

Anatomy of the metacercaria and adult of *Euclinostomum heterostomum* (Rudolphi, 1809) (Trematoda: Clinostomatidae)

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Anatomical descriptions are presented of *Euclinostomum heterostomum* (Rudolphi, 1809) metacercariae, collected from the muscles of the Mocambique tilapia *Oreochromis mossambicus* (Peters); as well as of an egg-bearing adult trematode. The latter was obtained by experimentally infecting a hand-reared grey heron *Ardea cinerea* L. chick with metacercariae from the same fish species.

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Anatomiese beskrywings is gemaak van metaserkarië en 'n volwasse wurm van *Euclinostomum heterostomum* (Rudolphi, 1809). Geïnsisteerde metaserkarië is versamel vanuit die spiere van die bloukurper *Oreochromis mossambicus* (Peters). Die volwasse wurm is verkry deur eksperimentele besmetting van 'n pasuitgebroeide bloureier-kuiken *Ardea cinerea* L. met metaserkarië afkomstig vanuit dieselfde vissoort.

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Euclinostomum heterostomum (Rudolphi, 1809) has a wide geographical distribution and has been reported from southern Europe, Russia, India and various countries in Africa (Dönges 1974) including South Africa (Mönnig 1926; Prudhoe & Hussey 1977).

This trematode has a three-host life cycle. The adult inhabits the oral cavity and oesophagus of fish-eating birds, the metacercariae occur in the muscles or gill chambers of fish and the redia is carried by snails.

Descriptions of adults of *E. heterostomum* from birds have been given by Rudolphi (1809), Von Linstow (1886), MacCullum (1899), Braun (1901), Dollfus (1932), Dollfus (1950), Shigin (1954), Dennis & Sharp (1973) and Grabda-Kazubska (1974).

The metacercaria of this parasite has been described from fish by Mönnig (1926), Joyeux & Houdemer (1928), Van der Kuyp (1953), Fischthal & Kuntz (1963) and Ukoli (1966). Metacercariae collected from the Mozambique tilapia *Oreochromis mossambicus* (Peters) and adults obtained from a wild grey heron *Ardea cinerea* L. in the same locality, were described by Prudhoe & Hussey (1977). The intramolluscan stages of *E. heterostomum* were described by Dönges (1974).

Experimental infections of bird hosts with metacercariae collected from fish were carried out by Srivastava (1950), Agarwal (1959) and Ramanaiah & Agarwal (1969) mainly for the purpose of obtaining eggs and miracidia. Complete morphological descriptions of the adult and metacercarial stages were however not given by these authors.

Ukoli (1966) experimentally infected bird hosts with *E. heterostomum* metacercariae. However, the adults he obtained were stunted and did not bear eggs. No description of both the metacercaria and egg-bearing adult obtained from experimental infection of the bird host with these metacercariae, has previously been published.

In the present paper, the anatomy of the *E. heterostomum* metacercaria from the muscles of *O. mossambicus* is described; as well as that of an egg-bearing adult, obtained by experimentally infecting a newly hatched hand-reared grey heron *Ardea cinerea* L. chick with these metacercariae.

Materials and Methods

Fish examined for *E. heterostomum* metacercariae were collected by means of gill-nets at Lepellane Dam, Lebowa, Northern Transvaal. The gills, gill chambers and muscles of the freshly killed fish were examined at the collecting localities by means of a dissection microscope. If fish were infected, the cysts were removed, manually excysted, fixed in hot AFA and stored in 70% ethyl alcohol. A newly hatched, hand-reared

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grey heron *Ardea cinerea* chick was infected experimentally by feeding it *E. heterostomum* metacercariae which had been removed from the muscles of *O. mossambicus* collected at the same locality. After nine days, an egg-bearing adult was recovered from the oesophagus of this bird.

