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THE LARVAE OF SOME MONOGENETIC TREMATODE PARASITES OF PLYMOUTH FISHES

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(Text-figs. 1–28)

The Order Monogenea of the Class Trematoda contains (Sproston, 1946) upwards of 679 species, but in only twenty-four of these species has a larval form been described. Of these larvae, fourteen belong to adults that parasitize fresh-water fishes, amphibians or reptiles, and ten to adults that parasitize marine fishes. *Udonella caligorum* is known (Sproston, 1946) not to have a larval stage in its life history. In the present study, accounts will be given of eleven hitherto undescribed larvae which belong to adults that parasitize marine fishes at Plymouth, and which represent seven of the eighteen families (Sproston, 1946) of the Monogenea.

For the most part the literature on larval monogeneans consists of isolated studies of individual species, and these have been listed by Frankland (1955), but the descriptions of four new larvae by Euzet (1955) appeared too late for inclusion in Frankland's list. More general observations on monogenean life cycles have been made by Stunkard (1937) and Baer (1951), and Alvey (1936) has speculated upon the phylogenetic significance of monogenean larvae.

Previous accounts of monogenean larvae have included but scant reference to culture techniques, presumably because the methods adopted have been simple and successful; in my hands, however, many attempts to rear larvae have been unsuccessful, and so some details of those procedures which have yielded successful cultures are included. At the same time it must be pointed out that the main object of the present study was to obtain specimens of monogenean larvae for morphological investigation, and properly designed experiments to determine factors influencing embryonic development were not attempted.

In addition to the studies made upon the eleven previously undescribed larvae, observations were made upon the larvae of *Diclidophora luscae* and *Polystoma integerrimum* in order to facilitate comparisons and to provide practice in techniques.

In past accounts of monogenean larvae it has been customary to describe the egg capsules, but the variations in the form of the capsules of the species studied here appear likely to be related more to variations in habits of oviposition than to larval characters, and so it is intended later to study the egg

capsules in relation to their respective adults. However, it seems worth while to record now that the thirteen species of monogeneans that I have studied, together with those in previous descriptions of larvae, all have operculate capsules. It is remarkable therefore that Dawes (1946, p. 13) should have stated that in Monogenea, operculate eggs are sufficiently rare to make this character of general diagnostic value in distinguishing between Monogenea (non-operculate eggs) and Digenea (operculate eggs).

MATERIAL AND TECHNIQUES

Specimens of adult trematodes were obtained from fishes at Plymouth in July and August, some in 1954 and 1955, but mainly in 1956. The gills of the fish hosts were examined in a manner described previously (Llewellyn, 1956), and in addition trematodes were obtained from the skin of the Common Sole and the Cuckoo Ray. A list of the parasites studied, named and classified according to Sproston (1946), is included in Table 1.

TABLE 1. CLASSIFIED LIST OF THE PARASITES STUDIED

		Host	
Monopisthocotylea			
Gyrodactyloidea			
Dactylogyridae	*Diplectanum aequans*	*Morone labrax*	Gills
Capsaloidea			
Capsalidae	*Entobdella soleae*	*Solea solea*	Skin
Acanthocotyloidea			
Acanthocotylidae	*Acanthocotyle lobianchi*	*Raja clavata*	Skin
Polyopisthocotylea			
Aviellloidea			
Polystomatoidea			
Polystomatidae	**Polystoma integerrimum*	*Rana temporaria*	Bladder
Hexabothriidae	*Rajonchocotyle emarginata*	*Raja clavata*	Gills
Diclidophoroidea			
Discocotylidae	*Plectanocotyle gurnardi*	*Trigla cuculus*	Gills
	Anthocotyle merluccii	*Merluccius merluccius*	Gills
Microcotylidae	*Gastrocotyle trachuri*	*Trachurus trachurus*	Gills
	Pseudaxine trachuri	*Trachurus trachurus*	Gills
	†Unidentified microcotylid species	*Trachurus trachurus*	Gills
	Microcotyle labracis	*Morone labrax*	Gills
Diclidophoridae	*Diclidophora merlangi*	*Gadus merlangus*	Gills
	**Diclidophora luscae*	*Gadus luscus*	Gills

* Described by previous authors, but studied from fresh material in the present investigation.

† This is the same trematode as referred to previously (Llewellyn, 1956, p. 117); a description of the adult will be published later.

Adult trematodes were transferred as soon as possible to dishes of fresh sea water and rinsed free of mucus with jets of sea water from a pipette. Even specimens that appeared moribund usually became active after this treatment, although it was sometimes several hours before egg-laying was resumed. The trematodes were rinsed in several changes of Berkefeld-filtered sea water and then placed at the various controlled temperatures that from time to time

became available. As stated previously, it was not possible to conduct properly designed experiments on the factors influencing the rate of egg-capsule production and the period of embryonic development, but the following general observations on about 480 adults and about 2600 capsules may be of use to future workers.

