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THE REDIA, CERCARIA AND EARLY STAGES OF *APOROCOTYLE SIMPLEX* ODHNER, 1900 (SANGUINICOLIDAE) – A DIGENETIC TREMATODE WHICH HAS A POLYCHAETE ANNELID AS THE ONLY INTERMEDIATE HOST

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ABSTRACT

An apharyngeate furcocercous cercaria which develops in the polychaete *Artacama proboscidea* Malmgren, 1865 (Annelida, Terebellidae), is shown experimentally to develop into *Aporocotyle simplex* Odhner, 1900 (Trematoda, Sanguinicolidae). *A. simplex* is a common blood fluke in *Hippoglossoides platessoides* (Fabricius), *Limanda limanda* (L.), and *Pleuronectes platessa* L. from Danish waters.

About 7 % of *A. proboscidea* from Øresund were infested. At least two redial generations occur. More than 1000 rediae were found commonly in individual polychaetes. The rediae, which occur free in the coelomic cavity, castrate the host.

The redia, cercaria and young specimens from fish are described. Both light microscopy and scanning electron microscopy were used.

The cercaria has a cephalic organ with 6-9 circlets of spines. Similar spines are common on the anterior half of the body. Five pairs of penetration glands occur in the body, and one pyriform gland occurs entirely within the cephalic organ. The digestive system consists of a long oesophagus and two short caeca. The anterior part of the cercaria has a pitted tegument similar to that of schistosome cercariae.

L. limanda, P. platessa and *Platichthys flesus* (L.) were used as experimental hosts. The cercaria penetrates the skin of the fishes, and up to six-month-old worms were found in the lymphatic system all over the fish body. Half of the 132-day-old specimens occurred in the branchial vessels. Six-month-old specimens from both the lymphatic and the blood system had uterine eggs.

During the first two-three months in the fish host, long pointed spines appear on tubercles laterally, and the body shape changes from cylindrical to lanceolate. The cephalic spines remain throughout the life of the worm.

The cercaria of *A. simplex* is compared with other marine sanguinicolid cercariae and especially with the three cercariae previously described from polychaetes. The holotype of *Cercaria hartmanae* Martin, 1952 was reexamined. The phylogeny of blood flukes (Sanguinicolidae, Spirorchiidae and Schistosomatidae) based on the developmental stages in the intermediate hosts is discussed.

INTRODUCTION

The blood fluke *Aporocotyle simplex* Odhner, 1900 is common in the following pleuronectid fishes from Danish waters: long rough dab *Hippoglossoides platessoides* (Fabricius), dab *Limanda limanda* (L.), and plaice *Pleuronectes platessa* L., but the cercaria has until now been unknown. The adult *A. simplex* has recently been redescribed (Thulin 1980a, b).

Marine cercariae suggested to develop into blood flukes of fish have previously been recorded in bivalve molluscs and polychaete annelids. Bivalves and polychaetes from Øresund have for several years been examined in order to find the larval stages of *A. simplex*. In December 1981 rediae containing apharyngeate furcocercous cercariae were found in the terebellid polychaete *Artacama proboscidea* Malmgren, 1865. The cercaria was proven experimentally to develop into *A. simplex*. It is the first known life-cycle of a marine fish blood fluke.

I thank Miss Harriet Hansen for the preparation of serial sections, and Dr J. R. Lichtenfels, United States National Museum, Helminthological Collection, Beltsville, for lending me type material of *Cercaria hartmanae* Martin, 1952.

The interference contrast microscope used in this study was financed by the Danish Natural Science Research Council.

MATERIAL AND METHODS

The intermediate host, *Artacama proboscidea* Malmgren, 1865 (Polychaeta, Terebellidae) was dredged in Øresund north of the island Veen at a depth of 40 metres.

Most polychaetes were dissected immediately after arrival to the laboratory. To study the emergence of the cercariae, heavily infested polychaetes – with rediae visible through the body wall – were placed in small glass containers without substratum.

Polychaetes intended for sectioning were kept for two-three days without substratum to empty their intestines for sand grains.

The rediae, cercariae and young developmental stages from fish were studied alive, unstained or stained *in vivo* with neutral red. The different developmental stages were fixed unpressed or slightly pressed in Bouin's fluid or 10 % formalin.

Material intended for scanning electron microscopical study was fixed for 2-3 hours in 2.5 % glutaraldehyde buffered with cacodylate to about pH 7.4, postfixed for about 1 hour in cacodylate-buffered osmium tetroxide, dehydrated in ethanol, transferred to benzene and freeze-dried. The specimens were studied in a Cambridge 600 Stereoscan microscope.

Specimens and pieces of uninfested and infested *A. proboscidea* and pieces of infected dabs *Limanda limanda* (L.) were fixed in Bouin's fluid and embedded in tissuemat. Serial sections (5 μm) were stained with Heidenhain's azan, a

0.02 % aqueous solution of toluidine blue, alcian blue in combination with the PAS-reaction, with or without Mayer's acid haematoxylin.

Rediae containing fully developed cercariae were fixed as the material intended for scanning electron microscopy, dehydrated in ethanol and propylene oxid and embedded in epon. Serial sections (2 μm) were stained with toluidine blue.

RESULTS

The redial generations

The sporocyst which occurs fully developed inside the miracidium (Thulin 1980a) was not found, although two whole and two anterior ends of the polychaete host harbouring young infestations were serially sectioned. The sporocyst probably dies after having produced a limited number of rediae.

Young infestations harbour a few rediae which contain small daughter rediae and undeveloped cercariae (Fig. 1B). The first-generation rediae may alternately produce rediae and cercariae as long as they live. Rediae of the second and perhaps subsequent generations may do the same until the polychaete is filled with them and thereafter only cercariae are produced. About 1000-1200 cercaria-producing rediae were found commonly in individual polychaetes.

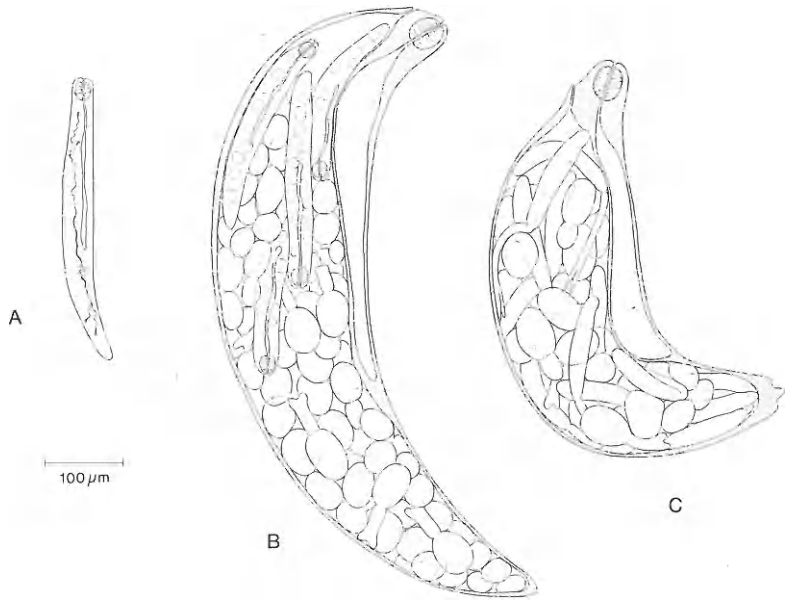

FIG. 1. Rediae of *Aporocotyle simplex*. Living, slightly flattened specimens. A, immature redia. B, redia containing daughter rediae and undeveloped cercariae (first-generation redia). C, redia containing cercariae only (second-generation redia).

In one *A. proboscidea* one redia differed from the remaining about 1000 cercaria-producing rediae. This redia contained one redia in addition to cercariae in all developmental stages, including fully developed cercariae. The enclosed redia which also contained fully developed cercariae had a large spherical, empty-looking caecum. The large redia may represent a redia which has not released one of the daughter rediae.

The rediae containing small rediae in addition to undeveloped cercariae, and rediae containing cercariae only were identical in shape and size.

Living slightly flattened rediae were 250 μm to 1.0 mm long and 40-280 μm wide. The pharynx measured 22-40 μm in length and 18-26 μm in width. Measurements of rediae fixed unpressed in formalin are given in Table 2.

Most rediae less than about 400 μm in length were cylindrical, contained germinal balls only and had a tubular, empty-looking caecum (Fig. 1A). The flame cell formula is $2[2+2] = 8$.

First-generation rediae contained up to 8 small rediae in the anterior part, and undeveloped cercariae and germinal balls in the posterior part of the brood chamber (Fig. 1B).

Second-generation rediae contained up to 30 apparently fully-developed cercariae distributed especially anteriorly and posteriorly in the brood chamber with the undeveloped cercariae in between. The smallest redia with apparently fully developed cercariae measured 380×160 μm.

The small rediae and the fully developed cercariae emerged through the birth pore opposite the caecum and close to the pharynx (Figs 1B, C, 2A).

Most large rediae were curved with the saccular caecum towards the concave side. The caecum was most often filled with a brown granular material. In some

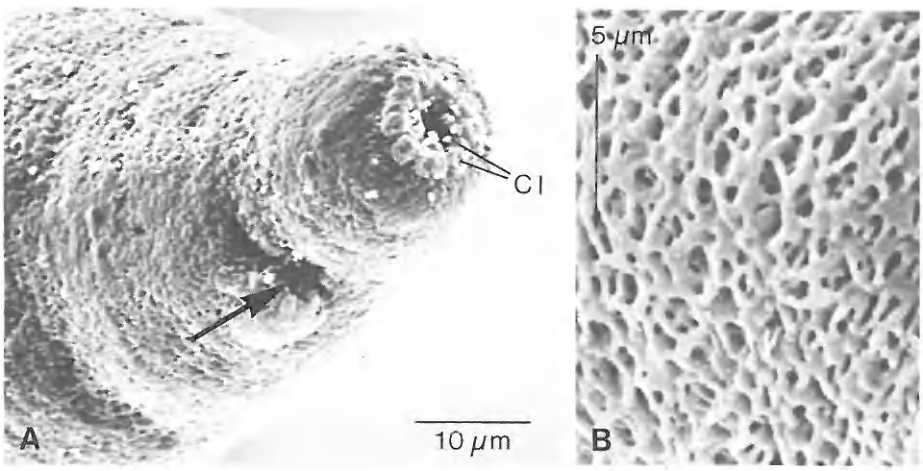

FIG. 2. Stereoscan micrographs of rediae of *Aporocotyle simplex*. A, anterior end of a large second-generation redia showing the mouth surrounded by cilia (Cl) and the birth pore (arrow). B, detail showing the lace-like tegument.

