STUDIES ON THE FRESHWATER COPEPOD FISH PARASITES OF KERALA

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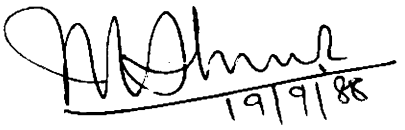
CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Shri Shaju Thomas, M.Sc. under my supervision and guidance in the Department of Industrial Fisheries, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY and that no part thereof has been submitted for any other degree.



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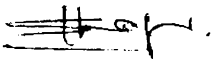
DECLARATION

I, Shri. Shaju Thomas, do hereby declare that the thesis entitled "STUDIES ON THE FRESHWATER COPEPOD FISH PARASITES OF KERALA" is a genuine record of research work done by me under the supervision and guidance of Dr. M. Shahul Hameed, Professor, Department of Industrial Fisheries, Cochin University of Science and Technology, and has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any university or institution.



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CONTENTS

PREFACE

The study of copepods parasitic on fishes was initiated by Linnaeus (1758) with the description of Lernaea cyprinacea. Since then, tremendous interest was shown in the study of this particular group of parasites and many species, new to science have been reported from different parts of the world. Parasitic copepods exhibit varying degrees of adaptation to parasitism, which culminates even in endoparasitic forms. Studies on copepods parasitic on fishes reveal information regarding the origin of parasitism, intricacies of host-parasite relationship and distribution of host-parasite populations.

At present, the number of copepods parasitic on fishes exceeds 1500 species. Majority of them are parasitic on marine fishes. Reports regarding freshwater species are less compared to that of marine forms. Some parasites are capable of infesting even amphibians and aquatic mammals.

In India, copepods parasitic on fishes have been studied to a great extent, mainly on systematics. Tripathi (1966), during the study of parasitic copepods from Indian waters, reported 22 species from freshwater fishes, 31 from estuarine fishes and 141 species from marine fishes. His survey was confined mainly to Eastern Indian region and partly to South India. Hameed (1972) reported 129 species of parasitic copepods from marine fishes from the coastal waters of Kerala.

Studies on parasitic copepods from freshwater fishes are still in its infancy. In recent years, there is a renewed enthusiasm in the study of freshwater fish parasites due to rapidly increasing aquaculture practices. The importance of diseases and their control assumes great significance because of the adverse impact of diseases on fish production and its economy. Copepods are one of the most harmful parasites of freshwater fishes. Reports on the damages caused by copepod parasites from different parts on the world are increasing alarmingly. But the information on parasitic copepods of freshwater fishes in India is quite meagre. Knowledge regarding this group of parasites, their biology and pathology from Kerala, is lacking. This is the main reason why Dr. Hameed had entrusted me to undertake this study.

Copepods parasitic on fishes include three suborders namely Poecilostomatoida, Cyclopoida and Siphonostomatoida. The first one includes five families, the second consists of a single family and the last one comprises fourteen families (Kabata, 1979). The suborder Cyclopoida consists of a single family viz; Lernaeidae, which is exclusively freshwater. Suborders Poecilostomatoida and Siphonostomatoida are represented by a few forms parasitic on freshwater fishes.

During the present study, parasitic copepods belonging to the family Lernaeidae (Cyclopoida) and Ergasilidae (Poecilostomatoida) were collected from freshwater fishes in Kerala. They were identified upto species level and described in detail. In addition to this, the life history

of a new Lernaeid copepod was carried out. Eggs were collected from adult females and allowed to hatch in controlled conditions. Then the larvae were released to different host fishes and observed the life cycle upto the emergence of egg-bearing females. Ecological studies with special reference to host-parasite relationship, prevalence and intensity of infection, were conducted for Lernaea osphronemi sp. nov. Eradication of parasite population from culture ponds is of utmost importance. So, prophylaxis and control measures were tried for the elimination of the new Lernaeid parasite.

The thesis consists of five chapters. The first chapter is a general introduction which deals with the review of literature on various aspects of parasitic copepods viz; systematics, life history, host-parasite relationship, ecology, pathogenicity, prophylaxis and control measures. Systematics of parasitic copepods of freshwater fishes collected during the present study forms the second chapter. The third chapter deals with the life cycle study of the new Lernaeid copepod, Lernaea osphronemi. The fourth chapter contains host-parasite relationship, ecology and treatment of the new species of Lernaea on Osphronemus goramy. General observations and a summary of the entire work constitute the fifth chapter.

CHAPTER - I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Copepods are commonly free-living but some of them are parasitic. They are parasites or associates on sponges, coelenterates, polychaete worms, molluscs, echinoderms and aquatic vertebrates. Copepods are capable of parasitising different parts of the body of the host. The site preferences of these parasites necessitate adaptation to a great extent, that leads to varying degrees of diversity in form and structure. The morphological variations exhibited by the adult parasitic copepods make it difficult to recognize them as true copepods, so in the past, some of them were identified as worms. Fish is one of the major hosts of copepods.

A survey of literature on parasitic copepods revealed that there are more marine forms than freshwater species. Though the present study is confined to freshwater parasitic copepods, it would be appropriate to mention the pioneers in the study of marine copepod parasites, because some of them were experts in both the fields. In addition to this, some members of the same family or genus parasitize both marine and freshwater fishes.

The first record of a parasitic copepod Lernaea cyprinacea by Linnaeus dates back to 1758. His study was succeeded by Muller (1785) on Caligus. The 19th century workers in the field of parasitic copepods were Hermann (1804), Risso (1816), Blainville (1822), Nordmann (1832), Dana (1853), Steenstrup and Lutken (1861), Kroyer

(1863), Heller (1865), Hesse (1873), Richiardi (1883) and Bassett-Smith (1899). C.B. Wilson (1905) spearheaded the study on both marine and freshwater parasitic copepods of America. He brought out a long series of publications for forty years. A & T Scott (1913) published a comprehensive work on British parasitic copepods. Leigh-Sharpe (1925, 1933) has also contributed to the study of parasitic copepods of British fishes. Kirtisinghe (1937) concentrated his studies on the copepod parasites in and around Ceylon and brought out several publications. It was followed by Heegaard (1943) and Shiino (1952).

Yamaguti (1963) proposed a classification and published a monograph on "Parasitic Copepoda and Branchiura of Fishes". Bocquet and Stock (1963) suggested a new approach in the study of parasitic copepods. Works by Kabata (1958-'87), Ho (1961-'85), Lewis (1963-'69), Hewitt (1964-'79), Roberts (1963-'70) and Cressey (1967-'83) are worth mentioning.

The important Indian scientists in the study of copepod parasites of marine fishes are, Gnanamuthu (1947-'60), Redkar (1949-'50), Kurian (1955-'61), Rangnekar and Murthy (1950-'64) ,Tripathi (1952-'69), Ranganekar (1955-'63), Pillai (1961-'83), Sebastian (1966), Reddiah (1970), Hameed (1972-'88) and Natarajan and James (1977).

Markevich's (1931-'78) studies on the copepod parasite fauna of the fishes of the U.S.S.R. brought out a lot of information on this subject. Hu's (1948) papers on parasitic copepods of China is worth

mentioning. He discussed in detail the taxonomy of the genus Lernaea and proposed five subspecies. Harding (1950) critically carried out the systematic study of genus Lernaea and prepared a key for the identification of this genus.

Fryer (1956-'82) conducted extensive studies on the freshwater copepod fish parasites of Africa. He described several new species of parasitic copepods. His approach to the problem was philosophical. He worked out the zoogeography and phylogeny of African freshwater copepod parasites. Causey (1957) reported the parasitic copepods from Louisiana freshwater fishes, which consist of four genera and twelve species. Bauer (1962) studied the ecology of parasites of freshwater fishes. Parasitic crustacea from inland water fishes of Israel was described by Paperna (1964). Sarig (1966) reviewed the diseases and parasites of fishes in warm-water ponds in the Near East and Africa and opined that pond-cultured fishes are greatly infected by ectoparasites. He suggested precautionary measures to prevent the hazards of parasitic infection.

Meyer (1966), in his review of the parasites and diseases of fishes in warm-water ponds in North America pointed out that parasitic copepods are more harmful than any parasites of cultured fishes. Hoffman (1967) described in detail the parasites of North American freshwater fishes and provided a key for the identification of parasitic copepods. Lernaea, Salmincola, Lepeophtheirus, Ergasilus, Lernaeocera and Achtheres were represented in the key.

The ectoparasitic infection of African freshwater fishes was studied by Paperna and Thurston (1968). They observed that the parasitic crustaceans in Africa were extremely rich in species and genera.

Rogers (1969) in a summary of fish disease cases received over a five year period at South Eastern Co-operative Fish Disease Laboratory, reported that 30% of the damage was caused by parasites. Lernaea was the most damaging of the parasitic crustaceans encountered.

Roberts (1970) carried out an extensive study on the genus Ergasilus in North America, discussed previous literature in detail and worked out a key for Ergasilus. A comprehensive account of the parasites of British freshwater fishes was brought out by Chubb (1970). He reported the occurrence of eight species of crustaceans from freshwater fishes. He concluded that the information on distribution, life-cycle, seasonal pattern of occurrence, long term cyclical changes in parasite abundance and other aspects of the biology of the parasites of freshwater fishes are necessary for a better management of fish populations. Johnson and Rogers (1973) worked out the distribution of the genus Ergasilus in several Gulf of Mexico drainage basins and suggested that some Ergasilus species are more hostspecific. A check list of British and Irish freshwater fish parasites with notes on its distribution was published by Kennedy (1974). It is a valuable contribution in the study of parasites of freshwater fishes.

Boxshall (1976) created a new genus to include the parasitic copepods coming under the family Lernaeidae. It is interesting to note that one of the species described under the new genus, Pseudolamproglena, was from India. Fish parasites of Wisconsin streams were studied by Amin (1977). Out of the fifteen species of parasites, copepoda was represented by L. cyprinacea. Kabata (1979) published a monograph on British Parasitic Copepoda . He corrected the errors and anomalies in the earlier descriptions, critically reviewed the status of genera and families and proposed a new classification. It was a milestone in parasitic copepod research.

Thatcher (1981) started studies on parasitic crustaceans of fishes from Brazilian Amazon and reported several new species. Ergasilid copepod parasites of Japanese freshwater fishes were studied by Do (1982). He prepared a valuable key for the identification of eleven species of Ergasilus reported from Japan. Kabata (1983) created two new genera of the family Lernaeidae for the description of two new species of copepod parasites on freshwater fishes from India. It clearly indicates that the freshwater copepod parasite fauna in India necessitates deep and detailed study. Kabata (1985) published a book entitled 'Parasites and Diseases of Fish Cultured in the Tropics'. This is a good guide to those interested in aquaculture practices. Byrnes (1986) reported the presence of new species of Ergasilids from Australian bream.

Studies on freshwater parasitic copepods from Indian Waters is fragmentary. Southwell and Prasad (1918) described copepod parasites

from Indian freshwater fishes. The occurrence of a new species of Ergasilus from Wallago attu was reported by Sundara Raj (1923). Contributions of Redkar et al. (1949), Rangnekar and Murthy (1950-'61) are quite significant. Karamchandani (1952) described a new species of Ergasilus and published a key for the identification of seven species of Ergasilus from India. Gnanamuthu (1951-'56) conducted studies on Lernaeid parasites infesting freshwater fishes and reported two new species of Lernaea.

Attention to the study of freshwater fish parasites is revived due to increased interest in aquaculture activities. Recently, several scientists are engaged in this field of study. Tripathy's (1966, 1969) work on the parasite fauna of freshwater fishes is noteworthy. Reports and reviews of Gopalakrishnan (1961, 1964a,b, 1966) gave good account to the damage of freshwater fishes by copepod parasites. Srinivasachar and Sundarabai (1974) conducted detailed studies on the crustacean parasites of freshwater fishes of Mysore and brought out several interesting observations on copepod parasites. Seenappa et al. (1980) described a new species of Lernaea parasitic on Catla catla (Ham) and Labeo rohita (Ham) from Karnataka.

CLASSIFICATION

The morphological variations exhibited by the adult parasitic copepods led to confusion in identifying the systematic position. Controversies and disputes regarding the position of species to a parti-

cular genus or family are not uncommon in the realm of parasitic copepods. Within fifty years, three different approaches to the classification of copepods were suggested. Wilson (1932) divided the order Copepoda into eight suborders viz; Arguloida, Calanoida, Harpacticoida, Cyclopoida, Nothodelphoida, Monstrilloida, Caligoida and Lernaeopoida. This classification was accepted by almost all workers in the field of copepod research.

Yamaguti (1963) upgraded the status of order Copepoda to that of a class and Wilson's suborders to orders. He has divided the subclass Copepoda into six orders viz; Cyclopidea, Caligidea, Philichthyidea, Andreinidea, Lernaeopodidea and Sarcotacidea.

Kabata (1979) proposed a new classification. He argued that free living copepods should also be taken into account for the classification of parasitic copepods. His suggestion is that from the point of systematics, morphological features can fall into two categories namely, primary or primitive and secondary or advanced. The differences between an ectoparasitic copepod and a planktonic copeopd are, mainly due to the accumulation of specilized features required in the process of adaptation to their respective modes of life. The primitive characters are less susceptible to parallelism and convergence. These include morphological features inherited from the ancestors and recognisably retained by the later generations. Kabata observed that the intersegmental articulation and the structure of mouth and mouth parts remain without much change in the evolutionary process of

copepods. These reliable primary characters can be taken as basic clues to the phylogeny of copepods.

According to the classification (Kabata, 1979), three suborders under the order Copepoda parasitize fishes. The suborders are Poecilostomatoida, Cyclopoida and Siphonostomatoida. Suborder Poecilostomatoida is divided into five families viz; Bomolochidae, Taeniacanthidae, Ergasilidae, Chondracanthidae and Philichthyidae. Cyclopoida consists of a single family - Lernaeidae. The families under Siphonostomatoida are Caligidae, Euryphoridae, Trebiidae, Pandaridae, Cecropidae, Dichelesthiidae, Eudactylinidae, Kroyeriidae, Pseudocynidae, Hatschekiidae, Lernanthropidae, Pennellidae, Sphyriidae and Lernaeopodidae. This classification is easy to follow, and also has advantages over the classifications suggested by Wilson and Yamaguti. It is accepted by majority of scientist in this field. So for the present study Kabata's classification is followed.

FAMILY : Ergasilidae and Lernaeidae

During the course of the present investigation parasitic copepods belonging to the genus Ergasilus von Nordmann 1832, Lamproglena von Nordmann 1832 and Lernaea Linnaeus 1758, were collected and studied in detail. The systematic position of the parasites are also discussed.

Family - Ergasilidae

Members of this family exceeds 100 species, which parasitize mainly on marine and freshwater teleost fishes. The parasitic Ergasilids

are morphologically least modified by their mode of life, resembles to free living Cyclopoid. Von Nordmann (1832) first described two species of Ergasilus; E. sieboldi and E. gibbus. Wilson (1911) published a collective account of the family Ergasilidae describing organ system, ontogeny and ecology from American waters. He suggested three subfamilies viz; Ergasilinae, Bomolochinae and Taeniacanthinae. Sars (1918) removed the genus Bomolochus from Ergasilinae and later Wilson (1932) came to the conclusion that the three subfamilies should be elevated to full family status. The important genera under Ergasilidae are, Ergasilus von Nordmann, 1832; Thersitina Norman, 1905; Pseudergasilus Yamaguti, 1936; Paraergasilus Markevich, 1937, Nipergasilus and Sinergasilus Yin, 1949; Neoergasilus Yin, 1956 and Diergasilus Do, 1981.

Genus - Ergasilus von Nordmann, 1832

It forms a major genus under the family Ergasilidae. The members of this genus exhibit broad range of morphological variations with respect to segmental boundary between the cephalosome and the leg bearing segments. The structure of appendages and number of abdominal segments are also varied. The morphology of the second antenna is quite variable and constitute one of the best characteristic features for different species. Adult Ergasilus females are usually attached to the outer surface or on the gills of the fishes whereas males remain free swimming throughout their life.

Family : Lernaeidae

The members of this family exhibit the highest degree of adaptation to parasitism. The genera under Lernaeidae belong to two different groups. Mesoparasitic group undergoes metamorphosis and their anterior part gets burried in the body of the host while the posterior part protrudes above the site of penetration. The other group that does not undergo metamorphosis are ectoparasitic, living mainly on the gills of their host. The former consists of the genus Lernaea Linnaeus, 1758; Lernaeogiraffa Zimmermann, 1922; Areotrachelus Wilson, 1924; Taurocheros Brain, 1924; Dysphorus Kurtz, 1924; Afrolernaea Fryer, 1956 and Opistholernaea Yin, 1960. The latter comprises Lamproglena Nordmann, 1832 and Lamproglenoids Fryer, 1964. Boxshall (1976) created a new genus Pseudolamproglena and described two new species, P. simplex from India and P. annulata from Iraq. Recently, two more genera were erected by Kabata (1983) for describing two new species from Indian waters. They are Pillainus volvicollis from Channa marulius and Indolernaea manohari from Mystus seenghala. At present, there are 13 genera under Lernaeidae, including Mesolamproglena Kuang, 1980.

Genus - Lamproglena von Nordmann, 1832

This genus, including more than twenty species, all parasitic on freshwater fishes, is the most primitive group of Lernaeidae. This genus was included in the family Dichelesthiidae by Fryer (1959) without

referring to the study of Sproston et al. (1950). Because of the cyclopoid nature of the developmental stages of Lamproglena, Sproston et al. (1950) removed it from Dichelesthiidae and included in Lernaeidae. Kabata (1979) also included this genus in Lernaeidae and discussed in detail the history and systematics.

Genus - Lernaea Linnaeus, 1758

It is the most popular and widely discussed genus under Lernaeidae. The members of this genus live on the body surface, gill cavity or mouth of their hosts. Reports and descriptions of the members of this genus from different parts of the world followed, the establishment of the type species Lernaea cyprinacea by Linnaeus (1758).

Hu (1948), in his paper "Studies on the Parasitic Copepods of China", discussed at length the specific and subspecific characters of L. cyprinacea and proposed five new subspecies. He used the relative position of the legs as a basis for subspecific identification and also the differences in first and second antenna. Harding (1950) recognised twentyeight species of Lernaea, of which fourteen were from the collections of the British Museum. Nine of them were new species. In addition, he synonimized seven species and prepared a key for the twentyeight recognized species. As the characters used to distinguish between different species of Lernaea were often ill-defined and not easily visible for identification, Harding suggested 'the shape of anchor and its arms' the most useful character for taxonomic purposes. The

structural differences and systematic position of Lernaeid copepod was analysed in detail by Fryer (1961a). He did not find any significance in the relative position of legs for subspecific differentiation. He proved that morphological variations exhibited by Lernaea depend mainly on the site of attachment; so, subspecific names are of no value.

Kabata (1979) reviewed the history and systematics of genus Lernaea and compiled the distribution of Lernaea in different parts of the world. He opined that seventeen species, the largest number, occur in Africa, eight species in North America, seven species in far-eastern Asia; only Europe and India have fewer species. The type species L. cyprinacea enjoys cosmopolitan distribution.

The morphological variability imposed by the host and site of attachment makes it difficult to delimit with precision, the boundaries of the species. This became quite evident after the publication of the work by Poddubnaya (1973, 1978). On Cyprinus carpio, Poddubnaya found the "classical" Lernaea cyprinacea which she referred to as "European", as well as another one, morphologically distinguishable from the first, which she named as "Asian". The latter is identical with Leigh-Sharpe's (1925) Lernaea elegans. L. cyprinacea has long 'T' shaped dorsal horns whereas in L. elegans the shape of the dorsal horns is 'Y' shaped. The first result of Puddudnaya's work was the recognition of the validity of L. elegans, which was considered synonymous with L. cyprinacea. She then conducted experiments to check the validity of some species of Lernaea employing different hosts

like Cyprinus carpio, Ctenopharyngodon idella and Carassius auratus and suggested that the name L. cyprinacea must be restricted to the parasite of the host fish Carassius auratus and all other cyprinids carry L. elegans.

Fratello and Sabatini (1972) examined the chromosome of Lernaea collected from Cyprinus carpio, Carassius auratus, Lepomis gibbosus and Gambusia affinis. The chromosomes were identical (2n=16) in all species. The authors concluded that all these hosts harboured the same species of Lernaea viz; Lernaea cyprinacea. This work appears to contradict some of Poddubnaya's findings. More work in this direction is needed for a better understanding of the systematics of genus Lernaea.

TERMINOLOGY OF CEPHALIC APPENDAGES

Homology of the cephalic appendages of parasitic copepods still remains an unsettled problem. Heegarrd (1945), Lang (1946) and Lewis (1969) studied the homology of the mouth parts of parasitic copepods, but they failed to arrive at an agreement. Kabata (1979) discussed in detail, the various aspects of copepod appendages and suggested that "we shall accept the existence of two pairs of antennae, mandible, two pairs of maxillae and one pair of maxillipeds in all the copepod species". This terminology is followed for the present study.

LIFE HISTORY

Life history studies are essential for developing methods to control copepods parasitic on fishes. Several workers contributed in this field of study. Gurney (1913) worked out the life cycle of Theristina gasterostei. Mirzoeva (1972, '73) studied the life history of Sinergasilus lieni and Zamerzlaya (1972) that of Ergasilus sieboldi. Urawa et al. (1980 a,b) successfully followed the naupliar development of Neoergasilus japonicus and compared the naupliar development of different species of Ergasilus. They suggested that there are six nauplii and five copepodid stages in the life history of Neoergasilus japonicus. Male and female could be first distinguished at the III copepodid stage and sexual maturation occurs in the adult stage. After copulation, females enter into a parasitic life.

Sproston (1942) worked out the complete series of developmental stages of Lernaeocera branchialis. One nauplius, one copepodid and four chalimus stages were followed by the adult stage. Lewis (1963) studied the life cycle of Lepeophterius dissimulatus, Wilkes (1966) that of Nectobranchia indivisa, Jones and Mathews (1968) that of Sphyrion lumpi and Izawa (1969) that of Caligus spinosus. Kabata (1972) described the life cycle of Caligus clemensi and Voth (1972) that of Lepeophtheirus hospitalis. Kabata and Cousens (1973), and Schram (1979) worked out the life cycle of Salmincola californiensis, and Lernaeenicus sprattae respectively. Kawatow et al. (1980) studied the life cycle of Alella macrotrachelus parasitic on cultured black sea bream. It

consists of one nauplius, one copepodid, four chalimus and the adult stage. Perkins (1983) worked out the life history of Cardiodectes medusaeus. Izawa (1986, 1987) described the development of Taeniacanthus lagocephali, which consists of two naupliar stages, six copepodid stages and sexual dimorphism became distinct during the third copepodid stage. He also worked out the phylogenetic implications found in the egg and naupliar stages of the Poecilostome cyclopoida.

The life cycle of commonly known species of Lernaea cyprinacea has been studied by several workers; Wilson (1917), Stolyarov (1936), Yashouv (1959), Kasahara (1962), Lahav and Sarig (1964), Rogers (1966), Bauer et al. (1973), Rukyani (1975), and Shields (1978). The most comprehensive and detailed description of the life history of L. cyprinacea has been given by Gradba (1963). The life cycle includes three naupliar, five copepodid and cyclopoid stages.

Gnanamuthu (1951 b) in his work on the life cycle of L. chackoensis suggested that the nauplius moulted only once to become metanauplius followed by the development of six copepodid stages. Fryer (1968) found that in lake Victoria, the copepodid stages of the race of L. cyprinacea infect the gills of Bagrus docmac Forskal, but in the adult stage, they parasitize Tilapia sp. In lakes Edward and George, L. barnimiana during larval stages were found on Bagrus sps., but in the adult stage lived on Tilapia sp. and Haplochromis sp. An experimental infection on Tilapia sp. proved that there is no need for an intermediate host for the completion of life cycle of L. barnimiana

(Thurston, 1969). Wilson (1917) had opined that members of the genus Lernaea needs a temporary host for the copepodid stage and a definitive host for the adult stage.

Kabata (1981) reviewed the reports and descriptions on the life cycle of parasitic copepods and suggested that most of the life cycles can be divided into four segments namely naupliar, postnaupliar, preadult and adult. With the exception of adult, these segments commonly consist of more than one stage. In order to eliminate the confusion existing in the terminology in labelling individual stages, the nauplii have been numbered I-V and the term metanauplius has been dropped. The postnauplii beginning with the first copepodid end with the last stage, earlier to the preadult. Those that remain free swimming and exhibiting no organogenetic changes foreshadowing parasitism, are designated by the name copepodid I-V. If they have become attached and enter the stage of "regressive reconstruction", they are given the name chalimus. The preadult is that part during which the copepodid either settles definitively on the host, or attain a definitive level of organization and reach the adult stage. Raibaut (1985) suggested that parasitic copepods have, in most cases, one host cycle (holoxenous), but there are species which use an intermediate host (heteroxenous) or a facultative host during their life cycle. Shariff and Sommerville (1986) studied the life cycle of L. polymorpha and L. cyprinacea and observed that the life cycles were similar irrespective of the host. The two species of parasites could not be differentiated morphologically at the larval stages. The first copepodid could not develop beyond

that stage in the absence of a host. The eggs from both species hatched into nauplii and required 14-15 days to form young female parasites at 24.3 to 29.0°C water temperature.

The life cycle of Lernaea bhadraensis was studied by Tamuli and Shanbhogue (1987). They reported that the first copepodid of this parasite can undergo successive moulting in the absence of a host and reach upto fifth copepodid stage within 17-18 days. This finding is quite interesting, since there are no reports on the survival of copepodids without host. The fifth stage lived in pond water for about 75 days without host and this also differs from earlier findings.

Nakai (1927) and Nakai and Kokai (1931) studied the development of Lernaea elegans and reported that at temperature higher than 36.5°C and lower than 10.1°C, the eggs stopped hatching and the larvae did not develop beyond the metanauplius stage at the temperature of 14°C. Kasahara (1962) observed that the newly hatched nauplii of L cyprinacea attain the sixth copepodid stage in 18 and 11 days at water temperature approximating 22 and 27°C respectively. The life span of the females is 45 days at 27°C. Rogers (1966) reported that the first copepodid stage was reached within three days after hatching in the case of L. cyprinacea. At 28°C, the time taken for the completion of life cycle is 17 days. Bird (1968) studied the life cycle of L. cyprinacea and noted that copulation took place at the fifth copepodid stage and was followed by further development, implantation and metamorphosis. The females which were unable to copulate, could not develop beyond the

fifth copepodid stage. Copulation is a trigger, activating development, but the mechanism is not clear. Thurston (1969) found that in L. barnimiana sexual dimorphism became distinct during the 5th copepodid stage and the adult female was visible to naked eye on 19th day at 21-26°C. The first egg sacs was formed on 23rd day and the life span was 32 days approximately.

According to Sarig (1971), the life cycle of Lernaea cyprinacea takes 25 days at 20°C, 20 days at 25°C, 17 days at 30°C and only 14 days at 35°C. He observed that the males and females can be differentiated during the fourth copepodid stage and copulation occurs in the cyclopoid stage, after which the males die. Al-Hamed and Hermiz (1973) showed that the life cycle of L. cyprinacea from egg to mature adult was completed in 13-14 days at 22-25°C and the first copepodid stage appeared 3-4days after hatching. Pan et al. (1979) reported that at 15-20°C, the development of L. polymorpha was completed within 14-16 days and at 26-31°C, it took only seven days. The life span of the adult female is about 20 days at temperatures of 25-37°C.

Lernaea in general has a complex life cycle, characterized by successive metamorphosis and moulting of the hard inflexible skeleton. Successive larval stages are characterised by increase in size and in number of body segments and appendages (Hoffman, 1976). Reports on the life cycle of Lernaea are contradictory in certain respcets; hence species-wise study is essential to establish suitable methods for the eradication of the parasites from fishes.

HOST - PARASITE RELATIONSHIP, PATHOGENICITY AND ECOLOGY

The copepod parasite, when it is free, must find a host and recognise whether it is suitable for infestation. The parasite has to select a precise microhabitat on the host before implantation. The susceptible mechanisms of infestation of the copepod parasites on their hosts are not known clearly. Many parasites enhance the chances of finding a new host by synchronizing the reproductive cycle with the abundance of host population. So far, this phenomenon has not been observed in the case of copepod parasites.

Knowledge regarding the mechanism of host finding by parasitic copepods are not clearly known. Fryer (1966) noted "a marked tendency for a fish which has acquired one parasite to acquire others". Shields and Tidd (1974) attributed the localization of Lernaea larvae in the mouth and branchial chambers of tadpoles associated with water currents occurring in those areas. 'Chemoreception' is suggested as a mechanism to detect the host, at least in Caligidae, by Kabata (1974, 1981). He also opined that the currents caused by the movements or respiration of the fish is one of the factors directing the copepod to the host.

Boxshall (1974a) observed that all the species of copepods, with the exception of members of the Lernaeopodidae which inhabit the gills, were attached with their anterior ends directed towards the gill arch and the body lying parallel to the primary gill filaments. Hanek and Fernando (1978 a) found that Ergasilus centrarchidarum was randomly distributed on the gills of Lepomis gibbosus, but it

preferred the dorsal and ventral sectors of the anterior halves of the hemi-branchs in Ambloplites rupestris. The preferred sites of most of the Lernaea sp. seemed to be the base of dorsal, pectoral and pelvic fins Shields and Tidd (1974) and Bulow et al. (1979). Amin et al. (1973) suggested that the site selection of L. cyprinacea was always related to body size of the host and stream conditions. As Kabata (1981) cited "site selection is undoubtedly determined by a set of morphological and physiological factors completely unknown at peresent".

Host specificity of parasitic copepods are reported by several workers (Fryer, 1968; Cressey and Collette, 1970; Hanek and Fernando, 1978 b; Kabata, 1979 and Shariff et al. 1986). Lewis et al. (1969) suggested that host preference can be indicated by the incidence of parasitism and the effect of the host is exerted not only on the sexually mature copepod but also on all stages in the life cycle, which infest the host. Although distinct host specificity among copepod occurs, further work of a statistical nature must be done with these crustaceans before we formulate significant generalizations about them (Noble and Noble, 1976).

Lernaea cyprinacea is usually reported as having a wide range of host susceptibility (Fryer, 1961 b; Demaree, 1967; Hoffman, 1967; Shields, 1968 and Poddubnaya, 1978). According to Kabata (1979), over 100 species of fishes have been recorded as hosts of this copepod. Shariff et al. (1986) reported that fish belonging to the Orders

Atheriniformes (Anabantidae and Cyprinodontidae) and Channiformes (Channidae) are uniformly susceptible to Lernaea. Among Cypriniformes only some species of Cyprinidae appear to be susceptible to Lernaea. Only one species of Perciformes, Oreochromis mossambicus (Peters) became infected by Lernaea cyprinacea.

There is a tendency that larger fishes have higher levels of paraiste infestation. This was reported by several workers; Dogeil (1961), Cressey and Collette (1970), Noble and Noble (1976), Kennedy (1975), Rawson (1977), Bortone et al. (1978), Kabata (1985) and Conneely and Mc Carthy (1985). Kabata (1981) opined that "the larger and older fish often carry great numbers of copepods is attributable mainly to the longer period of contact and larger attachment surface available for the parasite". Boxshall (1974 b,c) worked out the population dynamics of Lepeophtheirus Pectoralis (Muller) in relation to abundance, age structure and dispersion pattern.

Lower levels of parasitism in the largest size class is also observed by Noble et al. (1963), Bortone et al. (1978), Amin (1977) and Eiras (1986). Noble et al. offered a plausible explanation that older fish may develop some immunity to infestation. Shields and Goode (1978) and Shariff et al. (1986) also suggested the possible development of immunity among fishes against the infection of L. cyprinacea. Kabata (1970) reviewed the host-parasite relationship of copepod parasites and their host, remarked that the effect of parasite on host could be classified into local and general. Local effects are those limited to

the immediate vicinity of the copepod's attachment and feeding activities. The general effects are those which manifest themselves at site remote from the permanent habitat of the adult parasite.

The feeding habit of copepod parasites are also different. Ergasilus sieboldi feeds on gill epithelium, mucus gland, erythrocytes and white blood cells (Einszporn, 1965 a,b). Deeply penetrated copepods such as Leraneidae feed on tissue debris and blood (Fryer, 1968). Meyer (1966) observed that the sites of attachment of Lernaea cyprinacea are usually accompanied by acute haemorrhagic reactions which frequently become foci for secondary infections by bacteria and fungi. If the parasites are attached near nerve centres, such as brain or along the lateral line, the infested fish will frequently swim in a tight circle or exhibits convulsive movements. Shields and Goode (1978) described the formation of thickened whorls of hyperplastic epithelium and fibrous tissue around the parasite Lernaea cyprinacea on Gold fish. Radhakrishnan and Nair (1981) showed that Lernanthropus gibbosus and Lernanthropus koenigii were of serious concern to their hosts, causing irrepairable damage to the gills by way of their mode of attachment and feeding activity. Noga (1986) recorded Lernaea cruciata, as an important initiator of skin lesions in large mouth bass.

Thurston (1965) worked out the pathogenicity of crustacean parasites and suggested that parasitic infection occur most readily in crowded condition. Damages to aquaculture industry by Lernaea sp. have been reported by several workers, Nakai (1927), Fryer (1968),

Lahav and Sarig (1964), Paperna and Thurston (1968), Sarig (1971), Kabata (1985) and Shariff et al. (1986). High mortalities have occurred among cultured cat fish, gold fish, baitminnows, carp, trout and other fishes due to Lernaea infection (Post, 1983).

Srinivasachar and Shakuntala (1975) found that Lebistes reticulatus infested by Lernaea hesaragattensis consumed more oxygen than uninfected fish. It can be attributed to a 'stress reaction' due to parasitic infestation of the fish. Esch et al. (1975) analysed the relationship between stress and parasitism, suggested that the impact of stress upon the dynamics of host and parasite populations were varied. Such studies regarding copepods and their hosts are wanting.

The ecology of the host has a great significance in the variation of the rate of infestation by copepod parasites in terms of individual host. It is not merely the host but also the host's environment, that forms the environment of the parasite. This is especially true for the ectoparasites of aquatic animals. For parasite, the "microenvironment" is the host and the "macroenvironment", host's habitat (Dogiel, 1961). In the Victoria Nile, where the water was swift, Lernaea barnimiana occurred in abundance in the mouth, but on the same host in still-water conditions, most parasites were found at the base of the fins or flanks (Fryer, 1968). This indicated the influence of external environment in the selection of site with respect to host's habitat. Margolis et al. (1982) worked out definitions of a few terms used by parasitological ecologists.

The effect of temperature on the development of parasitic copepods has been reported by Grabda (1963), Shields and Tidd (1968), Pan et al. (1979) and Kabata (1985). According to Shields and Tidd (1968), in Lernaea cyprinacea, egg production will not occur at temperatures below 24°C, no naupliar development below 20°C and copepodid development and penetration to host not below 15-520°C. They suggested that the most successful laboratory cultures of Lernaea can be maintained between 24 and 29°C. Lernaea may overcome the winter seasons as larval females embedded within the tissue of the host (Hoffman, 1976).

