the average heart beat was 100 per minute, being 80 per minute at 29°C, 60 per minute at 24°C and 25 per minute at 16°C. At 16°C the animal became less active and below this, it was more or less inactive.

Sudden increase in temperature causes abrupt rise in heart beat slowly coming down to a normal.

In normal conditions, the pulse rate of the so called anterior heart was found to be nearly half to that of the heart proper. Pressure developed by the primary heart is apparently insufficiant and cor-frontale pulsates to supplement and insure adequate supply to all regions particularly the head. Contraction and expansion of cor-frontale is affected by the action of somatic muscles atttached on dorsal wall of the stomach. There does not seem to be any prominent muscle on the cor-frontale. However, a feeble muscle can be seen attached to the dorsal side of the cor-frontale. This muscle can help in expansion or dilation of cor-frontale.

RESPIRATORY SYSTEM

Respiration in almost all the Isopods is carried out by the abdominal appendages i. e. the pleopods. It has been established (Edney and Spencer, 1955), that respiration also occurs through the general body wall of the animal. This is also true for *Nichollsia kashiensis*. All the five pairs of pleopods are morphologically same and are respiratory in function.

Morphology and histology of the pleopods (Fig. 23D, E).

In Isopods, pleopods are the main organs of respiration. These are modified, flat and thin foliaceous abdominal appendages. They also

act as swimmerets. The branches of pleopods are so wide that they can not lie next to each other, so the exopodite lies infront of the endopodite. Pleopods are well vascularised structures. When at rest, they are turned posteriorly and lie somewhat flat at an acute angle to the body so that anterior surface is obliquely ventral and their morphologically posterior surface is dorsal in position. They are ventilated by beating forward and back, the speed of movement depends on several variables.

One simple lacune enters the peduncle (protopodite) and then divides into two, leading to the exo-and endopodites respectively, and are called as afferent vessels. In the same way the blood is carried from the pleopod by two lacunes fusing into one in the peduncle, they are called as efferent vessels. The afferent vessels run along the inner margin of the exopod and endopod and efferent along the outer margins. The efferent vessel is a continuation of afferent vessel. The flattened pleopod in between its anterior and posterior walls in cross section possesses three rows of compartments or lacunes, the anterior along the anterior wall, the posterior along the posterior wall and the middle lacunes being connected with the neighbouring lacunes. At the distal margins of the pleopods there may be only 2 lacunnar rows.

The cuticle of the pleopods is very thin and is less than 0.7μ (Fig. 23E cu.) in thickness. The hypodermis underlying the cuticle is hardly 0.3-0.4μ and is thus less than half of the thickness of the cuticle. In those parts where the two are not separated it is difficult to distinguish hypodermis from the cuticle but whenever hypodermal nuclei are present, one can easily establish its identity. The nucleus is very narrow (4.5μ) and elongated (11μ) lying adjacent to the cuticle. Similarly the septa forming the compartments in the lumen of the pleopods also possess nuclei and seem to be the extensions of the hypodermis. Septa of lacunes are flexible. When the blood enters from one lacune to another the latter gets expanded while the former is collapsed. In the whole mounts, the nerves are found traversing the pleopods and reaching to the setae present along the margins and on the surface. Microtrichs arranged in crescent form on the anterior and posterior faces are found on the exopods along their inner margins. Significance of these microtrichs, whether hydrostatic or else is not clear.

Movement of blood in Pleopods :

Blood enters in the exopod and endopod through the afferent vessels. From afferent it is distributed to central and surface lacunes and then to the afferent vessels. Quick blood flow in the afferent and efferent vessels were observed while it passes very slowly through the lacunes because it is distributed in many wider channels. The slow movement of blood facilitates the retention of blood and blood cells and exchange of gases through the skin under the respiratory surface.

Pleopod beat :

Pleopod beat is more or less a synchronised movement starting from the anterior ones and finishing to the posterior most. *i.e.*, fifth pleopod.

Pleopod beat in normal adult individuals is 80 to 120 per minute, but, it varies with the change of temperature and size and also the condition of moulting and reproduction. It is regular but not continuous and is interrupted at frequent intervals for a short or longer duration, causing variation in the beat rate in the same individual at the same temperature.

Effect of temperature and size (Fig. 25).

Effect of temperature within the range of 16-32°C was studied in these animals. It was found that at 16°C the animal becomes moer or less inactive with irregular pleopod beats. Gradually when the temperature is increased it regains its regular beating at 20°C. At higher temperatures the frequency increases in relation to the temperature. Similarly it was found that beat is inversely proportional to the size of the animal i.e., smaller the size higher is the frequency pleopod beat at a fixed temperature.

Deviation from the trend was noted in the females with embryos in brood pouch. They had higher frequency of pleopod beat per minute than those of normal females of the same size at the same time.

Very interesting results were achieved when a female of normal size was decapitulated. It lived for 3 hours. Its beat continued which was 70 per minute immediately after decapitulation. After 10-15 miuutes it went up to 150 per minute, later on going down to 110 and 20 till the animal died.

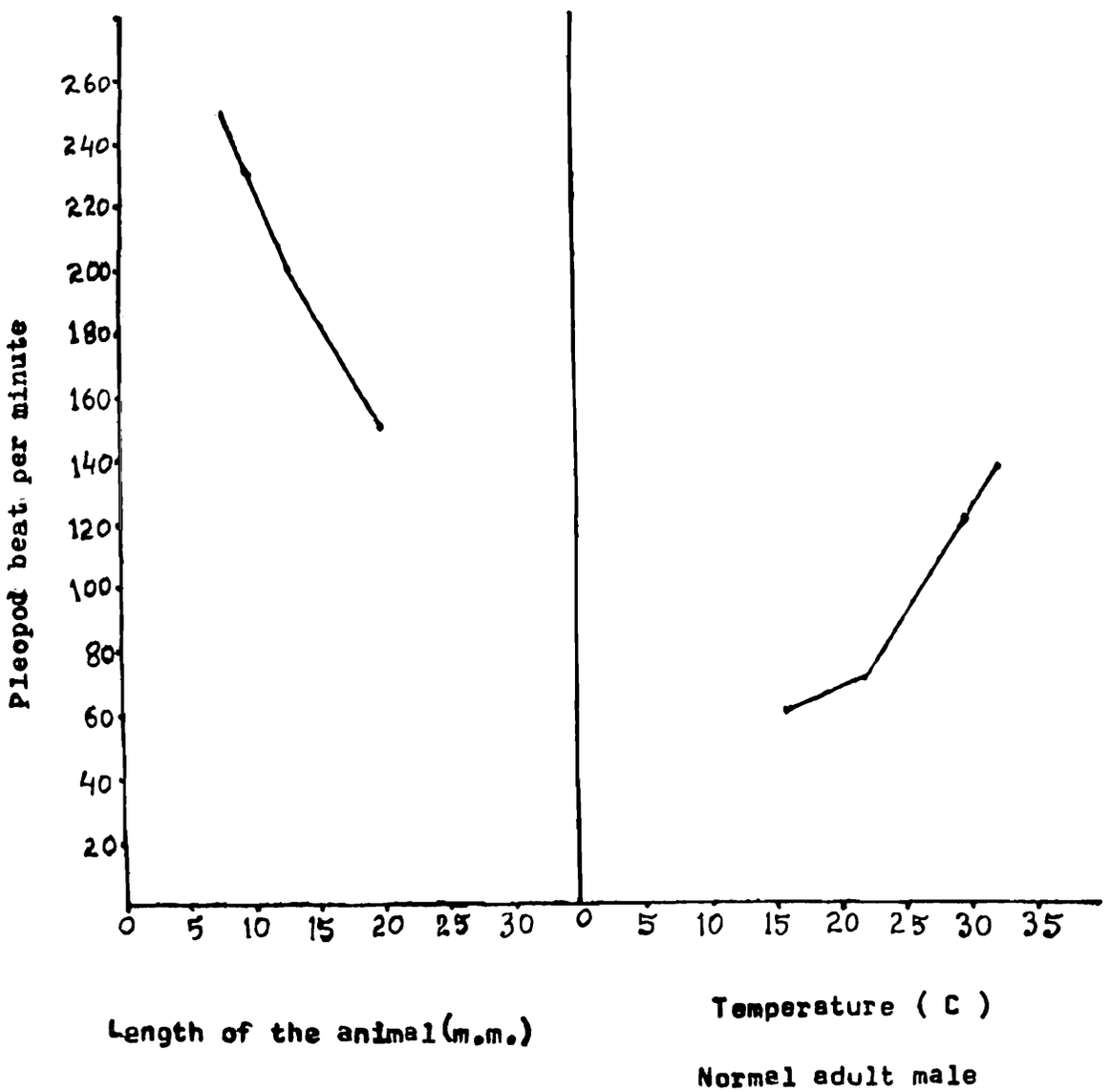


Fig. 25. Effect of temperature and body length on pleopod beat.

Oxygen consumption :

It was found that an average adult male consumed 0.044 to 0.446 cc. oxygen per gram per hour. It is a very low consumption in comparison to many other aquatic Isopods. In the above experiments the quality and temperature of the well water was more or less same to that of natural environment except the pressure existing at the bottom of the well. Since these animals move up to the surface during the winter season, it seems that the pressure in their natural environment does not play any vital role in respiration.

Apart from the pleopods, respiration occurs through the general integument (Edney and Spencer, 1955). The possible areas are the

segments of the body and appendages below which the circulation of blood is clearly marked. In *Ligia* and *Oniscus* nearly 50 per cent of the normal respiration takes place through the general integument (Edney and Spencer, loc. cit.). Flow of blood around the anus, while returning to the pericardium through abdominal vein, in *Nichollsia* indicates that the thin posterior wall of the telson around anal opening also acts as a respiratory surface.

Intestinal respiration :

Fox (1952) demonstrated that oral and anal intake of water takes place in many crustacea including Isopoda. This intake of water has been claimed to be of respiratory value. In *Nichollsia kashiensis* rhythmic gulping of water takes place through the anus particularly after faecal discharge, which may be of longer or shorter duration. However, this type of gulping may be for the clearance of faecal matter from the anus and not for respiration.

NERVOUS SYSTEM

Nervous system of *Nichollsia kashiensis* consists of a paired chain of ganglia. The first three ganglia form the brain or the central nervous system (Fig. 26, 27).

The nervous system may be divided into four parts :—

1. The Supra-oesophageal ganglia or the brain ;
2. The circumoesophageal connective ;
3. The suboesophageal ganglia ;
4. The ventral nerve cord.

1. *The Supra-oesophageal ganglia or the Brain* :

The brain is situated between the frontal spines, just below the body wall and immediately behind the origin of the antennules above the curvature of the oesophagus. A groove on the mid-longitudinal line divides the brain into two equal halves. Each half is composed of 3 lobes arranged vertically one below the other. Actually these are the continuations of the ventral nerve cord above the oesophagus. Thus we find that the brain has 3 paired ganglia arranged one below the other. The dorsal pair is called as protocerebrum (pr. c.), the middle

pair is deutocerebrum (de. c.) and the third pair as tritocerebrum (tr. c.). The lobes of one half are joined by transverse commissures to their counter parts in the other half (Pl. VII, fig. 1).

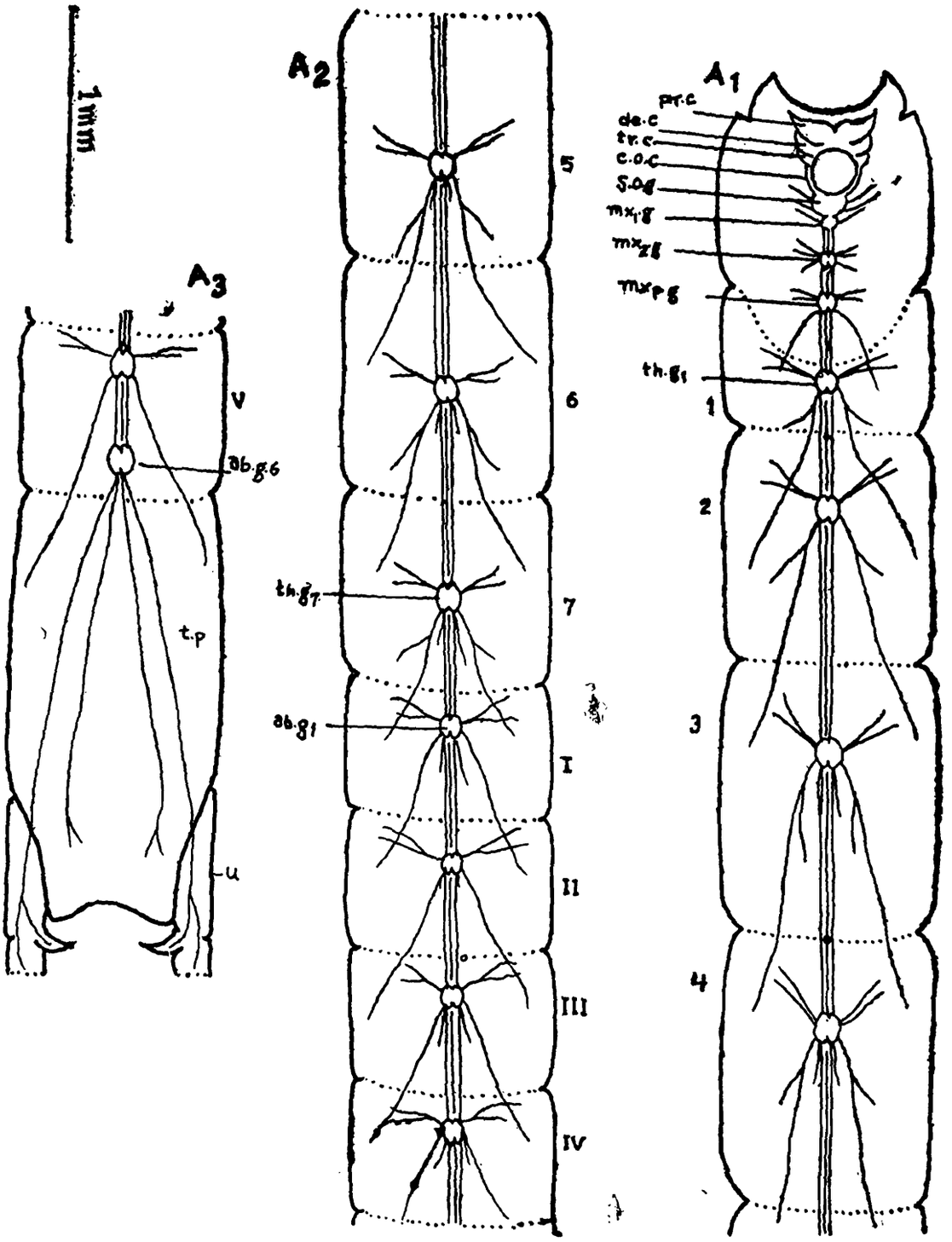


Fig. 26. $A_1A_2A_3$ Nervous system of *N. kashiensis.*

The protocerebrum is a large globular mass with small rudimentary conical termination to each frontal spine. These projections represent

the rudimentary optic nerves. It can be recalled here that the eyes are totally absent in *Nichollsia* resulting in the loss of optic ganglia.

The deutocerebrum sends nerves to the antennules while the tritocerebrum supplies to the antennae. A pair of thin nerves arise from

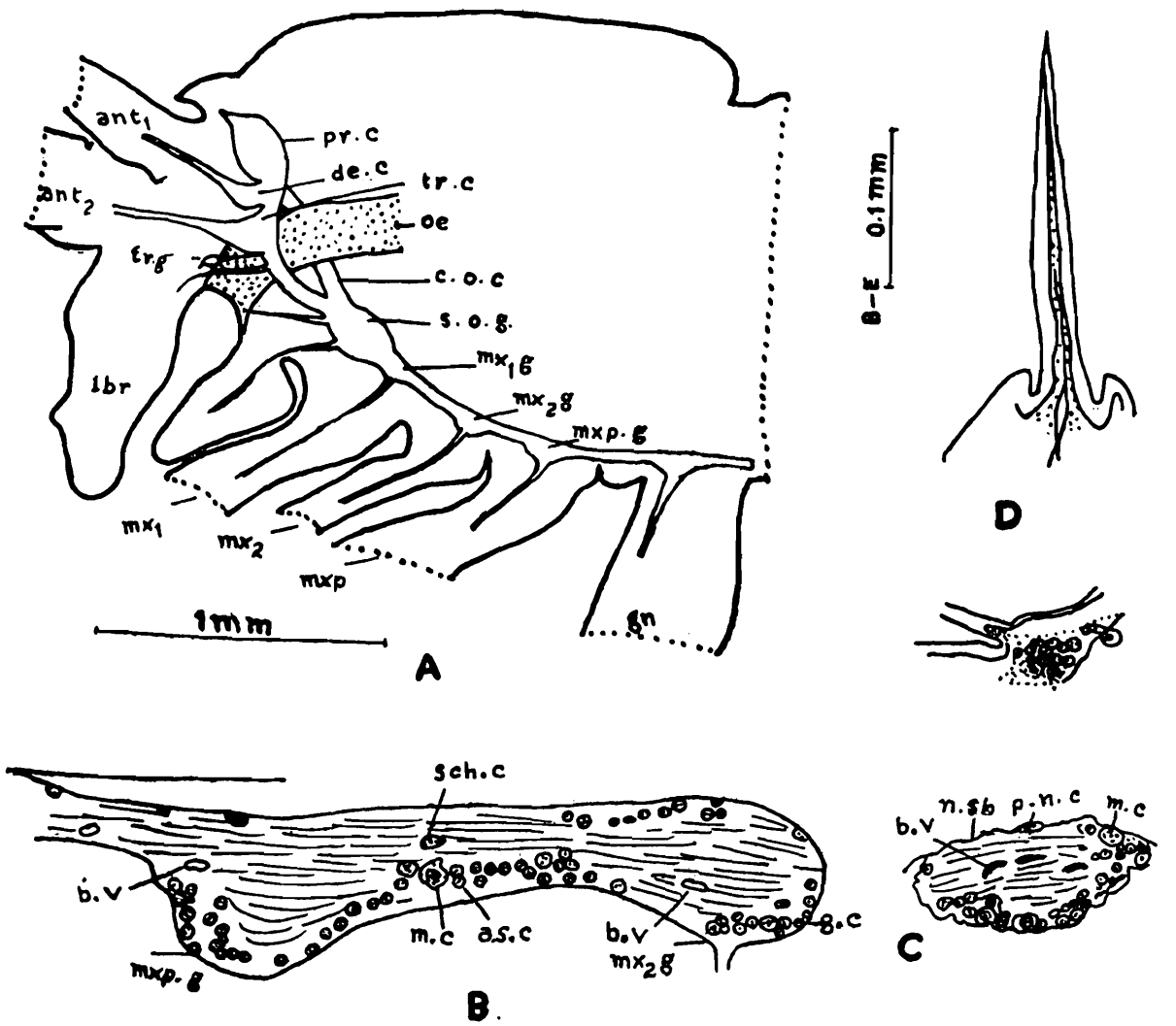


Fig. 27. A. Lateral view of nervous system in the head, B. L. S. Maxillary and maxillipedal ganglia. C. L. S. Maxillipedal ganglia. D. Single seta. E. Sensory seta on antenna (section).

the circumoesophageal connectives just below the antennal nerve and innervates the labrum. The antennal nerve is thick and prominent with a lobed base. Blood is supplied to the brain by a dorsal cerebral branch from the dorsal aorta.

2. *Circum-oesophageal connective* :

Circum-oesophageals descend down from the tritocerebrum on both sides of the oesophagus and join below the oesophagus to the suboeso-

phageal ganglia (s. o. g.). The connective is a thick nerve (Fig. 27A).

