

OBSERVATIONS ON THE HABITS AND POST-EMBRYONIC  
DEVELOPMENT OF A PARASITIC BRANCHIURAN *ARGULUS*  
*PUTHENVELIENSIS* RAMAKRISHNA

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INTRODUCTION

A FEW specimens of the cyprinid fishes *Esomus danrica* (Ham.) and *Puntius vittatus* Day collected from a freshwater pond at Udayamperoor, twelve miles south-east of Ernakulam, Kerala State, were found infested with an ectoparasite which was identified as an argulid. Later on, *Macropodus cupanus* Cuvier, an anabantid fish and *Panchax panchax blochii* (Arnold) a cyprinodontid were also found to carry the same parasite. On the advice of Dr. S. Jones, Director, Central Marine Fisheries Research Institute, two specimens were sent to the Zoological Survey of India and were identified as new by Shri G. Ramakrishna. He (1959) named the new species *Argulus puthenveliensis* as was suggested by the writer and described it. During the subsequent collections many mature specimens were obtained which facilitated the study of the habits and post-embryonic development of the parasite.

OBSERVATIONS

*Esomus danrica* carrying *A. puthenveliensis* was kept in a glass jar for observation. Two of these parasites were first detached from the host and kept in a specimen tube. On introducing a fish into this container, the parasites were quick in attaching itself to any part of the host's body, though at times they failed. After getting a hold on the host initially at such points as the caudal peduncle, the fins or the sides of the trunk, they tried to move to other regions of the body. For example, it was observed that they moved slowly from the place of initial attachment to other places such as the dorsal surface of the body posterior to the dorsal fin, the base of the pectoral fin, especially the posterior side and the dorsal surface of the head of the fish, thus showing certain preferences for the site of eventual attachment. The parasite clung to the host's body with its posterior end turned towards the rear end of the fish. Occasionally it pushed its anterior end between the scales, probably for a better and easier penetration by the sting.

It was interesting to note that the hosts always struggled to escape from the parasite *Puntius* and they even devoured the fully mature parasites. This peculiar behaviour was noticed twice during my observations. In both the cases the parasites were mature females measuring about 4.5 mm. and the hosts were *Macropodus cupanus* Cuvier of 37 mm. in length and *Puntius vittatus* Day 30 mm. long. On dissecting the fishes the parasites were recovered from their stomachs. It is probable that these fishes caught them by the head as only the heads were injured.

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**Copulation :** Two fully mature specimens of *A. puthenveli* (one male and one female) were transferred into a small petri dish containing a little water and observed under the binocular microscope. The male *A. puthenveli* clasped the female, on the dorsal side by the posterior thoracic legs. The abdomen of the male was twisted under that of the female, on either side, so that the male genital opening was brought in direct contact with the spermathecal openings of the female. This method of copulation of *A. puthenveli* agrees with that of *A. viridis* described by (Martin, 1932). The copulation was continued for 32 minutes after which the pair separated. From the study of subsequent copulations it was found that the duration of copulation varied from 32 to 37 minutes and was irrespective of the number of eggs subsequently laid by the female. In two out of five subsequent copulations observed, the pair did not separate, but the male continued to be on the female's back even while it was laying the eggs.

**Egg laying :** Five minutes after the completion of copulation, the egg laying was started which continued for 10-11 minutes. The eggs were laid on the side of the glass jar. But later, they were made to lay the eggs on a ring of transparent plastic sheet for easy examination.

The eggs were oval in shape and white in colour as noted in the case of *A. viridis* Martin (1932). The number of eggs varied from 140-204 in fully mature ones. But the young ones, soon after attaining maturity laid fewer eggs, about 20 to 25 eggs at a time. Later, the number of eggs increased. The eggs were found to be laid in definite rows. The number of rows varied from one to as many as five or six. In each row they were placed end to end. Each egg was enveloped in a gelatinous substance which hardened on coming into contact with water as was noticed by Wilson (1904) in *A. americanus*. Unlike *A. catostomi*, *A. americanus* and *A. maculosus* Wilson (1907) the envelope of the egg was uniform in *A. puthenveli*.

**Development :** The fertilized eggs were kept at 30°C and periodically observed until all were hatched out. These eggs did not fail to develop even when they were scraped off from the sides of the container for close examination. The eyes were developed after the 6th day and the eggs started hatching after 13 days and 21 hours. The duration of hatching was found to vary in the different sets from 30 to 48 hours irrespective of the number of eggs, the temperature being kept constant. The proportion between the total number of eggs and the number of unhatched eggs varied from 102 : 1 to 154 : 15 while in some sets all the eggs hatched out. The rate of hatching was found to be varying from 2.6 to 4.2 eggs per hour (Table I).