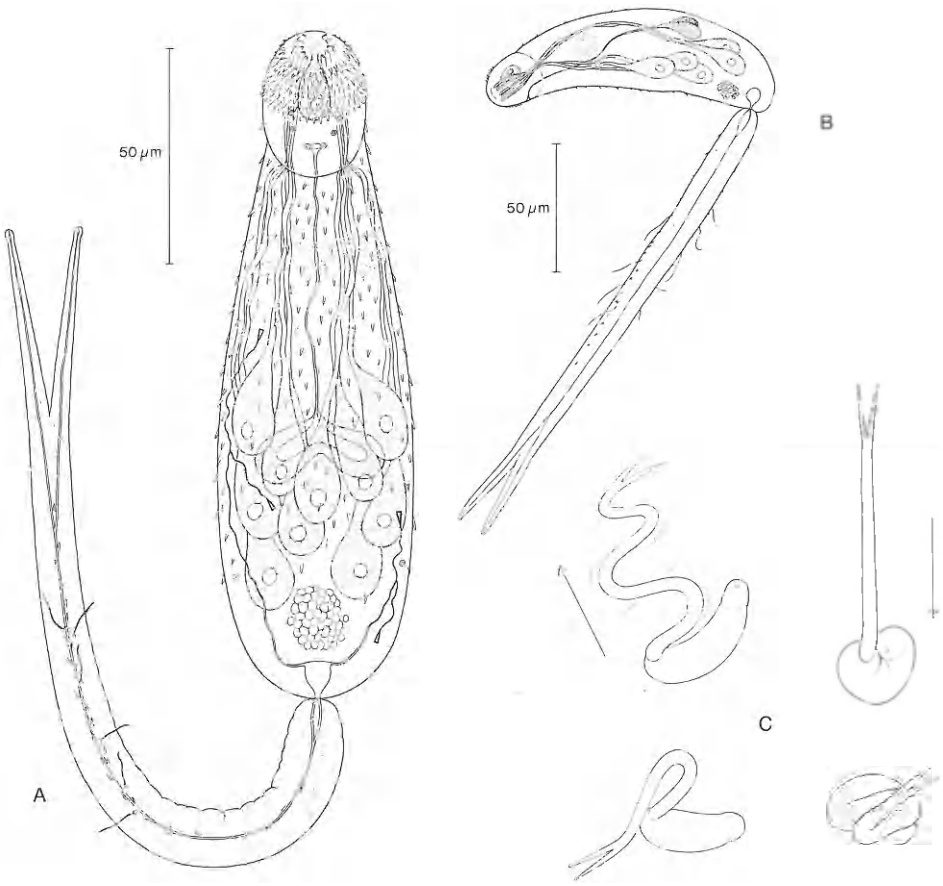

FIG. 3. Cercariae of *Aporocotyle simplex*. A, ventral view of living, slightly flattened specimen. B, lateral view of unpressed specimen. Only the left penetration glands are shown. C, swimming and resting positions of cercariae.

cases host cells were recognizable in the caecum. The length of the caeca of both small and fully developed rediae ranged from one half to one third of the length of the rediae.

The posterior end of both small and fully developed rediae varied between being pointed when extended and having small lateral processes when contracted.

The mouth is surrounded by about 12 annular-shaped elevations, from the center of which a short cilium projects. Shorter cilia projecting at the general surface level occur closer to the mouth opening (Fig. 2A).

The external surface of the rediae has a lace-like appearance (Fig. 2B). This enormous increase in the external surface indicates that absorption of soluble organic nutrients may play an important role for this redia which is surrounded by the nutrient-rich coelomic fluid.

Other redial surfaces known at the ultrastructural level have microvilli or ridges (see Køie 1971). The different surface topography may be explained by the different ecological conditions under which the different rediae live. Although nutrients are absorbed through the external surface of rediae in snails (Køie 1971), the microvilli or ridges may play an important role in the digestion of the surrounding snail tissue. The redial surface of *A. simplex* is probably not responsible for the disintegration of host tissue, as it rarely is in close contact with host cells contrary to rediae in snails.

The cercaria

As spontaneously emerged cercariae were unavailable the most developed cercariae from the rediae were used for the description. Some of these cercariae were infective and therefore supposed to be fully developed.

The cercaria has the general sanguinicolid features. It is apharyngeate, nonocellate, monostomatous, furcocercous and provided with a characteristic cephalic organ (= head organ, = penetration organ).

The body of living, slightly flattened cercariae is 140-170 μm (mean of ten specimens: 160 μm) long and 36-46 μm (mean: 40 μm) in maximum width (Figs 3A, 6A). The body of unpressed cercariae can be extended to a length of about 220 μm. Some measurements of cercariae fixed unpressed in formalin are given in Table 2.

The length of the tail of slightly flattened cercariae is 100-230 μm (mean: 190 μm) and the width is 12-20 μm (mean: 14 μm). The furcae are 34-46 μm (mean: 40 μm) long. During swimming the tail may be extended to several times the length of the body (Fig. 3C).

The body is cylindrical to pyriform depending on the state of contraction. A constriction appears between the cephalic organ and the remaining body.

The oval to spherical cephalic organ is 28-46 μm (mean: 34 μm) long. The anterior extremity is rounded or has a subterminal depression depending on the degree of contraction of the protrusible cephalic organ (Fig. 4). The cephalic organ is provided with six to nine circlets of spines about 3 μm long. There are about 44 spines in the posterior circlet and fewer in the more anterior circlets.

The subterminal ventral mouth appears as a small transverse slit (Fig. 4C, E). The anterior part of the long, narrow, slightly sinous oesophagus continues

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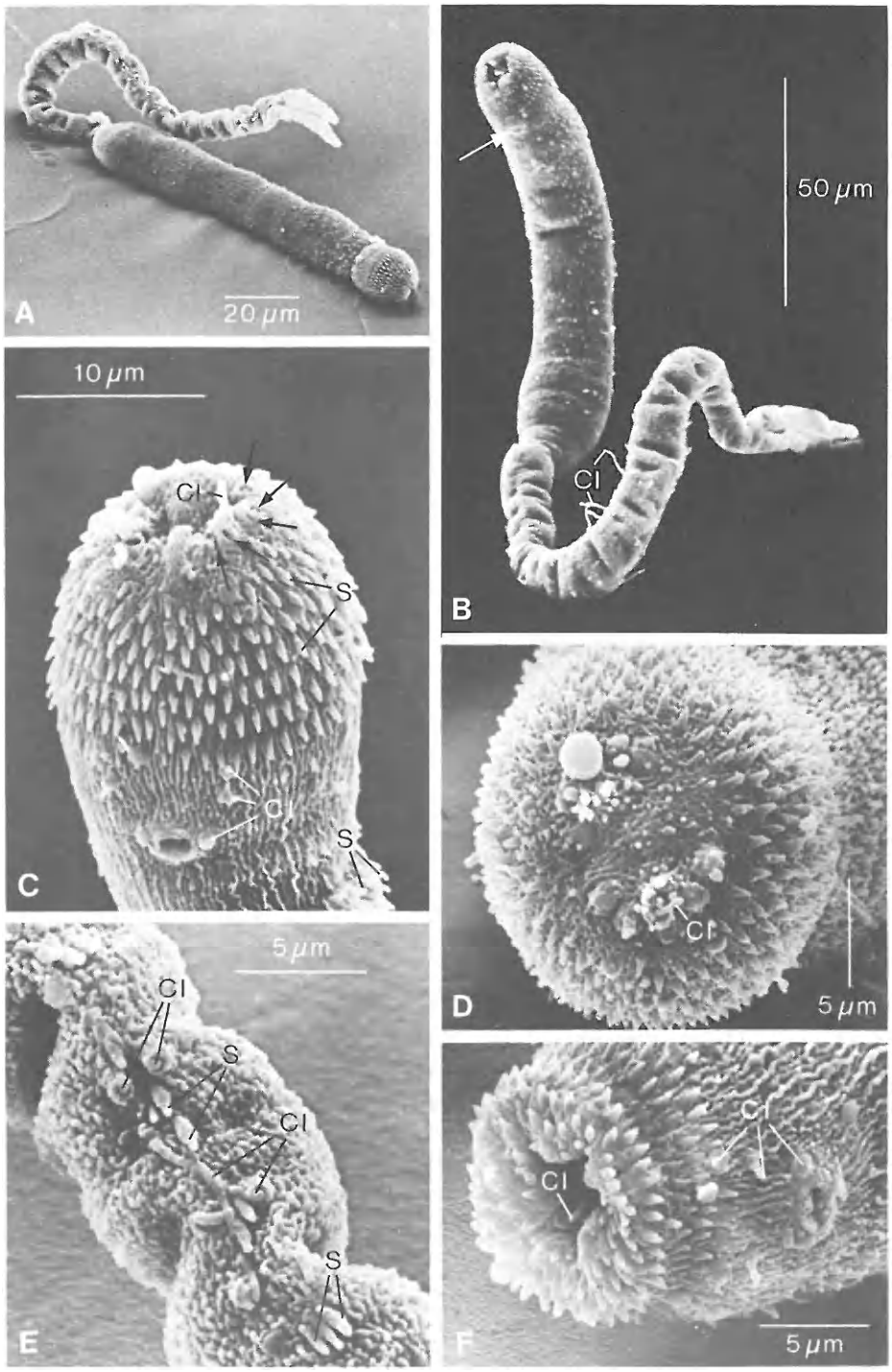
FIG. 4. Cercariae of *Aporocotyle simplex*. A, dorsal view. B, ventral view, arrow shows position of mouth. C, ventral view of cephalic organ. Note mouth, longitudinal ridges, cilia (CI), and cephalic and body spines (S). Apertures of the five left penetration glands marked by arrows. A droplet is secreted from one of the right glands. D, front view of cephalic organ. Ventral side of cercaria is towards lower left corner. Note the pitted tegument and the secreted droplet. E, detail of Fig. 4A showing dorsal view of tail with spines and cilia. F, cephalic organ with invaginated anterior tip.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ventrally, but posterior to the brain it occurs more dorsally (Fig. 3B). Just posterior to the middle of the body it bifurcates into short caeca, which run posteriad. The lumen of the caeca, which stains heavily with neutral red, (Fig. 6A) is surrounded by thick structureless walls. One, rarely two or three, highly refractile bodies occur at the end of the oesophagus or in the caeca. These bodies were in flattened cercariae released through the mouth and they may represent crystalline excretory products.

A pyriform, unicellular cephalic gland (= head gland) occurs medially, slightly dorsally and entirely within the cephalic organ. The contents are PAS-positive, stain blue with Heidenhain's azan and red with neutral red. The aperture was not seen, and it is supposed that the gland opens into the tegument. The tegument all over the cercarial body is PAS-positive and stains blue with Heidenhain's azan.

The greatest part of the body is occupied by the five pairs of unicellular penetration glands. The ducts of the three most anterior pairs occur close to the oesophagus, whereas the ducts of the two more posterior pairs occur more laterally. The nucleated parts of the anterior glands occur more ventrally than those of the posterior glands (Fig. 3B).

The contents of both types of glands are secreted through ten separated oblong apertures located anteriorly in two lateral crescents (Fig. 4C, D). The apertures of the anterior glands are located most ventrally and those of the posterior glands are located more dorsally. The contents of both types of glands are easily secreted from slightly pressed cercariae, but droplets were also released from unpressed specimens (Fig. 4C, D).

The contents of the anterior glands are PAS-positive and stain blue-violet with Heidenhain's azan. The contents of the posterior glands are PAS-negative, and stain blue with alcian blue and Heidenhain's azan. Both types stain with neutral red, but the posterior glands most heavily and nearly immediately (Fig. 6A).

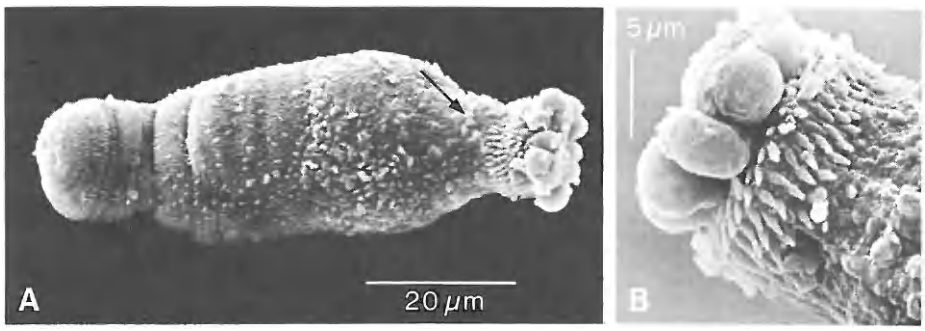


FIG. 5. Cercaria of *Aporocotyle simplex* which has shed the tail and secreted large droplets from all ten penetration glands. A, ventro-lateral view. Arrow indicates the position of the mouth. Note spination on posterior part of the body and secreted material which cover the longitudinal ridges and spines anteriorly. B, lateral view of anterior end.

