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142

Göran Bylund: The taxonomic significance of embryonic hooks in four European Diphyllobothrium species (Cestoda, Diphyllobothriidae)

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THE TAXONOMIC SIGNIFICANCE OF EMBRYONIC HOOKS IN FOUR EUROPEAN DIPHYLLOBOTHRIUM SPECIES

(CESTODA, DIPHYLLOBOTHRIIDAE)

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Abstract

BYLUND, GÖRAN (Parasitol. Inst., Åbo Akademi): The taxonomic significance of embryonic hooks in four European Diphyllobothrium species (Cestoda, Diphyllobothriidae). — Acta Zool. Fennica 142:1—22. 1975.

Comparative studies were carried out on oncosphere larvae of *Diphyllobothrium ditremum, D. vogeli, D. latum* and *D. dendriticum* to evaluate the taxonomic significance of their embryonic hook morphology. Species-specific features were established in each of the species studied. The shape of the hook is the most valuable character, the relative size of different pairs and parts of hooks has some value, while the absolute size is of limited value. Analysis of the embryonic hooks is a promising tool for the diagnosis of species in the genus *Diphyllobothrium*.

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Contents

Contribution No. 17 from the Parasitological Institute of the Societas Scientiarum Fennica.

INTRODUCTION

Numerous attempts have been made to recognize reliable species criteria and to improve the methods available for identification of species in the genus *Diphyllobothrium*. Nevertheless, our knowledge of the species-specific characters of these tapeworms is not yet sufficient for a comprehensive revision of their systematics. Again and again systematists have emphasized that the particular difficulty with this group of worms is their lack of solid structures — hooks, mouth parts, etc. — in the adult stage.

The embryonic hooks have received scant attention in cestode taxonomy, although these structures are even more universally present in cestodes than scolex hooks. There is a reason for this. As a rule the embryonic hooks of cestodes are small and delicate (7—20 µm). On trying to examine the hooks under the light microscope, one is faced with difficulties which make interpretation puzzling and which may explain our scanty and uncertain information about these structures.

As a consequence, data on the embryonic hooks of diphyllobothriids are sparse, and views regarding the usefulness of these structures for taxonomic purposes are conflicting. HILLIARD (1960) examined oncospheres of 8 different *Diphyllobothrium* species and concluded: "For the species of cestodes studied the measurements of the embryonic hooks appear to have no taxonomic value. In some species considerable variation in shape of hooks was noted in coracidia deriving from a single segment."

In the same year FRASER (1960) compared the embryonic hooks of four *Diphyllobothrium* species from Great Britain, at least two of which were identical with those examined by HILLIARD. She found "that the form of the larval hooks is constant and that hook types are related to different species. It is concluded that the hooks provide a sound basis for specific identification."

My interest in the matter was roused when I became familiar with the preparation methods and phase microscope techniques used by MALMBERG (1956, 1970) in his studies on monogenetic trematodes. I therefore made comparative studies on oncospheres of Finnish diphyllobothriids to evaluate the taxonomic significance of their hook morphology.

Acknowledgements: I am most grateful to Dr Göran Malmberg, of Stockholm, for his valuable advice during the progress of the work. I also wish to express my gratitude to

Prof. Bo-Jungar Wikgren for valuable discussions and to Mrs. Henna Lehti, Mr. Markku Wikström, M. Sc., and Mr. Julius Hindström for technical assistance.

MATERIAL AND METHODS

The studies were based on four different *Diphyllobothrium* species identified at the plerocercoid stage. Plerocercoids of *D. ditremum* (Creplin, 1825) were obtained from smelt, *Osmerus eperlanus* (L.), those of *D. dendriticum* (Nitzsch, 1824) from whitefish, *Coregonus lavaretus* (L.), and those of *D. latum* (Linnaeus, 1758) from pike, *Esox lucius* L., and from burbot, *Lota lota* (L.). Several articles on the occurrence of these three *Diphyllobothrium* species in Finland and on their identification at the plerocercoid stage have been published (Wikgren & Muroma 1956; Wikgren 1964; Wikgren & Bylund 1964; Bylund 1968, 1969, 1972, 1973).

The fourth species examined, *D. vogeli* Kuhlow, 1953, is also a well-established member of the Finnish fauna but has not attracted attention in previous reports (Bylund, in preparation). The plerocercoids of *D. vogeli* were obtained from the three-spined stickleback, *Gasterosteus aculeatus* L.

Plerocercoids of these four *Diphyllobothrium* species were reared to adults in golden hamsters *(Mesocrisetus auratus* Waterhouse). When the worms had reached the egg-producing stage 7—14 days after infection, strobilae were regained by killing the hamsters. Eggs were collected from gravid uterine coils of 4—6 strobilae per species. Aerated egg cultures were incubated at room temperature for 9—12 days with antibiotics added to the water to suppress microbial growth. The cultures were kept in darkness to encourage mass hatching at the end of the incubation period.

In the hatched coracidium the hooks lie obliquely in different planes, with the curved tips pointing in different directions. For proper phase contrast studies the larvae should be slightly depressed under the coverslip, which brings the hooks into one plane and allows them to be studied in lateral view. A suitable degree of depression was not easy to achieve with newly hatched coracidia. After hatching there is a pronounced swelling of the embryophore of the coracidium due to osmotic phenomena (Kuhlow 1953; Hilliard 1960). Coracidia which had been kept at room temperature for 24—36 hours after hatching, thus greatly increasing their volume, proved to be the best material for hook studies.

Several of these coracidia were transferred to a slide in a small drop of water, a small cover glass was applied and the specimens at once observed under the phase contrast microscope. As water evaporated from the edges of the cover glass the specimens were gradually depressed. This process was sometimes speeded up by carefully absorbing excess water from the edge of the cover glass with a piece of filter paper. When the specimens were sufficiently depressed a small drop of the fixative, ammonium picrate-glycerol (1:1) (Malmberg 1956, 1970), was placed at the edge of the cover glass. The fixative gradually penetrates beneath the cover glass as further water evaporates. Specimens fixed in this way may be stored for a long time, especially if the slide is sealed with paraffin or some other sealing medium.

