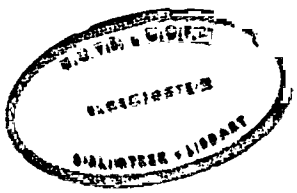

ASPECTS OF THE MORPHOLOGY AND LIFE HISTORY OF *Oculotrema hippopotami* (POLYSTOMATIDAE: MONOGENEA)

by

Itumeleng Amos Moeng

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Co-supervisor: Prof. D.J. Kok

With Love & Respect to my

Family.

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Chapter 1.

Introduction and historical overview

CHAPTER 1

INTRODUCTION AND LITERATURE OVERVIEW

Big fleas have little fleas upon their backs to bite 'em,

little fleas have lesser fleas and so, ad infinitum.

Swift

Parasitism is a common phenomenon among multicellular organisms. The large majority of animals are parasitic and most of those that are not parasites serve as hosts for other parasites. Kennedy (1983) put it that "Because parasitism is so widespread and parasites probably affect every living organism at some point in its lifetime, parasites must potentially influence the evolution of virtually every species of free living animal as well as the functioning of most populations and every ecosystem". Parasitism is not only a relationship between parasite and host, but also an association that must be quantified in terms of populations (Crofton, 1971). Because parasitism is such a diverse and complex field it allows scientists to study different aspects of this phenomenon and apply different techniques. Many studies focus on the effects of parasites on man while others may focus on its taxonomy or some other aspect. The study of parasites enables us to apply various scientific techniques which may include, apart from basic light microscopy, also scanning electron microscopy, transmission electron microscopy, fluorescence techniques, special staining techniques, molecular techniques and even mathematical modelling.

The term helminth is derived from the Greek word *helmins* or *helminthos*. Although it literally means 'worm', it has in zoological context a more precise connotation and is restricted mainly to members of the phyla Platyhelminthes, Nematoda and Acanthocephala (Smyth, 1994). Flatworms range in size from a millimetre or less to some of the tapeworms that are metres long. Their flattened bodies may be slender, broadly leaflike, or long and ribbonlike. As the name indicates flatworms are dorso-ventrally flattened, without definite anus, and a body cavity is lacking. Organs are embedded in specialised connective tissue known as parenchyma. Respiratory and circulatory systems are absent. The protonephridia contain flame cells (Smyth, 1994). The digestive system is incomplete and absent in some, while the reproductive system is complex, with fertilisation internal. The nervous system consists of a pair of anterior ganglia with longitudinal nerve cords connected by transverse nerves and located in the mesenchyme in most forms. The muscular system is primarily of a sheath form and of mesodermal origin, with layers of circular, longitudinal and sometimes oblique fibres beneath the epidermis (Hickman, Roberts & Hickman, 1984).

The Platyhelminthes comprises of four classes namely the non-parasitic, free-living Turbellaria and the three parasitic classes, namely, the Cestoda, the Monogenea and the Digenea. Monogenea was treated by early workers as an order of the class Trematoda within the Phylum Platyhelmithes. Bychowsky (1957) proposed that the Monogenea should be raised to the rank of class (*cf.* Schmidt & Roberts, 1985). Today most authors, though not all, refer to the Monogenea as a class in its own right (*cf.* Prudhoe & Bray, 1982; Schmidt & Roberts, 1985) and it is treated as such in this study.

The Monogenea is estimated to consist of about 20 000 species and is one of the largest group of Platyhelminthes (Rohde, 1996). The first monogenean was discovered in 1758 by Roesel von Rosenhof (Prudhoe & Bray, 1982). Zeder (1800) claimed that this parasite was in fact *Polystoma integerrimum* (Frölich, 1791).

Monogeneans are mainly parasites of marine and fresh water fishes, living on the skin, fins and gills, or exceptionally in the stomach or the intestine, in the cloaca. They however may be found in the uterus, body cavity, and the excretory system (Carus, 1863). Species found on or in the gills, buccal cavity, and urinary bladder feed mainly on blood, epithelium and mucus while skin forms feed on epidermal cells. Monogeneas are mostly host-specific and are generally restricted to a single host species, a single genus or a single family of host. The life cycle is direct without alteration of hosts (Smyth, 1994).

According to Bychowsky (1957) early studies were conducted by Zeller during 1872-1876, Cerfonteine during 1894-1900 and Seitaro Goto during 1891-1917. Between 1940 and 1954 Brinkman made a contribution on "Monogenoidea", specifically focusing on the morphology. Price (1939, 1943) and Sproston (1946) are among those who contributed to the taxonomy of Monogenea. A comprehensive work by Bychowsky in 1957 was published based on the phylogeny and taxonomy of the Monogenea. In 1963 Yamaguti published volume 4 of the series of "Systema Helminthum". Schmidt and Roberts (1985) refer to this work by Yamaguti (1963) as "an easy-to-use key to all genera of Monogenea."

In 1982 Prudhoe and Bray published the "Platyhelminth parasites of Amphibia". This excellent work deals with more than 30 families of flatworm parasites. In 1983 Smyth

and Halton published a book on the physiology of trematodes.

One monogenean family, the Polystomatidae, has radiated onto cold-blooded tetrapod vertebrates and are mainly found in anurans but also occurs on a diverse assemblage of hosts, including an Australian lungfish (*Neoceratodus forsteri)*, one urodele amphibian (*Onychodactylus japonicus*), terrapins and the hippopotamus (*Hippopotamus amphibius*). These hosts share one common feature: they all occur in water at certain times and this is the only opportunity when transmission of parasites can take place. In consequence, the life cycle of polystomatids shows a very close correlation with the ecology and behaviour of the host (Tinsley, 1990).

Polystomatidae occur, with the exception of Antarctica, on all continents and on several islands. The family is represented by 18 genera with the latest described as recently as 1993. Polystomatids are divided into 7 sub-families of which Polystomatinae Gamble, 1896 is by far the largest and best known. The sub-family Polystomatinae Gamble, 1896 includes nine genera. *Polystoma* Zeder, 1800, *Parapolystoma* Ozaki, 1935, *Eupolystoma* Kaw, 1950, *Protopolystoma* Bychowsky, 1957, *Riojatrema* Lamothe-Argumedo, 1964, *Metapolystoma* Combes, 1976, *Mesopolystoma* Vaucher, 1981 and *Wetapolystoma* Gray, 1993 are anuran parasites while *Parapseudopolystoma* Nasir and Fuentes, 1983 is found in an urodelid amphibian (*Onychodactylus japonicus)*. Polystomoidinae Yamaguti, 1968 is divided into three genera *Neopolystoma*, Price, 1939, *Polystomoides* Ward, 1917 and *Polystomoidella* Price, 1939, all of which infect terrapins. Diplorchinae Ozaki, 1931 which includes *Diplorchis* Ozaki, 1931 and *Pseudodiplorchis* Yamaguti, 1963, are both hosted by anurans. The Neodiplorchinae Yamaguti, 1963 and Pseudopolystomatinae Yamaguti, 1968 are monotypic sub-families including, respectively *Neodiplorchis*

Yamaguti, 1963 and *Pseudopolystoma* Yamaguti, 1968, both occurring in anurans. Oculotrematinae Yamaguti, 1968 is a monotypic sub-family and includes *Oculotrema* Stunkard, 1924, which is found on the hippopotamus (*Hippopotamus amphibius*). The Concinnocotylinae Pichelin, Whittington and Pearson, 1991, also a monotypic subfamily includes one genus *Concinnocotyla* Pichelin, Whittington and Pearson, 1991, found on an Australian lungfish (*Neoceratodus forsteri*).

Work on polystomatids in Africa was carried out mainly by French researchers, the involvement of whom must be seen against the background of the former French colonies, which were concentrated mainly in West and Central Africa. Many articles have been published on the taxonomy and biology of polystomatids in Africa. These reached a peak between the early seventies and the early eighties, the main period of the French involvement. Some of the leading French researchers that worked in Africa were Combes, Euzet, Bourgat, Salami-Cadoux, Vercammen-Grandjean and Murith. Among outstanding work done in Africa are a comprehensive study of the systematics of polystomes from the Ivory Coast (Murith, 1981a) and a study of the biology and development of polystomes (Murith, 1981b). During the seventies Tinsley focussed on Africa and did some outstanding work on polystomatids and also on other parasites of the clawed frogs (*Xenopus laevis.*).

Probably the most productive single contributor to the knowledge on Polystomatids in Africa is Tinsley, who published, with co-authors, on various aspects of the taxonomy and biology of *Eupolystoma*, *Polystoma*, and *Protopolystoma* (*cf.* Tinsley, 1973, 1974 a & b, 1978 a, b & c, 1980, 1983; Tinsley & Owen, 1975; Jackson & Tinsley, 1988).

The study of southern African polystomatidae began with the description of *Protopolystoma xenopodis*, originally described by Price (1943) as *Polystoma xenopi*. Combes and Channing (1979) described *Polystoma natalensis*, the first polystome described from South Africa. Since 1984 Kok and later Du Preez focussed on the polystomatids of southern Africa. New species that they described were *Polystoma australis* Kok & Van Wyk, 1986, *P. umthakati* Kok & Seaman, 1987, *P. sodwanensis* Du Preez & Kok, 1992, *Metapolystoma porosissimae* Du Preez & Kok, 1992, *P. marmorati* Van Niekerk, Kok & Seaman, 1993, *P. testimagna* Du Preez & Kok, 1993 and *P. claudecombesi* Du Preez & Kok, 1995. Du Preez (1986) conducted a study of *Polystoma australis* as part of a Masters degree. Van Niekerk (1992) focussed in her Masters on problems related to species identity and species diversity within the African Polystomatids. In his Doctoral dissertation Du Preez (1994) focused on host-specificity among Polystomatids.

Apart from *Polystoma, Protopolystoma* and *Metapolystoma*, polystomatids of anurans are also represented in South Africa by *Eupolystoma anterorchis* (Tinsley, 1978b).
Although there is a strong possibility that naturally occurring polystomatids of chelonians do occur in South Africa none has yet been found. *Pelomedusa subrafa*, a very common terrapin in South Africa, is known to harbour *Polystomoides choubudi* in Madagascar and in Uganda. *Polystomoides bourgati* is known from *Pelusins adamsoni* in Senegal and *Neopolystoma euzeti* is known from C*lemys caspica* in Tunisia.

Probably the most unusual parasite among all polystomatids is *Oculotrema hippopotami* Stunkard, 1924 from the eyes of the hippopotamus (*Hippopotamus amphibius*) in Africa. These worms are ectoparasites found in clusters around the nictitating membrane, under

the eyelids and on the anterior face of the eyeball of the hippopotamus (Thurston & Laws, 1965; Thurston, 1968a). Thus the name *Oculotrema*, where "Oculo" refers to eye and "trema" refers to hole or cavity.

In 1924 Stunkard described *Oculotrema* based on 5 specimens found in a museum collection and labelled "from the eye of hippopotamus". These specimens were apparently collected by a certain Professor A. Looss from a Nile hippopotamus in the Giza Zoological Gardens of Cairo, Egypt. If one takes the condition of the material into account Stunkard described the parasites very well. Measurements for *O. hippopotami* recorded in the present study differ to a large extent from that recorded by Stunkard. This was probably because of the limited material available as well as the poor condition of the material. For a period of four decades critics disagreed on the existence of such a parasite. Baer (1952) considered *O. hippopotami* as either an accidental infestation or mislabelled specimens. Yamaguti (1963) recognised this parasite and created a subfamily Oculotrematinae. It was only in 1965 that there was confirmation of the parasite's existence when Thurston recovered *O. hippopotami* from hippopotamus in Uganda (Thurston & Laws, 1965). In almost all other characteristics the specimens of *O. hippopotami* from Uganda correlate very closely to those of Stunkard's detailed description and measurements.

Two years later the parasite was recorded from the Kruger National Park in South Africa and this led to the second article on this parasite (McCully, Van Niekerk & Kruger, 1967). A year later two papers were published by Thurston, one describing the oncomiracidium (Thurston, 1968b) and the other the frequency distribution of *O. hippopotami* (see Thurston, 1968a). In 1970 Thurston conducted some tests, which

showed that the red colour of the parasite was due to the presence of haemoglobin. Because *O. hippopotami* feeds on mucus, Thurston suggested that haemoglobin must be of parasitic origin, since there is no blood found in the gut caeca. Except for a record of the presence of *O. hippopotami* in Zimbabwe (Jooste, 1990) and the record on the description of the haptoral suckers (Pichelin, 1995) no work on this parasite has been carried out for the past 26 years. A recent paper by Moeng & Du Preez (1997) dealt with the morphology of the oral sucker of *O. hippopotami* while the musculature of the parasite was studied by Moeng, Du Preez, Kruger & Cooper (1998). These two papers are included as Appendix A and Appendix B.

During 1996 Du Preez discovered this parasite in South Africa when hippopotami culled in Kwazulu-Natal were found to be infected with this parasite. Due to the high numbers of hippopotami in Ndumu Game Reserve, which is situated in the Northwest corner of Kwazulu-Natal, there was a need to control their population. Logistical problems ruled out the possibilities of translocating the hippopotami from this area and in 1997, a hippopotamus culling programme was undertaken. Despite the fact that the parasite was discovered almost eight decades ago, its description, based on limited material, justifies its resdiscription. Its life cycle is still a puzzle and many questions remain unanswered. The rediscovery of *O. hippopotami* posed a unique opportunity to continue studies on this parasite.

The present study was carried out against this background, and was directed at the following objectives:

- to study the relationship between *O. hippopotami* and the host, *Hippopotamus amphibius*.

- to study both the external and the internal morphology of *O. hippopotami*.
- to study the life history strategies of the parasite.

Following the **Introduction and historical overview,** the **Study area, material and methods** chapter briefly describes the study area, as well as material and methods regarding the collecting and laboratory preparation of material. A chapter on **The host *Hippopotamus amphibius*** is followed by the **Results** of the study. A **General discussion** serves to unite the study into an entity. This is followed by a **Summary** and the **Appendices**.

Chapter 2.

Study area, material and methods

CHAPTER 2

STUDY AREA, MATERIAL AND METHODS

2.1. Study area

Parasite specimens were obtained from hippopotami culled in the Ndumu Game Reserve. This reserve is situated in the North-west corner of Kwazulu-Natal ($26^0$53'S $32^0$16'E) (Fig. 2.1A.). Ndumu Game Reserve is situated at the junction of Usutu and Pongola floodplain system (Fig.2.1B). The Pongola River runs through the reserve from South to North while the Usutu River form the Northern border of the reserve and serves as the international boundary with Mozambique. There are two major shallow seasonal to permanent flood plain pans and many smaller ephemeral pans within the reserve. Ndumu Game Reserve covers an area of 10117 ha. Water masses may cover approximately 40% of the reserve in the wet summer months, while in the dry season this shrinks to approximately 15% of the reserve (Cowan & Marneweck, 1996). Due to extensive poaching in Mozambique hippopotami seek refuge in the Ndumu Reserve. This influx of hippopotami puts an additional demand on available vegetation. This in turn causes management problems. Due to logistical problems it was not possible to translocate hippopotami and surplus animals had to be culled. Between one and six animals were shot on a monthly basis during 1996 to 1997. In 1998 the culling programme was stoped after the hippopotami population was brought under control.

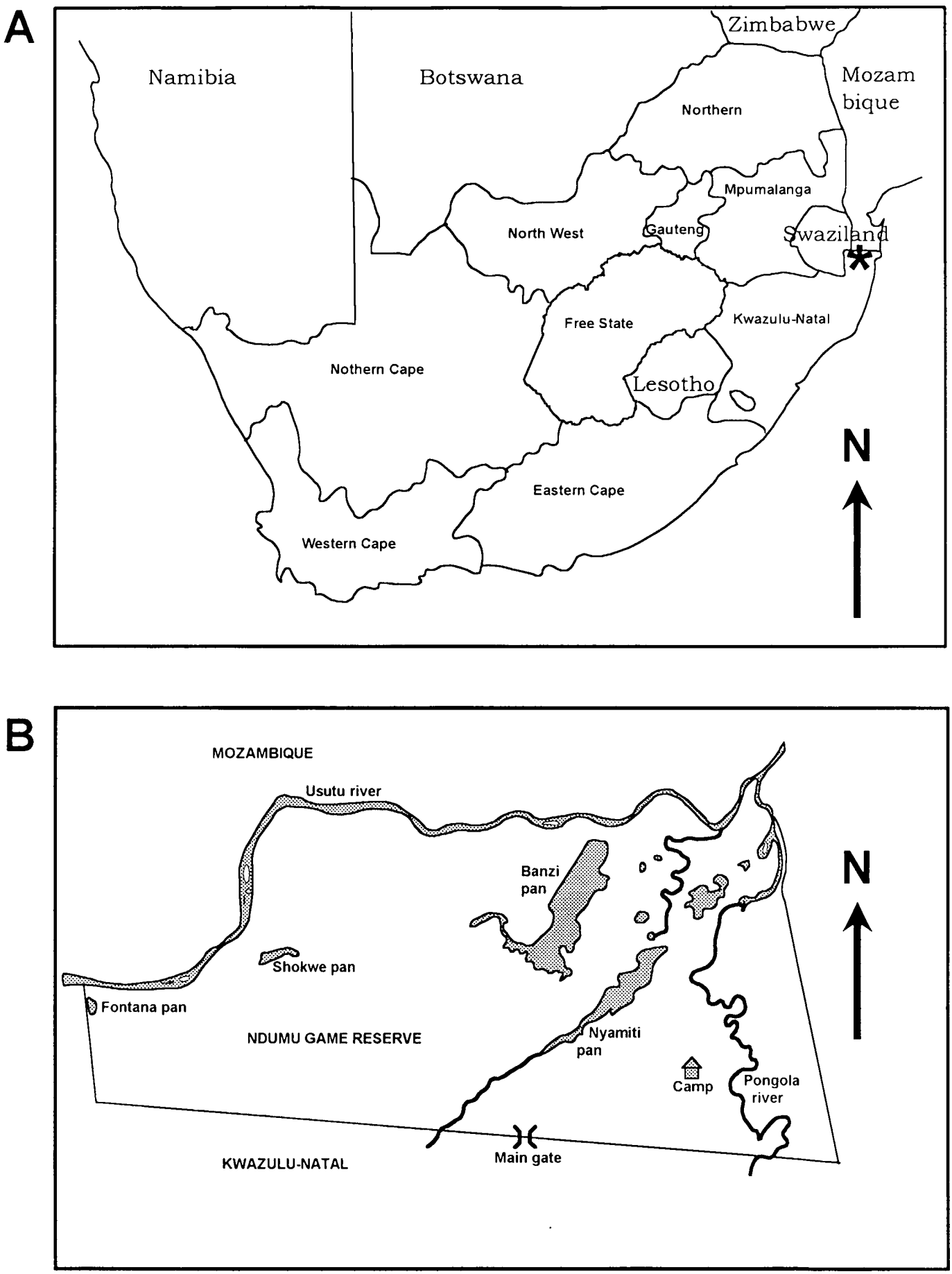
2.2. Collecting of material

Officers in the reserve agreed to collect the eyes of culled hippopotami with the eyelids intact and to keep them in a freezer until the eyes could be collected. In order to get hold

Figure 2.1

A) Map of South Africa to show the position of Ndumu Game Reserve on the Northern border of Kwazulu-Natal. The asterisk representing Ndumu Game Reserve.

B) Map of Ndumu Geme Reserve to show the location of the two border rivers and the larger pans.


A
Zimbabwe
Namibia
Botswana
Mozam
bique
Northern
Mpumalanga
North West
Gauteng
Swaziland
Free State
Kwazulu-Natal
Nothern Cape
Lesotho
Eastern Cape
Western Cape
N
B
MOZAMBIQUE
Usutu river
Banzi
pan
N
Shokwe pan
Fontana pan
NDUMU GAME RESERVE
Nyamiti
pan
Camp
Pongola
river
KWAZULU-NATAL
Main gate

of live parasites, two visits to Ndumo Game Reserve were undertaken, in June 1997 and in November 1997, respectively. Three hippopotami were shot on the 14th June and six on the 3rd November.

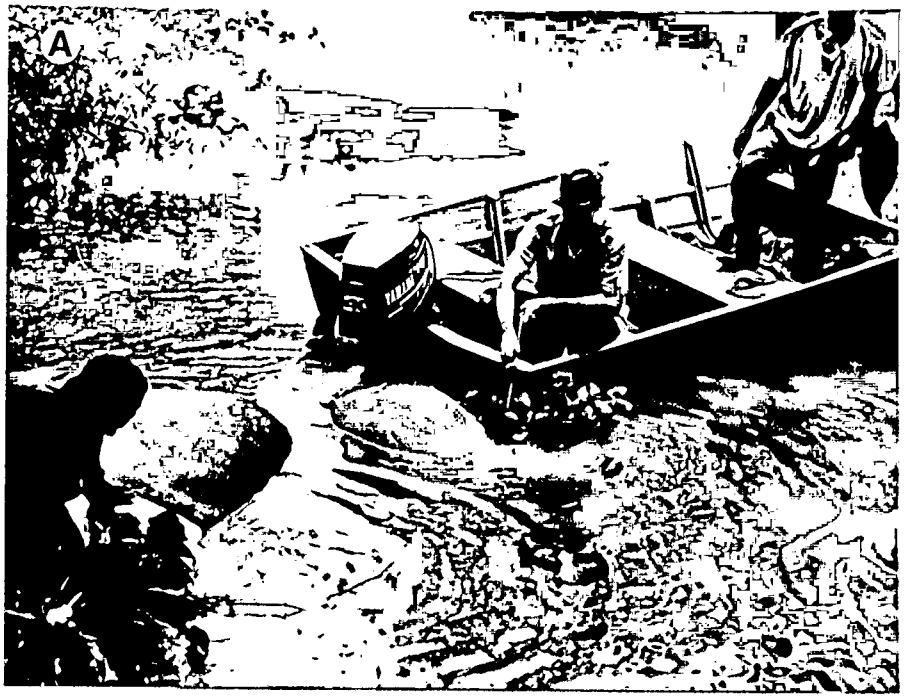
The hippopotami were shot shortly after sunrise. Carcasses sink to the bottom and surface again after about 30 minutes due to bloating. Carcasses were then tied with nylon ski rope and hauled to the loading area (Fig. 2.2A) using a motor boat. The carcasses were then loaded onto a trailer (Fig. 2.2B) using a winch, and transported to the abattoir. At the abattoir the carcasses were unloaded and the heads immediately removed (Fig. 2.3A). The eyes with the eyelids intact were removed and dissected, which is approximately one hour and thirty minutes after the shooting. The sites of attachment as well as the number of parasites were determined under a stereo microscope (Fig 2.3B). Live parasites were removed with a thin needle and care had to be taken not to damage the specimens because the parasites had a very firm grip on the host tissue. Eggs were flushed out of the eyes. The eggs and parasites were transferred to petri dishes containing dechlorinated tap water. Both the eggs and the parasites were fixed for different purposes.

In the case of the frozen eyes, of which some were collected over a period of the last six months of 1996, parasites and eggs were flushed out of the eyes after the eyes were defrosted. The water that was flushed through the eyes was poured through two plankton netting sieves with respective mesch size of 250 μm and 112 μm. The parasites remained on the 250 μm net and the parasite eggs on the 112 μm net. The eggs and parasites were transferred to petri dishes containing dechlorinated tap water to rinse them. Parasites were

Figure 2.2

A) *Hippopotamus amphibius* carcass at the loading area.

B) Loading of carcasses onto a trailer before transportation to the abattoir.


A


B

Figure 2.3

A) A head of a hippopotamus separated from the body shortly after culling.

B) Sites of attachment and number of parasites were determined with the aid of a dissecting microscope.


A
B

fixed for later use. Each parasite was allocated a unique reference number. Infection levels and size classes of parasites were determined for each eye. Most of the parasites and eggs from frozen eyes were permanenty mounted on slides.

2.3. Harvesting viable eggs

Live parasites were removed and placed in petri dishes with river water. No eggs were deposited. 49 Eggs were dissected out of live parasites of which four were placed in 0.6 % saline. The remaining 45 eggs were placed in petri dishes containing aerated river water at 30^0C. River water used was collected from the Pongola river. The river water was topped up with dechlorinated, aerated tap water. Nine oncomiracidia hatched from the petri dish with river water after 19-20 days. Three oncomiracidia were fixed in 2.5% glutaraldehyde, rinsed in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated and mounted for scanning electron microscopy. The remaining six were silver stained.

2.4. Fixation

Parasites were fixed for the preparation of whole mounts, histological sectioning, scanning electron microscopy, transmission electron microscopy, molecular analysis of DNA and lactophenol preparations. To prepare full mounts, parasites were fixed in 10% neutral buffered formalin (NBF) (Hayat, 1970) or in 70% ethanol (Humason, 1979). Small parasites were fixed under cover slip pressure but bigger parasites were fixed between two

microscope slides, which were clamped together using elastic bands. The clamping together of the microscope slides ensured that the parasites do not contract. Parasites were transferred individually from the petri dish to a drop of dechlorinated water on a microscope slide using a fine camel hair brush. Parasites were positioned and the cover slip or other microscope slide was carefully lowered to flatten it. Fixative was flushed through between the two glass slides by resting the temporary mount on its side on paper towelling and dropping fixative on the side of the preparation using a pasteur pipette. After thorough rinsing with fixative the temporary preparations were transferred to trays with fixatives and the parasites fixed over night. Parasites were then carefully removed and transferred to a small 50 µl glass duram vial with plastic stopper. The duram vial and data label were placed in a 25 ml glass vial with a tight sealing cap. Fixed material was stored until further use.

