Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 41 (1984)
Heft: 2

Artikel: An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance

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DOI: https://doi.org/10.5169/seals-313293

Acta Tropica *41*, 181–202 (1984)

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An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance

F. Frandsen, N. Ø. Christensen

I. Introduction

This introductory guide to the identification of cercariae from African freshwater snails contains identification keys, introductions to principles and methods in cercarial identification, a description of general cercarial morphology to the extend enabling the use of the keys included, and a description of the morphological and biological characteristics of each of the major cercarial types. By the aid of the keys and the other information included most cercariae known from African freshwater snails may be identified to the major type level and as far as the furcocercous cercarial type is concerned to the sub-type level. Special attention is paid to the identification of cercariae of trematode species being of medical and veterinary importance.

The keys in this guide are in contrast to the keys in existing guides and manuals based on the use of easily recognizable morphological characteristics, and they should therefore be of use for persons without any specialized training in cercarial identification. However, to reach the goal of simplicity some minor simplifications of existing systems for grouping cercariae have been made, and a few minor and not commonly encountered cercarial types belonging to trematode species being of neither medical nor veterinary importance have been deleted. On this background and in the light of the relatively few studies conducted in Africa the present guide may not be considered as being complete.

Editorial note: This article may seem to be somewhat misplaced in a scientific journal. We decided to publish it because to our knowledge there is no simple and practical guide for the identification of cercariae available, although there is a demand for one, especially from people working far from specialized libraries.

A. Degrémont

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Should more specific information be required the reader is referred to the more comprehensive and more complex keys and manuals available (see list of additional reading). Besides, the Danish Bilharziasis Laboratory in its capacity as WHO Collaborating Centre for Applied Malacology wellcomes any opportunity to assist in the identification of cercariae from freshwater snails from any part of the world.

We would appeal to readers to send us comments and criticisms, so that we could take account of them in the preparation of any further edition of this guide.

II. Principles in identification and naming of cercariae

The range of variation in cercarial morphology is considerable, and a classification system has on this background been created by Lühe (1909) for grouping cercariae into various types. Gross morphological characteristics like number and position of body suckers, shape and relative dimensions of the cercarial tail and the presence or absence of various specialized surface structures like stylet, spiny collar, etc., provide in this system the background for grouping cercariae into various major types, and each major cercarial type may be divided into sub-types on the basis of minor differences in the gross morphology[1].

Identification of cercarial specimens to the family level, and occasionally also to the genus level, is generally possible using morphological criteria alone, but the minor and not easily recognizable differences in cercarial morphology between different genera of trematodes and between different species within each trematode genus make it commonly necessary for the carrying out of a proper cercarial identification on the genus and species level to supplement observations on the morphology of the specimen with observations on its biology. Such biological characteristics of value for the identification include species of cercariae-producing snail, cercarial swimming behaviour and resting position, and further cercarial developmental scheme (encystation on an external substrate, encystation in a second intermediate host, direct penetration (infection) of the definitive host). The combination of observations on morphological and biological characteristics of a cercarial specimen may, however, not always enable a proper identification, especially on the species level, and it may therefore in the final end be necessary to conduct the identification on the basis of adult worm and/or egg morphology following infection of a proper definitive host.

The number of cercariae described and named on a morphological basis alone is very large and it is generally accepted to refer to such cercariae de-

[1] The system for grouping cercariae is purely descriptive with a rather limited systematic/taxonomic value, e.g. the same cercarial type may be found in more trematode families.

scribed in isolation from the rest of the life cycle as species using the genus name *Cercaria*. The term Cercaria may thus be regarded as a genetic name for all described "species" of cercariae, but the naming is changed to that of the adult trematode when the lifecycle, to which the cercaria belongs, becomes known, i.e. the cercaria originally named *Cercaria echinata* is now named *Cercaria Echinostoma revolutum*.

The system used in the present guide for grouping cercariae into major types is with small modifications that of Lühe (1909), and the system used for grouping the furcocercous cercarial type into sub-types is that of Miller (1926).

III. Harvest, preservation and staining of cercariae

Harvest of cercariae

Harvest of cercariae takes place by crushing the snail or by natural emergence. Harvest by natural emergence is, however, to be recommended because crushing of the snail may result in release of immature cercariae which are unsuitable for identification.

Harvest of cercariae by natural emergence is carried out by placing groups of snails (20 snails per 200 ml) under strong artificial illumination in containers with pond water or dechlorinated tap water at a temperature of 20–25 ° C for a period of 4–6 hours[2]. The possible emergence of cercariae must be checked at regular short intervals because emerged cercariae may encyst on external substrates or re-enter the snails rather quickly. In case of emergence of cercariae, the snails are subsequently transferred individually to small beakers, and the above scheduled procedure is repeated in order to obtain information concerning species of cercariae-producing snail(s), number and size of infected snail specimens, size of cercarial production, etc. Identification of the cercariae-producing snail(s) to species, or at least genus level, is very important from the viewpoint of cercarial identification, and this may be carried out using the "Field Guides to the Identification of African Freshwater Snails" published by Danish Bilharziasis Laboratory. These field guides may be obtained upon request from Danish Bilharziasis Laboratory.

At harvest of cercariae, and especially when using the crushing method, a number of non-cercarial organisms living on the snail shell, in the mantle cavity or in the tissue of the snail may be recovered. Various representatives of these organisms are depicted in Fig. 1. Besides, the different types of intramolluscan larval trematode stages, which may be found in the tissue of the snail when using the crushing method, are depicted in Fig. 2.

[2] This procedure ensures emergence of most "species" of cercariae including the cercariae of all trematode species being of major medical and veterinary importance in Africa. However, some "species" of cercariae emerge during darkness and it may therefore, depending on the goal of the study, be necessary to check for emergence of cercariae for a 24 hours period of 12 hours light and 12 hours darkness.