**Post-embryonic development :** The post-embryonic development of *A. puthenveli* was studied in detail.

The newly hatched larva was very active and swam about freely. When a young fish was introduced the larva promptly got attached to it. The length of the newly hatched larva varied from 0.525 to 0.56 mm. The carapace was oval. The posterior lobes of the carapace covered the first two pairs of thoracic legs. The carapace was fringed with chitinous ciliary processes and spinules, extending upto the level of the compound eyes. The first antenna was like that of the adult in general appearance. The proximal segment was present unlike *A. japonicus* Tokioka (1936) ; but it did not bear the posterior spines. The second segment had a long and pointed lateral hook and an anteriorly directed blunt spine which had not developed a hook. The third and fourth segments were distinct and bore three prominent setae at the distal end. The second antenna had only three well defined

segments, the basal one being the largest and bore a posterior spine. The distal segment had a strong curved claw. The proboscis had essentially the same structure as in the adult. The preoral sting was small. The first maxilla situated on the sides

TABLE I  
Details of observations on *A. puthenveli*ensis

	I	II	III
Number of the batches of eggs			
Duration of copulation (in minutes)	37	33	35
Duration of egg laying (in minutes)	10	11	11
Number of eggs	204	154	140
Duration of embryonic stage	13 days and 21 hours.	13 days and 21 hours.	13 days and 21 hours.
Time taken for complete hatching of the egg mass (in hours)	48	54	49
Number of unhatched eggs	2	15	nil
Average rate of hatching (per hour)	4.2	2.6	2.9
Sex ratio of young ones	11 : 6	11 : 7	11 : 6

of the proboscis was three segmented. The proximal segment was bulky and the distal one had two curved claws. The ventral of these two claws had four forwardly directed curved spines which decreased in size from the base of the claw to its tip. The dorsal claw had a single posteriorly directed curved spine. The basal segment of the first maxilla was provided with a pair of inwardly directed spines, one anterior and inner and the other posterior and outer in position. The second segment had a similar spine on its posterior inner margin. The five segmented second maxilla had developed a pair of spines at the posterior end of the basal segment, one outer and the other inner. These spines represented the inner and middle spines of the adult. The two pairs of post-maxillary spines were developed, the anterior pair being just posterior to the proboscis, on either side and the posterior pair in a level with the first thoracic legs. The thoracic appendage, were all well developed unlike *A. japonicus* Tokioka (1936). They had coxae and bases bearing segmented endopods, (except in the second pair) and unsegmented exopods. The first thoracic leg had an endopod of three segments with three small plumose setae at its tip. The third and fourth legs had endopods of two segments each ; but the second leg had one segment only. These thoracic legs had not developed distinct precoxae.

The abdomen was broader than long and the length was less than 1/9 of the total length of the body. The caudal rami were prominent and bore stiff and short setae. The sexes could be distinguished in this stage. The two pairs of respiratory areas were clearly visible. The anterior one which was club-shaped extended laterally around the posterior one.

No change in structure was noticed until the second day after hatching when the larva increased in size and further approached the adult shape. The ciliary processes were altogether absent unlike *A. japonicus* ; but the spinules were still present. The alae of the carapace did not reach the posterior end of the second leg. The basal

segment of the first antenna had developed two posterior spines, one outer and the other inner. The second antenna in this stage was four segmented as a result of the division of the basal segment of the earlier stage. The paired post-antennal spines