For histological sectioning parasites were fixed in 10% neutral buffered formalin or Bouin's fixative for 24 hours (Humason, 1979). After primary fixation those fixed in Bouin were transferred to 70% ethanol and stored.

Parasites and eggs earmarked for scanning electron microscopy were fixed for 24 hours in cold Flemming's solution (Van Niekerk, Els & Krecek, 1987) or cold buffered glutaraldehyde, dehydrated to 70% ethanol and then stored.

Specimens earmarked for transmission electron microscopy were fixed in 2.5% buffered glutaraldehyde for 24 hours at 4^0C (Hayat, 1970), washed overnight in 0.1 M sodium

cacodylate buffer, postfixed for 1 hour in 1% osmium tetroxide, rinsed in cacodylate buffer and dehydrated to 70% ethanol.

2.5. Cleared temporary preparations.

To fascilitate the study of larval sclerites a few small parasites were mounted in Lactophenol or in ammonium-picrate solution (Malmberg, 1956). Formalin fixed parasites were placed in a petri dish and the Formalin diluted to 5%. The dilution was left for 10 min. after which it was discarded. The parasites were then mounted in Lactophenol or ammonium picrate. A marking pen was used to mark the positions of parasites on the lactophenol preparations. This was necessary because the parasites became transparent and were then difficult to find under the microscope. Excess clearing fluid was removed by pressing the preparations between layers of tissue paper. Coverslips were sealed with clear nail vanish.

2.6 Silver staining

Six live oncomiracidia were stained in 0.5% silver nitrate and mounted in glycerine for chaetotaxy (Shinn, Sommerville & Gibson, 1993). Of the six, three were stained at 65^0C, and three were stained in an ice bath. Those on ice were exposed to sunlight for 10 minutes and those at 65^0C for 5 minutes stirring occasionally thus ensuring an even tan. Stained oncomiracidia were washed in several changes of distilled water to remove excess silver. All water was drawn off and a solution of absolute alcohol and glycerine in a ratio

of 9:1 was added and the alcohol was allowed to evaporate. The oncomiracidia were left in the solution to fix and after two hours mounted directly in glycerine.

2.7. Full mounts

Parasites fixed in 10% NBF were hydrated by transferring them to a dilution of water and 10% NBF in a petri dish. After 10 minutes the dilution was replaced with dechlorinated tap water for two hours. Parasites were then dehydrated to 70% ethanol in an ethanol series with intervals of 10%, 30%, 50% and 70% for 10 minutes each.

The parasites were stained in Alum Carmine for one to four hours. They were then dehydrated in an alcohol series of 70%, 80% and 96% with 10 min intervals and two changes of 100% ethanol at 20 min in each change. Half of the 100% ethanol was then discarded, and replaced with Xylene and left for 10 min. Parasites were cleared in two replacements of pure Xylene. The Xylene was then replaced for the third time and two drops of Canada Balsem or Eukitt mounting medium was added and left for about six hours.

Both the slide and a cover slip were carefully cleaned using a tissue. Two drops of mounting medium was applied in the middle of the cover slip. The cover slip was carefully lowered. Weights of about 13 g were put on the prepared slide to apply pressure to force out excess mounting medium and to keep the parasite flat. Weights were left overnight. Slides were labelled according to the tags. Excess mounting medium was left to dry and

was then removed with a scalpel. Slides were studied under a Nikon Alphaphot 2 or a Nikon Eclipse E800 compound microscope and measurements taken using an eyepiece micrometer.

2.8. Scanning electron microscopy

For further processing, the fixed material was dehydrated in an ethanol series to 100% ethanol. Dehydrated material was critical point dried in a Polaron Critical Point dryer. Dried material was mounted with the aid of epoxy resin (Pratley clear) on 12.5 mm aluminium stubs or brass stubs adapted to a cone shape. Specimens were gold-coated in a sputter coater (Polaron E5000) and examined in a Jeol Winsem 6400 Scanning electron microscope at 10 kV.

2.9. Transmission electron microscopy

For further processing, the fixed material was dehydrated in an ethanol series to absolute ethanol. For embedding, the specimens were placed in a 50 ml glass beaker and covered with a low viscosity epoxy embedding resin. The medium contained ERL, DER, NSA and S-1. The medium was left for nine hours under vacuum in a desiccator after which it was replaced for the second time and left overnight under the same conditions as the first. The specimens were transferred to a mounting tray and covered with epoxy embedding resin. The medium was left for nine hours at 70 ^{0}C to polymerise. The blocks were then removed. Using the LKB III Ultramicrotome 8802A, ultrathin sections were prepared.

Using water the ultrathin sections were collected and placed in 3.05 µm diameter 200 µm mesh copper grids and left to dry. The ultrathin sections were double stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and after 10 minutes viewed in the Philips CM 100 transmission electron microscope. Micrographs were taken on the microscope's 35 mm camera. Mss S. Cooper[1] & J. Kruger[1] assisted with the transmission electron microscopy techniques.

2.10. Histological sections

Material fixed in Bouin were transferred and stored in 70% ethanol. The material was dehydrated in an ethanol series (70, 80, 96 & twice in 100%). Dehydrated material was cleared in a Xylene-ethanol mixture for 10 min and finally in two replacements of pure Xylene for 20 min. each. Material was impregnated with paraffin wax at 60^0C for 24 hours. Impregnated material was embedded in paraffin wax with melting point of 65^0C in a Histocentre embedding machine. Material was sectioned at 6µm on a Reichert Yung motorised microtome. Sections were stretched on a stretching plate and left over night at 35^0C. Sections were stained in routine Harris' Haematoxylin and Eosin and cover slip mounted with Eukitt. Later on through the project Mrs M. Kruger[1] assisted with histological sectioning. Sections were then observed under the Nikon Alphaphot-2-(YS 2) compound microscope.

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[1]Mss S. Cooper, J. Kruger & M. Kruger, Department of Anatomical Pathology, Medical faculty, University of the Orange Free State, Bloemfontein.

2.11. Latex injection.

In order to study the digestive tract and reproductive system specimens were injected with fluid latex rubber (Boscotex). A Pasteur capillary pipet (150 mm) was heated and drawn out to create a very fine needle. A syringe was glued to the one end and latex of different colours (red, green & blue) were injected into the reproductive system and the digestive system of different parasites. Micrographs of latex injected parasites were taken on a Zeiss Tessovar ZTF1 photomicroscope. Specimens were then discarded.

2.12 Enzyme digestion

In order to study the harder skeletal structures the soft tissue of the parasite was digested using an enzyme digestion technique. The technique was adapted from a technique described by Harris, Cable, Tinsley & Lazarus (*in press*). Fixed individual parasites were placed in 70% ethanol in a watch glass and the body cut from the haptor using a new scalpel blade. The haptor was transferred onto 5 mm discs cut from an acetate sheet (overhead transparency) with a hole punch, and placed in a watch glass. An equal volume of distilled water was added to the ethanol droplet and the specimen allowed to rehydrate for 10 min. The fluid was removed using a finely drawn glass pasteur pipette and replaced with 25 µl distilled water. An approximate one-tenth volume of 10X digestion buffer (100 mM Tris-HCl pH 8.0, 10 mM EDTA, 5% SDS) and proteinase K (final concentration 100 µg/ml) were added to the specimen, which was then incubated at 50^0C for up to 10 min. The progress of digestion was monitored microscopically until lysis occured. The

incubation process was repeated four to five times. Surplus digestion buffer was carefully removed and replaced with distilled water. The water drop was carefully removed, and the specimen allowed to air dry for later observation under the Scanning Electron Microscope.

2.12 Photography

Micrographs of histological sections were taken on a H III Nikon microscope camera fitted to a Nikon Eclipse E 800 compound microscope.

Chapter 3.

The host,

Hippopotamus amphibius

CHAPTER 3

THE HOST, *Hippopotamus amphibius*

3.1. Introduction.

Originally, hippopotami ranged throughout Asia, Europe and Africa. Over the past several hundred years, even their remaining African range has decreased. In historic times, the hippopotami had been widely distributed over large parts of Africa and Palestine. Today, hippopotami are found mainly in parks or on reserves where they are protected from poachers. In these safe havens, hippopotamus numbers are slowly increasing (Grzimek, 1972).

3.2. Phylogeny.

The earliest origin of the hippopotamus is still unknown. Fossil hippopotamus teeth first appear in the East African Lower Miocene but the earliest cranium, of a relatively advanced hippopotamus, is from the Late Miocene deposits at Lothagam country. Their absence from the earliest fossil record may be because they were solitary forest animals (Kingdon, 1979).

The early Pliocene and Pleistocene hippopotami were more lightly built than the present-day *Hippopotamus amphibius*. They had longer limbs, but the softer, more herbaceous diets that are implied by low crowned teeth might have required more walking and greater agility without prejudice to their amphibious existence. Four functional toes with a wide splay and no protective hoofs could only have been retained by animals that were restricted to relatively soft ground (Kingdon, 1979).

Hippopotami have resemblances with pigs, peccaries and anthracotheres, and various authors have allied hippopotamus more closely with one or other of these three groups. It

is also possible that they evolved independently from a common suiform ancestor and that in this very early divergence they already tended to occupy the moister habitats (Kingdon, 1979). The pigs' and hippopotamus' common reliance on secure resting places might represent a carry-over from their origins as animals of the dense forest but the tendency to use swamps and waters as refuges has allowed it to develop a peculiarly successful ecological strategy. The modest appetite of the hippopotamus is correlated with great economy in the expenditure of energy and a specialised digestion and it is likely that all hippopotami shared these characteristics to some degree (Kingdon, 1979).

Hippopotami do not ruminate but they have a very slow rate of digestion and have evolved septa in the stomach and two accessory blind sacs that serve to direct and slow down the flow of food. One of these sacs might have developed before the mid-Eocene and these structures developed independently from similar sacs found in the living peccaries (Kingdon, 1979).

3.3. General morphology.

The proportion of the hippopotamus' body reflects its amphibious existence. The hippopotamus has a plumb and bulky build (Fig 3.1A). Males are usually larger than females. The neck is short and the body is barrel shaped. They weigh up to 3200 kg. The short legs, each with four only slightly spreadable toes, are webbed. The short round tail is partly flat near the tip. The thick skin has mucous glands and practically no hair (Grzimek, 1972). The animal's upper surfaces are purplish-grey to blue-black, while the lower surfaces and the skin around the eyes and ears tend to be brownish-pink in colour. Partial albinos have been seen, coloured bright pink with blotches of liver colour

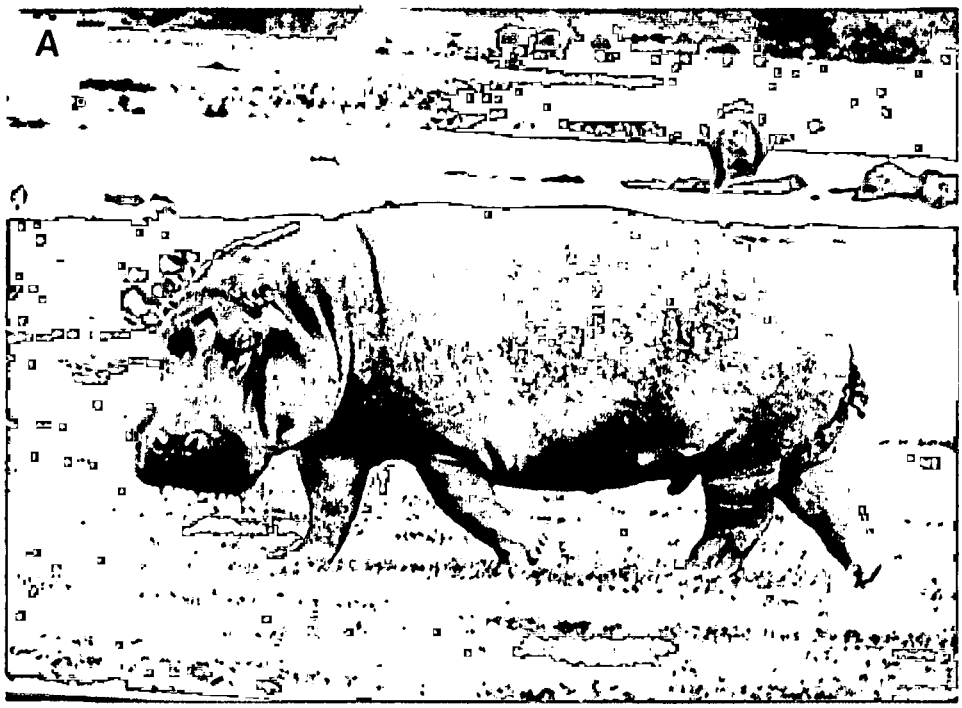
(Grzimek, 1972). The dermis varies considerably in thickness, being five to six centimetres on the back and rump and less than one centimetre thick on parts of the head and belly. By contrast, the outer horny layer of the skin is extremely thin everywhere (Kingdon, 1979).

The head is adapted to immersion in that the eyes, ears and nostrils have migrated to the top of the head and the latter are valvular and are opened and closed by small muscles along their margins. The broad, flat-fronted lips provide an efficient mechanism to seize and crop grass and the molar teeth, although relatively small, grind up fodder effectively. The canines and incisors, however, play little or no part in feeding and the heavily armoured lower jaw and great baggy jowl are developments entirely linked with ritualised fighting, which determines all relationships in the social life and courtship of the hippopotamus (Kingdon, 1979). With the nostrils, eyes and ears located on top of its head (Fig. 3.1B), the hippopotamus can stay safely under water, able to breathe, see, and hear the world above without exposing its body. If it submerges, it closes its nostrils and presses its ears flat against its head to prevent water from entering. Hearing, smell and sight are quite well developed. In areas where they are persecuted, hippopotami only emerge from their swampy refuges late on dark nights for a short period of intensive feeding and avoid coming out altogether on bright moonlight nights (Kingdon, 1979).

Figure 3.1

A) A fully grown male, *Hippopotamus amphibius*.

B) The head of a fully grown specimen of *H. amphibius* to show the position of the eyes.


A


B

3.4. The morphology of the hippopotamus eye.

No published information could be found on the hippopotamus eye. As part of this study the morphology of the eye was studied. In general the hippopotamus eye resemble a typical mammalian eye. The roughly spherical eyeballs are situated in a deep recesses on either side of the braincase.

The eyes, with a diameter of about 28 mm, can retract to the depth of about 25-mm into the orbits. This phenomenon ensures formation of very deep pockets under both the upper and the lower eyelids (Figs. 3.2A,B). A third eyelid (nictitating membrane) is present in the posterior corner of each eye (Fig. 3.2B). The membrane can cover the eye by sliding laterally over the eye. Mucus secreting cells ensure that the eyeball and the membranes are always lubricated. There is a lacrimal gland whose function is to furnish liquid for moistening and cleaning the cornea. The nasolacrimal duct is absent.

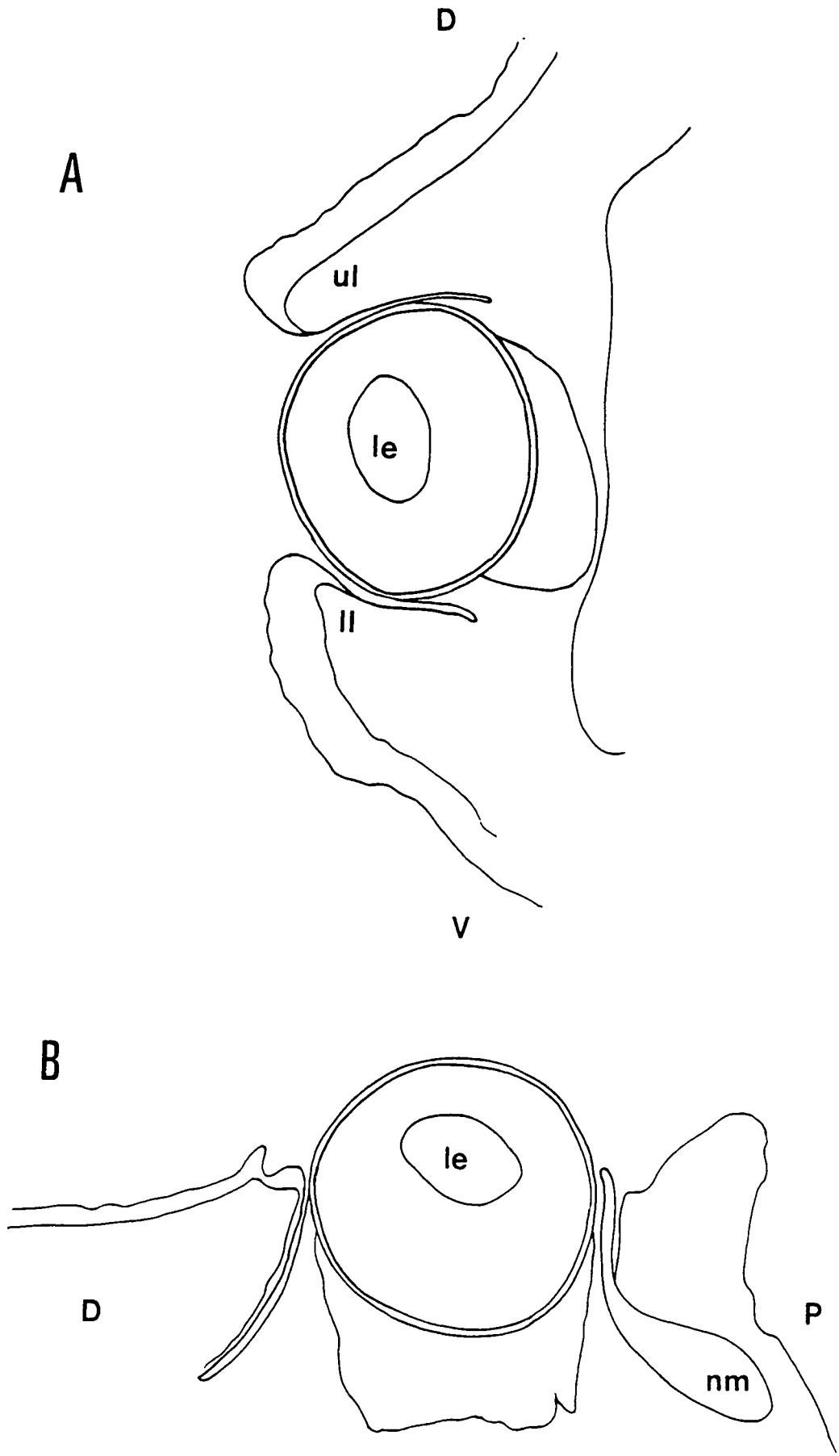
3.5. Behaviour.

The amphibious hippopotamus spends a great deal of its life in water. It is a gregasious animal who lives in the open and is well developed to life in water (Grzimek, 1972). Buoyancy allows this bulky animal to move easily through the water. It uses its short legs to propel itself forward, or to walk along the bottom in shallow water. A newborn must be able to swim because most of the time is spent in water. As an adult, a hippopotamus can stay underwater for 5-6 minutes. When it surfaces to breathe, it does so with loud snorts and hisses to expel stale air from its lungs through its nostrils (Grzimek, 1972).

Figure 3.2

A) Drawing of a mid saggital section through a hippopotamus eye.
Abbreviations: d, distal; le, lens; ll, lower lid; ul,upper lid; v, ventral.

B) Drawing of a horizontal section through a hippopotamus eye.
Abbreviations: d, dorsal; le, lens; nm, nictitating membrane; p, proximal.


A
D
ul
le
ll
V
B
le
D
P
nm

Hippopotami are not high performance swimmers. They prefer depths of only about one and half metres and places where there is either no current or only very slow moving water. In such places it does not even have to swim, it may walk on the bottom. During daytime when it is not too hot, they may sun themselves on sandbanks (Grzimek, 1972). They rely on water or mud to keep cool in hot, dry weather and on sunshine to warm them up when the water is cold, which suggests that thermoregulation is assisted by this behaviour to conserve energy. In normal circumstances this behaviour mentioned above is unimportant in the well-watered areas of the tropics and subtropics. The hippopotamus may be able to benefit directly from the sun's radiation so long as the compensatory effects of mud and water are available and it probably achieves thereby a considerable saving in metabolic energy. Indeed, a speciality of the hippopotamus might be its strategy for the conservation of energy; several lines of evidence point to this (Kingdon, 1979). Most of the night is spent grazing, they are herbivores, eating grass, aquatic and reed plants, leaves and fruits (Grzimek, 1972).

The basic social unit is the mother and her young and it is not uncommon to see a female closely followed by a file of up to four smaller animals, graded in size from a tiny infant of 24.5 kg to a three-quarter-grown young one (Grzimek, 1972). It is not known if these are necessarily her own offspring, for females apparently tolerate unrelated young and subadult animals, but the attachment of a baby to its mother is distinguished. These units are most readily recognised while the animals are grazing, but can sometimes be discerned when larger groups are scattered through a stretch of open water. Within a family unit the youngest animal is usually closest to the female. The mother often licks, nuzzles and scrapes her offspring with her lower incisors and even adults will groom

another full-grown animal, particularly when the partner is lying prostrate. The behaviour appears to have a reassuring function and it is sometimes followed by the groomer's resting its head on the other animal's back, which is also a common expression of sociability (Kingdon, 1979).

Hippopotami are seasonal breeders. There is a correlation between mating peaks and dry spells, at which time the hippopotamus population is most concentrated. The timing is due to the females' being seasonally polyoetrous, whereas the males show no evidence of sexual fluctuations (Kingdon, 1979). The gestation period is about 240 days and the female becomes very aggressive shortly before giving birth. The birth takes place on land or in the shallows. Newly born hippopotami are relatively small but can vary in weight from 25 to 55 kg. Suckling takes place on land or in the water. The young show suckling behaviour that is adapted to submersion irrespective of the animals' situation, and this can be seen when they are sucking from a bottle, during which the tongue rather than the horny lips hold the teat. Lactation lasts for about a year but the young hippopotami start to chew grass at about one month and to graze at five months (Kingdon, 1979).

Chapter 4.

Results

CHAPTER 4

RESULTS

4.1. MORPHOLOGY OF *Oculotrema hippopotami*

Although aspects of the external morphology of the egg and oncomiracidium were studied, the focus in this study was mainly on the adult parasite.

4.1.1. Egg

Eggs are oval, (Figs 4.1A, 4.2A) golden tan in colour and measure 0.23 (0.16-0.26) mm in length and 0.14 (0.09-0.18) mm in diameter (Table 4.3). In the present study, up to 58 eggs were observed *in utero* (Fig. 4.1B). The surface of the egg is smooth with no appendages. An operculum without smooth edges is present (Fig. 4.2B). The egg wall is exceptionally thick (Fig. 4.2C).

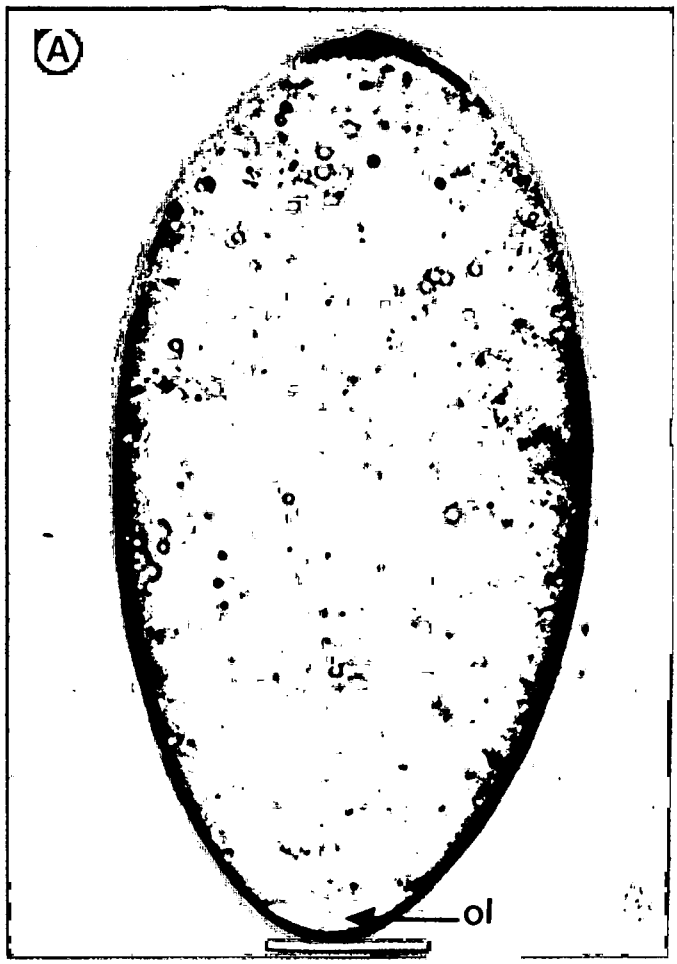
4.1.2. Oncomiracidium

The larval stage of *Oculotrema*, the oncomiracidium, has a more or less cylindrical body measuring 0.24 (0.23-0.27) mm in length, with a posterior haptor (Table 4.1, Fig. 4.3A,B). The mouth is situated, subterminally and is directed anteriorly. The oncomiracidium has two pairs of eyes. The smaller anterior pair is directed postero-laterally and the larger posterior pair faces antero-laterally.