Wholemounds were stained using Kirkpatrick's Carmalum or Mayer's Haemalum. Serial sections were microtomed at 8 μm and stained by the azan-azocarmine or Delafield's haematoxylin method. Xylene piling as described by Pusey (1939) was used to make graphic reconstructions of the metacercaria and adult trematode. Measurements were obtained by using a slide micrometer in conjunction with the eye-piece micrometer of the microscope. All measurements are given in mm; the arithmetic mean in parentheses follows minimum and maximum dimensions.

Table 1 Explanation of abbreviations used in Figures 1–6

at – anterior testis	np – ventro-lateral nerve
b – basement membrane	o – ovary
cm – circular muscle	ob – oblique muscle
cp – cirrus pouch	oc – ootype complex
dej – ductus ejaculatorius	od – oviduct
eb – excretory vesicle	oe – oesophagus
ec – recurrent vessel (excretory)	os – oral sucker
em – main excretory vessel	p – parenchyma
ep – excretory pore	pt – posterior testis
ex – excretory cavity	s – cuticular spine
g – gland cell	sng – supra-oesophageal nerve
gp – genital pore	ganglion
ic – intestinal caecum	te – tegument
l – longitudinal muscle	u – uterus
lc – Laurer's canal	us – uterine sac
m – metaterm	vd – vitelline duct
mg – Mehlis' gland	vef – vas efferens
mi – microtriches	vr – vitelline reservoir
na – nerve (anteriorly-directed)	vs – ventral sucker
ne – network of excretory vessels	vsem – vesicula seminalis
	x – seminal receptacle

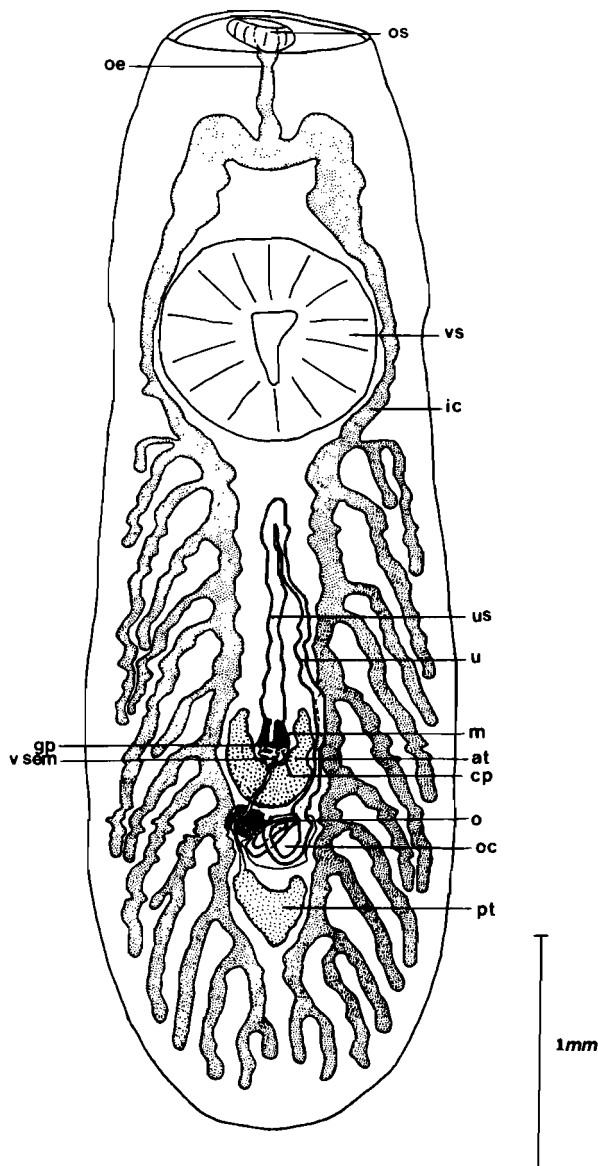


Figure 1 Graphic reconstruction of alimentary and reproductive systems of *E. heterostomum* metacercaria, ventral view. (See Table 1 for abbreviations.)

