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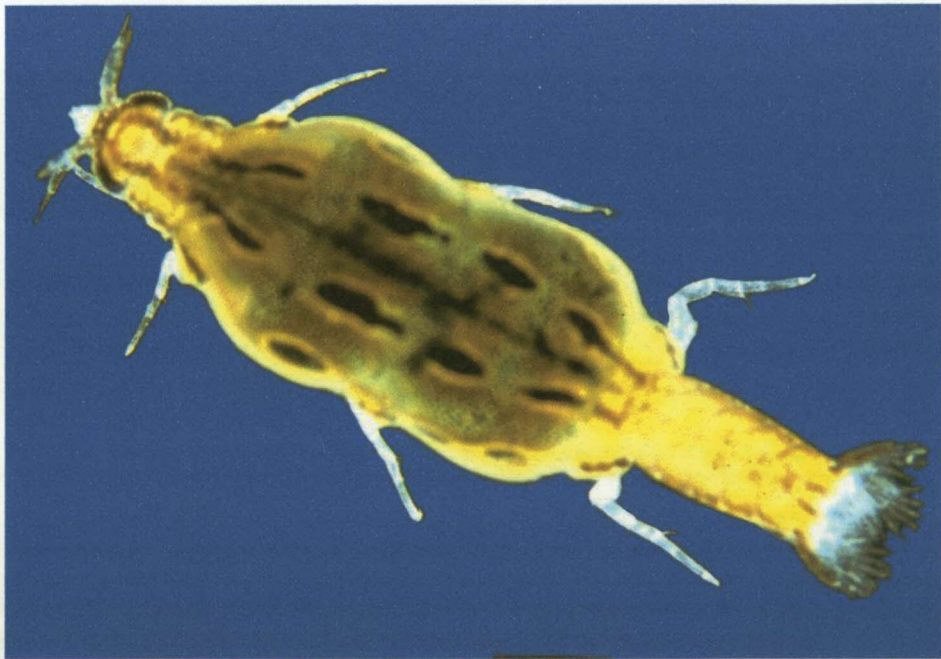
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“...throwing back their long antennae, they begin to beat the water with their false legs and propel themselves with the speed of lightning. By means of such manoeuvres they throw themselves forward like arrows; their pointed heads cut through the water easily while the beats of their false legs push their whole body forward with such force that the small body trembles like a string...”

Nicolas Wagner (1869)

**THE BIOLOGY OF GNATHIID ISOPOD
PARASITES AND THEIR ROLE AS
VECTORS OF FISH BLOOD PARASITES IN
SOUTH AFRICA**

by

Nicholas Jacobus Smit

*Thesis submitted in fulfilment of the requirement
for the degree Philosophiae Doctor
in the Faculty of Natural and Agricultural Sciences,
Department of Zoology and Entomology,
University of the Orange Free State*

**Promotor Prof. J. G. van As
Co-promotor Prof. Linda Basson
Co-promotor Prof. Angela Davies Russell**

December 2000

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1. Introduction

South Africa's coastline and marine environment constitutes one of the most unique and species diverse areas in the world. The southern African coastline, extending from northern Namibia around the South African coast eastwards to southern Mozambique forms, on its own, one of the 16 marine zoogeographical provinces of the world (Wye 1991). This region's marine fauna and flora consists of over 10 000 species or almost 15% of all coastal marine species known world-wide, with new species constantly being described. According to Branch, Griffiths, Branch and Beckley (1994) 12% of these known southern African species are endemic. Part of this uniqueness can be attributed to the presence of two totally different currents bathing the east and west coasts, forming an extreme contrast between these areas. One of the most powerful currents in the world, the Agulhus Current, brings warm water from the subtropics down the east coast and in contrast the waters of the west coast are chilled by the northward drifting cold water of the Benguela Current. All above mentioned factors make the study of marine organisms found alongside the southern African coast a very important and rewarding experience, even more so if the objects of the research belong to the family Gnathiidae Harger, 1880 of the crustacean order Isopoda.

Representatives of the isopod **family Gnathiidae** are unique in that they have only five pairs of walking legs or pereopods and not the usual seven pairs found in all other isopods. The gnathiid **male** is characterised by the great development of its forwardly-directed mandibles which are transformed into frontal forceps. In correlation with this, the development of the mandibular muscles leads to the formation of a more or less quadrangular-shaped cephalosome. Due to the enlargement of the cephalosome (particularly the increase in weight) a tendency is manifested for one or more post-cephalosome segments to enlarge and form an anterior somatic division, separated from the normal posterior division, by a more or less accentuated constriction. At this stage the taxonomical classification of gnathiids is based on the morphology of the male. This in itself presents some more problems in the taxonomy of gnathiids, since it makes it almost impossible to identify females and larvae when they are found in the absence of males. The **females** have more reduced mouthparts including a total absence of the mandibles. The thorax of the females is also characteristically swollen due to the presence of eggs or

juvenile larvae. Both the adult stages are non-feeding and can be found in a variety of habitats on the sea floor or in intertidal pools.

The **larvae** of gnathiids are blood sucking parasites infecting different species of marine, as well as estuarine, teleosts and elasmobranchs. The larvae can be divided into fed (pranizae) and unfed larvae (zupheae). The cephalosomes of the larvae are totally different from those of the adults and are characterised by large eyes and biting, sucking mouthparts.

Members of the Gnathiidae have been the cause of untold confusion in the scientific literature over the last two centuries. The reason for this can be found in the fact that, as already mentioned, different **life cycle** stages of the same species display enormous morphological differences. These differences led to the problem that early collectors failed to recognise the links between males, females and larvae, describing them as different species, even belonging to different genera.

The **first recognisable description** of a gnathiid was made by the Dutch zoologist Martin Slabber (1769) who drew a gnathiid larva. He was very excited about his find, but was not sure to which of the Linnean genera his specimens belonged. Thirty-five years later the first gnathiid male was described by Montagu (1804), but he was also uncertain to which genus the new species belonged. Due to the articulated tail and ten legs of the animal he named it *Cancer maxillaris* Montagu, 1804, but still felt that it might not truly belong to any of the known Linnaean genera. A few years later Montagu (1813) described a gnathiid larva from a fish host and named it *Oniscus caeruleatus* Montagu, 1813. Again he felt that it might better be placed in a new genus. Leach (1814) erected the genus *Gnathia* Leach 1814 and renamed *Cancer maxillaris* as *Gnathia termitoides* Leach, 1814. Amongst the male specimens he also found specimens resembling Montagu's *Oniscus caeruleatus*. Leach (1814) noted the similarity in leg number and antennal morphology of these two species and is thus the first person to suspect that they might be different life cycle stages of the same species.

In 1816 Risso created yet another genus for gnathiid males in describing *Anceus forficularius* Risso, 1816. Although the genus *Gnathia* already existed, the genus *Anceus* was still used until the turn of the 19th century. The third genus to be introduced for

gnathiids was the genus *Praniza* Latreille, 1817. Gnathiid larvae with a swollen gut (fed larvae) were placed in this genus. The fourth genus, *Zuphea* Risso, 1826 was described to accommodate the unswollen (unfed larvae) gnathiid larvae.

This **confusion in the literature** regarding the taxonomy of the gnathiids was partly clarified by Hesse (1864) when he accidentally discovered the true relationship between two of the different genera. A specimen of the genus *Praniza*, which he kept in sea water to draw at a later date, moulted into an *Anceus*. He suggested that these two genera must be considered as a single genus. However, his findings were not accepted without any resistance, especially from Bate (1858). Bate's opposition of Hesse's findings was, however, caused by misinterpretation of the results, rather than presenting evidence showing the opposite. In his paper Bate (1858) described *Praniza edwardsii* Bate, 1858. Without knowing it, Bate's species was actually the first accurate description of a female gnathiid, thus not belonging to the genus *Praniza* as defined by Latreille (1817). Because Bate found unborn larvae in his specimens he concluded that the specimens belonging to the genus *Praniza* are adults. Hesse, on the otherhand, regarded both males and females as *Anceus* and only the larvae as *Praniza*. When looking at this in retrospect, it is easy to see why Bate questioned Hesse's finding that representatives of *Praniza* can moult into an *Anceus*, and that *Anceus* can give birth to *Praniza*, because according to his findings members of the genus *Praniza* are adult forms.

The taxonomy and morphology of this group was finally sorted out by Théodore Monod, who published his extensive monograph on gnathiid biology, taxonomy and morphology in 1926. Monod (1926) clarified several taxonomic problems, the homology of mouthparts and somites, as well as the ontogeny. In this monograph he also described 66 species of gnathiids and gave all the synonyms of the already described species.

The life cycle and metamorphosis of gnathiids was first described by Smith (1904) and finally fully clarified by Mouchet (1928). A detailed discussion of the literature concerning the research on the life cycle of different gnathiid species will be presented in Chapter 3.

Another important **contribution to gnathiid taxonomy** was the recent revision of the classification of the Gnathiidae by Cohen and Poore (1994). They used phylogenetic

analysis to support their division of the family Gnathiidae into ten genera. This included the creation of two new genera, (*Monodgnathia* Cohen and Poore, 1994 and *Gibbagnathia* Cohen and Poore, 1994), the revival of another genus previously in synonymy (*Caecognathia* Camp, 1988), the elevation of a subgenus to generic status (*Elaphognathia* Monod, 1926) and the sinking of two genera and a subgenus in synonymy (*Akidognathia* Stebbing, 1912 as a synonym of *Bathygnathia* Dollfus, 1901; *Heterognathia* Amar and Roman, 1974 and *Perignathia* Monod, 1926 as synonyms of *Caecognathia*).

The importance of research on the biology, life cycles and specific host/parasite relationships of gnathiids is underlined by the findings of Davies (1982) that *Gnathia maxillaris* (Montagu, 1804) larvae may possibly be **vectors of the protozoan blood parasite** *Haemogregarina bigemina* Laveran and Mesnil, 1901 between blennies in Wales. According to Davies, Eiras and Austin (1994) this may also be the case with intertidal fishes in Portugal. The possibility of South African gnathiid larvae acting as vectors of *H. bigemina* was discussed by Smit and Davies (1999) and forms an integrated part of the current study.

Research in **Africa** on gnathiids is almost none existant. The only records are those of Barnard (1914a,b, 1920, 1925) describing four species from southern Africa [*Gnathia africana* Barnard, 1914; *Gnathia spongicola* Barnard, 1920; *Gnathia disjuncta* Barnard, 1920 and *Caecognathia cryptopais* (Barnard, 1925)], Daguerre De Hureaux (1971) describing a species from Morocco (*Gnathia panousei* De Hureaux, 1971) and a single record by Müller (1989a) from Kenya [*Elaphognathia wolffi* (Müller, 1989)]. Although Monod (1926) recorded some of the southern African species in his monograph, he only included Barnard's original descriptions without additional information. In Kensley's (1978) guide to the isopods of southern Africa he gave a key and a short description of the then described species of southern African gnathiids. Recent additions to this list of publications concerning gnathiids of southern Africa are a series of papers by Smit, Van As and Basson (1999a) redescribing *G. africana*, Smit, Basson and Van As (2000) redescribing *Caecognathia cryptopais* and Smit and Van As (2000) describing a new species (*Gnathia nkulu* Smit and Van As, 2000).

The author's attention was first drawn to gnathiids in 1995 during a fieldtrip of the Aquatic Parasitology Research group, Department of Zoology and Entomology of the University of the Orange Free State to Mc Dougall's Bay on the west coast of South Africa. The collection of *G. africana* during that trip and that of subsequent collections from other localities has led to the research and **completion of the author's MSc dissertation** entitled: Gnathiid isopod (Crustacea) parasites of marine fishes of southern Africa (Smit 1997). The aim of that study was to complete a comprehensive literature review of gnathiid research (not repeated here), redescribe the currently known southern African gnathiid species, to investigate the diversity of gnathiid species along the South African coast, to determine the life history and infectation pattern of *G. africana* and to re-run of the phylogenetical analysis of representatives of the family Gnathiidae of Cohen and Poore (1994), but including the southern African species previously omitted. Most of these objectives were met, but in doing so many new questions regarding gnathiid biology, ecology and taxonomy arose. One of the most intriguing of these questions is the possibility of gnathiids acting as vectors of some species of fish haemogregarines. The aim of the present study was thus to try and answer these questions and to contribute substantially to the knowledge on gnathiids and fish haemogregarines of southern Africa.

Against this background the present study was undertaken with the following specific objectives:

- to complete the life cycle of *G. africana* under laboratory conditions
- to study gnathiids as parasites of some elasmobranch species
- to determine the taxonomically important characteristics of females and larvae to serve as effective identification tools
- to research the possibility that *G. africana* is the vector of the fish blood parasite *H. bigemina* in South Africa
- to investigate the presence of other blood parasites in fish that serve as hosts of gnathiid larvae
- to continue the investigation into the diversity of gnathiid species along the South African coast.

As part of this research a number of **papers** have already been published in refereed journals and refereed conference proceedings (see Appendix I). These include a redescription of the adult male and pranzia larvae of *Gnathia africana* (Appendix I A: Smit *et al.* 1999a), a redescription of the adult male of *Caecognathia cryptopais* (Appendix I B: Smit *et al.* 2000) a description of a new species, *Gnathia nkulu* (Appendix I C: Smit and Van As 2000) and the use of mouthpart morphology in the taxonomy of larval gnathiids (Appendix I D: Smit, Van As and Basson 1999b). The preliminary results on the work on *H. bigemina* in southern Africa was also published (Appendix II: Smit and Davies 1999).

2. Materials and Methods

The southern African coastline can be divided into four distinct regions. These include the cold temperate west coast, warm temperate south coast, subtropical east coast and tropical east coast (Branch and Branch 1995). Of these four regions, the warm temperate south coast, stretching from Port St Johns in the east to Cape Point in the west, possesses the most unique fauna and flora, as already discussed in Chapter 1. Fieldwork for this study was conducted from June 1996 to October 1999 at two very different localities along the south coast region and at different times of the year. These two localities are the De Hoop Nature Reserve and Jeffreys Bay (Fig. 2.1A). The human impact on these two localities differs considerably. De Hoop Nature Reserve is a strictly controlled marine reserve, where no organisms, dead or alive, may be removed by the general public. Limited collection is allowed for research purposes. Jeffreys Bay is a popular holiday resort where the general public is allowed to remove certain organisms for bait as well as for consumption from the intertidal zone with the necessary permits.

2.1 Collection localities and collection of fish hosts

De Hoop Nature Reserve

Over the past six years, the Aquatic Parasitology Research Group has conducted research in the reserve. In order to collect intertidal organisms, collection permits were obtained from Cape Nature Conservation and Marine Coastal Management Office (see Appendix III). The conditions of these permits are that a detailed report of the collections and results be provided to the authorities on completion of each field trip. The two environmental education centers in the reserve, Koppie Alleen and Potberg, were made available by Western Cape Nature Conservation as base for the researchers during fieldtrips. Well-equipped laboratories were set up in the environmental centers for the duration of each field trip. These field laboratories contained, amongst other components, compound and dissection microscope photographic systems, chemicals for the use of staining and fixing of a wide range of parasites, as well as small aerated marine aquaria for the keeping of captured fishes.

Jeffreys Bay

The tidal pools at this locality consisted of small pools formed by rocks (Fig. 2.1C), in comparison to the huge tidal pools sunken into the rock plate at De Hoop (Fig. 2.1B). Field laboratories as described for De Hoop were also set up at Jeffreys Bay during each fieldtrip.

Collection of fishes

The initial aim of this project was to focus on residential tidal pool fishes. The residents include various species of the families Clinidae, Gobiidae and Blenniidae, of which a large number are endemic to South Africa (Branch *et al.* 1994). This initial aim was abruptly changed when we had the opportunity to dissect a single leopard catshark, *Poroderma pantherinum* (Smith, 1838) caught by a local fisherman at Jeffreys Bay in January 1999. The presence of gnathiid praniza larvae (see Chapter 4) on the gills of this shark led to the inclusion of elasmobranchs in our fish collection list.

During the first Jeffreys Bay field trip in January 1998, we also had the opportunity to collect fish by means of cast nets in the Seekoei River estuary. This estuary forms part of the Seekoei River Nature Reserve and is approximately 10 km west of Jeffreys Bay. We were only able to collect the flathead mullet, *Mugil cephalus* Linnaeus, 1758 during this one-off expedition.

Intertidal pool fishes at both localities were collected using the same techniques. The most successful methods were by the use of hand nets in small shallow pools and cast nets in deep tidal pools (Fig. 2.1D). Large adult specimens of some tidal pool species are found in the infratidal zone (see Bennett and Griffiths 1984). Hand lines were used to collect these fishes in order to obtain infestation data for large specimens as well.

Apart from the leopard catshark obtained from a fisherman at Jeffreys Bay, all the other sharks as well as a single ray, were collected at De Hoop Nature Reserve during the evening low tides. Bait consisted of dead fish from earlier dissections. This was used to lure the sharks into the gullies formed by the rocky banks. These sluggish, slow moving sharks were then easily caught using flash lights and hand nets. Great care was taken not to stress captured fish, as the gnathiid larvae tend to leave a stressed host, which could have resulted in inaccurate infestation data. During these night collection trips, a number

of specimens of the evileye pufferfish, *Amblyrhynchotes honkenii* (Bloch, 1795) were also collected.

The captured fish were placed in aerated marine aquaria in the temporary field laboratories. All fishes were identified using the well-illustrated Smith's Sea Fishes compiled by Smith and Heemstra (1986) and the Guide to the Common Sea Fishes of Southern Africa (Van der Elst 1995). The total length of each fish was measured from the tip of the snout to the tip of the caudal fin. In the field laboratory fishes were killed using high concentrations (2.5×10^{-5} g/l) of the anesthetic benzocaine (ethyl-4-aminobenzoate) and subsequently examined for parasite infections (Fig. 2.1E). Since this project forms part of a comprehensive survey of intertidal fish parasites, a complete autopsy of all the fishes was carried out by all members of the research group to search for a variety of parasites.

A total of 182 fishes belonging to seven families and 12 species were collected and examined during four excursions to Jeffreys Bay (June 1996, July 1997, January 1998, January 1999) and three to the De Hoop Nature Reserve (April 1997, April 1998, October 1999) (see Table 2.1).

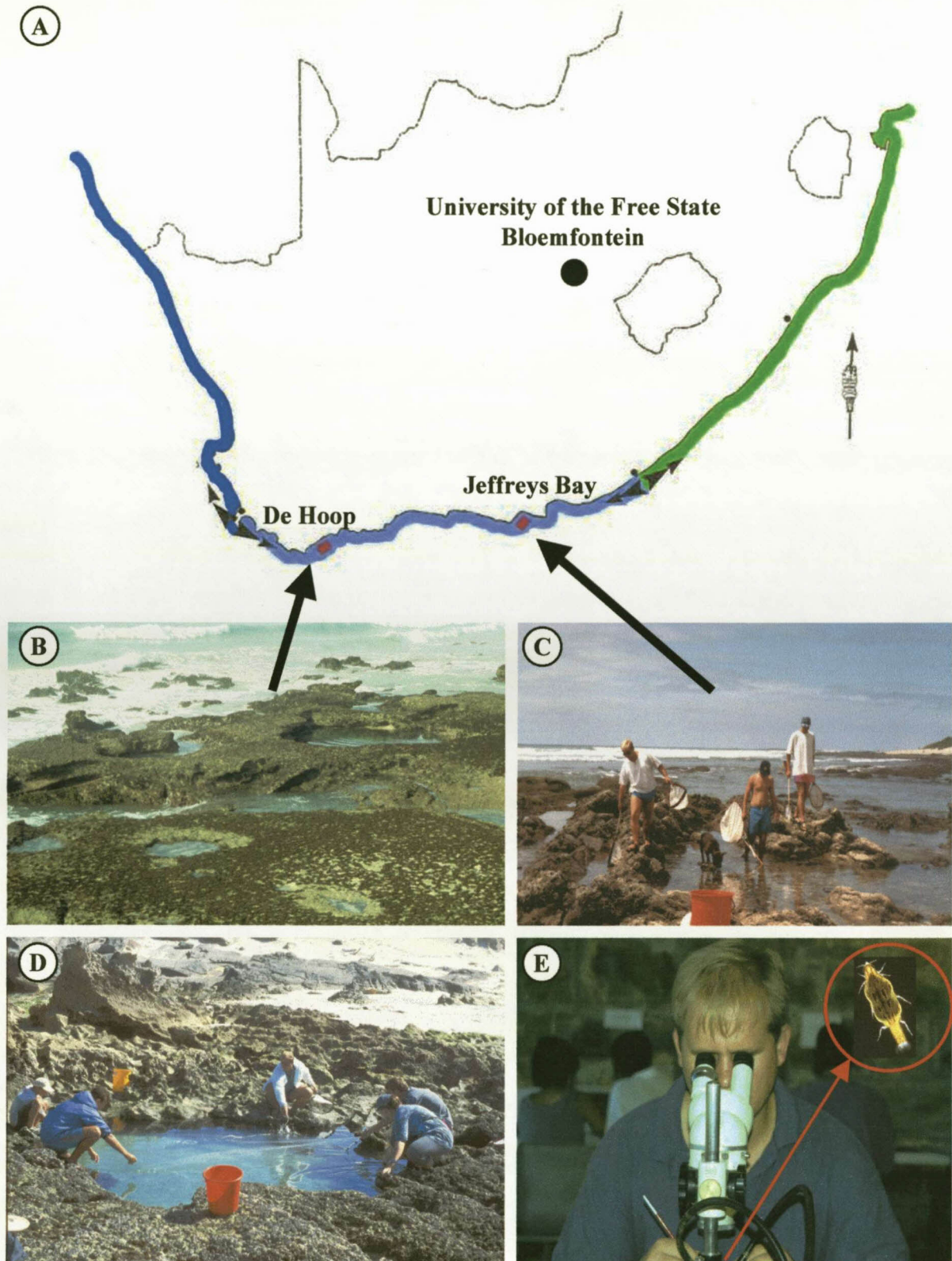


Figure 2.1. A. Map of South Africa showing the collection localities. B. Deep tidal pools at De Hoop Nature Reserve. C. Tidal pools at Jeffreys Bay. D. Collection with cast net at De Hoop Nature Reserve. E. Author busy with microscopy at De Hoop Nature Reserve.

Table 2.1. A summary of the fish species and families collected during seven different field excursions (N = total number of fish collected).

Fish species collected	Collection locality	N
CLASS OSTEICHTHYES		
CLINIDAE		
<i>Clinus cottoides</i> Valenciennes, 1836	De Hoop and Jeffreys Bay	22
<i>Clinus superciliosus</i> (Linnaeus, 1758)	De Hoop and Jeffreys Bay	97
<i>Clinus taurus</i> Gilchrist and Thompson, 1908	De Hoop	1
GOBIIDAE		
<i>Caffrogobius caffer</i> (Günther, 1874)	De Hoop and Jeffreys Bay	14
MUGILIDAE		
<i>Mugil cephalus</i> Linnaeus, 1758	Seekoei River Estuary	26
GOBIESOCIDAE		
<i>Chorisochismus dentex</i> (Pallas, 1769)	De Hoop and Jeffreys Bay	3
SPARIDAE		
<i>Diplodus sargus capensis</i> (Smith, 1844)	De Hoop	7
<i>Dichistus capensis</i>	De Hoop	1
TETRAODONTIDAE		
<i>Amblyrhynchotes honkenii</i> (Bloch, 1795)	De Hoop	4
CLASS CHONDRICHTHYES		
SCYLIORHINIDAE		
<i>Poroderma pantherinum</i> (Smith, 1838)	Jeffreys Bay	1
<i>Haploblepharus edwardsii</i> (Voight, 1832)	De Hoop	5
TORPEDINIDAE		
<i>Torpedo fuscomaculata</i> Peters, 1855	De Hoop	1

2.2 Taxonomy of gnathiids

In their study on the phylogeny and biogeography of the family Gnathiidae, Cohen and Poore (1994) provided a comprehensive summary of all the different taxonomically important characteristics of gnathiid males. These characteristics were also discussed in detail in the author's MSc. dissertation (Smit 1997). Throughout this thesis the terminology and numbering of pereonites and pereopods proposed by Cohen and Poore (1994) will be used (see Fig. 2.2). Problems, however, still exist regarding the terminology used for the different setae and spines, especially those found on the

pereopods. To minimise any further confusion, the terminology of setae and spines will be used as proposed in Figure 2.3A-H. The description of adult males will follow the format established by Cohen and Poore (1994) and recently used by Smit *et al.* (1999a), Smit *et al.* (2000) and Smit and Van As (2000).

The comprehensive description of adult females and larvae were largely neglected by previous authors and even totally omitted in Cohen and Poore's (1994) description of 25 new species from Australia. In order to establish a uniform format for the descriptions of females and larvae, most of the 72 taxonomically important characteristics defined for males by Cohen and Poore (1994) applicable to females and larvae were also used. In addition to these characteristics, those specifically unique to females and larvae were identified and included.

Preparation of material for scanning electron microscopy

All the scanning electron microscopy (SEM) work was done in the Department of Zoology and Entomology, University of the Free State in Bloemfontein. The fixed specimens were hydrated from 70 % ethanol to fresh water. The organisms were then washed and cleaned by brushing them with a soft sable hairbrush, under a dissection microscope, in order to get rid of salt crystals and debris. It was found that the best results were obtained when the specimens were already cleaned with a brush before fixing. Clean specimens were dehydrated through a series of ethanol concentrations and critical point dried using standard techniques. Dried specimens were mounted on inverted conical stubs made by the Department of Instrumentation in the Faculty of Natural and Agricultural Sciences. The aim of this particular design was to enable a tilt of the SEM stage of 90°, thereby ensuring an even black background on the micrographs. It was also possible to rotate specimens a full 360 degrees and take images from angles not available when the specimen was placed on a normal flat stub. Specimens were mounted on these stubs with a rapid-drying varnish (Japan Gold Size, Windsor and Newton), normally used in gilding. Specimens were sputter coated with gold and studied with the aid of a JEOL WINSEM JSM 6400 scanning electron microscope (SEM). Optimum results were obtained when SEM work was done at 10 kV with a working distance of 39mm and the stage tilted at 70° to 90°.

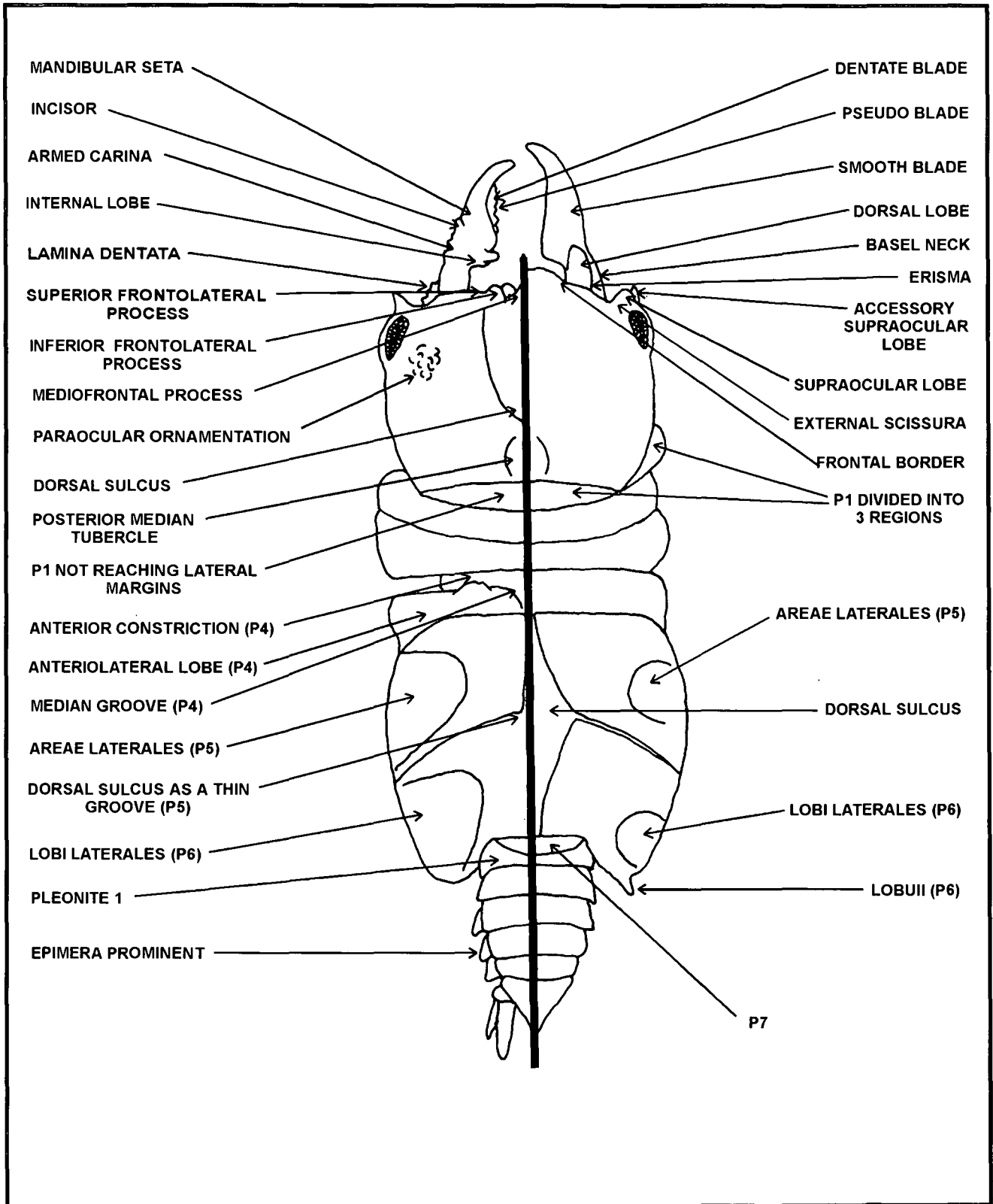
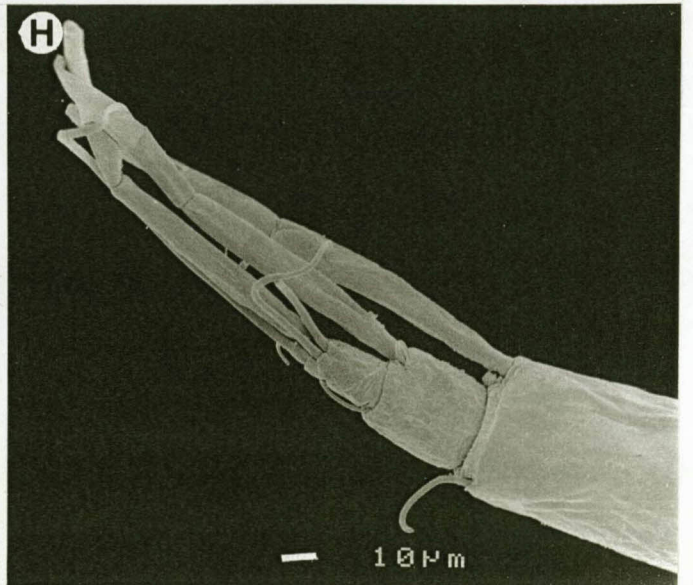
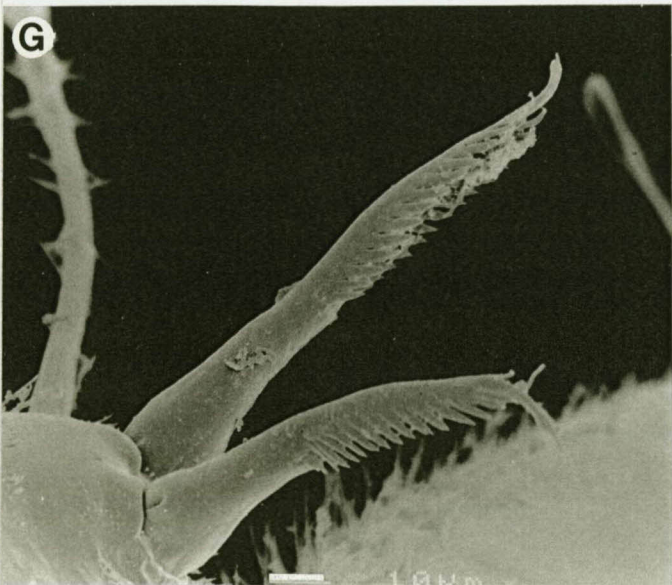
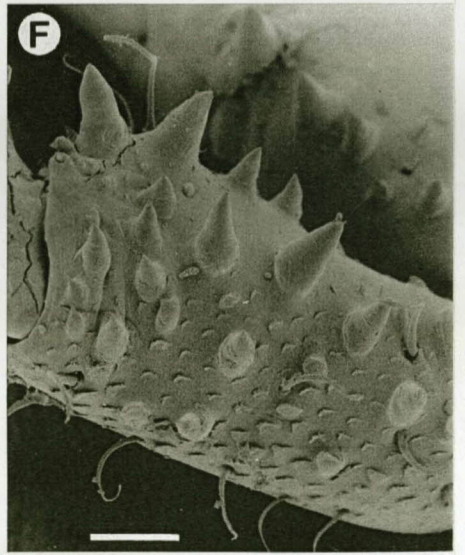
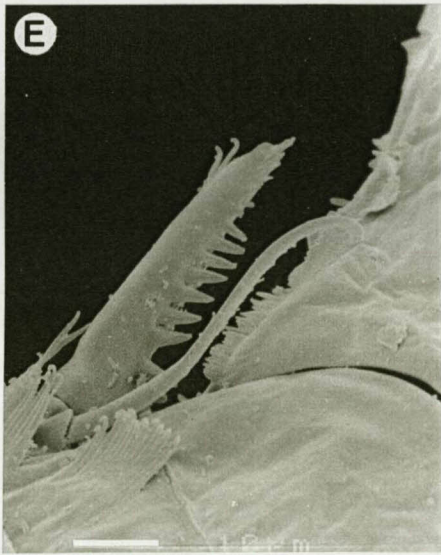
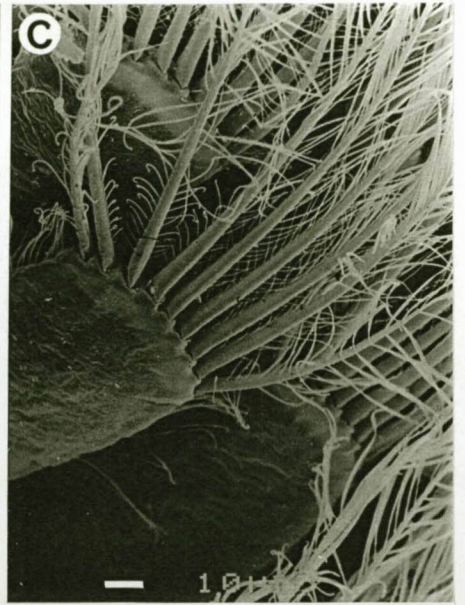
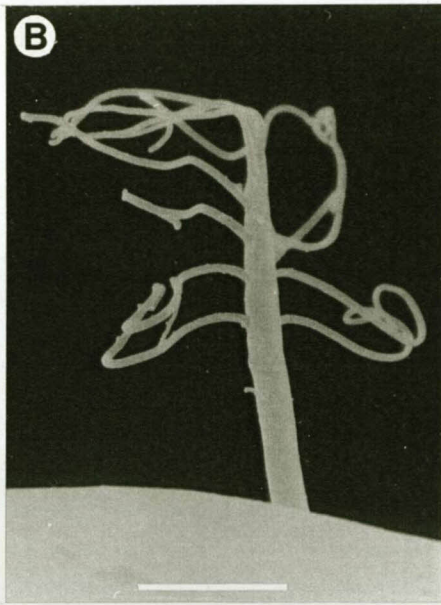


Figure 2. 2. An illustration of the morphological features of a stylised male gnathiid (dorsal view), redrawn from Cohen and Poore (1994)

Figure 2.3**Scanning electron micrographs of the different types of setae, spines and tubercles found on gnathiids**

- A. Simple setae
- B. Feather-like seta
- C. Plumose setae
- D. Pectinate scales
- E. Short denticulate compound spine usually found on propodus of pereopods
- F. Tooth-like tubercles
- G. Long denticulate compound spines
- H. Aesthetasc setae found on distal articles of antenna 1

Scale bars: A-E & G = 10 μm ; F = 100 μm ;



Light microscopy

The methods proposed for studying of the external anatomy of copepods by Humes and Gooding (1964) were successfully applied to work with gnathiids. Specimens used for illustrations were taken from 70% ethanol into 100% lactic acid (25 ml). Two grams lignin pink was dissolved in the lactic acid to stain the specimens, especially the fine structures and setae. Specimens were left for up to five hours in the stain. Temporary slides of stained specimens were prepared as whole mounts, as well as dissected cephalosome, pereon and pleon appendages. These were examined with a Leitz Laborlux D compound and a Wild M5 dissection microscope. Drawings were made from projections using drawing attachments on the microscopes. The combination of the lignin pink staining and the refractive index of the lactic acid enhanced the morphological features of the specimens, making it easy to make accurate line drawings.

Morphological measurements

The total length of all gnathiids was calculated from microscope projection drawings. In gnathiid males, total length was measured from the frontal border to the apex of the telson, and in females from the most anterior area of the produced frontal border to the apex of the pleotelson. In larvae total length was measured from the most anterior part of the labrum to the apex of the pleotelson.

2.3 Life cycle work on South African gnathiids

A marine aquarium was set up in the Department of Zoology and Entomology to maintain fish for use as hosts in feeding experiments with parasitic larval stages of *Gnathia africana*. The fish host used for these experiments was the super klipfish *Clinus superciliosus* (Linnaeus, 1758). This common intertidal fish species was found to be the preferred intertidal host for *G. africana* (Smit 1997). Initially, artificial sponges, as used by Wägele (1988) were built to serve as resting place for adults and resting larvae, but it was found that the animals survived perfectly well in 50ml screw-top containers of seawater alone. It was, however, important to exchange the water regularly (once every third day) and to clean each gnathiid with a small brush in order to remove debris from the body, especially from the pleopods and dorsal pereon. All animals were kept in seawater at temperatures between 20 - 25°C. Gnathiids were examined daily under a dissection microscope to monitor their condition and to look for signs of moulting to the

next instar or to an adult stage. The development of these larvae were carefully noted each day and video prints and color photographs were taken to build up a visual library of their development.

2. 4 Haemogregarine research in South Africa

Haemogregarines

Blood smears of fish were prepared on clean glass slides from heart blood. When possible, at least three smears per fish were made. Blood smears were immediately fixed in absolute methanol for 10 minutes and stained with Giemsa's stain (diluted 9:1 with a phosphate buffer of pH 7) for 25 minutes. Stained blood smears were screened for haemogregarines with a 100 X oil-immersion objective magnification using a Zeiss Axiophot photomicroscope. Measurements were made with an eyepiece graticule and stage micrometer. If no infection was detected after screening each slide for 10 minutes, it was assumed that the fish was not infected with a blood protozoan.

Life cycle of *Haemogregarina bigemina* Lavern and Mesnil, 1901

For the purpose of life cycle studies of *Haemogregarina bigemina*, the fish hosts *Clinus superciliosus*, *Clinus cottoides* Valenciennes, 1836 and *Chorisochismus dentex* (Pallas, 1769) were captured and identified, using methods already described. These species were specifically targeted, because earlier investigation showed that all three these fish species were infected with both *Gnathia africana* larvae and the fish blood parasite *H. bigemina* (see Smit and Davies 1999). The abundance of specifically *Clinus superciliosus* at both localities also supported the use of this specific fish host in *H. bigemina* life cycle studies. To determine which larvae had fed on fishes infected with *H. bigemina*, fishes were maintained singly in fresh, aerated seawater. Fully fed *G. africana* larvae started to leave their fish host at between two and 24 hours after capture. These free swimming fully fed larvae were removed with plastic pipettes and examined in watch glasses of seawater under a dissection microscope in order to classify them according to size. Others were transferred to small jars of fresh seawater, where they were kept in the dark at between 18-22°C for periods up to 28 days post feeding (as for life cycle experiments). Gnathiid larvae were prepared for screening either immediately or on each of 1-28 days post feeding on clinids and rocksuckers. They were removed individually from jars of seawater with a broad mouthed pipette, placed on paper towels to drain surface seawater,

crushed, and smeared whole between two glass slides. They were then fixed in absolute methanol, stained and screened as for fish blood films (see above).

2.5 General

The first reference to fishes caught during the present study will include the authorities in the species names. The authorities for hosts (fishes, sponges and leeches) referred to in literature will be omitted due the lack of availability of complete species names of hosts referred to in all cases.

For all the fish hosts and the description of parasites, the measurement values (in millimeters) will be presented as follow:

MIN – MAX (M ± STD) (N = ?)

MIN = minimum ; MAX = maximum , M = mean , STD = standard deviation, N = number of specimens measured

No means or standard deviations are provided where fewer than five specimens are measured.

All electron microscopy preparations, operation of the SEM, darkroom work, light microscope photography and line drawings of new species were done by the author.

3. The life cycle of *Gnathia africana* Barnard, 1914

The life history and ecology of members of the family Gnathiidae have intrigued scientists for more than two centuries. Even now the information available on these aspects of gnathiid biology is scanty. To the author's knowledge the life cycle of only the following six of the more than 170 described gnathiid species has been researched in any detail: *Gnathia maxillaris* by Smith (1904) and Mouchet (1928); *G. piscivora* Paperna and Por, 1977 by Paperna and Por (1977); *Paragnathia formica* (Hesse, 1864) by Monod (1926), Mouchet (1928), Stoll (1962, 1963), Amanieu (1963) and Upton (1987a,b); *Caecognathia calva* (Vanhöffen, 1914) by Wägele (1987, 1988); *C. abyssorum* (Sars, 1872) by Klitgaard (1991, 1997); and *Elaphognathia cornigera* (Nunomura, 1992) by Tanaka and Aoki (1998, 1999, 2000).

To compare the life cycle of *Gnathia africana* with that of the species described in the literature, the life cycle of each of these latter species, as presented by the different authors, will be summarised (Section 3.1). This will be followed by a detailed redescription of the female of *G. africana* (see Appendix I A) for the redescription of the adult male and praniza larva) (Section 3.2). This chapter will be concluded with the life cycle of *G. africana* constructed from field and laboratory work done during current research (Section 3.3).

3.1 Literature overview of the life cycle studies of gnathiids

The life cycle of *Gnathia maxillaris* (Montagu, 1804)

As mentioned in Chapter 1, the first description of the metamorphosis and life history of a gnathiid was by Smith (1904), who described aspects of the life cycle of *G. maxillaris*. In his work, Smith (1904) described the final metamorphosis of the praniza larvae into males and females in some detail, but speculated about the life history, since he was not able to persuade larvae to feed on fish in captivity. Although he found no distinction between male and female larvae, he did notice that the male larva prior to moulting underwent an enlargement of pereonite 2. He postulated that this "swollen region" is the chief formative region for the male cephalosome. In the female larvae, ova could be seen

developing as a narrow strip down the dorsal area of the pereon. This developed continuously until it filled the whole of the body. Smith (1904) disagreed with Hesse's (1864) statement that fertilisation must take place for the female larva to moult into an adult, because he was able to rear adult females in the absence of males. Unfortunately, Smith (1904) could not induce adults to mate and thus could only derive information on embryonic development from already fertilised females collected in the wild. Smith (1904) was puzzled by the presence of what he described as a "giant segmented larva" in his collection. Upon studying two praniza larvae in the process of moulting into these giant segmented larvae, he formulated a hypothesis that these giant segmented larvae developed from pranizae that had been brushed off their hosts at a time when they could not metamorphose into adults. Another intriguing question was the huge variation in size of the adult males (1-8 mm) and adult females (1-7 mm). Monod (1926) suggested that Smith (1904) was actually looking at a mixed population of at least two different species of males, and the females of probably three species. Smith (1904) concluded that the life cycle of *G. maxillaris* consisted of small segmented larvae that left the body cavity of the female, attached to a suitable fish host, on which they fed, and rapidly developed into the pranizae. After a period of unknown duration, these pranizae left the host and underwent metamorphosis into adult males or females.

Twenty-four years later, Mouchet (1928) was able to show the existence of three larval stages in *G. maxillaris*, thereby explaining the presence of a "giant segmented larvae" in a gnathiid population. Although he was unable to complete the life cycle under laboratory conditions, he did observe all the different development stages. From these observations he concluded that in *G. maxillaris* the fully fed praniza does not moult into an adult after its first blood meal as previously suggested by Smith (1904), but changes into a larger segmented larva (stage 2 zuphea). This segmented larva returns to a suitable fish host and feeds for the second time, becoming a stage 2 praniza. The stage 2 praniza moults again into a segmented larva (stage 3 zuphea or Smith's (1904) "giant segmented larva"). This stage 3 zuphea attaches to a fish host and the resulting stage 3 praniza is the last blood feeding stage in the life cycle. The stage 3 praniza moults into an adult after detaching from the fish host (Fig. 3.1A).

These two papers on the life cycle of *G. maxillaris* illustrate the basic life history of this species, but unfortunately give no information on the embryonic development, the

feeding behaviour, and length of the digestion period of the different larval stages, as well as the total time it takes for completion of the whole cycle.

The life cycle of *Gnathia piscivora* Paperna and Por, 1977

Paperna and Por (1977) claimed that they were able to reproduce the life cycle of *Gnathia piscivora* in the laboratory. They concluded that the larvae of this species possessed the three phases of feeding and moulting, as in the case of *G. maxillaris*. They also commented that they found the larvae to be indiscriminate in host selection and site of attachment. The larvae were found to feed on a wide variety of fish hosts belonging to the Mullidae, Lethrinidae, Sparidae, Carangidae, Tylosuridae and Mugilidae. Paperna and Por (1977) determined that the larvae attached to the skin of their hosts, became engorged and left their hosts again within two to four hours, but those attached to the gills and walls of the pharyngeal cavity left their hosts after at least one or more days. After seven to 10 days, at 24°C the final stage pranizae moulted into adults. Eggs could be seen developing inside the female larvae before the final moult. After 22-24 days of development in the adult female, up to 200 larvae was released through a slit in the brood pouch. A very interesting observation by Paperna and Por (1977) was that the emerging larvae first had to undergo a moult before they were able to feed.

This study (Paperna and Por 1977) gives some information on the parasitic stages in the life cycle of this species, but they are not very detailed. The description of the species also lacks any illustrations. Paperna and Por (1977) indicated that this was only a preliminary study on the gnathiids of the northern Red Sea, the Bitter Lakes and the eastern Mediterranean, but to the present author's knowledge no further data was published to confirm their preliminary data.

The life cycle of *Paragnathia formica* (Hesse, 1864)

The life history, morphology and taxonomy of *Paragnathia formica* are the most complete for any of the known gnathiid species. Monod (1926) used this species as a model for a detailed description of all the different morphological characters of the males, females and larvae. Monod (1926) was unable to establish the complete life cycle of *P. formica*, but provided detailed information on the moulting behaviour as well as the embryonic development. According to Monod (1926), the hatched larva is entirely segmented, and equipped with robust propulsion organs, which assure rapid movability as

it swims in search of a host. After fixing onto a fish host, feeding commences and soon, without moulting, the appearance of the larva changes profoundly through considerable dilation of pereonites 3 – 5. When sufficient reserves have accumulated in the distended intestine, the praniza (second larva) leaves the host and moults into an adult. Monod (1926) stated that this is the only post-embryonic moult of the whole development cycle. The three life-stages of gnathiids are, according to Monod (1926): the first larvae (free living, period of dissemination); second larvae (parasite, period of accumulation); and adults (free living, period of reproduction). Monod (1926) could not explain the existence of a “giant segmented larva” in *P. formica* as Smith (1904) found for *Gnathia maxillaris*. He did agree with Smith’s (1904) observation that the pranizae, which will transform into males, had a dilated region behind the cephalosome where the mass of the future cephalosome forms (Fig. 3.1B). Monod (1926) gave detailed information on the parasitic phase of the larvae and listed all the different hosts on which he was able to induce larvae to feed. The author also described the eggs and embryonic development in the female larvae and adult females respectively. He reported the ovaries in the female larva as two long strands stretching from pereonite 3 to the posterior border of pereonite 6, dorsal to the digestive tube (Fig. 3.1C). As the eggs became bigger, they distended and finally filled the whole pereon. Monod (1926) found that when the larva moulted into a female, its intestinal reservoir was already empty and the developing embryos occupied the entire pereon. He described three embryonic development stages (Fig. 3.2A-D) for *P. formica* and stated that the embryos are contained in the uterus and are not free in the general pereon cavity.

In his article on the life cycle of *Gnathia maxillaris*, Mouchet (1928) proposed that *P. formica* has three larval stages in its life cycle like *G. maxillaris*. The existence of the three larval stages in *P. formica* was confirmed by Stoll (1962), who provided a detailed description of the life cycle of this species. She concluded that *P. formica* larvae hatched in the form of a zuphea (1 mm in length) which grow through three larval stages during the course of their larval life, to the size of a praniza of 3.5 mm. Each stage comprises a segmented phase (zuphea) and a phase of thoracic extension (praniza). The zuphea Z1

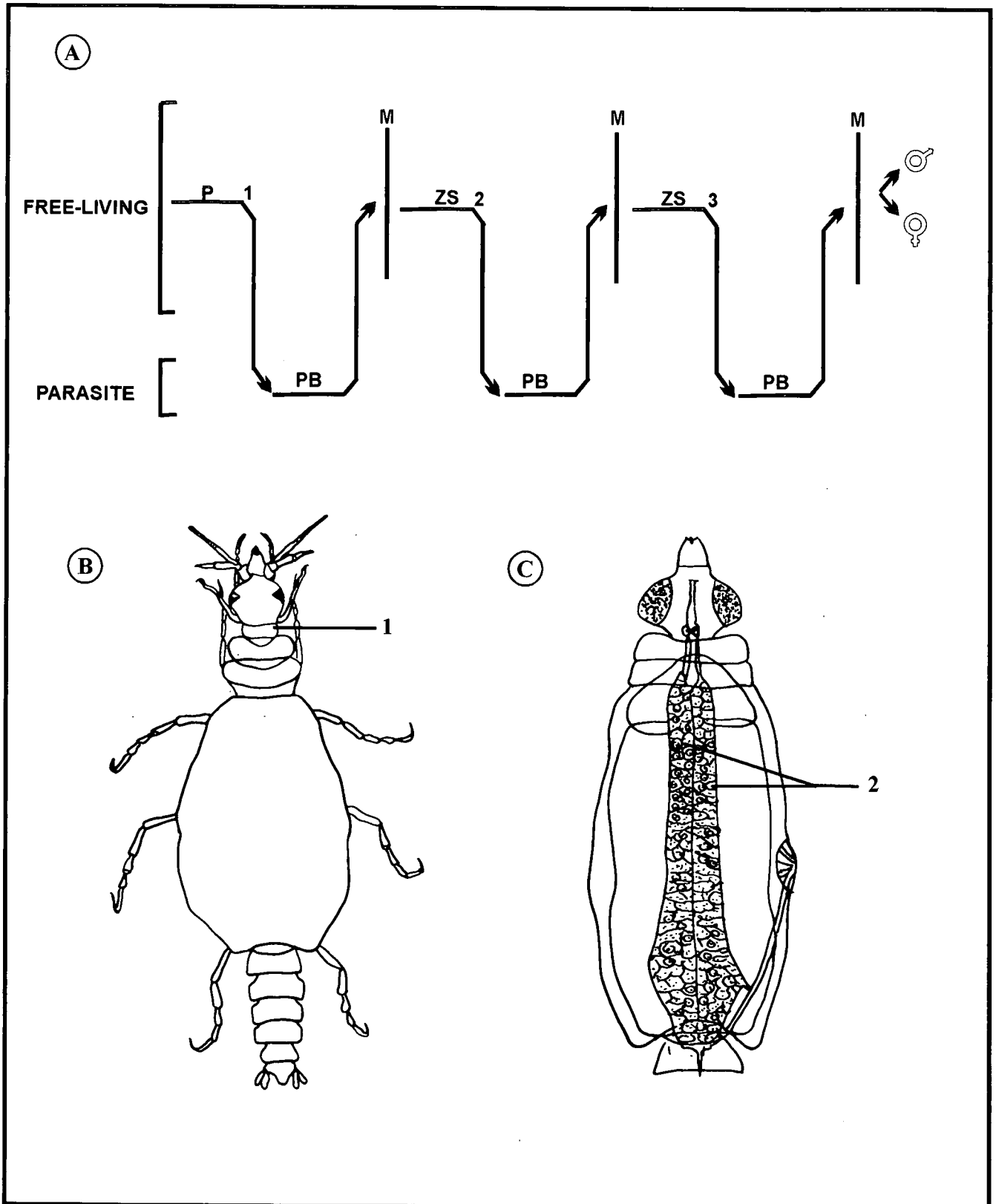


Figure 3.1. A. Schematic representation of the life cycle of *Gnathia maxillaris* (Montagu, 1804) by Mouchet (1928) (P- unfed larvae, PB- praniza larvae, ZS- zuphea larvae, M- moult, 1,2,3- stages) B. Male praniza larvae of *Paragnathia formica* (Hesse, 1864) with dilated region (1) behind cephalosome (redrawn from Monod 1926) C. Female praniza of *P. formica* with ovaries (2) visible as two long strands in pereon (redrawn from Monod 1926). Scale bars omitted by original author.

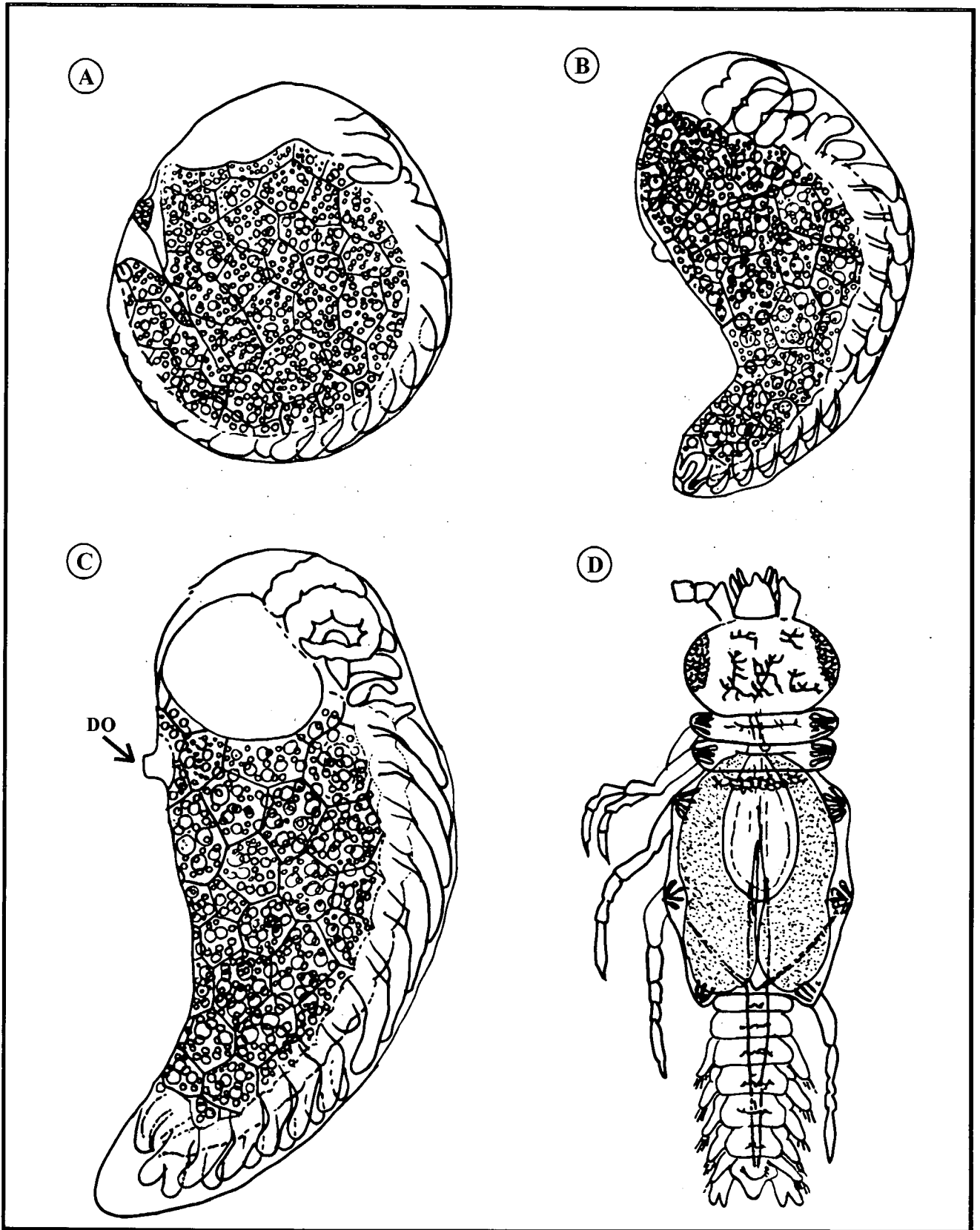


Figure 3.2. Different embryonic stages of *Paragnathia formica* (Hesse, 1864) according to Monod (1926) (redrawn from Monod 1926). **A.** Early first stage embryo **B.** Late first stage embryo (straightened) **C.** Second stage embryo with dorsal organ (DO) forming a conical knob **D.** Third stage embryo. Original author omitted scales.

(pullus), Z2 (second larval stage) and Z3 (third larval stage) live for some time in mud tunnels of 1 mm in diameter, until the depletion of their intestinal reserves (vitellus or blood) means that they fix onto a fish host. Feeding may keep the praniza away from the bank for some hours. After feeding they return to the bank, in the form of pranizae (P1, P2, and P3). Within a few weeks, praniza digest the ingested blood, and this passes from the intestine into the two caeca that dilate progressively. The P3 stage then undergoes a final metamorphosis into the adult form. Stoll (1962) gave detailed information about the time sequence of the feeding as well as the resting phases. She also highlighted the role that temperature plays in larval development, the seasonal rhythm of female metamorphosis, and the different cycles of males and females.

Amanieu (1963) studied the chronological evolution of a *P. formica* population by means of monthly collections over a two year period. He classified the specimens into seven different types and reported on the uninterrupted occurrence of males, time of year of female metamorphosis, length of gestation and season of hatching.

Work on the life cycle of *P. formica* was completed by Upton (1987a,b) who combined information from the literature with new observations and the results of an extensive field sampling program. He reported on the existence of an asynchronous male and female cycle, because of an almost total segregation in the settlement timing of female and male final stage larvae derived from the same generation. Upton (1987a) also distinguished 11 developmental phases amongst females (Fig. 3.3) and three developmental phases amongst males (Fig. 3.4).

To summarise the work done on the life cycle of *P. formica*, it is known that *P. formica* zuphea 1 larvae are released in autumn. These zuphea 1 stages attach to a suitable fish host on which they feed for 10-36 hours. The fully fed larvae (praniza 1) leave the fish and undergo a resting phase for six to 13 weeks. During this period digestion of the ingested blood or lymph fluid takes place and ends with the distinct two stage moult of isopods (anterior moult followed by a posterior moult). The resulting zuphea 2 feeds for an average of 13 hours to become a praniza 2. After a resting and digesting period of six to 12 weeks the praniza 2 moults into a zuphea 3 that feeds for an average of 48 hours. The resulting praniza 3 moults after seven to eight weeks into either an adult male or female. The females breeding phase varies from as little as four months to 10 months,

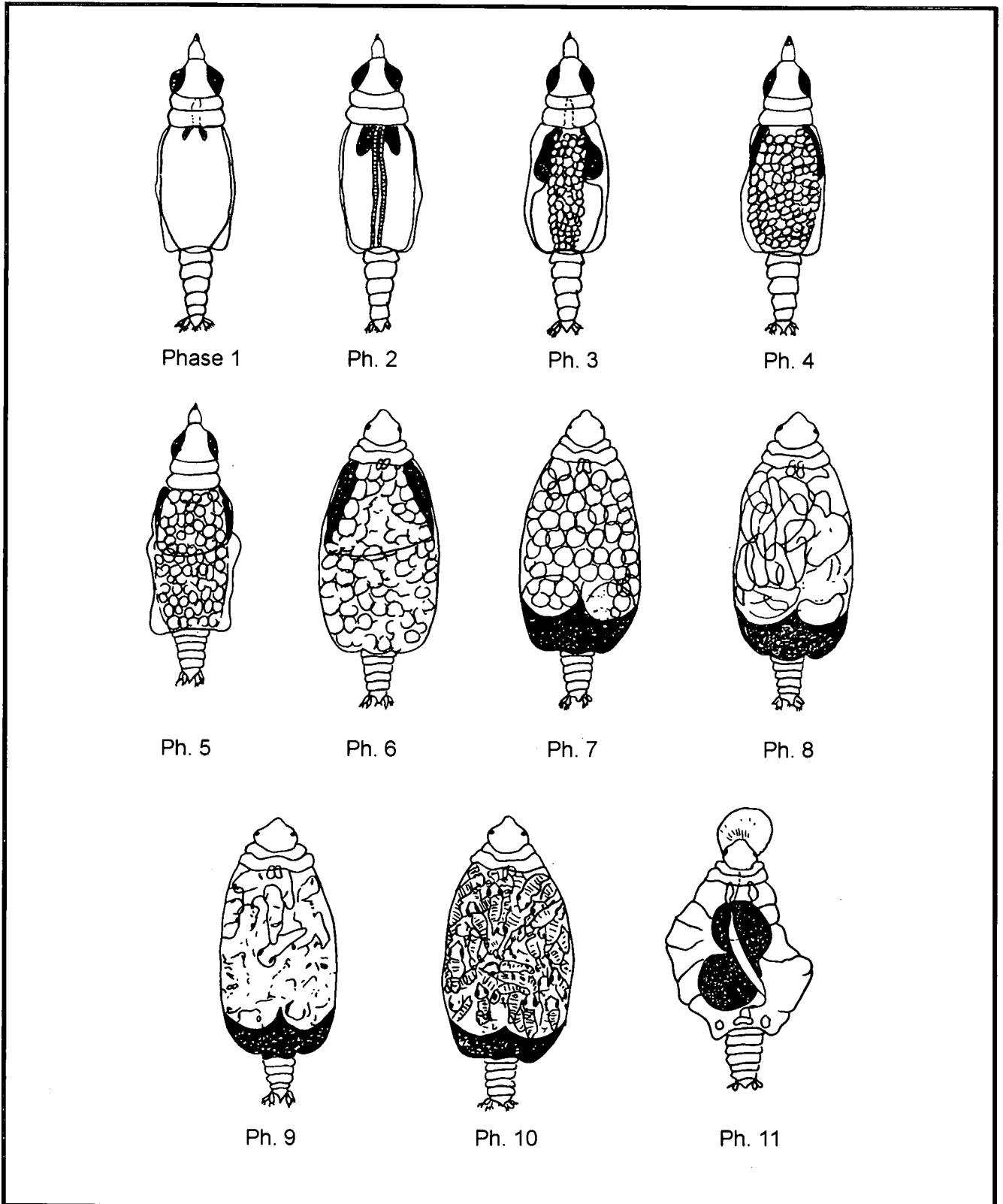


Figure 3.3. Female developmental phases of *Paragnathia formica* (Hesse, 1864) (redrawn from Upton 1987a) **Ph 1.** Recently settled P3 larva **Ph 2.** Eggs just visible in twin ovaries **Ph 3.** Ovaries partially obscure digestive caeca **Ph 4.** Ovaries spread across pereon **Ph 5.** Posterior moult completed **Ph 6.** Anterior moult completed **Ph 7.** Digestive caeca posterior to egg mass **Ph 8.** Embryos developing **Ph 9.** Larval eyes visible **Ph 10.** Fully developed larvae moving around inside bloated mother **Ph 11.** Female appears empty and crumpled after release of larvae

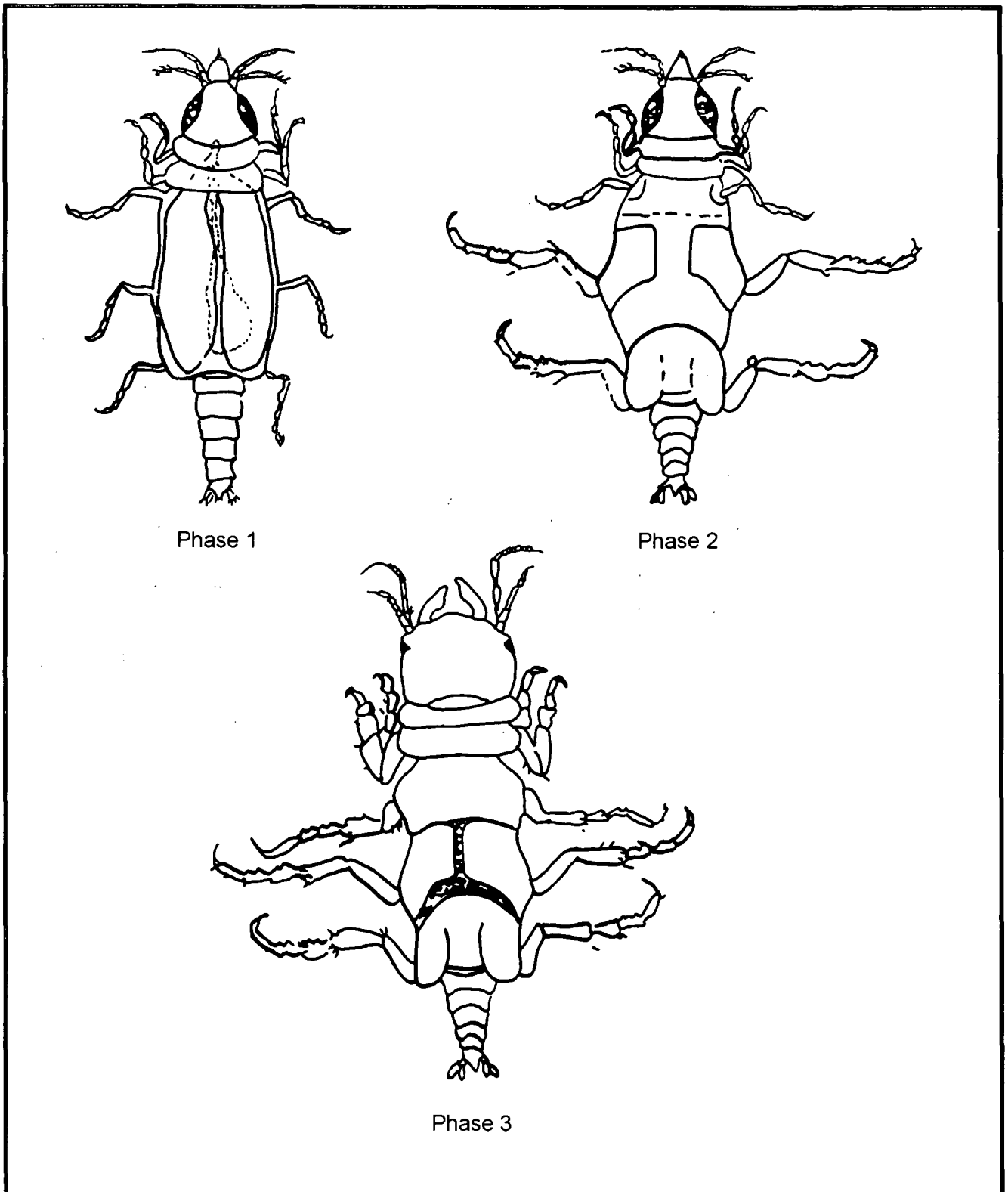


Figure 3.4. Male developmental phases of *Paragnathia formica* (Hesse, 1864) according to Upton (1987a) (redrawn from Upton 1987a) **Phase 1.** Recently settled P3 larvae **Phase 2.** Posterior moult completed **Phase 3.** Anterior moult completed. Scale bars omitted by original author.

with a total life span (Z1 to spent female) of one year. The total life span of males (Z1 to adult male) may be more than two years, thus overlapping successive generations. During the breeding season males are found with "harems" of up to 25 females. Females produce as many as 130 larvae that they release through a jugular opening formed by the opening of the gnathopods (pylopods).

The life cycle of *Caecognathia calva* (Vanhöffen, 1914)

A detailed description of the life history and morphology of the postembryonic stages of *C. calva* was presented by Wägele (1987, 1988). Wägele (1988) obtained the data on the life cycle of this species by keeping the live animals in the laboratory. They were raised in artificial sponges and filtered artificial seawater at -1°C . He determined the length frequencies of each stage by using measurements of the different resting larvae and adults collected from sponges. As in the case of the other species of gnathiids, three larval instars were discerned, each instar consisting of a zuphea (unfed) and a praniza (fed) stage. Wägele (1988) concluded that the first larvae (zuphea 1) are active swimming stages that search for the fish hosts on which they feed. Sucking the blood of the host (in this case Antarctic benthic fishes) led to an increase in length by the extension of the elastic cuticle of the central pereonite. The fully fed larvae (praniza 1) left the fish hosts and remained cryptic for an unknown period before moulting into a second feeding stage (zuphea 2). Wägele (1988) did not determine the exact length of each feeding or resting phase, but found that praniza 3 rested for up to two years. This process was repeated twice, and, upon completion of feeding, the third pranizae penetrated small hexactinellid sponges, where they moulted into mature females or immature males (Fig. 3.5). The presence of an immature male in the life cycle of *C. calva* is the only record of such a stage in gnathiids. According to Wägele (1987), the transformation of the praniza 3 into an immature male and the moulting of this into a mature male was observed in his aquarium. Although moulting could occur at any time of the year, an indication of seasonal reproductive activity was observed since ovigerous females had to live for more than a year before releasing an average of 129 larvae between February and May. The same harem phenomenon as described for *Paragnathia formica* was found in *C. calva*. Wägele (1988) found that a single male could guard up to 43 females, or immature specimens (an average of eight), and could live for more than two years. Combining all this data, Wägele (1988) calculated that the life cycle of *C. calva* might take four to five

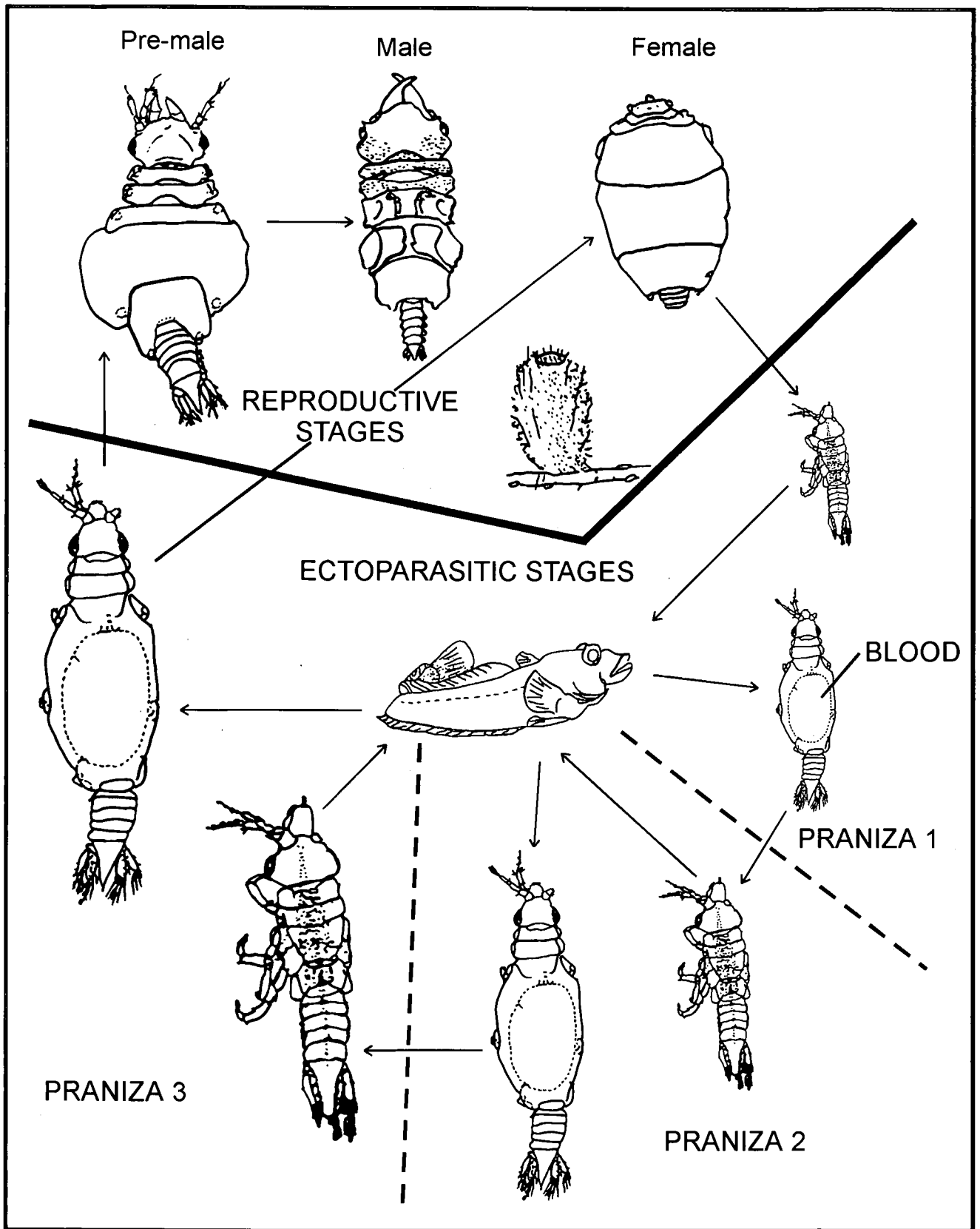


Figure 3.5. Schematic representation of the life-cycle of *Caecognathia calva* (Vanhoffen, 1914) (redrawn from Wägele 1988). Not drawn to scale

years to complete, that is, one year for embryonic development and possibly three to four years for the larval stages. He suggested that the retardation of the life cycle of this Arctic species is a product of the low temperatures in which they are found.

Although this account of the life cycle of *C. calva* by Wägele (1987, 1988) is very detailed, specifically regarding the morphology of the postembryonic stages, some gaps are evident due to the length of this cycle. These include data on the parasitic phase, the length of the digestion period of the different larval stages, and information on the embryonic development.

The life cycle of *Caecognathia abyssorum* (Sars, 1872)

Klitgaard (1991) used the measurements of larval and adult stages, collected during three different times of the year (May/June 1988-89, July/August 1987-89 and November 1988-89), to reconstruct the life cycle of *C. abyssorum*. All stages were collected from a variety of demosponges. Klitgaard's investigation indicated the existence of three larval stages, again each stage consisting of a zuphea (unfed) and praniza (fed) stage (Fig. 3.6). She assumed that these larvae were temporary ectoparasites of fish, because of their morphological resemblance to the larvae of other gnathiid species. This assumption was, however, not confirmed. Ovigerous, as well as spent females were found in only the May/June samples, thereby suggesting that embryonic development must have been in progress during that period. She also found first stage larvae (praniza 1) mainly in the May/June samples, with stage 2 and 3 larvae and males present in all three sampling periods. Klitgaard (1991) concluded that since the natural habitat of *C. abyssorum* shows a temperature variation only between 2 and 7°C throughout the year, continuous development without any seasonal interruption might be expected. Reproductive activity, however, did seem seasonal, starting in spring (March/April) and ending in about July/August. She also proposed that, although it was impossible to determine the life span of the males and females, the results pointed to an asynchronous male and female cycle, as described for *Paragnathia formica* (Upton 1987a). It seemed likely that the females complete their cycle in a year, while the males live for up to two years. Klitgaard (1991) suggested that the "harem" phenomenon described for *Paragnathia formica* was also found in members of *C. abyssorum*. A second investigation by Klitgaard (1997), on the reproductive biology of *C. abyssorum* and *C. robusta*, showed that the males are

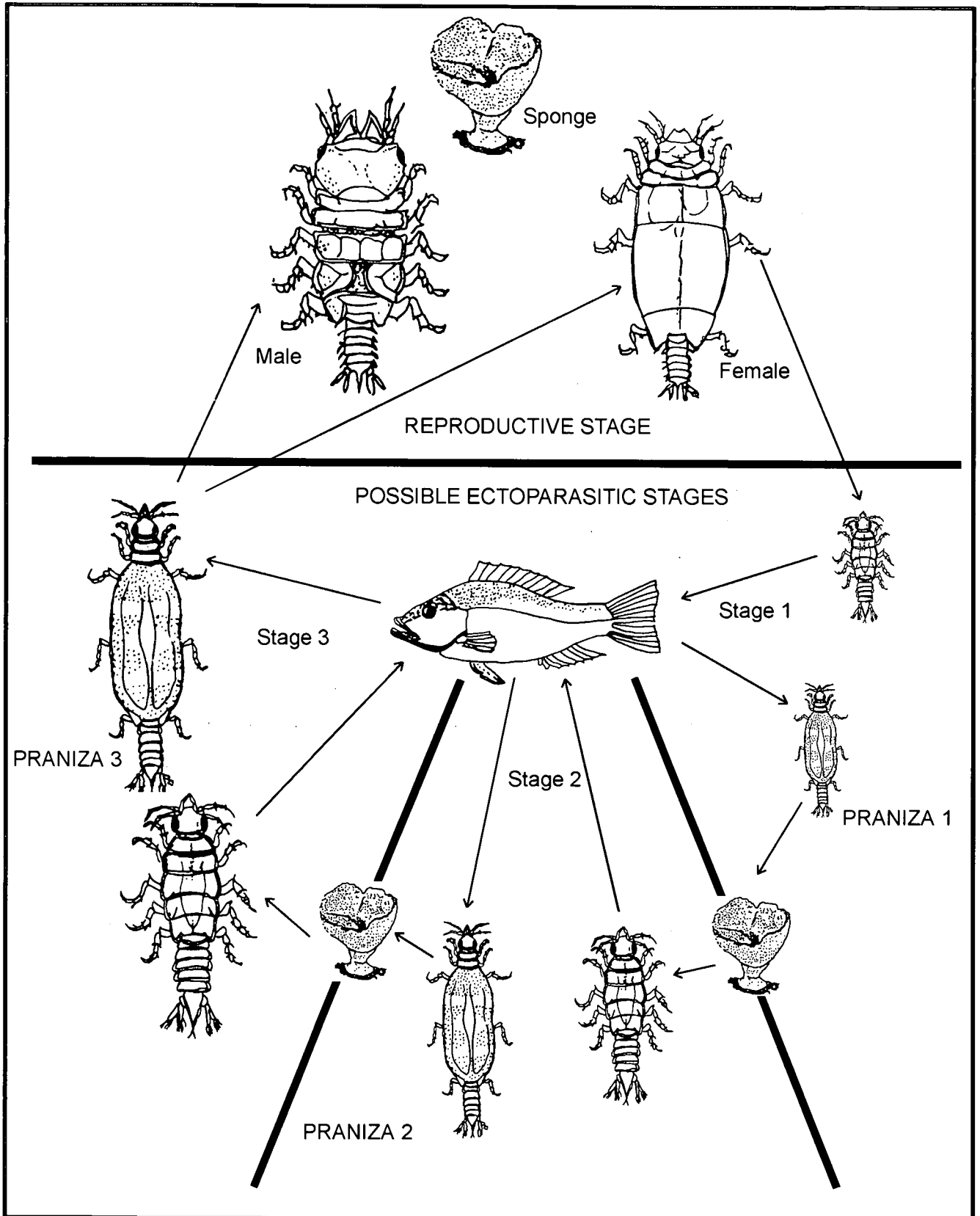


Figure 3.6. Schematic representation of the life-cycle of *Caecognathia abyssorum* (Sars, 1872) (redrawn from Klitgaard 1991). Not drawn to scale. The fish host is not known

primarily found with only one female and not with "harems" as previously suggested. This second study (Klitgaard 1997) was also done by assessing the gnathiid populations collected from demosponges. On the examination of seven females, Klitgaard (1997) found a range of 16 to 58 embryos per female (average of 33). This is far fewer than the figures for *Gnathia piscivora* (up to 200 embryos per female), *Paragnathia formica* (up to 130), *Caecognathia calva* (average of 129) and *C. robusta* (average of 110) females. On comparing the average length of a newly released larva in relation to the average length of the female, Klitgaard (1997) found that a female *C. abyssorum* produces fewer but bigger larvae than a female *C. robusta*, thus compensating for the low number of larvae.

This work by Klitgaard (1991, 1997) on the biology of *C. abyssorum* provided much information on the resting larva and adult stages of this species. The possibility of reconstructing the life cycle by using methods other than keeping live animals and cultivating them through their life stages was also introduced. Unfortunately, this method (length frequencies) did not allow collection of data on the parasitic stages of this species or the specific duration of the life cycle.

The life cycle of *Elaphognathia cornigera* (Nunomura, 1992)

In a series of papers, Tanaka and Aoki (1998, 1999, 2000), gave very valuable information on the biology and ecology of the intertidal species *Elaphognathia cornigera* found on the rocky shores of Nabeta Bay, Japan. Their interest in this gnathiid species started, as in the case with Klitgaard (1991), through the initial study of the crustacean infauna of a demosponge species.

In the first paper of this series, Tanaka and Aoki (1998) proposed a possible life cycle for a gnathiid species found inhabiting the demosponge, *Halichondria okadai*. This gnathiid was subsequently identified as *E. cornigera* (see Tanaka and Aoki 1999). The proposed life cycle was based on the presence of three peaks in the size distribution of the zuphea and praniza larvae and a single one for the adults, all found in the sponges. This led them to conclude that *E. cornigera* also has three larval stages and one adult stage (male and female) as described for other gnathiid species (Fig. 3.7). They also assumed that this species has fish parasitic larval stages, and the intertidal goby *Chasmichthys dolichognathus* was considered as the main host. In the second paper (Tanaka and Aoki 1999), the authors concentrated on the spatial distribution patterns of *E. cornigera* in the

intertidal zone. Their survey showed that this species occurred throughout the tidal zone and its distribution could be linked to its preferred hiding place, the sponge *H. okadai*. Tanaka and Aoki (1999) could not find a particular pattern in the vertical distribution of the adults, but did find that the larvae were more concentrated at mid-tide level. A possible reason for this might be the distribution of the fish host and the fact that the mid-tide level is where the water surface crosses over most frequently. They also found a correlation between the density of the adult males and the size of the sponge colony as well as an equal distribution of males throughout the sponge colony. Adults were non-feeding, thus food availability did not play any role in their distribution. Tanaka and Aoki (1999) postulated that the intraspecific competition between the males might cause this equal distribution throughout the sponge. No such correlation was found amongst females and larvae. Larvae were also found in small sponge colonies in contrast to the adults who mainly occupied large colonies (Tanaka and Aoki 1999).

In their most recent paper, Tanaka and Aoki (2000), investigated the seasonal traits of reproduction of *E. cornigera*. They were able to distinguish six phases for adult females based on the development of the eggs and embryos in the females. These phases consisted of (1) females before ovulation; (2) females after ovulation; (3) females with embryos with eyes in irregular shape; (4) females with embryos with distinguishable cephalon, thorax and abdomen; (5) females carrying fully developed larvae moving in the brood pouch; and (6) empty females after release of larvae. The authors also found a positive correlation between the body length and brood sizes of the females. Using the peaks in female numbers during the year, Tanaka and Aoki (2000), postulated that there exists between three and four generations of females per year with a life span of two months (from embryo through three larval stages to phase 6 female). They attributed the much shorter life span of *E. cornigera* in comparison to the yearly life cycle of the European species, *P. formica*, and the two year cycle of the Arctic species, *C. calva* to the warmer water temperature found in Japan.

This in-depth study by Tanaka and Aoki (1998, 1999, 2000) on the biology and ecology of *E. cornigera* is the first on a species of the genus *Elaphognathia*. It also contributes extensively to the knowledge of gnathiid biology in general, but unfortunately lacks any information on the parasitic stages of this species.