Studies by shields and Sperber (1974) revealed that Lernaea has a limited tolerance to increased salinities. Hoffman (1976) reported that adult Lernaea were not found in waters with salinity greater than 1.8% and larvae proved even more sensitive. In addition, they were not found in water with pH lower than 7. Seenappa et al. (1985) observed mortality and reduced hatchability of Lernaea at acidic pH ranges. Lernaea is considered as a typical freshwater form but its occurrence was noticed in Philippines on a brackish water fish Chanos chanos (Kabata, (1985).

Srinivasachar and and Sundarabai (1974) observed that incidence of Lernaea hesaragattensis, parasitic on Lebistes reticulatus was highest during July and lowest in January. Viljoen (1985) carried out seasonal investigation of the genus Lernaea and established that infestation and

site preference are related to host and season; body dimensions of the parasite also varied seasonally.

Timmons and Hemstreet (1980) studied the prevalence rate of L. cyprinacea on Micropterus salmoides (Lacepede) and reported that no fish smaller than 25 mm. or longer than 99 mm. were infected. Uehara et al. (1984) observed that Lernaea infection was different for the four species of fishes, Carassius auratus L., Salmo gairdneni Richardson, Micropterus salmoides Lacepede and Tinca tinca L. The prevalence rate was 80%, 78%, 12% and 0% respectively.

TREATMENT AND CONTROL

Kabata (1985) defined therapy as treatments intended to restore normal health to fish that have contracted disease. It is an interplay of three factors namely the pathogen, the fish and the therapeutic agent or medication. The choice of medication is based on the nature of the pathogen, that must be lethal to the pathogen but harmless to the fish.

Efforts for the control of crustacean parasites of fishes probably date back to fish culture practices. The first therapeutic measures were natural organic compounds. They were followed by chemicals and synthetic compounds (contact insecticides) of ever increasing complexity. The compounds used for the control of parasitic crustacea fall into D.D.T. Group, B.H.C. Group and Organophosphate group.

The therapy can be divided into three categories: (I) addition of chemicals to water, (2) addition of chemicals to feed, (3) application of chemicals directly to fish. Method of large scale treatment of pond has been considered best-suited for controlling parasites like Argulus and Lernaea. Sarig (1971) and Roberts and Shepherd (1974) have documented the requirements and precautions, while applying chemicals to pond water.

Eradication of crustacean parasites has been achieved by bath treatment. It can be divided into three categories: dips, short baths and long baths. Dips involve immersion for not less than five minutes, short baths last 5-60 minutes and longer treatments are considered as long baths. Fairly comprehensive reviews of measures against Lernaea have been published by Kabata (1970, 1985) and Hoffman and Meyer (1974).

Various chemicals have been tried to control and eliminate Lernaea. The easily obtainable and cheap common salt (NaC1) is one of them. This can be used for fishes which are able to tolerate salt concentrations required to kill the parasite. It has been reported to be quite useful against free swimming larval Lernaea, but less effective against attached females.Putz and Bowen (1964) suggested the use of 0.8 to 1.1% salt solution for three days for the eradication of Lernaea. Shilo et al. (1960) showed that salt solutions in 25,000 ppm. concentration have an adverse effect on the juveniles of Lernaea, but not harmful to them beyond the point of recovery.

Potassium permanganate ($KMnO_4$) has been used to control Lernaea in many parts of the world, applied in a multiplicity of ways. But, it has not been proved uniformly successful due to several reasons. The standard method of treatment using ($KMnO_4$) has been compiled by Sarig (1971). Carp infected with Lernaea can be treated in tanks in which weight of fish to water ratio is not less than 1:2.5. The concentration of $KMnO_4$ is slowly built up by gradual additions of the stock solution until it reaches 25 ppm. in 15 minutes (Kabata, 1970). After an interval of 20 minutes this procedure is repeated. Bath of 10 ppm. for periods between 50-60 minutes was recommended by some authors. A concentration of 2 ppm. can be used for indefinite period (Putz and Bowen, 1964). Sarig (1971) treated infected carps with 20 ppm. of $KMnO_4$. After 60-120 minutes of exposure, 90-100% of the adult Lernaea found on fish were killed. He also reported that $KMnO_4$ affects only the adult Lernaea and does not kill young parasites embedded in the skin of the fish. Pan et al. (1979) reported that bathing fish in a solution containing 12.5 pp. of potassium permanganate at water temperature 24-30°C was effective in killing the adult parasite without serious injury to host.

Formalin treatment is found to be successful only on the free swimming larval stages of Lernaea. Putz and Bowen (1964) reported the use of 30 - 60 minute baths at the concentration of 250 ppm. Baths were repeated every three weeks as long as necessary. Ravindranath et al. (1985) suggested a unique method of treatment of Lernaeosis using formalin and lime.

The use of D.D.T., B.H.C. and organophosphates were proposed by Schaperclaus (1954), Shilo et al. (1960), Meyer (1966), Sarig (1971), Gopalakrishnan (1964 b), Lahav et al. (1964), Pan et al. (1979), Kabata (1985), .Ali (1986) and Shariff et al. (1986).

Several plant-derived substances were used for the eradication of Lernaea. The oldest known plant remedy is the teaseed cake or meal used in China for many years. Chen (1933) questioned its efficiency, though some Chinese farmers still use them. Bundles of castor plants kept immersed in water are also considered effective against Lernaea (Kabata, 1985).

Chinese experts have reported the successful use of another plant Gelsenium elegans (cited by Kabata, 1985) for the eradication of Lernaea. Similarly, use of Acanthopanax spinosus (75-100 Kg/acre) resulted in complete eradication of Lernaea.

Though, biological control measures have been developed for the eradication of several pests, it has not been explored for the elimination of parasitic copepods. Kasahara (1962) observed that a planktonic copepod, Mesocyclops, was a main predator of free swimming larval stages of Lernaea. Recent Indonesian work (Kabata, 1985) on the susceptibility of various fish species to Lernaea suggests that Tilapia nilotica is more resistant to infection than other fishes tested. Carp, along with T. nilotica, reared in a pond were less infected than those reared alone. Further investigation in this direction is essential to find out a cheap and harmless method for the eradication of Lernaea.

CHAPTER - II

SYSTEMATICS

SYSTEMATICS

INTRODUCTION

Parasitic copepods of freshwater fishes in India were studied by Southwell and Prasad (1918), Gnanamuthu (1951 a, 1956), Karamchandani (1952), Tripathi (1966), Srinivasachar and Sundarabai (1974), Seenappa et al. (1980) and Nandeesha et al. (1984, 1985). Knowledge regarding the copepod parasites of freshwater fishes in Kerala is scanty. The aim of the present study is to investigate the parasitic copepods of freshwater fishes in Kerala.

The specimens described in this chapter were collected by examining fishes from freshwater fish landing centres and fish markets in different parts of Kerala. The present collection consists of five new species which are described in detail. Holotypes and allotypes will be deposited in the National Museum, Calcutta and paratypes will be deposited in the Museum of the Department of Industrial Fisheries, Cochin University of Science and Technology, Cochin.

CLASSIFIED LIST OF SPECIES

Order Copepoda

Suborder Poecilostomatoida

Family Ergasilidae

Genus Ergasilus von Nordmann, 1832.

Ergasilus thammani sp. nov.

Ergasilus vembanadi sp. nov.

Ergasilus kabati sp. nov.

Suborder Cyclopoida

Family Lernaeidae

Genus Lamproglena von Nordmann, 1832

Lamproglena krishnai sp. nov.

Genus Lernaea Linnaeus, 1758

Lernaea osphronemi sp. nov.

LIST OF HOSTS AND THEIR PARASITES

Channa striatus (Bloch)

Lamproglena krishnai sp. nov.

Mugil cephalus Linnaeus

Ergasilus thammani sp. nov.

Ergasilus kabati sp. nov.

Osphronemus goramy Lacepede

Lernaea osphronemi sp. nov.

Puntius sarana (Hamilton-Buchanan)

Ergasilus thammani sp. nov.

Wallago attu (Bloch and Schneider)

Ergasilus vembanadi sp. nov.

DESCRIPTION OF SPECIES

Order Copepoda

Suborder Poecilostomatoida

Family Ergasilidae

Genus Ergasilus von Nordmann, 1832

Ergasilus thammani sp. nov.

Figs. 1-12

Material:

Twentytwo females were collected from the gills of Mugil cephalus Linnaeus, from the freshwater fish landing centres at Thevara, Vaikom and Trivandrum and nine females from the gills of Puntius sarana (Hamilton-Buchanan) at Thammanam fish market.

Female:

Cophalothorax longer than broad, nearly rounded anteriorly and concave posteriorly with a medio-lateral constriction. Second to fifth leg bearing segments gradually decreasing in size posteriorly. Fifth segment very short and narrow. Genital segment broader than long and cup-shaped. Abdomen three-segmented, each segment somewhat narrower than the preceding one and the terminal segment with a medial cleft. Uropod squarish each with four terminal setae, the innermost one highly elongated, the other three smaller and subequal in length.

First antenna: Five-segmented, segments distinct, tapering terminally with several setae of varying length. Basal segment stout, longer than

broad and bearing the maximum number of setae. Second segment broader than the third and fourth segments. Fifth segment narrow compared to all other segments.

Second antenna: Four-segmented, first segment broad, stout and devoid of any armature. Second segment very long with a sensillum on the distal half of the medial margin. Third segment long and slender, slightly curved with a sensillum on the medial margin of the proximal half. Distal segment a strong stout claw, bearing small spinule on the disto-lateral margin.

Mandible: Unsegmented, subrectangular with two distal falciform blades; inner blade extends from a rectangular stalk-like structure and curved anteriorly. The inner and outer blades provided with rows of short setae on the ventral margins.

First maxilla: Very small, orbicular, with two setae of unequal length.

Second maxilla: Two-segmented, basal segment broader than long and stout. Distal segment falciform, long and thickly beset with spiniform setae dorsally.

Maxilliped: Absent.

First Leg: Sympod two-segmented. Coxa unarmed, basis with a single plumose seta on the lateral margin to the base of the exopod. Exopod

three-segmented. Basal segment broad, longer than the second and third segments combined, with a short spine distally. Second segment longer than the third bearing plumose seta on the inner margin. Terminal segment short with two spines and five plumose setae. Endopod three-segmented, basal segment longer than the second, bearing one plumose seta. Second segment slightly shorter than distal with a single plumose seta. Terminal segment longer than broad with two broad, spatulate spines unequal in length and four plumose setae. The inner margin of the basal segment of exopod and the outer margin of the basal and second segments of endopod with fine hairs.

Second leg: Coxa unarmed. Basis with single plumose seta lateral to the base of the exopod and a denticular patch in front of the endopod. Exopod three-segmented, first segment long and stout with a single spine distally. Second segment slightly longer than third, bearing a single plumose seta on the inner margin. Third segment short carrying six plumose setae distally. Endopod also three-segmented. Basal segment with an inner plumose seta, second segment with two plumose setae and distal segment with one spine and four plumose setae. The inner margin of the basal segment of exopod and outer margin of the first and second segments of endopod bearing fine hairs.

Third leg: Coxa unarmed. Basis bearing a small plumose seta on the outer margin and a denticular patch on the inner margin. Exopod three-segmented. Basal segment long and stout, with an outer spine distally and a row of fine hairs on the inner margin. Second and third

segment subequal in length. Second segment with a single plumose seta and distal segment carrying six plumose setae. Endopod also three-segmented. The outer margin of the first and second segments bearing hairs. The segments are subequal in length, basal segments with a single plumose seta, second segment carrying two plumose setae and distal segments with one spine and four plumose setae.

Fourth leg: Coxa unarmed. Basis with a single plumose seta to the base of the exopod. Exopod two-segmented. First segment long with a single spine on the distal margin and fine hairs on the inner margin. Second segment short, carrying five plumose setae distally. Endopod three-segmented and segments decreasing in length distally. First segment with a single plumose seta, second segment bearing two plumose setae and terminal segment carrying one spine and three plumose setae. First and second segments with fine hairs on the outer margin.

Fifth leg: Two-segmented. Basal segment very small carrying a plumose seta. Second segment long, suboval in shape with two plumose setae distally.

Armature of the rami as follows

Arabic numerals denote setae: Roman numerals denote spines.

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-0	0-1	II-5
II Leg	0-1	0-2	I-4	I-0	0-1	0-6
III Leg	0-1	0-2	I-4	I-0	0-1	0-6
IV Leg	0-1	0-2	I-3	I-0	0-5	-

Ergasilus vembanadi sp. nov.

Figs. 13-24.

Material:

Twenty females were obtained from the gills of Wallago attu (Bloch and Schneider) from Ernakulam fish market.

Female:

Cephalothorax longer than wide and dome shaped anteriorly. Cephalic fusion with the first thoracic segment marked by a groove dorsally. Cephalon broader at the region of fusion and provided with a pair of tiny setules dorso-laterally. Second to fifth legs bearing segments gradually diminishing in size posteriorly. Ventrally each segment except the fifth leg bearing segment carrying single row of fine spinules anteriorly. Genital segment barrel shaped, ventrally with several rows of fine spinules. Abdomen three-segmented. Each segment ventrally bearing a row of fine spinules, extending near anterior margin. Uropods squarish in shape, each with four terminal setae.

First antenna: Six-segmented, basal segment broader than long, second segment stout and thick, succeeding segments decreasing in width, each segment with numerous simple setae of varying length.

Second antenna: Four-segmented. Basal segment short and stout. Second segment longer with a sensillum on the distal half of the medial margin.

Third segment slender and curved. Distal segment is a sharp and strong claw.

Mandible: Indistinctly two-segmented. Basal segment massive, from the base of the distal segment arises an elongated spine, bearing hairs ventro-laterally; terminal spine beset with fine hairs, posterior to the spine a large falciform blade fringed with hairs. The mandibular palp attached to the basal segment with hairs on its inner margin.

First maxilla: Round in shape, armed with two long setae.

Second maxilla: Two-segmented. Basal segment broad and thick and the terminal segment thickly packed with spiniform setae dorsally.

Maxilliped: Absent.

First Leg: Sympod two-segmented. Coxa devoid of ornamentation. Basis with a plumose seta on the lateral margin and fine spinules on the anterior margin. Exopod three-segmented. Basal segment longer than broad with a spine distally, the spine and distal half of the segment denticulated. Second segment about half the length of the basal segment with a single plumose seta on the inner margin and a row of denticles on the inner and outer side of the outer border. Terminal segment small, bearing two spines with serrated flange and five plumose setae. The inner margin of the first and second segment with fine hairs. Endopod three-segmented. Basal segment longer than broad with small

denticles and fine hairs on the outer margin and a plumose seta on the inner margin. Second segment smaller than basal with a row of fine hairs on the outer margin and single plumose seta on the inner margin. Distal segment equal in size to the second segment, bearing two subequal spine having denticular flange and four plumose setae. The outer margin with a row of denticles.

Second leg: Coxa devoid of armature. Basis with a plumose seta on the lateral margin and fine spinules on the anterior margin. Exopod three-segmented. Basal segment longer than broad carrying a single spine on the outer margin and fine hairs on the inner margin. Second and third segments with very thin serration on the outer margin. The second segment carrying a single seta and fine hairs on the inner margin. Terminal segment small with six setae. Endopod three-segmented. First segment stout and long with a single seta. Second and third segment almost equal in size, second segment with two setae and distal segment carrying a strong spine and four setae. The outer margin of all the segments with a row of spinules and fine hairs. All setae are plumose.

Third leg: Sympod two-segmented. Coxa without any armature. Basis with a small plumose seta lateral to the base of the exopod and carrying fine spinules on the anterior margin. Exopod three-segmented. Basal segment longer than the second and third segment combined together, with a distal spine. Second segment with a single seta on the inner margin. Distal segment short with a single spine and six

setae. The inner margin of the basal and second segment carrying fine hairs. Endopod three-segmented. Segments decreasing in length distally. Basal segment with a single seta, second segment bearing two setae and distal segment with a strong spine and four setae. The outer margin of all the segments with a row of spinules and fine hairs. All setae are plumose.

Fourth leg: Coxa unarmed, basis with a lateral plumose seta and fine spinules on the anterior margin. Exopod two-segmented. Basal segment long and stout with an outer spine. Distal segment short and carrying one spine and five setae. Endopod three-segmented, segments subequal in length. Basal segment with a single seta, second segment with two setae and terminal segment with one long spine and three setae. The inner margin of the first exopodal segment and the outer margin of all the endopodal segments bearing fine hairs. All setae are plumose.

Fifth leg: Single-segmented, longer than broad and carrying two subequal plumose setae.

Armature of the rami as follows:

Arabic numerals denote setae: Roman numerals denote spines.

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-0	0-1	II-5
II Leg	0-1	0-2	I-4	I-0	0-1	0-6
III Leg	0-1	0-2	I-4	I-0	0-1	I-6
IV Leg	0-1	0-2	I-3	I-0	I-5	-

Uropod: Squarish with an elognated medial plumose seta and three subequal setae, the central one elongated and plumose.

Total length: 0.8 mm - 0.9 mm.

Male: Unknown

Remarks:

Ergasilus vembanadi sp. nov. resembles Ergasilus thammani sp. nov. in its general body shape. In E. vembanadi, the cephalothroax provided with a pair of tiny setules dorso-laterally, which is absent in E. thammani. The first antenna in the present specimen is six-segmented, but in E. thammani, it is five-segmented. The second antenna in E. thammani bearing sensillum on the second and third segments and a spinule on the claw, whereas in E. vembanadi, only the second segment of the second antenna carrying a sensillum. A leaf like maxillary palp present in the new species is absent in E. thammani. The first leg endopodal spines in E. thammani are spatulate whereas in E. vembanadi, the spines are with serrated flanges. The ornamentation of thoracic legs in both these species are also different. In E. vembanadi all the thoracic segments except the fifth leg bearing segment, the genital segment and the abdominal segment carrying fine spinules ventrally, whereas spinules are absent in E. thammani. The present species can be separated from all other species in this genus by the general shape of the body, presence of a single sensillum on the second segment of

the second antenna, structure of the first maxilla, first leg exopodal spines with serrated flange and the ornamentation on the body and thoracic legs.

Ergasilus kabati sp. nov.

Figs. 25 - 36.

Material:

Twelve females were collected from the gills of Mugil cephalus Linnaeus from Murinjapuzha fish landing centre.

Female:

Cephalon broader than long and rounded on either sides anteriorly and distinct from the posterior part by a dorsal groove. Cephalothorax longer than broad and laterally with a constriction in the middle. Second to fifth thoracic segments gradually decreasing in size, fifth segment comparatively very small. Genital segment longer than broad and slightly expanded anteriorly. Abdomen three-segmented. First segment double the length of the other two, distal segment short. Uropod short, inner distal corner produced into an elongated seta, laterally a stout spine-like process and two setae of subequal length.

First antenna: Six-segmented with several setae of varying length. First segment broader than long, second segment very stout, succeeding

segments gradually decreasing in size and terminal segment slightly longer than broad with apical setae.

Second antenna: Five-segmented, basal segment squarish, second segment longer than broad, third segment more than double the length of the second, fourth segment slender and slightly curved and fifth segment a curved claw. Second, third and fourth segments enveloped in a thin sheath, which is wrinkled randomly.

Mandible: Slender, elongated with indistinct segmentation, terminally bears a large and a small falciform blade with fine hairs. From the postero-medial part of the mandible arises an elongated palp with short hairs. A protruberance from the middle of the mandible bears a leaf-like mandibular palp.

First maxilla: Semicircular with two setae of unequal length.

Second maxilla: Indistinctly two-segmented, basal segment broad and subrectangular. The terminal segment anteriorly curved and thickly beset with spiniform setae on the dorsal surface.

Maxilliped: Absent.

First leg: Sympod two-segmented, coxa unarmed and basis carrying a single plumose seta on the lateral margin. Exopod three-segmented. Basal segment longer with an outer spine. Second segment shorter than

first and bearing a single plumose seta on the inner margin. Third segment broader than long with two spines serrated on the outer margin and five plumose setae. The outer border of the first and second segments with row of small denticles. Endopod three-segmented. Basal segment with a single plumose seta, second segment shorter than first and carrying a single plumose seta, distal segment equal in size to the second, bearing four plumose setae and two subequal spine surrounded by serrated membrane.

Second leg: Coxa unarmed, basis with a plumose seta posterior to the base of the exopod. Exopod three-segmented. First segment longer with a distally placed spine. Second and third segments almost equal in size. Second segment bearing a single plumose seta and the distal segment with six plumose setae terminally. The outer margin of each segment with a single row of small denticles. Endopod three-segmented. Basal segment comparatively long with a single plumose seta. Second and third segments equal in length, second segment carrying a single plumose seta and distal segment bearing a single spine and four plumose setae.

Third leg: Coxa without oranmentation, basis with a single plumose seta on the lateral margin. Exopod three-segmented. Basal segment long with a distal spine. Second segment broad with a single plumose seta. Third segment short with six plumose setae distally. Endopod three-segmented. Basal segment long bearing a single plumose seta. Second and third segment equal in size. Second segment carrying a single plumose seta and distal segment carrying a spine and four plumose setae.

Fourth leg: Basis carrying a plumose seta posterior to the base of the exopod. Exopod two-segmented. Basal segment slightly longer than the distal with a single spine. Distal segment short carrying five plumose setae. Endopod three-segmented. Segments subequal in length, basal segment carrying a single plumose seta, second segment with two plumose setae and terminal segment bearing one spine and three plumose setae.

Fifth leg: Single segmented, longer than broad with two subequal plumose setae posteriorly and a small naked seta postero-laterally.

Armature of the rami as follows:

Arabic numerals denote setae: Roman numerals denote spines.

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-0	0-1	II-5
II Leg	0-1	0-1	I-4	I-0	0-1	0-6
III Leg	0-1	0-1	I-4	I-0	0-1	0-6
IV Leg	0-1	0-2	I-3	I-0	0-5	-

Upropod: Roughly squarish, bearing an inner elongated plumose seta, a stout spine-like structure and two subequal setae in the distolateral margin.

Total length 0.9 mm - 1 mm.

Male: Unknown.

Remarks:

Ergasilus kabati sp. nov. Though having all the characters of the genus Ergasilus lacks close morphological resemblance with any of the species in this genus. The anterior portion of the cephalon broad and rounded laterally. Cephalothorax longer than broad with a lateral constriction in the middle. The genital segment differs from all other known species. This species can easily be identified by the peculiar nature of the second antenna with sheathing and the prominent spine-like structure on the uropod.

Suborder CYCLOPOIDA

Family Lernaeidae

Genus Lamproglena von Nordmann, 1832

Lamproglena krishnai sp. nov.

Figs. 37 - 46

Material:

Eight females were collected from the gills of Channa striatus (Bloch) from a freshwater pond at Cochin.

Female:

Body elongate, cylindrical, narrowing towards the hind end. Head comparatively small, antero-lateral parts expanded and fused with the thoracic segment, having a pair of fleshy lobe at the anterior end. First thoracic segment free, broader than long. Second, third and fourth

thoracic segments are stout, forming a pear-shaped trunk. Fifth thoracic segment broader than long, free from the trunk and genital segment. Genital segment clearly separated from the fifth thoracic segment, also longer than broad, but gradually tapering posteriorly, having a prominent lateral constriction near the center. Abdomen clearly three-segmented. First and second segment cylindrical, distinctly separated and equal in length. Third segment gradually narrowing posteriorly and having the combined length of the first two segments. Posterior end of the third segment bifurcated to form the uropod.

First antenna: An elongated structure with indistinct segmentation having five naked setae on the middle and four on the tip.

Second antenna: Shorter than the first antenna having two terminal naked setae.

Maxilla: Basal segment broad and stout having a slender winged claw pointed towards the tip.

Maxilliped: Two-segmented, basal segment very stout. Distal segment short, but slightly longer than broad. Terminally the segment bears four stout and strong distally curved subequal claws.

First leg: Biramous, basipod stout, with a pectinate ridge near the postero-ventral margin. Exopod distinctly three-segmented, first segment with an outer spine distally and an inner naked seta in the middle.

Second segment having an inner naked seta. Third segment with two subterminal stout spines and four elongated naked setae. Endopod three-segmented, outer margin of the segments with toothed ridges. First and second segments bearing a single seta and the third segment with four setae of subequal length on the disto-lateral margin.

Second leg: Biramous, exopod three-segmented, the basal segment of the exopod is comparatively very stout with an outer spine and an inner naked seta. Second and third segments subequal in length.. Second segment with an inner median naked seta. Terminal segment with three naked setae and a terminal spine. Endopod three-segmented, outer margin of the endopod with toothed ridges and inner margins armed with setae. First and second having one each and third having three naked setae.

Third leg: Biramous, exopod three-segmented, basal segment stout, other two segments comparatively short. First segment with a spine on the outer margin and a naked seta on the inner margin. Second segment with an inner naked seta. Terminal segment with two stout claw-like spine and four naked setae. Endopod three-segmented. First and second segments with single naked seta on its inner margin. Third segment with four naked setae antero-laterally. The outer margin of all the segments toothed.

Fourth leg: Biramous, exopod three-segmented, basal segment of the exopod long and stout, armed with an outer spine and an inner naked seta. Second and third segments subequal in length. Second segment

with a naked seta on its inner margin. Terminal segment with two stout terminal claw-like spine and the inner margin with three naked setae. Endopod three-segmented, outer margin of all segments toothed. Inner margin of the first and second segments each with a naked seta. Terminal segment with three naked setae.

Fifth leg: Uniramous, two-segmented; basal segment with two naked setae. Terminal segment armed with two naked distal setae.

Uropod: Fused with the abdomen, conical in shape, diverging each other from the postero-median line of the abdomen, having a spine on the base and distally with three setae of which the central one is longer.

Total length: 3 - 4 mm.

Remarks:

Lamproglena krishnai sp. nov. shows resemblance to L. ophiocephali Yamaguti (1939) in its general body shape. But the present species differs clearly in various other details. In L. ophiocephali the genital segment is fused with the thoracic segment and the abdomen is indistinctly sgemented, whereas in L. krishnai sp. nov. the genital segment is distinctly separated from the thoracic segment and the abdomen clearly three segmented. The uropod of the new species completely fused with the abdomen and conical in shape; but in L. ophiocephali the uropod is separated from the abdomen and oval in shape. In L. ophiocephali, in all the thoracic legs both the exopod and endopod are two-segmented,

whereas in L. krishnai in all the legs these are distinctly three-segmented. Postero-ventral margin of the basipod of the first leg alone is pectinate in the present species, whereas in L. ophiocephali basipods of all the legs carry pectination. In the present case, the arrangement of setae and spines on the legs are entirely different from L. ophiocephali. L. krishnai sp. nov. differs from all other known species of the genus by its three-segmented nature of leg rami, distinctly segmented abdomen and the number of spines and setae present on the legs.

Genus Lernaea Linnaeus, 1758

Lernaea osphronemi sp. nov.

Figs. 48 - 64.

Material:

Large number of femals were collected from the body surface of Osphronemus goramy Lacepede, from a freshwater fish pond of State Fisheries Department at Pannivelichira, Kerala, India.

Post-metamorphosis adult female:

Cephalothorax hemi-spherical, small, anterior to hold fast, bearing antennae and mouth parts. Holdfast consisting of two pairs of horns dorso-ventrally placed, ventral branches slightly longer than dorsal. The branches are simple with blunt tips. Variations in the structure

of holdfast is noticed among infrapopulations. Body subcylindrical, unsegmented and gradually expanding in breadth posteriorly. Neck comprising of second to fourth leg-bearing segments and circular in cross section. At the region of the second pair of leg, there is a node-like swelling, while at the places of third and fourth legs very slight constrictions are visible laterally. Behind the fifth pair of legs lies and pregenital prominence which is hemispherical and bilobed. Abdomen sub-conical with two constrictions ventrally giving the appearance of three segmented nature and carrying small, single-segmented uropods distally.

First antenna: Uniramous, three-segmented, basal segment is very long and equal in length of the second and third segments combined, bearing nine short setae and a long one on the distal part. The second segment is short, bearing three small and a long setae. The third segment longer than the second, carrying ten setae of varying length, seven are apical in position, out of which two are thicker and longer than the others.

Second antenna: Uniramous, two-segmented, segments subequal in length. Basal segment unarmed. Distal segment with two small setae along the posterior margin, a claw-like spine and four slender setae distally.

Labrum: Small flattened semicircular plate, overlying the mandible and first maxillae.

Second maxilla: Two-segmented, the basal segment very stout and broad, distal segment small with two curved strong claws.

Maxilliped: Two-segmented, basal segment broad and stout with a small papilla armed with an apical setule, and slightly projected on the median margin of the distal part. Terminal segment comparatively very short bearing one short curved and four large subequal strong claws at its anterior margin.

Thoracic legs: First four pairs of legs are biramous. Rami three-segmented. Sympod two-segmented. Proximal segment with a small plumose seta on the inner margin distally. Distal segment with a fine seta lateral to the base of the exopod. In the first leg a curved spine-like structure present at the medio-lateral margin of the distal segment.

Fifth leg: Reduced, one-segmented with four small setae of unequal length on the distal margin.

Sixth leg: It is represented as a single plumose seta.

Armature of the rami as follows:

Arabic numerals denote setae: Roman numerals denote spines.

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-1	I-1	II-5
II Leg	0-1	0-2	II-4	I-1	I-1	III-5
III Leg	0-1	0-2	II-4	I-1	I-1	III-5
IV Leg	0-1	0-2	II-3	I-1	I-1	III-5

Uropod: Subcylindrical, each bearing a long straight plumose seta extended posteriorly, two small setae on either side and another small seta on the medio-lateral margin.

Total length: 6 - 9 mm.

Remarks:

A total of nine species of Lernaea have been recorded from India and South-East Asia. They are L. cyprinacea Linnaeus (1758), L. oryzophila Monod (1932), L. polymorpha Yu (1938), L. lophiara Harding (1950), L. chackoensis Gnanamuthu (1951) a) L. bengalensis Gnanamuthu (1956), L. arcuata Soejanto (1965), L. hesarangattensis Srinivasachar and Sundarabai (1974), L. bhadraensis Seenappa, Manohar and Shetty (1980). The present new species shows resemblence to L. lophiara and L. bengalensis in its general body shape. The ventral arms of the holdfast slightly shorter than dorsal in L. lophiara, whereas in L. osphronemi the dorsal arms are shorter than ventral and also differs in other characters. L. osphronemi resembles L. bengalensis in the shape of the holdfast, but the holdfast arms are non-variable in shape in L. bengalensis whereas in the new species, holdfast exhibit variations among the members of infrapopulation (Margolis, et al. 1982). In the present species variations such as bifurcations and branching of the tip of the arms, differences in the length-breadth proportions of the arms were observed. In addition, it differs from L. bengalensis in the segmentation and arrangement of spines and setae of first and second antennae. Fifth and sixth legs are absent in L. bengalensis whereas it is present in L. osphronemi. The pregenital prominence in L. bengalensis is pressed

together to form a 'heel', but in the present species, the pregenital prominence is bilobed and distinct. Considering the structure of the holdfast, segmentation and setation of appendages, the present one differs from all other known species of Lernaea.

CHAPTER - III

LIFE HISTORY OF *LERNAEA OSPHRONEMI* sp. nov.

LIFE HISTORY OF LERNAEA OSPHRONEMI sp. nov.

INTRODUCTION

Studies on the life cycle of L. cyprinacea was carried out by several workers such as Wilson (1917), Yashouv (1959), Grabda (1963), Lahav and Sarig (1964), Bauer et al. (1973), Rukyani (1975), Shields (1978) and Shariff and Sommerville (1986). In India, the life history of L. chackoensis was worked out by Gnanamuthu (1951 b). Tamuli and Shanbhogue (1987) recently published an account on the larval stages of L. bhadraensis.

The present study is to establish the life cycle of L. osphronemi sp. nov. and to compare various aspects of its life history with that of other Lernaeid species. The morphological changes in each stage were studied in detail to understand the growth of larval stages. The effect of temperature on larval development is also discussed. The time taken for the completion of life cycle, the life span of the adult parasite and the breeding activity of the female parasite were studied. This information is inevitable for the effective control of this parasite in fish culture ponds.

MATERIALS AND METHODS

Copepod parasite Lernaea osphronemi with mature egg sacs were collected from Osphronemus goramy from the fish pond of the Fisheries Department

of the Government of Kerala at Pannivelichira in Pathanamthitta district and brought to the laboratory for the life cycle study. The parasites were transferred to petridishes and egg sacs were separated. The egg sacs collected were kept in petridishes containing dechlorinated tapwater for hatching. The larvae from the petridishes were released into large fiber glass aquariam tanks containing Osphronemus goramy of 8-15 cm. length and were allowed to infest the fish. This host-parassite system formed the stock population. If an infected fish died, it was replaced by another fish. Heavily infected fishes were taken from the tanks and placed separately in glass aquariam of size 75 x 30 x 30 cm. The matured egg sacs, greenish in colour, were plucked from the parasites. For convenience five pairs of egg sacs were put together in petridishes containing 60 ml. of dechlorinated water. The eggs were observed constantly under a stereo-dissection microscope to study the hatching and larval development upto I copepodid stage. After 12 hours all unhatched eggs and remanants of the hatched egg sacs were removed from the petridishes.

Previous studies on the life cycle of the genus Lernaea indicate that I copepodid is the infective stage. To test whether the I copepodid of L. osphronemi can develop without a host, about hundred larvae were released into a breaker containing water without host fish and observed every 24 hours.

To study the further sequence of moult and larval stages, two methods were followed. In the first method, one litre glass beakers (total Nos.

36) each with a single host fish of 2-4 centimetres in length were employed. Fifty numbers of I copepodid were released into each beaker. At the time of larval release, the beakers were filled with only 250 ml. of water to observe the activities of the larvae in the container by using a stereo-dissection microscope. Within 20 minutes after the release, the larvae were found attached on the fins as well as on the body surface of the hosts. Large number of larvae were found to be attached on the fins. After this observation, some more water was added into the beakers for the survival of the fish. The fishes in the beakers were fed with prepared fish food and kept in the laboratory for further examinations.

Every twenty-four hours, half the water from the beakers were removed by siphoning for cleaning and freshwater was added to keep the level. After 24, 30, 36 and 40 hours, each fish was sacrificed for collecting larvae. The fishes were dipped in 10% formalin several times. As a result, a good number of attached larvae got separated from the host and they were collected with a Pasteur pipette and preserved in 10% formalin for detailed study. After nine days of the release of I copepodids, immature females were seen on the fishes in the beakers. They were collected at every 24 hour duration by killing the fish, to study the sequence of growth of the parasite. This procedure was continued till collecting ovigerous females from the fishes.

In the other procedure, about 200 to 300 I copepodid larvae were freed into two aquariam tanks of 75 x 30 x 30 cm., each with a single host

fish of 18 cm. and 20 cm. in length. A specially designed plankton net made of bolting silk No. 20 was used for collecting any free-swimming larvae in the aquariam. The aim of this procedure was to check whether the larvae can detach from the host and swim freely during development. The net was drawn gently across the fish tank at every 24 hour duration and the larvae collected were preserved in 10% formalin. Collection of larvae by using the net was decreased gradually with the advancement of developmental stages.

Nine days after the release of the I copepodid, immature females were seen on the fishes in the aquariam. They were kept undisturbed for noticing the life span of the parasite. Temperature was noted during the course of the present study.