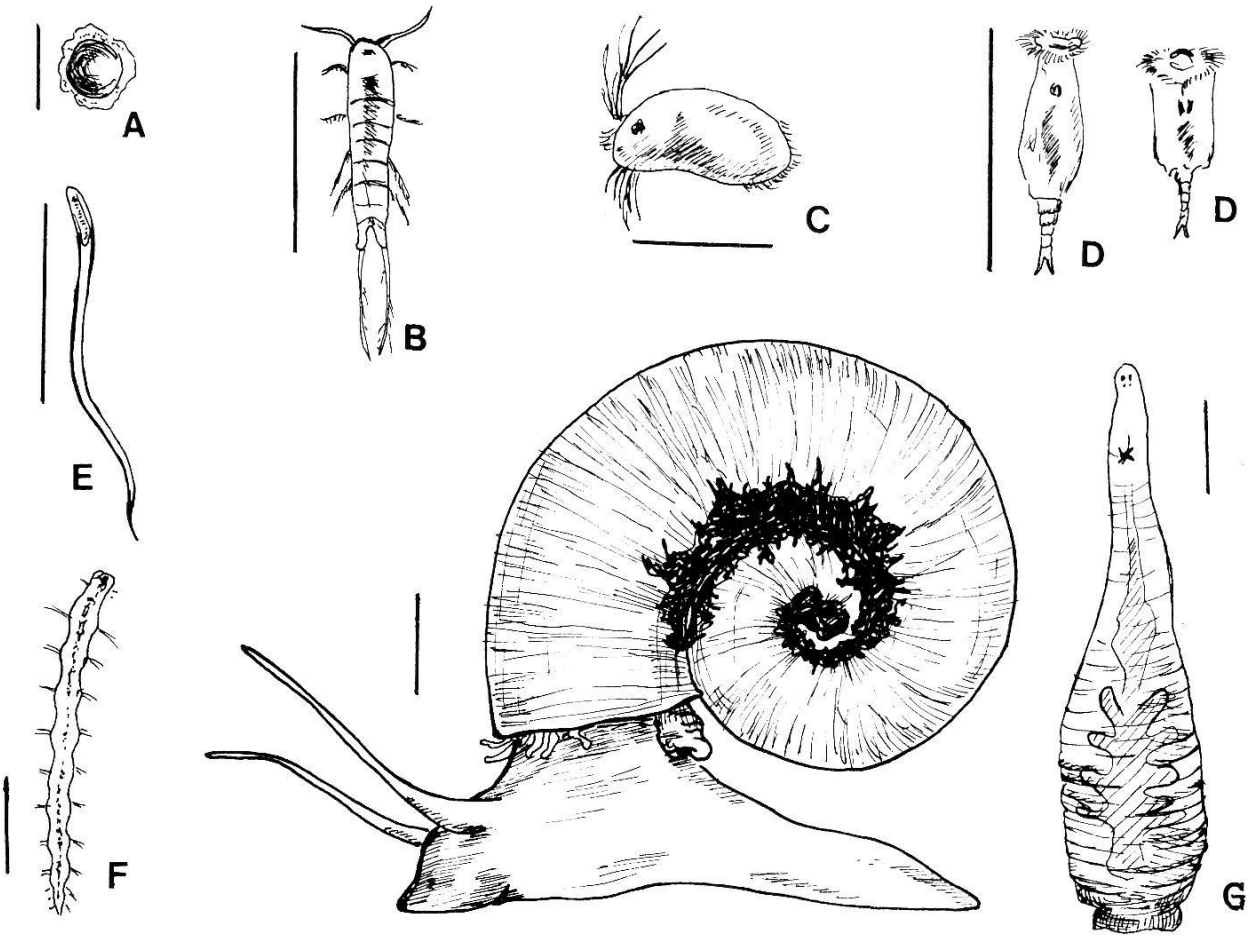


Fig. 1. Representative types of organisms living on the snail shell, in the mantle cavity and in the snail tissue.
A: Metacercaria; B and C: Crustaceans; D: Rotifer; E: Nematode; F: *Chaetogaster* sp.; G: Leech (A–E on the shell and occasionally as far as B, C and E are concerned in the snail tissue, F and G in the mantle cavity). Scale in A and D: 0.5 mm. Scale in B, C, E, F, G and in snail drawing: 1.0 mm.

Observations on living, unstained cercariae

Observations on swimming patterns, resting positions, etc., are conducted using cercarial specimens placed in a water volume allowing a normal behavioural pattern at a temperature of 20–25° C using a stereomicroscope of 8–40 times of magnification. The pattern of further cercarial development may be studied by introducing a piece of cellophane or plant material (for cercarial encystation on external substrates) and various potential second intermediate host organisms.

Identification of cercariae to the major type level (and occasionally also to lower levels) may normally be carried out using living and unstained cercarial specimens transferred to slides. The procedure is the following: remove a few cercariae from the emergence beaker using a pipette, transfer the cercariae in a drop of water onto a glass slide, add carefully a coverslip and draw off excess

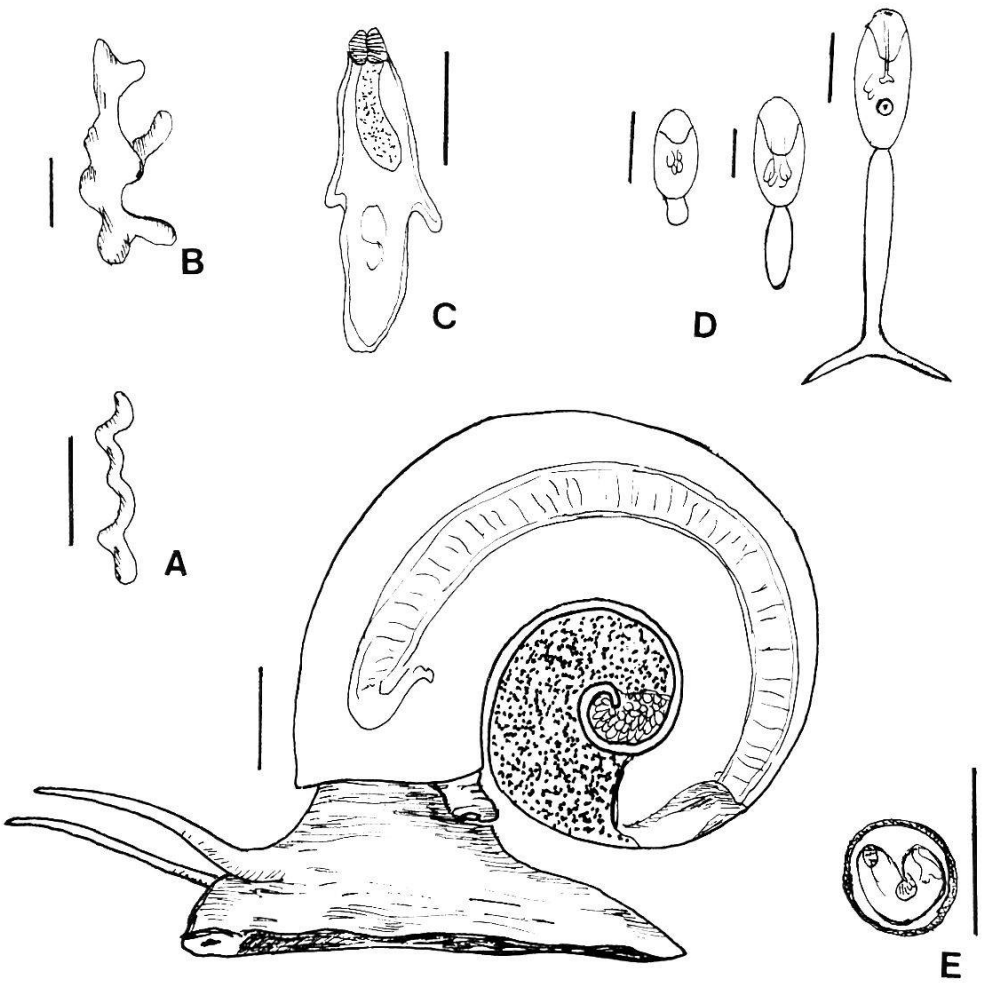


Fig. 2. Intramolluscan larval stages of trematodes.
A: mother sporocyst in the head-foot region; B: daughter sporocyst in the digestive gland; C: redia in the digestive gland; D: cercariae in various stages of development in the digestive gland; E: metacercariae in the digestive gland, pericardium, head-foot region or on the shell. Scale in drawings of trematode larvae: 0.1 mm. Scale in snail drawing: 1.0 mm.

water using a strip of filterpaper applied to one side of the coverslip. Removal of excess water gives rise to immobilization of the cercariae and normally also to a slight flattening, whereby external and internal structures become more easily observable. Immobilization may, however, also be achieved by adding a drop of a 2.5% methylcellulose solution to the preparation prior to adding the coverslip. The preparation is observed using a compound microscope of 160–400 times of magnification.