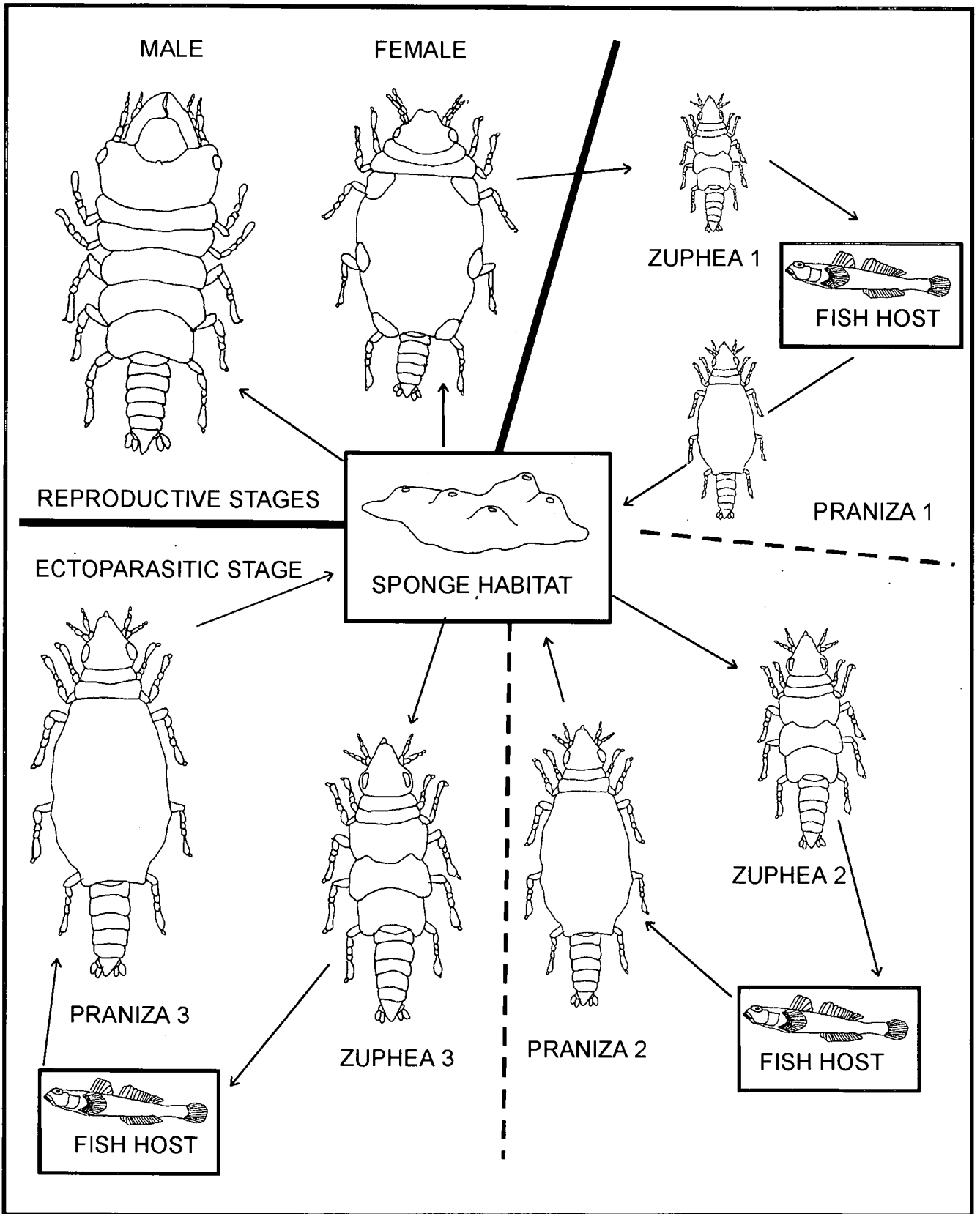


Figure 3.7. Schematic representation of the possible life-cycle of *Elaphognathia cornigera* (Nunomura, 1992) (redrawn from Tanaka and Aoki 1998). Not drawn to scale

3.2 Redescription of the adult female of *Gnathia africana* Barnard, 1914

Barnard (1914a) was not able to collect females to include in his original description of *Gnathia africana*, but was able to find them at a later stage and briefly described them in Barnard (1914b). As in the case of the males and larvae, Barnard's description did not provide detailed information and illustrations (see Figs. 3.8A,B). This problem is evident in the fact that the illustration of a *G. africana* female by Kensley (1978), in his book on isopods from South Africa, is actually that of a larva (Fig. 3.8C). The specimen he drew was most probably a female larva with eggs and therefore mistaken for an adult female. Smit *et al.* (1999a) were also not able to collect females and therefore only provided a redescription of the male and pranzia larva of *G. africana*.

Since the taxonomy of gnathiids is based solely on the morphology of the adult male, most authors either ignored the females in their species descriptions or just described their basic morphology in a few sentences. Fortunately there are some very good descriptions of females (see Monod 1928, Wägele 1987, Brandt and Wägele 1991, Müller 1993a), but only for a very small percentage of the known gnathiid species (less than 5%). The aim of this redescription is to provide a detailed record of the female of *G. africana*. This will make it possible to identify them in the absence of the males, to establish a format that can be used in future for the description of female gnathiids of new species, as well as for the redescrptions of females of known species.

Adult female

Figs. 3.9 -3.13

Description: Total length of material examined: 3.2-4.3 mm (3.74 ± 0.3 mm, $n = 11$).

Cephalosome. Broadened, short. Rectangular, 1.5 times as wide as long; two to four pairs of short simple setae on dorsal cephalosome, median area of posterior margin slightly concave (Figs. 3.9A,B, 3.12A,B). Well developed oval-shaped, bulbous, compound eyes on lateral margin of cephalosome, length of eye two thirds of cephalosome (Fig. 3.12D). No paraocular ornamentation, only three to five short simple setae.

Frontal border. Broadly rounded, produced, with four short simple setae on mid dorsal area (Fig. 3.12C).

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles increasing in length distally with third article as long as first and second articles combined. A single feather-like seta and few short simple setae on distal end of articles 1 and 2, two feather-like setae and two to five short simple setae on article 2. Five to nine short simple setae on article 3. Flagellum with five articles, article 2 largest, articles 3 and 4 with one aesthetasc seta each, article 5 terminating in one aesthetasc and three simple setae (Figs. 3.9C, 3.12E). Antenna 2 with five peduncle articles, article 5 largest, a single short simple seta on article 2 and three to seven short simple setae and a single feather-like seta on distal ends of articles 4 and 5 respectively. Articles 4 and 5 covered with pectinate scales. Flagellum with seven articles, article 1 largest, article 7 terminating in three to four simple setae (Fig. 3.9D).

Mandible. Reduced.

Maxilliped. Consists of basis, oostegite and four articulated palp (Figs. 3.10A, 3.13A). Endite short, setose, not reaching article 2 of palp. Lateral margins of basis fringed with three long plumose setae. Palp bearing plumose setae on lateral margins in order of 3-8-5-5, article 1 of palp with single short simple and a single long simple seta on the mesial border (Fig. 3.10A). Distal article of palp with four to five short simple setae (Fig. 3.13B). Oostegite broader and almost as long as palp. Mesial borders of basis, palp and oostegite densely setose.

Pylopod. Four articles, articles 1 and 2 fused. Article 1 broad, robust, curved anteriorly, with a single simple seta mid dorsally and a short curved spine dorso-laterally (Figs. 3.10B, 3.13C). Article 2 with two to six four simple setae distally. Article 3 with two to four simple setae distally (Fig. 3.13D). Article 4 small with one to two simple setae. Surface of articles 2 and 3 covered with pectinate scales and lateral borders with short hair-like simple setae. Oval-shaped oostegite, 2 times longer than broad, covers maxillipedes ventrally, not surpassing frontal border (Fig. 3.13C). Three to five short simple setae on posterior surface of oostegite, lateral and anterior borders with short hair-like simple setae.

Maxilla. Reduced.

Pereon. Swollen round, sutures between pereonites 5-7. One and a half times as long as wide, wider than cephalosome (Figs. 3.9A, 3.12F), short simple setae on lateral areas. Pereonites 5-7 form thin plate-like oostegites, enclose brood pouch, oostegites overlapping. Pereonite 7 dorsally visible, small with rounded posterior margin,

overlapping first pleonite. Ventral area of pereonite 6 with slit which appears to be genital opening (Fig. 3.13E).

Pleon. Pleon and pleotelson less than a quarter of total length (Fig. 3.9A). Five subequal pleonites dorsally visible, epimera not distinct, short hair-like setae and short setose setae randomly distributed on pleonites.

Pleotelson. Triangular, base as wide as or wider than length, lateral margins straight, dorsal surface with two pairs of simple setae and pectinate scales, distal apex terminating in pair of long simple setae (Figs. 3.9F, 3.13F).

Pereopods. Pereopod 2 basis elongated oval shaped with two to three feather-like setae and short simple setae anteriorly, two to five posterior simple setae (Fig. 3.11). Ischium two thirds length of basis, three to five anterior short simple setae, three short simple setae posteriorly. Merus half the length of ischium with anterior bulbous protrusion, three simple setae on bulbous protrusion, posterior margin with two to three tooth-shaped tubercles as well as two short and a single long simple seta. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion, posterior margin with eight to ten tooth-shaped tubercles, simple setae and a single feather-like seta. Propodus about twice the length of carpus, tooth-shaped tubercles on posterior margin, two elongated denticulated compound spines ending in sharp points situated on middle and distal part of posterior margin respectively, single simple seta and one feather-like seta antero-distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 similar to pereopod 2 in basic form, differ in setation, shape and number of tubercles (Fig. 3.11). Pereopod 6 with a single strong denticulated compound spine on posterior bulbous protrusion of merus. Dorsal surface of ischium, merus, carpus and propodus of all pereopods covered with pectinate scales (not shown in illustrations).

Pleopod. Endopod slightly shorter and wider than exopod. Both fringed distally with seven to eight short plumose setae (Fig. 3.9E). No coupling hooks visible. Sympodite with retinacula, single simple seta on lateral margin.

Uropod. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, both with long simple setae, pectinate scales on dorsal area of uropods (Figs. 3.9F, 3.13F). Endopod with three feather-like setae and three simple setae on dorsal surface. A pair of short simple setae on uropodal basis.

Remarks: The lack of detailed descriptions of females of other gnathiids species makes it difficult to provide a comprehensive comparison of this species with those of already described ones. For example, no detailed description exists for any of the females of the gnathiids described from Australia, an area with species that seems closely related with the males described from South Africa (Smit and Van As 2000).

According to Monod (1926) the flagellum of antenna 2 of *Paragnathia formica* females from France, consists of eight articles and the pylopod of six articles, this was also found to be true for *P. formica* females collected in Wales (Smit unpublished data). These characteristics, as well as the shape of the frontal border, clearly separate it from *G. africana* (antenna 2 flagellum with seven articles and pylopod with four articles). According to Brandt and Wägele (1991) the flagellum of antenna 1 of the females of a species in another genus, *Euneognathia gigas* (Beddard, 1886), also consists of eight articles as in the case of *P. formica*, but the pylopod of only four. The pleotelson of the female *E. gigas* is almost twice as long as wide in comparison to those of *G. africana* females that are almost as broad as long. *Gnathia africana* females can also be distinguished from those of *Caecognathia calva* by the shape of the frontal border, the number and basic form of the pylopod articles (two articles with first one broadened in *C. calva*) and the presence of plumose uropodal setae (see Wägele 1987). The pylopods of *Caecognathia polaris* (Hodgson, 1902) females are very similar to those of *G. africana*, but differ in the shape of the cephalosome, frontal border, pleotelson and the presence of long plumose setae on the distal margins of the uropods.

The males of *Gnathia firingae* Müller, 1991 are very similar to *G. africana* males (Müller 1991, Smit *et al.* 1999a). The females also show remarkable similarities, specifically in the presence of a produced rounded frontal border and the shape of the pleotelson. Unfortunately the description of the *G. firingae* female by Müller (1991) are not sufficiently detailed and therefore a comprehensive comparison is impossible.

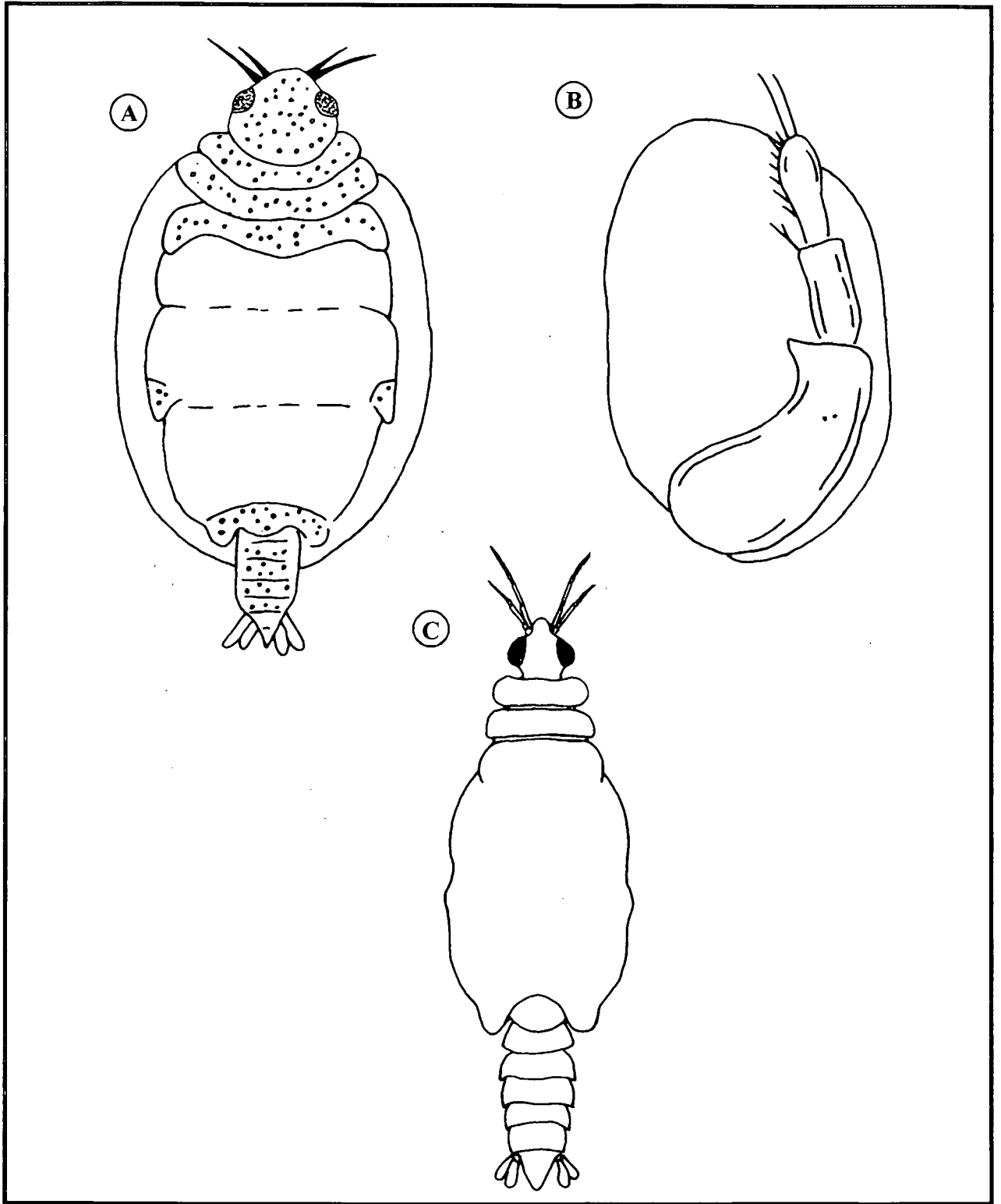


Figure 3.8. A. *Gnathia africana* Barnard, 1914 female. B. Pylopod of *G. africana* female (A and B redrawn from Barnard 1914b). C. Kensley's (1978) illustration of a *G. africana* female. Scale bars omitted by original authors.

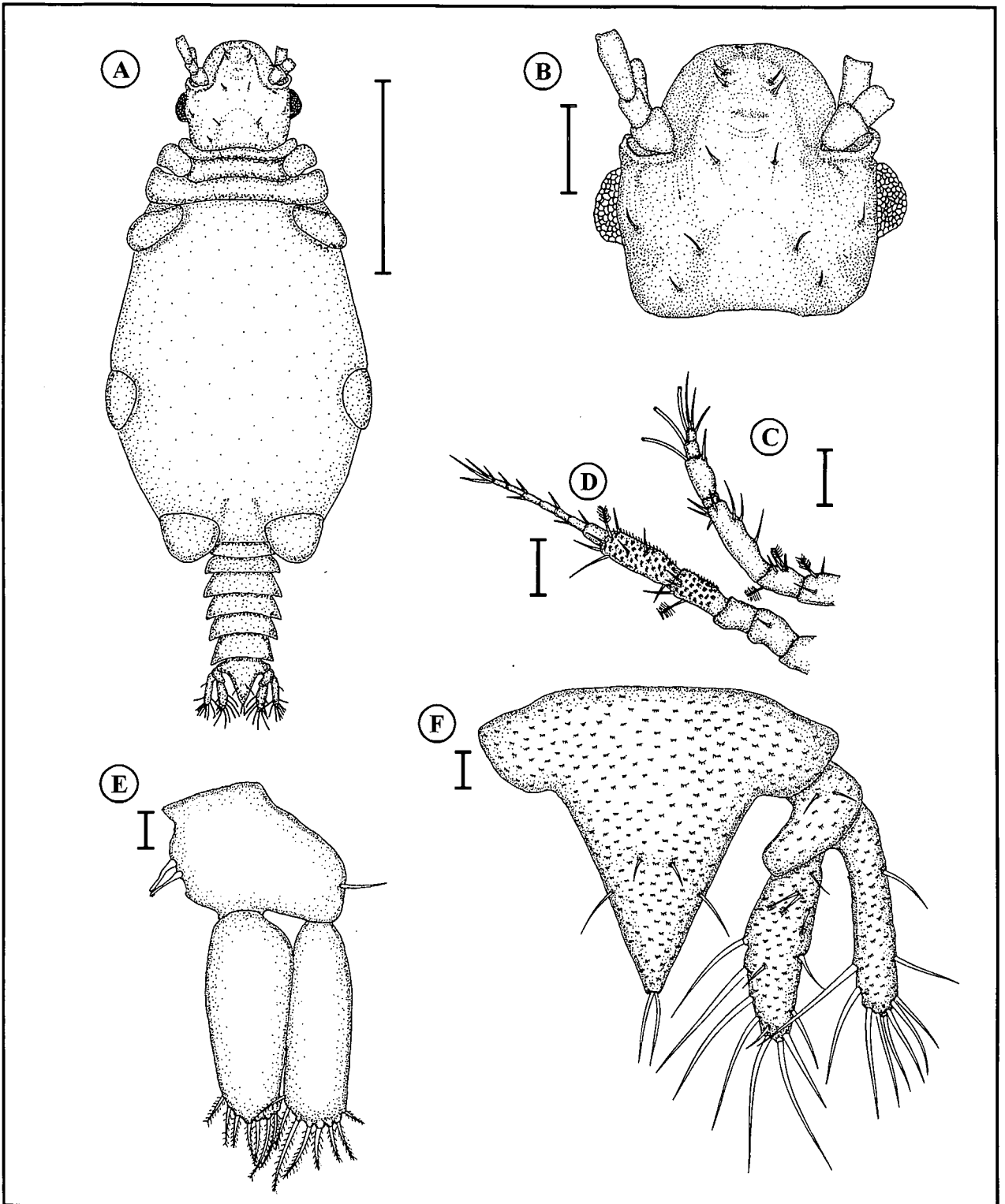


Figure 3.9. Microscope projection drawings of a female *Gnathia africana* Barnard, 1914
A. Full length dorsal view B. Dorsal cephalosome C. Antenna 1 D. Antenna 2 E. Pleopod 1
F. Pleotelson and right uropod. Scale bars: A = 1mm, B = 250 μ m, C-F = 100 μ m

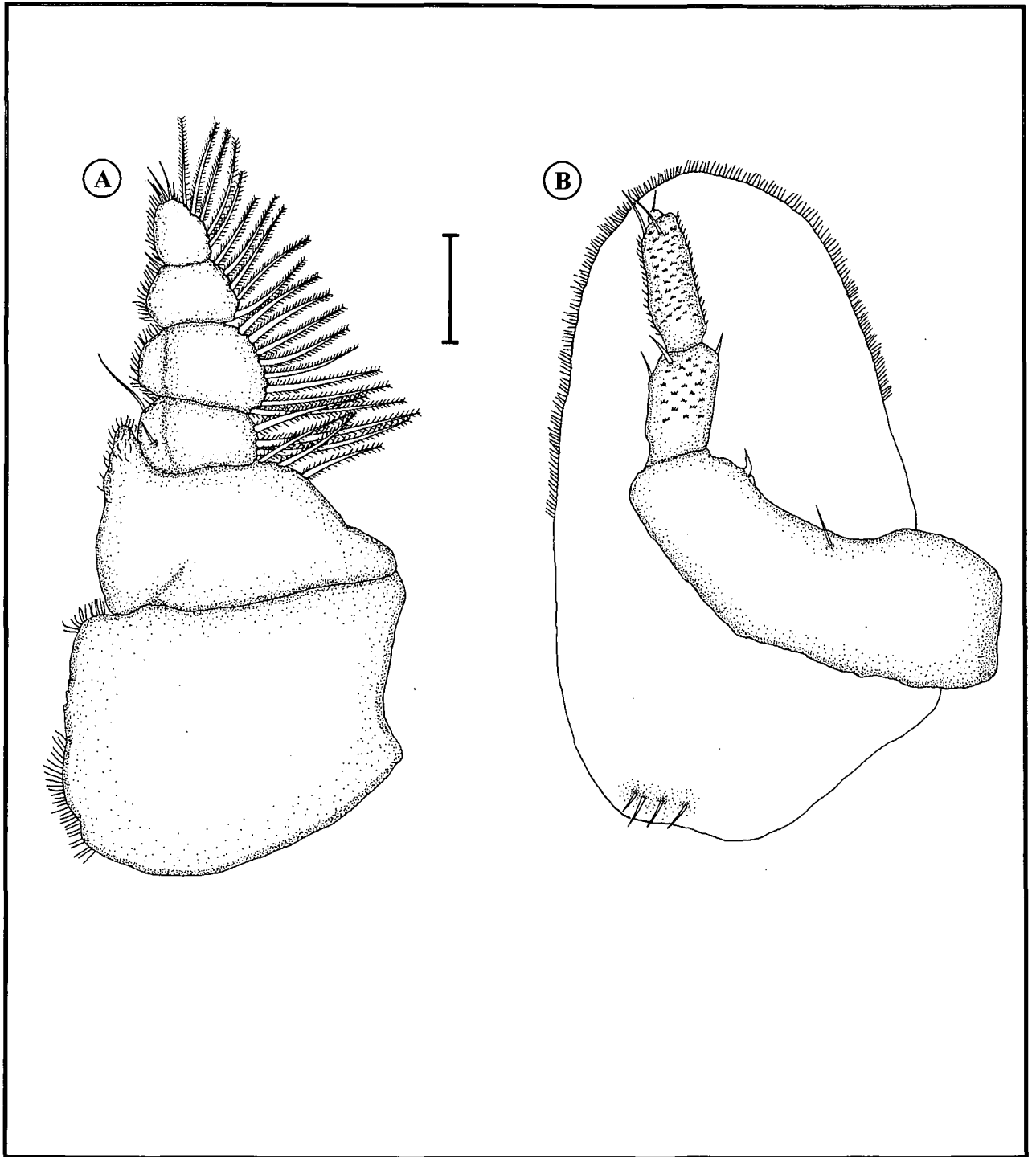


Figure 3.10. Microscope projection drawings of the cephalosome appendages of a female *Gnathia africana* Barnard, 1914. **A.** Left maxilliped. **B.** Left pylopod. Scale bar: 100 μ m.

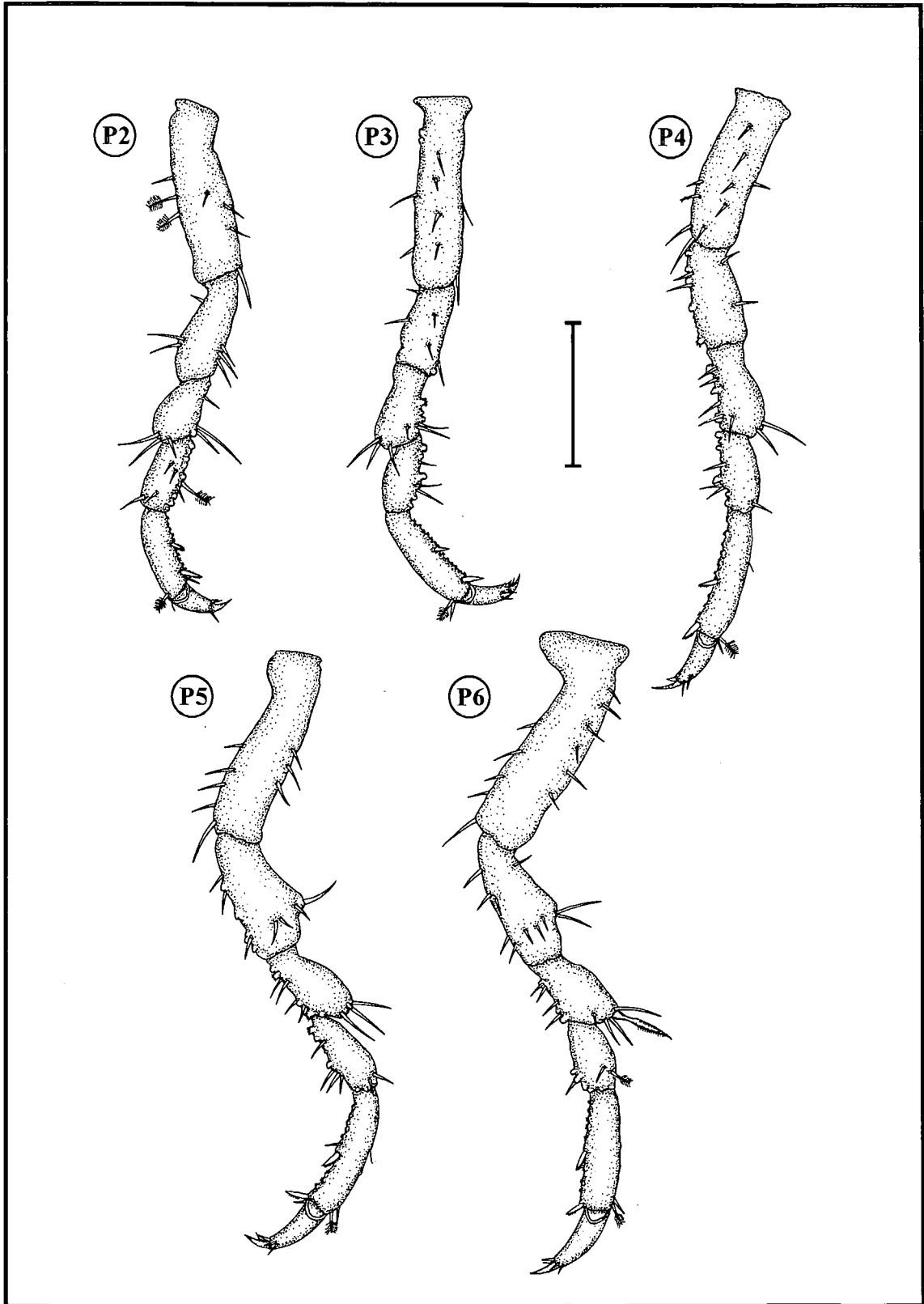


Figure 3.11. Microscope projection drawings of pereopods 2 to 6 (P2-P6) of a female *Gnathia africana* Barnard, 1914. Scale bar: 200 μ m

Figure 3.12

Scanning electron micrographs of a *Gnathia africana* Barnard, 1914 female (female larva collected at Jeffreys Bay and moulted into an adult female in the laboratory)

- A. Anterio-dorsal view of cephalosome
- B. Dorsal view of cephalosome and frontal border
- C. Dorsal view of frontal border
- D. Lateral view of cephalosome
- E. Distal flagellum articles of Antenna 1 with aesthetasc setae
- F. Dorsal view of pereonites 1 to 4

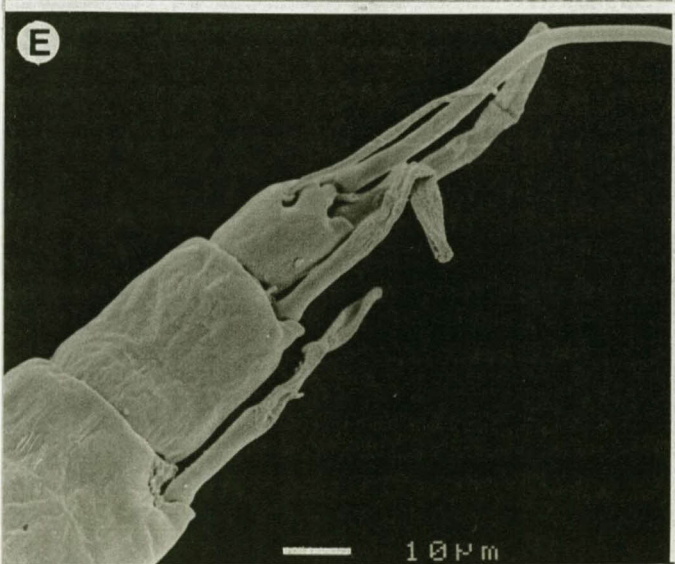
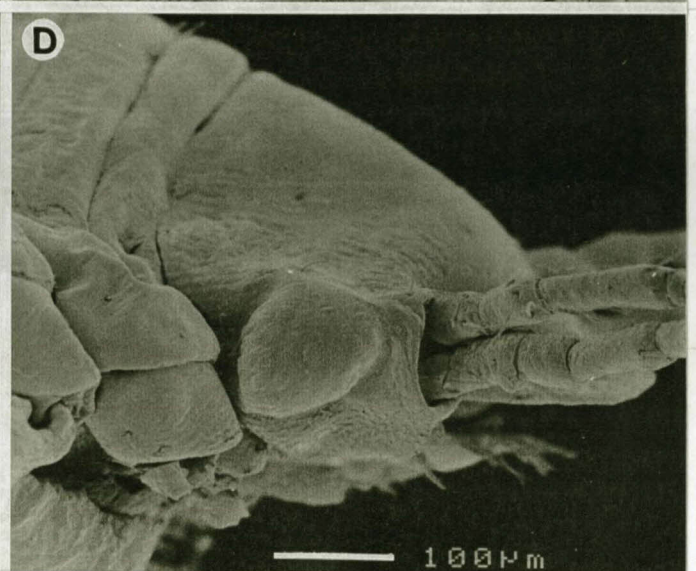
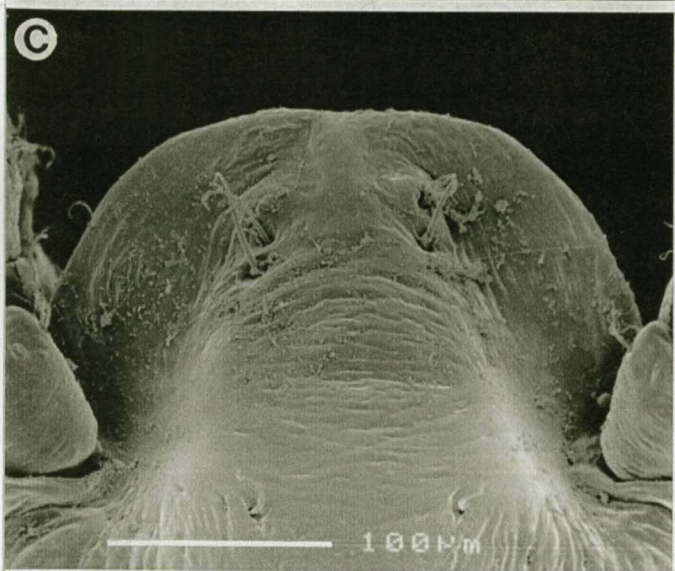
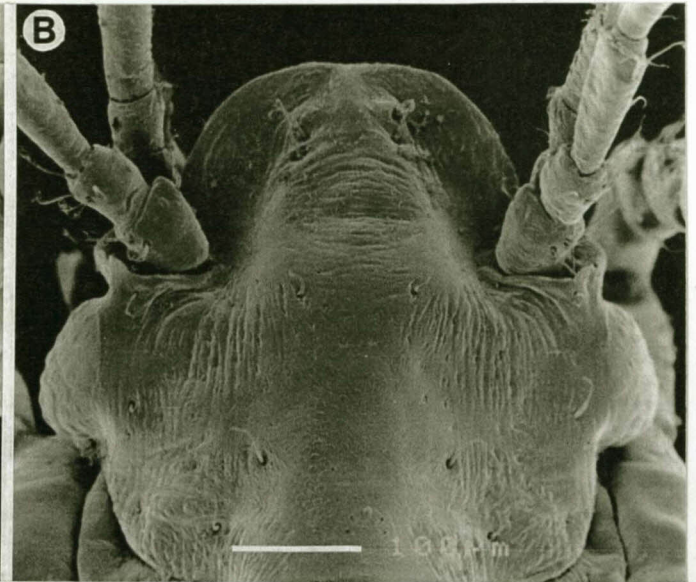
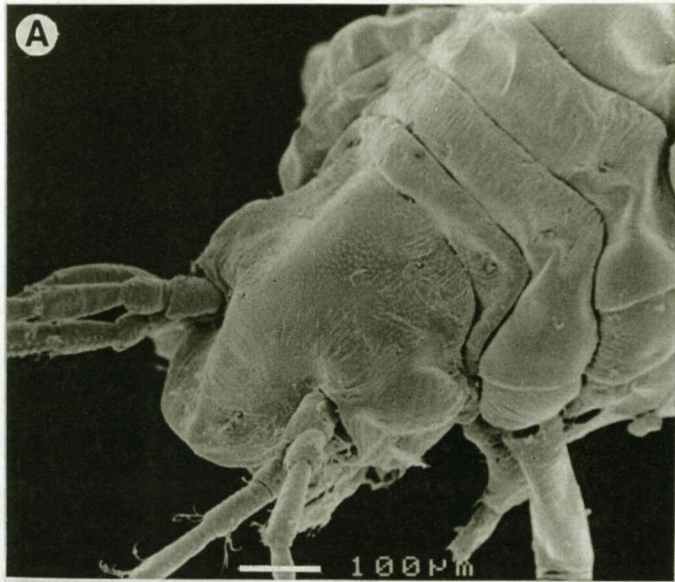
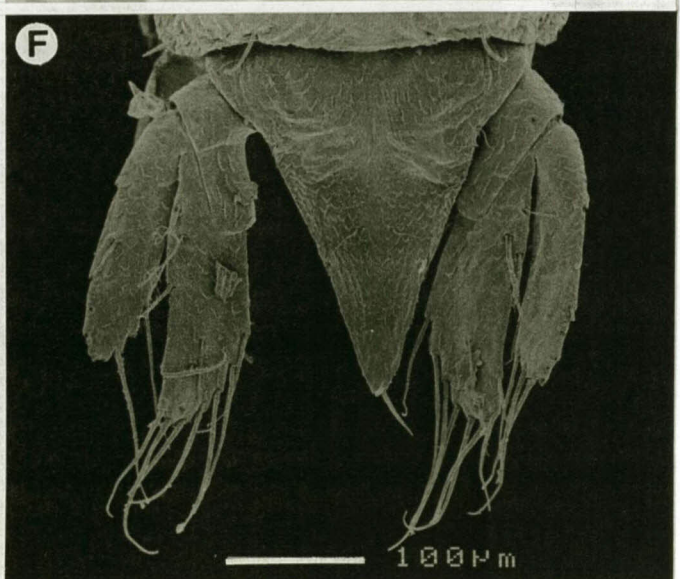
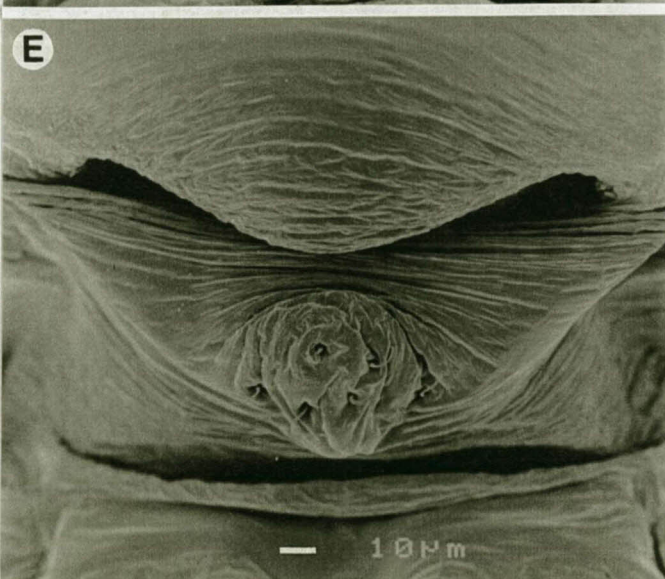
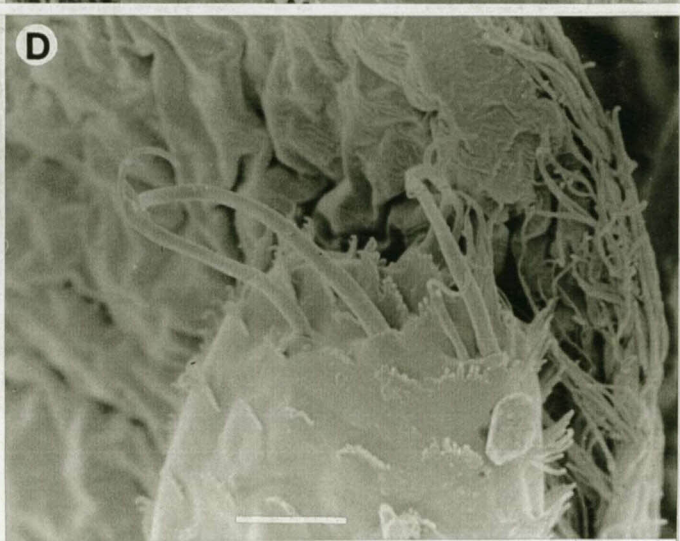
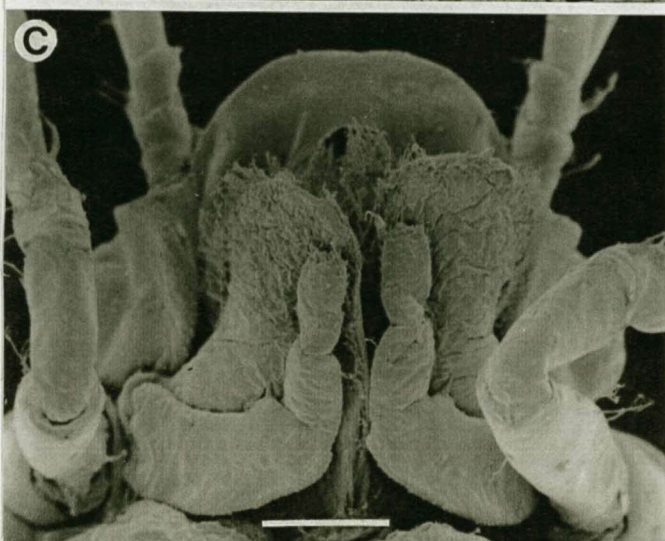
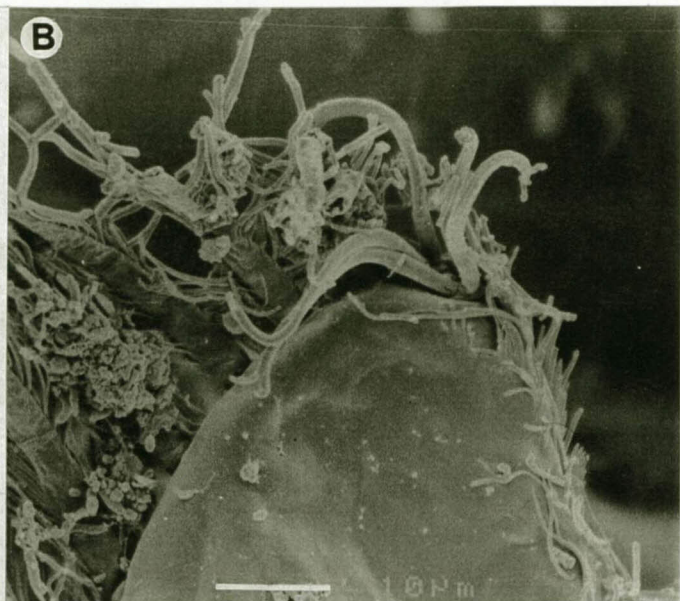
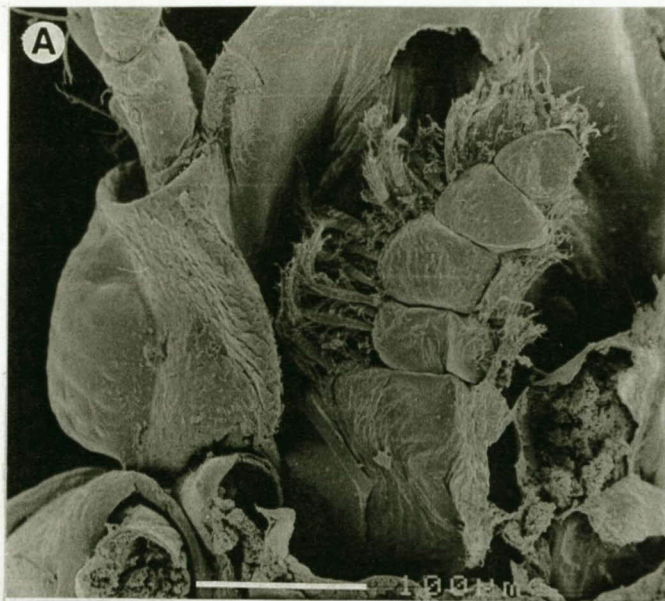


Figure 3.13

Scanning electron micrographs of a *Gnathia africana* Barnard, 1914 female (female larva collected at Jeffreys Bay and moulted into an adult female in the laboratory)

- A. Ventral view of right maxilliped
- B. Setae on distal article of right maxilliped
- C. Ventral view of pylopods
- D. Setae on distal article of right pylopod
- E. Ventral view of genital opening on pereonite 6
- F. Dorsal view of pleotelson and uropods with some setae missing

Scale bars: C = 100 μm ; D = 10 μm



3.3 The life cycle of *Gnathia africana* Barnard, 1914: field and laboratory experiments

Gnathia africana was the first gnathiid species to be described from southern Africa (Barnard 1914a,b). This species is very common along the South African west and south coasts where the parasitic larvae feed on the blood and lymph fluid of residential intertidal fishes (Smit and Davies 1999, Smit *et al.* 1999a). The resting larvae and adult stages are found in a variety of sponges, tunicates and tubes of serpulid worms (Smit *et al.* 1999a, Barnard 1914b). The abundance of these gnathiids in the intertidal zone and the availability of their fish hosts make them perfect research specimens, especially for life cycle experiments. Research on the life cycle of *G. africana* began with work done for the author's MSc. (Smit 1997). During that research, the life cycle of *G. africana* was reconstructed using the same methods as Klitgaard (1991, 1997) for *Caecognathia abyssorum*. To provide background information for the present study, knowledge of the life cycle of *G. africana* from Smit (1997) is summarised below, after which extensive information on the laboratory experiments will be presented.

Preliminary study on the life cycle of *Gnathia africana* Barnard, 1914 (from Smit 1997)

To establish the number of larval stages, the total length of each resting larva (praniza and zuphea) from two populations were measured to the closest 0.1mm. The Mc Dougall's Bay data was collected during September 1995 (spring) and Jeffreys Bay material in December 1996 (summer). All these larvae were collected from sponges and tunicates. The results were plotted as histograms (see Figs. 3.14A,B), from which it was concluded that the life cycle of *G. africana* follows the same three larval stage patterns proposed for other gnathiids by previous authors (Mouchet 1928, Upton 1987a,b, Wägele 1987, 1988, Klitgaard 1991). Each larval stage comprised a zuphea (unfed) and a praniza (fed) stage. It appeared that stage 1 zuphea larvae left the maternal marsupium during late summer in search of a suitable residential tidal pool fish.

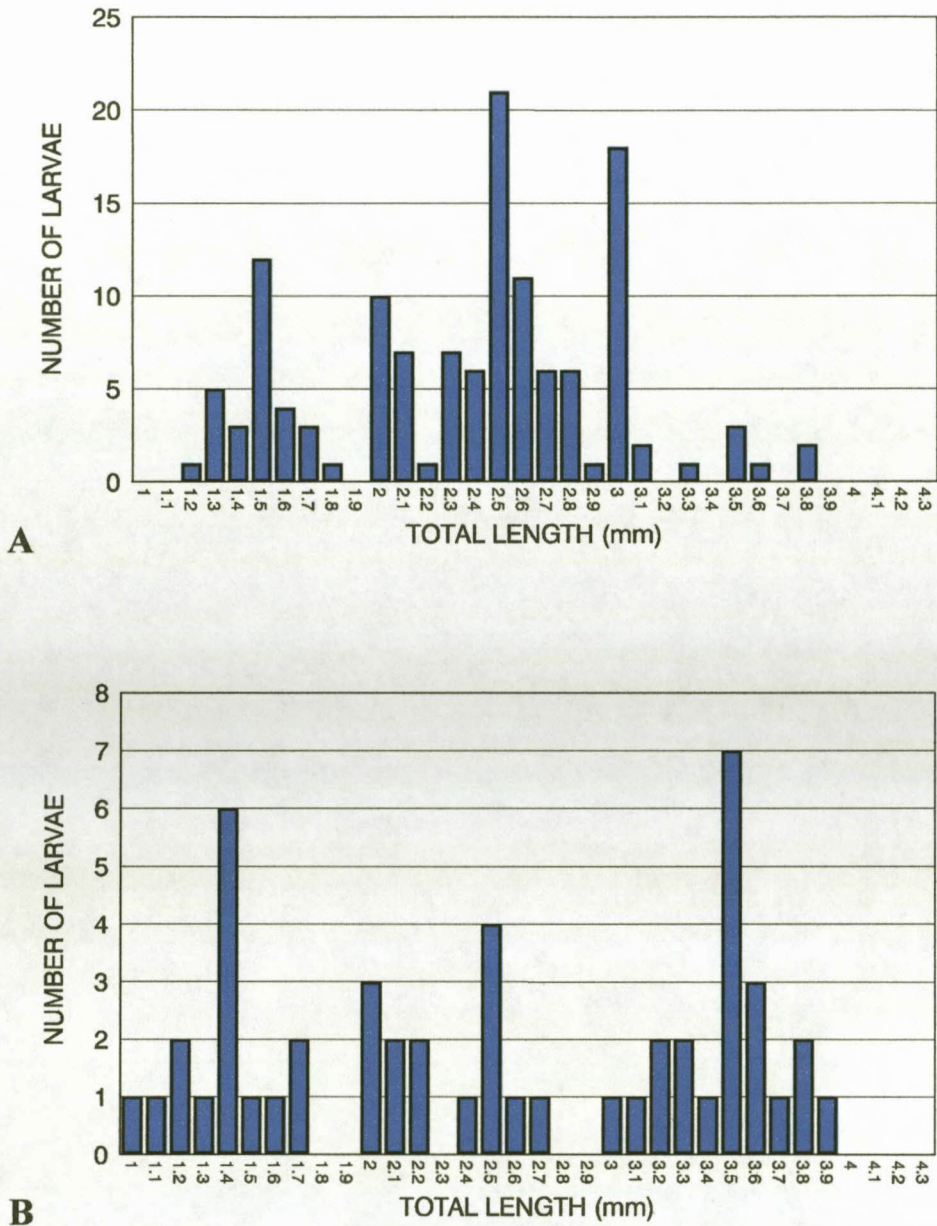


Figure 3.14. Histograms of the length frequencies of *Gnathia africana* Barnard, 1914 pranzia larvae. **A.** Pranzia larvae from Mc Dougall's Bay (September 1995). **B.** Pranzia larvae from Jeffreys Bay (December 1996). [Histograms from Smit (1997)].

While feeding, zuphea 1 larvae underwent the transition into the praniza 1 phase. After feeding, it was assumed that these praniza larvae detached from their fish hosts and sought protection in sponges and tunicates, where they survived on ingested blood and also moulted into stage 2 zupheae during the winter. The rest of the life cycle was presumed to be a repetition of the above with the larvae increasing in length during each feeding period. After the second and third stages, the stage 3 praniza was thought to moult into an adult male or female at the beginning of the summer. No gravid females were found in the sponges or tunicates, and only a few spent females were discovered during the December survey. From this it was assumed that the embryonic development in females might be less than two months and that they probably die shortly after the release of the young.

Laboratory research on the life cycle of *Gnathia africana* Barnard, 1914

Material used in the laboratory experiments during the present study of the life cycle of *G. africana* was collected during October 1998 at Jeffreys Bay. The results of the laboratory experiments were verified in the field by the collection of more material on a further two occasions, at Jeffreys Bay (January 1999) and De Hoop Nature reserve (October 1999). The main objective of the first Jeffreys Bay (1998) fieldtrip was to collect final stage larvae in order to let them moult into adults. These adults were then used to complete the life cycle of *G. africana* in the laboratory. During feeding experiments, the feeding period of 20 zuphea larvae of each stage (Z1-Z3) was measured to the closest five minutes (Table 3.1). The positions of attachment of each larva were also recorded. Twenty larvae of each stage were also measured to the closest 0.1mm in order to determine the growth lengths between different stages (Table 3.2). The time of digestion of 20 praniza larvae of each stage (P1-P2) was measured from the day of detachment to the day on which they moulted into the next stage (Table 3.3).

A total of 33 final stage larvae were collected from their fish hosts and 14 from sponges at Jeffreys Bay (1998). Since the exact time that larvae collected from sponges detached from the fish was not known, they were used only in determining male/female ratios. Of the 33 larvae collected from fish, 16 moulted into males and 17 into females. Of these females, 11 survived the whole term of the experimental period (from detachment of fish host to release of stage 1 zuphea larvae) (see Table 3.4).

Parasitic larvae of *Gnathia africana* Barnard, 1914

a. Zuphea 1 (Z1)

The larvae (zuphea 1) released by females started to feed immediately on the fish hosts in the laboratory. The cohorts of Z1 larvae from different sized females were almost uniform in size (1.0–1.1mm, Table 3.2). During feeding experiments, Z1 larvae attached to the host fish for between 1 hour 15 minutes and 4 hours 10 minutes (mean 2h 18min.). On comparing the length of the feeding period of Z1 larvae attached to different areas on the fish host, a correlation was evident between the attachment site on the host and the length of the feeding period. Larvae attached to the body of the host were able to finish their feeding much faster than those attached to the fins. No differences were found between the length of feeding periods of larvae attached to different fins. Upon completion of feeding the larvae left the fish host as praniza 1 larvae.

b. Praniza 1 (P1)

The detached P1 larvae ranged in length from 1.4–1.6 mm (mean 1.5 ± 0.06 mm). This means that the first feeding period led to an average increase of 0.5 mm in length. The P1 larvae started to moult into zuphea 2 larvae at seven to nine days (average 8 days) post feeding. The moulting process was a characteristic isopod moult, consisting of a posterior moult followed by an anterior moult (Fig. 3.15A). The moulting process took between 40 and 90 minutes to complete. The newly moulted larvae were able to use their limbs after 15 minutes and could swim 30 minutes after completion of the moult.

c. Zuphea 2 (Z2)

On completion of the moult, the remainder of the blood/lymph meal was still visible in the anterior hindgut of the Z2 larvae. The length of the Z2 larvae ranged from 1.5–1.8 mm (mean 1.7 ± 0.09 mm), thus suggesting an increase of 0.2 mm in length as a result of the first moult. The Z2 larvae were again ready to feed two to three days post-moulting. At that stage none of the previous meal was visible in the anterior hindgut. The feeding period of the Z2 larvae ranged from 1 hour 15 minutes to 4 hours (mean 2h 43min), not much longer than that of the Z1 larvae. As in the case of the Z1 larvae, Z2 larvae that attached to the body of the host fish were able to complete their feeding more rapidly than those attached to the fins of the host (see Table 3.1). The results of the second feeding phase were praniza 2 larvae.

d. Praniza 2 (P2)

The P2 larvae showed a mean length of 2.4 ± 0.1 mm (range 2.2–2.6 mm). The average increase in length of 0.7 mm produced by the second feeding period was almost one and a half times more than that of the first feeding stage. The size range of the larvae also increased. The P2 larvae spent between seven and 16 days (mean 10 ± 2.5 days) in a resting and digesting phase before they moulted into the next stage larvae. Moulting again followed the same pattern as for the P1 larvae, taking 40–100 minutes to complete. The resulting larvae (Zuphea 3) were the final feeding stages.

e. Zuphea 3 (Z3)

The Z3 larvae ranged in size from 2.3–2.8 mm, with a mean of 2.6 ± 0.16 mm. As in the case of the Z2 larvae, the Z3 larvae gained an average of 0.2 mm in length through moulting. Five days after moulting, the last of the blood meal was digested and the larvae were ready to feed again. The feeding period of the Z3 larvae (mean 10h 8min) was, on average, almost five times as long as those of the Z1 and Z2 larvae (Table 3.4). In some cases a feeding period of more than 20 hours was recorded. The feeding process resulted in an average increase of 1.2 mm in length. The now P3 larvae varied in length from 3.1–4.5 mm (mean 3.8 ± 0.35 mm). The P3 larvae (Fig. 3.16B) were the final larval stages and could be separated into male and female larvae.

Development of the male of *Gnathia africana* Barnard, 1914

In the P3 larvae that were going to moult into males, the testes could be seen situated in the dorsal pereon between three to five days after feeding (Fig. 3.15C). The male larvae of *G. africana* were also characterised by the elongation of the first pereonite forming a dilated region behind the cephalosome (Figs. 3.15E, 3.19A) as described by Monod (1926) for *Paragnathia formica*. After eight to 10 days, the male larva underwent the final moult into an adult male. In a single case the testes were observed 10 days post feeding in the male larva and the moulting only commenced after 19 days (see Table 3.3). The moulting process of *G. africana* P3 larvae also consisted of a posterior moult followed by an anterior moult, as described for other gnathiid species. The posterior moult took about one to two hours to complete. During this process formation of each adult pleonite could be seen (Figs. 3.15D). Upon completion of the posterior moult, the new adult's pereopods were unused for at least eight hours. At that stage, the adult posterior could easily be distinguished from the larval anterior (Figs. 3.15E, 3.19B).

About 14 hours after the posterior moult, the anterior moult commenced (Fig. 3.19C). This was a much more complex moult and took up to four hours to complete with the end result being a totally different looking cephalosome. The newly formed cephalosome (Fig. 3.15F) is soft and without the distinct *G. africana* pigmentation. This is the most vulnerable stage in the life cycle of the male. Because of the complexity of this moult, defects could occur resulting in deformation of the cephalosome (Fig. 3.19D). All sixteen P3 male larvae collected from fish hosts completed their moult successfully, but two of the P3 larvae collected from the sponges were not able to complete the anterior moult. In the laboratory, these deformed males stayed alive for a week and two weeks respectively, but it is assumed that they would not be able to survive long in nature. The total time of the moulting process varied from 13 to 29 hours with a mean of 20 hours (Table 3.3). The young adult male retained a great deal of fluid in its anterior hindgut and was able to survive for extended periods on it. Approximately 4 days after moulting, the cuticle of the male exoskeleton hardened and the distinct pigmentation of *G. africana* males could be observed (Fig. 3.16A). When these males were placed with females they were able to fertilise their eggs and were thus sexually fully developed. The average length of the male life cycle (from Z1 to sexually mature male) in a temperature range of 20–25°C was therefore 36 days.

Development of the female of *Gnathia africana* Barnard, 1914

The ovaries could be seen developing as two strings filled with immature eggs in the dorsal pereon of the female larvae five days post feeding (Fig. 3.16B). Due to the transparency of the larvae, the development of the eggs in the ovaries could be followed easily. The ovaries increased in size, and at nine days post feeding the eggs filled almost half (Fig. 3.16C) and at 11 days post feeding almost three-quarters of the pereon (Fig. 3.16D). At day 13 post feeding, the eggs filled the whole dorsal area of the pereon and the female larva was ready to moult into an adult (Fig. 3.16E). At this stage the difference between the first three pereonites of the male and female larvae could be observed. Those of the female (Fig. 3.19E) were broader and stouter compared with the elongated ones of the male larvae (Fig. 3.19A). The moulting of the female larvae into an adult female took place at 16 to 17 days after feeding, and it followed the same pattern as that of the male, a posterior moult (Fig. 3.19F) followed a few hours later by an anterior moult. Although the cephalosome of the adult female is also totally different to that of the larvae, the moulting process was not as complex as that of the males. One of sixteen female larvae

could not complete the anterior moult and died five days later. The total time of the moulting process varied from eight to 52 hours with an average of 22 hours (Table 3.3).

On completion of the moult, the eggs were still in the dorsal ovaries of the female (Figs. 3.17A,B). At that stage, internal fertilisation of the eggs by the male took place. The male penis [see Fig. 29 of Smit et al. 1999a, (Appendix I A)], consisting of two papillae seems to be a perfect match for the genital opening of the female (Fig. 3.13E). After fertilisation at 19 days post feeding, the eggs were transferred into the ventral marsupium. The anterior hindgut with the last of the blood meal was then visible dorsally (Fig. 3.17D). Two females were kept separately from males. In both cases the unfertilised eggs were also transferred into the ventral marsupium. The eggs could be seen degenerating after a few days and the females died ten and fifteen days later respectively. In the remaining females, the fertilised eggs were round (Fig. 3.17C) and no longer the oval shape (Fig. 3.17B) as they were before. Seven days after fertilisation (19 days post feeding) cell division took place and the eggs consisted of numerous cells (Fig. 3.17E). At 15 days after fertilisation (34 days post feeding), eyespots (Fig. 3.17F), as well as the cephalosome (Fig. 3.18A) of the embryos were visible. The formation of the pereon and pleon (Fig. 3.18B) was completed by day 17 after fertilisation (36 days post feeding) and the embryos then contained yolk in their anterior hindguts as a nutrition source during their last development stages (Fig. 3.18C). No more yolk was visible in the embryos' anterior hindgut at 21 days after fertilisation and the distinct *G. africana* pigmentation was also visible (40 days post feeding) (Fig. 3.18D). Development was concluded and the embryos were ready to leave the female as zuphea 1 larvae (Fig. 3.18E). During the release of the larvae, most left the female through the opening of the oostegites. However, on two occasions at the end of the releasing process, some larvae left the female through the genital opening and through an opening between the pylopods. The trigger for release is unknown, but once the process started, it took only between 10 and 45 minutes for all the larvae to be set free (Table 3.5). An average of 52 larvae (range of 32 to 68) was released from a single female (Table 3.5). A positive correlation was found between the number of larvae and the size of the female, smaller females producing fewer larvae than the bigger ones (Fig. 3.20). Upon release, the zuphea 1 larvae immediately search for a suitable fish host on which they subsequently feed, completing the life cycle (Fig. 3.18F).

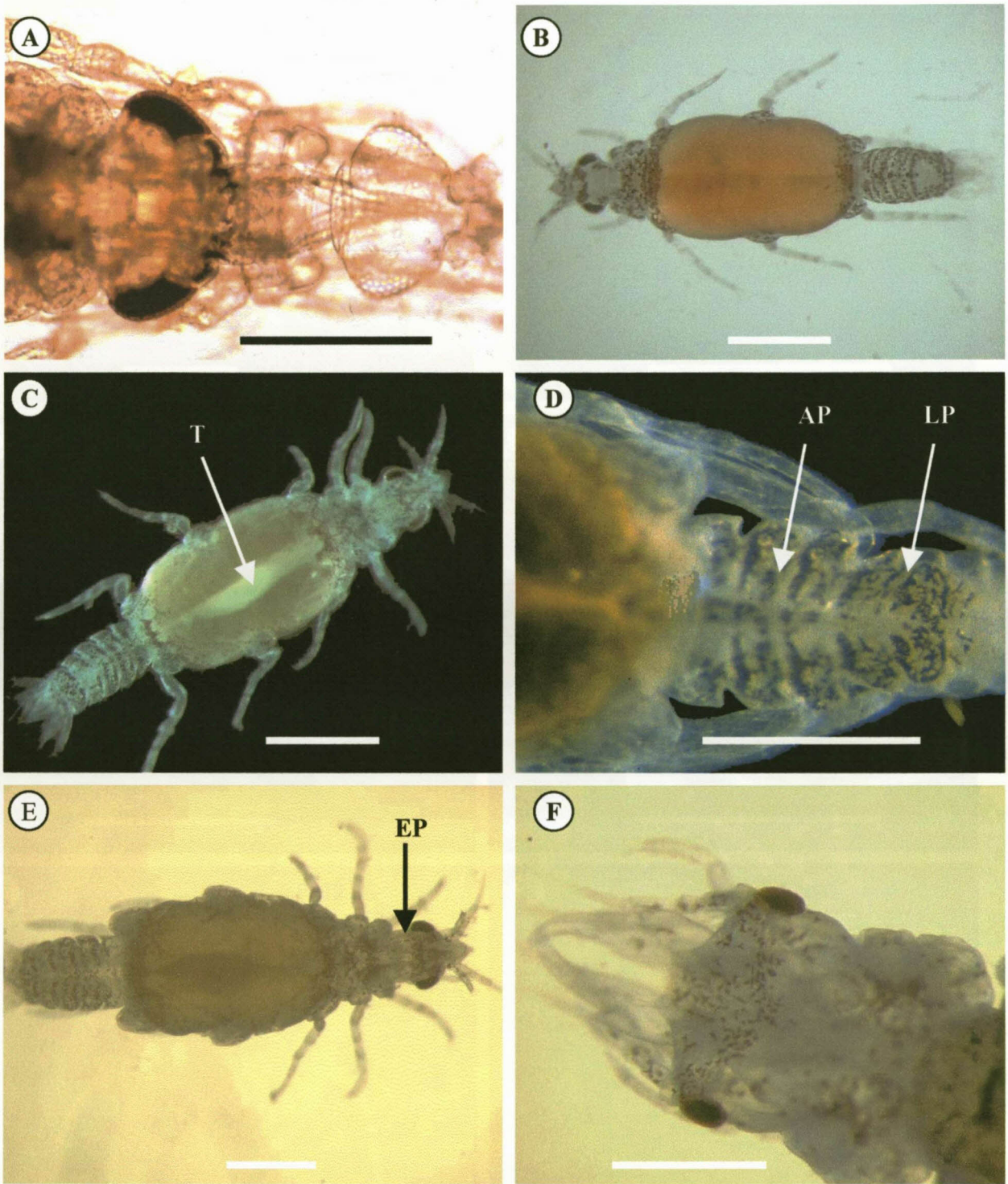


Figure 3.15. Light micrographs of the development stages of *Gnathia africana* Barnard, 1914. A. Praniza 1 busy with anterior moult. B. Dorsal view of a fully fed praniza 3. C. Dorsal view of a male praniza with testes (T). D. Dorsal view of male praniza busy with posterior moult (AP-Adult pleonites, LP- larval pleonites). E. Male praniza with posterior moult completed (EP- elongated pereonite 1). F. Cephalosome of recently moulted male. Scale bars: 1 mm.

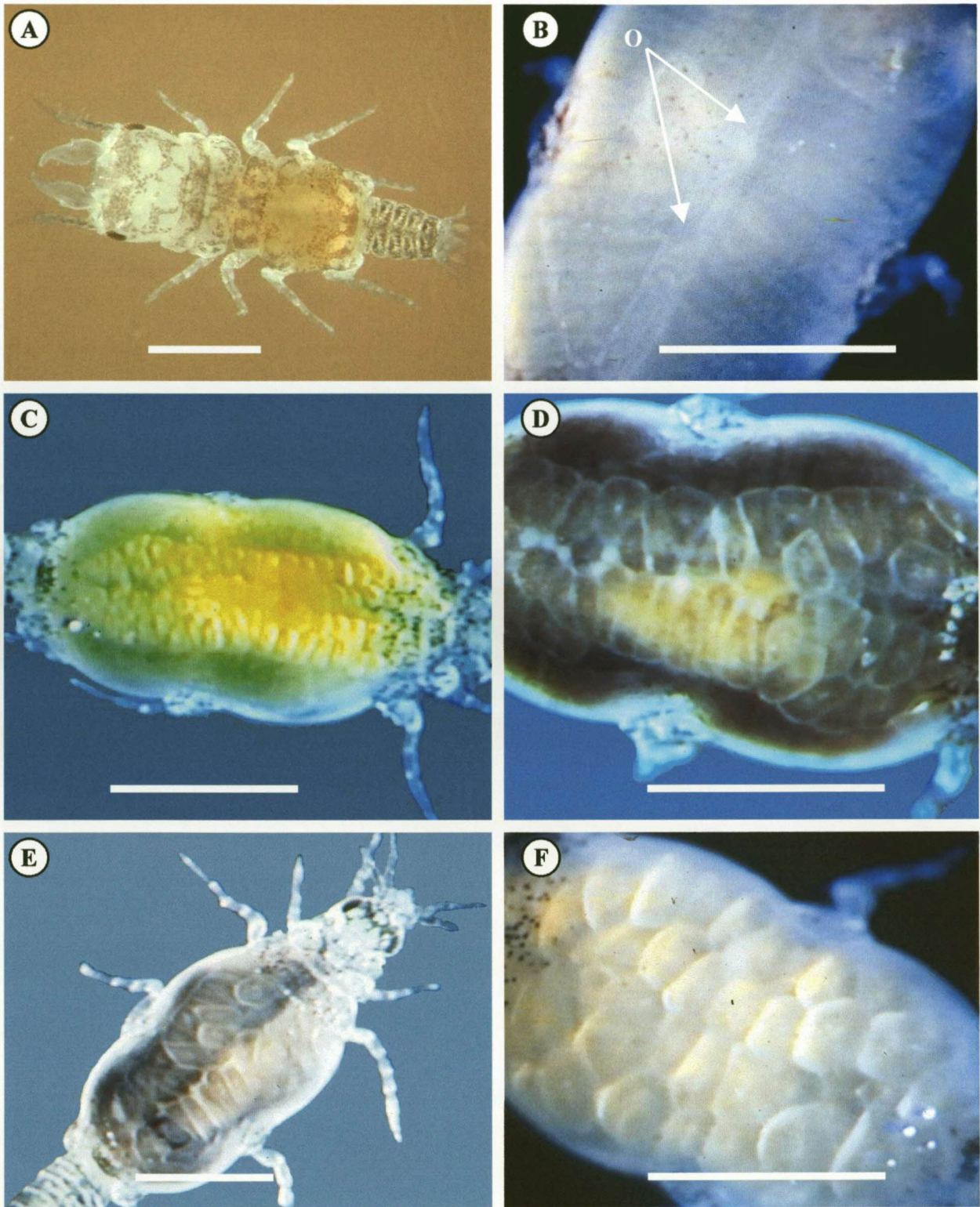


Figure 3.16. Light micrographs of the development stages of *Gnathia africana* Barnard, 1914. **A.** Adult male with characteristic pigmentation. **B.** Pereon of female praniza with ovaries (O). **C.** Female larva with eggs filling half of dorsal pereon. **D.** Female larva with eggs filling three quarters of dorsal pereon. **E.** Female larva with eggs filling complete dorsal pereon. **F.** Irregular shaped eggs in the ovaries of a female larva ready to moult into an adult. Scale bars: 1 mm.

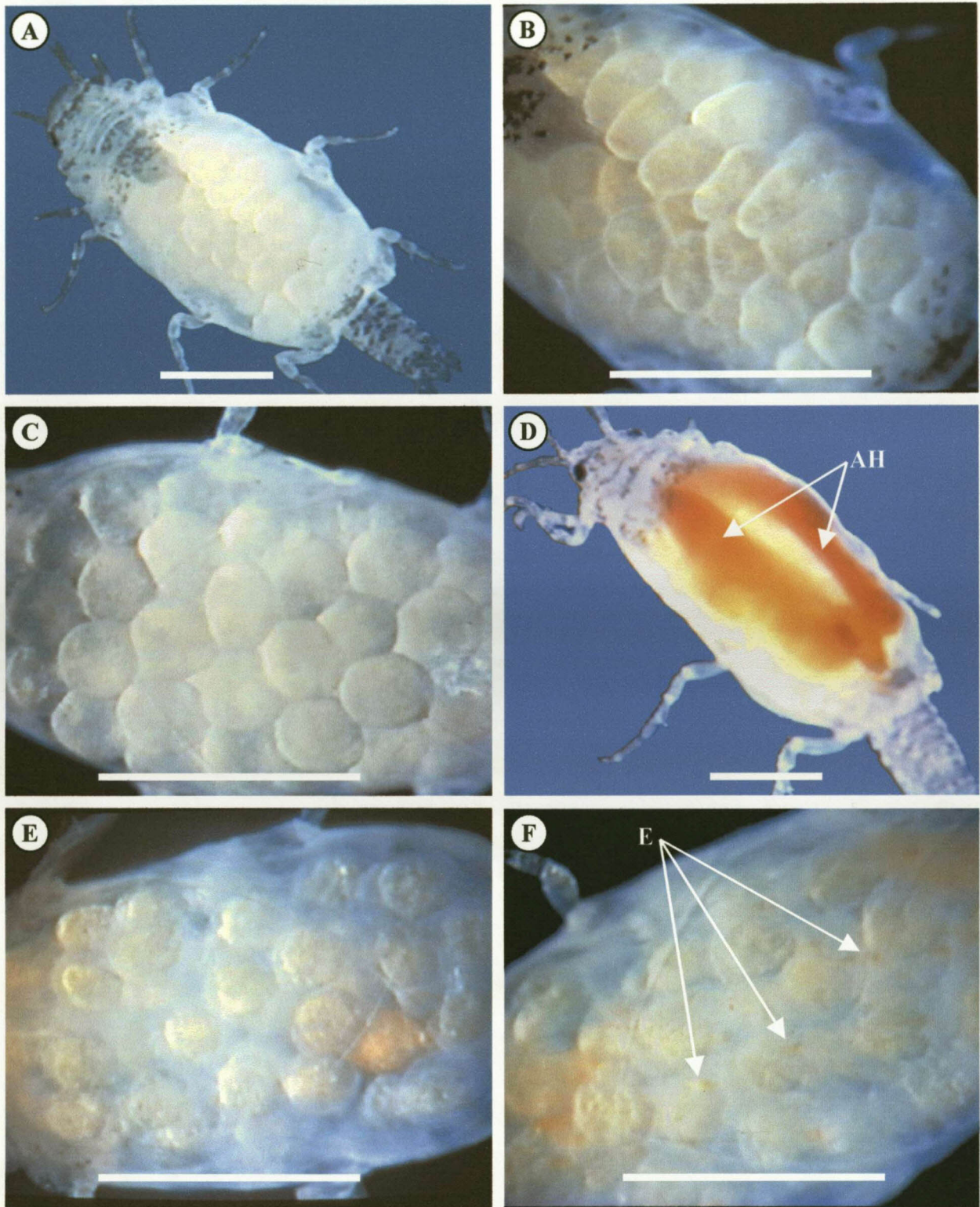


Figure 3.17. Light micrographs of the development stages of eggs and embryos in an adult *Gnathia africana* Barnard, 1914 female. **A.** Dorsal view of recently moulted adult female. **B.** Unfertilised eggs in dorsal ovaries of female. **C.** Round fertilised eggs in ventral marsupium. **D.** Anterior hindgut (AH) with remaining blood meal dorsally visible. **E.** Eggs in marsupium undergoing cell division. **F.** Eyespots (E) of embryos visible. Scale bars: 1 mm.

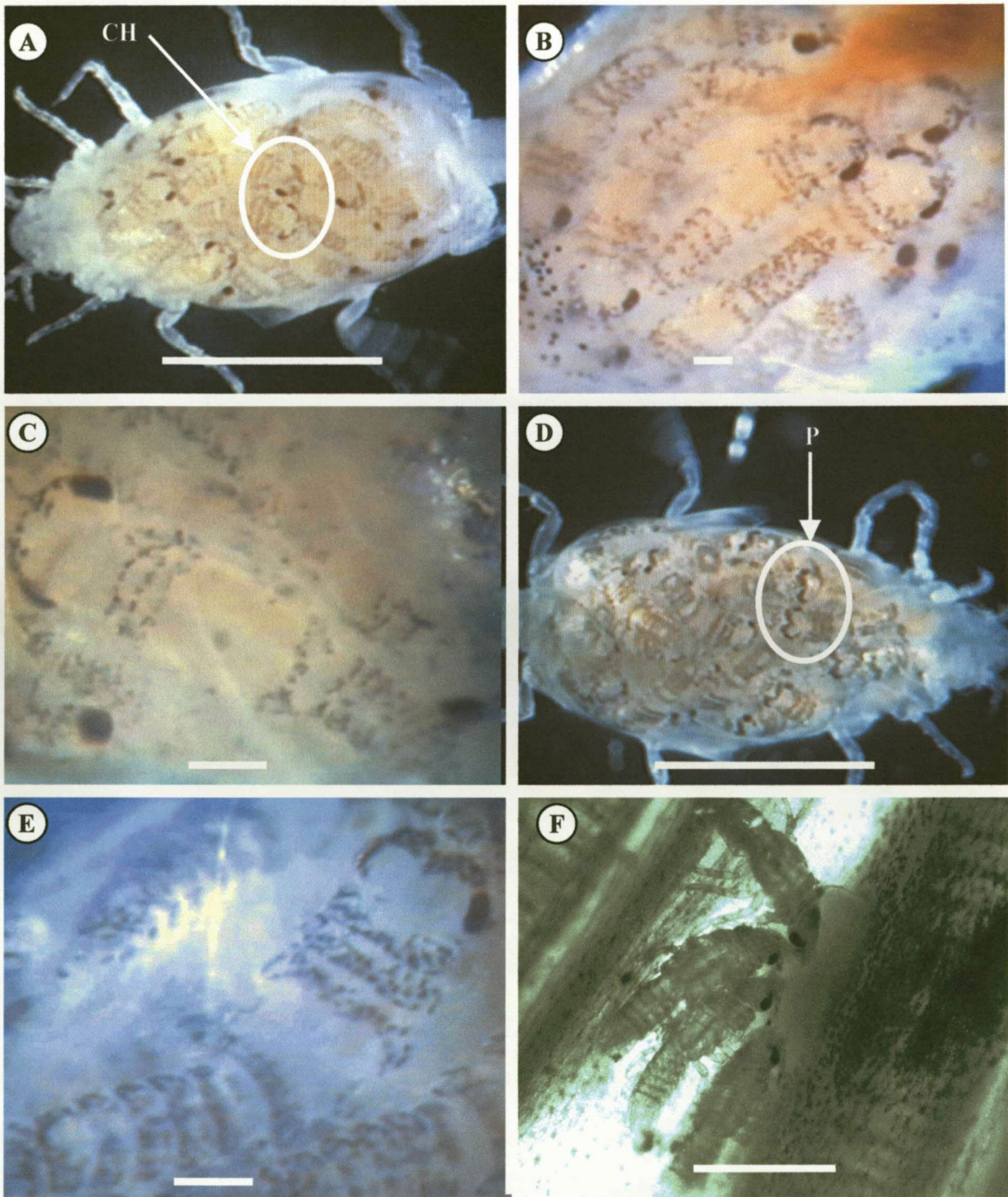


Figure 3.18. Light micrographs of the development stages of embryos in an adult *Gnathia africana* Barnard, 1914 female. **A.** Cephalosomes (CH) of embryos visible. **B.** Pereonites and pleonites of embryos visible. **C.** Yolk in anterior hindgut of embryos. **D.** Distinct pigmentation (P) of *G. africana* larvae visible in embryos. **E.** Embryos with no yolk, ready to leave maternal marsupium as zuphea 1 larvae. **F.** Zuphea 1 larvae attached to fins of host fish immediately after leaving maternal marsupium. Scale bars: A, D & F = 1 mm; B, C & E = 100 μ m.

Figure 3.19

Scanning electron micrographs of different stages in the moulting of *Gnathia africana* Barnard, 1914 male and female larvae

- A. Elongated pereonite 1 of a male praniza larva
- B. Male praniza larva after completion of posterior moult
- C. Male in the process of anterior moult
- D. Cephalosome of a deformed male caused by unsuccessful moulting
- E. Broadened first 3 pereonites of a female praniza larva
- F. Female praniza larva after completion of posterior moult

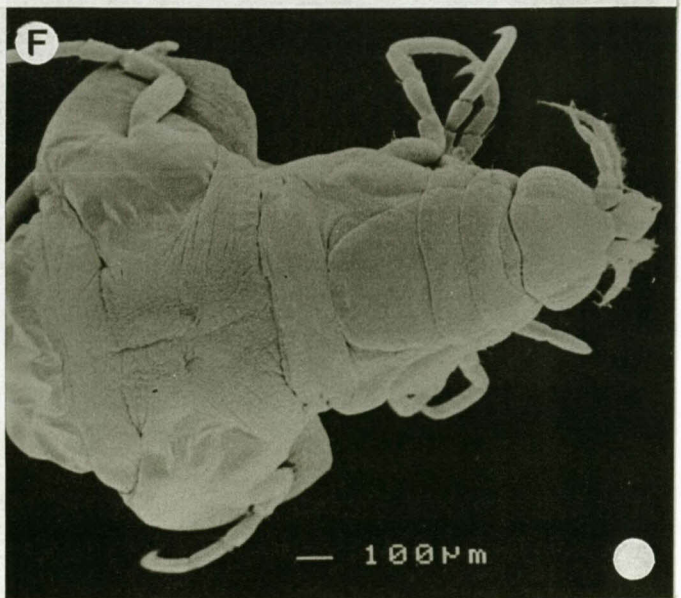
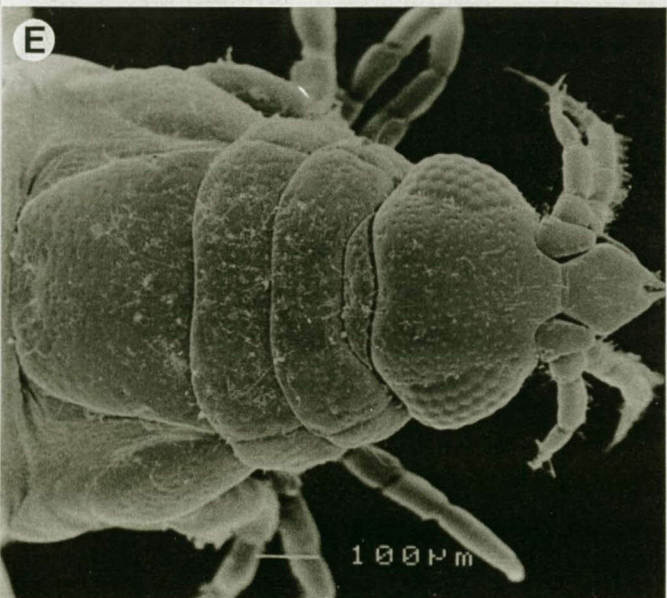
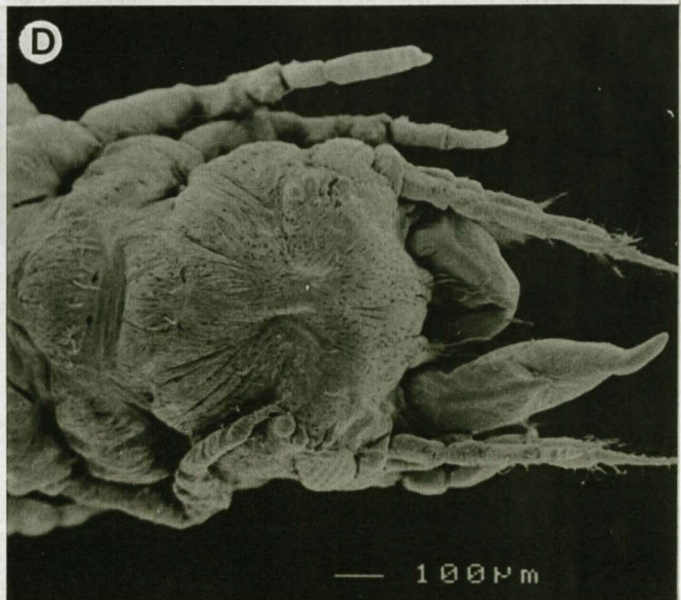
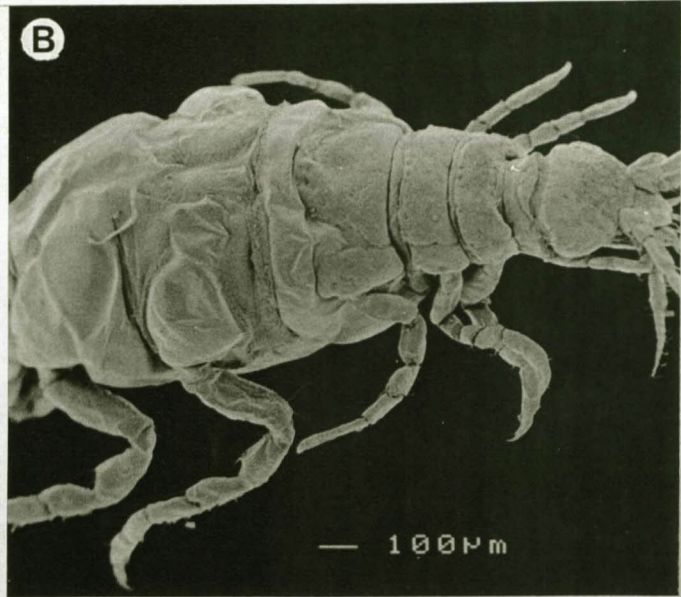
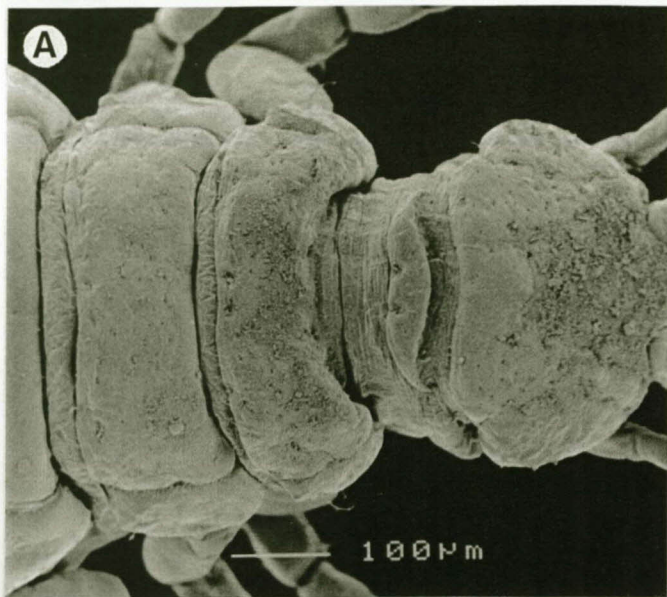


Table 3.1. Feeding time and attachment position of 20 of each different larval stages (Z1, Z2 & Z3) of *Gnathia africana* Barnard, 1914 on *Clinus superciliosus* (Linnaeus, 1758) under laboratory conditions in 20-25°C seawater.

	Zuphea 1		Zuphea 2		Zuphea 3	
	Time	AP	Time	AP	Time	AP
1	1 h 45 min	PF	3 h 45 min	AF	19 h 55 min	DF
2	1 h 55 min	PF	1 h 45 min	B	21 h 15 min	PF
3	1 h 55 min	AF	2 h 45 min	PF	22 h 45 min	PF
4	2 h 40 min	DF	1 h 15 min	B	12 h 35 min	AF
5	4 h 5 min	PeF	3 h 5 min	DF	7 h 15 min	B
6	1 h 15 min	B	2 h 55 min	B	20 h 5 min	DF
7	2 h	AF	2 h 10 min	AF	5 h 15 min	AF
8	2 h 40 min	PF	1 h 25 min	B	3 h 30 min	B
9	4 h 10 min	PeF	2 h	B	5 h 45 min	DF
10	1 h 30 min	B	1 h 45 min	DF	4 h 15 min	B
11	1 h 35 min	B	3 h 20 min	PF	4 h	B
12	1 h 35 min	B	4h 5 min	PF	25 h 5 min	DF
13	1 h 55 min	AF	3 h 45 min	AF	7 h 45 min	DF
14	2 h 40 min	PF	5 h 5 min	PF	6 h 10 min	DF
15	2 h 5 min	DF	2 h 15 min	CF	7 h 30 min	DF
16	3 h 15 min	CF	2 h 45 min	DF	11 h 45 min	PF
17	2 h 15 min	AF	1 h 55 min	B	3 h 20 min	B
18	2 h 25 min	PF	3 h 20 min	PeF	4 h 55 min	CF
19	2 h 20 min	DF	3 h	DF	4 h 50 min	CF
20	1 h 55 min	PF	2 h 5 min	DF	4 h 35 min	B
Mean	2 h 18 min		2h 43 min		10h 8min	

AP – Attachment position

PF - Pectoral fin

CF – Caudal fin

B – Body

PeF – Pelvic fin

DF – Dorsal fin

AF – Anal fin

Table 3.2. Length range of different larval stages and adult males of *Gnathia africana* Barnard, 1914 cultivated under laboratory conditions in 20-25°C seawater. (M- Arithmetic mean. S- Standard deviation.)

