

AN ABSTRACT OF THE THESIS OF

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1920 (COPEPODA: LERNAEOPODOIDA) A PARASITE OF THE STAR-
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The stages in the life history of the parasitic copepod Necto-
brachia indivisa Fraser, 1920, have been described.

The adult female lives permanently attached to the gills of the starry flounder, Platichthys stellatus (Pallas, 1811) Girard, 1854. The adult male is a dwarf, which usually lives attached to the genital process of the female.

The eggs hatch into free-swimming, but relatively inactive nauplii. They usually molt after 20-40 minutes into vigorous free-swimming copepodid larvae.

The copepodid either remains in the gill chamber of the original host or migrates to a new one of the same species. After about three days it attaches itself by means of a chitinous disc and filament to the cuticle of an adult female copepod, usually Nectobrachia indivisa but occasionally Acanthochondria rectangularis (Fraser, 1920) Oakley,

1927, or Lepeophtheirus parvicrurus Fraser, 1920.

A withdrawal of the protoplasm from the extremities into the cephalothorax takes place in the copepodid. A new skin forms around the protoplasmic mass and within the old copepodid skin. This marks the advent of the pupal stage. The old copepodid skin may or may not be cast, but the pupa continues to use the original attachment filament.

The pupa molts into either an immature female or a nearly mature male. The male soon seeks out an attached female of the same species, matures, and copulates. The post-pupal female remains connected to the pupal case for a period and then attaches permanently to a gill filament of the host by means of an eight-branched bulla.

After changing considerably, both in size and morphology, the female becomes sexually mature. Mating occurs and fertile eggs are deposited into the egg sacs.

The orientation of embryos in the egg sacs is with head to the inside, opposite to that of Achtheres ambloplitis Kellicott, 1880. Wilson proposed that oxygen supply is the factor in the latter, while ease of hatching may be the advantage of this arrangement for Nectobranchia indivisa.

The nauplius differs from all other lernaeopodid nauplii in having mandibles and non-plumose setae.

A long club-like process is present on each of the first antennae of the copepodid stage. It may have a sensory function involving taste

or smell or both.

The attachment of the copepodid appears to require a chitinous substrate. Possible methods of attachment are discussed.

The formation of a discrete pupal skin is recognized in this family and the maxillipedal sleeves are described, both for the first time. The latter function as a holdfast for the post-pupal female.

The first maxillipeds appear to play an active part in the removal of the bulla from the cephalic region of the post-pupal female. Possible attachment methods are considered and growth after the pupal molt is discussed. There is some evidence in favor of partial molting.

THE LIFE HISTORY OF NECTOBRACHIA INDIVISA FRASER, 1920
(COPEPODA: LERNAEOPODOIDA)
A PARASITE OF THE STARRY FLOUNDER

by

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INTRODUCTION

The subclass Copepoda includes in excess of 4,500 species, making it the largest of all the entomostracous crustacean groups. Although perhaps best known for its free-living forms, it contains several orders comprised exclusively of parasitic and commensal species. In addition, there are also a few parasitic and commensal species in some of the other copepod orders. These animals occur in association with a wide variety of aquatic organisms including sponges, coelenterates, molluscs, annelids, sipunculids, echiurids, arthropods, echinoderms, tunicates, elasmobranchs, fishes, and mammals. The great majority of the strictly parasitic forms belong to the orders Caligoida and Lernaepodoida, the species of which are found chiefly on marine and freshwater fishes. The copepod classification used in this paper is that of Wilson (1932).

Copepods parasitizing fishes have been known since antiquity. The writings of Aristotle (330 B. C.) and others record that tunnyfish and swordfish may be annoyed by a species of worms which fasten themselves under the fins and so irritate the fish that it often leaps out of the water and falls on board ships. This so-called worm was almost certainly a parasitic copepod belonging to the genus Penella.

The first recorded species, however, was not described until 1746 when Linnaeus assigned the name Lernaea tentaculatus quatour to a copepod parasitizing the carp, Cyprinus carassius. In the tenth edition of the *Systemae Naturae*, Linnaeus (1758) changed this name to the binomial Lernaea cyprinacea.

Despite this early awareness of the parasitic Copepoda by many workers, comparatively little research has been done on the life histories of these animals and that in only a few families. Outstanding as the classic of the early development work in this group is the study of Claus (1868). This investigator succeeded in working out the entire life cycle of Lernaeocera branchialis (Linnaeus, 1767) Blainville, 1822. Metzger (1868) had observed both male and female copepodid stages attached to the flounder, Pleuronectes (=Platessa) flesus, as well as on the lumpsucker, Cyclopterus lumpus. He noticed similarities between some of these copepodids and the adult female of L. branchialis and concluded that they were larval stages of this copepod. Later Metzger's material was given to Claus. Beginning with a gravid female, Claus was able to hatch the eggs into nauplius larvae which were followed by a copepodid stage. He observed that these copepodids attached themselves to the gills of the above mentioned flounder by means of their prehensile second antennae. Although he did not see how the attachment took place, Claus did record that the next four stages were attached to the flounder host by means of a chitinous

filament. After attaining sexual maturity, the fourth stage shed the attachment filament along with the cuticle of its frontal portion and began a free-living period during which copulation took place. The male then died and the female sought out its final host, usually a gadid fish. Claus then observed the drastic morphological change that takes place in the female after it has attached to the definitive host. This work marked the first mention of an intermediate host in the life cycle of a parasitic copepod, a phenomenon which has since been shown to be limited to only a few genera of the family Lernaeidae.

In the years since 1868 there have been some important contributions in the area of parasitic copepod development, but until Heegaard's work in 1947 on Acanthochondria cornuta (O. F. Muller, 1777) Oakley, 1927, and Caligus curtus O. F. Muller, 1785, Lernaeocera branchialis remained as the only species of which the entire life history was known. Life cycles completed since 1947 include those of Lepeophtheirus dissimulatus Wilson, 1905, by Lewis (1963) and Lernaea cyprinacea Linnaeus, 1758, by Grabda (1963).

The following historical resumé' of developmental studies will be limited to the Lernaeopodidae, the family in which Nectobranchia indivisa Fraser, 1920, has been most often placed.

The first mention of larval stages in the Lernaeopodidae (=Lernaeopodidae of Yamaguti, 1963) appears in the classic work of Nordmann (1832). In that paper he described several new species of

parasitic copepods including Achtheres percarum from the common perch, Perca fluviatilis, and Tracheliastes polycolpus from a carp, Cyprinus jesus. For both of these species, Nordmann was successful in hatching nauplii from the eggs and subsequently obtaining the first copepodid stages. As has since proved common among lernaeopodid copepods, the naupliar stage lasted only about 30 minutes.

Vejdovsky (1877) also worked with Tracheliastes polycolpus and he too succeeded in hatching the eggs, but the emergent stage was the copepodid rather than the nauplius.

The first significant research on the development of the Lernaeopodidae did not appear until 1911 when Wilson published the results of his study on the life history of Achtheres ambloplitis Kellicott, 1880, a gill parasite of the common freshwater rock bass, Ambloplites rupestris. Wilson found that, with few exceptions, the first stage to emerge from the egg was the copepodid. He estimated that about five percent of the eggs hatched out as metanauplii which, however, soon molted into copepodids. Wilson noted that the copepodid was a good swimmer which sought out a host immediately. Having reached the gill arches, the larva hung on first with its well developed maxillipeds, but in a short while became attached in a more permanent manner. This took place by means of a chitinous filament which Wilson had seen in the preceding stage as a structure coiled about the eye. However, rather than being connected to the larva at the cement gland

(= frontal gland) as in the Caligidae, the filament was grasped by the claws of the second maxillae (= first maxillipeds of Heegaard, 1947 = second maxillipeds of Scott and Scott, 1913, and others). Wilson never witnessed either the attachment of the distal end of the filament to the host tissue or the transfer of the proximal end from the cement gland to the maxillipeds. He conjectured that, once transferred, the filament remains in this position during the life of the female, being gradually shortened down to the length found in the adult. In the male it remained until the union of the sexes. At that time, the male withdrew its claws and from then on clung to the body of the female.

The next stage seen by Wilson was what he called the second copepodid but which later workers referred to as a pupa (Gurney, 1934; Heegaard, 1947). In this stage, the sexes could be distinguished and they bore a considerable resemblance to their adult counterparts which followed after the next molt.

Gurney (1934), in his study on Clavella uncinata (O. F. Muller, 1776) Oken, 1815, noticed that many of the copepodid appendages were visible through the naupliar cuticle. He was the first to see a pupal stage in which the skin was formed of the old copepodid cuticle. In this type of pupa, the proximal end of the attachment filament was fastened to the old copepodid skin and had no direct connection with the larvae as Wilson (1911) found with Achtheres ambloplitis. The next stage Gurney found was an adult with a fully-formed attachment

button or bulla. He thought, perhaps, that he had missed a free-living stage following the pupa because of the apparent difficulties extant in deriving the adult from the pupal form. He also theorized that the bulla was a new and independent process as opposed to Wilson (1911), who maintained that it was merely a transformation of the attachment filament of the preceding second copepodid (= pupa) stage.