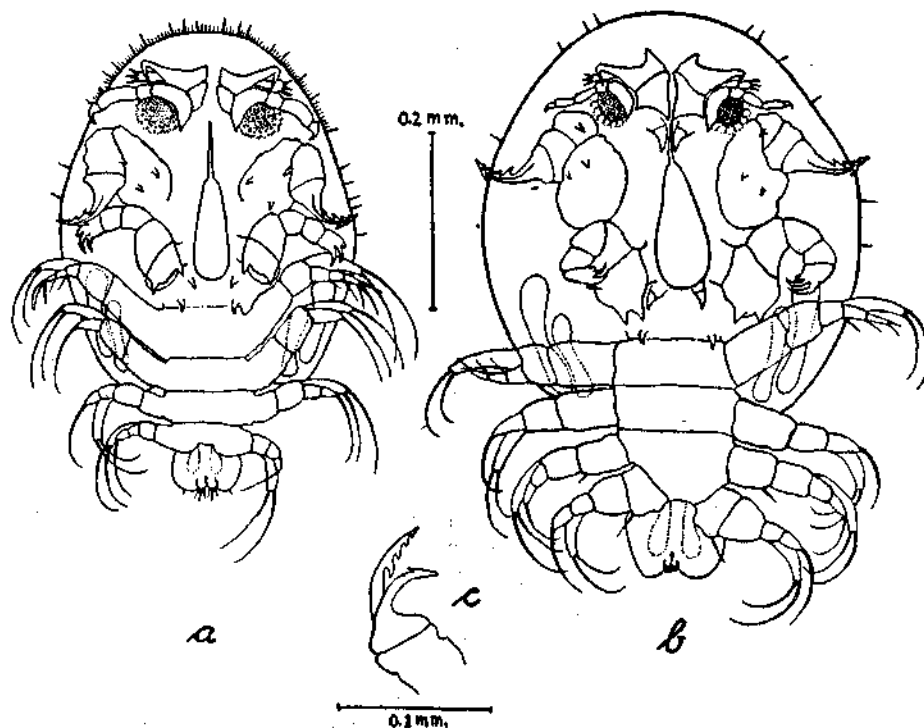


FIG. 1. *Argulus puthenveliensis*.

(a) Newly hatched larva, ventral view; (b) 2 days old larva, ventral view; (c) Dorsal view of the tip of the first maxilla of 2 days and 12 hours old larva showing the claws.

also appeared. The first maxilla became four segmented due to the division of the basal segment. The second segment was large and bore two posterior spines. The second maxilla remained unchanged. There was no modification in the swimming legs except for the increase in size.

When the larva was 4 days old the carapace had scattered spinules on the outer margin. The carapace was nearly as broad as it was long and the alae just reached the second pair of legs. The anterior spine of the second segment of the antenna developed a hook. The second antenna remained four segmented; but the claw at its tip was reduced in size. The second segment of the first maxilla enlarged and the first maxilla attained maximum development as clasp ing organs. The suction cup could be seen formed inside the second segment of the first maxilla. The second maxilla developed an outer spine at its base—the outer one of the adult.

The legs developed distinct precoxae and in the female the coxae of the last pair of legs began to enlarge posteriorly to form the natatory lobe of the adult. A number of spines appeared on the ventral surface in front of the anterior marginal groove and

along the margin of the carapace especially adjacent to the respiratory areas. The abdomen was a little more than  $1/7$  of the total length. The caudal rami remained the same.