It seems likely that although adult parasites survive for at least 2 or 3 weeks, and probably longer at temperatures of 3–7° C, very few or no egg capsules are produced below about 8° C. At 13° C, which is approximately the temperature of the bottom water in the localities from which the hosts were trawled, the rate of capsule production per parasite per hour varies according to species between about 1·0 and 0·5 on the first day or two, falling to about 0·25 on the third day, and ceasing altogether on the fourth day excepting in *Entobdella*, which continues to lay at gradually decreasing rates for another 2 or 3 days. At 18° C the rate increases to about 3 or 4 capsules per hour for the first 12 h, but the parasites do not survive longer than about 24 h. At 20° C in most species there was no egg production, and the parasites died within about 12 h, but *Plectanocotyle*, *Pseudaxine*, *Gastrocotyle*, *Microcotyle* and *Diplectanum* were able to lay about 0·5 per hour before dying.

An attempt was made to collect eggs from parasites still attached to a living host by keeping isolated specimens of living *Trachurus trachurus* in small tanks, but a collection of only fifty-seven capsules in 7 h from a host subsequently found to be harbouring thirty specimens of *Gastrocotyle trachuri* was disappointing, and collecting from isolated parasites was thought to be more profitable.

Culture dishes were examined daily with a stereomicroscope, and egg capsules were transferred by means of a pipette to dishes of fresh, filtered sea water. Dishes of various shapes and sizes were tried, and eventually it was found that covered Petri dishes of 4 cm diameter and 2 cm depth provided the most acceptable compromise between the conflicting desiderata of a small volume of water to facilitate searching for and capturing larvae with a pipette, and a large volume to minimize the frequently fatal osmotic effects due to evaporation from the sea water. The use of filtered sea water, combined with incubation in darkness, helped to restrict contamination of the cultures, for while the presence of extraneous organisms appeared to have little effect upon actual embryonic development, conditions for observation were made more difficult. The chief disadvantage of contamination, however, was the damaging attack by ciliates upon larvae during the period of their actual emergence from the capsule.

Little or no embryonic development took place in any species at temperatures of 8° C and below. At 13° C the period of embryonic development in *Plectanocotyle* was found to be something between 21 and 30 days, precise determination being complicated by the habit of the adult of accumulating the capsules in the uterus for 3 or 4 days before laying, and thus the exact

age of the capsules at the commencement of incubation was not known. Embryos of *Anthocotyle*, *Gastrocotyle* and *Diclidophora merlangi* all failed to complete development at 13° C in the 28-day period available to me for observation. At 18° C *Plectanocotyle* hatched after 13–16 days' incubation, and *Anthocotyle* after 21 days. At 20° C the periods in days of embryonic development were as follows: *Diplectanum*, 5; *Plectanocotyle*, 8–11; 'microcotylid species', 10; *Acanthocotyle*, 12; *Entobdella*, *Microcotyle labracis*, *Gastrocotyle* and *Pseudaxine*, 14–16; *Rajonchocotyle*, 25; and *Diclidophora merlangi*, 27.

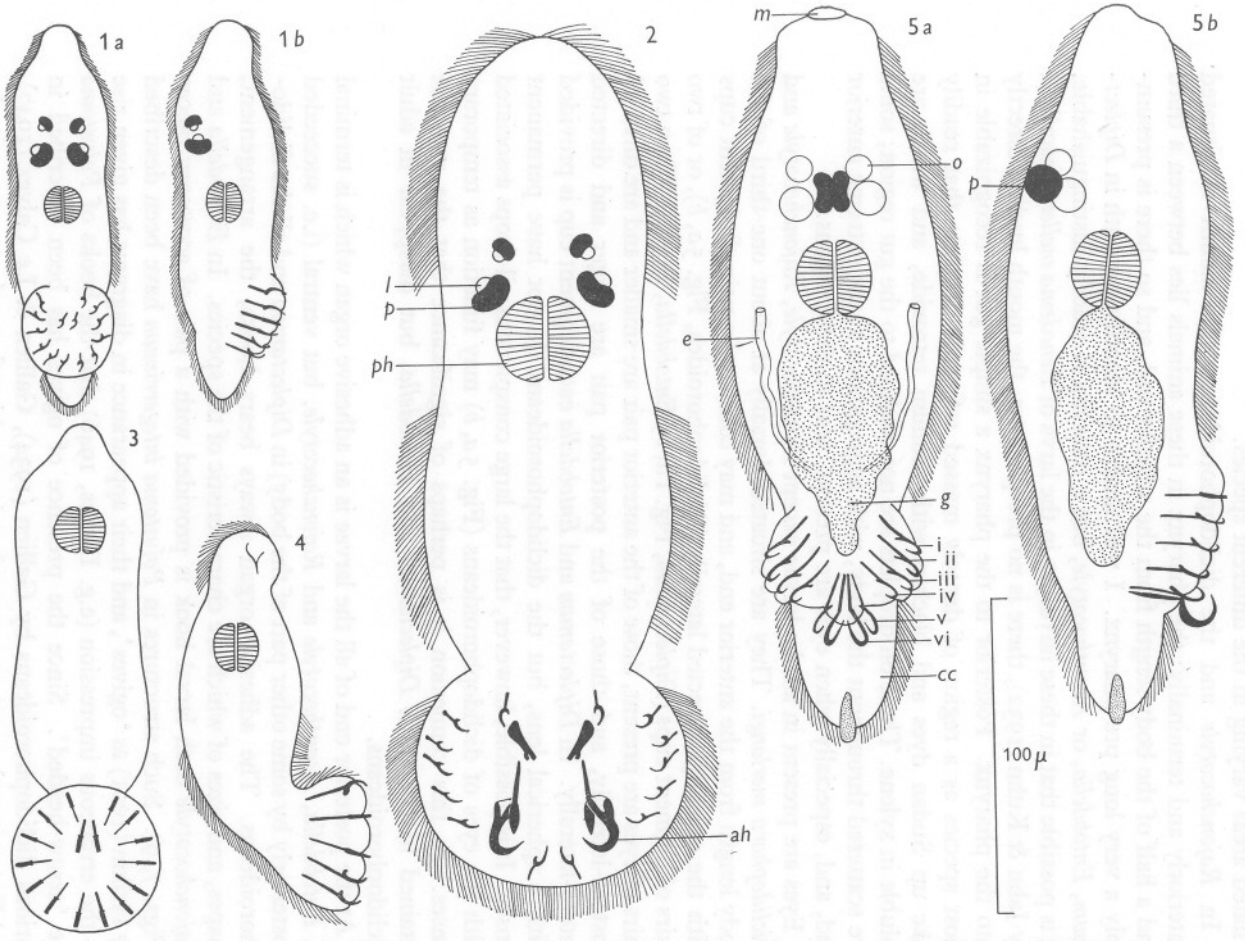
Hatching in all species appeared to be brought about entirely by repeated pressure against the operculum by muscular extension of the larvae as described by Frankland (1955) for *Diclidophora denticulata*, and in no case was there present a 'viscous cushion' mechanism as has been described in *Fasciola hepatica* by Rowan (1956).