Figure 4.1

A) Light micrograph of an *Oculotrema hippopotami* egg. Scale: 40 µm.
Abbreviation: ol, operculum lid.

B) Light micrograph showing intrauterine eggs of *O. hippopotami*. Scale: 400mm.
Abbreviation: eg, egg.


A
ol

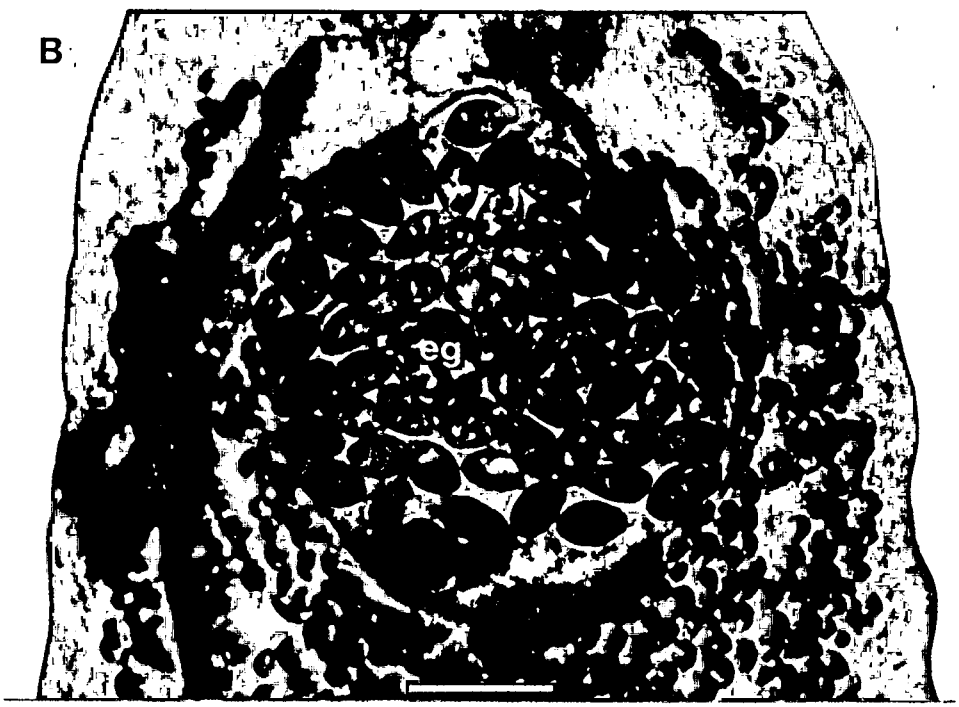

B
eg

Figure 4.2

A) Scanning electron micrograph of an *O. hippopotami* egg. Scale: 40µm.

B) Scanning electron micrograph showing the operculum. Scale: 10 µm.
Abbreviation: op, operculum.

C) Scanning electron micrograph showing the thickness of the eggshell. Scale: 5 µm.

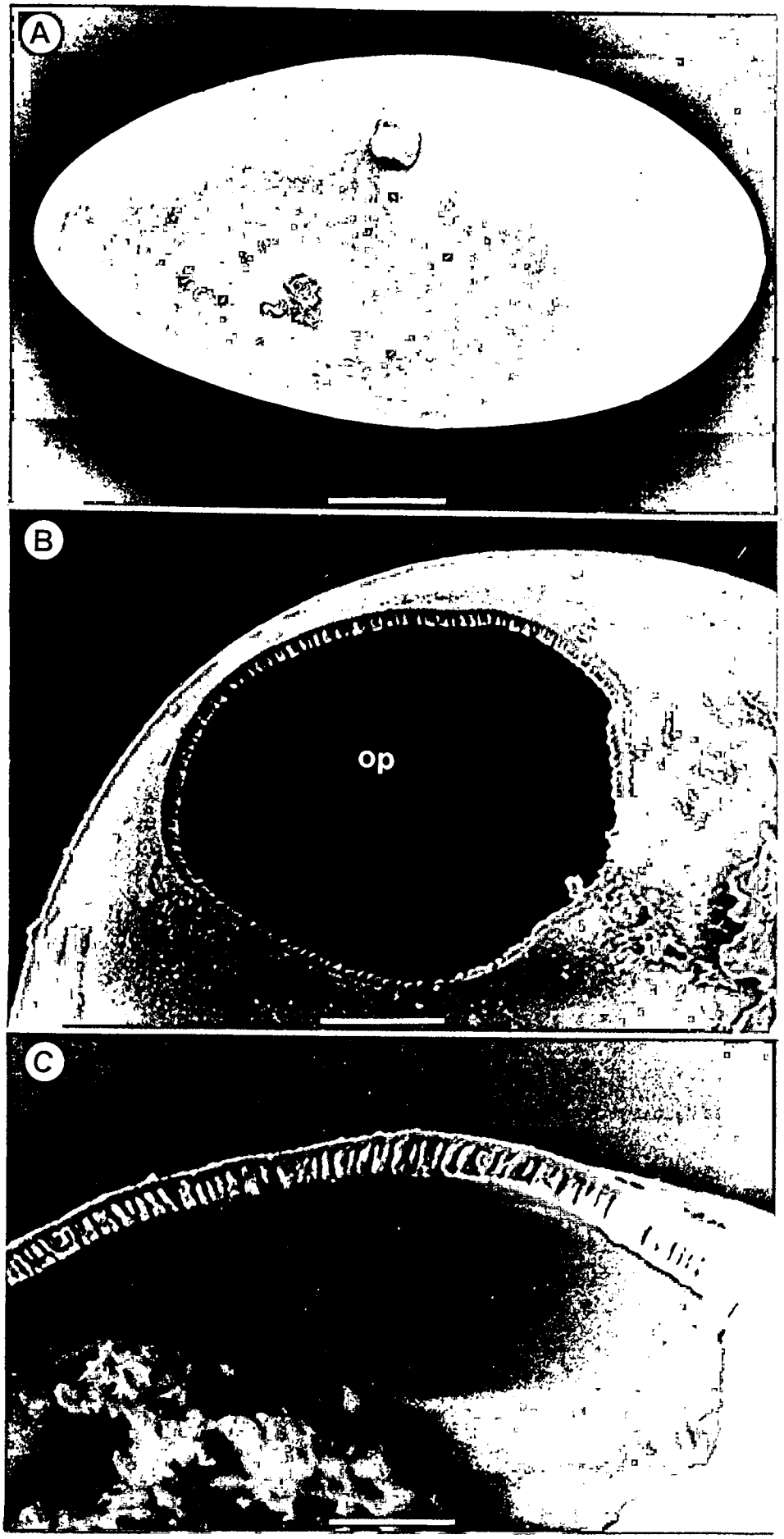
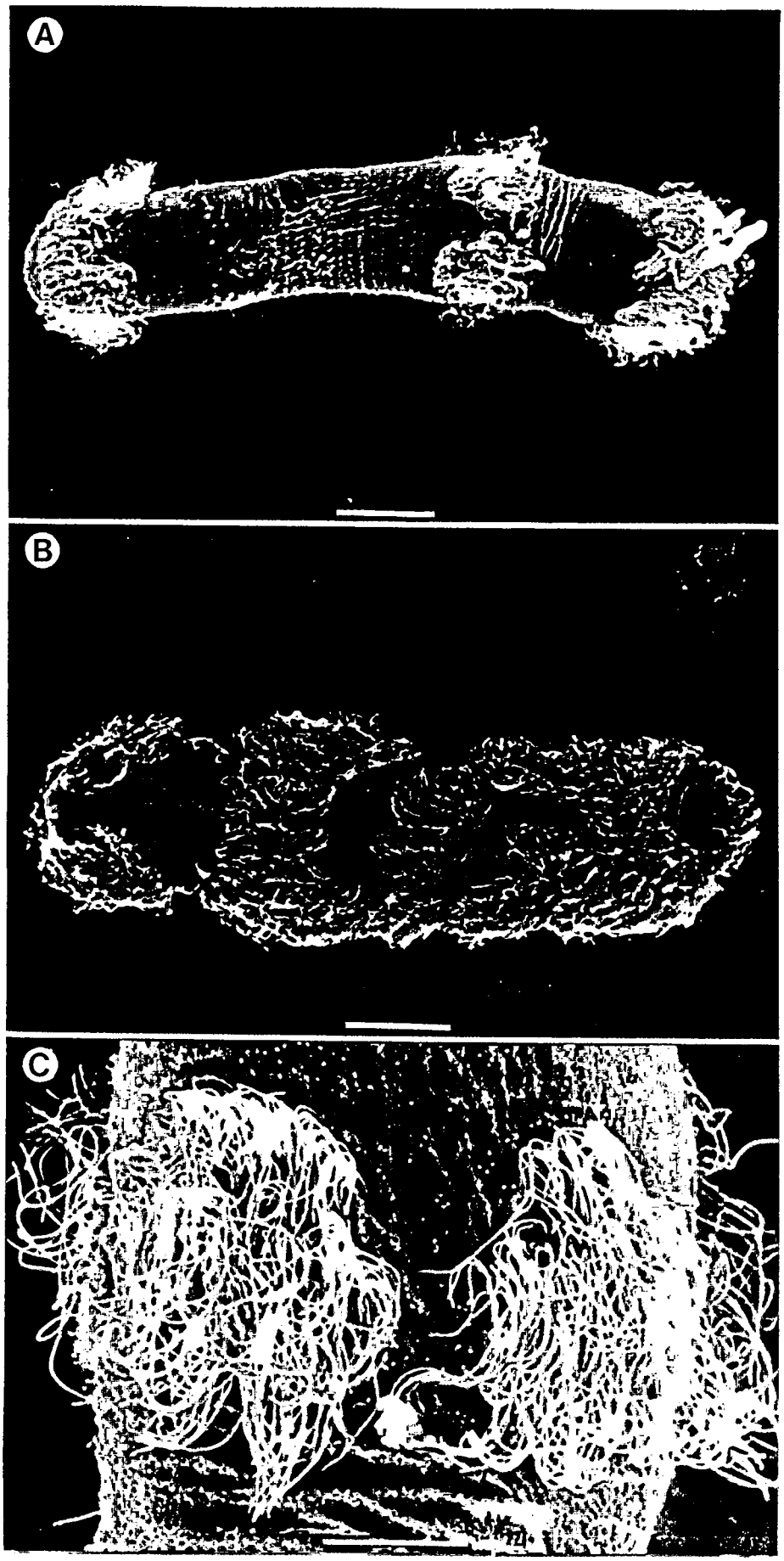

A
B
op
C

Figure 4.3

A) Scanning electron micrograph showing the dorsal view of an *O. hippopotami* oncomiracidium. Scale: 30 µm.

B) Scanning electron micrograph showing the ventral view of an *O. hippopotami* oncomiracidium. Scale: 30 µm.

C) Scanning electron micrograph showing the ciliated cells of an *O. hippopotami* oncomiracidium. Scale: 10 µm.


A
B
C

The body surface has a total number of 64 tegumental ciliated cells (Fig. 4.3C) that are arranged in transverse rows on the ventral, dorsal and lateral surfaces (Fig. 4.4A,B). These cells are arranged in 5 distinct groups (Fig. 4.4A,B):

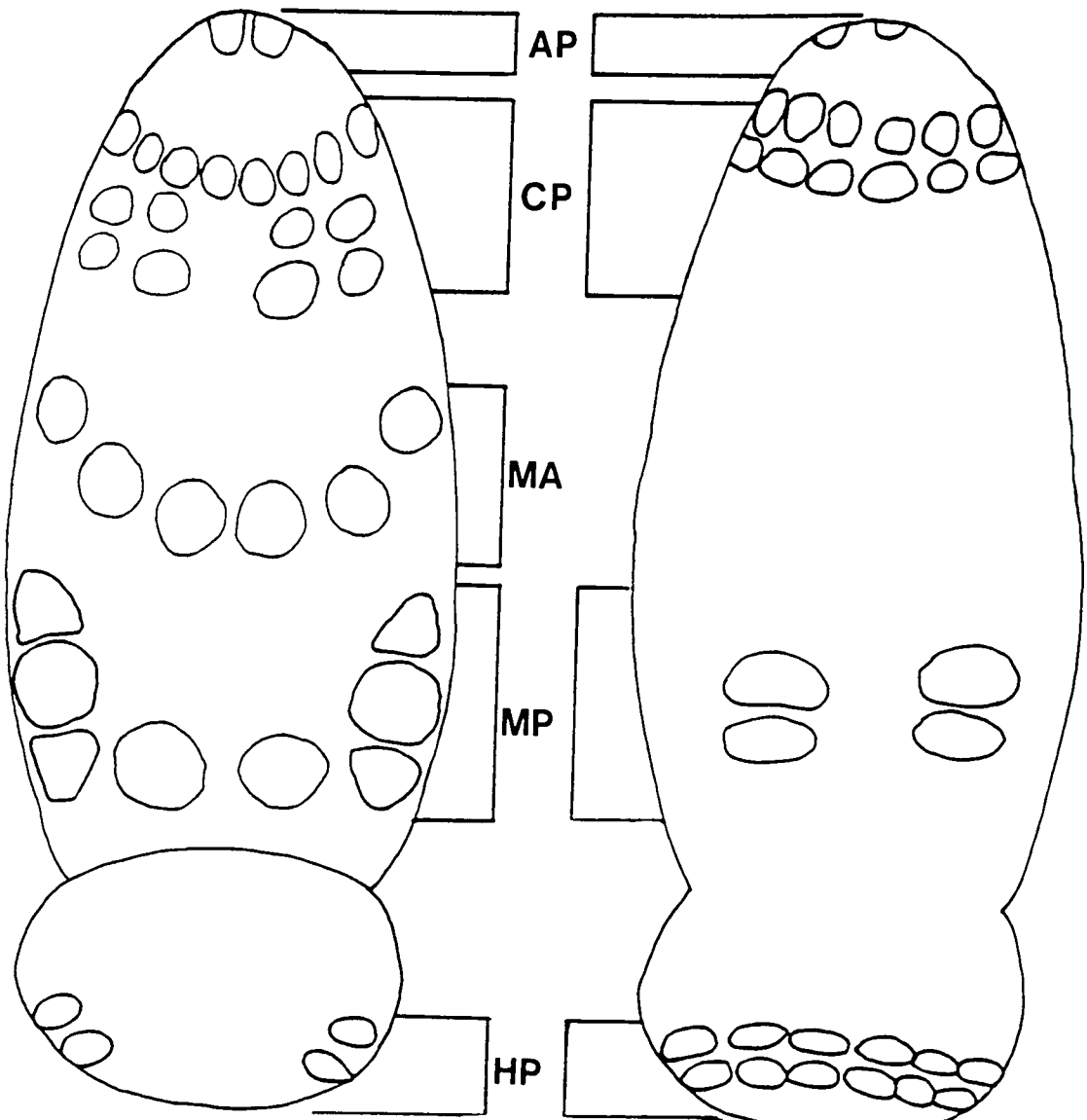
- Apical group: 2 cells, anterior.
- Cephalic group: 2 x 14 cells, dorsal lateral and ventral.
- Medio-anterior group: 2 x 3 cells, ventral.
- Medio-posterior group: 2 x 6 cells, dorsal and ventral.
- Haptoral group: 2 x 8 cells, dorsal and lateral.

The shape and size of the ciliated cells vary in different regions: medially situated cells are often broadly rounded whilst lateral cells are elongated and ellipsoidal. The tegument between the ciliated cells bears a series of epidermal sensillae, which occupy relatively constant positions with respect to the ciliated cells (Fig. 4.5A,B,C). Two types of epidermal sensillae can be distinguished: small circular 'buttons' with a relatively thin encircling wall and larger ellipsoidal, thick walled sensillae. The oval haptor is approximately one-third of the total body length. It is cup-shaped, opening ventrally and bears a total of 16 larval hooks (Fig. 4.6A). The postero-medial pair of hooks is referred to as larval hooks C1. Larval hook C 1 has a length of 15.11 μm (Table 4.2). Ratio of total length against handle length (Fig. 4.6B) is 1.68. These larval hooks are retained in the adult specimen. No hamulus premordia are present.

Figure 4.4

A) Drawing to show the positioning of the ciliated cells on the ventral side of an *O. hippopotami* oncomiracidium.
Abbreviations: AP, apical group; CP, cephalic group; HP, haptoral group; MA, medio-anterior; MP, medio-posterior.

B) Drawing to show the positioning of the ciliated cells on the dorsal side of the *O. hippopotami* oncomiracidium.
Abbreviations: AP, apical group; CP, cephalic group; HP, haptoral group; MP, medio-posterior



A

B

Figure 4.5

A) Scanning electron micrograph showing the ventral mouth with sensillae of an *O. hippopotami* oncomiracidium. Scale: 10 µm.

B) Scanning electron micrograph showing sensillae on the dorsal surface of an *O. hippopotami* oncomiracidium. Scale: 10 µm.
Abbreviation: se, sensilla.

C) Scanning electron micrograph showing high magnification of sensillae on the dorsal surface of the oncomiracidium of *O. hippopotami*. Scale: 5 µm.

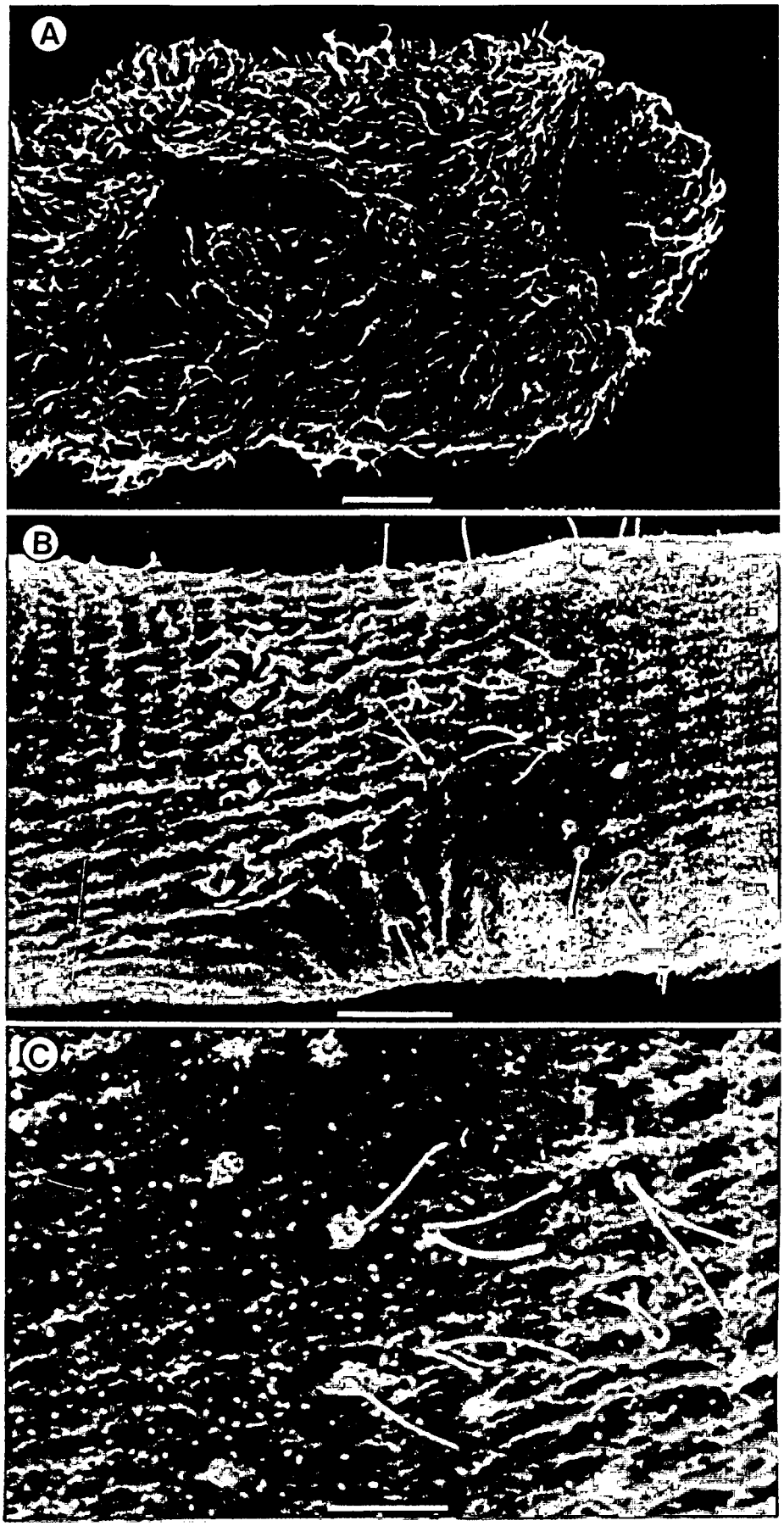
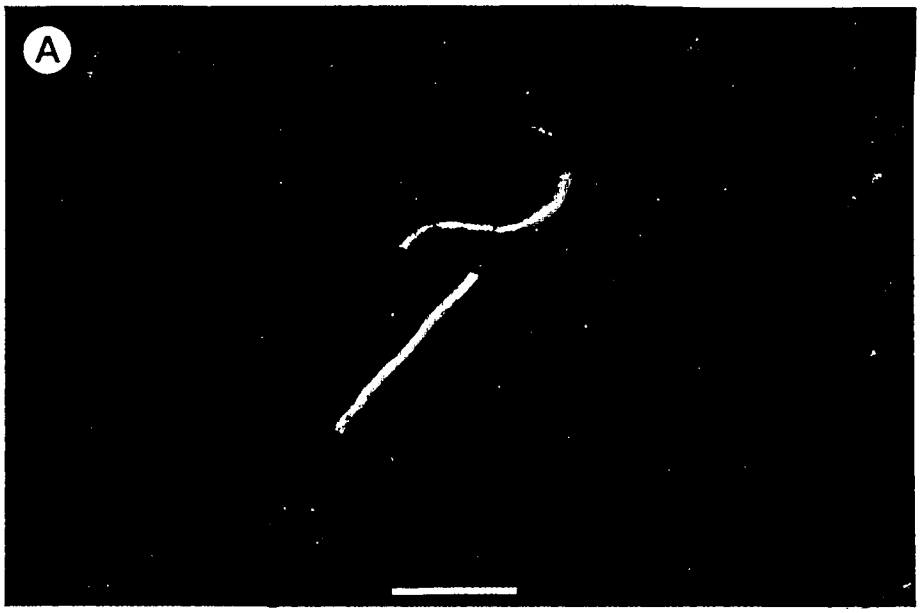

A
B
C

Figure 4.6

A) Scanning electron micrograph showing the larval hook C_1 of *O. hippopotami*. Scale: 5 µm

B) Drawing of the larval hook C_1 of *O. hippopotami*. Scale: 2.5 µm
Abbreviations: a, total length; b, handle length.


A

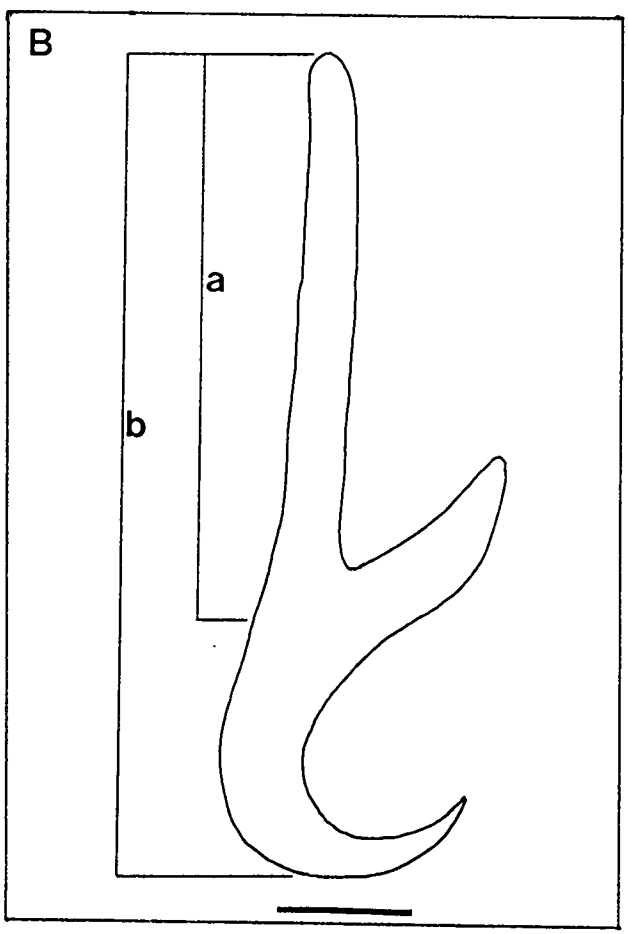

B
a
b

Table 4.1: Measurements of the oncomiracidium, *Oculotrema hippopotami.*

Structure	Mean	Range	SD	n	CV%
Body length (mm)	0.242	(0.226-0.270)	0.015	6	6.200
Greatest width (mm)	0.104	(0.097-0.117)	0.012	6	11.538
Haptor length (mm)	0.042	(0.31-0.43)	0.041	6	48.800
Haptor width (mm)	0.029	(0.023-0.052)	0.027	6	46.30

Table 4.2: Measurements of the hooklets C1of *Oculotrema hippopotami.*

Hooklet	Mean	Range	SD	n	CV%
Total length (a) (µm)	15.115	(14.700-15.680)	0.320	13	2.117
Handle length (b) (µm)	8.963	(0.699-9.996)	2.567	13	28.640

4.1.3. Adult parasite.

4.1.3a. Body size and shape.

