Studies of the Helminth Fauna of Norway XXXIV:

The morphological stability of *Diphyllobothrium* Cobbold. A comparison of adult *D. dendriticum* (Nitzsch), *D. latum* (L.) and *D. ditremum* (Creplin) developed in different hosts

KARIN ANDERSEN

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Adult *D. dendriticum* from common gull *(Larus canus),* herring gull *(Larus argentatus)* and arctic fox *(Alopex lagopus), D. latum* from arctic fox, and *D. ditremum* from merganser *(Mergus merganser)* were studied. A comparison with worms reared in golden hamster *(Mesocrisetus auratus)* was conducted. Morphological characters of reliable diagnostic value (characters independent of host and worm population size) are: neck length, type of boundary between neighbouring segments, position of the cirrus-sac in sagittal sections, position of seminal vesicle in relation to the cirrus-sac, and shape of ovary (ovary with or without anterior horns).

Karin Andersen, Zoological Museum, Sars gt. 1, Oslo 5, Norway.

INTRODUCTION

Some of the problems in the taxonomy of adult *Diphyllobothrium* have arisen due to the fact that different authors (Magath 1924, Wardle & McColl 1937, Markowski 1949, Kuhlow 1953b, c, Vik 1957, Bylund 1969, Rausch & Hilliard 1970 and others) have described worms developed in different final hosts. The stability and diagnostic value of morphological characters in use in this genus is rather uncertain (Vik 1964b and Stunkard 1965). In order to find morphological characters of reliable diagnostic value, Andersen (1971 and 1972a) has described and compared the morphology of adult *D. dendriticum, D. latum* and *D. ditremum* reared in golden hamster. For *D. dendriticum* the morphological effect of 'crowding' was investigated earlier (Andersen 1972b). The hamster, which is used as experimental host, is, however, not a host for *Diphyllobothrium* under natural conditions. The results achieved in previous experiments (Andersen 1971 and 1972a, b) therefore need confirmation based on further investigations of these tapeworm species developed in some of their natural final hosts. The present investigation was therefore carried out to compare each of these three *Diphyllobothrium* species developed in some of their natural final hosts with worms of the same species reared in golden hamster.

MATERIAL AND METHODS

In total 93 gulls were fed with 2–10 *D. dendriticum* plerocercoids each, while each of 6 arctic foxes were given three and one 15 larvae. Fifteen *D. latum* plerocercoids were fed to 5 arctic foxes, 3 larvae to each fox. In addition to this 6 foxes, 17 gulls and 15 rats were fed with 12–15 *D. ditremum* plerocercoids each (Table I).

To administer the larvae into the gulls, a stomach tube was used, while the foxes ate the plerocercoids wrapped in ground meat voluntarily.

The *D. dendriticum* plerocercoids which managed to establish in the alimentary canal

Table I. Results of attempts to infect gulls, arctic fox and rat with <u>D. dendriticum</u>, <u>D. latum</u> and <u>D. ditremum</u>

Final host	Number of plerocercoids given to each host	Number of hosts infected	Number of hosts with egg in faeces	Number of hosts expelling their worms . . days after eggs appeared in faeces: 1-4 days	5-9 days	10 days	Number of worm examine
<u>D. dendriticum</u>							
Common gull	2	30	0				
	5	42	7	1	2	1	5
	10	20	7	3			11
Herring gull	10	1	1				2
Fox	3	6	5		2		4
Fox	15	1	1				10
<u>D. latum</u>							
Fox	3	4	4		1		6
Fox	10	2	2				12
<u>D. ditremum</u>							
Fox	15	8	0				
Common gull	12	15	0				
Herring gull	15	5	0				
Rat	15	15	0				
Merganser*	?	?	?	?	?	?	7

* shot in southern Norway

of gulls and arctic foxes had gravid proglottids and started to produce eggs after 7–8 days, while *D. latum* in arctic fox first started to produce eggs 14–21 days after administration of the larvae.

D. ditremum, however, did not manage to establish in either of these hosts, and adult worms from natural infected merganser, shot in southern Norway, were therefore used in the present investigation.

