A Contribution to the Life History of *Bucephalus longicornutus* (Manter, 1954)

by

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A Contribution to the Life History of *Bucephalus longicornutus* (Manter, 1954)*

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Abstract

The sporocyst, cercaria, metacercaria and adult of *Bucephalus longicornutus* (Manter, 1954) (synonym: *Alcicornis longicornutus* Manter, 1954) are described. No redial generation occurs in the life history. Attempts to obtain miracidia from the eggs of experimentally induced adults were unsuccessful.

Sporocysts were recovered from the visceral mass, gills and pericardium of the New Zealand mud-oyster, *Ostrea lutaria* Hutton, 1873. These give rise to free-living cercariae, liberation of which could be up to 10,000 per oyster per day, but was intermittent. Behaviour of the cercaria, including attachment, penetration and encystment, is described. Encysted metacercariae, mature after 75–80 days, were recovered from the fin web, muscles, orbit and branchial chamber of experimentally infected specimens of *Tripterygion* sp. and *Acanthoclinus quadridactylus* (Forster). Adult flukes were recovered from the intestine of *Scorpaena cardinalis* Richardson, 35 days after feeding mature metacercariae, and from the intestine and pyloric caeca of *Kathetostoma giganteum* Haast, the definitive host.

All previous reports of bucephalid cercariae are listed. Reference is made to some cercariae which have been incorrectly assigned to adult genera. Possible taxonomic implications arising from variations found in adult fluke characters are discussed.

INTRODUCTION

Bucephalus longicornutus (Manter, 1954) is of considerable economic importance because its sporocysts infect the New Zealand mud-oyster, *Ostrea lutaria* Hutton, 1873, which is extensively fished in Foveaux Strait and marketed throughout New Zealand. This report of part of its life history confirms experimentally, for the first time, the main features of the life history of a marine member of the family Bucephalidae Poche, 1907.

Members of the family Bucephalidae, commonly referred to as bucephalids or gasterostomes, constitute an aberrant group of digenetic trematodes which, in their adult stage, infect the pyloric caeca, intestine, and more rarely the stomach and body cavity of both marine and fresh-water teleosts. The diagnostic feature of the family is that the mouth is on the mid-ventral surface and leads into a saccular intestine. Other morphological features are essentially the same as for the prosostome digenea (*vide* Dawes, 1946).

The presence of the ventral mouth and sac-like gut have served to link the family Bucephalidae with the Turbellaria, and the family is considered by some authors (e.g., Yamaguti, 1958) to stand apart from other digenetic trematode families in the Order Gasterostomata. On the other hand, the method of formation of the excretory bladder, the type of cercaria and its method of penetration into the second intermediate host has led La Rue (1957) to believe that the family

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has closer affinities with a number of other digenetic trematode families including the Strigeidae, Brachylaemidae, Fellodistomidae, Azygiidae and Schistosomatidae. Dawes (1946) stated (p. 508) that "the phylogeny of the Trematoda is a problem which cannot be solved in the present state of our knowledge" and this still holds true today. Thus the merits of either view given above cannot be evaluated at the present time.

The life history of members of the group is complex, involving three parasitic and two free-living phases, and has been determined for three fresh-water species only. A similar life history is postulated for marine species on the basis of work by Tennent (1906), and Carrere (1937), and stages recovered from marine hosts by various authors. The adults are generally intestinal parasites of predaceous fish. They produce eggs which hatch in water (or possibly within the rectum of the host) liberating free-swimming miracidia. These are small, pear-shaped organisms, and unique among trematode miracidia in that the cilia are borne on rod-like appendages. If they penetrate a suitable bivalve mollusc they develop into long, much branched germinal sacs or sporocysts in the visceral mass and eventually cause "parasitic castration" of the bivalve. Germinal cells in the sporocyst wall are proliferated into the sporocyst lumen and give rise to gasterostomate cercariae which are characterised by long, contractile furcae. After liberation from the sporocyst the cercariae commence a short free-living phase which culminates in attachment to small fish, and each becomes a metacercaria if it successfully penetrates the skin of the fish and encysts. When infected fish are eaten by predaceous fish the metacercariae excyst and develop into sexually mature adults.

The systematics of the family is based entirely on adult morphology. To date, some 140 adult species distributed among 12 genera are known. Hopkins (1956) has suggested that the generally accepted system of classification at the generic level may not be a natural one and the excretory system has much more phylogenetic significance than has been attributed to it in the past. However, until such time as excretory system details of all species are known, the only feasible system to follow is that set out by Yamaguti (1958).

The general uniformity of internal structure found among the adult members of the Bucephalidae has necessitated the use of minor characters to separate species. Judging by the variability of egg size and the relative positions of internal organs exhibited by some species, the validity of established species using such characters is questionable.

Yamaguti (1958) cited authors who have referred bucephalid metacercariae to various adult species of bucephalid. However, this latter practice is questionable because metacercariae are immature and, although they may show generic characters, they do not show the specific characters used to differentiate between adults. The adult status of a metacercaria must be determined experimentally.

To date, 23 bucephalid cercariae have been described. Of these, the adult status of three fresh-water North American species has been determined experimentally and that of one fresh-water European species has also been established on the basis of a solitary recorded species in Europe. The remainder cannot be assigned their adult status under the present system of classification since adult characters are not exhibited by cercariae. Nevertheless, many authors have placed cercariae in adult genera without experimental evidence to substantiate their views.

Many bucephalid cercariae are inadequately described for comparative purposes. *Bucephalopsis haimeanus,* for example, has been reported from 14 different species of bivalve molluscs. Regarding this, Hopkins (1950, p. 12) stated "On the basis of what is now known about the host specificity of trematodes in the precercarial

stages, it seems most probable that each of the bucephalid cercariae reported from a different molluscan host will eventually be found to be a distinct species."

Hitherto, bucephalid sporocysts have been recovered from eight commercially exploited species of marine bivalve molluscs, and it is perhaps surprising to find that the life histories of all of these are unknown although they may at times affect seriously the host stock. It is hoped, therefore, that the present study will stimulate further life history work on this important group of trematodes.

REVIEW OF PREVIOUS LIFE HISTORY STUDIES

Von Siebold (1848) was the first to suggest a possible relationship between the cercaria *Bucephalus polymorphus* von Baer, 1827, from European fresh-water bivalves, and the adult trematode *B. fimbriatus* (von Siebold, 1848) (synonym *Gasterostomum fimbriatum*), from European fresh-water predaceous fishes, on the basis of the ventral mouth and sac-like intestine in each case.

Wagener (1858) outlined the probable life history of *B. polymorphus.* He pointed out that the sporocysts occurred in fresh-water bivalves; cercariae were formed in the sporocyst and when mature were liberated and became free-swimming; encysted, immature bucephalids occurred on the gills of small cyprinid fishes and *B. fimbriatus* occurred in fresh-water perches. Ziegler (1883) also suggested that *B. fimbriatus* was a synonym of *B. polymorphus.*

Only one species of bucephalid is recognised at present from European fresh-water fishes and, therefore, there is no reason to doubt the validity of the suggested life history of *B. polymorphus.* In fact, Poche (1907) formally declared *B. fimbriatus* a synonym of *B. polymorphus.*

Rudolphi (1819) described several bucephalid species from marine predaceous fish and Lacaze-Duthiers (1854) described the first marine sporocysts and cercariae. When Maddox (1867) made the first discovery of encysted immature bucephalids in a marine fish, it appeared likely that marine bucephalids followed a similar life history to that postulated for the fresh-water *B. polymorphus.*

Tennent (1905, 1906, 1909) was the first to attempt to prove experimentally the connections between the various stages in the bucephalid life history. His work was based on the sporocysts and cercariae of *B. cuculus* McCrady, 1874, the sporocysts of which infect *Crassostrea virginica* (Gmelin). However, Tennent disregarded McCrady's name for the cercaria by calling it *B. haimeanus* Lacaze-Duthiers, 1854, even though McCrady had shown that it differed from *B. haimeanus.* The close similarity between the known bucephalid cercariae on the one hand, and adults on the other, led Tennent to believe that all forms were merely "physiological varieties" of the one species. It is clear from the systematic work of many earlier and subsequent authors on the group that this opinion held by Tennent is erroneous.

Hopkins (1954) critically reviewed Tennent's work and stated (p. 354) that "Tennent found immature encysted bucephalids (metacercariae) in *Menidia* and adults in *Strongylura* and assumed they were the same species as the oyster cercariae. He never obtained experimental evidence of a connection between these forms. He did prove that encysted forms in *Menidia* would excyst, live and grow when fed to predaceous fishes but he never completed these experiments . . . Tennent did prove that some unknown gasterostome in *Lepisosteus* was capable of infecting oysters and developing in oysters at least to the sporocyst stage." However, Tennent did not describe the adult from *Lepisosteus,* assuming it to be the same species as other bucephalid adults previously described.

Hopkins (1954) has shown that *Strongylura* harbours three species of bucephalids whose excretory systems exclude any possibility that they might develop from the

cercaria *B. cuculus*. He also described *Rhipidocotyle lepisostei* from *Lepisosteus spatula* and on the basis of the excretory system and Tennent's work, states that this species could possibly develop from *B. cuculus*. However, no infection experiments have been made to confirm this probable relationship.

Levinsen (1881) described the cercaria *B. crux* from the marine mussel *Modiolaria discors*. This cercaria was distinct from *B. haimeanus* in having a posterior, sucker-like extension of the tail-stem. Odhner (1905) considered that this was a stage in the life history of *Prosorhynchus squamatum* Odhner, 1905, and furthermore, he stated that immature specimens of this species had been found encysted in *Cottus scorpio* by Levinsen but identified by this author as *G. armatum* Molin.

Cole (1935) described the cercaria *B. mytili* (which resembles *B. crux*) and, although he was apparently unaware of Levinsen's *B. crux* and Odhner's suggested relationship between *B. crux* and *P. squamatum*, he considered that the identity of *B. mytili* "with a species of *Prosorhynchus* might yet be established."

Chubrik (1952) pieced together various stages in the life history of *P. squamatum*. He followed Odhner by assuming that a cercaria of the *B. crux*-type developed into a species of *Prosorhynchus*.

There is no experimental evidence to substantiate any of these views. Hopkins (1954, p. 355) stated "So far, there is no way to tell which genus of the Bucephalidae a given cercaria belongs to until the life cycle is worked out by means of experimental infections."

Carrere (1937) discovered bucephalid metacercariae in *Atherina* sp. from the Mediterranean Sea. These developed experimentally into a new species, *Dolichoenterum lamirandi*, in *Labrax lupes* and *Hyla arborea*. This, along with Tennent's observation that an unidentified bucephalid in *Lepisosteus* sp. could infect oysters and develop to the sporocyst stage, constitutes the only published experimental proof of a connection between the various stages in the postulated life history of marine bucephalids.

Yamaguti (1958) stated that the life history of the marine bucephalid *Prosorhynchus uniporus* Ozaki, 1924, was determined experimentally by Ozaki (1954). This work has not been found by the author despite an intensive search of the literature.

Three life histories of North American fresh-water bucephalids have been determined experimentally. They are *Rhipidocotyle papillosum* (Woodhead, 1929), and *B. elegans* Woodhead, 1930, determined by Woodhead (1929, 1930); and *R. septpapillata* Krull, 1934, determined in part by Krull (1934), and completely by Kniskern (1952).

MATERIALS AND METHODS

LIFE HISTORY STUDIES

Recovery of cercariae

Live oysters for experimental purposes were obtained from Foveaux Strait between June and October, 1963, and March and July, 1964. Each oyster, right valve uppermost, was kept alive in a 7in diameter finger bowl two-thirds filled with sea-water. Daily examinations of the water were made for liberated cercariae. The greatest concentration of cercariae was usually found on the bottom of the finger bowl opposite the exhalent chamber of the oyster.

Oysters showing infection were transferred to new bowls and those showing no infection had their water changed daily. If the oysters did not liberate cercariae within a week they were opened and a superficial examination was made of their visceral mass for the presence of sporocysts. If sporocysts were absent, the oysters were dissected or sectioned for any deep-seated infection.

Infection experiments

The term infection experiment, used throughout the text, denotes an experiment in which cercariae were used to infect second intermediate hosts to obtain metacercariae. Infection experiments were carried out with small specimens of *Tripterygion* sp., *Acanthoclinus quadridactylus* (Forster), *Helcogramma medium* (Gunther), and *Trachelochismus* sp. as experimental hosts. These fish were collected from Island Bay, Wellington, a locality where, because there are few bivalve molluscs, the chance of prior infection of these fish with bucephalid metacercariae was thought to be unlikely. Confirming this, examination of the fin webs of the fish for encysted metacercariae gave no evidence of infection. However, the fin webs of *A. quadridactylus* are not transparent and consequently no easy check on prior infection could be made.

One to three fish were transferred to each finger bowl containing cercariae on the basis of one fish to every 1,000 cercariae (see p. 6 for method of estimation). The water was stirred to disperse the cercariae. Attachment of cercariae was checked and a note made of the approximate numbers attached to various parts of the fish. The finger bowls were then stacked on shelves, aerated, and left undisturbed for 18 to 24 hours. After this period the water was changed daily and the fish fed on chopped mussel (checked as free from bucephalid sporocysts).

Four invertebrate species comprising a crab, *Cyclograpsus lavauxi*, a bivalve, *Mytilus edulis*, a coelenterate, *Phlyctenactis retifera*, and a polychaete, *Nereis* sp., were collected from rock pools at Island Bay, placed in finger bowls containing cercariae and treated in a similar manner to the experimental fish hosts in attempts to obtain metacercariae.

Feeding experiments

The term feeding experiment, as used here, denotes an attempt to obtain the adults of the metacercariae established experimentally in the small fish listed above by feeding these small fish to larger fish.

The larger fish selected as experimental hosts for feeding experiments were those in which bucephalid infections had not been reported by Manter (1954) or recorded by the author during the course of independent studies. These included *Helcogramma medium, Acanthoclinus quadridactylus, Tripterygion* sp. and *Trachelochismus* sp. collected with a dip net from rock pools at Island Bay; *Geniagnus monopterygius* (Bloch and Schneider) and *Pseudolabrus celidotus* (Forster) collected by otter-trawl in Wellington Harbour; *Scorpaena cardinalis* Richardson and *Pseudolabrus coccineus* (Forster) caught on a hand line at Island Bay. These fish were kept in aquaria supplied with running sea-water from the open sea at Island Bay.

Helcogramma medium, Tripterygion sp. and *Trachelochismus* sp. were fed chopped up pieces of fish that had been experimentally infected with metacercariae. *Geniagnus monopterygius, P. celidotus* and *P. coccineus* would not feed naturally on live or recently killed infected fish and had to be force fed. *Acanthoclinus quadridactylus* and *S. cardinalis* readily took live infected fish placed in the aquaria.

Attempts to obtain miracidia

Eggs were recovered from gravid adult specimens of *Bucephalus longicornutus* that had been established experimentally in *Scorpaena cardinalis*. The eggs were divided into two samples and placed in separate syracuse watch glasses, one of which contained sea-water and the other, sea-water diluted by two and a-half times

its volume with distilled water to make it isosmotic with fish body fluids. The latter is termed diluted sea-water in the remainder of the text. It was unknown whether the eggs might hatch in sea-water or in the rectum of the host and therefore the two possibilities were considered for this experiment. The eggs were examined daily.

Formation of the cyst wall by the cercaria

(a) Experiments were made to determine the reaction of cercariae to increasingly diluted sea-water. Twenty 4in diameter finger bowls were set up, each containing approximately 100 cercariae in 40cc of sea-water. Five cc of distilled water were added to the first finger bowl, 10 to the second, 15 to the third, etc.

(b) The body wall muscles of a freshly killed *Tripterygion* sp. were ground up with a mortar and pestle. The few cc of liquor obtained were decanted from the residue into a syracuse watch glass. Approximately 10 cercariae were added to this liquor with care to exclude as much sea-water as possible.