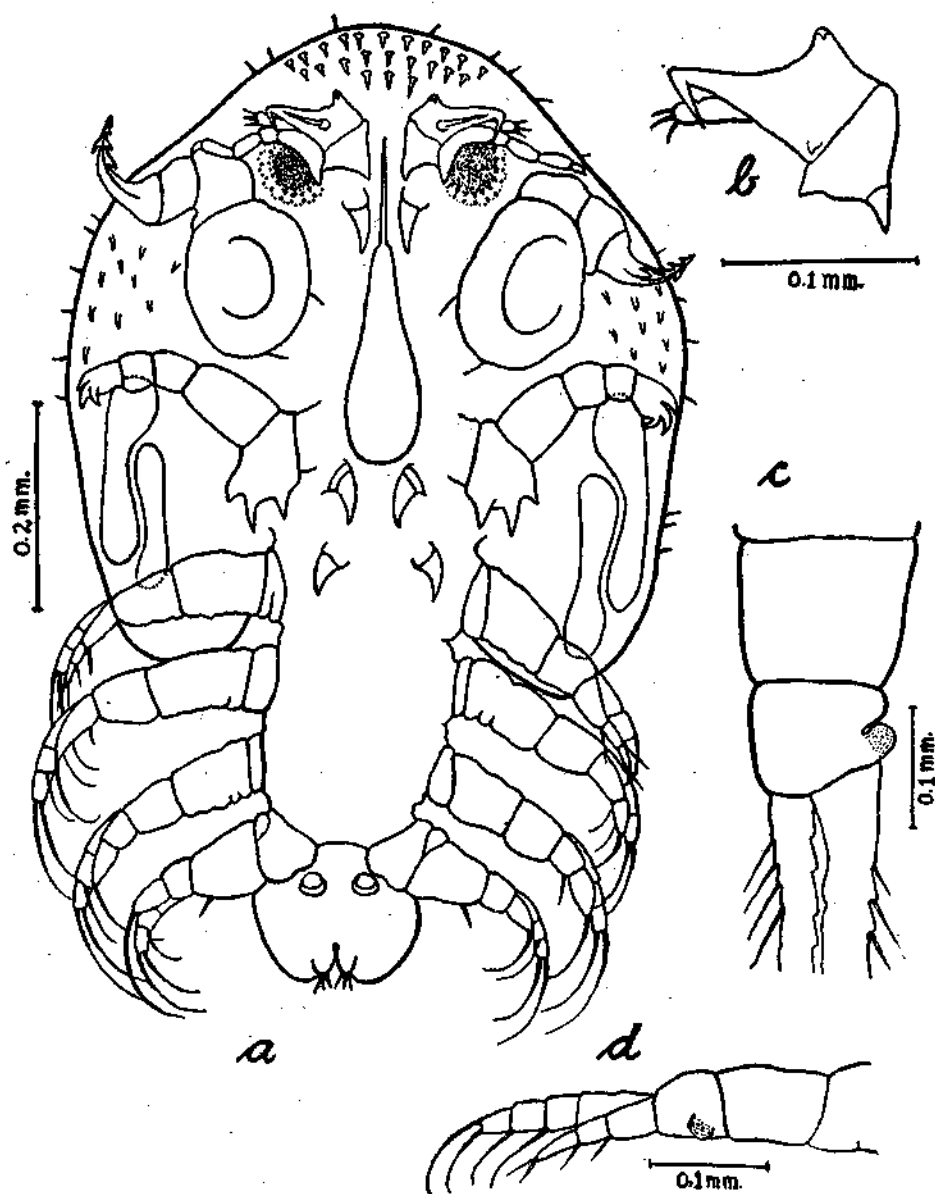


FIG. 2. *Argulus puthenveliensus*.

- (a) 4 days old larva, ventral view ; (b) First antenna of 4 days and 12 hours old larva ;  
 (c) Second leg of 5 days old larva showing the rudiment of flagellar process (lateral view) ;  
 (d) First leg of 5 days and 12 hours old larva showing rudiment of flagellar process.

On the 5th day the suction cup was better developed. In this stage the rudiment of the flagellar process of the adult appeared in the form of small processes at the distal end of the base of the first and second pair of legs. When the larva was 5 days and 12 hours old the triangular processes at the margin of the ribs of the suction cup and its musculature could be clearly seen within.

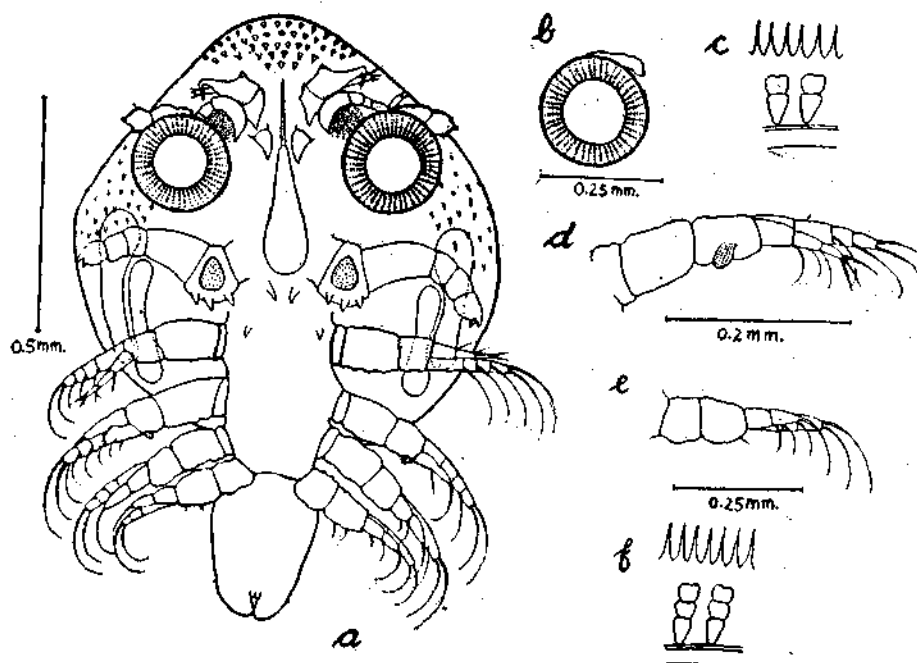


FIG. 3. *Argulus puthenveliensis*.

(a) 6 days old larva, ventral view; (b) Suction cup with residuum of the first maxilla of 6 days and 12 hours old larva; (c) Rib of suction cup of 6 days and 12 hours old larva; (d) First leg of 6 days and 12 hours old larva showing the club-shaped rudiment of the flagellar process; (e) Fourth leg of the same larva; (f) Rib of suction cup of 7 days and 12 hours old larva.