Observations were made with a Leitz Orthoplane microscope equipped with a Heine phase contrast condensor. The drawings of the hooks were made with a Leitz drawing prism (60° prism) using a x90 oil immersion objective and x25 eyepieces. The microphotographic uptakes were made with Wild photo-equipment (size of negative 24×36 mm).

OBSERVATIONS

The basic morphology of the hooks

In a morphological as well as a functional sense the coracidium comprises two distinct parts: the outer ciliated embryophore giving the organism an almost spherical symmetry and inside this structure the bilaterally symmetrical oncosphere. The most obvious structures in the oncosphere are the embryonic hooks. The larva has six hooks arranged in three pairs, lateral, medio-lateral and medial. Following HILLIARD's (1960) system they have been numbered 1 to 3, number 1 being the lateralmost and number 3 the medial hook pair. Each medial hook is orientated along the antero-posterior axis of the larval body with the curved blade directed posteriorly, i.e. in the opposite direction to the direction of swimming.

The hook consists of a curved blade and a straight handle (Fig. 1). The curved distal end of the blade tapers into a more or less sharp-pointed tip, while the proximal part consists of an enlargement, the guard. The handle is joined to the proximal part of the blade without an articulation. The free end of the handle is often swollen into a more or less prominent, bulbous knob. This structure, which varies greatly in degree of development, forms one of the two main points of attachment of the hook musculature.

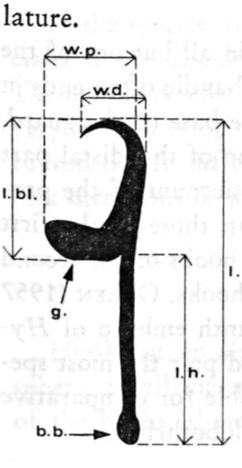

FIG. 1. The morphology of the *Diphyllobothrium* hook.
l. = total length of the hook
l.h. = length of the handle
l.bl. = length of the blade
w.p. = proximal width of the blade
w.d. = distal width of the blade
g. = guard
b.b. = basal bulb

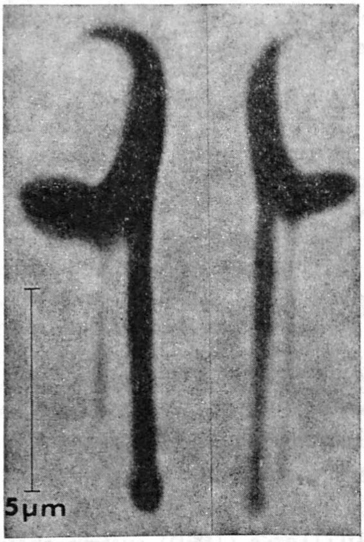

FIG. 2. Hooks of *D. dendriticum,* showing extended protrusions ventrally on the guard.

Ventrally on the guards some hooks may exceptionally have one or more small barblike protrusions (Figs. 2, 5 A, 6 A and B). Occasionally these protrusions are greatly extended and very distinct and may reach almost the length of the handle. The nature of these structures will be dealt with later on (p. 20). They seem to be of little taxonomic value.

The three pairs of hooks show marked differences in structure. This is in contrast to the statement by FRASER (1960) that the three pairs of hooks are identical in size and shape. The hooks of the first pair are the shortest and most delicate. The handle is very thin with the basal swelling poorly developed. The blade is narrow and sickle-shaped with a slightly curved tip and poorly developed guard.

The hooks of the second pair are the stoutest and in all but one of the species examined the longest (Table 1). The very stout handle often ends in a well-developed basal bulb. The blade is broad at the base of the guard, tapering uniformly towards the tip, the delicate portion of this distal part being relatively short and not very much curved. On account of the prominent guard the blade has a deeper sickle shape than those of the first pair. The stoutness of both handle and blade gives the hooks of the second pair a clumsy appearance as compared with the other hooks. OGREN (1957 and 1961), examining hook morphology in the hexacanth embryo of *Hymenolepis diminuta,* considered the hooks of the second pair the most specific. He suggested that these would prove most valuable for comparative purposes. This suggestion does not hold true of diphyllobothriids.

TABLE 1. Dimensions of the hooks in the four *Diphyllobothrium* species studied. Mean ± S.E. and range given (in µm).
1. = total length of the hook
1.bl. = length of the blade
w.p. = proximal width of the blade
w.d. = distal width of the blade

		D. ditremum	*D. vogeli*	*D. latum*	*D. dendriticum*
1st hook pair	1.	11.0±0.05 (10.0—11.8)	10.8±0.06 (10.3—12.0)	11.7±0.07 (10.5—12.3)	11.1±0.06 (10.3—12.0)
	1.bl.	4.0±0.03 (3.5—4.3)	4.1±0.03 (3.8—4.5)	4.3±0.03 (4.0—4.8)	4.5±0.03 (4.3—5.0)
	w.p.	1.6±0.02 (1.5—1.8)	1.5±0.03 (1.3—1.8)	1.7±0.03 (1.3—2.3)	1.7±0.02 (1.5—1.8)
	w.d.	1.4±0.03 (1.0—1.8)	1.4±0.03 (1.0—1.8)	1.9±0.03 (1.5—2.3)	1.8±0.03 (1.5—2.3)
2nd hook pair	1.	12.6±0.06 (11.8—13.3)	12.7±0.05 (12.0—13.8)	13.0±0.07 (12.0—14.3)	12.7±0.06 (12.0—13.8)
	1.bl.	4.5±0.03 (4.0—4.8)	4.7±0.03 (4.3—5.3)	4.5±0.06 (3.8—5.3)	4.8±0.03 (4.5—5.0)
	w.p.	2.9±0.03 (2.3—3.5)	2.4±0.03 (2.0—2.8)	2.5±0.04 (2.3—3.3)	2.9±0.03 (2.5—3.3)
	w.d.	1.6±0.03 (1.3—2.0)	1.6±0.03 (1.3—2.0)	1.7±0.02 (1.5—2.0)	1.8±0.03 (1.5—2.3)
3rd hook pair	1.	12.4±0.06 (11.5—13.3)	12.0±0.06 11.3—12.8)	13.0±0.06 (12.0—13.8)	12.1±0.07 (11.3—13.0)
	1.bl.	4.5±0.02 (4.3—4.8)	4.8±0.04 (4.3—5.5)	4.7±0.04 (4.3—5.3)	4.9±0.03 (4.5—5.3)
	w.p.	3.0±0.03 (2.5—3.3)	2.5±0.03 (2.0—2.8)	2.9±0.04 2.3—3.5)	3.1±0.03 (2.8—3.8)
	w.d.	2.1±0.04 (1.8—2.5)	2.1±0.03 (1.8—2.5)	2.2±0.03 (1.8—2.5)	2.5±0.04 (2.0—2.8)

In the species studied here, the hooks of the third pair are the most specialized and should be considered the most valuable for taxonomic use. The handle is mostly slender, without a prominent basal bulb. The blade is very well developed with the distal end extended into a delicate, sharply curved tip. In different species the prominent guard has diverged in different directions in shape, stoutness, main angle with the handle, etc.

