SNAIL BORNE LARVAL TREMATODES OF THE OKAVANGO DELTA, BOTSWANA

By

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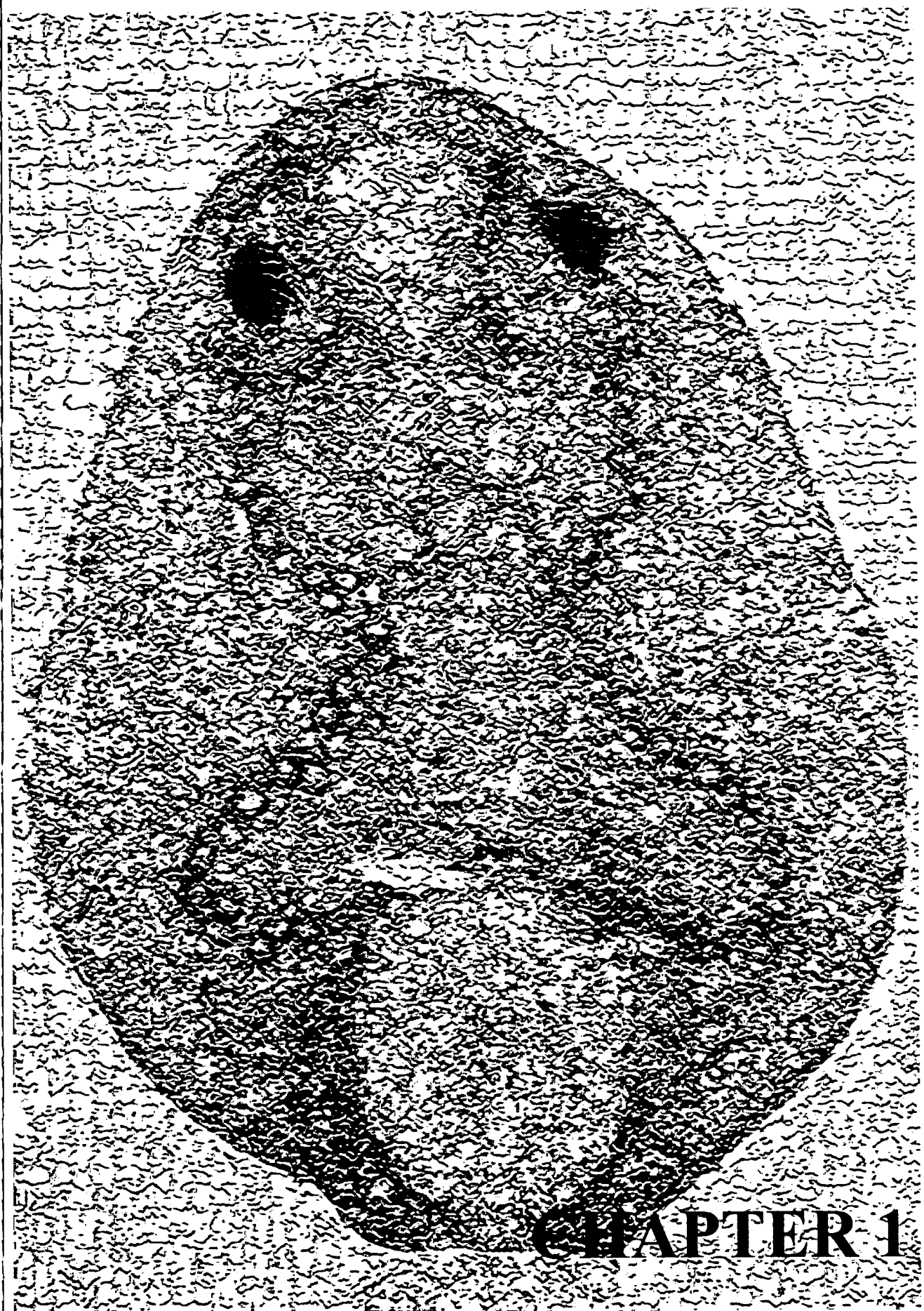


CHAPTER 1

INTRODUCTION

The Okavango Delta, which is situated in northwestern Botswana, is a vast and beautiful expanse of waterways and floodplains. The Okavango River has its origin in the Angolan Highlands as two streams namely the Cuito and Cubango. These streams which first flow south and south eastwards finally join to form the Okavango River that enters Botswana as a single wide meandering river near Mohembo at the Namibian border (Bailey 1998). As the gradient levels out at the edge of the Kalahari, the river spreads out as it reaches the Gumare fault, into an enormous alluvial fan. Within the fan is a mosaic of limpid streams, fringes with papyrus reeds and tall grasses, pools adorned with water lillies, and islands on which riverine forests abut against dry–land savannnah.

The Okavango River and Delta is increasingly being recognised as an important resource for the region and the world. More than 150 000 people living in Namibia and more than 100 000 living in Botswana depend on the Okavango in an otherwise harsh landscape. Over 70 percent of riparian community households collect water directly from the Delta in the dry season, 75 percent of households collect fish, edible or medicinal plants from the Delta, and nearly 20 percent of households conduct farming in the Delta floodplains. The Delta also supplies materials for building homes and making tourist crafts.

Due to its remoteness from modern developments and its variety of habitats, the Okavango Delta draws an extensive and diverse array of life forms, from the tiniest insect to the largest mammal. There are 68 known fish species of which 23 are endemic to the upper Zambezi System. During the past few years there have been reports by local fisherman and fisheries scientists of the decline of fish populations and massive fish kills within lagoons (Merron 1991). Due to this problem, scientists from the University of the Orange Free State, under the leadership of Prof. J.G. van As, submitted a project proposal to the Minister of Agriculture, Botswana expressing their concern for the declining fish populations.

In 1997 the Okavango Fish Parasite Project was approved by this Ministry and financial assistance was given by the donation fund of the DEBSWANA Diamond Company, Botswana. Additional financial aid was given by the National Research Foundation, South Africa under the inland resources programme. Vehicles were sponsored by Land Rover South Africa.

The aim of the project was to determine the biodiversity and distribution of fish parasites within the system, and whether these parasites may affect the health status of the fish. This study on fish parasites is the first work ever to be done in the Okavango Delta.

A number of publications have already appeared in well-known journals which form part of the results of the Okavango Fish Parasite Project and are as follows: Van As & Van As (1999); Smit, Davies & Van As (2000); Two masters dissertations: Christison (1998); Reed (2000). A number of conference contributions have also been made: Christison & Van As (1999); Christison, Van As & Basson (1999); Christison, Reed, Smit, Basson & Jansen van Rensburg (1999); Jansen van Rensburg, Basson & Van As (1999); Reed & Van As (1999); Reed, Kruger, Van As & Basson (1999); Van As, Van As & Basson (1999); Jansen van Rensburg, King & Van As (2000); Reed, Basson & Van As (2000); Reed, Smit, Christison & Basson (2000).

When studying these fish parasites, one can't overlook the snail populations, which also form part of the ecosystem. These snails are known to be vectors for a variety of trematode parasites. Digeneans are heterogenous groups of parasites, which have more than one host within their life cycle (Smyth 1994). Snails serve as the intermediate hosts while the final hosts are usually vertebrates, in which adult trematodes develop. Due to the various forms within the cercariae, grouping of these digeneans into families and genera has been a great source of frustration to many taxonomists, and still today the systematics of this group is a conundrum.

One of the most important trematode diseases affecting humans is, schistosomiasis, formerly known as bilharzia which is the second most important disease after malaria, occurring in subtropical Africa. Schistosomiasis is a parasitic infection of various mammals including man and domestic livestock, caused by 'blood flukes' of the

genus *Schistosoma*. *Schistosoma haematobium* (Bilharz, 1852), which causes urinary schistosomiasis and *Schistosoma mansoni* Sambon, 1907 causing intestinal schistosomiasis in man are the most common forms occurring in southern Africa (Brown 1994).

Besides these two forms of schistosomiasis, there are other trematode diseases, although not as well known, that occur in southern Africa and infect birds, wildlife and domestic animals. Liver fluke disease in sheep caused by *Fasciola hepatica* and paramphistomiasis in cattle caused by *Calicophoron microbothrium,* are also known to be of economical importance to humans.

The main reason therefore in studying the snail faunas of the Okavango Delta is to get an overview of the kinds of larval trematodes that are present in such an unique system

The present study therefore was undertaken to address the following specific objectives:

- **To determine which species of freshwater snails occur in the system**
- **To compile a data base on the occurrence of cercariae infecting freshwater snails in the Okavango system and southern Africa**
- **To determine whether human schistosomiasis occurs in the system**
- **To contribute to the results and findings of the Okavango Fish Parasite Project.**

The layout of this dissertation will be in the format where results and discussions will be given throughout the thesis: In **Chapter 2** the Okavango delta will be discussed with respect to the source of the river, the annual flood, limnological characters, ecological regions and biodiversity of animals and plants occurring in the system. **Chapter 3** explains the material and methods used. Collection localities will be discussed and represented by micrographs and maps, various methods employed to collect the cercariae and snails will be discussed as well as the further preparation of material for various microscope techniques. **Chapter 4** gives a brief history of digenean classification, some biology and general characteristics of the subclass Digenea and the different life cycle stages will be discussed with special references to

the different types of cercariae. In **Chapter 5** the freshwater snails of the Okavango delta and factors which influence the distribution of snails in a specific area will be discussed. In **Chapter 6** cercarial descriptions of the various cercarial types found in the delta will be presented and discussed, mention will also be made of the respective families. In **Chapter 7** the statistical information obtained with respect to cercarial infections will be addressed to try to come to some form of conclusion about the parasite-host associations. In conclusion **Chapter 8** will give the probable life cycles of each of the different cercarial species and schistosomiasis will be discussed in brief.

CHAPTER 2

THE OKAVANGO RIVER & DELTA

The Okavango Delta, situated in the northern reaches of Botswana, is one of Africa's last great-unspoiled wildernesses. It is an unexpected oasis, a glittering expanse of crystal clear waterways, lush green papyrus and fertile floodplains, surrounded on all sides by arid semi-desert and Kalahari sandveld.

The Okavango Delta System is hydrologically unique, the largest inland delta in sub-Saharan Africa after the inner delta of the Niger. Since it lies in a semi-arid area, 97% of the annual flow of between 7000 and 15000 million cubic meters is lost through evapotranspiration and seepage. Only 3% of the water is discharged from the delta.

THE SOURCE OF THE OKAVANGO DELTA

Rising on the Benguela Plateau in the highlands of central Angola is the Okavango, southern Africa's third largest river, and the largest in the world that does not flow into the sea. It begins life as two tributaries, the Cuito and Cubango. Spurred by huge subtropical storms, the Cubango River rises in central Angola, flows through Namibia as the Kuvango and finally enters Botswana as the Okavango River at Mohembo in the north (Fig. 2.1). The Cuito also rises in the Angolan Highlands and joins the mainstream before it flows across and forms the western boundary of the Caprivi Strip (Balfour 1996).

Upon entering Botswana the Okavango River is funnelled through parallel faults of the Panhandle as a deep fast flowing river before being confronted by another perpendicular fault, the Gumare fault, with a sudden increase in gradient. This slows the flow considerably as it spreads into relatively shallow sediment with a fall of only 62 metres over approximately 250 kilometres (Bailey 1998).

In the Panhandle, the Okavango is a mighty river, more than a kilometre wide in some places. Winding its way southwards, it flows alternately through acres of luxuriant papyrus floating in its own root mass or beneath towering wild fig and ebony trees offering shade on the high riverbanks.

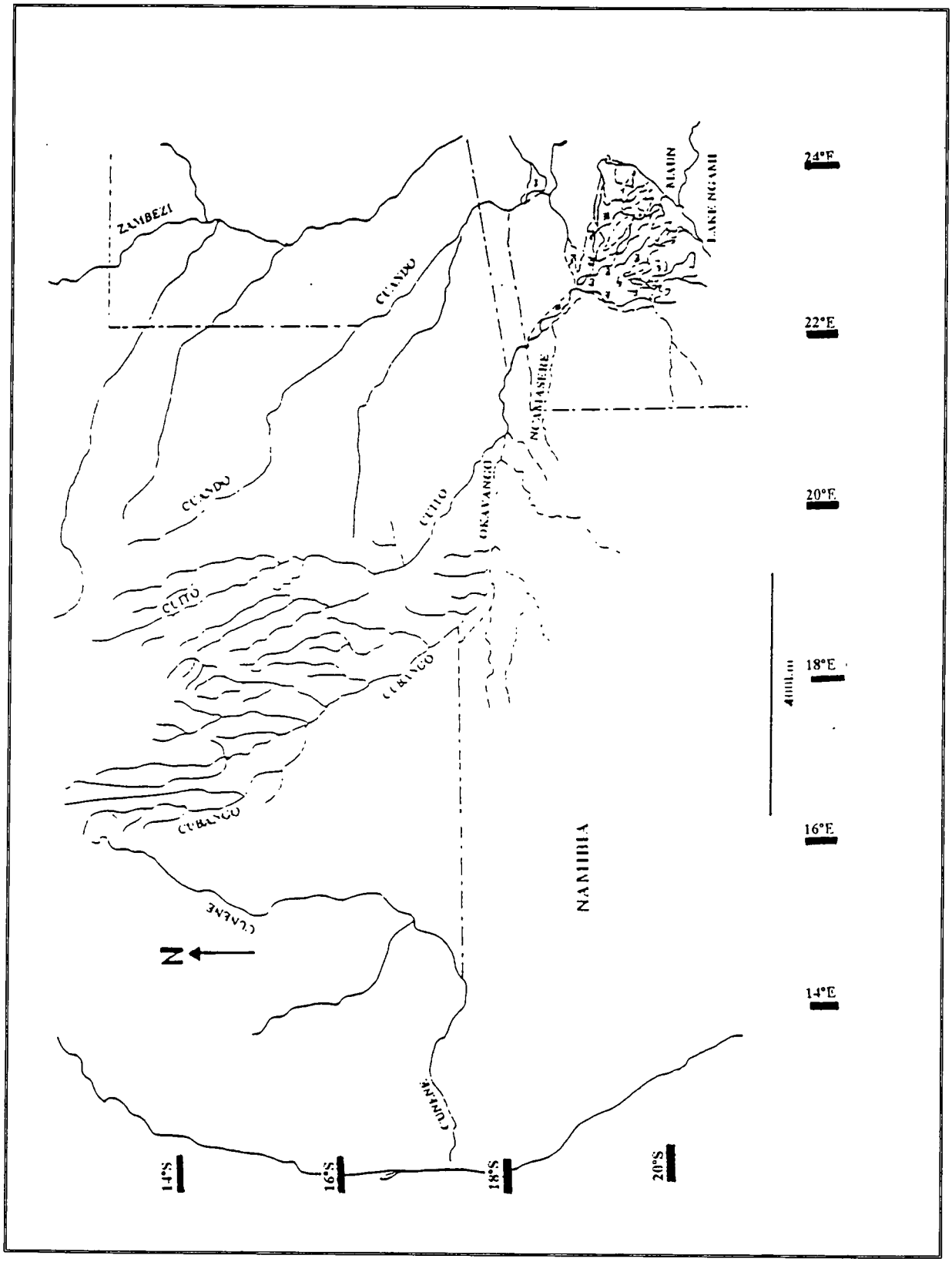


Figure 2.1: Map of the Okavango River and Delta drainage system showing the two main tributaries, the Cubango and the Cuito (adapted from Skelton, Bruton, Merron & Van der Waal 1985).

The Okavango slows its pace as it meanders further down the Panhandle, becoming shallower as its load of silt and sediment is deposited. It has been claimed that the river is slowly choking in its own sediment, with as much as 600 000 tons of silt deposited yearly (Balfour 1996). The journey through the Panhandle is the last time that the Okavango flows as a single river by that name, for hereafter it fans out to become the Okavango Delta, a 16 000 square kilometre wilderness of sparkling waterways and enchanted islands. In actual fact the Okavango is not, strictly speaking, a delta at all but rather an alluvial fan: instead of discharging into a body of water, as an authentic delta does, the Okavango's channels filter into the thirsty sands of the Kalahari (Bailey 1998).

THE OKAVANGO'S SEASONAL FLOODS

An important feature of the Okavango is the seasonal flooding, which commences in mid-summer in the north and ends about six months later in the south. This results in a cyclical motion of water rising in the north as it recedes in the south during summer, and rising in the south as it drops in the north during winter. The timing, magnitude and duration of the flood is not constant from year to year and fluctuates widely depending on the rainfall history in southern Angola. This slow pattern of inundation is due to the extremely low gradient (1: 36 000), which causes the water to spread out to form the delta (Bailey 1998).

The nature of the annual floods is gentle with floodplains and islands disappearing under water and then reappearing in an ever-changing landscape at the end of each season. This is particularly pronounced in the central Okavango. In terms of hydrology the Okavango is more stable in the northern regions and less stable in the southern regions (Merron 1991).

Floods result in a five-fold increase in the total surface area of the delta, from 3120km^2 in December to 17000km^2 in June (Wilson & Dincer 1976). The region receives approximately 430mm rainfall per annum. The rainfall is out of phase with the flood cycle in most places, except in the northern reaches of the system.

The rise and fall of the annual flood is thus one of the most important driving forces of the Okavango. It is intimately associated with habits of the fish and also assists in

their distribution as well as the clearing of blockages in the system caused by, for example, floating beds of papyrus (Skelton, Bruton, Merron & Van der Waal 1985).

ECOLOGICAL REGIONS WITHIN THE OKAVANGO DELTA

The delta can be divided between a permanently flooded zone in the north and a seasonally flooded zone in the south (Fig. 2.2).

- The northern zone includes the panhandle with its riverine forest fringes immediately adjacent to arid Kalahari woodlands and depending on the inflow from Angola a vast wetland of up to 12 000 square kilometres of islands, reed beds, channels, forest banks and permanent waterways.
- The seasonally flooded zone has large Kalahari sandveld islands with dry and deciduous woodlands fringed by wide grassy floodplains that are heavily influenced by seasonal floods.

Five major ecological regions are recognised in the Okavango Delta namely the riverine floodplain, perennial swamp, seasonal swamp, drainage rivers and sump lakes (e.g. Lake Ngami). These ecological regions can be regarded as ecotones because they grade into, and are dependent on, one another (Merron 1991).

The riverine floodplain and perennial swamps cover about two thirds of the delta and have surface waters that are 3m deep and covered with dense growth of papyrus (*Cyperus papyrus*), reeds (*Phragmites australis*), bulrushes (*Typhalati folia* subsp. *capensis*) and the fern (*Cyclosorus interruptus*) (Merron 1991).

Numerous tributaries and ox bow lagoons are associated with the mainstream channel. These areas are lined with dense islands of aquatic macrophytes including *Nymphaea capensis*, *Potamogeton thumbergi* and *Elodea densa.* The adjacent sawgrass floodplains and isolated lagoons are flooded between February and June each year.

Past Seronga the river splits into three main distributary systems: the Thaoge to the south, the Jao-Boro system in the centre and the Nqoga-Maunachira-Mboroga-Santantadibe system to the east. These channels serve as the delta's arteries, providing an essential supply of water that sustains the permanent swamp areas.

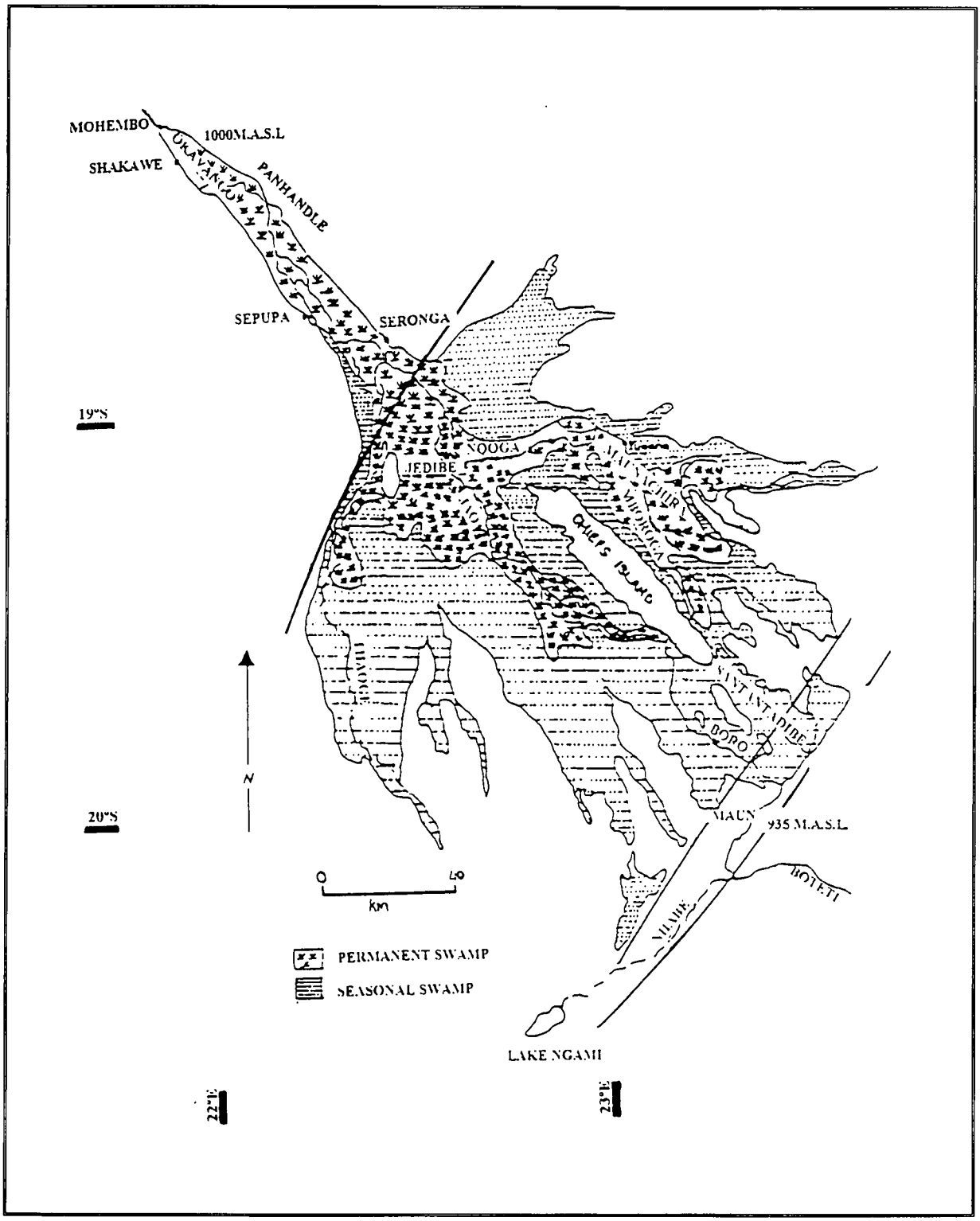


Figure 2.2: Map of the Okavango River and Delta in Botswana showing the panhandle, delta, faults and main distributary rivers (redrawn from Skelton, Bruton, Merron & van der Waal 1985).

The Thaoge used to flow strongly to the south, diverting much of the delta's water to the massive Lake Ngami. Today, according to Bailey (1998) the flow to the distal end of the Thaoge has ceased. Many say it is as a result of numerous blockages due to seismographic shifting. Described in the 1930's as a 'wasteful channel', the Boro River began to flow strongly in 1952, an event which may have been caused by a major earthquake that occurred nearby in that year. The Jao-Boro System takes about a quarter of the Okavango's flow and is the main channel to the west of Chief's Island in the central delta area. The remainder of flow is concentrated in the Nqoga-Maunachira-Mboroga-Santantadibe system. At present this is the Delta system's major distributary, but this channel is also undergoing change (Bailey 1998).

The southern seasonal swamp covers about one – third of the area of the Delta and is characterised by shallow grass and sedge-covered floodplains. The southern swamp is a seasonally inundated swamp that varies markedly in area, depending on the magnitude of the annual flood from Angola and the amount of local rainfall. Wilson and Dincer (1976) state the increase in the area covered by water during the flood is in the order of 1 to 2 in the northern permanent swamp and 1 to 10 in the southern seasonal areas in the delta.

At the lower (southeast) end of the Delta the main drainage channels, the Boro and the Santandadibe, re-unite along a fault line to form the southwest flowing Thamalakane River.

LIMNOLOGICAL CHARACTERS

Wetlands like the Okavango Delta are dynamic ecosystems, which have high biological productivity. The Delta is, however, low in available nutrients when compared to other wetlands and has a **water conductivity** less than 100μS cm^{-1}.

Water temperature ranges from 9-38°C depending on the season and site (Merron & Bruton 1988). The pH of the river was found to be higher in the northern reaches with a value of 5.8-6.7 while in the southern reaches the pH is more alkaline ranging between 7.1–8.2. Southern reaches are more alkaline as a result of large amounts of bicarbonate and carbonate salts that are inundated each year with the flood.

Oxygen values are low in certain areas, especially in mainstream channels and ox bow lagoons during receding and low water phase with values as low as 2.8p.p.m have been recorded (Merron & Bruton 1988). The low oxygen concentrations may be as a result of the abundant decomposing vegetation on the floodplains.

ISLANDS

The islands are perhaps the Okavango's greatest feature: it is estimated there are more than 50 000 of them, some little more than termite mounds rising above the surrounding waters. Others may be large enough to support permanent herds of game, sprawling tourist camps and centuries old trees and forests. The largest of them all, Chief's Island, is part of the Moremi Game Reserve and extends more than 50 kilometres from top to bottom and as much as 20 kilometres across. This is the only major landmass in the heart of the Okavango waterways (Balfour 1996).

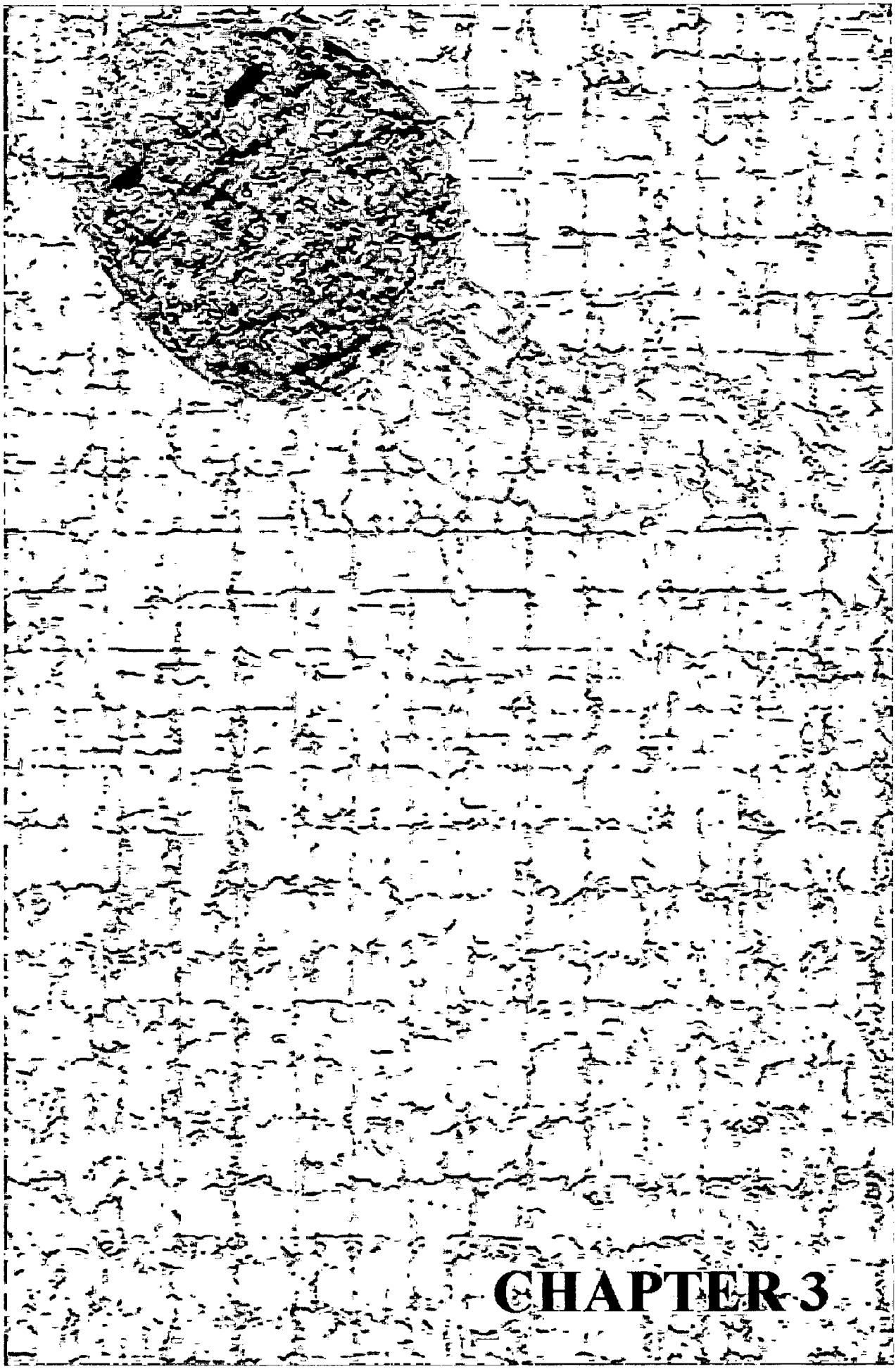
Because of its remoteness from modern developments and its variety of habitats, the Okavango Delta has great biodiversity and can be regarded as a treasure trove. It has a large population of sitatunga (*Tragelaphus spekei*) and red lechwe (*Kobus leche*), a significant population of wild dog (*Lycayon picatus*), and 72 small mammal species, as well as 95 species of reptiles and amphibians. Many terrestrial herbivores, including buffalo, zebra, elephant, blue waterbuck, and common duiker, inhabit the place, as well as lion, spotted hyena, cheetah, and leopard, which depend upon high concentration of herbivores near permanent water bodies.

There are also an estimated 68 species of fish in the Delta ecosystem and some 1061 different plant species. In other words, its long isolation, and the juxtaposition of waterways with dry land, has allowed a complex and intricate web of interdependent species of plants and animals to develop within the system. These animals in turn influence their habitats, e.g. hippos have a role in clearing waterways, and termites are instrumental in the formation of new islands.

The human presence though has had its impact on the environment, with a decline in wildlife numbers along the periphery of the Okavango. Although aquatic animals such as crocodile and hippo can be found in the greater Okavango River system, of

the antelope in the area it is only the more elusive, such as the swamp dwelling sitatunga, that still survive in the panhandle.

The Okavango Delta can therefore be regarded as a unique system, rare, beautiful and many faceted.



CHAPTER-3

MATERIALS AND METHODS

FIELD LABORATORIES/FIELDWORK

Since the Okavango Delta is such a great distance from Bloemfontein, preparations have to be made well in advance. Field laboratories are usually set up at camping sites along the river. In 1999 a barge (Fig. 3.1A) was kindly given to our study group to use by Dr Tim Liversedge. This barge was large enough to put up six tents, set up a fully equipped field laboratory and kitchen area. With the barge we were able to sample isolated localities which were previously inaccessible to us by land.

During the June 2000 field trip to the Okavango Delta, the camp was set up at Shakawe Fishing Lodge, situated in the Panhandle. The mobile field laboratory of the Zoology & Entomology department of the University of the Orange Free State was used. Since most of the fixation and processing of material takes place in the field, the methods are kept as simple as possible.

COLLECTION LOCALITIES

A number of different types of habitats and localities were sampled during both surveys (Fig. 3.2) .

Floodplains can be recognised as shallow temporary water masses on the marginal land which are inundated with water during the floods in winter and dry out during the hot summer months.

Backwaters are associated with the mainstream habitats and are represented by adjacent channel-like water bodies in which there is no current or water flow. These water bodies are distinguished from floodplains by being permanent.

Lagoons are deep, large open bodies of water and are usually associated with channels or mainstream habitats. These lagoons might sometimes become isolated from the mainstream when the channels leading to it clog up.

Mohembo floodplains (Fig. 3.1B): Situated in the upper region of the delta (riverine panhandle/floodplain), most northern part where collections took place. This

characteristic floodplain pool becomes separated from the mainstream environment when water levels are low. The locality is in the near vicinity of the town Shakawe(Fig. 3.2), and there is a pontoon that constantly transports people across the river.

Mohembo backwaters: Situated in the riverine panhandle just adjacent to the mainstream and was completely isolated from the mainstream. Permanent body of water that exhibits no flow of water. Decaying matter lying on bottom of this locality. In close proximity to village near Shakawe (Fig. 3.2).

Xaro Mainstream Lagoon: Upper region of panhandle just past the town of Shakawe (Fig. 3.2). Situated just off the mainstream; is a type of inlet (lagoon) with characteristic papyrus and sawgrass in the lagoon. Water flow not strong here, but is neither stagnant. Frequented by birds and wild animals and occasionally humans.

Nxamasere (Fig. 3.1C): Situated in the upper regions of the panhandle of the Okavango Delta. Is a floodplain habitat that consists of narrow channels, surrounded by sawgrass marsh. The channels are lined with dense stands of macrophytes including *Nymphea*, sawgrass and *Cyperus papyrus*. This locality is frequented by animals (Fig. 3.2).

Etsatsa mainstream: Situated at the beginning of the upper permanent swamp. Fast flowing waters, snails were collected just off the mainstream on the banks of the river; is a floodplain habitat adjacent to main river channel. Main route of transport so it is constantly in contact with human populations and even some wild animals and crocodiles (Fig. 3.2).

Etsatsa floodplains: Vast expanse of floodplains characterised by grasslike vegetation (sawgrass) adjacent to Etsatsa mainstream (Fig. 3.2). Frequented by animals.

Guma lagoon: Large, deep, open body of water connected to mainstream by a narrow channel, relatively stagnant body of water (Fig. 3.2). Frequented by humans.

Guma channel: Channel that leads into Guma Lagoon from the north (Fig. 3.2). Waterflow slightly strong, this body of water is shallower and narrower, characterised by papyrus on both sides of the channel and by grasslike vegetation just beyond papyrus beds. Frequented by birds, animals and humans.

Guma floodplains (Fig. 3.1D & E): Large area of floodplain adjacent to Guma lagoon and mainstream and is characterised by grass like vegetation. Frequented by humans and animals.

Seronga floodplains: Large shallow open floodplains with hippo-like paths in between. Also associated with small islands and grass like vegetation. Situated near the town Seronga so it is constantly in contact with humans and animals (Fig. 3.2).

Seronga fisheries camp: In vicinity of the town Seronga (Fig. 3.2). On banks of mainstream near the fisheries camp. Shallow areas floodplain habitat. Situated at the fisheries camp so in constant contact with humans.

Seronga polars camp: Banks of the river, floodplain habitat and associated with grasslike vegetation found in the vicinity of Seronga (Fig. 3.2). Frequented by humans and animals.

Willies camp: On the banks of river near Seronga (Fig. 3.2), type of floodplain area. Shaded area under trees. Some small isolated islands. Decaying matter on banks of river. Frequented by humans.

Duba Lagoon: A number of connected lagoons together with floodplain habitats that are characterised by their papyrus beds and grasslike vegetation. Situated in the centre of permanent swamp and forms part of Little Duba (Fig. 3.2). Frequented by birds and humans.

Kwihom island: Floodplain situated adjacent to Jao (Fig. 3.2). Upper swamp river. Relatively isolated from human contact but may be frequented by birds.

Jao island: Floodplain habitat with characteristic vegetation situated in the vicinity of Jao village (Fig. 3.2). Relatively isolated from human contact.

Jao channel (Fig. 3.1F): Narrower than Guma channel characterised by grasslike vegetation and papyrus beds with adjacent floodplain habitats. Frequented by birds and humans.

Thaoge Lagoon: Seasonal swamp situated in the vicinity of Thaoge Channel (Fig. 3.2). Similar to Guma lagoon but is not as deep and as large and is closer to mainstream. Also with decaying plant matter. Relatively isolated, occasionally frequented by humans.

Upper Thaoge Lagoon: Seasonal swamp situated in the Thaoge channel (Fig. 3.2). Smaller than Thaoge Lagoon. Occasionally frequented by humans.

Lechwe Island (Fig. 3.1G): Situated adjacent to hippo paths and narrow open channels. Floodplain type of habitat situated near to Makwena (Fig. 3.2). Seasonal swamp. Main route of transport so in constant contact with humans and also animals.

Figure 3.1 Collection method at some of the various collection localities within the Okavango Delta, Botswana. **A.** Barge **B.** Collection at Mohembo Floodplains **C.** Collection at Nxamasere **D & E.** Guma floodplains **F.** Collection done from the boat in Jao channel **G.** Sampling at Lechwe Island **H.** Microscope work in mobile field laboratory.

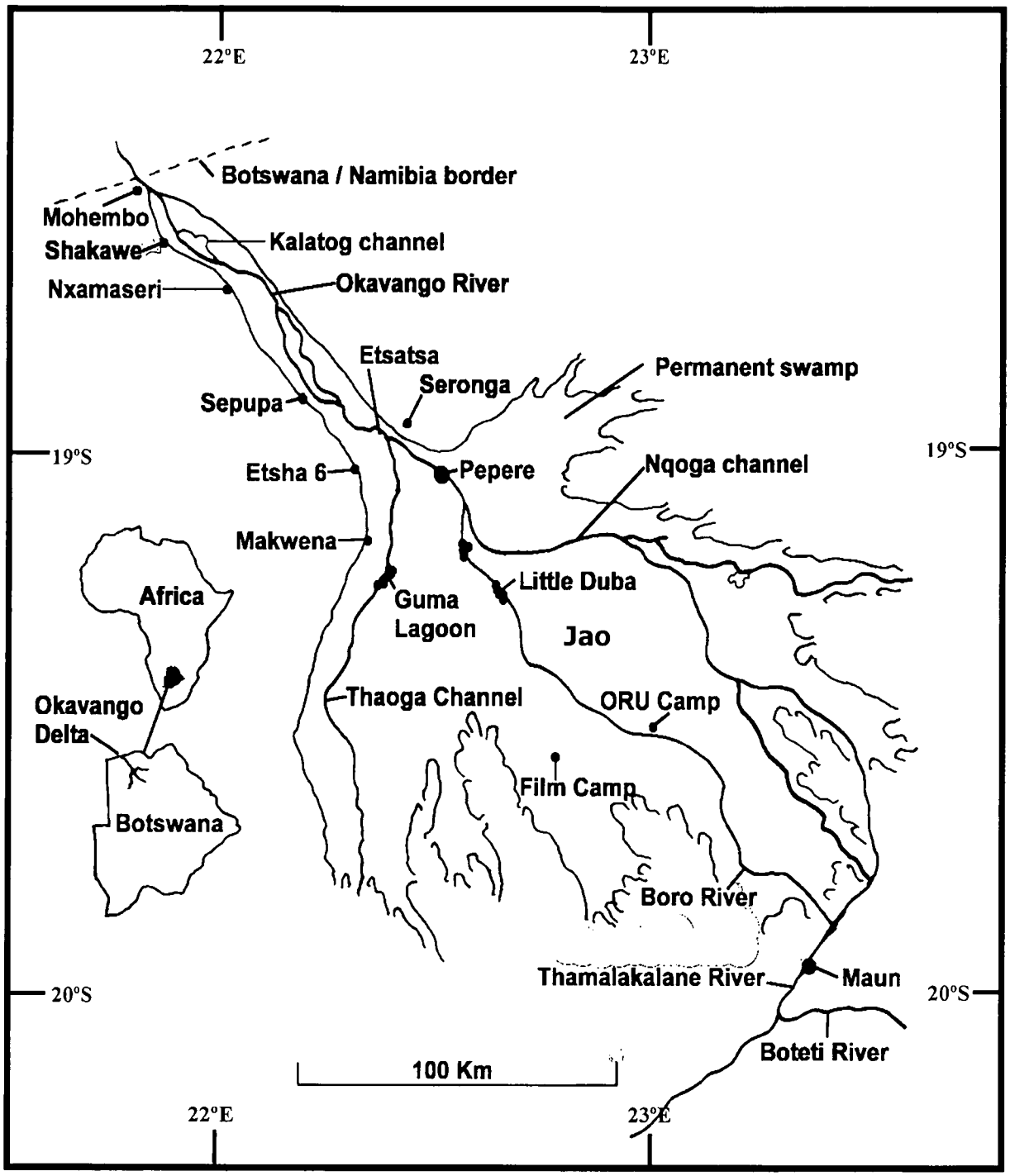


Figure 3.2: Map of the Okavango Delta illustrating the various sampling localities during the two surveys, June-August 1999 and June 2000.

COLLECTION OF SNAIL HOSTS AND CERCARIAE

Molluscs were collected from the different localities by means of a shallow long handled scoop net according to the specifications made by Van Eeden (1960).

The different snail species were placed in separate holders back at the field laboratory and placed in light but not direct sunlight so that natural shedding of cercariae could be observed. Some of the emerged cercariae were filtered through a 5 or 10μm nucleopore filter and fixed in four different fixatives, namely 4% buffered neutral formalin, 70% ethanol, 2.5% gluteraldehyde and Karnovskey's solution in preparation for scanning electron microscopy back at the laboratory in Bloemfontein. The remaining live cercariae were immediately prepared for light microscopy.

PREPARATION OF SPECIMENS FOR SCANNING ELECTRON MICROSCOPY

In the field laboratory the specimens that were fixed in gluteraldehyde and Karnovskey's were left in the solution overnight after which they were placed in a phosphate buffer (Millonigs buffer) for approximately ten minutes. The specimens were then dehydrated in a series of alcohols until 70% and placed in plastic test tube holders until further preparations.

Specimens that were fixed in 4% formalin were also placed in holders. Back at the laboratory in Bloemfontein the specimens were first placed in water and then dehydrated in a graded ethanol series of increasing concentrations (30% - 100%). The specimens that were in 70% ethanol were first taken down in alcohol concentrations from 70% to 30%, washed in distilled water and then dehydrated.