Oculotrema hippopotami has a cylindrical body with an anteriorly situated mouth and a posterior opishaptor with six suckers (Fig. 4.7C). The body is dorsoventrally flattened oval to pyriform in shape (Fig. 4.7C, 4.8), pointed anteriorly and attenuated posteriorly. In live specimens the anterior two-thirds of the body is dark red in colour (Fig. 4.7A).

On the host and when not feeding, worms are contracted and the body wall is folded (Fig. 4.9A,B,C). However, specimens removed from the eyes that were collected and stored in a freezer were relaxed and extended, which shows that in living conditions they are capable of enormous extension. Measurments taken during the present study indicated a mean total body length of 16.96 (11.00-29.15) mm and a mean greatest width of 2.50(1.41-4.95) mm for a mature worm (Table 4. 3).

Figure 4.7

A) Light micrograph showing the dark red colour of the anterior two-thirds of sexually mature *O. hippopotami*.

B) Light micrograph showing mature *O. hippopotami* on the hippopotamus eye.

C) Light micrograph of a mature *O. hippopotami*. Scale: 2mm.
Abbreviations: mo, mouth; oh, opishaptor.

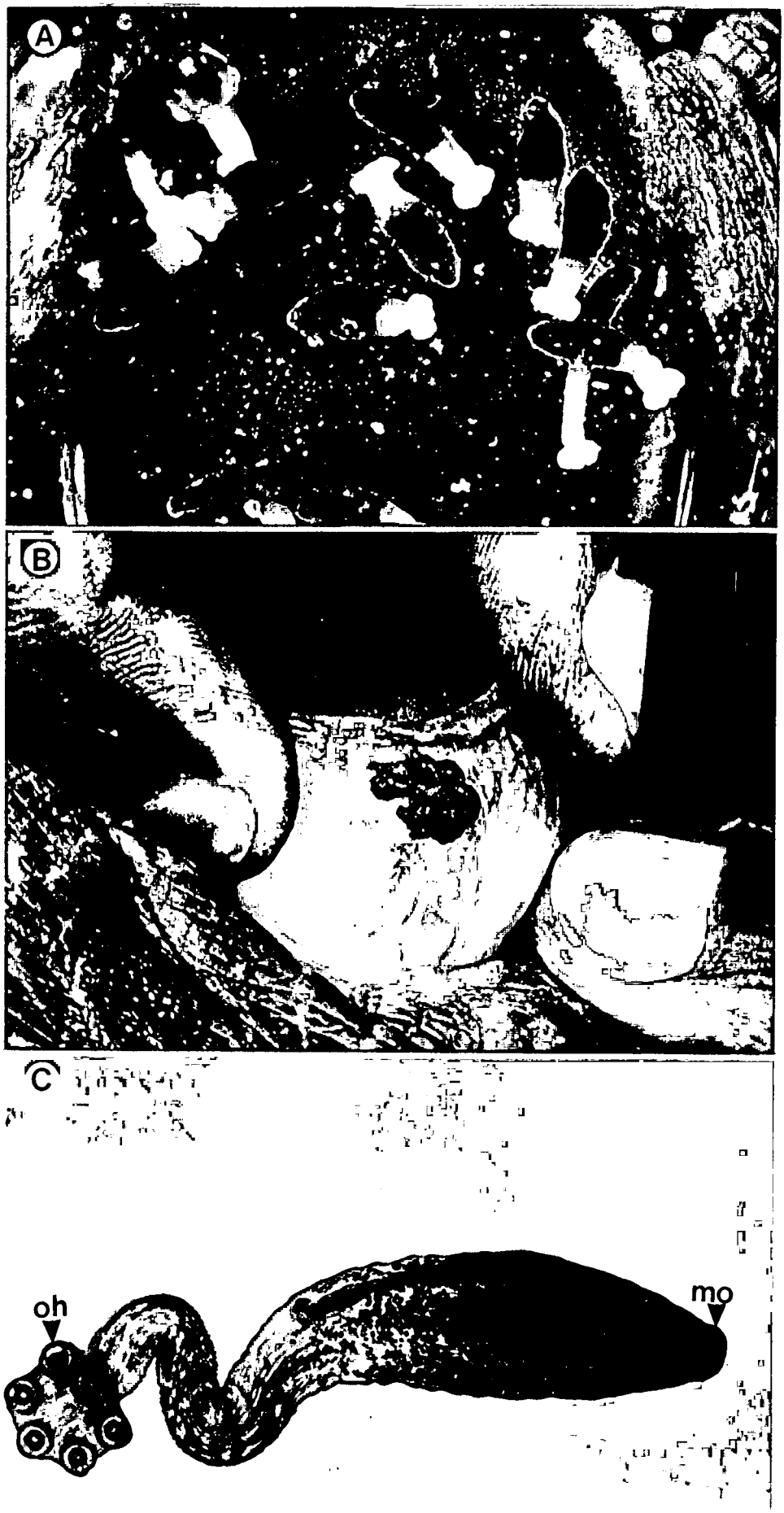

A
B
C
oh
mo

Figure 4.8

Oculotrema hippopotami. Ventral view of mature specimen. The dotted line indicates the outline of the vitelline system. Scale: 1mm.

Abbreviations: eg, egg; ic, intestinal caecum; mo, mouth; oh, opishaptor; ot, öotype; ov, ovary; ph, pharynx; su, sucker; sv, seminal vesicle; te, testis; ut, uterus; vi, vitelline system.

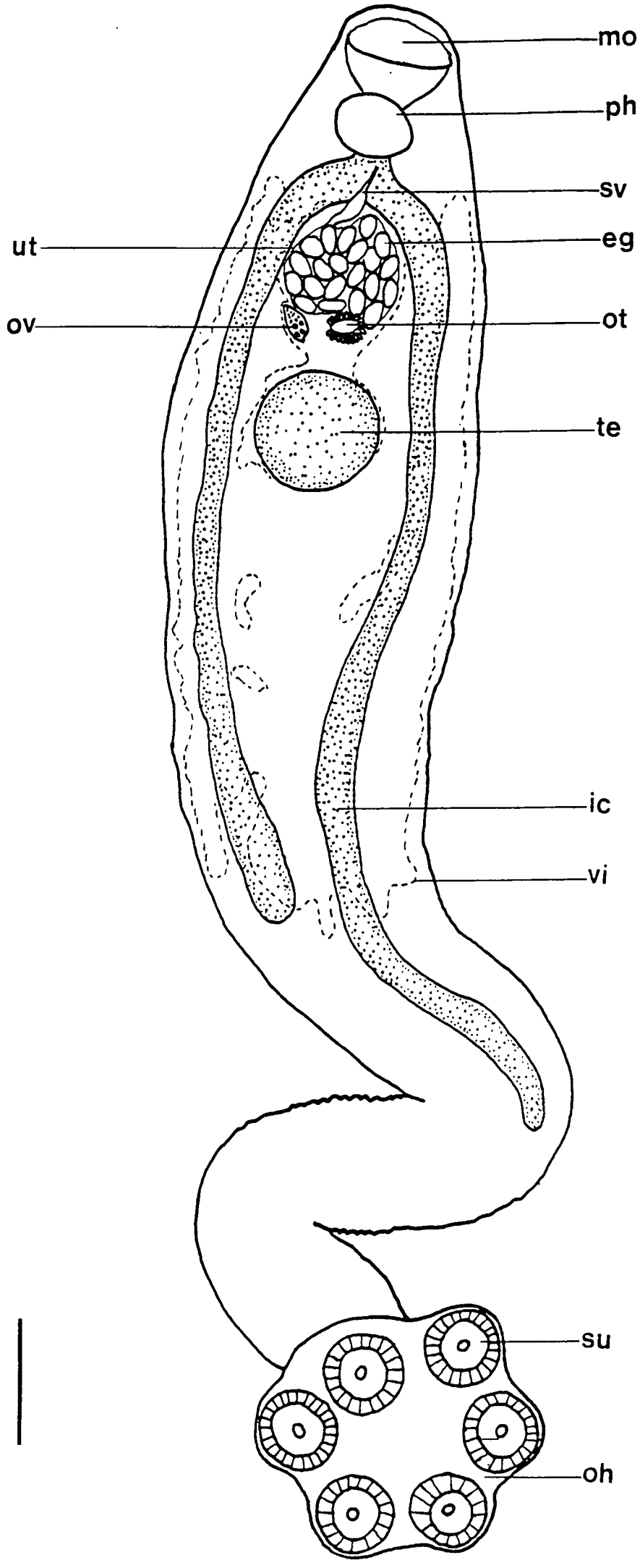

mo
ph
sv
ut
eg
ov
ot
te
ic
vi
su
oh

Figure 4.9

A) Scanning electron micrograph showing a contracted specimen of *O. hippopotami*. Scale: 1mm.
Abbreviations: mo, mouth; oh, opishaptor.

B) Scanning electron micrograph showing a specimen of *O. hippopotami* attached to the eye of its host. Scale: 400μm.
Abbreviation: mo, mouth; su, sucker.

C) Scanning electron micrograph showing high magnification of the sucker attached to the hippopotamus eye. Scale: 100 μm.
Abbreviation: su, sucker.

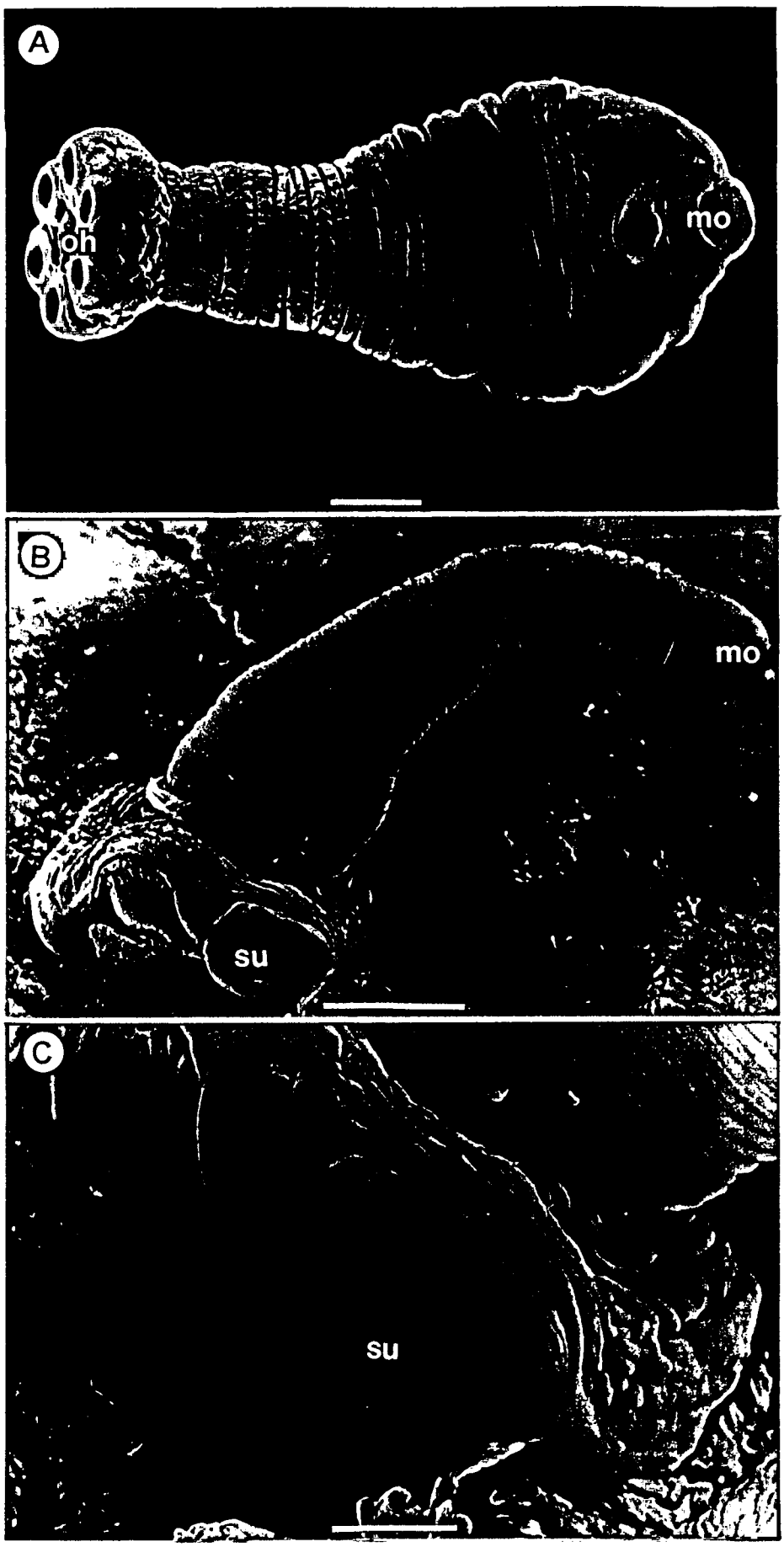

A
oh
mo
B
mo
su
C
su

Table 4 3: Measurements of sexually mature, egg-producing *Oculotrema hippopotami.*

Structure	Mean	Range	S.D.	n	CV%
Body length (BL) (mm)	16.955	(10.961-29.149)	5.125	30	30.227
Greatest width (mm)	2.495	(1.407-4.947)	0.775	30	31.062
Haptor length (HL) (mm)	2.198	(1.692-4.947)	0.695	29	31.620
Haptor width (mm)	2.568	(1.552-5.384)	0.909	29	35.397
Caecum length (CL) 1	4.312	(2.765-6.887)	1.11	23	25.742
Caecum length 2	2.058	(1.50-4.414)	1.432	27	69.582
HL/BL (ratio)	0.007	(0.003-0.013)	0.002	29	28.571
CL1/HL (ratio)	2.104	(0.588-3.276)	0.709	23	33.698
CL2/HL (ratio)	1.352	(0.304-2.293)	0.555	23	41.050
Oral sucker diameter (mm)	0.758	(0.388-1.067)	0.169	30	22.295
Pharynx length (mm)	0.514	(0.388-0.667)	0.062	29	12.062
Pharynx width (mm)	0.679	(0.534-0.776)	0.064	28	9.426
Uterus length (mm)	1.204	(0.872-1.940)	0.289	29	24.003
Uterus width (mm)	1.029	(0.385-1.552)	0.343	29	33.333
Ovary length (mm)	0.592	(0.190-0.950)	0.254	29	42.905
Ovary width (mm)	0.368	(0.095-1.102)	0.261	30	70.924
Testis length (mm)	0.814	(0.105-1.060)	0.208	30	25.553
Testis width (mm)	0.858	(0.513-1.040)	0.14	29	16.317
No. of intrauterine eggs	26.053	(1.00-58.00)	19.427	30	74.567
Egg length (mm) (Intrauterine eggs)	0.227	(0.162-0.257)	0.021	249	9.251
Egg diameter (mm (Intrauterine eggs)	0.139	(0.094-0.176)	0.019	262	13.670
Sucker diameter (mm)	0.601	(0.388-1.237)	0.211	174	35.108

4.1.3b. Digestive system.

The oral sucker is dorso-ventrally flattened, slightly subterminal and not sharply separated from the parenchyma (Fig. 4.10A). The anterior lip is thicker and much longer than the lower lip (Fig. 4.10B). The oral sucker measures 0.76 (0.39-1.07) mm (Table 4.3). The oral sucker extends to the pharynx, which is approximately the same size, the length measuring 0.51(0.39-0.67) mm and the width 0.68(0.53-0.78) mm (Table: 4.3). The pharynx is ellipsoidal (Fig. 4.10B). The pharynx leads to a bifurcate intestine with the caeca not confluent posteriorly. Although the caeca appear slightly knobby (Fig. 4.11A,B) diverticula and anastomoses are completely lacking. The caecae are of unequal length (Fig. 4.11C) and the longer caecum was in 85 % of the specimens on the same side as the ovary.

4.1.3c. Reproductive system.

Polystomatids are hermaphroditic. The female reproductive system consists of a small, pyriform ovary (Figs 4.8; 4.12A,B). usually situated on the right side of the parasite. It is confined to the anterior two thirds of the body. The ovary is situated just prior to the testis and measures 0.59 (0.19-0.95) mm in length, and 0.37(0.10-1.10) mm in width (Table: 4.3). The vitelline follicles (Fig. 4.12C) fill a large part of the anterior body and occupy most of the space dorsal and ventral to the gut caeca. The vitelline duct joins the oviduct to form an ovovitelline duct. A genito-intestinal canal runs from the joint vitelline duct to the longer one of the gut caeca and joins it at a position just anterior to the ovary. The oviduct runs from the ovary and joins the vitelline duct just anterior to the position where the genito-intestinal canal meets the vitelline duct (Fig. 4.13).

Figure 4.10

A) Scanning electron micrograph showing the mouth of *O. hippopotami*. Scale: 50 µm.

B) Light micrograph of a mid sagittal section through the oral region of *O. hippopotami*. Scale: 30 µm.
Abbreviations: mo, mouth; ph, pharynx.


A

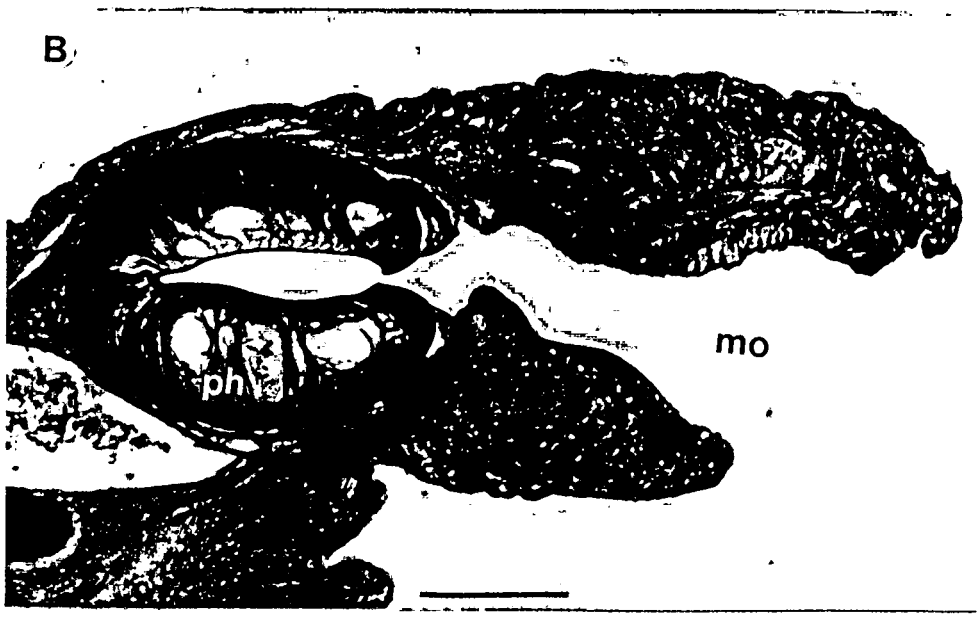
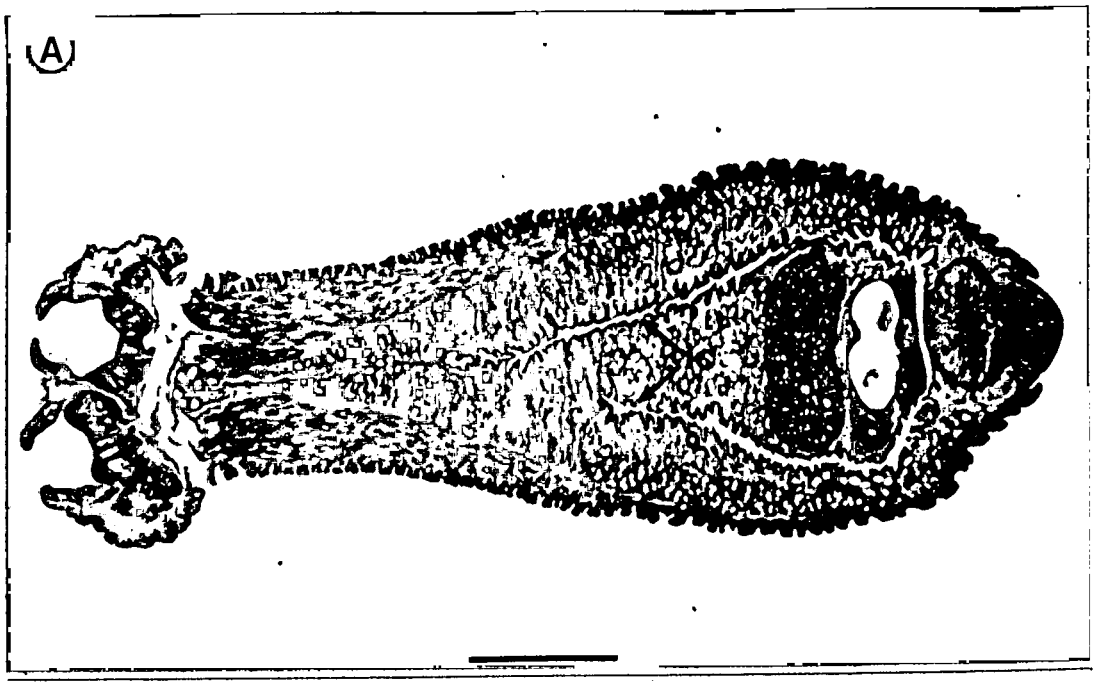

B
mo
ph

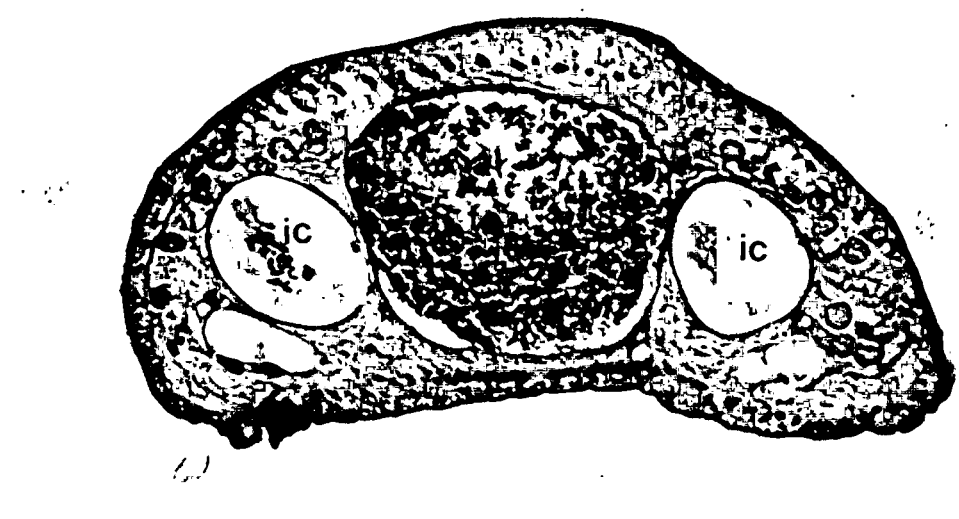
Figure 4.11

A) Light micrograph of a plane section of *O. hippopotami*. Scale: 1 mm.
Abbreviation: ic, intestinal caeca.

B) Light micrograph of a cross section through the caeca and the testis of *O. hippopotami*. Scale: 2 mm.
Abbreviation: ic, intestinal caeca; te, testis.

C) Light micrograph showing the unequal length of the latex injected caeca of *O. hippopotami*. Scale: 2 mm
Abbreviation: ic, intestinal caeca; te, testis.