	Z1 (mm)	P1 (mm)	Z2 (mm)	P2 (mm)	Z3 (mm)	P3 (mm)	Males (mm)
1	1.1	1.6	1.6	2.3	2.5	3.5	4
2	1	1.4	1.7	2.4	2.3	3.4	3.8
3	1.1	1.5	1.8	2.4	2.5	3.6	5
4	1.1	1.4	1.6	2.5	2.4	3.4	4.1
5	1.1	1.5	1.6	2.4	2.7	3.6	3.5
6	1.1	1.6	1.8	2.5	2.6	3.8	3.8
7	1.1	1.4	1.6	2.2	2.3	4	3.7
8	1	1.5	1.5	2.3	2.6	3.8	3.5
9	1.1	1.5	1.8	2.4	2.9	4.2	4
10	1.1	1.4	1.7	2.5	2.5	3.8	3.7
11	1	1.4	1.8	2.4	2.6	3.1	3.2
12	1.1	1.4	1.7	2.4	2.5	3.5	3.7
13	1	1.5	1.6	2.6	2.8	3.7	3.4
14	1	1.5	1.6	2.5	2.4	3.8	3.9
15	1	1.4	1.8	2.4	2.7	4	3.8
16	1	1.5	1.7	2.5	2.8	3.9	3.6
17	1	1.5	1.8	2.5	2.5	4.1	4.6
18	1	1.5	1.8	2.6	2.4	3.2	4.2
19	1	1.5	1.8	2.4	2.7	4.3	3.4
20	1	1.4	1.7	2.3	2.7	4.5	3.4
M ± S	1.0 ± 0.05	1.5 ± 0.06	1.7 ± 0.09	2.4 ± 0.10	2.6 ± 0.16	3.8 ± 0.35	3.8 ± 0.42

Table 3.3. Digestion and moulting periods of different praniza 1 & 2 and male praniza 3 larvae of *Gnathia africana* Barnard, 1914 under laboratory conditions in 20-25°C seawater. Digestion time measured from detachment from host to completion of anterior moult. Moulting length measured from start of posterior moult until completion of anterior moult. (PV- Pigmentation visible. M- Arithmetic mean. S- standard deviation.)

	Praniza 1	Praniza 2	Male Praniza 3 from fish hosts			
	Digestion (days)	Digestion (days)	Testes visible (days)	Moult complete (days)	PV (days)	Length of moult (hours)
1	8	8	6	10	2	22
2	9	10	6	10	3	21
3	9	9	5	8	6	21
4	8	12	6	9	5	19
5	9	10	5	8	4	22
6	7	12	5	9	4	18
7	7	13	10	19	3	19
8	8	16	5	9	4	16
9	9	12	3	10	4	18
10	8	15	4	10	5	20
11	9	11	4	9	2	13
12	7	9	4	10	5	21
13	7	9	5	11	4	18
14	8	8	3	8	5	17
15	9	12	4	10	3	29
16	7	11	5	10	5	23
17	8	7	-	-		-
18	8	8	-	-		-
19	7	8	-	-		-
20	8	8	-	-		-
M ± S	8 ± 0.8	10.4 ± 2.5	5 ± 1.6	10 ± 2.6	4 ± 1.2	20 ± 4

Table 3.4. Length of egg, moulting and embryonic development periods of female praniza 3 larvae (P3) and female adults of *Gnathia africana* Barnard, 1914 under laboratory conditions in 20-25°C seawater. [Development (in days) from time of detachment from host and moult length (in hours) from start of posterior moult to completion of anterior moult.]

	Female Praniza 3				Adult female							ML (hours)
	D1	D2	D3	D4	F1	F2	F3	F4	F5	F6	F7	
1	7	10	13	16	17	20	27	34	37	40	50	22
2	7	9	13	14	15	16	22	31	34	37	58	23
3	6	10	13	15	16	18	25	34	38	44	49	28
4	4	7	11	13	14	17	31	32	36	41	52	17
5	4	10	14	17	19	23	28	32	35	39	54	52
6	5	8	10	13	18	15	23	38	33	37	49	18
7	3	7	14	16	17	18	25	33	37	42	54	14
8	6	10	15	17	18	20	26	35	38	41	55	28
9	3	9	14	17	17	19	25	34	39	42	46	10
10	2	8	15	17	17	19	24	32	37	40	47	8
11	4	10	13	16	17	20	25	30	35	40	52	21
12	5	9	13	15	17	20	†					
13	3	11	13	17	19	26	†					
14	4	10	15	15	16	†						
15	6	10	11	18	19	†						
16	5	8	10	16	†							
17	7	†										
M ± S	5±2	9±1	13±2	16±2	17±1	19±2	26±3	33±2	36±2	40±2	51±4	22±12

D1 - Ovaries visible	F2 - Eggs ventral	F7 - Spent female dies
D2 - Eggs fill half of pereon	F3 - Egg division visible	ML - Moulth length
D3 - Eggs fill complete pereon	F4 - Eye spots visible	M - Arithmetic mean
D4 - Posterior moult starts	F5 - Embryos fully developed	S - Standard deviation
F1 - Anterior moult complete	F6 - Release of larvae	† - Dead

Table 3.5. Length frequencies of females with the number of embryos and the total time to release the young larvae of *Gnathia africana* Barnard, 1914 under laboratory conditions in 20-25°C seawater. (M- Arithmetic mean. S- standard deviation.)

	Females		
	Length (mm)	Number embryos	Time release (minutes)
1	3.2	32	30
2	3.5	38	35
3	3.6	44	15
4	3.6	52	45
5	3.6	50	40
6	3.7	49	20
7	3.8	56	35
8	3.9	53	40
9	3.9	61	10
10	4.1	63	25
11	4.3	67	20
M ± S	3.75 ± 0.3	51 ± 10.5	29 ± 11.4

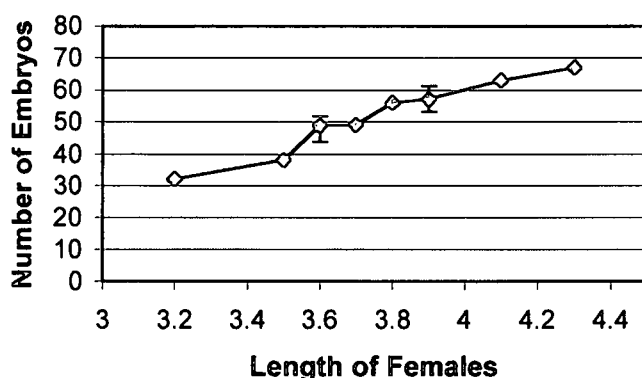


Figure 3.20. Line graph showing the relationship between the lengths of 11 *Gnathia africana* Barnard, 1914 females and the number of embryos produced under laboratory conditions in seawater of 20-25°C (error bars = range).

3.3 Discussion

Feeding

It is assumed that the feeding behaviour of the zuphea larvae during the feeding experiments is similar to that found under natural conditions, since one of the natural fish hosts (*Clinus superciliosus*) of *G. africana* was used. The preferred attachment position in the laboratory did, however, vary from the information obtained from wild populations. During field observations, most larvae were found attached to the pectoral and anal fins, followed in preference by the caudal, dorsal and pelvic fins, with only a small percentage of the parasites being attached to the body surface (Smit 1997). The laboratory experiments showed that the body of the fish was preferred, followed by the dorsal fin, pectoral fin and anal fin, with only a few attached to the caudal and pelvic fins (Fig. 3.21). The reason for these differences may be that, in order to experimentally infect the fish, the larvae were introduced into the fish bucket from the top. Larvae would then swim down towards the fish and immediately attach to the first area of the fish they came into contact with. This would explain their preference for the body and dorsal fin. In the case of natural populations, the larvae would attack the fish from the bottom of the pool, thus first coming into contact with the anal, pectoral and pelvic fins of the host. This indicates that the larvae probably do not have a preferred site of attachment, but rather attach to the first part of the host's body that they come in contact with. The success of

their attachment is illustrated by the fact that only five out of the 60 larvae needed a second attempt to attach successfully. To get the data for 60 larvae, 73 larvae were used. The reason for this was that the fish killed 13 of the larvae. Of these larvae, the fish removed six from their bodies (in most cases from the caudal fin) with their teeth, and ate them, while the remaining seven were spat out.

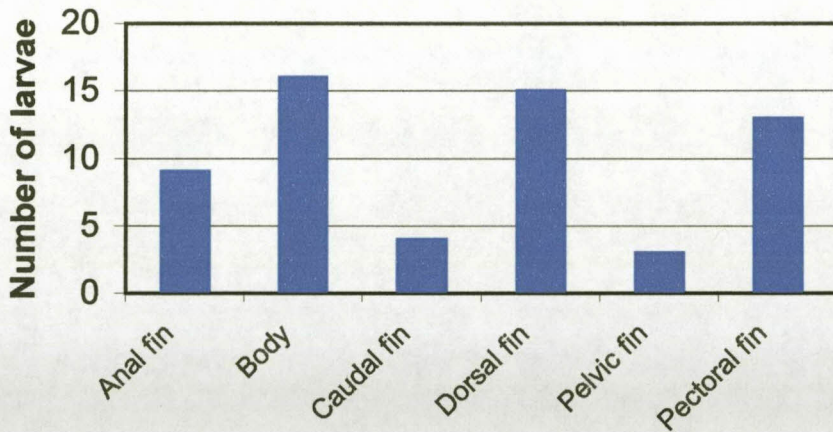


Figure 3.21. Histogram illustrating the number of *Gnathia africana* Barnard, 1914 per attachment site on *Clinus superciliosus* (Linnaeus, 1758) under laboratory conditions in 20–25°C seawater (Data from 60 Z1, Z2 and Z3 larvae pooled).

Life cycle

The data compiled from laboratory investigations combined with verification from natural populations, indicates that the life cycle of *Gnathia africana* can be summarised as follows (see Fig. 3.22). At a temperature range of 20–25°C the eggs develop after 21 days inside the female into stage 1 zuphea larvae. Immediately after leaving the maternal marsupium these larvae search for a suitable residential tidal pool fish host such as the super klipfish *Clinus superciliosus*. At this stage all the larvae are almost the same size. Most of the zuphea 1 attach to the fins of the host fish where they feed on blood or lymph fluid for a period of about two hours. While feeding, the elastic part of the larva's body between pereonites 3 to 5 extends by 0.5 mm and this marks the transition into the praniza 1 phase. After feeding, these praniza larvae detach from their fish hosts and seek protection in sponges and tunicates, where the praniza larvae survive on the ingested

blood and moult into stage 2 zuphea larvae after eight days. Two days after moulting, the zuphea 2 larvae feed again on a fish host for between two to three hours, increase in length by 0.7 mm and leave the fish host as praniza 2 larvae. After a period of 10 days, they moult into zuphea 3 larvae that feed again for three to 23 hours and subsequently become praniza 3 larvae. During the last feeding stage the larvae again increase in length by 1.2 mm, almost double that of the Z2. Three to six days after feeding the sex of the P3 larvae can be determined. The testis or ovaries can be seen developing in the dorsal pereon. Male larvae moult into adult males 10 days post feeding. The development of the female larvae can be divided into four phases and that of the adult females into seven phases (Fig. 3.23). Female larvae filled with eggs, moult at 17 days into adult females. Fertilisation of the eggs by the male takes place within 24 hours of completion of the female moult. The development of the embryos and subsequent release of the young larvae complete the cycle. This entire cycle takes approximately 62 days.

It seems that the life cycle of *G. africana* does not follow a seasonal pattern as was suggested in the preliminary study, but instead takes place continuously throughout the year. This contrasts with the life cycle of *Paragnathia formica*, which follows a yearly cycle as well as that of *Caecognathia calva* which has a cycle of two years, but similar to that of *Elaphognathia cornigera* which appeared to have three to four generations of females per year. Tanaka and Aoki (2000) attributed the shorter life cycle of *E. cornigera* in comparison to that of *P. formica* and *C. calva* to the warmer water temperature of Japan. It is thus interesting to note that in the distribution area of *Gnathia africana*, the water temperature differs from 5°C on the west coast of South Africa to 25°C on the south coast. Further experiments whereby gnathiids are reared at different temperatures are needed to establish the role of temperature on the life cycle length of *G. africana*.

During the collection of specimens from sponges, no females were found. This led to our earlier assumption that females are only found in late summer (January to February), because that was the only time we did not collect specimens from sponges (Smit 1997). This assumption is, however, possibly incorrect, because from the laboratory experiments it can be concluded that females will be present all year round. The reason for the absence of females in sponges and tunicates may be explained in that these habitats do not constitute the resting-place of the females. Barnard (1914a,b) was also not able to find females in sponges, but did describe them from tubes of serpulid worms. It is also

interesting that no female larvae were found amongst the P3 larvae collected from sponges. All these moulted into males and could thus not be used in establishing the male/female ratio in a natural population. From the 33 larvae used in this study a male/female ratio of almost one to one has been found. Since no males and females were collected together it is difficult to comment on the possibility of the males of this species living in a monogamous relationships with a single female instead of forming a "harem" as described for *Paragnathia formica* and *Caecognathia calva*. However, if the male/female ratio of almost one to one, found under laboratory conditions, reflects the situation in natural populations, it can be assumed that *Gnathia africana* males only live with one female each.

It is evident that the assumptions made from the preliminary investigation, where only fixed material was used, were not correct in all cases. This shows that life cycle studies on gnathiids based on length frequency measurements as recommended by Klitgaard (1991) do contribute to the knowledge of gnathiid biology, but must be verified with live animals in laboratory experiments in order to make a final conclusion.

This study also shows that the males and females of *G. africana* do not follow asynchronous life cycles as been documented for *Paragnathia formica*. The males of *G. africana* are able to reach sexual maturity fast enough in order for the males and females of the same generation to mate. Whether the males of *G. africana* are able to survive long enough to mate and guard consecutive females is not known.

Although the life cycle of *G. africana* follows the same basic pattern as described for the other five species, it does differ in some aspects. The zuphea 1 larvae of *G. africana* immediately start to feed and do not need to moult after they have been released, as described for *G. piscivora* by Paperna and Por (1977). The P3 larvae of *G. africana* moult directly into adult males and do not go through an immature phase as described by Wägele (1987, 1988) for *Caecognathia calva*.

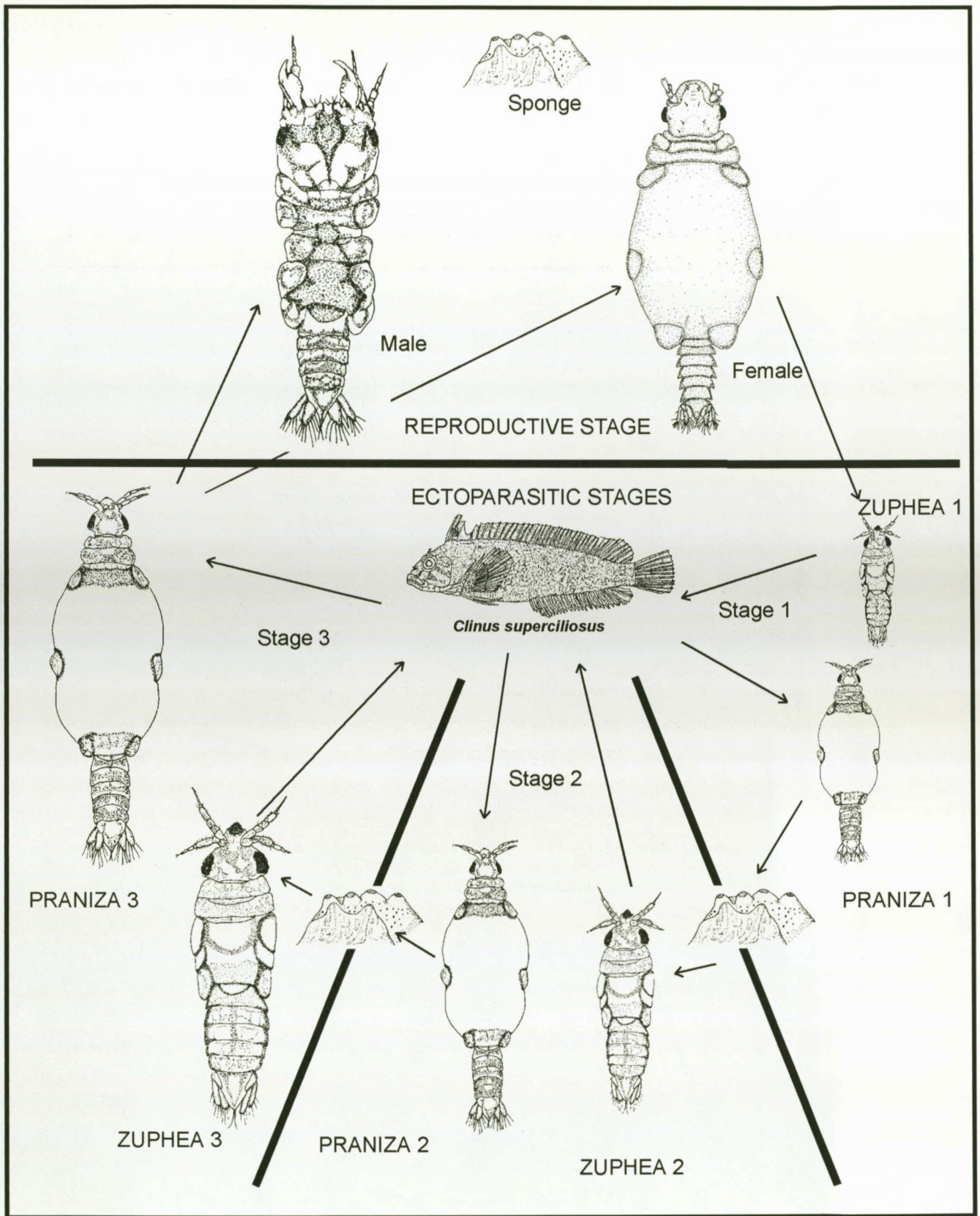


Figure 3.22. Schematic representation of the life-cycle of *Gnathia africana* Barnard, 1914. Male, Pranita and Zuphea larvae redrawn from Smit *et al.* (1999a), *Clinus superciliosus* (Linnaeus, 1758) redrawn from Penrith (1969). Not drawn to scale.

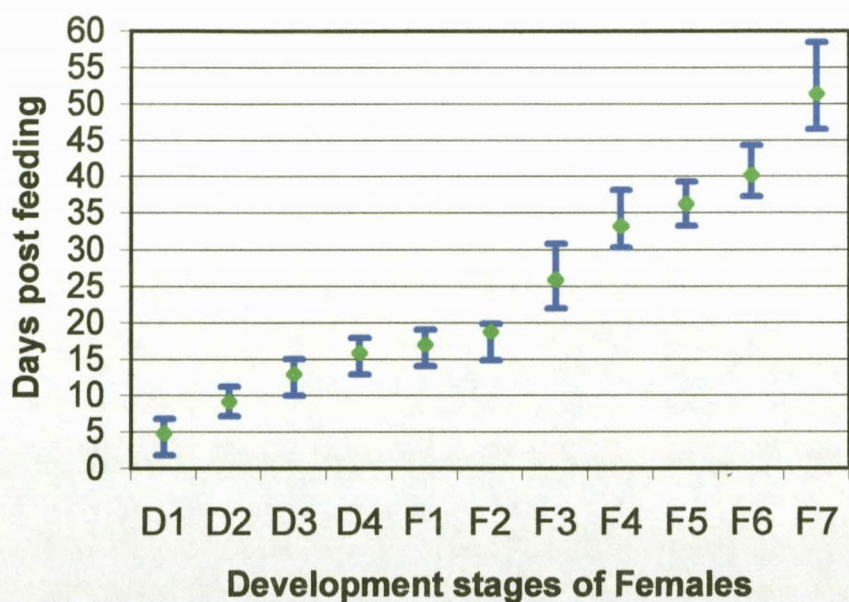


Figure 3.23. Line graph showing the number of days for each development stage of *Gnathia africana* Barnard, 1914 females (n = 17) under laboratory conditions in 20-25°C sea water (error bars = range). (D1- Ovaries visible. D2- Eggs fill half of pereon. D3- Eggs fill complete pereon. D4- Posterior moult starts. F1- Anterior moult complete. F2- Eggs ventral. F3- Egg division visible. F4- Eye spots visible. F5- Embryos fully developed. F6- Release of larvae. F7- Spent female dies.)

When the life cycle of *G. africana* is compared to those of members of another family of fish parasitic isopods, the family Cymothoidae, some similarities are found, but considerable differences exist. The major difference is that both the larval and adult stages in all species of the Cymothoidae are attached to a fish host (in some species the attached female is non-feeding), in comparison to the gnathiids where larvae feed on fish and adults are free-living and non-feeding. As in the case of most isopods, the female cymothoid also possesses a marsupium in which the eggs develop into larvae. In his monograph on the cymothoids of the eastern Pacific, Brusca (1981) provides a summary of the life history of this family. He reported that marsupial development progresses through a number of distinct stages. Brusca (1978a) described five marsupial egg stages and two distinct pre-hatch stages for the embryos of *Nerocila californica* Schioedte and Meinerte, 1881. In the first pre-hatch stage, the pereonites are visible and in the second, pereopods are seen. The second stage moults into a manca stage within the marsupium.

Adlard and Lester (1995) described this same intermarsupial moulting for *Anilocra pomacentri* Bruce, 1986, as did Brusca (1978b) for *Lironeca vulgaris* Stimpson, 1857. In most of the species investigated, a positive correlation was found between the female length and brood number (Brusca 1978a, 1978b, 1981, Maxwell 1982, Adlard and Lester 1995). The female subsequently releases the manca stages, which are able to immediately infect host fishes. Manca larvae only possess six pereopods and on developing the seventh pereopod, they are referred to as juveniles. According to Brusca (1981), possibly all members of the family Cymothoidae are protandrous hermaphrodites, thus meaning that the juveniles first moult into a male and eventually into a female. Although the exact trigger for the development of males into females is not known, it appears to be under neuro-endocrine control. The number of consecutive broods produced by an adult female is not known, but Brusca (1981) predicted that non-feeding females will have a single brood, and feeding females, two or three during their life-span.

Thus to summarise, the main differences and similarities between *G. africana* and members of the family Cymothoidae are as follows. Firstly, no intermarsupial moult exists in *G. africana*. In the parasitic larvae of *G. africana*, moulting takes place not on the fish host, but in the sponges while resting and digesting. In cymothoids all the moults take place while attached to the host. Furthermore, *G. africana*, is not a protandrous hermaphrodite, because larvae either moult into males or females, not first into a male and then into a female as in the case of cymothoids. Lastly, the females of *G. africana* only produce one brood, after which they die.

Similarities between *G. africana* and the cymothoids include the following. Firstly the larvae released by the females immediately begin to feed on host fish. Both have a positive correlation between the size of the females and the number of embryos. It is interesting to note that the presence of only six pereopods in the manca larvae of cymothoids indicates why gnathiids, with the absence of pereopod seven, are referred to as being neotenic (see Seed 1979).

This study on the life cycle of *G. africana* constitutes the most complete study on the life cycle of all the known gnathiids species. Despite this, questions regarding the determination of the sex in larvae (is the sex determined in the zuphea 1 larvae or only in the P3 larval stage?), the effect of temperature on the life span, and the trigger for the release of the zuphea 1 larvae by the females, still remain unanswered.

The importance of research into the life cycle and history of gnathiids will be further illustrated in Chapter 6, which implicates *G. africana* as the vector of the fish blood parasite *Haemogregarina bigemina*.

4. *Gnathia pantherinum* sp. n., an ectoparasite of some elasmobranch species

All current descriptions of gnathiids are based on the morphology of free living adult males. Most of these specimens were found in sponges (Barthel and Brandt 1995, Klitgaard 1997), on the ocean floor (Cohen and Poore 1994), associated with coral substratum (Müller 1989b,c 1991, 1993b) or as in the case of *Paragnathia formica* in micro cliffs of estuaries (Upton 1987a). In this chapter, a species of gnathiid is described for the first time from adult males and females obtained by moulting pranzia found parasitic on elasmobranch hosts. Following this method enables the description of the male, female and larva, as well as allowing insight into gnathiids as parasites of some elasmobranch species. Several authors have documented gnathiid larvae from elasmobranchs (Barnard 1926, Monod 1926, Paperna and Por 1977, Honma and Chiba 1991, Honma, Tsunaki, Chiba and Ho 1991, Grutter and Poulin 1998, Heupel and Bennett 1999). Some of these larvae were described as new species, but they are not valid, since descriptions should not be based on the parasitic larvae. Table 4.1 summarises all published records of gnathiids that parasitise elasmobranchs.

4.1 Description of *Gnathia pantherinum* sp. n.

The material used in this study was collected during field work in January 1999 at Jeffreys Bay, and during April and October 1999 at the De Hoop Nature Reserve. A single live specimen of a leopard catshark, *Poroderma pantherinum* (Fig. 4.1A) was obtained from a local fisherman at Jeffreys Bay. At the De Hoop Nature Reserve five puffadder shysharks, *Haploblepharus edwardsii* (Voight, 1832) (Fig. 4.1B) and a single blackspotted electric ray, *Torpedo fuscomaculata* Peters, 1855 (Fig. 4.1C) were collected as described in Chapter 2. Live gnathiid larvae were removed from the hosts (Fig. 4.1D) and their position, numbers and feeding status were determined. Mostly pranziae were found. All larvae were kept alive in 50ml specimen jars filled with seawater in order for them to moult into adults. The adult males obtained through the moulting of the male

larvae did not conform to the description of any known gnathiid males and are thus described as a new species.

Table 4.1. Previous records of gnathiid larvae that parasitise elasmobranchs. [References marked with an asterisk are from Monod (1926)].

Reference	Host	Family	Gnathiid species
Hesse (1864) *	"Squales"	Squalidae	No species name allocated
	<i>Squatina angelus</i>	Squatinae	
Kossmann (1877)*	<i>Rhinobatus halavi</i>	Rhinobatidae	<i>Anceus rhinobatis</i> Kossmann, 1877
Walter (1885)*	<i>Torpedo</i> sp.	Torpedinidae	<i>Anceus torpedinis</i> Walter, 1885
Willey (1897)*	<i>Aetobatis narinari</i>	Myliobatidae	No species name allocated
Stebbing (1900)*	<i>Aetobatis narinari</i>	Myliobatidae	<i>Gnathia aureola</i> Stebbing, 1900
Seurat (1904)*	<i>Aetobatis narinari</i>	Myliobatidae	No species name allocated
Nobili (1907)*	<i>Aetobatis narinari</i>	Myliobatidae	<i>Gnathia aureola</i> Stebbing, 1900
Scoenichen (1908)*	"Squales"	Squalidae	<i>Gnathia aldabrensis</i> Scoenichen, 1908
Monod (1926)	<i>Ginglymostoma concolor</i>	Orectolobidae	<i>Praniza javana</i> Kllr.
Barnard (1926)	<i>Aetobatis narinari</i>	Myliobatidae	<i>Gnathia aureola</i> Stebbing, 1900
	<i>Dasybatus pastinaca</i>	Dasyatidae	<i>Gnathia aureola</i> Stebbing, 1900
Paperna and Por (1977)	<i>Dasyatis uarnak</i>	Dasyatidae	No species name allocated
	<i>Isurus oxyrinchus</i>	Lamnidae	
Honma, Tsunaki, Chiba and Ho (1991)	<i>Dasyatis akajei</i>	Dasyatidae	
	<i>Dasyatis matsubarae</i>	Dasyatidae	
	<i>Raja kenoei</i>	Rajidae	
	<i>Mustelus manazo</i>	Carcharhinidae	
Grutter and Poulin (1998)	<i>Dasyatis kuhlii</i>	Dasyatidae	
	<i>Himantura fai</i>	Dasyatidae	
	<i>Hemiscyllium ocellatum</i>	Hemiscyllidae	
	<i>Chiloscyllium punctatum</i>	Brachaeluridae	
	<i>Rhinobatus typus</i>	Rhinobatidae	
	<i>Rhynchobatus djiddensis</i>	Rhynchobatidae	
Heupel and Bennett (1999)	<i>Hemiscyllium ocellatum</i>	Hemiscyllidae	

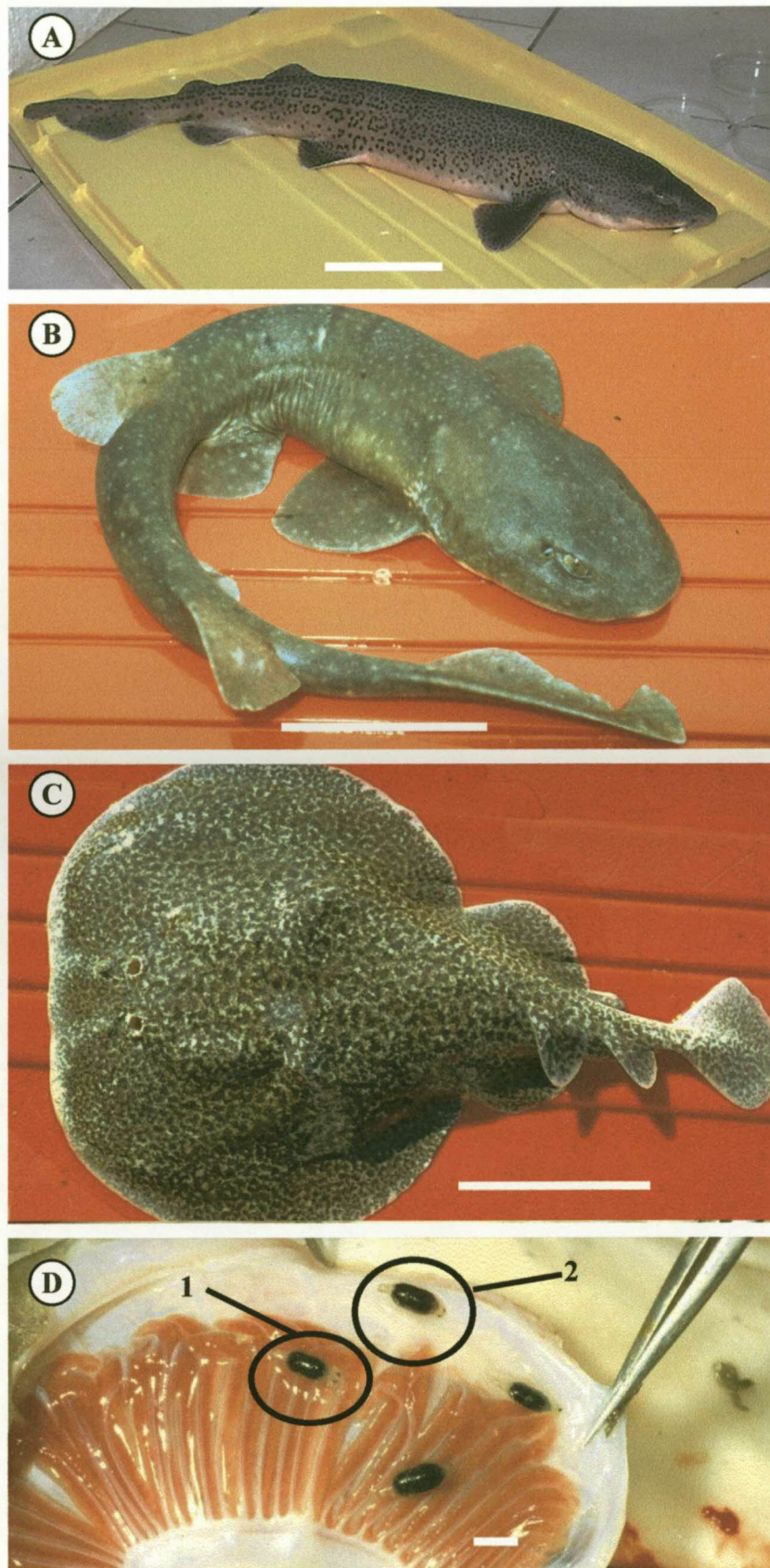


Figure 4.1. A-C. The three elasmobranch hosts of *Gnathia pantherinum* sp. n. A. *Poroderma pantherinum* (Smith, 1838). B. *Haploblepharus edwardsii* (Voight, 1832). C. *Torpedo fuscomaculata* Peters, 1855. D. *Gnathia pantherinum* sp. n. pranita larvae attached to the gill filaments (1) and gill septum (2) of *H. edwardsii*. Scale bars: A-C = 100mm; D = 2 mm.

Gnathia pantherinum sp. n.**Adult male**

Figs. 4.2-4.8

Description: Total length of holotype: 5.6 mm. Total lengths of paratypes: 4.9-6.1 mm (5.4 ± 0.43 mm, $n = 6$). Total lengths of other material: 3.7-6.8 mm (5.4 ± 1.0 , $n = 13$).

Cephalosome. Rectangular, 1.4 times as wide as long, deep dorsal sulcus, narrower than width of median process, extending half length of cephalosome (Fig. 4.5A), lateral margins convex, row of long setose setae and tubercles extending laterally from under the eyes posterior medially almost reaching median tubercle (Figs. 4.2C, 4.5C), posterior margin concave (Fig. 4.2A). Sensory pits, some with short setose sensory seta (Fig. 4.6B), and short hair-like simple setae distributed randomly over dorsal and lateral surface of cephalosome (Figs. 4.5A,C), no sensory pits in dorsal sulcus. Setose setae, sensory pits and short simple setae ventrally on lateral sides of buccal cavity (Fig. 4.5E). Well developed oval-shaped, bulbous, sessile compound eyes on lateral margin of cephalosome, length of eye slightly less than a third of cephalosome. Eight to ten paraocular tubercles with long setose setae (Fig. 4.5C). Elliptical posterior median tubercle present (Fig. 4.5A).

Frontal border. Slightly produced, superior frontolateral process conical, directed antero-laterally, with seven to nine long setose setae on inner border (Figs. 4.2B, 4.5B). Mediofrontal process inferior, deeply divided into two acute lobes, no frontolateral process (Figs. 4.2B, 4.5B). Lamina dentata with eight to eleven tubercles visible (Fig. 4.5A). External scissura shallow. Supraocular lobe not pronounced with eight to ten setose setae and four to five tubercles dorsally (Fig. 4.5D).

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles increasing in length distally with third article as long as first and second articles combined, all three peduncle articles covered with very short hair-like simple setae (Fig. 4.2E). Flagellum with five articles, article 3 largest, articles 3 and 4 with one aesthetasc seta each, article 5 terminating in one aesthetasc and three simple setae, few setae on each article (Fig. 4.2E). Antenna 2 with five peduncle articles covered with very short hair-like simple setae, article 4 and 5 largest, flagellum with seven articles, article 1 largest, article 7 terminating in five simple setae (Fig. 4.2D).

Mandible. Long, two-thirds length of cephalosome, twice as long as wide, broad basal neck, curved inwards with seven to eight processes on dentate blade, tussle of setae between processes, (Fig. 4.5F). Apex cylindrical, distally raised in lateral view. Slight

incisor present. Single setose mandibular seta extending from base of incisor process. Carina armed, forming ridge on lateral margin extending from basal neck to a third along mandible (Fig. 4.5F). Short simple hair-like setae distributed randomly on dorsal surface of blade. Internal lobe and pseudoblade absent.

Maxilliped. Five-articled (Fig. 4.3C), proximal article largest with slender mediodistal endite reaching article 3 (Figs. 4.7D,E). Outer margin of proximal article densely setose. Distal four articles bearing plumose setae on lateral margins in order of 5-7-5-7, mesial border with short simple setae. Distal article with 4 short simple setae. Palp 1.5 times as long as wide. No coupling hooks.

Pylopod. Three articles (Fig. 4.3A). First article greatly enlarged, convex mesial border fringed with plumose setae, lateral and proximal setae short and simple, a pair of feather-like setae near lateral border and five setose setae distally on posterior surface (Figs. 4.3A, 4.7A). Single small areolae. Second article oval, 1.5 times as long as wide, margins setose, six setose setae distally on posterior surface (Figs. 4.3B, 4.7B). Third article minute with fringing setae (Fig. 4.7C).

Maxilla. Reduced, kidney-shaped, on lateral sides of the buccal orifice (Fig. 4.7F).

Pereon. One and a third time as long as wide, wider than cephalosome (Fig. 4.2A), covered with numerous long setose setae and short simple hair-like setae (Fig. 4.6A). Pereonite 1 fused with cephalosome, dorsally visible, not reaching lateral margins, anterior border convex, posterior margin slightly concave (Fig. 4.6C). Pereonite 2 and 3 of similar size, widest part of body, lateral margins pointing anteriorly (Fig. 4.6C). Pereonite 4 with prominent anterior constriction separating it from pereonite 3 (Fig. 4.6D). Tubercles as well as long setose setae on anterior lateral lobe of pereonite 4, median groove present. Pereonite 5 with *areae laterales* and dorsal sulcus as thin groove. Pereonite 5 and 6 not fused. Pereonite 6 at least twice as long as other pereonites, 1.6 times as long as wide, posterior margin deeply concave, with *lobi laterales*, no *lobi*. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Long setose setae on anterior, lateral and posterior margins of pereonites.

Pleon. Pleon and pleotelson less than a third of total length (Fig. 4.2A). Five subequal pleonites dorsally visible, epimera dorsally visible, long setose setae randomly distributed on pleonites (Fig. 4.6E).

Pleotelson. Triangular, base wider than length, lateral margins slightly concave, dorsal surface with four pairs of long setose setae and proximal pair of feather-like setae,

many pectinate scales on dorsal surface, distal apex terminating in pair of setose setae (Figs 4.2G, 4.6F).

Pereopods. Pereopod 2 basis elongated, oval shaped with ten to 12 setose setae and feather-like setae anterior, five to eight posterior setose setae (Fig. 4.4). Ischium two thirds length of basis, three to five anterior setae, posterior tubercles with simple and setose setae in between. Merus half the length of ischium with anterior bulbous protrusion, setose and simple setae on bulbous protrusion, posterior margin with tubercles as well as setose setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion, posterior margin with tooth-shaped tubercles and setose setae. Propodus about twice the length of carpus, one tooth-shaped tubercle on proximal posterior margin, two elongated denticulated compound spines ending in sharp points situated on middle and distal part of posterior margin respectively, only a few short simple setae anteriorly with one feather-like seta distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 similar in basic shape to pereopod 2 (Fig. 4.4), differ in setation as well as distribution of tubercles. Pereopod 4 with distinct tooth-shaped tubercles on anterior margin of basis (Fig. 4.8A), ischium (Fig. 4.8B) and merus (Fig. 4.8C). Pereopod 6 with two strong denticulated compound spines on posterior bulbous protrusion of merus (Fig. 4.8D). Dorsal surface of all pereopods covered with pectinate scales (only showed in illustration of Pereopod 6).

Pleopod. Endopod longer than exopod. Both fringed distally with five to seven short setose setae, short simple setae on lateral and posterior margins of sympodite. Sympodite with retinaculæ on medial margin. Pleopod 2 endopod with appendix masculina, appendix masculina three quarters length of rami (Figs. 4.2F, 4.8E).

Uropod. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, both with long setose setae, pectinate scales on lateral areas of uropods (Figs. 4.2G, 4.6F). Uropodal basis covered with pectinate scales.

Penes: Prominent with two contiguous papillae, wider than long (Fig. 4.8F).

Remarks. The two different populations of *G. pantherinum* (De Hoop Nature Reserve and Jeffreys Bay) did not show any notable variation in body morphology, but differed in body size. Larger specimens were collected at De Hoop Nature Reserve.

The current four described species of the genus *Gnathia* from southern Africa can be divided into two groups, that is, species with pereonite 5 completely separated into two halves by pereonite 6 (*G. spongicola* and *G. disjuncta*) and those with a normal pereonite morphology (*G. africana* and *G. nkulu*). *Gnathia pantherinum* sp.n. falls into the latter group. The *G. pantherinum* males can be distinguished from these two species by means of its deeply concave divided mediofrontal process and the dorsally visible lamina dentata. Both *G. pantherinum* and *G. nkulu* have a row of long plumose setae and tubercles extending from laterally under the eyes posteriorly medially almost the reaching median tubercle. *Gnathia pantherinum* differs from *G. nkulu* in the shape of the frontal border, morphology of pylopod article 2 and the length of the appendix masculina.

The deeply concave mediofrontal process of *G. pantherinum* is very similar to that of the Caribbean species, *Gnathia beethoveni* Paul and Menzies, 1971. *Gnathia pantherinum* differs however from *G. beethoveni* in the shape of the pleotelson, the presence of long setose setae on the pereon and pleon and tubercles on the pereopods. According to the original line drawing by Paul and Menzies (1971) as well as the one in the redescription of *G. beethoveni* by Müller (1989b), the plumose setae on the palp articles of the maxillipedes are in the order of 3-6-5-7. This differs from the 5-7-5-7 formula of *G. pantherinum*. Müller (1989b) also pointed out that the antenna 1 of *G. beethoveni* terminates in two aesthetascs. In *G. pantherinum* the distal three articles of antenna 1 have a single aesthetasc each. The males of *G. pantherinum* are also much larger (3.7-6.8 mm) than *G. beethoveni* males (3 mm).

Another species with a concave divided mediofrontal process is *Gnathia margaritarum* Monod, 1926 described from the Gulf of Panama. *Gnathia pantherinum* differs from this species in the absence of a granular cephalosome and pereonites 1-3, and the presence of a characteristic row of long setose setae and tubercles extending laterally from under the eyes posteriorly medially. The plumose setae of the palp articles of *G. margaritarum* (in order of 4-5-5-6) (see Müller 1989b) is also different to that of *G. pantherinum* (in order of 5-7-5-7). As with *G. beethoveni*, *G. pantherinum* males are also much larger than those of *G. margaritarum* (2.3 mm).

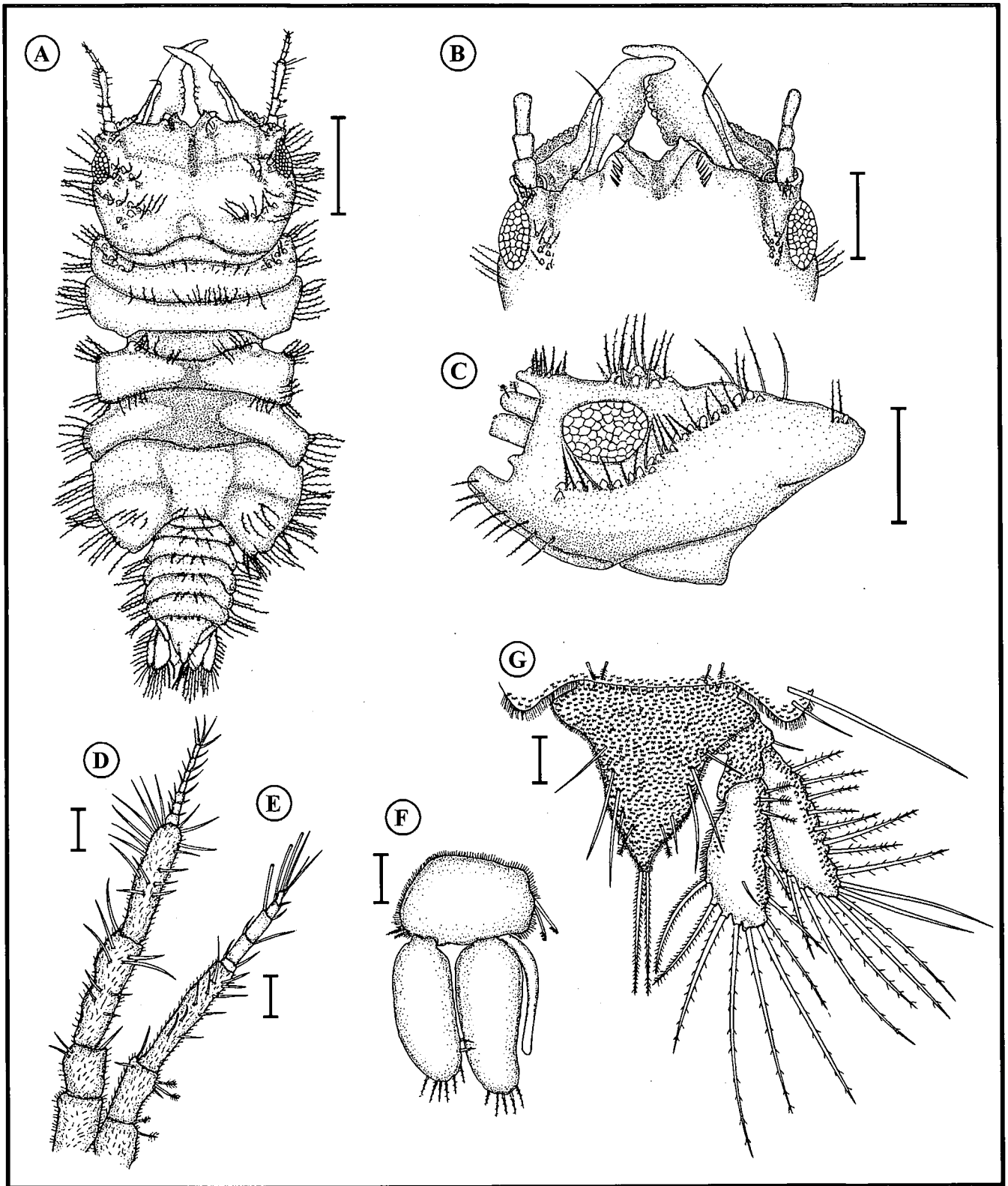


Figure 4.2. Microscope projection drawings of a *Gnathia pantherinum* sp. n. male. **A.** Full length dorsal view. **B.** Frontal border and mandibles. **C.** Lateral view of cephalosome. **D.** Second antenna. **E.** First antenna. **F.** Left pleopod 2 with appendix masculina. **G.** Pleotelson and uropods. Scale bars: A = 1 mm, B & C = 500 μ m, D-G = 100 μ m

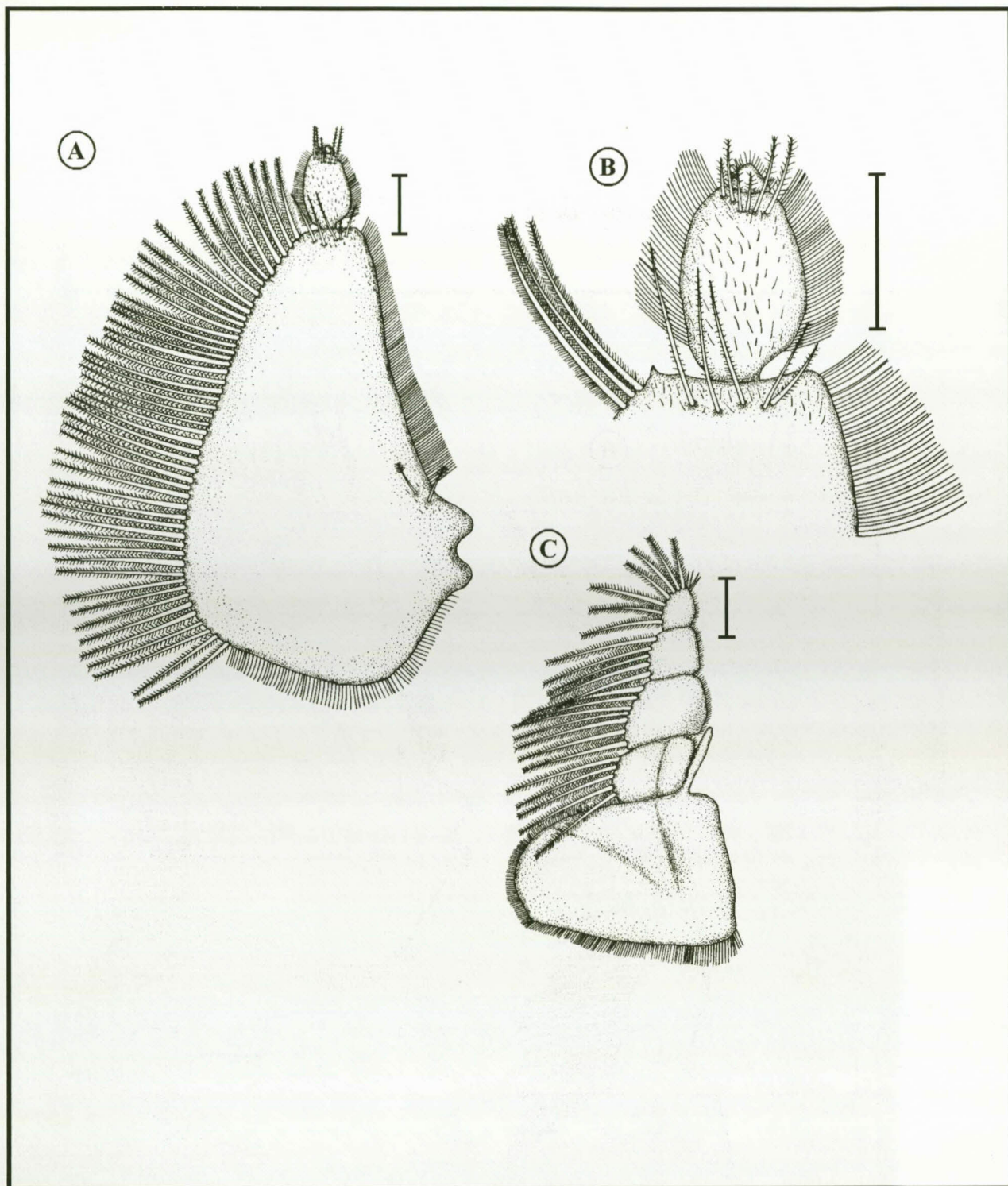


Figure 4.3. Microscope projection drawings of the cephalosome appendages of a *Gnathia pantherinum* sp. n. male. **A.** Left pylopod. **B.** Articles 2 and 3 of pylopod. **C.** Right maxilliped. Scale bars: 100 μm

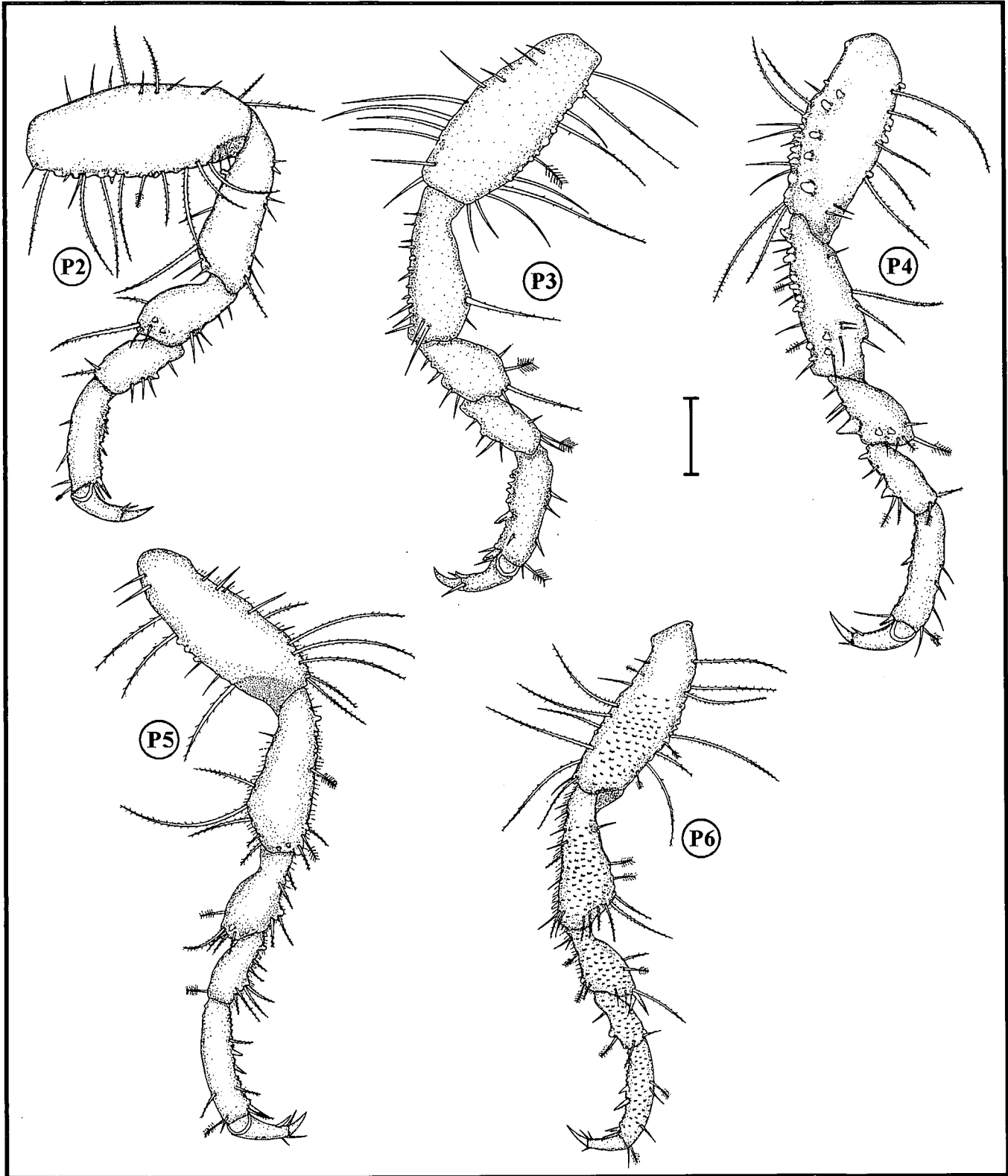


Figure 4.4. Microscope projection drawings of pereopods 2 to 6 (P2 - P6) of a *Gnathia pantherinum* sp. n. male. Scale bar: 200 μ m

Figure 4.5

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. male
(male larva collected at Jeffreys Bay and moulted into an adult male in the
laboratory)

- A. Dorsal view of cephalosome
- B. Dorsal view of frontal border
- C. Lateral view of cephalosome
- D. Lateral view of supraocular tubercles and setae
- E. Ventral view of areas lateral to mouthparts
- F. Dorsal view of left mandible

Scale bars: B = 10 μm ; C-F = 100 μm ;

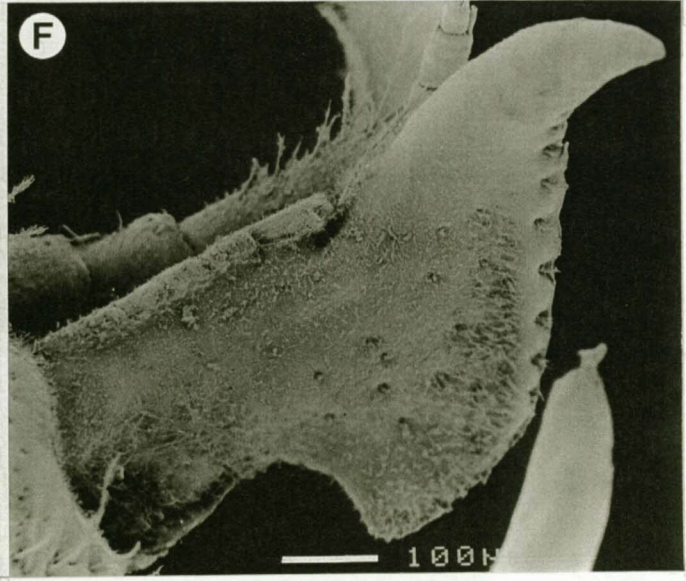
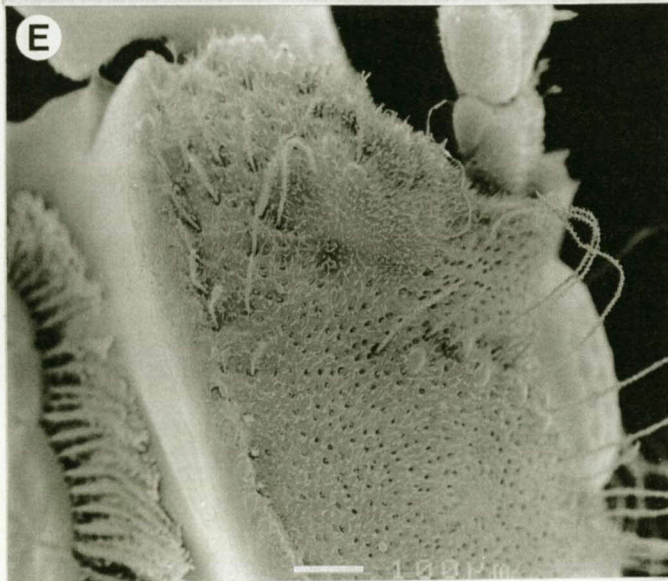
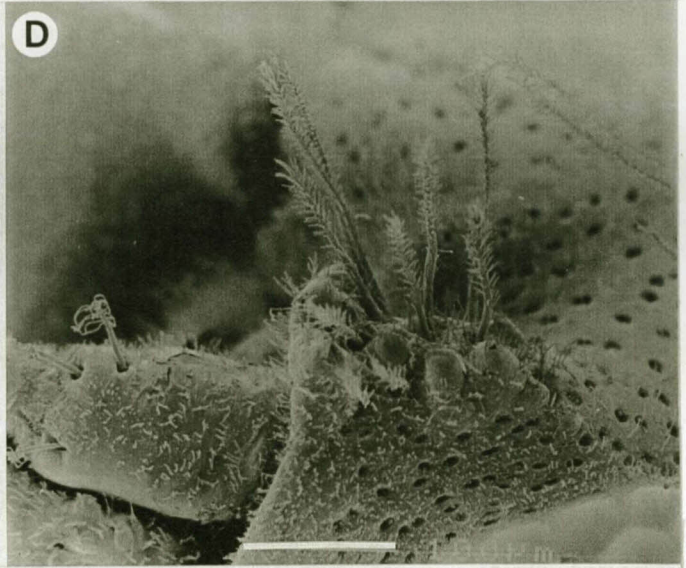
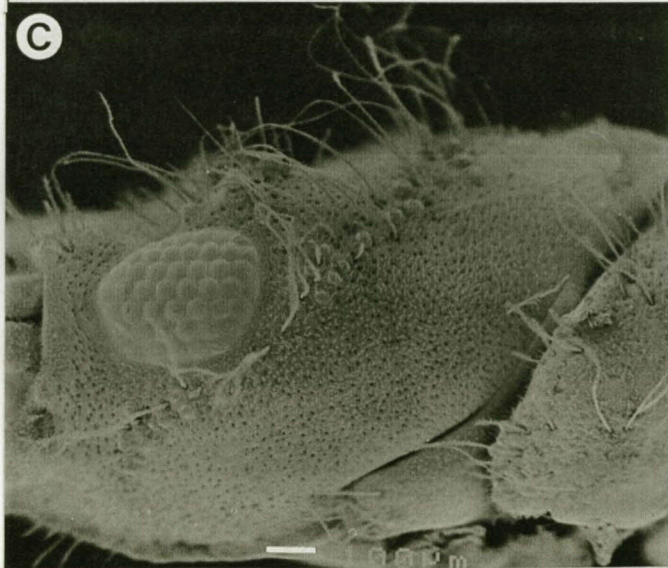
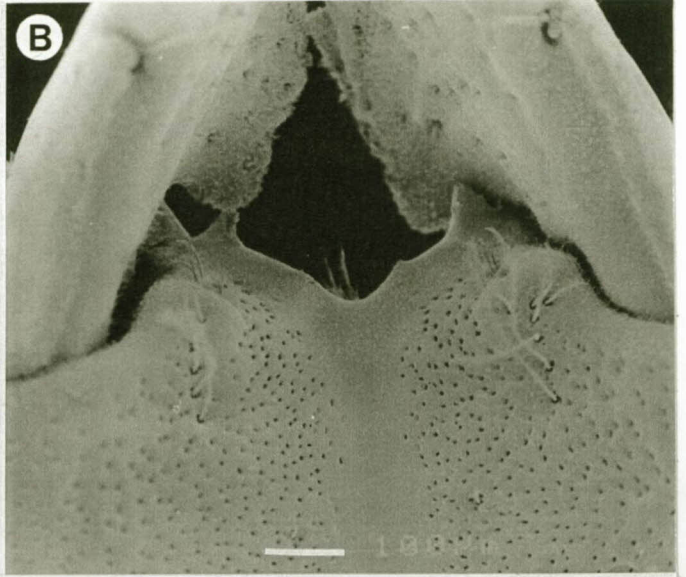
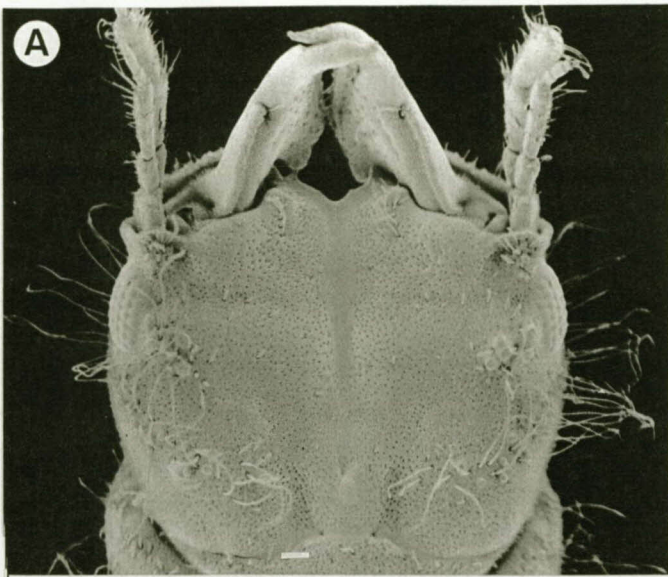


Figure 4.6

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. male
(male larva collected at Jeffreys Bay and moulted into an adult male in the
laboratory)

- A. Long setose seta on cephalosome
- B. Strong short setose seta sunken into cephalosome
- C. Dorsal view of pereonites 1-3
- D. Dorsal view of pereonites 4-6
- E. Dorsal view of pereonite 7, pleon and pleotelson
- F. Dorsal view of pleotelson and uropods

Scale bars: A & B = 10 μm ; C-F = 100 μm

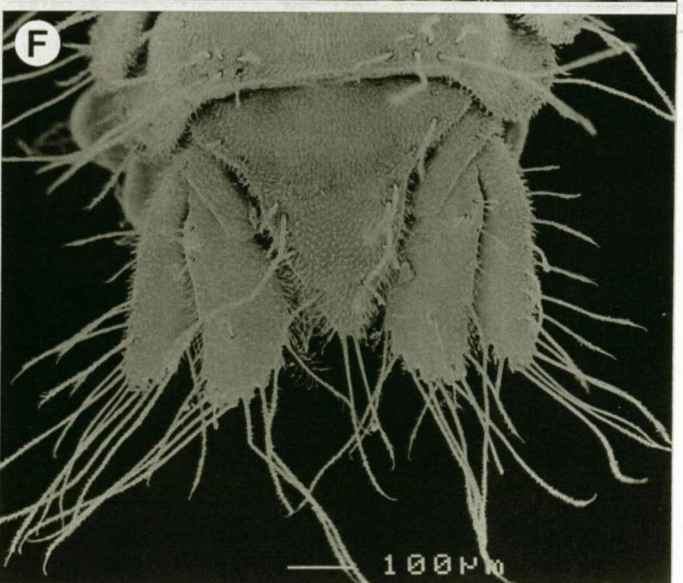
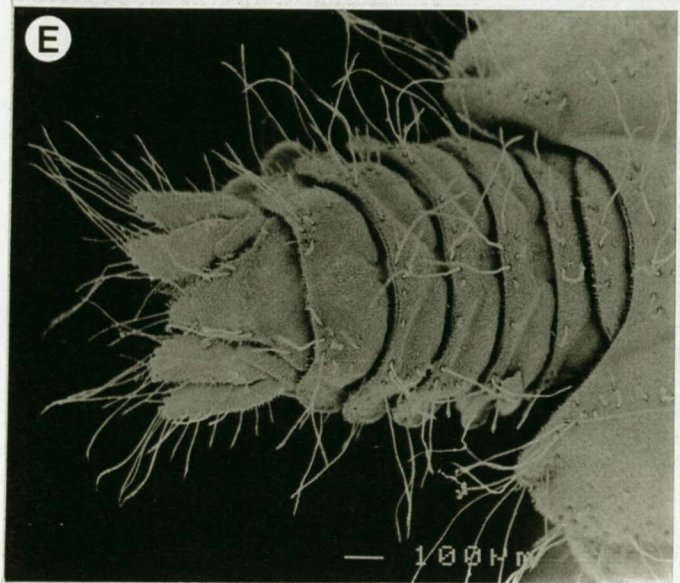
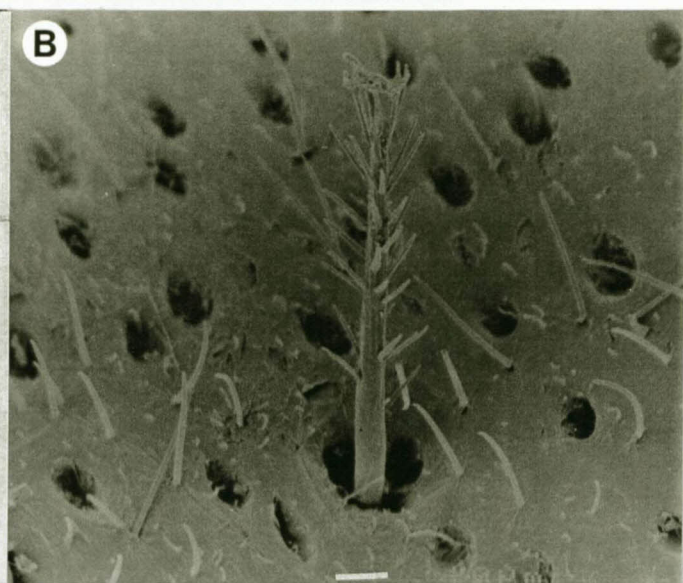
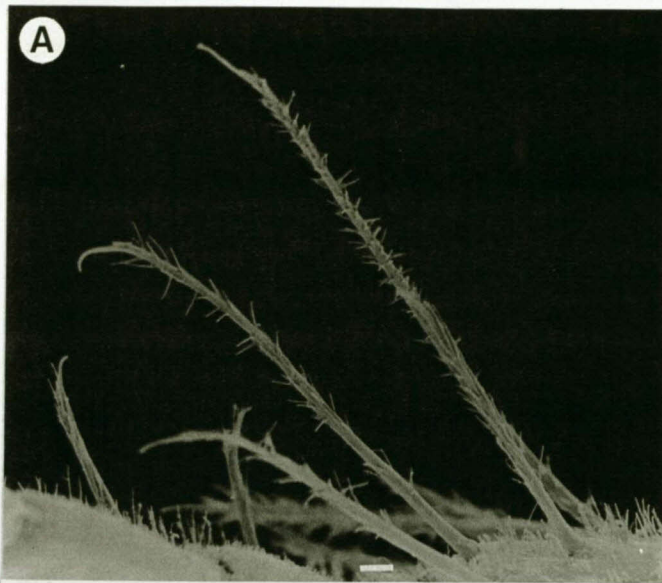


Figure 4.7

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. male
(male larva collected at Jeffreys Bay and moulted into an adult male in the
laboratory)

- A. Ventral view of left pylopod
- B. Article 2 and 3 of left pylopod
- C. Article 3 of pylopod
- D. Ventral view of maxillipedes
- E. Endites of proximal article of maxillipedal palp
- F. Ventral view of reduced maxilla and buccal orifice

Scale bars: A, B & D = 100 μm ; C, E & F = 10 μm

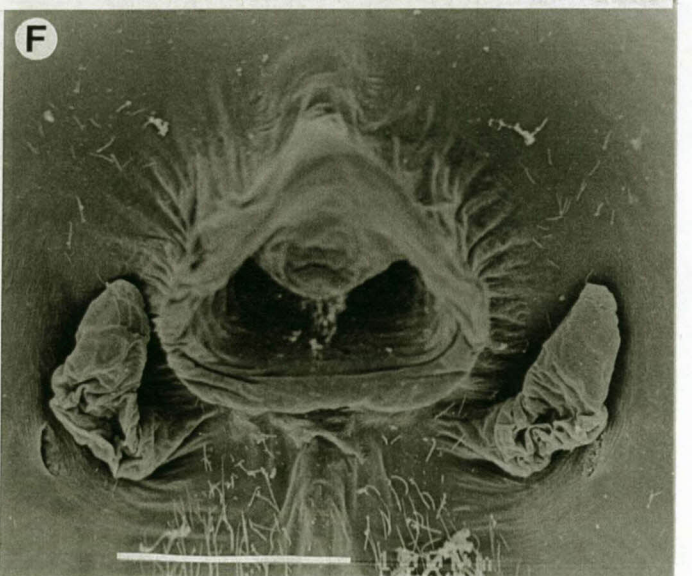
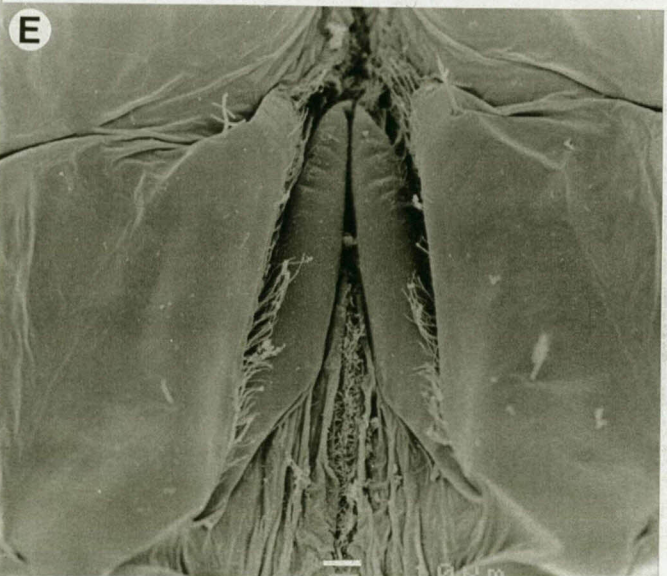
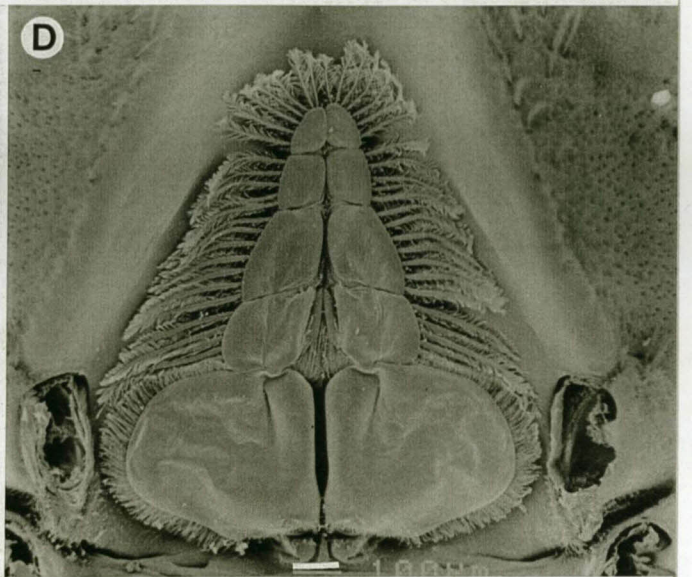
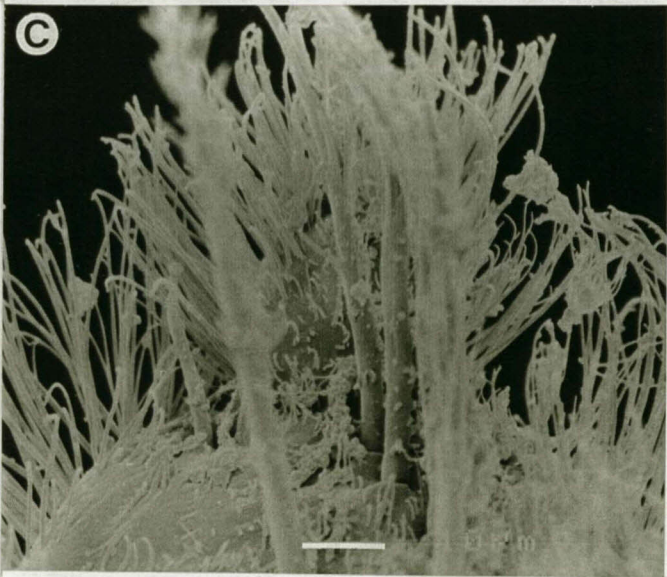
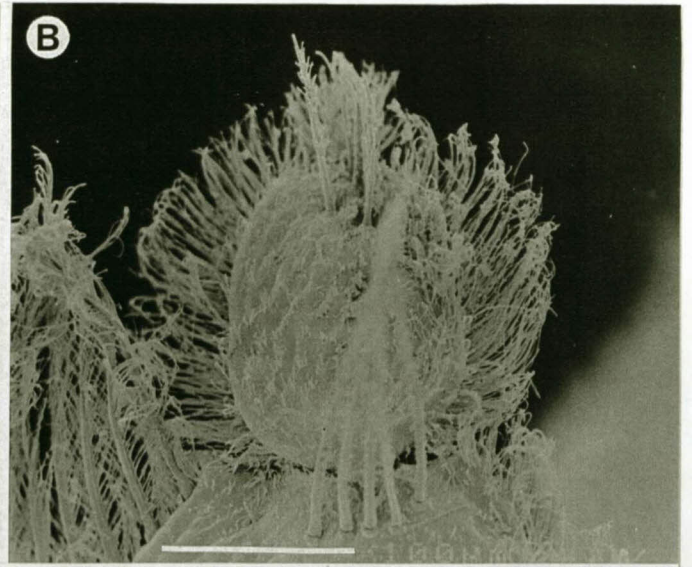
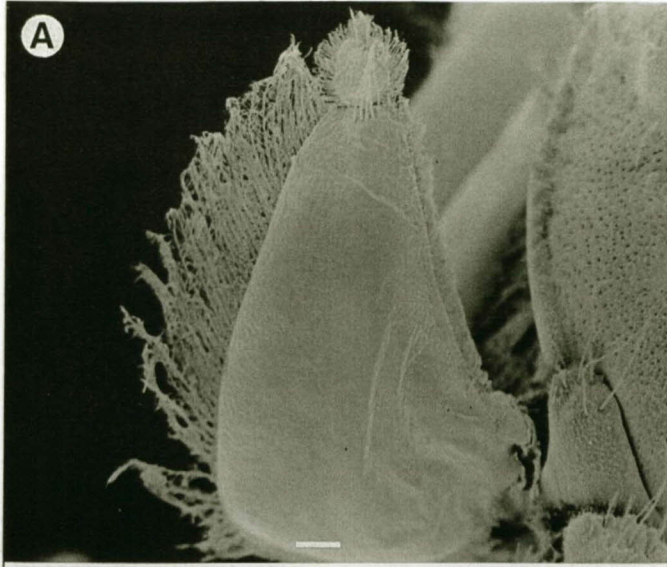
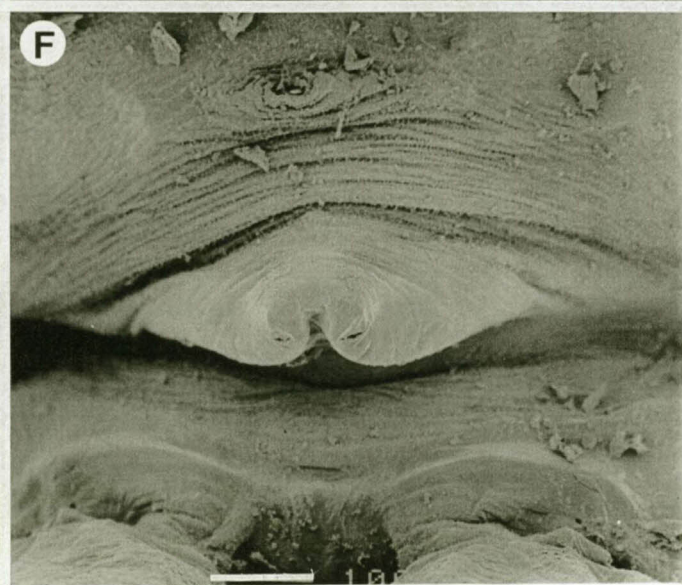
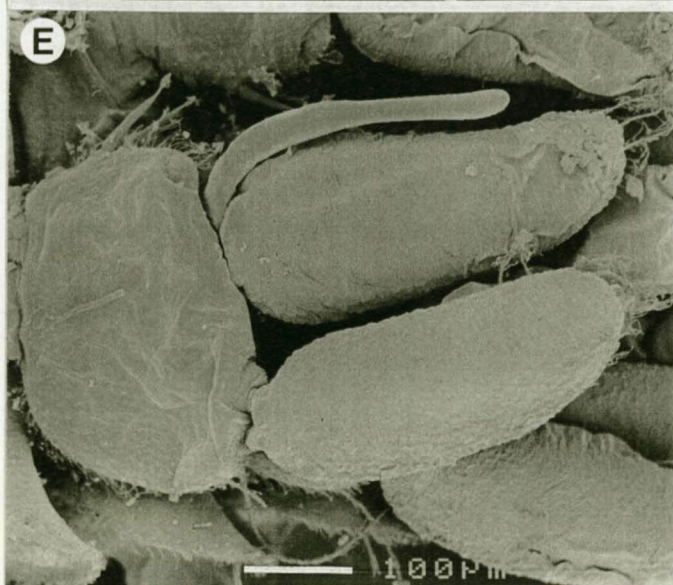
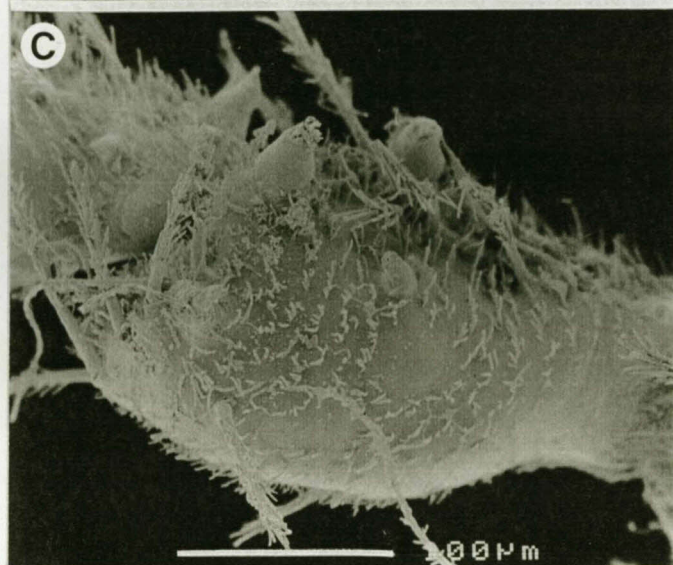
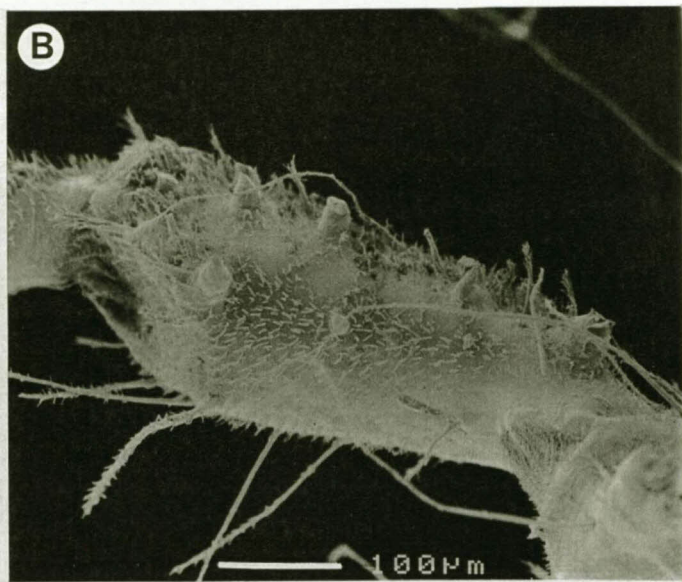
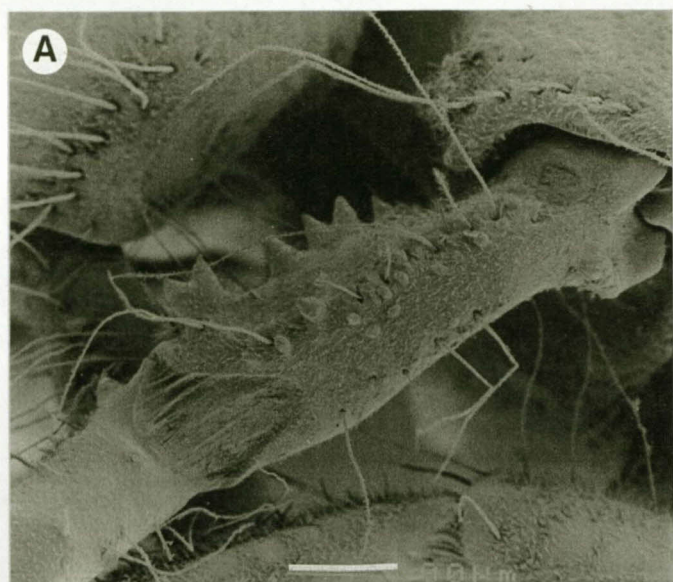


Figure 4.8

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. male
(male larva collected at Jeffreys Bay and moulted into an adult male in the
laboratory)

- A. Tooth shaped tubercles, setae and pectinate scales on the basis of pereopod 4
- B. Tooth shaped tubercles, setae and pectinate scales on ischium of pereopod 4
- C. Tooth shaped tubercles, setae and pectinate scales on merus of pereopod 4
- D. Paired strong denticulated spines on merus of pereopod 6
- E. Ventral view of right pleopod 2 with appendix masculina
- F. Ventral view of penes on pereonite 6

Scale bars: D = 10 μm ; F = 100 μm



Adult female

Figs. 4.9-4.14

Description: Total length of paratypes: 4.3-5.1 mm (4.7 ± 0.37 mm, $n = 6$). Total length of other material: 3.7-6.8 mm (5.2 ± 0.54 , $n = 10$).

Cephalosome. Broadened, short. Rectangular, 1.25 times as wide as long, short simple setae on dorsal, lateral and ventral cephalosome, posterior margin straight (Figs. 4.9B, 4.12A,D). Well developed oval-shaped, bulbous, compound eyes on lateral margin of cephalosome, length of eye three quarters of cephalosome (Figs. 4.9B, 4.12C). No paraocular ornamentation, only four to six short setose setae.

Frontal border. Broadly rounded, produced, slight concave anteriorly, with six to eight short setose setae on mid dorsal area (Fig. 4.12B).

Antennae. Antenna 2 longer than antenna 1 (Figs. 4.9C,D). Antenna 1 with three peduncle articles increasing in length distally with third article as long as first and second articles combined, few short setose setae on distal end of articles 1 and 2 and four to seven short setose and feather-like setae on article 3. Flagellum with five articles, article 2 largest, articles 3 and 4 with one aesthetasc seta each, article 5 terminating in one aesthetasc and three simple setae (Figs. 4.9C, 4.14A). Antenna 2 with five peduncle articles, article 4 largest, short setose setae on distal end of articles 4 and 5, flagellum with seven articles, article 1 largest, article 7 terminating in three to four simple setae (Fig. 4.9D). Peduncle articles of both antennae covered with very short hair-like simple setae and pectinate scales.

Mandible. Reduced.

Maxilliped. Consists of a basis, oostegite and palp of four articles (Figs. 4.10A, 4.13C). Endite short, not reaching article 2 of palp (Fig. 4.13D). Lateral margins of basis fringed with three long plumose setae. Palp bearing plumose setae on lateral margins in order of 8-7-5-10, article 1 and 3 of palp each with single short setose seta anterior-dorsally. Oostegite as broad but longer than palp, (Fig. 4.10A). Mesial borders of basis, palp and oostegite densely setose.