From time to time it was found convenient to delay hatching by storing capsules containing active larvae at a temperature of about 4° C. Such specimens could subsequently be induced to hatch by returning them to a temperature of about 20° C, following which hatching usually took place in the course of some 6–12 h.

Free-living larvae and active larvae still enclosed in their capsules were examined under the microscope by mounting them on slides in sea water under cover-glasses supported by petroleum jelly in the manner generally practised for the study of digenean larvae. These fresh preparations, pressed to the required degree by the withdrawal of sea water with filter paper, were found to be much more useful than more permanent preparations, the best of which were made by mounting specimens either directly in Farrant's Medium, or, after fixation in formaldehyde and staining in borax carmine, in Canada Balsam. With temporary preparations, an apochromatic water-immersion objective was found more suitable than an oil-immersion objective since there was less tendency for the cover-glass to be moved with the objective during focusing. Extensive use of photography was made in recording the shapes of the larvae, and Figs. 1–28 were prepared from such photographs.

DESCRIPTIONS OF THE LARVAE (Figs. 1–28)

The larvae of the monogeneans studied are all cylindrical or ovoid organisms between 100 and 300 μ long, and between 30 and 100 μ in diameter. Although belonging to the 'flatworm' phylum, the degree of dorso-ventral flattening in these larvae is at most only very slight (compare Fig. 1*a* with *b*, and Fig. 5*a* with *b*). The anterior end of the body is tapered to form a conical region that may be marked off from the rest of the body by a shoulder region, and the posterior end is similarly tapered (*Diplectanum*, Fig. 1*a*, *b*, and the diclidophoroideans, Fig. 5*a*, *b*), or ends in a terminal adhesive organ (*Entobdella*, *Acanthocotyle*, *Rajonchocotyle*, Figs. 2–4 respectively).



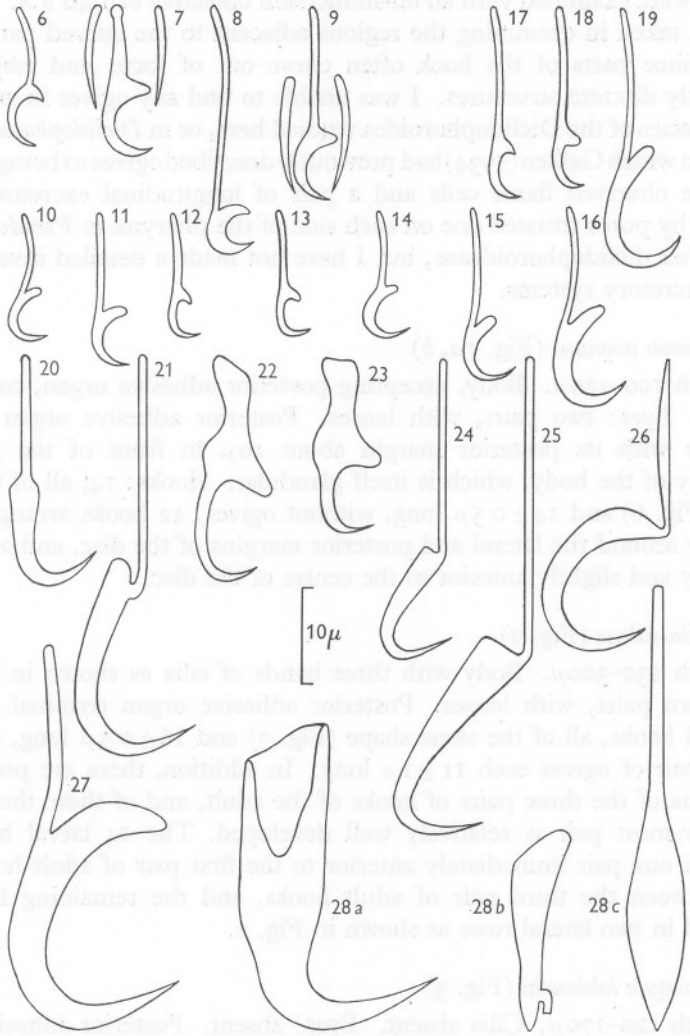
Figs. 1–5. Monogenean larvae, all drawn to same scale (ventral view unless otherwise stated). Fig. 1*a*, *Diplectanum aequans*. Fig. 1*b*, *D. aequans* (side view). Fig. 2, *Entobdella soleae*. Fig. 3, *Acanthocotyle lobianchi*. Fig. 4, *Rajonchocotyle emarginata* (side view). Fig. 5*a*, *b*, schematized larva of the superfamily Diclidophoroidea (Fig. 5*b* side view). *ah*, primordia of hooks of adult; *cc*, ciliated cone; *e*, excretory canal; *g*, gut; *l*, lens of eye; *m*, mouth; *o*, oil droplet; *p*, pigment cup of eye; *ph*, pharynx; i–iv, lateral hooks; v, postero-lateral hook; vi, posterior hook.