Specimens that were first fixed in gluteraldehyde and Karnovskey's were dehydrated further in a graded ethanol series (70%-100%), left in 100% ethanol for approximately 10-15 minutes were critical point dried for an hour and a half in a Biorad critical point dryer. Afterwards the filters with specimens were mounted on aluminium stubs and coated with gold in an Emscope SC500 sputter coater for approximately 4 minutes.

The specimens were examined using a JEOL WINSEM JSM 6400 scanning electron microscope at an accelerating voltage of 10kV and a working distance of 8mm. The same procedure was used for the specimens that were fixed in 4% buffered neutral formalin and 70% ethanol.

PREPARATION OF SPECIMENS FOR LIGHT MICROSCOPY

Once shedding of cercariae took place, a few cercariae were placed in Nile blue sulphate in watchglass (which proved most effective when staining) and left for 5 minutes to allow stain to be absorbed. Afterwards 1-2 cercariae were placed on a microscope slide covered with a coverslip, moved once or twice over a bunsen burner to slow down cercarial movement, and then observed under a Zeiss Microscope with attached drawing tube (Fig. 3.1H).

LIGHT MICROSCOPY STUDIES AND MORPHOLOGICAL MEASUREMENTS

Cercarial descriptions were made by studying live specimens under the light microscope. Microscope drawings were made by making use of a drawing tube attached to the microscope.

Measurement of the specimens was done as illustrated in Fig. 3.3. The measurements of at least 20 specimens were made of each of the different species from the Okavango Delta and used for cercarial descriptions.

Micrographs were made of the living material by using the automatic camera system mounted on the microscope. All measurements made in the study are referred to further in the text in μm and are presented in the description of cercariae as follows:

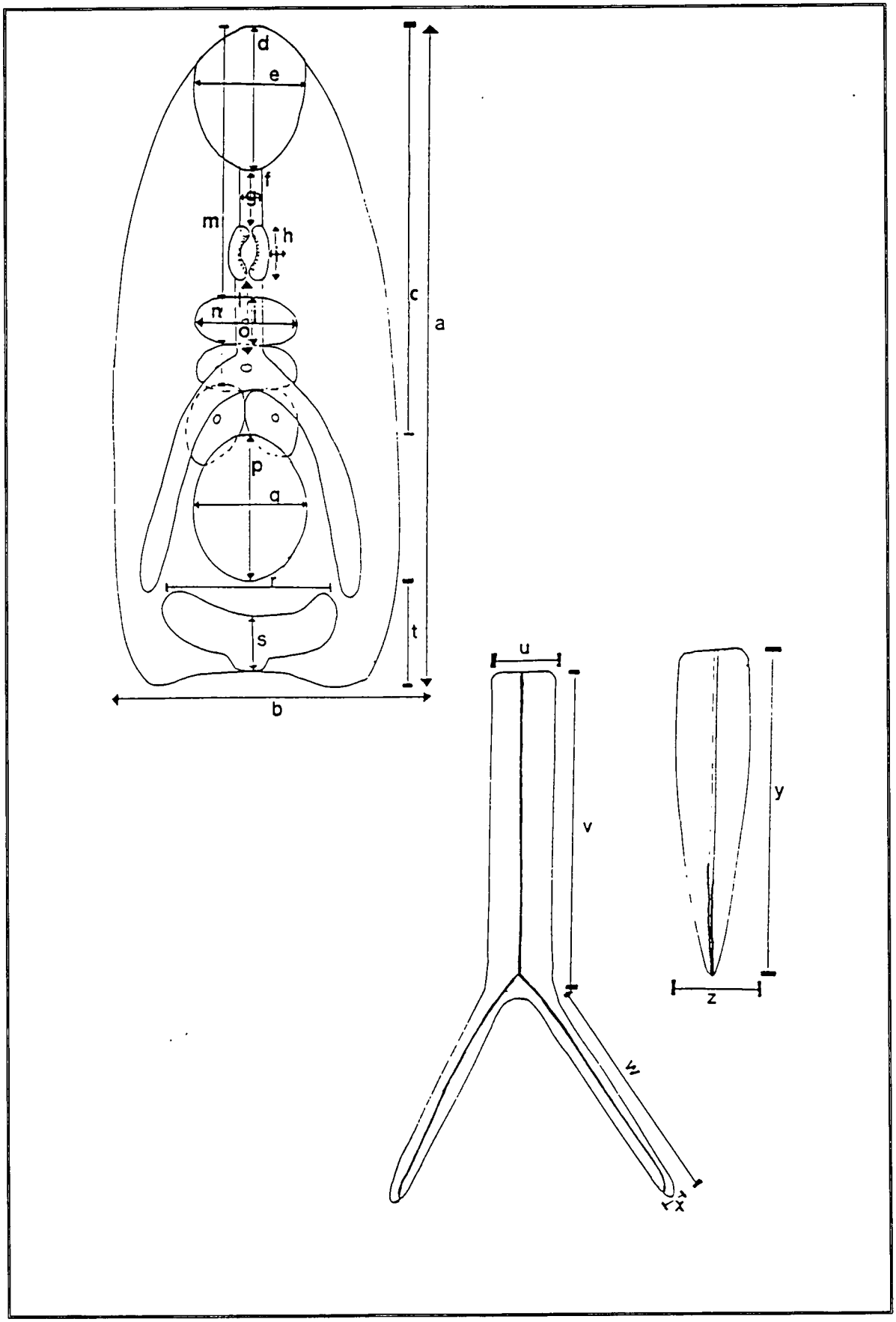
Minimum-Maximum (Average±Standard deviation)

All material collected and used in the study is deposited in the parasite collection of the Aquatic Parasitology study group, of the Department of Zoology & Entomology, U.O.F.S.

Figure 3.3

Diagram illustrating the morphological measurements used in the cercarial descriptions of the different types of cercariae.

a- Body length
b- Body width
c- Distance between anterior border and acetabulum
d- Oral sucker length
e- Oral sucker width
f- Prepharynx length
g- Prepharynx width
h- Pharynx length
i- Pharynx width
j- Penetration gland length
k- Penetration gland width
l- Oesophagus length
m- Distance between anterior border and 1st penetration gland
n- Distance between anterior border and 2nd penetration gland
o- Oesophagus width
p- Acetabulum length
q- Acetabulum width
r- Excretory bladder width
s- Excretory bladder length
t- Distance of acetabulum from posterior end of body
u- Fork-tail width
v- Stem length of fork-tail
w- Furcal rami length
x- Furcal rami width
y- Single tail length
z- Single tail width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CHAPTER 4

FRESHWATER SNAILS

Molluscs form the second largest invertebrate phylum with about 100 000 living species. Most of these species occur in great numbers in the sea. Two of the six classes which make up the phylum, namely the class Gastropoda (snails and limpets) and the class Bivalvia (mussels and oysters) occur in fresh and brackish waters.

There are about 350 species of gastropods and 110 species of bivalves in African freshwaters but precise numbers are not actually known (Appleton 1996). In southern Africa south of the Kunene, Okavango and Zambezi rivers, there are some 51 gastropod species, including 43 indigenous, eight exotic and 23 bivalve species, all of which are indigenous.

Snails are economically important because they serve as intermediate hosts for the parasites causing bilharzia in humans and animals, liver-fluke disease in cattle, sheep and horses, and conical fluke disease in cattle.

Two sub-classes of gastropods were recorded from the Okavango, the first subclass being the Prosobranchia. Prosobranchs are known to have a true gill and need to be submerged to be able to breathe. They are usually associated with sandy or muddy substrata and may form a major component of the bottom-dwelling animal community. Their lifespan is approximately from one to two years. Pulmonates on the otherhand are associated with aquatic plants and are therefore often found among marginal or submerged vegetation (Appleton 1996).

The second subclass, the Pulmonata, have the ability to breathe air and extract dissolved oxygen from water. This physiological plasticity enables them to survive fluctuations in water levels and exposure to air. The pulmonates may live for only six months to a year.

A precise description of conditions favouring snails is impossible because most freshwater snails can tolerate a wider range of physical conditions than marine or terrestrial species (Hubendick 1958). Freshwater snails inhabit a great variety of

different habitats, both natural and man-made, ranging in size from small temporary puddles to extensive lakes, and from minute seepages to large rivers (Sturrock 1974). Due to seasonal and climatic factors, many habitats, especially in Africa and the Americas are subject to change. Hence snails have evolved a high reproductive potential to allow residual populations to reinfest sites at some later stage.

The best habitats are said to be relatively shallow, with a moderate organic content but little turbidity. The substrate needs to be rich in organic matter supporting submerged, emergent or intrusive vegetation, though not so dense that it prevents moderate light penetration required for the growth of rich microflora. Physical conditions vary with pH in the range of five to nine and water temperatures from 18°C to 30°C or more, depending on the altitude and latitude (Sturrock 1974).

A number of opinions are held on the relative importance of the various physical and chemical factors that may affect freshwater snails. It is, however, accepted that temperature is one of the most important environmental factors, which can, amongst others, determine the geographical distribution of organisms.

In almost all cases the first intermediate host in a digenean life cycle, is a mollusc. It has been said that digeneans display more host specificity to their molluscan hosts than they do to their vertebrate hosts. This may imply that they established themselves as parasites of molluscs first, then added a vertebrate host as a later adaptation.

In the rest of the chapter I will provide a brief literature background of the limiting environmental factors which influence the distribution of snails. This will be done in order to understand why snails will only be found in certain areas in the delta. This will be followed by a compendium of snails occurring in the Okavango Delta.

FACTORS INFLUENCING THE OCCURRENCE AND DISTRIBUTION OF SNAIL FAUNAS IN A SPECIFIC AREA

TOTAL DISSOLVED CHEMICAL CONTENT

Conductivity is used to express a complex of chemical and physical variables and is nothing more than a guide to the factors that influence an organism (Brown 1994). It is difficult to say precisely what conductivity different snails prefer, since it may differ from snail to snail. Jennings, De Kock and Van Eeden (1973) proved that *Biomphalaria pfeifferi* Krauss, 1848 can survive and multiply at a conductivity range of 100 to 750 µS. It does, however, thrive better at higher conductivities, notably 300, 350 and 400µS. It has also been shown that in stagnant soft water bodies (below 25p.p.m $CaCO_3$) *B. pfeifferi* was found to be having badly pitted shells and some of them had no umbilicus (Frank 1964).

TURBIDITY

Turbid waters have adverse effects on snails. Studies done on *Biomphalaria pfeifferi* in Zimbabwe showed that these snails could not hatch in streams with 360 mg^{-1} suspended solids. Brown (1994) showed that *Bulinus globosus* (Morelet, 1868) and *Lymnaea natalensis* (Krauss, 1848) were not adversely affected at this concentration, and eggs of all three species hatched normally when the water was diluted to 190mg^{-1}. Turbidity can sometimes be beneficial, it has been observed that *Bulinus nasatus* (Martens, 1979) freely layed eggs in thick muddy water after emerging from aestivation, while *Bulinus senegalensis* Müller, 1781 was most abundant during a period of high turbidity in a reservoir in Nigeria.

OXYGEN

It has been shown that in field and laboratory experiments snail distribution is limited by low levels of dissolved oxygen (Brown 1994). Some species are capable of reducing their oxygen consumption to lower levels during aestivation. They possibly do this by using another metabolic pathway (Heeg 1977).

Pulmonates are capable of taking in atmospheric air into the mantle cavity, though oxygen is also absorbed through the general body surface, especially the pseudobranch, which is particularly elaborate in members of the genus *Bulinus*. It is

also said that the presence of haemoglobin in the blood of representatives of the Planorbidae further increases efficiency in respiration.

An important influence on distribution within habitats may be the level of oxygenation. Wright (1956) showed that there is a zone of high oxygen concentration available to snails immediately beneath the floating leaves of the water lilies (*Nymphaea* spp.). Conditions can become unfavourable when dense vegetation prevents snails from reaching the surface when there is a shortage of dissolved oxygen. McCullough (1957) noticed the death of many *Bulinus globosus* snails as a result of dense mats of *Azolla.*

Papyrus swamps are extensive areas of habitat with little if any dissolved oxygen (Jones 1964), and *Biomphalaria sudanica* (Martens, 1870) is the only planorbid to occur abundantly among the papyrus beds, though *Pila ovata* (Olivier, 1804) may be common at the margins.

TEMPERATURE

Snails may be killed by temperatures above or below lethal limits. Schiff (1966) stated that this direct mortality is not easy to assess in the field since there are fluctuating temperatures within even a small waterbody, which snails will actively seek out.

Temperature appears to be a major factor in the distribution of freshwater snails in Africa and it seems that the decline in ambient temperature with increasing altitude and, in southern Africa, latitude, limits the geographical range of species that are adapted to tropical conditions (Brown 1994). Too warm conditions on the other hand, restrict the occurrence of *Biomphalaria pfeifferi*, even though it is widely distributed in the tropical region.

Studies done by Joubert, Pretorius, de Kock and Van Eeden (1986) showed that *Bulinus africanus* (Krauss, 1848) and *Biomphalaria pfeifferi* die off at temperatures where *Bulinus globosus* can survive successfully, therefore it can be assumed that *B. globosus* is better adapted to deal with high temperature conditions.

Brown (1980) showed that these three snail species occur in hotter parts of the country where suitable habitats exist. De Kock (1973) observed that the reproduction of *B. africanus* was adversely affected at temperatures above 26°C while *B. globosus* is only affected at temperatures above 29°C. Frank (1964) stated that snails are unable to withstand temperatures near the freezing point of water.

WATER CURRENT

The shells of some snails are modified to resist dislodgment in areas where the river is fast flowing. The genus *Sierraia* Connolly, 1929 in West African rivers and *Burnupia* Walker, 1912 are two of the snails capable of inhabiting fast flowing rivers and streams. In small rivers and streams that are slow flowing or stagnant for most of the year, sudden spates following heavy rainfall sweep away many snails and cause major fluctuations in population density (Brown 1994).

Snails are seldom found in streams, which have a velocity greater than 0.3m/sec and also seek quieter backwaters, when they are available. The most important and probably fundamental effect of water velocity is the type of substrate that it allows to settle. Cobblestone substrates are rare where the average flow of water is high. The most common type of substrate is undoubtedly the sand to mud type, which supports a rich and varied algal flora on which snails feed. Those bodies of water, mostly man-made, which are immune to flushing, are better habitats and support flourishing colonies (Frank 1964).

LIGHT AND SHADE, CIRCADIAN RHYTHMS

Appleton (1978) observed that snail hosts for schistosomes are capable of surviving periods of total darkness, although adverse effects were also noted by El-Emam & Madsen (1982). There is evidence that regular activity patterns are related to the 24 hr cycle of day and night (circadian rhythms). Kuma (1975) and Appleton (1978) observed that *B. globosus* laid eggs mainly at night while Morgan and Last (1982) found that *B. africanus* laid eggs only by day.

It has also been reported that shaded sights are a means of controlling *B. pfeifferi.* Brown (1994) reported that dense shade beneath mats of floating vegetation is generally unfavourable for snails.

In conclusion, man is without doubt the snail's greatest asset. Where habitats have been altered one could surely expect to find snails. Frank (1964) found that in the Kruger National Park, where natural conditions prevail, snails were only found in areas where dam walls had been erected and other devices constructed to conserve water for the game during dry season. In this way man has interfered in natural habitats. The increase of snail populations will thus not be uncommon and will thereby increase the chances of trematode infections.

Appleton (1978), when reviewing the effects of physiochemical factors on snail hosts for schistosomes, concluded that temperature and current velocity were largely responsible for their distribution and abundance in southern Africa. Low oxygen concentrations can be limiting especially in polluted areas. Desiccation is a major factor limiting snail fauna. Pulmonates that thrive in temporary habitats prevent desiccation by going into aestivation. They also have the ability to quickly repopulate when water returns to the area (Brown 1994).

The remaining pages deals with the systematics of freshwater snail faunas (Table 4.1) and records of the species found within the Okavango Delta. To make the descriptions more understandable a generalised sketch of a snail is included showing different features/ morphology (Fig. 4.1).

Table 4.1: Freshwater Snails occurring in Botswana (compiled from Brown 1994).

FAMILY VIVIPARIDAE	
Bellamya capillata (Frauenfeld, 1865)	Previously belonging to genus *Vivipara*
Bellamya monardi (Haas, 1934)	Previously belonging to genus *Viviparus*
FAMILY AMPULLARIIDAE	
Pila occidentalis (Mousson, 1887)	Previously belonging to genus *Ampullaria*
Lanistes ovum Peters, 1845	
FAMILY HYDROBIIDAE	
Lobogenes michaelis Pilsbry & Bequaert, 1927	
FAMILY BITHYNIIDAE	
Gabbiella kisalensis (Pilsbry & Bequaert, 1927)	Previously belonging to genus *Bulimus*
FAMILY THIARIDAE	
Melanoides victoriae (Dohrn, 1865)	Previously belonging to genus *Melania*
Cleopatra elata Dautzenberg & Germain, 1914	
Cleopatra nsendweensis Dupuis & Putzeys, 1902	
FAMILY LYMNAEIDAE	
Lymnaea natalensis Krauss, 1848	
FAMILY ANCYLIDAE	
Ferrissia victoriensis Walker, 1912	
FAMILY PLANORBIDAE	
Afrogyrus coretus (De Blainville, 1826)	
Gyraulus costulatus (Krauss, 1848)	Previously belonging to the genus *Planorbis*
Segmentorbis angustus (Jickeli, 1874)	Previously belonging to the genus *Segmentina*
Segmentorbis kanisaensis (Preston, 1914)	Previously belonging to the genus *Segmentina*
Biomphalaria pfeifferi (Krauss, 1848)	Previously belonging to the genus *Planorbis*
Bulinus scalaris (Dunker, 1845)	Previously belonging to the genus *Physa*
Bulinus globosus (Morelet, 1868)	Previously belonging to the genus *Physa*
Bulinus depressus Haas, 1936	
Bulinus tropicus (Krauss, 1848)	Previously belonging to the genus *Physa*

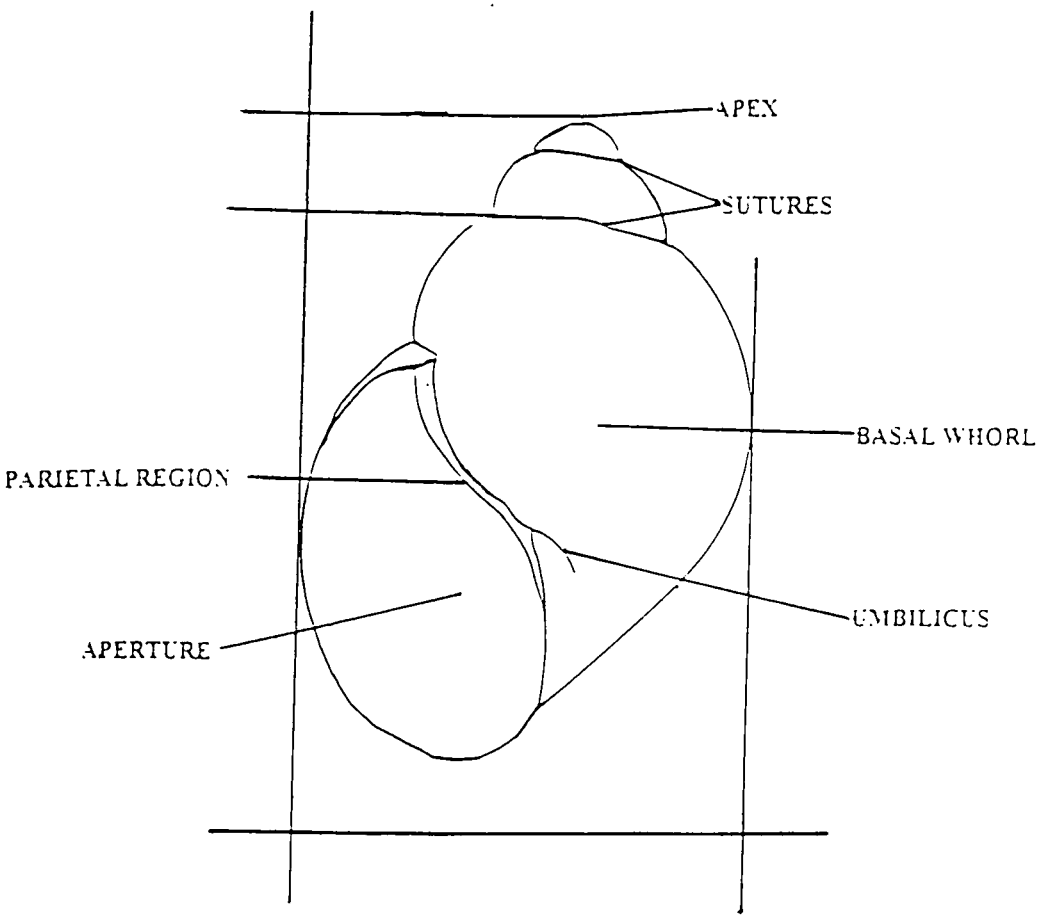


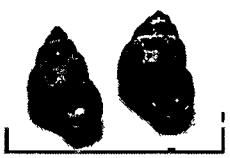
Figure 4.1: Main features of a snail shell (redrawn from Appleton 1996).

FRESHWATER SNAILS FOUND IN THE OKAVANGO DELTA AND RIVER, BOTSWANA

COMPENDIUM OF FRESHWATER SNAILS INHABITING THE OKAVANGO DELTA, BOTSWANA (compiled from Brown, Curtis, Bethune & Appleton 1992; Appleton 1996; Brown 1994).

SUBCLASS 1: STREPTONEURA (PROSOBRANCHIA)

FAMILY I: VIVIPARIDAE

	***Bellamya capillata* (Frauenfield, 1865)**
Descriptive notes 	Length 35 mm, narrowly umbilicate, with/without blunt angulation around umbilical opening. Fine microsculpture spiral ridges present, most strongly in umbilical area, bristles of periostracum may be present. Shell surface glossy, greenish brown, sometimes with darker spiral bands; large shells have dark rim. First whorl with two strong spiral ridges, each with periostracal crest, divides into bristles in later whorls.
Distribution	Range large, localities scattered. Tanzania southwards towards north-eastern Kwa-Zulu Natal; westwards into lower Zaire, Angola and Okavango River.
Habitat	Lakes, rivers, smaller waterbodies. Shallow pans on coastal plain of Kwa-Zulu Natal. Benthic species.
Okavango Habitat	Xaro Mainstream Lagoon, Guma Lagoon, Seronga Fisheries Camp, Seronga Polars Camp, Lechwe Island.

	***Bellamya monardi* (Haas, 1934)**
Descriptive notes 	Narrowly shouldered near suture, flattened above strongly curved or bluntly angular periphery. Surface with fine spiral ridges, not as smooth as *B. capillata*. Yellowish brown colour.
Distribution	Southern Angola and Northern Namibia; Cunene River and tributaries; Okavango River from Rundu to Popa Falls.
Habitat	Restricted to large lakes.
Okavango Habitat	Not found

FAMILY II: AMPULLARIIDAE

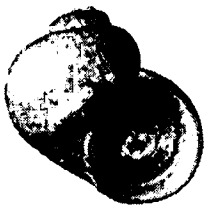
	***Pila occidentalis* (Mousson, 1887)**
Descriptive notes 	Shell large, 60 x 60 mm, globose, dextral snail, green/brown colour with about 10 darker, spiral bands. Operculum corneous with inner calcareous layer. Shell low spired, broadly perforated.
Distribution	Western Zambia; Eastern Caprivi; Okavango River in Namibia and Botswana; Southern Angola; Most southerly records near Maun and at Nyae Nyae Pan, Bushmanland.
Habitat	Temporary pools and papyrus swamps.
Okavango Habitat	Mohembo Floodplains and Backwaters, Nxamasere Floodplains, Seronga Floodplains, Etsatsa Floodplains and Mainstream, Kwihom Island, Lechwe Island.
Remarks	Shell varies in shape. It is also sometimes hardly distinguishable from *Pila ovata* (Olivier, 1804).

	***Lanistes ovum* Peters, 1845**
Descriptive notes 	Largest shell 66 mm in length x 54 mm width. Size and spire height varied; whorls evenly convex, rarely flattened and bluntly angular; uniform brown shell. Operculum corneous.
Distribution	Distributed from Okavango River System in northwest to lowlands of east. Southwards to Pongola River floodplain in Kwa-Zulu Natal.
Habitat	Highly varied, standing and flowing waters, with muddy bottoms and vegetation, seasonal pans, rain pools.
Okavango Habitat	Mohembo Floodplains and Backwaters, Seronga Floodplains, Duba Lagoon, Thaoge Lagoon; Etsatsa Floodplains and from a channel near Guma Lagoon.
Remarks	The name *Lanistes olivaceus* (Sowerby) was used by some authors. *L. procerus* Martens, 1866 is said to be synonymous with *L. ovum* (Brown 1994). A parasitic water mite *Unionicola macani* Gledhill lives in the mantle cavity (Fashuyi 1990).

FAMILY III: HYDROBIIDAE

	***Lobogenes michaelis* Pilsbry & Bequaert, 1927**
Descriptive notes 	Shell 4.5 mm in length, higher spire, imperforate. Retractable thin corneous paucispiral operculum.
Distribution	South-eastern Zaire; Lumbumbashi area and Lake Katebe. Zambia.
Habitat	Highly varied, including streams flowing over gravel and muddy pools.
Okavango Habitat	Guma Channel

FAMILY IV: BITHYNIIDAE

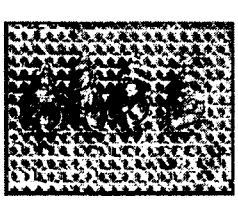
	***Gabbiella kisalensis* (Pilsbry & Bequaert, 1927)**
Descriptive notes 	Small, globose, whorls rapidly increasing. Operculum with spiral occupying half width of shell, concentric with spiral nucleus, calcareous and lodging at peristome.
Distribution	South-eastern Zaire; Angola; northern Mozambique; Zambia and 'Caprivi strip' at Kwena; East Caprivi and Okavango Delta. Widespread in Okavango River.
Habitat	Brook with a gravelly bottom, on sticks and debris in rivers, slow flowing water, residual pools and floodplains.
Okavango Habitat	Not found
Remarks	The shell is distinguished from *Lobogenes michaelis* by its small size, lower spire and open umbilicus.

FAMILY V: THIARIDAE

	***Melanoides victoriae* (Dohrn, 1865)**
Descriptive notes 	Whorls flat, sculpture weak or almost lacking on last whorl, strong ribs and tubercles may be present above. Apex generally decollate, most large shells have hardly more than five remaining whorls, which are weakly sculptured, flat sided and sometimes concave below suture.
Distribution	North and Eastern Transvaal; middle Zambezi Basin; North-eastern Nambia (East Caprivi and Okavango River); Cunene

	River.
Habitat	Rivers with sandy or muddy bottoms in the East Transvaal lowveld, rivers and floodplains in North-eastern Namibia.
Okavango Habitat	Not found

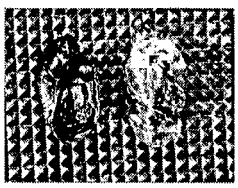
	***Cleopatra nsendweensis* Dupuis & Putzeys, 1902**
Descriptive notes 	Lower whorls smooth, somewhat flattened. Shells mostly decollate; maximum size 16 x 10 mm. Brown bands strong, comprising basal and subsutural band with 1 to 4 peripheral bands.
Distribution	Zaire: South-eastern region also Ubangi and Kinshasa. Zambia: Zambezi River above Victoria Falls and Kafue River. Angola; Northern Namibia (Cunene and Okavango Rivers) and Northern Botswana (Chobe).
Habitat	Uncertain
Okavango Habitat	Not found

	***Cleopatra elata* Dautzenberg & Germain, 1914**
Descriptive notes 	13 x 6 mm. Shell small, slender, whorls convex with deep sutures; spire may reach from 2-3 times height of aperture; 3-5 fine spiral ridges; slender brown bands on yellowish ground colour.
Distribution	South-eastern Zaire; Upper Zambezi River; East Caprivi; Okavango River and Delta. Possibly also Middle Zambezi Basin; Zimbabwe, Chirundu.

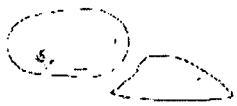
Habitat	Small stagnant slowly flowing waterbodies, some briefly seasonal, also in lakes, especially on muddy substrata.
Okavango Habitat	Mohembo Floodplains and Backwaters, Etsatsa Floodplains and Mainstream; Xaro Mainstream Lagoon; Seronga Floodplain and Willies camp (Seronga); Jao Island.

SUBCLASS 2 EUTHYNEURA: ORDER BASOMMATOPHORA

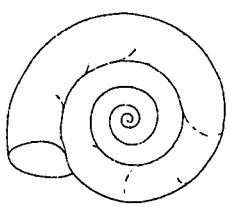
FAMILY I: LYMNAEIDAE

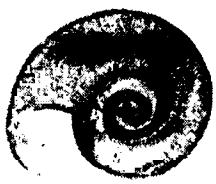
	***Lymnaea natalensis* Krauss, 1848**
Descriptive notes 	Largest shells from Okavango region reached 20mm in length. Basal whorl markedly swollen. Sculpture consisting of growth lines only. Shell spire less high than aperture, surface often with spiral rows of short transverse grooves.
Distribution	Found over the eastern half of the sub-continent north of latitude 20°S as well as the Orange and Okavango River Systems and the south–eastern coastal strip.
Habitat	Permanent waterbodies, including reservoirs, drains, very shallow though constantly seeping water; rarely in seasonally filled pools.
Okavango Habitat	Mohembo Floodplains and Backwaters; Xaro Mainstream Lagoon; Xaro Backwaters and Island off Xaro Mainstream; Sepopa Floodplains; Etsatsa Floodplains; Seronga Floodplains, Seronga Fisheries Camp, Channel near Guma and Guma Floodplains; Duba Lagoon; Jao Island; Thaoge Lagoon and Willies Camp.
Remarks	Serves as major intermediate host of the giant liver fluke *Fasciola hepatica* Linnaeus, 1758. Van Someren (1946) showed that this snail has a comparatively high requirement for oxygen and is usually tolerant of desiccation.

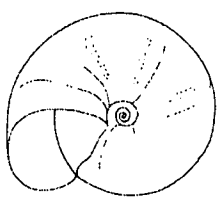
FAMILY II: ANCYLIDAE

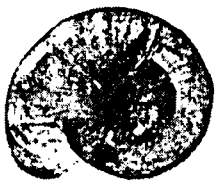
	***Ferrissia victoriensis* (Walker, 1912)**
Descriptive notes 	Small, rarely reaching 5mm long, very fine radial ridges on apex.
Distribution	Wide spread in tropical region and probably present throughout Africa. Namibia; East Caprivi; Kwando River bridge at Kongola.
Habitat	Varied habitats including streams, lakes, seasonal pools and irrigation channels; often attached to vegetation, especially underside of lily-leaves.
Okavango Habitat	Not found
Remarks	Easily overlooked because of its small size and close attachment to vegetation.

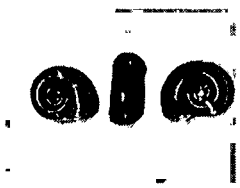
FAMILY III: PLANORBIDAE

	***Afrogyrus coretus* (de Blainville, 1826)**
Descriptive notes 	Largest shells 3.0 mm in diameter with 3.5 whorls; periphery is almost evenly curved, transverse sculpture weak, irregular.
Distribution	Widespread in Africa and tropical Africa. Southern limit in Namibia and Eastern Kwa-Zulu Natal. Apparently present in Zanzibar and possibly in Cape Verde Islands; Comores and Madagascar.
Habitat	Permanent waters with rich aquatic vegetation, commonly found on underside of floating lily-leaves, amongst leaf litter on bottom.
Okavango Habitat	Not found
Remarks	One species in southern Africa.

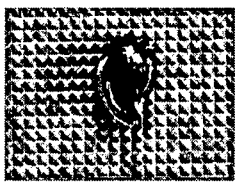
	***Gyraulus costulatus* (Krauss, 1848)**
Descriptive notes 	Shell depressed, whorls rapidly increasing. Largest shells 3.8 mm in diameter with 3.3 whorls. Have typical ribs, angular periphery with fringe of periostracum.
Distribution	Africa: mainly in the tropical region. Ethiopia; Sudan and southwards into Angola; Namibia (Okavango River and East Caprivi); Botswana (Okavango Delta) and lower Orange River.
Habitat	Aquatic vegetation, marginal grass and stones in slow flowing streams and rivers, large dams and lakes. Tolerant of shade and favourable of organic pollution (Ndifon & Ukoli 1989). Absent from habitats that regularly dry out.
Okavango Habitat	Not found

	***Segmentorbis angustus* (Jickeli, 1874)**
Descriptive notes 	Fully grown shell about three times broader than high, no more than three sets of septa.
Distribution	Widespread in tropical Africa, found as far south as Kwa-Zulu Natal. Nigeria; extends southwards into Okavango Delta and Natal coast.
Habitat	Vegetation in permanent marshes, on rocks in streams and stony beaches of Lake Victoria.
Okavango Habitat	Not found

	***Segmentorbis kanisaensis* (Preston, 1914)**
Descriptive notes 	Shell less than 5 mm in diameter, depressed, sharply carinate. Stronger spiral sculpture than other species.
Distribution	Tropical areas in Africa. Ethiopia and Southern Sudan; westwards through Niger and Mali into Gambia. Southwards to coastal plain of Angola; Okavango River and Durban (S.A.).
Habitat	Permanent marshes, temporary rainpools.
Okavango Habitat	Not found

	***Biomphalaria pfeifferi* (Krauss, 1848)**
Descriptive notes 	5.2 x 13 mm. Umbilicus occupies about one third of shell diameter, whorls somewhat bluntly angular below. Long slender tentacles and reddish blood that contains haemaglobin.
Distribution	Distributed mainly in tropical regions of Africa; South-western Arabia and Madagascar.
Habitat	Streams, irrigation channels, reservoirs, dams, some seasonal waters.
Okavango Habitat	Etsatsa Floodplain, Willies Camp, Seronga Floodplains, Channel near Guma Lagoon and Guma Floodplains; Duba Lagoon; Jao Island and Thaoge Lagoon.
Remarks	Most important intermediate host in tropical Africa. Serves as an intermediate host for *Schistosoma mansoni*. Natural infections with 11 species of larval trematodes were found in North-western Tanzania (Loker, Moyo & Gardner 1981).

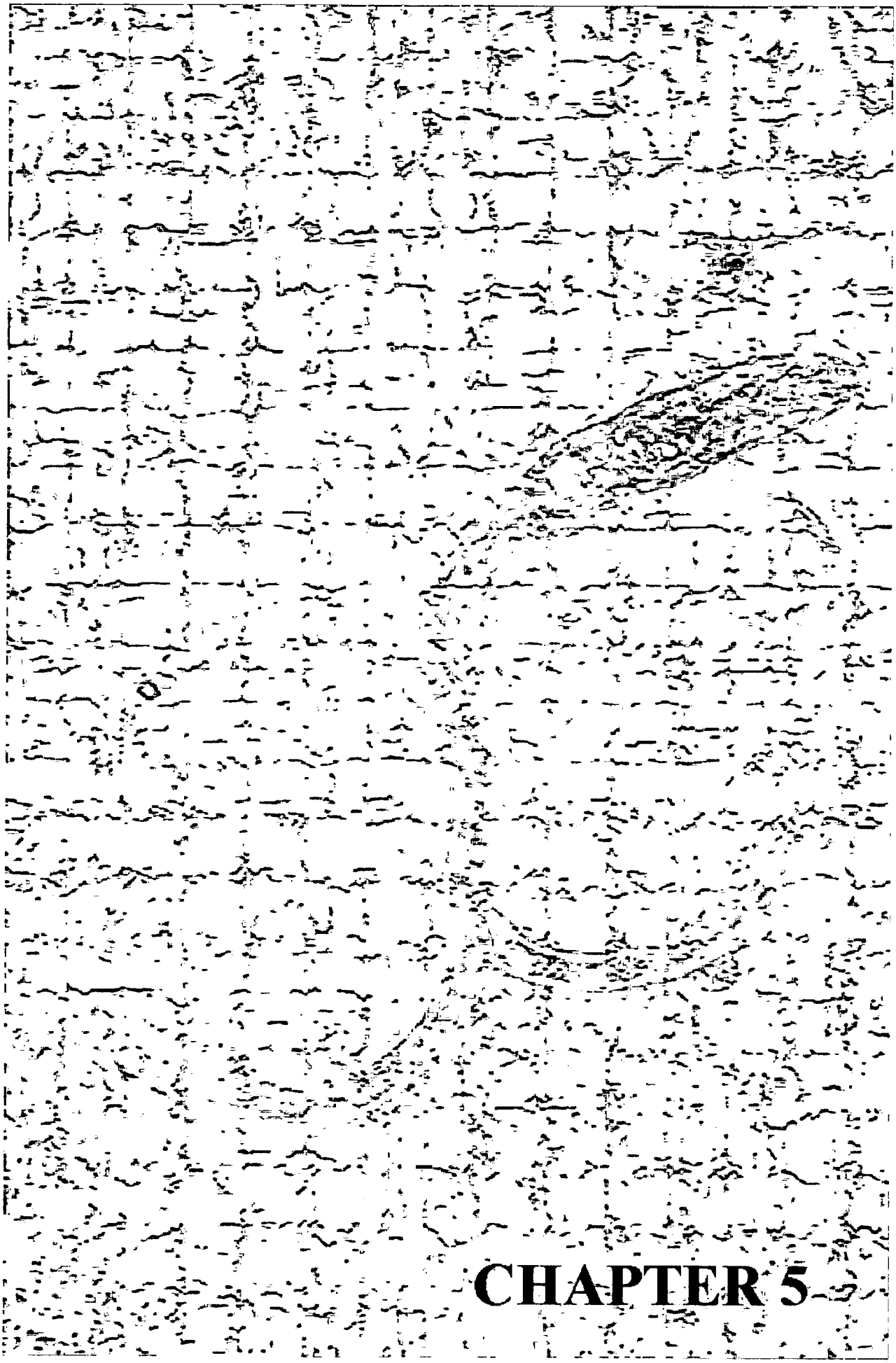
	***Bulinus scalaris* (Dunker, 1845)**
Descriptive notes 	Broad shell, lower whorls evenly convex, upper whorls somewhat shouldered, sometimes weakly carinate. Ribs present, though become progressively weaker, last whorl almost smooth.
Distribution	Eastern and southern Africa; Ethiopia highlands (Brown 1965); Uganda. Extends from Ethiopia southwards into Zimbabwe; Namibia; Botswana; Okavango floodplain and East Caprivi.
Habitat	Seasonal pools without vegetation, Ethiopia, Western Kenya. Habitats in Namibia and Angola include concrete-lined irrigation channels. Main channels in Okavango Floodplain, though snail occurs more frequently in isolated pools.
Okavango Habitat	Not found
Remarks	Synonym *Bulinus benguelensis* (Sowerby, 1873).

	***Bulinus globosus* (Morelet, 1868)**
Descriptive notes 	22.5 x 14 mm. Spire varies widely in proportional height. Columellar fold generally strong, narrowly umbilicate or less commonly imperforate. Corrugated microsculpture present to varying degree on all whorls.
Distribution	Greatest range of any member of species-group, occupying much of Africa south of Sahara. Southern limits are Okavango Delta and warmer part of coastal plain of eastern South Africa.
Habitat	Streams, rivers, seasonal pools, lakes, earth dams, irrigation systems and older rice paddies. Shallow water, where it may occur on bare substrate, more common among aquatic plants (Thomas & Tait 1984).
Okavango	Mohembo Floodplains and Backwaters; Xaro Backwaters Etsatsa

Habitat	Floodplains and Mainstream; Sepopa Floodplains; Seronga Floodplain, Seronga Fisheries Camp; Channel near Guma and Guma Floodplains; Duba Lagoon; Jao Channel and Thaoge Lagoon; Willies Camp.
Remarks	Known to be intermediate host for schistosomiasis. During the study, we collected *B. globosus* in the area and not *B. africanus* since the latter has a more easterly distribution and because of the habitat it was found in.

	***Bulinus depressus* Haas, 1936**
Descriptive notes 	Shells small, low spired, narrowly reflected columellar lip and membranous ribs. Largest shells, 6.5mm in length with 3.3 whorls. Whorls bluntly shouldered; lamellate, columellar margin of aperture is straight, slightly twisted, only narrowly reflected.
Distribution	Northern Transvaal; westwards down basins of Vaal and Orange Rivers (Hamilton-Atwell & Van Eeden 1969). Okavango River and Delta; East Caprivi.
Habitat	Rivers, pools, lakes, temporary marshes. Cement-lined reservoirs, earth dams and rivers (Schutte 1966; Hamilton-Atwell & Van Eeden 1969)
Okavango Habitat	Not found
Remarks	Snails from Northern Transvaal are not susceptible to infection with either *Schistosoma haematobium* or *S. mattheei* from South eastern Transvaal (Schutte 1966).

	***Bulinus tropicus* (Krauss, 1848)**
Descriptive notes 	12.3 x 7.8 mm (slender form), 10.6 x 8.3 mm (more globose form); rarely 20mm. Whorl shape varied from shouldered to almost evenly curved. Lamellate ribs generally strong, widely spaced. Columellar side of aperture varies from concave to straight. Shells perforate, with narrow to large umbilicus.
Distribution	Ethiopia; much of eastern Africa, southwards to Botswana; large part of South Africa; Lesotho; Namibia. Found up to altitudes of 2700 m in Kenya and 3100 m in Lesotho.
Habitat	Common in small earth dams, residual pools, seasonally flowing streams, highlands of eastern Africa and highveld of southern Africa (Brown 1994).
Okavango Habitat	Not found
Remarks	Serves as intermediate hosts for the following parasites: *Schistosoma bovis* in Kenya; *S. margrebowiei* in Zambia; *Calicophoron microbothrium* south of the Sahara.



CHAPTER 5

SYSTEMATICS OF THE SUBCLASS DIGENEA

EARLY DIGENEAN CLASSIFICATION

Digenean classification is rather unstable, particularly in the higher categories. The reason for this may be as a result of the great diversity of morphological features between species, as well as new species being described every year. The biology and ontogeny of most species was unknown in earlier years, therefore systematists had to depend on adult morphology for their basis of classification (Schmidt & Roberts 1977).