Description of metacercaria

(Based on nine specimens)

External features (Figure 1)

Body elongate, broad, slight lateral constriction in acetabular region, dorsally convex, ventrally concave, both ends bluntly rounded; 5,75–6,88 (6,01) long. Forebody width at acetabulum 1,50–2,20 (1,83), maximum width between anterior and posterior testes 2,15–2,98 (2,49). Oral field is thickened margin of anterior end of body; oral sucker 0,23–0,32 (0,26) long, 0,39–0,45 (0,41) wide, situated ventrally in centre of oral field, subterminal. Ventral sucker large, muscular, 1,13–1,30 (1,16) long, 1,13–1,35 (1,19) wide, triangular aperture with apex of triangle directed posteriorly, anterior border of ventral sucker 0,90–1,03 (0,99) from anterior end of body. Ratio of length of oral sucker to length of ventral sucker 1 : 4,53, that of breadth 1 : 4,12.

Structure of body wall and parenchyma (Figure 2)

Body covered by non-cellular tegument or syncytium, no spines in cuticle; immediately below tegument are basement membrane and outer longitudinal muscles; fibres few, small, evenly-spaced in single layer where present, absent in some parts of

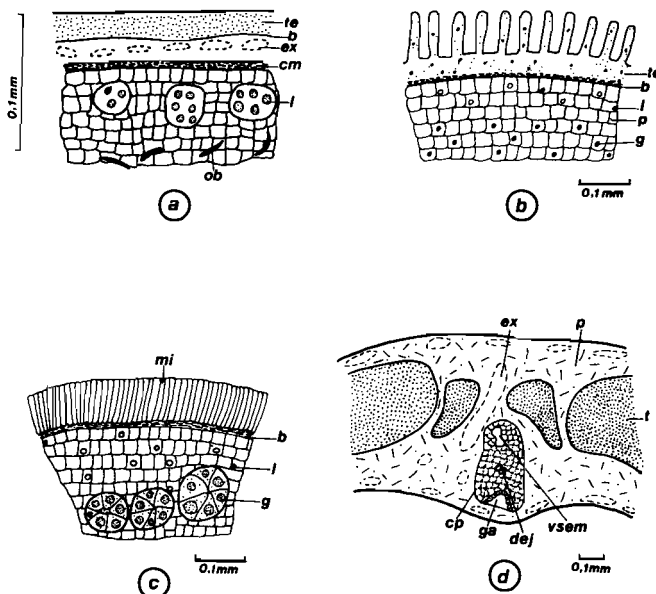


Figure 2 Transverse sections through *E. heterostomum* metacercaria. (a) Body wall; (b) Intestine; (c) Posterior portion of oesophagus; (d) Cirrus pouch. (See Table 1 for abbreviations.)

body wall. Circular muscle layer situated between outer longitudinal muscle layer and subcuticular excretory cavities, consists of numerous fibres. Inner longitudinal muscles below and sometimes interrupted by subcuticular vessels of excretory system, fibres grouped in bundles, evenly distributed as observed in transverse sections, muscle fibres vary in size. Oblique muscles in parenchyma adjacent to inner longitudinal muscles; parenchyma fills spaces within body between internal organs, longitudinal and transverse (dorso-ventral) muscles in parenchyma, in many cases transverse muscle fibre reaches from dorsal to ventral surface of body, nucleated myoblasts observed in connection with these muscles. No papillae on dorsal and ventral body surfaces, few papillae on lateral surface at level of oral sucker and acetabulum.

Alimentary system (Figures 1, 2b & 2c)

Mouth opening in centre of oral sucker; leads into oesophagus; pharynx absent; oesophagus 0,20–0,30 (0,28) long; anterior oesophagus narrow, tubular, short cuticular microtriches extend into lumen; posterior oesophagus with thick heavy parenchymatous wall; few longitudinal and circular muscles, thick mass of gland cells surrounds posterior bulbous dilatation, mass of brush-like microtriches extends into lumen of posterior oesophagus, microtriches 0,01–0,03 long. Oesophagus runs posteriorly along ventral body surface, proceeds dorso-posteriorly to enter transverse bar of intestine, from which two main intestinal caeca extend posteriorly to level of excretory bladder. Between posterior border of ventral sucker and excretory vesicle, each intestinal caecum gives off 10 to 13 caecal diverticula, postero-laterally directed, roughly parallel to each other in diagonal plane; diverticula occasionally branched, narrow or distended with intestinal contents, ends pointed or rounded.