From inner margin of the tritocerebrum at its frontal side arise thin small nerves which run inward from both sides and meet below the dorsal aorta with a small (Fig. 27A fr. g.) ganglion which represents the frontal ganglia of the animal.

3. *The suboesophageal ganglia* (Fig. 27, & Pl. VII fig. 3).

The suboesophageal is a large round mass of two fused ganglia. From the suboesophageal ganglia arise two pairs of nerves, the anterior pair going to the mandible while the posterior pair innervate the hypopharynx. The mandibular nerves arise near the junction of the two circumoesophageal connectives. Just behind the suboesophageal ganglia and very close to it is a small swelling of maxillular ganglia (mx_1. g.). It is so close to it that it looks like a part of suboesophageal ganglia. From this small ganglion arises a pair of thin nerves innervating the maxillulae. Further behind, there are two ganglia in the head. The anterior one is lying above the maxilla and the other above the maxilliped innervating the corresponding appendages.

4. *The ventral nerve cord* (Fig. 26).

Beyond and behind the head, the ventral nerve cord with paired nerves runs upto the fifth abdominal segment. There are seven thoracic and six abdominal ganglia. The first thoracic ganglion is one and a quarter times larger than the maxillipedal ganglion. The thoracic ganglia are larger than the abdominals except the last abdominal ganglion which is larger than other abdominals. Thoracic ganglia from first to fourth segment are positioned in the anterior half of the segment but fifth to seventh are placed in the posterior half of their segments. This shifting may be due to posterior shifting of the appendages in these segments. The abdominal ganglia are located in the anterior half of the segments, except the sixth abdominal which is lying near the posterior (ab. g. 6.) end in the posterior half of the fifth segment. Thus the fifth bears 2 ganglia *i. e.* fifth and sixth abdominals.

Each thoracic ganglion gives rise to two pairs of nerves. The anterior pair is a thick nerve which divides and redivides to supply the appendages and other visceral organs of the same segment. The posterior pair is thin and runs to the posterior segment innervating its muscles. Similarly each abdominal ganglion gives rise to two pairs of nerves and a third very small pair arises from the posterior extremity

of the ganglion. It runs along the nerve cord and supply to the ventral muscles of the same segment. The sixth abdominal ganglion gives rise to two pairs of thick nerves. The anterior pair innervates the posterior muscles of the telson and the hind intestine, while the posterior pair goes to the uropods. As already mentioned above this ganglion is larger than the remaining abdominals.

Histology of the nervous system :

The brain is bound by an outer thin membrane called neurolemma or neuralsheath with flat elongated nuclei in some places. Below the neurolemma, each lobe is composed of two parts, the outer, densely nucleated peripheral region is known as Cortex (cort.) and the middle region as medulla (med. Pl. VII Fig. 1).

Cortex :

It is the region of neurons where the nuclei are prominent with cytoplasm very much reduced. This region also includes blood vessels with blood cells. With Azan's it takes orange to red colour. The neurocytes can be differentiated into two types. Association cells (as.c.) with small and dense nuclei and sensory cells (s.c.) which are larger in size than association cells.

Medulla :

This region is the central portion of the brain just below and surrounded by the cortex. It is called neuropile and consists of axon fibres, blood vessels and blood cells in the periphery. This region is stained light blue with Azan and pink with eosin.

From its antero-dorsal border, each protocerebral lobe of the protocerebrum gives off a small pointed terminal projection. The projection does not show any nerve tract but only a net work of loose dendrites. At the base of this projection small association cells (as.c.) are arranged forming the inner boundary of these cones. Such reducation in this blind animal is an adaptive modification in relation to its subterranean habitat.

Protocerebral lobes and deutocerebral lobes are connected by protocerebral commissural tracts and deutocerebral commissural tracts respectively. Glomeruli are present in deuto and tritocerebral lobes,

Tritocerebral tracts are clear and glomeruli are visible in the longitudinal section of the brain. Schwan cells are visible in the transvease commissural tracts (sch.c.).

The motor neurocytes in the brain region are confined to the middle junction of the protocerebral lobs in the dorsal groove, and on the lateral grooves between the deutocerebrum and tritocerebrum and also the dorsal margin of the root of antennal nerves. Similar neurocytes have been located on the lateral sides of the suboesophageal ganglia near its junction with the circumoesophageal connectives.

All the ventral ganglia including suboesophageal have similar arrangement of nerve cells. The nerve cells are arranged on the ventral and lateral sides in the peripheral regions. Presence of transverse tracts between the component ganglia is very closely visible in the sections (Pl. VII tr.t.) the blood capillaries are also visible in the regions as empty round spaces. There are very few motor cells in each ventral ganglion (except last abdominal) with very large nucleus containing only peripheral chromatin (Pl. VII m.c.).

Between the neurolema (which takes blue stain in Azan) and the neurocytes at the fromtodorsal corner of the protocerebrum is a mass of fibrillar spongy structure. Inside the neuropile mass some cells with irregular shape have taken deep red stain, these are perhaps Schwan or neuroseeretory cells. It is possible that these are blood cells passing through the blood capillaries.

In the deutocerebrum on its posterior dorsal surface, below the protocerebrum, is a large cell with large nucleus having two nucleoli. It may be a neurosecretory or motor cell.

The last abdominal ganglion has the accumulation of large number of motor neurons (Pl. VII fig. m.c.). All the neurons are aggregated on the ventral and anterior dorsal region of the ganglion.

The ganglia of this ventral nerve cord, their connectives and the stout nerves to the appendages are covered by nerve sheath (Fig. 27B, C. n.sh.) formed of long, dorso-ventrally flattened cells with elongated nuclei. These nuclei are clearly visible in the nerves entering the antennae. Their dispositions only towards the periphery of the ganglia and nerve cords suggest that they are perineural cells (p.n.c.) forming the neural lamella or neurolemma. In general in between the nerve tracts of the ganglia as well as nerves, there are Schwan cells present

throughout the ventral nerve cord. But in *Nichollsia kashiensis* the ventral nerve cord, between the maxillipedal and maxillary ganglia has a number of sensory cells and association cells and a few (Fig. 27B, m.c.) motor cells on the ventral side just inner to the neural sheath. This interesting feature of presence of sensory and motor neurons between the two ganglia in *Nichollsia kashiensis* is not found in other parts of the ventral nerve cord behind the head and is a significant and unique feature and seems a primitive character. The shortest connection between the maxillipedal and first thoracic ganglion does not show this feature. In cross section a nerve cord may show a central nucleus.

REPRODUCTIVE SYSTEM

There is little information on the reproductive system of Phreatoicid isopods. These are on *Phreatoicus assimilis* by Chilton (1894). *Mesamphisopus* (*Phreatoicus*) *capensis* by Barnard (1927), *Colubotelson thomsoni* by Engemann (1964) and *Nichollsis* by Tiwari (1962). Of the above, only Barnard's description is little elaborate while Tiwari (1962) has given the outline of testes without going into further detail of the system.

In *Nichollsia kashiensis* the sexes are separate. External characters, which distinguish the sexes, begin to develop when the young animals attain a length of 6 mm. to 7 mm. At this stage male can be distinguished by the presence of a pair of small bud like penis (genital apophyses) on the ventral side of the seventh thoracic segment and a small bud like outgrowth of appendix masculina (Penial stylet) on the medial side of the endopod of second pleopod. In adult males the outer ramus of uropod is longer than the telson, while in females it is smaller than the telson. Males attain greater length than the females. In breeding females (Pl. I) the oostigites develop during breeding season on first to fourth peraeonic segments. Small oostegites are present at the bases of the maxillipeds.

Secondary sex characters of isopods show remarkable variations in different orders. For example, oostegites do not appear in the suborder Gnathiidea (Monod, 1926 ; pp. 202-210), although the area of the ventral pad of the body surrounding the genital atrium might be considered homologus. Oostegites as mentioned for *Limnoria* (Menzies, 1954), are present in most members of the suborder, e. g.,

Anthuroidea, Flabellifera, Bopyroidea, Valvifera, Asellota and Oniscoidea.

Male Reproductive System (Fig. 28, 29 & Pl. VIII fig. 1, 2).

Male reproductive organs of *Nichollsia kashiensis* are comparatively simple, consisting of a pair of testes, lying on either side of the intestine in trunk segments 4 and 5 and vas deferentia that open into the penis. Each testis (t) normally consists of 6 lobes within the range of 0.34-0.45 mm in length and 0.14-0.16 mm in breadth, the anterior

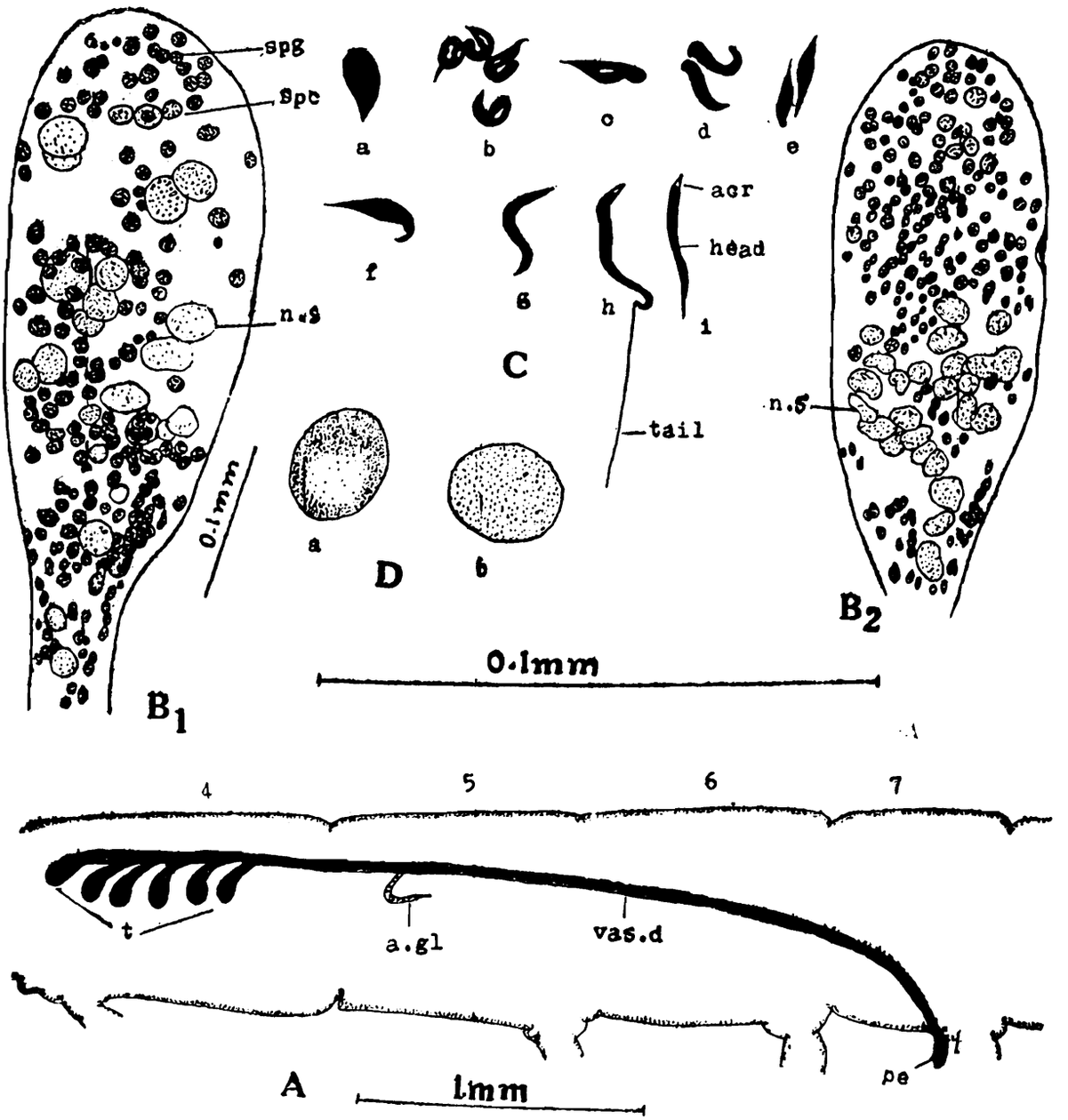


Fig. 28. A. Male reproductive system (lateral view). B. B_1B_2 whole mount of consecutive testicular lobes. C. Some stages in sperm formation. D. Two stages of nurse cell.

most of which is situated at the terminal end of the vasdeferens, while the other lobes are lateral in position lying on outer side of the vasdeferens into which they open. Each testis lobe is hollow and club-shaped with a long neck. Number of testicular lobes varies from 2-9 on each side, where the number is more the size of lobes is small. In most cases the numbers on two sides do not correspond and is usually one more or one less than the other side. There is no any relation between the number of lobes and length of the animal. The testis occupy the posterior 3/4th of the fourth segment extending behind to 1/3rd of the fifth peraeonic segment. The vasdeferens is a simple tube of almost uniform thickness running backward alongside the intestine to the posterior part of the seventh peraeonic segment, where it curves downwards and inwards to open into the penis. Penis is a short chitinous tube, perforated at the tip, (Fig. 29 D) lying at the base of the seventh peraeopod and separated from its counterpart on the other side by the median sternal ridge. Before entering the penis the vasdeferens dilates slightly.

A thin strand like organ the androgenic gland (Pl. VIII fig. 1, a. gl.) is attached to the vasdeferens in the anterior half of the fifth thoracic segment. The terminal end of this gland is connected to the base of the fifth leg by a connective strand. The vasdeferens is swollen where the androgenic gland is attached to it.

Histology of the testicular lobes :

The wall of the testis (Fig. 29A) consists of an outer narrow epithelium (ept.) and an inner germinal layer (g. l.). The epithelium is a thin layer of continuous protoplasm without cell limits but widely scattered elongated nuclei. General germinal layer is formed of syncitial protoplasm with large prominent deeply staining nuclei. On one side the germinal epithelium is thickened to form a longitudinal ridge called as germinal ridge (g. r.). While the general germinal layer is only one cell thick the germinal ridge is three to four cells in thickness. Towards the distal end of the testicular lobes, the germinal ridge is enlarged in width and thickness and almost fills the lumen of the lobe. In the germinal ridge, two types of nuclei are seen, namely small, shperical deep staining nuclei and slightly large, irregular nuclei which pick up light stain. The latter seem to resemble the nurse cells (n. s.) in the testis, observed in some of the crustaceans. Such nurse cells, as they are supposed, have been found in the germinal epithelium

where the spermatophores have already been formed and proceeded to the vasdeferens. In *Nicholllsia* spermatogenesis starts first in the terminal lobe in its proximal zone and then subsequently in other lobes. The large nuclei or the so called nurse cells appear in the proximal zone first, then subsequently in the distal zone (Fig. 28B_1 B_2). This suggests that these cells may take part in the secretion of seminal fluid or else provide nourishment to the spermatocytes. Formation of nurse cells is a later stage in the process of spermatogenesis as is evident in the different lobes of the same testis. Nurse cells extend to the vasdeferens. In Younger forms only spermatogonial cells are seen and no visible differentiation into spermatocytes and spermatids is found. Morphologically matured spermatophores however were found in the the lumen through out the length of the testis.

Fasten (1914) in *Cambarus*, Komai (1920) in *Squilla* Rathnavathy (1941) in *Clibanarius* and John (1968) in *Sphaeroma terebrans*, found similar nurse cells as reported in *Nicholtsia*, with irregular nuclei among the spermatogonial cells. Morphological changes in the nurse cells (Fig. 28D) indicates that they undergo transformation and finally disintigration.

Histology of the vasdeferens :

It is not easy to say where the testis ends and the vasdeferens begins, because the vasdeferens seems to extend to the root of the terminal lobe. As already stated above, the vasdeferens is tubular and nearly uniform in diameter throughout its entire length. It is swollen in the region of attachment of the androgenic gland (Pl. VIII fig. 1). The wall of the vasdeferens consists of two distinct layer, an outer muscular layer and an inner epithelial layer (Fig. 29 B, C) also called as myoepithelial layer (my. ep.) and epithelial layer (ept.) (Newstead & Dornfield, 1965).

The outer muscular layer is composed of more or less flattened cells with fibres crossing one another at places longitudinally. Contraction of this layer brings about the breaking of the continuous sperm flow. More posteriorly the muscular layer becomes thinner but thickened again at the terminal portion of the vasdeferens. The nuclei look as if folded in the muscular layer.

The inner epithelial layer is composed of two types of cells the small and the large cells, also known as prismatic (pri. c.) and

giant cells (gi.c) ; (Newstead & Dornfield, 1965). The small cells are more or less cuboidal with small nuclei possessing larger chromatin masses. The larger cells are few and are elongated with long flat nuclei. These large cells are confined to the anterior region of the vasdeferens and, as already mentioned, above are similar to the nurse cells of the testis. However the epithelial cells possess clear vacuoles in their cytoplasm. It is a presumption that the secretion of these cells help in binding of sperms and formation of spermatophores. In some of the preparations of whole mount bunches of spermatophores are seen arising directly from the testicular lobe, but at the same time it is sure that nurse cells play a role in secreting seminal fluid and maintain a pressure inside the vasdeferens.

The lumen of the vasdeferens is not straight but follow a spiral course which is evident from the photographs of the vasdeferens (Pl. VIII fig. 2) showing wavy arrangement of spermatophores.

This is further confirmed when the spermatic fluid comes out in spiral manner after cutting the vasdeferens in a freshly caught animal.

Histology of the penis (Fig. 29D).

As already stated above, the penis or style, like *Asellus*, (Necdham, 1938), is a chitinus tube perforated at the tip. Its wall is continuous with that of the body wall. In adult male it consists of a short basal (pr.s.) and a long distal (dis.s.) segment. The vasdeferens traverses the length of the penis and has a slight enlargement which may be in the nature of vesicula seminalis in the tubular segment. It opens some what laterally to the floor of the terminal ectodermal pit which broadens into a lateral pit near its aperture. The margin of the aperture is divided into a small median and a large lateral lip. Muscles move the styles (penis) as a whole and the distal on the proximal (pr. s.) segment. An intrinsic muscle system of longitudinal (l.m.) and transverse (t.f.) muscles compresses the distal segment and expel spermatophores from the vesicula seminalis. In *N. kashiensis* however, a sphinctor (or muscular) pad is formed at the terminal end, providing a valve closing the mouth of the vesicula seminalis. The intrinsic muscles originate from the outer surface of the epithelium and are inserted to the hypodermis of the wall. The cells of the lateral lip are columnar.

