# ON SOME INTERESTING LARVAL STAGES IN THE LIFE-HISTORY OF A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *ARYTHMORHYNCHUS*, FROM THE FROG, *RANA TIGRINA* (DAUD) FROM INDIA.

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(Plate VI—IX.)

CONTENTS.

## INTRODUCTION.

Of the few species of Acanthecephala whose life histories are known, the life history of the common acanthocephalan, *Macracanthorhynchus hirudinaceus* (Pall)=(*Echinorhynchus gigas*, Bloch, 1782), from the pig has been investigated by many authors: Schneider (1871), Hamann (1891), Kaiser (1893, 1913), Meyer (1931 et seq.) and Kates (1934).

Meyer who gave a detailed account of the life history of *Macracanthorhynchus hirudinaceus* called the newly hatched individual the 'larva' and all later stages 'juvenile' Van Cleave (1935, 1937) suggested a different terminology for various developmental stages found in the acanthocephalan life history. He termed the newly hatched individual 'acanthor' and the succeeding larval stages 'acanthella'; he restricted the term juvenile, only to the fully metamorphosed young forms of these worms. Van Cleave (1947) lays emphasis on the cytological and histological changes in the larval stages and considers them as important indications of metamorphosis taking place till the later larval stages, the acanthellae, are completely metamorphosed into juvenile forms. He (1947) says, "Meyer's observations on cytological changes accompanying transformation of the acanthella to the juvenile are among the best available evidences that a true metamorphosis is here involved"; yet Meyer failed to differentiate between larval stages and the fully metamorphosed juveniles.

Van Cleave, on the basis of his terminology, does not consider Kates 'acanthella Stage VI' in the life history of *Macracanthorhynchus hirudinaceus*, as a larva, but a fully metamorphosed juvenile, because it has acquired a functional proboscis and the structures and organs of the adult worm. Van Cleave (1947), however, agreed with Kates (1943) in calling the newly hatched individual in the gut of a suitable intermediate invertebrate host, the *first stage*, and after it penetrates the wall of the gut the *second stage*.

Other species of Acanthocephala, life histories of which are known, are *Monoliformis dubius*, Meyer, 1932 *and Macracanthorhynchus ingens* Von Linstow, 1879 investigated by Moore (1946). In both his papers, Moore introduced a new term 'Preacanthella' But according to Van Cleave those developmental stages which Moore called preacanthella should, in fact be called acanthella, and those which he labelled 'acanthella' and are fully metamorphosed individuals should be called juveniles. Sita (1949) in a note describes a juvenile form of *Monoliformis monoliformis*, and calls it 'Infective larvae (Acanthella)'. It should neither be called an 'Infective larva' nor an 'acanthella', as it possesses fully developed functional proboscis and has completely metamorphosed. It should be termed a 'juvenile' form.

The known life histories of acanthocephala are only of those species which belong to Meyer's order Archiacanthcephala. Very little is known of the design of development in the order Palaeacanthocephala. A few developmental stages of only two of Centrorhynchus species have been recorded. Gupta (1950), describes the gross anatomy of a few larval stages of *Centrorhynchus ptyasus*, (Gupta, 1950). She coins a new terminology for those stages. I described (Das, 1952) the acanthor and acanthella stages of *Centrorhynchus batrachus*, and gave an account of the histological changes which take place in the various stages leading to the development of adult organs. The present study was undertaken with a view to elucidating the life history of one species of the Palaeacanthocephala. It is an attempt to describe the various histological changes which take place during the development of the acanthor and acanthella of a new species of the genus Arythmorhynchus, (Palaeacanthocephala).

*Material and Methods.*—My attention was attracted by some orange-pink minute spots on the wall of the intestine of the common frog, ***Rana tigrina*** (Daud). On close examination they were found to be acanthocephalan cysts. I examined carefully the peritoneum of about 500 frogs, dissected in the laboratory during one session. In one specimen, I not only found the orange-pink cysts embedded in the wall of the intestine, but a cluster of them enveloped in the peritoneum of the duodenal loop of the frog, ***Rana tigrina.*** I freed them in the normal saline solution ; they numbered over forty and were of various sizes. I fixed half of the larvae thus obtained, and these included individuals of various sizes representing different stages of development. The remaining ones were kept in normal saline, in which they lived for fifteen days. I sectionised specimens showing all stages of development and also made whole mounts of the corresponding stages. This has enabled me not only to describe the external changes which take place during the larval history of this parasite, but also the histological changes in development leading to the differentiation of organs in the juvenile forms.