Estimation of cercariae liberated

Estimations of the number of cercariae liberated from infected oysters were made during June and July, 1964. The procedure was as follows: 11 infected oysters were set up in separate bowls each with one litre of sea-water. Bowls were examined daily. The oyster was first removed from the bowl, the water agitated vigorously to disperse the cercariae and then allowed to settle. The total number of cercariae in the field of view of a Zeiss "Opton" microscope using a ×10 eyepiece and 1.6 objective was counted and averaged over 10 counts. This gave the average number of cercariae in roughly 5cc of water. This method gave a means of repeatable, relative approximations. When very few cercariae were present they were counted individually.

ANATOMICAL STUDIES

Sporocysts

Portions of sporocysts were teased out of the visceral mass of infected oysters and mounted in sea-water. 0.5% neutral red and 0.5% methyl green in sea-water were used as vital stains but these were of little value in clarifying details of structure as stain tended to concentrate in the sporocyst wall and embryonic cercariae. Portions of sporocysts were fixed in warm formol-acetic-alcohol (FAA), and stained in acetic-acid-alum-carmine (AAAC), cleared in xylol and mounted in balsam.

Histological details of the sporocyst were obtained by sectioning portions of the gills and visceral mass of infected oysters. The best fixative was found to be Dubosq-Brasil followed by treatment with Lenoir's fluid for picric acid removal (Gray, 1953). Sections were stained with Heidenhain's haemotoxylin with alcoholic eosin, or Weigert's haematoxylin with picro-ponceau S.

Cercariae

Free-swimming cercariae were examined alive and 0.5% neutral red was occasionally used as an intra vital stain.

Some cercariae were fixed in FAA and stained in AAAC. A tangled cluster of approximately 50 cercariae was fixed in warm FAA and sectioned at 3 to 5μ. No care was taken to orientate the cercariae before embedding since adequate numbers ensured that at least some would be suitably orientated. Sections stained in Delafield's haematoxylin and alcoholic eosin gave the best results.

Metacercariae

Experimentally infected small fish were used as a source of metacercariae for study. After killing the fish, the fins were severed from the body, the two halves

of each fin web separated, and the relative abundance of cysts on the various fins recorded. The skin was then removed from the head, and the underlying tissues, together with the eyes, pharynx and gills, were examined for cysts. Finally, the skin was removed from the remainder of the body, and the body wall muscles and viscera were examined. A similar dissection procedure was used for small fish obtained from the Foveaux Strait oyster beds and Wellington Harbour in attempts to find natural infections of the metacercaria of *B. longicornutus*.

Cysts were freed from host tissue and mounted in diluted sea-water. The metacercariae were readily released from cysts by applying light pressure to the coverslip. Metacercariae were occasionally dissected out of their cysts before being examined, and a few mature metacercariae were found to excyst spontaneously in diluted sea-water.

Permanent mounts of metacercariae were made after fixing under slight pressure in warm FAA and staining in AAAC.

Adults

The technique suggested by Manter (1954) was followed in the examination of all fish for adult worms. Unfortunately, specimens of naturally infected definitive hosts, *Kathetostoma giganteum*, from Foveaux Strait were fixed in formalin before being sent to Wellington and those from Cook Strait were delayed in transit. As a result, of the 75 adult specimens of *B. longicornutus* obtained from this material only 24 were suitable for making whole mounts. These were washed and flattened under slight pressure of a coverslip. Warm FAA was drawn under the coverslip and after approximately five minutes, the slide, still covered by the coverslip, was immersed in a dish of 70% iso-propyl alcohol and left for 18 to 24 hours. This usually ensured that specimens remained flat during staining. Flattened specimens were stained with AAAC using standard procedures. Three adult worms were sectioned, and stained in Delafield's haematoxylin and alcoholic eosin.

Living material examined was limited to the few adults recovered from successful feeding experiments.

THE SPOROCYST

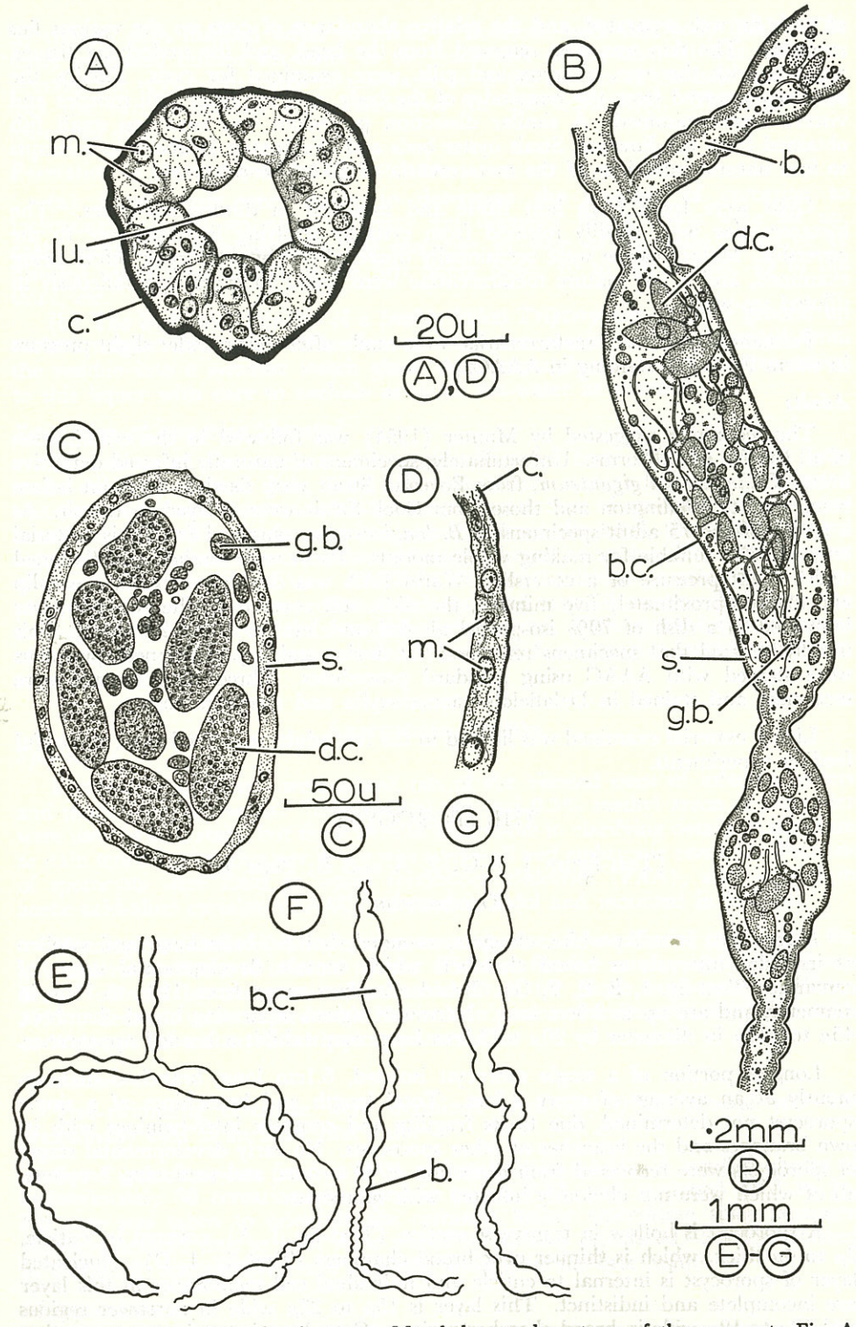
(Text-figure 1, A-G; 2, A and B; Plate 1.)

DESCRIPTION

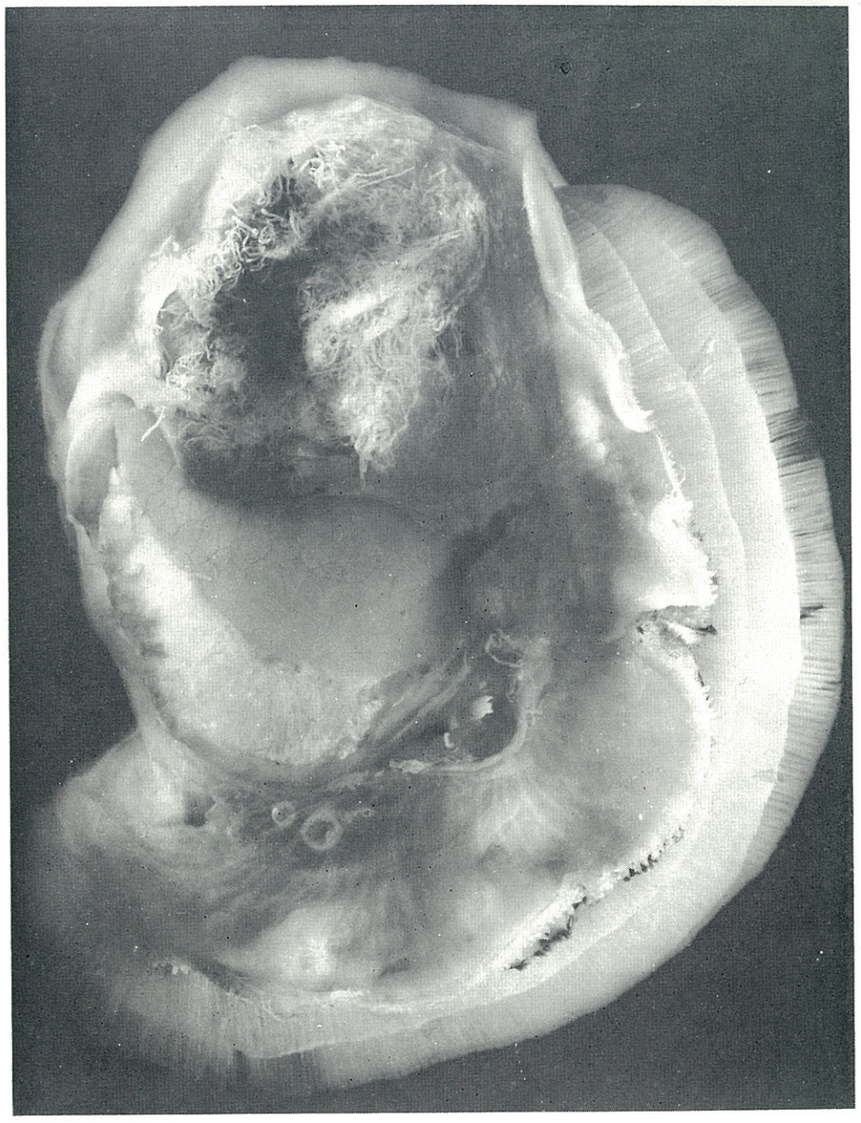
A sporocyst is milky-white, elongate, rounded, hollow, branching, and swollen at irregular intervals as brood chambers which contain developmental stages of cercariae (Text-fig. 1, B, E, F, G). Brood chambers range from 120μ to 750μ in diameter, and are up to 3.5cm long. Narrower regions separating brood chambers, 45μ to 110μ in diameter by 20μ to 3.5cm long, may exhibit a beaded appearance.

Longest portion of a single sporocyst isolated, 6.1cm long, branched dichotomously at an average of every 2.2cm. Total length and branchings of a single sporocyst not determined, due to its fragility and complex inter-twinings with its own branches and the branches of other sporocysts. No early developmental stages of sporocysts were recovered from dissection of 44 oysters, and sectioning 8 oysters, all of which were not obviously infected with sporocysts.

A sporocyst is hollow in transverse section (Text-fig. 1, A), covered by cuticle, 2μ to 4μ thick, which is thinner over brood chambers (Text-fig. 1, D). Nucleated layer of sporocyst is internal to cuticle and individual cell membranes in this layer are incomplete and indistinct. This layer is 15μ to 22μ wide in narrower regions and 10μ to 12μ wide in brood chamber regions. Cytoplasm is continuous suggesting a syncytium, and contains three types of nuclei. Ovoid to spherical vesicular nuclei,



TEXT-FIG. 1.—*Bucephalus longicornutus*. Morphology and anatomy of the sporocyst: Fig. A, T.S. of a sporocyst; Fig. B, whole mount of a portion of a sporocyst; Fig. C, T.S. of a brood chamber; Fig. D, portion of a T.S. through the sporocyst wall of a brood chamber; Fig. E, outline sketch of a branched portion of a sporocyst; Figs. F and G, variations in the morphology of the sporocyst.



Right lateral view of a specimen of *Ostrea lutaria* Hutton, from Area B, Foveaux Strait, infected with the sporocysts of *Bucephalus longicornutus* (Manter, 1954). The visceral mass has been teased open to display the sporocyst tubules more clearly.

5μ to 6μ in diameter, usually with one distinct, eccentrically or centrally situated nucleolus and some peripheral chromatin; and ovoid nuclei, 3μ to 4μ in diameter, containing scattered chromatin granules which may resemble nucleoli, are considered as mesenchymal (somatic) nuclei. In narrower regions, larger of these nuclei lie, in general, nearer cuticle (Text-fig. 1, A). Both types are common throughout all regions of sporocyst; in representative cross section, between 28 and 38 mesenchymal nuclei are generally present. Germinal nuclei are found in syncytium of sporocysts from gill interribs, in and near terminal regions of sporocyst, usually numbering 1 to 2 per cross section. They are spherical, 5μ to 7μ in diameter, with a conspicuous, centrally situated nucleolus, and many small chromatin threads and granules dispersed throughout the nucleoplasm, and stain more intensely with haemotoxylin than mesenchymal nuclei (Text-fig. 2, A).

Germinal nuclei lie in clusters in syncytium after several divisions, and, after further divisions, bulge into sporocyst lumen as a germinal cyst (Text-fig. 2, B). Germinal cysts are analogous with ovaries. Mitotic figures only located in those parts of sporocyst wall where germinal nuclei occur.

Cytoplasm of syncytium essentially hyaline, faintly granular in irregular patches. Cytoplasm forming a distinct band approximately 1μ to 2μ wide around germinal nuclei, is more densely granular than granular patches of cytoplasm in other parts of syncytium (Text-fig. 2, A).

Terminal region of sporocyst is densely nucleated, containing both germinal and mesenchymal nuclei. Cell membranes are more distinct in this region.

No muscle cells found in sporocyst wall and no movements of sporocyst were observed. No indications of an excretory system, testes or specialised feeding or nutritive branches of the sporocyst were observed.

Discussion of Sporocyst

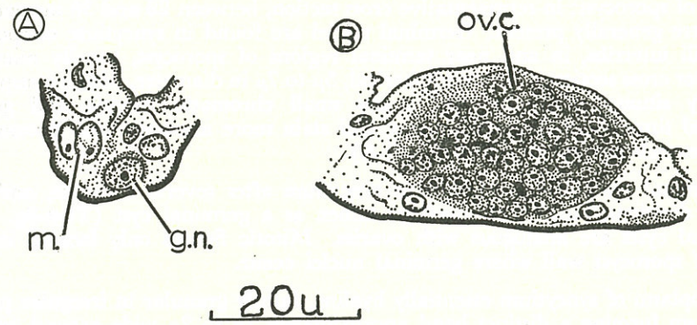
Published accounts of the sporocysts of bucephalids show that there are slight differences in form and structure among the different species.