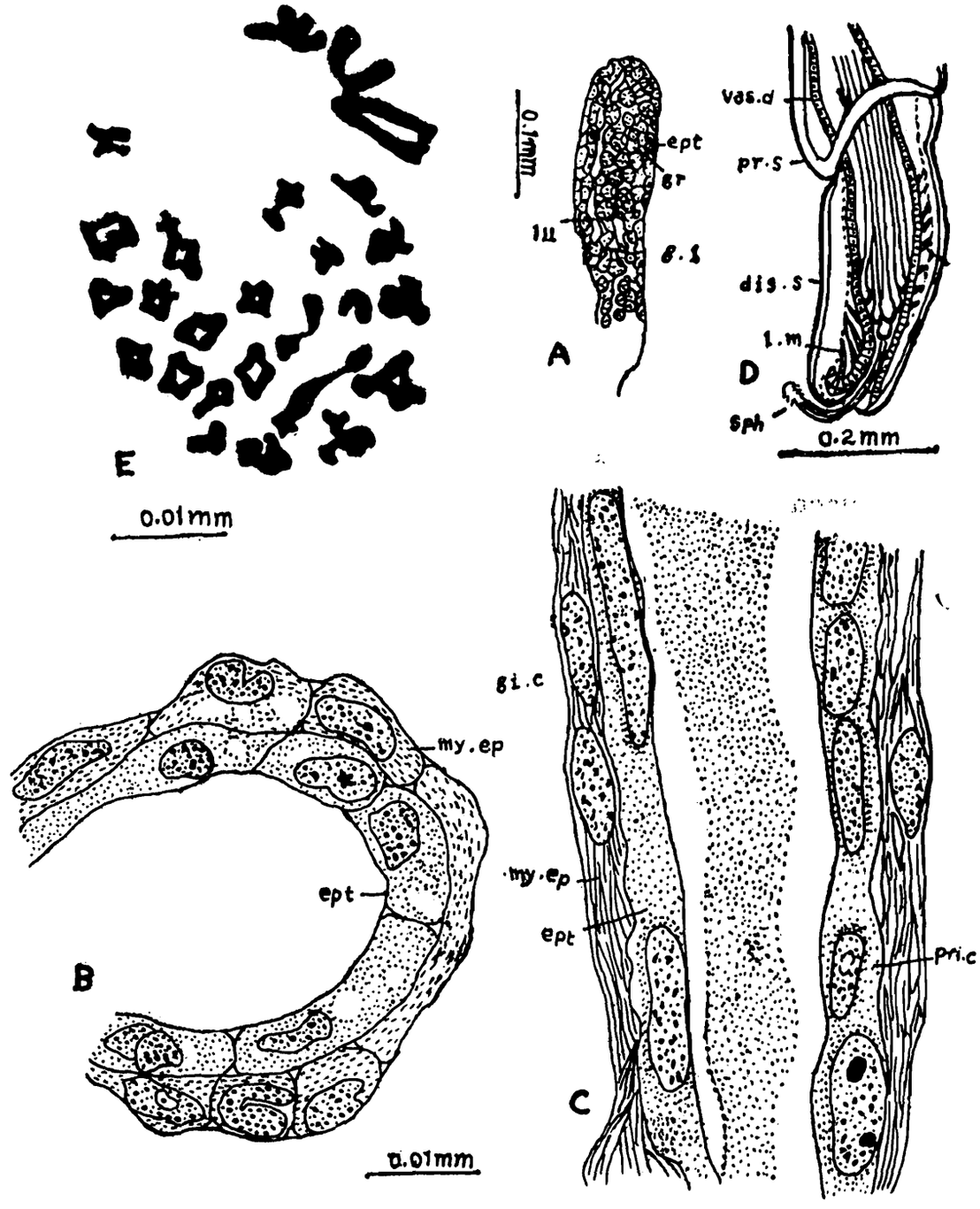


Fig. 29. A. L. S. of immature testis. B. T. S. vasdeferens in distal region. C. L. S. vasdeferens in middle region. D. L. S. penis. E. Metaphase stage in spermatogenesis.

The spermatozoa :

Spermatids show a variety of structures during their maturation to sperms (Fig. 28C). The head gradually lengthens and in the nature spermatozoon it is a long, narrow flexible structure pointed at both the ends. The elongated head has a cap called acrosome (a. cr.) which is

very lightly stained while rest of the head is deeply stained in Ehrlich's haematoxylene or orcein. The head is connected with a long tail. Thus a mature sperm has a head with a lightly stained cap (acrosome) and a long flagella like tail. The head is deep blue while tail is light red in haematoxylene, eosin staining. No neck or middle region is descernible. Further the sperm head is approximately 26μ in length and 2μ in breadth, while the tail is 830μ in length. Morphologically the spermatozoon of *Nichollsia* is very much similar to that of *Gammarus*, (Cussans, 1904) and *Asellus militaris*, (Reger, 1964).

As usual the sperms in *Nichollsia* are not released individually but in compact bundles of 24-36 sperms glued together. Each bundle is called as "Spermatophore". The spermatophore (sph.) has a cap enclosing the head of all the sperms together. In cross section of the spermatophore the heads are seen arranged radially around a cytoplasmic axis. The sperms are released from the testis to the vasdeferens in the form of spermatophores and it is possible that they are glued together by the secretion of giant cells or nurse cells as we prefer to call them. The sperms are non motile. Usually the sperms participating in the formation of spermatophores in Isopoda are non motile (Barnard, 1927 ; Menzies, Loc. cit ; Mathur, 1961 ; Reger, 1964 and Cotelli et. al, 1976).

Though detailed study of spermatogenesis does not form part of the present investigation, different stages shown in the figures indicates that process and structure of the sperm formation is more or less same as described for *Oniscus asellus* (Nicholls, 1902) ; *Phreatoicus assimilis*, (Chilton, 1894) ; *P. capensis*, (Barnard, 1927) and *Limnoria* (Menzies, 1954). The occurrence of spermatophores among crustacea has been emphasized by Calman (1909). He also refers to the presence of spermatophores in Isopods, even though he was not prepared to accept hypodermic impregnation of spermatophores as reported in *Jaera* (McMurrich, *loc. cit.*). However, it has been shown that there is considerable variation in Isopods in the plan of female genital system resulting changes in the modes of fertilization and breeding (Menzies, Loc. cit. and Veuille, 1978).

Orientation of spermatophores :

The mechanism by which spermatophores are transported from their place of origin in the testes to the vasdeferens and to the penis is not clearly understood and has led to considerable speculations. In most

of the Isopods spermatophores are passive and are being either carried by a fluid current through the vasdeferens or passed towards the distal regions of the vasdeferens by undulating movements of the tube. In *Nichollsia* it was noted that spermatophores are oriented in a spiral manner in the whole mount of the vasdeferens during breeding season. There seems to be a strong pressure within the tube, because on rupture the seminal fluid with spermatophores is thrown out with strong force in spiral manner. All the spermatophores have their heads directed towards the penis.

Two questions arise in this context :

1. Where does the fluid in a vasdeferens come from ?
2. What propels the fluid through the vasdeferens ?

It is suggested that the large quantity of cytoplasm which is sloughed from the spermatids during spermatogenesis, may form the major bulk of this fluid and the cytoplasm of the degenerating giant cells or the so-called nurse cells may also contribute to this mass. The peristaltic movement caused by the alternate contraction and expansion of the muscular (myoepithelial) layer of vasdeferens are responsible for the propulsion of the fluid through it. The Rotary movement of the discharging seminal fluid is caused by the pressure developed inside the tube by accumulation of spermatophores and fluid and a somewhat spiral lumen of the vasdeferens.

Androgenic gland (Pl. VIII, fig. 1).

Androgenic gland in *N. kashiensis* is an elongated thin ribbon like organ situated in the anterior half of the fifth (5th) thoracic segment attached to the vasdeferens. There are 2-4 rows of cells and at places it is even one cell thick. It is not a hollow structure. In an individual collected in the month of late November, the gland shows hypertrophy. However, it varies with the length of the animal.

The gland is diffuse and is a part of testis as can be seen in the photograph (Pl. VIII, fig. 1). The gland is attached terminally to the base of the fifth leg of its side by connective strand. There is no doubt about the holocrine secretion of this gland in Crustacea (Malacostraca) as a whole including *Nichollsia*.

The androgenic gland has the ability not only to determine the development of male characteristics, including the spermduct (vasdeferens) but also to determine the differentiation of the gonad itself

(Charniaux-Cotton, 1960). Transplantation of this gland in any part of a female brings total transformation into a male, externally as well as internally.

Female reproductive system (Fig. 30 & Pl. VIII, fig. 3).

The ovaries are paired organs located below and on either side of the tubular heart, above the intestine and hepatopancreas. Each ovary, when mature, extends from the fourth peraeonal somite to the fourth abdominal somite. The two ends of the ovary are drawn out into short thin strands suspected of carrying out endocrine function (Menzies, loc. cit ; John, loc. cit.) in *Limnoria* and *Sphaeroma* sp. The two ovaries are attached to the neighbouring organs by connective tissue strands.

The oviducts (Ovd.) originate in mid-lateral region of the ovary in the fifth thoracic segment as a funnel. The neck of the funnel runs

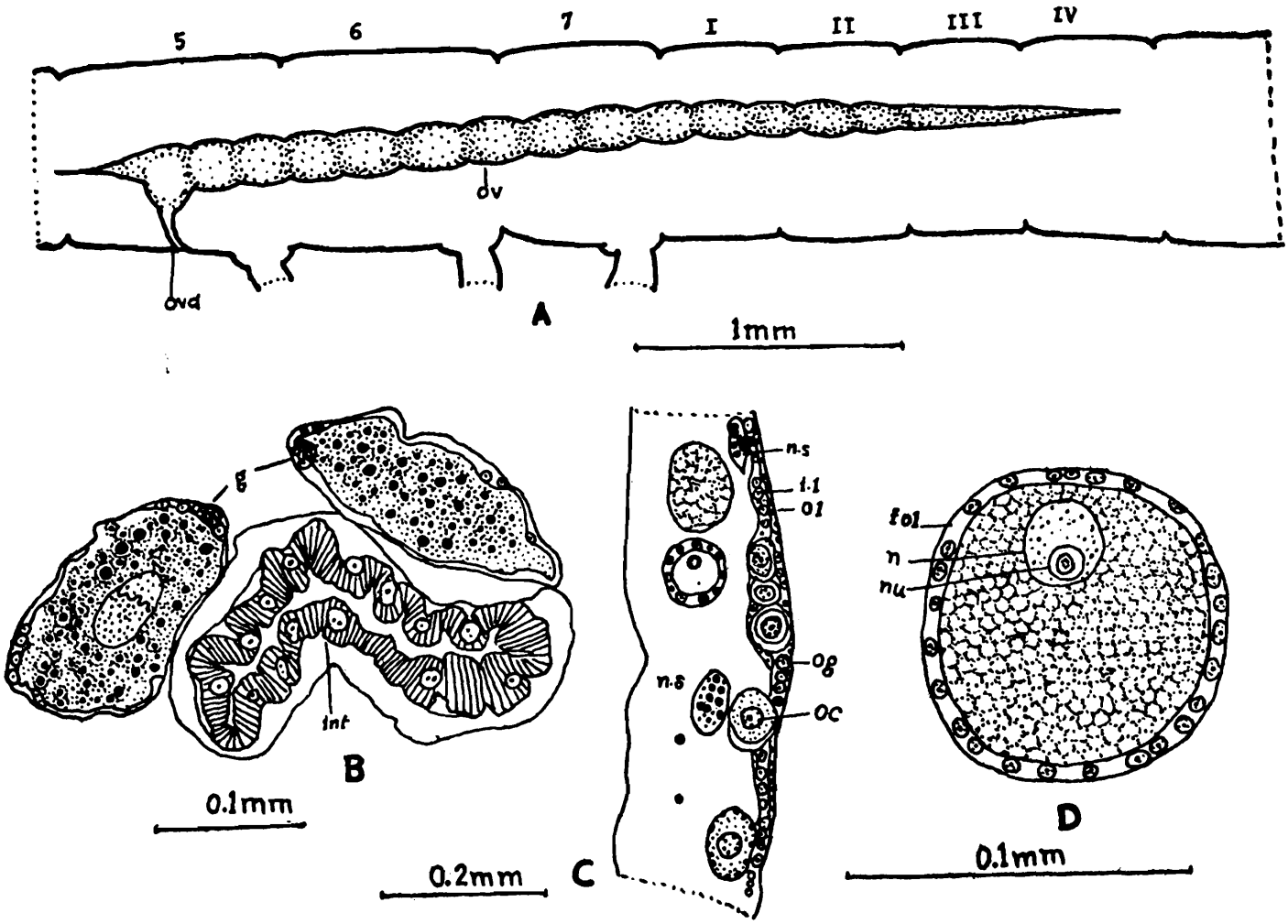


Fig. 30. A. Female reproductive system (lateral view). B. T. S. through ovary. C. Whole mount of ovary. D. Magnified view of oocyte.

downwards and inwards which on reaching the ventral side open separately in a gonopore (during the breeding season) situated on the mid-ventral line of the fifth thoracic segment.

Usually the ova are large, oval in shape and contain a large amount of yolk and are not of the same size, but the oldest eggs are nearer the oviduct.

Since the animals are semitransparent the matured ovary can be very well seen, during the breeding season, as white thick long bands on the lateral sides of the body. Matured ova could be counted. In such females 12-14 were counted, but in mounted ovary 18-20 ova are seen arranged in two rows of which only one the outer row contains the matured ova. The inner row possess oocytes in different stages of (Fig. 30C) development.

Histology of the ovary :

The wall of the ovary (Fig. 30 B, C) consists of an outer layer of thin flattened cells with small elongated and deeply stained scattered nuclei and an inner layer of more or less cuboidal cells with large round and deeply stained nuclei. These two layers are separated from each other by structurless lamina which contain blood spaces.

On the inner side of each lobe of the ovary, *i. e.*, the side facing its fellow of the opposite side there is a longitudinal thickening of the ovarian wall, from which the ova are budded off. This thickening is called as 'germogen' (Fig. 30B, g) and rest of the ovarian wall has been termed as 'vitellogen'. The matured eggs are first formed in the anterior region so that at the time of fertilization and oviposition they are discharged first and are not interrupted by immature oocytes.

The young oogonia in places appear to degenerate and slip away from the wall into lumen of the ovary, their nuclei becoming shrivelled and staining darkly showing dark (n. s.) granules. These cells probably contribute to the nourishment of the oocytes, and are found close to them (n. s.), but never in a definite investing shealh. The young oocytes (oc.) enclosed in the follicle (foll.) slip into the lumen of the ovary and start growing in size. All sizes of oocytes are found free in the lumen of the ovary, but in later stages when they are of considerable size, they tend to fill up the whole lumen, so that the follicle cells are pushed to the periphery of the lumen.

Histology of the oviduct (Pl. VIII, fig. 3).

The proximal end of each oviduct is in the form of a funnel. The mouth of the funnel is continuous with that of the ovary without any visible demarcation. The oviduct is formed of the same two layers as the ovarian wall with only slight modification in structure, chiefly in the inner epithelial layer. In the oviduct the inner epithelial layer is thick and the cells become columnar in its distal portion. A chitinous intima has been found bordering the lumen of the oviduct in distal parts. This intima is an invagination of the body cuticle.

In matured ovary, the structure of the cells forming the inner epithelial layer of the oviduct changes and the cells become larger. Vandel (1925) in terrestrial Isopods Identified the large cells as gland cells. However, Leichmann (1891) did not mention any such structure in *Asellus aquaticus*.

I have been unable to locate the so called sternal gland in the females of *N. kashiensis* which is so important in *Asellus aquaticus* (Charniaux-Cotton, 1954).

In an young ovary one can see the eggs (oocytes) in different stages of development. The germogen becomes prominent and in subsequent stages follicle enclosing the egg is formed with the deposition of yolk material.

The external opening of the oviducts become very prominent in sexually mature females during the breeding seasons and after the eggs are deposited in the brood pouch or marsupium. The openings are two but have a common outer margin which swells up after fertilization and forms a vulva. This vulva can be seen inclosed by marsupium till a short time before the young ones leave the pouch, afterwards it is closed by a thin delicate membrane.

Brood pouch or brood chamber (Pl. I, fig. 3, 4).

In *N. kashiensis* during the breeding season there arise close to the bases of coxae, of the first four peraeon segments, four pairs of functional oostegites. Associated with these in *Nichollsia* are the coxal lobes upon the maxilliped. The coxal lobes are vestiges of oostegites. The four pairs of oostegites which are plate like structures participate in the formation of marsupium or the brood chamber. All four pairs have a somewhat opaque central part, surrounded by a transparent border. The anterior and distal margins of all the oostegites are armed

with simple rod setae. The first pair are similar to the first pair in *Asellus aquaticus* in being bent in the middle so as to form a larger posterior lobe and a smaller anterior lobe. The posterior lobe resembles the other lamellae and forms part of the brood pouch. The anterior lobes, however, embrace the projecting mass formed by the coxal lobes of the maxilliped. The anterior margins of the anterior lobes are furnished with a row of close set plumose setae, which allow the lobes to be closely applied to the coxal lobes of the maxilliped.

Leichmann (1891) described four types of brood lamellae in isopods on the basis of pattern of vascularisation. Interestingly the posterior brood lamellae of *N. kashiensis* are similar to those of *Anthura gracilis*. In the arrange ment of oostegites it has been observed that posterior oostegite overlap the oostegite anterior to it, similarly the right one overlaps its counterpart from the left side. The posterior margins of fourth oostegites are folded inward to close the marsupium from behind which extends and cover the fifth segment from below. Thus the genital pore which is formed only during the breeding season, opens directly into the pouch.

The coxal lobes on the maxilliped are armed with plumose setae along their margins and while projecting into the pouch they form an interlocking which prevents the flow of dirt particles, entering the brood chamber, alongwith the water current.

The oostegites develop as small plate like lobes before the nuptial moult. They are evaginations of the sternal cuticle and hypodermis and consequently enclose a cavity, which is in free communication with the body cavity. The sternal ridges, in the segment 1-4 participating in the formation of brood pouch, disappear during the existence of brood pouch. These ridges reappear gradually as the brood plates are withdrawn after the young ones have been released.

Usually the oostegites are on the first to fourth free thoracic segments in Phreatoicids, in *Mesamphisopus capensis* and few other species there is a small pair in the fifth thoracic segment (Barnard, 1927, Nicholls, 1943). Some arcturidae have only one pair, while in certain Cymothoidae and Epicaridea a pair of oostegites is present on each of the seven free thoracic somites, the last two pairs, however, being very small.

Concerning the homologies of the oostegites, Claus (1886) has suggested that they may perhaps be modified epipodites, whereas Nicholls (1943) suggested them as modified gnathobases.

Pairing, Mating and Egg deposition :

These three phenomena are very difficult to observe in the natural environment of *Nichollsia kashiensis*, because they inhabit wells and underground waters. The present observations were made in the laboratory on the specimens maintained in aquarium. The first pair was observed in about middle of March 77 (25°C) recurring in May and September, 77 (27°C), but the complete behaviour was observed in the last week of July, 78. The male was considerably (2.5 cm.) larger than the female (1.4 cm) and held her with his gnathopods around the head and first peraeon and carrying her, here and there. The matured eggs in the ovary were clearly visible through the moulted posterior half of female. Pairing continued for 8-10 days. The female was lying under male's grip. The posterior segments of peraeon and the pleon of female remain free, and female keeps her abdomen bent downward while the male remains more or less straight. A pairing male did not allow any other male to approach near him or her kicking or repelling by his telson. Pairing is preceded by chasing and seizure of the female by male.

Copulation :

The male strengthens its grip over the female by involving his second and fourth thoracic legs around her second and sixth thoracic segments respectively, subsequently the male taps on the abdomen of the female by his abdomen, particularly telson, resulting in the straightening of her abdomen. This is followed by actual copulation in which the male fertilizes her from each side by arching his body in the posterior thoracic region and bending his posterior around to herside. The seventh thoracic segment was seen approaching and facing the female genital opening from below, when the male made strokes. This act, of copulation was repeated. Fertilization is internal. Careful observation by a hand lens did not reveal the involvement of appendix masculina in copulation. However, the direct observation of this organ was obstructed by the large pleopods and bending of the abdomen. There does not seem to be any reason why the penis should not be involved in the direct introduction of spermatophores in to the female gonopore. The penis as has already been illustrated is quite a long and tubular structure and the ventral or the sternal portion of the seventh thoracic segment is bulging out. The side plates or the pleural extension are absent. Further, the thoracopods are so much flexible that they do

not cause any obstruction to the penis in reaching the gonopores which is placed on the sternal ridge. Help by the appendix masculina in pushing the spermatophores inside the gonopore may be a possibility, which was not observed. Chances of inert spermatophore reaching to the appendix masculina could not be established and justified when position and morphology of the anterior pleopods was taken into consideration. Role of other appendages like antenna during copulation was not found of any significance. Uchida (1930) found in *Asellus*, that amputation of 4th pair of peraeopods prevents successful copulation. In *N. kashiensis* too, it seems that 4th thoracopod in male is important as it is involved at the time of copulation. In *Asellus communis*, Engemann (1964) has observed that first and second pleopods quiver violently and move back and forth rapidly but no transfer of of material could be seen.