## DESCRIPTION OF LARVAL STAGES.

### (a) *Early acanthella stages.*

*Stage I.*—The smallest of the larvae in this collection is rounded in shape (pl. VI, fig. 1). It measures 0·34m × 0·37mm. It is devoid of rostellar hooks and presents a mass of embryonic cells with active nuclei ; which shows that the larva has passed the acanthor stage and is now the early acanthella. The central cells aggregate to form an elongated rod like structure (pl. VI, fig. 1, fa), which detrmines the future axis of the body of the larva.

*Stage-II.*—In the next stage, the larva has attained an oval shape characteristic of an acanthella and in its sagittal section (pl. VI, fig. 2). the nuclei are seen in a state of division. The nuclei seem to have aggregated in definite regions and four of such regions are shown in the figure (N1-4). These nuclear aggregates are the dynamic centres or primordia where the various parts of the body of the parasite will, in course of development differentiate. The aggregation of nuclei at the periphery (pl. VI, fig. 2, N1), suggests the material which goes into the formation of the body wall. The aggregate of nuclei at one of the polar ends (pl. fig. 2, N2) is the primordium of hooks and proboscis. The nuclear mass at the other end (pl. VI, fig. 2, N3) is the primordium of the genitalia. The central mass of the nuclei (pl. fig. 2, N4) is the Anlage of the central nerve ganglion and the proboscis sheath.

*Stage-III.*—The acanthella at this stage appears to be a thick leaf-like structure, thicker at its more convex side. In a toto mount (pl fig. 3) the entire larva has increased in dimensions probably owing to the flattening by the cover slip. In the clearing medium it measured 0·30 mm× 0·55 mm. The nuclei are smaller in size than those of the previous stages. They occur uniformly except in two regions of the larval body : at the broader end a slight aggregation of nuclei marks a wedge-shaped area (pl. VI, fig. 3, wa), which indicates the position where the retracted neck and part of the body bearing spines of the juvenile will be formed, (pl. IX, fig. 4, rn). Secondly, a major part of the central region of the body (pl. VI, fig. 3 crb), which takes a slightly darker stain indicates the position of the proboscis and its sheath.

(*b*) *Late acanthella stages.*—The late acanthella stages include those acanthellae, in which only the proboscis and its hooks have developed but other tissues and organs have still not commenced to differentiate.

*Stage-IV.*—The acanthella attains a bean shaped form and has the same dimensions as in the previous stage, except that it becomes markedly rounded in its girth. In a sagittal section (pl. VI, fig. 4), the formation and differentiation of the proboscis can be made out. The proboscis (pl. VI, fig. 4, pr) runs practically throughout the length of the body. The cuticle (pl. VI, fig. 4, c) of the probosics is formed but the wall of the proboscis is still in a nebulous stage of development. The muscles of the wall appear as wavy bands (pl. VI, fig. 4, wb). The hooks have appeared. Nuclear fragments (pl. VI, fig. 4, nf) are present at the base of the hooks indicating that it is a site of developmental activity which will result in the formation of the roots and, if rudimentary roots have been formed, they take a deeper stain, like the nuclear masses, indicating their newly formed nature. There is as yet no indication of the development of body spines. The rudimentary proboscis sheath (pl. VI, fig. 4, ps) lies curved up against the distal end of the proboscis. The wall of the proboscis sheath and the retractor muscles (pl. VI, fig. 4, wps) are represented by wavy muscle bands. Within the region of the proboscis sheath, a partially differentiated cellular mass (pl. VI, fig. 4, cng) represents the rudimentary central nerve ganglion. The nuclear masses (pl. VI, fig. 4, nm) present at the distal end of the proboscis sheath will differentiate into the genital ligament and the reproductive organs during metamorphosis. The proboscis sheath and the proboscis occupy the whole of the body of the acanthella at this stage. At the anterior end of the developing proboscis which in this stage is retracted, there is an inverted U-shaped space (marked with X) which opens to the exterior in the next stage and through which the proboscis may be inverted (pl. VI, fig. 6, op).