Abnormal hooks

Hooks of an abnormal shape were rather frequently found in oncospheres of all the species studied. Mostly the defects concerned the handles of the hooks, these structures being very short, very thin or abnormally bent. Sometimes hooks without handles were seen. Several specimens were found with malformations of the hook blades. Thus most of the malformations pictured by MICHAILOV (1933) from hooks of *Triaenophorus nodulosus* were also recognized in the diphyllobothriid hooks.

Several larvae had abnormal numbers of hooks. Almost exclusively the erroneous number concerned hooks of the first pair. In some larvae these were lacking, but more frequently there were specimens with an excess of hooks, all the additional hooks being morphologically similar to those of the lateralmost pair. Larvae with 10 hooks were most common.

Species-specific characters of the hooks

Table 1 contains data on the hook dimensions of the species studied.

D. ditremum (Figs. 3, 7 and 8)

First pair. Very conspicuous specific characters are lacking. The slender handle is almost always without a distinct basal bulb. The small guard, usually projecting slightly upwards, broadens towards the tip. The pointed tip of the blade projects to the level of the tip of the guard. In dimensions they differ significantly ($p<0{,}001$) only from the corresponding hooks of *D. latum.*

Second pair. The hooks have a prominent guard, slightly bent and always pointing upwards. The guard is broadest close to the tip. The tip of the guard is mostly bluntly pointed, very seldom rounded. The stout handle frequently broadens towards the basal end but a conspicuous bulb structure is rare. The hooks show marked differences in size only when compared with those of *D. latum.*

Third pair. The hooks reach almost the same length as the second pair. They are characterized by well-developed, straight guards projecting at right angles (90°) from the handles or, more rarely, slightly upwards. The guard broadens somewhat towards the distal end, which is almost never rounded but with a bluntly pointed contour. The nature of the guard and the rather sharply curved tip of the blade give the latter a relatively deep, hooked sickle shape. The handle may be distinctly swollen basally, but a prominent bulb is lacking. In size these hooks differ significantly from the corresponding hooks of *D. vogeli* ($p<0{,}001$) and *D. latum* ($p<0{,}001$), those of the former species being shorter and those of the latter longer than the hooks of *D. ditremum.*

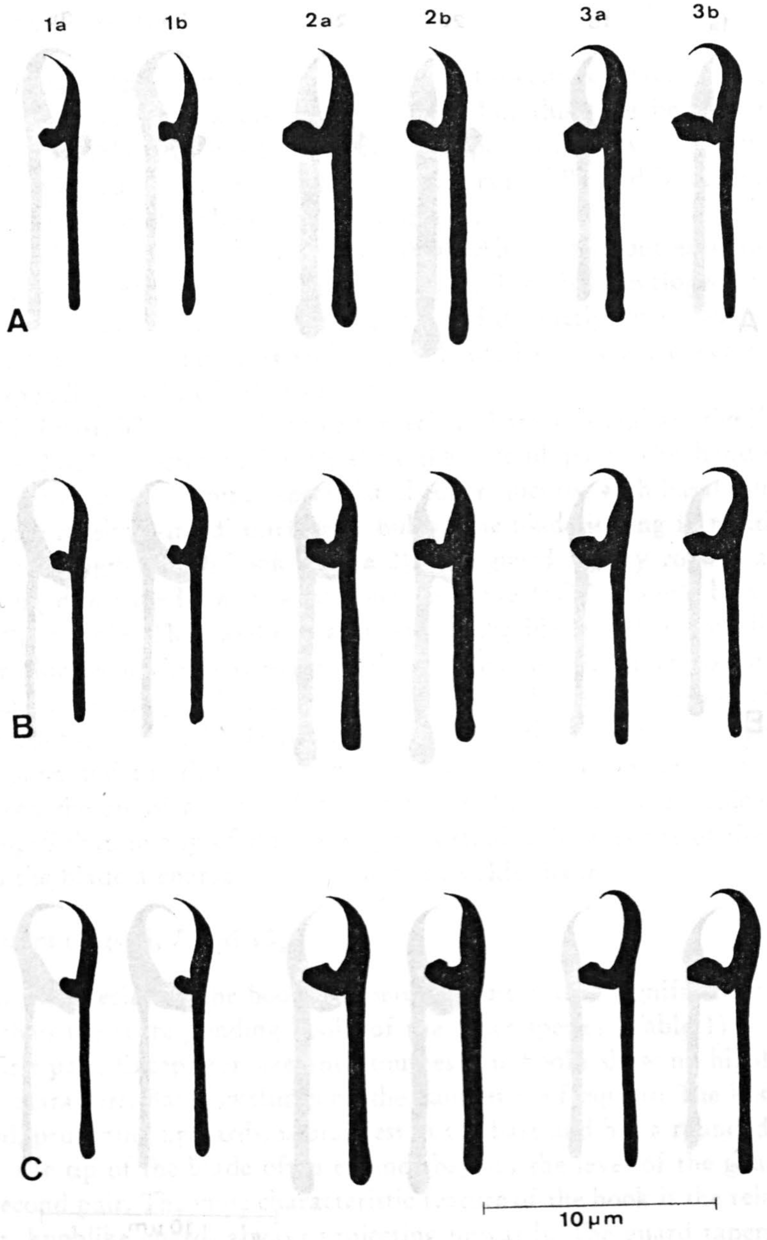

FIG. 3. Oncospheral hooks in three specimens (A—C) of *Diphyllobothrium ditremum* (drawn with a camera lucida).