During observations on pairing in laboratory, it was noticed that pairing period normally varies from 10-15 days but it may extend further. Act of copulation was stopped for some time if the couples were disturbed, but not the pairing.

Copulation and fertilization was followed by moulting of anterior half and sudden release of the full grown oostegites forming the brood chamber. At this time the female was relased by her partner, which sought some convenient hiding place near or under stone chips or in in the corner of the aquarium. Due to formation of the brood pouch during anterior moult the female was found creeping on its side and moving to a hiding place.

Transfer of eggs to the brood pouch :

This process was completed within 10-20 hours after copulation and formation of the brood chamber. The female was lying on its side with very transparent brood chamber showing the deposited eggs. Transfer of eggs was found to be painful job. The female was putting pressure on the ovary and oviduct from the posterior to anterior by jerks of her bent thorax and abdomen behind her fifth thoracic segment. This resulted in the extrusion of eggs through the gonopore directly to the chamber.

Size of the brood :

Number of eggs laid in the brood chamber was found between 6-24 (Table III) in different females either collected from the wells or

observed in the aquarium. There was difference in the maturity of eggs. Those eggs laid first in the marsupium were older than those laid afterwards. Size of the eggs varies from 0.4-0.5 mm (400-500 microns) in diameter. The eggs are spherical.

TABLE—III

Size of the Female in mm.	Number of eggs	Month	Remarks
12 mm	6	July-Aug.	In aquarium
16 mm	9	,,	,,
17 ,,	10	,,	,,
18 ,,	12	,,	,,
15 ,,	7	October	From well
16 ,,	6+3	June	,, ,,
18 ,,	9	September	,, ,,
20 ,,	10	April	,, ,,
24-25 mm	24	January	,, ,,

From the table there seems to be a positive correlation between size of females and the number of youngs in the brood pouch in this species.

In some instances it was found that female with already formed brood pouch was mounted by a male and a vigorous attempt of copulation was made. This was observee at least in 3 females.

Cannibalistic behaviour was found to play an active role. The gravid female was found eating its own eggs from the marsupium through the anterior end of the pouch, and eggs of the female were also eaten by their male counterparts.

In laboratory during July to September the temperature varied between 27-28° C in the aquarium during the above breeding observations. Higher temperature caused drastic and adverse effect on the gravid females. A gravid female under observation at 32-35°C temperature expelled all its eggs and ate them. It is possible that cannibalistic activity increases at higher temperatures.

Incubation and Release of brood :

Nichollsia kashiensis incubates its youngs within its external brood pouch. The incubation period as observed in laboratory was 35 to 40 days at 28-30°C temperature. The females release the young by flexing their abdomen upwards, reflecting the posterior pair of oostegites outwards and causing the brood pouch to gape. The young Isopods then exiit from this opening. These youngs are between 4 to 4.5 mm. in length. No maternal care was exercised over the youngs after liberation, where as instances of cannibalism during development of the embryos was observed, where the mother devoured some of her egggs or nymphs directly from the brood pouch.

As with other Isopods (Nusbaum, 1886), the young resembles the adult but have one fewer pair of legs and fewer antennal segments. The outer and inner ramii of uropods are equal, the ventral margin of 5th, 6th and 7th thoracic segments are more or less rounded. Although 7th peraeopod is absent it is visible in the form of a bud through the transparent cuticle of the 7th peraeon. A considerable quantity of yellow material presumably unabsorbed yolk (Text fig. 8A r. y.) could be seen in the thoracic (posterior) region of the young. The youngs released from the brood pourh become active immediately and start walking on the bottom. The first moult occurred approximately after 7 days of their hatching from the marsupium at 31-32°C. The 7th peraeopod is regained most probably after the first moult.

In *Porcellio scaber* (Anderson unpublished quoted by Sutton, 1972) and in *Porcellio laevis*, Nair (1976) and *Porcellionides pruinosus* snd *Cubaris* sp. Menon & Rait (1981) noted the absence of 7th thoracic segment and mentioned about similar conditions in the early free living stages of other common Isopods. In this regard the nymph released from the marsupium in *N. kashiensis* is in advanced stage of its development. Further, in *Porcellio laevis* first moult occurred within one day of liberation from the marsupium.

It is not intended to give detail of the development, but merely to draw attention to the fact that it is typically Isopodan, and that the embryo bears a striking resemblance to that of *Mesamphisopus capensis* (Barnard, 1927) and *Asellus aquaticus*. The embryo has the dorsal curvature, a characteristic of the Isopoda. Presence of the dorsal appendage could not be confirmed as the detailed embryology was not taken up during this study.

Release of the youngs from the brood pouch is followed by gradual withdrawal of the oostegites (Pl. I, fig. 4), reducing to a pregravid size after subsequent moult. It follows, then, that females with pregravid oostegites can be either virgin females or females which have already produced one or more broods. Such females were collected from the natural habitat during the month of September.

The growth rate was observed in a young specimen to average 1.mm increase in its length.

Reproductive Cycle :

Percentage composition of length groups of *N. kashiensis* has been

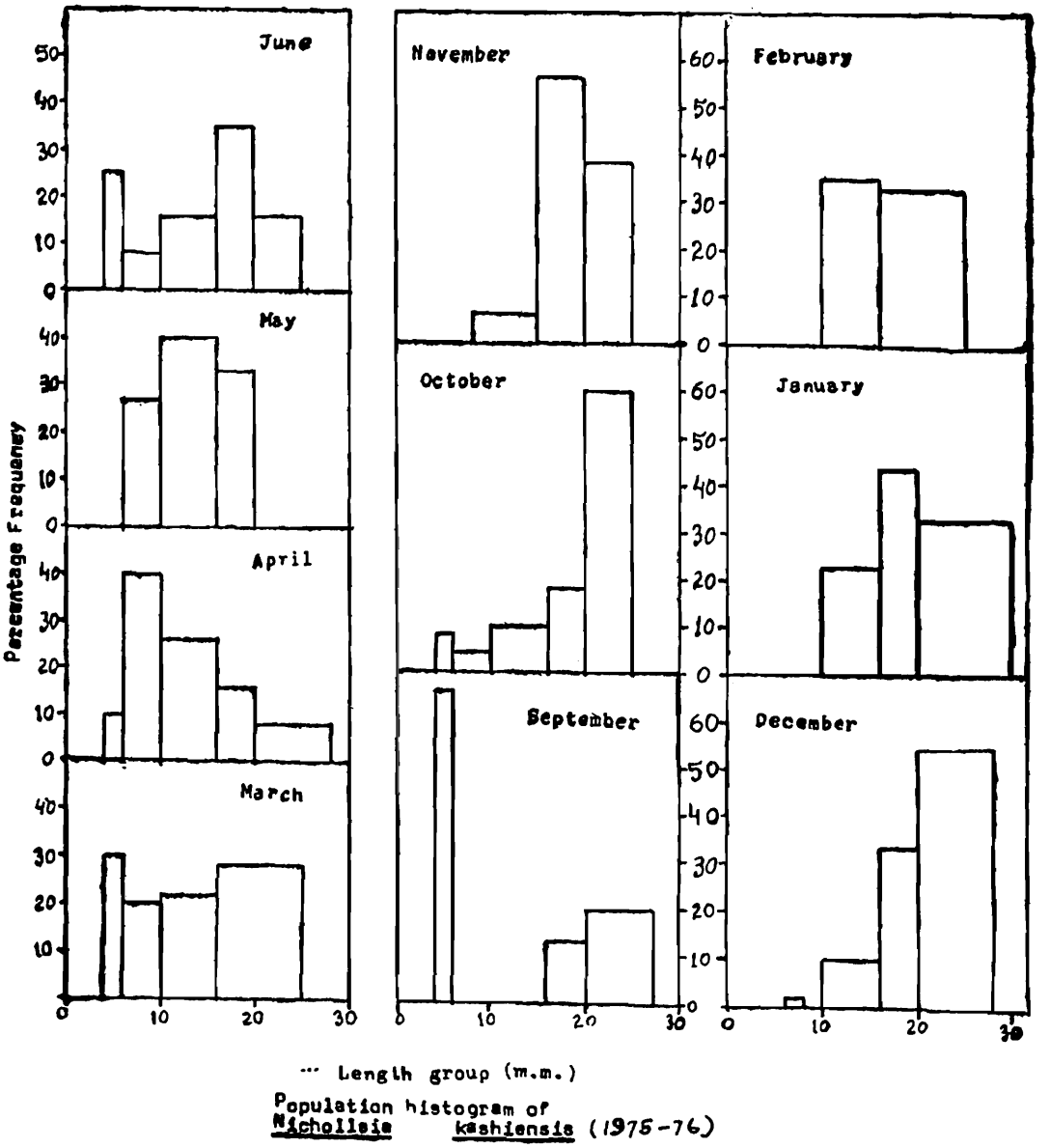


Fig. 31. Population histogram of *N. kashiensis.*

presented in the histograms of collections made over a year between

March, 1975 to February, 1976. Samples were divied into five size groups and their percentage is presented in the form of histograms (Fig. 31). It does not indicate the male and female populations separately. Data for the two consecutive months (July & August) are missing yet some general remarks on the reproductive biology can be inferred. Measurement was nearest one millimetre from the anterior edge of the head capsule to the posterior end of the telson (excluding uropods).

The histogram shows two breeding cycles per year in *N. kashiensis*. Pairing had been observed in two seasons in the laboratory. The large influx of youngs into the natural populations are one during the month of March extending upto June and the other during the mont of September. These observations have been confirmed in the laboratories. Pairing was observed in March and continued upto May in different females. Pairing does not last long as is seen in other Pbreatoicids e.g. *Colubotelson thomsoni*, (Engemann, loc. cit.). The youngs are most common in these periods. Pairing of the next breeding starts at the end of July and continues upto October, the youngs being very large in number during September and October. However, smallest individuals may be seen in some of the wells during December and only once a gravid female with recently laid eggs was collected in the month of January which is rare. The largest female and male collected so far attain the size of 2.5 cm and 3.2 cm respectively. On the basis of their approximate average increase at each moult it is estimated that the length of life of *Nichollsia kashiensis*, is $1\frac{1}{2}$ to 2 years, with probable production of 4 broods of youngs. Since the interval between moults varies from 15 days in youngs to 2 months in the largest specimen it was estimated that average intermoult period is about a month- Since the temperature in their natural habitat remains within a very narrow range (25°-27°C) of variation, it is presumed that breeding and to some extent moulting observed in laboratory, did not differ much from its natural habitat.

Examination of results in the histogrnm indicates that the females producing in early summer may produce a second brood in the early winter. The matured males were found with spermatophores in most part of the year. The spermatogenesis or meiotic division in testis is more frequent during the early part of winter. The large and intermediate size classes are although found throughout the year one or the other group is more prevalent in a particular month.

Sex Ratio :

Usually the two sexes are in the ratio of 1 : 1 but during the breeding season when the gravid females tend to hide in the crevices, their number in the collections decreases.

Month	Sex Ratio of *Nichollsia kashiensis*		
	Male	Matured Female	Youngs
April	39	36	210
June	10	8	6
September	8	3	—
October	69	28	3

Chromosomes (Fig. 29E & Pl. VIII, fig. 4).

The haploid number of chromosomes during meiotic division (Fig. 29 E) is 25 and most of chromosomes are metacentric. The diploid number of chromosomes was found by staining the developing embryos from the brood pouch by Aceto-orcein method. The diploid number (Pl. VIII, fig. 4) in *N. kashiensis* is 50. However, the detailed study will form the part of later work in this species.

DISCUSSION

A species or an individual is by and large the expression of its genotype under the influence of its environment in which it exists. This influence is expressed in its morphology, anatomy and to large extent its physiology.

From the present studies it will be seen that *Nichollsia kashiensis* possesses many morphological and anatomical features whieh could be considered as direct effect of its subterranean mode of life. Significance of some morphological peculiarities of this genus are a result of convergent evolution reflected in different systems and consequently the life history of the animal.

External Morphology and Affinities :

These two are interrelated. Thus basing their observations on the external morphology, Chopra & Tiwari (1950) and Tiwari (1955b) made the following remarks on the affinities of *Nichollsia*.

"With Amphisopidae this family agrees in the general structure of mouth parts. They are clearly amphisopine. The presence of lacinia mobilis on both the mandibles is a distinctive feature of Amphisopidae which it shares with the Indian family. Again the condition of cervical groove, multi-joined filament of antennule, presence of several plumose setae on the proximal endite of maxillula and the structure of maxilla, link Nichollsidae with Amphisopidae.

The general facies of *Nichollsia kashiensis* is however, more phreatoicine, than amphisopine. As in Phreatoicidae, the first peraeon segment in Nichollsidae is free from the head, the coxae of peraeopods are not fused with the pleurae of related segments and the bases of peraeopoda are not flattened".

However, the occurrence of clawed uropodal ramii, in the newly born young brings *Nichollsia* closer to the Amphisopidae in its ontogenetic relationship.

As already mentioned earlier *Nichollsia kashiensis* has got some unique features of its own. The uropods, with much longer outer ramii show a condition like that in Syncarida and reverse of Phreatoicoidea. Similarly emarginate postero-dorsal edge and the crennulate postero-lateral margin of the telson and the ridge patterns on the two molars of the mandibles are without parallel in the suborder. The mesial cleft of endopodite of all the pleopods and exopodites of four posterior pleopods with extreme lateral position of lobe is unique in this family. The genus *Nichollsia*, shows some similarity with two subterranean amphisopid genera, *Hyperoedesipus* and *Phreatoicoides*, where the two latter genera show a tendency towards lateral displacement of the lobes on four exopodites of pleopods and also in the absence of epipodites on pleopods. Absence of coupling hooks on sympodites of pleopods is only found in this family of the suborder.

The subterranean Phreatoicids, including *Nichollsia*, show certain features in common. All the three known subterranean genera of the family Amphisopidae viz., *Hyperoedesipus* (Sub. fam. Hypsimetopinae), are blind, have a slender vermiform body, reduced pleural extension in the abdomen, tendency towards the loss of setae on pleopods and body surface, lateral displacement of the lobe on the inner margin of exopodite of pleopods and reduction of the endopodite of the same. *Nichollsia kashiensis* however, exhibits all these characters in a more or less aggravated form. Apart from these characters the accumulation of

plumose setae on the inner margin, presence of microtrichs and other setae only along the inner margin and reduction on the outer margin also seen to represent the subterranean adaptation in *Nichollsia kashiensis*. The Hypsymetopines probably took this habitat somewhat later, and *Hyperoedesipus* was perhaps the last to go down.

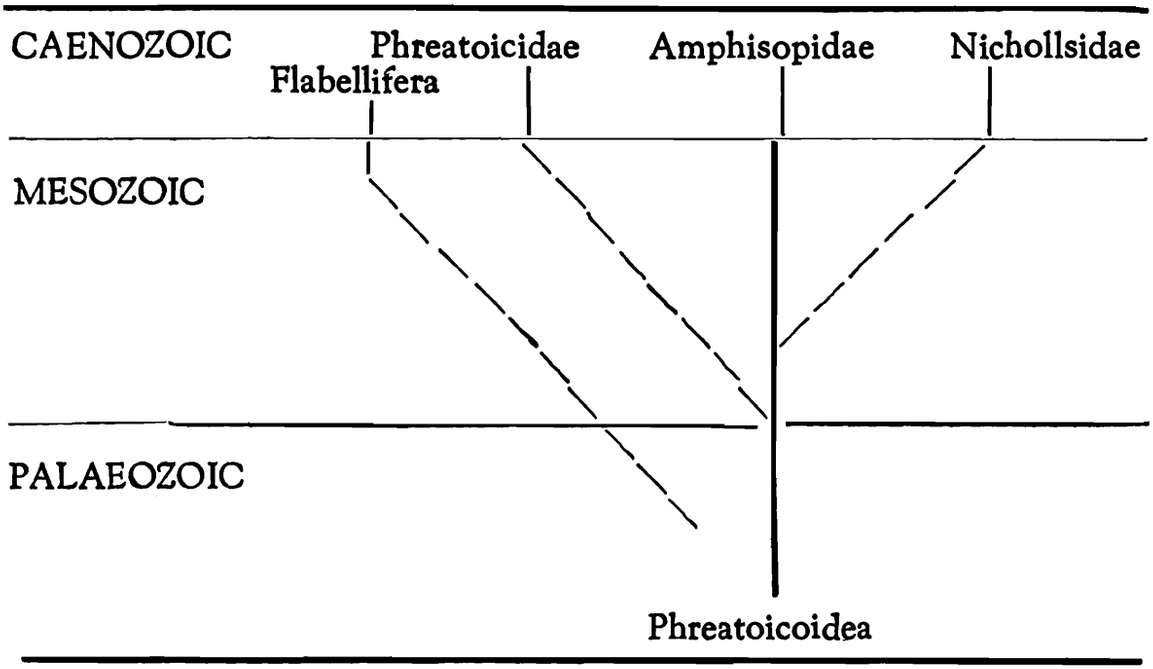
Chopra & Tiwari (1950) consider the position of *Nichollsia*, intermediate between *Hyperoedesipus* and Hypsimetopine genera, and that all these forms have evolved from a common Gondwanaland ancestor, it appears likely that divergence from the ancestral type and independent development along different directions must have started very early.

Changes in characters, such as elongation of the antennae, has taken place either by spontaneous mutations or mutation caused by other agencies like chemical and physical factors prevailing during those days of transgression from one habitat to another. Natural selection works mostly on those characters which are already present called as "pre-adaptive characters" and it seems probable that elongated antennae being a primitive character is preadapted character present in the early stages of evolution of the group or the life history of the animal and persisted unaltered. The obsolescence of a posterior process of the head and perhaps of the cervical groove have also been referred as preadaptive characters by Nicholls (1943 part II, page 16.). Secondarily modified characters such as loss of eyes, loss of pigments and pigment effector organs are post adaptive characters. The retention of longer antennae is as it seems, due to the absence of strong current in underground waters inhabited by them.

The vermiform elongation of the body affecting noticeably head and tail, elongation and slenderness of uropods are post-adaptive characters acquired in relation to subterranean habit (Nicholls, *op. cit.*).

While discussing the significance of uropodal ramii, Dahl (1955) remarked "There are, however, spines which may be of phylogenetic importance in that they represent the last vestiges of appendages or parts of appendages in the process of reduction. Nicholls (*loc. cit.*) suggested that the terminal spines of the uropod of *Mesamphisopus* should represent such vestiges of a free terminal segment, which occurs in various Peracarida. There are however, no indication of any free terminal segment of the uropod ramii in the embryo of *M. capensis* and there are no spines. Thus the ontogenetic evidence contradicts the suggestions made by Nicholls. "Presence of claws on the ramii of

uropod in the embryo or young of *N. kashiensis*, is an ontogenetic evidence supporting Nicholls's contention that it (the claw) represents the terminal segment which occurs in various peracarida and in adults of *Mesamphisopus*, *Phreatoicus* and *Notamphisopus*. This ontogenetic evidence supports the view expressed by Tiwari (1955b) that Amphisopidae is lower than Nichollsidae in evolutionary series and that Amphisopidae is rateher more primitive than what Nicholls (1943, pt. I, p. 32) considered. From the above discussions on the external morphology of *Nichollsia kashiensis* it can be reasonably assumed that this genus defected much earlier from a common stalk of Nichollsidae and Amphisopidae in the suborder. Similarity of characters with subterranean members of Phreatoicidae is the visual effect of convergent evolution in pre and postadaptive characters. Glaessner (1957) and Birstein (1962) have given the origin of Phreatoicoidea, sometimes during Palaeozoic period, but it seems that Nichollsidae separated during late Mesozoic period and adapted to this mode of life.



