Studies on Gill Trematodes from Oklahoma Fishes

AARON SEAMSTER

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STUDIES ON GILL TREMATODES FROM OKLAHOMA FISHES

BY

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BACHELOR OF SCIENCE OKLAHOMA AGRICULTURAL & MECHANICAL COLLEGE 1937

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Introduction

This investigation which deals with ectoparasitic flukes from Oklahoma fishes was begun in October, 1937. Problems involving technical laboratory procedures requisite for permanent mounts, the validity of the genus *Haplocleidus* Mueller, 1937, control methods, and new distributional records of hosts are considered.

Host specimens, which constitute one hundred thirty-seven fish belonging to the families Centrarchidae and Ameiuridae, were collected from streams and ponds in the vicinity of Stillwater, Oklahoma. After identification the hosts were either preserved by freezing or kept in laboratory aquaria pending experimentation. Parasites were collected and preserved according to the method described by Mizelle (1938b).

In order to determine combinations of fixatives and stains for optimal structural differentiation, five fixatives and five stains were employed. Twenty-five preparations were treated with each fixative and twenty-five (five to each fixer) with each stain.

The genus *Haplocleidus* Mueller, 1937, was split off the genus *Onchocleidus* Mueller, 1936, to include forms which exhibit a marked discrepancy in size of the dorsal and ventral anchors. This genus is considered invalid since a gradation of anchor lengths ranging from those characteristic of the genus *Cleidodiscus* Mueller, 1936, to those of *Haplocleidus* Mueller, 1937, has been observed in *Cleidodiscus pricei* Mueller, 1936.

Experiments on control measures employing chlorine, advocated by Laird (1927) and Laird and Embody (1931) (by use of Zonite), were performed in laboratory aquaria. Chlorinated water prepared by the local water plant was found reliable because of its constant chlorine content and availability.

This work is the first of its kind to be undertaken in the state of Oklahoma. Many new distribution records are reported. A preliminary report of the investigation was presented in December, 1937, at a meeting of the Oklahoma Academy of Science (Seamster, 1938).

Nomenclature for structural parts is used as proposed by Price (1934), Mueller (1936), and Mizelle (1936).

The author wishes to express his thanks and appreciation to Dr. John D. Mizelle of the Department of Zoology, Oklahoma Agricultural and Mechanical College, for his advice and direction of the problem.

* A contribution from the Zoological Laboratory of the Oklahoma Agricultural and Mechanical College.

Systematics

Johnston and Tiegs (1922) in Australia first gave comprehensive consideration to ectoparasitic flukes of the superfamily Gyrodactyloidea. Specific taxonomic work on this group of parasites in North America began with the description of *Gyrodactylus fairporti* Van Cleave, 1921, from *Cyprinus carpio* and *Ameiurus melas*. Later, Van Cleave and Mueller (1932) described *Ancyrocephalus aculeatus* from *Stizostedion vitreum* and Mueller and Van Cleave (1932) described *Gyrodactylus cylindriformis* from *Umbra limi* and *Dactylogyrus extensus* from *Cyprinus carpio*. Mueller (1934, 1936a, 1936b, 1937, 1938) described nine new genera, namely, *Cleidodiscus, Urocleidus, Onchocleidus, Leptocleidus, Tetracleidus, Aristocleidus, Haplocleidus, Pterocleidus* and *Actinocleidus* and many new species belonging to these and other genera of the Dactylogyridae and Gyrodactylidae. In 1934 Mueller assigned *Ancyrocephalus aculeatus* to the genus *Urocleidus* and in 1936 he restricted *Ancyrocephalus* to permanently include marine species and retained it provisionally for fresh-water forms of uncertain generic standing. Mizelle (1936, 1937, 1938a, 1938b) described several new species of Dactylogyrinae and Tetraonchinae from Illinois fishes. Price (1937) described *Gyrodactylus gurleyi* from goldfish ("Japanese fan-tail") and reviewed the superfamily Gyrodactyloidea. Mizelle and Hughes (1938) reduced the nine Muellerian genera to the following: *Cleidodiscus, Urocleidus,* and *Actinocleidus*. This paper deals mainly with North American fresh-water Tetraonchinae.