The adult worms were obtained by flushing the alimentary canal with physiological saline after killing and dissection of the hosts.

All the living worms were transferred to tap water, a few drops of chloroform were added and then placed in a refrigerator (temp. 4° C) for 2–3 hours. The furth treatment of the worms is described els where (Andersen 1971 and 1972a, b). A measurements presented were carried o after killing, but before fixing the worm The segment length is measured at t widest part of the strobila. The neck length calculated from the posterior margins of t bothria to the first interproglottid boundar

The number of adult worms investigat is given in Table I. In addition, two 'crowd worm populations of *D. latum* from gold hamster were studied and for further co parison *D. dendriticum, D. latum* and *ditremum* reared in hamster (Andersen 19 1972a, b and 1973) were used.

RESULTS

Feeding experiments

Table I gives the results of attempts to infect gulls with *D. dendriticum*. The first attempts were made with two plerocercoids given to each gull; no worms were recovered. Then attempts with 5 and 10 larvae given to each bird were made. The common gull has a very low take of *D. dendriticum* (Table I). In addition to this a great percentage of the gulls expelled their worms only a few days after the worms started to produce eggs (Table I).

The arctic fox, however, had a very high take both of *D. dendriticum* and *D. latum* (Table I).

The morphology of D. dendriticum *reared in common gull, herring gull, arctic fox and hamster*

General external morphology: The general appearance is given by Andersen (1971). Table II gives the morphometric measurements of *D. dendriticum* reared in the four above-mentioned hosts. There is no distinct difference in the morphometric measurements of the scolex between worms from these hosts, the variance within each host taken into consideration. Neither is there any difference in the neck length between worms from different hosts. However, the total length of worms developed in gulls, foxes or hamster differs a lot.

The longest worms were found in fox, then came those developed in gulls while the shortest worms were found in hamster. The number of segments anterior to primordia and gravid uterus and the total number of segments in each worm show the same trend as the total length (Table II). The longest worms had more segments anterior to primordia and gravid uterus and the total number of segments was higher.

The length of segments and its degree of variance is nearly the same in worms from hamster, fox or gull.

The greatest relative difference between

Table II. Morphometric measurements of *D. dendriticum* reared in hamster, fox and gull (one to three worm populations)

	60 worms from hamster	Average	9 worms from common gull	Average	2 worms from herring gull	4 worms from foxes	Average
Scolex length mm	1.33-2.08	1.63	1.42-2.08	1.79	1.78-1.80	1.25-1.83	1.58
Scolex dorso-ventral height mm	0.75-1.20	0.90	0.75-0.91	0.81	0.81-0.91	0.75-0.83	0.80
Neck length mm	1.00-4.20	2.23	1.91-3.75	2.45	2.20-2.51	1.00-3.33	1.61
Total length cm	23-72	43.6	42-74	57.8	72-72	106-148	120.0
Number of segments anterior to primordia	33-104	69.1	57-114	88.4	79-82	112-120	114.6
Number of segments anterior to gravid uterus	61-170	113.5	90-169	135.5	91-152	151-168	160.6
Total number of segments	164-497	294.6	203-379	307.3	285-399	384-611	483.0
Maximum width of the strobila mm	5.80-18.31	11.2	5.00-7.08	5.80	5.50-6.60	7.50-8.75	7.91
Length of segments mm	1.00-3.56	1.81	1.33-3.33	2.00	1.75-2.08	1.60-2.50	2.08

Table III. *D. dendriticum.* Morphometric measurements of a 7 worm population in gull compare[d] with two 8 worm populations and one 6 worm population in hamster and a 10 worm population i[n] fox (average)

	8 worm population from hamster	6 worm population from hamster	7 worm population from common gull	10 worm population from arctic fo[x]
Scolex length mm	0.88	0.95	0.88	0.88
Scolex dorsoventral height mm	1.46	1.47	1.95	1.65
Neck length mm	1.98	2.51	1.70	1.81
Total length cm	48.5	50.5	52.5	101.0
Maximum width of segments mm	6.78	8.12	3.74	6.33
Length of segments mm	0.96	0.97	1.50	2.88

worms developed in these four final hosts is, however, the maximum width of segments (Table II). The worms from hamster are nearly twice as broad as those from common gull, while the worms from arctic fox and herring gull lie somewhat in between.