Phylogenetic relationship of Nichollsidae

Body wall and moulting :

Beatty (1949) found that in three species of cavernicolous amphipod genus *Niphargus*, the carotenoid pigments are completely absent while in epigean species pigmentation is very common. This was considered by him as effect of subterranean life. Regarding absence of a distinct pigment layer in the cuticle of *Nichollsia* a similar conclusion can be made here.

Workers on crustacea have classified the setae, differently (Needham, 1942b ; Menzies, 1956, E. Silva, 1965 ; Thomas, 1970 and Fish, 1972). E, Silva (*op. cit.*) has indicated the taxonomic importance of setae in *Sphaeroma*. Needham (*op. cit.*) has illucidated the functional importance of microtrichs. In *Nichollsia* distribution of microtrichs is restricted to head and abdominal appendages.

Moulting cycle has been classified in to different stages in various groups Crustecea. The criteria for classification was basically devised by Drach (1939). These were followed by many workers (Pike, 1947 ; Passano, 1960 etc.). Many others (Drach, 1944 ; Hiatt, 1948 ; Charniaux-Cotton, 1957 ; Scheer, 1960 ; Travis, 1960a & b ; 1955 ; Stevenson, 1961, 1964 & 1968 ; Skinner, 1962) were of the opinion that different sets of criteria must be developed for different groups of Crustacea. Carlisle (1960) was of the opinion that the situation will improve if additional criteria, having universal applicability, could be found. In *Nichollsia* general rules can be applied for classifying different stages of moulting.

Digestive system :

According to Hewitt (1907) trituration occurs in *Ligia*, but this idea was not favoured by Nicholls (1931b.). Tait (1917) considered the foregut of *Glyptonotus* as a simple propelling organ. In *Sphaeroma terebrans* on the other hand, John (1968) believed that the lateral ampullae function as an efficient triturating mill. There is no doubt that trituration occurs in the foregut of *Nichollsia*.

Contrary to the authors's observations and interpretation of function of the ventral valve in *Nichollsia*, Kanneworff and Nicolaisen (loc. cit.) do not think that it has any function as a valve in *Bathyporeia sarsi* Watkin, an amphipod. According to them this has the function of keeping the funnel and intestinal tissue in position and forms the ventral and posterior sides of the connective cavity between the two hepatopancreatic caeca.

Hepatopancreas in Isopods have been called, enteric glands, Salivary glands, digestive glands, caecae and livers, midgut diverticula, midgut caeca, digestive glands or liver by various authors (Frenzel, 1884 ; Fahrenback, 1959 ; Strunk, 1959, Lane, 1959 and Tuzet *et al.* 1959).

Regarding controversial terminology of the hepatopancreas, Yonge (1926) states "Because of the superficial resemblance of the digestive

diverticula of the lamellibranchs and of many other invertebrates, to the liver of the vertebrates and the discovery in them of glycogen by Bernard (1855), they became known as the "liver". Weber (1880) later introduced the name 'hepatopancreas' as a result of his discovery of the secretory powers of the diverticula in crustacea. Inspite of the fact that none of the constituents of bile has ever been discovered in invertebrates, and that the digestive diverticula are in no way analogous to the liver of the vertebrates as Jordan (1913) has shown in his review of the subject, the terms "liver" and "hepatopancreas" as well as the less questionable designation "digestive gland" are still generally used.

Vonk (1960) observed that these structures in crustacea, fulfil the role of liver and pancreas in vertebrates and that the term "hepatopancreas" will give the best, though not entirely adequate idea of its many functions.

In all the isopods hitherto studied, the hepatopancreas tubules are symmetrical in their arrangement, though their number vary, three pairs in *Ligia oceanica* Hewitt loc. cit.) (Nicholls, 1931b) ; *Bathynomus giganteus*, (Lloyd, 1908) ; *Eurydice* Jones, 1968) ; and *Colubotelson thomsoni*, (Engemann, 1964) ; two pairs in *Ligia exotica*, *Armadillio elevatus*, (Chandy, *loc. cit.*) ; *Limnoria*, (Lane, *loc. cit.* ; Fahrenbach, *loc. cit.*) ; *S. terebrans* (John, 1968) ; *Porcellio laevis*, *Metoponorthus pruinosus*, (Alikhan, 1968, 1969) and *Asllus communis*, (Engemann, 1964).

It seems that the number of hepatopancreas depends upon the habitat and nature of food and feeding of the animal in Isopods. Those which are voracious and regular feeders, have three, while other Isopods, like *Asellus*, *Nichollsia* and most of the terrestrial forms have only two pairs.

In most crustaceans the midgut and the hindgut, which together form the intestine, have different origins. The midgut or part of the intestine extending from opening of the hepatopancreas to the rectum is generally believed to be endodermal in origin, whereas the hindgut or rectum is ectodermal and consequently lined internally by a chitinous layer. McMurrich (1896) showed that in Isopods, part of the alimentary canal usually termed midgut is actually the part of the proctodeal invagination. This view was supported by all subsequent authors, since in all the types of Isopods examined by them, the entire alimentary tract behind the stomach was found to be lined by an internal

layer of chitin (Schonichen, 1898 ; Ide, 1892 ; Murlin, 1902 ; Hewitt, 1907, Nicholls, 1931 ; Chandy, 1938 ; Tuzet *et al.*, 1959 ; John, 1968 ; Holdich, 1973). It was further supported by embryological evidences advanced by Goodrich (1939) and Nair (1956).

In *Nichollsia* a similar condition prevails and the midgut is confined to the hepatopancreas and its junction with foregut and hindgut.

Gupta (1961) has compared the rectal pads of woodlice with the glands of insects described by Wigglesworth (1932) and she denoted it as rectal gland. There is no doubt that heavy absorption occurs in this region in *Nichollsia*, before the faecal matter is discharged in the form of pellets, this pad in Isopods is not secretory but absorptive, hence the term rectal gland is untenable.

Ide (*loc. cit.*) observed that in *Oniscus murarius*, cells forming epithe lial layer are delimited by boundaries, but McMurrich (*loc. cit.*) and Schonichen (*loc. cit.*) put forward the view that epithelium is a syncitial layer of protoplasm, and boundary of individual cell is demarcated by cytoplasmic fibres. Later authors, viz., Hewitt (*loc. cit.*), Nicholls (*loc. cit.*) ; Chandy (*loc. cit.*) confirmed this observation. In *Nichollsia kashiensis* as already mentioned, cuticular folds of intima give the impression of cell individuality.

Stevenson (1961) reported the absence of chitinase in *Limnoria*. For long it was considered that cellulase is absent (Yonge, 1927) in *Limnoria* but later studies (Ray, 1951 ; Ray and Julian ; 1952) confirmed its presence in the animal in traces. It is possible that further study on this aspect may reveal the presence of chitinase in *Nichollsia*.

Excretory system :

Many workers have described the 'Salivary glands' in various groups of crustacea as ordinary racemose glands, with central ducts that unite to form a common trunk. Huet (1882) extended this theory to the point of suggesting that the small connective tissue cells attached to the surface of the glands are replacement cells, as in vertebrate salivary glands. He believed the glands to be present without exception throughout the Isopoda.

The first detailed account of these glands in Isopoda was given by Ide (1882). He described their structure quite different from that of the racemose type, each gland consisting of a regular mass of cells open-

ing by chitinous canals into a central duct. He observed considerable considerable variation in the size and number of glands in related species and in different parts of the same individual.

In his monograph on *Ligia*, Hewitt (1907) described two pairs of Salvary glands" on each side of, and opening into, the oesophagus, each being made up of a large number of rosettee glands. Ter-Poghossian's description (1909) agrees in general with that of Ide, except where he points out that in the terrestrial forms the rosette glands have a less restricted distribution than either Huet or Ide, supposed. They are found, he states, he states, throughout the whole of the head and its appendages. He found histological studies very difficult in land Isopods and failed to determine the course of the ducts.

According to Needham (*loc. cit.*) the histological resemblence of the rosettes to the development stage of the end sac of the maxillary organ of *Asellus* and the origin of both from scattered mesoderm cells might indicate true homology between them. But end sac is a segmental organ and would therefore scarcely be homologus with the rosettes in the non-segmental labrum. Further, he states that the rosettes develop later than the end sac. The similarity of rosette glands to the 'tegumental' glands of Decapoda (Herrick, 1895 ; Farkas, 1927 ; Yonge, 1932) merits consideration and also their possible affinity to others of the few celled glands of crustacea (Ide, 1892) and the labral glands of Entomostraca (Cannon and Manton, 1927).

Gorvett (1946) while dealing with the segmental glands and rosette glands considered all of them to be modified tegumental glands and has named differently. In *Nichollsia* I have preferred to call the suboesophageal rosetts as 'mandibular' glands. Rosette glands can be concluded to be modified tegumental (or cutaneous) glands, but the same conclusion can not be made for the coxal glands of head and thorax in *Nichollsia*. They are definitely modified segmental glands resorting to incretory function other than the maxillary glands. Parry (1960) called the thoracic coxal glands as "Kidneys of accumulation."

Isopods adapted for terrestrial life possess more number of tegumental glands while fresh water forms possess less and subterranean forms the least. In *Nichollsia* there is no organ like zenker's as described in *Asellus*, but 'end sac' of maxillary gland is large and duct is much elongated and coiled indicating that most of the excretory function is performed by this gland.

Circulatory system :

Important contribution on circulatory system have been mude by Audouin & Milne Edwards (1827), Rathke (1843) ; Kowalevsky (1864), Wagner (1865) ; Sars (1867) and Dohrn (1870). The culmination of this era is represented by Delage's (1881) fine work on the circulation in the "Edriophthalmes" still the main source of information concerning the present subject. Schneider (1891) also gave some clear conception of the system. Latest contributione on the subject are those of Silen (1954) on oniscoids and John (1968) on *Sphaeroma*. A variety of types were dealt with by these authors. Audouin & Milne Edwards and Delage (*loc. cit.*) examined *Ligia*, *Anilocra* and *Sphaeroma serratum* and the sole treaties on a true terrestrial form is that of Wagner (*op. cit.*) on *Porcellio*.

In *Ligia* (Hewitt, 1907) the pericardial chamber communicates with the anterior sinus while in *Sphaeroma terebrans* it does not. In *Nichollsia kashiensis* it is continuous with anterior sinus as well as posterior sinus which has been named here as abdominal vein. Among the isopods which delage (1881) examined he noticed that in *Anilocra*, *Conilera* and *Sphaeroma serratum* the pericardial cavity communicates with the sinuses on the dorsal side. Presence of numerous small sinuses over the roof of the pericardial cavity was also reported by John (1968) in *Sphaeroma terebrans*, but he concluded that they do not communicate with the pericardium. In *Nichollsia kashiensis* presence of numerous small sinuses over the roof of the pericardial cavity have been noticed but it does not seem to communicate with it. It is also observad, that these small sinuses are not throughout the length of the segments. Pericardial cavity laterally communicates with branchio-pericardial vein independently in each of the five abdominal segments on both sides of the body.

Presence of abdominal vein as in *Nichollsia*, receiving blood from the abdominal region and central lacuna has also been reported by Delage (*op. cit.*) in *Ligia* and *Anilocra*. It is also shown in circulatory system of a hypothetical primitive isopod.

Tubular heart is a common character of Isopods. According to to Silen (*op. cit.*) if consider the origin of heart as a differentiation of dorsal vessel, an elongated heart might be said to be a primitive condition, but the aquatic Isopods possess shorter type of heart. While elongated heart is a feature of terrestrial Isopods, at the same time

reduction in the arterial system in them is an advanced stage. Such combination of characters are perplexing and an elongated heart as a primitive condition needs further analysis. According to Silen (*loc. cit.*) the oniscoid heart can not be derived from a state found as far as known, in any recent aquatic form and hence the oniscoids have been placed at the side of the recent aquatic groups without direct connections with any of them. But here in *Nichollsia* which is an aquatic form and an old group we find the elongated heart to suggest that the oniscoids might have arisen from a phreatoicid group like *Nichollsia* if not directly from it and have retained this primitive character matched with the terrestrial mode of life.

Only 3 arteries running simultaneously, one median and two laterals, arise from the anterior end of the heart in *Sphaeroma* and *Ligia*. Gadzikiewicz (1905) found three vessels, intimately connected but with wholly separate lumina, issue from this part of the heart in the three genera viz., *Procellio*, *Idothea* and *Gnathia*. Silen (*op. cit.*) in *Ligia* and other Isopods and John (1968) in *Sphaeroma terebrans* confirmed the presence of the first lateral arteries running along the median aorta. The first lateral (second of Silen) in *Nichollsia* originates as a lateral branch of the median aorta in fourth thoracic segment and not from the anterior end of the heart. Similarly the 2nd lateral artery supplying to 5th thoracic segment also originates from the median aorta in the segment. The rest *i.e.*, 3rd and 4th lateral arteries arise directly from the heart in respective segments. This arrangement of the lateral arteries seems to represent a more primitive condition because the heart is basically a part of the median aorta.

Though the presence of subneural artery is a common feature of aquatic Isopod a distinct subneural artery is absent in *Nichollsia*. This indicates that though *Nichollsia* is a purely aquatic from it may be representing an intermediate stage between the aquatic and terrestrial forms, because in terrestrial forms the subneural artery is absent while the ventral branch of the lateral artery is reduced.

Regarding absence of first pair of lateral arteries in *Asellus*, Silen (*loc. cit.*) writes "the correspondence in extension of the anterior most lateral arteries of *Asellus*, with the 2nd lateral arteries in the remaining Isopods leads to the likely interpretation that the 1st lateral arteries are simply, absent in that form". Similar conclusions could be made in case of *Nichollsia*. But a fact omitted or overlooked in many of the

Isopods already dealt with is the arterial supply to the maxilliped which is (as in *Ligia*, Hewitt, 1907) supplied by the terminal branch of the so called second lateral artery in *Nichollsia*. This confirms the view that maxillipedal segment is the part of the thorax fused with the head.

In *Nichollsia* the number of ostia is four while in Oniscoids it is only two. However, in *Ligia*, Silen (*loc. cit.*) has reported the 3rd ostium and John (1968) in *Sphaeroma terebrans* reported four. More number of ostia also signify primitiveness of *Nichollsia*.

The vessel system in *Nichollsia* is much more complete than that of the true terrestrial Isopods. In this connection, however, the reservation must be made that the pictures of the vessel system given by the older authors cannot be relied upon fully concerning the extension of the syatem. Silen (*loc. cit.*) writes "Nevertheless, concerning the system of arteries we get a type series from forms with a well developed system represented by the aquatic Isopods via such with a less complete system, represented by the amphibious *Ligia* and possibly other ligiids and trichoniscids, to such with an even less complete system represented by the fully terrestrial oniscoides". Regarding the position of *Nichollsia* to be placed in the above statement it is coming in the first system. The absence of subneural artery may be due to convergence in evolution.

It has been a matter of discussions as to whether only oxygenated blood enters the pericard in the Isopods (*cf. eg.* delage. 1881 ; Brucke, 1923 ; Zimmer, 1927). According to the earlier opinion all the blood of the abdomen was thought to pass through the pleopods and the only openings into the pericard to be those of the lateral lacunes (or Branchio-pericardial veins). Thus it was mostly thought that the pericard contained oxygenated blood, a little mixed with venous blood from the abdominal pleurae. However, as we have seen here, the blood of the central lacune does not, largely pass through the pleopods but enters the pericard at its posterior end in different ways in *Nichollsia*. Similar interpretation was given by Silen (*loc. cit.*) for *Liga* and remaining oniscoids. As already discussed above, it is possible that also the blood passing by the pleopods, without entering them, receives oxygen to some degree round the rectum and anus. However, it seems improbable that this oxygenation could reach such proportions for the large quantities of blood in question to be characterised as arterial.

From the above account it will be seen that the vascular system in *Nichollsia kashiensis* resembles to those of other aquatic Isopods. Silen (1954) considered the vascular system in aquatic Isopods as a primitive one and *Nichollsia* represents more or less a similar condition. Delage (1881) gave a basic plan for the circulatory system of a hypothetical primitive aquatic Isopod to which *Nichollsia* is quite similar.

Respiratory system :

The volume of water which flows over a a crustacean's respiratory surfaces depends on several controlling factors. Basic are the frequency and amplitude of the appendage beats which produce the current concerned. The frequency of appendage movements propelling the respiratory current has repeatedly been studied by Fox (1952) and Walshe-Maetz (1956), in *Asellus aquaticus*, *Ligia*, *Idotea* etc. In *Asellus aquaticus*, it has been found to vary between 52 to 148 per minute at 12°C. However, in *Nichollsia* it is 50-120 in normal individuals under normal conditions. As already mentioned, the pleopod beat is regular but may not be continuous and is interrupted at frequent intervals.

Size of pleopod, temperature, salinity, oxygen concentration show significant influences on the rate of pleopod beat. Thus Ellenby (1951) noted decrease of pleopod beat in relation to increase in size of the animal. Nicholls (1938a) noted that in *Ligia oceanica*, the change in rate of beating is proportional to the change in temperature between 5°-35°C and also noted that reduction in salinity was accompanied by an increase in the rate of pleopod beat.

Fox (*loc. cit.*) and Walsche-Maetz (*loc. cit.*) found that when O_2 concentration in the water is decreased, the frequency of pleopod beat increases. It is possible that at higher temperatures the metabolic activity increases and the need for O_2 is more in the animal. To make up the supply of oxygen, frequency of pleoped beat increased.

In *Nichollsia kashiensis* the pleopod beat is inversely proportional to the size of the animal while it is directly proportional to the temperature.

The details of the lacunar system of the pleopod endopodite in the oniscoids and other Isopods have been treated by Bernecker (1909) ; Modlinger (1931) ; Unwin (1932) ; Silen (1954) and Ellis & Williams (1970).

Accorring to Silen (*loc. cit.*), Unwin (1932) described lacunes in

pleopods as running immediately below the ventral cuticle, thus without hypodermis separating them from the latter. However, it is not the case either in Oniscoids (Silen, *loc. cit.*) or *Nichollsia kashiensis* as already described above.

Subterranean and deep sea crustacea and also the caverni-colous forms are similar in their ecological stability in the scarcity of food, and in the absence of sunlight, and to cope with this situation they have a lower metabolic rate than their epigean relatives. Derouet (1953) found that the effects of temperature increase are greater in subterranean than in surface species of Amphipoda and Isopoda.

Burbanck, Edwards and Burbanck (1948) compared the survival rates of cavernicolus *Cambarus setosus* Foxon with an epigeal species of the same genus *C. rusticus* Giard. Derouet (1949) has likewise compared the respiratory exchanges of two aquatic amphipods, the epigeal *Gammarus pulex* L. and the cavernicolous *Niphargus virei* Chevraux.

These differences in the respiratory metabolism of cavernicolous and epigeal aquatics are emphasized by their opposite reactions to variations of salinity. Further studies by Derouet (1952) show that in cavernicolous amphipods (*Caecosphaeroma virei* Dollfus and *Niphargus orcinus* Joseph) the respiratory metabolism is increased in response to greater salinity, while in an epigeal species (*Gammarus pulex*) kept under the same conditions the respiratory motabolism is reduced.

Fage (1955) has summerised the characteristics of subterranean habit and its influence on the fauna.

Nervous system :

Available literature on Isopod brain is scanty. The account of some of the earlier workers like Sars (1867) ; Packard (1884, 1885) and Dollfus and Vire (1905) and others are drawn on a small scale and are in some cases rather indifferently drawn to represent a dorsal view. The only reference dealing with description of nervous system in Phreatoicoids is that of Barnard (1927) in *Mesamphisopus* (*Phreatoicus*) *capensis*. But it is also a mere outline. Some more recent literatures on the subject is that of Hanstrom, 1928 and 1947 ; Graber, (1933) ; Walker, (1935) ; Bullock & Horridge, (1965) ; John (1968) ; Satija and Ahuja (1977) and Sharma (1980). Satiza and Ahuja, concluded their findings on *Nerocila* with the following remarks "the observation on *Nerocila* and the above considerations suggests that we are yet not in a position to say as to what are the salient characters of the brain of an Isopod.