Staining and preservation of cercariae

Identification of cercariae to the sub-type, family, genus, and species level is normally carried out using stained cercariae in which the morphological features are more easily observable than in unstained specimens. Two staining methods exist, namely staining of living cercariae using vital dyes and staining

of fixed and preserved specimens using various stains like for example Ehrlich's Hematoxylin.

A number of vital dyes exist, but very dilute solutions of neutral red and Nile blue sulphate, which are prepared by adding one or two drops of a 0.1% solution of the dye to 50 ml of water, are normally recommended. The procedure for staining cercariae using vital dyes is the following: transfer the cercariae in a drop of water onto a slide, add a drop of the staining solution, cover carefully with a coverslip and draw of excess water using a strip of filterpaper. Alternatively, transfer the cercariae in a drop of water onto a slide, cover carefully with a coverslip, draw of excess water, place a drop of the staining solution at the edge of the coverslip and allow the stain to run under slowly. Using these procedures the cercariae become relaxed and sufficiently stained for observation after 10–15 minutes.

Cercariae are preserved in either 70% alcohol or 4% formalin and staining of preserved cercariae using stains like Ehrlich's Hematoxylin, Mayer's Paracarmine or Gower's Acetic Carmine may be carried out following an even prolonged storage in these fixatives. Staining with Ehrlich's Hematoxylin[3] is normally recommended and comprises six successive stays in different solutions (it is recommended to use a single staining unit (salt cellar or other glassware), replacing the six solutions successively instead of transferring the cercariae between units containing the different solutions). The six solutions and the approximate time periods to be used comprise the following:

1) 1 part hematoxylin and 3 parts glacial acetic acid (up to 10 minutes),
2) glacial acetic acid (5–10 minutes),
3) 3 parts glacial acetic acid and 1 part methyl salicylate (5–10 minutes),
4) 1 part glacial acetic acid and 1 part methyl salicylate (5–10 minutes),
5) 1 part glacial acetic acid and 3 parts methyl salicylate (5–10 minutes),
6) methyl salicylate (at least 10 minutes).

Permanent slides may be prepared following staining of preserved cercariae. The procedure comprises transference of the stained cercariae to a drop of mounting medium (Eukitt, Canada balsam) placed on a slide, followed by carefully covering with a coverslip. Mounted in this way the cercariae will keep the stain for a prolonged period of time. The preparation is to be labelled with information concerning locality and date of collection, species of cercariae-producing snail, type of cercaria and, if possible, family, genus and species name.

[3] Ehrlich's Hematoxylin Stain is prepared as follows:

Powdered hematoxylin	2 g
Ethyl alcohol (100%)	100 ml
Glycerol	100 ml
Glacial acetic acid	10 ml
Distilled water	100 ml
Aluminium potassium sulphate	10 g

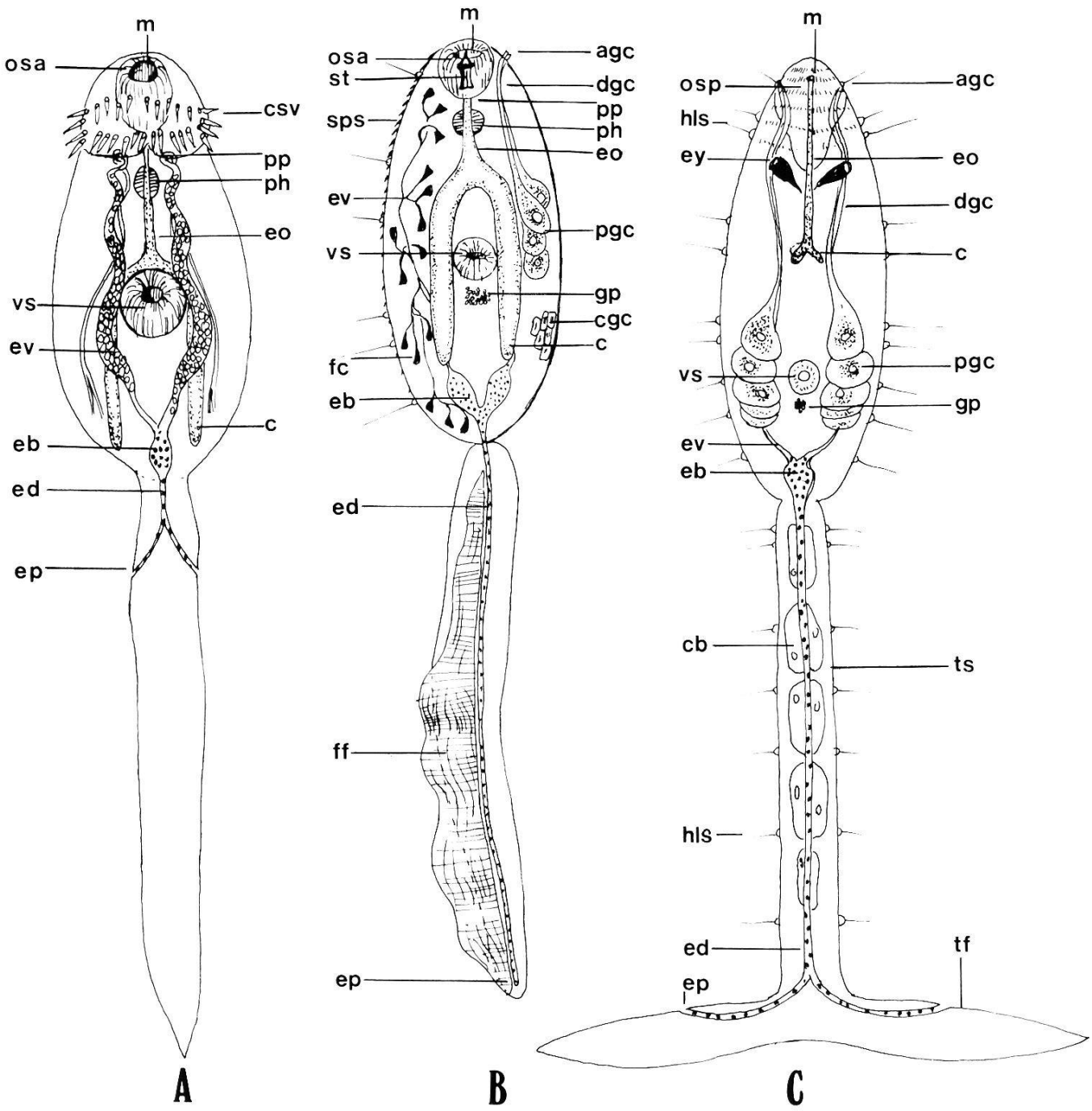


Fig. 3. General cercarial morphology. Diagrammatic illustrations, ventral views. A: Echinostome cercaria; B: Xiphidiocercaria; C: Furcocercous cercaria.
agc: aperture of penetration gland cells; c: caecum; cb: caudal bodies; cs: collar of spines; cgc: cystogenous gland cells; dgc: ducts of penetration gland cells; eb: excretory bladder; ed: excretory duct; eo: eosophagus; ep: excretory pore; ev: excretory vessel; ey: eyespot; fc: flamecells; ff: finfold; gp: genital primordium; hls: hair-like structure; m: mouth; osa: oral sucker, acetabulum-like; osp: oral sucker, modified to a pear-shaped penetration organ; ph: pharynx; pp: prepharynx; pgc: penetration gland cells; sps: spines; st: stylet; tf: tail furca; ts: tail stem; vs: ventral sucker.