Previous to 1933 the Tetraonchinae were included in the family Gyrodactylidae Cobbold, 1877. Bychowsky (1933) created the family Dactylogyridae which now includes the following sub-families: Dactylogyrinae Bychowsky, 1933; Tetraonchinae Monticelli, 1903; Diplectaninae Monticelli, 1903; and Bothitrematinae Price, 1936. The following is a classification of the North American fresh-water Tetraonchinae with a list of species.

Phylum.—PLATYHELMINTHES
Class.—TREMATODA
Order.—MONOGENEA
Suborder.—MONOPISTHOCOTYLEA
Superfamily.—GYRODACTYLOIDEA
Family.—DACTYLOGYRIDAE
Subfamily.—TETRAONCHINAE

Genus.—*Tetraonchus* (Wagener, 1857) Diesing, 1858.

Species.—1. *Tetraonchus monenteron* Wagener, 1857. Syn. *Dactylogyrus monenteron* Wagener, 1857; *Gyrodactylus cochlea* Wedl, 1857; and *Monocoelium monenteron* (Wagener, 1857) Wegener, 1910.

2. *Tetraonchus alaskensis* Price, 1937.

Genus.—*Murraytrema* Price, 1937.

Species.—1. *Murraytrema copulata* Mueller, 1938.

Genus.—*Cleidodiscus* Mueller, 1934. (*Leptocleidus* Mueller, 1936, in part)

Species.—1. *Cleidodiscus robustus* Mueller, 1934. Syn. *Cleidodiscus incisor* Mizelle, 1936.

2. *Cleidodiscus megalonchus* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Leptocleidus megalonchus* Mueller, 1936.
3. *Cleidodiscus capax* Mizelle, 1936.
4. *Cleidodiscus longus* Mizelle, 1936.
5. *Cleidodiscus uniformis* Mizelle, 1936.
6. *Cleidodiscus vancleavei* Mizelle, 1936. Syn. *Onchocleidus formosus* Mueller, 1936, and *Cleidodiscus formosus* (Mueller, 1936) Price, 1937.
7. *Cleidodiscus bedardi* Mizelle, 1936.
8. *Cleidodiscus floridanus* Mueller, 1936.
9. *Cleidodiscus pricei* Mueller, 1936.
10. *Cleidodiscus nematocirrus* Mueller, 1937.
11. *Cleidodiscus mirabilis* Mueller, 1937.
12. *Cleidodiscus stentor* Mueller, 1937.
13. *Cleidodiscus brachus* Mueller, 1938.
14. *Cleidodiscus alatus* Mueller, 1938.
15. *Cleidodiscus diversus* Mizelle, 1938.

Genus.—*Urocleidus* Mueller, 1934. Syn. *Onchocleidus* Mueller, 1936, in part; *Tetracleidus* Mueller, 1936, in part; *Aristocleidus* Mueller, 1936, in part; *Haplocleidus* Mueller, 1937, in part; *Pterocleidus* Mueller, 1937, in part.

Species.—1. *Urocleidus aculeatus* (Van Cleave and Mueller, 1932) Mueller, 1934. Syn. *Ancyrocephalus aculeatus* Van Cleave and Mueller, 1932.

2. *Urocleidus ferox* Mueller, 1934. Syn. *Onchocleidus ferox* (Mueller, 1934) Mueller, 1936.
3. *Urocleidus angularis* Mueller, 1934. Syn. *Ancyrocephalus angularis* (Mueller, 1934) Mueller, 1936.
4. *Urocleidus adspectus* Mueller, 1936.
5. *Urocleidus similis* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus similis* Mueller, 1936.
6. *Urocleidus mimus* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus mimus* Mueller, 1936.
7. *Urocleidus helicis* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus helicis* Mueller, 1936.
8. *Urocleidus acer* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus acer* Mueller, 1936, and *Pterocleidus acer* (Mueller, 1936) Mueller, 1937.
9. *Urocleidus dispar* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus dispar* Mueller, 1936, and *Haplocleidus dispar* (Mueller, 1936) Mueller, 1937.
10. *Urocleidus banghami* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Tetracleidus banghami* Mueller, 1936.
11. *Urocleidus principalis* (Mizelle, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus principalis* Mizelle, 1936, and *Onchocleidus contortus* Mueller, 1937.
12. *Urocleidus interruptus* (Mizelle, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus interruptus* Mizelle, 1936.
13. *Urocleidus mucronatus* (Mizelle, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus mucronatus* Mizelle, 1936.
14. *Urocleidus acuminatus* (Mizelle, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus acuminatus* Mizelle, 1936, and *Pterocleidus acuminatus* (Mizelle, 1936) Mueller, 1937.