*Stage-V.*—The larva (pl. VI, fig. 5) is bigger in size with pronounced convexity on one side. It measured in the clearing medium ·34×·65mm. Under the weight of the cover slip it got more flattened and ruptured at two places (pl. VI, fig. 5, r1 & r2). The hooks and proboscis are clearly seen in a whole mount (pl. VI, fig. 5, h, & pr) lying along the convex side of the larva. It is possible to see the histological changes which have taken place. The body wall (pl. VI, fig. 6, bw) is better differenttiated though the muscles of the body wall are still represented by wavy bands. A space between the body wall and the wall of the proboscis has appeared, which is the future pseudocoel of the worm (pl. VI, fig. 6, pse). The rest of the body is almost fully occupied with proboscis (pl. VI, fig. 6, pr) and the proboscis sheath (pl. fig. 6, ps). The U-shaped space on the anterior end is more pronounced. It encaps the proximal end of the retracted proboscis and opens at the anterior end (pl. VI, fig. 6, op). The skin shows two solid buds, one on each side of the proximal end of the retracted proboscis and these are the beginning of the lemnisci (pl. VI, fig. 6, lmn), which in the previous stage were represented by just a cellular mass, (pl. VI, fig. 4, lmn). The hooks of the proboscis almost attained their full size. The shoe-shaped roots measure ·05 mm. and the hookblades measure ·04mm. The spines (pl. VI, fig. 6, bsp) of the body also appear at this stage. The proboscis sheath (pl. VI, fig. 6, ps) occupies the same place as in the previous stage. The Anlage of the circular muscles (pl. VI, fig. 6, cm) of the proboscis sheath is present. The part of the body which elongates later and contains the genitalia is represented by a cellular mass (pl. VI, fig. 6, g), covered over by rudimentary body wall (pl. VI, fig. 6, bw).

*Stage-VI.*—The acanthellae (pl. VI, fig. 7 and pl. VII, fig. 1) in this stage measure 5·6 mm. × 5·2 mm. The larva cannot be flattened easily by cover slip. The hooks are now fully developed and are seen clearly in a whole mount (pl. VI, fig. 7, h). The proboscis is functional and it can evert (pl. VII, fig. 1, pr) on a slight stimulation with acidulated water. The proboscis, in the Archiacanthocephala does not become functional until the acanthella is completely metamorphosed into a juvenile form: an important difference with similar stages of the acanthella of Archiacanthocephala which will be discussed later. The rudimentary musculature of the proboscis sheath takes a deeper stain in whole mounts and is represented by nuclear aggregates in the form of spirals (pl. fig. 7 rmps) throtgh out the proboscis sheath. The primordia of the genitalia and posterior part of the body lie in close proximity of the proboscis sheath, posterior to it (pl. VI, fig. 7, g).

A sagittal section of the acanthella of this stage shows the hooks (pl. VII, fig. 2, h) fully developed. They are of the same size as those of the juvenile forms: the hook blade measure ·045mm and the hook root ·05mm. The muscular layer of the proboscis is not any more represented by wavy bands, but by a compact layer. The insertion of the hook roots clearly seen at a higher magnification in the same section (pl. VII, fig. 3, hr) to abut against the musclar layer (pl. VII, fig. 3, ml) while another layer of muscles seem to reinforce the attachment between the hook roots of the same row of hooks, which curves so as to lie between the roots of the hooks (pl. VII, fig. 3, m12) in the retracted proboscis. Since the proboscis is functional and possesses the musculature to effect eversion, some of the hooks lie on the outside of the proboscis wall in the space between proboscis and the body walls, (pl. VII, fig. 2, eh). The U-shaped space of the previous stages has deepened and serves as space through which the proboscis everts. The tissue (pl. VII, fig. 2, ht) above this space is the hcst tissue. At the posterior ends of the invagination where the neck and the body wall meet, buds of the rudimentary lemnisci, (pl. VII, fig. 2, lmn) have elongated and distinctly differentiated. All other structures described in the various stages are seen clearly in a sagittal section, though they may show further development, but no organ has finally differentiated and is formed except the proboscis and its hooks. The skin is still in a primitive state, and the musculature presents wavy bands, which have become more prominent. The skin and the body wall are still in a primitive state.