FIG. 4. Oncospheral hooks in three specimens (A—C) of *D. vogeli* (c. 1.).

D. vogeli (Figs. 4, 7 and 9)

First pair. The hooks show no pronounced specific characters. The slender handle often has a distinct basal bulb but this may be absent. The guard is weakly developed and frequently points downwards, giving the blade a wide curve. The tip of the blade tapers rapidly and is less extended than in corresponding hooks of other species.

Second pair. The hooks are very robust. Almost without exception the stout handle ends in a prominent basal bulb. The well-developed guard is bent like a crook with the broader distal end distinctly upcurved. The tip of the blade is not much extended. The blade has a wider curve than in corresponding hooks of other species.

Third pair. The hooks show very specific characters and are significantly ($p<0,001$) shorter in length than the second pair. The handles are slenderer than in any other species studied, frequently with basal swellings or, more rarely, with distinct basal bulbs. The blade is long in relation to the total length of the hook (Table 2). The guard is very robust, always pointing downwards, with a conspicuous acute ($<90°$) angle between it and the handle. The handle is attached to the blade rather dorsally, the latter sloping backwards more distinctly than in the other species. The guard widens like a club towards its distal end, which is truncate. The inner contour of the blade is softly rounded with no sharp limit between the guard and the distal portion of the blade. Thus the transition point between the guard and the distal part of the blade is less conspicuous in *D. vogeli* than in any of the other species studied. The nature of the guard gives the blade a characteristic wide-open sickle shape.

D. latum (Figs. 5, 7 and 10)

In this species all the hooks are more robust and of significantly larger size than the corresponding hooks of the other species (Table 1).

First pair. Except for size and stoutness the hooks show no highly specific characters. Basal swellings on the handles are frequent. The knob-like guard, projecting upwards, is broadest at the base and has a rounded distal end. The tip of the blade often extends beyond the level of the guard tip.

Second pair. The most characteristic feature of the hook is the relatively short, knoblike guard, always projecting upwards. The guard tapers from the base towards the tip. The blade is relatively short (Table 2). The handle is swollen at the base and a distinct bulb is sometimes seen. Their pronounced stoutness often gives the hooks a very clumsy appearance.

Third pair. These hooks are also stouter than the corresponding pair in

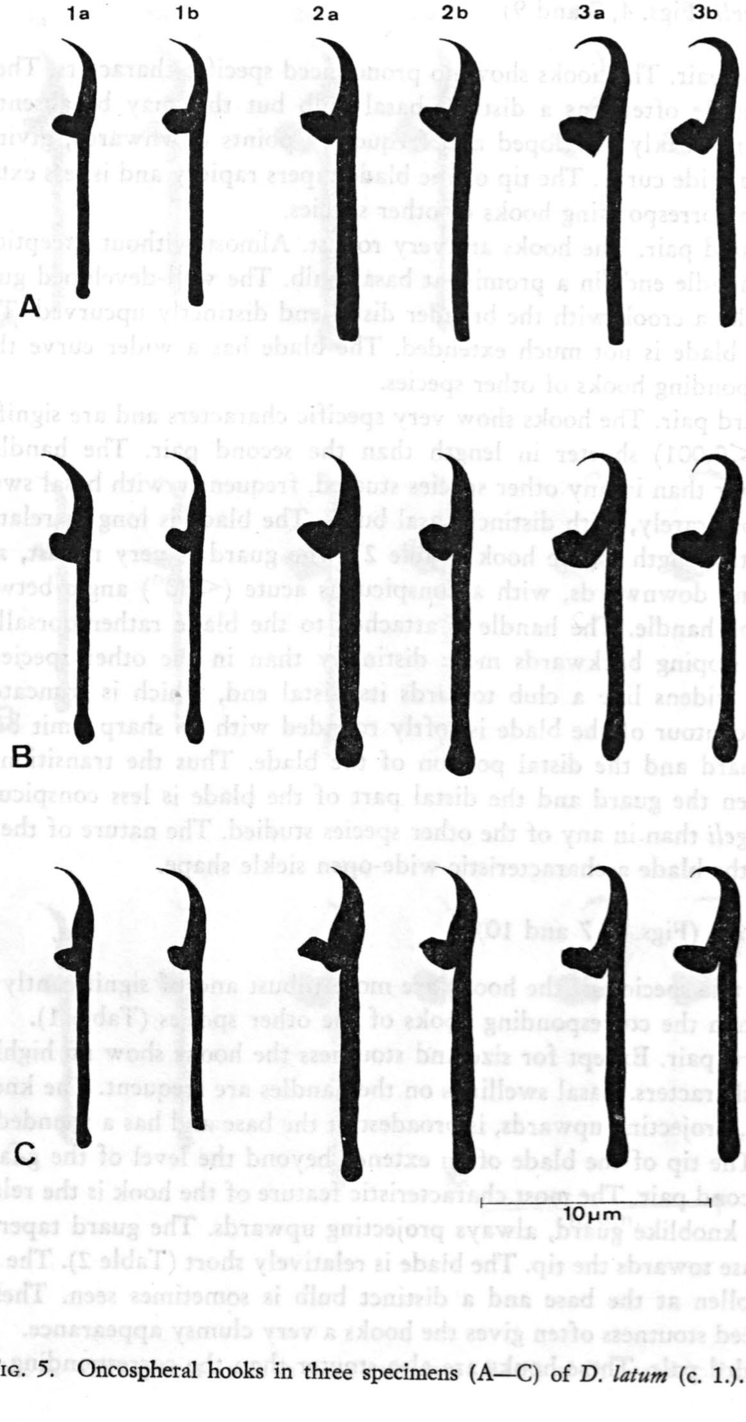

FIG. 5. Oncospheral hooks in three specimens (A—C) of *D. latum* (c. 1.).

other species. Sometimes they are even more robust than those of the second pair. The length equals that of the second hooks, a character shared with *D. ditremum.* The prominent guard is bent like a "knee". The short, narrow proximal part projects downwards from its junction with the handle like a shaft, the knob-like distal part being upturned. The point of attachment of the handle is rather dorsally on the blade, leaving a very characteristic open, acute angle between the handle and the proximal "shaft" of the guard. The distal part of the blade is relatively broad close to the guard and then tapers more rapidly than in other species. This portion is also rather straight, a marked curvature beginning only close to the tip. Basal swellings or even distinct bulbs occur frequently at the end of the handle.