The larvae, immature females and mature females preserved in 10% formalin, were mounted on slides and observed under a compound microscope. The larvae were dissected and temporary slides were prepared in lactic acid. All the drawings were made by using a mirror-type cameralucida.

OBSERVATIONS ON THE LIFE HISTORY

The mature egg sacs separated from the parasites contained an average of 150 eggs. The eggs were oval in shape and measuring about 0.113 mm. in diameter, the range being 0.099 mm. to 0.127 mm. (the average obtained by measuring thirty eggs from three different sacs). The eggs

were white and opaque in earlier stages but during incubation, they became transparent and swollen.

The egg sacs in the petridishes began hatching within hours, and the larvae were first released from the postero-lateral part, and the eggs that hatched last were from the anterior part of the egg sac. Nauplii have been observed to wriggle within the egg sacs for five to ten minutes before hatching. It took an average time of 60 to 90 minutes for the complete hatching of the eggs and hatching percentage was 80 to 90 within this period and the rest usually remained unhatched. When nauplii escape from the egg sac, they settle on the bottom of the petridish for a few moments before they start the characteristic short jerky movements. The hatching time was noted and successive development was observed carefully. It is found that the life cycle of Lernaea osphronemi consists of four phases: 1) a free-living naupliar phase during which the larvae live with the yolk present in the body. 2) a parasitic post naupliar phase in which the larvae can cling to the body of their host and free to move over the body. 3) a preadult phase during which the copepod settles permanently on the host and entered a period of metamorphosis and 4) the adult phase. The life history graph (Fig. I), shows the average length of time taken by each developmental stage of Lernaea osphronemi. The temperature fluctuation was between 27-32°C during the period of this study.

The first nauplius moves in the petridish and settles to the bottom.

Between 10 to 14 hours after hatching the nauplius undergoes first moulting. The second nauplius can easily be recognised by the presence of two pairs of bristles on the posterior part of the body. After 18 hours the second nauplius moulted to third, which bears three pairs of bristles and were bigger in size. During the naupliar stages, the larvae subsist on the stored food material in the body. The third nauplius underwent moulting within 32 to 36 hours to form the I copepodid. It was entirely different from the naupliar stages in morphology and move rapidly in the petridishes.

The I copepodid left in 100 ml. beaker without host started dying from second day onwards and by fourth day, all copepodids perished. This clearly indicates that Lernaea osphronemi cannot develop beyond the I copepodid stage in the absence of the host. The length and breadth of the free-swimming larval stages of L. cyprinacea, L. barnimiana, L. chackoensis and L. osphronemi are shown in Table I.

The I copepodid was followed by the II copepodid and the time taken for the moulting was between 32 - 38 hours. It was found that the successive stages also followed this time pattern. It was realised that the copepodid larvae need not leave the host at the time of moulting, as the moulted integuments of all copepodid stages were observed to be attached to the body of the host. All the copepodid larvae were capable to swim and often release their hold on the host, quickly dart through the water and take up new position on the host, when the hosts were disturbed. It was found that during any of these stages, the larvae

were capable of surviving four or five days in the absence of a host. They swim freely in water to find out a new host and if failed, they would perish.

Sexual dimorphism became pronounced on the V copepodid stage and during this time, copulation takes place. The males do not survive after copulation. The inseminated females moulted to cyclopoid and started burrowing and penetrate into the flesh of the host. Then they undergo metamorphosis, the body became elongated, four cephalic horns grew out and anchored the parasite in the body of the host. The first pair of egg sac was formed within three or four days of penetration. Relative increase in body length of different larval stages is represented in Fig. 2. The increase in length of the cephalothorax is represented in Fig. 3. Increase in body width of nauplius amnd cephalothoracic width of copepodids are shown in Fig. 4. Fig. 5 represents body length without uropod. Fig. 6 depicts the percentage of relative distance between the thoracic legs in relation to total length to show the diphasic growth of Lernaea osphronemi.

The first pair of egg sacs was comparatively small. The number of eggs contained in the egg sacs increased gradually and then decreased in number towards the end of the breeding period. Fig. 7 shows the number of eggs collected from a single adult parasite which was detached from the host during plucking of the egg sacs on the 16th day. The total number of egg sacs and eggs produced by the parasite during its life span could not be observed. It was found that the life span of the postmetamorphosis female was 20 - 25 days on an undisturbed host in the aquarium.

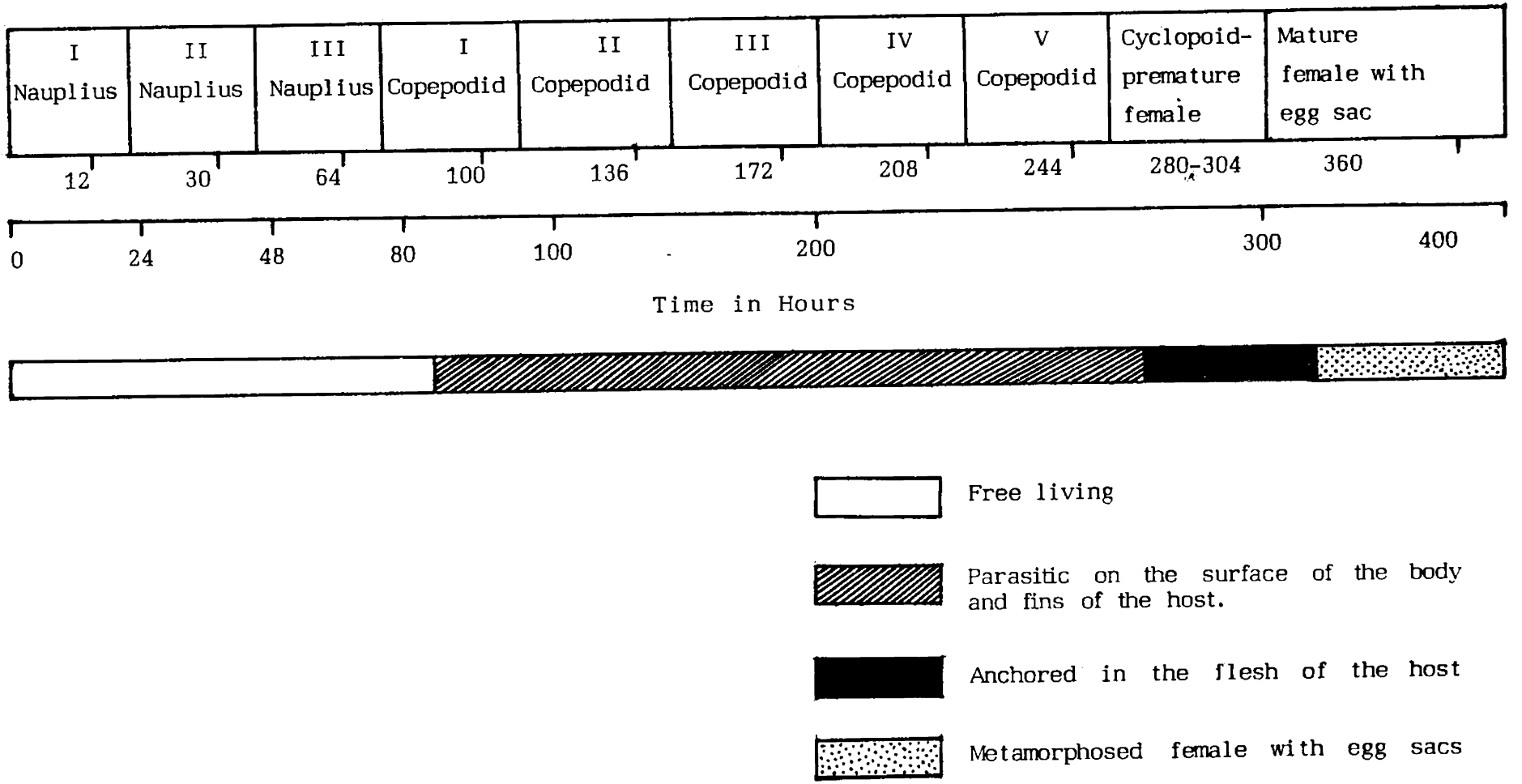

Fig 1. Life history of Lernaea osphronemi sp. nov. observed in the laboratory on the host Osphronemus goramy. It shows the average length of time taken by each developmental stage. Temperature ranges between 27 - 32°C.

Table 1 - The length and breadth of free-swimming larval stages of *L. cyprinacea*, *L. barnimiana*, *L. chackoensis* and *L. osphronemi*.

		L. cyprinacea (Lahav and Sarig, 1964) Size in micron	*L. barnimiana* (Thurston, 1969) Size in micron	*L. chackoensis* (Gnanamuthu, 1951 b) Size in micron	*L. osphronemi* Sp. nov. Size in micron
I	Nauplius	100 x 50	145 x 94	186 x 130	120 x 81
II	Nauplius	--	170 x 100	--	127 x 84
III	Nauplius	120 x 60	200 x 100	220 x 147	164 x 87
I	Copepodid	300 x 400	330 x 115	325 x 136	408 x 127

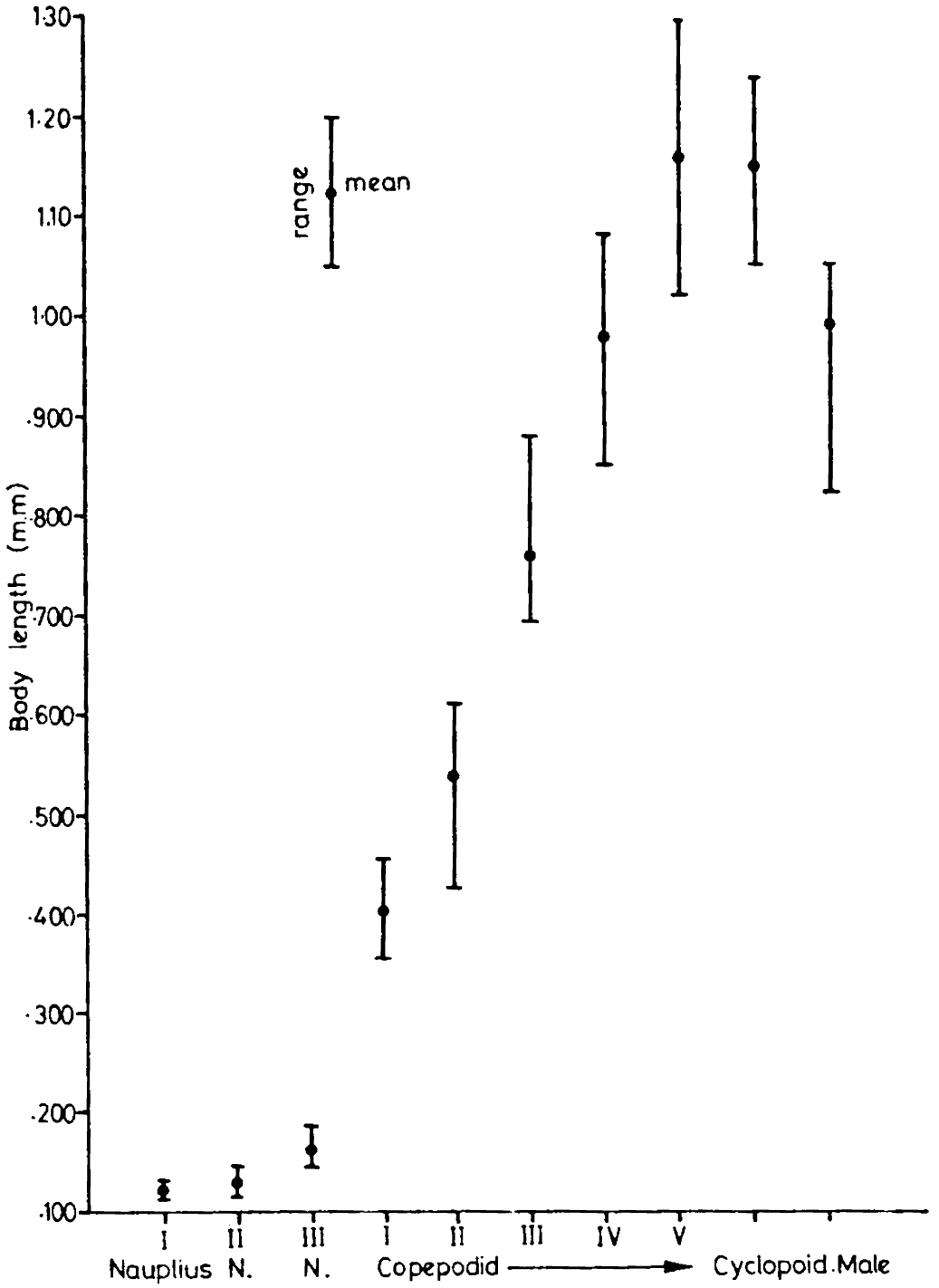

Fig. 2. Relation between body length and developmental stages of Lernaea osphronemi sp. nov.

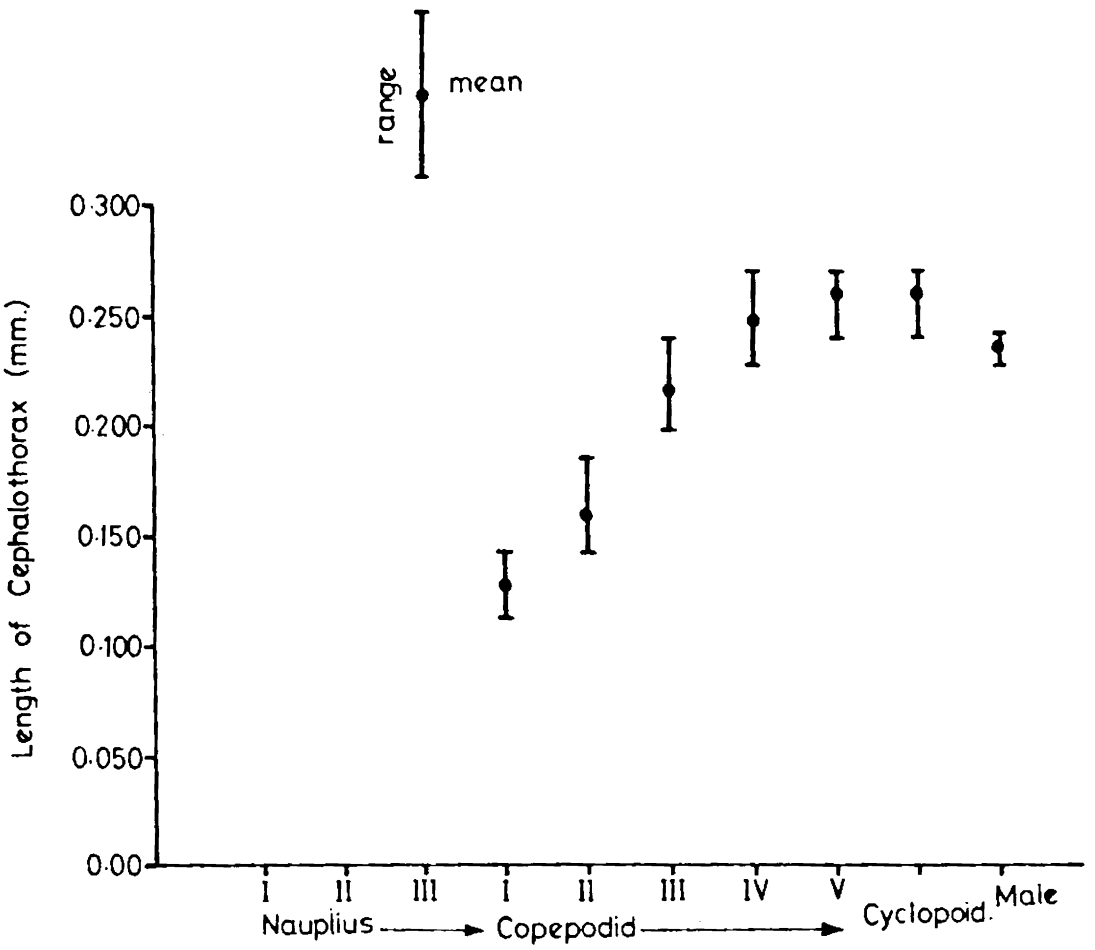

Fig. 3 Relation between cephalothorax length and developmental stages of Lernaea osphronemi sp. nov.

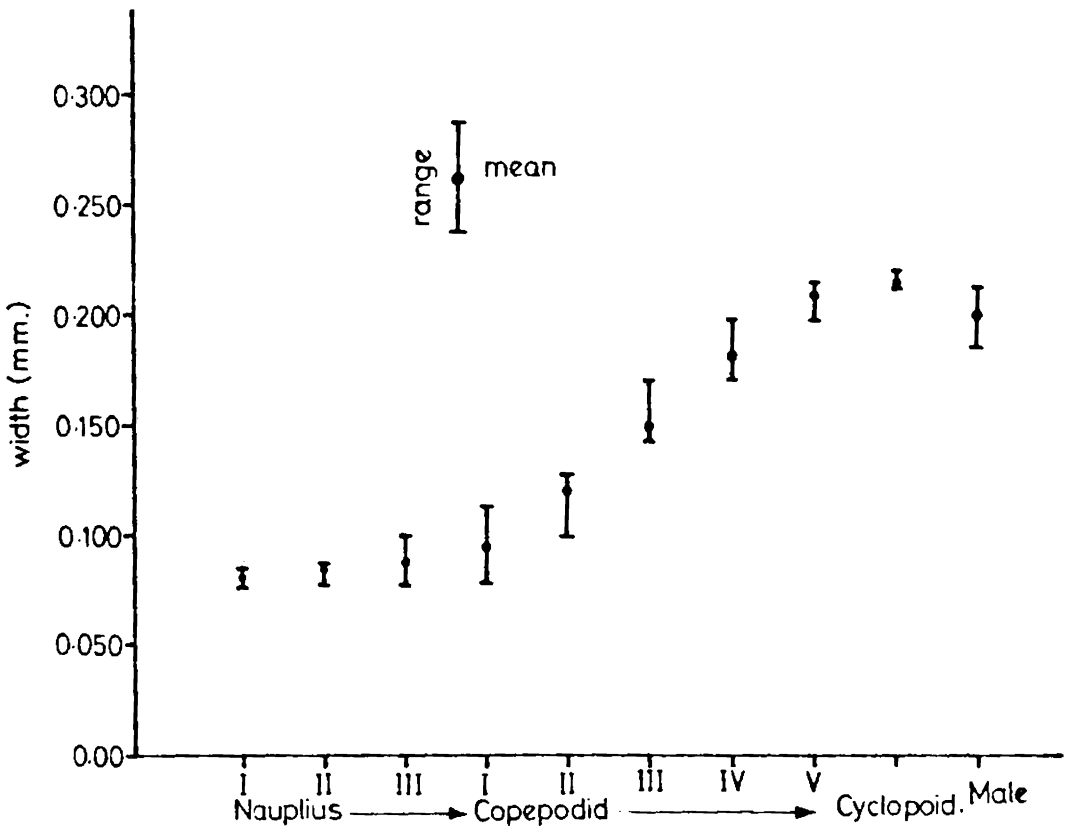

Fig. 4. Relation between body width of nauplius and cephalothoracic width of copepodid stages of Lernaea osphronemi sp. nov.

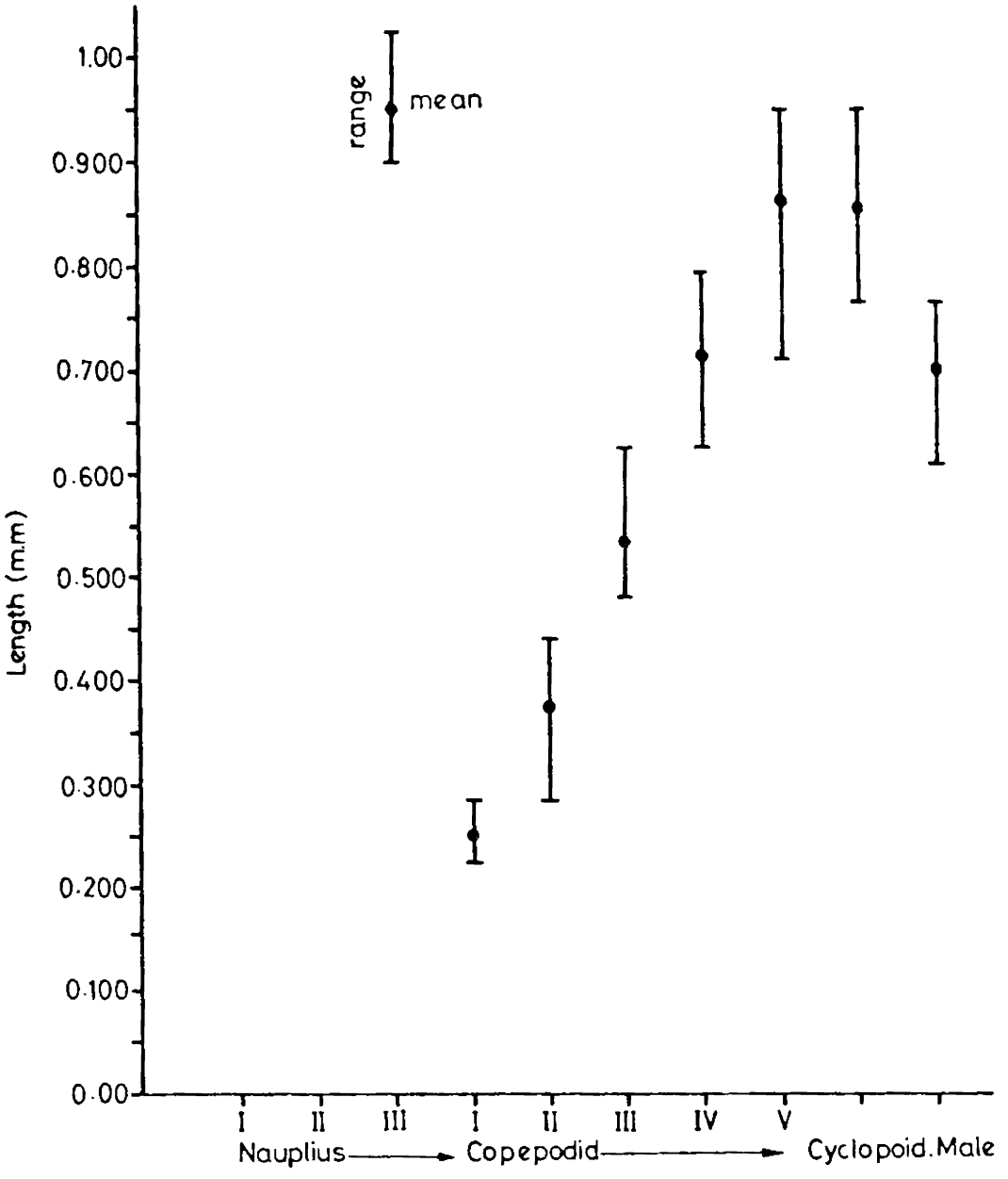

Fig. 5. Relation between body length without uropod and developmental stages of Lernaea osphronemi sp. nov.

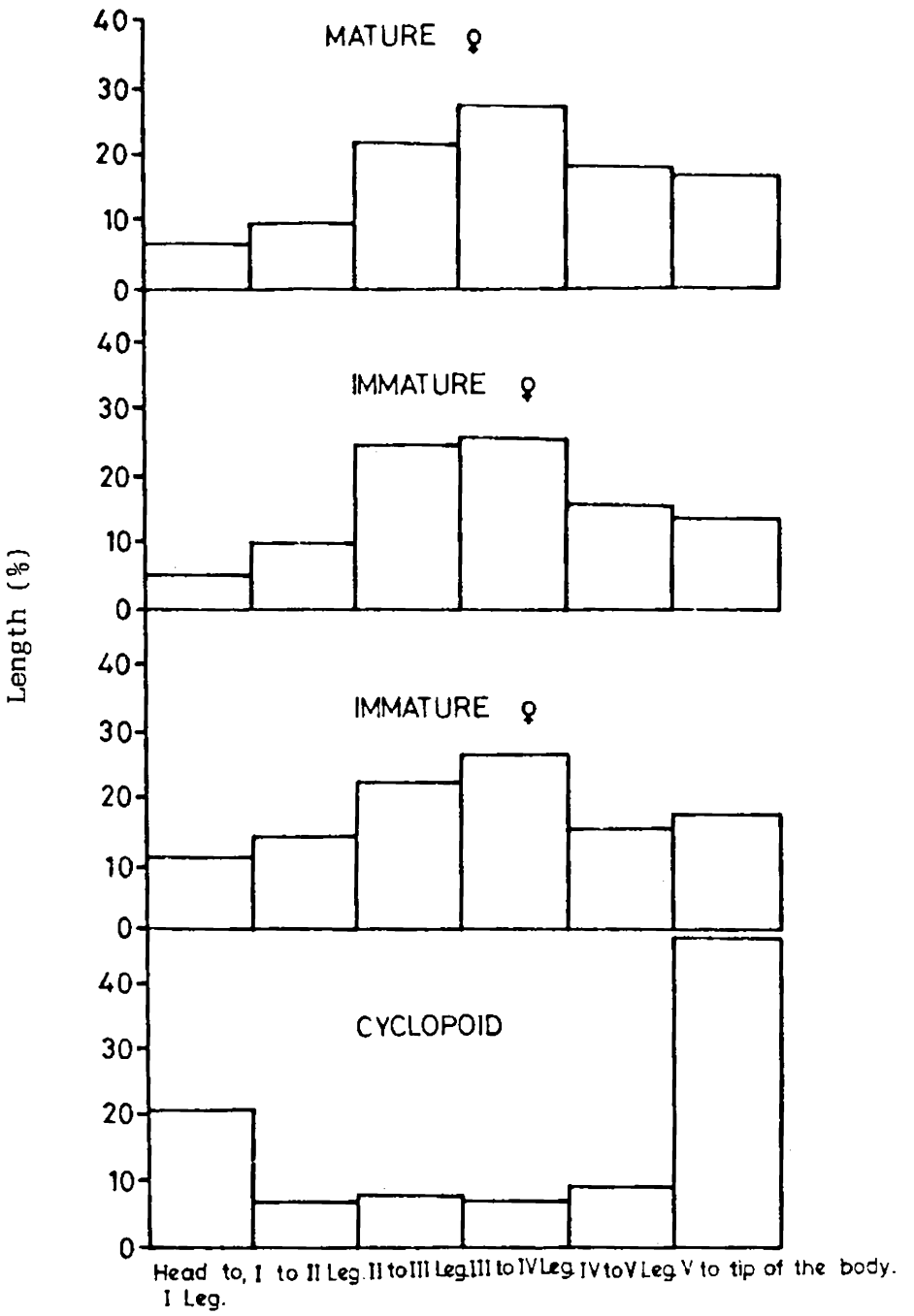

Fig. 6. Diphasic growth of Lernaea osphronemi sp. nov. during development.

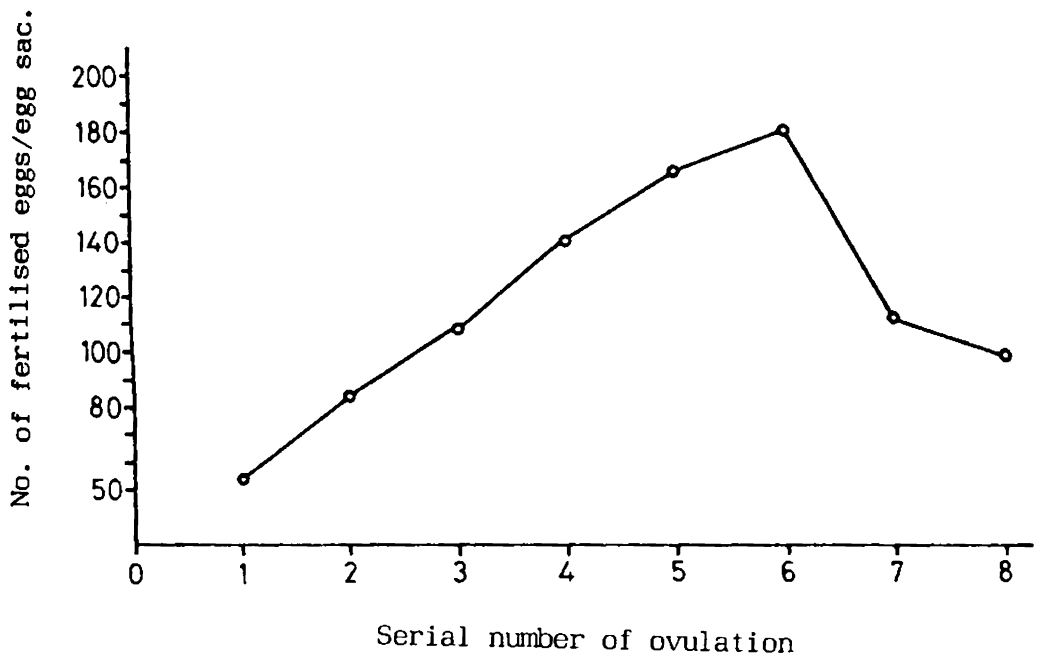

Fig. 7. Number of fertilized eggs laid by a female of Lernaea osphronemi sp. nov. in each ovulation.

FIRST NAUPLIUS

Figs. 1 - 4

The newly hatched nauplius was transparent, light greenish in colour and elliptical in shape. Major portion of the body was filled with yolk globules. On the midline of the dorsal side of the body close to the anterior margin was the 'X'-shaped red eye. The posterior end of the body with one pair of bristles.

There were three pairs of appendages. The first antenna uniramous, with two segments of unequal length. The basal segment possessed a long plumose seta at its distal end. The terminal segment with two-plumose setae and two very small spines.

Second antenna biramous. Exopod with of four segments, the first segment largest, second, third and fourth segments gradually tapering distally. Each segment carrying one plumose seta laterally. The fourth segment with two slender spines on either side of the seta. Endopod indistinctly two-segmented, bearing two plumose setae distally and a small spine medio-laterally.

The mandibles were biramous, smaller than the second antenna. Exopod consisted of four segments, the basal segment largest, second, third and fourth segments diminishing in size distally and each segment with a long plumose seta. Endopod one-segmented with two unequal plumose setae distally.

The following measurements were made from twenty specimens.

Mean length	-	0.120 mm.
Range	-	0.110 -0.127 mm.
Mean width	-	0.081 mm.
Range	-	0.078 - 0.085 mm.

SECOND NAUPLIUS

Figs. 5 - 8

Body size slightly increased than the first nauplius and the shape of the body was ovate. The yolk globule gradually decreased in quantity and rudiments of the first maxillae appeared as two spines posterior to the mandible.

The first antenna two-segmented, similar to the first nauplius, but the basal segment bearing a small spine posterior to the seta.

The exopod and the endopod of the second antenna resembled that of first nauplius, but increased in size.

The mandibles were similar to that of the first nauplius.

The posterior end of the body with two-pairs of bristles.

The following measurements were made from twenty specimens:

Mean length	-	0.127 mm.
Range	-	0.113 - 0.142 mm.
Mean width	-	0.084 mm.
Range	-	0.078 - 0.085 mm.

THIRD NAUPLIUS

Figs. 9 - 12

The body became longer and increased in size, oval in shape and slightly flattened on the ventral surface. The median eye was distinctly visible and the anterior margin of the cephalothorax was projected slightly. The amount of yolk globules highly reduced.

The first antenna uniramous and rami two-segmented. The two segments were almost equal in size. The basal segment with a long plumose seta and a very small spine. The distal segment bearing two long plumose setae and six spines.

The second antenna biramous, exopod similar in structure to that of the second nauplius. The one-segmented endopod armed at its distal end with a long plumose seta, a short non-plumose seta and three spines.

The mandibles were similar to that of second nauplius.

Three pairs of bristles present at the posterior end of the body.

The following measurements were made from twenty specimens:

Mean length	-	0.163 mm.
Range	-	0.142 - 0.184 mm.
Mean width	-	0.087 mm.
Range	-	0.078 - 0.099 mm.

FIRST COPEPODID

Figs. 13 - 22

The body was transparent and the intestine became conspicuous in its entire length. Body consisted of cephalothorax, three thoracic segments and an abdominal segment. The thoracic segments diminishing gradually in width posteriorly. The abdominal segment rectangular in shape with a pair of uropod.

The first antenna, second antenna, mandible, maxilla, maxilliped and first pair of thoracic legs were borne on the cephalothorax. The red eyes were visible on either side of the mid-line of the antero-dorsal part of the cephalothorax.

First antenna uniramous and three-segmented. The basal segment unarmed, second segment longer than broad, with two short setae proximally and a long seta distally. The terminal segment shorter than the second with two highly elongated setae on the distal margin and seven setae subequal in length on the disto-lateral margin.

Second antenna two-segmented, segments subequal in size. Basal segment unarmed, distal segment with two small setae medio-laterally, a stout claw-like spine and three subequal setae apically.

Since the structure of mandibles, maxillae and maxillipeds were similar

throughout all the copepodid stages, the description of these appendages will be included along with lthe fifth copepodid stage.

Two pairs of swimming legs were present. The sympod two-segmented, coxa unarmed and basis with a small seta lateral to the base of exopod. Exopod and endopod one-segmented. The exopod of the first leg carrying four spines and four long plumose setae. The endopod bearing two spines and five plumose setae. The second leg exopod provided with four spines and three long plumose setae. The endopod with two spines and four long plumose setae. The outer margin of the endopod with a row of fine hairs.

Uropod squarish, each armed with a very long inner plumose seta and four small setae subequal in length on the distal and lateral margins.

The following measurementes were made from twenty specimens:

Mean length	-	0.408 mm.
Range	-	0.355 - 0.454 mm.
Mean length of cephalothorax	-	0.137 mm.
Range	-	0.133 - 0.142 mm.
Mean width of cephalothorax	-	0.095 mm.
Range	-	0.078 - 0.113 mm.
Mean length without uropod and setae	-	0.251 mm.
Range	-	0.227 - 0.284 mm.

SECOND COPEPODID

Figs. 23 - 33

Body consisted of anteriorly rounded cephalothorax with four thoracic segments and an abdominal segment. The rami of the first two pairs of legs became two jointed.

First antenna three-segmented. Basal segment with two setae on the outer margin. Second segment equal to the basal segment with three setae unequal in length. The distal segment long, bearing two long and seven unequal setae.

Second antenna similar to that of the first copepodid.

First leg sympod two-segmented, coxa with a small plumose seta on the inner margin distally, basis with a small seta lateral to the base of the exopod and a curved spine at the medio-lateral inner margin. Exopod two-segmented, first segment short, armed with one spine on the outer margin and second segment with three spines and five plumose setae. Endopod two-segmented, basal segment with a plumose seta and distal sgement bearing two spines and four plumose setae.

Coxa of the second leg unarmed and basis with a small seta lateral to the base of the exopod. Both rami two-segmented. Basal segment of the exopod armed with a spine, distal segment carrying three spines and four plumose setae. The proximal segment of the endopod short

with a single plumose seta and distal segment long, bearing two spines and four plumose setae. The outer margin of the endopod having fine hairs.

Third leg sympod two segmented, coxa unarmed and basis bearing a small seta posterior to the exopod. Exopod and endopod single-segmented. Exopod with three spines and three plumose setae. Endopod having two spines and four plumose setae.