The most thorough work on freshwater lernaeopodid development is that of Friend (1941) who succeeded in completing most of the life cycle of Salmincola salmonea (Gissler, 1751) Wilson, 1915, a parasite from the gill filaments of the Atlantic salmon, Salmo salar. He found that hatching occurred about two weeks after the egg laying and that the first copepodid is the emergent stage. He observed that this stage attached to the gills of the salmon host. The means and method of attachment were similar to those described by Wilson (1911) for Achtheres ambloplitis. However, in the case of S. salmonea, the transfer of the attachment filament from the cement gland to the second maxillae (= first maxilliped of Heegaard, 1947, = second maxilliped of Scott and Scott, 1913, and others) did not take place until this species molted into the second copepodid stage (= pupa).

The second copepodid, in which the sexes are distinguishable, lost its attachment filament during the succeeding molt. The resultant unattached stage included both sexually mature males and what Friend referred to as first stage females. During this stage, copulation took

place and the male disappeared. Fasten (1914), working with Salmincola edwardsii (Olsson, 1869) Wilson, 1915, reported that the male dropped off and died following mating.

Meanwhile the cement gland in the female had been elaborating an attachment bulla which was planted on the gill tissue in its final position. The female then molted and the distal ends of each second maxillae (= first maxilliped) were forced as plugs into corresponding sockets in the bulla and a permanent connection was achieved. The subsequent growth of the female, which amounted to a tenfold increase in size, took place without any further molts. According to Friend (1941), this growth occurred merely by an expansion of the cuticle.

In 1947, Heegaard was able to work out most of the developmental details of Clavella uncinata. He chose to call the larval stage which emerged at hatching a pseudonauplius rather than a nauplius because a number of the usual naupliar appendages were either rudimentary or lacking. Heegaard went on to point out that this reduced condition was characteristic of all of the lernaeopodid nauplii described previous to that time. It was his opinion that this was a secondary reduction and that it represented a transitional step on the way to total elimination of the nauplius stage. He found support for this idea in the work of Fasten (1913) and Friend (1941) in which no naupliar stages were observed for two species of Salmincola.

Heegaard (1947) noted that the pseudonauplius stage of Clavella

uncinata lasted only about twenty minutes before molting into the free-swimming copepodid stage. The copepodid fairly soon sought out a gadoid host to which it attached by means of a frontal filament. He also observed the formation of the pupa within the copepodid cuticle as reported by Gurney (1934).

Following the pupal stage, Heegaard saw the "missing intermediate stage" postulated by Gurney (1934). However, it was an attached form rather than a free-living one as Gurney had predicted. This larva was a young female which was grasping the proximal end of a partly developed bulla with its maxillipeds while the old pupal attachment filament was still present. However, in this case the filament extended clear to the claw of the maxilliped and not just to the pupal skin as indicated by Gurney (1934) and Heegaard (1947) for the preceding pupal stage. Although the discovery of the above stage definitely demonstrated that the bulla and the attachment filament were discrete structures, Heegaard was unable to establish the point of origin of the bulla in the body of the larva.

Once the young female had attached to the host by means of its bulla, no further molting was observed. Heegaard (1947) cited some evidence that the extensive morphological changes which must occur to produce the definitive adult female are accomplished by means of partial molts.

The purpose of the following research was to work out the life

history of Nectobrachia indivisa Fraser, 1920, a lernaeopodid copepod which parasitizes the starry flounder, Platichthys stellatus (Pallas, 1811) Girard, 1854.

METHODS AND MATERIALS

Specimens of the starry flounder, Platichthys stellatus, were caught throughout the year in Yaquina Bay and Estuary, Lincoln County, Oregon, by means of a 15 foot gulf try net. This gear, which is a small otter trawl, was towed slowly along the bottom by a skiff powered with an outboard motor.

Following each tow, all host animals were examined for Nectobranchia indivisa. Records were kept on percent of infection. All females of N. indivisa bearing darkly pigmented eggs were removed from the host, placed in vials of sea water, and kept cool in an ice chest. At first all ovigerous females were collected in the above manner, regardless of the stage of egg development, but it was soon discovered that only those eggs which were fairly darkly pigmented would hatch within a few days. Varying numbers of host fishes infected with N. indivisa were also taken back to the laboratory for further examination. Some of these were killed and iced while others were brought back alive.

Upon return to the laboratory, some three to seven hours later, the ovigerous females previously selected for the advanced development of their eggs were transferred to petri dishes containing filtered sea water and refrigerated at 10-12^o C, the approximate bottom water temperature in Yaquina Bay. The water was changed at least once

every three days and care was taken to prevent overcrowding. The above procedure was followed for all other specimens kept in vitro during this investigation. This included adults and attached larval forms. All dead fish were examined within eight hours.

Observations were made on the developmental stages issuing from the egg and from subsequent molts. This was supplemented by observations on specimens removed from naturally infected fish. Notes were taken on the morphology, coloration, and behavior of the living larvae and adults. Specimens of each life history stage were also preserved for further study.

Studies on the experimental infection of the flounder with larval Nectobranchia indivisa were carried out. Parasite-free flounders were placed in small aquaria filled with well aerated, filtered sea water and exposed to both nauplius and copepodid stages of N. indivisa. After about three days the fish were transferred to larger containers where they fared much better physically. Four days after exposure, one fish a day was sacrificed and examined for N. indivisa. Water was changed at three day intervals.

The adult copepods collected in this investigation were fixed in Lavdowsky's solution (alcohol-formalin-acetic acid) and stored in 70 percent glycerin-alcohol or in 85 percent lactic acid. All larval stages were fixed in hot commercial formalin or in hot Lavdowsky's solution. Specimens to be dissected were placed in 85 percent lactic acid to

soften and clear them, stained with methyl blue dissolved in 85 percent lactic acid, and dissected in clear 85 percent lactic acid (Lewis, 1963). Best results were obtained by overstaining and then destaining with ten percent HCl. These stained specimens were stored in 85 percent lactic acid.

All drawings were made by the author. The major features of the whole animals and appendages were drawn with the aid of camera lucida attachments used in conjunction with Wild compound and dissecting microscopes. A Leitz microprojector was also used for the same purpose. The finer details of the drawings were made freehand following microscopic observation. Drawings of the whole animals were made from specimens temporarily mounted in 85 percent lactic acid. Appendages were usually drawn in situ from whole mounted specimens. When better definition was required, however, they were removed and mounted permanently in Hoyer's medium.

All measurements are in millimeters. They were taken from freshly-fixed specimens with an ocular micrometer. The average measurement, based on ten specimens, unless stated otherwise, is listed first with the size range following in parentheses.

LIFE HISTORY RESUME'

The adult female of Nectobrachia indivisa lives permanently attached to the gill filaments of the starry flounder, Platichthys stellatus. The adult male is a dwarf and is usually seen attached to the genital process of the female. After mating occurs, fertilized eggs pass out of the oviducts into the egg sacs where they go through the various cleavage stages.

After a time the eggs hatch and a free-swimming, though relatively inactive, nauplius emerges. Within a very short time, the nauplius molts into another free-swimming stage, the copepodid. This very active stage either remains with the original host or reaches a new one of the same species.

The copepodid then attaches itself by means of a chitinous disc and filament to the cuticle of an adult female Nectobrachia indivisa. Occasionally, however, these attached stages are also found on Lepophtheirus parvicruris, Fraser, 1920 and Acanthochondria rectangularis, (Fraser, 1920) Oakley, 1927, two other parasitic copepods which inhabit the gill cavity of the starry flounder.

After attachment, a general withdrawal of the organic parts from the extremities into the cephalothorax takes place in the copepodid. The subsequent formation inside of the old copepodid skin of a cuticle, which envelopes the seemingly structureless mass, marks the

beginning of the pupal stage.

The copepodid cuticle may or may not drop off, however, the pupa continues to utilize the original attachment filament. Shortly after the formation of the pupal cuticle, appendages begin to form. The mature pupa molts either into a mature male or an immature female. The males soon seek out attached females, but the immature females remain connected to the pupal skin by means of long extensions of the first maxillipeds. After some time, the female sheds the maxilliped extensions and is completely free on the gills of the host.

In a few hours these young females attach permanently to the gill filaments with a new structure called a bulla which they grasp with the first pair of maxillipeds. The female probably attains its definitive size and morphology through differential growth or a series of partial molts. No molting was observed among any of the post pupal forms.

DESCRIPTION AND BEHAVIOR OF LIFE HISTORY STAGES

Egg

Egg bearing females occur throughout the year, however, they are in greater evidence during the period August through January. The relatively large eggs (0.22-0.32) are arranged in three staggered rows. Early in embryology the eggs are about spherical but become oval as development proceeds.

An indication of approaching maturity is an increasing amount of pigment in the eggs. In eggs of advanced development this pigment, which is light brown in color, is placed laterally and assumes a reticular pattern. Occasional movements by the embryo may be seen in eggs at this stage of development. The dark brown retinal pigment is readily seen in its anterior median dorsal position. It has already taken the double-cupped shape typical of the definitive copepod eye, however, no lenses are evident.

The eggs are oriented in such a manner that their longitudinal axes are perpendicular to the length of the egg sac. In addition, the anterior ends of the eggs are always pointed inwardly. At maturity, the egg sac ruptures lengthwise exposing the individual eggs to the water. The eggs, which are attached by their membranes to the outer wall, are now ready for hatching.

In the in vitro cultures, the eggs most commonly hatch while still

connected to the egg sac, however, sometimes the eggs drop out of the sac prior to hatching. In the latter case, it appears that it is more difficult for the larva to rid itself of the egg membrane. Examination of specimens taken from wild caught flounders indicates that the former method is probably also the most common in nature.

The rupture of the egg membrane is probably caused by osmotic pressure in conjunction with increased activity on the part of the embryo.