Table III gives some morphometric measurements of a 'crowded' worm population from gull compared with 'crowded' populations from hamster and arctic fox. The same phenomena as described for 'crowded' *D. dendriticum* populations in hamster (Andersen 1972a) are found in gull and fox. The worms were narrower, and the length of segments varied while the total length of the worms seemed to be less affected, but with a relatively high percentage of the worms possessing primary strobilae (Andersen 1973).

Internal morphology: The internal morphology of adult *D. dendriticum* from hamster and its degree of variance is described by Andersen (1971 and 1972b). It was not possible to point out any host dependent difference in the internal morphology of worms developed in hamster, gulls or arctic fox.

The morphology of D. latum *reared in hamster and arctic fox*

General external morphology: The general appearance is given by Andersen (1971). Table IV gives the morphometric measurements of worms from hamster and fox.

There is no marked difference in scole[x] length or dorsoventral height nor in nec[k] length or length of segments.

The total length of the worms differs, th[e] worms developed in fox averaging 100 c[m] longer than those from hamster and possessin[g] a higher number of segments anterior t[o] primordia and gravid uterus. Further, a pronounced difference in the maximum widt[h] of segments was found (Table IV).

Two populations of five and seven worm[s] respectively of *D. latum* from hamster (Tabl[e] IV) were also studied. The same phenomen[a] as observed in 'crowded' populations of *D. dendriticum* from hamster, common gull an[d] arctic fox were registrated for *D. latum* a[s] well, and here for the first time the autho[r] observed primary strobilae of *D. latum* (Andersen 1973).

Internal morphology: The internal morph[ol]ogy of *D. latum* from hamster and its vari[]ance is described earlier (Andersen 197[1,] 1972b and 1973). No host dependent differ[]ence in the internal morphology betwee[n] worms developed in hamster and arctic f[ox] was noticeable.

The morphology of D. ditremum *develope[d] in hamster and merganser*

General external morphology. The gener[al] appearance has been given previously (A[n]dersen 1972a). Table VI presents the morph[]

metric measurements of worms from hamster and merganser. The morphometric measurements of the scolex and neck are relatively stable and host independent while as regards all the other external measurements there is a more or less pronounced host dependence and the individual variance is greater.

Internal morphology: No difference could be found between the morphology of worms from merganser and that of the same species described earlier from hamster (Andersen 1972a).

DISCUSSION

The systematics and the taxonomic characters of the genus *Diphyllobothrium* have been much disputed in previous years.

Vik (1964a, p. 6) states: 'Although the genus *Diphyllobothrium* Cobbold, 1858, has been revised several times (Lühe 1910, Cohn 1912, Stunkard 1949, Wardle and McLeod 1952 and Yamaguti 1959) since the type species was described by Cobbold in 1858, the systematics of the group are still in a state of confusion and identification is made difficult by discrepancies in original and subsequent descriptions.'

Stunkard (1965) points out that variation within the genus *Diphyllobothrium* results from difference among individuals in age and degree of maturity; from disparate physiological status owing to nutritional dissimilarities; from extent of crowding; from type and condition of the hosts; and other factors.