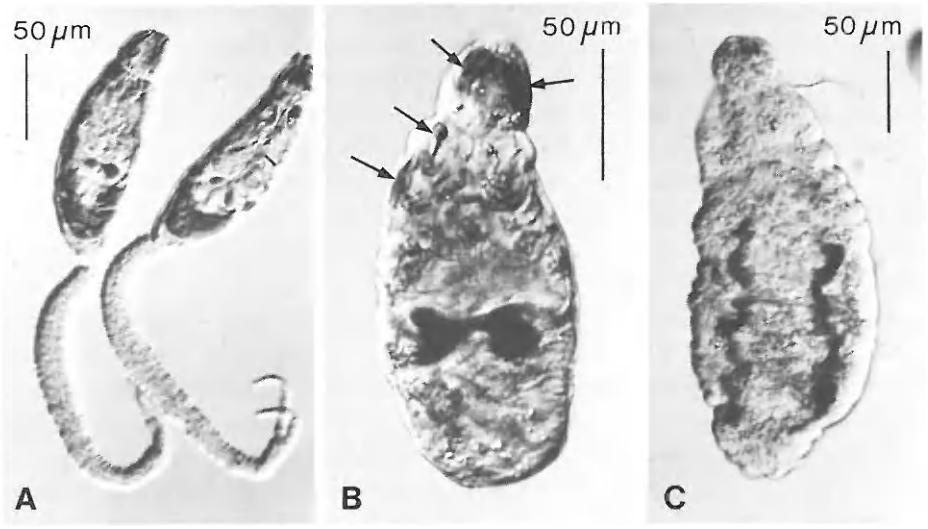


FIG. 6. Cercariae and young specimens of *Aporocotyle simplex* stained *in vivo* with neutral red. A, two cercariae. B, 30-day-old specimen. Note remnants of penetration glands (arrows). C, 50-day-old specimen.

The natural shedding of the tail after long periods of swimming, or mechanical separation of the tail from the body, stimulates the release of material from the ten penetration glands (Fig. 5). Simultaneously the tegument of the anterior part of the body is covered by a secreted material. This material stains the same way as the contents of the cephalic gland and may originate from this gland. The function might be to protect the tegument during the initial stay in the fish host.

Spines identical with the cephalic spines are scattered on the cercarial body. Dorsally the spines are limited to the anterior half of the body (Fig. 4A), ventrally they occur nearly to the posterior end but most abundant anteriorly. However, close to the mouth no spines are found.

A papilla, from the center of which an about 0.5-1.0 μm long cilium projects, occurs on each side of the mouth. Similar papillae are found ventrally and anterior to the mouth, between the cephalic spines and at the extreme anterior end. A few similar papillae occur laterally on the posterior third of the body. Between the apertures of the penetration glands are found a few cilia about 2 μm long.

The cercarial surface has longitudinal ridges. The tegument of the anterior end and especially that of the cephalic organ is increased in area by numerous infoldings which give the tegument a pitted appearance (Fig. 4D).

A genital primordium is situated ventrally close to the spherical to pyriform excretory vesicle. The right excretory duct is longer than the left, resulting in an asymmetrical arrangement of the flame cells. The flame cell formula is $2[1 + 1] = 4$.

Proximally the caudal excretory system is separated into two ducts. A single duct extends through most of the tail stem, but bifurcates before the bifurcation of the tail itself. The ducts discharge through minute vesicles at the tip of each furca.

The surface of the tail is granulated (Fig. 4E) and provided with transverse lateral folds (Fig. 4A, B). Medially, both dorsally and ventrally, the tail has a row of pointed spines. Proximally and distally the spines occur singly, but at about the middle third of the tail two or three spines may occur close to each other laterally (Fig. 4E). In the same area are up to 15 μm long cilia found slightly lateral to the spines.

The cercariae showed no orientation with respect to light. They are relative poor swimmers which rarely rise more than a few cm above the bottom of the container. The violent vibration of the tail carries the cercaria upwards with the tail in advance. The short period of activity is followed by a rest period during which the cercaria slowly sinks with the tail stretched out. Still free in the water, or after having reached the bottom, the cercaria curls up (Fig. 3C). It remains in this position until disturbed. The most developed cercariae from dissected rediae could be maintained alive for about three days (6 °C). It is unknown whether they remain infective for all this period.

The cercaria of *A. simplex* has several features in common with the schistosome cercariae, the only other cercariae of blood-flukes studied using scanning electron microscopy. Both the cercaria of *A. simplex* and schistosome cercariae have a pitted tegument, a feature which is not found in cercariae maturing in the alimentary tract of vertebrates.

The arrangement of the apertures of the penetration glands of the cercaria of *A. simplex* is similar to that of schistosome cercariae, except that the apertures of the latter are surrounded by tegumentary ridges (Robson & Erasmus 1970, Short & Cartrett 1973, Sakamoto & Ishii 1978).

Schistosome cercariae also have two types of penetration glands. However, the histochemical reactions of these glands differ from those of the cercaria of *A. simplex* (see Stirewalt 1974). Schistosome cercariae have a cephalic gland very similar to that of the cercaria of *A. simplex*. The schistosome cephalic gland opens into the tegument at the anterior end of the oral sucker (Dorsey 1976).

The intermediate host, Artacama proboscidea

A. proboscidea, a sedentary tube-dwelling terebellid polychaete, is common on muddy bottom in the northern and middle parts of Øresund. It is extremely common north of the island of Veen at a depth of 40 m where it is the dominating polychaete.

Infested *A. proboscidea* were first found in Dec. 1981, but due to ice the next dredge haul was not made before March 1982. In all 704 *A. proboscidea* (15-60 mm long) were examined (Table 1). The average incidence of infestation was

TABLE 1. *Artacama proboscidea* (15-60 mm long) from Øresund, 40 metres depth, infested with larval stages of *Aporocotyle simplex*.

Date	Number examined	First-generation rediae only	First-, and second-generation rediae w. undevel. cercariae	Most rediae with fully developed cercariae	Number infested	Incidence %
14.12.1981	22	1	1	1	3	(13.6)
4. 3.1982	44	2		1	3	(6.8)
24. 3.1982	76	2		4	6	(7.9)
14. 4.1982	49	1		2	3	(6.1)
24. 5.1982	183	1	3	5	9	(4.9)
15. 6.1982	160	4	2	11	17	(10.6)
16. 7.1982	17			1	1	(5.9)
8. 9.1982	153	1		5	6	(3.9)
	704				48	6.8

6.8 % with no essential seasonal variations being observed. Young infestations were found throughout the period studied (Table 1). Small, intermediate and large *A. proboscidea* showed the same incidence of infestation.

The shape of infested polychaetes did not differ from that of uninfested worms, but in heavy infestations the rediae were visible from the outside.

Even though intact infested polychaetes often were placed in glass containers without substratum for several days (5-10 °C) only a single free-swimming cercaria was observed. No rediae emerged from these polychaetes. Exposure of infested polychaetes to alternating periods of dark and light, or to constant light, failed to stimulate emergence of cercariae.

Uninfested *A. proboscidea* kept as mentioned above were observed to spawn. The large eggs (Fig. 7G) were released through the nephridiopores anteriorly. The cercariae may similarly be released through the five pairs of metanephridia which occur in the anterior nine segments. However, the small size of the cercariae also makes it likely that they may emerge through the body wall of the host.

No extraredial cercariae were found in the serially sectioned polychaetes. It is suggested that possible extraredial cercariae have been shed during the dredging and subsequent washing, and that the unfavourable conditions in the laboratory result in the cessation of release of cercariae from the rediae.

Uninfested *A. proboscidea* contain sexual products throughout the year, and spawning apparently occurs all the year round. In infested *A. proboscidea* with a few (less than about 50) first-generation rediae sexual products were still found (Fig. 7G). During growth and multiplication of the rediae the gonads and

free sexual products of the host atrophy, presumably due to competition by the rediae for the available nutrients. As a consequence all *A. proboscidea* harbouring rediae became sterile due to the castration by the parasites.

Even in young infestations the rediae were found in the coelomic cavity throughout the polychaete body, showing that they may perforate the thin septa posteriorly.

Both uninfested and heavily infested *A. proboscidea* had abundant coelomocytes free in the coelomic cavity. The coelomocytes were spherical to discoid with transitional stages suggesting that they may represent stages in a single cell line (Fig. 7E). It was not evident whether the parasites stimulate the cellular defence system of the host as the total number of coelomocytes appeared to be less in infested than in uninfested polychaetes.

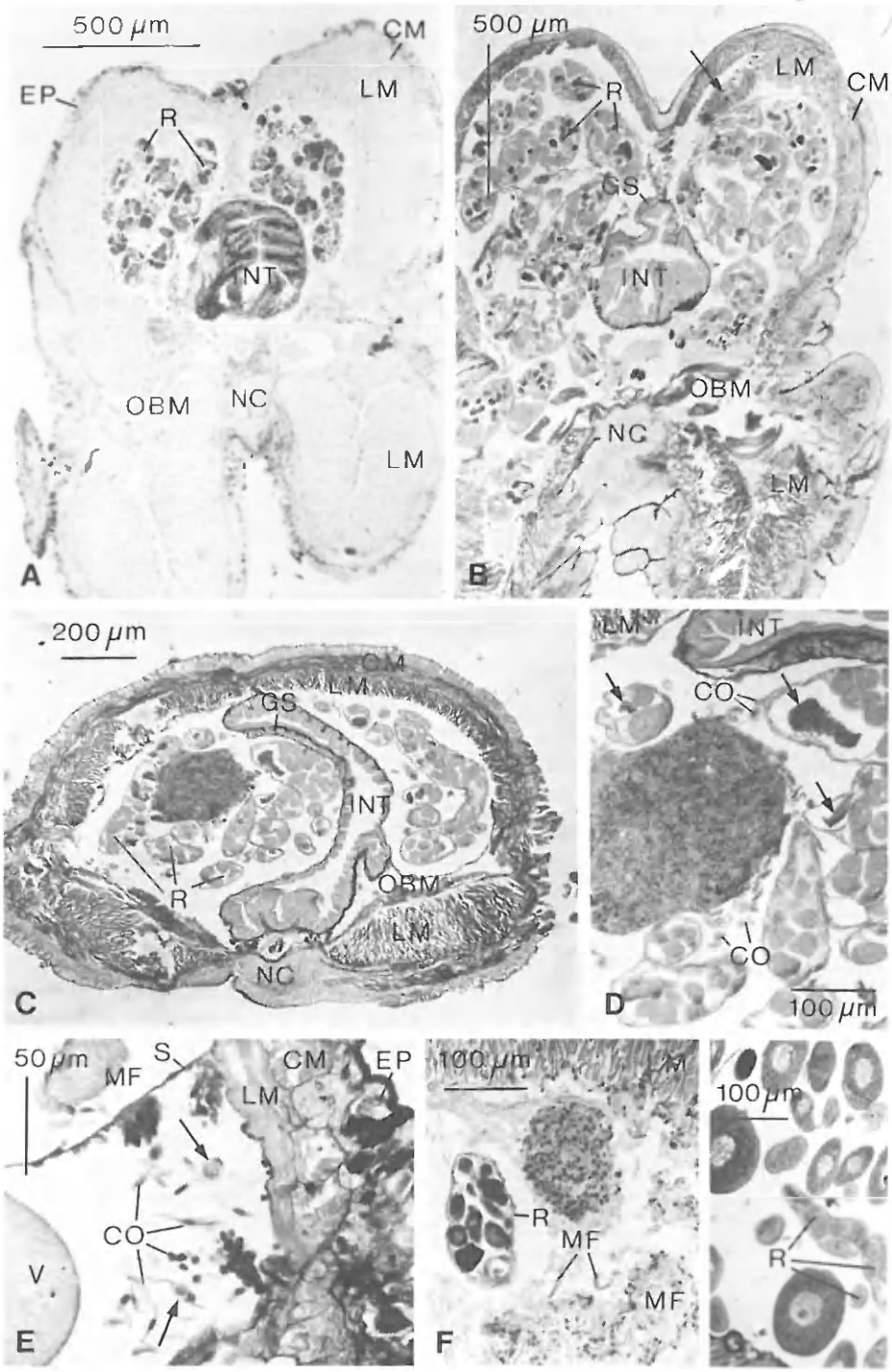
Uninfested *A. proboscidea* and polychaetes with a young infestation have a thick muscular body wall (Fig. 7A). In infested *A. proboscidea* the rediae cause atrophy of muscles, especially the longitudinal muscles, with the result that the body wall of polychaetes with old infestations becomes thin (Fig. 7B, C). The more or less disintegrating muscle fibres were found free in the coelomic cavity (Fig. 7F) and coelomocytes functioning as phagocytes aggregated between and around the necrotic host tissue, but no regular granulomata were found. Large irregular aggregations of more or less disintegrated host cells were occasionally found in the coelomic cavity (Fig. 7C, D). These aggregations were often closely surrounded by rediae. An apparently identical material was found in the lumen of the redial caeca, suggesting that they ingest the disintegrated tissue as well as the coelomocytes.