IV. Introduction to cercarial morphology

This section comprises an introduction to cercarial morphology to an extend enabling the use of the keys included in this guide. For a support to the description see the diagrammatic illustrations in Fig. 3.

General appearance

The cercaria consists of a cercarial body and a cercarial tail. The body is globular, cylindrical or oval in shape, but the shape is subject to considerable changes, i.e. shows great plasticity. The cercarial tail is generally a well developed and complex structure, but it may be reduced to a short knob-like appendix and even absent.

Tegument

The body is covered by a tegument which is normally smooth but occasionally granular in appearance, and which may be equipped with spines. The spines are normally backwardly pointing and either fine or stout, and they are generally most frequent in the region of the oral sucker. However, heavily spined caudal pockets may be present on either side of the body at the point of insertion of the tail, and a distinctive collar of very stout spines may be arranged around the oral sucker. Besides, hair-like structures arising from small papilla are commonly present, and the body may bear finfolds.

Body suckers

Suckers are normally present on the cercarial body. The basic scheme consists of an oral sucker surrounding the mouth and a ventral sucker, but one or even both suckers may be absent. The oral sucker is usually well-developed, muscular and acetabulum-like in appearance with or without a stylet in the anterior (dorsal) lip and occasionally equipped with ridges and papilla along the edges, but it may be modified to a pear-shaped penetration organ heavily armed with both recurved and forwardly directed spines. The ventral sucker is normally simple and acetabulum-like and exhibits little morphological variation. It may be posterior or central in position on the mid-ventral surface, and it may be large or small and even absent.

Alimentary system

The alimentary system consists basically of mouth, prepharynx, muscular pharynx, oesophagus, oesophageal glands emptying into the oesophagus and two intestinal caeca. Parts of the alimentary system may be absent as for example the pharynx, and the two intestinal caeca may be reduced to minute sacs at the end of the oesophagus.

Excretory system[4]

The excretory (protonephridial) system consists basically of an excretory bladder located in the posterior part of the body and a system of primary, secondary and tertiary collecting vessels in either side of the body. The tertiary collecting vessels terminate in flame-cell equipped capillaries.

The excretory bladder opens to the exterior via excretory ducts in (an) excretory pore(s). The excretory pore(s) may be situated terminally at the posterior end of the body or near the ventral sucker, sub-terminally or terminally at the tip of the unforked tail, terminally or sub-terminally on the tail furca or sub-terminally on the tail-stem of the forked tail.

Gland cells

Three types of gland cells may exist, namely mucoid gland cells, cystogenous gland cells and penetration gland cells, and a reservoir for secretions from the mucoid gland cells, named the virgula organ, may also be present. This organ is located in the region of the oral sucker and opens to the exterior by short ducts on each side of the mouth. The mucoid gland cells and the cystogenous gland cells are placed subtegumentally, and the dense granular content of the cystogenous gland cells makes the internal organs more or less invisible when they occur in high numbers. The penetration gland cells occur in pairs and are situated anterior, lateral or posterior to the ventral sucker. They may vary in number from two to six pairs, and they may be divided into an anterior and posterior group on the basis of their optical appearance and histochemical characteristics. The ducts from the penetration gland cells run anterior and open through apertures in the oral sucker.

Nervous system and sensory organs

The nervous system consists of a cerebral ganglion complex in the region of the pharynx and a number of longitudinal trunks which branch to innervate suckers, eyespots, tail, etc.

Sensory organs may include two or occasionally three darkly pigmented eyespots and hair-like structures arising from a small papilla.

Genital primordium

A genital primordium, which is an undifferentiated group of cells more or less well-defined in form, may be present in the posterior part of the body.

[4] The variations in morphology and development of the excretory system are many, and the number and arrangement of the flame-cells and the structure of the bladder wall are of great importance in trematode taxonomy and at identification of cercariae. These morphological details are, however, not easily observable and are therefore not used in the keys in the present guide.

Cercarial tail

The cercarial tail is generally a very well developed and complex structure, but it may be reduced to a short knob-like structure and may even be absent. The tail may be many times the length of the body and may bear finfolds, it may be forked or unforked and may be equipped with spines and hair-like structures. The tail consists in the forked-tailed type of cercaria of a tail-stem and two furcae, and the tail furcae may be either slender and tapered or lobe-like, and may be equipped with furcal finfolds. The brevifurcate forked-tailed cercarial type has furcae which are less than one-half the length of the tail-stem, and the longifurcate forked-tailed cercarial type has furcae which are longer than one-half the length of the tail-stem. Both types of forked-tailed cercariae may in the tail-stem contain vesicle-like structures named caudal bodies.

V. Key to identification of cercariae from African freshwater snails to the major type level

The procedure for using the key is very simple. In each numbered "couplet" (i.e. in each set of two opposing statements), two statements are made of which only one conforms to the cercarial specimen to be identified. The correct answer leads either to another numbered set of opposing statements or assigns the cercaria to the definite major type. A diagrammatic illustration and a description of the morphological and biological characteristics of each major type of cercariae are found in section VI. The various sub-types within the furcocercous group of cercariae are dealt with in section VII.

1	A	Tail absent	*Cercariaeum cercariae*
	B	Tail present	*2*
2	A	Tail bulbous and cystlike, having appendices	*Cystophorous cercariae*
	B	Tail not as described above	*3*
3	A	Tail short, knoblike or cupshaped	*Microcercous cercariae*
	B	Tail as long or longer than body	*4*
4	A	Tail forked	*Furcocercous cercariae* (see section VII)
	B	Tail not forked	*5*
5	A	Ventral sucker vestigial or absent	*6*
	B	Ventral sucker present	*7*

6	A	Ventral sucker absent; eyespots present; a pair of adhesive organs present at posterior end of body; many cystogenous glands in body; tail without finfolds	*Monostome cercariae*
	B	Ventral sucker vestigial or absent; eyespots present; adhesive organs at posterior end of body not present; tail provided with finfolds	*Parapleurolophocercous cercariae*
7	A	Ventral sucker large and located at posterior end of body	*Amphistome cercariae*
	B	Ventral sucker on midventral surface of body	*8*
8	A	Stylet in oral sucker present	*9*
	B	Stylet in oral sucker not present	*11*
9	A	Tail provided with a dorso-ventral finfold; ventral sucker smaller than oral sucker	*Ornatae xiphidiocercariae*
	B	Tail without a dorso-ventral finfold	*10*
10	A	Virgula organ present; ventral sucker smaller than oral sucker	*Virgulate xiphidiocercariae*
	B	Virgula organ not present; suckers of equal size or ventral sucker larger than oral sucker	*Armatae xiphidiocercariae*
11	A	Oral sucker surrounded by a spiny collar ..	*Echinostome cercariae*
	B	Spiny collar around oral sucker not present	*Gymnocephalous cercariae*

VI. Morphological and biological characteristics of major types of cercariae from African freshwater snails

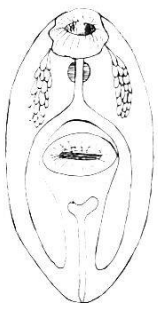
This section contains a short description of the morphological and biological characteristics of each major cercarial type recovered from African freshwater snails, a list of snail genera from which they have been recovered, and a list of trematode families to which they belong. The information available concerning genera of snails from which the various major cercarial types have been recovered is in a summary form presented in Table 1, but the number of studies carried out in Africa is too limited to allow the summary to be considered as being complete. The furcocercous group of cercariae is dealt with separately in section VII. Scale in drawings of cercariae: 0.1 mm.