Uropod rectangular in shape, each bearing an inner elongated plumose seta with two short setae on either side and another seta on the antero-lateral margin.

The following measurements were made from twenty specimens:

Mean length	-	0.543 mm.
Range	-	0.426 - 0.610 mm.
Mean length of cephalothorax	-	0.159 mm
Range	-	0.142 - 0.184 mm.
Mean width of cephalothorax	-	0.121 mm.
Range	-	0.099 - 0.127 mm.
Mean length without uropod and setae	-	0.374 mm.
Range	-	0.284 - 0.440 mm.

THIRD COPEPODID

Figs. 34 - 45

Cephalothorax elongated with five free thoracic segments and one abdominal segment. The first three pairs of legs biramous and a new pair of one-segmented leg, the fourth pair appeared on the third thoracic segment. The abdomen slighlty enlarged bearing uropod.

First antenna uniramous and three-segmented. Basal segment longer than broad, with six setae of unequal length. Second segment shorter than the basal and bearing three small and a long setae. The distal segment longer than the second with ten setae of varying length, seven apical in position out of which two were thicker and longer than the others.

Second antenna resembled to that of second copepodid, but increased in size.

First leg sympod two-segmented, similar to the first leg of second copepodid. Exopod two-segmented, basal segment equal in length to the distal segment, bearing a spine on the outer margin and a single plumose seta on the inner margin. Distal segment with three spines and five plumose setae. Endopod two segmented, basal segment short with a single plumose seta and the distal segment large with two spines and five plumose setae.

The coxa of the second leg carrying a short plumose seta on its inner margin and basis with a small seta on the outer margin. Exopod

two-segmented and segments equal in length. First segment with an inner plumose seta and an outer spine. Second segment with four spine and five plumose setae. Endopod two-segmented, basal segment short, bearing a single plumose seta and the distal segment with two spines and five plumose setae. The outer margin of segments with fine hairs.

Third leg biramous, sympod similar to second leg. Exopod two-segmented, basal segment with a single spine and distal segment bearing three spines and four plumose setae. Endopod two-segmented, basal segment short with a single plumose seta and distal segment large with two spines and four plumose setae. Endopod having fine hairs on the outer margin.

Coxa of the fourth leg unarmed, basis with a small seta to the base of the exopod. The exopod and endopod one-segmented. Exopod bearing four spines and three plumose setae and the endopod with two spines and four plumose setae.

Posterior to the fourth pair of legs the rudiments of the fifth pair of legs were seen in the form of two fine setae.

Uropod similar in structure to that of the second copepodid.

The following measurements were made from twenty specimens:

Mean length	-	0.761 mm.
Range	-	0.695 - 0.880 mm.
Mean length of cephalothorax	-	0.216 mm.
Range	-	0.198 - 0.241 mm.
Mean width of cephalothorax	-	0.149 mm.
Range	-	0.142 - 170 mm.
Mean length without uropod and setae	-	0.534 mm.
Range	-	0.482 - 0.624 mm.

FOURTH COPEPODID

Figs. 46 - 58

Body consisted of cephalothorax, five thoracic segments and two abdominal segments with the uropod.

First antenna similar to third copepodid, but the only difference was in the elongation of the basal segment.

Second antenna resembled that of the previous stage, with the exception of one more seta on the apical margin of the distal segment.

The first two pairs of thoracic legs were similar to that of the third copepodid stage.

Sympod of the third leg two-segmented, coxa bearing a short plumose seta and basis with a small seta lateral to the base of the exopod. Rami two-segmented. First segment of the exopod with a spine on the outer margin and a plumose seta on the inner margin. Second segment long with four spines and five plumose setae. Endopod two-segmented, basal segment small, bearing a single plumose seta and distal segment comparatively long with two spines and six plumose setae. Outer margin of the endopod having fine hairs.

Fourth leg sympod similar to the third leg. Exopod two-segmented, basal segment with an outer spine and distal segment bearing four spines and five plumose setae. The basal segment of the endopod carrying

a single plumose seta and distal segment with two spines and four plumose setae. The outer margin of the endopod bearing fine hairs.

Fifth leg single-segmented, with three small plumose setae subequal in length.

Uropod similar to the third copepodid but slightly larger in size.

The following measurements were made from fifteen specimens:

Mean length	-	0.980 mm.
Range	-	0.852 - 1.079 mm.
Mean length of cephalothorax	-	0. 248 mm.
Range	-	0.227 - 0.269 mm.
Mean width of cephalothorax	-	0.183 mm.
Range	-	0.170 - 0.198 mm.
Mean length without uropod and setae	-	0.714 mm.
Range		-0.624 - 0.795 mm.

Uropod: Squarish, inner seta very long, plumose and three small subequal setae laterally, with the medial one plumose.

Total length: 0.8 mm. - 1 mm.

Male: Unknown.

Remarks:

The present new species resembles Ergasilus spatulus Cressey (1970)in the general shape of the body. Exopod and endopod of the first leg are three-segmented and the endopod with two spatulate spines in both specimens, but in the present species, the spatulate spines are comparatively broad. The first antenna of E. spatulus is six-segmented whereas in E. thammani sp. nov., it is distinctly five-segmented. Second antenna devoid of armature in E. spatulus whereas in E. thammani second antenna bearing sensillum on the second and third segments. The thoracic appendages exhibit some similarity, but differ in the ornamentation of basis and rami. In E. spatulus several rows of spinules present on the genital and abdominal segments, but in the present species, such structures are absent. E. thammani sp. nov. can be identified from all other known species of the genus by the broad spatulate endopodal spines of the first leg, five-segmented nature of first antenna and the arrangement of sensillum on the second antenna.

FIFTH COPEPODID

When the fourth copepodid larvae passed into the fifth copepodid stage, sexual dimorphism became distinct. Body consisted of cephalothorax, five thoracic and three abdominal segments.

The Female Copepodid

Figs. 59 - 71

Body elongated and diminishing in width from the posterior part of the cephalothorax to the tip of the uropod. The paired spherical eyes situated upon the median line in the anterior margin of the head.

First antenna similar in structure to that of the fourth copepodid stage with the exception of an increase in size and the number of setate. The basal segment bearing ten setae.

Second antenna increased in size compared to the preceding stages and having one more small seta on the posterior margin of the distal segment.

Mandible short, one-segmented with an apical claw.
First maxilla very small, one-segmented and wedge-sdaped.

Second maxilla two-segmented, basal segment stout and broad and terminal segment with two strong curved claws.

Maxilliped two-segmented, basal segment broad and stout with a papilla armed with an apical setule and slightly projected on the median margin of the distal part. Terminal segment short with one short curved and four large subequal strong claws at its distal margin.

Thoracic legs: First four pairs of legs biramous. Sympod two-segmented, proximal segment of the sympod with a small plumose seta on the inner margin and distal segment with a small seta lateral to the base of the exopod. Rami three segmented. In the first leg a curved spine-like structure present at the medio-lateral margin of the distal segment. The outer margin of the endopods having fine hair-like structures.

Arrangements of spines and setae are given below:

Arabic numerals denote setae; Roman numerals denote spines

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-1	I-1	II-5
II Leg	0-1	0-2	II-4	I-1	I-1	III-5
III Leg	0-1	0-2	II-4	I-1	I-1	III-5
IV Leg	0-1	0-2	II-3	I-1	I-1	III-5

Fifth leg small, one-segmented with four small plumose setae unequal in length on the distal margin. Sixth leg not present.

Uropod resembled with that of the fourth copepodid but increased in size.

The following measurements were made from fifteen specimens:

Mean length	-	1.157 mm.
Range	-	0.994 - 1.292 mm.
Mean length of cephalothorax	-	0.260 mm.
Range	-	0.241 - 0.269 mm.
Mean width of cephalothorax	-	0.209 mm.
Range	-	0.198 - 0.213 mm.
Mean length without uropod and setae	-	0.862 mm.
Range	-	0.7120 - 0.951 mm.

THE MALE COPEPODID

Figs. 72 - 85

The body of the male was shorter and broader than that of female. Paired eyes distinct. The first free thoracic segment as broad as cephalothorax. The next three thoracic segments diminish in width posteriorly. The genital segment large and cup-shaped. Abdomen three-segmented, gradually decreasing in size and bearing the uropod posteriorly.

First antenna distinctly four-segmented, longer than that of the female and heavily armed with setae of varying length along the outer margin.

Second antenna longer than that of female and bearing two more setae on the distal segment lateral to the claw-like spine.

The mandibles, maxillae and maxillipeds were similar to that of the female.

Thoracic legs: First four pairs of thoracic legs with two-segmented sympod and three segmented rami. Coxa with a small plumose seta on the inner margin distally and basis carrying a small seta lateral to the base of the exopod. The basis of the first leg armed with a large double-hooked claw on its inner margin. In the same position on the other three pairs of legs a group of fine hairs present. The outer margin of the endopod of the four pairs of legs having numerous hair like structure.

Arrangement of spines and setae are given below:

Arabic numerals denote setae; Roman numerals denote spines

	Endopod			Exopod		
	1	2	3	1	2	3
I Leg	0-1	0-1	II-4	I-1	I-1	II-5
II Leg	0-1	0-2	II-4	I-1	I-1	III-5
III Leg	0-1	0-2	II-4	I-1	I-1	III-5
IV Leg	0-1	0-2	II-3	I-1	I-1	III-5

Fifth leg two-segmented, the basal segment bearing a small papilla armed with a small seta. The distal segment broad with six plumose seta of varying length.

Sixth leg highly reduced bearing three setae, subequal in length.

Uropod similar to that of the female.

The following measurements were made from ten specimens:

Mean length	-	0.994 mm.
Range	-	0.823 - 1.050 mm.
Mean length of cephalothorax	-	0.236 mm.
Range	-	0.227 - 0.241 mm.
Mean width of cephalothorax	-	0.200 mm.
Range	-	0.184 - 0.213 mm.
Mean length without uropod and setae	-	0.702 mm.
Range	-	0.610 - 0.766 mm.

CYCLOPOID FEMALE

Figs. 86 - 99

Total length of the body slightly decreased and the cephalothoracic width increased compared to the fifth copepodid. Body consisted of cephalothorax, five thoracic and three abdominal segments. The genital segment larger in size and the uropod similar to that of the fifth stage.

First antenna increased in length, basal segment longest, bearing ten setae, the disto-median seta elongated. Second segment half the size of the first, with four setae, three small and one long. Distal segment longer than the second with twelve setae of varying length, two terminal setae stout and highly elongated.

Second antennae, mandibles, maxillae and maxillipeds increased in size without any structural change.

The thoracic appendages were exactly similar to that of the fifth female copepodid.

Sixth leg a single plumose seta mounted on a papilla like structure, just posterior to the fifth leg.

The following measurements were made from ten specimens::

Mean length	-	1.125 mm.
Range	-	1.05 - 1.235 mm.
Mean length of cephalothorax	-	0.259 mm.
Range	-	0.241 - 0.269 mm.
Mean width of cephalothorax	-	0.214 mm.
Range	-	0.213 - 0.220 mm
Mean length without uropod and setae	-	0.861 mm.
Range	-	0.766 - 0.951 mm.

METAMORPHOSIS OF THE FEMALE

Figs. 100 - 104

The cyclopoid females, penetrated into the host tissue and underwent metamorphosis to become the adult. The major events observed during the process of metamorphosis were rapid lengthening of the body and formation of holdfast from the cephalothorax. The thoracic and abdominal region became narrow and elongated. The segmentation of the body gradually disappeared.

During the process of metamorphosis, the dorsal horns first erupted out followed by the ventral horns and in due course, the ventral horns became longer than the dorsals.

The metamorphosing female attained about 3 mm. in body length within 24 hours and within three to four days, they became 6 - 8 mm. long, with first pair of egg sacs. Plate.I. shows ovigerous Lernaea osphronemi on the host Osphronemus goramy.

DISCUSSION

The life cycle of Lernaea osphronemi sp. nov. was successfully worked out in the laboratory. The eggs just before hatching became transparent and larger in size. Davis (1968), while reviewing the mechanisms of hatching in aquatic invertebrates pointed out that an outer membrane, the chorion, splits by pressure from within caused by expansion of the inner (larval) membrane. The inner membrane expands until it is much larger than the original egg, and within this blister membrane, the unhatched nauplius begins to swim actively around and finally comes out. Davis suggested the change in the osmotic value of the fluid within the membrane was responsible for the rupturing of the eggs in copepods. In the present study, it is observed that the nauplii struggled inside the egg membrane for a few minutes before hatching and certain mechanical pressure is exerted during the process. Lewis (1963) briefly mentioned hatching in the Caligoid Lepeophtheirus dissimulatus, "as the movement of the larva within the egg increases, the egg expands and finally ruptures..... The first nauplius by sporadic violent movements of its appendages, makes its way out of the egg case". Though other factors may influence the process of hatching, the present observation coincides with that of Lewis.

Wilson (1917) suggested the need for an intermediate host to complete the life cycle of Lernaea. Fryer (1968) observed that the copepodid stages of L. cyprinacea needed Bagrus docmac as an intermediate host before they infect Tilapia sp. Thruston (1969) reported that L. barnimiana

lived on Tilapia sp. and Haplochromis sp. as an adult, required Bagrus sp. as an intermediate host for the completion of life cycle. These observations were from studies in the natural habitat. However, in the present study, it is found that the L. osphronemi sp. nov. can complete its life cycle on a single host. Lernaea chackoensis completed the life cycle on one host alone, but it was capable of changing its location on its host till it metamorphoses into adult (Gnanamuthu, 1951 b). The present study clearly indicates that L. osphronemi can complete the life cycle on a single host and it is able to change its location or find out another individual of the host species, if the first host dies. Shariff and Sommerville (1986) opined that Lernaea sp. can complete the life cycle on a single host species, and the use of an intermediate host by some forms was flexible and revealed the adaptability of the parasites.

The result of the present study pointed out that the development of nauplius to adult parasite took an average of 12 - 15 days at 27 - 32°C, is in accordance with the findings of Shariff and Sommerville (1986). They noted that in the case of L. polymorpha and L. cyprinacea, the nauplii required 14 - 15 days to develop into a young female parasite at the mean temperature of 27°C. Yashouv (1959) reported that at 26 - 29.5°C, the development of L. cyprinacea from nauplius to cyclopoid stages was attained within 12 - 16 days and according to Shields (1978), this took between 11 - 15 days at 25 - 32°C. The finding of the present study is slightly different, that at 27 - 32°C, the development of L. osdphronemi from nauplius to cyclopoid stages is accomplished in 9 - 11

days. Kasahara (1962) also observed the same rate of growth at 27°C in the case of L. cyprinacea.

The observations during the present study revealed that the first nauplii moulted to second nauplii within 10 - 14 hours, second nauplii to third nauplii within 16 - 20 hours and third nauplii to first copepodid within 32 - 36 hours. So on the third day, the first copepodid appeared. This finding contradicts with that of Shariff and Sommerville (1986). They suggested that each nauplius stage lasted for 24 hours before moulting. Tamuli and Shanbhogue (1987) reported that the nauplii metamorphosed into successive stages and reached first copepodid after 65 - 67 hours at 27.5°C and this agrees with the present study. Thurston (1969) suggested that at 21 - 26°C, the first nauplii moulted and entered the second stage 19 hours after hatching and the third stage nauplii developed 42 hours after hatching and first copepodid about 97 hours after hatching. The variations of these findings with that of the present study may be due to the difference in temperature at which development takes place.

Thurston (1969) observed that in L. barnimiana, sexual dimoirphism became distinct during the fifth copepodid stage. Sarig (1971) reported that males and females of L. cyprinacea can be differentiated during the fourth copepodid stage and copulation occured in the cyclopoid stage, after which the males die. In the present study, it is observed that the males and females of L. osphronemi can be differentiated during the fifth copepodid stage. After copulation, the males die and the females

undergo metamorphosis. According to Bird (1968), in the life cycle of L. cyprinacea, copulation took place at the fifth copepodid stage, followed by further development, implantation and metamorphosis.

During the present study, it is observed that the first copepodid appendages viz., the mandible, maxillae and maxillipeds maintained the same structure in all the later stages of development except size. The other cephalic and thoracic appendages became larger and complex in all the succeeding stages. The males can easily be distinguished during the fifth copepodid stage from the females by the shape of the body and differences in cephalic and thoracic appendages.

According to Kasahara (1962), the females, after burrowing into the flesh of the host, began laying eggs within 4 or 5 days. Egg sacs appeared on the parasite 4 days after the parasite became visible to naked eye (Thurston, 1969). But in the present study, it was observed that egg sacs were visible 3 - 4 days after the parasites were seen.

Gnanamuthu (1951 b) observed that the first copepodid of L. chackoensis always lived only upto 3 - 5 days in the absence of a host. Shields and Tidd (1968), Thurston (1969) and Shariff and Sommerville (1986) observed the same phenomenon in the first copepodid of Lernaea sp. It is in accordance with the present observation. Thurston (1969) reported that the copepodid stages I - IV were living in the gill chamber of host fish, while V copepodid and cyclopoids on the body surface. In the present investigation, it is observed that all copepodid stages and cyclopoid lived on the body surface of the host and not in the gill chamber.

Tamuli and Shanbhogue (1987) reported that the first copepodid underwent successive moulting in the absence of a host to reach the fifth copepodid stage. This finding is quite interesting, because in the present species and several of the already described species the first copepodid is the infective stage and in the absence of a suitable host, it dies within a few days.

The result of the present study revealed that to complete the life cycle, i.e. from hatching of the egg to the emergence of the adult with egg sacs, took 13 - 15 days at 27 - 32°C. According to Lahav and Saring (1964), Shields (1978) and Shariff and Sommerville (1986), the life cycle of L. cyprinacea took 18 - 25 days at 25 - 32°C and 14 - 15 days at 27°C respectively. Bauer et al. (1973) reported that the life cycle of L. cyprinacea took 25 days at 30°C and Rukyani (1975) opined that at 28°C, it took 21 - 23 days. Though some of these findings are contradictory, it is clear that the development rate of Lernaea sp. is definitely influenced by temperature variation.

Kasahara (1962) observed that the breeding period of the female of L. cyprinacea lasts for about 3 weeks at 27°C and the number of fertilized eggs contained in a pair of egg sacs does not exceed 100 at the beginning for a breeding period, but increases to 400 - 600 in the middle of the breeding period and then decreases towards the end of the breeding period. A similar phenomenon is observed during the present study; (Fig. 7), shows that the female produces 8 sets of eggs before getting separated from the body of the host. Shields

(1978) reported that a single female may produce 8 sets of egg sacs before death or rejection by the host. The capacity of the female to produce how many egg sacs during its life span could not be estimated in the present study.

As per Kasahara (1962), the life span of the adult L. cyprinacea was 28 days at 27°C, whereas Pan et al. (1979) suggested 20 days at 26 - 30°C. In the present study, it is observed that the female parasite lived an average of 20 - 25 days at 27 - 32°C.

Kabata (1981) opined that like other crustaceans, the parasitic copepods increases in size by a series of moults and on reaching sexual maturity, they cease to moult. The metamorphosed individual will be entirely different form the larval forms and this ultimate difference in shape is due to differential growth of the body. The initial phase of development associated with feeding and anchoring is restricted to the anterior part of the body and the later phase associated with reproduction is centred to the posterior part of the body. Kabata referred this as "diphasic growth". Fig. 6 shows that in the cyclopoid stage, the cephalothoracic and abdominal region constitute about 67% of the total length of the body and the thoracic region comprises only 33%. But in the later stages of development, this tendency is changed and in the adult female, the cephalothoracic and abdominal region constitute only 23% of the total length of the body, whereas the thoracic region forms about 77%. This shows that the thoracic region of the parasite, which is associated with reproductive function underwent rapid growth after burrowing into

the body of the host. The growth of the various body parts are hence differential and this observation establishes that the growth of the parasite is "diphasic".

Successive larval stages exhibit an increase in length and breadth (Figs. 2,3,4&5). However, the female cyclopoid stage in the present species is slightly shorter than the fifth copepodid. But the cepahlothorax is broader. Though the difference is not very great, it is to be noticed that upto fifth copepodid stage, the larvae attach to the host almost permanently. The cyclopoid has to swim freely in search of a new host. So, this morphometric change might have some advantage for the better distribution of the parasite during host finding.

CHAPTER - IV

HOST-PARASITE RELATIONSHIP, ECOLOGY AND TREATMENT

HOST-PARASITE RELATIONSHIP, ECOLOGY AND TREATMENT

INTRODUCTION

The study of host-parasite relationship involves physiological and ecological factors which are of great importance for understanding some of the basic problems in biology.

Knowledge regarding host finding, site selection and host specificity are almost lacking in the case of parasitic copepods. Seasonal variation, prevalence, intensity of infection and ecological factors affecting parasitic copepods were studied by Fryer (1968), Shields (1968), Boxshall (1974 a,b), Srinivasachar and Sundarabai (1974), Hoffman (1976), Timmons and Hemstreet (1980), Kabata (1981), Uehara et al. (1984) and Viljoen (1985).

The present study intends to explore the dynamics of host-parasite relationship and also certain ecological aspects of the host-parasite system.

Treatment and control measures against Lernaea were compiled by Kabata (1970, 1985) and Sarig (1971). During the course of the present study, three chemicals, sodium chloride (NaCl). Potassium Permanganate ($KMnO_4$)) and Formalin were tested to find out the efficiency of these chemicals against Lernaea. These chemicals were selected, since they are cheap, easily available and harmless compared to D.D.T. group, B.H.C. group and organophosphate group. Oreochromis mossambicus (Tilapia) was

used to find out whether it can act as a 'Biological Control' to Lernaea.

MATERIALS AND METHODS

Osphronemus goramy Lacepede, was found to be the host of the parasitic copepod Lernaea osphronemi sp. nov. To test whether this parasite can infest other fishes, the following experiments were conducted. Five glass aquarium tanks of 75x30x30 cm. were filled with 50 litre dechlorinated tap water. Three pairs of Carassius auratus, Oreochromis mossambicus, Cyprinus Carpio and Osphronemus goramy with an average length of 7 cm. and Poecilia spherops with 4 cm. length were used for the experiment. The fishes were observed in the aquariam tanks for fifteen days to find out any infection. First copepodid larvae of Lernaea osphronemi sp. nov. were released into each tanks for infecting the fishes, which were reared in petridishes. The fishes were fed with prepared fish food. Faecal matter and remanents of food particles were removed from the bottom of the fish tanks by siphoning and fresh water was added to maintain the level of water in the aquariam. The fishes were left undisturbed for fifteen days and observed every twenty-four hours.

Fifteen osphronemus goramy were examined on a monthly basis for eight months from the fish pond at Pannivelichira to find out seasonal variations of L. osphronemi infection (Table I). Drag net and cast net were employed to catch the fishes. The number of fishes infected, number of parasites on each fish and site of attachment of the parasites were recorded. Prevalence and mean intensity of parasites were calculated as suggested by Margolis et al. (1982).

To find out the site preference by the parasite, a total of 96 infected O. goramy were examined from the fish pond at Pannivelichira (Table I). The number and distribution of parasites on the body of the host fishes were recorded. The total length of all the fishes examined were noted for establishing any correlation between size of the host and prevalence and mean intensity of parasite infection (Table 2).

Experiments were conducted to find out whether the commonly used chemicals against fish diseases, such as $KMnO_4$, Formalin and NaCl are useful for the control of Lernaea osphronemi infection on O. goramy.

The efficiency of different concentrations of $KMnO_4$ was tested against L. osphronemi in aquarium tanks of size 30x20x20 cm. For the experiment, O. goramy with more than three parasites on each individual was used. Fishes were given bath treatment in 30 ppm. and 20 ppm. $KMnO_4$ solution for 30 minutes twice daily. The time interval between the two treatments were 60 minutes. Aquaria were aerated continuously throughout the treatment period. The treatment was conducted for seven days continuously. (Table 3 and 4). Fresh solution of $KMnO_4$ was prepared before starting every treatment.

Nauplii and copepodids were also subjected to 30 ppm. and 20 ppm. $KMnO_4$ treatment. These larvae, hundreds in number, were placed in 100 ml. petridishes and treated for a period of 30 minutes. Treatment was given twice a day and the time gap between two treatments was 60 minutes. After each treatment, the larvae were collected from the petridishes and placed in fresh water for further observation.

Formalin (40% Formaldehyde) was used for Lernaea eradication experiments. Infected O. goramy, with at least three parasites, were subjected to treatment. Formalin solution of 200 ppm. and 400 ppm. were prepared in 30x20x20 cm. aquarium tanks and the fishes were given bath treatment for 30 minutes and 15 minutes respectively. Two treatments were given daily, the time interval was 60 minutes and the treatment continued for seven days (Table 5 and 6). After each treatment, fishes were transferred to aerated tap water.

Nauplii and copepodids were subjected to 400 ppm, and 200 ppm. formalin treatment. These larvae, 50 in each group, were released into 100 ml. petridishes containing formalin solution and treated separately. Untreated nauplii and copepodid, fifty in each dish were kept as control. The time of treatment was 5 and 10 minutes respectively.

Two aquaria 75x30x30 cm., were filled with 50 litre 1% NaCl solution and a third one of the same size was filled with freshwater. Fifteen number of infected O. goramy of length ranging from 5 - 10 cm. and each with more than three parasites were selected. They were divided into three groups of five individuals and released into these tanks. The fishes were fed with prepared fish food and kept undisturbed.

Sodium chloride at the concentration of 1% was treated against nauplii and copepodids. Petridishes of 100 ml. capacity and 50 nauplii and 50 copepodids were used. Similar sets with freshwater acted as control. Continuous observations were made under the microscope at one hour interval.

Oreochromis mossambicus (Tilapia) measuring 5-8 cm. in length were used to find out whether they have any role in eradicating Lernaea osphronemi infection on O. goramy. Fibre glass tanks of 100 litre capacity with soil bed were used for the experiment. In one tank, ten O. goramy measuring 5-8 cm. in length were released. In another tank, ten O. goramy and ten Tilapia of about the same size were put together. The fishes were fed with prepared fish food. In each tank, two hundred first copepodid larvae were released and the system was left undisturbed. Fishes were collected and examined on 10th, 12th and 15th day after stocking. The experiment was repeated twice.

TABLE - I

Osphronemus goramy examined from the fish pond at Pannivelichira to find out seasonal variations and site preferences of the parasite L. osphronemi sp. nov.

No	Month	Total fish examined	No. of fishes infected	Dorsal finbase	Pectoral finbase	Pelvic finbase	Caudal finbase	Sides of the body	Lower jaw & operculum	Total No. of parasites
1	August	15	15	49	48	47	48	35	8	235
2	September	15	15	37	43	22	26	26	4	158
3	October	15	15	48	43	17	18	40	14	180
4	November	15	13	47	51	7	18	17	6	146
5	December	15	12	24	29	12	23	15	5	108
6	January	15	10	12	10	4	5	3	3	37
7	February	15	8	4	5	2	3	2	-	16
8	March	15	8	4	8	3	2	1	-	18
		120	96	225	237	114	143	139	40	898

TABLE - 2

Length of O. goramy in relation to the infection L. osphronemi.

Length group (length in cm)	Total fish examined	Number of fishes infected	Parasites collected
0 - 5	26	20	39
5 - 10	11	8	18
10 - 15	13	8	8
15 - 20	8	5	7
20 - 25	17	14	74
25 - 30	15	13	157
30 - 35	12	12	224
35 - 40	18	16	371
	120	96	898

TABLE - 3

Experiment for the eradication of

L. osphronemi on *O. goramy* using 30 ppm KMn04.

Trial No.	Average weight of fish (gm)	Fish:water ratio (Kg:Lit)	Mean no of parasites per fish	KMn04 (ppm)	Duration of bath (Minutes)	Time interval between two treatment	No. of treatment per day	No. of days of treatment	% of parasite killed	No. of fish killed
1	89 (10 fish)	1 : 25	3.8	30	30	60	2	7	100	1
2	128 (10 fish)	1 : 25	4.2	30	30	60	2	7	100	-

Figures in parenthesis indicate number of fishes.

TABLE - 4

Experiment for the eradication of
L. osphronemi on O. goramy using 20 ppm KMnO4.

Trial No.	Average weight of fish (gm)	Fish:Water ratio (Kg:Ltr)	Mean No. of parasites per fish	KMnO4 (ppm)	Duration of bath (Minutes)	Time interval between 2 treatments (Minutes)	No. of treatment per day	No. of days of treatment	% of parasites killed	No. of fishes killed
1	110 (10 fish)	1 : 25	3	20	30	60	2	7	96.6	--
2	90.5 (10 fish)	1 : 25	3.6	20	30	60	2	7	88.8	--

Figures in parenthesis indicate number of fish.

TABLE - 5

Experiment for the eradication of
L. osphronemi on *O. goramy* using
200 ppm formalin.

Trial No.	Average weight of fish (gm)	Fish:Water ratio (Kg:Ltr.)	Mean No.of parasites per fish	Formalin (ppm)	Duration of bath (Minutes)	Time interval between 2 treatment (Minutes)	No. of treatment per day	No. of days of treatment	% of parasite killed	No. of fishes killed
1	95 (10 fish)	1 : 25	3.2	200	30	60	2	7	46.8	1
2	86 (10 fish)	1 : 25	3.4	200	30	60	2	7	52.9	2

Figures in parenthesis indicate number of fish.

TABLE - 6

Experiment for the eradication of
L. osphronemi on O. goramy using
400 ppm formalin.

Trial No	Average weight of fish (gm)	Fish:Water ratio (Kg:Ltr)	Mean No.of parasites per fish	Formalin (ppm)	Duration of bath (Minutes)	Time interval between 2 treatment (Minutes)	No. of treatment per day	No. of days of treatment	% of parasite killed	No. of fishes killed
1	106 (10 fish)	1 : 25	3.5	400	15	60	2	7	51.4	2
2	98 (10 fish)	1 : 25	4.0	400	15	60	2	7	52.5	2

Figures in parenthesis indicate number of fishes.

RESULTS AND DISCUSSION

Of the five sets of fishes maintained for infestation experiment, O. goramy and P. spherops were found to be infected. The other fishes were free from Lernaea infection. This clearly indicates that L. osphronemi is not having a wide range of hosts. Lernaea cyprinacea is not host specific and about 100 species of fishes have been recorded as hosts of this copepod (Kabata, 1979). Carassius auratus and Cyprinus carpio are the common hosts of Lernaea cyprinacea whereas the infestation experiments showed that these fishes are not susceptible to L. osphronemi. Tilapia sp. is the host of several species of Lernaea like L. cyprinacea, L. tilapiae, L. barnimiana and L. lophiara (Harding, 1950; Thurston, 1965; Fryer, 1968; and Shariff, et al. 1986). But the present study establishes that Tilapia is not susceptible to L. osphronemi. Two or three parasites were observed on the base of the pectoral fin and on the body surface of infected P. spherops. But the fish died before the parasites produced egg sacs. This indicates that though the parasite is capable of infesting P. spherops, the fish cannot withstand infestation. This type of infection is definitely of no value for the survival of the parasite. Another observation from the field is also noteworthy. The fish pond owned by the State fisheries department is for fish seed production. In that pond in addition to O. goramy breeders of Catla, Rohu, Mrighal and Carps were reared. Examination of the fishes revealed that they were not infected by Lernaea. The conclusion of the present study is that L. osphronemi is host specific to O. goramy. The susceptibility of other fishes to this parasite can be established only after conducting detailed investigation. Further

observations from wild fish populations may reveal the host range of L. osphronemi.

Knowledge regarding host finding by parasitic copepods is meagre. During infestation experiments, it was observed that caudal, pectoral and dorsal fins of the hosts were heavily infected than other parts of the body by the larvae. The head region was almost free from larvae and adult parasites. Shields and Tidd (1974) attributed the localization of Lernaea larvae in the mouth and branchial chambers of tadpoles due to the water currents occuring in those areas. Kabata (1981) opined that currents caused by the movements or respiration of the fish are among the factors directing the copepod to the host. The increased occurrence of larval forms of L. osphronemi on the fins compared to the body and head of the host may be due to the movements of water currents produced by these organs, and it is in accordance with the suggestion of previous authors.

After attachment of the larvae, the fishes have been observed dashing about in the aquarium, rubbing their head and sides of the body against the wall of the aquarium and even jumping out of water. This may be one of the reasons why the anterior part of the body was less infected than the bases of fins and posterior part of the body.

Fryer (1966) reported that a fish which has acquired one parasite continues to attract others. He also observed that if two or more parasites were present, often they were situated in very close proximity to one another. During the present study, it is observed that O. goramy

ranging from 0-5 cm. in length, carried only two or three parasites on their body and the parasites were usually anchored at the base of the pectoral fin and deeply penetrated into the body.

Bulow et al. (1979) reported that on the body of Black burn fork fishes L. cyprinacea incidence was highest on the base of the dorsal fin (27.9%) followed by pectoral fin (19.7%), pelvic fin (19.7%), caudal fin (14.8%), gills (8.2%), body surface (4.9%) and operculum (1.5%). According to Timmons and Hemstreet (1980), the most common sites for attachment of L. cyprinacea on the host fish, Large mouth bass, were below the dorsal fin (41.6%), behind the pectoral fin (25.0%), pelvic fin (16.7%), on the side of the body (8.3%), the operculum (4.2%) and on the lower jaw (4.2%). In the present study, the distribution of L. osphronemi on the host's body was slightly different from the above observations. The base of the pectoral fin (26.4%) was the most preferred site. It was followed by dorsal fin base (25.0%), caudal peduncle (15.9%), sides of the body (15.5%), pelvic fin base (12.7%), lower jaw and operculum (4.5%). Al-Hamed and Hermiz (1973) observed the maximum number of L. cyprinacea behind the pectoral fins (86), in the buccal cavity (56) and on the base of the caudal fin (46) on carps. The parasite showed a definite affinity for the body near the base of fins. Mc. Neil (1961) studying infections of hatchery rainbow trout observed that L. cyprinacea prefers locations which offered maximum protection from water currents. Protection from water currents would be especially important for parasites of fishes living in streams. The fins also provide protection from the effects of scraping and tissues near fin bases may be more easily penetrated by the parasite.

During the present investigation, it was observed that older and larger fishes have higher levels of prevalence and mean intensity of infection (Fig. 1,2). Similar observations were made by several authors such as Dogiel (1961), Cressey and Collette (1970), Kennedy (1975), Rawson (1977), and Conneely and Mc Carthy (1985). But, in the present study, prevalence of infection was more on 0-5 cm. size group whereas in 5-10 cm., 10-15 cm. and 15-20 cm. size groups, the prevalence was less. Mean intensity of infection was higher in 0-10 cm. length group than 10-15 cm. and 15-20 cm. length group. Both prevalence and mean intensity were higher in large size groups. Kabata (1981) suggested that larger and older fish often carry great number of copepods. It is mainly due to the longer period of contact and larger attachment surface available for the parasite.

Some authors noted lower levels of parasitism in the older and larger size host (Noble et al. 1963; Bortone et al. 1978). The explanation is that older fish may develop some immunity against infestation. But in the present observation, such possibility is ruled out, since older and larger fishes have highest level of parasitic intensity and prevalence.