Nauplius

The stage which emerges at hatching is a nauplius larva (Figure 1). Although fairly active during the hatching period and for a short time thereafter, this stage soon becomes quiescent, making only an occasional feeble movement. The nauplius is oval and has a length of 0.42 (0.38 - 0.43). Its widest point is about one third the distance from the anterior end and measures 0.25 (0.24 - 0.26). The length of the cephalothorax is 0.33 (0.31 - 0.35).

The light brown pigment is spread out from its lateral position in the egg and now covers the body of the nauplius with a loose, but continuous reticulate pattern. From the dorsal aspect, the shiny dark reddish brown eye pigment is conspicuous. It has increased somewhat in size over that in the egg and now the clear round lenses can be seen in their definitive position, seated in the double retinal

cups.

Through the dorsal cuticle of the cephalothorax, one can see many centrally located globules of variable size. These globules probably represent remnants of yolk material. An occasional nauplius is seen to have a larger structure in among the globules. This may be the stomach. There is no indication that any other parts of the digestive tract are present. A small slightly tapered abdomen is located posterior to the cephalothorax and tends to curve dorsad.

The nauplius has three pairs of locomotory appendages, the first antennae (= antennules), the second antennae, and the mandibles. All of these limbs are located ventrally on the anterior one third of the cephalothorax. The setal armature of these three pairs of appendages is non-plumose.

The first antennae are uniramous appendages consisting of two approximately equal segments. They originate just ventral to the anterolateral margins of the cephalothorax. There are two long medial and two minute lateral setae on the free end of the distal segment. The proximal segment is armed with two long setae which arise medially near the distal end.

Just posterior and lateral to the first antennae are the biramous second antennae. Each of these appendages consists of a large undivided protopodite, a two-jointed endopodite, and an unjointed exopodite. The exopodite bears five long terminal setae, the endopodite

has two, and the protopodite is bare.

The mandibles are located posterior and lateral to the second pair of antennae. They are somewhat smaller than the second antennae, but otherwise differ only in regard to the exopodite. The mandibular exopodite is the longer of the two and possesses four setae instead of five. There are no balancers on this nauplius.

The duration of the naupliar stage of Nectobranchia indivisa is very short, usually lasting only from about 20 to 40 minutes before molting into the subsequent copepodid stage. Some individuals did, however, remain as nauplii for extended periods, but often these failed to develop further.

The succeeding copepodid larva is almost fully developed at the hatching of the nauplius. At that time several of the copepodid structures can be seen through the naupliar cuticle (Figure 2).

Copepodid

Molting takes place through an anterior dorsal split in the naupliar cuticle. In contrast to the sluggish nauplius, the copepodid which emerges darts rapidly about (Figures 3 and 4).

The changes occurring from the nauplius to the copepodid are the most spectacular of any single molt during the development of Nectobranchia indivisa. In one brief interval this copepod has been transformed from a relatively simple organism with only three pairs

of appendages into quite a complex one which possesses nine pairs of appendages as well as several other innovations.

The elongate copepodid has a body plan which includes a cephalothorax, three free thoracic segments, two pairs of swimming appendages, and a fused genito-abdominal segment.

The copepodid has an overall length of 0.63 (0.58 - 0.66) and a cephalothorax length of 0.39 (0.37 - 0.41). The width of the cephalothorax is 0.25 (0.22 - 0.29).

The anteriormost structure of the ellipsoidal cephalothorax is an adhesive attachment disc which lies just beneath the cuticle (Figure 3). A short chitinous filament links it to a bilobed basal part located in front of the eye and above the mouth cone. The cement gland, from which the above structures originate, was not seen.

The eye appears to be the same as in the nauplius stage, as is the arrangement and color of the body pigment. A number of the globules mentioned in the naupliar description can be seen in the posterior half of the cephalothorax of the copepodid. As in the nauplius, an occasional copepodid larva possesses a larger structure among the globules which may be the stomach. Except for this and the mouth cone which will be described later, there are no indications of the presence of any other parts of the digestive tract.

Looking at the cephalothorax from the ventral aspect, it can be seen that several appendages originate there. The first antennae

arise just lateral to and slightly behind the lower part of the attachment disc. These structures are uniramous as in the nauplius, but now consist of four segments rather than two. The four joints are approximately equal, each being slightly longer than wide. Beginning distally, the first segment bears six medium-length and two short setae. The next segment has a long, club-shaped process which bears no resemblance to any structure seen in either earlier or later life history stages of this species. It is longer than the entire antenna and may be merely an extension of the second segment. Gurney (1934) pictures a similar structure on a copepodid tentatively assigned to Epibrachiella impudica (Nordmann, 1832) Wilson, 1915. He thought that it might be an aesthete (= light sensitive structure). At the base of this process is a single short seta. The third segment has one small seta which is placed distally on the leading edge while the basal joint bears two fairly long flattened setae in the same relative position.

The second antennae are biramous processes located posterior and lateral to the first antennae. The protopodite has two segments for certain and possibly three. The segmental boundaries are often unclear in this and other instances. The chelate endopodite consists of three joints including the claw. The second joint bears a short spine which opposes the claw. The long basal joint is unarmed. On the inner face of this segment are present striations which possibly function as adhesive organs. The exopodite is one-segmented and

bears three short setae on its distal margin.

The mouth cone (= mouth tube) is located midway between the bases of the second antennae. It projects downward and forward, being broader than long at the base. This process is formed largely from the lower lip (= labium) which in cross section is roughly horse-shoe shaped. The top of the cone is closed off by the upper lip (= labrum), the rounded distal end of which overlaps the frontal groove of the lower lip. At this stage of development the tube within the mouth cone ends blindly, hence is not used for food intake.

Posterior and lateral to the second antennae is a pair of uniramous tapering transparent chitinous flaps. These are the remnants of the biramous mandibles of the nauplius.

Just medial to the mandibular flaps are the maxillae. These appendages each consist of a lobe armed with two pointed fleshy palps. According to Heegaard (1947) the maxillae are biramous appendages but in the Lernaepodidae have lost their articular boundaries.

The first maxillipeds are broad, slightly tapering lobes, which arise ventrolaterally on the cephalothorax about one third of the way from the anterior end. Near the distal end is a fold which may indicate a segmental boundary.

Just medial to the first maxillipeds and often obscuring them are the strongly chelate second maxillipeds, the most prominent of all the copepodid appendages. Basally there is a large folded segment which

serves as a pedestal for the powerful chela. When the maxilliped is extended, this basal segment is folded out and is anchored by a sclerotized rod which is connected to the sternal region. The next segment is large and heavily chitinized. It is distinguished by a series of broad striations on its inner surface. These are similar to those found in the same relative position on the second antennae and likewise probably function as adhesive organs. In addition, this segment possesses a cushion upon which the claw rests when it is closed. This cushion is located on the proximal end of the leading edge of the joint and is covered with small hairs which are probably sensory in function. A well developed knee separates this segment from the three that follow. Beyond the knee come two short segments, the division of which is not always apparent. The most distal of these bears an accessory spine which opposes the terminal claw.

Behind the maxillipeds, near the posterior margin of the cephalothorax, is the first pair of swimming appendages. Each bears the usual three divisions of a biramous appendage; however, these divisions are each reduced to a single flattened segment. There is one small seta on the distal margin of the protopodite adjacent to its junction with the exopodite. The exopodite bears six setae, a small one on the lateral margin and four long plumose ones and a medium-sized flattened one located distally. The endopodite is armed with one medium and six long plumose setae arranged palmately on the terminal

margin.

Abutting the cephalothorax along its entire posterior margin is the first and largest of the three free thoracic segments. This segment bears the second pair of swimming appendages which is identical with the first pair described above. The limbs of each pair are united by a chitinous plate referred to as a "coupler" (Marshall and Orr, 1955).

The globose second free thoracic segment follows. It is less than one half the width of the first and gives rise to the vestigial third pair of swimming appendages. These each consist of a small fleshy lobe armed with two setae.

Behind the second segment is the slightly smaller third which bears no limbs.

Following the free thoracic segments is the combined genito-abdominal segment. Quadrilateral in shape, this segment bears a pair of well developed anal laminae located posterolaterally. Each lamina bears two long, two short, and two minute setae. The two long ones are plumose except that the lateral surface of the outer one is bare.

The above observations have been made on life history stages of Nectobranchia indivisa reared in vitro in the laboratory. Numerous eggs were hatched, but none appeared to develop beyond the copepodid stage. This fact, coupled with the generally prevalent belief that the

copepodid is the infective stage, led to some experimental infection studies. The procedures used are outlined in the section on methods and materials. All attempts to establish this parasite in the flounder failed.

During the course of examining naturally infected hosts, a number of specimens of a minute organism were found attached to adult females of Nectobranchia indivisa. A closer examination of these forms revealed that some of them were almost identical to the copepodids of this species, both in size and morphology. All were attached by means of a chitinous disc and filament which, although partially pushed out, could surely be homologized with the attachment structure seen in the cephalothorax of the copepodid. The outstanding difference seen in these attached forms was that the cuticle was completely or partially devoid of protoplasm except in the region of the cephalothorax. In certain specimens, the cephalothorax appeared to be filled with a shapeless mass, while in others definite form was observed. The cuticle of one of the attached specimens was broken open, revealing an almost mature male of N. indivisa.

A re-examination of in vitro culture material indicated that some of the stages had indeed developed beyond the copepodid (Figure 5). These were identical with some of the attached forms. Heegaard (1947) found that the stage which followed the copepodid was an attached one very similar in many respects to that discussed above.