15. *Urocleidus distinctus* (Mizelle, 1936) Mizelle and Hughes, 1938. Syn. *Onchocleidus distinctus* Mizelle, 1936.
16. *Urocleidus hastatus* (Mueller, 1936) Mizelle and Hughes, 1938. Syn. *Aristocleidus hastatus* Mueller, 1936.
17. *Urocleidus perdix* (Mueller, 1937) Mizelle and Hughes, 1938. Syn. *Onchocleidus perdix* Mueller, 1937.
18. *Urocleidus spiralis* (Mueller, 1937) Mizelle and Hughes, 1938. Syn. *Onchocleidus spiralis* Mueller, 1937.
19. *Urocleidus affinis* (Mueller, 1937) Mizelle and Hughes, 1938. Syn. *Haplocleidus affinis* Mueller, 1937.
20. *Urocleidus furcatus* (Mueller, 1937) Mizelle and Hughes, 1938. Syn. *Haplocleidus furcatus* Mueller,
21. *Urocleidus biramosus* (Mueller, 1937) Mizelle and Hughes, 1938. Syn. *Pterocleidus biramosus* Mueller, 1937.
22. *Urocleidus malleus* (Mueller, 1938) Mizelle and Hughes, 1938. Syn. *Cleidodiscus malleus* Mueller, 1938.
23. *Urocleidus chautauquaensis* (Mueller, 1938) Mizelle and Hughes, 1938. Syn. *Tetracleidus chautauquaensis* Mueller, 1938; and *Cleidodiscus chautauquaensis* (Mueller, 1938) Mueller, 1938.
24. *Urocleidus umbraensis* Mizelle, 1938.
25. *Urocleidus cyanellus* (Mizelle, 1938) Mizelle and Hughes, 1938. Syn. *Onchocleidus cyanellus* Mizelle, 1938.

Genus.—*Actinocleidus* Mueller, 1937.

Species.—1. *Actinocleidus fusiformis* (Mueller, 1934) Mueller, 1937. Syn. *Cleidodiscus fusiformis* Mueller, 1934.
2. *Actinocleidus oculatus* (Mueller, 1934) Mueller, 1937. Syn. *Cleidodiscus oculatus* Mueller, 1934.
3. *Actinocleidus bursatus* (Mueller, 1936) Mueller, 1937. Syn. *Ancyrocephalus bursatus* Mueller, 1936.
4. *Actinocleidus articularis* (Mizelle, 1936) Mueller, 1937. Syn. *Cleidodiscuss articularis* Mizelle, 1936.
5. *Actinocleidus gracilis* Mueller, 1937.
6. *Actinocleidus maculatus* Mueller, 1937.
6. *Actinocleidus maculatus* Mueller, 1937.
7. *Actinocleidus triangularis* Summers, 1937.
8. *Actinocleidus longus* Mizelle, 1938.
9. *Actinocleidus fergusoni* Mizelle, 1938.

Technical Procedure

Little success in preparing differentially stained mounts of Tetraonchinae has been accomplished. Mizelle (1937) experienced difficulty in obtaining a good differential stain and recommended unstained mounts for accurate study.

In order to discover an optimal fixer-stain combination for these forms a comparative study of fixing and staining procedures was attempted. Five fixers, Gilson's, formalin (ten percent), alcohol (seventy percent), Karpenchenko's, and Bouin's; and five stains, Harris' haemotoxylin, Delafield's haemotoxylin, Heidenhain's haemotoxylin, picrocarmine, and borax carmine were employed.

In order to insure relatively uniform results, preparations of *Cleidodiscus pricei* Mueller, 1936, were used throughout the experiment.

It was observed that the formalin-Heidenhain's haemotoxylin combination produced the best results and that the Bouin's-Harris' haemotoxylin yielded the poorest. The head organs were differentiated by all the stains. Although the ovary was best differentiated by Heidenhain's haemotoxylin, the testis failed to differentiate plainly. Haptoral and copulatory complex parts were usually obscured by the stained surrounding tissues. Karpenchenko's and alcohol (seventy percent) gave the least desirable results as fixers.