In all larvae except *Acanthocotyle* the body is ciliated, the extent of the ciliated areas varying in the different species.

In *Rajonchocotyle* and the diclidophoroideans, the mouth is situated anteriorly and terminally; the pharynx in these animals lies between a third and a half of the body length from the anterior end, and so there is presumably a very long pre-pharynx. I was unable to identify a mouth in *Diplectanum*, *Entobdella*, or *Acanthocotyle*, but a pharynx is readily distinguishable. It is possible that in these larvae, as in the larva of *Benedenia melleni* described by Jahn & Kuhn (1932), there is no pre-pharynx, the mouth leading directly into the pharynx. Posterior to the pharynx a simple gut is recognizable in most species as a region of densely massed refractile droplets that readily take up Sudan dyes and blacken with osmium tetroxide, and which are soluble in xylene. These oil droplets are not confined to the gut region; some are scattered throughout the body, with aggregations at the extreme anterior end, and, especially when eyes are present, near to these organs.

Eyes are present in all the larvae except *Acanthocotyle*, *Rajonchocotyle* and *Diclidophora merlangi*. They are situated dorsally at about one-third of the body length from the anterior end, and may consist of a pair of pigment cups with the openings directed laterally (Diclidophoroidea, Fig. 5*a*, *b*), or of two pairs of pigment cups (*Diplectanum*, Fig. 1*a*, *b*; *Entobdella*, Fig. 2). When two pairs of eyes are present, those of the anterior pair are smaller and are directed postero-laterally, and those of the posterior pair are larger and directed antero-laterally. In *Diplectanum* and *Entobdella* each pigment cup is provided with a spherical lens, but the diclidophoroideans do not have permanent lenses. It is possible, however, that the large conspicuous oil drops associated with the eyes of diclidophoroideans (Fig. 5*a*, *b*) may function as temporary lenses. In this connexion it is perhaps of significance that the eyes are retained in the adult *Diplectanum* and *Entobdella*, but disappear in adult diclidophoroideans.

At the posterior end of all the larvae is an adhesive organ which is terminal in *Entobdella*, *Acanthocotyle* and *Rajonchocotyle*, but ventral (i.e. succeeded posteriorly by some other part of the body) in *Diplectanum* and all the diclidophoroideans. The adhesive organ always bears hooks, the arrangement, shapes, and sizes of which are characteristic of the species. In *Entobdella* and *Rajonchocotyle* each larval hook is provided with a pair of accessory pieces (Figs. 7, 9). Such structures in *Polystoma integerrimum* have been described by Halkin (1901) as 'ogives', and their appearance in diagrams has given rise to the erroneous impression (e.g. Dawes, 1946) that the hooks of *Polystoma* are 'arrow-headed'. Since the presence of ogives has been described in various diclidophoroideans by Gallien (1934), Gallien & Le Calvez (1947), and Euzet (1955), great care was taken in searching for such structures in the diclidophoroideans studied in the present investigation. Thoroughly flattened specimens, mounted in sea water, glycerin, Farrant's Medium, or Canada



Figs. 6–28. Hooks of larval monogeneans, all drawn to same scale. Fig. 6, *Diplectanum aequans*. Fig. 7, *Entobdella soleae* (with ogives). Fig. 8, *Acanthocotyle lobianchi*. Fig. 9, *Rajonchocotyle emarginata* (with ogives). Figs. 10–16, typical lateral hooks of diclidophoroidean larvae. Fig. 10, *Plectanocotyle gurnardi*. Fig. 11, *Anthocotyle merluccii*. Fig. 12, *Gastrocotyle trachuri*. Fig. 13, *Pseudaxine trachuri*. Fig. 14, unidentified microcotylid species. Fig. 15, *Microcotyle labracis*. Fig. 16, *Diclidophora merlangi*. Figs. 17–19, postero-lateral hooks of diclidophoroideans. Fig. 17, *Gastrocotyle trachuri*. Fig. 18, *Pseudaxine trachuri*. Fig. 19, *Diclidophora merlangi*. Figs. 20–26, posterior hooks of diclidophoroideans. Fig. 20, *Plectanocotyle gurnardi*. Fig. 21, *Anthocotyle merluccii*. Fig. 22, *Gastrocotyle trachuri*. Fig. 23, *Pseudaxine trachuri*. Fig. 24, unidentified microcotylid species. Fig. 25, *Microcotyle labracis*. Fig. 26, *Diclidophora merlangi*. Figs. 27–28, primordia of adult hooks present in larva at time of hatching. Fig. 27, *Microcotyle labracis*. Fig. 28 *a*, *b*, *c*, 3rd, 1st and 2nd hooks respectively of *Entobdella soleae*.