This was the same larva that Gurney (1934) saw and called a pupa, the name which will subsequently be used herein.

Pupa

Using size and form as criteria, a logical series was established from the several pupae collected. The following chronology of pupal development has been derived from this series.

The copepodid either remains with the original host or reaches another of the same species. Within about three days it attaches itself to the exoskeleton of a female copepod of the same or other species within the gill cavity of the flounder. It does this by means of its attachment disc and filament. Prior to effecting this attachment, the copepodid very likely uses the second maxillipeds and perhaps the second antennae to grasp the copepod host.

There is a progressive withdrawal of the protoplasmic constituents from the copepodid extremities. Once the retreating protoplasm is completely within the confines of the cephalothorax, a cuticle is formed about it inside of the old copepodid skin. The formation of this new cuticle marks the actual beginning of the pupal stage. The old skin may or may not be retained; however, the pupa continues to utilize the original attachment filament. The pupal cuticle is characterized by some peculiar outgrowths that appear to match the position and shape of the old copepodid appendages. At any rate, they bear no

resemblance at all to the pupal appendages which soon appear from out of the once seemingly shapeless mass of the early pupa.

After an undetermined period, the sexes can be distinguished (Figures 6 and 7). At this time the pupal male closely resembles the adult male. It can be easily separated from the pupal female by its first maxillipeds which are short and broad in contrast to the long narrow ones of the female. In addition, the mouth tube of the male is much more prominently displayed than that of the female. There is no apparent connection between pupa and case. The pupa has a length of 0.42 (0.35 - 0.53).

As the pupa nears the end of its development, it begins to move within the case. The movements become stronger and finally the case splits open. The male which leaves the pupal skin is mature or nearly so, being identical with males which have been recovered from the genital process of females (Figure 15). A description of the male will be given later. The newly emerged male presumably seeks out an attached female within a short time. At any rate, it does not remain near the pupal case for very long. On the other hand, the female remains attached for some time. The means of attachment are elongated, chitinous sleeve-like structures which completely cover the first maxillipeds (Figure 9). These transparent sleeves extend back into the pupal case where each is anchored by a sclerotized hook. Along most of their length, the sleeves have a crenulated appearance,

a fact which probably indicates that they are extensible. This opinion is supported by comparing the length of these structures in Figure 8, a newly emerged post-pupal female with that of those in Figure 9, a post-pupal female several hours after the pupal molt.

The recently emerged female swings about on the maxillipedal sleeves, moving its appendages continually. Although not actually observed, it seems reasonable to assume that, during this time, the young female comes into contact with a gill filament to which it clings with its chelate second maxillipeds and perhaps the second antennae. After some time, the young female is able to extricate its maxillipeds from the sleeves and is then completely free upon the gills of the host.

Post-Pupal Female

The female which leaves the pupal case is still immature and differs considerably in size and morphology from the mature female. This young female (Figure 8), with its slightly tapering, sac-like hind body, resembles the pupa of a honey bee. This stage has a length of 0.51 (0.48 - 0.56) and a width of 0.20 (0.18 - 0.21).

Beginning anteriorly, the large attachment organ occupies about one-fifth of the body. From the dorsal and frontal aspects, this structure presents a bilobed appearance.

About halfway back on the body, just below the dorsal surface, one can see the remnant of the retinal pigment of the eye. It no longer

has a double-cupped shape nor are the lenses present. The color and the pattern of the pigment are about the same as that of the nauplius and copepodid stages.

The stomach is definitely present in this stage. It can be seen in the forepart of the last half of the body when viewed from the ventral aspect. Posteriorly, an intestine connects with the stomach and leads back to the, as yet, unclosed anal groove. This groove extends from the intestine to the posterior margin on the ventral surface and a short way up the dorsal surface. No connection between the stomach and mouth cone is visible.

On each side and close to the hindmost point is a minute protuberance which bears two short setae.

From the ventral aspect, the first pair of antennae can be seen to originate just posterior to the attachment organ. Each is composed of four segments as in the copepodid stage, but are smaller and have relatively fewer setae. There are three short and three minute setae, all on the distal segment.

The biramous second antennae are very similar to those of the copepodid both in morphology and position; however, they are relatively much larger.

The mouth cone extends out considerably further than that of the copepodid and now is provided with some short hairs or setae around the opening of the tube. These probably have a sensory function.

When looking straight into the oral opening, one sees a pair of tooth-like structures which arise from the floor of the mouth (Figure 10). Nothing is known about the function of these structures, however; considering their shape and position, it is likely that they are used in piercing blood vessels when the post-pupal female starts to feed.

Lateral to the mouth cone are the minute mandibles. Because the bases of these appendages are obscure, it is not possible to determine whether they arise near the base of the mouth cone or actually from the sides of the mouth cone itself. Each mandible bears six or seven hooked teeth. The mandibles of this stage are entirely different in shape, size, and position, from their antecedents in the copepodid.

The maxillae are little changed from those of the copepodid stage; however, they occupy a more medial position just lateral to the mandibles.

Even without the long extension sleeves mentioned earlier, the first maxillipeds are by far the most prominent appendages of the newly emerged female. They bear little resemblance to their broad-lobed counterparts of the copepodid stage. Near the end of these appendages is an elbow-like bend. At this point they taper down into a narrow terminal portion which bears a sclerotized groove in the medial surface.

Some changes have also taken place in the second maxillipeds which are now considerably larger than those of the copepodid. There

is now a small spine present on the trailing edge of the second segment after the claw. The basal segment is divided into two and the large segment of the chela proper is more elongated. The cushion opposite the claw is not as prominent and no hairs are visible around this structure. In one of the post-pupal females, there was observed through the cuticle of both second maxillipeds the definite outline of another maxilliped, as if in anticipation of a future molt. The second maxillipeds of this stage seem to originate farther behind the first pair than in the copepodid.

The attachment of the post-pupal female to the gill filament of the flounder has not been observed during this investigation. Because of the difficulties inherent in witnessing such a phenomenon, it is possible that it will never be seen. However, enough observations have been made on the behavior of post-pupal females kept in vitro to be able to suggest essentially what happens. The following account of the attachment process is based on these observations with an occasional guess compatible with available evidence.

The attachment process begins shortly after the post pupal female sheds the maxillipedal extensions. After this occurs, the first maxillipeds are repeatedly brought into contact with the region of the attachment organ. Within a few hours, the anterior cuticle splits and the attachment organ is exposed. The attachment structure proper is a bulla shaped somewhat like a flower. A complete description of the

bulla is included in the section on the adult female. At this time the bulla is enclosed within a sticky flesh-like substance. The stickiness of this outer covering becomes evident when one manipulates specimens in which the attachment organ is exposed.

Post-pupal females have been observed with the attachment organ in various stages of removal, including two specimens in which it was completely free of the head region. In both of these instances, the attachment organ was being grasped by the first maxillipeds, the definitive adult position. The base of the bulla fits into the grooves in the ends of the maxillipeds which then grow together. For lack of other evidence, it appears that the maxillipeds are instrumental in the removal of the attachment organ, either by pulling or squeezing it from the cephalic region.

All that remains is for the copepod to bring the attachment organ into contact with a gill filament. Upon examining host tissue where female Nectobranchia indivisa have been attached for some time, it is readily seen that the bulla is firmly entrenched beneath the surface of the filament. Just how this attachment is accomplished is not certain; however, it appears likely that both the bulla and the sticky outer substance are actively involved. Some possible alternatives are suggested in the discussion.

Further Development of the Female

Once attached, the female copepod spends the rest of its life in this position. It can be seen from Figures 11, 12, and 13 that the female undergoes further changes in size and morphology before reaching maturity. Just how these changes occur is not clear at this time. Molting, the usual method of growth in the Arthropoda, has not been demonstrated for any stages of Nectobranchia indivisa beyond the post-pupa.

The available specimens of immature attached females have been segregated into three fairly distinct categories. Based on size and morphology, these stages are herein referred to as stages A, B, and C. They have been selected to demonstrate trends as well as to introduce new structures which occur with advancing maturity.

On examining a specimen of stage A, (Figures 11 and 12) which is a newly attached female, one can see that it fairly closely resembles the post-pupal female. The major difference stems from the fact that the attachment organ has been removed from the head, fused to the first maxillipeds, and anchored to the host. The removal of this organ gives the illusion that the mouth cone and the paired appendages have moved forward. In addition, the genital process is beginning to form in a median dorsal position, on the posterior margin. The eye and body pigments have diminished to some extent over that of the

previous stage. The second maxillipeds have moved forward, but are still located slightly behind the first pair. The basal segment of the second maxillipeds is enlarged over that of the preceding stage. There is now only one segment between the claw and the base of the chela proper. With the exception of the changes listed above, the remainder of the description given for the post-pupal female will suffice for stage A. Based on three specimens, the length measures 0.69 (0.58 - 0.81) and the width 0.25 (0.23 - 0.29). The length of the first maxilliped is 0.59 (0.55 - 0.66).