D. dendriticum (Figs. 6, 7 and 11)

First pair. In this species rather distinct specific features are also seen in this pair of hooks. The guards of these hooks are more extended than in the corresponding hooks of the other species. They are uniformly narrow and always project sharply upwards. Mostly the guards project in a direction parallel to the distal tip of the blade. The blade is long in proportion to the total length of the hook (Table 2), its tip extending beyond the level of the end of the guard. Basal swellings are very rarely seen on the handles.

Second pair. These hooks are also clearly characterized by the guards, which project sharply upwards and parallel to the tip of the blade. Thus the angle between the guard and the handle always clearly exceeds 90°. Another very characteristic feature is the straight ventral edge of the guard. The guard is of uniform, rather narrow width. Frequently there is

TABLE 2. Relative dimensions of the different hook parts. A = length of blade as a percentage of total hook length. B = length : width (proximal) ratio of the blade.

		D. ditremum	*D. vogeli*	*D. latum*	*D. dendriticum*
1st hook pair	A	36.7	38.1	36.5	40.8
	B	2.47:1	2.76:1	2.56:1	2.68:1
2nd hook pair	A	35.2	36.5	34.6	37.8
	B	1.54:1	1.95:1	1.78:1	1.64:1
3rd hook pair	A	36.5	40.0	36.5	40.1
	B	1.53:1	1.92:1	1.66:1	1.55:1

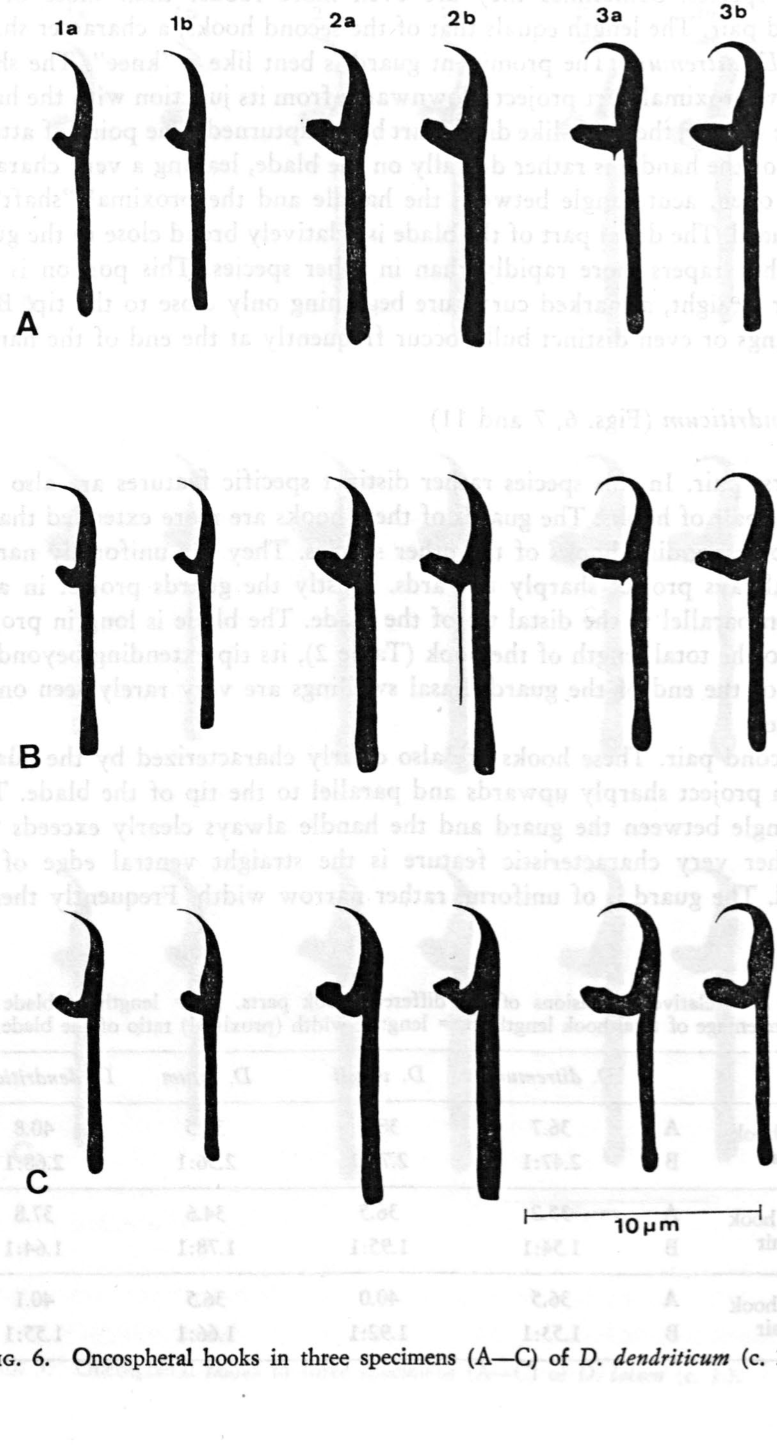

Fig. 6. Oncospheral hooks in three specimens (A—C) of *D. dendriticum* (c. 1.).