Pylopod. Comprises three articles, articles 1 and 2 fused. Article 1 broad, robust, curved anteriorly, with two feather-like setae mid dorsally, article 2 with five to six short setose setae distally (Figs. 4.10B, 4.13A). Article 3 with eight to ten long setose setae distally (Figs. 4.10B, 4.13B). Oval-shaped oostegite, 2.5 times longer than broad, covers mouthparts ventrally, not surpassing frontal border (Fig. 4.10B). Posterior surface of article 2 and distal half of article 1 covered with pectinate scales and short hair-like

simple setae, proximal halve of article 1 and lateral borders of all articles and oostegite with short hair-like simple setae.

Maxilla. Reduced, kidney-shaped (Fig. 4.13F), on postero-lateral sides of ventral cephalosome (Fig. 4.13E).

Pereon. Swollen round, sutures between pereonites 5-7. One and a half times as long as wide, wider than cephalosome (Figs. 4.9A, 4.12E), covered with numerous long setose setae and short simple hair-like setae. Pereonites 4-6 form thin plate-like oostegites, enclose brood pouch, oostegites overlapping (Fig. 4.12F). Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Ventral area of pereonite 6 with slit which appears to be genital opening (Fig. 4.14F). Most setae on anterior and lateral margins of pereonites.

Pleon. Pleon and pleotelson less than a quarter of total length (Fig. 4.9A). Five subequal pleonites dorsally visible, epimera not distinct, short hair-like setae and short setose setae randomly distributed on pleonites (Fig. 4.14C).

Pleotelson. Triangular, base wider than length, lateral margins slightly concave, dorsal surface with five pairs of feather-like setae and many short simple setae and pectinate scales, distal apex terminating in pair of long setose setae (Figs. 4.9F, 4.14D).

Pereopods. Pereopod 2 basis elongated oval shaped with four to six feather-like setae and simple setae anteriorly, two feather-like setae and four to eight posterior simple setae (Fig. 4.11). Ischium two thirds length of basis, three to five anterior feather-like setae and simple setae, posterior tubercles with three simple setae and four feather-like setae. Merus half the length of ischium with anterior bulbous protrusion, three simple setae on bulbous protrusion, posterior margin with tooth-shaped tubercles as well as feather-like setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion, posterior margin with large tooth-shaped tubercles. Propodus about twice the length of carpus, tooth-shaped tubercle on posterior margin, two elongated denticulate compound spines ending in sharp points situated on middle and distal part of posterior margin respectively, single simple seta anteriorly with one feather-like seta distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Posterior margin of all articles densely setose. Pereopods 3 and 6 similar to pereopod 2 in basic form, differ in setation, shape and number of tubercles (Fig. 4.11). Pereopod 6 with two strong denticulated compound spines on posterior bulbous

protrusion of merus (Fig. 4.11). Dorsal surface of all pereopods covered with pectinate scales (Fig. 4.14B)

Pleopod. Endopod longer than exopod. Both fringed distally with four to eight short setose setae (Figs. 4.9E, 4.14E). No coupling hooks visible. Sympodite without retinacula, single feather-like seta on lateral margin, all margins setose.

Uropod. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, both with long setose setae, pectinate scales on anterio lateral areas of uropods (Fig. 4.9F). Five feather-like setae on dorsal endopod. Uropodal basis with two feather-like setae.

Remarks. In the remarks of the redescription of *Gnathia africana* (see Chapter 3) the difficulty of comparing females due to the lack of comprehensive species descriptions are discussed. The differences between *G. africana* females and those belonging to other genera were also provided in Chapter 3 and therefore will not be repeated here.

The rounded produced frontal border of *G. pantherinum* females is very similar to *G. africana*, but in all *G. pantherinum* specimens examined, it appears to be slightly concave anteriorly. *Gnathia pantherinum* females can be distinguish from *G. africana* females by the following characteristics. The lateral margins of the pleotelson of *G. pantherinum* are concave and those of *G. africana* females are straight. The sympodite of the pleopods of *G. pantherinum* females lack a retinacula, found in *G. africana* females. The plumose setae on the lateral margins of the maxillipedal palp articles of *G. pantherinum* are in the order 8-7-5-10 (from proximal to distal) and that of *G. africana* is 3-8-5-5. *Gnathia pantherinum* females (3.7-6.8 mm) are also larger than *G. africana* females (3.2-4.3 mm).

There are unfortunately no descriptions available for the females of the two species, *G. beethoveni* and *G. margaritarum*, whose males show some similarities to the males of *G. pantherinum*.

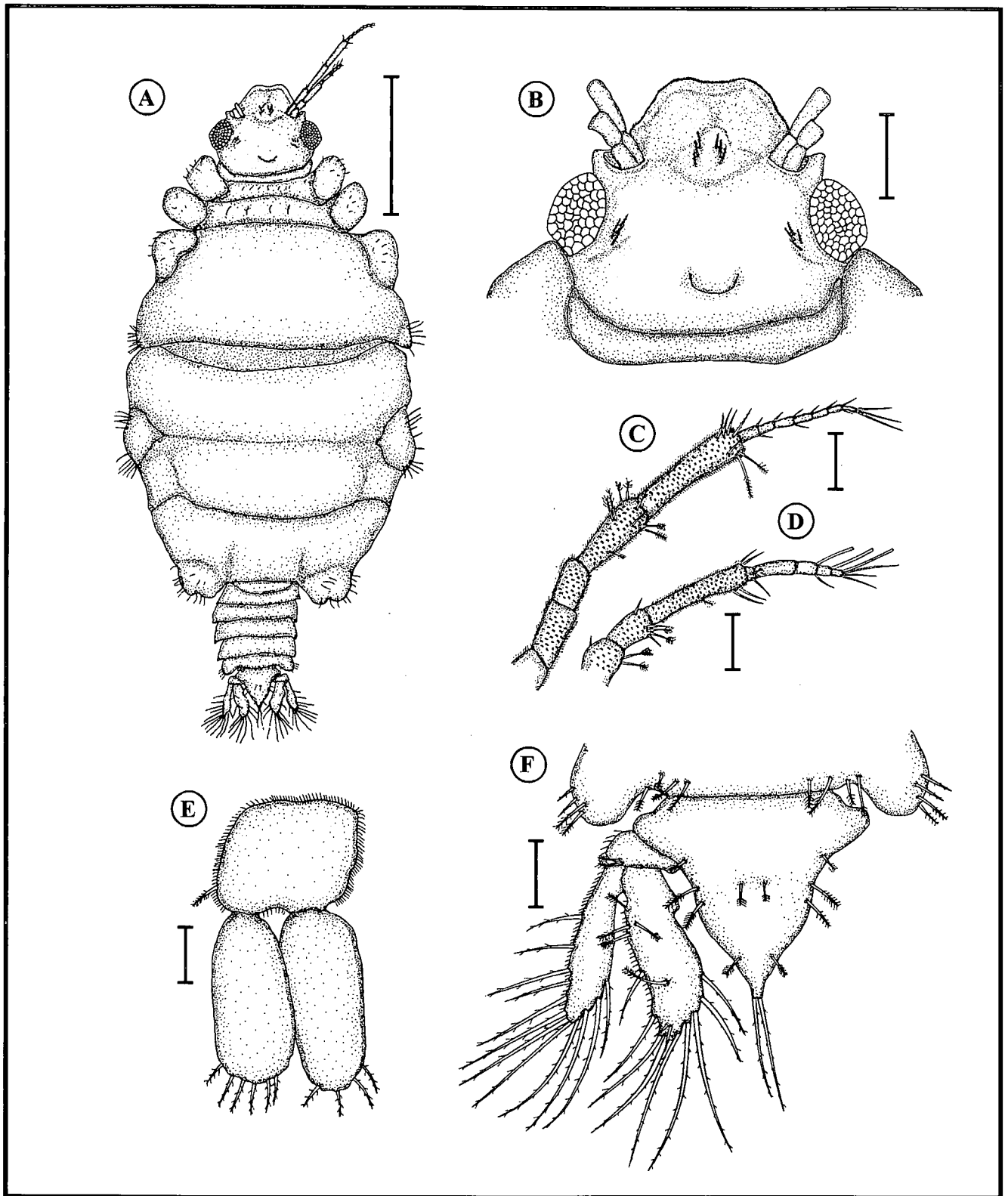


Figure 4.9. Microscope projection drawings of a *Gnathia pantherinum* sp. n. female. **A.** Full length dorsal view. **B.** Cephalosome and frontal border. **C.** Second antenna. **D.** First antenna. **E.** Left pleopod 1. **F.** Pleotelson and uropods. Scale bars: A = 1 mm, B = 250 μ m, D-F = 100 μ m

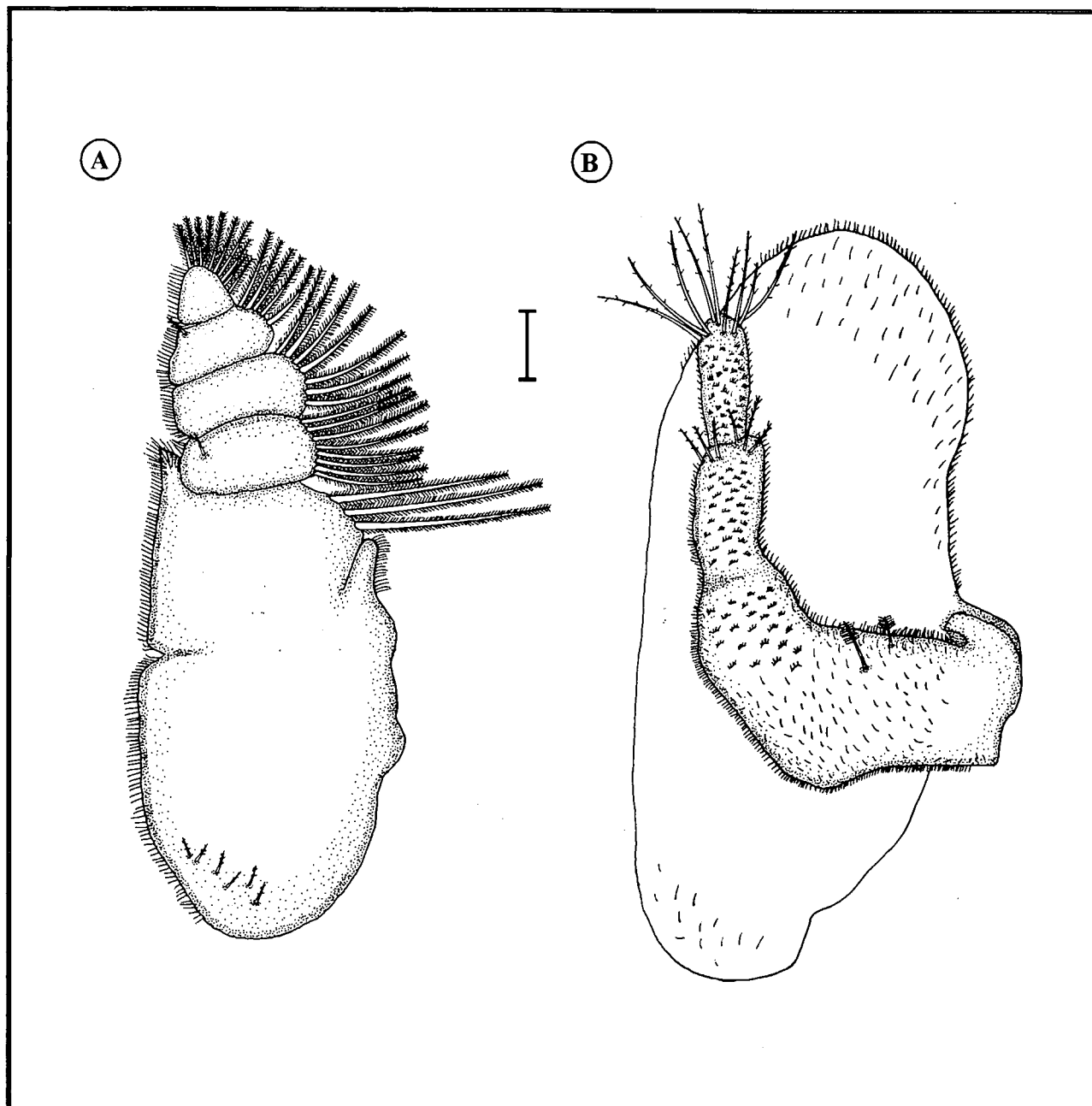


Figure 4.10. Microscope projection drawings of the cephalosome appendages of a *Gnathia pantherinum* sp. n. female. **A.** Maxilliped. **B.** Pylopod. Scale bar: 100 μm

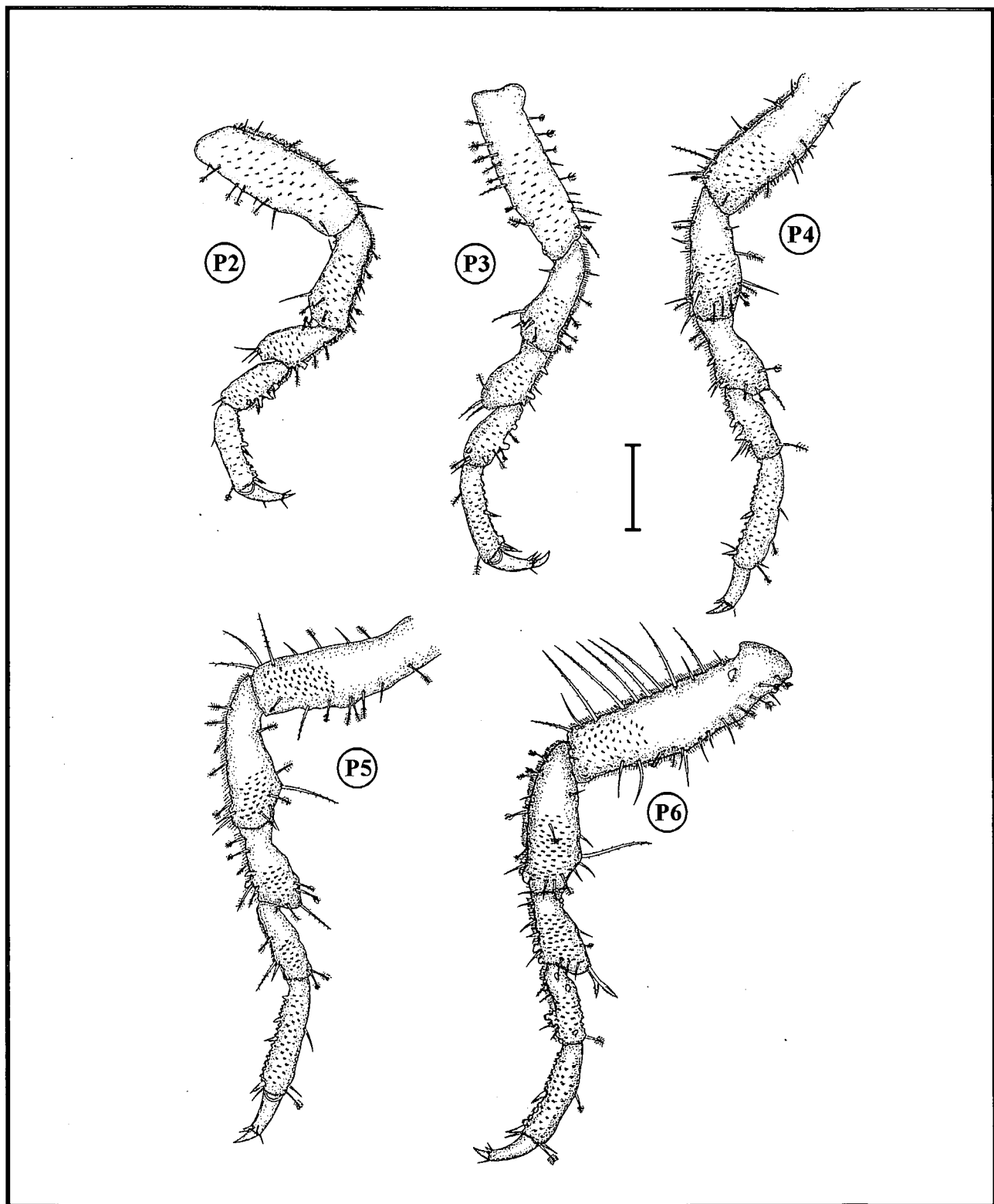


Figure 4.11. Microscope projection drawings of pereopods 2 to 6 (P2 - P6) of a *Gnathia pantherinum* sp. n. female. Scale bar: 200 μ m.

Figure 4.12

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. female (female larva collected at Jeffreys Bay and moulted into an adult female in the laboratory)

- A. Dorsal view of cephalosome
- B. Dorsal view of frontal border
- C. Lateral view of cephalosome
- D. Ventral view of left eye
- E. Dorsal view of cephalosome and pereonites 1-3
- F. Ventral view of pereon with overlapping oostegites

Scale bars: A-E = 100 μ m

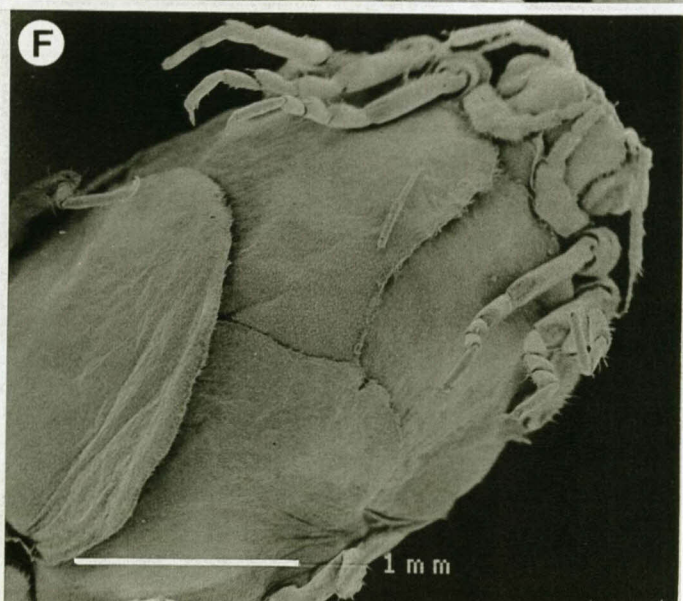
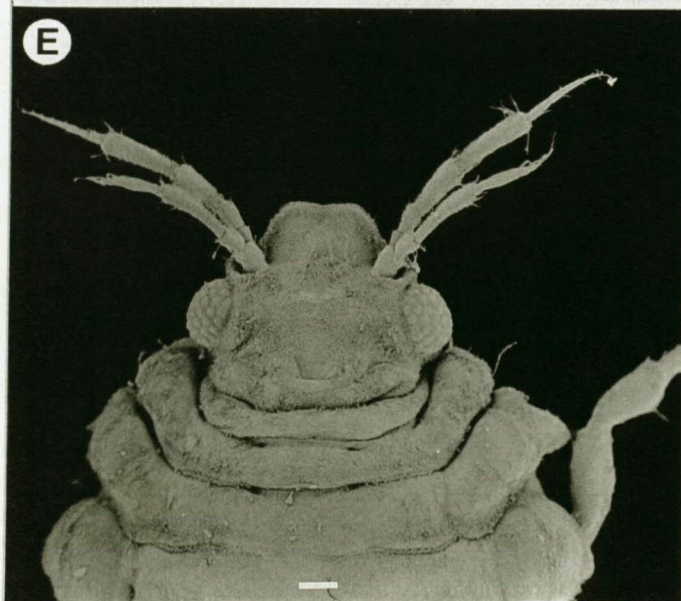
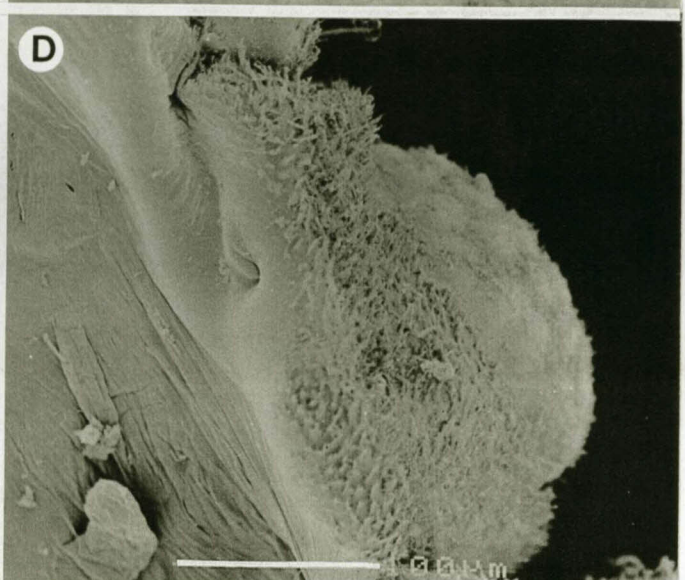
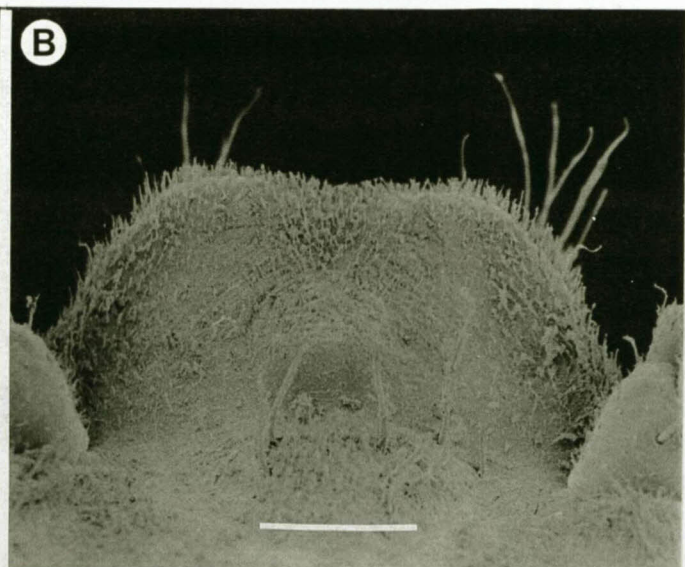
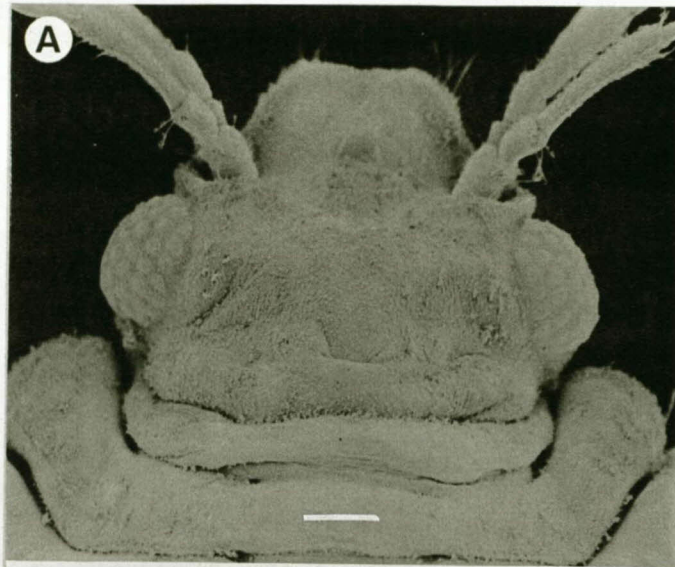


Figure 4.13

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. female
(female larva collected at Jeffreys Bay and moulted into an adult female in the
laboratory)

- A. Left pylopod
- B. Articles 2 of the pylopods
- C. Maxillipedes
- D. Endites of the maxillipedes
- E. Cephalosome with the pylopods and maxillipedes removed
- F. Reduced maxilla

Scale bars: A-C & E = 100 μm ; D & F = 10 μm

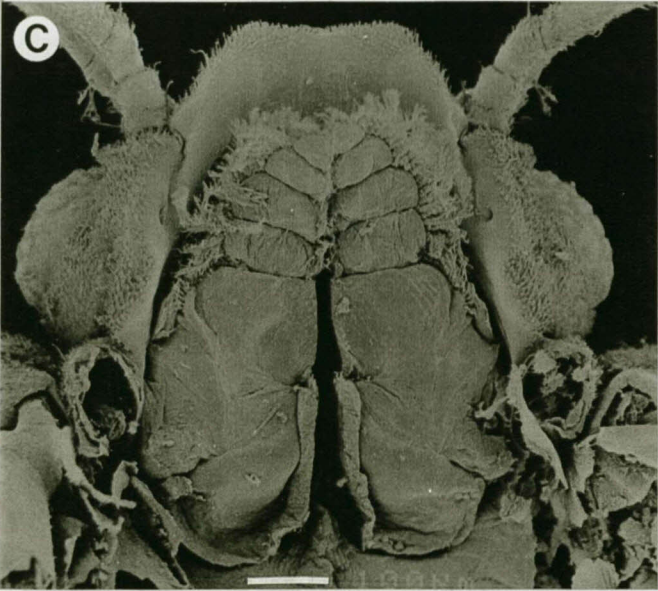
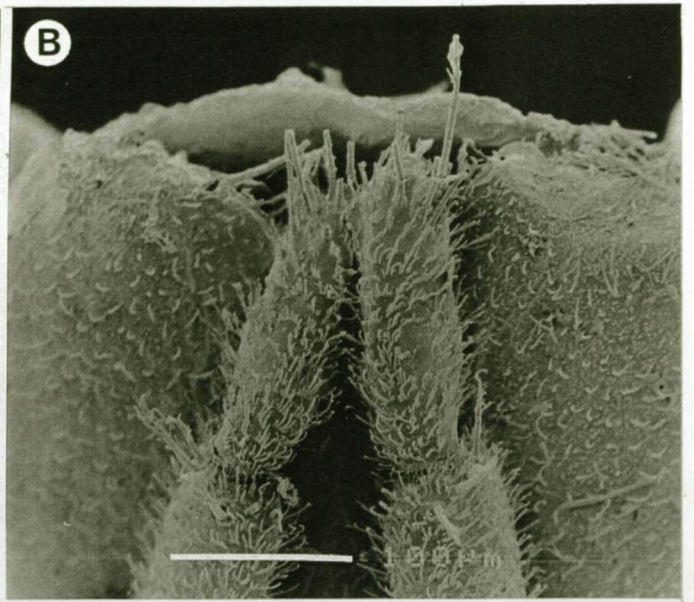
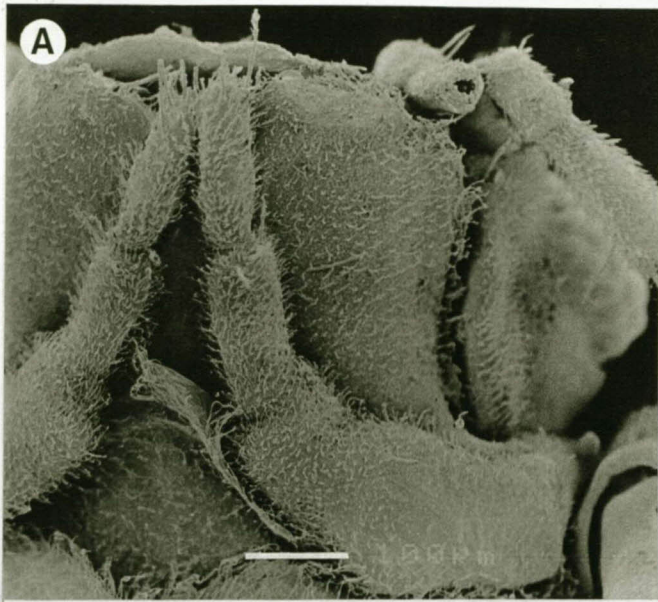
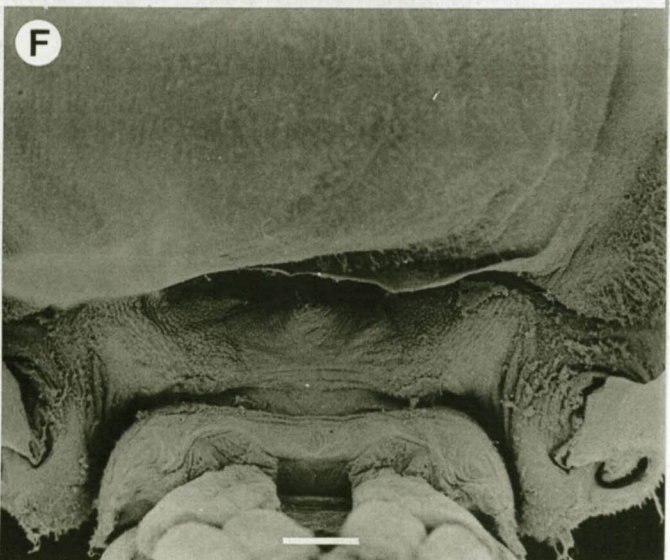
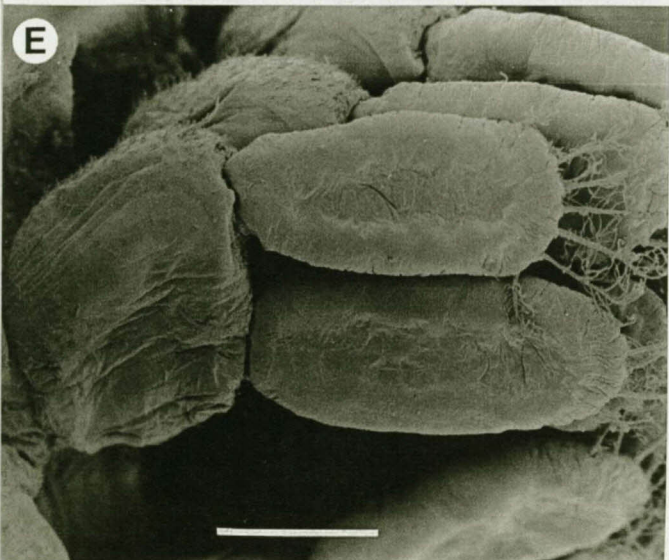
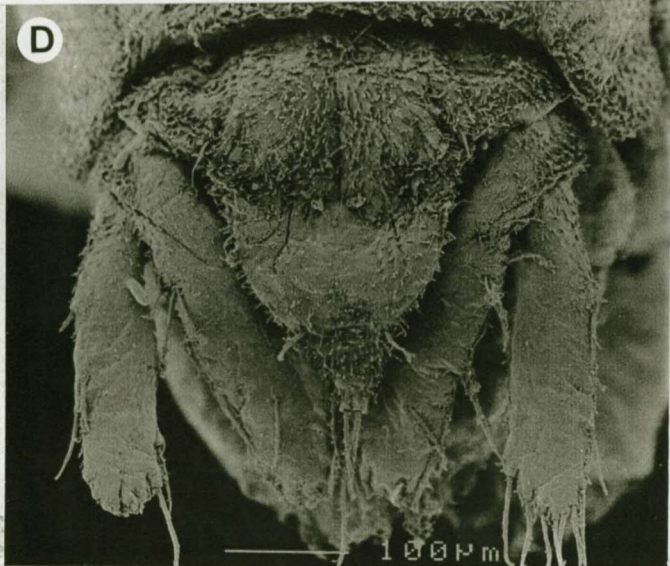
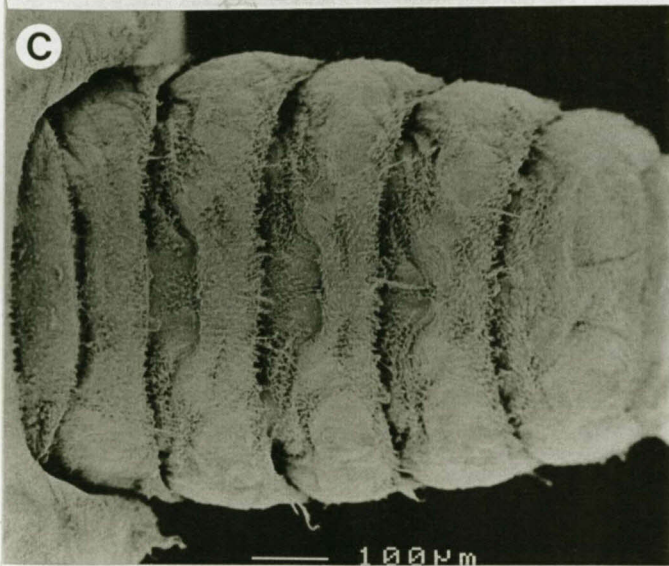
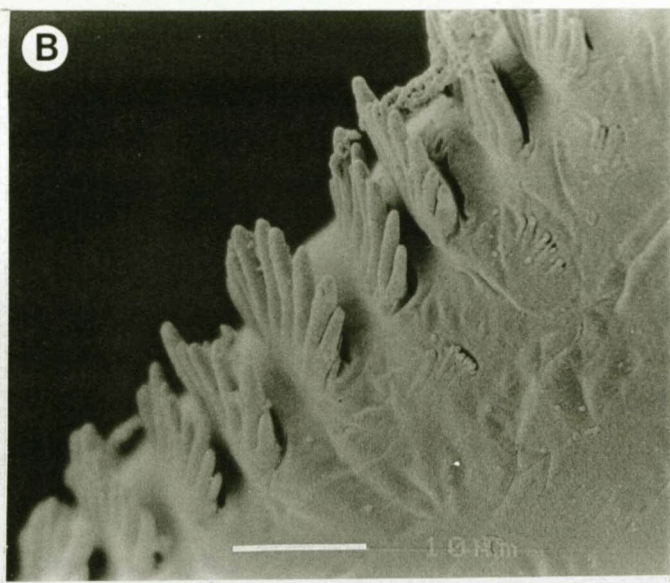
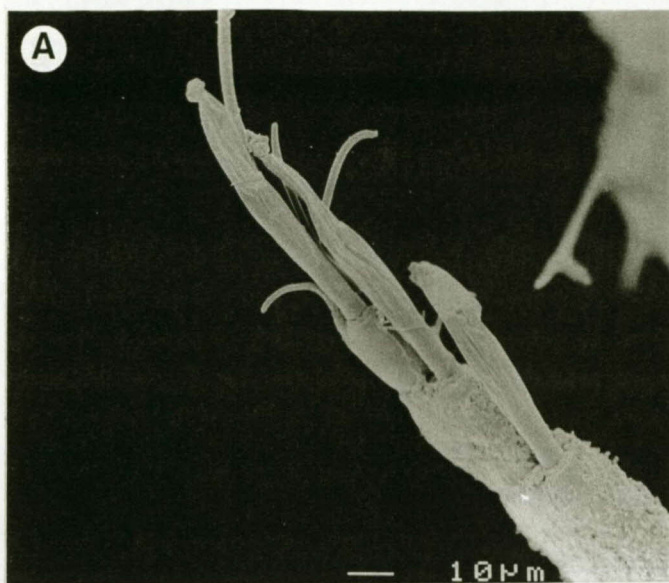


Figure 4.14

**Scanning electron micrographs of a *Gnathia pantherinum* sp. n. female
(female larva collected at Jeffreys Bay and moulted into an adult female in the
laboratory)**

- A. Antenna 1 flagellum articles 3-5, with one aesthetasc each
- B. Pectinate scales on propodus of pereopod 2
- C. Dorsal view of pleon
- D. Dorsal view of pleotelson and uropods
- E. Ventral view of left pleopod 1
- F. Ventral view of genital opening on pereonite 6

Scale bars: E & F = 100 μ m



Praniza larva

Figs. 4.15-4.20

Description: Total lengths of paratypes: 3.9-5.8 mm (5.1 ± 1.1 mm, $n = 10$). Total lengths of other material: 3.8-6.0 mm (5.2 ± 1.0 mm, $n = 30$)

Cephalosome. Posterior margin straight, slightly wider than anterior margin, almost as wide as long, lateral margins slightly convex (Figs. 4.15B, 4.18A). Many sensory pits and three pairs of short simple setae on dorsal surface of cephalosome. Compound eyes large, well developed, oval-shaped, bulbous, on lateral margins of cephalosome, length of eye almost same as cephalosome (Fig. 4.18C). No sulcuses or tubercles on dorsal cephalosome. Medio-anterior margin of cephalosome straight with lateral concave excavations to accommodate first articles of antennae.

Labrum. Prominent, half length of cephalosome, semicircular with apical process, truncated posterior margin, anterior margin concave (Fig. 4.18B). Ventral part of labrum gutter-like with central groove, covers mandibles dorsally and laterally.

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles, a pair of feather-like setae on articles 1 and 2 respectively, article 3 largest with short hair-like setae and pectinate scales on anterior border. Flagellum with four articles, first article with two to three short simple setae mid dorsally, article 2 largest, articles 2 and 3 with one aesthetascs setae each, article 4 terminating in one aesthetascs and three simple setae, few setae on each article (Figs. 4.15C, 4.18D). Antenna 2 with four peduncle articles, article 4 largest, flagellum with seven articles, article 7 terminating in three to four simple setae, few setae on distal end of each article (Fig. 4.15D). Antennae slightly bent.

Mandible. Stout, swollen at base, distal margin styliform with eight teeth on mesial margin, two teeth small and situated at tip of mandible, six larger, triangular and backwardly directed, increasing in size from anterior to posterior (Figs. 4.16A, 4.18E).

Gnathopod. Smaller than pereopods, seven articles, single feather-like seta on basis, only few simple setae on other articles (Figs. 4.16B, 4.18F). Dactylus strongly hooked (Fig. 4.19A).

Maxilliped. Large, cylindrical, elongated base with pectinate scales and short hair-like seta laterally, endite almost reaching palp with single long simple seta coupling hook (Figs. 4.16C, 4.18F). Palp with three articles, first article acute with three to five small teeth and a single simple seta mesially (Fig. 4.19B), articles 3 with five to seven simple setae (Fig. 4.16C).

Maxillae. Not visible.

Maxillule. Long, slender, swollen base, stretching past distal margin of labrum (Figs. 4.16D, 4.19C). Seven small teeth on distal inner margin (Fig. 4.19D).

Paragnaths. Elongated, gutter-like, terminates in sharp point, no teeth (Figs. 4.16E, 4.19C).

Pereon. Almost twice as long as wide, wider than cephalosome (Fig. 4.15A). Pereonite 1 fused with cephalosome, dorsally visible (Fig. 4.18A). Pereonite 2 with anterior constriction separating it medianly from pereonite 1. Pereonite 4 twice as wide as long, lateral sides tapering towards rounded posterior margin, posterior margin stretching over pereonite 5 (Fig. 4.15A), lateral shields at leg attachment. Pereonite 5 consists of elastic membrane fully expanded in praniza stage with blood meal, bulbous shields present on lateral sides at leg attachment. Pereonite 6 rectangular, posterior margin slightly concave, lateral shields at leg attachment. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite (Fig. 4.15A). Sensory pits randomly distributed over all pereonites.

Pleon. Pleon and pleotelson slightly half length of pereon. Five pleonites dorsally visible (Fig. 4.15A). Single simple setae, on each posterior lateral side of each pleonite.

Pleopod. Endopod larger than exopod. Both fringed distally with seven to nine long plumose setae, short hair-like setae on all margins (Fig. 4.15E). Sympodite with retinacula, single simple seta on lateral margin and short hair-like setae on dorsal surface and margins.

Pleotelson. Triangular, longer than wide, anterior half of lateral margins slightly concave, posterior half straight, a pair of simple setae on posterior dorsal surface, distal apex terminating in pair of simple setae, dorsal surface covert with pectinate scales (Fig. 4.15F).

Uropod. Endopod extending beyond apex of pleotelson, exopod reaching apex. Endopod longer and wider than exopod, both with long fringing setae, endopod with mesial six setae plumose, exopod with mesial four setae plumose, rest of setae simple (Fig. 4.15F). Lateral dorsal areas of both endo- and exopods with pectinate scales, all margins with short hair-like seta. Uropodal basis with two simple setae.

Pereopods. Pereopod 2 basis elongated with single feather-like bristle and two to four simple setae anterior, single simple seta posteriorly (Fig. 4.17). Ischium three quarters length of basis and almost as wide, two to four anterior setae and two to three posterior. Merus half the length of ischium with anterior bulbous protrusion, single long denticulated compound spine and a single simple seta on bulbous protrusion, posterior

margin with simple setae. Carpus of almost same size and shape as merus, but without anterior bulbous, tubercles and single feather-like seta on posterior margin. Propodus about twice the length of carpus, two short denticulated spines ending in sharp points situated on middle and distal part of posterior side respectively, only a few simple setae anteriorly with single feather-like seta antero-distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, two to three simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 (Fig. 4.17), similar to pereopod 2 in basic form, differ in setation and number and presence of spines. Pereopods 3 to 5 without long denticulated compound spines, pereopod with two on bulbous protrusion of merus and a single one on anterior margin of carpus. Pereopods 2 and 3 are directed posteriorly and pereopods 4 to 6 anteriorly. All articles of pereopods with pectinate scales and short hair-like setae (Fig. 4.17).

Remarks. No morphological differences were found between the zuphea and praniza stages, except that in the praniza stages the elastic membrane between pereonites 3 and 6 were fully stretched due to the presence of the blood meal in the anterior hindgut (Fig. 4.19E). As in the case of gnathiid females, not a large number of comprehensive descriptions of gnathiid larvae exist. On comparing the descriptions of the known species it is easy to initially agree with different authors who consistently suggest that there does not really exist morphological differences between the larvae of different species (see Cohen and Poore 1994). However, on closer examination it was found that taxonomically important characteristics, such as the shape of the pleotelson and the number and shape of teeth on the mandible, maxillule and maxilliped, can be used in the separation of the larvae of different species. To illustrate this point, a detailed comparison of these taxonomically important characters will be made between the praniza larvae of *G. pantherinum* and those fully described in literature. Of the South African species, a detailed description of only the larvae of *G. africana* is available (see Smit *et al.* 1999a, Appendix 9.1). Other comprehensive descriptions have been done for the larvae of *Gnathia maxillaris* by Davies (1981), *Caecognathia calva* by Wägele (1987), *Paragnathia formica* by Charmantier, Euzet and Davies (1987), *Euneognathia gigas* by Brandt and Wägele (1991), and *Caecognathia bicolor* (Hansen, 1916) by Svavarsson (1999).

The basic morphology of *G. pantherinum* larvae is very similar to that of *G. africana* larvae. *Gnathia pantherinum* can, however, be distinguished from *G. africana* by the following morphological characteristics. The pleotelson of *G. pantherinum* larvae have concave antero-lateral margins in comparison to the straight margins of those of *G. africana* larvae. The mandibles of *G. africana* have nine to 10 teeth on the mesial margin, in comparison to the eight teeth of *G. pantherinum*. The first palp article of the maxilliped of *G. africana* has five to seven teeth distally and *G. pantherinum* three to five small teeth distally. The maxillipedal endite of *G. africana* lacks the coupling hook, which is present in *G. pantherinum*. Praniza 3 larvae of *G. pantherinum* are also much larger than those of *G. africana*.

The pleotelson of *G. pantherinum* larvae is very similar to that of *G. maxillaris*. Although it appears, from the electron micrograph of Davies (1981), to be rather slightly broader than long, in comparison to the longer than broad pleotelson of *G. pantherinum*. The pereon and pleon of *G. maxillaris* larvae are also covered with pectinate scales in comparison to the smooth surface of *G. pantherinum*. *Gnathia maxillaris* mandibles have twelve teeth, with the maxillules and the first article of the maxillipedal palp bearing nine teeth respectively. This differs from *G. pantherinum*, which has mandibles with six, maxillules with seven and a maxillipedal palp with three to five teeth. The endite of the maxilliped of *G. maxillaris* does not possess a coupling hook.

The slightly longer than broad pleotelson of *G. pantherinum* larvae differs considerably from that of *C. calva* larvae. The lateral margins of the pleotelson of *C. calva* larvae are concave and the pleotelson is almost twice as long as broad with the uropods not surpassing the apex of the pleotelson (see Wägele 1987). The mandibles of both these species have eight teeth, while the maxillule of *C. calva* has eight compared to the seven of *G. pantherinum*. The endite of the maxilliped of *C. calva* surpasses the insertion of the palpal endites, whereas in *G. pantherinum*, the endite only reaches the palp.

The larvae of *G. pantherinum* can easily be distinguished from those of *P. formica* by the number of flagellum articles of antenna 2. *Gnathia pantherinum* has seven flagellum articles and *P. formica* nine. These two species also differ considerably in the number of the mandible teeth. Those of *P. formica* have 12 or 13 teeth, compared to *G. pantherinum*, which only has eight. The gnathopods of *P. formica* larvae bear a small

tooth near the base of the dactylus, a characteristic which is absent in *G. pantherinum* larvae. The first article of the maxillipedal palp of *P. formica* has 11 or 12 teeth compared to the three to five of *G. pantherinum*. When comparing *G. pantherinum* larvae to the larvae of another genus, *Euneognathia gigas*, the most distinct difference is again the shape of the pleotelson. The pleotelson of *E. gigas* larvae is narrow and more than twice as long as wide, with the uropods also not surpassing the pleotelson apex. In the case of *G. pantherinum* the pleotelson is slightly longer than broad with the endopod of the uropods surpassing the apex of the pleotelson. Of all these species *G. pantherinum* can easily be distinguished from the larvae of *Caecognathia bicolor* since the larvae of *C. bicolor* does not possess eyes and the flagellum of antenna 2 consists of only six articles.

The small size of the mouthparts and cephalosome appendages of most of the gnathiid larvae species makes them difficult to study. It is, however, a worthwhile aspect to study, because when used in combination with the morphology of the pleotelson and the number of antenna 2 flagellum articles, it proves possible to distinguish between the larvae of different species. Unfortunately, due to the lack of information on these specific characteristic of larvae of other species, it is difficult to be certain that the above mentioned characteristics are suffice to distinguish between the larvae of all species. However, until further information proves otherwise, it appears as if these characteristics may very well be used in distinguishing between different larvae.

Taxonomic summary of *Gnathia pantherinum* sp. n.

Type-material: Holotype: In the collection of the National Museum, Bloemfontein (1 male) (No specimen number available yet). Paratypes: In the collection of the National Museum, Bloemfontein (5 males, 6 females, 10 praniza larvae) (No specimen number available yet)

Type-locality: Jeffreys Bay (34°2.2'S, 24°56.5'E).

Other localities: De Hoop Nature Reserve (34°28'S, 20°30'E).

Hosts: *Poroderma pantherinum* (Smith, 1838) (type host), *Haploblepharus edwardsii* (Voight, 1832) and *Torpedo fuscomaculata* Peters, 1855.

Other material: In the collection of the author (13 males, 10 females, 30 praniza larvae).

Etymology: The species is named for the specific name of the type host *Poroderma pantherinum*.

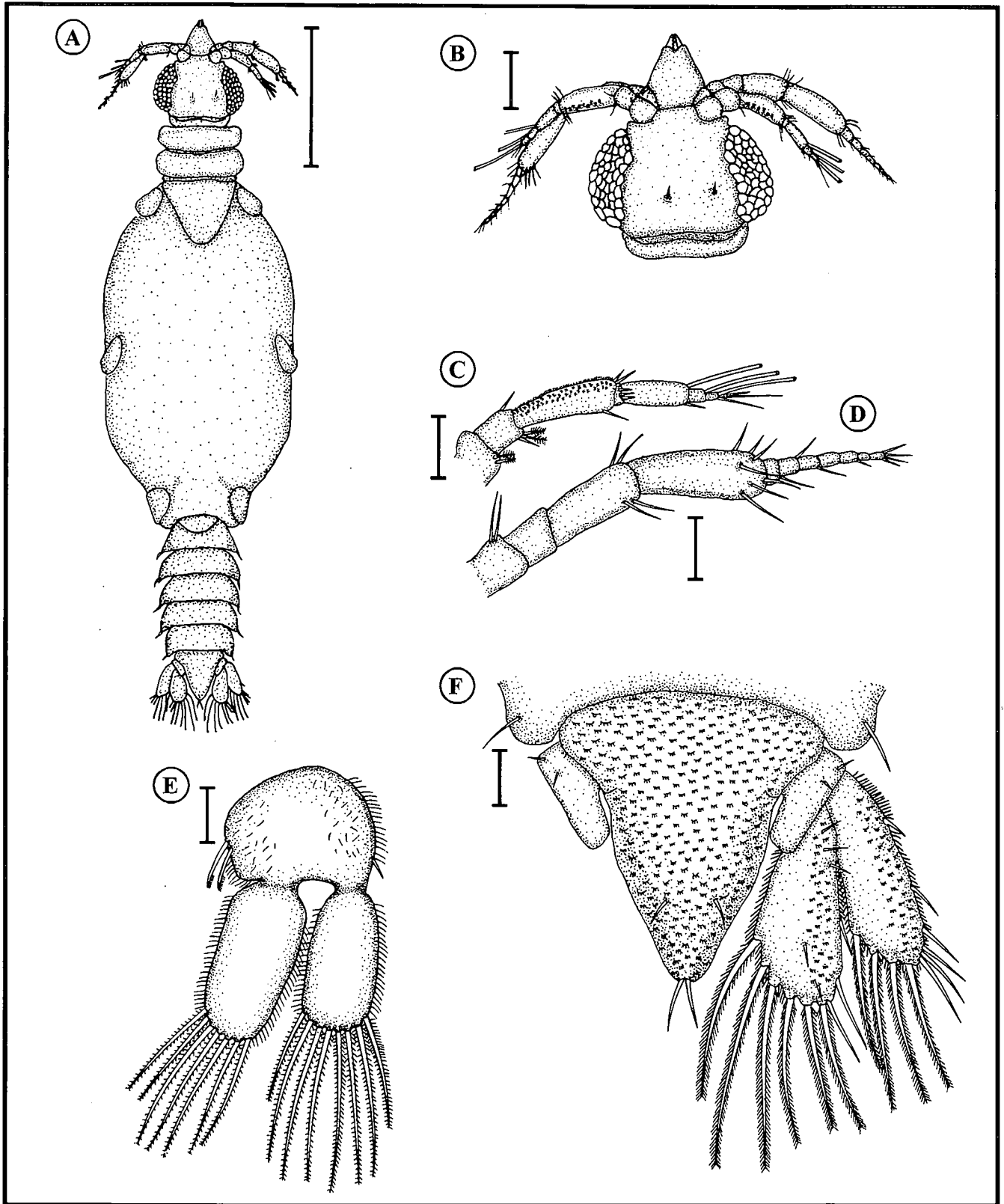


Figure 4.15. Microscope projection drawings of a *Gnathia pantherinum* sp. n. praniza larva. **A.** Full length dorsal view. **B.** Dorsal cephalosome. **C.** First antenna. **D.** Second antenna. **E.** Left pleopod 1. **F.** Pleotelson and uropods. Scale bars: A = 1 mm, B = 250 μm , C-F = 100 μm .

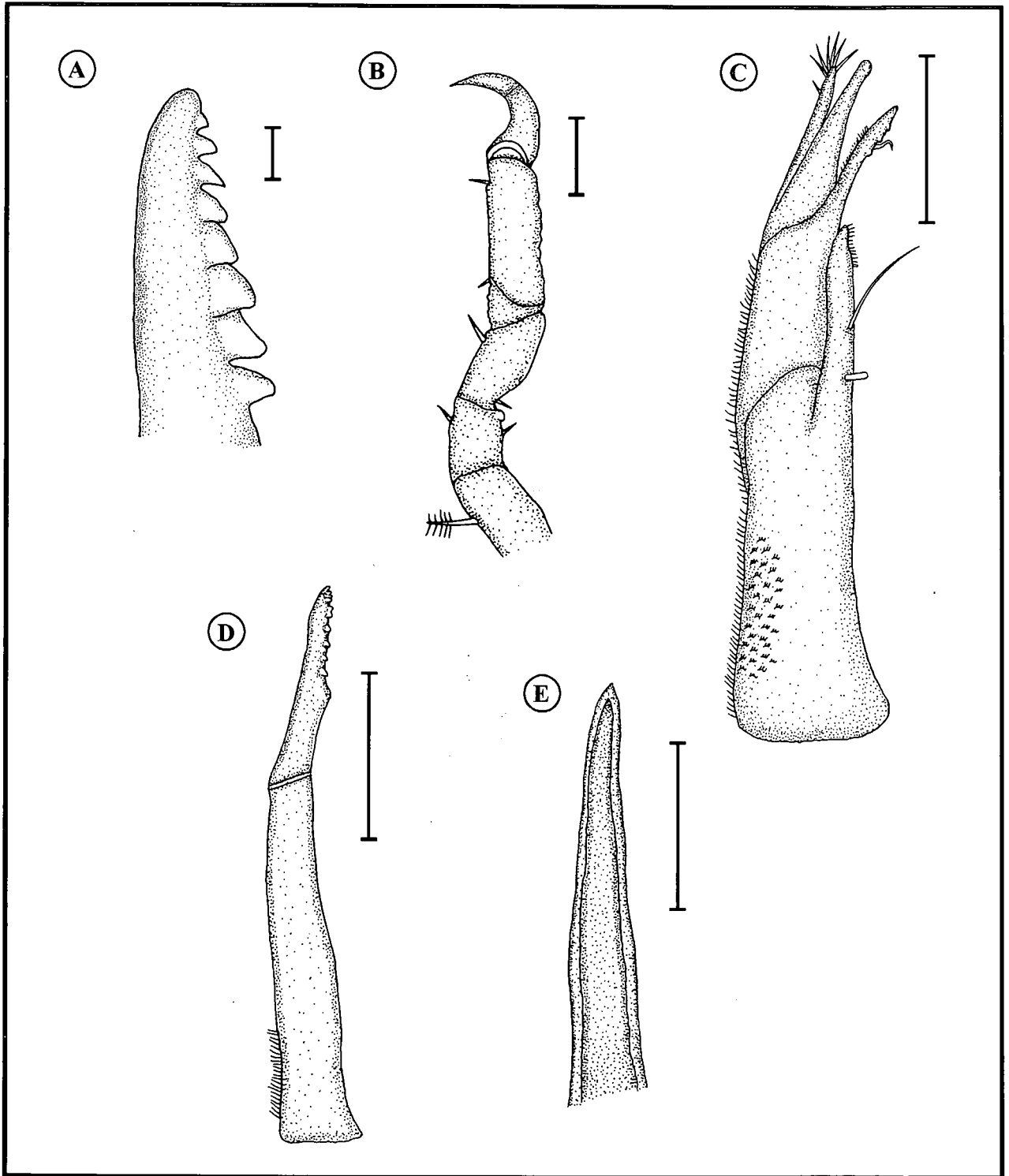


Figure 4.16. Microscope projection drawings of the cephalosome appendages of a *Gnathia pantherinum* sp. n. praniza larva. **A.** Mandible **B.** Gnathopod **C.** Maxilliped **D.** Maxillule **E.** Paragnath. Scale bars: A = 10 μm , B-E = 100 μm .

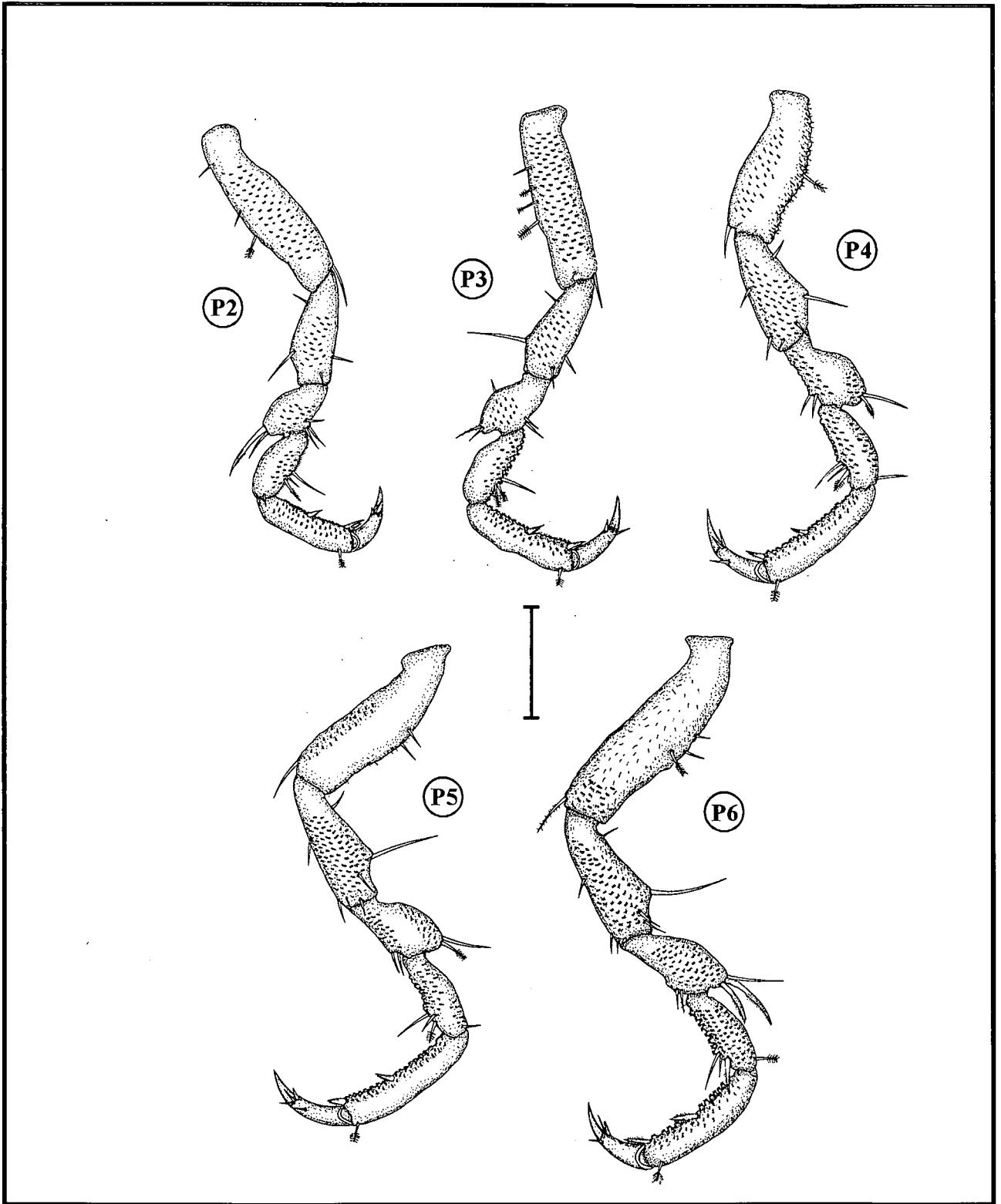


Figure 4.17. Microscope projection drawings of the pereopods 2 to 6 (P2 - P6) of a *Gnathia pantherinum* sp. n. praniza larva. Scale bar: 200 μ m

Figure 4.18

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. praniza larva,
collected from the leopard catshark at Jeffreys Bay

- A. Dorsal view of cephalosome
- B. Dorsal view of labrum
- C. Lateral view of cephalosome and eye
- D. Distal flagellum articles of Antenna 1 with aesthetasc setae
- E. Apex of mandible (m) with eight teeth
- F. Ventral view of cephalosome with gnathopods (g) and maxillipedes (mx)

Scale bars: A & B = 100 μ m; E = 10 μ m

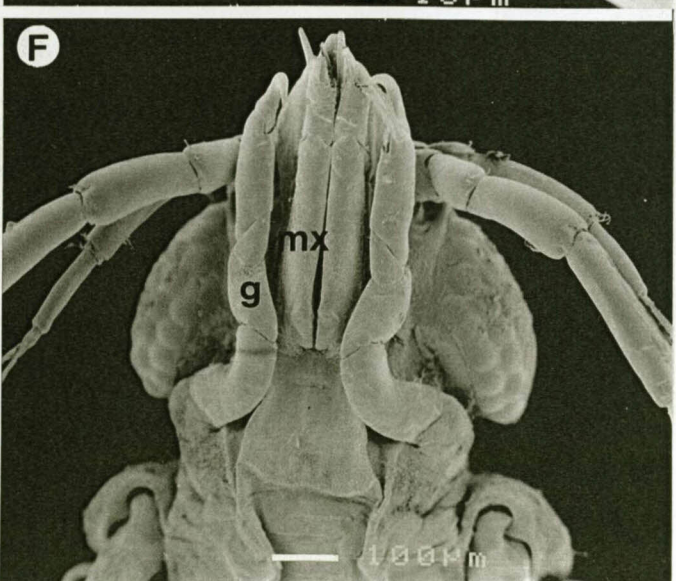
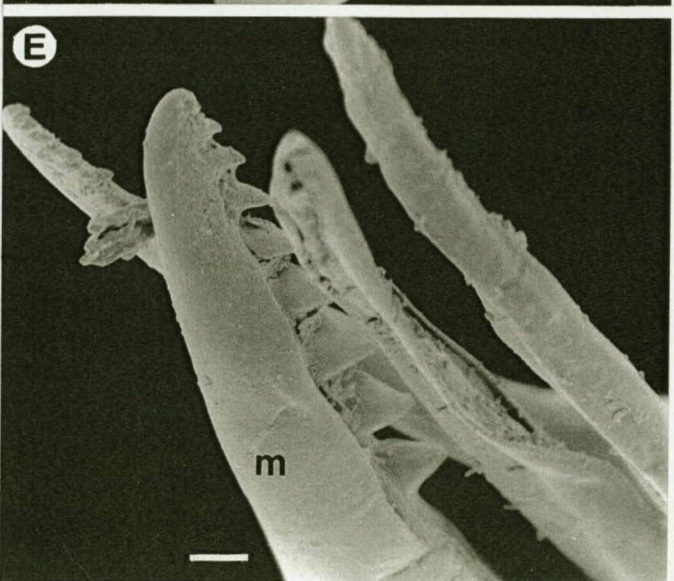
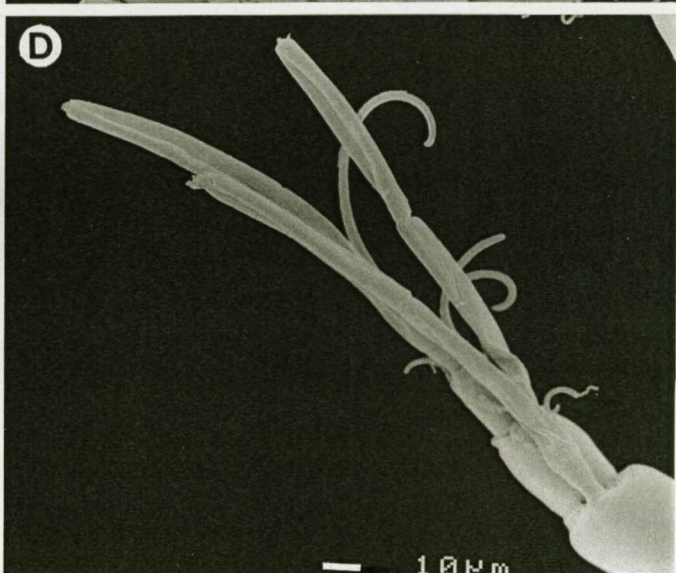
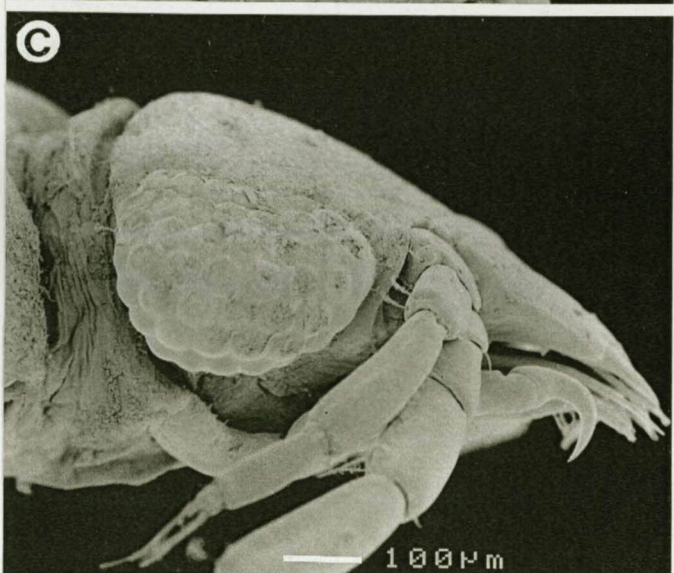
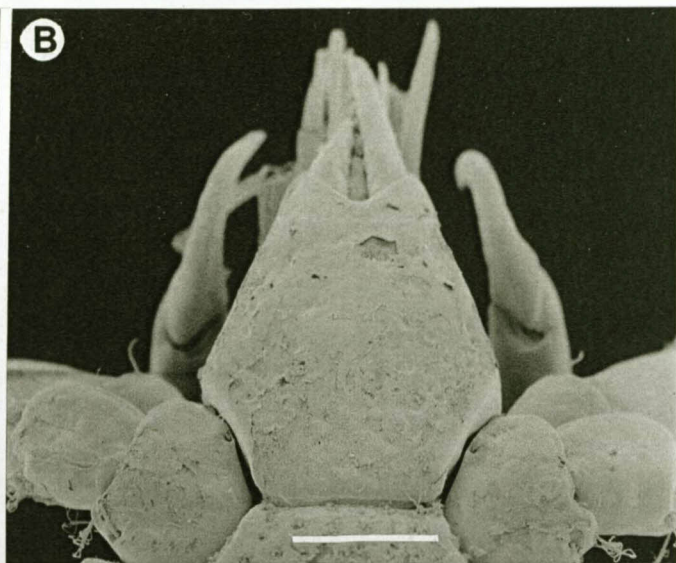
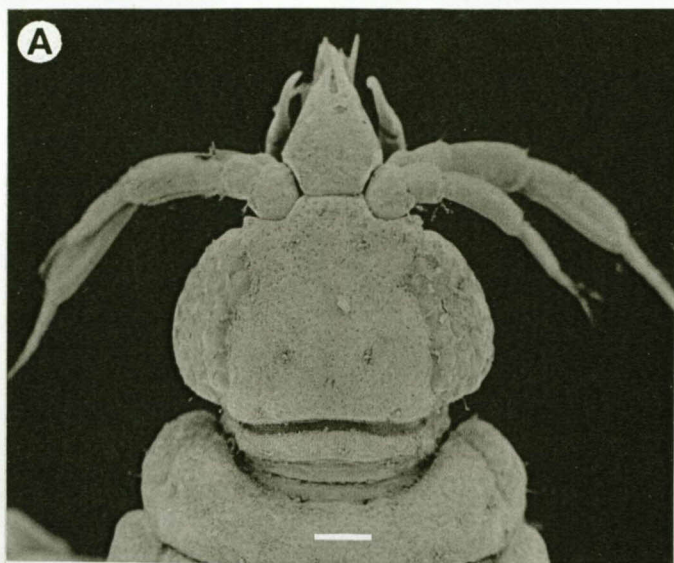
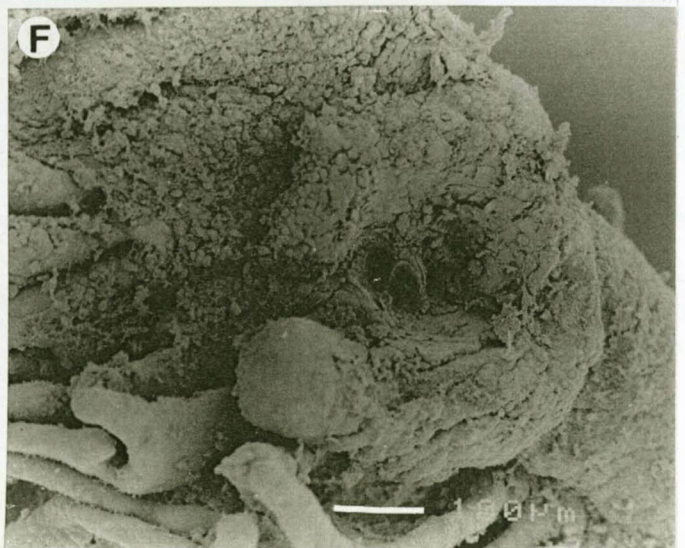
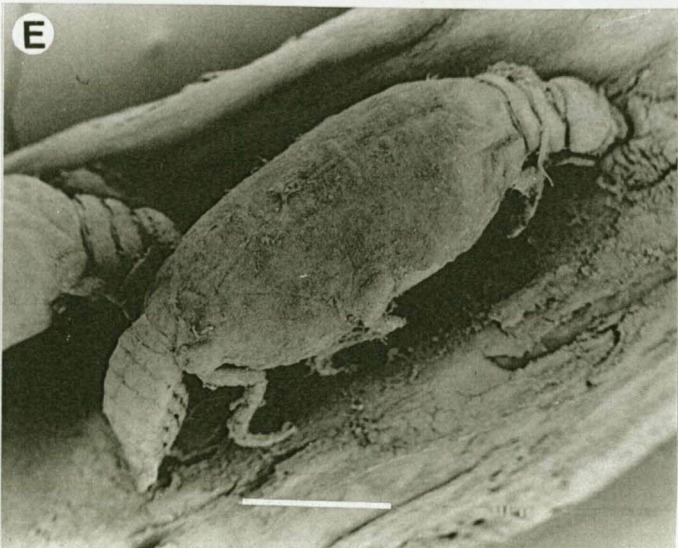
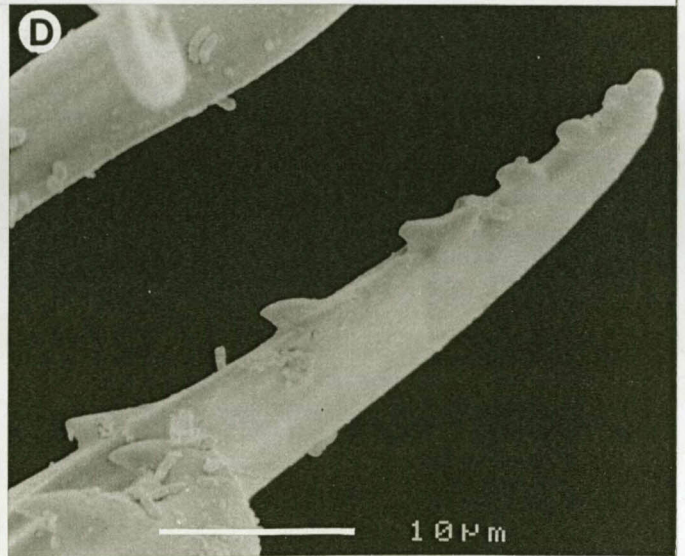
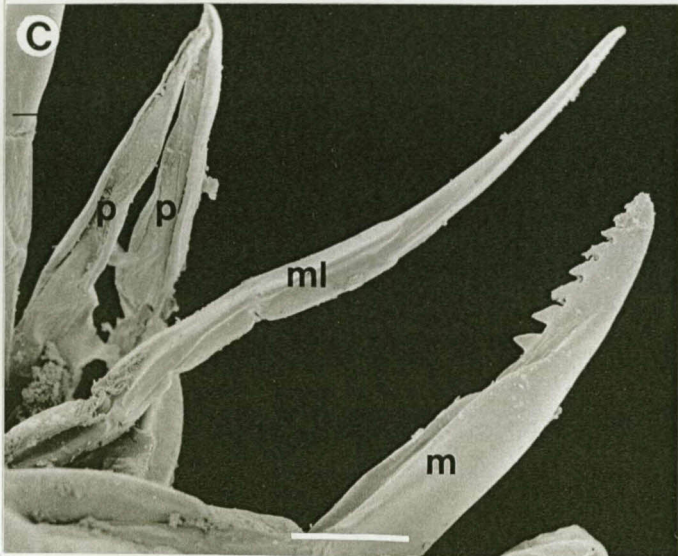
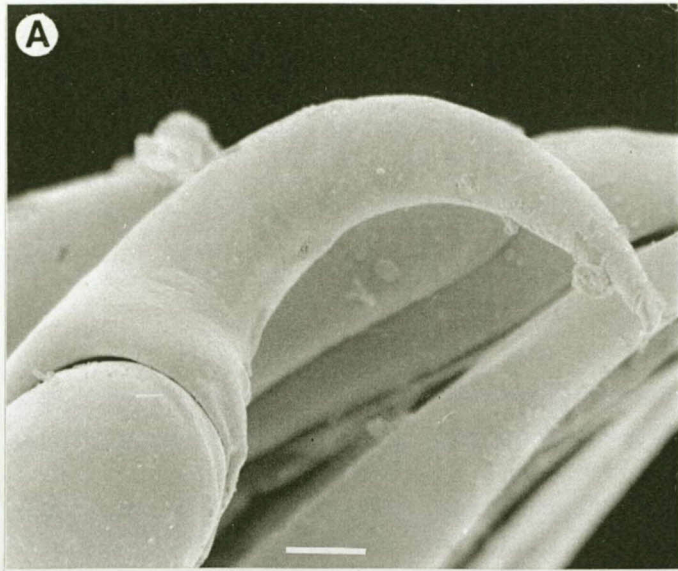


Figure 4.19

Scanning electron micrographs of a *Gnathia pantherinum* sp. n. praniza larva, collected from the puffadder shyshark at the De Hoop Nature Reserve

- A.** Ventral view of the hooked dactylus of left gnathopod
- B.** Article 1 (a), 2 (b) and 3 (c) of the maxillipedal palp
- C.** Lateral view of a mandible (m), maxillule (ml) and paired paragnaths (p)
- D.** Distal point of maxillule with small teeth
- E** Fully fed praniza larvae attached to the gill septum of host
- F** Lesions left by the feeding of larvae on the gill septum of host

Scale bars: A & B = 10 μm ; C = 100 μm ; E = 1 mm



4.2 Parasitic larvae of *Gnathia pantherinum* sp. n.

The study of the parasitic gnathiid larvae was recently revived in Australia and New Zealand through an ongoing in-depth study of cleaning symbiosis by scientists from that region (Grutter 1994, 1996, 1999a,b, Grutter and Poulin 1998). Grutter (1996, 1997a) found that gnathiid larvae constitute the largest portion of ectoparasites removed by cleaner fish from client fish, with the cleaners showing a preference for larger gnathiid specimens (Grutter 1997b). This led to a number of papers being published on different aspects of the biology of gnathiid larvae, such as the relationship between host size and abundance of larvae (Grutter and Poulin 1998), the diurnal variation in abundance of gnathiid larvae on host fish (Grutter and Hendrikx 1999), the infestation dynamics of gnathiid larvae found parasitic on a coral reef fish species (Grutter 1999c) and the pathology of gnathiid larvae infecting a shark species (Heupel and Bennet 1999). The importance of the ability to identify gnathiid larvae, resulted in the work by Grutter, Morgan and Adlard (2000), introducing the use of molecular techniques to distinguish between different species of larvae and to subsequently match them with the adult stages. The work on cleaner fish and their main food source, gnathiids larvae, is not confined to Australasia. Investigations by Sikkel, Fuller and Hunte (2000) in the Caribbean also showed that gnathiid larvae were the most common crustacean parasite on client fish (71.25%). In many of the above mentioned case studies on cleaning symbiosis, elasmobranchs were found to be one of the hosts of these gnathiid larvae. To quantify all the results, more information is thus needed on the parasitic larvae of gnathiids including those found parasitising elasmobranchs.

Most of the larvae in the present study were found inside the branchial cavity where they were attached to the gill filaments and the interbranchial septa of the host fish (Table 4.2). The same kind of lesions as described by Heupel and Bennet (1999) were found on the gills and gill septa where larvae had been attached (Figs. 4.19E,F). In the case of one puffadder shyshark, three larvae were attached to the inside of the left nostril and a single specimen to the floor of the buccal cavity. Of the 148 larvae collected from all the hosts, only four (from the leopard catshark) were zuphea larvae (Fig. 4.21A). In all cases, the hosts were kept alive for 24 hours after capture, to see whether fed larvae would detach. In only three out of the 148 larvae collected, did the larvae detach while the hosts were still alive. This suggested that the feeding period of the final stage larvae of *G.*

pantherinum must be longer than 24 hours. This is much longer than the feeding period of *G. africana* (see Chapter 3) as well as that of unidentified gnathiid larvae used by Grutter and Hendrikx (1999) in laboratory experiments (4 hours). All the larvae found in the present study were final stage larvae. This means that, if the life cycle of *G. pantherinum* follows the same three larval stage found in the current study for *G. africana*, one of two possibilities exist. Firstly that the elasmobranch hosts investigated do not constitute the hosts for the first and second stage larvae of this species or secondly that the life cycle follows strict seasonal patterns with no overlapping of larval stages. Since the material was collected in three different seasons (summer, autumn and spring), the second possibility can probably be ruled out. The question of the hosts for the first two stages are unanswered at present and this needs further investigation.

A positive correlation was found between the number of larvae and the size of the five puffadder shysharks investigated (Fig. 4.20). Grutter and Poulin (1998) found that the same is true for a variety of teleost and elasmobranch species infected with gnathiid larvae collected on Lizard and Heron Island in the Great Barrier Reef. This also agrees with the results of Heupel and Bennett (1999), who found that the abundance of gnathiids parasitising the epaulette shark, *Hemiscyllium ocellatum* correlates positively to the host size.

Table 4.2. Number and site of attachment of parasitic larvae of *Gnathia pantherinum* sp. n. on three species of elasmobranchs investigated.

Fishes			Larvae				
Fish species	n	Length (mm)	NO	BC	G	T	MB ± S (Range)
<i>Poroderma pantherinum</i>	1	580	0	0	34	34	-
<i>Torpedo fuscomaculata</i>	1	388	0	0	3	3	-
<i>Haploblepharus edwardsii</i>	5	448, 510, 610, 620, 640	3	1	107	111	22 ± 19.2 (2-45)

n – number

NO – nose opening

BC – buccal cavity

G - Gills

T - Total

MB - mean burden.

S- Standard deviation

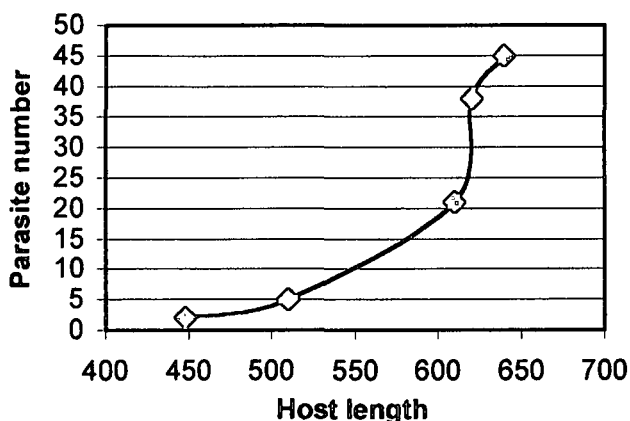


Figure 4.20. Line graph of the number of *Gnathia pantherinum* sp. n. parasitising *Haploblepharus edwardsii* (Voight, 1832), plotted against total host fish length.

4.3 Final life cycle stages of *Gnathia pantherinum* sp. n.

The collection of final stage larvae from the elasmobranch hosts permitted the study of the development of these larvae into the adult stages, as well as the development of the eggs and embryos in the females. This study follows the same pattern as the study on *G. africana* and will be compared with it. As already mentioned, the larvae of *G. pantherinum* exclusively feed on blood, thus lacking the transparency of *G. africana*, making the observation of egg and embryonic development more difficult.

Of the 148 larvae collected, 30 were fixed for scanning electron microscopy and morphological studies. Two larvae died before their sex could be determined. Of the remaining 116 larvae 73 were females and 43 males. The length of the moulting periods of both males and females were not determined.

Development of the male of *Gnathia pantherinum*

The development times of male larvae to adult males of 20 specimens from all the different hosts and from the same host collected at different times of the year were determined (see Table 4.3). All three larvae collected from the electric ray were male larvae and moulted successfully into adult males. After four to 12 days post feeding

(mean 7 ± 2 days) the testis could be seen in the dorsal pereon of the male larvae. These larvae moulted into adults after eight to 17 days post feeding (mean 12 ± 3 days). The moulting process was similar to that of other isopods and *Gnathia africana*, consisting of a posterior moult (Fig. 4.21B) followed by an anterior moult. As in the case of the males of *G. africana*, the visibility of the distinct pigmentation of the young males (Fig. 4.21C) and specifically the bright red colouring of the pleonites (Fig. 4.21D) indicated that the young males had reached sexual maturity. This happened at 11 to 20 days post feeding (mean 14 ± 3), approximately two days after moulting.

Development of the female of *Gnathia pantherinum*

The complete development of 14 female larvae was followed from their detachment from the fish hosts to the release of the young. Females from all three the different hosts were used in these experiments (see Table 4.4). Ten female larvae died before they reached the point in development where they could moult into adults. Thirty-five female larvae and adult females were fixed during different development stages for light and electron microscopy, as well as for use in the morphological studies.

The developing ovaries of the female larvae became visible at four to 14 days post feeding (mean 9 ± 2 days) (Fig. 4.21E). The eggs filled half of the pereon from day six to day 20 post feeding (mean 14 ± 4 days) and the female larvae (completely filled with eggs) moulted after 13 to 28 days (mean 20 ± 5 days) into adult females. The process followed the same pattern as described for the males and *G. africana* females. The mortality of females during moulting (7/41; 17%) was high in comparison to that of the males (none) and of the females of *G. africana* (1/16; 6%). On completion of the anterior moult the unfertilised eggs could still be seen in the dorsal ovaries (Fig. 4.21F). Fertilisation of the eggs occurred approximately 2 days post moulting after which they were transferred into the ventral marsupium. Fertilisation always took place when a mature male was present. As in the case of *G. africana* females, movement of the eggs from the ovaries to the marsupium occurred regardless of whether the eggs were fertilised or not. The fertilised eggs started to divide at 19 to 34 days post feeding (Figs. 4.22 A,B), with the eye spots of the larvae visible at 21 to 38 days post feeding (Fig. 4.22C). Embryos with yolk in their anterior hindguts were visible at 23 to 42 days post feeding (12 days post moulting) (Fig. 4.22D). Development of the embryos was completed between 25 and 48 days post feeding. At this stage the distinct pigmentation of the

cephalosome of the embryos was the same as that of the larvae (Fig. 4.22E). The larvae started to move around in the marsupium and were subsequently released by the females. The larvae left the female through the opening of the marsupial plates. The females released an average of 105 ± 16 larvae (range 78-128). As in the case of *G. africana*, a positive correlation was found between the size of the female and the number of larvae produced (Table 4.5, Fig. 4.23). All the zuphea larvae released by the different size females were of approximate equal size (range 1.0-1.1 mm for larvae from all females). The spent females (Fig. 4.22F) were able to survive for an average of 13 days after the release of the young.

Unfortunately no sharks were available for feeding experiments with the newly hatched zuphea 1 larvae, to enable the completion of the live cycle as was done for *G. africana* (see Chapter 3). A few cliniids (*Clinus superciliosus*) used in the life cycle studies of *G. africana* were still available. More than one hundred *G. pantherinum* larvae were placed with the cliniids and they immediately swam towards the fish. The larvae were seen walking around on the fish, but not a single larva attached and fed on the cliniids. Three to four days later the starved larvae started to die. No conclusive evidence regarding the host specificity of gnathiid larvae exists, and because of their abundance on a large variety of fish hosts it was assumed that gnathiids are not host specific. Monod (1926) reported that he was able to let the larvae of *Paragnathia formica* feed on more than 15 different fish hosts, even on a frog, *Rana temporaria*. During the study on *G. africana*, the larvae were also able to feed on aquarium fish such as the black molly, *Poecilia sphenops*. Although this feeding experiment with the elasmobranch gnathiid, *G. pantherinum*, is not conclusive, it appears that the larvae of this species do not feed on intertidal teleosts.

Table 4.3. Length of digestion of male praniza 3 larvae of *Gnathia pantherinum* sp. n. collected from their respective fish hosts under laboratory conditions in 20-25°C sea water (PV – Pigmentation visible).