Almost three centuries ago, trematodes were first classified in the publication of Linnaeus' *Systema Naturae* in 1758. The system recognised only one genus namely *Fasciola* Linnaeus, 1758, with two species of which only one, *Fasciola hepatica* Linnaeus, 1758 was a trematode. During the first century of trematode systematics only a few genera were erected and a small number of species were characterised based on organs of attachment (La Rue 1957).

Larval stages were first described in the 19th century, although many authors recognised them as being adult trematodes (La Rue 1957). O.F. Müller (1773) called them "Cercaria", while Lamarck (1815) named them "Furcocercaria" and Bory de St. Vincent (1823) named them "Histrionella".

The major landmark in the classification of trematodes, was the division of the Trematoda by Van Beneden (1858) into two major groups, Monogénèses (Monogenea of Carus) and the Digénèses (Digenea of Carus). The division was based on the fundamental differences in the life history strategies of these two groups. Van Beneden (1858) applied the term Monogénèses to trematodes having a direct development and Digénèses to those having "an indirect development, a double reproduction, agamic and sexual." Important to note is that Van Beneden stressed that essential differences in reproduction were important, and not the numbers of hosts involved as suggested by some authors. Van Beneden based his foundation on the concept that life history is an important criterion for the separation of trematodes into two major groups, now ranked as subclasses.

DIVISION OF DIGENEA INTO FAMILIES AND SUBORDERS IN THE 19TH CENTURY

Diesing (1850) erected a suborder for cercariae, which he actually recognised as adult trematodes. His revision of the cercariae in 1855 recognised 20 species and nine genera. Although he accepted the larval nature of cercariae, he still assigned generic names to them. Looss (1899) published his work on the trematode fauna of Egypt, this report contained the results of an investigation on the natural classification of the genus *Distomum*. He based his conclusions on anatomical studies of adult worms and erected many natural genera with twelve subfamilies (La Rue 1938).

Monticelli (1888) followed Van Beneden in recognising the Monogenea and Digenea as two separate groups. He divided the Digenea into four families: Amphistomeae Monticelli, 1888, Diplostomeae Monticelli, 1888, Distomeae, Monticelli, 1888 and Monostomeae, Monticelli, 1888. Monticelli (1892) rejected Van Beneden's classification system and developed one based on organs of attachment and method of development.

Although accepted by Braun (1893) in his work on trematodes, Monticelli's system did not survive. Braun (1893) questioned whether we are warranted in basing a system of the trematodes exclusively on their different methods of development. In his monograph, Braun (1893) divided the suborder Malacocotylea Monticelli 1888 into two groups, Metastatica to include the Holostomidae, and Digenea to include the Amphistomidae, Distomidae, Didymozoonidae and Monostomidae (La Rue 1938).

The nineteenth century was important in that many investigators tried to relate generations of digenetic trematodes in snails to adult worms (La Rue 1938). In doing this they rendered a valuable service to the taxonomy of the digenetic trematodes. The discovery of the life history of the sheep liver fluke, *Fasciola hepatica*, by Leuckart (1882) and Thomas (1883) respectively, was an important contribution and led the way to subsequent solution of other life histories.

STUDIES IN THE 20TH CENTURY

Lühe (1909) designed a system of classification, which was based largely on external characters such as suckers, stylets, collar and collar spines, and types of tail. The system was largely artificial since it did not take internal characters into consideration. Although artificial, the system laid the foundation for cercarial classifications down to the present (La Rue 1957).

Odhner (1905) made a very significant contribution to the development of the modern system of the Digenea. He wrote a series of articles entitled "Zum natürlichen system der digenen Trematoden". He also rendered a great service in reducing the great group of digenetic trematodes to an orderly system (La Rue 1938). Odhner (1905), in developing his ideas placed great emphasis on the arrangement of the reproductive system, the excretory system and other anatomical structures.

THE EXCRETORY SYSTEM USED AS AN IMPORTANT TAXONOMIC TOOL/CHARACTER IN THE CLASSIFICATION OF THE DIGENEA

Cort (1917) considered the excretory system to be of great value in establishing a natural system of classification. While studying several fork-tailed cercariae he saw close similarities in the arrangement of the excretory canals and differences in other organ systems, especially the gut. Faust (1918, 1919 & 1924), Sewell (1922), Szidat (1924), La Rue (1926) and others developed this idea further, which led to the establishment of the flame cell formula by Faust (1919) (Dawes 1968). This valuable contribution is still currently being used today.

In the earlier century strigeids were regarded as being metastatic in development. During the years 1920-24 three important investigations, namely those of Lutz (1920), Ruszkowski (1921) and Szidat (1924), proved without doubt that strigeids were in fact true digenetic trematodes. The authors showed that the miracidium in this family possess two pairs of flame cells; the cercariae have forked tails with slender tail stems; pharynges are present and the oral sucker bears no stylet. Their work made evident that the fork-tailed cercariae with pharynges described by Cort (1917) belonged in fact to species of strigeids.

La Rue (1926) first showed the probable relationship between representatives of the Strigeidae and the Schistosomatidae. The statement was not only based on the similarities of the excretory systems but also because the miracidia of these two groups possess two pairs of flame cells while other miracidia known at that time had only one pair (La Rue 1938).

Faust (1929) presented an outline of the classification of the digeneans based primarily on fundamental excretory patterns, but also used life history criteria, while in the same year Stunkard (1929) showed that by using only larval characters and single systems of organs one could easily make the wrong assumptions.

Stunkard (1929) made a detailed comparison of the excretory systems of three genera, i.e. *Heterophyes* Cobbold, 1866, *Microphallus* and *Cryptocotyle* Lühe, 1899 all belonging to the same family Heterophyidae. In this comparison special emphasis was placed on the flame cell formula and he concluded that the excretory systems and flame cell formulas of these three genera were markedly different. The excretory patterns of *Microphallus* and *Heterophyes,* however, were more similar than *Heterophyes* and *Cryptocotyle* (La Rue 1938).

The form of the excretory vesicle has been said to be an important taxonomic character. Baer (1924) and Mehra (1931) showed that the excretory vesicle can be represented by different forms, namely cylindrical, Y-shaped and V-shaped, sometimes a network type of excretory system may also be found (La Rue 1938).

McMullen (1937) brought the relationships of the three main forms of excretory systems together in the form of a plate. He showed that the vesicle might change from the Y to V form when the body extends and contracts. The taxonomic value of the excretory vesicle in the adult therefore depends on the ability of the adult to retain its original form, in other words its ability to retain the form it had in the cercariae. In order for the investigator to determine what the form was, he needs to know what the life history is and sometimes this may be impossible.

A character of considerable value, is the pharynx (Miller 1926). Its absence characterises the entire group of blood flukes, both adults and cercariae, while its presence characterises the Strigeoidea.

The development of a natural taxonomic system for the Digenea must be based upon comparative anatomy of all stages in the life history (La Rue 1938). When looking at the systematics of a group, the following should be taken into consideration (Fig. 5.1):

- **Miracidium:** Number of flame cells present. Number and arrangement of epidermal plates.
- **Cercaria:** Form or type of cercaria. Number of penetration glands. Tail and external characters. Presence or absence of pharynx. Excretory system including flame cell pattern, form and proportions of the excretory vesicle.
- **Adult:** Arrangement of reproductive organs.

As mentioned above the classification of the subclass Digenea is a difficult undertaking. Personal communication with Dr. David Gibson from the Natural History Museum has revealed that he and his colleagues will be releasing a new, and as of yet, unpublished classification system. The classification system will be released in a series of volumes. Dr. Gibson has kindly given me permission to use this scheme in my thesis. The scheme will appear in my thesis exactly as I received it via e-mail from Dr. Gibson. Since I only received the scheme, I will not be commenting on any of the divisions he has made, neither will I add authors to families and subfamilies (Table 5.1).

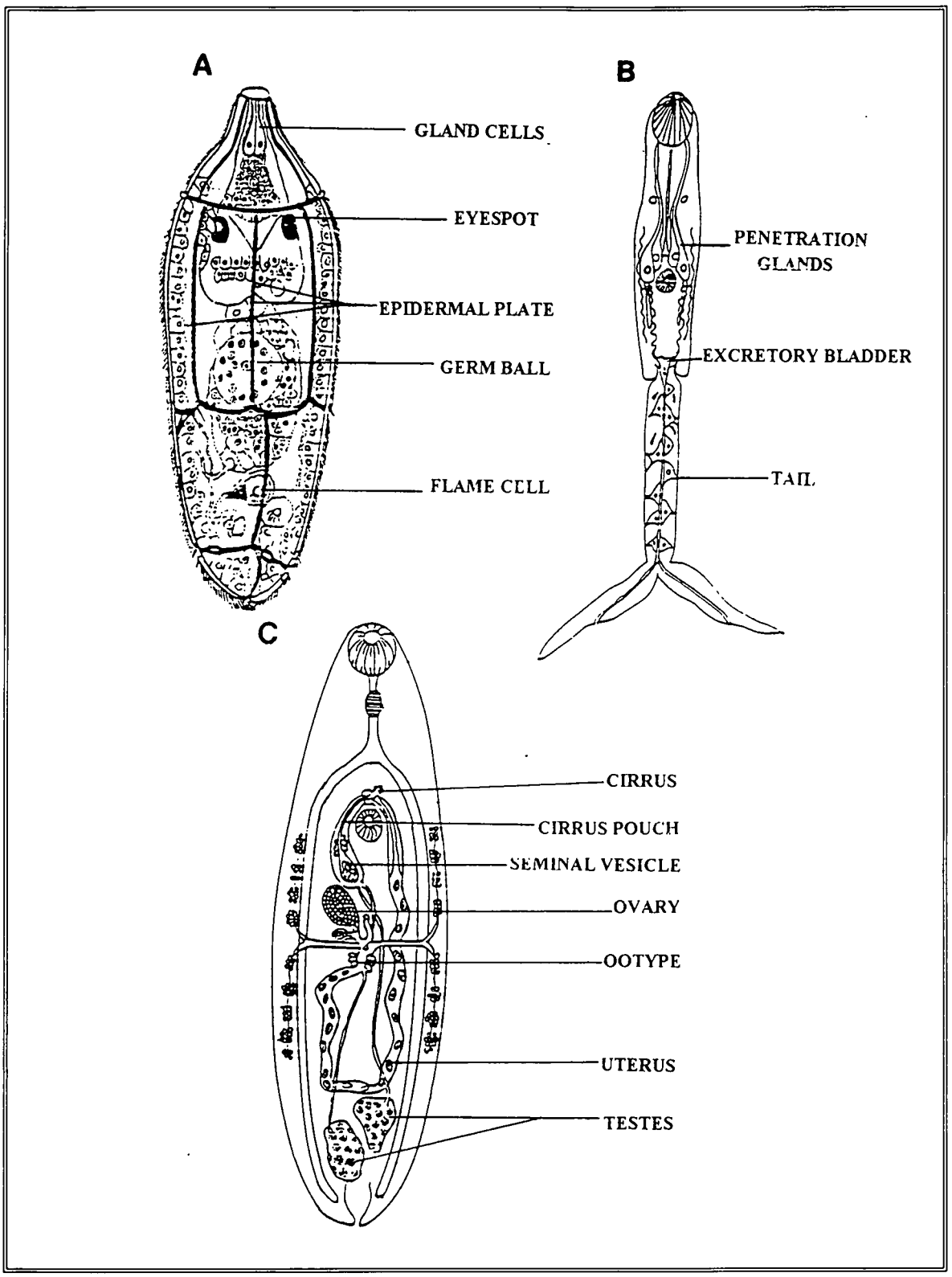


Figure 5.1: Showing the characteristics of (**A**) Miracidium (**B**) Cercaria and (**C**) Adult, when considering the systematics of the digeneans (compiled from Smyth 1994, Noble & Noble 1982).

Table 5.1 : New Classification Scheme of the subclass Digenea as proposed by Gibson, Bray & Jones (In press).

SUBCLASS	ORDER	SUPERFAMILY	FAMILY
Digenea Carus, 1863	Strigeida	Azygioidea	Azygiidae
		Bivesiculoidea ?	Bivesiculidae
		Brachylaimoidea	Brachylaimidae
			Hasstilesiidae
			Leucochloridiidae
			Leucochloridiomorphia
			Moreauiidae
			Panopistidae
			Ovariopteridae
			Thapariellidae
		Bucephaloidea	Bucephalidae
		Clinostomatoidea	Clinostomatidae
			Liolopidae
		Cyclocoeloidea	Cyclocoelidae
			Bothriogastridae
			Eucotylidae
			Typhlocoelidae
		Diplostomoidea	Bolbocephalodidae
			Brauninidae
			Cyathocotylidae
			Diplostomatidae
			Proterodiplostomatidae
			Strigeidae
		Gymnophalloidea	Botulosaccide
			Callodistomidae
			Fellodistomidae
			Gymnophallidae
			Tandanicolidae
		Hemiuroidea	Accacoeliidae
			Bathycotylidae
			Bunocotylidae
			Derogenidae
			Dictysarcidae
			Didymozoidae
			Hemiuridae
			Hirudinellidae
			Isoparorchiidae
			Lecithastteridae
			Ptychogonimidae
			Sclerodistomidae
			Sclerodisromoididae
			Syncoeliidae
		Schistosomatoidea	**Schistosomatidae**
			Sanguinicolidae
			Spirorchidae
		Transversotrmatoidea	Transversotrematidae

Table 5.1 continued: New Classification Scheme of the subclass Digenea as proposed by Gibson, Bray & Jones (In press).

SUBCLASS	ORDER	SUPERFAMILY	FAMILY
Digenea Carus, 1863	Echinostomida	Echinostomatoidea	Atractotrematidae (?)
			Balfouridae (?)
			Echinostomatidae
			Fasciolidae
			Haploporidae (?)
			Haplosplanchnidae (?)
			Ommatobrephidae (?)
			Philophthalmidae
			Psilostomatidae (incl. Cathaemasiidae)
			Rhopaliidae
			Rhytidodidae (?)
			Waretrematidae (?)
		Notocotyloidea	Charaxicephalidae
			Labicolidae
			Notocotylidae
			Nudacotylidae
			Opisthotrematidae
			Pronocephalidae
			Rhabdopoeidae
		Renicoloidea (?)	Renicolidae
		Paramphistomatoidea	Angiodictyidae
			Balanorchiidae
			Brumptiidae
			Cladorchiidae
			Diplodiscidae
			Gatrodiscidae
			Gastrothylacidae
			Heronimidae (?)
			Megasoleniidae(?)
			Mesometridae (?)
			Microscaphidiidae
			Paramphistomidae
			Stephanopharyngidae
			Zonocotylidae
			Zygocotylidae
	Plagiorchiida	Allocreadioidea	Allocreadiidae
			Opecoelidae
			Opistholebetidae
		Lepocreadioidea	Acanthocolpidae
			Deropristidae
			Diplangidae (?)
			Enenteridae (?)
			Gorgocephalidae (?)
			Gyliauchenenidae (?)
			Apocreadiidae
			Lepocreadiidae
			Liliatrematidae
			Megaperidae
			Pleorchiidae

Table 5.1 continued: New Classification Scheme of the subclass Digenea as proposed by Gibson, Bray & Jones (In press).

SUBCLASS	ORDER	SUPERFAMILY	FAMILY
Digenea Carus, 1863		Opisthorchioidea	Acanthocollaritrematidae (?)
			Cryptogonimidae (incl. Acanthostomidae)
			Heterophyidae
			Jubilariidae (?)
			Opisthorchiidae
			Pachytrematidae
			Ratziidae
			Tetracladiidae (?)
		Microphalloidea	Anenterotrematidae (?)
			Microphallidae
			Taiwantrematidae (?)
		Plagiorchioidea	Allassogonoporidae
			Anchitrematidae (?)
			Auridistomidae (?)
			Brachycoelidae
			Braunotrematidae (?)
			Calycodidae (?)
			Cephalogonimidae (?)
			Choanocotylidae
			Dicrocoeliidae
			Dolichoperidae (?)
			Echinoporidae (?)
			Eumegacetidae (?)
			Exotidendriidae
			Gekkonotrematidae
			Gorgoderidae
			Laterotrematidae (?)
			Lecithdendriidae
			Macroderidae
			Macroderoididae
			Meristocotylidae (?)
			Mesotretidae (?)
			Ocadiatrematidae (?)
			Ochetosomatidae
			Omphalometridae
			Pachypsolidae (?)
			Plagiorchiidae (incl. Haplometridae)
			Prosthogonimidae (?)
			Stomylostrematidae (?)
			Teleorchiidae
			Urotrematidae
		Troglotrematoidea	Achillurbaniidae
			Collyriclidae (?)
			Cortrematidae (?)
			Orchipedidae (?)
			Paragonimidae
			Prouterinidae (?)
			Troglotrematidae (incl. Nanophyetidae)
		Zoogonoidea	Cephaloporidae
			Chelogonimidae (?-inquirendae)
			Faustulidae
			Lissorchiidae
			Monorchiidae
			Octotestidae (?)
			Zoogonidae

CLASS TREMATODA RUDOLPHI, 1800

Trematodes are flatworms, which originally evolved as parasites of molluscs and virtually all species, retain a molluscan element in their life history (Gibson 1998). All trematodes are parasitic and may live on the skin, gills, buccal and cloacal surfaces of aquatic hosts, in the alimentary tract and its accessory structures or in the reproductive, excretory, respiratory, blood and nervous system.

SUBCLASS DIGENEA CARUS, 1863

GENERAL CHARACTERISTICS AND BIOLOGY

Digenetic trematodes, or flukes, are the most common and abundant of parasitic worms, second only to nematodes in their distribution. These parasites are found in the intestine, gall bladder, urinary bladder, blood, oesophagus, and practically in every other major organ within their hosts (Schmidt & Roberts 1977).

The digenetic trematodes are microscopically distinguished from the monogeneans by their simple external structure, in particular the absence of complicated adhesive organs; only simple organs are present. Adult digenetic trematodes usually possess two prominent suckers on their body surface – the anterior sucker, often referred to as the oral sucker when it surrounds the mouth, and a ventrally located holdfast sucker called the acetabulum (Fig. 5.2) (Cheng 1986). The digenea not only possess by far the most complex life cycles among the Platyhelminthes, but also among the Animal Kingdom. During the life cycle, the worm utilizes two, three, four, or more hosts – one being the definitive and the other intermediate or paratenic hosts. In each host the parasite takes on a different form. Digenetic trematodes are all monoecious, except the schistosome blood flukes and certain members of the suborder Didymozoida (Cheng 1986). Sexual reproduction may be brought about by either self-fertilization or cross fertilization between two individuals.

This heterogenous group of parasites exhibit a strong and persistent association with aquatic habitats. General biotic factors associated with aquatic environments influence the appearance and survival of these parasites. Therefore the survival of both adult and larval trematodes is susceptible to climatic and seasonal changes in the environment (Erasmus 1972).

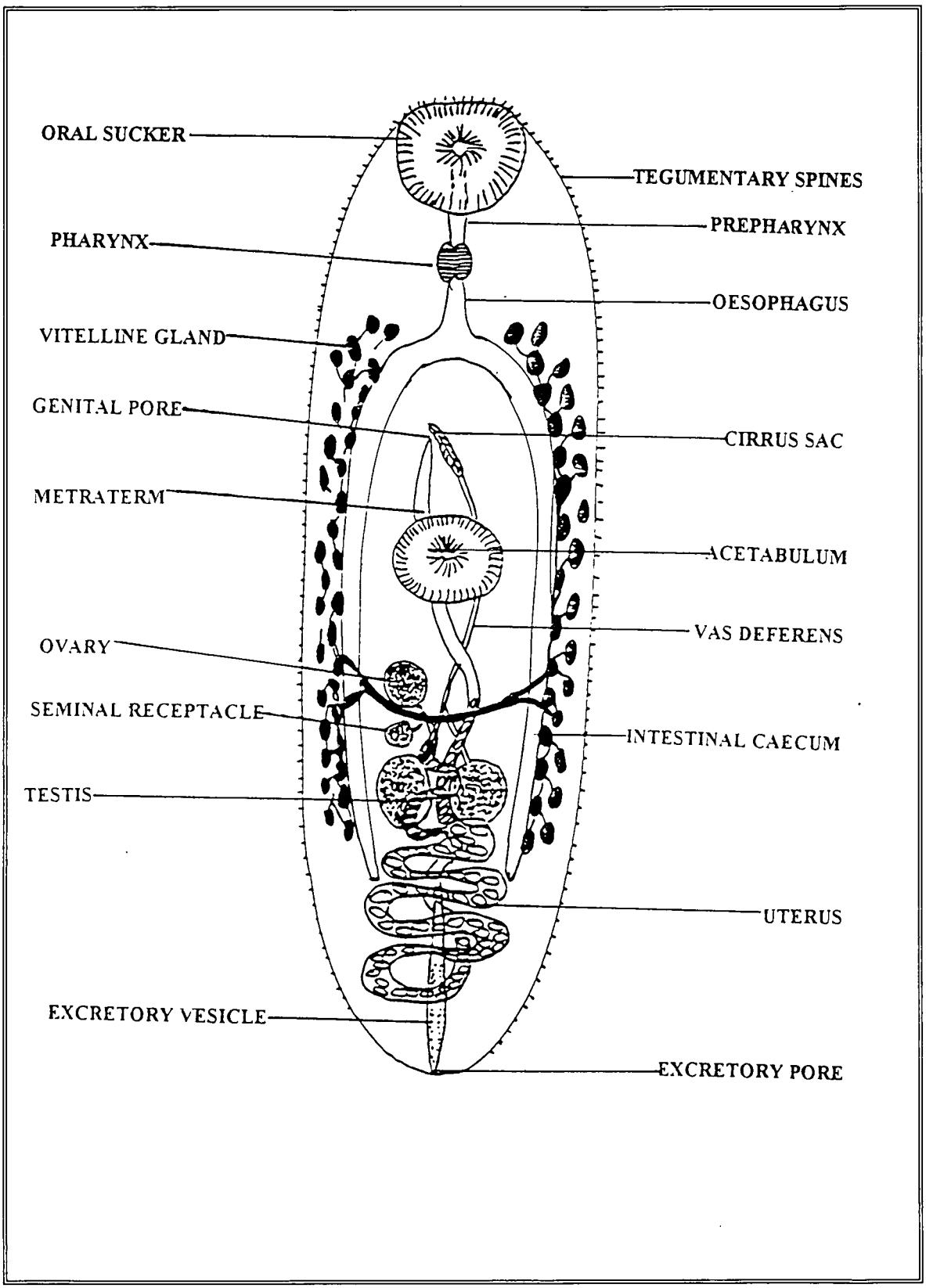


Figure 5.2: Generalised diagram of a digenetic trematode (redrawn from Cheng 1986).

The first intermediate host, in most cases, is a mollusc. The exception being an annelid which is also used as the first intermediate host. Larval stages undergo polyembryony so that enormous numbers of larvae may result from small infections (Smyth 1994). The extensive sequence of multiplication within the molluscan first intermediate host culminates in the production of cercariae. The basic function of this stage is dissemination, although in many cycles the ability to enter and infect a host directly is combined with this function (Erasmus 1972).

Several species cause economic losses to society through infections of domestic animals, while others are medically important parasites of humans. Since these parasites are of great importance, they have stimulated vast amounts of research, and the literature available on the Digenea is immense (Schmidt & Roberts 1977).

Fried and Graczyk (1997) compiled a very interesting table showing significant contributions made by various authors to the biology of Trematodes. Some of the selected milestones are shown in Table 5.2:

Table 5.2: Selected Milestones in the Biology of Trematodes (compiled from Fried & Graczyk 1997).

Date	Author	Contribution
1688	Redi	Published the first figure of a liver fluke, probably *F. hepatica*; rejected the idea of spontaneous generation
1690	Faber	Observed that liver fluke is found in bile ducts and not in blood vessels
1699-1700	Van Leeuwenhoek	Reported the occurrence of *F. hepatica* in sheep
1737	Swammerdam	First observed cercariae in snails
1758	Linnaeus	Classified *F. hepatica* as a worm infecting humans
1773	Müller	Gave the word, cercaria, for tailed microscopic animals
1807	Nitzsch	First report of cercarial encystment
1808-1810	Rudolphi	Flukes and leeches were originally grouped together until he established Trematoda for the flukes

Table 5.2 continued: Selected Milestones in the Biology of Trematodes (compiled from Fried & Graczyk 1997).

DATE	AUTHOR	CONTRIBUTION
1818	Bojanus	Reported rediae in snails, noted the escape of cercariae from the snails and also noted the resemblance between rediae, cercariae and adults
1837	Filippi	Named rediae after Francesco Redi and recognised them as larval flukes
1837	Von Siebold	Noted encystment of cercariae in snails
1842	Steenstrup	Described alternation of generations that had important implications on trematode research
1850	Diesing	First classification of cercariae (did not recognise them as larval trematodes)
1852	Bilharz	Discovered *Distomum haematobium*
1854	Filippi	Used term, sporocyst
1882-1883	Thomas (in England) and Leukart (in Germany)	Independently described the first life history of a digenean, *F. hepatica*, using experimental infections; independently discovered that *Lymnaea* snails were the intermediate hosts of *F. hepatica*
1892	Lutz	Demonstrated the development of *F. gigantica* adults from the metacercarial stage; recovered adult flukes from guinea pig that were fed cysts
1904	Katsurada	Described *Schistosoma japonicum*
1909	Fujinami and Nakamura	Showed that *S. japonicum* infections in cows were acquired through the skin
1909	Lühe	First comprehensive classification of cercariae
1911	Lebour	Major revision of cercarial classification
1913	Miyairi and Suzuki	Described the life cycle of *S. japonicum* in Japan
1915	Leiper and colleagues	Described the life cycle *S. haematobium*
1916	Leiper	Showed that *S. mansoni* developed in *Biomphalaria* snails and was distinct from *S. haematobium*
1934	Wesenberg-Lund	Showed that echinostome rediae preyed on rediae and sporocysts of other digeneans in the same snail host.
1946	Dawes	First major treatise on trematodes
1959	Smyth and Clegg	Described eggshell formation in trematodes
1961	Vernberg	Studied oxygen consumption in digenetic trematodes
1964	Halton	Described nutrition of digeneans

Table 5.2 continued: Selected Milestones in the Biology of Trematodes (compiled from Fried & Graczyk 1997).

DATE	AUTHOR	CONTRIBUTION
1965-1966	Dixon	Described encystment and excystment of *F. hepatica* metacercariae
1966	Smyth	First account of *The physiology of Trematodes*
1966	Von Brand	Reviewed biochemistry of larval and adult digeneans in *Biochemistry of Parasites*
1968	Jennings	Discussed nutrition and digestion in trematodes
1972	Erasmus	Published classic text, *The Biology of Trematodes*
1972	Eveland	Described conversion of cercariae of *Schistosoma mansoni* to schistosomula
1973	Hockley	Described ultrastructure of the tegument of schistosomes
1975	Lackie	Described the activation of larval stages of digeneans in vertebrate hosts
1976	Smithers	Described immunity to trematode infections with special reference to schistosomiasis and fascioliasis
1977	Erasmus	Discussed the concept of the host-parasite interface in trematodes
1980	Jourdane and Theron	Cloned *S. mansoni* by microsurgical transplantation of sporocysts
1981	Simpson	Isolated and characterised the schistosome tegument
1983	Smyth and Halton	Second edition of *The Physiology of Trematodes*
1987	Rollinson and Simpson	First book on the biology of schistosomes, *Genes to Latrines*
1993-1994	Bayne	Cultured cells from larval *S. mansoni in vitro*
1994	Halton	Reviewed studies on regulatory peptides in trematodes
1995	Haas	Described chemical cues in snail-host finding by miracidia and cercariae

GENERALISED LIFE CYCLE OF A DIGENETIC TREMATODE

The life cycle of a digenean is said to be unique in that it usually involves two asexual generations in a mollusc and a sexual generation in a vertebrate host. The life cycle is represented in **Fig. 5.3** and the various life cycle stages will be explained below.

DESCRIPTION OF THE VARIOUS LIFE CYCLE STAGES:

The Digenean Egg (Fig. 5.4A):

- Egg is a developing embryo enclosed by shell
- Once in ootype, egg becomes surrounded by a predetermined number of vitelline cells
- Scleritin, which is an inert and yellow substance, makes up the egg shell
- Different species have different colouring and thickness
- Egg operculate, except schistosome species, which are non-operculate and ornamented with spines
- All cases eggs hatch to release miracidium

The Miracidium (Fig. 5.4B)

- Is a multicellular free swimming larval stage
- Similar to the larvae of monogeneans, the oncomiracidium
- Exhibit special behavioural responses, so as to locate hosts
- Covered by ciliated epidermal plates
- Epidermal plates have a definite arrangement, being placed in four to five transverse rows
- Arrangement differs from species to species
- Have sensory organs to locate host
- Takes approximately 30 minutes to penetrate molluscan host
- Once the miracidium has entered the host, it sheds its ciliated plates and transforms into the sporocyst

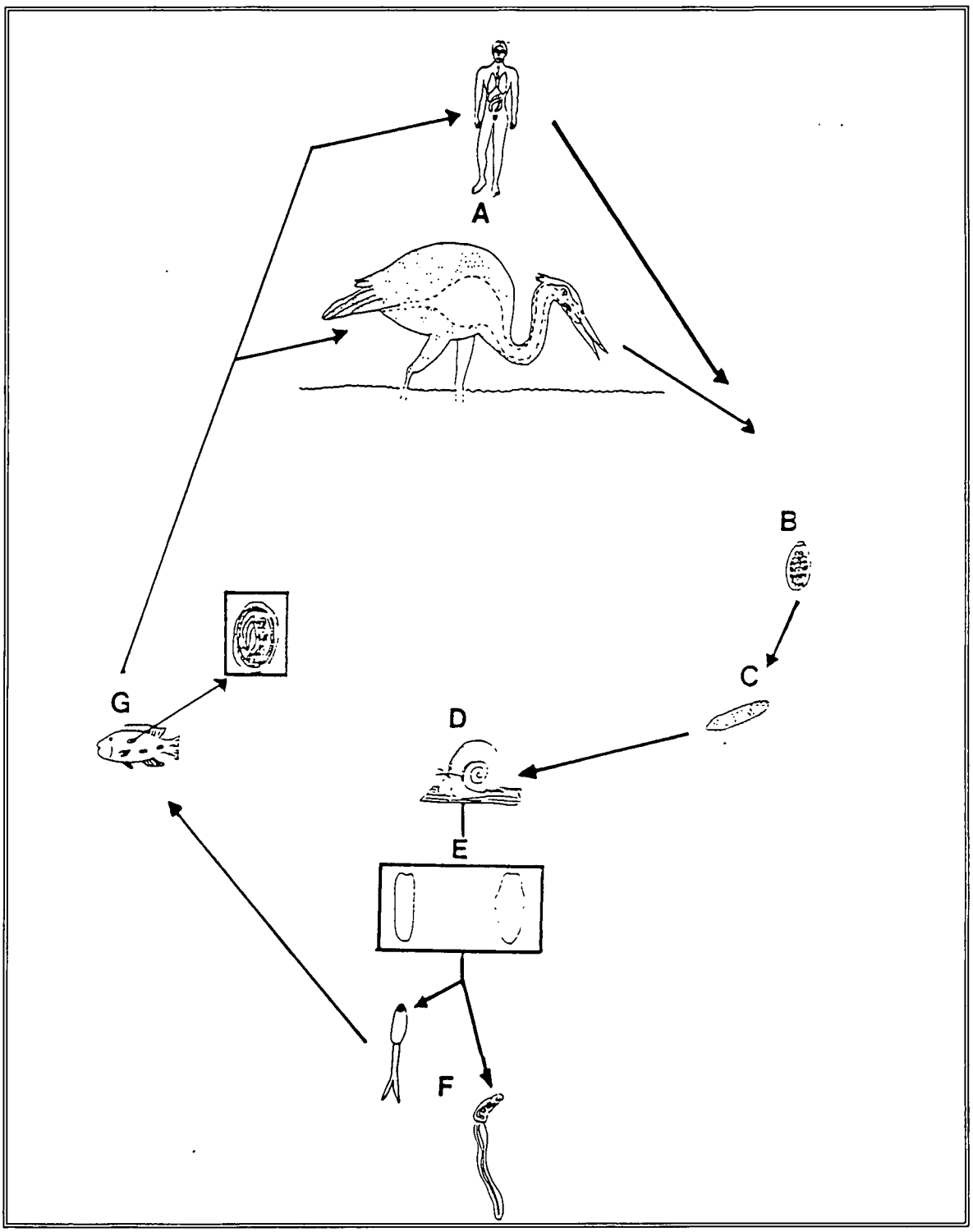


Figure 5.3: Generalised life cycle of a digenetic trematode. (**A**) Final Vertebrate Host/Definitive Host (**B**) Digenean Egg (**C**) Free Swimming Miracidium (**D**) 1st Intermediate Host (**E**) Sporocyst and Redia develop in snail which give rise to (**F**) Cercariae (**G**) 2nd Intermediate Host with encysted metacercariae.

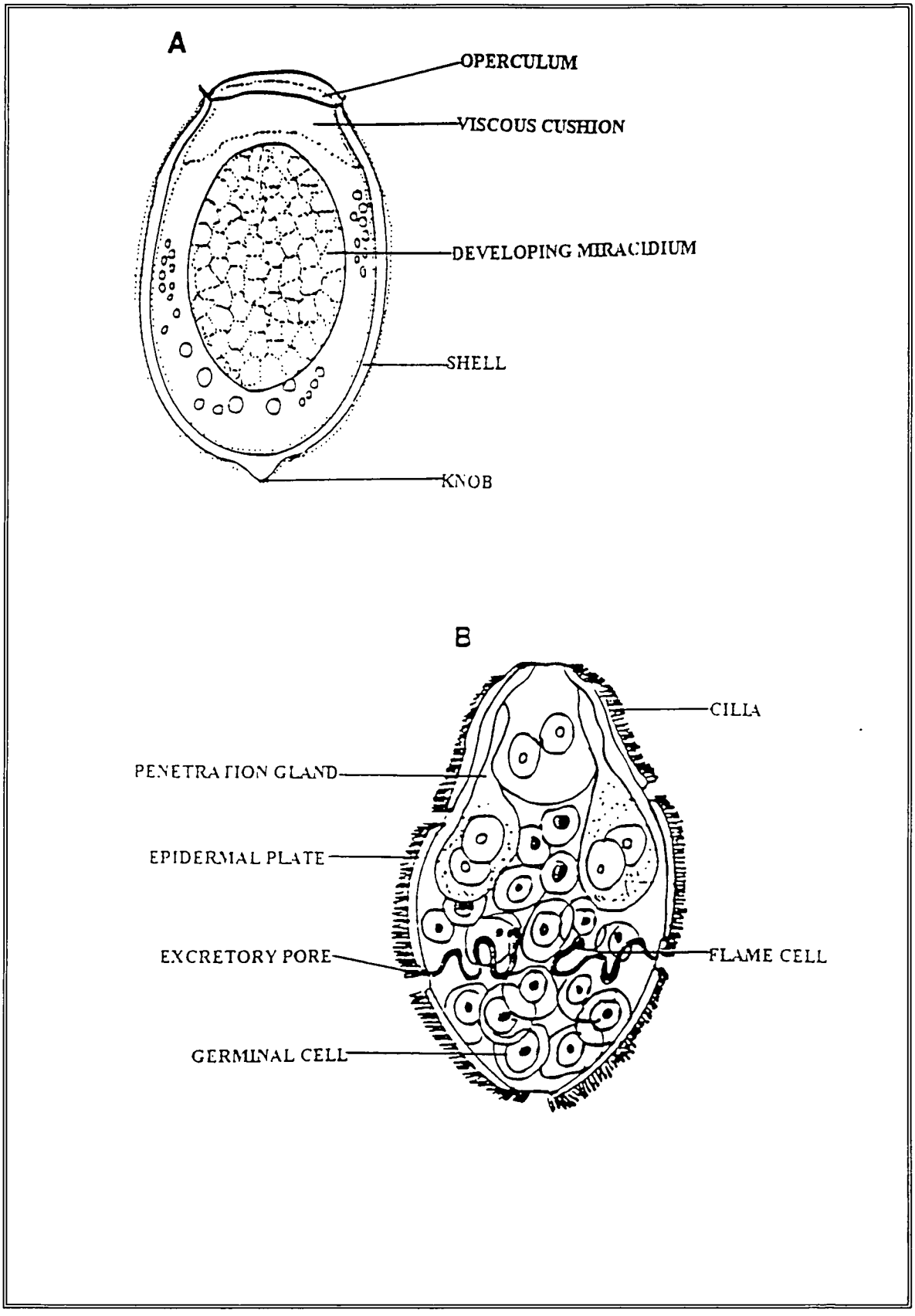


Figure 5.4: Illustrating (**A**) Digenean egg (**B**) Miracidium (redrawn from Cheng 1986).

The Sporocyst (Fig. 5.5A)

- Is a hollow fluid filled germinal sac into which the germinal masses protrude
- Larvae emerge from the birth pore at the anterior end of the sporocyst body
- Germinal masses develop internally into daughter sporocysts or into a second larval stage, namely the redia
- Different species of trematodes undergo different patterns of development
- Miracidium always develops into a sporocyst to start with, if daughter sporocysts are formed, redia don't develop
- In the lung fluke *Paragonimus* Braun, 1899 the parent sporocyst produces two generations of redia
- When daughter sporocysts emerge from parental sporocysts they migrate through host tissue and localise near the molluscs' digestive diverticulum
- Sporocyst stage obtains nutrients by passage of soluble material across sporocyst tegument (Erasmus 1972)

The Redia (Fig. 5.5B)

- Rediae burst through birth pore and migrate to the hepatopancreas or gonad of molluscan host (Schmidt and Roberts 1977)
- Similar to sporocyst also contains germinal masses within fluid filled sac
- May develop into either a second generation daughter redia, or into final larval stage within mollusc, the cercaria
- Redia are more active than the sporocyst and possess a simple gut
- Feed on tissue that is molluscan in origin
- Has been shown redia of echinostomes will actively seek out schistosome developing stages and feed on them (Lie, Basch and Umathevy 1965)
- Redia have gut which consists of mouth opening into large pharynx which inturn opens into a simple rhabdocoel like intestine
- Have a ridge-like collar, below which the birth canal opens, from which cercariae or daughter rediae emerge **(Fig. 5.5C).**
- Along the body lobe-like extensions are found which aid in the movement of the parasite in the host's tissue

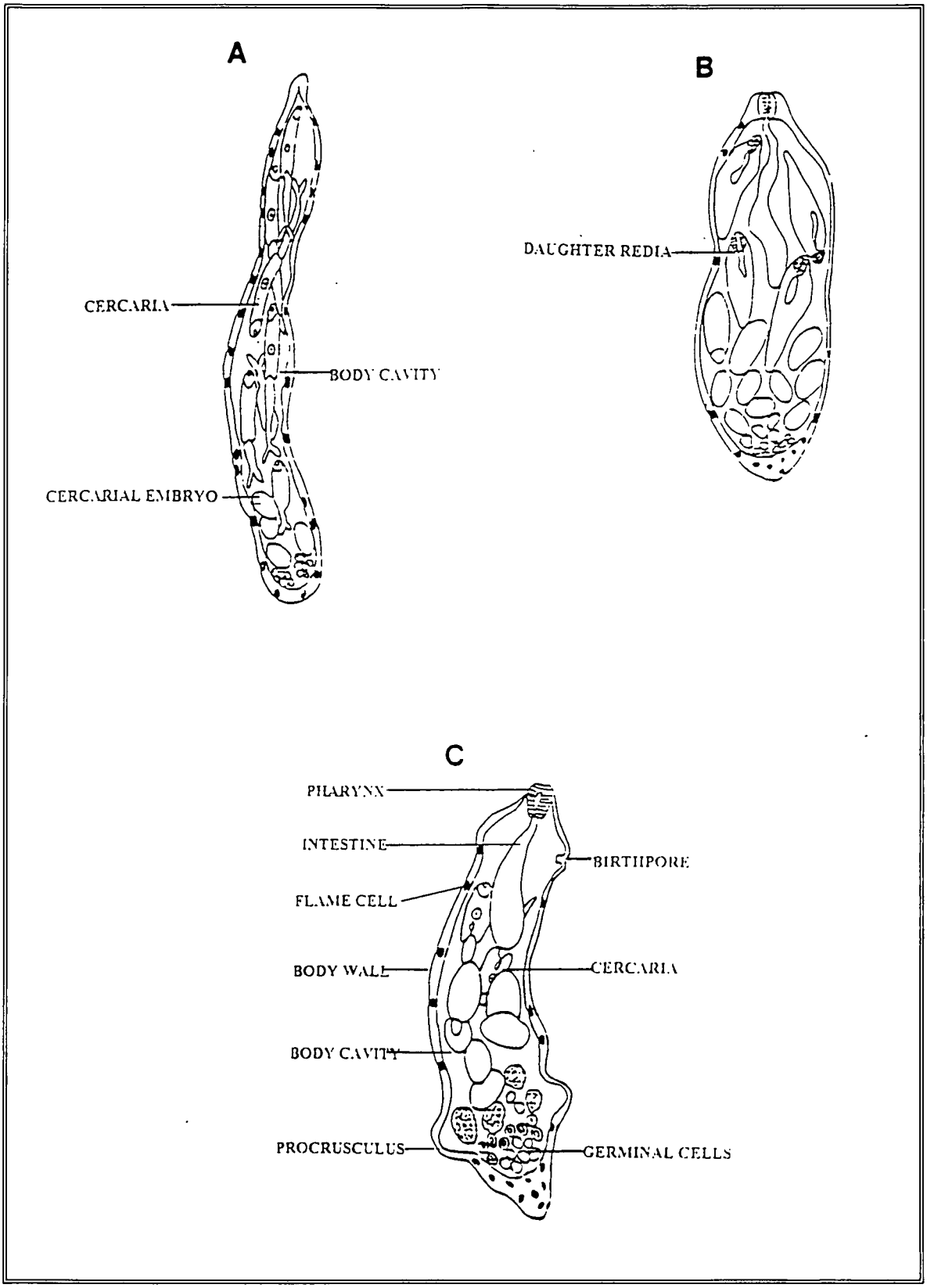


Figure 5.5: Illustrating the (**A**) Sporocyst (**B**) Mother Redia (**C**) Daughter redia (redrawn from Schell 1970).