Table 1. A survey of cercarial types recovered from various African freshwater snails* **

Snail genus	Cercarial type
Bellamya	Xiar
Biomphalaria	Amph, BAD, BAM, Cer, Cyst, Ech, Gymn, LPD, Mono, Xiar, Xior
Bulinus	Amph, BAD, BAM, Cyst, Ech, Gymn, LPD, Loph, Xiar
Ceratophallus	Amph, BAM, Cyst, Ech, Gymn, Loph, LPD, Para, Xiar, Xior
Cleopatra	LPM
Gabbiella	Cer, Gymn, LPM, Para, Xiar
Gyraulus	Amph, BAD, BAM, Ech, Gymn, LPD
Lentorbis	Amph, Ech
Lymnaea	BAD, BAM, Cer, Cyst, Ech, Gymn, LPD, Mono, Xiar, Xivi
Melanoides	BAD, Gymn, LPD, LPM, Para, Xiar, Xivi
Melanopsis	LPM
Pila	Ech
Pirinella	Para
Potadoma	Micro?
Segmentorbis	Amph, BAD, Ech, Xiar

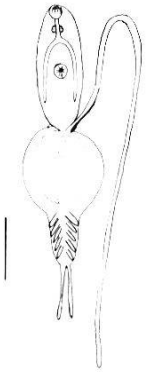
* Abbreviations used:
Amph: Amphistome cercariae
BAD: Brevifurcate-apharyngeate distome cercariae
BAM: Brevifurcate-apharyngeate monostome cercariae
Cer: Cercariaeum cercariae
Cyst: Cystophorous cercariae
Ech: Echinostome cercariae
Gymn: Gymnocephalous cercariae
Loph: Lophocercous-apharyngeate cercariae
LPD: Longifurcate-pharyngeate distome cercariae
LPM: Longifurcate-pharyngeate monostome cercariae
Micro: Microcercous cercariae
Mono: Monostome cercariae
Para: Parapleurolophocercous cercariae
Xiar: Armatae xiphidiocercariae
Xior: Ornatae xiphidiocercariae
Xivi: Virgulate xiphidiocercariae

** The number of studies carried out in African freshwater is too low to allow the survey to be considered as being complete.



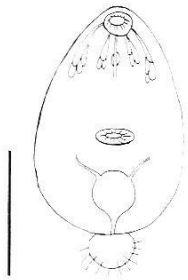
Cercariaeum cercariae

Tail absent. Develop in rediae. Encyst in snails. Produced by species of the families Monorchiidae (intestinal parasites of fish) and Cyclocoelidae (parasites of the respiratory tract of birds). Of no economical importance.
Produced by species of the genera *Biomphalaria*, *Gabbiella* and *Lymnaea*.



Cystophorous cercariae

Tail bulbous and cystlike, having appendices. Develop in rediae. Encyst in copepods after peroral ingestion. Produced by species of the family Hemiuridae (parasites of fish and amphibians). Of no economical importance.
Produced by species of the genera *Biomphalaria, Bulinus, Ceratophallus* and *Lymnaea.*



Microcercous cercariae (several sub-types exist)

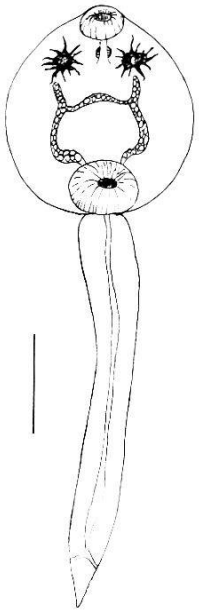
Tail short, knoblike or cupshaped. Develop in rediae. Encyst in crayfish and crabs. Produced by species of the family Troglotrematidae and possibly also other families. Species of the family Troglotrematidae of medical importance (see section IX).
Probably produced by species of the genus *Potadoma.*

Monostome cercariae

Tail unforked, ventral sucker absent, finfolds on tail not present, two or occasionally three eyespots present, a pair of adhesive organs present at posterior end of body, numerous cystogenous glands in body. Develop in rediae. Encyst on external substrates. Produced by species of the family Notocotylidae (intestinal parasites of birds and occasionally mammals). Of no economical importance.
Produced by species of the genera *Biomphalaria* and *Lymnaea.*

Parapleurolophocercous cercariae

Tail unforked and with well developed finfolds, ventral sucker vestigial or absent, eyespots present, adhesive organs at posterior end of body not present, cystogenous glands in body few in number. Develop in rediae. Encyst in fish and amphibians. Produced by species of the family Heterophyidae (intestinal parasites of birds and mammals). Some species of the family of medical importance (see section IX).
Produced by species of the genera *Ceratophallus, Gabbiella, Melanoides* and *Pirinella.*



Amphistome cercariae

Tail unforked, ventral sucker large and located at posterior end of body, eye-spots commonly present. Develop in rediae. Encyst on external substrates or in the skin of tadpoles. Produced by species of the family Paramphistomatidae. Two types of amphistome cercariae exist:

1. Diplocotylea: cystogenous glands in body few in number. Produced by amphistome species being intestinal parasites of frogs. Of no economical importance.
2. Pigmentata: cystogenous glands in body numerous. Produced by amphistome species being intestinal parasites of mammals, especially ruminants. Some species of great veterinary importance (see section IX).

Produced by species of the genera *Biomphalaria*, *Bulinus*, *Ceratophallus*, *Gyraulus*, *Lentorbis* and *Segmentorbis*.

Xiphidiocercariae

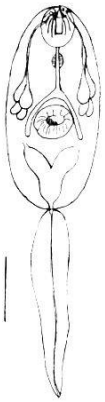
Tail unforked, ventral sucker on mild-ventral surface of body, stylet in oral sucker present. Develop in sporocysts. Encyst in invertebrates, amphibians and reptiles. Of no economical importance. Three sub-types are presented here.

Ornatae xiphidiocercariae

Tail provided with a dorso-ventral finfold, virgula organ not present, ventral sucker smaller than oral sucker. Produced by species of the families Macroderoididae (intestinal parasites of fish and amphibians) and Haplometridae (lung parasites of amphibians).
Produced by species of the genera *Biomphalaria* and *Ceratophallus*.

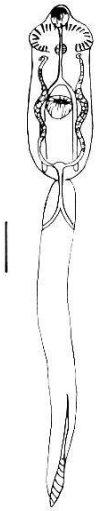
Virgulate xiphidiocercariae

Tail without a dorso-ventral finfold, bilobed or pyriform virgula organ present in the region of the oral sucker, ventral sucker smaller than oral sucker. Produced by species of the family Lecithodendriidae (intestinal parasites of bats, birds and amphibians).
Produced by species of the genera *Lymnaea* and *Melanoides*.