Male Praniza 3 larvae collected from <i>Poroderma pantherinum</i>				
	Testes visible (days)	Moult complete (days)	PV (days)	Length (mm)
1	4	10	11	5.6
2	5	10	12	5.2
3	9	19	20	5.5
4	7	13	15	5.6
5	8	11	13	6.1
Male Praniza 3 larvae from <i>Torpedo fuscomaculata</i>				
6	7	12	15	5.1
7	8	12	14	4.9
8	12	17	19	4.6
Male Praniza 3 larvae from <i>Haploblepharus edwardsii</i> (04/1999)				
9	7	11	12	6.0
10	7	11	13	5.2
11	6	10	12	5.4
12	4	8	10	6.6
13	6	11	13	6.8
Male Praniza 3 larvae from <i>Haploblepharus edwardsii</i> (11/1999)				
14	7	14	17	6.1
15	5	9	11	5.8
16	8	13	15	6.0
17	4	9	11	5.4
18	9	14	15	3.7
19	11	16	18	3.8
20	8	15	17	4.3
Mean ± STD	7 ± 2	12 ± 3	14 ± 3	5.4 ± 0.84

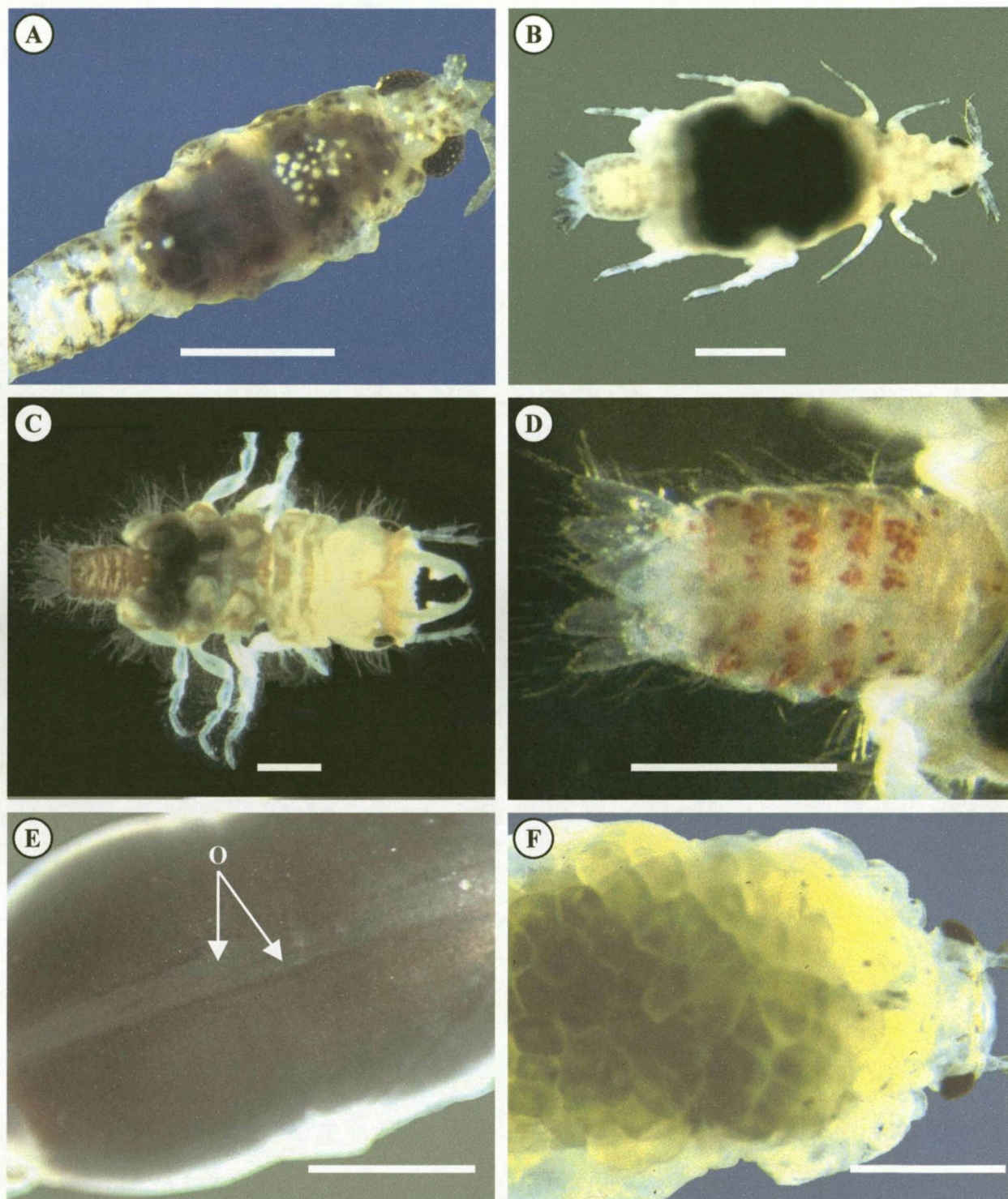


Figure 4.21. Light micrographs of the development stages of *Gnathia pantherinum* sp. n. **A.** Dorsal view of a zuphea 3 larva. **B.** Dorsal view of a male larva with posterior moult complete. **C.** Dorsal view of a mature male. **D.** Ventral view of the pleopods of a mature male. **E.** Ventral view of the pereon of a female larva with developing ovaries (O) visible. **F.** Dorsal view of a female with unfertilised eggs. Scale bars: 1 mm.

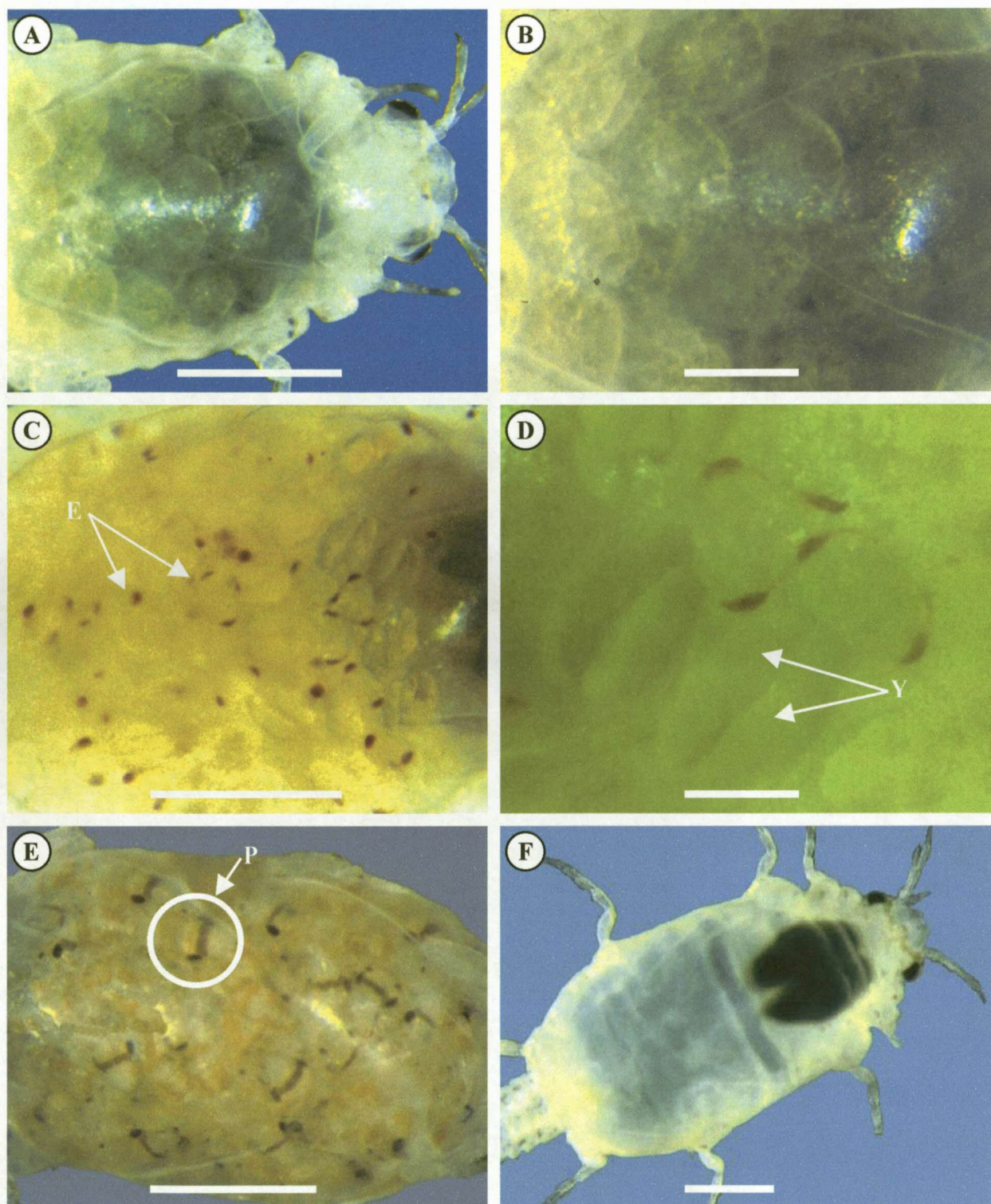


Figure 4.22. Light micrographs of the development stages of *Gnathia pantherinum* sp. n. females. **A.** Ventral view of a female with dividing eggs. **B.** Dividing eggs. **C.** Ventral view of a female with the eyespots (E) of embryos visible. **D.** Young embryos with yolk (Y) in their anterior hindguts. **E.** Ventral view of the female with fully developed embryos. Distinct pigmentation (P) of *G. pantherinum* larvae visible. **F.** Dorsal view of a spent female. Scale bars: A, E & F = 1 mm; B & D = 100 µm; C = 500 µm

Table 4.4. Length of egg and embryonic development periods of female praniza 3 larvae (P3) and female adults of *Gnathia pantherinum* sp. n. under laboratory conditions in 20-25°C seawater [Development (in days) measured from time of detachment from host].

Female Praniza 3 larvae from <i>Poroderma pantherinum</i>											
	D1	D2	D3	D4	F1	F2	F3	F4	F5	F6	F7
1	4	6	9	10	13	15	19	21	23	25	30
2	8	10	15	16	17	19	21	23	25	30	38
3	10	15	19	21	22	23	26	29	33	36	49
4	6	10	13	16	17	19	21	23	25	31	45
Female Praniza 3 larvae from <i>Haploblepharus edwardsii</i> (04/99)											
5	11	14	19	21	22	23	26	29	33	35	53
6	9	15	18	21	22	24	27	29	31	34	48
7	12	19	22	25	26	27	32	35	38	41	55
8	10	12	19	21	22	24	28	32	36	39	52
9	11	19	25	27	28	29	34	38	41	45	60
Female Praniza 3 larvae from <i>Haploblepharus edwardsii</i> (11/99)											
10	9	14	16	16	17	20	26	29	33	37	52
11	8	11	14	16	17	18	24	27	32	38	49
12	14	20	24	26	27	29	34	37	42	48	61
13	9	11	14	15	15	16	20	23	27	32	48
14	10	13	16	17	18	21	25	30	34	36	49
M ± S	9 ± 2	14 ± 4	17 ± 4	19 ± 5	20 ± 5	22 ± 4	26 ± 5	29 ± 5	32 ± 6	36 ± 6	49 ± 8

D1 - Ovaries visible

D2 - Eggs fill half of pereon

D3 - Eggs fill complete pereon

D4 - Posterior moult starts

F1 - Anterior moult complete

F2.- Eggs ventral

F3 - Egg division visible

F4 - Eye spots visible

F5 - Embryos fully developed

F6 - Release of larvae

F7 - Spent female dies

M - Mean

S - Standard deviation

Table 4.5. Length frequencies of *Gnathia pantherinum* sp. n. females and the number of embryos released under laboratory conditions in 20-25°C seawater (M = Arithmetic mean; S = standard deviation).

	Females	
	Length (mm)	Number embryos
1	4.3	78
2	4.3	92
3	4.4	89
4	4.5	94
5	4.9	87
6	4.9	104
7	5	101
8	5.1	103
9	5.2	107
10	5.3	117
11	5.4	119
12	5.4	128
13	5.8	121
14	6	128
M ± S	5 ± 0.53	105 ± 16

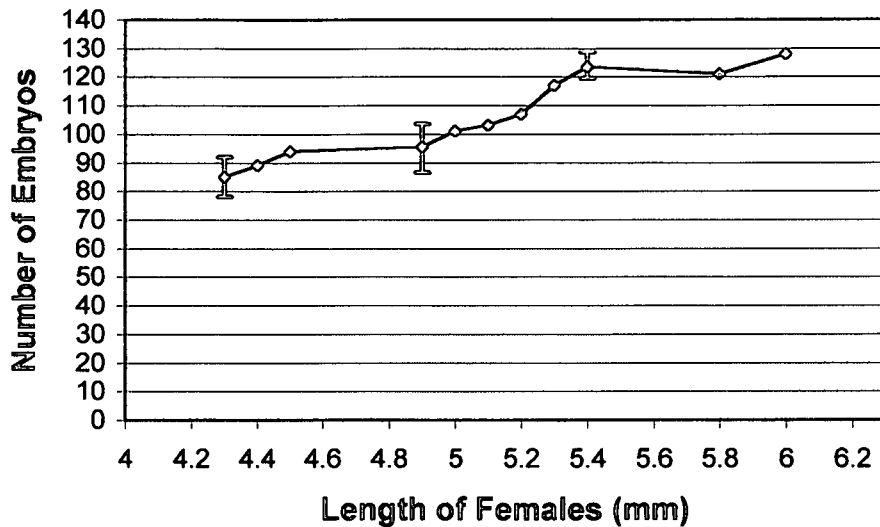


Figure 4.23. Line graph showing the relationship between the lengths of 14 *Gnathia pantherinum* sp. n. females and the number of embryos produced under laboratory conditions in 20-25°C seawater (error bars = range).

4.4 Discussion

Taxonomy

As already mentioned, the description of the male, female and larvae of *G. pantherinum* is the first species description of a gnathiid that parasitises an elasmobranch. This species description not only introduces a new technique for obtaining of males and females for a species description, but also provides a detailed record of the larvae and females enabling them to be positively identified in the absence of the males. This is in contrast to previous methods where new species were described from free living adult males. The latter method always left uncertainty about whether the larvae and females found in near proximity to the males actually belong to the same species. This uncertainty has led to numerous cases where the description of females and larvae were omitted, rather than risk incorrect identifications. Detailed descriptions of all life cycle stages are especially important for the larvae, because positive identification of the larvae, especially when found on a fish host, will contribute to the knowledge of the parasitic stage in the life cycle of gnathiids.

Specific morphologically important characters for the identification of the larvae of different species were also discussed (see remarks of the praniza larvae). It was concluded the number of antenna 2 flagellum articles differ between some genera and the morphology of the pleotelson and cephalosome appendages, separates species of the same genus. The number of teeth on the mandible also proved to be a very reliable characteristic. Another taxonomical question still remains unanswered i.e. what morphological characters can be used to link the female and larval stages to the male? This detailed study of all three life cycle stages made it possible to pay close attention to all the different characteristics of the three stages and compare them with one another. The huge difference in the morphology of the cephalosome and its appendages between male, females and larvae diminish the possibility of linking characteristics even further. The pereopods and pleopods of the different life cycle stages also differ greatly. It was found that the most constant morphological characteristic between the three different stages is the pleotelson (see Figs. 4.2G, 4.9F, 4.15F). This was also found to be true for *G. africana*. Once again, the lack of descriptions of the females and larvae of other species make it impossible to make conclusive assumptions. This hypothesis will be further investigated once more comprehensive descriptions of all the life cycle stages of gnathiids become available.

Final life cycle stages

The development of the final life cycle stages of *G. pantherinum* is very similar to that of *G. africana*. The same development sequence is followed where the development of male larvae is much faster than that of female larvae. Adult males appear approximately six to seven days before adult females. This makes it possible for males to fertilise females of the same generation. The male/female ratio of *G. pantherinum* in the present study was almost 1:2 (43:73) in comparison to the almost 1:1 ratio of *G. africana*. This might suggest that males of *G. pantherinum* will have more than one female at the same time, but investigations into natural populations are necessary to confirm this. As with *G. africana*, it was possible to identify four developmental phases for female larvae and seven for adult females (see Fig. 4.24). Although *G. pantherinum* is a larger species, with the females producing on average of almost twice as many larvae than *G. africana*, the development time of females under the same temperature conditions is almost the same. This might suggest that temperature, as mentioned by Tanaka and Aoki (2000), is the most important factor in the determining development period of gnathiids and not their

size or the number of larvae produced. If this is the case, then it can be assumed that the life cycle of *G. pantherinum* follows the same pattern and time span as the one elucidated for *G. africana*. This would also explain the all year round presence of pranzia 3 larvae on the fish hosts.

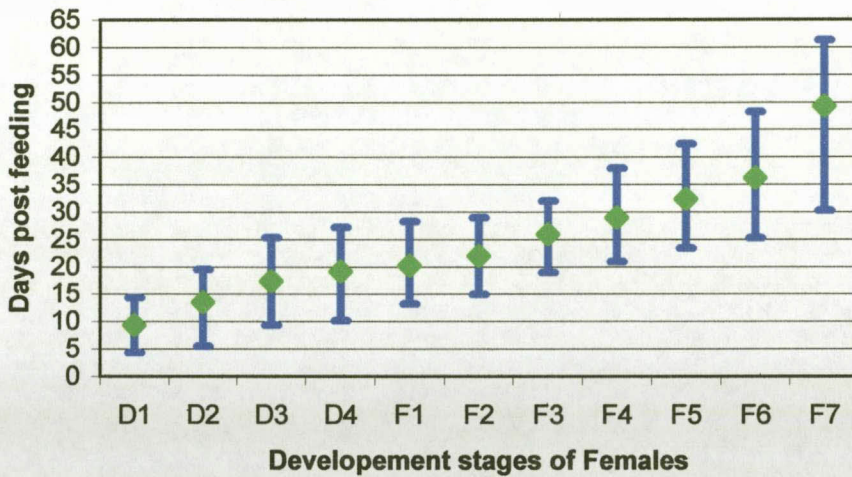


Figure 4.24. Line graph showing the number of days for each development stage of *Gnathia pantherinum* sp. n. females (n = 14) under laboratory conditions in 20-25°C sea water (error bars = range). (D1- Ovaries visible. D2- Eggs fill half of pereon. D3- Eggs fill complete pereon. D4- Posterior moult starts. F1- Anterior moult complete. F2- Eggs ventral. F3- Egg division visible. F4- Eye spots visible. F5- Embryos fully developed. F6- Release of larvae. F7- Spent female dies.)

5. *Gnathia pipinde* sp. n., a temporary parasite of the evileye pufferfish

In Chapter 4 a new method for obtaining adult gnathiids for species descriptions was introduced. This method, whereby praniza larvae collected from the fish host were reared into adults, was again successfully used for the description of another new species. A total of 14 praniza larvae were collected from a single evileye pufferfish, *Amblyrhynchotes honckenii* (Fig. 6.5B), caught at the De Hoop Nature Reserve during October 1999. All the larvae were attached to the dorsal and lateral areas of the body just posterior to the cephalon. Most of the larvae were already fully engorged when the host fish was captured. The fish were kept alive as described earlier and the fully fed larvae detached six to eight hours after capture. In order to rear the larvae into the next stage, they were kept alive as described for *Gnathia africana* and *G. pantherinum*. Three of the larvae moulted into adult males nine days post feeding. These males did not conform to the description of any known males from South Africa or elsewhere in the world, and are thus described as a new species. No females were found.

5.1 Description of *Gnathia pipinde* sp. n.

Gnathia pipinde sp. n.

Adult male

Figs. 5.1 - 5.6

Description: Total length of holotype: 5.1 mm. Total lengths of paratypes: 4.8 mm & 4.6 mm.

Cephalosome. Rectangular, 1.2 times as wide as long, deep dorsal sulcus, narrower than width of median process, extending two thirds length of cephalosome (Fig. 5.4A), lateral margins convex, posterior margin concave. Long setose setae and sensory pits randomly distributed over dorsal and lateral surface of cephalosome (Figs. 5.1A,C, 5.4C), no sensory pits in dorsal sulcus. Setose setae, sensory pits and short simple setae ventrally on lateral sides of buccal cavity (Fig. 5.4E). Well developed oval-shaped, bulbous, sessile compound eyes on lateral margin of cephalosome, length of eye slightly more than a third of cephalosome. Four to eight paraocular tubercles with long setose setae (Fig. 5.4C). Elliptical posterior median tubercle present (Fig. 5.4A). Single tubercle present between median tubercle and dorsal sulcus.

Frontal border. Slightly produced, superior frontolateral process conical, directed antero-laterally, with five to seven long setose setae on inner border (Figs. 5.1B, 5.4D). Mediofrontal process inferior, divided into two conical lobes, no frontolateral process (Figs. 5.1B, 5.4D). External scissura shallow. Supraocular lobe not pronounced with two to three prominent tubercles dorsally (Fig. 5.4C).

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles increasing in length distally with third article as long as first and second articles combined. Article 1 and 2 of flagellum with two feather-like setae each. Flagellum with five articles, article 3 largest, articles 3 and 4 with one aesthetasc seta each, article 5 terminating in one aesthetasc and three simple setae, few setae on each article (Figs. 5.1D, 5.4F). Antenna 2 with four peduncle articles, proximal article covered with very short simple hair-like setae and pectinate scales, article 3 and 4 largest, article 3 with 11 to 14 simple setae and a single feather-like seta, article 4 with 40 – 50 simple and two feather-like setae. Flagellum with seven articles, article 1 largest, article 7 terminating in four simple setae (Fig. 5.1E).

Mandible. Long, two-thirds length of cephalosome, twice as long as wide, broad basal neck, curved inwards with eight to ten processes on dentate blade, tussle of setae between processes (Fig. 5.5B). Apex cylindrical, distally raised in lateral view. Slight incisor present. Single simple mandibular seta extending from base of incisor process. Carina smooth, forming ridge on lateral margin extending from basal neck to halfway along mandible (Fig. 5.5B). Sensory pits with short simple hair-like setae distributed randomly on dorsal surface of blade. Internal lobe and pseudoblade absent.

Maxilliped. Five-articled (Fig. 5.2C), proximal article largest with slender mediodistal endite reaching article 3 (Figs. 5.5C,D). Outer margin of proximal article densely setose. Distal four articles bearing plumose setae on lateral margins in order of 5-7-6-7, mesial border of palp with short simple hair-like setae. Distal article with 5 short simple setae. Palp 2.5 times as long as wide. No coupling hooks.

Pylopod. Three articles (Fig. 5.2A), operculate. First article greatly enlarged, convex mesial border fringed with plumose setae, lateral and proximal setae short and simple, a single feather-like and two simple setae near lateral border, a single simple seta mid mesially and six simple setae distally on posterior surface (Figs. 5.2A, 5.5E). Three distinct areolae. Second article oval, 1.35 times as long as wide, margins setose, two simple setae on mid posterior surface and seven distally (Figs. 5.2B, 5.5F). Third article minute with fringing setae and four short simple setae (Fig. 5.2C).

Maxilla. Reduced, kidney-shaped, on lateral sides of the buccal orifice (Fig. 5.5C).

Pereon. One and a half times as long as wide, slightly wider than cephalosome (Figs. 5.1A, 5.5A), covered with numerous long setose setae and short simple hair-like setae (Fig. 5.5A). Pereonite 1 fused with cephalosome, dorsally visible, not reaching lateral margins, anterior border convex, rounded posterior margin (Fig. 5.1A). Pereonite 2 and 3 of similar size, widest part of body, lateral margins pointing anteriorly (Fig. 5.5A). Pereonite 4 with prominent anterior constriction separating it from pereonite 3. Long setose setae on anterior lateral lobe of pereonite 4, median groove present. Pereonite 5 with *areae laterales* and dorsal sulcus as thin groove. Pereonite 5 and 6 not fused. Pereonite 6 almost twice as long as other pereonites, posterior margin deeply concave, with *lobi laterales*, no *lobii*. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Long setose setae on anterior, lateral and posterior margins of pereonites.

Pleon. Pleon and pleotelson less than a third of total length (Fig. 5.1A). Five subequal pleonites dorsally visible, epimera distinct, long setose setae randomly distributed on posterior margins of pleonites (Fig. 5.6A).

Pleotelson. Triangular, base as wide as or wider than length, lateral margins slightly concave, dorsal surface with eight simple setae antero dorsally and a pair of long simple setae postero dorsally, many pectinate scales on dorsal surface, distal apex terminating in pair of simple setae (Figs. 5.1G, 5.6B).

Pereopods. Pereopod 2 basis elongated, oval shaped with 25 to 30 long simple setae and six to eight short simple setae (Fig. 5.3). Ischium two thirds length of basis, five to seven simple and a single long setose setae anteriorly, posterior with short simple setae and short hair-like setae inbetween. Merus half the length of ischium with anterior bulbous protrusion, three to four simple setae on bulbous protrusion, posterior margin with tubercles as well as long simple and short hair-like setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion, posterior margin with tooth-shaped tubercles, simple setae and a single long denticulated spine. Propodus about twice the length of carpus, two elongated denticulated compound spines situated on middle and distal part of posterior margin respectively, three simple setae anteriorly with one feather-like seta distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 similar in basic shape to pereopod 2 (Fig. 5.3), differ in setation as well as distribution of tubercles. Pereopod

3-5 lack the long denticulated spine on carpus, pereopod 6 with a single long denticulated spine on merus. Dorsal surface of ischium, merus, carpus and propodus covered with pectinate scales (only illustrated on Pereopod 2).

Pleopod. Endopod longer than exopod. Both fringed distally with eight to ten plumose setae (Figs. 5.1F, 5.6C). Sympodite with a single short simple seta on lateral margin and retinaculæ on medial margin (Fig. 5.6D). Pleopod 2 endopod without appendix masculina (Fig. 5.1F).

Uropod. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, endopod with six long plumose setae and exopod with three long plumose seta, both fringed with short hair-like setae (Figs. 5.1G, 5.6B). Uropodal basis with a single short seta dorsally.

Penes: Long, prominent, pointing anteriorly, more than a third length of pereon (Figs. 5.4B, 5.6E). Anterior end ending in sac-like extension dorsal to opening (Fig. 5.6F).

Remarks: When compared to the South African gnathiid species the shape and size of the frontal processes of *Gnathia pipinde* are very similar to those of *G. africana* and *G. nkulu*. This species can, however, easily be distinguished from the other South African species by the shape and size of its elongated penes.

To the author's knowledge there are only two other species of the genus *Gnathia* with elongated penes. They are *Gnathia falcipenis* Holdich and Harrison, 1980 and *Gnathia cooki* Müller, 1989. According to Holdich and Harrison (1980) the mediofrontal process of *G. falcipenis* is conical and thus distinctly different from the deeply divided mediofrontal process of *G. pipinde*. The frontal processes and shape of the pleotelson of *G. cooki* described by Müller (1989b) are very similar to those of *G. pipinde*. The South African species differ in the number of antenna 2 flagellum articles (seven instead of six) and the number of plumose setae on the distal four articles of the maxillipedes (in the order of 5-7-6-7 instead of 5-6-5-7 as in *G. cooki*).

The presence of the frontal processes, 3 articulated operculated pylopods, and denticulated mandibles with incisors, supports the placement of this species in the genus *Gnathia* (see Cohen and Poore 1994).

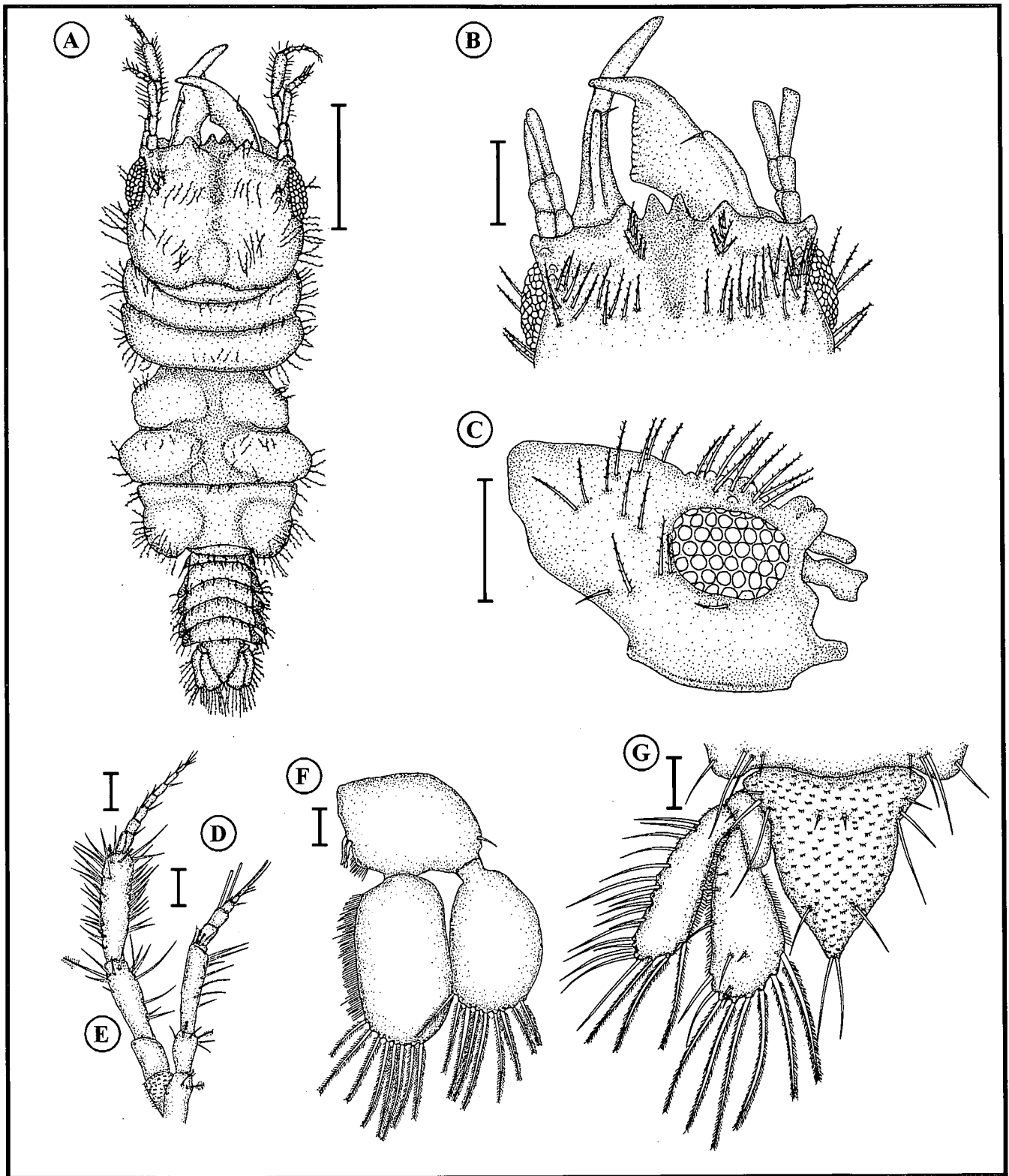


Figure 5.1. Microscope projection drawings of a *Gnathia pipinde* sp. n. male. **A.** Full length dorsal view. **B.** Frontal border and mandibles. **C.** Lateral view of cephalosome. **D.** First antenna. **E.** Second antenna. **F.** Left pleopod 2 with appendix masculina. **G.** Pleotelson and uropods. Scale bars: A = 1 mm, B & C = 500 μ m, D-G = 100 μ m.

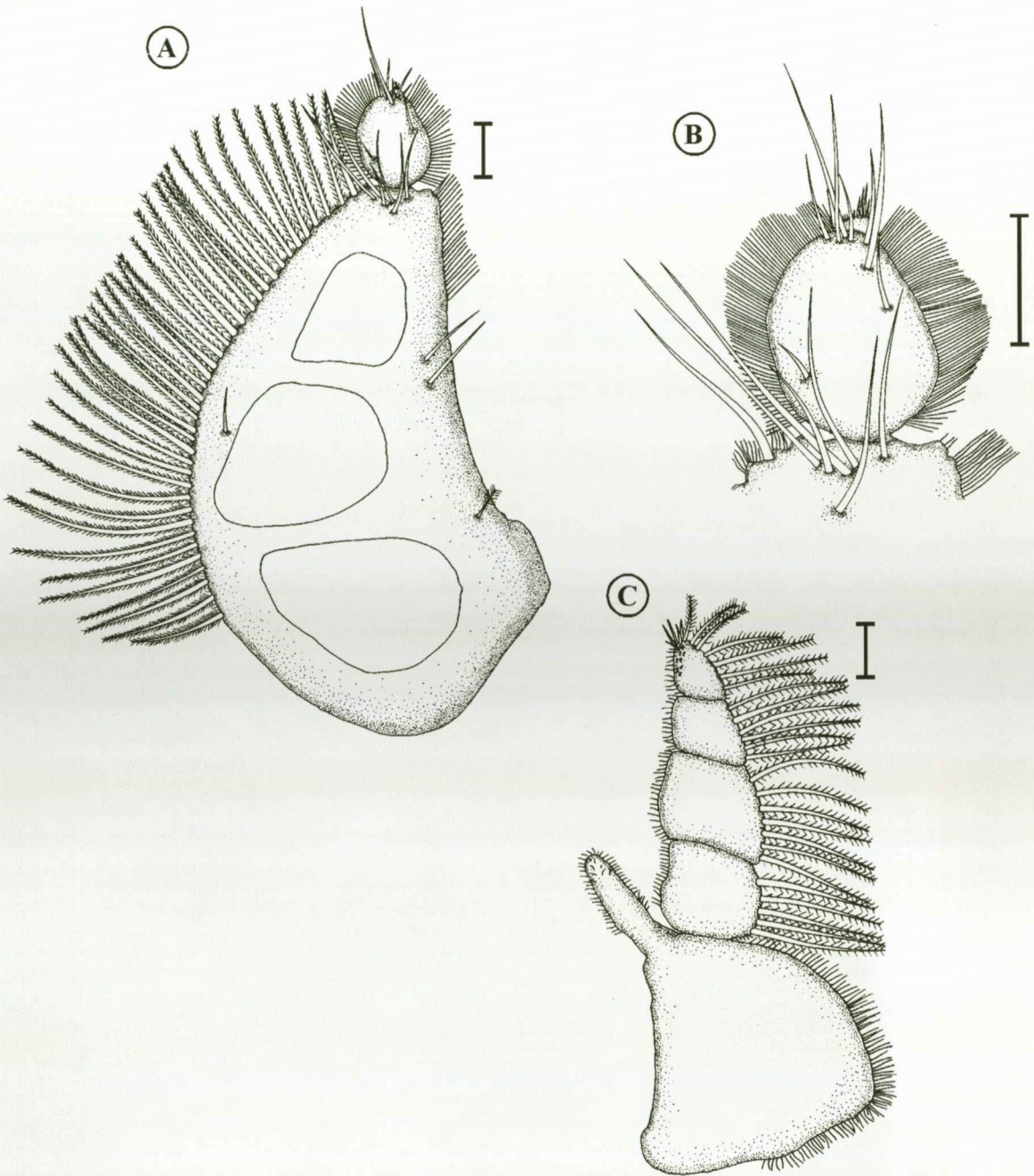


Figure 5.2. Microscope projection drawings of the cephalosome appendages of a *Gnathia pipinde* sp. n. male. **A.** Left pylopod. **B.** Articles 2 and 3 of pylopod. **C.** Left maxilliped. Scale bars: 100 μ m

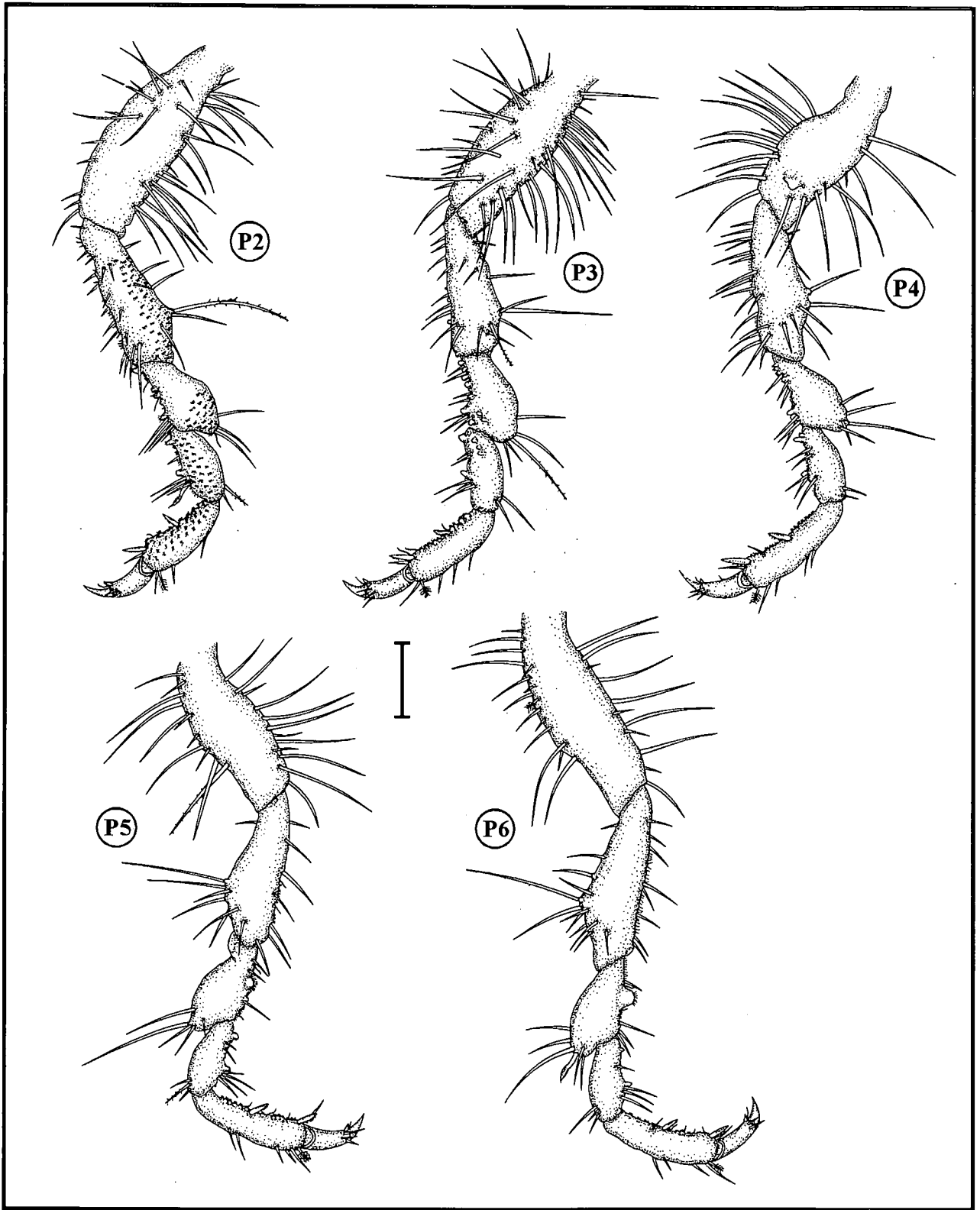


Figure 5.3. Microscope projection drawings of pereopods 2 to 6 (P2 - P6) of a *Gnathia pipinde* sp. n. male. Scale bar: 200 μ m

Figure 5.4

**Scanning electron micrographs of a *Gnathia pipinde* sp. n. male
(male larva collected at De Hoop Nature Reserve and moulted into an adult
male in the laboratory)**

- A. Dorsal view of cephalosome and pereon
- B. Ventral view of cephalosome and pereon
- C. Dorso-anterior view of cephalosome
- D. Dorsal view of superior frontolateral and mediofrontal processes of frontal border
- E. Lateral view of cephalosome
- F. Flagellum of right Antenna 1 with aesthetasc setae

Scale bars: C-E = 100 μ m

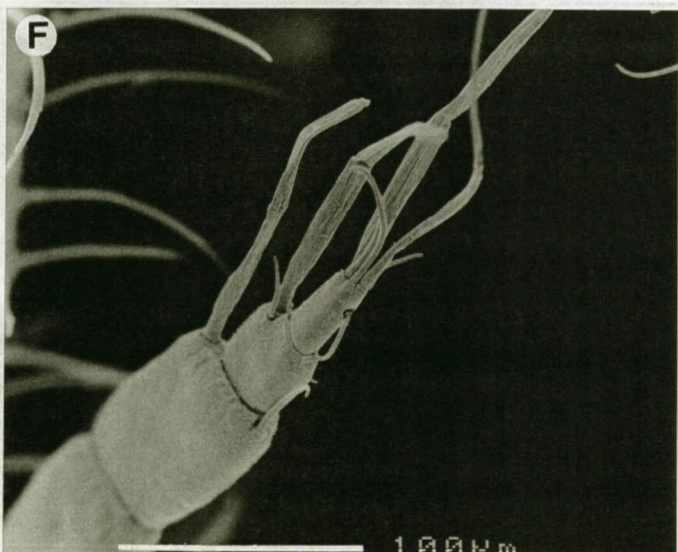
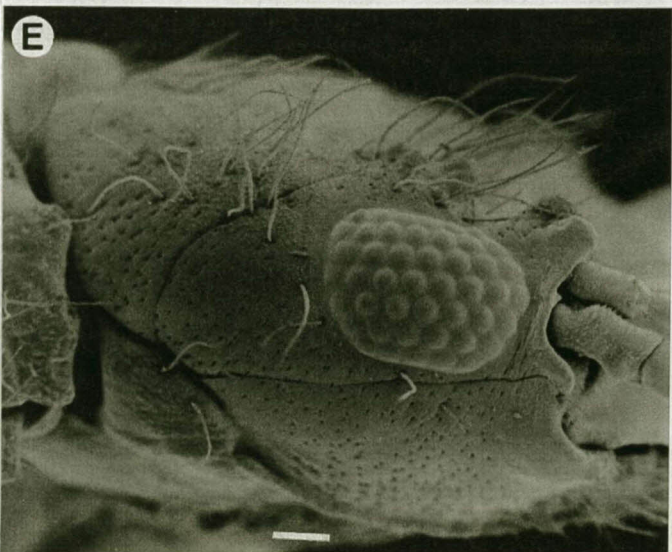
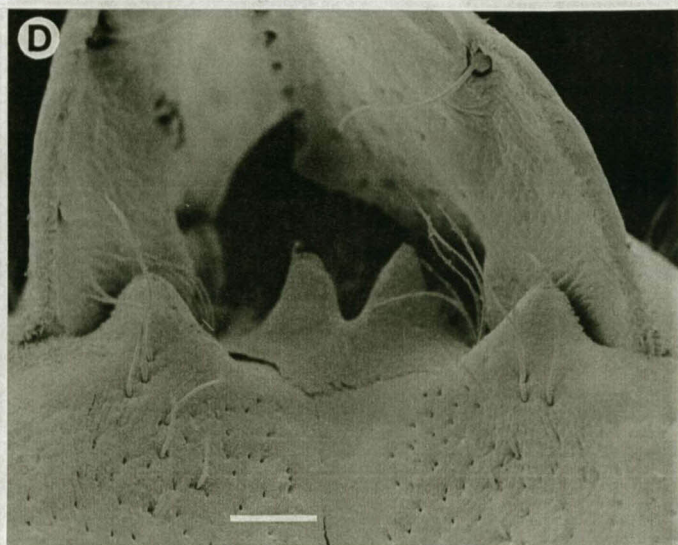
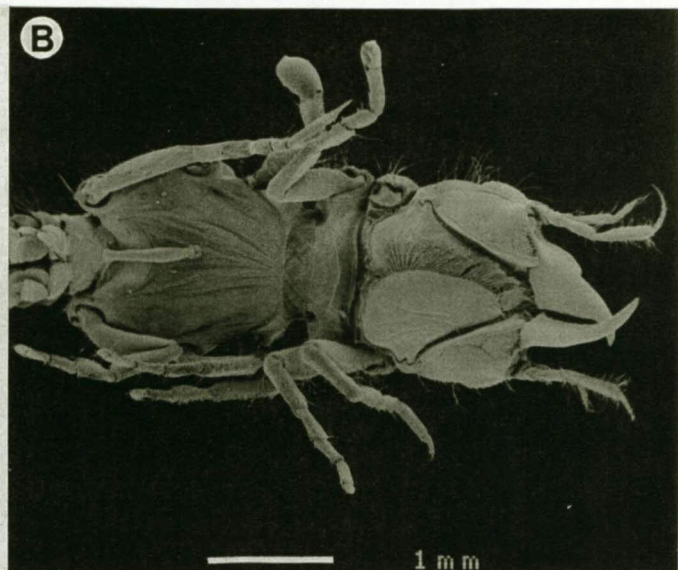
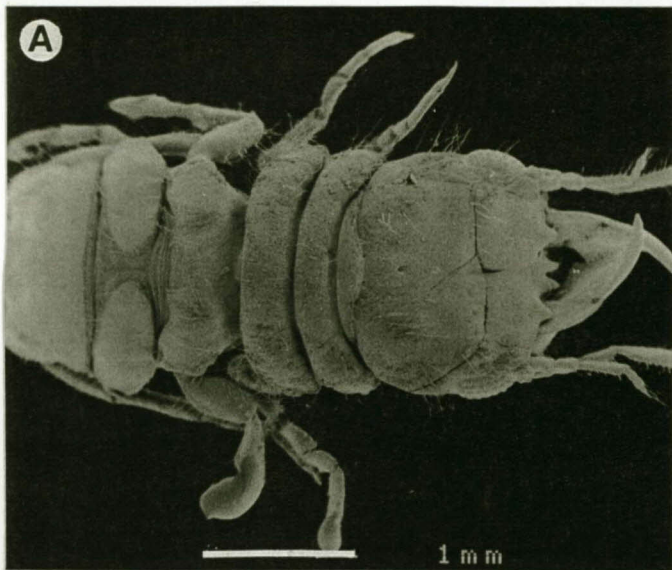


Figure 5.5

**Scanning electron micrographs of a *Gnathia pipinde* sp. n. male
(male larva collected at De Hoop Nature Reserve and moulted into an adult
male in the laboratory)**

- A.** Example of the long setose seta found on cephalosome, pereon and pleon
- B.** Dorsal view of left mandible
- C.** Ventral view of left maxilliped
- D.** Dorsal view of article 1 and endite of left maxilliped
- E.** Ventral view of overlapping pylopods
- F.** Ventral view of article 2 and 3 of left pylopod

Scale bars: E & F = 100 μ m

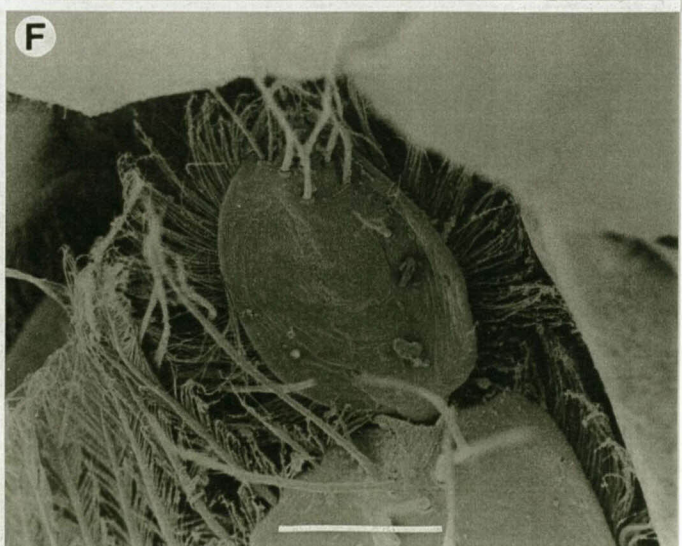
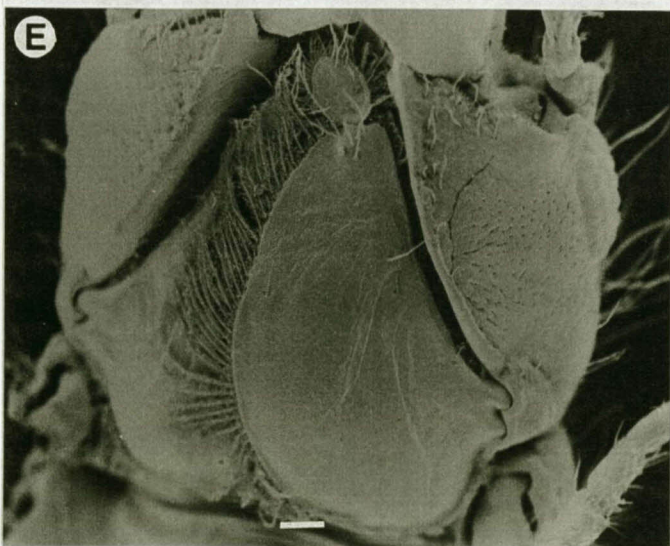
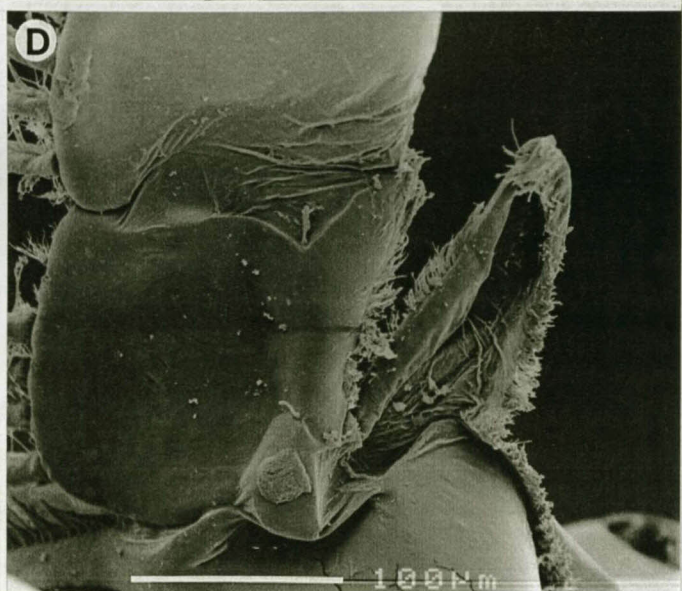
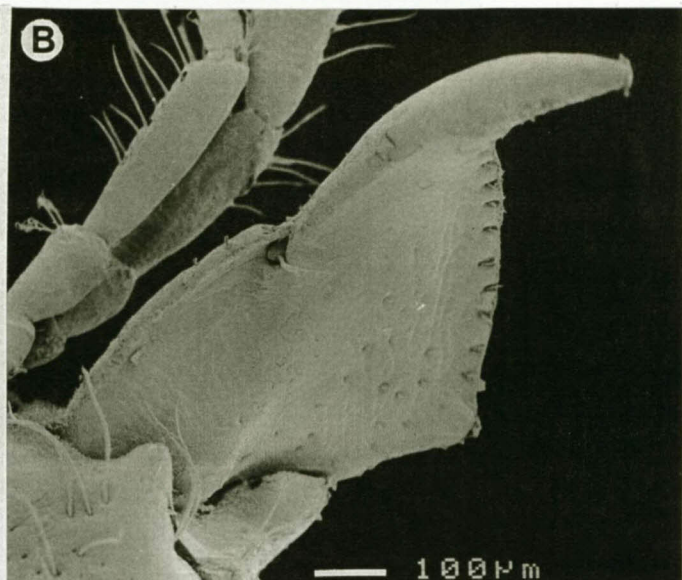
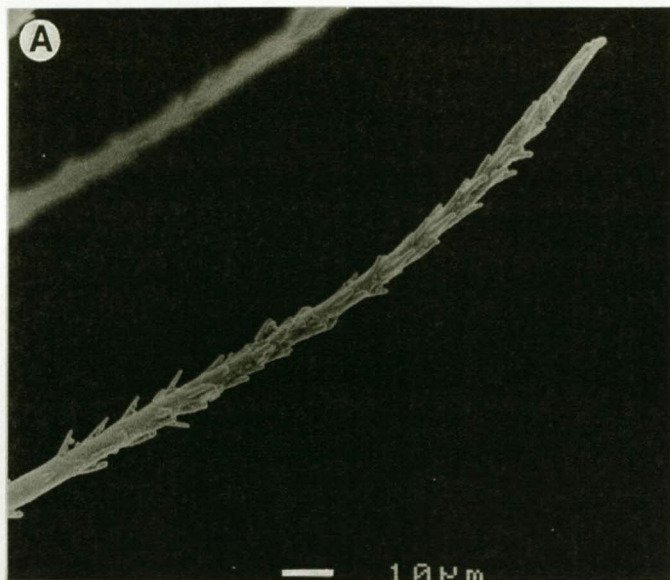
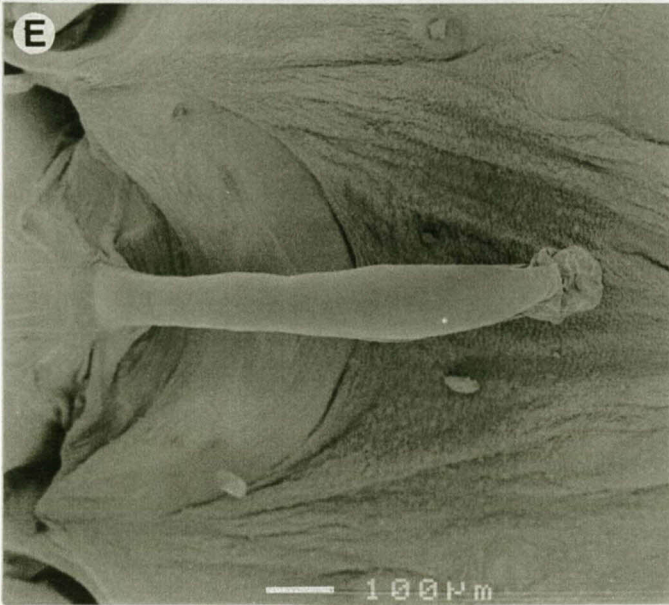
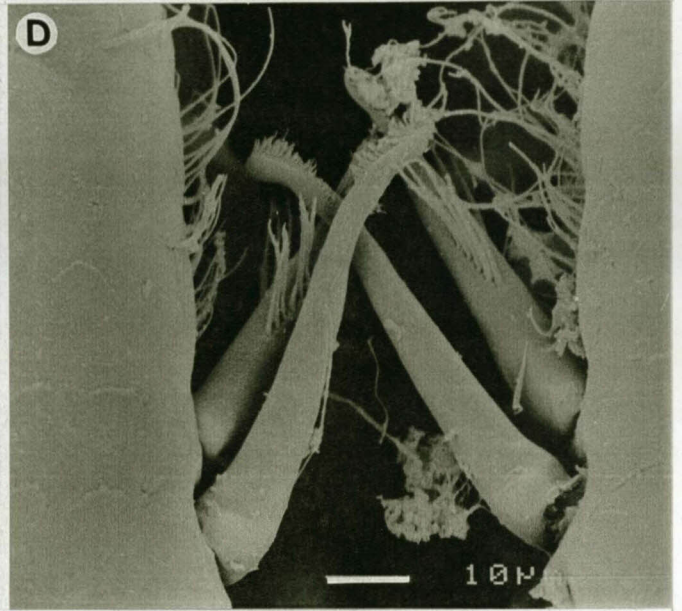
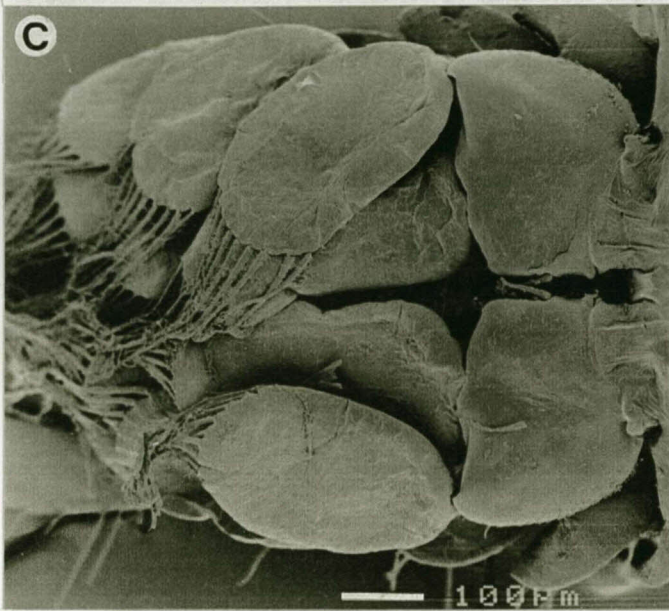
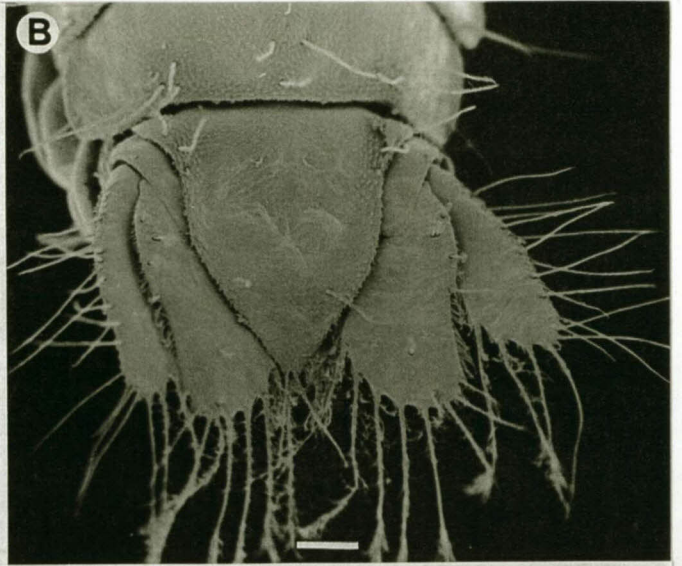
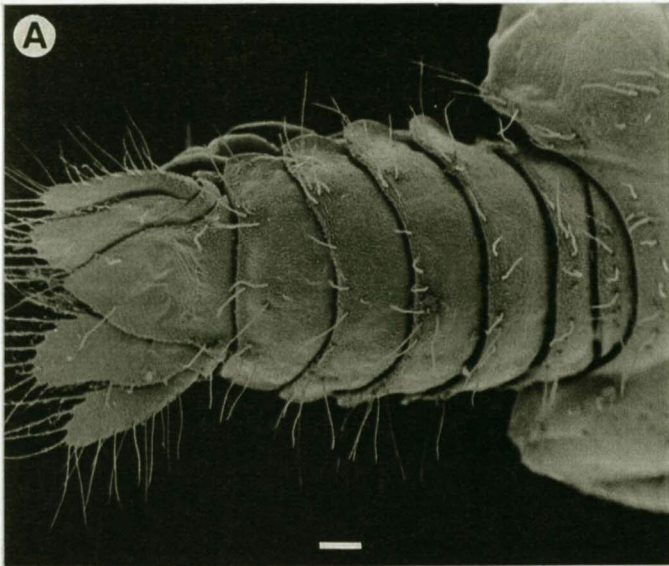


Figure 5.6

Scanning electron micrographs of a *Gnathia pipinde* sp. n. male
(male larva collected at De Hoop Nature Reserve and moulted into an adult
male in the laboratory)

- A. Dorsal view of pereonite 7 and pleon
- B. Dorsal view of telson and uropods
- C. Ventral view of first pair of pleopods
- D. Ventral view of the paired retinaculæ on sympodites of pleopods
- E. Ventral view of penes
- F. Ventral view of anterior end of penes

Scale bars: A & B = 100 μm ; F = 10 μm



Praniza larva

Figs. 5.7 – 5.11

Description: Total length of paratypes: 3.8-4.6 mm. (4.3 ± 0.8 ; $n = 11$)

Cephalosome. Posterior margin slightly concave, wider than anterior margin, longer than wide, lateral margins slightly concave (Figs. 5.7B, 5.10B). A few sensory pits and no setae on dorsal surface of cephalosome. Compound eyes large, well developed, oval-shaped, bulbous, on lateral margins of cephalosome, length of eye almost same as cephalosome (Fig. 5.10C). No sulcuses or tubercles on dorsal cephalosome (Fig. 5.10A). Medio-anterior margin of cephalosome straight with lateral concave excavations to accommodate first articles of antennae.

Labrum. Prominent, less than half length of cephalosome, semicircular with apical process, truncated posterior margin, anterior margin concave (Fig. 5.7B). Ventral part of labrum gutter-like with central groove, covers mandibles dorsally and laterally.

Antennae. Antenna 2 longer than antenna 1 (Fig. 5.10A). Antenna 1 with three peduncle articles, a single feather-like setae on article 3, short hair-like setae on anterior borders of all three articles. Flagellum with four articles, long first article with two to three short simple setae mid dorsally, article 2 largest, articles 2 and 3 with one aesthetascs setae each, article 4 terminating in one aesthetascs and three simple setae, few setae on each article (Figs. 5.7C, 5.10D). Antenna 2 with four peduncle articles, article 4 largest, very long prominent hair-like setae on anterior margins of all four peduncle articles (Fig. 5.7D, 5.10D). Flagellum with seven articles, article 7 terminating in three to four simple setae, few setae on distal end of each article (Fig. 5.7D). Antennae slightly bent.

Mandible. Stout, swollen at base, distal margin styliform with 16 teeth on mesial margin, three teeth small and situated at tip of mandible, 13 larger, triangular and backwardly directed, increasing in size from anterior to posterior (Figs. 5.8A, 5.10E).

Gnathopod. Smaller than pereopods, seven articles, only few simple setae on articles (Figs. 5.8B, 5.10F). Dactylus strongly hooked (Fig. 5.11A).

Maxilliped. Large, cylindrical, elongated base, endite almost reaching palp with single long simple seta coupling hook (Fig. 5.8C). Palp with three articles, first article acute with six prominent teeth mesially, article 3 with five to seven simple setae (Fig. 5.11B).

Maxillae. Not visible.

Maxillule. Long, slender, swollen base, six to eight small teeth on distal inner margin (Figs. 5.8D, 5.11C).

Paragnaths. Elongated, gutter-like, terminates in sharp point, no teeth (Figs. 5.8E, 5.11C).

Pereon. Elongated, almost three times longer than wide, wider than cephalosome (Fig. 5.7A). Pereonite 1 fused with cephalosome, dorsally visible (Fig. 5.10B). Pereonite 4 twice as wide as long, lateral sides tapering towards rounded posterior margin, posterior margin stretching over pereonite 5 (Fig. 5.7A), lateral shields at leg attachment. Pereonite 5 consists of elastic membrane fully expanded in praniza stage with blood meal, bulbous shields present on lateral sides at leg attachment. Pereonite 6 rectangular, posterior margin slightly concave, lateral shields at leg attachment. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Pereonites smooth with no setae and sensory pits.

Pleon. Pleon and pleotelson half length of pereon. Five pleonites dorsally visible (Fig. 5.7A). Pleonite 5 almost twice the length than the other articles.

Pleopod. Endopod larger than exopod. Endopod fringed distally with seven long plumose setae and exopod with eight, plumose setae same length or longer than exopod (Figs. 5.7E, 5.11E). Mesial borders of endo- and exopod with short hair-like setae. Sympodite with retinacula, single simple seta on lateral margin.

Pleotelson. Triangular, longer than wide, posterior two thirds of pleotelson convex, two pairs of simple setae on dorsal surface, distal apex terminating in pair of simple setae, dorsal surface covert with pectinate scales (Figs. 5.7F, 5.11D).

Uropod. Endopod extending beyond apex of pleotelson, exopod not reaching apex. Endopod longer and wider than exopod, both with long fringing setae, endopod with mesial six setae plumose, exopod with mesial four setae plumose, rest of setae simple (Figs. 5.7F, 5.11D). All margins of both endo- and exopods fringed with short hair-like seta. Uropodal basis with two simple setae.

Pereopods. Pereopod 2 basis elongated with a few hair-like seta anterior and two simple setae posteriorly (Fig. 5.9). Ischium three quarters length of basis and almost as wide, single short simple seta and a single long simple seta anteriorly and one to three posterior. Merus half the length of ischium with anterior bulbous protrusion, two long denticulated compound spine and a single simple seta on bulbous protrusion, posterior margin with simple and hair-like setae. Carpus of almost same size and shape as merus, but without anterior bulbous, tubercles and single long denticulated compound spine on posterior margin. Propodus about twice the length of carpus, two short denticulated spines ending in sharp points situated on middle and distal part of posterior side

respectively, only a few simple setae anteriorly with single feather-like seta antero-distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, two to three simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 (Fig. 5.9), similar to pereopod 2 in basic form, differ in setation and number and presence of spines. Pereopods 3 without long denticulated compound spines, pereopod 4 with a single long denticulated compound spines on bulbous protrusion of merus, pereopod 5 with a single one on the anterior margin of carpus and pereopod 6 with two on the bulbous protrusion of the merus and a single one on the anterior margin of carpus. Anterior margin of pereopod 5 and 6 with round tubercle fringed with many hair-like setae (Fig. 5.11F). Pereopods 2 and 3 is directed posteriorly and pereopods 4 to 6 anteriorly. All articles, except basis, of all pereopods with pectinate scales and short hair-like setae (Fig. 5.9).

Remarks. The differences between larvae of different genera were discussed in the remarks of the description of *G. pantherinum* larvae (see Chapter 4) and will not be repeated here. It seems that the known larvae of the genus *Gnathia* share common features such as the number of antenna 2 flagellum articles and the basic morphology of the labrum, cephalosome, pereon, pleon and pleonites. As mentioned in Chapter 4, a combination of the morphology of the pleotelson and cephalosome appendages can be used to distinguish between these larvae.

The larvae of *G. pipinde* can be distinguished from the described larvae of the South African species, *G. africana* and *G. pantherinum*, by the presence of 16 teeth on the mandible (*G. africana* with nine to ten and *G. pantherinum* with eight). The shape of the pleotelson of *G. pipinde* (posterior two thirds convex) also differs from that of *G. africana* (lateral margins straight) and *G. pantherinum* (anterior half of lateral margins slightly concave).

Gnathia pipinde differs from the European and British intertidal species, *Gnathia maxillaris*, in the number of teeth on the mandibles, maxillules and the first article of the maxillipedal palp. According to Davies (1981) *G. maxillaris* has 12 mandibular teeth and nine on both the maxillules and the first article of the maxillipedal palp. The smooth surface of the pereon of *G. pipinde* larvae separates it further from the pectinate covered pereon of *G. maxillaris*.

Taxonomic summary of *Gnathia pipinde* sp. n.

Type-material: Holotype: In the collection of the National Museum, Bloemfontein .

Type-locality: De Hoop Nature Reserve (34°28'S, 20°30'E).

Type host: *Amblyrhynchotes honckenii*

Deposition of types: Holotype: In the collection of the National Museum, Bloemfontein.

(1 male,) (No number available yet). Paratypes: In the collection of the author (2 males; 11 larvae)

Etymology: The Xhosa word "pipinde" was chosen, because "pipi" means penis and the post "nde" means long in the language of this southern African tribe and thus describing the most distinct characteristic of this species. Pronounced as pie-pie-n-dê.

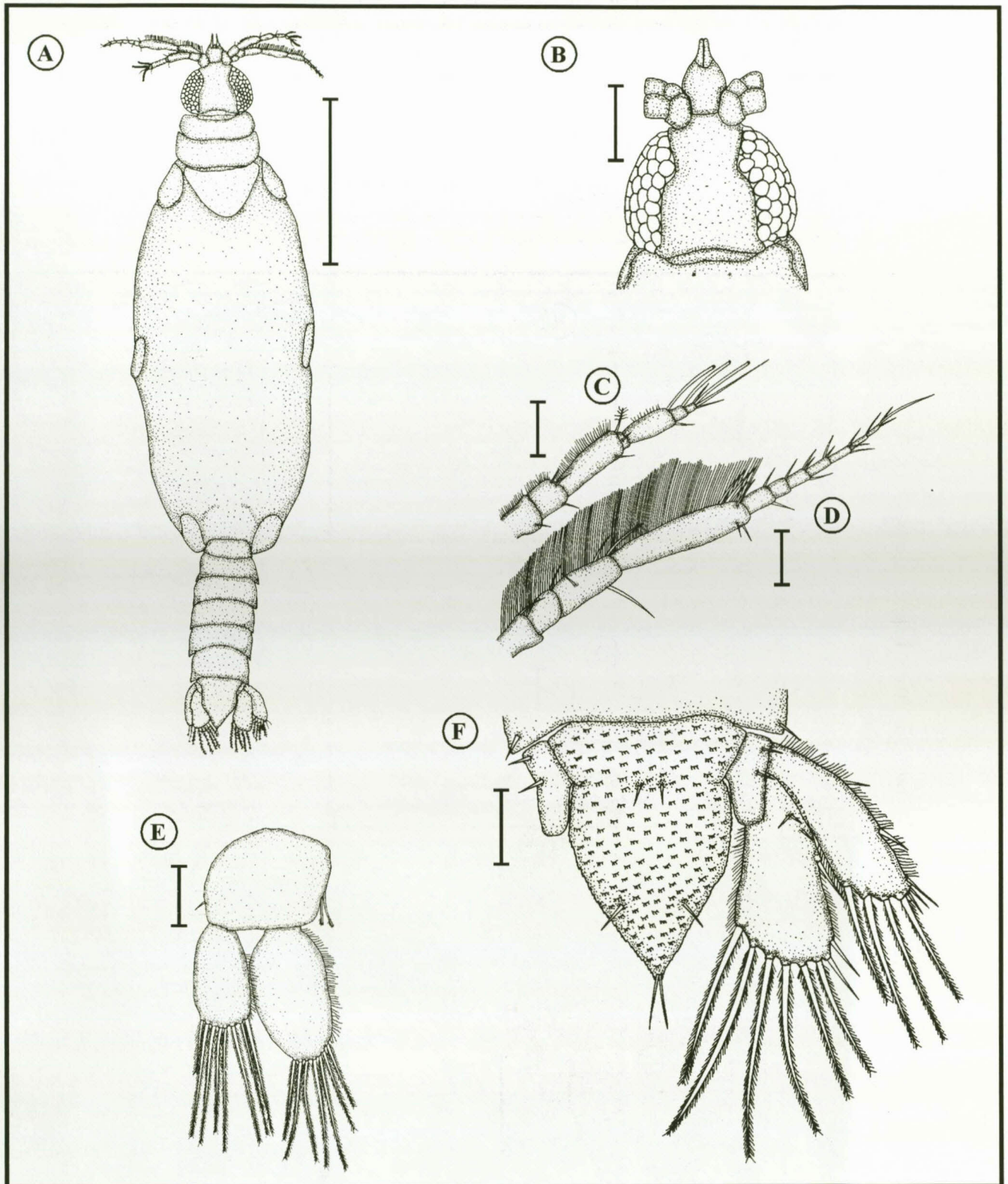


Figure 5.7. Microscope projection drawings of a of *Gnathia pipinde* sp. n. praniza larva. **A.** Full length dorsal view. **B.** Dorsal cephalosome. **C.** Antenna 1. **D.** Antenna 2. **E.** Pleopod 1. **F.** Pleotelson and uropods. Scale bars: A = 1 mm; B = 250 µm; C-F = 100 µm

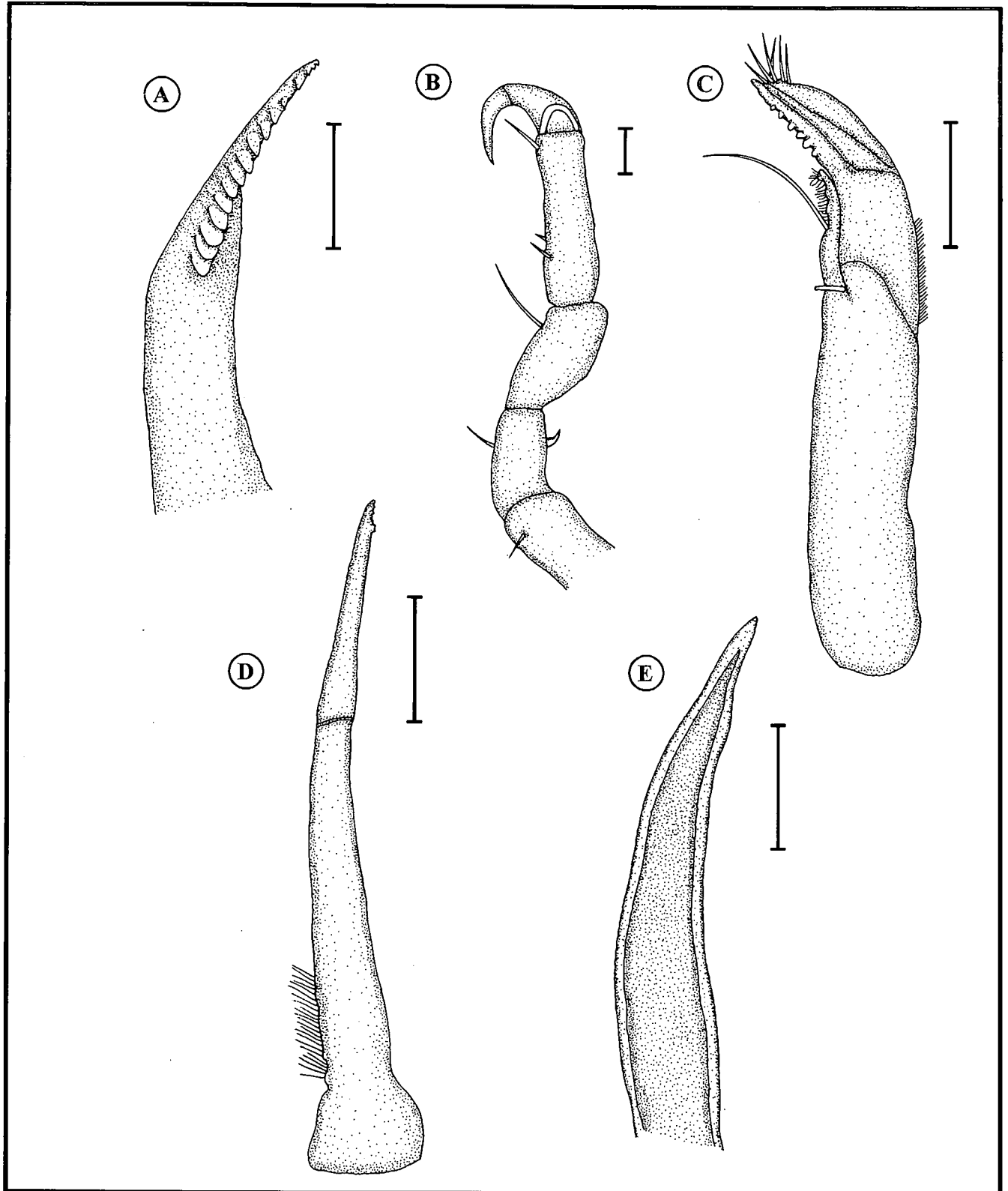


Figure 5.8. Microscope projection drawings of the cephalosome appendages of a *Gnathia pipinde* sp. n. praniza larva. **A.** Mandible. **B.** Gnathopod. **C.** Maxilliped. **D.** Maxillule. **E.** Paragnath. Scale bars. 50 μ m

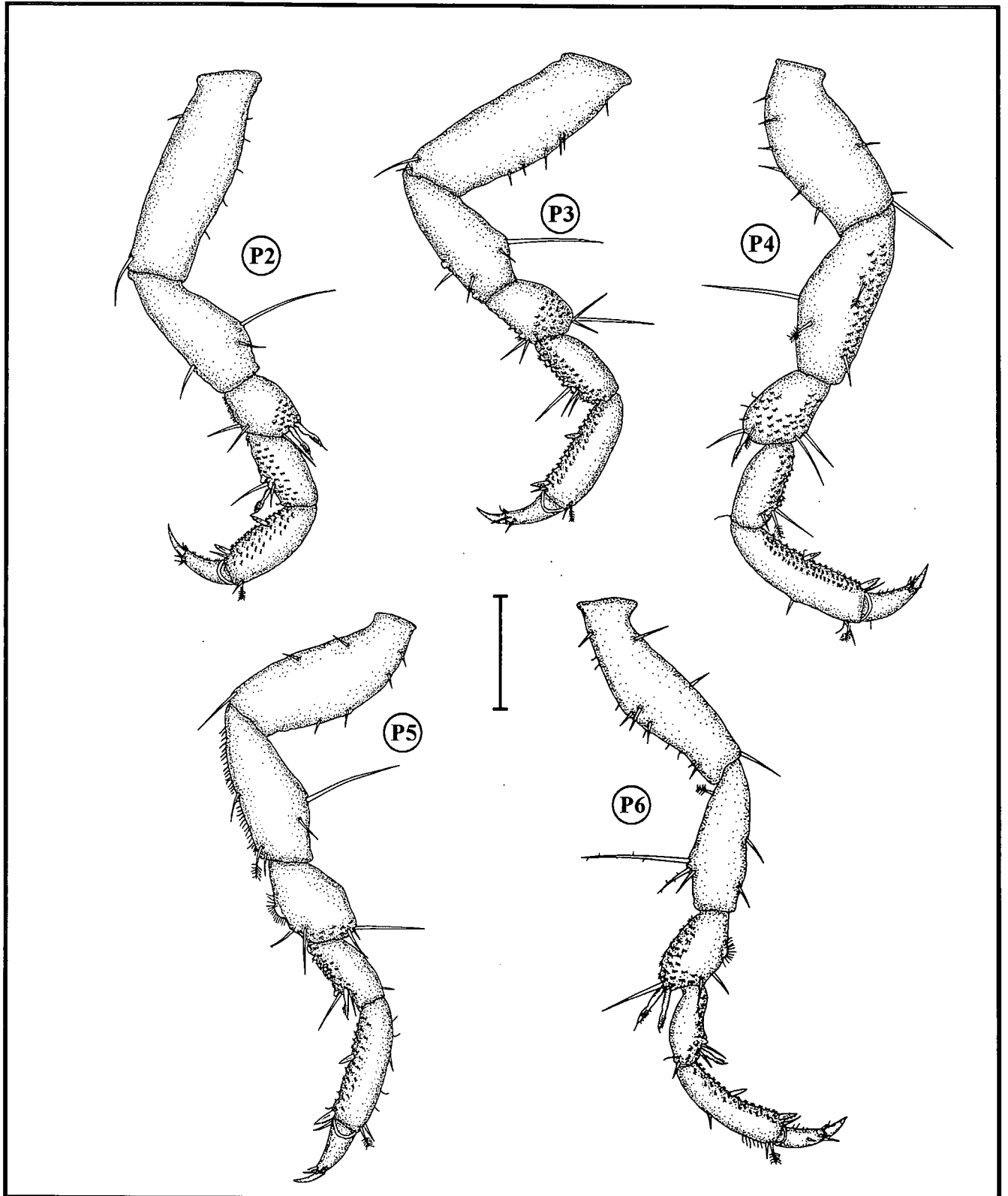


Figure 5.9. Microscope projection drawings of pereopods 2 to 6 (P2 - P6) of a *Gnathia pipinde* sp. n. praniza larva. Scale bar 200 μ m

Figure 5.10

Scanning electron micrographs of a *Gnathia pipinde* sp. n. praniza larva,
collected at the De Hoop Nature Reserve

- A. Anterio-lateral view of labrum, cephalosome and antennae
- B. Dorsal view of cephalosome and eyes
- C. Lateral view of cephalosome and eye
- D. Ventral view of antenna 1 and 2
- E. Lateral view of distal point of mandibles (m) with 16 teeth (proximal tooth not clearly visible)
- F. Ventral view of cephalosome with gnathopods and eyes

Scale bars: A-D & F = 100 μ m

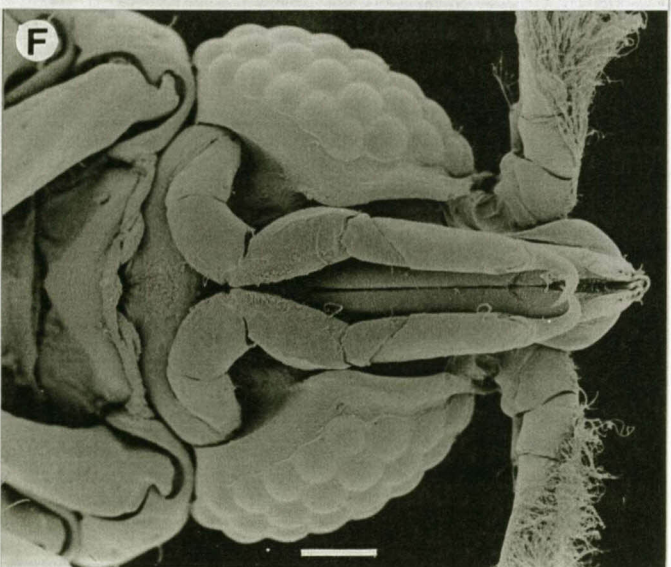
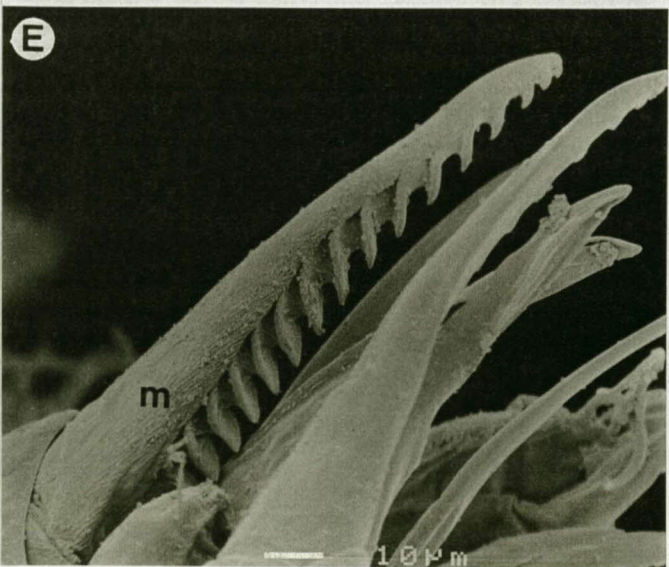
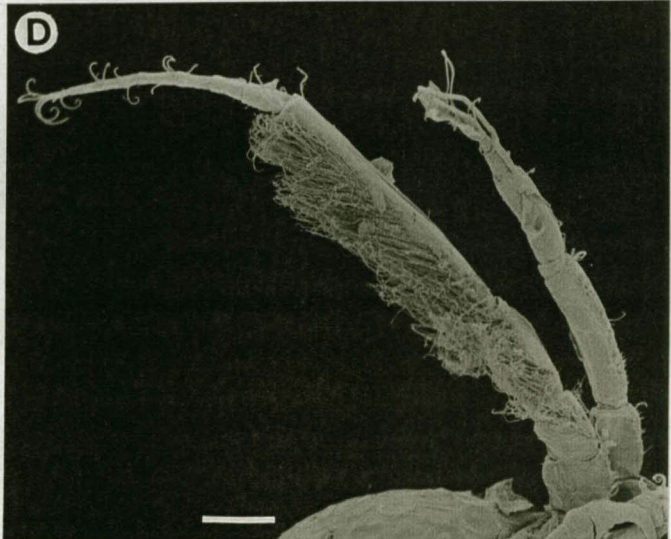
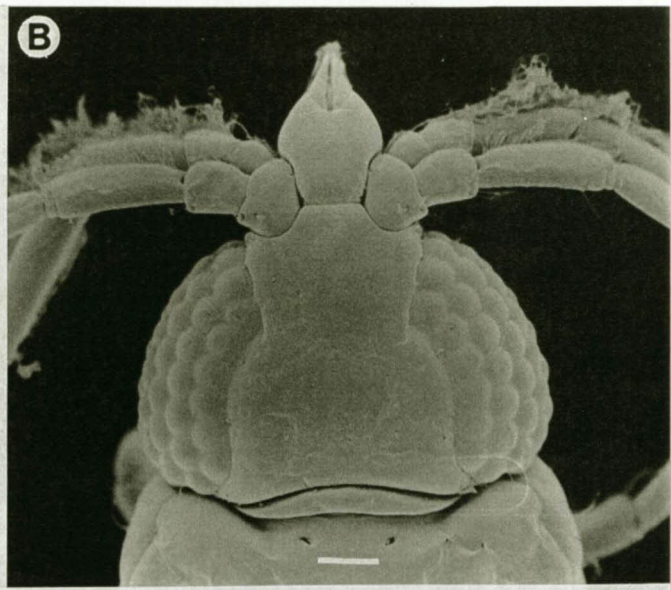
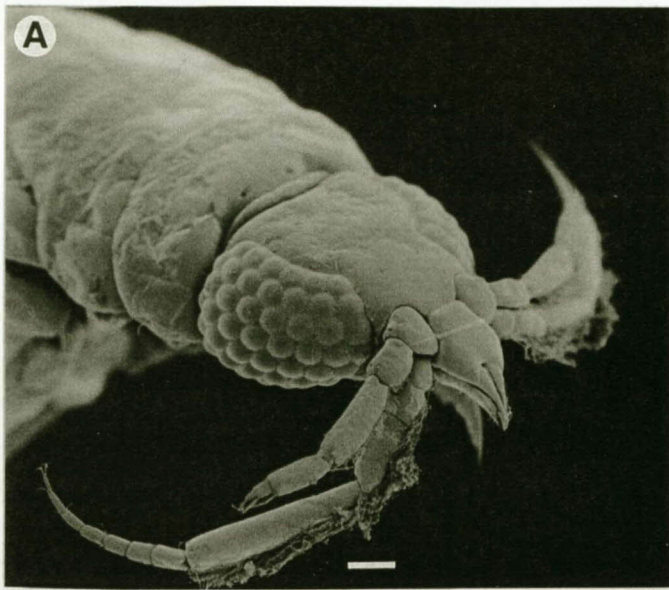
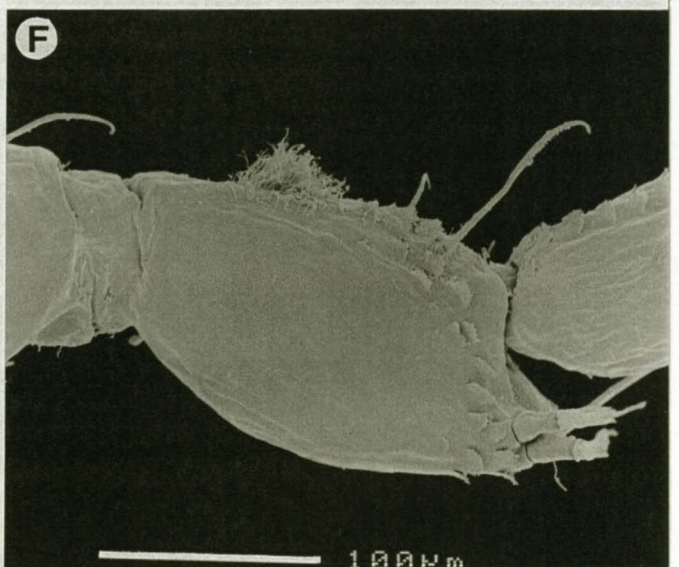
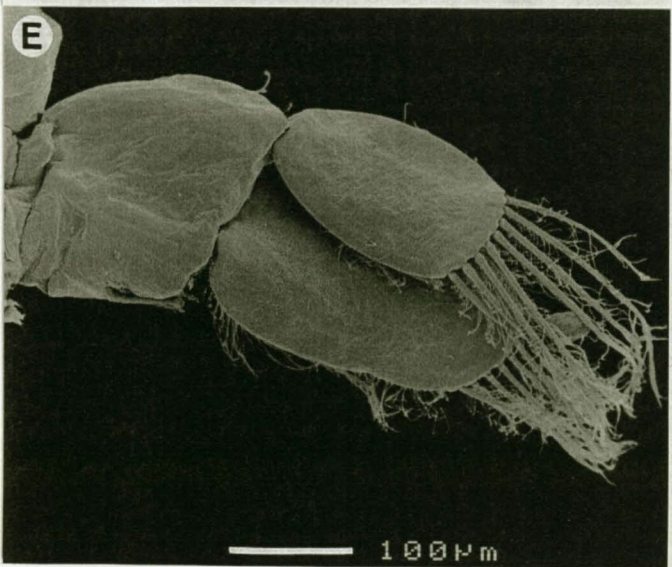
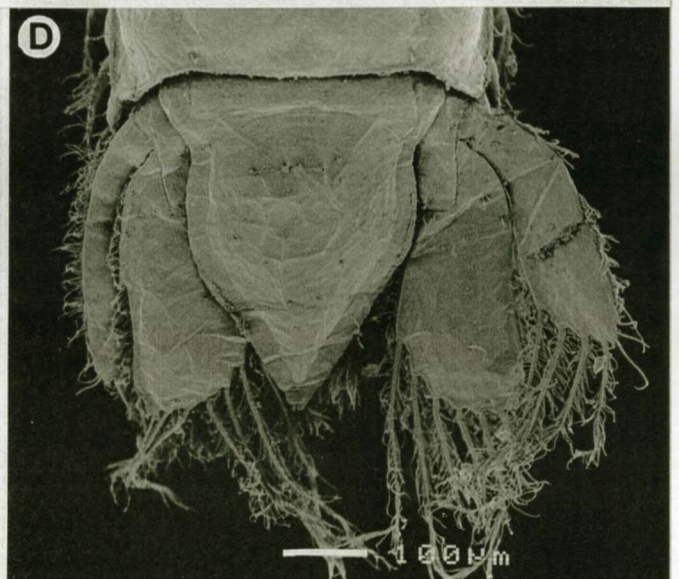
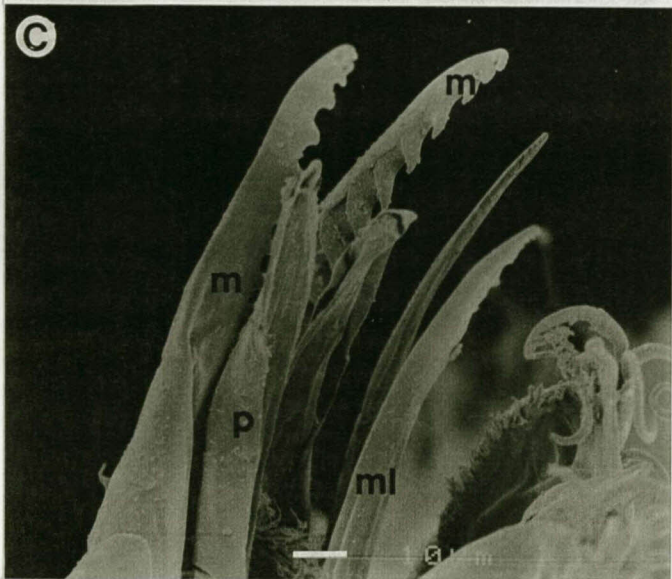
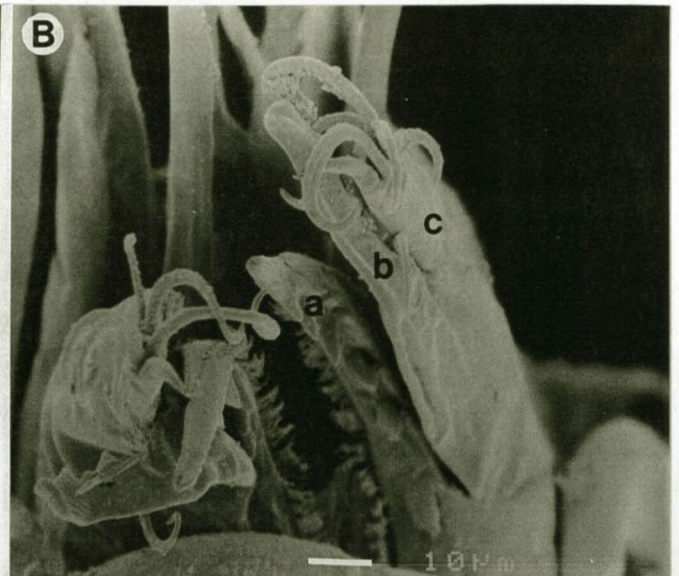
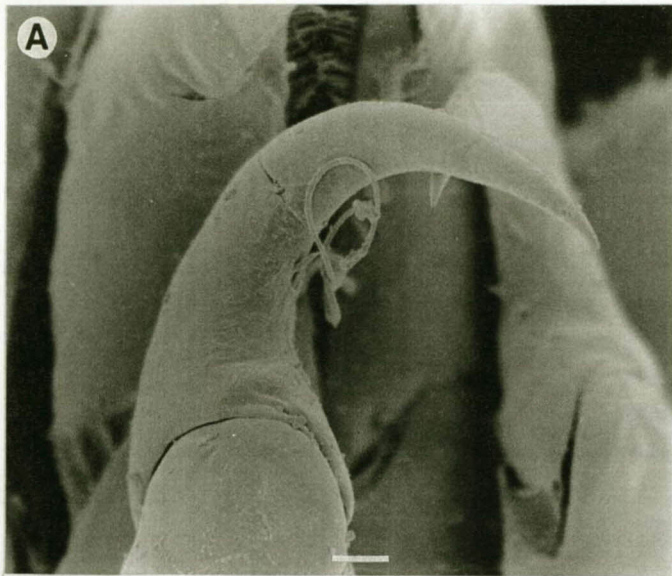


Figure 5.11

Scanning electron micrographs of a *Gnathia pipinde* sp. n. praniza larva,
collected at the De Hoop Nature Reserve

- A. Ventral view of the hooked dactylus of the right gnathopod
- B. Article 1 (a), article 2 (b) and article 3 (c) of palp of maxilliped
- C. Lateral view of mandibles (m), paragnaths (p) and maxillules (ml)
- D. Dorsal view of the pleotelson and uropods
- E. Ventral view of left pleopod 1
- F. Merus of pereopod 5 with round tubercle fringed with hair-like seta

Scale bars: A-C = 10 μ m



6. Haemogregarines from South African fish and their possible vectors

The phylum Apicomplexa (Sporozoa) Levine, 1970 is represented by parasitic protozoans equipped with a specialised apical complex, a group of structures believed to aid in the process of host cell invasion (Lom and Dyková 1992). Within the phylum, some members of the class Coccidea Leuckart, 1879 are found parasitising fishes. The focus of this chapter is on South African representatives of one of the families of the class Coccidea, the family Haemogregarinidae Léger, 1911. According to Lom and Dyková (1992), haemogregarines are characterised by a non-motile zygote, secreting a flexible membrane that is stretched during sporogony. Their life cycle involves vertebrate and invertebrate hosts with merogony and gamogony in various cells of vertebrates and sexual development, sporogony and further merogony in invertebrates (Davies 1995).