It is apparent that unstained preparations are best for study since the essential structures (anchors, hooks, bars, cirrus, and accessory piece), for classification, are obscured by staining.

Validity of the Genus *Haplocleidus* Mueller, 1937

The genus *Onchocleidus* Mueller, 1936, was created to include Tetraonchinae with the following characteristics: "Intestine bifurcate, confluent posteriorly. Ovary anterior to testes, near center of body. Vagina present, on right; small seminal receptacle present. Cirrus a chitinous slender tube, at times with spiral fins, at times coiling. Accessory piece generally absent. Vitellaria from pharynx to posterior end of intestine. Haptor wedge-shaped. Four anchors may or may not be of equal size, with short roots, and long shafts and points. Fourteen marginal hooks. Two supporting bars, not joined, approximately similar in size and shape. . . .

"This genus is distinguished from *Cleidodiscus* and *Urocleidus* in lacking the accessory piece for the cirrus. It is distinguished from the latter in the presence of a vagina, and from the former in having the vagina on the right"* *Onchocleidus* is distinguished from *Leptocleidus* Mueller, 1936, by the presence of a relatively simple cirrus, from *Tetracleidus* Mueller, 1936, by the absence of an accessory piece in the copulatory complex, and from *Aristocleidus* Mueller, 1936, by the possession of dorsal and ventral anchors of similar size and shape. Original members of these genera (except *Aristocleidus*) possess anchors of nearly equal size except *Onchocleidus dispar* Mueller, 1936. In 1937 Mueller named the genus *Haplocleidus* to include *Onchocleidus*-like forms which possess a marked difference in size of dorsal and ventral anchors (dorsals larger); *Pterocleidus* to apply to *Onchocleidus*-like species which have a spur-like projection on each anchor shaft; and *Actinocleidus* to include *Cleidodiscus*-like species with all four anchors on the same side of the haptor and articulate or fused bars. Mizelle and Hughes (1938) recognized the sporadic absence of vaginae in several genera besides *Urocleidus*, the insignificance of spines and spurs on haptoral parts, the presence of an accessory piece in the copulatory complex of nearly all species of *Onchocleidus*, a size and shape discrepancy of the two pairs of anchors in the later described species belonging to genera other than *Haplocleidus* and reduced Mueller's nine genera to three, namely, *Cleidodiscus*, *Urocleidus*, and *Actinocleidus*. Support for suppressing the genus *Haplocleidus* was partially obtained from data presented in this paper.

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* Mueller, 1936a.

In addition to the previously recorded *Cleidodiscus pricei* Mueller, 1936, from *Ameirus melas* there was found a *Haplocleidus* form, on this host, with a copulatory complex identical with that of *Cleidodiscus pricei* (Figs. 4 and 5). Examination of a large number of specimens revealed an almost complete gradation of anchor sizes between those of *Cleidodiscus pricei* and the *Haplocleidus*-like form. The cirrus measurements for the two forms were nearly the same (Chart). This difference in size of the two pairs of anchors is interpreted to be a normal variation in this species and lends support to the invalidity of the genus *Haplocleidus* as contended by Mizelle and Hughes (1938).

CHART I.

Structural Variation in *Cleidodiscus pricei* Mueller, 1936.

Specimen	*Dorsal Anchors*	*Ventral Anchors*	*Cirrus*	
1	0.038 mm.	0.032 mm.	0.028 mm.	
2	0.038 mm.	0.032 mm.	0.027 mm.	
3	0.038 mm.	0.033 mm.	0.025 mm.	
4	0.038 mm.	0.033 mm.	0.028 mm.	
5	0.040 mm.	0.033 mm.	0.028 mm.	
6	0.040 mm.	0.033 mm.	0.027 mm.	
7	0.042 mm.	0.033 mm.	0.028 mm.	
8	0.042 mm.	0.033 mm.	0.028 mm.	
9	0.042 mm.	0.033 mm.	0.028 mm.	
10	0.042 mm.	0.033 mm.	0.028 mm.	
11	0.040 mm.	0.035 mm.	0.028 mm.	
12	0.042 mm.	0.037 mm.	0.025 mm.	
13	0.042 mm.	0.037 mm.	0.032 mm.	
14	0.042 mm.	0.038 mm.	0.025 mm.	
15	0.043 mm.	0.038 mm.	0.028 mm.	Av. 0.275 mm.
16	0.049 mm.	0.041 mm.	0.025 mm.	
17	0.051 mm.	0.041 mm.	0.033 mm.	
18	0.053 mm.	0.041 mm.	0.033 mm.	
19	0.054 mm.	0.041 mm.	0.033 mm.	
20	0.054 mm.	0.041 mm.	0.030 mm.	
21	0.056 mm.	0.041 mm.	0.025 mm.	
22	0.058 mm.	0.041 mm.	0.026 mm.	
23	0.058 mm.	0.041 mm.	0.029 mm.	
24	0.058 mm.	0.043 mm.	0.033 mm.	
25	0.058 mm.	0.043 mm.	0.026 mm.	
27	0.056 mm.	0.044 mm.	0.031 mm.	
28	0.056 mm.	0.044 mm.	0.031 mm.	
29	0.054 mm.	0.046 mm.	0.028 mm.	Av. .0297 mm.