On the 6th day the suction cup was exposed by the rupture of the second segment of the first maxilla. The distal segments of the first maxilla got reduced considerably. The rudiment of the flagellar process increased in size and became club-shaped. The exopods became segmented and had developed long plumose setae along their posterior surface. The endopods of the second pair of legs remained unsegmented. The lobes of the carapace reached the third pair of legs. In the male the abdomen elongated. The anal sinus became deeper and was more than  $1/5$  the length of the abdomen. At the age of 6 days and 12 hours the larva had a small residual portion of the distal segments of the first maxilla without any trace of segmentation. The first antenna developed a posterior spine on the inner side of the second segment. The second antenna had a highly reduced claw at its tip. The female had better developed natatory lobes. The rudiments of the flagella increased in size; but did not develop any setae. The rib of suction cup had one big piece and a small one.

The larva after 7 days and 12 hours developed more spines on the carapace. The second antenna bore setae on the second and third segments. The fourth one had three stiff setae instead of the claw of the earlier stages. The residuum of the first maxilla got further reduced. The rib of the suction cup had one more smaller piece. The flagellum in this stage was well developed and bore two short plumose setae.

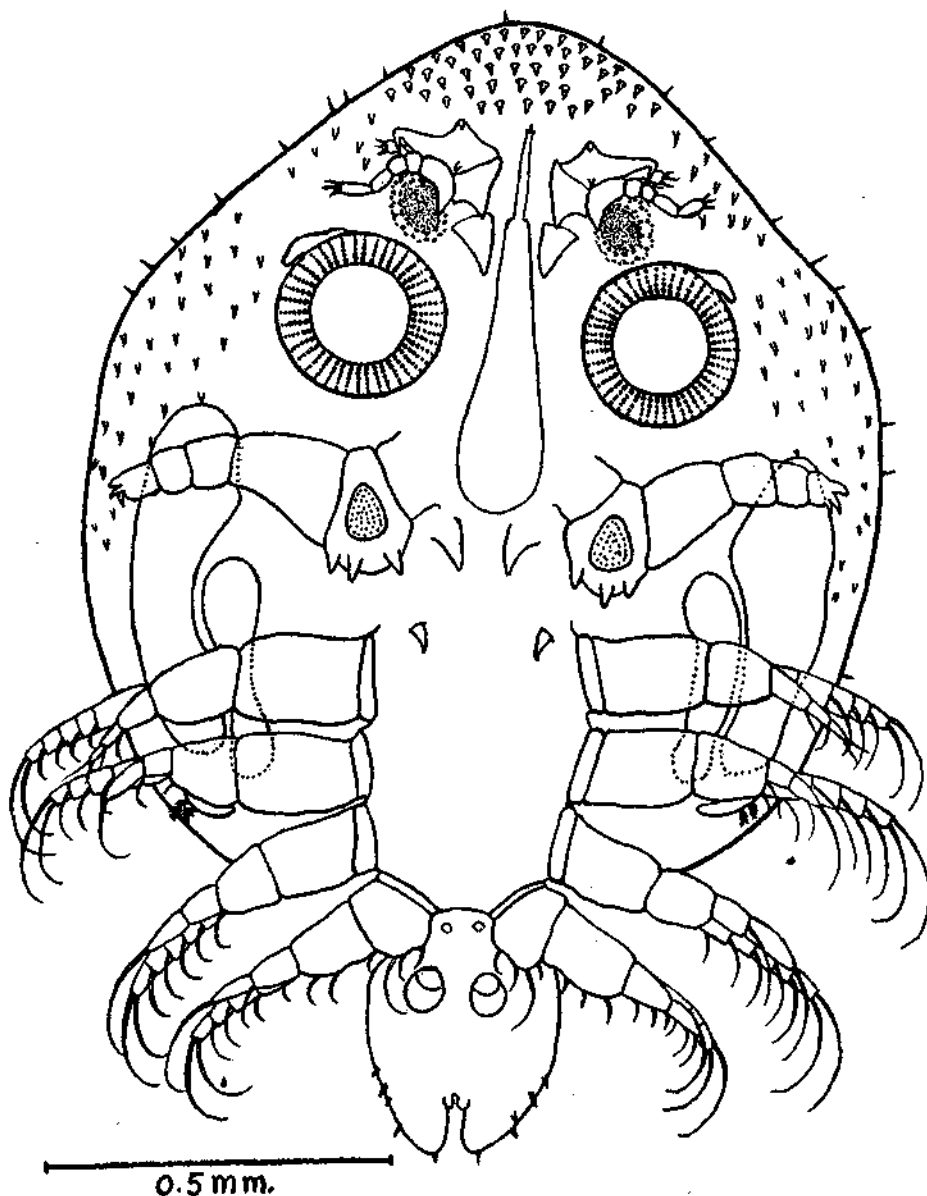


FIG. 4. *Argulus puthenveliensis*.