The intestine, the nerves and the closed vascular system were not affected by the rediae.

No dead rediae were found, and in no cases were rediae encapsulated by coelomocytes as has been described for other polychaetes infested with metacercariae and other parasites (see Dales 1978).

FIG. 7. *Artacama proboscidea* infested with rediae of *Aporocotyle simplex*. A, cross section through the middle part of the body of a polychaete with a young infestation. Toluidine blue. B, similar section of a heavily infested polychaete. Note thin and disintegrating (arrow) longitudinal muscles. Heidenhain's azan. C, cross section of heavily infested polychaete. Rediae and an aggregation composed of necrotic host cells and coelomocytes are seen in the coelomic cavity. Heidenhain's azan. D, detail of Fig. 7C showing the aggregation surrounded by rediae. Note the material in the lumen of redial caeca (arrows). Heidenhain's azan. E, part of a septum and peritoneum from which it is suggested that the coelomocytes are derived. Coelomocytes of different shapes occur free in the coelomic cavity. Some of the cells are apparently phagocytic (arrows). Alcian blue-PAS. F, more or less disintegrated muscle fibres and coelomocytes in the coelomic cavity. Alcian blue-PAS. G, a young infestation where rediae and eggs occur together. Heidenhain's azan.

Abbreviations: CM, circular muscle; CO, coelomocyte; EP, epithelium; GS, gut sinus; INT, intestine; LM, longitudinal muscle; MF, muscle fibre; NC, ventral nerve cord; OBM, oblique muscle; R, redia; S, septum; V, vessel of closed vascular system.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Due to the thin body musculature the infested polychaetes were more vulnerable than uninfested polychaetes. The body wall of heavily infested polychaetes ruptured easily, giving rise to loss of coelomic fluid and projection of the intestine through the wound. As a consequence these polychaetes soon die. Large *A. proboscidea* do not show a higher incidence of infestation than younger specimens. This indicates that infested polychaetes do not survive for years.

Experimental infection of the fish host

The potential hosts for *A. simplex* in Øresund are long rough dab *H. platessoides*, dab *L. limanda*, plaice *P. platessa*, and flounder *Platichthys flesus* (L.). As *H. platessoides* is difficult to keep alive in aquaria the three last-mentioned fishes were used as experimental hosts. The fishes, which measured 6-12 cm in length, were kept in aquaria for six months to one year before they were exposed to cercariae.

As naturally emerged cercariae were unavailable, cercariae from dissected rediae were used. Two to four fish were placed for about 20 hours (6 °C) in a glass container (20 cm ∅) without substratum together with thousands of cercariae. After exposure the fish were transferred to aquaria with recirculating water from the closed water system (10 °C). Two dabs harbouring 3 and 5 large *A. simplex* from natural infestations in the branchial vessels died during the exposure. All fish without a natural infestation survived the exposure.

Dabs were used to study the growth and development of *A. simplex*. They were dissected 4, 17, 30, 42, 50, 62, 72, 83, 94, 132, and 180 days after exposure (Fig. 9).

Two dabs were exposed twice with an interval of 42 days. They were examined 50 and 94 days after exposure.

To study whether the cercariae showed a preference for either plaice or flounder two specimens of each species were exposed to the cercariae in the same container and examined 42 and 132 days after exposure.

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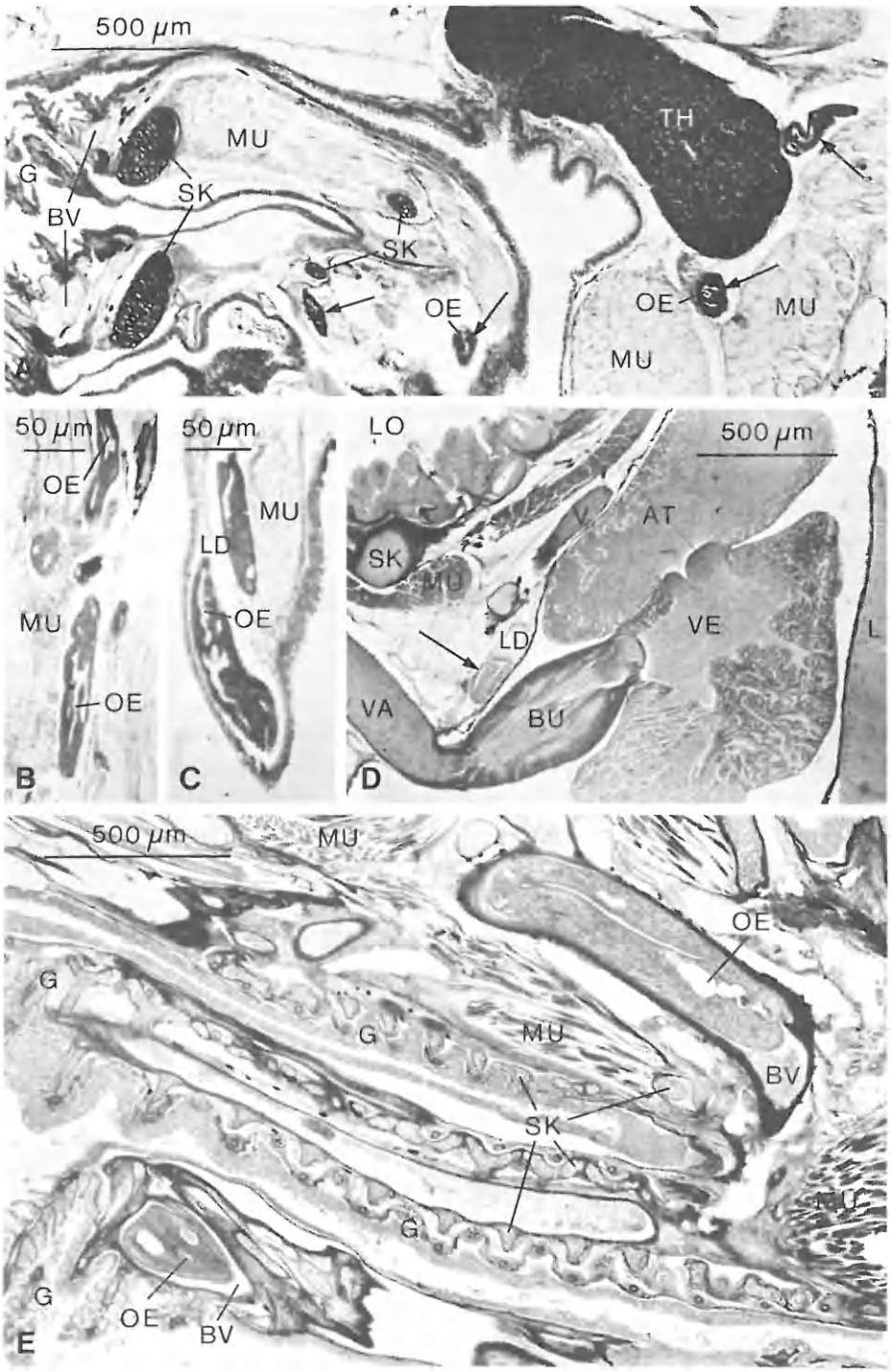
FIG. 8. *Aporocotyle simplex* in dab *Limanda limanda* (7-10 cm long). A, oblique section through head of dab harbouring a 62-day-old infection. All the parasites (arrows) occur in the lymphatic system. Toluidine blue. B, five 72-day-old worms in cephalic lymph ducts and muscles. Toluidine blue. C, two 83-day-old worms in the operculum. Toluidine blue. D, the heart without parasites and a 83-day-old worm (arrow) in a lymph duct. Heidenhain's azan. E, two more than ten-month-old *A. simplex* from natural infestations. The worms occur in highly distended branchial vessels. The longitudinally sectioned specimen at the top is bended ventrally. It is apparently ingesting cells of the inner wall of the blood vessel, and similar cell material is found in its oesophagus. Heidenhain's azan.

Abbreviations: AT, atrium; BU, bulbus; BV, branchial vessel; G, gills; LD, lymph duct; L, liver; LO, lumen of oesophagus; MU, muscle; OE, oesophagus of parasite; SK, skeletal structure; TH, thymus; V, blood vessel; VA, ventral aorta; VE, ventricle.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All three flatfishes showed the same intensity of infection. About 60 to 100 *A. simplex* were found in each fish exposed once. The largest fish harboured slightly more *A. simplex* than the smallest. The intensity was lower than should be expected considering the large number of cercariae available. Probably only one or two cercariae from each redia were fully developed and therefore infective.

The dabs infected twice had about the double number of worms than those exposed only once, and the size and development of the worms did not differ from worms of identical age from dabs exposed once. This indicates that the host immune system has no effect on the early development of *A. simplex*.

The cercariae of *A. simplex* penetrated flounders as easily as plaice. The development of *A. simplex* in these fishes was identical to that of *A. simplex* in dabs.

The following results are from experimentally infected dabs. Four days after infection the small worms were found under the skin and between the muscles throughout the body of the fish, indicating that the cercariae penetrate the skin all over the fish body. Approximately the same number occurred in the posterior as in the anterior half of the body. The head contained a few worms, but none were found in the blood system.

The habitats of all or nearly all 17- to 83-day-old *A. simplex* were the lymphatic system and the muscles of the dabs (Fig. 8A-D). The worms were evenly distributed, no more worms occurred in the anterior part – the head not included – than in the posterior part of the fish body. About one sixth to one third of the worms were found in the lymphatic system of the head, the greatest number being found in the oldest infections. The worms probably enter the head lymphatic system via the neural lymph duct (see Wardle 1971). No worms were found in the blood system.

In the fish body most 62- to 94-day-old worms were found in the interspinal lymph ducts and the neural lymph duct (see Wardle 1971). In newly killed dabs cut into halves the worms were seen emerging from the neural lymph duct in great numbers.