Male reproductive system (Figures 1, 2d & 3a)

Testes tandem, intercaecal, in posterior third of body; anterior testis U- to crescent-shaped, 3,70–4,50 (4,00) from anterior end of body, 0,40–0,45 (0,41) long, 0,58–0,70 (0,65) wide. Posterior testis Y-shaped to triangular, with apex posteriorly directed 0,40–0,48 (0,45) from anterior testis, 0,25–0,35 (0,28) long, 0,45–0,55 (0,51) wide; vas efferens of posterior testis long, leaves right anterior margin of testis, extends anteriorly along dextral border of intertesticular space, passes ovary and anterior testis ventrally to join vas efferens of anterior testis, latter very short, leaves anterior testis left of vas efferens from posterior testis; vasa efferentia unite immediately after entering the cirrus sac to form short vas deferens which dilates to form vesicula seminalis, then narrows to form ductus ejaculatorius. Cirrus sac 0,20–0,28 (0,24) long, 0,15–0,25 (0,21) wide, within arms of anterior testis, vesicula seminalis occupies posterior portion of cirrus sac, prostate gland cells present; cirrus enclosed by anterior part of cirrus sac, which opens into genital atrium; latter leads to genital pore, which is 3,48–4,25 (3,78) from anterior end of body.

Female reproductive system (Figures 1, 3b & 3c)

Ovary small, spherical, 0,13–0,23 (0,16) long, 0,10–0,23 (0,19) wide, on dextral side of intertesticular space; oviduct short, leaves ovary mesially to enter dorso-dextral side of ootype complex; oviduct enlarges to form seminal receptacle, which narrows dorsally to form Laurer's canal, latter opens to exterior on dorsal surface of body. Vitelline duct from small vitelline reservoir enters oviduct; ootype surrounded by radially

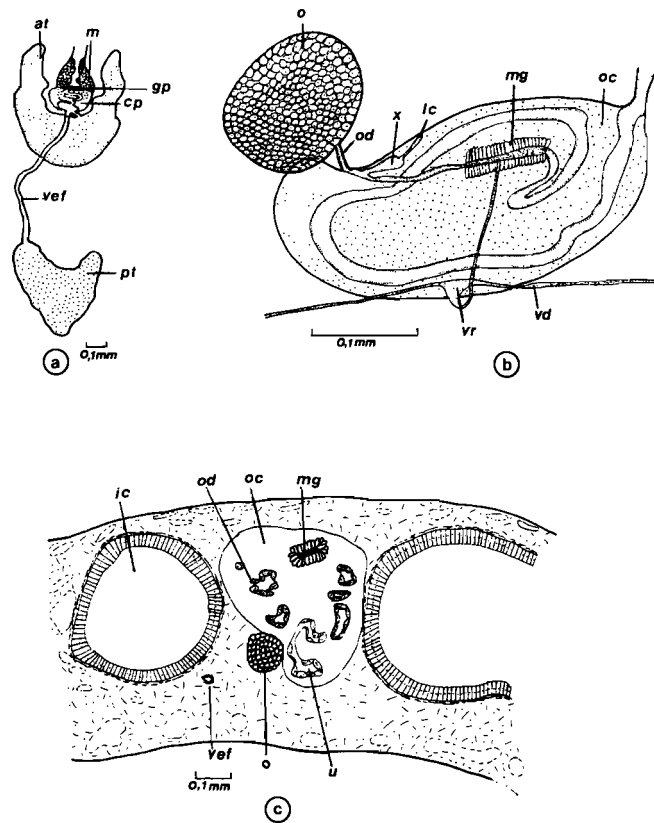


Figure 3 *E. heterostomum* metacercaria. (a) Ventral view of male reproductive system; (b) Ventral view of ootype complex; (c) Transverse section through ootype complex. (See Table 1 for abbreviations.)