In stage B, (Figure 13) the eye and body pigment are no longer visible. The genital process has reached its maximum size and within this posterior outpouching can be seen the paired vaginae. Each of these structures leads from a laterally placed genital pore (= vulva) into the posterior trunk region. The anal groove has closed up except for the anus which is situated dorsal and anterior to the genital process. The small setae-bearing postero-lateral protuberances have disappeared, as has the yolk material of the digestive tract. There is still no apparent connection between the mouth and the stomach. In some specimens of this stage, the second maxillipeds have moved up even with and, in some cases, ahead of the first maxillipeds. The basal segment of the second maxillipeds continues to enlarge. The mouth parts are about the same size as in the previous stage, but much smaller in relation to the body size. Two specimens of this

stage were observed with attached males. The males were in the mating position upon the genital process. Based on the measurements of eight specimens, the length is 1.09 (0.96 - 1.23) and the width 0.38 (0.30 - 0.51). The length of the first maxillipeds amounts to 1.09 (0.90 - 1.23).

The posterior margin of stage C is flattened except for the genital process which now occupies a relatively small area. The body is often wider at the posterior end than at the level of the maxillipeds, as it was in the previous stages. The bases of the second maxillipeds have become quite swollen. In all of the specimens examined in this stage, the second maxillipeds had moved up ahead of the first pair. More of the genital apparatus is visible, including portions of the oviduct, cement glands, and occasionally what may be the spermatheca. The length of this stage measures 1.51 (1.36 - 1.70) and the width 0.71 (0.44 - 0.89). The length of the first maxillipeds is 1.51 (1.35 - 1.69).

Adult Female

The adult female (Figure 14) was described by Fraser (1920) from specimens taken from the gills of Platichthys stellatus caught in the Strait of Georgia near Nanaimo, British Columbia, Canada. This description is as follows:

Body short, stout, dorso-ventrally compressed. Head

forming a tapering projection from the anterior end of the body, without any indication of separation or any constriction to form a neck. Body, second maxillae and egg strings of the same length. No carapace. Body nearly rectangular, but slightly broader near the posterior end. A slight swelling posteriorly with a knob attached to the swelling. No abdomen, no anal laminae. Egg strings the same length as the body and over one-third of its width. Eggs large, 25 to 30 in number, in three longitudinal rows. First antennae short, two-jointed; the second joint curved, with a spine on the inner surface; terminal with five tubercles, three of which bear claw-like spines. Second antennae small, one-jointed, terminating in two spines, one of which is much larger than the other. Mouth tube longer than the antennae and first maxillae. Mandible with inner surface slightly irregular but not toothed. First maxillae terminating in two long tail-like rami, the outer one somewhat curved near the tip, the inner more nearly straight. Second maxillae placed well to the side but still on the ventral surface, passing outward and upward at an acute angle to the body, come together and are fused for two-thirds of their length. Bulla single. Maxillipeds with stout base; second joint much more slender and narrowing suddenly at the distal end; the terminal claw slightly curved; a small accessory claw present. There are no body appendages. Length of body equals length of second maxillae equals length of egg strings equals 2.2 mm, width of body 2.2.

Yu and Wu (1932), in their description of Nectobranchia wilsoni from the gills of Microstomus stelleri, stated that the first antennae were three-jointed, the second antennae were biramous, and that the egg sacs were short and elliptical. Otherwise their description agrees with that of Fraser (1920) for N. indivisa. This is mentioned here because Markevich (1946) has synonymized N. wilsoni with N. indivisa. Specimens examined during this study indicate the following: the first antennae are three-jointed but otherwise conform to Fraser's

description, the second antennae are biramous, but are not accurately described by anyone, and the egg sacs do occur as described above, but are atypical. A complete description of the second antennae based on specimens used in this research is given here. Each of these biramous appendages consists of a two-jointed protopodite, a four-jointed endopodite, and an unjointed exopodite. The terminal joint of the endopodite is a small claw, as in all of the life history stages after the nauplius. This claw is opposed by two accessory spines, which are located on the second most distal segment. The exopodite bears two slender setae.

Gussev (1951) has given the only adequate description of the unique bulla of Nectobranchia indivisa. The essentials of this description follow. The bulla consists of a stem and a curved plate which is divided into two parts. Each of these parts is further divided into four upturned petal-like branches. There are two canals in the stem each of which branches into 17-20 smaller canals. Three to five of these smaller canals run out into each branch of the curved plate. This bulla is unlike any other as yet described for a parasitic copepod. Gussev's description of the mandible seems to be more accurate than that of Fraser (1920). He reported that there are four large and four small teeth on each of these appendages whereas Fraser said there were no teeth.

The description of Fraser (1920), as amended in the above two

paragraphs, now agrees quite well with the specimens of Nectobrachia indivisa used herein. There is little obvious difference between stage C and the adult female. The adult female is usually somewhat larger and is seen to contain developing eggs. A complete digestive tract is visible. It consists of a straight, more or less uniform tube with two short club-shaped lateral branches in the posterior one-third of the body. Posterior to the lateral branches, the tube bends dorsal and terminates at the anus. It was noticed that the mouth cone and associated appendages seem to be mounted on retractile tissue and on occasion are completely withdrawn. Even after adulthood is reached, it appears that growth continues. The following measurements reflect somewhat the variability in the size of the adult. Based on 25 specimens, the length of the body with mouth tube extended measures 2.41 (2.00 - 2.75). The width is 1.27 (1.13 - 1.46) and the length of the first maxillipeds is 2.41 (1.89 - 3.02). The egg cases are 2.57 (1.67 - 3.08) long by 0.53 (0.38 - 0.65) wide.

Adult Male

As mentioned previously, the male (Figure 15) which emerges at the pupal molt is externally indistinguishable from the adult, even though it probably is not yet sexually mature. The following description is based on specimens collected during this study. It agrees fairly well with that of the original description by Markevich (1946),

but is somewhat more detailed.

The cephalothorax is large, rounded posterodorsally, and occupies the greater part of the body. It is covered by a carapace. The rest of the body appears like a small appendix on the posteroventral margin. Anteriorly there is a small cephalic prominence. The remnant of the retinal pigment of the eye is visible about halfway back on the body, slightly below the dorsal surface. The body pigment is similar to that of the copepodid. The digestive tract is not visible.

From the ventral aspect, the first pair of antennae can be seen to originate slightly behind the cephalic prominence. Each of these short uniramous appendages bears four segments, the middle two of which are the longest. Armature is present only on the distal margin of the terminal segment and is indistinct. It appears to consist of three or four small setae and one or more minute tubercles.

The second antennae are biramous and about the same length as the first pair. They are much less prominent than those of the copepodid. They arise posterior and slightly ventral to the first pair. The segmentation of these appendages is indistinct. The protopodite consists of two segments and bears no setae. It gives rise to an unsegmented exopodite and a three-segmented endopodite. The exopodite bears three small setae and the terminal segment of the endopodite is a strong claw.

Medial and ventral to the second antennae are the maxillae which

lie along the sides of the mouth cone. As in the copepodid, they consist of a single fleshy lobe with two slender pointed palps.

The mouth cone is very prominent in the male. It occupies a median ventral position between the bases of the second antennae. It extends out further than in the copepodid and, as in the female bears minute hairs or setae around the opening of the tube. Mandibles are not apparent.

Posterior to the mouth cone and arising ventrally at about mid-body are the broad first maxillipeds. These inflated-appearing appendages have changed drastically from the simple chitinous flaps of the copepodid. They taper considerably toward the distal end and are separated from the strong terminal claw by a well developed knee. When in a closed position, the tip of the claw rests between two projections on the broad basal segment. These projections are each covered with several small tubercles.

Posterior to the first maxillipeds are the much more slender and somewhat shorter second maxillipeds. These appendages have also changed from those of the copepodid. Including the claw, there are now two segments distal to the knee, rather than the three of the previous stage. This has resulted from a fusion of the two small segments between the claw and the knee of the copepodid. This fused segment and the claw are joined so closely that they give, at first glance, the illusion of one large claw. They actually do function

together as the movable member of the well developed chela. The fused segment still bears the combined armature of the former two segments. When the claw is closed, it rests upon a conspicuous raised process located on the distal end of the posterior margin of the large base of the chela proper. This is probably a modification of the cushion described for the copepodid. It is tipped with three chitinous teeth of equal size. The base of the chela is the most prominent segment of the appendage. It is sub-quadrate in shape and like that of the copepodid bears striations on its inner face. There does not appear to be a large folded basal segment as in the copepodid. Instead the base of the chela proper attaches directly to the body of the male. This connection is bolstered by a sclerotized supporting rod.

Just lateral to the junction of the small hind body with the cephalothorax, two pairs of rudimentary legs are present. These are the remnants of the well developed swimming appendages of the copepodid. Because of their minute size, they are observed only with difficulty. According to a drawing from Markevich (1946) each member of both pairs bears one small and one minute seta.

The length of the male is 0.32 (0.31 - 0.35).

DISCUSSION

Egg

The orientation of the embryos of Nectobranchia indivisa appears to be diametrically opposite to that of Achtheres ambloplitis as reported by Wilson (1911). In that study, the head of the embryo was always directed toward the surface of the egg sac and Wilson theorized that this was because of the better oxygen supply there.

The eggs of lernaeopodid copepods are almost spherical and are usually packed rather loosely within the egg sac. Therefore it seems improbable that the oxygen supply would differ markedly from exterior to interior, especially when the numbers of eggs are not great.

If, however, one does assume that the oxygen supply is greater at the surface, then, perhaps, the difference in the life histories of the above two copepods can account for the different orientation. Without having access to any pertinent data on the subject, it might be logical to assume that the developmental time within the egg of Achtheres ambloplitis is longer than that of Nectobranchia indivisa. The reasoning behind this is that A. ambloplitis hatches as a copepodid, a more advanced stage than the nauplius, the stage which emerges from the egg of N. indivisa. Perhaps the oxygen demand of the very active copepodid is greater than that of the sluggish nauplius as hatching approaches. It might be added here that in the case of the copepodid of

A. ambloplitis, there are at least three and possibly four membranes for oxygen to pass through as opposed to two for N. indivisa. So perhaps even very subtle differences in the amount of this element would account for the outward orientation of the embryos of A. ambloplites.