A few 94-day-old worms were found in the afferent branchial vessels, but none were found in other parts of the blood system. The major lymph spaces in the head of teleosts are linked to the ducts of Cuvier (see Wardle 1971). It is suggested that the parasites – as the lymph – enter the heart and the afferent branchial vessels via these ducts. About half of the 132- and 180-day-old specimens occurred in the branchial vessels. One fourth of the worms were found in the interspinal lymph ducts and neural lymph duct throughout the fish body and the remaining fourth occurred in the cephalic lymph sinuses and ducts. About 50 worms were found in the branchial vessels of one of these dabs. It is unlikely that it would survive this infection for long (see Fig. 8E showing two large *A. simplex* from natural infestations in the branchial vessels of a small dab). However, no dabs died during the 180 days where the infections were

followed and the parasites had no visible effect on the dabs. No dead worms were found. No host defence reaction was observed. The worms which occurred free in the muscles were sometimes surrounded by more or less disintegrated host tissue (Fig. 8B).

Young developmental stages of A. simplex in the fish host

Fig. 9 shows the length of *A. simplex* during the first six months of life in the lymphatic system of dabs. During the first weeks the growth was slow, and after about 50 days the length was only twice that of the cercaria. Subsequent growth was faster. A marked increase in length occurred after two months. However, worms of identical age varied greatly in length and development.

Four-day-old worms were of similar shape as the tailless cercariae. The cephalic gland had disappeared. The penetration glands were still recognizable, although most of the contents had been secreted. The intestine was unchanged. Numerous spherical bodies, probably lipid droplets, occurred all over the body.

In some of the 30-day-old worms growth of the anteriorly directed caeca had started (Fig. 6B). In some 42-day-old worms the four caeca were equally long (Fig. 9). In older specimens both the anteriorly and posteriorly directed caeca

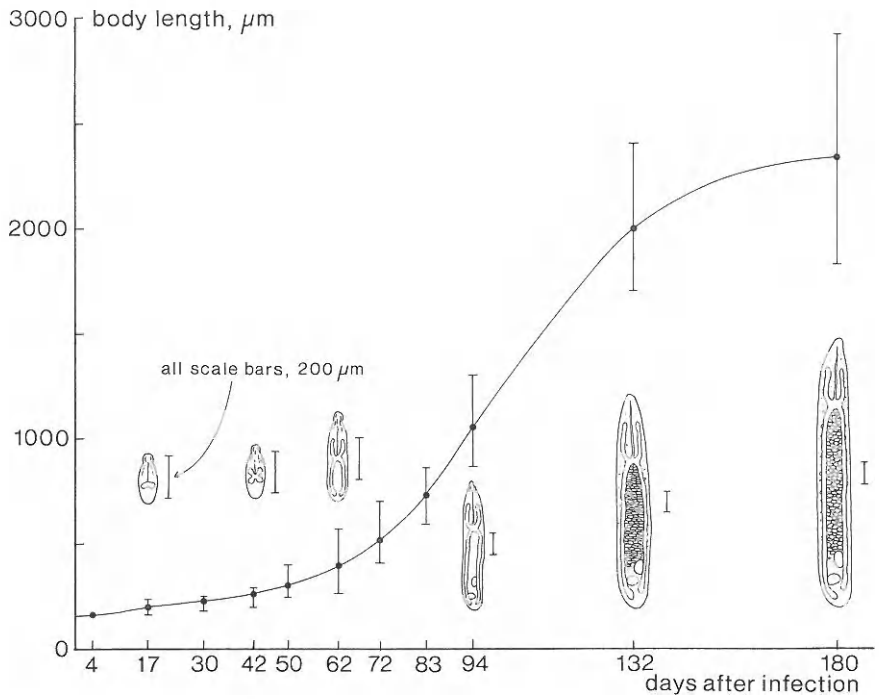

FIG. 9. Diagram showing the growth of *Aporocotyle simplex* living in the lymphatic system of experimentally infected dab *Limanda limanda*. Measurements based on living, slightly flattened specimens. Mean size and range of ten randomly selected specimens.

showed a rapid increase in length. In most 50-day-old worms the posterior caeca nearly reached the posterior end of the body (Fig. 6C). During subsequent growth the relative length of the different parts of the digestive system changed enormously. In most 62-day-old worms the length of the oesophagus was about half the length of the body, whereas in most 180-day-old worms it was about one fourth of the body length.

Remnants of the penetration glands was still recognizable 94 days after infection; they stain *in vivo* with neutral red.

The body shape gradually changes from pyriform-cylindrical to flat and elongate (Fig. 9).

The cirrus sac and the ovary were clearly observed in most 94-day-old worms. The reproductive system of most 132-day-old worms was apparently fully developed, but uterine eggs were only found in 180-day-old worms. However, most eggs were abnormal which seems to be a common feature of *A. simplex* in dabs (see also Thulin 1980a). No difference in development was found between specimens living in the lymphatic system and specimens living in the blood system of the dabs. The lymph of plaice has a composition similar to blood plasma (Wardle 1971). This may explain why the growth of *A. simplex* is identical in the two habitats.

The worms living in the blood system were easily recognized by the yellow-brown contents of the intestinal caeca, whereas the contents of worms from the lymphatic system were light-yellow except for the dilated ends of the four caeca where the contents were darker yellow. Remnants of host cells were found in the oesophagus and caeca of worms more than 62-day-old, but, as could be expected due to the small size of the mouth, whole cells (lymphocytes, erythrocytes) were only found in 132- and 180-day-old worms.

Stereoscan micrographs of four-day-old worms revealed that all the papillae with short cilia situated anteriorly persisted, whereas the apical cilia had disappeared.

The external surface of 17-day-old worms was more corrugated than younger stages with deeply folded ridges. The cephalic spines were unchanged, but the body spines had regressed, being reduced to about half the original length.

The 30-day-old worms had completely lost the body spination. The deeply folded tegument anteriorly still had the pitted appearance. The tegument of most 42-day-old and 50-day-old worms was covered by a material which probably had been secreted by the parasite.

Most 62-day-old worms were slightly flattened. They had apparently lost the pitted tegument anteriorly, and the whole surface was covered by irregular, mostly longitudinal ridges. Small tubercles or bosses appeared on the lateral margins (Fig. 10A-C). Initially they are without spines. As development proceeds, the number of spines on the tubercles increases, as does the size of the tubercles.

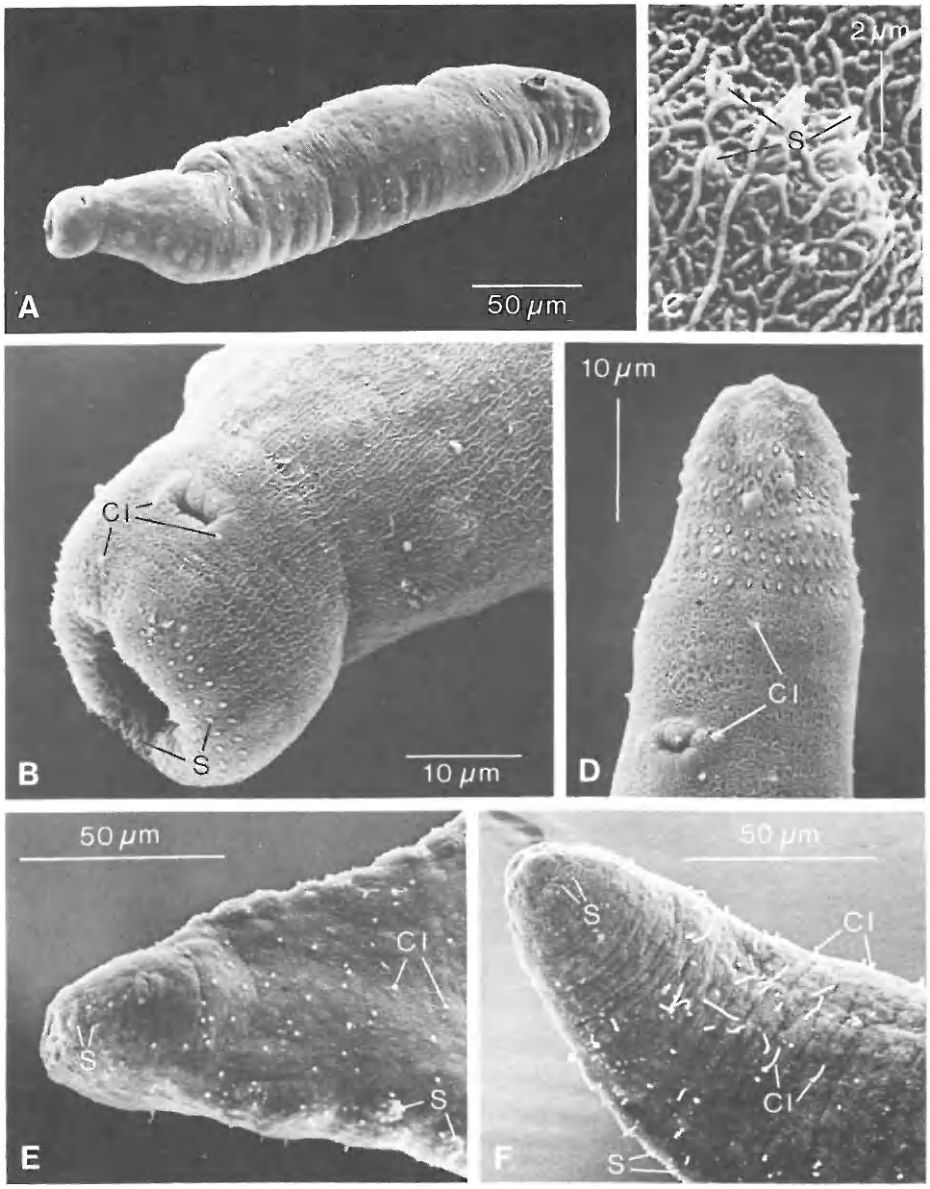

FIG. 10. Stereoscan micrographs of *Aporocotyle simplex* from the lymphatic system of experimentally infected dab *Limanda limanda*. A, ventral view of 62-day-old specimen. Note small tubercles on anterior lateral margin. B, detail og Fig. 10A. The anterior tip is invaginated, forming a sucker-like structure apically. Note cephalic spines (S), mouth, sensory cilia (CI) and longitudinal ridges. C, detail showing undeveloped spinous tubercle of a 62-day-old worm. D, the anterior end of a 72-day-old worm with protruded anterior tip. E & F, 180-day-old worms. E, ventral view showing cephalic spines, mouth, ventral cilia and ventro-lateral spinous tubercles. F, dorsal view showing long cilia.

In 180-day-old worms the tubercles had also appeared ventro-laterally. The cephalic spines remained unchanged. Papillae, each with a short cilium, were found ventrally (Fig. 10E), and up to 15 μm long cilia were symmetrically arranged dorsally on the anterior part of the body (Fig. 10F).

Thulin (1980b) describes the surface structures of large *A. simplex* from the gill arteries of *H. platessoides*.

Both the cephalic and the body spines may assist in penetrating the skin of the fish. This may be the only function of the body spines as they disappear shortly after penetration. The main function of the cephalic spines may be to assist migration in the lymphatic vessels, but they may also function as anchors before the development of the lateral spines.

The migration of *A. simplex* may be compared with that of schistosomula in the mammalian host. The schistosomulum of *S. mansoni* uses the cephalic spines – in addition to spines posteriorly – as anchorage during movement in blood vessels (Crabtree & Wilson 1980). However, the cephalic spines of *S. mansoni* disappear as the cephalic organ develops into the oral sucker. The cephalic spines of *A. simplex* remain throughout its life, showing that they must have a function even after the development of the lateral spines.

The lateral spinous tubercles may play an important role as providing purchase on the wall of the lymphatic vessels or blood vessels. The development and function of the spinous tubercles of *A. simplex* are comparable to similar structures on the dorsal surface of male *Schistosoma* spp. (see e.g. Voge *et al.* 1978, Mansour & Voge 1981).