The present study shows that prevalence (Fig. 3) of parasite infection was 100% in August, September and October. Then it decreases slowly; during February and March, the rate was below 60%. During January, February and March, smaller fishes were more infected and the site of attachment of the parasite were mainly at the base of the pectoral fin. The prevalence rate of parasitic infection of larger fishes decreased

during this period and reddish lesions were found on their body. The mean intensity (Fig. 4) exhibit a different pattern. It was highest in August, low in September, again increased in October, gradually decreased and very low during February and March. Bulow et al. (1979) reported a similar observation in the case of L. cyprinacea. The authors examined 13 species in August and observed that 8 species were infected. The intensity of infection was highest in August. Srinivasachar and Sundarabai (1974) observed that incidence of infestation of L. hesaragattensis on Lebistes reticulatus was highest during July and minimum during January. Viljoen (1985) reported that prevalence and abundance of L. barnimiana on Labeo sp. was highest during July. This variation in prevalence and mean intensity may be related to temperature and breeding cycle.

Haemorrhagic lesions followed by inflammatory responses were seen on O. goramy infected with L.osphronemi. Gold fish infested by L. cyprinacea shows minute lesions and tumors at the site of penetration on the skin (Tidd and Shields, 1963). Noga (1986) observed many lesions without Lernaea and suggested that the host may have harboured the parasite and then rejected it.

The detrimental effects of Lernaea sp. on fish population have been reported by several workers such as Fryer (1968), Sarig (1971), Al-Hamed and Hermiz (1973), Kabata (1979), Post (1983) and Shariff et al. (1986). During the present study, mortality of smaller fishes was observed due to Lernaea infection. The haemorrhagic lesions, caused

by Lernaea infection, providing site for infection by bacteria and fungus was also noticed. The exact influence of Lernaeosis on the growth rate and weight loss of the host O. goramy is not studied.

Experiments conducted for the eradication of Lernaea osphronemi, using different chemicals gave good result. KMnO4 is one of the most commonly applied chemical against Lernaea and has been used in many parts of the world. But it hasn't been uniformly successful (Kabata 1985). The standard method of treatment of Lernaea infected fishes by KMnO4 is given by Sarig (1971). He suggested that carps survived treatments with 20 ppm. KMnO4, for upto 120 minutes and 90-100% of the adult Lernaea found on the fish were killed within 60 to 120 minutes of exposure. But O. goramy exhibited great distress in 20 ppm. KMnO4 solution and some of them died after 60 minutes exposure. So a modified procedure was applied. The present study has established that, 30 minutes bath in 30 ppm. KMnO4 twice daily at one hour interval for seven days is the most effective method for the eradication of Lernaea from O. goramy (Table 3). KMnO4 of 20 ppm. was also effective, about 90% of the attached parasites were killed on the 7th day of treatment and the remaining parasites were found to be degenerated within 2-3 days after treatment. (Table 4).

This concentration of KMnO4 was not detrimental to the host fish. Lahav et al. (1964) reported that the sensitivity of fish to KMnO4 increases along with the decrease in age and weight, and it was not possible to use KMnO4 for fish weighing less than 25 gms. But in the present

experiment, the fishes treated were less than 25 gms. in weight. They were unaffected and mortality rate was almost nil. $KMnO_4$ affect only the adult Lernaea and does not kill young parasites embedded in the skin of the fish. These young parasites develop quickly in summer and appear on the skin of the fish 50-70 hours after treatment (Sarig, 1971). Such observation was lacking in the present investigation. This may be because of 7 days prolonged treatment of the fish for Lernaea eradication. Spraying $KMnO_4$ directly into pond has not been satisfactory, because differences in climatic conditions and in the level of organic content of the ponds unpredictably modify its effects on the fish and copepod (Kabata, 1985).

The nauplii and copepodid were unaffected by $KMnO_4$ treatment. They survived the treatment, moulted in time and infested the untreated fish.

Putz and Bowen (1964) reported the use of 30-60 minutes baths at 250 ppm. formalin, repeated every three weeks, to control Lernaea infection. In the present study, it was observed that the use of formalin (200 ppm and 400 ppm) against attached Lernaea was not satisfactory (Table 5 and 6). Only 50% of the parasites were destroyed after 7 days continuous treatment and fish mortality was also high. So, it is of no applicability.

The larval forms were highly sensitive to formalin treatment. Within 5-10 minutes, all the larvae exposed to 200 ppm. and 400 ppm. were dead. Kabata (1985) reported that larval stages were killed by formalin at 166-250 ppm. within one hour.

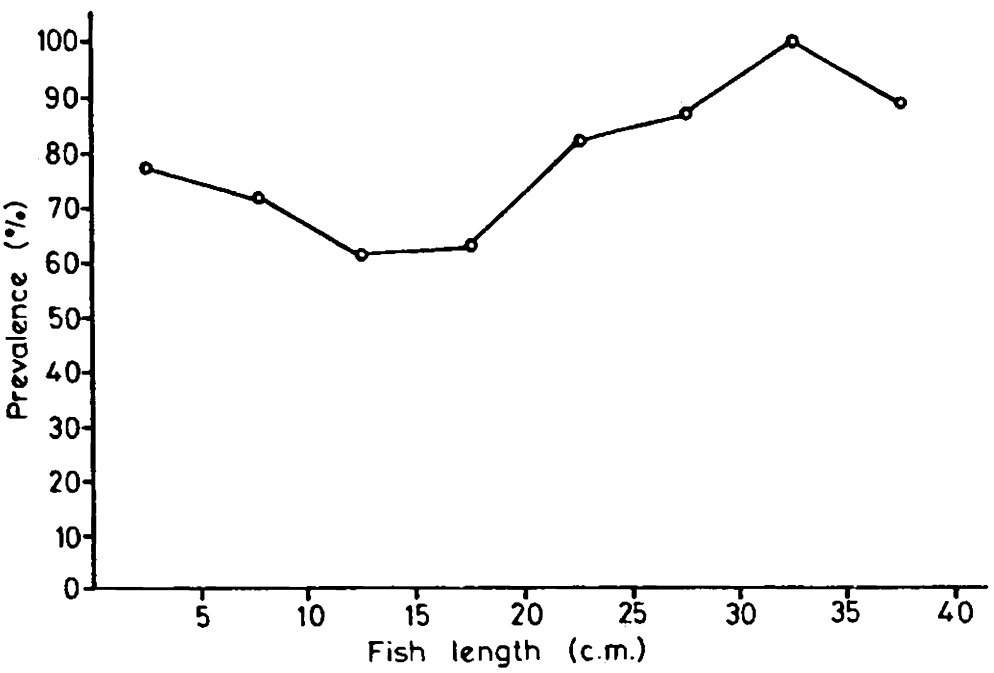

Fig. 1. Prevalence of L. osphronemi in relation to length of O. goramy

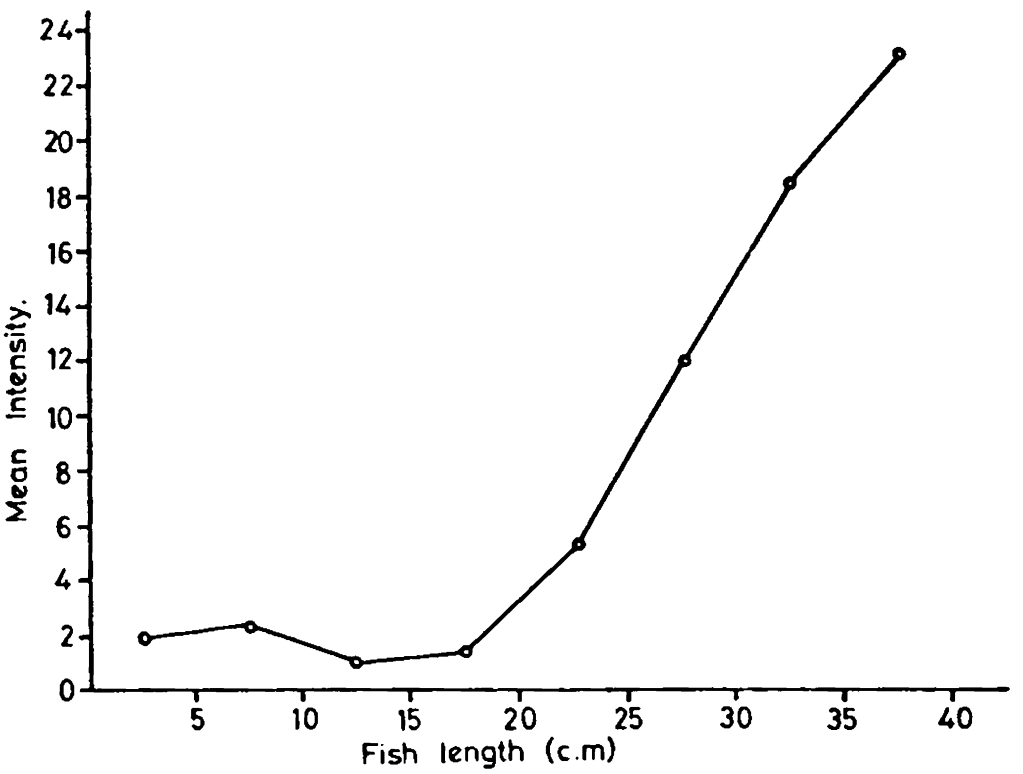

Fig. 2. Mean intensity of L. osphronemi in relation to length of O. groamy.

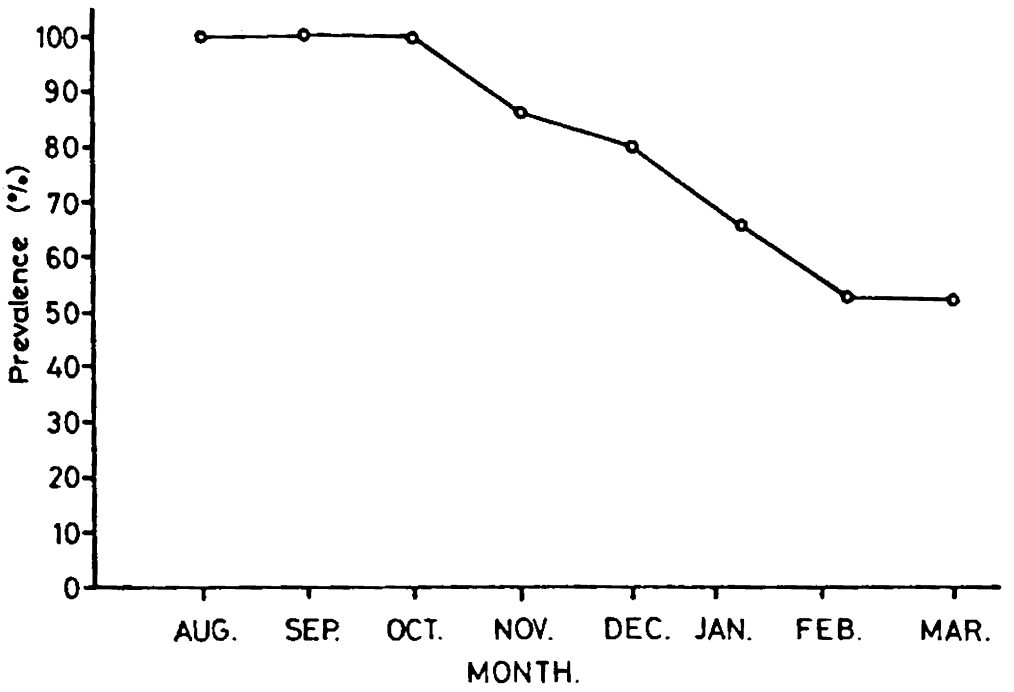

Fig. 3. Monthly prevalence of L. osphronemi on Osphronemus goramy.

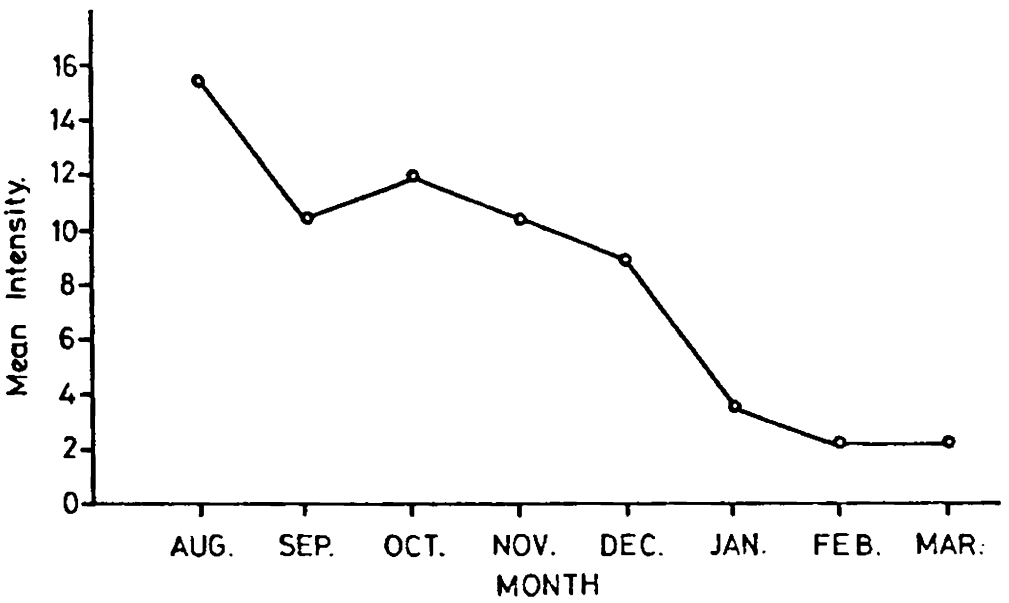

Fig. 4. Monthly mean intensity of L. osphronemi on Osphronemus goramy.

Commonsalt (NaCl), the cheapest and easily available chemical used against Lernaea infection, was found to be very effective. It was observed that 72 hours bath in 1% NaCl was capable of eradicating all the attached Lernaea from the fish. The fishes were able to tolerate 1% NaCl solution without any difficulty. Putz and Bowen (1964) suggested the use of 0.8-1.1 % salt solution for three days. Though Sarig (1971) has not recommended salt whereas in the present investigation, it was found to be useful against both adult and larval forms of L. osphronemi.

Biological control against Lernaea using O. mossambicus was tried in the course of the present study as suggested by Kabata (1985). Of the two sets of experiments conducted, O. goramy alone was reared in one tank and both O. goramy and O. mossambicus in the ratio 1:1 was reared in the other. It was interesting to note that the individuals of the tanks containing O. goramy alone showed greater intensity of infection (mean No. of parasites on 20 fishes - 56) than the O. goramy reared along with O. mossambicus (mean No. of parasites on 20 fishes - 13). Oreochromis mossambicus were uninfected by the parasite. This clearly indicates the possibility of predation of larval Lernaea by O. mossambicus. Detailed investigation in this direction is needed to establish a solid base for the use of O. mossambicus as a biological control against Lernaea.

CHAPTER - V

GENERAL OBSERVATIONS AND SUMMARY

GENERAL OBSERVATIONS

The study of copepods parasitic on fishes is of great importance, because of the adverse impact of these parasites on aquaculture paracites. Earlier works were mainly on the classification of this group of parasites. Now, this trend has changed and it is a highly diversified field of study with many branches of equal importance like life cycle, host-parasite relationship, ecology, pathology and therapy. Parasitic copepods as a whole exceed any other group of parasites in number and structural modification. They are ideal for understanding the impact of parasitism on an organism. The structural as well as functional modification attained by these group of parasites range from the mere closeness with their free-living relatives, to extreme modification which leads to the loss of all prominent diagnostic characters.

Copepods parasitic on fishes belong to three suborders; Poecilostomatoida, Cyclopoida and Siphonostomatoida. The members of these suborders are widely distributed and found to be parasitising almost all types of fishes. They are more abundant on marine than freshwater fishes. During the course of the present study, parasitic copepods belonging to two families and three genera were obtained from the freshwater fishes of Kerala. The families are Ergasilidae and Lernaeidae and the genera are Ergasilus, Lamproglena and Lernaea.

Ergasilus belongs to the suborder Poecilostomatoida and exhibits primitive cyclopoid morphology of free-living copepods and are also adapted to parasitism. The first antennae are sensory in function but the second

antennae have become modified into powerful organs for prehension. Female members of Ergasilus are usually found clinging by their antennae on the gill filaments of the host fish, for which the terminal segment of second antennae is typically in the form of a sharp claw. Thirty-two species of freshwater fishes were examined during the present study and only three species of fishes were found to be infected with Ergasilus. The new species of Ergasilus recorded are E. thammani, E. vembanadi and E. kabati. Roberts (1970) suggested that the second antenna of Ergasilus is quite variable and consititue one of the best characteristic features for species identification. During the identification of Ergasilus, it is found that the structure of second antennae in the three species are variable. But the mandibles and maxillae maintain a basically similar structure without much variations in these species.

Family Lernaeidae is more specialised and highly evolved than Ergasilidae. Genus such as Lernaea exhibit greater modification for parasitism. In the present study, Lamproglena krishnai sp. nov. and Lernaea osphronemi sp. nov. are recorded, and established that family Lernaeidae is more specialised for parasitism. L. krishnai is a gill parasite on Channa striatus. They attach themselves to the gills of the host by means of hook-like spines on the maxillae and maxillipeds. L. osphronemi penetrates into the flesh of O. goramy and gets attached by the anchor-like expansion of the cephalothoracic region.

The feeding mechanism of parasitic copepods is not adequately known and the elucidation of this mechanism is a matter of detailed investigation, particularly in parasite, such as Lernaea, whose head is completely

buried in the tissue of the host. One can assume that the mode of feeding mainly depends on the site of attachment and nature of mouth parts. The nature of mouth parts indicate that Ergasilus and Lamproglena probably feed on the gill tissues of the host and Lernaea subsists on tissue or body fluid.

It is difficult to suggest any basic mechanism for the specific host finding and site selection of parasitic copepods. During the present study, it is observed that out of the 32 species of fishes examined only five species were infected. Except E. thammani, all parasites were found on specific hosts. Definitely, the chances of exposure of these parasites to other fishes are almost equal. Reasons for this host specificity are unknown. Chemoreception is considered to be a possible mechanism of finding a specific host. Several authors suggested the role of tactile sensation as a possible way to find the hosts. But, this also is a conjecture, because any report on direct observation in this regard is lacking and future studies may throw light on this unknown aspect of copepod parasites.

Site specificity of copepod parasites are observed in this study. Ergasilus and Lamproglena are gill parasites whereas Lernaea is anchored in the flesh of the host fish. These parasites are structurally and functionally adapted to the specific sites, but how this site specificity evolved is not known.

Another observation regarding selection of host is that older and larger hosts have larger number of parasites. L. osphronemi sp. nov. infesting

Osphronemus goramy is found to be larger in number on older and larger hosts. But, the medium size fishes are less infected than the smaller size group. Cressey and Collette (1970) found that specialized groups of parasites permanently attached on the host increase in number with the increase in size of the host. This may be due to the increase in attachment area with the increasing size of the host.

The life cycle study of Lernaea osphronemi sp. nov. shows that it consists of four stages, a naupliar stage, post-naupliar stage, preadult stage and adult stage. Wilson (1911) suggested that the reduction of free-living stages reflected the degree of intimacy achieved within the host-parasite system. In the case of Lernaea it has only three free-living naupliar stages whereas free-living copepods have six naupliar stages. The reduction in free-living naupliar stages may be attributed to an advancement towards parasitism. Kabata (1981) while reviewing the life cycle of parasitic copepods has not agreed to Wilson's suggestion.

One of the most characteristic features of the life cycle of parasitic copepods is metamorphosis. Prior to metamorphosis, the copepod develops by moulting from stage to stage, in time-honoured crustacean fashion. The onset of metamorphosis is marked by complex physiological and structural changes as in the case of Lernaea. But, the mechanism behind the metamorphosis of copepods is still elusive and requires deep and detailed study.

Kabata (1979) discussed the phenomenon of metamorphosis from the point of view of differential growth. The ultimate difference in shape

of the body is due to the fact that some parts of the copepod's body grow faster or more vigorously than others. The periods of accelerated growth might also differ from one part of the body to another. The initial morphological changes following the attachment to the host, are mainly directed towards strengthening of the parasite's prehensile and feeding abilities. As a result, the early post-attachment growth is more active in the anterior part of the body associated with this function. When the parasite is safely established on the host's body, the next phase follows, that of development of reproductive capacity and consequent growth of the posterior part of the body. Finally the definitive shape of the adults body is attained. Hence, the growth consists of two phases and is referred to as "diphasic growth". The present study on the development of L. osphronemi agrees with the above suggestion.

The effect of temperature on the development of parasitic copepods are well established. Comparison of the life history of L. osphronemi with other species of Lernaea indicates the possible influence of temperature on development.

The life span of L. osphronemi is observed to be 20-25 days and the first pair of egg sacs contained lesser number of eggs than the succeeding pairs of egg sacs. This is in accordance with other reports.

Study on the ecology of parasitic copepods is a developing branch. An eight month study on seasonal variation, prevalence and intensity of infection of L. osphronemi on O. goramy brought out several interesting

observations. It is found that prevalence and intensity of infection are, to a certain extent, related to host size and also related to particular seasons.

Literature on the treatment and control measures against parasitic copepods are abundant. Lot of chemicals are found to be effective against copepod parasites, but some of them are very expensive and capable of biological magnification and pollute the environment. So, the least harmful and cheap chemicals were selected for the treatment and control of L. osphronemi sp. nov. on Osphronemus goramy. The results are quite encouraging. But, the point is that for each host-parasite system, we have to find out which type of treatment is effective. It will be easy, if we have a generalised idea of the parasite involved and the chemicals used.

SUMMARY

The present study includes systematics of the freshwater copepod parasites, life history of a new Lernaeid copepod parasite, host-parasite relationship, ecology and treatment.

Copepods parasitic on fishes include three suborders, namely poecilostomatoida, cyclopoida and siphonostomatoida. Parasitic copepods of freshwater fishes for the present study are collected from different freshwater fish landing centers and fish markets in Kerala. The collected parasites are identified to species level and described in detail.

It is found that, the five species collected are new to science and they belong to the genera Ergasilus von Nordmann, 1832, Lamproglena von Nordmann, 1832 and Lernaea Linnaeus, 1758. They are named as Ergasilus thammani, E. vembanadi, E. kabati, Lamproglena krishnai and Lernaea osphronemi.

The parasites exhibit host and site specificty and they are well adapted for parasitism. It can be inferred from the structural and functional modification of the various body parts of the parasites.

The life history of a new lernaeid copepod parasite, L. osphronemi has been successfully worked out in the laboratory. It has been found that no intermediate host is required for the completion of the life cycle of L. osphronemi. The life cycle consists of four phases namely :

1) free-living naupliar phase,

2) parasitic postnaupliar phase,

3) Pre adult phase and

4) an adult phase.

There are nine larval stages in the life cycle of L. osphronemi. They are: I Nauplius, II Nauplius, III Nauplius, I Copepodid, II Copepodid, III Copepodid, IV Copepodid, V Copepodid and Cyclopoid. Sexual dimorphism is distinct during V Copepodid stage. During each larval stage the length and breadth increases except in the cyclopoid female, which is slightly shorter but broader than the preceding stage. The morphometric measurements are made for each larval stage and represented graphically.

Based on the data obtained by rearing L. osphronemi on O. goramy in the laboratory, a life history graph has been made. It represents the average length of time taken by each developmental stage together with the relative lengths of time spent by the parasite during development. The parasite takes an average of 15 days at 27-32°C for the completion of life cycle. The 'diphasic growth' of the Leaneid parasite is observed during the present study. The life span of the adult parasite is found to be 20-25 days and the first pair of egg sacs contain less number of eggs than later stages. Afterwards the number of eggs decreases during the breeding period.

Lernaea osphronemi sp. nov. is found to be hostspecific and the preferred sites of attachment are the base of the pectoral fin, dorsal

fin, caudal fin, sides of the body, pelvic fin base, lower jaw and operculum respectively. Monthly prevalence and mean intensity of infection are varied. The prevalence rate is 100% in August, September and October and below 60% during February and March. Mean intensity of infection is highest in August and lowest in February and March. Mortality of smaller Osphronemus goramy is observed due to lernaeosis.

Experimnents conducted for the eradication of L. osphronemi from its host, O. goramy using cheap and easily available chemicals are found to be effective. Treatment with 30 ppm. $KMnO_4$ twice daily for 30 minutes at one hour interval for 7 days is found to be the efficacious method for the eradication of Lernaea. Long bath in 1% salt solution is also effective. Use of 200 ppm and 400 ppm formalin against adult Lernaea is less satisfactory, whereas the same concentration of formalin is highly useful for eradicating nauplii and copepodids of Lernaea osphronemi.

Studies conducted with Oreochromis mossambicus (tilapia) as a biological control against Lernaea on O. goramy gave encouraging results, but needs further detailed investigation for establishing its practice in culture systems.

A brief discussion on certain aspects of parasitism arising mainly out of the present study is also included.

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* Not referred to in original.

EXPLANATION OF FIGURES

Ergasilus thammani sp. nov.

Figs. 1 - 12

1 Female, dorsal view

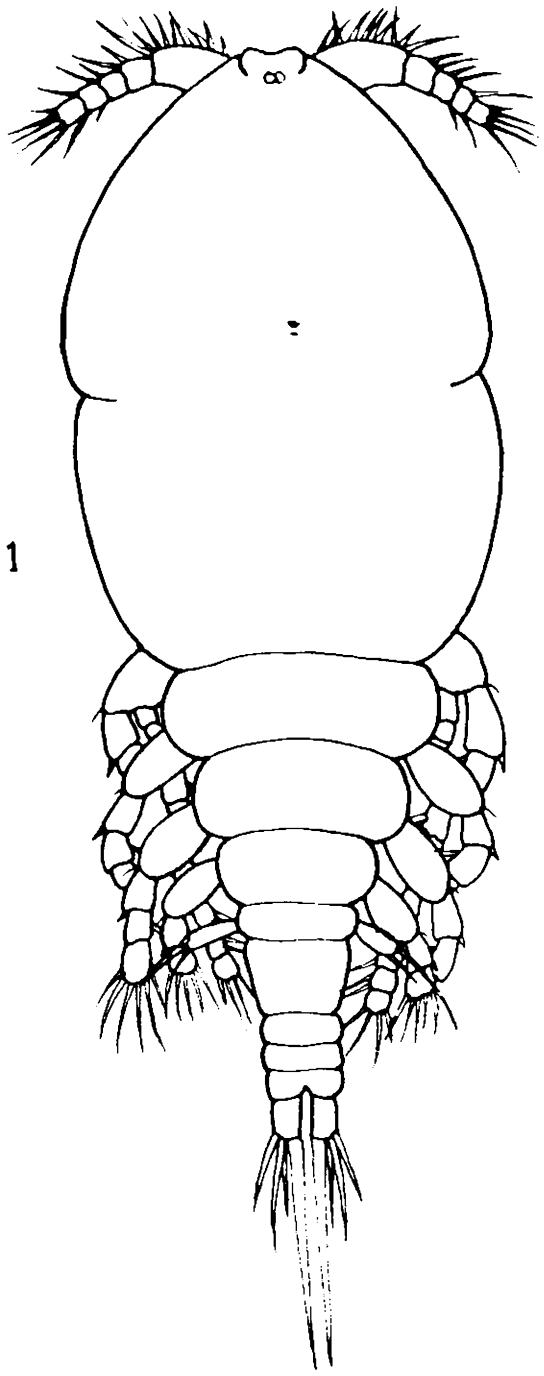
2 First antenna

3 Second antenna

4 Mandible

5 First Maxilla

6 Second Maxilla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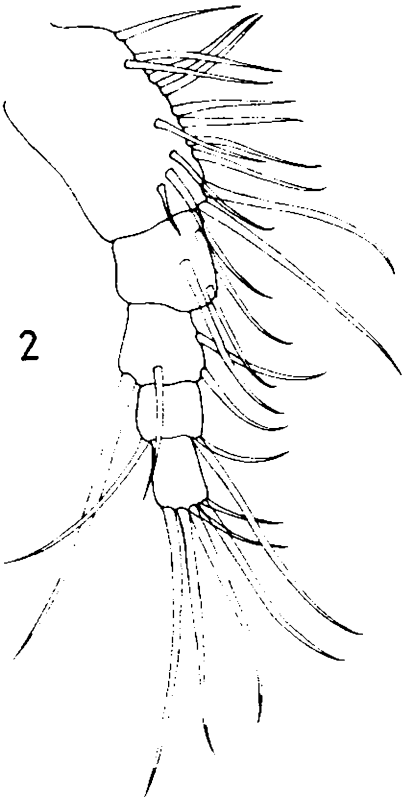
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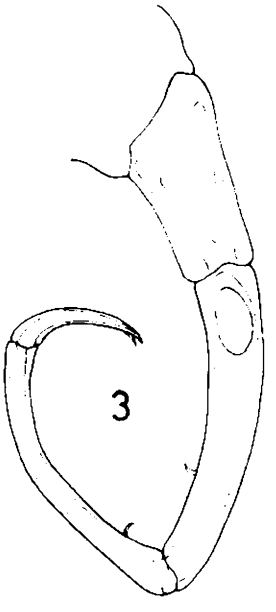
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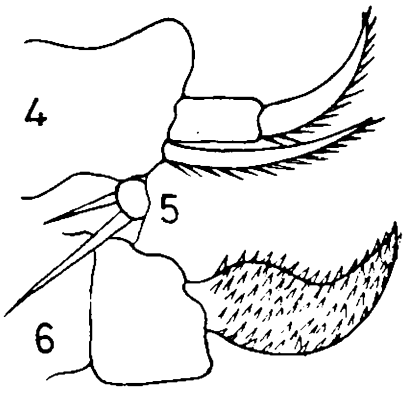
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EXPLANATION OF FIGURES

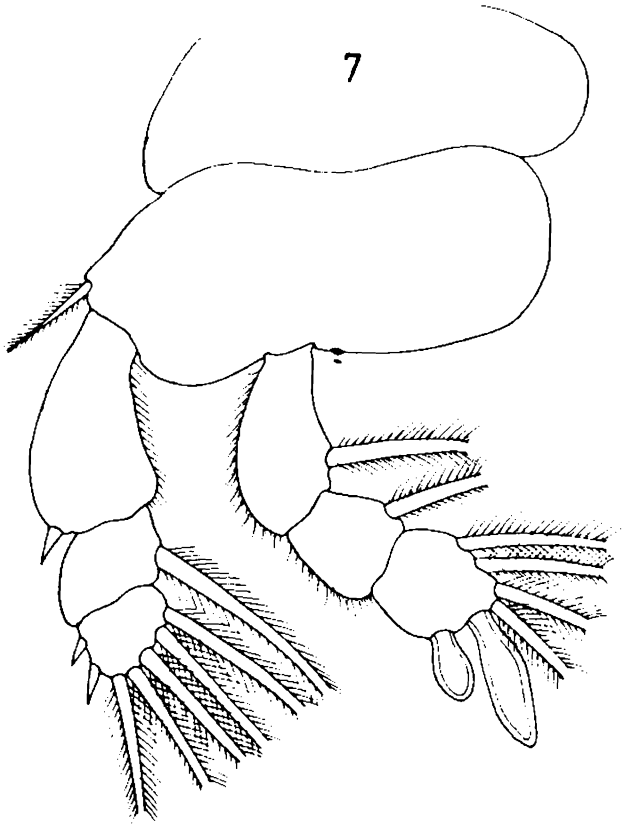
Ergasilus thammani sp. nov.

7 First leg

8 Second leg

9 Third leg

10 Fourth leg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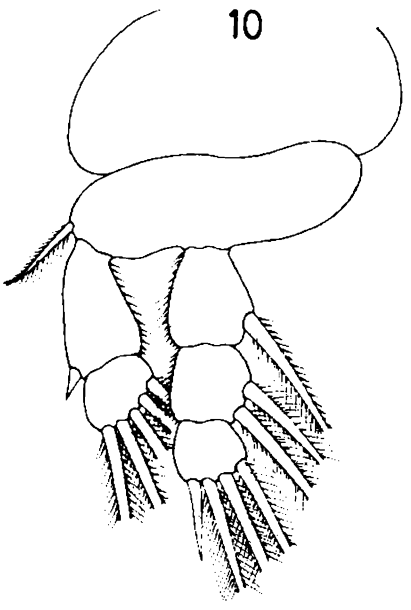
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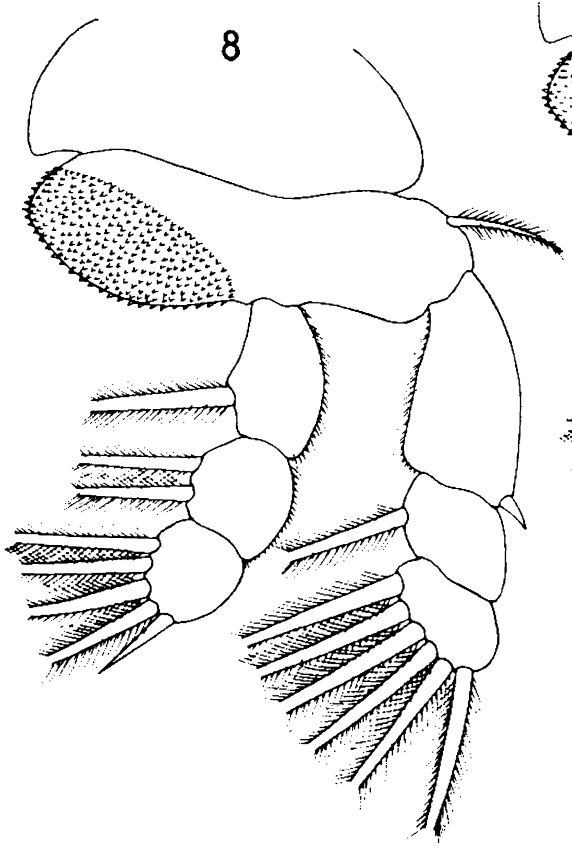
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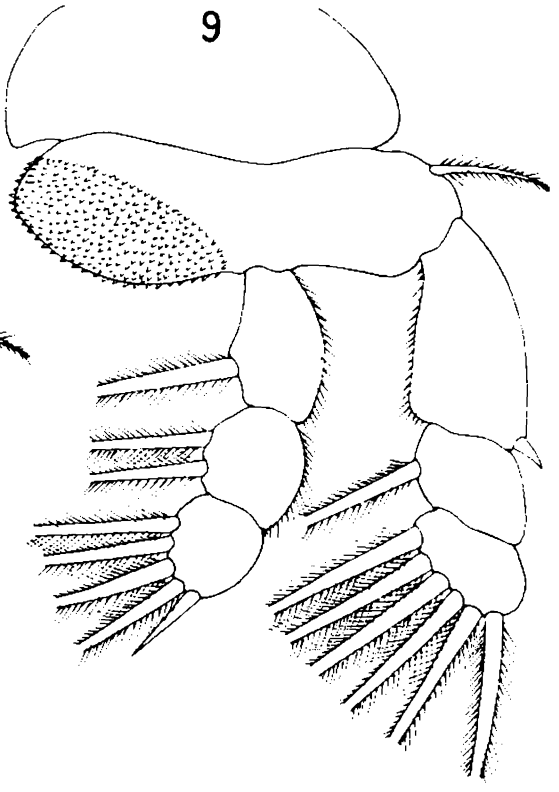
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EXPLANATION OF FIGURES

Ergasilus thammani sp. nov.