The Cercaria (Fig. 5.6)

- Emerge from the mollusc, and is the infective form for the vertebrate host
- Exhibit considerable variations in structure which make them taxonomically important
- These variations reflect in many cases the adaptations that cercariae have to specific life cycles.
- A **system of cercarial classification** has evolved and is based on that of Lühe (1909), the system is descriptive rather than systematic in content (**Fig. 5.7A-O**).

1. **Gasterostome cercariae**. Two symmetrical tail furcae arising from posterior end of body. Mouth central on ventral surface. Intestine sac-like. Develop in sporocysts.
2. **Monostome cercariae**. No ventral sucker. Pigmented two or three eyes. Two excretory canals in body uniting near eyes, one in simple tail. No pharynx. Dense cystogenous glands. Develop in rediae.
3. **Amphistome cercariae**. Ventral sucker at root of slender tail. Develop in rediae.
4. **Distome cercariae**. Commonest form of cercaria with ventral sucker some distance from posterior end of body.
5. **Rhopalocercous cercariae**. Tail when contracted is wider than body.
6. **Cystocercous cercariae**. Body can be withdrawn into a pocket at base of well-developed tail. Tail may be forked or not. Remainder of anatomy very variable.
7. **Gymnocephalous cercariae**. Two almost equal suckers, no stylet, well-developed pharynx, oesophagus and intestine, tail simple. A heterogenous collection.
8. **Xiphidocercariae**. Boring stylet with single point. Stylet glands, tail simple. Develop in sporocysts.
9. **Echinostome cercariae**. Collar of spines around anterior end, tail simple.
10. **Trichocercous cercariae**. Tail with rings of fine bristles.
11. **Furcocercous cercariae**. Tail forked distally containing branches of excretory duct. Flame cells in tail stem.

(a) Blood fluke cercariae without pharynx, e.g. *Schistosoma mansoni*

(b) Strigeid cercariae with pharynx, e.g. *Diplostomum phoxini*

12. **Mirocercous cercariae**. Tail vestigial.
13. **Cercariaea**. Tail absent.

14. **Rattenkönig' cercariae**. Cercariae tangled by tails to form colony. Marine, little known.

- Body of cercariae resembles adult trematode both internally and externally e.g. the ring of spines found at the anterior end of echinostome cercariae is also present in adult fluke
- Cercariae have number of different gland cells
- Include cystogenous glands which are used in forming the cyst wall during metacercarial stage, penetration glands used by cercariae to penetrate next host
- Once released from molluscan host, cercariae locate next host, the final or definitive host which they actively penetrate, or find suitable solid substrate to encyst upon and be ingested by definitive host
- Equipped with variety sensory organs to locate their targets
- Eyespots, touch receptors allow specialised behaviour, designed to bring the cercariae into an environment where they have the maximum probability of infecting next host
- Schistosomes for e.g. exhibit negative phototrophy, positive thermotrophy and thigmotrophy
- Cercariae exhibit definite patterns of circadian rhythms, being shed at times optimal for bringing them into contact with their next host

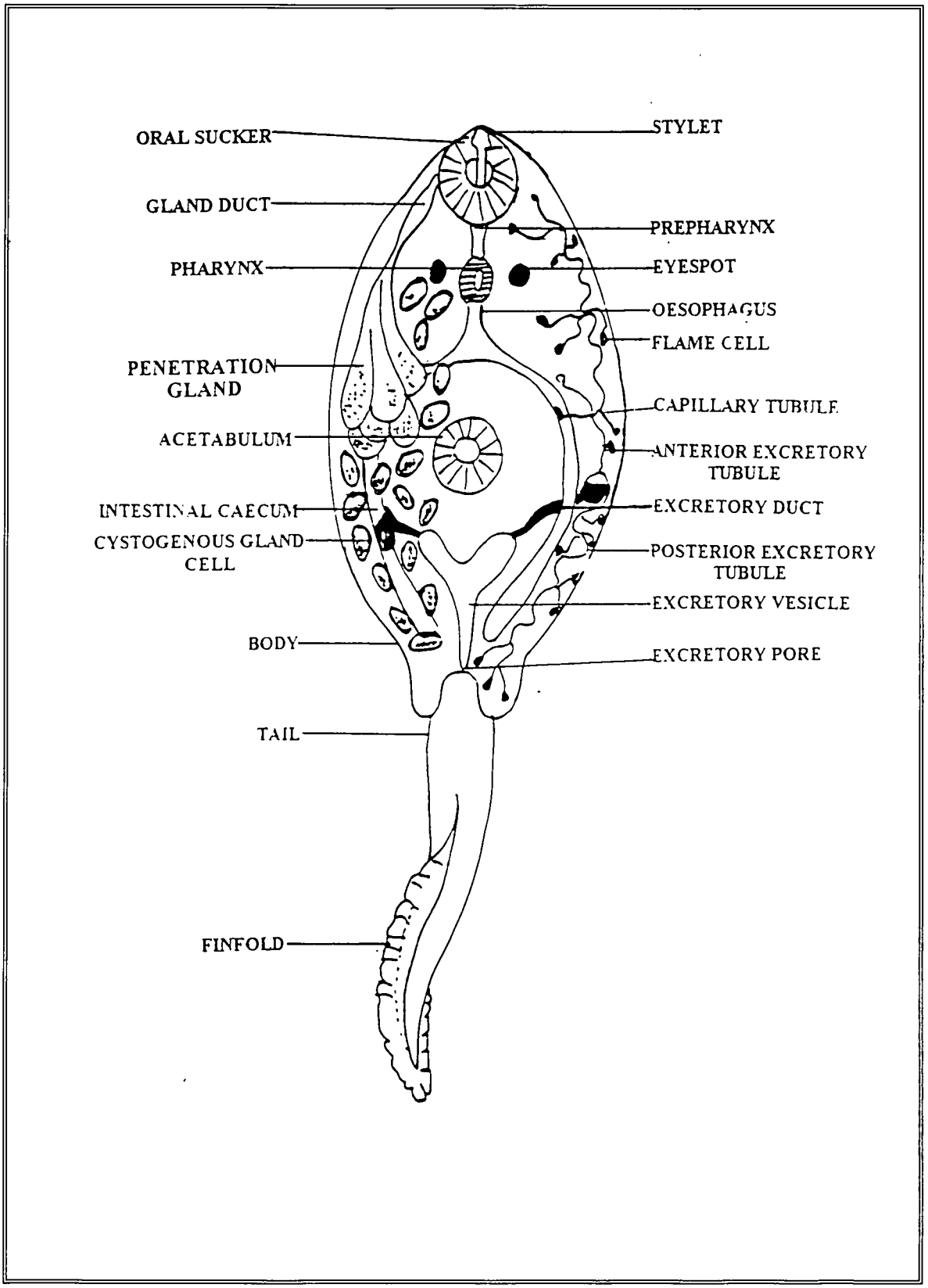


Figure 5.6: Generalised diagram showing the internal and external characteristics of a cercaria (redrawn from Schell 1970).

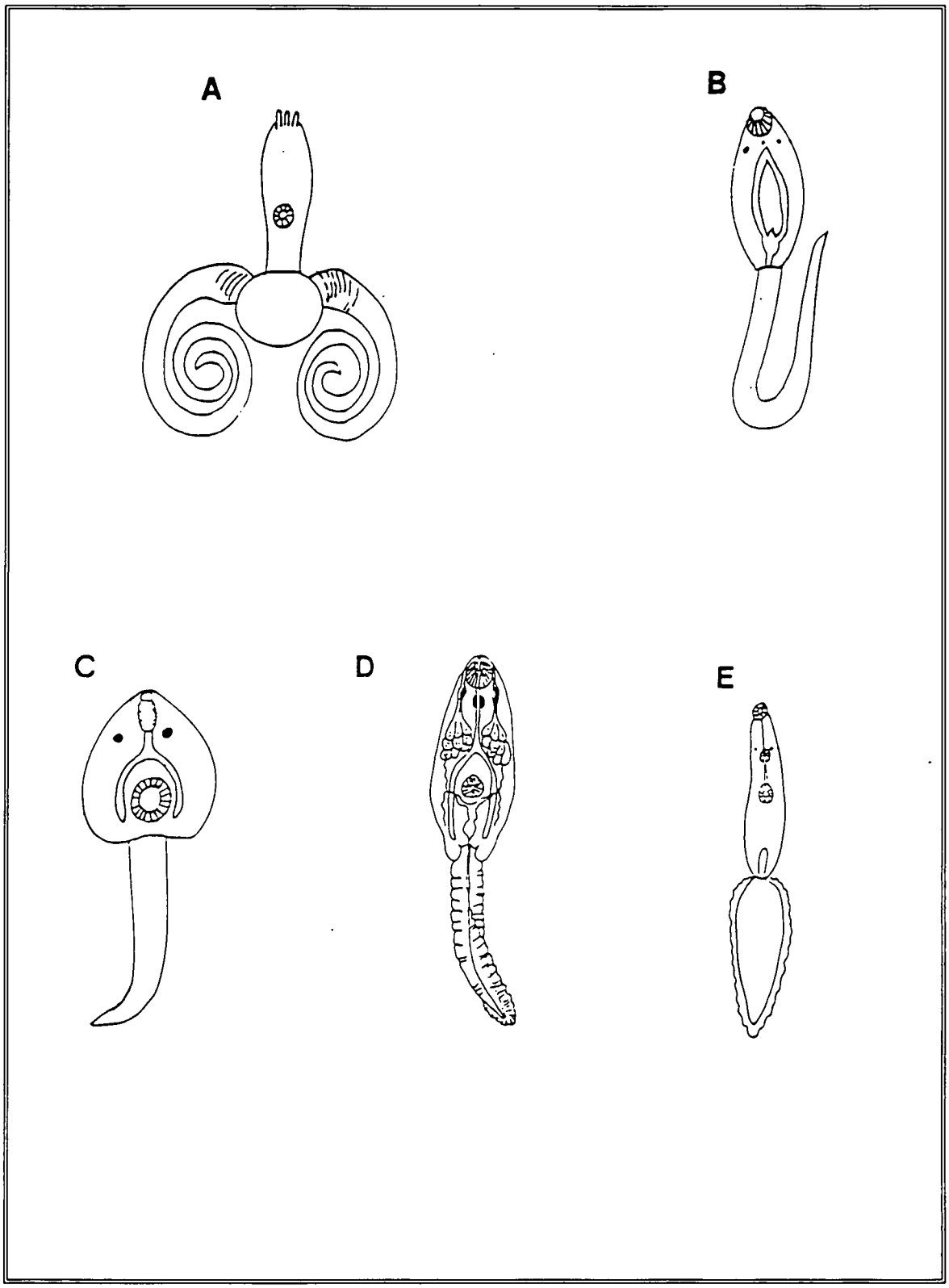


Figure 5.7: Illustration of the different types of cercariae. (**A**) Gasterostome cercaria (**B**) Monostome cercaria (**C**) Amphistome cercaria (**D**) Distome cercaria (**E**) Rhopalocercous cercaria (redrawn from Schmidt & Roberts 1977).

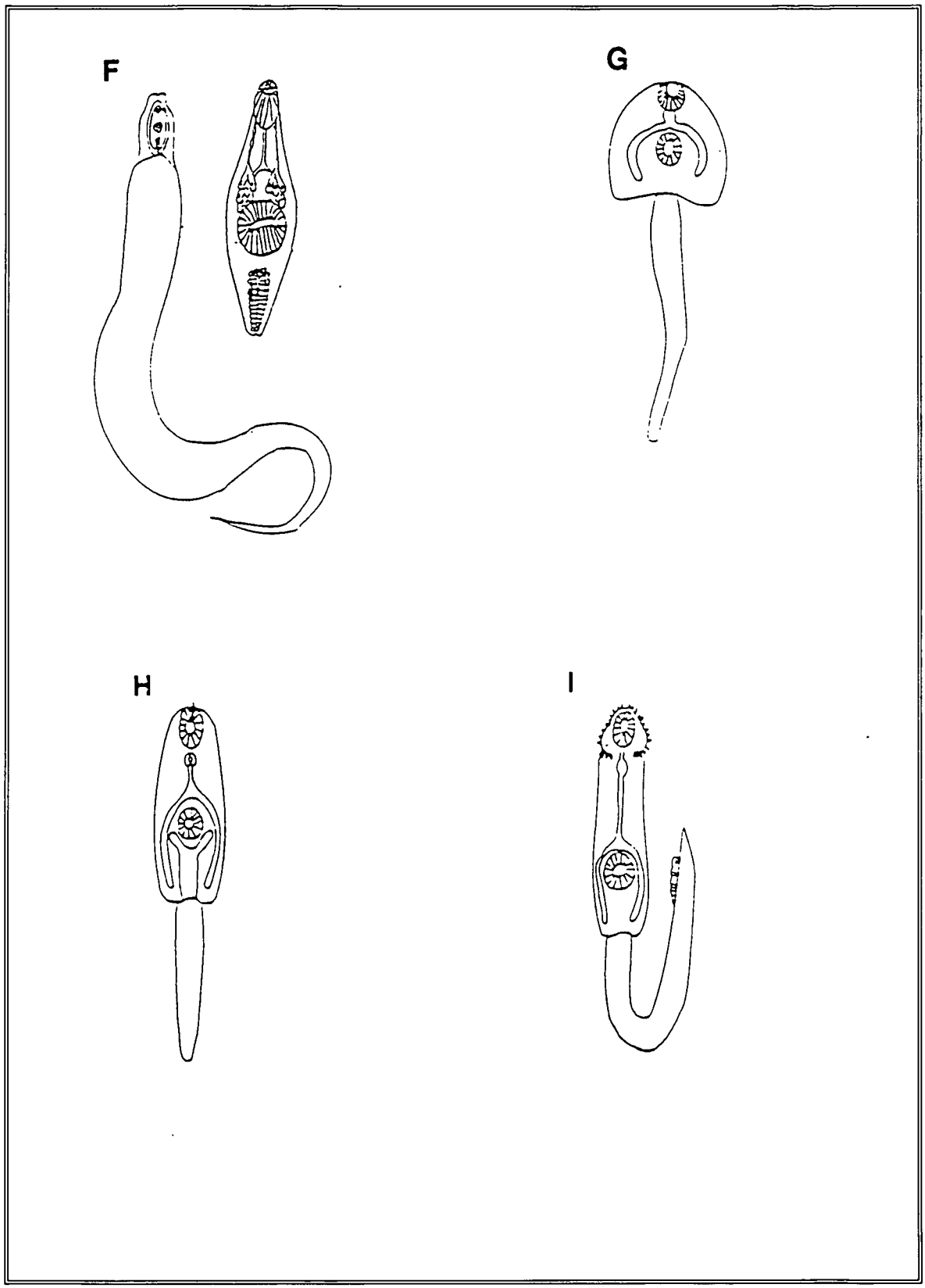


Figure 5.7 continued: Illustration of the different types of cercariae. (**F**) Cystocercous cercaria (**G**) Gymnocephalous cercaria (**H**) Xiphidio cercaria (**I**) Echinostome cercaria (redrawn from Schmidt & Roberts 1977).

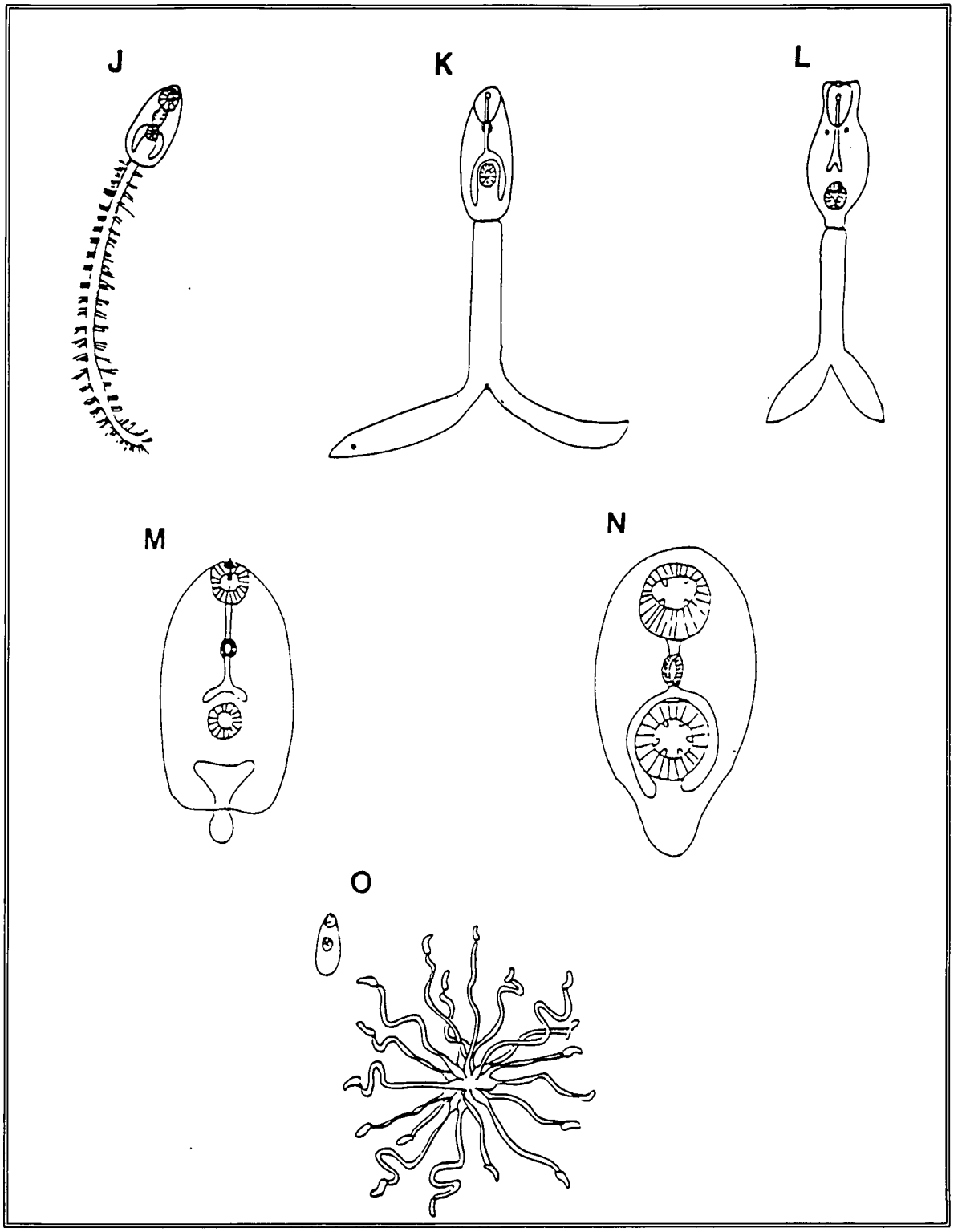


Figure 5.7 continued: Illustration of the different types of cercariae. (**J**) Trichocercous cercaria (**K**) Pharyngeate furcocercous cercaria (**L**) Apharyngeate furcocercous cercaria (**M**) Microcercous cercaria (**N**) Cercariaea (**O**) Rattenkönig cercaria (redrawn from Schmidt & Roberts 1977).

The Mesocercaria

- Is a resting stage within the parasite life cycle and uses a second intermediate host in a parasite life cycle, utilising four hosts

Pearson (1956) defined Mesocercaria as follows: *The mesocercaria is a definite prolonged stage in the adult generation of strigeate trematodes, which closely resembles the cercarial body, from which it develops in the second intermediate host, and which does not possess metacercarial features; it develops in turn into the metacercaria in another host*

- Parasites having this larval stage are capable of surviving within a very wide range of paratenic host which ingest a second intermediate host
- This ability therefore increases the number of hosts which the parasite may use in its life cycle

The Metacercaria (Fig. 5.8A)

- Is a resting larval stage of the trematode parasitic life cycle, formed either in a final intermediate host, or on a solid substrate in the external environment
- Final intermediate host may be a fish, arthropod or another mollusc
- Some trematodes don't have a second intermediate host but encyst on aquatic vegetation or on shells of other organisms, which will in turn be ingested by the parasites definitive host
- Cyst wall made up of tanned proteins, lipids and polysaccharides

The Juvenile Adult Stage

- On ingestion the metacercaria must transform into adult
- This process varies considerably, e.g. in some species the adult flukes are found within the alimentary tract
- In these cases metacercarial cyst wall is broken down and releases young fluke, which only has to migrate a short distance to reach preferred site within the hosts body
- In other groups however the adult forms are located in other sites within the hosts' body and must undergo a different migratory path
- The most common migratory paths are shown in Table 5.3.

Table 5.3: Common Migratory Paths of Trematodes within the Definitive Host

Heterophyes sp. *Metagonimus* sp *Echinostoma* sp.	**Adult Forms** in Duodenum (No migration						
Opisthorchis sp.	Duodenum	Bile Duct- **Adult Forms**					
Dicrocoelium sp.	Duodenum	Bile Duct	Liver - **Adult Forms**				
Fasciola hepatica	Duodenum	Gut wall (penetrates)	Peritoneal Cavity (more rarely the bloodstream)	Liver	Bile Duct – **Adult Forms**		
Paragonimus sp.	Duodenum	Gut wall (penetrates)	Peritoneal Cavity	Diaphragm (penetrates)	Thoracic Cavity	Lungs – **Adult Forms**	
Schistosoma sp.	External environment	Skin (penetrates)	Blood vessels	Heart	Lungs	Liver	Mesenteric Veins – **Adult Forms**

The Adult Digenean Fluke (Fig. 5.8B)

- Adults develop in specific sites within vertebrate host
- Body takes on a number of forms, but are generally dorsoventrally flattened and usually hermaphroditic
- Most adults found in vertebrate alimentary tracts
- Have tegument which is important for nutrient uptake and protection from the host environment
- Excretory system is based on a protonephridial system with so called flame cells located distally and connecting by series of canals to the excretory bladder which empties via the excretory pore
- Depending on species their diet consists of blood, mucus and surface epithelial cells

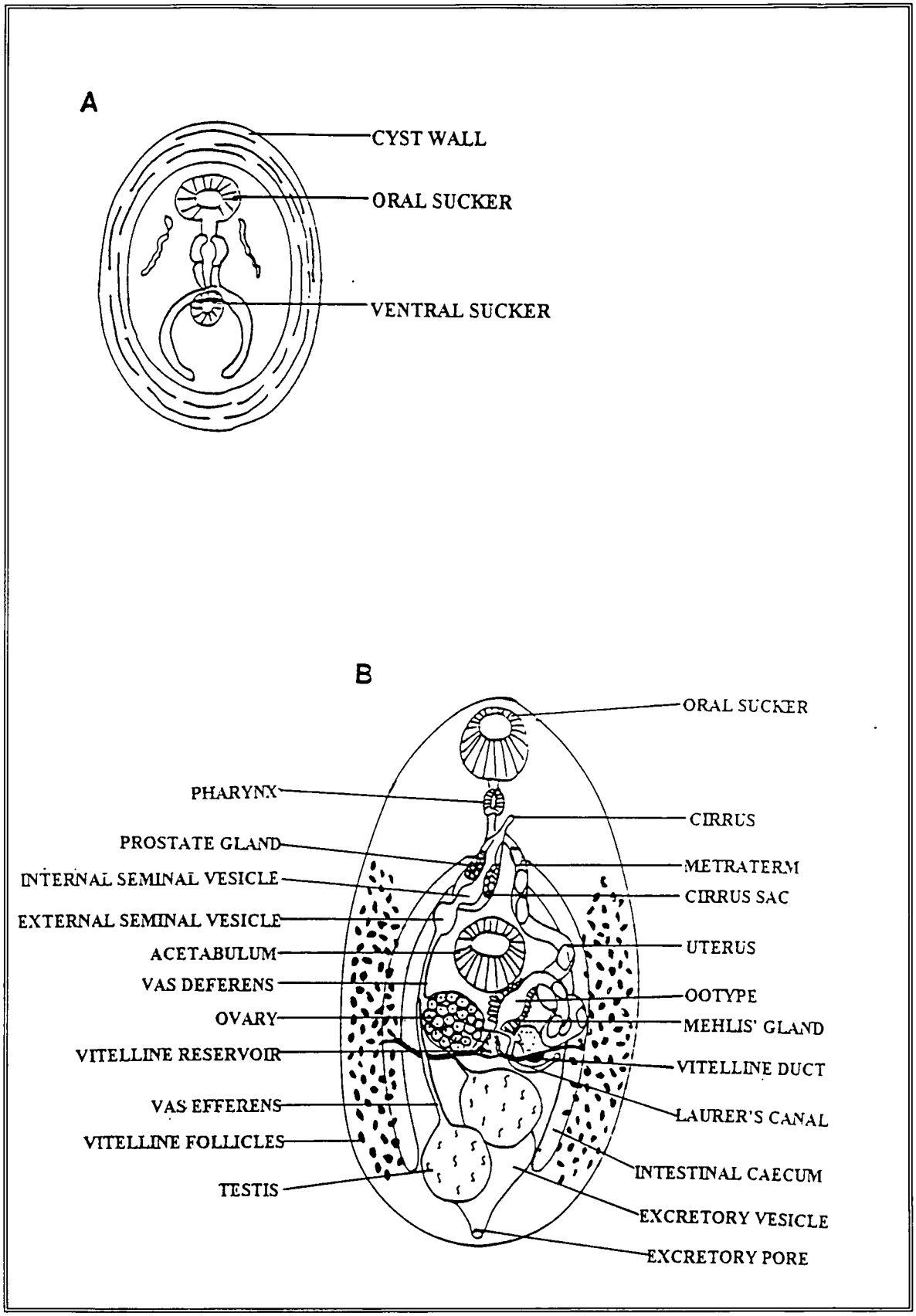
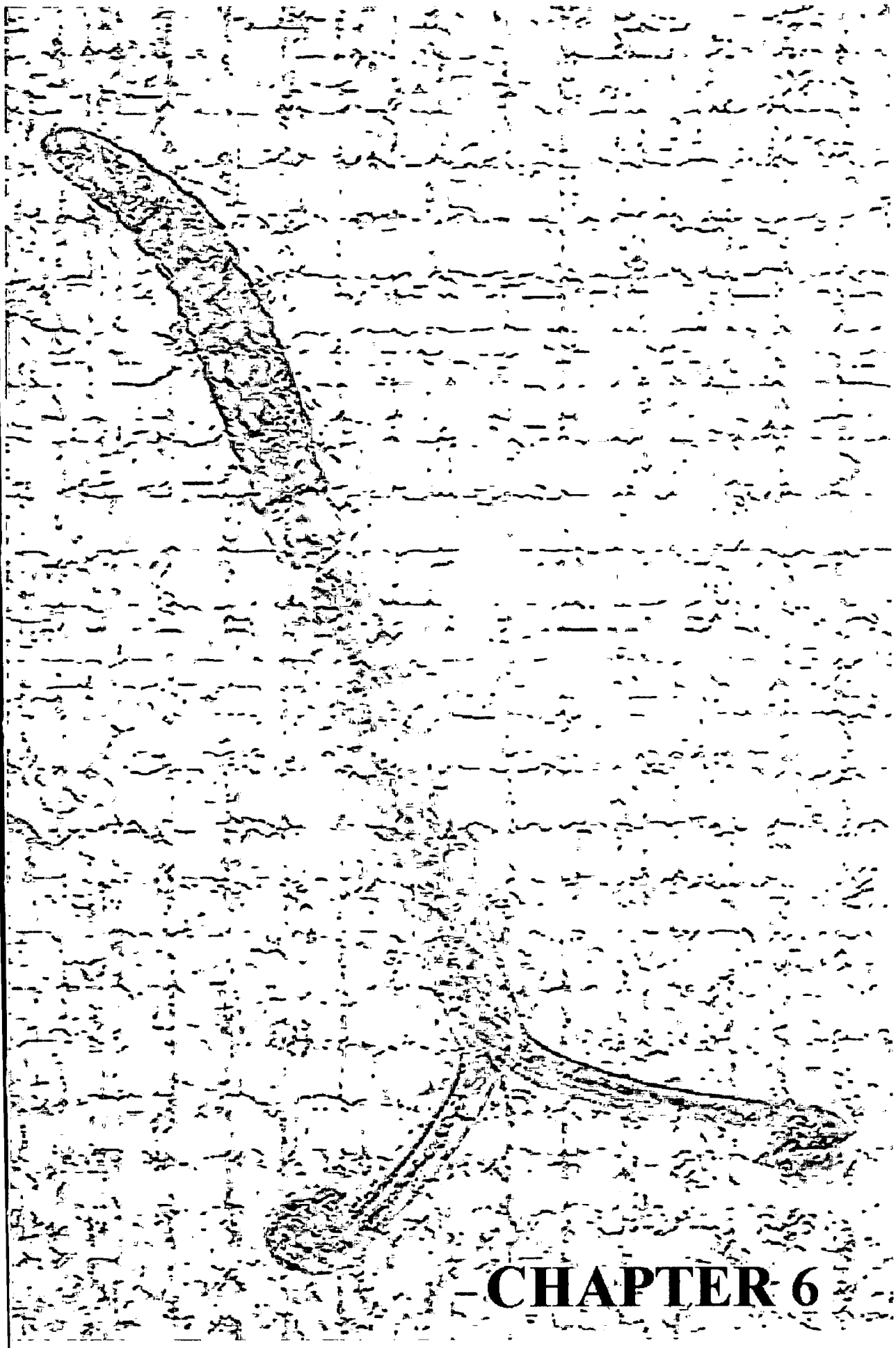


Figure 5.8: Illustrating the (**A**) Metacercaria (**B**) Adult (compiled from Schell 1970, Noble & Noble 1982).



CHAPTER 6

CERCARIAE OCCURRING IN THE OKAVANGO DELTA, BOTSWANA

A total of eight different types of cercariae were shed by six different snail species from the Okavango Delta, Botswana (Fig. 6.1). Descriptions of the cercariae will be given in the chapter together with the families to which these cerariae belong.

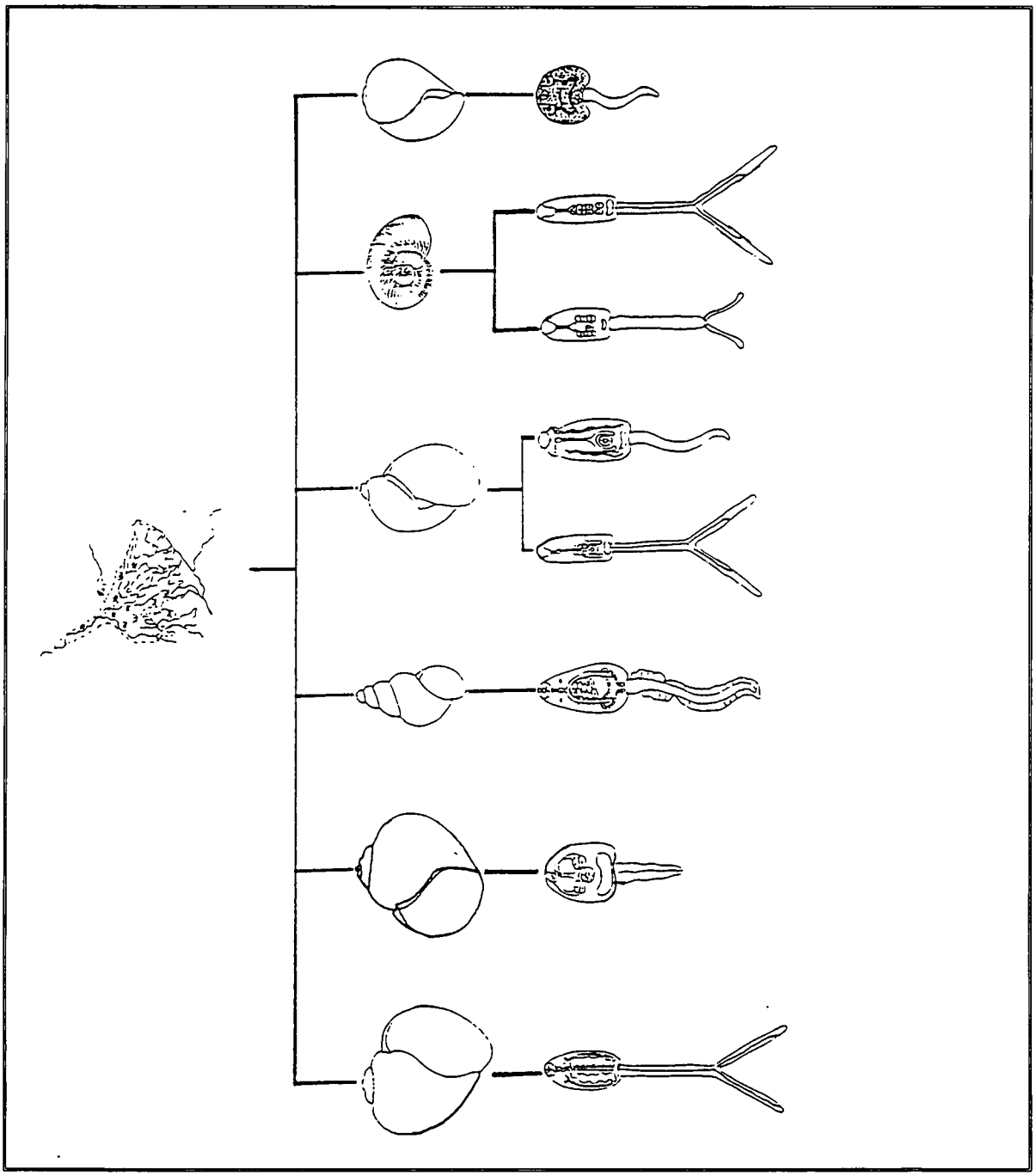


Figure 6.1: Cercariae shed by freshwater snails from the Okavango Delta, Botswana.

HOST: *Pila occidentalis* (Mousson, 1887)

LOCALITY: Mohembo Floodplains

TYPE OF CERCARIAE: Fork tailed cercaria A

(All measurements in μm)

SPOROCYST/REDIA: Not found

CERCARIA:

Body of cercaria elongate to oval (Figs. 6.2A, 6.10A), 370-550 (458±51.9) x 180-330 (267±47.0). Tail longer than body measuring 480-680 (562±55.8) x 50-120 (78.3±25.7) (Figs. 6.2B, 6.10B). Stem forks into two long laterally flattened furcae 250-490 (423±54.3) x 30-90 (57.2±22.2). Approximately 22 setae are found evenly distributed on either sides of tail stem (Fig. 6.3A) and fifteen setae on inside of each furcae (Fig 6.2B). Setae also evenly distributed on either side of body. Finfolds absent.

Round, pyriform protrusible oral sucker, (Fig. 6.3B) wider than long situated at anterior end of body. Oral sucker measures 40-100 (57.8±16.3) x 20-110 (67.2±25.4). Papillae present on oral sucker (Fig. 6.3B). Acetabulum not clearly visible under light microscope but visible under SEM (Fig. 6.3C). Oral sucker opens into globular pharynx (Fig. 6.3D), 20-50 (30.6±10.6) x 10-40 (26.7±10.8), leading into an oesophagus, 23-40 (32.5±5.4) x 10-20 (14.5±3.5). Caeca long, broad, bifurcation of intestine situated in the first third of the body (Figs 6.2A, 6.3D, 6.10A) ending at posterior end of body just above excretory bladder. Penetration glands not visible.

Excretory bladder broad, three-lobed (Fig. 6.2A), main excretory ducts four in number, two median ones between caeca uniting to form a median canal, which is connected with lateral canals by lateral vessels at level of oesophagus (Figs. 6.3D & E), posterior canal dividing into two branches extending one along each furcal ramus opening to the outside on the inner furcal rami (Figs. 6.2B, 6.3F). Flame cell formula as follows 2[(3+2+1+1+2+2+1+1+3+{10}]=42.

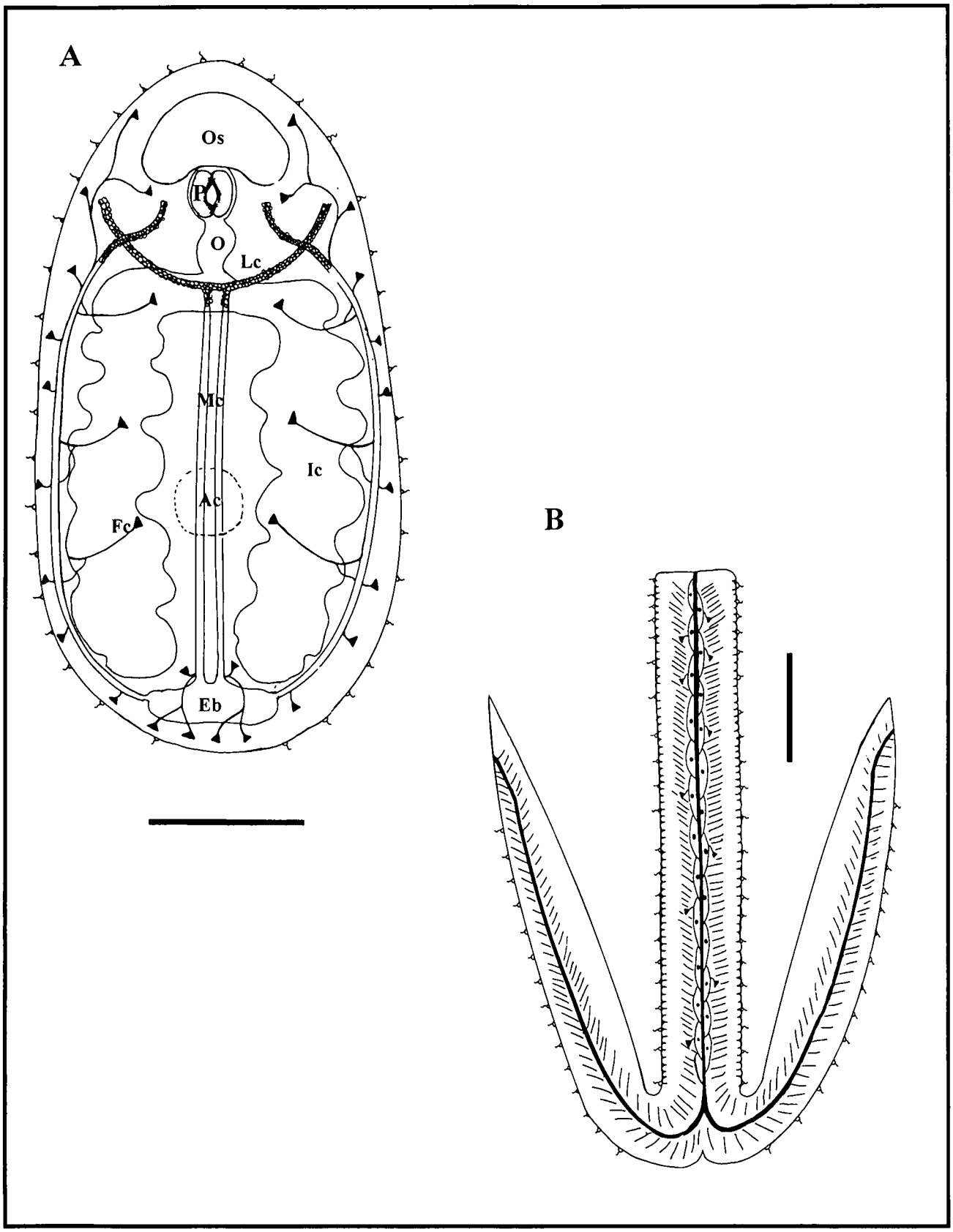


Figure 6.2: Light microscope projection drawings of the morphology of the fork-tailed cercaria shed by *Pila occidentalis* (Mousson, 1887) collected from the Okavango Delta, Botswana. **A.** Body of cercaria. **B.** Fork tail. **Os**- Oral sucker, **P**- Pharynx, **O**-Oesophagus, **Lc**- Lateral canal, **Mc**- Median canal, **Ac**- Acetabulum, **Ic**- Intestinal caeca, **Fc**- Flame cell, **Eb**- Excretory bladder. **Scale bar**: 100μm.

FIGURE 6.3

Scanning electron micrographs (A, B, C, F, G, H) and light micrographs (D, E) of the external and internal morphology of the fork-tailed cercaria shed by *Pila occidentalis* (Mousson, 1887) collected from the Okavango Delta, Botswana.