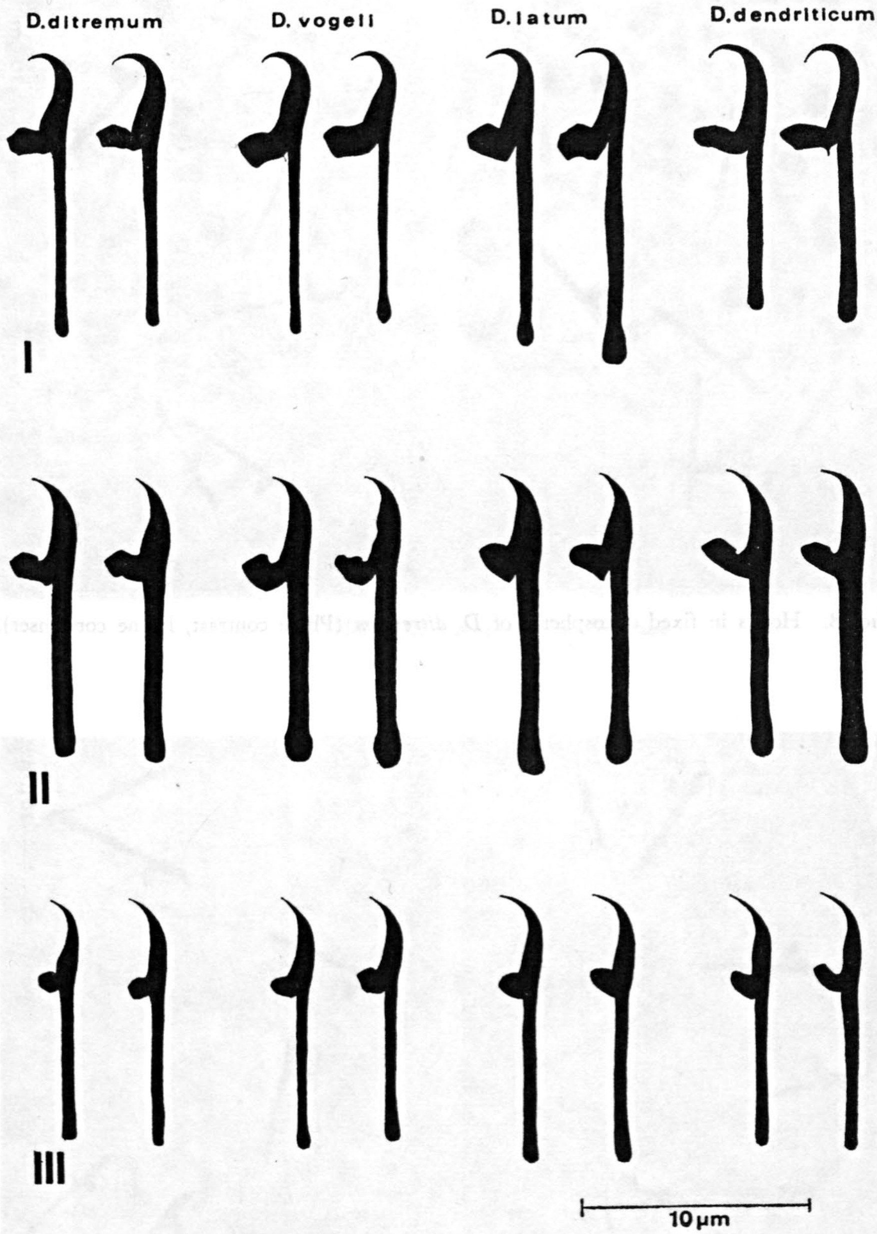

FIG. 7. Hooks of the four species compared.
I = middle pair of hooks;
II = medio-lateral hooks;
III = lateral hooks.

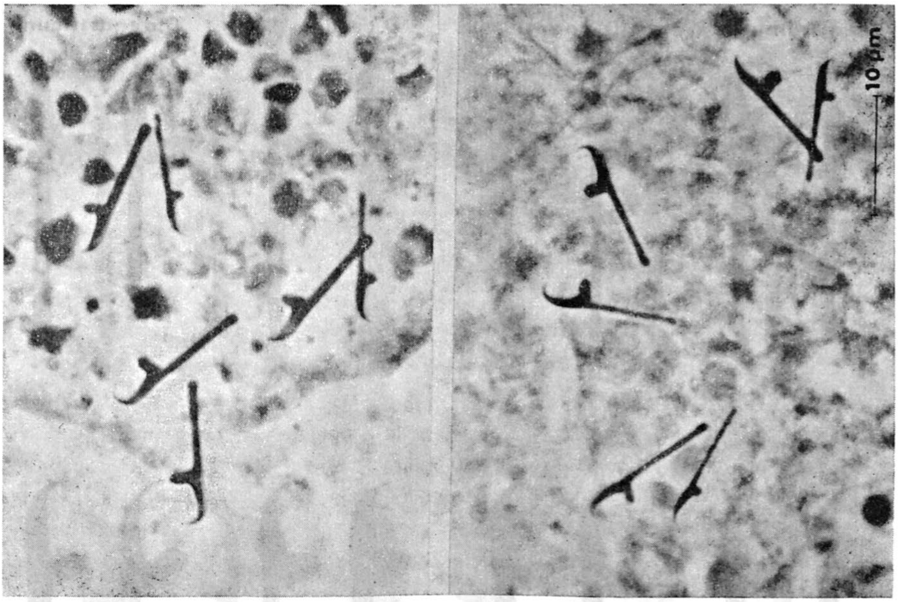

FIG. 8. Hooks in fixed oncospheres of *D. ditremum* (Phase contrast, Heine condenser).

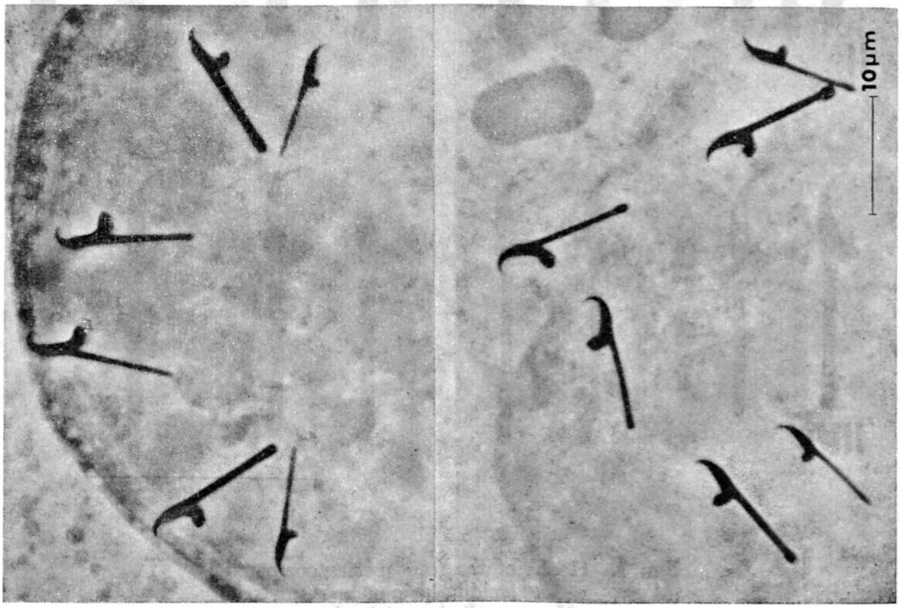

FIG. 9. Hooks in oncospheres of *D. vogeli*.