Although the first reported haemogregarine species, *Haemogregarina stepanowi* Danilewsky 1885, was described in 1885, the first record of haemogregarines from the blood of marine fishes was reported 16 years later by Lavern and Mesnil (1901). Since then, more than 400 species have been recorded from fish, amphibia, reptiles, birds and mammals (see Levine 1988, Desser 1993, Davies 1995, Smith 1996). Most of these species are known only by their gamonts found in peripheral blood smears. Desser (1993) reported the problems associated with haemogregarine taxonomy in the early 1990s. He pointed out that the reasons for this lie in the difficulty to differentiate among species and genera, on the basis of gamonts within circulating blood cells. Another reason is the habit of authors to name new species solely on the basis of the morphology of the gamonts. Desser (1993) suggested that taxonomic confusions could only be resolved when more basic information on the life cycles of haemogregarines became available. Recent revisions of the taxonomy of haemogregarines by Siddall (1995), Smith (1996) and Smith and Desser (1997), have improved this position, but taxonomic problems remain. For the purpose of this work, the classification of haemogregarines as summarised by Davies and Johnston (2000) will be followed.

In order to give some background information on fish blood protozoa, a review of specific aspects of the research in this field will be provided. This short review draws strongly on information from the partial systematic revision of the haemogregarine complex by Siddall (1995), and the reviews of the biology of fish haemogregarines and of some intraerythrocytic parasites of fishes, amphibia and reptiles by Davies (1995) and Davies and Johnston (2000).

6.1 Literature review of the taxonomy and life cycle studies of fish haemogregarines

The family Haemogregarinidae currently consists of six genera, *Cyrcilia* Lainson, 1981, *Desseria* Siddall, 1995, *Haemogregarina* Danilewsky, 1885, *Hemolivia* Petit, Landau, Baccam and Lainson, 1990, *Hepatozoon* Miller, 1908 and *Karyolus* Labbé, 1894. In their analytical systematic review of the haemogregarine complex, Mohammed and Mansour (1959) recommended the use of the qualifier “*sensu lato*” for *Haemogregarina* species for which sexual development has not yet been described and “*sensu stricto*” for those with a known sexual development. Siddall (1995), in a partial systematic revision of the Adeleiorina, assigned fish haemogregarines to the genera *Cyrcilia*, *Desseria* and *Haemogregarina* (*sensu lato*). Only these three genera will therefor be discussed.

The genus *Cyrcilia* Lainson, 1981

Type species: *Cyrcilia* (*Haemogregarina*) *lignieresesi* (Laveran, 1906) Siddall, 1995

Type host: The fish *Synbranchus marmoratus*

Although Lainson (1981) originally allocated two species, *Cyrcilia* (*Haemogregarina*) *gomesi* (Neiva and Pinto, 1926) Lainson, 1981 and *Cyrcilia* (*Haemogregarina*) *unicinata* (Khan, 1978) Lainson, 1981 to this genus, Siddall (1995) reduced the genus to a single species by relegating *Cyrcilia uncinata* to *Haemogregarina* (*sensu lato*). Siddall (1995) suggested that *C. gomesi* was a subjective junior synonym of *C. lignieresesi*. With the recent addition of *Cyrcilia* (*Desseria*) *nili* (Wenyon, 1909) NegmEldin, 1999 to the genus (NegmEldin 1999), there are again two species within *Cyrcilia*.

Members of *Cyrcilia* are characterised by the presence of an oocyst with 20, although

according to Siddall (1995) 16, or more sporozoites. Sporogony is intracellular in leech intestinal epithelial cells and infection of the intermediate host is presumably by salivary transmission. Vermicular intraerythrocytic meronts as well as gamonts are present in the fish host. The location of gametogenesis and of primary merogony as well as the number of microgametes formed from microgametogenesis are unknown.

Lainson (1981) described how in *C. lignieresii* the meronts were found within a conspicuous vacuole, mostly in kidney erythrocytes of the fish host. Gamonts were also within conspicuous vacuoles, but in erythrocytes of peripheral blood. Micro- and macrogamonts were found in the gut of the definitive host, the leech *Haementeria lutzi*, 15 hours after feeding (Fig. 6.1A-V). Macrogamonts, attached to the brush border of the caeca, were fertilized by the microgametes. The oocyst produced 20-30 sporozoites, but their presumed invasion of the proboscis was not followed.

In a study of *C. nili*, NegmEldin (1999) described two successive types of merogonic cycles, the first of which comprised large meronts producing eight small merozoites and a second, involving small meronts producing four merozoites each. Gamonts then developed from the small merozoites. Syzygy took place in the crop of the definitive host, the leech *Batracobdeloides tricarinata*. Oocysts subsequently produced up to 60 sporozoites within the intestinal tissue. Sporozoites migrated towards the salivary and proboscis tissues. Biological transmission between leech vector and fish hosts was experimentally obtained (NegmEldin 1999), though the fish used in these experiments were wild-caught.

The genus *Desseria* Siddall, 1995

Type species: *Desseria (Haemogregarina) myoxocephali* (Fantham, Porter, and Richardson, 1942) Siddall, 1995

Type host: The fish *Myoxocephalus octodecemspinus*

In erecting this genus, Siddall (1995), moved 41 species mainly from the genus *Haemogregarina* to *Desseria*. Smith (1996) added another species by moving *Desseria (Haemogregarina) marshallairdi* (Khan, Threlfall and Whitty, 1992) Smith, 1996 from the genus *Haemogregarina* to its present taxonomic position. In contrast Cruz and Davies (1998) showed that *Desseria (Haemogregarina) bettencourti*

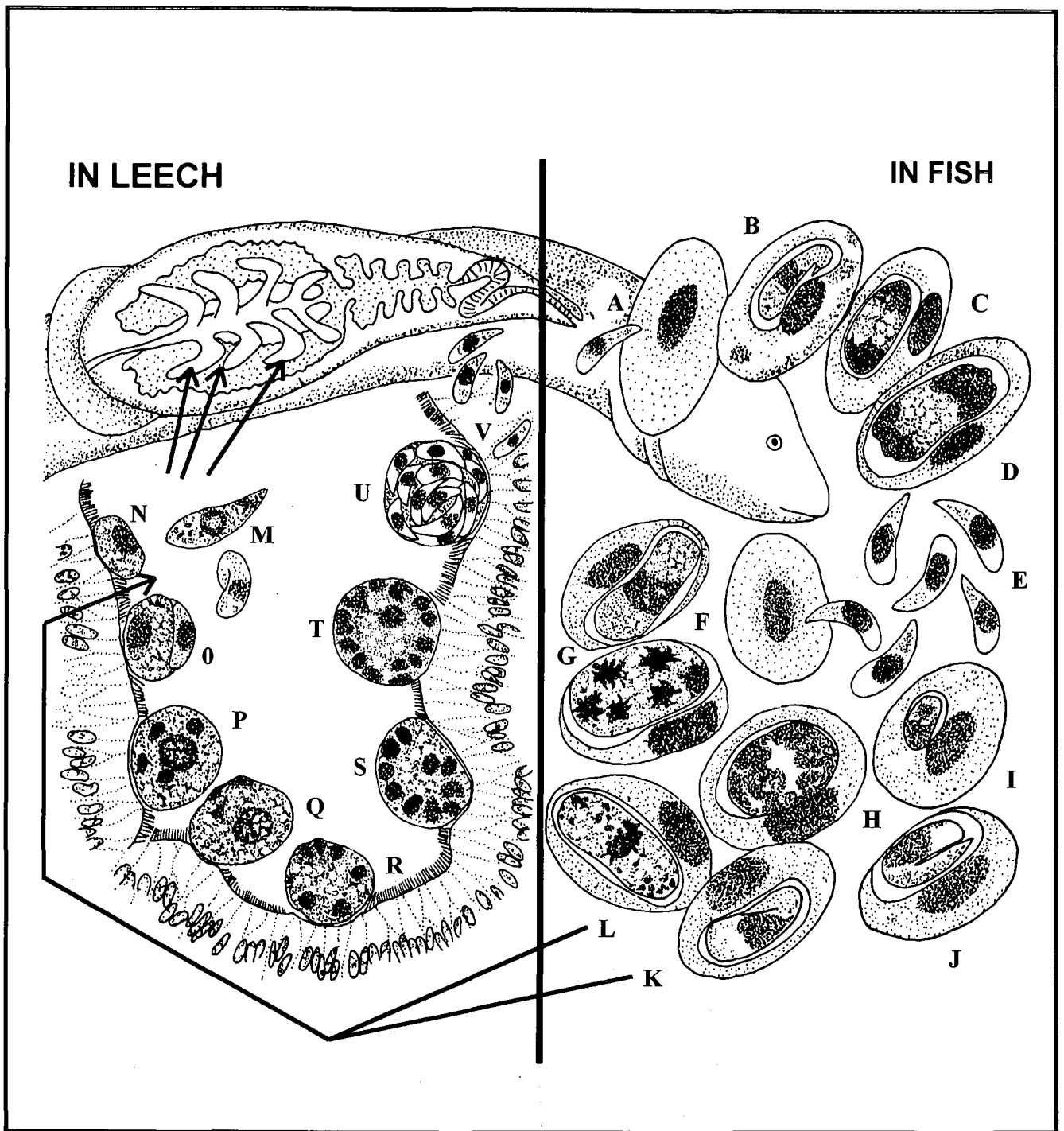


Figure 6.1. Diagram illustrating the life cycle of *Cyrilia ligmeresi* (Laveran, 1906) in the fish *Synbranchus marmoratus* and the leech *Haementeria lutzi* (redrawn from Lainson 1981). A. Sporozoites from leech enter red blood cell of fish B-H. Successive generations of schizonts in erythrocytes I, J. Gamonts in erythrocytes K. Mature microgametocyte L. Mature macrogametocyte M. Freed micro- and macrogametocytes in leech N. Attachment and growth of macrogamete O. Syzygy P. Fertilisation Q-U. Division V. Release and migration of sporozoites from intestinal epithelium to proboscis of leech.

(Franca, 1908) Siddall, 1995 is actually a babesiosome and rectified its taxonomic status accordingly. NegmEldin (1999) not only moved *Desseria nili* to the genus *Cyrlia* (see above), but also synonymised *Desseria (Haemogregarina) tilapiae* (Légér and Légér, 1914) Siddall, 1995 with it, thus to the present author's knowledge there are now 39 species in this genus. According to Davies and Johnston (2000) this number might still change, because there are *Desseria* species that are possibly not haemogregarines, and others are suspected to be junior synonyms of other fish haemogregarines.

According to Siddall (1995), sporogony of members of the genus *Desseria* occurs epicellularly on intestinal cells of leeches with a single sporogonic germinal centre producing 16 or more sporozoites. Transmission of primary merozoites to intermediate hosts is via the salivary gland. Secondary merogony takes place in the internal organs of the fish host. An important characteristic of this genus is the absence of merogony in the erythrocytes. Gamonts are, however, present in the host's erythrocytes.

The life history of the type species, *D. myoxocephali* is one of the best known of all the members of this genus (Figs. 6.2A-K). This is largely due to the detailed descriptions of Khan, Barrett and Murphy (1980) and Siddall and Desser (1992, 1993). The leech, *Malmiana scorpii* while feeding on an infected fish host, ingests intraerythrocytic gamonts. The gamonts are released in the gastric caeca where micro- and macrogamonts associate in pairs epicellularly on intestinal cells. One of the four aflagellated microgametes produced by the microgamont fertilizes the macrogamete. The oocyst undergoes multiple sporogonic divisions and produces 16 to 32 sporozoites. The sporozoites are released into the lumen and reinfect the epithelium, where they transform into uninucleate meronts. Merogony produces four elongated merozoites, which are also released into the lumen. Merozoites are transported via the blood sinus to the salivary cells from which they presumably infect host fish during subsequent blood feeding by the leech.

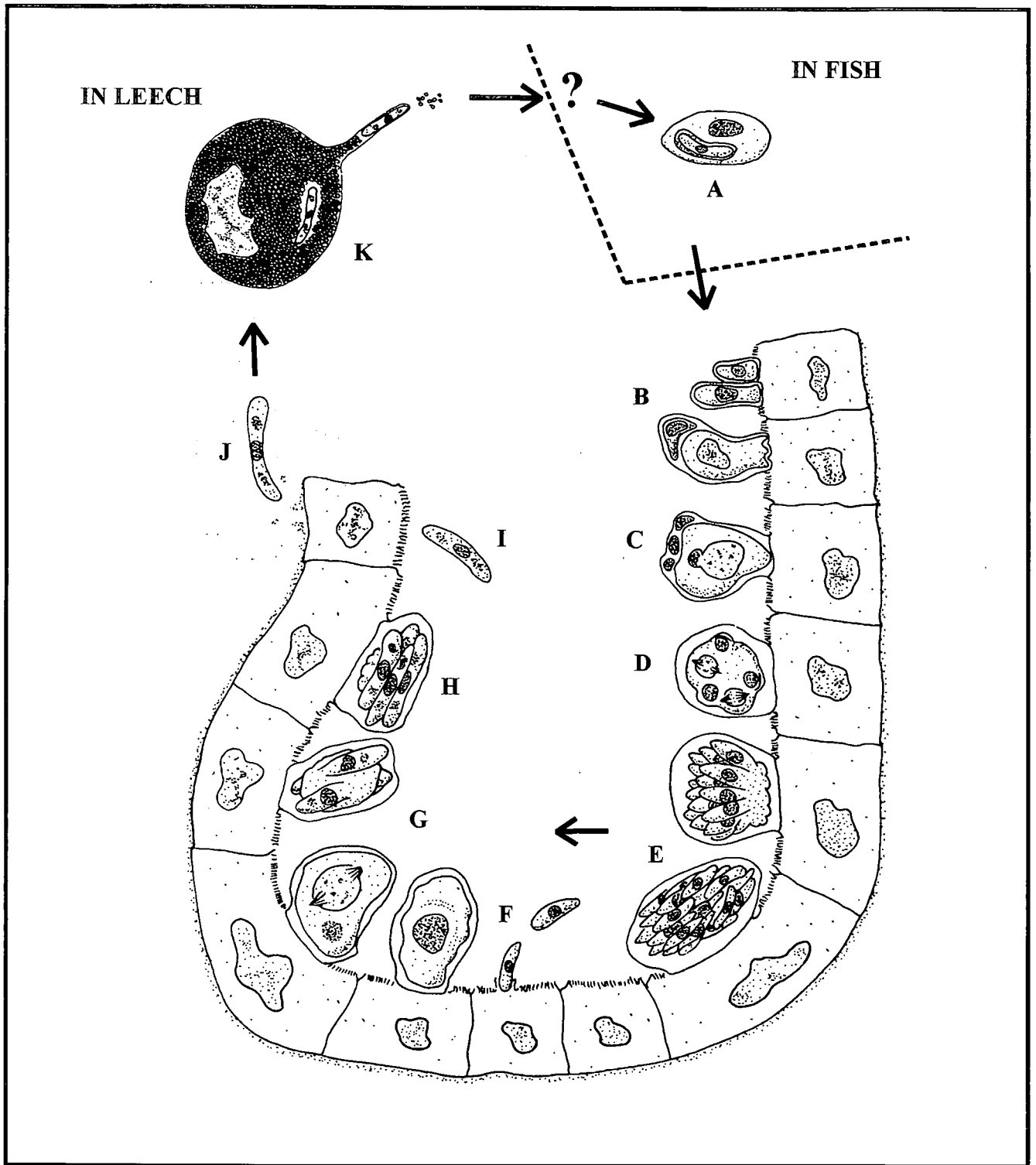


Figure 6.2. Diagram of the life cycle of *Desseria myxocephali* (Fantham, Porter and Richardson, 1942) redrawn from Siddall and Desser (1993). A. Intra-erythrocytic gamonts B. Pairing of micro- and macrogamonts on intestinal cells C. Microgametogenesis D. Multiple sporogonic division of zygote E. Zygote producing 16-32 sporozoites F. Release of sporozoites into the lumen and transformation into meronts G. Merogony H. Formations of four elongate merozoites I. Release of merozoites J. Penetration of epithelium by merozoites K. Transport of merozoites to the salivary glands from where infection occurs during subsequent feeding. Scale bars omitted by original authors.

The genus *Haemogregarina* (*sensu lato*) Danilewsky, 1885

The type species of the genus *Haemogregarina* is assigned to *Haemogregarina* (*sensu stricto*). The type host is also a chelonian and not a fish.

Type species: *Haemogregarina stepanowi* Danilewsky, 1885

Type host: The chelonian *Emys orbicularis*

Siddall (1995) allocated 13 species to the genus *Haemogregarina* (*sensu lato*) and Smith (1996) added another three previously omitted by Siddall to bring the total number of species to 16.

The development of members of *Haemogregarina* (*sensu lato*) in the fish host is well documented for several species, especially the cosmopolitan species *Haemogregarina* (*sensu lato*) *bigemina* (Laird 1953, Davies and Johnston 1976, Davies 1982, Eiras and Davies 1991, Smit and Davies 1999). According to Davies (1995) two different early development stages of *H. bigemina* in its vertebrate host have been described. In European fish the earliest detectable stages were small intraerythrocytic trophozoites, which enlarged to form meronts. Meronts then divided to form paired intra erythrocytic immature gamonts. Paired mature gamonts were also found in erythrocytes with rarely more than one pair per red cell. Laird (1953) described early development of *H. bigemina* in New Zealand fish hosts, which took place in basophil erythrocytes, small and large lymphocytes and monocytes to form merozoites that invaded erythrocytes. The rest of the development was the same as described above. Intraleucocytic stages have also been described for *Haemogregarina sachai* Kirmse, 1978 and *Haemogregarina simondi* (Laveran and Mesnil, 1901) by Kirmse (1978, 1979).

Haemogregarina (*sensu lato*) *bigemina* and *H. uncinata* are the only species of this group of fish haemogregarines where information exists on their possible developmental stages in the invertebrate host.

Khan (1978) recorded gamonts emerging from fish host cells in leeches (*Johanssonia* sp.) from one to 12 days after feeding on Laval's eelpouts infected with *H. uncinata*. These gamonts then presumably associated in pairs, producing two to four microgametes and a single macrogamete respectively. Although Khan (1978) did not observe fertilization, oocysts were found developing intracellularly within the

intestinal epithelial wall of the leech five days post feeding. In a leech examined 28 days post feeding, he found mature oocysts as well as several sporozoites. During a second experiment with the same two host species, Khan (1978) recorded mature oocysts with less than 100 sporozoites in the intestinal lumen of infected leeches 61 to 62 days post feeding. At 112 days post feeding he found some sporozoites in the proboscises of infected leeches, thus postulating that transmission could be via the bite of the leech. Khan (1978) was unfortunately not able to obtain experimental transmission of *H. uncinata*.

Studies on the transmission of *H. bigemina* in the northern hemisphere conclude that praniza larvae of the genus *Gnathia* are probably involved (Davies 1982). Davies (1995) has considered much of the evidence for this to be circumstantial. In Wales in 1976, the haemogregarine was located in all fishes, *Lipophrys (Blennius) pholis*, over 5.0 cm long and in none under 3.5 cm in length (Davies and Johnston 1976), but no leeches were found. It was difficult to account for the high prevalence of infection among fishes, except that gnathiid pranizae were common and they were found all year round. Some pranizae were found to contain stages looking remarkably like development stages of haemogregarines. *Haemogregarina bigemina* was also found in *Gnathia* pranizae in Wales in 1982. Davies (1982) found that the blood meal of two stage 1 gnathiid larvae contained gamonts resembling those of *H. bigemina* (Figs. 6.3A,B). Six days after feeding, young oocysts as well as larger oocysts with two to eight nuclei in each were found free in the blood meal of two stage 2 larvae (Figs. 6.3C-G). Some of the oocysts looked as if they were dividing into up to eight structures, tentatively identified as sporoblasts by Davies (1982) (Figs. 6.3H-J). Davies (1982) also found a few hundred free sporoblasts of which some showed evidence of division into perhaps two sporozoites (Figs. 6.3K,L). In another study in the northern hemisphere, Davies *et al.* (1994) investigated the transmission of *H. bigemina* by *Gnathia maxillaris* larvae between intertidal fishes in Portugal. The authors found numerous gamonts in the gut contents of 25% of praniza larvae of *G. maxillaris* and possible syzygy in a stage 2 larvae three days after feeding on fish infected with *H. bigemina*. Davies *et al.* (1994) also found gamonts as well as structures resembling oocysts in another stage 2 praniza examined five days after capture. The discovery of these development stages lent further support for gnathiids acting as vectors of *H. bigemina*.

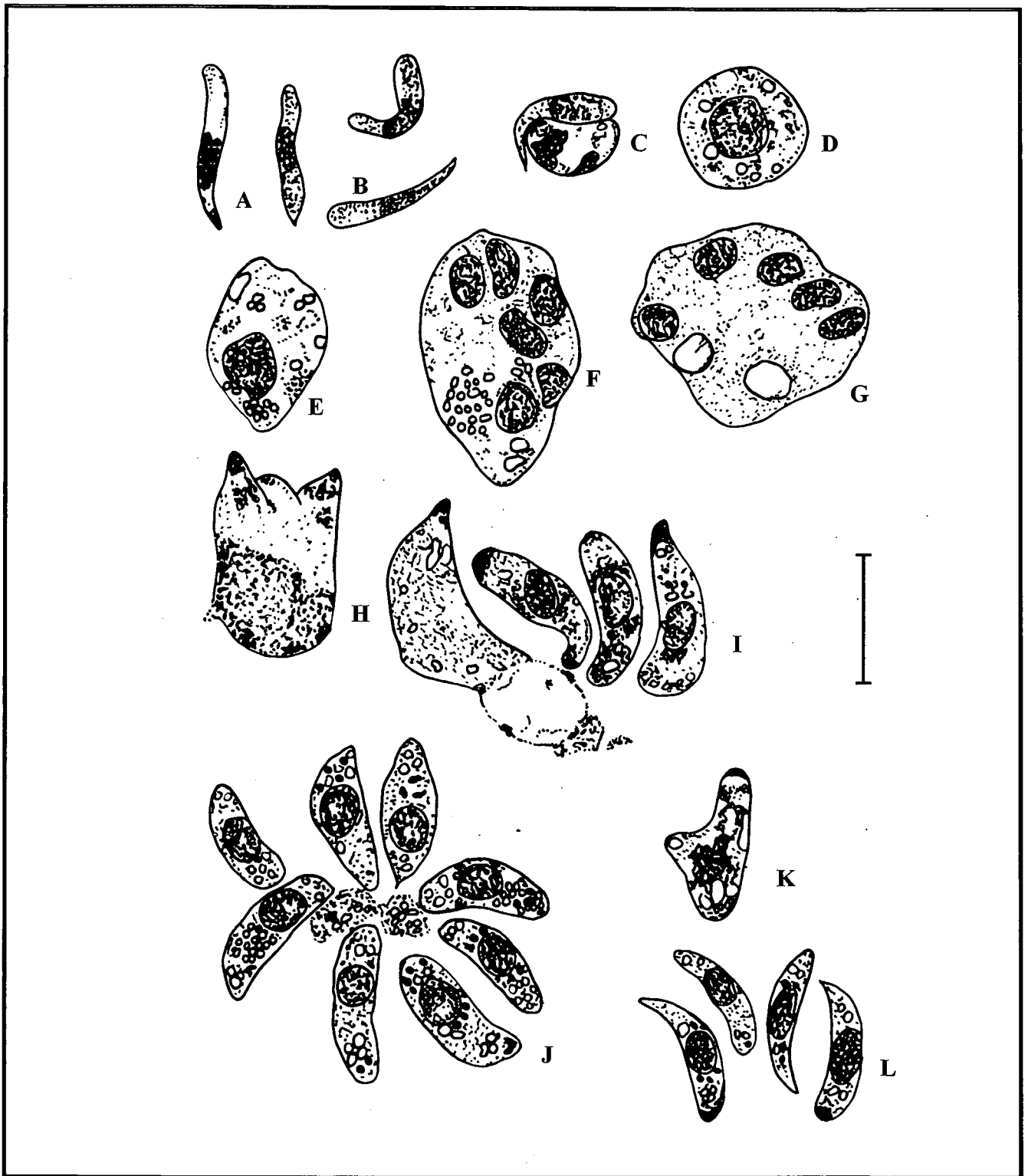


Figure 6.3. Diagram of the presumed development of *Haemogregarina bigemina* Laveran and Mesnil, 1901 in the anterior hindgut of the isopod *Gnathia maxillaris* (Montagu, 1804) (redrawn from Davies 1995). A, B. Gamonts. C. Possible syzygy. D, E. Zygotes or young oocysts. F, G. Large oocysts with several nuclei. H-J. Oocyst division, possible sporoblasts. K. Possibly sporoblast division. L. Sporozoites. Scale bar: 10 μ m.

Haemogregarina bigemina is a cosmopolitan fish blood parasite and is found in a wide variety of fish hosts from a number of geographical regions (see Davies 1995). In comparison, *Gnathia maxillaris* are confined to the United Kingdom and the west coasts of France, Spain and Portugal (Davies 1981, Monod 1926). If *H. bigemina* is transmitted by gnathiids, then other species than *G. maxillaris* must be able to act as intermediate hosts of this fish blood parasite. During a preliminary study on *H. bigemina* in South Africa, Smit and Davies (1999) found haemogregarine gamonts in 50 % of examined praniza larvae of *Gnathia africana* as well as gamonts undergoing syzygy. Recently, *H. bigemina* has been examined in detail in some marine fishes and gnathiids at De Hoop Nature Reserve and at Jeffreys Bay, South Africa. The results of that study will be discussed later on in this chapter (see 6.3).

Haemogregarine research in southern Africa

The haemogregarines of marine fishes from southern Africa had been largely ignored until recently when Smit and Davies (1999) published a paper as part of the current research project, describing *Haemogregarina bigemina* from fish hosts caught on the south coast. A reprint of the paper is bound into this thesis, and it contains a summary of haemogregarine research in southern Africa (see Appendix II). Rather than repeat the full content of the paper here, some facts relevant to this section are highlighted below.

Between 1918 and 1932 Fantham produced a number of papers in which he described the parasitic protozoa of the area, recording haemogregarines from a number of marine fish hosts. Fantham (1918) noted intraerythrocytic haemogregarines in the bull klipfish *Clinus taurus* Gilchrist and Thompson, 1908, from St. James Marine Aquarium, Cape Town. Later, (Fantham 1919) he also recorded haemogregarines from the panga, *Pterogymnus (Pagurus) laniarius* (Cuvier, 1830), the harder, *Liza richardsonii* (syn. *Mugil capito*) (Smith, 1846), and the Hottentot fish, *Pachymetopon (Cantharus) blochii* (Valenciennes, 1830) caught at Kalk Bay, south coast, and what might have been haemogregarines from the leucocytes of three species of marine fishes. Fantham (1930) reported a haemogregarine which occurred in the heart blood of a blenny, *Parablennius (Blennius) cornutus* (Linnaeus, 1758), captured on the south coast at St. James. He reported recent schizogony in heart blood, but was unclear whether this haemogregarine, *Haemogregarina fragilis* Fantham, 1930, truly underwent merogony in erythrocytes. Fantham's work presents a variety of problems

to the taxonomist because of his brief descriptions and his failure to conclude, for the most part, whether his parasites are known species or new ones. Much later, Siddall (1995) moved Fantham's *H. fragilis* to the genus *Desseria*, naming it *Desseria (Haemogregarina) fragilis* (Fantham, 1930) Siddall, 1995. Smit and Davies (1999) reported *Haemogregarina bigemina* in two new host fish and in larvae of *Gnathia africana*, caught on the south coast of South Africa (see Appendix 10.1). They concluded that *D. fragilis* and *H. bigemina* are the only two named species of haemogregarines from South Africa and discussed the possibility that the two species might, in fact, be identical.

6.2 Some haemogregarines from South African marine fish

Over the past four years, blood smears of 178 fishes belonging to 10 different species were collected at Jeffreys Bay and at Koppie Alleen, De Hoop Nature Reserve on the South African south coast (Table 6.1). Of these fishes, individuals of seven species, *Clinus superciliosus* (Fig. 6.4A), *C. cottoides* (Fig. 6.4B), *Clinus taurus* (Fig. 6.4C), *Chorisochismus dentex* (Pallas, 1769) (Fig. 6.4D), *Mugil cephalus* (Fig. 6.5A), *Amblyrhynchotes honckenii* (Fig. 6.5B) and *Haploblepharus edwardsii* showed haemogregarine infections. Four different types of haemogregarines were found, two known species, *Haemogregarina bigemina* and *Desseria (Haemogregarina) mugili* (Carini, 1932) Siddall, 1995, and two previously undescribed species, which will be referred to as *Haemogregarina* sp. A and *Haemogregarina* sp. B respectively, until further material from these two infections can be examined, and their taxonomy assessed fully. The description of the known species will be followed by the description of the species new to science.

Table 6.1. Statistical data of the different haemogregarine species collected from a variety of fish hosts from De Hoop Nature Reserve (DH) and Jeffreys Bay (JB). (N = number, ML = mean length).

Year	Site	Fishes			Haemogregarine	
		Fish species	N	ML(Range) mm	N (%)	Species
1996	JB	<i>Clinus superciliosus</i>	7	102.1 ± 11.5 (87-120)	7/7 (100)	<i>H. bigemina</i>
		<i>Clinus cottoides</i>	3	74, 84, 95	2/3 (66.7)	<i>H. bigemina</i>
		<i>Chorisochismus dentex</i>	1	165	1/1 (100)	<i>H. bigemina</i>
		<i>Caffrogobius caffer</i>	3	63, 76, 88	0/3 (0)	
1997	JB	<i>Clinus superciliosus</i>	23	115.1 ± 31.9 (65-174)	18/23 (78.2)	<i>H. bigemina</i>
		<i>Clinus cottoides</i>	2	94, 98	2/2 (100)	<i>H. bigemina</i>
1997	DH	<i>Clinus superciliosus</i>	7	115.0 ± 40.9 (78-180)	6/7 (85.7)	<i>H. bigemina</i>
		<i>Caffrogobius caffer</i>	4	73, 80, 100, 115	0/4 (0)	
1998	JB	<i>Mugil cephalus</i>	26	154.0 ± 40.3 (96-251)	5/26 (19.2)	<i>D. mugili</i>
		<i>Caffrogobius caffer</i>	3	67, 80, 96	0/3 (0)	
1998	DH	<i>Clinus superciliosus</i>	22	127.2 ± 37.6 (71-219)	22/22 (100)	<i>H. bigemina</i>
		<i>Clinus cottoides</i>	9	80.9 ± 10.5 (57-96)	9/9 (100)	<i>H. bigemina</i>
		<i>Diplodus sargus</i>	4	234, 285, 285, 295	0/4	
1999	JB	<i>Clinus superciliosus</i>	24	126.0 ± 24.8 (100-195)	22/24 (91.7)	<i>H. bigemina</i>
		<i>Clinus cottoides</i>	1	102	1/1 (100)	<i>H. bigemina</i>
		<i>Chorisochismus dentex</i>	2	85, 201	1/2 (50)	<i>H. bigemina</i>
1999	DH	<i>Clinus superciliosus</i>	14	100.6 ± 36.3 (47-155)	9/14 (64.3)	<i>H. bigemina</i>
		<i>Clinus cottoides</i>	7	84.6 ± 7.4 (75-95)	5/7 (71.4)	<i>H. bigemina</i>
		<i>Clinus taurus</i>	1	145	1/1 (100)	<i>H. bigemina</i>
		<i>Caffrogobius caffer</i>	4	73, 80, 100, 115	0/4 (0)	
		<i>Diplodus sargus</i>	3	250, 300, 340	0/3 (0)	
		<i>Dichistus capensis</i>	1	320	0/1 (0)	
		<i>Amblyrhynchotes honckenii</i>	4	155, 170, 175, 180	3/4 (75)	<i>Haemogregarina</i> sp. A
<i>Haploblepharus edwardsii</i>	3	448, 510, 610	3/3 (100)	<i>Haemogregarina</i> sp. B		
Total			178		106/123 (86.2)	

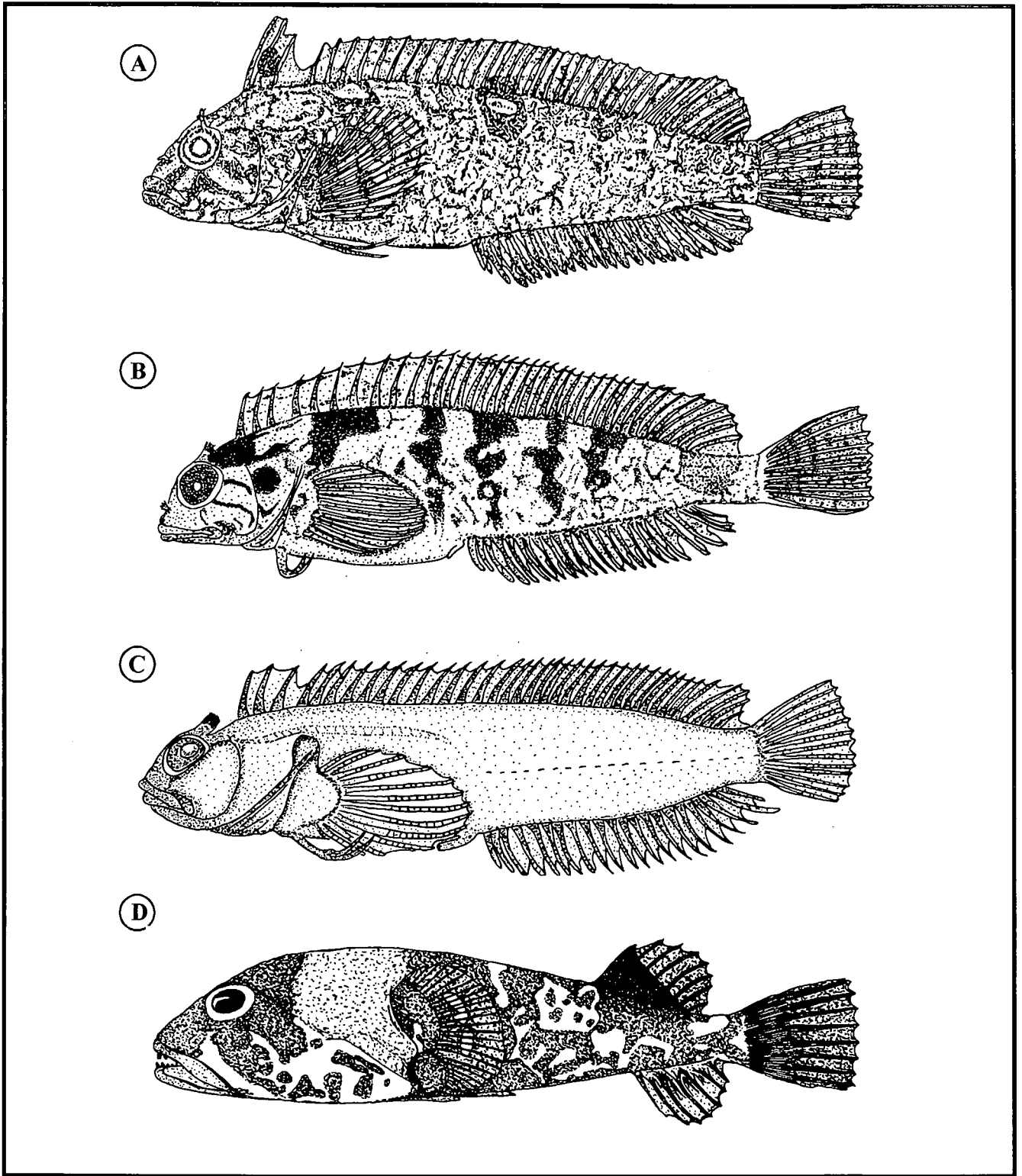


Figure 6.4. Fish hosts of *Haemogregarina bigemina* Laveran and Mesnil, 1901 in South Africa
A. *Clinus superciliosus* (Linnaeus, 1758) B. *Clinus cottoides* (Valenciennes, 1836) C. *Clinus taurus* (Gilchrist and Thompson, 1908) D. *Chorisochismus dentex* (Pallas, 1758) (A-C redrawn from Penrith 1969, D redrawn from Smith and Heemstra 1986) Not drawn to scale

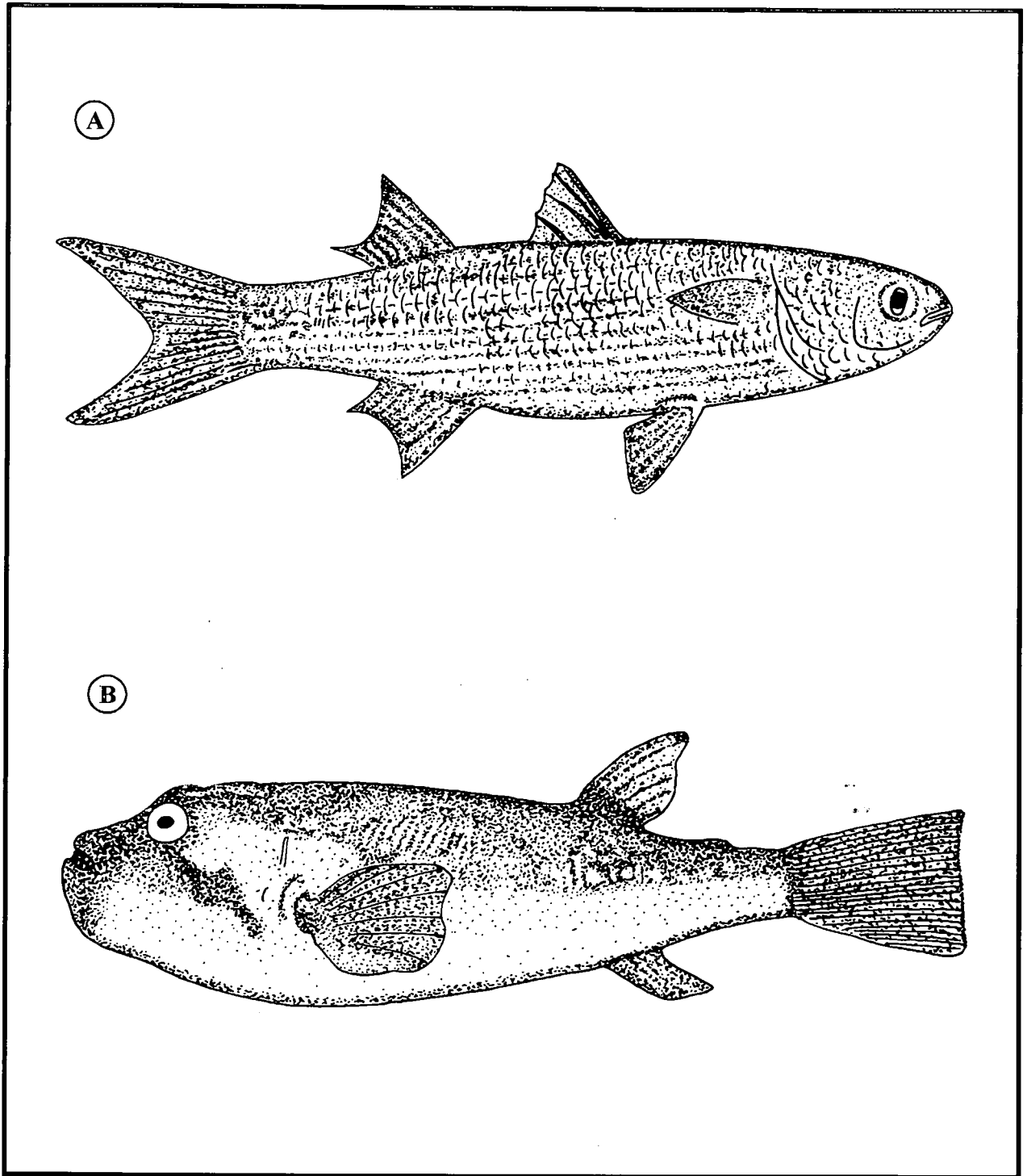


Figure 6.5. Some of the fish hosts of haemogregarines in South Africa. **A.** *Mugil cephalus* Linnaeus, 1758 **B.** *Amblyrhynchotes honckenti* (Bloch, 1795) (Redrawn from Smith and Heemstra 1986). Not drawn to scale

***Haemogregarina (sensu lato) bigemina* Laveran and Mesnil, 1901**

Different life cycle stages of *H. bigemina* were found in the blood of four residential intertidal fish species, *Clinus superciliosus* (super klipfish), *C. cottoides* (bluntnose klipfish), *C. taurus* (bull klipfish) and *Chorisochismus dentex* (rocksucker) collected at De Hoop Nature Reserve and Jeffreys Bay (Table 6.1). Of these species *H. bigemina* had the highest prevalence in *C. superciliosus*, with 84/97 (86.6%) of the specimens infected.

Small elongated trophozoites (Figs. 6.6A, 6.11C) were found individually in the erythrocytes of only infected *Clinus superciliosus* and *C. cottoides* specimens. The overall dimensions for trophozoites were 4.9 ± 0.6 (4.2-5.5) μm long by 2.1 ± 0.2 (1.9-2.6) μm wide ($n = 20$). The cytoplasm stained light blue with Giemsa and the centrally placed nuclei were deep purple.

Meronts, which presumably arose from trophozoites, were found more readily in erythrocytes (Figs. 6.6B, 6.11D,E). These stages were found in all four host fishes. Meronts were located individually in mature erythrocytes adjacent to the nucleus and measured 6.7 ± 1.0 (4.5-8.3) μm long by 3.3 ± 0.8 (2.1-4.5) μm wide ($n = 50$). One end of these elongated meronts was broader than the other, with the nucleus situated slightly towards the narrower end. Cytoplasm stained mostly light blue, except at the periphery of the meront, which was darker blue (Fig. 6.6C). No vacuoles were present in the cytoplasm. Meront nuclei appeared granulated and stained deep purple. The nuclei of larger meronts (longer than 7 μm) were in division, with the two daughter nuclei appearing at opposite sides or ends. The divisions of the cytoplasm were therefore either diagonal (Figs. 6.6D, 6.11F) or lateral (Fig. 6.6E).

Two vermicular immature gamonts seemed to result from division of a single meront (Figs. 6.6F, 6.11G). These measured 8.6 ± 0.4 (8.1-9.2) μm long by 1.4 ± 0.3 (1.0-1.7) μm wide ($n = 50$). Nuclei of immature gamonts were centrally placed and stained deep purple. Mature gamonts were characterised by a slender elongated body with a broad anterior and narrow pointed posterior. These forms were 11.6 ± 1.4 (9.0-13.8) μm long by 2.0 ± 0.3 (1.2-2.5) μm ($n = 100$) wide. The deep purple stained nuclei were situated in the narrow posterior half of the body, approximately 7 μm from the anterior and 2 μm from the posterior end and each measured 3.3 ± 0.6 (2.0-

4.2) μm long by 1.5 ± 0.4 (1.0-2.2) μm ($n = 100$) wide. The nuclei tapered with the body towards the posterior end. The cytoplasm of mature gamonts stained bluish purple, with posterior granules staining deep purple present in some specimens. The paired monomorphic gamonts were mostly situated next to one another in close proximity of the nucleus of the red cell (Figs. 6.6H, 6.11H). In some cases however, the gamonts were found on opposite sides of the erythrocyte nucleus (Fig. 6.6G). No erythrocytes were found infected with more than a single pair of gamonts. All the different stages were found in mature erythrocytes with some free gamonts present in some of the infected blood smears (Figs. 6.6I, 6.11I).

Remarks: The intraerythrocytic stages of *H. bigemina* seen in blood smears from the various species of fishes agreed in size and morphology with those previously recorded for this species (see Laird 1953, Davies 1982, 1995; Smit and Davies, 1999). No intraleucocytic stages were seen. A discussion concerning the similarities between *H. bigemina* and *D. fragilis* was recorded by Smit and Davies (1999) (see Appendix II) and the reader is referred to this text for the comparison. Overall, however, it is impossible to reach firm conclusions about the affinity between *D. fragilis* and *H. bigemina*, except that their gamonts are of similar size. Capture and examination of *Parablennius cornutus* would help resolve this problem.

The intertidal fishes *Chorisochismus dentex* and *Clinus taurus* are yet another new host records for *H. bigemina* in South Africa.

Taxonomic summary

Haemogregarina (*sensu lato*) *bigemina*

Synonym: *Haemogregarina salariasi* Laird, 1951

Type host: *Lipophrys (Blennius) pholis*

Type locality: Cap de la Hague, Northern France

Present study:

Hosts: *Clinus superciliosus* (Linnaeus, 1758), *Clinus cottoides* Valenciennes, 1836, *Chorisochismus dentex* (Pallas, 1769), *Clinus taurus* Gilchrist and Thompson, 1908

Localities: De Hoop Nature Reserve (34°28'S, 20°30'E), Jeffreys Bay (34°2.2 'S, 24°56.5'E)

Prevalence: 86.2% (106/123)

Intensity of infection: 1/1000 (0.1%) of erythrocytes infected. Only intraerythrocytic infections observed.

Measurements (data from all different host fishes combined):

Trophozoites: 4.9 ± 0.6 (4.2-5.5) μm by 2.1 ± 0.2 (1.9-2.6) μm (n = 20)

Meronts: 6.7 ± 1.0 (4.5-8.3) μm by 3.3 ± 0.8 (2.1-4.5) μm (n = 50)

Immature gamonts: 8.6 ± 0.4 (8.1-9.2) μm by 1.4 ± 0.3 (1.0-1.7) μm (n = 50)

Mature gamonts: 11.6 ± 1.4 (9.0-13.8) μm by 2.0 ± 0.3 (1.2-2.5) μm (n = 100)

Gamont nuclei: 3.3 ± 0.6 (2.0-4.2) μm by 1.5 ± 0.4 (1.0-2.2) μm (n = 100)

Vector: The haematophagous larvae of *Gnathia africana* (see 6.3)

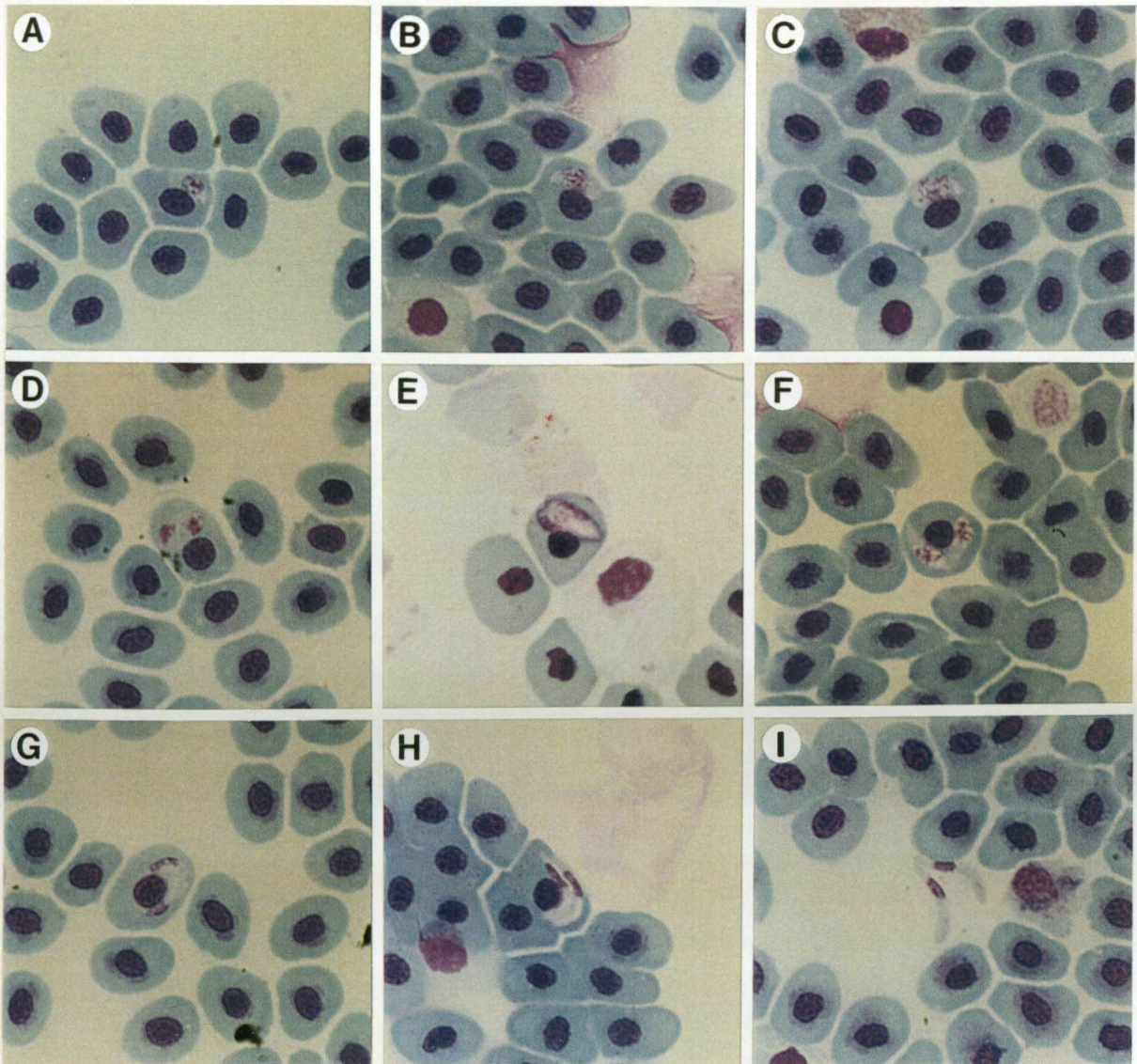


Figure 6.6. Light micrographs of the different life cycle stages of *Haemogregarina bigemina* Laveran and Mesnil, 1901 in its fish host *Clinus superciliosus* (Linnaeus, 1758) (X1000). **A.** Trophozoite. **B.** Young meront. **C.** Larger meront. **D.** Lateral dividing meront. **E.** Diagonal dividing meront. **F.** Young gamonts. **G.** Gamonts on opposite sides of erythrocyte nucleus. **H.** Paired gamonts. **I.** Extracellular gamonts.

Desseria mugili (Carini, 1932)

Three distinctly different intraerythrocytic stages of a haemogregarine were found in 19.2% (5/26) of flathead mullets, *Mugil cephalus* caught in the Seekoei River estuary near Jeffreys Bay during January 1998. None of the stages were undergoing division, and all forms occurred individually in erythrocytes.

The smallest stages measured 5.5 ± 0.4 (4.9-6.2) μm length by 1.9 ± 0.1 (1.7-2.1) μm breadth ($n = 15$) and were provisionally identified as merozoites (Figs. 6.7A,B). Mostly they were situated half way between the nucleus and cell periphery of the erythrocyte. These merozoites were elongated with in some cases a slightly broader, possibly anterior end. Their nuclei, which were apparently situated posteriorly, had ill defined margins and appeared to consist of a number of reddish stained granules. Nuclei measured 2.1-2.6 μm by 1.4-1.9 μm . Merozoite cytoplasm stained light blue with one or two vacuoles in the anterior half.

Immature gamonts, probably resulting from the growth of merozoites, measured 7.4 ± 0.4 (6.8-7.9) μm in length by 2.1 ± 0.1 (1.9-2.3) μm in breadth ($n = 15$). In most of the cases, immature gamonts were situated closer to the cell membrane than to the nuclei of erythrocytes (Fig. 6.7C). Like merozoites, they were also elongated, and vermicular, with what was presumed to be the anterior end, slightly broader than the posterior end. The margins of the nuclei were better defined than in those of the merozoites and stained dark red. The nuclei were situated in the posterior half of the cell almost reaching the posterior end and they measured 2.7-3.8 μm by 1.8-2.2 μm . The cytoplasm of these stages stained slightly darker blue than that of the merozoites with no obvious vacuoles present.

The immature gamonts were presumed to enlarge to form monomorphic mature gamonts with a overall dimension of 10.1 ± 0.7 (9.2-11.3) μm length by 2.5 ± 0.3 (2.1-2.9) μm breadth ($n = 15$). The gamonts were elongated, and vermicular with their anterior and posterior ends slightly curved towards the nucleus of the erythrocyte (Figs. 6.7D-I). In all the specimens, what was presumed to be the anterior end was always slightly broader than the posterior end (Figs. 6.7G,H). Nuclei had well defined margins, stained dark purple and were situated in the posterior two thirds of the gamont, 3.0-5.5 μm from the anterior and 1.5-3.0 μm from the posterior ends

respectively. They measured 3.9 ± 0.3 (3.5-4.5) μm in length by 1.9 ± 0.1 (1.7-2.1) μm in breadth ($n = 15$). Their cytoplasm stained light purple, with a prominent dark stained anterior cap (Figs. 6.7H,I). Dark staining granules were present at the posterior end of mature gamonts, as well as a few vacuoles between the anterior cap and nucleus.

Remarks: This is the first time that three different stages are described for this parasite. Previous records do not differentiate between these stages (Carini 1932, Saunders 1964, Laird 1958, Eiras, Ranzani-Paiva and Davies 1995). Although the mature gamonts are larger, the dimensions of the merozoites (4.9-6.2 by 1.7-2.1 μm) and immature gamonts (6.8-7.9 by 1.9-2.3 μm) described here fall within the size ranges described by the original author Carini (1932) (2 by 6-9 μm) as well as those found by Saunders (1964) (2.5-9.5 μm) for *Desseria mugili*. The mature gamonts (2.1-2.9 by 9.2-11.3 μm), however, do fall within the size range reported for *Desseria mugili* by Laird (1958) (1.8-2.8 by 8.2-10.5 μm), and Eiras *et al.* (1995) (2.6-3.1 by 8.3-10.4). The peculiar large (2.1-3.6 by 10.4-18.3 μm) C shaped stages found by Eiras *et al.* (1995) were not detected in any of the South African mullets.

Mullets are known to be parasitised by a variety of blood protozoa from the genera *Trypanosoma*, *Desseria* and *Dactylosoma* (Becker and Overstreet 1979, Paperna and Overstreet 1981, Eiras *et al.* 1995). The only records of blood protozoa infecting mullets in South Africa are that of Fantham (1918) and Paperna (1981). Fantham (1918) only mentioned the presence of a haemogregarine from *Liza richardsonii* (syn. *Mugil capito*), but did not allocate a species name to it. Paperna (1981) reported the presence of *Trypanosoma mugilicola* Becker and Overstreet, 1979 (which Eiras *et al.* 1995 considered likely to be *Trypanosoma froesi* Lima, 1976), as well as describing a dactylosome, *Dactylosoma hannesii* Paperna, 1981 from *Mugil cephalus*, *Liza richardsonii* and *Liza dumerili* (Steindachner, 1869). In his review on parasites, infections and diseases of fishes in Africa, Paperna (1996) also cited *T. mugilicola* and *D. hannesii* as the only known blood protozoans from South African mullets. This is therefore the first report of *Desseria mugili* from South Africa.

Taxonomic summary

Desseria mugili

Type host: *Mugil brasiliensis*

Type locality: Guarajá

Present study:

Host: *Mugil cephalus* Linnaeus, 1758

Locality: Seekoei river estuary, Jeffreys Bay (34°2.2 'S, 24°56.5'E)

Prevalence: 19.2% (5/26)

Intensity of infection: 1/10000 (0,01%) of erythrocytes infected. Only
intraerythrocytic infections observed

Measurements:

Merozoites: 5.5 ± 0.4 (4.9-6.2) μm by 1.9 ± 0.1 (1.7-2.1) μm (n = 15)

Immature gamonts: 7.4 ± 0.4 (6.8-7.9) μm by 2.1 ± 0.1 (1.9-2.3) μm (n = 15)

Mature gamonts: 10.1 ± 0.7 (9.2-11.3) μm by 2.5 ± 0.3 (2.1-2.9) μm (n = 15)

Gamont nuclei: 3.9 ± 0.3 (3.5-4.5) μm by 1.9 ± 0.1 (1.7-2.1) μm (n = 15)

Vector: unknown

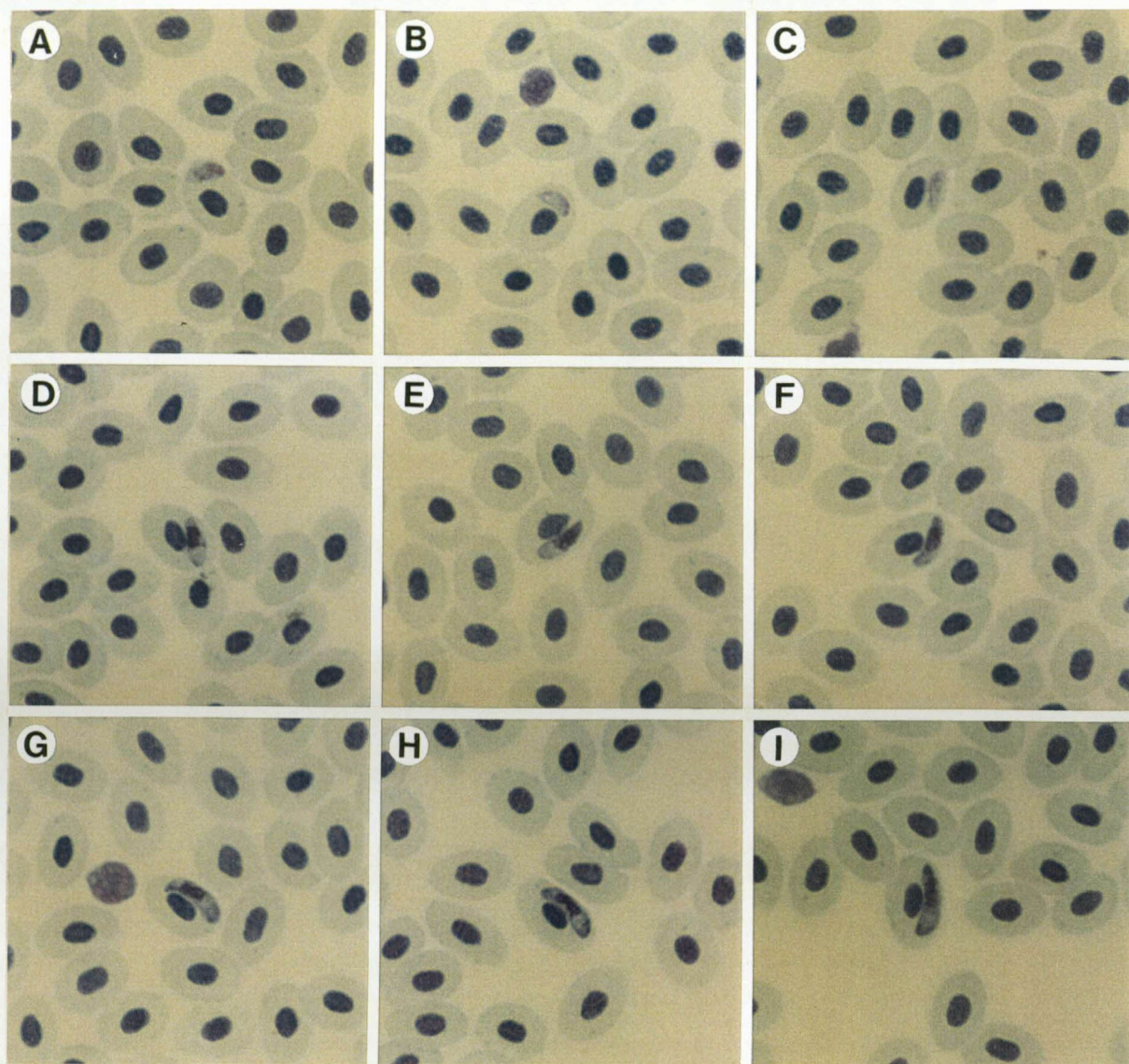


Figure 6.7. Light micrographs of the different life cycle stages of *Desseria mugili* (Carini, 1932) in its fish host *Mugil cephalus* Linnaeus, 1758 (X1000). A, B. Merozoites. C. Immature gamonts. D-I. Different size mature gamonts with prominent dark stained anterior cap.

Haemogregarina sp. A

Different life cycle stages of a haemogregarine species were found in the heart blood of three out of four specimens of *Amblyrhynchotes honckenii* (evileye pufferfish) caught in October 1999 at De Hoop Nature Reserve.

Small elongated haemogregarines with a rounded presumed, anterior, end and a pointed posterior, were identified as trophozoites (Figs. 6.8A,B). They measured 5.8 ± 0.6 (5.0-6.4) μm long by 2.6 ± 0.3 (2.3-3.0) μm wide ($n = 6$) and were found individually in erythroblasts and erythrocytes of all three infected fish. The cytoplasm of these stages stained light pinkish blue with Giemsa. The centrally placed nuclei were diffuse and were always deeply stained. Trophozoites in erythroblasts were situated close to the nuclei of the host cell, some times in contact with host nuclei. Where these were found infecting erythrocytes, the trophozoites were closer to the limiting membrane of the cytoplasm than the nucleus.

Large meronts, which presumably arose from trophozoites, were found singly adjacent to the nucleus of primarily erythroblasts in all three infected fish (Fig. 6.8C). These oval-shaped parasites measured 6.8 ± 0.6 (6.1-8.0) μm long by 2.7 ± 0.2 (2.3-3.0) μm wide ($n = 6$). Cytoplasm stained pale magenta, except at the periphery of the meront, which was darker blue. The diffuse nucleus appeared granulated, filled almost two thirds of the meront and stained deep purple. No vacuoles were present in the cytoplasm. The nuclei of larger meronts (longer than 7 μm) were in division, with the two daughter nuclei appearing at opposite ends of the body of the parasite (Fig. 6.8D). Four to seven vacuoles were distributed randomly in the cytoplasm of the dividing meronts.

A third form was also found in the erythroblast of all three infected fish. These haemogregarines, identified as merozoites, presumably derived from the division of the meronts, were larger than the trophozoites, but more slender than the meronts, with one end broad (anterior) and the other pointed (posterior) (Fig. 6.8E). These occurred individually in host cells and measured 6.7 ± 0.9 (5.5-8.4) μm long by 2.4 ± 0.3 (2.0-2.7) μm wide ($n = 8$). Their cytoplasm stained light pinkish blue and a single vacuole was present between the nucleus and the broad anterior end. The nucleus

situated in the posterior two thirds of the merozoite, was not as diffuse as in the previous stages and stained darkish pink.

The largest intraerythrocytic forms seen were identified as gamonts, and they always occurred singly in mature erythrocytes. They measured 7.3 ± 0.9 (6.1-9.8) μm long by 2.8 ± 0.4 (2.0-3.6) μm wide ($n = 20$). These specimens were the dominant form with an average of 0.5% of erythrocytes in all three infected fish parasitised. The gamonts were characterised by their elongated body that seemed to be encapsulated (Figs. 6.8F,G). The capsule stained dark blue and the parasite cytoplasm light blue. A few randomly distributed vacuoles were present in the cytoplasm of some of the specimens. Another characteristic of the gamonts was the deep purple staining anterior cap present in almost all of the specimens. The nuclei were situated in the posterior half of the body, approximately 4.5 μm from the anterior and 1 μm from the posterior end and each measured 2.5 ± 0.3 (2.3-3.2) μm long by 2.1 ± 0.2 (1.8-2.5) μm wide ($n = 20$). Nuclei were diffuse and consisted of deep purple stained granules. The gamonts were situated in close proximity to the nucleus of the red cell and only in a few cases did they seem to distort the host cell. Five gamonts were also seen to be in the process of escaping from the erythrocyte (Fig. 6.8H). In these cases no prominent capsule was detected. A number of extracellular gamonts conforming to the description of the intraerythrocytic stages were also found, but without the capsule and the deep staining cap (Fig. 6.8I). These stages were slightly larger than the intraerythrocytic stages and measured 9.7 ± 0.4 (8.9-10.2) μm long by 2.3 ± 0.4 (1.8-3.0) μm wide ($n = 10$).

In one of the infected fish a second kind of extracellular gamont was found. This type was characterised by an elongated body with a pointed anterior end and a long recurved tail more than half the length of the body of the haemogregarine (Figs. 6.8J,K). The bodies of these stages were 9.7 ± 0.4 (8.9-10.7) μm and the tails were 5.5 ± 0.9 (4.1-6.6) μm long, making a total length 15.2 ± 1.0 (13.6-16.6) μm . At their widest point, these specimens were 2.1 ± 0.2 (1.8-2.5) μm . The diffuse nucleus of this stage consisted of deep staining granules and was situated in the anterior half of the parasite approximately 5.5 μm from the anterior and 7.5 μm from the posterior ends respectively. The cytoplasm stained bluish purple, with purple staining granules lying anterior to the nucleus in some specimens. A single vacuole was present near

the posterior end of the tail in some of the specimens. In the blood film of one of the infected fish, three extracellular gamonts with straightened tails were also found (Fig. 6.8L).

The vector of this parasite is unknown, but one of the three infected fish were parasitised by the haematophagous larvae of *Gnathia pipinde*, a new species described as part of this study (see Chapter 5). Two of the three infected fish also harbored a few specimens of an unidentified *Caligus* sp.

Remarks: At first it appeared that there were two kinds of gamonts (encapsulated intracellular forms and extracellular forms with a recurving tail). Closer examination of the morphometrical data revealed that they are actually the same form, with the tail tucked underneath the body in the capsulated intracellular stages but released in the extracellular form. The larger size of the body of the extracellular forms (range of 8.9-10.7 μm) in comparison with that of the encapsulated ones (range of 6.1-9.8 μm) is also expected. In encapsulated haemogregarines from other vertebrates, it has been found that the intracellular forms could gain up to 60% in size upon escaping from the host cell (Ball 1958, see Davies and Johnston 2000). The correlation between the size of the nucleus and the distance of the nucleus from the anterior end (see description) of the two different forms also support this assumption. It can thus be concluded that the gamonts of this species are monomorphic.

This is a very remarkable haemogregarine, showing characteristics that are not usually found amongst fish haemogregarines, but normally associated with haemogregarines infecting reptile erythrocytes. These characteristics include encapsulated intraerythrocytic gamonts and the presence of a recurving tail. According to Davies and Johnston (2000), encapsulated gamonts are only present in a small number of fish haemogregarines, such as *Desseria (Haemogregarina) londoni* (Yakimoff and Kohl-Yakimoff, 1912) Siddall, 1995 and *Desseria (Haemogregarina) lepidosirensis* (Jepps, 1927) Siddall, 1995. In comparison, a large number of encapsulated haemogregarines infect toads, lizards, tortoises and snakes (see Davies and Johnston 2000). Good examples were described by Sambon and Seligmann (1907) who recorded more than 20 haemogregarines with capsules infecting snakes from a variety of different localities.

To the author's knowledge the only encapsulated fish haemogregarine with a recurving tail is that of *D. lepidosirens* from the primitive South American lungfish. In *D. lepidosirens* the recurving tail was visible in the intraerythrocytic stages and as many as four parasites were found infecting a single host red cell (see Jepps 1927). Jepps (1927) did not give measurements to her specimen, but the gamont in her drawing had a body length of 13 μm and a tail of 6.21 μm (total length = 19.21 μm), thus much larger than *Haemogregarina* sp. A. The two species also differ in the position of the gamont nucleus.

The presence of a deeply stained anterior cap in the present species places it in the "rovignensis group" as proposed by Laird (1952). This group includes species such as *Cyrlia uncinata*, *H. aeglefini*, *H. anarhichadis*, *H. coelorhynchi* and *H. rovignensis* (see Davies 1995).

The observation of division of the mature meronts in the erythrocytes supports the placement of this haemogregarine in the genus *Haemogregarina* (*sensu lato*) (see Siddall 1995).

Taxonomic summary

Haemogregarina sp. A

Type host: *Amblyrhynchotes honckenii* (Bloch, 1795)

Type locality: De Hoop Nature Reserve (34°28'S, 20°30'E)

Prevalence: 75% (3/4)

Intensity of infection: 5/1000 (0.5%) of erythrocytes infected. Only intraerythrocytic and extracellular infections observed.

Measurements:

Trophozoites: 5.8 ± 0.6 (5.0-6.4) μm by 2.6 ± 0.3 (2.3-3.0) μm (n = 6)

Meronts: 6.8 ± 0.6 (6.1-8.0) μm by 2.7 ± 0.2 (2.3-3.0) μm (n = 6)

Merozoites: 6.7 ± 0.9 (5.5-8.4) μm by 2.4 ± 0.3 (2.0-2.7) μm (n = 8)

Encapsulated intraerythrocytic gamonts: 7.3 ± 0.9 (6.1-9.8) μm by 2.8 ± 0.4 (2.0-3.6) μm (n = 20)

Nuclei of encapsulated intraerythrocytic gamonts: 2.5 ± 0.3 (2.3-3.2) μm by 2.1 ± 0.2 (1.8-2.5) μm (n = 20)

Extracellular gamonts with recurved tail: 15.2 ± 1.0 (13.6-16.6) μm by 2.1 ± 0.2 (1.8-2.5) μm (n = 15)

Nuclei of extracellular gamonts with recurved tail: 3.3 ± 0.3 (2.7-4.1) μm by 1.8 ± 0.1 (1.6-2.0) μm (n = 15)

Extracellular gamonts with straightened tail: 14.2 ± 0.1 (14.1-14.3) μm by 2.3 ± 0.1 (2.3-2.5) μm (n = 3)

Nuclei of extracellular gamonts with straightened tail: 3.63 ± 0.5 (3.2-4.1) μm by 2.0 ± 0.2 (1.8-2.3) μm (n = 3)

Vector: unknown

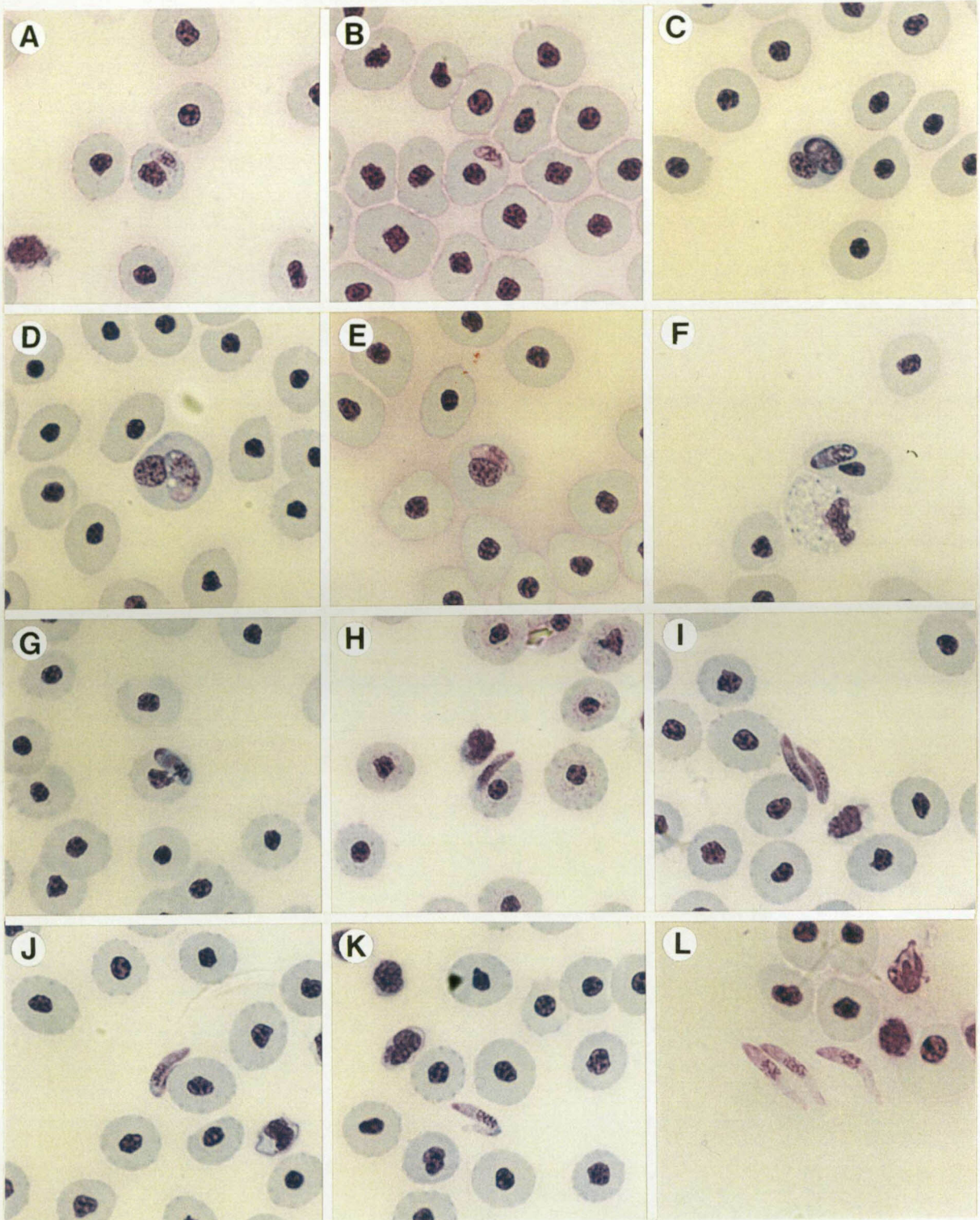


Figure 6.8. Light micrographs of the different life cycle stages of *Haemogregarina* sp. A in its fish host *Amblyrhynchotes honckenii* (Bloch, 1795) from the De Hoop Nature Reserve (X1000). A. Young trophozoite. B. Older trophozoite. C. Meront. D. Dividing meront. E. Merozoite. F. Young gamont. G. Older gamont. H. Gamont in the process of escaping from the host erythrocyte. I. Extra-erythrocytic gamonts. J. Extra-erythrocytic gamont with recurved tail just visible. K. Extra-erythrocytic gamont with recurved tail clearly visible. L. Extra-erythrocytic gamonts with straightened tail.

Haemogregarina sp. B

Three distinctly different intraerythrocytic stages of a haemogregarine were found in 100% (3/3) of puffadder shysharks, *Haploblepharus edwardsii*, caught at De Hoop Nature Reserve in October 1999.

The smallest stages measured 6.1 ± 0.3 (5.7-6.4) μm long by 4.0 ± 0.2 (3.6-4.1) μm wide ($n = 5$) and were identified as trophozoites or merozoites (Fig. 6.9A). These stages did not show a preference for a specific position in the host cell and were either in close proximity to the nucleus or next to the cell periphery. They also showed differences in form, the larger ones ($>6 \mu\text{m}$) being more elongated, with a slightly broader anterior end in some cases (Fig. 6.9B). The nuclei of the smaller specimens ($<6 \mu\text{m}$) were stained purple and were centrally situated. The nuclei of the larger ones had ill defined margins and consisted of a number of deeply stained granules. The cytoplasm stained light blue with a few vacuoles distributed randomly in some specimens.

Large oval to round meronts were found singly, adjacent to the nucleus of erythrocytes of all three infected fish (Figs. 6.9C,D). These specimens measured 9.0 ± 1.0 (7.3-10.0) μm long by 6.9 ± 0.5 (6.1-7.7) μm wide ($n = 20$) and their cytoplasm stained light blue with the periphery of the cytoplasm staining darker blue. One or two vacuoles were present in the cytoplasm of some of the specimens. The compact nuclei were centrally placed in some of the round meronts, but in the posterior two thirds of the oval forms. The nuclei stained deep purple and most were in division, with two daughter nuclei visible (Figs. 6.9E-H).