Balsam, were examined with an oil-immersion objective of 1·40 N.A. Especial care was taken in examining the regions adjacent to the curved parts of the hooks, since parts of the hook often curve out of focus and reappear as apparently discrete structures. I was unable to find any ogives in any of the seven species of the Diclidophoroidea studied here, or in *Diclidophora luscae*, a species in which Gallien (1934) had previously described ogives as being present.

I have observed flame cells and a pair of longitudinal excretory canals opening by pores situated one on each side of the pharynx in *Pseudaxine* and some other diclidophoroideans, but I have not made a detailed investigation of the excretory systems.

Diplectanum aequans (Fig. 1 *a*, *b*)

Length 100–130 μ. Body, excepting posterior adhesive organ, completely ciliated. Eyes: two pairs, with lenses. Posterior adhesive organ situated ventrally with its posterior margin about 10 μ in front of the posterior extremity of the body, which is itself glandular. Hooks: 14, all of the same shape (Fig. 6) and $14 \pm 0{\cdot}5\,\mu$ long, without ogives; 12 hooks arranged equidistantly around the lateral and posterior margins of the disc, and 2 situated medianly and slightly anterior to the centre of the disc.

Entobdella soleae (Fig. 2)

Length 230–300 μ. Body with three bands of cilia as shown in diagram. Eyes: two pairs, with lenses. Posterior adhesive organ terminal. Hooks: 14 larval hooks, all of the same shape (Fig. 7) and $16 \pm 0{\cdot}5\,\mu$ long, and each with a pair of ogives each $11 \pm 1\,\mu$ long. In addition, there are present the primordia of the three pairs of hooks of the adult, and of these the third or posterior-most pair is relatively well developed. The 14 larval hooks are arranged one pair immediately anterior to the first pair of adult hooks, one pair between the third pair of adult hooks, and the remaining five pairs arranged in two lateral rows as shown in Fig. 2.

Acanthocotyle lobianchi (Fig. 3)

Length 120–170 μ. Cilia absent. Eyes: absent. Posterior adhesive organ terminal. Hooks: 16, all of same shape (Fig. 8) and $26 \pm 0{\cdot}5\,\mu$ long, without ogives, with 14 arranged peripherally and equidistantly and two situated more centrally. An adhesive organ exactly similar to this larval structure is found immediately posterior to the large adhesive organ of the adult, thus it seems extremely probable that the larval adhesive organ survives unaltered throughout life.

Rajonchocotyle emarginata (Fig. 4)

Length 100–140 μ. Body with two bands of cilia as shown in diagram. Eyes: absent. Posterior adhesive disc terminal. Hooks: 10, all of the same

shape (Fig. 9), $20 \pm 0{\cdot}5\,\mu$ long, each provided with a pair of ogives $12 \pm 1{\cdot}0\,\mu$ long, and arranged approximately equidistantly round the periphery of the adhesive organ. All of my preparations were slightly distorted, and I was unable to recognize the bilateral arrangement of hooks illustrated by Euzet (1955) for *Neoerpocotyle catenulata* (Hexabothriidae), but it is possible that such arrangement is present in *Rajonchocotyle*.

Diclidophoroidea (Fig. 5*a*, *b*)

Body, excepting adhesive organ, completely ciliated. Eyes: a single pair of laterally directed pigment cups without permanent lenses but with conspicuous oil droplets that probably function as lenses; paired nature of eyes easily recognizable in some, e.g. *Plectanocotyle*, but the two eyes so closely adpressed as to appear as one in others (e.g. *Pseudaxine*); eyes absent in *Diclidophora*. Body terminating posteriorly in a ciliated cone, the apex of which is glandular and from which a drop of viscous material in the course of being secreted may frequently be observed. This ciliated cone is probably a deciduous organ, since it is not recognizable after the shedding of the ciliated epidermis. Posterior adhesive organ ventral and bears a pair of lateral 'wings' on which are borne some of the larval hooks. Hooks: 12, without ogives, arranged in 6 pairs that exhibit serial differentiation into two or three kinds (Figs. 10–26). The hooks of the first four pairs (=lateral hooks) are invariably all alike in size and shape (Figs. 10–16), and are borne on the lateral wings of the adhesive organ; the hooks of the fifth pair (=postero-lateral hooks) are similar in shape to the lateral hooks, but may be larger (Figs. 17–19), and are borne more medianly than the lateral hooks; the hooks of the sixth pair (=posterior hooks, Figs. 20–26), are invariably larger than and of a different shape from the first five pairs, the particular shape and size varying with the species (Figs. 20–26).