A. Setae and spines on tail

B. Oral sucker showing papillae

C. Acetabulum

D. Pharynx, oesophagus and lateral canal

E. Intestinal caeca and median canal

F. Opening of excretory pore

G. Body showing backward directed spines

H. Sensory receptors covering body and acetabulum

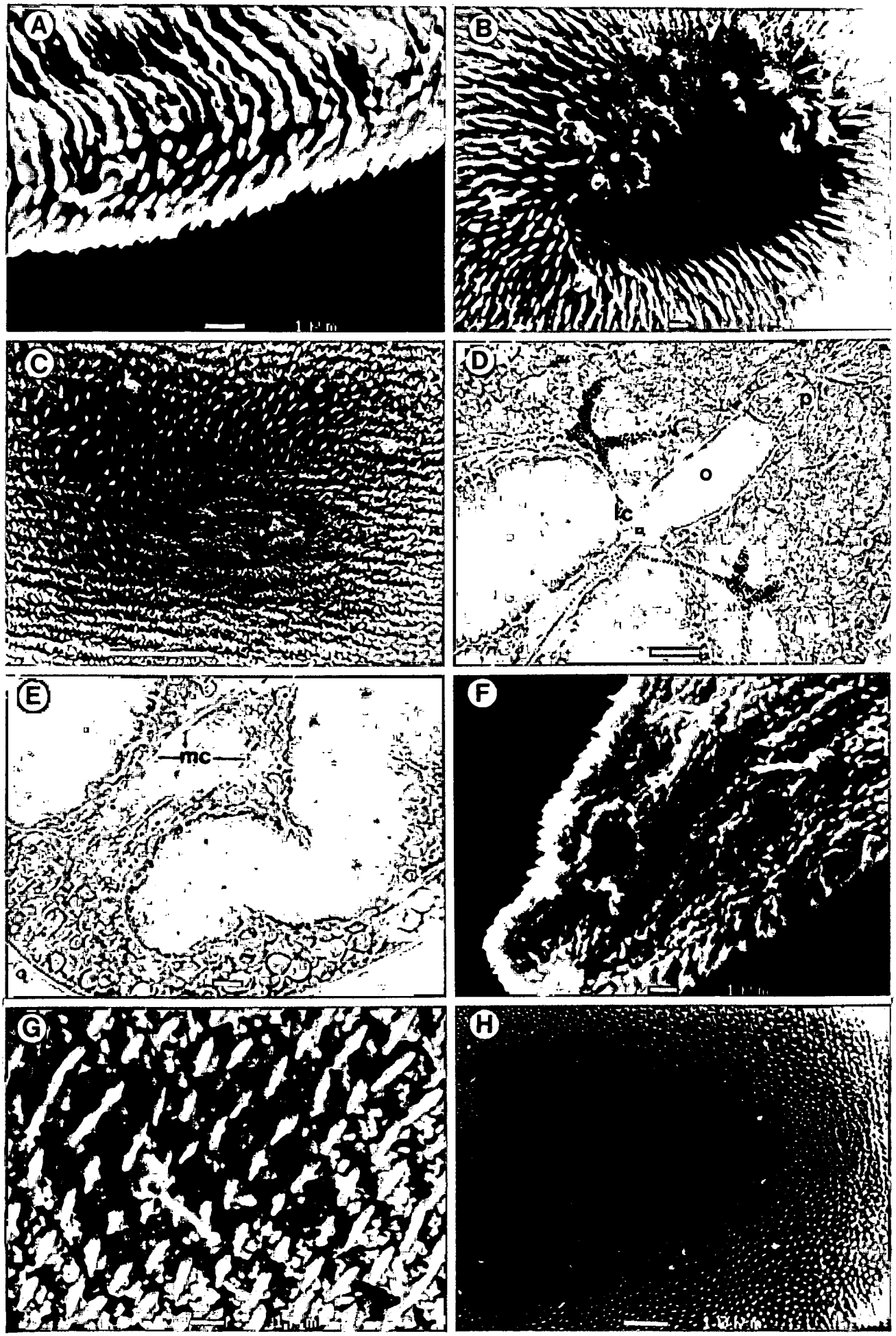
p- Pharynx

o- Oesophagus

lc- Lateral canal

mc- Median canal

Scale bar: A, B, C, F, G- 1μm. D, E, H- 10μm.


A
B
C
D
p
o
lc
E
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H

Body of cercaria covered with spines (Fig. 6.3G). Tail covered by backwardly directed spines on sides (Fig 6.3B). Sensory receptors concentrated on entire body and acetabulum (Fig.6.3H).

REMARKS:

This cercariae can be placed in the longifurcate – pharyngeate monostome cercariae (Vivax cercariae) according to the key proposed by Frandsen & Christensen (1984). Porter (1938) described a vivax cercaria, *Cercaria theodoxa* from Kwa–Zulu Natal from the snail *Neritina natalensis.* The cercaria in the present study conforms to the one described by Porter in that it has no penetration glands, having the same type of excretory system and the body and tail are also covered with spines. It differs in that it doesn't have the same number of flame cells and differs in size. It is also similar to *Cercaria indica* XXXIII described by Sewell (1922) from India. The most representative family accommodating vivax cercariae is the family Cyathocotylidae.

FAMILY CYATHOCOTYLIDAE

This group gets its name from the type species, *Cercaria vivax Sonsino*, 1892, and according to Sewell (1922), the chief characteristics are a small rudimentary ventral sucker, and an excretory system, which comprises twelve pairs of flame cells in the body and three pairs in the tail. Dubois (1929) regarded this group of cercariae as transitional between Distome and Monostome Furcocercariae. Looss (1900) gave a full description of specimens of *C. vivax*, which he obtained from *Cleopatra bulimoides* in Egypt. The vivax cercariae from the present study differs from *C. vivax* in that the number of penetration glands and size of the body differs.

Szidat (1936) erected a new subfamily namely Pharyngostomatinae with the genus *Pharyngostomum* occurring only in mammals (Dawes 1968). Adults of this family have a body that is small (less than 2mm), rounded, oval or elongate (Schell 1985). The ventral sucker may be absent in some genera and the oral sucker and pharynx are present. The caeca terminate at or near the posterior extremity (Yamaguti 1958). Schell (1985) erected a key for the genera of the family Cyathocotylidae and discussed various life cycles of some of the genera. The cercariae from this family

are known to develop in sporocysts and encyst in fish. They are parasitic in the digestive tract of reptiles, birds and mammals.

HOST: *Lanistes ovum* Peters, 1845

LOCALITY: Mohembo Floodplains; Thaoge Lagoon

TYPE OF CERCARIA: Short single tailed cercaria B

DAUGHTER SPOROCYSTS:

Found distributed throughout body of snail. Sporocysts colourless, measuring 280-450 (340±60.7) x 180-260 (222.5±31.9). Average number of cercaria in sporocyst ±7. Cercaria measure 100-200 (150±37.4) x 40-80 (55±13.6). Approximately three germ cells in sporocyst with diameter of 40-60 (52.5±7.5) (Fig. 6.4A, 6.19A).

CERCARIA:

Xiphidio cercariae are shed in large numbers by the snails. Body and tail of cercariae capable of changing shape when body extends and contracts (Fig. 6.10C). Cercariae relatively small with body oval (Figs. 6.4B, 6.5A, 6.10D), measuring 70-140 (94.2±17.5) x 58-85 (67.0±8.0). Tail shorter than body measuring 60-110 (81.5±17.1) x 19-30 (24.8±3.0) (Figs. 6.4C, 6.5B, 6.10D).

Oral sucker rounded, 28-40 (31.6±3.0) long and 24-38 (31.5±3.6) broad (Fig. 6.5C). No pharynx. Oral sucker leads to oesophagus that bifurcates anterior to acetabulum into two short caeca ending midway between penetration glands. Stylet measuring 21-31 (26.1±2.8) x 3-8 (5.2±1.2) originates from oral sucker (Fig. 6.5D).

Acetabulum situated 8-40 (24.1±9.7) from oral sucker thus in posterior half of body (Fig. 6.4B, 6.5E). Acetabulum round, measuring 16-30 (22.3±3.6) x 14-30 (22.5±3.8), smaller than oral sucker. Acetabulum wall covered by sharp spines (Fig. 6.5E).

Two pairs of penetration glands (Figs. 6.4B, 6.5D) stretch from mid body to about acetabulum. First gland pair measures 7-18 (12.3±3.0) x 13-28 (17.6±3.4); second gland pair measures 9-22 (15.6±3.5) x 13-25 (18.5±3.5). From these glands, fine tubes stretch to anterior border of oral sucker and open to outside (Fig. 6.4B).

Excretory bladder situated at the posterior end of body measures 9-20 (15.4±3.4) x 32-64 (43.0±7.7).

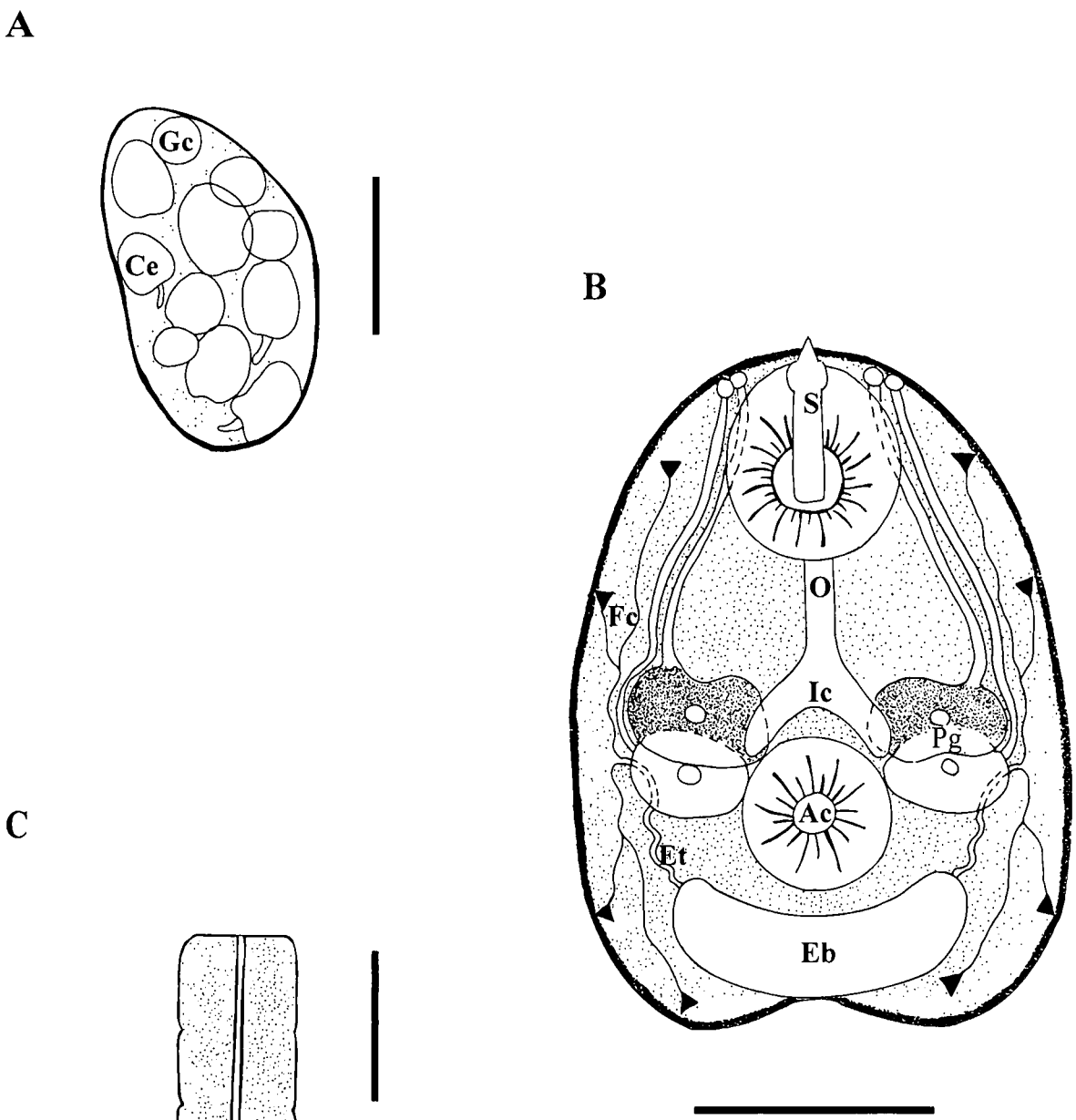


Figure 6.4: Light microscope projection drawings of the morphology of the single tailed cercaria shed by *Lanistes ovum* Peters, 1845 collected from the Okavango Delta, Botswana. **A**. Daughter sporocyst. **B**. Body of cercaria. **C**. Single tail. **Ce**- Cercaria, **Gc**- Germ cell, **S**- Stylet, **O**- Oesophagus, **Ic**- Intestinal caeca, **Pg**- Penetration gland, **Ac**- Acetabulum, **Fc**- Flame cell, **Et**- Excretory tubule, **Eb**- Excretory bladder. **Scale bar**: A- 100µm. B, C- 33µm.

FIGURE 6.5

Scanning electron micrographs (A, B, C, E, F, G, H) and light micrograph (D) of the external and internal morphology of the single-tailed cercaria shed by *Lanistes ovum* Peters, 1845 collected from the Okavango Delta, Botswana.

A. Body of cercaria

B. Short contractile tail showing the opening of excretory pore

C. Oral sucker

D. Stylet and penetration glands

E. Acetabulum

F. Short backwardly directed spines on body

G. Ciliated papillae on dorsal surface

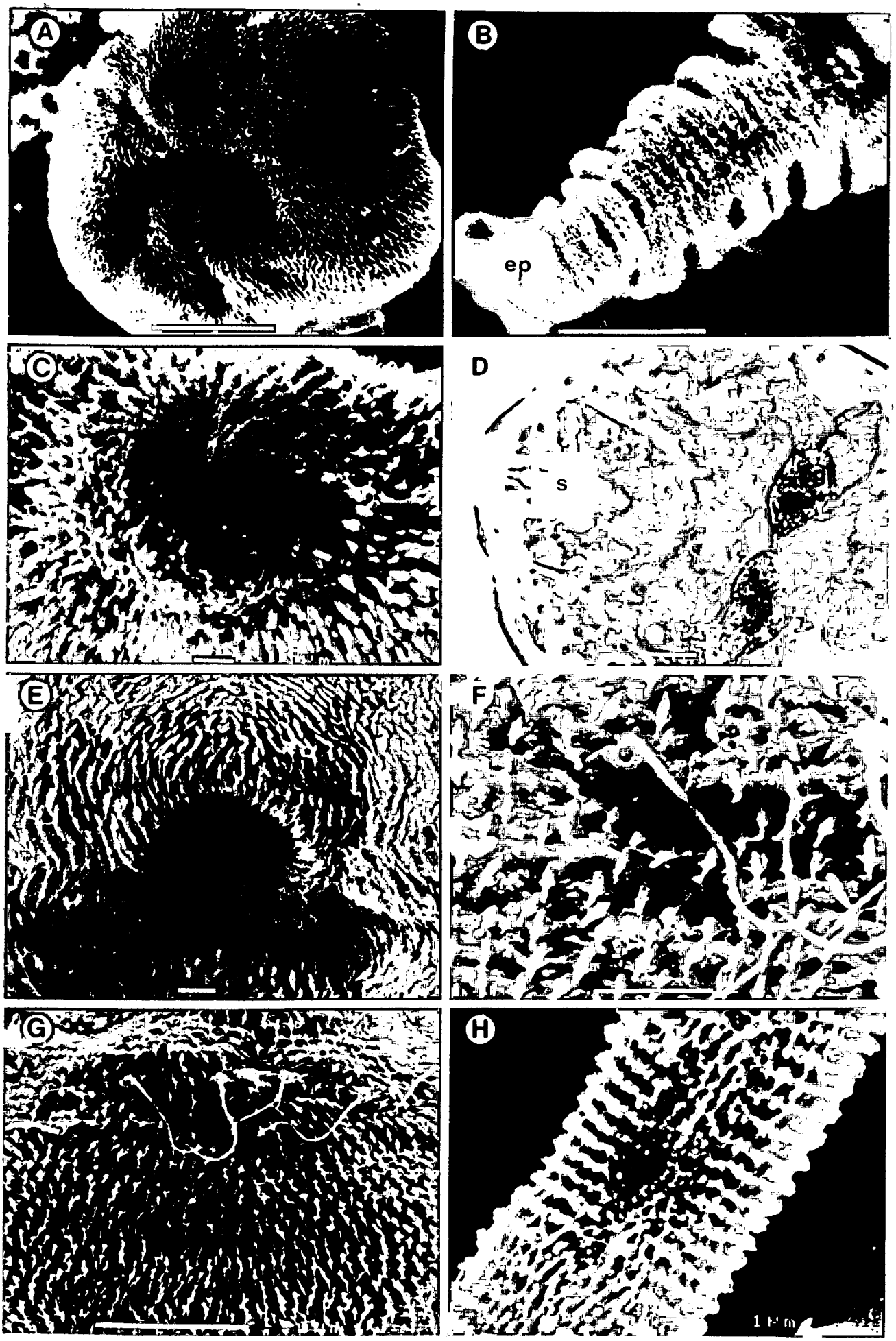
H. Tail surface

s- Stylet

ep- Excretory pore

pg- Penetration gland

Scale bar: A, B, D, G- 10μm. C, E, F, H- 1μm.


A
B
ep
C
D
s
E
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G
H
1 µm

Excretory bladder shape may vary from V-shape when body contracts to oval shaped when relaxes (Fig. 6.4B). Main excretory duct on either side of bladder extends anteriorly until acetabulum where it divides into anterior and posterior collecting ducts (Fig. 6.4B). Anterior collecting duct running anteriorly gives rise to two capillaries, each ending in flame cells. Similarly, posterior collecting duct on either side of body runs posteriorly and divides into two capillaries, all ending in flame cells. Flame cell formula as follows: $2[(1+1) + (1+1)] = 8$. Posterior to excretory bladder a caudal excretory duct arises in tail stem running to end, opening to outside by means of pore (Fig. 6.5B).

Body of cercariae completely covered by spines that are short, sharp and backwardly directed (Fig. 6.5F). Between spines, few ciliated papillae are found. Dorsal surface also spinous and covered with ciliated papillae arrange in a row near oral sucker (Fig. 6.5G). No finfolds or caudal bodies present on tail. No spines present on tail (Fig. 6.5H). Surface of tail rough and few sensory receptors present.

REMARKS:

The cercaria in the present study is similar to the cercariae found by Nasir (1971) from the snail *Pomacea urceus*, *Cercaria farakhanweri*. It has the same flame cell formula, the body size and body structures are similar, and the tail is also aspinose, as well as the characteristic two pairs of penetration glands that correspond in size and location. It is also similar to *Cercaria cellulosa* Looss (Wesenberg-Lund, 1934) that also has the same flame cell formula and same number of penetration glands. The morphological features of this cercaria correspond with that of the family Plagiorchiidae.

FAMILY PLAGIORCHIIDAE

Xiphidio cercariae were first described by Diesing (1855), while Lühe (1909) described xiphidio cercariae as belonging to the distome group with a short tail and stylet at the anterior end of the body. Work done by later authors showed that although the stylet was characteristic of the xiphidio cercariae, the stylet also occurs in other cercarial groups for example, Microcercous-, Rhopalocercous- and Cystocercous cercariae.

Lühe (1909) recognized four groups within the Xiphidio cercariae called *Cercaria microcotylae*, *C. virgulae*, *C. ornatae* and *C. armatae*. Lebour (1911) placed a number of forms in another group namely *Spelotrema,* and Cort (1915) added another group called *Polyadenous.*

Sewell (1922), to reclassify the xiphidio cercariae, subdivided the four groups of Lühe: *Microcotylaea* into '*Cellulosa*, *Pusilla*, *Parapusilla* and *Vesiculosa*; *Virgulae* into *Virgula* and *Paravirgula* ; *Ornatae* into *Prima* and *Cercaria ornata*; and *Armatae* into *Polyadena* and *Daswan* (Dawes, 1968).

According to a key of Frandsen & Christensen (1984) this xiphidio cercaria found in the Okavango Delta, falls into the Armatae group.

Cercaria armatae

Group diagnosis:

Lühe (1909) described this group of xiphidio cercariae as having a tail and body length of almost equal length. This group has no finfolds present on the tail. No virgula organ present. The oral and ventral suckers are of equal size, or the ventral sucker is larger than oral sucker. The acetabulum is situated posterior to the middle of the body. Produced by species of the family Plagiorchiidae, which are intestinal parasites in all groups of vertebrates.

HOST: *Cleopatra elata* Dautzenberg & Germain, 1914

LOCALITY: Mohembo Floodplains; Xaro Mainstream Lagoon

TYPE OF CERCARIA: Long singled tailed cercariae C

REDIA: Redia found within the digestive gland of the snail. Slightly orange to white in colour. Measures 385-450 (414±23.6) in length x 120-140 (128±7.8) in breadth. Spherical pharynx 40-50 (48.1±4.9) x 30-45 (36.9±4.6) present at anterior end (Fig. 6.6A).

CERCARIA:

Body of cercariae oval and pigmented (Fig. 6.6B). Body very contractile. Body measures 165-430 (265±80.2) long and 90-190 (130±31.1) broad (Fig. 6.10E). Tail single, muscular and striated (Fig. 6.7A), measures 340-650 (505±83.4) x 30-150 (61.4±25.2). Finfold present (Figs. 6.6C, 6.7B, 6.10F). No sensory receptors present on tail.

Oral sucker round measures 30-80 (47.9±13.2) x 20-60 (42±9.9). Mouth opens by means of oral sucker to outside. Oral sucker surrounded by three rows of specialised, spearheaded spines (Fig. 6.7C). First row nearest to mouth consists of 3-5, the second row of 7-9 and third row of 7-9 spines. Points of spines directed forward. Oral sucker leads to short prepharynx 5-30 (13.6±5.9) x 5-15 (10±1.6). Pharynx measures 10-30 (15.2±5.1) x 10-40 (14.7±7.9). Pharynx leads to short oesophagus 5-15 (8.7±2.3) x 5-15 (9.1±2.4) which bifurcates into two intestinal caeca that extend to posterior half of the body (Fig. 6.6B). Six setae present on either side of body (Fig. 6.6B)

Two dark eyespots occur in the anterior third of body (Fig. 6.10E). Eyespots are 10-22 (12.5±4.4) long and 10-20 (15.1±4.0) broad. Fourteen penetration glands present situated in middle of body below branching of caeca and above excretory bladder (Figs. 6.6B, 6.7D). Cell bodies of penetration glands contain coarse granules and conspicuous nuclei. Ducts of penetration glands open to the outside at oral sucker, arranged in four bundles of 3-4-4-3 ducts (Fig. 6.6B).

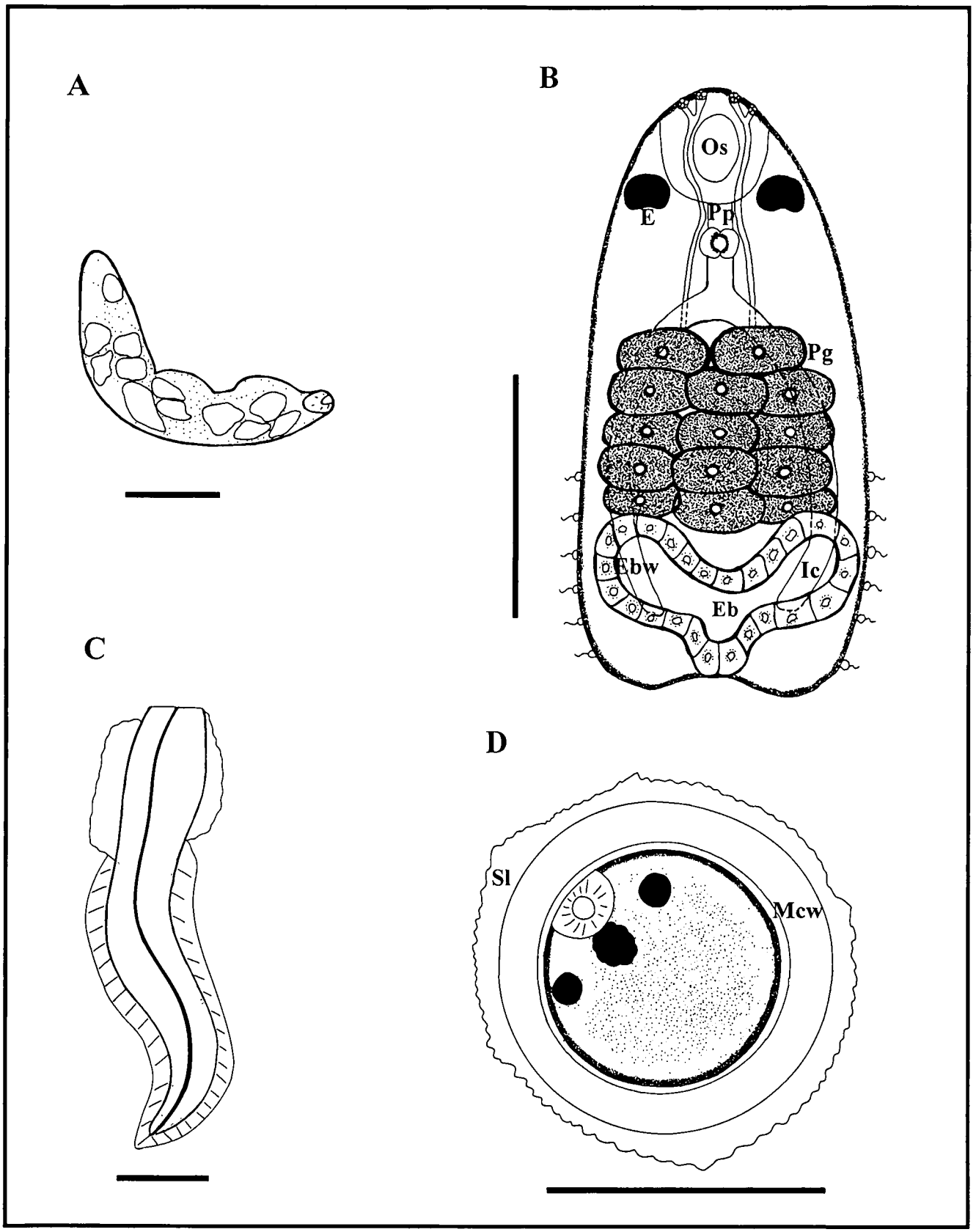


Figure 6.6: Light microscope projection drawings of the morphology of the single-tailed cercaria shed by *Cleopatra elata* Dautzenberg & Germain, 1914 collected from the Okavango Delta, Botswana. **A**. Mother redia. **B**. Body of cercaria. **C**. Single tail. **D.** Metacercarial cyst. **Os**- Oral sucker, **Pp**- Prepharynx, **Ic**- Intestinal caeca, **Eb**- Excretory bladder, **Ebw**- Excretory bladder wall, **Pg**- Penetration gland, **Sl**- Slime layer, **Mcw**- Metacercarial cyst wall. Scale bar: 100µm.

FIGURE 6.7

Scanning electron micrographs (A, B, C, E, F) and light micrograph (D) of the external and internal morphology of the long single-tailed cercaria shed by *Cleopatra elata* Dautzenberg & Germain, 1914 collected from the Okavango Delta, Botswana.

A. Tail surface

B. Finfold on tail

C. Oral sucker with rows of spines

D. Penetration glands, excretory bladder wall and excretory bladder

E. Tiny spines at the anterior end of the body

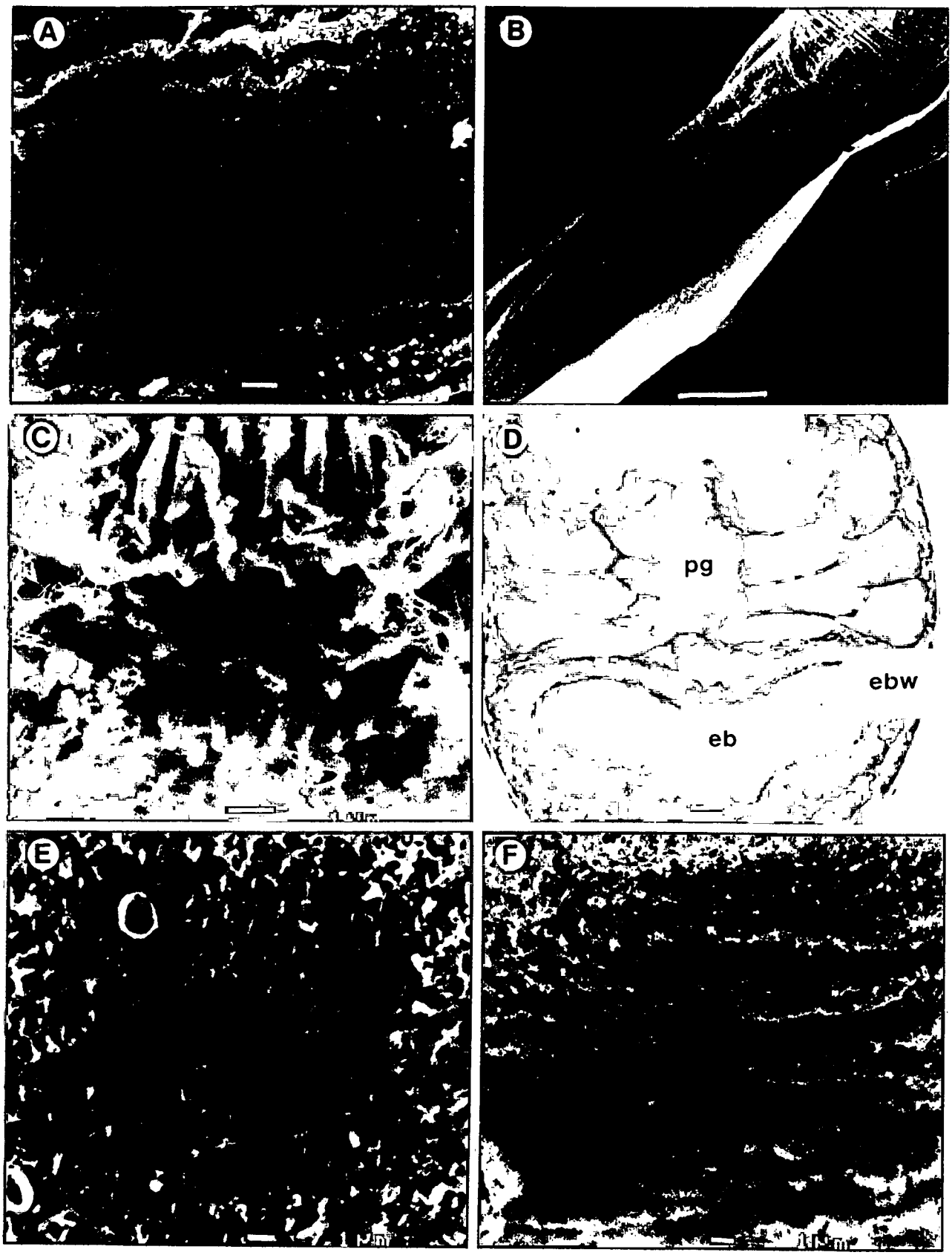
F. Posterior body surface

pg- Penetration glands

eb- Excretory bladder

ebw- Excretory bladder wall

Scale bar: A, C, E, F- 1μm. B, D- 10μm.


A
B
C
D
pg
ebw
eb
E
F

The excretory system consists of an excretory bladder at the posterior end of the body which is capable of extending and contracting at the same time as the body, changing from Y-shaped to oval (Figs. 6.6B, 6.7D). Wall of excretory bladder composed of large cells with granular cytoplasm and large nuclei (Figs. 6.6B, 6.7D). Unable to see flame cells due to dark pigmentation of body. Body of cercaria covered with tiny spines at anterior end of body (Fig. 6.7E), which decrease in size toward posterior end (Fig. 6.7F).

METACERCARIAE

Cercariae encysted within two hours on sides of the glass container after being released by the snail. Metacercarial stage found within a cyst (Figs. 6.6D, 6.19B). Round cysts measure 100-135 (120.1±10.3) in diameter. Cyst is surrounded by thick cyst wall that measures 10-16 (12.5±2.0) in thickness. Around this wall another layer occurs which could be some sort of slime layer. Slime layer measures 2-10 (5.5±2.8) in thickness.

REMARKS:

This cercaria was identified as a parapleurolophocercous cercariae according to the key of Frandsen & Christensen (1984). Porter (1938) described two different cercariae namely *Cercaria melanoides* and *Cercaria britsiae* from *Melanoides tuberculata* respectively. The cercaria from the present study is similar to *Cercaria melanoides* in that it possesses large spines around the oral sucker and has a spinous body without a ventral sucker, while *C. melanoides* differs in that it lacks intestinal caeca.

It is also similar to *Cercaria britsiae* in that it possesses a pharynx, intestinal caeca and the same number of penetration glands. It differs from *Cercaria britsiae* in that it doesn't possess a ventral sucker and it possesses spines around the oral sucker.

Martin & Kuntz (1956) described a cercaria, namely a *Heterophyes* sp. from *Pironella conica* from Egypt. The cercaria in the present study is similar to the cercariae of the *Heterophyes* sp. in that it has the same spine arrangement around the oral sucker,

same number of penetration glands and similar excretory bladder. It differs in that it has an intestinal caeca.

The spear headed spines that surround the oral sucker are supposedly used in the penetration of the second intermediate host and possibly also in leaving the snail.

FAMILY HETEROPHYIDAE

The family Heterophyidae Odhner, and its individual members, have repeatedly attracted the notice of many writers. Zoologists have called attention to their peculiar anatomical structure, while medical men have studied these worms because some of them are parasites of man and domestic animals.

In spite of a rather large literature, including several monographs devoted to heterophyidae, there still remains many obscure points in their anatomical structure, life history and classification.

The name Heterophyidae was given by Odhner (1914) to replace the former incorrect names Cotylogonimidae and Coenogonimidae. Odhner (1914) included in this family the genera, which have been attributed to it by previous authors and several others the systematic position of which had been uncertain. The first attempt to gather all known species into a closed group was made by Ransom (1920), who gave a new modified diagnosis but divided the family only into genera, though the outlines of three subfamilies had been already defined by previous authors.

Ciurea (1924) was the first who defined the division of the Heterophyidae into subfamilies: Heterophyinae, Metagoniminae, Centrocestinae, Apophallinae, and Cryptocotylinae, basing his division on the terminal portion of the genital ducts. Later Faust and Nishigori (1925) added a sixth subfamiy Monorchitreminae. Nicoll (1923) included Microphallinae and Gymnophallinae as well as some genera, which up to this date have not been attributed to it.

The life history of several species of Heterophyidae has already been studied by different authors (Witenberg 1932). Cercaria develop in snails, from snail cercariae reaches a fish, encysts in the organs and becomes metacercariae. After being

swallowed by final host becomes an adult, which parasitizes the intestine. Adults parasitize the intestine of mammals, birds and rarely fish (*Haplorchis*). Metacercariae in fish and cercariae develop in operculated molluscs.

HOST: *Lymnaea natalensis* Krauss, 1848

LOCALITY: Xaro Mainstream Lagoon

TYPE OF CERCARIA: Fork-tailed cercariae D

REDIA: Not found

CERCARIA:

Cercarial body elongate to oval (Figs. 6.8A, 6.10G), 120-190 (155±19.4) x 30-50 (39.4±7.7). Tail longer than body measuring 100-230 (192±34.6) x 20-50 (32.3±8.4), no spines present on tail (Figs. 6.8B, 6.10H). Stem forks into two long laterally flattened furcae 130-250 (193±40.3) x 3-30 (13.8±5.6). Four anterior caudal setae and five posterior caudal setae present on either side of tail stem. Sensory receptors with long cilia also present on tail (Figs. 6.8A, 6.9A). Single setae present posteriorly on either side of body (Fig. 6.9B). Caudal furcae with two setae approximately in middle on both sides of each furcae (Fig. 6.9C). Finfolds absent.

Protrusible, pear shaped oral sucker (Fig. 6.9D) longer than wide situated at anterior end of body. Oral sucker measures 15-40 (26.5±7.4) x 15-40 (24.3±7.3). Surface of rim covered with small backwardly directed sharp spines arranged in rows, large receptors with short cilia present around oral sucker (Fig. 6.9E). Acetabulum spherical, smaller than oral sucker, 12-27 (18.3±4.2) x 10-30 (19±5.0) situated in posterior third of the body. Acetabulum situated 40-90 (63.3±13.4) from the oral sucker. Acetabulum edge displays 24 to 25 well-developed spines, bears five large sensory receptors (Fig. 6.9F). Oral sucker opens into a short prepharynx 2-10 (6.4±2.2) x 3-10 (5.6±2.3) followed by a pharynx, 5-15 (9.5±1.8) x 5-15 (9.7±2.8) that leads into short oesophagus, 32-38 (35±1.6) x 1.2-1.9 (1.8±0.4). Oesophagus bifurcates in mid-body region into two well-developed intestinal caeca, which extend to half way between acetabulum and posterior end of body (Fig. 6.8A).

Four large round to oval penetration glands present anterior to acetabulum (Fig. 6.8A). Cytoplasm of all glands finely granulated, stain light blue when coloured with Nile blue sulphate. Penetration glands arranged two in tandem and two symmetrically, anterior to acetabulum

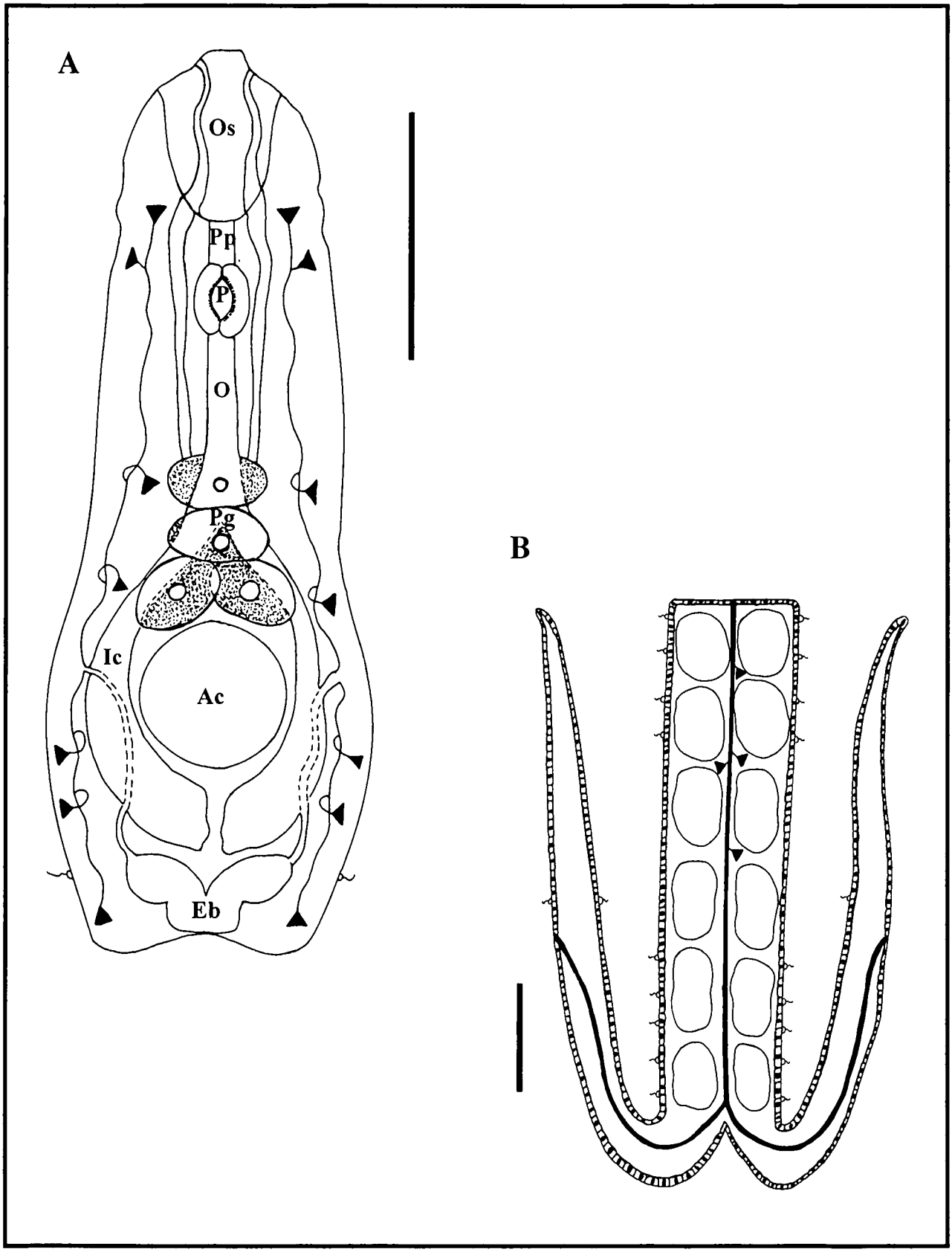


Figure 6.8: Light microscope projection drawings of the morphology of the long fork-tailed cercaria shed by *Lymnaea natalensis* Krauss, 1848 collected from the Okavango Delta, Botswana. **A**. Body of cercaria. **B**. Fork tail. **Os**- Oral sucker, **Pp**- Prepharynx, **O**- Oesophagus, **Pg**- Penetration glands, **Ac**- Acetabulum, **Ic**- Intestinal caeca, **Eb**-Excretory bladder. **Scale bar**: 33µm.

FIGURE 6.9

Scanning electron micrographs of the external morphology of the long fork-tailed cercaria shed by *Lymnaea natalensis* Krauss, 1848 collected from the Okavango Delta, Botswana.