7 days and 12 hours old female larva, ventral view.

TABLE II.

*Measurements (in mm.) of the Larval Stages and Adults of Argulus puthenveliensis Ramakrishna*

	Larval Stages												Adults		
Age in days	0	1	2	3	4	5	6	7	8	9	10	11	12	13♀	13♂
Total Length	0.525	0.525	0.593	0.703	0.725	0.841	1.291	1.363	1.363	1.457	1.740	2.088	2.204	3.143	2.928
Length of Carapace	0.319	0.319	0.377	0.522	0.522	0.667	0.667	0.986	0.986	0.995	1.305	1.218	1.305	2.286	1.857
Breadth of Carapace	0.268	0.283	0.326	0.464	0.493	0.587	0.725	0.841	0.899	0.841	1.189	1.16	1.131	2.114	1.571
Length of Abdomen	0.065	0.065	0.065	0.087	0.131	0.116	0.319	0.348	0.293	0.319	0.435	0.754	0.812	0.571	1.714
Breadth of Abdomen	0.068	0.073	0.087	0.116	0.102	0.152	0.232	0.276	0.232	0.261	0.293	0.334	0.348	0.914	0.429
Sucker Diameters	..	..	..	..	..	..	0.179	0.203	0.203	0.218	0.261	0.261	0.261	0.400	0.400
	..	..	..	..	..	..	0.189	0.203	0.189	0.218	0.261	0.232	0.261	0.400	0.400



In the female the natatory lobes were fully developed and had seven plumose setae. The abdomen had spinules on its outer margin. The anal sinus was deep and the anal papillae were small and situated at the anterior end of the sinus. In the male the abdomen was longer. Its length was a little less than  $1/3$  the total length of the body. The number of spinules on the outer margin was more than that of the female. The deep anal sinus was  $1/4$  of the length of abdomen. The anal papillae were placed near the posterior end of the sinus.