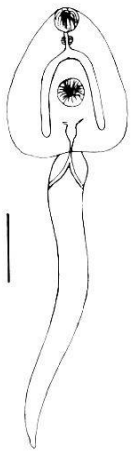
Armatae xiphidiocercariae

Tail without a dorso-ventral finfold, virgula organ not present, oral and ventral suckers of equal size or ventral sucker larger than oral sucker. Produced by species of the family Plagiorchiidae (intestinal parasites in all groups of vertebrates).
Produced by species of the genera *Bellamya, Biomphalaria, Bulinus, Ceratophallus, Gabbiella, Lymnaea, Melanoides* and *Segmentorbis.*



Echinostome cercariae

Tail unforked, ventral sucker on mid-ventral surface of body, oral sucker without a stylet but surrounded by a spiny collar, cystogenous glands in body numerous, no eyespots. Develop in rediae. Encyst in invertebrates, fish and amphibians. Produced by species of the family Echinostomatidae (intestinal parasites of birds, reptiles and mammals). Some species may be of some economical importance.
Produced by species of the genera *Biomphalaria, Bulinus, Ceratophallus, Gyraulus, Lentorbis, Lymnaea, Pila* and *Segmentorbis.*



Gymnocephalous cercariae

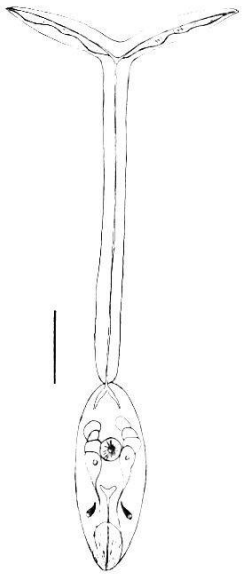
Tail unforked, ventral sucker on mid-ventral surface of body, neither stylet nor spiny collar present, no eyespots, numerous cystogenous glands in body. Develop in rediae. Encyst on external substrates. Produced by species of the family Fasciolidae (intestinal and liver parasites in herbivorous mammals) and by species of numerous other families. Species of the family Fasciolidae of great veterinary importance (see section IX).
Produced by species of the genera *Biomphalaria, Bulinus, Ceratophallus, Gabbiella, Gyraulus, Lymnaea* and *Melanoides.*

VII. Key to identification of furcocercous cercariae to the sub-type level and a description of morphological and biological characteristics of each sub-type

Common for all furcocercous cercariae is a forked tail and an oral sucker having a pear-shaped appearance, and the morphological criteria for dividing the furcocercous group of cercariae into subtypes comprise the presence or absence of pharynx[5], presence or absence of oral and ventral suckers and type of furcation of the tail, i.e. being either brevifurcate or longifurcate. Five subtypes of furcocercous cercariae are presented here, and the brevifurcate-apharyngeate distome furcocercarial sub-type, which among others includes the cercariae of species of the genus *Schistosoma*, is dealt with in more details in section VIII. Scale in drawings of cercariae: 0.1 mm.

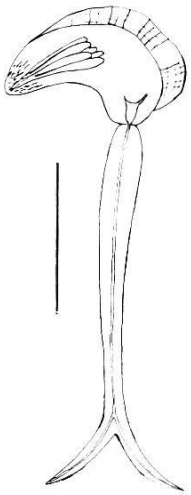
1	A	Tail brevifurcate; (pharynx absent)	*2*
	B	Tail longifurcate; (pharynx present)	*4*
2	A	Ventral sucker well developed	*Brevifurcate-apharyngeate distome cercariae* (see section VIII)
	B	Ventral sucker vestigial or absent	*3*
3	A	Oral sucker absent; eyespots not present; body finfold present	*Lophocercous-apharyngeate cercariae*
	B	Oral sucker present; body finfold present; eyespots sometimes present	*Brevifurcate-apharyngeate monostome cercariae*
4	A	Ventral sucker present; furcal finfolds not present; caudal bodies in tailstem present; excretory pores located on sides of furcae .	*Longifurcate-pharyngeate distome cercariae (Strigea cercariae)*
	B	Ventral sucker vestigial or absent; furcal finfolds present; caudal bodies in tailstem not present; excretory pores located at tips of furcae	*Longifurcate-pharyngeate monostome cercariae (Vivax cercariae)*

[5] The presence or absence of pharynx is not easily observable, and this criterium is therefore not used in the key in the present guide.



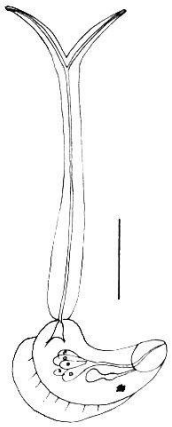
Brevifurcate-apharyngeate distome cercariae

Tail brevifurcate, pharynx absent, oral and ventral suckers present, penetration gland cells of two types, furcal finfolds and eyespots sometimes present. Develop in sporocysts. Penetrate the definitive host directly. Produced by species of the families Spirorchiidae (blood parasites of reptiles) and Schistosomatidae (blood parasites of birds and mammals). Species of the family Schistosomatidae of great medical and veterinary importance (see section VIII).
Produced by species of the genera *Biomphalaria*, *Bulinus*, *Gyraulus*, *Lymnaea*, *Melanoides* and *Segmentorbis*.



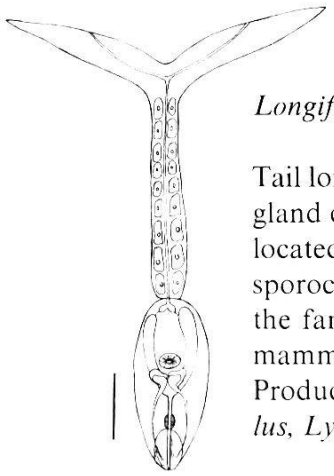
Lophocercous-apharyngeate cercariae

Tail brevifurcate, pharynx absent, ventral sucker vestigial or absent, oral sucker absent, penetration gland cells all of one type, eyespots not present, body finfold present. The body is in the resting position above the tail. Develop in sporocysts. Penetrate the definitive host directly. Produced by species of the family Sanguinicolidae (blood parasites of fish). Some species of some economical importance.
Produced by species of the genera *Bulinus* and *Ceratophallus*.



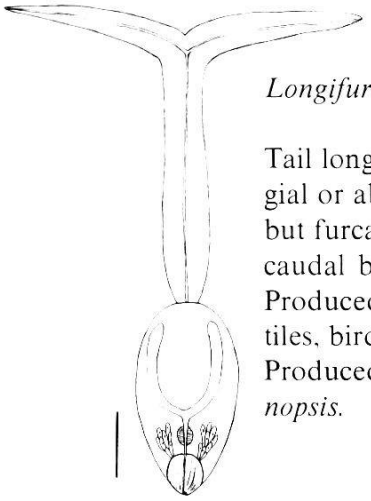
Brevifurcate-apharyngeate monostome cercariae

Tail brevifurcate, pharynx absent, oral sucker present but ventral sucker absent, penetration gland cells all of one type, eyespots sometimes present, body finfold present. The body is in the resting position below the tail. Develop in rediae. Encyst in fish. Produced by species of the family Clinostomatidae (parasites in mouth and oesophagus of birds). Of no economical importance.
Produced by species of the genera *Biomphalaria*, *Bulinus*, *Ceratophallus*, *Gyraulus* and *Lymnaea*.



Longifurcate-pharyngeate distome cercariae (Strigea cercariae)

Tail longifurcate, pharynx present, oral and ventral suckers present, penetration gland cells all of one type, body and furcal finfolds not present, excretory pores located on sides of furcae, caudal bodies present in the tailstem. Develop in sporocysts. Encyst in snails, tadpoles, reptiles and fish. Produced by species of the families Strigeidae and Diplostomatidae (intestinal parasites of birds and mammals). Of no economical importance.
Produce by species of the genera *Biomphalaria*, *Bulinus*, *Ceratophallus*, *Gyraulus*, *Lymnaea* and *Melanoides*.