Plectanocotyle gurnardi

Length 100–150 μ. Lateral and postero-lateral hooks all of same shape (Fig. 10) and all $13 \pm 0{\cdot}5\,\mu$ long. Posterior hooks (Fig. 20) $23 \pm 0{\cdot}5\,\mu$ long, with the shaft joined to the middle of the proximal border of the blade.

Anthocotyle merluccii

Length 160–200 μ. Lateral and postero-lateral hooks all of same shape (Fig. 11) and $16 \pm 1{\cdot}0\,\mu$ long. Posterior hooks (Fig. 21) $40 \pm 2\,\mu$ long, with a long slender shaft attached to the blade at some little distance down the inner border of the blade.

Gastrocotyle trachuri

Length 160–200 μ. Postero-lateral hooks ($21 \pm 1\,\mu$ long, Fig. 17) longer than the lateral hooks ($13 \pm 0{\cdot}5\,\mu$, Fig. 12). Posterior hooks with shaft region expanded into a stout curved base (Fig. 22).

Pseudaxine trachuri

Length 160–200 μ. Lateral hooks (Fig. 13) indistinguishable from those of *Gastrocotyle*, postero-lateral hooks ($19 \pm 1\,\mu$, Fig. 18) with shafts slightly shorter than those of *Gastrocotyle*. Posterior hooks ($19 \pm 1\,\mu$, Fig. 23) similar in shape to those of *Gastrocotyle*, but smaller.

Microcotylid species from *Trachurus*

Length 100–140 μ. Lateral hooks and postero-lateral hooks all identical with each other ($13 \pm 0{\cdot}5\,\mu$, Fig. 14). Posterior hooks ($32 \pm 1\,\mu$, Fig. 24) with long slender shaft attached at the junction of the proximal border of the blade to the inner border of the blade.

Microcotyle labracis

Length 200–240 μ. Lateral and postero-lateral hooks all identical with each other ($18 \pm 1\,\mu$, Fig. 15). Posterior hooks similar in shape to those of previous microcotylid species, but larger ($51 \pm 1\,\mu$, Fig. 25). In the newly hatched larva of *M. labracis* there are present the primordia of one of the pairs of hooks of the adult (Fig. 27).

Diclidophora merlangi

Length 210–250 μ. Postero-lateral hooks ($27 \pm 2\,\mu$, Fig. 19) similar in shape to the lateral hooks ($21 \pm 2\,\mu$, Fig. 16), but larger. Posterior hooks ($31 \pm 2\,\mu$, Fig. 26) with outer margin of the proximal region of the blade sloping to meet the shaft.

COMPARISONS WITH OTHER MONOGENEAN LARVAE

Diplectanum aequans

The larva of *Diplectanum aequans*, in possessing 2 pairs of eyes and having 14 larval hooks, resembles the following larval gyrodactyloideans: *Dactylogyrus vastator*, described by Kulwieć (1929); *D. anchoratus*, described by Kulwieć (1927); *Neodactylogyrus macracanthus*, described by Wilde (1936); and *Acolpenteron ureteroecetes*, described by Fischthal & Allison (1942). *Diplectanum* differs from another gyrodactyloidean *Ancyrocephalus vistulensis*, described by Siwak (1932), only in that the latter is reported as having only 12 hooks, although Siwak's illustration of the larva appears to show 13 hooks. *Ancyrocephalus* was illustrated as having a posterior ciliated cone such as is present in *Diplectanum* and also in *Neodactylogyrus macracanthus*.

Entobdella soleae

The larva of *Entobdella soleae* bears a very close resemblance to the larva of *Benedenia melleni*, described by Jahn & Kuhn (1932), and differs from it only

in the presence in the larval *Benedenia* of the anterior suckers which characterize the adult. The larvae of *Entobdella* and *Benedenia*, both capsaloideans, resemble the previously discussed gyrodactyloidean larvae in having 2 pairs of eyes and 14 larval hooks, but in the capsaloideans primordia of the 3 pairs of adult hooks are also present.

Acanthocotyle lobianchi

The larva of *Acanthocotyle lobianchi*, as described in the present study, resembles the embryo of *A. pugetensis*, as described by Bonham & Guberlet (1938), and thus differs slightly in the arrangement of its 16 hooks from the embryonic *A. pacifica*, which was also described by Bonham & Guberlet. Of other young forms of Monogenea, the embryos and larvae of *Acanthocotyle* with their 16 hooks and absence of eyes and cilia resemble most the embryo of *Gyrodactylus elegans*, described by Katheriner (1904). The significance of this similarity may, however, be slight when the clearly abnormal polyembryonic development of *Gyrodactylus* is considered.

Rajonchocotyle emarginata

The larva of *Rajonchocotyle emarginata*, with its absence of eyes and its 10 hooks, resembles very closely the larva of *Neoerpocotyle catenulata* described by Euzet (1955) and belonging to the same family, the Hexabothriidae.

Diclidophoroidea

Among the Diclidophoroidea the only previously known larval discocotylid is *Diplozoon paradoxum*, described by Zeller (1872*a*). This species differs from *Plectanocotyle gurnardi* and *Anthocotyle merluccii* in that the newly hatched young does not have a larval adhesive organ, the clamps of the adult being already present. One pair of hooks is present, and these persist in the adult, but insufficient is known of their development to permit comparisons with the larval hooks of other species. In common with other diclidophoroideans, the newly hatched *Diplozoon* has 2 eyes which disappear in the adult.