The morphological change of the external tegument of the anterior end, from being pitted and ridged to being ridged only, coincides with the development of the four large caeca. The increased surface may have a function in the uptake of soluble organic nutrients during the initial stay in the fish host before the digestive system is fully developed. *A. simplex* differs in this respect from *Schistosoma* spp. where the pitted tegument of the cercaria becomes more deeply pitted and even lace-like in mature specimens (see e.g. Hockley 1973, Voge *et al.* 1978, Mansour & Voge 1981).

McLaren & Hockley (1977) compared the external membrane of different blood flukes, viz. *Schistosoma* spp., *Spirorchis* sp., and *A. simplex* and *A. spinosicanalis* Williams, 1958 with that of digeneans belonging to families which inhabit the gut and associated body cavities of the host. All the blood flukes studied had a double outer membrane in contrast to the single membrane of non-blood flukes. The authors conclude that the outer membrane of blood flukes assists in protecting the parasite against the immunological response of the host.

Natural infestation of the fish hosts

As *A. simplex* has been found mainly in the heart, the ventral aorta and the gill arteries (see references below) only these parts of the fishes were examined. The potential hosts for *A. simplex* in Øresund, i.e. *P. flesus, P. platessa, L. limanda* and *H. platessoides*, were studied. Only the three latter were infested. Only plaice more than three years old were infested, whereas both 0-group dabs and 0-group long rough dabs were infested. Long rough dabs were most heavily infested, showing a 100 % incidence of infestation, and often with more than 50 mature worms in the above mentioned habitats. About 80 % of dabs more than about 15 cm long were infested, and the intensity of infestation was lower than that of long rough dab. 30 lemon sole *Microstomus kitt* (Walbaum) (25-45 cm long) from the same area in Øresund where the infested flatfishes were caught were uninfested.

A. simplex in long rough dab and plaice were up to about 10 mm long and they had most often hundreds of spindle-shaped eggs, whereas specimens in dab rarely became more than six mm in length and most often had abnormal eggs (see also Thulin 1980a).

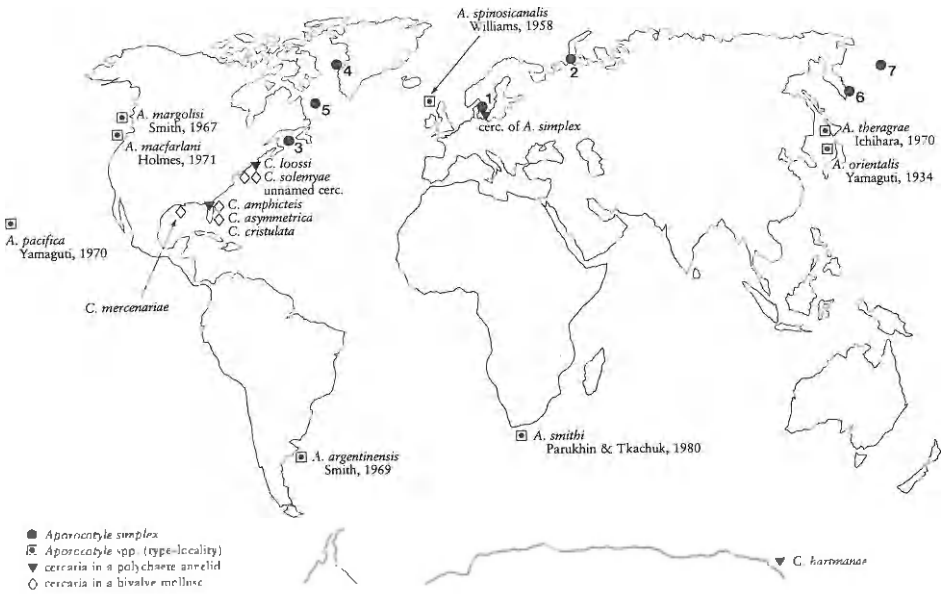

FIG. 11. Records of marine sanguinicolid cercariae and *Aporocotyle* spp.

A. simplex appears to be specific to pleuronectids. It has been recorded in gadoid and macrourid fishes as well (e.g. by Grabda 1977, Zubchenko 1981), but these specimens probably belong to *A. theregra* Ichihara, 1970 (see Smith 1972, Thulin 1980a).

A. simplex has an arctic-boreal distribution. It has been recorded from seven areas on the northern hemisphere (Fig. 11): (1) in *H. platessoides, L. limanda, P. platessa,* and *P. flesus* from the type-locality, Kristineberg, western Sweden, and Øresund, Denmark (Odhner 1900, 1911, Thulin 1980a, and present study, (2), (3) and (4) in *H. platessoides* from the Barents Sea, the Gulf of St. Lawrence, and Disco Bay, western Greenland (Isaichikov 1933, Ronald 1960, and own observations, July 1978, respectively), (5) in *H. platessoides* and *Glyptocephalus cynoglossus* (L.) from the northwest Atlantic (Zubchenko 1980) and (6), (7) in *Hippoglossus hippoglossus* (L.), *Hippoglossoides elassodon* Jordan & Gilbert, and *Reinhardtius hippoglossoides matsuurae* Jordan & Evermann from the Bering Sea (Mamaev *et al.* 1963, Mamaev 1965).

Nine species of the genus *Aporocotyle* have been described (Fig. 11) (see references in Yamaguti 1971, Smith 1972, in addition Parukhin & Tkachuk 1980).

Redescription of Cercaria hartmanae Martin, 1952

Cercaria hartmanae, which has a terebellid polychaete as intermediate host, is very similar to the cercaria of *A. simplex* (Table 2) suggesting a close relationship between the two cercariae. However, according to Martin (1952) *C. hartmanae* has a ventral sucker, which is not a sanguinicolid feature. Hence a reexamination of the holotype of *C. hartmanae* seemed to be necessary. The stain had faded and it was impossible to distinguish the penetration glands. Apart from the 'ventral sucker' the observations by Martin (1952) were confirmed. The structure interpreted as the ventral sucker appears as an empty-looking spherical vacuole about 6 μm in diameter. An identical structure occurs in the same part of the body of immature cercariae of *A. simplex* and represents the end of the undeveloped digestive system. Later the spherical structure divides into the two caeca. Comparing the two cercarial species it is evident that the spherical structure in *C. hartmanae* represents the undeveloped caeca.

Martin had only one infested polychaete at his disposal, and although it contained a large number of rediae most of these contained germinal balls and, in a few instances, cercariae. The description was made on cercariae dissected out of the rediae, and they were probably not fully developed.

DISCUSSION

Odhner (1900) found one *A. simplex* on the gill of a flounder *P. flesus*, but since that it has never again been recorded in flounders, although a large number of flounders has been examined (Odhner 1900, 1911, Thulin 1980a, present study). The single record in flounder may be due to the different distributions of the flounder and the intermediate host *A. proboscidea*, since flounders prefer shallower and most often less saline water than the polychaete. The different distribution of the two hosts may also explain why small plaice were not found infested in nature, although they – as the flounders – were easily infected in the laboratory.

A. proboscidea occurs in the Arctic, in the northern Atlantic Ocean, in the northern Pacific Ocean, in the northern part of the North Sea and in Danish waters in Skagerak, Kattegat, Great Belt, Øresund and the western part of the Baltic. In addition, it has been recorded from antarctic and subantarctic areas. The vertical distribution is from the upper part of the sublittoral down to more than 3000 m depth (Hartmann-Schröder 1971). In Øresund it occurs as south as Copenhagen. Here it has apparently not been recorded from depths less than about 15 m (Eliason 1962).

The distribution of *A. proboscidea* coincides with that of *A. simplex* except that *A. simplex* has not been recorded from the southern hemisphere. This indicates that *A. proboscidea* is the only intermediate host for *A. simplex*.

The geographical distribution of *H. platessoides* nearly coincides with that of *A. proboscidea* and both occur on muddy bottom at great depths. This may explain why *H. platessoides* in Øresund and off the western coast of Sweden shows the greatest incidence and intensity of infestation, and that it has been mentioned as the main host for *A. simplex* (Odhner 1911, Thulin 1980a). However, *H. platessoides* does not live in the northern Pacific Ocean, and here other flatfishes function as hosts.

New observations on the penetration of cercariae of *Sanguinicola* spp. show that they penetrate the external surface, especially in soft or less heavily scaled areas (Meade & Pratt 1965, Meade 1967). The cercaria of *Sanguinicola* sp. similarly penetrates the skin of carp fry and remains in the skin where it continues to develop to maturity (Iqbal & Sommerville 1982). This observation and the fact that six-month-old specimens of *A. simplex* were found everywhere in the lymphatic system of the fish body indicate that the lymphatic system may be an important habitat for sanguinicolids – as also pointed out by Smith (1972) – but that they remain to be found there in natural infestations. However, since the hatching of the eggs takes place in the gill filaments and the miracidia should be released into the water only worms maturing in the blood system may propagate.

Eight marine cercariae which are supposed to develop into blood flukes of fishes have been described previously (Table 2). These cercariae are in the literature referred to as sanguinicolid or aporocotylid cercariae. They are all monostomatous, apharyngeate, non-ocellate and, with one exception, basically furcocercous.

Most authors (see review article by Smith 1972) refer all blood flukes of fishes to the Sanguinicolidae von Graff, 1907, which has priority over Aporocotylidae Odhner, 1912. Yamaguti (1971) maintained the family Aporocotylidae, containing only the genus *Aporocotyle*, as distinct from Sanguinicolidae. He wrote that cercariae of the family Aporocotylidae develop in serpulid, terebellid or ampharetid polychaetes. He had, however, no proof for this as no life-cycles of cercariae developing in polychaetes were known. According to Yamaguti (1971) the cercariae of Sanguinicolidae develop in molluscs, never in annelids. Yamaguti (1975) included *C. asymmetrica* and *C. cristulata* from marine bivalves in the family Aporocotylidae without giving any reasons.

Following Skrjabin (1951), Van der Land (1967) and Smith (1972) I regard all blood flukes of fishes as belonging to the family Sanguinicolidae.

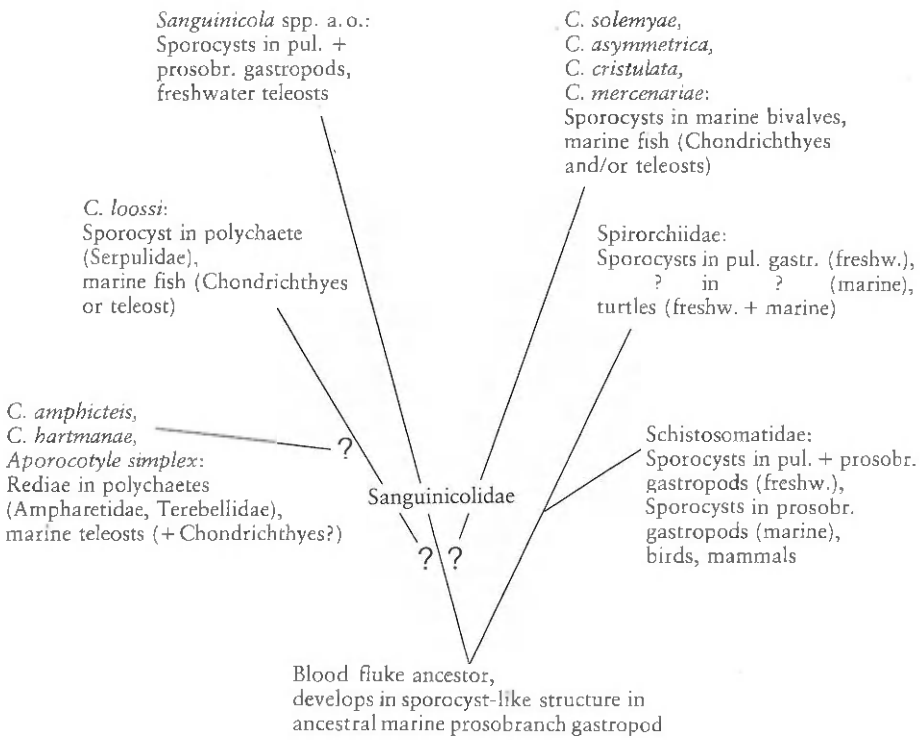

FIG. 12. Suggested phylogenetic relationships of blood flukes based on developmental stages in the intermediate hosts.