In describing the nervous system of crustacea the terms 'commissures and connectives' have been used to signify different parts. For example in *Cancer* (Pearson, 1908) and in *Panulirus* (George *et al.*, 1955) the term connectives is used for the transverse connections and commissures for the longitudinal connections, while earlier workers like Bellonci (1881), Packard (1884) and later Patwardhan (1937) have described "commissure" for both longitudinal and transverse connections. Calman (1909), Pike (1947) and John (1968) have described the longitudinal strands as "connectives" and transverse fibres as 'commissures'. The same terminology has been followed here also.

In general all the arthropods have a central nervous system with a supraoesophageal ganglionic mass or brain united by circumoesophageal connectives with a double ventral chain of segmentally arranged ganglia. In Isopods however, various degrees of concentration and coalescence of the ganglia distinguish the different genera. In *Sphaeroma* (John *loc. cit.*) even the paired connectives are partially fused or coalesced along the median line.

In *Nichollsia*, the first thoracic segment is fused with the head and it is represented by maxilliped and maxillipedal ganglion in the posterior cephalic region. The second to eighth (treated here as first to seventh thoracic) ganglia are located in the corresponding segments. The connectives are longest between the fourth and fifth thoracic ganglia as the fourth is situated in the anterior half of the segment. Connectives in the abdominal segments are shorter with the corresponding length of the segment. Finally, the sixth abdominal ganglion is placed in the fifth segment along with the fifth ganglion and thus it is the shortest connective in the abdominal region. The shortest connective in the ventral chain is between the maxillipedal and first thoracic segments. There is no further forward shifting of ganglia as has been reported by John (1968) in *Sphaeroma*. In *Sphaeroma* the connectives between all the succeeding ganglia are fused together along the median line but in *Nichollsia* the connectives of the two sides are separate and distinct throughout the entire length of the ventral cord like *Ligia*. This fussion of connectives is a more evolved stage. Thus *Nichollsia* and *Ligia* are primitive in this respect. Fusion of ganglia in *Ligia* may not be of much significance from evolutionary point of view but it may be an adaptation because fusion of segments do not occur here.

Hewitt (1907), in *Ligia*, reported the presence of a median nerve along the thoracic nerve cord running between the two parallel nerve

cords. This he suspected to be sympathetic nerve. In *Nichollsia* this type of nerve is absent.

In more evolved Isopods, the first thoracic ganglion which supplied nerves to the maxilliped is fused with the suboesophageal ganglia, similarly a distinct ganglion of the telson is not distinguishable. But in *Nichollsia* the presence of independene ganglion in the maxilla and maxilliped segments (the so called first thoracic ganglion of many authors) and the presence of a free sixth abdominal ganglion of the telson seem to indicate a much more primimitive condition than those of *Sphaeroma* and *Ligia*.

The nervous system of *Nichollsia* is in close similarity with the nervous system of *Mesamphisopus* (=*Phreatoicus*) *capensis* described by Barnard (1927). Both have 7 thoracic and abdominal ganglia with the 6th abdominal lodged in fifth segment behind the fifth abdominal ganglion. Both are lacking the optic ganglia.

Packard (1884, 1885 & 1886) and Dollfus and Vire (1905) have given some effective changes in the nervous system of subterranean Isopods of the genera *Caecidotea*, *Asellus* and *Vireia* respectively. Both authors have found that optic ganglia is absent in totally blind forms, and *Nichollsia* is not exception to it. Vandel (1965) has described different stages in the reduction of eyes and gradual loss of optic ganglia and its nerve components in regressive evolution in subterranean crustaceans. Thus subterranean life has deeply effected the morphology of *Nichollsia kashiensis*.

Reproductive system :

Usually the number of follicles in Isopods does not exceed 3 in each testis, in *Limnoria* there is only one lobe, but in most of the Phreatoicids the number is much higher. Chilton (1894) reported 5-6 oval lobes in *P. assimilis* while in *P. capensis* the number of lobes is still greater varying from 8-10 on each side and extend forwards as for as about the junction between the 2nd and 3rd segments.

The shape of the testicular lobes are very variable in different genera of Isopoda, but in Phreatoicids, studied so far, there seems to be a generalised oval or club-shaped outline of the lobes. In *Nichollsia*, like other Phreatoicids a more or less similar trend in shape and higher number of lobes (2-9) is maintained.

Early workers studying the reproductive tracts of male Isopods were

interested primarily in the course of spermatogenesis and remarked only incidentally on the structure of the vasdeferens (Gilson, 1884, 1886 ; Nicholls, 1902 ; Hewitt, 1907). A feature common to the vasdeferens of Isopods examined by these workers is the presence of giant cells to which was ascribed a role in secretion of the seminal fluid. Nicholls (1902) suggested that the giant cells in *Oniscus asellus* are derived by hypertrophy of the smaller cells. Radu (1930) described a gradual hypertrophy of the smaller epithelial cells in the vasdeferens of *Armadillidium vulgare*. Mathur (1961) re-examined the vasal epithelium of *Oniscus asellus* with particular reference to the cytochemical characteristics of component cells. He concluded that the giant cells are secretory and that their secretions are acidic mucopolysaccharides which cement sperms stored in the lumen of the vasdeferens into spermatophors. Newstead and Dornfield (1965) have investigated the epithelial structures in the anterior segment of the vasdeferens of *Porcellio scaber* (Latreille) and say that the giant cells are probably secretory in nature. However, the whole mounts of testis of *Nichollsia* reveals that the gaint cells are not permanent structures at least in the testicular lobes.

The occurrence and physiological function of the androgenic gland was discovered in the amphipod' *Orchestia gammarellus* by Charniaux-Cotton in 1954. This gland has now been observed in all orders of the Malacostraca including Isopoda. Balesdent-Marquet (1958) for the first time discovered this gland in *Asellus aquaticus* filling the lacuna, otherwise for long time it was considered absent in the group Isopoda. The gland varies in its morphology and location in the body in different groups. They are paired, mesodermal, solid cellular bodies. In amphipods it is located near the testis but not as a part of it, while in Isopods the gland is partly included in the testis.

While dealing with the effect of subterranean life on the androgenic gland, Husson and Graf (1961a, b.) reported that the androgenic gland of *Niphargus* is much smaller than that of *Gammarus* and shows reduced activity. However, such conclusions can not be generalized (Lane 1977). According to Vandel (1965), Legrand and Juchault (1961) have shown the presence of two androgenic glands per gonad (one in the fifth segment and the other in the sixth or seventh), in the marine *Sphaeroma*, *Dynamene* and *Cymodocea*, while the cavernicolus species *Caecosphaeroma burgundum* possesses only one gland in the fifth segment. In *Nichollsia kashiensis* the androgenic gland

is filamentous and is situated in the fifth thoracic segment behind the testicular lobes. How far, such variations in size and number of androgenic glands are related to the subterranean life needs further investigation.

Although the whole credit for establishing the existence of androgenic gland in Crustacea has been given to Charniaux-Cotton (1954), it is necessary to point out here that Leichmann (1891) in his paper "Beitrage zur Naturgeschichte der Isopoden. *Bibliotheca zoologica* Heft. 10 : 1-44" has already figured the male reprodnctive system of *Sphaeroma rugicauda* (Plate II, fig. 6. f.) and has shown a filamentous structure with its cytological details which is very much similar in its morphology and position on the vasdeferens (=sperm duct) to the present androgenic glands of sphaeroma or Isopoda in general. If this is true the credit of discovery signifying a specific structure should go to him.

The yolk globule of the matured ova seem to be of mitochondrial origin (King, 1926 ; Bhatia and Nath, 1931). In *Oniscus asellus*, King (1926) did not favour the presence of a true follicle, while in *Nicholl-sia*, follicle can easily be seen investing the oocyte even at younger stages. Similar follicles have been observed in *Asellus aquaticus* (Leichmann, 1891) and *Limnoria tripunctata* (Menzies, 1954).

In typical case the crustacean ovary is a hollow sac from the inner surface of which ova are budded off into the cavity. As these ova grow and reach maturity they fill up the cavity of the sac (Calman, 1909). In these simple types the entire inner surface of the overian wall acts as a formative layer, but very often the formative layer is restricted to a definite region and may be called as a second stage (*Oniscus asellus*, King, 1926) in evolution, or becomes modified as in-pushings into the cavity of the ovary, third stage in evolution is found in *Sphaeroma terebrans* (John (*loc. cit.*) where only the germinal ridge is produced into folds at places longitudinally. The fourth stage is found in Palaemonidae (Parameswaran, 1954) where continuous longitudinal infolding is present. However, *Nichollsia* falls under the second category of this evolutionary series.

In female of *N. kashiensis* a seminal receptacle is absent, though it is present in many other isopods such as *Trichoniscus*, *Asellus*, *Jaera*, *Porcellio* and *Armadillium* (Schoebl, 1880 ; Vandel, 1925, quoted by Menzies loc. cit.). It is absent in *Limnoria tripunctata* (Menzies loc. cit.) and *Sphaeroma terebrans* (John, loc. cit.). Menzies has observed

that the absence of seminal receptacle in certain Isopods can be correlated with the prolonged association of the members of the opposite sex during pairing. For example a male and female *Limnoria* occupy a single burrow throught their life and in the female of this species a seminal receptacle is absent, similarly in the Bopyrid, *Epipinaeon* and in the commensal parasitic *Cymothoa*, *Anilocra physodes* a seminal receptacle is absent (Hiraiwa, 1936 ; Montalenti, 1941 ; quoted by Menzies, *loc. cit.*). From these observations, it seems likely that the absence of a seminal receptacle in *Nichollsia kashiensis* may be correlated with its long association and copulation which is immediately followed by formation of marsupium and egg deposition. In the terrestrial Isopods which have been extensively studied in this regard and which bear a seminal receptacle, a single copulation has been found sufficient for the production of two broods (Howard, 1939, 1940 and Lueken, 1963). Although storage of functional sperms in their seminal receptacles for longer time is an adaptation in the terrestrial Isopods, the great majority of broods are multipaternal in *Porcellio scaber* (Sassaman, Clay 1978).

The number of membranes surrounding the egg and their origin in Crustacea is a disputed point. In *Jaera*, *Asellus*, *Porcellio* and *Armadillidium*, McMurrich (1895) found that the egg when extruded is enveloped by a membrane which is secreted by the follicular cells surrounding the egg. A chorion has been described for the egg in *Irona* (Nair, 1956). Korschelt and Heider (1899) however, observed that in some cases the eggs pass into the brood pouch without an outer membrane. This condition is noticed in *Hemimysis* and *Nebalia* (Manton, 1928b, 1934). In *Moina cetochilus* and *Gammarus* the egg does not possess a membrane at the time of extrusion (Grobbon, Della Valla, quoted by McMurrich, 1895). According to Cannon (1921) in *Simocephalus* the egg membrane is formed only after fertilisation. In *Porcellio* and *Armadillidium* a chorion is said to be developed only after the sperms enter the oviduct (Goodrich, 1939). In *Sphaeroma terebrans*, John (1968) noticed a non staining membrane in the sections of oocytes. In *Nichollsia kashiensis* a similar membrane is found around the oocytes and seems to show close similarity with *Sphaeroma terebrans*.

Although a number of Isopods show a positive correlation between size of female and the number of youngs in the brood pouch (*Armadillidium vulgare*, as described by Hatchett, 1947 and Paris and Pitelka, 1962 ; *Cylisticus convexus*, Hatchett, 1947 ; *Asellus intermedius*, Ellis,

1961 ; *Asellus aquaticus*, and *A. meridianus*, Steel, 1961 ; *Ligidium japonica*, Saito, 1965 ; *Dynamene bidentata*, Holdich, 1968 ; *Asellus tomalensis*, Ellis, 1971 ; *Jaera albifrons* group, Jones and Naylor, 1971 and *Porcellio laevis*, Nair 1976), the relationship between these parameters is not clear in *Limnoria tripunctata*, Menzies, 1954 and *Ligia pallasii*, Carefoot (1973b). In the species *Limnoria lignorum* (Rathke), Somme (1940) reported that broods produced during the summer were on an average larger by 10-12 eggs than Autumn broods. Coker (1923) conversely found spring broods larger than summer and fall broods by several eggs. In *N. kashiensis* there are winter and summer broods but seasonal relation with the number of youngs and size of the animals could not be established, clearly.

Egg number among the Isopods shows no pronounced phylogenetic correlation or pronounced correlation with size of the species. Thus within the suborder Oniscoidea, egg number per brood varies between 3 and 200, and in the suborder Flabellifera between 32 and 350 (Menzies, 1954). In the suborder Phreatoicoidea the largest species *Phreatoicopsis terricola* (39 mm length), produced 30 eggs and a much smaller species *Phreatomerus latipes* produced 109 eggs per brood (Nicholls, 1943), whereas *Colubotelson thomsoni* (length 12 mm) products maximum 17 eggs per brood (Engemann, 1964). Engeman (*loc. cit.*) has correlated more eggs with higher mortality rate. In *N. kashiensis*, 6-24 eggs have been recorded.

Vandel (1965) has summerised the effect of cavernicolus and subterranean habit on the number and size of the eggs in different groups of animals. One of the most remarkable facts recorded by biospeologists is the reduction in the number of eggs deposited by cavernicoles. This number is generally much less than that of epigeous species belonging to the same group. This decrease in the number of eggs is related to an increase in their size, which is more pronounced in those cavernicoles which are more specialised. Such reduction in egg number in Isopods is reported in Anthuridae (Karaman, 1940) ; Parasellidae (Chappuis, 1951 & 1956) Karaman, 1934 & 1940 ; Chappuis and Delamare-Deboutteville, 1954 & Chappuis and Paulian, 1956 ; Gnanamuthu, 1954, Siewing, 1959) ; Asellids (Husson and Daum, 1955 ; Kaullbersz, 1913).

The marsupium of *Asellus cavaticus* contains 10-20 embryos (an average of 15), while that of *Asellus aquaticus* an epigeous form, con-

tains 50-60 (Kaullbersz, 1913) and *A. communis* contains 95-168 (Engemann, 1964).

The fact that different species have differing brood size is of considerable interest indicating that perhaps the ecological factors associated with the reproductive capabilities of various species are also different. In *Nichollsia kashiensis* it is the subterranean mode of life which has influenced the reproductive capability of the animal.

Summary

1. The distribution, general ecology, vertical migration predators and parasites have been described.
2. In natural envirnment the parasites and predators are very very few. Only one instance of gregarine infection has been recorded.
3. Detailed external morphology of adult and also a newly hatched young has been given.
4. A newly hatched young differs, apart from other morphological variations, from the adult in the absence of 7th peraeopod.
5. Sexual dimorphism is apparent only after the male attains the length of 0.6-0.7 mm.
6. Appendix masculina in male is a very complicated structure.
7. The structure of body wall, muscular, digestive, excretory, circulatory, respiratory, nervous and reproductive systems have been worked out and compared with other Isopods and Amphipods.
8. Moulting occurs in two stages. Posterior half is cast off first, after 24 hours of which, in normal conditions, the anterior half is shed. Moulting is affected by temperature and other factors.
9. Hypodermis, hepatopancreas, and athrocytes work as storage and release organs for reserve materials during moulting.
10. Compared to the musculature of *Ligia*, in *Nichollsia* the musculature is simple.
11. In its natural habitat *Nichollsia* feeds on detritus but in aquarium they feed on plants and animal matters. Coprophagus and cannibalism is very common. Composition of detritus in its natural habitat is given.

12. Different methods of feeding mechanism of *Nichollsia* have been detailed. Food from the molar processes is directly transferred to the oesophagus by pushing.
13. The oesophagus is short with tetraradiate lumen and without tegumental glands.
14. Chitinous ridges forming lamellae and ampullae are very similar to *Asellus aquaticus*. The whole cuticle is internally provided with fine microtrichs, hooks and bristles.
15. Trituration occurs in the stomach.
16. Liquid food is filtered through filter I and II of stomach and passes to hepatopancreas. Filter II has inner and outer filter channels, the opening of inner filter chamber is guarded by a ventral valve.
17. The midgut in *Nichollsia* is reduced and restricted to the junction of foregut and hindgut.
18. The hindgut is straight and is underlined by a chitinousintima throughout its length. This chitinousintima is absorptive. A typhlosole is absent.
19. The epithelium of intestine or hindgut is syncitial.
20. The rectal pad, also called by some workers as rectal gland, is absorptive, not secretory.
21. The anus is provided with valves and radial dilator muscles.
22. There are two pairs of hepatopancreas ; the two types of cells generally seen in the epithelium have been observed with the third type as replacement cells. Histological details show that secretion is primarily holocrine and probably the same cell works as absorptive in the beginning.
23. Anal uptake of water has been observed.
24. pH of the digestive system always remains acidic.
25. The main excretory organ is maxillary gland, it has large endsac, long coiled duct and a large bladder. Flow from endsac to atrium is guarded by a sphinctor valve. The secretion in endsac is holocrine. There is a small valve at the junction of the duct of bladder.
26. Other segmental glands are coxal glands and branchio-pericardial organs which are more active during moulting.

27. The structure of the heart, pericardium and the distribution of the arteries and blood sinuses and course of blood have been studied.
28. Distinct subneural artery is absent.
29. Blood from the caudal region is collected by the abdominal vein without going to pleopods and remain (possibly) unoxygenated.
30. Bronchio-pericardial sinus is wide and present in all the five abdominal segments.
31. Effect of temperature on heart beat, which is directly proportional to temperature, was studied.
32. All the five pairs of pleopods are respiratory in function. Morphology and histology of the pleopods has been studied.
33. Effect of temperature and size on the pleopod beat has been studied.
34. Oxygen consumption of *Nichollsia kashiensis* is very low *i. e.* it is 0.04 to 0.046 c. c. of oxygen per gm. per hour.
35. Behind suboesophageal ganglia, there are 3 in head, 7 in thorax and 6 ganglia in abdomen.
36. The optic ganglia is absent although proto-cerebral lobe is quite large.
37. There are large number of motor neurocytes aggregated in the sixth abdominal ganglion.
38. Perineurial cells, Schwan cells and glia cells have been noticed in the ventral nerve chain. Few motor cells (neurosecretory cells ?) between maxillary and maxillipedal ganglia are present.
39. Secondary sexual characters, and detailed structure of the male and female reproductive organs and reproduction biology have been studied.
40. A germinal ridge, nurse cells and two types of cells in vasdeferens have been noticed.
41. A filamentous androgenic gland is present.
42. A clear vesicula seminalis is absent.
43. Germogen in ovary and follicle around oocytes have been noticed. Different stages of oocyte formation have been shown.

44. A distinct sperm storage organ is absent.
45. Pairing, mating and act of copulation have been described. It is similar to *Asellus aquaticus*, and *Gammarus pulex*.
46. Female genital opening is formed only during the breeding season. A common vulva is formed.
47. Spermatophore introduction to female gonopores is directly by penis. Penial stylet did not seem to take part in transfer of sperms.
48. Release of youngs takes about 35-40 days after the eggs are laid out into the brood pouch.
49. There are two breeding periods in a year.
50. The 2n, number of chromosomes in *Nichollsia kashiensis* is 50.
51. Effect of subterranean life on different systems has been discussed.
52. Relationship of the family Nichollsidae with other Phreatoicids has been discussed.