The larvae of *Microcotyle labracis* and of the 'unidentified microcotylid species' from *Trachurus trachurus* resemble the larvae of *Microcotyle chrysophrii* and *Axine bellones*, described by Euzet (1955), in that they are diclidophoroideans having the lateral and postero-lateral hooks all similar to each other, and in that the shapes of the posterior hooks in all four species are all very similar. In all these microcotylideans except the one from *Trachurus*, a seventh pair of hooks, which appears to be primordia of adult hooks, is present. Such hooks are also present in the larva of *Microcotyle spinicirrus*, described by Remley (1942), and very probably in *Diplasiocotyle johnstoni* also, but Sandars's (1944) description of the latter species was insufficiently

detailed to be certain about this point. *Microcotyle spinicirrus* differs from all the other known microcotylid larvae in that it has *six* pairs of lateral and postero-lateral hooks in addition to its posterior hooks and its primordial adult hooks.

The larvae of *Gastrocotyle trachuri* and *Pseudaxine trachuri* resemble each other very closely indeed, and in fact are only distinguishable from each other in the slight differences in size of their hooks (see pp. 251–2). Both species differ from the other members of the Microcotylidae, in which they are at present classified, in the differences between lateral and postero-lateral hooks, in the very different shapes of the posterior hooks, and in the absence of primordia of adult hooks.

A comparison of the larva of *Diclidophora merlangi* with those of *D. denticulata*, described by Frankland (1955), and *D. luscae*, described by Gallien (1934), reveals no differences excepting in the relative sizes of the lateral and postero-lateral hooks. Gallien (1934), without giving a separate description, stated that the structure of the adhesive organ of the larva of *D. pollachii* was identical with that of *D. luscae*.

The above comparisons have included reference to all the known larval monogeneans excepting the two diclidophoroideans *Kuhnia scombri*, described by Gallien & Le Calvez (1947), and *Hexostoma thynni*, described by Euzet (1955), and the Polystomatidae *Polystoma integerrimum*, described by Zeller (1872*b*), Halkin (1901), and Gallien (1935); *Sphyranura oligorchis*, described by Alvey (1936); *Polystoma nearcticum* and *Polystomoidea oris*, both described by Paul (1938); and *Diplorchis scaphiopodis*, described by Rodgers (1941).

Kuhnia scombri differs from other larval diclidophoroideans in that the hooks are all similar, there being no differentiation into lateral, postero-lateral and posterior hook types, and in that no eyes are present. *Hexostoma thynni* resembles the discocotylideans *Plectanocotyle* and *Anthocotyle* in its equipment of hooks, but differs from all other diclidophoroideans in that it has 2 pairs of eyes and a curious asymmetrical longitudinal band of pigment. The five larval Polystomatidae all resemble each other in having 16 larval hooks with one or 2 pairs of primordial adult hooks. Four of the species have two pairs of eyes, but in the fifth species *Sphyanura osleri*, which hatches in an advanced state of development, eyes (and cilia) are absent. The polystomatid larvae are thus quite similar to the larval gyrodactyloideans and capsaloideans from which they differ in having 16 instead of 14 larval hooks.

DISCUSSION

Monogenean larvae were in the past variously referred to as 'miracidia' or merely as 'ciliated larvae', until Gallien (1934) proposed the term 'gyrodactyloid larva' for the larvae of *Diclidophora* and *Polystoma*, on account

of their resemblance to *Gyrodactylus*. However, *Gyrodactylus* has a very abnormal embryonic development, and the adult does not have the cilia or eye-spots characteristic of most monogenean larvae. Moreover, the use of a term which is also used to refer to the members of one particular superfamily of the Monogenea could cause confusion when it is desired to refer to the larvae of species belonging to other superfamilies. Thus in place of Gallien's 'gyrodactyloid larva' as a name for monogenean larva, I propose the substitution of 'oncomiracidium' (Greek, *onkos*, hook; *meirakidion*, youth), which suggests affinity with the familiar digenean miracidium and reflects the presence of the characteristic monogenean hooks.

The contribution that studies of larvae may make to the study of the phylogeny of the Monogenea will now be considered. If the 33 oncomiracidia now known (*Diplozoon* and *Sphyranura* are omitted because of their very advanced development on hatching) are arranged according to their complements of eyes and hooks, the following groups readily emerge.

1. Gyrodactyloideans (except *Gyrodactylus*) and capsaloideans, both with 14 larval hooks and 2 pairs of eyes with lenses (9 spp.).
2. Polystomatidae with 16 larval hooks and 2 pairs of eyes with lenses (4 spp.).
3. Diclidophoroideans with 6 pairs of larval hooks exhibiting serial differentiation, and with 0, 1, or 2 pairs of eyes without permanent lenses (16 spp.).
4. Hexabothriidae with 10 larval hooks and without eyes (2 spp.).
5. *Acanthocotyle* and *Gyrodactylus*, both with 16 hooks and without eyes. On account of the abnormal development of *Gyrodactylus* and the difference in hosts (one on elasmobranchs, the other on teleosts), the resemblance of these species may be no more than superficial (2 spp.).