*Stage-VII.*—There is no change in the external appearance of the acanthella of this stage. But the internal histological changes as seen in a sagittal section (pl. VII, fig. 4) show final differentiation of tissues in their adult positions. In higher magnification the skin shows a deeply stained cuticle (pl. VII, fig. 4, c), lined with cells which are seen to differentiate into the fibrillar subcuticula (pl. VIII, fig. 5 fsc) punctuated with spaces (pl. VIII fig. 5, sp) which will form the lacunar system of the juvenile and the adult. The circular muscles of the body wall line the subcuticula (pl. VIII, fig. 5, cm), whereas the longitudinal muscles are seen to lie next to the sub-cuticula in wavy bands (pl. VIII, fig. 5, lm). The genitalia are not yet differentiated. I think that this is the final stage of acanthella and that henceforth the larva has only to grow and its tissues have to become finally differentiated. But no change in their histology is involved. Pl. VII fig. 5 shows the growing phase of acanthellae. Later the body wall and the proboscis sheath get so well differentiated that they are even visible in a whole mount (pl. VII, fig. 6, bw and ps). This is the early juvenile which further grows in size (pl. VIII, fig. 1) and becomes a fully developed juvenile. The sagittal section of juvenile forms with retracted and protracted proboscis (pl. VIII, fig. 2 and pl. VIII, fig. 3) respectively, indicate the structure of the functional tissue and organs. The morphology of the worm will be described in a separate paper.

## Development of some Organs.

From the sagittal sections of the various stages of acanthella it is possible to study the differentiation of the various tissues of the larvae leading to the formation of the functional tissue of the juveniles. The following account includes only those stages of differentiation and formation in which there is an obvious developmental change in the structure of the tissues.

(i) *Body-wall.*—There are three stages which depict the formation of the body wall satisfactorily : acanthella stage IV, acanthella stage VII and a newly metamorphosed juvenile. A part of the sagittal section of acanthella stage IV under high magnification (pl. VIII, fig. 4) shows the body wall made of cellular structure (pl. VIII, fig. 4, cl) the outer boundary of these cells is invested with cuticle (pl. VIII, fig. 4,c) probably a product of the cellular layer. On the inner side of the cellular layer, elongated cells are seen which from the wavy bands of muscles. Further marked changes in body wall are visible in the sagittal section of acan thella VII, when seen at a higher magnification (pl. VIII, fig. 5). The cuticle becomes prominent (pl. VIII, fig. 5, c) and the cells lining it differentiate into fibrillae (pl. VIII, fig. 5, fsc) of the subcuticula, though their appearance is not yet like the fine fibrillae of the adult. These fibrillae-like structures are interspersed with nuclei and small spaces and the nuclei are not of the nature of aggregates but resemble the ones of the adult tissue. The spaces will form the lacunar system of the adult. Lining the subcuticula is the muscular layer formed of wavy bands, which elongate ultimately to occupy the position in the adult. It is yet difficult to differentiate between the circular and the longitudinal layers of muscles. In the newly metamorphosed juvenile, the cuticle is thick (pl. IX, fig. 1, c) the subcuticula represented by the fibrillar layer (pl. IX, fig. 1, fsc) with prominent nuclei (pl. IX, fig. 1, n) situated at almost equal intervals. In the proximity of each nucleus there is a lacunar space (pl. IX, fig. 1, lsp). The circular muscles (pl. IX, fig. 1, cm) line the subcuticula, whereas the longitudinal muscles (pl. IX, fig. 1, lm) are still in the process of formation.

(ii) *Lemnisci.*—Lemnisci are the prolongations of the inner layer of the epidermis. Their earliest appearance is indicated by a cellular bud (pl. IX, fig. 2, cb) at the root of the invaginated neck. The bud has arisen by the proliferation of the cellular subcuticula of that region and shows prominent nuclei (pl. IX, fig. 2, n). The next significant stage in the development of the lemnisci is indicated in acanthella VI, where the bud (pl. IX, fig. 3, cb) elongates and the cells do likewise at the same time attaining a diffused appearance. The nuclei are in an active state of division. Later the cells give rise to fibrillae typical of the subcuticula of the body wall, with nuclei (pl. IX, fig. 4, n) lying almost at equal intervals in a line. The bud increases in length and width and becomes fully developed lemnisci which hangs down from the root of the neck in the body cavity (pl. IX, fig. 4, pse) (pseudocoel) as is shown in the sagittal section of a juvenile form (pl. VIII, fig. 2, lmn) and (pl. XI, fig. 4, lmn).

(iii) *Central nervous system.*—The central nerve ganglion becomes prominent in acanthella stage IV, and commences to differentiate into cells (pl. VI, fig. 4, cng) and continues differentiating till the juvenile stage is reached where it appears as rounded mass of nerve cells (pl. VIII, fig. 3, cng), from which nerve fibres originate. These nerve fibres innervate the muscles of the proboscis and the body wall.