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List of Abbreviations

a. d. l.—anterior dorsal lamella
a. ext.—anterior extensor
a. gl.—androgenic gland
a. l. CI-II—anterior lateral constrictor—I-II
a. lev. m.—anterior levator muscle
a. v. c.—anterior ventral constrictor
a. v. l.—anterior ventral lamella
ab. art.—abdominal artery
ab. c.—absorption cell
ab. gl-6—abdominal ganglia 1-6
ab. v.—abdominal vein
ace.—Acetabulum
acr.—acrosome
aes.—aesthetasc
af. v.—afferent vessel
al. c.—alae cordis
an.—anus
an. pl.—anal plate
an. sl.—anal slit
ant_1.—antennule
ant_2—antenna
ant_1art—antennal artery
as. c.—association cell
athr.—athrocyte
atr.—atrium.

b. c.—blood cell
b. m.—basal membrane
b. p.—basal plate
b. p. v.—branchiopericardial vessel
b. v.—blood vessel
ba.—basis
bl.—bladder
br.—brain
br. b.—brush border
br. se.—brush setae

c. f.—corfrontale
c. gr.—cervical groove.
c. la.—central lacuna
c. m.—circular muscle
c. o. c.—circum oesophageal connective.
c. t.—connective tissue
c. v.—central valve.
ca.—carpus
cep. art.—cephalic artery
co.—coxa
co. h.—coupling hook
cort.—cortex
cr. art.—crural artery
ct. f.—connective fibre.
cu.—cuticle
cyt. f.—cytoplasmic fibre

d—duct
d. a.—dorsal ampulla
d. an. d.—dorsal anal dilator
d. c.—dorsal constrictor
d. d. I-III dorsal dilator I-III
d. fl.—dorsal flexor
d. la.—dorsal lacuna

d. l. m.—dorso longitudinal muscle
d. m.—dorsal muscle
d. o. d. I-II—dorsal oesophageal dilators I-II
d. o. m.—dorsal oblique muscle
da.—dactylus
de. c.—deutocerebrum
dis. end.—distal endite of maxillula
dis. end_2—distal endite of maxilla
dis. S—distal segment
dpr. m—depressor muscle.

e. s.—end sac.
ef. v.—efferent vessel
end—endopodite
end. c.—endocuticle
ep. epipodite
ep. c.—epicuticle
ept—epithelium
ex. d.—excretory duct
exo. l—lateral lobe of exopod
ext. c—extrusion cell
ebt. m—extensor muscle

f. art—facial artery
f. m.—food mass
f. pr.—fulcral process
fil. se.—filtratory setae
fl. m.—flexor muscle
fol.—follicle
fr. g.—frontal ganglia
fr. lam.—frontal lamina
fr. p.—frontal process

g.—germogen
g. c.—glia cell
g. l.—germinal layer
gen. gr.—genal groove
gi. c.—giant cell
gi. c—giant cell
gn—gnathopod
gr.—germinal cells

hep—hepatopancreas
ht—heart
hyp—hypodermis

i. b. la—intra branchial lacuna
i. f. c.—inner filter channel
i. l.—inner layer
i. pr—incisor process
i.r.—inner ramus
int.—intestine
int. ep.—intestinal epithelium.
is—ischium

l. a.—lateral ampulla
l. an. d—lateral anal dilators
l. art_{1-4}—lateral artery 1-4
l. d. I-II—lateral dilators I-II
l. ext.—lateral extensor
l. l.—lateral lamella
l. m.—longitudinal muscle
l. o. d.—lateral oesophageal dilator.
l. o. m_{1-3}—lateral oblique muscle 1-3

la. m.—lacinia mobilis
lbm.—labium

lbr—labrum
lev. m.—levator muscle
lip. gr.—lipoid granules
l. ma—lateral margin
lu-lumen

m—muscle
m. a.—median aorta
m. abd.—mandibular abductor
m. add.—mandibular adductor
m. c.—motor cell
m. f.—myofibril
m. pr.—molar process
m. w.—median wedge
ma. lin—marginal line
me—merus
med—medulla
med. b—median bar
mes—mesial border
mnd—mandible
mnd. p—mandibular palp
mtr.—microtrich
mx_1—maxillule
mx_2—maxilla
mx_1. g—maxillular ganglia
mx_2. g—maxillary ganglia
mx_2. gl.—maxillary gland
mx_1. scl—maxillular sclerite
mx_2 scl—maxillary sclerite
mxp—maxilliped
mxp.—maxilliped
mxp. g—maxillipedal ganglia
my. ep.—myoepithelium

n—nucleus
n. c.—nerve cord
n. s.—nurse cell
n. sh—neural sheath
nu—nucleolus

o—ostium
o. cav.—oral cavity
o. f.—ostial fibre
o. f. c.—outer filter channel
o. l.—outer layer
o. r.—outer ramus
o.v. m.—outer ventral muscle
oc—oocyte
occ-gr.—occipital groove
oe—oesophagus
og—oogonium
op. art.—opthalmic artery
ov—ovary
ovd—oviduct

p. c.—pericardial chamber
p. d. l—posterior dorsal lamella
p. fl.—posterior flexor
p. l. c. I-II—Posterior lateral constrictor I-II
p. n. c.—perinureal cell
p. s.—pericardial septum
p. v. c.—posterior ventral constrictor
p. v. l.—posterior ventral lamella
par.—paragnath
par. sk.—paragnath skeleton
pct.—pectinate setae

pe.—penis
pe. st.—penial stylet
per. I—peraeon I
ph. pr.—pharyngeal process
pl. se.—plumose setae
pl. pd.—pleopod
po. ma.—posterior margin
pr.—propodus
pr. c.—protocerebrum
pr. end_1—proximal endite of maxillule
pr. end_2—proximal endite of maxilla
pr. s.—proximal segment.
pri. c.—prismatic cell
pty.—maxillopterygoid process

r. c.—replacement cell
r. m.—reserve material
r. pd.—rectal pad
r. y.—reserve yolk
ri.—ridge

s. c.—sensory cell
s. m.—spiral muscle
s. o. g.—suboesophageal ganglia
s. se.—simple setae
s. v. m.—superficial ventral muscle
sch. c.—schwan cell
scr.—secretion
scr. c.—secretory cell
ser. se.—serated setae
sp.—spines
spc.—spermatocyte
spg.—spermatogonia
Sph.—Spermatophore
Sph. v.—Sphinctor valve
St.—Stomach
st. al.—sternal alae
st. s.—sternal sinus
ste—sternite of maxilla
sub. orb.—suborbital notch
Sym.—Sympodte

t.—testis
t. f.—transverse fibre
t. p.—telson
t. z.—terminal zone
th. g 1-7—thoracic ganglia 1-7
tr. t.—transverse tract

u. art.—uropodal artery
u. p.—uropod peduncle
u. so.—uropodal socket
u. v.—cellular valve

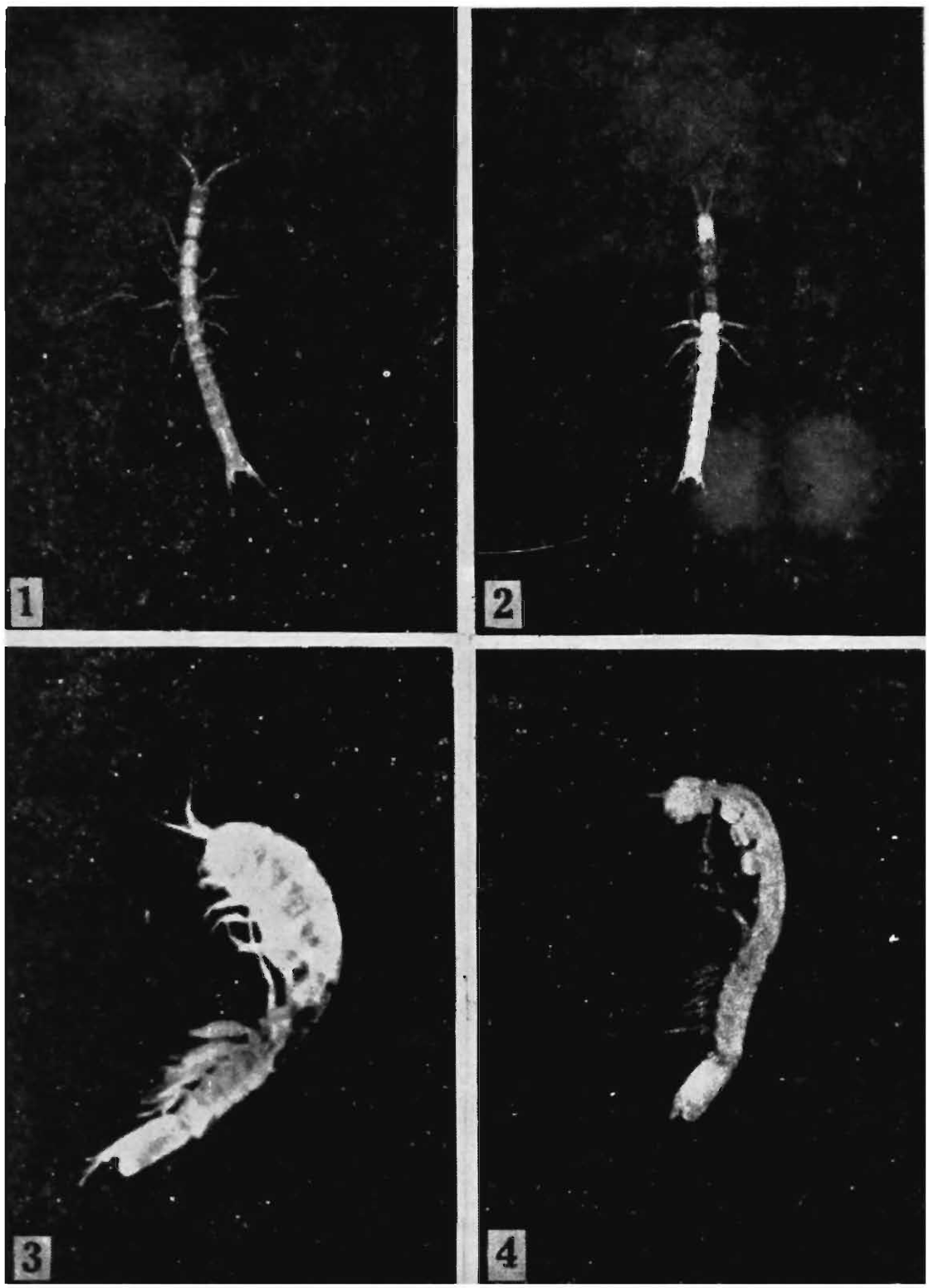
v.—valve
v. a.—ventral ampulla
v. an. d.—ventralanal dilator
v. d. I-II—ventral dilators I-II
v. dpr.—Ventral depressor
v. n. c.—Ventral nerve cord
v. o. d.—ventral oesophageal dilators I-II
v. s.—ventral septum
v. s. m.—ventral segmental muscle
v. v.—ventral valve
Vac—vacuole
Vas. d.—vasdeferens
vis. art.—visceral artery

W. C.—Wandering cell

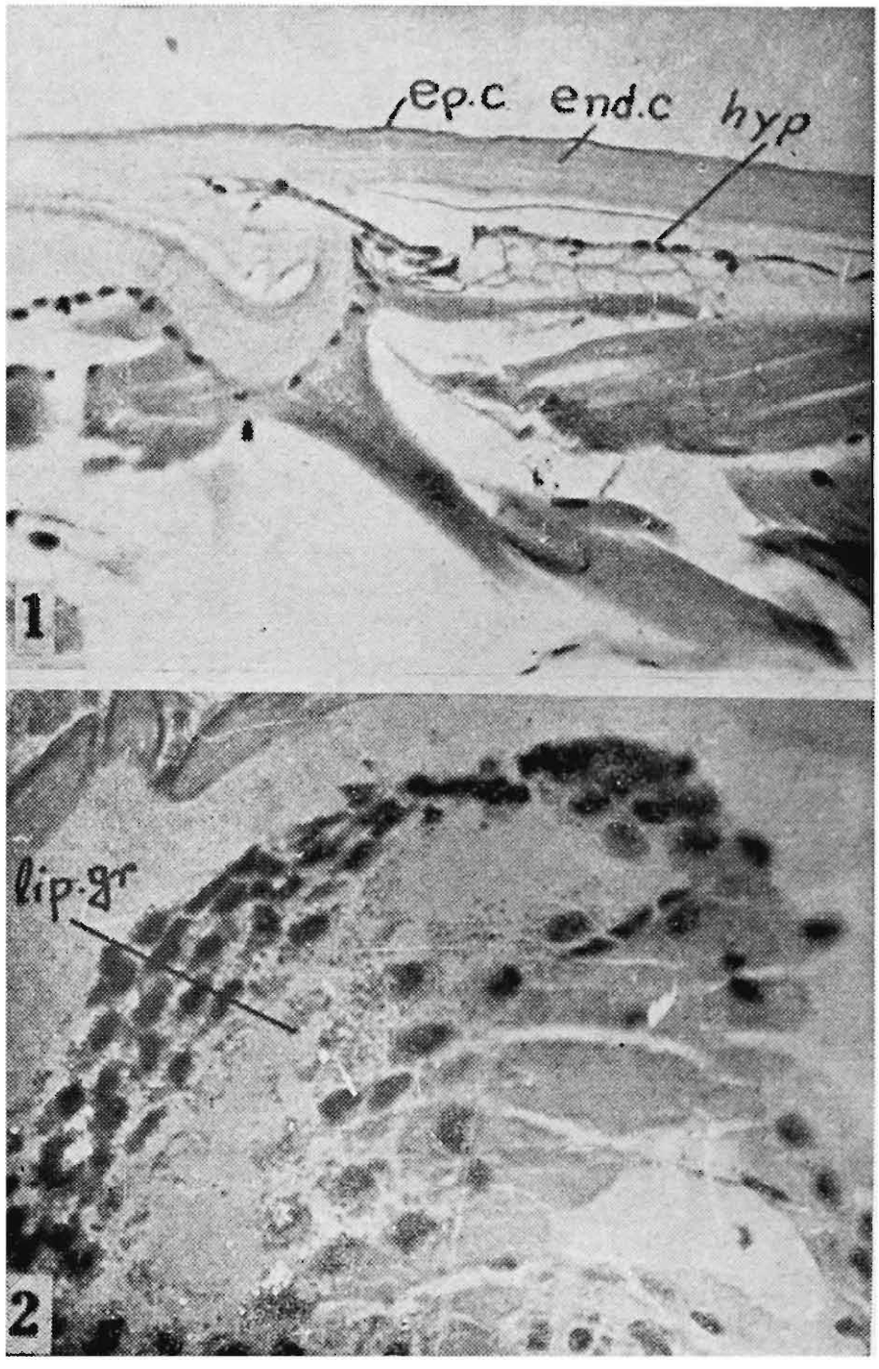
z. g.—Zymogen granules

1-7—Peraeon segments
I-V pleon segments

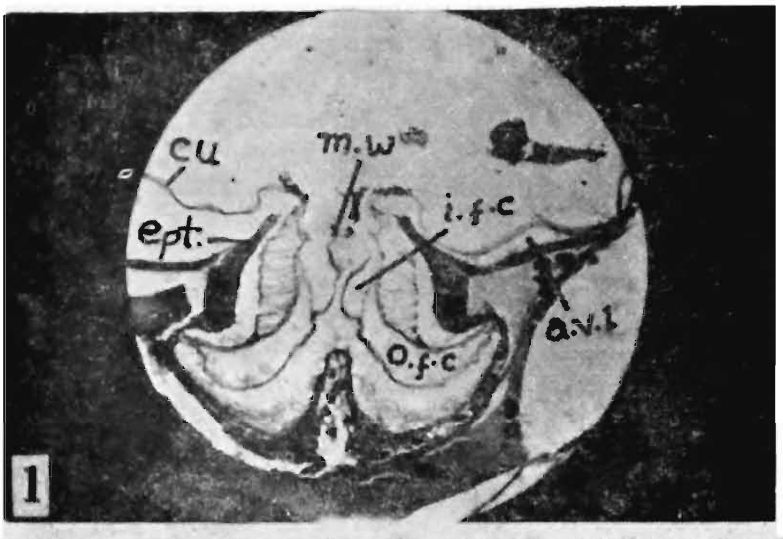
PLATES

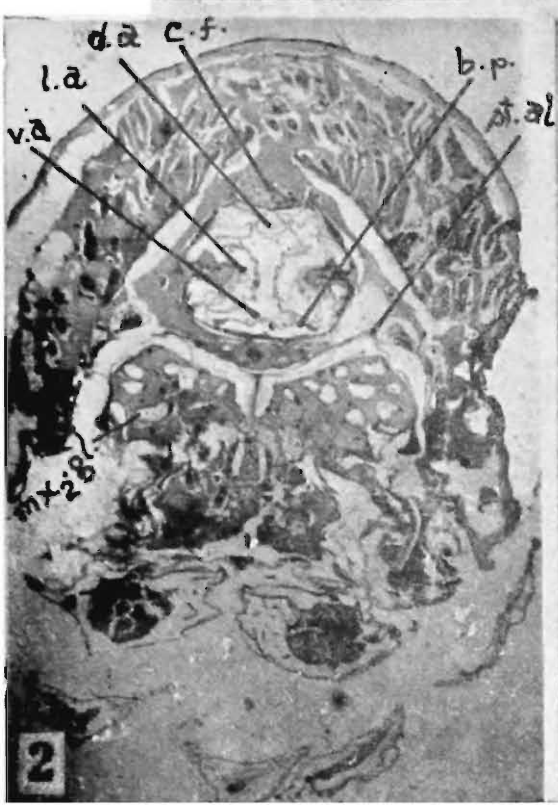


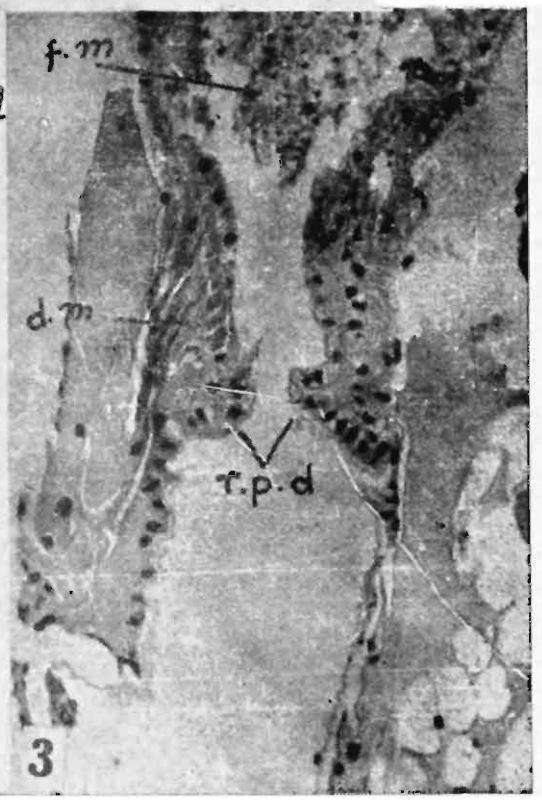
Figs. 1-4. 1. *Nichollsia kashiensis* dorsal view of male.
2. *Nichollsia kashiensis* dorsal view of female (Posterior half moulted).
3. *Nichollsia kashiensis* lateral view of female with brood chamber.
4. *Nichollsia kashiensis* lateral view with reduced brood plates.



Figs. 1&2. 1. L. S. dorsal body wall.
2. Tangential section of body wall showing hypodermis with lipoid granules.