The description of adult *D. latum* given by Magath (1929), Wardle & McColl (1937) and Rausch & Hilliard (1970) disagrees due

Table IV. Morphometric measurement of *D. latum* reared in hamster and arctic fox

	20 worms from hamster (single worm infections)		12 worms from hamster (a 5 and a 7 worm population)		6 worms from fox (one to 3 worm populations)	
		Average		Average		Average
Scolex length mm	1.59-2.50	2.03	2.08-2.25	2.14	2.08-2.25	2.16
Scolex dorsoventral height mm	0.92-1.66	1.08	0.80-1.09	0.98	0.83-1.08	0.97
Neck length mm	4.1-30.0	12.7	8.3-10.0	9.5	10.0-20.5	10.2
Total length cm	61-138	91.6	52-98	82.5	91-260	190.9
Number of segments anterior to primordia	130-256	180.3	157-314	183.7	198-280	229.3
Number of segments anterior to gravid uterus	211-500	324.0	272-443	343.3	345-440	391.6
Total number of segments	385-651	495.8	412-607	480.3	588-760	689.3
Maximum width of the strobila mm	6.7-14.1	9.3	3.6-6.4	5.4	4.6—9.3	7.3
Length of segments mm	2.4- 5.0	3.03	2.5-5.0	3.90	2.5—4.2	3.33
No. of worms with primary strobilae	0		5		0	

Table V. Comparison of morphometric measurements for D. dendriticum given by Markowski (1949), Kuhlow (1953b), Vik (1957) and Bylund (1969)

	Markowski 1949	Kuhlow 1953b	Vik 1957	Bylund 1969	
Host or hosts	Different gull species and Pelecanus erythrorhynchus	Different gull species, cat, mouse and rat	Man, different gull species, magpie, gray heron, rat, fox and cat	Larus ridibundus	Man
Worms killed in	-	tap water	tap water	-	-
Scolex length mm	1.2-2.0	1.4-1.8	0.73-1.75	-	-
Scolex dorso-ventral height mm	0.6	0.6-1.0	0.41-0.77	0.7	0.6
Neck length mm	2-4	1.1-4.6	1.58	1.1	1.6
Maximum total length cm	42	109	40-220	38.6	61.0
Maximum width mm	6	3.2-8.6	2.0-9.7	4.1	5.3
Length of segments mm	2	-	-	-	-
Total number of segments	-	658	332-877	347	650

to the worms having been developed in different final hosts and different killing and fixing methods being used.

Magath (1929) gives a neck length of 8 mm and a total length of 100–200 cm in younger worms (worms with 'primary strobilae') while the neck length in 'old' *D. latum* varied and the total length ranged between 200–900 cm. Wardle & McColl (1937) reported that the neck region of 23 worms, developed in the same dog, varied in length between 1.4 and 2.6 mm while the mean strobilar length in superinfected dogs varied from 25.6 cm (32 days after initial infection) to 169 cm (98 days after initial infection). Rausch & Hilliard (1970) in their description of *D. latum* from man report a neck length of 8.4 to 14.0 mm and a maximum total length of 1140 cm.

Also for *D. dendriticum* and *D. ditremum*, divergencies may be pointed out (Bylund 1969, 1970 and 1973, Kuhlow 1953a, b, Markowski 1949 and Vik 1957). For *D. dendriticum* some morphometric measurements given by different authors are listed in Table V.

According to Vik (1964b), far too little is known about the variation and constancy of morphological features hitherto used in the taxonomy of *Diphyllobothrium*. A common trend within the taxonomy of this group has been that when maximum or average external morphometric measurements were given (Magath 1929, Markowski 1949, Kuhlow 1953a, b and others) measurements from individuals from different final hosts and worms from populations of different sizes were mixed together.

Such measurements, however, are unsuitable for species description and of little value as characters for identification.

Andersen (1971, 1972a and 1973) compared the morphology of adult *D. dendriticum*, *D. latum* and *D. ditremum* reared in golden hamster and found several morphological characters to be of taxonomic value for worms from this host. However, as the hamster is an experimental or a pure laboratory host, the present comparison includes worms reared in natural hosts.

Table VI gives the average measurements of *D. dendriticum, D. latum* and *D. ditremum* developed in different hosts. The measurements of the scolex and neck of all three species, seem to be relatively stable and host independent. The segment length appears to be host independent, too, but varies within the same host (Andersen 1973) and with worm population size (Andersen 1972b and Table III).

For the rest of the measurements given in Table VI there is a parallel change in worms of the same species reared in different hosts. The difference between *D. dendriticum, D. latum* and *D. ditremum* developed in the same kind of final host does, however, persist.