A


B
ic
ic

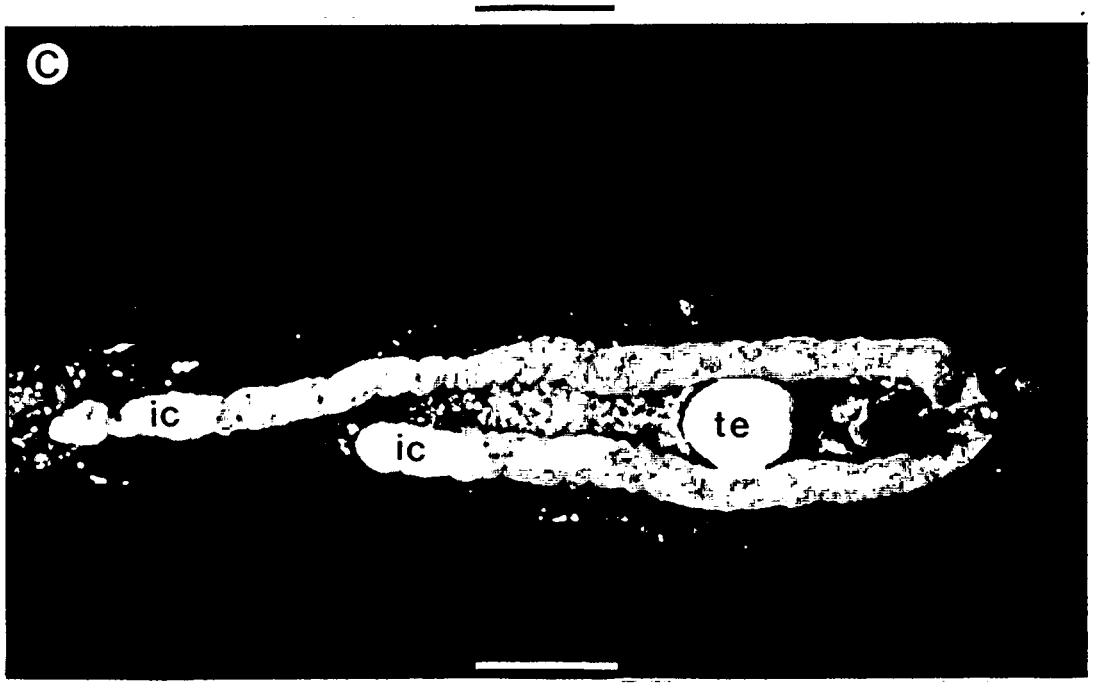

C
ic
ic
te

Figure 4.12

A) Light micrograph of a plane section through *O. hippopotami* showing the position of the ovary. Scale: 50 μm. Abbreviations: eg, egg; ov, ovary; ut, uterus.

B) Light micrograph of a section through the ovary of *O. hippopotami* using NOMARSKI DIFFERENTIAL INTERFERENCE CONTRAST. Scale: 20 μm. Abbreviation: om, ovum.

C) Light micrograph showing vitelline follicle of *O. hippopotami* using NOMARSKI DIFFERENTIAL INTERFERENCE CONTRAST.
Scale: 20 μm.

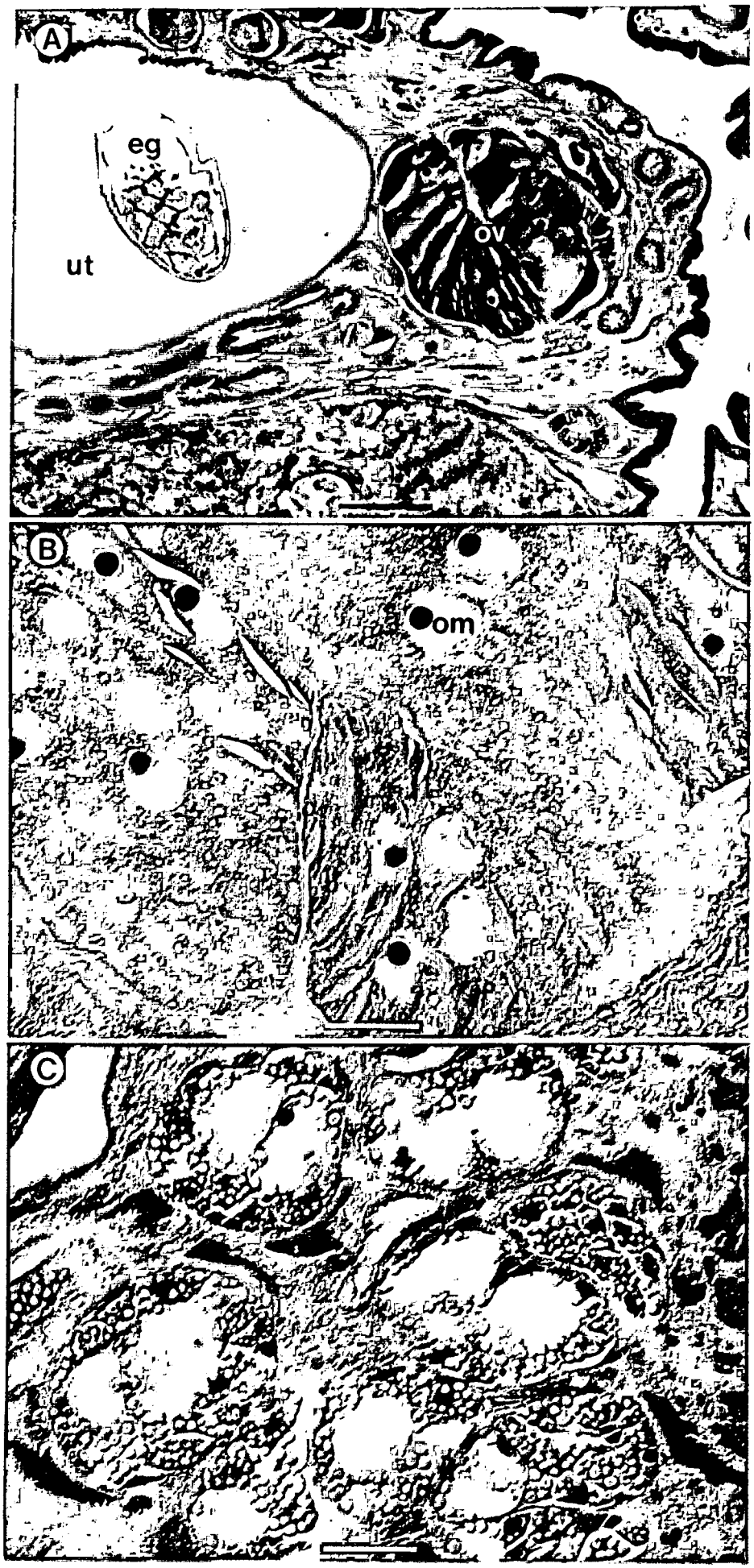
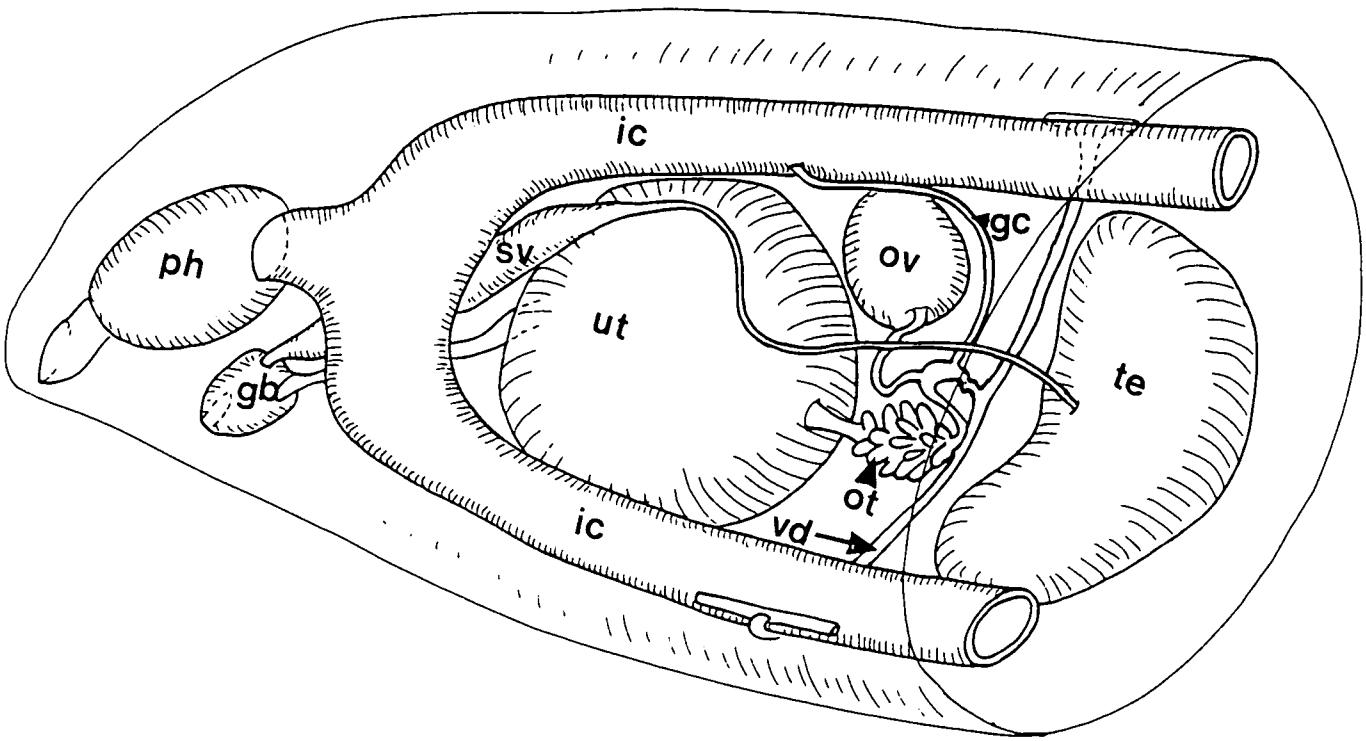

A
eg
ut
ov
B
om
C

Figure 4.13

Drawing of the reproductive system of *O. hippopotami*.

Abbreviations: gc, genito-intestinal canal; gb, genital bulb; ic, intestinal caeca; ot, öotype; ov, ovary; ph, pharynx; sv, seminal vesicle; te, testis; ut, uterus; vd, vitelline duct.


ic
ph
sv
ov
gc
ut
te
gb
ot
ic
vd

In the male system a single testis is located at the ventro-medially in the first one-third of the body. It is slightly oval in shape, longer in the lateral axis of the body measuring 0.81 (0.11-1.06) mm in length, and 0.86 (0.51-1.04) mm in width (Fig. 4.14A,B,C). The testis is filled with sperm and bound by a lamellate membranous sheath and is surrounded by parenchyma (Figs 4.14B). The vas deferens spring antero-dorsal on the testis and widens to form a semen vessicle. The semen vessicle proceeds to the penial bulb where it joins the uterine duct in the genital artrium (Fig. 4.13). The sperm duct is surrounded by circular and longitudinal muscle layers. The ducts are lined with epithelial cells (Fig. 4.16B). Penial spines are completely lacking. The mature spermatozoon consists of an elongate nucleus and mitochondrion, two axonemes and cortical microtubules in a linear array surrounded by a smooth plasma membrane (Fig. 4.16A). The nucleus, with a densely packed chromatin, forms a rodlike structure, which is tapered at both ends. The axonemes have a general basic structure found in platyhelminths, whereby the microtubules have a "9+1" arrangement (Figs 4.15B(5), 4.15C, 4.16F). The nine peripheral tubules each consist of doublets, usually designated A and B. Tubule B lies in close association with tubule A, which bears two lateral arms that are directed clockwise when viewed antero-posteriorly (Fig. 4.15C). The two lateral arms are referred to as the outer and the inner arms. Tubule A is connected to a central hub by a single filament. These spokes connecting the doublets with the core often lie in contact with microtubules. The central hub of the "9+1" structure is not a microtubule but consists of a dense core surrounded by an electron-lucent intermediate zone and its enclosed by a cortical sheath. The elongate mitochondrion is moderately electron-dense with oblique cristae (Figs 4.15B(3,4); 4.16C,D,E).

Figure 4.14

A) Light micrograph showing a cross section through the testis of *O. hippopotami*. Scale: 2mm.
Abbreviation: te,testis.

B) Light micrograph showing the position of the testis of *O. hippopotami*. Scale: 200 µm.

C) Light micrograph showing high magnification of the testis of *O. hippopotami* using Nomarski differential interference contrast. Scale: 20 µm.
Abbreviations: ov, ovary; te, testis; ut, uterus.

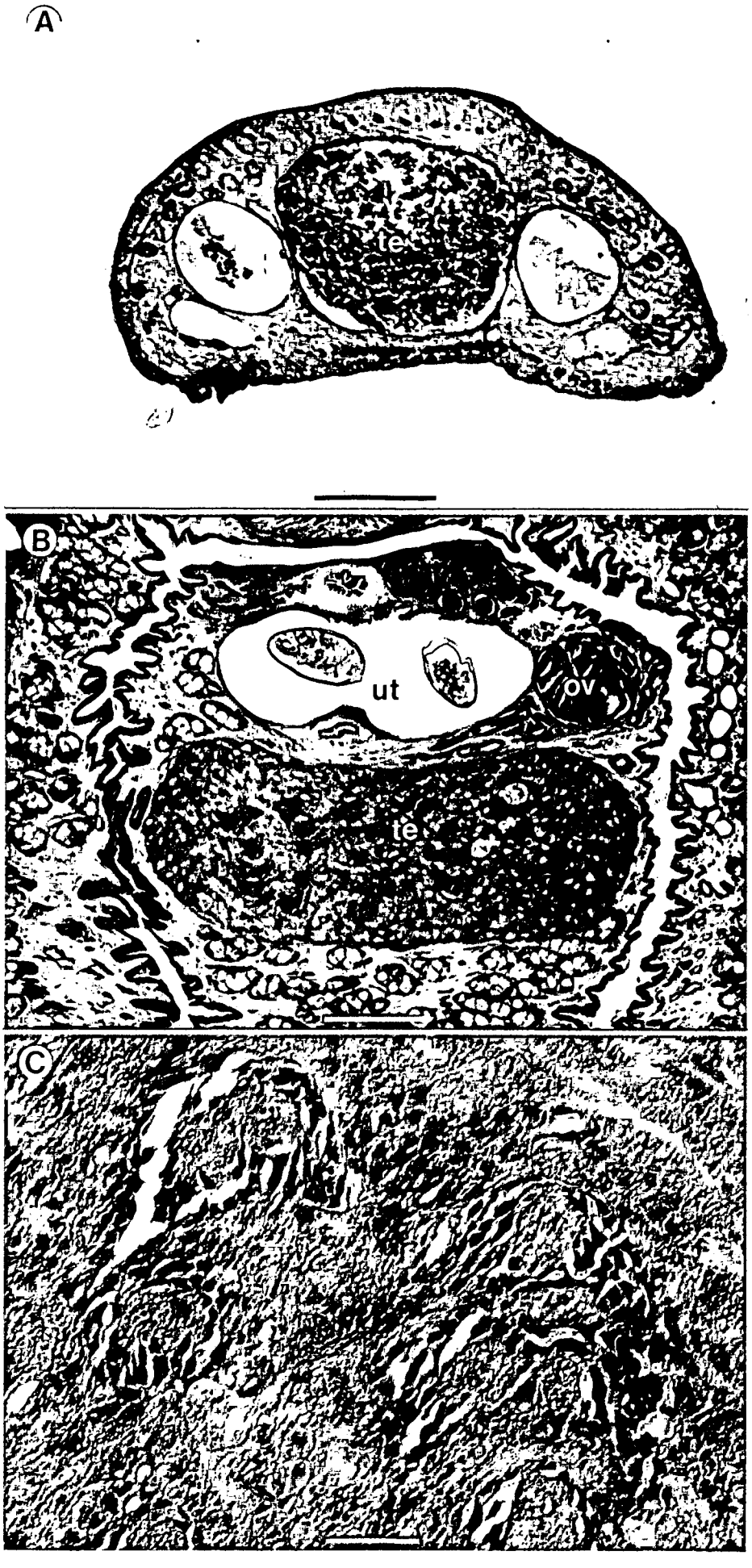

A
te
B
ut
ov
te
C

Figure 4.15

(A) Diagram of a mature spermatozoon.
Abbreviations: ax, axonema; mc, mitochondrion; nu, nucleus.

(B) Cross sections through mature spermatozoon at levels indicated in Fig. 4.15 A.

C) Drawing of the classical platyhelminth "9+1" arrangement of microtubule.
Abbreviations: am, A-microtubule; bm, B-microtubule; ch, central hub; cs, central sheath; ia, inner arm; nl, nexin link; oa, outer arm; sh, spoke head; sp, spoke.

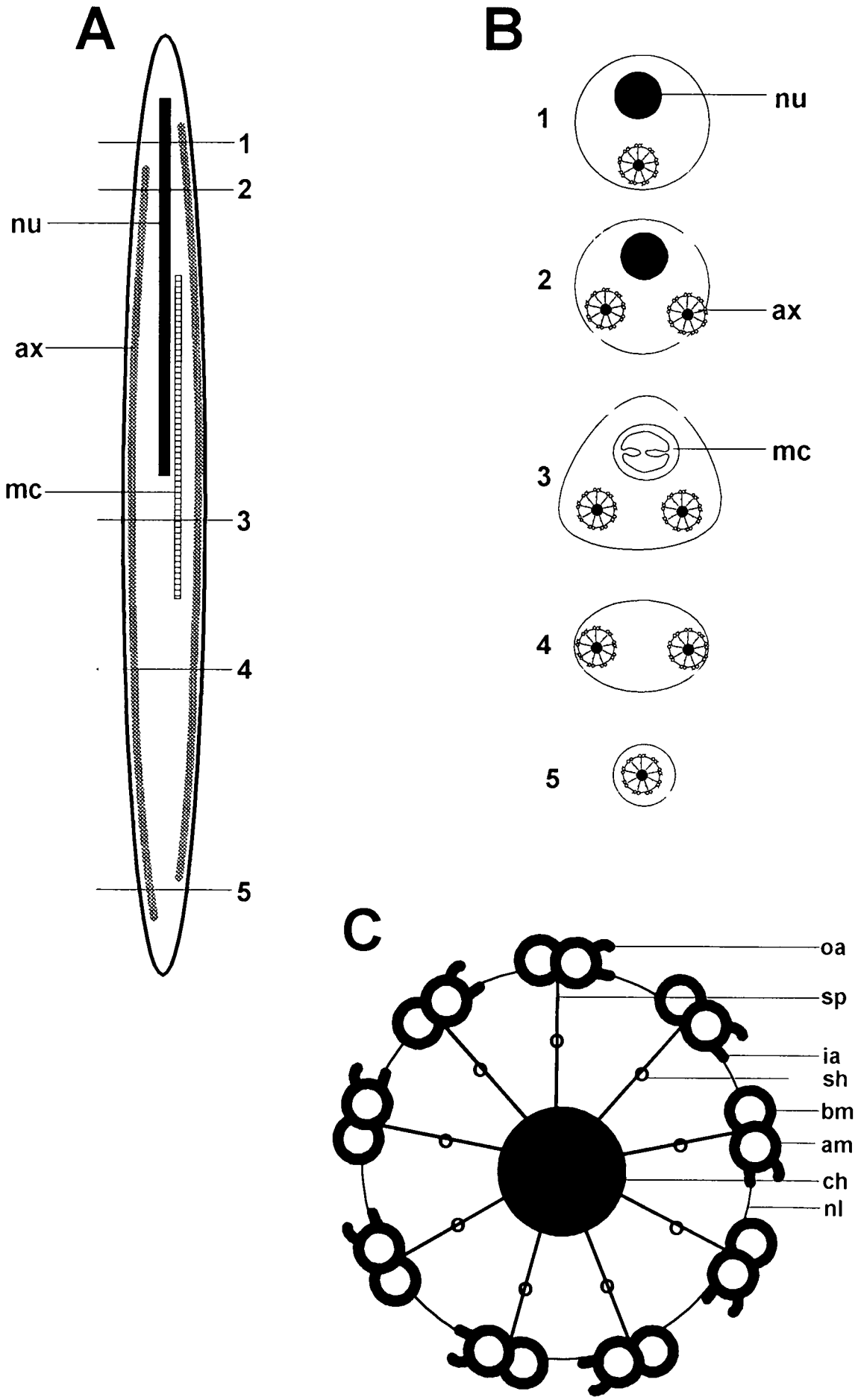

A
1
2
nu
ax
mc
3
4
5
B
1
nu
2
ax
3
mc
4
5
C
oa
sp
ia
sh
bm
am
ch
nl

Figure 4.16

Transmission electron micrographs of sections through the testis and mature spermatozoa of *O. hippopotami*.

A) The testis with sperm duct. Scale: 15 µm.

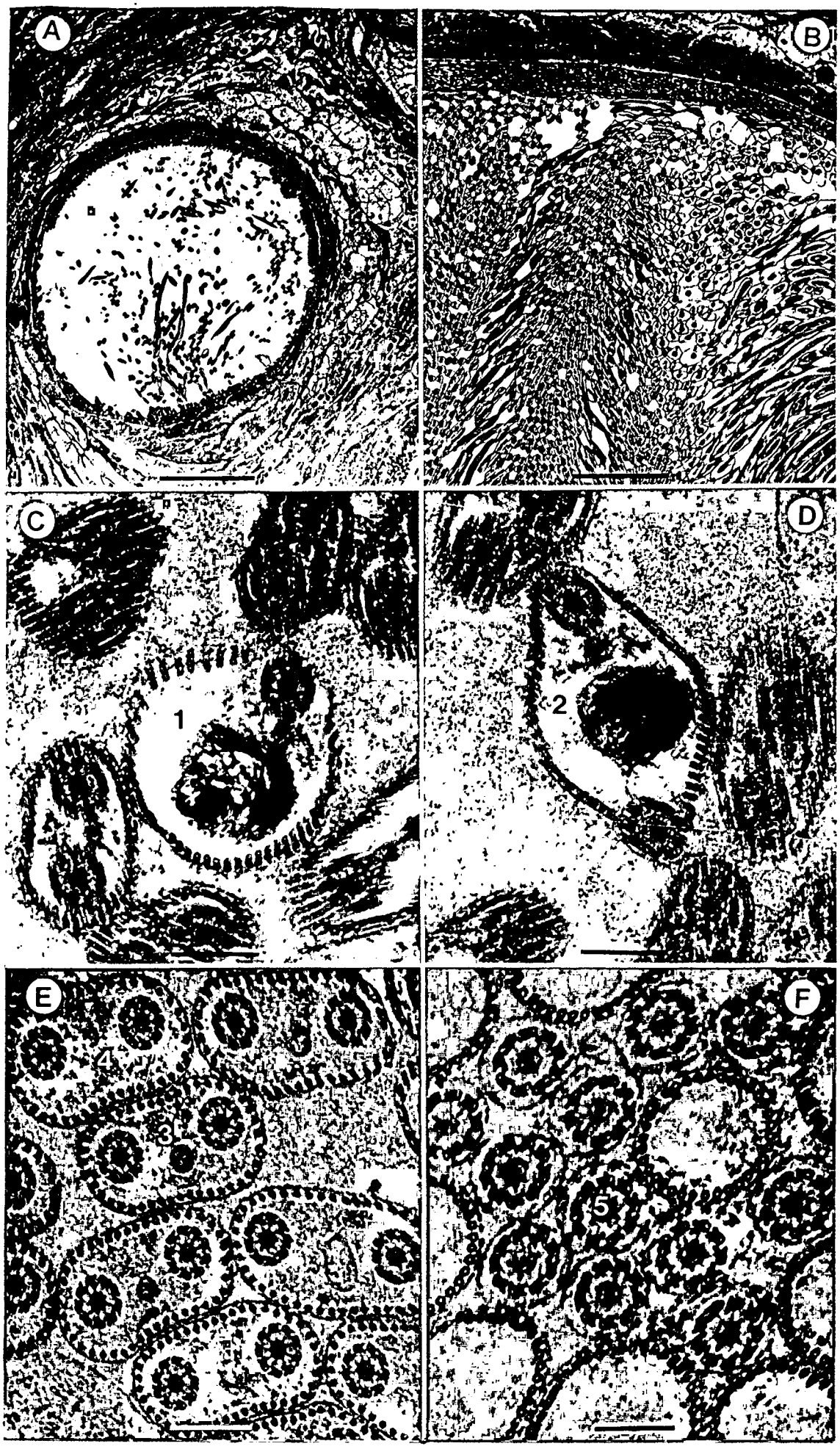
B) Cross section through a sperm duct. Scale: 5 µm.

C) Transverse section through a sperm showing the nucleus and proximal end of the first axoneme. Scale: 0.2 µm.
Abbreviation: 1, position 1in Fig. 4.15 B.

D) Transverse section through a sperm showing the nucleus and two axonemes. Scale 0.2 µm.
Abbreviation: 2, position 2 in Fig. 4.15 B.

E) Transverse section through a sperm showing the mitochondria and two axonemes. Scale: 0.2 µm.
Abbreviation: 3, position 3in Fig. 4.15 B; 4, position 4 in Fig. 4.15 B.

F) Transverse section through a sperm tail tip showing one axoneme. Scale: 0.2 µm.
Abbreviation: 5, position 5 in Fig. 4.15 B.


A
B
C
1
D
2
E
4
3
F
5

4.1.3d. Integument and musculature.

Three levels of muscle fibres are present directly under the integument. The body wall muscles are powerfully developed with both circular and longitudinal fibres (Fig. 4.17A,B,C). The external level of the muscle is formed by circular or annular fibres and the internal level is formed by a sheath of longitudinal fibres. Between the external and the internal levels there is a sheet of diagonal muscle fibres (Fig. 4.17B,C).

Between the anterior body and the opishaptor the thinner and highly flexible mid-piece is present (Fig. 4.18A). The mid-piece has a column of well-developed bundles of longitudinal muscles gradually tapering to the sides (Fig. 4.18A). At the position where the mid-piece meets with the opishaptor, bundles of well-developed circular muscles were observed. Ultrastructural examination of the mid-piece revealed muscle bundles (Figs 4.18B,C,D,E). The muscle bundles are approximately 7μm in diameter. The muscle has thick filaments with dense bodies randomly spaced (Figs 4.18B,C,D,E)

Figure 4.17

A) Light micrograph showing the anterior body muscles of *O. hippopotami*. Scale: 500 µm.
Abbreviation: ml, muscle layer.