arranged cells of Mehlis' gland; uterus originates as terminal coil of oviduct in ootype complex, leaves left anterior border of ootype complex, extends anteriorly between left intestinal caecum and anterior testis, enters anterior tip of uterine sac, latter extends posteriorly; metraterm short, leaves posterior part of uterine sac, opens into genital atrium, uterine sac 1,20–1,35 (1,30) long, 0,10–0,28 (0,18) wide; genital pore covered by thin layer of cuticle, leads to genital atrium. Vitellaria present or absent, follicles immature when present, extend posteriorly in two lateral bands from posterior level of ventral sucker, overlap caeca and diverticula, converge at posterior end of body, behind posterior testis.

Nervous system (Figure 4)

Dorsal commissure connects two large supra-oesophageal nerve ganglia dorsal and posterior to oral sucker; from ganglionic complex, two well-defined nerves extend posteriorly, pass ventral sucker and intestinal caeca ventro-laterally; several small nerves extend anteriorly from ganglia, two anteriorly directed nerves innervate oral sucker.

Excretory system (Figure 4)

Excretory vesicle Y-shaped, leads to excretory pore which opens on dorsal posterior part of body. Two main collecting vessels, one on each side of body, leave cornua of excretory vesicle and extend anteriorly, parallel and ventro-lateral to intestinal caeca. Collecting vessels receive many tubules along length; transverse tubules and communicating vessels anastomose to form network extending throughout parenchymatous tissue; recurrent vessels run dorso-laterally to collecting vessels which they join in the oral sucker region. Recurrent vessels converge behind excretory vesicle at posterior end of body, narrower than collecting vessels, do not receive transverse or communicating vessels, vibratile organs attached to wall of recurrent

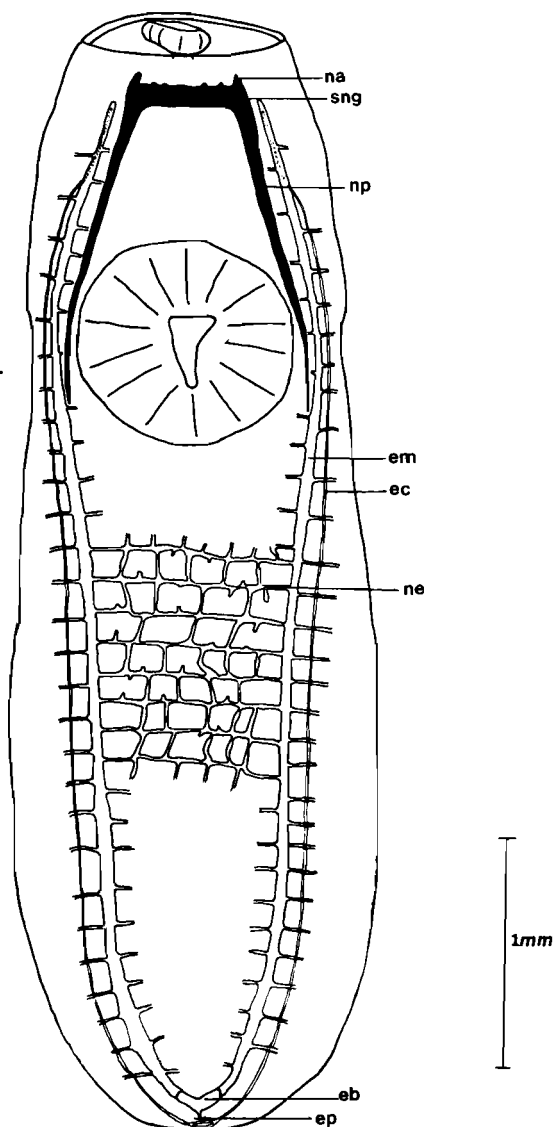


Figure 4 Graphic reconstruction of nervous system and portion of excretory system of *E. heterostomum* metacercaria, ventral view. (See Table 1 for abbreviations.)

vessels along length at close intervals and extend into lumen of vessels.