The beaded appearance of narrow regions alternating with dilated regions or brood chambers has been noted for *Bucephalus cuculus* by Tennent (1906), *Rhipidocotyle papillosum* by Woodhead (1930), and for the present species. Narrower regions of more or less uniform diameter merging with brood chambers has been noted or figured for *Bucephalopsis haimeanus* by Lacaze-Duthiers (1854), *Bucephalus elegans* by Woodhead (1930), *R. septpapillata* by Kniskern (1952), and

List of Abbreviations for all Figures

a., anterior sucker; a.m., anterodorsal musculature of sucker; a.s., axial strand; b., beaded region; b.c., brood chamber; bo., body; c., cuticle; c.f., cuticular flap; c.g., cystogenous granules; ci., rudimentary cirrus sac; c.o., cystogenous organ; c.p., cirrus sac; c.t., constriction; c.w., cyst wall; cy., cyst; d., duct of cystogenous organ; d.c., developing cercariae; e., excretory vesicle; eg., egg; e.p., excretory pore; f., furca; fi., fin web of host fish; fl., flame cell; fp., flap of cuticle; f.r., fin ray; g., intestine; g.a., genital anlagen; g.at., genital atrium; g.b., germ ball; g.c., gland cells; g.d., gland cell ducts; ge., genital lobe; gm., gap between groups of muscle fibres; g.n., germinal nucleus; g.p., genital pore; gr., granules; i.m., undifferentiated mass of cells; l., four lipped anterior extremity; l.c., Laurer's canal; l.d., longitudinal excretory tubule; lu., lumen; m., mesenchymal nuclei; m.c., mucus cells; mo., mouth; mu., muscle layer; mus., core of tentacle; n., ganglionic mass; o., oblique muscle fibres; oe., oesophagus; oo., ootype; ovi., oviduct; ov.c., germinal cyst; oy., ovary; p., pharynx; pa., parenchyma; pap., papilla; p.d., posterior longitudinal excretory tubule; pr., prostate cells; pv., pars prostatica; r., semi-retracted tentacle; r.v., left vitelline duct; s., syncytial layer; s.g., Mehlis's gland; s.v., seminal vesicle; t.d., diagonal excretory tubule; t.du., tentacular duct; te., testes; th., thorn of tentacle; ti., tissue of host origin; tn., tentacle; t.s., tail stem; u., uterus; v., vacuolate intestine; v.f., vesiculate floor of sucker; vit., vitellaria; v.r., vitelline reservoir.

R. papillosum by Ciordia (1956). It is notable that although Woodhead and Ciordia independently examined the same species they have described differences in form, so the external appearance of the sporocysts of the same species is not necessarily constant.



TEXT-FIG. 2.—*Bucephalus longicornutus*. Germinal nuclei and cyst in the wall of the sporocyst: Fig. A, T.S. of a portion of the sporocyst wall near the growing point showing a germinal nucleus; Fig. B, T.S. of a portion of the sporocyst wall near the growing point showing a germinal cyst. For abbreviations see p. 9.

Both Woodhead (1930) and Kniskern (1952) have noted the presence of two types of sporocyst tubules in *R. papillosum* and *R. septpapillata* respectively. The former observed brownish, empty tubules which he interpreted as being spent sporocysts of the previous year. Kniskern observed tubules devoid of cellular content which he termed nutritive branches. However, polymorphism of the sporocyst tubules has not been noted in the present species.

The general structure of the sporocyst wall, namely, cuticle, cellular layer and lumen, is most similar to the sporocyst of *R. septpapillata* as described by Kniskern (1952). However, the present species differs in having two kinds of mesenchymal nuclei. Kniskern's figures do not permit any closer comparisons to be made and he fails to give any description of nuclear detail.

Muscle cells were not observed in the sporocyst wall of the present species. However, Tennent (1906) and Ciordia (1956) have noted "muscle fibres" immediately within the cuticle of the sporocysts of *Bucephalus cuculus* and *R. papillosum* respectively, but Ciordia's figures do not substantiate the presence of such fibres. Apart from Woodhead (1931), who noted undulations in the tips of the sporocysts of *R. papillosum* but did not show muscle cells in his figures, there seems to be general agreement that no appreciable movement of the sporocysts takes place and accordingly, the presence of "muscle fibres" in these two species is unusual. It seems probable that either Kniskern (1952) or Ciordia (1956) is in error since they were both dealing with the sporocysts of related species of *Rhipidocotyle*.

The various interpretations of the cellular layer of bucephalid sporocysts differ markedly. Haswell (1903) inferred that the entire cellular layer of the sporocyst of *Bucephalus* sp. was a "germinal epithelium" and any part of it was capable of proliferation to give rise to cercariae.

Tennent (1906) found two types of nuclei in the cellular layer of the sporocyst wall of *B. cuculus*. The larger of these, which he termed propagatory cells, occurred in the tip of the sporocyst and in parts of the wall nearer the tip. They resemble the germinal nuclei of the present species. These nuclei, as they appear in his

figures, are surrounded by dense cytoplasm. The other type of nuclei in Tennent's figures resembles the larger mesenchymal nuclei of the present species. He stated (p. 649) "There is little doubt that the germ-cells arise in the wall of the sporocyst." However, he gives no evidence to substantiate this statement. The distinctly cellular nature of the terminal region, as described by Tennent, is comparable with the present species.

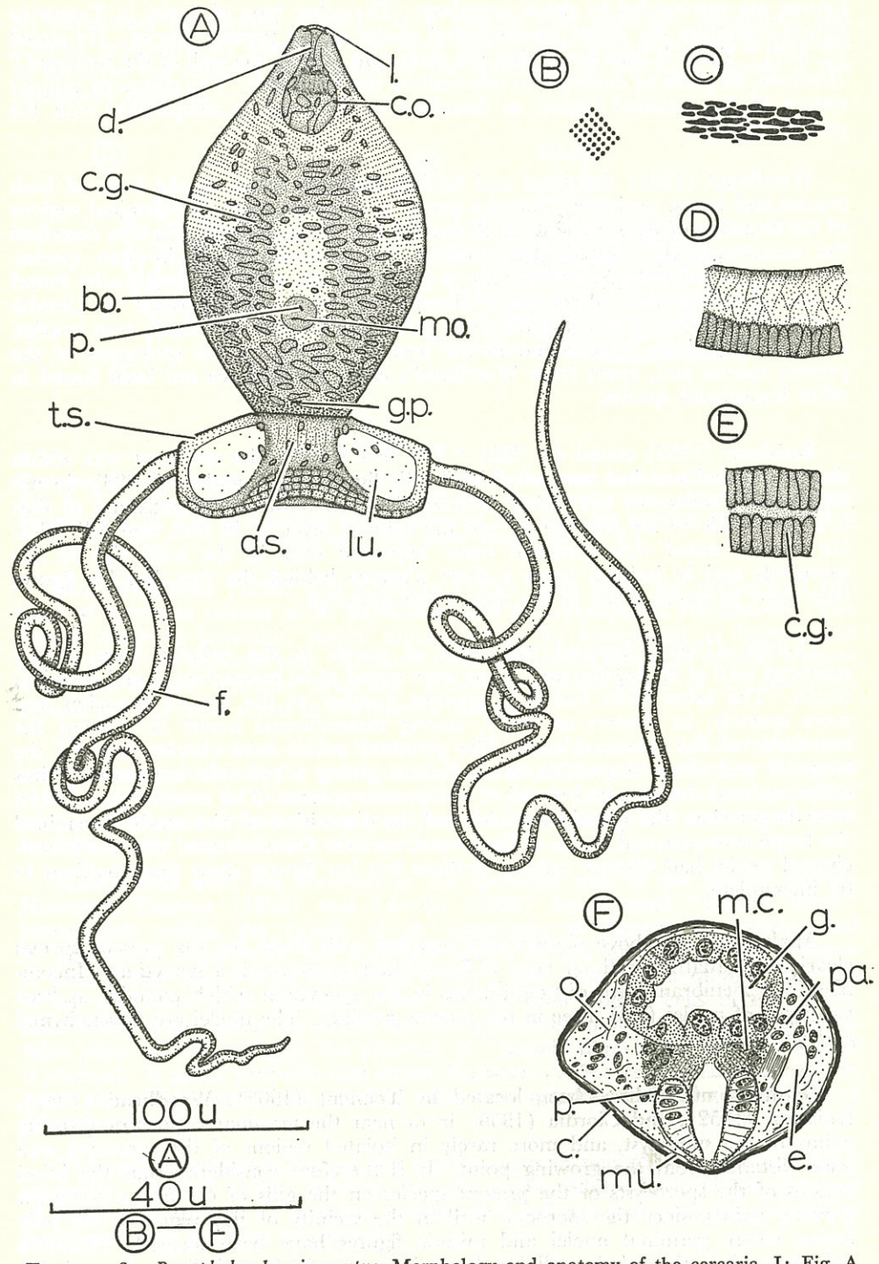
Woodhead (1931) described and figured germinal tissue, in the form of both ovaries and testes, projecting into the lumen as processes from the terminal regions of the branches of the sporocysts of *B. pusillus* and *R. papillosum*. He also described the occurrence of a redial generation in the sporocyst lumen in both species. However, he did not give any details or figures of the other types of nuclei found in the sporocyst wall. Woodhead's observations were not confirmed by Ciordia (1956) who repeated Woodhead's work on *R. papillosum*. No testes, true ovaries, or redial generation were found in the terminal regions of the sporocysts of the present species and, apart from Woodhead's observations, have not been found in other bucephalid species.

Kniskern (1952) stated (p. 322) "The true wall of the sporocyst was within the cuticle and was but one cell thick." His figures (1, 2 and 4, p. 323) do not support this statement but rather indicate that cell outlines are indistinct so that it is impossible to say whether or not the cellular layer is, in fact, one cell thick. He further stated that germinal tissue appeared only in the solid tips of the sporocysts and in isolated zones a short distance behind the tips, but his figures only show germinal tissue in the tips of the sporocyst.

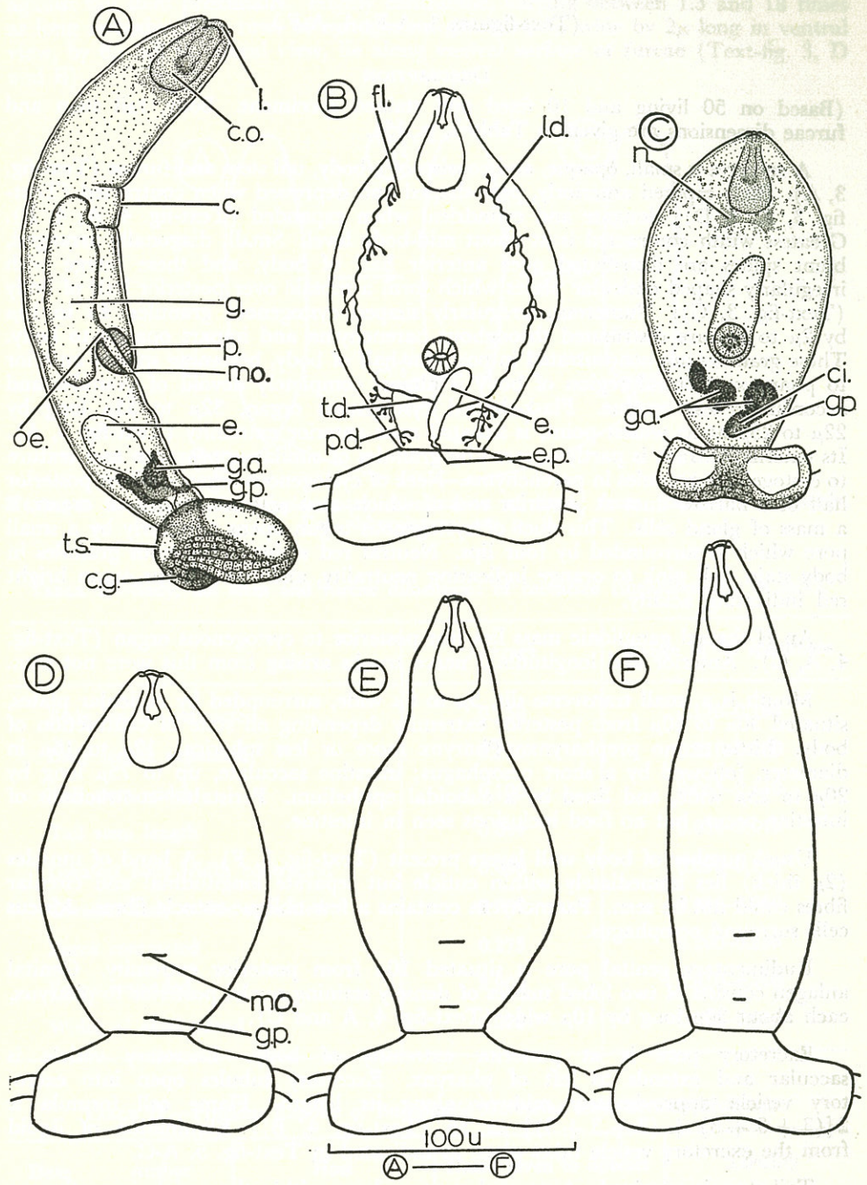
Ciordia (1956) observed two types of nuclei in the cellular layer of the sporocyst wall of *R. papillosum*. These were stated to be mesenchymal and germinal nuclei. The latter were found in greatest number at the tips of branches and more sparsely, in clusters or singly, among mesenchymal nuclei throughout the remainder of the sporocyst wall. The germinal nuclei were characterised by the irregular and dense disposition of chromatin giving a pyknotic appearance, thus differing from the germinal nuclei of the present species. The mesenchymal nuclei were larger than the germinal nuclei and, in disposition of chromatin, resembled the larger mesenchymal nuclei of the present species. Ciordia stated that the mesenchymal nuclei had distinct cellular outlines but her figures show these outlines to be incomplete.

Analysis of the above observations coupled with those for the present species clearly show that the cellular layer of bucephalid sporocysts is a syncytium. Incomplete cell membranes are distinguishable in the syncytium which contains at least two types of nuclei (and three in the present species). The nuclei are mesenchymal and germinal.

Germinal nuclei have been located by Tennent (1906), Woodhead (1931), Kniskern (1952), and Ciordia (1956) in or near the terminal regions or growing points of the sporocyst, and more rarely in isolated regions of the sporocyst wall some distance from the growing point. It is therefore considered that the blind regions of the sporocysts of the present species on the gills of *O. lutaria* represent growing points, since the sporocyst wall in the vicinity of this region is the only region where germinal nuclei and mitotic figures have been located. Haswell's inference, that the whole cellular layer is a germinal epithelium, is probably incorrect. Characterisation of nuclei in descriptions and figures for many species is inadequate and does not permit close comparisons to be made with the present species, and it is evident that the sporocysts of most species need critical restudy.



TEXT-FIG. 3.—*Bucephalus longicornutus*. Morphology and anatomy of the cercaria. I: Fig. A, ventral view of a living specimen; Fig. B, cuticular spines from the anterior region of the body; Fig. C, cuticular plates from the posterior region of the body; Fig. D, lateral view of a furca of a living specimen; Fig. E, ventral view of a furca of a living specimen; Fig. F, T.S. of the cercaria at pharynx level. For abbreviations see p. 9.



TEXT-FIG. 4.—*Bucephalus longicornutus*. Morphology and anatomy of the cercaria. II: Fig. A, lateral view of a fixed and stained extended specimen; Fig. B, diagrammatic representation of the excretory system; Fig. C, ventral view of a fixed and stained contracted specimen; Figs. D to F, body movements in a living specimen. For abbreviations see p. 9.

THE FREE-SWIMMING CERCARIA

(Text-figures 3, A-F; 4, A-F.)

DESCRIPTION

(Based on 50 living and 10 fixed and stained specimens. Body, tail stem and furcae dimensions are given in Table I, p. 15).

A cercaria is small, opaque, and consists of a body, tail stem and furcae (Text-fig. 3, A). Body tapered anteriorly, pear-shaped and depressed when contracted (Text-fig. 3, A; 4, D), elongate and cylindrical when expanded (Text-fig. 4, A; 4, F). Greatest width contracted is at about mid-body level. Small, diagonally arranged, blunt spines are distributed over anterior half of body, and these merge with irregularly shaped cuticular plates which form a mosaic over posterior half of body (Text-fig. 3, C). Numerous, irregularly shaped cystogenous granules, 4μ to 15μ by 2μ to 9μ, are distributed throughout parenchyma and impart opacity to body. These granules are concentrated in posterior half of body, becoming sparser anterior to pharynx. Central region of body is almost completely devoid of granules and accordingly less opaque. Flask-shaped cystogenous organ, 32μ to 35μ long by 22μ to 25μ at its widest point, is situated near anterior extremity (Text-fig. 3, A). Its external surface is partly covered by granules of similar appearance and texture to cystogenous granules in parenchyma. Neck of cystogenous organ encloses posterior half of a narrow duct at posterior end of which, and within cystogenous organ, is a mass of gland cells. This duct of cystogenous organ opens anteriorly by a small pore which is surrounded by four lips. Neutral red stains cystogenous granules in body stain dull pink to orange indicating neutrality, and cystogenous organ bright red indicating acidity.