The largest intraerythrocytic stages, identified as gamonts, were found individually or paired in erythrocytes and they measured 11.1 ± 0.9 (9.1-13.6) μm long by 5.8 ± 0.5 (5.0-7.3) μm wide ($n = 30$). The gamonts were elongated, with the presumed anterior end slightly broader than the posterior end (Fig. 6.9J,K). Their compact nuclei had well defined margins, consisted of a large number of dark purple stained granules and were situated in the posterior two thirds of the gamont, 3.5-5.5 μm from the anterior and 2.0-3.0 μm from the posterior end respectively. The nuclei were almost round and in most cases slightly broader than long and measured 4.0 ± 0.4 (2.7-4.5) μm long by 4.5 ± 0.6 (3.6-5.9) μm wide ($n = 30$). Gamont cytoplasm stained the same light

blue colour as in meronts and the peripheral cytoplasm was also darker blue. Few deep staining granules were present in the cytoplasm anterior to the nucleus and a single characteristic vacuole occurred posterior to the nucleus. In all the erythrocytes infected with this haemogregarine, the nucleus was situated towards the one end of the red cell (Fig. 6.9K) in comparison with non-parasitised host cells where the nucleus was more centrally placed. This was especially evident in the cells infected with two parasites (Fig. 6.9I). In most of the cells that were parasitised, deep staining granules of uncertain identity were found attached to the outer membrane of the parasite. Although these granules were mostly situated adjacent to the nucleus, they were also found at the anterior and posterior ends of the parasites. A large number of extracellular gamonts were found in all three infected fish (Fig. 6.9L). The gamonts were monomorphic.

The vector of this species is unknown, but large numbers of the haematophagous larvae of *Gnathia pantherinum* were found attached to the gills of all three infected fish (see Chapter 4).

Remarks: Currently five species of haemogregarines belonging to two genera are known to infect elasmobranchs worldwide. These are: *Desseria (Haemogregarina) dasyatis* (Saunders, 1958) Siddall, 1995, *Desseria (Haemogregarina) heterodonti* (von Prowazek, 1910) Siddall, 1995, *Desseria (Haemogregarina) torpedinis* (Neumann, 1909) Siddall, 1995 (syn. *H. lobianci* Yakimov and Kohl-Yakimov, 1912) *Haemogregarina carcharias* Laveran, 1908, (syn. *H. hemiscylli* Mackerras and Mackerras, 1961) and *H. delagei* Laveran and Mesnil, 1902.

Although the gamonts of *Haemogregarina* sp. B show some similarity in basic form with those of *H. carcharias* (20–27 X 7–10 μm) and its synonym *H. hemiscylli* (16–19 X 5–8 μm), they are much smaller than the species described from sharks in Australia (see Laveran 1908, Mackerras and Mackerras 1961). The only other species of the genus *Haemogregarina*, *H. delagei*, is probably the best described of all the elasmobranch species with information available on its distribution, hosts and life cycle stages in the vertebrate hosts (see Laveran and Mesnil 1902, Laird and Bullock 1969, So 1972, Khan 1972, Becker and Overstreet 1979, Khan *et al.* 1980). Although *Haemogregarina* sp. B gamonts do overlap in length with some measurements of *H. delagei* (6.3–13.7 X 1.6–3.6) the current parasite is generally almost twice as wide.

None of the dividing stages described for *H. delagei* by So (1972) and Khan (1972) were found in the South African species and this also separates the two species.

In many of the cases where haemogregarines were described from elasmobranchs, the same fishes were also parasitised by trypanosome species (Mackerras and Mackerras 1961, So 1972, Daily 1978). It is thus interesting to note that all three catsharks infected with *Haemogregarine* sp. B in this study, were also parasitised by an unidentified trypanosome.

Taxonomic summary

Haemogregarina sp. B

Type host: *Haploblepharus edwardsii* (Voight, 1832)

Type locality: De Hoop Nature Reserve (34°28'S, 20°30'E)

Prevalence: 100% (3/3)

Intensity of infection: 8/1000 (0.8%) of erythrocytes infected. Only intraerythrocytic and extracellular infections observed.

Measurements:

Trophozoites or merozoites: 6.1 ± 0.3 (5.7-6.4) μm by 4.0 ± 0.2 (3.6-4.1) μm (n = 5)

Meronts: 9.0 ± 1.0 (7.3-10.0) μm by 6.9 ± 0.5 (6.1-7.7) μm (n = 20)

Mature gamonts: 11.1 ± 0.9 (9.1-13.6) μm by 5.8 ± 0.5 (5.0-7.3) μm (n = 30)

Gamont nuclei: 4.0 ± 0.4 (2.7-4.5) μm by 4.5 ± 0.6 (3.6-5.9) μm (n = 30)

Vector: unknown

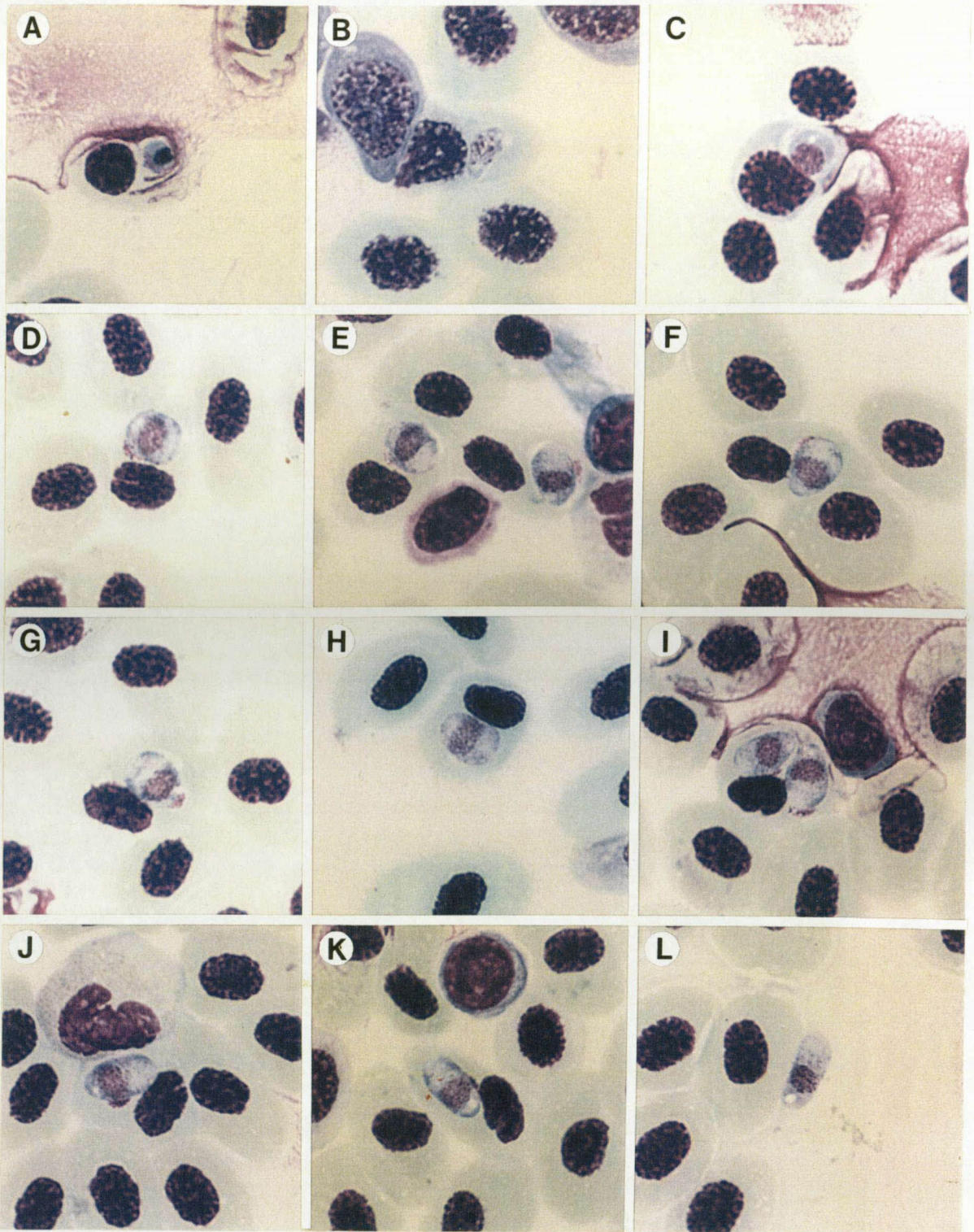


Figure 6.9. Light micrographs of the different life cycle stages of *Haemogregarina* sp. B in the elasmobranch host, *Haploblepharus edwardsii* (Voight, 1832), from the De Hoop Nature Reserve (X1000). A. Small trophozoite or merozoite. B. Large trophozoite or merozoite. C-E. Meronts. E-H. Dividing meronts. I. Paired gamonts. J. Gamont with deep staining granules on outer membrane. K. Gamont displacing erythrocyte nucleus. L. Extra-erythrocytic gamont.

6.3 Studies on the life cycle of *Haemogregarina bigemina* Laveran and Mesnil, 1901

This section has also been written up as a paper which has been accepted for publication in *Folia Parasitologica*. The content of the following paragraphs are therefor similar to those that appear in the paper, but are worded differently and presented in a different format.

In Chapter 3 it was shown how the abundance of *Gnathia africana* in intertidal pools and the relative ease with which it could be kept in laboratories made it a perfect crustacean parasite for life cycle studies. These facts, and the knowledge that almost 100% of some intertidal fish hosts are infected with *H. bigemina*, also allowed investigation of the possible role of *G. africana* as a vector of this fish blood parasite. For the purpose of the study presented in this section, a large number of fully fed *G. africana* pranzia larvae were collected from their fish hosts during April 1998 and 1999 from Koppie Alleen, De Hoop Nature Reserve and in January 1999 from Jeffreys Bay.

Results

During the two De Hoop excursions (1998 & 1999), a total number of 97 of 168 pranziae collected from *Clinus superciliosus* and *Clinus cottoides* were screened for stages of *H. bigemina* using methods described in detail in Chapter 2. The rest of the pranziae were used in life cycle (see Chapter 3) and morphological studies of *G. africana* (Smit *et al.* 1999a,b). At Jeffreys Bay, 106 of 243 pranziae were screened for haemogregarine-like stages. These pranziae were collected from *C. superciliosus* and *C. cottoides* as well as from *Chorisochismus dentex*. Remaining gnathiids from Jeffreys Bay were also used for life cycle and morphological studies as at De Hoop.

Except for gamonts, the stages of the haemogregarine seen in smears taken from *G. africana*, and their relative sizes are recorded in Figures 6.10A-Q, 6.11J-V and Table 6.2. In smears taken from *G. africana* at De Hoop and Jeffreys Bay free gamonts were present (Figs. 6.10A, 6.11J) like those seen in the erythrocytes of fishes with *H. bigemina*. Some were more deeply stained with a pronounced cap. Gamonts were found either immediately or for up to six days after pranziae had fed on fishes with *H.*

bigemina. All three sizes of pranizae (P1, P2, P3) were affected. Unchanged gamonts were also found 18 days after two third-stage larvae (P3) had fed, but this seemed exceptional. Syzygy, or pairing of gamonts, was recorded for up to six days after feeding (Figs. 6.10B, 6.11K). The macrogamont was haemogregarine-like initially, but then appeared to become broader. Its cytoplasm stained deeply and the nucleus was quite prominent. Microgamete nuclei were difficult to distinguish but three or four were visible in each microgamont (Figs. 6.10B, 6.11K). Syzygy was only seen in gnathiids that had fed on *C. superciliosus* with *H. bigemina*.

Immature oocysts (Table 6.2, Figs. 6.10C, 6.11L) were seen in smears taken from *G. africana* praniza larvae from seven days to 19 days after feeding. They were quite numerous in some cases and were found in praniza stage 1, 2 and 3 larvae. These forms were round in shape and their cytoplasm pale blue and vacuolated. Their nuclei, which stained deep purple, lay centrally.

Fourteen days post feeding, oval or round mature oocysts were found (Figs. 6.10D, 6.11M). Very good examples of these stages were found in stage two praniza larvae that had fed on *C. superciliosus*. The cytoplasm of these mature oocysts stained blue, and the intensity of this staining increased with size (age) of the oocyst. Vacuoles were also found in the cytoplasm. Deep magenta staining nuclei were either single or, when sporogony had begun, there were from two to eight within a single oocyst (Figs. 6.10E, 6.11N, Table 6.2). Most of the dividing oocysts had five nuclei.

The formation of at least five sporozoites through sporogony was observed in pranizae 11 days after they had fed on both on *C. superciliosus* and *Chorisochismus dentex* (Figs. 6.10F, 6.11O) although free sporozoites were found to be present in smears of larva nine days after they had fed. These deeply-blue stained sporozoites were broad, and their more or less centrally placed nuclei stained deep magenta (Figs. 6.10G, 6.11P, Table 6.2). Deep staining granules of various sizes as well as one or two distinct vacuoles, occurred either anterior or posterior to the nucleus.

Small first generation meronts were formed from rounded sporozoites at 11 days after feeding (Figs. 6.10H, Table 6.2). They possessed similar staining characteristics and vacuolation to sporozoites. These meronts appeared to divide (Figs. 6.10I, 6.11Q) to form small numbers (probably between two and four) of merozoites. These

merozoites were characterised by sharply pointed or rounded extremities (Figs. 6.10J,K, 6.11R, Table 6.2). Only a few vacuoles were observed in the cytoplasm of these stages. One quarter to a third of the cytoplasm of the first stage merozoite stained pale cream. The remaining cytoplasm stained pale blue (Figs. 6.10J,K). The nucleus was found much nearer to one end of the parasite than the other, thus dividing the blue cytoplasm from the cream area. The nucleus consisted of loosely arranged chromatin.

Large second generation meronts were found in the praniza larvae 11 days after detachment from host fish. These stages were fan-shaped and produced at least 16 slender, immature merozoites (Figs. 6.10L, 6.11S). At 18 days post feeding, mature, second generation merozoites were observed (Figs. 6.10M,N, 6.11T, Table 6.2). They were more numerous as well as larger than first generation merozoites. Large groups were often found enclosed by gut tissue (Fig. 6.10O). This suggested that this stage of merogony might exist in the gut (anterior hindgut?) lining of the praniza larvae. The cytoplasm of second generation merozoites stained pale blue and were slightly vacuolated. The chromatin of the centrally placed nuclei was loosely arranged as in first generation merozoites.

At 18 days post feeding second generation merozoites containing two nuclei (= third generation meronts) were observed (Figs. 6.10P, 6.11U). The division of these stages (binary fission) presumably formed third generation merozoites. Third generation merozoites were the shortest merozoites observed (Table 6.2). These small merozoites (Figs. 6.10Q, 6.11V) were only found in preparations made at De Hoop and were thought to be the final stage of *H. bigemina* in *G. africana* larvae. Like second generation merozoites, these final stages were also found in gut tissue. Two stage 2 praniza larvae collected at De Hoop harboured a mixed population of sporozoites, and first, second and third generation merozoites.

Table 6.2. The different stages of *Haemogregarina bigemina* Laveran and Mesnil, 1901 in *Gnathia africana* Barnard, 1914 pranzia larvae collected from De Hoop Nature Reserve (DH) and Jeffreys Bay (JB), (PF = post feeding, No. = number measured, ML = mean length, MW = mean width).

Stage	Site	Days PF	No.	ML (Range)	MW (Range)
Immature oocysts	DH	7 - 19	15	9.4 ± 0.8 (8.3 - 10.8) in diameter	
	JB	7 - 19	15	10.3 ± 2.2 (6.0 - 14.0) in diameter	
Mature oocysts	DH	14 - 28	10	18.0 ± 4.6 (14.0 - 24.0)	11.5 ± 1.7 (10.0 - 16.8)
	JB	14 - 28	4	14.3 ± 0.4 (14.0 - 15.0)	11.8 ± 1.1 (10.0 - 13.0)
Sporozoites	DH	9 - 28	20	8.7 ± 0.7 (7.6 - 9.2)	4.4 ± 0.4 (3.4 - 5.0)
	JB	9 - 28	25	8.0 ± 0.8 (7.0 - 9.5)	4.4 ± 0.6 (3.0 - 5.5)
First generation meronts	DH	11 - 28	7	9.6 ± 0.8 (8.4 - 10.9)	8.4 ± 1.2 (5.9 - 10.1)
	JB	11 - 28	6	8.6 ± 1.2 (7.0 - 11.0)	6.75 ± 0.4 (6.0 - 7.0)
First generation merozoites	DH	11 - 28	15	6.6 ± 0.4 (5.9 - 7.6)	2.5 ± 0.2 (2.1 - 2.9)
	JB	-	0	-	-
Second generation merozoites	DH	18 - 28	20	8.0 ± 0.5 (7.1 - 9.2)	2.6 ± 0.2 (2.5 - 3.4)
	JB	18 - 28	10	7.9 ± 0.6 (6.5 - 8.5)	2.4 ± 0.2 (2.0 - 2.5)
Third generation merozoites	DH	18 - 28	15	5.9 ± 0.2 (5.0 - 6.3)	2.5 ± 0.2 (2.1 - 2.9)
	JB	-	0	-	-

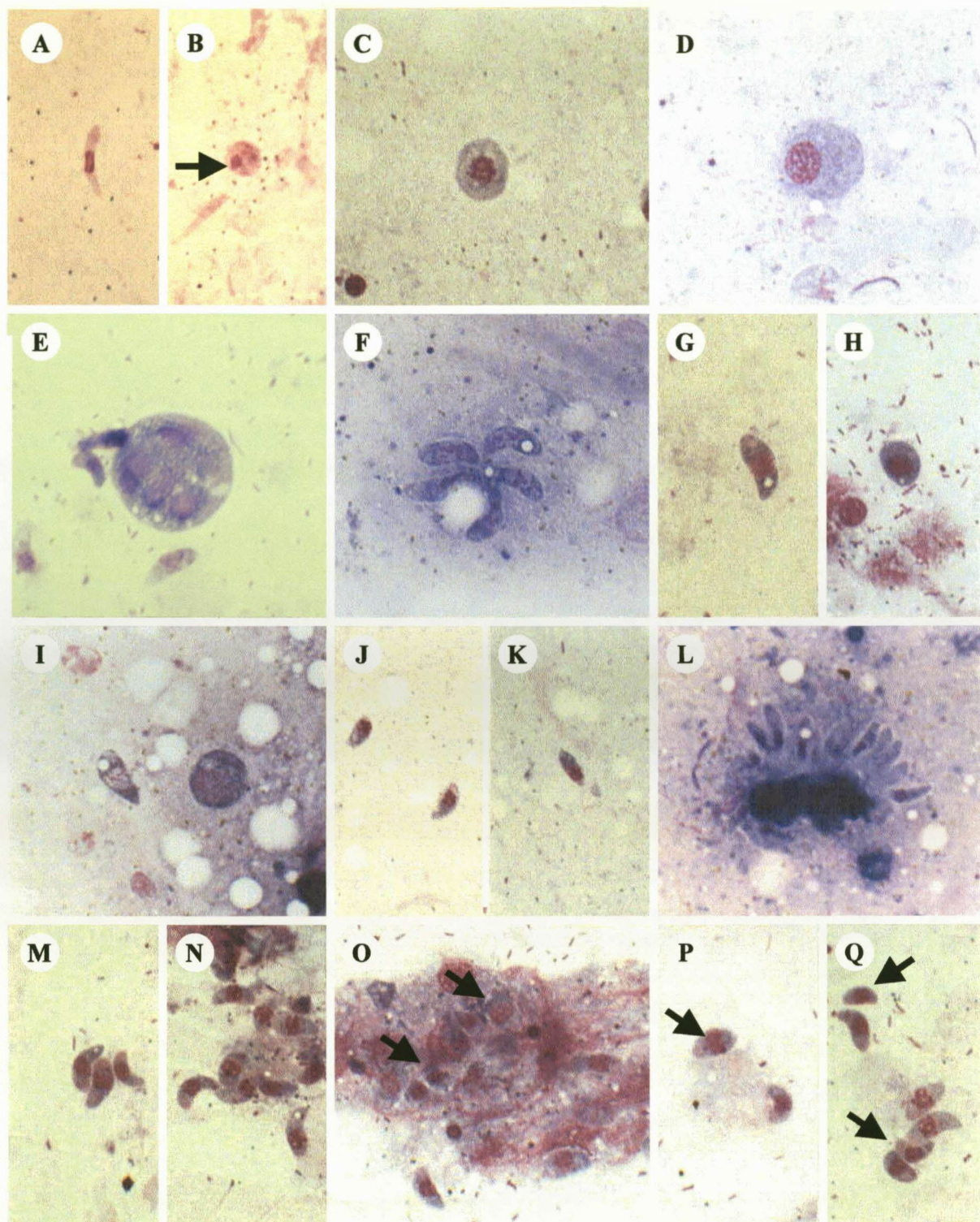


Figure 6.10. Giemsa-stained smears from *Gnathia africana* Barnard, 1914 taken from fishes with *Haemogregarina bigemina* Laveran and Mesnil, 1901 (X1000). **A.** Free gamont resembling those seen in blood films from fishes. **B.** Syzygy. Macrogamont (arrow) closely associated with three visible microgamete nuclei. **C,D.** Immature oocysts at different stages of development. **E.** Maturing oocyst with at least four nuclei. A few second generation merozoites lie near the oocyst. **F.** Sporogony leading to the formation of five sporozoites. **G.** Free mature sporozoite. **H.** First generation meront. **I.** Free sporozoite (left) and dividing first generation meront (right). **J,K.** First generation merozoites. **L.** Second generation meront producing slender, immature merozoites. **M,N.** Mature second generation merozoites in clusters. **O.** Second generation merozoites enveloped by gut tissue (arrows). **P.** Dividing third generation meront (arrow). **Q.** Third generation merozoites (arrows).

6.4 DISCUSSION

Haemogregarines from South African fish hosts

Work on fish haemogregarines in South Africa is almost non-existent, as discussed earlier. That there is much research necessary on haemogregarines in South Africa is evident from this pilot study. During the current research only specific fish species were targeted for examination (fish that are known hosts of gnathiids) and already two known species, *Haemogregarina bigemina* and *Desseria mugili*, as well as two species new to science were found. This report on *H. bigemina* and *D. mugili* provided new distribution as well as new host records for these two cosmopolitan parasites. The description of *Haemogregarina* sp. A records the presence of a very unique fish haemogregarine, (encapsulated gamonts with recurved tails) from South Africa. The description of *Haemogregarina* sp. B is only the third species of this genus to be described from elasmobranch hosts.

The life cycle of *Haemogregarina bigemina* Laveran and Mesnil, 1901

To date the intricate relationships among *Haemogregarina bigemina*, intertidal fishes, and blood-feeding, larval, gnathiid isopods have been examined in Wales and Portugal. In all cases a high prevalence of the haemogregarine in the fish populations examined has been demonstrated, and these fishes have been shown to act as hosts for large numbers of gnathiids (Davies and Johnston 1976, Davies 1982). The present study confirms that this is the same in South Africa, where large numbers of *G. africana* larvae were found on three species of intertidal fishes (*Clinus superciliosus*, *C. cottoides* and *Chorisochismus dentex*). Fifty-eight specimens of these fishes examined carried a total of 348 *G. africana* praziza stage 1, 2 and 3 larvae. Individuals of all three these fish species were also infected with *H. bigemina*. Although in a preliminary report from South Africa, three *Caligus* sp. and one unidentified leech were found on clinids at Jeffreys Bay (Smit and Davies 1999), no other ectoparasites, such as leeches, that could possibly act as vectors for *H. bigemina* were found in this study.

The stages noted in Giemsa-stained smears from *G. africana*, taken at different times following feeding, included syzygy, oocysts, sporozoites, and what appeared to be three generations of merozoites. These results contribute to the existing evidence that a relationship between haemogregarines and gnathiids does exist. The characteristic

haemogregarine-like morphology of the stages, and the fact that they were found in gnathiids collected from fishes infected with *H. bigemina*, leads to the conclusion that they must be the development stages of this haemogregarine in its invertebrate host.

Traditionally, leeches have been regarded as the vectors of haemogregarines from aquatic hosts, especially in the transfer between fishes. It is an important finding to discover that arthropods transmit *H. bigemina* between intertidal fishes in both the northern and southern hemispheres. Arthropods usually act as vectors for the haemogregarines of semi-aquatic and terrestrial vertebrates (see Davies and Johnston 2000). *Haemogregarina bigemina* in gnathiids resembles *Hemolivia* sp. in ticks, *Amblyomma* sp. and *Hyalomma aegyptium* (Petit, Landau, Baccam and Lianson 1990, Smallridge and Paperna 1997, Landau and Paperna 1997), and *Hepatozoon lygosomarum* Allison and Desser, 1981 in the mite, *Ophionyssus scincorum* (Allison and Desser 1981), in that sporogony appears to be confined to the gut of an arthropod host. Its oocysts are not irregular or star shaped, as in *Hemolivia* sp. (Petit *et al.* 1990), and its oocysts are not large and located in the haemocoel as in many *Hepatozoon* (see Smith 1996). Furthermore, *H. bigemina* has not been seen to produce sporokinetes or sporocysts, as do *Karyolysus*, *Hemolivia*, and *Hepatozoon* (Svahn 1975, Petit *et al.* 1990, Smith 1996).

Although the development of *H. bigemina* in gnathiids more closely resembles the stages of members of the genera *Cyrcilia* and *Desseria* that have been observed in leeches, differences do exist. In *Cyrcilia*, 20 or more sporozoites are reported developing from a single oocyst, and in *Desseria* 16 to 32 sporozoites. Both these numbers are much higher than the four to eight found in this study for *H. bigemina*. In *Cyrcilia* these sporozoites apparently migrate to the salivary glands of the leech, where they are infectious to a new host (Lainson 1981). The occurrence of merogony in members of *Desseria* is similar to what was found for *H. bigemina*. The result of merogony in *Desseria* is only four merozoites, whereas in *H. bigemina* evidence for the presence of at least three generations of distinct morphological types of merozoites were found.

The use of the crush/smear techniques in this study showed no evidence of the presence of haemogregarines in the salivary glands of the gnathiids. However, it is impossible to be certain that the haemogregarine never enters the salivary cells. All

gnathiids collected from the haemogregarine-infected fish hosts showed a relatively high infection of the parasite, and in the laboratory, as in Welsh studies, intertidal fishes of all ages readily ate larval gnathiids (see Davies 1982, see Chapter 3). This behavior of fishes makes it thus possible that *H. bigemina* is transmitted when infected gnathiids are ingested by fishes, rather than when the gnathiids feed on the fish host. It is presently not clear whether infection with *H. bigemina* in gnathiids can accumulate over their life stages between successive feeds and moults. According to Smallridge and Bull (1999), this has been suggested to occur in ticks with *Hemolivia mariae*. To establish whether this is the case in gnathiids, biological transmission in the laboratory would be necessary.

All the information gathered on the development of *H. bigemina* in the fish as well as isopod hosts during this study was used to compile the first complete life cycle for this species and is illustrated in Figure 6.11C-V.

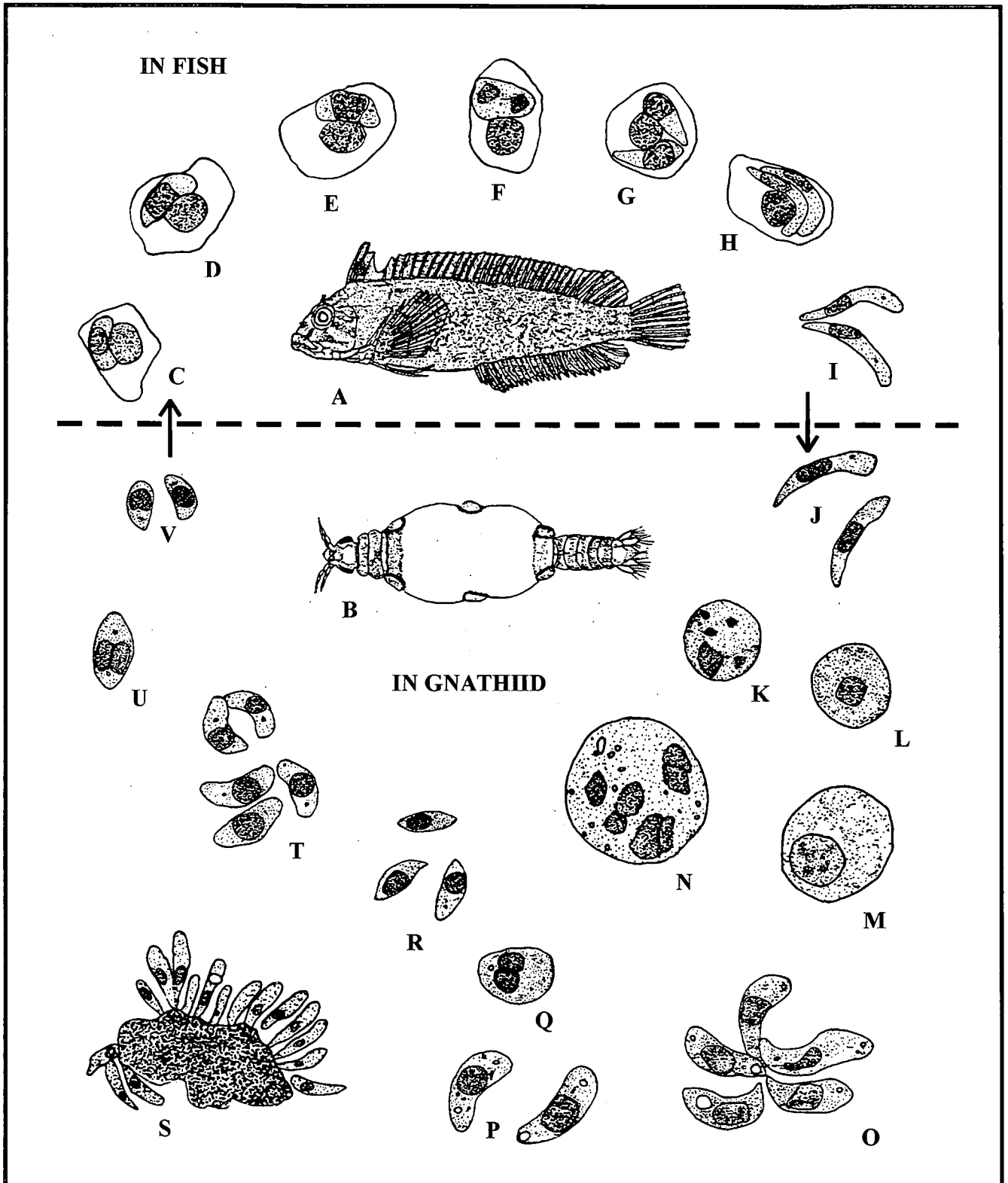


Figure 6.11. A. *Clinus superciliosus* (Linnaeus, 1785) redrawn from Penrith (1969) (not drawn to scale) B. Praniza larva of *Gnathia africana* Barnard, 1914 redrawn from Smit *et al.* (1999a) (not drawn to scale) C-V. Microscope projection drawings to illustrate a proposed life cycle for *Haemogregarina bigemina* (X 1500). C. Intraerythrocytic trophozoite. D,E. Intraerythrocytic meronts. F. Pregamontic binary fission. G. Immature, paired, intraerythrocytic gamonts. H. Mature, paired, intraerythrocytic gamonts. I. Extraerythrocytic gamonts. J. Free gamonts in *G. africana*. K. Syzygy. L,M. Immature oocysts at different stages of development. N. Maturing oocyst. O. Sporogony leading to the formation of five sporozoites. P. Free mature sporozoites. Q. Dividing first generation meront. R. First generation merozoites. S. Second generation meront producing slender, immature merozoites. T. Mature second generation merozoites. U. Dividing third generation meront. V. Third generation merozoites

7. Concluding remarks

Although the first gnathiid species was described almost 200 years ago in 1804, and the life cycle of this group elucidated approximately a 100 years ago, in depth research into the taxonomy and biology of the family Gnathiidae is still in its infancy. The main reasons for this is that most of the work conducted on gnathiids over the past two centuries concentrated on taxonomy and then only on the males (see Chapter 4). Even though most of the papers published through the years have been on the taxonomy of this group, these still do not reflect the true distribution of gnathiids around the world, but rather the distribution of scientists interested in this group. The main aim of this research project on gnathiids in southern Africa was thus to contribute extensively to the knowledge of gnathiid biology in general, by completing a specific case study on the common intertidal gnathiid, *Gnathia africana*. This study included the completion of the life cycle under laboratory conditions, observations on the feeding behaviour and the development of embryos in the females.

That the distribution of gnathiids is merely a reflection of the distribution of scientists researching them, is well illustrated in South Africa, because it is highly unlikely that a region known for its rich diversity of marine fauna only has five species of gnathiids. The ongoing study into the biodiversity of gnathiids along the South African coast is therefore essential and forms an integral part of this study.

While working on the taxonomy of new species found along the coast of South Africa, the opportunity arose to investigate the different taxonomically important characteristics of the female and larvae. This type of study is essential for gnathiid research because recent research on cleaner fish symbiosis (Grutter 1994, 1996, 1997a,b, 1999a,b,c, Grutter and Poulin 1998), gnathiid pathology (Paperna and Por 1977, Mugridge and Stallybrass 1983, Honma *et al.* 1991, Heupel and Bennet 1999), and the possibility of gnathiids as vectors of diseases (Davies and Johnston 1976, Davies 1982, 1995, Smit and Davies 1999, current study) has demonstrated the necessity to identify the parasitic larvae in the absence of males.

The work on the biology of *G. africana* provided the opportunity to investigate the possibility of this gnathiid as possible definitive host of fish haemogregarines. Before vector work could be conducted it was, however, first necessary to document the presence of the different haemogregarines infecting the fish hosts of gnathiids found in this study, as well as in any other fish collected. This was made very difficult, because the knowledge of fish blood parasites in southern Africa is very scanty (see Chapter 6). So, before the life cycle work could be concluded, these haemogregarines first had to be identified and described.

Each of the chapters in this thesis has its own detailed discussion on the particular topic addressed. To conclude, the new contributions of the present study to science and the questions arising from it will be highlighted under the sub-headings of “New contributions” and “Topics for future research”.

7.1. Life cycle of *Gnathia africana* Barnard, 1914

New contributions

- ◆ First ever, successful completion of a gnathiid life cycle under laboratory conditions.
- ◆ Establishment of the feeding behaviour and exact length of the feeding process of all three larval stages of *G. africana*.
- ◆ First complete photographic record of the different development phases of male and female larvae, adult males and adult females of *G. africana*.

Topics for future research

- ◆ Determine the effect of temperature on the development and life cycle of *G. africana* and other intertidal gnathiid species.
- ◆ Establish internal or external factors that determine the sex of the larvae as well as the specific stage when this process takes place.

7.2 Biodiversity of southern African gnathiids

New contributions

- ◆ Redescription of two known species (Appendix I A & I B),
- ◆ Description of a new species from museum material (Appendix I C)

- ◆ Description and naming of two new species collected on the south coast of South Africa (Chapter 4 & 5).
- ◆ Establishment of a new technique for obtaining adult males and females, whereby the pranzia 3 larvae collected from the fish hosts are allowed to moult into adults.
- ◆ Description of the first valid gnathiid species, *Gnathia pantherinum* from elasmobranch hosts.

Topics for future research

- ◆ Research into the biodiversity of gnathiids in Africa is an ongoing necessity.

7.3 Taxonomy of gnathiids

New contributions

- ◆ Identification of specific morphological characteristics which distinguish between the females, as well as between the larvae, of different gnathiid species.
- ◆ Extensive use of scanning electron microscopy in the species descriptions contributes substantially to the understanding of the different morphological characteristics of gnathiids.
- ◆ A new standard for the line drawings of specimens.

Topics for future research

- ◆ Description of more females and larvae of known species, as well as the inclusion thereof in the description of new species.
- ◆ Verifying the use of the identified taxonomically important characteristics for identifying females and larvae once more descriptions become available.

7.4 Biodiversity of fish haemogregarines in southern Africa

New contributions

- ◆ New distribution and host records for two known species.
- ◆ Description, without naming, of two new species.
- ◆ Description of, rarely observed, encapsulated haemogregarine from fishes.
- ◆ Species described from elasmobranchs comprises only the third species of the genus *Haemogregarina* described from elasmobranch hosts throughout the world.

Topics for future research

- ◆ Naming of the species described as *Haemogregarina* sp A and *Haemogregarina* sp B, following further study.
- ◆ Ongoing research into the biodiversity of fish haemogregarines.
- ◆ Investigations into the presence of other fish blood protozoans in southern Africa.

7.5 Life cycle of *Haemogregarina bigemina* Laveran and Mesnil, 1901

New contributions

- ◆ Elucidation of the complete, two-host life cycle of *H. bigemina*.
- ◆ The first proven record that implicates an arthropod as vector of fish blood parasites.

Topics for future research

- ◆ To determine whether *H. bigemina* enters the salivary glands of gnathiids.
- ◆ To achieve laboratory transmission of *H. bigemina*.
- ◆ To establish the correct taxonomical position of *H. bigemina* considering the new information obtained regarding its life cycle.
- ◆ Using the same methods applied in this study to further investigate the possible role of other gnathiid species in the transmission of different species of fish haemogregarines.

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Reference incomplete

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ACKNOWLEDGEMENTS

The author would like to acknowledge and express his sincere appreciation to the following persons and institutions:

My mentors, Prof. Jo van As and Prof. Linda Basson: For the opportunity to work in their laboratory, the unforgettable days on fieldtrips, the support and guidance during my study and especially for teaching me much more than just science.

My co-promotor Prof Angela Davies: For introducing me to the weird and wonderful world of haemogregarines and all her help and support during my study, specifically the writing up of my thesis.

My parents: For their love, interest and support throughout my entire undergraduate and post graduate years.

Cecilé Reed: For her sincere love support and help, especially during the last few months of late night working.

Dr. Liesl van As, and all Aquatic Parasitology students: For all their support and assistance during laboratory and field work.

Dr. Mike Johnston: For all his help during the De Hoop 1998 fieldtrip, his enthusiastic support of my research and the unforgettable visits to him in Wales.

My sister Hanlie: For all the help during the final stages of this study.

All my friends: For their interest, company and sincere friendship.

Prof. Gerrit van Wyk, Dean of the Faculty of Natural and Agricultural Sciences: For financial support of my overseas visits as well as his enthusiasm for my work.

Trevor Farr and all the staff at Potberg Environmental Education Center: For making available there wonderful facilities for fieldtrips and their interest in my research.

Prof. Reg Davis, Dean of the Faculty of Natural Sciences and Dr. Ralph Manly, Head of the School of Life Sciences, Kingston University: For inviting me as Research Fellow to Kingston University and the financial support during those visits.

National Research Foundation (NRF): For the bursary received in support of my study.

Cannon Collins Trust for southern African Education: For funding my two research visits to the United Kingdom.

The Department of Zoology and Entomology, University of the Orange Free State, South Africa: For the opportunity to do my study in this dynamic department and for the use of departmental facilities.

Abstract

Research on marine fish parasites has been largely neglected in South Africa. This is especially true for the temporary fish parasites of the isopod family Gnathiidae and fish blood parasites of the family Haemogregarinidae. In this study, surveys were carried out to determine the presence of gnathiid isopods and fish haemogregarines associated with different intertidal and sub-tidal fish hosts over a period of four years, at two different localities on the South African south coast. The study also focussed on the relationship between gnathiids and haemogregarines, specifically the possibility that the gnathiid *Gnathia africana* Barnard, 1914 might be a vector of the fish blood parasite *Haemogregarina bigemina* Laveran and Mesnil, 1901. Laboratory work was conducted to elucidate the life cycle of *G. africana*. It was found that *G. africana* has three larval stages, consisting of three unfed (zuphea) and three fed (praniza) stages, with the final stage praniza larvae moulting into either male or female adults. Forty days after their last feed as praniza 3 larvae, mature females release stage 1 zuphea larvae. Detailed information was also supplied on the feeding and digestion length for each larval stage. The females of *G. africana* were re-described from the material collected. Furthermore, other gnathiid larvae, not resembling any of the known species from South Africa, were collected from three species of elasmobranchs as well as the evileye pufferfish. Both groups of larvae were kept in the laboratory, where they moulted into adult males that did not conform to the description of any other gnathiid species in South Africa, or world-wide. These specimens were described as new species (*Gnathia pantherinum* sp. n. and *G. pipindae* sp. n.), using light and scanning electron microscope observations. Extensive information was also provided on the final life cycle stages of *G. pantherinum* sp. n. Special attention was given to finding distinguishing characteristics to identify females and larvae of various species in the absence of males. Comparing material in this study with information from the literature showed that the morphology of the pleotelson and cephalosome appendages of females and larvae could be successfully used as differentiating characteristics. The research conducted on fish haemogregarines led to new distribution and host records for two known species, and the description of two new, but un-named species. Development stages of *H. bigemina* were found in the gut of *G. africana* larvae that had fed on *H. bigemina*-infected host fish. The data obtained from these results was used to compile a complete life cycle for *H. bigemina* in both the fish and the arthropod host.

Key words: South Africa, fish parasites, marine hosts, Isopods, gnathiids, life cycle, morphology, taxonomy, haemogregarines, vector,

Opsomming

Navorsing op mariene visparasiete in Suid Afrika is grootliks nagelaat. Dit is veral waar vir die tydelike visparasiete van die isopood familie Gnathiidae en die visbloedparasiete van die familie Haemogregarinidae. Die teenwoordigheid van isopode van die genus *Gnathia* en vishaemogregarienes, was oor 'n periode van vier jaar by twee verskillende lokaliteite aan die Suid-Afrikaanse suidkus ondersoek. Hierdie studie fokus ook op die interaksie tussen verteenwoordigers van die genus *Gnathia* en haemogregarienes, en spesifiek die moontlikheid dat die isopood, *Gnathia africana* Barnard, 1914 as vektor van die haemogregarien, *Haemogregarina bigemina*, Laveran and Mesnil, 1901 kan optree. Laboratoriumeksperimente was op die lewensiklus van *G. africana* onderneem. Daar was bevind dat die lewensiklus van *G. africana* uit drie verskillende larwaalfases bestaan. Die finale praniza 3 larf vervel in 'n volwasse mannetjie of wyfie. Wyfies gee geboorte aan fase 1 larwes, 40 dae nadat sy as praniza 3 larf die visgasheer verlaat het. Gedetailleerde inligting oor die voeding en vertering van elke larwaalfase word ook verskaf. Die wyfies van *G. africana* word hierin vanaf die versamelde materiaal herbeskryf. Isopood larwes, wat nie aan die beskrywings van die larwes van enige bekende Suidelike-Afrika spesies voldoen het nie, is vanaf drie kraakbeenvisspesies en die groenoogblaasop versamel. Beide hierdie groepe larwes is in die laboratorium aangehou waar hulle na volwasse mannetjies vervel het. Hierdie mannetjies het nie dieselfde eenskappe as enige ander beskryfde spesie in Suid Afrika of die res van die wêreld besit nie en is gevolglik as nuwe spesies, *G. pantherinum* sp. n. en *G. pipinde* sp. n. beskryf. Die spesiebeskrywing is op lig-en skandeer-elektronemikroskoop waarnemings gebaseer. Uitgebreide inligting oor die finale lewensiklusstadiums van *G. pantherinum* is ook verskaf. Spesiale aandag is aan die ondersoek na morfologiese kenmerke om wyfies en larwes van verskillende spesies in die afwesigheid van mannetjies te identifiseer, geskenk. Die materiaal gedurende hierdie studie versamel, sowel as informasie uit literatuur, is vergelyk en daar is gevind dat die morfologie van die pleotelson en die kefalosoomaahangsels suksesvol as onderskeidende kenmerke gebruik kan word. Die navorsing op vishaemogregarines het tot nuwe verspreidings- en gasheerreords van twee bekende spesies gelei, sowel as die beskrywing van twee nuwe spesies. Verskillende ontwikkelingstadiums van *H. bigemina* was in die maag van *G. africana* larwes, wat op *H. bigemina* geïnfecteerde visse gevoed het, gevind. Die data van bogenoemde resultate was gebruik om 'n volledige lewensiklus van *H. bigemina* vanuit beide die vis-en arthropodgashere saam te stel.

Appendix I A

A redescription of the adult male and pranzia of *Gnathia africana* Barnard, 1914 (Crustacea, Isopoda, Gnathiidae) from southern Africa

Nico J. Smit, Jo G. Van As and Linda Basson

Department of Zoology and Entomology, University of the Orange Free State, PO Box 339, Bloemfontein, 9300, South Africa

Key words: *Gnathia africana*, redescription, taxonomy, morphology

Abstract. A redescription of the adult male and pranzia of *Gnathia africana* Barnard, 1914 is provided from material collected at three localities along the South African coast and from syntypes and other material deposited by the original author. This redescription is based on light and scanning electron microscopy.

Knowledge of the gnathiids of the world reflects the location of scientists interested in these isopods rather than their actual distribution. Almost one quarter of the known species are described from Australia (Cohen and Poore 1994). Africa is poorly represented. The only records are those of Barnard (1914a,b, 1920, 1925) describing four species from southern Africa (*Gnathia africana* Barnard, 1914; *Gnathia spongicola* Barnard, 1920; *Gnathia disjuncta* Barnard, 1920; and *Gnathia cryptopais* Barnard, 1925) and a single record by Müller (1989) from Kenya (*Gnathia wolffi* Müller, 1989). Although Monod (1926) recorded the southern African species in his monograph, he included Barnard's original descriptions without additional information. None of these works provided sufficient information on the morphology of the southern African species for their inclusion in the phylogenetic analysis of Cohen and Poore (1994), who supported Müller's (1991) suggestion that the African material needed redescription.

In the present study, males and larvae of *Gnathia africana* were collected from the intertidal zone of the west and south coasts of southern Africa and this material is used to redescribe the species. No females were found. Three populations of *G. africana* were compared with the type material in the South African Museum, Cape Town.

MATERIALS AND METHODS

Field work was undertaken during low tide at McDougall's Bay, De Hoop Nature Reserve and Jeffreys Bay. Sponges (possibly of the genera *Hymeniacidon* and *Polymastia*) and colonial tunicates were collected with a spatula and examined in a field laboratory under a dissection microscope for the presence of gnathiids. Fish were collected from intertidal pools with hand nets, cast nets and hand lines and placed in an aerated aquarium. Fish were anaesthetised with benzocaine (ethyl-4-aminobenzoate), identified using Smith and Heemstra (1986) and Branch et al. (1994), measured and examined for

gnathiid larvae. Larvae were removed from fish, measured, categorised according to size and feeding status, and fixed in 70% ethanol. Both zupheae (with distinct segmentation) and pranziae (with a swollen intersegmental membrane) were taken.

For scanning electron microscopy specimens were re-hydrated in a descending sequence of ethanol and washed in tap water in order to get rid of salt crystals and debris. The specimens from the sponges and tunicates were cleaned with a soft sable hair brush. They were then dehydrated to absolute ethanol and critical point dried. Specimens were mounted on aluminium stubs, sputter coated with gold and studied with the aid of a JEOL WINSEM JSM 6400 at 10 kV.

Type and other material examined by Barnard (1914a, b) was borrowed from the South African Museum, Cape Town.

RESULTS

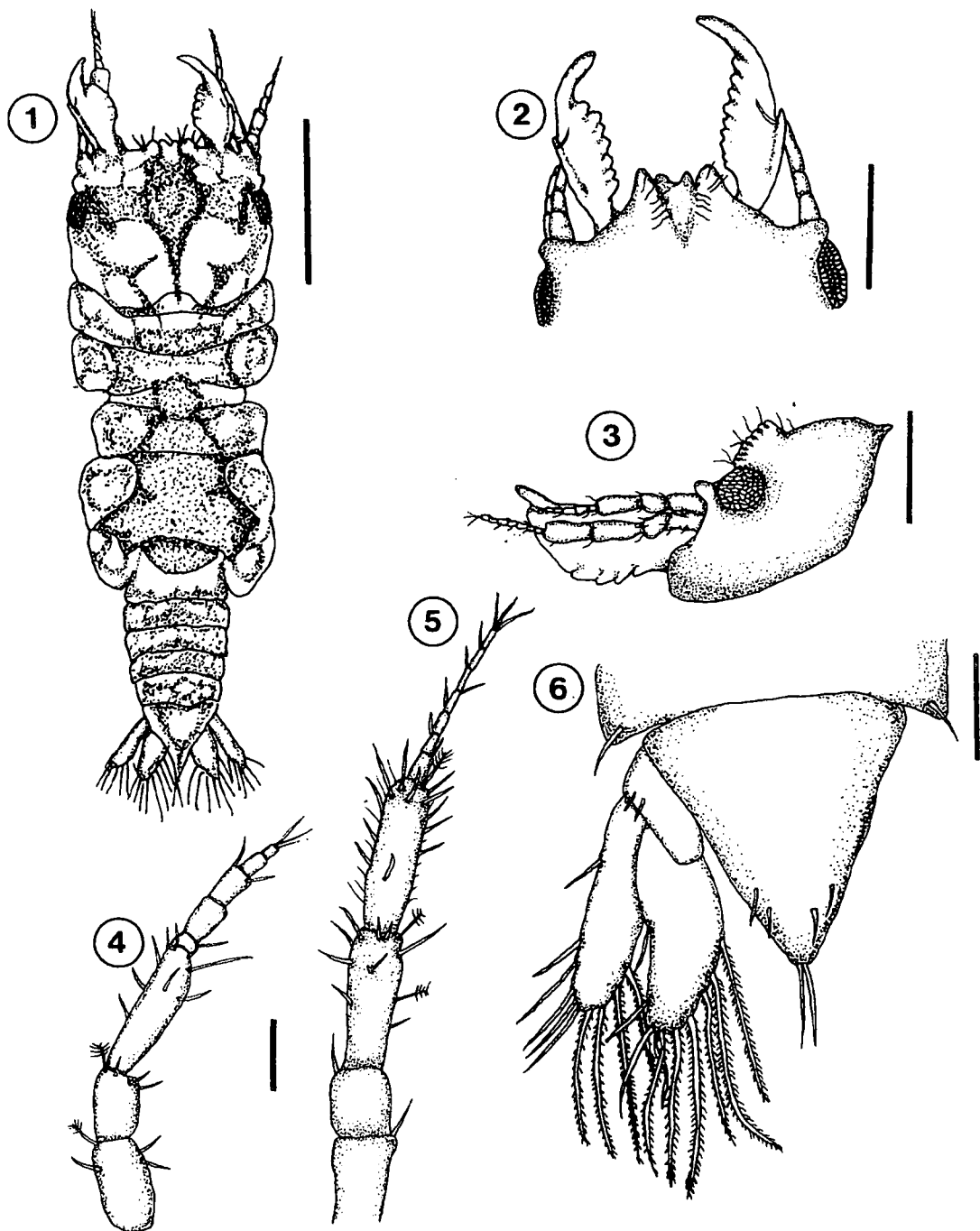
Gnathia africana Barnard, 1914

Adult male Figs. 1-10, 23-29

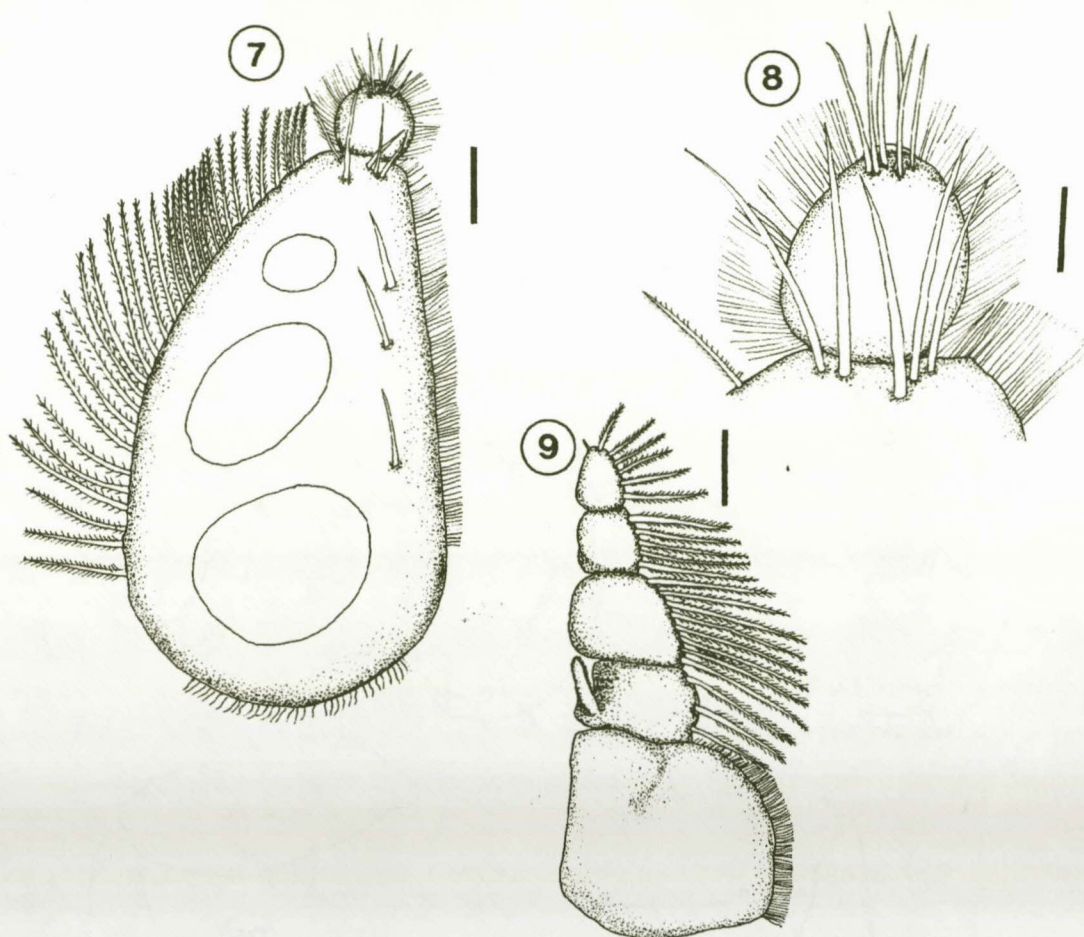
Description: Total lengths of new material: 3.7-5.1 mm (mean 4.8 mm, n = 15) (McDougall's Bay); 3.0-4.4 mm (4.0 mm, n = 15) (De Hoop Nature Reserve); 2.9-4.2 mm (3.75 mm, n = 15) (Jeffreys Bay).

Cephalon. Rectangular, 1.4 times as wide as long, deep dorsal sulcus (Fig. 23), same width as median process, extending to median tubercle, lateral margins convex, few setae on dorsal posterior cephalon, posterior margin concave (Fig. 1). Sensory pits distributed randomly over dorsal surface of cephalon (Fig. 23). Setae as well as sensory pits ventrally on lateral sides of buccal cavity (Fig. 26). Well developed oval-shaped, bulbous, compound eyes on lateral margin of cephalon, length of eye slightly less than a third of cephalon (Fig. 24). Prominent paraocular ornamentation with five to seven tubercles forming ridge above eye, same length as eye, stretching from middle of eye

Address for correspondence: N.J. Smit, Department of Zoology and Entomology, University of the Orange Free State, PO Box 339, Bloemfontein, 9300, South Africa. Phone: ++27 51 401 2440; Fax: ++ 27 51 448 8711; E-mail: smitn@dre.nw.uovs.ac.za



Figs. 1-6. Microscope projection drawings of a male *Gnathia africana* Barnard, 1914 collected at McDougall's Bay. Fig. 1. Full length dorsal view. Fig. 2. Frontal border and mandibles. Fig. 3. Lateral view of cephalon, antennae and mandible. Fig. 4. First antenna. Fig. 5. Second antenna. Fig. 6. Telson and uropods. Scale bars: Fig. 1 = 1 mm; Figs. 2, 3 = 500 μ m; Figs. 4-6 = 100 μ m.



Figs. 7-9. Microscope projection drawings of mouthparts of a male *Gnathia africana* Barnard, 1914 collected at McDougall's Bay. Fig. 7. Pylopod. Fig. 8. Articles 2 and 3 of pylopod. Fig. 9. Maxilliped. Scale bars: Figs. 7, 9 = 100 μ m; Fig. 8 = 50 μ m.

posteriorly, ridge adorned with setae (Figs. 3, 24). Posterior median tubercle present, elongated (Fig. 23).

Frontal border. Produced, superior frontolateral process conical, with seven to ten long simple setae on outer border and three on inner ventral border (Figs. 2, 25). Mediofrontal process inferior, divided concavely into two triangular lobes with obtuse points, no inferior frontolateral process (Fig. 25). External scissura deeply excavated. Supraocular lobe prominent, extending laterally.

Antennae. Antenna 2 longer than antenna 1 (Fig. 28). Antenna 1 with three peduncle articles, with third one largest, flagellum with five articles, article 2 and 3 largest, articles 3 and 4 with one aesthetascs setae each, article 5 terminating in one aesthetascs and two to three simple setae, few setae on each article (Fig. 4). Antenna 2 with four peduncle articles, article 3 and 4 largest, flagellum with seven articles, article 1 largest, article 7 terminating in three to four simple setae, few setae on each article, except article 4 of peduncle with 20 to 25 setae (Fig. 5). Antennae slightly curved inwards.

Mandible. Long, same length as cephalon, twice as long as wide, curved inwards with eight to ten processes on dentate blade, tussle of setae between processes (Fig. 27). Apex cylindrical, distally raised in lateral view at 45°. Prominent incisor, terminating in sharp point (Fig. 2). Single mandibular seta extending from base of incisor process. Carina unarmed, forming ridge on lateral margin extending from basal neck to halfway along mandible (Fig. 27). Few short simple setae distributed randomly on dorsal surface of blade. Internal lobe and pseudoblade absent.

Maxilliped. Five-articled, proximal article largest with slender endite (Fig. 9). Endite contiguous with article 2, almost reaching article 3. Distal four articles with plumose setae on lateral margin in order of 3-8-5-6. Palp 2.5 times as long as wide. No coupling hooks.

Pylopod. Three articles (Fig. 7). First article greatly enlarged, mesial border fringed with plumose setae, lateral setae short and simple, three simple setae near lateral border and five distally on posterior surface (Fig. 7). Three areolae. Second article oval, 1.25 times as

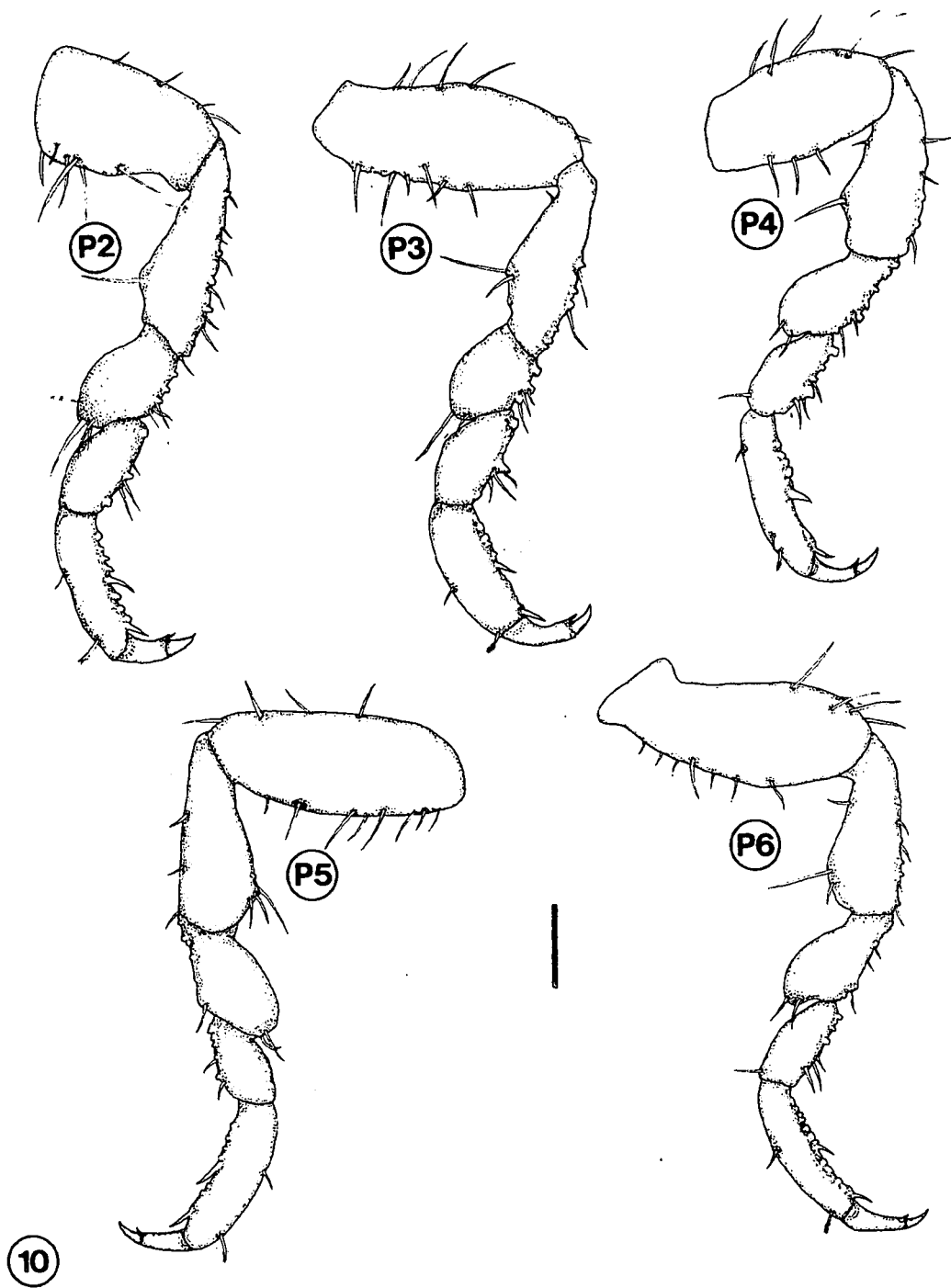
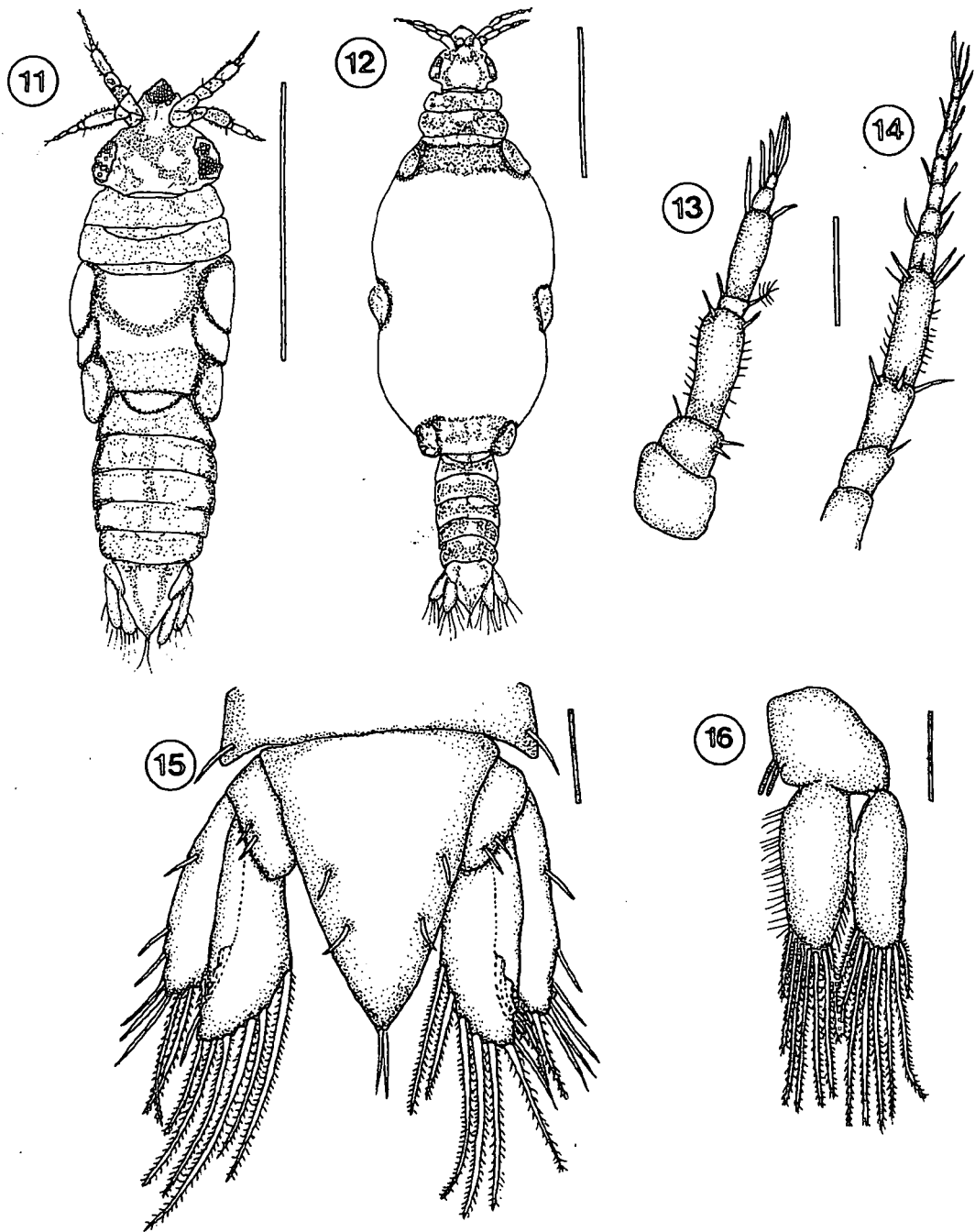
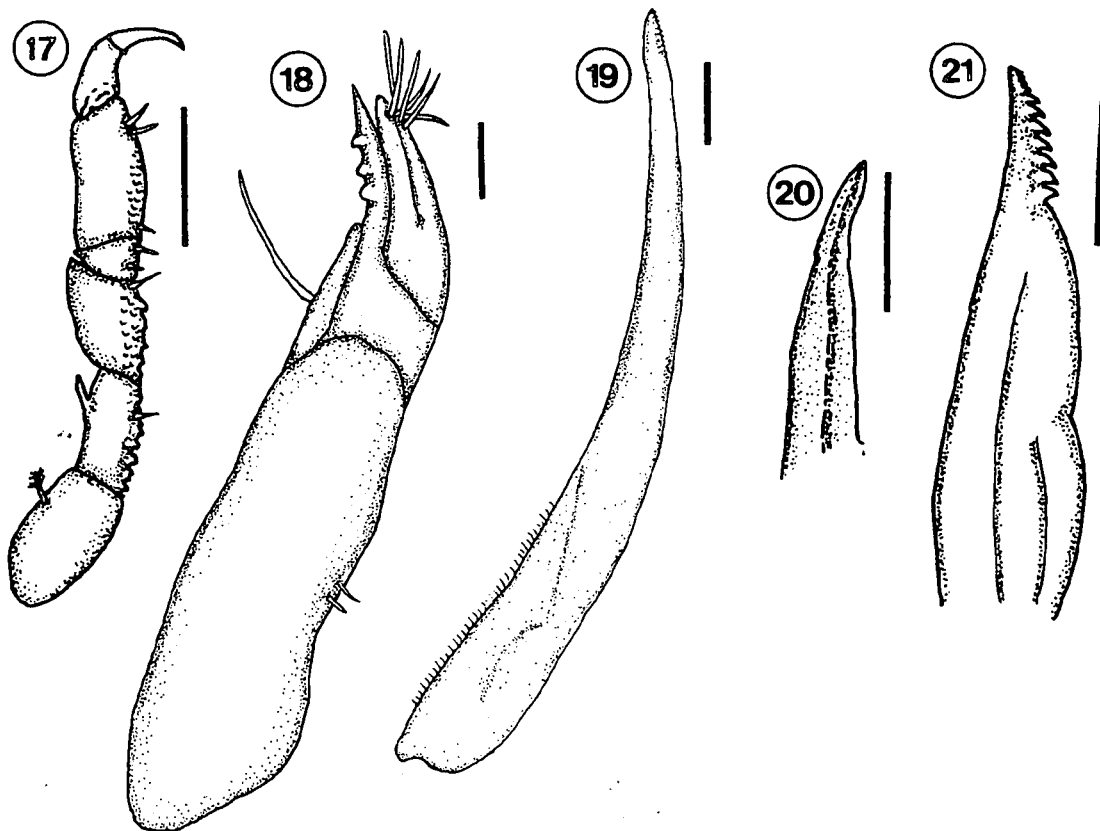


Fig. 10. Microscope projection drawings of pereopods 2 to 6 (P2-P6) of a male *Gnathia africana* Barnard, 1914 collected at McDougall's Bay. Scale bar = 200 μ m.



Figs. 11-16. Microscope projection drawings of *Gnathia africana* Barnard, 1914 larvae collected at McDougall's Bay. Fig. 11. Full length dorsal view of zuphea. Fig. 12. Full length dorsal view of praniza. Fig. 13. First antenna. Fig. 14. Second antenna. Fig. 15. Telson and uropods with fringing setae. Fig. 16. Left pleopod. Scale bars: Figs. 11, 12 = 500 μ m; Figs. 13-16 = 100 μ m.



Figs. 17-21. Microscope projection drawings of mouthparts of *Gnathia africana* Barnard, 1914 praniza larva collected at McDougall's Bay. Fig. 17. Gnathopod. Fig. 18. Maxilliped. Fig. 19. Maxillule. Fig. 20. Paragnath. Fig. 21. Mandible. Scale bars: Figs. 17, 20 = 100 μ m; Figs. 18, 19, 21 = 50 μ m.

long as wide, margins setose, six simple setae distally on posterior surface (Fig. 8). Third article minute with fringing setae.

Pereon. One and a half times as long as wide, wider than cephalon (Fig. 1). Pereonite 1 fused with cephalon, dorsally visible, not reaching lateral margins, divided into three regions by posterior margin of cephalon, anterior border convex, posterior margin straight (Fig. 1). Pereonite 2 and 3 of similar size, widest part of body, lateral margins pointing anteriorly. Pereonite 3 with anterior constriction, but not separating pereonite 2 and 3. Prominent anterior constriction of pereonite 4 separating it from pereonite 3. Pereonite 4 with shallow median groove extending as dorsal sulcus to pereonite 6. Pereonite 5 with areae laterales. Pereonite 5 and 6 not fused, but separation not distinct. Pereonite 6 at least twice as long as other pereonites (Fig. 1), 1.3 times as long as wide, posterior margin deeply concave, with lobi laterales, no lobii. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Setae on anterior margins of pereonites 1, 2 and 3, rest of pereon sparsely setose. Sensory pits randomly distributed on pereonites 1, 2 and 3.

Pleon. Pleon and pleotelson slightly less than a third of total length (Fig. 1). Five pleonites dorsally visible, epimera not distinct.

Pleotelson. Triangular, base as wide as length or wider, lateral margins straight, four simple setae on dorsal surface near apex, distal apex terminating in a pair of simple setae (Fig. 6).

Pereopods. Pereopod 2 basis elongated with eight to twelve simple setae anterior, four to seven posterior simple setae (Fig. 10). Ischium as long as basis but not as wide, three to five anterior setae, posterior tubercles with simple setae in between. Merus half the length of ischium with anterior bulbous protrusion, simple setae on bulbous protrusion, posterior margin with tubercles as well as simple setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion. Propodus about twice the length of carpus, prominent tubercles fringe posterior side, two elongated spines ending in sharp points situated on middle and distal part of posterior side respectively, only a few simple setae anteriorly with one plumose seta distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side

proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 similar to pereopod 2 (Fig. 10), differ only in direction, pereopods 4 to 6 directed posteriorly and pereopods 2 and 3 anteriorly.

Pleopods. Exopod and endopod almost of similar size. Both fringed distally with seven to nine long plumose setae, few shorter simple setae on lateral margins. Plumose setae as long as pleopod.

Pencs. Prominent with two contiguous papillae, as long as wide (Fig. 29).

Uropod. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, both with long fringing setae, endopod with inner six setae plumose, exopod with inner four setae plumose, rest of setae simple (Fig. 6). Uropodal basis with two simple setae.

Praniza larva

Figs. 11-22, 30-36

Description: Total length of material examined: 1.1-3.9 mm (n = 50)

Cephalon. Posterior margin slightly wider than anterior margin, almost as wide as long, lateral margins straight parallel, few setae on dorsal posterior cephalon, posterior margin straight (Fig. 33). Many sensory pits distributed randomly over dorsal surface of cephalon. Compound eyes large, well developed, oval-shaped, bulbous, on lateral margins of cephalon, length of eye almost same as cephalon (Fig. 32). No sulcuses or tubercles on dorsal cephalon. Medio-anterior margin of cephalon straight with lateral concave excavations to accommodate first articles of antennae.

Labrum. Prominent, two fifths length of cephalon, semicircular with apical process, truncated posterior margin, anterior margin concave (Fig. 33). Ventral part of labrum gutter-like with central groove, covers mandibles dorsally and laterally.

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles, third one largest, flagellum with four articles, article 2 largest, articles 2 and 3 with one aesthetascs setae each, article 4 terminating in one aesthetascs and two simple setae, few setae on each article (Fig. 13). Antenna 2 with four peduncle articles, article 4 largest, flagellum with seven articles, article 1 largest, article 7 terminating in three to four simple setae, few setae on distal end of each article (Fig. 14). Antennae straight.

Mandible. Stout, swollen at base, distal margin styliform with nine to ten teeth on mesial margin, two teeth small and situated at tip of mandible, seven to eight teeth larger, triangular and backwards directed (Figs. 21, 36).

Gnathopod. Smaller than pereopods, seven articles, dactylus strongly hooked, only few simple setae, many pectinate scales on inner and lateral sides (Figs. 17, 34).

Maxilliped. Large, cylindrical, elongated base, edite reduced (Fig. 35). Palp with three articles, first article acute with five to seven teeth distally and single

long simple seta ventrally, articles 2 and 3 with many long simple setae (Fig. 18). Basis with many large pectinate scales ventrally.

Maxillae. Not visible.

Maxillule. Long, slender, swollen base, stretching past distal margin of labrum (Fig. 19). Six to eight teeth on distal inner margin.

Paragnaths. Elongated, terminates in sharp point, no teeth (Fig. 20).

Pereon. Almost twice as long as wide, wider than cephalon. Pereonite 1 fused with cephalon, dorsally visible, anterior and posterior borders shallow convex (Fig. 33). Pereonite 2 with anterior constriction separating it medianly from pereonite 1 (Figs. 30, 31). Pereonite 3 widest part of body. Pereonite 4 twice as wide as long, lateral sides tapering towards rounded posterior margin, posterior margin stretching over pereonite 5 (Figs. 12, 31), lateral shields at leg attachment. Pereonite 5 consists of elastic membrane with bulbous shields on lateral sides at leg attachment (Fig. 30). Pereonite 6 rectangular, posterior margin slightly concave. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Many sensory pits randomly distributed over all pereonites.

Pleon. Pleon and pleotelson same length as pereon. Five pleonites dorsally visible (Figs. 11, 12). Single simple setae, on each posterior lateral side of each pleonite.

Pleotelson. Triangular, longer than wide, lateral margins straight, four simple setae on dorsal surface, distal apex terminating in pair of simple setae (Fig. 15).

Uropod. Endopod extending beyond apex of pleotelson, exopod reaching apex. Endopod longer and wider than exopod, both with long fringing setae, endopod with mesial six setae plumose, exopod with mesial four setae plumose, rest of setae simple (Fig. 15). Uropodal basis with two simple setae.

Pleopods. Exopod and endopod of almost similar size. Both fringed distally with seven to nine long plumose setae, few shorter simple setae on lateral margins (Fig. 16). Plumose setae almost as long as pleopod.

Pereopods. Pereopod 2 basis elongated with single feather-like bristle and three to four simple setae anterior, two to three posterior simple setae (Fig. 22). Ischium three quarters length of basis and almost as wide, three to five anterior setae. Merus half the length of ischium with anterior bulbous protrusion, single serrated spine and two simple setae on bulbous protrusion, posterior margin with simple setae. Carpus of almost same size and shape as merus, but without anterior bulbous, tubercles and single serrated spine on posterior margin. Propodus about twice the length of carpus, prominent tubercles and pectinate scales on posterior side, two elongated serrated spines ending in sharp points situated on middle and distal part of

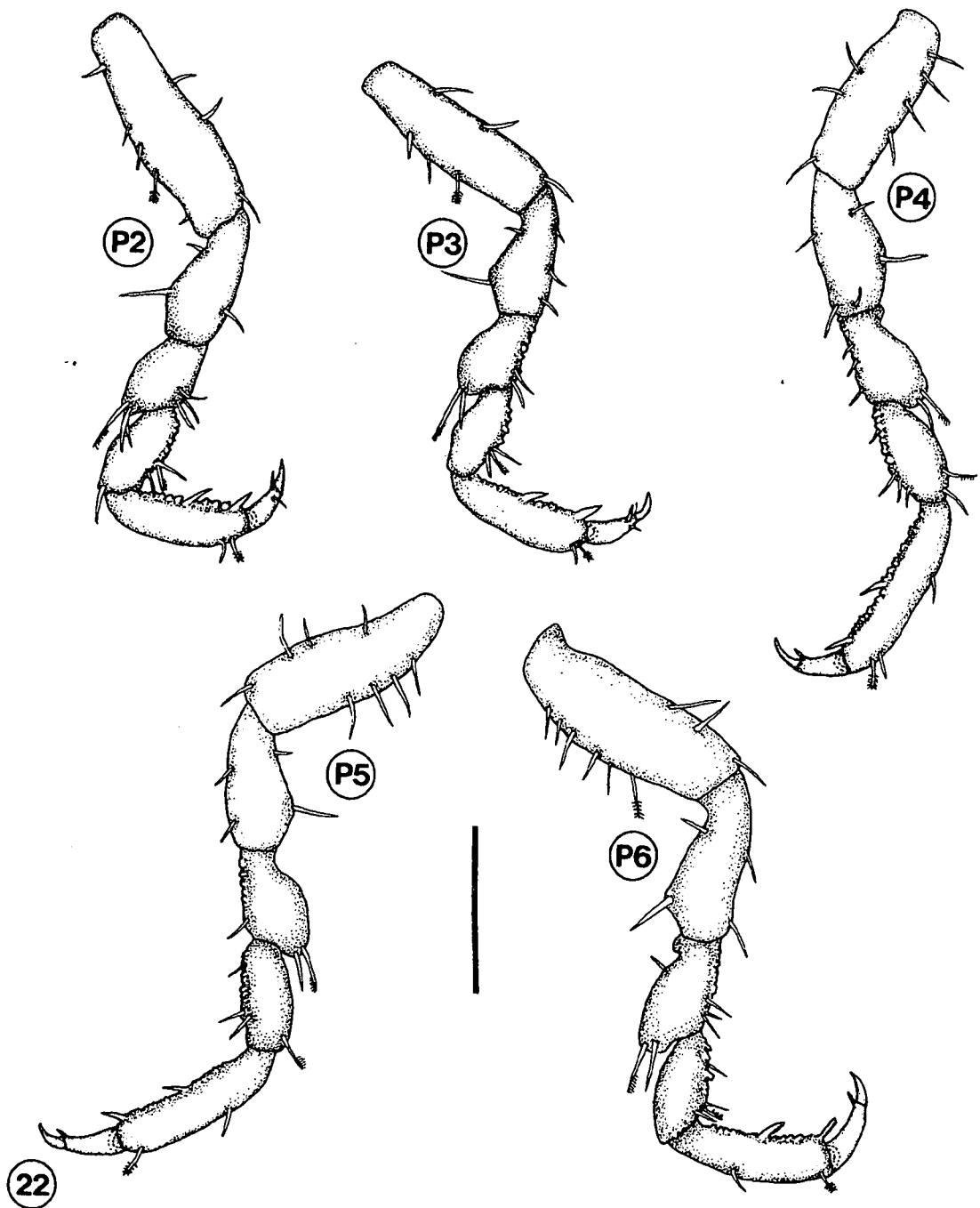
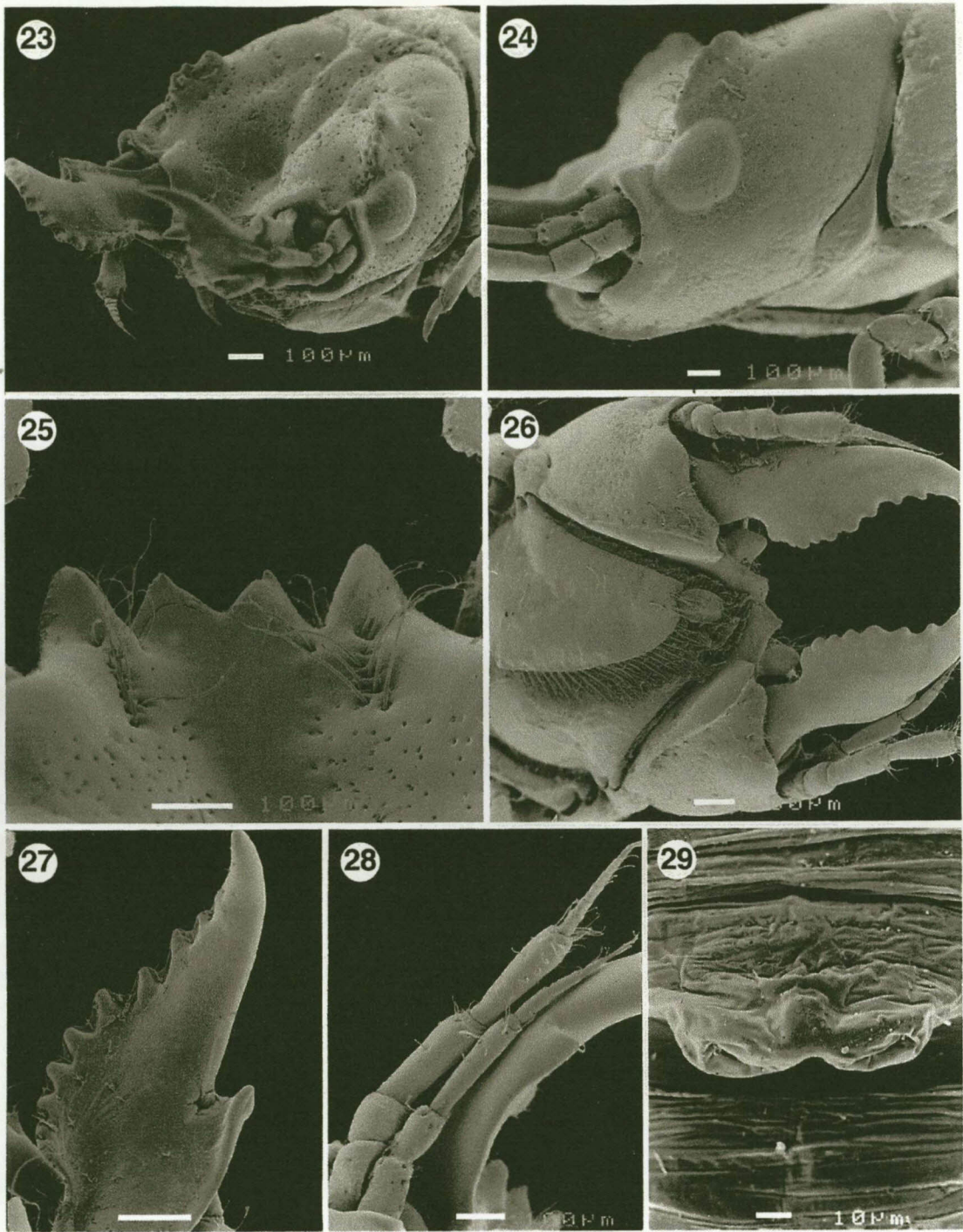
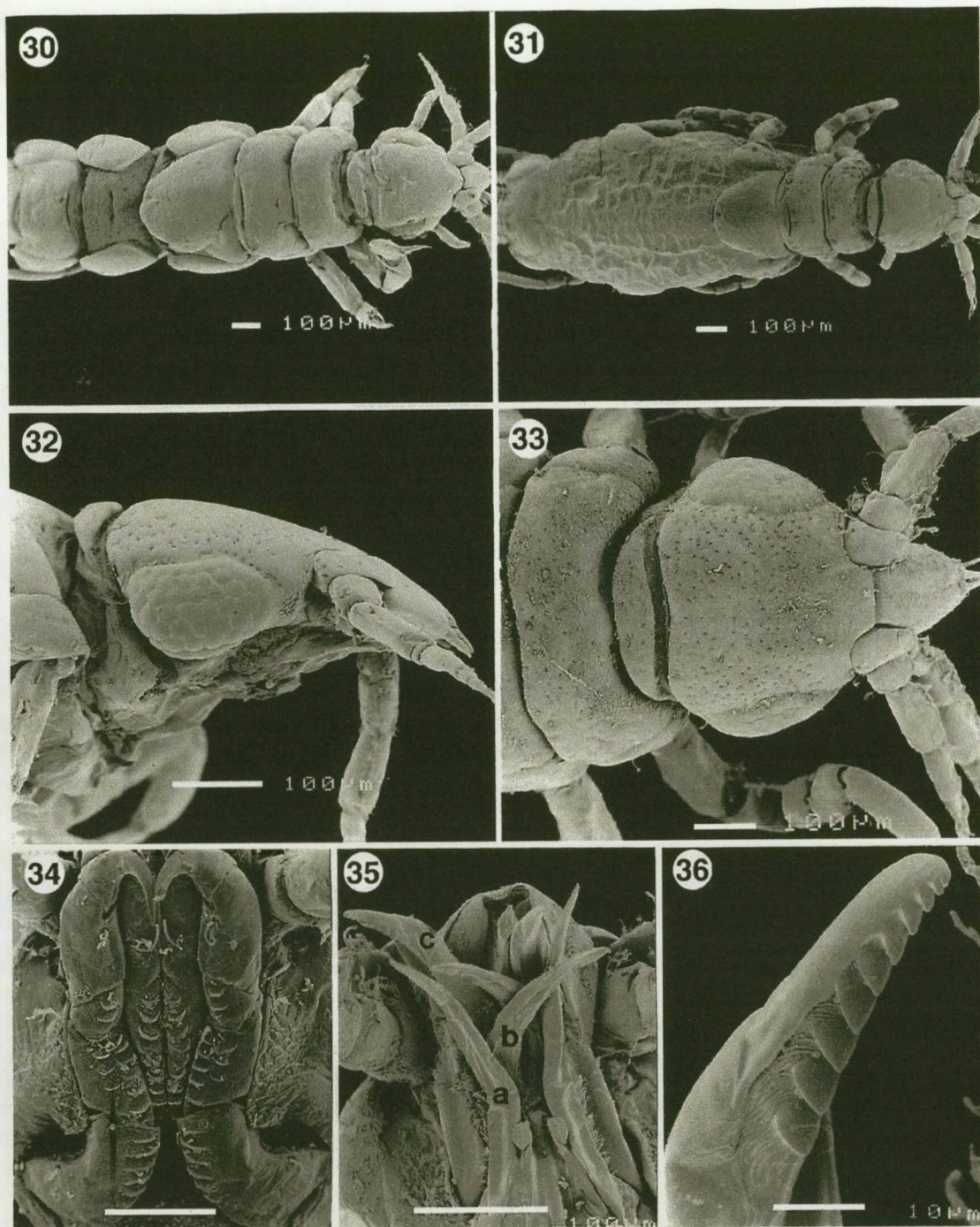


Fig. 22. Microscope projection drawings of pereopods 2 to 6 (P2-P6) of *Gnathia africana* Barnard, 1914 praniza larva collected at McDougall's Bay. Scale bar = 200 μ m.