B) Light micrograph showing the diagonal muscle fibres under the integument of *O. hippopotami*. Scale: 300 µm.

C) Light micrograph showing high magnification of the diagonal muscle fibres under the integument of *O. hippopotami*. Scale: 200 µm.

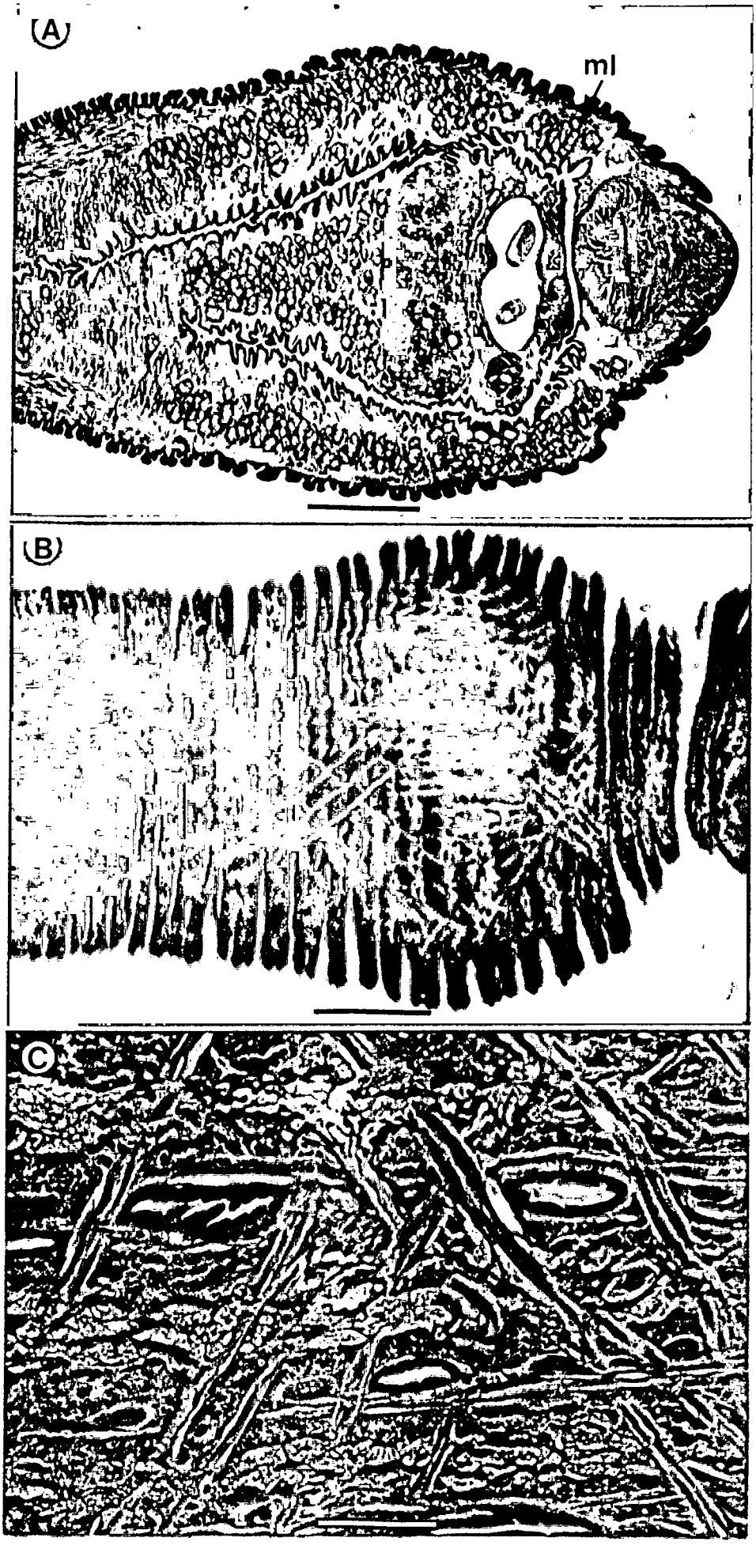

(A)
ml
(B)
(C)

Figure 4.18

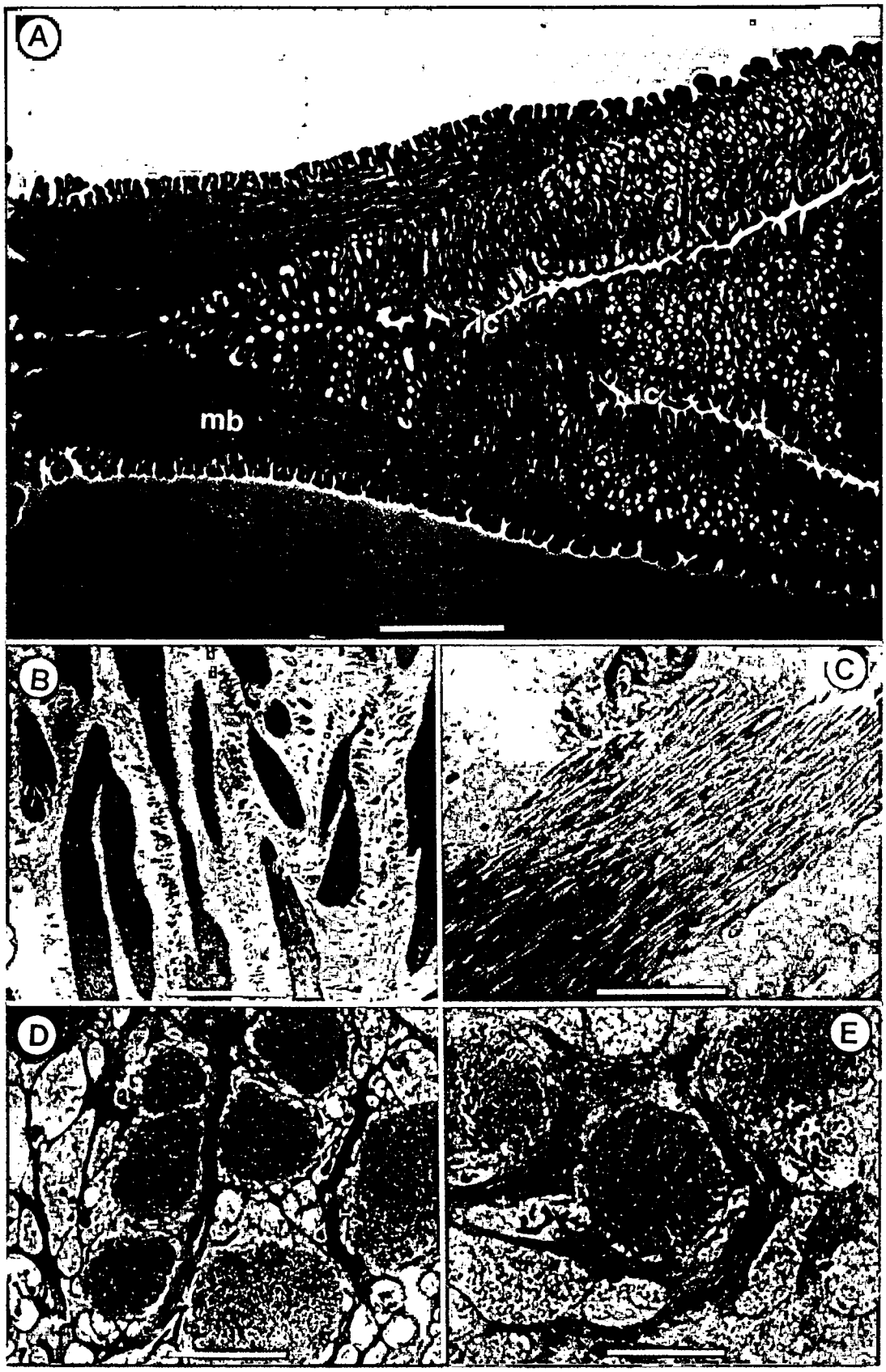
A) Light micrograph of a plane section through *O. hippopotami* showing the mid-piece muscle fibres. Scale: 400 μm.
Abbreviation: ic, intestinal caeca; mb, muscle body.

B) Transmission electron micrograph of longitudinal muscles under low magnification. Scale: 10 μm.

C) Transmission electron micrograph of longitudinal muscles under high magnification. Scale: 2 μm.

D) Transmission electron micrograph of a cross-section through the muscle bundles. Scale: 4μm.

E) Transmission electron micrograph of a cross-section through the muscle bundles. Scale: 4μm.


A
mb
ic
ic
B
C
D
E

4.1.5.3e. Opishaptor

The opishaptor measures 2.20 (1.70-5.00) mm long and 2.57 (1.55-5.39) mm wide when flattened (Table 4.3). It has six well-developed suckers arranged in a circle (Fig. 4.19A). The suckers are positioned in such a way that they open outwards. The central portion of the disc surrounded by the suckers is small and hardly as large as one of the suckers. The suckers are almost spherical (Figs 4.19A,B) and measures 0.60 (0.39-1.24) mm in diameter. They are muscular cups with a continuous lining (Fig. 4.19C). The sucker is supported by a skeletal complex. Based on this skeletal complex the sucker was divided into three annular zones namely the peripheral zone, intermediate zone and the central zone (Figs 4.21A, 4.22A,B,C, 4.23A). The central zone is completely chitinous and consists of a ring of brick-like structures that tightly fit into one another (Figs 4.22G,H). Between each two brickslike structures a canal connects the peripheral zone with the central zone (Figs 4.22E,G,H). The skeletal structure of the peripheral zone consists of inner and outer chitinous digiti protruding from the intermediate zone skeletal ring (Figs 4.21B,C; 4.22E,G,H). These digiti are not solid but consist of segments giving them some flexibility (Fig.4.21B,C). The skeletal structure of the central zone consists of the inner and the outer costi that connect the central ring to the skeletal funnel (Figs 4.22E,G,H). The costi also consist of segments giving them flexibility. The skeletal funnel consists of a solid structure (Fig 4.22D,F). The peripheral zone and cuticular skeleton have continuous alternating perforations (Figs 4.22E,G,H). The ultrastructure shows the perforations to open to the outside of the sucker (Fig. 4.22E).

The sucker has a combination of muscular fibres and collageneous fibres that gives it high manoeuverability (Fig 4.23A). In the peripheral zone bundles of muscle fibres connect the digitae to the central ring (Fig 4.23A). These digiti are covered with muscles and other tissue and have the appearance of parallel rays that are visible in Fig. 4.19C. Muscle fibre rings are also present in the peripheral zone (Fig. 4.23A). The central zone is a very muscular zone with numerous bundles of muscle fibres connecting the inner and the outer costae, and the outer costae and skeletal funnel with the rest of the opishaptor (Fig. 4.23A). The muscle fibres connected to the skeletal funnel are extremely well developed.

No muscle fibres were observed in the central zone. Through each of the canals running through the central zone, a bundle of collageneous fibres connect the skeletal digitae in the peripheral zone with the inner costae in the central zone.

Based on the morphology of the sucker we hypothesise that the mechanism of the sucker is as follows: When the parasite attaches to the host, the skeletal funnel is moved towards the peripheral zone (Fig. 4.23C). The inner costae are thus bend inwards with the result that tension is placed on the band of collagenous fibres. This in turn will bent the skeletal digitae outwards, which will open the peripheral zone while the intermediate remains in position. After contact is made with the host tissue the skeletal funnel is pulled back. The result is that the host tissue is drawn into the sucker after which the rings of the muscle fibres in the peripheral zone contract to close around the host tissue (Fig. 4. 20A,B,C). A bud of host tissue is thus grabbed inside the sucker.

Figure 4.19

A) Scanning electron micrograph showing the opishaptor of *O. hippopotami*. Scale: 200 µm.

B) Scanning electron micrograph showing the parallel rays in the peripheral zone of the sucker of *O. hippopotami*. Scale: 50 µm.

C) Scanning electron micrograph showing the peripheral zone of the sucker of *O. hippopotami*. Scale: 30µm.

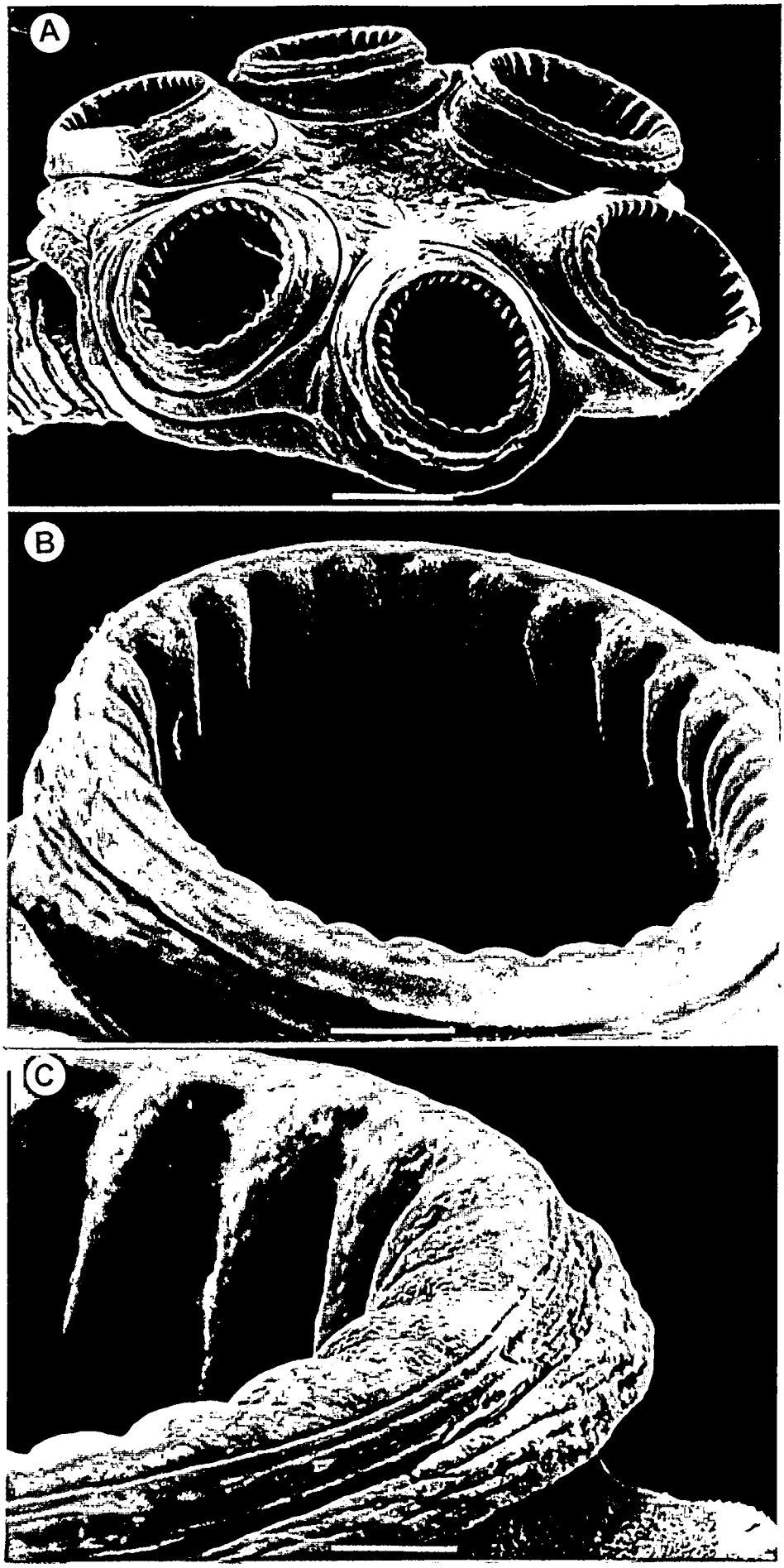

A
B
C

Figure 4.20

A) Scanning electron micrograph showing *O. hippopotami* attached to the hippopotamus eye. Scale: 500 μm.

B) Scanning electron micrograph showing buds of host tissue formed by *O. hippopotami* suckers. Scale: 200 μm.

C) Scanning electron micrograph showing a single bud of host tissue formed by a *O. hippopotami* sucker. Scale: 300 μm.

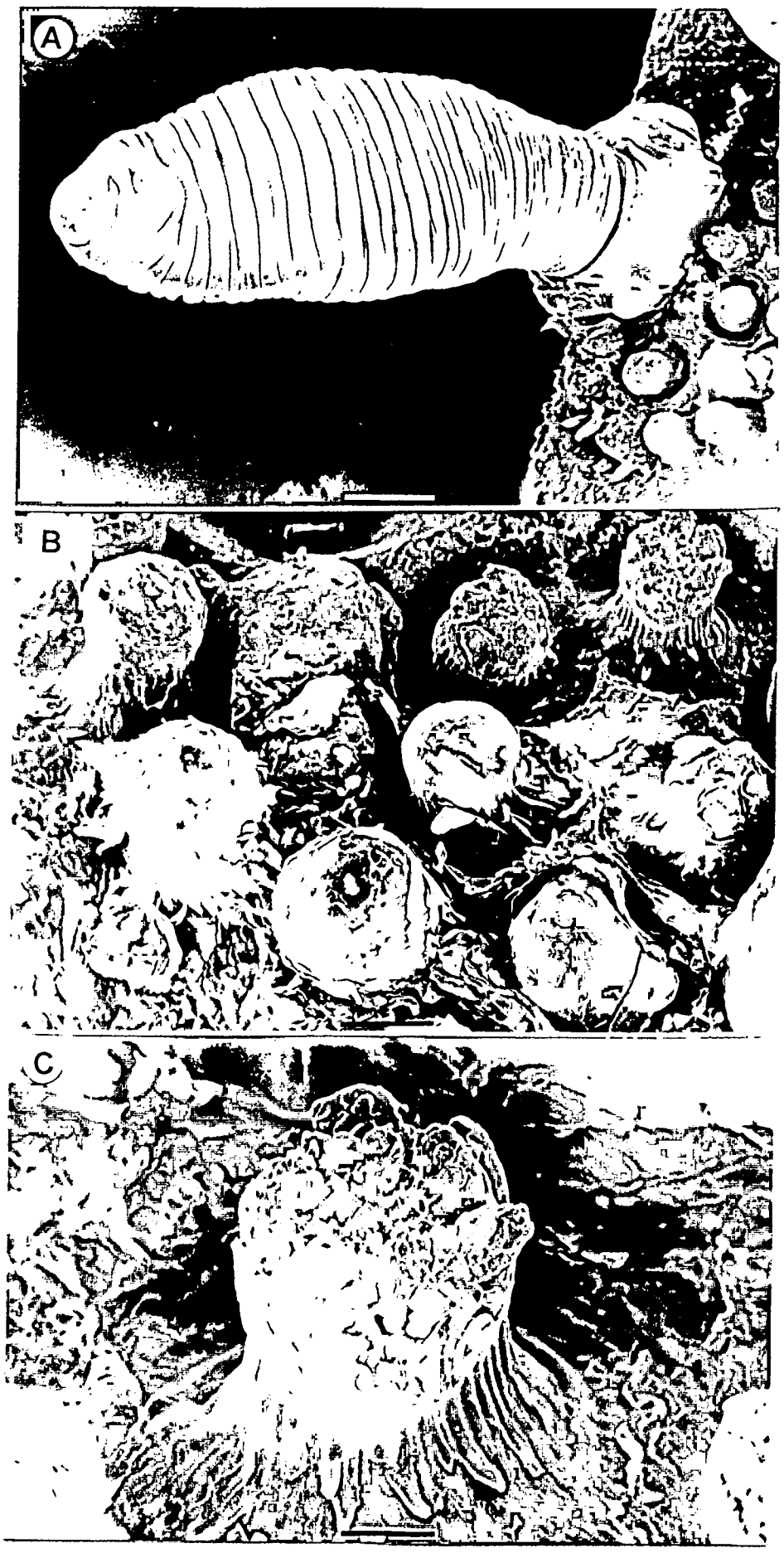

A
B
C

Figure 4.21

A) Light micrograph of a lactophenol preparation showing three annular zones of the haptoral sucker of *O. hippopotami*. Scale: 200 µm.
Abbreviations: cz, central zone; iz, intermediate zone; pz, peripheral zone.

B) Light micrograph of a section through a sucker of *O. hippopotami*, low magnification. Scale: 100 µm.
Abbreviations: ec, external costae; ic, internal costae; sd, skeletal digitae; sf, skeletal funnel; sr, skeletal ring.

C) Light micrograph of a section through a sucker of *O. hippopotami*, high magnification. Scale: 60 µm.
Abbreviation: cf, collageneous fibres; ic, internal costae; sd, skeletal digitae; sr, skeletal ring.

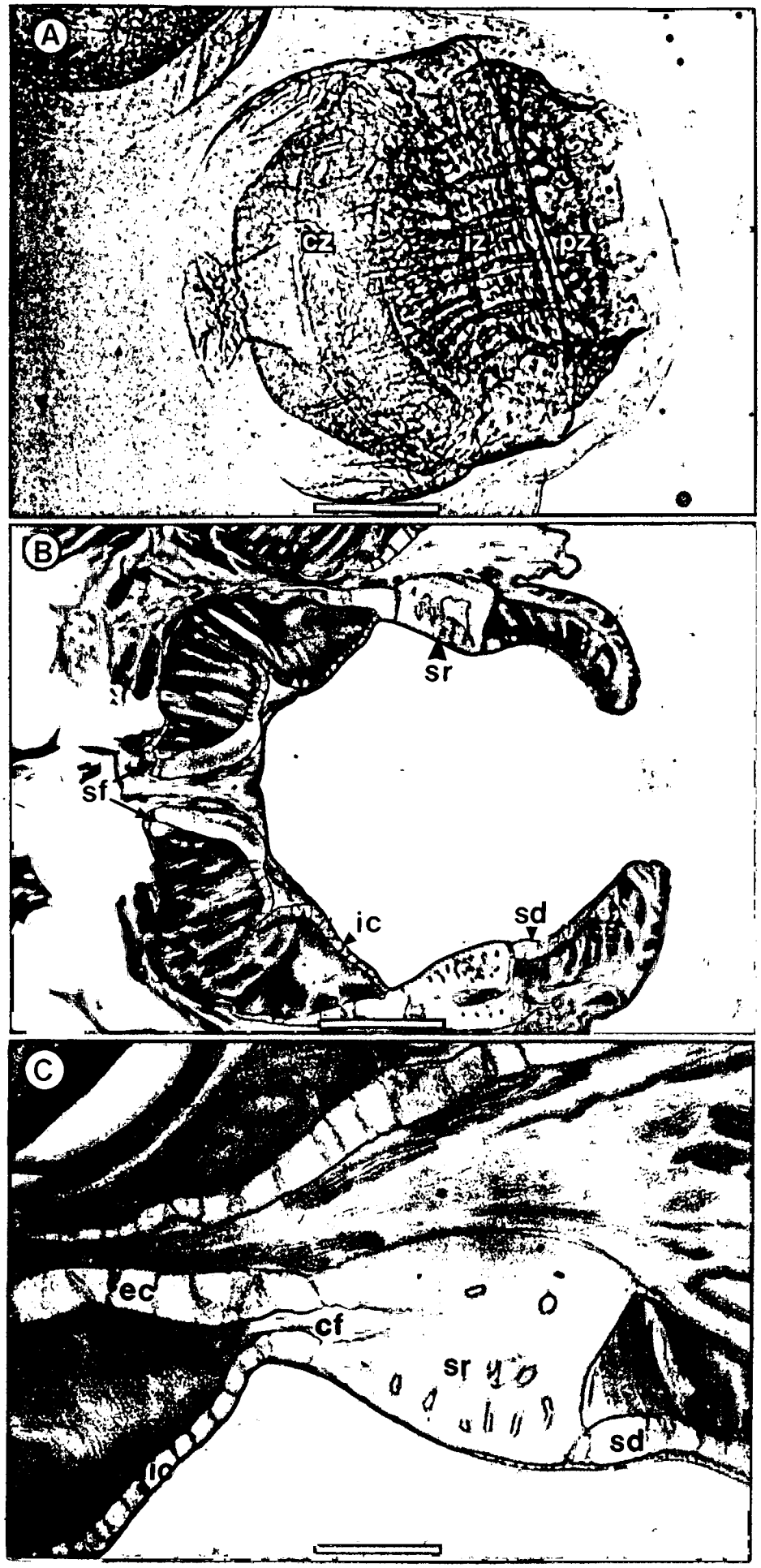

A
cz
iz
tpz
B
sr
sf
ic
sd
C
ec
cf
sr
sd
lo

Figure 4.22

Scanning electron micrographs showing aspects of haptoral suckers of *O. hippopotami* in various stages of digestion.

A) Partially enzyme digested suckers with peripheral zone still intact.. Scale: 200 µm.
Abbreviations: iz, intermediate zone; pz, peripheral zone.

B) Partially digested suckers showing perforations on the intermediate zone. Scale: 100 µm.
Abbreviations: iz, intermediate zone; pz, peripheral zone.