On the other hand, it appears that the inward orientation of the embryos of Nectobranchia indivisa makes it easier for them to hatch. In this position, these relatively weak nauplii are able to make full use of their appendages against the single wall of the egg membrane rather than against both the egg membrane and the egg sac which are glued together on the outside wall.

The comparative activity of the nauplius and the copepodid stages may also determine how hatching takes place in the above two species. The eggs of Achtheres ambloplitis drop out of the egg sacs and hatch in the water. Apparently the vigorous copepodid accomplishes this with little trouble. When the eggs drop out of the egg sac of Nectobranchia indivisa, the nauplii emerge with difficulty, if at all. In the more common occurrence of hatching within the ruptured egg sac, the nauplii seem to have considerably more success in escaping from the egg membrane. In the latter case it appears that, even though the nauplii are not very active, they succeed because the egg membrane is being held in place while they work against it.

Nauplius

The nauplius of Nectobranchia indivisa, in many respects, is similar to the other known nauplii of the Lernaeopodidae. It has the same general shape, a relatively short existence, poor locomotory ability, and it lacks balancers. A striking difference, however, is the possession of the third or mandibular pair of locomotory appendages. This is characteristic of all copepod nauplii except those of the Lernaeopodidae. Other less apparent features which distinguish this species involve differences in setal armature. The first antennae of N. indivisa bears two long setae on the basal segment, whereas other lernaeopodids have none. In addition, the exopodite of the second antennae bears five long terminally-placed setae instead of the usual four. Perhaps of more significance than these numerical differences, is the fact that all of the setae are of the non-plumose variety. This may be one of the contributing factors to the poor swimming ability of this stage of N. indivisa, but even so, the absence of plumose setae has never been reported in the Lernaeopodidae, even in forms in which the naupliar stage passes in the egg. Heegaard (1947) felt that the naupliar stage will probably entirely disappear eventually in this family. Perhaps then, the presence of non-plumose setae is one step further toward elimination of this stage. It does seem, however, that the presence of the third pair of locomotory appendages indicates that N. indivisa

is not too closely related to the main line of lernaeopodid evolution.

Copepodid

The copepodid of this species likewise shows a great deal of similarity with the known copepodids of other lernaeopodid species; however, there are some differences which stand out.

Because the first antennae are usually associated with sensory functions, it is probably reasonable to assume that the long processes arising from these appendages in Nectobranchia indivisa are likewise sensory in function. Whether or not these structures are light sensitive, as suggested in the description, is open to question. The flounder host is a marine fish which usually lives in and around bays and estuaries often at considerable depths. It is a relatively sluggish animal and often lies motionless on the bottom. Because of this it would seem unlikely that light would be a factor in the host to host migrations of the copepodid larvae. It is possible, however, that the ability to reach the surface might expose the larvae to some of the more far reaching currents, thereby enhancing the overall distribution of the parasite. Observations on the effect of light on larval Nectobranchia indivisa have not been carried out during this investigation; however, general observations made on several larval copepods kept in vitro indicate that the nauplii of caligid copepods are much more positively phototropic than are the copepodids of this species. Fasten (1913)

and Wilson (1911) have noted that the copepodid larvae of two lernaepodid species swim to the surface in the daytime and actively seek out their hosts which are feeding there. Fasten (1913) demonstrated that the copepodids of Salmincola edwardsii were attracted by strong light; the stronger the light, the stronger the attraction. Although the above-mentioned larvae have been shown to be positively phototropic, none possesses the specific structure being discussed here.

It is the opinion of the author that the so-called aesthetes of N. indivisa have a sensory function other than light perception; perhaps involving touch, taste, or smell or a combination of the latter two. It must be kept in mind that the copepodid must find a host in a comparatively short time, so any migration would of necessity have to be a relatively short one. If the tactile sense alone were involved, it would seem that this parasite would be found in many more species of fish out of the high numbers which abound within the distributional ranges of the starry flounder, and which in many cases are closely related to this fish. Nevertheless, N. indivisa seems to be very host specific, being known to parasitize only the starry flounder in the Northeastern Pacific and one or two additional species in the Northwestern Pacific.

In experiments with S. edwardsii, Fasten (1913) found that the choice of host was made by some chemical means, smell or taste, or a combination of the two. Wilson (1915) also reached the same

conclusion. It seems that something similar is involved in the attraction of N. indivisa to the starry flounder. The reason to suspect this is that this fish has a very unique aroma which sets it apart from all other fish with which the writer has come into contact. It seems quite possible that the so-called aesthetes are chemoreceptors which are capable of either smelling the odor or of tasting the material which produces the odor.

The first maxillipeds which are represented by chitinous flaps in the copepodid of Nectobranchia indivisa are quite different from any others so far described for copepodids of the Lernaeopodidae. Although there appears to be a considerable amount of variation in this appendage among different species, this is the only one not bearing a claw of some sort. A claw does develop on this appendage during the pupal stage, but its function appears to be somewhat different than that of other lernaeopodid copepods. This is discussed further in the section dealing with the pupa.

All lernaeopodid copepods apparently have attachment organs. The one in the copepodid of this species has by far the shortest filament of any yet described. In addition, it appears that the copepodid is able to attach the disc only to adult copepods. The fact that it is not specific in its choice of copepod "host" indicates probably that the choice depends on the type of substrate more than anything else. The reason that more of the attached stages are found on adult females of

Nectobranchia indivisa is probably because they provide the closest suitable attachment surface. The other two copepods found in the same proximity, Lepeophtheirus parvicruris and Acanthochondria rectangularis, are found just inside the gill cover, often some distance from the gills.

It is not definite as to just how the attachment organ is removed from its position in the anterior part of the copepodid body. It may be pushed out from within in some, as yet, unknown manner or pulled out in one or the other of two ways. The first of these two ways would be for the copepodid to press the supposedly sticky disc against a copepod and then to push away from the attachment surface once a firm connection had been achieved. Pushing away would probably be done with the strong second maxillipeds. The second way of pulling out the filament would be for the copepodid to use either its chelate second antennae or second maxillipeds to strip out the filament once the disc has been firmly attached to the host. Heegaard (1947) saw this happen in the case of a caligid copepodid. In that instance, the copepodid bored into the host with its clawed first maxillipeds, placed the attachment disc into the resultant hole, and then used the chelae of the first maxillipeds to grasp the filament and subsequently strip it out. On the basis of copepodids recovered from the in vitro cultures with their attachment filaments out, it would appear that the method of pushing out the disc and filament from within may be used in the case of N.

indivisa. The method of pushing away from the attachment surface could also possibly be used. However, there might be some difficulty in reaching the attachment filament with either the second antennae or the second maxillipeds, so this might preclude the use of the third method as far as N. indivisa is concerned.

Pupa

It appears that Heegaard (1947), working with the lernaeopodid Clavella uncinata, may have been under some misconception when he stated that the old copepodid skin also serves as the pupal skin. True, the old copepodid skin may remain surrounding the pupa, even throughout the pupal period, but it is quite probable that a new skin had formed under this and should be regarded as the true pupal skin. The present work on Nectobrachia indivisa indicates that this is the case. In this species, the copepodid skin may or may not remain throughout the pupal period. Further evidence supporting the existence of a discrete pupal skin is found in the work of Gurney (1934), who also worked with C. uncinata. In that study, Gurney pictured a pupa with a case which is definitely different than the copepodid skin and bears a considerable resemblance to the pupal case of N. indivisa.

The long maxillipedal sleeves which connect the newly emerged post-pupal females to the pupal case have never been mentioned in the literature. It is possible that Heegaard (1947) saw these in his study

on Clavella uncinata, but was unaware of it. He shows a figure of a post-pupal female which has just attached by means of its bulla and is already grasping the bulla with its first maxillipeds. This copepod is also still holding on with these appendages to what Heegaard calls the pupal attachment filament. This filament leads to the old pupal skin and from there to where it is attached to the host. Since Heegaard stated that the pupa is free in the pupal case, it seems that there would be no opportunity for the pupa to grasp the pupal attachment filament in the manner shown. It may be possible that the part of the so-called pupal filament extending between the maxilliped and the pupal case is actually the remnant of one of the maxillipedal sleeves. The segment between the case and the host tissue probably is the pupal filament. There is nothing in the present work which might support the above interpretation of Heegaard's drawing. Observations on post-pupal females of Nectobranchia indivisa kept in vitro indicate that they shed the maxillipedal sleeves and undergo a short free living period before starting the fusion of the first maxillipeds with the base of the bulla.

The purpose of the maxillipedal sleeves may be one or both of two things. It may be to let the female develop somewhat further prior to permanent attachment; however, it would seem more likely that it would be used as a holdfast, thereby preventing the post-pupal female from being swept out of the gill cavity of the host before a

secure position is attained on a gill filament. The adult females, to which the pupae are attached, are themselves usually affixed near the distal end of the gill filament and consequently hang down into the gill cavity where an unsecured post-pupal female would be in peril. The male, which emerges at the time of the pupal molt, has no maxillipedal sleeves, but does have chelate first maxillipeds. These appendages, along with the second maxillipeds, provide the male with adequate means for holding on as well as migrating via the gills to another female.