11 Fifth leg

12 Uropods

Ergasilus vembanadi sp. nov.

Figs. 13 - 24

13 Female, dorsal view

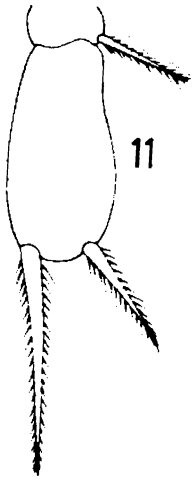
14 First anetnna

15 Second antenna

16 Mandible

17 First maxilla

18 Second maxilla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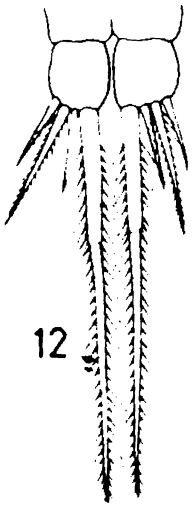
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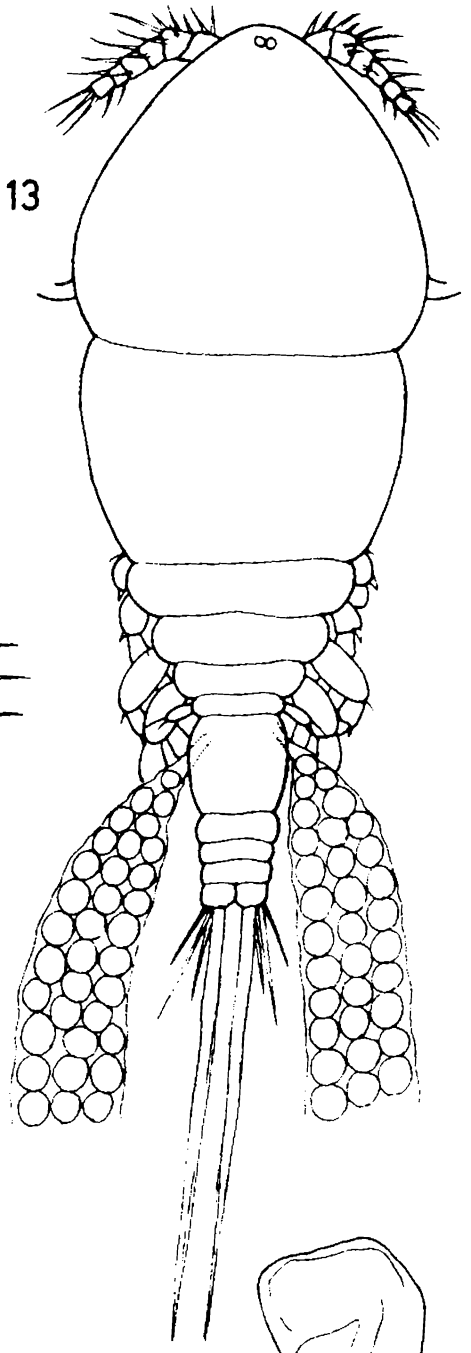
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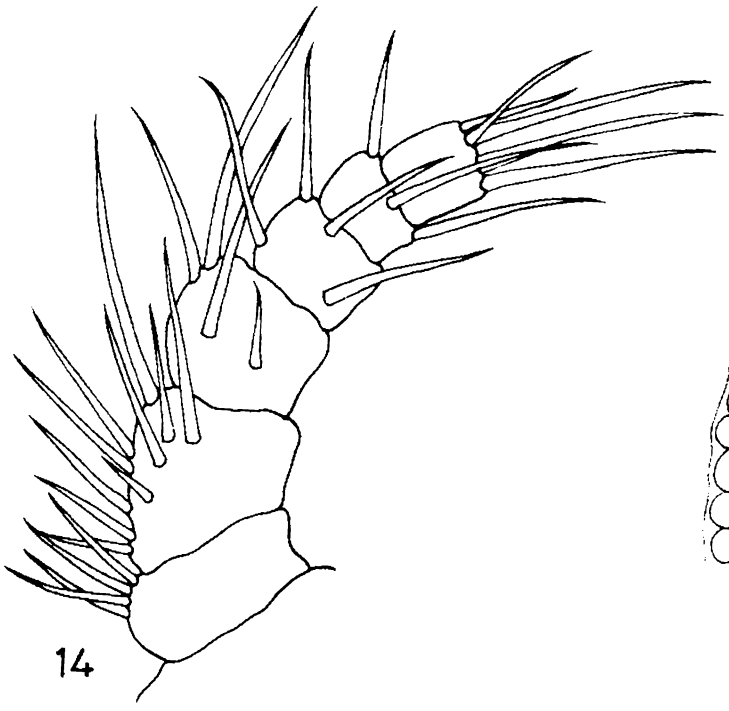
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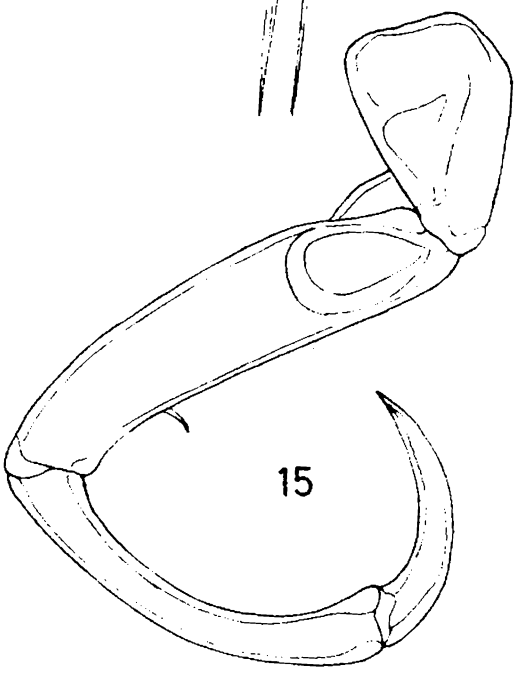
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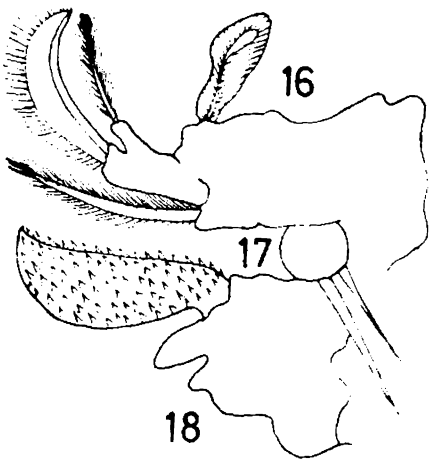
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EXPLANATION OF FIGURES

Ergasilus vembanadi sp. nov.

19 First leg

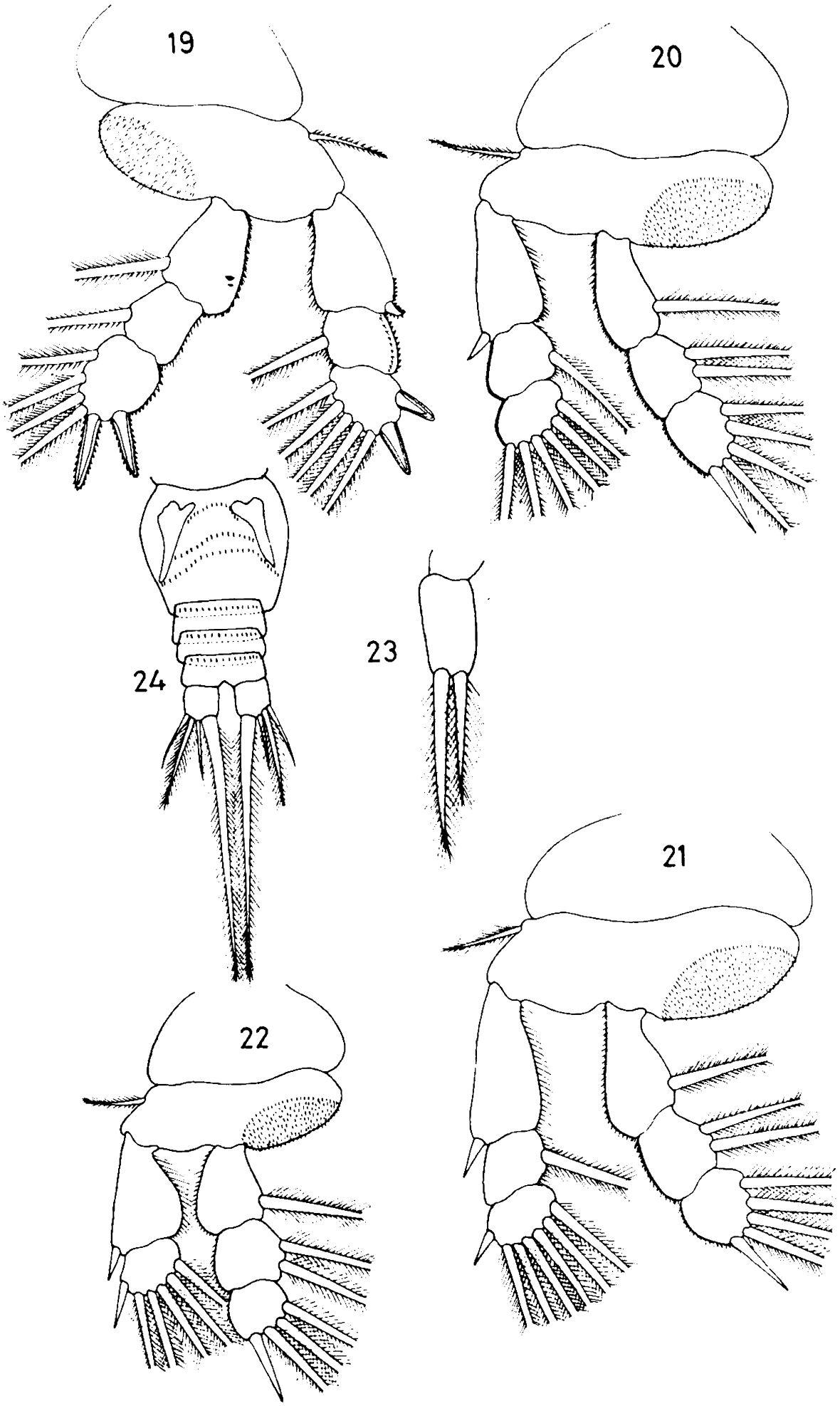
20 Second leg

21 Third leg

22 Fourth leg

23 Fifth leg

24 Ventral view of genital segment, abdomen and uropods.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EXPLANATION OF FIGURES

Ergasilus kabati sp. nov.

Figs. 25 - 36

25 Female, dorsal view

26 First antenna

27 Second antenna

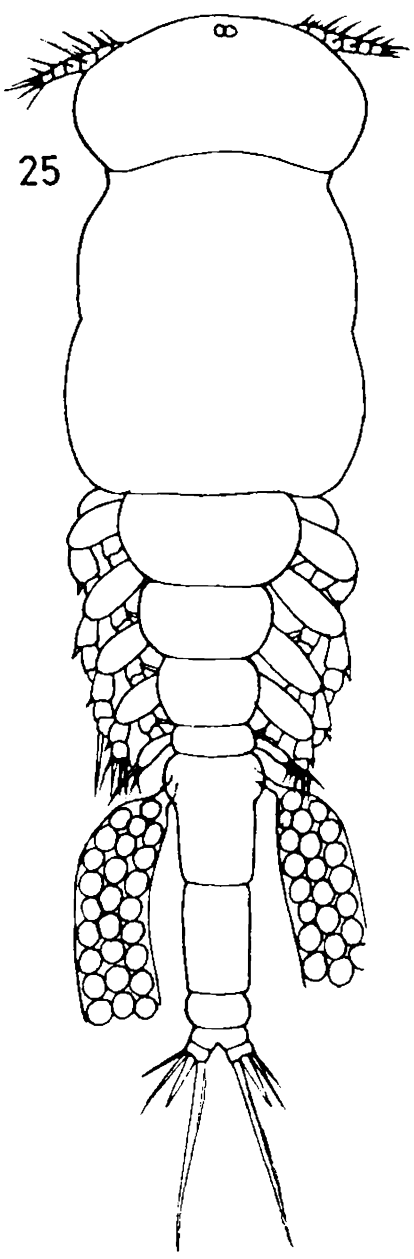
28 Mandible

29 First maxilla

30 Second maxilla

31 First leg

32 Second leg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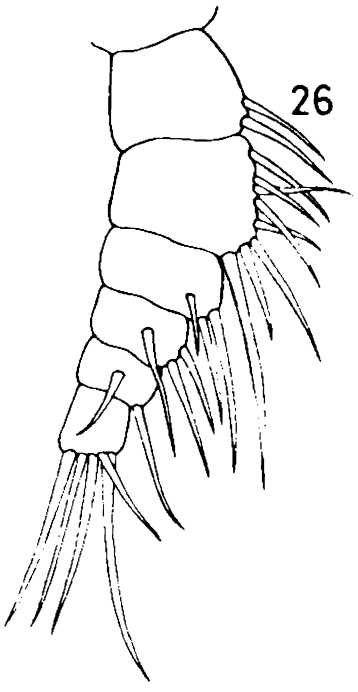
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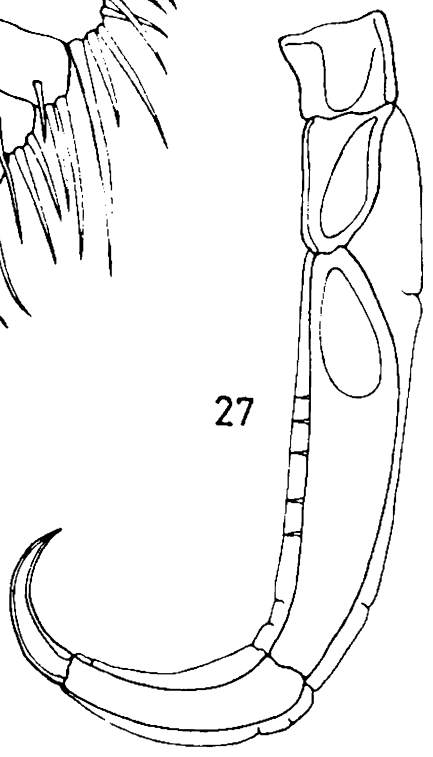
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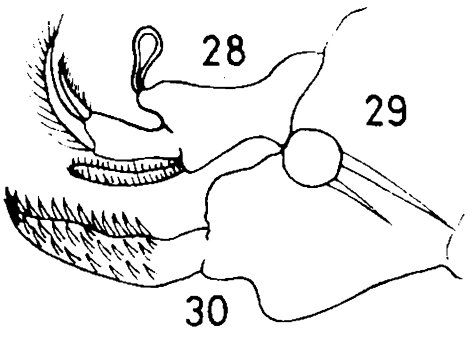
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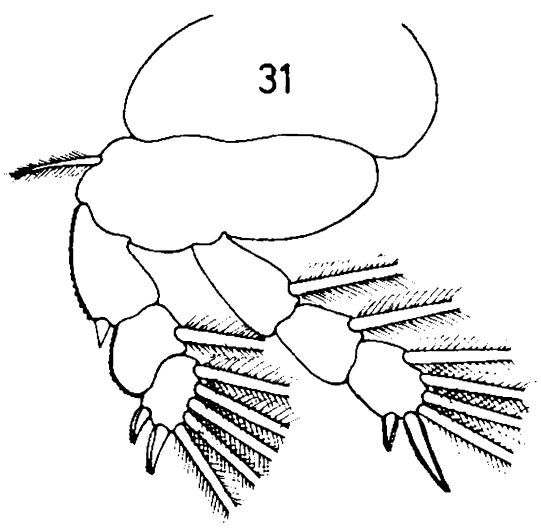
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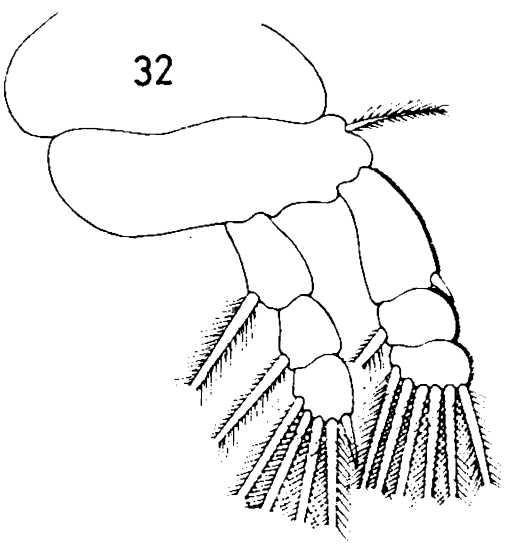
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EXPLANATION OF FIGURES

Ergasilus kabati sp. nov.

33 Third leg

34 Fourth leg

35 Fifth leg

36 Uropods

Lamproglena krishnai sp. nov.

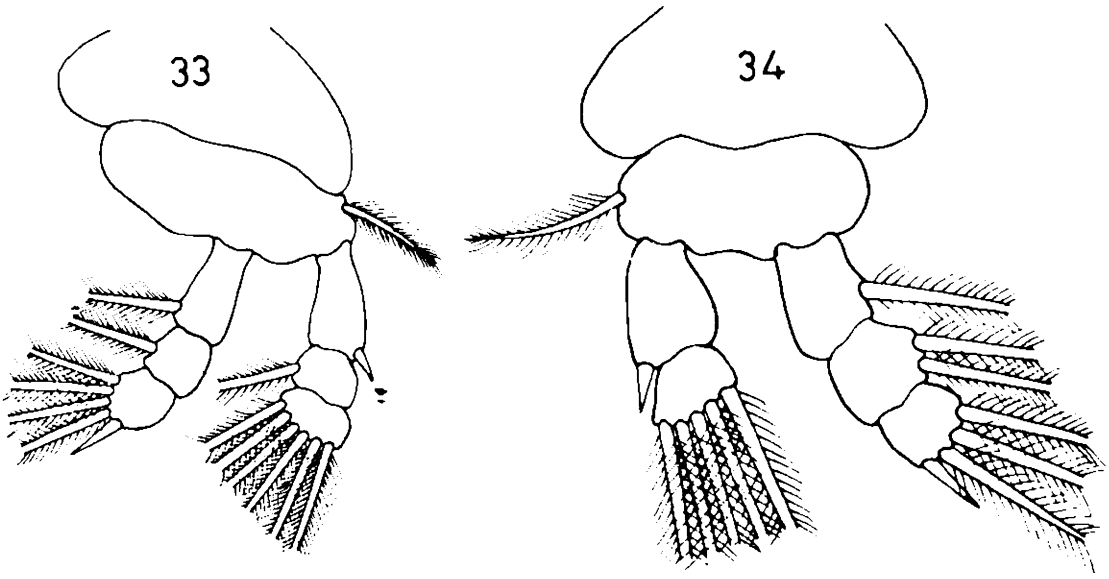
Figs. 37 - 46

37 Female, dorsal view

38 First antenna

39 Second antenna

40 Maxilla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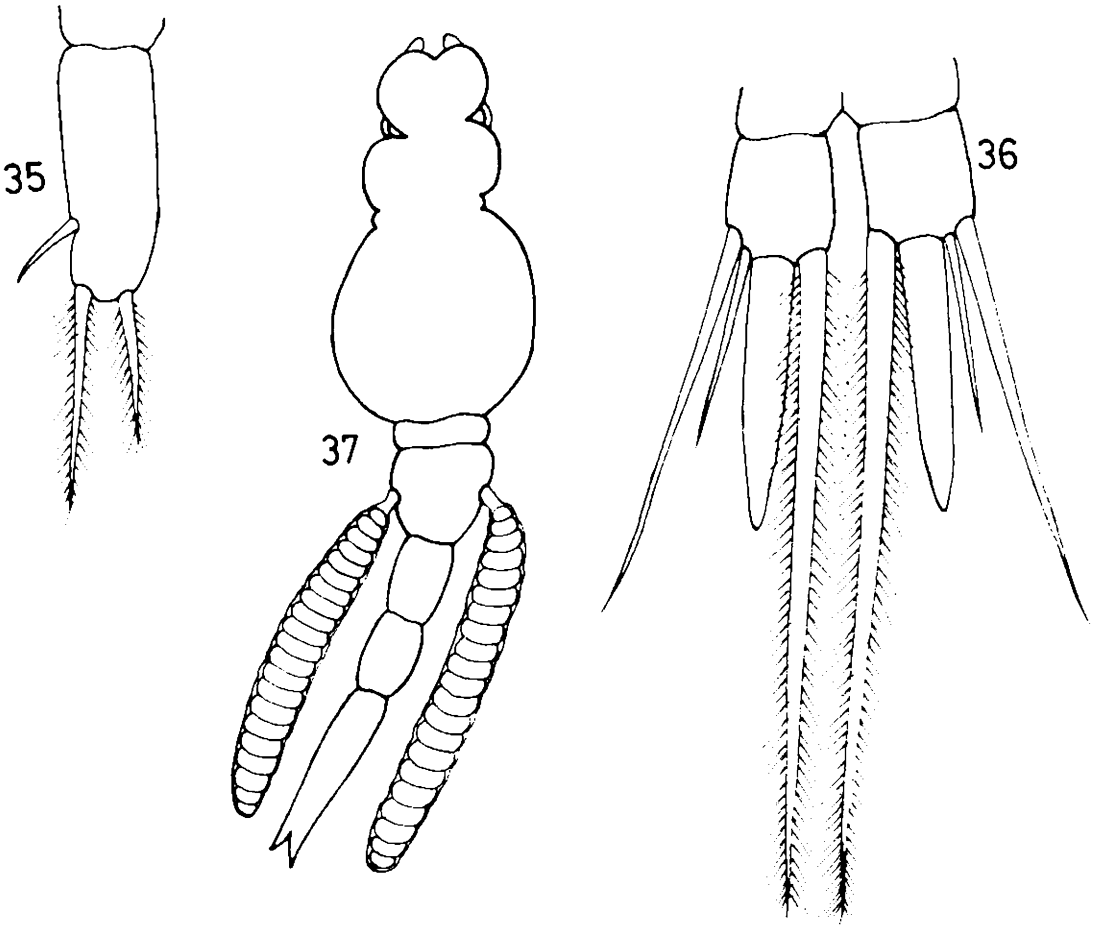
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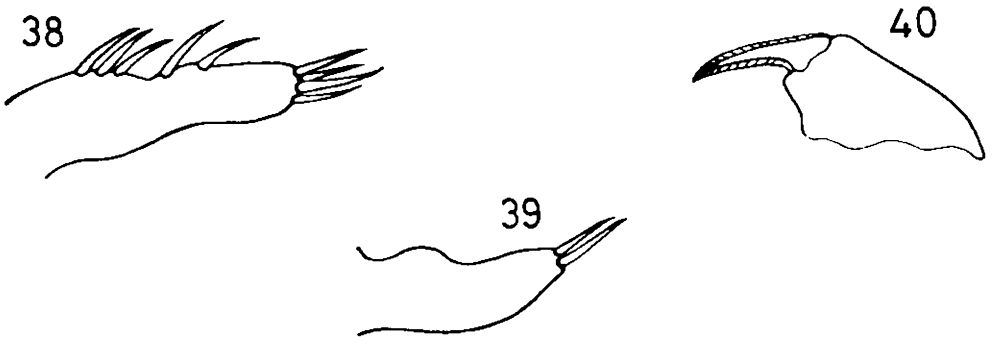
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EXPLANATION OF FIGURES

Lamproglena krishnai sp. nov.

41 Maxilliped

42 First leg

43 Second leg

44 Third leg

45 Fourth leg

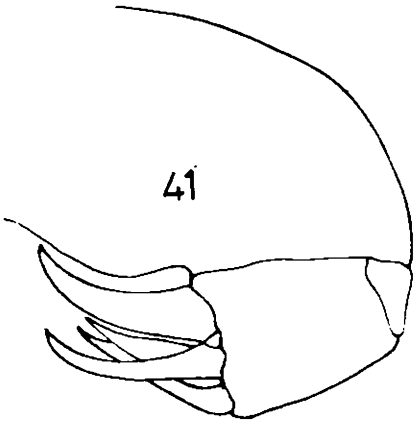
46 Fifth leg

47 Uropods

Lernaea osphronemi sp. nov.

Figs. 48 - 64

48 Female, dorsal view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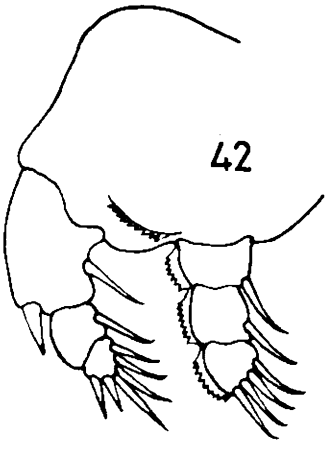
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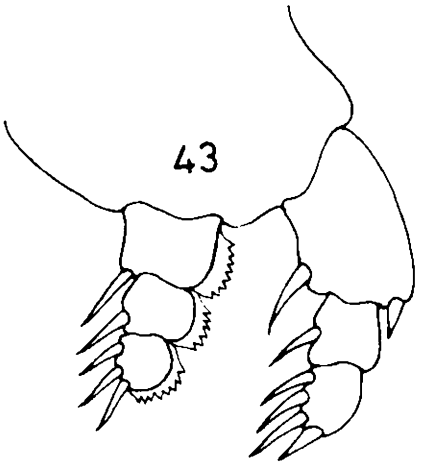
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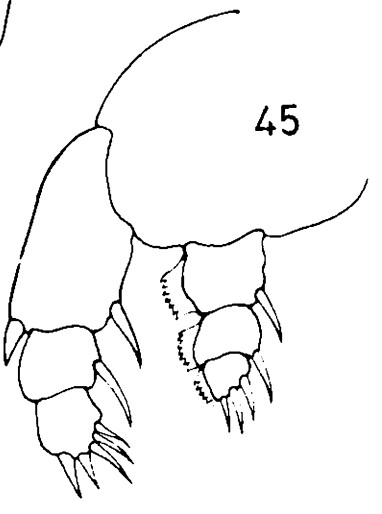
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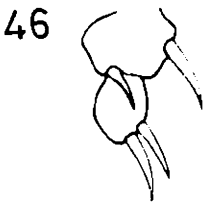
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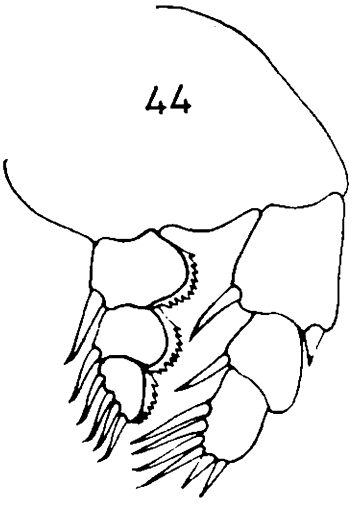
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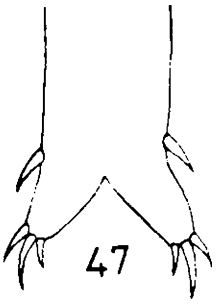
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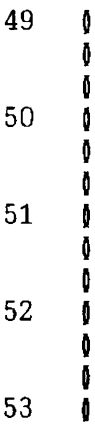
EXPLANATION OF FIGURES

Lernaea osphronemi



49 50 51 52 53 variations in the structure of holdfast.

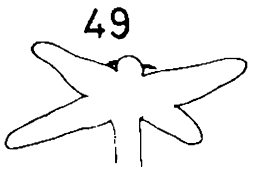
54 First antenna

55 Second antenna

56 Second maxilla

57 Maxilliped

58 First leg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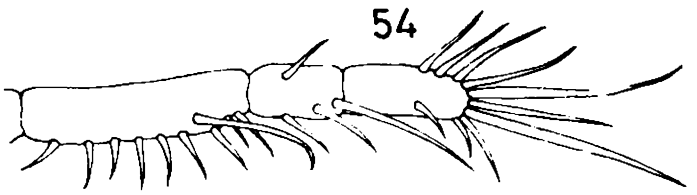
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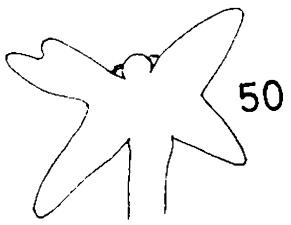
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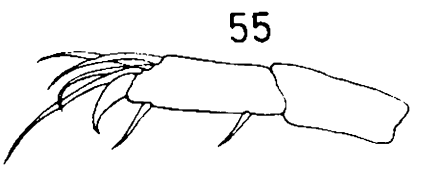
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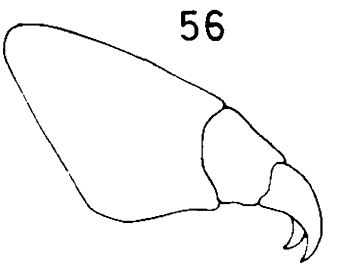
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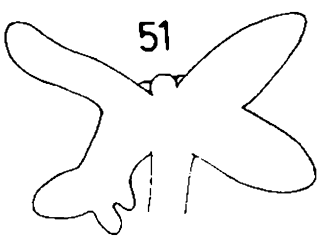
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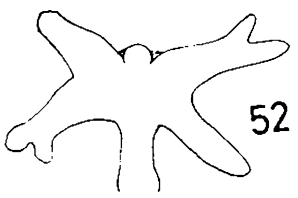
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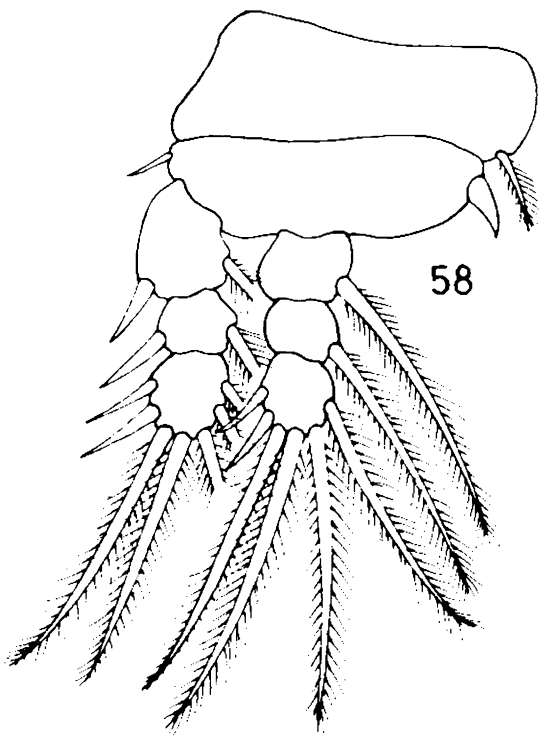
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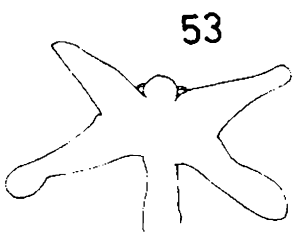
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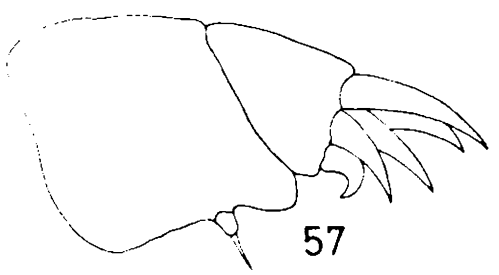
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EXPLANATION OF FIGURES

Lernaea osphronemi sp. nov.