Description of adult

(Based on one specimen)

External features (Figure 5)

Body linguiform, anterior end truncated, posterior end broadly rounded, 8,02 long, dorsally convex, ventrally concave, fore-body width at acetabulum 2,39, maximum width between anterior and posterior testes 3,78; oral field protrusible, dome-shaped with thickened margin; oral sucker in centre of oral field, oral sucker 0,24 long, 0,29 wide, subterminal, ventrally situated; ventral sucker large, muscular, triangular apex with apex of triangle pointing posteriorly, ventral sucker 1,37 from anterior end of body. Ratio of length of oral sucker to length of ventral sucker 1 : 5,20 and that of breadth 1 : 4,25.

Structure of body wall and parenchyma

Body covered by non-cellular tegument, cuticle devoid of spines; very few outer longitudinal muscle fibres; circular muscle layer composed of numerous fibres, lies between outer longitudinal muscles and subcuticular excretory cavities; subcuticular excretory system not as well developed as in metacer-

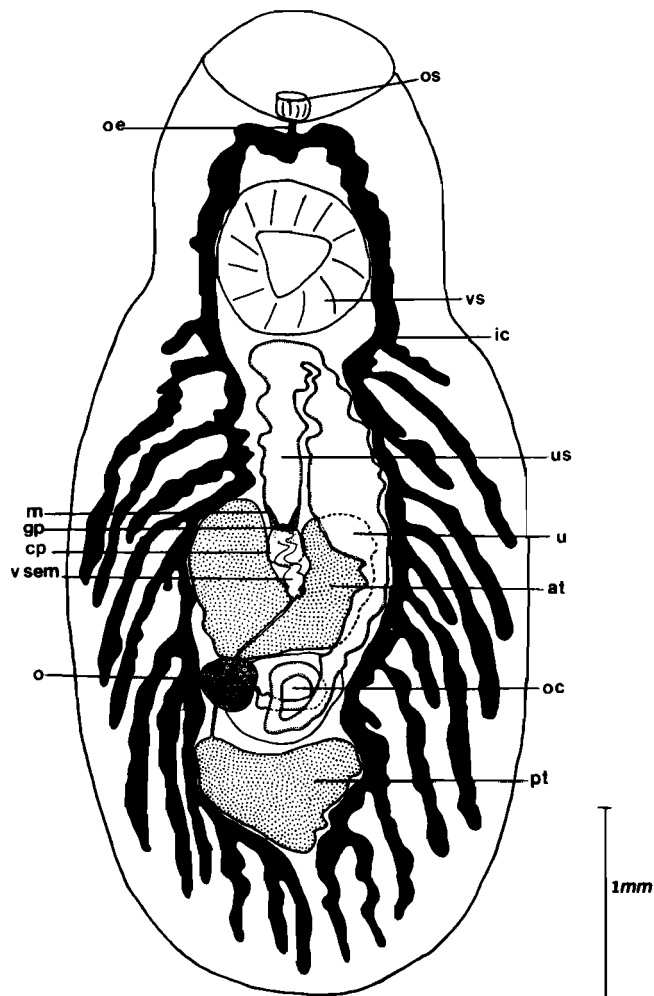


Figure 5 Graphic reconstruction of alimentary and reproductive systems of *E. heterostomum* adult, ventral view. (See Table 1 for abbreviations.)

caria; inner longitudinal muscle fibres grouped in bundles which are evenly distributed as seen in transverse sections, muscle fibres vary in size; oblique muscles located in parenchyma on inner side of inner longitudinal muscles. Parenchyma occupies spaces in body between organs, syncytial in mature adults, longitudinal and transverse (dorso-ventral) muscles present. Dorsal and ventral body surfaces do not have papillae; few papillae on lateral sides of oral sucker and in acetabular region.