(iv) *Reproductive organs.*—The nuclear masses which form the primordium of reproductive organs as shown in acanthella stage IV, gradually divide to form smaller masses which later form the nuclei of cells. These lie at the base of the proboscis sheath. In later stages these cells could not be made out from the muscle cells, till the acanthella fully metamorphosed into the juvenile form. But in the juvenile form some of the cells investing the base of the proboscis sheath, proliferate and give rise to a small pouch (pl. IX, fig. 5, p) which hangs in the body cavity, its wall is made up of a single layer of cells, some of these cells multiply further and give rise to ovaries (pl. IX, fig. 5, ov) or testis. The walls of the pouch elongate and form the genital ligament.

## Discussion.

(*a*) *Comparison of the foregoing developmental stages with those of other species of the order Palaeacanthocephala.*—There are only two species of the order Palaeacanthocephala in which development of larval stages has been investigated. Gupta (1950) described the larval stages of *Centrorhynchus ptyasis* (Gupta, 1950) from whole mounts. I described in detail (1953) the various histological changes, which take place during the larval history of acanthella species of the same genus, *Centrorhynchus batrachus*, (Das, 1952). Van Cleave lays considerable importance on the histological changes which take place during development. He (1947) states, " Students who are not familiar with metamorphosis in its varied aspects are inclined to place too much emphasis upon the external transformation in shape of body when in reality cytological and histological changes are much more significant than the gross external evidences of changes " The observation recorded on the histological changes during the larval development of yet another species of the order Palaeacanthocephala correspond to those of *Centrorhynchus batrachus in* essential particulars and variations in the developmental stages of species of the same genus are unlikely. And yet they differ from those described by Gupta (1950) on *Centrorhynchus ptyasis*. Since Gupta (1950) based her observations on the study of toto mounts only, a correct appreciation, therefore, of histological changes leading to the development of the various organs cannot be made and the differences cannot be explained.

In *Arythomorhynchus tigrinus* (sp. nov.) whose larval stages are described in this paper, nuclear masses though not so prominent as in *Centrorhynchus batrachus*, never the less play the same role in development since they are located in the regions where the gradient of developmental activity is high (pl. VI, fig. 4, nm). As in *Centrorhynchus batrachus*, they are seen in succeeding stages of development to fragment into smaller masses and finally into the nuclei of the functional tissues (pl. IX, fig. 1, n). Examination of sagittal sections of acanthellae in various stages of development clearly show the histological changes taking place, which are peculiar to acanthella stages only and lead to the differentiation and formation of organs. These changes detailed in the foregoing account are of the same nature as changes described in *Centrorhynchus batrachus* (Das, 1953). In both cases, the hooks and proboscis are the first among the organs to appear. The hooks attain their full development by the time the acanthella reaches the stage VI (pl. VII, fig. 2, h) and are of the same size as the hooks of the juvenile form, (pl. VIII, fig. 2, h). The muscles of the proboscis wall (pl. VII, fig. 3, ml) are not represented any more by wavy bands but by compact muscular layer, in which the roots of the hooks are inserted, hence it is obvious that the proboscis and hooks complete their development, where as the other organs are still in the process of differentiation : the body wall is in very early stage of development, the muscles of the body wall are represented by wavy bands (pl. VII, fig. 2, wb) still spread all over the future body cavity, the pseudocoel. It is clearly evident from the histological condition of the various organs that the larva is an acanthella and though the Anlage of various organs is clearly noticeable, the appearance of the tissues is entirely different from that of the functional tissue of the juvenile. It clearly indicates that the metamorphosis is far from complete. The proboscis is not only fully developed earlier than the rest of the tissue of the body, but that it also becomes functional, which was evident when, on slight stimulation with dilute acidulated water it everted its proboscis, an imporant fact which compares with the acanthella stage of *Centrorhynchus batrachus* (Das 1952) where also the hooks and the proboscis develop and become functional far earlier than the rest of the organs.