59 Second leg

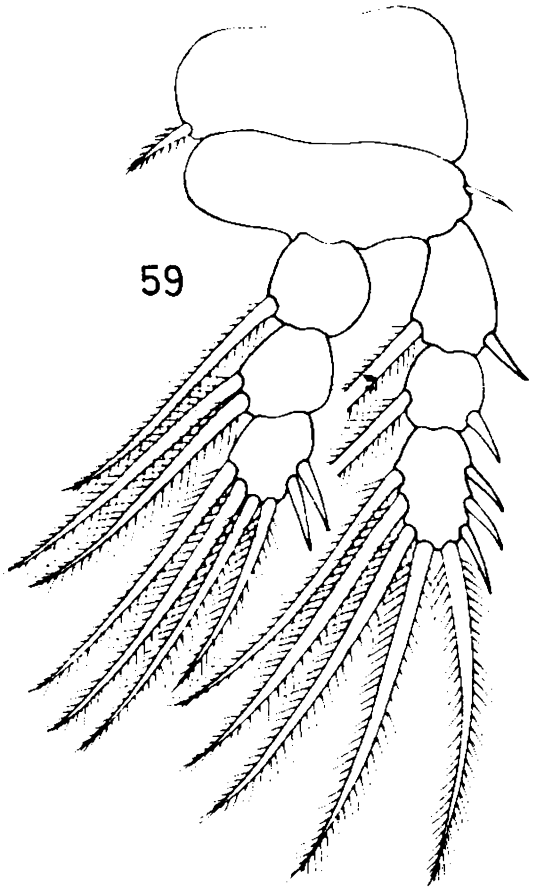
60 Third leg

61 Fourth leg

62 Fifth leg

63 Sixth leg

64 Uropods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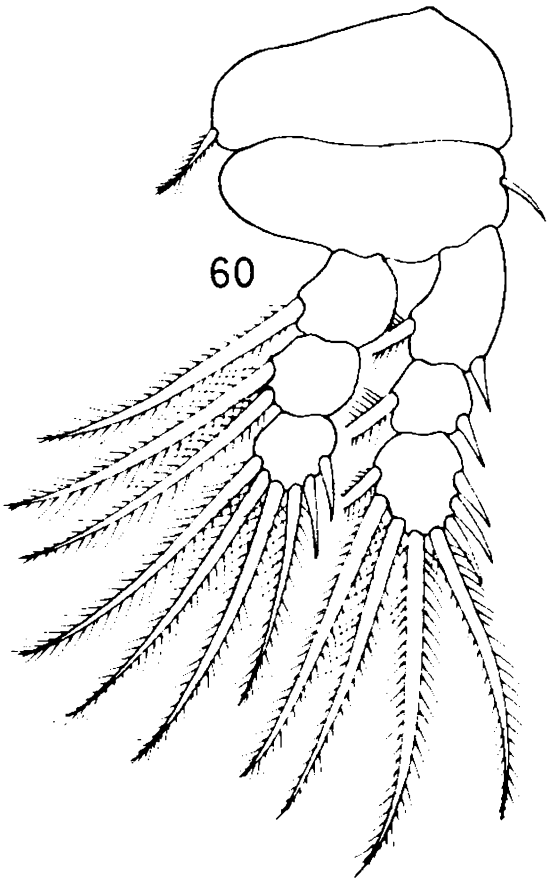
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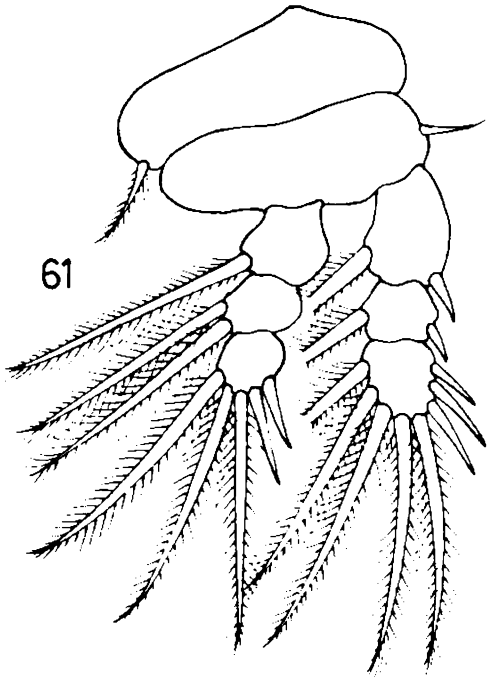
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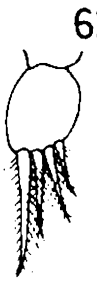
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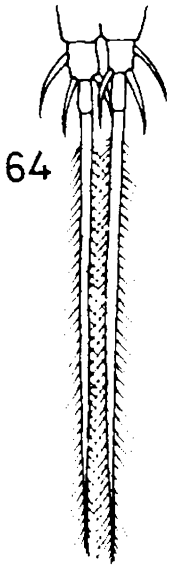
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EXPLANATION OF FIGURES

First Nauplius

Figs. 1 - 4

1 I Nauplius, dorsal view

2 First antenna

3 Second antenna

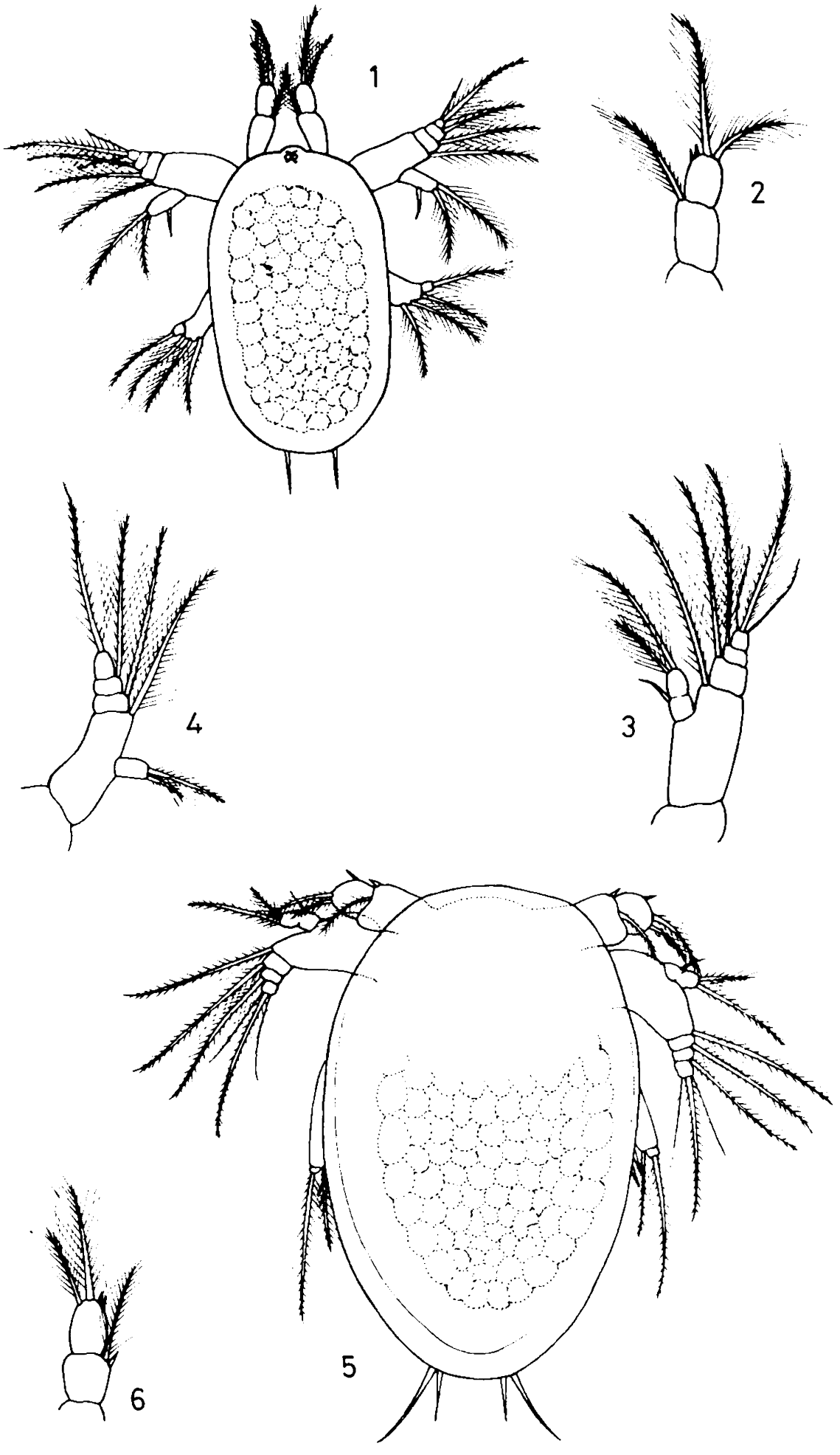
4 Mandible

Second Nauplius

Figs. 5 - 8

5 II Nauplius, dorsal view

6 First antenna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EXPLANATION OF FIGURES

II Nauplius

7 Second antenna

8 Mandible

Third Nauplius

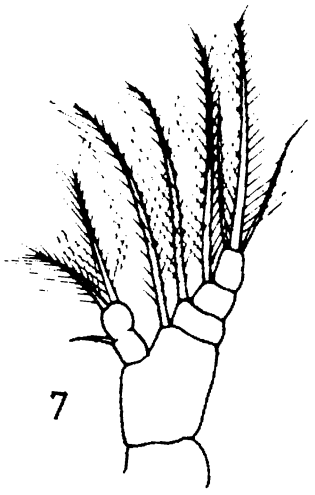
Figs 9 - 12

9 III Nauplius, dorsal view

10 First antenna

11 Second antenna

12 Mandible



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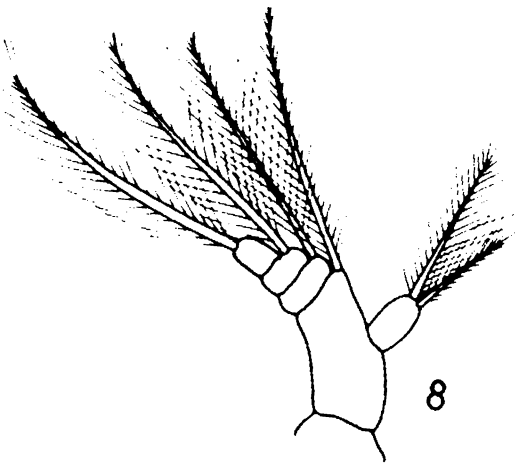

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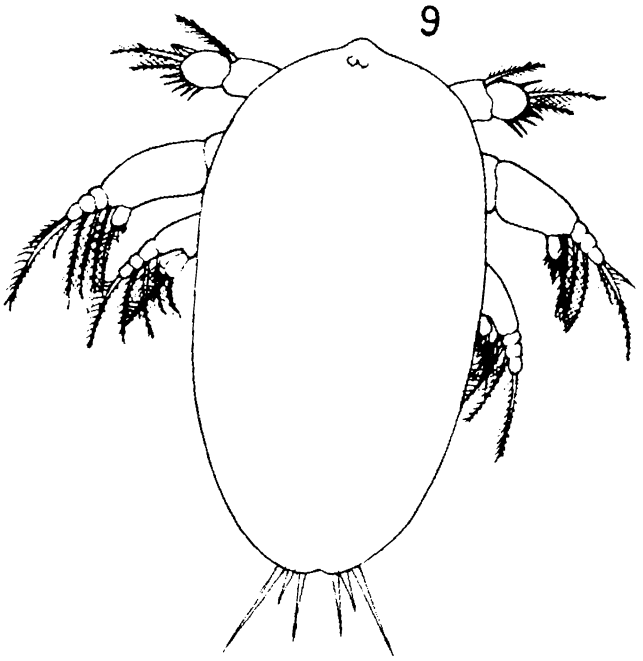

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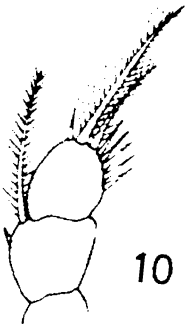

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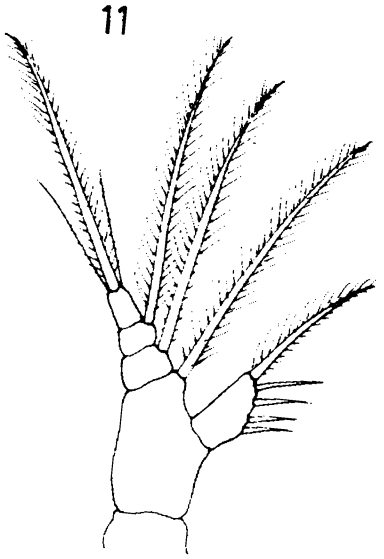

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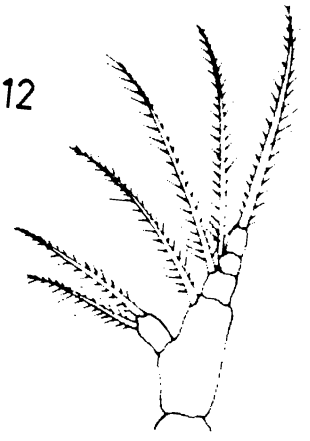


12

EXPLANATION OF FIGURES

First Copepodid

Figs. 13 - 22

13 I Copepodid, dorsal view

14 First antenna

15 Second antenna

16 Mandible

17 First maxilla

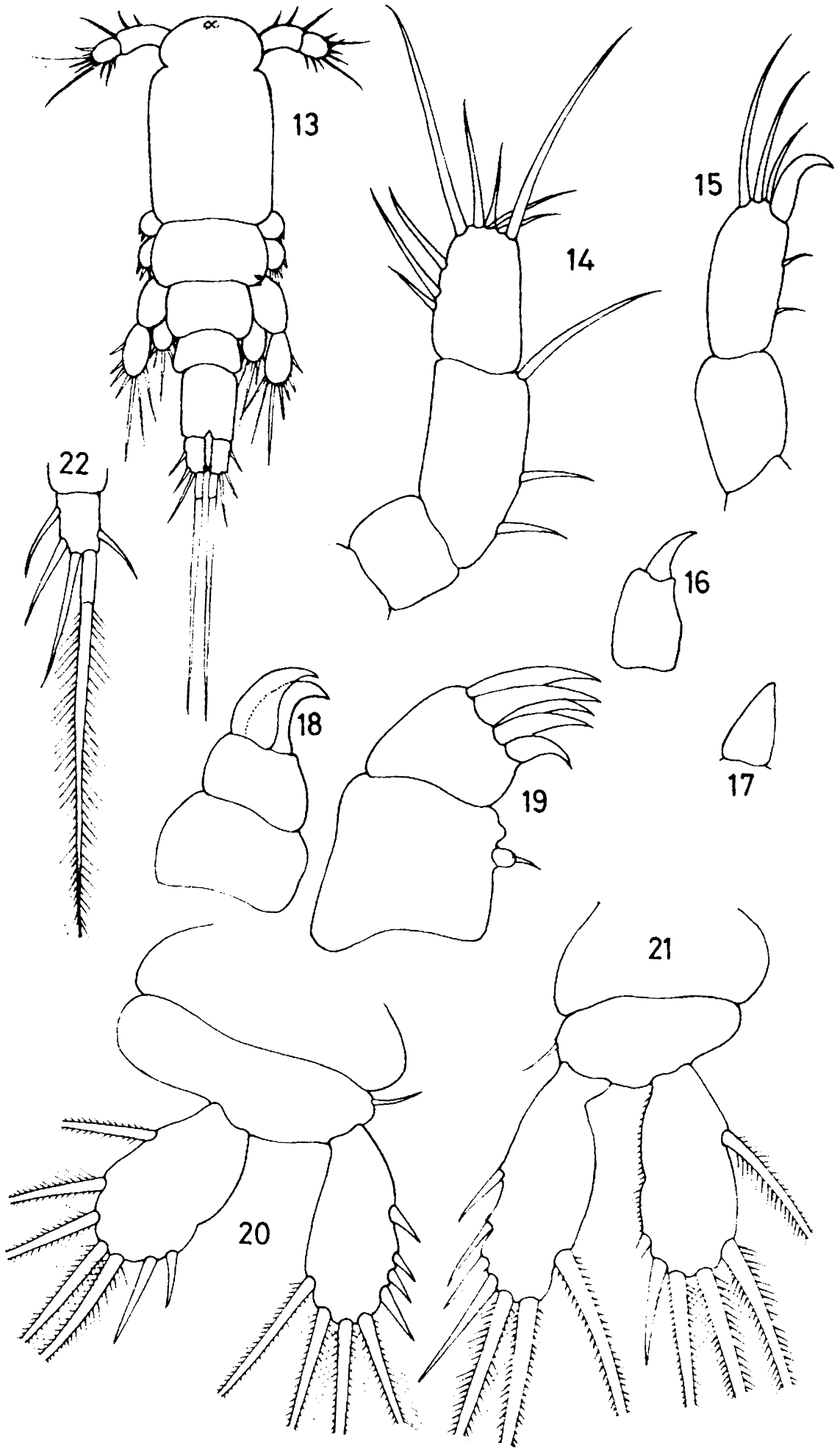
18 Second maxilla

19 Maxilliped

20 First leg

21 Second leg

22 Uropod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EXPLANATION OF FIGURES

Second Copepodid

Figs 23 - 33

23 II Copepodid

24 First antenna

25 Second antenna

26 Mandible

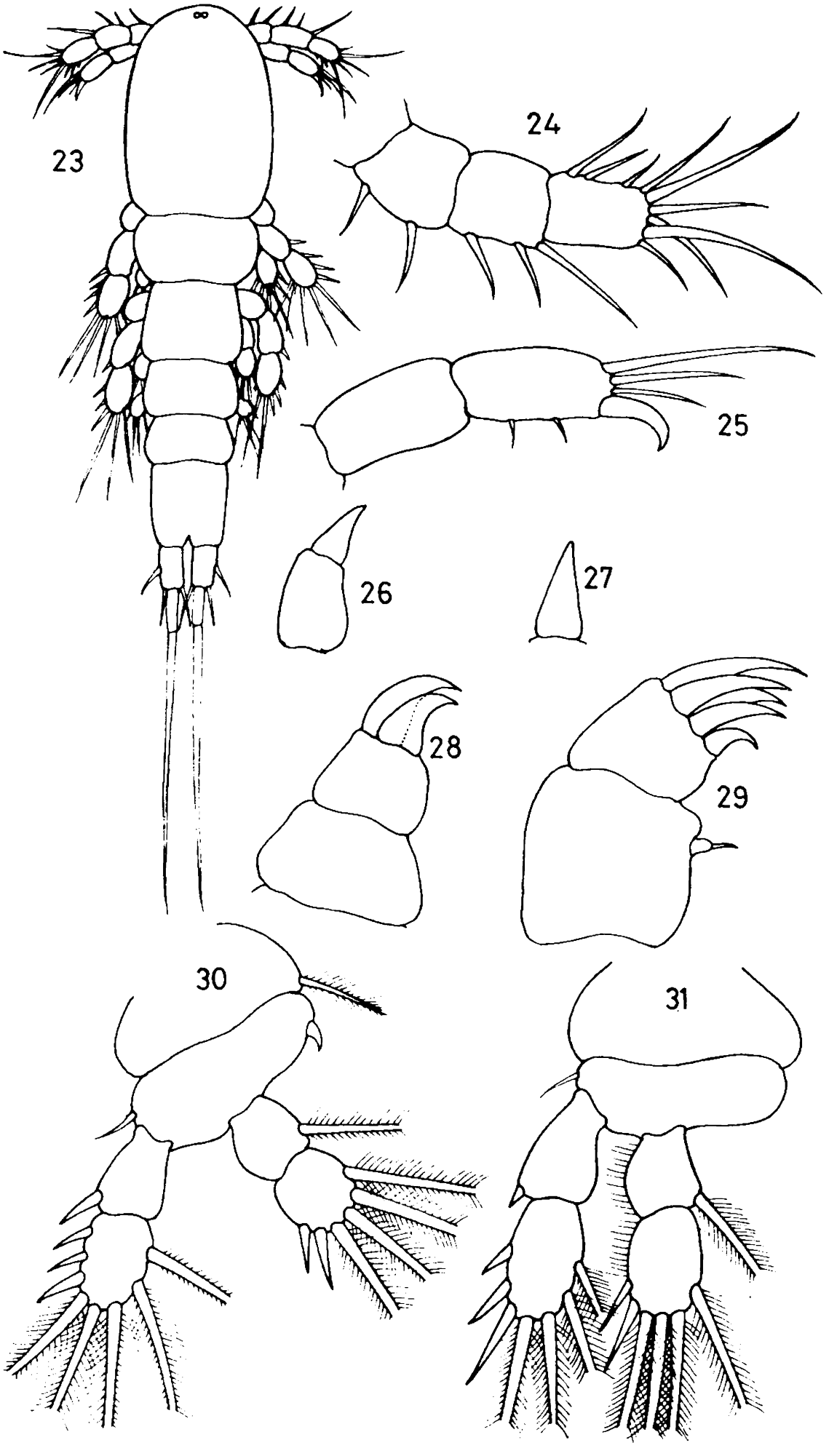
27 First maxilla

28 Second maxilla

29 Maxilliped

30 First leg

31 Second leg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EXPLANATION OF FIGURES

II Copepodid

32 Third leg

33 Uropod

Third Copepodid

Figs. 34 - 45

34 III Copepodid, dorsal view

35 First antenna

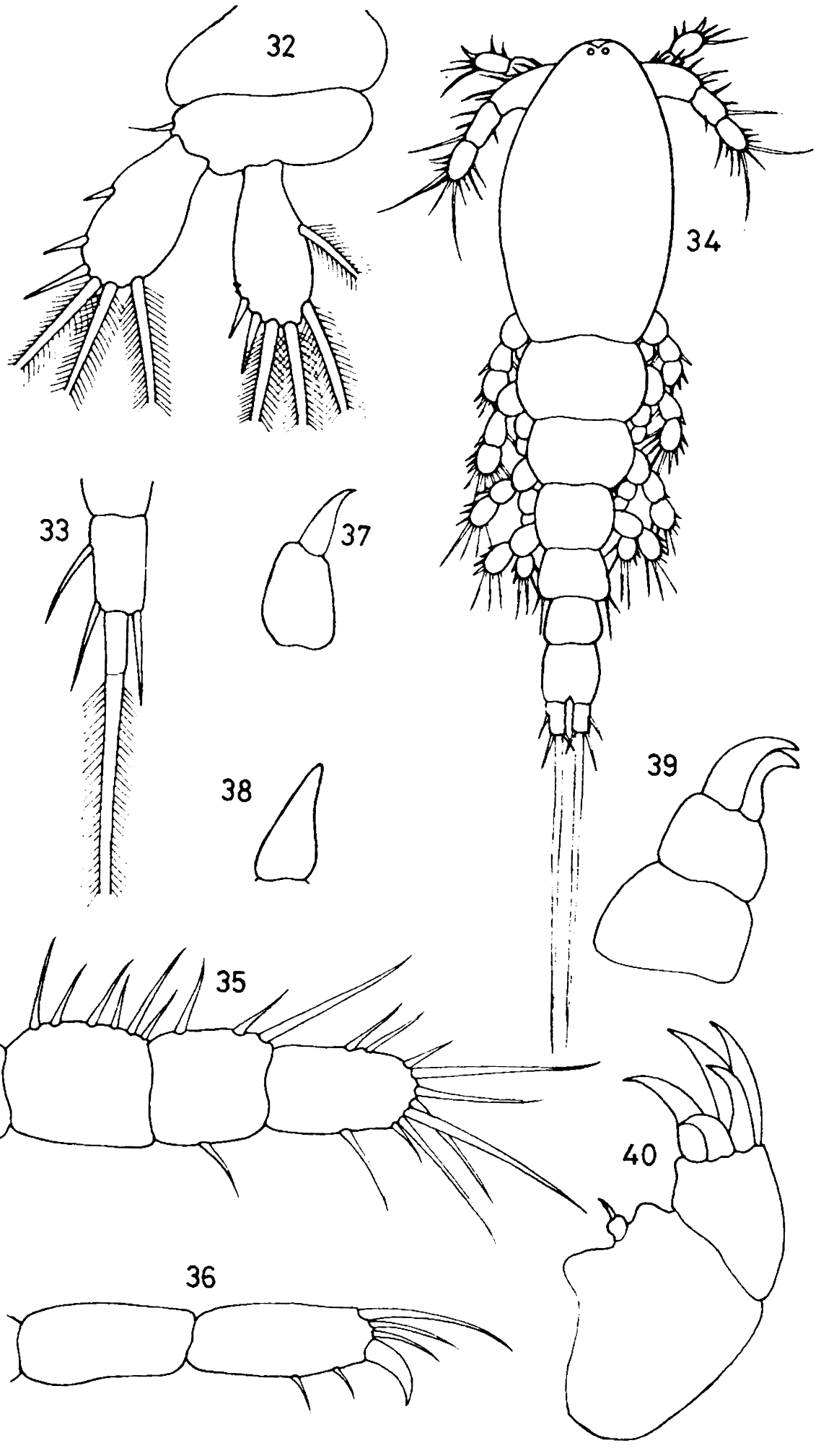
36 Second antenna

37 Mandible

38 First maxilla

39 Second maxilla

40 Maxilliped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EXPLANATION OF FIGURES

III Copepodid

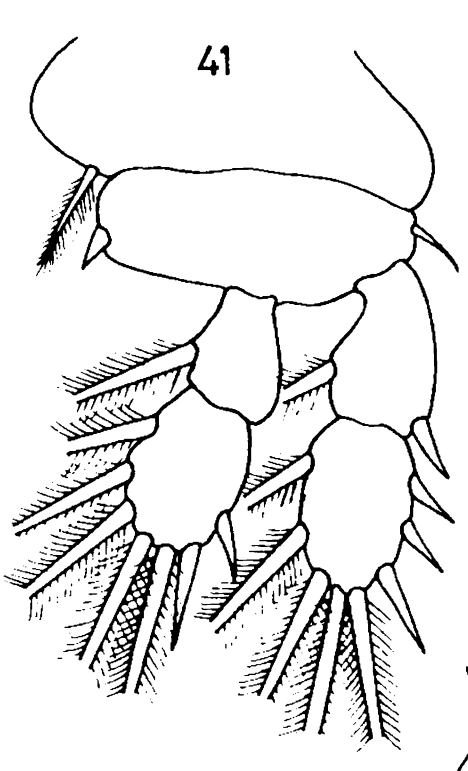
41 First leg

42 Second leg

43 Third leg

44 Fourth leg

45 Uropod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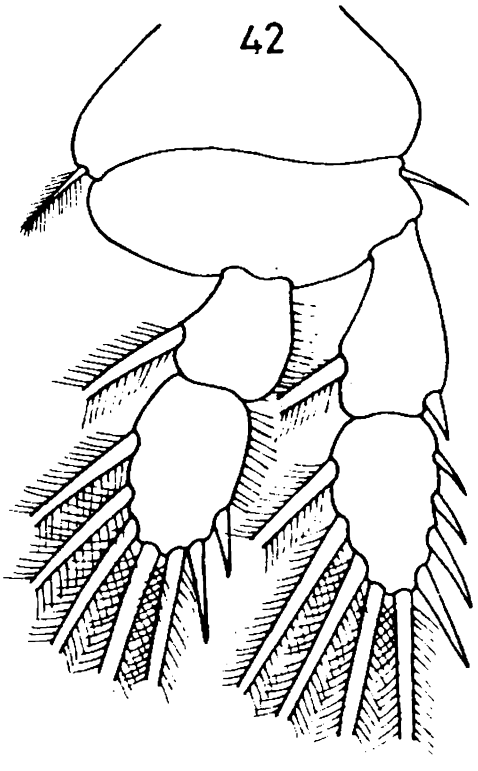
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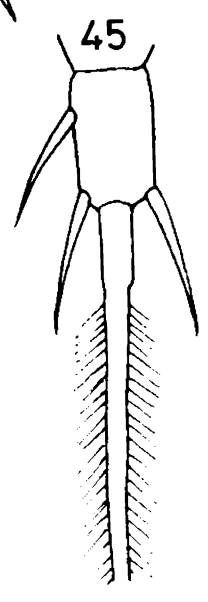
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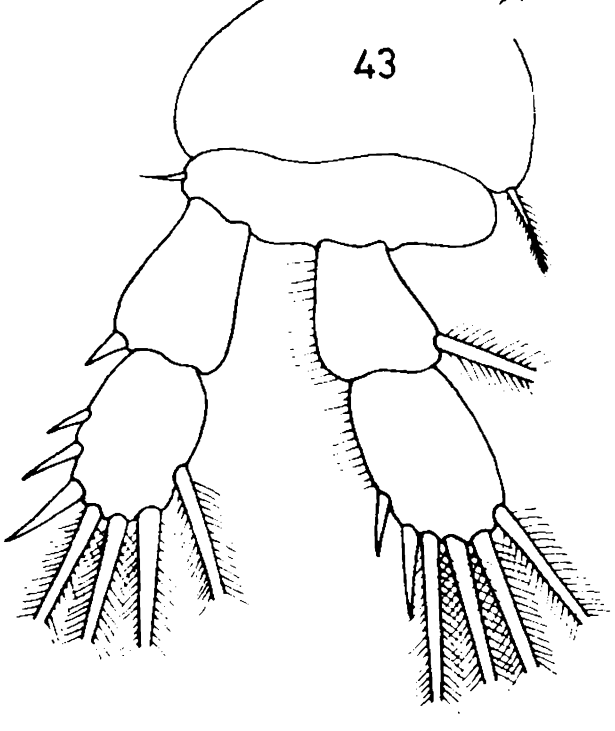
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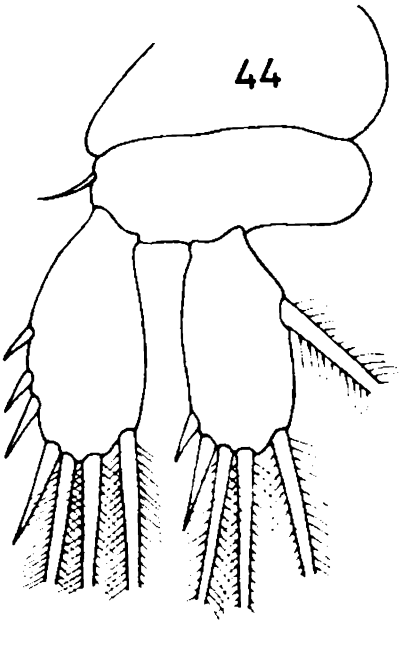

44

EXPLANATION OF FIGURES

Fourth Copepodid

Figs. 46 - 58

46 IV Copepodid, dorsal view

47 First antenna

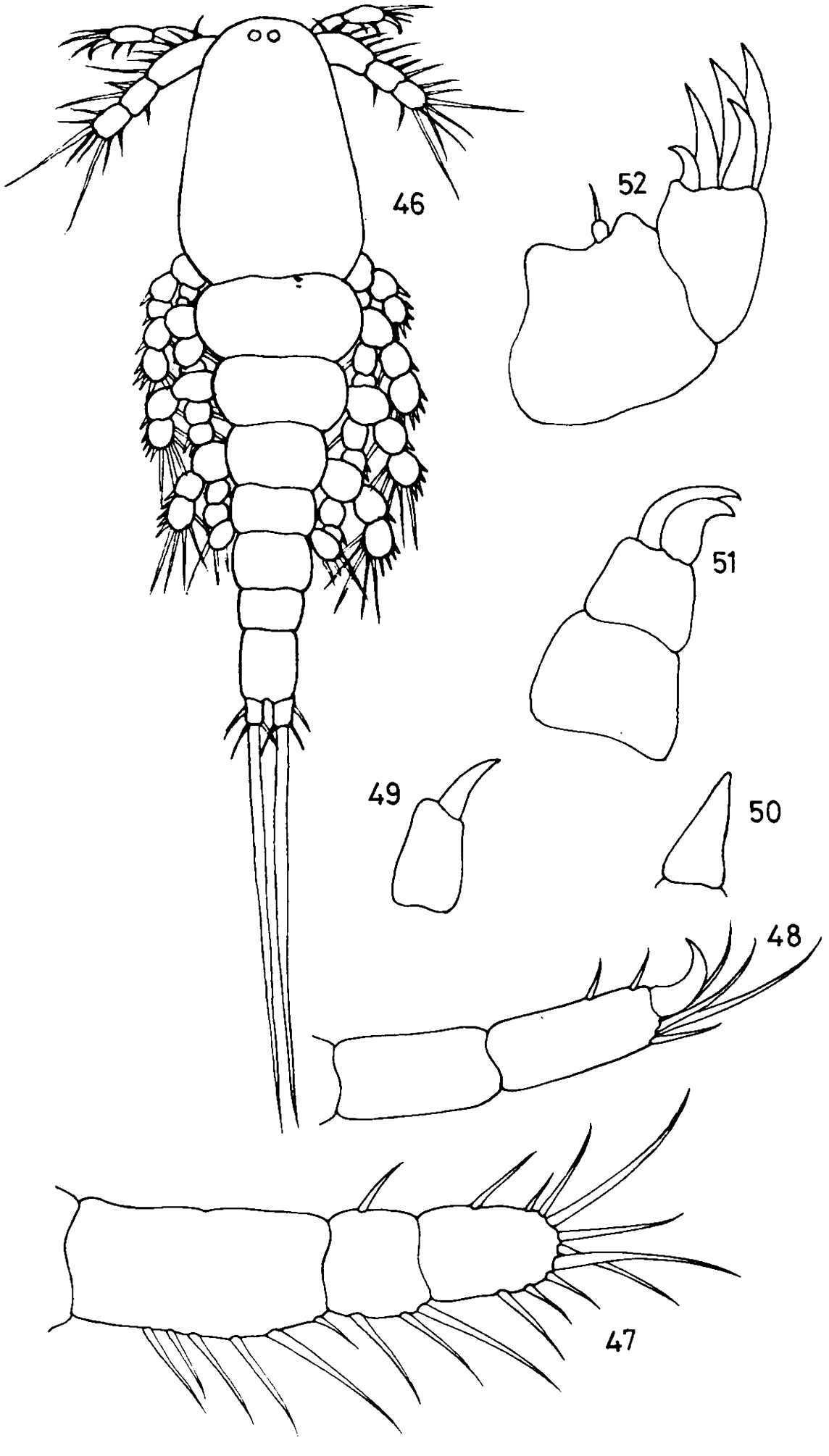
48 Second antenna

49 Mandible

50 First maxilla

51 Second maxilla

52 Maxilliped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EXPLANATION OF FIGURES

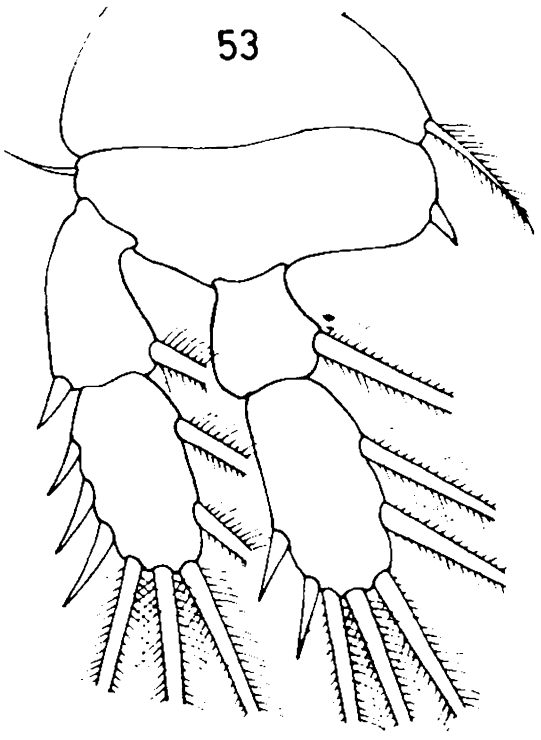
IV Copepodid

53 First leg

54 Second leg

55 Third leg

56 Fourth leg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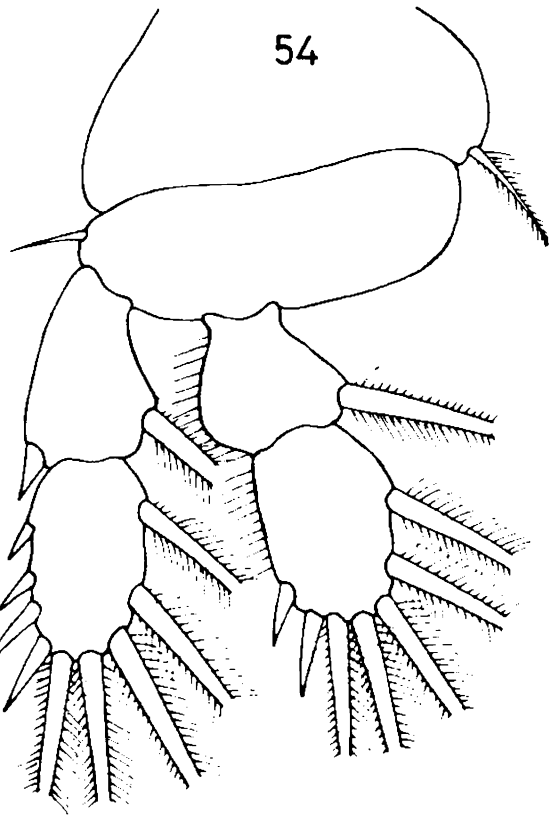
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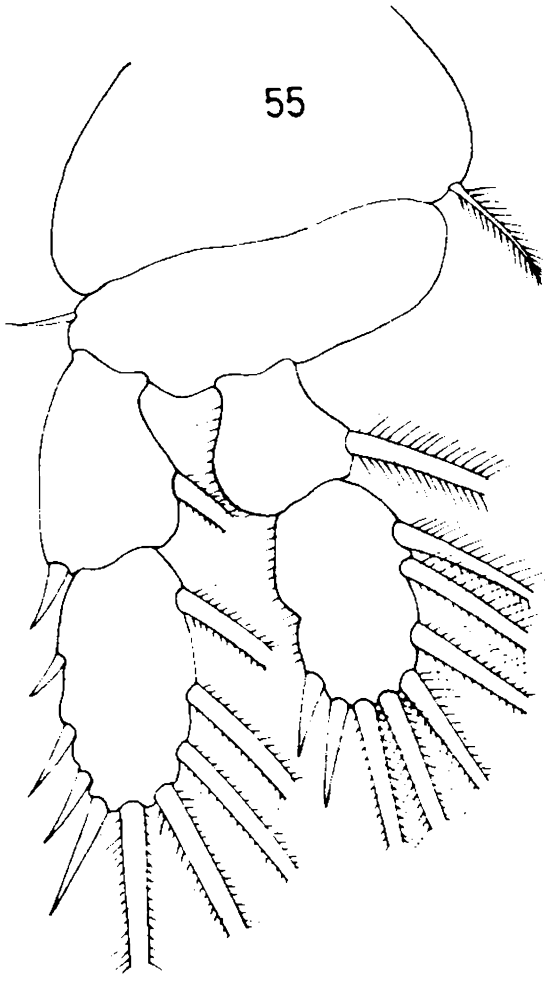
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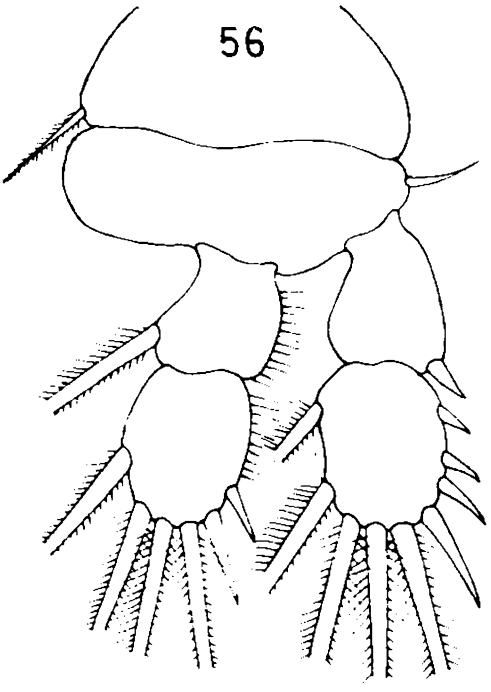
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EXPLANATION OF FIGURES