Longifurcate-pharyngeate monostome cercariae (Vivax cercariae)

Tail longifurcate, pharynx present, oral sucker present but ventral sucker vestigial or absent, penetration gland cells all of one type, body finfold not present but furcal finfolds sometimes present, excretory pores located at tips of furcae, caudal bodies in tailstem not present. Develop in sporocysts. Encyst in fish. Produced by species of the family Cyathocotylidae (intestinal parasites of reptiles, birds and mammals). Of no economical importance.
Produced by species of the genera *Cleopatra*, *Gabbiella*, *Melanoides* and *Melanopsis*.

VIII. Guide for assignment of the brevifurcate-apharyngeate distome furcocercarial type to the genus Schistosoma, and instructions for assignment of cercariae of the genus Schistosoma to the species level

The brevifurcate-apharyngeate distome furcocercarial type is in freshwater in Africa only found in the families Spirorchiidae (blood flukes of reptiles) and Schistosomatidae (blood flukes of birds and mammals). The family Schistosomatidae comprises in Africa the genera *Trichobilharzia* and *Gigantobilharzia* (blood flukes of birds) and the genera *Schistosoma* and *Bivitellobilharzia* (blood flukes of mammalians). However, the cercariae of all species of the family Spirorchiidae and of the family Schistosomatidae *except* the cercariae of species of the genus *Schistosoma* have eyespots. Thus, the *Schistosoma* sp. cercaria is the only brevifurcate-apharyngeate distome furcocercarial type without eyespots. Besides, the *Schistosoma* sp. cercaria has in contrast to all other brevifurcate-apharyngeate distome cercariae the tail furcae curved backwardly in the resting position when observed in open water. The backwardly curvation of the tail furcae is, however, only rarely observed in preserved cercariae or in living cercariae under pressure of the coverslip on the slide. Diagrammatic illustrations of cercariae of a spirorchid trematode, an avian schistosome and a *Schistosoma* species are found in Fig. 4.

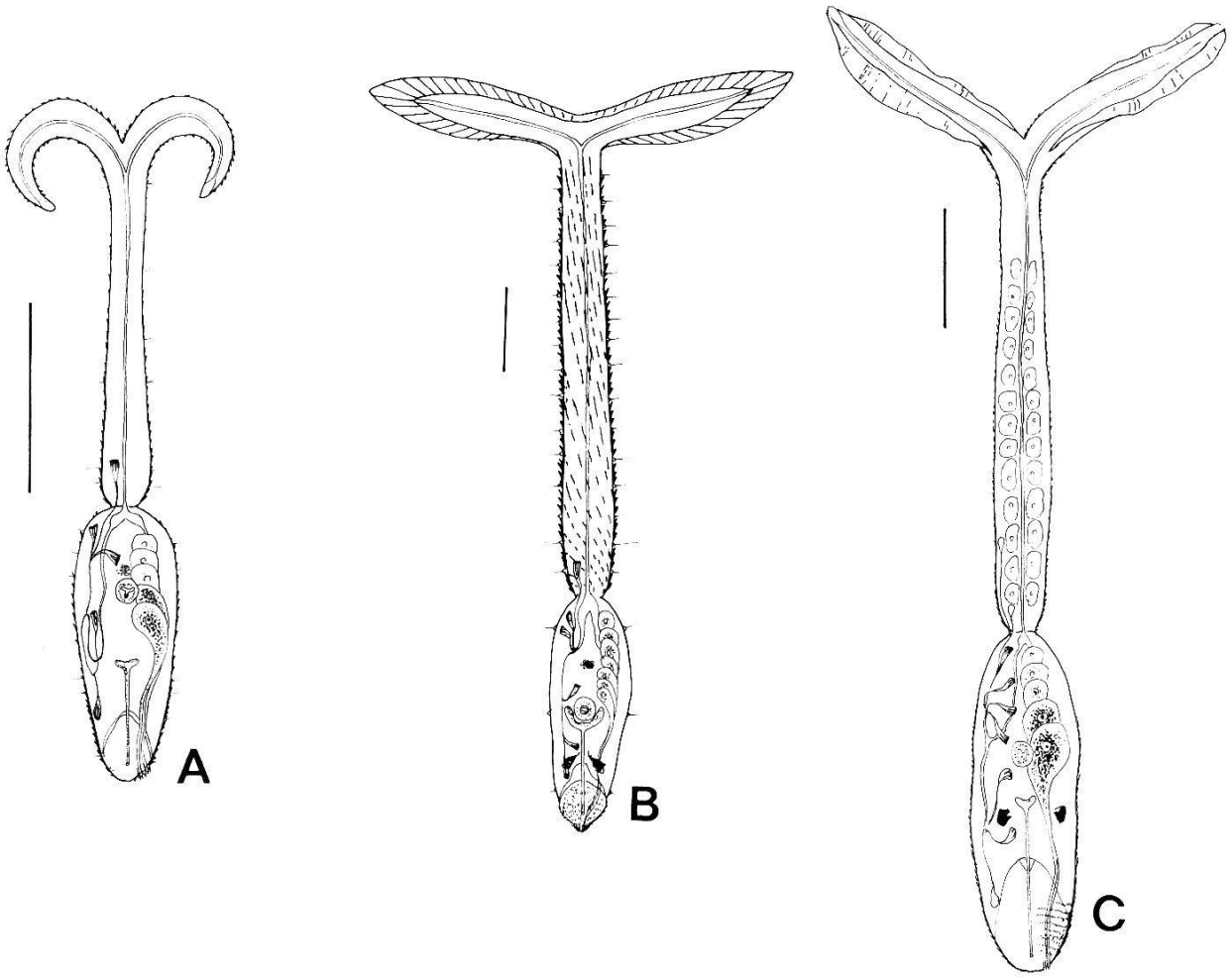


Fig. 4. A: Cercaria of a *Schistosoma* species. B: Cercaria of a spirorchid trematode. C: Cercaria of an avian schistosome species. Scale: 0.1 mm.

Numerous species of the genus *Schistosoma* exist in Africa (see Table 2) and assignment of a *Schistosoma* sp. cercaria to the species level is for the non-specialist not possible on the basis of morphological criteria because morphological differences between the cercariae of the different species are extremely small[6]. Biological characteristics as for example species of cercariae-producing snail are also insufficient for identification on the species level because the same snail species may be host for more *Schistosoma* species within the same geographical area (Table 2). Besides, the normally poorly defined local transmission conditions and the limited information available concerning the distributional patterns and definitive host spectra, especially as far as some of the non-human *Schistosoma* species are concerned, provide the basis for epidemiologi-

[6] The number and distributional pattern of argentophilic papilla on the cercarial surface are relatively constant within each *Schistosoma* species and sufficiently different from those in other species to be taken as characteristic. Isoenzyme techniques might also show promise in differentiating *Schistosoma* cercariae.