(*b*) *Comparison with the developmental stages of the species of Archiacanthocephala.*—Van Cleave (1947) states, " There have been but a few species of ACANTHOCEPHALA for which details of development have been published. To the present time most of the species on which detailed information is available belong to the ARCHIACANTHOCEPHALA. There is *no necessary assurance* (italics are mine) that in representatives of the EOACANTHOCEPHALA and the PALAEACANTHOCEPHALA the pattern of development is identical " With data now available of the histological details of the larval stages of two species of Palaeacanthocephala, *Arythmorhynchus tigrinus* (sp. nov. described in the present paper) and *Centrorhynchus batrachus* (Das, 1952) the assurance can be given that Van Cleave's terminology (1935, 1937) employed for the various developmental stages of an acanthocephalan worm, based on the studies of Archiacanthocephala can with advantage be used for the various representatives of the order Palaeacanthocephala. The general pattern of development is the same as in the Archiacanthocephalan forms, but the order of the differentiation of organs varies. Varaiations in other minor details are also likely. According to Van Cleave (1947) in the Archiacanthocephala, the proboscis becomes functional only when the larva gets completely metamorphosed into a juvenile form. Whereas in the representatives of the Palaeacanthocephala, which have been investigated the proboscis becomes functional much earlier, when the larva is in an acanthella stage. However, the presence of the functional proboscis in an acanthella stage, need not change the aspect of the terminology, as Van Cleave (1947), states, " In the light of detailed studies on life histories such as those conducted by Meyer (1933, 1938), by Kates (1934) and by Moore (1946), it is now obvious that the cytological changes accompanying metamorphosis of the acanthella to the juvenile stage are more significant as evidence of metamorphosis (in species that have been investigated) than the attainment of a functional proboscis as originally maintained "

## CONCLUSION.

(*a*) *Functional proboscis.*—The occurence of a functional proboscis in the acanthella stages of the species of Palaeacanthocephala which have been investigated makes them better suited for their parasitic mode of life, and thus seem to be more highly evolved than their allies of the Archiacanthocephala because an early functional proboscis in the devepolmental stages gives a greater range of infective period, and a better chance of self preservation. These acanthella stages with a functional proboscis if ingested by a vulnerable host, will evert their proboscis on reacting with the acidic gastric juice of its stomach, and will secure attachment to the lining of the intestine.

(*b*) *Intermediate host.*—In all the species of Archiacanthocephala, life histories of which are known, the developmental stages from acanthors to juveniles are completed in the invertebrate intermediate host, which may be swallowed by a second intermediate host, usually a vertebrate, also called a transport or a reservoir host in its body cavity the juvenile parasite reencyst and remains quiescent to mature only when ingested by the definitive vertebrate host. Whereas in some forms of Palaeacanthocephala which have been investigated, the primary intermediate host may either be an invertebrate (arthorpod) or a vertebrate (amphibia). Hyman (1951) records that encysted juveniles have been found in fresh water shrimp (*Palaemon squilla* Porta, 1905) with fish as transport host. As detailed in the present paper, it is obvious that a large number of larval stages have been found embedded in the peritoneum of the Indian frog, *Rana tigrina*, which clearly indicates that *Rana tigrina* is the primary intermediate host, having been infected directly without the invertebrate host intervening.

Referring to the genus centrorhynchus Hyman (1951) writes, " The primary intermediate host for this genus appears to be *unknown* but frogs, lizards and snakes act as transport hosts ". Nevertheless, Das (1952) gave an account of developmental stages, from the acanthors to the juveniles of *Centrorhynchus batrachus* (Das, 1953), which were found embedded in the peritoneum of *Rana tigrina*. Gupta (1950) found larval stages (acanthors and acanthellae) of *Centrorhynchus ptyasis* (Gupta, 1950) encysted in the peritoneum of *Ptyasis mucosus*. Subramaniam (1927) found the larvae of *Centrorhynchus aluconis* (Muller, 1780). encysted in the peritoneum of toads *Bufo melanostictus*. He observes, " Almost all the toads he dissected were heavily infected with these larvae, few being free from this infection ". Probably Subramaniam meant *Juveniles*, when he used the term larva according to the accepted terminology for the developmental stages of the acanthocephalan life histories. However, the heavy infection of all the toads shows great possibility of having been infected directly, hence serving as primary intermediate hosts.

From the foregoing account of the occurrence of Centrorhynchus larvae (acanthors and acanthellae) in various vertebrate intermediate hosts, it is evident that these *hosts are the primary intermediate hosts for the Centrorhynchus species*. Therefore it must be presumed that in some forms of Palaeacanthocephala the vertebrate intermediate host is the primary intermediate host, and can be infected directly without the invertebrate host intervening, which can be taken as another evidence of the fact that the Palaeacanthocephala are more highly evolved forms with enhanced chance of the larvae injecting a vertebrate intermediate host in the absence of the invertebrate intermediate host.

I propose to describe the morphology of this species in a separate paper.

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