Figs. 23-29. Scanning electron micrographs of a male *Gnathia africana* Barnard, 1914 collected at McDougall's Bay. Fig. 23. Anterio-lateral view of cephalon. Fig. 24. Lateral view of cephalon. Fig. 25. Dorsal view of superior frontolateral and mediofrontal processes with simple setae. Fig. 26. Ventral view of cephalon. Fig. 27. Dorsal view of right mandible. Fig. 28. Lateral view of left antennae. Fig. 29. Ventral view of penes. Scale bar: Fig. 27 = 100 µm.



Figs. 30-36. Scanning electron micrographs of *Gnathia africana* Barnard, 1914 larvae collected at McDougall's Bay. Fig. 30. Dorsal view of cephalon and pereon of zuphea larva. Fig. 31. Dorsal view of cephalon and pereon of praniza larva. Fig. 32. Lateral view of cephalon. Fig. 33. Dorsal view of cephalon. Fig. 34. Ventral view of gnathopods of a praniza larva. Fig. 35. Ventral view of (a) maxillipedes, (b) maxillule and (c) mandibles of a praniza larva. Fig. 36. Teeth on mandible of a praniza larva. Scale bar: Fig. 34 = 100 μ m.

posterior side respectively, only a few simple setae anteriorly with single feather-like bristle distally. Dactylus half the length of propodus, terminates in sharp posterior-pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 (Fig. 22), similar to pereopod 2, differ only in direction, pereopods 4 to 6 directed posteriorly and pereopods 2 and 3 anteriorly.

Type material: Syntype: In the collection of the South African Museum, Cape Town (SAM-A2553). Original author designated no holotype.

Type locality: St. James, False Bay (34°8.2'S, 18°27.4'E)

Other localities: Lüderitz (26°40'S, 15°3'E), McDougall's Bay (29°45'S, 16°45'E), De Hoop Nature Reserve (34°28'S, 20°30'E) and Jeffreys Bay (34°2.2'S, 24°56.5'E).

Material examined: Material in the collection of the South African Museum, Cape Town, collected from the tubes of serpulid worms (SAM-A2693).

New material: 95/09/09-01, 96/12/20-01 and 98/04/01-01 in the collection of the authors, all material collected from sponges.

Other material: In the collection of the South African Museum, Cape Town (SAM-A12614).

DISCUSSION

Populations of *Gnathia africana* differed in body size. The largest specimens were found at McDougall's Bay (west coast) with a progressive decrease in the size southwards. The specimens from De Hoop were intermediate in size with the smallest individuals collected at Jeffreys Bay (south east coast). This is in line with the phenomenon that animals on the southern African west coast tend to grow larger than the same species on the south and east coasts (Branch et al. 1994).

Gnathia africana adult males can be distinguished from the other southern African species by means of its

medianly divided mediofrontal process, sensory pits and prominent paraocular process. According to Müller (1991), *G. africana* is similar to *Gnathia fringae* Müller, 1991 from Réunion Island in overall body proportions, in its frontal border and in the presence of the anterior constriction of pereonite 4 separating pereonite 4 from pereonite 3. *G. africana* differs from *G. fringae* in possessing prominent paraocular ornamentation, sensory pits randomly distributed over the cephalon and in lacking long simple setae distributed over both the cephalon and pereon. *G. africana* (3.8-5.2 mm) is also almost twice the size of *G. fringae* (1.7-2.1 mm).

The zuphea larva (Figs. 11, 30) differs from the praniza larva (Figs. 12, 31) only in the absence of the elastic membrane between pereonites 4 and 6. Segmentation between pereonites 3 and 7 is therefore indistinct. The praniza larvae are larger due to the stretched pereon.

The abundance of sensory pits on the dorsal body of *G. africana* larvae appears to be similar to those found by Davies (1981) on larvae of *Gnathia maxillaris* (Montagu, 1804). According to Charmantier et al. (1987), *Paragnathia formica* (Hesse, 1864) lacks these sensory pits. The bodies of *G. maxillaris* larvae are covered with scales, in contrast to the smooth body surface of *G. africana*. The larvae of *Caecognathia calva* (Vanhöffen) as illustrated by Wägele (1987) have narrower uropods than *Gnathia africana*. Although the larvae of *G. africana* appear to be similar in basic morphology to those of *G. maxillaris*, *Caecognathia calva* and *Paragnathia formica*, differences in the number of teeth on the mouthparts were also found.

Acknowledgements. The authors thank Ms. Michelle van der Merwe of the South African Museum, Cape Town, for making the gnathiid material available for examination and Dr Angela Davies Russell, Kingston University, UK, for reading the draft copy. This study was funded by the marine resource program of the Foundation for Research Development (FRD) of South Africa.

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Received 8 December 1998

Accepted 11 March 1999

Appendix I B

A redescription of the adult male of *Caecognathia cryptopais* (Barnard, 1925) (Crustacea: Isopoda: Gnathiidae) from southern Africa

Nico J. Smit, Linda Basson and Jo G. Van As

Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa

Key words: *Caecognathia cryptopais*, redescription, taxonomy, morphology

Abstract. A redescription of the adult male of *Caecognathia cryptopais* (Barnard, 1925) is provided from syntypes and other material deposited in the South African Museum. The generic status of *Caecognathia cryptopais* is also revised. This redescription is based on light and scanning electron microscopy.

From their phylogenetic analysis of the family Gnathiidae, Cohen and Poore (1994), established 10 genera for the family. One of their significant taxonomic changes was the resurrection of the genus *Caecognathia* Dollfus, 1901. This genus is closely related to the genus *Gnathia* Leach, 1814. Cohen and Poore (1994) transferred many of the species described as belonging to the genus *Gnathia* to *Caecognathia*. Most of the species of Monod's (1926) *Section Productae* of the genus *Gnathia* were also moved to *Caecognathia*. The main taxonomic character distinguishing species of *Caecognathia* from those of *Gnathia* is the presence of a produced frontal border lacking any frontal processes.

All four gnathiid species from southern Africa have been described by Barnard (1914a,b, 1920, 1925a,b) as belonging to the genus *Gnathia* (*Gnathia africana* Barnard, 1914; *Gnathia spongicola* Barnard, 1920; *Gnathia disjuncta* Barnard, 1920 and *Gnathia cryptopais* Barnard, 1925). Due to the lack of sufficient information on their morphology, the southern African species were not included in Cohen and Poore's (1994) phylogenetic analysis and thus their generic status was not revised.

In the present study the type material in the South African Museum, Cape Town as well as other material in their collection was used to do a redescription of *Caecognathia cryptopais* and a revision of its generic status.

MATERIALS AND METHODS

For scanning electron microscopy specimens were rehydrated in a descending sequence of ethanol and washed in tap water in order to get rid of debris. They were then dehydrated to absolute ethanol and critical point dried. Specimens were mounted on aluminium stubs, sputter coated with gold and studied with the aid of a JEOL WINSEM JSM 6400 at 10 kV.

Temporary slides were prepared of whole mounts as well as dissected mouthparts and pereopods. These were examined with the aid of a Leitz Laborlux D compound as well as a Wild M5 dissection microscope and drawings made from projections using drawing attachments.

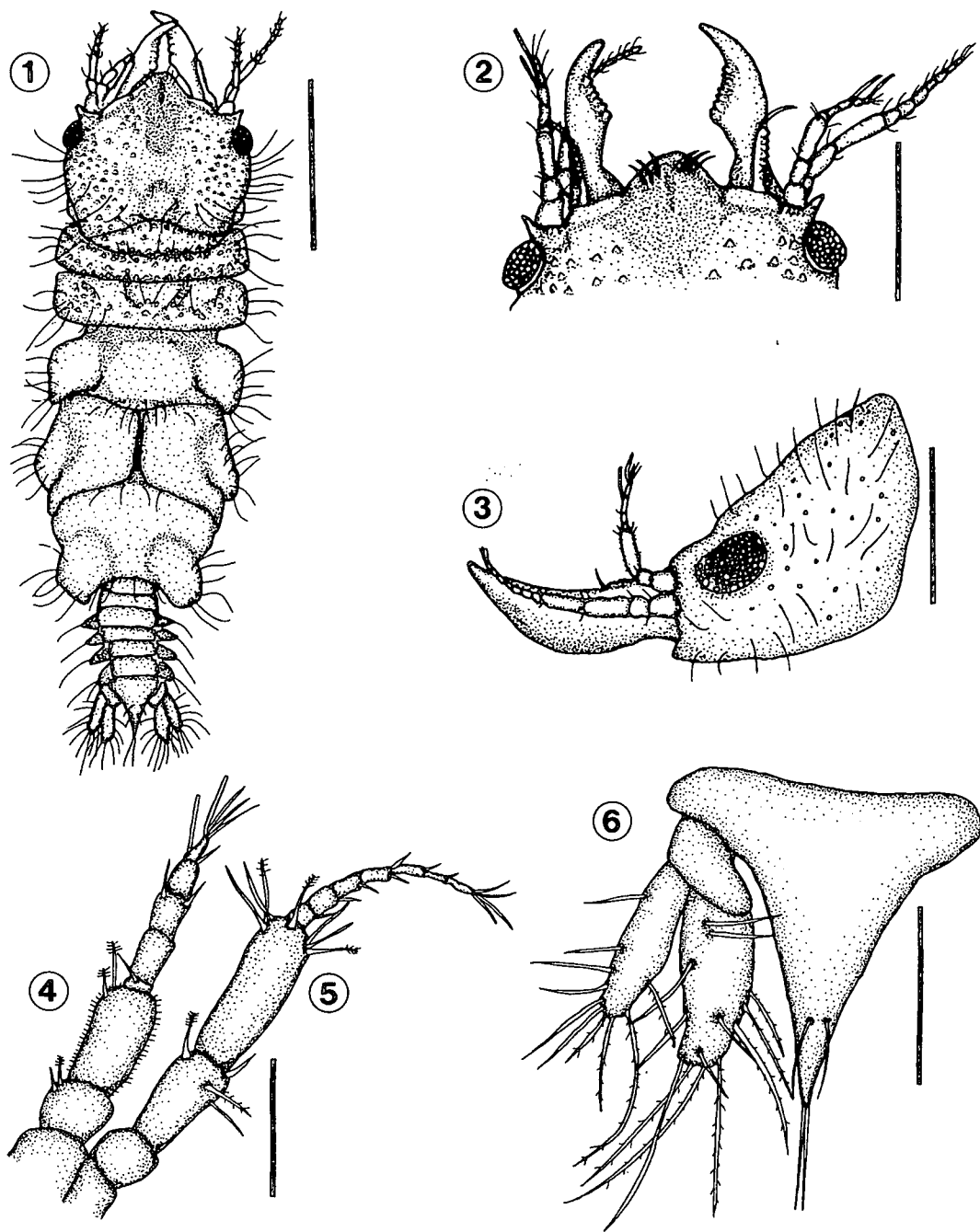
RESULTS

Caecognathia cryptopais (Barnard, 1925) Figs. 1-17
Syn.: *Gnathia cryptopais* Barnard, 1925

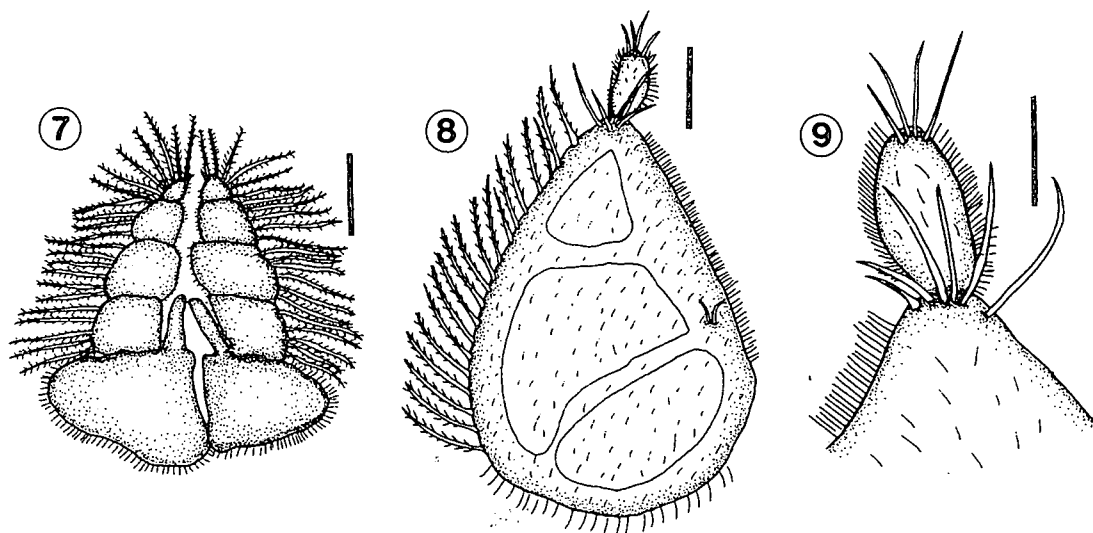
Description: Total length of syntype: 2 mm (SAM-A6051). Total lengths of other material: 2-2.35 mm (mean 2.15 mm, n = 4) (SAM-A43161), 2.05 and 2.15 mm (n = 2) (SAM-A19310), 3.80 mm (n = 1) (SAM-A19311), 3.65 and 3.75 mm (n = 2) (SAM-A19312), 3.74 and 3.94 mm (n = 2) (SAM-A14602).

Cephalon. Rectangular, one and a half times as wide as long, posterior margin concave, lateral margins convex (Figs. 1, 12). Shallow dorsal sulcus, extending to median tubercle (Figs. 1, 11). Dorsal and ventral cephalon armed with numerous randomly distributed wart-like tubercles closely associated with long simple setae. Well developed oval-shaped, prominent bulbous, compound eyes on lateral margin of cephalon, slightly elevated and encircled by a smooth rim, length of eye slightly more than a fifth of cephalon (Figs. 3, 15). No paraocular ornamentation (Figs. 3, 11). Posterior median tubercle from middle of cephalon to posterior margin.

Frontal border. Rounded, produced for a fifth of cephalon's length, lacking any frontal processes (Figs. 2, 13). Median groove on produced border, six to seven long simple setae on dorsal surface of median producing frontal border, rest of border covered with short simple setae. External scissura deeply excavated (Fig. 2). Supraocular lobe prominent, convex, extending laterally with five to seven tubercles.



Figs. 1-6. Microscope projection drawings of a male *Caecognathia cryptopais* (Barnard, 1925) (SAM-A43161). Fig. 1. Full length dorsal view. Fig. 2. Frontal border and mandibles. Fig. 3. Lateral view of cephalon, antennae and mandible. Fig. 4. First antenna. Fig. 5. Second antenna. Fig. 6. Telson and uropods. Scale bars: Fig. 1 = 1 mm; Figs 2, 3 = 500 μ m; Figs. 4-6 = 100 μ m.



Figs. 7-9. Microscope projection drawings of the mouthparts of a male *Caecognathia cryptopais* (Barnard, 1925) (SAM-A43161). Fig. 7. Maxillipede. Fig. 8. Pylopod. Fig. 9. Articles 2 and 3 of pylopod. Scale bars: Figs. 7, 8 = 100 μ m; Fig. 9 = 50 μ m.

Antennae. Antenna 2 longer than antenna 1. Antenna 1 with three peduncle articles, with third one largest and covered with short simple setae, flagellum with five articles, article 1 small, 2, 3 and 4 largest, article 4 with one aesthetascs setae, article 5 terminating in one aesthetascs and two to three simple setae, few setae on each article (Fig. 4). Antenna 2 with four peduncle articles, article 4 largest, flagellum with seven articles, article 1 largest (Fig. 5). Few long simple and feathered setae on each article.

Mandibles. Two thirds length of cephalon, twice as long as wide, outer border curved inwards, inner border straight with sparsely dentate blade (Figs. 2, 16). Apex cylindrical, curved inwards, distally raised in lateral view at 40°. Prominent incisor not present. Single mandibular setae extending from base of incisor process. Carina armed with 11 to 13 tubercles, forming ridge on lateral margin extending from basal neck to halfway along mandible (Figs. 2, 13). Internal lobe and pseudoblade absent. Short simple setae on ventral surface of blade.

Maxillipedes. Five-articled, proximal article largest, prominent endite of proximal article reaching article 3, endite not contiguous with article 2 (Fig. 7). Lateral margins of distal four articles with plumose setae in order of 5-6-5-6 (Fig. 7). Mesial margins of distal four articles fringed with short simple setae. Palp three times as long as wide. No coupling hooks.

Pylopods. Three articles. First article greatly enlarged, pear-shaped, mesial and lateral borders convex, mesial border fringed with plumose setae, lateral and posterior fringing setae short and simple (Figs. 8, 14). Three distinct areolae. Ventral surface

covered with short simple setae and six long simple setae distally. Second article oval, twice as long as wide, outer margin setose, ventrally also covered with short simple setae, three to five simple setae distally on ventral surface (Fig. 9). Third article minute.

Pereon. Twice as long as wide, wider than cephalon (Fig. 1). Pereonite 1 fused with cephalon, dorsally visible, not reaching lateral margins, anterior border convex, posterior border straight (Figs. 1, 12). Pereonites 2 and 3 of similar size, forming widest part of body. Pereonite 3 with anterior constriction but not separating it from pereonite 2. Prominent anterior constriction of pereonite 4 separating it from pereonite 3. Pereonite 4 with distinct median groove extending to pereonite 6, median groove forming oval tubercles on lateral margins of pereonites 4 to 6 (Fig. 1). Pereonite 5 and 6 not fused. Pereonite 6 narrowest, but longest, 1.6 times as long as wide, posterior margin deeply concave, without lobuli. Pereonite 7 dorsally visible, very small with rounded posterior margin, overlapping first pleonite. Dorsal and lateral pereon with wart-like tubercles closely associated with long simple setae.

Pleon. Pleon and pleotelson slightly less than a fifth of total length (Fig. 1). All five pleonites dorsally visible, epimera distinct. Single long medio-posterior simple seta on border of each pleonite, single long simple seta on epimera of all posterior four pleonites.

Pleotelson. Triangular, base not as wide as long, lateral margins slightly concave, ventral surface with many short simple setae and two to four long simple setae, distal apex terminating in pair of simple setae (Fig. 6).

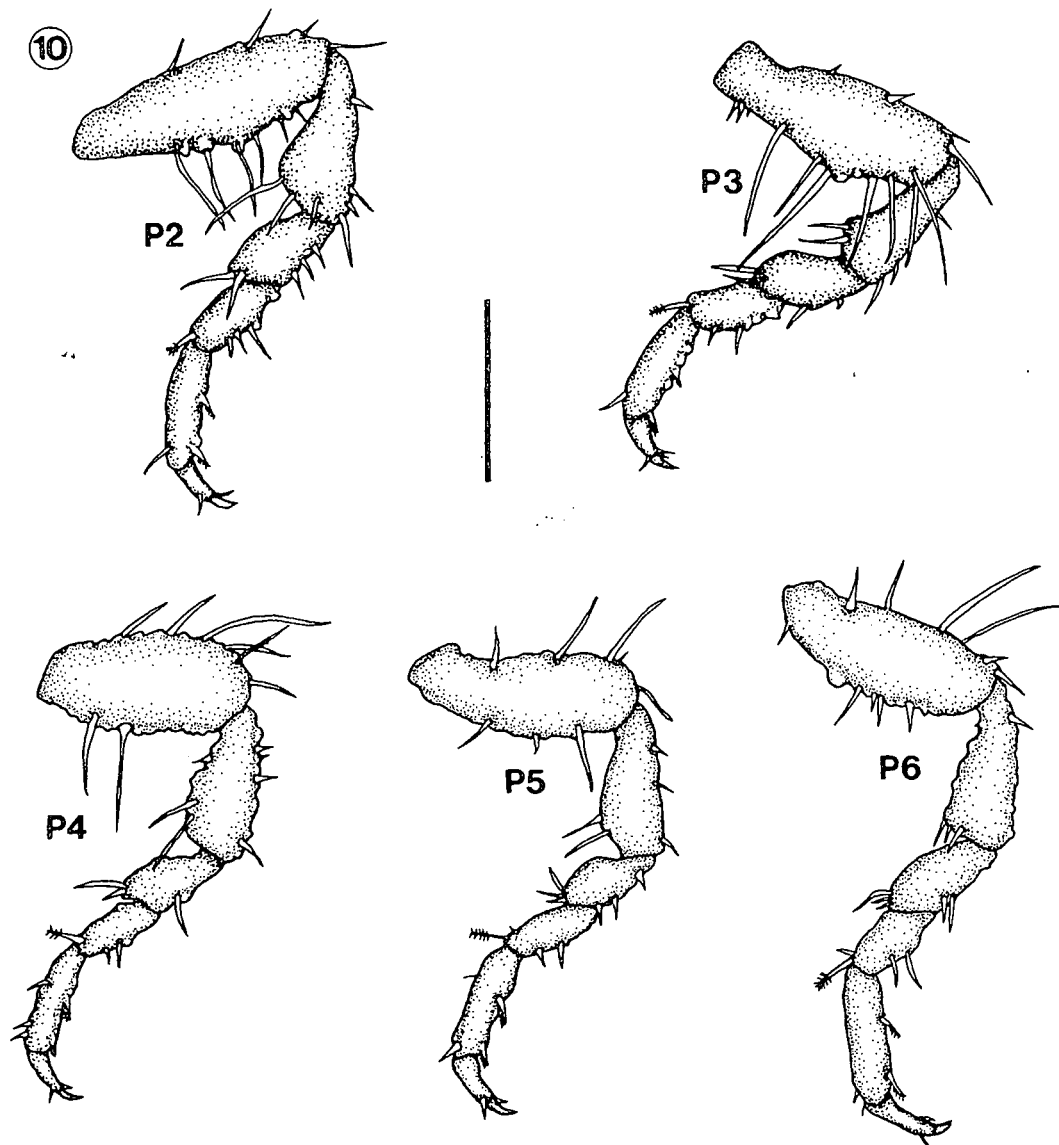
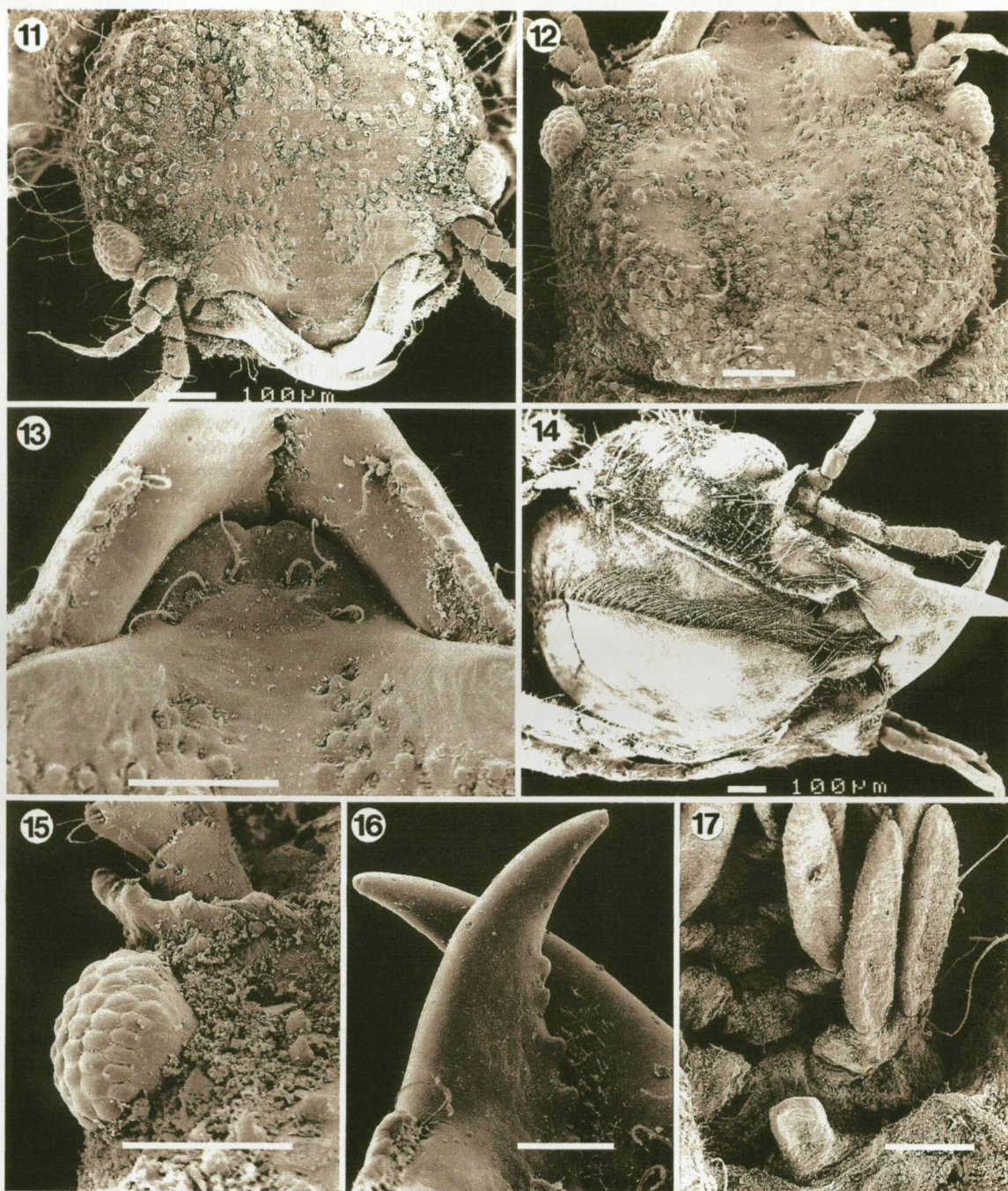


Fig. 10. Microscope projection drawings of pereopods 2 to 6 (P2-P6) of a male *Caecognathia cryptopais* (Barnard, 1925) (SAM-A43161). Scale bar = 200 μ m.

Uropods. Extending to apex of pleotelson, endopod longer and wider than exopod, both with long fringing setae, endopod with inner six plumose setae, four to six simple setae on dorsal surface, exopod with inner four plumose setae, rest of setae simple (Fig. 6).

Pereopods. Pereopod 2 consists of elongated basis with nine to eleven simple setae anterior and four to seven on posterior side, ischium two thirds length of basis, with three to five anterior setae and posterior tubercles with simple setae in between (Fig. 10). Merus is half the length of ischium with anterior bulbous

protrusion, simple setae on bulbous protrusion, posterior margin with tubercles as well as simple setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion single plumose seta on distal part of anterior margin. Basis, ischium, merus and carpus covered with short simple setae. Propodus about twice the length of carpus, prominent tubercles fringe posterior side, two elongated denticulated compound spines ending in sharp points situated on middle and distal part of posterior margin respectively, only a few simple setae anteriorly. Dactylus half the length of



Figs. 11-17. Scanning electron micrographs of a male *Caecognathia cryptopais* (Barnard, 1925) (SAM-A43161). **Fig. 11.** Anterio-lateral view of cephalon. **Fig. 12.** Dorsal view of cephalon. **Fig. 13.** Dorsal view of produced frontal border with simple setae. **Fig. 14.** Ventral view of cephalon and pylopods. **Fig. 15.** Dorsal view of left eye and supraocular lobe. **Fig. 16.** Dorsal view of left mandible. **Fig. 17.** Ventral view of penes and first pleopods. Scale bars: Figs. 12, 13, 15, 17 = 100 µm; Fig. 16 = 30 µm.

propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis with few simple setae on dorsal and ventral sides. Pereopods 3 to 6 similar to pereopod 2 (Fig. 10), differing only in direction, pereopods 4 to 6 are directed posteriorly and pereopods 2 and 3 anteriorly.

Pleopods. Five pairs of similar exo- and endopods, without fringing setae, short simple setae on distal borders (Fig. 17).

Penes. Large, one and a half times as long as wide, consist of two contiguous papillae (Fig. 17).

Type material: Syntypes: In the collection of the South African Museum, Cape Town (SAM-A6051). Original author designated no holotype.

Type locality: Duminy Point, off Saldanha Bay (E. x N. 0.5 N., distant 8 nautical miles)

Other localities: Off East London (32°14.9'S, 29°10.43'E), (32°29.5'S, 28°57.1'E) and (32°28.6'S, 28°58.8'E). Off Port Alfred (33°39.3'S, 27°11.6'E).

Type host: Unknown.

Material examined: Type material in the collection of the South African Museum, Cape Town (1 male, 1 larva, SAM-A6051).

Other material: In the collection of the South African Museum, Cape Town (2 males, SAM-A14602) (2 males, SAM-A19310) (1 male, SAM-A19311), (2 males, SAM-A19312), (4 males, SAM-A43161).

DISCUSSION

According to Cohen and Poore (1994) the genus *Caecognathia* is characterised by a produced frontal border without frontal processes, cephalon without paraocular ornamentation, pereonite immersed in cephalon, pylopod two- or three-articled, with article 1 enlarged and article 3 small or absent. *Caecognathia cryptopais* conforms to all these specifications for the

genus and hence its transfer from the genus *Gnathia* to *Caecognathia*.

Caecognathia cryptopais differs from the other southern African species in that it is the only species with a rounded produced frontal border without any frontal processes, many wart-like tubercles on the body, no long simple setae on the distal margins of pleopods and very big penes in comparison with the other southern African species. According to Barnard (1925a,b, 1940), *C. cryptopais* is closely related to *Caecognathia elongata* (Kröyer, 1847) (syn. *Gnathia cerina* Stimpson, 1853) but differs in the presence of wart-like tubercles associated with long simple setae all over the dorsal and lateral surface of the body. *Caecognathia elongata* also has no prominent dorsal sulcus on pereonites 4 to 6 forming oval lobi laterales on pereonites 4 to 6. Monod (1926) as well as Barnard (1940) pointed out that *C. cryptopais* seems to be similar to *Caecognathia antarctica* (Studer, 1883). On examination of a single specimen of *C. antarctica* deposited in the South African Museum (SAM-A16158) it was found that it can be distinguished from *C. cryptopais* by its different shaped mandible, broader cephalon, a more prominent median tubercle and the indistinct epimera of the pleon. All the above mentioned species related to *C. cryptopais* were also transferred by Cohen and Poore (1994) from the genus *Gnathia* to *Caecognathia*.

Acknowledgements. The authors thank Ms. Michelle van der Merwe of the South African Museum, Cape Town, for making the gnathiid material available for examination and Dr. Gary Poore, Museum of Victoria, Australia, for confirming the new generic status of *Caecognathia cryptopais*. This study was funded by the marine resource program of the National Research Foundation (NRF) of South Africa.

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Received 27 May 1999

Accepted 14 July 1999

Appendix I C

A new species, *Gnathia nkulu* sp. n. (Crustacea: Isopoda: Gnathiidae) from southern Africa

Nico J. Smit and Jo G. Van As

Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa

Key words: *Gnathia nkulu*, description, taxonomy, morphology

Abstract. A new species, *Gnathia nkulu* sp. n. is described from material collected off the South African coast at 80-200m depth. It differs from the intertidal species *Gnathia africana* Barnard, 1914 in that the mediofrontal process is not deeply divided into two lobes, article 2 of the pylopod is rounded and small wart-like tubercles and long simple setae are present on both the cephalosome and pereon.

The coastline of southern Africa is known for its unique and diverse vertebrate and invertebrate fauna with many species being endemic to this region. So far only four species of temporary fish parasites of the isopod family Gnathiidae have been described from southern Africa, thus not reflecting this diversity. A similar situation occurred in Australia in the late 1970s. Holdich and Harrison (1980) attributed the low number of described species at that time to poor sampling effort and ignorance of the smaller elements of the macrofauna. After extensive sampling Cohen and Poore (1994) proved them right by describing 29 species from southeastern Australia. The same situation may also be found in southern Africa.

While working on the gnathiid collection of the South African Museum, Cape Town, specimens of the genus *Gnathia* Leach, 1814 that do not correspond to any description of the known species from South Africa (see Kensley 1978) as well as other parts of the world were found. These specimens, wrongly identified as *Gnathia africana* Barnard, 1914, will be described herein as a new species. This description brings the total number of gnathiids found from southern Africa up to five with the other records being those of Barnard (1914a,b, 1920, 1925) describing *Gnathia africana*; *Gnathia spongicola* Barnard, 1920; *Gnathia disjuncta* Barnard, 1920 and *Caecognathia cryptopais* (Barnard, 1925).

MATERIALS AND METHODS

The specimens used in this study were collected from various localities along the South African south coast between 1972 and 1978 by means of dredges of 80-200m depth. Since most of the material was fixed in 70% ethanol for more than 20 years before examination, special attention was given to the cleaning of the specimens. For light as well as scanning electron microscopy, specimens were prepared following the same methods described elsewhere (see Smit et al. 1999). For the purpose of this description, we followed the anatomical terminology used by Monod (1926) and the numbering of perconites and pereopods adopted by Cohen and Poore (1994).

RESULTS

Gnathia nkulu sp. n.

Figs. 1-15

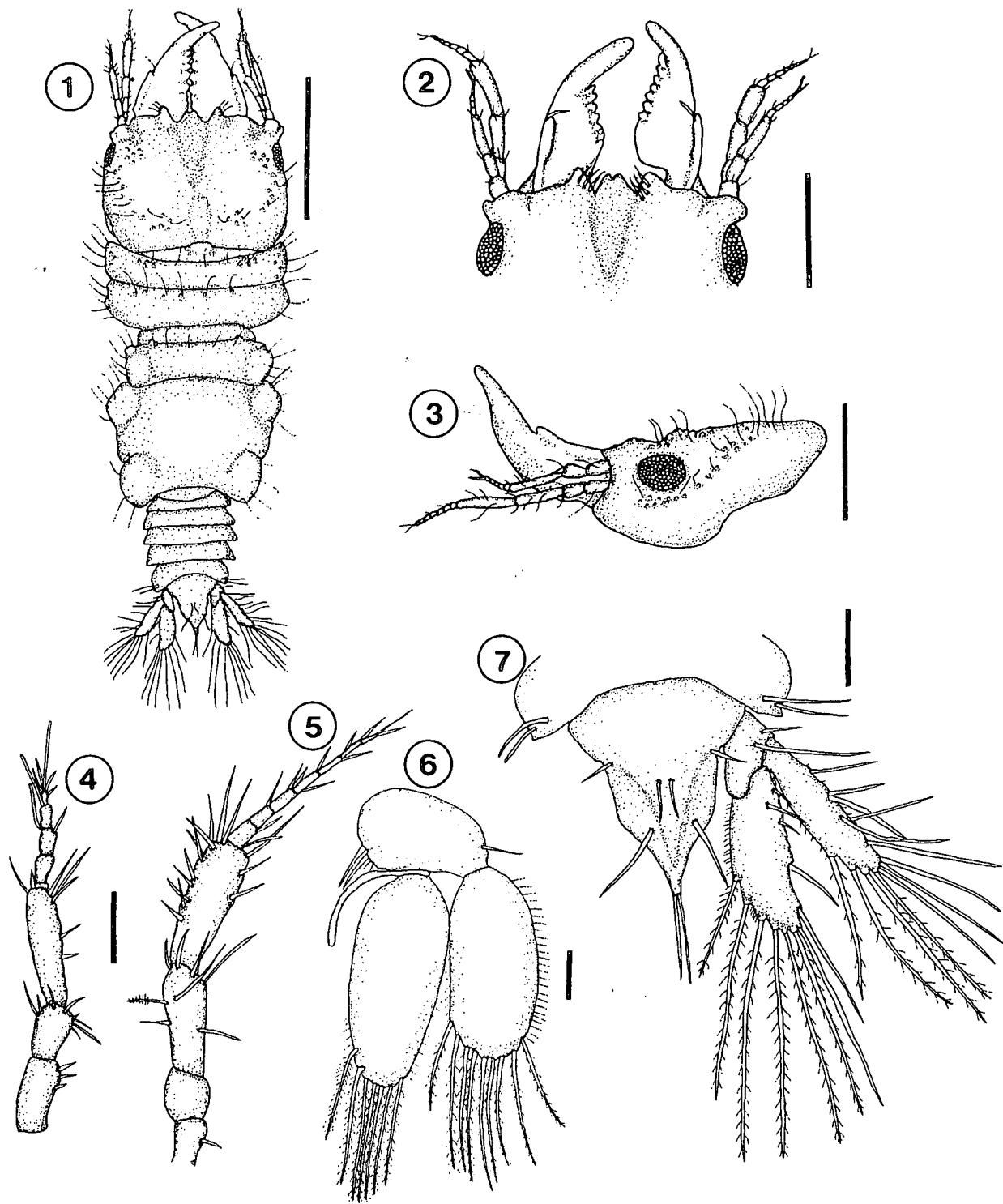
Adult male

Description: Total length of holotype: 3.7 mm. Total lengths of paratypes: 3.35-4.9 mm (mean 4.1, n = 20). Total lengths of other material: 3.3 mm (n = 1), 4.9 mm (n = 1).

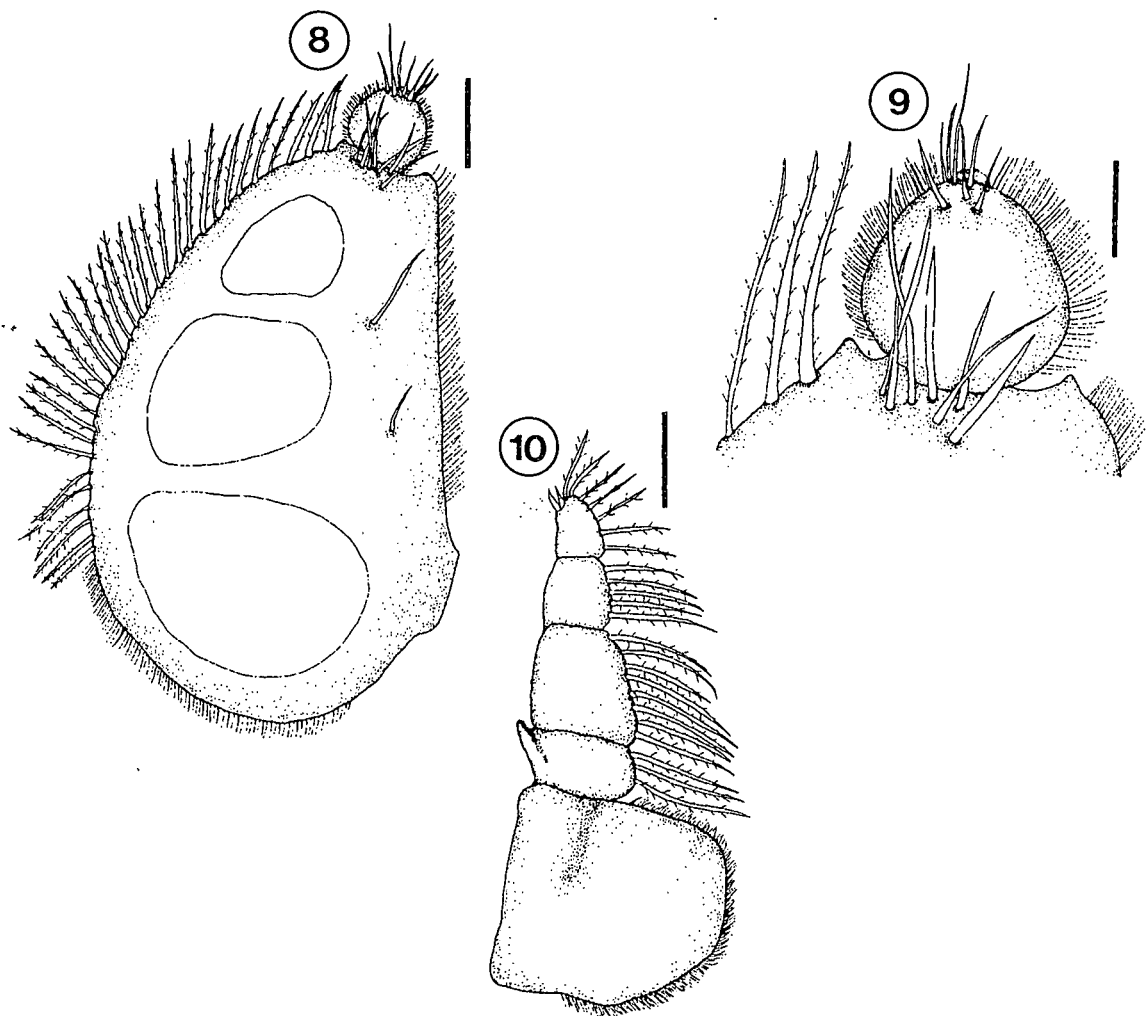
Cephalosome. Rectangular, 1.3 times as wide as long, very deep V-shaped dorsal sulcus (Fig. 12), extending medianly to almost middle of cephalosome, lateral margins straight, posterior margin slightly concave, cephalosome with few dorsal setae (Fig. 1). Sensory pits and small wart-like tubercles distributed randomly over dorsal surface as well as ventrally on lateral sides of buccal cavity of cephalosome (Fig. 12). Long simple setae sparsely distributed over cephalosome. Well-developed oval-shaped, compound eyes on lateral margin of cephalosome, length of eye less than a third of cephalosome (Fig. 3). Paraocular ornamentation consisting of eight to ten small tubercles with simple setae dorsal to eye (Fig. 13). Semicircular row of tubercles on lateral sides and posterior half of dorsal cephalosome. Elongated posterior median tubercle present.

Frontal border. Slightly produced superior fronto-lateral process conical, with three long simple setae on process (Fig. 2). Mediofrontal process inferior, small concave median notch, minute tubercles on lateral margins (Fig. 14). No inferior frontolateral process. External scissura deeply excavated. Supraocular lobe prominent, extending laterally.

Antennae. Antenna 2 longer than antenna 1 (Fig. 2). Antenna 1 with three peduncle articles, with third one largest, flagellum with five articles, article 2 and 3 largest, articles 3 and 4 with one aesthetascs setae each, article 5 terminating in one aesthetascs and three to five simple setae, few setae on each article (Fig. 4). Antenna 2 with five peduncle articles, article 5 largest, flagellum with seven articles, article 1 largest, article 7



Figs. 1-7. Microscope projection drawings of a male *Gnathia nkulu* sp. n. (SAM-A19314). Fig. 1. Full length dorsal view. Fig. 2. Frontal border and mandibles. Fig. 3. Lateral view of cephalosome, antennae and mandible. Fig. 4. First antenna. Fig. 5. Second antenna. Fig. 6. Left pleopod 2 with appendix masculina. Fig. 7. Telson and uropods. Scale bars: Fig. 1 = 1 mm; Figs. 2, 3 = 500 μ m; Figs. 4-7 = 100 μ m.



Figs. 8-10. Microscope projection drawings of the mouthparts of a male *Gnathia nkulu* sp. n. (SAM-A43160). Fig. 8. Pylopod. Fig. 9. Articles 2 and 3 of pylopod. Fig. 10. Maxilliped. Scale bars: Figs. 8, 10 = 100 μ m; Fig. 9 = 50 μ m.

terminating in three to four simple setae, few setae on each article, except article 5 of peduncle with 15 to 20 setae (Fig. 5).

Mandible. Long, almost same length as cephalosome, more than twice as long as wide, curved inwards with seven to nine processes on dentate blade, tussles of setae between processes (Figs. 2, 15). Apex conical, distally raised in lateral view at 45° (Fig. 3). Incisor present, terminating in acute point. Single mandibular seta extending from base of incisor process. Prominent unarmed carina, forming ridge on lateral margin extending from basal neck to halfway along mandible. Basal neck quarter of total mandible length. Pits on dorsal and ventral sides of mandible. Internal lobe and pseudoblade absent.

Maxilliped. Five-articled, proximal article the largest, distal four articles with plumose setae on external margin in order of 3-8-5-7 (Fig. 10). Palp 3.5

times as long as wide. Endite contiguous with article 2, reaching article 3. Simple setae on external border of endite. No coupling hooks.

Pylopod. Three articles, flat, overlapping. First article greatly enlarged, internal border fringed with plumose setae, external setae short and simple, two simple setae posteriorly near external border and seven distally (Fig. 8). Three areolae. Second article round, as long as wide, margins setose, seven simple setae distally on ventral surface (Fig. 9). Third article minute, with fringing setae.

Pereon. Almost one and a half times as long as wide, wider than cephalosome (Fig. 1). Pereonite 1 fused with cephalosome, dorsally visible, not reaching lateral margins, divided into three regions by posterior margin of cephalosome, anterior border with convex median evagination, posterior margin straight (Fig. 1). Pereonite 2 and 3 of similar size, widest part of body, lateral

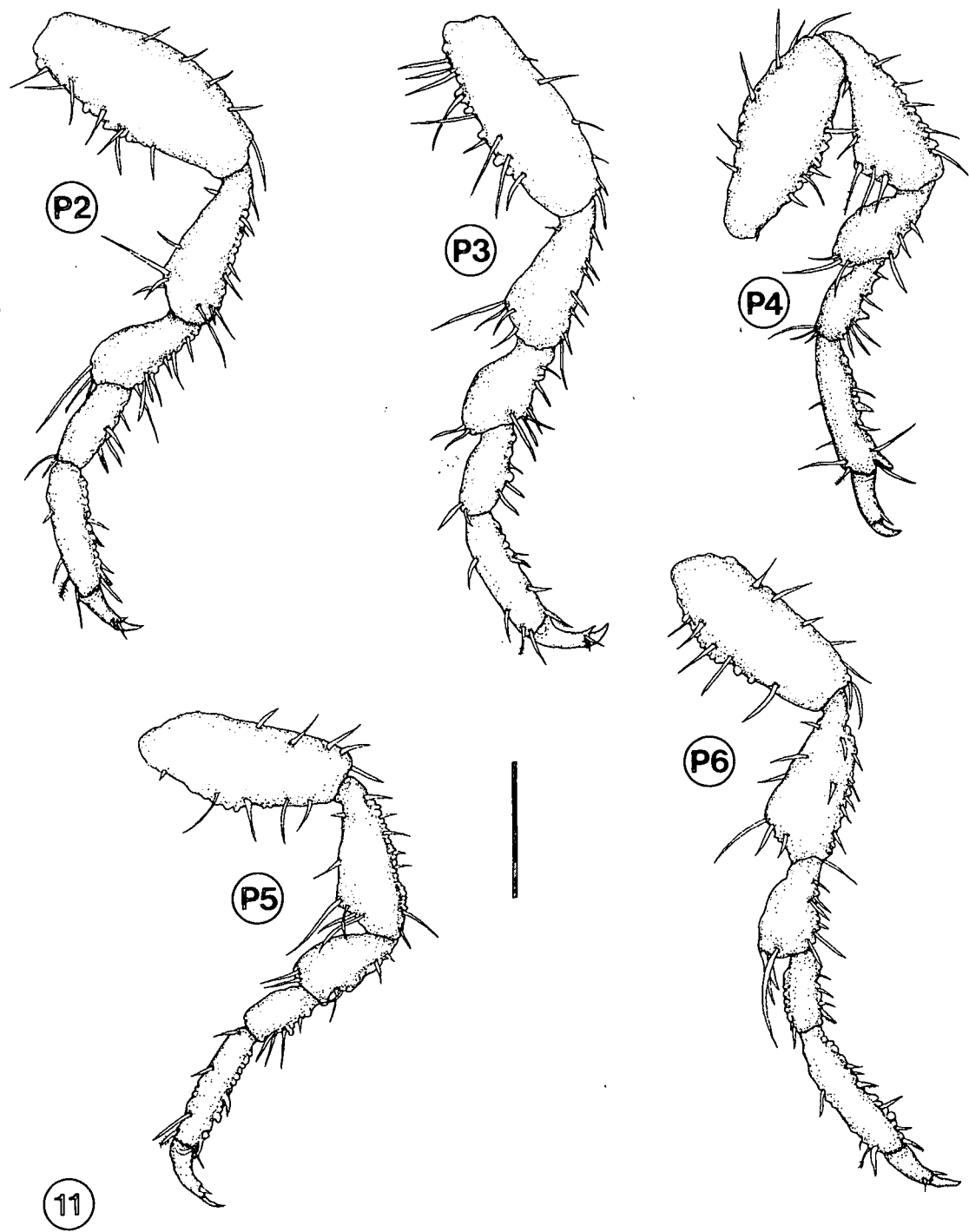
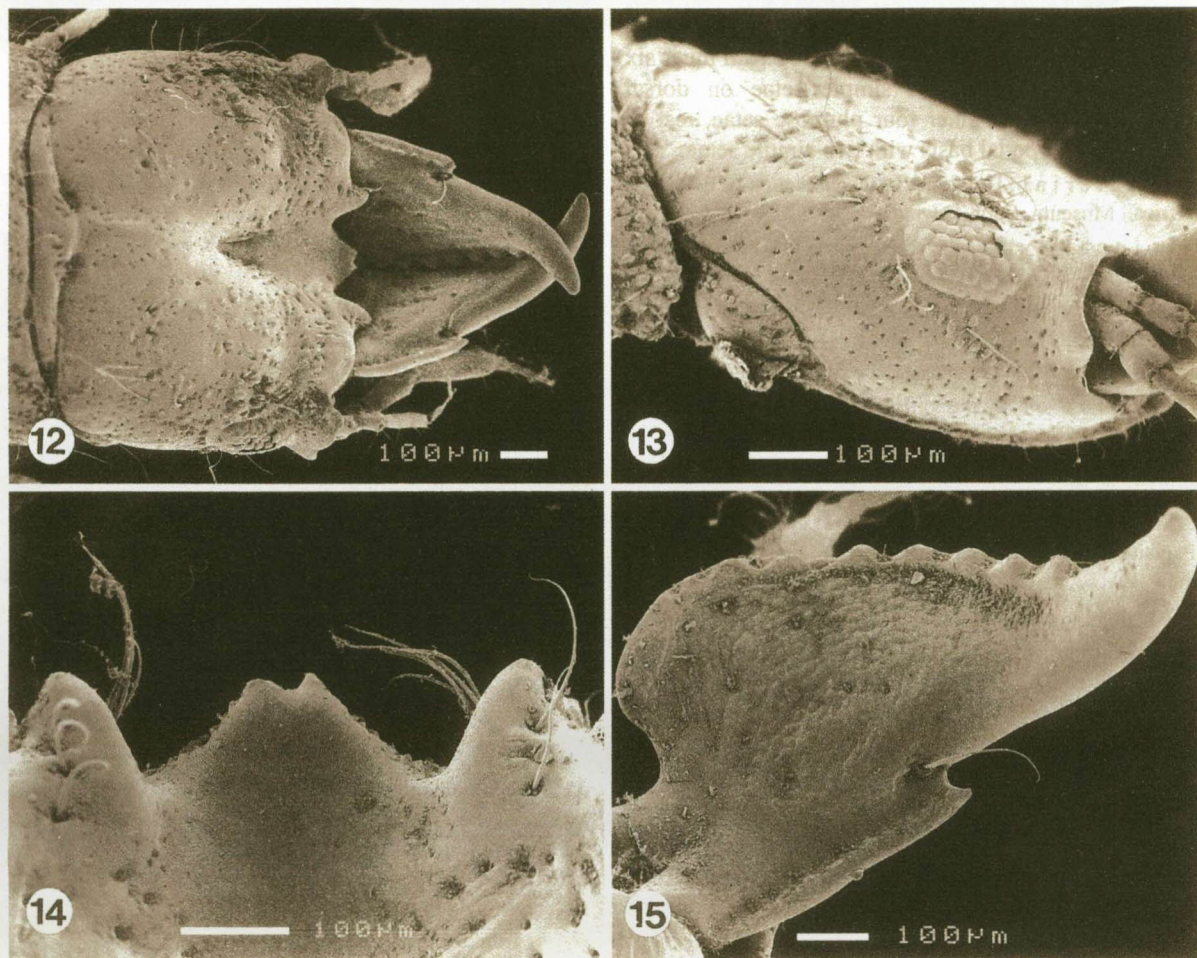


Fig. 11. Microscope projection drawings of pereopods 2 to 6 (P2-P6) of a male *Gnathia nkulu* sp. n. (SAM-A43160). Scale bar: 200 μ m.

margins pointing anteriorly. Pereonite 3 with anterior constriction, but not separating lateral sides of pereonites 2 and 3. Prominent anterior constriction of pereonite 4 separating it from pereonite 3. Pereonite 4 with shallow median groove. Pereonite 5 and 6 not fused, but separation not distinct. Pereonite 6 at least

twice as long as other pereonites, 1.3 times as long as wide, posterior margin deeply concave, without lobes. Pereonite 7 dorsally visible, small with rounded posterior margin, overlapping first pleonite. Sensory pits, simple setae and small wart-like tubercles randomly distributed on pereon.



Figs. 12-15. Scanning electron micrographs of a male *Gnathia nkulu* sp. n. Fig. 12. Dorsal view of cephalosome. Fig. 13. Lateral view of cephalosome. Fig. 14. Dorsal view of frontal border with simple setae. Fig. 15. Dorsal view of right mandible.

Pleon. Pleon and pleotelson slightly less than a fifth of total length (Fig. 1). All five pleonites dorsally visible, epimera not distinct.

Pleotelson. Triangular, longer than wide, lateral margins tapering in two steps towards apex, three pairs of simple setae on dorsal surface, distal apex terminating in pair of simple setae (Fig. 7).

Pereopods. Pereopod 2 basis elongated with eight to twelve simple setae as well as a few tubercles on anterior and four to seven simple setae on posterior side. Ischium as long as basis but not as wide, three to five anterior setae and posterior tubercles with simple setae in between (Fig. 11). Merus half the length of ischium with anterior bulbous protrusion, simple setae on bulbous protrusion, posterior margin with tubercles as well as simple setae. Carpus of almost same size and shape as merus, but without anterior bulbous protrusion. Propodus about twice the length of carpus, prominent tubercles fringe posterior side, two elongated spines

ending in sharp points situated on middle and distal part of posterior side respectively, only a few simple setae anteriorly with single plumose seta distally. Dactylus half the length of propodus, terminates in sharp posterior pointing unguis, prominent spine on posterior side proximal to unguis, few simple setae on dorsal and ventral sides of spine. Pereopods 3 to 6 similar in basic form to pereopod 2, but differ in number and placement of setae (see Fig. 11) as well as in direction, pereopods 4 to 6 directed posteriorly and pereopods 2 and 3 anteriorly.

Pleopods. Exopod and endopod of almost similar size. Both fringed distally with seven to nine long plumose setae, few shorter simple setae on lateral margins. Plumose setae almost as long as pleopod. Pleopod 2 endopod with appendix masculina almost half the length of rami (Fig. 6).

Penes. Prominent with two contiguous papillae, as long as wide.

Uropods. Rami extending beyond apex of pleotelson, endopod longer and wider than exopod, both with long fringing setae (Fig. 7). Endopod with inner six plumose setae, four to six simple setae on dorsal surface. Exopod with inner four plumose setae, rest of setae simple. Uropodal basis with two simple setae.

Type material: Holotype: In the collection of the South African Museum, Cape Town (SAM-A19314).

Type locality: Off the south coast, east of Port Alfred (33°39.3'S, 27°11.6'E)

Other localities: Off Port Alfred (33°39.3'S, 27°11.6'E) and (35°22'S, 22°31'E).

Type host of parasitic larvae: Unknown.

Deposition of types: Holotype: In the collection of the South African Museum, Cape Town (1 male, SAM-A19314). Paratypes: In the collection of the South African Museum, Cape Town (19 males and 7 larvae, SAM-A43160) and in the collection of the Institute of Parasitology, ASCR, České Budějovice, Czech Republic (1 male, PAŮ AVČR 1981).

Other material: In the collection of the South African Museum, Cape Town (1 male, SAM-A19313), (1 male, SAM-A14859).

Etymology: The Xhosa word "nkulu" was chosen as this means large in the language of this southern African tribe.

DISCUSSION

Of all the southern African species, *Gnathia nkulu* is very similar to *Gnathia africana* in size and overall body proportions, particularly the mandibles, pereon

and pleotelson. *Gnathia nkulu* differs in that the medio-frontal process is not deeply divided into two lobes, article 2 of the pylopod is rounded and small wart-like tubercles and long simple setae are present on both the cephalosome and pereon.

Gnathia nkulu and *G. africana* populations do overlap in their distribution, but there is a big difference in the depth at which they occur. *Gnathia africana* are found in the intertidal zone (Barnard 1914a, Smit et al. 1999) and *G. nkulu* in water of 80-200m deep.

Gnathia nkulu shows some resemblance to the Australian species *Gnathia biorbis* Holdich et Harrison, 1980, especially in the shape of the frontal border. Both species have conical superior frontolateral processes and an inferior mediofrontal process with a slightly indented apex (Holdich and Harrison 1980). Our species differs from the Australian species in the shape of the pleotelson, the presence of a semicircular row of tubercles on the lateral sides of the cephalosome, the presence of long plumose setae on the rami of the pleopods and in the endopod of pleopod 2 having an appendix masculina.

Acknowledgements. The authors thank Ms. Michelle van der Merwe of the South African Museum, Cape Town, for making the gnathiid material available for examination. This study was funded by the marine resource program of the National Research Foundation (NRF) of South Africa.

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Appendix I D

Appendix II

SHORT COMMUNICATIONS

New host records for *Haemogregarina bigemina* from
the coast of southern Africa

N.J. Smit and A.J. Davies*†

Department of Zoology and Entomology, Faculty of Natural Sciences, University of the Orange Free State, Bloemfontein,
South Africa. *School of Life Sciences, Faculty of Science, Kingston University, Kingston, Surrey, KT1 2EE.

†To whom correspondence should be sent.

One hundred and fifty intertidal fishes were captured at Jeffreys Bay in July 1996 and February 1997, and at De Hoop Nature Reserve in April 1997, South Africa. *Caffrogobius caffer*, *Chorisochismus dentex*, *Clinus superciliosus*, and *Clinus cottoides* were all parasitised by haematophagous larvae of the isopod, *Gnathia africana*, and between 22 and 81% of fishes were infested with 1 to 76 larvae each. Of those fishes examined for blood parasites, 35/46 (76.1%) had an intraerythrocytic haemogregarine identified as *Haemogregarina (sensu lato) bigemina*, but only *Clinus superciliosus* and *Clinus cottoides* were infected. Gamonts of the haemogregarine, some undergoing syzygy, were also present in 50% of stained batches of the anterior hindgut contents of *G. africana* larvae which had fed on *Clinus superciliosus*, but were absent from larvae taken from *Clinus cottoides* and *Chorisochismus dentex*. This study provides new host records for *H. bigemina* and further evidence that gnathiid isopods play a role in the transmission of the haemogregarine.

Haemogregarines (Apicomplexa: Adeleina) were reported first from the blood of marine fishes by Laveran & Mesnil (1901) in northern France. They have been shown since to be widely distributed in both marine and freshwater fishes. The taxonomy of fish haemogregarines was revised recently to include the genera *Cyrtia*, *Desseria* and *Haemogregarina (sensu lato)*, while the genus *Haemogregarina (sensu stricto)* was reserved for haemogregarines infecting chelonians (Siddall, 1995). Although biological transmission has not been achieved, leeches are the likely invertebrate hosts of some fish haemogregarines whereas ectoparasitic copepods and isopods may transmit others (see Davies, 1995; Siddall, 1995).

Current knowledge of the haemogregarines of marine fishes from South Africa comes from a series of papers written by Fantham between 1918 and 1932. However, *Desseria (Haemogregarina) fragilis* (Fantham, 1930) Siddall, 1995 appears to be the only named fish haemogregarine from the region, which Fantham (1930) recorded from the heart blood of the blenny, *Parablennius (Bleinnius) cortuus* (L.), captured at St James.

As part of a study of intertidal parasites and symbionts of South Africa, the diversity, prevalence, and transmission of some blood parasites of fishes is being investigated. This short communication reports on *Haemogregarina (sensu lato) bigemina* Laveran & Mesnil, 1901 in two new host fishes and in the haematophagous praniza larvae of the isopod, *Gnathia africana* Barnard, 1914.

Intertidal pool fish (N=150) were collected by hand nets, cast nets or hand lines at Jeffreys Bay in July 1996 and February 1997, and at De Hoop Nature Reserve in April 1997. Captured fish were maintained in tanks of aerated seawater until examination, when they were anaesthetized with benzocaine. Fish were identified using Branch et al. (1994) and Smith & Heemstra (1995). They were examined microscopically for ectoparasites, which were identified, removed gently, or allowed to detach by themselves. Heart blood smears, fixed in absolute methanol and stained with phosphate-buffered Giemsa (pH 6.8), were examined from about one third (N=46) of the

fish captured, to identify common blood parasites and to estimate prevalence of infection.

Replete, ectoparasitic praniza larvae were examined within an hour of their detachment from fishes. They were placed on absorbent paper to drain off surface seawater and decapitated. Blood-filled, anterior hindgut contents from batches of two to four larvae were squashed and smeared between glass slides. Sixteen such batches [14 batches from *Clinus superciliosus* (Linnaeus, 1758), one from *Clinus cottoides* Valenciennes, 1836, and one from *Chorisochismus dentex* (Pallas, 1769)] were fixed in absolute methanol and stained with Giemsa's stain as for blood smears, and examined for parasites. Photomicrographs of parasites in fish blood smears and in smears from pranizae were taken on a Zeiss Axioskop photomicroscope. These parasites were measured with an eyepiece graticule and stage micrometer.

The 150 tidal pool fishes captured belonged to four species (Table 1). One species, *Caffrogobius caffer* (Günther, 1874), was found only at Jeffreys Bay. All four species of fishes were parasitised by pranizae of *G. africana* (Table 1), infested fish bore between 1 and 76 (mean=8.2±14.4) pranizae each, and these were attached to the fins and body surfaces. Two other species of ectoparasites which were recorded (not shown in Table 1), were a single specimen of the crustacean parasite, *Caligus* sp., found on each of three specimens of *Clinus superciliosus* at Jeffreys Bay in 1996, and an unidentified leech discovered on *C. cottoides* at the same site in 1997.

Of the fishes examined for blood parasites, *Caffrogobius caffer* and *Chorisochismus dentex* had no detectable infections but *Clinus superciliosus* and *C. cottoides* were infected with a single species of intraerythrocytic haemogregarine and prevalence of infection was high in the two clinids (Table 1). Intensity of infection was low for all *C. cottoides* and for most *C. superciliosus* (<0.1% of erythrocytes infected), except in two specimens (75 and 84 mm total length) collected at Jeffreys Bay in July 1996 and two others (70 and 117 mm long) captured in February 1997 at the same site, which showed a high intensity of infection (1–2.5%

Table 1. Identity and number of fishes collected during the three excursions; prevalence of gnathiid larvae and haemogregarines.

Fish species	Jeffreys Bay July 1996		Jeffreys Bay February 1997		De Hoop April 1997		Total (prevalence %) All Sites	
	G	H	G	H	G	H	G	H
<i>Clinus superciliosus</i>	15/21	7/7	18/27	18/23	50/54	6/7	83/102 (81.4)	31/37 (83.8)
<i>Clinus coltooides</i>	6/11	2/3	1/2	2/2	7/15		14/28 (50)	4/5 (80)
<i>Caffrogobius caffer</i>	2/9	0/3					2/9 (22.2)	0/3 (0)
<i>Chorisochismus dentex</i>	1/1	0/1			2/3		3/4 (75)	0/1 (0)
Total	24/42	9/14	19/29	20/25	59/72	6/7	102/143 (71.3)	35/46 (76.1)

G, fishes with gnathiid larvae/ fishes examined; H, fishes with haemogregarines/fishes examined. All fishes with haemogregarines (H) bore gnathiids. Prevalence in parentheses.

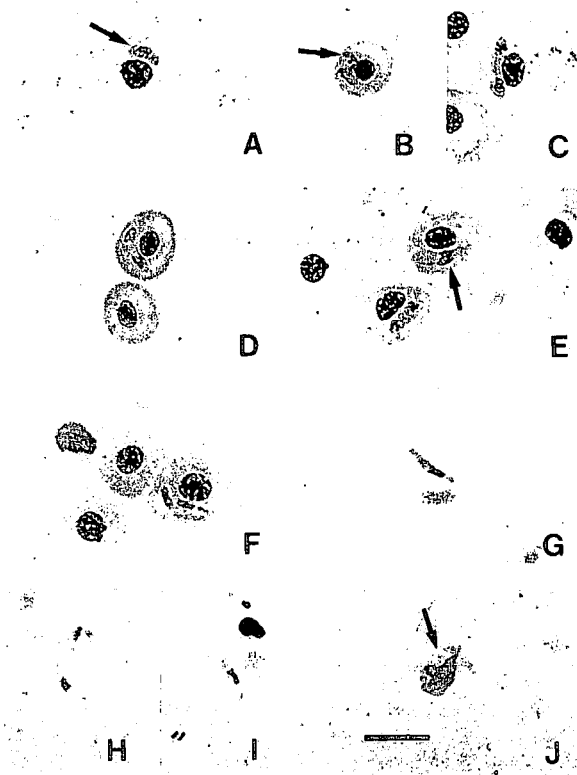


Figure 1. (A–G) are Giemsa-stained blood films from *Clinus superciliosus* showing intraerythrocytic (A–F) and extracellular (G) stages of *Haemogregarina bigemina*. (A) trophozoite (arrow); (B) meront (arrow); (C–E) pregamontic binary fission leading to the formation of paired immature gamonts (arrow, in E); (F) paired mature gamonts; (G) single mature gamont. (H–J) are Giemsa-stained smears from the anterior hindgut of *Gnathia africana* pranizae. (H & I) single mature gamonts; (J) syzygy. Scale bar: 10 μ m.

of erythrocytes infected). No intraleucocytic stages of the haemogregarine were detected in any of the blood smears examined.

The haemogregarine present in blood smears from *C. superciliosus* and *C. coltooides* was indistinguishable morphologically from *Haemogregarina bigemina* (see Laird, 1953), showing the intraerythrocytic trophozoites, meronts, pregamontic binary fission and paired gamonts characteristic of the species (Figure 1A–F). Average length and breadth measurements for gamonts

from *C. superciliosus* were 10.8 ± 1.3 (9.0–13.2) μ m by 1.8 ± 0.3 (1.2–2.2) μ m (N=30) and from *C. coltooides*, 11.8 ± 0.7 (9.8–12.3) μ m by 2.1 ± 0.4 (1.6–2.5) μ m (N=12). Gamont nuclei measured 3.2 ± 0.5 (2.5–4.1) μ m by 1.4 ± 0.2 (1.1–1.8) μ m (N=30) from *C. superciliosus* and 3.3 ± 0.1 (3.1–3.5) μ m by 1.3 ± 0.1 (1.2–1.6) μ m (N=12) for *C. coltooides*. Similar gamonts measuring 10.3 ± 1.4 (8.4–13.0) μ m by 1.6 ± 0.4 (1.2–2.5) μ m (N=20), with nuclei measuring 2.7 ± 0.6 (2.0–4.0) μ m by 1.2 ± 0.3 (1.0–2.2) μ m (N=20) were also present within 7/14 (50%) of stained batches of *G. africana* which had fed on *C. superciliosus* with *H. bigemina* (compare Figure 1G with Figure 1H & I). Some gamonts were undergoing syzygy (Figure 1J). Gamonts were not detected in the two stained batches of pranizae which had fed on *C. coltooides* and *Chorisochismus dentex*.

The average length and breadth measurements of the gamonts found in fishes and gnathiids in South Africa fall well within the size range for *H. bigemina* (9.0–14.9 by 1.0–2.4 μ m, nuclei 2.1–4.4 by 1.0–1.8 μ m) from an extensive study of New Zealand fishes (Laird, 1953), although Smith (1996) warns that for adeleorines, gamont measurements may be unreliable taxonomic discriminators. However, the high prevalence of *H. bigemina* in clinids, its morphology and development in erythrocytes, and its apparent lack of intraleucocytic stages, also agrees with the descriptions of *H. bigemina* from Welsh and Portuguese fishes (see Davies, 1995). Levine (1988) listed 85 species of marine fishes in 59 genera as hosts for *H. bigemina*, including both intertidal and offshore hosts. *Clinus superciliosus* and *C. coltooides* do not appear on this list and therefore they appear to be new host records for this haemogregarine.

Haemogregarines recognizable as *D. fragilis* were not detected in any fishes in this study, although its type host, *P. cornutus*, was not examined. Laird (1953) noted similarities between *D. fragilis* and *H. bigemina*, especially in the size of extra- and intracellular gamonts. Fantham (1930) did not record the number of gamonts within each parasitized erythrocyte, although he deduced that merogony had occurred because of the number of extracellular haemogregarines in the heart blood of the blenny. Given this brief description of *D. fragilis*, it is difficult to draw conclusions about its affinity with *H. bigemina*, except that their gamonts are of similar size. Furthermore, *D. fragilis* was recently transferred from *Haemogregarina* to the genus *Desseria*, presumably because of, 'erythrocytic merogony absent; gamonts in erythrocytes' (Siddall, 1995). If the two haemogregarines are not the same parasite, then South Africa is a new locality for *H. bigemina*.

Laveran & Mesnil (1902) were the first to note gnathiid pranizae on fishes, *Lipophrys pholis* (L.), which were also hosts for

H. bigemina in France: Much later (see Davies, 1995), likely development of the haemogregarine to sporozoite stages was recorded in isopod pranizae of *Gnathia maxillaris* (Montagu, 1804) taken from Welsh fishes (*L. pholis*) with *H. bigemina*, and to oocysts, in pranizae taken from infected Portuguese fishes, *L. pholis* and *Coryphoblennius galerita* (L.). In the present study, the majority of ectoparasites on fishes were the larvae of *G. africana* and fishes were hosts for up to 76 larvae each. A high percentage of batches (50%) of smears of gnathiids from infected *Glinus superciliosus* contained haemogregarine gamonts with dimensions similar to those of *H. bigemina*, and syzygy was observed. The absence of development of the haemogregarine beyond syzygy in *G. africana* pranizae was not surprising because all smears were made a relatively short time after their detachment from their fish hosts. The similarities between the observations in Wales, Portugal and the present study are remarkable. These findings are interpreted as additional evidence that gnathiids play some role in the transmission of *H. bigemina* between fishes and we are pursuing this possibility further in South Africa.

One of us (N.J.S.) was funded in this study by the Canon Collins Educational Trust for Southern Africa and the South African Foundation for Research Development. Assistance on fishing expeditions was kindly provided by Professors J. van As and Linda Basson, and by other members of the Aquatic Parasitology Research Group, University of the Orange Free State.

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Submitted 16 July 1997. Accepted 16 March 1999.

Appendix III

NAVRAE
ENQUIRIES Mev M S Liebenberg

TELEFOON
TELEPHONE (021) 483 3584

FAKS
FAX (021) 483 4158

VERWYSING
REFERENCE ANO 2/17

DATUM
DATE 10 Februarie 1997

PROVINCIAL ADMINISTRATION: WESTERN CAPE

Cape Nature Conservation

PROVINSIALE ADMINISTRASIE: WES-KAAP

Kaapse Natuurbewaring

Departement Dierkunde/Entomologie
Fakulteit Natuurwetenskappe
Universiteit van die Oranje-Vrystaat
Posbus 339
BLOEMFONTEIN
9300

Geagte Mnr N Smit

**PERMIT NO. 2/1997: PERMIT VIR VERSAMELING VAN GETYPOEL VISSIE EN
GESELEKTEERDE INTERGETY INVERTEBRATA VIR
PARASIE STUDIES**

1. Ingevolge artikel 73 van die Ordonnansie op Natuur- en Omgewingsbewaring, 1974 (Ordonnansie 19 van 1974) word magtiging aan u verleen vir die versameling van getypoolvisse en geselekteerde intergety invertebrata vir navorsingsdoeleindes aan die De Hoop Natuurreservaat kus.
2. Die voorwaardes van hierdie permit is as volg:
 - 2.1 Nie meer as 20 individue per spesie mag versamel word nie.
 - 2.2 Alle ander organismes wat nie vir die projek benodig word nie, moet onmiddelik en onbeseerd in die water teruggeplaas word.
 - 2.3 'n Volledige versalg t.o.v. die navorsing en versameling van organismes moet binne 'n redelike tyd na afloop van die ekskursie aan hierdie kantoor voorsien word.
 - 2.4 Hierdie permit is geldig vanaf 28 Maart 1997 tot 30 April 1997.

Die uwe



h DIREKTEUR: NATUURBEWARING



ENQUIRIES
NAVRAE Grietha Liebenberg

TELEPHONE
TELEFOON (021) 483 3584

FAX
FAKS (021) 483 4158

REFERENCE
VERWYSING ANO 2/17

DATE
DATUM 16 Maart 1998

PROVINCIAL ADMINISTRATION: WESTERN CAPE
Cape Nature Conservation

PROVINSIALE REGERING: WES-KAAP
Kaapse Natuurbewaring

Prof JG van As & Medewerkers
Universiteit van die Oranje-Vrystaat
Departement Dierkunde/Entomologie
Fakulteit Natuurwetenskappe
Posbus 339
BLOEMFONTEIN
9300



Geagte Prof van As & Medewerkers

PERMIT NO. 2/1998: PERMIT VIR VERSAMELING VAN GETYPOEL VISSE EN GESELEKTEERDE INTERGETY INVERTEBRATA VIR PARASIEET STUDIES

1. Ingevolge artikel 73 van die Ordonnansie op Natuur- en Omgewingsbewing, 1974 (Ordonnansie 19 van 1974) word magtiging aan u en u medewerkers verleen vir die versameling van getypool visse en geselekteerde intergety invertebrata vir navorsingsdoeleindes aan die De Hoop Natuurresewaat kus.
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 - 2.3 'n Volledige verslag t.o.v. die navorsing en versameling van organismes moet binne 'n redelike tyd na afloop van die ekskursie aan hierdie kantoor voorsien word.
 - 2.4 Hierdie permit is geldig vanaf 27 Maart 1998 tot 30 April 1998.

M. Liebenberg
waarnemende **DIREKTEUR**

KAAPSE NATUURBEWARING

ADDENDUM TOT PERMIT NO. 2/1998

PROJEKLEIERS :

Prof. J. G. Van As D.Sc. *PU CHO*
Prof. L. Basson *PhD. RAU*

MEDEWERKERS:

Dr. L.L. van As *PhD. UOVS*
N.J. Smit *Msc. UOVS*
K.W. Christison *BSc.Hons. UOVS*
H. Botes Bsc. *Hons. OUVS*
N.J. Grobler Bsc. *Hons. OUVS*

M. de Beer

waarnemende DIREKTEUR



ENQUIRIES
NAVRAE Grietha Liebenberg

TELEPHONE
TELEFOON (021) 483 3584

FAX
FAKS (021) 483 4158

REFERENCE
VERWYSING ANO 2/17

DATE
DATUM 19 Maart 1999

PROVINCIAL ADMINISTRATION: WESTERN CAPE
Cape Nature Conservation

PROVINSIALE REGERING: WES-KAAP
Kaapse Natuurbewaring

Prof. JG van As en Medewerkers
Universiteit van die Oranje-Vrystaat
Department Dierkunde/Entomologie
Fakulteit Natuurwetenskappe
Posbus 339
BLOEMFONTEIN
9300

Geagte Prof. van As & Medewerkers

PERMIT NO. 1/99: PERMIT VIR VERSAMELING VAN GETYPOEL VISSE EN GESELEKTEERDE INTERGETY INVERTEBRATA VIR PARASIT STUDIES

1. Ingevolge artikel 73 van die Ordonnansie op Natuur- en Omgewingsbewaring, 1974 (Ordonnansie 19 van 1974) word magtiging aan u en u medewerkers verleen vir die versameling van getypool visse en geselekteerde intergety invertebrata vir navorsingsdoeleindes aan die De Hoop Natuurreservaat kus.
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 - 2.3 'n Volledige verslag t.o.v. die navorsing en versameling van organismes moet binne 'n redelike tyd na afloop van die ekskursie aan hierdie kantoor voorsien word.
 - 2.4 Hierdie permit is geldig vanaf 19 Maart 1999 tot 30 April 1999.

M. Liebenberg
DIREKTEUR

KAAPSE NATUURBEWARING

ADDENDUM TOT PERMIT NR. 1/1999

PROJEKLEIERS:

Prof. JG van As D.Sc. PŪ CHO
Prof L Basson PhD RAU

BESOEKENDE WETENSKAPLIKE:

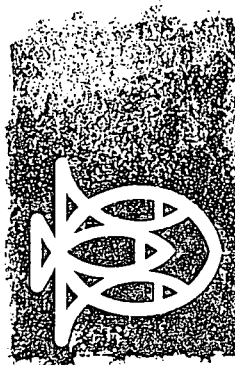
Prof. I Dykova (Czech Republic)

NAVORSERS/MEDEWERKERS:

Dr. LL van As PhD UOVS
NJ Smit Msc UOVS
KW Christison Msc UOVS
H Botes Bsc Hons UOVS
NJ Grobler Bsc Hons UOVS
CC Reed Bsc Hons UOVS
C Jansen van Resnburg Bsc Hons UOVS
G Visagie - Tegnikus

M. de Vries
DIREKTEUR





Reference: V1/1/5/1
Telephone: 021-4023136
Enquiries: K VAN ZYL

SEA FISHERIES
SEEWISSERYE
EEOKUDOBA
OEVANDLE
EEOKULOBA
EUVANDLE

Boer van Eeden
Marine Living Resources Act
Telephone: 021-4023136
Private Bag 112
Pretoria 0001
Tel: 012 345 6789
Fax: 012 987 6543

EXEMPTION FOR PURPOSES OF SCIENTIFIC RESEARCH

1. In terms of Section 81 (1) of the Marine Living Resources Act, 1998 (Act No 18 of 1998), exemption is hereby given to **Dr L.L van As of the Department of Zoology and Entomology, University of the Free State**, from those relevant provisions and restrictions of the Act in order to collect, possess, transport and dispose of any marine fish regardless of the size or condition for research purposes.
2. This exemption is subject to the following conditions:
 - (a) Collections in terms of this exemption may only be made for the purposes of *bona fide* research projects of the Department of Zoology and Entomology at the University of the Free State, as authorized by the Head of the Department.
 - (b) A certified copy of this exemption shall be carried by staff during collections and must be shown to a Fishery Control Officer or any other authorized person on demand. Staff and students undertaking collections shall identify themselves, if requested to do so, as staff members and students of the Department of Zoology and Entomology at the University of the Free State by means of an identification document issued by the Head of the Department.
 - (c) Collections may only be made at the De Hoop and De Mond Nature Reserves.
 - (d) A maximum of 20 specimens per species may be collected, and returned to its original location wherever possible.
 - (e) The holder of this exemption shall inform the relevant regional authority responsible for law enforcement under the Marine Living Resources Act of the sampling and survey date (s) and place (s) prior to each collection. (Mr G van Eeden, tel: 021-4307000)
 - (f) Research report (s) that include descriptions of all collections that took place must be



submitted to the Director of Research, Private Bag X2, Roggebaai, 8012 and to Cape Nature Conservation Private Bag x9086, Cape Town, 8001 Attention: Mr D Hignett within six months after the expiry date of this exemption.

(g) Fish caught in terms of this exemption shall not be sold or offered for sale.

3. This exemption is valid from date of issue to 31 December 1999.



MINISTER OF ENVIRONMENTAL AFFAIRS AND TOURISM

DATE: 1999 -10- 08



Reference: VI/1/5/1
Telephone: 021-4023036
Enquiries: Mrs B Taylor

Chief Directorate:
Marine and Coastal Management
Private Bag X2
8012 ROGGEBAAI

EXEMPTION FOR PURPOSES OF SCIENTIFIC RESEARCH

In terms of Section 81 (1) of the Marine Living Resources Act, 1998 (Act No. 18 of 1998), exemption is hereby given to DR L L VAN AS OF THE DEPARTMENT OF ZOOLOGY AND ENTOMOLOGY, UNIVERSITY OF THE FREE STATE, from those relevant provisions and restrictions of the Act to collect, possess, transport and dispose of any marine fish regardless of the size or condition for research purposes.

This exemption is subject to the following conditions:

1. Collections in terms of this exemption shall only be made for the purposes of *bona fide* research projects of the Department of Zoology and Entomology at the university of the Free State, as authorized by the Head of the Department.
2. A certified copy of this exemption shall be carried by staff during collections and must be shown to a Fishery Control Officer or any other authorized person on demand. Staff and students undertaking collections, shall identify themselves, if requested to do so, as staff members and students of the Department of Zoology and Entomology at the University of the Free State by means of an identification document issued by the Head of the Department.
3. Collections may only be made at the DE HOOP AND DE MOND NATURE RESERVES.
4. A maximum of 20 specimens per species may be collected, and returned to its original location wherever possible.
5. The holder of this exemption shall inform the relevant regional authority responsible for law enforcement under the Marine Living Resources Act of the sampling and survey date(s) and place(s) prior to each collection (Mr G van Eeden at telephone 021-4307000).
6. Research report(s) that include descriptions of all collections that took place must be submitted to the Director of Research, Private Bag X2, Roggebaai 8012 and to Cape Nature Conservation, Private Bag X9086, Cape Town 8000 for attention Mr D Hignett, within 6 months after the expiry date of this exemption.
7. Fish caught in terms of this exemption shall not be sold or offered for sale.
8. This exemption is valid from date of issue to 31 DECEMBER 2000.


MINISTER

DATE: 2000-03-24