An H-shaped ganglionic mass lies just posterior to cystogenous organ (Text-fig. 4, A, C). Anterior and longitudinal nerve trunks arising from this were not seen.

Mouth is a small transverse slit, 5μ to 6μ wide, surrounded by cuticular plates, situated 30μ to 40μ from posterior extremity depending on state of contraction of body. Little or no prepharynx. Pharynx more or less spherical, 12μ to 16μ in diameter, followed by a short oesophagus; intestine sacculate, up to 75μ long by 20μ to 25μ wide, and lined by a cuboidal epithelium. Peristaltic contractions of intestine occur, but no food inclusions seen in intestine.

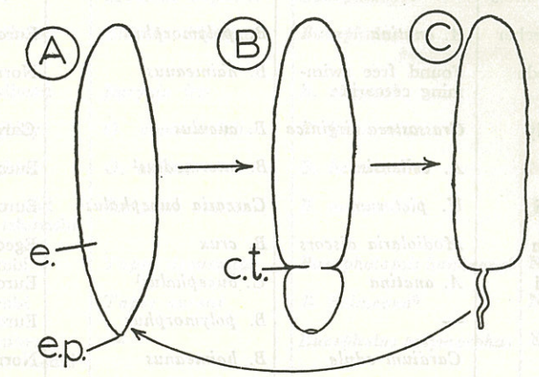
Usual number of body wall layers present (Text-fig. 3, F). A band of muscles (2μ thick) lies immediately within cuticle but separate longitudinal and circular fibres could not be seen. Parenchyma contains a few oblique muscle fibres. Mucus cells surround oesophagus.

Rudimentary genital pore is situated 10μ from posterior extremity. Genital anlagen consists of two lobed masses of densely staining nuclei posterior to pharynx, each about 30μ long by 10μ wide (Text-fig. 4, A and C).

Excretory pore is at posterior extremity of body. Excretory vesicle is saccular and extends to left of pharynx. Excretory tubules open into excretory vesicle approximately midway along its length. Flame cell formula is $2[(3+3+3)+(3+3+3)] = 36$ (Text-fig. 4, B). Elimination of liquid from the excretory vesicle takes place as indicated in Text-fig. 5, A-C.

Tail stem is unspined. A parenchymatous layer within the cuticle is continuous with an axial strand of parenchyma and muscle fibres. A single row of cystogenous granules lies within cuticle along posterior surface of tail stem, apart from axial strand region where granules may be four or five rows deep. A few scattered granules are distributed through axial strand. Lumen of tail stem contains a few oily droplets.

Furcae are sub-circular in section, unspined, tapered distally and flattened against tail stem proximally. Highly contractile, varying between 1.3 and 18 times as long as body. Two rows of cystogenous granules, 6μ wide by 2μ long in ventral view, by 6μ tall in lateral view, lie along ventral surface of furcae (Text-fig. 3, D and E).



TEXT-FIG. 5.—*Bucephalus longicornutus*. Elimination of liquid from the excretory vesicle. For abbreviations see p. 9.

TABLE I.—Body, tail stem and furcae dimensions of cercariae (measurements in mm).

	Average	Range of 50 specimens
Body length contracted	0.150	0.120–0.168
Body length expanded	0.250	0.210–0.290
Tail stem width	0.100	0.085–0.110
Tail stem length	0.044	0.036–0.049
Greatest body width contracted	0.096	0.090–0.108
Greatest body width expanded	0.060	0.056–0.068
Furca contracted	0.275	0.200–0.286
Furca expanded	3.900	3.810–4.100
Width of axial strand	0.026	0.020–0.032

TABLE II.—Previous Reports of Bucephalid Cercariae from Bivalve Molluscs.

Date	Author	Host	Name given to Species	Locality
1827	von Baer	*Unio pictorum*	*Bucephalus polymorphus*	Europe (F)
1827	von Baer	*Anodonta anatina*	*B. polymorphus*	Europe (F)
1827	von Baer	*A. cellensis*	*B. polymorphus*	Europe (F)

1848	Siebold	*A. cellensis*	*B. polymorphus*	Europe (F)
1854	Lacaze-Duthiers	*Ostrea edulis*	*B. haimeanus*	Mediterranean (S)
1854	Lacaze-Duthiers	*Cardium rusticum*	*B. haimeanus*	Mediterranean (S)
1857	Pagenstecher	*A. anatina*	*B. polymorphus*	Europe (F)
1863	Claparede	Found free swimming cercaria	*B. haimeanus*	Normandy (S)
1874	McCrady	*Crassostrea virginica*	*B. cuculus*	Carolina (S)
1878	Ulicny	*A. cellensis*	*B. intermedius*[1]	Europe (F)
1881	Ercolani	*U. pictorum*	*Cercaria bucephalus*[1]	Europe (F)
1881	Levinsen	*Modiolaria discors*	*B. crux*	Egedesminde (S)
1882	Ercolani	*A. anatina*	*C. bucephalus*[1]	Europe (F)
1883	Ziegler	—	*B. polymorphus*	Europe (F)
1888	Huet	*Cardium edule*	*B. haimeanus*	Normandy (S)
1890	Nelson	*Crassostrea virginica*	"Gregarinoid parasites"	New Jersey, U.S.A. (S)
1893	Huet	*Mactra solida*	*B. haimeanus*	Normandy (S)
1893	Nelson	*Crassostrea virginica*	*B. cuculus*	New Jersey, U.S.A. (S)
1894	Vaullegeard	*Tapes pullastra*	*B. haimeanus*	Europe (S)
1894	Vaullegeard	*Tapes decussatus*	*B. haimeanus*	Europe (S)
1899	Kelly	Unionidae	*B. polymorphus*	Mississippi R. (F)
1903	Haswell	*Mytilus latus*	*B.* sp.	New Zealand (S)
1904, 05	Johnstone	*Cardium edule*	*B. haimeanus*	England (S)
1905, 06, 09	Tennent	*Crassostrea virginica*	*B. haimeanus*[2]	East Coast, U.S.A. (S)
1906	Pelseneer	*Syndosmya alba*	*B. haimeanus*	Europe (S)
1906	Pelseneer	*Cardium edule*	*B. haimeanus*	Europe (S)
1907	Pelseneer	*Mactra solida*	*B. haimeanus*	Europe (S)
1907	Pelseneer	*Mactra subtruncata*	*B. haimeanus*	Europe (S)
1907	Pelseneer	*Donax trunculus*	*B. haimeanus*	Europe (S)
1909	Sinitzin	*Dreissensia polymorpha*	*B. polymorphus*	Warsaw (F)
1909	Sinitzin	*A. mutabilis*	*B. polymorphus*	Warsaw (F)
1909	Sinitzin	*Tapes rugatus*	*B. haimeanus*	Black Sea (S)
1911	Sinitzin	*Tapes rugatus*	*C. hydriformis*[3]	Black Sea (S)
1911	Lebour	*Cardium edule*	*B. haimeanus*	England (S)

1911	Lebour	*S. alba*	*B. syndosmyae*	England (S)
1924	Wunder	—	*B. polymorphus*	Europe (F)
1925	Miller	*Pinna carnea*	*Cercaria* N	Tortugas (S)
1928	Faust	*Modiola capensis*	*Bucephalopsis modiolae*	Sth. Africa (S)
1929	Woodhead	*Elliptio dilatatus*	*Bucephalus papillosum*[4,8]	Michigan (F)
1930	Woodhead	*Eurynia iris*	*B. elegans*[8]	Michigan (F)
1933	Roughley	*O. commercialis*	*B. haimeanus*	N.S.W. (S)
1933	Roughley	*O. angasi*	*B. haimeanus*	N.S.W. (S)
1934	Ozaki and Ishibashi	*Pinctada martensi*	*B. margaritae*	Japan (S)
1934	Palombi	*Tapes decussatus*	*Bucephalopsis haimeana*[6]	Naples (S)
1934	Palombi	*Tapes aureus*	*B. haimeana*[6]	Naples (S)
1934	Wesenberg-Lund	—	*Bucephalus polymorphus*	Europe (F)
1935	Cole	*Mytilus edulis*	*B. mytili*	England (S)
1936	Woodhead	*A. grandis*	*C. argi*	Michigan (F)
1936	Woodhead	*Lampsilis siliquoidea*	*C. basi*[5]	Michigan (F)
1936	Woodhead	*Eurynia iris*	*C. scioti*	Michigan (F)
1939	Rees	*Cardium edule*	*B. haimeanus*	England (S)
1949	Andreu	*Tapes aureus*	*Bucephalopsis haimeana*[6]	Spain (S)
1952	Chubrik	*Mytilus latus*	*Prosorhynchus squamatus*[7]	Arctic? (S)
1952	Kniskern	*L. siliquoidea*	*Rhipidocotyle septpapillata*[8]	Michigan (F)
1954?	Ozaki	?	*Bucephalus itabo?*	Japan (S)
1954	Hopkins	*Crassostrea virginica*	*B. cuculus*	U.S.A. (S)
1956	Cable	*Donax denticulatus*	*C. caribbea* XLII	Puerto Rico (S)
1956	Cable	*Tellina lintea*	*C. caribbea* XLII	Puerto Rico (S)
1958	Hopkins	*Donax variabilis*	*B. loeschi*	Mustang Is. (S)
1960	Ozaki	*Caecella chinensis*	*P. caecellae*	Japan (S)
1960	Ozaki	*Gryphaea gigas*	*P. magakii*	Japan (S)
1961	Angel	*Velesunionis ambiguus*	*C. velesunionis*	Australia (F)
1961	Holliman	*Mulinia lateralis*	*C. apalachiensis*	Florida (S)
1961	Laird	*Ostrea belcheri*	unidentified sp.	Pakistan (S)

KEY TO TABLE II

F Fresh water species.
S Marine species.
1 Considered as synonyms of *B. polymorphus.*

2 Synonymous with *B. cuculus*
3 ≡ *B. haimeanus* of Sinitzin, 1909.
4 Now *R. papillosum*
5 Synonymous with *R. septpapillata*
6 ≡ *Bucephalopsis haimeanus*
7 Adult status not determined experimentally but based on morphological resemblances.
8 Adult status determined experimentally.

DISCUSSION OF CERCARIA

All previous descriptions or reports of bucephalid cercariae known to the author are listed in Table II. Of the descriptions of marine species, only those given by Levinsen (1881), Haswell (1903), Ozaki and Ishibashi (1934), Cole (1935), Chubrik (1952), Hopkins (1954), Cable (1956), Hopkins (1958) and Holliman (1961) are adequate for close comparisons with the present species. *Bucephalus crux, B. mytili* and the cercaria of *Prosorhynchus squamatus* described by Levinsen, Cole and Chubrik respectively have a posterior sucker-like extension of the tail stem and are thus quite distinct from the present species; *B. margaritae* Ozaki and Ishibashi differs in details of spination, the absence of an axial strand and has "dermal gland cells" rather than cystogenous granules distributed through the body; *B.* sp. Haswell differs in the shape of the intestine and has two slender processes at the anterior end of the body; *B. cuculus* McCrady, as described by Hopkins (1954), has a flame cell formula of $2[(2+2+2)+(2+2+2)]=24$; the behaviour, spination and granulation, number of lips surrounding the opening of the cystogenous organ pore, and shape and structure of the tail stem of *Cercaria caribbea* XLII Cable and *C. apalachiensis* Holliman differ from the present species; *B. loeschi* Hopkins has a shorter excretory bladder and a smaller intestine.

As stated elsewhere (p. 2) and as can be seen from Table II, many authors have placed cercariae in adult genera without any experimental evidence to substantiate their views. It is therefore recommended that all bucephalid cercariae described as species of *Bucephalus* or *Prosorhynchus* without experimental proof be transferred to the group name *Cercaria* to agree with the usual practice regarding cercariae whose adult status is unsure. Furthermore, as shown by Hopkins (1954), *Bucephalopsis* can only be used for the cercaria *Bucephalopsis haimeanus* (Lacaze-Duthiers, 1854). Thus *Bucephalopsis modiolae* Faust, 1928, must now become *C. modiolae* (Faust, 1928).

The probable error of many authors in synonymising the cercariae they have found with *Bucephalopsis haimeanus* has already been referred to (p. 2). This practice is a reflection on the inadequate descriptions that have been given and it is obvious that the majority of different cercariae need re-describing to rectify this situation.

NUMBER OF CERCARIAE LIBERATED FROM INFECTED OYSTERS

Data concerning the number of cercariae liberated from 11 infected oysters are given in Table III, which covers a 53-day period during June and July, 1964. There was a general tendency for peak liberations of more than 1,000 cercariae to be followed by lulls with few or no cercariae liberated. The duration of peaks and lulls in liberation varied considerably in the individual oysters under observation. A peak was maintained for 11 days in oyster 4 (June 10–20), while peak liberation lasted for only 24 hours in all oysters on various occasions. Peaks were generally, however, of the order of 1.5 days in duration. A lull persisted for 13 days in oyster 8 (July 3–15) but only for 24 hours in many instances. Lulls were generally more variable in duration than peaks.

Oyster	Number of days on which liberations occurred	Total number liberated.	Number of peak liberations (>1,000 liberated/24 hours).	Range (in days) in lengths of peak liberations.	Mean duration (in days) of peak liberations.	Range in numbers liberated/24 hours during peak liberations.	*Mean number liberated/24 hours during peak liberations.	Range (in days) in lengths of lulls in liberation (<1,000 liberated/24 hours).	Mean duration (in days) of lulls in liberation.	Range in numbers liberated/24 hours during lulls in liberation (excluding nil liberations).	*Mean number liberated/24 hours during lulls in liberation (excluding nil liberations).
1	37	31,360	7	1–3	1.4	1,000– 8,000	2,700	1–16	6.5	5–750	150
2	36	68,875	12	1–4	1.6	1,000–10,000	3,800	1–8	2.5	5–800	140
3	29	25,785	9	1–2	1.1	1,000– 4,000	2,200	1–11	4.5	5–600	110
4	39	126,545	10	1–11	2.5	1,000–10,000	4,700	1–5	2.7	5–250	80
5	34	89,200	9	1–5	1.9	1,000– 8,500	5,100	1–6	3.1	5–400	120
6	28	19,445	7	1–2	1.1	1,000– 4,000	1,800	2–7	4.3	5–600	280
7	35	22,325	8	1–2	1.1	1,000– 4,000	2,000	1–7	4.7	10–750	200
8	35	9,605	4	1–2	1.3	1,000– 1,200	1,100	3–13	8	5–750	170
9	28	18,270	6	1–2	1.3	1,000– 2,000	1,400	1–12	4.4	5–750	330
10	25	59,125	7	1–3	1.6	1,000–10,000	5,600	1–12	5.1	5–750	190
11	17	41,800	6	1–3	1.5	1,000–10,000	4,500	1–15	6.6	10–400	90

* Figures in these columns are given to the nearest 100 or 10.

TABLE III.—Numbers of Cercariae Liberated from 11 Infected Oysters over a 53-day period During June and July, 1964.

The maximum number of cercariae liberated from one oyster over a 24-hour period was 10,000, and this occurred on one occasion in oysters 2 and 11, and on two occasions in oysters 4 and 10.