Post-Pupal Female

The removal of the attachment organ of Nectobranchia indivisa may take place in a manner somewhat different than that suggested by the observations on the post-pupal females kept in vitro. Friend (1941) stated that the bulla of Salmincola salmonea is affixed to the gill filament first and then grasped by the second maxillae (= first maxillipeds). This may be the normal sequence for N. indivisa also, although it would appear that the maxillipeds do play a more active part in the removal of the attachment organ than in S. salmonea.

The precise roles played by the bulla and its fleshy outer layer in the attachment process are not known for Nectobranchia indivisa. However, by virtue of its position and sticky nature, it seems reasonable to assume that the outer layer has an adhesive function. If

so, it would probably serve to fasten the bulla to the gill filament until permanent attachment has been achieved. At the same time it might also serve to glue the maxillipeds to the base of the bulla until they grow together. This outer layer may also be responsible for digesting away the surface of the gill filament preliminary to the insertion of the bulla. Or perhaps, it might cause an irritation of the host tissue to the extent that it would be stimulated to proliferate new tissue around the bulla, thus effecting permanent attachment. This last alternative seems particularly attractive because, as mentioned earlier, the bulla does in fact become established within the tissue of the gill filament. It may be possible that this type of function may be directly assumed by the bulla after the outer layer has prepared the way. The system of branching canals, as shown in Figures 16 and 17, may be utilized in the transport and secretion of an enzyme bearing substance. There is support for the kind of reactions described above in the findings of Kurz (1877). He noted that the viscid secretion which fastens the bulla to the host acts as an irritant and causes the tissue to fester and swell into a fold which rises up around the bulla and finally envelops it.

Further Development of the Female

It has been noted that considerable growth takes place in the

female of Nectobranchia indivisa from the time it emerges from the pupal case until and even after sexual maturity. However, despite the relatively large increase in size, no evidence of molting has been observed.

Compared with other copepods, the advanced stages of lernaeopodids have much softer body tissues, or at least softer integuments. In light of this, it may not be too extreme to suspect that there is some modification of the typical arthropod molting process in these animals.

In his work with Salmincola salmonea, Friend (1941) concluded that no further molting took place after the first female stage developed its bulla. Basing his opinion on the findings of Wigglesworth (1933), he explained all subsequent growth as an expansion of the cuticle.

Heegaard (1947), rejecting the idea of cuticle expansion, maintained that the drastic transformation which took place in Clavella uncinata could only occur at a molt, or at least, a partial molt. He then cited instances in which he observed advanced female stages which appeared to have a double cuticle. Some of these were seen in which the outer cuticle had ruptured and was hanging down in flakes. He conjectured that C. uncinata, as well as other Lernaeopodidae, could thus change their cuticle without shedding it all at once. Heegaard felt that this form of ecdysis was compatible with the fact that the bulla is formed only once and is not cast off and regenerated.

In the present work there is some evidence favoring the theory of partial molting; however, it is quite different from that described above. The phenomenon of shedding the maxillipedal sleeves, which is a regular occurrence following the pupal molt, might be considered an example of a partial molt. Admittedly, this does not explain the considerable growth which takes place following this event, but it does suggest the possibility that this type of thing may occur many times on a smaller and less conspicuous scale. Additional evidence which has already been mentioned is the instance in which another set of second maxillipeds was observed through the cuticle of a post-pupal female. This might imply that a partial molt was going to occur at some future time.

SUMMARY

The stages in the life history of the parasitic copepod Nectobranchia indivisa have been described.

The adult female lives permanently attached to the gills of the starry flounder. The adult male is a dwarf, living attached to the female.

The eggs hatch into free-swimming, but relatively inactive nauplius larvae. They molt after 20-40 minutes into vigorous free-swimming copepodid larvae.

The copepodid either remains in the gill chamber of the original host or migrates to a new one of the same species. Within about three days it attaches to the female of any of three species of copepods which inhabit the gill cavity of the host. It does this by means of a chitinous disc and filament.

A withdrawal of the protoplasm from the extremities into the cephalothorax takes place. A new skin forms around this concentrated protoplasm and the pupal stage begins. During development, the pupal skin remains attached by the old copepodid filament.

The pupa molts into either immature females or nearly mature males. The males seek out an attached female of the same species, mature, and copulate. The post-pupal females remain connected to the pupal case for a period and then attach permanently to a gill

filament of the host by means of a bulla.

After considerable change in size and morphology, the female becomes sexually mature. Mating occurs and fertile eggs are deposited into the egg sacs.

The orientation of the embryos in the egg sac is with head to the inside, opposite to that of Achtheres ambloplitis. Wilson (1911) proposed that oxygen supply is the factor in the latter, while ease of hatching may be the advantage of this arrangement for Nectobranchia indivisa.

The nauplius differs from all other lernaeopodid nauplii in having mandibles and non-plumose setae.

A long club-like process is present on each of the first antennae of the copepodid stage. It may have a sensory function involving taste or smell or both.

The attachment of the copepodid appears to require a chitinous substrate. Possible attachment methods are discussed.

The formation of a discrete pupal skin is recognized in this family and the maxillipedal sleeves are described, both for the first time. The latter function as a holdfast for the post-pupal female.

The first maxillipeds appear to play a role in the removal of the bulla from the cephalic region of the post-pupal female. Possible attachment methods are considered and growth after the pupal molt is discussed. There is some evidence in favor of partial molting.

BIBLIOGRAPHY

1. Aristoteles. De historia animalium (as quoted by Wilson, Charles Branch. North American parasitic copepods belonging to the Lernaeidae, with a revision of the entire family. Proceedings of the U. S. National Museum 53:1-150. 1917.)
2. Claus, Carl. Beobachtungen über Lernaeocera, Peniculus, und Lernaea. Ein Beitrag zur Naturgeschichte der Lernaeen. Schriften Gesellschaft Beförderung Gesamt Naturwissenschaft 9(Supplement 2):1-32. 1868.
3. Fasten, Nathan. The behavior of a parasitic copepod, Lernaeopoda edwardsii Olsson. Journal of Animal Behavior 3:36-60. 1913.
4. _____ . Fertilization in the parasitic copepod, Lernaeopoda edwardsii Olsson. Biological Bulletin 27:115-121. 1914.
5. Fraser, C. McLean. Copepods parasitic on fish from the Vancouver Island region. Transactions of the Royal Society of Canada, ser 3, 13 (Section V):45-68. 1920.
6. Friend, G. F. The life history and ecology of the salmon gill maggot, Salmincola salmonea (L.) (copepod crustacean). Transactions of the Royal Society of Edinburgh 60:503-541. 1941.
7. Grabda, Jadwiga. Life cycle and morphogenesis of Lernaea cyprinacea L. Acta Parastologica Polonica 11:169-198. 1963.
8. Gurney, Robert. The development of certain parasitic Copepoda of the families Caligidae and Clavellidae. Proceedings of the Zoological Society of London, 1934, p. 177-217.
9. Gussev, A. B. Parasitic Copepoda of some marine fishes. Parazitologicheskii Sbornik 13:394-463. 1951. (In Russian)
10. Heegaard, Poul. Contribution to the phylogeny of the arthropods. Copepoda. Spolia Zoologica Musei Hauniensis 8:1-227. 1947.

11. Kurz, Wilhelm. Studien über die Familie der Lernaepodiden. Zeitschrift für Wissenschaftliche Zoologie 29:380-428. 1877.
12. Lewis, Alan G. Life history of the caligid copepod Lepeophtheirus dissimulatus Wilson, 1905 (Crustacea: Caligoida). Pacific Science 17:195-242. 1963.
13. Linnaeus, Carolus. Fauna suecica. 1st ed. Lugduni Batavorum 1746. 1 vol.
14. _____ . Systema naturae. 10th ed. Stockholmiae 1758.
15. Markevich, A. P. Nectobrachia indivisa Fraser (Copepoda parasitica), peculiarities of structure and systematic position. Naukovi Zapyskŷ, Kyivsk' skŷi Derzhavnyi Universytet 5:215-220. 1946. (In Russian)
16. Marshall, S. M. and A. P. Orr. The biology of a marine copepod. Edinburgh, Oliver and Boyd, 1955. 188 p.
17. Metzger, A. Ueber das Männchen und Weibchen der Gattung Lernaea vor den Eintritt der sogenannt ruckschreitenden Metamorphose. Archiv Naturgeschichte 34:106-110. 1868.
18. Nordmann, Alexander von. Mikroskopische Beiträge zur Naturgeschichte der wirbellosen Tiere. 2d Heft. Berlin, G. Reimer, 1832. 150 p.
19. Scott, Thomas and Andrew Scott. The British parasitic Copepoda. London, Ray Society, 1913. 2 vols.
20. Vejdovskŷ, Franz. Untersuchungen über die Anatomie und Metamorphose von Tracheliastes polycolpus Nordmann. Zeitschrift für Wissenschaftliche Zoologie 29:15-46. 1877.
21. Wigglesworth, V. B. The physiology of the cuticle and ecdysis in Rhodnius prolixus. Quarterly Journal of Microscopical Science 76:269-318. 1933.
22. Wilson, Charles Branch. North American parasitic copepods. Part 9. The Lernaepodidae. Proceedings of the U. S. National Museum 39:189-226. 1911.