Alimentary system (Figure 5)

Oral sucker leads to oesophagus which is 0,34 long, no pharynx; oesophagus extends ventrally, bends dorsally to form bulbous chamber, surrounded by gland cells of which function is uncertain; microtriches extend into lumen of posterior oesophagus, 0,005 – 0,010 long, fewer and shorter microtriches than in metacercaria; anterior oesophagus narrow, tube-like, very few microtriches; from transverse section of intestine, two main intestinal caeca extend posteriorly, end blindly at level of excretory vesicle, give rise to 12 to 13 intestinal diverticula of unequal length, branch occasionally, less distended than in metacercaria.

Male reproductive system (Figure 5)

Testes tandem, in posterior half of body; anterior testis U-shaped, 1,02 long, 1,24 wide, anterior margin 4,78 from anterior end of body; posterior testis 0,83 long, 1,34 wide, latter triangular with apex of triangle posteriorly directed,

anterior margin of posterior testis 0,83 from posterior margin of anterior testis. Vasa efferentia follow same path as those in metacercaria, unite after entering cirrus sac to form vas deferens; latter dilates to form seminal vesicle then narrows to form ductus ejaculatorius; prostate glands present; cirrus sac 0,59 long, 0,29 wide, located between horns of anterior testis; cirrus either in cirrus sac or inserted in metraterm; genital atrium leads to genital pore, which is 4,24 from anterior end, genital pore opens directly to exterior and is not covered by layer of cuticle as in metacercaria.

Female reproductive system (Figure 5)

Ovary spherical, 0,44 long, 0,49 wide, on right side of median line in intertesticular space; oviduct arises mid-dorsally from ovary, enters ootype complex, gives off Laurer's canal which opens on mid-dorsal body surface; ootype surrounded by gland cells to collectively form Mehlis' gland; common vitelline duct and vitelline reservoir better defined than in metacercaria. Uterus leaves sinistral anterior border of ootype complex, continues anteriorly, ventral to left side of anterior testis, enters anterior tip of uterine sac, latter extends posteriorly, short metraterm, which opens into genital atrium, latter leads to genital pore; uterine sac 1,54 long, 0,34 wide, uterine sac, uterus and oviduct distended with eggs; many vitellaria present, well defined, extend from posterior border of ventral sucker to posterior end of body, overlap intestinal caeca and diverticula dorsally and ventrally. Vitelline follicles confluent in median line behind posterior testis, no vitellaria in region of excretory pore.

Nervous system (Figure 6)

Dorsal commissure connects two supra-oesophageal nerve ganglia, as in metacercaria; two well-defined nerves extend posteriorly from ganglionic complex and two anteriorly directed nerves innervate oral sucker.

Excretory system (Figure 6)

Excretory vesicle Y-shaped, opens into excretory pore in dorsal median position near posterior end of body; excretory system consists of pair of collecting vessels, pair of recurrent vessels, and capillary network as described for metacercaria; collecting vessels receive transverse and communicating vessels; recurrent vessels have vibratile organs along length, connected to collecting vessels at oral region, recurrent vessels converge in median line, posterior to excretory vesicle.

Discussion

Metacercariae of *E. heterostomum* recovered from wild fish have been described and measured by Van der Kuyp (1953), Fischthal & Kuntz (1963), Ukoli (1966) and Prudhoe & Hussey (1977). The anatomical and morphological features of the metacercariae in the present study conform to these descriptions, although minor variation in dimensions exists. These differences are probably due to the age of the metacercaria. Dönges (1974) presented measurements of different age groups of metacercariae, obtained after experimentally infecting fish with cercariae. From this information it appears that there is a gradual increase in size of the metacercaria with age. Measurements of metacercariae in the present study fall within the upper limits of the range for the 62-day-old metacercariae described by Dönges (1974).