This grouping differs from the present classification of adults (Sproston, 1946), mainly in the divorce of the family Hexabothriidae from its place alongside the Polystomatidae in the superfamily Polystomatoidea, and in the inference of a closer affinity than is now recognized between the Polystomatidae on the one hand, and the Gyrodactyloidea and Capsaloidea on the other. A consideration of the affinities between the respective hosts yields strong support for the re-arrangement since the Hexabothriidae parasitize Chondrichthyes, the Polystomatidae parasitize Amphibia and Reptilia, and both the Gyrodactyloidea and Capsaloidea parasitize Teleostii.

It becomes pertinent therefore to examine the criteria used at present in the classification (Sproston, 1946) of the Monogenea. The main diagnostic features separating the two suborders Monopisthocotylea and Polyopisthocotylea (including the Hexabothriidae, Polystomatidae and Diclidophoroidea)

are the relationship of the adhesive organs of the adult to those of the larva, and the presence or absence of a genito-intestinal canal.

The use of the first of these characters is of extremely doubtful validity. The Polyopisthocotylea were defined as being Monogenea in which (among other characters) the functional haptor (=adhesive organs) of the adult is developed immediately anterior to the larval haptor. So far as I can discover, this conception can only be based upon what appears to be a misinterpretation of some rather confused observations by Remley on the development of *Microcotyle spinicirrus*. Remley (1942, p. 151) stated that the larval haptor of *M. spinicirrus* bore 6 pairs of hooklets (=lateral and postero-lateral hooks in my descriptions) along the lateral and posterior border, and that 2 pairs of large anchor hooks (=posterior hooks and primordial adult hooks in my descriptions) were medianly located near the posterior end. Later on p. 151, and on p. 152, Remley used the term 'larval haptor' in a different sense—to include only that part bearing the 'anchor hooks', and when he stated that by the 20 to 30-clamp stage the haptor was lost, he was referring only to the anchor-bearing part. There are no grounds then for supposing that the adhesive organs of the adult do not develop around and replace the larval hooks as they have been described to do so in *Diclidophora denticulata* by Frankland (1955) and in several polystomatids by various authors.

The use of the second character appears to be more valid: a genito-intestinal canal is invariably present in the Polyopisthocotylea, but has been reported as being present also in the Protogyrodactylidae and in some turbellarians, and so it is possible that its occurrence throughout the Polyopisthocotylea is due to convergence. However, the genito-intestinal canal in the Polyopisthocotylea may be associated with another feature that may be shown eventually to be exclusive to the group: I have shown that in eight representative species the gut epithelium consists of scattered 'pigment cells' associated with a blood-feeding habit (Llewellyn, 1954), and it is possible that there may be a connexion between an obligatory blood-feeding habit and the presence of a genito-intestinal canal. It is known (see Caullery, 1952) that there is a close correlation between strict haematophagy and the presence of intestinal symbionts, and that such blood-feeding animals are obliged to have some means of transferring the symbionts to succeeding generations. I have observed cells laden with bacteria (=mycetocytes?) in the intestine of adult specimens of *Axine bellones*, in the egg capsules of *Polystoma integerrimum*, and in the egg capsules of most of the diclidophoroideans that I have described in this paper. The genito-intestinal canal *could* be the pathway whereby such mycetocytes may be transferred from the gut to the egg capsules. If this hypothesis be shown eventually to be correct, the inference would be of a common origin for those monogeneans having a blood-feeding habit, gut pigment cells, and a genito-intestinal canal. This in turn would mean that the similarity between the larvae of the Polystomatidae and those of the

Capsaloidea is superficial unless it be due to the present-day Polystomatidae being in fact those Polyopisthocotylea which have diverged least from an ancestral stock that is common also to the Capsaloidea.

Within the present suborder Monopisthocotylea, the inference from larval studies is that there is a closer affinity between the Gyrodactyloidea (excepting the Gyrodactylidae) and the Capsaloidea than is suggested by the present classification, and this is supported by the widespread occurrence of 14 marginal hooks (=persistent larval hooks?) in many adults of both the Gyrodactyloidea and the Capsaloidea.

With regard to the Diclidophoroidea, while an insufficient number of species is known yet to permit a classification of the group on larval characters, it is possible to make the following observations: (1) In *Kuhnia scombri* (Mazocraeidae) the presence of undifferentiated hooks appears to be the survival of a primitive character, and Sproston (1945) has already regarded the clamp structure of the adult *Kuhnia* to be the basic type from which other diclidophoroidean clamps have evolved—a view which I have opposed (Llewellyn, 1957). (2) The larvae of *Gastrocotyle trachuri* and *Pseudaxine trachuri* (at present classified in the Microcotylidae) appear to have less in common with microcotylid larvae than the microcotylids have in common with the discocotylids, diclidophorids, and hexastomatids. This would support Price's (1943) proposal, on the grounds of the clamp structure of the adults, of a new family Gastrocotylidae.

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SUMMARY

Techniques for the rearing of monogenean larvae are described.

The new term 'oncomiracidium' is proposed for the larvae of monogenetic trematodes.

Eleven new oncomiracidia of fish parasites are described, bringing the total now known to thirty-five.

These oncomiracidia are tentatively classified, and the classification is compared with the existing classification of adult monogeneans.

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