Towards the end of the observation period there was a marked tendency for peaks in liberation to become less frequent. This might suggest that the infection was abating but this has been discounted elsewhere (Howell, 1966, in press). More probably, only one change of water per day for each oyster is insufficient to provide the necessary food requirements to maintain both oyster and parasite and this is reflected in fewer cercariae produced and liberated. It is difficult to adjudge from the results obtained any effect of temperature (which fluctuated between 10–15°C) on the production and liberation of cercariae. However, despite these points, the net results suggest that liberation of cercariae is neither cyclic nor continuous but essentially an intermittent phenomenon.

It should be noted that it was possible to determine the state of the infection in individual oysters when they were opened after completing the above observations. As expected, the number of cercariae liberated from a given oyster could be correlated with the state of the infection. Oysters 3, 6, 7, 8 and 9 proved to be relatively lightly infected; oysters 1 and 11 moderately heavily infected; and oysters 2, 4, 5 and 10 heavily infected.

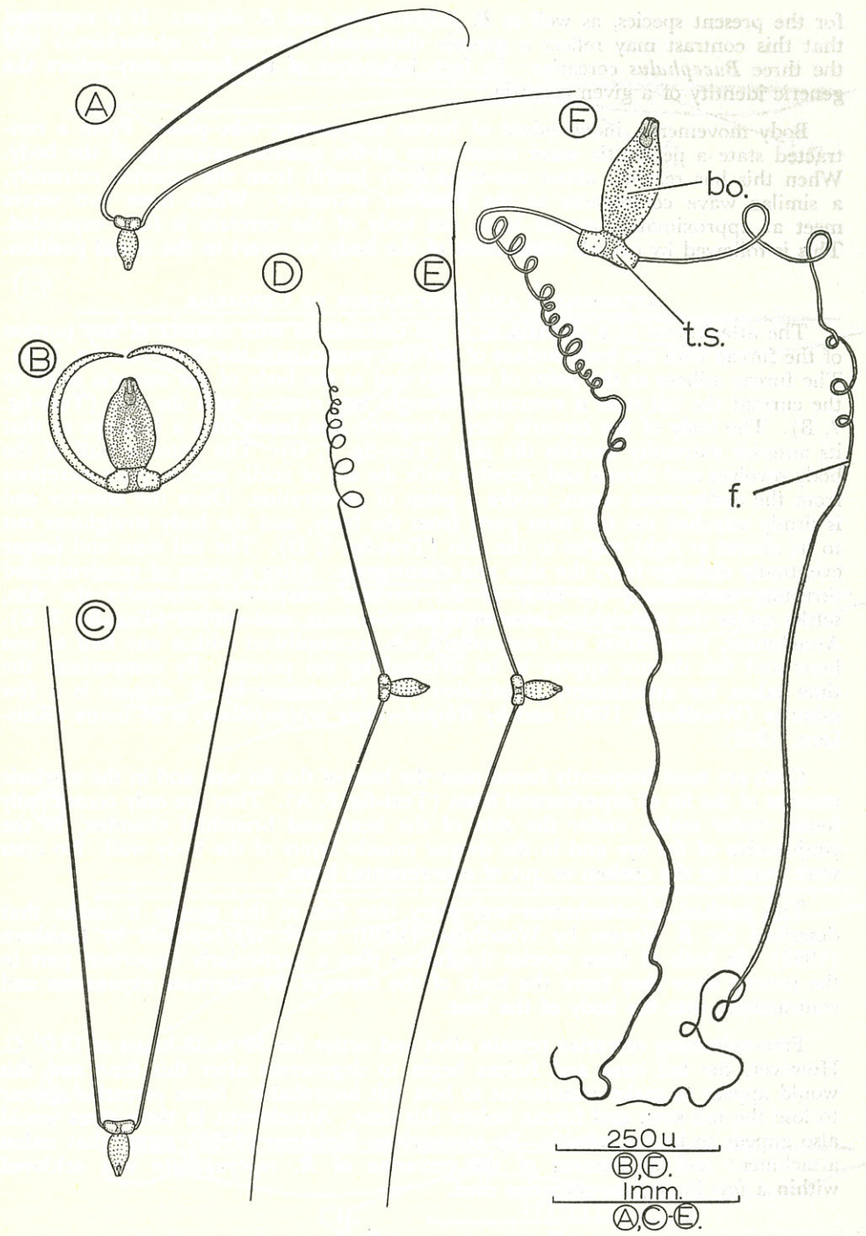
MOVEMENT AND BEHAVIOUR OF CERCARIAE

Examination of liberated cercariae in an undisturbed finger bowl shows that they are located in greatest numbers opposite the exhalent chamber. The cercariae are generally in a resting position with the furcae contracted, encircling the body, and almost meeting in front of the anterior extremities (Text-fig. 6, B).

The furcae rapidly expand and tend to coil on stimulation by light (Text-fig. 6, F). If the water is then agitated any one of the positions illustrated in Text-figs. 6, A, C or E is assumed with the body suspended vertically and the furcae streaming above. The angle between the furcae is acute when the cercaria is nearer the bottom of the bowl, but obtuse nearer the surface. If strong currents are set up in the water the furcae bend with the current (Text-fig. 6, A). The sudden flexure and coiling of one furca of a suspended cercaria results in horizontal movement in calm water (Text-fig. 6, D). A cercaria may remain suspended for at least one hour in calm water, but after this time slowly sinks to the bottom of the bowl and coils the furcae which are eventually fully contracted to revert to the resting position (Text-fig. 6, B).

Light tapping of a finger bowl containing cercariae with the furcae expanded causes immediate and complete contraction of the furcae. The furcae normally revert to their expanded state when the tapping ceases. A similar reaction to mechanical shock has been described by Woodhead (1930) for the cercaria *Bucephalus elegans*. By comparison with the present species, however, *B. elegans* requires about three minutes to recover from the shock. Dawes (1946) does not describe the reaction of *B. polymorphus* to mechanical shock but he does describe the position of the furcae in a resting cercaria which is identical with that of the present species. Both Woodhead and Dawes stated that *B. elegans* and *B. polymorphus* respectively, are able to swim by alternate contractions and expansions of the furcae, but this was not observed in the present species.

Hopkins (1954) and Holliman (1961) stated that true swimming movements of *Cercaria cuculus* and *C. apalachiensis* do not occur and the cercariae in each case rely on turbulence in the water to remain suspended. With mechanical shock, the furcae of the latter species violently contract to about twice body length and are temporarily orientated at about 60° to the body in a rod-like manner. The resultant appearance of this cercaria can be contrasted with that described above



TEXT-FIG. 6.—*Bucephalus longicornutus*. Behaviour of the cercaria: Fig. A, suspended cercaria (semi-diagrammatic) in a strong current; Fig. B, resting position or position induced by light mechanical shock; Fig. C, suspended cercaria some distance below the surface of the water; Fig. D, suspended cercaria undergoing lateral movement by flexure of one of the furcae; Fig. E, suspended cercaria near the surface of the water; Fig. F, relaxed position assumed with the furcae expanded and partly coiled. For abbreviations see p. 9.

for the present species, as well as *B. polymorphus* and *B. elegans*. It is suggested that this contrast may reflect a generic distinction between *C. apalachiensis* and the three *Bucephalus* cercariae. In fact, behaviour of the furcae may reflect the generic identity of a given cercaria.

Body movements, independent of furcae movements, take place. From a contracted state a peristaltic wave commences at the anterior extremity of the body. When this has reached about one-third body length from the anterior extremity, a similar wave commences at the posterior extremity. When these two waves meet at approximately mouth level, the body of the cercaria is fully expanded. This is followed by overall contraction of the body to revert to the initial position.

ATTACHMENT AND PENETRATION OF CERCARIAE

The attachment of a cercaria to a fish commences with contact of any portion of the furcae with the body surface of the fish, particularly the fins (Text-fig. 7, A). The furcae adhere at the point of contact and as the body of the cercaria drifts in the current the tail stem is eventually brought into contact with the skin (Text-fig. 7, B). The body of the cercaria then elongates, and bends into a U-shape so that its anterior extremity touches the skin (Text-fig. 7, C). The anterior half of the body revolves and thrusts and, possibly with the aid of acidic and enzymic secretions from the cystogenous organ, erodes a point of penetration. Once the anterior end is firmly attached the tail stem parts from the body, and the body straightens out to lie almost at right angles to the skin (Text-fig. 7, D). The tail stem and furcae eventually dislodge from the skin and disintegrate. After a series of revolving and thrusting movements, the body of the cercaria completely penetrates the skin, settles under the malpighian layer or in muscle tissue, and encysts (Text-fig. 7, E). Attachment, penetration and encystment are accomplished within one half to one hour and fish do not appear to be irritated by the process. By comparison, the time taken for attachment, penetration and encystment by *B. elegans* is a few minutes (Woodhead, 1930) and by *Rhipidocotyle septpapillata,* is 24 hours (Kniskern, 1952).

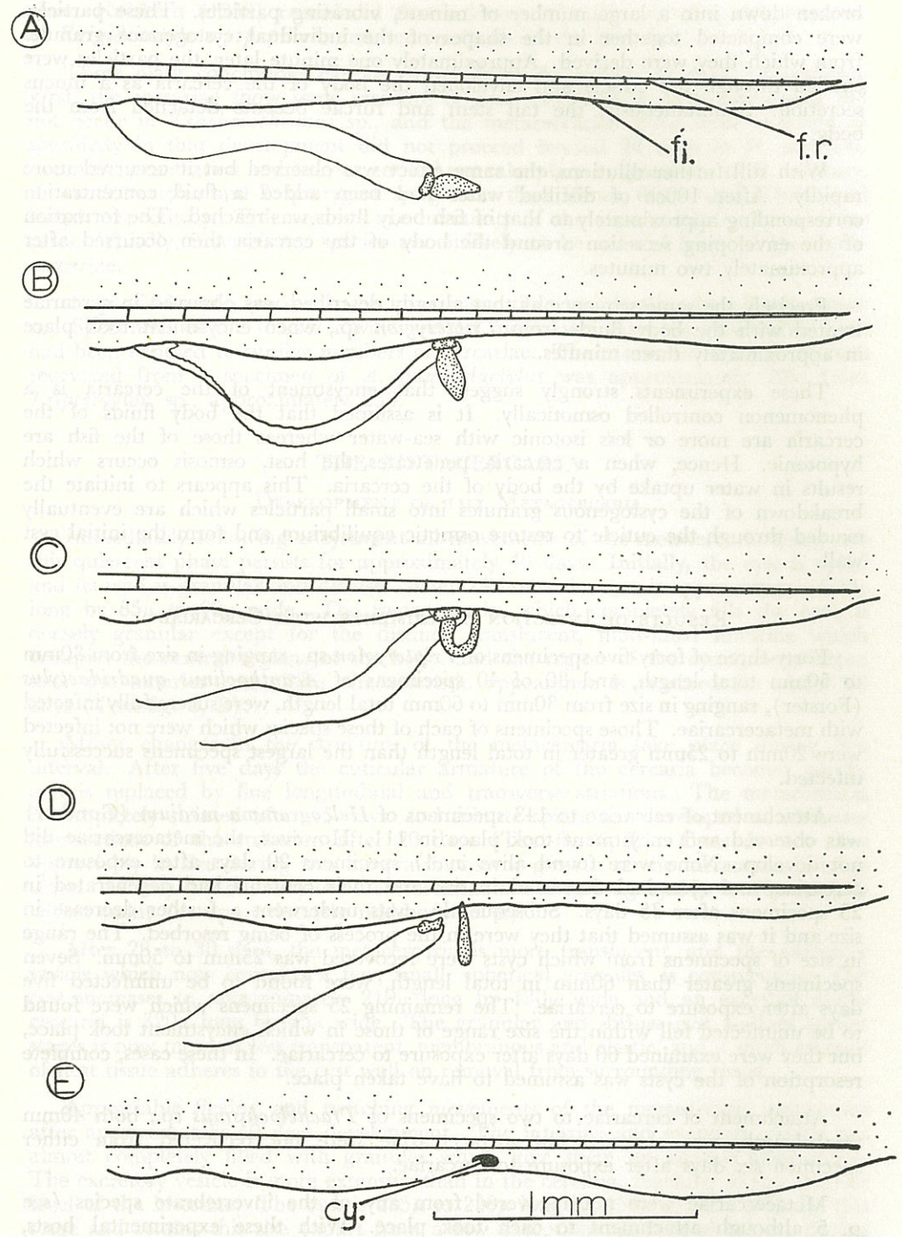
Cysts are most frequently found near the base of the fin web and in the extrinsic muscles of the fin of experimental hosts (Text-fig. 8, A). They are only occasionally found under scales, under the skin of the head and branchial chamber, in the conjunctiva of the eye and in the deeper muscle layers of the body wall. No cysts were found in the coelom or gut of experimental hosts.

The method of attachment and entry into fish in this species is unlike that described for *B. elegans* by Woodhead (1930) or *R. septpapillata* by Kniskern (1952). In both of these species the furcae play a particularly important part in the process since they force the body of the cercaria, by alternate expansions and contractions, into the body of the host.

Free-swimming cercariae remain alive and active for 36 to 38 hours at 13.0° C. However, the tail stem and furcae begin to degenerate after this time and this would appear to make attachment to host fish improbable. Some cercariae appear to lose the tail stem and furcae before this time. Attachment in these cases would also appear to be improbable. By comparison, Kniskern (1952) stated that unless attachment and encystment of the cercariae of *R. septpapillata* was achieved within a few hours, the cercariae died.

ENCYSTMENT OF CERCARIAE

Observations were made to determine the effect of successive dilutions of sea-water on cercariae. There appeared to be no visible effects until the sea-water was diluted 50% by distilled water. At this dilution, the body of the cercaria expanded slightly, and within five minutes, individual cystogenous granules were



TEXT-FIG. 7.—*Bucephalus longicornutus.* Attachment and method of entry of the cercaria into the fish intermediate host (semi-diagrammatic): Fig. A, adherence of furcae; Fig. B, attachment of tail-stem; Fig. C, U-shape assumed by body as the enterior end is brought into contact with the skin of host; Fig. D, penetration of the anterior end of the cercaria and dislodgement of the tail stem and furcae from the body; Fig. E, complete penetration and encystment. For abbreviations see p. 9.

broken down into a large number of minute, vibrating particles. These particles were compacted together in the shapes of the individual cystogenous granules from which they were derived. Approximately one minute later, the particles were exuded through the cuticle and enveloped the body of the cercaria as a mucus secretion. Simultaneously, the tail stem and furcae become detached from the body.

With still further dilutions, the same effect was observed but it occurred more rapidly. After 100cc of distilled water had been added a fluid concentration corresponding approximately to that of fish body fluids was reached. The formation of the enveloping secretion around the body of the cercaria then occurred after approximately two minutes.

Precisely the same sequence as that already described was observed in cercariae treated with the body fluids from *Tripterygion* sp., when encystment took place in approximately three minutes.

These experiments strongly suggest that encystment of the cercaria is a phenomenon controlled osmotically. It is assumed that the body fluids of the cercaria are more or less isotonic with sea-water whereas those of the fish are hypotonic. Hence, when a cercaria penetrates the host, osmosis occurs which results in water uptake by the body of the cercaria. This appears to initiate the breakdown of the cystogenous granules into small particles which are eventually exuded through the cuticle to restore osmotic equilibrium and form the initial cyst wall.

RESULTS OF INFECTION EXPERIMENTS WITH CERCARIAE

Forty-three of forty-five specimens of *Tripterygion* sp., ranging in size from 30mm to 50mm total length, and 30 of 40 specimens of *Acanthoclinus quadridactylus* (Forster), ranging in size from 30mm to 60mm total length, were successfully infected with metacercariae. Those specimens of each of these species which were not infected were 20mm to 25mm greater in total length than the largest specimens successfully infected.