A. Sensory receptors

B. Setae on body

C. Setae on furcal rami

D. Oral sucker surrounded by rows of spines

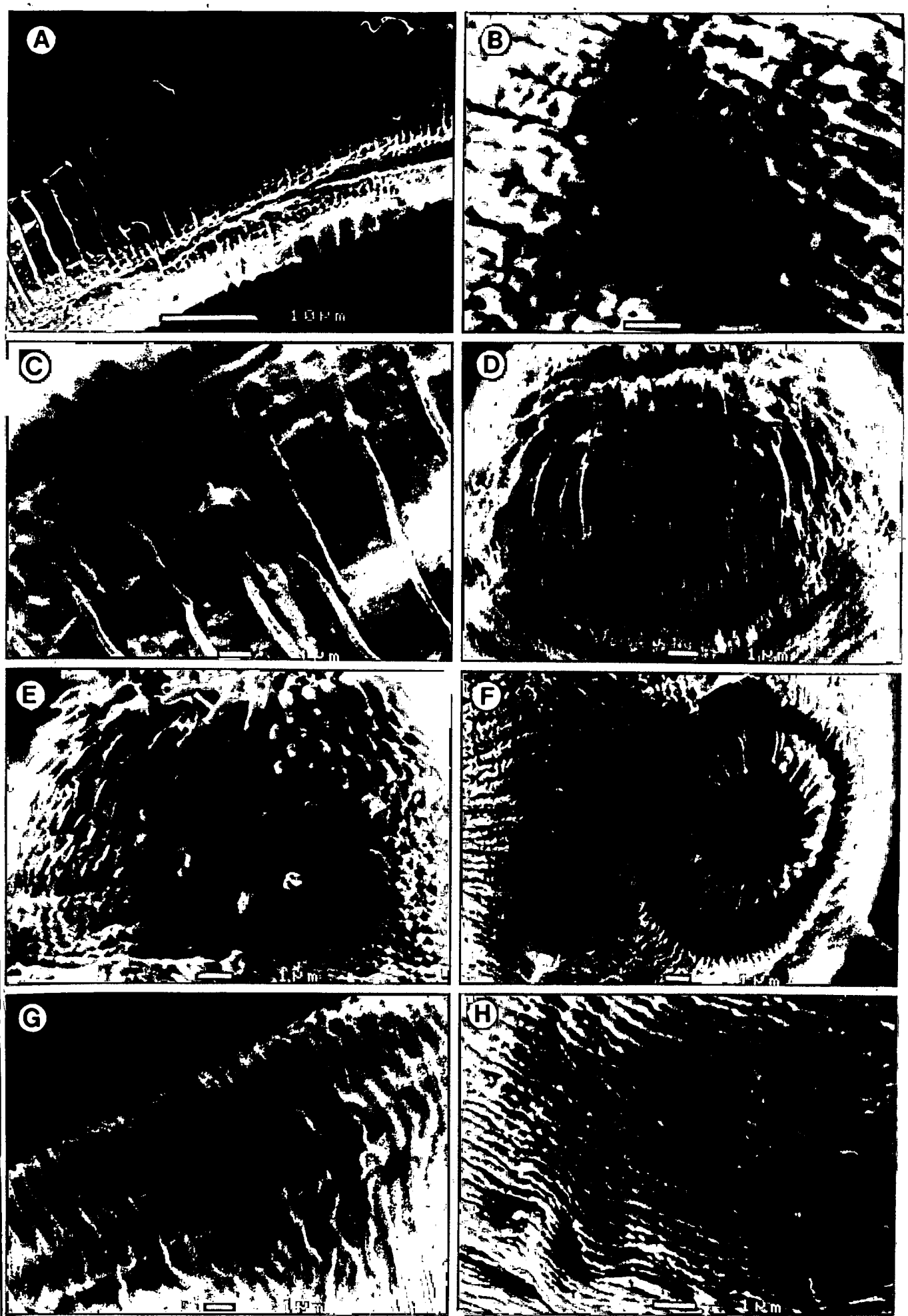
E. Papillae present around oral sucker

F. Acetabulum showing spines and papillae

G. Opening of excretory pore

H. Body surface

Scale bar: A- 10μm. B-H- 1μm.


A
B
C
D
E
F
G
H

First gland measures 6-10 (7.8±1.3) x 9-20 (11.7±3.7); second gland 5-10 (7.1±1.9) x 10-16 (12±2.5); remaining two glands 8-15 (11.1±2.5) x 5-15 (9.1±3.0). Ducts arising from these glands open anteriorly on either side of oral sucker (Fig. 6.8A).

Excretory bladder relatively small, triangular, measuring 5-15 (8.8±2.6) x 9-20 (11.5±2.9), situated at posterior end of body (Fig. 6.8A). Main excretory duct on either side of bladder extends anteriorly in zigzag manner. On reaching middle of body, it turns posterior again till reaching acetabulum where it divides into anterior and posterior collecting ducts. Anterior collecting duct running anteriorly that gives rise to four capillaries, each ending in flame cells. Similarly, posterior collecting duct on either side of body runs posteriorly and divides into three capillaries, all ending in flame cells. Posterior collecting duct on either side of body also extends into tail stem, forming four capillaries in anterior half of tail stem. Flame cell formula as follows: 2[(1+1+1+1) + (1+1+1)] +2[(1+1)]=18. Posterior to excretory bladder a caudal excretory duct arises which divides at base of tail stem into two branches, each running into caudal furcae and opens halfway to outside by means of a pore. Pore situated on inner side of furcae (Fig. 6.9G). Six pairs of caudal bodies present in tail stem measuring 10-20 (15.8±3.3) x 6-16 (12.2±2.7).

Body of cercaria not covered with spines (Fig. 6.9H). Posteriorly body is not covered with spines.

REMARKS:

The cercaria was classified as a pharyngeal, longifurcous, distome cercariae according to the key proposed by Frandsen & Christensen (1984). General features show that the cercaria belongs to the strigeid group of parasites. These cercariae will ultimately result in an adult trematode, which will either belong to the Family Strigeidae or Diplostomidae.

In her work, Porter (1938) described three different pharyngeal longifurcous distome cercariae shed by *Lymnaea natalensis*: *Cercaria maritzburgensis* collected in Pietermaritzburg, Kwa-Zulu Natal, *Cercaria scheepoortia* and *Cercaria magaliensia* collected from the Hartebeespoort Dam, Transvaal (North West Province). None of the above mentioned cercariae conform to the fork-tailed cercaria presented here.

They differ in a number of respects, the size of the body structures, number and position of penetration glands, presence of pharynx and in the number and arrangement of the flame cells.

The cercariae in the present study seem to possess similar characteristics as those collected by King & Van As (1997) from *Bulinus tropicus* in the Free State region, namely same external morphological features, internal structures such as the number and arrangement of penetration glands, number and arrangement of flame cells. Because of the similarity to the cercariae found by King & Van As (1997), the cercariae can possibly be placed in the family Diplostomidae.

FAMILY DIPLOSTOMIDAE

The family Diplostomidae is a large group, which are intestinal parasites of both mammals and birds. A number of genera have already been identified in this family, namely *Alaria* Schrank, 1788, *Uvilifer* Yamaguti, 1934, *Bolbophorus* Dubois, 1935 and the most well known, *Diplostomum* von Nordmann, 1832 (King 1991).

One species of the genus *Neodiplostomum* Railiet, 1919, which is more commonly a parasite of rats, has been recorded in the intestine of humans in Korea on several occasions (Gibson 1998). Diplostomids are recognised by the bipartate nature of the body and a wide ventrally concave forebody.

Larval forms (metacercariae) parasitize fish being located in various organs and some species are known to cause serious diseases in fish (Markevich 1963).

Eggs of adult diplostomids leave with the faeces. If they enter water the miracidium is released and after penetrating an aquatic snail it transforms into a mother sporocyst. The mother sporocyst produces daughter sporocysts, which release fork-tailed cercariae. Cercariae leave the snail and usually encyst as metacercaria on fishes, but in the case of *Neodiplostomum* the metacercariae may be found in frogs (Gibson 1998). Humans may become infected when eating frogs.

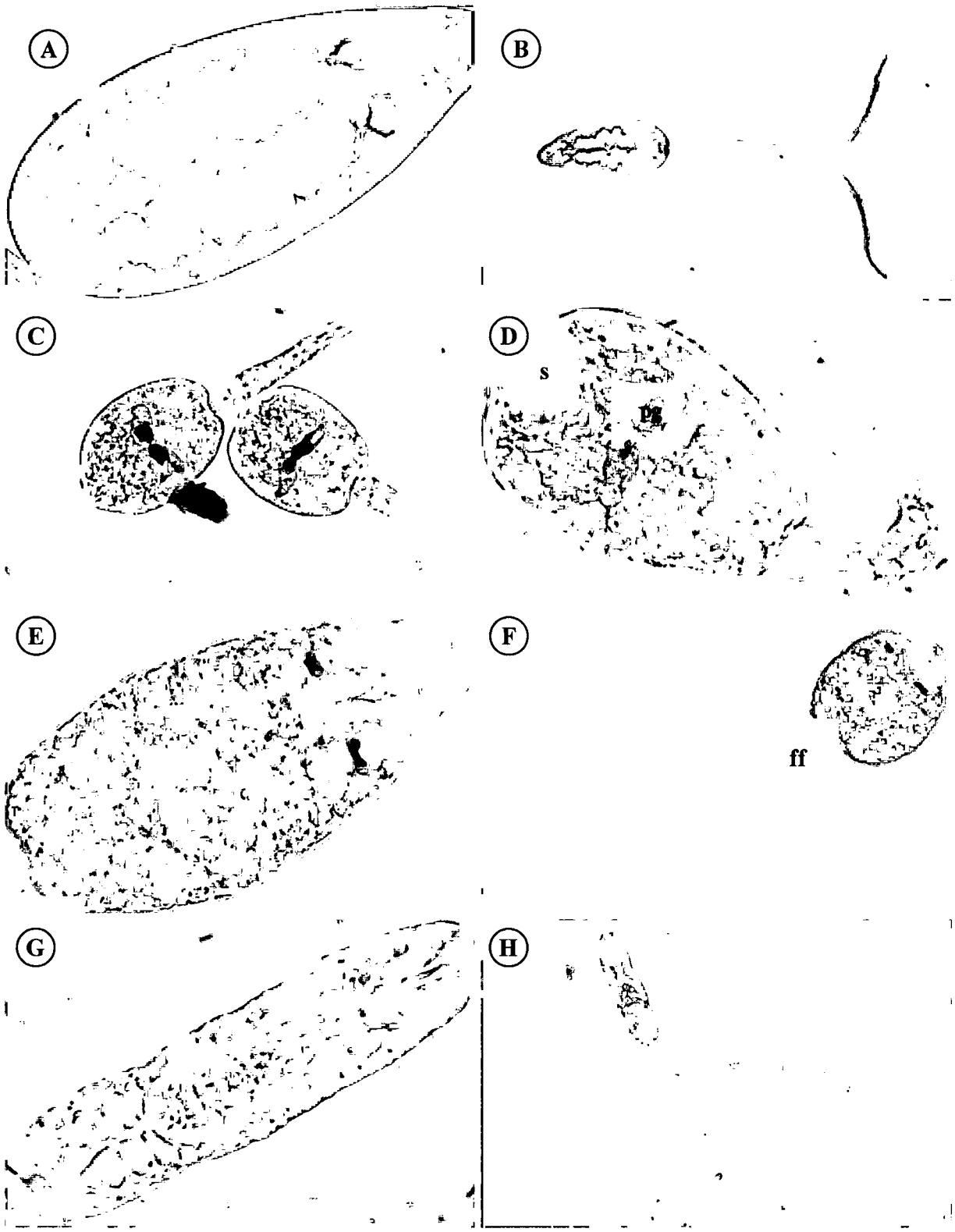


Figure 6.10: Light micrographs of cercariae from different digenean families. **A.** Vivax cercaria body (Cyathocotylidae). **B.** Vivax cercariae showing body and tail. **C.** Xiphidio cercariae (Plagiorchiidae). **D.** Xiphidio cercaria body showing stylet (s) and penetration glands (pg). **E.** Parapleurolophocercous cercaria body (Heterophyidae). **F.** Parapleurolophocercous cercaria showing finfold on tail (ff). **G.** Diplostomatid cercarial body (Diplostomidae). **H.** Diplostomatid cercaria showing long fork-tail. Scale bars: A, B, C & F = 100μm D, E, G & H = 33μm.

HOST: *Lymnaea natalensis* Krauss, 1848

LOCALITY: Mohembo Floodplains; Guma Floodplains; Seronga Floodplains, Seronga Polars Camp; Thaoge Lagoon; Upper Thaoge Lagoon

TYPE OF CERCARIA: 27-spined single-tailed echinostomatid cercaria D

REDIA: Redia found within the digestive gland of the snail (Fig. 6.11A). Orange in colour. Measures 450-500 (477±21.0) x 140-150 (146±4.6). Pharynx spherical 40-50 (25.7±4.3) x 20-35 (25.7±4.8), situated at anterior end of body.

CERCARIA:

Body of cercariae oval (Figs. 6.11B, 6.20A), 180-360 (262±61.0) long x 100-200 (153±33.9) broad. Characteristic collar present anteriorly around oral sucker measuring 50-70 (58.6±7.8) broad (Figs. 6.11C, 6.12A, 6.20A). Arrangement of spines on collar as follows: four corner spines 14-20 (16.7±2.3) x 4-6 (4.83±0.6), arranged on either side in area between oral sucker and pharynx. Corner spines succeeded by five lateral spines (Fig. 6.12B), 14-30 (19.7±6.3) x 4-10 (6.11±1.7). Rest of collar spine arrangement consists of nine dorsal spines 12-18 (14.4) x 3-7 (4.7) arranged in two alternating rows of four oral 10-15 (11.8±1.2) x 3-6 (4.4±1.0) and five aboral spines 14-21 (16.9±1.8) x 3-8 (5±1.0) (Fig. 6.11C).

Oral sucker (Fig. 6.12C) situated, at anterior end of body 20-61 (45.4±12.1) x 40-70 (58.4±9.7), followed by prepharynx measuring 10-51 (19.9±11.4) x 5-14 (9.67±2.5), followed by pharynx, 10-41 (18.8±8.5) x 5-36 (16.6±8.4). Oesophagus measures 5-9 (7.4±1.3) x 3-5 (4.5±1.0). Oesophagus bifurcates directly anterior to acetabulum into two intestinal caeca, each extending to posterior end of body where they terminate on either side of excretory bladder (Fig. 6.11B).

Acetabulum 70-180 (118±35.8) from oral sucker and 100-220 (167±36.4) from anterior end of body (Fig. 6.11B). Acetabulum protrusible (Fig. 6.12D), 17-74 (49.5±18.6) x 18-84 (60.6±19.3) situated posteriorly to middle of body.

Excretory system stenostomate (Fig. 6.11B). Main ducts extend anteriorly from both sides of small bipartite bladder measuring 10-35 (16.2±7.9) x 35-72 (50.1±13.5) and 5-24 (11.2±4.7) x 15-48 (27.1±11.0).

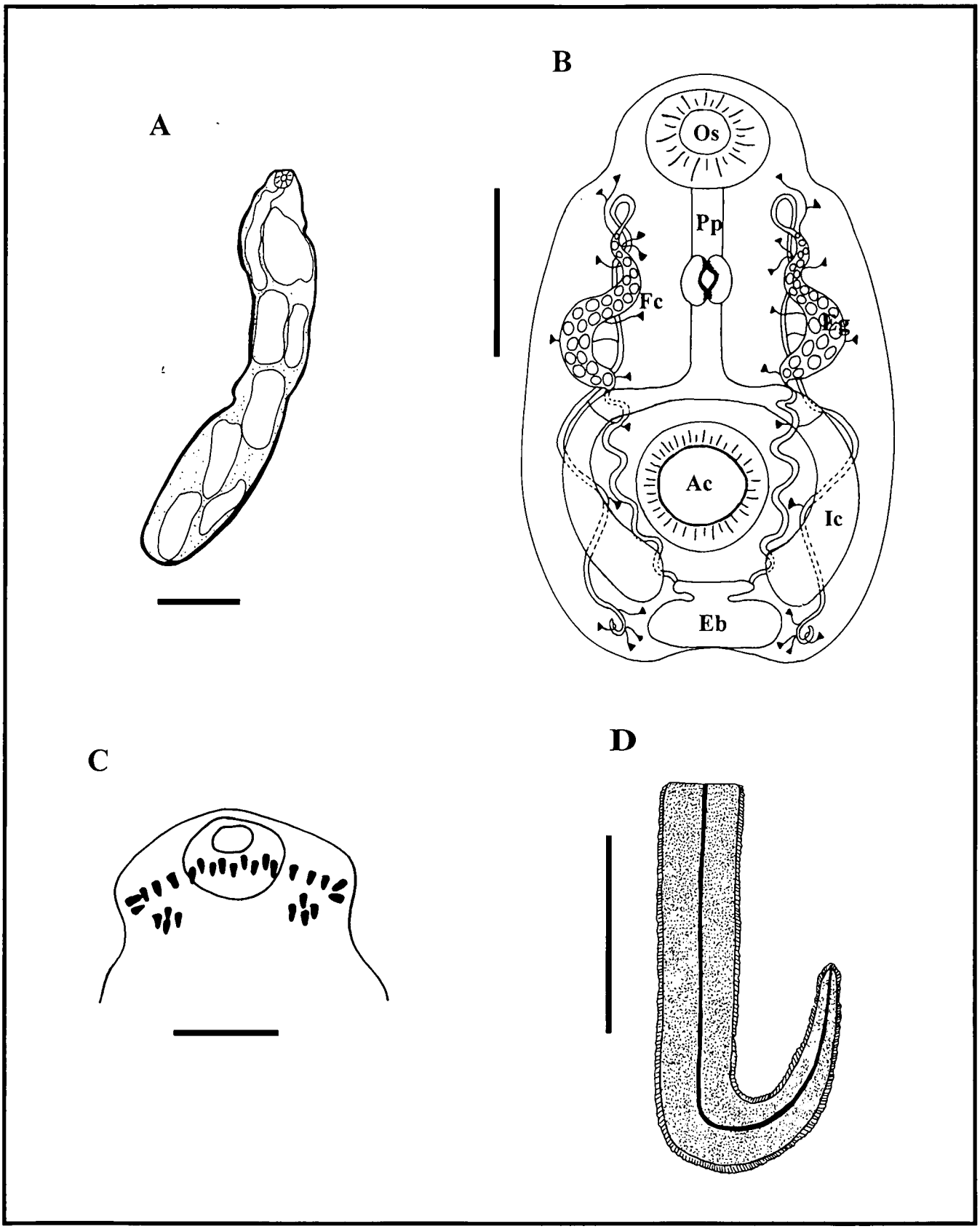


Figure 6.11: Light microscope projection drawings of the morphology of the single tailed cercaria shed by *Lymnaea natalensis* Krauss, 1848 collected from the Okavango Delta, Botswana. **A**. Mother redia. **B**. Body of cercaria. **C**. Collar of spines. **D**. Single Tail. **Os**- Oral sucker, **Pp**- Prepharynx, **Eg**- Excretory granules, **Fc**- Flame cell, **Ac**- Acetabulum, **Eb**- Excretory bladder.
Scale bar: A, B, D- 100μm. C- 25μm.

FIGURE 6.12

Scanning electron micrographs (C, D, E, F) and light micrographs (A, B) of the external and internal morphology of the long single-tailed cercaria shed by *Lymnaea natalensis* Krauss, 1848 collected from the Okavango Delta, Botswana.

A. Characteristic collar of spines around oral sucker

B. Corner spines

C. Oral sucker

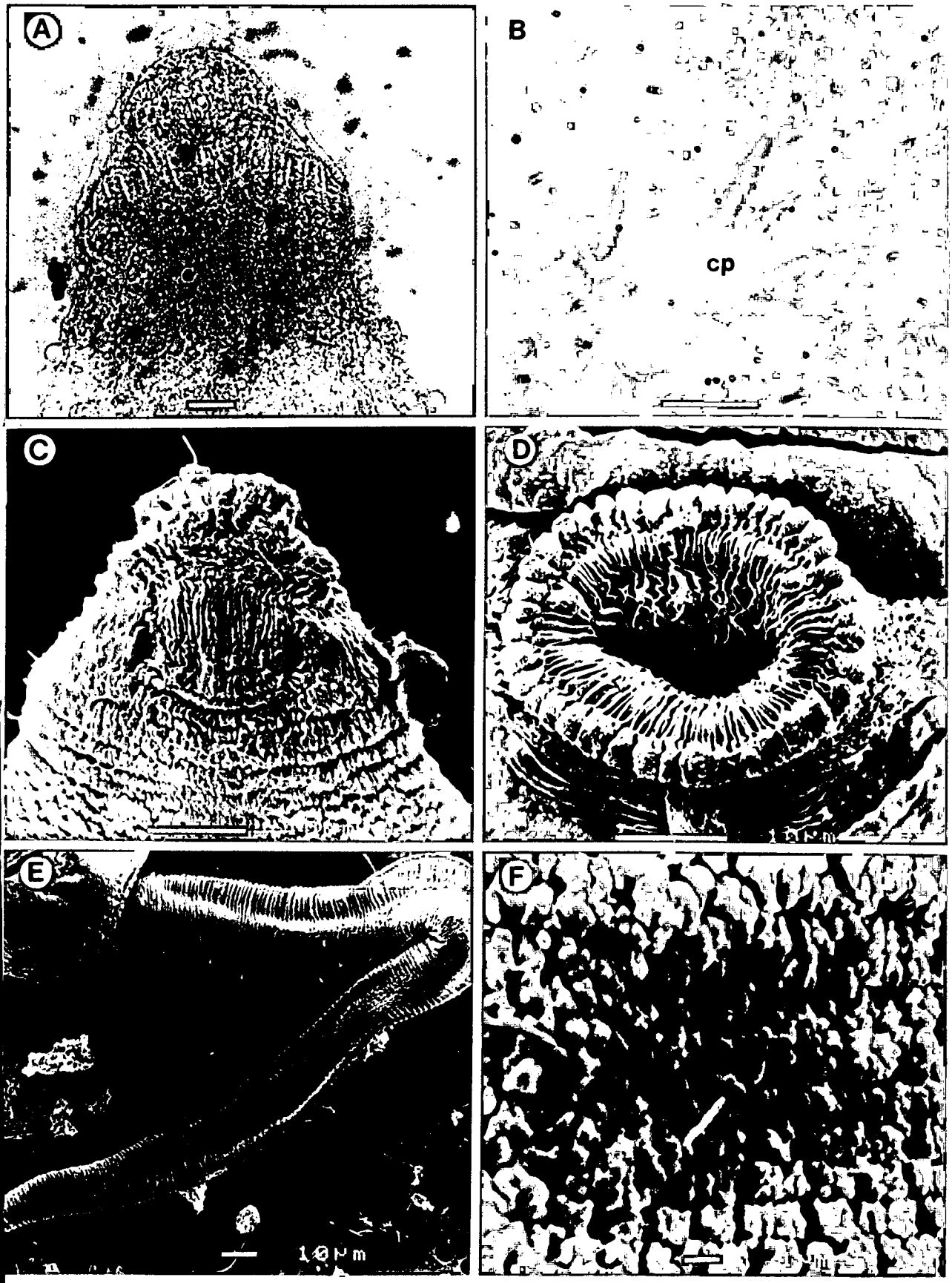
D. Acetabulum

E. Tail

F. Body surface

cp- Corner spines

Scale bar: A-E- 10μm. F- 1μm.


A
B
cp
C
D
E
10μm
F

At point where duct reaches bifurcated caeca, it widens to form broad "S" shape tube which extends to a point posteriorly of pharynx where it again narrows, extending to a point just posterior to oral sucker. At this point it folds back, extending all the way to bladder, terminating at posterior end of body. "S" shaped ducts filled with numerous refractile granules, measuring 2-8 (5.11±2.0) in diameter (Fig 6.20A). At various points along excretory system short ducts extend from main excretory duct to terminate as flame cells. Eight flame cells anterior and six flame cells posterior to acetabulum on both sides of body, total of 14 pairs of flame cells were observed. Flame cell formula: 2[(2+2+2+2) + (2+2+2)]=28. Flame cells absent in tail stem. Caudal branch of excretory system opens at tip of tail. Tail single, measures 170-360 (297±41.2) long and 30-80 (44.2±10.9) broad without finfolds (Figs. 6.11D, 6.12E, 6.20B). Body of cercaria not covered by spines (Fig. 6.12F).

REMARKS:

This is a typical echinostome cercaria characterised by a collar of spines surrounding the oral sucker and a stenostomate excretory system. Porter (1938) described a cercaria, *Cercaria middelburgensis* with a collar of 26 spines, from the snail *Lymnaea natalensis* Krauss, 1848 from a lake at Middelburg, Transvaal (Mpumalanga). This cercaria differs in many morphological characteristics from the cercaria in the present study, for example, the size and detail of all body structures, the number and arrangement of the collar of spines and number of flame cells.

The present cercaria is similar though to the 27 spined echinostomatid cercaria, *Cercaria veldskoonensis* described in an unpublished thesis by Taplin (1964). She found the cercariae from *B. tropicus* collected from Westdene Dam and Albertskroon, as well as *Bulinus africanus* (Krauss, 1848) collected from Veldskoon. It is also similar to the cercaria described by King and Van As (1997). It differs though in that it does not possess a prepharyngeal sac. Therefore it may be proposed that the cercariae in the present study conform to the cercariae described by Taplin (1964). This cercaria falls therefore within the morphological descriptions of the family Echinostomatidae.

FAMILY : ECHINOSTOMATIDAE

The family Echinostomatidae is a large family, which are parasites of reptiles, birds and mammals. A number of different subfamilies occur in this group, one of these being the subfamily Echinostomatinae Faust, 1929. Within this group there are some well known genera including *Echinostoma* Rudolphi, 1809 and the largest and most well known genera, *Echinoparyphium* Dietz, 1909. Other genera include *Petasiger*, 1909; *Nephrostomum* Dietz, 1909; *Paryphostomum* Dietz, 1909 and *Euparyphium*, Dietz, 1909.

According to Taplin (1964) larval echinostomes were first described from South Africa by Cawston (1917, 1918) who described two cercaria from Kwa-Zulu Natal, namely *Cercaria catenata* and *Cercaria arcuata* from *Physopsis africana* and *Lymnaea natalensis* respectively. Cawston (1917) remarked on the 'chains of cystogenous vesicles in the former and the 'chain of blackish granules' in the latter, where he was presumably referring to the excretory granules within the excretory canals. Faust (1919, 1920) and Cort (1915) criticized Cawston's descriptions and redescribed the two species together with a new species, *Cercaria constricta.*

Faust (1920, 1921, 1926) further described four new species from South Africa, namely *Cercaria 30-acanthostoma* from *Physopsis africana* Krauss, 1848, *Cercaria isodorae* from *Bulinus forskali* (Ehrenberg, 1851), *Cercaria caudadena* from *Planorbis pfeifferi* (Krauss, 1848), and *Cercaria cucumeriformes* from *Lymnaea natalensis* Krauss 1848. More anatomy was described in these later accounts but because the material was fixed, insufficient information was available about essential characters such as the collar spines and excretory systems and thus these early accounts could not be used for diagnostic purposes, as descriptions and diagrams were incomplete (Taplin 1964). The most comprehensive study on cercarial taxonomy was done by Porter (1938) whose work is still being used today.

No 27 spined echinostome cercaria had been described but Porter (1938) described an echinostome cercaria having 26 spines (actually 27 spine echinostome since all spine numbers are of an uneven number) namely *Cercaria middelburgensis* Porter, 1938 which was shed by *Lymnaea natalensis* found in Middleburg, Transvaal

(Mpumalanga). Years later Taplin (1964) described a 27 spined echinostome cercaria from Westdendam, Albertskroon, Veldskoon, and Johannesburg. The cercaria *C. veldskoonensis* Taplin, 1964 was shed by the snails, *B. tropicus* and *B. africanus* respectively.

Most of the echinostome parasites with 27 spines are placed under the genus *Euparyphium* (King, 1991). The genus *Euparyphium* is a relatively small group having representatives in both birds and mammals. The genotypic species *E. capitaneum* Dietz, 1909 from *Plotus anhinga* was described from Brazil. Further species described were *E. longitestis* (Verma, 1936) from India and *E. melis* (Shrank 1788) Dietz, 1909 from the duodenum of *Mustela vision* as described by Gupta (1962) from the U.S.A.

The presence of 27 collar spines is not only limited to the genus *Euparyphium*, but can also occur in other genera. *Paryphostomum testrifolium* was described by Gogate (1934) from *Dendrocygna javanica* from Rangoon and another species, namely *P. segregatum* Dietz, 1909 whose life cycle was described by Lie & Basch (1967) also has 27 spines. Lie & Basch (1967) found that *Biomphalaria glabrata*, *Biomphalaria striminea* and *Biomphalaria tenagophila* serve as first intermediate hosts, while fish and tadpoles serve as second intermediate hosts and the vulture *Coragyps atratus* serves as the final host.

The cercaria usually enter a second snail host or a fish or an amphibian to encyst, while the adult parasites are usually located in the intestine and sometimes in the bile ducts (Dawes 1968). Mostly parasites of birds and mammals.

HOST: *Biomphalaria pfeifferi* (Krauss, 1848)

LOCALITY: Xaro Mainstream Lagoon; Duba lagoon; Thaoge Lagoon

TYPE OF CERCARIAE: Fork tailed cercaria E

REDIA: Not found

CERCARIA:

Body of cercaria elongate (Figs. 6.13A, 6.14A, 6.20C), 110-180 (141±19.4) x 35-80 (52.8±12.3). Tail longer than body 150-300 (235±37.0) x 10-50 (24.5±10.3) (Figs. 6.13B, 6.14B, 6.20D). Stem forks into two short furcae 50-150 (77.8±24.2) x 5-20 (10.2±4.6). Finfolds absent.

Pear shaped protrusible oral sucker (Figs. 6.13A, 6.14C) longer than wide situated at anterior end of body. Oral sucker measures 20-80 (34.6±18.5) x 15-42 (28.2±7.5). Acetabulum spherical, (Fig. 6.14D), 15-25 (17.7±3.9) x 15-25 (17.71±3.3). No pharynx present. Long oesophagus, bifurcates into two small intestinal caeca which end above penetration glands (Fig. 6.13A).

Two groups of five penetration glands situated on either side of acetabulum (Fig. 6.13A). First three gland pairs finely granulated stain light blue when coloured with Nile blue sulphate. First gland measures 10-15 (11.4±2.0) x 10-20 (11.5±3.3); second gland 9-12 (10.9±1.4) x 10-23 (12.2±4.3); third gland pair 6.8-10 (9.8±1.0) x 10-22 (11.6±3.7); fourth gland pair 10-15 (10.8±1.7) x 10-14 (10.7±1.4) and fifth gland 10-15 (10.3±1.6) x 10-15 (10.6±1.6). Ducts arising from these glands open anteriorly on either side of oral sucker (Fig. 6.13A).

Excretory bladder small, oval, situated at posterior end of body. Main excretory duct on either side of bladder extends anteriorly. On reaching area just above acetabulum it divides into anterior and posterior collecting ducts. Anterior collecting duct running anteriorly gives rise to two capillaries, each ending in flame cells. Similarly, posterior collecting duct on either side of body runs posteriorly and divides into two capillaries, also ending in flame cells. Posterior collecting duct on either side of body also extends into tail stem, forming one capillary in anterior end of tail stem (Fig 6.13A). Flame cell formula as follows: $2[(1+1) + (1+1+\{1\})] = 10$.

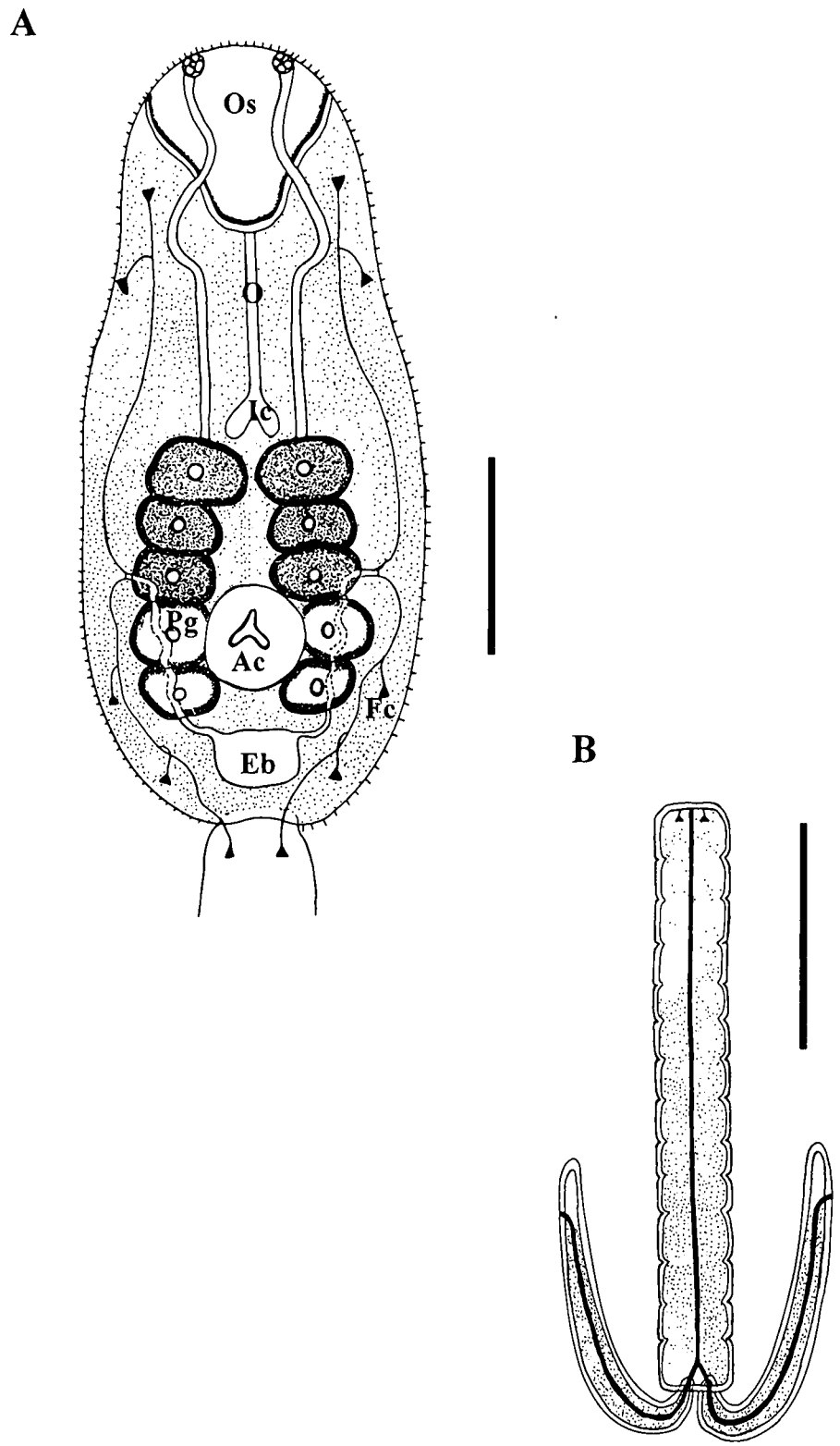


Figure 6.13: Light microscope projection drawings of the morphology of the short fork-tailed cercaria shed by *Biomphalaria pfeifferi* (Krauss, 1848) collected from the Okavango Delta, Botswana. **A**. Body of cercaria. **B**. Fork Tail. **Os**- Oral sucker, **O**- Oesophagus, **Ic**- Intestinal caeca, **Fc**- Flame cell, **Ac**- Acetabulum, **Eb**- Excretory bladder, **Pg**- Penetration glands. **Scale bar**: A- 33μm. B- 100μm.

FIGURE 6.14

Scanning electron micrographs of the external morphology of the short fork-tailed cercaria shed by *Biomphalaria pfeifferi* (Krauss, 1848) collected from the Okavango delta, Botswana.

A. Body of cercaria

B. Short tail

C. Oral sucker

D. Acetabulum

E. Opening of excretory pore

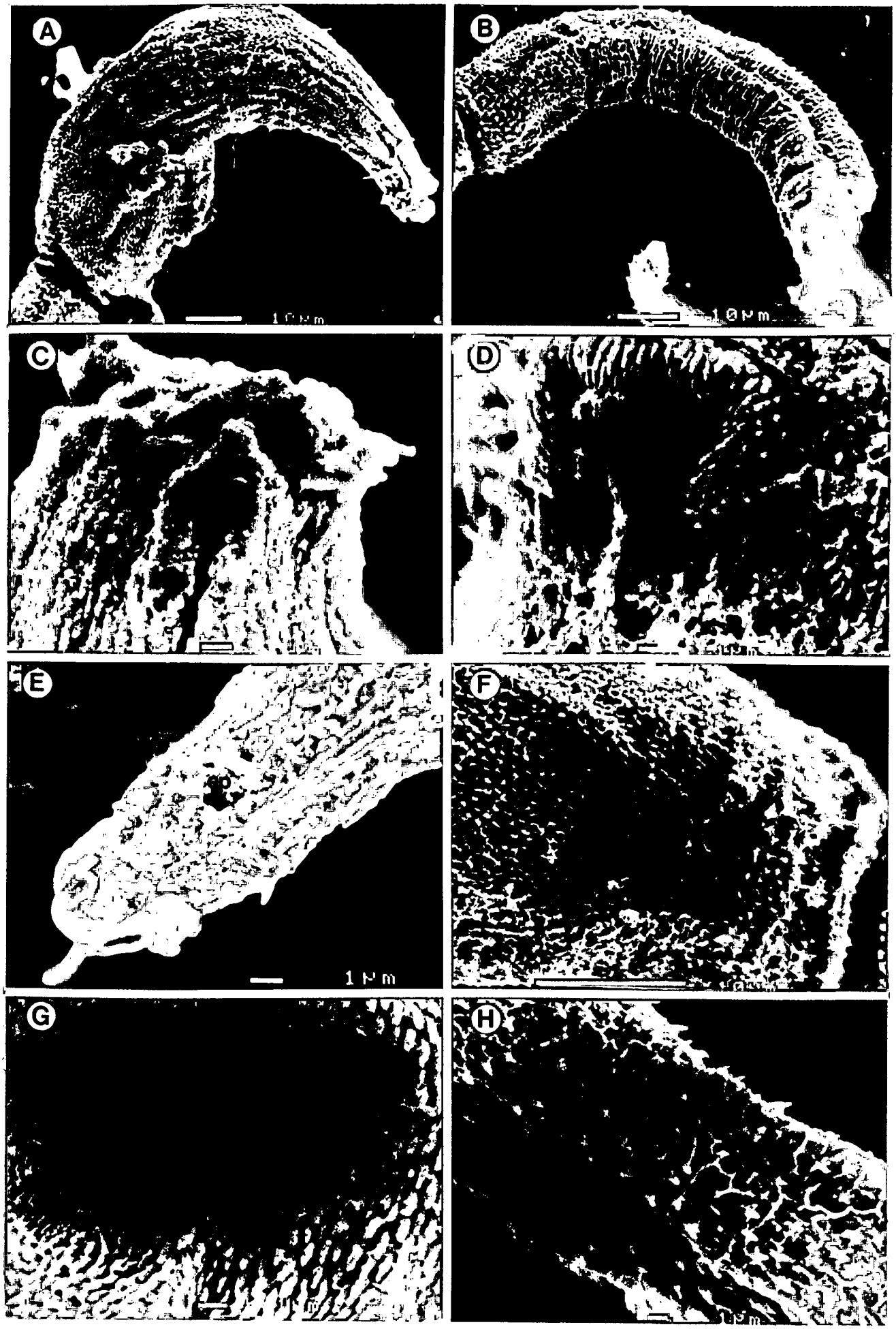
F. Body surface covered with spines

G. Papillae on body surface

H. Spiny tail surface

ep- Excretory pore

Scale bar: A, B, F- 10μm. C, D, E, G, H- 1μm.


A
B
C
D
E
1 µm
F
G
H

Posterior to excretory bladder a caudal excretory duct arises which divides at base of tail stem into two branches, each running into caudal furcae and opens to outside by means of a pore, at the end of furcae on the inside (Fig. 6.14E).

Body of cercaria covered with spines (Fig. 6.14F), these spines are short but sturdy with sharp points, face same direction. Sensory receptors with long cilia present on body (Fig. 6.14G). Tail also covered by a number of spines that are spread evenly over surface at base of tail stem (Fig. 6.14H).

REMARKS:

The cercaria in the present study was identified as an apharyngeal, distome, brevifurcate fork-tail cercaria giving an indication that it is a schistosome cercaria. In her work Porter (1938) described a number of schistosome species. The cercaria in the present study is similar to *Schistosoma spindale* described by Porter in that it has the same number and arrangement of penetration glands, short ending intestinal caeca and body covered by spines, however the oral suckers are not similar and the number of flame cells are also not the same.

It is uncertain to which species this cercariae belongs but since it was shed between 17:00 and 18:00 which gives an indication that it would not be a human schistosome species since these cercariae are usually shed at midday. It is suspected that the hippopotamus may serve as the final vertebrate host, since the cercariae are shed at a time when hippos are active, and schistosome eggs were found in hippo faeces. The cercaria could therefore conform to the species *Schistosoma edwardiense*. The cercaria belongs to the family Schistosomatidae.

FAMILY SCHISTOSOMATIDAE

Three schistosome species are of vast medical importance: *Schistosma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum* – all parasites of humans since antiquity. Bloody urine was a well-recognised disease symptom in northern Africa in ancient times. The first Europeans to record contact with *S. haematobium* were surgeons with Napoleon's army in Egypt (1799-1801). They reported that hematuria (bloody urine) was prevalent among the troops, although the cause, of course, was

quite unknown. Nothing further was learned about *Schistosomiasis haematobia* for over 50 years, until a young German parasitologist, Theodor Bilharz discovered the worm that caused it.

The first genus of this family was discovered by Bilharz in 1852 when he recovered some worms from the mesentric veins of a native from Cairo and named the parasite *Distomum haematobium.* This genus was reported by other workers under different names: *Gynaecophorous* Diesing, 1858; *Bilharzia* Cobbold, 1859; Thecosome Moquin-Tandon, 1860. The name "*Distomum*" for this blood fluke was a misnomer and was replaced by *Schistosoma* by Weinland in 1858.

According to Behari Lal (1937), Looss (1899) created the family Schistosomatidae for the genus *Schistosoma* and added the second genus *Bilharziella* to the family.

There is also a wide knowledge of *Schistosoma* species occurring in wild animals, domestic animals as well as in birds. Records of *Schistosoma* species from game animals have recently been listed by Van den Berghe (1939), Amberson and Schwarz (1953), Yamaguti (1958) and Nelson (1960). Schistosomes have been described from several antelopes, buffalo, zebra, camel, elephant ad monkeys. Thurston (1963, 1964) described *Schistosoma hippopotami* and *Schistosoma edwardiense* from the hippopotamus in Uganda.

HOST: *Biomphalaria pfeifferi* (Krauss, 1848)

LOCALITY: Willies camp

TYPE OF CERCARIA: Fork-tailed cercaria F

REDIA: Not found

CERCARIA:

Body of cercaria elongate to oval (Figs. 6.15A, 6.20E), 130-260 (202±35.6) x 40-74 (51.7±12.7). Tail slightly longer than body measuring 160-280 (218±39.9) x 20-70 (47.3±21.5) (Figs. 6.15B, 6.20F). Stem forks into two long laterally flattened furcae 130-250 (181±41.8) x 10-50 (31.8±18.3). Four anterior caudal setae and eight posterior caudal setae present on either side of tail stem. Caudal furcae with two setae present on the posterior half on both sides of each furcae (Figs. 6.15A, 6.16A). Single setae also present posteriorly on either side of body (Fig. 6.16B). Finfolds absent.