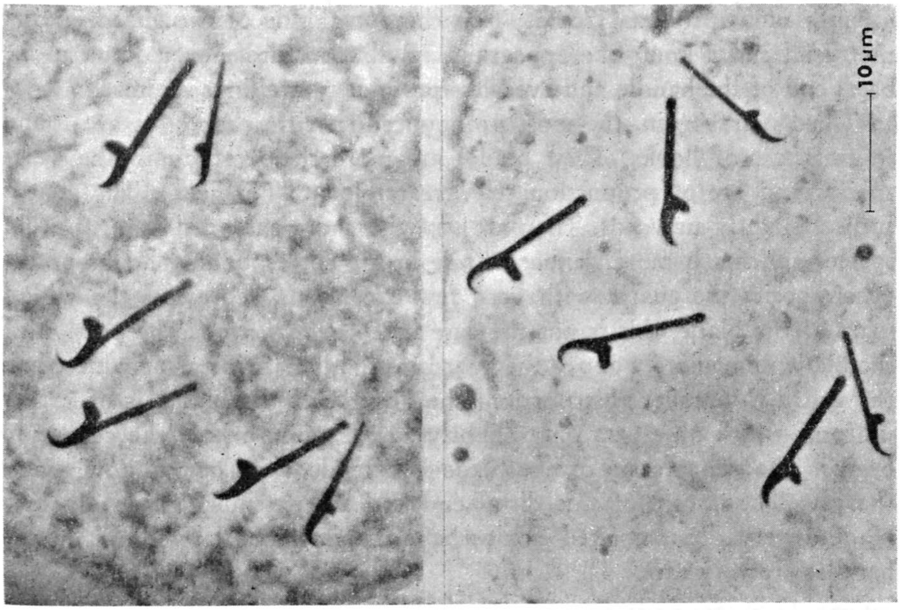

FIG. 10. Hooks in oncospheres of *D. latum*.

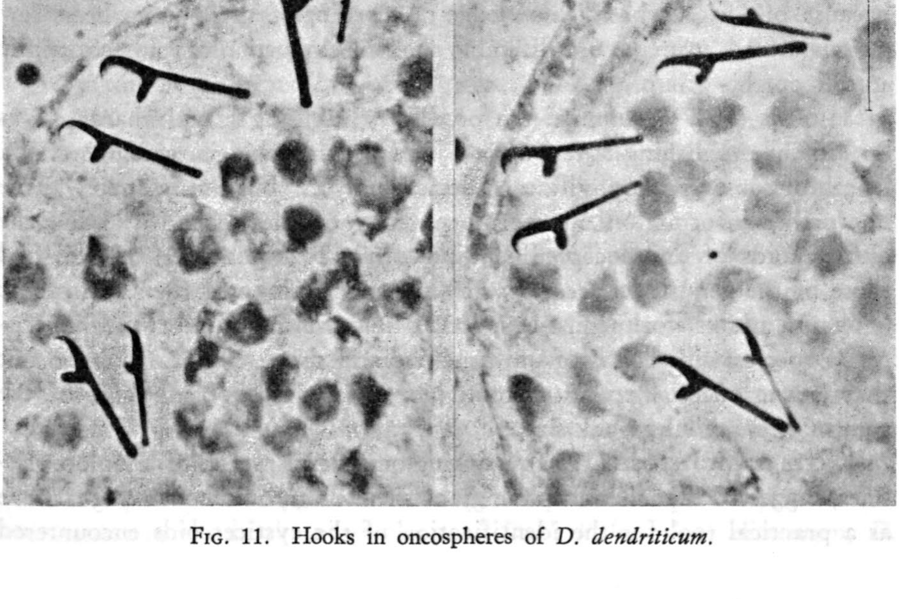

FIG. 11. Hooks in oncospheres of *D. dendriticum*.

a conspicuous proximal "cavity" on the dorsal side of the guard, giving the distal end a knob-like appearance. Bulb structures never occur at the basal end of the handle and even less prominent swellings are rare.

Third pair. Again, the most pronounced specific characters are found in this pair of hooks. Their blades are more slender than in the other species and are of proportionately greater length (Table 2). The guards project slightly upwards. They are straight, more extended than in other species and much more slender. While in the corresponding hooks of the other species the guard, with very few exceptions, represents the widest part of the blade, this is not the case in *D. dendriticum.* The portion of the blade immediately above the guard is usually as wide as or wider than the guard. Moreover this portion, the "back" of the blade, is of rather uniform width or tapers only slightly to the point where the curvature begins. The delicate tip of the blade is extended and curves down very sharply. The nature of this distal end and the extended guard give the blade a very deep, hooked sickle shape. Distinct basal swellings on the handles are very rare.

DISCUSSION

Embryology has not greatly influenced cestode classification, largely because of lack of basic knowledge. Accordingly, there have been few serious efforts to make use of embryonic hook morphology in taxonomic studies on this group.

THOMAS (1947) examined the possibility of using larval characters as an aid in establishing species. He found considerable variation in hook length of some pseudophyllidean species analysed, but unfortunately gave no further information on the morphology of the hooks. Studying the cysticercoids of anoplocephaline tapeworms, FREEMAN (1949) identified those of the genus *Monoecocestus* by the morphology of their embryonic hooks. In a later work (FREEMAN, 1952), however, he found two *Monoecocestus* species with hooks of uniform size and shape. He also pointed out that larvae of *Monoecocestus* could not be separated from those of the genus *Cittotaenia* by hook size.