Attachment of cercariae to 143 specimens of *Helcogramma medium* (Gunther) was observed and encystment took place in 111. However, the metacercariae did not develop. None were found alive in 47 specimens 20 days after exposure to cercariae, and cysts had decreased in size and their contents had degenerated in 25 specimens after 35 days. Subsequently, cysts underwent a further decrease in size and it was assumed that they were in the process of being resorbed. The range in size of specimens from which cysts were recovered was 25mm to 50mm. Seven specimens greater than 60mm in total length, were found to be uninfected five days after exposure to cercariae. The remaining 25 specimens which were found to be uninfected fell within the size range of those in which encystment took place, but they were examined 60 days after exposure to cercariae. In these cases, complete resorption of the cysts was assumed to have taken place.

Attachment of cercariae to two specimens of *Trachelochismus* sp., both 40mm total length, was observed but metacercariae were not recovered from either specimen six days after exposure to cercariae.

Metacercariae were not recovered from any of the invertebrate species (see p. 5 although attachment to each took place. With these experimental hosts, examination of the soft parts was made three days after exposure to cercariae.

A total of 12 fish died at various intervals after exposure to cercariae but there was no evidence to suggest that this was due to the infection. In fact, fewer cysts were recovered from those that died than from the majority of those that remained

alive. Kniskern (1952) considered that a heavy infection with the metacercariae of *Rhipidocotyle septpapillata* resulted in death of the host fish.

Three main conclusions can be drawn from the results of these experiments. First, the cercariae show some measure of host specificity in that encystment did not occur in *Trachelochismus* sp., and the metacercariae show some measure of specificity in that development did not proceed beyond 20 days in *H. medium*. Secondly, penetration and encystment did not take place in specimens of *H. medium*, *A. quadridactylus* and *Tripterygion* sp. greater than 50mm, 60mm and 50mm total length respectively. This suggests age immunity of the experimental hosts in these cases. Thirdly, invertebrate species are unlikely to be natural hosts of the metacercariae.

A. quadridactylus proved to be more susceptible to infection than *Tripterygion* sp. Specimens of the former always yielded more cysts than the latter after both had been exposed to similar numbers of cercariae. The maximum number of cysts recovered from a specimen of *A. quadridactylus* was approximately 325; from *Tripterygion* sp., approximately 250.

THE METACERCARIA

DEVELOPMENT OF THE METACERCARIA

Immediately following encystment all movements of the metacercaria cease and this quiescent phase persists for approximately 40 days. Initially, the cyst is ovoid and its wall is granular and diffuse. After 24 hours the cyst is approximately 200μ long by 85μ to 90μ wide. The metacercaria, which completely fills the cyst, is densely granular except for the distinct, translucent, fluid-filled intestine which occupies the central regions of the body, and the region of the cystogenous organ near the anterior end. This characteristic appearance is retained for about 26 days (Text-fig. 8, B).

Minor changes in the structure of the metacercaria take place during this interval. After five days the cuticular armature of the cercaria becomes patchy and is replaced by fine longitudinal and transverse striations. The metacercaria is completely striated after 15 days, and minute spines are developed at alternate intersections of the striations after 20 days (Text-fig. 8, E). Cysts do not alter appreciably in size during this interval but some growth of the metacercaria takes place. Twenty-day-old excysted metacercariae are 230μ long by 55μ to 60μ at their widest point (at approximately mid-body level).

After 28 to 30 days, the metacercaria is more translucent, and the excretory vesicle which now contains a few, small, spherical granules, is conspicuous. The cyst increases to approximately 210μ long by 130μ wide and an excysted metacercaria is 320μ long by 65μ wide. The granular and diffuse cyst wall of earlier stages is now more or less transparent, membranous and elastic, and a small amount of host tissue adheres to the cyst wall on removal from surrounding tissue.

Appreciable flexing and twitching movements of the metacercaria commence after approximately 40 days development. The intestine and excretory vesicle are almost completely filled with granules which give them an opaque appearance. The excretory vesicle is more extensive than in the cercaria, reaching to the anterior level of the intestine. The cyst is 235μ to 250μ long by 150μ to 185μ wide at this stage and retains this size (apart from a few exceptions mentioned below) during subsequent development. Cyst walls are thicker by 5μ in cysts recovered from muscle tissue than in those from the fin web.

After approximately 50 days, both the anterior sucker and cuticular spination are well developed. Activity of the metacercaria, which at this stage consists of

rotating movements within the cyst, gradually increases and reaches a peak after 75 to 80 days.

The maximum size of the metacercaria is attained after approximately 80 days development, irrespective of the experimental host in which it develops. Expanded metacercariae range from 700μ to 715μ long by 80μ to 90μ wide; contracted, from 270μ to 275μ long by 125μ to 130μ wide. The reproductive system does not undergo any further development beyond that observed in specimens of this age.

A thin band of brown pigment is laid down on the inner surface of the cyst wall of many cysts recovered from *Tripterygion* sp. after 85 to 90 days. Metacercariae are still alive within these cysts but their activity is greatly diminished by comparison with 80-day-old specimens. After 95 to 100 days, metacercariae within these pigmented cysts are either dead or very inactive. Of the cysts that remain unpigmented, many contain granular masses resulting from the degeneration of the enclosed metacercariae, and they are smaller than normal cysts. The remainder contain normal, active metacercariae which, in addition to spherical granules, contain needle-shaped crystals, 10μ long, in the intestine.

After 110 days development, all metacercariae in either pigmented or unpigmented cysts recovered from *Tripterygion* sp. are either dead or degenerate. Dead metacercariae in pigmented cysts are themselves pigmented brown and their major morphological features are still recognisable.

Cysts of up to 120 days of age recovered from *Acanthoclinus quadridactylus* do not become pigmented but some metacercariae degenerate into a granular mass within the cyst after this time, and the activity of many others is diminished.

Cysts from *Tripterygion* sp. and *A. quadridactylus,* older than 110 days and 120 days development respectively, have not been examined.

Two, three or four metacercariae are occasionally confined within the same cyst (Text-fig. 8, J). These cysts, which are larger than normal cysts containing one metacercaria, were recovered from both experimental hosts.

Between two and eight normal cysts are frequently recovered from *A. quadridactylus,* and these are joined to one another in a chain-like fashion (Text-fig. 8, I).

Occasionally, groups of three or four larger cysts, embedded in an opaque, granular and fibrous matrix, presumably of host origin, are recovered from *A. quadridactylus* (Text-fig. 8, K). Apart from this phenomenon, tissue reactions induced by large numbers of cysts in both experimental hosts are slight.

Spontaneous excystment of 75- to 95-day-old metacercariae takes place from approximately 10% of the cysts recovered from *A. quadridactylus* and only occasionally in cysts recovered from *Tripterygion* sp.

Kniskern (1952) found that the metacercariae of *Rhipidocotyle septpapillata* reached maximum development after 12 days. By comparison, the time taken for maximum development in the present species is prolonged since it is not reached until after 75 to 80 days. Kniskern found that the contents of the cysts degenerated into a "cheesy mass" after 84 days in *R. septpapillata.* The appearance of these cysts may be comparable with those of the present species which contain degenerate metacercariae. Pigmentation of cysts has not been described for other bucephalid metacercariae.

Kniskern also reported that spontaneous excystment of all mature metacercariae of *R. septpapillata* took place; this is in striking contrast to the 10% noted in the present species obtained from *A. quadridactylus.*

Schurmans-Stekhoven (1934) for *Bucephalus polymorphus* and Kniskern (1952) for *Rhipidocotyle septpapillata* have both noted that cysts may contain two or occasionally more metacercariae. With the same phenomenon recorded for the present species, it may be a characteristic feature of metacercariae of this family.

Woodhead (1929, 1930) has given no details of development of the metacercariae of either *R. papillosum* or *B. elegans.*

The fact that metacercariae remain alive longer in *A. quadridactylus* than in *Tripterygion* sp. suggests that the former may be a natural host or may be more akin to the natural host or hosts of the metacercariae than the latter.

THE MATURE METACERCARIA

(Text-fig. 8, A-L.)

The natural fish host or hosts of the metacercariae were not discovered during the course of this study. In general, attempts were made to obtain and examine fish that fell within the size range of those successfully infected experimentally. Three attempts were made to obtain likely fish hosts from Balaena Bay, Wellington Harbour, where infected oysters are known to occur, but no fish were recovered from the fish traps used. Ten specimens of *Tripterygion* sp., collected with a dip net from the same locality, were not infected with metacercariae of the present species.

Small fish are not common in oyster "cultch" from oyster dredges working in Foveaux Strait. In one day spent dredging on the vessel *Kumea,* only 11 specimens of *Trachelochismus* sp. and four specimens of *Acanthoclinus trilineatus* (Forster) were recovered by the author but none of these proved to be infected. A day spent trawling over the oyster beds in Foveaux Strait yielded, in addition to the larger fish species listed elsewhere (p. 33), 30 small fish comprising 25 specimens of *Trachelochismus* sp., three specimens of *Tripterygion* sp. and two specimens of *A. trilineatus.* None of these specimens proved to be infected.

Experimentally infected specimens of *A. quadridactylus* and *Tripterygion* sp. provided the only source of metacercariae for study. For reasons stated elsewhere (p. 26), the following description is based on 80-day-old and older specimens.

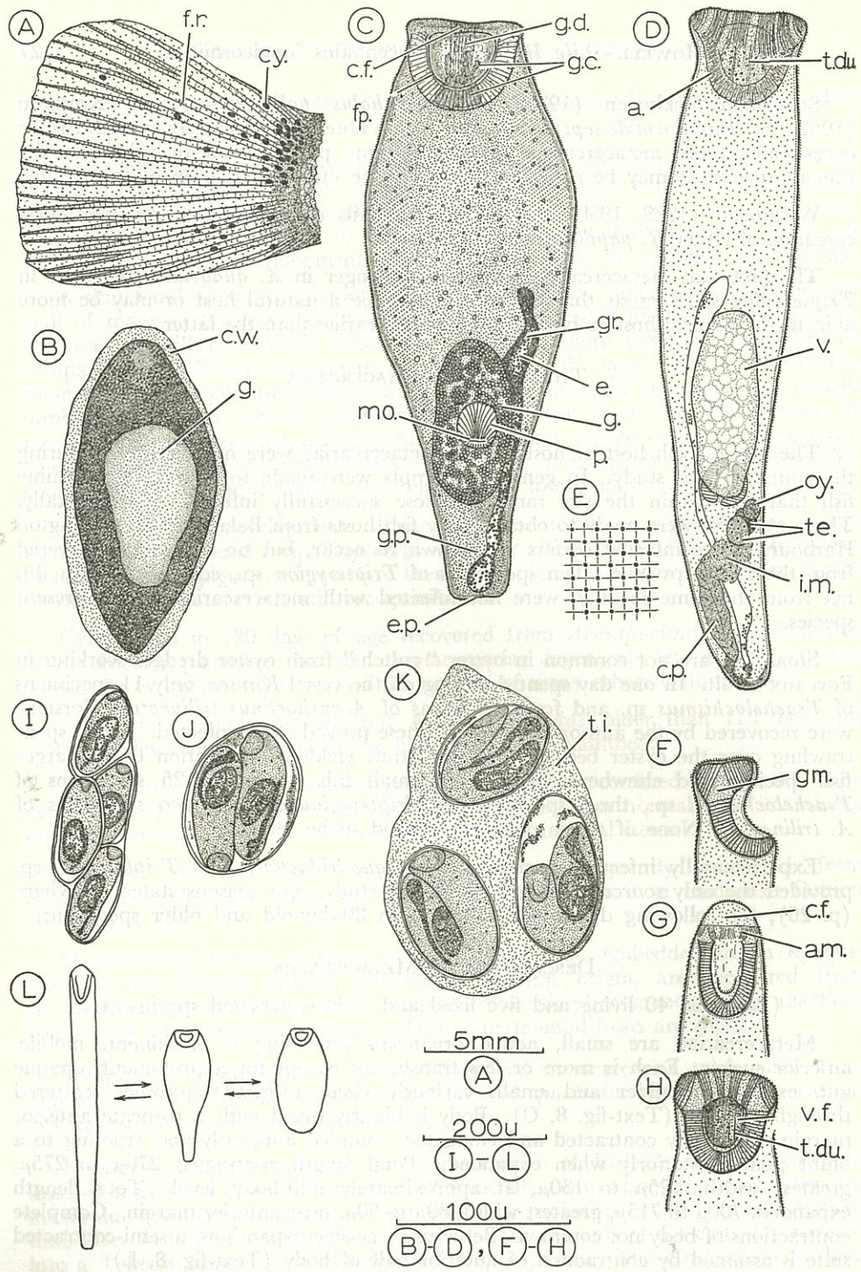
DESCRIPTION OF METACERCARIA

(Based on 40 living and five fixed and stained excysted specimens).

Metacercariae are small, active organisms possessing a prominent, mobile, anterior sucker. Each is more or less translucent except for a prominent, opaque gut, excretory bladder and small, variously sized, refractile granules scattered throughout body (Text-fig. 8, C). Body is bluntly ovoid with a truncate anterior margin when fully contracted and elongated, rounded anteriorly and tapering to a blunt point posteriorly when expanded. Total length contracted 270μ to 275μ, greatest width, 125μ to 130μ, at approximately mid-body level. Total length expanded, 700μ to 715μ, greatest width, 80μ to 90μ, near anterior margin. Complete contractions of body not common. Between successive expansions, a semi-contracted state is assumed by contraction of anterior half of body (Text-fig. 8, L).

Body is covered by a thin cuticle, 3μ to 4μ thick. Small, diagonally arranged spines are embedded in cuticle except within concavity of anterior sucker. Spines are not as prominent posterior to level of pharynx as over anterior region of body.

A distinctive, mobile sucker is situated at anterior end of body. Its shape is variable, depending on its degree of contraction. Sucker is truncate anteriorly, rounded posteriorly, when contracted, with a deep anteroventral concavity shaped



TEXT-FIG. 8.—*Bucephalus longicornutus.* Morphology and anatomy of the metacercaria: Fig. A, lateral view of the caudal fin of *Tripterygion* sp. showing the location of cysts; Fig. B, ventral view of encysted, 24-hour-old, living specimen; Fig. C, ventral view of excysted, 85-day-old, living specimen; Fig. D, dorsal view of excysted, 91-day-old, fixed and stained specimen; Fig. E, diagrammatic representation of cuticular spines showing how diagonal arrangement is achieved; Fig. F, right lateral view of the contracted sucker of a 91-day-old, fixed and stained specimen; Fig. G, ventral view of the expanded sucker of a 91-day-old, living specimen; Fig. H, ventral view of the contracted sucker of a 91-day-old, fixed and stained specimen; Fig. I, aggregation of four, 38-day-old metacercarial cysts from experimentally infected *Acanthoclinus quadridactylus;* Fig. J, multiple cyst from *A. quadridactylus;* Fig. K, three larger cysts embedded in host tissue, recovered from *A. quadridactylus;* Fig. L, body movements of the metacercaria. For abbreviations see p. 9.

like a quarter-segment of a sphere (Text-fig. 8, C), and is 65μ to 70μ wide at its anterior margin (which is slightly lobed laterally) and 50μ to 55μ from anterior margin to posterior limit. Musculature of sucker is essentially scoop-shaped with open and directed anteriorly. Thin flap of cuticle at anterior end of body closes off open end of musculature and marks anterior border of sucker concavity. In living specimens, this flap of cuticle obscures muscle fibres along anterodorsal margin of sucker. Band of radial muscles, approximately 10μ wide, prominent around posterior, semi-circular margin of sucker concavity.

When sucker is expanded, musculature is more elongated. Sucker is 40μ to 45μ wide, approximately 60μ to 65μ long, and its concavity is relatively inconspicuous (Text-fig. 8, G). Anterodorsal muscle fibres of musculature are now relatively conspicuous, and consist of several oblique groups separated by more or less triangular areas devoid of muscle fibres (Text-fig. 8, G). These groups of fibres can be traced some distance posteriorly into dorsal musculature of sucker in fixed and stained specimens only (Text-fig. 8, D). Five areas devoid of muscle fibres are seen in ventral view; three are dorsal, two ventral (Text-fig. 8, H). Thin flap of cuticle which closes off open end of musculature when sucker contracted, is now projected anterior to musculature as a semi-circular lobe which gives anterior end a rounded appearance (Text-fig. 8, G). However, anterior end of body proper is distinctly demarcated, and flap of cuticle projects beyond this.