23. _____ . North American parasitic copepods belonging to the family Lernaeopodidae, with a revision of the entire family. Proceedings of the U. S. National Museum 47:565-729. 1915.
24. _____ . The copepods of the Woods Hole region Massachusetts. Washington, 1932. 635 p. (U. S. National Museum. Bulletin 158)
25. Yamaguti, Satyu. Parasitic Copepoda and Branchiura of fishes. New York, Interscience 1963. 1104 p.
26. Yü, S. C. and H. W. Wu. Parasitic copepods of the flatfishes from China. Bulletin of the Fan Memorial Institute of Biology 3:55-75. 1932.

APPENDIX

Figure 1. Dorsal view of nauplius.

Figure 2. Ventral view of nauplius with copepodid appendages showing within.

Figure 3. Dorsal view of copepodid.

Figure 4. Ventral view of anterior part of copepodid.

Figure 5. Late copepodid with attachment filament extended.

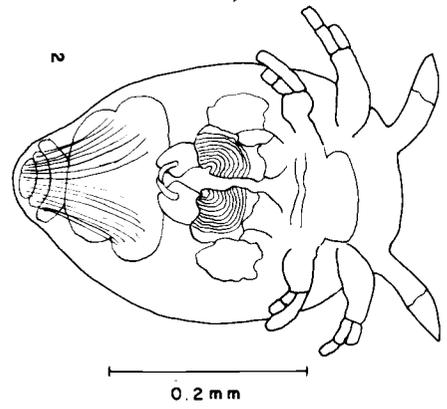
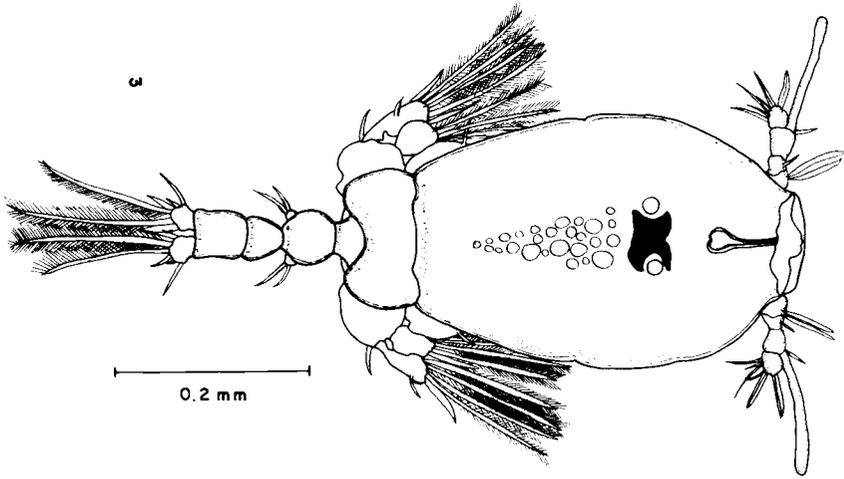
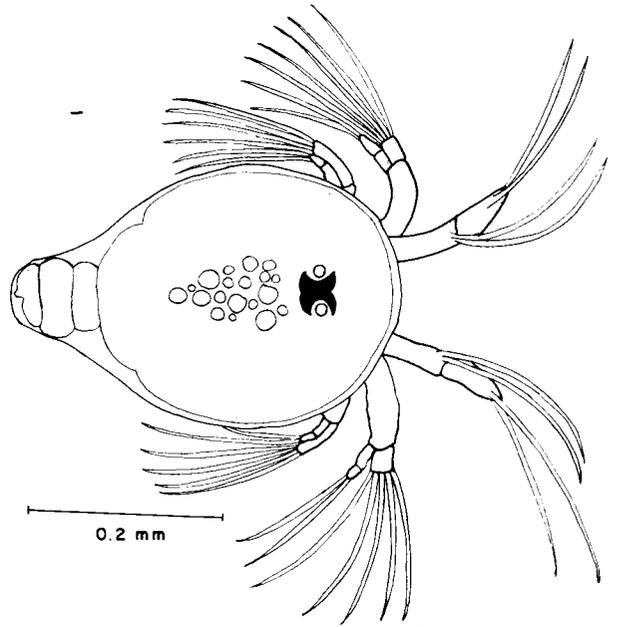
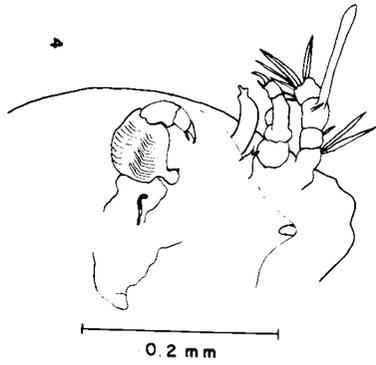
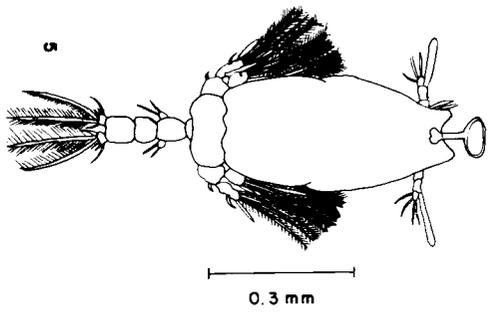


Figure 6. Pupal female.

Figure 7. Pupal male.

Figure 8. Post-pupal female before maxillipedal sleeves are fully extended.

Figure 9. Post-pupal female with maxillipedal sleeves extended and anchored to pupal case.

Figure 10. Oral view of mouth cone.

Figure 11. Dorsal view of Stage A.

Figure 12. Lateral view of Stage A.

Figure 13. Lateral view of Stage B.

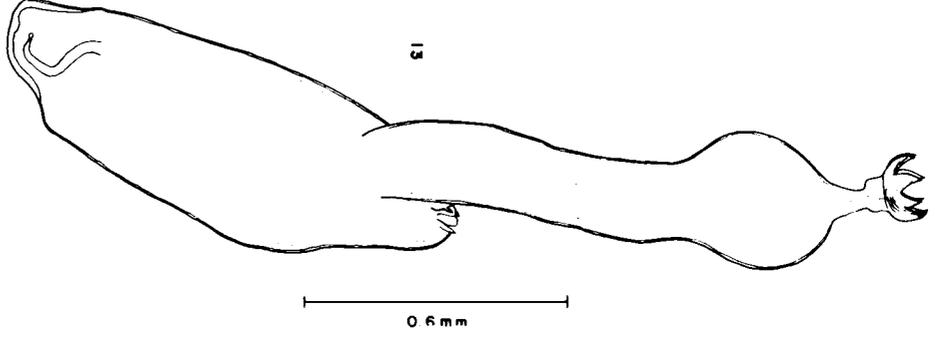
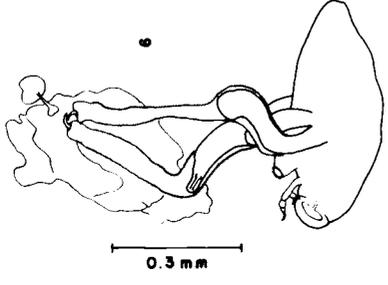
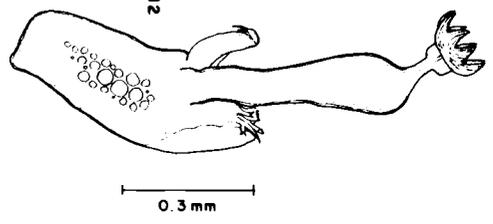
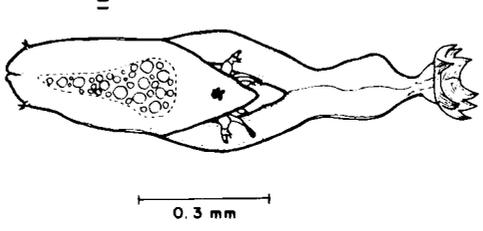
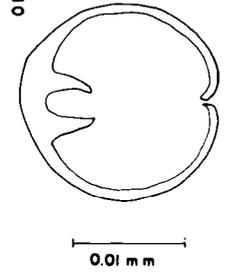
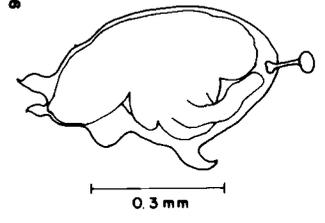
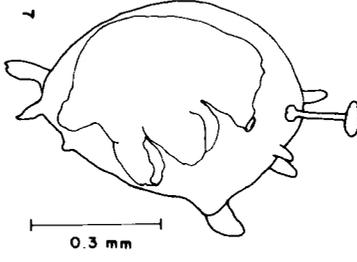
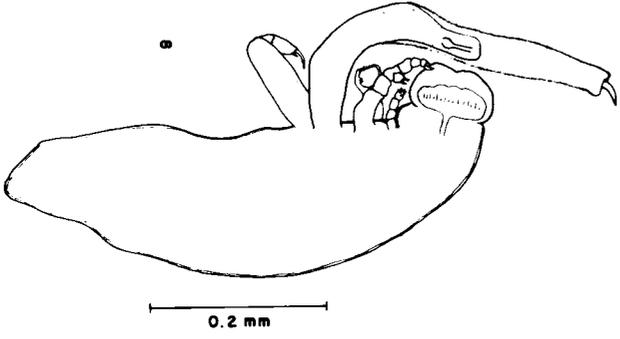


Figure 14. Adult female.

Figure 15. Adult male.

Figure 16. Anterior view of bulla. (After Gussev, 1951)

Figure 17. Lateral view of bulla. (After Gussev, 1951)