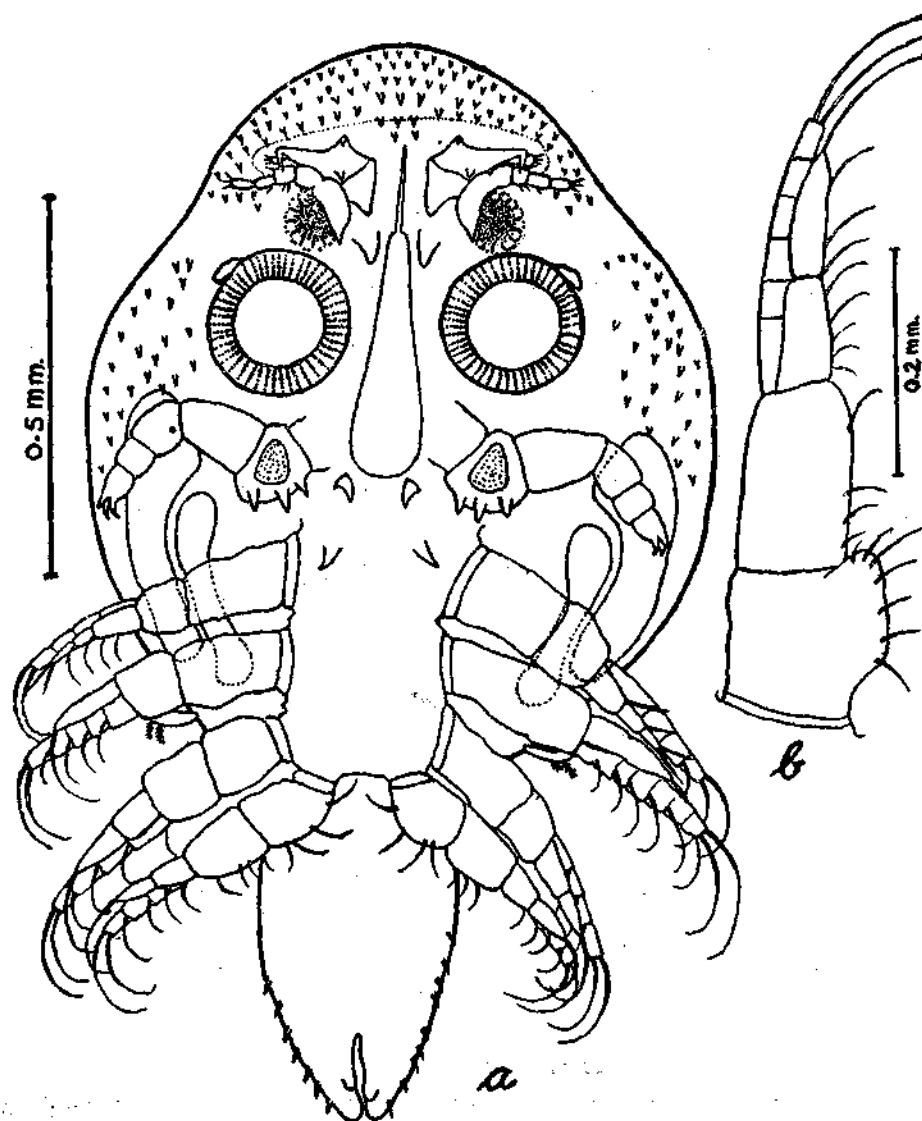


FIG. 5. *Argulus puthenveliensis*.

(a) 9 days old male larva, ventral view; (b) Last leg of 10 days old larva, ventral view.

At the age of 9 days and 12 hours the male young one had developed a cleft on the posterior surface of the third leg between the coxa and the basis which later became the socket of the adult. At the same time, the rudiment of the peg of the adult also originated in the form of a small prominence on the anterior surface of the basis of the last thoracic leg. In this stage the rib of the suction cup had a long piece and four smaller pieces.

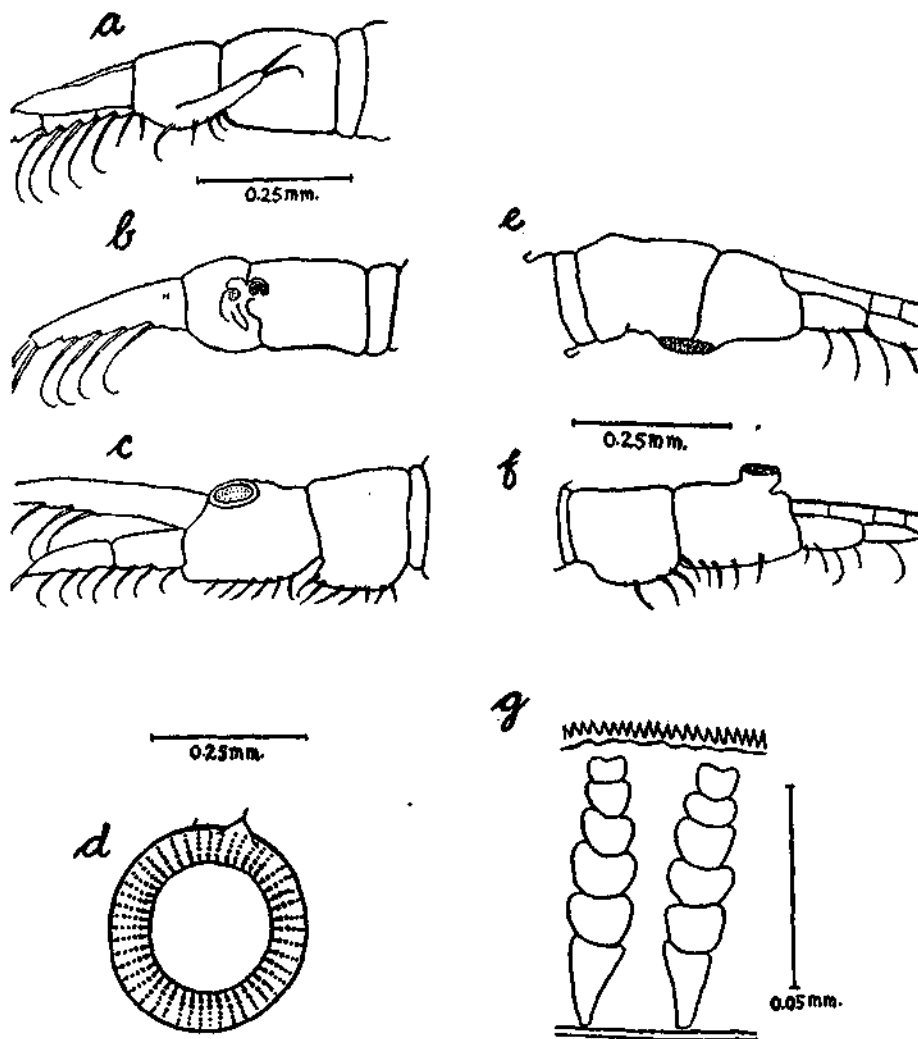


FIG. 6. *Argulus puthenveliensis*.