Floor of the sucker concavity is vesiculate, and in ventral view three, narrow, longitudinal ducts, one median and one on either side of it, can be observed in floor (Text-fig. 8, H). These ducts are continuous with non-muscular areas between groups of oblique muscle fibres along the anterodorsal margin of the sucker and are designated tentacular ducts.

Sucker musculature is essentially C-shaped in lateral view (Text-fig. 8, F). Anterior portion thickened, consisting of dorsal and ventral groups of oblique fibres which run towards dorsal and ventral surfaces of the musculature respectively from anterior margin of sucker. Posterior arm of C consists of radial muscles. Triangular area, devoid of muscles, between these three groups of fibres. Five non-muscular areas between groups of muscle fibres seen in ventral view, combined with each lateral area make a total of seven. Tentacles are not developed in 120-day-old metacercariae.

An undetermined number of gland cells are situated near anterior end of body, and comprise a conical-shaped mass dorsal to posterior half of sucker musculature, and a few scattered irregularly anterior to this mass (Text-fig. 8, C). A number of narrow, parallel ducts run anteriorly from gland cells and open at anterodorsal margin of body by several pores.

Mouth is posterior to mid-body level, varying between 75μ and 120μ from posterior extremity depending on degree of contraction of body. Pharynx 28μ to 30μ long by 23μ to 25μ wide; oesophagus indistinct, approximately 35μ to 40μ long; intestine ovoidal and directed posteriorly. Intestine almost completely filled with spherical granules (approximately 3μ in diameter) in life but in mounted specimens these granules are inconspicuous and cavity of intestine has a vacuolate appearance (Text-fig. 8, D).

Reproductive system situated in hind-body posterior to pharynx (Text-fig. 8, D). Ovary and testes on right side of body and together, form three ovoid to spherical masses of cells lying slightly obliquely to longitudinal body axis. Most anterior mass, at approximately mouth level, is ovary which is 10μ to 12μ in diameter. It overlaps the anterior testis dorsally. Posterior testis overlaps the anterior testis dorsally. Testes are 15μ to 18μ in diameter.

Cirrus pouch is on left side of body, 60μ long and 15μ wide at its anterior end, tapering to a blunt point posteriorly; extending from 12μ to 15μ behind posterior testis to level of genital pore which varies between 18μ to 25μ from posterior extremity. Mass of cells comprising cirrus pouch not differentiated into seminal vesicle and pars prostatica and genital atrium not developed.

Gap between cirrus pouch and posterior testis occupied by an irregularly-shaped mass of cells which merges with, and is similar in appearance to, cells of the cirrus pouch. Significance of these cells not clear. No evidence of gametogenesis, and vitelline follicles, uterine coils and Mehlis's gland not observed.

Excretory pore situated at posterior extremity. Excretory bladder long, running to left of intestine and terminating approximately 20μ to 30μ anterior to intestine. Small spherical granules almost completely fill bladder in life but most of these disappear after fixation. Flame cell formula as for cercaria.

DISCUSSION OF METACERCARIA

Several differences between the anatomy of the metacercaria and cercaria are evident. These include for the metacercaria the possession of the sucker and gland cells in place of the cystogenous organ and four lips at the anterior end of the body; a more elongate excretory bladder; granulation of the excretory bladder and intestine; spination, and some further differentiation of the reproductive system. The relationship between the cystogenous organ and four lips at the anterior end of the body of the cercaria and the sucker and gland cells of the metacercaria was not determined. Accordingly, it is not known whether the sucker is a completely new development or develops by modifications of pre-existing structures at the anterior end of the body.

Although tentacles are not developed in the metacercariae examined, the present species can be confidently assigned to the genus *Bucephalus* even though the flap of cuticle at the anterior extremity is suggestive of the "hood" of *Rhipidocotyle*. The ducts observed in the sucker musculature and their confluence with gaps between the groups of muscle fibres along the anterior margin of the sucker correspond in position with the three most dorsal tentacular ducts of adult specimens of *Bucephalus longicornutus* (Manter, 1954) and other species of *Bucephalus*. Furthermore, the nature of the sucker musculature is typical of many species of *Bucephalus* (*vide* Ziegler, 1883; Manter, 1940). In *Rhipidocotyle*, however, tentacular ducts are not present, and the musculature of the sucker differs from *Bucephalus* species (*vide* Nagaty, 1937).

Two other *Bucephalus* metacercariae are known. The metacercaria of *B. elegans*, figured but not described by Woodhead (1930), is insufficiently characterised for it to be closely compared with the present species. The metacercaria of the present species shows some resemblance to the metacercaria of *B. polymorphus*, described by Ziegler (1883) and Schurmans-Stekhoven (1934), with regard to the shape of the sucker and position of the gland cells, shape of the cyst, and granulation of the intestine and excretory vesicle. However, the cirrus sac is not as fully differentiated in the present species as indicated by Ziegler for *B. polymorphus*. It is not clear from Schurmans-Stekhoven's description or figures whether the longitudinal ridges in the sucker concavity of *B. polymorphus* are comparable with the tentacular ducts of the present species.

RESULTS OF FEEDING EXPERIMENTS WITH METACERCARIAE

A total of 14 fish belonging to eight different species were fed on one or more occasions with specimens of *Tripterygion* sp. and *Acanthoclinus quadridactylus* that had been experimentally infected with metacercariae.

TABLE IV.—Feeding Experiments.

	Experimental Host	Number of Times Fed	Age (in days) of Metacercariae Fed	Days After First Feeding When Examined	Bucephalids Recovered	Other Intestinal Parasites
1.	*Geniagnus monopterygius*	7	75, 81, 84, 78, 81, 91, 94	21	Nil	*Neocreadium geniagni* Howell, 1966, and cestode larvae.
2.	*Geniagnus monopterygius*	3	58, 63, 75	24	Nil	
3.	*Geniagnus monopterygius*	2	89, 101	40	Nil	
4.	*Acanthoclinus quadridactylus*	2	58, 88	5	Nil	*Opegaster* sp. and cestode larvae.
5.	*Acanthoclinus quadridactylus*	8	82, 68, 74, 75, 90, 94, 78, 86	42	Nil	
6.	*Acanthoclinus quadridactylus*	3	63, 75, 80	10	Nil	
7.	*Helcogramma medium*	1	68	5	Nil	*Opegaster gobii.*
8.	*Tripterygion* sp.	3	25, 35, 30	14	Nil	
9.	*Tripterygion* sp.	2	85, 87	28	Nil	Nil.
10.	*Trachelochismus* sp.	1	45	7	Nil	
11.	*Pseudolabrus celidotus*	3	65, 75, 85	35	Nil	*Decemtestis pseudolabri.*
12.	*Pseudolabrus coccineus*	3	75, 80, 81	15	Nil	*Plagioporus interruptus.*
13.	*Scorpaena cardinalis*	4	80, 80, 81, 89	35	**Bucephalus longicornutus*	Nil.
14.	*Scorpaena cardinalis*	1	85	35	**Bucephalus longicornutus*	Nil.

* Gravid specimens were recovered from the intestine.

The experimental hosts were examined between five days and six weeks after feeding for the presence of juvenile or adult worms. Two gravid specimens of *Bucephalus longicornutus* were recovered from the intestine of one specimen of *Scorpaena cardinalis* Richardson, the scarpee or red rock cod. The posterior half of a bucephalid, considered to be *B. longicornutus* by the nature of the structure of the cirrus sac and genital atrium, was recovered from another specimen of *S. cardinalis*. This latter specimen was damaged during examination of the host's intestinal contents and despite a careful examination, its anterior half could not be found. Details of all experiments conducted are summarised in Table IV.

It was stated elsewhere (p. 26) that metacercariae do not reach maximum development until after approximately 80 days. Accordingly, the feeding of sub-mature metacercariae of only 25 to 75 days development in the above experiments might be questioned assuming that metacercariae are not infective until they have reached maximum development. The reason for feeding with sub-mature metacercariae was that some experiments were conducted before a satisfactory indication was obtained of when maximum development of the metacercariae was reached. Furthermore, the feeding of metacercariae of different ages to the one experimental host in some experiments might be questioned. Again, without the necessary knowledge of when metacercariae reached maximum development it was hoped to establish an infection and establish the age limits within which the metacercariae were infective. This was not successful. Only two experiments were performed, with host species 3 and 14, in which the metacercariae used were known beforehand to have reached maximum development.

It is of interest to note that successful results were obtained only with *Scorpaena cardinalis* and that this species (apart from *Trachelochismus* sp. which, however, was fed sub-mature metacercariae) was the only species used that was free from intestinal parasites other than the specimens of *Bucephalus longicornutus*. Whether there is any correlation between the successful experimental establishment of the bucephalid and the absence of other intestinal parasites can, as yet, only be conjectured.

It should be pointed out that *S. plumieri* from Florida which is related to *S. cardinalis* is infected with *Bucephalus scorpaenae* Manter, 1940. Therefore, it is possible that *S. cardinalis* could harbour a natural *Bucephalus* infection which would invalidate the experimental results. However, Manter (1954) examined nine specimens of *S. cardinalis* from Wellington and Portobello, and the author has, during the course of this study, examined 15 from Island Bay, Wellington, and no natural bucephalid infections have yet been found in this species. This supports the view accepted here, that the specimens of *B. longicornutus* recovered from the intestine of *S. cardinalis* were established experimentally.

THE ADULT

Two gravid specimens and the posterior half of another gravid specimen of *Bucephalus longicornutus* (Manter, 1954) were recovered from experimentally infected *Scorpaena cardinalis* Richardson. The definitive host was found to be *Kathetostoma giganteum* Haast, the monkfish, and examination of several specimens from different localities combined with the experimental material provided numbers of *B. longicornutus* for study as follows: from the intestine of *Scorpaena cardinalis* used as an experimental host, two complete specimens in one host, and the posterior half of a specimen in another host; from the intestine and pyloric caeca of *Kathetostoma giganteum* from Foveaux Strait, 74 specimens from 10 of 13 hosts; from *K. giganteum* from Cook Strait, one specimen from one of four hosts.

K. giganteum from Foveaux Strait showed a 77% incidence of infection, and in 38% of the infected specimens, 5 or more adult worms were recovered.

K. giganteum from Cook Strait showed a 25% incidence of infection and only one adult worm was recovered. This is a similar incidence of infection to that obtained by Manter (1954), who examined monkfish from Cook Strait.

A number of fish species were obtained from Foveaux Strait in order to determine whether the range of host species for *B. longicornutus* could be extended. These included three specimens of *Physiculus bachus* (Bloch and Schneider); two *Trigla kumu* (Lesson and Garnot); three *Neophrinichthys latus* (Hutton); one *Genypterus blacodes* (Bloch and Schneider); two *Parapercias colias* (Forster); and two *Arnoglossus scapha* (Forster). However, no bucephalid infections were found. It was not possible to obtain a large number of each host species and thus the zero infection found may not be significant.

Bucephalus longicornutus (Manter, 1954)

(Text-fig. 9, A-J.)

1954. *Alcicornis longicornutus* Manter, p. 482.

HOST: *Kathetostoma giganteum* Haast, monkfish; family Uranoscopidae.

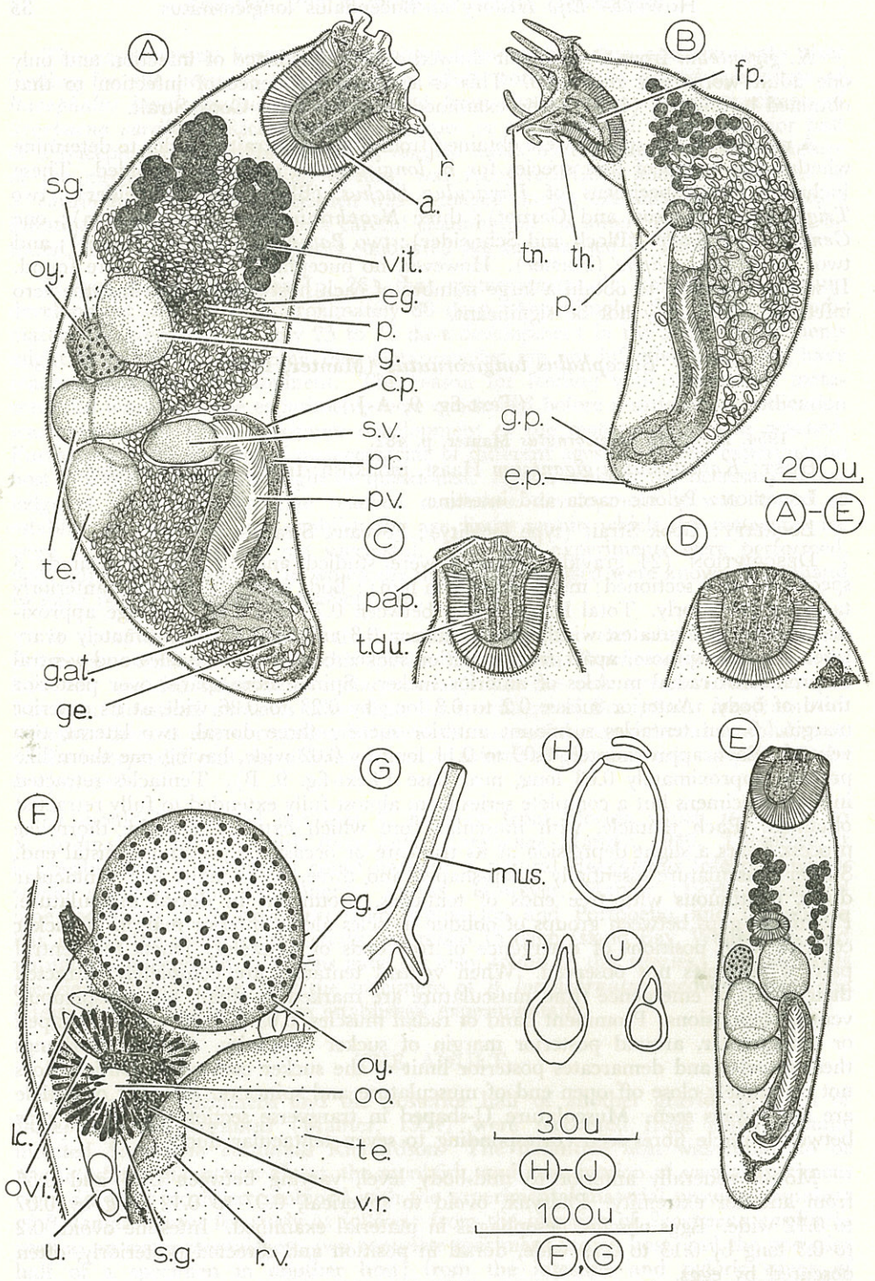
LOCATION: Pyloric caeca and intestine.

LOCALITY: Cook Strait (type locality); Foveaux Strait.