The adult *E. heterostomum* described in this study was recovered from an *A. cinerea* chick, nine days after experimental infection. Only one of the four specimens recovered

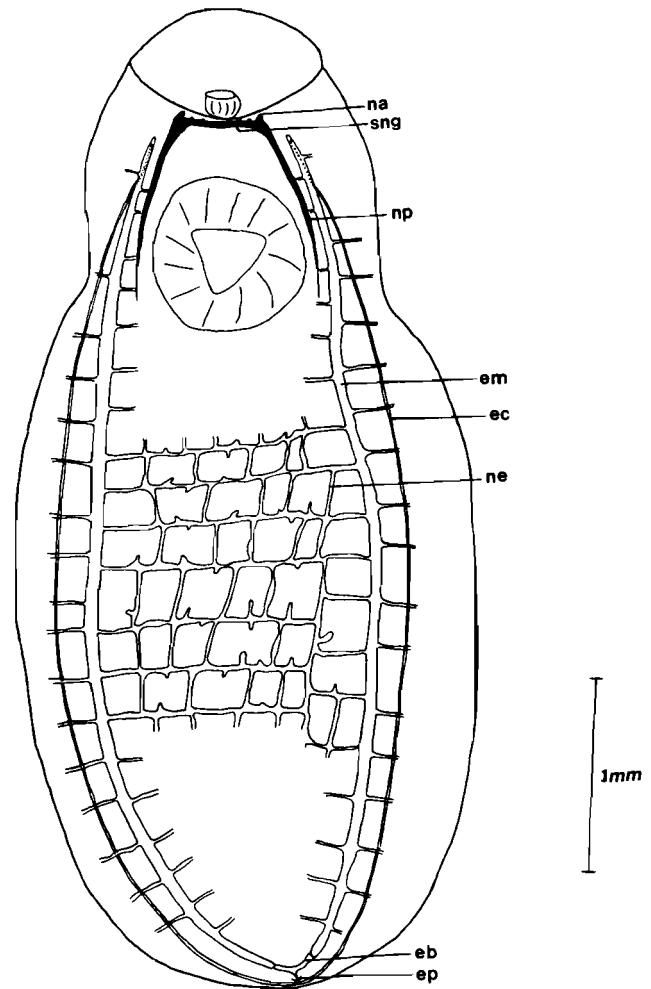


Figure 6 Graphic reconstruction of nervous system and portion of excretory system of *E. heterostomum* adult, ventral view. (See Table 1 for abbreviations.)

was microtomed for descriptive purposes, as external morphological differences were not noted amongst the specimens. Since the egg was incubated, and the chick hand-fed, there was no possibility of infection by other trematodes. Anatomically the metacercarial and adult forms are very similar, with the exceptions of the relative size of the gonads (which are better developed in the latter case) and the presence of a cuticle covering the genital pore of the metacercaria. According to Paperna (1980) specific diagnosis using metacercariae only is usually not feasible owing to the fact that the gonads (which are the most important criteria for diagnosis in trematodes) are normally not sufficiently developed in larval stages. In the case of *E. heterostomum* it appears that specific diagnosis can successfully be carried out using the metacercariae only, owing to the presence of well-developed gonads. The similarity between the metacercariae in the present study and metacercarial descriptions presented by Van der Kuyp (1953), Fischthal & Kuntz (1963), Ukoli (1966) and Prudhoe & Hussey (1977) without obtaining adults, confirms their specific diagnosis as *E. heterostomum*. The anatomical dimensions of the adult presented in this study fall within the range of measurements of adults from wild birds presented by Van der Kuyp (1953), Dennis & Sharp (1973), Grabda-Kazubska (1974) and Prudhoe & Hussey (1977). The minor differences in dimension are probably due, as in the case of the metacercaria, to differences in age.

Ukoli (1966) presents the only description of an adult *E. heterostomum* obtained from experimentally infecting a bird

host. This specimen was stunted, however, and did not have eggs. This may be due to the fact that a cattle egret *Bubulcus ibis* L. was used as the experimental host. Sexually mature adults collected from wild *A. cinerea* have been described by Van der Kuyp (1953), Grabda-Kazubska (1974) and Prudhoe & Hussey (1977). Experimental infection of the same species in the present study also resulted in the development of fully mature adults. It would thus appear that *Bubulcus ibis* used by Ukoli (1966) is not the definitive host, and this resulted in inhibition of normal development.

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