In nature the overlapping between the final hosts of *D. ditremum* and the two other species is limited (except for merganser, which was once found to be infected with *D. dendriticum). D. latum* and *D. dendriticum,* however, have a lot of mammalian hosts in common (Kuhlow 1953a, Vik 1957).

The present results indicate that besides the general appearance (Andersen 1972b, Fig. 6) the neck length is the best external morphological criterion for separating *D. dendriticum, D. latum* and *D. ditremum.* There is a distinct difference in neck length between these species. The neck length is relatively stable and host independent (Magath 1929, Kuhlow 1953a, b, Vik 1959, Bylund 1969 and 1973, Rausch & Hilliard 1970 and the present results given in Tables II, IV and VI).

The short neck length given for *D. latum* by Wardle & McColl (1937) may be caused by the method of killing, resulting in a special state of contraction of the worms. The mea-

Table VI. Comparison of morphometric measurements of D. dendriticum and D. latum from hamster and from arctic fox and D. ditremum from hamster and merganser (one to three worm populations) (average)

	D. dendriticum from hamster	D. latum from hamster	D. ditremum from hamster	D. dendriticum from fox	D. latum from fox	D. ditremum from merganser
Scolex length mm	1.63	2.03	1.75	1.58	2.16	1.80
Scolex dorso-ventral height mm	0.90	1.08	1.15	0.80	0.97	1.12
Neck length mm	2.23	12.7	0.24	1.61	10.2	0.50
Total length cm	43.6	91.6	23.0	128.0	190.9	33.1
Number of segments anterior to primordia	69.1	180.3	43.8	114.6	229.3	65.4
Number of segments anterior to gravid uterus	113.5	324.0	67.1	160.6	291.6	90.1
Total number of segments	294.6	495.8	167.0	483.0	689.3	261.1
Maximum width of the strobila mm	11.2	9.3	4.5	7.9	7.3	3.9
Length of segments mm	1.81	3.03	1.47	2.08	3.33	1.41

surements of the scoleces are also quite stable but of little use as identification characters because they are too similar.

Other external measurements such as total length, maximum width, segment length or total number of segments may not be used to separate these species unless the final host or hosts and the worm population size are known and taken into consideration.

The internal morphology of mature segments in *D. dendriticum, D. latum* and *D. ditremum* does not show any host or 'crowding' dependent variance (Andersen 1971, 1972a, b, and 1973).

The best morphological characters of reliable diagnostic value to separate relaxed adult worms of these species are therefore as the following:

1. Neck length.
(*D. dendriticum* averaging 2 mm, *D. latum* 8 mm or more and *D. ditremum* 0.75 mm or less.)
2. Type of boundary between neighbouring segment.
(In *D. dendriticum* and *D. ditremum* there is no distinct boundary between neighbouring segments; vitellaria and testes are confluent from one segment to another. In *D. latum* there is a constriction between the segments and often an area without testes and vitellaria.)
3. Position of the cirrus-sac in sagittal sections.
(In *D. dendriticum* and *D. ditremum* the cirrus-sac is situated obliquely while in *D. latum* it is situated horizontally.)
4. Position of seminal vesicle in relation to the cirrus-sac.
(In *D. dendriticum* the seminal vesicle is situated dorsally to the cirrus-sac and cannot be seen from the ventral side (Andersen 1971, Fig. 16).
In *D. latum* (Magath 1929, Figs. 16 and 17) and in *D. ditremum* (Andersen 1972a, Fig. 2) the seminal vesicle is situated dorsally and caudally to the cirrus-sac and is usually seen also from the ventral side.)
5. Shape of ovary.
(In *D. dendriticum* and *D. latum* the ovary varies somewhat in shape (Andersen 1971, 1972a and 1973) but with no anterior horns seen.
In *D. ditremum* the ovary is always 'butterfly shaped' with distinct anterior horns (Andersen 1972a).)

As supporting characters and when the final host and the worm population size are taken into consideration, segment width and length, total length and total number of segments may be used.

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