DESCRIPTION (24 gravid specimens were studied and 12 were measured; 3 specimens were sectioned; measurements in mm): Body elongate, truncate anteriorly tapering posteriorly. Total length varies between 0.73 and 2.86 (average approximately 1.6) and greatest width varies between 0.3 and 0.66 at approximately ovary level. Cuticle spinose, apart from anterior sucker concavity, tentacles and ventral to prominent radial muscles of anterior sucker. Spines more sparse over posterior third of body. Anterior sucker 0.2 to 0.3 long by 0.23 to 0.26 wide at its anterior margin. Seven tentacles surmount anterior sucker; three dorsal, two lateral, two ventral. Each approximately 0.09 to 0.11 long by 0.02 wide, having one thorn-like process, approximately 0.03 long, near base (Text-fig. 9, B). Tentacles retracted in most specimens but a complete series from almost fully extended to fully retracted obtained. Each tentacle, with muscular core which extends into the thorn-like process, bears a slight depression at its truncate or occasionally rounded distal end. Sucker musculature essentially scoop-shaped and three, occasionally five, tentacular ducts, continuous with free ends of tentacles, prominent in dorsal musculature. Prominent gaps between groups of oblique muscles along anterior margin of sucker coincide with positions of emergence of free ends of tentacles. Ducts for ventral pair of tentacles not observed. When ventral tentacles are completely retracted their points of emergence from musculature are marked by small, but conspicuous, ventral depressions. Prominent band of radial muscles, 0.05 to 0.06 wide, U-shaped or semicircular, around posterior margin of sucker concavity. Cuticle overhangs these muscles and demarcates posterior limit of the sucker concavity. Cuticle does not completely close off open end of musculature and spines on this part of cuticle are not always seen. Musculature U-shaped in transverse section and seven gaps between muscle fibres seen corresponding to seven tentacular ducts.

Mouth generally anterior to mid-body level, varying between 0.33 and 0.56 from anterior extremity. Pharynx, ovoid to spherical, 0.07 to 0.12 long by 0.07 to 0.12 wide. Eggs obscure oesophagus in material examined. Intestine ovoid, 0.2 to 0.3 long by 0.13 to 0.19 wide, dorsal in position and directed posteriorly, often obscured by eggs.

Testes, ovoid to spherical, 0.13 to 0.2 long by 0.1 to 0.2 wide, on right side of the body, generally between intestinal and mid cirrus sac levels, diagonal in most specimens but occasionally tandem. Anterior testis usually touches posterior margin of ovary but in one specimen it overlaps ovary ventrally by approximately



TEXT-FIG. 9.—*Bucephalus longicornutus*. Morphology and anatomy of adult specimens from *Kathetostoma giganteum* Haast: Fig. A, ventral view of a specimen with the tentacles partly retracted; Fig. B, left lateral view of a specimen with the tentacles extended; Fig. C, ventral view of the anterior sucker with the tentacles almost completely retracted; Fig. D, ventral view of the anterior sucker with the tentacles completely retracted; Fig. E, ventral view of an immature specimen; Fig. F, ventral view of the Mehlis's gland complex; Fig. G, detail of a tentacle; Fig. H, normal operculate egg; Figs. I, J, abnormal eggs. For abbreviations see p. 9.

0.08 and in two specimens it is separated from ovary by approximately 0.02. Posterior testis usually touches anterior testis but in two specimens, it slightly overlaps anterior testis dorsally and in two specimens it is separated from anterior testis by a narrow gap. Vasa deferentia not observed. Cirrus sac, 0.4 to 0.64 long by 0.09 to 0.15 wide, extends to about mid-body level. In one specimen, proximal third of its length is bent at right angles to distal two-thirds. In remaining specimens it varies from straight, to crescent-shaped to sigma-shaped (Text-figs. 9, A and B). Cirrus sac consists of ovoid seminal vesicle, 0.09 to 0.13 long by 0.08 to 0.1 wide, and long pars prostatica. Genital atrium, 0.19 to 0.3 long, contains a smooth or corrugated genital lobe, 0.1 to 0.17 long. Gland cells surround genital atrium. Genital pore varies between 0.08 and 0.11 from posterior extremity.

Ovary generally spherical (but in five specimens ovoid), varies between 0.1 and 0.13 in diameter or 0.1 to 0.11 long and 0.12 wide, on right side of body at approximately intestinal level. Oviduct emerges from right side of ovary, opens into ootype, 0.01 to 0.02 posterior to ovary and gives off Laurer's canal approximately one-third from its proximal end. Laurer's canal runs obliquely towards dorsal midline and opens to exterior at approximately anterior testis level. Uterus emerges from posterior margin of ootype and was traced posteriorly to intertesticular level. Remaining coils indistinct.

Vitellaria arranged in two lateral groups between sucker and pharynx. Individual follicles clumped or spread and this can be related to number of eggs in uterus. Most often, 16 follicles on each side but this number can vary between 14 and 18. Each follicle more or less spherical, between 0.04 and 0.06 in diameter. A difference of 0.005 generally exists between extremes in sizes of follicles in a given specimen. Left vitelline duct runs obliquely to posterior testis level then loops and runs anteriorly on right side of body to enter vitelline reservoir immediately median to ootype. Right vitelline duct runs slightly obliquely and enters vitelline reservoir. Short duct connects vitelline reservoir with ootype (Text-fig. 9, F).

Eggs occupy most of body in most specimens. Their anterior extent is variable. In two specimens they extend just beyond pharynx level but in most they reach to within 0.1 to 0.18 of posterior limit of sucker. Eggs extend posteriorly to level of genital pore. They are ovoid, distinctly operculate, 0.021 to 0.035 long by 0.015 to 0.022 wide (Text-fig. 9, H). Two specimens contained a few abnormal eggs (Text-fig. 9, I, J).

Excretory pore terminal and saccular excretory bladder extends just anterior to pharynx. Flame cell formula not determined.

DISCUSSION OF ADULT

Compared with the metacercaria, adult *B. longicornutus* have tentacles surmounting the anterior sucker and show considerable development of the reproductive system. The development of elements of the latter in the hindbody has a considerable bearing on the position of the mouth and intestine, a feature which differs considerably between metacercaria and adult. Remaining differences are relatively minor. Spines are absent from the cuticle overlying the band of radial muscles of the adult sucker; and the flap of cuticle closing off the open end of the sucker musculature in the metacercaria, while still present in the adult, is not conspicuous. Both of these differences may possibly result from the effects of attachment to the intestinal wall of the definitive host.

Only three immature worms were recovered from the material examined. Two of these had their tentacles partially extended and all three, apart from the absence of eggs, were very similar to sexually mature adults.

Manter (1954) placed the above species in the genus *Alcicornis* McCallum, 1917. The single specimen he recovered from one of four *Kathetostoma giganteum* from Cook Strait was mounted in lateral view and the sucker-like nature of the anterior end was not evident, appearing instead rhynchus-like, and this is diagnostic of the genus *Alcicornis*. Manter compared the species with other known species of *Alcicornis*. However, because of the change in generic status proposed here it is now necessary to compare *Bucephalus longicornutus* with related species of *Bucephalus*.

In the possession of one thorn-like process at the base of the tentacle, *B. longicornutus* resembles *B. polymorphus* von Baer, 1827, *B. introversus* Manter, 1940, and *B. scorpaenae* Manter, 1940. However, it differs from *B. polymorphus* in that the cirrus sac is much longer in relation to the total length, the tentacles do not taper to a point and the thorn-like process on the tentacle is situated nearer the base of the tentacle; from *B. introversus* in that the anterior sucker does not introvert into the anterior end of the body and the structure of the genital atrium is not as complex; and from *B. scorpaenae* in that the excretory vesicle is shorter, the pars prostatica is not subdivided and the genital lobe is simpler in structure.

B. longicornutus does show some resemblance to *B. kathetostomae* (Manter, 1934) (a species in which the structure of the tentacles is unknown), from a related host, *Kathetostoma albigutta* Bean. However, it differs from *B. kathetostomae* in the absence of the muscular sphincter surrounding the genital pore, shorter genital atrium, the absence of processes on the eggs and considerably shorter excretory vesicle.

There are two main differences between Manter's description of *B. longicornutus* and that given above. These are the bipartite rather than ovoid seminal vesicle, and the longer tentacles. Both of these characters are considered to be subject to variation. The shape of the seminal vesicle is probably dependent on the amount of sperm present and the length of the tentacles on their degree of contraction.

The variations noted for *B. longicornutus* are considerable. The position of the internal organs is apt to be governed by the degree of contraction of the body on fixation and by the number of eggs in the uterus. The arrangement of the vitelline follicles, which has been mentioned above, illustrates this latter point. Egg size is extremely variable between specimens from different hosts but is generally more or less constant within a group of specimens from the same host. A similar phenomenon has been noted by Manter (1940a) for *B. varicus* Manter, 1940 (synonym: *B. polymorphus* of Nagaty, 1937). Slight variations of from 0.002 to 0.004 exist between egg sizes in a given specimen as was also noted by Manter (1954) in the one specimen of *B. longicornutus* which he examined. Egg size cannot be correlated with the length of the specimen, e.g., a specimen of 1.718 long had eggs 0.021 by 0.016 while a specimen 1.818 long had eggs 0.032 by 0.020; but can, however, be correlated with the number of eggs present, e.g., a specimen 1.110 long had few eggs and these were large, being 0.035 by 0.022 while a specimen 1.496 long had many eggs and these were relatively small, being 0.024 by 0.016.

B. longicornutus parallels *B. varicus* in the range of variation exhibited. Such a range has not been noted for other species of the genus. However, many of these species are either insufficiently described (*vide* van Beneden, 1870) or known only from a few specimens (*vide* Velasquez, 1959). Characters such as total length, relative position of internal organs and minor differences of only 2μ to 3μ in egg size have, in some cases, been used to separate species (*vide* Velasquez, 1959). In view of the variations of these characters known for *B. varicus* and *B. longicornutus*, two species which have been collected from a number of different host species and the same host species respectively, these specific distinctions should be regarded as tentative and possibly subject to alteration after reference to further material.

The most constant features noted in those specimens of *B. longicornutus* examined were the nature of the genital atrium and genital lobe, and the floor of the anterior sucker. Unfortunately, descriptions of these features are absent from many systematic papers dealing with species of *Bucephalus* and they may, in future, prove more satisfactory criteria for differentiating between species than those used in the past.

THE MIRACIDIUM

The eggs of adult specimens of *Bucephalus longicornutus*, obtained and set up as described elsewhere (p. 5), were examined on 20 consecutive days in order to detect miracidia. None was found and no eggs showed signs of embryonation. The eggs may not have been viable but this point needs further investigation.

GENERAL DISCUSSION

The major features of the life history of *Bucephalus longicornutus* agree with those described for fresh-water bucephalids by Woodhead (1929, 1930), and Kniskern, 1952) and it would now seem probable that the type of life history displayed by these species is typical of all members of the family, whether fresh-water or marine.

A consideration of the life history of *B. longicornutus* shows there is still no way of determining the generic status of a cercaria other than by infection experiments although an alternative means has been suggested (p. 22). The latter means is hypothetical and yet to be tested.

Generic identity can be assigned to the metacercaria but its specific identity must be obtained from experimental infections.

It should be stressed again that future investigators must give more accurate descriptions of sporocysts, cercariae and metacercariae than have previously been given by many authors. Because of the close morphological similarity of bucephalid cercariae, information on flame cell formulae, behaviour and all essential details of spination and granulation will be necessary for separation of species. The variability of adult *B. varicus* and *B. longicornutus* noted on p. 36 may also apply to other species and genera of the family, and systematists are urged to avoid the tenuous criteria that have, hitherto, been used to differentiate between species.

SUMMARY

1. With this contribution to the life history of *Bucephalus longicornutus* (Manter, 1954), of which the sporocyst, cercaria, metacercaria and adult are described, life history studies on marine, digenetic trematodes are introduced to New Zealand workers, and life history studies of the economically important family Bucephalidae Poche, 1907, are reintroduced to overseas workers.

2. An analysis of previous life history studies of marine members of the family reveals that, hitherto, the experimental proof of the adult status of bucephalid sporocysts infecting bivalve molluscs has not been achieved. The major features of the bucephalid life history, ascertained previously from experimental studies of fresh-water species, are herein confirmed in a marine species for the first time.

3. Sporocysts of *B. longicornutus* infect the visceral mass, pericardium and gills of the mud-oyster, *Ostrea lutaria* Hutton, 1873. Terminal regions of the sporocyst are found on the gills adjacent to the visceral mass. It was not possible to isolate a complete sporocyst and therefore the number infecting an individual oyster can only be speculated.

4. The structure of the sporocysts of many species of bucephalid are imperfectly known. The nucleated layer of those species for which histological details are available and the present species is syncytial and contains one or two types of mesenchymal nuclei in addition to germinal nuclei. The former occur throughout the sporocyst wall while the latter are only in or near the terminal regions or growing points.

5. All previous reports of bucephalid cercariae are listed and it is considered that many descriptions of these are inadequate for comparative purposes. Cercariae described as species of *Bucephalus* or *Prosorhynchus* without experimental proof are herein transferred to the group name *Cercaria*. *Bucephalopsis* is valid only for *B. haimeanus* (Lacaze-Duthiers, 1854) and therefore *B. modiolae* Faust, 1928, becomes *C. modiolae* (Faust, 1928).

6. Upwards of 10,000 cercariae may be liberated from an infected oyster over a 24-hour period; over several weeks, peak liberations of cercariae occur intermittently with lulls of varying duration during which few or no cercariae are liberated. Cercariae do not actively swim but can remain suspended in calm water for at least an hour. They depend on turbulence in the water for initial dispersion from a resting position in the bottom of a finger bowl. Lateral movement is achieved by flexure of one of the furcae. It is postulated that the behaviour of the furcae (which in this species resembles *B. polymorphus* and *B. elegans* and contrasts with *C. apalachiensis*) may reflect the generic identity of the cercaria.

7. The method of attachment and penetration of the cercaria into the second intermediate host depends initially on accidental contact of the furcae with the body of the host; the cercaria drifts in the current and the tail stem attaches to the skin; a point of penetration is eroded with the aid of acidic and enzymic secretions of the cystogenous organ. The method of cyst wall formation from cystogenous granules (which are broken down and exuded through the cuticle), is governed by osmotic phenomena.

8. Encystment in experimental hosts occurs in the web of all fins, in the extrinsic muscles at the base of the fins, body wall muscles, under the skin of the head and branchial chamber and rarely in the conjunctiva of the eye. *Acanthoclinus quadridactylus* (Forster) and various unidentified species of *Tripterygion* were successfully infected with metacercariae. Age immunity is exhibited by some specimens of these hosts. Host specificity of the cercaria towards *Trachelochismus* sp. is recorded. The natural hosts of the metacercaria were not discovered.

9. Metacercariae are host specific against *Helcogramma medium* (Gunther), in which development does not proceed beyond 20 days after exposure to cercariae. The majority of cysts are resorbed after 35 days.

10. Invertebrates are unlikely hosts of the metacercariae.

11. Metacercariae reach maximum development after approximately 80 days in both experimental hosts. Spontaneous excystment of at least some mature metacercariae occurs. Cysts in *Tripterygion* sp. tend to become pigmented after 85 to 90 days and are either dead or degenerate after 110 days. Cysts from *A. quadridactylus* do not become pigmented and the metacercariae are still active after 120 days. It is suggested that *A. quadridactylus* is more closely related to the natural host or hosts of the metacercaria than *Tripterygion* sp. Mature metacercariae bear an anterior sucker (devoid of tentacles) whose scoop-shaped musculature is characteristic of species of *Bucephalus*. Tentacular ducts are located in the sucker musculature.

12. Gravid specimens of *B. longicornutus* (Manter, 1954), were recovered from the intestine of *Scorpaena cardinalis* Richardson, the scarpee, 35 days after feeding

fish infected with metacercariae of 80 days or more development. In addition to this material, 75 specimens of *B. longicornutus* were recovered from naturally infected specimens of *Kathetostoma giganteum* Haast, the monkfish, the definitive host, from Foveaux Strait and Cook Strait. Although a few species of fish, other than *K. giganteum,* have been examined from Foveaux Strait no other natural hosts of *B. longicornutus* adults have been found.

13. The range of variation in egg size, and the relative positions of the internal organs is considerable and is comparable to that exhibited by *B. varicus* Manter, 1940. It is considered that to define species on the basis of a few specimens is unwise. Egg size, and the relative positions of internal organs is of doubtful value in differentiating between species, and some species of *Bucephalus* will possibly fall as synonyms when further material is examined and the range of variation for the various species is determined.

14. Attempts to obtain miracidia from the eggs of experimentally induced adults were not successful. The eggs may not have been viable.

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