C) Ventral view of the sucker. Peripheral zone already detached. Scale: 100 µm.
Abbreviations: cz, central zone; sf, skeletal funnel; sr, skeletal ring.

D) Two partially digested suckers. Only the skeletal ring and skeletal funnel of the sucker on the left are visible. Scale: 200 µm.
Abbreviations: cz, central zone; sf, skeletal funnel.

E) The alternating perforations on the intermediate zone. Scale: 50 µm.
Abbreviation: pe, perforations; iz, intermediate zone.

F) Skeletal ring and skeletal funnel. Scale: 50 µm.
Abbreviation: sf, skeletal funnel.

G) The chitinous skeletal ring. Scale: 100 µm.
Abbreviation: sr, skeletal ring.

H) The chitinous skeletal ring. The canal running through the skeletal ring is clearly visible. Scale: 50 µm.
Abbreviation: ca, canal; sr, skeletal ring.

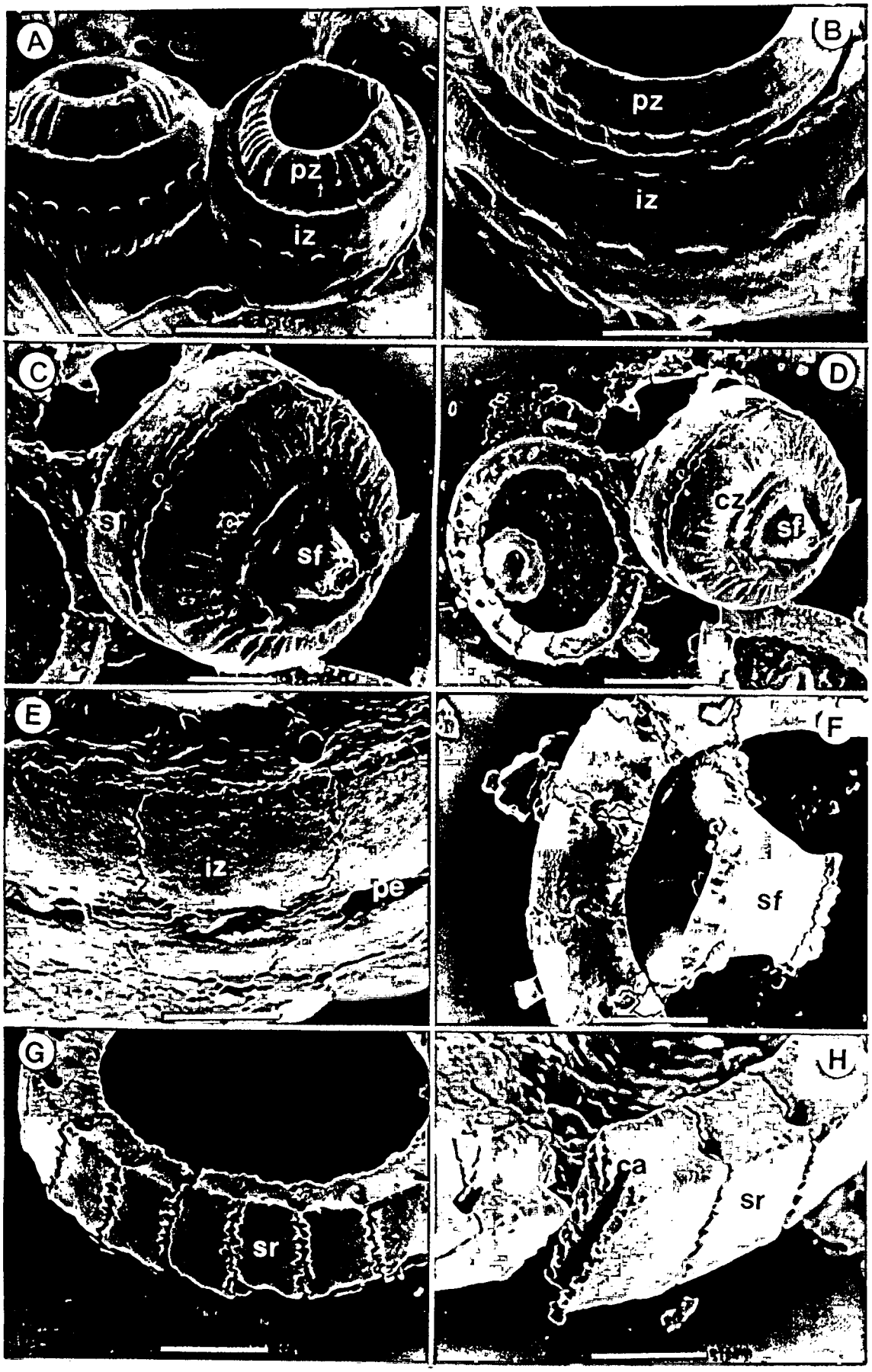

A
pz
iz
B
pz
iz
C
sf
D
cz
sf
E
iz
pe
F
sf
G
sr
H
ca
sr

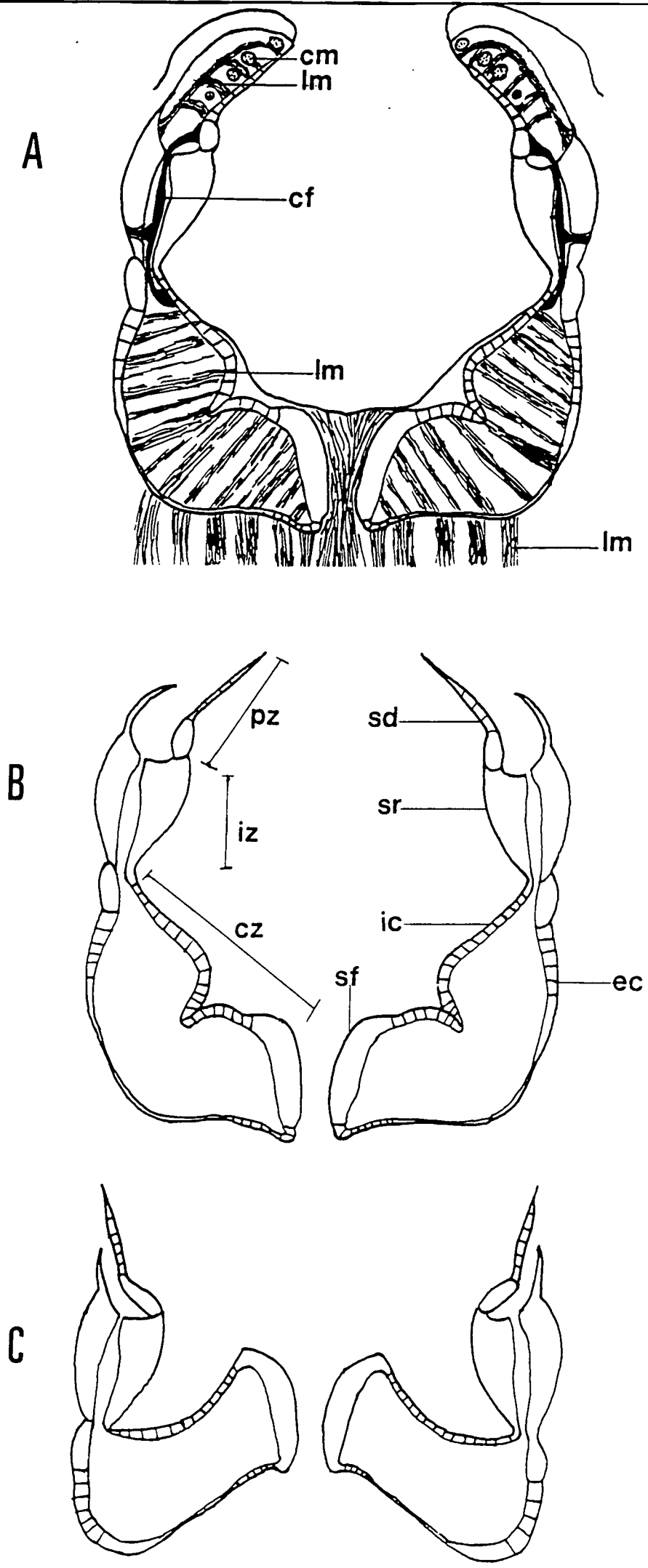
Figure 4.23

Drawings of longitudinal sections through haptoral sucker of *O.hippopotami*.

A) Drawings to show position and attachment of muscles.
Abbreviations: cf, collageneous fibre; cm, circular muscles; lm, longitudinal muscles.

B) The skeletal complex of the sucker showing the three annular zones.
Abbreviations: cz, central zone; ec, external costae; ic, internal costae; iz, intermediate zone; pz, peripheral zone; sd, skeletal digitae; sf, skeletal funnel; sr, skeletal ring.

C) Hypothesised articulation and position of skeletal complex prior to attachment to host tissue.


A
cm
lm
cf
lm
lm
B
pz
iz
cz
sd
sr
ic
sf
ec
C

4.2. LIFE HISTORY STRATEGIES OF *Oculotrema hippopotami.*

4.2.1. General.

Each living organism has a life expectancy of a certain period of time, and in this period a variety of events take place. For all these to occur, certain survival strategies are needed, and these vary between species. The key life history traits influence reproduction and survival, including growth, fecundity, life span, age to maturity and population energetics. In 1976 Stearns identified key life-history traits as brood size, size of young, the age distribution of reproductive effort, the interaction of reproductive effort with adult mortality and the variation in these traits among and individual's progeny. According to Stearns (1976) the central biological problem is not survival as such but design for survival.

4.2.2. Infection levels

Based on the examination of 38 eyes collected from 25 hippopotami culled, 92% of the hippopotami were infected in at least one of the eyes (Tables 4.4 - 4.8). A maximum of 37 parasites and 35 eggs were removed from individual hippopotami. The prevalence per eye (mean number of parasites per infected eye) for the mature parasites was 6.17 (Table 4.8), 5.19 for immature parasites and 9.85 for the total parasite sample. The abundance per eye (mean number of parasites per total number eyes examined) for the mature parasites was 4.87, 3.68 for immature parasites and 8.56 for the total parasite sample (Table 4.8). Some of the examined eyes harboured eggs, immature and mature worms.

Of the three hippopotami shot during the June 1997 visit to Ndumo, one eye was severely damaged by a bullet (Table 4.5). The lack of parasites in some eyes of hippopotami collected during December 1997 can be explained by the fact that eyes were damaged with mud during the loading process (Table 4.7). Areas where parasites had been attached could be seen on the surface of the eyeball after the mud was removed.

Table 4.4: The numbers of *Oculotrema hippopotami* in 10 eyes of dissected eyes of hippopotami collected from Ndumo Game Reserve over the period June to November 1996. These eyes were not kept in pairs.

Eye no	Number of parasites and eggs on each eye			
	Mature	Immature	Total of Parasites	Eggs
1	1	17	18	0
2	0	7	7	0
3	3	7	10	0
4	13	24	37	0
5	10	6	15	0
6	1	13	14	0
7	7	1	8	1
8	10	7	17	0
9	4	7	11	6
10	9	6	15	6
Total eyes examined	10	10	10	10
Total eyes infected	9	10	10	3
Total eggs or parasites	58	95	153	13
Prevalence	6.44	9.50	15.30	4.33
Abundance	5.8	9.5		1.30

For the total sample the prevalence for eggs per eye was found to be 10.2 and the abundance 1.34. For the hippopotami culled during June 1997 the prevalence for eggs per eye was 19.00 (Table 4.5) compared to the 0% reported for both November 1997 (Table 4.6) and December 1997 (Table 4.7). This indicated that *O. hippopotami* lays eggs during the cooler winter months.

Table 4.5: The numbers of *Oculotrema hippopotami* on eyes of hippopotami collected from Ndumu Game Reserve on 14 June 1997.

Hippopotamus no.	Sex	Eye	Number of parasites and eggs on each eye			
			Mature	Immature	Total of parasite	Eggs
1	Female	Left	5	6	11	3
		Right	7	8	15	35
2	Male	Left: damaged	-	-	-	-
		Right	0	1	1	0
3	Male	Left	0	1	1	0
		Right	0	0	0	0
Total eggs or parasites			12	16	28	38
Prevalence			6.00	4.00	7.00	19.00
Abundance			2.00	2.66	4.67	0.60

Table 4.6: The numbers of *Oculotrema hippopotami* on eyes of hippopotami collected from Ndumu Game Reserve on 03 November 1997.

Hippopotamus no.	Sex	Eye	Number of parasites and eggs on each eye			
			Mature	Immature	Total of parasite	Eggs
1	male	Left	11	3	14	0
		Right	14	3	17	0
2	male	Left: partially	4	0	4	0
		damaged	5	1	6	0
		Right				
3	male	Left	0	0	0	0
		Right	0	0	0	0
4	female	Left	1	0	1	0
		Right	3	1	4	0
5	female	Left	3	2	5	0
		Right	4	0	4	0
6	male	Left	7	3	10	0
		Right	0	0	0	0
7	male	Left	5	3	8	0
		Right	8	1	9	0
Total eggs or parasites			65	17	82	0
Prevalence			5.91	2.13	7.45	0
Abundance			4.64	1.21	5.85	0

Table 4.7: The numbers of *Oculotrema hippopotami* on eyes of hippopotami collected from the Ndumu Game Reserve on 4 December 1997.

Hippopotamus no.	Sex	Eye	Number of parasites and eggs on each eye			
			Mature	Immature	Total of parasite	Eggs
1	male	Left	2		2	0
		Right	3	1	4	0
2	male	Left	18	5	23	0
		Right	1	1	2	0
3	male	Left:damaged	-	-	-	-
		Right	0	0	0	0
4	female	Left	5	0	5	0
		Right	1	0	1	0
5	female	Left	9	3	12	0
		Right	11	2	13	0
Total eggs or parasites			50	12	62	0
Prevalence			2.25	1.33	7.75	0
Abundance			5.56	1.20	6.88	0

Table 4.8: Cumulative numbers of *Oculotrema hippopotami* collected from hippopotami culled at the Ndumu Game Reserve over the study period.

	Number of parasites and eggs on eyes			
	Mature	Immature	Total of parasite	Eggs
Total eyes infected	30	27	33	5
Total eggs or parasites	185	140	325	51
Prevalence	6.17	5.19	9.85	10.2
Abundance	4.87	3.68	8.56	1.34

4.3. BEHAVIOURAL STUDIES OF *Hippopotamus amphibius*

Due to logistic problem collection of data on the behaviour of hippopotami in the wild or under captivity conditions proved to be very difficult. A behavioural study of the relationship between a newborn hippopotamus and both parents was undertaken at the Bloemfontein Zoological Garden. The aim of this behavioural study was to observe and quantify physical contact between the adults and a newborn calf. The observations were conducted during daytime between 8h30 and 14h30. Observation periods varied from 180 minutes to 360 minutes. Observations were conducted on the first Thursday of each month for a period of six months. Results obtained during this study are presented in tables 4.9 and 4.10.

From tables 4.9 and 4.10 indications are that more time is spent in water during warm days, while during cooler days more time is spent on dry land of which at times 100% of the time is spent on dry land basking in the sun. A linear correlation was found between temperature and the time spent in water for both male and female hippopotami (Fig. 4. 24). During warmer days time spent on land was mostly during feeding. The feeding stall is on dry land and under a canopy so that feeding took place in the shade. Feeding took between 40 to 60 minutes with the male feeding the longest, at times up to about 70 minutes a day. The animals were always fed between 11h00 and 11h30 and immediately when food was placed in the feeding stall they would go and feed. After feeding the hippopotami, including the calf, always became active, moving and jumping in water for about 15 minutes. Defecation was always in water, mostly after feeding.

Table 4.9: Physical orientation and contact between one mother and calf.

DATE	TEMP (°C)	OBSERVATIONAL DURATION (min)	TIME SPEND (min)		TIME & DISTANCE BETWEEN MOTHER & CALF (min)			
			IN WATER	ON LAND	PHYSICAL CONTACT	~1m	~3	~5
09-03-98	28	360	310.49 86%	49.51 14%	8.05 2%	285.07 79%	50.07 14	17.21 5%
12-03-98	29	360	317.41 88%	42.59 12%	48.25 13%	257.19 72%	44.07 12%	10.49 3%
16-03-98	29	360	321.50 89%	38.50 11%	44.33 12%	223.00 62%	41.06 11%	52.01 15%
02-04-98	26	240	183.44 76%	56.56 24%	31.27 13%	150.02 62%	49.59 21%	9.12 4%
07-05-98	23	240	48.00 20%	192.00 80%	34.41 14	137.06 58%	51.29 21%	17.24 7%
04-06-98	20	180	- -	180 100%	-	180 100%	-	-
02-07-98	21	180	18.48 10%	161.52 90%	29.49 16%	100.27 56%	39.07 22%	11.17 6%
06-08-98	21	180	11.00 6%	169.00 94%	31.16 17%	115.33 64%	21.42 12%	12.09 7%
AVERAGE	**25**	**262**	**151**	**111**	**28**	**181**	**37**	**16**
AVERAGE %			**47**	**53**	**11**	**69**	**14**	**6**

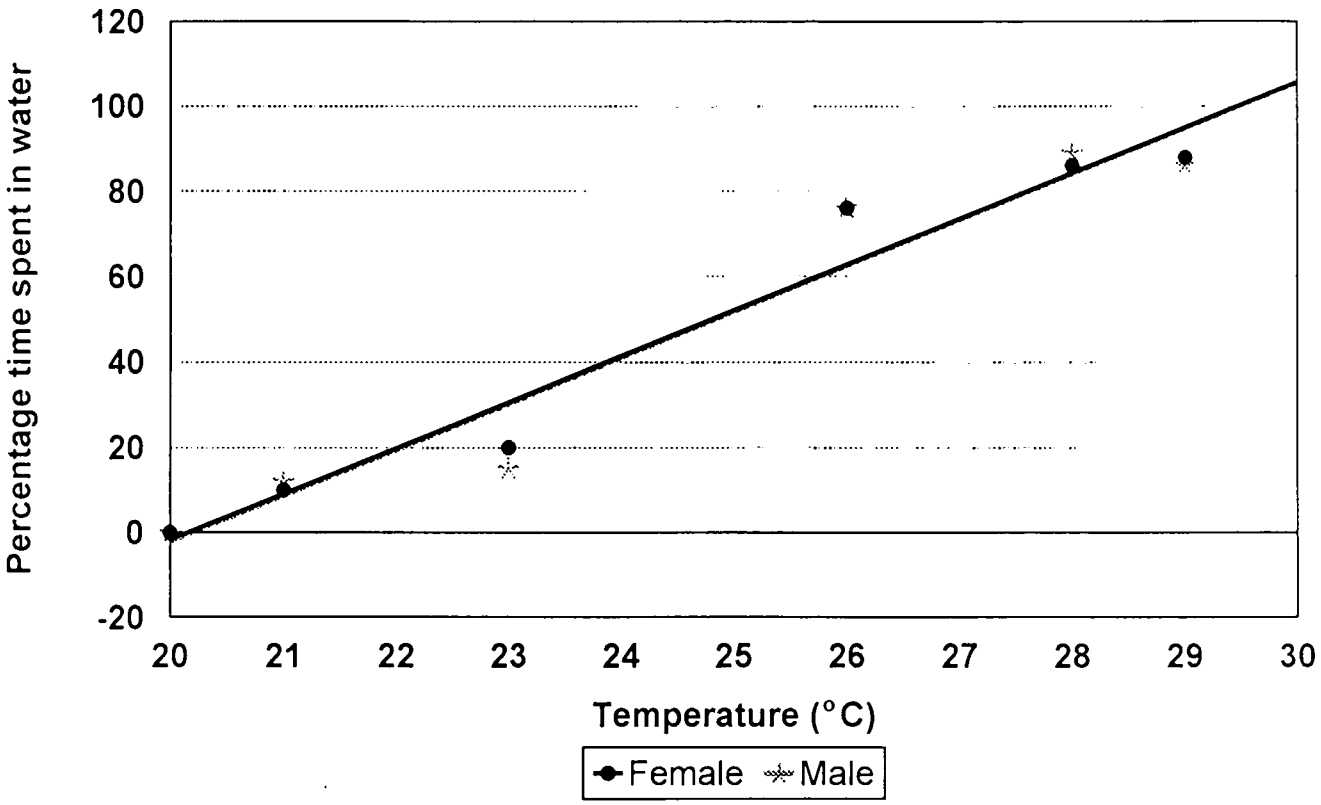
Table 4.10: Physical orientation and contact between one father and calf.

DATE	TEMP. (°C)	OBSERVATIONAL DURATION (min)	TIME SPEND (min)		TIME & DISTANCE BETWEEN FATHER & CALF (min)			
			IN WATER	ON LAND	PHYSICAL CONTACT	~1m	~3	~5
09-03-98	28	360	321.56 89%	38.44 11%	0	17.24 5%	0	343.16 95%
12-03-98	29	360	301.54 84%	58.46 16%	0	11.29 3%	0	349.11 97%
16-03-98	29	360	309.44 86%	50.56 14%	0	12.01 3%	0	348.39 97%
02-04-98	26	240	181.55 76%	58.45 24%	0	9.12 4%	0	231.28 96%
07-05-98	23	240	37.43 15%	202.57 85%	0	17.24 7%	0	223.17 93%
04-06-98	20	180	-	180 100%	0	180 100%	0	0
02-07-98	21	180	21.57 12%	158.43 88%	0	11.17 6%	0	169.23 94%
06-08-98	21	180	24.53 14%	155.47 86%	0	12.07 7%	0	168.33 93%
AVERAGE	25	262	149	113	0	34	0	229
AVERAGE %			47	53	0	17	0	83

For both male and female hippopotamus the average time spent in water was recorded as 47% compared to 53% spent on dry land feeding or basking in the sun. The average time spent by the mother close to the calf for approximately 1m was 69% of which 11% was in close physical contact (Table 4.9). On the otherhand the father was mostly within 1m distance from both the mother and the calf, of which on average time spent by the father between the two was 17% (Table 4.10). In almost all cases the calf was always in the middle of the parents.

Figure 4.24

A linear correlation graph between temperature and time spent in water for male and female hippopotami .


Percentage time spent in water
120
100
80
60
40
20
0
-20
20
21
22
23
24
25
26
27
28
29
30
Temperature (°C)
Female
Male

Chapter 5.

Discussion

CHAPTER 5

DISCUSSION

5.1 MORPHOLOGY

5.1.1 Egg

According to Tinsley (1983) the eggs of most monogeneans are exceptionally large in size and in ratio to the size of the parasite compared to the egg of other platyhelminth in general. Eggs of monogeneans vary in shape from round, ovoid, elliptical, fusoid to tetraedric. The shape of the egg is determined by the walls of the öotype (Schmidt & Roberts, 1985).

The egg of *Oculotrema hippopotami* is ovoid with no specific sculpture or attachments. Contrary to what both Stunkard (1924) and Thurston (1968b) results the present study revealed that the eggs are operculated. The operculum is however not spherical and the egg wall is thicker than in other polystomatids. The thickness of the egg-shell might be a very important adaptation, taking into account the ectoparasitic mode of living of *O. hippopotami*. The fact that the eggs were recovered from the surface of the hippopotamus' eye indicates that eggs are not necessary washed out, but may remain on the eye. The thickness of the egg wall may play a role in lowering the chances of breaking during eye-blinking.

According to Thurston (1968b) up to 62 intrauterine eggs were found in *O. hippopotami* which closely corresponds to the 58 found in the present study. Eggs were not expelled when worms were transferred from the eye to a petri dish containing river water. Present studies on the hatching of *O. hippopotami* eggs showed that eggs developed and hatched in water but not in saline. This is supported by Thurston (1968b) who also found that eggs failed to develop in saline.

5.1.2. Oncomiracidium

The oncomiracidium studied in the present study had 64 ciliated cells. This is consistent with what Thurston (1968b) reported. Tinsley (1981) reported that the number of ciliated cells varies from 55 in *Polystoma, Eupolystoma* and *Metapolystoma* to 59 in *Diplorchis* and 64 in *Protopolystoma, Polystomoides* and *Oculotrema*. Sixty four cells were also reported for *Neopolystoma* (see Pichelin, 1995).

5.1.3. Adult parasite

Morphologically *Oculotrema* is the largest known polystomatid and may reach a length of 30 mm in extended form. Among the distinguishing characteristics that are restricted to this polystomatid are the unequal length of the intestinal caecae, the restriction in distribution of the vitelline follicles and the absence of vaginae and genital spines.

Unlike other mucous feeding polystomatids, *O. hippopotami* has a dark red colour on the anterior two-thirds of its body. Thuston (1970) reported that tests indicated that the red colour was due to the fact that the parasite had haemoglobin. The haemoglobin may facilitate the transport of oxygen to tissues, and it may also act as a store of oxygen, which might be necessary for *O. hippopotami* if the worm retreats under the eyelids of the hippopotamus to escape from drying out or from excessively high temperatures (Thurston, 1970).

Indications are that *O. hippopotami* feeds on epithelial cells and mucous and that its gastroderm consists of a monotypic layer of digestive cells (Halton & Jennings, 1965). The oral morphology reflects the mode of feeding of *O. hippopotami* which probably,

like other mucous feeding polystomatids, scoops up the mucous and epithelial cells. Its subterminal flat mouth allows the parasite to feed at an angle and, in so doing, covers a large surface area.