Table 2. Survey of intermediate host snails for African species of human and bovine schistosomes of the genus *Schistosoma**

S. haematobium				*S. intercalatum*	*S. mansoni*	
North Africa	East Africa	West Africa	Central Africa		Egypt	Africa exc. Egypt
*B. truncatus***	*B. globosus***	*B. truncatus***	*B. globosus***	*B. forskalii***	*B. alexandrina***	*B. pfeifferi***
	B. africanus	*B. senegalensis*		*B. globosus***		*B. sudanica*
	B. nasutus	*B. globosus*				*B. camerunensis*
	B. abyssinicus					*B. choanomphala*
S. bovis				*S. mattheei*	*S. margrebowiei*	*S. leiperi*
North Africa	East Africa	West Africa	Central Africa			
*B. truncatus***	*B. truncatus*	*B. forskalii***	*B. forskalii?*	*B. africanus***	*B. forskalii***	*B. africanus***
	*B. forskalii***	*B. senegalensis*	*B. truncatus?*	*B. globosus***	*B. scalaris*	*B. globosus?*
	B. abyssinicus	*B. globosus?*	*B. globosus?*		*B. tropicus?*	
	B. nasutus	*B. truncatus?*			*B. truncatus?*	
	*B. africanus***					

* Host snail genus for *S. mansoni: Biomphalaria;* host snail genus for other schistosome species: *Bulinus.*

** indicates the overall quantitatively most important snail species. Other species listed may, however, locally be of primary importance.

? indicates that the status of the snail species as host is unknown but strongly suspected.

cal observations like for example species of mammalians having contact with a given snail habitat being of only limited value. The final and proper assignment of a *Schistosoma* sp. cercaria to the species level therefore for the non-specialist comprises infection of a proper experimental animal with subsequent determination of the cercarial species on the basis of adult worm and egg morphology. Instructions concerning methods to be used may be obtained upon request from the Danish Bilharziasis Laboratory.

IX. Comments concerning the identification of cercariae of non-schistosome trematodes of medical and veterinary importance

Paragonimus uterobilateralis (Troglotrematidae, a lung-fluke of man) and *Heterophyes heterophyes* (Heterophyidae, an intestinal fluke of man) are found in parts of West Africa and in Egypt, respectively. The cercaria of *P. uterobilateralis*, probably being produced by species of the genus *Potadoma*, belongs to the Microcercous cercarial group, and that of *H. heterophyes*, which is produced by *Pirinella conica*, to the Parapleurolophocercous group of cercariae, but the information available concerning the occurrence of these two cercarial types

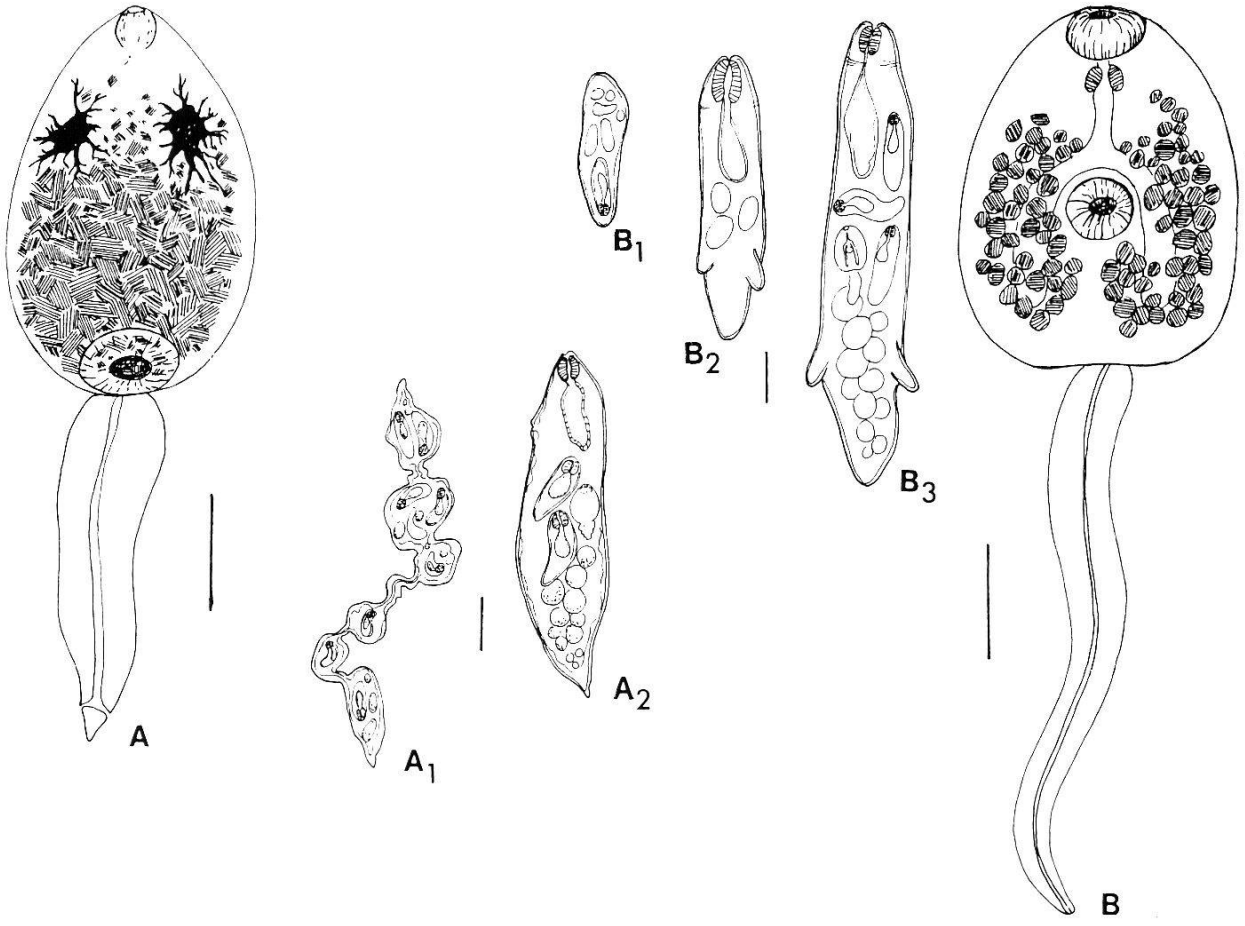


Fig. 5. Intramolluscan larval stages and cercariae of *Paramphistomum microbotrium* (A) and *Fasciola* sp. (B). A and B: Cercariae; A_1 and B_1: Sporocysts; A_2, B_2 and B_3: Rediae. Scale: 0.1 mm.

and their different sub-types in African freshwater is too limited to provide the background for a proper identification by a non-specialist of the cercariae of these two medically important species of trematodes. A specialist will therefore have to be consulted.

The cercariae of *Paramphistomum microbotrium* and of other species of veterinary important paramphistomes (Paramphistomatidae, parasites of ruminants) belong to the Pigmentata sub-type of the Amphistome group of cercariae, and those of species of the genus *Fasciola* (Fasciolidae, liver-flukes of domestic stock animals) to the Gymnocephalous group of cercariae. The cercaria of *P. microbotrium* is produced by species of the genus *Bulinus,* those of other paramphistome species by species of the genera *Biomphalaria, Ceratophallus* and *Segmentorbis,* and the cercariae of species of the genus *Fasciola* are produced by species of the genus *Lymnaea.* However, a very large number of African trematode species without medical or veterinary importance have cercariae belonging to either the Amphistome or the Gymnocephalous group of cercariae, and it is therefore not possible for the non-specialist on a morphological basis alone to identify properly the cercariae of the above mentioned veterinary important species of trematodes. In this case it is therefore also necessary to consult a specialist. Drawings of the intramolluscan larval stages and the cercariae of *P. microbotrium* and *Fasciola* sp. are found in Fig. 5, but it is important to stress that these drawings must not provide the basis for a cercarial identification.

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