(a) Second leg of 12 days old larva showing fully formed flagellum, dorsal view; (b) Third leg of 12 days old male larva showing the socket with the folds, posterior view; (c) Fourth leg of 12 days old larva showing the peg; (d) Suction cup of 12 days and 12 hours old larva with highly reduced residuum; (e) Third leg of 12 days and 12 hours old male larva showing the socket, lateral view; (f) Fourth leg of the same larva with fully developed peg; (g) Rib of suction cup of the adult.

In the subsequent stages the median and lateral folds of the socket were developed and the peg assumed the adult nature. The flagella of the first two pairs of legs were in the form of long, finger-shaped processes with a pair of long setae at their tips unlike the whip-like flagella of *A. americanus* Meehan (1940). The residuum of the first maxilla became very much reduced in size. By the twelfth day it became a mere projection with a small spinule at its tip. On the thirteenth day after hatching the residuum completely disappeared and the parasite attained the mature stage.

The two pairs of respiratory areas remained in more or less the same shape throughout the development. The anterior one was club-shaped and curved around the posterior one which was dumb-bell shaped. The respiratory areas of *A. puthenveli* resembled those of *A. siamensis* Wilson in the general shape and the orientation of the two areas; in *A. puthenveli* they were slightly broader than those of *A. siamensis*. The number of the ribs of the suction cups varied from 38 to 49 in different individuals, irrespective of the stages. It was interesting to note that none of the larvae had the same number of supporting ribs in both the suction cups.

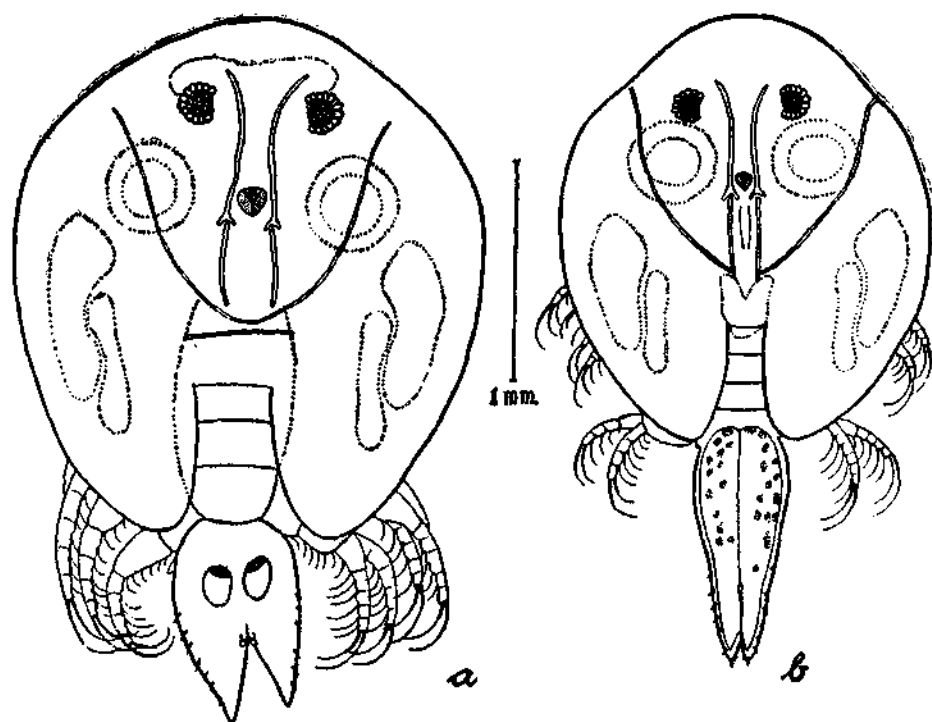


FIG. 7. *Argulus puthenveli*.

(a) Adult female, dorsal view; (b) Adult male, dorsal view.

During the study of the development the successive moultings could not be followed and hence not referred to in the present paper. It was observed that for every eleven female young ones there were six to seven males. After attaining maturity they started reproducing themselves within a week's time.

## SUMMARY

*Argulus puthenveli* Ramakrishna was found infesting the freshwater fishes of Udayamperoor, Kerala State. Its behaviour was observed, especially the method of attachment to the host, copulation and egg-laying. The fertilized eggs laid in masses were allowed to continue the development. They hatched out after 13 days and 21 hours and the newly hatched larvae were very active. They were allowed to get attached to small fishes and the post-embryonic development was studied in detail until the young ones reached maturity. They started reproducing themselves within a week.

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