Numerous attempts have been made to described the systematic and phylogenetic relations of blood flukes based on the morphology of adult specimens. It is generally accepted that the blood flukes of birds and mammals are related to those of fish and turtles (Smith 1972, Cable 1974). All blood flukes have a two-host cycle; the cercariae penetrate the final vertebrate host directly. As the vertebrate host is regarded as secondary the most reliable phylogenetic relationships may be elucidated by studying the developmental stages in the intermediate hosts (see Cable 1974). In addition, the digeneans are more specific for their molluscan than for their vertebrate hosts, and in certain instances the final hosts are apparently so recent that constant and permanent relations have not been established.

It is suggested that the blood fluke ancestor was furcocercous, distomatous, apharyngeate and provided with a specialized cephalic organ (Fig. 12). It probably developed in a sporocyst-like structure in an ancestral marine prosobranch gastropod. The furcate tail is common in cercariae and has probably evolved more than once. The specialized cephalic organ may be regarded as an advanced feature; it is found in related but pharyngeate families (e.g. Diplostomidae and Strigeidae (see also Cable 1974)). The cephalic organ of Spirorchiidae and Schistosomatidae may in adult worms develop into an oral sucker, whereas it in adult Sanguinicolidae disappears or is very weakly developed. Apharyngeate cercariae occur in a few other families which, however, are not closely related to the blood flukes. According to Yamaguti (1971) some adult spirorchiids have a pharynx. However, the descriptions are not convincing. The oesophagus of these parasites are often surrounded by gland cells; these cells may in some species have been interpreted as a poorly developed pharynx.

At some stage the blood flukes split into two groups; one remained distomatous (Schistosomatidae and Spirorchiidae), whereas the other lost the ventral sucker and became monostomatous. Some adult spirorchiids and schistosomes have a reduced ventral sucker, others lack a ventral sucker completely, but the ventral sucker is always present in the cercarial stage. Blood flukes of birds and mammals separated from those of turtles and became gonochoristic, whereas the other blood flukes remained hermaphroditic. The monostomatous branch developed into the blood flukes of fishes.

Several life-cycles of freshwater sanguinicolids are known (see Smith 1972), but the only marine sanguinicolid life-cycle known is that of *A. simplex*. All freshwater sanguinicolid cercariae develop in sporocysts (with one questionable exception, see Wales 1958) in gastropods.

The marine sanguinicolid cercariae differ from all other known blood flukes in that they do not develop in gastropods but in bivalves and polychaetes. However, it can not be excluded that they occur in marine prosobranchs although they have not yet been found there.

Four cercariae have polychaetes as the only intermediate hosts (Table 2). These polychaetes belong to three related families, Serpulidae, Ampharetidae and Terebellidae, the two last-mentioned being the most closely related (see Fauchald 1977). The three cercariae which develop in Ampharetidae and Terebellidae differ from other sanguinicolid cercariae in that they develop in rediae which in all three species are very similar in shape and size (Table 2). The cercariae have no dorsal or furcal fin-folds. The lack of furcae in *C. amphicteis* may be an adaptive feature in its life-history with no systematic significance. *C. amphicteis* and the cercaria of *A. simplex* have apparently identical asymmetric flame cell systems; that of *C. hartmanae* is unknown.

It is obvious that *C. amphicteis* and *C. hartmanae* are closely related to the cercaria of *A. simplex*, and it is likely that they all belong to the genus *Aporocotyle* which has been recorded from all over the world (Fig. 11).

The morphology of *C. loossi*, which develops in sporocysts in a serpulid polychaete, suggests that it is more closely related to the sanguinicolid cercariae which develop in sporocysts in molluscs than to those developing in rediae in terebellid or ampharetid polychaetes. *C. loossi* is apparently morphologically more similar to the sanguinicolid cercariae which develop in freshwater gastropods (see e.g. Erickson & Wallace 1959, Meade 1967) than to those which develop in marine bivalves (Table 2).

Nobody has attempted to explain the presence of cercariae in polychates although the problem often has been commented on (e.g. Wright 1971, Margolis 1971, Smith 1972).

Turbellarians, especially Rhabdocoela, often form associations with molluscs, and it is generally accepted that digeneans are primary parasites of molluscs and developed from marine rhabdocoel-like turbellarians (Jennings 1974, Cable 1974).

Endocommensal or endoparasitic turbellarians have never been recorded in polychaetes (Myzostomidae not included) (Jennings 1974) indicating that the larval digeneans in polychaetes probably have another origin. In addition, it is unlikely that some sanguinicolids arose from turbellarian-like ancestors living in association with polychaetes and other sanguinicolids arose from turbellarian-like ancestors living in association with molluscs. It is obvious that the sanguinicolids which develop in polychaetes and the sanguinicolids which develop in molluscs have the same ancestor and that their morphological and ecological similarities are not due to parallel development or convergence.

It is well known that some cercariae are able to aquire new snail hosts and that they may change hosts with different ecological situations (Stunkard 1957). However, a change from a molluscan host to a polychaete and from a sporocyst to a redia is not easily explained.

The change might have happened in two steps. First a change of hosts from gastropod to polychaete as examplified by *C. loossi*, whereafter the changed

TABLE 2. Marine sanguinicolid cercariae. Measurements (in μm) are based on fixed specimens.

Name	Host	Develop in sporocyst (S) redia (R)	Body length, Body width	Tail length, Furca length	No. of penetration glands	Intestine	Dorsal fin-fold	Furcal fin-fold	Cephalic organ	Spines on ant. end	Body spination	Locality, Reference
Cercaria solemyae Martin, 1944	*Solemya velum* (Solemyidae)	S	59-78 22-34	16-19 (non-furcate)	2 × 5	4-5 pouches	–	(non-furcate)	–	–	2 longitudinal rows	Woods Hole, Mass, USA Martin 1944a
unnamed	*Aequipecten irradians* (Pectinidae)	S	85 27	115 ?	?	?	+	+	?	?	?	Woods Hole, Mass, USA Linton 1915a, b
Cercaria asymmetrica Holliman, 1961	*Donax variabilis* (Donacidae)	S	92-121 25- 32	219-265 right 33 left 10	right 4 left 3	sac-shaped caecum	+	–	–	about 7 circlets	2 longitudinal rows	Florida, USA Holliman 1961
Cercaria cristulata Holliman, 1961	*Chione cancellata* (Veneridae)	S	240-275 28- 36	265-316 63- 75	4 × 2	2 short caeca	+	+	–	about 6 circlets	2 longitudinal rows	Florida, USA Holliman 1961
Cercaria mercenariae Wardle, 1979	*Mercenaria campechiensis* (Veneridae)	S	240-260* 30- 70	280-390* 60-140	2 × 5	2 short caeca	+	+	–	spines coarser on ant. end	spines on ant. half of body	Galveston, Texas, USA Wardle 1979
Cercaria loossi Stunkard, 1929	*Hydroides dianthus* (Serpulidae)	S	mean 129 mean 30	mean 323 mean 37	2 × 5	un-developed	+	+	+	12-13 circlets	–	Woods Hole, USA Linton 1915a Stunkard 1929 Martin 1944b
Cercaria amphicteis Oglesby, 1961	*Amphicteis gunneri floridus* (Ampharetidae)	R[1]	78-127 13- 17	70-97 (non-furcate)	2 × 5	4 pouches	–	(non-furcate) lateral fin-folds on tail stem	+	5 circlets	–	Florida, USA Oglesby 1961
Cercaria hartmanae Martin, 1952	*Lanicides vayssierei* (Terebellidae)	R[2]	99-143 28- 37	133-195 15- 28	2 × 5	?	–	–	+	about 6 circlets	?	Ross Island, Antarctic Martin 1952
Cercaria of *Aporocotyle simplex* Odhner, 1900	*Artacama proboscidea* (Terebellidae)	R[3]	120-160 30- 50	180-220 40- 60	2 × 5	2 short caeca	–	–	+	6-9 circlets	spines most common on ant. half of body	Øresund, Denmark present study

*Measurements based on living specimens.

1. Length 255-637, width 87-178, pharynx 30 Ø.
2. Length 294-938, width 28-210, pharynx 19-40 × 12-28.
3. Length 230-940, width 30-240, pharynx 20-38 × 16-24.

ecological conditions in some intermediate hosts might have favoured a development of a caecum, thus transforming the sporocysts into rediae.

The occurrence of rediae in several families which are not closely related shows that rediae have developed independently several times during the evolution of the digeneans.

The wide geographical distribution of the cercariae developing in rediae in polychaetes (Fig. 11) indicates that the change of hosts may have taken place in ancient geological time.

The cercariae which use polychaetes as the only intermediate host are apparently just as specific regarding their host as most cercariae which use molluscs. Rankin (1946) studied a large number of polychaetes from Woods Hole, Massachusetts, but only *Hydroides dianthus* was infested. The host specificity of these parasites may be of value in resolving systematic or phylogenetic problems in the different host groups.

Only few observations have been made on the influence of sanguinicolid larvae on the polychaete hosts. Martin (1944b) observed that in some segments of *H. dianthus* infested with sporocysts of *C. loossi* the muscle tissue of the ventral region was invaded by sporocysts and varying degrees of muscle atrophy occurred. Martin (1952) observed that the only infested *Lanicides vayssierei* studied was abnormally swollen due to rediae of *C. hartmanae*.

Martin (1944b) and Oglesby (1961) noticed that respectively entire sporocysts and rediae emerged through pores in the polychaete body wall. The sporocysts and rediae contained cercariae of various developmental stages. Cercariae emerged by rupturing the sporocyst wall. The emergence of the sporocysts and rediae is apparently an artefact as it would result in loss of the immature cercariae which seems unlikely.

The sanguinicolid cercariae in marine bivalves differ from all blood fluke cercariae in that they have no cephalic organ although the cephalic spines often exist (Table 2). In addition, all known sanguinicolid cercariae in bivalves (apart from *C. mercenariae* which was inadequately described) have one lateral row of spines on each side and, apart from the cephalic spines, no other body spination. It thus appears that the sanguinicolid cercariae in bivalves are closely related, although it is unknown whether they form a monophyletic group.

C. cristulata differs from the remaining sanguinicolid cerariae in having flame cells in the tail stem. This is a characteristic feature of spirorchiid and schistosome cercariae. *C. solemyae* differs from the remaining sanguinicolid cercariae in bivalves by having a short tail where only the shape of the caudal excretory duct reveals its furcate origin. *C. solemyae* has the protobranch bivalve *Solemya velum* as intermediate host. This bivalve is of ancient origin and stands rather isolated systematically. It has both primitive and advanced features, as has its parasite.

Marine fish blood flukes occur in both elasmobranchs, *Chimaera monstrosa* and teleosts (see review by Smith 1972). All nine known species of *Aporocotyle* have teleosts as final hosts. The remaining marine sanguinicolid cercariae may have Chondrichthyes and perhaps also teleosts as final hosts. Wardle (1979) mentions that a shark is the most likely final host for *C. mercenariae*.