Numbers 1-15 inclusive represent typical *Cleidodiscus* forms; numbers 16-29 represent *Haplocleidus*-like forms.

CONTROL EXPERIMENTS

During the early part of the investigation it was observed that fishes kept in laboratory aquaria for more than twenty-four hours prior to examination were negative for gill parasites. Chlorine effects were suspected since this element was known to be present in the local water supply and had been used previously by Laird (1927) and Laird and Embody (1931) (by use ofZonite) to destroy Gyrodactyloidea. The water was tested, using the orthotolodine method, and found to contain 0.1 ppm. of free chlorine.

In order to rule out possible detrimental effects to Gyrodactyloidea by materials in solution, other than chlorine, the following experiment was performed. One fish *(A. melas)* infested with *Cleidodiscus pricei* Mueller, 1936, was placed in chlorine-free distilled water, one in chlorinated distilled water, one in chlorine-free tap water, and one in chlorinated tap water. Chlorine-free distilled water proved more toxic to *C. pricei* than chlorinated distilled water, and the latter was more toxic than chlorinated tap water. Dechlorinated tap water had no effect on this species of parasite. Determination of possible complimentary effects of dissolved salts and chlorine became complicated since the chlorine-free distilled water was more toxic to *C. pricei* than was chlorinated distilled water. Due to lack of time and equipment, determinations of this nature were not attempted. Further experiments were devised to test the interval of exposure necessary to kill Gyrodactyloidea by use of chlorinated tap water.

In each of the following experiments 0.1 ppm. of chlorine in tap water was used as a vermicide. Controls showed that parasites and hosts were unaffected by chlorine free tap water and that that hosts were unaffected by chlorinated tap water. Bullheads and crappies were kept in chlorinated water for periods of two weeks without evident harm. Brownian movement within parasites was used as a criterion of death.

Two bullheads *(A. melas)* infested with *C. pricei* were placed in chlorine solution. At the end of the first hour all parasites on one host were alive. At the end of the second hour eighty-eight percent of the parasites on the second host were dead. Four additional experiments, using the same experimental set up, showed the minimal lethal time of exposure for *C. pricei* to vary from three to six hours. Parasites that died during the shorter intervals were taken from a local pond. Fish harboring parasites that required upper ranges of exposure for lethal effect were taken from a slowly-moving stream (Boomer Creek).

Two black crappies *(Pomoxis sparoides)* infested with *Cleidodiscus vancleavei* Mizelle, 1936, were placed in an aquarium containing chlorinated water. Microscopic examinations were made on each fish at alternate hours. Six, and six and one-half hours, respectively, were required to kill all the gill trematodes present on the two hosts.

There remains much work to be done on control measures utilizing chlorine as a vermicide. Experiments show that dissolved chlorine in small amounts (0.1 ppm.) is lethal to Gyrodactyloidea, but that the time required to kill parasites is variable. The author realizes that these experiments are preliminary in scope but believes them suitable as a basis for further study.

Gyrodactyloid Host Records From Oklahoma

Host: *Helioperca macrochira* (Rafinesque)
Urocleidus mucronatus (Mizelle, 1936) Mizelle and Hughes, 1938.
Actinocleidus fergusoni Mizelle, 1938.
Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938.
Urocleidus acer (Mueller, 1936) Mizelle and Hughes, 1938.

Ictalurus punctatus (Rafinesque)
Cleidodiscus pricei Mueller, 1936.