Round protrusible oral sucker (Figs. 6.15A, 6.16C) longer than wide situated at anterior end of body. Oral sucker measures 21.2-55 (33.8±12.0) x 17-61 (30.2±13.1), surrounded by tiny blunt spines. Oral sucker opens into oesophagus, 7-10 (8.7±1.2) x 5-9 (6.7±1.5). Oesophagus bifurcates into two very short intestinal caeca which extend a ¼ of the way down body (Fig. 6.15A). Acetabulum not observed.

Six large round to oval penetration glands present in posterior half of body (Figs. 6.15A, 6.16D). Cytoplasm of glands finely granulated stain light blue when coloured with Nile blue sulphate. Penetration glands arranged two tandemly, four symmetrically. First gland measures 5-15 (11.1±2.4) x 8-20 (16.1±3.2); second gland 8-18 (10.7±2.3) x 12-25 (17.1±3.7); third gland pair measures 9-28 (15.7±4.4) x 10-20 (11.4±2.5) remaining gland pair 8-20 (14.9±4.2) x 10-20 (12.1±3.1). Ducts arising from these glands open anteriorly on either side of oral sucker.

Excretory bladder small, rectangular, situated at posterior end of body measuring 5-15 (10.6±3.2) x 20-50 (26.5±10.0). Main excretory duct on either side of bladder extends anteriorly. On reaching second last pair of penetration glands, it divides into anterior and posterior collecting ducts. Anterior collecting duct running anteriorly gives rise to six capillaries, each ending in flame cells.

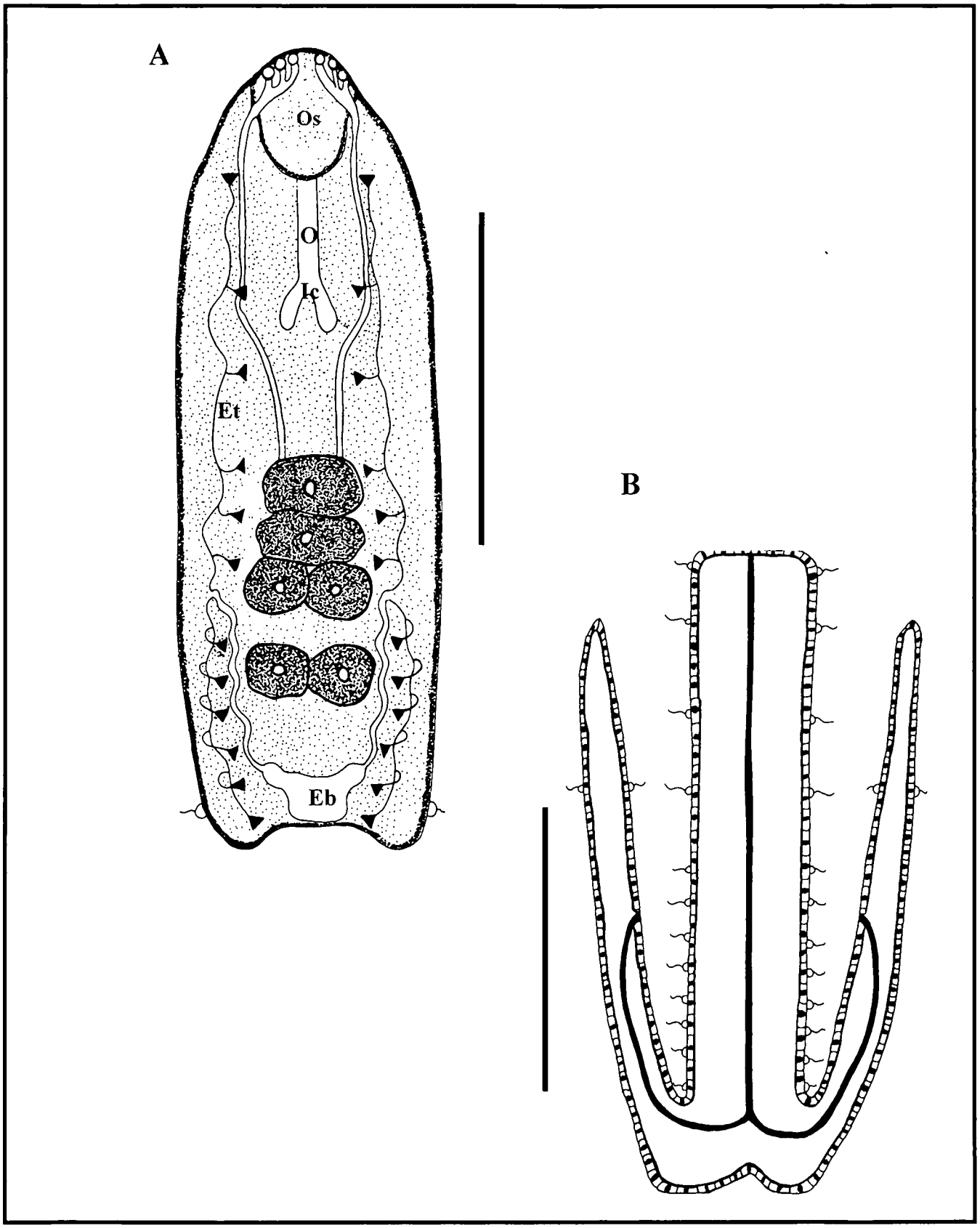


Figure 6.15: Light microscope projection drawings of the morphology of the fork- tailed cercaria shed by *Biomphalaria pfeifferi* (Krauss, 1848) collected from the Okavango Delta, Botswana. **A.** Body of cercaria . **B**. Fork Tail. **Os**- Oral sucker, **O**- Oesophagus, **Ic**- Intestinal caeca, **Et**- Excretory tubules, **Eb**- Excretory bladder. **Scale bar**: 100μm.

FIGURE 6.16

Scanning electron micrographs (A, B, C, E, F, G, H) and light micrograph (D) of the external and internal morphology of the long fork-tailed cercaria shed by *Biomphalaria pfeifferi* (Krauss, 1848) collected from the Okavango Delta, Botswana.

A. Setae on furcal rami

B. Single setae on body

C. Oral sucker

D. Penetration glands

E. Opening of excretory pore

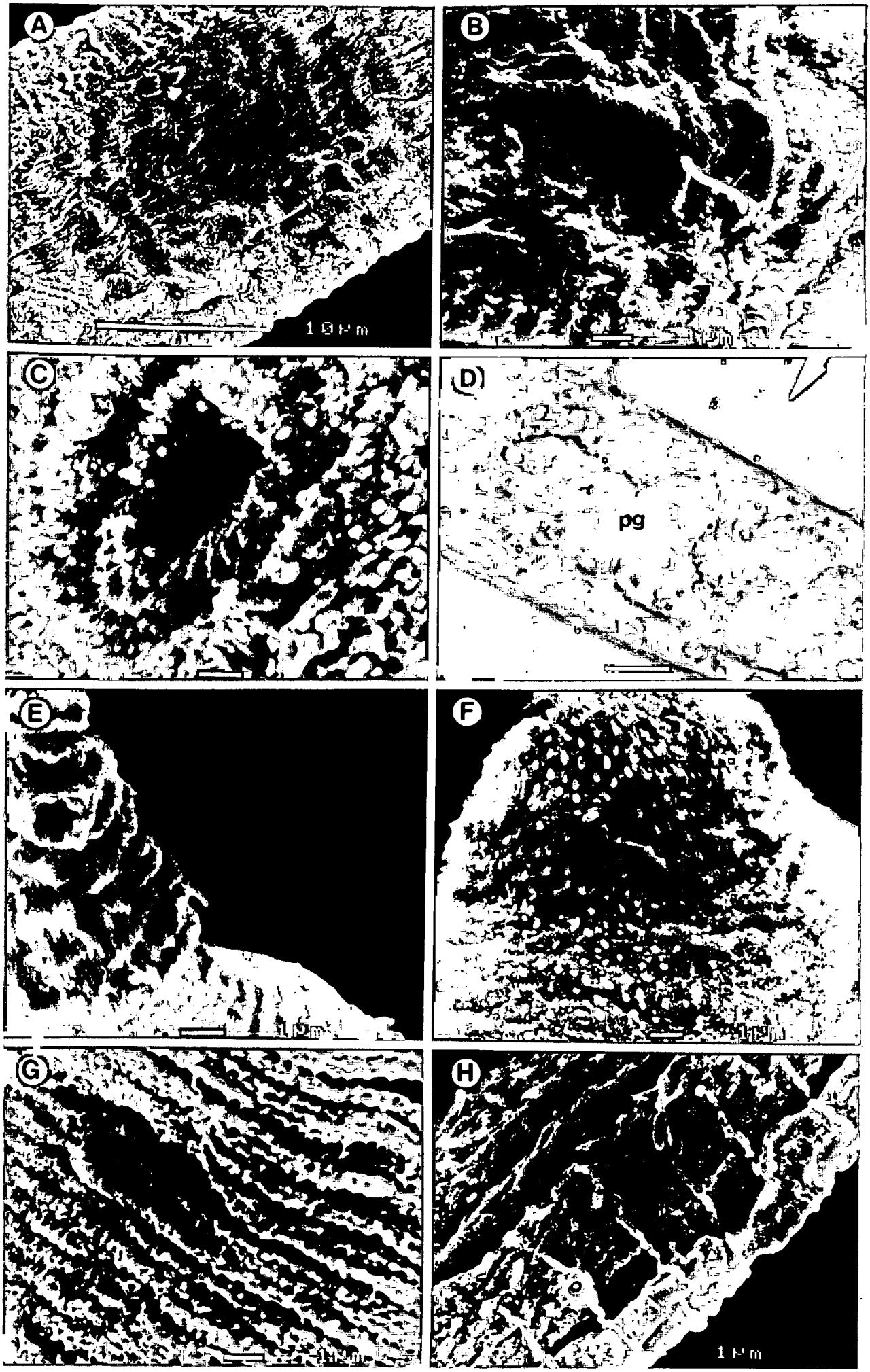
F. Spines present on dorsal side of oral sucker

G. Body surface

H. Tail surface with backwardly directed spines in the centre of tail

pg- Penetration glands

Scale bar: A, D- 10μm. B, C, E, F, G, H- 1μm.


A
1 µm
B
C
D
pg
E
F
G
H
1 µm

Similarly, posterior collecting duct on either side of body runs posteriorly and divides into six capillaries, all ending in flame cells (Fig. 6.15A). Flame cell formula as follows: 2[(1+1+1+1+1+1) + (1+1+1+1+1+1)]= 24. Posterior to excretory bladder a caudal excretory duct arises which divides at base of tail stem into two branches (Fig. 6.15B), each running into caudal furcae and opens halfway to outside by means of a pore (Fig. 6.16E).

Body of cercaria not covered with spines (Fig. 6.16F). Small blunt spines present on anterior dorsal side of body (Fig. 6.16G). Posteriorly body is not covered with spines. Tail covered in centre by backwardly directed spines (Fig. 6.16H). Sensory receptors with short cilia present on tail stem.

REMARKS:

This cercariae was identified as a pharyngeal, longifurcate distome cercariae according to the key of Frandsen and Christensen (1984). This cercariae belongs to the strigeid group of parasites, which may be representative of either the family Diplostomidae or Strigeidae.

Johnston & Angel (1942) described a strigeid cercaria, *Cercaria metadena* from Australia with similar characteristics as the cercaria in the present study. It is similar in that it possesses the same number of penetration glands but arrangement of the penetration glands differs. It has the same type of intestinal caeca and has no ventral sucker. It differs in that it doesn't have the same number of flame cells, and doesn't have eyespots.

Lengy & Wolff (1971) described a strigeid cercariae from Israel occurring in the snail, *Bulinus truncatus*. The cercaria in the present study is similar to *Cercaria levantina* in that it possesses the same number of penetration glands and is also without eyespots. *Cercaria levantina* differs from the cercaria in the present study in that possesses a ventral sucker and does not have the same number of penetration glands.

The cercaria in the present study differs from the cercaria found by King & Van As (1997) in that the external body structure, the number of flame cells and the number

and arrangement of the penetration glands are not similar. Porter (1938) described a cercaria, *Cercaria elvaeformis* also with six penetration glands and with a small ventral sucker but arrangement of the glands are not the same and no ventral sucker was observed in the cercaria from present study.

FAMILY DIPLOSTOMIDAE

* Refer back to Fork-tailed cercariae shed by *Lymnaea natalensis*

HOST: *Bulinus globosus* (Morelet, 1868)

LOCALITY: Mohembo Floodplain; Xaro Mainstream Lagoon; Guma Lagoon; Seronga Floodplains; Upper Thaoge Lagoon

TYPE OF CERCARIA: Amphistome cercariae G

REDIA: Not found

CERCARIA:

Body of cercariae oval, concave (Fig. 6.18A), and darkly granulated (Figs. 6.20G, 6.19A). Body also muscular and very contractile measuring 290-400 (344±41.2) x 280-380 (330±24.2). Muscular tail (Figs. 6.17B, 6.20H) measures 500-680 (581±63.2) x 80-100 (91±7.1).

Oval oral sucker is situated sub-terminally 11-30 (18.6±7.2) from the anterior border of the body. Oral sucker measures 30-80 (60.9±15.6) x 30-80 (52.4±12.0) (Figs. 6.17A, 6.18B). Acetabulum situated at posterior end of body and is 90-280 (150±42.6) from the oral sucker. Acetabulum measures 50-130 (98.1±23.9) x 60-130 (108±17.4) (Figs. 6.17A, 6.20G). Mouth opens by means of an oral sucker to the outside. No oesophagus, pharynx and intestinal caeca could be seen due to the dark pigmentation of the cercariae.

Two dark eyespots occur in anterior third of the body. Eyespots are 20-45 (35.5±6.6) long and 20-40 (31.9±7.7) broad (Fig. 6.17A, 6.20G).

Excretory system consists out of an excretory bladder at the posterior end of the body, capable of extending and contracting at same time as body. Main excretory ducts on either side of bladder extend from apical point of bladder after which the ducts extend laterally in an anterior direction (Fig 6.17A). In middle of body these ducts turn inwards and then extend laterally to once again extend inwards posterior of eyespots. Here ducts bend round eyespots where they then extend backwards in posterior direction. Excretory ducts are connected to each other in middle of body. Throughout the body these ducts are filled with dark excretory granules (Fig. 6.17A).

Body of cercariae is not covered with spines, but by mass of protrusions (Fig. 6.18C). Protrusions are found on both ventral and dorsal sides of body.

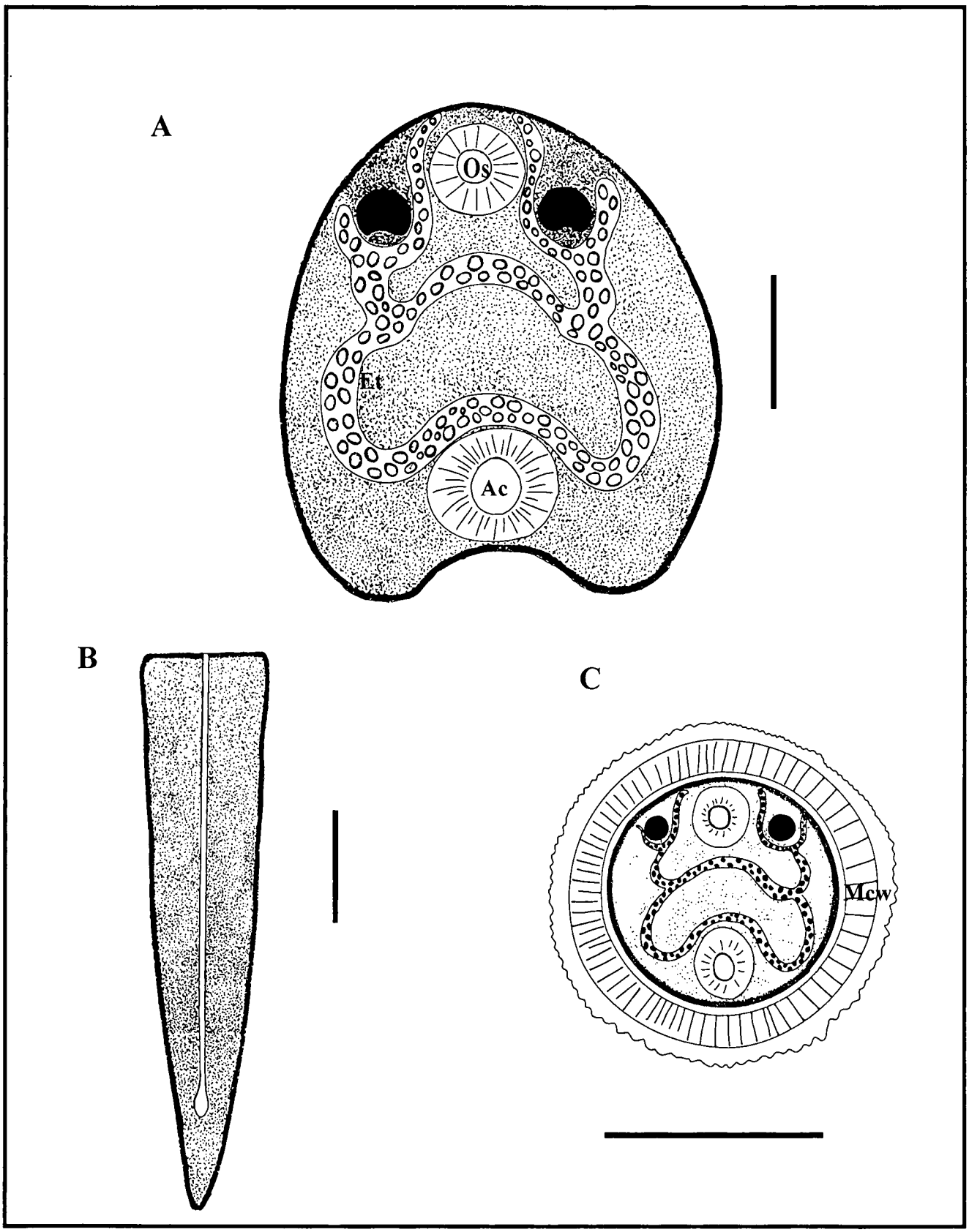


Figure 6.17: Light microscope projection drawings of the morphology of the amphistome cercaria shed by *Bulinus globosus* (Morelet, 1868) collected from the Okavango Delta, Botswana.
A. Body of cercaria. **B**. Single Tail. **C**. Metacercarial cyst. **Os**- Oral sucker, **Et**- Excretory tubule, **Ac**- Acetabulum, **Mcw**- Metacercarial cyst wall. Scale bar: 100μm.

FIGURE 6.18

Scanning electron micrographs of the external morphology of the amphistome cercaria shed by *Bulinus globosus* (Morelet, 1868) collected from the Okavango Delta, Botswana.

A. Concave cercarial body

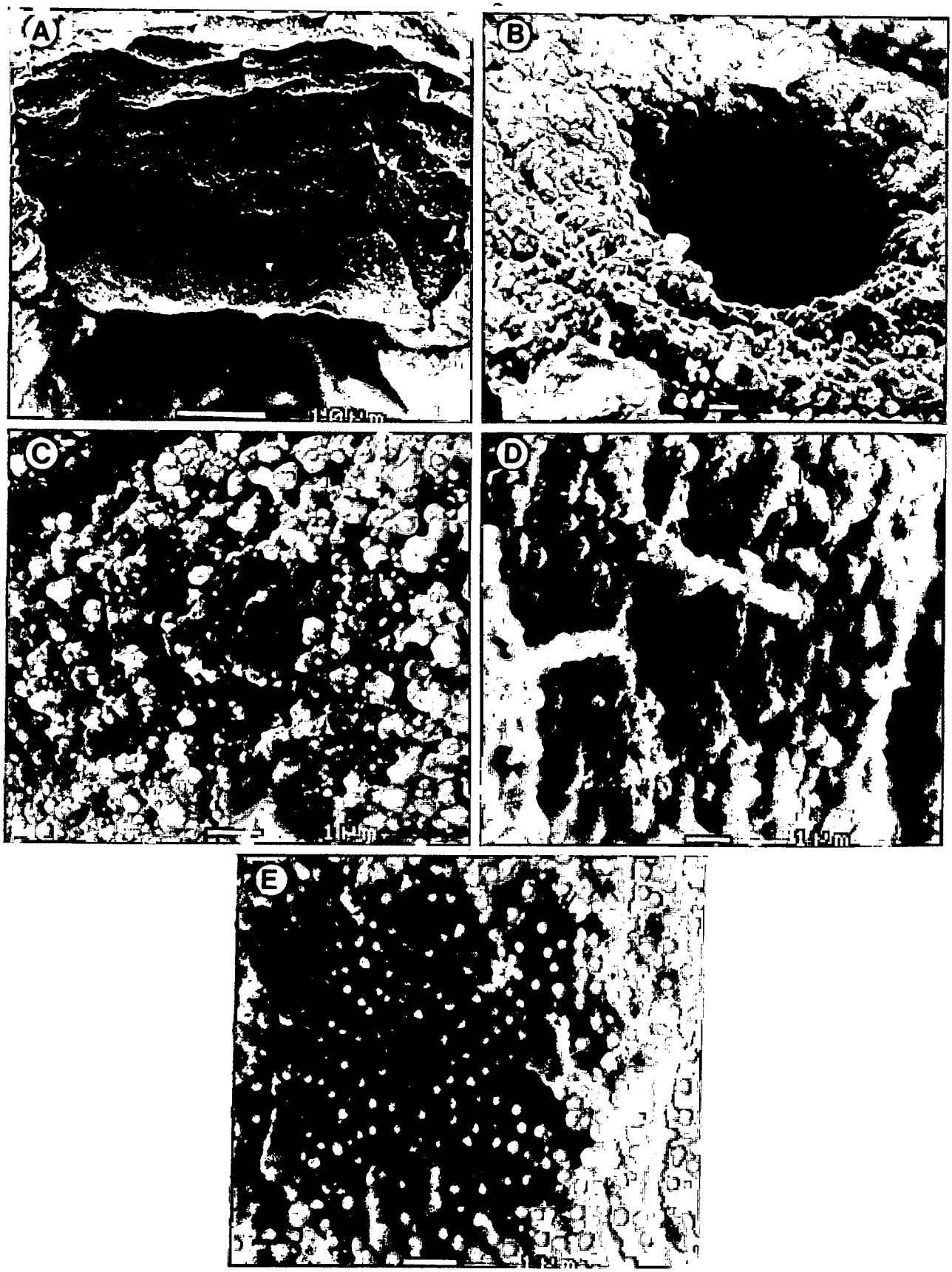
B. Oral sucker

C. Protrusions on body

D. Sensory receptors around oral sucker

E. Tail surface

Scale bar: A- 10μm. B-E- 1μm.


A
B
C
D
E

Sensory receptors are limited to oral sucker where they occur around the sucker (Fig. 6.18D). Receptors are mainly ciliated with ciliums that are long in length. Surface of tail covered by protrusions (Fig. 6.18E).

METACERCARIAE

Cercariae encysted within 2-3 hours after being shed by the snail. Metacercarial stage is found within a cyst (Figs. 6.17C, 6.19C). Fully formed cyst is round when viewed from above and dome shaped in lateral view. The slightly flared base acts as a support for the cyst and attaches it to the substratum, usually plant material or any other surfaces in a water body. Round cysts measure 111-130 (118.5±10.3) in diameter. Metacercaria retains its heavy pigmentation, and therefore cysts look like black spots when they are examined. Cyst is surrounded by thick cyst wall, which is 10-16 (12.7±2.0) in thickness, colourless and transparent. Around this wall another layer occurs which could be some sort of slime layer. Slime layer measures 2.1-5.5 (4.11±1.1) in thickness and could most probably protect the cyst from dehydrating.

REMARKS:

This cercaria is similar to the cercaria found by Porter (1920 and 1938) known as *Cercaria paramphistomi calicophorum.* Presently this cercaria is known as *Calicophoron microbothrium* a parasite of cattle and sheep. Dinnik and Dinnik (1954) described the life cycle of *Paramphistomum microbothrium* now known as *Calicophoron microbothrium*, occurring in cattle from Nairobi and Nakuru in Kenya. The cercariae from the present study conforms to *P. microbothrium* in having more or less the same body size, the same internal structures and also is heavily pigmented. It differs however from *P. microbothrium* in having more prominent oral and ventral suckers and the eyespots also differ in shape. The morphological features of the cercaria from the present study correspond with that of the family Paramphistomidae.

FAMILY PARAMPHISTOMIDAE

The family Paramphistomidae Fischoeder, 1901 is divided into seven subfamilies. These subfamilies include Paramphistomatinae Fischoeder, 1901; Gastrothylacinae Stiles and Goldberger, 1910; Cladorchiinae Fischoeder, 1901; Balanorchiinae

Stunkard, 1917; Zygocotylinae Stunkard, 1916; Diplodiscinae Cohn, 1904 and Gastrodiscinae Stiles and Goldberger, 1910 (Dawes 1968).

The subfamily Paramphistomatinae can be divided into a number of genera namely, *Paramphistomum* Fischoeder, 1901 and *Stephanopharynx* Fischoeder, 1901 (Dawes 1968). A third genera namely *Cotylophoron* Stiles and Goldberger, 1901 can be separated from the other two genera by the presence of a rudimentary sucker around the genital pore. Dawes (1968) regarded this characteristic as too small to erect a third genera and regarded the genera *Paramphistomum* and *Cotylophoron* as synonymous.

According to Yamaguti (1975) and Eduardo (1982) the subfamily Paramphistomatinae can now be divided into five genera. These include the genera *Paramphistomum* (Syn. *Liorchis, Srivastavaia*); *Calicophoron* Näsmark, 1937 (Syn. *Bothriophoron*); *Gigantocotyle* Näsmark, 1937; *Explanatum* Fukui, 1929; as well as *Cotylophoron* Stiles and Goldberger, 1910.

This group of parasites has long since been in existence. The adult trematodes occur mostly in cattle and sheep. The life cycle of this parasite group is relatively simple, with a mammal that serves as the final host. The parasite produces eggs, which leave the animals body via the faeces. Only if these eggs land in water will the life cycle continue and the miracidium breaks from the egg.

The next host is a freshwater mollusc namely *Bulinus globosus* as found in the Okavango Delta and *B. tropicus* in other places like the Free State and North West Province. After the miracidium penetrates the snail further development takes place within the snail, which eventually gives rise to the cercariae. Once cercariae leave the snail it does not penetrate another host but encysts on water plants. The infective stages are the water plants that the animals eat. From here the metacercariae develops into an adult, after which the cycle starts again.

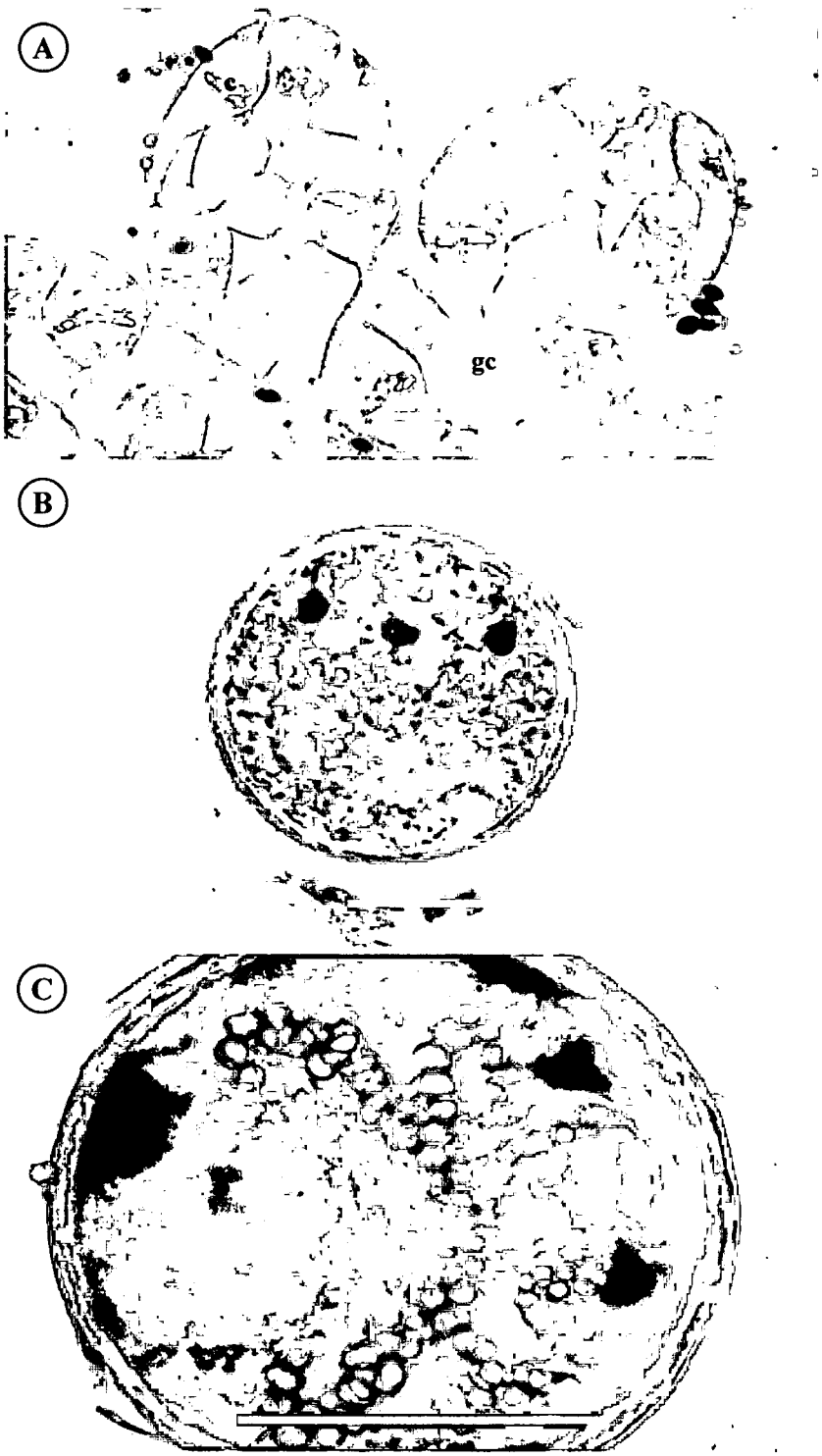


Figure 6.19: Light micrographs of different larval stages of different cercariae found within the different snails. **A.** Daughter sporocyst with cercaria (c) and germ cells (gc). **B.** Metacercarial cyst of the parapleurolophocercous cercaria. **C.** Metacercarial cyst of the amphistome cercaria. Scale bar: 50µm

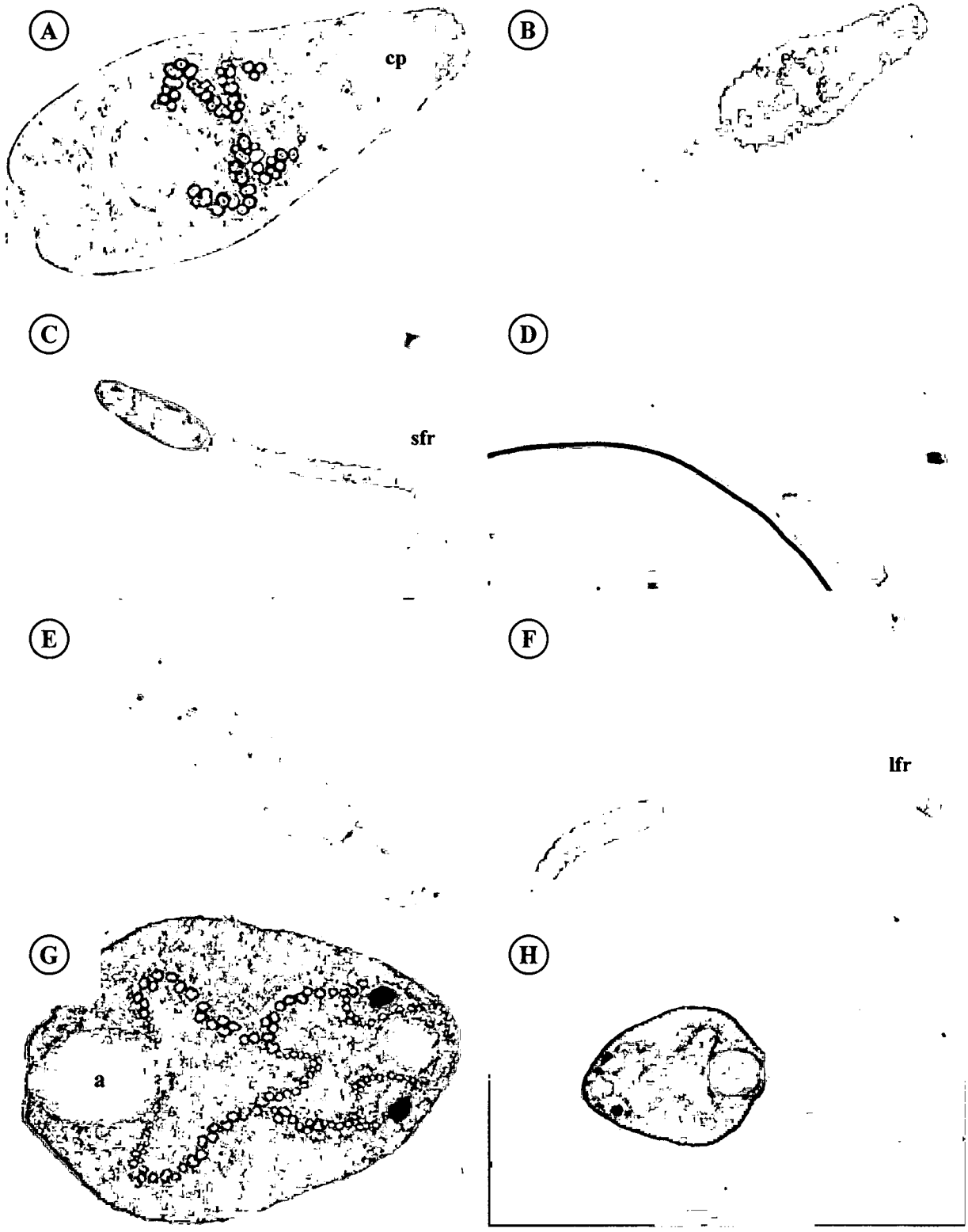
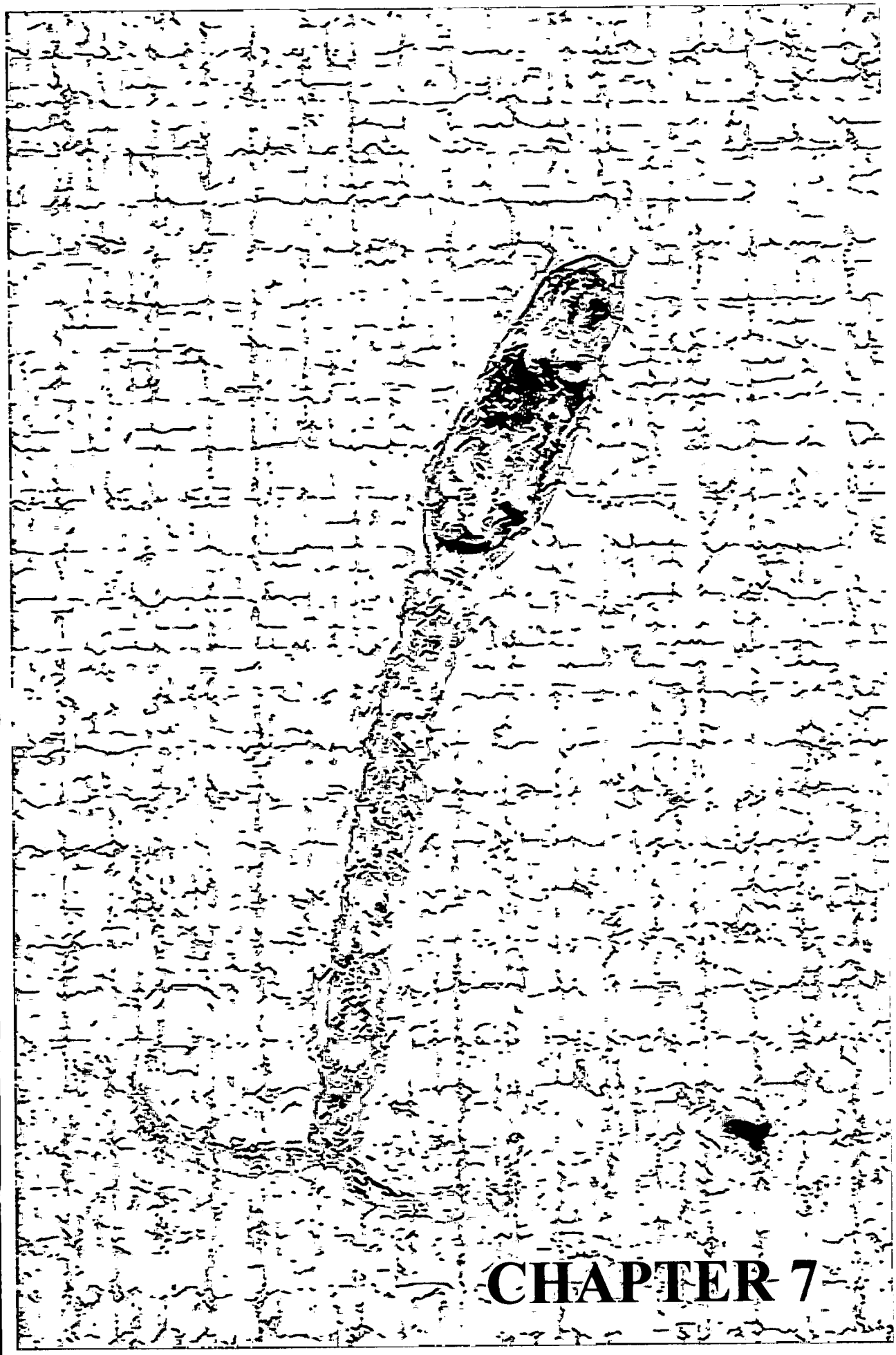


Figure 6.20: Light micrographs of cercariae from different digenean families. **A.** 27-Echinostomatid cercaria body (Echinostomatidae). **B.** Echinostome cercaria showing body and tail. **C.** Schistosome cercaria (Schistosomatidae) showing short furcal rami (sfr). **D.** Schistosome cercaria. **E.** Strigeid body (Strigeidae). **F.** Strigeid cercaria showing long furcal rami (fr) **G.** Amphistome cercaria showing dark pigmented body, acetabulum (a). **H.** Amphistome cercaria showing single tail. Scale bars: 100 μm.



CHAPTER 7

PARASITE HOST ASSOCIATIONS

This chapter will be devoted to provide some information on the occurrence and distribution of freshwater snails in the Okavango Delta collected during a survey in 1999 and in 2000. Furthermore infestation statistics will be provided of snail borne larval trematodes in the Okavango Delta, Botswana.

Data of the snail hosts that were collected and infected with different larval trematodes is represented in Table 7.1 and 7.2.

Of the 20 snail species present in the Delta only seven were collected during this study. Despite considerable amounts of efforts to collect the other species they were not found. This may be attributed to the fact that the right localities may not have been sampled or the snails may not occur in great abundance in the areas that were sampled. Since sampling was done during the winter season, the season may also have played an important part in the collection findings.

Except for two of the snail species, namely *Bellamya capillata* and *Lobogenes michaelis* all of the other snail species were found to be infected, at most, in one or more of the sampling localities. *Bellamya capillata* was collected during both surveys, namely seven specimens in the 1999 survey and fifteen specimens in the 2000 survey, therefore it would be difficult to conclude whether any trematodes were associated with snails because of the very low numbers of specimens that were collected. Ten specimens of *L. michaelis* were collected during the 2000 survey and none were collected during the 1999 survey therefore it would also be meaningless to come to any conclusions about infections and numbers.

Pila occidentalis was collected during both surveys. In 1999, 40 specimens of *P. occidentalis* were collected from six localities but none of these specimens were infected. During the 2000 survey 123 specimens of *P. occidentalis* were collected from three localities. Four of these specimens were infected from one locality. Important to note is that all the infected snails were from the Mohembo Floodplains.

Table 7.1: Summary of the different snail species collected from different localities in the Okavango Delta, Botswana in 1999 and 2000. Infected snails indicated by ✓ and uninfected snails indicated by × .