As in the case of the chelonian parasites the single compact testis is prominent. The seminal vessicle is very prominent and packed with sperm. *O. hippopotami* spermatozoa is of typical platyhelminth form, namely, filiform and contain two axoneme (with a characteristic 9+1 microtubule arrangement), a nucleus, a mitochondrion and a cortical longitudinal microtubule. It is unclear how far each axoneme extend. One axoneme may extend farther than the other, so that the spermatozoon tapers to a point containing a single axoneme. Alternatively, the two axonemes may separate and become free from each other at their posterior tip, giving the spermatozoon a forked tail (Cable & Tinsley, 1993). From the seven sections observed by Cable and Tinsley (1993) only five sections could be identified in the present study. Sections through mitochondia only and through the mitochondria, nucleus and two axonemes could not be observed with the present transmission electron microscopical studies.

Although the sequence of events during spermatogenesis in platyhelminths is well documented, much controversy surrounds the mechanisms by which the organelles change in shape and position (Troyer & Cameron, 1980). The mitochondrial element may play a role in chromatin condensation and reshaping of the nucleus (Martinucci & Felluga, 1979). In platyhelminths, microtubules are never directly associated with the nucleus so it is unlikely that they are involved in nuclear shaping, but Erwing and Halton (1983) suggested that the cortical microtubules of *Bucephaloides gracilescens* may play a role in the movement of the nucleus and the mitochondria. The only other record of the

mitochondria associated with the nucleus of the platyhelminth spermatozoa is the capsalids and dionchids (Justine & Mattei, 1983, 1987). However, Justine and Mattei (1985) considered that tubules are involved in the motility of aflagellate spermatozoa are still doubtful (Justine, Ponce De Leon & Mattei, 1987).

The ovary was found to be very small and inconspicuous with relatively few oocytes. In both the present and Thurston and Laws' 1965 studies, the ovary was found to be situated mostly at the same side of the longer caecum whereas in Stunkard's 1924 original description the opposite was found.

The presence of muscle fibres directly under the tegument enhances the flexibility of the general body of the organism. The muscle arrangement shows signs of working antagonistically. This is probably an advantage because the flexibility of the parasite may help in positioning itself or change its shape to avoid being washed off during eye-blinking of the host.

The remarkable character which is unique to this parasite is its ability to stretch out which enables the parasite to feed on mucous in a fairly large surface area surrounding it. The mid piece has well developed muscle fibres which enhance the flexibility and the elasticity of the parasite considerably. At the position where the mid-piece meets the opishaptor the presence of well developed circular muscles enhance flexibility. This extensive musculature in the mid piece of the parasite is unique among polystomatids. This, together with the complete absence of any other organs in the pre-haptoral region, enables the parasite to stretch out and feed over a large surface area. *O hippopotami* is usually found in clusters on the eye and indications are that they do not detach the

opishaptor when feeding. Large hamuli are completely absent and attachment is achieved by the suckers, which also contain a supportive skeletal complex. It was also noticed that the parasites have a very firm grip on the host tissue and it was quite difficult to remove parasites. The skeletal support in the sucker and the hypothesised mechanism involved, ensure a very firm grip on the host tissue.

In 1961 Williams pointed out that *O. hippopotami* and the polystomatids of chelonians differ from the parasites of Anura, in possessing elaborately cuticularized suckers which are divided into three annular zones. She suggested that this may reflect a common original stock, distinct from that ancesteral to the parasites of Anura. With the new knowledge of the sucker of *O. hippopotami* it remains to be seen if the chelonian parasites and *Concinnocotyla* have the same type of suckers.

5.2 NATURAL OCCURRENCE OF *O. hippopotami.*

In the present study 92% hosts examined were infected with *O. hippopotami* in at least one of the eyes. This is consistent with Thurston (1968a) who found that of 42 hippopotami investigated in Uganda 90.5% were infected. This high infection level is exceptional among polystomatids and indicate a very effective mode of parasite transfer. No intra-uterine development of the eggs were observed. Incubation took 20 days at 30^0C and will probably be longer in natural waterbodies. The chance of an oncomiracidium finding a hippopotamus within its limited life span is extremely remote. If an oncomiracidium does make contact with a hippopotamus the chance to locate the eye on the massive host seems to be very remote.

Eggs found imbedded in the mucous on the eye indicate that eggs may develop on the eye of the host. As many as 35 eggs were found on a single eye during the present study. Emerging oncomiracidia then probably attach to the surface of the eye. In 1968a Thurston suggested that where both mature and immature worms are found on the same eye, the small immature worms are the result of subsequent re-infections and not of the development *in situ* of eggs from the mature *O. hippopotami*. If this was possible, how is the parasite then transferred to another host? Observations in the Zoological Garden revealed a very close contact in water between both parents and the calf. According to Tinsley and Owen (1975) the period spent by the host in water provides the only opportunity for the release of parasite infective stages and for invasion of new hosts. Mother and calf were observed to spend long intervals in direct physical contact of the heads. In the present study it is hypothesised that the parasite may crawl out of the eye along the water line to another host individual and establish on the eye. This hypothesis may sound far fetched but is supported by observed time spent between hippopotami and the calf, close physical contact and by Thurston (1968a) who reported a very high infection level for hippopotamus calves. This could indicate that hippopotami may not be as susceptible to infection when they become older or that the transfer of the parasites from mother to calf is extremely effective. Heavy infections appear to irritate the host, which reacts by blinking its eyes frequently (Thurston, 1968a).

5.3 PHYLOGENY

Because of a lack of palaeontological evidence it is exceedingly difficult, if not impossible to follow the evolution of parasitic platyhelminths. Estimates of the antiquity of monogeneans have been made by Bychowsky (1957) and by Llewellyn (1982). Bychowsky regarded the main groups of monogeneans as having arisen in the Mesozoic period while Llewelllyn offered evidence for much earlier divergence in the Palaeozoic era. Thus, there is every likelihood that monogeneans appeared very early in the history of vertebrates and certainly long before the modern groups of fishes emerged.

According to Prudhoe & Bray (1982) platyhelminths probably evolved from rhabdocoelid- like turbellarians having facultative commensal associations with various aquatic invertebrates. This association possibly became obligatory. It can be accepted that fishes, as the first vertebrates, preyed on these infected invertebrates and became infected themselves. These rhabdocoelid- like turbellarians probably genetically already had the potential to survive in the intestinum, buccal cavity, gill chambers or on the surface of the host. The next important event in the evolutionary development probably was the development of a posteriorly situated attachment organ, the opishaptor.

Attachment may have been achieved at first by the secretion of adhesives, which are in widespread use for temporary attachment to substrates in a range of modern, free-living platyhelminths, especially those inhabiting the interstitial environment of the bottom sediment (Rieger, Tyler, Smith and Rieger, 1991). Bychowsky (1957) postulated that the first accessory attachment organ was in the form of sclerotised hooks while Llewellyn (1970) suggested that suckers developed first. However, both authors agreed that the

important developments took place in the opishaptor and that the larval sclerites are important as systematic character. Perhaps initially the larval sclerites served as firm internal attachment sites for muscles, later becoming partly external and acquiring a secondary role for attachment to the host. Whatever their origins, the acquisition of hooks was the single most important achievement of these early parasites and they became the "hallmark" of monogeneans and central to their subsequent evolutionary expansion and success.

The development of permanent associations between early monogeneans and their hosts had a number of important consequences. First, it conserved energy, since parasites no longer needed to search for a host every time they needed a meal. Another consequence of permanent attachment to the host was that the parasite provoked the host's anti-parasite defences. If the host is capable of generating a localised response then sedentary parasites might be particularly vulnerable and this may have exerted additional pressure on monogeneans to move regularly to different sites within the host.

In the phylogeny of the Polystomatidae a strategy developed which enabled the parasite on the aquatic tadpole to leave the aquatic habitat when the host undergoes metamorphosis. This evolutionary event led to further adaptive radiation in the various hosts with varying degrees of water dependency. This diversity of survival strategies from primarily aquatic to arid adapted hosts make the Polystomatidae a unique group of parasites to study.

The chelonians have acquired their polystomatids by the phylogenetic route from their amphibian ancestors and this would imply closer affinities between anurans and

chelonians than either of these groups has with the urodeles, as Llewellyn (1963) has pointed out. However, Tinsley (1981) took a different view, advocating host-switching from primitive, aquatic, pipid anurans to the chelonians and to the hippopotamus. A feature shared by the reptilian and mammalian polystomatids is the abandonment of the haematophagous habits of their ancestors and adoption of a diet of epithelial cells and mucus, a change that appears to reflect increased thickness and toughness of the epithelia lining, their habitats and the inaccessibility of blood capillaries (Allen & Tinsley, 1989). Alternatively, this epithelial diet, common to the polystomatids of reptiles and that of the hippopotamus, might reflect a common ancestry for the parasites, with *Oculotrema* owing its existence to host-switching from chelonians rather than from anurans. Indeed, epithelial-feeding polystomatids may have been better placed to survive on the hippopotamus than blood-feeders.

The striking similarity of the haptoral sucker between *O. hippopotami* and the Polystomoidinae genera, suggests a very close relationship between the two. They both have what Pichelin term "haptoral sucker type 2" (Pichelin, 1995), which consists of muscular cups with a continuous lining. The lining projects into the side of the cup, dividing the musculature into halves and forms an inner equatorial groove. The composition of the lining is unknown but appears to be soft keratin because it is slightly yellow and opaque (Pichelin, 1995). In addition to the haptoral sucker, another similarity is the presence of 64 ciliated cells on both *Protopolystoma xenopodis* (see Tinsley, 1976, 1981) and *Oculotrema hippopotami* (see Pichelin, 1995). Thus it was already suggested that the arrangement of these cells points towards a relationship between polystomatids from turtles, the hippopotamus and pipid toads (Tinsley, 1983). It remains to be determined if the number of ciliated cells on the oncomiracidia is more informative than

the morphology of the haptoral sucker and thus if *O. hippopotami* is more closely related to frog polystomatids or turtle polystomatids.

A hypothesised close relationship between chelonian parasites and *O. hippopotami* is supported by a molecular study conducted by Neeta Sinnappah[1]. (personal communication, 1998).

Based on the above, and reports of serrated hinged terrapin resting on the back of a sleeping hippopotamus (Pienaar, Haacke & Jacobsen, 1983), one may argue the possibility of transfer of a terrapin polystome to the hippopotamus which later evolved and adapted to the particular environment, provided by the hippopotamus' eye.

The hosts of polystomatids vary in degree of ecological association with water. The period spent by the host in water provides the only opportunity for the release of parasite infective stages and for invasion of new hosts. Variation in the frequency and duration of entry into water govern the life cycle strategies of various polystomatid genera. At one extreme *Xenopus*, the African clawed toad , is totally aquatic, so transmission of its polystomatid (*Protopolystoma xenopodis*) can occur virtually continuously (Tinsley and Owen, 1975). For amphibious hosts, such as many chelonians and the hippopotamus, there is a regular diurnal alteration of aquatic and terrestrial periods (the hippopotamus out of water at night to browse, turtles out of water by day to bask in the sun), so transmission is likely to be a diurnally process. For *Polystoma*, in species of *Rana* etc., transmission is typically vertical with parasites in adult frogs depositing eggs during host spawning but the eventual targets of invasion being the tadpole offspring. In most cases

[1] Ms Neeta D. Sinnappah, Laboratoire de Biology Animale , Université de Perpignan , Perpignan, France.

where the ecology of the anuran host is likely mesic, the transmission of its polystomatid parasites is tied to the one period when the host can be guaranteed to enter water, i.e. for spawning (Tinsley, 1990).

5.4. POSSIBLE FURTHER STUDIES.

Aspects that need to be studied in more depth include:

- The morphology of the egg wall of *O. hippopotami*. The egg wall is exceptionally thick and the nature and selective advantage of this need to be investigated.
- The morphology and chemical nature of the intestinal gastrodermis needs to be studied at transmission electron microscopical level. This might reveal valuable information on the diet of *O. hippopotami.*
- Sperm morphology needs to be studied at TEM level in order to be able to study the phylogeny of the parasite.
- A comparative study of the haptoral sucker morphology within the Polystomatidae will most certainly reveal very interesting results.
- Development of the *O. hippopotami* oncomiracidium.
- Longevity, swimming performance and tactile behaviour of the *O. hippopotami* oncomiracidium might shed some light on the host infection strategies of this parasite.
- The physiological conditions including pH, temperature, and salinity on the eye of a hippopotamus needs to be established. This might assist us to understand the interhost transfer of the parasite.

Chapter 6.

Summary

CHAPTER 6

SUMMARY

Being the only monogenean known from a warm-blooded animal and from a mammal *Oculotrema hippopotami* Stunkard (1924) took a major leap in monogenean evolution. After its description in 1924 various researchers rejected the claim that it came from the hippopotamus and made it out as a mislabeled specimen. It was only 40 years later that this parasite received full recognition. In spite of the fact that it was described more than seven decades ago, only a few papers on this parasite have seen the light. During 1996 this parasite was rediscovered in South Africa. A hippopotamus culling program in Kwazulu-Natal gave an opportunity to study this parasite.

The present study is the first detailed attempt to study the morphology and life history of *Oculotrema hippopotami.* The approach in this study was as follows:

1. Background on the host's morphology, behaviour and phylogeny is given. The hippopotamus' eye is situated deep in the orbit with the result that a deep crevice is present all around the eye, which serves as habitat for the parasites.

2. The external morphology of the egg, oncomiracidium and adult parasite was studied using scanning electron microscopy. This is the first ever scanning electron microscopical study of *O. hippopotami* and this study revealed many new information.

3. The internal morphology of the adult parasite was studied histologically using wax sections. This revealed unique musculature in the mid piece that has never before been reported for any polystomatid parasite.

4. Sperm morphology and the ultrastructure of the musculature in the mid piece was studied at transmission electron microcope level. Indications are that the sperm morphology is very similar to that reported for other polystomatids.

5. Infection levels for different seasons were compared. In contrast with most other polystomatids that reproduce during the warmer summer months, *O. hippopotami* lays eggs during the cooler winter months.

6. The parental care and behaviour of a pair of hippopotami with a newborn calf were studied. A very close bond with long periods of physical contact was observed. This could give an ideal opportunity for parasite transmission from mother to calf.

Chapter 7.

References

CHAPTER 7

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Chapter 8.

Appendices

APPENDIX 1

Moeng, I.A. & Du Preez, L.H. (1997). Oral sucker morphology of two polystomatids (Monogenea): One blood feeding and one mucous feeding. *Microscopy Society of Southern Africa*. **27**: 116.

ORAL SUCKER MORPHOLOGY OF TWO POLYSTOMATIDS (MONOGENEA): ONE BLOOD FEEDING AND ONE MUCOUS FEEDING

I.A. Moeng and L.H. du Preez

Department of Zoology and Entomology, University of the Orange Free State, Bloemfontein, South Africa

Monogeneans are primarily parasites of "cold blooded" vertebrates. The great majority infest marine and freshwater fishes, living on the skin, fins and gills, or exceptionally, in the stomach, intestine, cloaca or body cavity. One family, the Polystomatidae, occurs on a diverse assemblage of hosts, including lungfish, anuran and urodele amphibians, chelonians and one mammal, namely, the hippopotamus.

Mucous and blood feeding polystomatids differ fundamentally in terms of diet and gastrodermal organisation. Mucous feeding polystomatids feed on epithelial cells and mucus[1] and their gastroderm consists of a monotypic layer of digestive cells[2]. By contrast blood feeding polystomatids feed on blood[1,2] and the gastroderm consists of two components, namely, digestive cells and the connecting syncytium which forms a sheet-like lining over the caecal wall[3]. The divergence in diet amongst polystomatid monogeneans may reflect differences in habitat conditions. The blood feeding polystomatids occur in a delicate well-vasculated urinary bladder and feed on blood. The habitat occupied by mucous feeding polystomatids has few capillaries accessible to a surface-browsing parasite. The difference in diet may also be associated with the major difference in intestinal morphology in blood-feeding and epithelium-feeding groups within the Polystomatidae. Blood feeding polystomatids have more or less profusely branched intestinal caeca with diventricula[3]. In contrast the mucous feeding polystomatids possess two simple unbranched tubular caeca.

The mouth of blood feeding polystomatids is circular, terminal, slightly ventrally orientated and separated from the body parenchyma (Figs. 1 & 2). The anterior lip is a slightly larger and thicker but both lips are more or less of equal length (Fig 1). Comparatively the oral sucker of a mucous feeding polystomatid is dorsoventrally flattened, subterminal and not sharply separated from the body parenchyma (Figs. 3 & 4). The anterior lip is thicker and much longer than the lower lip (Fig. 4).

The oral morphology closely reflects the mode of feeding. Blood feeding polystomatids suck a part of the host tissue into the oral cavity, the tissue ruptures and blood is drawn from the tissue. This requires a firm grip and suction which can only be acquired with a circular oral sucker. Mucous feeding polystomatids scoop up mucous and epithelial cells. The subterminal flat mouth allows the parasite to feed at an angle and, in so doing, cover a large surface area. One would expect that feeding on mucous would not require a firm grip and suction.

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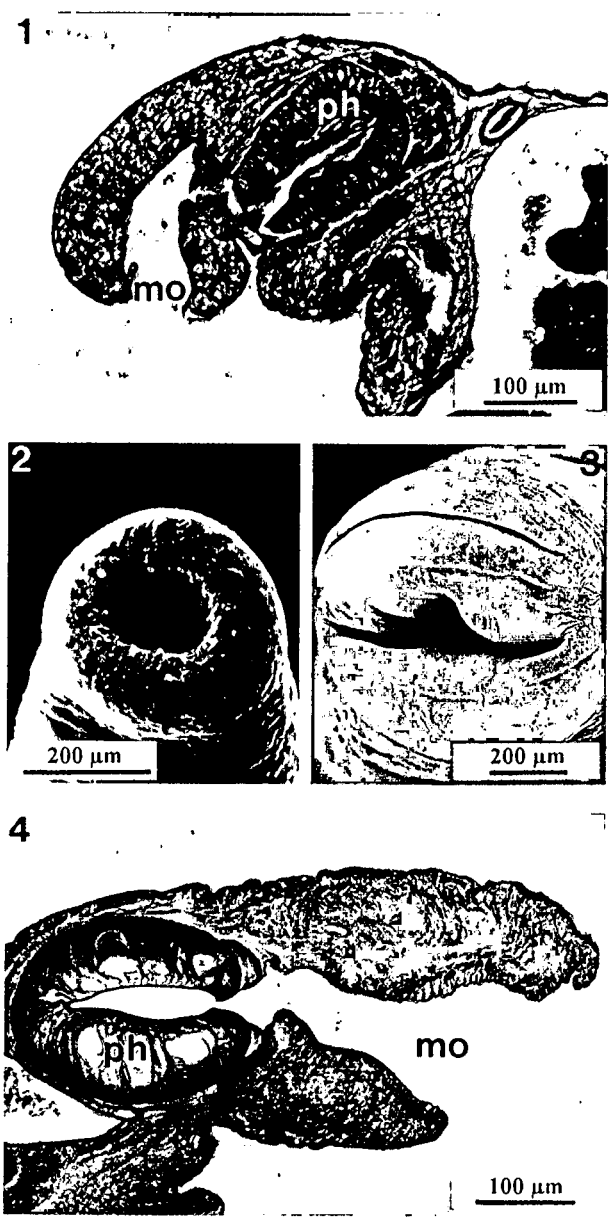

Fig. 1. Longitudinal section through oral region of *Eupolystoma sp.* Mouth (mo), pharynx (ph).
Fig. 2. Mouth of *Polystoma australis.*
Fig. 3. Mouth of *Oculotrema hippopotami.*
Fig. 4. Longitudinal section through oral region of *O. hippopotami.* Mouth (mo), pharynx (ph).

APPENDIX 2

Moeng, I.A., Kruger, J., Cooper, S. & Du Preez, L.H. (1998). Unique musculature found in *Oculotrema hippopotami* (Monogenea: Polystomatidae). . *Microscopy Society of Southern Africa.* **28**: 83

UNIQUE MUSCULATURE FOUND IN *Oculotrema hippopotami* (MONOGENEA: POLYSTOMATIDAE)

I.A. Moeng[1], J. Kruger[2], S. Cooper[2] and L.H. du Preez[1]

[1] Department of Zoology and Entomology, [2] Department of Anatomical Pathology,
University of the Orange Free State, Bloemfontein, South Africa

Monogeneans are mostly parasitic in fish but one family, the Polystomatidae, has radiated onto the tetrapods. Most polystomatids are found in amphibians but they also infect chelonians, the Australian lungfish and the hippopotamus.

Oculotrema hippopotami (Fig 1) is a monotypic genus and the only monogenean known from a warm-blooded animal namely the hippopotamus[1]. *O. hippopotami* is found under the nictitating membrane, under the eyelids and on the anterior face of the eyeball of the hippopotamus where they often occur in clusters[2]. These parasites are believed to feed on the mucous on the surface of the eye.

To avoid being whipped off during blinking of the eye the parasites do not wander around but attach firmly and apparently remain in that position. *O. hippopotami* is equipped with six extremely well developed suckers (Fig. 1) which ensures a firm grip[3].

For histological sectioning live parasites were fixed in Bouin's fixative, imbedded in paraffin wax and routinely sectioned at 4 µm. Sections were stained in hematoxylin and eosin. In order to study the nature of tissue of the mid-piece, live *O. hippopotami* were fixed in buffered glutaraldehyde, rinsed in sodium cacodylate buffer and post-fixed in osmium tetroxide. Material was embedded in Spurr's epoxy resin. Ultra-thin sections (80 nm) were cut, stained and examined in a Philips 301 transmission electron microscope.

A remarkable character of this parasite is its ability to stretch out. In contracted form *O. hippopotami* has a length of about 11 mm, while it can stretch out to a length of more than 30 mm. This enables the parasite to feed on the mucous in a fairly large area surrounding it. In all other known polystomatids the intestine extends up to the opisthaptor. In some parasites the testis or the ovary, may be situated posteriorly in the body. However in the case of *O. hippopotami* the mid-piece area just anterior to the opisthaptor (Figs. 2,3) lacks any organs and is extremely elastic.

Just anterior to the opisthaptor the parasite has a column of well developed bundles of longitudinal muscles gradually tapering to the sides (Figs. 2,3). At the position where the mid-piece meets with the opisthaptor bundles of well developed circular muscles were observed.

Ultrastructural examination of the mid-piece revealed structures resembling bundles of smooth muscle (Fig. 4). The muscle bundles are approximately 7 µm in diameter. The muscle has thick filaments with dense bodies randomly spaced (Fig. 5).

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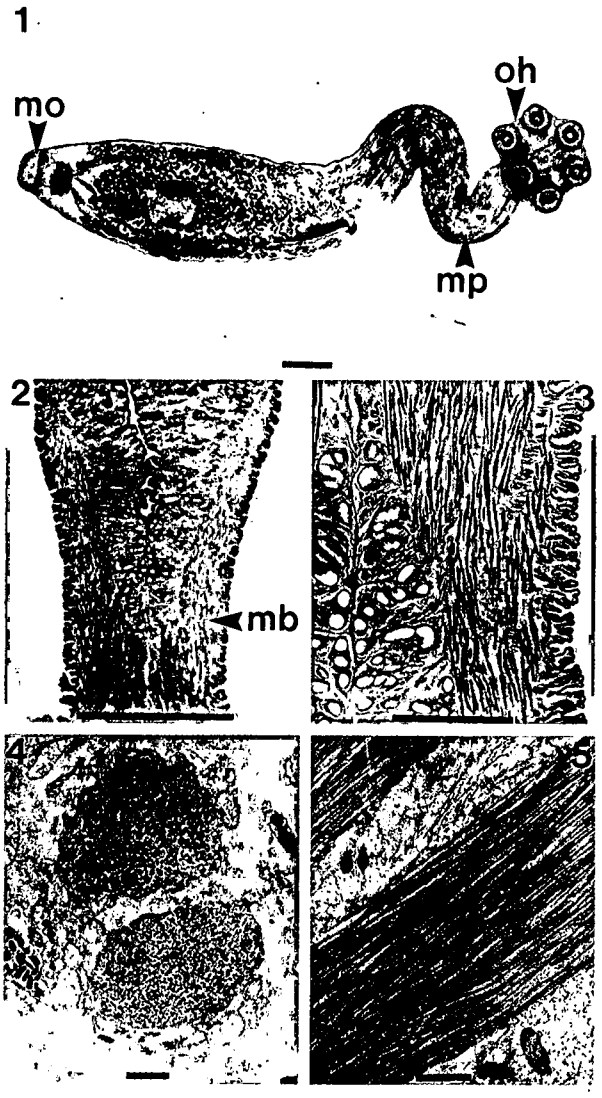

Fig. 1. Light micrograph of *O. hippopotami* showing the mouth (mo), mid-piece (mp) and opisthaptor (oh).
Fig. 2. Light micrograph of a plain section through the parasite showing the column of longitudinal muscle bundles (mb).
Fig. 3. Light micrograph of the mid-piece.
Fig. 4. TEM micrograph of a cross-section through muscle bundles.
Fig. 5. TEM micrograph of longitudinal section through muscles.
Scale bar: 1mm (1,2), 30µm (3), 1 µm (4,5).