Several authors have analysed the morphology of adult species of *Aporocotyle* and other marine sanguinicolids to elucidate their taxonomic or phylogenetic relationships. Smith (1969) discussed the phylogeny of three species of *Aporocotyle* in three species of hake (*Merluccius*) and concluded that they form an evolutionary series. Holmes & Price (1980) increased the genealogical analysis to comprise all eight species of *Aporocotyle*. The allometric growth of the digestive system of the worms in the fish host, the changed habitat in the host with age, and the morphological variation with age of e.g. the body spination should, however, be taken into account when these features are used for systematic on phylogenetic analysis (cf. e.g. Van der Land 1967, Smith 1969, Holmes 1971, Brooks 1980, Holmes & Price 1980).

REFERENCES

BROOKS, D.R., 1980. Allopatric speciation and non-interactive parasite community structure. – Syst. Zool. **29**: 192-203.

CABLE, R.M., 1974. Phylogeny and taxonomy of trematodes with reference to marine species. – *In* W.B. Vernberg (ed.): Symbiosis in the Sea, pp. 173-193. Univ. S.C. Press, Columbia, USA.

CRABTREE, J.E. & R.A. WILSON, 1980. *Schistosoma mansoni*: a scanning electron microscope study of the developing schistosomulum. – Parasitology **81**: 553-564.

DALES, R.P., 1978. Defence mechanisms. – *In* P.J. Mill (ed.): Physiology of Annelids, pp. 479-507. Academic Press.

DORSEY, C.H., 1976. *Schistosoma mansoni*: Description of the head gland of cercariae and schistosomules at the ultrastructural level. – Expl Parasit. **39**: 444-459.

ELIASON, A., 1962. Weitere Untersuchungen über die Polychaetenfauna des Öresunds. (Unders. över Öresund 41). – Acta Univ. lund., N.F., 2. Ser. **58**(9): 1-98.

ERICKSON, D.G. & F.G. WALLACE, 1959. Studies on blood flukes of the genus *Sanguinicola*. – J. Parasit. **45**: 310-322.

FAUCHALD, K., 1977. The Polychaete Worms. Definitions and Keys to the Orders, Families and Genera. – Publs Los Ang. Mus. (Zool.) **28**: 1-190.

GRABDA, J., 1977. Studies on parasitisation and consumability of Alaska pollack, *Theragra chalgogramma* (Pall.). – Acta ichthyol. piscat. **7**(2): 15-34.

HARTMANN-SCHRÖDER, G., 1971. Annelida, Borstenwürmer, Polychaeta. – Tierwelt Dtl. **58**: 1-594.

HOCKLEY, D.J., 1973. Ultrastructure of the tegument of *Schistosoma*. – Adv. Parasit. **11**: 233-305.

HOLLIMAN, R.B., 1961. Larval trematodes from the Apalachee Bay area, Florida, with a checklist of known marine cercariae arranged in a key to their superfamilies. – Tulane Stud. Zool. **9**: 1-74.

HOLMES, J.C., 1971. Habitat segregation in sanguinicolid blood flukes (Digenea) of scorpaenid rockfishes (Perciformes) on the Pacific coast of North America. – J. Fish. Res. Bd Can. **28**: 903-909.

HOLMES, J.C. & P.W. PRICE, 1980. Parasite communities: The role of phylogeny and ecology. – Syst. Zool. **29**: 203-213.

IQBAL, N.A.M. & C. SOMMERVILLE, 1982. Development of the blood fluke *Sanguinicola* sp. (Digenea: Sanguinicolidae) in cultured carp fry (*Cyprinus carpio* L.). – *In* M. Müller, W. Gutteridge & P. Köhler (eds): Molecular and Biochemical Parasitology. Suppl., pp. 163-164. Elsevier Biomedical Press.

ISAICHIKOV, I.M., 1933. Contributions to knowledge of parasitic helminths of some groups of vertebrates in the Russian Arctic. A. Trematodes (Part II). – Trudy gos. okeanogr. Inst. 3: 3-36. (In Russian.)

JENNINGS, J.B., 1974. Symbiosis in the Turbellaria and their implications in studies on the evolution of parasitism. – *In* W.B. Vernberg (ed.): Symbiosis in the Sea, pp. 127-160. Univ. S.C. Press, Columbia, USA.

KØIE, M., 1971. On the histochemistry and ultrastructure of the redia of *Neophasis lageniformis* (Lebour, 1910) (Trematoda, Acanthocolpidae). - Ophelia 9: 113-143.

LINTON, E., 1915a. Sporocysts in an annelid. – Biol. Bull. Woods Hole 28: 115-118.

LINTON, E., 1915b. Note on trematode sporocysts and cercariae in marine mollusks of the Woods Hole region. – Biol. Bull. Woods Hole 28: 198-209.

MCLAREN, D.J. & D.J. HOCKLEY, 1977. Blood flukes have a double outer membrane. – Nature, Lond. 269: 147-148.

MAMAEV, Y.L., 1965. Helminths of fish from the Bering Sea. – *In* A.A. Sobolev (ed.): Parasitic Worms of domestic and wild Animals, pp. 168-187. Dalnevostochni Gos. Univ., Vladivostok. (In Russian.)

MAMAEV, Y.L., A.M. PARUKHIN & O.M. BAEVA, 1963. Parasitic worms of flatfishes from far eastern seas. – *In* P.G. Oshmarin (ed.): Helminths of Animals of Primore and the Pacific Ocean, pp. 82-113. Moscow. (In Russian.)

MANSOUR, N.S. & M. VOGE, 1981. Changes in the tegumental surface of *Schistosoma haematobium* during development in the mammalian host. – Am. J. trop. Med. Hyg. 30: 127-134.

MARGOLIS, L., 1971. Polychaetes as intermediate hosts of helminth parasites of vertebrates: a review. – J. Fish. Res. Bd Can. 28: 1385-1392.

MARTIN, W.E., 1944a. *Cercaria solemyae* n.sp., probably a blood fluke, from the marine pelecypod, *Solemya velum*. – J. Parasit. 30: 191-195.

MARTIN, W.E., 1944b. Studies on trematodes of Woods Hole IV. Additional observations upon *Cercaria loossi* Stunkard developing in an annelid. – Trans. Am. microsc. Soc. 63: 237-243.

MARTIN, W.E., 1952. Another annelid first intermediate host of a digenetic trematode. – J. Parasit. 38: 356-359.

MEADE, T.G., 1967. Life history studies on *Cardicola klamathensis* (Wales, 1958) Meade & Pratt, 1965 (Trematoda: Sanguinicolidae). – Proc. helminth. Soc. Wash. 34: 210-212.

MEADE, T.G. & I. PRATT, 1965. Description and life history of *Cardicola alseae* sp. n. (Trematoda: Sanguinicolidae). – J. Parasit. 51: 575-578.

ODHNER, T., 1900. *Aporocotyle simplex* n.g.n.sp., ein neuer Typus von ektoparasitischen Trematoden. – Zentbl. Bakt. ParasitKde, Abt. I, 27(2): 62-66.

ODHNER, T., 1911. *Sanguinicola* M. Plehn – ein digenetischer Trematode! – Zool. Anz. 38(2): 33-45.

OGLESBY, L.C., 1961. A new cercaria from an annelid. – J. Parasit. 47: 233-236.

PARUKHIN, A.M. & L.P. TKACHUK, 1980. New trematode species from the Indian Ocean fishes. – Biol. Nauki 6: 41-44. (In Russian.)

RANKIN, J.S., 1946. Examination of tube-dwelling polychaete annelids for larval trematode infections. – J. Parasit. 32: 92.

ROBSON, R.T. & D.A. ERASMUS, 1970. The ultrastructure, based on stereoscan observations, of the oral sucker of the cercaria of *Schistosoma mansoni* with special reference to penetration. – Z. ParasitKde 35: 76-86.

RONALD, K., 1960. The metazoan parasites of the Heterosomata of the Gulf of St. Lawrence. VI. Digenea. – Can. J. Zool. **38**: 923-937.

SAKAMOTO, K. & Y. ISHII, 1978. Scanning electron microscope observations on miracidium, cercaria, and cercarial papillar patterns of *Schistosoma japonicum*. – J. Parasit. **64**: 59-68.

SHORT, R.B. & M.L. CARTRETT, 1973. Argentophilic 'papillae' of *Schistosoma mansoni* cercariae. – J. Parasit. **59**: 1041-1059.

SKRJABIN, K.I. (ed.), 1951. Trematodes of Animals and Man. Principles of Trematodology. Vol. V. Izdatelstvo Akademii Nauk SSSR, Moscow. (In Russian.)

SMITH, J.W., 1969. On *Aporocotyle argentinensis* n.sp. (Digenea: Sanguinicolidae) from *Merluccius hubbsi*, and the phylogeny of *Aporocotyle* Odhner, 1900 in hake. – J. Helminth. **43**: 371-382.

SMITH, J.W., 1972. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates and some comparison with the schistosomes. – Helminth. Abstr. Ser. A. **41**: 161-204.

STIREWALT, M.A., 1974. *Schistosoma mansoni*: cercaria to schistosomule. – Adv. Parasit. **12**: 115-182.

STUNKARD, H.W., 1929. Further observations on the cercaria which occurs in the marine annelid, *Hydroides dianthus*. – J. Parasit. **16**: 106.

STUNKARD, H.W., 1957. Intraspecific variation in parasitic flatworms. – Syst. Zool. **6**: 7-18.

THULIN, J., 1980a. A redescription of the fish blood-fluke *Aporocotyle simplex* Odhner, 1900 (Digenea, Sanguinicolidae) with comments on its biology. – Sarsia **65**: 35-48.

THULIN, J., 1980b. Scanning electron microscope observations of *Aporocotyle simplex* Odhner, 1900 (Digenea: Sanguinicolidae). – Z. ParasitKde **63**: 27-32.

VAN DER LAND, J., 1967. A new blood fluke (Trematoda) from *Chimaera monstrosa* L. – Proc. K. ned. Akad. Wet., Ser. C **70**: 110-120.

VOGE, M., Z. PRICE & D.A. BRUCKNER, 1978. Changes in tegumental surface during development of *Schistosoma mansoni*. – J. Parasit. **64**: 585-592.

WALES, J.H., 1958. Two new blood fluke parasites of trout. – Calif. Fish Game **44**: 125-136.

WARDLE, C.S., 1971. New observations on the lymph system of the plaice *Pleuronectes platessa* and other teleosts. – J. mar. biol. Ass. U.K. **51**: 977-990.

WARDLE, W.J., 1979. A new marine cercaria (Digenea: Aporocotylidae) from the southern quahog *Mercenaria campechiensis*. – Contr. mar. Sci. **22**: 53-56.

WRIGHT, C.A., 1971. Flukes and Snails. George Allen and Unwin Ltd., London, 168 pp.

YAMAGUTI, S., 1971. Synopsis of Digenetic Trematodes of Vertebrates. Keigaku Publish. Co., Tokyo, Vol. I: 1074 pp.; Vol. II: 349 pls.

YAMAGUTI, S., 1975. A synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates with special Reference to the Morphology of their larval Forms. Keigaku Publish. Co., Tokyo, 590 pp., 219 pls.

ZUBCHENKO, A.V., 1980. Parasite fauna of Anarhichadidae and Pleuronectidae families of fish in the Northwest Atlantic. – Sel. Pap. ICNAF **6**: 41-46.

ZUBCHENKO, A.V., 1981. Parasitic fauna of some Macrouridae in the Northwest Atlantic. – J. Northw. Atl. Fish. Sci. **2**: 67-72.