B.c *- Bellamya capillata,* ***P.o-*** *Pila occidentalis,* ***L.o-*** *Lanistes ovum,* ***L. m*** *- Lobogenes michaelis,* ***C.e-*** *Cleopatra elata,* ***L.n-*** *Lymnaea natalensis,* ***B.p*** *- Biomphalaria pfeifferi* ***B.g-*** *Bulinus globosus*

	1999									2000								
LOCALITY*	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	**TOTAL**	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	**TOTAL**
Mohembo floodplains		×	✓		✓	✓		✓	47		✓	✓					✓	70
Mohembo backwaters		×			×	×		×	18									
Xaro backwaters						×		×	25									
Xaro mainstream lagoon										×				✓	✓	✓		62
Etsatsa mainstream		×			×	×		×	13									
Etsatsa floodplains		×	×		×	×	×	✓	13									
Nxamasere											×							76
Guma channel												×	×		×	×	×	71
Guma lagoon	×		×					✓	10									
Guma floodplains						✓	×	×	69									
Guma backwaters																		
Seronga floodplains						✓		✓	100									
Seronga fisheries camp															×		×	64
Seronga polars camp										×	×	×		×	×	×	×	130
Willies Camp										×				×	✓	✓	×	108
Duba Lagoon			×			×	✓	✓	117									
Kwihom Island		×	×				×	×	49									
Jao Island					×	×	×		6									
Jao channel							×	×	11									
Thaoge Lagoon			✓			✓	✓	×	69									
Upper Thaoge Lagoon					×	✓	×	×	25									
Lechwe Island	×	×	×			×	×	✓	138									
TOTAL	7	40	53	0	62	141	156	258	717	15	123	21	10	79	89	169	75	581
NUMBER INFECTED	0	0	2	0	2	9	3	9	25	0	4	8	0	4	7	5	1	29
PREVALENCE	0	0	2.7	0	3.2	5.6	1.8	3.5	3.6	0	3.2	38.1	0	5	7.8	2.9	1.3	4.9
	B. c	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	**TOTAL**	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	**TOTAL**

* For description of localities see Chapter 3 Materials and Methods

Table 7.2: Summary of the total number of snail species collected and infected with different larval trematodes from various localities within the Okavango Delta, Botswana in 1999 and 2000

B.c *Bellamya capillata,* ***P.o****. Pila occidentalis,* ***L.o.*** *Lanistes ovum,* ***L. m*** *Lobogenes michaelis,* ***C.e*** *Cleopatra elata,* ***L.n*** *Lymnaea natalensis,* ***B.p*** *Biomphalaria pfeifferi,* ***B.g*** *Bulinus globosus*

	1999									2000								
LOCALITY*	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	TOTAL	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	TOTAL
Mohembo floodplains		6(0)	3(1)		19(2)	10(2)		9(1)	47		30(4)	13(8)					27(1)	70
Mohembo backwaters		3(0)			4(0)	2(0)		6(0)	18									
Xaro backwaters						18(0)		7(0)	25									
Xaro mainstream lagoon										5(0)				14(4)	23(6)	20(1)		62
Etsatsa mainstream		1(0)			9(0)	1(0)		2(0)	13									
Etsatsa floodplains		2(0)	3(0)		1(0)	5(0)	1(0)	1(1)	13									
Nxamasere											76(0)							76
Guma channel												5(0)	10(0)		5(0)	43(0)	8(0)	71
Guma lagoon	5(0)		1(0)					4(1)	10									
Guma floodplains						31(2)	6(0)	32(0)	69									
Guma backwaters																		
Seronga floodplains						22(1)		78(2)	100									
Seronga fisheries camp															43(0)		21(0)	64
Seronga polars camp										5(0)	17(0)	3(0)		22(0)	9(0)	63(0)	11(0)	130
Willies Camp										5(0)				43(0)	9(1)	43(4)	8(0)	108
Duba Lagoon			3(0)			24(0)	41(1)	49(3)	117									
Kwihom Island		1(0)	1(0)				41(0)	6(0)	49									
Jao Island					1(0)	1(0)	3(0)		6									
Jao channel							10(0)	1(0)	11									
Thaoge Lagoon			12(1)			6(1)	42(2)	9(0)	69									
Upper Thaoge Lagoon					1(0)	11(3)	8(0)	9(0)	25									
Lechwe Island	2(0)	27(0)	30(0)			2(0)	4(0)	45(1)	138									
TOTAL	7	40	53	0	62	141	156	258	717	15	123	21	10	79	89	169	75	581
NUMBER INFECTED	0	0	2	0	2	9	3	9	25	0	4	8	0	4	7	5	1	29
PREVALENCE	0	0	2.7	0	3.2	5.6	1.8	3.5	3.6	0	3.2	38.1	0	5	7.8	2.9	1.3	4.9
	B. c	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	TOTAL	*B. c*	*P. o*	*L. o*	*L.m*	*C. e*	*L. n*	*B. p*	*B. g*	TOTAL

* For descriptions of localities see Chapter 3 Materials and Methods

#() indicates the number of infected snails

During the 1999 survey 53 specimens of *Lanistes ovum* were collected from seven localities. Two infected specimens were collected from two of the seven localities. The localities where infections occurred were from the Mohembo Floodplains and Thaoge Lagoon. These two types of localities differ considerably from each other. In the 2000 survey a total of 21 specimens of *L. ovum* were found from three localities. Eight of these specimens were infected from a single locality, namely Mohembo floodplains. Although fewer specimens were collected during the 2000 survey more infected snails were found. The role of locality where this high infection was found seems to play an important role.

Cleopatra elata was found during both surveys. A total of 62 *C. elata* specimens were collected from six localities from which two infected specimens were found during the 1999 survey. The infected snails were once again found from Mohembo Floodplains. In the 2000 survey a total of 79 *C. elata* specimens were collected from three localities and four infected snails were found in a single locality, namely Xaro Mainstream Lagoon. This locality is near areas where some wildlife is present, which obviously play role in parasite transmission in the area.

A total of 141 specimens of *Lymnaea natalensis* were collected from 12 localities during the 1999 survey. Nine infected snails were found from five localities, while in the 2000 survey 89 *L. natalensis* specimens were collected from five localities where seven infected snails were found from two of these localities. The localities where the infected specimens from both surveys were found are mostly from shallow floodplains and lagoons.

Biomphalaria pfeifferi was found during both surveys. A total of 156 *B. pfeifferi* specimens were collected from nine localities during the 1999 survey. Three infected snails were found from two of the nine localities. The two localities being Duba Lagoon and Thaoge Lagoon. During the 2000 survey, a total of 169 *B. pfeifferi* specimens were collected from four localities and five infected snails were found from two of the localities, namely Xaro Mainstream Lagoon and Willies Camp. These three localities are very busy in terms of human and animal activities meaning that they are always in contact with humans and animals either by humans using the river for transport or for fishing and animals coming to drink water from the river.

During the 1999 survey on the other hand some of the localities were not situated near human settlements thereby resulting in higher infections during the 2000 survey.

During the 1999 survey, 258 specimens of *Bulinus globosus* were collected from 14 localities. Of these, nine specimens were found to be infected. In the 2000 survey a total of 75 *B. globosus* snails were collected from five localities and a single infected specimen was found. Infections were therefore higher in the 1999 survey because more specimens were collected and therefore more infected snails were found. The specimens from both surveys were collected only from floodplain habitats. Large numbers of animals and domestic livestock were also present in localities from the 1999 survey which could have resulted in the presence of more infected snails.

During both surveys a wide variety of localities were sampled, but Mohembo Floodplain was the only locality that was sampled during both surveys. Interesting to note is that *Lymnaea natalensis* and *Cleopatra elata* were not collected in the 2000 survey from this particular locality. This may be due to the higher floods during 2000, where the water levels were higher thereby making the pools where collections usually took place inaccessible.

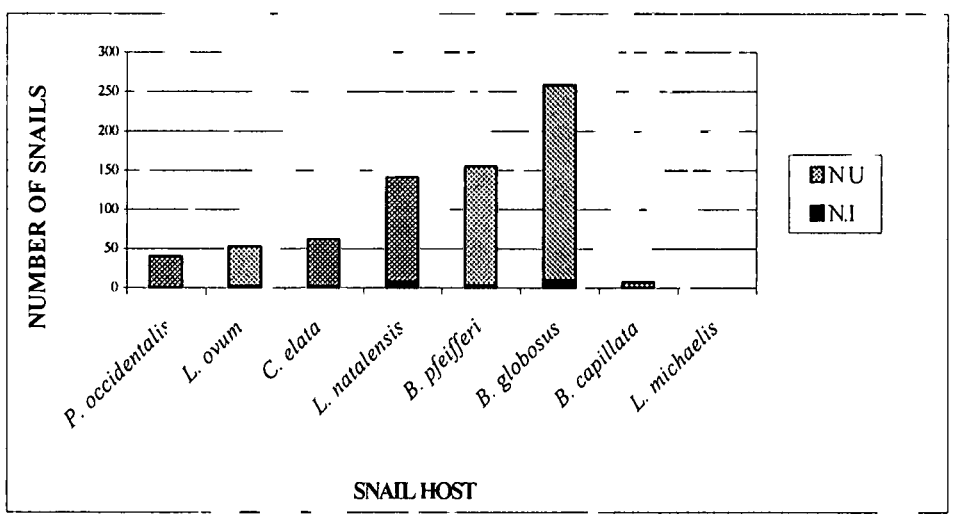


Figure 7.1: Histogram illustrating the total number of each snail species collected, the number of specimens infected and uninfected for the year 1999 from the Okavango Delta, Botswana (N.I= Number of snails infected, N.U= Number uninfected).

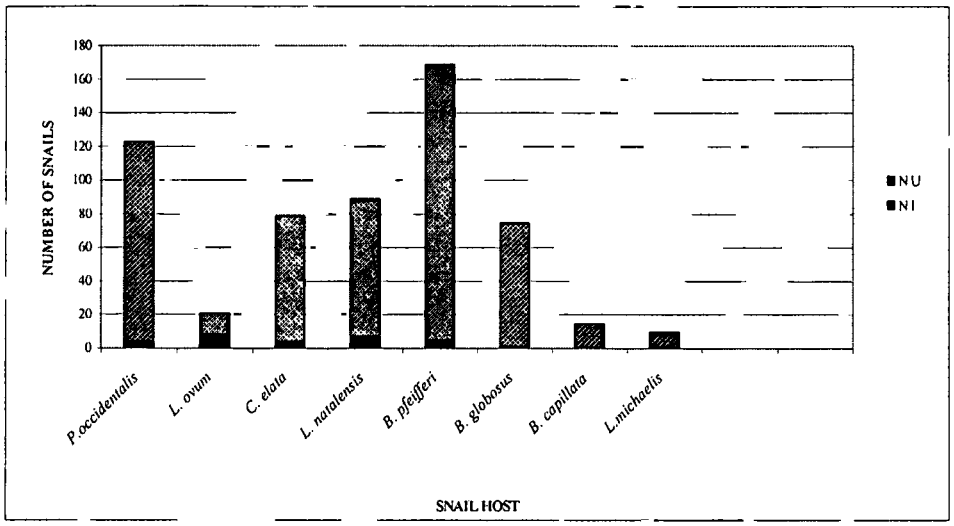


Figure 7.2: Histogram illustrating the total number of each snail species collected, the number of specimens infected and uninfected for the year 2000 from the Okavango Delta, Botswana (N.I= Number of snails infected, N.U= Number uninfected).

If sampling of specimens during both surveys was random enough and can be assumed as correct, the following conclusion may be made:

From Table 7.1 and Figures 7.1 and 7.2 three snails seem to stand out namely *Bulinus globosus*, *Biomphalaria pfeifferi* and *Lymnaea natalensis*. A total of 333 *Bulinus globosus* specimens were collected from 19 localities during both surveys, 325 *B. pfeifferi* specimens were collected from 13 localities during both surveys and 230 *L. natalensis* specimens were collected from 17 of the localities. It therefore appears that *B. globosus* is the dominant species and the most widespread. Although fewer *L. natalensis* snails were collected, it has a wider distribution than *B. pfeifferi* since it was collected from 17 localities compared to *B. pfeifferi*, which was only collected from 13 localities.

At Lechwe Island the most specimens namely 138 individuals were found, followed by Duba Lagoon where 117 specimens were found in the 1999 survey, while 130 specimens were collected from Seronga Polars Camp followed by Willies Camp where 108 specimens were collected during the 2000 survey. Six different snail species were collected from Lechwe Island and Etsatsa Floodplains in the 1999

survey, while in the 2000 survey seven different snail species were collected from Seronga Polars Camp. It would seem that Lechwe Island can be regarded as a favourable snail habitat since the most specimens and species were collected here during 1999. This habitat might have an abundance of nutrients, and slow flowing water, which are suitable for snail life. The same applies to Seronga Polars Camp for the 2000 survey.

In Mohembo floodplains the five different snail specimens that were collected during both the 1999 and 2000 survey were infected. Once again the importance of this locality can be seen and will be explained later in the Chapter.

No infected snails were found in the 1999 survey from Etsatsa Mainstream, Kwihom Island, Jao Island and Jao channel. The reason for this may be because fewer snails were collected from these localities. In the 2000 surveys no infected snails were found from Nxamasere, Guma Channel and Seronga Fisheries Camp. Here on the other hand large numbers of snails were collected but no infected snails were found so there might be another reason for uninfected snails occurring here. The reason could be that conditions are not favourable in these areas for infections to take place, in other words intermediate and final host may not be present in these areas.

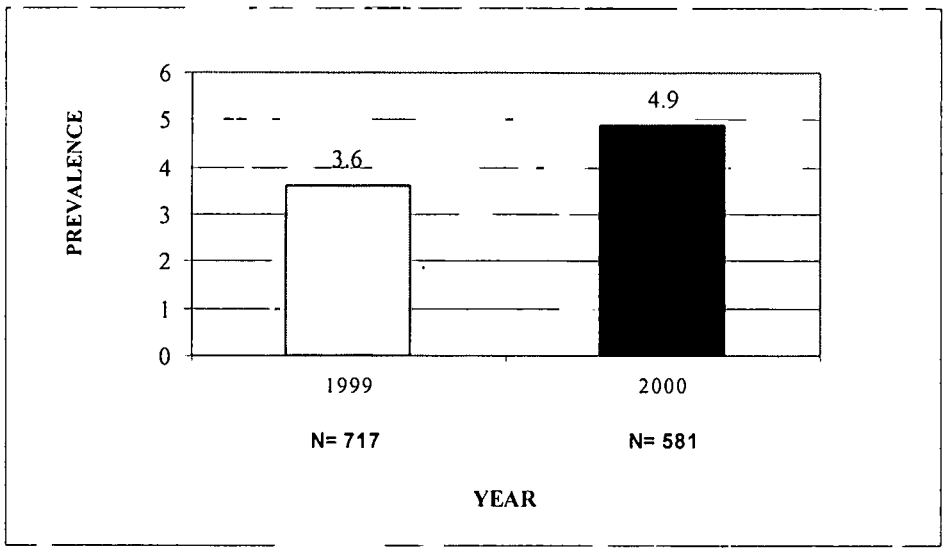


Figure 7.3: Histogram showing the prevalence of larval trematode infections in freshwater snails in 1999 and 2000 from the Okavango Delta, Botswana (N=Total number of snails collected).

During the June-July 1999 field trip to Okavango Delta, a total of 717 snails were collected with an infection prevalence of 3.6%, while in June 2000, a total of 581 snails were collected with an infection prevalence of 4.9% (Fig. 7.3). The reason for the higher prevalence in 2000 may be due to the fact that collections were concentrated in a specific region compared to 1999 where more sites were sampled over a greater range and less infected snails were collected. In 1999 collections took place from Mohembo Floodplains in the north to Duba Lagoon in the south. Some of the localities sampled during 1999 were also isolated from any human interference so chances of finding infections in these areas were considerably low.

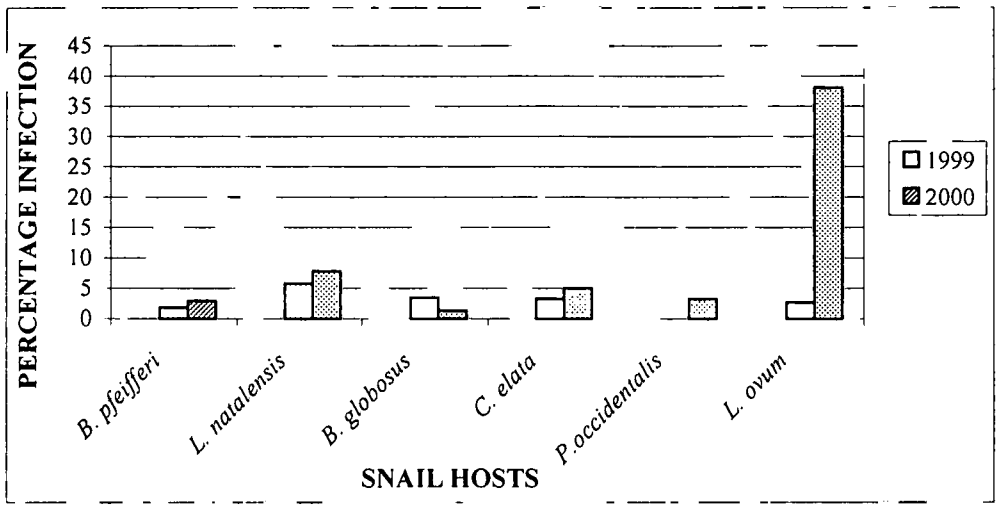


Figure 7.4: Histogram illustrating the percentage infection of larval trematodes in the different snail hosts, for the 1999 and 2000 surveys from the Okavango Delta, Botswana.

From Fig. 7.4 it can be seen that the infection prevalence of the different snail specimens ranges from between 2% and 38.1%. The prevalence of the various snail specimens did not differ significantly during both surveys and this is what one would expect to find in any natural environment. But there is an exception though, namely that of *Lanistes ovum* where 38% of the specimens were infected from a single locality, giving an indication that something may be happening in the locality where this high infection was found. It seems difficult to explain why this snail would be heavily infected. A reason for this could be that these snails are known to grow very large over a number of years and get very old. In addition to this, the snail is known to have an operculum, which enables it to survive harsh conditions, and so it can therefore withstand repeated infections over a few years. An explanation might be that since the cercaria shed are small, they might not stress the snail to such an extent that haemorrhaging of tissue takes place resulting in the death of the snail.

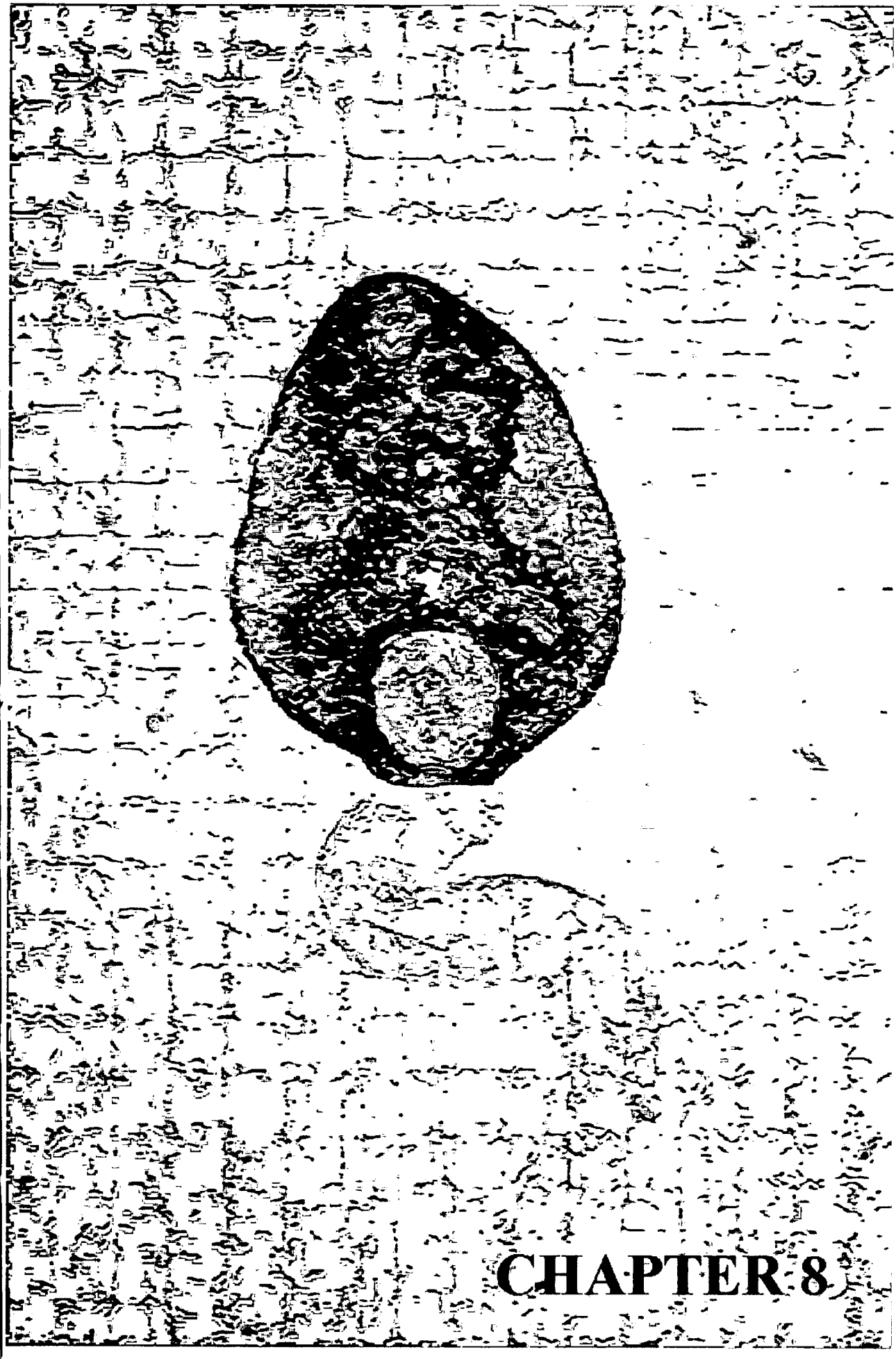
Cleopatra elata and *Lymnaea natalensis* had a higher infection prevalence during the 2000 survey because more infected snails were found. Except for *Bulinus globosus* all the other snail species had a higher percentage infection of larval trematodes in the 2000 survey. In this instance *B. globosus* had a prevalence of 3.5% during the 1999 survey and 1.3% during the 2000 survey. This may be because one snail was infected from one locality during the 2000 survey while in the 1999 survey ten infected snails

were found from five localities. It may also be because it was early in the season, water was flowing into the system and water level was still high in the floodplains

Finally it may be said that the most favourable locality for infections to be found was at Mohembo Floodplains, where all snail species that were collected during both surveys were infected with cercariae. This can easily be explained in that this area is where the most human contact and interference occur. At Mohembo there is a ferry, which constantly transports humans and occasionally their livestock across the river. Cattle, sheep, goats and donkeys freely roam these floodplains and are most probably the final hosts for many parasite species. The more final hosts that are present in these areas, increases the chances of finding a higher number of infected snails.

It may also be concluded that floodplains and lagoons seem to be the most favourable habitats for finding cercarial infections in this study. These habitats are known to have fish and other animals in them, or in close vicinity to them, which could serve as second and final hosts for these parasites. These habitats also seem to be in areas where humans are in close proximity. Floodplains are known to be favourable habitats for snails (Porter 1938). Lagoons may be favourable since there aren't any strong currents within them so snails can't be swept away and they also have substrates to which snails can attach.

Mention can also be made that although more snails were collected during the 1999 field trip, there was a lower percentage infection than in the 2000 survey where fewer snails were collected but a higher percentage infection was found. It may therefore be assumed that chances of infection with cercariae may be more likely in the northern reaches of the Delta where more human settlements are present and interference with the system occurs more regularly.



CHAPTER 8

LIFE CYCLE POSSIBILITIES

The eight different cercarial species, as classified under their proposed families give some indication of the diversity of digeneans that may be found in the Okavango Delta (Table 8.1). The species that were found are probably just the tip of the iceberg. The Okavango Delta is world renown for its bird population especially piscivorous birds, which feed mainly on fish. Due to the Okavango Delta being such a huge wetland and having so many birds, one would expect to find quite a number of snails to be infected. But the season may have greatly influenced the results of this study.

In order to positively identify the cercaria from the present study, elaboration of the life cycles of each of these specific digeneans would need to be done under laboratory conditions. Since there was a time limitation to the study this was not possible. This does not mean that speculations on the various life cycles can not be made since there is a great amount of published work on trematode life cycles.

Brief speculations on the life cycles of each of the cercariae from the present study is given below:

Pila occidentalis shed a vivax cercaria belonging to the family Cyathocotylidae. This family is known to parasitize birds, reptiles and mammals (Dawes 1968). The following life cycle is suggested for the cercariae from the present study: The snail, *P. occidentalis* serves as the first intermediate host. Cercariae shed by *P. occidentalis* will most likely penetrate fish, probably a cichlid, e.g. *Sargochromis carlottae,* which is known to be found in floodplain habitats (Skelton 1993). The cercariae may then encyst to form metacercariae in the muscles of the fish. Yamaguti (1975) found *Cyathocotyle orientalis* encysted in the muscle of *Pseudorasbora parva.* The fish might then be eaten by a bird, most likely a Goliath Heron, *Ardea goliath* or a darter *Anhinga melanogaster*, or a little egret, *Egretta garzetta* and develop into an adult in the intestine of the bird. This cercaria might only have been found from Mohembo since this family appears to be host specific for this snail species.

Table 8.1: Summary of the larval trematode families shed by the different snail host species from different localities in the Okavango Delta Botswana in both surveys

❶ *Pila occidentalis* ❷ *Lanistes ovum* ❸ *Cleopatra elata* ❹ *Lymnaea natalensis* ❺ *Bulinus globosus* ❻ *Biomphalaria pfeifferi*

	DIGENEAN FAMILIES						
LOCALITY*	**Cyathocotylidae**	**Plagiorchiidae**	**Heterophyidae**	**Echinostomidae**	**Diplostomidae**	**Schistosomatidae**	**Paramphistomidae**
Mohembo floodplains	❶	❷	❸	❹			❺
Mohembo backwaters							
Xaro backwaters							
Xaro mainstream lagoon			❸		❹	❻	
Etsatsa mainstream							
Etsatsa floodplains							❺
Nxamasere							
Guma channel							
Guma lagoon							❺
Guma floodplains				❹			
Guma backwaters							
Seronga floodplains				❹			❺
Seronga fisheries camp							
Seronga polars camp							
Willies Camp				❹	❻		
Duba Lagoon						❻	
Kwihom Island							
Jao Island							
Jao channel							
Thaoge Lagoon		❷		❹		❻	
Upper Thaoge Lagoon				❹			❺
Lechwe Island							

* For descriptions of localities see Chapter 3 Materials and Methods

Lanistes ovum shed a Xiphidio cercariae belonging to the family Plagiorchiidae. Some of these parasites are known to have more complex life cycles compared to the other families. The cercariae could most likely follow one of two different life cycles in the Okavango Delta. The following life cycle/s are suggested: Cercaria shed by *L. ovum* will most likely find and penetrate a water insect larva like a dragon fly, where it will encyst in or on the body. The dragon fly nymph develops into an adult still containing metacercarial cysts. King (1991) found encysted metacercariae in water insects. An amphibian might eat this insect, probably a frog where it will develop into an adult in the anterior end of the rectum as suggested by King (1991) as well as Smyth (1994). The final host might also be a bird, for example a pied king fisher, *Ceryle rudis* or cattle egret, *Bubulcus ibis* eating infected insects.

The other suggested life cycle may be as follows: Cercariae shed by *L. ovum* may repenetrate another snail of the same species and form metacercarial cysts in the body of the snail. King (1991) found metacercarial cysts of a Xiphidio cercaria within snails that he was studying. The snail might be eaten by a water bird, e.g. a saddle-billed stork (*Ephippiorhynchus senegalensis*), which are known to occur in floodplain type of habitats and feed on molluscs, especially *Pila* spp. and *L. ovum* (MacLean 1993).

Cleopatra elata shed a parapleurolophocercous cercaria belonging to the Family Heterophyidae. This cercaria will most likely have the following life cycle: Cercariae will find and penetrate a suitable second intermediate host, most likely a tilapia, for example *Tilapia ruwetti*, since this fish occurs in these types of habitats (Skelton 1993). Cercariae will probably encyst in the muscles of the fish as was found by Stunkard (1930). The fish will most likely be eaten by a bird, e.g. a Blackheaded heron, *Ardea melanocephala* whose diet mainly consists of fish (MacLean 1993). The trematode will develop into an adult in the intestine of the bird. Dawes (1968) reported *Cryptocotyle lingua* from the intestine of a night heron. Cercariae resulting in parasites of this family were found from two localities. On some occasions humans may be infected. If this happens the following life cycle could be followed: Cercariae will find and penetrate fish and encyst in the fish under the scales. Martin and Kuntz (1955) found *Heterophyes* spp. encysted in *Gambusia affinis*. Man might then eat this fish raw, where the adult will mature in the intestine.

Lymnaea natalensis shed two types of cercariae from different localities (Table 8.1). The first cercaria, namely a 27-echinostomatid cercaria is known to be a parasite of birds and mammals (Smyth 1994). The following life cycle may be suggested: Cercariae shed by *L. natalensis* may find and penetrate a second intermediate host which will most likely be a tadpole of a *Xenopus* spp. Metacercaria will develop just under the skin of the tadpole. A 27-echinostomatid cercaria, *Petasiger variospinosus* shed from *B. tropicus* was described by King and Van As (2000) and followed a similar life cycle. The tadpole might then be eaten by a water bird, most likely a reed cormorant, *Phalacrocorax africanus* (King & Van As 2000). The family Echinostomatidae seems to be prominent in a number of localities. The reason for this may be because the 2nd intermediate as well as the final hosts may be more widely spread throughout the delta and the adult parasite may not be entirely host specific.

The second cercaria shed by *Lymnaea natalensis* from Xaro Mainstream Lagoon belongs to the family Diplostomidae. Cercariae shed will most likely follow the life cycle as suggested by Tinsley and Sweeting (1974) as well as by King and Van As (1997). Cercaria shed by the snail host will penetrate a tadpole, most probably a *Xenopus* spp. and form metacercarial cysts under the skin. The tadpole develops into an adult frog with encysted metacercaria found in the pericardial cavity (King & Van As 1997). The frog will most likely be eaten by a bird, probably a Grey heron, *Ardea cinerea.* The reason for the diplostomatid cercaria only being found in one locality may be because this cercaria may follow a very specific life cycle. The intermediate and final hosts may only be found in this particular locality within the Okavango delta.

Biomphalaria pfeifferi also shed two types of cercariae from different localities. The first one, a schistosome cercaria will find and penetrate the final host, which will most likely be a mammal, e.g. hippopotamus. The reason for this suggestion is because cercariae were shed between 17:00 and 18:00 and in the early hours of the morning. During the course of this study, fresh hippo faeces were scanned for the presence of schistosome eggs. Four schistosome eggs were found from the faeces of the hippo. These eggs measured 199µm x 51µm and 14µm width 40µm anterior to spine. This egg closely resembles the schistosome egg found by Pitchford (1974) from a waterbuck occurring in Kazangula ranch, Zimbabwe. He suggested that it could be

the egg of *Schistosoma bovis* or it could be a hybrid between *Schistosoma mattheei* and *Schistosoma leiperi*. This egg, however, differs significantly in size and shape from the eggs of the two schistosome species previously found in hippos namely *Schistosoma hippopotami* and *Schistosoma edwardiense*. It therefore seems that another definitive host must be used, which could be a hippo. The reason for the schistosome cercariae only being shed from the three localities may be because the final hosts may be more abundant and active in these areas.

The second cercaria belongs to the Family Diplostomidae and the following life cycle is suggested. Cercaria will find and penetrate a second intermediate host, most likely a cichlid, for example *Serranochromis angusticeps*. The metacercariae will encyst under the scales of the fish as was found by Paperna (1997). The fish will probably be eaten by a pied kingfisher, *Ceryle rudis* as was found by Preble & Harwood 1944 or it may be eaten by a fish eagle, *Haliaeetus vocifer*.

Bulinus globosus shed an amphistome cercaria belonging to the family Paramphistomidae. This cercaria may have the following life cycle: Cercaria shed from *B. globosus* will encyst on plants forming metacercarial cysts as was found by Grobbelaar (1922). Plants will be eaten by sheep, cattle or goats. This specific cercariae was shed from five localities in the delta, where livestock was in close vicinity. The adult parasite is most probably known as *Calicophoron microbothrium*.

These parasites are found associated with hosts that are in constant contact with water or partly in contact with water for most of their lifespan. Without the water medium these digenean parasites might not be able to survive.

In this type of study there are always limitations. In this case there was a time and distance limitation, which meant that the data that was collected had to be used conservatively and carefully since once material that was collected was utilised there was no going back for more. It was not possible to sample during the whole year since other obligations had to be met. The ideal situation of course would be to sample throughout the year to obtain a wider and overall picture of what is happening in the system.

This study has therefore laid the foundation for future studies where the life cycle of each different cercariae will be investigated and perhaps even new cercariae may be found.

One of the few places where human and animal bilharzia infection has recently spread in southern Africa is the seasonal part of the Okavango delta Botswana (Appleton 1996). There have been reports by Andersen, Magnussen, Wouters, Berczy, Friis and Ali (1984) of human schistosomiasis occurring in Ngamiland, Botswana. The MMWR weekly also received reports of 12 travellers returning from Botswana who were infected with two different schistosome species namely *Schistosoma mansoni* and *Schistosoma rodhaini* after their three week stay in Botswana.

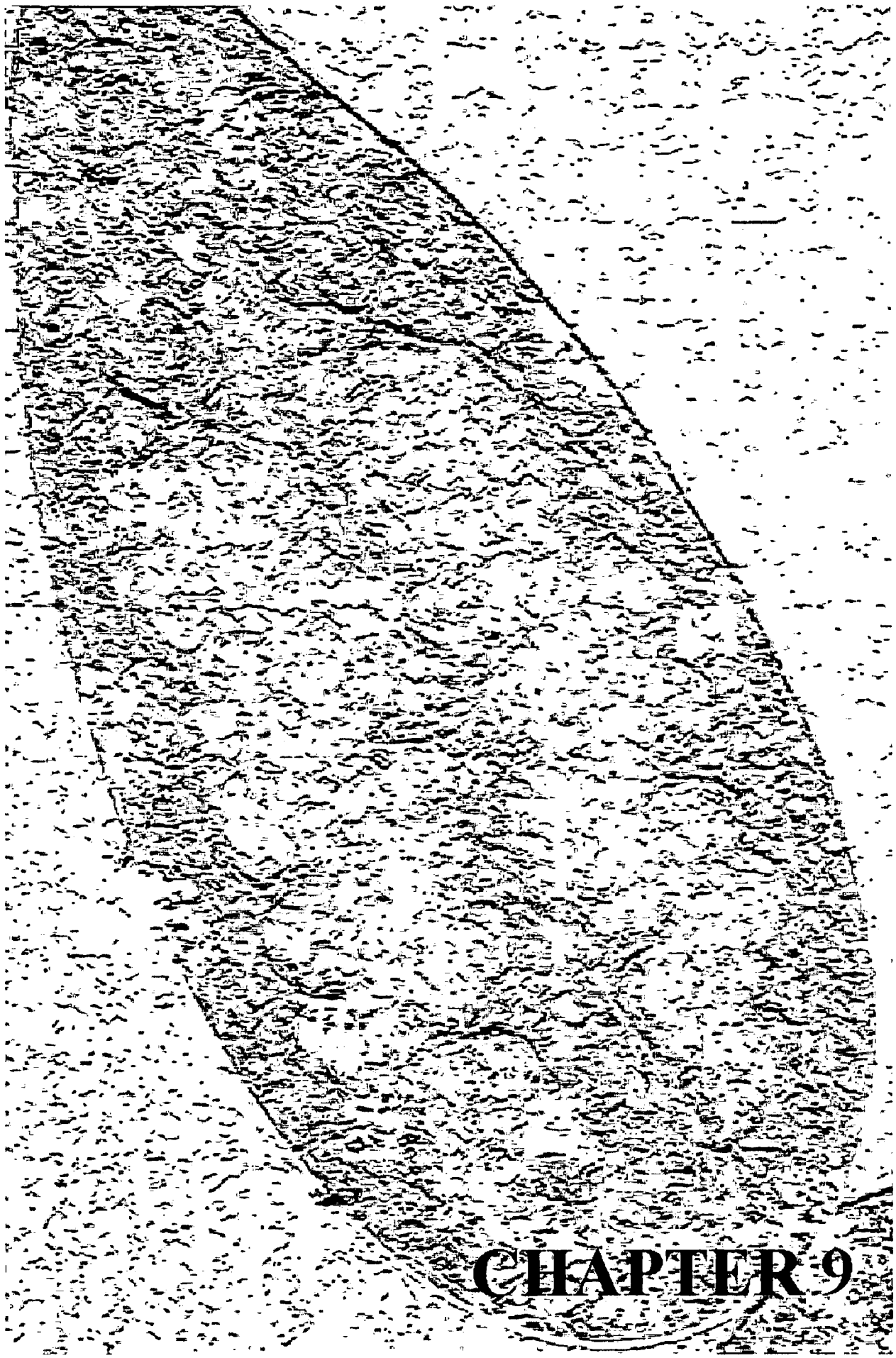
It has been suggested that the dramatic rise in *S. mansoni* infection taking place in the delta from the 1960's is because of ecological changes (Appleton 1996). These changes could include the shooting out of crocodiles, thereby making the water more accessible to people, or the shooting of lechwe, a once plentiful water-loving antelope. Lechwe are said to harbour other species of *Schistosoma* which may also have given immunity to people living there and thereby protecting them from infection by *S. mansoni* (Appleton 1996).

Both the intermediate hosts for schistosomiasis namely *Bulinus globosus* and *Biomphalaria pfeifferi* are present in the delta, so chances of finding human bilharzia is likely. In our studies no snails were found infected with larval trematodes which could result in human schistosomiasis. We sampled over a great range and if infections were present we were sure to have found them. But of course we have to bear in mind that there might have been some isolated pools that we overlooked. The season may also have played an important role in the results of this study. During both surveys snails were collected during the winter months. It is suspected that if collections took place during the summer months a whole new picture may be seen.

In the northern reaches of the delta, as already mentioned *B. pfeifferi* is the most dominant snail species. There is therefore a great risk of infection occurring in these areas since the primitive villages have no proper ablution facilities and all the system needs is for one person to be infected.

This study not only contributes to the identity of a number of larval trematodes that are found in the Okavango Delta, but it will eventually lead to the identification of a number of different trematode life cycles within the Okavango Delta System.

Schistosomiasis is a major threat hanging over the Okavango Delta System. With the increase of tourists visiting the delta, the potential of a major catastrophe increases. Studies like this contribute greatly to making people aware of not only the threat of human related diseases but also the unique and interesting trematode life cycles associated with wild animals in a pristine ecosystem.



CHAPTER 9

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ABSTRACT

The Okavango Delta, situated in northwestern Botswana is one of the worlds largest inland delta systems formed by the Okavango River, flowing in a southeasterly direction from Angola. The snail fauna comprises 20 species occurring in the Okavango Delta and surrounding areas. The aim of this project was to determine what types of cercaria were found infecting freshwater snails in the system as well as to determine whether human schistosomiasis was present in the system. Snails are economically important because they serve as the intermediate hosts for a number of parasitic diseases such as schistosomiasis in humans and animals and paramphistomiasis in cattle. During two consecutive field trips to the Okavango Delta in 1999 and 2000 freshwater snails were collected from various localities within the delta. A total of eight different cercariae were shed from six different freshwater snail species. Cercariae were described and placed into their respective families: *Pila occidentalis* shed a vivax cercaria belonging to the family Cyathocotylidae which are common parasites of birds, reptiles and mammals. *Lanistes ovum* shed a xiphidio cercariae belonging to the family Plagiorchiidae and are known to parasitize amphibians and birds. *Cleopatra elata* shed a parapleurolophocercous cercaria belonging to the family Heterophyidae which parasitize fish. *Lymnaea natalensis* shed two types of cercaria from different localities within the delta, the first type being a strigeid cercaria belonging to the family Diplostomidae and parasitising birds. The second type was a 27-echinostomatid cercaria belonging to the family Echinostomatidae and are known to be parasites of birds. *Biomphalaria pfeifferi* also shed two types of cercariae, a schistosome cercaria belonging to the family Schistosomatidae known to parasitise mammals and another strigeid cercaria belonging to the family Diplostomidae. *Bulinus globosus* shed a dark bodied amphistome cercaria belonging to the Family Paramphistomidae and are known to be parasites of livestock. The prevalence of infection was higher in the 2000 survey than in the 1999 survey since the localities that were sampled in 2000 were closer to human settlements. No snails were found to be infected with larval trematodes, which could result in human schistosomiasis. This study of snail borne larval trematodes provided insight into the different kinds of larval trematodes that are present in this unique system and has laid the foundation for further research of the different trematode life cycles occurring in the Okavango Delta.

OPSOMMING

Die Okavango Delta is een van die wêreld se grootste binnelandse deltas. Die Okavango Rivier het sy oorsprong in die hooglande van Angola en vloei in 'n suid oostelike rigting na Botswana. Die varswaterslak fauna van die Okavango Delta en omgewing bestaan uit 20 spesies. Die doel van hierdie projek wat om vas te stel watter tipe serkarieë infekteer varswaterslakke en of menslike schistosomiasis in hierdie stelsel teenwoordig is. Slakke is van ekonomiese belang aangesien dit as tussengasheer vir 'n verskeidenheid van parasitiese siektes dien, bv. schistosomiasis by mens en dier en paramphistomiasis by beeste. Tydens twee geleenthede, in 1999 en 2000, is veldwerk die Okavango Delta uitgevoer waar varswaterslakke by verskillende lokaliteite versamel is. 'n Totaal van agt verskillende serkarieë is deur ses verskillende slak spesies afgeskei. Serkarieë is beskryf en tot familie vlak geidentifiseer: *Pila occidentalis* het 'n vivax serkarie afgeskei wat aan die familie Cyathocotylidae behoort en wat 'n algemene parasiet by voëls, reptiele en soogdiere is. *Lanistes ovum* het 'n xiphidio serkarie afgeskei wat aan die familie Plagiorchiidae behoort en daarvoor bekend is dat dit amfibieë en voëls parasiteer. *Cleopatra elata* het 'n serkarie afgeskei wat aan die familie Heterophyidae behoort en waarskynlik visse parasiteer. *Lymnaea natalensis* het twee tipes serkarieë afgeskei, die eerste hiervan behoort aan die familie Diplostomidae en parasiteer voëls. Die tweede, ' n 27-stekel serkarie behoort aan die familie Echistomatidae en parasiteer ook voëls. *Biomphalaria pfeifferi* het ook twee tipes serkarieë afgeskei, een behoortende aan die familie Schistosomatidae parasiteer waarskynlik soogdiere, die ander serkarie van die familie Diplostomidae parasiteer voëls. *Bulinus globosus* het 'n donker gekleurde serkarie van die familie Paramphistomidae afgeskei. Hierdie parasiet is bekend daarvoor dat dit plaasdiere infekteer. Die persentasie besmetting van slakke tydens die 2000 opname was hoër as gedurende die 1999 opname. Geén slakke is aangetref wat besmet was met larwale trematode wat die mens kan infekteer nie. Hierdie studie het die grondslag vir verdere navorsing oor lewenssiklusse van parasitiese platwurms in die Okavango Delta gelê.

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