Ameiurus melas (Rafinesque)
Cleidodiscus pricei Mueller, 1936.
Gyrodactylus elegans von Nordmann, 1832.

Apomotis cyanellus (Rafinesque)
Actinocleidus longus Mizelle, 1938.
Cleidodiscus diversus Mizelle, 1938.
Urocleidus cyanellus (Mizelle, 1938) Mizelle and Hughes, 1938.

Pomoxis annularis (Rafinesque)
Cleidodiscus vancleavei Mizelle, 1936.
Cleidodiscus longus Mizelle, 1936.

Pomoxis sparoides Lacépède
Cleidodiscus vancleavei Mizelle, 1936.

Micropterus dolomieu Lacépède
Urocleidus principalis (Mizelle, 1936) Mizelle and Hughes, 1938.

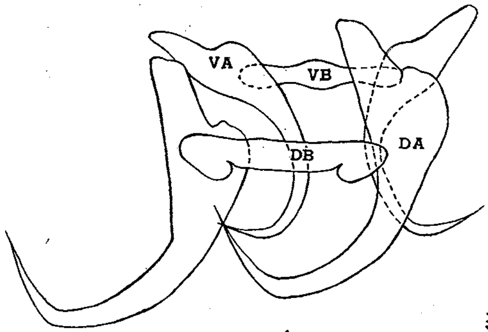
Aplites salmoides (Lacépède)
Actinocleidus fusiformis (Mueller, 1934) Mueller, 1937.

Allotis humilis (Girard)
Urocleidus mucronatus (Mizelle, 1936) Mizelle and Hughes, 1938.
Urocleidus dispar (Mueller, 1936) Mizelle and Hughes, 1938.
Actinocleidus fergusoni Mizelle, 1938.

PLATE 1

Comparative figures of *Cleidodiscus pricei* Mueller, 1936. Figs. 2, 5, and 7 are of typical forms. Figs. 1, 3, 4, and 6 are of the *Haplocleidus* type which show increased size of dorsal anchors. Original anchor size described for this species (Mueller, 1936b) is 0.058 mm. This measurement is the maximum dorsal anchor size recorded in this investigation. For further variation in haptoral and copulatory structures see Mueller, 1936b, plate 57, figs. 12-15.

D.B.—Dorsal Bar
V.B.—Ventral Bar
D.A.—Dorsal Anchor
V.A.—Ventral Anchor
A.P.—Accessory Piece
C. —Cirrus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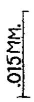
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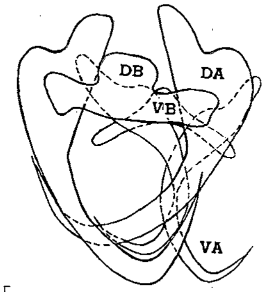
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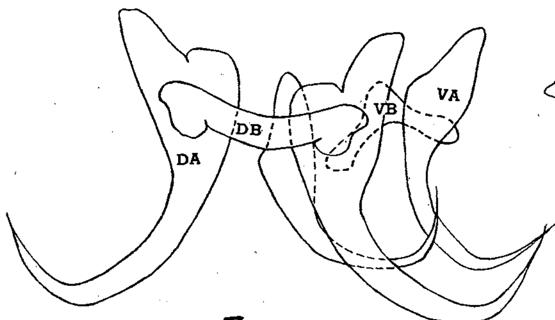
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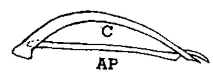
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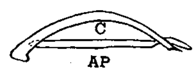
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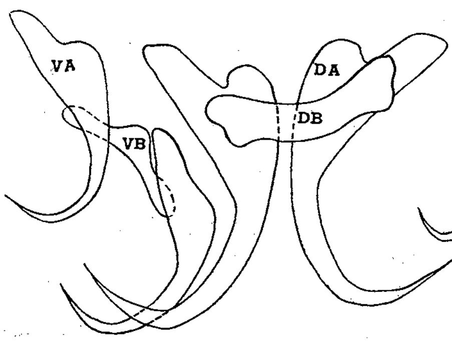
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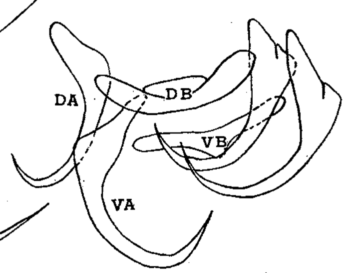
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