KATES & McINTOSH (1950), working on several species of anoplocephalines, suggested that the morphology of their embryonic hooks might serve as a practical tool for the identification of the cysticercoids encountered in intermediate hosts. Moreover, they predicted that hook morphology would prove indicative of phylogenetic relationships in studies based on specimens from a great variety of species.

HWANG & KATES (1956) briefly summarized the variations in size and morphology of the embryonic hooks in several cestodes. They drew attention to the considerable variation in the appearance of the guards of the hooks. The diagrams of the hooks in different cestode groups presented by OGREN (1957) also reveal a conspicuous variation in the size and shape of these structures.

FRASER (1960) and HILLIARD (1960) investigated the possibilities of identifying diphyllobothriids by their hook morphology. As pointed out above, their conclusions were different. FRASER stated that a characteristic type of hook is associated with each of the species studied. In her opinion, reliable identification of species can be made only by examination of the hooks of the larvae. But she did not recognize the dissimilarities between the three pairs of hooks of a single coracidium. Hence her conclusions appear somewhat rash.

Judging from the diagrams published by FRASER, the hooks of *D. dendriticum* and *D. latum* on which she based her opinion correspond to the third pairs of the same species in my material. The hooks of *D. ditremum* as represented by FRASER, resemble those of *D. vogeli* in my material. FRASER did not, however, mention the source of her material of this or of the other species she studied. Thus the validity of her conclusion cannot be evaluated.

HILLIARD (1960), after examining the hooks in 11 species of diphyllobothriid cestodes, concluded that hook measurements could not serve as valid taxonomic characters. Moreover he considered hook shape to be of restricted taxonomic value. For four of the species he studied he found the hook form fairly diagnostic, but in seven other species the hook structures were in his opinion too variable to be of taxonomic value. Specific characters, when present, he found in the third pair. The hooks of a species identified by HILLIARD as *D. ditremum* show very close conformity to the hooks of *D. ditremum* in my material.

In a study on the procercoid protonephridial systems of some *Diphyllobothrium* species, MALMBERG (1971) also drew attention to the larval hooks. He briefly described the basic morphology of these and suggested that they are of taxonomic interest. MALMBERG also stressed the importance of using refined methods for hook studies.

In the present investigation I was able to establish species-specific characteristics in the hook morphology of *Diphyllobothrium ditremum, D.*

vogeli, D. latum and *D. dendriticum.* The specific features are not very conspicuous in living or imperfectly handled larvae, but are unmistakable when the hooks are depressed into a lateral position.

The absolute size of the hooks seems to be of restricted value in species diagnosis. Of the four species studied here only *D. latum* could be easily separated from the others on the basis of a significantly larger hook size.

The relative size of the hooks of different pairs in a single larva, however, appears to be a character of considerable interest. In two of the species studied above *(D. dendriticum* and, especially, *D. vogeli)* the hooks of the second pair are significantly ($p<0{,}001$) longer than those of the third pair. In the other two species *(D. latum* and *D. ditremum)* the hooks of the second and third pairs are of about equal length. In all species studied the hooks of the first pair are about 9—12 % shorter than those of the third pair.

Also the relative dimensions of the different parts of the hook, especially those of the third pair, are of some taxonomic value, e.g. the length of the blade in proportion to the total length of the hook, the length-width ratio of the blade, etc. The blades are relatively longer in *D. vogeli* and *D. dendriticum* than in the other two species examined (Table 2). The hooks of *D. vogeli* significantly ($p<0{,}001$) differs from those of the other species in the length-width ratio of the blade (Table 2).

The species-specific features, however, are reflected very conspicuously only in the shape of the hooks, i.e. characters which cannot be represented by numerical values. The shape of the blade and the guard, the main angle of the guard to the handle and to the distal part of the blade, the point of attachment of the handle to the blade and the stoutness of the handle and blade are the characters of greatest importance for the species diagnosis. As pointed out earlier, the specific features are most pronounced in the hooks of the third pair, although specific characters are also found in the other hooks. Thus an analysis should comprise the whole assortment of hooks in a larva.

The nature of the small barblike protrusions sometimes found ventrally on the guards of the hooks remains to be investigated. These protrusions sometimes reach a considerable length but usually they are very short. Sometimes there are several such processes on the same guard. In some hooks they protrude proximally on the guard close to the handle, in others almost at the tip of the guard. Most frequently the protrusions are found on hooks of the third pair but they are also seen on other hooks, although very sparsely on the lateralmost pair.

Malmberg (1971) regarded the protrusions as rudiments and suggested that they might be rudimentary handles. In my opinion this suggestion is ruled out by their varying position on the guards and the presence of several protrusions in some hooks. The ventral zone of the guard is the site of insertion of the hook musculature (Vogel 1930; Ogren 1961). A massive layer of connective tissue is present between the hook substance and the muscle at the insertion sites (Collin 1968; Swiderski 1971). I suppose the protrusions may be fragments of this connective tissue. In oncospheres of *Hymenolepis diminuta* ogren (1961) found short barbs projecting from the guards in all mature hooks.

A number of observations were also made on hooks of the procercoid larva. The morphology of the hooks is, of course, similar in these two larval stages. With the procercoid, however, it is more difficult to apply suitable pressure on the specimen, i.e. to bring the hooks into the lateral position necessary for proper examination. Procercoid hooks were studied most successfully on preparations of isolated cercomeres.

The present study revealed distinct specific features in the hook morphology of each of the four *Diphyllobothrium* species studied. As the observations are so far based on larvae of a restricted number of species, interpretations must be made with care. Nevertheless it seems reasonable to expect that analysis of the embryonic hooks will prove a very valuable tool for the diagnosis of species in all diphyllobothriids and perhaps in other troublesome cestode groups as well. Thus it is suggested that future studies dealing with descriptions and redescriptions of these and related species should include characters of their hook morphology.

The achievement of reliable species identifications on procercoids obtained from their natural intermediate hosts also offers extremely good possibilities for studies on the biology of the pseudophyllids, for example.

There is reason to believe that refined methods of studying the hooks will reveal subtle details of their structure not hitherto recognized. Stereoscan methods would probably facilitate observations on these hard structures. Efforts in this respect are in progress.

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