IV Copepodid

57 Fifth leg

58 Uropod

Female Copepodid

Figs. 59 - 71

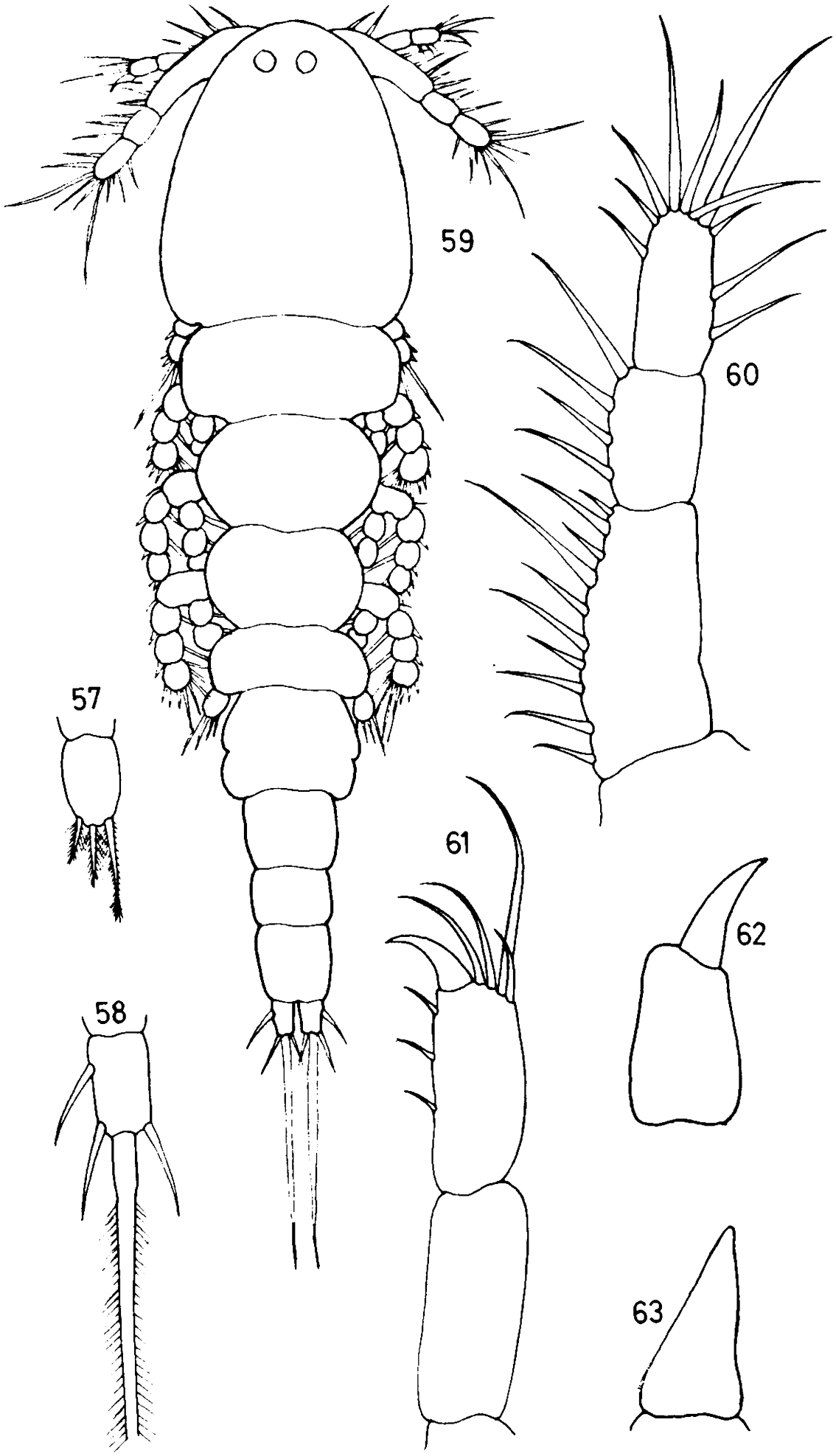
59 Female Copepodid, dorsal view

60 First antenna

61 Second antenna

62 Mandible

63 First maxilla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EXPLANATION OF FIGURES

Female Copepodid

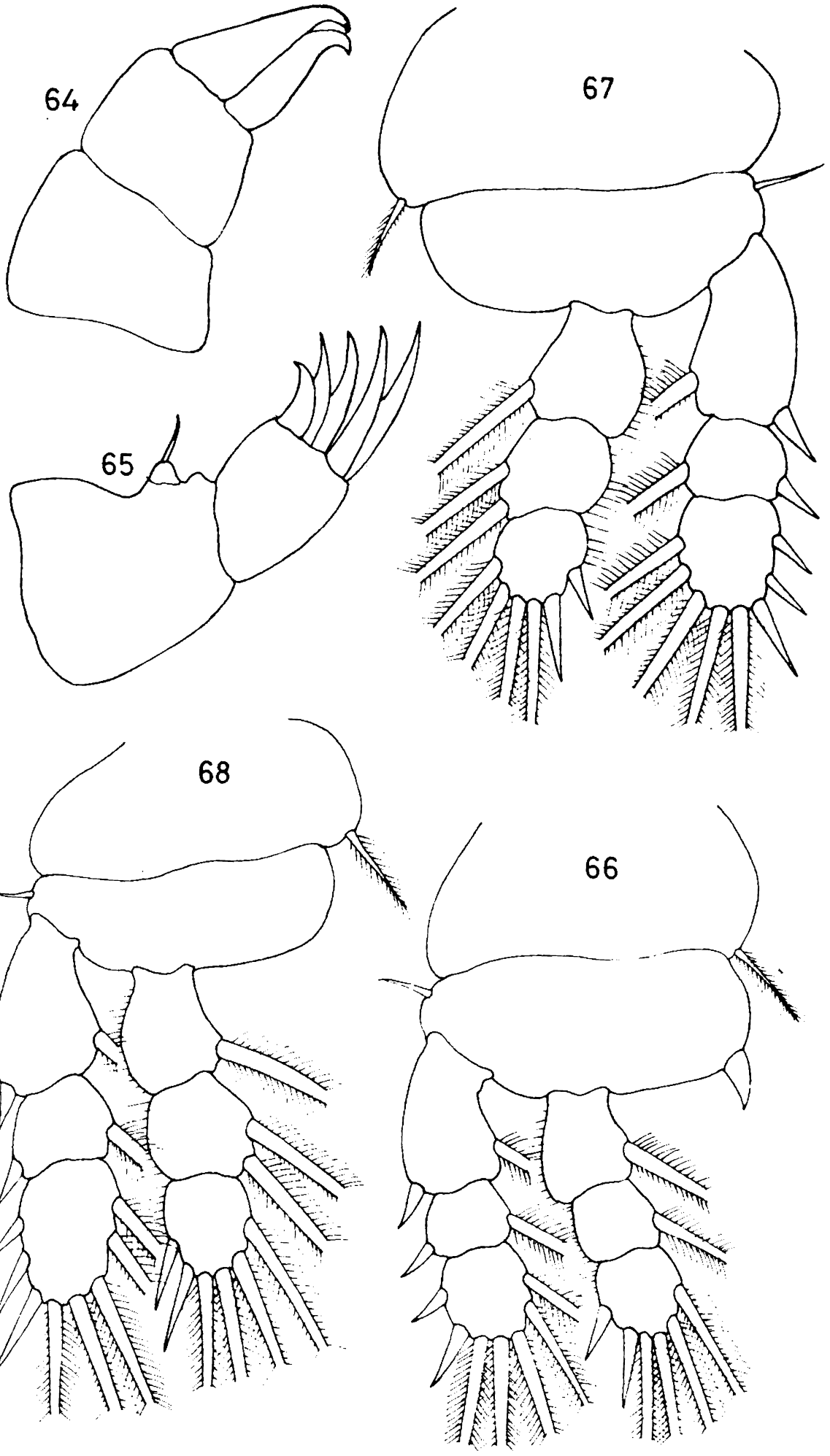
64 Second maxilla

65 Maxilliped

66 First leg

67 Second leg

68 Third leg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EXPLANATION OF FIGURES

Female Copepodid

69 Fourth leg

70 Fifth leg

71 Uropod

Male Copepodid

Figs. 72 - 85

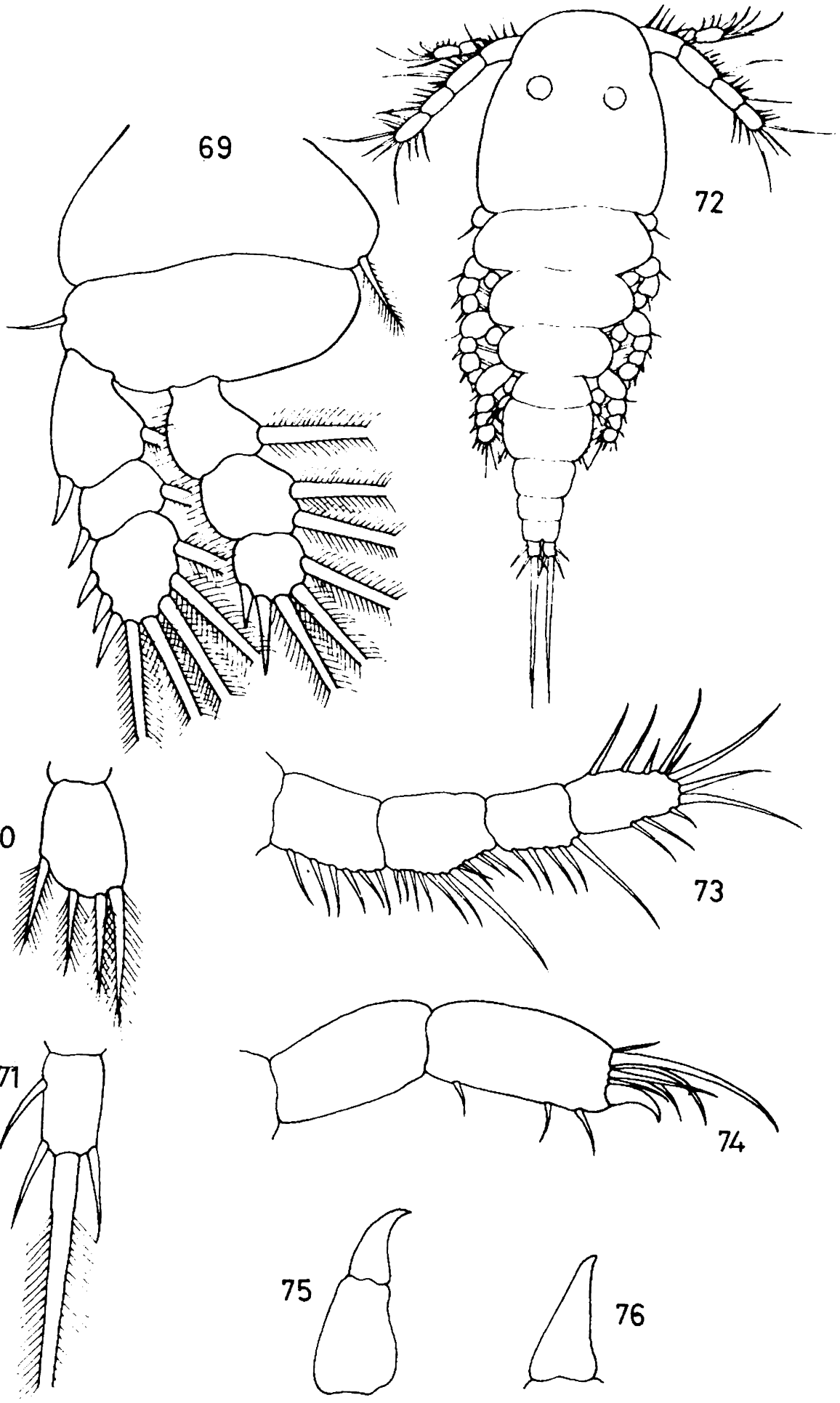
72 Male copepodid, dorsal view

73 First antenna

74 Second antenna

75 Mandible

76 First maxilla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EXPLANATION OF FIGURES

Male Copepodid

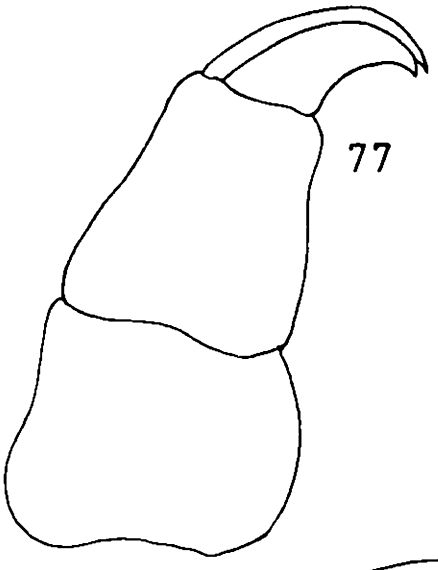
77 Second maxilla

78 Maxillped

79 First leg

80 Second leg

81 Third leg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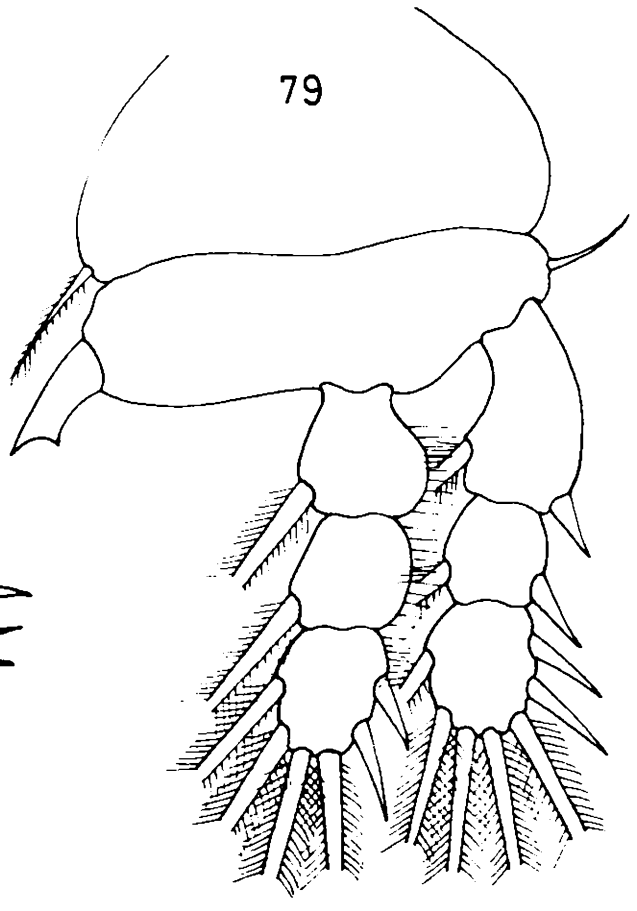
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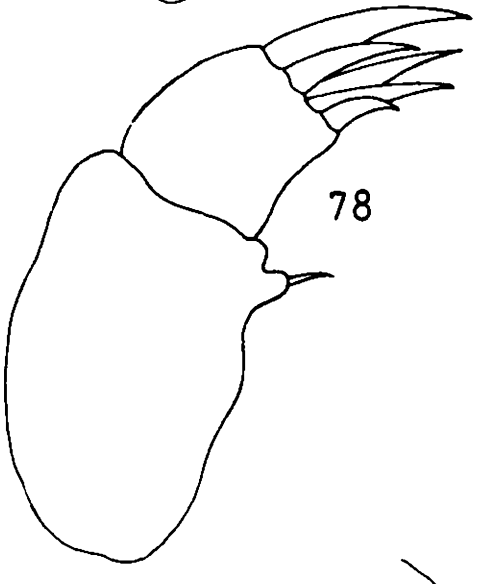
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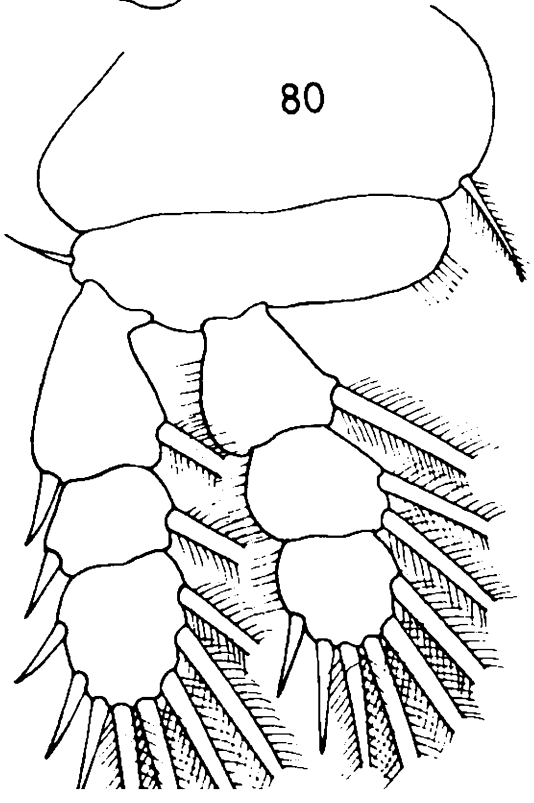
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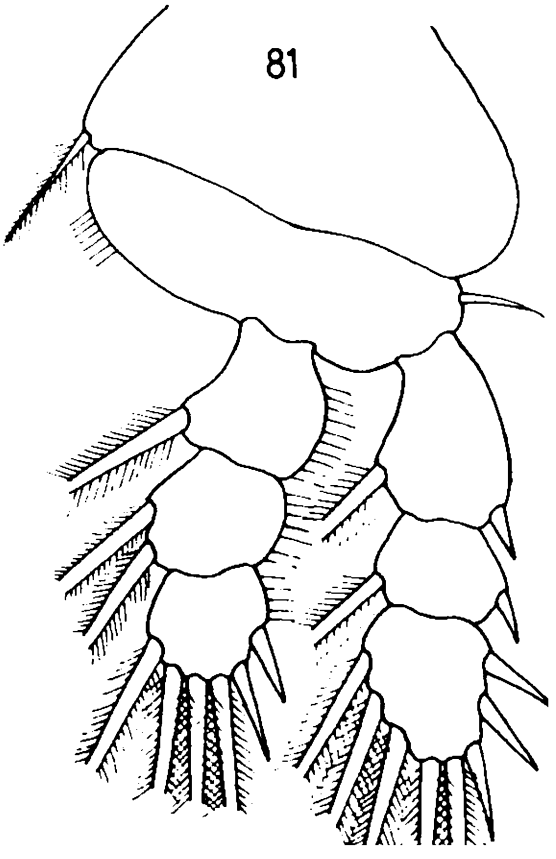
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EXPLANATION OF FIGURES

Male Copepodid

82 Fourth leg

83 Fifth leg

84 Sixth leg

85 Uropod

Cyclopoid Female

Figs. 86 - 99

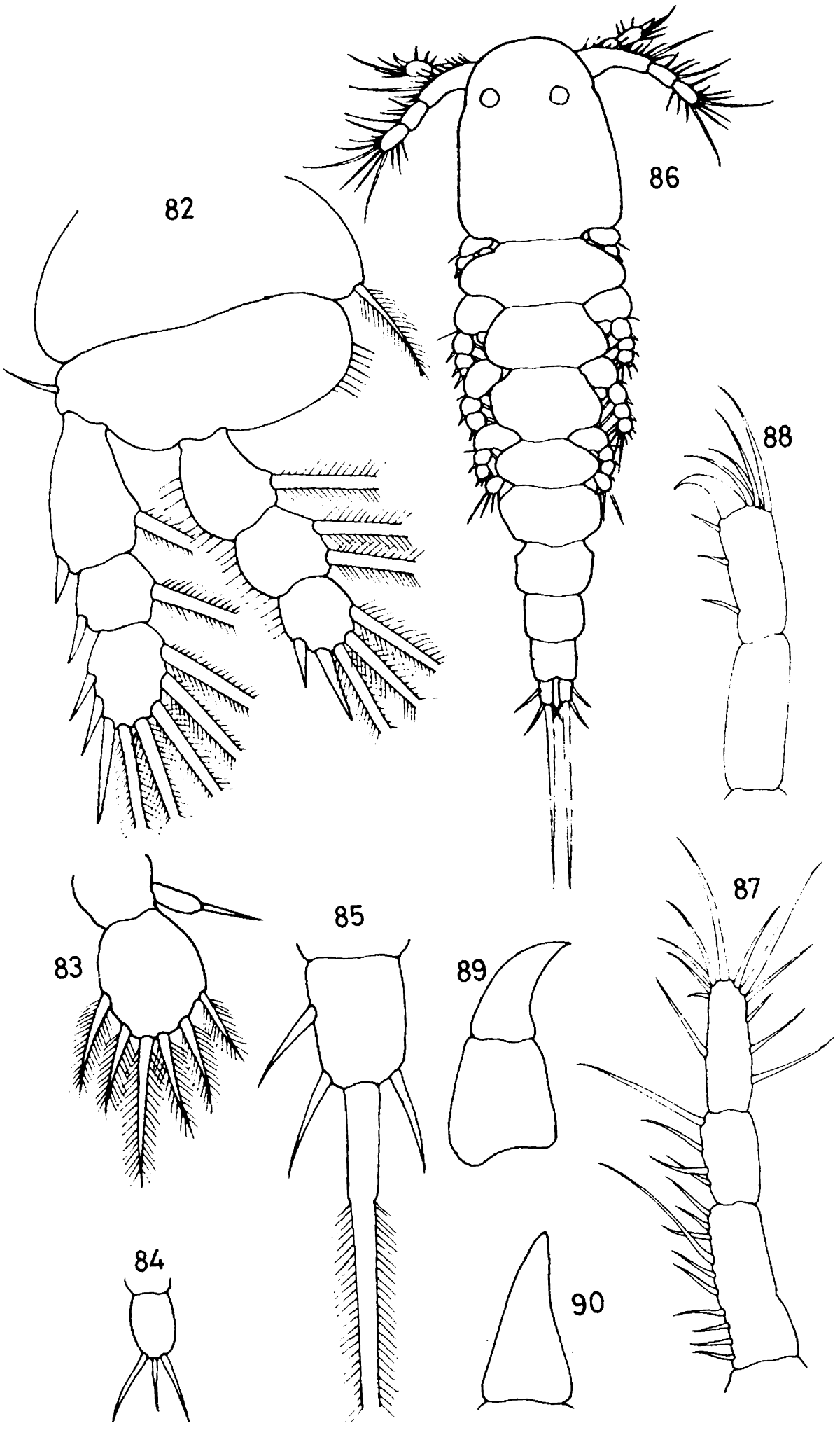
86 Cyclopoid female, dorsal view

87 First antenna

88 Second antenna

89 Mandible

90 First maxilla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EXPLANATION OF FIGURES

Cyclopoid female

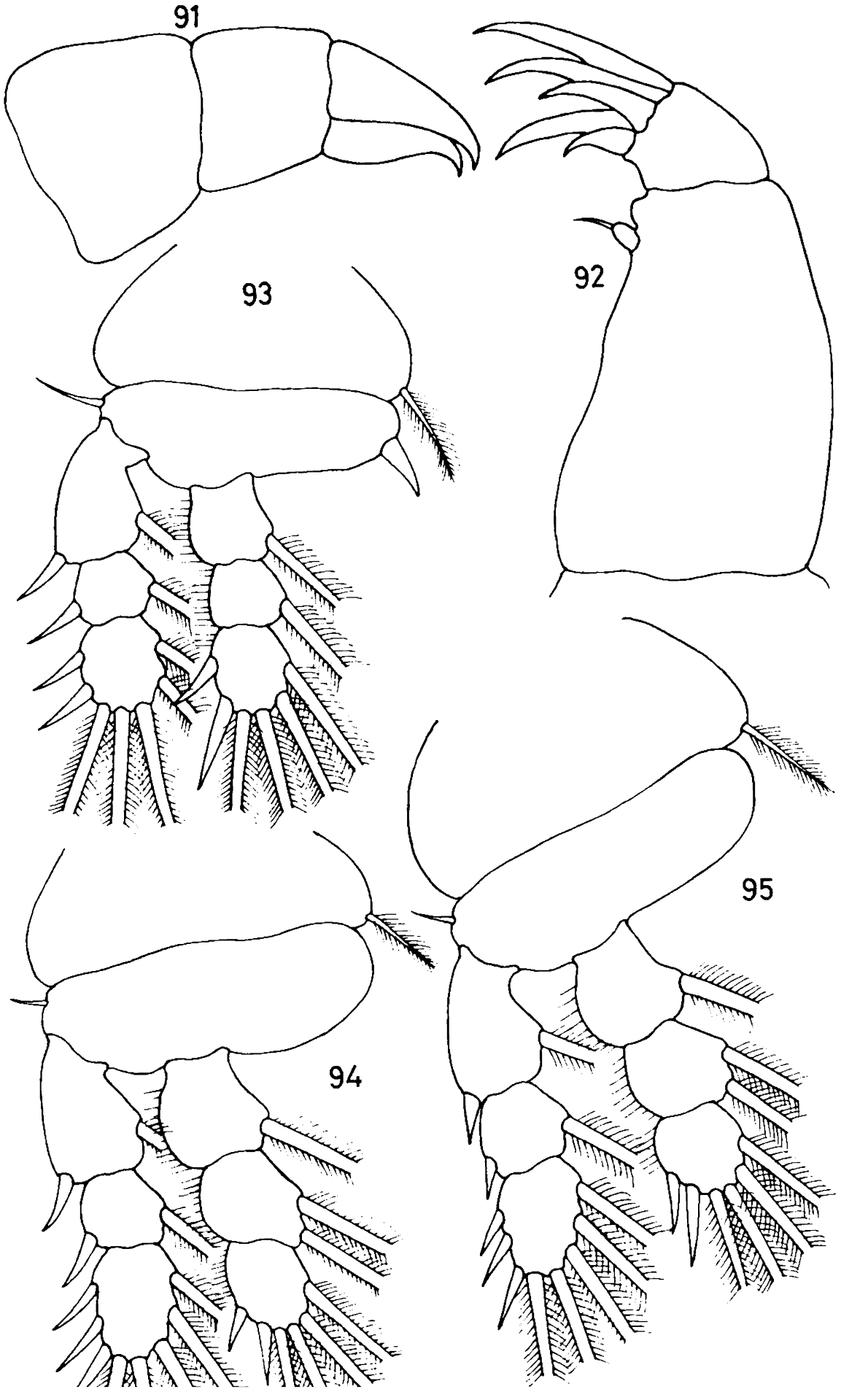
91 Second maxilla

92 Maxilliped

93 First leg

94 Second leg

95 Third leg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EXPLANATION OF FIGURES

Cyclopoid female

96 Fourth leg

97 Fifth leg

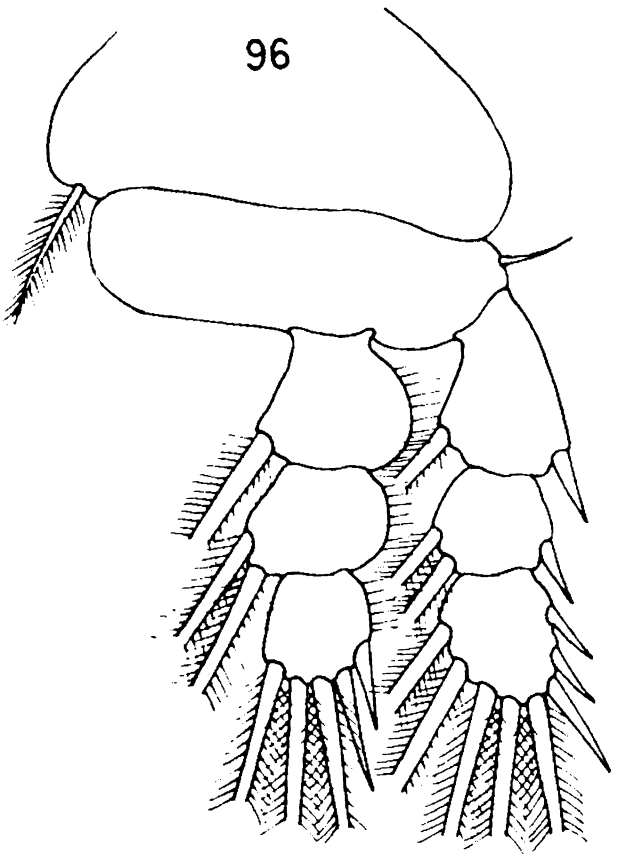
98 Sixth leg

99 Uropod

Metamorphosis of Female

Figs. 100 - 104

100 Immature female,
24 hours after penetration
into the body of the host.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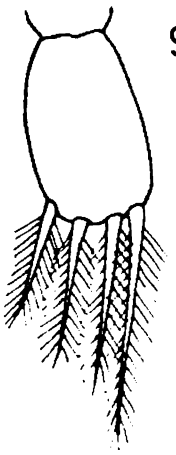
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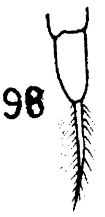
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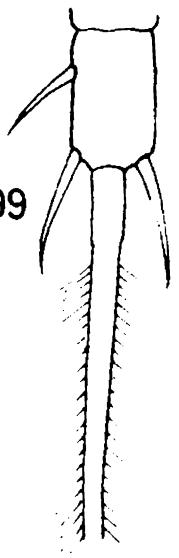

99


100

EXPLANATION OF FIGURES

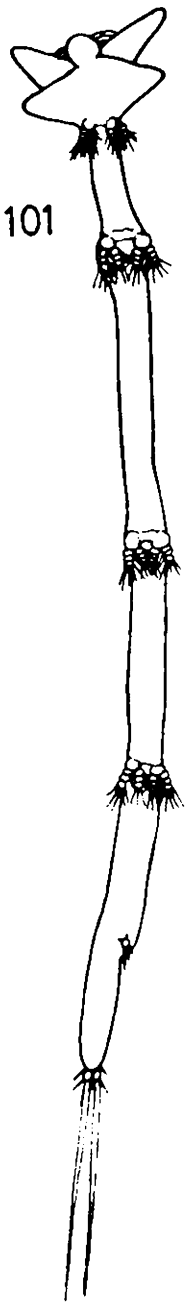
Metamorphosis of female

101 Immature female,
48 hours after penetration.

102 Cephalic region of immature
female, 60 hours after penetration.

103 Cephalic region of immature
female, 72 hours after penetration.

104 Mature female, 80 hours
after penetration.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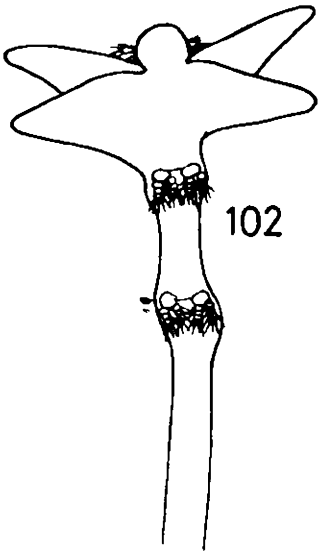
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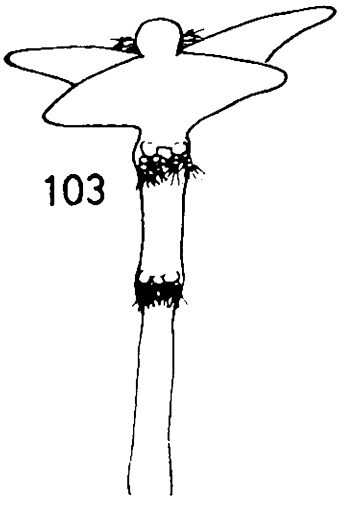
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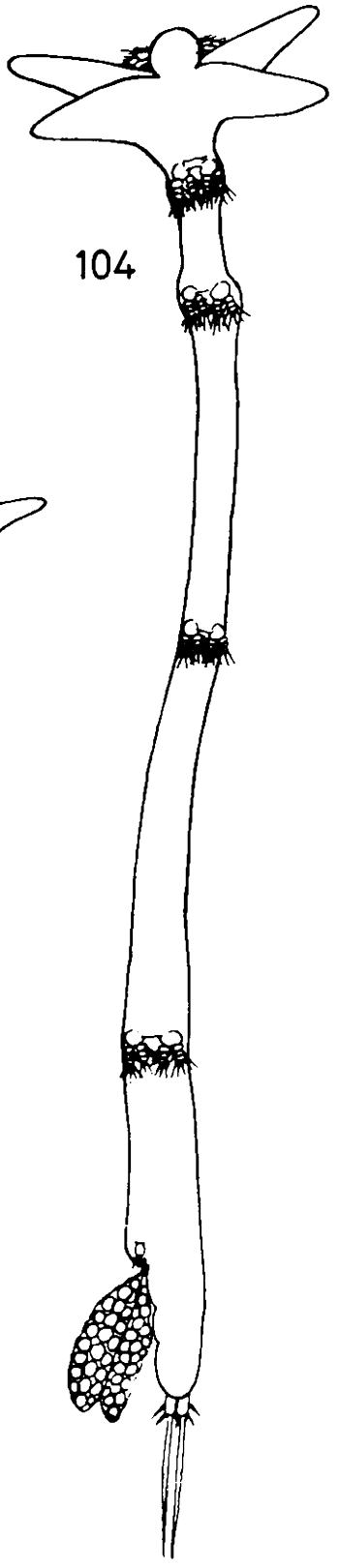
104

Plate No.1. Ovigerous Lernaea osphronemi Sp.nov.
infested on Osphronemus goramy.