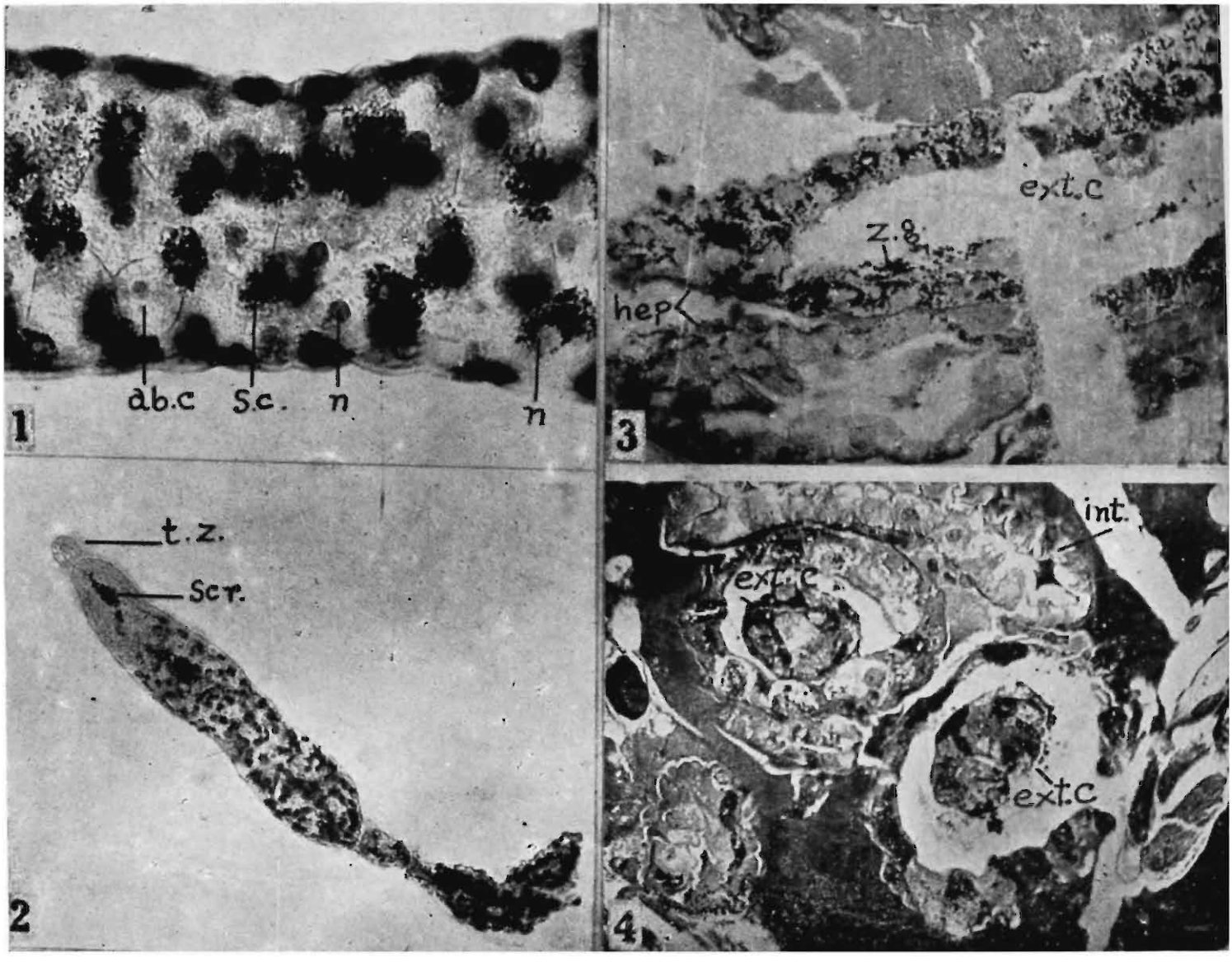




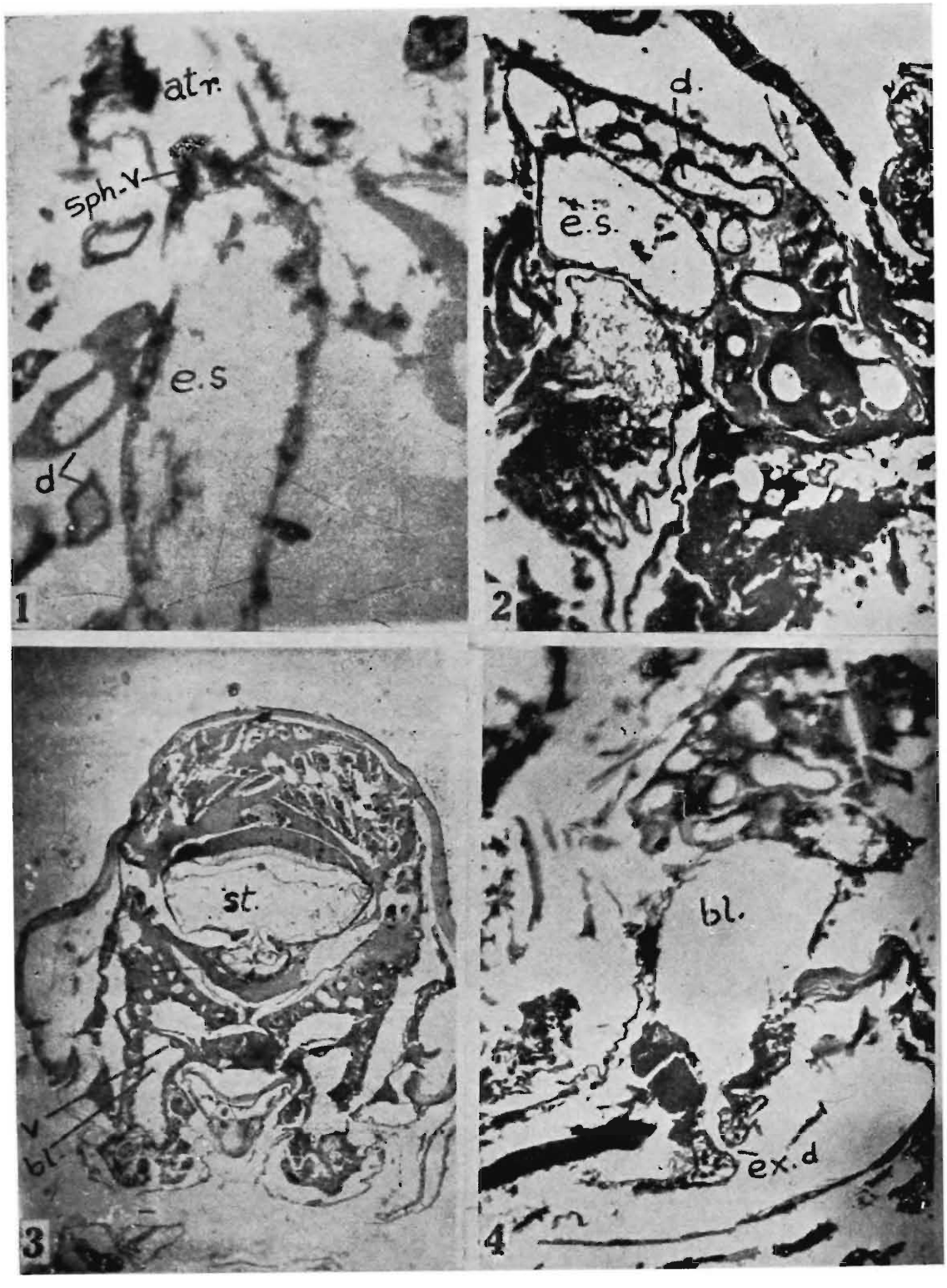
Figs. 1-3. 1. T. S. Stomach through filter II.

2. T. S. Head through stomach and Maxillary gland.

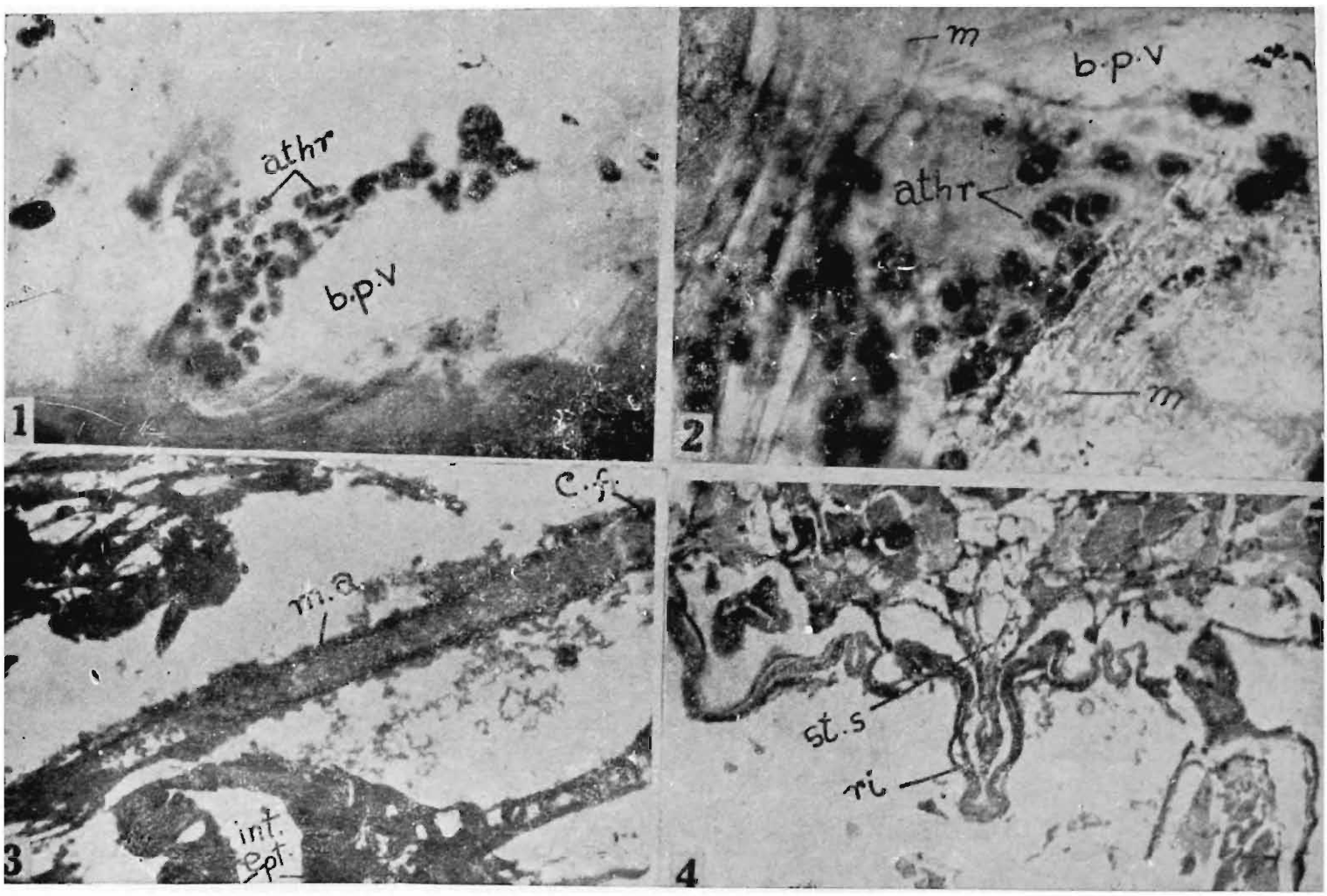
3. L. S. Telson showing rectal pad.



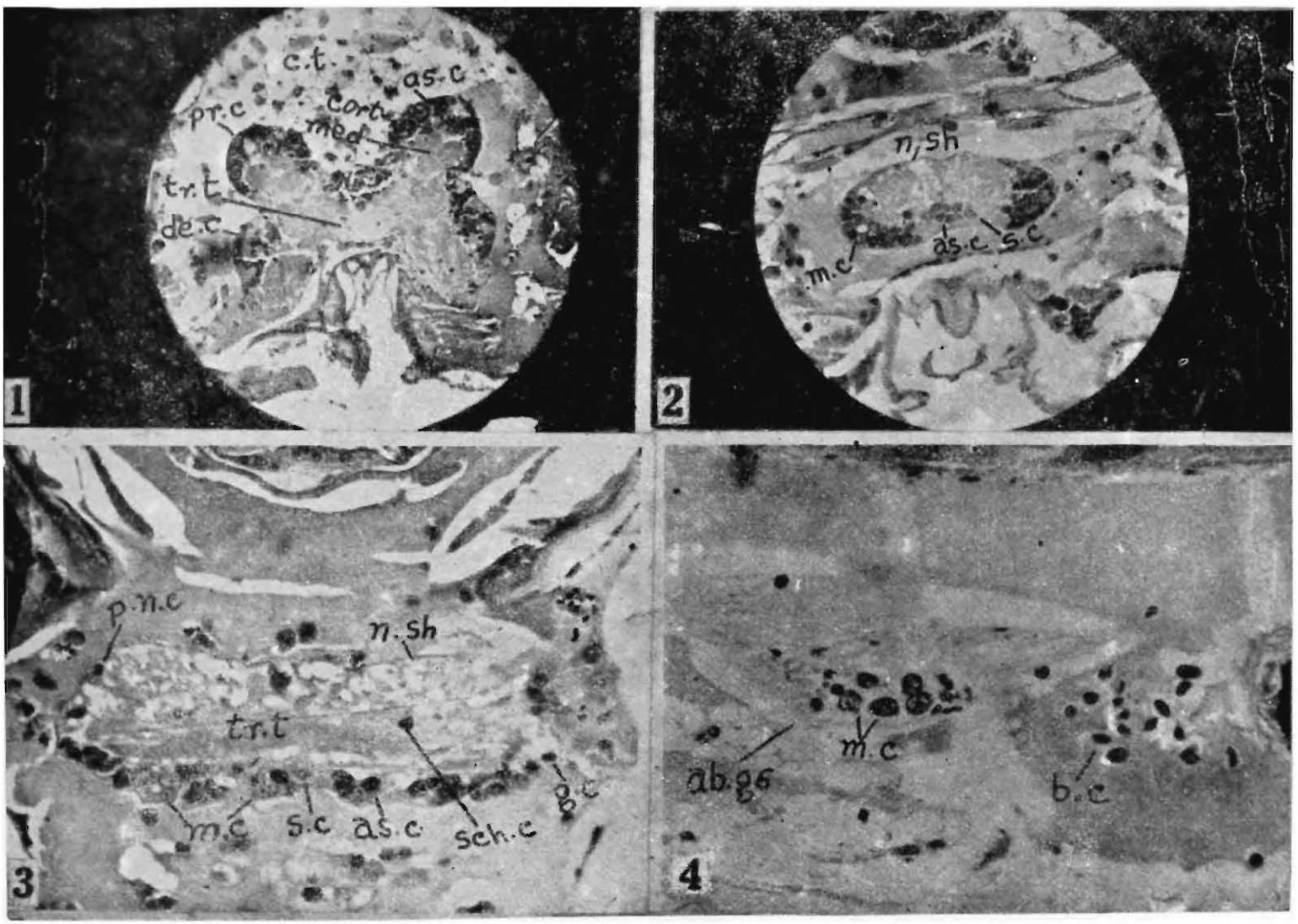
Figs. 1-4 1. Whole mount of middle zone of hepatopancreas.
2. Whole mount of distal zone of hepatopancreas.
3. L. S. Hepatopancreas.
4. T. S. Intestine and hepatopancreas.



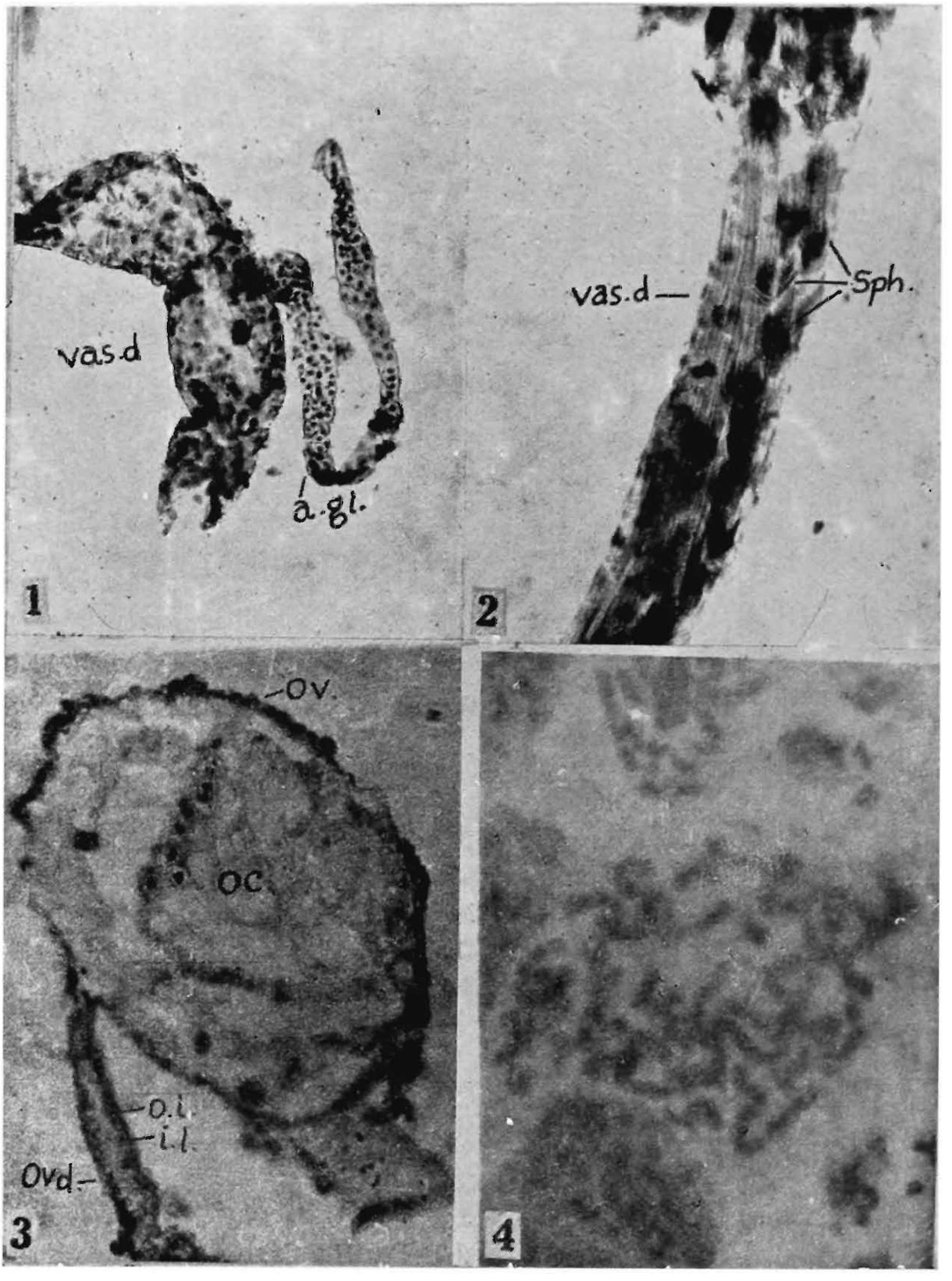
Figs. 1-4. 1. L. S. maxillary gland.
2. L. S. maxillary gland (duct with brush border).
3. T. S. head through bladder of maxillary gland.
4. T. S. head through exit duct of maxillary gland.



Figs. 1-4. 1. Branchio-pericardial veins with athrocytes.
2. Magnified view of Athrocytes.
3. Corfrontale in L. S. head.
4. T. S. through sternal sinus and ridges.



Figs. 1-4. 1. T. S. Head through brain.
2. T. S. Head through maxillipedal ganglia.
3. T. S. Head through suboesophageal ganglia.
4. L. S. 6th Abdominal ganglia.



Figs. 1-4. 1. Whole mount vasdeferens and androgenic gland.
2. Spermatophores in whole mounts of vasdeferens.
3. T. S. of ovary through oviduct.
4. Chromosome plate of an embryo.